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The effects of furosemide, surfactant, and positive end-expiratory pressure on gas exchange and lung impedance in a PMA dog model of acute lung injury

Waugh, Jonathan Byron, Ph.D.

The Ohio State University, 1994
THE EFFECTS OF FUROSEMIDE, SURFACTANT, AND POSITIVE END-EXPIRATORY PRESSURE ON GAS EXCHANGE AND LUNG IMPEDANCE IN A PMA DOG MODEL OF ACUTE LUNG INJURY

DISSERTATION

Presented in Partial Fulfillment of the Requirements for the Degree Doctor of Philosophy in the Graduate School of the Ohio State University

By

Jonathan Byron Waugh, Ph.D.

****

The Ohio State University

1994

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Advisor
Interdisciplinary Program
For my parents, John & Sharroll
ACKNOWLEDGEMENTS

It is with enthusiasm and great pleasure I state my thanks and deep appreciation for the efforts of my research advisor Dr. Thomas L. Clanton on my behalf. His investment of personal time, energy, and resources in me provided an opportunity for successful scientific endeavor that may otherwise not have been possible given my unique program. I am thankful for the many excellent opportunities he has provided me access to. I am appreciative of the support, guidance, and patience that all of my committee members have extended to me over the past years, especially in regard to navigating this one-of-a-kind program to completion. Special thanks to Dr. Lynne Olson and Dr. David Lamb for the loan of measuring equipment. My thanks go to Dr. James Gadek for his generous support of my work and to the other members of the Division of Pulmonary and Critical Care Medicine for their excellent feedback and allowing me to participate in their various activities.
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CHAPTER I

Introduction

There is no universal agreement on the criteria for the diagnosis of acute respiratory distress syndrome (ARDS). According to Dal Nogare, five criteria are typically described as being essential for a confident diagnosis of ARDS.

1) There is a rapid onset of respiratory distress in a patient with a predisposing condition, typically progressing over 72 hours to overt respiratory failure that requires mechanical ventilation.¹

2) Radiographic findings include diffuse opacification due to accumulation of edema fluid and recruitment of inflammatory cells to the lung parenchyma. This early interstitial pattern of edema ultimately evolves into a homogeneous alveolar pattern that is nearly indistinguishable, radiographically, from cardiogenic pulmonary edema.

3) Severe hypoxemia, defined as an arterial oxygen partial pressure (PaO₂) < 50 mm Hg with a fractional inspired oxygen (FIO₂) ≥ 0.6, indicates the presence of serious intrapulmonary shunting and/or low ventilation/perfusion matching. The proportion of shunt flow (Qs/Qt) is often ≥ 30%.
4) Total respiratory compliance (lungs, chest wall, and diaphragm), as measured by occluding the expiratory portion of a ventilator circuit at the end of a tidal volume inspiration and reading the airway pressure (compliance = exhaled tidal volume/peak airway pressure), is typically less than 30 ml/cm H2O.

5) The final diagnostic criterion is a pulmonary capillary wedge pressure (PCWP) ≤ 12 mm Hg, which is perhaps the most helpful discriminator for differentiating between ARDS and fulminant cardiogenic edema. Lloyd et al., objected to this as an absolute criterion because it would exclude the diagnosis of ARDS when it coexisted with a condition that caused an increased left atrial pressure.2

Table 1. Abbreviated Terms.

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<tr>
<td>AaDO2</td>
<td>Alveolar-arterial Oxygen Difference</td>
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<td>ARDS</td>
<td>Acute Respiratory Distress Syndrome</td>
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<td>CI</td>
<td>Cardiac Index</td>
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<td>CO</td>
<td>Cardiac Output</td>
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<td>CVP</td>
<td>Central Venous Pressure</td>
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<tr>
<td>DDLW</td>
<td>Double-dilution Lung Water</td>
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<tr>
<td>DO2</td>
<td>Oxygen Delivery</td>
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<tr>
<td>EVLW</td>
<td>Extravascular Lung Water</td>
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<tr>
<td>FIO2</td>
<td>Fractional Inspired Oxygen</td>
</tr>
<tr>
<td>O2EXT</td>
<td>Oxygen Extraction Ratio</td>
</tr>
<tr>
<td>PaO2</td>
<td>Arterial Oxygen Partial Pressure</td>
</tr>
<tr>
<td>PaCO2</td>
<td>Arterial Carbon Dioxide Partial Pressure</td>
</tr>
<tr>
<td>PAP</td>
<td>Pulmonary Artery Pressure</td>
</tr>
<tr>
<td>PCWP</td>
<td>Pulmonary Capillary Wedge Pressure</td>
</tr>
<tr>
<td>PMN</td>
<td>Polymorphonuclear Leukocytes</td>
</tr>
<tr>
<td>PVR</td>
<td>Pulmonary Vascular Resistance</td>
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<tr>
<td>Qs/Qt</td>
<td>Shunt Fraction</td>
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The American-European consensus conference on ARDS (1993) agreed upon the following basic diagnostic criteria for ARDS: bilateral infiltrates on chest x-ray, a PaO\textsubscript{2}/FI\textsubscript{O}\textsubscript{2} < 200, and a PCWP value < 18 mm Hg.\textsuperscript{3}

Many conditions are associated with and predispose one to ARDS, these include sepsis, aspiration, pneumonia, inhaled toxins, trauma, shock, drug overdose, head injury, transfusion reactions, burns, and near drowning.\textsuperscript{4,5} Polymorphonuclear leukocytes (PMNs) are involved in the pathogenesis of most ARDS cases, and according to a multicenter study by Fowler et al., the incidence of ARDS associated with sepsis was 38 percent of the total ARDS cases reviewed.\textsuperscript{6} Mortality in ARDS that follows septicemia tends to be around 80%, much higher than the average mortality from ARDS.\textsuperscript{7,8}

The acute (exudative) phase of the diffuse alveolar damage that initiates ARDS has a stereotypic morphologic finding.\textsuperscript{9-10} A marked loss of endothelial and Type I epithelial cells occurs from day 1 to day 7 in this acute, diffuse, alveolar disease process.\textsuperscript{1,4,10} In vitro studies have shown that when endothelial cells are killed, they rupture and release significant amounts of prostacyclin, a prostaglandin that inhibits platelet aggregation and increases capillary permeability.\textsuperscript{11} A hemorrhagic edema (also accounting for up to an eight-fold increase in polymorphonuclear leukocytes (PMN) in both the interstitial and alveolar spaces leads to the formation of thick hyaline membranes composed of fibrin and cellular debris lining the alveoli.\textsuperscript{5,12-14} Complement C5a is thought to be the primary chemotactic agent responsible for directing formed destructive substances onto the pulmonary
endothelium. A stimulus such as bacterial endotoxin activates complement C5a that in turn causes neutrophils to firmly adhere to endothelium and more importantly, triggers the neutrophils to inappropriately release their lysosomal contents.

At least four groups of substances released by the neutrophils are thought to cause damage to the endothelium. Oxygen radicals can damage by lipid peroxidation and by inactivating alpha-1-antitrypsin, a glycoprotein that is the major protease inhibitor of human serum. The second group consists of proteolytic enzymes, such as the especially damaging elastase, that alter elastin fibers which attract monocytes and macrophages and cause direct damage to the endothelial cells. Attracted mononuclear cells release cytokines such as tumor necrosis factor, a peptide that produces severe physiologic changes, including profound hypotension and metabolic acidosis. A third group of substances released by neutrophils is comprised of arachidonic acid metabolites, histamine, and other leukotrienes and prostaglandins, that cause pulmonary venoconstriction in damaged lung areas. This increases capillary leakage, increases vascular permeability, and attracts neutrophils. Finally, the fourth group includes platelet-activating factors associated with the injury process that cause intravascular coagulation. Blood proteins such as albumin and fibrin that leak into the alveolar spaces have been shown to interfere with the action of lung surfactant and may also contribute to the tissue damage. The consequences of this profound lung edema include decreased lung volumes, decreased compliance, and most importantly, large intrapulmonary shunts.
During the proliferative stage (beginning around day 7), fibroblasts that have accumulated in the interstitial and alveolar spaces respond to chemotactic factors released during the acute phase of ARDS, resulting in fibrosis. Destroyed alveolar Type I cells are replaced with Type II cells, which are not functional for gas exchange. The secondary scarring alterations that occur in the proliferative stage add to the initial injury and appear to be contingent upon the degree of damage in the acute stage of ARDS. Theoretically, if aggressive intervention during the acute stage of ARDS could occur, it reasonably follows that not only would acute damage be minimized, but the damage occurring during the proliferative and fibrotic phases would also be lessened by diminishing the various chemotactic triggers available.

It is well-established that ARDS is a syndrome with a high mortality rate and is characterized by a fairly rapid evolution from interstitial and alveolar edema to end-stage fibrosis, and ultimately, permanent injury of the alveolar-capillary unit. This progression of diffuse alveolar damage occurs in the order of exudative, proliferative, and fibrotic phases. Although ARDS frequently produces fibrosis, this fibrosis is unusual in that it typically disappears within a short time. The dominant pathological influence is edema and cellular debris that are spread non-homogeneously throughout the lungs. From the beginning to the end-stage of the syndrome, the involvement of the pulmonary vasculature is an important element of ARDS.
Adult Respiratory Distress Syndrome (ARDS) is often cited as having an annual incidence of up to 150,000 cases in the United States based on a National Heart and Lung Institute task force report published in 1972.\textsuperscript{21} This number has been challenged in recent years as being an overestimate but continues to be quoted, largely because no better predictions have been agreed upon.\textsuperscript{22} Considerable research has yielded a better understanding of the mechanisms of ARDS, but therapeutic progress has been much less fruitful and mortality remains high. Sources report mortality rates from 40-80\%, depending upon the diagnosis criteria used.\textsuperscript{1,6,8} A review of the literature reveals a need for data to support the various therapies presently in use and to elucidate the mechanisms by which they work.

The diffuse collection of extra fluid in the lungs and body tissues (edema) has a profoundly negative impact on oxygen delivery and extraction in the body. Clinicians differ in their preference for a body fluid management strategy when treating ARDS patients. One camp supports the belief that body fluid balance should be maintained at normal or supranormal levels in order to support cardiac output and, hopefully, oxygen delivery to the tissues. The other group prefers to maintain a negative body fluid balance in an effort to keep the lungs and tissues non-edematous, thereby enhancing gas exchange.

Although initial edema patterns may be similar for ARDS and cardiogenic edema, the treatment approaches are profoundly different because of the different etiologies. Cardiogenic edema is due to a high pulmonary capillary pressure that results from blood backing up in the lungs because of poor left heart function. Lung
edema in ARDS results from damage in the form of many microscopic holes in the pulmonary capillary walls that allow for leakage of vascular fluid into the lung spaces. Cardiogenic edema happens when the blood pressure in the capillaries exceeds the epithelial membrane's capacity to contain it. Fluid is literally squeezed out of the capillaries into the interstitium and eventually through the alveolar epithelium into the lung spaces. This can generally be corrected by giving drugs or providing interventions that improve heart function and by administering diuretic drugs to remove edema. These therapies work because the membrane barrier between the blood and alveoli is intact. A principal reason ARDS is so difficult to treat is because the integrity of the alveolar-capillary membrane has been compromised; it allows fluid to leak into the lungs even at low capillary pressures.

Models of ARDS Lung Injury

Many models of lung injury are described in the literature, and several exhibit most of the pathophysiologic changes that occur in ARDS. Although the exact mechanisms by which ARDS develops are unknown, the previous section cited evidence suggesting that neutrophils typically participate in its pathogenesis. Weiland et al. reported a correlation between the magnitude of neutrophil influx into the lungs and the severity of pulmonary edema. Furthermore, neutrophil-derived enzymes capable of injuring lung tissue have been detected in bronchoalveolar lavage fluid of ARDS patients. Hudson's recent review of the literature concluded,
"Although some clinical and animal model data suggest that lung injury can occur without major involvement of neutrophils, a general consensus among investigators supports the neutrophil hypothesis—that is, that neutrophils play a central role in inflammatory lung and other organ injury in most clinical circumstances."

A suitable model of ARDS should exhibit the characteristic accumulation of fluid that has escaped from the vasculature into the lung parenchyma due to damaged pulmonary endothelium. The endothelial damage that occurs in the lungs of ARDS patients also happens in the rest of the body. Indeed, many researchers believe that the lung damage seen in ARDS is really an early sign of a larger disease process termed multiple organ system failure or dysfunction (MOSD). The tissue damage in an ARDS model should result mostly from an excessive response of the body's own immune system. A titratable stimulus should start with the triggering of the neutrophils, the immune system's primary early response cells, causing them to migrate to lungs and then to marginate. Because the blood volume has the opportunity to move relatively slowly through the pulmonary capillaries, many of the neutrophils will adhere easily to the lung endothelium. Next, the model of the lung injury should cause these neutrophils accumulated from the vasculature, along with resident alveolar leukocytes, to release enzymes such as lysoenzymes and proteases, and to release the highly destructive molecules, hydrogen peroxide, oxygen radicals, hydroxyl ions, and ultimately hypochlorous acid. The spillage of these enzymes and oxygen metabolites into the surrounding area will cause an inflammatory reaction and proteolytic injury of the tissues.
Both neutrophil adherence and the consequent production of radical oxygen species appear to be necessary for lung edema to occur. Studies that blocked the "respiratory burst" of neutrophils or used oxygen radical scavengers showed that lungs were protected against the development of edema and increase in epithelial permeability to blood proteins.\textsuperscript{30-32} However, procedures that inhibit neutrophil adherence without inhibiting oxygen radical production or degranulation can also provide protection from lung injury.\textsuperscript{32-33}

More than anything else, it appears that the initial burst of activity from the neutrophils produces the damage to capillary endothelium that allows for escape of vascular fluid.\textsuperscript{34} Damage continues as an inflammatory response is mounted, causing even more escape of plasma fluid and particles into the lung interstitial space. Eventually, the egress of vascular fluid overwhelms the pulmonary lymphatic system's capacity to remove the fluid and the interstitial edema moves into the alveolar spaces. The arachidonic acid cascade also plays a role in the diffuse inflammatory reaction in the lung's microvasculature, but is not yet known to what degree.\textsuperscript{19} Prostaglandins and thromboxane A\textsubscript{2} produced by this cascade increase pulmonary artery pressure and pulmonary vascular resistance.\textsuperscript{35-36} Such increases can cause profound changes in the ventilation/perfusion relationship, cardiac output, and, consequently, arterial oxygenation.

ARDS patients often have coagulation and platelet abnormalities, but they are unreliable predictors of ARDS. It has been proposed that these abnormalities may cause an increased flow through precapillary channels, effectively shunting blood
away from capillary beds.\textsuperscript{37} Despite promising clinical findings, no increase in precapillary blood flow or flow to organs with low oxygen extraction fractions have been observed in animal models of ARDS.\textsuperscript{38-41}

When microvascular damage in the lung parenchyma becomes so severe that red blood cells are able to freely escape from the capillaries and move into the alveoli, the outcome is almost certain death. Therefore, the goal for a model of ARDS is to produce an injury via an immune response that exhibits the signs and symptoms of ARDS but not severe enough to produce death. It is difficult to cause such an injury by provoking the immune system; therefore, some investigators have selected models utilizing a more direct mechanism of injury (e.g., air emboli, acid installation, saline lavage, etc.) that tends to be easier to titrate. However, when research questions involve evaluation of a treatment or require analysis of systemic injury as well as lung injury, it is preferable to use a model that causes broad injury via the immune system.

Some of the animal models of acute ARDS lung injury use the following induction agents: oleic\textsuperscript{42} or hydrochloric acids,\textsuperscript{43} bacterial endotoxin\textsuperscript{44} or live bacterium,\textsuperscript{45} ethchlorvynol,\textsuperscript{46} nitrosourethane,\textsuperscript{47} and phorbol myristate acetate (PMA or TPA).\textsuperscript{48} For the purpose of comparison it may be helpful to classify insults to the alveolar-capillary membrane as being either direct and indirect insults. Indirect insults would act by triggering the immune response described previously, whereas direct insults would injure without the aid of secondary agents. Some agents cause both direct and indirect insults, others predominantly one type. Of the many agents
predisposing to ARDS, the most prominent, sepsis, works primarily through an indirect immune-mediated to the alveolar-capillary membrane.

The persistence of injury in a model is as important as the rate of injury development. Although many agents can be given to produce the endothelial damage, the method that most closely mimics the process that occurs in the body is most desirable. Consequently, lung injury models that generated an ARDS-like injury via a neutrophil-mediated response were given preference during the model selection process for the present study.

Endotoxin and oleic acid, two of the most common agents used to induce lung injury, have been compared by different assays. Winn et al. calculated the change in the Starling fluid filtration coefficient (Kf) of perfused lungs before and after endotoxin and oleic acid infusion and concluded that endotoxin does not cause injury to isolated lung endothelium for at least five hours. Additionally many researchers find endotoxin or live bacterium dosing a troublesome procedure with a long time course for development of serious injury. Schuster et al., demonstrated that oleic acid injury tends to have its effect by "first pass" contact with lung endothelium, indicating less of a neutrophil-mediated injury and more of a direct vascular injury. Shiue and Thrall were able to reverse oleic acid lung injury, as measured by reduced pulmonary inflammation and increased lung compliance, by giving corticosteroids. Although pre-injury corticosteroid treatment does provide protection in some models of lung injury, such a post-injury treatment response is not typical for clinical ARDS nor most injury models. These findings raise doubts
about endotoxin or oleic acid being the best choices for injury models of ARDS, especially when treatment response is being tested.

Ethchlorvynol and N-nitroso-N-methylurethane (nitrosourethane) are two agents that have been described in the literature as being capable of producing acute lung edema by increasing pulmonary capillary permeability.\textsuperscript{53-55} Ryan and colleagues reported that nitrosourethane provided a stable acute alveolar injury practically indistinguishable by light and electron microscopy from that seen in humans with ARDS.\textsuperscript{47} They also conceded that because the mechanism of nitrosourethane-induced lung injury is unknown, the model has no known etiologic relevance to the human injury. Ethchlorvynol, a nonbarbiturate sedative hypnotic, is known to produce edema in humans as well as animals by increasing capillary permeability, but the injury is reversible, uncharacteristic of ARDS.\textsuperscript{56-57} Also uncharacteristic of most ARDS injury is the ability of ethchlorvynol to produce lung injury in the absence of neutrophils.\textsuperscript{58}

Some other lung injury agents, namely, glass microspheres, air emboli, and hydrochloric acid, tend to produce stable, titratable alveolar injury, but primarily through direct contact with tissue rather than by triggering an immune system PMN-mediated injury.\textsuperscript{59-61} Sepsis constitutes the largest predisposing agent for ARDS but many of the risk factors for ARDS (e.g., trauma, massive transfusion, disseminated intravascular coagulation) have been directly or indirectly linked to endothelial damage and pulmonary edema consequent to PMN cell involvement.\textsuperscript{62} Again, an injury development that mimics the natural etiology of ARDS is most desirable when
the evaluation of a treatment is planned.

PMA is an ester derivative of croton oil that has been shown to be an effective PMN activator capable of producing a pulmonary edema by increasing the permeability of pulmonary capillaries. The neutrophil is thought to be the primary inflammatory cell responsible for the lung injury in the PMA model of ARDS.\textsuperscript{40,63-65} Although in some studies, PMA appears to cause lung injury independent of neutrophils.\textsuperscript{66-68} The often cited study by Shasby et al. found that neutrophil presence was essential to produce a degree of injury similar to that in ARDS.\textsuperscript{69} The strongest evidence for neutrophil dependence is likely the study by Perry and Taylor\textsuperscript{70} that demonstrated the requirement for neutrophils to produce PMA-induced endothelial permeability. This same study found that PMA-induced changes in vascular resistance did not require neutrophils. It appears that PMA can produce some injury without neutrophils, but the extent of such injury is minor and certainly not comparable to that in ARDS. Given the evidence to date, it is reasonable to claim that the PMA model is neutrophil-dependent when used to produce an ARDS-like injury.

