PULMONARY HEMODYNAMICS IN ACUTE PNEUMONIC PASTEURELLOSIS IN NEONATAL CALVES

By

Shalal A. Alnoor

A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Physiology

1986

ABSTRACT

PULMONARY HEMODYNAMICS IN ACUTE PNEUMONIC PASTEURELLOSIS OF NEONATAL CALVES

By

Shalal Alnoor

I tested the hypothesis that acute pneumonia induced by Pasteurella haemolytica causes a decrease in pulmonary vascular resistance and that pneumonic hypoxemia is due to the regional vasodilation and increased pulmonary blood flow in the pneumonic lung regions. I studied the effects of acute pneumonic pasteurellosis on pulmonary and systemic vascular parameters, gas exchange and the hemodynamic responses to hypoxia. Pneumonia was induced in treated calves by intratracheal inoculation of $2X10^9$ P. haemolytica bacteria suspended in bovine fetal serum (BFS). Control animals were inoculated with BFS. Acute pneumonia caused arterial hypoxemia accompanied by a decrease in pulmonary arterial pressure which was attributable to the decrease in pulmonary vascular resistance (PVR). Three hours after inoculation and after inhalation of 10% oxygen in nitrogen, PVR increased in the control animals. In the treated group, hypoxia did not increase PVR. These data indicate that the hypoxic vasoconstrictor mechanism is attenuated in acute pneumonic pasteurellosis.

Secondly, I investigated the regional distribution of pulmonary blood flow in local pneumonia induced by P. haemolytica. Regional pneumonia was induced in neonatal calves by local inoculation of P. haemolytica suspended in BFS. The control group received BFS only. Regional distribution of pulmonary perfusion was measured with (microspheres) before (baseline), 1.5 and 3 hours after inoculation. Acute local pneumonia resulted in arterial hypoxemia and increased pulmonary perfusion in the pneumonic lung regions. The increase in regional blood flow was due to the decrease in regional pulmonary vascular resistance (rPVR). There was no significant changes in pulmonary perfusion or rPVR in the normal lung regions. This indicates that the decrease in PVR is due to local vasodilation of the diseased lung regions.

Finally, I examined the role of arachidonic acid (AA) metabolites in the regional pulmonary vasodilation induced by P. haemolytica using the AA cascade blocker 5,8,11,14-eicosatetraynoic acid (ETYA). By increasing rPVR, ETYA prevented the increase in regional blood flow which results from local inoculation of P. haemolytica. I concluded that 1) arachidonic metabolites released in the pneumonic lung mediate pulmonary vasodilation and increased blood flow, and 2) hypoxic vasoconstriction is overcome by the vasodilator agents and becomes ineffective in directing blood flow away from the pneumonic lung regions, resulting in arterial hypoxemia.

To the memory of my brother Yousif

ACKNOWLEDGMENTS

I wish to express my deep appreciation to my major advisor, Dr. N. Edward Robinson, for his advice, support and continual encouragement during my graduate training at Michigan State University.

I also wish to extend my gratitude to my committee members Dr. John Chimosky, Dr. Frederik Derksen, Dr. Ronald Slocombe, and Dr. William Spielman, for their academic guidance and support.

To Dr. Basim Toma, my friend whom I could always depend on for encouragement and personal advice, I extend a special thanks. I wish also to thank my teacher and friend at Baghdad University, Iraq, Dr. A.W.R. Mehdi, for his help and continual support.

I wish to thank Roberta Milar for her technical assistance, as well as other members of the pulmonary laboratory, Dr. Richard Broadstone, Dr. Cheryl Killingsworth, Dr. Jacqueline Scott, Cathy Berney, Stephanie Yang, Heidi Immegart, and Elizabeth Rozanski.

I would like to thank Eileen Salmond for the typing of this dissertation.

I would like to thank Dr. Gregory Fink for helping me to use the mixed ANOVA program and Dr. Mark Gorman for teaching me to use the three spheres program.

I appreciate the opportunity to complete my doctorate studies at Michigan State University and wish to thank the Iraqi government and the University of Baghdad for making this possible.

Finally, I would like to thank my family, my wife, Aida and my daughters, Zainab and Zeena, for their patience and support.

TABLE OF CONTENTS

TABLE OF CONTENTS--continued

TABLE OF CONTENTS--continued

LIST OF TABLES

LIST OF FIGURES

LIST OF FIGURES (continued)

LIST OF FIGURES (continued)

CHAPTER 1.

INTRODUCTION

The balance between pulmonary blood flow and alveolar ventilation in the lung must be efficient and rapidly adaptable in order to meet the various long-term and sudden changes in gas exchange. In particular, an adequate blood flow to a ventilated lung region is of considerable importance.

Pneumonia is associated with severe arterial hypoxemia but the mechanism of hypoxemia remains incompletely understood. (Sadie 1919; Colp et al. 1962; Alexander et al. 1963; Slocombe et al. 1983a). Important changes in the ventilation-perfusion relationships take place during various stages of pneumonia. Studies on different species of experimental animals and on human beings show persistence of, or increase in, blood flow through right-to-left shunts in acute pneumococcal pneumonia (Alexander et al. 1963; Goldzimer et al. 1973; Wagner et al. 1975; Light et al. 1981). However, other investigators of both human and animal models of pneumonia have demonstrated a decrease in perfusion to consolidated lung and preservation of the hypoxic vasoconstrictor mechanism in pneumococcal pneumonia. The latter serves to decrease blood flow to the diseased lung region and, thus assists to maintain normal arterial oxygen tension (Goldzimer et al. 1974; Hiser et al. 1975; Kyellman 1976).

Acute pneumonia induced by P. haemolytica is associated with alterations in pulmonary mechanics and gas exchange (Slocombe et al. 1984b). Recent investigation has shown that neutropenia prevents the

arterial hypoxemia and the typical pneumonic lesions of pasteurellosis (Slocombe et al. 1985). It is also known that neutrophils, when activated, produce lysosomal enzymes and oxygen radicals and can generate metabolites derived from arachidonic acid. Several investigators have stressed the importance of neutrophils in acute lung inflammation and proposed that the changes in pulmonary perfusion in pneumonia could be due to vasoactive substances, particularly prostaglandins, released by the neutrophils or by the inflammatory process initiated by bacteria. The mechanisms of acute lung injury and the effect of neutrophils and their products on pulmonary hemodynamics in pneumonia will be considered in further detail in the Review of Literature.

The effects of acute pneumonia induced by Pasteurella haemolytica on pulmonary hemodynamics and the distribution of pulmonary blood flow, and the role of arachidonic acid metabolites in pulmonary perfusion have not been investigated. This study was designed to investigate the effect of acute pneumonic pasteurellosis on the pulmonary hemodynamics and regional distribution of pulmonary blood flow and to determine the influence of cyclooxygenase and lipoxygenase blockade with 5,8,11,14-eicosatetraynoic acid (ETYA) on pulmonary perfusion in neonatal calves. The dissertation includes three main studies (reported in Chapters 3, 4, and 5), in which the pulmonary hemodynamics were assessed after intratracheal inoculation of P. haemolytica (Chapter 3), pulmonary hemodynamics and regional distribution of blood flow to the diseased and healthy lung regions were measured (Chapter 4) and the effect of ETYA on the pulmonary and regional hemodynamics was assessed after local inoculation of P.

haemolytica (Chapter 5). Each study has its own introduction and methods, a results section and a discussion of the specific findings. Chapter 6 is a general discussion of the three studies which includes a recapitulation and summary of the major findings and their interpretation.

CHAPTER 2.

REVIEW OF LITERATURE

The Pulmonary Circulation in the Normal Lungs

One of the major exceptions to the parallel arrangement of the vasculature of the body is the pulmonary circulation. The entire cardiac output flows from the right heart through the pulmonary circulation. Within a few days after birth, the pulmonary circulation changes from a high pressure, high resistance system to the low pressure, low resistance vascular bed which persists throughout life (Assali et al. 1962). The lung also receives a bronchial circulation which provides nutritional blood flow to the bronchi and visceral pleura. The bronchial circulation is a branch of the systemic circulation and receives a small proportion of cardiac output (1% to 2%). It is therefore a high pressure, high resistance vascular bed (McLaughlin et al. 1961).

The major function of the lung is to provide a surface for exchange of gases between blood and alveolar gas. To accomplish this, blood passes through low resistance capillaries in which every red blood cell can be exposed to an alveolar wall and through it, alveolar gas. The anatomy of the pulmonary circulation is well suited for this purpose because it consists of inflow and outflow vessels, extra-alveolar vessels that supply and drain an extensive capillary bed, the alveolar vessels (West 1977).

The pulmonary circulation has additional functions beside gas exchange (Heinemann and Fishman 1969) such as modification of the pharmacologic properties of a variety of circulating substances by

biochemical transformation. It also acts as an endocrine organ due to the continuous synthesis and release of prostaglandins, particularly prostacyclin from the vascular endothelium (Gryglewski et al. 1978).

Pulmonary perfusion is not uniform throughout the lung (West 1964). Because of gravitational forces, the vascular transmural pressures are greater in the ventral than in the dorsal regions of lung. The pressure on the outside of the alveolar capillaries is that of the alveolar air; on the average, atmospheric pressure. The alveolar capillary transmural pressure is the difference between intravascular pressure and intra-alveolar pressure. In the normal condition, pulmonary capillary pressure is quite low and many capillaries are unperfused during at least part of expiration (West 1975). Because intravascular pressure increases down the lung, distension of the vessels in the ventral regions of lung by the higher transmural pressure lowers the resistance of vessels in this area and keeps them open during expiration. Thus, in resting mammals, blood flow through the ventral part of the lung is much higher than blood flow through dorsal areas.

The transmural pressure of extra-alveolar vessels is the difference between intravascular pressure and pressure in the perivascular space. The perivascular pressure of the extra-alveolar vessel is slightly subatmospheric and decreases during inspiration (Goshy et al. 1979).

The pulmonary vascular bed is innervated by both sympathetic and parasympathetic nerve fibers (Dowing and Lee 1980; Nandiwada et al. 1983). Despite considerable variation in the number, distribution, and concentration of nerve endings among species, adrenergic and

cholinergic components have been identified in the pulmonary vasomotor nerves of all species that have been studied (Hebb 1969). Both alpha- and beta-adrenergic receptors are present, but the alpha receptors clearly predominate in number and function (Porcelli and Bergofsky 1973). In contrast to the evidence for involvement of the adrenergic mechanism, the function of the cholinergic system in the control of pulmonary circulation has not been identified. Studies with neurogenic blocking drugs have led to the conclusion that the autonomic control of pulmonary circulation is species specific. For example, in humans, the autonomic nervous system exerts little or no control on the pulmonary circulation (Widdicombe et al. 1970). In cattle, the response to autonomic stimulation is vigorous, suggesting the presence of a highly reactive pulmonary vascular bed in these animals (Reeves et al. 1962).

In general, the active vascular responses in the pulmonary circulation are relatively weak compared with the systemic vascular bed. One exception to this is an important local control mechanism: hypoxic pulmonary vasoconstriction. The pulmonary arteries and arterioles constrict in response to alveolar hypoxia and, to a much lesser extent, to arterial hypoxemia (Fishman et al. 1960; Fishman 1976). The decrease in alveolar oxygen tension results in pulmonary vasoconstriction and reduces blood flow to the hypoxic area of the lung. Blood flow is then directed to another area of the lung where oxygenation can occur (von Euler and Liljestrand 1946). The alveolar hypoxia-induced pulmonary vasoconstriction is very important because it affects the balance between alveolar ventilation and perfusion in the normal subject (Haas and Bergofsky 1968). Hypoxic

vasoconstriction also contributes to the pulmonary hypertension and gas exchange abnormalities occurring in patients with significant pulmonary parenchymal injury (Rubin 1984).

Cattle are known for their vigorous pulmonary vascular response to alveolar hypoxia. In a comparative study Tucker et al. (1975) found that cattle developed a greater pulmonary hypertensive response to hypoxia than dogs, cats or sheep. The larger amount of medial smooth muscle in the small pulmonary arteries of the bovine species (Alexander 1963) may contribute to this phenomenon.

Although the details of transduction of the hypoxic signal to the pulmonary vasculature remain unclear (Weir 1984), contraction of pulmonary vascular smooth muscle is obviously the final common pathway for this response. Recent observations with calcium channel potentiators (Tolins et al. 1986) as well as previous studies using calcium channel blockers (McMurtry et al. 1976; Tucker et al. 1976), support the concept that the mechanism of hypoxic vasoconstriction may involve altered calcium channel activity. However, none of these observations can indicate whether this is a direct effect of hypoxia on the cell membrane of the smooth muscle cell, or whether the smooth muscle cell responds to signals sent by hypoxic endothelium or other cells (Voelkel et al. 1984). Finally, the precise mechanism of this response is obscure but it occurs in excised isolated lung and so does not depend on central nervous connections (Glazier and Murray 1971).

Mechanisms of Pulmonary Vascular and Lung Tissue Injury

Many functional disorders of the lung, heart and respiration are associated with reversible or irreversible alterations of the

pulmonary circulation. Most of these conditions are not as closely related in pathogenesis to the pulmonary vasculature as acute, diffuse lung injury. Because pulmonary vascular endothelium is a key and early target of acute lung injury, much of the study of the mechanisms mediating lung injury focuses on pulmonary vascular endothelium and smooth muscle. Studies have also emphasized the importance of the production, metabolism, and biological effects of agents that can modify the structure and function of pulmonary vessels (Said 1986).

The association of acute lung injury with infection, embolism, trauma and hyperoxia prompted speculation that a wide variety of factors in vascular or alveolar sites might contribute to the mechanisms mediating acute lung injury.

The role of neutrophils. It is well established that the intrapulmonary pool of granulocytes (neutrophils) is normally large. Several investigators have proposed possible explanations for the presence of excess granulocytes in the lungs microcirculation. Schmid-Schoenbeim et al. (1980) presented convincing evidence that physical factors would favor retardation of granulocytes relative to the smaller erythrocytes in small pulmonary vessels. Additional evidence was reported by Martin et al. (1982) who demonstrated that the number of neutrophils present in the lungs is inversely related to pulmonary blood flow. Some studies have suggested that there is a specific interaction between vascular endothelium and neutrophils occurring by means of a chemotactic peptide (Beesley et al. 1978; Pearson et al. 1980; Hoover et al. 1980). Endothelial cells have surface receptors for chemotactic peptides and incubation of endothelial cells with unstimulated granulocytes increases leukocytic

adherence (Buchanan et al. 1981). This in vitro interaction between endothelial cells and granulocytes is not affected by prostacyclin (Gimbrone and Buchanan 1982), but is inhibited by blockers of the lipoxygenase pathway of arachidonic acid metabolism (Buchanan et al. 1981; Gimbrone and Buchanan 1982). Boxer et al. (1980) reported that mobilization of the marginated neutrophil pool by epinephrine is the result of 3,5-cyclic adenylate (CAMP) generation by endothelial cells after beta-adrenergic stimulation. These findings suggest that there is humoral communication between granulocytes and vascular endothelium which affects the presence of granulocytes in the lungs. In normal conditions, presence of neutrophils has little effect on the important functions of the lungs, but if neutrophils are activated or frustrated in their attempts to ingest large materials, considerable lung damage can occur (Tate and Repine 1983). Theoretically, when activated, neutrophils could release at least three groups of products that could cause tissue damage: lysosomal enzymes, toxic species of reduced oxygen, and products of arachidonic acid metabolism.

Considerable evidence suggests that neutrophils contribute to the development of lung injury in humans with acute respiratory disorders (Repine 1985). Increased numbers of neutrophils are commonly found in lung lavage fluid of patients during the early part of the disease process (Lee et al. 1981). Moreover, neutrophil accumulation frequently occurs adjacent to damaged endothelial cells in patients with acute lung diseases (Cochrane et al. 1983) and this is also true in several experimental forms of lung injury (Staub et al. 1982; Johnson and Ward 1982; Loyd et al. 1983; Newman et al. 1983).

Acute pneumonia and endotoxemia, airway diseases, microembolism

and pulmonary oxygen toxicity in animals and humans are all associated with granulocyte accumulation in the lungs (Brigham and Meyrick 1984). In acute pneumonia induced by Pasteurella haemolytica in newborn calves, lungs are invaded by a large number of neutrophils (Slocombe et al. 1984b). The intrapulmonary presence of P. haemolytica causes severe arterial hypoxemia, decreased dynamic compliance and increased pulmonary resistance in addition to the typical pneumonic lung lesions. Neutrophil depletion with hydroxyurea prevents the development of hypoxemia, tachypnea and the characteristic pneumonic lesions in newborn calf lungs (Slocombe et al. 1985). Slocombe et al. (1985) indicated that the clinical signs of lung injury were neutrophil mediated, rather than due to the direct effects of P. haemolytica. In their study, they also reported that the chemotactic factors for neutrophil migration into the lungs in acute pneumonic pasteurellosis are unknown. Several possibilities were raised. First, Pasteurella may die in the lungs and liberate endotoxin, which activates the complement system. Second, Pasteurella cytotoxins may directly damage alveolar tissue and resident granulocytes, and these injured cells may release chemotactic factors and third, gram negative organisms release peptides that are similar to complement and may attract neutrophils.

Previous studies have attempted to investigate the role of neutrophils in pulmonary vascular injury induced by endotoxin (Pingleton et al. 1971; Kux et al. 1972; Gaynor 1973). Infusion of Escherichia coli endotoxin into unanesthetized sheep causes transient pulmonary hypertension followed by a long period of increased lung vascular permeability to fluid and protein (Brigham et al. 1979).

During the early phase of endotoxemia, Pingleton et al. (1971) and Mlezoch et al. (1978) reported that leukocytes, primarily granulocytes, are sequestered in pulmonary vessels of dogs. Meyrick and Brigham (1983) reported a rapid and profound accumulation of granulocytes in the lungs of sheep infused with gram-negative endotoxin. Granulocyte accumulation started with margination of leukocytes in the small vessels and degranulation of neutrophils. Later, there is migration of neutrophils between endothelial cells and finally endothelial injury with gaps appearing between endothelial cells.

To see whether circulating granulocytes are necessary for the lung vascular reaction to endotoxin, Heflin et al. (1981) measured the endotoxin response in chronically instrumented sheep before and after granulocyte depletion with hydroxyurea. They found that circulating neutrophils are necessary for the development of increased lung vascular permeability to fluid and protein following endotoxin infusion. They also reported that pulmonary vasoconstrictor effects of endotoxin in sheep are independent of neutrophils. In a similar model, other workers showed attenuation of endotoxin-induced, late-phase pulmonary hypertension in sheep after granulocyte depletion with nitrogen mustard, but the early phase of pulmonary hypertension was not abolished (Huttemeier et al. 1982). Thus, it appears that in this experimental model of acute lung disease, granulocytes are important in the lung injury which increases vascular permeability, but do not seem essential in the vasoconstrictor response to endotoxin. However, a central role for leukocytes in the mechanism of lung injury has not been shown in all animal models of endotoxemia.

Leukopenia does not prevent the hemodynamic and blood gas changes after E. coli endotoxemia in baboons (Guenter 1971). These data suggest that the mechanism of lung injury is different in baboons than in sheep.

In addition to the role of neutrophils in the acute lung injury induced by endotoxin, granulocytes also accumulate in the lungs in other animal models of diffuse lung injury, for example extensive microembolism (Staub et al. 1982b). Acute pulmonary vascular injury after microembolization and the effect of neutropenia has been investigated in sheep. Flick et al (1981) studied the effects of uneven pulmonary artery obstruction by microemboli on transvascular fluid and protein exchange in normal and leukopenic sheep. With normal levels of circulating leukocytes, these investigators reported that, sheep developed an increased protein-rich lymph flow from the lung, a characteristic of increased permeability and vascular injury.

They also demonstrated that the pulmonary vascular permeability and increased lymph flow following embolization was significantly attenuated by neutrophil depletion. Similar observations were reported in the study of Johnson and Malik (1982) that pulmonary vascular injury induced by bead microembolization is a neutrophil mediated response.

Hyperoxia induced lung injury is a well recognized clinical and pathological condition characterized by massive pulmonary edema. In the histological studies of Kistler et al. (1967) and Crapo et al. (1980) using rat lungs, the massive pulmonary edema was consistent with vascular endothelial cell injury caused by oxygen. There is an increase in the number of neutrophils in the rat lung during oxygen

toxicity, especially in perivascular locations (Crapo et al. 1980). Several studies have demonstrated that neutrophils contribute to the pathogenesis of oxygen induced lung injury (Fox et al. 1981; Shasby et al. 1982; Campbell and Wald 1983). Shasby et al. (1982) examined the relationship between neutrophils in the lung and the edema of acute hyperoxic lung injury in rabbits. They found that the degree of lung injury was correlated with the number of granulocytes in lung lavages from rabbits exposed to hyperoxia for 72 hours. In addition, when neutrophils were depleted with nitrogen mustard, there was a decrease in the amount of lung injury.

Most studies of the role of granulocytes in acute lung injury have concentrated on microvascular injury. Evidence is accumulating that granulocytes are essential participants in the abnormalities of lung mechanics in both human and animal models of acute lung injury (Petty and Ashbaugh 1971; Brigham and Meyrick 1984). For example, early in the response to endotoxemia in sheep, there are marked reductions of lung compliance and marked increases in airway resistance in the lungs (Esbenshade et al. 1982; Snapper et al. 1983). However, unlike the vasoconstriction which is unaffected by neutropenia changes in lung mechanics after endotoxemia in sheep are attenuated by neutrophil depletion with hydroxyurea (Hinson et al. 1983).

Attempts have been made by several investigators to elucidate the mechanisms mediating lung injury and how neutrophils damage the endothelium. It has been suggested that alterations in vascular endothelial membranes cause neutrophils to adhere to endothelium (Cross and Hyde 1978), and that adherence and activation may be caused

by complement (Craddock et al. 1977). Another group of investigators have thought that neutrophils may damage the endothelium physically as they migrate through the endothelium into the lung's interstitium (Henson et al. 1979), providing a channel for fluid and protein. However, recent investigations by Meyrick (1986) showed that neutrophil migration through pulmonary vascular endothelium does not produce either functional or structural evidence of endothelial damage in vitro. Thus, the margination and migration of granulocytes across intact endothelial cells does not seem to be a sufficient explanation for the severe and persistent endothelial injury caused by endotoxemia and other agents of acute lung injury.

Despite the fact that bacteria and endotoxin or other agents can directly injure pulmonary endothelium, activated neutrophils may exaggerate the injury by their interaction with the altered endothelium. Therefore, acute lung injury may be mediated by the release of superoxide radicals, prostaglandins, leukotrienes, or other arachidonic acid metabolites produced by neutrophils.

Role of oxygen-derived free radicals in lung injury. Neutrophils and the rapidly generated oxygen radicals are considered to be the central mechanism mediating acute lung injury. In response to activation by particulate and/or specific soluble inflammatory mediators, neutrophils respond with large increases in their oxygen consumption (Fantone and Ward 1982). In conjunction with an increase in oxygen consumption, neutrophils secrete superoxide anion (O_2^-) and hydrogen peroxide (H_2O_2) (Drath and Karnovsky 1975; Babior 1981). The enzyme system responsible for the increased oxygen consumption and superoxide generation has been identified as a membrane-associated

nicotinamide adenine dinucleotide (NADPH), NAD(H) oxidase (McPhail et al. 1976; Babior et al. 1978). Precise information is not available concerning the location and biochemical characterization of the oxidase system in phagocytic cells. Several investigators have suggested that the oxidase enzyme of the neutrophils is located at least in part on the external surface of the plasma membrane, accounting for the release of superoxide anion into the extracellular space (McPhail et al. 1976 and Fantone and Ward 1982).

Several additional highly reactive oxygen derived metabolites have been identified or predicted to exist as a result of activation of phagocytic cells. These include hydroxyl radical (OH·) (Tauber and Babior 1978; Weiss et al. 1978), singlet oxygen (O_2^-) (Rosen and Klebanoff 1977; Piatt and O'Brien 1979), and hypochlorous acid (HOCl) (Harrison and Schultz 1976). The proposed mechanism of OH formation is by interaction of O_2^- and H_2O_2 in the modified Haber-Weiss reaction, also known as the Fantone reaction (McCord and Day 1978; Till and Ward 1986):

$$Fe^{3+} + O^-_2 \rightarrow Fe^{2+} + O_2$$

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + OH\cdot$$

Small quantities of superoxide anion are normally produced in the tissues. A number of intracellular enzymes, including superoxide dismutase (SOD), catalase (CAT) and peroxidase inactivate free radicals to prevent cellular injury. However, in the extracellular space, SOD and CAT are present in very low concentrations making cell membranes adjacent to this location susceptible to free radical-mediated toxicity (McCord and Fridovich 1978).

Several studies have shown that these oxygen metabolites, as well

as products of the myeloperoxidase reaction, can have toxic effects on a variety of cell types including vascular endothelial cells (Sacks et al. 1978; Clark and Klebanoff 1979; Simon et al. 1981). In vitro studies provide evidence that toxic oxygen species mediate endothelial cell injury (Sacks et al. 1978; Weiss et al. 1981), and that the human endothelial cell may be uniquely sensitive to injury caused by H_2O_2 (Weiss et al. 1981). For example, BCG-activated neutrophils have the ability to lyse endothelial cells through an oxygen-dependent mechanism (Nathan et al. 1979). Catalase is effective in inhibiting this reaction. However, SOD, scavengers of singlet oxygen and/or hydroxyl radical, and inhibitors of heme enzymes, have no inhibitory effects, suggesting that H_2O_2 is the toxic metabolite mediating cell lysis in humans (Nathan et al. 1979). In contrast, human neutrophils activated by opsonized zymosan cause cytolysis of the cell by a mechanism that is inhibited by cyanide, azide (heme enzyme inhibitor) and catalase. Because oxygen metabolites have been shown to cause damage of endothelial cells in vitro, it is possible that the local production of these metabolites by neutrophils is responsible for the acute respiratory syndrome observed in many clinical conditions.

Although the precise mechanism of lung injury induced by oxygen metabolites in vivo is not known, several investigators have hypothesized that pulmonary vascular injury is caused by oxygen radicals derived from activated neutrophils. Preliminary evidence in rats following intravascular infusion of cobra venom factor indicates that pulmonary edema and focal intra-alveolar hemorrhage are associated with neutrophil sequestration. This is associated with evidence of endothelial cell damage which can be prevented by

treatment of animals with catalase (Fantone and Ward 1982). Superoxide dismutase treatment was also shown to significantly attenuate pulmonary injury in sheep that had received repeated intravenous injection of complement activated plasma (Perkowski et al. 1983). Additional study has shown that the intratracheal installation of specific enzyme substrate systems known to generate oxygen metabolites cause acute lung injury (Johnson et al. 1981). When xanthine and xanthine oxidase are administered intratracheally in rats, there is increased vascular permeability and focal hemorrhage at 4 hours. These pathologic changes can be inhibited with simultaneous instillation of SOD. It has also been shown that when glucose oxidase (which generates H_2O_2) is instilled into the airways, there is a marked increase in vascular permeability, edema and pronounced neutrophils influx (Johnson et al. 1981). These changes are consistent with the diffuse alveolar damage associated with adult respiratory distress syndrome in humans (Rinaldo and Rogers 1982). Johnson et al. (1981) reported that the pathological changes induced by glucose enzyme system are inhibited with catalase. The lung's response to hyperoxia is believed to be mediated by release of oxygen radicals from a variety of cellular sources. Neutrophil depletion, administration of antioxidants and liposome-encapsulated SOD and CAT result in less lung injury and edema following acute hyperoxia (Shasby et al. 1982; Campbell and Ward 1983). These studies demonstrate that oxygen-derived free radicals and their metabolites have the capacity to cause acute lung injury.

To further evaluate the nature of the oxygen metabolites and possible mechanism or mechanisms involved in the pathogenesis of acute

lung microvascular injury, the effects of OH· scavengers and iron chelators were tested by Repine et al. (1979) and Ward et al. (1983). These interventions were chosen because there is considerable evidence that generation of OH from H_2O_2 in the presence of iron ions can result in lipid peroxidation, which has been shown to be involved in tissue damage (Barber and Bernheim 1967). Pretreatment of rats with the potent OH scavengers, dimethyl sufoxide provided significant protection against complement and neutrophil-mediated acute pulmonary injury (Ward et al. 1983). The same protective effects were seen with iron chelators such as iron-free lactoferrin and deferoxamine mesylate (Till and Ward 1986). Furthermore, infusion of ferric chloride into the cobra venom-injected rats increased pulmonary injury in a dose-dependent fashion (Ward et al. 1983). Similar observations, i.e. lung protective effects of OH scavengers and an iron chelator, were seen in thermally injured rats (Till et al. 1984). Although the mechanism of OH -related injury to lung vascular endothelium is not known, recent experimental evidence suggests that lipid peroxidation may be involved (Till et al. 1984). This hypothesis is supported by the observations that plasma from thermally injured rats contained increased levels of lipid peroxidation products that were dependent on the availability of neutrophils. The increased levels of lipid peroxides were blocked by OH· scavenger and iron chelators (Till et al. 1984).

Because stimulated neutrophils are able to release other potentially toxic products, it could not be determined exactly from the above experiments if oxygen-derived products could cause pulmonary injury in the absence of other factors produced by neutrophils. To

address this issue, Tate and associates (1982) examined the effects of oxygen products generated by either xanthine oxidase or glucose oxidase on isolated salt-perfused rabbit lungs. Using this experimental model, they possibly differentiated the effects of oxygen products on the lung from the effects of other toxic properties of stimulated neutrophils. Reduced species of oxygen generated by the xanthine-xanthine oxidase reaction caused severe pulmonary edema and vascular injury. Additional experiments using oxygen radical scavengers suggested that these changes were mediated by H_2O_2 or H_2O_2 products (Johnson et al. 1981). It now appears that toxic oxygen products are capable of causing lung microvascular injury in the absence of other products which are released by stimulated neutrophils.

Neutrophil-derived free radicals may augment the inflammatory responses by the inactivation of serum protease inhibitors, thereby causing destruction of the cells (Matheson et al. 1979). Additional in vitro studies have also shown that locally produced oxygen radicals have the capacity to generate chemotactic factors (Petrone et al. 1980). Although most of the reports suggest a proinflammatory role of the oxygen metabolites, the precise in vivo effects of these substances is not clear and awaits further investigation.

In summary, acute lung injury induced by bacteria, endotoxin, hyperoxia and complement results in a series of events leading to activation and infiltration of neutrophils in pulmonary capillaries. It also appears that toxic oxygen products of activated neutrophils are largely responsible for the damage to vascular endothelial cells.

Arachidonic acid metabolites. Arachidonic acid (AA) is a long

chain unsaturated fatty acid located in the extracellular and intracellular membranes of the cell. Activation of phospholipase enzymes in the lung by inflammatory, mechanical, or other stimuli can convert AA rich phospholipid membrane into three major derivatives, thromboxanes, prostaglandins, and leukotrienes (Moncada and Vane, 1979; Samuelsson, 1983).

It has been suggested that AA metabolites are among the most potent agents affecting the functions of the pulmonary circulation. Cyclooxygenase products which include both vasoconstrictors and potent vasodilators may have important consequences for lung function in diffuse lung injury (Malik et al. 1985). Lipoxygenation products of arachidonate may also affect the function of both airways and blood vessels, although the evidence for this is less convincing than for the cyclooxygenase products (Brigham 1985; Malik et al. 1985). Several investigators have shown that the concentrations of lipoxygenation and cyclooxygenase products are higher in the pulmonary circulation after acute lung injury induced by endotoxin infusion into sheep (Brigham and Ogletree 1981; Ogletree et al 1983, 1982). The same investigators also believe that the increased concentrations of these metabolites may originate from the neutrophils recruited into the sheep lung. This suggests that neutrophils, in addition to being the source of free oxygen radicals, may also be a potent source of other inflammatory mediators, in particular, the derivatives of arachidonic acid. This hypothesis is supported by recent investigations of Brigham (1985) who demonstrated some of the AA metabolites released from sheep lungs after neutrophil infiltration subsequent to diffuse lung injury induced by E. coli endotoxin. This

investigator also showed that there is no close correlation between acute lung injury induced by endotoxin and endogenous generation of arachidonate metabolites in sheep. This indicates that the active arachidonic acid metabolites are involved but may not initiate the pathological changes in the lungs. However, previous investigation has shown that the vasodilator prostaglandins generated during the neutrophilic inflammatory reaction, enhance the chemotactic-factor-mediated neutrophil infiltration and increase the extent of vascular injury in rabbits (Issekutz and Movat 1982). This suggests that prostaglandins, especially of E type and prostacyclin may have proinflammatory actions. Several lipoxygenation products, in particular leukotriene B_4, also appear to be potent chemoattractants for neutrophils (Nagy et al. 1982). Because of their chemotactic properties, AA metabolites, particularly the lipoxygenase products, may provide the link betyween the initial phase of inflammation and the subsequent diffuse lung injury in which more neutrophils are recruited. It is also possible that the production of these substances is directly linked with oxygen radical mediated lung injury. This hypothesis is supported by the fact that lipid peroxides, which are involved in prostaglandin synthesis, are elevated in acute lung injury caused by oxygen radicals (Till et al. 1984).

Among the possible effects of lipoxygenation products that could be important in diffuse lung injury are bronchoconstriction, vasoconstriction (Yokochi et al. 1982), and increased capillary permeability (Brigham et al. 1982). In acute lung injury, when neutrophils are activated, the vascular endothelium becomes more permeable to protein and there is a leakage of fluid into the

interstial space and eventually into the alveoli. Although the increased vascular permeability appears to be initiated by oxygen radicals (Till et al. 1982), it may also involve leukotrienes because it can be prevented by lipoxygenase and cyclooxygenase blocking agents such as corticosteroids (Brigham et al. 1981; Olson 1985). Besides causing increased vascular permeability and being chemotactic agents, leukotrienes particularly C_4 and D_4 are potent smooth muscle contracting substances. Therefore they may mediate inflammatory reactions by producing changes in blood flow and increases in vascular permeability (Ford-Hutchinson 1985). Evidence is now accumulating that elevated levels of leukotrienes may be found in various human lung diseases (Ford-Hutchinson 1985) which suggests a pathological role of these mediators.

It has been proposed that thromboxane A_2 mediates lung vascular injury by causing pulmonary vasoconstriction, platelet aggregation and neutrophil adherence (Moncada and Vane 1979; Garcia-Szabo et al. 1983a). It has also been shown that thromboxane generation occurs after thrombin-induced pulmonary injury in sheep (Garcia-Szabo et al. 1983b). Thromboxane A_2 generation precedes the increase in lung vascular permeability in complement (McDonald et al. 1983) and endotoxin (Miyazawa et al. 1982) models of acute pulmonary vascular injury. Garcia-Szabo et al. (1983b) reported that the thromboxane synthetase blocker, diazoxiben, prevented the thrombin-induced increase in pulmonary vascular permeability in sheep which suggests the involvement of thromboxane in pulmonary vascular injury. The same investigators also indicated that the protective effect of diazoxiben is due to the inhibition of platelet aggregation and independent of

the pulmonary arterial pressure changes. Moreover, thromboxane synthetase inhibition with diazoxiben reduces the chemotaxis of sheep neutrophils to thrombin, which suggests that thromboxane generation is involved in pulmonary sequestration of neutrophils (Spagnulo et al. 1980).

