NEGATIVE PRESSURE PULMONARY EDEMA:

A CLINICAL REVIEW AND STUDY OF ITS PATHOPHYSIOLOGY

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In Partial Fullfillment of the Requirements for

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by

M. Peter Tauriainen

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M. PETER TAURIAINEN

A Thesis/Practicum submitted to the Faculty of Graduate Studies of The University of Manitoba in partial fulfillment of the requirements of the degree of

MASTER OF SCIENCE

ABSTRACT

Abnormally negative pleural pressures from upper airway obstruction or re-expansion of a lung can result in pulmonary edema and its sequelae . This thesis reviews this problem and the experiment studies the pathophysiology of negative pressure pulmonary edema.

Laser colorimetry, weight transients, hematocrit and plasma protein changes were used to determine fluid and protein flux in 3 groups of isolated blood perfused left lower canine lung lobes. Each group (n=12) was subjected to an initial 50 minute control period with a capillary pressure (P_c) of 15 cm H_2O and pleural pressure (P_{pl}) of -5 cm H_2O. Group 1 was subjected to a further 50 minutes of perfusion with the same conditions. Transpulmonary pressure was increased in the other two groups from 20 to 30 cm H_20 for 50 minutes by decreasing P_{pl} to -15 cm H_2O in group 2 (preferentially affecting extra-alveolar vessels) and by increasing P_c to 25 cm H_2O in group 3 (affecting alveolar and extra-alveolar vessels).

There were no differences in fluid flux between initial and final filtration periods in Group 1 by laser colorimetry (0.333 vs. 0.431 ml/gm dry weight/hour). Group 2 had a significant increase in filtration compared to all other groups during the final filtration period increasing from 0.420 to 1.383 ml/gm dry weight/hour ($p<0.001$). Group 3 filtration increased from 0.381 to 0.816 ml/gm dry weight/hour ($p<0.001$). These findings were confirmed by edema calculations based on hematocrits and weight transients. Weight transients overestimated edema compared to hematocrit and laser

colorimetry derived values (0.66 vs. 0.40 vs. 0.26 ml/gm dry weight/hour, $p< 0.05$). Filtrate to plasma protein concentrations (CF/CP) decreased in Groups 2 and 3 (from 1.143 to .921 and 1.099 to .914 respectively, $p<0.05$) but did not change significantly in Group 1 (1.176 vs. 1.041). No differences in CF/CP could be detected based on plasma protein concentrations.

Negative pleural pressures significantly increased pulmonary fluid filtration with the majority of the increase taking place in the extra-alveolar vessels under these experimental conditions. Filtrate protein concentration changes suggest that the increased filtration is due to increased surface area and/or increased hydrostatic forces, but not from a permeability change.

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INTRODUCTION

Pulmonary edema has many possible etiologies including altered capillary permeability (e.g. infections, toxins, endotoxemia, diffuse intravascular coagulation, radiation, uremia, drowning, aspiration, smoke inhalation, ARDS), increased capillary pressure (cardiac failure, pulmonary venous disease, over hydration), decreased plasma oncotic pressure (malnutrition, renal disease, liver disease), lymphatic insufficiency, increased negative interstitial pressure (re-expansion pulmonary edema ,upper airway obstruction), and mixed or unknown causes (high altitude pulmonary edema, neurogenic pulmonary edema, pulmonary embolism, eclampsia, cardiopulmonary bypass). The principles governing fluid exchange in the lung and in particular negative pressure pulmonary edema (NPPE) due to lung re-expansion or upper airway obstruction are reviewed. Experimental data will be presented to help elucidate the mechanism of increased filtration and the location of the filtering vessels during increased negative pleural pressures .

Fluid Movement Through Capillary Walls

The idea that tissue fluids were formed by filtration of blood through capillary walls was not recognized until the 1850's by Ludwig[1]. He thought that the main factor determining lymph flow was capillary blood pressure. Later, Cohnheim studied the effects of dilute salt solutions injected into the femoral artery of dogs with variable plasma albumin concentrations.[2] He believed hypoalbuminemia was not the cause of

edema but that it would develop more rapidly as a result of mild inflammation or increased capillary pressure. In 1896, Starling postulated that fluid flow through capillary membranes was a passive process and that the rate of this flow depended on the differences in the hydrostatic and oncotic pressures of plasma and the tissues.[3] Quantitative evidence to support these theories took over 30 years to accrue because of the technical difficulties involved.

Landis was finally able to confirm some of Starling's theories by his study of single capillaries of the frog mesentery.[4] By following capillary red blood cell (RBC) movement he was able to determine that a linear correlation existed between capillary pressure and fluid movement. When transcapillary fluid movement was zero, capillary pressure was in the range of protein osmotic pressure for that species of frog. These findings were confirmed in other species, and in each case the capillary pressure correlated closely with estimates of plasma protein oncotic pressure.

Among others, Pappenheimer and Soto-Rivera provided further evidence to support Starling's theories.[5] They studied the isolated hindlimbs of cats and dogs and estimated fluid flux by following the weight changes of the preparation. Increase in weight was thought to represent net filtration from the intravascular space to the interstitial space, while weight loss was thought to represent resorption. By varying capillary pressure (P_c) and plasma oncotic pressure (Π_p), they were able to establish the relationship between capillary fluid movement and hydrostatic and oncotic forces.

The principle's introduced by Starling can be described by the various forms of the Starling equation, for example:

$$J_v/A=K\{(P_c-P_i)-\sigma(\Pi_c-\Pi_i)\} \quad (1)$$

where J_v/A is the filtration per unit area of membrane, P_c and P_i are the hydrostatic pressures in the capillary and the pericapillary interstitial fluid respectively, Π_c and Π_i are the protein osmotic pressures of the capillary plasma and the pericapillary fluid respectively, K is the membrane coefficient (a measure of the hydraulic conductivity of the membrane), and σ is the protein reflection coefficient (a measure of how easily solute and solvent pass through the membrane). A few points are worth mentioning. This is of course a simplification of physiology. If the membrane is perfectly permeable (σ=1), then all of the solute is separated from the solution during filtration (i.e. no solute passes through the membrane) and the full osmotic pressure opposes filtration. If the membrane is completely permeable to solute (σ=0), then the solution's osmotic pressure has no effect on filtration. Equation 1 only applies to a single perfect solute with a linear relationship between osmotic pressure and concentration (not true for proteins and macromolecules). It should be noted that in most tissues including the lung, σ approaches 0 for ions and low molecular weight solutes, and thus is only influenced to a significant degree by plasma proteins (σ for albumin is approximately 0.8).[6] Furthermore, any filtration will change the terms in the equation. For example, P_i would be expected to increase, and Π_i would be expected to decrease. The equation does not take into account the compliance of the interstitial or intravascular spaces, nor does it consider the effect of the lymphatic removal of fluid and protein from the interstitium.

Despite these and other limitations, the equation nonetheless helps conceptualize factors affecting filtration.

METHODS OF MEASURING FILTRATION

Gravimetric Techniques

The first attempts to determine filtration rates in lungs by gravimetric techniques were by Guyton in 1959.[7] Left atrial pressure was increased in intact animals prior to removal of the lungs. The difference in wet to dry weights was used to calculate edema formed by the increase in left atrial pressure. This technique and modifications thereof have been used by hundreds of investigators. A common modification, often accepted as the "Gold Standard", is to continuously monitor weight changes in an isolated lung preparation taking the weight changes to represent fluid flux, such as done by Gaar in 1967.[8] In these studies the initial change in weight after an intervention is interpreted as the change in vascular volume followed by a slow secondary change due to transcapillary filtration or resorption. Stress relaxation of the pulmonary vasculature is known to occur.[9] Unfortunately, the precise time course of the vascular volume changes is unknown. Because most of these experiments have not independently assessed vascular volume changes, controversy exists regarding the validity of this technique.[10-13] Results from these experiments are valid perhaps only if the filtration rate remains constant for a

long time after an intervention.[14-15] Furthermore, weight changes cannot differentiate changes in cellular volumes from changes in interstitial volume. Although gravimetric techniques remain the most commonly used method of measuring fluid filtration in the lung, this technique has a number of drawbacks making interpretation of data difficult.

Lymphatic Techniques

At steady state conditions, net filtration is equal to lymphatic flow. Thus, lymphatic flow can be used to estimate filtration. This method involves either cannulation of a major lymphatic or creation of a chronic lymph fistula. [16-18] The volumes obtained by these techniques have tended to be more variable than those obtained by other techniques, since there is a possibility of contamination of the lymph or loss of lymph from other channels. Furthermore, steady state may not have been achieved. Even with the use of steady-state data and the analysis of responses to increased fluid filtration pressures, the interpretation of lymph data is difficult. Drake et al[105] demonstrated that the lymph collected from the mediastinal node in sheep can be heavily contaminated by contributions from systemic sources. This has been found to be especially true if the node is injured.[106] Investigators using this model have been careful to acknowledge the possibility of systemic contamination in the interpretation of their data. Some investigators have cannulated the afferent lymphatic in dogs to avoid the problems associated with modification of the lymph in the node.[107, 108] Unfortunately, this is technically difficult, has a low success rate, and can only be used with acute

preparations.[109] Thus results using lymphatic techniques have often been highly inaccurate and variable.

Hematocrit and Protein Changes

Attempts have also been made to measure fluid shifts between plasma and the tissues by measuring changes in plasma proteins and hematocrit.[19-20] Weiser and Grande (1974) published an equation describing the relationship between filtered volume and hematocrit change :

$$V_t = Pv_i \times (1-Hct_i/Hct_t) \quad (2)$$

where V_t is the volume exchanged at time t, Pv_i represents the initial plasma volume, and Hct_i and Hct_t represent hematocrits at the initial time point and time t respectively. Therefore, if multiple hematocrit determinations are made, filtered volumes can be estimated. This method circumvents the problem of stress relaxation as it is independent of vascular volume changes. However, it assumes that significant hemolysis, loss of red cells through the vessel wall or red cell trapping is not occurring. Furthermore, there must not be any significant loss of perfusate from the system, either from leakage or from evaporation.[22]

Laser Colorimetry

Laser colorimetry is used to follow hematocrit changes on a continual moment-by-moment basis. When a laser (monochromatic light beam) strikes an object, the beam may be absorbed, scattered, transmitted, reflected or it may excite fluorescence. Hemoglobin's absorbance is dependent on oxygen at all wavelengths except 815 nm (the isobestic point) where the absorption of hemoglobin and oxyhemoglobin overlap.[21] With this technique, monochromatic light is aimed at the circuit tubing of an isolated organ experiment. Transmission of this light through the solution is governed by the Beer-Lambert law:

$$I/I_o = 10^{-kCl} \quad (3)$$

where I represents the light transmitted through the tubing, I_o represents the incident light, C represents the concentration of the solute, k is the extinction coefficient of the solute to a particular wavelength, and l is the distance the signal must travel through the solution. This relationship is complicated by hemoglobin being packed in discrete cells allowing light to pass between red blood cells without absorption (sieving), and by scattering of the light. Fortunately, none of these effects is of concern as a linear relationship exists between hematocrit and $\log_{10}I$. Thus an 815 nm laser colorimetric device can be calibrated to measure changes in hematocrit which may reflect fluid

exchange. More detail regarding calibration and use of this device will be discussed later.

