PULMONARY TRANSVASCULAR FLUID FLUX: ITS MEASUREMENT AND MANIPULATION

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by

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Abstract

The forces governing fluid movement were investigated, for which an optical method to measure transvascular fluid flux independently from weight changes was developed. Validation of this method was performed and the results indicated that there was no significant difference between filtration measured by laser colorimetry or weight changes. Transvascular fluid filtration was then compared in excised canine lobes by three methods; weight, sample microhematocrit, and laser colorimetry. In Section I, measurements were made before and after lung injury had been induced with oleic acid (0.15ml). When rates of linear weight gain were compared to measured filtration from sample microhematocrit or laser colorimetry there was no significant difference between methods either with or without oleic acid added to the system. It was concluded therefore that transvascular fluid flux could be characterized by the relationship constant fluid flux (Jv), to vascular pressure (Pc), and that Jv could be obtained from either laser colorimetry or linear weight gain. In section II, the relative contribution of alveolar and extra-alveolar vessels to fluid exchange was investigated. Pc(crit), the point at which the forces across the membrane are in balance, was affected by

changes in alveolar pressure. The significance of the extra-alveolar vessels in transvascular fluid flux was questioned. This suggested the importance of the alveolar vessels in transvascular fluid flux, and suggested the possibility of fluid exchange with the alveolar space from the onset of a Pc change. In Section III, the effect of colloid force manipulation on fluid flux was examined in normal and injured lobes in which the macromolecule Heyastarch was used to increase the "effective" oncotic pressure. Hetastarch increased the Pc(crit) of normal lobes from 10.1 ±1.0 to 14.2 ±0.6 cmH2O ($p<0.01$). Following oleic acid Pc(crit) fell from 10.1 ±1.0 to 4.5 ±1.9 cmH2O ($p<0.01$), with edema forming at very low capillary pressures. With the addition of Hetastarch Pc(crit) rose to 12.6 ±0.3cmH2O ($p<0.001$), with reabsorption of edema occurring at normal capillary pressures. The results suggest that oncotic therapy which increases Pc(crit) above Pc may play a role in producing reabsorption of pulmonary edema in the clinical situation.

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Finally, this work is dedicated to Elizabeth whose support and understanding has been unconditional throughout this undertaking.

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INTRODUCTION

Historical Background

Transvascular fluid flux in biological systems has been the focus of much investigation in the past one hundred years, however, many areas of controversy still remain. The clinical correlates of pulmonary edema, resulting from either elevated hydrostatic pressures or altered permeability continue to be a significant cause of patient morbidity and mortality. In order to effect further developments in the treatment of pulmonary edema one must first have a thorough understanding of the factors that govern fluid movement in biological systems.

The initial postulates of how fluid moved through capillary membrane systems were qualitatively outlined by Starling in 1892. He suggested that fluid flow through capillaries was a passive process, and that the energy for this flow resulted from the difference between hydrostatic and oncotic pressures existing in the plasma and tissues. The magnitude and direction of fluid flux reflected these imbalances. Starling also demonstrated that fluid reabsorption from the tissues was determined primarily by the transvascular oncotic gradient with tissue hydrostatic pressure playing only a minor role. These novel hypotheses remained qualitative for

some thirty years until quantitative experimental evidence to support these hypotheses emerged.

1) Fluid Movement in Single Capillaries

Fluid filtration was first studied in single capillaries. Landis described transvascular fluid flux in the capillaries of frog mesentery (1927). Fluid movement was determined in single vessels that had been cannulated at one end to allow occlusion. The capillary hydrostatic pressure was determined directly by micropuncture technique using a glass micropipette. Landis observed that in some capillaries red cell movement was towards the site of occlusion while in others it was observed to be away from the site of occlusion. He concluded that the observed movement of red cells was due to the transudation of fluid out of or into the capillary. Landis then measured the initial rate of red cell movement, before the protein concentration within the capillary changed, and plotted this against the measured capillary pressure for a group of individual experiments. When the transvascular flux was expressed per unit area of the vessel as a function of the capillary pressure a linear relationship was obtained. This was cast into the equation summarizing the forces that Starling had previously described:

Jv = Kf [hydrostatic pressure gradient - oncotic gradient] (1)

He had also demonstrated that flux was bidirectional, either into or out of the capillary. The pressure intercept of the filtration profile represented the point where the hydrostatic capillary pressure and opposing oncotic gradients were in balance, with no flux occurring. This pressure was between 7-14 cmH2O, and corresponded to the colloid osmotic pressure which had previously been determined by independent investigators in that species. (White, 1924)

2) Fluid Movement in Mammalian Capillary Beds

Extension to perfused mammalian tissue was first presented by Pappenheimer and Soto-Rivera using isolated cat and dog hindlimbs. (Pappenheimer, 1948) They used the concept of continuously weighed preparations of isolated tissue. Any measured increase in hindlimb weight was taken to reflect formation of edema and conversely net weight loss taken to represent edema reabsorption. (Danielli, 1940) Their data supported the contention that transvascular fluid flux was a linear function and was bidirectional in nature around a single isogravimetric capillary pressure that could be determined for each experiment.

3) Starling Equation

These principles elucidated theoretically by Starling and supported by experimental data have been cast into various numerical forms. Volume flow (Lv) across an exchanging area was summarized by Kadem and Katchalsky as:

$$Lv = Lp(\Delta P - \sigma \Delta \pi) \qquad (2)$$

in which Lp described the hydraulic conductivity of the membrane (cm5/dyn/s), ΔP, $\Delta \pi$ the average protein and oncotic gradients across the membrane with si being the average reflection coefficient to protein of the membrane. For any biological system the Lp is directly dependant on the surface area for exchange, and when considered it may be cast the most familiar form of the Starling equation:

$$Jv = Kf\ [(pc - pt) - \sigma d(\pi c - \pi t)] \qquad (3)$$

The opposing hydrostatic and oncotic gradients are again modified by two membrane properties the filtration coefficient, denoted Kf, and the membrane reflection coefficient to protein.

3) Membrane Properties and Fluid Flux in Pulmonary Tissue

i) Fluid Filtration Coefficient (Kf)

The quantitative measurement of Kfs in the lung has been the subject of much research. Various methods have been described to measure Kf with a 1,000 fold range in the results obtained.

a) Gravimetric techniques

The first determination of Kf was obtained using a gravimetric method in intact animals (Guyton, 1959). Left atrial pressure was increased for 30-180 minutes after which the lungs were removed. Edema was calculated from pulmonary wet weight dry weight measurements. The rate of edema was obtained from dividing the edema accumulated by the time of the pressure elevation. A plot of rate of edema formation versus left atrial pressure demonstrated that edema formation was zero unless the left atrial pressure exceeded πc. Above the "critical pressure" edema formed in a linear fashion and Guyton and Lindsay calculated Kf as the change in rate of edema formation divided by the change in left atrial pressure. Since that time other investigators have used modifications of this method in both excised lobes (Ehrhart, 1984) and in-situ preparations (Drake, 1980). These experiments involved

continuously weighed preparations in which step changes in pressure (Pc) were introduced and constant rates of weight gain at varying Pc determined. Kf was obtained from the change in rate of constant weight gain divided by the change in Pc. One disadvantage of these methods was that long time periods, sometimes exceeding 30 minutes, were required to ensure linear rates of weight gain. Another potential problem was that relatively large filtered volumes were accumulated during Kf determinations using this method. With greater than 40-50% initial wet weight fluid flux the measured Kfs have been found to increase significantly compared with those obtained from smaller filtered volumes (Drake, 1988, Hancock et al, 1989).

b) Weight Transient Techniques

These techniques attempted to measuring fluid flux (Jv) before any change in Pt, or πt had occurred. These techniques utilized excised continuously weighed preparations (Gaar et al, 1967, Drake et al., 1978). The method was based on two assumptions. First, that excised lobar preparations under baseline conditions were in an isogravimetric (no net weight change) state following readjustment in Starling forces. This has recently been questioned by the demonstration that lobar preparations continue to gain weight, taken to represent transvascular fluid flux, under baseline conditions (Hancock et al., 1989). The second assumption was that following a step

increase in Pc vascular volume changes occurred, which were relatively short lived, after which fluid filtration occurred as a monoexponential function until a new isogravimetric state was reached. Extrapolation of this function to time zero was used to calculate the lobar Kf. However, stress relaxation of the pulmonary vasculature has been shown to occur (Sarnoff et al., 1952), and any vascular volume changes that occurred during this time period would have led to a significant overestimation of the Kf measured.

c) Lymphatic Techniques

This method involved cannulation of a major lobar lymphatic channel and assumed that the measured lymph flow equalled Jv (Erdmann et al., 1975, Michael et al., 1977). This method used assumptions regarding transmembrane gradient values to solve the Starling equation for Kf but unfortunately has yielded inaccurate, highly variable results.

ii) Pc(crit)

Both Landis, and Pappenheimer had described bidirectional flux around a point at which the Starling forces were in balance. This was modified somewhat by other investigators using the pulmonary model. In the lung Guyton and Lindsay (1959) had noted that at left atrial pressures below plasma

oncotic pressure little edema accumulated in the lung. However, above what they called the critical pressure, which was related closely to plasma oncotic pressure, edema accumulated in a linear fashion. Drake (1980) defined the Pc(critical) as the maximum capillary pressure at which lung weight could be maintained constant. In the in-situ preparation total lung weight change with time (S) equalled the difference between Jv and lymph flow (Jl):

$$S = Jv - Jl \qquad (4)$$

Pc(critical) resulted when S=0, and was shown to be related to three components:

$$Pc(crit) = (Jl(max)/Kf) + pt + \sigma(\pi c - \pi t) \qquad (5)$$

The first component Jl/Kf was the pressure difference across the pulmonary membrane that led to transvascular fluid flux equal to the maximal lymph flow rate. The second was the maximum downstream pressure assumed to be pt. The third component was related to the "effective" oncotic pressure gradient across the pulmonary membrane. The relative importance, and magnitude of each component has not yet been addressed.

This analysis must be modified for determinations of Pc(crit) in excised lobes. Differences in measured Pc(crit) in excised lobes (Ehrhart, 1984) as well as those to be presented can be explained in large part by maximal lymphatic flow rates (Erdmann, 1975, Drake, 1980) that would offset transvascular fluid flux occurring in the in-situ preparations despite isogravimetric conditions. In the isolated condition only two of the components described above would be present:

$$Pc(crit) = pt + \sigma(\pi c - \pi t) \qquad (6)$$

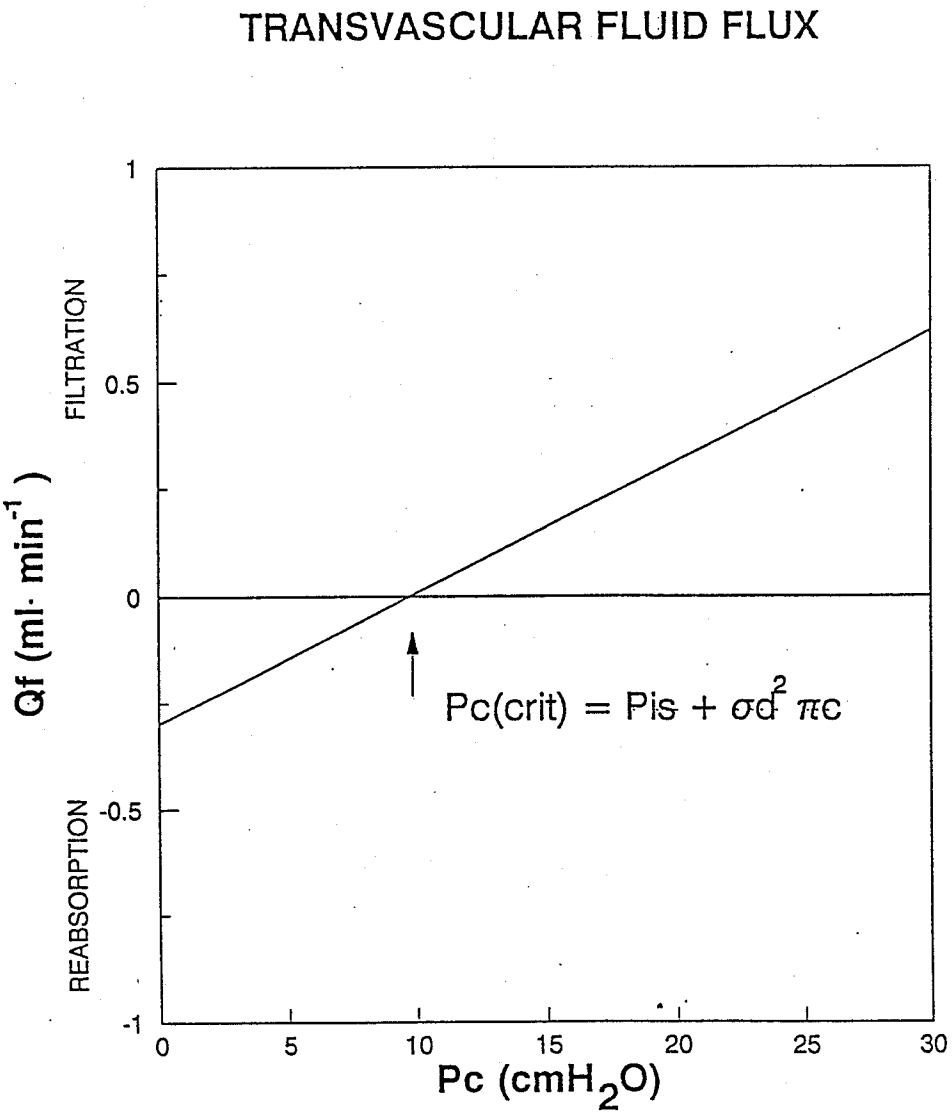
Under filtration independent conditions, during which diffusion would be small relative to convective component of fluid flux, πt should be equal to $(1 - \sigma)$ times the plasma protein concentration (Drake, 1988). Substituting into equation (6) for πt:

$$Pc(crit) = pt + \sigma^2 \times \pi c \qquad (7)$$

The importance of the bidirectional nature of transvascular fluid flux around the Pc(crit) described initially by Landis (1934), and supported by Pappenheimer (1948) has been overlooked in the study of pulmonary physiology. As shown in Figure 1 in the excised lobar preparation filtration and reabsorption occur around Pc(crit) determined by the above

equation. Therefore to induce reabsorption of accumulated edema Pc(crit) must be greater than the lobar hydrostatic capillary pressure (Pc). The experimental and clinical implications of this hypothesis have not previously been tested.

Figure 1



iii) Fluid Filtration Measured by Hematocrit Changes

Transvascular fluid flux was shown to be related to changes in hematocrit using a equation described by Weiser and Grande (1974) where:

$$Vft = CirVol(iblood) \times (1 - hcti/hctt) \qquad (8)$$

with Vft representing the fluid exchanged at any given time, CirVol(iblood) initial circuit blood volume, hcti, and hctt are the initial hematocrit and the hematocrit at any time respectively. By obtaining multiple microhematocrit samples this equation has allowed accurate estimates of filtered volumes relative to red cell concentration changes.

Using the principle described by Weiser and Grande a method was developed to measure transvascular fluid flux that circumvented the requirements of large filtered volumes and was independent of continuous weight measurement. This served to obviate the possibility that stress relaxation of the pulmonary vasculature, leading to increased vascular volume, would significantly alter the measurements of transvascular fluid filtration obtained from continuously weighed preparations.