Prostaglandins of the E series are vasodilator agents produced by neutrophils, platelets, and macrophages (Moncada and Vane 1979). The concentration of PGE increases in lung perfusate and arterial blood after lung vascular injury induced by microembolization and endotoxin challenge (Ogletree 1982; Malik 1983). Previous investigation by Wedmore and Williams (1978) postulated that PGE_2 induced dilation of precapillary vessels enhances neutrophil migration to the downstream vessels after the chemoattractant stimulus. The neutrophils are then capable of inducing vascular injury when activated. In vitro studies indicate that prostaglandins of the E series do not cause aggregation of human neutrophils (O'Flaherty et al. 1979). However, PGE_2 causes an increase in neutrophil infiltration in skin microvessels when administered in combination with leukotrienes (Issekutz and Movat 1982). Thus prostaglandins of the E series may play a permissive role in acute lung injury by causing vasodilation. Prostaglandin F_{2alpha} is produced by neutrophils but does not appear to be involved in pulmonary vascular injury (Naxano and Cole 1979).

Prostacyclin (PGI_2) is a powerful vasodilator produced by pulmonary endothelium in response to various forms of endothelial injury (Weksler et al. 1978). In vitro studies indicate that PGI_2 inhibits platelet aggregation and neutrophil adherence to human endothelial cells (Boxer et al. 1980). Prostacyclin also reduces the

degree of thrombocytopenia as well as neutropenia after complement or platelet activating factor injection in rabbits (Camussi et al. 1981). Therefore, PGI_2 may not be important in neutrophil-dependent lung vascular injury. However, in acute lung injury induced by thrombin infusion, PGI_2 does not modify the degree of neutrophil infiltration in the lung, but does inhibit the thrombocytopenia associated with thrombin challenge (Malik et al. 1985). The in vitro observation that PGI_2 inhibits adherence of human neutrophils to endothelial cells (Boxer et al. 1980) is not supported by the in vivo studies. Despite the lack of the effect of PGI_2 on neutrophil infiltration induced by thrombin infusion, the pulmonary vascular permeability was reduced by 50% in a PGI_2-treated group of sheep (Malik et al. 1985). These investigators also suggested that the reduced pulmonary vascular permeability in PGI_2-treated animals was attributable to PGI_2-mediated reduction in the release of pulmonary vasoactive agents from platelets or other cells in the lung.

Recently it has been shown that experimentally induced streptococcus pneumonia in rabbits results in an increase in the concentration of thromboxane A_2 (TxA_2) and prostacyclin in the blood (Goldblum et al. 1986). Pneumococcal challenge also induced neutropenia, thrombocytopenia as well as pulmonary leukostasis. Thromboxane A_2 synthetase inhibition blocks the pneumococcus-induced elevation in TxA_2 in blood without altering pulmonary leukostasis and PGI_2 concentration in the blood. This may indicate that the immediate elevation in the level of PGI_2 induces pulmonary vasodilation, enhances pulmonary leukostasis and increases the inflammatory reaction in pneumococcal pneumonia in rabbits.

In summary, there is a close relationship between endogenous generation of AA metabolites and the pathophysiological response in diffuse lung injury. However, clear establishment of cause and effect relationships is difficult. It is quite possible that endogenous generation of some arachidonate products is a result of the complicated sequence of events eventuating in microvascular injury rather than causing those changes. For example, inhibition of pulmonary vascular permeability by high doses of corticoids could prevent rises in the concentration of PGI_2 and lipoxygenation metabolites if those changes were a result of microvascular injury rather than the cause (Brigham et al. 1981).

Pulmonary Vascular Effect of AA Metabolites

Effects of AA metabolites on pulmonary hemodynamics. The pulmonary circulation is a low pressure, low resistance system. However, it is not clear how the resistance is kept low and what the regulatory factors are. In addition to the passive vasodilation and recruitment of reserve capillaries in this circulation, there could be substances intrinsic to the lung that are constantly released and act as pulmonary vasodilators. It is well known that lung tissue can generate substantial quantities of prostaglandins and thromboxane A_2, especially during pathophysiological responses. For example, pulmonary vascular endothelium is a rich source of prostacyclin (PGI_2) which is not inactivated in the pulmonary circulation (Dey et al. 1981). Therefore, the balance between the vasodilatory effects of prostaglandins and the vasocoonstrictor effect of thromboxane A_2 must be considered in the determining the overall level of vascular tone in

pulmonary circulation (Ogletree 1982).

The AA metabolites are considered by many investigators to modulate pulmonary vascular resistance in the lung. In the pulmonary circulation, low levels of intravascular pressure may be maintained, at least in part by the continuous intrapulmonary production of PGI_2 (Gryglewski et al. 1978). When pulmonary arterial pressure rises due to alveolar hypoxia (Hamasaki et al. 1982), or when the pulmonary vascular endothelium is directly stretched by hyperinflation (Said 1982), the lungs increase their production of PGI_2 returning the pulmonary arterial pressure toward normal. Walker et al. (1982) and others (Kadowitz et al. 1975; Wennmalm, 1978) found that inhibitors of prostaglandin synthesis, such as meclofenamate and indomethacin increase baseline pulmonary arterial pressure and PVR in conscious dogs as well as in the isolated lung. This indicates that PG synthetase products contribute to regulation of lung vessels in the normal condition. However, more recently Rubin et al. (1985) evaluated the effects of the acute intravenous administration of 3 cyclooxygenase inhibitor drugs, indomethacin, meclofenamate, and ibuprofen in intact anesthetized dogs. The administration of indomethacin produced an increase in mean pulmonary arterial pressure from 8 to 13 mmHg and PVR 1.2 to 2.7, whereas meclofenamate and ibuprofen had no effect. They also reported that indomethacin given during hypoxic ventilation slightly, but insignificantly, increased Ppa and PVR compared with hypoxia alone and with the vehicle or meclofenamate treated groups. Treatment with indomethacin or meclofenamate given subcutaneously had no effect on normoxic or hypoxic pulmonary tone. These authors concluded that PGs do not

appear to play a major physiological role in modulating normoxic pulmonary vasomotor tone in anesthetized dogs and that the indomethacin-increases in pressure and resistance are independent of prostaglandin inhibition.

In the study of Lock et al. (1980), the effects of prostaglandins PGD_2, PGE_1, PGE_2, PGF_{2alpha}, and PGI_2 on pulmonary vascular tone were investigated in normoxic and hypoxic unsedated newborn lambs. Prostaglandin D_2 and PGF_{2alpha} were found to be local pulmonary vasoconstrictors during normoxia, confirming a previous study of Kadowitz et al. (1974) in sheep and Wasserman et al. (1977) in dogs. It is also reported that hypoxia diminished the local vasoconstrictor effects of PGD_2 and abolished the early local effects of PGF_{2alpha}. The mechanism by which hypoxia attenuates these constrictor effects is not known. Nevertheless, the clinical (Harris et al. 1956), and experimental (Silove et al. 1968) studies suggest that baseline oxygenation may have a considerable effect on the pulmonary response to other agents such as acetylcholine and sympathomimetic drugs. Lock et al. (1980) also found that PGE, PGE_2 and PGI_2 were local pulmonary vasodilators during both normoxia and hypoxia. There are conflicting reports concerning the effect of PGE_2 on the pulmonary vascular tone. The vasodilator effects of PGE_2 in newborn lambs in the study of Lock et al., support the results of Tyler et al. (1975) in newborn goats, but are at variance with those of Kadowitz et al. (1975) in adult animals. These differences in PGE_2 effects may be attributed to the age of the animals (Lock et al. 1980).

Unlike PGE_1 and PGE_2, PGI_2 lowers PVR in all animal species studied so far when administered in either the pulmonary or systemic

circulation (Lock et al. 1980). Prostacyclin also produces a greater fall in PVR than in systemic vascular resistance in hypoxic newborn lambs. This indicates that PGI_2 is a relatively more specific pulmonary vasodilator than the E type prostaglandins. Furthermore, the importance of baseline conditions in determining the effects of an agent was especially apparent for PGI_2, in that hypoxia lowered the threshhold for the direct effects of PGI_2 on the pulmonary vasculature.

Although the response of large vessels to prostaglandins has been well studied, little information is available on their pulmonary microvascular effect. Gunther et al. (1982) studied the effect of PGI_2 infusion on the pulmonary microcirculation. These investigators used lung lymph flow and lymph to plasma protein ratio as a sensitive indicator of net fluid (QF) and protein flux (CP). They found an increase in QF and CP which was felt to be due to an increase in the surface area of fluid exchange vessels rather than increased permeability. The increase in lymph flow and decrease in lymph to plasma ratio indicate an increase in pulmonary microvascular pressure by PGI_2.

These results suggest that prostaglandins can act locally on pulmonary smooth muscle tone in vivo and that the level of oxygenation of baseline tone influences the pulmonary responses to prostaglandins. These findings are consistent with the previous study of Wicks et al. (1976). These authors compared the effects of AA, the prostaglandin precursor on the PVR with those of PGF_{2alpha} and norepinephrine in the presence of sympathetic and parasympathetic blockers and serotonin receptor blocking agents in canine lungs. The effect of AA on the

pulmonary vascular pressure gradient was not blocked by pretreatment with the alpha blocker, phentolamine, the beta blocker, propranolol, the serotonin receptor blocker, cyproheptadine, or the cholinergic blocker, atropine. However, aspirin (25 mg/kg) completely blocked the pulmonary vascular effect of AA, but did not affect the response to PGF_{2alpha}. Therefore, these experiments again suggest that AA is converted into vasoactive intermediates or the prostaglandins which act directly on precapillary pulmonary vascular smooth muscle rather than through platelets, plasma, adrenergic, or cholinergic mechanisms.

Ellsworth et al. (1983) evaluated the effect of sympathetic nerve stimulation on the pulmonary production of PGI_2 and thromboxane A_2 in isolated canine left lower lobes. Stimulation of the sympathetic ganglia resulted in an increase in pulmonary arterial pressure which then declined over the next 5 minutes. Associated with this decline was a 0.24 ng/ml increase in PGI_2 in the pulmonary perfusate. These investigators suggested that the decline in Ppa is due to the synthesis and release of PGI_2 by the vascular endothelial cells. The mechanism by which PGI_2 attenuates the sympathetic increase in pressure is unclear. It has been reported that in addition to their direct vasoactive properties, prostaglandins may inhibit the release of norepinephrine prejunctionally and interact postjunctionally with the released neurotransmitter at the vascular smooth muscle receptor (Brody and Kadowitz 1974; Hedqvist 1977).

In another study, the effects of different doses of AA on the feline pulmonary vascular bed were investigated by Hyman et al. (1980). These experiments were conducted under conditions in which pulmonary blood flow naturally varied or was controlled, and when

vascular tone was at basal levels or elevated. In approximately half of the animals studied, AA infusions caused small decreases in pulmonary arterial pressure, and the depressor response was enhanced when the PVR was actively increased. In the other animals AA infusion increased pulmonary arterial pressure. In all experimental animals, infusion of large amounts of the precursor acid, as well as bolus injections, increased Ppa and PVR. The responses to AA were attenuated by meclofenamic acid, a cyclooxygenase inhibitor. Furthermore, these experiments also showed that in half of the animals studied the pressor response to hypoxia was decreased by AA, whereas in the others, hypoxic vasoconstriction was enhanced. Hayman et al. (1980) suggest that both vasodilator and vasoconstrictor products in the cyclooxygenase pathway can be formed from AA in the lung of intact animals. These authors also believed that when delivered at low concentrations, AA is converted into vasodilator products that have PGI_2-like activity, but when delivered at high concentrations, substances such as pGD_2 and PGF_{2alpha} are formed which have pulmonary pressor activity. This hypothesis is supported by the observations of Nugteren and Hazelhof (1973) and Hamberg and Fredholm (1976). They reported that in the presence of a high dose of AA it is possible that excess PGH_2 is formed and the PGI_2 synthetase may be saturated resulting in isomerization of PGH_2 into a vasoconstrictor prostaglandin. These assumptions are supported by the finding that the PGI_2 synthetase from the lung is saturated at low substrate (PGH_2) concentration (Sun et al. 1977).

The formation of TxA_2 probably plays little, if any, role in the response of pulmonary vasculature to AA because the enzyme that forms

this substance has its highest activity in the platelets (Hamberg et al. 1975; Needleman et al. 1976). Under normal conditions, platelets remain in the blood and do not stick in the lung.

It has been reported that the alternative fate of arachidonate is peroxidation by lipoxygenase to form hydroxyperoxyarachidonate and subsequently the leukotactic agent hydroxyarachidonate (Turner et al. 1975). It is also possible that inhibition of the cyclooxygenase pathway of AA may increase biosynthesis of lipoxygenase products. Ogletree et al. (1980) tested the effect of AA on the pulmonary vascular bed in sheep in the presence of indomethacin (a cyclooxygenase blocker) and ETYA, a cyclooxygenase and lipoxygenase inhibitor. These authors designed this experiment to determine whether the effect of AA on the pulmonary vascular bed is mediated via cyclooxygenase and/or lipoxygenase products. Since both indomethacin and ETYA blocked the increase in lymph flow initiated by arachidonate, it was concluded that cyclooxygenase products caused the pulmonary hypertension and increased lung lymph flow in sheep.

In the study of Leffler et al. (1984), intravenous administration of leukotriene D_4 caused dose-dependent increases in PVR in neonatal piglets. Neither treatment with lipoxygenase inhibitor nor with the leukotriene receptor antagonist altered any baseline cardiovascular parameters measured. These investigators concluded that endogenous leukotrienes do not appear to have an influence on resting pulmonary vascular function, although exogenous leukotrienes are capable of producing cardiovascular effects.

It appears that lipoxygenase products do not play a significant role in the pulmonary vascular reaction to AA infusion. However, this

does not exclude the possibility that in abnormal conditions these products of arachidonate metabolism may influence the release or activity of other mediators in pulmonary circulation. Furthermore, pulmonary vascular responses to the AA metabolites depend on the concentration as well as the animal species, route of administration, and basal level of tone of the pulmonary vascular bed.

Effect of eicosanoids on the pulmonary microvasculature and their relationship to hypoxic vasoconstriction. It has been forty years since hypoxia was first reported to cause pulmonary vasoconstriction (von Euler and Liljestrand 1946). Various substances, such as histamine, 5-hydroxytryptamine, and norepinephrine, have subsequently been proposed to explain the mechanism by which alveolar hypoxia causes pulmonary vascular smooth muscle constriction (Weir et al. 1976). It has been reported that during hypoxia, changes in calcium permeability may modify AA metabolism (Haack et al. 1975). Alternatively, hypoxia may directly inhibit oxygen-dependent enzymes in the prostaglandin pathway. Early reports by Said et al. (1974) suggested that synthesis of a vasoconstrictor prostaglandin such as PGF_{2alpha}, is stimulated by acute hypoxia and contributes to hypoxic pulmonary vasoconstriction. Although alveolar hypoxia-induced vasoconstriction occurs to some degree in all animal species, there is variability within the same species in the strength of the reaction (Hales et al. 1978). This variation probably affects the ability of the lung to direct blood flow away from poorly oxygenated regions.

Recently, several investigators have proposed that hypoxia stimulates pulmonary production of vasodilator substances. Hales et al. (1978) were the first to suggest that vasodilator prostanoids

contributed to inter-individual variability in the strength of hypoxic vasoconstriction. These investigators examined the influence of aspirin and indomethacin in two groups of dogs, those with weak alveolar hypoxic vasoconstriction (AHV), and those with vigorous AHV. A double-lumen endotracheal tube was used to allow ventilation of one lung with nitrogen as an alveolar hypoxic stimulus and ventilation of the other lung with oxygen to maintain systemic oxygenation. Pulmonary perfusion to each lung was measured with xenon-133 and external counters. In dogs with weak responses to alveolar hypoxia, both aspirin and indomethacin induced a four-fold increase in the magnitude of hypoxic vasoconstriction, whereas these drugs had no significant influence in dogs with vigorous responses to hypoxia. It is also reported that cyclooxygenase inhibitors produce enhanced reactivity in the isolated lung to alveolar hypoxia and PGF_{2alpha} but not to angiotensin II and norepinephrine. These results suggest that the weak hypoxic vasoconstriction may be due to an excess of vasodilator prostaglandins. These studies also indicated that the strength of AHV is variable even within the same species. Garrett and Thomas (1983) used meclofenamate, a cyclooxygenase inhibitor, in dogs with lobar atelectasis to further assess the extent to which the variation in the strength of hypoxic vasoconstriction is due to the effects of vasodilator prostaglandin. After intravenous infusion of meclofenamate (2 mg/kg followed by 2 mg/kg/hr), shunt fraction fell from 18 to 11%. The pulmonary pressor response to hypoxia rose from 9 to 13 torr. Thus, meclofenamate by increasing the strength of hypoxic vasoconstriction, decreased shunt fraction substantially. These authors suggested that prostanoid effects during hypoxia tend to be

uniform and are superimposed on hypoxic vasoconstriction.

Based on this information, it seems reasonable to suggest that the products of cyclooxygenase-mediated AA metabolism attenuate the pulmonary vascular response to alveolar hypoxia. It is also believed that PGI_2 plays a central role in mediating these responses, because PGI_2 is not only synthesized in the lung (Gryglewski et al. 1978) but when administered, it also decreases PVR (Kadowitz et al. 1978).

Sprague et al. (1984) used inhibition of cyclooxygenase activity and responses to exogenous PGI_2 to identify the contribution of this prostaglandin to the support of blood flow to hypoxic areas of the dog lung. These authors reported a decrease in arterial oxygen tension (PaO_2) after PGI_2 administration. Since mixed venous oxygen tension was not affected by PGI_2, the reduction in PaO_2 was not related to any increased tissue uptake of oxygen but rather to an increase in venous admixture. The investigators concluded that PGI_2 attenuates hypoxic vasoconstriction which allows flow to be maintained to hypoxic alveoli resulting in reduced PaO_2.

In another study Voelkel et al. (1981) reported that PGI_2 is produced by the lungs in response to vasoconstriction. In rat lungs perfused with ^{14}C-arachidonate, these authors found an increase in effluent radioactivity after angiotensin II-induced pulmonary vasoconstriction. The chromatographic analysis of lung effluent showed that 6-keto-prostaglandin F_{1alpha} (PGI_2 metabolite) was the major AA metabolite released during pulmonary vasoconstriction. Whatever the mechanism regulating PGI_2 production, it may act as a local hormone that helps to maintain a low pulmonary arterial pressure and it appears to be important as a defense against excessive

pulmonary vasoconstriction.

Other vasodilators derived from the metabolism of AA could possibly participate in the variability of hypoxic vasoconstriction. Phillips et al. (1983) studied the effects of both pulmonary and systemic infusions of PGD_2 on the acute vascular response to one minute of hypoxia in newborn lambs. Infusion of 1 ug/kg/min PGD_2 into the left atrium or inferior vena cava prevented the change in PVR in response to hypoxia, but not the change of systemic arterial pressure. Thus, PGD_2 specifically prevents hypoxic pulmonary vasoconstriction while maintaining systemic pressures, regardless of infusion site.

To further assess the role of prostanoids in the HPV, Garrett et al. (1985) measured prostanoid metabolites as well as shunt fraction and pressor response to alveolar hypoxia in anesthetized dogs with acute left lower lobe atelectasis. In this study, prostanoid metabolites, 6-keto-PGF_{1alpha} averaged 88 pg/ml prior to atelectasis and 81 pg/ml after atelectasis. Likewise, thromboxane B_2 values were normal. It seems that the baseline level of prostaglandin, in particular PGI_2, is sufficient to attenuate hypoxic vasoconstriction without raising blood levels of their metabolites. These authors also reported that the low level of 6-keto-PGF_{1alpha} did not correlate with shunt values which averaged 21%. This lack of correlation was justified by the presence in blood of variable amounts of vasodilator prostanoids produced elsewhere than the lung which were not assessed (Garrett et al. 1985).

Moon et al. (1983) also studied the effects of acute hypoxia (FIO_2=0.09-0.11 for 20 minutes) on transpulmonary plasma prostaglandin concentration in anesthetized dogs. Compared to normoxic conditions,

acute hypoxia did not affect transpulmonary plasma prostaglandin concentration. This experiment suggests that acute hypoxia does not alter net plasma prostaglandin metabolism.

Garrett et al. (1985) suggested that the increase in the tone of the hypoxic vessels after cyclooxygenase inhibition may be due to both inhibition of PGI_2 production and to an increase in leukotriene production. It has been proposed that cyclooxygenase inhibition not only decreases production of vasodilator prostanoids, but also may divert arachidonic acid into the lipoxygenase pathway (Voelkel et al. 1983).

Finally, the limited role of vasodilator prostanoids in controlling the pulmonary circulation in response to regional or global hypoxia contrasts with the major effects of prostanoids in the presence of significant lung injury. Thus, airway hypoxia may be a factor regulating in vivo prostaglandin metabolism and may contribute to abnormal prostaglandin concentrations. In addition, other nonspecific lung injuries such as inflammation or trauma may cause AA release and increase prostaglandin metabolism. If massive injuries occur with large amounts of AA release, the concentration of vasodilator prostaglandins may increase and may have detrimental effects on regional blood flow and ventilation-to-perfusion relationships in the diseased lung.

Pathophysiologic Changes of Acute Pneumonia

Pneumonia is an inflammation of the parenchyma (alveolar space and/or interstitial tissue) of the lung. Involvement of an entire lobe is called lobar pneumonia; of parts of the lobe only, lobular

pneumonia; and of alveoli contiguous with bronchi, bronchopneumonia. Pneumonia can also be classified according to its specific etiologic agent. The pathophysiologic changes which occur during acute pneumonia are complex, involving alterations in gas exchange such as arterial hypoxemia, lung mechanics, pulmonary hemodynamics particularly regional distribution of blood flow, and cardiac function.

Arterial hypoxemia. The effectiveness of gas exchange by the lungs is usually assessed through measurement of arterial oxygen and carbon dioxide tensions (PaO_2 and $PaCO_2$, respectively) (Murray 1986). However, additional information about the mechanisms that underlie any abnormality of gas exchange is obtained by measuring the alveolar-arterial oxygen difference (Murray 1986), $\dot{V}/\dot{Q}$ disturbances using multiple inert gases, and measurement of shunt fraction (Wagner et al. 1975). Nevertheless, measurements of arterial oxygen tension provide a useful clinical guide to the overall adequacy of respiratory function.

Although previous investigations have stressed the importance of arterial hypoxemia associated with pneumonia, the etiology of the pneumonic hypoxemia remains incompletely understood. Theoretically, the possible causes of pneumonic hypoxemia are hypoventilation, diffusion impairment, ventilation perfusion inequality and right-to-left shunt (West 1979).

Despite the fact that alveolar oxygen tension in the consolidated lung regions may be low, hypoventilation is ruled out as a possible cause for the arterial hypoxemia in pneumonia. Acute pneumococcal pneumonia in dogs (Light et al. 1981) and acute pneumonic

pasteurellosis in calves (Slocombe et al. 1984b) cause severe arterial hypoxemia with no change in arterial CO_2 tension. These investigators suggested that the pneumonic hypoxemia was not due to hypoventilation because arterial CO_2 tension did not change.

Optimal gas exchange in the normal lung requires the matching of ventilation and blood flow. The collateral ventilation and hypoxic vasoconstriction of the pulmonary vessels are thought to be important homeostatic mechanisms for matching ventilation with blood flow (Robinson 1982). Wagner et al. (1975) used the multiple inert gas technique to study distributions of ventilation-perfusion ratios in dogs with normal lungs and during lobar pneumonia, pulmonary embolism and hemorrhagic pulmonary edema. Six different inert gases, dissolved in 5% dextrose, were infused into a peripheral vein at 2 to 5 ml/min for about 15 minutes. At the end of this time, samples of systemic arterial blood, mixed venous blood, and mixed expired gas were collected for analysis of the 6 gases. Cardiac output (dye dilution) and minute ventilation were also measured. Using computer analysis, the relationship between arterial and mixed venous concentration ratio against blood:gas partition coefficient was drawn (the retention-solubility curve) and transformed into a plot of blood flow against $\dot{V}/\dot{Q}$ ratio. Similar calculations were made for the relationship between blood:gas partition coefficient and the ratio of mixed expired-to-mixed venous concentrations (excretion-solubility curve) to construct a plot of ventilation against $\dot{V}/\dot{O}$ ratios. These investigators showed that in acute lobar pneumonia, the major gas exchange abnormality was right-to-left shunt 48 hours following bacterial inoculation in dogs. The same authors also reported that

when pneumonia resolved, shunt fraction decreased and units with low ventilation-perfusion ratios developed 3 days after pneumococcal inoculation. In acute pneumococcal pneumonia, Light et al. (1981) reported that arterial hypoxemia was due to increased shunt and venous admixture fractions in the infected regions of dog lung. They also proposed that local hypoxic vasoconstriction was in most instances ineffective in directing blood flow away from the diseased regions of lung. In acute lung injury induced by pulmonary embolism, Wagner et al. (1975) demonstrated that in moderate pulmonary embolism there was little change in distribution of ventilation-perfusion ratios. In severe lung injury induced by pulmonary embolism, shunt as well as low $\dot{V}/\dot{Q}$ ratios appear and the arterial oxygen tension is in the range of 40 to 60 mmHg 48 hours after microemboli injection in dogs. The same investigators reported that in acute hemorrhagic pulmonary edema produced by injection of oleic acid into dogs right ventricle, arterial hypoxemia developed 1 hour after injection and was due to a large veno-arterial shunt. They also showed that the distribution of blood flow and ventilation to unaffected lung regions was similar to that in normal dogs. These findings seem to indicate that in acute lung injury caused by bacteria or other agents, the homeostatic mechanisms such as collateral ventilation and hypoxic vasoconstriction are overridden, so that some areas of lung receive ventilation and little blood flow, whereas other regions receive little or no ventilation but continue to receive blood flow. This mismatching of ventilation and blood flow may account for the severe hypoxemia in acute lung injury.

Recent investigations by Slocombe et al. (1984b) have shown that

acute pneumonia induced by P. haemolytica results in arterial hypoxemia 1 hour after intratracheal inoculation of $2X10^9$ organisms in neonatal calves. Hypoxemia is not due to alveolar hypoventilation because $PaCO_2$ remains unchanged. Pneumonic hypoxemia must therefore be due to either shunt, ventilation-perfusion mismatching, of diffusion impairment. In another study, the same investigators suggested that pneumonic hypoxemia probably results from the persistence of perfusion to poorly ventilated pneumonic regions of lung (Slocombe et al. 1983a). An increase in dead space/tidal volume ratio develops 6 hours after P. haemolytica exposure (Slocombe et al. 1984b). Increases in dead space/tidal volume ratio were probably caused by increased alveolar dead space resulting from a mismatching of ventilation and blood flow (Slocombe et al. 1983b).

There are a few studies on human beings and in experimental models of pneumonia that have demonstrated a decrease in, but still significant, perfusion to consolidated lung regions 3 to 7 days after pneumococcal infection (Goldzimer et al. 1974; Bjork and McNeil 1976; Kyellman 1976). Using perfusion scintiphotography, regional lung perfusion in dogs during pneumonia was assessed using sulfur colloid macroaggregates labeled with technetium 99m and human serum albumin labeled with iodine 131 (Goldzimer et al. 1974). In this study there was a slight decrease in pulmonary perfusion in the diseased lung regions which was attributed to local hypoxic pulmonary vasoconstriction. In this investigation, the complete disappearance of the perfusion defect was achieved by pulmonary vasodilating agents, isoproterenol and PGE_1. In these investigations of pneumonia, the decrease in PaO_2 was explained by low $\dot{V}/\dot{Q}$ ratio of the infected lung.

There is general agreement that pulmonary perfusion is maintained or increased in the diseased lung during pneumococcal pneumonia. However, the decrease in pulmonary blood flow during pneumonia may be attributable to the technique or time in which the measurement is taken. It also appears that bacteria and/or the inflammatory process they elicit in certain phases of inflammation may override hypoxic vasoconstriction and maintain blood flow in consolidated regions of lung.

Hemodynamics and regional distribution of blood flow in pneumonia. There are only a few studies on the effects of acute pneumonia on pulmonary and systemic hemodynamics. Hiser et al. (1975) studied the relationship between the severity of pneumonia and pulmonary vascular resistance in dogs before and within 36 to 72 hours after intrapulmonary administration of type II pneumococcus. The severity of pneumonia was documented by chest roentgenogram and confirmed subsequently by post mortem examination of most dogs. These authors found that PVR was increased and the increase was related to the severity of the pneumonic lesion. The increase in resistance was attributed to the vasoconstriction which caused an increase in pulmonary arterial pressure and a decrease in blood flow. Hemodynamic effects of pneumonia were also investigated in patients within 24 hours of pneumococcal infection (Benson et al. 1970). Patients were considered to be in the acute stage of pneumonia if they were febrile and if the studies were performed within 24 hours of admission to the hospital. In these patients during the acute phase of pneumonia, cardiac output was decreased and total peripheral resistance (TPR) and hematocrit were increased. The increase in TPR was attributed to the

passive vasoconstriction resulting from decreased intravascular blood volume. In another study Benson et al. (1970) showed that after expansion of plasma volume by infusion of 10% dextran in saline, all patients had a normal or near normal hemodynamic profile. These authors also suggested that the hypodynamic state associated with acute pneumonia may be due to a depressed myocardial contractility.

Several investigations have studied regional distribution of pulmonary blood flow during acute pneumonia using chest angiography, multiple inert gases, or the radiolabeled microsphere techniques. Wagner et al. (1975) studied regional distribution of blood flow in acute pneumococcal pneumonia using the multiple inert gas technique and showed an increase in blood flow through right-to-left shunt in dogs 24 hours post inoculation. These authors also reported that 3 days after infection, shunt flow decreases and areas with low $\dot{V}/\dot{Q}$ develops. Investigation using angiographic techniques have indicated that there is a decrease, but significant pulmonary perfusion in the diseased lung regions 3 days after pneumococcal infection in dogs (Goldzimer et al. 1974). In acute lobar pneumonia, distribution of pulmonary perfusion was investigated within 24 to 72 hours of pneumococcal infection in dogs using radiolabeled microspheres (Light et al. 1981). Pulmonary blood flow perfusing the diseased lung increased by a mean of 15% and venous admixture fraction increased from 5 to 20%. These investigators believed that the increased shunt and venous admixture fraction was due to the failure of hypoxic vasoconstriction to divert blood away from the diseased and nonventilated lung regions.

It has been proposed that the failure of hypoxic vasoconstriction

and increased blood flow to the pneumonic lung, may be attributable to the presence of viable bacteria or to the local inflammatory reaction. For example, Light et al. (1981) demonstrated a variable change in pulmonary perfusion to the pneumonic lung that correlated with the number of viable bacteria isolated by swabbing the cut surface of the lobe over an agar plate. Inflammation is associated with increased production of prostaglandins, one of which, the pulmonary vasodilator PGI_2, may oppose hypoxic vasoconstriction (Reeves and Grover 1974).

Cardiac function in pneumonia. Pneumonia may pose an excessive load upon the myocardial function since cardiac failure has been noted to be the single most frequent cause of death in pneumonia (Herzog et al. 1959; Adler et al. 1964). Electrocardiographic studies on patients with pneumococcal pneumonia were conducted by Thomson et al. (1946). Deviations such as inverted T-wave, prolonged P-R period and elevated S-T segments were frequently found in the electrocardiographic tracing during pneumonia. These investigators proposed that change in T-wave may be of importance in the differential diagnosis of pneumococcal pneumonia and myocardial diseases in patients in whom clinical evidence was not definitive. However, post mortem examination of the hearts of 9 patients who died from pneumococcal pneumonia showed no significant structural abnormalities (Thomson et al. 1946).

Boucher et al. (1974) studied the mechanical performance of myocardial muscle during diplococcus pneumonia infection in monkeys. They observed a decrease in myocardial contraction associated with hyperthermia and tachycardia 16 hours after inoculation of the pneumococcus. They also reported that all parameters returned to

relatively normal levels 4 days post inoculation. These investigators did not explain why myocardial depression occured in coincidence with the febrile response and tachycardia. However, previous studies by Benson et al. (1970) showed that 39% of patients who died of pneumococcal pneumonia exhibited inflammatory changes in the myocardium compatible with an acute myocarditis. Therefore, these studies suggest that myocarditis may be an important factor in the depressed cardiac contractility reported by Boucher et al. (1974).

Decreased cardiac output was found to be one of the cardiovascular disturbances in some patients with acute pneumococcal pneumonia (Benson et al. 1970; Kumar et al. 1970). The primary mechanism for the low cardiac output was thought to be a depression of myocardial activity. However, in the study of Kumar et al. (1968), it was suggested that the decreased intravascular blood volume may contribute to the inadequate cardiac output during pneumonia.

Alder et al. (1964) studied the pulmonary and systemic circulations in 25 patients with acute lobar pneumonia. They found that mean values for cardiac output, stroke volume, and heart rate were all normal compared to 25 control subjects. They also reported that left ventricular output exceeded right ventricular output in 6 patients by a mean of 10%. The increased left ventricular output was attributed to an elevated bronchial blood flow in pneumonia.

More recent investigations by Light et al. (1981) showed no significant differences in central hemodynamics between a control and a pneumonic group of dogs. It appears that the results of the studies on the cardiac output response in pneumonia are still conflicting. Although the myocardial muscle may be affected during pneumonia, most

studies have not found a decrease in cardiac output during the early stage of the disease.

A final conclusion cannot be drawn on how microorganisms affect pulmonary hemodynamics and regional distribution of pulmonary blood flow in the diseased lungs. It appears that blood flow through pneumonic regions of lung varies with the stage of inflammation and type of bacteria. Robinson (1982) suggests that the decreased right-to-left shunt later in pneumonia may be because the products of inflammation are no longer overriding the hypoxic vasoconstriction or because ventilation returns to the previously consolidated lung.

