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Pulmonary microvascular sites of liquid filtration

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PULMONARY MICROVASCULAR SITES OF LIQUID FILTRATION

by

MICHAEL ALLAN GROPPER
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DISSERTATION

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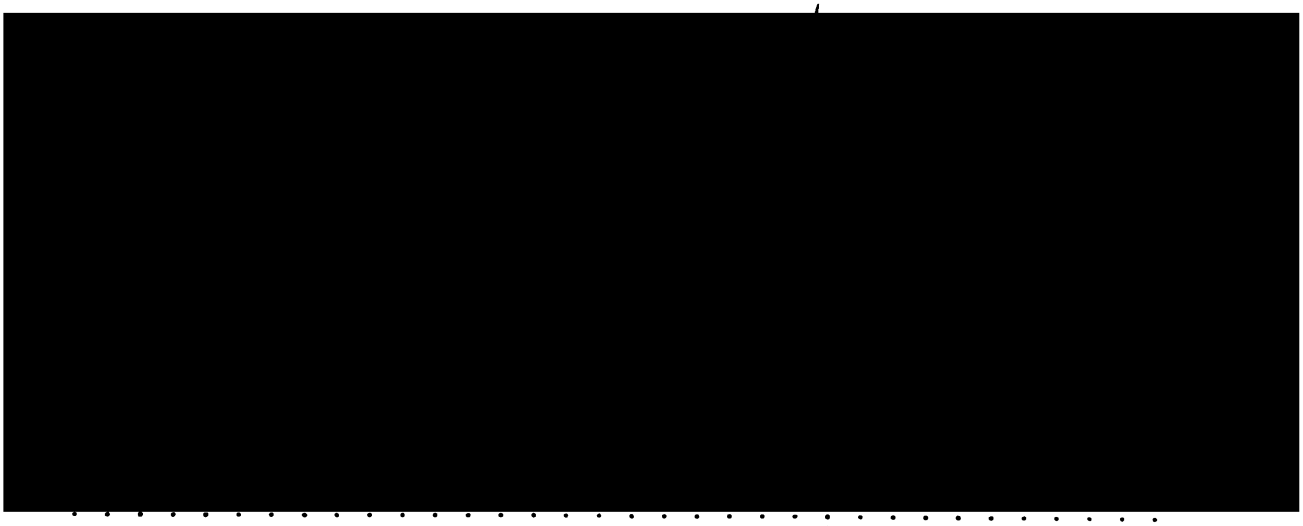

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ABSTRACT

Little is known about the exact site of liquid filtration in the lung. Although traditional thought would suggest that the bulk of filtration occures across the huge surface area (70 m^2) of the alveolar wall capillaries, there is little evidence to support this view. The structure of the pulmonary interstitium suggests that filtration may occur in vessels proximal and distal to the alveolar wall.

I studied the contribution of the extraalveolar arterial and venous beds to total filtration by compressing the alveolar capillary bed with alveolar pressure greater than vascular pressure (Zone I). I found that the arterial and venous beds had identical filtration characteristics, and as a whole, contributed 43% of the total filtration seen from the pulmonary vascular bed. The shape of the filtration profile obtained by increasing vascular pressure at constant alveolar pressure suggests that recruitment of filtration surface area occurs while the lung is still in Zone I. When the lung is in Zone III (vascular pressure > alveolar pressure), filtration is only 3-5 times greater than that seen in Zone I, although filtration surface area has increased 50-70 times. These experiments show the substantial contribution of extraalveolar vessels to lung liquid filtration.

The effect of alveolar pressure on filtration rate is controversial. Studies have shown that increasing alveolar pressure can increase, decrease or have no effect on Zone I filtration rate. I found that alveolar pressure had no effect on filtration rate in either isolated or open thorax, intact dog lung lobes. This result suggests

that interstitial pressure at the site of filtration is closely related to pleural pressure. In my experiments, pleural and vascular pressures were maintained constant, relative to each other, therefore there was no change in filtration rate when alveolar pressure was changed.

The final component of my dissertation studied albumin flux into the hilar interstitium induced by hydrostatic edema. I developed a method for the measurement of albumin concentration in 10-20 nl samples of interstitial liquid collected by micropuncture. I found that there was no difference in interstitial albumin flux whether edema was induced in the arterial or venous beds. Interstitial albumin concentration was 83% of the plasma albumin concentration. The similar albumin permeabilities of the arterial and venous beds agrees with my earlier gravimetric studies.

The experiments in my dissertation provide a detailed study of liquid and protein flux from the extraalveolar vascular bed. Further studies should be done examining regional interstitial albumin and total protein concentrations to gain a clearer picture of the sites of filtration in the lung.

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INTRODUCTION

In order to carry out their primary function of gas exchange, the lungs move the entire right ventricular output through a vast capillary system. The walls of vascular structures of the lung must be thin enough to allow rapid gas exchange, while having the strength and integrity to keep liquid from flooding the alveoli. The pulmonary vessels branch extensively to maximize vascular and gas exchanging surface area. The microvascular bed is so extensive that Fung and Sobin (21) likened it to a continuous sheet of blood interrupted by a few supporting posts. Weibel (78) estimates the pulmonary capillary surface area in man to be approximately 70 m^2, with only a slightly smaller value found in a large dog. Another morphologic study of the pulmonary circulation showed the capillaries to be a high-density network of tubules (28).

In their anatomic studies of the lung, both Miller and Drinker (17,49) found an extensive lymphatic system and suggested that there must be substantial liquid filtration. There must be structural and physiological specializations in the alveolar epithelium, capillary endothelium and interstitium to maintain normal liquid balance. This is also important in the arterioles and venules of the lung, which have very thin walls, unlike their counterparts in muscle.

Lung liquid balance

Liquid balance in the lung, as in any tissue, is accurately described by the Starling equation. It was Starling (68) who first developed the concept that hydrostatic pressure in the capillaries is balanced by the osmotic pressure of plasma proteins. This relationship is most commonly written as:

$$J_v = K_f [(Pmv - Ppmv) - (\Pi mv - \Pi pmv)]$$

where:

J_v = transvascular volume flux

K_f = intrinsic permeability of the vascular barrier

Pmv = microvascular hydrostatic pressure

Ppmv = perimicrovascular (interstitial) hydrostatic pressure

σ = reflection coefficient for protein

Π mv = plasma protein osmotic pressure

Π pmv = perimicrovascular protein osmotic pressure

The four Starling pressures are shown in Figure 1.

Under normal conditions, there is net filtration out of the lung microvascular lumen into the interstitium. The filtered liquid is carried away by lymphatics and returned to the systemic circulation via

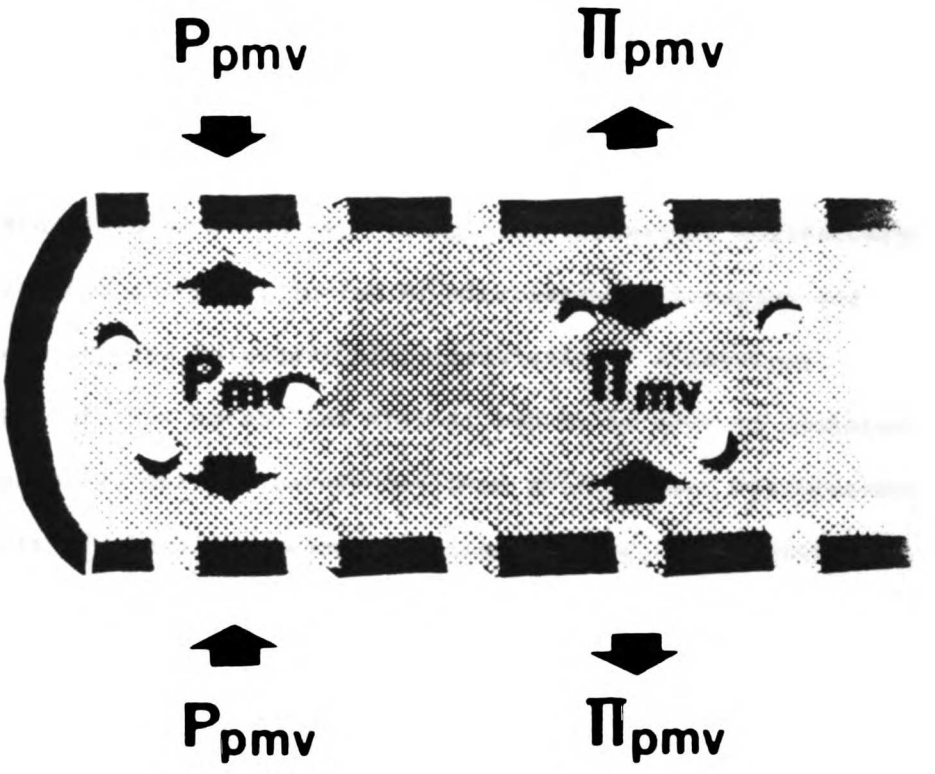


Fig. 1 The Starling forces acting across the microvascular membrane. Microvascular hydrostatic pressure (Pmv) and interstitial protein osmotic pressure (π pmv) favor filtration while interstitial hydrostatic pressure and microvascular protein osmotic pressure favor reabsorption.

the thoracic duct or right lymphatic duct.

When using the Starling equation as the basis for studies of lung liquid balance, some factors are more easily determined than others. For example, it is possible to determine Pmv. The most accurate measurement of Pmv is to directly micropuncture pulmonary blood vessels using the servo-null pressure measuring system (6,52,80). This device allows measurement of microvascular pressure under various conditions of vascular and alveolar pressure. Using this technique, Bhattacharya and Staub determined the distribution of microvascular pressure and resistance along the pulmonary microcirculation. A previous disadvantage of this method was that it had to be done in isolated lungs. Recently, our laboratory has been able to make micropuncture measurements of vascular pressure in open-chested, anesthetized dogs (65) with the bronchial circulation and lymphatic drainage of the lobe intact.

Measurement of Ppmv is difficult. The first attempts at such measurements were made with surgically implanted, porous capsules connected to a catheter leading outside the animal (30). This method is controversial, as scar tissue formation and inflammation around the capsule may give misleading results. Bhattacharya et al (7,8,10) used the servo-null micropuncture method to measure interstitial liquid pressure at various sites in isolated dog lungs. This is a less traumatic procedure, as the pipettete tip is only 2-3 microns in diameter and is less likely to disrupt or distort the tissue as much as a larger surgically-implanted tissue capsule.

Determining π_{mv}, the protein osmotic pressure of plasma is not difficult. The pressure can be measured across a selective membrane in

an osmometer. Nitta et al (54) and more recently Yamada and co-workers (81) have developed a nomogram for determination of protein osmotic pressure, if the total protein and the albumin fraction are known.

The most difficult measurement of all the pressure variables in the Starling equation is Π_{pmv}, the perimicrovascular protein osmotic pressure. The pulmonary interstitium is composed of liquid mixed with a gel containing collagen fibrils and a tangled network of glycosaminoglycans. Although there is albumin in the free liquid, it is restricted from a certain volume of the interstitium, because of its molecular radius and the proximity of the collagen fibers. The volume of interstitium into which albumin cannot penetrate is referred to as the excluded volume. This results in solute (plasma proteins) and solvent having different volumes of distribution (55). The net effect of solute exclusion is to increase the concentration, hence the osmotic pressure of the interstitial proteins by restricting them to a smaller solvent volume. The structure of the interstitium makes sampling interstitial liquid very difficult. Most investigators think that the structural elements of the interstitium have little osmotic activity (27).

The dynamic nature of protein and liquid movement through the interstitium makes it particularly difficult to make steady-state physiologic measurements of Π_{pmv}. By collecting the lymph draining the lung, one can get an estimate of the protein concentration of the interstitial fluid. The most frequently used technique is the lung lymph fistula, as developed by Staub (73).

Many of the workers studying lung liquid balance have relied on observing the effect of altering one or more Starling variables. For

example, increasing Pmv by inflating a balloon catheter in the left atrium will increase Jv, resulting in net filtration and subsequent lung edema, if the capacity of lymphatics is exceeded (20). If Πmv is decreased by dilution of plasma proteins, the result is similar to increasing Pmv (16,40). This is a difficult experiment, as removal of plasma protein results in a rapid flux of protein from the interstitium back into the vascular space. It is also not easy to manipulate the other two Starling variables, Πpmv and Ppmv. The difficulty in measuring pmv makes its role in lung liquid balance controversial. Most certainly, with edema interstitial proteins are diluted resulting in more favorable forces for liquid reabsorption (20). This is sometimes referred to as an edema "safety factor". Although Ppmv increases with lung edema, the magnitude is not great (7,10,67), and its importance would depend on where the pressure increase is occurring, i.e. if interstitial pressure increases at the site of filtration, or at some site downstream along the interstitial liquid drainage pathway.

The relationship between blood flow and alveolar pressure in the lung was systematically studied by West et al. (79). They determined three conditions in the lung, based on the relationship between alveolar and vascular pressures throughout the lung:

Zone I: Alveolar > pulmonary arterial > pulmonary venous pressure

Zone II: Pulmonary arterial > alveolar > pulmonary venous pressure

Zone III: Pulmonary arterial > pulmonary venous > alveolar pressure

In Zone I there is no blood flow, as the higher alveolar pressure compresses the alveolar capillaries. In Zone II there is blood flow but, since alveolar pressure is greater than pulmonary venous pressure, the relevant driving pressure determining blood flow is the difference between pulmonary arterial and alveolar pressure. This phenomenon is sometimes referred to as a Starling Resistor, where the flow is independent of the final downstream pressure. In Zone III, blood flow is determined solely by the difference between pulmonary arterial and venous pressures, and the entire pulmonary vasculature is perfused.

Classes of pulmonary vessels

The lung can be divided into two functional interstitial compartments based on the effects of alveolar pressure on the blood vessels within each. The pioneering studies in this area were done by Macklin in 1945 and by Permutt and Howell in the early 1960's (36,46,61). Macklin studied isolated dog lungs filled with saline or latex. The artery and vein of the lungs were connected to liquid-filled burettes. Saline filled all the blood vessels of the lung including the alveolar capillaries, whereas the latex could not enter these latter small vessels. Macklin observed the height of liquid in the burettes

connected to the pulmonary artery and vein while inflating the lungs with positive pressure. He found that when the lungs were saline filled, the vascular volume would increase at low alveolar pressure, and then vascular volume would decrease at high alveolar pressure. Conversely, with latex filling only larger vessels, vascular volume increased continuously as alveolar pressure increased. Based on these findings, Macklin concluded that the alveolar capillaries were compressed by high alveolar pressure and the liquid within them pushed back into the burettes. The larger vessels were apparently pulled open mechanically or by the surrounding tissue interstitial pressure as the lung was inflated.

Permutt and Howell (36,61) repeated these studies and defined the two compartments as "alveolar", containing the alveolar wall capillaries and their interstitium, and "extraalveolar" containing the arteries, veins, airways and their interstitia. They suggested that the interstitial liquid pressure (P_{pmv}) in the extraalveolar compartment might be determined by pleural pressure, while P_{pmv} in the alveolar compartment is more closely related to alveolar pressure. Nicolaysen and Hauge (53) examined the effects of varying pleural pressure, alveolar pressure, and lung volume on liquid filtration in Zone I lungs. They found that filtration decreased when alveolar pressure and lung volume was increased, and that filtration increased when lung volume was increased by decreasing pleural pressure. Histologically, the alveolar capillaries were compressed, but they found that the alveolar junctional or "corner" vessels (vessels located at the junction of 2 or 3 alveolar walls) remained patent, even far into Zone I (where alveolar pressure is much greater than vascular pressure). They suggested that there might

be a third vascular and interstitial compartment termed "transitional" that may respond in an intermediate manner to a combination of alveolar and pleural pressures. The corner vessels would be located in this compartment, and could be responsible for much of the filtration seen in Zone I.

Many investigators have tried to determine the importance of interstitial liquid pressure in lung liquid balance. Staub, Nagano and Pearce (70) described the sequence of liquid accumulation in dog lungs exposed to high microvascular pressure or injury. They found that the first histological sign of edema was the formation of liquid filled interstitial "cuffs" around small extraalveolar arteries, veins and airways. As liquid accumulation continued, they found thickening of the alveolar wall interstitium followed ultimately by flooding of the alveoli with edema fluid. These leakage pathways are illustrated in Figure 2. Based on these findings, Staub postulated that there was a gradient of interstitial liquid pressure from the alveolar wall toward the perivascular interstitium around larger blood vessels and airways and finally toward the hilum. Bhattacharya and his associates (8) recently confirmed this pressure gradient via direct micropuncture of the interstitium at three sites. In the alveolar wall junctions, in the adventitia around arterioles and venules, and at the hilum. This gradient is maintained, even in severe edema. These data are shown in Figure 3.

The results of Staub et al and Bhattacharya et al illustrate a mechanism whereby the lung can remove excess liquid from the gas exchanging regions and store it more centrally until it can be removed. Edema liquid flows along the hydrostatic pressure gradient from the

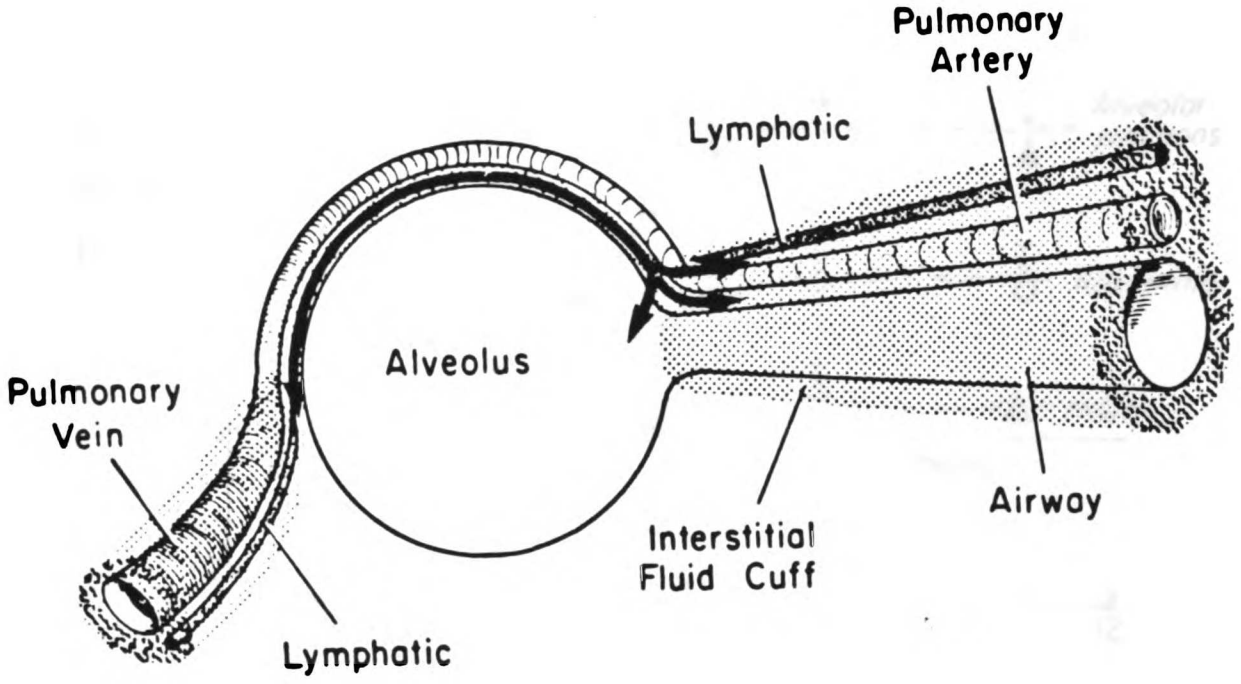


Fig. 2. Pathways of edema liquid movement. Liquid moves from the site of filtration into interstitial "cuffs" surrounding airways and blood vessels. With continuing edema, liquid swells the alveolar walls and finally floods the alveoli.

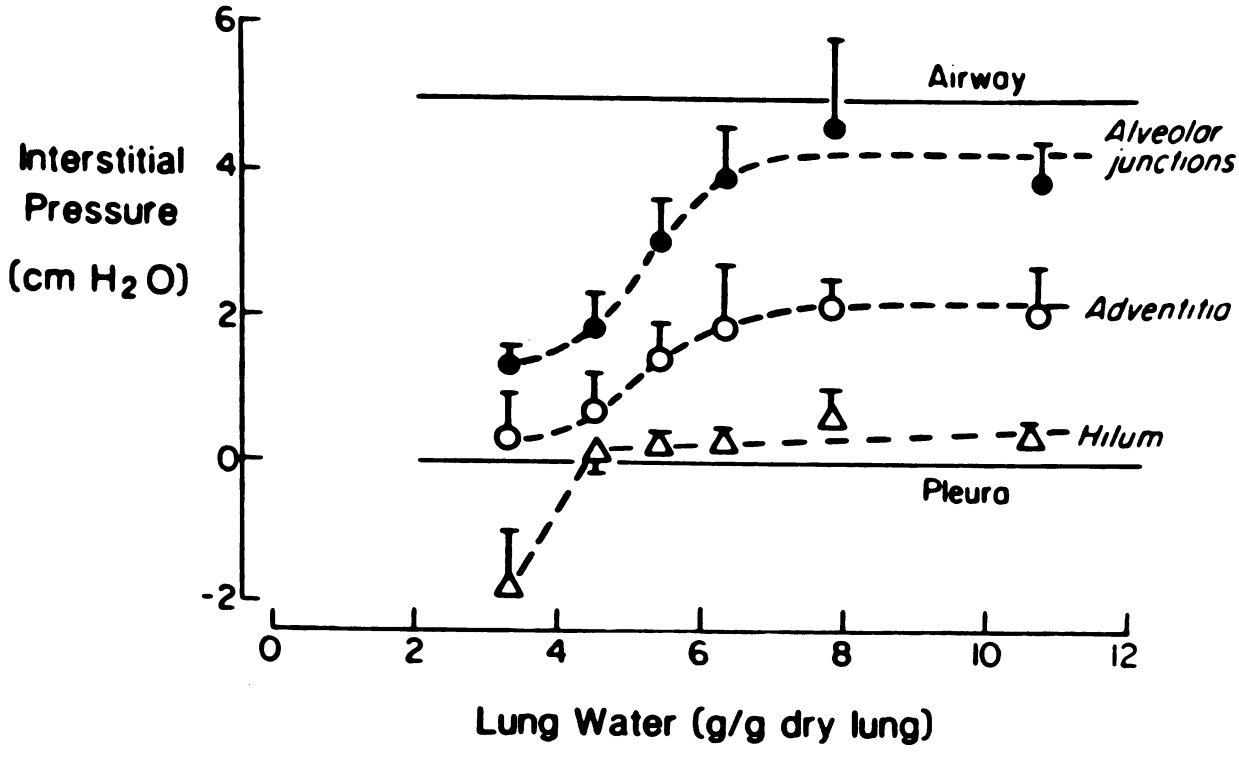


Fig. 3. Interstitial liquid pressures in lobes with differing degrees of edema measured by micropuncture from Bhattacharya et al (8). There is a gradient of interstitial pressure from the alveolar junctions to the hilum, even with severe edema.

alveolar wall to the hilum.