Laser Colorimetric System:

Using optical theory, a method was devised in the laboratory in which this work was done for the sensitive measurement of concentration changes of red cells contained within a column of blood. When a beam of radiation strikes any object the energy may be modified in a number of ways. The radiation can be absorbed, scattered, transmitted, reflected, or it can excite fluorescence. The device used for the quantitative determinations of fluid flux makes use of the first two principles. As edema forms in a hemoperfused lobar circuit, sieving will occur at the pulmonary exchange membrane. The edema formed under normal circumstances will largely exclude red cells from the transvascular exchange liquid. Therefore, as egress of fluid occurs the concentration of red cells in the circuit (hct) will be increasing, and conversely as reabsorption occurs the hematocrit will fall. Accurate measurement of these changes in hct would allow determination of transvascular fluid flux. Quantitative analysis of concentration changes can be obtained from absorbance measurements. The Beer-Lambert Law states that the concentration of a substance in solution is directly proportional to the absorbance of that solution. This law applies only to monochromatic light and is described

mathematically as follows:

$$A = \log\%T = kcl \quad (9)$$

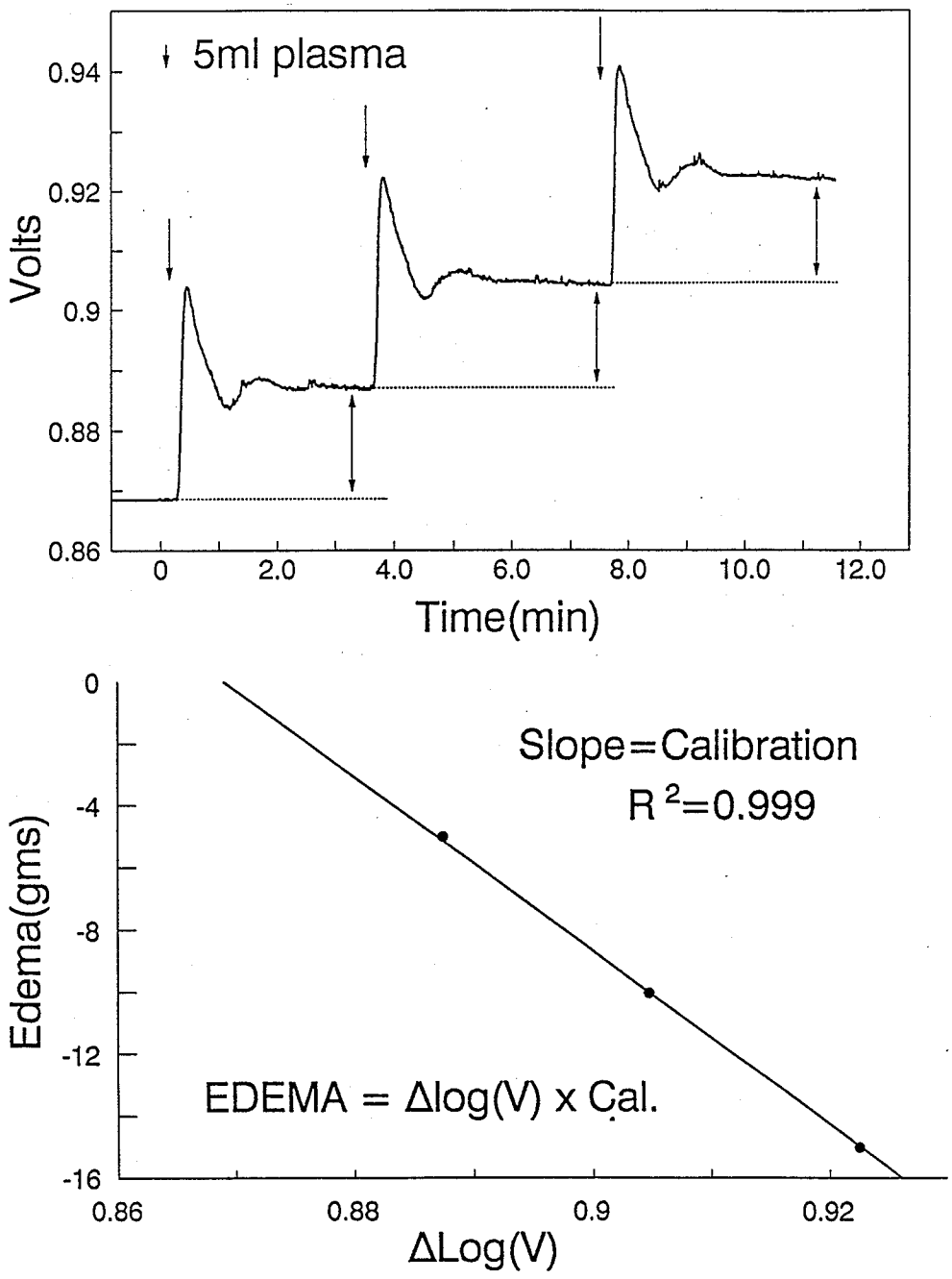
where A is absorbance for any substance in this case hemoglobin, T represents transmittance, k the coefficient of extinction, l the length of the light path, and c the concentration of absorbing solution. The situation for hemoglobin is somewhat more complex because saturation of the hemoglobin moiety will influence the observed absorbance changes. For this reason an infra-red laser diode producing monochromatic light at a wavelength of 815nm was chosen as this is the isobestic point where the spectra for absorption of hemoglobin and oxyhemoglobin overlap (Oppenheimer, 1987). The isobestic point represents the wavelength at which changes in saturation will not affect the absorbance changes. Scatter will also play a significant role due to the corpuscular nature of the red cell but provided the changes are linear the signal calibration would be unaffected. A 100 mW cw laser diode (Spectra Diode Laboratories) generating coherent light at a wavelength of 815nm was used to track changes in hematocrit. In order to allow quantitative tranvascular fluid flux measurements a calibration procedure that was performed prior to every experiment was devised.

Calibration of Laser Signal

The laser signal was allowed to stabilize to a steady drift free signal, which required approximately thirty minutes. Aliquots of five mls. of plasma were obtained from canine centrifugated blood. A series of three boluses were introduced into the circuit via the reservoir producing a dilution of the red cell concentration. Once the infusion became well mixed with the perfusate, as evidenced by a steady laser output signal at an increased level, the voltage change in the transmitted laser light was recorded. The procedure was repeated for each bolus. A regression was then performed relating mean change in the log of the transmitted voltage from the laser (abscissa) to the dilution (ordinate) as represented by Figure 2. This calibration procedure allowed on-line changes in hematocrit to be determined, with the calibration factor obtained denoted the IR Cal. A series of experiments were then undertaken to validate the technique.

Figure 2

(Laser Calibration Procedure)



Validation of the Laser Colorimetric Method

1) Validation as a Method to Measure on-line Changes in Hematocrit

Previous work had demonstrated the validity of the laser device in the measurement of changes in hematocrit (Hancock et al., in press).

2) Measurement of Fluid Flux

Using the principle described previously and the hematocrit validation, overall transvascular flux was then calculated from concentration changes relative to red cell mass (hct) in the following manner:

$$Vf(815) = \log(815) \times IR\ Cal \qquad (10)$$

where Vf(815) refers to the filtered volume relative to the 815nm signal. A hemoperfused circuit with an interposed dialysis hemofilter was used to simulate bidirectional fluid exchange. Quantitative filtration measurements obtained simultaneously from hemofilter weight and circuit hct changes determined from the laser were compared.

Methods

An Amicon filter was placed in a hemperfused circuit and suspended from a calibrated force transducer to allow continuous weight determinations. Calibration of the laser was then performed. Filtration was induced by raising the height of the filter outflow reservoir and allowing fluid to accumulate in the filter and attached tubing. Filtration was allowed to continue for 20 minutes. The reservoir was then lowered to induce reabsorption over a similar time period. Fluid exchange as determined by colorimetric and weight measurements was then compared.

Results

The results have been summarized for both filtration and reabsorption in figures 3 and 4. Figure 3 represents the correlation of on-line determinations of filtration from weight changes (abscissa) and laser colorimetry (ordinate). Individual points were obtained at 5 minute intervals from both methods and plotted as shown the and the resulting linear correlation was highly significant to $p<0.01$ (laser(ml) = weight(ml) x 1.002 - 0.122(ml), $r2 = 0.998$).

Figure 3

(Colcrimetric Filtration Validation)

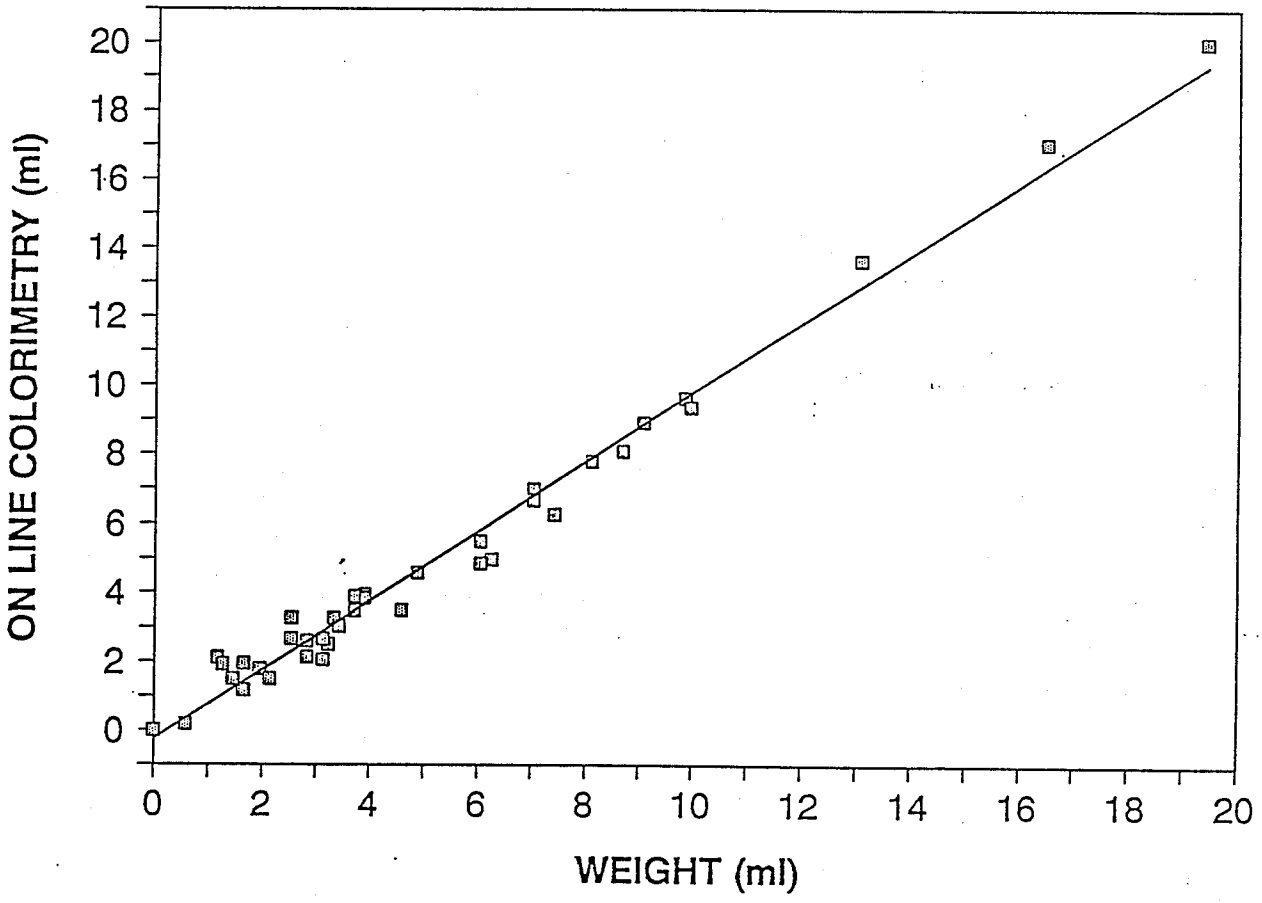
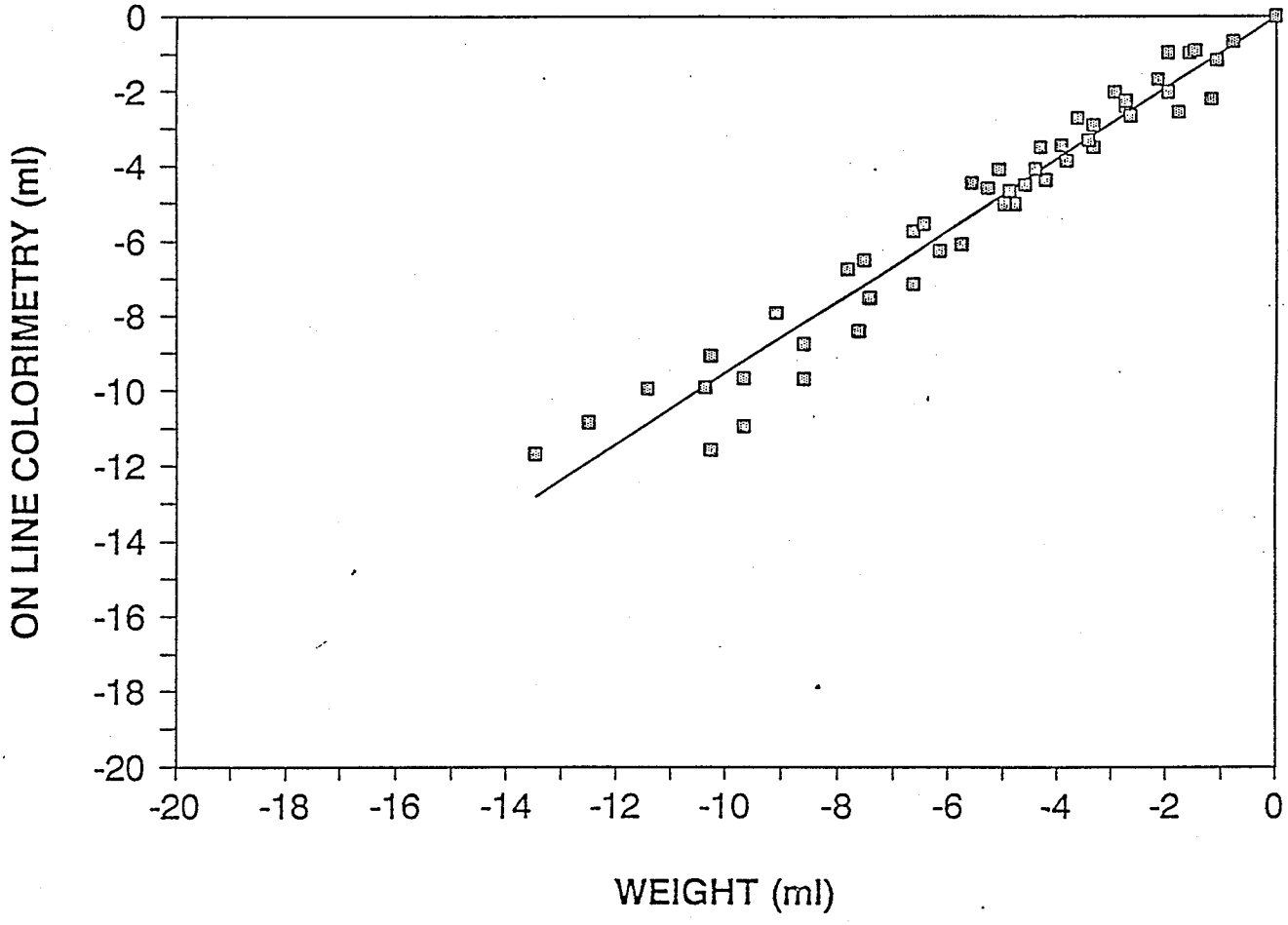


Figure 4 data was collected in a similar fashion for fluid reabsorption from the filter. Again note the highly significant linear correlation to $p<0.01$ (color(ml) = weight(ml) x 0.96 + 0.002(ml), r2 = 0.987).

Figure 4

(Colorimetric Reabsorption Validation)



Consequently, the device was capable of not only detecting small changes in hematocrit but to the extent that hematocrit change was an accurate measure of transvascular fluid flux laser colorimetry offered an independent continuous measure of bidirectional fluid exchange. The filter experiments demonstrated that there was good agreement when pure filtration, achieved using an Amicon hemofilter, was measured by standard weight or laser colorimetry.

Sources of Error

Although the development of the laser colorimetric device resulted in a highly sensitive reliable method for transvascular fluid flux determination one must consider some of the potential shortcomings.