Lung Function and Pathological Changes in Acute Pneumonic Pasteurellosis

The anatomy of the bovine lung, in health and with pasteurellosis, has received more attention than its function. The bovine lung is a completely lobulated organ characterized by a lack of collateral ventilation (McLaughlin et al. 1961). Each lobule has only one pathway to supply ventilation during respiration and as a result, airway obstruction in cattle may have profound effects on gas exchange because there are no alternative pathways for gas to enter the obstructed lobules (Slocombe et al. 1981). The lobular structure of the bovine lung may also facilitate atelectasis subsequent to airway obstruction because the loose connective tissue surrounding each lobule may reduce interdependence forces that would assist in preventing collapse of the lobule (Mead et al. 1970). It is also reported that cattle have a relatively small gas exchange surface area in relationship to their metabolic rates and minute ventilation (Veit and Farrell 1978). This may facilitate the exposure of the lung to

environmental hazards and jeopardize gas exchange with minor disturbances in respiratory structure. Other structural properties of the bovine lung may also influence function and predispose to pneumonic pasteurellosis. The bovine lung has relatively few alveolar macrophages (Rybicka et al. 1974) and these are thought to require high oxygen tensions for normal function (Stossel 1974). If terminal bronchiolar obstruction occurs in the bovine lung, the phagocytic function of macrophages may be impaired due to hypoxia thus facilitating growth of bacteria and initiation of acute lung injury. It is generally accepted that local blood flow in the lungs is matched to local ventilation by the vasoconstrictor response to alveolar hypoxia. The pulmonary vascular response to hypoxia is characterized by interspecies as well as intraspecies differences (Kuida et al. 1962). Cattle are known for their highly reactive pulmonary vascular response to alveolar hypoxia. It has been found that cattle develop a greater pulmonary hypertensive response to hypoxia than do dogs, cats or sheep. The larger amount of medial smooth muscle in the small pulmonary arteries of the bovine species may contribute to this phenomenon (Alexander 1963).

These anatomical and functional properties appear to place cattle in a disadvantaged position against pathogens arriving to the lung. It is also believed that such structural disadvantages have an important role in predisposing to shipping fever (acute pneumonic pasteurellosis).

Shipping fever is a disease syndrome of cattle which remains an economically important disease in North America. It is characterized by high fever, anorexia, respiratory distress, and coughing following

a period of stress. The difficulty with eliminating shipping fever as a problem of cattle has been due in part to the inability to identify a causative agent responsible for the lesions. Although numerous microbiological organisms have been identified in cattle affected with the clinical and pathologic changes compatible with shipping fever, P. haemolytica plays a central role in causing the pulmonary damage associated with this disease condition. The development of serologic tests for the various strains of Pasteurella and their association with shipping fever indicate that P. haemolytica serotype 1A is the principle agent involved in extensive pulmonary injury (Rehmtulla and Thomson 1981). However, P. multocida is also an important pathogen of cattle, sheep and pigs and a cause of pneumonia and hemorrhagic septicemia (Prodjoharjono et al. 1974). Pasteurella haemolytica causes septicemia and pneumonia only in domesticated sheep, pigs and cattle (Smith et al. 1972). Therefore, P. haemolytica is considered to be an important cause of the severe, life threatening lesions of shipping fever, although a variety of predisposing factors and initiating viruses can be involved.

Pasteurella haemolytica is a gram negative bacterium, considered a normal resident of the nasal cavity of bovine species (Lillie 1974). Following periods of stress as a result of shipping or cold weather, aerosols containing these microorganisms are generated and deposited in the lung of these animals (Pass and Thomson 1971). Stresses may impair phagocytic responses by means of a variety of immunosuppressive mechanisms and may facilitate growth of bacteria and initiation of pneumonia (Jericho and Magwood 1977; Kelley 1986). Pasteurella haemolytica predominates in the upper respiratory tract of stressed or

pneumonic calves (Thomson et al. 1975) as well as the lower respiratory tract of cattle dying of shipping fever (Rehmtulla and Thomson 1981).

Gross investigation of the lungs infected with P. haemolytica shows congestion, consolidation, and fibrinous pleuritis (Slocombe et al. 1984a; Ames et al. 1985). Suppurative lymphadenitis of bronchial and anterior mediastinal lymph nodes are also reported (Slocombe et al. 1984a).

Histopathological studies show that the most obvious lesions are areas of fibrin and edema fluid accumulation in interlobular septa and/or within alveoli. In the early stages of disease, the alveolar architecture may either remain normal, or there may be coagulative necrosis of the entire area (Ames et al. 1985). Accumulation of a mixed inflammatory cell exudate, erthyrocytes, degenerating neutrophils and bacteria in the lumen of bronchioles, alveolar ducts and alveoli are demonstrated in acute pneumonic pasteurellosis of neonatal calves (Slocombe et al. 1984a). Intratracheal inoculation of P. haemolytica in calves causes lesions in the pulmonary vascular bed characterized by congestion of pulmonary veins and capillaries with margination of leukocytes, principally neutrophils, 12 hours post inoculation (Slocombe et al. 1984a).

Acute pneumonic pasteurellosis causes alterations in pulmonary mechanics and gas exchange, but the mechanisms by which the organisms initiate lung damage are not understood. Slocombe et al. (1984b) demonstrated an increase in minute ventilation, frequency of respiration, dead space/tidal volume ratio and dead space volume in neonatal calves intratracheally challenged with P. haemolytica. These

changes indicate the early deterioration of lung function. They also reported a severe arterial hypoxemia concurrent with pronounced changes in dynamic compliance 3 hours after inoculation and an increase in airway resistance by 12 hours after intratracheal inoculation of $2X10^9$ P. haemolytica organisms. The decrease in dynamic compliance and increase in airway resistance may be indicative of diffuse peripheral airway obstruction with exudates. Pasteurella haemolytica resulted in a decrease in circulating white blood cells by 3 hours after inoculation. The decrease in circulating leukocytes was attributed to the accumulation of neutrophils in the lung (Slocombe et al. 1984a). Because neutrophils are thought responsible for several forms of vascular injury in the lung (Slocombe et al. (1985), neutrophils depleted calves by using 5-hydroxyurea protected against the development of hypoxemia, hypercarbia, tachypnea, and the development of characteristic pneumonic lesions subsequent to P. haemolytica challenge. Therefore, the functional changes and clinical signs of lung injury were neutrophil-mediated, rather than due to the direct effect of P. haemolytica. To ensure that the protection was due to neutrophil depletion and not a non-specific effect of 5-hydroxyurea, these investigators evaluated the effect of Pasteurella inoculation before the development of neutropenia following administration of 5-hydroxyurea. These animals developed gas exchange impairment, hypoxemia and lung lesions typical of pneumonic pasteurellosis. This observation indicates that the protective effects of 5-hydroxyurea were related to neutrophil depletion and not to direct effects of hydroxyurea on bacterial growth.

Statement of Objectives

In the literature there are extensive descriptions of the pathological changes in the lung of cattle with acute pneumonic pasteurellosis and descriptions of gas exchange abnormalities. The pulmonary vascular endothelium and smooth muscle are a key and early target of acute lung injury induced by bacteria or other agents. In addition, many disorders of the lung, heart, and respiration are associated with alterations of the pulmonary circulation. For these reasons and because there are no descriptions of circulatory function in pasteurellosis, I investigated the pulmonary circulation during acute pneumonic pasteurellosis. The first objective of this dissertation was to investigate the effects of intratracheal inoculation of $2X10^9$ P. haemolytica organisms on the pulmonary and systemic circulation. In addition, I determined if P. haemolytica challenge altered the response of the pulmonary circulation to alveolar hypoxia.

The mechanism of the arterial hypoxemia associated with acute pneumonic pasteurellosis has never been investigated. Slocombe et al. (1984a) suggested that pneumonic hypoxemia was due to persistence of pulmonary blood flow to the consolidated and non-ventilated regions of lung. Since optimal gas exchange in the normal lung requires the matching of ventilation and blood flow, the second objective of this study was investigation of the distribution of pulmonary blood flow to both healthy lung regions and regions inoculated with $5X10^8$ P. haemolytica organisms.

Much of the study of the mechanisms of lung injury focuses on pulmonary vascular endothelium and smooth muscle, and on the production, metabolism, and biological effects of AA metabolites that can modify the structure and function of pulmonary vessels and microvessels. Acute pneumonic pasteurellosis may cause pulmonary vascular damage either directly and/or indirectly by activation of neutrophils, resulting in production of AA metabolites. The third objective was therefore to assess the role of AA products in regional pulmonary hemodynamics following local inoculation of P. haemolytica in the lung.

CHAPTER 3.

HEMODYNAMIC EFFECTS OF ACUTE PNEUMONIA EXPERIMENTALLY INDUCED IN NEWBORN CALVES INOCULATED WITH *PASTEURELLA HAEMOLYTICA*

SUMMARY

Hemodynamic responses to acute pneumonia and to hypoxia were investigated in 10 newborn calves. Experiments were performed on heparinized, anesthesized and ventilated calves. Control calves were inoculated intratracheally with bovine fetal serum. Pneumonia was induced in treated calves by intratracheal inoculation of *P. haemolytica* suspended in bovine fetal serum. Before inoculation (baseline), at the time of inoculation (T = 0), and at 30-minute intervals for 3 hours, pulmonary arterial pressure (Ppa), systemic arterial pressure (Psa), cardiac output (CO), arterial blood gases, and pulmonary and systemic vascular resistances (PVR and SVR, respectively) were determined. At T = 0, calves in both groups became hypoxemic, alveolar-arterial oxygen difference, PVR, and Ppa increased, and CO and SVR remained unchanged. At subsequent measurement intervals, all values returned to baseline values in control calves, whereas treated calves had progressive hypoxemia associated with a decrease in Ppa and PVR, with no change in CO. Three hours after inoculation and after inhalation of 10% oxygen in nitrogen, PVR increased significantly in the control calves. In the treated group, hypoxia did not increase the resistance as compared with baseline and 3-hour values. The data indicate decreased Ppa during pneumonic pasteurellosis is due to a decrease in PVR and that

pneumonia may attenuate the normal pulmonary hypoxic vasoconstrictor response.

INTRODUCTION

Abnormalities in pulmonary mechanics suggest hypoxemia caused by Pasteurella haemolytica in newborn calves is due to ventilation/perfusion ($\dot{V}/\dot{Q}$) mismatching (Slocombe et al. 1984a; 1984b). Pulmonary arteries of newborn calves constrict vigorously in response to hypoxia (Kuida et al. 1962; Will et al. 1975). and should direct blood flow away from diseased and poorly ventilated lung regions toward the normal regions of lung (Hiser et al. 1975). Hypoxic vasoconstriction should therefore protect calves against the development of $\dot{V}/\dot{Q}$ inequality. The objectives of the experiments described in this chapter were to identify the cardiovascular changes in acute pneumonia induced by intratracheal inoculation of P. haemolytica and to determine if the pulmonary vascular response to hypoxia is altered by pneumonia.

METHODS

Ten 1- to 2-week-old calves (5 control, 5 treated), weighing 40 to 43 kg, were anesthetized with chloralose (80 mg/kg of body weight) and urethane (500 mg/kg), placed in sternal recumbency, and mechanically ventilated using a constant-volume ventilator (Harvard pump, model 615, Harvard Apparatus Co., Inc., Millis, MA) with a tidal volume of 20 ml/kg. Respiratory rate was adjusted to maintain an end-expired CO_2 fraction between 4.8 and 5%. Minute ventilation ($\dot{V}_E$) was monitored by connecting a wedge spirometer (model 570 wedge

spirometer, Medical Science Electronics Inc., St. Louis, MO) to the expiratory port of the ventilator. Rectal temperatures were determined, using a rectal thermometer. A polyethylene catheter was placed into the left carotid artery and used to collect arterial blood samples and measure systemic arterial pressure (Psa). The left jugular vein was surgically exposed, and a balloon-tipped catheter (Swan-Ganz flow-directed monitoring catheter, model 93-110-5F, American Edwards Laboratories, Irvine, CA) was inserted and advanced, with continuous pressure monitoring, into the pulmonary artery to obtain the pulmonary arterial pressure (Ppa). Systemic and pulmonary arterial pressures were measured by use of transducers calibrated against a mercury manometer and adjusted to be level with the point of the left shoulder.

Polyethylene catheters also were introduced into the right femoral artery and vein. The femoral artery catheter was connected to the inlet of the arterial cuvette of a continuous arteriovenous oxygen difference ($A\text{-}VO_2$) analyzer (Avox, Avox Systems, Inc. San Antonio, TX) (Shepherd and Burger 1979). The femoral vein catheter was advanced, with continuous pressure monitoring, into the right ventricle to obtain mixed venous blood and then was connected to the inlet of the venous cuvette of the Avox. A smaller catheter (Venocath 14 g I.V. catheter, Abbott Laboratories, North Chicago, IL), was introduced into the right jugular vein and received the outflow of the Avox system. Immediately after catheterization, each calf was given heparin (500 u/kg, IV) and blood was circulated through the Avox cuvettes, using a double-roller pump (Masterflex pump, model 7564-00 with 2 xx 7014 heads, Cole-Palmer Instruments Co., Chicago, IL) with a constant flow

rate of 10 to 12 ml per minute. The zero of the Avox was determined by running arterial blood through both arterial and venous cuvettes, using a shunt circuit. The Avox signal, the wedge spirometer output, and all pressure signals were displayed on a 12-channel physiograph.

After baseline measurements were taken, each calf was disconnected from the ventilator. Pneumonia was induced in the treated calves by intratracheal inoculation of 2×10^9 P. haemolytica suspended in 20 ml bovine fetal serum (BFS). The method used for preparation of P. haemolytica, inoculation of each calf, and verification of pneumonia have been described previously (Slocombe et al. 1984a). Control calves were inoculated with 20 ml of BFS. Calves were reconnected to the ventilator and all measurements were taken immediately after inoculation with BFS or the P. haemolytica suspension (T = 0). Measurements were then repeated every 30 minutes for 3 hours.

At each measurement period, expired gas composition was determined by use of an oxygen analyzer (model OM-14 oxygen analyzer, Beckman Instruments Inc., Fullerton, CA) and a carbon dioxide analyzer (model 1L-713, digital pH/blood gas analyzer, Instrumentation Laboratory Inc., Lexington, MA) and was used to calculate oxygen consumption ($\dot{V}O_2$) (West 1979). Arterial blood samples were obtained at the midpoint of a 5-minute gas collection period and were analyzed for oxygen tension (PaO_2), carbon dioxide tension ($PaCO_2$) and pH. Alveolar gas tension (PAO_2), dead space/tidal volume ratio (VD/VT), alveolar-arterial oxygen difference (PAO_2-PaO_2) and carbon dioxide production ($\dot{V}CO_2$) were calculated, using standard respiratory gas equations (West 1979; Cohn 1985). Cardiac output (CO) was

calculated, using the Fick principle. Pulmonary vascular resistance (PVR) was computed as (Ppa-8)/CO and systemic vascular resistance (SVR) was calculated as (Psa-5)/CO. Left and right atrial pressure were assumed to be 8 mm of Hg and 5 mm of Hg, respectively.

Each calf was exposed to a hypoxic gas mixture of 10% oxygen in nitrogen 3 hours after intratracheal inoculation. All measurements were repeated when pulmonary and systemic pressures reached steady state.

Data were analyzed by use of a factorial analysis of variance (split plot design). Tukey's omega multiple comparison test (Steel and Torrie 1980) was used to compare the means of each measurement period when the calculated F-values were significant at $P<0.05$.

RESULTS

Changes in PaO_2 of control calves were not significant except at T = 0, when PaO_2 decreased transiently (Figure 1). In treated calves, PaO_2 decreased at T = 0, increased again at post-inoculation minute (PIM) 30, and then decreased progressively. The PAO_2-PaO_2 remained unchanged in control calves except at T = 0, when PAO_2-PaO_2 increased significantly above baseline values. In treated calves, PAO_2-PaO_2 also increased at T = 0, decreased again, and then increased progressively.

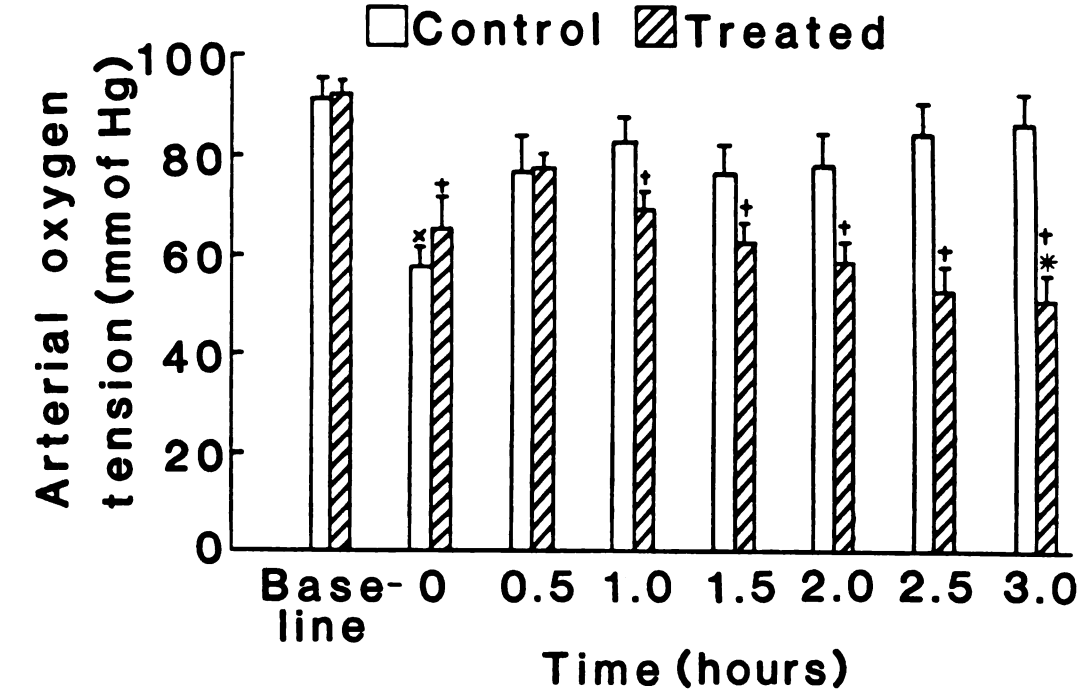
Mean pulmonary arterial pressure increased in both groups of calves at T = 0 and returned to baseline values in the control calves. In treated calves, Ppa was significantly less than in control calves from T = 0 to post-inoculation hour (PIH) 3 (Figure 2). Cardiac output did not change in control or treated calves (Figure 2).

Figure 1. Arterial oxygen tension and alveolar-arterial oxygen difference in control and treated calves. Calves were inoculated with Pasteurella haemolytica (treated) or with bovine fetal serum (control) at T = 0.

*Significant difference between groups ($P<0.05$).

xSignificant difference within control group between preinoculation (baseline) values and values of the calves at subsequent evaluation periods after inoculation.

+Significant difference within treated group between baseline values and values of the calves at subsequent evaluation periods.


Control
Treated
Arterial oxygen tension (mm of Hg)
100
80
60
40
20
0
Base-line
0
0.5
1.0
1.5
2.0
2.5
3.0
Time (hours)

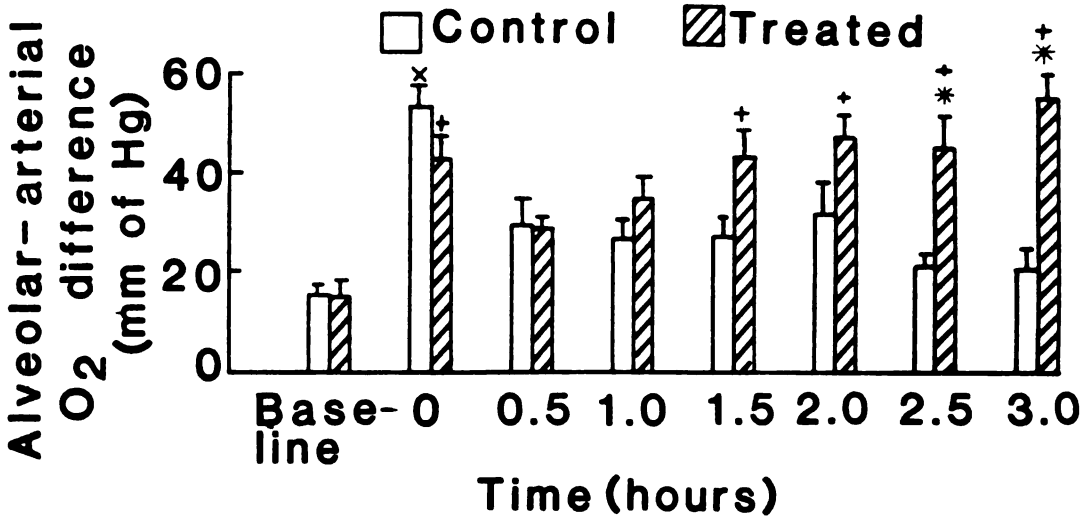
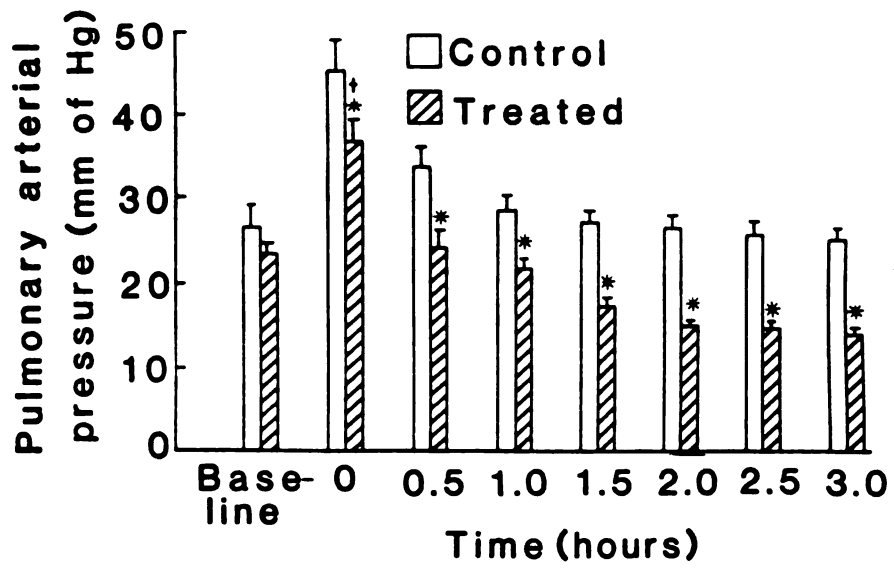

Control
Treated
Alveolar-arterial O_2 difference (mm of Hg)
60
40
20
0
Base-line
0
0.5
1.0
1.5
2.0
2.5
3.0
Time (hours)

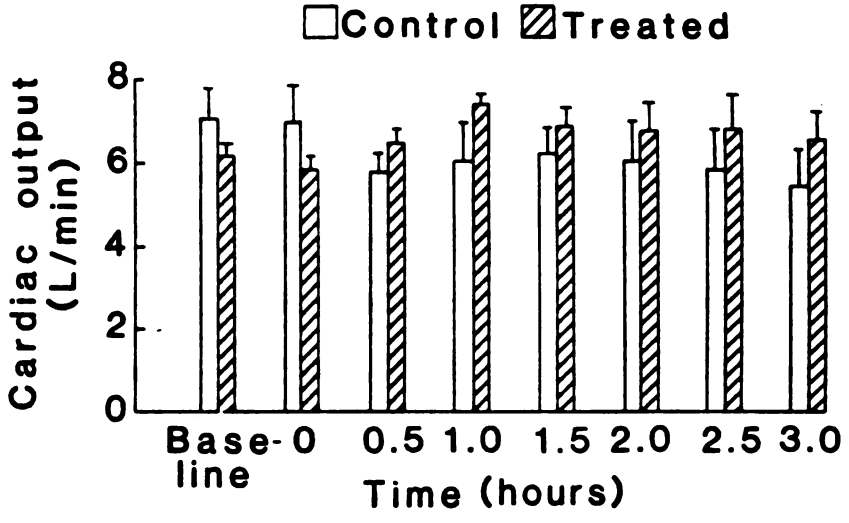
Figure 2. Pulmonary hemodynamics of control and treated calves. Calves were intratracheally inoculated with Pasteurella haemolytica (treated) or with bovine fetal serum (control) at T = 0.

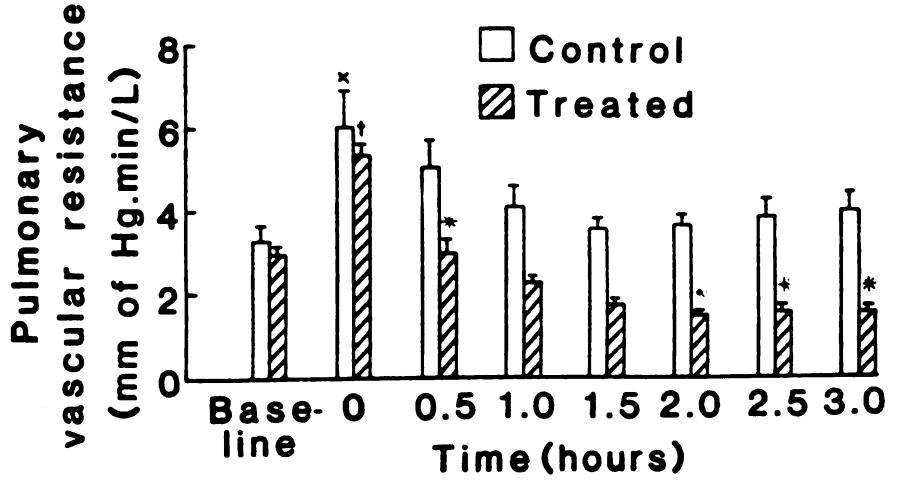
*Significant difference between groups ($P<0.05$).

xSignificant difference within control group between preinoculation (baseline) values and values of the calves at subsequent evaluation periods.

+Significant difference within treated groups between baseline values and values of the calves at subsequent evaluation periods after inoculation.


Pulmonary arterial pressure (mm of Hg)
50
40
30
20
10
0
Control
Treated
Base-line
0
0.5
1.0
1.5
2.0
2.5
3.0
Time (hours)


Control
Treated
Cardiac output (L/min)
8
6
4
2
0
Base-line
0
0.5
1.0
1.5
2.0
2.5
3.0
Time (hours)


Pulmonary vascular resistance (mm of Hg.min/L)
8
6
4
2
0
Control
Treated
Base-line
0
0.5
1.0
1.5
2.0
2.5
3.0
Time (hours)

Pulmonary vascular resistance increased significantly in control and treated calves at T = 0, returned to baseline values in control calves, and declined significantly in treated calves at PIM 30 and at PIH 2, 2.5, and 3 as compared with values for control calves (Figure 2).

As PaO_2 decreased from 92 mm of Hg (baseline) to approximately 60 mm of Hg at (T = 0) in both groups, PVR increased from 3.24 to 6.07 mm of Hg min/L in control calves and from 2.8 to 5.3 mm of Hg min/L in treated calves. In control calves, PaO_2 was 86.6 mm of Hg and the PVR remained slightly above the baseline value at PIH 3. A linear relationship was found between PaO_2 and PVR in control calves, which was maintained for the entire study (Figure 3). In treated calves at PIH 3, hypoxemia of similar magnitude to that observed at T = 0 was not associated with increased PVR and PVR decreased to 1.45 mm of Hg min/L (a decrease of approximately 50%) as PaO_2 decreased to 52 mm of Hg. Alveolar oxygen tension, VD/VT, $PaCO_2$, $\dot{V}O_2$, $\dot{V}CO_2$, Psa, SVR, stroke volume (SV), and HR did not change significantly during the study (Tables 1 and 2).

Immediately after the 3-hour measurement period, the hemodynamic response to hypoxia was evaluated in the control and treated calves and the results were compared with the baseline and PIH 3 values. In control calves, Ppa increased significantly during hypoxia. A significant change in Ppa was not found in treated calves (Figure 4). In control calves, PVR increased significantly as the PaO_2 decreased to 30 mm of Hg. In treated calves with pneumonia, hypoxia did not significantly increase the resistance as compared with baseline and PIH 3 values. The difference in response to hypoxia was not

Figure 3. Relationship between arterial oxygen tension (PaO_2) and pulmonary vascular resistance in calves inoculated with bovine fetal serum (controls, ●) or with Pasteurella haemolytica (treated, x) before inoculation (baseline), at the time of inoculation (T_0), and 3 hours after inoculation (T_3).

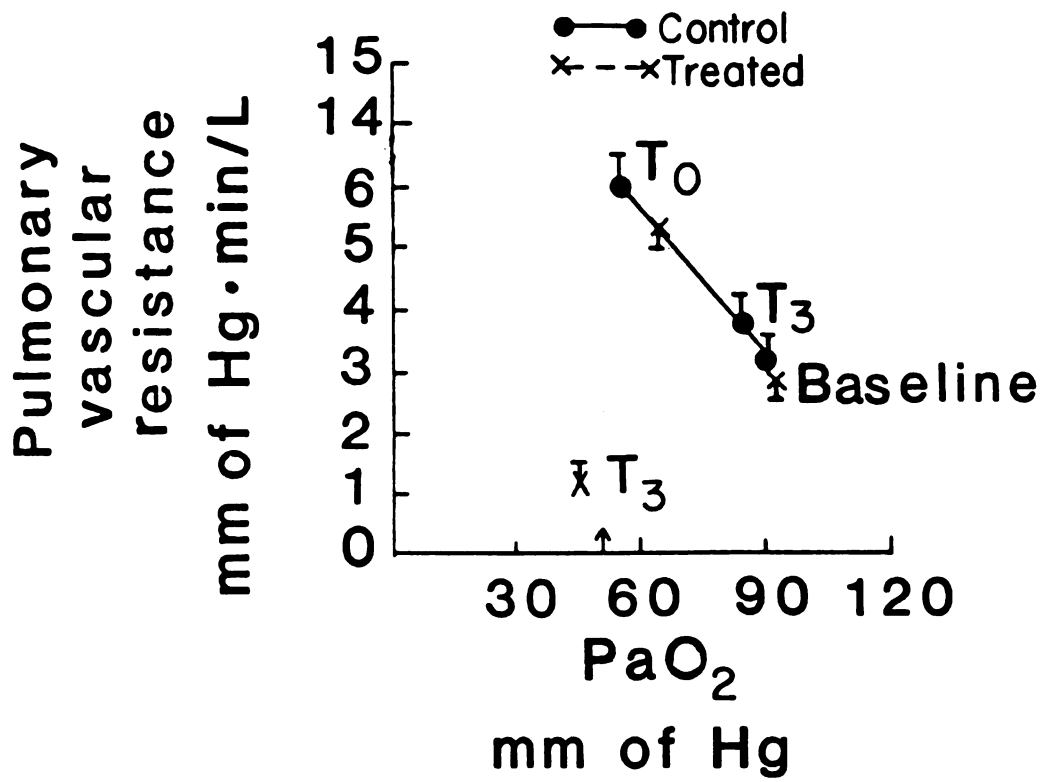

Control
Treated
Pulmonary vascular resistance
mm of Hg·min/L
15
14
6
5
4
3
2
1
0
T_0
T_3
Baseline
T_3
30 60 90 120
PaO_2
mm of Hg

Table 1. Alveolar oxygen tension (PAO_2), arterial CO_2 tension ($PaCO_2$), dead space/tidal volume ratio (VD/VT), carbon dioxide production ($\dot{V}CO_2$), and O_2 consumption ($\dot{V}O_2$) in calves intratracheally inoculated with bovine fetal serum (controls) or with Pasteurella haemolytica (treated calves)

Hours after inoculation	PAO_2 (mm of Hg)		$PaCO_2$ (mm of Hg)		VD/VT		$\dot{V}CO_2$ (ml/min)		$\dot{V}O_2$ (ml/min)	
	Control	Treated	Control	Treated	Control	Treated	Control	Treated	Control	Treated
Baseline	107.0±4.1	108.0±2.4	34.8±2.0	34.6±1.0	0.31±.03	0.29±.03	204.0±11.6	197.0±.9	281.0±18.0	247.0±9.0
0	110.0±2.2	108.0±2.8	34.4±2.0	35.8±1.0	0.33±.03	0.29±0.01	209.0±12.0	199.0±3.8	270.0±7.0	253.0±9.0
0.5	106.0±2.2	107.0±3.0	36.6±2.0	37.3±1.7	0.27±.06	0.29±.03	194.0±8.0	197.0±10.2	257.0±9.0	250.0±13.0
1.0	109.0±2.0	104.0±3.0	35.7±.64	37.0±2.0	0.30±.04	0.28±.02	201.0±7.0	199.0±12.0	257.0±15.0	271.0±9.0
1.5	104.0±2.0	106.0±2.2	38.5±1.2	37.3±1.3	0.32±.03	0.27±.03	192.0±4.4	199.0±6.5	257.0±12.0	261.0±12.0
2.0	110.0±3.0	105.0±2.8	36.2±1.0	38.6±1.3	0.30±.03	0.32±0.01	200.0±5.7	195.0±11.4	240.0±13.0	241.0±8.0
2.5	106.0±4.0	99.0±3.9	38.6±2.5	40.6±1.7	0.31±.04	0.40±0.04	185.0±4.7	206.0±21.0	232.0±7.0	274.0±30.0
3.0	108.0±3.4	106.0±1.6	37.2±1.2	39.1±2.1	0.29±.02	0.44±.08	187.0±6.6	170.0±10.5	235.0±18.0	216.0±13.0

Data are expressed as the mean ± SE (n=5/group).

Table 2–Mean systemic arterial pressure (Psa), systemic vascular resistance (SVR), heart rate (HR), and stroke volume (SV) in calves intratracheally inoculated with bovine fetal serum (controls) or with Pasteurella haemolytica (treated calves).