PMA is a known to activate the calcium-dependent enzyme, protein kinase C, which activates or deactivates target enzymes via phosphorylation.\textsuperscript{71} In vitro, PMA stimulates neutrophils to adhere, aggregate, degranulate, and release toxic oxygen metabolites.\textsuperscript{72-73} Lungs from an in vivo study of PMA-treated dogs exhibited a moderate, diffuse pneumonitis that included the presence of fibrin, red blood cells, and granulocytes in the alveolar lumina.\textsuperscript{62} Johnson and Ward observed acute and
progressive lung injury after contact with PMA. Studies of PMA injury in rabbits also mirrored the human ARDS progression of injury from acute pneumonitis to the final stage of fibrosis.

In addition to appropriate lung injury, PMA appears to approximate the systemic abnormalities of multiple organ system dysfunction typically associated with ARDS. Data from Mizer and associates suggested that lung injury and nonpulmonary organ injury occurred concomitantly in their PMA dog model of ARDS. The nonpulmonary organs from the majority of those PMA-treated dogs were found to have a three-to-four-fold increase in neutrophils compared to the controls. Consensus is found with other studies that the most striking histopathologic alterations occurred in the lungs and livers of animals with PMA injury. This type of injury is consistently characteristic of other established animal models of neutrophil-mediated injury. For these reasons, a PMA-induced lung injury model was selected for the present study.

**Problems of Fluid Balance in ARDS**

Nearly a century ago E. H. Starling asserted that the rates of fluid formation and removal are the same in normal lungs. Fluid balance in the lung is basically achieved by maintaining just enough liquid volume in the space between cells, the interstitium, to allow for proper hydration of tissues and diffusion of dissolved substances. Too much fluid in the interstitium impairs diffusion and compresses delicate alveolar structures. It is normal for a small amount of fluid from blood in
the pulmonary circulation to escape into the interstitium, but most of the fluid that is moved out at the arterial end of the capillary is drawn back in at the venous end.

Fluid movement across the pulmonary capillaries is similar to fluid movement in the other capillary beds throughout the body except that the pulmonary capillary pressure is much lower than in systemic capillaries. The well-regulated accumulation and removal of this fluid is a result of competing physical forces and the lymphatic drainage system. The relationship of physical forces acting on fluid exchange is represented by the Starling equation: $Q = Kf[(Pc - Pi) - \sigma (\pi c - \pi i)]$, where $Q =$ volume flow across the capillary wall (a positive value for $Q$ represents movement of fluid from the capillary to the interstitium; conversely, a negative $Q$ reflects movement of fluid into the capillary); $Kf =$ filtration coefficient that represents volume flow per unit time per unit pressure per 100 g tissue; $Pc =$ hydrostatic pressure of the capillary; $Pi =$ hydrostatic pressure of the interstitial space; $\sigma =$ the osmotic reflection coefficient, a value that expresses the degree of permeability of the membrane to plasma proteins relative to water; $\pi c =$ oncotic pressure of the capillary; and $\pi i =$ oncotic pressure of the interstitial spaces. Using Guyton's exemplary values for oncotic and hydrostatic pressures, a summation of forces yields a very small net force tending to move fluid out of the capillary (Table 1). This small amount of filtrate is returned to the circulation through the lymphatic vessels.
Table 2. The Starling equilibrium of capillary fluid exchange.

<table>
<thead>
<tr>
<th>Forces tending to move fluid outward:</th>
<th>mm Hg</th>
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<tr>
<td>Mean capillary pressure</td>
<td>17.0</td>
</tr>
<tr>
<td>Negative interstitial fluid pressure</td>
<td>7.0</td>
</tr>
<tr>
<td>Interstitial fluid colloid osmotic pressure</td>
<td>4.5</td>
</tr>
<tr>
<td><strong>Total Outward Forces</strong></td>
<td><strong>28.5</strong></td>
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<table>
<thead>
<tr>
<th>Forces tending to move fluid inward:</th>
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<tr>
<td>Plasma colloid osmotic pressure</td>
</tr>
<tr>
<td><strong>Total Inward Forces</strong></td>
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<table>
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<tr>
<th>Summation of forces:</th>
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<tr>
<td>Outward</td>
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<tr>
<td>Inward</td>
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<td><strong>Net Outward Force</strong></td>
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Fluid is normally drawn from the pulmonary capillaries into the interstitium by the subatmospheric pulmonary interstitial pressure resulting from lymphatic drainage and the recoil pressure of the lung. This happens because larger lymphatic vessels can constrict and, with the aid of one-way valves and the motion of the respiratory cycle, can produce intra-lymphatic pressures of 25 to 30 cm H2O. The fluid is filtered through a fibrous basement membrane and then diffuses through a gel-like interstitial "ground" substance composed of extracellular fluid, mucopolysaccharides, and collagen before arriving at larger, more compliant perivascular spaces. The alveolar-capillary barrier doesn't swell much when fluid enters the pulmonary interstitium because the compliant perivascular spaces quickly accept large volumes of fluid with only small changes in pressure. Because of this shifting of edema within the interstitium away from gas exchange sites, only minimal impairment of oxygen exchange occurs during normal fluid exchange. Only after very large amounts of fluid have entered the interstitium (a 50% expansion of the interstitial space) does fluid begin to finally enter the alveoli. The route by which fluid enters the alveoli is not known, but openings at the junction of the alveolar ducts or at the alveolar epithelial membrane and/or holes in the airways can serve as pathways for fluid movement.

The alveolar epithelial lining has an intricate fluid transport balance in addition to the pulmonary endothelial/lymphatic fluid exchange. Several mechanisms produce a physiologic salt solution in the large airways, and Clara cells in the epithelium are hypothesized to be the primary sources of fluid secretion. Just as
there is a net fluid movement out of the pulmonary capillaries, a net removal of fluid from the alveolar lumen occurs by active transport of sodium.\textsuperscript{83-85} It is not known whether this active transport system continually counters a tendency for the alveoli to fill with fluid or if it merely responds "on-demand" when fluid accumulates.\textsuperscript{82} The sodium transport can be accelerated by certain beta-adrenergic agonists, which may offer an additional course of treatment for pulmonary edema.\textsuperscript{85-86}

Normal lungs with an intact alveolar-capillary barrier will not exhibit edema accumulation until left atrial pressure exceeds about 23 mm Hg.\textsuperscript{86} Lung edema begins accumulating at a much lower left atrial pressures (or pulmonary capillary pressures) when the pulmonary endothelium is damaged, as in ARDS. This edema collection at lower pressures occurs for at least two reasons: 1) the lymph system is less effective at fluid removal, and 2) tissue osmotic pressure is not as low as it would normally be because plasma proteins leak into the interstitium through the damaged capillary endothelium. This low-pressure edema found in ARDS is sometimes referred to as "leaky lung syndrome" and occurs even at normal capillary pressures.

The lung is designed such that it can tolerate a considerable amount of edema collection before gas exchange is seriously compromised. The most important goal related to perserving gas exchange in ARDS-stricken lungs is to prevent fluid from moving into and collecting in the alveolar spaces. This goal forms the foundation for rationales underlying therapies such as negative body fluid balance, pharmacologic stimulation of epithelial sodium-ion transport, and, to some degree, the application of
positive end-expiratory pressure.

**Actions of Furosemide**

Furosemide (aka. frusemide, fursemide, Lasix) is categorized as a high-ceiling or loop diuretic, identifying it as both potent and working its effect on the loop of Henle in the kidney nephrons. Investigations have shown that furosemide not only blocks electrolyte and fluid reabsorption in the loop of Henle but in the proximal and distal tubules as well. The action on the distal tubule is independent of any inhibitory effect on carbonic anhydrase and aldosterone. The major site of action in the loop of Henle is the less permeable, thick ascending limb. Inhibition of the sodium-potassium-chloride active transporter in this segment causes fluid to remain in the tubule and eventually be excreted. Furosemide possesses diuretic activity in either the presence of acidosis or alkalosis.

Loop diuretics are more potent than other diuretic agents for at least two reasons. First, the loop of Henle is responsible for the largest proportion of sodium reabsorption. Drugs that work by inhibiting only proximal tubule sodium and fluid reabsorption are countered by the ability of the ascending limb to increase the rate of sodium reabsorption in the presence of an increased tubular sodium load. Furthermore, the distal tubule and collecting ducts in the kidney nephrons have relatively limited capacity for sodium reabsorption and so are unable to recapture much of the large quantity of tubular sodium and fluid presented when ascending limb reabsorption is blocked.
Second, furosemide is known to possess vasoactive qualities that precede the diuretic effect. In a study reported in the New England Journal of Medicine, Dikshit and associates found venous capacitance rose in humans and both peripheral vascular resistance and left ventricular filling pressure fell within five minutes of furosemide injection. Unfortunately their study design did not determine whether the change in left ventricular filling pressure was due to a direct vasodilation effect on the pulmonary vasculature or peripheral vasculature or both. McGowan et al., observed that furosemide produced a concentration-dependent relaxation of venous smooth muscle without any significant effect on arterial smooth muscle. A sheep study measuring pulmonary microvascular fluid filtration rate in normal lungs recorded a 30% decrease in lung lymph flow after 80 mg of furosemide. The majority of the decrease occurred before the onset of diuresis, demonstrating that furosemide significantly decreased the fluid filtration rate by a non-diuretic effect.

High-pressure pulmonary edema (non-ARDS) has long been treated effectively by furosemide. As a treatment for ARDS, furosemide is thought to act by decreasing central blood volume and therefore reducing the capillary hydrostatic pressure, in turn, reducing the amount of edema. However the use of furosemide for the prevention and treatment of ARDS is controversial. Some attribute pulmonary improvements seen with furosemide to "the mobilization of interstitial fluid caused by the pronounced diuresis," but often a significant diuresis is not seen after furosemide therapy because of MSOD, hypoalbuminemia, and generalized tissue edema. Some clinicians believe that furosemide therapy will likely produce a
hypovolemia that compromises adequate blood flow and oxygen delivery to the body, thereby resulting in further injury.

It is important to determine if the non-diuretic effects of furosemide occur in the presence of lung injury. An important initial consideration is that although furosemide tends to have vasodilating properties, it acts differently on pulmonary vasculature than do other vasodilators. Pulmonary vasodilators such as nitroprusside typically worsen intrapulmonary shunt, presumably by negating the alveolar hypoxic vasoconstriction response. Furosemide typically improves intrapulmonary shunting before a decrease in lung edema, suggesting a different mode of action. Ali and associates reported data from an oleic acid (OA) lung injury model in dogs that suggested an effect of furosemide on redistributing blood flow away from flooded alveolar units within the edematous lung. Even in nephrectomized animals, Ali et al. observed that furosemide prevented the increase in intrapulmonary shunt seen between two and four hours after OA administration in dogs with injury.

Furosemide's effect on lung edema caused by OA was measured in one study using both indicator-dilution technique and gravimetric (wet/dry weights) analysis of excised dog lungs. The investigators concluded that neither method clearly demonstrated a reduction in canine OA-induced edema and that furosemide may even worsen that type of low pressure edema. A comparison of pulmonary vascular pressures and protein concentrations indicated an increased capillary hydrostatic pressure after giving furosemide during OA injury. The capillary oncotic pressure was not different after furosemide. Therefore, edema should have increased, which
seemed to be borne out by trends (though not significant) in larger wet-dry lung weight ratios.

An increase in pulmonary capillary hydrostatic pressure after furosemide is a troublesome observation because it is not consistent with the data from indicator-dilution measurements of lung water. One possible explanation of the reduced shunt and reduced edema with furosemide in low-pressure edema is the possibility that furosemide may cause vasoconstriction in edematous regions of the lung. In Chernicki's study, pulmonary artery pressure tended to increase after furosemide, a finding consistent with a smaller vascular bed available for perfusion due to selective vasoconstriction in edematous regions. Although some drugs are known to produce opposite effects, e.g., constriction or dilation, depending on what vascular area they act on, affirming this hypothesis for furosemide would be difficult to justify with current data. A more plausible answer is that blood flow is indeed directed away from leaky portions of the lung capillary bed but due to furosemide's general vasodilatory effect, only non-edematous lung vasculature can respond. That is, the presence of edema fluid may inhibit the normal vasodilatory response to furosemide.

Additional mechanisms may contribute to the over-all effect of furosemide on reducing shunt and edema. The mechanical pressure of edema may compress surrounding capillaries, rendering them less responsive to a vasodilator. A possible contributing factor may be that furosemide also affects Kapanci cells. These contractile cells in the lung interstitium may respond to furosemide by relaxing, conceivably resulting in additional vasodilation. Kapanci cell relaxation in response
to furosemide stimulation may be minimized by the presence of edema resulting in preferential vasodilation and perfusion in non-edematous areas. In addition, furosemide may have an effect on the sodium-potassium ATPase pumps in the alveolar epithelium. Basset and others have demonstrated that alveolar epithelium performs rapid fluid transport from the alveoli to the interstitial space by active transport of sodium.\textsuperscript{83-85,105} Since it has been shown that the activity of these sodium pumps can be increased by some beta-adrenergic agonists, it is possible that furosemide may also exert such an effect.\textsuperscript{86,85}

**General Principles of Gas Exchange in the Lung and Tissues**

The primary function of the lung is to deliver sufficient oxygen to the blood at an ample rate to supply the metabolic needs of the tissues and remove carbon dioxide at the rate it is produced by the tissues. This is accomplished in the lung by two physical forces: diffusion and convection. Convection is the dominant form of gas transport in the large airways of the lung, and diffusion becomes more important in the smaller airways (respiratory bronchioles and beyond). Fick's first law of diffusion states that the flow of a material (in this case a gas) per unit area in a given time occurs from regions of higher to lower concentration at a rate that is proportional to the difference in concentration between the two regions.\textsuperscript{106} This is an over-simplification because the driving force for diffusion is more accurately termed the chemical potential difference of the substance in the two regions (this applies to regions of solid, liquid, or gaseous phases).\textsuperscript{107}
The chemical potential of a gas in a solution depends on both its concentration and its solubility. Chemical potentials of a given concentration of a gas may vary with solubility, but the chemical potential at a given partial pressure is the same for any ideal gas. Concentration differences are used to describe diffusion within a single phase, and the diffusivity coefficient is usually measured using them but equilibrium between phases occurs when partial pressures (thus, chemical potentials), not simply concentrations, are equal. Therefore, the rate of gas transfer across the alveolar-capillary membrane is given by an approximation of Fick's law for diffusion: \( R = D \cdot \lambda \cdot A \cdot \Delta P / H \) (where \( R \) is the rate of gas transfer across the membrane, \( D \) is the diffusion coefficient or diffusivity of the membrane, \( \lambda \) is the solubility of the gas in the membrane, \( A \) is the surface area available for diffusion, \( \Delta P \) is the pressure difference across the membrane, and \( H \) is the thickness of the membrane).106

Blood flow is proportional to the difference in mean pressure between the arterial and venous blood vessels supplying and draining a given organ and is inversely related to the resistance of the vessels within the organ. These relationships are described by the equation: Organ blood flow = \((BP_{\text{arterial}} - BP_{\text{venous}}) / \text{Resistance}\).106 Resistance is inversely proportional to the square of the cross-sectional area of the vessels, i.e., doubling the vessel area decreases resistance by a factor of four (a very close approximation that discounts non-laminar flow and changes in blood viscosity and velocity). This equation is helpful for understanding the role of cardiac output in determining organ blood flow. Total cardiac output is
not a part of the equation because it does not directly affect organ blood flow. For example, if a patient's cardiac output were to decrease due to blood loss it would impact organ blood flow only to the degree that it decreased mean arterial pressure and affected vascular autoregulatory (stretch, neural, and humoral) mechanisms. Cardiac output increases with exercise, but blood flow to some organs (kidneys and splanchnic viscera) actually decreases below basal rate. Consequently, it is not reliable to base inferences about changes in blood flow largely on changes in cardiac output.

Gas transport in the normal lung is so rapid that under most conditions the surrounding capillary blood has the same partial pressures as the alveolus before it leaves the alveolar capillary. That means prolonged contact time would not increase net transfer of gas. Indeed, a normal individual can lose nearly one-eighth of alveolar surface area or decrease blood transit time fivefold without the rate of diffusion affecting gas exchange.\(^{106}\) This gas exchange "reserve" is advantageous under demanding conditions such as exercise (decreased alveolar-blood contact time) or lung disease (diminished alveolar surface area). However, under normal conditions, arterial partial pressures are less than alveolar partial pressures. Many clinicians attribute this to a "shunt," the passage of some venous blood into the arterial circulation without exposure to ventilated alveoli, but this does not appear to account for the discrepancy. Some theorize that alveolar-arterial oxygen partial pressure difference (\(P(A-a)O_2\)) and the differences for all other lung gases are most likely due to inhomogeneity in the ratio of ventilation to perfusion (\(V/Q\)) among
some alveoli.

**Normal mechanisms of \( \dot{V}/\dot{Q} \) matching and the Effect of Blood pH**

It is important to understand the \( \dot{V}/\dot{Q} \) relationship because it has profound implications for the ARDS scenario. The lung is often modeled as having multiple compartments or zones, each having its own \( \dot{V}/\dot{Q} \) value, dependent primarily upon the effect of gravity on alveolar inflation and perfusion. Alveoli at the relative apices of the lungs are more inflated at end expiration but experience less volume change than do those at the bottom. Gravity causes perfusion to be greatest for the alveoli in the lowest compartment, so the most ventilated alveoli would receive the greatest perfusion and vice-versa. Keeping in mind the previously described primary constraints on gas exchange, i.e., gas solubility, partial pressures, and other determinants of diffusion rate, the less than ideal combined \( \dot{V}/\dot{Q} \) of the theoretical lung model can also contribute to the alveolar-arterial pressure difference because the different \( \dot{V}/\dot{Q} \) ratios of the compartments are combined. All alveoli in the model's three compartments (top, middle, and bottom of the lung) have the same initial concentration of a given gas. Since it is the partial pressures that equilibrate between alveoli and capillary, an equal amount of blood going to an alveolus with half the ventilation of another will result in a lower oxygen partial pressure in the blood exiting the smaller alveolus relative to the larger one. Likewise, for two alveoli of equal ventilation, a doubling of blood flow to one would result in a lower gas partial pressure at equilibrium.
True shunt (perfusion without ventilation) is often muddled together with \( \dot{V}/\dot{Q} \) mismatch (abnormal ventilation-to-perfusion ratio) when discussing arterial oxygenation problems. The differences between the two are that the blood leaving an alveolus with even a very low \( \dot{V}/\dot{Q} \) will have a higher \( PO_2 \) than venous blood and the \( PO_2 \) of this blood can be increased by raising the inspired oxygen concentration. True shunt is refractory to oxygen therapy and difficult to measure. Therefore, a more feasible, less discriminating index like the shunt fraction which counts both true shunt and \( \dot{V}/\dot{Q} \) mismatching, is used to evaluate gas exchange efficiency in the whole lung, which.

The lung responds to changing conditions, e.g., ARDS, that alter \( \dot{V}/\dot{Q} \) in the lung units primarily by hypoxic vasoconstriction. The effect is predominately induced by reductions in the alveolar \( PO_2 \) but also in the pulmonary capillary \( PO_2 \). Pulmonary arterioles constrict in a non-linear response to such hypoxia, diverting blood flow away from alveoli with low \( O_2 \) tensions to regions with higher \( O_2 \) tensions. High alveolar \( PCO_2 \) levels tend to enhance the hypoxic vasoconstrictor response. Low blood \( pH \) increases pulmonary vascular resistance and accordingly decreases blood flow. Not only does \( pH \) alter blood flow on its own, it has been shown to strongly potentiate the hypoxic vasoconstrictor response. Taylor states the relationship as, "The hypoxic vasoconstrictor response is three times more potent when there is a simultaneous decrease in \( pH \) of 0.1 to 0.2 unit." Therefore, \( pH \) can be an important variable in understanding the lung's response to \( \dot{V}/\dot{Q} \) mismatching.
Assessing the adequacy of tissue oxygenation in ARDS patients has been problematic because only crude clinical methods exist, and measurements at the tissue level are generally not available or feasible in a patient care setting. Therefore, more global measures such as oxygen delivery (DO₂) and the oxygen partial pressure of mixed venous blood (PvO₂) or venous saturation (SvO₂), or venous blood lactate have been utilized. Defined as the volume of oxygen leaving the left ventricle per minute, DO₂ can be calculated from the cardiac output and arterial oxygen content. Unfortunately, both theoretical considerations and clinical studies cast doubt upon the utility of changes in PvO₂ or SvO₂ serving as measures of tissue oxygenation. Because of the considerable heterogeneity of the ratio of individual organ oxygen consumption to blood flow, the global values of PvO₂ and SvO₂ can be poor indicators of the overall adequacy of tissue oxygenation. In addition, using vasopressors and inotropic drugs to increase cardiac output will also alter flow distribution to the tissues.