1) Calibration Error

The regression line obtained from the calibration was certainly subject to possible error as was accurate determination of plasma bolus volume. However, the highly significant r^2 value, which was seen to be very reproducible, as well as the fact that the calibration was done prior to each experiment served to minimize this error.

2) Hemolysis

Significant hemolysis in hemoperfused circuits has been described that influenced the filtered volumes calculated (Maron, 1987). Any loss of red cells would be seen by the laser device as dilution from reabsorption. At the same time accumulation of free hemoglobin may affect the laser signals obtained. The stability of the 815nm laser signal was observed in six experiments to 40 minutes with no change in the measured voltage. Qualitative verification that accumulation of small amounts of free hemoglobin would have little effect on the signal obtained was determined in 6 experiments. A bolus of autologous plasma obtained prior to the beginning of each experiment was added to the circuit and the voltage change measured. This was then compared to an equal bolus of autologous plasma obtained from the circuit at the end of each experiment after hemolysis was anticipated to have occurred. There was no difference in the voltage change obtained from either bolus signifying minimal effect on the fluid flux determined by laser signals even when hemolysis was maximal for that experiment. This was validated spectrophotometrically (Shimadzu) at 815nm and there was virtually no change in the absorbance spectra due to free hemoglobin at that wavelength, although the presence of some hemoglobin was noted by absorbance changes at 600nm on the spectrophotometer.

Quantitative analysis of the magnitude of hemolysis that occurred in our hemoperfused preparation was also addressed. However, the highly significant correlation between weight and laser filtered volumes determined with the Amicon filter experiments suggested relatively low rates of hemolysis. Free hemoglobin was measured spectrophotometrically (Shimadzu) using the Cyanomethemoglobin technique (Wolf et al., 1989) in three circuit (without Amicon filter), and three lobar experiments. Free hemoglobin was measured at 420nm by mixing 200 µl of plasma with 5 ml of reagent. Calibration curves were constructed for cyanmethemoglobin standards (Fisher Diagnostics) at the same wavelength. The experiments performed lasted for three hours with hourly samples taken for microhematocrit and free hemoglobin concentration (FHC) determinations. These results are summarized in Table 1.

Table 1

Hourly Free Hemoglobin Concentration ($g \cdot dl^{-1}$)

Circuit Experiments	Number	0	1	2	3
	1	0.116	0.144	0.202	0.280
	2	0.131	0.168	0.205	0.268
	3	0.158	0.165	0.187	0.253
	MEAN	0.135	0.159	0.198	0.267
	S.D.	0.017	0.011	0.007	0.011
Lobar Experiments	1	0.170	0.151	0.194	0.287
	2	0.123	0.177	0.220	0.273
	3	0.137	0.182	0.235	------
	MEAN	0.143	0.170	0.216	0.280
	S.D.	0.020	0.014	0.017	0.007

From these measurements the total free hemoglobin (FH) released can be determined:

$$FH = FHC \times CirVol \times (1 - hct) \qquad (11)$$

where CirVol was the circuit volume used.The mass of hemoglobin contained in the red cell volume (RCVH) was also calculated:

$$RCVH = BH/hct \qquad (12)$$

with BH representing the blood hemoglobin concentration (gm/dl) which was obtained from spectrophotometric measurements. The measured free hemoglobin (FH) was then related to the hemoglobin content of the red cell volume. The red cell volume destroyed by hemolysis (RCVH) was then determined:

$$RCVH = FH \times CirVol \times (1-hct) \times hct / BH \qquad (13)$$

If the RCVH is expressed as a fraction of the CirVol then the volume of hemolyzed red cells as a fraction of CirVol (Hhct) can be calculated:

$$Hhct = FH \times (1 - hct) \times hct / BH \qquad (14)$$

From Table 1 average free hemoglobin increased by 0.044±0.009 gm/dl/hr. The average blood hemoglobin concentration in the experiments was 12.24±1.35 gm/dl with a corresponding average hct of 42.7±2.25%. The average Hhct was calculated to be 0.089%. This would have resulted in underestimated transvascular fluid flux of approximately 0.8ml/hr.

These determinations were then repeated in three isolated hemoperfused lobes. Pc was held constant and the lobes allowed to gain weight for three hours with a mean weight gain of 15.73 ±5.92 gms. Laser colorimetric, 17.74 ±3.59 gms, and sample microhematocrit, 18.0 ±5.0 gms, of filtered volumes similar. Estimates of free hemoglobin concentration changes were 0.046 ±0.006 gm/dl/hr (Table 1). This suggested that hematocrit calculations from either method should have underestimated fluid exchange again by 0.8 mls/hr. This phenomenon was obscured, when compared to weight measurements, by any evaporative loss from the lobar surface. Previous estimates have suggested that evaporative loss from this preparation was in the order of 2 mls/hr.

5) Hemmorhage

Another source of error would have resulted from significant hemorrhage. Gross inspection of the lobes at the completion of the experiments did not reveal any significant hemorrhagic component. However, loss of red cells would have had an effect on transmitted light from the laser, and subsequent edema calculations, if red cell concentration had also changed. If we assume that the edema formed was whole blood this then would have caused an underestimation of edema formation as measured by laser colorimetry, detected only by weight increases.

This possibility was explored using data obtained from the Section (I) experiments. There was a significant increase in the predicted DW/BW ratio (±S.E.) of 0.527 ±0.003 to the observed DW/BW ratio 0.858 ±0.100 which suggested an increase in some nonevaporable substance. We then supposed, for the purpose illustration, that the entire increase in dry weight was due to hemmorrhage. By multiplying the increase in dry weight by 4.0 (an average WW/DW for dog blood, Craven, 1979) it was found that hemorrhage could maximally account for only 29% of the increase in wet weight of the lobes making significant hemorrhage unlikely.

Using the same principle for protein there was much closer agreement to the measured increase in wet weight of the lobe which suggested accumulation of protein rich edema fluid rather than significant hemorrhage.

Despite these potential sources of error the laser technique allowed for determination of transvascular fluid flux independent from constant weight gain measurements. There was excellent agreement between these methods in simulated bidirectional flux using the Amicon hemofilter. These techniques were subsequently applied to a series of excised lobar experiments. Pulmonary transvascular fluid flux was first characterized by construction of Pc/Qf relationships from gravimetric and laser colorimetric measurements, with and without lung injury. The factors governing the balance of forces existing at the pulmonary membrane were also investigated including assessment of the downstream pressure that determined transvascular fluid flux, and the effects of oncotic pressure manipulation on altering transvascular fluid flux.

METHODS

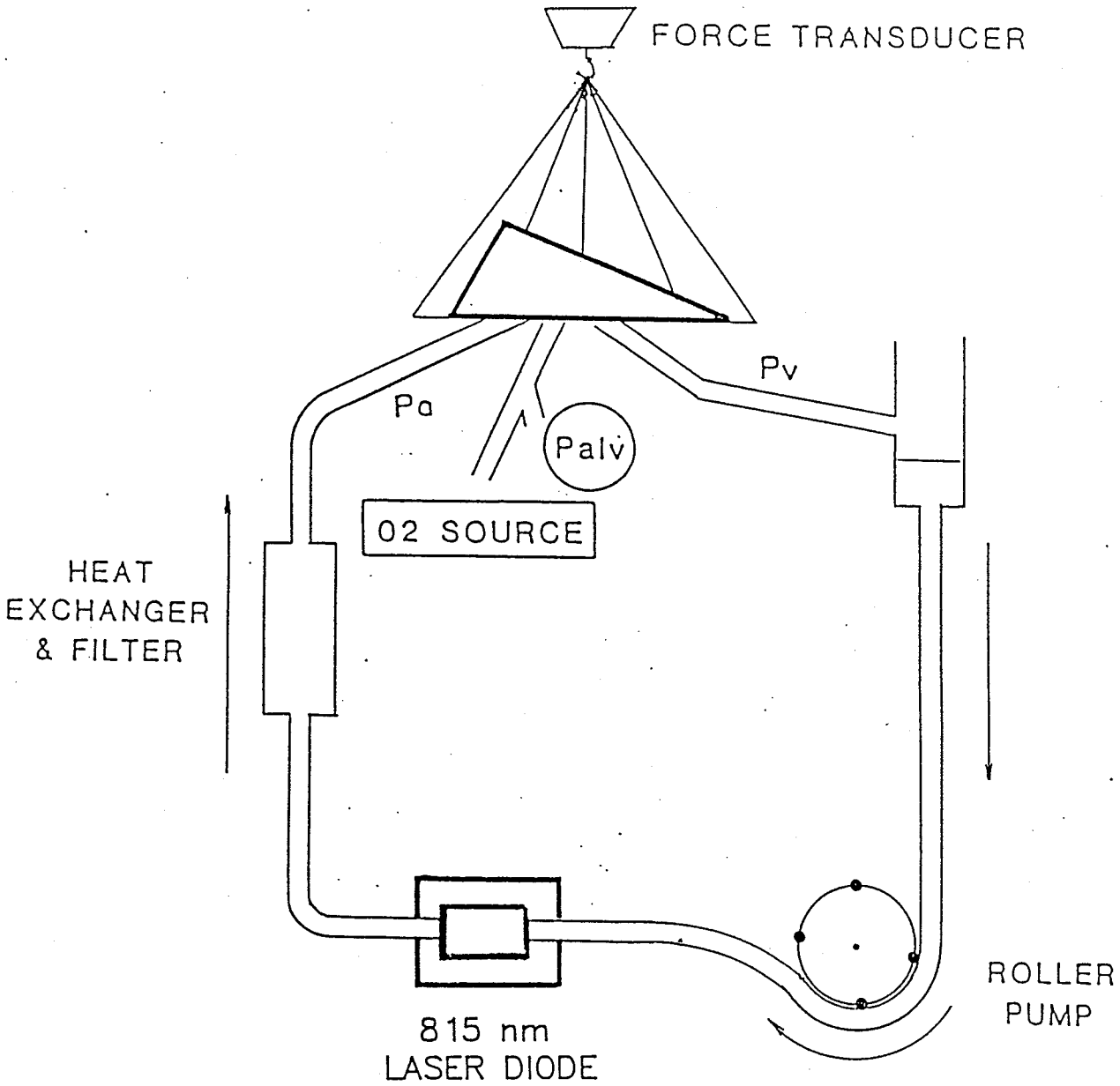
Animal Preparation:

The preparation that was used is diagrammed in Figure 5. Adult mongrel dogs of either sex weighing between 15 and 28 kilograms were used for this study. Anaesthesia was induced with intravenous pentobarbitol sodium in a dose of 30mg/kg following which the animals were intubated and ventilated. Following intravenous access and muscle relaxation using succinylcholine (20mg) a left thoracotomy was performed through the bed of the fifth interspace. After heparinization the left lower lobe artery, vein, and bronchus were cannulated. The animal was then exsanguinated and the left lower lobe excised.The lobe was then supported on a mesh trampoline with the hilum in the dependant position.The lobe was then additionally suspended using EKG pads affixed to the surface of the lobe with cyanoacrylate adhesive. A suture was then secured to the centre of each pad following which the lobe was wrapped loosely in plastic.

The entire preparation was then suspended from a Stratham force transducer for continuous weight determination and placed in a plastic tent into which humidified air flowed to minimize evaporation. The force transducer was connected to a Validyne CD-19 carrier demodulator, and the signal displayed on a Brush Gould 260 oscillographic recorder.The bronchial cannula was attached to a humidified oxygen source and the lobe was statically inflated to a fixed transpulmonary pressure (5cmH20). Lobar arterial and venous pressures were continuously measured via pressure port catheters advanced through the cannulas to the hilum. Pressures were measured using Validyne MP45-1 guages, with all signals displayed on the Brush-Gould 260 oscillographic recorder. The perfusion circuit consisted of a single reservoir of adjustable height to which the venous cannula was attached. A Masterflex Digistaltic pump was used to return blood from the venous reservoir through a heat exchanger, that was set at a fixed temperature of 38C, macropore filter and then back into the arterial cannula. The animal preparation was similar for all investigations with slight differences that will be described in the context of individual protocols.

Figure 5

(Excised Lobar Preparation)



EXPERIMENTS

A series of experiments, divided into three sections, were performed to address the following hypotheses:

1) Could pulmonary transvascular fluid flux be characterized in an accurate and reproducible manner using laser colorimetry ?

2) What are the factors that govern the balance of forces that exist at the pulmonary membrane, and could these forces be manipulated ?

SECTION (I)

Characterization of Transvascular Fluid Flux

Background

It is generally accepted that isolated lobe and lung preparations reach isogravimetric conditions in which Starling forces acting across the microvascular membrane (hydrostatic and colloid osmotic gradients) are assumed to be in equilibrium. The introduction of a step change in microvascular hydrostatic pressure (Pc) results in a rapid change in weight, assumed to represent pulmonary vascular volume change, followed by a slow exponential phase of weight change, which is thought to represent transvascular fluid exchange (Jv). The exponential character of the slow weight gain is attributed to readjustment of Starling forces opposing further filtration. A new isogravimetric state is achieved if changes in tissue pressure, associated with fluid accumulation in the pulmonary interstitium, successfully balance the change in Pc. If Pc is now returned to baseline, the process is reversed resulting in fluid reabsorption from the interstitium (Gaar et al.,1967, Drake et al.,1978, Taylor, 1978). A Pc may be reached where this buffering capacity of the tissues is overwhelmed and filtration continues at a constant rate after the slow exponential weight time course is completed (Drake et al,1980). Further increases in Pc result in proportionally

faster constant Jv. When Pc is plotted against Jv, a linear relationship is obtained. The intercept when Jv=0 is interpreted as the critical value of Pc (Pcrit) at which Starling forces are overwhelmed. The slope is considered a measure of the lobar conductance to transvascular fluid flux (Kf).

Using the colorimetric device described to continuously measure perfusate hematocrit from changes in light transmission, and assuming that changes in hematocrit reflect transvascular fluid exchange, a continuous record of Jv independent of vascular volume changes was obtained. In previous work in which the Pc was increased 11cmH20 from baseline in one step, Jv, as measured colorimetrically increased immediately to a constant rate (Hancock et al., 1990). Slow exponential weight gain persisted for 20 minutes; thereafter, weight gain continued at a constant rate which agreed well with colorimetric estimates of Jv. It was speculated that slow exponential weight changes may include slow vascular volume changes and transvascular exchange at constant rates.

The objective of the present study was to explore the pulmonary filtration properties following a series of step increases in hydrostatic pressure both before and after the addition into the system of oleic acid, a compound known to increase pulmonary microvascular permeability (Ehrhart, 1984).

Experimental Protocol

Following the preparation and calibration procedures 13 lobes were inflated transiently to 25-28 cmH20 to reduce atelectasis and the reservoir was raised to 30 cmH20 to recruit the lobar vasculature. The lobar arterial (Pa), venous (Pv), and alveolar (Pl) pressures were manipulated to place the lobe in Zone III. (Pa=15, Pv=10, Pl=5) The lobes were then allowed a period of stabilization (30mins) after which baseline weight and colorimetric signals were recorded. Two sequential step increases in capillary hydrostatic pressures of approximately 5 cmH20 were introduced by raising the height of the venous reservoir. Recording of the above continuous signals began with the pressure increase and continued until the rate of weight gain became constant. Interval measurements of tube microhematocrit (8 samples), protein concentration by refractometry (Atago, 8 samples), and free hemoglobin from the

perfusate were performed on samples from the reservoir at baseline and following the second step increase in hydrostatic pressure. The reservoir was then returned to baseline and oleic acid was added to the circuit (0.15ml) through a pressure port between the macropore filter and the lobe. A period of 1 hour was allowed to ensure onset of permeability injury. Identical measurements of both the continuous and interval parameters were made as described above. Following completion of the experiment the blood was drained from the lobe and a final wet weight determination performed. The lobe was inflated, and dried for final dry weight determination.