PULMONARY PROTEIN FLUX

Measurement of protein flux across the pulmonary capillary membrane is crucial to understanding the pathophysiology of pulmonary edema in its various forms. Human studies have documented that the protein content (especially the ratio to serum values) of edema fluid aspirated from the airway or fluid obtained by bronchoalveolar lavage is relatively high during adult respiratory distress syndrome (ARDS) compared to normal controls and patients with pressure-induced pulmonary edema.[81,82] Transvascular flux of protein is thought to occur through gaps between capillary cells[23] and also via endothelial cell vesicles.[24] Furthermore, this may be as a result of diffusion (down a concentration gradient) or convection (protein may be dragged by the fluid).

Attempts to measure protein flux have generally been unsatisfactory. Chinard and Enns (1954) followed the loss of low molecular weight tracers to estimate endothelial integrity in the lung .[25] Aviado and Schmidt (1957) used ^{131}I labeled albumin (diffusable) and ^{32}P labeled RBC (nondiffusable) to follow alloxan-induced pulmonary edema.[65] Emissions from the radiolabels in the lung were counted with a Geiger counter inserted in the pleural space. Because of technological limitations, only one tracer could be used in each animal. However, the authors were able to observe an increase in ^{131}I

counts in the lung occurring agonally, at a time when ^{32}P counts were known to fall. They suggested that this reflected increased extravasation of plasma albumin into the lung parenchyma. Other investigators have reported the transfer rates of intravenously injected radioactive molecules into the bronchoalveolar space and aspirated edema fluid as a measure of alveolocapillary permeability.[83-85]

Measurement of lymph protein has also been popular despite the required assumption of filtered protein, lymphatic protein and interstitial protein being equal.[26] Attempts have been made to validate this technique. Raj and Anderson determined lung interstitial fluid albumin concentration in lambs with hydrostatic pulmonary edema and correlated it with lymph and plasma albumin concentrations.[94] Interstitial fluid was collected from interlobular septal pools and peribronchial, periarterial, and perivenous liquid cuffs near the hilum with micropipettes. They found regional differences in albumin concentrations. Specifically, lymph, periarterial, and peribronchial albumin concentrations were significantly higher than in the perivenous or interlobular spaces.[94] Furthermore, lymph protein may be a poor measure of Π_i as it is argued that the interstitial matrix excludes plasma proteins from some regions of the interstitial space.[28] Lung lymph may also contain protein washed from the tissue spaces.[95,96] Because lymph must flow through nodes, postnodal lymph protein may differ from the filtered protein. Many investigators have used dyes to label plasma proteins to measure their flux across membranes.[27]

Flux of plasma proteins labeled with Evans blue dye (EBD) may be followed with a laser colorimeter (spectrophotometer) by using two separate wavelengths (815 and 670

nm).[72] This technique was used in the experiment described in this thesis. This technique needs further validation. Much of the time and effort spent on this experiment was used to further develop this method of following protein flux.

STARLING FORCES IN THE LUNG

The Starling forces in the lung differ from those in other organs for a number of reasons. The lung accepts the entire cardiac output at much lower than systemic pressures. Since the P_c in the lungs of all mammals is in the order of only 10 cm H_2O and Π_c is about 30 cm H_2O, [6] the net intravascular force favours reabsorption of fluid. Thus extravascular forces are important in determining steadystate fluid filtration in the lung . Overall evidence indicates that the interstitial fluid pressure (Pi) is negative [29] in the order of -10 cm H_2O, and is more negative near the extra-alveolar vessels and near the top of the lung. Π_i has never been directly measured in normal lung tissue. Its value has only been estimated using many of the models discussed above and is generally thought to be less than Π_c. [86]

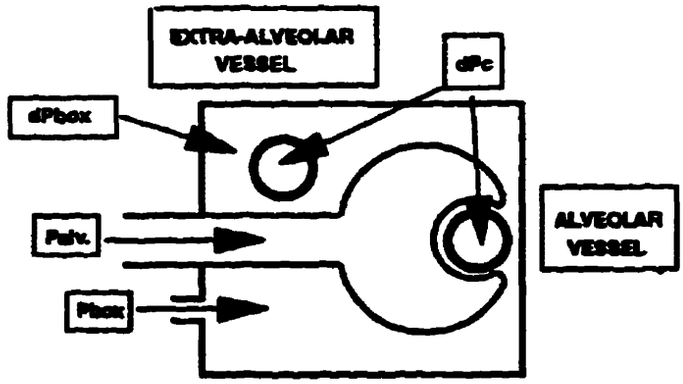
ALVEOLAR VERSUS EXTRA-ALVEOLAR VESSELS

The presence of two types of pulmonary vessels, intra-alveolar (commonly called alveolar, IAV) and extra-alveolar (EAV), was first shown by Macklin[30] using burettes filled with a latex suspension to separate large from small vessels. The exact definition and differentiation of the two types of vessels is confusing because they are defined differently by anatomists and physiologists. For the purposes of this thesis, the IAVs are defined as capillaries that are contained within the walls that separate adjacent alveoli. They are surrounded by interstitium that varies in thickness and in the nature and content of cells, collagen and elastic fibers. Their caliber decreases with increased alveolar pressure.[87] As the lung expands, the alveolar pericapillary pressure is less than the alveolar pressure (largely because of the surfactant lining of the alveoli) but higher than the pressure surrounding EAVs.[88] EAVs by definition are larger vessels in the interstitium (including veins, venules, arteries, and precapillaries) that are not affected by changes in alveolar pressure but do enlarge with lung inflation.[88] Surrounding the EAVs is an interstitial space that is bounded by extensions of the fascial sheaths that envelop the trachea and esophagus. Within this space lies loose areolar tissue, collagenous fibers, and lymphatics. The alveolar and extra-alveolar interstitial spaces are generally pictured as a continuous pathway for fluid movement toward the lymphatics.[88]

Figure 1 is a simplified diagrammatic representation of the alveolar and extra-alveolar vessel model used in the experiment described in this thesis. By definition, IAVs would be affected by changes in alveolar pressure, while EAVs would be more susceptible to

Figure 1: Theoretical model. This diagram depicts a simplification of the alveolar and extra-alveolar vessels in the lung. Note that dPbox= change in box pressure, dPc= change in capillary pressure, and Palv= alveolar pressure.

THEORETICAL MODEL:



1. **Changes in capillary pressure (dPc) should affect all vessels.**
2. **Changes in box pressure (dPbox) should preferentially affect extra-alveolar vessels.**

changes in the interstitium and lung inflation (i.e. changes in the box or pleural pressure). Howell et al studied the pressure-volume characteristics of isolated canine lungs with the vessels containing either dextran (which was present in IAVs and EAVs) or kerosene (present mainly in the EAVs).[101] They concluded that the larger vessels (EAVs) were enlarged by inflation of the lobe regardless of the vascular pressure, and the smaller (IAVs) were reduced in volume by inflation regardless of the vascular pressure.

Numerous investigators have studied the relative contributions of these vessel types to edema in the lung. Initially, the IAVs were thought to be the major site of fluid flux in both hydrostatic and increased permeability pulmonary edema, primarily because they have a greater surface area per unit membrane thickness.[31] Staub estimated the vascular surface area of the alveolar vessels to be 10-10000 times that of all the other pulmonary vessels combined.[31] For his thesis, Landolfo performed experiments using the same apparatus described later in this paper comparing excised canine lobes during two filtration periods. The alveolar pressure was maintained at 5 cm H_20 for both periods while the box pressure (equivalent to decreasing pleural pressures) was decreased from atmospheric to -10 cm H_20 for the final filtration period. Weight transients demonstrated an increase in weight between the two periods. However, there was no change in edema based on colorimetry. He concluded that increased negative pleural pressures resulted in increased EAV volume with no change in filtration. He also stated this was strong evidence that the extra-alveolar vessels contributed little to the total transvascular fluid exchanged under normal physiologic conditions.[50] Nonetheless, a number of investigators have found that the EAVs contribute significantly to edema. Using isolated

canine lungs, Iliff demonstrated 63% of edema fluid originated from the EAVs.[32] Albert et al using in situ dog lungs embolized with beads estimated that 41% of filtration was from the extra-alveolar arteries and 32% from the extra-alveolar veins.[33] This issue deserves further study.

CLINICAL CONSEQUENCES OF HIGHLY NEGATIVE PLEURAL PRESSURES

This thesis examines the effects of increased negative pleural pressures on fluid and protein flux in the lung. There are two clinical situations where this is important. The first (re-expansion pulmonary edema) occurs when a lung is rapidly re-expanded (with or without applied negative pleural pressures). In the second situation, pulmonary edema occurs during or following upper airway obstruction.

Re-expansion Pulmonary Edema

Re-expansion pulmonary edema (REPE) following evacuation of pleural fluid or air has been recognized since 1853. Riesman[34] and Hartley[35] described cases of "albuminous expectoration" following re-expansion of the chest at the beginning of this century. In a review by Mahmood et al only 47 cases had been reported in the world literature between the years 1958 to 1987. [41] Although REPE is thought to be a rare

complication, [74,75] there are very few studies documenting its incidence. Rozenman et al studied all patients presenting to their institution between January 1986 and January 1994 with a spontaneous pneumothorax. Diagnosis of REPE was based on classical radiological findings of pulmonary edema. REPE was diagnosed in only 3 of 180 patients (1.7%). [74] Other studies have documented a higher incidence. In a study by Matsuura et al, twenty-one of 146 cases (14%) of spontaneous pneumothorax that were treated by thoracentesis or continuous low negative pressure suction drainage (- 12 cm H_20) of the pleural space developed REPE.[40] Their criteria used to diagnose REPE included: (1) clinical status with a serious cough, foaming sputum, agitation, tachycardia, and tachypnea; and (2) extensive ground glass-like shadow in the chest x-ray film following re-expansion. Hosakawa reported that they experienced three cases of REPE among 12 treated cases of spontaneous pneumothorax,[76] and Takamura found a 27 percent incidence of REPE in an experimental study.[77] In a more recent paper, Trachiotis et al reported an incidence of one case per two years at their institution.[73] Overall, the evidence presented suggests that REPE is a much more common problem than is generally appreciated.

