THE UNIVERSITY OF MANITOBA

TRANSVASCULAR FLUID FLUX IN THE LUNG

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ABSTRACT

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Studies of pulmonary transvascular fluid flux have suggested that, in a standard isolated perfused canine left lower lobe preparation, the microvascular membrane may be unusually permeable resulting in overestimations of the microvascular filtration coefficient (K_f) and production of edema at abnormally low capillary pressures. In Part I, the stepwise pressure elevation technique was used to study the relationship between constant weight gain (Q_f) and microvascular pressure (P_c) in eight isolated perfused canine left lower lobes. Lobar conductance to filtration (K_f), obtained from the slope of this relationship, was 0.0022 ± 0.0003 $ml \cdot min^{-1} \cdot cmH_2O^{-1} \cdot g$ dry wt^{-1}. The intercept when $Q_f = 0$, representing the critical capillary pressure to filtration (Pcrit), was 9.53 ± 1.18 cmH_2O. At capillary pressures greater than 9.53 cmH_2O, isogravimetric conditions were never achieved. In 12 additional experiments, the perfusate's colloid osmotic pressure (II) was increased with albumin from 12 to 37 mmHg. On the average, Pcrit increased from 12.2 to 23 cmH_2O. Using the changes in Pcrit and II, the microvascular drag reflection coefficient for albumin (σ_d) was estimated to be 0.67. After the addition of albumin, capillary pressures less than Pcrit induced constant weight loss along the same Q_f-to-P_c relationship. The only

point at which the lobe attained an isogravimetric state was at Pcrit. To control for time, five additional lobes were observed at constant P_c for 100-180 minutes. Slight acceleration of the rate of weight gain occurred after lobar weight increased by 30-40%. The low K_f and high σ_d suggest that the microvasculature is well preserved during the time course of these experiments. The low Pcrit may reflect obliteration of lymphatic channels.

It is generally assumed that, following an increase in capillary pressure, the initial rapid weight gain represents primarily vascular volume changes and is followed by a slow exponential weight change representing transvascular flux. However, vascular volume changes may persist for longer periods influencing measurements of transvascular fluid exchange and K_f. In Part II, a laser colorimetric device was utilized to directly measure fluid exchange using continuous measurements of hematocrit. In 12 isolated excised canine left lower lobes, transvascular volume exchange (V_E) determined from continuous colorimetric measurements correlated well with simultaneous calculations of V_E obtained from changes in microhematocrit. Following increases in P_c, weight signals showed an initial rapid weight gain followed by a slow exponential time course whereas the calculated V_E using hematocrit changed linearly. V_E reflected about 60% of the slow weight gain. When P_c was decreased, weight signals

decreased exponentially, whereas V_E continued to increase linearly but at a slower rate. These results suggest that a significant component of the slow weight signal represents slow vascular volume changes. Contrary to what the weight signal suggested, edema was never reabsorbed over the range of P_c measured.

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LIST OF ABBREVIATIONS

Q_f	weight change (grams/minute)
K_f	microvascular filtration coefficient; lobar conductance to filtration
P_c	capillary hydrostatic pressure
P_t	tissue hydrostatic pressure
σ_d	osmotic reflection coefficient
II_c	capillary oncotic pressure
II_t	tissue oncotic pressure
P_a	arterial pressure
P_v	venous pressure
P_A	alveolar pressure
V_E	transvascular volume exchange
Hct	hematocrit
V_{Fmhct}	filtered volumes determined from microhematocrit
V_{Fclmtr}	filtered volumes determined from colorimetric measurements of hematocrit

REVIEW OF THE LITERATURE

FLUID FILTRATION

The filtration of fluid across any membrane is governed by a balance of forces and capillary membrane permeability according to the Starling equation:

$$Q_f = K_f (P_c - P_t - \sigma(II_c - II_t)) \quad (1)$$

where Q_f is transvascular fluid flux, P_c and P_t the capillary and tissue hydrostatic forces, respectively, II_c and II_t capillary and tissue oncotic forces, respectively and σ the capillary membrane reflection coefficient to protein. K_f is the lobar conductance to filtration and is a measure of the rate at which fluid filters across a membrane for a given imbalance of hydrostatic and oncotic forces.

Experimental techniques used to study fluid filtration involve increasing perfusion pressure and measuring the resulting rate of organ weight gain. A step increase in pulmonary capillary hydrostatic pressure (P_c) produces a biphasic weight time course. The initial rapid increase in weight is presumed to represent vascular and extravascular volume changes while the slow exponential time course is assumed to represent transvascular fluid flux (Q_f) exclusively (Drake et. al., 1978; Pappenheimer and Soto-Rivera, 1948; Guyton and Lindsey, 1959). The

exponential character of the slow phase of weight change is attributed to readjustment of Starling's forces across the microvascular membrane as edema fluid accumulates in the pulmonary tissues. When a new balance of forces is achieved, edema accumulation stops and the preparation becomes isogravimetric (no change in weight with time). With further increases in P_c, the lung fails to return to an isogravimetric state and continues to gain weight at a constant rate. Constant Q_f is assumed to represent an imbalance in Starling's forces that can no longer be counteracted because edema fluid would not only exchange with the perimicrovascular interstitium but also with distant compartments around major hilar structures and, at some point, the alveolar space (Drake and Gabel, 1981; Drake et. al., 1980).

MEASUREMENTS OF LOBAR CONDUCTANCE (K_f)

Ideally, precise measurement of the pulmonary filtration coefficient would involve direct measurement of all other factors in the Starling equation. However, tissue forces have been difficult to measure and, thus, indirect measurements using changes in organ weight resulting from a change in perfusion pressure have been utilized. In addition, the biphasic time course of weight gain following a step increase in pulmonary hydrostatic

pressure creates difficulties in separating changes in weight due to vascular volume and those due to fluid filtration alone. To avoid the initial contribution of vascular volume changes to the weight gain curve, Drake et. al. (1978) have applied an extrapolation technique to estimate the filtration coefficient (weight transient technique). Assuming that vascular volume changes are complete within the first few minutes following a change in capillary pressure, the logarithm of the rate of lung weight gain ($\Delta wt/\Delta t$) during the slow component of the curve can be plotted as a function of time. The initial rate of fluid filtration ($\Delta wt/\Delta t, t=0$) is estimated by extrapolating to the time the capillary pressure was changed ($t=0$). At this point, the total pressure gradient acting across the microvascular membrane is ΔP_c. The filtration coefficient is given by the following equation:

$$K_f = (\Delta wt/\Delta t)_{t=0} / \Delta P_c \qquad (2)$$

Thus, the rate of fluid flux may be determined before changes in tissue hydrostatic pressure and oncotic forces have had time to adjust to prevent further filtration. However, it is difficult to determine when the vascular component no longer contributes to the weight gain curve and measurements of K_f vary according to which portion of

the weight gain curve is measured after an increase in capillary pressure (Drake et. al., 1978). If flux is measured too soon after an increase in P_c, part of the weight gain will reflect vascular volume distension and K_f will be overestimated but, if measured too late, changes in P_t and II_t will have occurred to oppose further edema formation, resulting in underestimates of K_f.

Drake, Smith and Gabel (1980) developed a stepwise vascular pressure elevation technique to gravimetrically measure K_f in intact dog lung and this technique has been used successfully in the isolated lung (Ehrhart et. al., 1984; Drake and Gabel, 1981; Morriss et. al., 1980). By elevating capillary pressure in small steps, changes in lymph flow, P_t and II are minimized since, for a constant P_c, the rate of weight gain is constant if P_t, II_t and lymph flow are also constant. As rate of weight gain is constant once P_c exceeds a critical capillary pressure, K_f can be measured from the slope of the line relating rate of weight gain to capillary pressure. This represents total lobar conductance to fluid exchange through the microvascular membrane, interstitium and alveolar membrane (Drake et. al., 1980; Drake et. al., 1981). Rate of weight gain often increases once lobar weight is 40-50% of normal necessitating measurement of K_f from the Q_f-to-P_c relationship before the lobe gains 50% of its initial

weight (Drake et. al., 1980). Drake and Gabel (1981) have shown that the extrapolation technique gives significantly higher values of K_f as compared to the stepwise pressure elevation technique. Comparison of intact and isolated lobes has shown that K_f is three-fold higher in isolated lobes suggesting that the microvascular membrane is more permeable in this preparation (Morriss et. al., 1980). Other investigators have challenged this concept as similar measurements of K_f produced values in isolated preparations within the range reported for intact lobes (Gaar et. al., 1967a, 1967b; Ehrhart et. al., 1984).

CRITICAL CAPILLARY PRESSURE (Pcrit)

In an isogravimetric state, lung preparations neither gain nor lose weight. However, elevation of capillary pressure causes the lobe to gain weight until Starling's forces have rebalanced. At some point, a P_c is reached at which the lobe can no longer achieve an isogravimetric state and lung weight increases continuously (Guyton and Lindsey, 1959; Gaar et. al., 1967a). The maximum capillary pressure at which the lung weight can be maintained constant is defined as the critical capillary pressure (Pcrit). This critical P_c has been shown to vary linearly with capillary oncotic pressure (II_c) (Drake et. al., 1980). Pcrit may be determined from stepwise pressure

elevation measurements of rate of weight gain by extrapolating the slope of the line relating rate of weight gain to P_c back to the pressure intercept where rate of weight gain is zero (Drake et. al., 1980). This pressure is assumed to represent the P_c at which Starling's forces are overwhelmed and edema fluid can no longer be contained in the interstitium (Drake et. al., 1980; Drake et. al., 1981). Measurements of Pcrit have shown that the critical capillary pressure is much lower in isolated lobes as compared to intact lobes suggesting that damage to the microvascular membrane may occur during the isolation technique (Morriss et. al., 1980; Ehrhart et. al., 1984).

OSMOTIC REFLECTION COEFFICIENT (σ)

The osmotic reflection coefficient measures the selectivity of the pulmonary microvascular membrane to the passage of plasma proteins. Since the microvascular membrane contains pores that allow the passage of some plasma proteins into the interstitial space, measurements of σ range between 0 (which reflects a membrane that is freely permeable to protein) and 1 (a perfect semipermeable membrane which restricts the passage of protein allowing only filtration of fluid). Consequently, only a portion of the osmotic gradient ($II_c - II_t$)

contributes to transvascular fluid flux. σ has been used as a measure of microvascular membrane permeability (Drake and Laine, 1988).