Hours after inoculation	Psa (mm of Hg)		SVR (mm of Hg min/L)		HR (beat/min)		SV (ml)	
	Control	Treated	Control	Treated	Control	Treated	Control	Treated
Baseline	126.8±7.6	121.0±5.3	17.7±2.8	18.7±1.4	165.6±11.0	155.0±3.5	34.7±5.5	34.3±4.0
0	150.0±7.6	147.0±9.7	21.7±3.0	24.7±2.7	177.6±15.0	177.6±9.0	34.7±5.9	36.9±2.1
0.5	119.0±11.2	135.0±7.0	19.8±2.9	19.7±1.9	157.0±16.0	171.0±6.7	32.5±5.0	31.7±3.8
1.0	126.0±7.0	140.4±9.0	21.0±3.0	18.2±1.5	169.0±10.0	177.6±4.4	29.8±4.6	33.2±5.1
1.5	121.4±11.1	140.4±14.0	18.5±2.7	20.2±3.5	166.8±14.0	178.8±5.8	31.8±4.7	34.5±5.0
2.0	113.2±14.5	137.4±16.6	17.8±3.3	21.3±4.7	162.0±15.9	181.0±9.2	27.4±4.5	35.7±5.0
2.5	112.0±14.4	127.0±12.0	20.6±4.7	18.5±2.3	162.0±14.9	181.0±7.4	29.9±6.0	36.5±5.0
3.0	110.6±12.8	117.0±13.4	21.0±4.5	17.0±1.8	158.0±13.9	172.8±7.4	30.6±5.8	36.0±4.2

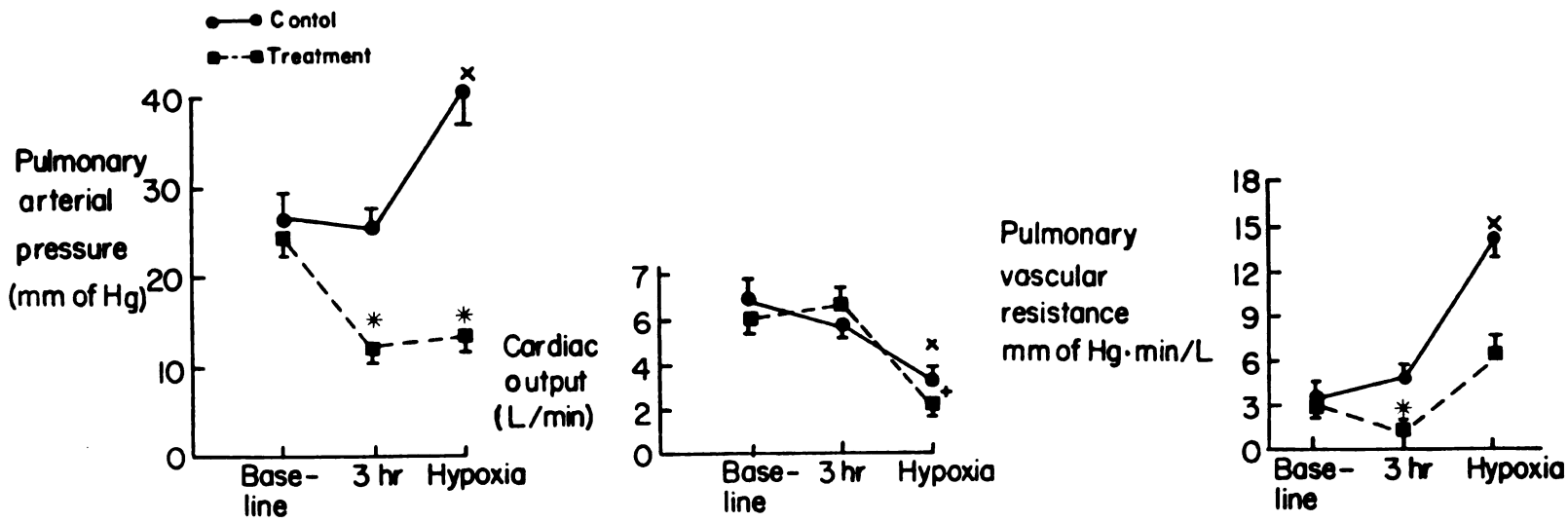
Data are expressed as the mean ± SE (n=5/group).

Figure 4. Pulmonary hemodynamic responses to hypoxia in calves intratracheally inoculated with bovine fetal serum (control, ●) or with Pasteurella haemolytica (■).

*Significant difference between groups ($P<0.05$).

xSignificant difference ($P<0.05$) within the control group between the hypoxia value and both the preinoculation (baseline) and postinoculation hour (PIH) 3 values.

+Significant difference ($P<0.05$) within the treated group between the hypoxia value and both the preinoculation (baseline) and PIH 3 values.


Contol
Treatment
Pulmonary arterial pressure (mm of Hg)
40
30
20
10
0
Base-line
3 hr
Hypoxia
Cardiac output (L/min)
7
6
4
2
0
Base-line
3 hr
Hypoxia
Pulmonary vascular resistance mm of Hg·min/L
18
15
12
9
6
3
0
Base-line
3 hr
Hypoxia

significant between groups (Figure 4). Therefore, pulmonary response to hypoxia was diminished during pneumonic pasteurellosis. Cardiac output decreased in both groups after breathing 10% oxygen. Differences in PaO_2 and PAO_2-PaO_2 between control and treated calves were decreased during hypoxia (Figure 5), as compared to the same measurements at PIH 3.

DISCUSSION

Pneumonic pasteurellosis caused substantial hypoxemia accompanied by pulmonary hypotension. Pulmonary hypotension was caused by the decrease in PVR, because CO did not change. These findings may have been due to vasodilation in all lung vessels or to a local decrease in PVR in the diseased regions of lung. Pneumonic hypoxemia was not due to alveolar hypoventilation because $PaCO_2$ was unchanged. The increased PAO_2-PaO_2 indicated that hypoxemia may have been due to $\dot{V}/\dot{Q}$ inequality, shunt, or diffusion impairment. The $\dot{V}/\dot{Q}$ inequality may have resulted from persistence of, or increase in, perfusion to the diseased regions of lung, in which ventilation had been impaired.

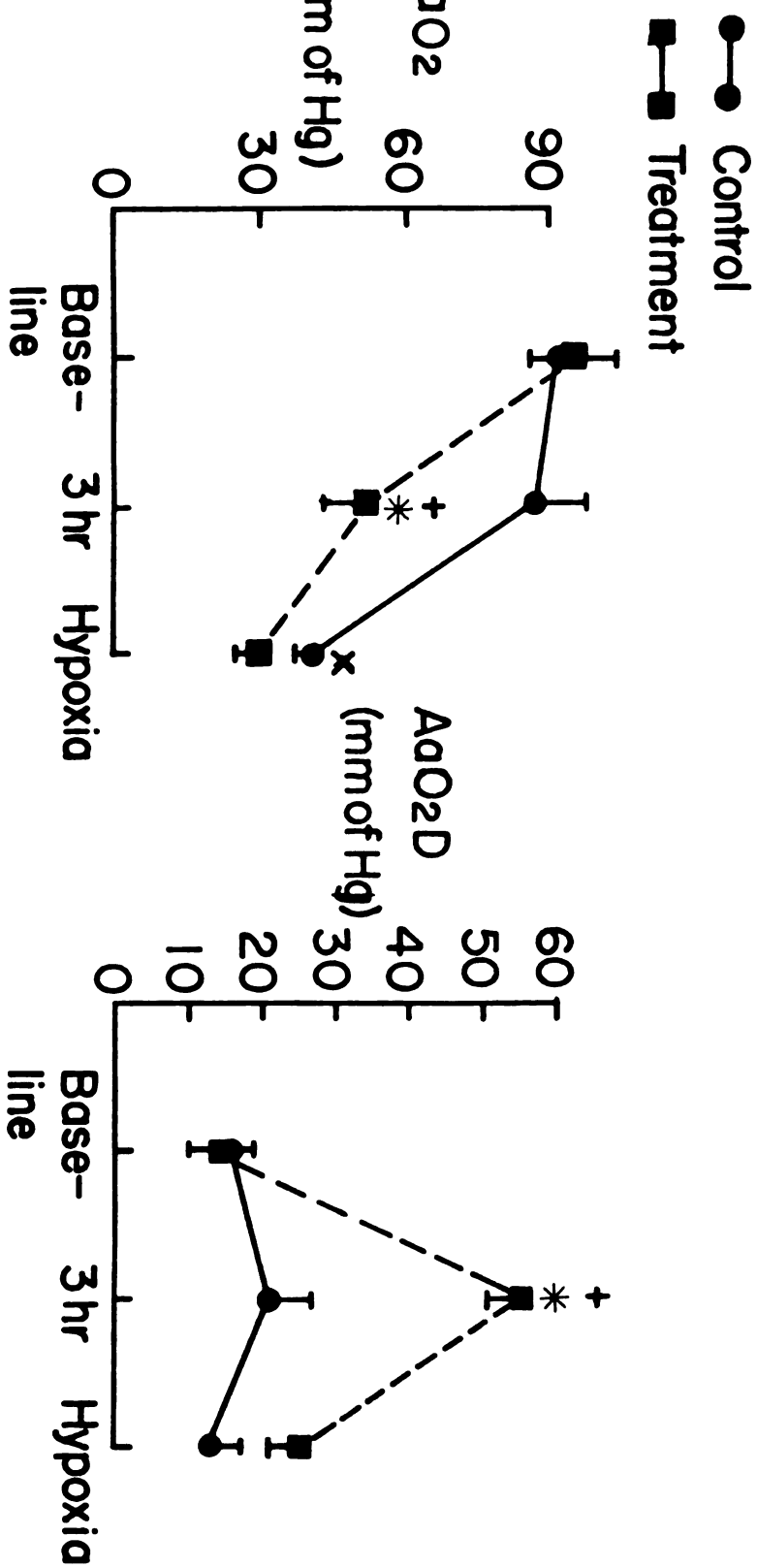
The pulmonary artery of healthy calves constricts vigorously in response to hypoxia and this phenomenon is thought to divert blood flow away from poorly ventilated lung regions (Hiser et al. 1975). Integrity of this response was evidenced in both groups of calves at T = 0, when PVR and Ppa increased and PaO_2 decreased. During pneumonia, hypoxemia developed, but was not accompanied by an increase in Ppa or PVR; Ppa and PVR decreased during pneumonia. Redistribution of blood flow, accounting for the ($\dot{V}/\dot{Q}$) inequality, would be possible if blood vessels were more vasodilated in the inflammed area than in healthy

Figure 5. Effect of hypoxia on arterial oxygen tension (PaO_2) and alveolar-arterial oxygen difference (PAO_2-PaO_2) in calves intratracheally inoculated with bovine fetal serum (controls) or with Pasteurella haemolytica (treated calves).

*Significant difference between groups ($P<0.05$).

xSignificant difference within control group between the hypoxia value and both the preinoculation (baseline) values and post-inoculation hour (PIH)-3 values.

+Significant difference within treated group between the hypoxia value and both the baseline and PIH-3 values.


Control
Treatment
PaO2
(mm of Hg)
90
60
30
0
Base-line
3 hr Hypoxia
AaO2D
(mm of Hg)
60
50
40
30
20
10
0
Base-line
3hr Hypoxia

regions of the lungs. Because total blood flow to the lung (cardiac output) did not change, local vasodilation in inflamed regions probably resulted in reduced blood flow to normal regions of the lungs. Although not significant, VD/VT generally increased at PIH 2.5 and 3 in treated calves. This increase in VD/VT may have resulted from increased alveolar dead space due to reduced perfusion in healthy lung regions.

Lack of vasoconstriction, despite hypoxemia, may have been the result of humoral factors produced by the inflammatory process initiated by P. haemolytica. During acute pneumonic pasteurellosis, the lung is invaded by large numbers of polymorphonuclear leukocytes. Because hypoxemia of pasteurellosis is prevented by neutropenia, neutrophils are thought to mediate pulmonary injury (Slocombe et al. 1985). When activated, neutrophils produce proteases and oxygen radicals, and activate the arachidonic acid cascade. Prostaglandin E_2 (PGE_2) is a powerful vasodilator that may originate from neutrophils. Concentrations of PGE_2 are increased in arterial blood after microembolism-induced pulmonary injury (Malik 1983) and PGE_2 may be responsible for abolition of the pulmonary hypoxic vasoconstriction by E coli endotoxin (Reeves and Grover 1974). Therefore, PGE_2 may be released during lung injury induced by P. haemolytica and cause pulmonary vasodilation. Results of the present study indicated a significant difference in PVR between control and treated calves at PIM 30; therefore, P. haemolytica may have an immediate effect on the pulmonary vasculature. Vasodilation may enhance neutrophil and macromolecule supply to the down-stream vessels at the site of pulmonary injury (Issekutz 1981; Issekutz and Movat 1982). When

activated, these neutrophils are capable of inducing vascular injury and releasing more vasodilator substances. However, results of the present study do not dismiss the possibility of a vasodilator substance within our P. haemolytica inoculum.

Prostacyclin (PGI_2) is produced by the pulmonary endothelium in response to inflammatory and/or mechanical stimuli or in response to various forms of endothelial injury (Weksler et al. 1978). In the present study, prostacyclin may have been released in the injured regions of the lung, thus inducing vasodilation (Gunther et al. 1982). Although prostanoids probably are produced in injured regions of the lungs and probably cause local vasodilation, these and other arachidonic acid metabolites also may circulate in the blood and cause generalized vasodilation within the lungs.

In addition to the possible effect of chemical mediators, mechanical factors may also be the cause of local changes in blood flow. When a region of the lungs does not inflate, mechanical distending forces are accentuated on this region (Mead et al. 1970). Enjeti et al. (1982) suggested that the mechanical interdependence between the sublobar region and the surrounding structures may decrease the pressure around the vessels within the sublobar region, thereby dilating blood vessels, increasing vascular conductance and blood flow, and even overriding the hypoxic vasoconstriction. Acute pneumonic pasteurellosis causes abnormalities in pulmonary mechanics that probably limit inflation of the diseased region (Slocombe et al. 1984b). Therefore, in pasteurellosis, pulmonary interdependence may participate in increasing vascular conductance and pulmonary blood flow through pneumonic regions of lung. This interdependence may

oppose other mechanical and hypoxic effects of pneumonia, which may reduce blood flow and vascular conductance in the diseased region.

To confirm that hypoxic vasoconstriction was impaired in acute pneumonic pasteurellosis, each calf was exposed to a hypoxic gas mixture. The results indicate that the response to hypoxia was much less vigorous in treated calves than control calves. Therefore, the intrapulmonary presence of P. haemolytica may not have completely abolished this major control mechanism for pulmonary vessels completely or only the healthy lung region may have responded to the hypoxia. Hiser et al. (1975) demonstrated that the response to hypoxia was retained in canine pneumococcal pneumonia, 18 and 36 hours after inoculation of the organisms. Because the hypoxic vasoconstrictor mechanism in the experimental model of pneumonia used in the present study was tested 3 hours after inoculation, this contradiction between our results and those of Hiser et al. (1975) could be accounted for by the duration of the disease or the type of bacterium.

Alexander et al. (1963) characterized the gas exchange defect of people with pneumonia by ventilating the patient first with air and then with a hypoxic gas mixture. They observed a narrowing of the PAO_2-PaO_2 gradient, concluding that a major portion of the gas exchange defect was due to right-to-left vascular shunts rather than due to diffusion impairment. In the present study, PAO_2-PaO_2 decreased markedly when calves were ventilated with a hypoxic gas mixture. Thus, in our experimental model of pneumonia, hypoxemia was due in part to right-to left shunts and probably also was due, in part, to regions of lung having $\dot{V}/\dot{Q}$ ratios <1 and >0.

CHAPTER 4.

REGIONAL DISTRIBUTION OF PULMONARY BLOOD FLOW IN ACUTE LOCAL PNEUMONIA INDUCED BY *PASTEURELLA HAEMOLYTICA*

SUMMARY

Acute pneumonic pasteurellosis causes severe arterial hypoxemia accompanied by pulmonary hypotension. We have tested the hypothesis that the decreased pulmonary vascular resistance (PVR) and the arterial hypoxemia associated with pasteurellosis, is caused by regional vasodilation and increased blood flow in the diseased lung regions. In 5 anesthetized calves, regional pneumonia was induced in 15 to 20% of the lung by local inoculation of *P. haemolytica* suspended in bovine fetal serum (BFS). A control group received BFS. Measurements of pulmonary hemodynamics, gas exchange, and distribution of pulmonary perfusion with radiolabeled microspheres, were made before (baseline), 1.5 and 3 hours after local intrapulmonary inoculation of *P. haemolytica*. Acute local pneumonic pasteurellosis resulted in arterial hypoxemia and increased pulmonary perfusion in the pneumonic regions. The increase in regional blood flow was due to a decrease in regional pulmonary vascular resistance (rPVR). The percentage of cardiac output perfusing the diseased lung regions increased by 7% 3 hours after Pasteurella inoculation, whereas venous admixture ($\dot{Q}va/\dot{Q}t$) increased by 15% which suggests that the increase in venous admixture cannot be explained totally by the increased blood flow to the diseased regions of lung. There was no significant change between groups or with time in perfusion or rPVR in the apparently normal lung regions. We conclude that the decrease in PVR is due to local vasodilation of the diseased regions of lung. These data

suggest that pneumonic hypoxemia results from increased shunt and venous admixture, and that hypoxic vasoconstriction is ineffective in directing blood flow away from the pneumonic regions.

INTRODUCTION

We have previously demonstrated that acute pneumonia induced by P. haemolytica causes severe arterial hypoxemia accompanied by pulmonary hypotension. Several studies in humans and in animal models of pneumonia have proposed that pneumonic hypoxemia is largely due to right-to-left vascular shunts through pneumonic regions of lung (Alexander et al. 1963; Wagner et al. 1975) while others suggest hypoxemia is due to ventilation/perfusion ($\dot{V}/\dot{Q}$) imbalance (Clop et al. 1962; Slocombe et al. 1984a).

Because there is a reduction in pulmonary perfusion to the consolidated lung regions in certain types of pneumonia, there are some investigators who advocate the view that pneumonic hypoxemia also stems from abnormal gas exchange in regions of lung that are not grossly consolidated (Goldzimer et al. 1974; Light et al. 1981). The reduction in blood flow to the diseased regions is attributed to local hypoxic vasoconstriction (Hiser et al. 1975). If pulmonary blood flow to the diseased lung regions is reduced by vasoconstriction, this would help to limit hypoxemia whereas the maintenance or increase in perfusion to the diseased areas would result in an increased shunt fraction and depressed arterial oxygen tension. Therefore, the additional mechanism for the pneumonic hypoxemia could be the gas exchange abnormality in regions of lung that are not grossly consolidated.

In experimentally induced streptococcal pneumonia of dogs, Light et al. (1981) reported that hypoxemia is due to an increased shunt as well as venous admixture. These investigators also proposed that the pulmonary hypoxic vasocontrictor mechanism was ineffective in directing blood flow away from the diseased lung regions. We have previously reported a decrease in pulmonary arterial pressure in acute pneumonic pasteurellosis due to a decrease in pulmonary vascular resistance (PVR). Previous studies on pneumonic pasteurellosis have not investigated the relative contribution of pulmonary perfusion in the diseased lung regions to the overall gas exchange abnormality. Therefore, we studied regional distribution of pulmonary blood flow in local pneumonia induced by P. haemolytica to determine 1) whether the observed reduction in PVR is due to pulmonary vasodilation and an increase in blood flow to the diseased lung region, or is due to a generalized vasodilation through the lung vascular bed, and 2) whether pulmonary perfusion to the pneumonic regions completely or partially accounts for the hypoxemia that occurs in this type of pneumonia.

METHODS

Measurements of pulmonary and systemic hemodynamics, gas exchange, and distribution of pulmonary perfusion were made in ten neonatal calves weighing between 30 and 36 kg, divided into two groups, control and treated. Each animal was anesthesized with 80 mg/kg chloralose and 500 mg/kg urethane, given intravenously. Animals were intubated via a midcervical tracheostomy and mechanically ventilated (Harvard pump, model 615, Millis, Mass) using a constant

tidal volume of 20 ml/kg. Respiratory rate was adjusted to give an end expired carbon dioxide fraction of 4.8% to 5.0%. Minute ventilation ($\dot{V}E$) was measured by connecting a wedge spirometer (Medical Science Electronics, Inc., model 570, St. Louis, MO) to the expiratory port of the ventilator. Body temperature was monitored during the experiment. Arterial blood samples were obtained and systemic arterial pressure (Psa) was measured from a femoral artery catheter connected to a pressure transducer (Statham Instruments P23Db, Hato Rey, Puerto Rico). A balloon-tipped catheter (American Edwards Laboratories, model 93-110-5F, Irvine, CA) connected to a second pressure transducer, was introduced into an exposed left jugular vein and advanced, with continuous pressure monitoring, into the pulmonary artery until both pulmonary arterial pressure (Ppa) and wedge pressure (Pw) were obtained. Both transducers were calibrated against a mercury manometer and adjusted to be level with the point of the left shoulder.

Catheters were also introduced into the right femoral artery and vein. The femoral artery catheter was connected to the inlet of the arterial cuvette of the continuous arterio-venous oxygen difference (CaO_2-CvO_2) analyzer (Avox Systems, Inc., San Antonio, TX) (Shepherd and Burger 1977). The femoral vein catheter was advanced, with continuous pressure monitoring, into the right ventricle to obtain mixed venous blood and then connected to the inlet of the venous cuvette of the Avox. A smaller catheter (Venocath 14g IV catheter, Abbott Laboratories, North Chicago, IL) introduced into the right jugular vein received the outflow of the Avox system. Immediately after catheterization, each calf received 50 units/kg heparin

intravenously, and blood was circulated through the Avox cuvettes using a double roller pump (Master flex pump, model 7564-00, Chicago, IL) at a constant flow of 12 ml/min. The Avox signal, the wedge spirometer output and all pressure signals were displayed on a 12-channel physiograph (VR12, Electronics for Medicine, White Plains, NY).

Production of Pneumonia

Local pneumonia was induced in the treated group by inoculating P. haemolytica into the lung by means of a 5.4 mm diameter bronchoscope (Pentax, model FB-15H, Orangeburg, NY). The lobulated lung and lack of collateral ventilation in calves allowed local infusion of organisms and creation of pneumonia restricted to a few lobules. The method we used to induce local pneumonia was a modification of the procedure described by Terrell et al. (1933). Briefly, the bronchoscope was advanced down the tracheobronchial tree and a 2.43 mm diameter catheter was passed through the biopsy channel of the bronchoscope and wedged into the distal part of the following lobes: the posterior part of the right cranial lobe (region 1), the right caudal lobe (region 2), the accessory lobe (region 3), and the left caudal lobe (region 4). An inoculum containing 5×10^8 P. haemolytica organisms suspended in 5 ml bovine fetal serum (BFS) was injected into each lobe. The control group of calves were inoculated with BFS in the same manner as the treated group. India ink (0.5 ml) was added to the BFS to identify the inoculated lobules at necropsy.

We have shown that hypoxemia develops at 1 to 2 hours and the mean pulmonary vascular resistance decreases three hours after

inoculation of P. haemolytica (Chapter 3). We therefore measured gas exchange and distribution of pulmonary perfusion before and 1.5 and 3 hours after inoculation.

Measurement of Gas Exchange

At each measurement period, expired gas composition was determined with oxygen (Beckman, model OM-14, Fullerton, CA) and carbon dioxide analyzers (Beckman, model LB-2, Fullerton, CA) and used to calculate oxygen consumption ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$). Arterial blood samples were obtained at the midpoint of a 5-minute gas collection period and analyzed for oxygen and carbon dioxide tension (PaO_2 and $PaCO_2$), pH, hemoglobin concentration (Hb) and percentage saturation of Hb (% sat) (Instrumentation Laboratory, Inc., model 1L-713, Lexington, MA). Alveolar gas tension (PAO_2), dead space/tidal volume ratio (VD/VT), and alveolar-arterial oxygen difference (PAO_2-PaO_2) were calculated using standard respiratory gas equations. Cardiac output was calculated using the Fick principle.

Measurements of venous admixture $\dot{Q}va/\dot{Q}t$ were made at the 3 measurement periods. Arterial oxygen content (CaO_2) was calculated as CaO_2 = (Hb X %sat X 1.34) + (PaO_2 X 0.003). The mixed venous oxygen content ($C\bar{v}O_2$) was calculated as CaO_2 minus the arterio-venous oxygen difference. End capillary oxygen content ($C\bar{c}O_2$) was calculated from the above equation replacing PaO_2 by calculated PAO_2. Since the PAO_2 values were more than 100 mmHg, the per cent saturation of hemoglobin was assumed to be (100%). The venous admixture was then calculated as $\dot{Q}va/\dot{Q}t = (C\bar{c}o_2-CaO_2)/(C\bar{c}O_2-C\bar{v}O_2)$.

Measurement of Pulmonary and Systemic Hemodynamics

Pulmonary vascular resistance was computed as (Ppa-Pw)/CO, and systemic vascular resistance was calculated as (Psa-5)/CO. The right atrial pressure was assumed to be 5 mmHg. When wedge pressure could not be measured it was assumed to be the pulmonary arterial diastolic pressure minus 3 mmHg (Buchbinder 1976). Stroke volume and heart rate were also obtained in both groups of calves.

Distribution of Pulmonary Perfusion

Regional distribution of pulmonary blood flow was determined in both groups of calves at the three measurement periods. Microspheres (15 μm in diameter) labeled with one of three radionucleotides Ce^{141}, Sr^{85}, and Cr^{51} were injected at baseline, 1.5 hours, and 3 hours after inoculation. The number of microspheres per injection was selected to provide at lease 400 and usually in excess of 900 microspheres in any region of lung selected for counting (Heymann et al. 1977). The stock microsphere suspension (3M Company, St. Paul, MN) had an initial activity of 0.05 mci/ml and the suspending agent was 10% dextran. For fifteen minutes prior to use, the stock suspension was thoroughly mixed using a sonicator and Vortex mixer. Approximately 0.5 ml of the stock solution ($1x10^6$ spheres) was drawn into a syringe and infused into the right atrium through a catheter introduced into the right jugular vein. The infusion catheter was then flushed with 20 ml saline.

At the end of the experiment after the calf was killed with sodium pentobarbital, the lung was exposed and the pulmonary artery and veins were ligated. The lung was excised and the inoculated lung

lobules were separated from those which appeared grossly normal. The inoculated and normal lung regions were weighed. The lung tissues were cut into pieces, placed in vials and the activity was determined using a gamma counter. Using the regional activity, total activity of the whole lung and CO determined by the Fick method, the fraction of the cardiac output perfusing each part of lung at the time of each isotope injection was calculated as ml/min/100 gm. The total flow and per cent of cardiac output going to diseased and normal regions of each lobe was also calculated. Regional pulmonary vascular resistance (rPVR) was computed as the pressure difference across the pulmonary circulation divided by the regional blood flow/100 gm.

Data were analyzed using a factorial analysis of variance. Tukey's omega test was used to compare the means at each measurement period when the calculated F-values were significant at $P<0.05$ (Steel and Torrie 1980).

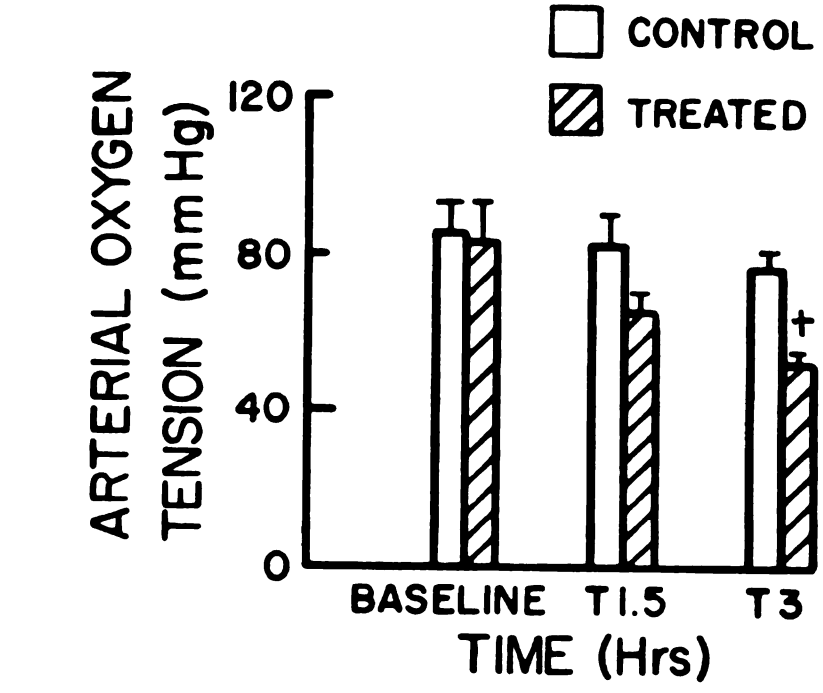
RESULTS

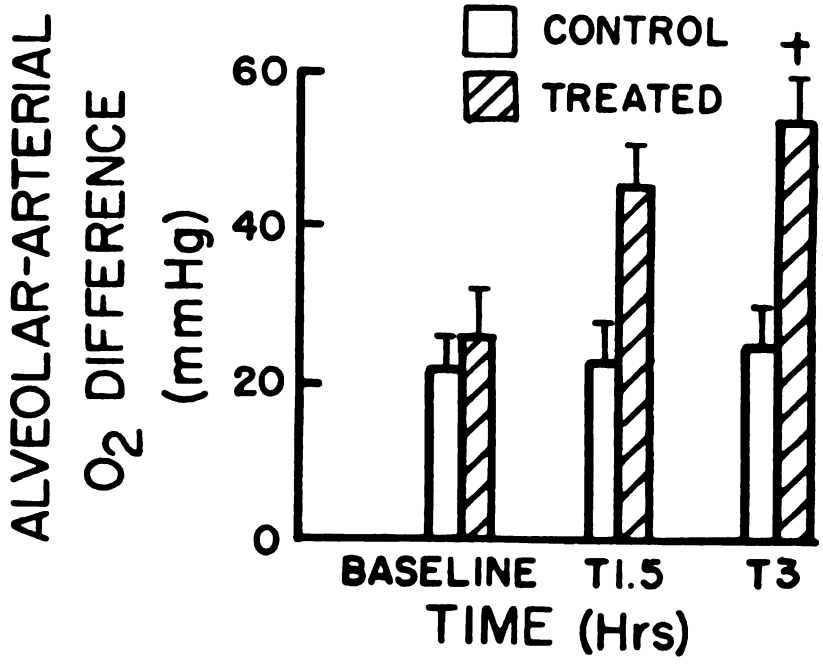
The lung regions inoculated with P. haemolytica had a severe local pneumonia. The infected regions were consolidated and hemorrhagic compared with the healthy regions of lung in the same group of animals or with those which received BFS.

Figure 6 shows PaO_2 and PAO_2-PaO_2 of control and treated animals. PaO_2 decreased substantially in all animals with local pneumonia, whereas no significant change occurred in the control group. Alveolar-arterial oxygen difference remained unchanged in the control group. In the treated group, PAO_2-PaO_2 increased significantly after 3 hours compared with the baseline value in the same group. Alveolar

Figure 6. Arterial oxygen tension and alveolar-arterial oxygen difference before (baseline), 1.5 and 3 hours after local intrapulmonary inoculation of Pasteurella haemolytica (treated group) and bovine fetal serum (control group).

+Indicates statistically significant difference within treated group between baseline and subsequent periods ($P<0.05$).


CONTROL
TREATED
ARTERIAL OXYGEN TENSION (mmHg)
120
80
40
0
+
BASELINE
T1.5
T3
TIME (Hrs)


CONTROL
TREATED
ALVEOLAR-ARTERIAL O_2 DIFFERENCE (mmHg)
60
40
20
0
+
BASELINE
T1.5
T3
TIME (Hrs)

oxygen tension, VD/VT, $PaCO_2$, $\dot{V}O_2$ and $\dot{V}CO_2$ did not change significantly over the course of the experiment (Table 3).

Figure 7 shows the calculated $\dot{Q}va/\dot{Q}t$ values for the two groups of animals. Venous admixture of both groups averaged approximately 11% at baseline. The $\dot{Q}va/\dot{Q}t$ of calves with local pneumonia increased by a mean of 8% by 1.5 hours and 15% by 3 hours after inoculation with P. haemolytica. In the control group, $\dot{Q}va/\dot{Q}t$ did not change significantly with time. There were no radioactive counts in the peripheral blood, heart, or kidneys indicating the absence of vascular shunts in the lungs larger than 15 μm in diameter.

Although mean pulmonary arterial pressure increased at the 1.5 and 3 hour measurement periods in the control group, the change was not significant (Figure 8). In the treated animals, Ppa did not change significantly with time, but after 3 hours, Ppa was significantly less than in the control group at the same measurement period (Figure 8). Cardiac output/kg and PVR did not change in the control and treated group (Figure 8). There were no significant differences between groups of animals or with time in Psa, SVR, stroke volume (SV) and heart rate (Table 4).

Regional Blood Flow

A nested factorial analysis of variance showed significant differences between P. haemolytica and BFS-treated calves but no significant differences between inoculated regions within each treatment group. The data of the individual regions were therefore combined and the treatment groups compared for significant differences. The percentage of lung tissue affected by local

Table 3. Alveolar oxygen tension (PAO_2), dead space/tidal volume ratio (VD/VT), arterial CO_2 tension ($PaCO_2$), carbon dioxide production ($\dot{V}CO_2$) and oxygen consumption ($\dot{V}O_2$) in control and treated animals.

Parameters	Baseline	Hours after inoculation 1.5	3
PAO_2 (mmHg)			
Control	108.5±1.5	106.2±2.7	104.9±2.0
Treated	113.0±1.2	110.2±2.3	111.0±2.9
VD/VT			
Control	0.27±.03	0.30±.03	0.29±.02
Treated	0.29±.04	0.27±.04	0.26±.04
$PaCO_2$ (mmHg)			
Control	35.2±1.7	35.1±1.4	36.8±1.7
Treated	34.0±1.1	36.7±1.8	38.0±1.8
$\dot{V}CO_2$ (ml/min)			
Control	164.0±9.2	156.0±12.7	159.2±16.3
Treated	170.6±10.2	164.8±15.2	162.0±16.2
$\dot{V}O_2$ (ml/min)			
Control	208.6±15.0	194.6±8.3	194.4±21.9
Treated	199.6±12.5	186.6±14.8	175.6±14.3

Data are expressed as the mean ± SE (n=5/group).

Figure 7. Venous admixture ($\dot{Q}va/\dot{Q}t$) before (baseline), 1.5 and 3 hours after local intrapulmonary inoculation of Pasteurella haemolytica (treated group) and bovine fetal serum (control group).

+Indicates statistically significant difference within treated group between baseline and subsequent periods ($P<0.05$).