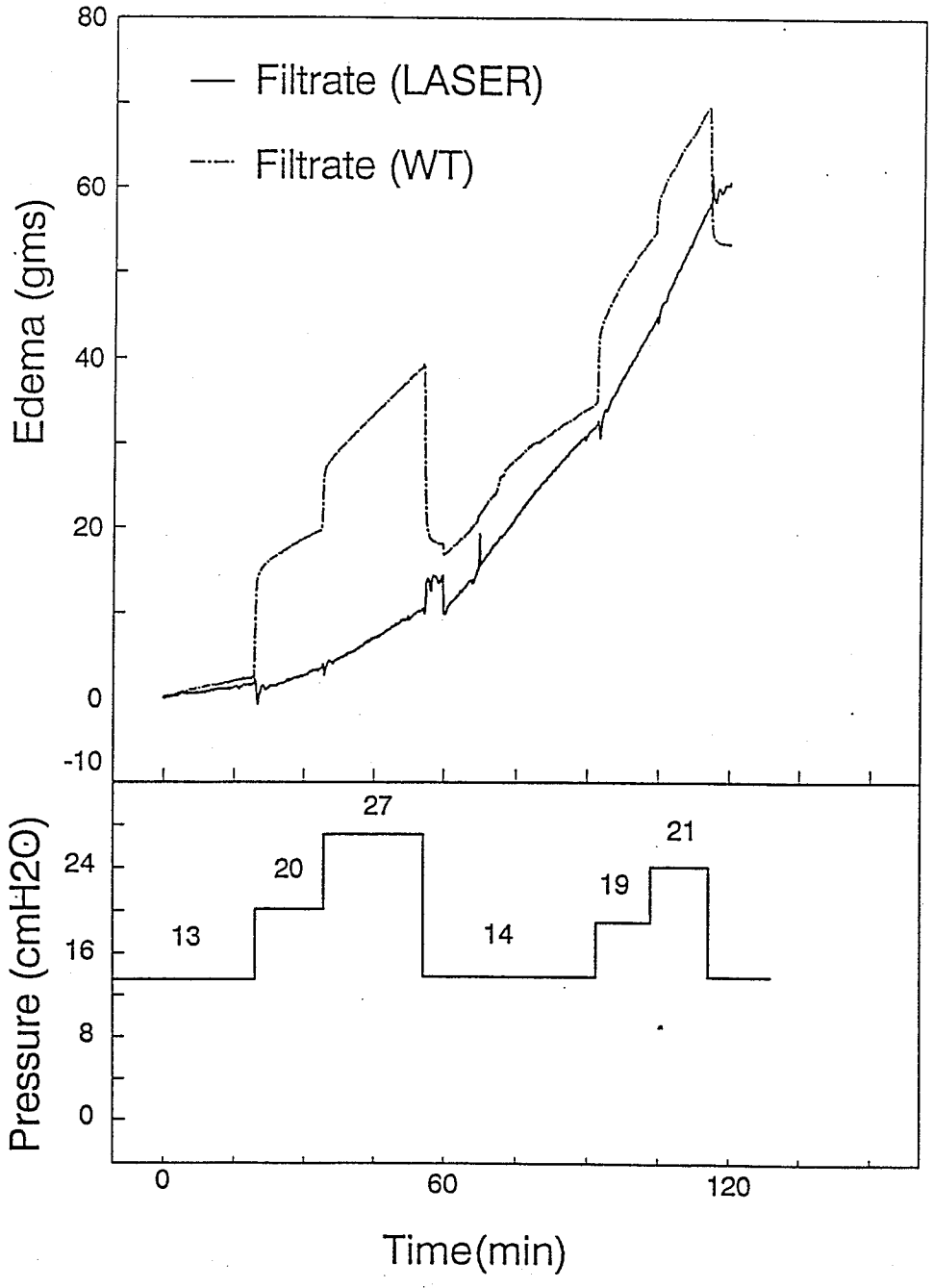
Statistical Analysis

Lobar comparisons before and after oleic acid were made using paired t-tests, with group comparisons being made using one way analysis of variance. Significance was considered to be at the $p<0.05$ level. Standard deviations as well as standard errors were calculated for all group means as indicated in the results.

Results

The time course of weight and colorimetric data is illustrated below in Figure 6 for an entire experiment.

Figure 6



Both, weight changes and Vft from 815nm colorimetry are represented. The mean capillary hydrostatic pressures (± S.E.) were calculated by the equation described by Gare (Gare et al., 1967). Before oleic acid these pressures averaged 12.8 ±1.2cmH2O, 19.6 ±2.3cmH2O, and 26.9 ±2.6cmH2O for baseline and the two the sequential step increases respectively. Following oleic acid administration the capillary pressures were not significantly different at 13.5 ±2.3cmH2O, 18.9 ±1.7cmH2O, 21.4 ±2.7cmH2O.

The experimental protocol was considered in two parts, before and after addition of oleic acid into the system. Two groups of control lobes, considered part three, were also performed.

Part I:Pre-oleic acid

This section summarizes the results of 13 isolated lobar experiments. The filtration rates with S.E. bars, as determined by weight gain and laser colorimetry, have been summarized in Figure 7. Note the correlation between 815nm colorimetric signal and constant rate of weight gain at varying capillary pressures (no statistical difference). The mean Kfc values (±S.E.) were 0.119 ±0.027 ml/min/mmHg/100gm and 0.131 ±0.037 ml/min/mmHg/100gm, and Pcrit values (±S.E.) were 8.27 ±0.75mmHg, and 7.65 ±0.81mmHg from weight gain and 815nm laser colorimetry respectively. These are summarized in Table 2. The overall filtered volumes determined by the three methods are illustrated in the histogram in Figure 8. Note the correlation of filtered volumes between calculations from weight, and hematocrits by sample microhematocrit and laser colorimetry.

Figure 7

(Filtration Rates from Laser and Weight)

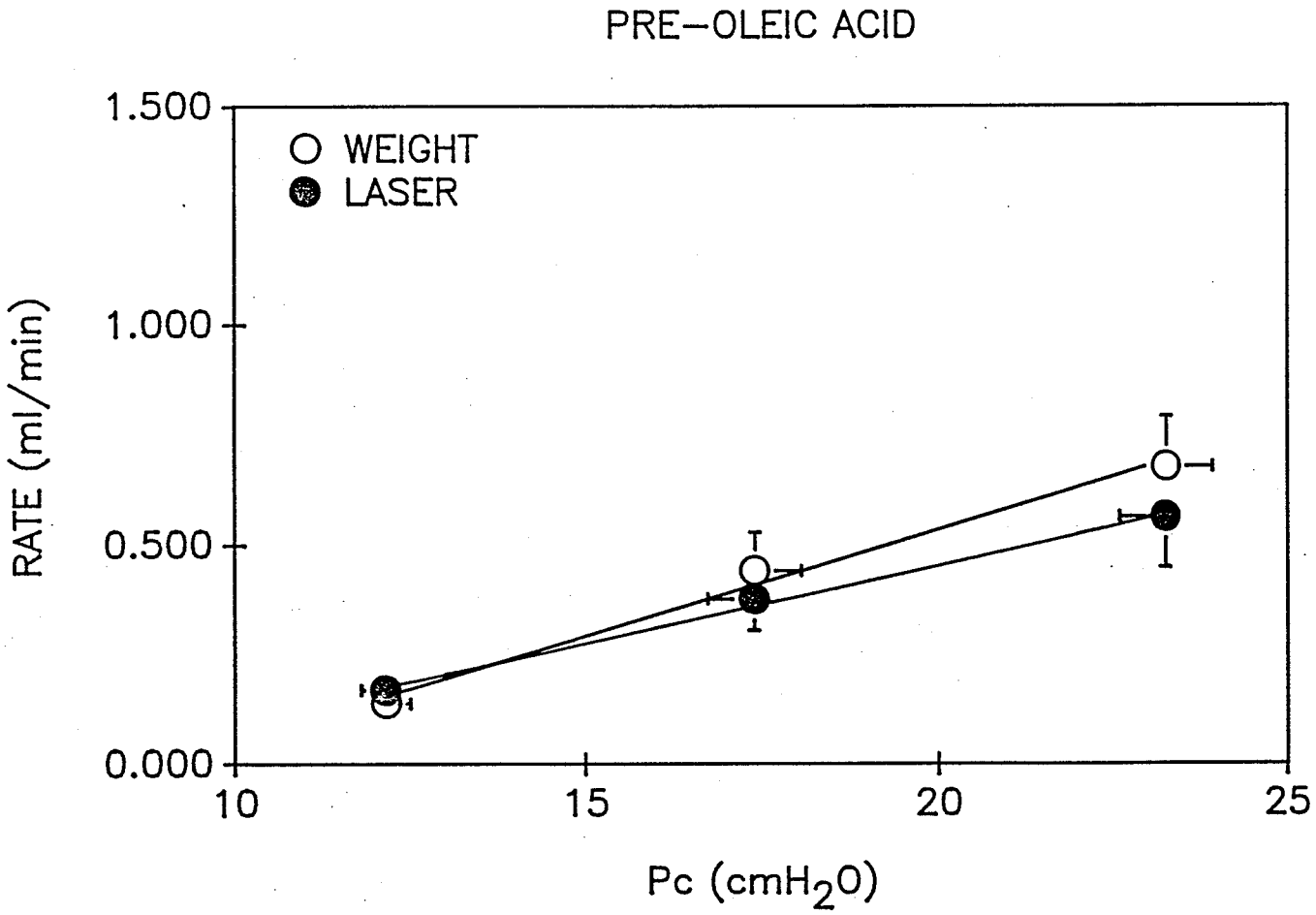


Figure 8

(Filtered Volumes from Three Methods)

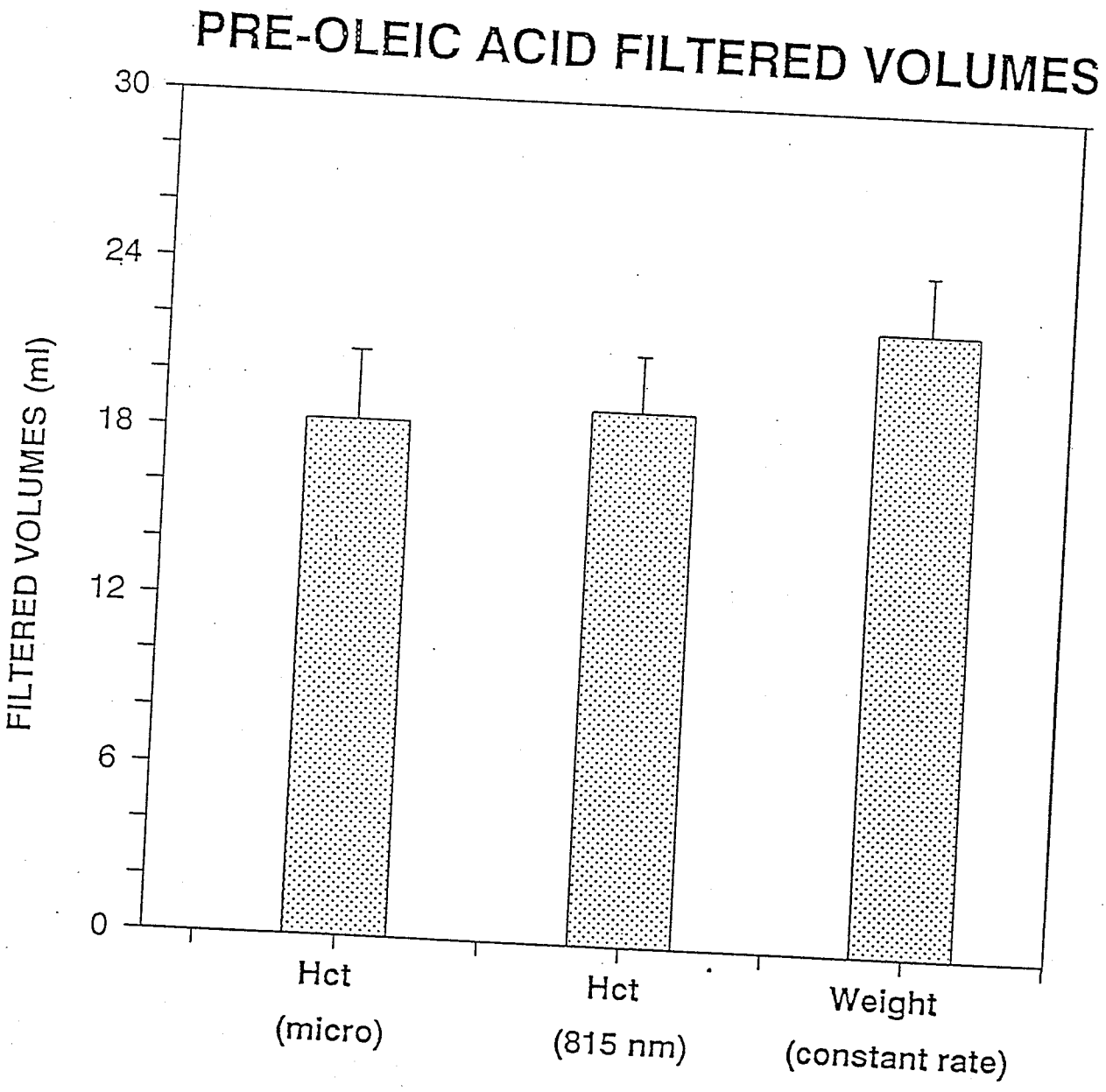


Table 2

PRE-OLEIC ACID

	Kf ($ml \cdot min^{-1} \cdot mmHg^{-1} \cdot 100g^{-1}$)		Pc_{crit} (mmHg)	
Experiment	Weight	Laser	Weight	Laser
1	0.0621	0.0323	7.73	5.91
2	0.0725	0.0365	6.37	7.84
3	0.107	0.105	5.32	5.95
4	0.0516	0.0425	7.79	6.61
5	0.0976	0.0813	6.65	3.01
6	0.0501	0.0526	9.17	5.28
7	0.336	0.251	8.22	8.43
8	0.0731	0.0286	4.45	4.87
9	0.294	0.431	6.46	8.30
10	0.229	0.187	8.29	7.57
11	0.0686	0.0295	13.21	12.79
12	0.0610	0.0472	10.98	9.74
13	0.0490	0.0513	12.99	13.11
Mean	0.119	0.131	8.27	7.65
S.E.	0.027	0.037	0.75	0.81

Part II:Post oleic acid

The filtration rates (±S.E. bars) are shown in Figure 9. Note the increase in filtration following the addition of oleic acid represented by the weight and 815nm signals. Again no statistical difference exists between rate of constant weight gain and rate determined by 815nm laser colorimetry. Mean Kfc values (±S.E.) were 0.151 ±0.024 ml/min/mmHg/100gm and 0.187 ±0.052 ml/min/mmHg/100gm, with mean Pcrit values (±S.E.) being 4.76 ±1.17mmHg and 5.08 ±0.61mmHg for constant rate of weight gain and 815nm laser signal respectively, seen in Table 3. Filtered volumes are seen in Figure 10 where again there was no difference between hematocrit, 815nm colorimetry, and weight determined filtered volumes.