VASCULAR VOLUME CHANGES

In measurements of transvascular fluid flux, it is generally assumed that the initial rapid increase in organ weight following a step increase in capillary pressure represents primarily vascular volume changes. In determining the microvascular filtration coefficient, most investigators assume that the vascular changes are complete within the first 3-4 minutes (Drake and Gabel, 1981, Drake et. al., 1978). However, as the rapid component of the weight gain curve has been observed to continue for up to 20 minutes (Drake et. al., 1978; Drake and Gabel, 1980; Drake et. al., 1980; Lee and Lee, 1979; Chang et. al., 1979), this assumption may not be entirely valid. Prolonged blood volume expansion due to vessel distension or recruitment would influence estimates of transvascular fluid exchange and K_f.

Since measurements of transvascular flux using changes in organ weight alone may lead to erroneous conclusions, investigators have searched for alternate methods to quantitate volumes of filtered fluid. Based on the knowledge that hematocrit and protein concentration

increase as water moves from the capillaries into the interstitium, Drake et. al. (1978) calculated filtered volumes utilizing hematocrit and protein concentration changes following an increase in P_c and compared the results to filtered volumes calculated from weight alone. They found that the different methods of calculating filtered volumes were not statistically different.

Other investigators have utilized ^{51}Cr-labelled red cells to measure the contribution of vascular volume changes to the weight gain curve (Lee and Lee, 1979; Lunde and Waaler, 1968). However, the sensitivity of these experiments did not allow conclusive evidence of prolonged vascular volume expansion. Chang et. al. (1979), using dextran blue as a vascular indicator, demonstrated that pulmonary vascular volume (PVV) increased for 15 minutes following an increase in P_c and similar slow declines in PVV were observed following a return of P_c to control level. Oppenheimer et. al. (1983, 1987) have developed a colorimetric device which measures flux directly using intravascular dyes. The colorimetric data in plasma perfused lobes suggested that, with changes in P_c, an early phase of fluid exchange with the perimicrovascular tissues was followed by a second phase of redistribution within the interstitium (Unruh et. al., 1984).

STATEMENT OF THE PROBLEM

Measurement of the microvascular filtration coefficient using gravimetric techniques is well established. However, several areas warrant further study. Firstly, values of K_f appear to vary according to the preparation used. Although similar values of K_f have been obtained in isolated lobe preparations as compared to intact lobes, there may be a three-fold increase in K_f in some isolated lobes. As well, extrapolated values of Pcrit reported for isolated excised lobes are lower than the range of P_c used for most weight transient techniques with lobes in Zone III of West. These results suggest that the integrity of the microvascular membrane may be altered during the isolation procedure affecting subsequent measurements of fluid and protein filtration.

In part I, the stepwise pressure elevation technique was used to study the relationship between rate of constant weight gain (Q_f) and microvascular pressure (P_c) in eight isolated canine left lower lobes with particular attention to the range of pressures close to Pcrit. Continuous weight determinations were obtained at 10 times the sensitivity of previous reports to enable measurements of very small rates of constant weight gain. To ensure that straight line extrapolations in Pcrit were valid, in 12 additional experiments, the relationship of rate of

weight gain to P_c was examined by increasing the colloid osmotic pressure in order to obtain Pcrit within the range of pressures tested and still maintain Zone III conditions within the lung (West et. al., 1964). In addition, the change in Pcrit produced was used to calculate the osmotic reflection coefficient (σ) of the microvascular membrane. Other factors that may influence Q_f include time and lobar hydration. Deterioration of the lobe with time may result in increasing permeability of the microvascular membrane while transvascular transport of protein may gradually decrease the colloid osmotic gradient. Increasing rates of weight gain have been observed once the lobe gains 50% of initial weight suggesting a more permeable membrane. The effects of time and hydration were evaluated in five control lobes (group 3) by maintaining each lobe at a constant P_c for 100-180 minutes and measuring rates of weight gain at several intervals.

Another area of continuing controversy is the effect of vascular volume changes on measurements of the rate of weight gain following a change in capillary pressure. The possibility exists that ongoing slow vascular volume changes may continue for some time thus influencing measurements of fluid filtration. In part II, laser colorimetry was used to separate vascular volume changes from ongoing transvascular fluid exchange. In 12 isolated

lobe preparations, transvascular fluid exchange was measured directly by following on-line changes in hematocrit in the perfusate. These measurements were correlated with microhematocrit determinations obtained at intervals throughout the experiments. The volume of fluid exchange calculated from laser hematocrits and microhematocrits were compared to the measured lobar weight change during four pressure maneuvers.

METHODS

ISOLATED LOBE PREPARATION

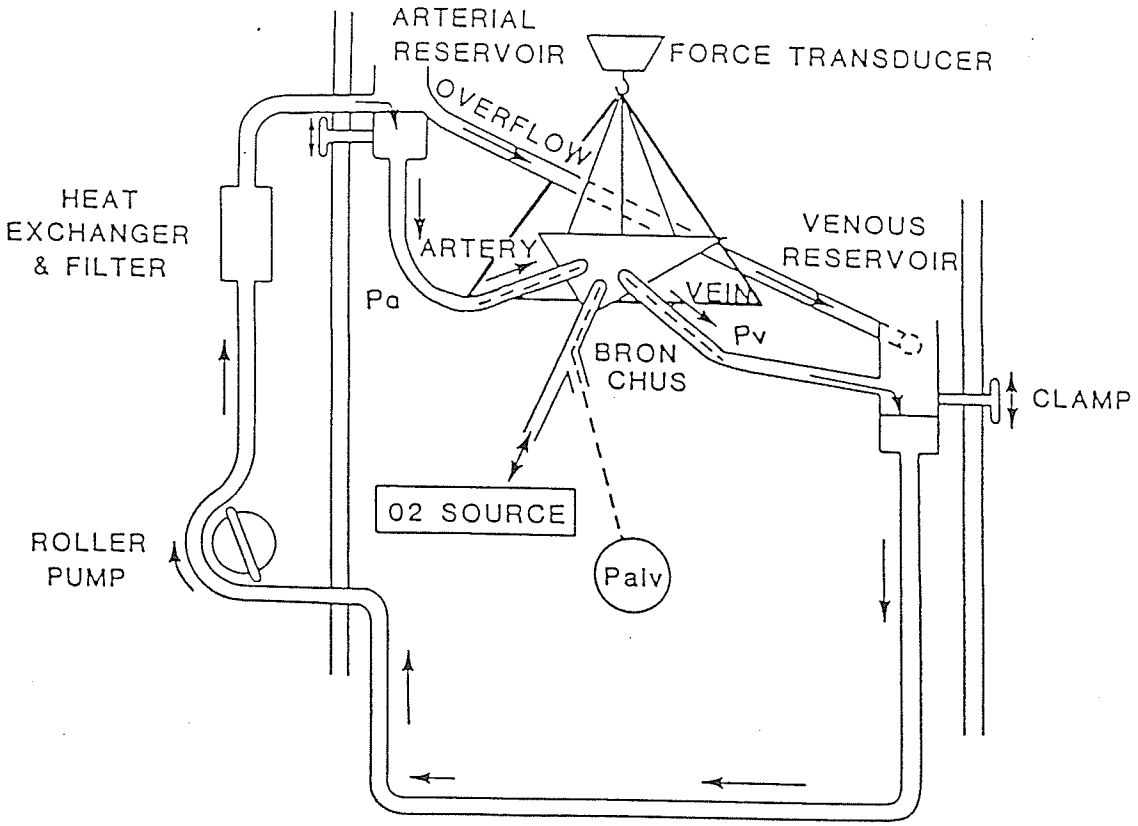
Adult mongrel dogs of either sex weighing 16-38 kg were anesthetized with pentobarbital sodium (30 mg/kg,iv), intubated and ventilated with 100% oxygen on a Harvard respirator (model 560) set at a tidal volume of 10-15 ml/kg and 12 breaths/minute. With the dog placed in the right lateral decubitus position, paralysis was induced with intravenous succinylcholine (20 mg) and the chest was entered through a thoracotomy in the fifth intercostal space. Once the chest was entered, positive end-expiratory pressure at 5 cmH_2O was added. The animal was given heparin sodium (500 U/kg) intravenously and, when fully anticoagulated, a cannula was inserted and tied into the artery of the left lower lobe taking care not to admit air bubbles into the circulation. The cannula was allowed to fill in a retrograde manner from the lobe and then clamped. The pericardium was incised and a second cannula was inserted into the left atrium, secured temporarily, allowed to fill with blood from the atrium, and clamped. The dog was quickly exsanguinated through a femoral artery cannula and the blood saved to perfuse the system. The venous cannula was advanced into the left lower lobe vein and securely tied in place. The left lower lobe bronchus was then exposed, incised and a cannula was inserted and tied into the airway. The lobe was carefully excised,

perfused under low pressure with autologous blood and inflated to remove areas of atelectasis. Three circular foam pads, 3 cm in diameter, each with a string tied to the center, were glued onto the surface of the inflated lobe with cyanoacrylate adhesive. To prevent sagging of the lobar hilum with time, a light metal frame was constructed with a net at its base. The lobes were tied to the apex of the frame using the pad strings and the hilar structures were directed through an opening in the net. The cannulas were secured to the frame to prevent tension. The frame was suspended from a Statham force transducer with the hilum of the lobe in a dependent position. The force transducer was connected to a Validyne CD-19 carrier demodulator and continuous weight signals were amplified, filtered and displayed on a Brush Gould 260 oscillographic recorder such that a change of 1 gram resulted in a 25 division deflection on the chart paper. A combination of suspension with adhesive pads and support on the netting with the inflow and outflow cannulas tied to the structure resulted in a stable isolated lobe preparation using highly sensitive weight measurements. The lobe was statically inflated with 100% O_2 at a transpulmonary pressure of 5 cmH_2O. The arterial and venous cannulas were connected to constant level reservoirs of adjustable height. Blood from the venous reservoir was pumped

(Masterflex Digistaltic pump) through a heat exchanger at 38°C and filtered through a 20 um high capacity transfusion filter (MC2131, Fenwal Laboratories of Canada). An overflow tube from the arterial to venous reservoirs kept the arterial level constant. Small catheters advanced just beyond the arterial and venous cannula tips were connected to Validyne MC1-10 pressure transducers by small fluid-filled catheters for inflow (P_a) and outflow (P_v) pressure measurements. These were zero referenced to the hilum of the lobe and the signals displayed on the oscillographic recorder. The system was enclosed in a 15 ft^3 plastic bag containing warm humidified air (Figure 1).