Attempts have been made to identify risk factors and the etiology of REPE. This is thought to be most likely to occur when the lung has been collapsed for three days or more, and when lung re-expansion is accomplished rapidly.[36,37] In the review by Trachiotis et al, 83% of cases of REPE were as a result of a chronically collapsed lung from a pneumothorax, 83% of the time the pulmonary edema was on the ipsilateral side, 6.7% of the time the edema was bilateral, occasionally the edema occurred only in the

contralateral lung, and 33% of the cases occurred when re-expansion was accomplished without pleural suction.[73]

Most of the data in the literature comes from case reports with only a few laboratory experiments in the literature. In one experimental paper, Miller *et al* found ipsilateral pulmonary edema within 2 hours in 80-100% of monkeys when a pneumothorax had been maintained for three days and when rapid re-expansion was performed using -10 cmHg suction.[37] In contrast, pulmonary edema did not develop if underwater drainage alone was applied after three days collapse or if the lung was re-expanded after only one hour of collapse even if negative pleural pressure was applied. Slow re-expansion has been suggested to prevent REPE but was not successful in 21 of 146 cases treated by Matsuura et al.[40] The rate of REPE has been found to be higher in the group aged 20-39 years than other age groups ($p<0.001$) possibly due to changes in the lung with aging that protect it from REPE.[40] Other possible factors affecting formation of edema include hypoxic pulmonary vasoconstriction and capillary leak, highly negative pleural pressure, excessive intrathoracic suction, rapid increase in pulmonary blood flow, increased pressure gradient between the alveolus and pulmonary capillary, disturbed lymphatic flow, destruction of surfactant, and possible vasoactive substances (to explain why edema occasionally occurs in the opposite lung).[36-41] Few of these have ever been studied. Pavlin *et al* suggested that extra-alveolar vessels might leak as non-compliant lungs are forced to conform to the chest cavity.[36] In another paper, they found both re-expansion and oleic acid-induced pulmonary edema increased albumin flux across the lung.[38] They concluded that re-expansion edema is due to increased pulmonary vascular

permeability caused by mechanical stresses applied to the lung during reinflation. Others have found a high protein content in pulmonary edema fluid during REPE suggesting increased pulmonary capillary permeability.[39,66,67]

The mainstay of therapy for REPE remains oxygenation, mechanical ventilation with positive end-expiratory pressure, diuresis, and hemodynamic support.[73] In their series, Matsuura et al aggressively treated their 21 cases with a variety of modalities including oxygen, steroids, inotropic agents, sedatives and diuretics without a fatal outcome.[40] Others have used anti-inflammatory and cytoprotective agents such as ibuprofin, indocin, and misoprostil to try to prevent the sequelae of REPE.[73] Currently, there are no studies comparing different treatment modalities.

The clinical manifestations of REPE can vary significantly. In some cases, symptoms may be absent and REPE may only be manifested by typical radiographic findings. In other case it may be associated with severe cardiorespiratory insufficiency and circulatory shock. Three cases of hypotension following rapid evacuation of long-standing pneumothorax were reported by Pavlin.[80] Cases of fatal REPE have been reported in the literature. Fanning et al reported a woman with Stage IV ovarian carcinoma who died of REPE following thoracentesis.[78] Sautter et al had a similar experience with a 69 year old woman after treatment of a spontaneous pneumothorax.[79] In a review by Mahmood and associates, 11 of 53 patients (20.7%) with REPE died.[41] In view of these facts, REPE should be viewed as a potentially lethal complication of lung re-expansion and warrants efforts to reduce its occurrence and ameliorate its consequences.

Pulmonary Edema Due to Upper Airway Obstruction

Negative pressure pulmonary edema (NPPE) due to upper airway obstruction (UAO) has also been a poorly recognized phenomenon. The pulmonary sequelae of acute inspiratory obstruction was first recognized by Moore in 1927 who demonstrated NPPE in dogs.[42] Swann reported the incidence of pulmonary edema in sudden asphyxial deaths[43] and later described pulmonary edema in nine of ten rats strangled to death.[44] Although Oswald is credited with the first case report of NPPE due to UAO (1977),[45] others had described "heart failure" in children with chronic upper airway obstruction (e.g. enlarged tonsils).[46] In a review by Willms and Shure (1988), only 26 cases were identified in the literature.[87] Since that time there has been a dramatic increase in the number of case reports as this complication has become more recognized. In addition to the above causes, the list of inciting causes of UAO resulting in NPPE is long and includes the following: laryngospasm, endotracheal tube occlusion, croup, epiglottitis, goitre, asthma, tonsillitis, tumour, foreign body, vocal cord paralysis, acromegaly, and sleep apnea.[45-49,52-58,87-92] NPPE due to UAO is thought to be more common in children because of a more compliant chest wall with 85% due to croup, epiglottitis and laryngospasm. Overall, laryngospasm after extubation appears to be the most common cause in the literature.[87] Olsson and Hallen reviewed 136,929 patients that underwent anasthesia.[89] They noted an incidence of laryngospasm of 8.7 per 1000 patients receiving general anesthesia. This places a large population of patients at risk for NPPE.

The incidence of this complication is underestimated. Tami *et al* found 3 out of 27 (11%) patients with UAO developed NPPE [47], compared to 3 of 43 (9%) [48] and 4 of 34 (12%)[49] in other reviews.

The pathogenesis of NPPE due to UAO remains controversial. Most of the data are from case reports and clinical series with little or no experimental or clinical data to collaborate theories regarding the pathophysiology of this problem. Most authors have hypothesized that the development of NPPE is directly or indirectly a consequence of the extremely negative pleural pressure generated during inspiration through an obstructed airway. [41,53,54] The proposed consequences of extremely negative intrapleural pressures include decreased pulmonary (perivascular) pressure, [45,53,54,55] increased venous return and pulmonary hyperemia, [53,54,56] altered right and left ventricular function, [49,53,54,56] and altered capillary permeability. [45,57] Other factors that may contribute include hypoxic damage to pulmonary capillaries, hypoxic vasoconstriction resulting in increased pulmonary capillary pressures, increased sympathoadrenergic activity with peripheral vasoconstriction and increased venous return, and altered lymphatic drainage. [49,54,57,58]

The normal mean pleural pressures are -8 cm H_2O during inspiration and -3.4 cm H_2O during expiration decreasing to -54 cm H_2O during forced inspiration against a closed glottis.[63] Pleural pressures during asthma have been estimated at -51 to -67 cm H_2O.[62] Even more extreme pressures can be developed with maneuvers such as chest tube stripping leading to -400 cm H_2O in the pleural space.[64] A patient with severe airway obstruction fighting for air can produce very negative pleural pressures.

NPPE has often been found to occur after relief of upper airway obstruction. Expiration against a partially occluded airway is thought to result in intrinsic positive end expiratory pressure (auto-PEEP) which tends to mask or oppose NPPE.[47] Relief of obstruction may result in loss of auto-PEEP resulting in pulmonary edema. Increased intrathoracic pressure during expiration may decrease venous return which can increase dramatically following relief.[47]

Hyperinflation of the lungs with UAO was recognized by Masa-Jimenez et al.[92] Although not mentioned in the clinical literature on NPPE, increased lung volumes have been shown to result in decreased extra-alveolar perimicrovascular pressure.[93] This could potentially result in increased filtration from the EAVs.

The cardiac effects of UAO were studied by Buda *et al* .[53] They found that negative pleural pressures resulted in decreased ejection fraction and increased left ventricular end systolic volume which favoured increased pulmonary capillary pressures. Robotham *et al* found that dogs subjected to the Mueller maneuvre (inspiration against a closed glottis) had increased left ventricular pressures.[59] Thus *in vivo* cardiac effects are likely to be important for the formation of NPPE with UAO in the clinical situation.

Laboratory experiments looking at the effects of negative pressures have been inconsistent. Hansen *et* al [61] studied 2-4 week old lambs with lymph fistulas over a 2 hour baseline period followed by 2-3 hours of inspiratory obstruction. They found no effect on lymph flow or protein concentration. They concluded that any increase in interstitial pressure was offset by a decrease in microvascular hydrostatic pressure, so that pulmonary edema in the clinical setting was most likely due to hypoxia-induced

vasoconstriction or left ventricular dysfunction. Unfortunately, their study has all the problems associated with lymph fistula studies (such as loss of edema fluid through lymph channels that have not been cannulated). Furthermore, filtration may have increased without a change in lymph flow if lymphatic flow is disturbed by negative intrathoracic pressures.[49,54,57,58] Landolfo studied isolated dog lungs using the colorimetric technique described in this thesis and found that increases in negative pleural pressure resulted in increases in extra-alveolar vascular volume, but did not change filtration.[50] In contrast to these two studies, Smith-Erichsen and Bo found an 8-fold increase in pulmonary edema when intrapleural pressure decreased 5 cm H_2O against a closed glottis.[51] Furthermore, when Algren *et al* [60] subjected 7 dogs to inspiratory obstruction for three hours, six dogs developed histologic evidence of pulmonary vascular congestion, of which four developed histologic pulmonary edema. Mean inspiratory intrapleural pressure was -28 mmHg in dogs with pulmonary edema. All dogs had an increase in central venous pressure. Dogs with pulmonary edema also had decreased cardiac output and decreased alveolar ventilation. They concluded that the pathogenesis of pulmonary edema in UAO remains uncertain and that further studies are needed. Since that time, little has been added to our knowledge of this problem.

Just as much of the literature grossly underestimates the incidence of this complication, its treatment has not been well outlined.[91] Relief of the obstruction should be the first step. Intubation with mechanical ventilation is usually required, often also requiring positive end expiratory pressures and high FIO_2.[47] In milder cases, oxygen

without intubation may be all that is required. Diuretics and steroids are frequently used but their role is unproven.[90] Cardiovascular instability may result with relief of the airway obstruction.[104] Thus the clinician must be aware that the patient may deteriorate after relief of the obstruction and require hemodynamic support.

EXPERIMENT

During his surgical residency the author was exposed to several patients with either re-expansion pulmonary edema or negative pressure pulmonary edema due upper airway obstruction. This lead to an interest in the pathophysiology of NPPE. The possible causes discussed in the literature have been presented earlier and include changes in the hydrostatic forces, hypoxic pulmonary vasoconstriction, rapid changes in pulmonary blood flow, disturbed lymphatic flow, destruction of surfactant, secretion of vasoactive substances, mechanical stress failure of the capillaries, and altered ventricular function. Very little experimental evidence exists to support that any of these factors are important in the pathphysiology of NPPE. An experimental preparation is described that can examine the effects of interstitial forces on an isolated lobe and can find out if negative interstitial pressures can result in increased filtration in the absence of hemodynamic changes or tissue hypoxia. Changes in pleural and interstitial pressures may result in both increased filtration and vascular volume changes that cannot be differentiated with weight transients. The 815 nm laser was used to help measure filtration independent of vascular volume changes. Since changing the transvascular pressure could result in stretching of tight junctions and a change in permeability of the vessels, a method of following permeability to protein was required. Ideally the technique should be simple, accurate and could be used on-line with the 815 nm signal. The method using the 670 nm laser colorimetric technique had shown promise with unpublished data in our laboratory and was chosen to follow protein flux.