Liquid filtration in Zone I lungs

The effect of lung inflation on filtration in lungs in Zone I conditions is quite controversial. Iliff (37) found no effect of changing lung volume on edema formation from Zone I isolated greyhound lungs. In two studies, Albert found that filtration increased as lung volume (alveolar pressure) increased (2,3). Nicolaysen and Hauge's findings (described in the previous section) were different; filtration increased or decreased depending on how lung volume was increased, either by lowering pleural pressure or increasing alveolar pressure.

Not only is the nature of interstitial liquid pressure in the lung just beginning to be understood but there is also relatively little known about the site of leakage of liquid from the pulmonary microcirculation. Experiments to identify this site have included vascular tracer studies, which have not provided definitive data, and more recent studies using the technique of liquid instillation into the alveoli and following its course through the interstitium (13,19,24).

That extraalveolar vessels might contribute substantially to liquid filtration was first suggested by Iliff in 1971. Since then, many workers have confirmed the hypothesis that significant leakage can occur from these vessels (1,2,3,11,50,53).

My dissertation is concerned with the role of extraalveolar vessels in liquid filtration in the isolated and intact dog lung. I have

examined the relative permeabilities of the arterial and venous extraalveolar beds and the contribution of these vessels to total lung filtration. I have also determined the effect of alveolar pressure on filtration from extraalveolar vessels in isolated and in situ dog lungs. Finally, I have studied albumin flux into the interstitium from extraalveolar vessel filtration induced by hydrostatic pressure. From these studies I have developed a reasonable hypothesis about the nature of filtration from extraalveolar vessels in isolated and intact dog lungs.

SERIES 1: THE ROLE OF EXTRAALVEOLAR VESSELS IN LIQUID FILTRATION

In this series of experiments, I examined the separate contributions of the arterial and venous extraalveolar vascular beds. By inflating isolated lung lobes to high alveolar pressure (Palv), I functionally isolated the extraalveolar vessels from the alveolar wall microcirculation (so-called Zone I condition). Since in Zone I, alveolar pressure is greater than vascular pressure (Pvasc), and there is no blood flow due to compression of the alveolar wall capillaries. Any liquid filtration under these conditions necessarily represents filtration from vessels not compressed. Histologic studies show compression of the main alveolar wall capillaries, with blood forced out from the alveolar wall microvessels into larger arteries and veins (12,69). Nicolaysen et al showed similar pictures in isolated rabbit lungs (53). As there is no blood flow, the arterial and venous extraalveolar vascular beds can be studied separately simply by raising vascular pressure only in the side of interest.

Weighing is a simple, accurate and sensitive method for determining filtration rate. Pappenheimer and Soto-Rivera introduced the isogravimetric method in 1948 for studying systemic capillary permeability (57). This technique was introduced to measure the sum of all pressures opposing vascular pressure. This method is based on the assumption that the vascular pressure at which the organ becomes isogravimetric (constant weight) and is equal to the sum of opposing forces. The isogravimetric technique and variations of it have been

used by many investigators to determine filtration characteristics of many different microvascular barriers (74). The gravimetric method I have used is somewhat different in that I begin in an isogravimetric condition and then induce a change in vascular or alveolar pressure to induce filtration.

The gravimetric method entails weighing the organ before, during and after a step change in vascular pressure. Fig 4 shows an example of a filtration measurement in an isolated dog lung lobe. The initial large increase in weight is due mainly to vascular recruitment plus some initial filtration. One of the shortcomings of this method is to distinguish between these two phenomena early in the filtration period (45,56). The most systematic evaluation of this technique in the lung was carried out by Lunde and Waaler (45). They used isolated rabbit lungs perfused with blood containing ^{51}Cr-red blood cells. By simultaneously measuring lung weight and total lung radioactivity, Lunde and Waaler were able to separate the weight gain due to vascular volume change from that due to filtration. If the lung continued to gain weight while total lung radioactivity remained constant, they attributed the weight gain to filtration. Lunde and Waaler found that after a brief period (20 seconds) of recruitment, weight increase was due solely to filtration. An example of a filtration measurement is shown in Figure 4.

I determined filtration rate during the last 2 minutes of a 5 minute vascular pressure step. The five minutes allowed 3 minutes for the initial adjustments in vascular volume and 2 minutes for pure filtration. The duration is a compromise between the need for accurate measurement of filtration and desire to keep edema formation minimal.

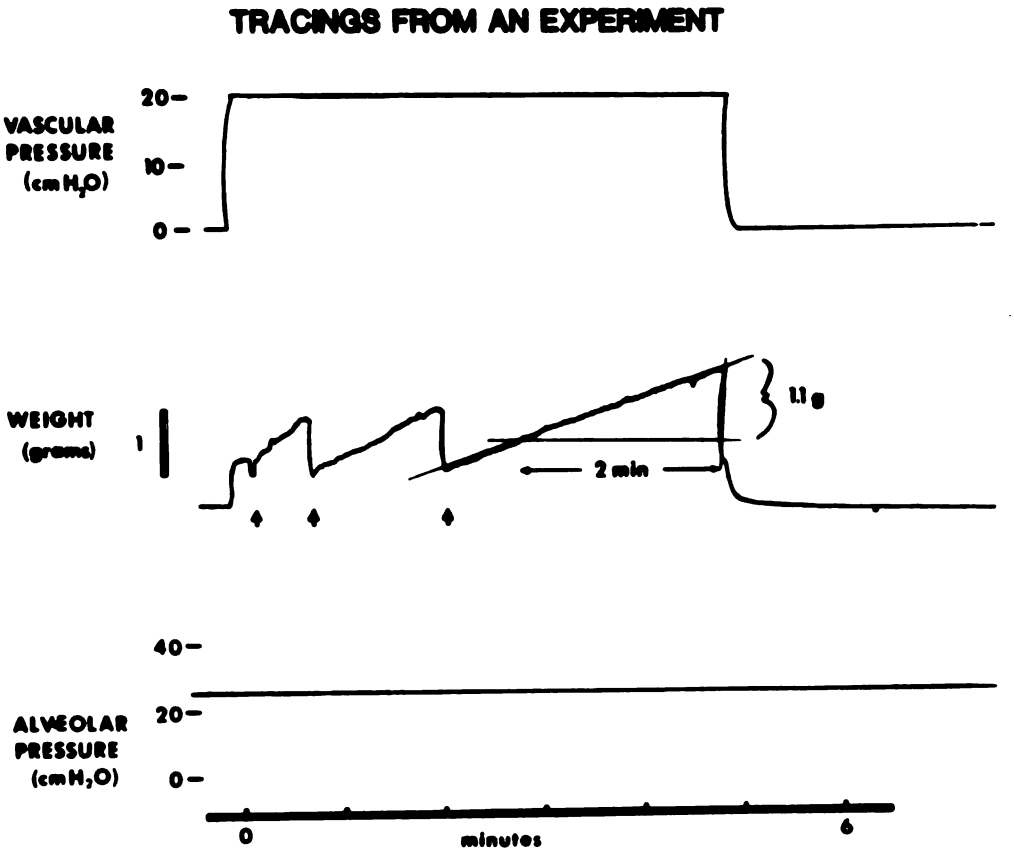


Fig. 4. Example of a gravimetric filtration measurement. Weight recording sensitivity before first arrow was one-tenth that for the rest of the measurement due to vascular volume increase as vascular pressure was increased. Filtration rate is measured over the last 2 minutes of a 5 minute vascular pressure transient.

In the final 2 minute period I feel confident that all vascular recruitment changes had already occurred. Thus the filtration rate is an accurate representation of total filtration from extraalveolar vessels. There may be changes in this rate with increasing edema (5,26,59), including decreased filtration due to increased interstitital pressure or increased filtration due to dilution of interstitial protein or increased filtration due to increased interstitial hydraulic conductivity. This is an area of controversy. For example, Parker found decreased filtration with edema, but Bhattacharya found continually increasing filtration with edema. Bhattacharya, Gropper and Staub (8) found that alveolar junctional interstital pressure did not increase substantially, even with severe edema. I kept filtration to a minimum to avoid possible changes in Starling variables.

METHODS

Mongrel dogs of either sex, 10-25 kg, were anesthetized using pentobarbital-sodium (30 mg/kg). Following endotracheal intubation, the dogs were ventilated with air using a Harvard ventilator at a tidal volume of 15 ml/kg at a rate of 12-15 per minute to maintain blood gases within normal limits during surgery. I cannulated the femoral artery, injected heparin intravenously (500 u/kg), then exsanguinated the dog. I opened the chest via a midline sternal-splitting incision, and removed the heart and lungs en bloc. I used two matching lower lung lobes from each animal. This permitted paired experiments for each animal. The upper lobes and 25 ml of blood were saved for determination of control extravascular lung water. The remaining blood was centrifuged at 2300 x G for 10 minutes to obtain autologous plasma for perfusion.

I tied plastic cannulas into the artery, vein and bronchus of each lower lobe, taking care to avoid the introduction of air. The lobes were then cut away from the heart and other mediastinal tissue.

Each lobe was placed on the pan of a Mettler balance (Model 2000) with a digital to analog signal converter (Mettler GC 47) to allow a record of weight changes on a Grass Model 7 polygraph. This is different from the usual method of suspending the lung from a force transducer, which may require counterbalancing of the lung to obtain sufficient sensitivity of the transducer. Initial lung weight was recorded and the arterial and venous cannulas connected to plasma reservoirs using silastic tubing (Dow-Corning). The reservoirs could be

set at various heights relative to the lung to give any desired vascular pressure. PE-90 tubing was inserted into the silastic tubing and connected to a pressure transducer for measurement of vascular pressure within the artery and vein. The bronchus was connected to a constant pressure gas system containing 30% O_2, 5% CO_2 and 65% N_2. All pressures were measured with Gould-Statham pressure transducers. Vascular pressures were measured relative to the base of the lung, and to pleural (atmospheric) pressure. The experimental set up is shown in Figure 5.

The lung blood vessels were flushed with 200-300 ml of plasma until the venous effluent ran clear. This is important because erythrocytes may interfere with filtration (Hansen, Lunde, Julien). This is of particular concern under Zone I conditions where there is no blood flow. Filtration would allow the hematocrit in the exchange vessels to increase, slowing or stopping filtration. A sample of the perfusate was collected for determination of albumin and total protein concentrations. Vascular pressures were then set to 0 cmH_2O and alveolar pressure set to 25 cmH_2O, after an initial inflation to 30 cmH_2O. As the lungs averaged 8 cm in height when fully inflated (25 cmH_2O), vascular pressure in blood vessels at the top of the lung was 8 cmH_2O lower than that recorded at the bottom, thus assuring that the entire lung lobe was in Zone I condition. If the lung was in Zone I at the bottom, then it was 8 cmH_2O further into Zone I at the top. This avoided any heterogeneity of zonal conditions.

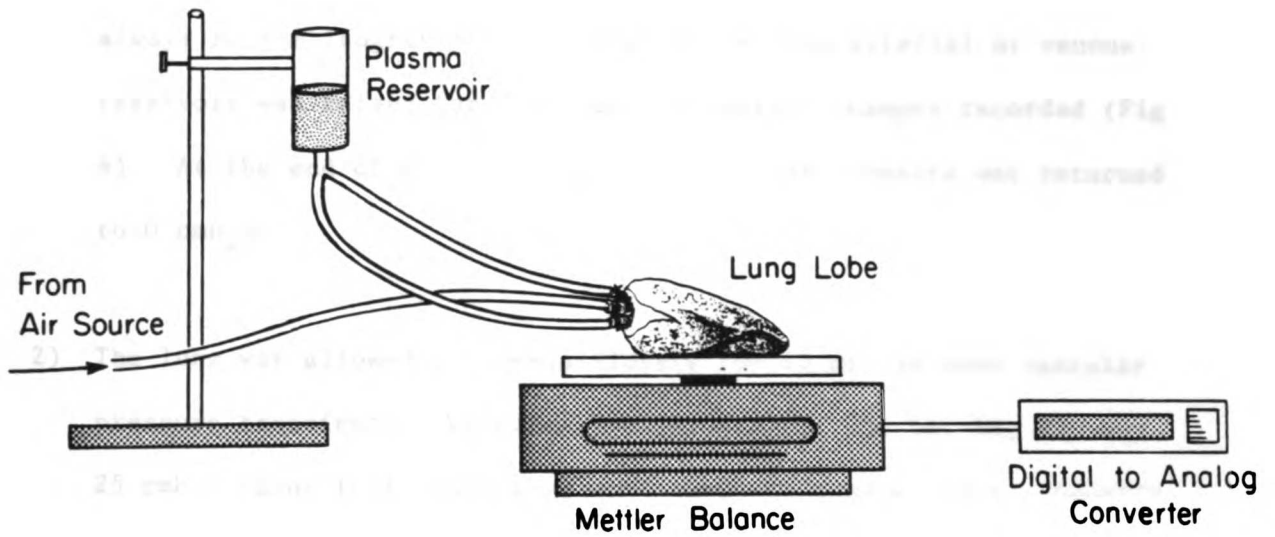


Fig. 5. Experimental set up for isolated lung filtration measurements. Lobes are weighed on a Mettler balance with a digital to analog convertor. Vascular pressure is changed by raising the height of the reservoir.

Experimental protocol

For the first series of experiments the following protocol was used:

1) After Pvasc had been set to 0 cmH_2O and Palv to 25 cmH_2O, I waited 10 min to allow the lung to stabilize. In this time, the lungs always became isogravimetric. Then either the arterial or venous reservoir was raised for 5 min and the weight changes recorded (Fig 4). At the end of the 5 min period, vascular pressure was returned to 0 cmH_2O.

2) The lung was allowed to re-equilibrate for 10 min between vascular pressure transients. Vascular pressures of 5, 10, 15, 20, 23, and 25 cmH_2O (Zone I/III boundary) were used, in random order. Usually, I tested 4 or 5 pressures in each lobe and repeated each pressure.

3) Filtration rate was calculated from the weight increase over the last 2 min of the 5 min pressure change and expressed as grams/(min x 100g wet weight). The wet weight was the lobe weight as described above.

4) At the end of the experiment, the lobes were perfused with autologous whole blood and rapidly frozen in liquid nitrogen for subsequent determination of extravascular lung water. The whole blood was necessary, since the hemoglobin is used as the marker of intravascular volume. The complete method for extravascular lung water determination is described in Appendix 1.

Statistics

All values are reported as mean $\pm$ SD. Differences in filtration rate were analyzed by paired Student's t-test. A p value of $< .05$ was accepted as a significant difference.

RESULTS

In Table 1 I have listed the values obtained for filtration from the pulmonary arterial or venous extraalveolar vascular beds at four different vascular pressures.

In six lobes, there was no filtration from the extraalveolar arterial segment when vascular pressure was set = 5 cmH_2O. Filtration began when Pvasc = 10 cmH_2O. Filtration rates averaged .16$\pm$.24, .50$\pm$.17 and .60$\pm$.20 g/(min x 100g wet weight) when Pvasc was increased to 10, 15 and 20 cmH_2O, respectively.

Similar filtration rates were found for the venous extraalveolar vessels. Filtration rates averaged .01, .16$\pm$.12, .61$\pm$.41 and .53$\pm$.36 g/(min x 100g wet weight) for Pvasc = 5, 10, 15 and 20 cmH_2O, respectively.

Filtration rates between the extraarterial arterial and venous beds were not significantly different ($p < .05$).

TABLE 1

FILTRATION RATE FROM ARTERIES AND VEINS

VESSELS	VASCULAR PRESSURE (cm H_2O)			
	5	10	15	20
Arterial bed	0	-0.018	0.333	0.730
	0	0.222	0.607	0.497
	0	0.050	0.369	0.674
	0	0.552	0.695	0.857
	0	0.020	-	0.308
	0	-	-	0.510
mean ± SD	0	.165±.235	.499±.174	.596±.196
Venous bed	0.043	0.368	1.046	0.261
	0	0.138	0.878	0.323
	0	0.082	0.300	0.336
	0	0.161	0.214	0.857
	0	0.063	-	0.324
	0	-	-	1.105
mean ± SD	.007±.001	.162±.122	.610±.414	.534±.356

Filtration rates are g/(min x 100g wet weight).
Alveolar pressure = 25 cm H_2O

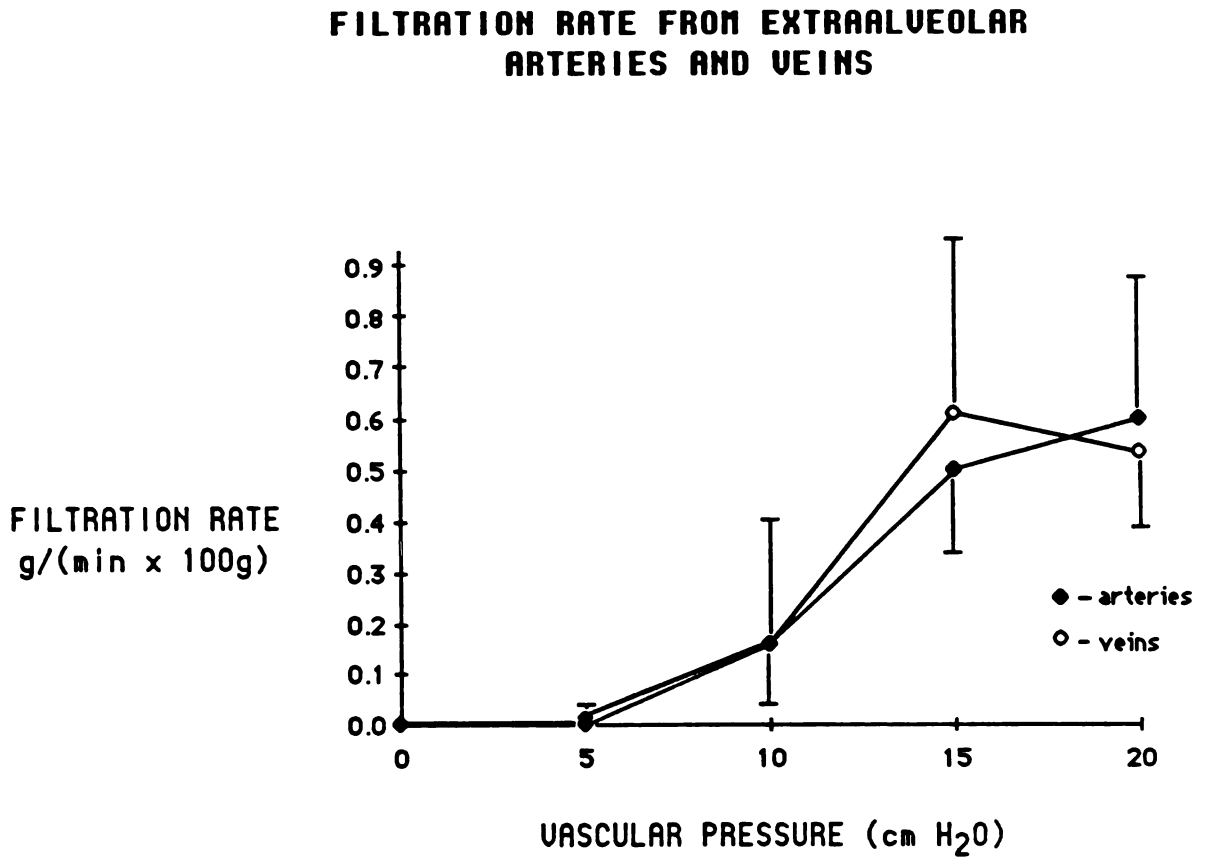


Fig. 6. Plot of filtration rates from Zone I extraalveolar arterial or venous bed filtration. Alveolar pressure = 25 cmH_2O. Filtration rates were not different from the arterial or venous beds.

DISCUSSION

There are two important aspects to my findings in these experiments. First there is filtration from extraalveolar vessels in Zone I, whereas one would expect that most of the filtration should come from the vessels with the greatest surface area, i.e. the alveolar capillaries. In Zone I the alveolar capillaries are mainly compressed. Either there is a population of extremely leaky vessels open in Zone I or some of the alveolar wall capillaries remain at least partially patent up to 15 cmH_2O into Zone I.

I examined thin histologic sections of a glutaraldehyde fixed lung in Zone I conditions (Pvasc = 20 cmH_2O and Palv = 25cmH_2O). All the alveolar wall capillaries appeared to be collapsed, with no red blood cells visible within them. This is similar to the findings of Bruderman and of Staub and of Mazzone and West, (12,47,69) as described in the previous section. A photograph of the histology is shown in Figure 7.

Morphologically, Bennett et al found no difference between muscle and pulmonary capillaries (4), so there is no structural basis to expect these vessels to behave uniquely. The most likely explanation for my findings is that there are alveolar wall microvessels open at the junctions between three or more alveoli that are structurally similar to capillaries (no smooth muscle) and which filter liquid readily. This was also suggested by Nicolaysen and Hauge (53).