Perfusion was established under Zone III conditions (on the average, P_a = 16, P_v = 12, and alveolar pressure (P_A) = 5 cmH_2O). In an effort to recruit lobar vessels, the reservoirs were raised to a P_c of 25 cmH_2O for approximately 2 minutes. The lobe was inflated to P_A = 20 cmH_2O three times and allowed to stabilize for approximately 30 minutes. After this time, lobes consistently appeared to be in isogravimetric conditions at the sensitivity used in most isolated lobe preparations (1 gram = 2.5 chart paper divisions) but were always observed to be gaining weight at a constant, although very slow, rate when the sensitivity was increased 10-fold.

FIGURE 1. Isolated lobe preparation.



I. STEPWISE PRESSURE ELEVATION

The lobes were prepared as described above. After the period of stabilization, the experiments were conducted as follows:

GROUP 1. STEPWISE PRESSURE ELEVATIONS. In eight isolated lobe preparations, P_a and P_v, starting at P_a = 15.6 ± 0.3 and P_v = 12.6 ± 0.5 cmH_2O, were elevated by equal amounts in steps of approximately 2 cmH_2O. Weight was continuously recorded and when a constant rate of weight gain was established (approximately 15-20 minutes), P_c was obtained by the double occlusion technique (Dawson et. al., 1982; Townsley et. al., 1986). All data obtained during the exponential phase of weight gain were excluded. Determinations were made over a range of P_c between 12.5 and 25.5 cmH_2O.

GROUP 2. INCREASED COLLOID OSMOTIC PRESSURE. In 12 lobes, rates of constant weight gain were determined at three capillary pressures. Thereafter, the colloid osmotic pressure (II) of the perfusate was increased with 100 ml of 25% human serum albumin (Cutter Laboratories) over approximately 20 minutes. After the lobe was allowed to restabilize, rates of constant weight gain were again measured at three capillary pressures. Plasma samples were drawn at base line and at the end of each pressure maneuver for measurements of total serum protein by

refractometry (Atago) and II using a Wescor 4400 colloid osmometer.

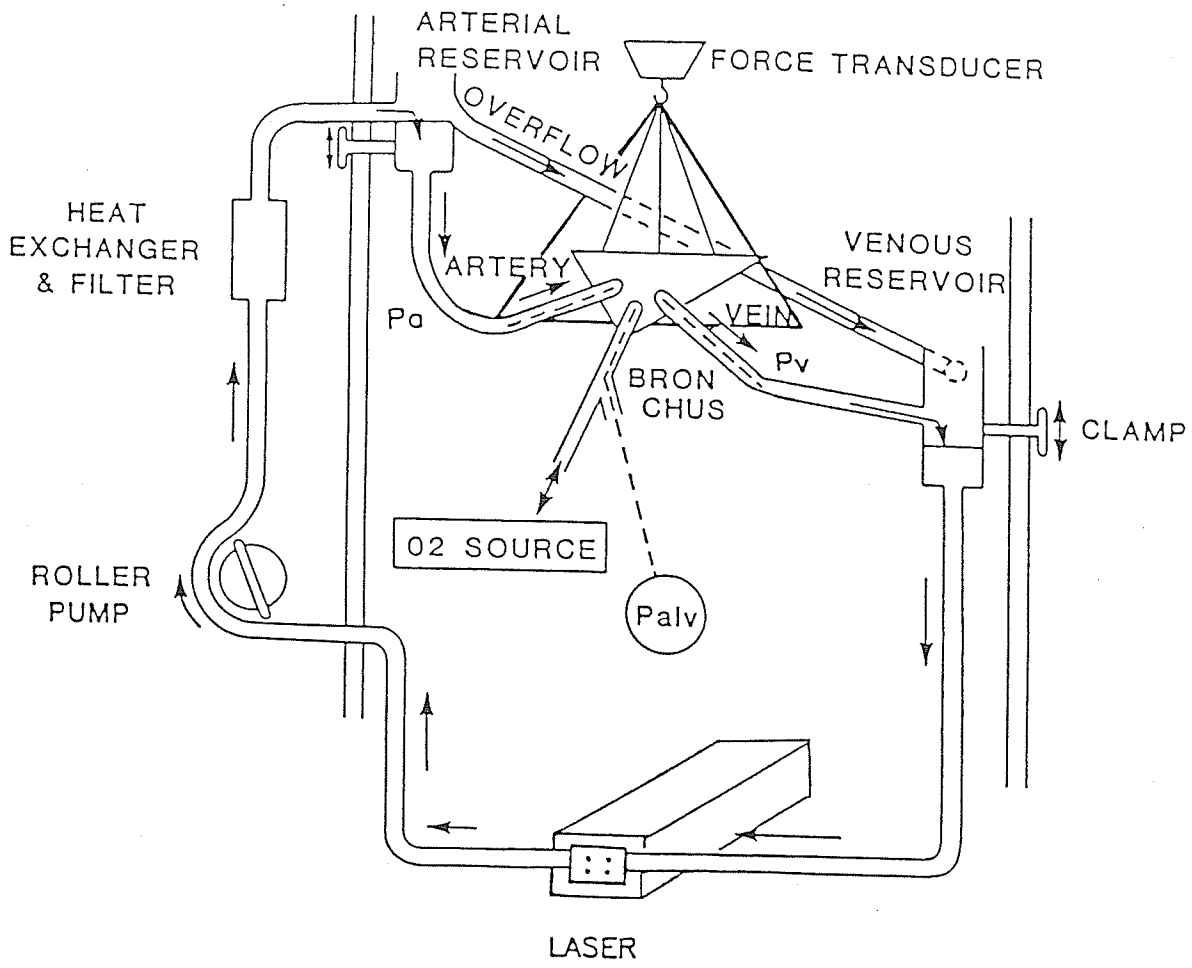
GROUP 3. CONTROLS. In five isolated perfused lobes, after the period of stabilization, P_c was set somewhere between 14 and 24 cmH_2O (determined by double occlusion) and kept constant for the duration of the experiment. Rates of weight gain were evaluated at intervals of 5-10 minutes over 100-180 minutes.

All lobes were dried to a constant weight.

II. LASER COLORIMETRY

The lobes were excised, perfused and stabilized as described above. Figure 2 illustrates the isolated lobe preparation with the laser interposed within the circuit for continuous on-line colorimetric measurements of hematocrit. Continuous weight recordings were obtained such that a 1 gram weight change produced a 50 mV deflection. Masterflex 6409-17 tubing was used to minimize hemolysis. To prevent evaporation, the preparation was enclosed in a plastic environment containing warm humidified air and the openings of the reservoirs were covered with latex gloves.

FIGURE 2. Laser colorimetry preparation. The laser is interposed in the circuit before the roller pump to ensure constant flow through the cuvette.



(a) COLORIMETRIC DEVICE: This device is based on the Beer-Lambert law of absorption spectroscopy:

$$I/I_o = 10^{-kcl} \qquad (3)$$

where I_o and I represent the incident and transmitted light intensities, respectively, c the concentration of the substance responsible for the light transmission changes (in this instance, the hematocrit), k the extinction coefficient of the substance, and l the length of the light path through the solution. The device generates a voltage proportional to I/I_o. The laser device is a modification of that described by Oppenheimer et. al. (1987) and consists of a 50 mW continuous wave laser diode (Spectra Diode) which emits light at 815 nm. The laser was interposed in the circuit immediately before the roller pump to ensure constant flow through the cuvette (Figure 2). The cuvette is a black acrylic plastic mount which holds Tygon tubing across the laser beam. To obtain the best signal to noise ratio possible given the power of the laser diode, path length, l, (Equation 3) was set at 0.5 cm (internal diameter of the tubing). Directly across from the incident light, three photodiodes were mounted along the surface of the tubing ensuring detection of the widely scattered beam transmitted through the column of blood.

The photodiodes were connected in parallel to the photodetector circuit as described by Oppenheimer et. al. (1987). The laser diode is provided with a built-in photodiode capable of measuring light fluctuations in the laser diode chamber. This photodiode was connected to a separate photodetector circuit to correct for changes in incident light intensity. The laser diode is also provided with a thermoelectric cooler capable of modifying its operating temperature. Changes in the operating temperature allowed modification of the wavelength of the emitted light such that an isosbestic point was reached (a specific wavelength in which the oxygen saturation of hemoglobin ceased to influence light transmission). To minimize power fluctuations, the laser current supply was connected to an isolation transformer. In addition, small signal drifts related to fluctuations in the incident light were corrected continuously using the signal from the built-in photodiode:

$$V_{c(t)} = V_{D,R} \times (V_{(t)}/V_{D(t)}) \qquad (4)$$

where $V_{c(t)}$ represents the corrected voltage at any time (t), $V_{D,R}$ the reference voltage measured by the laser diode photodetector at the beginning of the experiments, and $V_{(t)}$ and $V_{D(t)}$, the voltages registered in the cuvette

and the laser diode photodetector circuits at any time (t). The validity of this equation was confirmed as follows: the lobe was clamped off to prevent fluid exchange while circulation continued through the overflow tubing. For a constant hematocrit, the current through the laser diode was varied to produce changes in emitted light. The changes in $V_{(t)}$ were of similar magnitude to those observed in actual experiments but, because both $V_{(t)}$ and $V_{D(t)}$ changed in the same proportion, $V_{C(t)}$ remained unchanged. Occasionally, and particularly at the beginning of the experiments, a slow drift unrelated to fluctuations in emitted light or hemoglobin saturation could be detected. This problem was resolved once all possible sources of evaporation were minimized as much as possible.