Figure 9

(Filtration Rates from Laser and Weight)

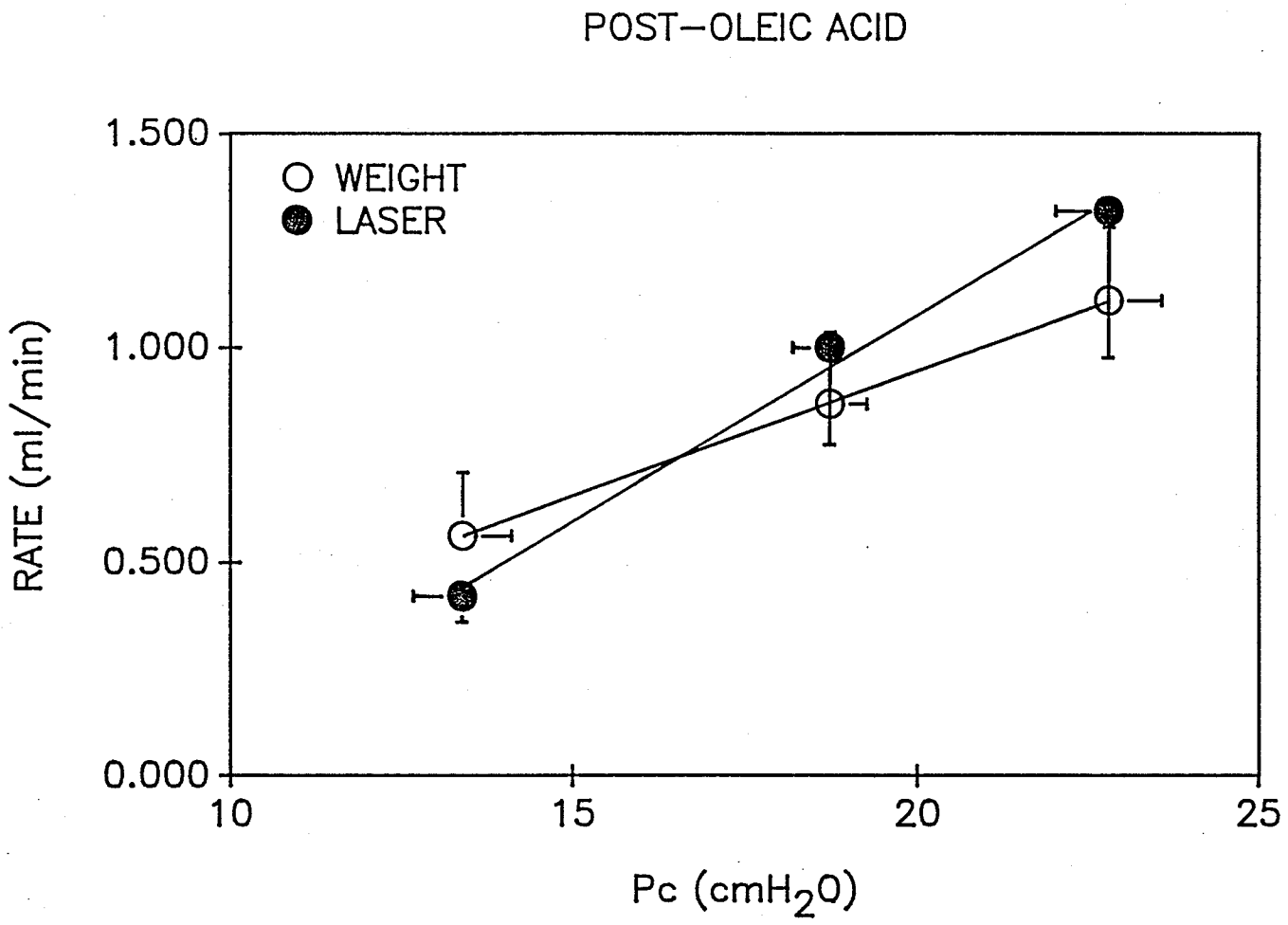


Figure 10

(Filtered Volumes from Three Methods)

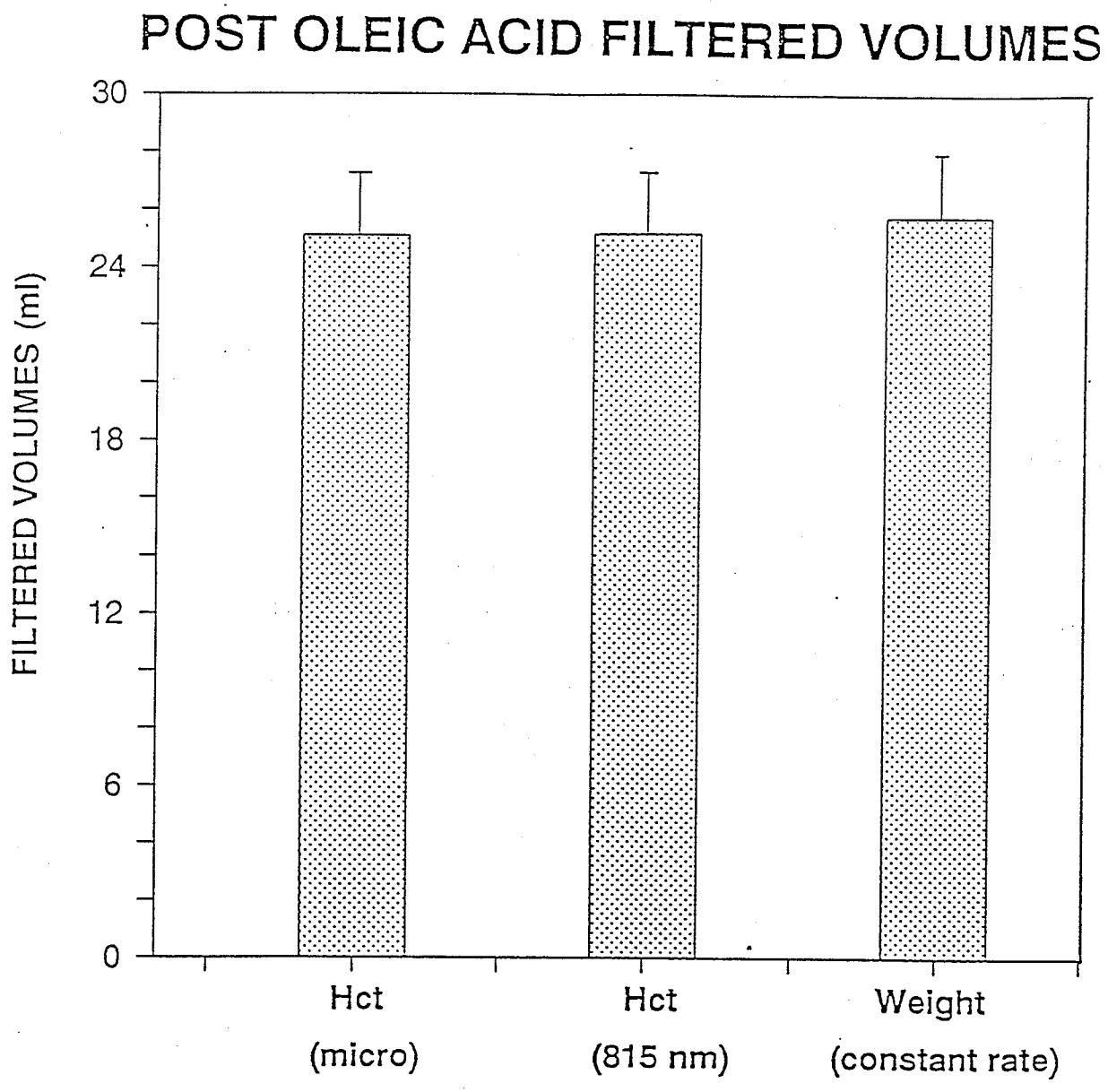


Table 3

POST-OLEIC ACID

	Kf ($ml \cdot min^{-1} \cdot mmHg^{-1} \cdot 100g^{-1}$)		Pc_{crit} (mmHg)	
Experiment	Weight	Laser	Weight	Laser
1	0.0980	0.0393	7.54	3.64
2	0.0950	0.0607	6.43	5.26
3	0.0981	0.125	3.90	0.57
4	0.129	0.153	6.79	2.24
5	0.0992	0.769	-6.5	8.90
6	0.130	0.149	7.39	3.59
7	0.158	0.269	8.00	5.64
8	0.146	0.132	3.67	5.64
9	0.368	0.274	0.31	6.26
10	0.301	0.174	7.73	5.21
11	0.0534	0.0502	5.14	5.32
12	0.163	0.172	8.99	7.76
13	0.123	0.0764	2.48	6.12
Mean	0.151	0.187	4.76*	5.08*
S.E.	0.024	0.052	1.17	0.61

* $p < 0.05$ vs pre-oleic acid

Part III: Controls

Tables 4 and 5 demonstrate the results of five control lobes in which Kfs and Pc(crits) were determined over a similar time period without the addition of oleic acid. There was no significant change in either measure in these control lobes.

To ensure that the lung injury produced by oleic acid was constant and not reversible three additional control experiments were performed. Once the oleic acid (0.15ml) was added to the circuit the preparation was left for three hours without a change in Pc being introduced. This corresponded to the maximal duration experiments following the addition of oleic acid to the circuit. There was a significant increase in the rate of filtration observed ($p<0.05$) from baseline and this increased rate persisted for the entire three hours. There was no statistical difference, analyzed by one way anlysis of variance, between either control group or the pre-oleic acid group with respect to the Kf or Pc(crit) values calculated.

Table 4

CONTROL INITIAL

	Kf ($ml \cdot min^{-1} \cdot mmHg^{-1} \cdot 100g^{-1}$)		Pci (mmHg)	
Experiment	Weight	Laser	Weight	Laser
1	0.0487	0.0581	8.81	8.71
2	0.0840	0.0574	6.65	6.34
3	0.0688	0.0556	8.00	5.27
4	0.2386	0.0609	7.22	6.30
5	0.0865	0.0395	7.77	5.01
Mean	0.105	0.054	7.69	6.33
S.E.	0.0034	0.0038	0.36	0.65

Table 5

CONTROL FINAL

	Kf ($ml \cdot min^{-1} \cdot mmHg^{-1} \cdot 100g^{-1}$)		Pci (mmHg)	
Experiment	Weight	Laser	Weight	Laser
1	0.0479	0.0423	5.97	4.18
2	0.0687	0.0861	6.63	7.80
3	0.0595	0.0538	6.84	6.35
4	0.1806	0.0862	6.79	7.86
5	0.0798	0.0852	5.58	4.74
Mean	0.087	0.0707	6.63	6.19
S.E.	0.0239	0.0094	0.25	0.76

Discussion

Difficulty in separating vascular volume changes from true filtration using weight gain alone has been recognized (Sarnoff, 1952). Laser colorimetry offered a method of measuring pulmonary transvascular fluid flux that was independent of changing vasculature. Comparisons of weight gain and 815nm colorimetric signals confirmed the hypothesis that rates of constant weight gain agreed well with rates of transvascular fluid flux (Jv), and no slow exponential time course was identified colorimetrically. This held true at baseline and following each step change in Pc introduced. Comparisons under baseline conditions as well as following permeability injury with oleic acid were similar. When, using laser colorimetry, filtration was measured independently from vascular volume change fluid exchange was found to be a linear function relating rates of constant Jv to Pc above Pc(crit).

Transvascular fluid flux can be characterized by a series of step changes with measurements determined at constant rates of weight gain at each pressure as has been suggested by other investigators (Drake et al., 1980) . A regression line of least squares difference through these points is measure of the total lobar conductance of the pulmonary membrane to fluid flux. The extrapolate to a Jv = 0 represents the point at

which the Starling forces are in balance. Colorimetric determinations were advantageous because relatively short periods of filtration at each Pc were needed for measurement of fluid flux. Gravimetric determinations often required greater than 30 minutes to achieve a constant rate. The observed measures of Kf under baseline conditions agreed well with those described by others (Drake et al.,1980, Ehrhart, 1984) as measured by either weight or laser colorimetry. The characterization of fluid exchange using the construction of Pc/Qf relationships was valid at baseline as well as following a permeability injury with oleic acid.

In a previous study, reported from this laboratory, Pcrit was found to be lower than baseline Pc and consequently the lobes were found to accumulate edema at a constant rate from the outset (Hancock, 1989). Slow exponential changes in the weight time course likely represent slow vascular volume changes that require a much longer time period to be completed than has been previously appreciated. With each increase in Pc, Jv increased and became constant immediately which suggested that force imbalance for Jv, summarized by the Starling equation, remains constant. Unless the hydrostatic and oncotic pressure gradients readjust continuously to maintain a constant imbalance our results suggest fluid accumulates in a compartment where no back pressure forms as

fluid flux occurs. If one assumes that diffusional protein transport is not significant then for Jv to remain constant, Pt must also remain constant or change very slowly. One explanation is that the pulmonary interstitium is more compliant than previously thought. Alternatively, in isolated lobar preparations where the clearing mechanisms have been interrupted, fluid could accumulate in the alveolar space as soon as the force imbalance develops.

Some investigators have contended that excised canine preparations are not comparable to the in-situ condition and that damage occurred in the excision process (Morriss et. al.,1980). Others have validated the use of excised preparations and demonstrated its stability (Ehrhart, 1984). The present data support these latter findings and swow comparable membrane properties to those described in in-situ preparations. Stability over time of the excised preparation was also demonstrated in the control experiments performed in this study.

SECTION (II)

Which is the Downstream Pressure to Transvascular Fluid Flux?

Background

A second series of experiments were done in order to investigate the effective downstream pressure to pulmonary transvascular fluid flux. The presence of two types of pulmonary vessels, intra-alveolar and extra-alveolar, were first demonstrated by Macklin (1946). The relative contribution of each type of vessel in the genesis of edema and their behaviour at varying alveolar pressures and lung volumes has been an area of much interest. Iliff (1971) used excised canine lobes under zone 1 conditions, and was the first to suggest that the extra-alveolar vessels contributed to edema formation, with greater than 60% of fluid flux resulting from these vessels. In-situ studies that used glass bead microembolization of the alveolar vessels, as well as Zone 1 conditions, supported the significance of the extra-alveolar vessels in transvascular fluid exchange (Albert, 1978). In previous studies, however, although specific

isolation of the extra-alveolar vessels for study had been achieved, those experiments were done under non-physiologic conditions. Another major deficiency was that single weight measurements were used to quantitate filtration from 2 to 5 minutes following a vascular pressure change. As was previously discussed, ongoing slow vascular changes persist for much longer than the time period encompassed by these measurements, casting doubt upon their accuracy.

In the isolated isolated lobe, as alveolar pressure is increased relative to surrounding surface pressure transpulmonary pressure increases in a concomitant fashion. In this caes, it is reasonable to assume that an increase in the alveolar pressure would result in an increase in the pressure of the alveolar interstitium. At the same time, however, the volume of the extra-alveolar interstitial space would also increase while the extra-alveolar interstitial pressure drops (Permutt, 1974, Lai-Fook, 1979, Inoue, 1980). Previous attempts to have been made to manipulate pleural and alveolar pressures independently to assess the relative transvascular fluid flux following pressure change to each interstitial component. However, these results have been inconsistent (Bo et al.,1977).