The experiment consists of three groups. Changes in filtration in any of the groups could be due to changes in hydrostatic pressure, changes in surface area of the filtering vessels, or changes in permeability (which should also result in increased protein loss). The first group is a control arm to look at time related changes. Isolated organ experiments are inherently unstable over the long term so that possible time related changes must be looked at. Based on previous experience with this preparation[50] we did not anticipate significant changes in the preparation during the time course of this experiment. This group is compared to itself during two filtration periods and also to the two intervention groups. In the second group of the experiment, increased negative pleural pressure is applied to the lobes with constant airway pressure. This should decrease the interstitial pressure in the extra-alveolar space but have less effect on the alveolar space. Although the experimental evidence looking at the effect of increased negative interstitial pressure has been contradictory[50,51,59,60,61] , an increase in pulmonary edema was anticipated primarily from the EAVs. Clinical experience with NPPE had suggested a capillary leak problem.[45,57] Changes in capillary permeability due to leak from tight junctions has not been investigated as well with highly negative interstitial pressures as it has with increased capillary pressures. In the third group of the experiment, capillary pressures were increased resulting in a change in transvascular filtration pressures affecting both EAVs and IAVs with an anticipated increase in filtration. Despite the equal change in transpulmonary pressure between groups 2 and 3, a greater change in filtration was expected with group 3 since EAVs and IAVs were

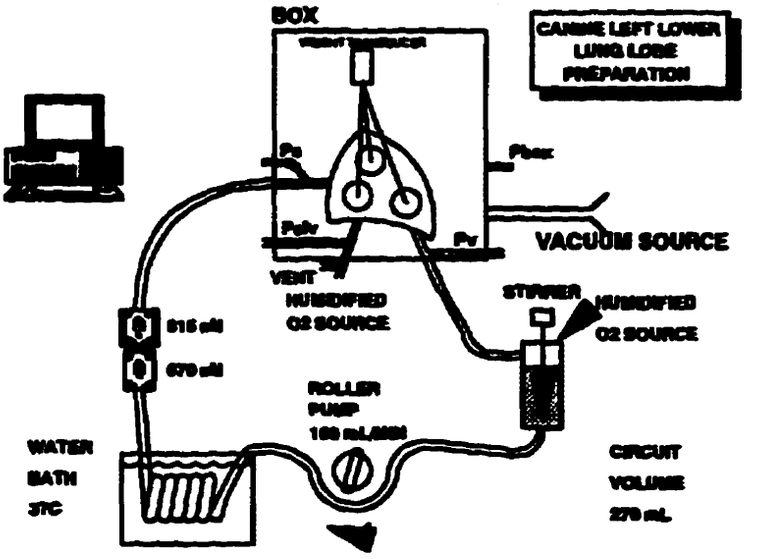
affected. No change in capillary permeability was expected with the increase in the capillary pressure to 25 cm H_2O as has been seen with higher pressures.[102]

METHODS

Animal preparation

Adult mongrel dogs of either sex weighing between 15 and 25 kilograms were used for the study. University Animal Care Committee approved protocols were followed and all animals were managed by CCAC guidelines. Twenty-four hours prior to the experiment each animal received an intravenous injection of Evans blue dye (EBD, 10 mg/kg) to label plasma proteins. Prior to the experiment the dogs received intravenous pentobarbital in a dose of 30 mg/kg for anesthesia and were intubated and ventilated with a Harvard apparatus respirator (15 ml/kg at 16 breaths per minute). The femoral artery was cannulated with IV tubing for later exsanguination to collect blood for the circuit. Paralysis was achieved with intravenous succinylcholine (20 mg), and a thoracotomy was performed through the left fifth interspace. After heparinization (400U/kg), the left lower lobe pulmonary artery, vein and bronchus were cannulated. The animal was exsanguinated with the blood collected for use in the circuit. The lobe was then supported on a mesh trampoline with the hilum in the dependent position as in Figure 2. The lobe was additionally suspended using EKG pads affixed to the surface of the lobe with cyanoacrylate adhesive. A suture was attached to each pad and the lobe was loosely wrapped in plastic. The entire preparation was then suspended from a

Figure 2: The excised canine left lower lobe preparation. (P_{alv}=alveolar pressure, P_a=arterial cannula pressure, P_v=venous cannula pressure, P_{box}= applied negative box pressure)



1. **The dog is given Evan's blue to label plasma proteins 24 hours prior to excision of the left lower lobe.**
2. **The excised lobe is perfused with blood and subjected to experimental conditions.**

Stratham force transducer for continuous weight determination and placed in a humidified airtight box that was constructed to house the entire preparation. Ports were constructed on the box to allow force transducer measurement of weight changes, arterial, venous, and airway cannulas as well as box pressure. The lobar pleural pressure was manipulated by the application of suction to the box. A box pressure of -5 cm H_2O (pleural pressure) was used for all lobes during the baseline period and then altered according to the protocol. The force transducer was connected to a Validyne CD-19 carrier demodulator and the signal displayed on a Brush Gould 260 oscillographic recorder. Data was also transmitted to an attached PC computer every 10 seconds. Rates of edema formation were calculated from the linear portion of the curve usually starting 10-20 minutes into the perfusion lasting to 50 minutes (see Figure 5 in the results). The bronchial cannula was attached to a humidified oxygen source (100% O_2) with an alveolar pressure of 0 cm H_2O. Lobar arterial and venous pressures were continuously measured with Validyne MP45-1 gauges with all signals displayed on the recorder and computer. The perfusion circuit consisted of a single reservoir of adjustable height to which the venous cannula was attached. This allowed modulation of the venous pressure (P_v) and thus the resulting capillary pressure (P_c) according to the following equation:

$$P_c=P_v+0.4(P_a-P_v) \qquad (11)$$

where P_a is the arterial cannula pressure. A Masterflex Digistaltic pump (model 7021-20) was used to return blood to the lobe at a constant rate of 100 mL/min. The blood went through a heat exchanger set at a constant 37°C prior to a macropore filter and returning to the arterial cannula. The circuit tubing went through a device called a cuvette which contained 815 and 670 nm laser diodes and detectors. Transmittance data went to the recorder and computer. The change in voltage was used as described elsewhere to calculate edema based on the 815 nm signal. Rates were calculated from the linear part of the curve (see Figure 6 for data from a typical experiment based on the 815 nm signal). Data from the 670 nm signal combined with the 815 nm signal were then used to estimate protein concentration changes and thus CF/CP (the ratio of filtrate to plasma protein concentrations). Figure 7 in the results section shows the blue labeled protein concentration data for a typical Group 2 experiment.

The lobes were then transiently inflated to 25-28 cm H_2O to reduce atelectasis. The lobes were then allowed a period of stabilization (30 minutes) with P_v=0 cm H_2O. This resulted in a P_a of about 10 cm H_2O and a P_c of about 2 cm H_2O. The box pressure was -5 cm H_20. The laser signals were then calibrated as described in the section on calibration.

The Laser Colorimetric Device

The laser colorimetric device was designed and built to accurately follow changes in hematocrit and protein concentration on-line with isolated organ experiments. Figure 2

shows the excised canine left lower lobe preparation used in this experiment. Two laser diodes are placed in the cuvette surrounding the circuit tubing with detectors opposite the laser sources. The 815 nm (isobestic point) laser is used to follow changes in hematocrit. As edema forms in the hemoperfused lung lobe, the concentration of red cells will increase in the circuit as they are largely excluded from the filtrate. This results in decreased transmittance of the signal, which is measured as a change in voltage at the detector, as described by the Beer-Lambert law (equation 3). Equation 2, introduced earlier in this paper, relates hematocrit to fluid exchange. Changes in hematocrit followed by the 815 nm signal allow accurate measurements of transvascular fluid exchange. Validation of this technique has been published by Hancock et al.[10] The second laser diode is at 670 nm, a wavelength that is absorbed by the Evans blue dye (EBD) labeled protein and also by hemoglobin. If the hemoglobin effects are removed by using data from the 815 nm signal, the 670 nm signal can be used to follow changes in EBD labeled protein in the circuit. Weight transients, plasma hematocrits, protein concentrations and colloid osmotic pressure were measured to compare to the data from the two signals.

Calibration of the Laser Signal

The laser signal was initially allowed to stabilize to a steady drift free signal over thirty minutes. Three five ml aliquots of plasma were obtained by centrifuging blood obtained from the dog . The dog had received Evans blue dye 24 hours prior to plasma collection. Thus, the plasma proteins were labeled with the blue dye. A series of three

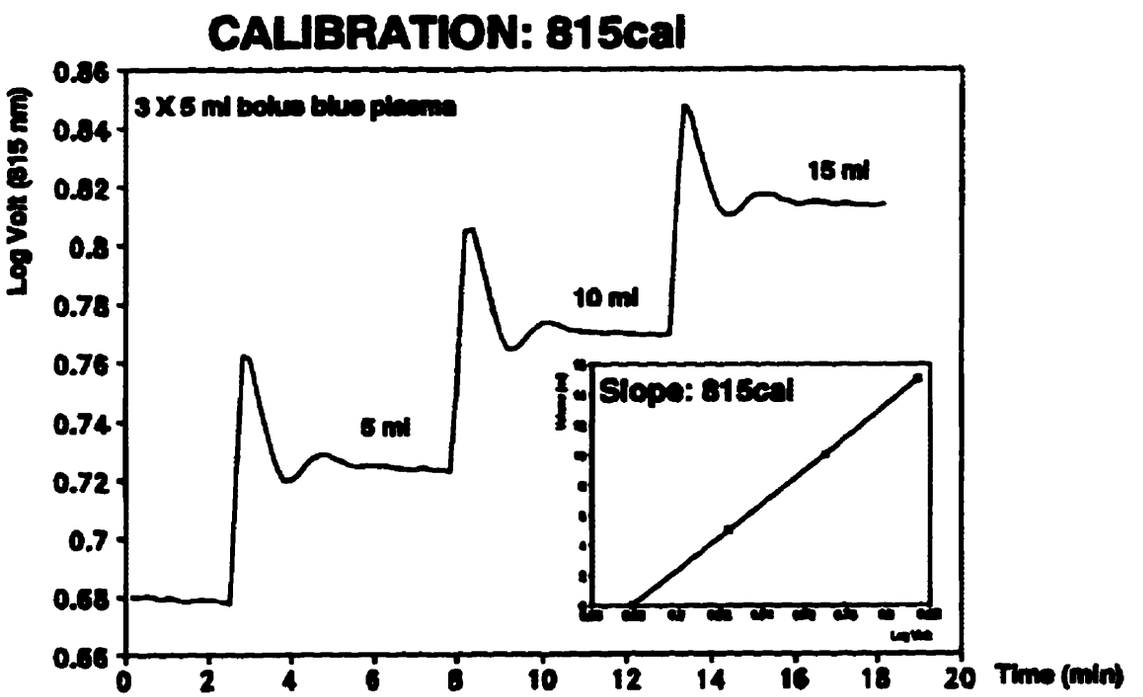
boluses were introduced into the circuit via the reservoir resulting in dilution of the red cells, but not the EBD labeled proteins. The effect of these three boluses on the logarithm of the 815 nm signal over the 20 minute calibration period can be seen in Figure 3. It should be noted that changes in the 815 and 670 nm signals after these boluses are entirely due to changes in the red cell concentration. This fact is used later in calibration for following protein concentration changes. Since the dilution of the red cells is equivalent to fluid resorption, changes in the 815 nm signal could then be used to estimate fluid exchange. A regression equation was then made relating the equivalent of the fluid exchanged (0, 5, 10, and 15 ml) to the mean logarithm of the 815 nm signal at the respective four time points (prior to the first bolus and following stabilization of the signal after each bolus). The following regression equation was obtained:

$$Qc_f = \log(815) \text{ X } 815cal + constant \quad (4)$$

where Qc_f represents volume exchanged based on the 815 nm signal, log(815) is the logarithm of the 815 nm signal at any point in time, and 815cal is the slope of the regression line. The linear correlation was excellent in all cases with R^2 in the order of 0.99 or greater. This calibration technique allowed on-line changes in hematocrit to determine fluid filtration. Experiments to validate this technique are described elsewhere.[50]