Light transmission across the cuvette was assumed to be proportional to the perfusate's hematocrit. Because the hematocrit change produced by a unit volume of exchanged fluid is proportional to the total volume in the circuit, the size of the reservoirs and tubing were reduced as much as possible such that the circuit volume was about 400 ml. In addition, both reservoirs were provided with magnetic stirrers to optimize mixing and ensure a uniform hematocrit throughout the circuit. To validate the device, periodic samples of blood were obtained and hematocrit

measured using eight 75 mm long microhematocrit tubes (Fisher) for each determination. The tubes were centrifuged for 5 minutes at 8,000 revolutions per minute in an Autocrit II centrifuge (Clay Adams), and the proportion of red cell to total blood volume determined visually with the help of the built-in hematocrit scale and magnifying glass. The determinations were not corrected for the volume of other cells and plasma that could be trapped between red cells. This volume has been estimated to be 3% of the packed cell volume (Bauer, 1982). Considering that the hematocrit changes during the experiments were less than 5%, this error would be small. Periodic determinations of free hemoglobin were obtained spectrophotometrically (Baush and Lomb, Spectronic 200 UV).

b) PROTOCOL

In order to have measurable light transmission through the blood column in the cuvette (light path 0.5 cm), it was necessary to reduce the perfusate hematocrit to less than 50%. This was accomplished with the dog's own plasma. The roller pump was set at 100 rpm (about 280 ml per minute in animals). Because hemofilters offer significant resistance to the flow of perfusate, the caliber of the tubing used in the pump was decreased to reduce flow to 120 ml/minute. The pulsatile oscillations

in light transmission were electronically filtered.

(i) CALIBRATION OF THE COLORIMETRIC DEVICE: Once a stable, drift free signal was obtained, the device was calibrated as follows:

a) 50 ml of perfusate was drawn and centrifuged to obtain packed cells (approximately 20 ml with a hematocrit of 95%).

b) after measuring the the initial perfusate's hematocrit, the packed cell volume was divided into 4 aliquots of 5 ml. Each aliquot was added sequentially to the perfusate.

c) after each packed cell aliquot had thoroughly mixed with the perfusate, as reflected by a steady colorimetric signal, the voltage produced by the colorimetric device was recorded and 8 small samples of perfusate obtained for microhematocrit determinations. On the average, each aliquot increased hematocrit by 0.8% and produced a decrease in the colorimetric signal of about 250 mV (slightly smaller with each aliquot due to the exponential relationship between hematocrit and light transmission). The signal noise was about 20 mV.

d) the separated plasma (25-30 ml) and excess blood drawn but not used in microhematocrit measurements were returned to the circuit to bring the perfusate's hematocrit back to baseline. Consequently, the

calibrations spanned changes in hematocrit which would result from filtered volumes of 25-30 ml.

e) the logarithms of the voltages ($V_{c(t)}$) produced by the colorimetric device (ordinate) were plotted against hematocrit determinations obtained by averaging the 8 microhematocrit measurements after each packed cell aliquot (abscissa). In each experiment, a least-squares linear best fit regression was drawn through 5 points (initial and after each of 4 aliquots) and used to convert the on-line colorimetric voltage to hematocrit. In each experiment, the correlation between $\log(V_{c(t)})$ and hematocrit determinations was excellent. The r^2 values ranged from 0.9294 to 0.9964, with an average value (±S.D.) of 0.9724 ± 0.0264. Because there was significant variability in the slopes (range -0.0604 to -0.3231 with an average (±S.D.) of -0.1479 ± 0.0919), a calibration curve was constructed for each experiment. The calibration factor was used to calculate on-line hematocrit ($Hct_{(t)}$) at any given time:

$$Hct_{(t)} = \log(V_{c(t)}) - \text{intercept/slope} \qquad (5)$$

f) the volume of perfusate used to fill the circuit and cannulae was carefully recorded. In addition, to account for initial blood volume present in the lobar

vasculature, the circuit volume (V_I) was calculated as follows:

$$V_I = V_s \times (Hct_s - Hct_2)/(Hct_2 - Hct_1) \qquad (6)$$

where V_s and Hct_s represent the volume and Hct of the total packed cell sample, and Hct_1 and Hct_2 the circuit hematocrit before and after the packed cells were added. On the average, V_I was about 10 ml larger than the volume of blood used to fill the circuit.

(ii) CALCULATION OF TRANSVASCULAR EXCHANGE: Voltages proportional to light transmission obtained colorimetrically were converted to hematocrits using the regression described in equation 5. These measurements were used to estimate the volume of fluid exchanged at any given time ($VE_{(t)}$) with a modification of a formula previously used by Drake et. al. (1978):

$$VE_{(t)} = V_I \times (1 - Hct_I/Hct_{(t)}) \qquad (7)$$

where V_I and Hct_I represent the initial circuit volume and hematocrit, respectively, and $Hct_{(t)}$ the hematocrit measured at any given time (t). V_I was obtained using equation 6.

(iii) DETERMINATION OF FREE HEMOGLOBIN: Significant hemolysis, with the release of free hemoglobin, could affect colorimetric determination of weight gain in 2 ways: firstly, the presence of free hemoglobin could change the light transmission for any given hematocrit and secondly, hemolysis could produce a change in hematocrit independent of filtration. To address the first concern, spectrophotometric scans of plasma samples drawn at the beginning and end of 6 experiments were obtained. Whereas there were significant changes in absorbance at wavelengths lower than 600 nm, indicating the presence of some free hemoglobin, absorbance at 815 nm was always zero. Thus, this wavelength was not absorbed significantly by the concentrations of free hemoglobin present in these experiments. To address the second concern, samples of blood were obtained, hematocrit measured and the sample was diluted with water (to produce complete hemolysis) to five predicted hematocrits ranging between 0.5 and 5%. The light absorbance of these samples was measured at a wavelength of 540 nm (Baush & Lomb, Spectronic 200 UV) and calibration curves were obtained by plotting these results against the predicted hematocrits. At the end of the experiments, the light absorbance of initial and final circuit plasma was measured and the degree of hemolysis estimated using these calibration curves. This method

proved very variable. Additional experiments using the cyanomethemoglobin technique to measure free hemoglobin were performed. As described by Wolf et. al. (1987), Drabkin's solution was used to convert hemoglobin into cyanomethemoglobin. Two sets of spectrophotometric calibration curves were constructed using cyanomethemoglobin standards (Fisher Diagnostics) at 420 and 540 nm. Free hemoglobin was measured at 420 nm mixing 200 ul of plasma with 5 ml of reagent. Total perfusate hemoglobin concentration was measured at 540 nm mixing 20 ul of blood with 5 ml of reagent. Since measurements of free hemoglobin were < 0.5% of the hematocrit change over 3 hours, hemolysis was not a significant factor in these experiments.

(iv) PRESSURE MANEUVERS: After the calibration procedure was completed, lobes were inflated transiently to 25 cmH_2O to reduce atelectasis and both reservoirs were raised to 25 cmH_2O for 3 minutes to recruit the lobar vasculature. Thereafter, arterial (P_a), venous (P_v), and airway (P_A) pressures were manipulated to place the entire lobe in Zone III of West (West et. al., 1964). Specifically, P_A = 3 cmH_2O, P_v = P_A + height + 2 cmH_2O and P_a = P_v + 4 cmH_2O. The lobe was allowed to stabilize for 30 minutes. After the period of stabilization was completed, pulmonary capillary hydrostatic pressure (P_c)

was increased by an average of 9 cmH_2O by simultaneously raising both reservoirs for about 40 minutes. Both reservoirs were then returned to baseline for a comparable time (first P_c increase and decrease, respectively). Weight and light transmission signals were measured continuously. This entire procedure was repeated for 30 minute periods (second P_c increase and decrease, respectively). Before and after each pressure change, P_c was measured with the double occlusion method (Dawson et. al., 1982; Townsley et. al., 1986) and samples were drawn for microhematocrit measurements. To rule out the presence of hemorrhage, which could produce weight changes without concomitant changes in hematocrit, the lobes were flushed thoroughly with 1.5 liters of Dextran 70 at the end of each experiment. After flushing, all lobes appeared completely white except for small hemorrhagic areas under the foam pads and around the hilum. They were also incised in multiple sites in an effort to visually detect hemorrhages. This was never the case. In the first 5 experiments, the lobes were homogenized. Spectrophotometric analysis of the homogenate as described above failed to demonstrate any appreciable amounts of hemoglobin, suggesting minimal hemorrhage. Thereafter, absence of hemorrhage was accepted on visual inspection only.

RESULTS

PART I: STEPWISE PRESSURE ELEVATION

Figure 3, page 36 illustrates the results obtained in group 1. For each lobe, the rate of constant weight gain normalized for dry weight ($g \cdot min^{-1} \cdot g$ dry wt^{-1}) was plotted against P_c in cmH_2O. The filtration coefficient (K_f, $ml \cdot min^{-1} \cdot cmH_2O^{-1} \cdot g$ dry wt^{-1}) and Pcrit (cmH_2O) were determined from the slope and the zero weight change intercept of the least-squares linear regression relating constant rate of weight gain to P_c for each lobe. The results are summarized in Table 1, page 37. To facilitate comparison with other studies, K_f and Pcrit are also expressed in $ml \cdot min^{-1} \cdot mmHg^{-1} \cdot 100g^{-1}$ and mmHg, respectively. Mean K_f was 0.0022 ± 0.0003 $ml \cdot min^{-1} \cdot cmH_2O^{-1} \cdot g$ dry wt^{-1} (0.0853 ± 0.0127 $ml \cdot min^{-1} \cdot mmHg^{-1} \cdot 100g^{-1}$) and mean Pcrit was 9.53 ± 1.18 cmH_2O (7.01 ± 0.87 mmHg). The correlation coefficients for the regressions, summarized in column 3, averaged 0.89 ± 0.05.

The data points were pooled and Figure 4 (page 38) illustrates the least-squares linear regression obtained by relating all normalized rates of constant weight gain in group 1 lobes to P_c: Q_f ($ml \cdot min^{-1} \cdot g$ dry wt^{-1}) = 0.002 P_c - 0.021, $r = 0.75$, $df = 69$, $P<0.01$. The calculated K_f and Pcrit from this regression were 0.0021 ± 0.0002 $ml \cdot min^{-1} \cdot cmH_2O^{-1} \cdot gm$ dry wt^{-1} and 10.3 cmH_2O (7.57 mmHg),

respectively, very close to the values obtained by averaging K_f and Pcrit from individual experiments.

The results from the 12 lobes in group 2 are summarized in Figure 5 (page 39). The solid squares represent constant rates of weight gain obtained before II was increased. Three determinations of Q_f at increasing P_c were obtained for each lobe. The open squares represent rates of constant weight gain after II was increased with the infusion of 100 ml of 25% albumin. The solid lines represent least-squares linear regressions relating rates of constant weight change and P_c using all determinations obtained in the 12 experiments. Table 2 (page 40) contains the measurements of K_f, Pcrit, correlation coefficient of the relationships, protein concentration, and II before and after the albumin infusion. Mean K_f increased from 0.0021 ± 0.0006 $ml \cdot min^{-1} \cdot cmH_2O^{-1} \cdot g$ dry wt^{-1} to 0.0046 ± 0.0025 $ml \cdot min^{-1} \cdot cmH_2O^{-1} \cdot g$ dry wt^{-1}. Pcrit increased from 12.21 ± 2.41 to 22.98 ± 1.76 cmH_2O. Total protein increased from a mean of 5.00 ± 0.37 to 9.61 ± 0.58 g/dl, and II increased from 12.03 ± 2.41 to 36.97 ± 5.79 mmHg.