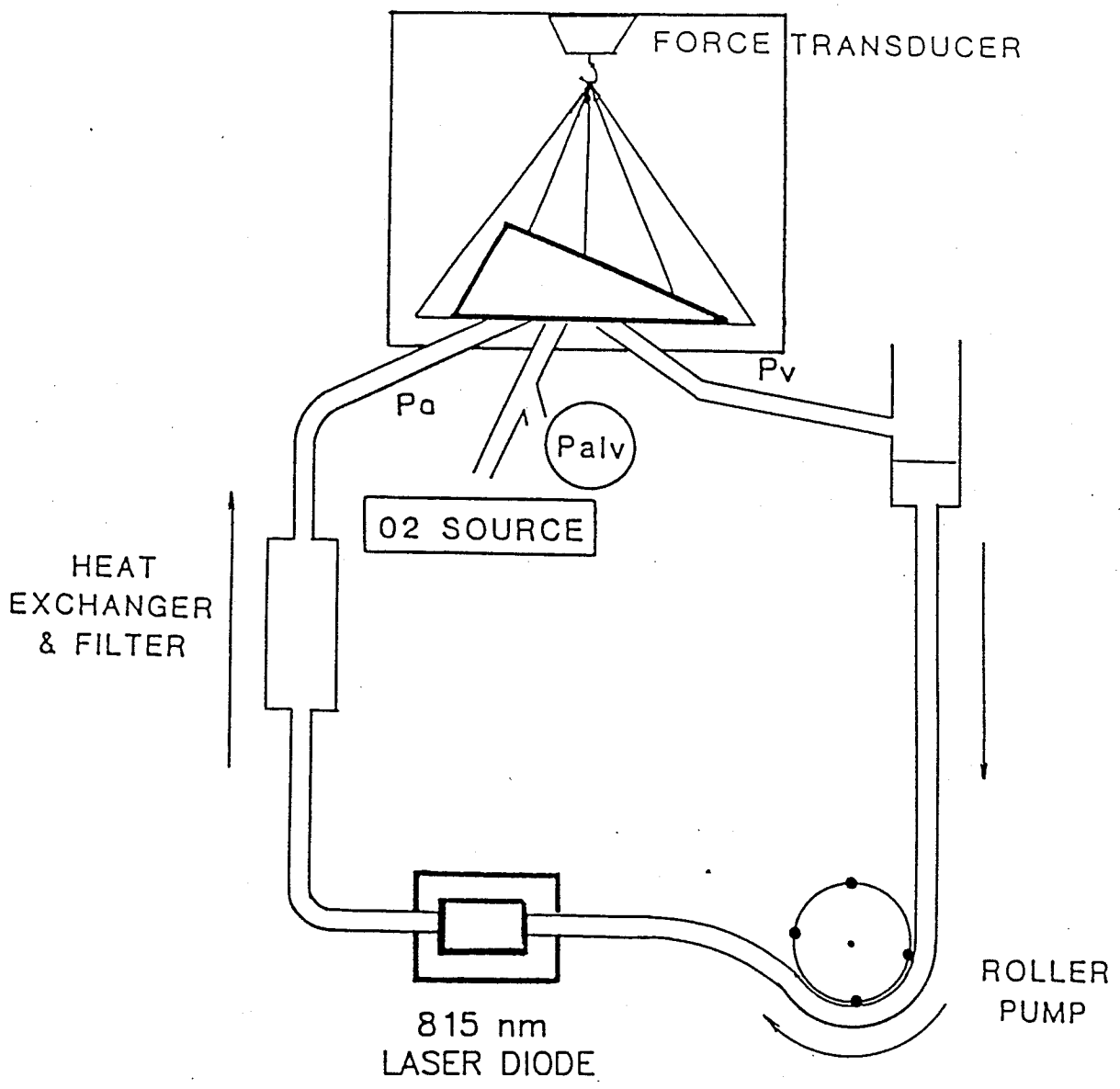
Another area of controversy surrounds the effect of positive end expiratory pressure (PEEP) on the formation

of pulmonary edema. Some investigators have demonstrated increased filtration with the application of PEEP (Demling, 1975, Albert, 1980), while others have shown no change (Bo et al.,1977). One proposed explanation for increased fluid flux with PEEP has been the notion that the contribution of the extra-alveolar vessels to edemagenesis would increase, since pressure would increase along these vessels favouring edema formation (Albert, 1980).

In order to study the contribution to transvascular fluid flux of these vessels and compartments, alveolar and transpulmonary pressures were manipulated independently, under physiologic Zone 3 conditions. The effects of these independent changes in alveolar and transpulmonary pressures on the Pc/Qf relationships and Pc(crit) were determined. The data also demonstrated a significant effect of PEEP on transvascular fluid filtration.

Methods

The standard preparation has been discussed in detail previously, but for these experiments some modifications were developed. The preparation is shown below in Figure 11.



Isolated, hemoperfused canine left lower lobes were used. The entire preparation was placed in a sealed humidified acrylic 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 to pass from the lobe to the circuit as well as a box pressure measurement port. The lobar pleural pressure was manipulated by the application of suction or humidified air to allow independent manipulation of surface and alveolar pressures.

Protocol

The protocol consisted of initial preparation stabilization in West's Zone 3 with arterial, venous and static airway pressure maintained at 15, 10, and 5cmH2O respectively. Calibration of the on-line laser signal was then performed. The lobes were randomized to one of three groups and filtration was characterized under two conditions in each group:

GroupI: Changes in alveolar pressure were introduced while constant lobar volume was maintained.

This was achieved by increasing the box pressure by the same amount as the alveolar pressure increase. In this group transpulmonary pressure therefore remained constant. The two conditions investigated were an alveolar pressure of 5 cmH2O with a box pressure of 0 cmH2O, and an alveolar pressure of 15cmH2O with a box pressure of 10cmH2O.

GroupII: The alveolar pressure was held constant while the transpulmonary pressure was increased. This was accomplished by applying suction to decrease the box pressure. An alveolar pressure of 5 cmH2O was maintained while the box pressure was changed from atmospheric pressure to -10cmH2O.

Group III: In this group of experiments the alveolar pressure was increased with a concomitant and equal rise in transpulmonary pressure. To accomplished this the box was exposed to ambient atmospheric pressure. This was a combination of the conditions introduced in Groups I and II and is analogous to the clinical situation in which positive end expiratory pressure (PEEP) is used. Alveolar pressure was altered from 5 to 15 cmH2O while the box pressure remained atmospheric.

With the lobe under West's Zone 3 conditions two Pc/Qf relationships were constructed before and after the changes outlined in each group. The order that the Pc/Qf relationships were performed was also randomized. Filtration was determined by on-line weight and laser signals at three pressures.

Statistical Analysis

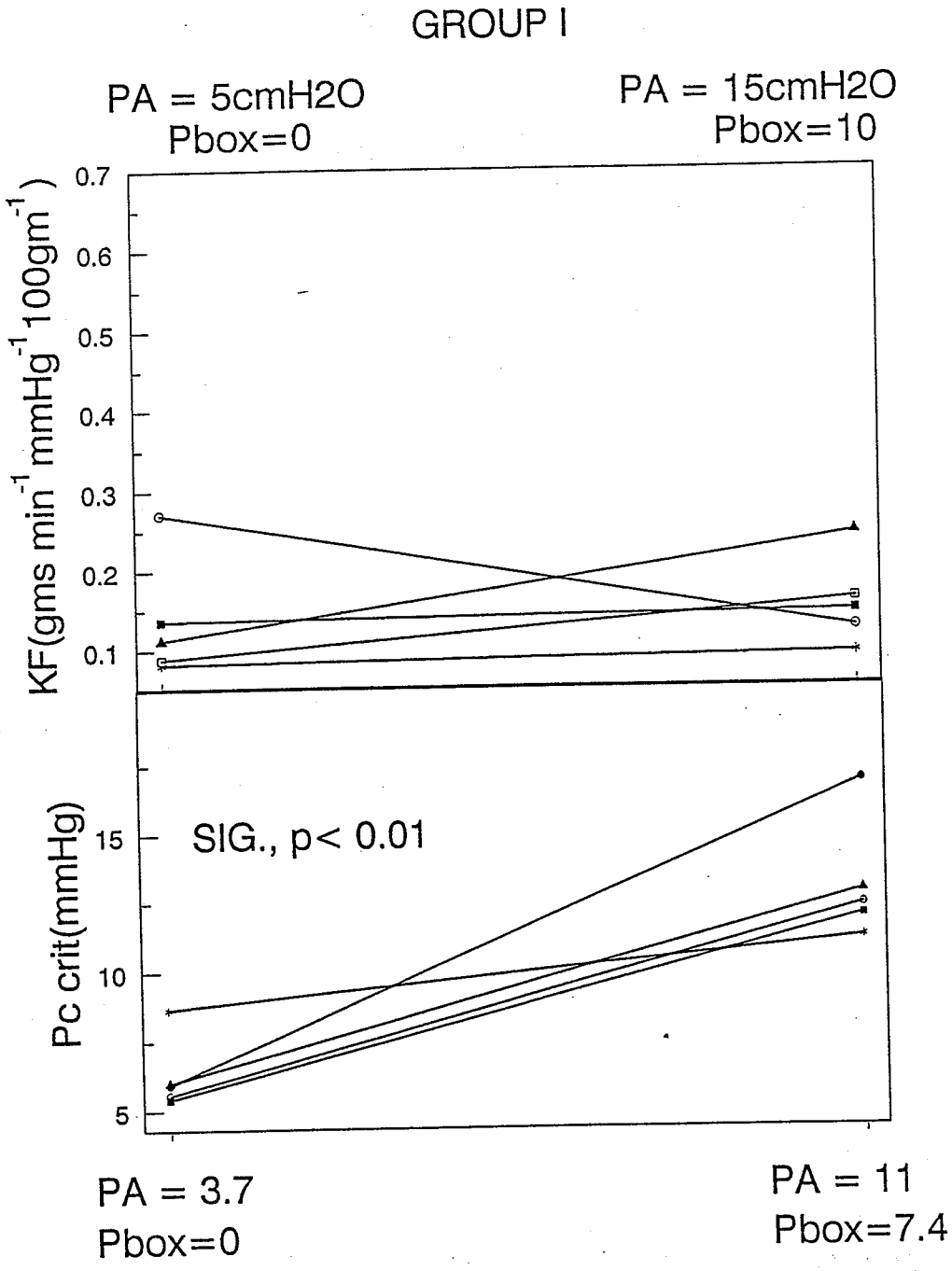
Paired t-tests were used for the analysis within each group, and a one way analysis of variance to compare the results between all groups.

Results

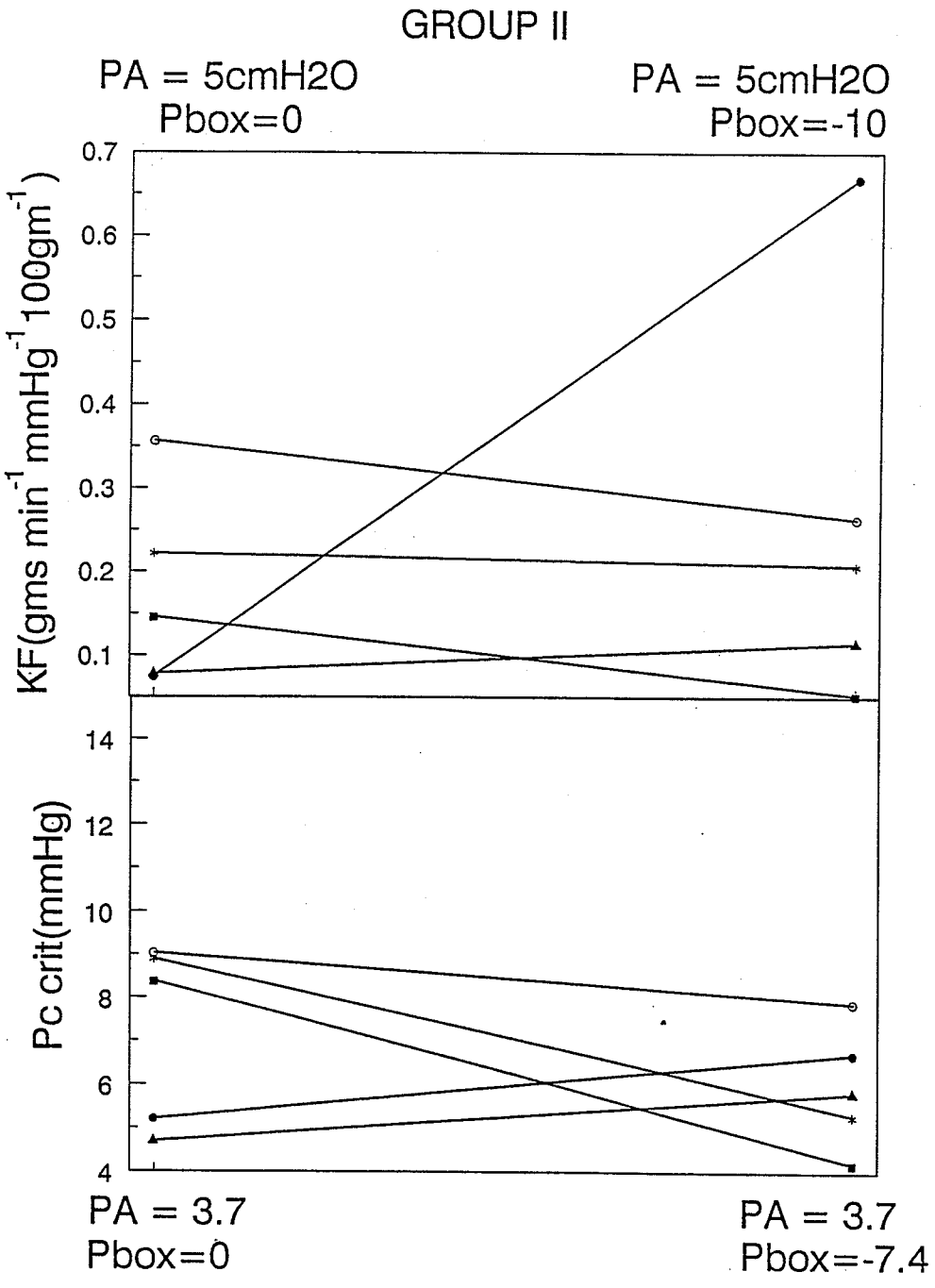
The results of experiments in Group I are seen in Figure 12. The pressures are expressed along the lower abscissa in mms of Mercury. The order that the Pc/Qf relationships were done in each experiment for all groups was randomized. All Kf's were expressed in mls/ min/ mmHg/ 100gm initial wet weight. Under these conditions there was no significant change in the slope of the Pc/Qf relationship, or Kf, between the two conditions. However, there was a significant rise in Pc(crit) to $p < 0.01$ at higher alveolar

and box pressure. This corresponded to a mean increase in Pc(crit) of 9.04cmH2O (6.64 mmHg), for an increase in alveolar pressure of 10cmH2O.

Figure 12

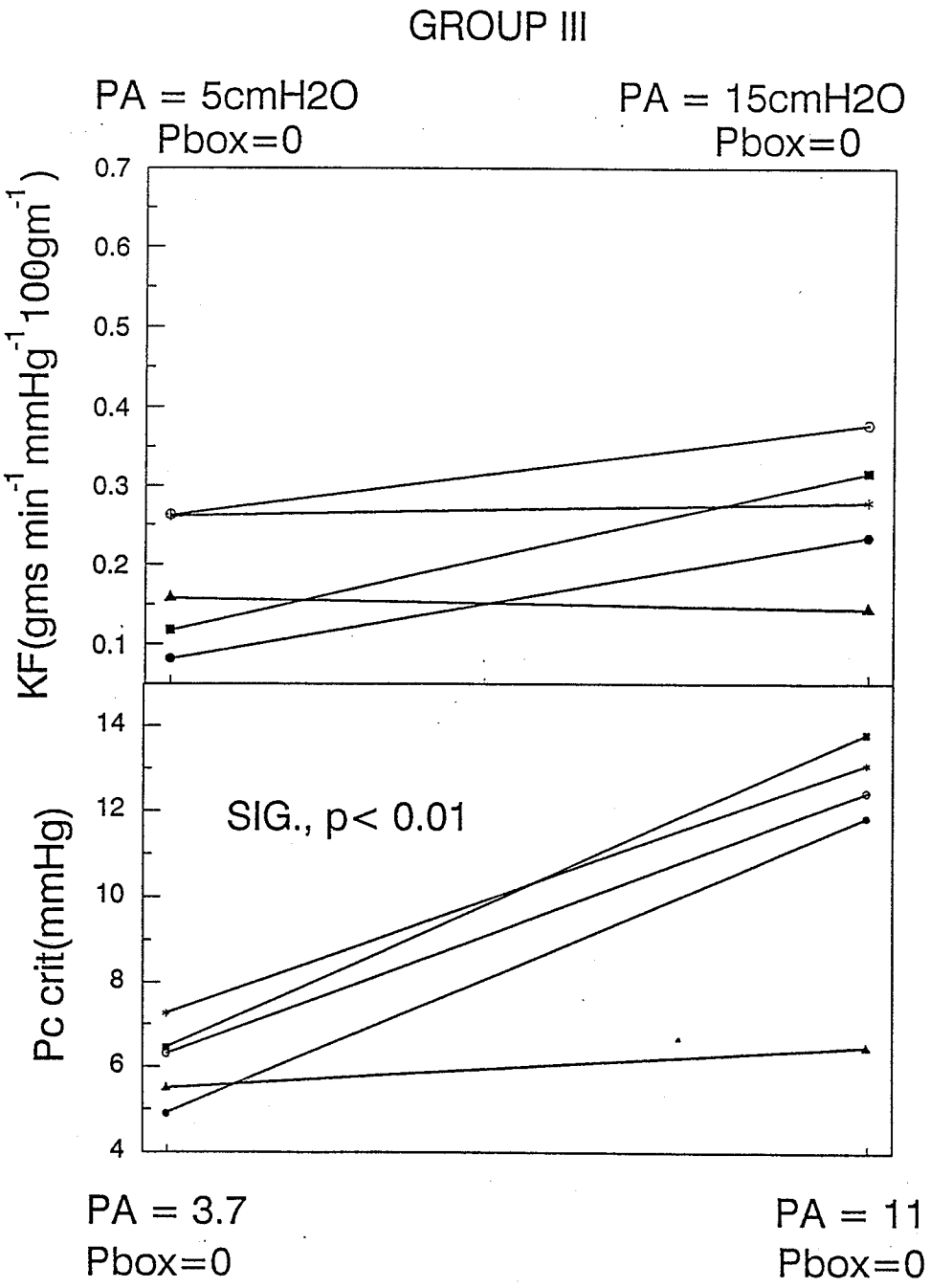


The Group II results are summarized in Figure 13. The Kf's determined in each condition were not significantly different nor was there any significant change in Pc(crit) between a transpulmonary pressure of 5cmH2O and 15cmH2O.