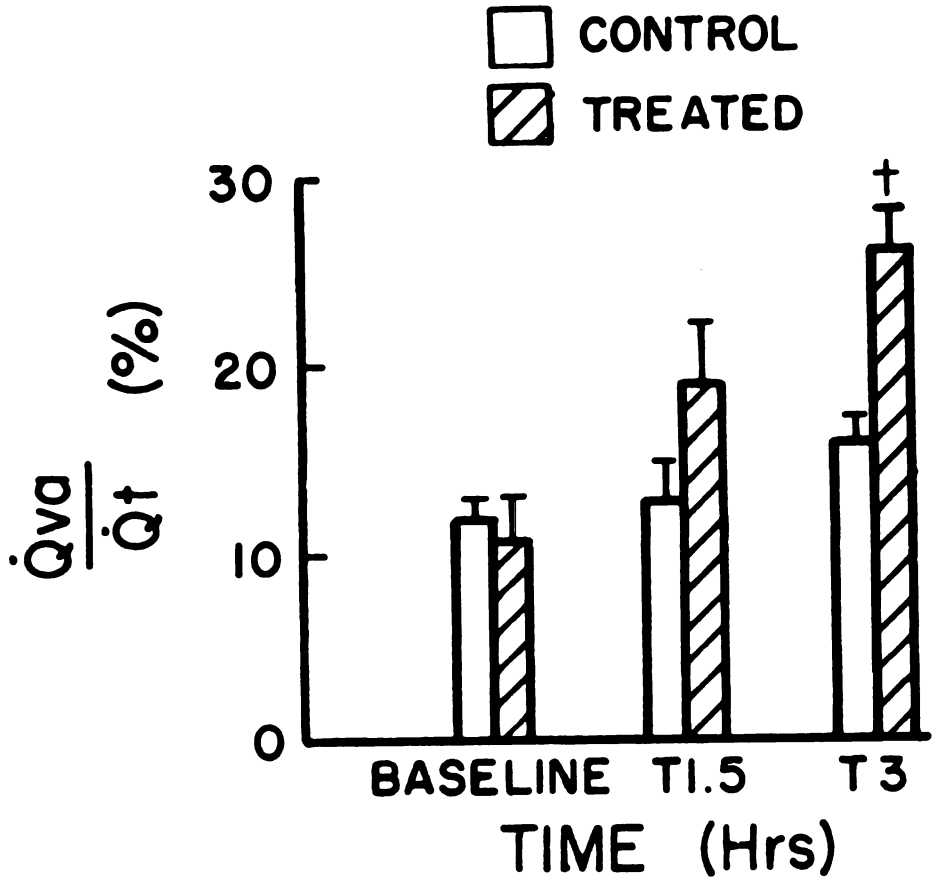

CONTROL
TREATED
30
20
10
0
†
$\frac{\dot{Q}va}{\dot{Q}t}$ (%)
BASELINE
T1.5
T3
TIME (Hrs)

Figure 8. Mean pulmonary arterial pressure, cardiac output/kg body weight, and pulmonary vascular resistance before (baseline), 1.5 and 3 hours after local intrapulmonary inoculation of Pasteurella haemolytica (treated group) and bovine fetal serum (control group).

*Indicates statistically significant difference between groups ($P<0.05$).

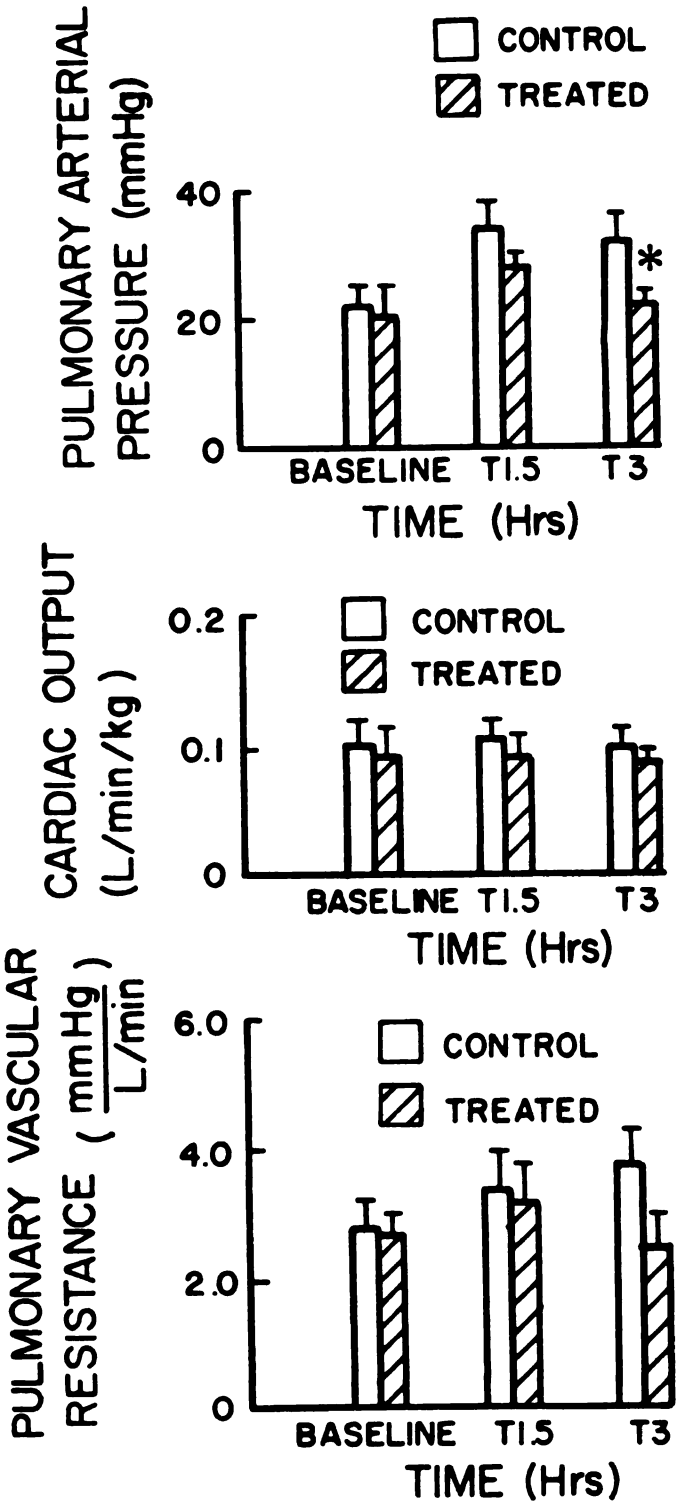

PULMONARY ARTERIAL PRESSURE (mmHg)
CONTROL
TREATED
40
20
0
*
BASELINE
T1.5
T3
TIME (Hrs)
CARDIAC OUTPUT (L/min/kg)
0.2
0.1
0
CONTROL
TREATED
BASELINE
T1.5
T3
TIME (Hrs)
PULMONARY VASCULAR RESISTANCE (mmHg / L/min)
6.0
4.0
2.0
0
CONTROL
TREATED
BASELINE
T1.5
T3
TIME (Hrs)

Table 4. Mean systemic arterial pressure (Psa), systemic vascular resistance (SVR), heart rate (HR), and stroke volume (SV) in the control and treated animals.

Parameters	Baseline	Hours after inoculation 1.5	3
Psa (mmHg)			
Control	106.8±7.8	113.4±8.9	111.2±9.8
Treated	114.8±6.8	122.6±7.8	107.8±10.5
SVR (mmHg min/L)			
Control	28.8±2.0	28.0±2.3	31.5±1.26
Treated	33.3±2.3	35.1±3.6	36.4±4.0
Heart rate (beat/min)			
Control	168.0±6.5	184.8±10.4	177.6±15.3
Treated	168.0±5.3	169.8±7.11	167.4±5.8
SV (ml)			
Control	23.0±2.2	21.1±1.9	19.2±3.1
Treated	17.5±2.9	17.5±2.5	16.5±3.6

Data are expressed as the mean ± SE (n=5/group).

inoculation of P. haemolytica was 18 ± 4.3. The regional PVR (rPVR) of both groups is shown in Figure 9. In the control group, rPVR in the BFS-inoculated regions increased significantly at the 3-hour period compared with the baseline. Whereas 3 hours after inoculation, rPVR decreased significantly in the P. haemolytica-inoculated regions compared with control (BFS inoculated) regions at the same time period. Regional vascular resistance was significantly lower in the treated regions compared with the healthy lung regions of the control and treated animals. Figure 10 shows the calculated rPVR for the healthy regions of lung. Both groups showed a slight but insignificant increase in rPVR at 1.5 and 3 hours, but there was no significant difference between groups at any measurement period.

Acute pneumonic pasteurellosis caused a substantial increase in pulmonary perfusion in the pneumonic regions (Figure 11). In the treated group there was a significant increase in pulmonary perfusion 1.5 hours and 3 hours post inoculation compared with the control. While in the control animals, there was no significant change in pulmonary perfusion (Figure 11). Correlation analysis was performed to test whether the increased venous admixture 3 hours after inoculation was correlated with the increased perfusion to the diseased lung regions. There was a significant correlation between the change-in venous admixture and the change in pulmonary perfusion 3 hours post-inoculation ($r=0.8$) (Figure 12). The per cent CO perfusing the healthy lung regions was not significantly different between groups or over time (Figure 13).

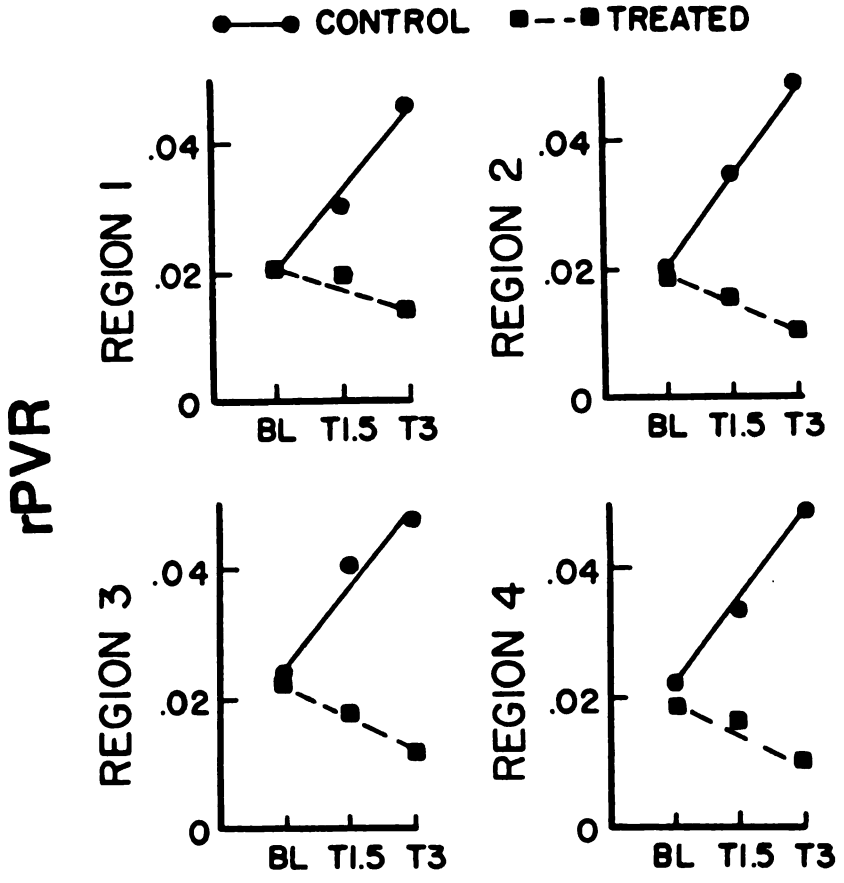
Figure 9. Regional pulmonary vascular resistance (rPVR) (mmHg min/ml/100 gm) before (baseline), 1.5 and 3 hours after local intrapulmonary inoculation of Pasteurella haemolytica (treated group) and bovine fetal serum (control group).

The upper panels show regional vascular resistance of individual regions of lung.

The lower panel shows regional vascular resistance of combined lung regions.

*Indicates statistically significant difference between groups ($P<0.05$).

xIndicates statistically significant difference within the control group ($P<0.05$).


CONTROL
TREATED
rPVR
REGION 1
REGION 2
REGION 3
REGION 4
.04
.02
0
BL T1.5 T3

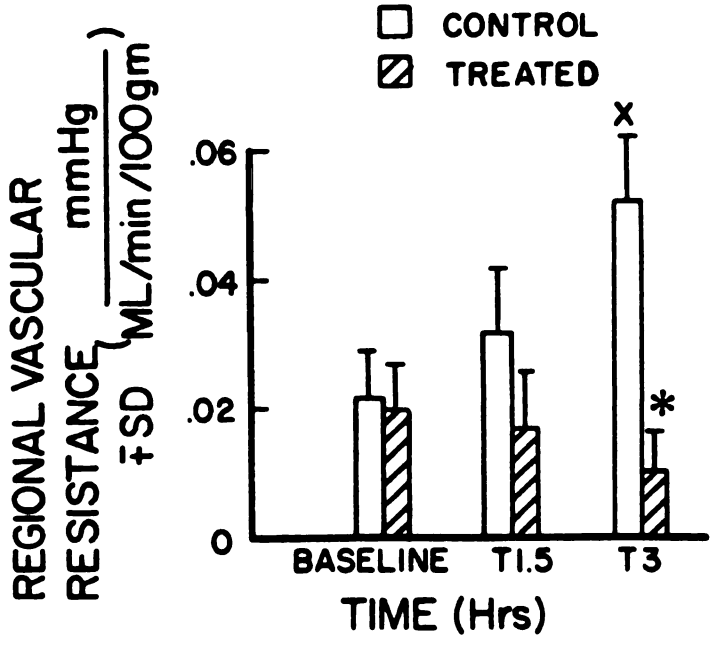

CONTROL
TREATED
REGIONAL VASCULAR RESISTANCE ± SD (mmHg / ML/min/100gm)
.06
.04
.02
0
X
*
BASELINE
T1.5
T3
TIME (Hrs)

Figure 10. Regional vascular resistance (rPVR) (mmHg min/ml/100 gm) of the healthy lung regions before (baseline), 1.5 and 3 hours after local intrapulmonary inoculation of Pasteurella haemolytica (treated group) and bovine fetal serum (control group).

The upper panel shows regional vascular resistance of individual lung regions.

The lower panel shows regional vascular resistance of combined lung regions.

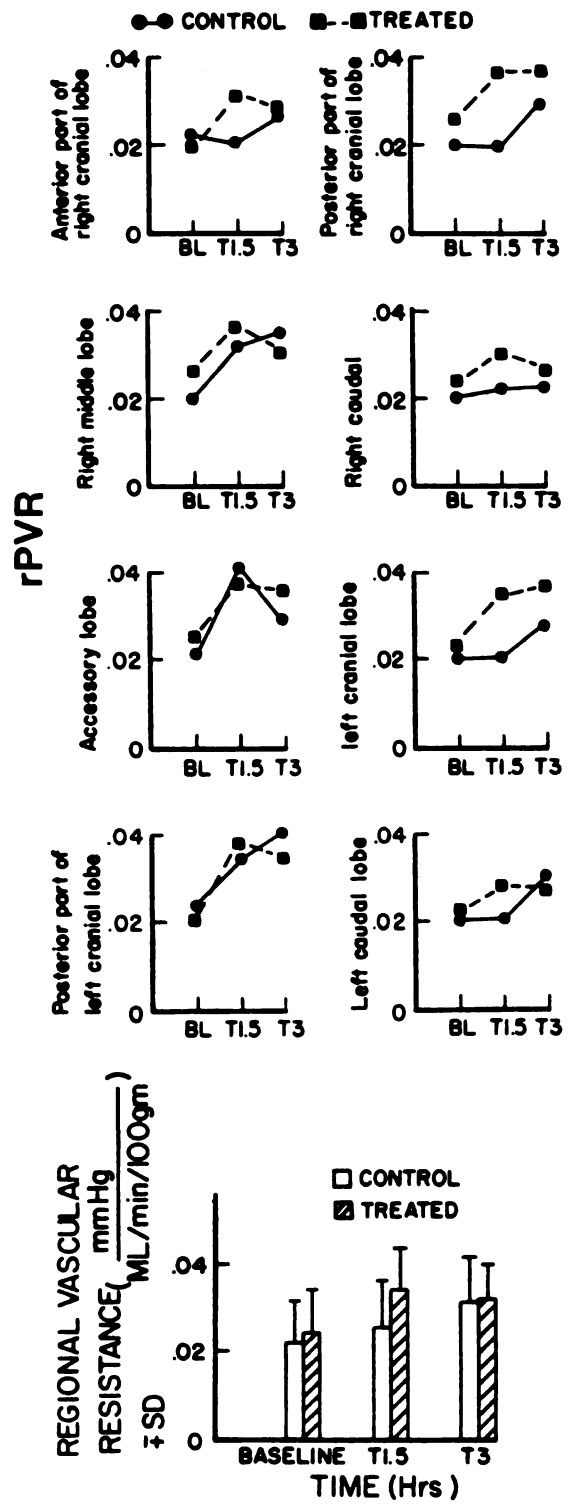
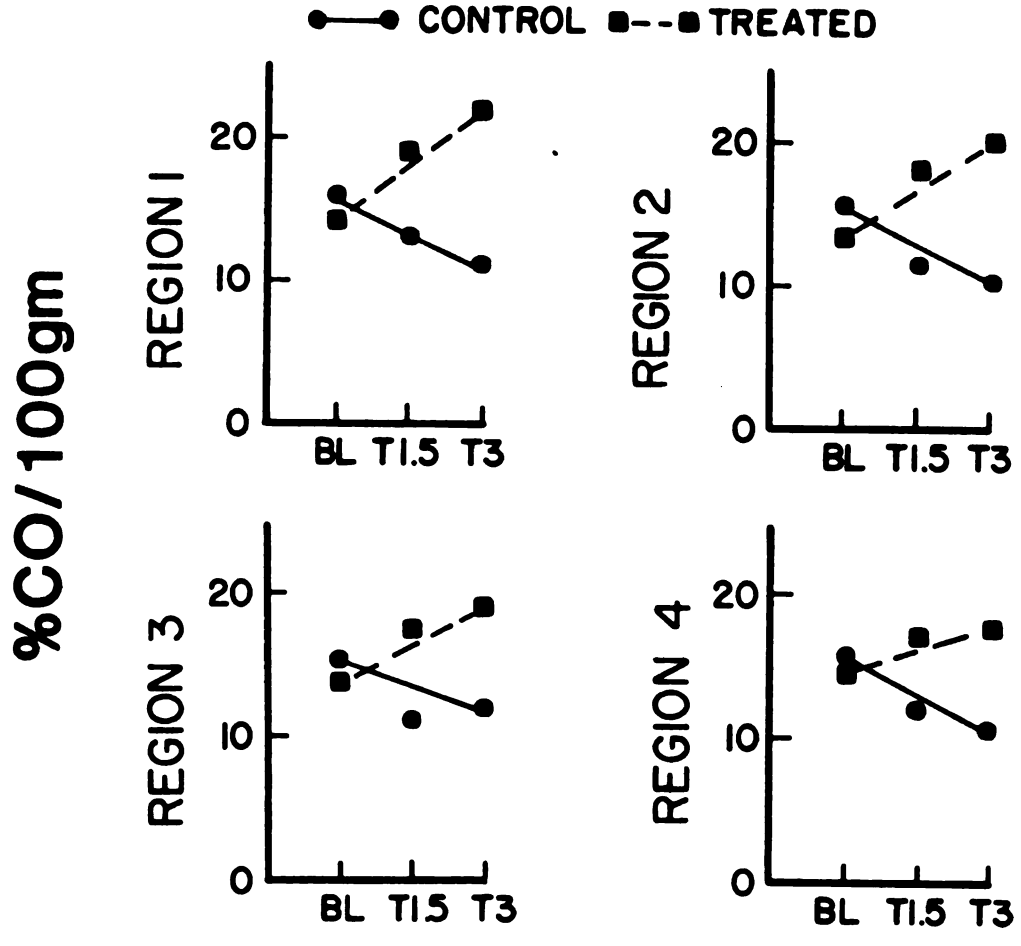

CONTROL
TREATED
rPVR
Anterior part of right cranial lobe
Posterior part of right cranial lobe
Right middle lobe
Right caudal
Accessory lobe
left cranial lobe
Posterior part of left cranial lobe
Left caudal lobe
.04
.02
0
BL T1.5 T3
REGIONAL VASCULAR RESISTANCE (mmHg / ML/min/100gm)
± SD
CONTROL
TREATED
BASELINE
T1.5
T3
TIME (Hrs)

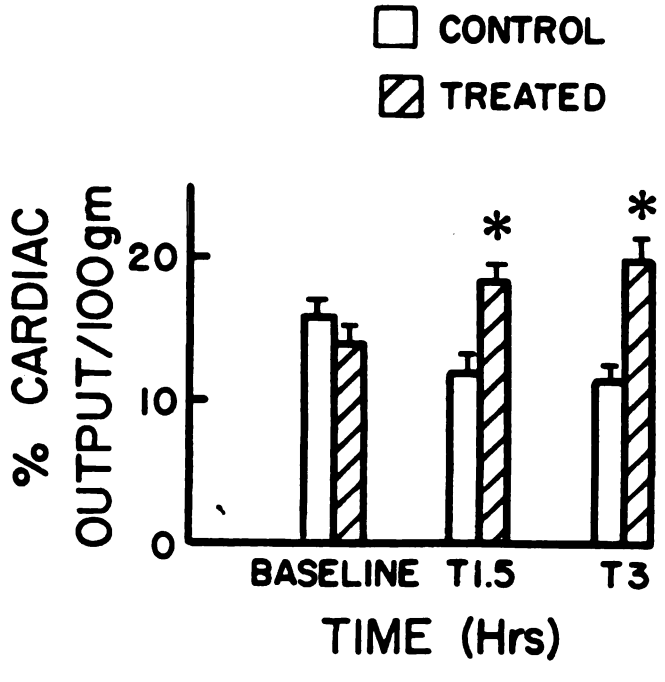
Figure 11. The percentage cardiac output/100 gm (% CO/100 gm) perfusing the lung regions before (baseline), 1.5 and 3 hours after intrapulmonary inoculation of Pasteurella haemolytica (treated regions) and bovine fetal serum (control regions).

The upper panel shows pulmonary perfusion of individual lung regions.

The lower panel shows pulmonary perfusion of combined lung regions.

*Indicates statistically significant difference between groups ($P<0.05$).


CONTROL
TREATED
%CO/100gm
REGION 1
REGION 2
REGION 3
REGION 4
20
10
0
BL T1.5 T3


CONTROL
TREATED
% CARDIAC OUTPUT/100gm
20
10
0
*
*
BASELINE T1.5 T3
TIME (Hrs)

Fig

Figure 12. The relationship between the change in venous admixture ($\dot{Q}va/\dot{Q}t$) and the change in percentage cardiac output perfusing diseased lung regions (% CO) (r=0.8). Each data point represents the changes occurring in each treated calf between baseline and 3 hours following local intrapulmonary inoculation of Pasteurella haemolytica.

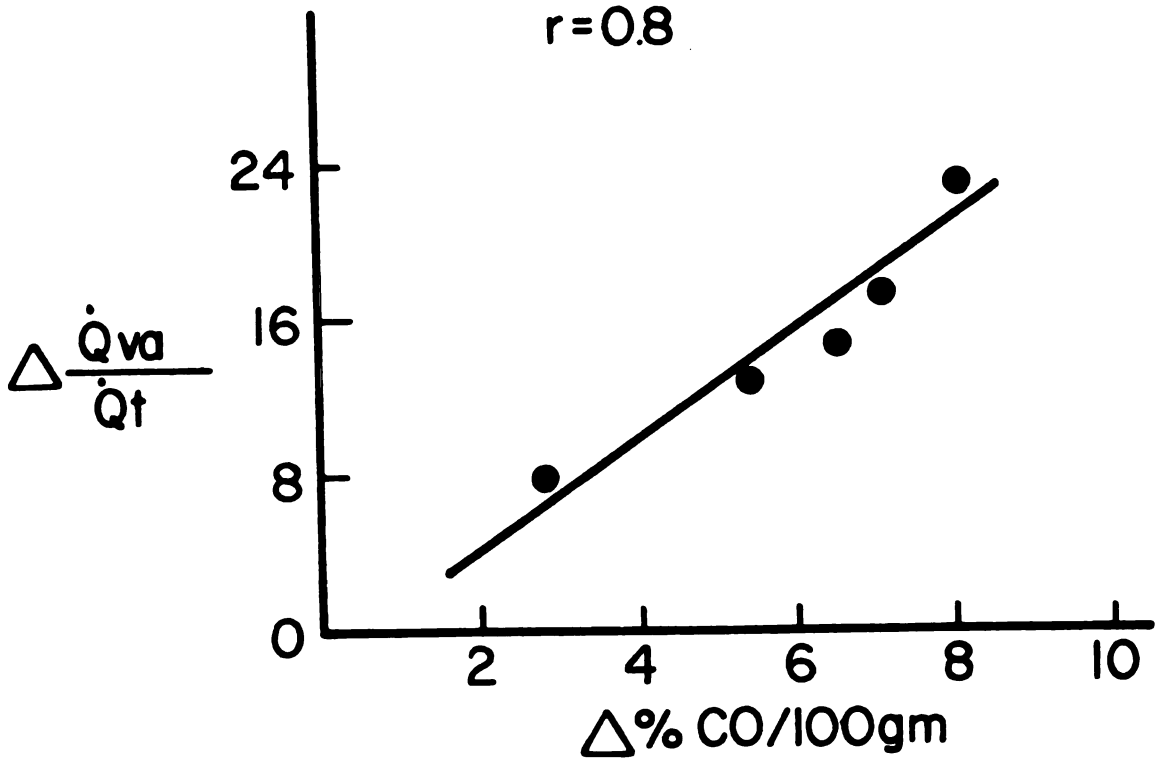
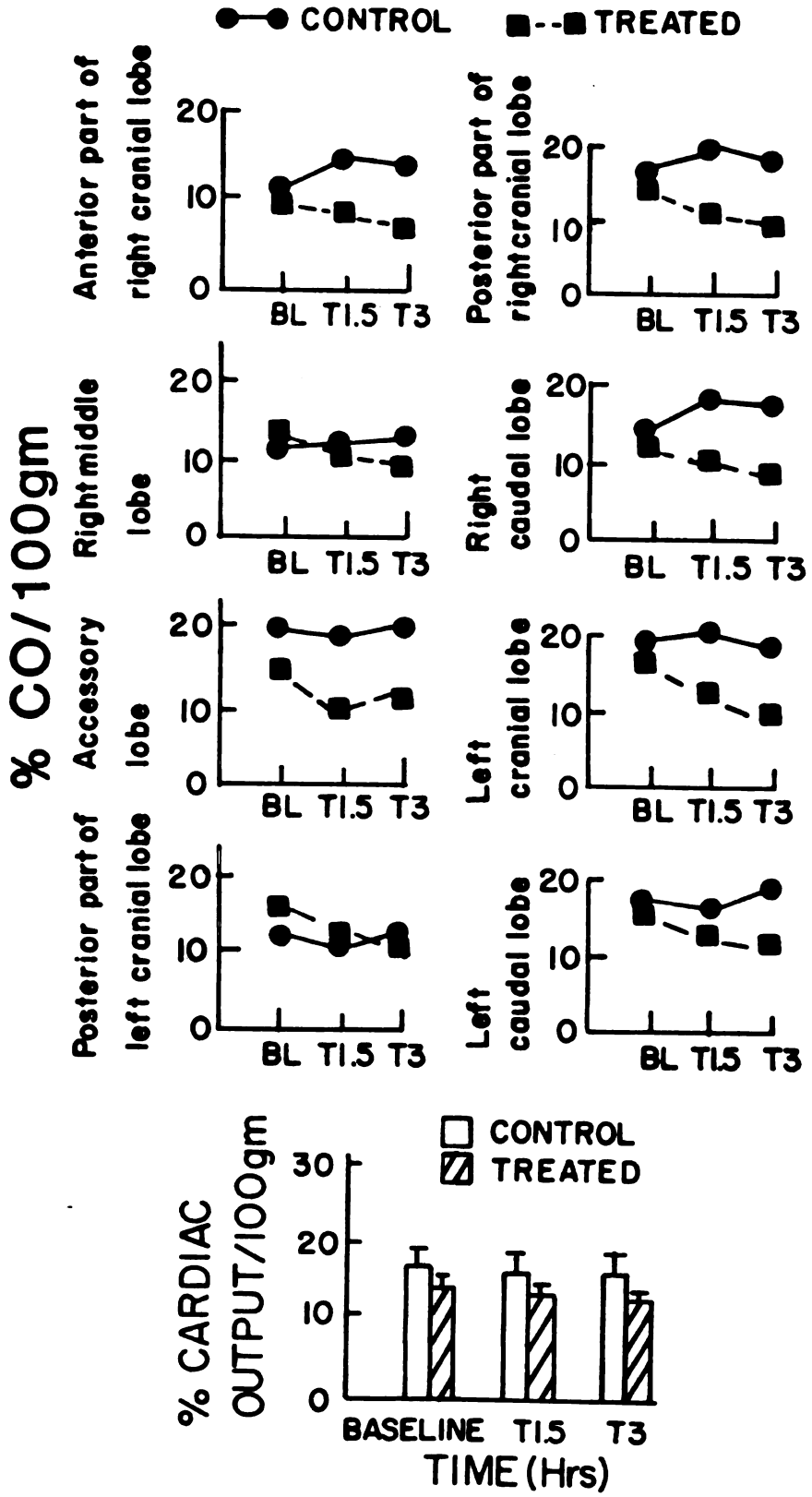

r=0.8
24
16
8
0
2
4
6
8
10
Δ% CO/100gm
Δ Q̇va / Q̇t

Figure 13. The percentage cardiac output/100 gm (% CO/100 gm) perfusing the healthy lung regions before (baseline), 1.5 and 3 hours after intrapulmonary inoculation of Pasteurella haemolytica (treated group) and bovine fetal serum (control group).

The upper panel shows pulmonary perfusion of individual lung regions.

The lower panel shows pulmonary perfusion of combined lung regions.


CONTROL
TREATED
% CO/100gm
Anterior part of right cranial lobe
Posterior part of right cranial lobe
Right middle lobe
Right caudal lobe
Accessory lobe
Left cranial lobe
Posterior part of left cranial lobe
Left caudal lobe
20
10
0
BL T1.5 T3
% CARDIAC OUTPUT/100gm
30
BASELINE T1.5 T3
TIME (Hrs)

DISCUSSION

Our data demonstrate that local pneumonia induced by P. haemolytica and affecting nearly 20% of lung caused a significant decrease in arterial oxygen tension 3 hours after inoculation of organisms into the lung. The results also indicate that the mean resistance to blood flow was decreased in the diseased regions relative to the regions inoculated with BFS and to the rest of the lung. Arterial hypoxemia in a local lung disease depends in part on the fraction of pulmonary blood flow perfusing the diseased lung regions. Therefore, the decrease in local resistance which resulted in the increase in pulmonary perfusion to the consolidated lung regions could have accounted in part for the arterial hypoxemia.

The increased PAO_2-PaO_2 in this experimental preparation suggests that hypoxemia is due to either ventilation/perfusion ($\dot{V}/\dot{Q}$) inequality, right-to-left shunt or diffusion impairment. Pneumonic hypoxemia was not due to alveolar hypoventilation because $PaCO_2$ was unchanged. We have previously characterized the gas exchange abnormality of calves with acute pneumonia by ventilating the animal first with air and then with a hypoxic gas mixture (Chapter 3). We observed a narrowing of the alveolar-arterial oxygen difference which was interpreted to mean that a major portion of the gas exchange defect was due to shunt rather than diffusion impairment (Chapter 3). This latter observation does not eliminate the possiblity that hypoxemia also results from $\dot{V}/\dot{Q}$ ratios greater than zero and less than 0.8. It is highly probable that the $\dot{V}/\dot{Q}$ inequality in this experimental model results from the increase in perfusion to the diseased lung in which ventilation has been impaired.

The percentage of cardiac output perfusing the diseased lung regions increased by 7% 3 hours after Pasteurella inoculation whereas $\dot{Q}va/\dot{Q}t$ increased by 15% which suggests that the increase in venous admixture cannot be explained totally by the increased blood flow to the diseased regions. Therefore we propose that part of pneumonic hypoxemia is attributable to venous admixture from poorly ventilated lung units in the apparently healthy lung regions. If the highest value of the venous admixture in our experimental model of local pneumonia had been precisely matched with the highest blood flow to the diseased lung, the hypothesis that $\dot{Q}va/\dot{Q}t$ of the diseased lung regions is totally responsible for the hypoxemia would have received strong support. A similar lack of correlation between venous admixture and shunt values was reported in the study of Light et al. (1981) in which the shunt fraction was increased by 8% while the increase in $\dot{Q}va/\dot{Q}t$ was 14% three days after pneumococcal infection in dogs. A previous study by Wagner et al. (1975) also indicated that the pneumonic hypoxemia is due to the increase in blood flow through right-to-left shunts as well as to $\dot{V}/\dot{Q}$ mismatching in dogs with acute pneumococcal pneumonia. The latter investigators also reported that when pneumonia resolves, shunt flow decreases and units with low ventilation perfusion ratios predominate.

The pulmonary artery of neonatal calves constricts vigorously in response to hypoxia (Kuida et al. 1962). This phenomenon is thought to direct blood flow away from poorly ventilated regions of lungs and thereby serves to match local ventilation to blood flow (Hiser et al. 1975). The integrity of this response was demonstrated in the control animals by the increase in rPVR caused by inoculation of BFS. The

intrapulmonary presence of BFS alone did not cause hypoxemia nor increase the alveolar arterial oxygen difference further suggesting that the hypoxic vasoconstrictor response was effective in redistributing blood flow to better ventilated regions of lung.

In this experimental model of local pneumonia, we clearly documented that the development of arterial hypoxemia is associated with a decrease in regional vascular resistance. This decrease in regional resistance in acute pneumonic pasteurellosis results in the maintenance of blood flow to consolidated regions of lung. A similar observation was made by Light et al. (1981) who documented perfusion to the consolidated lung regions was maintained 3 days after infection of dogs with pneumococcal pneumonia.

Our previous investigations showed that the pulmonary vascular response to hypoxia was much less vigorous in a pneumonic group of calves compared with a control group receiving BFS suggesting that the intrapulmonary presence of P. haemolytica attenuates the hypoxic vasoconstrictor mechanism in the pulmonary circulation (Chapter 3). Other investigators suggest that bacteria or the inflammatory process they elicit may override hypoxic vasoconstriction and maintain blood flow in the diseased region with pneumonia (Goldzimer et al. 1974; Light et al. 1981). Therefore, it seems reasonable to postulate that the vasodilation and hypoxemia are attributable either to the presence of the viable bacteria or to a humoral factor produced by the inflammatory process initiated by bacterial organisms.

Previous investigation has shown that lungs with acute pneumonic pasteurellosis are invaded by a large number of neutrophils (Slocombe et al. 1984a) and pneumonic hypoxemia is prevented by neutrophil

depletion (Slocombe et al. 1985). Activation of neutrophils could cause the release of lysosomal enzymes, superoxide radicals and initiation of the arachidonic acid cascade (Staub et al. 1985a). In addition, the pulmonary vascular endothelium can metabolize arachidonic acid to various prostaglandins during inflammation or other forms of endothelial injury (Weksler et al. 1978; Johnson et al. 1981). Therefore, we hypothesize that the vasodilator prostaglandins, generated during the inflammatory reaction are a primary factor responsible for the increased perfusion to the diseased lung regions. Previous investigations have shown that the vasodilator prostaglandins, in particular PGE_1 and PGE2 are generated in neutrophilic inflammation, modulate local leukocytic infiltration and may also increase the severity of vascular damage (Johnston et al. 1979; Higgs and Salmon 1979; Issekutz and Movat 1982).