Table 3 (page 41) includes the changes in Pcrit and plasma colloid osmotic pressure (II_p) as measured in the twelve group 2 lobes. This data was used to calculate the membrane's drag reflection coefficient (σ_d) as proposed by Drake and Laine (1988), where

$$\sigma_d = \sqrt{\Delta Pcrit / \Delta II_p} \qquad (8)$$

Assuming that the change in Pcrit would be the consequence of the change in transmural II, then

$$\Delta Pcrit = \sigma_d \Delta (II_p - II_t) \qquad (9)$$

where II_t represents the interstitial colloid osmotic pressure. At rates of filtration induced in these experiments, tissue protein concentration would reflect the composition of the filtrate, hence,

$$II_t = (1-\sigma_d) II_p \qquad (10)$$

Equation 8 is obtained by substituting equation 10 into equation 9. Changes in II_p were measured directly and also calculated ($II_p{}^1$) using the equation derived by Landis and Pappenheimer (1963) relating II to plasma albumin concentration (C_A). It was assumed that the change in C_P responsible for the change in Pcrit was exclusively the result of the albumin infused, hence

$$\Delta II_p = 2.8C_A + 0.18C_A{}^2 + 0.012C_A{}^3 \qquad (11)$$

where C_A is concentration of albumin in gm/100ml and II_p

is plasma oncotic pressure in mmHg. From Table 3, the mean σ_d values were 0.57 ± 0.10 using direct measurements of II_p and 0.66 ± 0.08 using changes in plasma protein concentration.

In Figure 6 (page 42), change in lobar weight as a percent of initial weight is plotted against time for the five control lobes (group 3). The P_c at which the measurements were obtained is indicated next to each set of data. The solid lines represent least-squares linear regressions obtained through the rectilinear portion of the curves as proposed by Drake et. al. (1980). In three experiments, there was a slight acceleration in the rate of weight gain which always occurred after the lobes had gained 30-40% of initial weight. When P_c was held at 24 cmH_2O, the acceleration occurred in approximately 1 hour, when weight had increased by 40%. At lower P_c, lobes remained stable for approximately 2.5 hours.

FIGURE 3. Rate of constant weight gain vs. capillary pressure for each lobe in group 1.

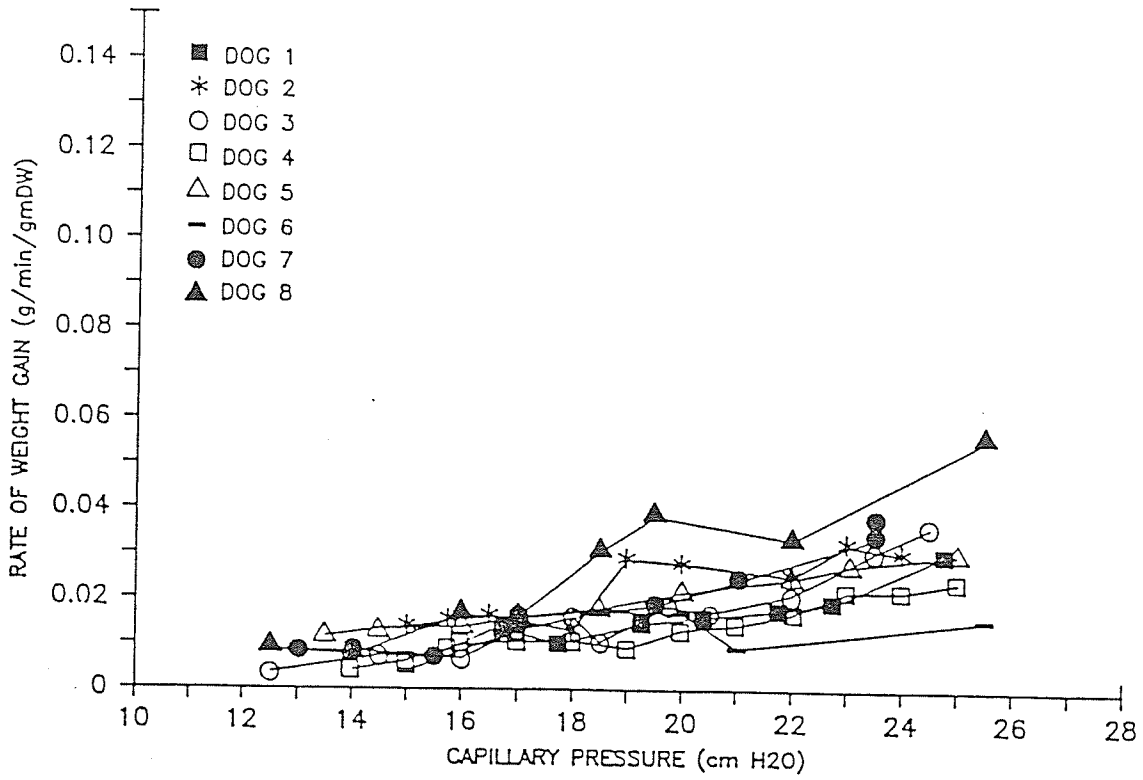


TABLE 1. Summary of K_f and Pcrit determinations.

Dog No.	K_f $ml \cdot min^{-1} \cdot cmH_2O^{-1} \cdot g$ dry wt^{-1}	K_f $ml \cdot min^{-1} \cdot mmHg^{-1} \cdot 100g^{-1}$	r	Pcrit mmHg
1	0.0021	0.1056	0.91	8.88
2	0.0021	0.0670	0.86	6.05
3	0.0025	0.1141	0.94	9.05
4	0.0017	0.0815	0.96	8.29
5	0.0017	0.0507	0.99	5.31
6	0.0007	0.0245	0.56	1.94
7	0.0027	0.1091	0.96	8.33
8	0.0037	0.1296	0.95	8.21
Mean	0.0022	0.0853	0.89	7.01
± SD	±0.0003	±0.0127	±0.05	±0.87
Regression	0.0021 ±0.0002		0.75	7.57

K_f, filtration coefficient; Pcrit, critical capillary pressure. Regression denotes least-squares linear regression relating rate of constant weight gain to capillary pressure in group 1.

FIGURE 4. Least-squares linear regression relating rate of constant weight gain (Q_f) to capillary pressure (P_c) using all measurements obtained in group 1.

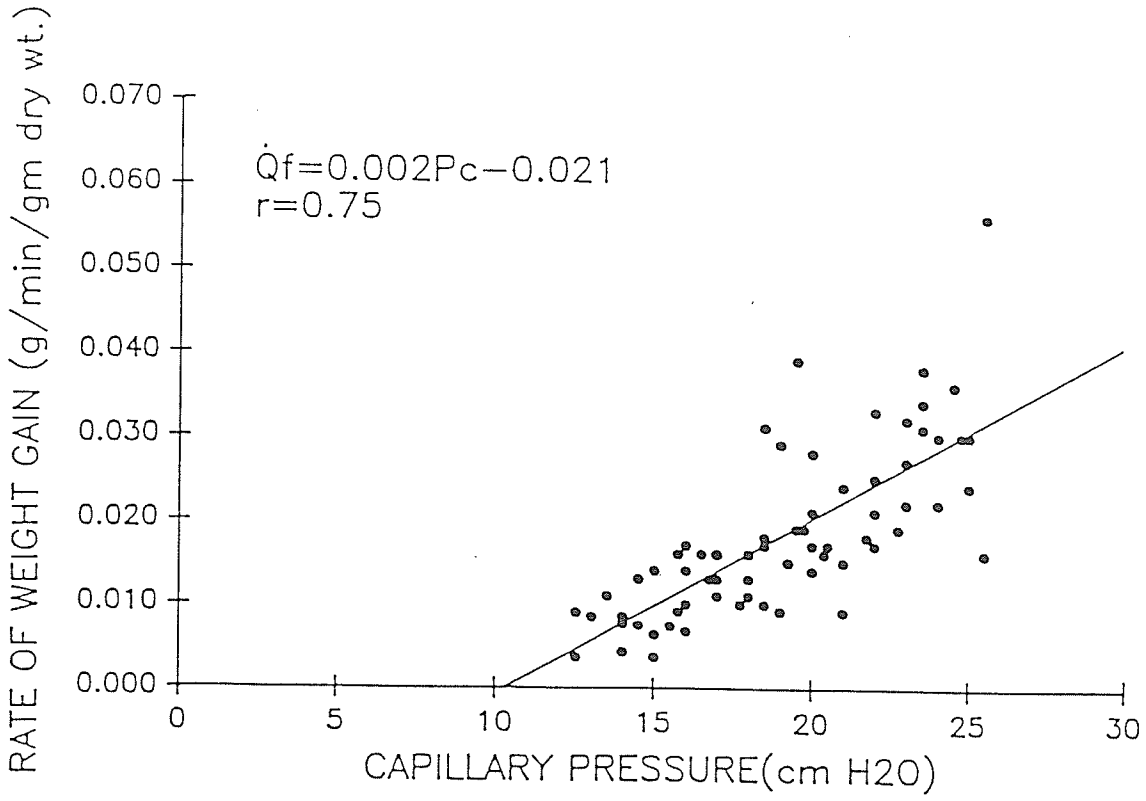


FIGURE 5. Rates of constant weight gain (Q_f) before (solid squares) and after (open squares) addition of albumin vs. capillary pressure (P_c). The solid lines are least-squares linear regressions for each set of data.