Iliff (37) was the first to report that in isolated dog lungs under Zone I conditions, there was considerable weight gain. When compared to filtration in Zone III lungs (vascular pressures are greater than

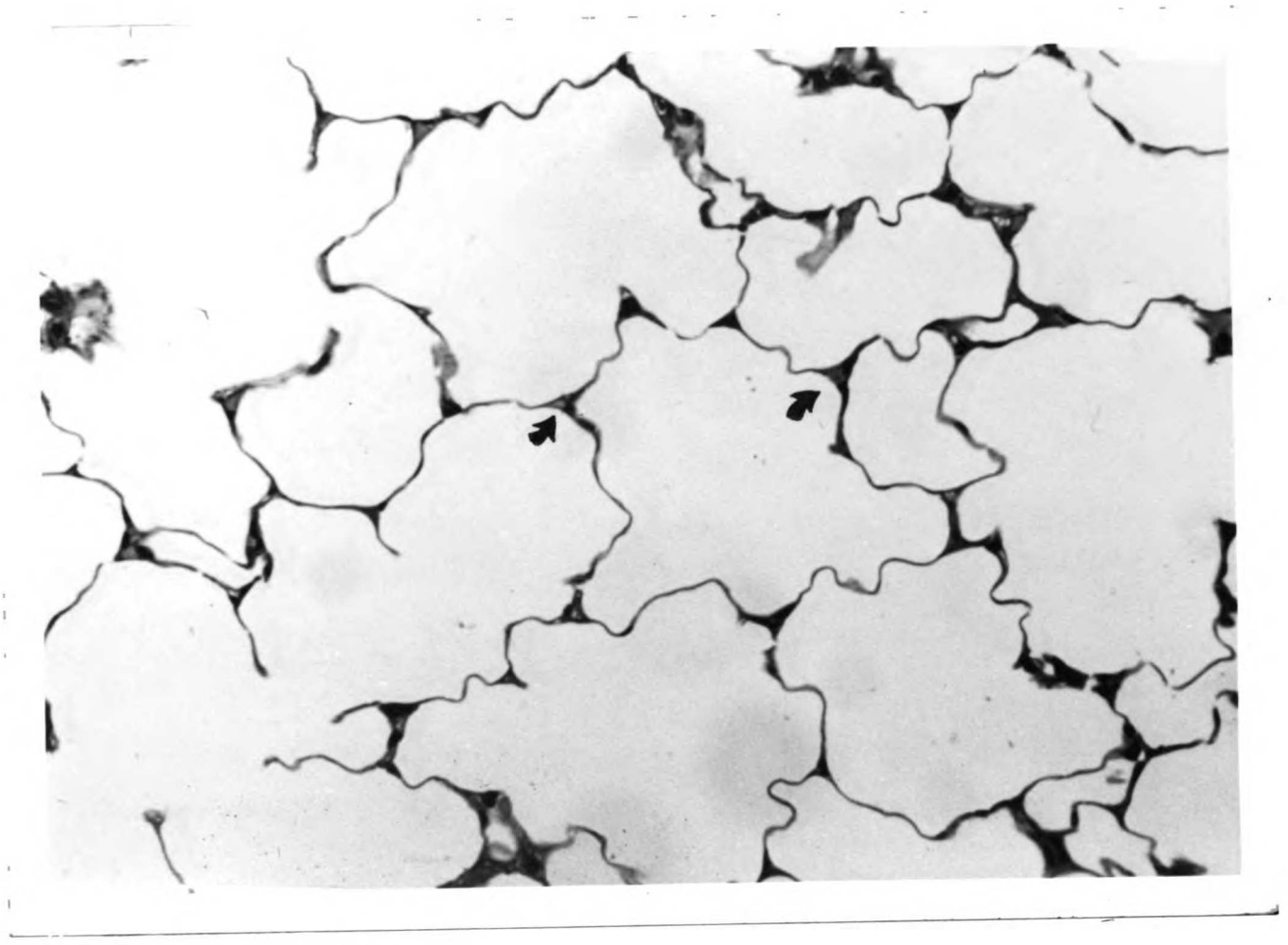


Fig. 7. Histologic section of a Zone I dog lung fixed at 25 cmH_2O alveolar pressure and 20 cmH_2O vascular pressure. Fixation was in glutaraldehyde. Note blood vessels patent at the alveolar wall junction (arrow). Magnification 30X.

alveolar pressure and the alveolar capillary bed is fully recruited), she reported that extraalveolar vessels contributed 63% of the total liquid filtration. Based on surface area considerations, if I assume that the alveolar capillaries are completely compressed in Zone I, the population of vessels that are filtering liquid make up only 2 to 3% of the total vascular surface area. If 63% of the total filtrate comes from this small surface area, then their hydraulic conductivity must be very high (10 to 20 times that of the capillaries).

Another more likely possibility is that some of the alveolar wall capillaries remain open and able to filter even in Zone I. This is possible even though by light microscopy, alveolar walls appear compressed. The alveolar junctional vessels are open far into Zone I and filtration could be coming from these vessels. The combination of these vessels and the extraalveolar vessels may account for the total filtration. A third possibility is that the alveolar wall interstitium somehow prevents leakage from the capillaries.

My results differ from those of Iliff in one way. She found that the pulmonary venous segment was leakier than the arterial by a factor of two. This was similar to the findings of Rous et al (64), who found a gradient of increasing vascular permeability from systemic arteries to veins by filling blood vessels with dye and watching extravasation. I failed to confirm Iliff's finding, as the arterial and venous filtration rates in my experiments were identical (Fig 6). A number of recent studies of pulmonary vascular filtration also found no difference in hydraulic conductivity between the arterial and venous extraalveolar circulations (3,50).

Although vascular conductance (filtration rate per unit driving

pressure) may be uniform, there may be a gradient of filtration along the vascular tree. Bhattacharya and Staub measured the distribution of pulmonary vascular pressure in isolated, perfused dog lungs (6). They found the majority of the pressure drops located between the arterial and venous capillaries. This pressure profile would result in greater filtration from arterial microvessels than veins under conditions of blood flow.

Conclusion

There is significant filtration from extraalveolar arteries and veins in Zone I, and there is no difference in filtration rate between arteries and veins. I conclude that in the normal lung there is greater filtration from the arterial rather than the venous extraalveolar vascular beds because of the gradient of microvascular pressure.

Series 2: VASCULAR RECRUITMENT IN ZONE I

In this group of experiments, I studied whether studied recruitment of vascular surface area occurred in Zone I as vascular pressure increases and Zone III is approached. The Zone I/III border is where vascular pressure reaches alveolar pressure. In Zone III, the entire vascular bed should be available for filtration. In Series 1 I had found that the filtration rate for the arterial and venous extraalveolar segments were identical, therefore in these experiments I examined the alveolar capillary and extraalveolar vascular beds as a whole, studying arteries and veins together. To do this, I used a single vascular reservoir for both the arterial and venous beds. As there is no blood flow in Zone I, this simplified the experiments, assuring that arterial and venous pressures were identical.

The procedure in this group was to change vascular pressure in Zone I isolated dog lungs and determine the shape of the filtration profile. The shape of the curve describing filtration rate vs. vascular pressure would give me two important pieces of information. First, by examining the filtration profile, I could determine if there was recruitment of vascular surface area in Zone I. This gave me an indication of the population of vessels contributing to filtration under these conditions. Second, I could determine the contribution of extraalveolar vessels to total lung filtration by studying lungs in comparable conditions in Zones I and III. This would give me a quantitative estimate of the contributions of the alveolar and extraalveolar beds to total filtration.

This series of experiments was done at constant alveolar pressure = 25 cmH_2O, with variations only in vascular pressure. Lung volume was high, and I studied a wide range of vascular pressures from 0 to 30 cmH_2O. If alveolar and pleural pressures are constant, then interstitial pressure should remain nearly constant throughout the lung. Interstitial pressure may increase a little as vascular pressure (and therefore vascular distension) increases. If this occured in the perimicrovascular interstitium of the filtering segment, it ought to decrease filtration in this segment, leading to an underestimation of filtration rate.

The first important piece of information obtained is the shape of the filtration profile as vascular pressure increases and the lung conditions are changed from Zone I to Zone III. If this profile is linear, then the surface area is most likely constant and filtration is increasing due only to increasing vascular hydrostatic pressure. Conversely, if the filtration profile is non-linear, then recruitment of vascular surface area is occurring. My hypothesis was that if no recruitment of vascular surface area occurred in Zone I then the rate of filtration ought to be a linear function of vascular pressure up to the Zone I/III boundary, then show a sudden jump in rate as the alveolar capillary surface area became available. The second piece of information obtained in this section is determined by extrapolation of the filtration profile, from Zone I into Zone III. From this projection, I can determine what the relative contributions of alveolar and extraalveolar vessels would be at the same microvascular and perimicrovascular interstitial pressures.

METHODS

In this group of experiments, I measured filtration in Zone I extending the range of vascular pressures up to the Zone I/III boundary and into Zone III. To determine the relative contribution to filtration of those vessels compressed by high Palv I included experiments where vascular pressure was set to 30 cmH_2O (Zone III) with Palv set to 25 cmH_2O. I used the same lung lobe preparation as Series I, except that I used a single plasma reservoir. Figures 8 and 9 show a schematic of Zone I and Zone III conditions, respectively.

The methods for determination of filtration rate and extravascular lung water were identical to those described for Series 1. The Zone III determination was always done last, as I was concerned that the large amount of liquid filtered might affect subsequent filtration measurements. The protocol was the same for these experiments as for those in Series 1, except for two exceptions. A single vascular reservoir was used, and a single determination was made for each lobe in Zone III conditions.

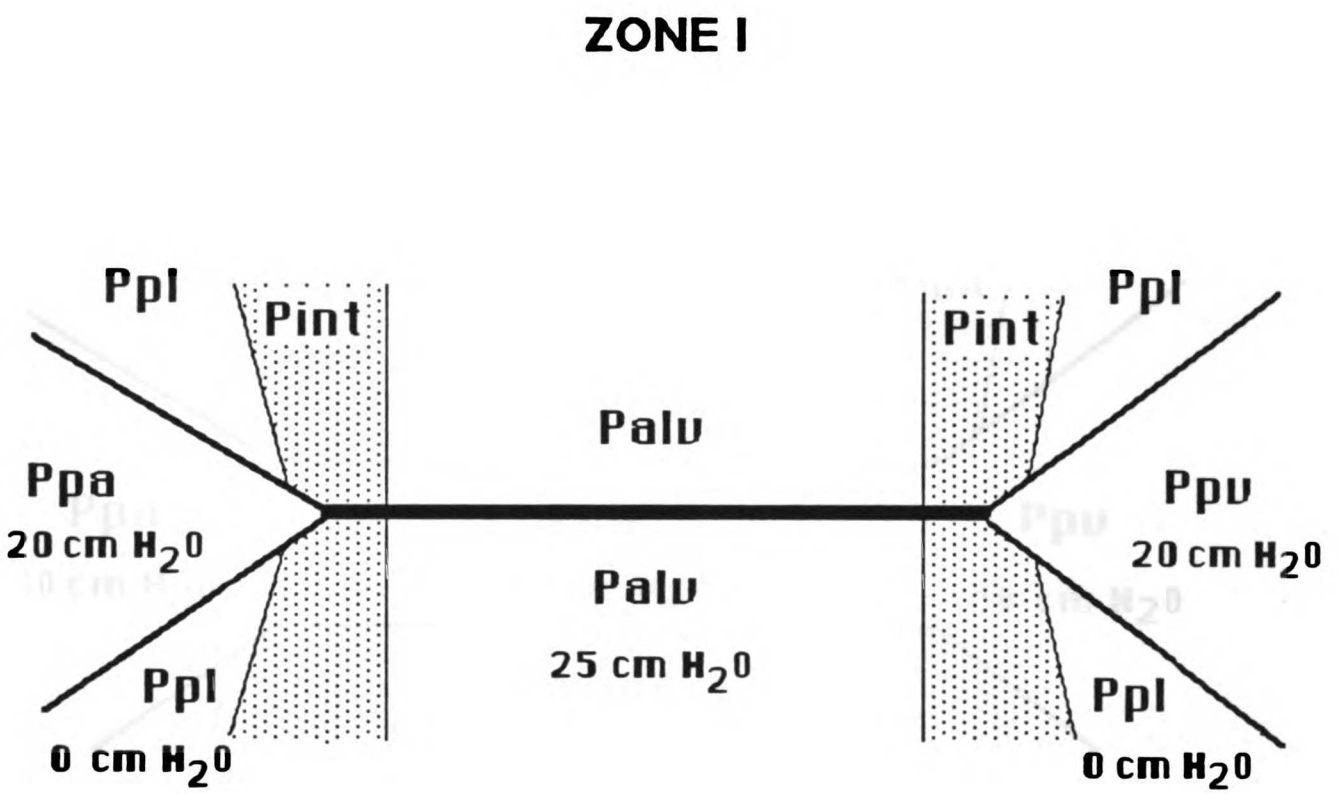


Fig. 8. Diagram of Zone I microcirculation. Interstitial pressure in the extraalveolar compartment is close to pleural pressure (Ppl). Interstitial pressure in the alveolar wall is close to alveolar pressure (Palv). The stippled area (Pint) represents interstitial pressure at the proposed filtration site. This pressure must be intermediate between Palv and Ppl.

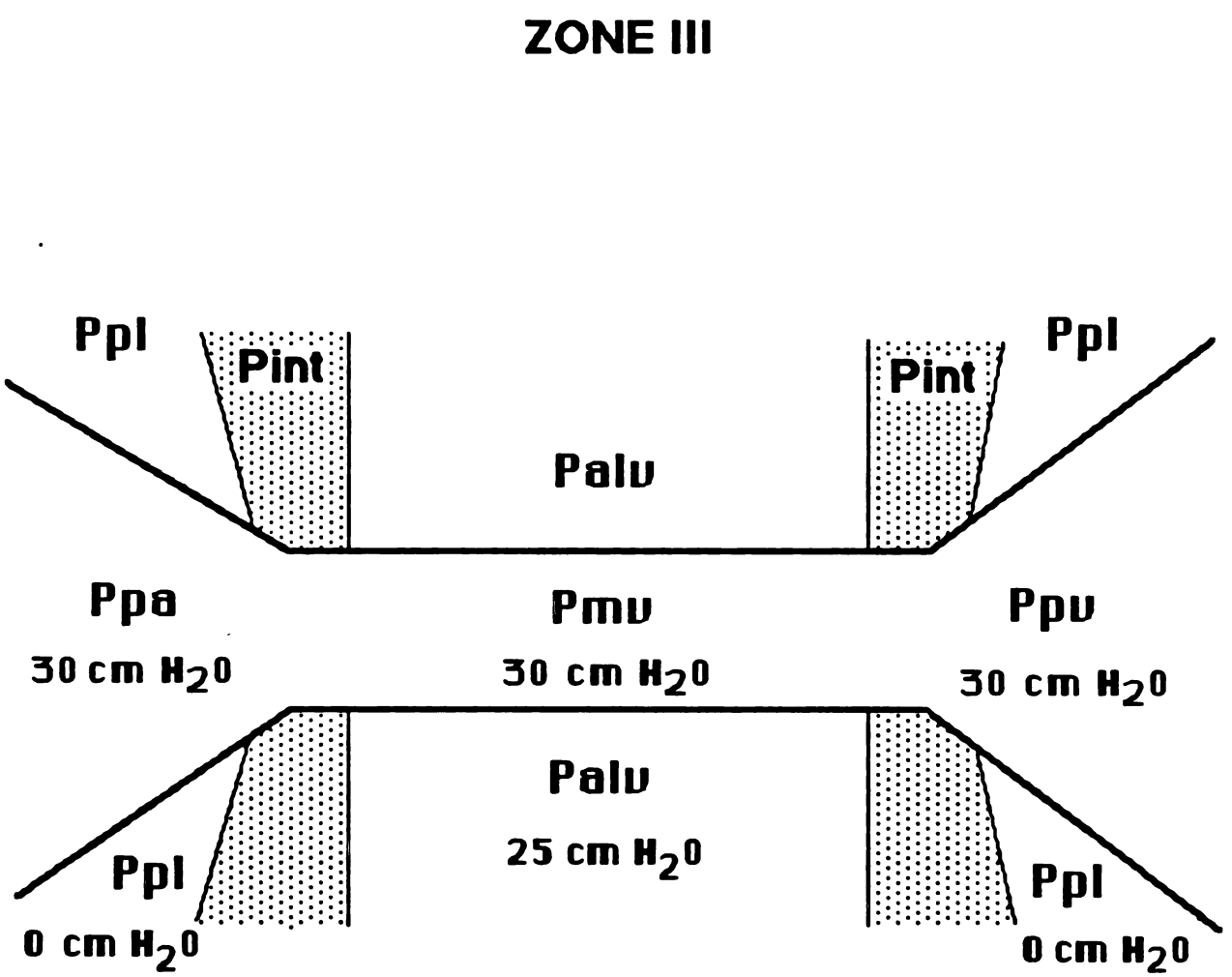


Fig. 9. Diagram of Zone III microcirculation. The alveolar capillary circulation is recruited since vascular pressure (Pvasc) is greater than alveolar pressure (Palv). Interstitial pressure in the extraalveolar compartment is close to pleural pressure (Ppl). Interstitial pressure in the alveolar wall is close to alveolar pressure (Palv). The stippled area (Pint) represents interstitial pressure at the proposed filtration site.

RESULTS

Contribution of extraalveolar vessels

In Table 2 I have listed the filtration rates at different vascular pressures expressed in grams/(min x 100g wet weight). Filtration rate increased linearly even while the lung was 10 to 15 cm into Zone I (Palv much greater than Pvasc), then increased non-linearly as vascular pressure increased toward the Zone I/III border (where Pvasc = Palv). Figure 10 shows the filtration profile.

Fig 11 shows the same filtration profile, except that the filtration rate in Zone I is projected to Zone III to allow estimation of the contributions of the alveolar and extraalveolar beds. The filtration rates are normalized as a percentage of the filtration rate at 20 cmH_2O (5 cm H_2O into Zone I) to account for variations in lung size, surface area and individual animals. The line projected from the linear part of the curve far in Zone I projects to an estimated filtration rate of 2.28 g/min x 100g wet weight at Pvasc = 30 cmH_2O. The projected line represents the increase in filtration expected due to increasing hydrostatic pressure, at constant exchange surface area or perimicrovascular interstitial pressure. Filtration measurements made at 30 cmH_2O vascular pressure in Zone III had a mean filtration rate of 5.256 g/(min x 100g wet). I divided the actual filtration rate by the projected rate to obtain a percent of total filtration from extraalveolar vessels. Forty three percent of the total filtration at 30 cmH_2O can be ascribed to vessels patent 10-15 cmH_2O into Zone I.

TABLE 2

EXTRAALVEOLAR FILTRATION RATE

VASCULAR PRESSURE (cm H_2O)						
5	10	15	20	23	25	30
0	0.654	0.415	1.191	1.970	3.253	7.949
0	0.514	1.063	1.502	3.367	4.555	0.950
0	-	0.726	0.679	2.825	4.352	4.260
0	-	0.190	2.075	2.947	3.695	2.088
0	-	2.000	1.632	0.570	0.912	5.283
-	-	0.288	1.808	1.163	1.825	2.513
-	-	1.038	0.342	3.150	1.113	-
-	-	0.314	0.888	0.788	5.283	-
-	-	-	2.750	4.717	1.706	-
-	-	-	0.713	-	-	-
-	-	-	2.032	-	-	-
-	-	-	0.673	-	-	-
0*	.58±.10	.75±.61	1.40±.79	2.39±1.37	2.97±1.6	3.84±2.54

Filtration rates are g/(min x 100g wet weight).
* mean ± SD

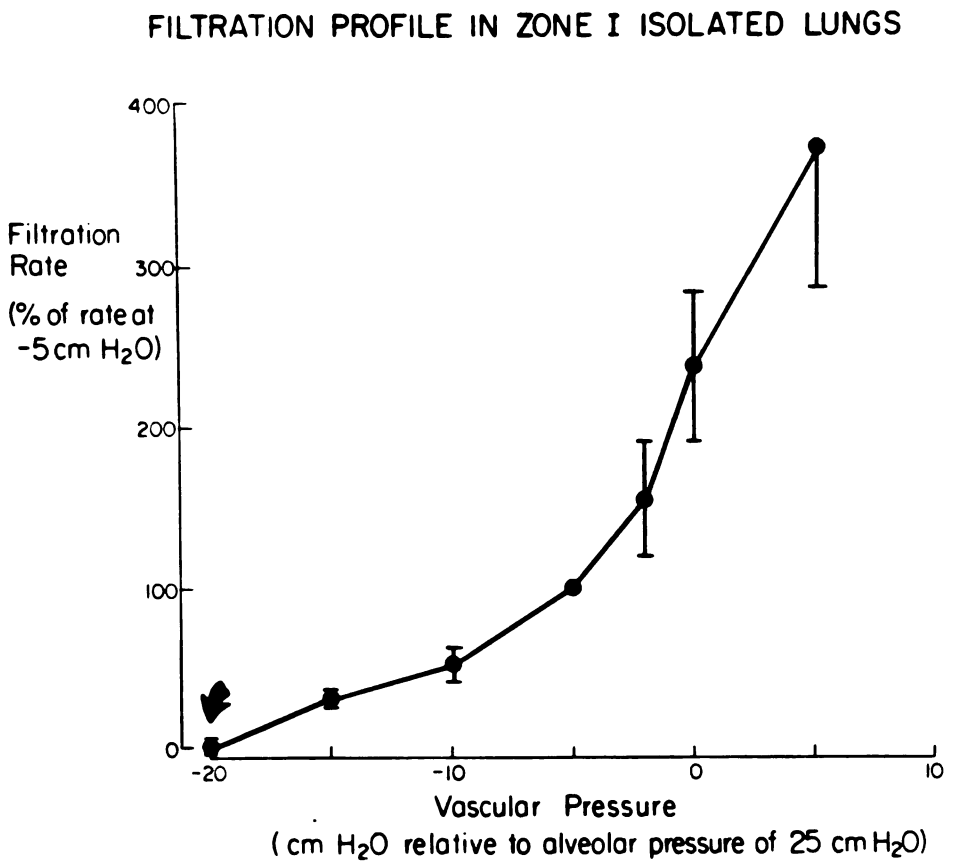


Fig. 10. Filtration profile in Zone I isolated lungs. Note filtration surface area recruitment while still in Zone I (vascular pressure below zero). The increase in filtration in Zone III is not as great as expected, given surface area considerations.

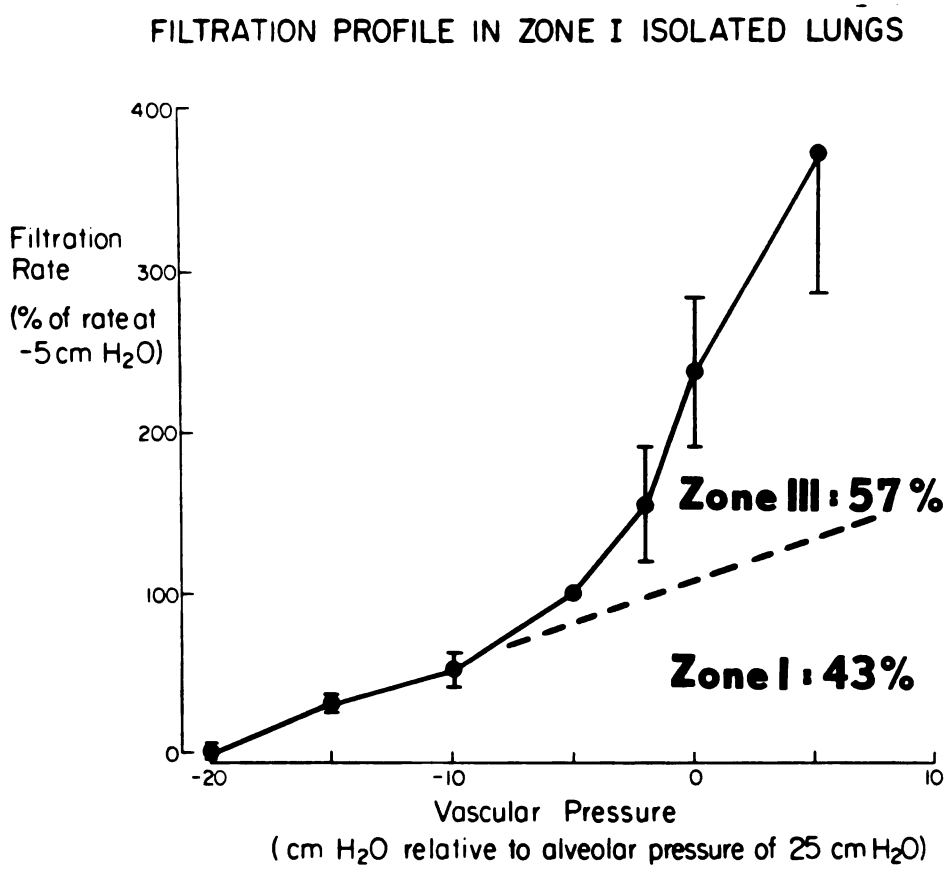


Fig. 11. Filtration profile in Zone I isolated lungs. The filtration rate from far in Zone I is projected into Zone III to determine the contribution of the extraalveolar vessels to filtration. 43% of the total filtration in Zone III is from extraalveolar vessels.