Other investigators have indicated that prostacyclin, the potent vasodilator, is released after the mechanical stimulation of the pulmonary vascular bed (Voelkel et al. 1981). Since pneumonic pasteurellosis can cause endothelial damage, prostacyclin may have been released in the injured lung and caused local vasodilation. Several investigators have reported that the vasodilator prostaglandins are responsible for the decrease in the net strength of hypoxic pulmonary vasoconstriction (HPV) and development of shunt in lobar atelectasis induced experimentally in dogs (Garrett and Thomas 1983; Garrett and Thomas 1985). The proposed mechanism would be compatible with the result of the present study: the regional vasodilation in acute pneumonic pasteurellosis would be due to the

release of prostaglandins, in particular prostacyclin, which are able to overcome the hypoxic vasoconstriction of the lung vessels.

Another explanation for the regional vasodilation may be the mechanical factors which can cause local changes in blood flow. Acute pneumonic pasteurellosis causes decreased lung compliance and increased pulmonary resistance which probably limits inflation of the diseased region (Slocombe et al. 1984b). Therefore, we might expect that inflation of the surrounding healthy lung tissue decreases pressure in the diseased regions causing a decrease in regional vascular resistance. Moreover, this interdependence may oppose other mechanical and hypoxic effects of pneumonia which would tend to reduce blood flow and vascular conductance in this diseased region.

CHAPTER 5.

THE EFFECT OF ARACHIDONIC ACID CASCADE BLOCKADE ON REGIONAL PULMONARY BLOOD FLOW IN ACUTE PNEUMONIC PASTEURELLOSIS

SUMMARY

The role of arachidonate metabolites in the pulmonary vasodilation occurring in acute pneumonic pasteurellosis, was examined in pneumonic calves after intraperitoneal administration of the arachidonic acid cascade blocking agent 4,8,11,14-eicosatetraynoic acid (ETYA). Experiments were performed on 15 anesthetized and ventilated neonatal calves, divided into 3 groups of 5 animals each. In the negative control group (group 1), 4 regions of lung were inoculated with bovine fetal serum (BFS) 30 minutes after intraperitoneal (IP) administration of ETYA. In group 2, 30 minutes after ETYA injection, regional pneumonia was induced in the treated animals by inoculation of P. haemolytica suspended in BFS. The positive control group (group 3) received 95% alcohol (15 ml IP) 30 minutes prior to inoculation with P. haemolytica. Measurements of pulmonary hemodynamics, gas exchange, and distribution of pulmonary blood flow with radiolabeled microspheres were made before (baseline), 1.5 and 3 hours after local inoculation of P. haemolytica or BFS. Local inoculation of P. haemolytica (group 3) caused severe arterial hypoxemia and increased pulmonary blood flow to the pneumonic lung regions. In the treated group (group 2), ETYA administration prevented the decrease in PaO_2 and increase in pulmonary perfusion to the pneumonic regions. The decrease in regional pulmonary perfusion was due to the increase in rPVR. In the negative control group (group

1), local inoculation of BFS did not significantly change regional pulmonary hemodynamics or gas exchange. In the healthy lung regions, pulmonary perfusion and rPVR were not significantly changed by ETYA treatment and/or P. haemolytica inoculation. We conclude that arachidonic acid metabolites maintain pulmonary blood flow in the pneumonic lung regions. These data suggest that the release of vasodilator eicosanoids is either greater in the pneumonic lung or the production of eicosanoids is normal but the vascular response is augmented in the diseased regions.

INTRODUCTION

We have previously demonstrated that local pneumonia induced by P. haemolytica causes severe arterial hypoxemia accompanied by pulmonary vasodilation in the diseased lung regions (Slocombe et al. 1984b; Chapter 4). The decrease in arterial oxygen tension is due in part to the increased blood flow to the pneumonic regions of lung. Recent investigations by Slocombe et al. (1985) have shown that lungs with acute pneumonic pasteurellosis are invaded by a large number of neutrophils and neutrophil depletion with hydroxyurea prevents the hypoxemia and the typical lesions of acute pneumonic pasteurellosis. These findings, coupled with the observation that prostaglandins are elevated in the neutrophilic inflammatory reactions (Johnston et al. 1979; Higgs et al. 1980), suggest that synthesis of vasodilator prostaglandins actively maintains neonatal pulmonary vessels in a dilated state in the pneumonic lung regions.

In agreement with this concept are data showing that inhibition of prostaglandin synthesis augments pressor responses to hypoxia

(Vaage et al. 1975; Weir et al. 1976) and other pulmonary vasoconstrictors, including those released in canine experimental asthma (Cohn et al. 1978). In addition, stimulation of prostaglandin synthesis in dogs given small amounts of endotoxin or of the prostanoid precursor, arachidonic acid, causes pulmonary vasodilation (Reeves et al. 1974; Gerber et al. 1980). However, arachidonic acid metabolism can produce both vasoconstrictor and vasodilator prostaglandins and other products, and in some species arachidonic acid products can cause pulmonary vasoconstriction (Kadowitz et al. 1977).

It has been reported that the endogenous release of lipoxygenase products, in particular leukotrienes, does not have an influence on resting pulmonary vasculature, neither do they appear to be necessary for hypoxic pulmonary vasoconsntriction in normal conditions (Leffler et al. 1984). However, it is possible that lipoxygenation products of arachidonic acid may influence the release and activity of other mediators in the diseased lung regions.

The purpose of the present investigation was to study the role of arachidonic metabolites in pulmonary vasodilation induced by P. haemolytica using 5,8,11,14-eicosatetraynoic acid (ETYA) as a cyclooxygenase and lipoxygenase blocker.

METHODS

Experimental preparation. Fifteen neonatal calves, 1 to 3 weeks old, weighing between 30 and 40 kg, were used and divided into three groups, control and treated groups receiving ETYA (groups 1 and 2, respectively), and a positive control group (treated group receiving

vehicle, 95% ethanol) (group 3). Each animal was anesthetized intravenously with 80 mg/kg chloralose and 500 mg/kg urethane. Animals were intubated via a midcervical tracheostomy and mechanically ventilated, using a constant-volume ventilator (Harvard, model 615, Millis, MA) with a tidal volume of 20 ml/kg. Respiratory rate was adjusted to give an end-expired carbon dioxide fraction of 4.8% to 5.0%. Minute ventilation ($\dot{V}_E$) was measured by connecting a wedge spirometer (Medical Science Electronics Inc., model 570 wedge spirometer, St. Louis, MO) to the expiratory port of the ventilator. Rectal temperature was measured throughout the experiment. Arterial blood samples were obtained and systemic arterial pressure (Psa) was measured from a femoral artery catheter connected to a pressure transducer (Statham Instruments, model P23Db, Hato Rey, Puerto Rico). A balloon-tipped flotation catheter (Swan-Ganz flow-directed monitoring catheter, model 93-110-5F, American Edwards Co., Irvine, CA) connected to a second similar pressure transducer, was inserted into the left internal jugular vein and advanced with continuous pressure monitoring into the pulmonary artery until both pulmonary arterial pressure (Ppa) and wedge pressure (Pw) were obtained. Both transducers were calibrated against a mercury manometer and adjusted to be level with the point of the left shoulder.

Polyethylene catheters were also introduced into the right femoral artery and vein. The femoral artery catheter was connected to the inlet of the arterial cuvette of a continuous arterio-venous oxygen difference (CaO_2-$C\bar{v}O_2$) analyzer (Avox, Avox Systems, Inc., San Antonio, TX) (Shepherd and Burger 1977). The femoral vein catheter was advanced into the right ventricle, pressure being monitored

continuously, to obtain mixed venous blood and then connected to the inlet of the venous cuvette of the Avox. A smaller catheter (Venocath 14 g I.V. catheter, Abbott Labs, North Chicago, IL), introduced into the right jugular vein, was used to receive the outflow of the Avox system and for the administration of the anesthetics. Immediately after catheterization, each calf received 50 units/kg heparin intravenously, and blood was circulated through the Avox cuvette using a double roller pump at a constant flow of 12 ml/min. The Avox signal, the wedge spirometer output and all pressure tracings were continuously displayed on a 12-channel physiograph.

Production of pneumonia. Local pneumonia was induced in the treated groups by inoculating the lung with P. haemolytica using a bronchoscope. The lobulated lung and lack of collateral ventilation in calves allowed local infusion of organisms and creation of pneumonia restricted to a few lobules. The method we used to induce local pneumonia was a modification of the procedure described by Terrell et al. (1933). Briefly, the bronchoscope was advanced down the tracheo-bronchial tree and wedged into the distal part of the following lobes: the posterior part of the right cranial lobe, the right caudal lobe, the accessory lobe, and the left caudal lobe. An inoculum containing 5×10^8 P. haemolytica, suspended in 5 ml BFS, was injected into each lobe. The control group of calves were inoculated with BFS in the same manner as the treated group. India ink (0.5 ml) was added to the BFS to identify the inoculated lobules at necropsy. The catheter and the bronchoscope were then removed and the animals remained under continuous observation until the end of the experiment.

Experimental protocol. Preliminary experiments were conducted on two calves to determine the required dose of ETYA to block physiologic effects of intravenous arachidonic acid infusion. A dose response curve was established to determine the effect of arachidonic acid on the pulmonary arterial pressure. The concentration producing the half maximum response (ED_{50}) for arachidonic acid was calculated and tested against the sodium salt of ETYA. The intraperitoneal injection of ETYA in a dose of (20 mg/kg), dissolved in ethanol (15 ml) 30 minutes prior to administration of arachidonic acid, was sufficient to block the increase in pulmonary arterial pressure produced by the ED_{50} dose of arachidonic acid.

We have shown that hypoxemia occurs at 1 to 2 hours and the mean pulmonary vascular resistance decreases three hours after inoculation of P. haemolytica. We therefore measured the distribution of pulmonary perfusion before, and 1.5 and 3 hours after inoculation. To evaluate the role of eicosanoids in the pulmonary vasodilation occurring in acute pneumonic pasteurellosis, an intraperitoneal bolus of (20 mg/kg) ETYA dissolved in 95% ethanol was given 30 minutes prior to inoculation of P. haemolytica (group 2). The positive control group (group 3) received vehicle, i.e. 95% ethanol, 30 minutes pre-inoculation. To evaluate the direct effect of ETYA on the pulmonary vasculature, the negative control group (group 1) received ETYA 30 minutes prior to inoculation of BFS. The timing of inoculation and measurement, after ETYA injection, was chosen because previous investigation has shown that the peak effect of this drug is reached within 30 to 60 minutes (Kadowitz et al. 1975).

Measurement of gas exchange. At each measurement period, expired gas composition was determined with oxygen and carbon dioxide analyzers and used to calculate oxygen consumption ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$). Arterial blood samples were obtained at the midpoint of a 5-minute gas collection period and analyzed for oxygen and carbon dioxide tension (PaO_2 and $PaCO_2$), pH, hemoglobin concentration (Hb) and percentage saturation of Hb (% sat). Alveolar gas tension (PAO_2), dead space/tidal volume ration (VD/VT), and alveolar-arterial oxygen difference (PAO_2-PaO_2) were calculated using standard respiratory gas equations. Cardiac output was calculated using the Fick principle.

Measurements of venous admixture ($\dot{Q}va/\dot{Q}t$) were made at the 3 measurement periods, baseline, 1.5 and 3 hours after inoculation. Arterial oxygen content (CaO_2) was calculated as CaO_2=(Hb X % sat X 1.34) + (PaO_2 X 0.003). The mixed venous oxygen content ($C\bar{v}O_2$) was calculated as CaO_2 minus the arterio-venous oxygen difference. End capillary oxygen content ($C\bar{c}O_2$) was calculated from the above equation replacing PaO_2 by calculated alveolar oxygen tension (PAO_2). Since the PAO_2 values were more than 100 mmHg, the per cent saturation of hemoglobin was assumed to be (100%). The venous admixture fraction was then calculated as $\dot{Q}va/\dot{Q}t = (C\bar{c}O_2-CaO_2)/(C\bar{c}O_2-C\bar{v}O_2)$.

Measurement of pulmonary and systemic hemodynamics. Pulmonary vascular resistance was calculated as the mean pulmonary arterial pressure minus the mean pulmonary artery wedge pressure (mmHg) divided by the cardiac output (CO) (liter/minute). Systemic vascular resistance was calculated as (Psa-5)/CO. The right atrial pressure

was assumed to be 5 mmHg. Heart rate was determined from physiograph trace and stroke volume was calculated.

Distribution of pulmonary perfusion. Regional distribution of pulmonary blood flow was determined in both groups of calves at the three measurement periods. Microspheres (15 um in diameter) labeled with one of three radionucleotides Ce^{141}, Sr^{85}, and Cr^{51}, were injected at baseline, 1.5 hours, and 3 hours after inoculation. The number of microspheres per injection was selected to provide at least 400 and usually in excess of 900 microspheres in any single lung region weighing approximately 10 gm (Heymann et al. 1977). The stock microsphere suspension as obtained from the 3M Company (St. Paul, MN) had an initial activity of 0.05 mci/ml and the suspending agent was 10% dextran. The stock suspension was agitated mechanically with a vortex mixer, then shaken with further intermittent agitation for at least 15 minutes prior to use. Approximately 0.5 ml of the stock solution (1×10^6 spheres) was infused into the right atrium through a catheter introduced from the right jugular vein. The infusion catheter was then flushed with 20 ml saline.

At the end of the experiment, after the calf was killed with sodium pentobarbital, the lung was exposed and the pulmonary artery and veins were ligated. The lung was excised and the diseased lung lobules were separated from those which appeared grossly normal. The diseased and healthy lung regions were weighed. The lung tissues were cut into pieces, placed in vials and the activity was determined using a gamma counter. Using the regional activity, total activity of the whole lung and CO determined by the Fick method, the fraction of the cardiac output perfusing each part of lung at the time of each isotope

injection was calculated as ml/min/100 gm. The total flow going to diseased and normal regions of each lobe was also calculated. Regional pulmonary vascular resistance (rPVR) was computed as the pressure difference across the pulmonary circulation divided by the regional blood flow/100 gm.

Data were analyzed using factorial analysis of variance. Tukey's omega test was used to compare the means at each measurement period when the calculated F-values were significant at $P<0.05$ (Steel and Torrie 1980).

RESULTS

The effect of acute pneumonic pasteurellosis and administration of the cyclooxygenase and lipoxygenase inhibitor ETYA on PaO_2 and PAO_2-PaO_2 are shown in Figure 14. Acute pneumonic pasteurellosis (group 3) caused a substantial decrease in PaO_2 1.5 and 3 hours after inoculation, whereas no significant change occurred in the treated and control groups receiving ETYA. Alveolar-arterial oxygen difference remained unchanged in the treated and control animals receiving ETYA, while in the P. haemolytica-inoculated animals receiving vehicle (group 3), PAO_2-PaO_2 increased significantly at 1.5 and 3 hours compared with the baseline values. Alveolar oxygen tension, VD/VT, $PaCO_2$, $\dot{V}O_2$ and $\dot{V}CO_2$ did not change significantly over the course of the experiment (Table 5).

Figure 15 shows the venous admixture for the 3 groups of animals. The venous admixture of all groups of calves averaged 9% at the baseline period. In acute pneumonic pasteurellosis (group 3), venous admixture increased by a mean of 20% by 1.5 hours and 30% by 3 hours

Figure 14. Arterial oxygen tension and alveolar arterial oxygen difference before (baseline), 1.5 and 3 hours after intrapulmonary inoculation of bovine fetal serum (BFS) (group 1) and Pasteurella haemolytica (group 2 and 3). Group 1 received ETYA 30 minutes prior to BFS inoculation. Group 2 received ETYA 30 minutes before Pasteurella haemolytica inoculation. Group 3 received vehicle (95% ethanol) 30 minutes before Pasteurella haemolytica inoculation.

+Indicates statistically significant difference within treated (group 3), between baseline, and subsequent periods ($P<0.05$).

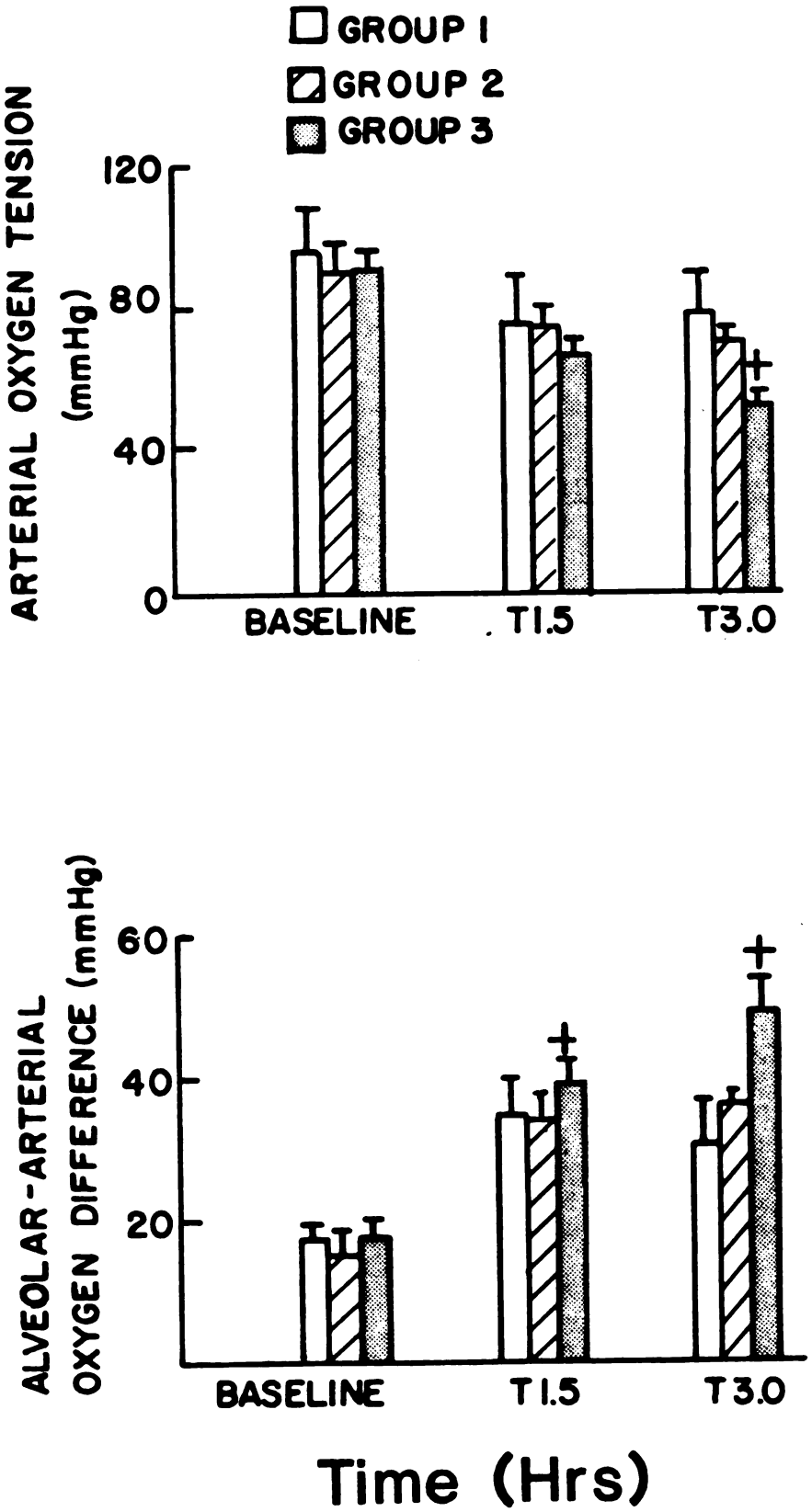

GROUP 1
GROUP 2
GROUP 3
ARTERIAL OXYGEN TENSION (mmHg)
120
80
40
0
BASELINE
T1.5
T3.0
+
ALVEOLAR-ARTERIAL OXYGEN DIFFERENCE (mmHg)
60
40
20
BASELINE
T1.5
T3.0
+
Time (Hrs)

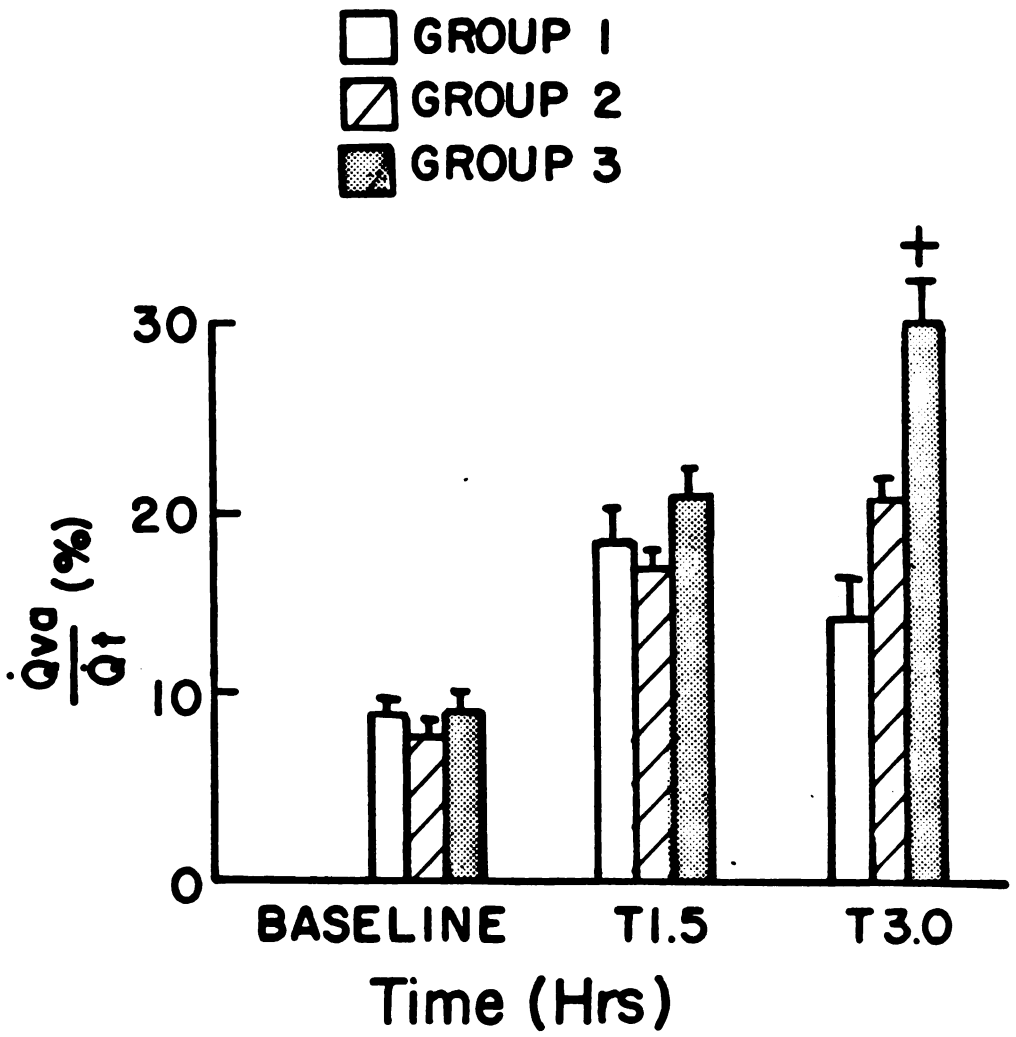
Table 5. Alveolar oxygen tension (PAO_2), dead space/tidal volume ratio (VD/VT), arterial CO_2 tension ($PaCO_2$), carbon dioxide production ($\dot{V}CO_2$) and oxygen consumption ($\dot{V}O_2$) in both control and treated animals receiving ETYA (group 1 and 2), and treated animals receiving vehicle (group 3). The control group was inoculated with bovine fetal serum. Treated groups were inoculated with Pasteurella haemolytica. ETYA was given 30 minutes before inoculation.

Parameters	Baseline	Hours after inoculation 1.5	3
PAO_2 (mmHg)			
Group 1	116.0±2.0	110.0±4.0	109.0±2.1
Group 2	106.0±2.8	106.0±2.0	107.0±2.0
Group 3	108.0±0.6	104.6±0.8	105.4±1.2
VD/VT			
Group 1	0.31±0.03	0.35±0.04	0.36±0.01
Group 2	0.36±0.04	0.38±0.02	0.38±0.03
Group 3	0.29±0.02	0.30±0.02	0.31±0.03
$PaCO_2$ (mmHg)			
Group 1	27.6±1.5	32.4±2.0	33.7±1.04
Group 2	35.5±1.7	36.6±1.5	37.6±1.4
Group 3	32.7±0.4	36.2±0.3	36.8±0.7
$\dot{V}CO_2$ (ml/min)			
Group 1	167.0±5.8	141.0±8.5	140.8±8.5
Group 2	155.8±3.5	138.0±8.7	133.6±10.4
Group 3	159.4±8.1	141.0±5.7	143.0±13.03
$\dot{V}O_2$ (ml/min)			
Group 1	213.4±7.3	186.4±8.6	177.8±4.2
Group 2	211.6±4.5	177.2±11.0	171.2±19.0
Group 3	214.6±12.0	186.0±6.7	177.6±14.0

Data are expressed as the mean ±SE (n=5/group).

Figure 15. Venous admixture ($\dot{Q}va)/(\dot{Q}t$) before (baseline), 1.5 and 3 hours after intrapulmonary inoculation of bovine fetal serum (BFS) (group 1) and *Pasteurella haemolytica* (group 2 and 3). Group 1 received ETYA 30 minutes prior to BFS inoculation. Group 2 received ETYA 30 minutes before *Pasteurella haemolytica* inoculation. Group 3 received vehicle (95% ethanol) prior to *Pasteurella haemolytica* inoculation.

+Indicates statistically significant difference within treated group between baseline and subsequent periods ($P<0.05$).


GROUP 1
GROUP 2
GROUP 3
+
30
20
10
0
$\frac{\dot{Q}va}{\dot{Q}t}$ (%)
BASELINE
T1.5
T3.0
Time (Hrs)

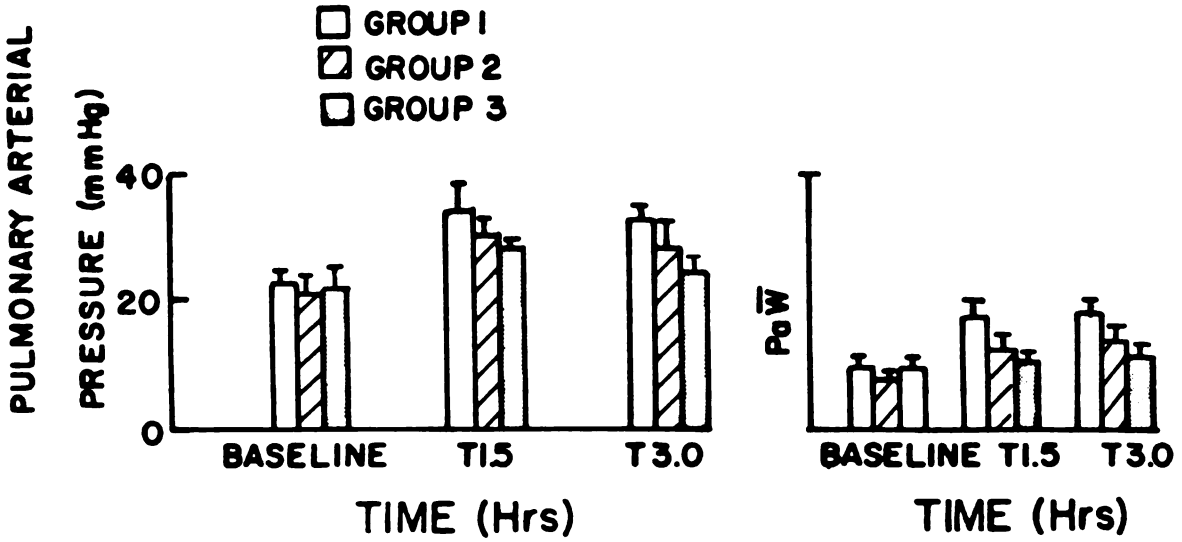
after inoculation compared with the baseline values. Administration of ETYA to calves with local pneumonia (group 2) decreased venous admixture by a mean of 10% compared with the treated group receiving vehicle (group 3) at the 3 hour period. In addition, in group 2 there was no significant increase in venous admixture at the 3 hour measurement period compared with the baseline values. In the negative control group (group 1), the increase in venous admixture at 1.5, and 3 hour periods was not significant compared with the baseline values. There was no significant difference between groups at any time period. There were no radioactive counts in the peripheral blood or tissues indicating the absence of vascular shunts larger than (15 um) in diameter.

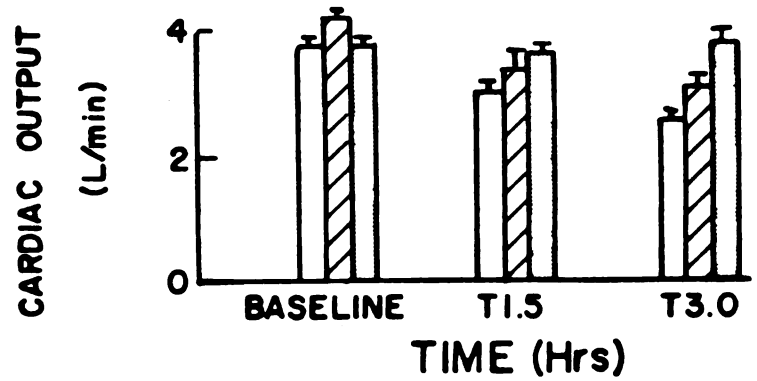
The effects of intraperitoneal administration of ETYA on pulmonary hemodynamics of the 3 groups of animals are shown in Figure 16. Although both mean pulmonary artery pressure and pulmonary vascular resistance rose at 1.5 and 3 hours postinoculation in the 3 treatment groups, the change was not statistically significant. There was no significant change in CO between groups or with time.

There were no significant systemic hemodynamic effects of ETYA administration, BFS, or P. haemolytica inoculations within each group when compared with its baseline control values or with other groups at the same time period (Table 6).

Distribution of pulmonary blood flow. A nested factorial analysis of variance determined the difference in regional blood flow between regions in each group of animals. Since there was a treatment effect with time, but no significant differences between lobes, the data of the individual regions in each group were therefore combined

Figure 16. Mean pulmonary arterial pressure, cardiac output, and pulmonary vascular resistance of neonatal calves before (baseline), 1.5 and 3 hours after intrapulmonary inoculation of bovine fetal serum (BFS) (group 1) and Pasteurella haemolytica (group 2 and 3). Group 1 received ETYA 30 minutes prior to (BFS) inoculation. Group 2 received ETYA 30 minutes prior to Pasteurella haemolytica inoculation. Group 3 received vehicle (95% ethanol) prior to Pasteurella haemolytica inoculation.


GROUP 1
GROUP 2
GROUP 3
PULMONARY ARTERIAL PRESSURE (mmHg)
40
20
0
BASELINE
T1.5
T3.0
TIME (Hrs)
PaW
BASELINE T1.5 T3.0
TIME (Hrs)


CARDIAC OUTPUT (L/min)
4
2
0
BASELINE
T1.5
T3.0
TIME (Hrs)

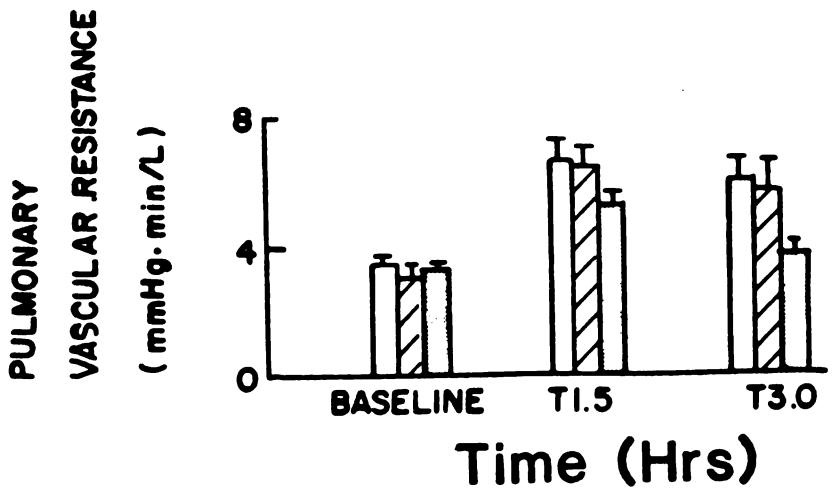

PULMONARY VASCULAR RESISTANCE (mmHg·min/L)
8
4
0
BASELINE
T1.5
T3.0
Time (Hrs)

Table 6. Mean systemic arterial pressure (Psa), systemic vascular resistance (SVR), heart rate (HR), and stroke volume (SV) in both control and treated groups receiving ETYA (group 1 and 2, respectively), and the treated group receiving alcohol (group 3). The control group was inoculated with bovine fetal serum. Treated groups were inoculated with Pasteurella haemolytica. ETYA was given 30 minutes before inoculation.

Parameters	Baseline	Hours after inoculation 1.5	3
Psa (mmHg)			
Group 1	105.0±6.3	89.0±11.0	86.0±7.3
Group 2	101.0±6.6	90.6±8.5	78.0±8.5
Group 3	98.0±4.6	104.0±8.8	78.6±5.8
SVR (mmHg)/(L/min)			
Group 1	26.79±3.24	28.78±4.38	32.58±2.17
Group 2	22.20±2.2	28.71±4.93	24.11±4.12
Group 3	24.42±0.8	27.12±2.59	20.10±0.99
Heart rate (beats/min)			
Group 1	156.0±3.8	177.6±5.8	158.4±12.2
Group 2	172.8±6.1	170.4±4.4	160.8±7.2
Group 3	183.0±4.4	201.0±2.3	189.0±5.8
SV (ml)			
Group 1	24.78±1.89	17.0±1.46	16.60±2.05
Group 2	25.34±1.16	20.0±3.86	19.27±3.65
Group 3	20.94±1.57	18.2±1.17	19.96±1.36

Data are expressed as the mean ± SE (n=5/group).

and compared for significant differences. The regional vascular resistance is shown in Figure 17. Although the regional vascular resistance rose in the negative control group (group 1), the change was not statistically significant. Administration of ETYA produced a significant increase in rPVR in the pneumonic group of calves (group 2) compared with the baseline values. Regional resistance did not differ between groups receiving ETYA (group 1 and 2). In the treated group receiving vehicle (group 3), local pneumonia caused a significant reduction in rPVR 3 hours post-inoculation compared with groups receiving ETYA (group 1 and 2). However, there was no significant difference compared with the baseline values in the same group of calves (group 3). Regional vascular resistance of the healthy lung regions did not change significantly between groups or with time (Figure 18).

The portion of pulmonary blood flow perfusing the inoculated lung regions, expressed as a percentage of CO is shown in Figure 19. In the group receiving vehicle (group 3) local pneumonia caused a substantial increase in pulmonary blood flow to the diseased lung regions 3 hours post-inoculation compared with the groups receiving ETYA (group 1 and 2). In contrast regional blood flow did not significantly change with time in the animals treated with ETYA (groups 1 and 2).