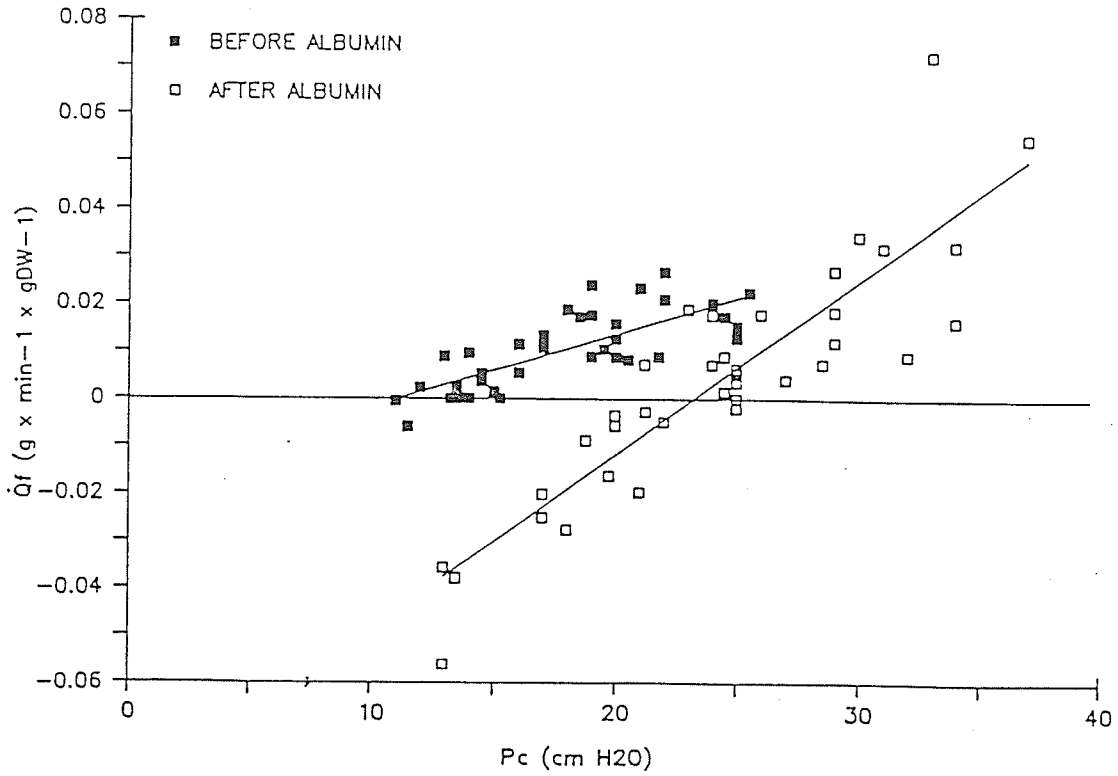


TABLE 2. Summary of group 2 results.

	Before Albumin					After Albumin				
Expt. No.	Kf, ml min-1 cmH2O-1 g dry wt-1	Pcrit, cmH2O	r	Cp, g/dl	Ilp, mmHg	Kf, ml min-1 cmH2O-1 g dry wt-1	Pcrit, cmH2O	r	Cp, g/dl	Ilp, mmHg
1	0.0017	12.55	0.966	5.25	8.0	0.0015	25.71	0.919	10.5	28.5
2	0.0022	14.47	0.999	5.3	11.9	0.0035	22.46	0.946	9.7	32.0
3	0.0025	12.63	0.952	5.1	12.8	0.0039	24.92	0.956	9.7	44.2
4	0.0016	14.29	0.942	5.8	14.9	0.0032	23.93	0.902	9.7	25.7
5	0.0018	13.93	0.994	4.4	12.7	0.0036	21.64	0.992	8.4	34.8
6	0.0015	15.88	0.998	4.8	13.1	0.0018	25.00	0.983	9.3	38.9
7*	0.0027	11.15	0.990	5.0	12.7	0.0076	20.27	0.994	9.2	37.9
8*	0.0033	13.79	0.859	4.5	12.5	0.0108	21.46	0.993	9.1	38.2
9*	0.0007	7.37	0.954	5.2	14.0	0.0038	24.73	0.992	9.4	37.6
10*	0.0017	10.63	0.967	4.8	9.7	0.0057	22.86	0.985	9.6	36.8
11*	0.0020	8.57	0.991	4.7	10.5	0.0053	20.66	0.969	10.2	43.4
12*	0.0240	11.22	0.980	5.2	11.6	0.0039	22.17	0.991	10.5	45.6
Mean	0.0021	12.21	0.969	5.00	12.03	0.0046	22.98	0.967	9.61	36.97
± SD	0.0006	2.41	0.038	0.37	2.41	0.0025	1.76	0.030	0.58	5.79

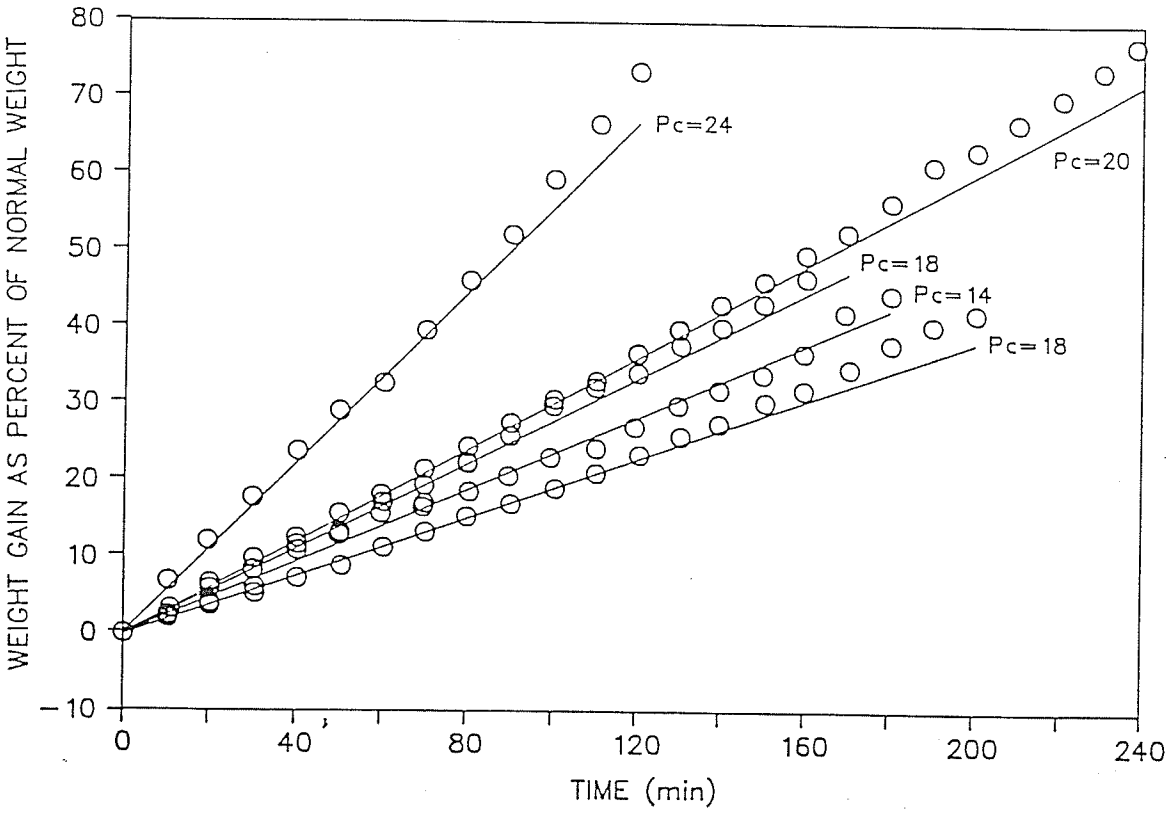
Kf, filtration coefficient; Pcrit, critical capillary pressure; Cp, plasma protein concentration; Ilp, plasma colloid osmotic pressure. * Experiments with constant rates of weight loss.

TABLE 3. Calculation of the reflection coefficient.

Expt. No.	Δ Pcrit, mmHg	Δ II_p, mmHg	Δ $II_p{}^1$, mmHg	σ_d	$\sigma_d{}^1$
1	9.7	20.5	21.4	0.69	0.67
2	5.9	20.1	16.1	0.54	0.59
3	9.0	31.4	17.9	0.53	0.71
4	7.1	10.8	14.4	0.81	0.70
5	5.7	22.1	14.8	0.51	0.62
6	6.7	25.8	17.3	0.51	0.62
7	6.7	25.2	15.8	0.52	0.66
8	5.6	25.7	17.9	0.47	0.56
9	12.8	23.6	15.8	0.74	0.90
10	9.0	27.1	18.9	0.58	0.69
11	8.9	32.9	22.8	0.52	0.62
12	8.0	34.0	21.7	0.49	0.61
Mean	7.9	24.9	17.9	0.57	0.66
± SD	±2.0	±6.1	±2.6	±0.10	±0.08

Pcrit, critical capillary pressure; σ_d, microvascular drag reflection coefficient for albumin; II_p, plasma colloid osmotic pressure; Δ $II_p{}^1$ and $\sigma_d{}^1$, values calculated using changes in protein concentration.

FIGURE 6. Rate of weight gain as percent of initial weight vs. time in controls (group 3). In 3 experiments, there is slight acceleration once weight has increased by 30-40% of initial weight. P_c, capillary pressure.



PART II. LASER COLORIMETRY

Using laser colorimetry, ongoing changes in perfusion hematocrit were followed in 12 isolated perfused canine left lower lobes during four pressure maneuvers. The first increase in P_c, measured by the double occlusion technique, averaged 9.0 ± 4.8 cmH_2O. It was maintained for 40 minutes and then returned to baseline for a similar time (first decrease in P_c). The second increase in P_c averaged 9.3 ± 5.1 cmH_2O. It was maintained for 30 minutes and then returned to baseline for 30 minutes (second P_c decrease).

Figure 7 (page 47) illustrates the time course of weight and colorimetric data from a representative experiment. Weight changes and $V_{E(t)}$ are depicted with squares and dots, respectively. There are two major discrepancies between the weight and colorimetric data:

1) following step changes in P_c, the weight time course follows a biphasic pattern, where initial rapid events, attributed to vascular volume changes, are followed by a slow exponential time course, which is assumed to represent transvascular fluid exchange and readjustment of Starling's forces across the membrane. However, the colorimetric data are considerably more linear, suggesting a rate of constant filtration;

2) following decreases in P_c, weights decrease over a

long time period, suggesting fluid reabsorption. However, the colorimetric data suggest ongoing filtration, although at a slower rate. This pattern was present in all experiments. To compare the time course of transvascular fluid exchange obtained by both methods, the first 3 minutes were discarded, where weight signals are thought to predominantly represent vascular volume changes. In figure 8 (page 48), the upper panel shows the average weight (circles) and the corresponding colorimetric data (squares) following increases in P_c, expressed in relation to 100 grams of initial lobar weight. The white symbols represent the first increase in P_c, the black, the second increase. For the first increase in P_c, standard deviations are shown above the mean data. For the second, they are placed below. When comparing weight and colorimetric data obtained at the same time, there was a significant difference after 10 minutes (paired Student's t test), weight exceeding colorimetric changes. Whereas, for the first 20 minutes, weight followed a slow exponential time course which was followed by constant weight gain, the colorimetric data suggested constant filtration throughout the period of observation. On the average, fluid accumulation calculated colorimetrically represented about 60% of weight increases. In the lower panel, signal changes following decreases in P_c are shown

using the same symbols described above. There was a slow, gradual decrease in weight, whereas colorimetric fluid flux continued at a constant rate.