Tissue oxygen consumption (\(\dot{V}O_2\)) under normal conditions is thought to be maintained at a relatively constant level independent of oxygen delivery (DO₂) for most of the DO₂ range (segment B-C in Figure 1). The threshold at which \(\dot{V}O_2\) becomes dependent on DO₂ is called the "critical point" (point B in Figure 1). ARDS appears to alter the \(\dot{V}O_2/DO_2\) relationship such that \(\dot{V}O_2\) remains dependent on DO₂ throughout the measurable range of DO₂ (dashed line in Figure 1). This is termed supply-dependent oxygen consumption (SDOC). It is believed by many
investigators that the SDOC phenomenon is indicative of inadequate oxygen delivery to the tissues. This is based on the assumption that VO$_2$ dependence on DO$_2$ denotes insufficient oxygen delivery to meet the metabolic demands of the tissues$^{40}$. Therefore, many clinicians argue that increasing DO$_2$ should improve an ARDS patient's outcome by lessening the oxygen insufficiency.
Figure 1. Normal and Supply Dependent Oxygen Consumption Relationships.
At least two studies involving humans have demonstrated that $\text{DO}_2$ values calculated from blood oxygen content and cardiac output are susceptible to measurement errors, perhaps more so than when calculated from respiratory gases.\textsuperscript{110} Snyder and Carrol suggested that SDOC may be an artifact because of the likelihood of mathematical coupling magnifying measurement errors when cardiac output is used both to calculate $\dot{\text{ VO}}_2$ and $\text{DO}_2$. Cardiac output is typically measured in the clinical setting by thermodilution technique via a catheter passed into the right side of the heart. A measurement error resulting in a spuriously high cardiac output would cause false increases in both $\dot{\text{ VO}}_2$ and $\text{DO}_2$, and, therefore in SDOC. At the very least these reports indicate the need for careful measurement and consideration of such data.

A review by Kacmarek found that most studies demonstrating SDOC also had a problem with marked spontaneous variations in $\dot{\text{ VO}}_2$ that occurred over the duration of the study.\textsuperscript{106} Villar and associates found a 7\% to 147\% (mean 38\%) variation in $\dot{\text{ VO}}_2$ and a 9\% to 189\% (mean 42\%) variation in $\text{DO}_2$ over a 2 h period in a group of critically ill patients.\textsuperscript{111} Another problem related to this is that most studies do not systematically change $\text{DO}_2$ and then measure $\dot{\text{ VO}}_2$. Furthermore, it is possible that the order of interventions affects the results, such as decreasing cardiac output first and then increasing it rather than the reverse that is typically done.\textsuperscript{112}

Many contemporary studies involving humans in various disease states have demonstrated a linear relation between $\dot{\text{ VO}}_2$ and $\text{DO}_2$ (SDOC), but most of these studies alter $\text{DO}_2$ by changing only cardiac output, not oxygen content.\textsuperscript{106} Instead of
relating $\dot{V}O_2$ to $DO_2$, $\dot{V}O_2$ is actually being related to cardiac output. Still, these studies clearly show evidence of the SDOC phenomenon, most commonly in sepsis or ARDS.

It is difficult to interpret the results of most studies that depict SDOC findings because pooled data rather than individual data are typically reported. Pooling individual trends can lead to the assumption of a plateau where none actually exists, as demonstrated by the example in Figure 2. To test reliably for a plateau, each patient must have enough data points to demonstrate individual plateaus.
Figure 2. False Oxygen Consumption Plateau Resulting from Pooled Data.
Elastic Properties of the Lung and the Effects of Surfactant

The lung is an elastic organ and will collapse upon itself when its exterior is exposed to atmospheric pressure. Lung movement is essentially a passive function that happens as a result of forces originating outside the lung. These forces are generated by the inspiratory muscles during spontaneous breathing or by a pressure gradient developed between the airway and the atmosphere during mechanical ventilation. The pattern of lung movement is determined by the impedance or "hinderance" of the respiratory system. Impedance falls mainly into two categories: 1) elastic resistance of lung tissue and of the alveolar gas/liquid interface to movement, and 2) frictional resistance to gas flow. The inertia of gas and of tissue and the friction related to tissue deformation are additional minor sources of impedance.

Work performed to overcome elastic resistance is stored as potential energy and then used to deflate the lungs during expiration. The elastic recoil of the lungs was originally thought to result entirely from the stretching of elastin filaments in lung tissue. By comparing the different elastance values for air-filled lungs versus lungs filled with and immersed in water, von Neergaard found that much of elastic recoil was due to surface tension related to the large air/water interface area of the lungs. The surface tension of a bubble-shaped, liquid-lined structure such as an alveolus can be predicted from a relationship described by Laplace, i.e., pressure inside the bubble equals twice the surface tension of the liquid lining divided by the bubble radius. Accordingly, if the alveolar lining fluid had the same surface
tension as water, the lungs would be very stiff, and the tendency for alveoli to collapse would be greater as lung volume decreased. Normal lungs do not behave in this fashion because of the extraordinary properties of lung surfactant.

Pulmonary surfactant performs several important mechanical functions. In contrast to the bubble model, pressure within an alveolus will decrease as the radius is decreased when surfactant is present. Surfactant decreases the recoil pressure of the lung as volume decreases, thus obeying Hooke's law for elastic bodies. Surfactant's effect on surface tension decreases as alveoli fill with air until it reaches a tension similar to that produced by plasma fluid. These attributes tend to stabilize alveolar volumes and promote more uniform filling of adjacent units. Lowered alveolar surface tension increases compliance, reducing an individual's work of breathing.

ARDS can cause problems in lung impedance by disrupting the surfactant lining of the lungs. Edema associated with the alveolar-capillary membrane injury can dilute and denature surfactant components which would lead to alveolar instability and may result in unequal alveolar ventilation and atelectasis. Ventilation-perfusion imbalance and intrapulmonary shunting are typical consequences of progressive atelectasis. Pulmonary compliance is also reduced when surfactant loses its ability to counter surface tension and causes an increased work of breathing for the patient. Mechanical ventilation of such patients requires higher airway pressures in order to maintain gas exchange, putting the patient at risk for barotrauma and compromising cardiac output. These factors tend to aggravate the permeability
edema already present in the ARDS lung.\textsuperscript{114}

Human and animal studies have shown that functional changes in surfactant occur in ARDS and in similar lung injuries. Holm and colleagues,\textsuperscript{118} by measuring air and saline pressure-volume curves on a rabbit lung injury model, demonstrated that abnormal surface forces directly contribute to abnormal lung mechanics. Gregory and associates recovered surfactant from the lungs of ARDS patients and found a four-fold increase in surface tension compared to normals. Other studies show that not only is surfactant abnormal in the early stages of ARDS, it also correlates with the syndrome severity.\textsuperscript{119-122}

Since surfactant impairment has been found to be significant in ARDS patients, it is logical to consider replacing it with functional surfactant. The surfactant-replacement product given as a treatment in the present study was Survanta\textsuperscript{®} (beractant) made by Ross Laboratories (Columbus, OH). It is a natural lung extract from New Zealand cattle that contains phospholipids, neutral lipids, fatty acids, and surfactant-associated proteins. Colfosceril palmitate (dipalmitoylphosphatidylcholine), palmitic acid, and tripalmitin are added to standardize the composition and to mimic surface-tension lowering properties of natural lung surfactant. It is suspended in 0.9% sodium chloride solution and heat sterilized, using no preservatives. The suspension contains the surfactant-associated proteins commonly known as SP-B and SP-C, but not SP-A. Survanta comes packaged in 8 mL vials with each milliliter containing 25 mg of phospholipids.
Survanta, containing longer-chain phospholipids and surfactant-associated proteins, is thought to be more effective at lowering minimum surface tension in lung units than products that contain shorter-chain phospholipids only. Surfactant replacement therapy is effective at improving lung compliance in premature lungs. The positive effects of surfactant in adult lungs are less pronounced and vary with the pre-existing condition of the pulmonary tissue.

**PEEP and the rationale for its application**

Positive end-expiratory pressure (PEEP) is an adjunct to mechanical ventilation intended to increase functional residual capacity (FRC). FRC can be thought of as the volume in the lungs at the end of a normal breath. ARDS patients typically exhibit a diminished FRC due to loss of functional lung tissue resulting from their lung injury. This loss of functional lung tissue causes V/Q mismatching and intrapulmonary shunting. As previously stated, hypoxemia in ARDS is generally refractory to supplemental oxygen. PEEP has been consistently used to improve oxygenation in ARDS, although patient response varies. PEEP may increase FRC in ARDS-compromised lungs in two ways: 1) greater inflation of marginally ventilated alveoli (including air movement through collateral passages) and/or 2) reopening of collapsed alveoli.

Although the influence of PEEP on oxygenation in ARDS is clear, its impact on mortality is less apparent. Nelson and associates found high versus moderate levels of PEEP made no difference in duration of mechanical ventilation or overall
The fact that PEEP does not decrease extravascular lung water has been shown by many investigators. Nonetheless, at least two studies have demonstrated that PEEP decreased intra-alveolar fluid volume. A prospective trial by Pepe and colleagues found that 8 cm H2O PEEP in patients at high risk for developing ARDS showed no benefit of PEEP in preventing ARDS. Whether or not PEEP proves to be prophylactic, it is still one of the few supportive therapies available that improves oxygenation in ARDS.

Application of PEEP is generally guided by three goals: 1) re-establishing a normal FRC, 2) maintaining an acceptable PaO2 at a minimum F1O2, and 3) achieving these goals with minimal cardiovascular compromise. The terms "best PEEP" or "optimal PEEP" have been used to describe the highest level of oxygen transport and minimum dead space possible at the lowest pressure. Many strategies have been proposed to determine best PEEP but none appear to accurately predict for all ARDS patients. Perhaps the most practical method for guiding PEEP application is the "least PEEP" strategy proposed by Nelson, Carroll, and others. This involves trying to reduce the F1O2 to a level no more than 0.5 while maintaining an arterial oxygen tension above 60 mm Hg. Some investigators have advocated application of prophylactic PEEP during the early stage of mechanical ventilation for ARDS patients. Early application of PEEP (before PEEP is indicated by blood gas values) may improve patient course by preventing the collapse of some alveoli in injured lung regions rather than trying to reopen them after the fact. Regardless of the strategy used, the application of PEEP for ARDS patients is accepted as
conventional therapy.
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CHAPTER II

A Model of Acute Lung Injury Using PMA in the Dog.

INTRODUCTION

The adult respiratory distress syndrome (ARDS) is an acute respiratory disorder that, despite all modern therapies developed since it was first described, still has a high mortality rate.\(^1\) Risk factors for development of ARDS include hemorrhagic, cardiogenic, septic, or anaphylactic shock, aspiration, smoke inhalation, pancreatitis, drug overdose, near drowning, or various traumas.\(^2\) Blood-borne infection (sepsis) constitutes the largest category of predisposing factors for ARDS.\(^3\) The massive immune response typically produced by the infected host often results in damage to the lungs and other organs that include edema, decreased lung compliance, diminished gas exchange, and an increased shunt fraction. Neutrophil-mediated injury is thought to be the primary instigator of ARDS injury.\(^4\)\(^7\) Weiland and associates reported a correlation between the magnitude of neutrophil influx into the lungs and the severity of pulmonary edema in ARDS patients.\(^8\)
Phorbol 12-myristate 13-acetate (PMA)
or 12-O-tetradecanoylphorbol-13-acetate

Figure 3. Chemical structure of PMA.
Phorbol 12-myristate 13-Acetate (PMA) is a chemical agent used to trigger an immune response capable of producing a similar injury to that found in ARDS (Figure 3). A phorbol ester derived from croton oil, it is thought to act as a protein kinase C-activator in neutrophils. PMA has been used to induce an early ARDS-like pulmonary edema in several types of animal models. Animal studies with PMA-induced lung injury show a rapid onset of neutrophil-mediated acute lung injury with a reported duration of at least six hours. The acute response after PMA injection includes intra-alveolar hemorrhage, fibrin deposits, and pulmonary edema within 5 hours. Hyperplasia of alveolar type II cells and interstitial inflammation occurs two to four days after injection. This proliferative stage culminates in an atypical fibrosis (that eventually clears); this is found in ARDS patients that survive the acute stage of the syndrome as well as in PMA-treated animals. A review of neutrophil-mediated lung injury by Glauser & Fairman concluded, "the preponderance of evidence indicates that PMA causes intrapulmonic neutrophil trapping, release of unstable oxygen radicals, damage to the pulmonary endothelium, and increased permeability pulmonary edema."  

The intention of this study was to develop a model of acute lung injury using PMA which resembled, as much as possible, the kind of lung injury which occurs in man in the acute phase of ARDS. The model developed paralleled that of Dorinsky, et al., except that the experiment duration was longer, metabolic acidosis was treated with sodium bicarbonate, and fluid input was controlled. The hypothesis tested was that PMA would induce an acute lung injury resulting in a rapid progression to
fulminant pulmonary edema and marked abnormalities in gas exchange typical of ARDS as described by the criteria for ARDS.

METHODS

The refinement process of the experimental procedure that produced the final representative PMA injury group (n=7) used for comparsion to treatment groups involved a total of twenty-two experiments. The first fifteen experiments examined variables such as control of blood pH, fluid management, PEEP, and PMA dosage and consistency. Mongrel dogs (20-30 kg) were premedicated with acepromazine, 0.25 mg/lb subcutaneously, and then anesthetized with pentobarbital sodium adjusted for the acepromazine (animal's weight in pounds/5 - 2 = milliliters of pentobarital concentrate), or about 20 mg/kg, through a percutaneous peripheral IV. The level of anesthesia was monitored using the standard indicators of heart rate (e.g., prevent sudden increases as a result of pinching between foot pads), movement, eye twitch, jaw resistance, and presence of lacrimation. Additional boluses of fifteen to thirty milligrams were administered every thirty minutes as needed in response to the above indicators to assure adequate anesthesia.

Immediately after the initial dose of pentobarbital was given, an artificial airway was established using either a 7 or 8 mm internal diameter endotracheal tube with patency and position verified by auscultation. A condensing humidifier was placed in-line between the endotracheal tube and the ventilator circuit to retain heat and moisture in the animal's airway. No additional heat or water was added to the
inspired gases. A Bourns Bear I volume ventilator set on constant flow and assist-control mode provided ventilatory support for all subjects. Mechanical ventilation was begun with a tidal volume of 15 cc/kg of body weight at a rate of 12 breaths/minute and an $F_1O_2$ of 0.21. Ventilator rate was adjusted to keep the $PaCO_2$ between 35-45 mm Hg until lung injury exceeded the limits of this compensatory technique.

Bilateral femoral cutdowns were performed to catheterize the femoral veins and arteries. Arterial blood samples and pressure measurements were taken from the right femoral artery and an Edwards lung water catheter was placed in the abdominal aorta via the left femoral artery for sampling blood temperature and densitometry to measure extravascular lung water by double-dilution technique (DDLW). Placement of a thermodilution, balloon-tipped, Swan-Ganz catheter in the right jugular vein was guided by continuous monitoring of pressure waveforms. Catheter ports were connected to Validyne and Statham pressure transducers which were referenced to the level of the right atrium. A continuous IV drip of 0.9% normal saline was administered at 20 ml/kg-hr during the first hour of instrumentation to insure the animal was adequately hydrated. The saline rate was decreased to 10 ml/kg-hr as a maintenance rate for the duration of the experiment. The amount of fluid added to the vasculature in the form of lung water & cardiac output injectate was recorded for fluid input/output calculations.

Arterial and mixed-venous blood gas samples were aspirated into heparinized plastic syringes and immediately placed on ice. The blood gas samples were quickly
taken to a blood gas laboratory and analyzed for pH, PO$_2$, and PCO$_2$ using an Instrumentation Laboratories blood gas analyzer (Model 1312, Instrumentation Laboratories Inc., Andover, MA). Blood gas machine calibration was performed immediately prior to each sample analysis. Intra-assay (n=9) and inter-assay (n=3) analyses were performed on the arterial blood gas analyzer to confirm acceptable precision and accuracy, using dog blood (Appendix 1). Daily quality control was performed on the machine using acidosis, normal, and alkalosis standardized control samples. Hematocrit measurements were performed by centrifugation of arterial and venous micropipette samples. Plasma protein values were obtained reading the supernate of centrifuged venous samples with a refractometer.

Sodium bicarbonate (8.4% solution) was administered according to blood gas results. Arterial pH values of 7.30 and lower associated with a low bicarbonate value were treated by substituting 25-50 ml doses of bicarbonate solution for normal saline in the maintenance infusion (10 ml/kg-hr) until the pH returned to a minimum value of 7.35. Blood pressure typically fell after PMA administration and if mean arterial pressure (MAP) approached 70 mm Hg, an intravenous bolus of 0.9% saline of up to 20 ml/kg body weight was given, followed by an intravenous dopamine drip (200 μg/ml) titrated according to a continuous MAP recording. Pulse oximetry provided continuous monitoring of heart rate and arterial hemoglobin saturation to allow for rapid mechanical ventilation adjustments. A catheter was placed in the urethra to monitor urine output and specific gravity. Heating pads were used to maintain a core temperature close to 38 degrees centigrade.
This study modeled PMA preparation and dosages after the methodology in two studies by Dorinsky, et al.\textsuperscript{13,17} The chemical agent PMA (Calbiochem Inc., LaJolla, CA) was dissolved in dimethylsulfoxide (DMSO) at a concentration of 5 mg/ml. The PMA was then stored in 200 microliter aliquots at -80 degrees C (200 microliter aliquot = 1000 milligrams PMA). Immediately prior to use, a 200 microliter aliquot of PMA was diluted in 3.0 ml of normal saline and 1.8 ml of DMSO to yield a final concentration of 200 \( \mu \)g/ml.

Two different lots of PMA were used in this study to produce a lung injury with a significant increase in lung edema. The first lot was given at a dose of 30 \( \mu \)g/kg and the second at a dose of 25 \( \mu \)g/kg. Five experiments were performed to determine the appropriate dose for the second lot to produce a similar injury to that of the first lot. The second lot dosage was lowered because it was found to cause death before the end of the seven hour measurement period if given at 30 \( \mu \)g/kg.

The lots were divided among the three originally proposed study groups of the thesis--PMA, PMA+furosemide, and PMA+surfactant, so that each group had four animals at 30 \( \mu \)g/kg and three animals at 25 \( \mu \)g/kg. The PMA+surfactant+PEEP group used only the 25 \( \mu \)g/kg dose lot since it was not originally planned and a mixing of dosages could not be provided at the near the end of data collection.

Once data collection started, some animals were not included as subjects either because of premature death or lack of injury. Premature death occurred in four animals that received a 30 \( \mu \)g/kg PMA dose (first lot). One animal that received a 25 \( \mu \)g/kg dose from the second lot of PMA died an hour before the
experiment was to have ended. Two animals that received a 25 μg/kg dose were excluded because serial measurements indicated no presence of injury. Post-mortem examination of the lungs of these two animals confirmed no presence of the highly visible injury pattern typically found in the gravity-dependent lung regions with PMA injury. Thus, during the consecutive sequence of 32 experiments (injury and injury+treatment) that included the refined PMA protocol, a total of seven animals were excluded.

Double-dilution extravascular lung water measurement was performed using the Edwards Model 9310 Lung Water Computer (American Edwards Laboratories, Irvine, CA). The double-dilution technique is a non-destructive means for serial measurement of extravascular lung water (pulmonary edema). This unit has been used for both animal and human studies of lung water and also performs thermodilution cardiac output measurement. Correct cardiac output values were especially important since they were used for calculating several other cardiorespiratory variables so intra- and inter-assay coefficients of variation were assessed at the beginning of the study to assure acceptable precision & accuracy (Appendix 2).