The non-linear nature of the relationship describing filtration in Zone I suggests recruitment of vascular surface area as vascular pressure is increased.

Calculation of K_f

Using the filtration rate I found at a vascular pressure of 20 cmH_2O (5 cmH_2O into Zone I), I calculated a Zone I filtration coefficient (K_f) of .070 g/(min x cmH_2O x 100g weight). This value assumes that perimicrovascular interstitial pressure is constant and equal to zero, and that the reflection coefficient and protein osmotic pressure difference is across the endothelium is zero. I made these assumptions in order to compare the leakiness of my preparation to those of other investigators.

In Zone III, Kf = .128 g/(min x cmH_2O x 100g), using the value for filtration at a vascular pressure of 30 cmH_2O.

DISCUSSION

My results confirm those investigators who found substantial filtration from extraalveolar vessels. Iliff found that extraalveolar vessels contributed 63% of the total liquid filtration. If we assume the alveolar capillaries are completely compressed in Zone I and only the extraalveolar vessels are patent, the population of vessels that are filtering liquid make up only 2 to 3% of the total vascular surface area (34,35,78). I found that 43% of the total filtration was coming from extraalveolar vessels. This is similar to the results of Mitzner and Robotham (50), who reported 50% of the total filtration coming from extraalveolar structures.

If 43% of the total filtrate originates from non-capillary vessels, then their hydraulic conductivity must be extremely high, as discussed in Series 1. Structural studies using silicone casts by Fung et al (22) have shown that venules remain patent even when alveolar pressure exceeds vascular pressure. Under these same conditions, the alveolar capillaries appear to be compressed. This is further evidence that there are small vessels capable of filtration that are open under Zone I conditions.

I found a filtration coefficient (Kf) of .13 g/(min x 100g weight x cmH_2O) in Zone III. This value is similar to the K_f obtained by Gaar et al, who found K_f = .051 g/(min x cmH_2O x 100g weight) in isolated dog lungs. Although my value is higher than that of Gaar et al, the difference may be due to differences in protein osmotic pressure in the two sets of experiments. Gaar et al measured a protein osmotic pressure

of 20 mmHg using a membrane osmometer. Using the Nitta nomogram (54), I calculated a mean protein osmotic pressure in my experiments of 15 mmHg. The lower intravascular protein osmotic pressure in my experiments may account for the higher filtration coefficient. Moriss et al (51) reported a somewhat higher value of .22 g/(min x cmH_2O x 100g). In Zone III, there ought to be much more surface area available for filtration. Nevertheless, the K_f value is only 1.5 to 3 times the value I find in the Zone I lung. This is much less than one would expect if filtration were determined only by surface area, the vast majority of which is in the alveolar wall capillaries.

These studies of filtration rate with increasing vascular pressure suggest two important points. First, the non-linear nature of the curve of filtration rate against vascular pressure suggests that there is recruitment of filtration surface area as the Zone III border is approached. The branching pattern of the pulmonary vasculature is such that surface area increases exponentially with longitudinal distance along the vascular bed (74). If there is any longitudinal creep of the liquid front into previously compressed capillaries as hydrostatic pressure increases, then a large surface area could be recruited, leading to an exponential increase in filtration rate.

Goldberg (26) reported no evidence for vascular recruitment within Zone I. This seems highly unlikely, based on my results in this group of experiments. It is difficult to explain the non-linear nature of the filtration profile without invoking increasing surface area. My finding of recruitment in Zone I is opposite to that of Goldberg. Although histologically the alveolar wall microvessels appear to be completely collapsed, there may be minute infractuosities in these vessels that

could contribute to filtration.

My finding of recruitment in Zone I is opposite to that of Goldberg. It is difficult to postulate a model for the non-linear increase in filtration rate seen in my data, other than invoking an increase in surface area.

Another possible way to explain my data is based on the gradient of interstitial pressure from the alveolar wall to the hilum. Bhattacharya, Gropper and Staub (8) measured interstitial liquid pressure at the subpleural alveolar wall junctions, the adventitia around 30-50 um microvessels and at the hilum. They found that there was always a gradient of interstitial hysdrostatic pressure that was highest at the alveolar wall junction and decreased toward the hilum. This gradient was maintained at different levels of lung inflation and lung edema. Interstitial pressure at the alveolar wall junction followed the change in alveolar pressure (7). Lai-Fook (42) found that interstitial pressure at the hilum decreased relative to vascular pressure as the lung was inflated by positive pressure.

Given these considerations, it seems likely that interstitial pressure is more negative around extraalveolar vessels than it is around alveolar wall capillaries, where interstitial pressure approximates alveolar pressure (66). The more negative interstitial pressure around extraalveolar vessels gives a greater hydrostatic gradient for filtration at this site. The structure of these vessels, thin walled and non-muscular, is consistent with this model. Recent measurement of filtration rate in single lung arterioles and venules using the split-drop method show that K_f in these vessels is similar to that of the alveolar capillaries (9). This is further evidence that there must be

recruitment of alveolar capillary surface area as vascular pressure increases.

The interstitium surrounding the alveolar wall capillaries may be the barrier limiting filtration from these vessels (14,70). If this were true, all filtration would have to occur in extraalveolar vessels and I would expect all vascular recruitment to occur before the Zone I/III border.

I conclude that there is substantial filtration from extraalveolar vessels, beyond what would be expected based on surface area. This can be partially accounted for by the more favorable hydrostatic pressure gradient at these vessels. I also find that vascular surface area is increased as the Zone I/III border is approached, as would be expected with longitudinal movement of the liquid front into exponentially increasing vascular surface area.

SERIES 3: EFFECT OF ALVEOLAR PRESSURE ON FILTRATION

In this group of experiments, I studied the effect of changing alveolar pressure on filtration rate at constant vascular pressure. Although the effect of lung inflation on filtration from extraalveolar vessels has been studied many times, there is no unanimity about its effect (2,37,53).

Albert et al had found that as they inflated the lung at constant vascular pressure and moved farther into Zone I, filtration rate increased. Iliff had found no effect of lung inflation on Zone I filtration rate. Nicolaysen and Hauge found decreasing filtration with increasing alveolar pressure and increasing filtration with decreasing pleural pressure. My experiments were stimulated by my finding of no filtration at high alveolar pressure and low vascular pressure (Palv = 25 cmH_2O and Pvasc = 5 cmH_2O) as shown in Figure 10. My results were quite different from those of Albert et al, however, I had limited alveolar pressure in my studies to one value.

Albert explained his findings by suggesting that the increase in filtration was due to the influence of pulmonary vascular interdependence as described by Mead, Takeshima and Leith (48). When the lung is inflated by positive alveolar pressure, hilar perivascular interstitial pressure decreases due to the differing elasticities of the larger blood vessels and their surrounding parenchyma. As the parenchyma is more compliant and expands to a greater degree than the blood vessels, Albert suggested that negative liquid pressure was created in the interstitium surrounding the vessels, resulting in an

increased outward gradient for filtration. A graph of Albert's results is shown in Figure 12.

In my experiments described in Series 2, there was no filtration when Palv was 25 cmH_2O and vascular pressure 5 cm H_2O, conditions where Albert found substantial filtration (see arrows). The trend in my results in this series was that filtration decreased as alveolar pressure was increased at constant vascular pressure (as the lung was put farther into Zone I). Iliff had found no effect of lung inflation Zone I filtration rate (Figure 13). Nicolaysen and Hauge found decreasing filtration with increasing alveolar pressure and increasing filtration with decreasing pleural pressure (Figure 14).

Based on these discrepancies, I set out to determine the effect of lung inflation on filtration from extraalveolar vessels in isolated Zone I dog lungs. The results of the isolated lung studies were that alveolar pressure had no effect on filtration rate. One possibility was that somehow the experiment in isolated lobes was different from intact lungs as used by Albert. The intact lung could behave differently from the isolated lung for the following reasons: the bronchial circulation is intact and could contribute to filtration or vascular volume changes; the lung is connected to the mediastinum at the hilum, which could be a pathway for liquid movement from the interstitium.

I was concerned that the results were dependent on how the experiments were done, not on the relation of lung volume (interstitial pressure) and vascular pressure. Because of this, I repeated Albert's experiments in the <u>in situ</u> dog lung.

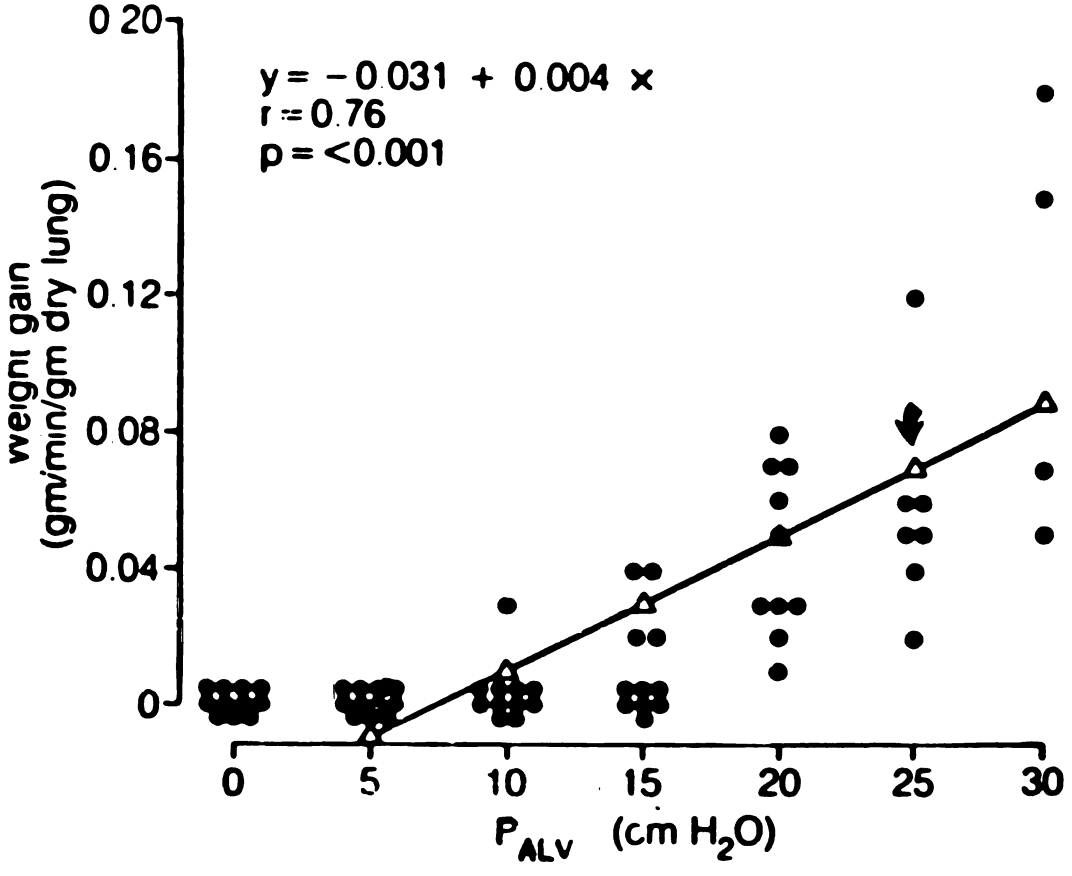


Fig. 12. Figure of filtration rate vs. alveolar pressure in intact, open thorax dog lungs from Albert et al (2). Note that filtration rate increases with alveolar pressure at constant vascular pressure (5 cmH_2O). Compare data point with arrow to data point shown in Figure 10. Both these points were obtained at 25 cmH_2O alveolar pressure and 5 cmH_2O vascular pressure.

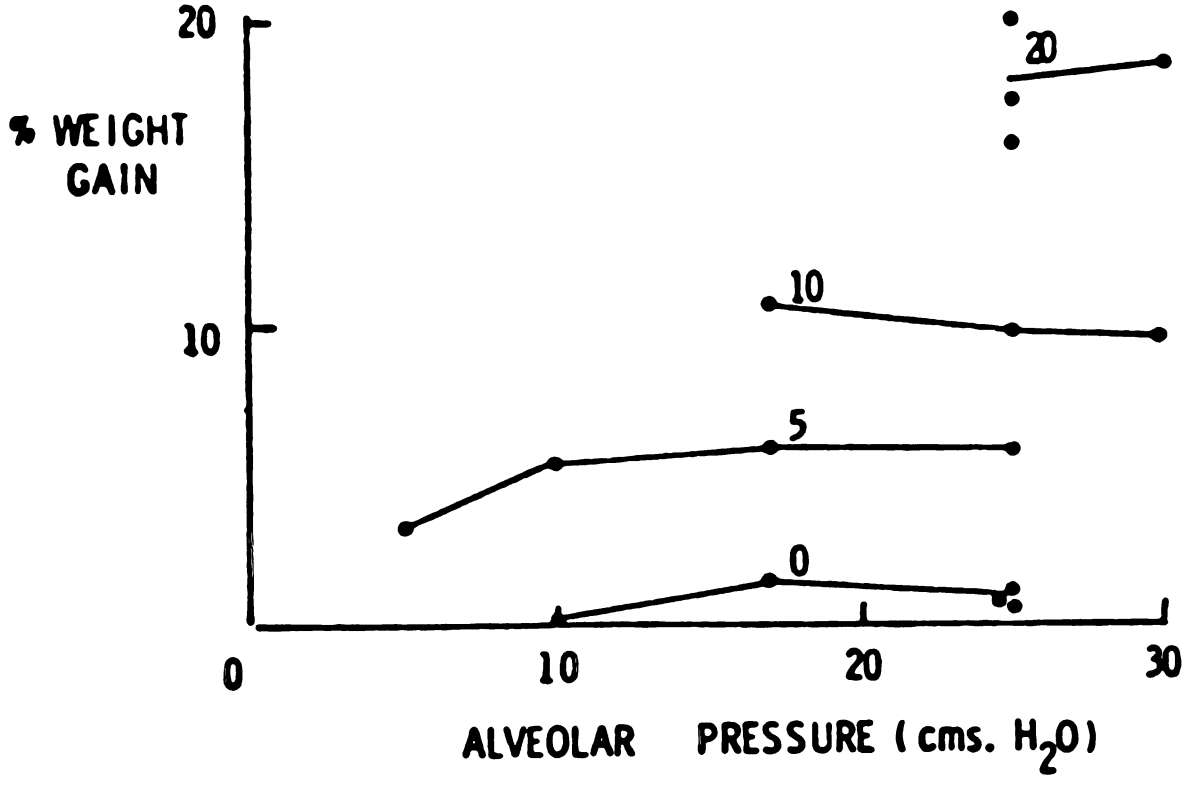


Fig. 13. Figure of weight gain vs. alveolar pressure over 2 hours in isolated dog lung lobes from Iliff (37). She found no effect of alveolar pressure on liquid accumulation. Numbers above each line represent vascular pressure in cmH_2O.

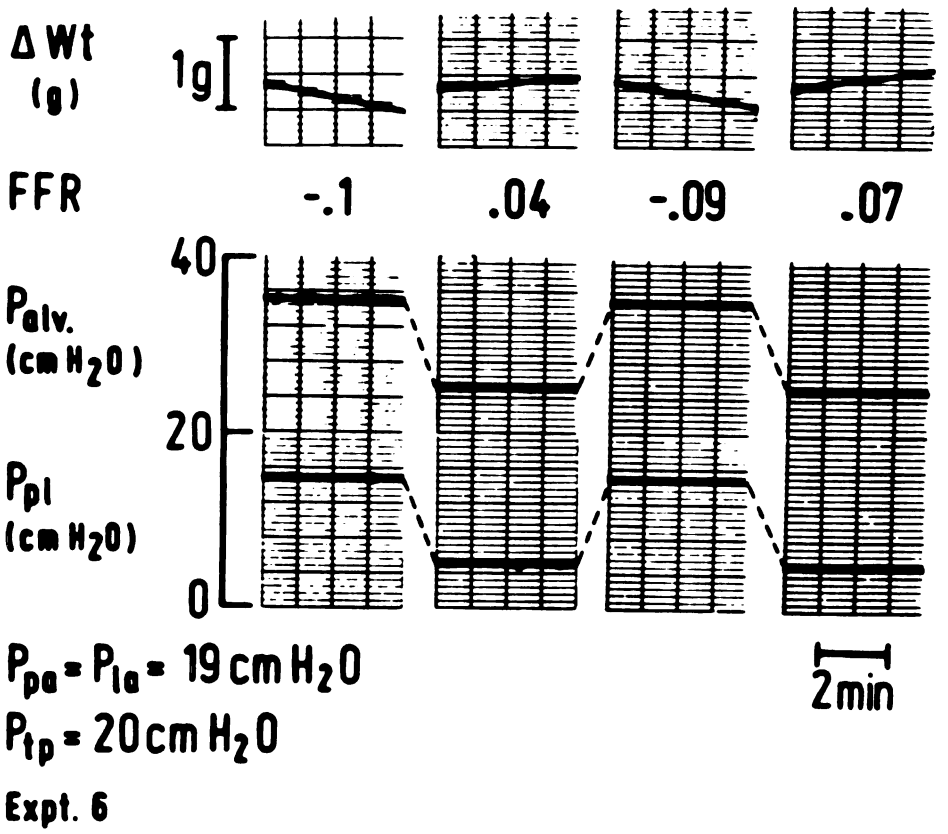


Fig. 14. Figure of weight gain in isolated rabbit lungs from Nicolaysen and Hauge (53). They varied alveolar and pleural pressures at constant transpulmonary pressure and found that filtration rate decreased as alveolar pressure was increased and that filtration rate increased as pleural pressure decreased. This suggested that interstitial pressure at the site of filtration might be closely related to pleural pressure. Note that vascular pressure in these experiments was high (19 cmH_2O).

METHODS

Isolated lobe

The lung lobes were prepared in the manner described in Series 1. To reproduce Albert's conditions, I set and maintained vascular pressure at 1 cmH_2O relative to the base of the lung and to pleural pressure (atmospheric). I varied Palv in steps, to 5, 10, 15, 20, 25 and 30 cmH_2O using a constant pressure overflow system. The gas used was 30% O_2, 5% CO_2 with the balance N_2. In each study the following protocol was used:

1) After cannulation, the lungs were allowed to equilibrate for 10 min, in which time they usually became isogravimetric. At no time was Pvasc greater than Palv. I followed the protocol of Albert (2). Before each randomly chosen Palv was set, I increased Palv to 30 cmH_2O to assure that there was no atelectasis, then returned it to 5 cmH_2O (Zone I). Palv would then be set to the chosen pressure, kept at this level for 5 min, then returned to 5 cmH_2O.

2) Filtration rate was measured over the last 2 min of the 5 min Palv transient. All pressures were studied, in duplicate, in each lobe, in duplicate, since I soon found that there was little, if any, filtration and therefore no edema.

3) At the end of each experiment, the lobes were perfused briefly with autologous whole blood and frozen for determination of extravascular

lung water.

Open thorax, intact lung

I used mongrel dogs of either sex, 10-25 kg, anesthetized with intravenous pentobarbital sodium, 30 mg/kg. After endotracheal intubation, the dogs were ventilated with a Harvard animal respirator at a tidal volume of 15 ml/kg and a rate of 12-18/minute. Blood gases were monitored periodically, and ventilatory adjustments made to keep pH, PO_2 and PCO_2 within normal limits.

The dogs were placed in the left lateral decubitus position. The left chest wall between the 5th and 10th ribs and the sternum and paraspinous muscles was removed to widely expose the left lower lobe. The left upper lobe was resected at the hilum. Stainless steel cannulae with side-ports for pressure measurement (Courtesy of T. Hakim, McGill University) were secured into the left lower lobe pulmonary artery and vein. The venous cannula was introduced via a purse-string suture through the left atrium and then tied into the lobar pulmonary vein. The metal cannulae were connected to silastic tubing with a reservoir system identical to that described for Series 2.

I isolated the airway of the left lower lobe using a tracheal divider. This allowed me to ventilate the left lower lobe independently from the right lung. In the case of hypoxemia or hypercapnia, adjustments were be made in the ventilation of the right lung. The left lower lobe was ventilated with 30% O_2, 5% CO_2 and the balance N_2.

Three small (2 cm dia.) corks were glued to the costal surface of

the left lower lobe using cyanoacrylate adhesive (Crazy Glue). Pins were inserted into the tops of the corks and thin strings tied from the pins to a Grass Model FT03C strain gauge. With this system I was able to continuously weigh the lung. The strain gauge was calibrated by placing weights on the lung after it had been suspended. Respiratory movement from the right lobes and cardiac motion made these filtration measurements less sensitive than those in isolated lungs. It was necessary to use signal filtration on the Grass recorder (frequency = .1 sec^{-1}). The experimental set-up is shown in Figure 15.

Once the lung had been suspended from the force transducer, I waited 10 min. Alveolar pressure was then increased to 30 cmH_2O and then lowered to 5, 10, 15, 20, 25 or 30 cmH_2O in random order, using a constant pressure overflow system to maintain steady airway pressures in the left arm of the tracheal divider.

I made measurements of filtration over the last 2 minutes of a 5 minute alveolar pressure step change. I waited 10 minutes between filtration measurements. At the end of the experiment, the left lower lobe was rapidly frozen in liquid N_2 and saved for determination of extravascular lung water content.

Statistics

All data are reported as means $\pm$ SD. I used a paired Student's t test for paired comparisons between filtration rates. I accepted $p < .05$ as a statistically significant difference.