Table 4 (page 49) summarizes the initial circuit volumes and hematocrits, estimates of fluid exchange calculated from on-line colorimetric signals, microhematocrits and weight changes (after 3 minutes) for each change in P_c. In the first four lobes, hematocrits were measured only at the beginning and end of the experiments. Consequently, only total filtered volumes calculated from these measurements are reported (final column, Hct(F)). On the average, estimates of filtered volumes from colorimetric and microhematocrit measurements represented 61 and 56%, and 60 and 56% of gravimetric determinations during the first and second elevations in P_c, respectively. There was good agreement when estimates of filtered volumes from microhematocrit (V_{Fmhct}) measurements were compared to colorimetric determinations (V_{Fclmtr}): V_{Fclmtr} (ml) = V_{Fmhct} (ml) - 0.17 ml, r = 0.985.

In Figure 9 (page 50), microhematocrit measurements (average of 8 samples per measurement) obtained during the course of the experiments (abscissa) are compared with simultaneous colorimetric determinations of hematocrit (ordinate). The correlation was excellent:

laser hematocrit = 1.04 x microhematocrit - 1.58, r = 0.996.

Table 5 (page 51) summarizes the microvascular filtration coefficients calculated using various techniques. K_{f1}, column 1, determined from lobar weight changes following the first pressure increase using the extrapolation technique, averaged 0.483 ± 0.190 $ml \cdot min^{-1} \cdot mmHg^{-1} \cdot 100g^{-1}$. To account for errors which may be introduced by slow vascular changes, K_f was also obtained by relating the rate of constant filtration to the applied force imbalance. K_{f2} (column 2), obtained when the rate of weight gain following the first pressure increase had become constant (30-40 minutes), averaged 0.121 ± 0.048 $ml \cdot min^{-1} \cdot mmHg^{-1} \cdot 100g^{-1}$. In column 3, K_{f3} was obtained using least-squares linear regressions through all colorimetric data points following the first pressure increase ($r > 0.95$ in all cases). Mean K_{f3} was 0.111 ± 0.022 $ml \cdot min^{-1} \cdot mmHg^{-1} \cdot 100g^{-1}$. K_{f4} (column 4) was determined by relating the change in constant Q_f obtained colorimetrically to the first change in P_c from baseline:

$$K_{f4} = (\text{const.}Q_f(1^{st}P_c\,\text{up}) - \text{const.}Q_f(1^{st}P_c\,\text{down}))/\triangle P_c \quad (12)$$

K_{f4} averaged 0.078 ± 0.029 $ml \cdot min^{-1} \cdot mmHg^{-1} \cdot 100g^{-1}$. K_{f1} was significantly different than K_{f2}, K_{f3} and K_{f4} ($p<0.001$).

FIGURE 7. Laser colorimetry: an illustrative experiment. Note how the weight tracing (squares) follows an exponential time course, suggesting edema formation and reabsorption, whereas the laser signal (dots) filters continuously at rates proportional to the microvascular pressure.

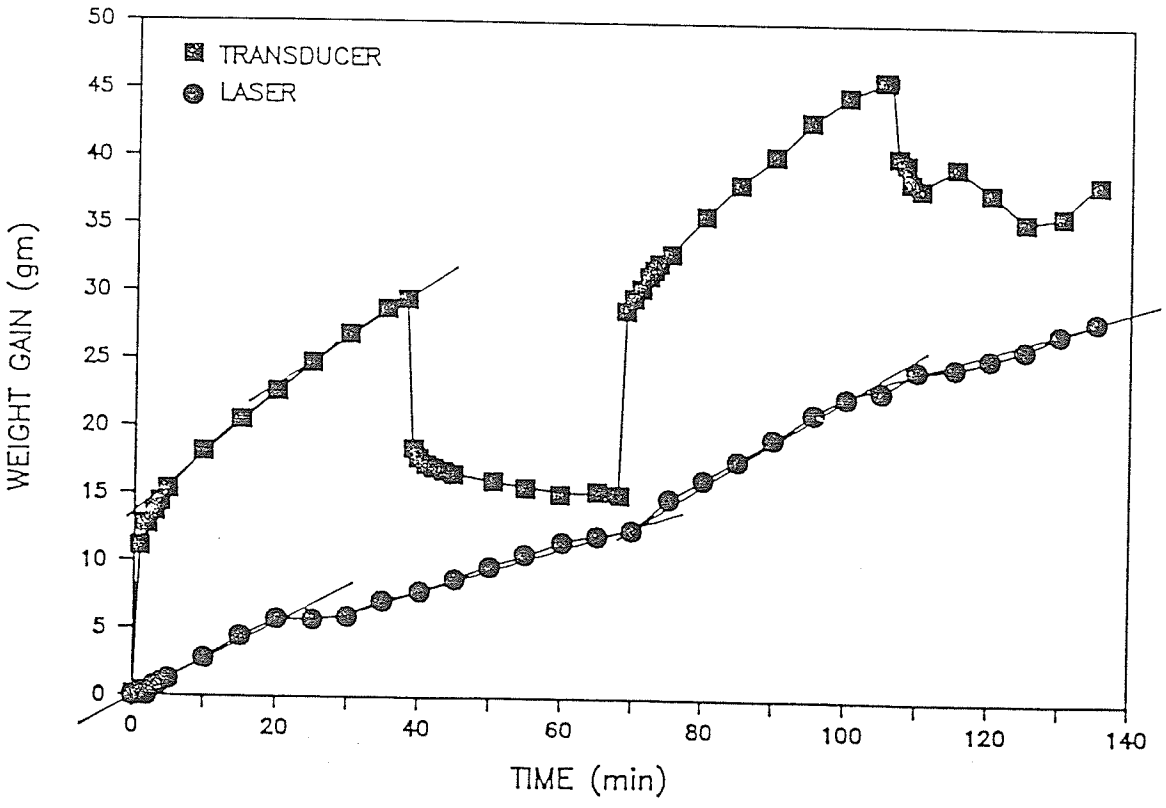


FIGURE 8. Summary of all weight changes determined from the force transducer (circles) and the laser (squares). The upper panel includes data from the first (white symbols) and second (black symbols) increases in P_c. The lower panel uses the same symbols to summarize data following the return of P_c to baseline.

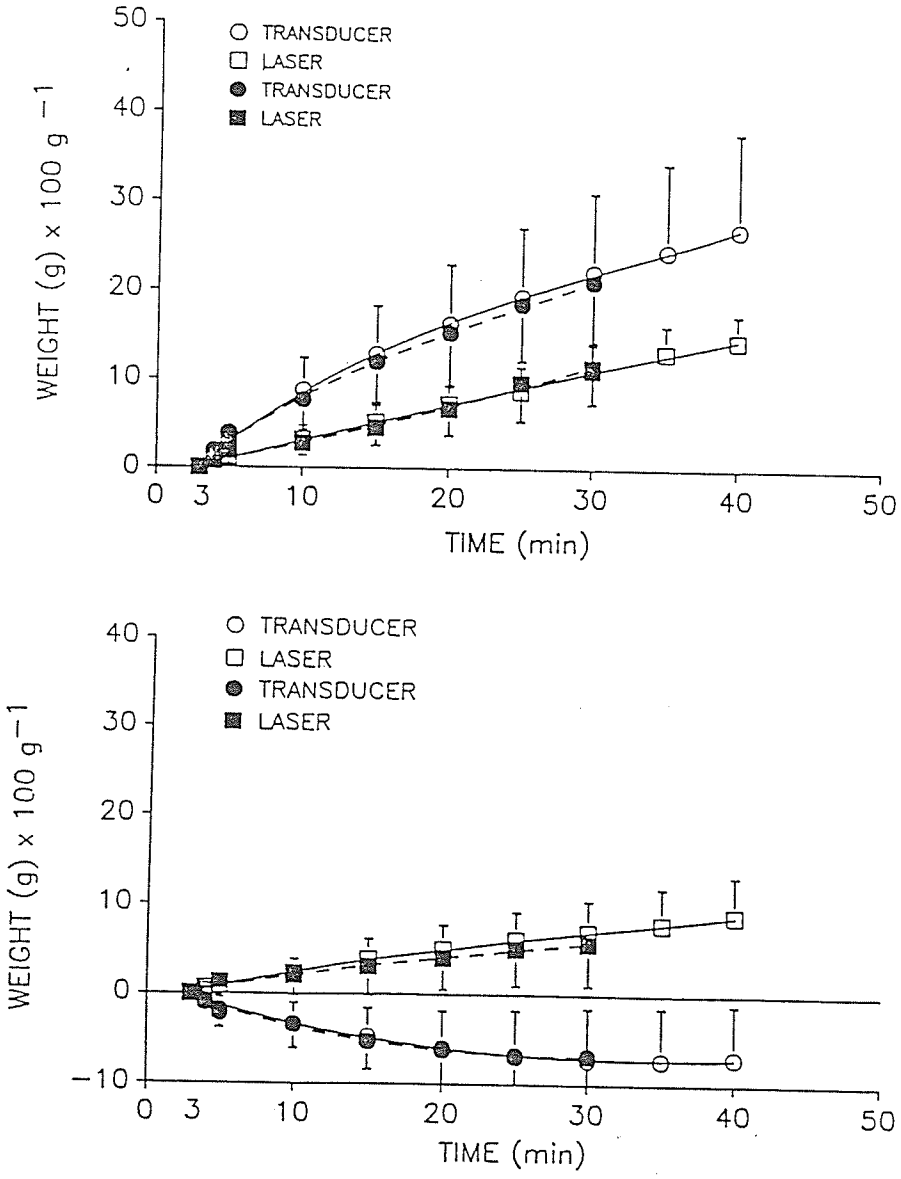


TABLE 4. Summary of lobar changes in weight, colorimetric hematocrits and microhematocrits.