Consistent with our previous observation, in the treated group receiving vehicle (group 3), there was a significant correlation between the change in venous admixture and the change in pulmonary blood flow perfusing the diseased lung regions 3 hours after inoculation ($r=0.90$) (Figure 20). Figure 20 also shows the data of

Figure 17. Regional pulmonary vascular resistance (rPVR) of neonatal calves before (baseline), 1.5 and 3 hours after local intrapulmonary inoculation of bovine fetal serum (BFS) (group 1) and Pasteurella haemolytica (group 2 and 3). Group 1 received ETYA 30 minutes before BFS inoculation. Group 2 received ETYA 30 minutes before Pasteurella haemolytica inoculation. Group 3 received vehicle (95% ethanol) 30 minutes before Pasteurella haemolytica inoculation.

The upper panel shows rPVR of individual lung regions.

The lower panel shows rPVR of combined regions.

‡ Indicates statistically significant difference within the treated group receiving ETYA (group 2) ($P<0.05$).

*Indicates statistically significant difference between groups ($P<0.05$).

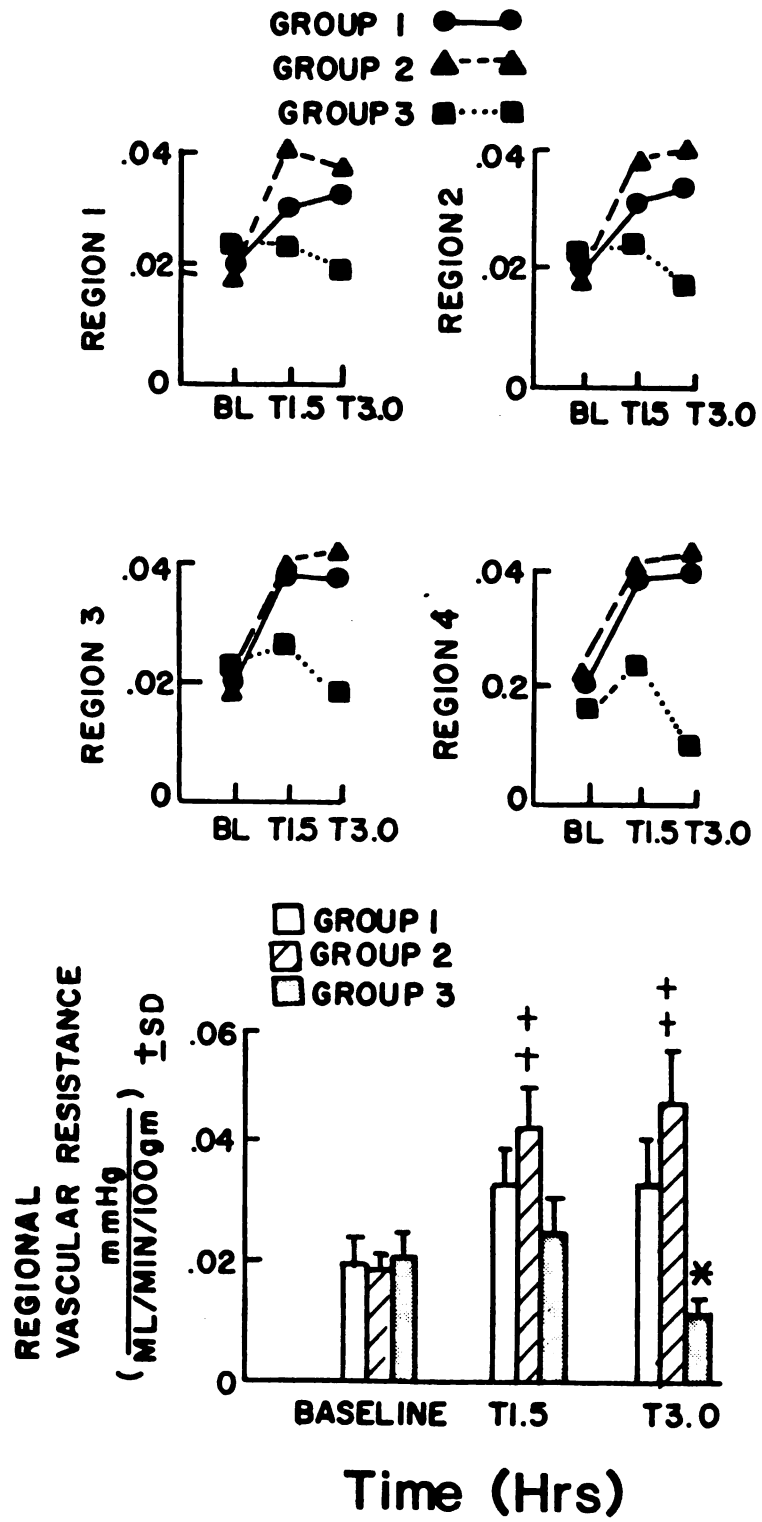

GROUP 1
GROUP 2
GROUP 3
REGION 1
REGION 2
REGION 3
REGION 4
.04
.02
0
.2
BL T1.5 T3.0
REGIONAL VASCULAR RESISTANCE
(mmHg / ML/MIN/100gm) ±SD
.06
+
*
BASELINE
T1.5
T3.0
Time (Hrs)

Figure 18. Regional vascular resistance (rPVR) of the healthy lung regions before (baseline), 1.5 and 3 hours after local intrapulmonary inoculation of bovine fetal serum (BFS) (group 1) and Pasteurella haemolytica (group 2 and 3). Group 1 received ETYA 30 minutes before BFS inoculation. Group 2 received ETYA 30 minutes before Pasteurella haemolytica inoculation. Group 3 received vehicle 30 minutes before Pasteurella haemolytica inoculation.

The upper panel shows regional vascular resistance of individual lung regions.

The lower panel shows regional vascular resistance of combined lung regions.

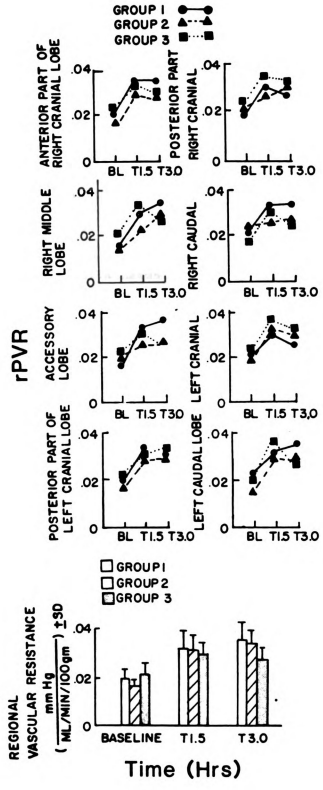

GROUP I
GROUP 2
GROUP 3
rPVR
ANTERIOR PART OF RIGHT CRANIAL LOBE
POSTERIOR PART RIGHT CRANIAL
RIGHT MIDDLE LOBE
RIGHT CAUDAL
ACCESSORY LOBE
LEFT CRANIAL
POSTERIOR PART OF LEFT CRANIAL LOBE
LEFT CAUDAL LOBE
.04
.02
0
BL T1.5 T3.0
GROUP I
GROUP 2
GROUP 3
REGIONAL VASCULAR RESISTANCE mm Hg (ML/MIN/100gm) ±SD
BASELINE
T1.5
T3.0
Time (Hrs)

Figure 19. Percentage cardiac output/100 gm perfusing the lung regions before (baseline), 1.5 and 3 hours after intrapulmonaary inoculation of bovine fetal serum (BFS) (group 1) and Pasteurella haemolytica (group 1 and 2). Group 1 received ETYA 30 minutes prior to inoculation with BFS. Group 2 received ETYA 30 minutes before Pasteurella haemolytica. Group 3 received vehicle (95% ethanol) 30 minutes before Pasteurella haemolytica.

The upper panel shows pulmonary perfusion of individual lung regions.

The lower panel shows pulmonary perfusion of combined lung regions.

*Indicates statistically significant difference between groups ($P<0.05$).

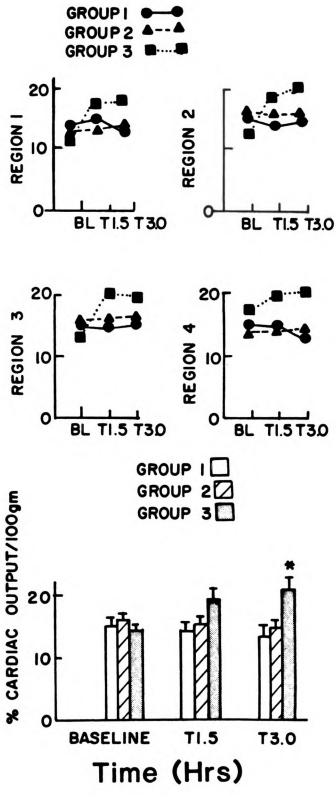
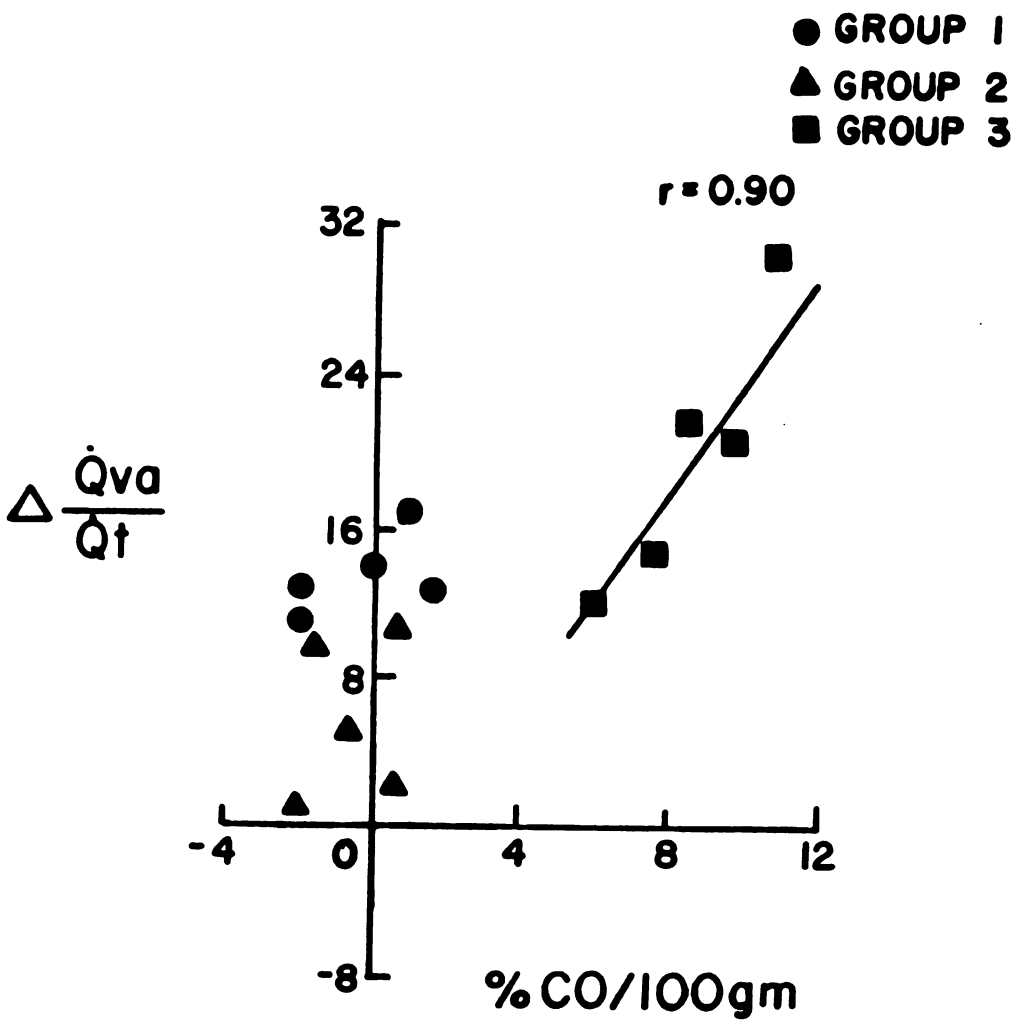

GROUP 1
GROUP 2
GROUP 3
REGION 1
REGION 2
REGION 3
REGION 4
20
10
0
BL T1.5 T3.0
GROUP 1
GROUP 2
GROUP 3
% CARDIAC OUTPUT/100gm
*
BASELINE
T1.5
T3.0
Time (Hrs)

Figure 20. The relationship between the change in venous admixture and the change in percentage cardiac output perfusing the inoculated lung regions for the 3 treatment groups. Each data point represents the changes occurring in each treated calf between baseline and 3 hours following local intrapulmonary inoculation of bovine fetal serum (BFS) (group 1) or Pasteurella haemolytica (group 2 and 3). Group 1 received ETYA 30 minutes prior to inoculation with BFS. Group 2 received vehicle (95% ethanol) 30 minutes before Pasteurella haemolytica inoculation. There is a sigificant correlation between venous admixture and pulmonary blood flow to the pneumonic regions in the treated group receiving vehicle (group 3) (r=0.90). There is no correlation between the change in venous admixture and the change in pulmonary perfusion in the inoculated regions in both control and treated groups receiving ETYA (group 1 and 2, respectively).


GROUP 1
GROUP 2
GROUP 3
r = 0.90
32
24
16
8
-8
-4
0
4
8
12
Δ Q̇va / Q̇t
% CO/100gm

the other groups of calves (group 1 and 2) in which there was no significant correlation between the increase in regional blood flow and the changes in venous admixture.

The percentage CO perfusing the healthy lung regions in the presence and absence of ETYA did not change with time in any group of animals (Figure 21).

DISCUSSION

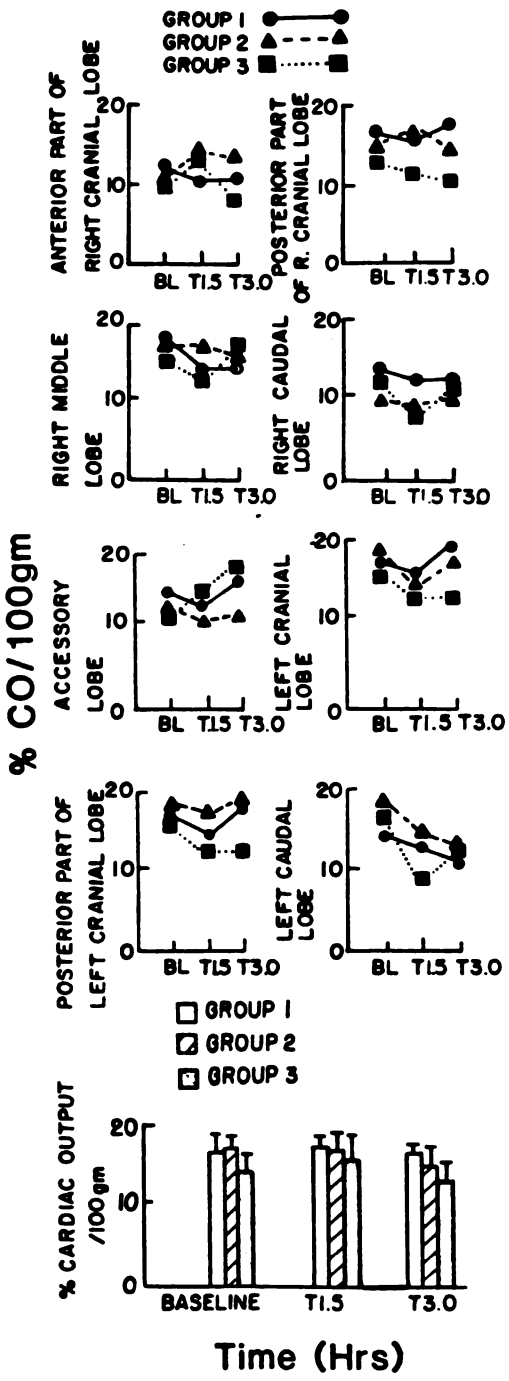
The results of the present study are consistent with the hypothesis that arachidonate metabolites maintain blood flow in the pneumonic regions of lung. Regional blood flow is determined by driving pressure and regional vascular resistance. Local inoculation of P. haemolytica resulted in a slight but insignificant decrease in rPVR, no change in pulmonary perfusion pressure, and consequently a modest increase in regional pulmonary blood flow at 3 hours. In contrast, administration of ETYA prior to P. haemolytica inoculation caused a dramatic increase in rPVR compared with the treated group of calves receiving vehicle. This finding suggests that the production of vasodilator eicosanoids is responsible for the maintenance of blood flow and low rPVR in the diseased regions of lung. The combination of P. haemolytica and ETYA caused more local vasoconstriction than ETYA alone, suggesting two possibilities. Firstly, P. haemolytica or the inflammatory process produces a vasoconstrictor agent, the actions of which are unmasked by arachidonic acid metabolism blockade. Secondly, the vascular smooth muscle becomes hyperreactive during pasteurellosis.

Figur

Figure 21. The percentage cardiac output/100 gm perfusing the healthy lung regions in the presence and absence of ETYA injection before (baseline), 1.5 and 3 hours after intrapulmonary inoculation of bovine fetal serum (BFS) (group 1) and Pasteurella haemolytica (group 1 and 2). Group 1 received ETYA 30 minutes prior to inoculation of BFS. Group 2 received ETYA 30 minutes before Pasteurella haemolytica inoculation. Group 3 received vehicle (95% ethanol) 30 minutes before Pasteurella haemolytica inoculation.

The upper panel shows pulmonary perfusion of individual lung regions.

The lower panel shows pulmonary perfusion of combined lung regions.


GROUP 1
GROUP 2
GROUP 3
ANTERIOR PART OF RIGHT CRANIAL LOBE
POSTERIOR PART OF R. CRANIAL LOBE
RIGHT MIDDLE LOBE
RIGHT CAUDAL LOBE
ACCESSORY LOBE
LEFT CRANIAL LOBE
POSTERIOR PART OF LEFT CRANIAL LOBE
LEFT CAUDAL LOBE
BL T1.5 T3.0
20
10
0
% CO/100gm
GROUP 1
GROUP 2
GROUP 3
% CARDIAC OUTPUT /100gm
BASELINE
T1.5
T3.0
Time (Hrs)

Several investigators have shown that cyclooxygenase inhibitors increase PVR and augment the response to alveolar hypoxia and proposed that the vasodilator eicosanoids maintain the normal low resistance of pulmonary vascular bed (Kadowitz et al. 1975; Alexander et al. 1977; Cohn et al. 1978). Our data do not allow us to confirm this hypothesis, because we were unable to demonstrate a significant increase in PVR or rPVR of the healthy regions of lung in calves receiving ETYA. The trend toward increased rPVR of healthy regions was observed in group 3 as well as in groups receiving ETYA. However, in the diseased regions of lung, vasodilator eicosanoids do appear to be important to maintain a dilated vascular bed.

The lung produces and releases prostaglandins in response to a number of physiological and pathophysiologic stimuli including hyperinflation, anaphylaxis and pulmonary embolization (Malik 1983; Hasan and Proulx 1983). Additionally, previous investigations have shown that the vasodilator prostaglandins, in particular PGE_1 and PGE_2, are augmented in neutrophilic inflammation, modulate local leukocytic infiltration and may also increase the severity of vascular damage (Higgs and Salmon 1979; Johnston et al. 1979; Issekutz and Movat 1982). In our experimental model of pneumonia, acute pneumonic pasteurellosis may induce regional vasodilation by stimulating the production of a vasodilator prostaglandin either in the diseased lung regions or at a distal site. Although measurements of prostaglandin metabolites in an experimental preparation similar to ours are needed to confirm this hypothesis, the vasoconstriction produced by ETYA in calves inoculated with P. haemolytica suggests that vasodilator

eicosanoids play a crucial role in the hemodynamic responses occurring in pasteurellosis.

Recent investigation has shown that lungs with acute pneumonic pasteurellosis are invaded by a large number of neutrophils. Neutrophil depletion with hydroxyurea prevented the hypoxemia and the acute lung injury induced by P. haemolytica (Slocombe et al. 1985). It is also established that when activated, neutrophils are capable of initiation of the arachidonic acid cascade. It is possible that other vasodilator prostaglandins produced by neutrophils such as PGE_2 or PGD_2 could participate in the regulation of pulmonary blood flow in neonatal calves with pneumonic pasteurellosis. In addition these two prostaglandins exhibit vasodilator properties solely in fetal and neonatal animals (Tyler et al. 1975; Phillips et al. 1983). The results of the present experiment indicate that the hemodynamic effect of acute pneumonic pasteurellosis is restricted to the pneumonic lung regions which suggests one of two hypotheses. Either the production of prostaglandins is normal throughout the lung but the response is augmented to produce substantial vasodilation only in the diseased lung regions. Alternatively, increased production of prostaglandins may have taken place in the pneumonic lung tissues.

The change in local blood flow in the 3 groups of calves between baseline and 3 hours can explain the change observed in $\dot{Q}va/\dot{Q}t$, PAO_2-PaO_2 and PaO_2. Group 3 had the highest blood flow in the inoculated regions, accompanied by the largest $\dot{Q}va/\dot{Q}t$ and PAO_2-PaO_2 and the lowest PaO_2. In group 1 and 2, regional blood flow was less than in group 3 resulting in lower $\dot{Q}va/\dot{Q}t$ and PAO_2-PaO_2 and higher PaO_2. In our previous studies (Chapter 4), we found a close

relationship between the change in venous admixture and pulmonary perfusion 3 hours after P. haemolytica inoculation. We also observed that 3 hours post inoculation with P. haemolytica, the increase in venous admixture could not be explained totally by the increased blood flow to the diseased lung regions. Similarly in the present study there was a significant correlation between the change in regional blood flow to the pneumonic regions and the change in $\dot{Q}va/\dot{Q}t$ between baseline and 3 hours in group 3 animals. However, the increase in $\dot{Q}va/\dot{Q}t$ was greater than could be explained by the increase in regional blood flow. Therefore, we propose that part of pneumonic hypoxemia may be attributable to venous admixture from poorly ventilated lung units in the healthy lung regions. Because the venous admixture slightly increased in group 1 and 2 unaccompanied by increased regional blood flow (Figure 20), the increased $\dot{Q}va/\dot{Q}t$ in these groups, as well as in group 3, could also be explained by a lack of ventilation in the inoculated lung regions.

CHAPTER 6.

SUMMARY AND CONCLUSIONS

In summary, acute pneumonia induced by intratracheal inoculation of $2X10^9$ P. haemolytica organisms caused pulmonary hypotension accompanied by severe arterial hypoxemia in neonatal calves (Chapter 3). The decrease in pulmonary arterial pressure was due to the decrease in PVR because CO did not change.

The intrapulmonary presence of P. haemolytica attenuated the hypoxic vasoconstrictor mechanism of the lung vessels 3 hours after inoculation (Chapter 3). A previous study by Hiser et al. (1975) demonstrated that the hypoxic vasoconstrictor mechanism was preserved in canine pneumococcal pneumonia 36 hours after inoculation. Since the response to hypoxia in this experiment was tested 3 hours after inoculation of gram negative bacteria (P. haemolytica), the differences between these experiments and those of Hiser et al. (1975) could be explained either by the duration of the disease or the type of microorganisms.

Local inoculation of P. haemolytica into sublobar regions of lung in neonatal calves caused an increase in pulmonary blood flow to the diseased lung regions. The increase in pulmonary perfusion was due to a decrease in regional vascular resistance (Chapter 4). The decrease in local resistance which resulted in the increase in pulmonary blood flow in the pneumonic lung regions could have in part accounted for the arterial hypoxemia with pasteurellosis.

The regional vasodilation and hypoxemia in this experimental model of pneumonia (Chapter 4) is attributable either to the presence

of P. haemolytica or to a vasodilator substance released from the neutrophils during the inflammatory reactions initiated by bacterial organisms. It has been suggested by many that bacteria or the humoral factors produced by the inflammatory process may override hypoxic vasoconstriction and maintain blood flow in the diseased region with pneumonia (Goldzimer et al. 1974; Hiser et al. 1975; Light et al. 1981). My results suggest this is true because the vascular response to hypoxia was attenuated by P. haemolytica.

In neonatal calves with local pneumonic there was a correlation between venous admixture and the percentage cardiac output perfusing the pneumonic lung regions (Chapter 4). However, the increase in venous admixture was greater than the increase in pulmonary perfusion to the diseased lung 3 hours post-inoculation. In canine lobar pneumococcal pneumonia, the venous admixture was also higher than the venoarterial shunt (Light et al. 1981). Therefore, the increase in venous admixture fraction in pneumonic pasteurellosis cannot be explained totally by the increased blood flow to the diseased regions. I propose that part of pneumonic hypoxemia is attributable to venous admixture from poorly ventilated lung units in the apparently healthy lung regions.

Intraperitoneal administration of the cyclooxygenase and lipoxygenase blocker ETYA, prior to challenge of calves with P. haemolytica, decreased blood flow to the diseased lung regions and improved arterial oxygen tension (Chapter 5) when compared with calves which did not receive ETYA. The decrease in pulmonary perfusion was associated with an increase in regional vascular resistance in the pneumonic regions. It has been shown that the concentration of

vasodilator prostaglandins increases after lung vascular injury with endotoxin and microembolization (Malik 1983; Hasan et al. 1983) and allows flow to be maintained to hypoxic alveoli resulting in hypoxemia. The inhibition of the regional vasodilation by ETYA suggests that vasodilator eicosanoids released by neutrophils and/or injured endothelium, play an important role in pulmonary hemodynamics in pasteurellosis.

LIST OF REFERENCES

LIST OF REFERENCES

Adler, L.N., L. Casella and W.H. Abelmann. Hemodynamics in lobar pneumonia. Clin. Res. 12:289, 1964.

Alexander, A. Normal structure of bovine pulmonary vasculature. Am. J. Vet. Res. 24:1083-1121, 1963.

Alexander, J.K., H. Takezaca, H.J. Abu-Nassar and E.M. Yow. Studies of pulmonary blood flow in pneumococcal pneumonia. Cardiovasc. Res. Cent. Bull. 1:86-92, 1963.

Alexander, J.M., M.D. Nyby and K.A. Jasberg. Prostaglandin synthesis inhibition restores hypoxic pulmonary vasoconstriction. J. Appl. Physiol. 42:903-908, 1977.

Ames, T.R., R.F. Markham, J.O. Leininger and S.K. Maheswaran. Pulmonary response to intratracheal challenge with Pasteurella haemolytica and Pasteurella multocida. Can. J. Comp. Med. 49:395-400, 1985.

Assali, N.S., N. Sehgal and S. Marable. Pulmonary and ductus arteriosus circulation in the fetal lamb before and after birth. Am. J. Physiol. 202:536-540, 1962.

Babior, B.M. Oxygen-dependent microbial killing by phagocytes. N. Engl. J. Med. 298:721-725, 1978.

Babior, B.L., R.E. Rosen, B.J. McMurrich, W.A. Peters and B.M. Babior. Arrangement of the respiratory burst oxidase in the plasma membrane of the neutrophil. J. Clin. Invest. 67:1724-1728, 1981.

Barber, A.A. and F. Bernheim. Lipid peroxidation: Its measurement, occurrence, and significance in animal tissues. Adv. Gerontol. Res. 2:355-401, 1967.

Beesley, J., J. Pearson, S. Carleton, A. Hutchings and J. Gordon. Granulocyte migration through endothelium in culture. J. Cell. Sci. 38:237-248, 1978.

Benson, H., M. Akbarian, L.N. Adler and W.H. Abelmann. Hemodynamic effects of pneumonia: Normal and hypodynamic responses. J. Clin. Invest. 49:791-798, 1970.

Boucher, J -H., D.E. Hilmas, C.T. Liu, W.P. Czajkowski and R.O. Spertzel. Myocardial depression during diplococcus pneumonia infection in monkeys. Proc. Soc. Exp. Biol. Med. 145:112-116, 1974.

Boxer, L., J. Allen and R. Bachner. Diminished polymorphonuclear leukocyte adherence: function dependent on release of cyclic AMP by endothelial cells after stimulation of B-receptors by epinephrine. J. Clin. Invest. 66:268-274, 1980.

Brigham, K., R. Bowers and J. Haynes. Increased sheep lung vascular permeability caused by E. coli endotoxin. Circ. Res. 45:292-297, 1979.

Brigham, K.L., R.E. Bowers and C.R. McKeen. Methylprednisolone prevention of increased lung vascular permeability following endotoxemia in sheep. J. Clin. Invest. 67:1103-1110, 1981.

Brigham, K.L. and M.L. Ogletree. Effects of prostaglandins and related compounds on lung vascular permeability. Bull. Eur. Physiopathol. Respir. 17:703-722, 1981.

Brigham, K.L., J.H. Newman, J.R. Snapper and M.L. Ogletree. Metabolites of arachidonic acid in the pathophysiology of the pulmonary circulation. In The Prostaglandin and Cardiovascular System, J.A. Oates, Ed., Raven Press, NY, 1982, pp. 355-364.

Brigham, K.L. and B. Meyrick. Interactions of granulocytes with the lungs. Circ. Res. 54:623-635, 1984.

Brigham, K.L. Metabolites of arachidonic acid in experimental lung vascular injury. Fed. Proc. 44:43-45, 1985.

Brody, M.J. and P.J. Kadowitz. Prostaglandins as modulators of the autonomic nervous system. Fed. Proc. 33:48-60, 1974.

Buchanan, M., M. Vazquez and M. Gimbrone. Influence of lipoxygenase on the adhesion of human leukocytes to cultured vascular endothelial cells. Blood 58:(Suppl. 70a):214-220, 1981.

Buchbinder, N. Hemodynamic monitoring. Anaesthesiology 45:146-155, 1976.

Campbell, E.J. and M.S. Wald. Hypoxic injury to human alveolar macrophages accelerates release of previously bound neutrophil elastase. Am. Rev. Resp. Dis. 127:631-635, 1983.

Camussi, G., C. Tetta, G. Segoli, M.C. Deregibus and F. Bussolino. Neutropenia induced by PAF-acether released from neutrophils: the inhibitory effect of PGI_2. Agents Actions 11:550-553, 1981.

Clark, R.A. and S.J. Klebanoff. Myeloperoxidase-mediated platelets release reaction. J. Clin. Invest. 63:177-183, 1979.

Cochrane, C., R. Spragg, S. Revak, A. Cohen and W. McGuire. The presence of neutrophil elastase and evidence of oxidant activity in bronchoalveolar lavage fluid of patients with adult respiratory distress syndrome. Am. Rev. Resp. Dis. 127:525-527, 1983.

Cohn, M.A., H. Baier and A. Wanner. Failure of hypoxic pulmonary vasoconstriction in the canine asthma models: The effect of prostaglandin inhibitors. J. Clin. Invest. 61:1463-1470, 1978.

Cohn, P.F. Clinical Cardiovascular Physiology, W.B. Saunders Co., Philadelphia, 1985, p. 106.

Colp, C.R., S.S. Park and M.H. Williams. Pulmonary function studies in pneumonia. Am. Rev. Respir. Dis. 85:808-815, 1962.

Craddock, P.R., J. Fehr, K.L. Brigham, R.S. Kronenberg and H.S. Jacob. Complement and leukocyte-mediated pulmonary dysfunction in hemodialysis. N. Engl. J. Med. 296:769-774, 1977.

Crapo, J., F. Barry, H. Foscue and J. Shelburne. Structural and biochemical changes in rat lungs occurring during exposures to lethal and adaptive doses of oxygen. Am. Rev. Respir. Dis. 122:123-143, 1980.

Cross, C.E. and R.W. Hyde. Treatment of pulmonary edema. In Lung Water and Solute Exchange, N.C. Staub, Ed., Marcel Dekker, New York, 1978, pp. 471-525.

Dey, R.D., W.A. Shannon, Jr. and S.I. Said. Localization of VIP-immunoreactive nerves in airways and pulmonary vessels of dogs, cats, and human subjects. Cell. Tissue Res. 220:231-238, 1981.

Dowing, S.E. and J.C. Lee. Nervous control of the pulmonary circulation. Ann. Rev. Physiol. 42:199-210, 1980.

Drath, D.B. and M.L. Karnovsky. Superoxide production by phagocytic leukocytes. J. Exp. Med. 141:257-261, 1975.

Ellsworth, M.L., T.J. Gregory and J.C. Newell. Pulmonary prostacyclin production with increased flow and sympathetic stimulation. J. Appl. Physiol. 55:1225-1231, 1983.

Enjeti, S., P.B. Terry, H.A. Menkes and R.J. Traystmas. Mechanical factors and the regulation of perfusion through atelectatic lung in pigs. J. Appl. Physiol. 52:647-654, 1982.

Fantone, J.C. and P.A. Ward. Role of oxygen-derived free radicals and metabolites in leukocyte-dependent inflammatory reactions. Am. J. Pathol. 107:397-418, 1982.

Fishman, A.P., H.W. Fritts and A. Cournand, Jr. Effects of acute hypoxia and exercise on the pulmonary circulation. Circulation 22:204-215, 1960.

Fishman, A.P. Hypoxia on the pulmonary circulation: How and where it acts. Circ. Res. 38:221-231, 1976.

Flick, M., A. Percel and N. Staub. Leukocytes are required for increased lung microvascular permeability after microembolization in sheep., Circ. Res. 48:344-351, 1981.

Ford-Hutchinson, A.W. Leukotrienes: their formation and role as inflammatory mediators. Fed. Proc. 44:25-29, 1985.

Fox, R., J. Hoidal, D. Brown and J. Repine. Pulmonary inflammation due to oxygen toxicity: involvement of chemotactic factors and polymorphonuclear leukocytes. Am. Rev. Respir. Dis. 123:521-523, 1981.

Garcia-Szabo, R., F.L. Minnear, R. Bizios, A. Johnson and A.B. Malik. Role of thromboxane in the pulmonary microembolization. Chest 835:765-785, 1983.

Garcia-Szabo, T., M.B. Peterson, W.D. Watkins, R. Bizios and D.L. Malik. Thromboxane generation after thrombin. Circ. Res. 53:214-222, 1983.

Garrett, R.C. and H.M. Thomas. Meclofenamate uniformly decreases shunt fraction in dogs with lobar atelectasis. J. Appl. Physiol. 54:284-289, 1983.

Garrett, R.C. and H.M. Thomas. Relation of prostanoids to strength of hypoxic vasoconstriction in dogs with lobar atelectasis. J. Appl. Physiol. 59:72-77, 1985.

Gaynor, F. The role of granulocytes in endotoxin induced vascular injury. Blood 41:797-808, 1973.

Gerber, J.G., N.F. Voelkel, A.S. Nies, I.F. McMurtry and J.T. Reeves. Moderation of hypoxic vasoconstriction by infused arachidonic acid: Role of PGI_2. J. Appl. Physiol. 49:107-112, 1980.

Gimbrone, M. and M. Buchanan. Interactions of platelets and leukocytes with vascular endothelium. In vitro studies. Ann. NY Acad. Sci. 401:171-183, 1982.