Another calibration procedure was then performed to follow EBD labeled protein concentration changes in the circuit. First of all, a second linear regression was done

Figure 3: Calibration of the 815 nm signal. This calibration involves giving three 5cc boluses of the dog's plasma labeled with Evans blue dye. These boluses change the red blood cell concentration without affecting the concentration of the blue labeled proteins. The inset depicts the regression curve obtained from the calibration, which allows accurate measurement of transvascular fluid exchange.



relating the logarithm of the 670 and 815 nm signals from the EBD labeled plasma boluses as follows:

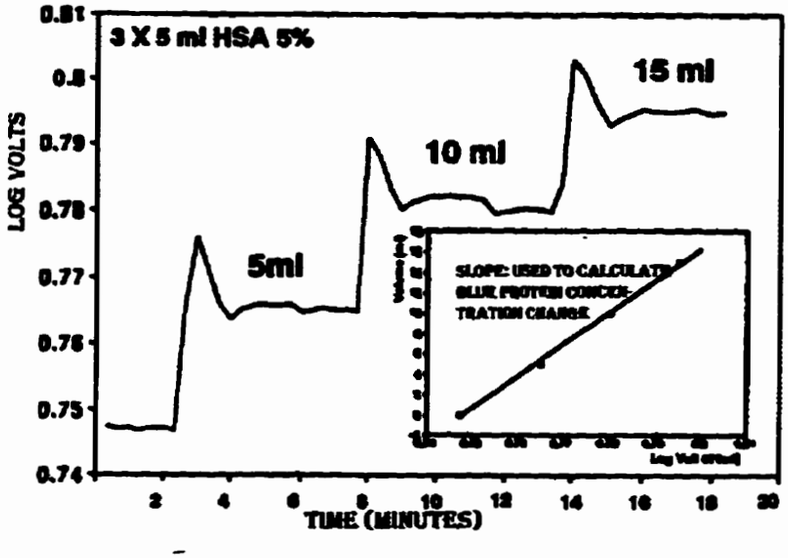
$$\log(670)=\text{RATIO X } \log(815)+\text{constant} \qquad (5)$$

where RATIO represents the slope of the regression line. Excellent linear correlation was present in all cases. Since these boluses only affect the red cells, this equation relates the red cell contribution to the 670 nm signal. Then three 5cc boluses of 5% human serum albumin (HSA) were added to the circuit as shown in Figure 4. This resulted in changes in concentration of both the red blood cells and EBD labeled protein. To find the effect on proteins, the red cell effects need to be removed from the 670 nm signal. The red cell contribution to the 670 nm signal was calculated by substituting the new logarithm of the 815 nm signal (called P815) into equation 5 for each of the new boluses. This was termed logadj670 and results in the following equation:

$$\text{logadj670}= \text{RATIO X } \log(\text{P815}) + \text{constant} \qquad (6)$$

The red cell contribution to the logarithm of the 670 nm signal (logadj670) was then subtracted from the logarithm of the 670 nm signal obtained following the HSA boluses (logP670) according to the following equation:

Figure 4: Calibrating for Evans blue labeled protein. Three boluses of 5% HSA are administered over a 20 minute period. The 670 nm signal as shown is affected by red blood cell and blue labeled protein concentration changes. The red cell contribution must be removed from the signal prior to the calibration regression shown in the inset.



$$BLU670 = logP670 - logadj670 \qquad (7)$$

where BLU670 represents the blue labeled protein changes contribution to logP670. Since the circuit volume (250 ml plus approximately 15cc of blood in the lobe prior to the start of perfusion) and the hematocrit are known (from samples drawn from the reservoir) plasma volume (V_p) can be calculated:

$$V_p = V_c \, X \, (1 - \text{hematocrit}) \qquad (8)$$

where V_c is the circuit volume. If an arbitrary concentration of 100 units is given as the initial protein concentration prior to the HSA boluses and we ignore the small contribution of the HSA to the protein in the circuit, the protein concentration (CP) following each of the three 5cc boluses can be calculated. If a linear regression of BLU670 and CP is done excellent correlation is obtained according to the equation :

$$CP = BLU670 \, X \, BLUCONC + constant \qquad (9)$$

where BLUCONC is the slope of the regression line. Changes in 815 and 670 nm signals can then be used to follow plasma blue labeled protein changes during perfusion of an isolated lung lobe. If the volume filtered across the lung is known as well as the changes in CP, then the concentration of blue labeled protein in the filtrate (CF) can be calculated:

$$CF=(PV_t X CP_t - Pv_{t+1} X CP_{t+1})/(PV_t - PV_{t+1}) \quad (10)$$

where t and t+1 are two time points, PV is the plasma volume and CP is the protein concentration of plasma. The ratio CF/CP is often used to estimate capillary leakage of protein as a high concentration of protein in the filtrate relative to plasma usually constitutes a permeability problem.

By carefully following this calibration procedure the device can be used to not only detect small changes in hematocrit but also changes in protein in both the plasma and filtrate.

Filtration periods

Arterial blood gases (ABG), hematocrits, colloid osmotic pressure (COP) measurements, and plasma protein levels were drawn serially at baseline and following each intervention. Equation 2 allowed calculation of filtered volumes on the basis of the hematocrit change. This was used as an attempt to validate the laser colorimetrically derived data. Changes in colloid osmotic pressure and plasma protein concentration were used to validate the colorimetric protein data.

Following the period of stabilization the venous reservoir was raised to achieve a capillary pressure of 15 cm H_20. Each lobe was perfused for a 50 minute baseline period with P_c =15 cm H_2O, P_{box} =-5 cm H_20, and P_{alv}=0 cm H_20. The lobes were assigned to

one of three groups (n=12 for each group) with a second 50 minute perfusion period as follows: Group 1 (control) with P_c =15 cm H_2O, P_{box} =-5 cm H_2O, Group 2 with P_c=15 cm H_2O, P_{box} =-15 cm H_2O, and Group 3 with P_c=25 cm H_2O, P_{box} =-5 cm H_2O. Note that for groups 2 and 3 the transpulmonary pressure was increased from 20 to 30 cm H_2O in both cases although by different mechanisms. From the simplified model of the lung in Figure 1, it can be seen that changes in P_c should affect alveolar and extra-alveolar vessels equally . Changes in P_{box} would be expected to affect only the extra-alveolar vessels as this pressure change would not reach the alveolar vessels that are subjected to the alveolar pressure which has not changed.

Any blood loss from the lobe was collected and measured at the end of the experiment.

Statistical Analysis

Statistical analysis was performed using commercial software (WINKS, Texasoft, Dallas, Texas). Data are presented as the mean ± standard error of the mean (SE). A significance level of less than 0.05 was chosen for all comparisons. When appropriate, paired t-tests were used for analysis. For multiple comparisons, where appropriate, two way repeated measures ANOVA was used with Newman-Keuls Multiple Comparisons.

Results

There was no difference in dog weights between the groups (19.58±0.91 vs. 19.58±0.43 vs. 19.25±0.70 kg for groups 1 to 3 respectively). Mean blood loss from the lobes was 15.42±6.65 ml vs. 20.17±6.93 ml vs. 20.42±6.65 ml respectively. This loss was not different between groups.

Figures 5 to 7 show some of the typical data obtained during the experiments. In Figure 5, change in weight of a typical control lobe is followed over time for two filtration periods. The rapid weight gain seen in the first few minutes is due to an increase in the capillary pressure (P_c) from about 2 cm H_2O to 25 cm H_2O at the start of the initial filtration period resulting in rapid weight gain from vascular volume changes. This is followed by a period of relatively constant period of weight gain which is interpreted by many investigators to represent transvascular fluid flux. Figure 6 demonstrates the transvascular fluid flux (edema) based on the 815 nm laser colorimeter for a typical group 3 experiment. The slope of the lines estimates rate of fluid flux which is linear for the two filtration periods. Note that during the second filtration period the box pressure had been decreased to -15 cm H_2O resulting in an increased rate of filtration. Figure 7 depicts changes in the relative concentration of blue labeled plasma proteins (RCp is the concentration relative to a baseline of 100) over time for a group 2 experiment. Note that the concentration of proteins is relatively constant during the initial filtration period with a slight increase in concentration over time at the more negative box pressure.

Figure 5: Data from weight transients. This is from a typical control experiment (Group 1). Following the initial rapid weight change in the initial filtration period, weight changes are considered to represent transvascular fluid flux.

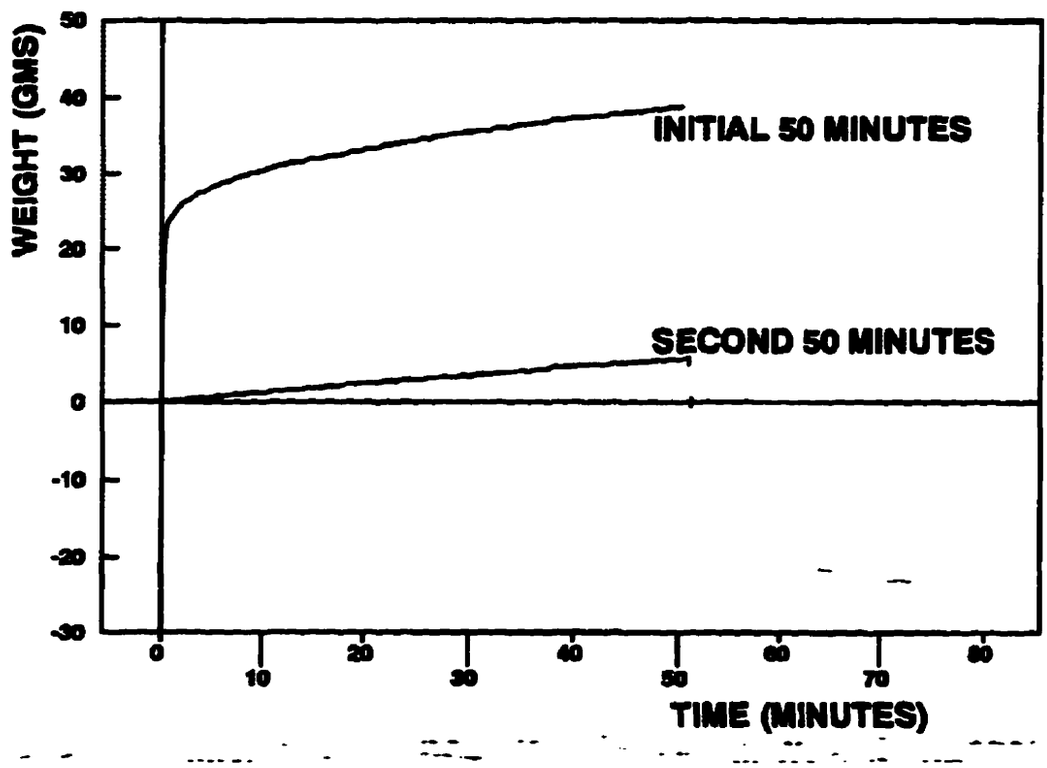


Figure 6: Fluid filtration based on the 815 nm signal. This represents fluid exchanged during the two filtration periods for a typical Group 3 experiment. This allows accurate continuous measurement of transvascular fluid exchange.