The double-dilution technique involves a simultaneous injection of two indicators into the superior vena cava or right atrium. One indicator remains entirely intravascular, while the other indicator diffuses freely across the pulmonary capillaries to mix with the extravascular water in the lungs. The resulting indicator-dilution curves are detected downstream from the lungs in the abdominal aorta. The
two indicators are indocyanine green dye for the intravascular compartment and a cold bolus of dextrose solution for the total vascular volume. The indocyanine green dye binds rapidly to albumin and remains in the blood during the first pass through the lungs while the thermal indicator diffuses freely and rapidly throughout the pulmonary extravascular spaces. The special femoral artery catheter is equipped with a thermistor tip to measure the thermal curve and a sampling port connected to a densitometer that measures changes in absorbance over the light range sensitive to green dye.

The simultaneously obtained indicator-dilution curves (lung water computer corrects for instrument response time and catheter volume) allow for computation of extravascular lung water by a computer program that calculates mean transit times for each curve and cardiac output (EVLW equals the product of cardiac output and the difference in mean transit times of the green dye and thermal curves). Each lung water measure is verified by direct observation of the dilution curves on a real-time video display to insure curve-shape integrity and two samples within 15% of each other are averaged and reported as the lung water value. Indocyanine green cocktails were prepared by cleansing a 25 mg bottle of dye into a 200 ml beaker with 40 ml of 5% dextrose solution. Sixty milliliters of distilled water were added and mixed well before aspirating into 50 ml syringes. The green-dye cocktails were then kept in an ice slurry until immediately before use.

Wet/dry weight measurement was patterned after an FDA approved wet and dry lung weights procedure (Dayton toxicology group). After the animal was
euthanized, the thoracic cavity was opened and the trachea was clamped two centimeters below the level of the cricoid cartilage. The pulmonary arteries and veins were cut so as to allow the lungs to passively exsanguinate. After removal from the thoracic cavity, the lung exterior was briefly rinsed with normal saline. A wet weight was obtained using a triple-beam balance scale. The lungs were placed in a pre-weighed foil tray and set in an oven for at least 48 hours at 110°C. Dry weights were measured on the balance scale for several consecutive days until no change in weight was observed. The wet and dry weights were used in the following formulas:

\[
\% \Delta H_2O = \frac{(WWc - DWc)}{WWc} \times 10^2
\]  
\[
g_{H_2O/g \text{ dry lung wt.}} = \frac{(WWc - DWc)}{\text{body wt. in g}} \times 10^3
\]

where WWc = [wet lung wt. (g) corrected for apparatus wt.] and DWc = [dry lung wt. (g) corrected for apparatus wt.]. To assess the degree of edema formation, a comparison is made of the above values (% H2O, g H2O/kg body wt, g solid/kg body wt) between the PMA-injury-only and the treatment groups. If a statistically significant difference in lung edema as measured by DDLW was found when comparing two groups, a similar significant difference in % H2O and gm H2O/gm dry lung wt. would be expected.

Each experimental trial consisted of three phases; an initial period allowing for the animal to stabilize on mechanical ventilation, instrumentation, and collection of baseline measurements (1.5 hours); an intermediate period during which PMA lung injury was induced (1.5 hours); and the final period during which treatments
RESULTS

Mechanical Ventilation Variables

Changes in the pressure required to ventilate the animals were followed by observing the peak airway pressures, and dynamic and quasi-static compliances during ventilator breaths (measured from the ventilator circuit). One-way ANOVA tests (with "time" as the independent variable) of each ventilation variable indicated significant changes from hour one values ($p < 0.05$) for peak inspiratory pressure (PIP) only at hours five and six (Figure 4).

Gas Exchange in the Lung

Damage to the A-C membrane of the lungs was assessed over time by measuring the shunt fraction ($Q_s/Q_t$) and alveolar-arterial oxygen difference ($AaDO_2$) (Figure 5). A brief decrease in $Q_s/Q_t$ ($p = 0.037$ when compared to hour one "baseline" value, not significant for $AaDO_2$ ) occurred after PMA was given, perhaps due to the concomitant increase in respiratory frequency (66% increase). Although minute volume was not directly measured, an increased frequency would produce a proportionately large increase in minute ventilation because the ventilator mode was such that it would assist each inspiratory effort of the animal with a full mechanical breath. During hours 3-7, the time period over which a treatment effect would be
measured, the $Q_s/Q_r$ values significantly increased at hours five ($p = 0.029$) and seven ($p = 0.008$) compared to hour three.
Figure 4. Changes in mechanical ventilation variables (error bars in standard deviation, "**" indicates p-value < 0.05 compared to hour one).
Figure 5. Changes in AaDO₂ and QS/Qt over time (error bars in standard deviation, "**" indicates p < 0.05 over hours 1-7, "☆" indicates p < 0.05 over hours 3-7).
Plots of the group averages for arterial pH, PaCO₂, and PaO₂ reveal the variables were kept fairly constant during the experiments via mechanical ventilation support and bicarbonate administration (Figure 6). Blood gases were sampled with the F₁O₂ set at 1.0 and zero PEEP, yet the greatest difference between any two mean values of oxygen tension was 87 mm Hg.

**Hemodynamic Responses**

Changes in the group means of central venous pressure (CVP), pulmonary capillary wedge pressure (PCWP), and mean pulmonary artery pressure (PAP) were modest but considerable variability was seen after PMA administration (Figure 7). The trends of CVP, PCWP, and mean PAP did not fluctuate more than 2-3 units of pressure during the experiment period. Particularly after PMA was given, the standard deviations associated with each mean greatly increased. As predicted, PCWP remained well below the previously stipulated criterion of 18 mm Hg pressure for low-pressure pulmonary edema.

Cardiac index (C.I.), the cardiac output indexed to m² of body surface area, and pulmonary vascular resistance (PVR) changed inversely with respect to each other, as C.I. decreased, resistance through the pulmonary circuit increased (Figure 8). Cardiac index decreased significantly from the baseline value starting at hour 3 and continuing throughout the remainder of the experiment, however; PVR did not exhibit significant decreases at an alpha-level of 0.05.
Figure 6. Changes in blood gas variables during the experiment (error bars in standard deviation).
Figure 7. Changes in PCWP, CVP, and PAP over time (error bars in S.D.)
Figure 8. Changes in pulmonary vascular resistance and cardiac index (error bars in standard deviation, "*" indicates p-value < 0.05 compared to hour 1).
Body Fluid Changes

By the end of the experiment, the overall body fluid balance (input/output) yielded a net positive gain of 39 mL/kg. Hematocrit, another indicator of fluid movement (or splenic emptying of erythrocytes) and a variable that impacts vascular resistance, rose to 43% by hour three, a significant hemoconcentration ($p=0.031$, per ANOVA post-hoc test--Tukey's Honestly Significant Difference) compared to the initial baseline value at hour one (Figure 9). Although the hematocrit values did not return to baseline, subsequent measures were not of significant difference from baseline. Plasma protein concentration did not change.

Changes in Peripheral Gas Exchange

Since ARDS-type injuries typically exhibit diminished gas exchange in both the lungs and the tissues, I predicted that oxygen delivery would decrease and oxygen extraction would decrease or at least remain the same after injury. Oxygen delivery rapidly decreased after PMA administration such that the third hour measurement yielded a p-value of 0.023 compared to hour one. Oxygen extraction ratio did not increase significantly during the experiment (Figure 10). Serum lactate, another indicator of tissue oxygenation, did not produce a significant difference, perhaps due in part to the marked variability in values observed after PMA was given (Figure 11).
Figure 9. Changes in venous hematocrit and plasma protein concentration (error bars in standard deviation, ** indicates a significant change from hour one).
Figure 10. Changes in oxygen delivery and extraction ratio (error bars in S.D.).
Figure 11. Changes in serum lactate over time (error bars in standard deviation).
Changes in Lung Water

A permeability edema lung injury should produce increases in lung edema (extravascular lung water). The double-dilution lung water measurement technique showed a trend of approximately 65% increase in lung water by hour three and a doubling of the baseline value by the end of the experiment (Figure 12). Even so, the variability of the data was such that no increase was observed using a one-way ANOVA test.

Changes in Lung Elastic Properties

The composite static pressure-volume curves generated from the lungs of both PMA group and the group not receiving PMA (normal) are shown in Figure 13. In pulmonary function terminology, the PMA group curve exhibits a "restrictive" pattern compared to the uninjured group due to the decreased TLC. Table 3 gives the group means and standard deviations of the minimal lung volume at zero airway pressure ($V_{\text{MIN}}$), maximal lung volume at 30 cm H$_2$O pressure (TLC), and expiratory compliance measured in the range of tidal breathing for the PMA and normal groups. Maximal lung volume for the PMA group was only 54% of the normal group, making TLC the only variable with a significant change ($p=0.012$). ANOVA results revealed no significant differences between groups for either expiratory compliance ($p = 0.105$) or $V_{\text{MIN}}$ ($p = 0.472$). PMA-injury did not significantly alter the lung hysteresis, as indicated by the circumscribed area between the inspiratory and expiratory curves. Even after correcting for the disparity in TLC values between the
two groups, the hysteresis area for the PMA group still remained within the boundries of the normal group (Figure 14).
Figure 12. Changes in lung water per double-dilution method (error bars in S.D.).
Figure 13. Pressure-volume curves for excised left lung (means±standard deviation listed in Appendix 3).
Table 3. Volume and compliance values from excised left lungs.

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>$V_{\text{MIN}}$</th>
<th>TLC</th>
<th>$C_L$ (1)</th>
<th>$C_L$ (2)</th>
<th>$C_L$ (3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>8</td>
<td>10.9±6.4</td>
<td>52.4±20.8</td>
<td>4.6±2.3</td>
<td>50.0±19.9</td>
<td>0.070±0.019</td>
</tr>
<tr>
<td>P</td>
<td>6</td>
<td>8.8±2.3</td>
<td>25.4±9.2</td>
<td>2.8±0.9</td>
<td>57.6±28.3</td>
<td>0.085±0.034</td>
</tr>
<tr>
<td>p-value</td>
<td></td>
<td>0.472</td>
<td>0.012</td>
<td>0.105</td>
<td>0.856</td>
<td>0.372</td>
</tr>
</tbody>
</table>

N = Normal, P = PMA, S = PMA+Surfactant, SP = PMA+Surfactant+PEEP; $C_L$ (1) = slope of pressure-volume curve between 45%-55% of TLC in units of mL/g/cm H$_2$O; $C_L$ (2) = slope of the pressure-volume curve between 5-8 cm H$_2$O; $C_L$ (3) = $C_L$ (2)/volume at 5 cm H$_2$O. Results listed as mean±S.D.
Figure 14. PMA pressure-volume curve corrected according to TLC.
DISCUSSION

The two primary findings for this model of lung injury were the lack of major decreases in gas exchange variables (AaDO₂ and Qₐ/Q₉) and the variability present in many of the dependent variables. The initial response to PMA involved large individual fluctuations as seen by the large standard deviations for hours two and three. Hemodynamic instability was such a problem that 10 cm H₂O of positive end-expiratory pressure could not be added during this time without fatal results. Some variables such as hematocrit and PVR showed partial recovery towards the end of the experiment. Lung water did not significantly increase over the course of the experiment.

The hallmark signs of the early phase of ARDS are: 1) diffuse pulmonary edema with a normal pulmonary artery wedge pressure, 2) a resulting hypoxemia from intrapulmonary shunting that significantly increases the alveolar-arterial oxygen difference and requires mechanical ventilatory support, and 3) a decrease in lung compliance. Although AaDO₂ and shunt fraction exhibited significant increases during the treatment comparison period, the actual values represented only modest injury. A shunt fraction approaching 0.3 was desired, but the highest value during the treatment period was only slightly greater than 0.1 (normal < 0.1). Although extravascular lung water increased throughout the experiment as expected with lung injury, PCWP remained below 18 mm Hg, the criterion used to differentiate between a low-pressure, permeability edema and a high-pressure, cardiogenic edema. Excised left lung compliance of the PMA-injured group was not significantly different from
that of the normal group.

It is not uncommon for PMA-treated animals to develop an acidic arterial pH over the course of an experiment.\textsuperscript{5,17} This can be compensated for to a limited degree by increasing alveolar ventilation with the ventilator. In the present study, the observed acidosis had a large metabolic component. Metabolic acidosis is most commonly treated by intravenous sodium bicarbonate replacement. It was necessary to use sodium bicarbonate to maintain pH within the target range of 7.35-7.45 because of the compounding factors of hypoxemia and hydrogen ion accumulation related to insufficient renal excretion. Bicarbonate administration often causes elevated carbon dioxide levels in the blood and therefore may confound measurements of that variable. Experiments of short duration (four hours or less) may not require bicarbonate supplements, but such support appears unavoidable for longer experiment periods.

It appears that the majority of experiments using PMA have a duration of only a few hours.\textsuperscript{5,13,23-24} As previously noted, PMA and other models that trigger a neutrophil-mediated injury can produce an acute lung injury within such a time period. Although this injury that includes the development of lung edema and egress of protein and other debris into alveoli is similar to the injury seen in the early stage of ARDS, it does not reflect the later stage of the syndrome, which includes fibrin accumulation and scar tissue deposition. This presents a dilemma for the investigator. Smaller serial doses of PMA can be given over several days to produce a lung injury that resembles the later stage of ARDS, but not without significant
discomfort for an unanesthetized animal. To satisfy ethical concerns it is desirable to keep the animal fully anesthetized once injury is initiated. The associated support required for this would require continuous monitoring and care similar to that provided in a veterinary hospital intensive care unit, and this is beyond the capability of many laboratories.

Problems encountered with variable PMA dose-response from one lot to another were similar to those described by Sprague and others.\textsuperscript{25} The best approach to date given by Sprague for maintaining as consistent an injury as possible across a sample group is to prepare a large number of individual doses from the same lot. As much as double the number of doses required for the intended sample size should be prepared to allow for some premature animal deaths.

Although PMA was found to be quite capable of causing injury and/or death, the animal responses tended to be variable at a sub-lethal dose. The Dorinsky model used a PMA dose of 30 $\mu$g/kg of animal body weight with a constant saline infusion of 7 ml/kg-hr, with measurements taken over a three hour period.\textsuperscript{17} The associated significant hemodynamic findings after PMA administration included a 50% decrease in cardiac output after 90 minutes, more than doubling of mean pulmonary artery pressure (PAP), and a 63% decrease in mean arterial pressure (MAP). The animals concluded the experiment in an acidotic state with a final arterial pH of 7.12 (7.40 initial pH). In another dog study using the same PMA dose and fluid support, alveolar-arterial oxygen partial pressure difference (AaDO$_2$) measured on an $F_iO_2$ of 1.0 increased from 110±8 mm Hg to 360±70 mm Hg over a six hour measurement
Other researchers using whole animal models have produced similar results with varying PMA doses. Sprague & Stephenson used two different doses of PMA in dogs and found varying dose-dependent responses. The two dosages given were a low-range of 10 or 15 μg/kg and a high-range of 20 or 30 μg/kg. After sixty minutes, the 20 or 30 μg/kg animals were found to have a 50% decrease in cardiac output, more than doubled PAP, a six-fold increase in pulmonary vascular resistance, and a drop in WBC count from 11.8 to 0.6 cells x 10^3/mm^3. The low dose group also demonstrated similar trends but of a lesser magnitude. Extravascular lung water thermal-dye (double-dilution method) measurements showed a 140% increase for the high-dose group but no significant change for the low-dose group.

The two dosages used in the Sprague study within the low and high-ranges represent adjustments due to a change in PMA potency. Two different lots of PMA were used during data collection and animal responses were found to be different to each lot. Therefore, the high-range dose in μg/kg was lowered from 30 to 20 for the second lot of PMA and the low-range was lowered from 15 to 10. In a personal communication with author, it was revealed that this variability among different lots was typical from his experience and that of his colleagues but rarely published. I found similar differences in potency in my investigation.

Fluid given to the animals was carefully controlled and monitored to minimize its effect on the measured dependent variables. All groups receiving PMA injury were maintained on a constant saline infusion to provide uniform volume
support. Even bicarbonate administration was done by substituting its volume as part of the constant saline infusion. Such fluid support is not unlike what ARDS patients require in a clinical setting. The nature of the injury involves the steady loss of vascular fluid and proteins through damaged capillary membranes. This was evidenced in this model by the steady decrease of plasma protein concentrations and the rise in blood hematocrit. Dopamine was used only to maintain a minimum mean arterial blood pressure greater than 70 mm Hg, and individual responses were quite variable, requiring constant adjustment.

Although the loss of subjects is undesirable, it was not surprising given the morbid nature of the syndrome that was modeled. While mongel dogs generally have a certain robustness and commonality related to their mixed breeding, the unknown histories of the animals were a limitation. Some animals may have prematurely died due to past/preexisting illness and/or hypersensitivity to the PMA agent. Regardless, some loss of subjects is expected in a scenario where the dose of injury agent was adjusted to in an attempt to simulate a "life-threatening" syndrome.

The following conclusions were made for this PMA model of acute lung injury in the dog: 1) PMA produces a variable injury that affects the lungs and other non-pulmonary organs: heart-decreased cardiac output, kidneys-low urine output. 2) Determining a dose to cause severe lung injury without causing a significant number of premature deaths is difficult, and it generally requires the sacrifice of several animals to establish a dose-response for each new lot of PMA agent. 3) The trends of many of the dependent variables appear to be bi-phasic with a partial recovery late
in the experiment. 4) Lung water steadily increases over the entire experiment and is independent of changes in AaDO$_2$ and shunt fraction. 5) Expiratory compliance as measured in excised left lungs did not significantly change compared to normals but TLC was markedly decreased.

Overall, PMA injury is difficult to control. The dosage required to produce a severe lung injury appeared to be very close to a lethal dose. This may have been due in part to varied, individual immune responses and the health/condition of the animal. In general I would not recommend this model for a short experiment period, but it may prove to be satisfactory if a longer time course for injury development is allowed, using a series of smaller doses.
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CHAPTER III

The Effects of Furosemide Diuretic on Gas Exchange and Lung Water in a PMA Model of Acute Lung Injury

INTRODUCTION

Diuretics such as furosemide have been used experimentally and clinically to treat both cardiogenic (high pressure) and non-cardiogenic (low pressure) pulmonary edema. 1 Cardiogenic edema generally results from a dysfunctional left heart causing vascular pressures within lung capillaries to rise until fluid is forced out of the vessels and into the interstitial spaces faster than the lymphatic system can drain it. Non-cardiogenic edema, e.g., ARDS, is also characterized by fluid leakage from the capillaries but is due to a damaged alveolar-capillary membrane at low capillary pressures. Aggressive diuretic therapy combined with drugs to support hemodynamic function is one approach used to manage fluid balance in ARDS. There has been considerable study of the diuretic effect of furosemide on pulmonary edema and its untoward effects, but much less information is available on the extrarenal impact. 2-3

Various investigators have labored to offer explanations of how furosemide works to produce therapeutic effects in ARDS in the presence of a damaged alveolar-
capillary membrane. First, some argue in support of the diuretic action that causes fluid loss and a decrease in the circulating blood volume. As fluid is removed from the blood, the blood becomes more concentrated, yielding a higher osmotic pressure. An increased plasma osmotic pressure would tend to counter fluid movement out the capillaries. Indeed, this is does appear to be the primary mechanism for the effect of furosemide in resolving high pressure lung edema.\textsuperscript{4} In ARDS however, this mechanism is confounded by two problems, the first being that as plasma is concentrated, it may simply become distributed across the damaged alveolar-capillary membrane, thereby frustrating the attempt to increase the osmotic pressure gradient between the capillary and the lung interstitium.\textsuperscript{5,6} In a study that measured the effect of both furosemide and albumin on canine lung injury induced by hydrochloric acid installation, treatment with both furosemide and albumin or furosemide alone prevented the increase in lung lymph flow associated with acid injury.\textsuperscript{7} An attempt to increase the protein transvascular osmotic pressure gradient by albumin alone did not slow lung lymph flow, indicating that attempts to create such osmotic gradients to treat ARDS may be of little value. Investigations using saline infusion to induce high pressure pulmonary edema concluded that furosemide was effective in mobilizing pulmonary edema by increasing plasma colloid osmotic pressure through diuresis.\textsuperscript{8} Although not understood, it is clear that furosemide influences gas exchange in ARDS by more than one mechanism.\textsuperscript{7,8}
Furosemide has been reported to decrease lung edema before the onset of diuresis, and to improve gas exchange in oleic acid-induced pulmonary edema (low-pressure edema) before there was any detectable change in the amount of lung edema. It has been suggested that these effects are mediated through a relaxation of pulmonary vascular tone, but, as reviewed by Leeman, most studies have generally found vasodilators impair gas exchange in ARDS, whereas furosemide often improves gas exchange (exception-nitric oxide).