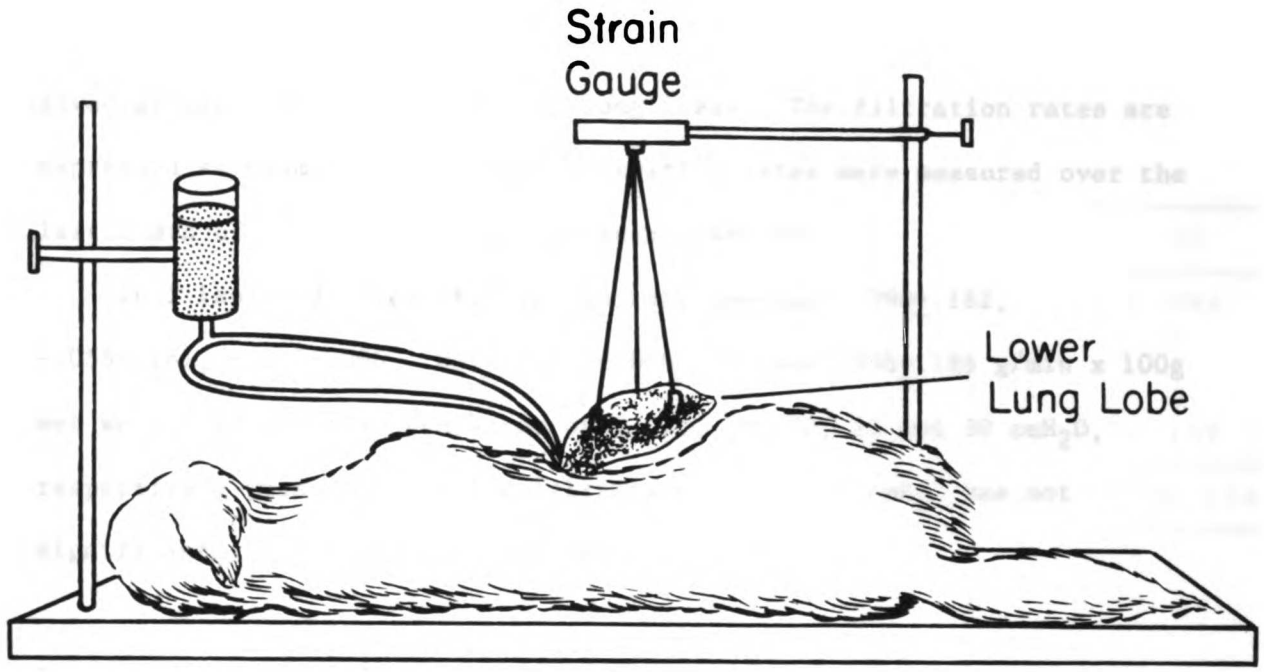


Fig. 15. Set-up for open thorax, intact lobe filtration measurements. The left chest wall was removed, and the left and right lobes ventilated separately using a divided endotracheal tube. The left lower lobe was suspended from a strain gauge for weight measurements.

RESULTS

Isolated lobes

I have listed in Table 3 and Figure 16 filtration rate as a function of alveolar pressure in isolated dog lung lobes. The filtration rates are expressed as grams/(min x 100g). Filtration rates were measured over the last 2 min of a 5 min alveolar pressure transient.

In 5 isolated lobes, filtration rate averaged .094±.162, -.055±.181, -.007±.098, .037±.056, -.048±.091 and -038±.186 g/min x 100g wet weight at alveolar pressures of 5, 10, 15, 20, 25 and 30 cmH_2O, respectively (mean ± SD). The filtration rate at 5 cmH_2O was not significantly different from the rate at 30 cmH_2O.

Open thorax, intact lobes

Filtration rates in four lobes averaged .11±.21, -.85±1.02, -.50±.80, -.35±.38 and .17±1.53 g/(min x 100g wet weight) at alveolar pressures of 5, 10, 15, 20, 25 and 30 cmH_2O, respectively (mean ± SD). The data for this set of experiments are shown in Table 4 and Figure 17. Extravascular lung water content was normal in all (isolated and intact) lobes. Figure 18 shows both the isolated and intact lobe filtration rates on the same axes.

TABLE 3

EFFECT OF ALVEOLAR PRESSURE ON FILTRATION RATE
ISOLATED LUNGS

ALVEOLAR PRESSURE (cm H_2O)					
5	10	15	20	25	30
-	0	-0.048	0.127	-0.063	0.094
-	-0.159	-0.127	0	0	-
0	-0.079	0	0	-0.183	-
0	-0.317	0	0	0.064	-
0.281	0.225	0.142	0.056	-0.156	-0.169
.094±.162*	-.055±.181	-.007±.098	.037±.056	.048±.091	-.038±.186

Filtration rates are g/(min x 100g wet weight).
* mean ± SD

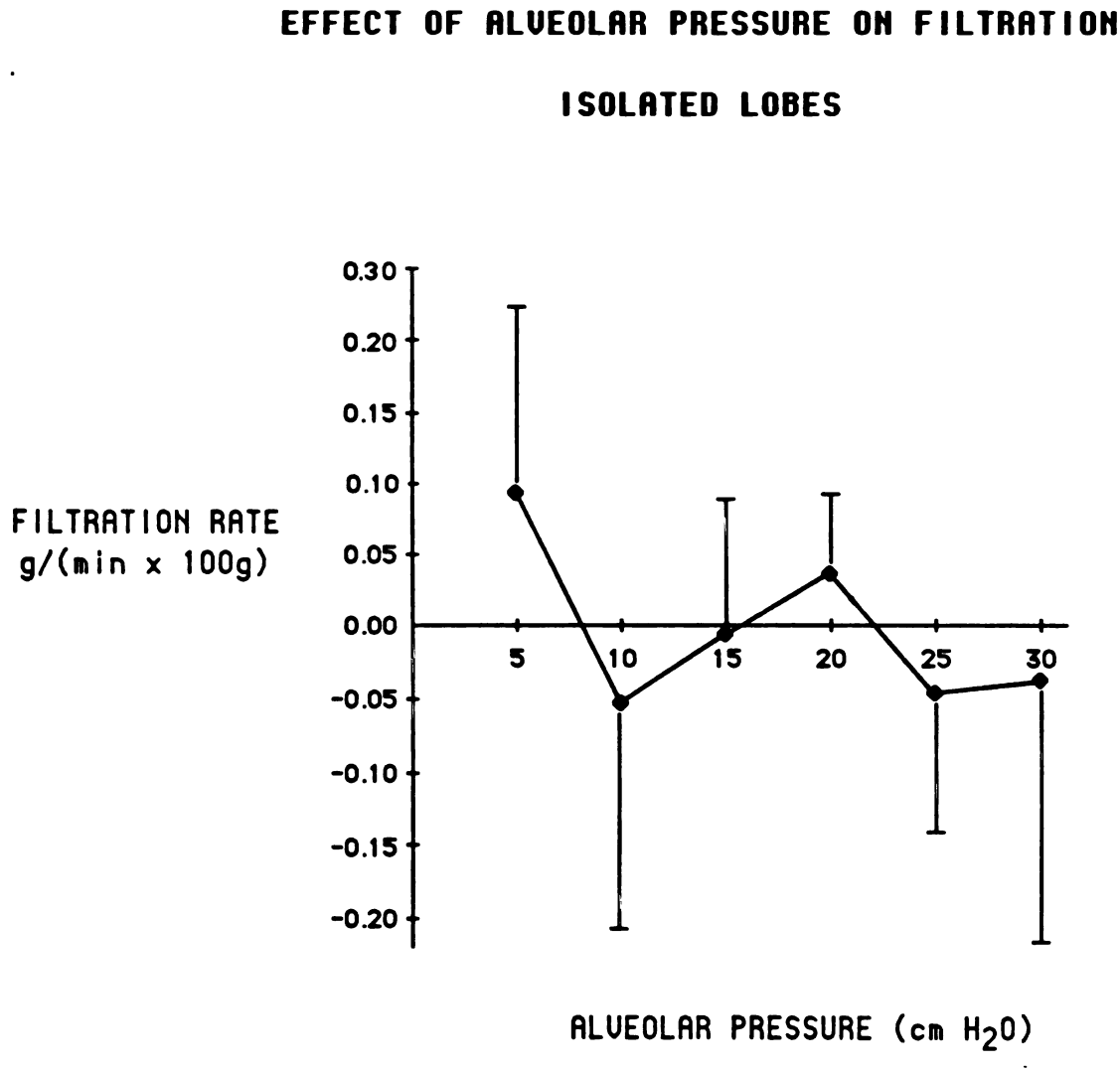


Fig. 16. Filtration rate vs. alveolar pressure in isolated, Zone I dog lung lobes. Increasing alveolar pressure at constant vascular pressure had no significant effect on filtration rate.

TABLE 4

EFFECT OF ALVEOLAR PRESSURE ON FILTRATION RATE
OPEN THORAX, INTACT LUNGS

ALVEOLAR PRESSURE (cm H_2O)					
5	10	15	20	25	30
0	-2.36	-1.65	-0.18	-0.89	2.26
0.42	-0.37	0	-0.27	0	-1.12
0	-0.13	0	-0.57	-0.32	-0.79
0	-0.53	1.18	-0.53	-0.20	0.33
.11±.21*	-.85±1.02	-.50±.80	-.39±.19	-.35±.38	.17±1.53

Filtration rates are g/(min x 100g wet weight).
* mean ± SD

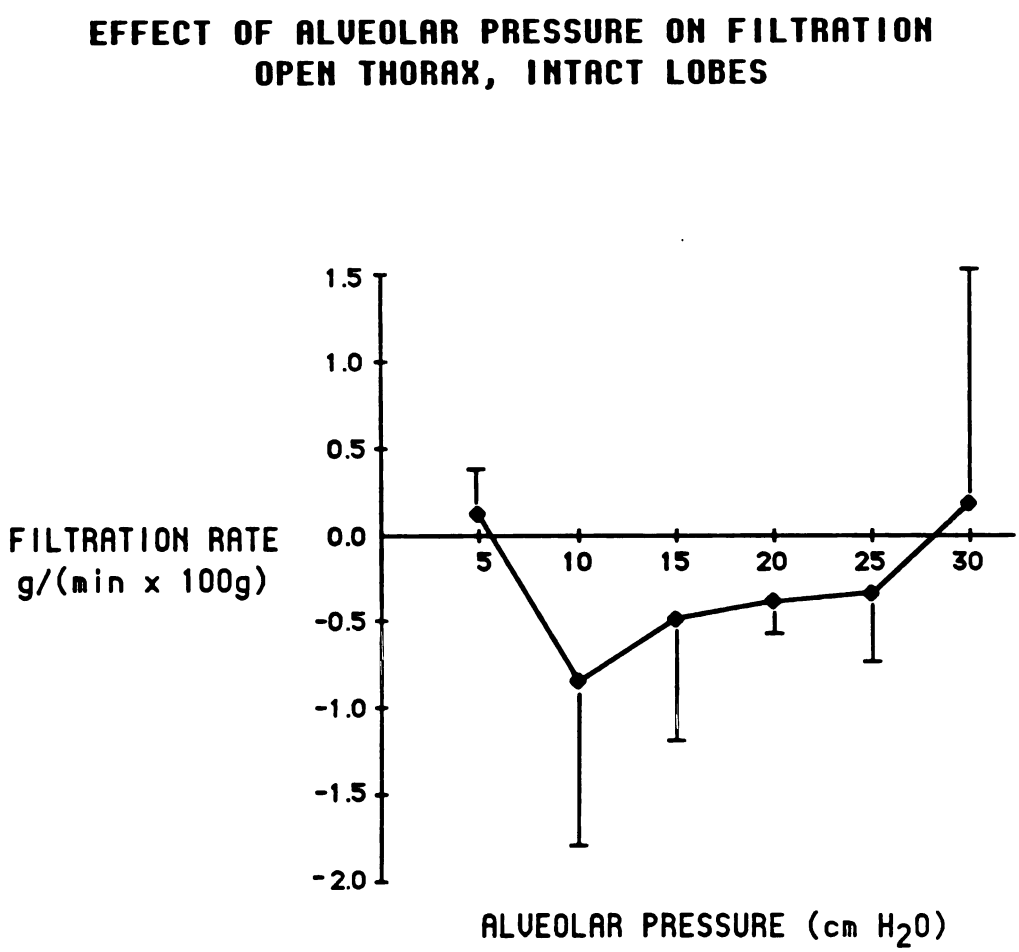


Fig. 17. Effect of alveolar pressure on filtration rate in open thorax, intact dog lung lobes. Alveolar pressure had no significant effect on filtration rate.

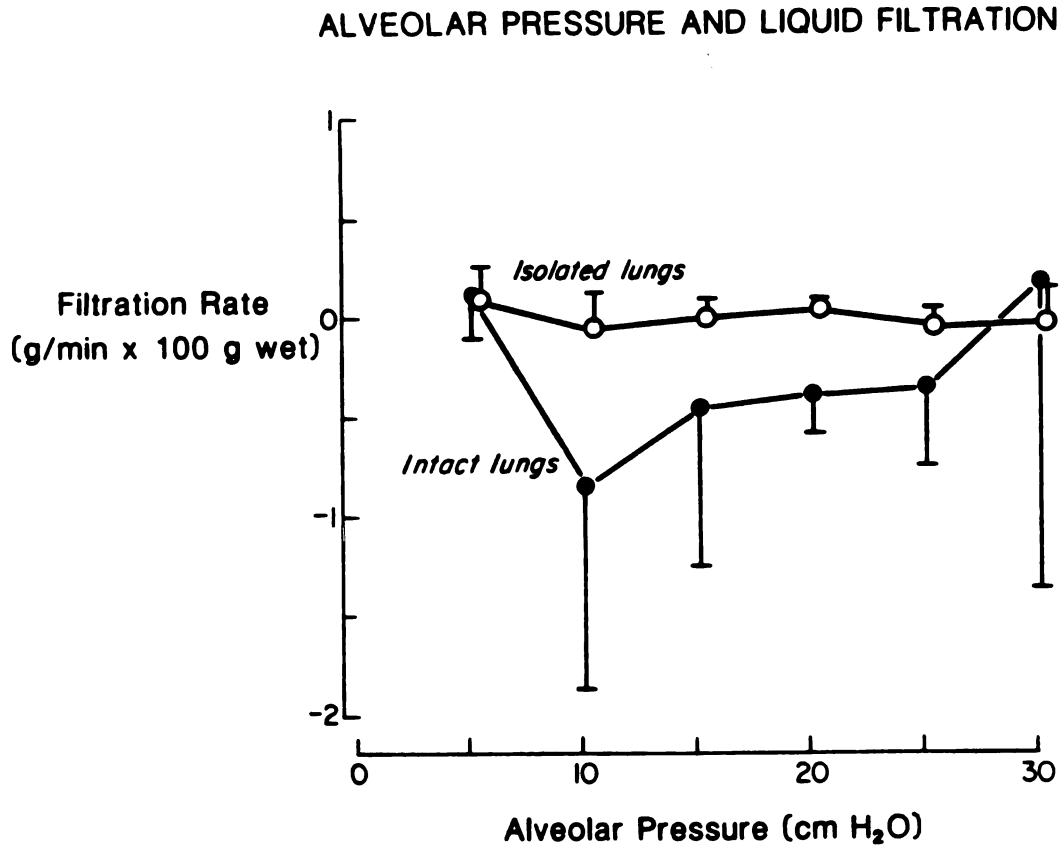


Fig. 18. Effect of alveolar pressure on filtration rate in both isolated and intact dog lung lobes plotted on the same scale. There was much greater variability in the intact measurements due to motion of the mediastinum caused by cardiac motion and ventilation of the contralateral lung.

DISCUSSION

I found no effect of positive pressure lung inflation on filtration rate in isolated or intact lungs. My results are similar to those of Iliff (37), who found no effect of alveolar pressure on lung edema in isolated dog lung lobes, even at different vascular pressures (see Figure 13).

I interpret my findings as indicating that perimicrovascular interstitial liquid pressure remains constant relative to vascular pressure at the sites of extraalveolar vessel filtration, even though alveolar pressure is increasing. This is likely be the case if the interstitial liquid pressure at the site of filtration is related to pleural pressure, as suggested by Nicolaysen and Hauge (53). In both the isolated and intact lungs, vascular and pleural pressures remained constant relative to each other as alveolar pressure changed.

Albert explained his finding of increasing filtration with lung inflation by invoking pulmonary vascular interdependence. He suggested that perimicrovascular liquid pressure decreased (becomes more negative) relative to a constant vascular pressure as lung volume increased, thereby increasing the hydrostatic gradient for filtration. Indeed, Lai-Fook (42) showed that interstitial liquid pressure at the hilum of isolated lungs, measured with micropipettes or wick catheters, becomes more negative with lung inflation. This data is shown in Figure 19.

It must be stressed that these measurements were made at the hilum of isolated lungs. It is unclear to what extent, or how much interstitial pressure in the alveolar walls changes with lung inflation,

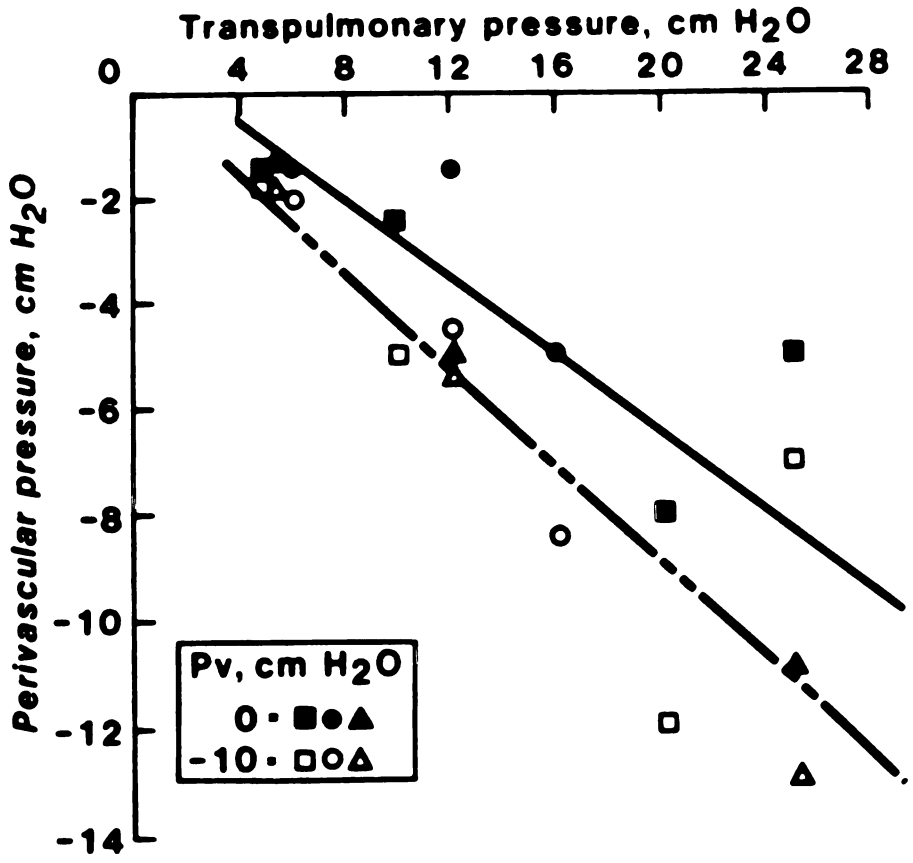


Fig. 19. Effect of transpulmonary pressure on perivascular interstitial pressure measured at the hilum with wick catheters from Lai-Fook and Toporoff (41). Note that hilar interstitial pressure decreases relative to vascular pressure with lung inflation.

although micropuncture measurements have recently been made at the alveolar wall junction. It is likely that the sites of liquid filtration are closer to the alveolar wall than they are to the hilum, and that they would be more effected by interstitial pressure nearer the alveolar wall. Although interstitial pressure becomes more negative at the hilum, it must become more positive in the alveolar wall, since alveolar wall capillaries are compressed with high alveolar pressure in Zone I as seen in Figure 7.

In fact, micropuncture measurements by Bhattacharya et al (7,10) have shown that peri-arteriolar and peri-venular adventitial pressure changes little with lung inflation. Bhattacharya found that interstitial pressure decreased from -0.6 to -2.1 cmH_2O in non-edematous dog lung lobes and increased from 2.0 to 3.6 cmH_2O in edematous lobes when alveolar pressure was increased from 7 to 23 cmH_2O relative to pleural (ambient) pressure. These are small changes in pressures, and would not cause a significant increase or decrease in filtration rate, especially if vascular pressure is held constant. This is further evidence that lung inflation may not change the hydrostatic pressure gradient driving filtration at the extraalveolar vessels.

Filtration in Zone I lungs most likely occurs proximal to alveolar wall capillaries, as these are collapsed. Interstitial pressure at the site of filtration probably behaves in a manner intermediate to that in the alveolar wall or at the hilum, i.e. if interstitial pressure increases at the alveolar wall and decreases at the hilum when the lung is expanded, then there must be no change in interstitial pressure at some intermediate point.

Since there was no effect of changing alveolar pressure on

filtration in the isolated lung series, I investigated the possibility that the lack of filtration in the isolated lobes might be explained by some difference between isolated lobes and Albert's intact lung preparation. In the latter, although the pulmonary artery and vein are cannulated, the bronchus and any other mediastinal connections are intact, including bronchial blood vessels.

Although there was no change in filtration with increasing inflation in my intact preparation, I did find substantial blood flow into the lobe and reservoir. This was determined to be bronchial blood by the fact that blood accumulated in the reservoir despite the fact that the pulmonary artery and vein were ligated in the lobe being weighed (this lobe was ventilated independently from the right lung using a tracheal divider and Harvard ventilator). When vascular pressure was set at 5 cmH_2O, the blood reservoir filled with blood. The only plausible explanation for blood flowing into the reservoir is that bronchial arterial blood was flowing into the lung and then into the perfusion circuit. It was shown by von Hayek (33) that there are broncho-pulmonary anastomoses where bronchial arteries join pulmonary veins. If the total pressure resisting filtration in these lungs was higher than 5 cmH_2O, as suggested by Gaar et al (23), who found an isogravimetric pressure of 7 mmHg, then blood would flow into the reservoir rather than filtering into the interstitium. Another indication that this blood was systemic in origin is that blood stopped accumulating in the perfusion circuit when the dog was killed. In a recent study Jindal et al (38) found that pulmonary arterial obstruction led to an increase in bronchial blood flow, measured in a preparation identical to mine and Albert's. If collapse of the alveolar capillaries

is analogous to pulmonary artery obstruction, then one would expect Zone I conditions to cause increased bronchial blood flow.

A partial explanation for Albert's different results may be that if his preparation was more permeable, blood or plasma entering the lung from the bronchial circulation may have filtered rather than flowing into the perfusion reservoir. There is also the possibility that the bronchial blood caused a gradual increase in vascular volume that might have been interpreted as filtration. Albert did not report on bronchial blood flow or extravascular lung water accumulation, although the small amounts of liquid filtered would be difficult to detect using blood-free wet to dry weight ratios.