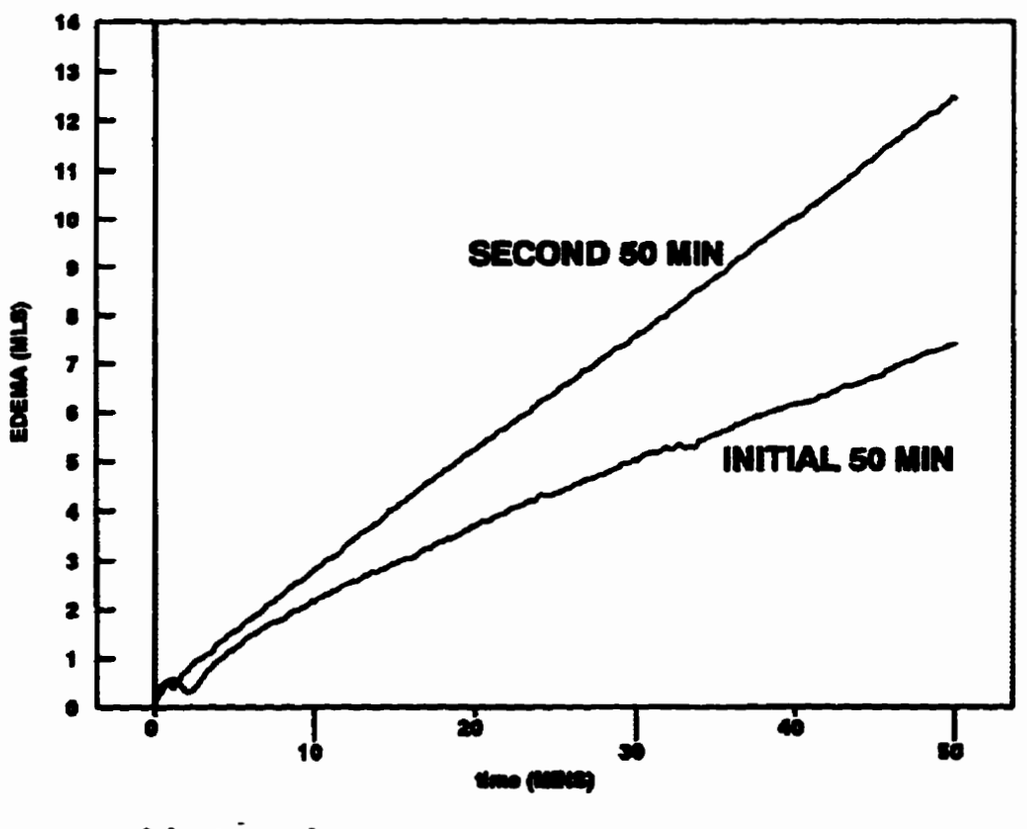
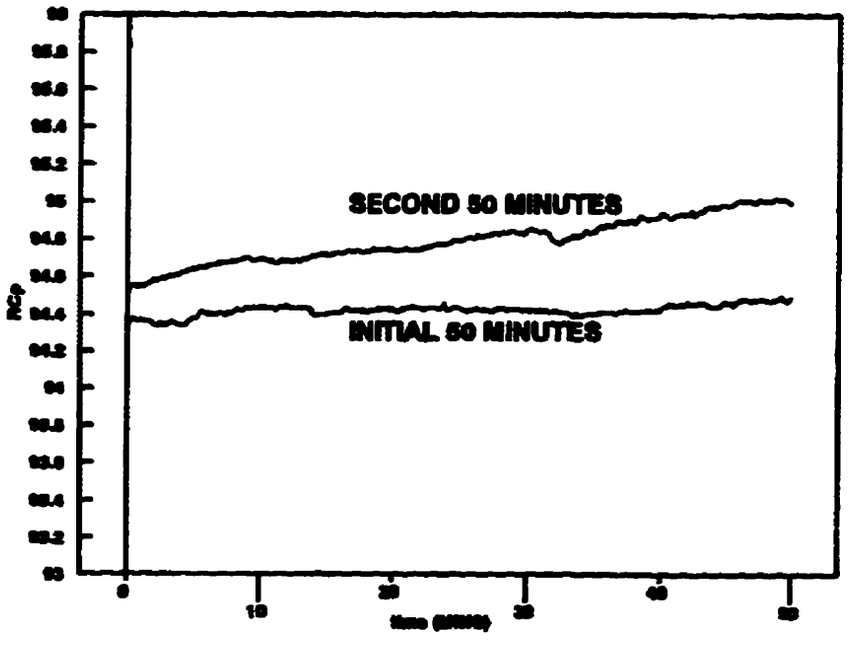


Figure 7: Blue labeled protein concentrations. Rcp represents the relative concentration of blue labeled plasma protein. These data are based on the 815 and 670 nm signals for a typical Group 2 experiment. This allowed continuous measurement of plasma and filtrate protein concentrations.



	EDEMA BASED ON WEIGHT TRANSIENTS(ml/hr/gm dry weight)		EDEMA BASED ON 815nm LASER SIGNAL (ml/hr/gm dry weight)		EDEMA BASED ON HEMATOCRIT CHANGES(ml/hr/gm dry weight)	
Filtration period	Initial	Final	Initial	Final	Initial	Final
GROUP 1 (control)	1.031±0.100	0.763±0.064 $p<0.02$	0.333±0.071	0.431±0.100 NS	0.568±0.088	0.766±0.061 NS
GROUP 2 (P_{box}=-15)	0.915±0.132	2.308±0.268 $p<0.001$	0.420±0.082	1.383±0.246 $p<0.001$	0.690±0.097	1.516±0.257 $p<0.01$
GROUP 3 (P_c=25)	0.875±0.093	1.830±0.188 $p<0.001$	0.381±0.048	0.816±0.087 $p<0.001$	0.745±0.086	1.062±0.126 NS

Table 1. Summary of filtration rate data. Significance is based on paired t-test comparing initial and final filtration periods. See text for details regarding comparisons between methods.

	CF/CP BASED ON LASER COLORIMETRY (averaged over each filtration period)		CF/CP BASED ON PLASMA PROTEIN MEASUREMENTS		CHANGE IN COP (units per hour)	
Filtration period	Initial	Final	Initial	Final	Initial	Final
GROUP 1 (control)	1.176±0.073	1.041±0.069 NS	1.004±0.316	1.246±1.430 NS	0.231±.099	0.578±0.130 NS
GROUP 2 (P_{box}=-15)	1.143±0.090	0.921±0.034 $p<0.05$	1.032±0.259	0.647±0.151 NS	0.521±0.109	0.848±0.107 $p<0.05$
GROUP 3 (P_c=25)	1.099±0.091	0.914±0.033 $p<0.05$	1.515±0.283	1.443±0.314 NS	0.536±0.115	0.874±0.064 $p<0.05$

Table 2. Summary of protein data. Significance is based on paired t-test comparing initial and final filtration periods. See text for details regarding comparisons between methods.

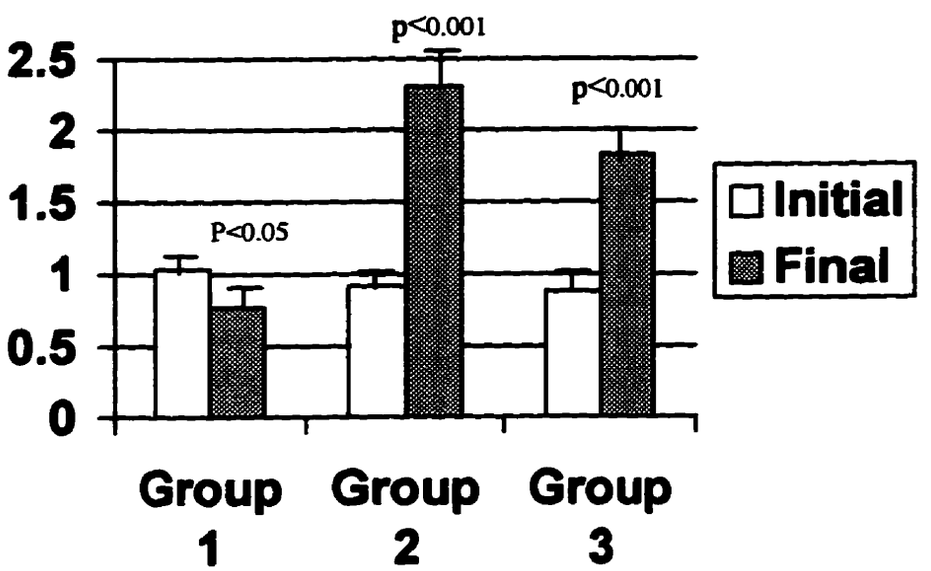


Figure 8: Filtration rates based on weight transients in ml/hour/gm lobe dry weight. Data are mean with standard error.

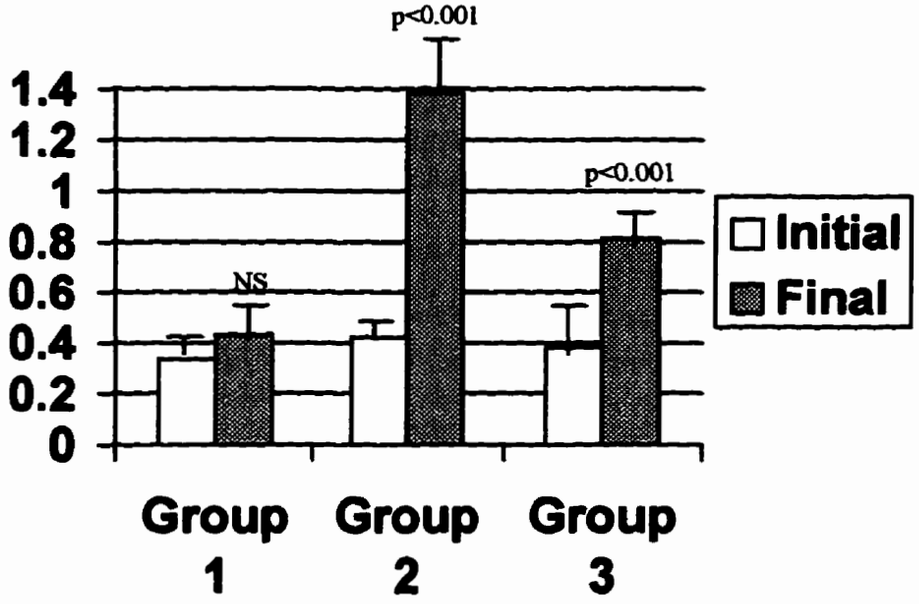


Figure 9: Filtration rates based on 815 nm signal in ml/hr/gm lobe dry weight. Data are mean with standard error.