A second concern when using furosemide to treat ARDS comes from studies of multiple organ system dysfunction (MOSD—typically found in ARDS patients) that show kidney damage which would result in a diminished renal response to diuretics. Such a condition would presumably place the patient almost solely under the influence of the extrarenal effects of the drug, whatever they may be. It is possible that the diuretic effect of furosemide is potentiated by extrarenal effects or that diuresis may mask negative extrarenal effects. ARDS patients often don't respond (due to MOSD) with increased urine output when given a diuretic so the extrarenal effects of furosemide may be of more significance in ARDS patients.

Inducing hypovolemia by furosemide treatment and thereby increasing capillary osmotic pressure avoids the problem that can occur when osmotic agents (e.g., albumin or macromolecular colloids) are given. That is, the osmotic agent eventually leaks across the damaged capillaries, pulling fluid with it. However, therapeutic hypovolemia is not without hazards. The ARDS scenario is frequently characterized by reductions in cardiac output, poor distribution of blood flow in
systemic capillary beds, and marked hypoxemia, all of which could be aggravated by a hypovolemia-induced diminished cardiac output. Reducing circulating volume via diuretics must be done with care to maintain acceptable oxygen delivery to the tissues.

The alveolar epithelial lining also has an intricate fluid transport balance in addition to the pulmonary endothelial/lymphatic fluid exchange. Several mechanisms produce a physiologic salt solution in the large airways, and Clara cells are hypothesized to be the primary sources of fluid secretion. Just as there is a net fluid movement out of the pulmonary capillaries, a net removal of fluid from the alveolar lumen occurs but by osmosis in response to the active transport of sodium. It is not known whether this active transport of sodium continually counters a tendency for the alveoli to fill with fluid or if it merely responds "on-demand" when fluid accumulates. The sodium transport can be accelerated by certain beta-adrenergic agonists, which may offer an additional course of treatment for pulmonary edema. Given that furosemide induces sodium transport in some extrapulmonary tissues, it may have a similar effect in the pulmonary alveoli, thus enhancing fluid removal by a non-renal mechanism.

Physicians in the Pulmonary and Critical Care Medicine Division at the Ohio State University Hospitals have been practicing conservative fluid support with their ARDS patients for several years and have produced a mortality rate lower than the national average. They theorize that their success is due at least in part to reducing fluids given and using diuretics to maintain a net body fluid balance that is
somewhat negative, on the other hand, other physicians prefer to put their ARDS patients into a positive fluid balance in an effort to increase oxygen delivery to the tissues. A major purpose of the present study was to document the effects of conservative fluid support in a controlled setting and to begin to elucidate the underlying mechanisms that caused an overall decrease in patient mortality.

We tested the following hypotheses in a dog model of ARDS: 1) Furosemide treatment will reduce lung water content in early ARDS when compared to a control. This will result in improved lung compliance and increased lung volumes. 2) Decreases in lung water after furosemide administration will be associated with a diuresis-induced hemoconcentration. 3) Furosemide treatment, by decreasing lung water, will produce a lower alveolar-arterial oxygen difference and shunt fraction. 4) Pulmonary vascular resistance will be lowered as a result of furosemide treatment, resulting in a reduction in pulmonary artery pressure, that will be associated with reduced lung water.

METHODS

Two groups of seven mongrel dogs weighing 20-31 kg were anesthetized and mechanically ventilated for a seven-hour measurement period (see PMA model chapter for procedural details). One group received PMA-injury only and the other received PMA+Furosemide treatment. The same procedures were followed for both groups except dogs in the furosemide treatment group were supported with a different protocol to maintain minimum mean arterial blood pressures (MAP). Blood
pressure typically fell after PMA administration, and if MAP approached 70 mm Hg, an intravenous dopamine drip (200 µg/ml) was started and titrated according to a continuous MAP recording in an attempt to maintain MAP above 70 mm Hg. Unlike the PMA-only group, no pre-dopamine saline bolus was given so as not to blunt any diuretic effect of the furosemide. Sodium bicarbonate (8.4%) was used to suppress metabolic acidosis and was administered in the same manner as described for the PMA injury-only group. A "normal" group of six that did not receive PMA injury or furosemide was used for ex vivo lung compliance comparisons.

Heparinized arterial and mixed venous blood gas samples were drawn hourly and immediately iced before blood gas analyses were performed on an IL 1312 blood gas machine (Instrumentation Laboratory Inc., Lexington, MA) that was tested daily for accuracy and reliability using whole-blood control samples. One and two-point calibrations were repeated throughout each experiment to insure measurement accuracy. Hematocrit was calculated after centrifugation of each arterial and venous blood sample by centrifugation of sample. Total hemoglobin was calculated using the relationship of 33 g of hemoglobin per 100 mL of packed red blood cells. Plasma protein was measured by reading centrifuged venous blood samples using an Atigo hand-held refractometer.

Core body temperatures were measured with a thermistor-tipped catheter positioned in the pulmonary artery. This catheter also allowed for measurement of cardiac output based on the thermal-dilution technique. The body surface area used to calculate cardiac index was obtained from a nomogram based on body weight.
Continuous monitoring of femoral arterial, pulmonary arterial, central venous, and airway opening pressures were done on a Gould four-channel recorder. Inflation of the balloon-tip of a Swan-Ganz catheter every thirty minutes allowed for measurement of pulmonary capillary wedge pressure, a reflection of left atrial pressure. In addition to peak airway opening pressures, static airway pressures were recorded by first giving several manual ventilator breaths and then occluding the exhalation valve so as to momentarily trap the volume of a ventilator breath in the lungs.

All indicator-dilution measurements of extravascular lung water were performed using a dedicated lung water computer (Edwards Laboratories Inc., Santa Ana, CA). The measuring technique involved injecting a 10 mL bolus of iced, green dye solution (2.5 mg indocyanine green dye in 10 mL 5% dextrose solution) into the right atrium via the injectate port of a Swan-Ganz catheter. Thermal and dye curves were detected downstream by a 14 french lung water catheter (Edwards Lab. Inc.) placed in the left femoral artery and guided into the abdominal aorta. A mean value derived from two measures within 10% of each other was indexed to intitial body weight. Thermal and dye dilution curve integrities were inspected using real-time video tracings in addition to the built-in error detection of the lung water computer. The lung water computer was also used for determining cardiac output by using iced injectate without dye.

Body fluid status was monitored by recording all fluids administered and all fluids removed including blood and urine. Urine output was measured every thirty
minutes, and urethral catheter patency was checked regularly. Fluid secretions
removed via suctioning were recorded and potential fluid loss from the
gastrointestinal tract was prevented by occluding the animals' rectum.

At the conclusion of the seven-hour experiment the animals were euthanized
with intravenous magnesium chloride to induce cardiac asystole. The heart and lungs
were quickly removed through a sternotomy after clamping the trachea. After rinsing
the lungs with normal saline and separating them from the heart, the trachea and
right mainstem bronchus were double-clamped to prevent fluid from entering or
exiting the lungs as the airways were cut. The trachea was trimmed to three inches
above the branching of the mainstem bronchi, and the right bronchus was cut distal
to the clamp, approximately one centimeter from the carina. Cotton twill suture was
used to secure the trachea, which was attached only to the left lung at this point, to
connecting tubing used for inflation and pressure measurement. The right lung was
removed because it was used for wet/dry weight measurements that would have been
affected by the lung wash procedure that followed the ex vivo compliance measures.

The left lung was suspended in air and inflated three times to 30 cm H₂O
pressure before pressure-volume measurements were started. Leaks were detected by
clamping the lung inflation tubing at 30 cm H₂O pressure and watching on a Gould
recorder to make sure an asymptotic airway pressure tracing was quickly achieved
(pressure change ≤ 1 cm H₂O over 10 s). Leaks in the tissue were repaired by
attaching small tissue patches with ethyl-cyanoacrylate adhesive. Inspiratory and
expiratory pressure-volume recordings were generated using increments of 25-100
97 mL of air. At the end of ex vivo compliance measures, the inflation tubing was clamped to trap zero-pressure volume in the lungs. This remaining gas volume was estimated by the water displacement method. The left lung weight minus the weight of attached clamps that were submerged with the lung was divided by a value for lung tissue density (1.065) and then subtracted from the displaced water volume.\textsuperscript{24}

The change in volume over the pressure range of 0-30 cm $H_2O$ was obtained by adding the previously recorded volume increments to the zero-pressure volume. The data were further standardized for comparison by calculating the volume at each 2 cm $H_2O$ pressure increment between 0-30 cm $H_2O$, using linear interpolation. The resulting data points were indexed to the dry weight of the right lung because the normal functional residual capacity (FRC) for each subject was not available. The dry weight was used because the wet weight of the lung-injured groups would be affected by the additional lung edema weight. Dry lung weight had lower variability than either whole body weight or body surface area and was therefore used as an indexing variable.

Wet and dry weights were measured and lung water directly determined to verify double-dilution lung water measures. Only the right lung was weighed because the maximal inflation/deflation and consequent lung wash done on the left lung would have altered lung fluid weight. After the animal was euthanized, the thoracic cavity was opened and the right main bronchus was clamped and cut immediately distal to the carina. Wet weight of the right lung was measured on an a triple-beam balance scale, and the lungs were then placed in an oven for at least 48 h
at 100°C. A dry weight was recorded after no change in consecutive weights was observed. The percent change in total lung water (% H\textsubscript{2}O) for PMA-only to PMA+furosemide treated dogs was used to assess the degree of edema formation. Comparisons were performed with the expectation that changes detected by both direct and indirect lung water measurement techniques would prove to be similar.

The Student's t-test for unpaired data, Pearson's correlation coefficient, analysis of variance (ANOVA), and ANOVA with repeated measures were performed with Systat 5.02 for Windows (Systat, Inc., Evanston, IL). Analysis of covariance (ANCOVA) with repeated measures was done using BMDP Software Version 6.0 (BMDP Statistical Software, Inc., Los Angeles, CA). The more complex BMDP application was used because it accommodates covariates that change across trials (over time in this case). The ANCOVA adjustment procedure is equivalent to artificially assuming a common covariate distribution based on the combined sample over all groups.\textsuperscript{25} Not only are the means assumed to be equal, but the entire distribution of the covariates in the combined sample is assumed to be the same as the distribution of the covariates in each group. The pH of blood is known to affect pulmonary vascular resistance, which in turn impacts indicators of V/Q such as shunt fraction and AaDO\textsubscript{2}.\textsuperscript{15} Arterial pH was evaluated as a covariate because, although vigorous efforts were made to maintain it within a normal range (7.35 to 7.45) using intravenous sodium bicarbonate and ventilator manipulation, it dropped below that range toward the end of many experiments. ANCOVA can take into account the possible extraneous variable "arterial pH" by treating it as a covariate and by
extracting its effect from the data so that final results can be compared and judged more fairly.

Criteria are available to determine if a variable should be treated as a covariate; however, such criteria are only needed if there are many variables under consideration or if it would be costly or difficult to run the additional ANCOVA test on a very large data set. If there is any doubt as to whether a variable should be treated as a covariate, the safest approach is to go ahead and perform an ANCOVA test. Since pH was the only variable under consideration (due to its aforementioned potential effects on hemodynamics), ANOVA and ANCOVA repeated measures tests were performed on the dependent variables that might be influenced by variances in pH over time. The ideal outcome would be for both tests to reveal similar p-values. Such agreement would remove concern that significant outcomes may be due in part to changes in blood pH rather than to drug treatment.

RESULTS

An overview of the changes in several important indicators that occurred over the course of the experiments is given in Figure 15. Peak airway pressures and quasi-static respiratory system compliance (measured on the mechanical ventilator) worsened after injury in both groups. The trends were similar even after treatment with furosemide and although the PMA+furosemide group did exhibit poorer values, the differences as measured by ANOVA with repeated measures were not significant for peak airway pressure (p = 0.348) or compliance on the ventilator (p = 0.113).
The group receiving furosemide treatment clearly showed much greater deterioration in arterial oxygen tensions compared to the injury-only group (p = 0.006).

The final cumulative urine outputs for PMA-only and PMA+furosemide were close, with respective group means of 39.0±28.7 ml/kg and 44.1±30.2 ml/kg (p=0.75). Only one of the diuretic animals concluded the experiment with a negative net body fluid volume. Clearly, furosemide did not produce significant diuresis in this PMA.
Figure 15. Compliance and arterial oxygen tension group means (S.D. error bars).
model of acute lung injury. A final "net fluid volume" was computed for both groups by totaling all fluids added or removed from each animal during the course of the experiments. Net body fluid volume comparisons proved to be similar to the urine output values in that there was little difference between groups. The PMA group mean was 53.0±35.3 mL/kg, and the PMA+furosemide group mean was 56.7±41.1 mL/kg (p=0.86). Group distributions for final fluid balance were also similar as displayed in a composite histogram showing both groups (figure 16). The pattern seen in Figure 16 shows that despite the administration of a diuretic and conservative fluid support, the furosemide group had nearly the same frequency of animals in each grouping of net fluid gain across the range of net fluid values as did the untreated group. The frequency distributions for each group were nearly the same, differing by only one subject on the low end.

Correlations were done to test the hypothesis that net body fluid balance and gas exchange efficiency were correlated in this model of acute lung injury (Figure 17). The resulting Pearson r-values (posted on the associated scatter plot) do not indicate a significant relationship of net fluid balance to either AaDO₂ nor Qs/Qt.

Although total body fluid volumes did not change as a result of furosemide compared to control, there was a possibility that fluid had shifted differently between the various body fluid compartments for each group. For example, it is possible that furosemide could have specific effects on fluid movement into the peritoneal compartment. To study this possibility, furosemide-induced changes in intravascular volume and concentrations of solids and solute were indirectly estimated directly by
Figure 16. Distribution of Final Net Body Fluid Results.
hematocrits and by plasma protein concentrations. During the treatment period (between hours 3 through 7), hematocrit data analyzed by ANOVA with repeated measures gave a main effect for group of $p = 0.054$ (the furosemide-treated animals having more concentrated blood), with a time effect of $p = 0.825$ and an interaction effect (group x time) of $p = 0.004$ (Figure 18). Mean hematocrits for the two groups tracked very closely until the start of the treatment period, when the PMA+furosemide group diverged from the PMA group and maintained an increased
Figure 18. Hematocrit, plasma protein, and cardiac index trends (S.D. error bars).
red cell concentration for the duration of the experiment (figure 18). The plasma protein concentration analysis revealed no significant effects for group ($p = 0.179$), time, or interaction effects. Therefore, though the blood was becoming more concentrated, as evidenced by the increase in hematocrit, plasma protein was not increasing, this is consistent with an increased capillary permeability to protein due to PMA.

Dependent variables measured hourly were analyzed using ANOVA with repeated measures (Table 4). The gas exchange variables, shunt fraction ($Q_s/Q_t$) and alveolar-arterial oxygen partial pressure difference ($A_aDO_2$), were also analyzed by ANCOVA with repeated measures (arterial pH as covariate) to correct for any effects of pH variability. The $Q_s/Q_t$ and $A_aDO_2$ followed similar patterns, as seen in Figure 19. Furosemide treatment produced deleterious trends for both variables in comparison to the PMA injury-only group.

Pulmonary vascular resistance (PVR) was measured hourly over the course of each experiment and was found to have significant main effects for both group and time when analyzed by ANOVA with repeated measures (Table 4). A plot of PVR changes over time reveal an interesting pattern (Figure 20). The two groups had similar mean PVR values at the beginning of the measurement period (hour one). After the PMA injury was initiated, the PVR trends quickly diverged reaching the greatest disparity at hour three (post-injury/pre-furosemide treatment). During the treatment period the PMA+furosemide group PVR trend essentially plateaued and maintained higher values for the rest of the experiment. Shunt fraction, and to a
Table 4. Repeated measures ANOVA for dependent variables measured hourly.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group Effect</th>
<th>Time Effect</th>
<th>Interaction Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>AaDO2</td>
<td>p = 0.006</td>
<td>p = 0.000</td>
<td>p = 0.000</td>
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<td></td>
<td>p = 0.014†</td>
<td>p = 0.000†</td>
<td>p = 0.001†</td>
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<tr>
<td>Qs/Qt</td>
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<td>p = 0.000</td>
<td>p = 0.085</td>
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<td></td>
<td>p = 0.017†</td>
<td>p = 0.000†</td>
<td>p = 0.102†</td>
</tr>
<tr>
<td>Pulmonary Vascular Resistance</td>
<td>p = 0.009</td>
<td>p = 0.011</td>
<td>p = 0.155</td>
</tr>
<tr>
<td></td>
<td>p = 0.021†</td>
<td>p = 0.041†</td>
<td>p = 0.217†</td>
</tr>
<tr>
<td>Cardiac Index</td>
<td>p = 0.889</td>
<td>p = 0.103</td>
<td>p = 0.543</td>
</tr>
<tr>
<td></td>
<td>p = 0.697†</td>
<td>p = 0.360†</td>
<td>p = 0.375†</td>
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<tr>
<td>Oxygen Delivery</td>
<td>p = 0.763</td>
<td>p = 0.347</td>
<td>p = 0.923</td>
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<tr>
<td>Oxygen Extraction Ratio</td>
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<tr>
<td>System Compliance on Ventilator</td>
<td>p = 0.113</td>
<td>p = 0.217</td>
<td>p = 0.878</td>
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<tr>
<td>Peak Airway Pressures</td>
<td>p = 0.348</td>
<td>p = 0.049</td>
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<td>PCWP</td>
<td>p = 0.577</td>
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<td>p = 0.720</td>
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<td>p = 0.054</td>
<td>p = 0.869</td>
</tr>
<tr>
<td>PAP</td>
<td>p = 0.024</td>
<td>p = 0.170</td>
<td>p = 0.081</td>
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</tbody>
</table>

† Denotes corresponding ANCOVA repeated measures with arterial pH as covariate.
Figure 19. Changes in shunt fraction and Alveolar-arterial oxygen partial pressure difference over time (S.D. error bars).
Figure 20. Changes in pulmonary vascular resistance over time (S.D. error bars).

lesser degree AaDO₂, did exactly the opposite at hour three, showing sudden
decreases in their trends. Cardiac index decreased over time but both groups did so
almost equally (Figure 18).

The trends for the two groups' mean values were similar for pulmonary
capillary wedge pressure (PCWP) and central venous pressure (CVP), as seen in
Figure 21. ANOVA results in Table 5 show no significant difference between
groups for either PCWP or CVP. Mean pulmonary artery pressure (PAP) did not
decrease as predicted (Fig. 21). The main effect for group was significant, but the
difference between groups was due to a sudden rise that occurred mostly at hour
three, before the furosemide was given. After furosemide, the PAP did decrease and
Figure 21. Changes in PCWP, CVP, and mean PAP over time (S.D. error bars).
essentially paralleled the PMA-only group but always remained at a higher level. T-tests for the final two measurements revealed no significant differences (p-values for hours 5, 6, and 7 were: 0.074, 0.154, 0.206, respectively).

Ex-vivo pressure/volume measures of the left lungs were compared for the normal (n = 8), PMA (n = 6), and PMA+furosemide (n = 7) groups using a one-way ANOVA with Tukey's post-hoc test. There was no significant difference (p = 0.076) for expiratory lung compliance measured at 45-55% of TLC indexed to dry lung weight. Data for this particular variable was available for only five of the seven PMA+furosemide animals; two animals had zero pressure lung volumes that occurred above 45% of TLC and therefore did not allow for calculation of a compliance at the near-tidal breathing range. The two lung volumes measured, minimum volume trapped in the lungs at zero airway pressure (Vmin) and total lung capacity (TLC)--the volume contained in the lung at an inflation pressure of 30 cm H2O, were obtained for all subjects in the three groups. One-way ANOVA testing revealed no significant differences between groups for Vmin (p = 0.803) but a highly significant difference for TLC (p = 0.008). Further post hoc analysis using Tukey's Honestly Significant Difference test revealed that PMA and PMA+furosemide groups were similarly different from the normal group (p = 0.013 for normal x PMA and p = 0.019 for normal x PMA+furosemide). PMA-injury did diminish functional lung tissue as indicated by TLC results, but furosemide diminished any effect on ventilatory variables that might provide an explanation for V/Q changes.
Lung water measures by double-dilution technique (DDLW) were started an hour and fifteen minutes into the experiment (pre-injury baseline). Subsequent measures were taken at: 2.9 hours (post-injury/pre-treatment), 4.2 hours (post-treatment), 5.2 hours, and 7 hours (final hour) into the experiment, that showed an increasing trend after injury (Figure 22). Unlike most of the other dependent variables, hourly measurements were not performed due to the high cost of the green dye tracer and the concern for keeping fluid input at a minimum. A repeated measures ANOVA revealed no significant main effect for group (p=0.741). However, the groups did exhibit significant increases in lung water over time as indicated by main effect for time (p=0.003). A comparison of the final hour DDLW measurement t-test result to that of wet/dry lung weight measures was performed to confirm DDLW measures. There was no significant difference between groups for DDLW (p = 0.625) or wet/dry weight ratios as measured by percent change in water (p = 0.970) or the difference in grams of water divided by dry right lung weight (0.344).