Nicolaysen and Hauge and Bo, Hauge and Nicolaysen (11,53) found in isolated rabbit lungs that increasing alveolar pressure at constant vascular and pleural pressures in Zone III caused a decrease in filtration rate, whereas I found no filtration at all. An explanation may be that they used a much higher vascular pressure (19 cmH_2O), which produced a net filtration that was then modified by the changes in alveolar pressure. Certainly their findings are directly in opposition to those of Albert et al. Interestingly, Nicolaysen and Hauge did find that when alveolar and pleural pressures were changed in parallel, i.e. transpulmonary pressure and therefore lung volume was kept constant, that filtration increased when pleural pressure was more negative. This would suggest that in Zone I, interstitial pressure at the site of filtration is closely related to, and changes in parallel with pleural pressure. Increasing alveolar pressure at constant pleural pressure and constant vascular pressure merely results in compressing more vascular surface area, which will decrease filtration, if it is occurring. This

is analogous to our results when we increased vascular pressure with constant alveolar pressure in Zone I. There was progressive recruitment of vascular surface area as the Zone I/III border was approached, as indicated in Figure 10.

In conclusion, my results support the concept that interstitial pressure at the site of liquid filtration in Zone I is determined primarily by pleural pressure. Although interstitial pressure at the hilum is known to decrease with lung inflation, it is not relevant to perimicrovascular pressure at the site where filtration is occurring. Using a preparation identical to that of Albert et al, I cannot reproduce his finding of increasing liquid filtration with increasing alveolar pressure. The discrepancy may be due to the fact that bronchial arterial blood can enter the intact lung lobe and cause an apparent increase in lobe mass, which might have been mis-interpreted as filtration. Personal communication with Albert has failed to resolve the discrepancy. Unfortunately, he has been on Sabbatical leave for one year, so that we were unable to do experiments together. I agree with Nicolaysen et al that the primary determinant of interstitial pressure at the site of filtration in Zone I is pleural pressure. I am also in agreement with Iliff, who found no effect of Palv on filtration in Zone I lungs.

My studies also agree with those of Bhattacharya et al (7,10), who found little change in peripheral interstitial pressure with lung inflation. Although interstitial pressure at the hilum decreases relative to vascular pressure with lung inflation, this change does not appear to occur at the site of extraalveolar vessel filtration.

SERIES 4: ALBUMIN FLUX INTO THE EXTRAALVEOLAR INTERSTITIUM

Having measured extraalveolar liquid filtration under several conditions, I developed an ultramicro method to measure the leakiness of extraalveolar (arteries or veins) for albumin. Liquid flux can be studied by changing hydrostatic pressure in Zone I isolated dog lung lobes and measuring the resultant rate of weight change but protein flux can only be studied by sampling the liquid in the interstitium. The structure of the pulmonary interstitium is such that only extremely small samples (several nanoliters) of interstitial fluid can be collected even in edematous lungs. To analyze these samples required that I develop a technique specifically suited to measuring albumin concentration in nanoliter samples of lung liquid.

Many investigators use the lung lymph fistula model to monitor changes in pulmonary microvascular liquid and protein flux. This technique, introduced by Staub (71), is based on the theory that by collecting the lymph draining the lung, one can observe steady state transvascular liquid and protein balance across the pulmonary microvascular endothelium. The liquid that is collected represents a weighted average of the net filtrate, biased toward those vessels with the greatest filtration.

The pulmonary interstitium is composed of collagen, reticular and elastic fibers enmeshed in a ground substance matrix consisting of hyaluronic acid, chondroitin sulfate, heparan sulfate and other complex glycosaminoglycans (25). The large liquid domains of the glycosaminoglycans and the fibrillar network effectively separates water into a

gel-like continuum. Even though water can flow through the interstitium, sampling liquid is very difficult.

Guyton Scheel and Murphree (31) measured interstitial tissue hydraulic conductivity by implanting two catheters in subcutaneous tissue and measuring the time required for liquid injected through one catheter to travel to the other. They found the interstitial resistance was even higher when suction was applied through the downstream catheter. This implies that any "sucking" procedure used to sample interstitial fluid will likely be unsuccessful, as interstitial resistance increases exponentially with dehydration. Conversely, the same group found that interstitial hydraulic conductivity increased substantially when the tissue became hydrated. Although these studies were done in subcutaneous tissue, the chemical and structural nature of the lung interstitium is similar, and would be expected to behave similarly.

Vreim et al (77) measured the protein concentration of interstitial fluid in dog lungs with increased permeability edema by rapidly freezing the lungs in liquid nitrogen and then chipping frozen interstitial liquid out of the perivascular liquid cuffs. These samples of a few microliters were then assayed for total protein by the Lowry method. This is a tedious and inexact procedure, as the cuffs are small and the liquid can be contaminated by blood during sampling. Vreim found that lung interstitial albumin concentration in sheep with increased pressure edema averaged 1.6 g/dl and 2.3 g/dl in lungs with increased permeability edema induced by pseudomonas bacteremia.

As the micropuncture technique has been used extensively in our laboratory for measurements of microvascular and interstitial pressure,

I devised a method to use this technique for relatively atraumatic sampling of interstitial fluid. I found that it was impossible to collect liquid when the lung tissue was normally hydrated. However, once the lung had gained 30-40% of its initial weight in edema fluid, it was possible to collect fluid from the tissue surrounding the artery, vein and bronchus at the lung hilum.

Even under the best of conditions, I was able to sample only extremely small volumes of interstitial fluid. It was therefore necessary to develop a method whereby I could measure albumin concentration in 10-30 nl samples of interstitial liquid collected by micropuncture.

I measured interstitial albumin concentration in 3 lobes with arterial filtration and 3 lobes with venous filtration. All experiments were done under Zone I conditions to assure that filtration was occurring only from extraalveolar vessels. From these studies, I was able to determine the albumin permeability of the extraalveolar arterial and venous beds in isolated lungs.

METHODS

The samples were collected under direct microscopic visualization using glass micropipettes with a tip diameter of 9-12 microns. The samples of lung liquid were drawn into the micropipette and then transferred with an inulin apparatus, which is A device consisting of 2 micromanipulators and a microscope set up to allow handling of extremely small samples. The samples were diluted and then transferred into an agarose gel where they were subjected to rocket immunoelectrophoresis for determination of albumin concentration.

Pipette manufacture

Pipettes were made from chromic acid cleaned, siliconized glass stock 2mm in diameter. Pipets were pulled (David Kopf Instruments pipette puller) to achieve optimum tip length and diameter prior to beveling. The pipettes were then beveled with a diamond-surfaced beveler (Sutter Instruments) which allowed me to make an extremely sharp pipette which easily penetrated the visceral pleura of the lung. After beveling, the pipettes were again cleaned with chromic acid, water and acetone. If the tip diameter was too small (less than 8u), liquid collection was extremely slow, conversely, if the pipette was too large, it was difficult to puncture the pleura.

Sample collection

Prior to micropuncture, the pipettes were filled with paraffin oil colored with Sudan Black dye. This was done to improve visualization of the pipette tip above and within the parenchyma of the lung. The oil also served to eliminate an air-water interface after the sample was collected. Eliminating the air interface decreased the amount of suction needed to collect a sample. The pipette was attached to a specially-made holder which was connected to an oil- filled glass syringe via polyethylene tubing to supply suction. The entire pipette holder was held by a Leitz micromanipulator. The lung surface structures could be seen with a Leitz stereomicroscope, (magnification 100x) with a long focal distance. Figure 20 shows an isolated lung with the micropuncture equipment.

The pleural surface of the lung was covered with silicon oil to prevent dehydration or hydration of the pleura and underlying interstitium. The lung surface was held steady with a suction ring that applied gentle suction (2-4 cmH_2O). The pipette was inserted into the area of interest and samples collected by gentle suction with the glass syringe. When sufficient liquid had been collected, the pipette was withdrawn to a point just above the pleura, but still within the oil layer. Additional oil was drawn into the pipette to trap the sample between two oil interfaces. This eliminated sample loss or evaporation.

Volume measurement with transfer pipet

Using the inulin apparatus, the sample was ejected into a clear

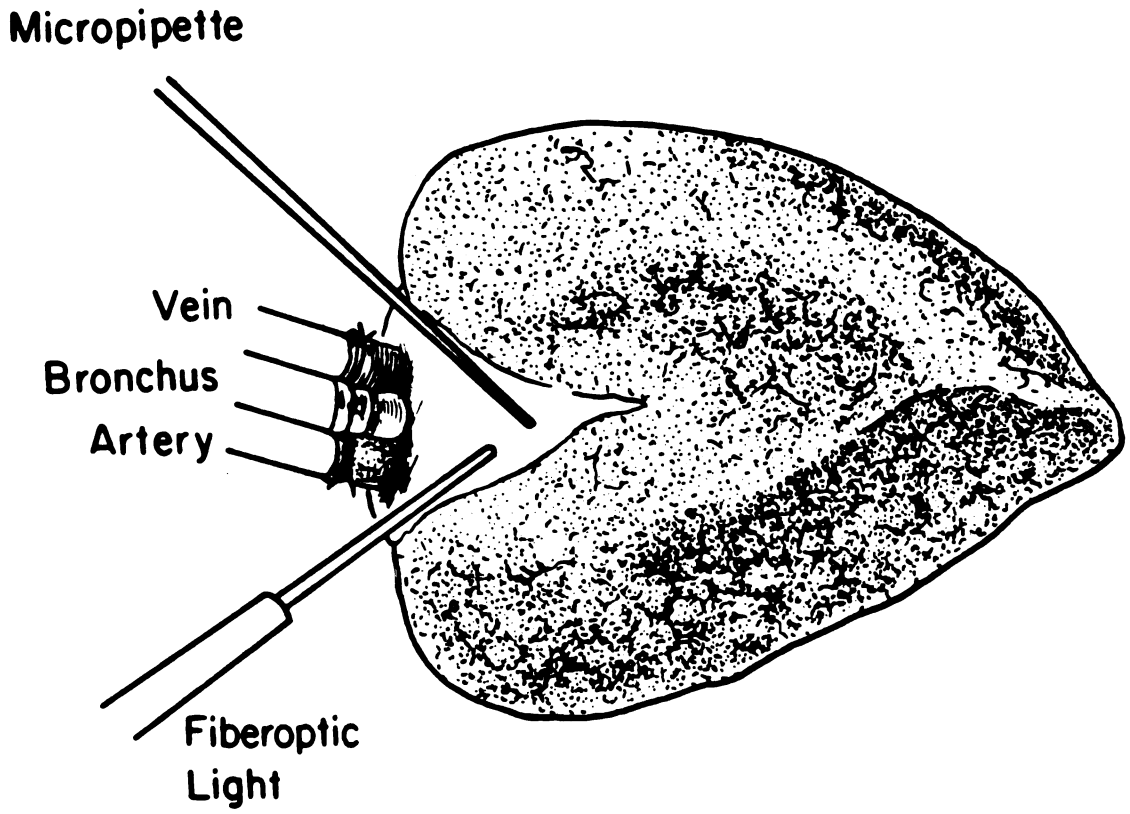


Fig. 20. Isolated dog lung lobe set up for interstitial liquid collection. Fiber-optic light source illuminates the hilar interstitium for micropuncture.

lucite trough containing water-equilibrated silicon oil. The sample was gently ejected onto the wall of the trough where it was held by surface tension. When all samples had been collected, they were transferred from the trough into plastic pipette tips containing tris-barbital buffer. To transfer the samples, we made a "transfer pipette" using 10u diameter, constant bore "omega" quartz glass. A marker was glued alongside the pipette to act as a constant volume marker. The exact volume of the pipette did not need to be determined as long as the same pipette was used for the entire analysis of unknowns and standards. The average volume was calculated to be 15-20 nanoliters. The tip of the transfer pipette was narrowed slightly using a flame to allow accurate control of the meniscus as it moved up and down the pipette. A transfer pipette and oil-filled plexiglass trough is diagrammed in Figure 21.

Measurement of albumin concentration

Using gentle suction, the samples were drawn into the transfer pipette up to the mark and then ejected into pipette tips (Eppendorf) containing 11.5 microliters of tris-barbital buffer at pH7.4. The buffer was held in the pipette tips by surface tension. The same procedure was followed for four dilutions of pooled dog plasma . Plasma was diluted to 75, 50 and 25% of normal using saline, and subjected to the same procedure with the transfer pipette. These served as standards for construction of a calibration curve for each experiment. Albumin concentration in larger samples of the pooled plasma dilutions was determined separately by the bromcresol green (BCG) colorimetric method (29). The pipette tips containing the samples in buffer were covered

TRANSFER PIPET AND OIL-FILLED TROUGH

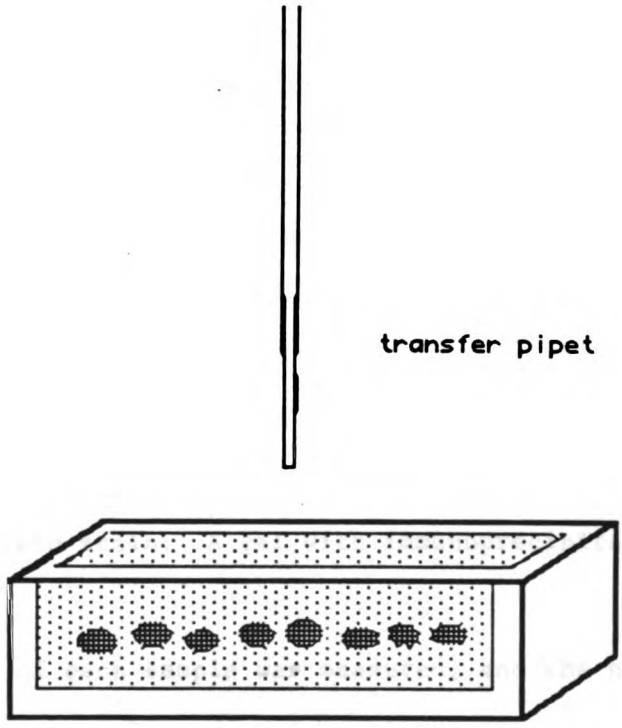


Fig. 21. Transfer pipet and oil-filled plexiglass trough. Samples are ejected from the collection micropipette and adhere to the side of the trough. The samples are then moved using the transfer pipet and ejected into 10 ul of buffer for electrophoresis.

with oil or plastic to prevent evaporation.

Rocket immunoelectrophoresis

I measured albumin concentration in the samples by the rocket immunoassay method of Laurell (44). The samples (unknowns and standards) were pipetteted into 15ul volume wells cut into an agarose gel made with 25ml of 1% agarose (SeaKem) in tris-barbital buffer. The gel contained 40-60ul of anti-canine albumin antiserum from rabbits (Cappel antisera). Sufficient antiserum was used to result in peaks that were sharp, and traversed 60-70% of the gel. The gel was formed on a thin, hydrophilic plastic (Gelbond, FMC). The samples were pipetteted into the wells of the gel while a field of 5 V/cm was applied. This minimized radial diffusion of sample during the pipetteting process. Samples were electrophoresed for 3 hours at 10 V/cm. Once electrophoresis was complete, the gels were dried and then stained with croceine scarlet protein stain. A gel with immunoprecipitate peaks is shown in Figure 22.

The peak height of each sample was measured, and the heights of the pool plasma dilution peaks plotted against albumin concentration determined by the BCG method. Although protein concentration is theoretically related by total rocket area, the rockets were symmetrical in shape, so height could be used. Linear regression analysis of this data generated a line whose slope described the relationship between albumin concentration in the pooled standards and rocket peak height. Albumin concentration in the unknowns was calculated from this relationship. Figure 23 shows a calibration curve plotted from the pool

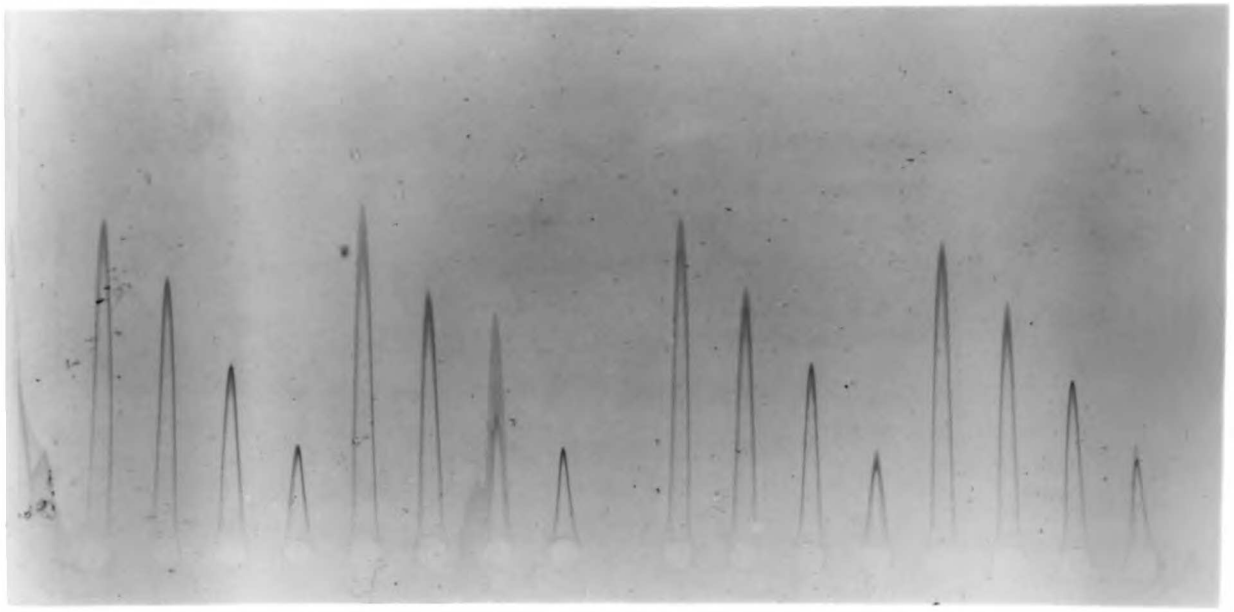


Fig. 22. Albumin immunoprecipitate rocket peaks formed from immunoelectrophoresis. These are four dilutions of pooled dog plasma and three duplicates. Peaks are stained with croceine scarlet. Rocket peak height is proportional to albumin concentration.

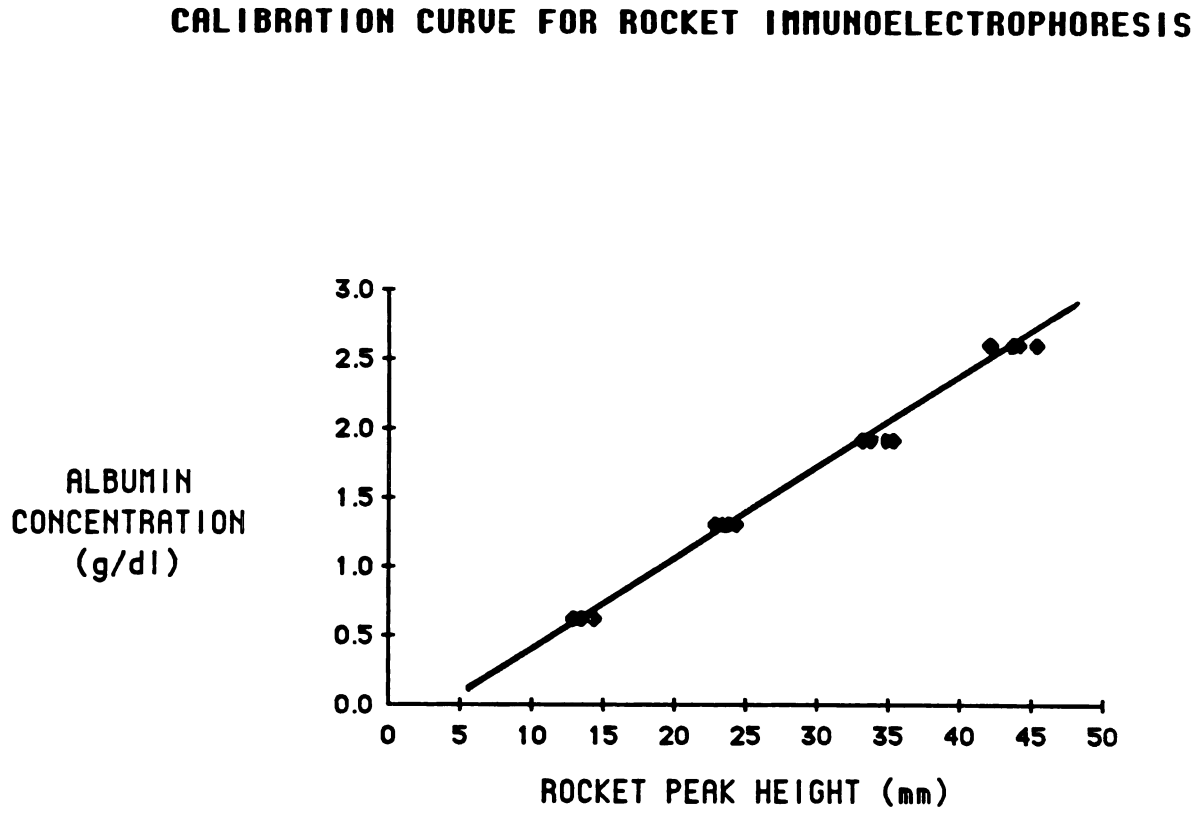


Fig. 23. Plot of rocket peak height vs. albumin concentration as determined by the BCG method. These are the samples shown on the gel in Fig. 22. Pooled plasma dilutions were run with each experiment to establish a standard curve from which albumin concentration in the interstitial liquid samples could be determined.

plasma dilutions used on the gel in Figure 22.

EXPERIMENTAL PROTOCOL

These experiments were done in isolated dog lung lobes. The isolation procedure was the same as described in Series 1. The lobes were perfused with autologous, heparinized plasma and inflated with 30% O_2, 5% CO_2 and 65% N_2.

I initially tried to do these experiments using whole blood, trying to simulate normal intravascular forces but found that under conditions where there had been filtration using plasma (Series 1: vascular pressure = 20 cmH_2 and alveolar pressure = 25 cmH_2O), there was little or no filtration, if the vessels were blood-filled. Other investigators have studied this phenomenon (32,45,39), most recently that of Julien et al who found decreasing filtration rate in Zone III dog lungs as hematocrit was increased. It is likely that red blood cells decrease the effective filtration surface area, an effect that would be most pronounced in small blood vessels and in Zone I conditions. The red blood cell membrane may superimpose its very low filtration coefficient on the microvascular barrier, as suggested by Hansen. Thus, in order to get edema formation in these experiments, I had to perfuse the lungs with autologous plasma. The effect of hematocrit on filtration rate will be studied in our laboratory, but is not a part of my thesis research.

The lungs were placed on the Mettler balance for continuous weight measurement. The lungs were not weighed during the interstitial liquid

collection period because we used a suction ring to stabilize the surface of the lobe for micropuncture.