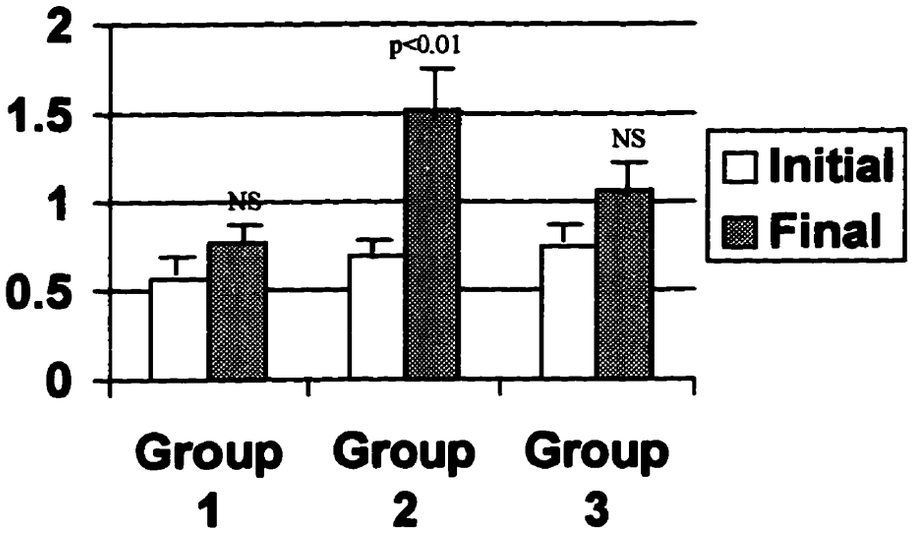


Figure 10: Filtration rates based on hematocrit changes(ml/hour/gm lobe dry weight. Data are mean with standard error.

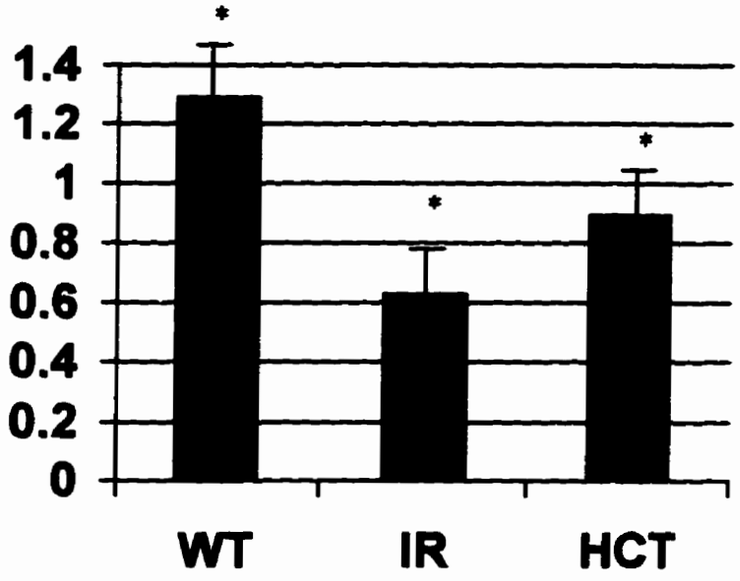


Figure 11: Comparison of edema based on weight transients(WT), laser colorimetry (IR), and hematocrit (HCT) in ml/hour/ gm dry lobe weight averaged across all groups. There is a significant difference between all three groups ($p<0.05$).

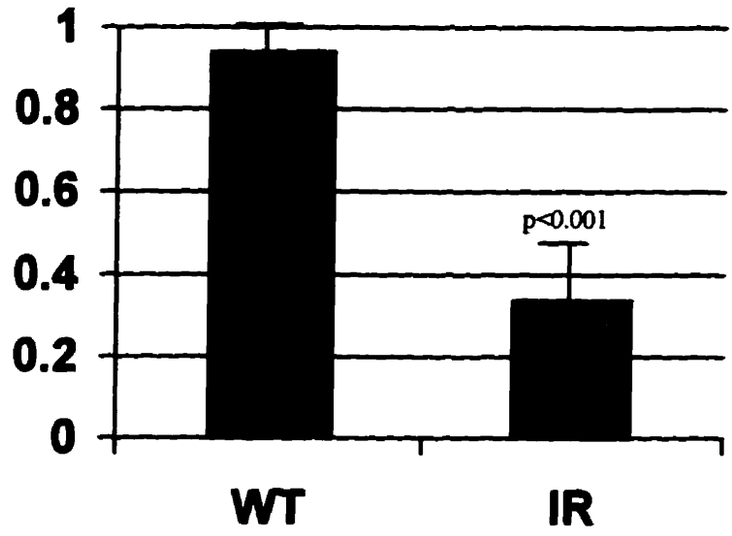


Figure 12: Comparison of edema based on weight transients (WT) and laser colorimetry (IR) during the initial perfusion period averaged across all groupsin ml/hour/gm lobe dry weight.

The initial baseline measurement of filtration did not differ between the three groups regardless of the method of determination (see Table 1). Using weight transients, the filtration rate during the initial period exceeded the final period in group 1 lobes (see Figure 8, $p<0.05$). Weight transients consistently significantly exceeded determinations based on colorimetry and hematocrit (see Figures 8 to 10). The overall filtration data combined for both periods for each method is further summarized in Figure 11 again demonstrating that weight transients overestimated edema relative to the other methods ($p<0.05$). This was especially evident during the initial filtration period. To increase the power of the comparison, rates calculated during the initial filtration period for all lobes are compared using weight transients versus colorimetric data (Figure 12). This demonstrates a large difference between the two methods with weight transients exceeding laser colorimetric data. A more negative box pressure (Group 2) resulted in a dramatically increased rate of filtration using any method of determination and exceeded all other groups ($p<0.05$ by two way repeated measures ANOVA), as shown in Figures 8-10. An increase in capillary pressure resulted in a less dramatic but nonetheless significant increase by colorimetry and weight transients ($p<0.05$ by two way repeated measures ANOVA) but not by hematocrit change.

Data on protein concentration change reported as CF/CP is summarized in Table 2. CF/CP did not differ significantly between groups 1 to 3 during the initial filtration period when analyzed by ANOVA using colorimetry or plasma protein concentrations. In control lungs, there was no difference between initial and final filtration periods.

Based on laser colorimetry, group 2 and group 3 lobes had a decrease in CF/CP during the final filtration period ($p<0.05$). There was no difference between group 2 and group 3 CF/CP during the final filtration period. No differences could be detected between the groups using protein measurements. COP increased significantly between initial and final filtration periods in group 2 and 3 lobes.

The ABGs confirmed adequate oxygenation of the preparation to eliminate the possibility of hypoxia affecting the results.

Discussion

Laser colorimetry is an elegant way to follow fluid and protein exchange in this isolated lung preparation. It can be used to accurately follow changes in fluid filtration moment by moment using an 815 nm laser diode. Filtration rates between the two 50 minute periods in control lobes were very consistent. There were no significant time related changes with respect to filtration or colorimetric CF/CP between the initial and final filtration periods. This demonstrates that the lobes were quite stable for the duration of the experiments. The initial filtration period for each group acted as a further control demonstrating no differences during this time between the groups. We then used this technique to observe differences between isolated lung lobes subjected to an equal change in transpulmonary pressure by either decreasing pleural pressure (group 2) simulating negative pressure pulmonary edema (such as with upper airway obstruction

or lung re-expansion), or increasing capillary pressure (group 3) simulating hydrostatic pulmonary edema seen with heart failure.

In group 2 lobes, the negative pressure in the box was decreased from -5 to -15 cm H_2O simulating the change in pleural pressures seen with negative pressure pulmonary edema. As was stated earlier, normal mean pleural pressures are about -8 cm H_2O during inspiration and -3.4 cm H_2O during expiration.[63] Thus the mean pleural pressure approximates the pressure used during the control period. Since pleural pressures of -54 cm H_2O have been measured during forced inspiration against a closed glottis,[63] the box pressure of -15 cm H_2O was a fairly small change. This change in box was chosen to match the change in transpulmonary pressure in group 3. The magnitude of change in group 3 was chosen to minimize the chance of developing a permeability leak while still expecting an increase in fluid filtration. The decrease in box pressure can result in not only changes in hydrostatic interstitial pressure but also an increase in lung volume. By definition the hydrostatic pressure change should preferentially affect the EAVs. As the alveolar vessels are generally thought to be the main filtering unit in the lung, a relatively small increase in filtration might be expected with this intervention, especially with this being a small change compared to changes in pleural pressures in clinical NPPE. Surprisingly, this resulted in a very dramatic increase in filtration from a baseline of 0.420 to 1.383 ml/hr/gm dry lobe weight by laser colorimetry exceeding the results obtained for group 3 lobes. How can this be explained? From the time of the studies by Bowditch and Garland (1879)[103] there has been nearly complete agreement that inflation of the lungs with negative pressures with vascular pressures constant relative to alveolar

pressure causes an increase in pulmonary blood volume. The evidence suggests that the increase in volume is largely in the EAVs. The transmural pressures of the EAVs increase both because of the rise in vascular pressure relative to pleural pressure and the fall in net stress on the outer surface of the EAVs. Macklin observed that the diameter and length of the intraparenchymal vessels (EAVs) increased with lung inflation.[30] These findings were confirmed by Howell et al who also found that variations in the extra-alveolar vascular pressure-volume behaviour with lung inflation indicated that the surrounding lung parenchyma applied axial and radial stresses on the EAVs.[101] Thus increased negative pleural pressures resulting in increased lung volumes will ultimately increase the surface area of these vessels. The pressure-volume relationship (compliance) of the interstitium surrounding vessels is also a major determinant of lung fluid balance.[29] The interstitial compliance around the EAVs is thought to be much higher than in other parts of the lung.[29] There is evidence to suggest that increasing lung volume with increasingly negative interstitial pressures makes the interstitial space more compliant.[28]

To find out if changes in filtration rates were caused by permeability changes we looked at protein flux with the 670 nm laser. CF/CP had a small but significant decrease from 1.143 to 0.921. This paralleled the change seen with group 3. Thus, under these conditions, increased negative pleural pressures resulted in a large increase in filtration of relatively protein poor filtrate, which contradicts some reports which measured proteins in edema fluid of patients with negative pressure pulmonary edema.[38,39,66,67] Our data suggests that this increase in filtration is not due to a capillary leak problem. Instead, it is

likely that the change in pleural pressures resulted in an increase in lobe volume (the lobe was not restricted from expanding) with a subsequent increase in surface area of the extra-alveolar vessels as result of distention of previously filtering vessels and/or recruitment of previously collapsed vessels. If this were the sole explanation, then CF/CP should have remained constant as a change in surface area will not change the relative amounts of fluid and protein flux unless there is an injury to the membrane (which would result in an increase in CF/CP, not a decrease). Because CF/CP decreased significantly, hydrostatic forces must also have had an effect on the filtering vessels as increased hydrostatic forces will result in excess fluid filtration relative to protein (unless very high hydrostatic pressures damage the filtering vessels).