The peculiar development of edema in the gut region seen in many of the dogs is worthy of mention. The abdominal circumferences of some animals were measured and were found to increase by as much as 11 cm, while other animals did not increase at all. This was true of both groups.
Figure 22. Lung water changes over time as measured by DDLW method.
DISCUSSION

The primary finding of this study was that in contrast to my hypothesis, furosemide administration had no significant effect on the accumulation of water in the lungs during the early stage of PMA-injury. It did not significantly improve lung compliance or mechanics of breathing on the ventilator. In addition, furosemide had a deleterious effect on gas exchange, significantly elevating AaDO₂ and shunt fraction. These findings must be interpreted with the understanding that in this model, PMA administration resulted in nearly complete loss of effective kidney function. This means that these deleterious effects may represent extra-renal influences of furosemide, particularly on the pulmonary circulation. The effects of furosemide may have been different had the kidneys been able to respond normally to furosemide treatment, reducing body water. The extra-renal effects of furosemide on gas exchange may be associated with the significant increases in pulmonary vascular resistance and hematocrit that were observed in the furosemide-treated animals.

The interpretation of these results is bounded by several limitations. First, the PMA lung injury was modest with regard to AaDO₂ and shunt fraction values. The considerable variability among individual subjects as to the degree of injury, was heightened by a small relatively sample size. Although there are many potential sources of variability, one source is the various degrees of gut edema seen in different animals. It is possible that in dogs, the gut may often bear the brunt of a PMA-triggered neutrophil injury rather than the lungs, hence the overall modest lung
injury observed. Third, while the measurement period was longer than many studies using a PMA model, it may have been too short to detect later important treatment effects. Finally, the dose of furosemide used in this study was relatively large, and although it has been used by other investigators, it is much higher than the typical clinical doses used on humans and in some animal experiments.

It is clear that for this study any effect of furosemide on lung function at this dose cannot be attributed to its diuretic effect. Furosemide did not attenuate fluid accumulation in the lungs or in the rest of the body, nor did it affect PCWP. Given the short time course over which injury occurred, changes in lung mechanics would be expected to result from the presence of edema. Fluid accumulation in the lungs was nearly the same for both groups, and this resulted in nearly the same significant decreases in TLC for both when compared to normals. Similarly, although cardiac index was nearly identical over time for both groups, the PVR was increased over the treatment period for the furosemide group. The resistance increase seen in the pulmonary circulation is opposite of the decrease in systemic vascular resistance typically produced by furosemide. Stimulation of the kidneys through changes in atrial natriuretic factor was unlikely since PCWP and CVP pressures were unremarkable.

In PMA+furosemide dogs, AaDO₂ and shunt fraction increased significantly compared to the PMA-only group. This diminution of gas exchange was opposite of expectations. Previous studies using furosemide to treat acute lung injury typically found improvement in these gas exchange variables. One of the studies included a
group of four dogs that received furosemide but did not experience diuresis, and the shunt fraction still decreased to a statistically significant level, although it was small and of short duration.\textsuperscript{1} The other study reported a group of six nephrectomized dogs that displayed similar significant improvements in shunt fraction after furosemide as those achieved by a their counterparts that had functional kidneys.\textsuperscript{11}

The various measures of lung impedance and volumes revealed no significant differences to explain the observed gas exchange anomalies. Furosemide has been shown to provide a protective effect against bronchoconstriction caused by allergen, exercise, nebulized distilled water, and sodium metabisulfite in asthmatic patients.\textsuperscript{27-29} The present study used two surpranormal doses of furosemide an hour apart, a dose clearly in the upper range of those used in related studies.\textsuperscript{7,30-31} However, even with this high dose, no significant changes in ventilatory variables were observed that might explain the gas exchange abnormalities.

The effects of furosemide on small pulmonary blood vessels are not well documented. McGowan and colleagues reported that furosemide produced a concentration-dependent relaxation in isolated pulmonary venous smooth muscle rings but had no effect on arterial smooth muscle.\textsuperscript{32} A representative model of blood flow in the lungs is given in Figure 23, that allows for conceptualizing several hypotheses. A hypothesis for such a venodilation at points C and D would predict pulmonary congestion, including an increase in lung water. There were no significant differences in lung water or PCWP pressures for the furosemide group. In response to edema injury, the protective hypoxic vasoconstriction reflex would
increase constriction at point B. The hypothesis that furosemide produces a
generalized vasodilation in the lungs would be consistent with the increases in shunt
fraction and AaDO₂ observed in this study. The furosemide would be expected to
reduce constriction at points B-D, allowing a greater portion of total blood flow to
pass by poorly ventilated alveoli. However with less vascular constriction, a drop in
the PVR would also be expected, and this was not observed.

![Figure 23. Schematic model of blood flow in the lungs.](image-url)
The available data do not appear to support a hypothesis that furosemide produced gas exchange embarrassment by altering the vascular tone by the aforementioned mechanisms. As previously noted, the hypothesis that furosemide worsens gas exchange by a general vasodilator effect that overrides the protective hypoxic vasoconstriction response of the lung is consistent with the data, except for the increased PVR in the furosemide group. The increase in venous hematocrit observed after furosemide treatment provides a congruent explanation. Changes in blood viscosity have dramatic effects on the small pulmonary arterial-venous pressure gradient. Hematocrits greater than 40 percent produce a relative resistance change of 4 percent per hematocrit unit.\textsuperscript{15} During the treatment period in the present study, hematocrits in furosemide animals ranged from 43% to almost 48%, therefore, a furosemide-induced abatement of the protective hypoxic arterial constriction reflex in combination with hemoconcentration by some mechanism other than diuresis may explain the rise in pulmonary vascular resistance in the face of perhaps local vasodilation and the decrease in gas exchange.

It is also possible that the dose of furosemide used in this study may have been sufficient to induce reflex responses in the pulmonary vasculature that are not commonly seen. The PAP trends show an initial disparity at hour three, immediately before treatment, but this might have been due to the fluid restrictive protocol for the furosemide group. The PAP values for each group quickly reach plateaus about 3 mm Hg pressure apart for the duration of the treatment period. This may be related to finding that at the end of the experiment, each group had very similar net body
fluid values, despite the withholding of a volume-expanding normal saline bolus available to the PMA-only animals. Regional changes in blood flow may have occurred which were not distinguished by the global dependent variables available.

In summary, the results suggest that furosemide has effects on both the pulmonary circulation and whole body fluid distribution in this PMA-model of ARDS, and these effects have a negative impact on gas exchange in the lung. The exact mechanism for these effects of furosemide is difficult to determine at this time and merits continued investigation.

The results suggest that other factors, including effective kidney function, may need to be present for furosemide to have clinical utility in ARDS patients. They also indicate that in some clinical situations, particularly in the absence of effective renal function, high furosemide dosing should be done with caution. Giving more than two serial doses of furosemide to ARDS patients without noting any diuresis may aggravate preexisting V/Q problems and put the patient at greater risk. Further work needs to be done to identify relevant clinical factors that make furosemide utilization the most advantageous to patients with ARDS.
REFERENCES


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CHAPTER IV

The Effects of Exogenous Surfactant and PEEP on Gas Exchange and Lung Mechanics in a PMA Model of Lung Injury

Introduction

Respiratory distress syndrome (RDS) is a condition that affects and is affected by the complex lung substance called "surfactant." When investigators first began describing a particular pattern of lung injury in adults that included atelectasis, hemorrhage, pulmonary edema, and hyaline membrane formation, it was thought to closely resemble findings in premature infants with advanced RDS so the name adult-RDS or "ARDS" was applied. The main predisposing factor that leads to infant RDS is that premature lungs are unable to produce surfactant, the detergent-like substance that is critical for normal lung inflation, stability, and certain metabolic processes that occur in the lung. Infants with RDS typically have a dramatically positive response to surfactant replacement therapy, and this is probably because the lungs are essentially normal except for the lack of surfactant. The ARDS scenario is much more complicated in that pre-existing lung surfactant is
altered by dilution and deactivation, as well as diminished synthesis. Many other simultaneous injury mechanisms further cloud the picture as to what is "cause versus effect" and to what degree each mechanism contributes to the overall injury.

Fundamentally, surfactant stabilizes the lung by lowering alveolar surface tension, particularly at low resting volumes, which would otherwise promote collapse of lung units. Lung alveoli that collapse as a result of edema and surfactant inactivation are difficult to re-open, requiring high transpulmonary pressures to do so. The thin surfactant film that coats the alveolar surface acts as an interface that allows for efficient air-breathing. It is a liquid composed mostly of two fractions, about 85-90% saturated phosphatidylcholine (dipalmitoylphosphatidylcholine or DPPC) and at least four surfactant proteins (SP) A, B, C, and D. While the DPPC component alone is able to reduce alveolar surface tension, the effect is greatly enhanced when combined with the proteins, SP-B in particular.

Abnormal surfactant function can affect the lungs in several ways, including non-mechanical influences such as altering immune cell activity. Impaired surfactant leads to alveolar instability which may result in unequal alveolar ventilation and atelectasis. Ventilation-perfusion imbalance and intrapulmonary shunting are typical consequences of progressive atelectasis. Pulmonary compliance is also reduced when surfactant loses its ability to counter surface tension and causes an increased work of breathing for the patient. Mechanical ventilation of such patients requires higher airway pressures in order to maintain gas exchange, putting the patient at risk for barotrauma (high inflation pressures sufficient to
rupture alveoli) and compromising cardiac output. These factors tend to aggravate the permeability edema already present in the ARDS lung. By forcing a progressively smaller portion of normally functioning lung to bear the load of ventilation, the injury of normal parenchyma is hastened.

There is consensus that the typical findings of ARDS: hypoxemia due to ventilation-perfusion mismatch and shunting, decreased lung compliance, increased work-of-breathing, and lung infiltrates are, in part, due to surfactant abnormalities. Since infant RDS and ARDS have several similar findings and surfactant therapy is known to ameliorate infant RDS symptoms, it is reasonable to suggest that surfactant therapy may effectively treat ARDS symptoms. It is unclear however, to what degree the additional injury associated with ARDS impact the efficacy of exogenous surfactant.

Human and animal studies have provided data that functional changes in surfactant occur in ARDS and similar lung injuries. Holm and colleagues demonstrated that abnormal surface forces directly contribute to abnormal lung mechanics by measuring air and saline pressure-volume curves in a rabbit lung injury model. Gregory and associates recovered surfactant from the lungs of ARDS patients and found a four-fold increase in surface tension compared to normals. Other studies show that not only is surfactant abnormal in the early stages of ARDS, it also correlates with the syndrome severity.

Given that surface tension is dependent upon surfactant and both are found to be abnormally altered in ARDS patients, it would be desirable to measure surface
tension as an indicator of surfactant replacement efficacy. Direct surface tension measurements are difficult to perform and destructive to tissue so other indirect measures are often used. Static recoil pressure is the summation of surface tension at the gas-liquid interface of the lung and tissue elastance (contingent mainly on elastin and collagen fibers) that corresponds to a specific lung volume.²¹ At high lung volumes tissue elastance is the primary component of total recoil pressure but surface tension becomes more important at lower lung volumes.²² Another indicator of surface tension is the "looping" or hysteresis observed on pressure-volume curves plotted from inspiration through expiration. Clements et al., noted hysteresis is accentuated by processes that deplete or inactivate surfactant.²³

ARDS is thought to alter endogenous surfactant primarily by dilution, inactivation, and reducing the rate of production.²³ Lung edema that enters the alveoli increases both the distance for gas diffusion and dilutes or washes out the surfactant film lining the alveoli. Fibrin, cellular debris, and toxic substances from the immune system's assault on the lung accompanies the edema fluid and denatures the surfactant components as well as disrupting the synthesis pathways. This disruption causes lower rates of surfactant production which cause lower output and generation of dysfunctional forms.²⁴-²⁵ These substances also affect surfactant replacement products. Artificial surfactant preparations in particular appear to be more sensitive to deactivation by fibrin and toxic metabolites than natural surfactant preparations.²⁶-²⁷
Surfactant replacement therapy is expensive, with a single adult treatment costing in excess of two thousand dollars. It is important to determine if surfactant administration elicits improvement in any of the previously mentioned clinical findings of ARDS. The rationale for surfactant therapy is straightforward; since acute lung injuries like ARDS are known to be associated with dysfunctional surfactant and abnormal surfactant contributes to the previously mentioned problems with lung mechanics and gas exchange, then replacing damaged surfactant with normal surfactant should reduce related symptoms. Can natural surfactant measurably improve lung compliance, diminish hysteresis (an indicator of surface tension), reclaim collapsed alveoli, and/or diminish V/Q mismatching, thereby improving gas exchange? If surfactant is shown to improve the above variables that influence pulmonary edema then a measurable change in lung water should be detected.

Finally, because of the clinical criteria that must be met before giving surfactant therapy to ARDS patients, positive end-expiratory pressure (PEEP) is always present at the time of administration. PEEP has long been used to increase arterial oxygen tension in acute lung injury but the mechanism of action is not clear. A dog study by Frank, et al., found lung water did not change as oxygen tensions increased in response to PEEP. Several studies suggest that PEEP improves gas exchange by preventing the closure of at least some alveoli that would otherwise collapse as a result of falling below some critical closing pressure in injured lung areas. The PEEP may function largely as a substitute force to counter surface
tension in place of surfactant that has become dysfunctional. With these things in mind, is PEEP necessary in part or without exception for the effectiveness of surfactant replacement in ARDS? Does it prime injured lung portions to better receive and distribute surfactant? If so, it may indicate the need for investigating an "optimal" PEEP for surfactant delivery and overall effectiveness for the therapy.

The purpose of this study was to test the hypothesis that surfactant administration would improve pulmonary gas exchange and lung mechanics in an animal model of a permeability edema injury. To pursue this, a phorbol myristate (PMA) model of lung injury was used to produce damage.

METHODS

Three groups of mongrel dogs (PMA-injury only n=7, PMA+Surfactant n=7, PMA+Surfactant+PEEP n=4) were anesthetized and mechanically ventilated for a seven hour measurement period (see PMA model chapter for procedural details). Animals received a tidal volume of 15 cc/kg at a minimum frequency of 10 breaths per minute with the $F_1O_2$ titrated to maintain a minimum arterial hemoglobin oxygen saturation of 90 percent. The two treatment groups followed the same protocol described for the PMA model of sodium bicarbonate to maintain pH and saline and dopamine to maintain a minimum mean arterial blood pressure of 70 mm Hg. A fourth group that did not receive PMA injury, surfactant, or PEEP served as the "normal" group (n=8). The PMA+surfactant and PMA+surfactant+PEEP groups received four, 25 mg/kg doses of purified bovine surfactant (Survanta®) in rapid
succession by tracheal installation 2.5 hours after PMA administration. The installation catheter was measured and marked to ensure proper positioning of the catheter tip just beyond the end of the artificial airway during surfactant delivery. Between each of the four doses the animal was alternately turned to the right and left sides to promote bilateral distribution and two sigh breaths (1.5 times the tidal volume) followed each dose.

In the PMA+surfactant+PEEP group, 10 cm H2O of PEEP was initiated 1.5 hours after PMA, one hour before surfactant. The PEEP was not started earlier because previous subjects in pilot trials would enter a hemodynamic crisis if additional positive end-expiratory pressure was present during the initial injury phase. PEEP was set according to the pressure measured at the patient y-connector of the ventilator circuit. All subjects were carefully monitored to insure adequate anesthesia but they were not paralyzed. Several manual breaths were given in rapid succession to diminish active expiratory efforts by the animals prior to plateau pressure measurements.

Ten minutes prior to each hourly blood gas sample, the animals were placed on 100 percent inspired oxygen. Arterial and mixed-venous blood gas samples were aspirated into heparinized plastic syringes and immediately placed on ice. The blood gas samples were quickly taken to a blood gas laboratory and analyzed for pH, PO2, and PCO2 using an Instrumentation Laboratories blood gas analyzer (Model 1312, Instrumentation Laboratories Inc., Andover, MA). Daily quality control was performed on the machine using acidosis, normal, and alkalosis standardized control
samples. Hematocrit measurements were performed by centrifugation of arterial and venous micropipette samples. Plasma protein values were obtained reading the supernate of centrifuged venous samples with a refractometer.

Sodium bicarbonate (8.4% solution) was administered according to blood gas results. Arterial pH values of 7.30 and lower associated with a low bicarbonate value were treated by substituting 25-50 ml doses of bicarbonate solution for normal saline in the maintenance infusion (10 ml/kg-hr) until the pH returned to a minimum value of 7.35. Pulse oximetry provided continuous monitoring of heart rate and arterial hemoglobin saturation to allow for rapid mechanical ventilation adjustments. A catheter was placed in the urethra to monitor urine output. Heating pads were used to maintain a core temperature close to 38 degrees centigrade.

This study modeled PMA preparation and dosages after the methodology in two studies by Dorinsky, et al.32-33 The chemical agent PMA (Calbiochem Inc., LaJolla, CA) was dissolved in dimethylsulfoxide (DMSO) at a concentration of 5 mg/ml. The PMA was then stored in 200 microliter aliquots at -80 degrees C (200 microliter aliquot = 1000 milligrams PMA). Immediately prior to use, a 200 microliter aliquot of PMA was diluted in 3.0 ml of normal saline and 1.8 ml of DMSO to yield a final concentration of 200 µg/ml.

At the conclusion of the experiment (after hour seven) the animals were euthenized with intravenous magnesium chloride to induce cardiac asystole. The heart and lungs were quickly removed through a sternotomy after clamping the trachea. After rinsing the lungs with normal saline and separating them from the
heart, the trachea and right mainstem bronchus were double-clamped to prevent fluid from entering or exiting the lungs as the airways were cut. The trachea was trimmed to three inches above the branching of the mainstem bronchi and the right bronchus was cut distal to the clamp, approximately one centimeter from the carina. The left lung (with trachea) was secured to a ribbed, plastic connector on the end of tygon tubing with one quarter-inch cotton suture for inflation and pressure measurement. The right lung was removed because it was used for wet/dry weight measurements that would have been affected by the lung wash procedure that followed the ex vivo compliance measures.

The lungs were suspended in air and inflated three times to 30 cm H$_2$O pressure before pressure-volume measurements were started. These pressures were maintained for 5-10 minutes to ensure the opening of closed or atelectatic regions of the lung. Leaks were tested for by clamping the lung inflation tubing at 30 cm H$_2$O pressure and watching the Gould recorder to make sure an asymptotic airway pressure tracing was quickly achieved (pressure change $\leq$ one cm H$_2$O over ten seconds). Leaks were repaired by attaching small tissue patches with ethyl-cyanoacrylate adhesive. Inspiratory and expiratory pressure-volume recordings were generated using increments of 25-100 ml of air. At the end of ex vivo compliance measures, the inflation tubing was clamped to trap zero-pressure volume in the lungs. This remaining gas volume was estimated by water displacement method (as per Archimedes). The left lung weight minus the weight of attached clamps that were submerged with the lung was divided by the value for lung tissue density (1.065) and
then subtracted from the displaced water volume.

The change in volume over the pressure range of 0-30 cm H₂O was obtained by adding the previously recorded volume increments to the zero-pressure volume. The data was further standardized for comparison by calculating the volume at each two cm H₂O pressure increment between 0-30 using linear interpolation. The resulting data points were indexed to the dry weight of the right lung because the normal FRC for each subject was not available. The dry weight was used instead because the wet weight of the lung-injured groups would be affected by the additional lung edema weight. This indexing variable was found to have the least amount of variability compared to whole body weight or body surface area. Each right lung was placed in an oven set at 100 degrees centigrade for two days, when a consecutive weighings showed no change in weight.