Once the lobe had been excised, its initial weight was recorded. The artery, vein and bronchus were cannulated and set on the Mettler balance. The lobe was perfused at low pressure with autologous plasma to avoid possible settling of red cells in the microvessels. The lung lobe was placed with the costal surface down to expose the hilar aspects of the artery, vein and bronchus. The Leitz microscope provided excellent visualization of the hilar tissue.

Once the lobe was set up, alveolar pressure was increased to 30 cmH_2O and then lowered to 25 cmH_2O to put the lung in Zone I. Vascular pressure was increased by raising a reservoir on either the arterial or venous side. Vascular pressure was set at 20 cmH_2O, enough to result in filtration of .5-1.0 g/min. Collection of interstitial liquid began approximately 1 hour after vascular pressure was set when the lobe had gained approximately 40% of its inital weight. Samples were collected at hourly intervals until lung weight had increased 2 to three times (3-4 hours). At the conclusion of the experiment, the lungs were perfused with autologous blood and then rapidly frozen in liquid nitrogen for subsequent determination of extravascular lung water.

RESULTS

I measured hilar interstitial albumin concentration in six lung lobes, three with only extraalveolar arterial filtration and three with venous filtration. Micropunctures were made every hour, beginning when the lobe had gained approximately 30 - 40% of its initial weight in edema fluid. The experiments were continued until lung weight had increased 2-3 times control.

Interstitial albumin concentration resulting from arterial filtration averaged 2.08±.10, 2.18±.26, 2.29±.26 and 2.15±.05 g/dl at 1, 2, 3 and 4 hours, respectively. Albumin concentration in the venous filtration studies was 2.40±.26, 2.32±.40, 2.20±.32 and 2.26±.30 at the same time intervals. All values are expressed as mean±standard deviation. These data are displayed in Table 5. Albumin concentrations in the arterial and venous filtration experiments were not significantly different from each other as determined by a student's t-test ($p > .05$). Plasma albumin concentration averaged 2.68±.28 g/dl. The interstitial fluid to plasma albumin concentration ratio averaged .83. Albumin concentration is plotted against time in Figures 24, 25 and 26.

Extravascular lung water content for the arterial filtration experiments averaged 8.70±.71 grams extravascular water per gram dry lung weight. Control lung water in these experiments was 3.49±.17. For the venous filtration experiments, extravascular lung water content was 9.05±1.26 g extravascular water per gram of dry lung with a control value of 3.36±01.

TABLE 5

HILAR INTERSTITIAL ALBUMIN CONCENTRATION

Experiment	1 hr	2 hrs	3 hrs	4 hrs	Qwl/dQl	
Arteries					control	final
DLG 1	2.18	2.47	2.56	-	3.60	8.52
DLG 5	1.99	1.97	2.05	2.11	3.29	8.09
DLG 7	2.08	2.09	2.27	2.18	3.57	9.48
mean ± SD	2.08±.10	2.18±.26	2.29±.26	2.15±.05	3.49±.17	8.70±.71
Veins						
DLG 3	2.34	2.34	2.05	2.05	3.36	8.17
DLG 4	2.18	1.91	1.98	-	3.37	8.49
DLG 6	2.68	2.71	2.56	2.47	3.36	10.50
mean ± SD	2.40±.26	2.32±.40	2.20±.32	2.26±.30	3.36±.01	9.05±1.26

Albumin concentrations are g/dl.

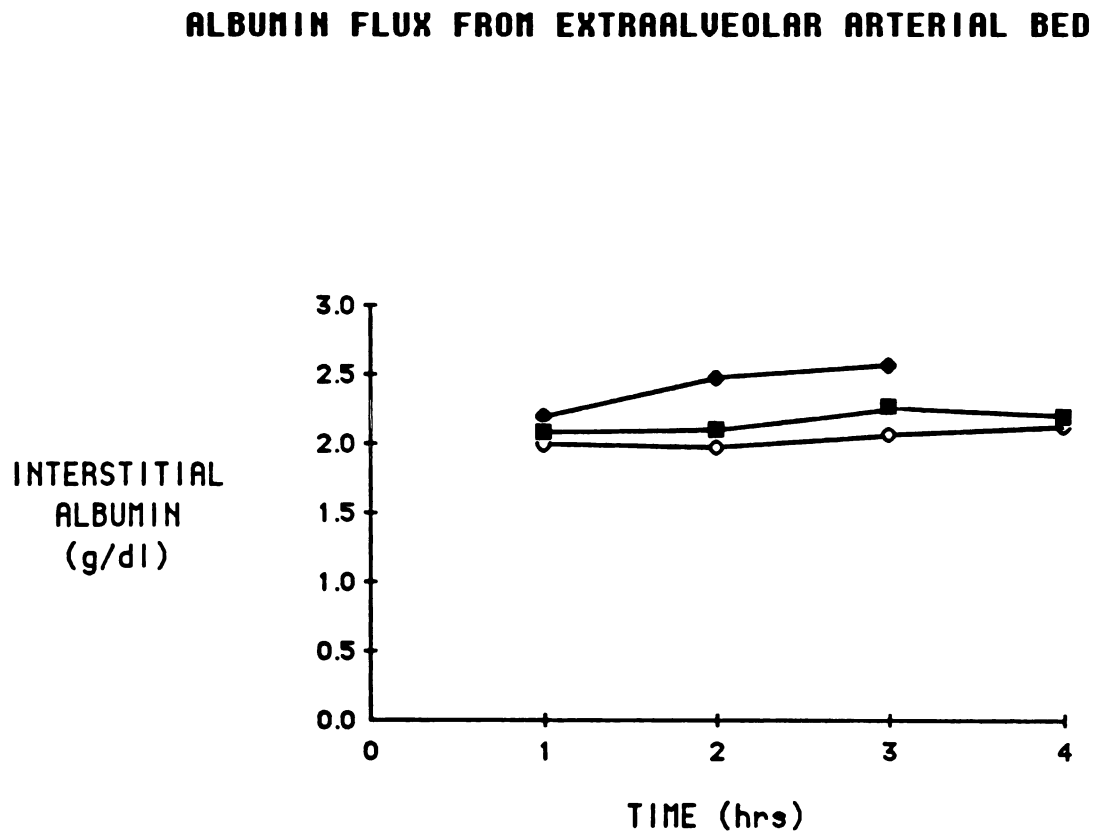


Fig. 25. Interstitial albumin concentration vs. time in samples collected by micropuncture from the hilum of dog lungs with hydrostatic edema in the extraalveolar arterial bed. Interstitial albumin concentration averaged 83% of plasma albumin concentration.

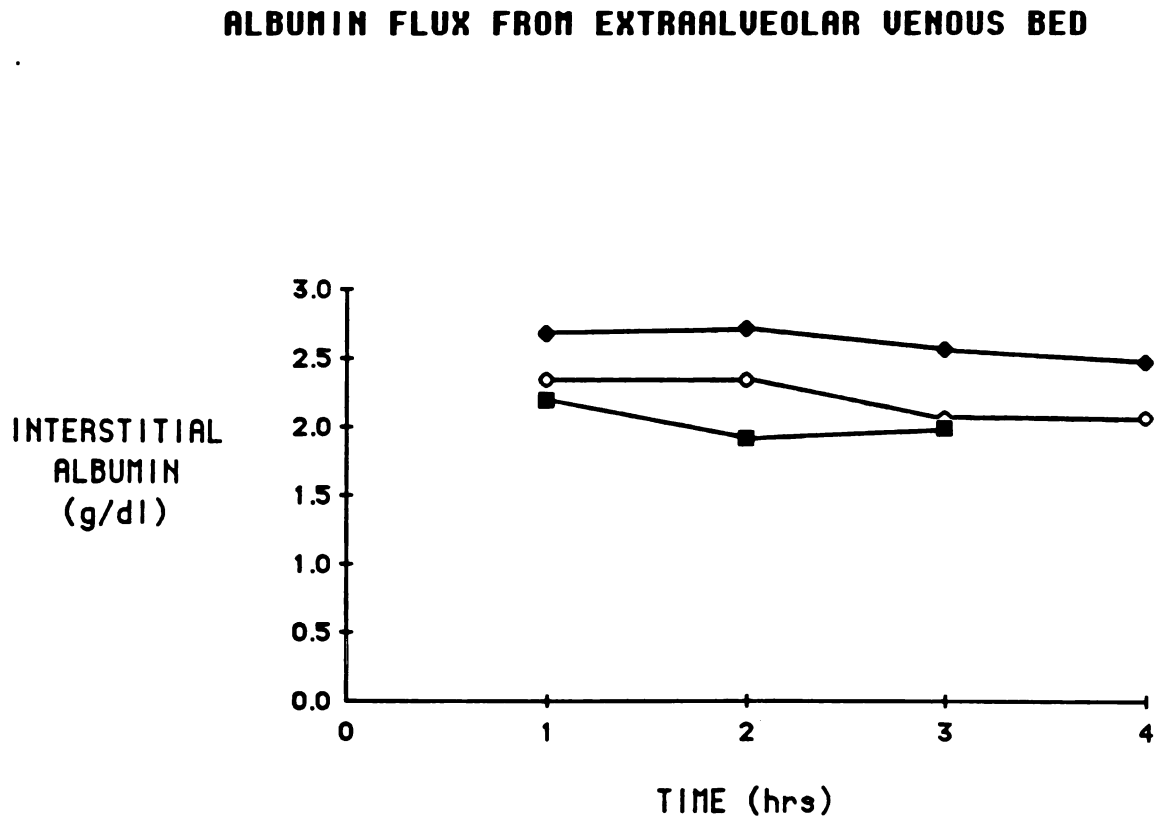


Fig. 25. Interstitial albumin concentration vs. time in samples collected by micropuncture from the hilum of dog lungs with hydrostatic edema in the extraalveolar venous bed. Interstitial albumin concentration averaged 83% of plasma albumin concentration.

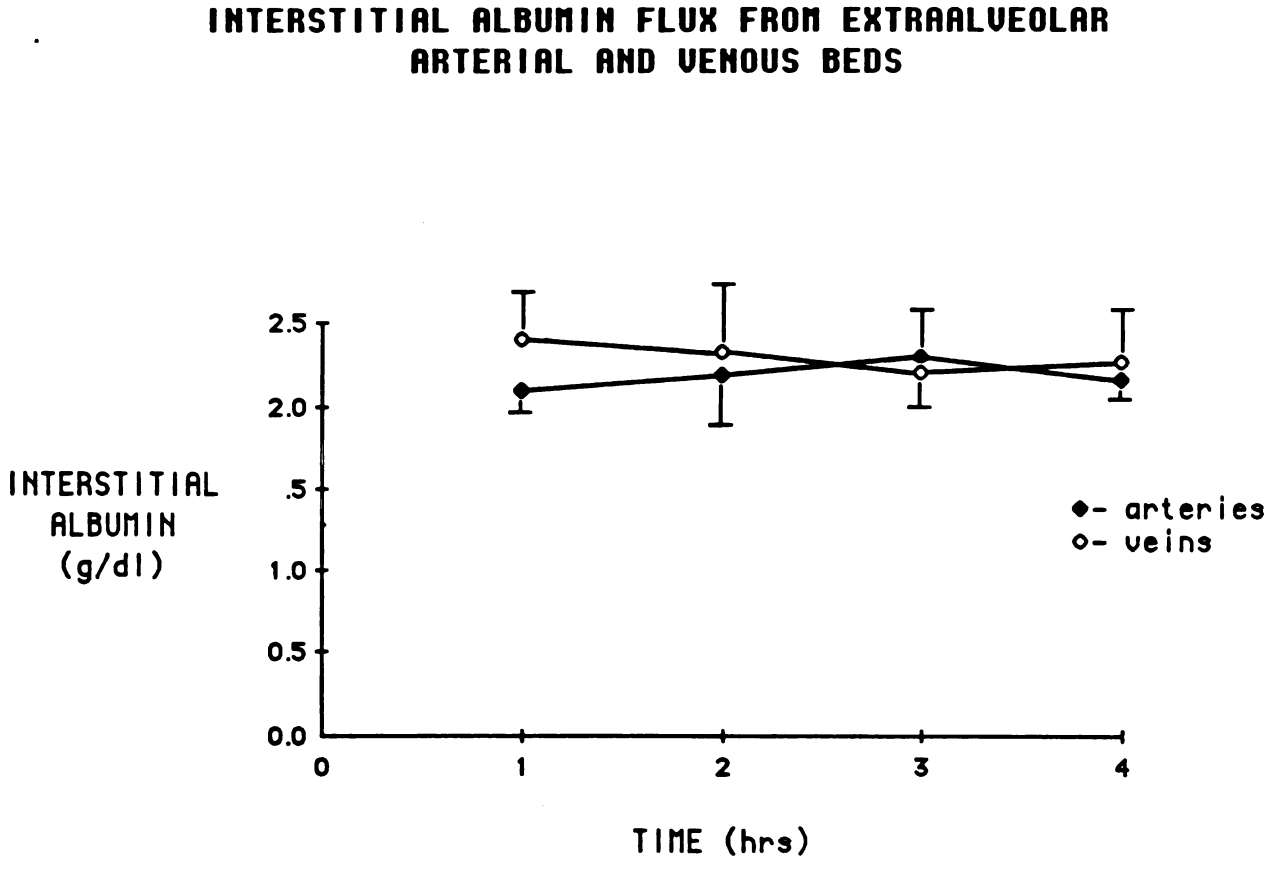


Fig. 26. Mean interstitial albumin concentration vs. time in samples collected by micropuncture from the hilum of dog lungs with hydrostatic edema. Although there was significant edema at 4 hours, there was not washout of interstitial albumin.

DISCUSSION

In recent years the predominant method used to study pulmonary microvascular permeability has been the sheep lung lymph fistula (71). This method involves collection of steady state lymph, as the measure of liquid and protein filtered into the pulmonary interstitium. It is useful for studies of both high pressure and increased permeability pulmonary edema. But this method measures only the net filtrate. It is unable to determine the individual contributions of different parts of the pulmonary microvasculature. Recent criticisms have been based on the fact that some systemic lymph may enter the caudal mediastinal lymph node efferent duct or that under some conditions lymph entering lymph nodes may be modified within the node (18). Extensive control experiments (15,63,73) show that such problems are minimal. But in theory one always prefers afferent lymph (pre-nodal). By collecting interstitial liquid directly from the tissue, the possibility of lymphatic or node modification or dilution by systemic sources is eliminated. As discussed previously, the nature of the interstitium is such that only extremely small samples of liquid can be collected and only under conditions of interstitial hydration. To collect and analyze these samples, I had to develop a technique for the measurement of albumin concentration in samples of liquid collected by micropuncture. These samples averaged 5-25 nl, necessitating special techniques for the handling and transfer of the samples once they had been collected. I used an immunoassay, as this provided the most accurate and sensitive method for albumin concentration determination.

Investigators have made interstitial pressure measurements at the hilum of isolated dog lungs using both wick catheters and micropipettetes (41,42). This is the largest and most accessible interstitial space in the lung. Once the lung lobe had become slightly edematous, it was relatively easy to collect interstitial liquid with micropipettes at the hilum. I initially attempted to collect liquid from more peripheral interstitial sites such as the adventitia around arterioles and venules. These attempts were unsuccessful due to the extremely small tissue space and amount of liquid at these locations, even in an edematous lung. When I attempted to collect liquid from peripheral sites nearer the alveolar wall microvessels, the alveoli surrounding the micropipettete collapsed.

As can be seen from the data in Table 5 and Figure 26, there was no difference in albumin concentration in the samples collected from the hilum whether filtration was from the extraalveolar arterial or venous vascular beds. This is consistent with my Zone I filtration studies described in section 1, where there was no difference in filtration rate measured gravimetrically when arterial or venous hydrostatic pressure was increased. This supports the hypothesis that the extraalveolar arterial and venous beds have similar permeabilities.

I have been unable to find other experimental data in which anyone has examined the individual contributions of the extraalveolar arterial and venous vascular beds to interstitial albumin concentration, so any comparisons must be extrapolated to somewhat different experimental techniques and conditions.

I cannot give a precise time at which the liquids I collected were filtered, except the cuffs appeared during the first hour of

filtration. Considering the relatively low hydraulic conductivity of the pulmonary interstitium, the liquid was probably filtered some time before it was collected. It is also likely that some of the albumin collected was resident in the hilum before the filtration began, and that it was only with increasing hydration that it could be collected, although one would expect the filtration to dilute this resident albumin.

Erdmann et al (20) measured liquid and protein flux by collecting lung lymph from the caudal mediastinal lymph node in unanesthetized sheep. They induced hydrostatic edema by inflating a balloon that had been placed in the left atrium. There was some variability of the values reported for lymph albumin concentration in the study of Erdmann et al, but most were in the range of 1.8 - 2.2 g/dl with increased left atrial pressure. This agrees quite closely with the values obtained by interstitial micropuncture listed in Table 5. One must be cautious in comparing these results, as the study of Erdmann et al was done in unanesthetized sheep under Zone III conditions, whereas my studies were in isolated lungs under Zone I conditions. This does, however, raise the possibility that extraalveolar filtration might be a primary determinant of interstitial albumin flux.

When Vreim et al measured the albumin concentration in interstitial fluid cuffs, they she found a slightly lower value of 1.7 g/dl in sheep with hydrostatic edema. This may be due to differences in experimental preparation or degree of extravascular water accumulation. She found no significant difference between lymph protein concentration and the protein concentration in the interstitial fluid cuffs. It may be that the isolated lung is leakier than the intact lung (66).

There are few studies of interstitial or lymph albumin concentration in dogs. Parker, Ryan and Taylor (59) measured lymph flow and protein concentration from a single hilar afferent vessel in dog. They found a lymph albumin concentration of 1.2 g/dl in baseline open thorax, anesthetized dogs. Plasma albumin in their experiments averaged 1.5 g/dl. Both these values are somewhat lower than I obtained and lower than reported by Vreim or Erdmann et al. However, the lymph/plasma albumin concentration ratio in their experiments was 80%, the same as the 83% I found.

Although my dog lungs had hydrostatic edema (see Table 5), , there was no "wash-out" of interstitial albumin. As it is impossible to sample interstitial liquid in a "dry" lung, I cannot say what interstitial albumin concentration might be when no edema is present. It might be feasible to tag the intravascular albumin at various times after the lung is perfused and then measure the concentration of the label as it is collected at the hilum. This would give a more detailed picture of the dynamics of protein movement across the endothelium and through the interstitium to the hilum.

The development of this technique for measurement of interstitial liquid collected by micropuncture gives us a powerful tool for further studies of protein flux in isolated and intact lungs.

GENERAL DISCUSSION

My experiments provide a comprehensive study of filtration from extraalveolar vessels in dog lungs. It is clear that there is substantial filtration from these vessels, although the exact site of leakage remains to be identified.

There is no difference in the liquid filtration or protein permeability characteristics of the arterial and venous extraalveolar beds (Series 1 and Series 4). In Series 1, I found that filtration rate measured gravimetrically was identical from the arterial or venous extraalveolar beds, while in Series 4 I found the interstitial albumin concentration from liquid filtration at these sites to be identical.

Series 1 gives convincing evidence of a difference between the systemic muscular and pulmonary vascular beds. Rous reported that the capillaries and venules of the muscle vasculature were leakier than the arterioles or larger vessels (64). I have found in the lung that larger vessels contribute substantially to filtration, far more than would be expected based on surface area considerations. I have also found that the arterial and venous ends of the pulmonary circulation have identical filtration characteristics, another departure from what Rous found in muscle. Undoubtedly, the distribution of interstitial pressures in the lung contribute to the distribution of filtration along the vascular bed. Whereas in skeletal muscle one would expect interstitial pressure to be fairly constant along the length of the circulation, the lung has two main interstitial compartments that have different interstitial pressures. This heterogeneity of interstitial pressure gives a

heterogeneity of hydrostatic pressure gradients for filtration. Whereas arterioles in skeletal muscle are heavily muscularized, they are not in the lung (62), which probably explains their similarity to venules in filtration characteristics.

Although it is suprising to find such a large fraction of the total filtration (43%) coming from a tiny fraction of the total surface area, considering the structure/function relationships in the lung, this might be desirable. If filtration is necessary for normal lung function, it is preferable that it occur in structures proximal or distal to the alveolar wall microvessels, so as not to interfere with gas exchange. If filtration occurs at the alveolar wall junctions or in larger arterioles of venules, excess liquid is kept away from the alveoli and has a relatively short path to the initial lymphatics (70), which are not found in the alveolar wall interstitium. Once liquid is filtered at these sites, there is a hydrostatic pressure gradient to lead it away from the alveoli and into lymphatics where it can be removed (8,24). In the case of excessive filtration (edema), the proximity of an extraalveolar leakage site to interstitial fluid cuffs would provide for rapid removal and storage of edema liquid.

There appears to be recruitment of filtration surface area as the lung moves down Zone I towards Zone III at constant alveolar pressure. This is not suprising. I envision longitudinal "creep" of the liquid front into exponentially greater surface area as the vascular pressure is increased toward alveolar pressure. I cannot explain the results of Goldberg, who found no recruitment within Zone I as vascular pressure was increased. Perhaps his preparation was becoming increasingly edematous, causing an increase in perivascular interstitial liquid

pressure which counteracted the effect of increasing surface area. If the leakage site in Zone I is near the alveolar wall junctions, as is suggested by the Zone I histology, then one would expect increasing vascular pressure to recruit filtration surface area as the alveolar wall microvessels begin to open.

In Series 3 I examined the effect of alveolar pressure on filtration in Zone I. This has been a controversial topic, but I feel that my studies have helped in our understanding. I found that there was no effect of alveolar pressure on filtration rate in either isolated or in situ dog lungs. Although previous studies have shown that interstitial pressure decreases relative to vascular pressure with lung inflation, these were all measurements made at the hilum (41,42). Interstitial pressure closer to the alveolar wall is probably some value between pleural and alveolar pressure (8). It appears that the interstitial pressure at the site of filtration is determined primarily by pleural pressure, as there was never any change in filtration rate as the lung was inflated. If interstitial pressure at this point were closer to alveolar pressure, then one would expect lung inflation to cause a decrease in filtration rate. If the pressure at this point were related to hilar interstitial pressure, then one would expect filtration rate to increase with lung inflation.

My findings in Series 3 agree most closely with those of Iliff and of Nicolaysen and Hauge (37,53). Iliff found no effect of alveolar pressure on weight gain in isolated dog lungs. There were some methodological differences between her experiments and mine in that she let the lungs gain weight for 2 hours, rather than a short period (5 min) that I used. Nevertheless, my results show the same trend as hers.