The third group of lobes was done to compare the group 2 lobes to a hydrostatic group with repect to filtration and permeability characteristics. Group 3 lobes had an increase in filtration from a baseline of 0.381 to 0.816 ml/hr/gm dry lobe weight by laser colorimetry. This intervention would be expected to have an equivalent effect on alveolar and extra-alveolar vessels. Increased capillary pressure pulmonary edema may result in stretching or disruption of endothelial membranes or intracellular tight junctions and thus affect permeability to protein.[100] We did not expect this to occur at the capillary pressures used in this experiment (15-25 cm H_2O). West et al found that in anesthetized rabbit lungs stress failure did not occur until capillary pressures exceeded 40 mmHg.[102] Since increasing capillary pressure (in the range used in Group 3) should not result in a capillary permeability problem, it would be expected that as more fluid is filtered it would be relatively protein poor compared to the baseline period. This is in fact borne

out by a small but significant change in CF/CP from 1.099 during baseline to 0.914 as obtained by laser colorimetry during increased capillary pressure.

Attempts were made to validate the laser colorimetric CF/CP data by following changes in plasma protein concentrations and colloid osmotic pressure. Plasma protein concentration measurements turned out to be a much less sensitive way to measure protein exchange and thus no significant differences could be found. This is because the equipment used to measure the protein concentration was not sensitive enough to measure the small changes present in this experiment. Plasma COP measurements also turned out to be unreliable in determining protein exchange although overall the data seems to support the colorimetric data. In group 3, COP increased with increased capillary pressure ($p<0.05$ by paired t-test).

The filtration data based on weight transients deserves further examination. These results consistently produced higher filtration rates than results based on either colorimetry or hematocrit changes. Figure 11 compares the combined data for edema based on weight transients, colorimetry and hematocrit changes. The data was combined to increase the statistical power of the comparison. Either weight transients overestimate edema or colorimetry underestimates it. It has been assumed in the literature that the effect of vascular volume changes on weight transients only lasts several minutes.[10,12,14] In Group 1 (control lobes), filtration based on weight transients decreased from a baseline of 1.031 to 0.763 ($p<0.02$) during the final period. If vascular volume changes were not taking place beyond the first few minutes these two periods should have had equivalent rates. This data strongly suggests that vascular volume

changes are in fact occurring for a much longer period than is generally accepted. Hancock *et al* [10] compared colorimetric measurements of transvascular fluid exchange with weight changes and also found that slow vascular volume changes persisted for 40 minutes. They found that colorimetric determinations averaged 60% of those based on weight transients. The data from this experiment is further proof that experiments relying on weight transients should be interpreted with caution as slow vascular volume changes may indeed persist for long periods of time.

CF/CP based on laser colorimetry during the baseline for all groups was greater than one. This contradicts values that are published in the literature. Steady-state lymph to plasma protein concentrations the literature are in the range of 0.6 to 0.7.[97,98,99] If CF/CP were greater than one, it would mean that the filtrate contained a higher concentration of protein than plasma. As was discussed earlier, protein transport across the membrane may be due to either diffusion (down a concentration gradient), convection (being carried with the fluid), or active transport. The interstitial protein concentration is about 60% of the plasma level[28] so that diffusion of protein across this gradient is expected. In a normal lung with low flux of fluid, diffusion is the most important factor in protein transport[29] which could result in a CF/CP greater than one. As transvascular fluid flux increases, convection begins to dominate.[29] Active transport is not thought to be a major factor. It is possible to have a CF/CP in the range obtained with this experiment but it is more likely that there is a consistent error in the method that elevates the values of CF/CP. If the CF/CP were falsely elevated, it implies that either the lobes were damaged during the preparation or there was a methodological

problem. Some investigators have contended that excised canine preparations are not comparable to the *in situ* condition and that damage occurs in the excision process.[68] Others have validated the use of excised preparations and demonstrated their stability.[69] Numerous experiments in our laboratory have also shown that these preparations are stable for a number of hours. In fact the slow rates of filtration regardless of the method of measurement in this experiment would support that these lobes were not in fact damaged. It is , however, more likely that there is a consistent error in the methodology that overestimates filtrate protein concentration. Dallal *et al* found that the use of Evans blue dye (EBD) as a marker of extravascular protein leakage resulted in a permeability five times higher than that obtained using ^{125}I-labeled albumin[71]. They concluded that EBD was not a reliable marker because it rapidly binds to lung tissue proteins. We have attempted to prevent this by saturating tissue proteins by administering the dye 24 hours prior to the experiment, but it is still possible that there is continued loss of the dye as a result of further binding to tissue proteins, circuit tubing, or HSA added during the calibration. The absolute value of the CF/CP obtained may not be accurate. Since the error should be consistent across all experiments, the comparison of groups and filtration periods remains valid.

One of the reasons for studying the group 2 lobes was to try to understand the clinical problem of negative pressure pulmonary edema. Can these results from an isolated lobe preparation be extrapolated to the clinical problems seen in intact patients? Using an isolated organ has advantages and disadvantages. The potential advantages include the ability to control for some of the factors involved. For example, in this

experiment we controlled for the hemodynamic variables by perfusing at a constant rate and pressure. We also tried to control for tissue hypoxia by ascertaining a high pO_2 for the duration of the experiment. The disadvantages of an isolated organ experiment include the fact that they are not very physiologic, they do not include neurohormonal factors that may be important, the organs may be damaged during removal, and the preparations are inherently unstable and have a limited life span. Certainly increased negative pleural pressures should result in increased filtration on the basis of hydrostatic gradients (ie increased pressure gradient between the lumen of the vessels and the interstitium, especially with respect to the EAVs and the extra-alveolar interstitial space). In the experiment, lobes were able to freely expand within the limits of the compliance of the lobes, but were not limited by the presence of a chest wall. In the clinical problem of REPE, the lung starts out collapsed and also re-expands for a period of time without being restricted by the chest wall. Certainly the forces attempting to re-expand the lung may preferentially affect the EAVs (as alveolar pressure is kept constant) with significant radial traction on these vessels. This will be even more important if the alveoli remain collapsed due to airway plugging and loss of surfactant as the forces expanding the lung will be expanding the extra-alveolar space preferentially. With NPPE due to UAO the chest wall and diaphragm attempt to expand the lung. Although the chest wall mechanics are certainly different with NPPE due to UAO than seen in group 2 lobes, there is evidence to suggest that these lungs (in the clinical scenerio) may also be hyperinflated.[92,104] Thus the negative forces created by the chest wall can still result in an increase in volume of the lung and EAVs. It has been suggested that negative pressure

pulmonary edema is a consequence of hemodynamic changes (effects on the heart chambers and venous return) or alveolar hypoxia resulting in damage to the lung. The lobes were perfused at a constant rate with the pump, and oxygen levels in the circulating blood were always greater than 300 mmHg. The lungs were ventilated with 100% O_2 prior to starting the experiment and a constant low flow source of 100% O_2 was attached to the bronchus to decrease the chances of alveolar hypoxia. It is unlikely that tissue oxygen levels were low during the duration of the experiments. Therefore in the clinical setting hemodynamic effects and alveolar hypoxia may be contributing effects but may not be absolutely necessary.

Further studies should be done to look at what effects the increased negative pressure has on lung volume. It would also be interesting to see what effect the increased negative pressures would have on filtration if the lung could be restricted from expanding in total volume. Another experiment could be done with obstructing the bronchus after the lung has been inflated and then increasing the negative pleural pressure. This intervention should affect the IAVs as well as the EAVs.

Potential Sources of Error

Although the laser colorimetric device appears to be an accurate and highly sensitive device for measuring transvascular fluid and protein flux, several potential sources of error exist and must be minimized.

Calibration of the device with the plasma and HSA is a potential source of error. The bolus volume needs to be accurate. The calibration needs to be done prior to each experiment. The highly significant linear correlation obtained between bolus volume and the logarithm of the laser signals seems to prove that this is not a major concern. The second calibration would ideally be done with the dog's own plasma drawn prior to administration of the Evans blue dye instead of the HSA as was done in the past. The HSA was used to simplify the experiment, but the unlabeled protein may lead to inaccuracies in the calculation of labeled protein concentrations since the new protein absorbs dye. The sodium load from the HSA may also affect cell volumes leading to inaccuracy. If we ignore the plasma volume of the lobe prior to adding blood to the circuit, the circuit volume was 250 ml. The mean hematocrit was 33.01 prior to start of filtration resulting in a plasma volume of 167.5 ml. 15 ml of EBD labeled plasma had been added to the circuit prior to the HSA, resulting in a circuit plasma volume of 182.5 ml. If the initial sodium concentration is assumed to be 140 meq/l, the sodium content of the plasma is 25.5 meq. Since the sodium content of 5% HSA is 154 meq/L, 15 ml of HSA would contain 2.31 meq of sodium. This would increase the plasma sodium to

141.1 meq/L, an insignificant change. If the plasma albumin concentration prior to addition of the HSA is assumed to be 40 gm/L, the albumin content of the 182.5 ml of plasma would be 8.0 gm. Fifteen ml of 5%HSA contains 0.75 gm of unlabeled albumin. The resulting plasma albumin would be 44.3 gm/L. Although the EBD is also bound to other plasma and tissue proteins, the addition of this unlabeled protein load could affect the results.

Significant hemolysis has been described in hemoperfused circuits.[70] Hemolysis would be seen by the colorimeter as dilution from resorption. Accumulation of free hemoglobin could also potentially affect the signal. The signal has been verified to be extremely stable for prolonged circuit experiments without organs in the circuit. Free hemoglobin levels when measured by Landolfo while using this experimental setup were minimal and the addition of free hemoglobin had no significant effect on the results. [50]

Significant hemorrhage into the lobe or from the circuit could affect the results. Hemorrhage into the lobe was avoided by careful handling of the lobe. Most lobes had little or no external evidence of hemorrhage. Loss of blood from the circuit was measured during the duration of the perfusion. Although most lobes had a small amount of blood loss (about 10 cc), this did not appear to be a major factor as the hematocrit was not significantly affected. Error due to evaporation was minimized by humidifying the box and using humidified oxygen.

Other sources of error such as red cell trapping in the lung are more difficult to identify. Overall, these do not seem to add up to much error. This has also been verified using circuits with hemofilters to measure losses. [50] With these experiments the volume

of filtrate removed from the circuit is known as it comes out of a hemofilter. These data have correlated well with simultaneous colorimetric data.

SUMMARY

This thesis has reviewed some of the theory concerning fluid and protein exchange in the lung. The clinical problem of highly negative pleural pressures due to pulmonary re-expansion and upper airway obstruction has been discussed. The pathophysiology of this problem has been poorly investigated in the past. Increased negative pleural pressures in the experiment described in this paper resulted in a dramatic increase in filtration of relatively protein poor fluid. This suggests that the increased filtration from the increased negative pleural pressures was due to a combination of increased surface area of extra-alveolar vessels and a change in hydrostatic pressure rather than a permeability problem. Hemodynamic consequences of negative pleural pressures and alveolar hypoxia do not appear to be mandatory for increased filtration, but may play a role in clinical NPPE.

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