RESULTS

Hourly measures of arterial oxygen tension (PaO₂), peak airway pressures, and static compliance measured on the ventilator showed a worsening trend over the course of the experiment (Figure 24). Of those three, the only variable found to have a significant main effect for group during the treatment period (hours 3-7) was PaO₂ (group effect p = 0.000, time effect = 0.000, interaction effect = 0.000). The co-application of PEEP with surfactant resulted in much higher PaO₂ levels immediately after surfactant installation (Figure 25). The main effect for group during the first 25 minutes after installation was highly significant (p = 0.003) as
well as for time (p = 0.000) and interaction effects (p = 0.000) for PMA+surfactant and PMA+surfactant+PEEP. The PMA injury given to each group was expected to cause a progressive deterioration in dependent variables and the treatments were predicted to slow or halt the pace but not necessarily reverse it. The PaO$_2$ values during the treatment period decreased for both treatment groups with surfactant+PEEP having the most decreased trend. One-way analysis of variance (ANOVA) for each hour during the treatment period (hours 3-7) yielded significant differences among the group PaO$_2$ means at hour 5 (all 3 groups different, p<0.01), hour 6 (all groups different, p<0.022), and hour 7 (PMA+surfactant different from PMA-only and PMA+surfactant+PEEP, p<0.000).
Figure 24. Changes in $\text{PaO}_2$ and compliance on the ventilator during across the groups (error bars in S.D.).
Figure 25. Arterial oxygen partial pressure changes the first twenty-five minutes after surfactant administration (error bars in S.D.).
Composite inspiratory & expiratory pressure-volume curves for each group obtained from excised left lungs are displayed in Figure 26. Clearly all groups that received PMA, regardless of whether treatment was received, were visibly deviated from the normal group tracing. The measurement of compliance was first addressed by assessing the differences between specific points and ranges on the expiratory curves. The volume trapped in the left lung at zero airway pressure or the minimum volume ($V_{\text{MIN}}$) was indexed to both the percent TLC and dry right lung weight (Table 5). The TLC or maximal lung volume was designated as the volume in the lungs at 30 cm H$_2$O airway pressure.

To test the hypothesis that surfactant would significantly increase lung compliance in PMA-damaged lungs, expiratory compliance ($C_e$) was measured in three ways, first by taking the slope of the pressure-volume curve between 45%-55% of TLC (Table 5). The second method involved dividing the volume difference occurring between the pressure points, 5 and 8 cm H$_2$O, by the pressure change between those same points. The final method calculated a specific compliance by dividing the values obtained with the second method by the associated volumes at 5 cm H$_2$O (ie., specific compliance). The first compliance measurement (between 45-55% of TLC) was chosen to examine the expiratory recoil at the volume region that would approximate FRC.$^{34}$ Measurements two and three were based on the finding that tidal breathing generally occurs between ~3 and 8 cm H$_2$O of transpulmonary pressure$^{35}$ and because slope of the curve is typically linear between 5-8 cm H$_2$O.
Figure 26. Composite inspiratory and expiratory compliance curves (see appendix 4 for standard deviations).
Table 5. Descriptive statistics for left lung $V_{\text{MIN}}$, TLC, and compliance.

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>$V_{\text{MIN}}$</th>
<th>TLC</th>
<th>$C_L$ (1)</th>
<th>$C_L$ (2)</th>
<th>$C_L$ (3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>6</td>
<td>10.9±6.4</td>
<td>52.4±20.8</td>
<td>4.6±2.3</td>
<td>50.0±19.9</td>
<td>0.070±0.019</td>
</tr>
<tr>
<td>P</td>
<td>6</td>
<td>8.8±2.3</td>
<td>25.4±9.2</td>
<td>2.8±0.9</td>
<td>57.6±28.3</td>
<td>0.085±0.034</td>
</tr>
<tr>
<td>S</td>
<td>7</td>
<td>4.9±2.5</td>
<td>19.1±8.1</td>
<td>1.2±0.6</td>
<td>33.8±15.4</td>
<td>0.081±0.016</td>
</tr>
<tr>
<td>SP</td>
<td>4</td>
<td>5.1±2.1</td>
<td>31.5±3.0</td>
<td>2.3±0.5</td>
<td>47.3±11.2</td>
<td>0.092±0.017</td>
</tr>
</tbody>
</table>

N = Normal, P = PMA, S = PMA+Surfactant, SP = PMA+Surfactant+PEEP; $C_L$ (1) = slope of pressure-volume curve between 45%-55% of TLC in units of mL/g/cm H$_2$O; $C_L$ (2) = slope of the pressure-volume curve between 5-8 cm H$_2$O; $C_L$ (3) = $C_L$ (2)/volume at 5 cm H$_2$O. Results listed as mean±S.D.

A One-way ANOVA was performed on the group means for $V_{\text{MIN}}$, TLC, and $C_L$ (1-3), to test the hypotheses that exogenous surfactant increases functional lung volumes and decreases elastic recoil in PMA-injured lungs. There was a significant difference among the four groups for $V_{\text{MIN}}$ indexed to dry right lung weight and a Tukey HSD test attributed it to the difference between the normal and PMA+surfactant groups (Table 6). A highly significant difference was found for the TLC means and further post hoc analyses revealed the disparity was between the normal group and the PMA as well as the PMA+surfactant groups. Of the three compliance measures, only method one ($C_L$ [1]), exhibited any difference between groups. The normal group mean was significantly different from PMA+Surfactant...
but not the other two groups.
Table 6. ANOVA results for groups N, P, S, and SP.

<table>
<thead>
<tr>
<th></th>
<th>V_{MIN} p = 0.041</th>
<th>TLC p = 0.001</th>
<th>C_L(1) p = 0.002</th>
<th>C_L(2) p = 0.227</th>
<th>C_L(3) p = 0.384</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>* Post Hoc Analysis below</td>
<td>* Post Hoc Analysis below</td>
<td>* Post Hoc Analysis below</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(V_{MIN} = \text{minimum volume indexed to dry right lung weight}\)

*Tukey HSD Matrix of Pairwise Comparison Probabilities: Test of TLC.

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>P</th>
<th>S</th>
<th>SP</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>0.007</td>
<td>1.000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S</td>
<td>0.001</td>
<td>0.841</td>
<td>1.000</td>
<td></td>
</tr>
<tr>
<td>SP</td>
<td>0.086</td>
<td>0.898</td>
<td>0.484</td>
<td>1.000</td>
</tr>
</tbody>
</table>

*Tukey HSD Matrix of Pairwise Comparison Probabilities: Test of C_L (1).

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>P</th>
<th>S</th>
<th>SP</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>0.143</td>
<td>1.000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S</td>
<td>0.001</td>
<td>0.233</td>
<td>1.000</td>
<td></td>
</tr>
<tr>
<td>SP</td>
<td>0.080</td>
<td>0.948</td>
<td>0.643</td>
<td>1.000</td>
</tr>
</tbody>
</table>

*Tukey HSD Matrix of Pairwise Comparison Probabilities: Test of V_{MIN}.

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>P</th>
<th>S</th>
<th>SP</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>0.801</td>
<td>1.000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S</td>
<td>0.051</td>
<td>0.346</td>
<td>1.000</td>
<td></td>
</tr>
<tr>
<td>SP</td>
<td>0.137</td>
<td>0.517</td>
<td>1.000</td>
<td>1.000</td>
</tr>
</tbody>
</table>
An ANOVA of the above groups without the normal group yielded significant P-values for compliance and minimum volume \( p = 0.003 \) for \( C_L(1) \) and \( p = 0.020 \) for \( V_{\text{min}} \) but not TLC. Tukey HSD analysis identified compliance for the surfactant group to be significantly lower than PMA-only \( (p = 0.003) \) and the surfactant group minimum volume to be significantly lower than PMA-only \( (p = 0.024) \). Therefore, lungs treated with surfactant-only can be characterized as less compliant with lower minimum gas volumes than normal or injury-only lungs.

An examination of hysteresis changes by Analysis of Variance was done to test the hypothesis that surfactant decreases hysteresis of air-filled PMA-injured lungs. Comparing the area circumscribed between the inspiratory and expiratory curves revealed significant differences between groups. The hysteresis areas for each group represent the difference between the area under each pair of inspiratory and expiratory curves indexed to the dry right lung weight, as measured by numeric integration (Table 7). The ANOVA yielded \( p = 0.001 \), so a post hoc analysis was performed to determine which groups differed and by how much (Table 8). Clearly, all groups were significantly different from the normal group except for the PMA+Surfactant+PEEP group. The decreased areas observed for the PMA and PMA+surfactant groups were decreased just as TLC values for these two groups were decreased compared to the other two groups.
Table 7. Group mean values for the areas circumscribed by the inspiratory & expiratory curves.

<table>
<thead>
<tr>
<th>Group</th>
<th>Area between Curves</th>
<th>Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>265</td>
<td>72.6</td>
</tr>
<tr>
<td>P</td>
<td>155</td>
<td>27.7</td>
</tr>
<tr>
<td>S</td>
<td>103</td>
<td>42.4</td>
</tr>
<tr>
<td>SP</td>
<td>180</td>
<td>36.6</td>
</tr>
</tbody>
</table>

(N = Normal, P = PMA, S = PMA+Surfactant, SP = PMA+Surfactant+PEEP)

Table 8. Post Hoc analysis of Hysteresis Area

Tukey HSD Matrix of Pairwise Comparison Probabilities: Post Hoc Test of Hysteresis Area.

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>P</th>
<th>S</th>
<th>SP</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>1.000</td>
<td>0.013</td>
<td>0.000</td>
<td>0.106</td>
</tr>
<tr>
<td>P</td>
<td>0.013</td>
<td>1.000</td>
<td>0.300</td>
<td>0.881</td>
</tr>
<tr>
<td>S</td>
<td>0.000</td>
<td>0.300</td>
<td>1.000</td>
<td>0.120</td>
</tr>
<tr>
<td>SP</td>
<td>0.106</td>
<td>0.881</td>
<td>0.120</td>
<td>1.000</td>
</tr>
</tbody>
</table>
The large difference in maximal volume (TLC) across groups (Table 5) indicates why the Normal group could have the largest circumscribed area; it has a much larger range over which volume changes. Perhaps if the other groups were corrected by applying a correction factor representing the disparity between each group's TLC and the Normal group's TLC, the order of magnitude for the circumscribed areas might turn out as predicted with the Normal group having the smallest circumscribed area. To test this proposition, the circumscribed area of the composite inspiratory & expiratory curves for the PMA group were corrected by adjusting them to the TLC of the normal group. That is, each point on the inspiratory and expiratory curves was multiplied by a correction factor derived from dividing the normal group TLC by the PMA group TLC, and the area between the two curves was recalculated (see Appendix 4). Although an increase in the PMA group mean area was obtained, the PMA group mean area still remained less than the Normal group mean area. Therefore, correcting group mean pressure-volume curves to the maximum volume of the normal group still did not provide evidence to reject the null hypothesis (pressure-volume hysteresis is not decreased with the addition of surfactant).

Another approach was proposed to better measure changes in hysteresis between the groups by measuring the horizontal width between the inspiratory and expiratory curves at 45% of TLC. This proved unfeasible because the y-intercepts (volume at zero pressure) of some curves were above 45% TLC. Therefore, the horizontal distance between inspiratory & expiratory curves at 50% of the range of
volume change were measured and compared. Again, the Normal and PMA-injury groups were compared to determine if it would be worthwhile to apply the method to all groups (Table 9). The results proved to be similar to the unaltered mean areas circumscribed by the inspiratory and expiratory curves so no further analysis was attempted.

Table 9. Horizontal distance between inspiratory & expiratory curves at 50% of the range of volume change.

<table>
<thead>
<tr>
<th></th>
<th>Normal Group</th>
<th>PMA Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interpolated expiratory pressure value at 50% of range of volume change</td>
<td>3.9</td>
<td>5.2</td>
</tr>
<tr>
<td>Interpolated inspiratory pressure value at 50% of range of volume change</td>
<td>13.1</td>
<td>10.4</td>
</tr>
<tr>
<td>Horizontal distance between inspiratory &amp; expiratory curves</td>
<td>9.2</td>
<td>5.2</td>
</tr>
</tbody>
</table>

To test the hypothesis that surfactant improves gas exchange in PMA-injured lungs, five variables that reflect gas exchange in the lungs or influence gas exchange values were measured hourly for the duration of the experiment. Alveolar-arterial oxygen difference ($\text{AaDO}_2$), shunt fraction ($Q_s/Q_T$), cardiac index (CI), oxygen delivery ($\text{DO}_2$), and oxygen extraction ratio ($O_2\text{EXT}$), were analyzed using Analysis
of Covariance (ANCOVA) with repeated measures for PMA and PMA+Surfactant groups. This particular test (2V module of Biomedical Data Processing® software) allowed for a covariate that changed across trials. The covariate in question was arterial blood pH since it is known to have considerable effect on vascular resistance, shunt fraction, and cardiac output. The pre-treatment pH values were not significantly different across groups with p values of 0.241 and 0.210 for hours one and three respectively. During the treatment period (hours 3-7) the pH varied significantly and was therefore treated as a covariate for analyses of the treatment period.

The trends for PMA-only and PMA+surfactant groups were unremarkable for $\text{DO}_2$ and $\text{O}_2\text{EXT}$ variables, and CI group means decreased over time in relative synchrony (Figure 27). The $\text{Q}_s/\text{Q}_r$ and AaDO$_2$ trends showed dramatic changes at the beginning of the treatment period and maintained them for the remainder of the experiment (Figure 28). The ANCOVA results showed a significant main effect for group for both AaDO$_2$ and $\text{Q}_s/\text{Q}_r$ during the treatment period but not for CI, $\text{DO}_2$, or $\text{O}_2\text{EXT}$ (Table 10). An ANOVA with repeated measures without a covariate was also performed and the p-values were nearly the same with no changes in which variables demonstrated statistically significant change. Comparing the mean cardiac index values for the small PMA+surfactant+PEEP group to the PMA+surfactant group yielded a p-value = 0.965 and nearly the same when compared to the PMA-only group (p = 0.986).
Figure 27. Changes in DO₂, O₂ EXT, and CL variables over time.

- Cardiac Index (L/min x m²)
- Oxygen Extraction Ratio
- O₂ Delivery (mL/min/kg)
Figure 28. Ordinal relationship of interaction effect for AaDO₂ and Qs/Qt.
Table 10. ANCOVA (ANOVA) results for AaDO₂, Qs/Qr, CI, DO₂, and O₂EXT during treatment period, hours three through seven († denotes ANOVA p-value).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group Effect</th>
<th>Time Effect</th>
<th>Interaction Effect</th>
</tr>
</thead>
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<tr>
<td>AaDO₂</td>
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<td>0.0000</td>
<td>0.0000</td>
</tr>
<tr>
<td></td>
<td>0.000†</td>
<td>0.000†</td>
<td>0.000†</td>
</tr>
<tr>
<td>Qs/Qr</td>
<td>0.0004</td>
<td>0.0000</td>
<td>0.0000</td>
</tr>
<tr>
<td></td>
<td>0.000†</td>
<td>0.000†</td>
<td>0.000†</td>
</tr>
<tr>
<td>CI</td>
<td>0.3286</td>
<td>0.1021</td>
<td>0.7070</td>
</tr>
<tr>
<td></td>
<td>0.974†</td>
<td>0.119†</td>
<td>0.786†</td>
</tr>
<tr>
<td>DO₂</td>
<td>0.6439</td>
<td>0.4516</td>
<td>0.5042</td>
</tr>
<tr>
<td></td>
<td>0.772†</td>
<td>0.445†</td>
<td>0.502†</td>
</tr>
<tr>
<td>O₂EXT</td>
<td>0.4104</td>
<td>0.2720</td>
<td>0.8462</td>
</tr>
<tr>
<td></td>
<td>0.389†</td>
<td>0.311†</td>
<td>0.758†</td>
</tr>
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</table>

Tukey's HSD post hoc test was performed to determine which particular groups were actually different at an alpha level of 0.05 when a significant main effect for group was encountered. The significant changes in AaDO₂ main effect for time occurred early in the treatment period with hours three and four significantly different from the remainder of the measurement period. Recalling that hour three measures constitute a post-injury, pre-treatment baseline measurement, it is clear that the rise in AaDO₂ and Qs/Qr for the group that received surfactant coincided with the surfactant instillation given at hour four. The interaction effect was ordinal in nature.
and hours five through seven maintained an elevated plateau (Figure 28).

The results for $Q_s/Q_T$ main effect for group and hour (time) closely followed the AaDO$_2$ results. Given the lack of significant change among groups for cardiac index and the tremendous changes in AaDO$_2$ and $Q_s/Q_T$, one might expect a concomitant increase in pulmonary vascular resistance (PVR). There was no significant difference in PVR between PMA-only, PMA+Surfactant, and PMA+Surfactant+PEEP groups at the beginning of experimental measure ($p = 0.835$) nor at hour three (after PMA-injury but before treatment, $p = 0.357$). An ANOVA with repeated measures over hours three to seven yielded no significant difference when all three groups were compared (group effect $p = .087$) but comparing just PMA and PMA+Surfactant indicated significant change between groups with a $p = 0.043$. Within-subjects comparisons yielded non-significant p-values for time and interaction effects regardless of whether PMA and PMA+surfactant or all three groups were compared (two groups/three groups: hour effect $p = 0.768/0.629$, interaction effect $= 0.971/0.937$). This indicates early plateaus were established for each group after injury and were maintained for the duration of the experiment (Figure 29).

The primary measurement for lung water in this study, the double-dilution method (DDLW), was checked against wet/dry lung weights (Table 11). The wet/dry weight method does not quantify the volume of extravascular lung water but it does indicate if the overall lung fluid amount in one group has changed in relation to another. If a significant difference were detected using the DDLW method, it should
be detected in the wet/dry data as well and vice-a-versa. The final double-dilution method measurements were compared to the right lung wet/dry measurements in two forms, grams of water difference between wet and dry lung indexed to dry right lung weight ($\Delta$gm H$_2$O) and the percent change in wet/dry weight ($\%\Delta$H$_2$O). One-way ANOVA results showed no significant differences in final lung water measurements between PMA, PMA+surfactant, and PMA+surfactant+PEEP groups for the three measurement methods (DDLW $p=0.890$, $\Delta$gm H$_2$O $p=0.562$, $\%\Delta$H$_2$O $p=0.878$).

Figure 29. Pulmonary vascular resistance changes across time (Error bars in S.D.).
Table 11. Individual data for three methods used to measure change in lung water.

<table>
<thead>
<tr>
<th>GROUP/#</th>
<th>DDLW</th>
<th>$\Delta$gm H$_2$O</th>
<th>%$\Delta$H$_2$O</th>
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<td><strong>PMA</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>7.0</td>
<td>3.7</td>
<td>88.7</td>
</tr>
<tr>
<td>2</td>
<td>19.4</td>
<td>4.5</td>
<td>82</td>
</tr>
<tr>
<td>3</td>
<td>10.4</td>
<td>4.5</td>
<td>81.3</td>
</tr>
<tr>
<td>4</td>
<td>13.8</td>
<td>4.9</td>
<td>83.1</td>
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<td>5</td>
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<td>3.2</td>
<td>76.3</td>
</tr>
<tr>
<td>6</td>
<td>7.1</td>
<td>3.3</td>
<td>76.8</td>
</tr>
<tr>
<td>7</td>
<td>11.3</td>
<td>4.5</td>
<td>81.8</td>
</tr>
<tr>
<td><strong>PMA+Surfactant</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>10.9</td>
<td>2.7</td>
<td>72.9</td>
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<tr>
<td>2</td>
<td>10.3</td>
<td>4.4</td>
<td>81.6</td>
</tr>
<tr>
<td>3</td>
<td>18.4</td>
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</tr>
<tr>
<td>4</td>
<td>5.3</td>
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<td>80.4</td>
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<td>9.9</td>
<td>4.1</td>
<td>80.6</td>
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<tr>
<td>7</td>
<td>8.7</td>
<td>3.6</td>
<td>78.3</td>
</tr>
<tr>
<td><strong>PMA+Surfactant+PEEP</strong></td>
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<tr>
<td>1</td>
<td>10.2</td>
<td>4.8</td>
<td>82.9</td>
</tr>
<tr>
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<td>10.8</td>
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<td>83.0</td>
</tr>
<tr>
<td>3</td>
<td>4.5</td>
<td>4.1</td>
<td>80.6</td>
</tr>
<tr>
<td>4</td>
<td>17.2</td>
<td>5.6</td>
<td>84.9</td>
</tr>
</tbody>
</table>

* Not available due to missing wet weight for right lung.
DISCUSSION

The primary finding of this study was that surfactant treatment without co-administration of PEEP had a deleterious effect on gas exchange over the short time frame studied in this model. The use of PEEP to prepare the lungs greatly improves PaO₂ immediately after surfactant installation compared to treatment without PEEP. This suggests that expanding the airways and alveoli to an inflated volume is a necessary prerequisite to effective surfactant distribution.