In Group III (Figure 14) concomitant changes in alveolar and transpulmonary pressure were introduced, a combination of the effects introduced in Groups I and II.

Figure 14



The Kf's determined during the two conditions were not significantly different from one another, but the Pc(crit) rose significantly to, $p < 0.01$, with the increase in alveolar pressure. The mean rise in Pc(crit) was (7.37cmH2O) or 5.43 mmHg.

To ensure no intergroup lobar differences a summary of similar conditions in each group is shown in the table below. There was no difference in the Kfs or the Pc(crits) between the three groups with comparable alveolar and surface pressures.

Table 6

PA = 5 : Pbox = 0

	Group I	Group II	Group III
Kf (gms min^{-1} $mmHg^{-1}$ 100gm wt^{-1})	0.140 ± 0.030	0.176 ± 0.047	0.177 ± 0.033
$Pc_{(crit)}$ (mmHg)	6.4 ± 0.5	7.7 ± 0.8	6.1 ± 0.4

(Results: mean ± standard error)

Discussion

The results of this study reconciled some of the discrepancies in the literature. In the Group I experiments, alveolar pressure was changed while pressure and volume changes in the extra-alveolar interstitial space were minimized. Although Kf remained unchanged the Pc(crit), representing the point of balance of the Starling forces, rose significantly. The magnitude of this increase was almost equal to the increase in the alveolar pressure that was introduced. This suggested that their was a direct relationship between Pc(crit) and alveolar pressure, that is, as alveolar pressure was increased the balance of Starling forces was shifted upwards so that a higher Pc was required before edema began to form. The transmission of alveolar pressure to the perimicrovascular space surrounding the vessels appeared to be nearly one to one in nature, and suggested that this alveolar pressure was being transmitted to the fluid exchange vessels in a similar fashion.

Morphometric data obtained by Staub (1974) showed that the vascular surface area per unit membrane thickness was 10-10,000 times greater for the alveolar capillaries than for any other segment, pointing to the largest filtration capacity for vessels within the alveolar walls. The results obtained

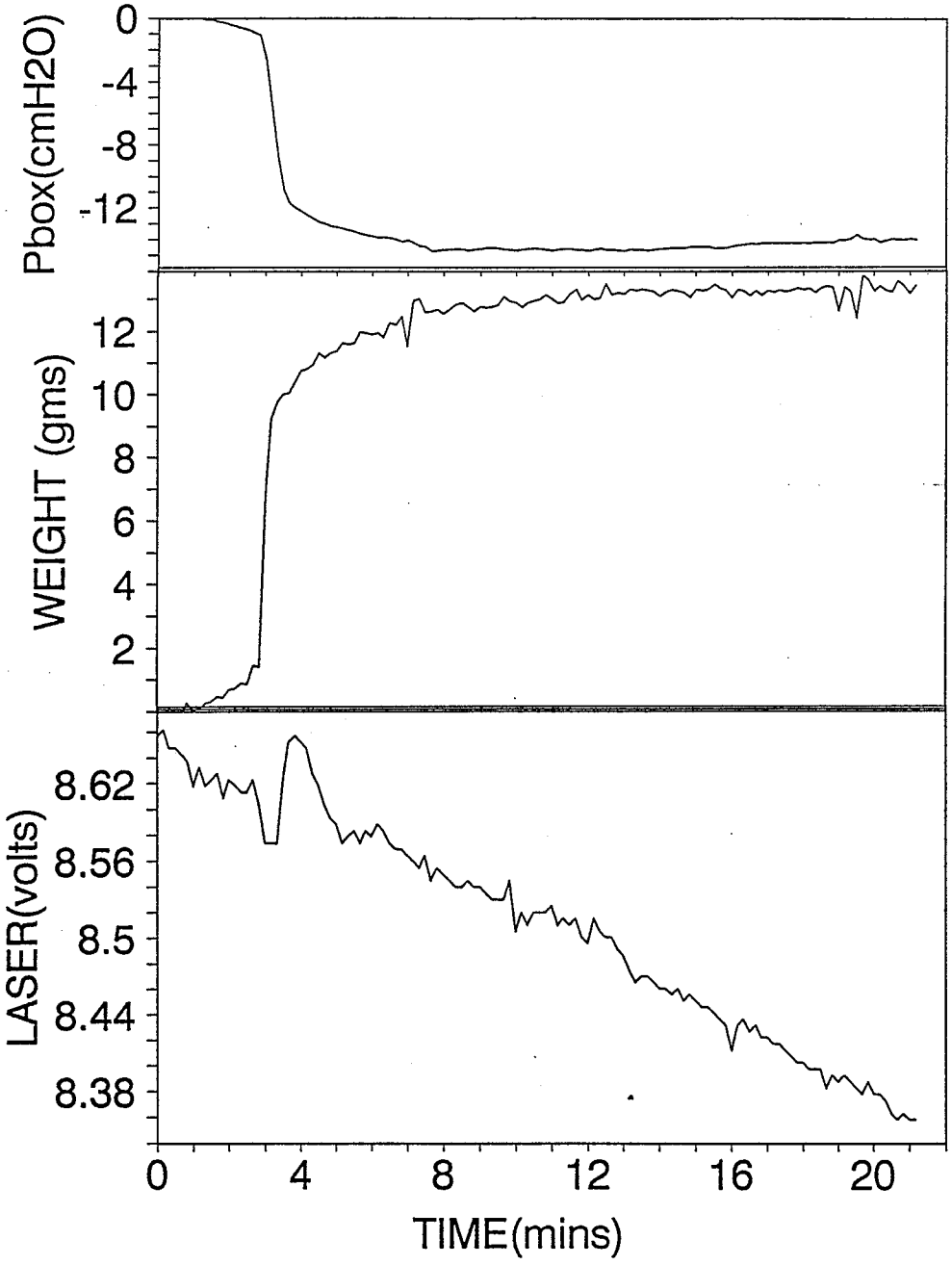
in this study offer support to this contention where isolated pressure changes in the alveolar compartment affected the balance of Starling forces. If the downstream compartment for transvascular fluid exchange was the alveolar space, or alveolar interstitium (provided there was alveolar pressure transmission of equal magnitude) then any alveolar pressure change introduced would affect the balance of forces by an equal amount. This in fact was the case as demonstrated by the Group I results. Additional studies done by Gee (1979) demonstrated that the perivascular cuff fluid of small to medium sized vessels (>1mm) was a relatively sequestered pool. That work suggested the possibility that an alternative site, possibly the alveolar space, was present where significant fluid exchange took place.

In the Group II experiments changes in the alveolar interstitium were minimized while a pressure drop in the extra-alveolar interstitial space was introduced. This group of experiments addressed the relative contribution of the extra-alveolar vessels to transvascular fluid flux. The increased volume in the extra-alveolar interstitial space would have increased the transmural hydrostatic gradient favouring edema formation. However, the results failed to demonstrate any significant change in the filtration characteristics, either Kf or Pc(crit).

Group II closely mimics the Zone 1 studies done by previous investigators who in fact demonstrated a significant filtration contribution of the extra-alveolar vessels (iliff, 1971, Albert, 1978, Mitzner, 1979). This seemingly contradictory result can be explained if one refers to Figure 15. The tri-panel graph demonstrates the effect of a pressure drop in the extra-alveolar interstitial space at constant alveolar pressure. The weight tracing demonstrated the characteristic biphasic response that would be measured as transvascular fluid exchange over the short time period that these weight changes were followed by previous investigators. The laser colorimetric signal remained unchanged following the introduction of an increased transpleural pressure gradient. This suggests that the vascular volume of the extra-alveolar vessels was increased but that there was no significant transvascular fluid exchanged. If weight signals were followed until rates of constant weight gain were achieved similar findings were demonstrated. This was strong evidence that extra-alveolar vessels contributed little to the total transvascular fluid exchanged under normal physiologic conditions.

Figure 15

(The effect of isolated extra-alveolar pressure drop on fluid flux)



In Group III, alveolar interstitial pressure was increased at the same time as the extra-alveolar interstitial pressure presumably fell. This group was therefore a combination of the effects introduced in Groups I and II, and was somewhat analogous to the clinical situation of instituting PEEP. Despite the findings of previous investigators (Demling, 1975, Albert, 1980) our findings suggested that institution of PEEP on the excised preparation altered the balance of forces at the pulmonary membrane. However, the Kf remained unchanged. Therefore once above Pc(crit), although this did not occur until a higher capillary pressure had been reached, transvascular fluid exchange occurred at a similar rate. Translation of this finding to the in-situ preparation or the clinical setting is not warranted from this data but the possibility that PEEP may shift the balance of forces to favour reabsorption at higher microvascular pressures merits further study.

Conclusions

These experiments supported the concept that transvascular fluid flux can be accurately described by means of Pc/Qf relationships obtained from rates of constant weight gain or laser colorimetry. The slopes of the Pc/Qf relationships, assumed to represent total lobar conductance, or Kf, were not altered significantly by changes in either alveolar or transpulmonary pressures. The balance of forces at the pulmonary membrane were significantly altered such that increasing alveolar pressure with or without changes in transpulmonary pressure resulted in a significant increase in Pc(crit). At constant alveolar pressure, changes in transpulmonary pressure had no effect on Pc(crit). Because Pc(crit) was seen to change only with alterations in alveolar pressure, fluid exchange must have occurred with the alveolar interstitium or more likely the alveolar space from the onset of transvascular fluid flux.

SECTION (III)

Oncotic Manipulation and its Effect on Fluid Flitration

Background

A third series of experiments were performed to investigate the effect of macromolecular colloid manipulation on transvascular fluid flux. Normal human body fluids contain a small number of large particles (MW > 30,000), mainly proteins, which contribute a small amount (5%) to the total plasma osmolality. However this component of plasma, referred to as the colloid component, is a major determinant of the distribution of water between the body fluid spaces because of the limited permeability of capillary membranes to this colloid component. Since the time of Starling it has been assumed that hydrostatic pressure differences between the plasma and tissues are effectively opposed by the plasma to tissue oncotic gradient. However the importance of maintaining or increasing oncotic forces for the treatment of pulmonary edema remains controversial. Edema formation would not occur provided net fluid filtration into the tissues was less than

the regional lymph flow capacity. This hypothesis was initially supported by Guyton and Lindsay (1959) and subsequent studies by a number of groups confirmed these observations (Gaar, 1967, Effros et al., 1981). However, confidence in the role of protein in pulmonary fluid balance has been eroded by other reports (Uter, 1967). There is to date little conclusive evidence that protein infusions effectively diminish lung edema content in animals or humans (Brown et al., 1973, Marty et al., 1975, Rackow, 1977, Virgilio et al., 1979 Sibbald et al., 1983)

In the present study, transvascular fluid flux was again characterized by the construction of Pc/Qf relationships. The pressure intercept of such a plot, as previously discussed, represents the balance of forces that exist at the pulmonary membrane. Bidirectional fluid flux occurs around this Pc(crit) as shown in Figure 1. To effect reabsorption of edema the hydrostatic capillary pressure must be lower than Pc(crit). Therefore, as previously demonstrated (equation 7) for reabsorption:

$$Pc(crit) = Pt + \sigma^2 \times \pi c > Pc \qquad (15)$$

Colloid manipulation for these experiments was effected using a large macromolecule, Hetastarch. The MW of Hetastarch

averages 450,000 (10,000-2,000,000) and therefore it was predicted that this molecule would have a high reflection coefficient when compared to albumin (MW 63,000). Hetastarch was also commercially available and came as an osmotically balanced 6% solution. It is licensed for use in both Canada and the United States. Indications for administration include leukophoresis and plasma volume expansion, although use in lung injury has never been described.

The objective of these experiments was to determine if the critical pressure, Pc(crit), could be manipulated to effect reabsorption of pulmonary edema at normal pressures in excised canine lobes. This hypothesis was tested under two conditions:

1) The normal excised lobar preparation.

2) The same preparation following a permeability injury induced by the addition of oleic acid into the system.

Methods

The study utilized excised canine left lower lobes which were set up in the manner described previously. The colorimetric device was not used for transvascular fluid flux determinations in these experiments.

Protocol

The protocol consisted of two arms:

In Arm 1 the lobes were initially excised and stabilized. Step changes in pressure were then instituted and weight gain followed to enable construction of the Pc/Qf relationship. Hetastarch was then added to the circuit. A volume of 250mls. of Hetastarch was added to the circuit such that the final concentration of the Hetastarch component was 2.7%. Following an equilibration period of 30 minutes the steps were repeated.

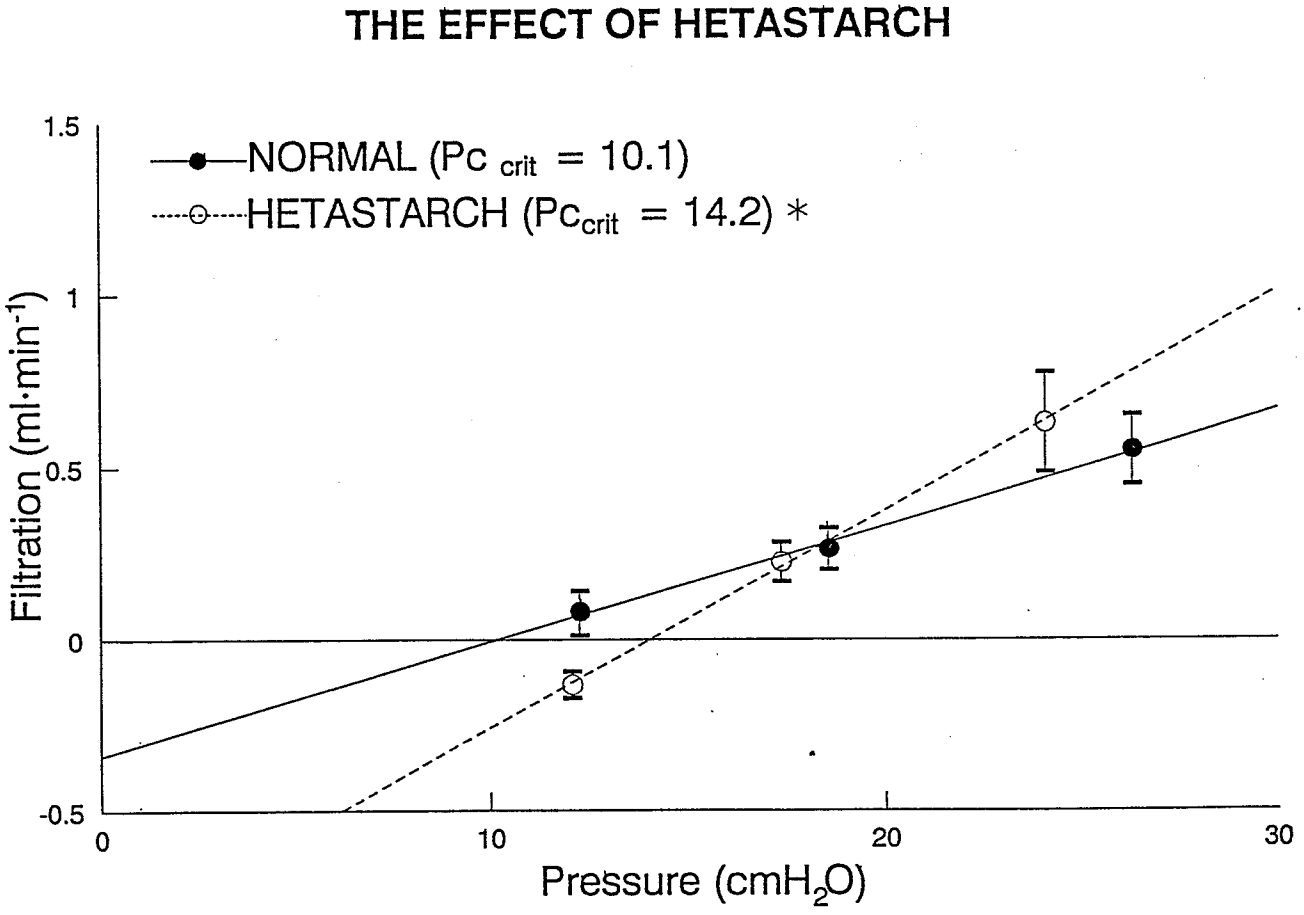
Arm 2 was done in an identical manner, however, oleic acid (0.15ml) was added to the circuit at the beginning of the experiment.