Nicolaysen and Hauge found that increased alveolar pressure decreased filtration rate in Zone I lungs. This is not inconsistent with my results because they used a much higher vascular pressure (19 cmH_2O) and therefore had recruited some surface area which was de-recruited when alveolar pressure was increased. If I increased alveolar pressure is increased under conditions of filtration, I would expect the filtration rate to decrease as vascular surface area decreases due to compression. In my experiments, however, vascular pressure was set to 1 cmH_2O. There was no filtration at any alveolar pressure therefore no change in filtration rate could be detected. More importantly, Nicolaysen and Hauge found that filtration rate increased when pleural pressure was made more negative and decreased when pleural pressure was more positive. This suggests that interstitial pressure at the site of filtration in Zone I is determined by pleural pressure. In my experiments in Series 3, vascular pressure was kept constant relative to pleural pressure, so one would not expect filtration to be affected by the changing alveolar pressure.

Not only are my data inconsistent with Albert's but also when I tried to do experiments exactly as he did, I could not reproduce his results. I detected considerable bronchial blood flow into the perfusion system during the experiment. It may be that his results are in some way explained by bronchial blood flow in his preparation. Studies should be done in which the bronchial blood supply to the lobe being weighed is eliminated. I expect that under these conditions, there would be no weight gain as long as pleural and vascular pressures remained the same.

In the final part of my dissertation (Series 4) I measured the

concentration of albumin in the hilar interstitial space as a result of hydrostatic edema in Zone I. I studied the individual contributions of the arterial and venous extraalveolar beds and found them to have identical albumin permeabilities. This is the result I would expect, based on my gravimetric studies in Series 1. The method I developed to measure albumin concentration in small (5-25 nl) samples of lung liquid collected by micropuncture from the hilar interstitium could be used to measure any soluble protein for which there is an antibody available.

I found a high albumin concentration in the interstitial fluid, approximately 80 - 85% of the plasma albumin concentration. This value is the same as that found by Parker et al in afferent lymph and similar to values reported in a study of sheep lung lymph collection (20).

These experiments suggest the importance of the extraalveolar vascular bed in normal liquid and solute exchange in the lung. One must be careful when making assumptions about relative contributions of vascular beds to filtration based on surface area alone. There may be longitudinal variations in interstitial pressure that overwhelm the effects of surface area. Many studies remain to be done before we fully understand the factors regulating liquid balance in this region of the lung.

REFERENCES

1. Albert, R.K, S. Lakshminarayan, T. Huang and J. Butler. Fluid leaks from extra-alveolar vessels in living dog lungs. J. Appl. Physiol. 44(5):759-762, 1978.

2. Albert, R.K., S. Lakshminarayan, W. Kirk and J. Butler. Lung inflation can cause pulmonary edema in Zone I of in situ dog lungs. J. Appl. Physiol. 49(5):815-819, 1980.

3. Albert, R.K., S. Lakshminarayan, N.B. Charan, W. Kirk and J. Butler. Extra-alveolar vessel contribution to hydrostatic pulmonary edema in in situ dog lungs. J. Appl. Physiol. 54(4):1010-1017, 1983

4. Bennett, H.S., J.H. Luft, and J.C. Hampton. Morphological classifications of vertebrate blood capillaries. Am. J. Physiol. 196(2):381-390, 1959.

5. Bhattacharya, J., K. Nakahara and N.C. Staub. Effect of edema on pulmonary blood flow in the isolated perfused dog lung lobe. J. Appl. Physiol. 48:444-49, 1980.

6. Bhattacharya, J. and N.C. Staub. Direct measurement of microvascular pressures in the isolated perfused dog lung. Science, 210:327-328, 1980.

7. Bhattacharya, J., M.A. Gropper and A. Eaton. Interstitial fluid pressure measured by micropuncture of isolated, edematous dog lungs at different levels of lung inflation. Physiologist, 26(4):A56, 1983.

8. Bhattacharya, J., M.A. Gropper and N.C. Staub. Interstitial fluid pressure gradient measured by micropuncture in excised dog lung. J. Appl. Physiol. 56(2):271-277, 1984.

9. Bhattacharya, J., M.A. Gropper and J.M. Shepard. Measurement of filtration rate in single lung microvessels by the split-drop technique. Microvasc. Res. 29(2):208, 1985.

10. Bhattacharya, J., M.A. Gropper and J. Shepard. Micropuncture measurement of interstitial pressures in excised dog lungs at different levels of lung inflation and lung edema. Fed. Proc 44(6):1911, 1985

11. Bo, G., A. Hauge and G. Nicolaysen. Alveolar pressure and lung volume as determinants of net transvascular fluid filtration. J. Appl. Physiol. 42(4):476-482, 1977.

12. Bruderman, I., K. Somers, W.K. Hamilton, W.H. Tooley and J. Butler. Effect of surface tension on circulation in the excised lungs of dogs. J. Appl. Physiol. 19:707-714, 1964.

13. Conhaim, R.L., M.A. Gropper and N.C. Staub. Effect of lung inflation on alveolar-airway barrier permeability protein permeability in dog lung. J. Appl. Physiol. 55:1249-1256, 1983.

14. Cottrell, T.S., O.R. Levine, R.M. Senior J. Wieber, D. Spiro and A. Fishman. Electron microscopic alterations at the alveolar level in pulmonary edema. Circ. Res. 21:783-798, 1967.

15. Demling, R.H., M. Manohar and J.A. Will. Relationship between the measured and calculated colloid osmotic pressure of plasma and lung lymph in the sheep. Lymphology, 13:18-23, 1980.

16. Dodek, P.M., T.W. Rice, M.R. Bonsignore, S. Yamada and N.C. Staub. Effects of plasmapheresis and of hypoproteinemia on lung liquid conductance in awake sheep. J. Appl. Physiol. In press, 1985.

17. Drinker, C.K. and E. Hardenbergh. Absorption from the pulmonary alveoli. J. Exp. Med. 86:7-17, 1947.

18. Drake, R., K.A. Gaar and A.E. Taylor. Estimation of the filtration coefficient of pulmonary exchange vessels. Am. J. Physiol. 234(3):H266-H274, 1978.

19. Effros, R.M., G.R. Mason, E. Reid, L. Graham and P. Silverman. Diffusion of labelled water and lipophilic solutes in the lung. Microvasc. Res. 29:45-55, 1983.

20. Erdmann, A.J., K.L. Brigham, W.C. Woolverton and N.C. Staub. Effect of increased vascular pressure on lung fluid balance in unanesthetized sheep. Circ. Res. 37: 271-284, 1975.

21. Fung, Y.C. and S.S. Sobin. Pulmonary alveolar blood flow. Circ. Res. 30(4):470-90, 1972

22. Fung, Y.C., S.S. Sobin, H. Tremer, M.R.T. Yen and H.H. Ho. Patency and compliance of pulmonary veins when airway pressure exceeds blood pressure. J. Appl. Physiol. 54(6):1538-1549, 1983.

23. Gaar, K.A., A.E. Taylor, L.J. Owens and A.C. Guyton. Pulmonary capillary pressure and filtration coefficient in the isolated prefused lung. Am. J. Physiol. 213:910-914, 1967.

24. Gee, M.H. and D.O. Williams. Effect of lung inflation on perivascular cuff fluid volume in isolated dog lung lobes. Microvasc. Res. 17:192-201, 1979.

25. Gil, J. Lung interstitium, vascular and alveolar membranes. In: Lung water and solute exchange. 1978, N.C. Staub, ed. Marcel Dekker Inc., New York, N.Y.

26. Goldberg, H.S., Pulmonary interstitial compliance and microvascular filtration coefficient. Am.J. Physiol. 239:H189-H198, 1980.

27. Grodzinsky, A.J., Electromechanical and physico-chemical properties of connective-tissue. C.R.C. CR Biomed. 9(2):133-199, 1983.

28. Guntheroth, W.G., D.L. Luchtel and I. Kawabori. Pulmonary microcirculation: Tubules rather than sheet and post. J. Appl. Physiol. 53(2): 510-515,1982.

29. Gustaffson, J.E.C. Improved specificity of serum albumin determination and estimation of acute phase reactants by use of the bromcresol green reaction. Clin. Chem. 22:616, 1976.

30. Guyton, A.C., Interstitial fluid pressure: II. Pressure-volume curves of interstitial space. Circ. Res. 16:452-460, 1965.

31. Guyton, A.C., K. Scheel and D. Murphree. Interstitial fluid pressure: III. Its effect on resistance to tissue fluid mobility. Circ. Res. 19:412-419, 1966.

32. Hansen, A.T.H., Osmotic pressure effect of the red blood cells - possible physiological significance. Nature, 190:504-08, 1961.

33. Hayek, H. von. The anastomoses between bronchial and pulmonary arteries. IN: The Human Lung, Hafner, New York, 1960, pp. 282-287.

34. Horsfield, K. Morphometry of the small pulmonary arteries in man. Circ. Res. 42(5):593-597, 1978

35. Horsfield, K., and I. Gordon. Morphometry of pulmonary veins in man. Lung, 159:211-218, 1981.

36. Howell, J.B.L., S. Permutt, D.F. Proctor and R.L. Riley. Effect of inflation of the lung on different parts of the pulmonary vascular bed. J. Appl. Physiol. 16(1):71-76, 1961.

37. Iliff, L.D., Extra-alveolar vessels and edema development in excised dog lungs. Circ. Res. 28:524-532, 1971.

38. Jindal, S.K., S. Lakshminarayan, W. Kirk and J. Butler. Acute increase in anastomotic bronchial blood flow after pulmonary arterial obstruction. J. Appl. Physiol. 57(2):424-428, 1984.

39. Julien, M., R. Vahi, H.K. Chang, N.C. Staub and T. Hakim. Effect of hematocrit on fluid filtration in isolated dog lungs. Fed. Proc. 44: 1904, 1985.

40. Kramer, G.C., B.A. Harms, B.I. Bodai, E.M. Renkin and R.H. Demling. Effects of hypoproteinemia and increased vascular pressure on lung fluid balance in sheep. J. Appl. Physiol. 55(5):1514-1522, 1983.

41. Lai-Fook, S.J. and B. Toporoff. Pressure-volume behavior of perivascular interstitium measured in isolated dog lung. J. Appl. Physiol. 48(6):939-946, 1980.

42. Lai-Fook, S.J. Perivascular interstitial fluid pressure measured by micropipettes in isolated dog lung. J. Appl. Physiol. 52(1):9-15, 1982.

43. Lai-Fook, S.J. and K.C. Beck. Alveolar liquid pressure measured by micropipettes in isolated dog lung. J. Appl. Physiol. 53(3):737-743, 1982.

44. Laurell, C.. Quantitative estimation of proteins by electrophoresis in agarose gel containing antibodies. Anal. Biochem. 15:45-52, 1966.

45. Lunde, P.K. M. and B.A. Waaler. Transvascular fluid balance in the lung. J. Physiol. 205:1-18, 1969

46. Macklin, C.C. Evidences of increase in the capacity of pulmonary arteries and veins of dogs, cats and rabbits during inflation of the freshly excised lung. Rev. Can. Biol. 5:199-232, 1946.

47. Mazzone, R.W., C.M. Durand and J.B. West. Electron microscopy of lung rapidly frozen under controlled physiological conditions. J. Appl. Physiol. 45(2):325-333, 1978.

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

49. Miller, W.S. The Lung. 2nd edition. Charles C. Thomas, Springfield, Il, 1947.

50. Mitzner, W. and J.L. Robotham. Distribution of interstitial compliance and filtration coefficient in canine lung. Lymphology, 12:140-148, 1979.

51. Moriss, A.W., R.E. Drake and J.C. Gabel. Comparison of microvascular filtration characteristics in isolated and intact lungs. J. Appl. Physiol. 48(3):438-443, 1980.

52. Nagasaka, Y., J. Bhattacharya, S. Nanjo, M.A. Gropper and N.C. Staub. Micropuncture measurement of microvascular pressure profile during hypoxia in cats. Circ. Res. 54:90-95, 1984.

53. Nicolaysen, G. and A. Hauge. Determinants of transvascular fluid shifts in Zone I lungs. J. Appl. Physiol. 48(2):256-264, 1980.

54. Nitta, S., T. Ohnuki, K. Ohkuda, T. Nakada and N.C. Staub. The corrected protein equation to estimate plasma colloid osmotic pressure and its development on a nomogram. Tohoku J. Exp. Med. 135:43-49, 1981.

55. Ogston, A.G. and C.F. Phelps. The partition of solutes between buffer solutions containing hyaluronic acid. Biochem. J. 78:827-833, 1961.

56. Oppenheimer, L., H.W. Unruh, C. Skoog and H.S. Goldberg. Transvascular fluid flux measured from intravascular water concentration changes. J. Appl. Physiol. 54(1):64-72, 1983

57. Pappenheimer, J.R. and A. Soto-Rivera. Effective osmotic pressure of the plasma proteins and other quantities associated with the capillary circulation in the hindlimb of cats and dogs. Am. J. Physiol. 152:471-491, 1948.

58. Parker, J.C., A. Guyton and A.E. Taylor. Pulmonary interstitial and capillaru pressures estimated from intraalveolar fluid pressures. J. Appl. Physiol. 44:267-76, 1978.

59. Parker, J.C., J. Ryan and A.E. Taylor. Plasma-lymph albumin kinetics, total lymph flow and tissue hematocrit in normally hydrated dog lungs. Microvasc. Res. 28:254-269, 1984.

60. Pearce, M.L., J. Yamashita and J. Beazell. Measurement of pulmonary edema. Circ. Res. 16:482-488, 1965.

61. Permutt, S., J.B.L. Howell, D.F. Proctor and R.L. Riley. Effect of lung inflation on static pressure-volume characteristics of pulmonary vessels. J. Appl. Physiol. 16(1):64-70, 1961.

62. Reid, L. Structural and functional reappraisal of the pulmonary artery system. Sci. Basis Med. Annu. Rev. 1:289-307, 1968.

63. Roos, P.J., J.P. Wiener-Kronish, K.H. Albertine and N.C. Staub. Removal of sources of caudal mediastinal node lymph in anesthetized sheep. J. Appl. Physiol. 55(3):996-1001, 1983.

64. Rous, P., H.P. Gilding, and F. Smith. The gradient of vascular permeability. J. Exp. Med. 51:807-830, 1930.

65. Shepard, J.M., M.A. Gropper, N.C. Staub and J. Bhattacharya. Micropuncture measurement of lung microvascular pressures in open thorax anesthetized dog. Fed. Proc. 44:1759, 1985.

66. Snashall, P.D., K. Nakahara and N.C. Staub. Estimation of perimicrovascular fluid pressure in isolated perfused dog lung lobes. J. Appl. Physiol. 46(5): 1003-1010, 1979.

67. Snashall, P.D., S.J. Keyes, B.M. Morgan and K.F. Chung. Pulmonary interstitial compliance: A function of the osmotic constituents of the interstitium. J. Appl. Physiol. 53(2):324-329, 1982.

68. Starling, E.H. On the absorption of fluids from the connective tissue spaces. J. Physiol. (Lond.), 19:312-326, 1896.

69. Staub, N.C., Effects of alveolar surface tension on the pulmonary vascular bed. Jpn. Heart J. 7:386-399, 1966.

70. Staub, N.C., H. Nagano and M.L. Pearce. Pulmonary edema in dogs, especially the sequence of fluid accumulation in lungs. J. Appl. Physiol. 22(2): 227-240, 1967.

71. Staub, N.C., Steady state pulmonary transvascular water filtration in unanesthetized sheep. Circ. Res. 28/29(Suppl. 1):135-139, 1971.

72. Staub, N.C., Pulmonary Edema. Physiol. Rev. 54(3):678-811, 1974.

73. Staub, N.C., R.D. Bland, K.L. Brigham, R.H. Demling and A.J. Erdmann III., Preparation of chronic lung lymph fistulas in sheep. J. Surg. Res. 19: 315-320, 1975.

74. Staub, N.C., Pathopohysiology of pulmonary edema. In: Edema. N.C. Staub and A.E. Taylor eds. pp. 719-746, Raven Press, New York, NY, 1984.

75. Taylor, A.E., Capillary fluid filtration, Starling forces and lymph flow. Circ. Res. 49(3):557-575, 1981.

76. Unruh, H.W., H.S. Goldberg and L. Oppenheimer. Pulmonary interstitial compartments and tissue resistance to fluid flux. J. Appl. Physiol. 57(5):1512-1519, 1984.

77. Vreim, C.E., P.D. Snashall, R.H. Demling and N.C. Staub. Lung lymph and free interstitial fluid protein concentration in sheep with edema. J. Appl. Physiol. 230(6):1650-1653, 1976.

78. Weibel, E.R., Morphological basis of alveolar capillary gas exchange. Physiol. Rev. 53(2):419-484, 1973.

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

80. Wiederhielm, C.A., and B.V. Weston. Microvascular, lymphatic and tissue pressures in the unanesthetized mammal. J. Appl. Physiol. 225(4):992-996, 1973.

81. Yamada, S., M.K. Grady and N.C. Staub. Measured osmotic pressure of albumin and non-albumin fractions of sheep plasma. Fed Proc. 44:1909, 1985.

APPENDIX

Extravascular lung water determination

To determine the extravascular lung water content in the lungs in series 3 and 4, I used the method of Pearce et al, as modified by Selinger et al.

The lungs are homogenized and weighed. A sample was taken, weighed and weighed again after drying to constant weight. A portion of the homogenate is centrifuged at 30000 x G for one hour. A sample of the supernatant is weighed and then weighed again after drying to constant weight. The supernatant is assayed for hemoglobin concentration by the cyanmethemoglobin method. Blood was collected at the beginning of the experiment for control blood water and hemoglobin determination. Another sample was taken at the end of the experiment. The blood was also weighed before and after drying to constant weight.

The calculation of lung water content was as follows:

(Hb) = hemoglobin concentration, b = blood, h = homogenate, s = supernatant
Qh = volume of homogenate
Qs = volume of supernatant
Db = density of blood
Fw = fraction of water, b = blood, h = homogenate, s = supernatant

A) Volume of blood in the lung, Qb:

assume that the density of blood/density of supernatant = 1.0385

$$Qb = \frac{(Hb)s}{(Hb)b} \times \frac{Qh}{Dh} \times Db$$

$$(Hb)h = (Hb)s \times \frac{Fwh}{Fws} \times \frac{Dh}{Ds}$$

$$Qb = \frac{(Hb)s \times \frac{Fwh}{Fws} \times \frac{Dh}{Ds}}{(Hb)b} \times \frac{Qh}{Dh} \times Db$$

simplifying,

$$Qb = \frac{(Hb)s}{(Hb)b} \times \frac{Fwh}{Fws} \times Qh \times \frac{Db}{Ds}$$

B) Volume of extravascular lung water, Qwl:

$$Qwl = (Qh \times Fwh) - (Qb \times Fwb)$$

C) Blood-free dry weight of lung, dQl:

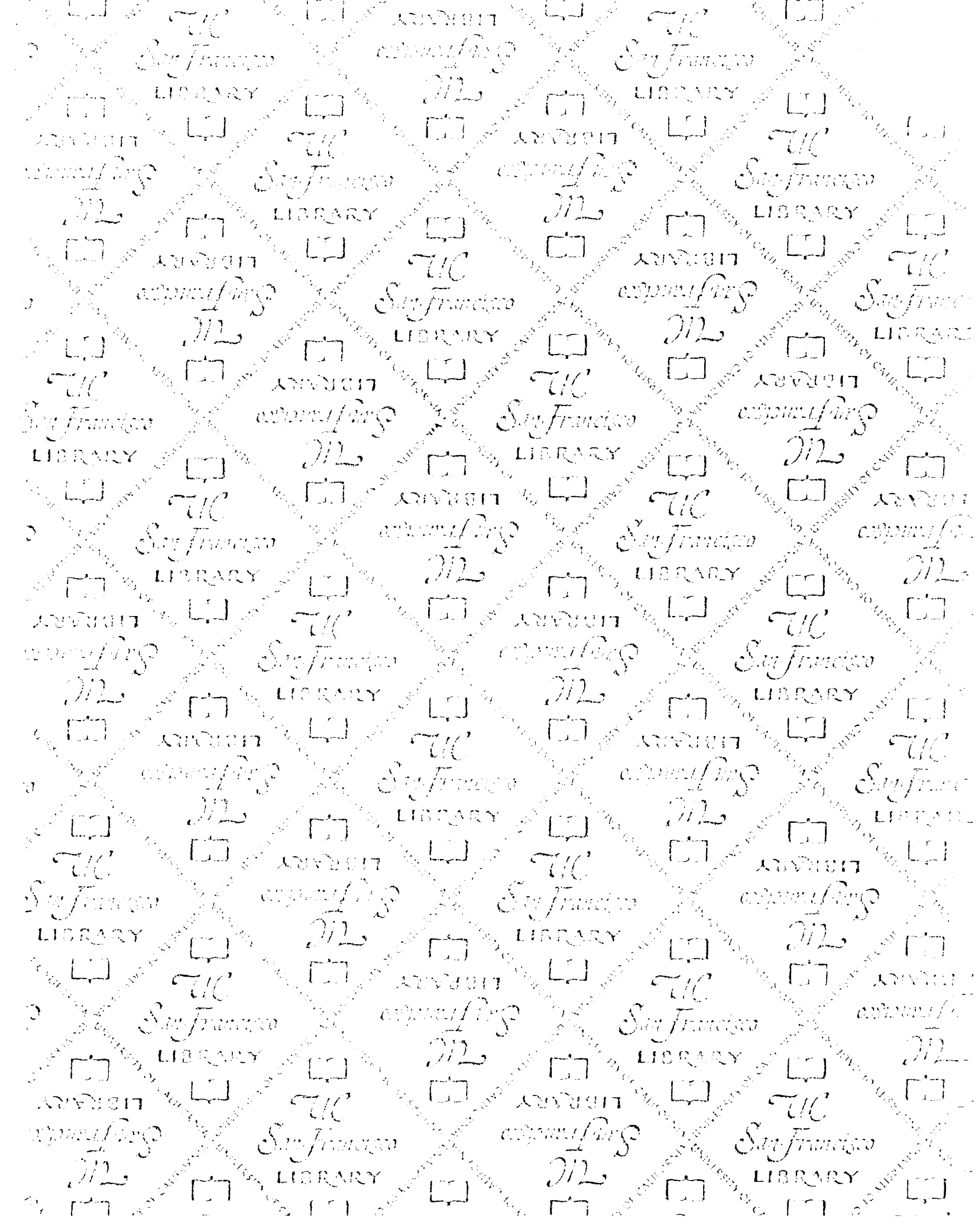
$$dQl = Qh - Qwl - Qb$$

The lung water measurement is expressed as $\frac{Qwl}{dQl}$ (g/g dry).

References:

Pearce, M.L., J. Yamashita and J. Beazell. Measurement of pulmonary edema. Circ. Res. 16: 482-488, 1965.

Selinger, S.I., R.D. Bland, R.H. Demling and N.C. Staub. Distribution volumes of albumin, sucrose and chloride in sheep lung. J. Appl. Physiol. 39(5): 773-779, 1975.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