It was evident that although PMA lung injury was present, it was modest, as described in the PMA model chapter. In spite of this, the treatments produced prominent effects on some of the dependent variables, most notably TLC. The initial negative impact of surfactant installation on lung recoil of RDS patients followed by rapid recovery to baseline is fairly well-recognized and was not unexpected. It is also well-known that PEEP has a profound influence on gas exchange however, response to PEEP is not consistent. For that reason the initial study design removed PEEP as a confounding variable, particularly since gas exchange was not compromised sufficiently in most animals to warrant application of PEEP. Only after it was evident that gas exchange variables were not returning to baseline after surfactant administration, let alone improving, was a follow-up group of four subjects that received surfactant+PEEP studied.

Compliance measurements done at 45%-55% of TLC on the expiratory curves were of particular interest because this range approximated where tidal breathing in the normal dog occurred. This ex vivo lung compliance, identified as method one,
was significantly decreased in all groups that received PMA injury except the group that also received surfactant+PEEP. This was most likely due to an edema-related restriction of maximal lung expansion which was further supported by significantly diminished TLC values in the same groups. The order of decreasing function was interesting in that the PMA-injury only group fared better than those treated with surfactant-only. The surfactant+ PEEP group exhibited a lower mean compliance and TLC than the normal group but not of statistical significance. These results indicate that PEEP is effective in counteracting these early effects of surfactant instillation on the lung. Indeed, for this mode of surfactant delivery (direct tracheal installation), PEEP may be essential for "priming" injured lungs to receive a surfactant dose.

Analysis of the circumscribed area between the inspiratory and expiratory pressure-volume curves supports the hypothesis that a component of the lung injury included a decrease in ventilatory function. The findings of Kolobow et al., Gattinoni et al., and others describe the ARDS lung as essentially a functionally small lung, not necessarily a large stiff one of near-normal dimensions. Changes in the circumscribed areas of the pressure-volume curves match the order of relationships between the groups for TLC. Quasi-static, open-chested lung pressure-volume curves for rabbits with hyperoxic lung injury that received instillations of surfactant or saline revealed the surfactant instilled lungs had higher pressure-volume hysteresis area compared with the saline-only animals. Normalizing the hysteresis area of each group using the maximal volume of the normal group resulted in nearly
equal areas, suggesting that hysteresis area was altered as a result of changes in TLC values. Unlike the maximum volumes, the minimum volumes were not significantly different. The alteration in lung function was such that the excised lungs could deflate to a comparable base volume but maximal volumes were different depending on what treatment accompanied injury. Thus, alveoli were able to derecruit similarly but recruitment was impaired to varying degrees in the non-normal groups.

Expiratory compliance of the excised lungs was greatest for the normal group and least for the PMA+surfactant-only group. Experiments that have measured expiratory pressure-volume curves using air-filling after saline lavage found normal inflating pressures at maximal lung volume but markedly higher transpulmonary pressures at lower volumes. This indicates that a loss of surfactant (such as caused by a saline lavage) was not the primary cause of lung dysfunction resulting from PMA injury and treatment combinations. Although the reduced maximal lung volumes could be explained by edema fluid filling portions of the alveolar spaces, this would also reduce minimal lung volumes, which was not observed. Rather, this is more consistent with lungs that are restricted by interstitial edema fluid and possibly attended by atelectasis with accompanying overdistension of normal lung units.

The circumscribed areas for PMA injury-only and surfactant+PMA were significantly smaller for two possible reasons. First, the presence of edema produced lower overall curve amplitudes (volume/lung weight) for the given inflation pressures. It is plausible that due to the short time period of the experiment, the
edema in the injured lung areas remained predominantly in the interstitial space, not yet having moved into the alveolar compartment. The interstitial edema would have a resistive effect, especially on maximal lung volumes. The restrictive effect would be less pronounced for intermediate lung volumes because uninjured lung portions would assume the work of injured portions by hyperinflating. This hyperinflation of normal lung units or "volutrauma" is a common problem in the treatment of ARDS patients. If pressure-volume measurements were performed by inflating the lung to some sub-maximal volume—a volume attainable in all groups that did not require more than 30 cm H$_2$O, rather than a maximal pressure, then the predicted relationship of injured lungs having the greatest circumscribed area may be observed.

It appears that PEEP was able to either maintain or recruit some alveoli in injured portions of the lung and allow surfactant to distribute more effectively. In the surfactant without PEEP group, the surfactant was probably delivered mostly to normally functioning lung units and so no improvement was noted. Any exogenous surfactant that remained in the airways could decrease lung function by acting as an obstruction to gas flow. UsingGattinoni's terminology to describe the various states of alveoli in acute lung injury, all the groups receiving PMA-injury had varying percentages of three types of lung units—normally inflated, poorly inflated, and non-inflated tissue. One effect of PEEP may have been to keep open a certain proportion of alveoli that would have otherwise collapsed as a result of the excess fluid from PMA injury. Without PEEP, the injured lung receiving surfactant would
have been comprised of largely normally inflating and non-inflating alveoli, resulting in overinflation of normal alveoli. The addition of PEEP changed the scenario to more of a three compartment model, due to the presence of more poorly inflated lung units. In a study specifically investigating the effect of PEEP on ARDS patients, CT scans showed an increase in the ratio of aerated to non-aerated tissue volume within minutes after application of moderate levels of PEEP (5-14 cm H2O). Thus, the increased volumes were likely a result of increasing the percent of abnormal but open alveoli, allowing distribution of exogenous surfactant to these alveoli. It is unlikely that the increase in TLC measured in the PMA+surfactant+PEEP group was due mainly to PEEP only because ex vivo lung measure preparations did not allow for maintaining a positive pressure in the lung until the start of measurement.

The unexpected decrease in PaO2 values following treatments was especially surprising in that the surfactant+PMA-injury group mean PaO2 did not even return to the level of the PMA-only group over the measurement period, let alone surpass it. This was in contrast to findings in other studies that reported PaO2 values markedly improving within one to two hours of administration. Important differences between this study and the others include a different type of lung injury compared to those used in previous studies (e.g., saline-lavage) and PEEP was not eliminated in one of the previous studies as a confounding variable. Regardless of the model, a review of animal studies makes it clear that animal models of ARDS have greater variability in physiologic responses than infant-RDS models.
Surfactant without PEEP clearly impeded gas exchange in the injured lungs as demonstrated by p-values of zero to four decimal places for shunt fraction and AaDO₂ main effects. The PMA+surfactant+PEEP group only had four subjects and although the statistical power of any test applied would be enhanced by the serial measures over time, the results of any statistical comparison to this group must be viewed with considerable skepticism. However, over the last two hours of the treatment period Figure 25 shows a clearly improving trend for AaDO₂ and Qₛ/Qᵣ in the group that received PEEP in addition to surfactant. Ideally a fifth group with lung-injury and PEEP-only would have been observed if not for time and funding constraints. However, one of the previously noted studies using saline-lavage lung injury in guinea pigs showed dramatic improvements in gas exchange immediately after surfactant and no improvement without surfactant, in spite of aggressive PEEP and 100% oxygen.⁴⁹

The decline in gas exchange after surfactant without PEEP cannot be explained by differences in cardiac index, which was nearly the same as the injury-only group over all points in time. Excluding total blood flow differences as a cause leaves the possibilities of true shunt resulting from collapsed alveoli and V/Q disturbances resulting from poor ventilation, impaired diffusion, or altered regional blood flow within the lungs. The ANOVA for Pulmonary vascular resistance gave a p-value < 0.05 for group main effect but this difference between groups was established before the treatment period began. The significant differences in Qₛ/Qᵣ and AaDO₂ did not occur until after surfactant was given.
Recalling the interpretation of ARDS as being a problem of a progressively smaller portion of functional lung having to bear the burden of increasing gas exchange requirements, a surfactant bolus could cause several problems. First, the fluid bolus would likely have been carried to portions of the lung that were best ventilated (ie., uninjured lobes with the highest compliance), regardless of attempts to alter body position. Thus, diffusion in essentially normal portions of the lung (already assuming a greater burden of gas exchange) would be hindered by the presence of additional fluid from the surfactant bolus. Alveoli in injured regions, as a result of being less ventilated due to edema fluid and obstruction, would be least likely to receive new surfactant and would probably inactivate any that did penetrate because of denaturing substances in the collected edema. The rate of surfactant distribution to the lung periphery given via bolus instillation varies depending on variables such as the volume of vehicle solution, the differing lung anatomy of various species, mode of ventilation, and the degree of edema fluid from injury present in the airways. Since surfactant exerts its primary mechanical benefit in the alveoli, it would likely cause deleterious effects for the time it remained in the airways. This might result from surfactant fluid further narrowing the diameter of the airways already contending with edema fluid from lung injury, resisting or obstructing gas flow.

The conclusions of this study are: (1) natural surfactant treatment without PEEP causes a persistent decrease in pulmonary gas exchange as measured by Qs/Qr and AaDO2, (2) surfactant therapy without PEEP causes persistent decreases in lung
compliance and maximal lung volume, (3) 10 cm H₂O PEEP is effective in counteracting the early untoward effects of surfactant instillation on gas exchange and lung mechanics in acute lung injury (indeed, for this mode of surfactant delivery-direct tracheal installation, PEEP appears to be essential for "priming" injured lungs to receive a surfactant dose), and (4) 10 cm H₂O of PEEP in conjunction with surfactant does not appear to compromise cardiac output.

The results suggest several questions for further investigation. A systematic method for application of PEEP in ARDS and the ability to predict its effect on gas exchange remain unperfected. Determining how to set PEEP levels for optimal surfactant distribution would be beneficial, especially given the cost of treatment and the morbidity of ARDS. It would be useful to determine if flushing the lungs to remove injury-related substances that might inactivate exogenous surfactant would improve its effect on gas exchange and lung impedance. Finally, would the altered lung mechanics of ARDS-afflicted lungs respond better to a small, constant surfactant administration (as with a continuous aerosol) instead of a bolus installation?
REFERENCES


46. Dreyfuss D, Saumon G. Barotrauma is volutrauma, but which volume is the one responsible? Intensive Care Med 1992;18:139-141.


SUMMARY

The experimental results presented in the preceding chapters indicate that the effects of the two treatments, furosemide diuretic and exogenous surfactant, on a PMA model of acute lung injury were highly dependent upon preexisting conditions in the body. Surprisingly, the treatments did not just fail to produce positive results under these specific conditions, but they clearly worsened the animals' pulmonary status. Marked, deleterious changes in shunt fraction and AaDO₂ were seen after surfactant or furosemide, despite the modest PMA injury. When compared to normal lungs, surfactant without PEEP was the only injury group that had a significant decrease in both expiratory compliance and TLC. On the other hand, the surfactant with PEEP group was the only injury group that did not have a significantly lower TLC than the normal group.

Based upon the available data, it appears that furosemide acted as a general vasodilator in the pulmonary capillary bed. The modest increase in intrapulmonary shunting seen in the PMA-only group was dwarfed by that observed in the PMA+furosemide group, apparently due to an overriding of the protective hypoxic vasoconstriction response. The hemoconcentration noted in the PMA+furosemide group was unexpected because no diuresis was attained, but this hemoconcentration
provided an explanation for the increased PVR, despite the presence of vasodilation. Investigation of the mechanism underlying this phenomenon is beyond the scope of this study but should be pursued.

It was clear that PEEP diminished the initial negative impact of surfactant on gas exchange and hastened the return towards pre-treatment injury levels. Surfactant may eventually improve gas exchange and lung impedance in a PMA injury model, but more than a 4 h measurement period for detecting treatment effects would be required. PEEP promoted better distribution of surfactant, as evidenced by the reclaiming of functional lung units in the form of increased TLC values. The question of what the optimal PEEP level is to promote surfactant distribution without compromising hemodynamic stability remains. The addition of the fourth group (PMA+surfactant+PEEP) was added midway in the study to better understand the effect of surfactant in this model. Ideally, a fifth group of PMA+PEEP-only would have been useful for further hypothesizing but time and funding limitations necessitated deferring such investigation to some future time.

Although the ability to generalize these results is limited due to small sample size and variability of the model, the data provide certain clear cautions for practitioners treating ARDS patients. The overall conclusions for this study were: 1) The PMA model in dogs produces a variable lung and gut injury that quite often results in no diuretic response to even high doses of furosemide diuretic, 2) administration of furosemide without an increase in urine output decreased gas exchange as measured by shunt fraction and the difference in alveolar-arterial oxygen
partial pressures, 3) furosemide does have significant extrarenal effects that may be masked when diuresis is present, 4) surfactant without the co-administration of PEEP decreases lung compliance and TLC in this model, 5) surfactant without PEEP can cause a sustained deterioration in gas exchange, and 6) even after surfactant is combined with PEEP, it appears that any positive effects on gas exchange above the pre-treated injury level require more than four hours before they can be detected.
APPENDIX A

Nine sequential analyses were run on the contents from a single blood sample to determine intra-assay variation. Inter-assay variation was determined from three serial samples that were collected (within two minutes) in three different syringes.

**Intra-assay coefficients of variation**

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<th>s.d.</th>
<th>mean</th>
<th>%CV</th>
</tr>
</thead>
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<td>PO2</td>
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**Inter-assay coefficients of variation**

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<th>%CV</th>
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<tr>
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<td>48.767</td>
<td>0.516 %</td>
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APPENDIX B

Coefficients of variation (CV) for the lung water computer measures were tested during the stable baseline period prior to lung injury. For five control subjects, the individual CVs ranged from 3.16 % to 13.44 % (CV = 100 \times \text{standard deviation/mean}).

The lung water computer accuracy at measuring thermal dilution curves was assessed by connecting the device to an artificial circuit with a constant flow provided by a roller-pump. The pump output was collected over a 90 second time period and measured in a graduated cylinder. The known flow rate of the circuit was 2.07 (± 0.08) L/min. The lung water computer's mean flow rate value over ten serial measurements was 2.19 L/min, with a CV of 3.83 %.
APPENDIX C

Group Means and Standard Deviations for Expiratory Volumes (mL) at Specific Transpulmonary Pressures Indexed to Dry Right Lung Weight (gm).

<table>
<thead>
<tr>
<th>Pressure (cm H₂O)</th>
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<th>6</th>
</tr>
</thead>
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<td>Normal</td>
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<td>35.8±14.7</td>
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<td>16.0±5.0</td>
<td>20.7±5.9</td>
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<td>7.0±2.8</td>
<td>9.8±4.1</td>
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<tr>
<td>Surfactant+PEEP</td>
<td>5.0±1.8</td>
<td>12.1±2.7</td>
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</tr>
<tr>
<td>8</td>
<td>40.0±16.6</td>
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</tr>
<tr>
<td>Surfactant+PEEP</td>
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<td>24.4±1.5</td>
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<tr>
<td>16</td>
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<td>49.1±18.6</td>
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<td>Surfactant</td>
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<td>29.0±8.3</td>
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<td>Surfactant</td>
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<tr>
<td>Surfactant+PEEP</td>
<td>30.7±2.5</td>
<td>31.0±2.6</td>
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</table>
Group Means and Standard Deviations for Inspiratory Volumes (mL) at Specific Transpulmonary Pressures Indexed to Dry Right Lung Weight (gm).

<table>
<thead>
<tr>
<th>Pressure (cm H₂O)</th>
<th>0</th>
<th>2</th>
<th>4</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>8.0±3.9</td>
<td>10.9±4.2</td>
<td>12.8±4.5</td>
<td>14.5±5.1</td>
</tr>
<tr>
<td>PMA</td>
<td>6.6±1.8</td>
<td>7.4±2.0</td>
<td>8.2±2.3</td>
<td>9.4±3.1</td>
</tr>
<tr>
<td>Surfactant</td>
<td>4.1±2.4</td>
<td>4.5±2.5</td>
<td>5.0±2.6</td>
<td>5.5±2.7</td>
</tr>
<tr>
<td>Surfactant+PEEP</td>
<td>4.2±1.0</td>
<td>5.5±0.6</td>
<td>6.8±0.6</td>
<td>7.9±0.6</td>
</tr>
<tr>
<td>Normal</td>
<td>16.9±6.9</td>
<td>20.5±9.2</td>
<td>25.0±11.4</td>
<td>30.9±12.4</td>
</tr>
<tr>
<td>PMA</td>
<td>11.1±4.3</td>
<td>13.7±6.1</td>
<td>17.0±7.4</td>
<td>19.8±8.1</td>
</tr>
<tr>
<td>Surfactant</td>
<td>6.3±2.9</td>
<td>8.0±3.7</td>
<td>9.8±4.7</td>
<td>11.9±5.6</td>
</tr>
<tr>
<td>Surfactant+PEEP</td>
<td>9.1±0.7</td>
<td>11.8±1.3</td>
<td>15.3±1.7</td>
<td>19.5±1.6</td>
</tr>
</tbody>
</table>

| Normal           | 35.6±13.0| 39.6±13.6| 43.2±14.4| 45.9±15.7|
| PMA              | 22.1±8.4 | 23.8±8.5 | 25.2±8.4 | 26.2±8.1 |
| Surfactant       | 13.5±6.0 | 14.8±6.5 | 16.0±6.9 | 16.8±7.2 |
| Surfactant+PEEP  | 22.3±1.2 | 24.7±0.9 | 26.9±1.1 | 28.0±1.4 |
| Normal           | 48.2±16.9| 50.2±18.5| 51.6±19.2| 52.4±19.5|
| PMA              | 26.8±8.5 | 27.5±8.5 | 28.4±8.4 | 29.0±8.3 |
| Surfactant       | 17.6±7.4 | 18.2±7.5 | 18.7±7.6 | 19.1±7.5 |
| Surfactant+PEEP  | 29.3±2.1 | 30.3±2.4 | 31.2±2.6 | 31.6±2.8 |
APPENDIX D

Method of hysteresis area correction and results.

PMA Group Correction

Normal group mean TLC = 43.7, PMA group mean TLC = 28.8, Correction factor = 43.7/28.8 or 1.52

Corrected mean values for Control group

<table>
<thead>
<tr>
<th>Pressure</th>
<th>Exp Corrected</th>
<th>Insp Corrected</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>13.4</td>
<td>10.0</td>
</tr>
<tr>
<td>2</td>
<td>18.2</td>
<td>11.2</td>
</tr>
<tr>
<td>4</td>
<td>24.3</td>
<td>12.5</td>
</tr>
<tr>
<td>6</td>
<td>31.5</td>
<td>14.3</td>
</tr>
<tr>
<td>8</td>
<td>35.0</td>
<td>16.9</td>
</tr>
<tr>
<td>10</td>
<td>37.2</td>
<td>20.8</td>
</tr>
<tr>
<td>12</td>
<td>38.6</td>
<td>25.8</td>
</tr>
<tr>
<td>14</td>
<td>40.0</td>
<td>30.1</td>
</tr>
<tr>
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<td>33.6</td>
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<tr>
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<tr>
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<td>40.7</td>
</tr>
<tr>
<td>26</td>
<td>43.5</td>
<td>41.8</td>
</tr>
<tr>
<td>28</td>
<td>43.8</td>
<td>43.2</td>
</tr>
</tbody>
</table>

Normal group mean area circumscribed by inspiratory & expiratory curves = 264
Corrected PMA group mean area circumscribed by inspiratory & expiratory curves = 237
Uncorrected PMA group mean area circumscribed by inspiratory & expiratory curves = 155.2
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