Statistical Analysis

Paired t-tests were done to compare each lobe in Arm 1 and Arm 2, with minimum significance recognized at the $p<0.05$ level.

Results

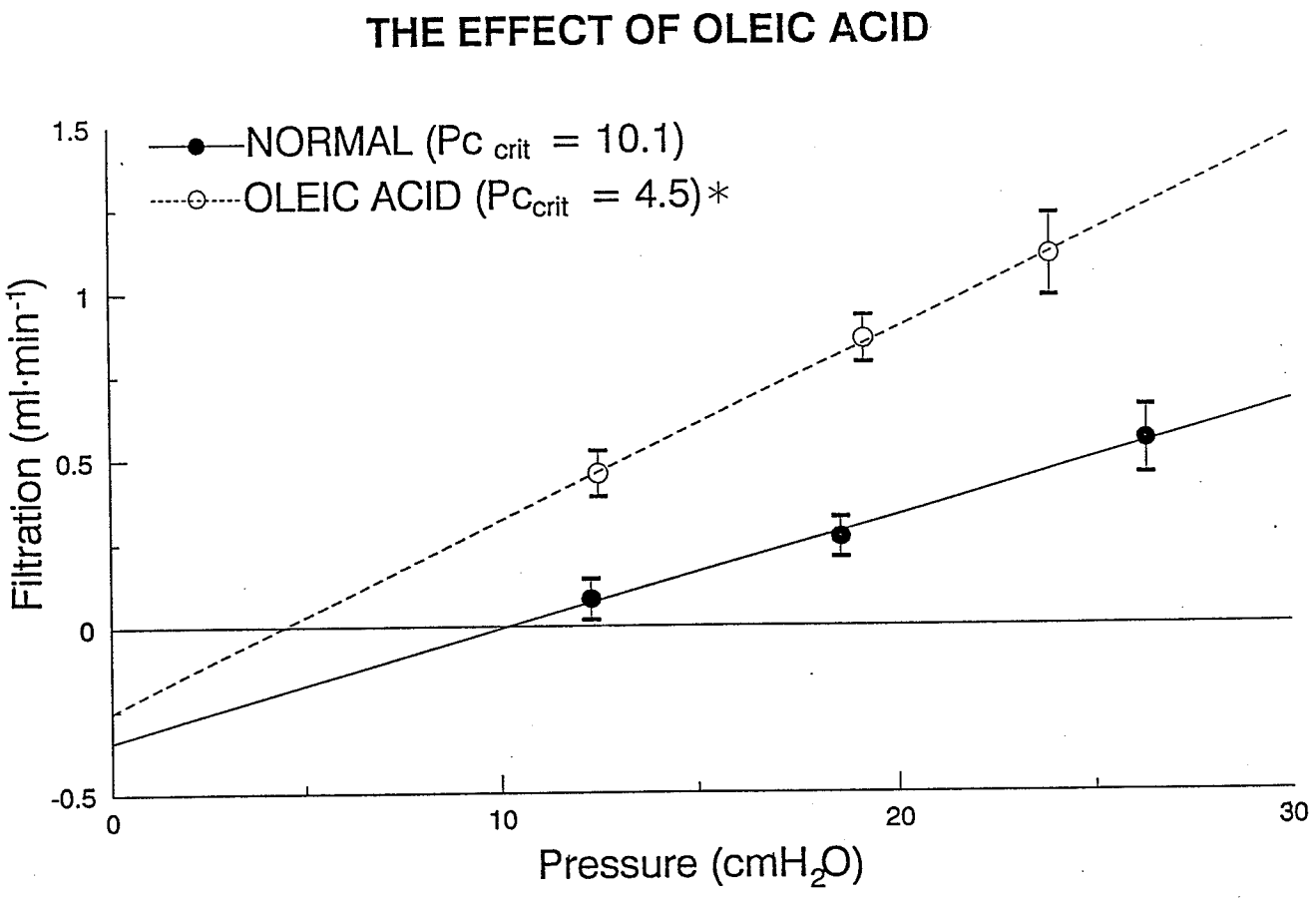
The effect of Hetastarch on normal lobes is shown below in Figure 16. The dotted and solid lines, with accompanying standard error bars, represent lobes perfused with and without Hetastarch in the circuit respectively.



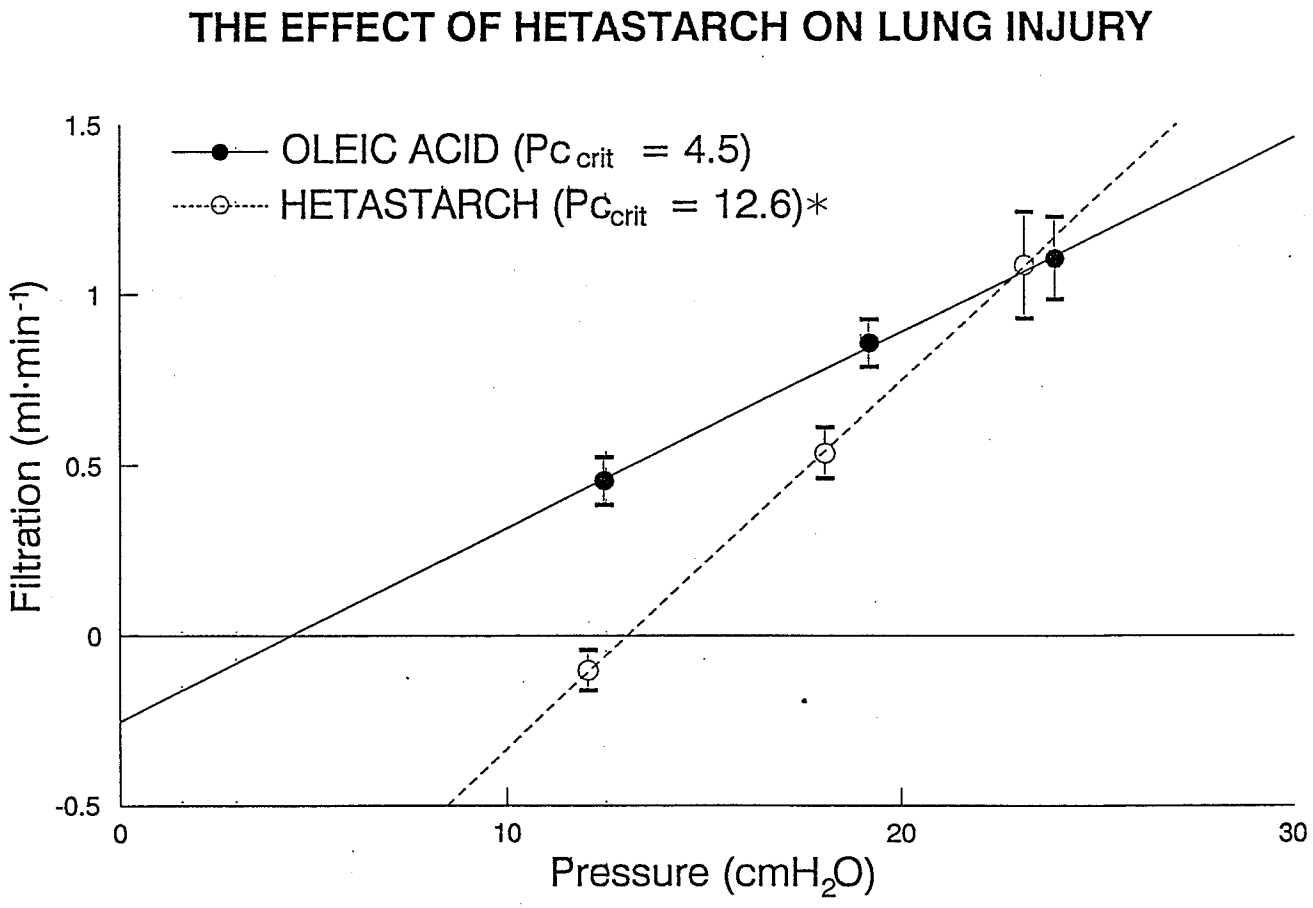
There was a significant increase in the Pc(crit), from 10.1 ±1.0 to 14.2 ±0.6 cmH20 following the addition of Hetastarch. This was significant to $p<0.01$. The increase was such that at the lobar baseline capillary pressure reabsorption was induced. The slope of the Pc/Qf relationship was also seen to increase following the addition of Hetastarch. The Kf (±S.E.) increased from 0.0889±0.013 to 0.1778±0.029 ml/min/mmHg/100gm with the addition of Hetastarch ($p<0.05$).

In Figure 17 the effect of the addition of oleic acid to the system is shown. Pc(crit) fell significantly ($p<0.01$), from 10.1±1.0 to 4.5±1.9 cmH20, and edema began to form at very low lobar Pcs. The total lobar conductance, represented by the slope of the Pc/Qf line, also increased significantly following the addition of oleic acid from 0.0889±0.013 to 0.1592±0.013 ml/min/mmHg/100gm ($p<0.01$). Both of these factors suggested that a significant permeability injury had been induced by oleic acid.

Figure 17


THE EFFECT OF OLEIC ACID
NORMAL (Pc crit = 10.1)
OLEIC ACID (Pccrit = 4.5)*
Filtration (ml·min-1)
1.5
1
0.5
0
-0.5
0
10
20
30
Pressure (cmH2O)

The effects of Hetastarch with lung injury are shown below in Figure 18. Note that from the baseline oleic acid lobes, represented by the solid line again with standard error bars, addition of Hetastarch to the circuit caused a rise in Pc(crit) from 4.5±1.9 to 12.6±0.3 cmH2O. This was highly significant to $p<0.001$. Again at baseline capillary hydrostatic pressures reabsorption was seen in all lobes. The Kfs determined also were seen to rise significantly from 0.1592±0.013 to 0.2761±0.029 ml/min/mmHg/100gm with Hetastarch in the circuit ($p<0.05$).



Discussion

The plasma colloid component is of paramount importance in determining the water distribution in biological systems. In the formation of pulmonary edema imbalance in Starling forces result from either increased hydrostatic forces, altered permeability which lowers the effective oncotic force at the pulmonary membrane or both. In these experiments a model of low pressure, or increased permeability, pulmonary edema was used. The addition of oleic acid into the preparation circuit had the effect of causing a significant imbalance in the Starling forces such that an edema forming state was present at very low capillary hydrostatic pressures.

Hetastarch administration resulted in significant protection from edema formation due to an increase in the Pc(crit) of both normal as well as lobes injured with oleic acid. The increase in Pc(crit) was such that reabsorption of fluid was seen at baseline hydrostatic pressure in both normal lobes as well as following lung injury. The inability of many previous investigators to demonstrate a benefit from albumin infusions can be explained by considering the factors that determine Pc(crit). The fraction of the oncotic pressure that is effective at the pulmonary membrane is determined by the

square of the reflection coefficient (equation 7). Previous data obtained from 13 lobes using the same model demonstrated a fall in the mean σ to total protein of 50%, from 0.58 ±0.09 to 0.30 ±0.04 (±S.E.) after oleic acid injury. The reflection coefficient was calculated using a method described by Pilati and Maron (1985). With lung injury the "effective" oncotic pressure ($\sigma^2 \times \pi c$) generated by albumin would be low even with large volume infusions. Although not measured directly the molecular weight difference between Hetastarch and albumin as well as its effectiveness even in the presence of low reflection coefficient to albumin with lung injury suggest the rise in Pc(crit) was likely due to maintanance of the reflection coefficient by Hetastarch.

The slopes of the Pc/Qf relationships rose with the addition of Hetastarch to the circuit under both normal conditions as well as with lung injury. One can speculate a number of possible explanations. Other investigators have demonstrated a rise in measured Kfs with >50% wet weight accumulation (Drake et al., 1980). The experimental design was such that all Hetastarch Pc/Qf relationships were obtained following some baseline determination. The average edema accumulation during Pc/Qf determinations was 40% of initial wet weight, therefore an increase in Kf independent of Hetastarch administration was possible.

Alternatively, the Hetastarch molecule may have had a effect on lobar conductance, although this has not been described for Hetastarch or compounds of similar biochemical composition.

Conclusion

By using the concept of Starling's balance of forces we have demonstrated that appropriate colloid therapy may be effective treatment for inducing reabsorption of pulmonary edema even in the presence of significant lung injury. With focus on Pc(crit) and not simply on increasing the plasma oncotic pressure, resolution of edema can be effected at normal capillary hydrostatic pressures.

GENERAL DISCUSSION AND SUMMARY

The objective of this preceding work was two fold. The initial hypothesis required a method to characterize transvascular fluid flux that was accurate, and independent of weight determinations. Laser colorimetry offered an alternative to standard weight measurements and was validated using a hemofilter device that ensured weight changes to be true filtration thus circumventing the possibility of any vascular volume change. Bidirectional flux, measured either by weight or laser yielded comparable results. This technique proved capable of accurate measurements of lobar transvascular fluid flux under normal conditions as well as with lung injury. The use of this method offered strong support to the concept that fluid flux is completely described by linear rates of filtration for a given Pc. Fluid flux is bidirectional around a single isogravimetric point at which the Starling forces are in balance. This simple approach to pulmonary fluid flux in the excised lobar preparation agrees well with early work in single capillaries and subsequently in other mammalian capillary beds. The monoexponential function that has in the past been used for characterizing transvascular fluid flux and studying membrane properties most likely represents a vascular artifact due to stress relaxation

of the pulmonary vasculature. Gravimetric methods agreed well with laser measurements provided adequate time allowed for linear rates of weight gain to be achieved.

Factors governing the balance of forces, or Pc(crit), was the second major focus of this work. The impact and manipulation of forces acting at the exchange surface was addressed in the second and third sections of experiments. The relative importance of the interstitium (extra-alveolar) as the downstream compartment to fluid flux has been challenged. The data demonstrated that pressure within the alveolar space was in large part transmitted to the exchange surface and suggested that fluid exchange with the alveolar space may be occurring from the outset of a pressure increase above Pc(crit). The role of PEEP in fluid exchange remains poorly defined, but it was shown to impact on the balance of forces at the pulmonary membrane. A protective role in the genesis of pulmonary edema was implied but extrapolation beyond the excised lobar preparation would not be valid.

The role of Pc(crit) and oncotic force manipulation was the focus the last series of experiments. The importance of the reflection coefficient, especially with lung injury, in determining the effectiveness of attempts to induce reabsorption should be emphasized. Adherence to this principle, as it pertained to increasing the Pc(crit) allowed

reabsorption of pulmonary edema even with lobar injury at normal hydrostatic capillary pressures. We have recently used this approach successfully in two patients, and these initial results in the management of established ARDS (low pressure pulmonary edema) appear promising.

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