Glazier, J.B. and J.F. Murray. Sites of pulmonary vasomotor reactivity in the dog during alveolar hypoxia and serotonin and histamine infusion. J. Clin. Invest. 50:2550-2558, 1971.

Goldbrum, S.E., K.M. Wu and H.H. Tai. Streptococcus pneumoniae-induced alterations in levels of circulating thromboxane and prostacyclin: Dissociation from granulocytopenia, thrombocytopenia and pulmonary leukostasis. J. Infect. Dis. 153:71-77, 1986.

Goldzimer, E.L., P.D. Wagner and K.M. Moser. Sequence of ventilation/perfusion alterations during experimental pneumococcal pneumonia in the dog. Chest 64:394-395, 1973.

Goldzimer, E.L., R.G. Konopka and K.M. Moser. Reversal of the perfusion defect in experimental canine lobar pneumococcal pneumonia. J. Appl. Physiol. 37:85-91, 1974.

Goshy, M., S.J. Lai-Fook and R.E. Hyatt. Perivascular pressure measurements by wick-catheter technique in isolated dog lobes. J. Appl. Physiol. 46:950-955, 1979.

Gryglewski, R.J., J. Korbut and A. Ocetkiewicz. Generation of prostacyclin by lung in vivo and its release into the arterial circulation. Nature (London) 273:765-767, 1978.

Gunther, R., C. Zaiss and R.H. Demling. Pulmonary microvascular responses to prostacyclin PG(I_2) infusion in unanesthetized sheep. J. Appl. Physiol. 52:1338-1342, 1982.

Haack, D.W., J.H. Abel and R.S. Jaenke. Effects of hypoxia on the distribution of calcium in arterial smooth muscle cells of rat and swine. Cell. Tiss. Res. 157:125-129, 1975.

Haas, F. and E.H. Bergofsky. Effect of pulmonary fasoconstriction on balance between alveolar ventilation and perfusion. J. Appl. Physiol. 24:491-497, 1968.

Hales, C.A., E.T. Rouse and J.L. Slate. Influence of aspirin and indomethacin on variability of alveolar hypoxic vasoconstriction. J. Appl. Physiol. 45:33-39, 1978.

Hamasaki, Y., H.H. Tae and S.I. Said. Hypoxia stimulates prostacyclin generation by dog lung in vitro. Prostaglandins Leukotrienes Med. 8:311-316, 1982.

Hamburg, M.J., J. Svensson and B. Samuelsson. Thromboxanes: a new group of biologically active compounds derived from prostaglandin endoperoxides. Proc. Natl. Acad. Sci. USA 72:2994-2998, 1975.

Hamberg, M. and B.B. Fredholm. Isomerization of prostaglandin H_2 into prostaglandin D_2 in the presence of serum albumin. Biochem. Biophys. Acta 431:189-193, 1976.

Harris, P., H.W. Fritts, R.H. Clauss, J.E. Odell and A. Cournand. Influence of acetylcholine on human pulmonary circulation under normal and hypoxic conditions. Proc. Soc. Exp. Biol. Med. 93:77-79, 1956.

Harrison, J.E. and J. Schultz. Studies on the chlorinating activity of myeloperoxidase. J. Biol. Chem. 251:1371-1374, 1976.

Hasan, F.M. and J. Proulx. Role of prostacyclin in endotoxin-induced lung injury. Fed. Proc. 42:1107, 1983.

Hebb, C. Motor innervation of the pulmonary blood vessels of mammals. In Pulmonary Circulation and Interstitial space, A.P. Fishman and H.H. Hecht, Eds., University of Chicago Press, IL, 1969, pp. 195-222.

Hedqvist, P. Basic mechanisms of prostaglandin action on autonomic neutrotransmission. Ann. Rev. Pharmacol. Toxicol. 17:259-179, 1977.

Heflin, A.C. and K.L. Brigham. Prevention by granulocyte depletioon of increased vascular permeability of sheep lung following endotoxemia. J. Clin. Invest. 68:1253-1260, 1981.

Heinemann, H.O. and A.P. Fishman. Non-respiratory function of mammalian lung. Physiol. Rev. 49:1-47, 1969.

Henson, P.M., K. McCarthy, G.L. Larsen, R.O. Webster, P.C. Gielas, R.B. Dreisin, T.E. King and J.O. Shaw. Complement fragment, alveolar macrophages and alveolitis. Am. J. Pathol. 97:93-105, 1979.

Herzog, H., H. Staub and R. Richterich. Gas analytical studies in severe pneumonia. Observations during the 1957 influenza epidemic. Lancet 1:593-596, 1959.

Heymann, M.A., B.D. Payne, J.E. Hoffman and A.M. Rudolph. Blood flow measurements with radionuclide-labeled particles. Prog. Cardiovasc. Dis. 20:55-79, 1977.

Higgs, G.A. and J.A. Salmon. Cyclo-oxygenase products in carrageenin-induced inflammation. Prostaglandin 17:737-746, 1979.

Higgs, G.A., K.E. Eakins, K.G. Mugridge; S. Moncada and J.R. Vane. The effects of non-steroid anti-inflammatory drugs on leukocyte migration in carrageenin-induced inflammation. Eur. J. Pharmacol. 66:81-86, 1980.

Hinson, J., A. Hutchison, M.L. Ogletree, K. Brigham and J. Snapper. Effects of granulocyte depletion on altered lung mechanics after endotoxemia in sheep. J. Appl. Physiol. 55:92-99, 1983.

Hiser, W., R.W. Penman and J.T. Reeves. Preservationn of hypoxic pulmonary pressor response in canine pneumococcal pneumonia. Am. Rev. Respir. Dis. 112:817-822, 1975.

Hoover, R., R. Folger, W. Haering, B. Ware and M. Karnovsky. Adhesion of leukocytes to endothelium: Roles of divalent cations, surface change, chemotactic agents and substrate. J. Cell. Sci. 45:73-86, 1980.

Huttemeier, P., D. Watkins, M. Peterson and W. Zapol. Acute pulmonary hypertension and lung thromboxane release after endotoxin infusion in normal and leukopenic sheep. Circ. Res. 50:688-694, 1982.

Hyman, A.L., E.W. Spannhake and P.J. Kadowitz. Divergent responses to arachidonic acid in the feline pulmonary vascular bed. Am. J. Physiol. 239:H40-H46, 1980.

Issekutz, A.C. The effect of vasoactive agents on polymorphonuclear leukocyte emigration in vivo. Lab. Invest. 45:234-240, 1981.

Issekutz, A.D. and H.Z. Movat. The effect of vasodilator prostaglandins on polymorphonuclear leukocyte infiltration and vascular injury. Am. J. Pathol. 107:300-309, 1982.

Jericho, K.W.F. and S.E. Magwood. Histological features of respiratory epithelial of calves held at differing temperature and humidity. Can. J. Comp. Med. 41:369-379, 1977.

Johnson, A.R., A.R. Callahan, S.C. Tsai and W.B. Campbell. Prostacyclin and prostaglandin biosynthesis in human pulmonary endothelial cells. Bull. Eur. Physiol. Pathol. Respir. 17:531-551, 1981.

Johnson, A. and P. Ward. Acute and progressive lung injury after contact with phorbol myristate acetate. Am. J. Pathol. 107:29-35, 1982.

Johnson, K.J., J.C. Fantone, J. Kaplan and P.A. Ward. In vivo damage of rat lungs by oxygen metabolites. J. Clin. Invest. 67:983-993, 1981.

Johnson, T. and A.B. Malik. Pulmonary edema after glass bead microembolization: protective effect of granulocytopenia. J. Appl. Physiol. 52:155-1161, 1982.

Johnston, M.G., J.B. Hay and H.Z. Movat. Kinetics of prostaglandin production in various inflammatory lesions, measured in draining lymph. Am. J. Pathol. 95:225-238, 1979.

Kadowitz, P.J., P.D. Joiner and A.L. Hyman. Influence of prostaglandins E_1 and F_{2alpha} on pulmonary vascular resistance in sheep. Proc. Soc. Exp. Biol. Med. 145:1258-1261, 1974.

Kadowitz, P.J., P.D. Joiner and A.L. Hyman. Effect of prostaglandin E_2 on pulmonary vascular resistance in intact dog, swine and lambs. Eur. J. Pharmacol. 31:72-80, 1975.

Kadowitz, P.J., B.M. Chapnick, P.D. Joiner and A.L. Hyman. Influence of inhibitors of prostaglandin synthesis on the canine pulmonary vascular bed. Am. J. Physiol. 229:941-946, 1975.

Kadowitz, P.J., E.W. Spannhake, L.P. Feigen, S. Greenberg and A.L. Hyman. Comparative effects of arachidonnic acid bisenoic prostaglandins and an endoperoxide analogue on the canine pulmonary vascular bed. Can. J. Physiol. Pharmacol. 55:1369-1377, 1977.

Kadowitz, P.J., B.M. Chapnick, L.P. Feigen, A.L. Hyman, P.K. Nelson and E.W. Spannhake. Pulmonary and systemic vasodilator effects of the newly discovered prostaglandin PGI_2. J. Appl. Physiol. 45:408-413, 1978.

Kelley, K.W. Stress and immune function: A bibliographic review. Ann. Rech. Vet. 11:445-478, 1980.

Kistler, G.S., P.R. Caldwell and E.R. Weibel. Development of fine structural damage to alveolar and capillary lining cells in oxygen-poisoned lungs. J. Cell. Biol. 32:605-627, 1967.

Kuida, H., A.M. Brown, J.L. Thorne, R.L. Lange and H.H. Hecht. Pulmonary vascular response to acute hypoxia in normal, unanesthetized calves. Am. J. Physiol. 203:391-396, 1962.

Kumar, R., W.A. Wallace and W.H. Abelmann. Reversal of hypodynamic circulatory state in acute pneumonia by volume expansion. Clin. Res. 16:237, 1968.

Kumar, R., W.A. Wallace, A. Ramirez, H. Benson and W.H. Abelmann. Hemodynamic effects of pneumonia. II. Expansion of plasma volume. J. Clin. Invest. 49:799-804, 1970.

Kux, M., J. Coalson, H. Massion and A. Guenter. Pulmonary effects of E. coli endotoxin: role of leukocytes and platelets. Ann. Surg. 26:175-183, 1972.

Kyellman, B. Regional lung function studies with Xe^{133} in children with pneumonia. Acta Paediatr. Scand. 56:467-476, 1976.

Lee, C.T., A.M. Fein, M. Lippman, H. Holtzman, P.Kimbel and G. Weinbaum. Elastolytic activity in pulmonary lavage fluid from patients with adult respiratory distress syndrome. N. Engl. J. Med. 304:192-196, 1981.

Leffler, C.W., J.A. Mitchell and R.S. Green. Cardiovascular effects of leukotrienes in neonatal piglets: Role in hypoxic pulmonary vasoconstriction. Circ. Res. 55:780-787, 1984.

Light, R.B., S.N. Mink and D.H. Wood. Pathophysiology of gas exchange and pulmonary perfusion in pneumococcal lobar pneumonia in dogs. J. Appl. Physiol. 50:524-530, 1981.

Lillie, L.E. The bovine respiratory disease complex. Can. Vet. J. 15:233-242, 1974.

Lock, J.E., P.M. Olley and F. Coceani. Direct pulmonary vascular responses to prostaglandins in the conscious newborn lamb. Am. J. Physiol. 238:H631-H638, 1980.

Loyd, J., J. Newman, D. English, M. Ogletree, B. Meyrick and K. Brigham. Lung vascular effects of phorbol myristate acetate in awake sheep. J. Appl. Physiol. 54:267-276, 1983.

Malik, A.B. Pulmonary microembolism. Physiol. Rev. 63:1114-1207, 1983.

Malik, A.B., M.B. Perlman, J.A. Cooper, T. Noonan, and R. Bizios. Pulmonary microvascular effects of arachidonic acid metabolites and their role in lung vascular injury. Fed. Proc. 44:36-42, 1985.

Martin, B., J. Wright, H. Thomason and J. Hogg. The effect of pulmonary blood flow on the exchange between the circulating and orginating pool of polymorphonuclear leukocytes (PMN) in dog lungs. J. Clin. Invest. 69:1277-1285, 1982.

Matheson, N.R., D.S. Wong and J. Travis. Enzymatic inactivation of human alpha-1-proteinase inhibitor by neutrophil myeloperoxidase. Biochem. Biophys. Res. Commun. 88:402-409, 1979.

McCord, J.M. and E.D. Day, Jr. Superoxide-dependent production of hydroxyl radical catalyzed by iron-EDTA complex. Febs Letter 86:139-142, 1978.

McCord, J.M. and I. Fridovich. The biology and pathology of oxygen radicals. Ann. Intern. Med. 89:122-127, 1978.

McDonald, J.W.D., M. Ali, E. Morgan, E.R. Townsend and J.D. Cooper. Thromboxane synthesis by sources other than platelets in association with complement-induced pulmonary leukostasis and pulmonary hypertension in sheep. Circ. Res. 52:1-6, 1983.

McLaughlin, R.F., W.S. Tyler and R.O. Canada. A study of the subgross pulmonary anatomy in various mammals. Am. J. Anat. 108:149-158, 1961.

McMurtry, I.F., A.B. Davidson, J.T. Reeves and R.F. Grover. Inhibition of hypoxic vasoconstriction by calcium antagonists in isolated rat lungs. Circ. Res. 38:99-104, 1976.

McPhail, L.C., I.R. Dechatelet and P.S. Shirley. Further characterization of NADPH oxidase activity of human polymorphonuclear leukocytes. J. Clin. Invest. 58:774-780, 1976.

Mead, J., T. Takishima and D. Leith. Stress distribution in lungs: a model of pulmonary elasticity. J. Appl. Physiol. 28:596-608, 1970.

Meyrick, B. and K. Brigham. Acute effects of E. coli endotoxin on the pulmonary microcirculation of anesthetized sheep. Structure:function relationships. Lab. Invest. 48:458-470, 1983.

Meyrick, B.O. Endotoxin-mediated pulmonary endothelial cell injury. Fed. Proc. 45:19-24, 1986.

Miyazawa, T., M. Hiramoto, T. Hiramoto and O. Nishida. Reversal of oleic acid-induced respiratory distress by prostacyclin (PGI_2). Respir. Physiol. 47:351-364, 1982.

Mlezoch, J., E. Weir, F. Grover and J. Reeves. Pulmonary vascular effects of endotoxin in leukopenic dogs. Am. Rev. Respir. Dis. 117:118-124, 1978.

Moncada, S. and J.R. Vane. Pharmacology and endogenous roles of PG endoperoxides, thromboxane A_2 and prostacyclin. Pharmacol. Rev. 30:293-331, 1979.

Moon, M.A., R.J. Lemen and S.F. Quan. Failure of acute hypoxia to alter pulmonary prostaglandin metabolism in dogs. Prostaglandins 25:615-627, 1983.

Murray, J.F. The Normal Lung, 2nd Ed. W.B. Saunders Co., Philadelphia, 1986, pp. 139-162.

Nandiwada, P.A., A.L. Hyman and P.J. Kadowitz. Pulmonary vasodilator response to vagal stimulation and acetylcholine in the cat. Circ. Res. 53:86-95, 1983.

Nagy, L., T.H. Lee, E.J. Goetzl, W.C. Pickett and A.B. Kay. Complement receptor enhancement and chemotaxis of human neutrophils and eosinophils by leukotrienes and other lipoxygenase products. Clin. Exp. Immunol. 47:541-547, 1982.

Nathan, C.F., L. Brukner, S.C. Silverstein and Z.A. Cohn. Extracellular cytolysis by activated macrophages and granulocytes. J. Exp. Med. 149:100-113, 1979.

Naxano, J. and B. Cole. Effect of prostaglandins E_1 and F_{2alpha} on systemic, pulmonary and splanchnic circulation in dogs. Am. J. Physiol. 217:222-227, 1979.

Needleman, P.S., S. Moncada, S. Dunting, J.R. Vane, M. Hamberg and B. Samuelsson. Identification of an enzyme in platelet microsomes which generates thromboxane A_2 from platelet endoperoxides. Nature (London) 261:558-560, 1976.

Newman, J.H., J.E. Loyd, D.K. English, M.L. Ogletree, W.J. Fulkerson and K.L. Brigham. Effect of 100% oxygen on lung vascular function in aware sheep. J. Appl. Physiol. 54:1379-1386, 1983.

Nugteren, D.H. and E. Hazelhof. Isolation and properties of intermediates in prostaglandin biosynthesis. Biochem. Biophys. Acta 326:448-461, 1973.

O'Flaherty, J.T., D.L. Kreutzer and P.A. Ward. Effect of prostaglandins E_1, E_2, and F_{2alpha} on neutrophil aggregation. prostaglandins 17:201-210, 1979.

Ogletree, M.L. and K.L. Brigham. Arachidonate raises vascular resistance but not permeability in lungs of awake sheep. J. Appl. Physiol. 48:581-586, 1980.

Ogletree, M.L. Pharmacology of prostaglandins in the pulmonary microcirculation. Ann. NY Acad. Sci. 384:191-206, 1982.

Ogletree, M., J. Oates, K. Brigham, and W. Hubbard. Evidence for pulmonary release of 5-hydroxyeicosatetraenoic acid (5-HETE) during endotoxin induced leukopenia and increased lung vascular permeability in unanesthetized sheep. Prostaglandins 23:459-468, 1982.

Ogletree, M.L., C.J. Begley and K.L. Brigham. Combination of steroidal and nonsteroidal anti-inflammatory agents inhibits early and late effects of endotoxin in awake sheep. Am. Rev. Respir. Dis. 4:303, 1983.

Olson, N.C., R.E. Meyer and D.L. Anderson. Effects of flunixin meglumine on cardiopulmonary responses to endotoxin in ponies. J. Appl. Physiol. 59:1464-1471, 1985.

Pass, D.A. and R.G. Thomson. Wide distribution of Pasteurella haemolytica type 1 over the nasal mucosa of cattle. Can. J. Comp. Med. 35:181-186, 1971.

Pearson, J., S. Carleton, J. Beesley, A. Hutchings and J. Gordon. Granulocyte adhesion to endothelium in culture. J. Cell. Sci. 38:225-235, 1979.

Perkowski, S.Z., A.M. Havill, J.T. Flynn and M.H. Gee. Role of intrapulmonary release of eicosanoids and superoxide anion as mediators of pulmonary dysfunction and endothelial injury in sheep with intermittent complement activation. Circ. Res. 53:574-583, 1983.

Petrone, W.F., D.K. English, K. Wong and J.M. McCord. Free radicals and inflammation: The superoxide dependent activation of a neutrophil chemotactic factor in plasma. Proc. Natl. Acad. Sci. 77:1159-1163, 1980.

Petty, T. and D. Ashbaugh. The adult respiratory distress syndrome: Clinical features, factors influencing prognosis, and principles of management. Chest 60:233-239, 1971.

Phillips, J.B., R.K. Lyrene, M. McDevitt, W. Perlis, C. Satterwhite and G. Cassady. Prostaglandin D_2 inhibits hypoxic pulmonary vasoconstriction in neonatal lambs. J. Appl. Physiol. 54:1585-1589, 1983.

Piatt, J.F. and P.J. O'Brien. Singlet oxygen formation by a peroxidase, H_2O_2 and halide system. Eur. J. Biochem. 93:323-332, 1979.

Pingleton, W., J. Coalson and C. Guenter. Significance of leukocytes in endotoxin shock. Clin. Res. 683:19-26, 1971.

Porcelli, R.J. and E.H. Bergofsky. Adrenergic receptors in pulmonary vasoconstrictor responses to gaseous and humoral agents. J. Appl. Physiol. 34:483-488, 1973.

Prodjoharjono, S., G.R. Carter and G.H. Conner. Serologic study of bovine strains of Pasteurella multicida. Am. J. Vet. Res. 35:111-114, 1974.

Repine, J.E., J.W. Eaton, M.W. Anders, J.R. Hoidal and R.B. Fox. Generation of hydroxyl radical by enzymes, chemicals and human phagocytes in vitro. Detection with the anti-inflammatory agents dimethyl sulfoxide. J. Clin. Invest. 648:1642-1651, 1979.

Repine, J.E. Neutrophil, oxygen radicals and the adult respiratory distress syndrome. In Pulmonary Circulation and Acute Lung Injury. S.I. Said, Ed., NY, 1985, pp. 249-282.

Reeves, J.T., R.F. Grover, D.H. Will and A.F. Alexander. Hemodynamics in normal cattle. Circ. Res. 10:166-171, 1962.

Reeves, J.T. and R.F. Grover. Blockade of acute hypoxic pulmonary hypertension by endotoxin. J. Appl. Physiol. 36:328-332, 1974.

Rehmtulla, A.J. and A.J. Thomson. A review of the lesions in shipping fever of cattle. Can. Vet. J. 22:1-8, 1981.

Reynolds, S.R. The fetal and neonatal pulmonary vasculature in the guinea pig in relation to hemodynamic changes at birth. Am. J. Anat. 98:97-102, 1956.

Rinaldo, J.E. and R.M. Rogers. Adult respiratory distress syndrome: changing concepts of lung injury and repair. N. Engl. J. Med. 306:900-909, 1982.

Robinson, N.E. Some functional consequence of species differences in lung anatomy. Adv. Vet. Sci. Comp. Med. 26:2-29, 1982.

Rosen, I.I. and S.J. Klebanoff. Formation of singlet oxygen by the myeloperoxidase-mediated antimicrobial system. J. Biol. Chem. 252:4803-4810, 1977.

Rubin, L.J. Pulmonary hypertension secondary to lung disease. In Pulmonary Hypertension. E.K. Weir and J.T. Reeves, Eds. Futura Publishing Co., NY, 1984, pp. 291-320.

Rubin, L.J., J.D. Hughes and J.D. Lazar. The effects of eicosanoid synthesis inhibitors on normoxic and hypoxic pulmonary vascular tone in dogs. Am. Rev. Respir. Dis. 132:93-98, 1985.

Rybicka, K., B.D.T. Daly, J.J. Migliore and J.C. Norman. Ultrastructure of pulmonary alveoli of the calf. Am. J. Vet. Res. 35:213-222, 1974.

Sacks, T., C.F. Moltow, P.R. Craddock, T.K. Bowers and H.S. Jacob. Oxygen radical-mediated endothelial cell damage by complement-stimulated granulocytes. An in vitro model of immune vascular damage. J. Clin. Invest. 61:1161-1167, 1978.

Sadie, W.C. The oxygen of the arterial and venous blood in pneumonia and its relation to cyanosis. J. Exp. Med. 30:215, 1919.

Said, S.I., T. Yoshida, S. Kitamura and C. Vreim. Pulmonary alveolar hypoxia: release of prostaglandins and other humoral mediators. Science 185:1181-1183, 1974.

Said, S.I. Metabolic functions of the pulmonary circulation. Circ. Res. 50:325-333, 1982.

Said, S.I. The Pulmonary Circulation and Acute Lung Injury. S.I. Said, Ed., Futura Publishing Co., NY, 1986, p. 3.

Samuelsson, B. Leukotrienes: mediators of immediate hypersensitivity reactions and inflammation. Science 330:568-575, 1983.

Schmid-Schoenbein, G.W., S. Usami, R. Skalak and S. Chien. Interaction of leukocytes and erythrocytes in capillary and post-capillary vessels. Microvasc. Res. 19:45-70, 1980.

Shasby, D.M., R.B. Fox, R.N. Harada and J.E. Repine. Reduction of the edema of acute hypoxic lung injury by granulocyte depletion. J. Appl. Physiol. 52:1237-1244, 1982.

Shepherd, A.P. and C.G. Burger. A solid state arteriovenous oxygen difference analyzer for flowing whole blood. Am. J. Physiol. 232:H437-H440, 1977.

Silove, E.D., T. Inoue annd R.F. Grover. Comparison of hypoxia, pH, and sympathomimetic drugs on bovine pulmonary vasculature. J. Appl. Physiol. 24:355-365, 1968.

Simon, R.H., C.H. Scoggin and D. Paterson. Hydrogen peroxide causes fatal injury to fibroblasts exposed to oxygen radicals. J. Biol. Chem. 256:7181-7186, 1981.

Slocombe R.F. and N.E. Robinson. Histamine H_1, H_2 receptor effects on mechanics of ventilation and gas exchange in neonatal calves. Am. J. Vet. Res. 42:764-769, 1981.

Slocombe, R.F., F.J. Derksen, N.E.. Robinson, A. Grapp, A. Gupta and J.P. Newman. Interactions of cold stress and Pasteurella haemolytica in the pathogenesis of pneumonic pasteurellosis in calves: Method of induction and hematologic and pathologic changes. Am. J. Vet. Res. 45:1757-1763, 1983a.

Slocombe, R.J., F.J. Derksen and N.E. Robinson. Interactions of cold stress and Pasteurella haemolytica in the pathogenesis of pneumonic pasteurellosis in calves: Changes in pulmonary function. Am. J. Vet. Res. 45:1764-1770, 1983b.

Slocombe, R.F., J. Malark, R. Ingersoll, F.J. Derksen and N.E. Robinson. Importance of neutrophils in the pathogenesis of acute pneumonic pasteurellosis in calves. Am. J. Vet. Res. 46:2253-2258, 1985.

Smith, H.A., T.C. Jones and R.D. Hunt. In Veterinary Pathology, 4th Edition. Lea and Febiger, Philadelphia, 1972, pp. 1100-1105.

Snapper, J., A. Hutchison, M. Ogletree and K. Brigham. Effects of cyclooxygenase inhibitors on the alterations in lung mechanics caused by endotoxemia in the unanesthetized sheep. J. Clin. Invest. 72:63-76, 1983.

Spagnuolo, P.J., J.J. Ellner, A. Hassid and M.J. Dunn. Thromboxane A_2 mediates augmented polymorphonuclear leukocyte adhesiveness. J. Clin. Invest. 66:406-414, 1980.

Sprague, R.S., A.H. Stephenson and A.J. Lonigro. Prostaglandin I_2 supports blood flow to hypoxic alveoli in anesthetized dogs. J. appl. Physiol. 56:1246-1251, 1984.

Staub, N., E. Schultz and E. Albertine. Leukocytes and pulmonary vascular injury. Ann. NY Acad Sci. 384:332-342, 1982a.

Staub, N.C., E.L. Schultz and K.H. Albertine. Number and distribution of neutrophils (PMNS) in sheep lungs after air embolization. Am. Rev. Respir. Dis. 123:235, 1982b.

Staub, N.E., E.L. Schultz, K. Koike and K.H. Albertine. Effect of neutrophil migration induced by leukotriene B_4 on protein permeability in sheep lung. Fed. Proc. 44:30-35, 1985.

Steel, R.G.D. and J.H. Torrie. Principles and Procedures of Statistics. McGraw-Hill Book Co., 1980, pp. 383-387.

Stossel, T.P. Phagocytosis (Parts I, II, III). New Engl. J. Med. 290:717-723; 774-780; 833-839, 1974.

Sun, F.F., J.P. Chapman and J.C. McGuire. Metabolism of prostaglandin endoperoxide in animal tissues. Prostaglandins 14:1055-1074, 1977.

Tate, R.M., K.M. Van Benthuysen, D.M. Shasby, I.F. McMurtry and J.E. Repine. Oxygen-radical mediated permeability edema and vasoconstriction in isolated perfused rabbit lungs. Am. Rev. Respir. Dis. 126:802-806, 1982.

Tate, R.M. and J.E. Repine. Neutrophils and the adult respiratory distress syndrome. Am. Rev. Resp. Dis. 128:552-559, 1983.

Tauber, A.I. and B.M. Babior. Evidence for hydroxyl radical production by human neutrophils. J. Clin. Invest. 147:316-324, 1978.

Terrell, E.E., O.H. Robertson and L.T. Coggeshall. Experimental pneumococcal lobar pneumonia in the dog. I, II and III. J. Clin. Invest. 12:394-493, 1933.

Thomson, K.J., D.D. Rutstein, D.M. Tolmach and W.H. Walker. Electrocardiographic studies during and after pneumococcus pneumonia. Am. Heart J. 31:565-569, 1946.

Thomson, R.G., S. Chander, M. Savan and M.L. Fox. Investigation of factors of probable significance in the pathogenesis of pneumonic pasteurellosis in cattle. Can. J. Comp. Med. 39:194-207, 1975.

Till, G.O., K.J. Johnson, R. Kunkel and P.A. Ward. Intravascular activation of complement and acute lung injury. Dependency on neutrophils and toxic oxygen metabolites. J. Clin. Invest. 69:1126-1135, 1982.

Till, G.O., W. Tourtellotte, M.J. Lutz, T. Annesley and P.A. Ward. Acute lung injury secondary to skin burns: evidence for role of hydroxyl radical and lipid peroxidation products. Circ. Shock 13:76-80, 1984.

Till, G.O. and P.A. Ward. Systemic complement activation and acute lung injury. Fed. Proc. 45:13-18, 1986.

Tolins, M., E.K. Weir, E. Chesler, D.P. Nelson and A.H.L. From. Pulmonary vascular tone is increased by a voltage-dependent calcium channel potentiator. J. Appl. Physiol. 60:942-948, 1986.

Tucker, A. Lung vascular smooth muscle as a determinant of pulmonary hypertension at high altitude. Am. J. Physiol. 228:762-767, 1975.

Tucker, A., I.F. McMurtry, R.F. Grover and J.T. Reeves. Attenuation of hypoxic pulmonary vasoconstriction by verapamil in intact dogs. Proc. Soc. Exp. Biol. Med. 151:611-614, 1976.

Turner, S.R., J.A. Tainer and W.S. Lynn. Biogenesis of chemotactic molecules by the arachidonate lipoxygenase system of platelets. Nature (London) 257:680-681, 1975.

Tyler, T., C. Leffler, R. Wallis and S. Cassin. Effects of prostaglandins of the E series on pulmonary and systemic circulation of newborn goats during normoxia and hypoxia. prostaglandidn 10:963-970, 1975.

Tyler, T., R. Wallis, C. Leffler and S. Cassin. The effects of indomethacin on the pulmonary vascular response to hypoxia in the premature and mature newborn goat. proc. Soc. Exp. Biol. Med. 150:695-698, 1975.

Vaage, J., I. Bjertnates and A. Hauge. The pulmonary vasconstrictor response to hypoxia: Effects of inhibitors of prostaglandin biosynthesis. Acta Physiol. Scand. 95:95-101, 1975.

Veit, H. The anatomy and physiology of the bovine respiratory system relating to pulmonary disease. Cornell Vet. 68:555-581, 1978,

Voelkel, N.F., J.G. Gerber, I.F. McMurtry, A.S. Nies and J.T. Reeves. Release of vasodilator prostacyclin, PGI_2, from isolated rat lung during vasoconstriction. Circ. Res. 48:207-213, 1981.

Voelkel, N.F., M. Morganroth, K. Stenmark, O.C. Feddersen, R.C. Murphy and J.T. Reeves. Leukotrienes in the lung circulation: Actions and interactions. In Hypoxia, Exercise and altitude. Proc. Third International Hypoxia Symp., Liss, NY, 1983, pp. 141-152.

Voelkel, N.F., K.R. Stenmark, J.T. Reeves, M.M. Mathias and R.C. Murphy. Action of lipoxygennase metabolites in isolated rat lungs. J. Appl. Physiol. 57:860-867, 1984.

von Euler, U.S. and G. Lilestrand. Observations on the pulmonary arterial pressure in the cat. Acta Physiol. Scand. 12:301-320, 1946.

Wagner, P.D., R.B. Laravuso, E. Goldzimer, P.F. Neumann and J.B. West. Distribution of ventilation-perfusion ratios in dogs with normal and abnormal lungs. J. Appl. Physiol. 38:1099-1109, 1975.

Walker, B.R., N.F. Voelkel and J.T. Reeves. Pulmonary pressor response after prostaglandin synthesis inhibition in conscious dogs. J. Appl. Physiol. 52:705-709, 1982.

Ward, P.A., G.O. Till, R. Kunkel and C. Beauchamp. Evidence for role of hydroxyl radical in complement and neutrophil-dependent tissue injury. J. Clin. Invest. 172:789-801, 1983.

Wassermans, M.A., D.W. Ducharmf, R.L. Griffin, G.L. Degraff and F.G. Robinson. Bronchopulmonary and cardiovascular effects of prostaglandin D_2 in the dog. Prostaglandins 13:255-269, 1977.

Weir, E.K., I.F. McMurtry, A. Tucker, J.T. Reeves and R.F. Grover. Prostaglandin synthesis inhibitors do not decrease hypoxic pulmonary vasoconstriction. J. Appl. Physiol. 41:714-718, 1976.

Weir, E.K. Acute hypoxic pulmonary hypertension. In Pulmonary Hypertension. E.K. Weir and J.T. Reeves, Ed., Futura Publishing Co., NY, 1984, pp. 251-289.

Weiss, S.S., P.K. Rustagi and A.F. Lobuglio. Human granulocyte generation of hydroxyl radical. J. Exp. Med. 147:316-324, 1978.

Weiss, J.J., J. Young, A.F. Lobuglio, A. Slivka and N.F. Minch. Role of hydrogen peroxide in neutrophil mediated destruction of cultured endothelial cells. J. Clin. Invest. 68:714-721, 1981.

Weksler, B.B., C.W. Ley and E.A. Jaffe. Stimulation of endothelial cell prostacyclin production by thrombin, trypsin, and the ionophore, A23187. J. Clin. Invest. 62:923-930, 1978.

Wennmalm, A. Influence of indomethacin on the systemic and pulmonary vascular resistance in man. Clin. Sci. Mol. Med. 54:141-145, 1978.

West, J. B., C.T. Collery and A. Naimark. Distribution of blood flow in isolated lung; relation to vascular and alveolar pressures. J. Appl. Physiol. 19:713-724, 1964.

West, J.B., A.M. Schneider and M.M. Mitchell. Recruitment in networks of pulmonary capillaries. J. Appl. Physiol. 39:976-984, 1975.

West, J.B. Respiratory Physiology-The Essentials, 2nd Edition. Williams and Wilkins Co., Baltimore, 1979, pp. 160-165.

Wicks, T.C., J.L. Rose, M. Johnson, P.W. Ramwell and P.A. Kot. Vascular response to arachidonic acid in the perfused canine lung. Circ. Res. 38:167-171, 1976.

Widdicombe, J.G. and G.M. Sterling. The autonomic nervous system and breathing. Arch. Intern. Med. 126:311-329, 1970.

Will, D. Influence of altitude and age on pulmonary arterial pressure in cattle. Proc. Soc. Exp. Biol. Med. 150:564-567, 1975.

Yokochi, K., P.M. Olley, E. Sideris, F. Hamilton, D. Huktanen and F. coceani. Leukotriene D_4: A potent vasoconstrictor of the pulmonary and systemic circulations in the newborn lamb. Adv. Prostaglandin Thromboxane Leukotriene Res. 9:211-214, 1982.