			First Pc up			First Pc down			Second Pc up			Second Pc down			
DOG	C.V.	Hct(I)	WT	COL	Hct	WT	COL	Hct	WT	COL	Hct	WT	COL	Hct	Hct(F)
1	409	33.3	14.8	9.1		-6.2	5.4		9.2	6.7		-5.6	2.5		21.8
2	415	33.7	20.4	10.5		-1.2	4.9		15.8	9.2		-2.8	4.3		25.8
3	407	33.6	10.5	7.8		-4.4	5.1		13.3	6.4		-4.4	4.6		23.4
4	410	34.3	22.4	10.8		-7.6	6.6		13.3	8.5		-6.4	4.2		32.5
5	411	34.4	12.3	8.4	7.5	-2.0	3.2	4.2	12.9	5.5	6.3	-0.3	1.9	2.3	
6	408	35.1	12.6	9.7	11.0	-1.5	3.2	3.9	8.1	4.5	5.5	-2.2	2.1	1.9	
7	411	36.0	11.8	6.2	7.9	-8.3	2.1	2.5	12.6	5.1	6.0	-8.1	1.7	1.1	
8	408	37.5	10.6	6.9	6.5	-4.9	1.4	4.0	8.8	5.6	4.7	-1.8	2.8	1.8	
9	414	37.4	9.6	9.9	9.5	-3.7	6.6	4.0	12.8	7.2	6.3	-3.8	4.8	4.2	
10	405	36.7	15.2	7.2	7.9	-1.4	3.8	5.0	13.5	9.2	10.2	-1.3	4.5	5.4	
11	412	38.4	13.8	10.2	11.2	-0.8	6.4	7.2	14.6	8.1	8.8	-2.6	5.5	4.7	
12	433	47.1	18.7	9.3	7.6	-2.3	4.2	3.6	11.2	5.2	6.4	-1.2	1.8	1.5	
Mean	411.9	36.4	14.4	8.8	8.6	-3.7	4.4	4.3	12.2	6.8	6.8	-3.4	3.4	2.9	
S.D.	6.9	3.6	3.9	1.5	1.6	2.5	1.7	1.3	2.3	1.6	1.7	2.2	1.3	1.5	

C.V., circuit volume (ml); Hct(I), initial hematocrit (%); WT, COL, and Hct, fluid exchanged from weight, colorimetric and microhematocrit changes (ml); Hct(F), final filtered volumes (ml)

FIGURE 9. Comparison of laser hematocrits and microhematocrits. Hematocrits calculated from the laser signal (abscissa) are plotted against microhematocrit determinations (ordinate). The solid line represents the least-squares linear best fit.

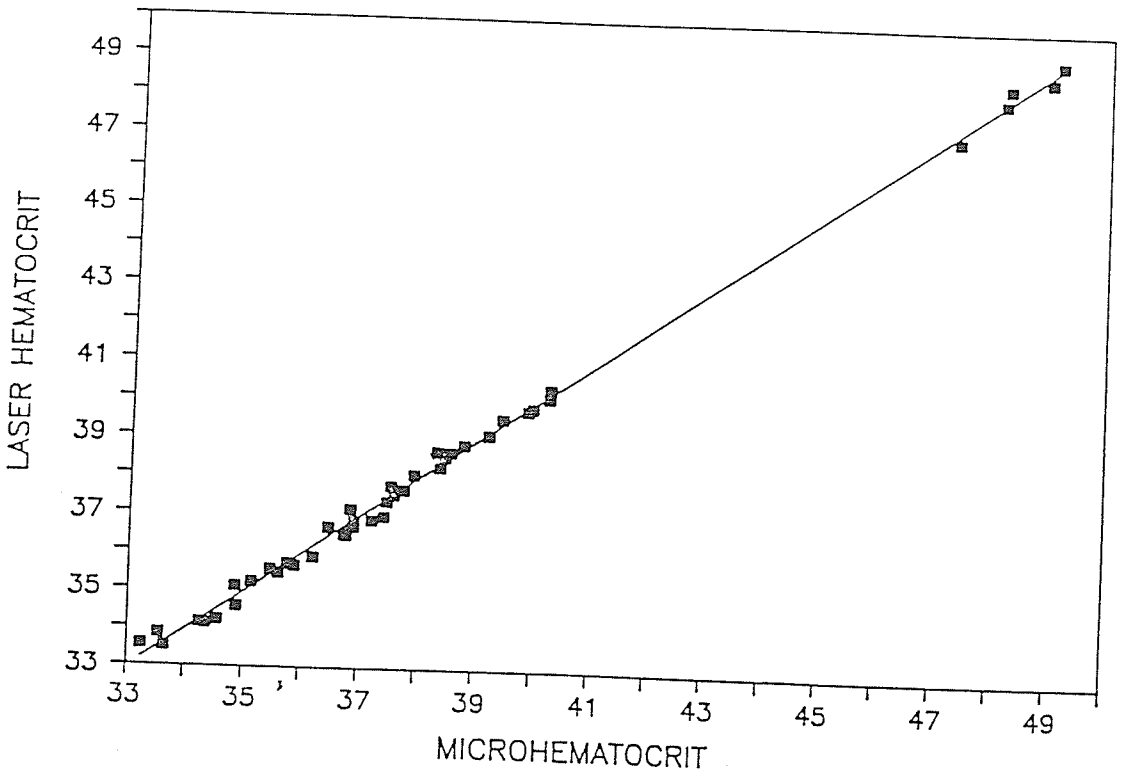


TABLE 5. Summary of microvascular filtration coefficients.

Dog No.	K_{f1}	K_{f2}	K_{f3}	K_{f4}
1	0.768	0.122	0.115	0.068
2	0.513	0.182	0.017	0.083
3	0.504	0.094	0.102	0.064
4	0.896	0.233	0.161	0.117
5	0.228	0.142	0.117	0.066
6	0.506	0.098	0.119	0.118
7	0.429	0.066	0.080	0.054
8	0.341	0.057	0.082	0.030
9	0.268	0.081	0.119	0.064
10	0.427	0.149	0.081	0.044
11	0.317	0.109	0.118	0.110
12	0.595	0.125	0.117	0.116
Mean	0.483	0.121	0.111	0.078
S.D.	0.190	0.048	0.022	0.029

All K_f values in $ml \cdot min^{-1} \cdot mmHg^{-1} \cdot 100g^{-1}$. K_{f1} and K_{f2}, from lobar weights using extrapolation and constant Q_f-to-P_c relationship, respectively. K_{f3} and K_{f4}, from colorimetric data using regressions through volumes exchanged and constant Q_f-to-P_c relationship, respectively.

DISCUSSION

I STEPWISE PRESSURE ELEVATION

The isolated lobe preparation follows a reproducible and characteristic pattern of weight change when step changes in P_c are introduced. Rapid changes in weight, dominated by adjustments in vascular volume, are followed by a slower phase of weight change along an exponential time course. This slow exponential weight transient has been attributed to transvascular fluid filtration (Drake, et. al., 1978; Goldberg, 1980). Fluid exchange with the pulmonary tissues would result in readjustment of Starling's forces across the microvascular membrane to oppose further filtration. The time constant of this exponential weight change would be determined by the microvascular filtration coefficient (K_f) and changes in tissue forces.

As tissue forces are difficult to measure, K_f has been measured indirectly by extrapolating the slow exponential weight transient to the time P_c was changed. At this point, tissue changes would be minimal (Drake et. al., 1978; Goldberg, 1980). This approach is based on the following assumptions: 1) an isogravimetric state exists before pressure changes are introduced and is reestablished once Starling's forces readjust and 2) the slow weight transient reflects fluid exchange between vascular and interstitial compartments exclusively. Using

the weight transient technique, K_f is frequently obtained after P_c is increased from a baseline of 10 to 15 cmH_2O, where the lobe is thought to be isogravimetric. However, Morriss et. al. (1980) and Ehrhart et. al. (1984) have reported rates of constant weight gain at $P_c < 10$ cmH_2O in isolated canine lobes. If this is so, isogravimetric conditions would not be present over the range of P_c usually used in isolated canine lobes. Fluid exchange could occur through structures other than the microvascular membrane and significantly influence estimates of K_f.

In part I, the experiments in group 1 were undertaken to examine this possibility. The results obtained confirmed the findings of Morriss et. al. (1980) and Ehrhart et. al. (1984). By means of a higher weight sensitivity and following the weight time course for long periods, rates of constant weight gain (constant for approximately 10 minutes) were observed at P_c less than 10 cmH_2O. As shown in Table 6 (page 57), the mean filtration coefficient of 0.085 $ml \cdot min^{-1} \cdot mmHg^{-1} \cdot 100g^{-1}$ was close to the K_f of 0.07 $ml \cdot min^{-1} \cdot mmHg^{-1} \cdot 100g^{-1}$ reported by Ehrhart et. al. (1984) but four-fold smaller than the value of 0.30 $ml \cdot min^{-1} \cdot mmHg^{-1} \cdot 100g^{-1}$ obtained by Morriss et. al. (1980) in isolated excised canine lobes. Presumably these differences reflect the variability of the preparation in

different laboratories. Using comparable units of K_f as reported by Drake and Laine (1981), estimated K_f values from group 1 lobes and group 2 lobes before the administration of albumin (Table 6) were found to be similar to determinations obtained from in situ isolated lobes (Drake and Gabel, 1981; Drake et. al. 1980;, Mink et. al., 1985) and intact animal preparations (Guyton and Lindsey, 1959). A significant difference does exist in the estimates of Pcrit between in vivo and excised preparations which may be related to either increased microvascular permeability or ligation of lymphatic vessels in excised preparations as suggested by Morriss et. al. (1980).

The potential effect of constant weight gain on weight transient determinations of K_f is illustrated in Figure 10, page 58, (an experiment from group 3). Initially, the lobe appears to be isogravimetric at baseline P_c but, when high sensitivities are used (Figure 10, bottom left), a rate of constant weight gain is present. In the top panel, the change in lobar weight following a step change in P_c from 12 to 22 cmH_2O is shown. After the initial fast weight changes, the rate of weight gain changes exponentially until it becomes constant at approximately 30 minutes. The effect of constant weight gain would be most significant if it were

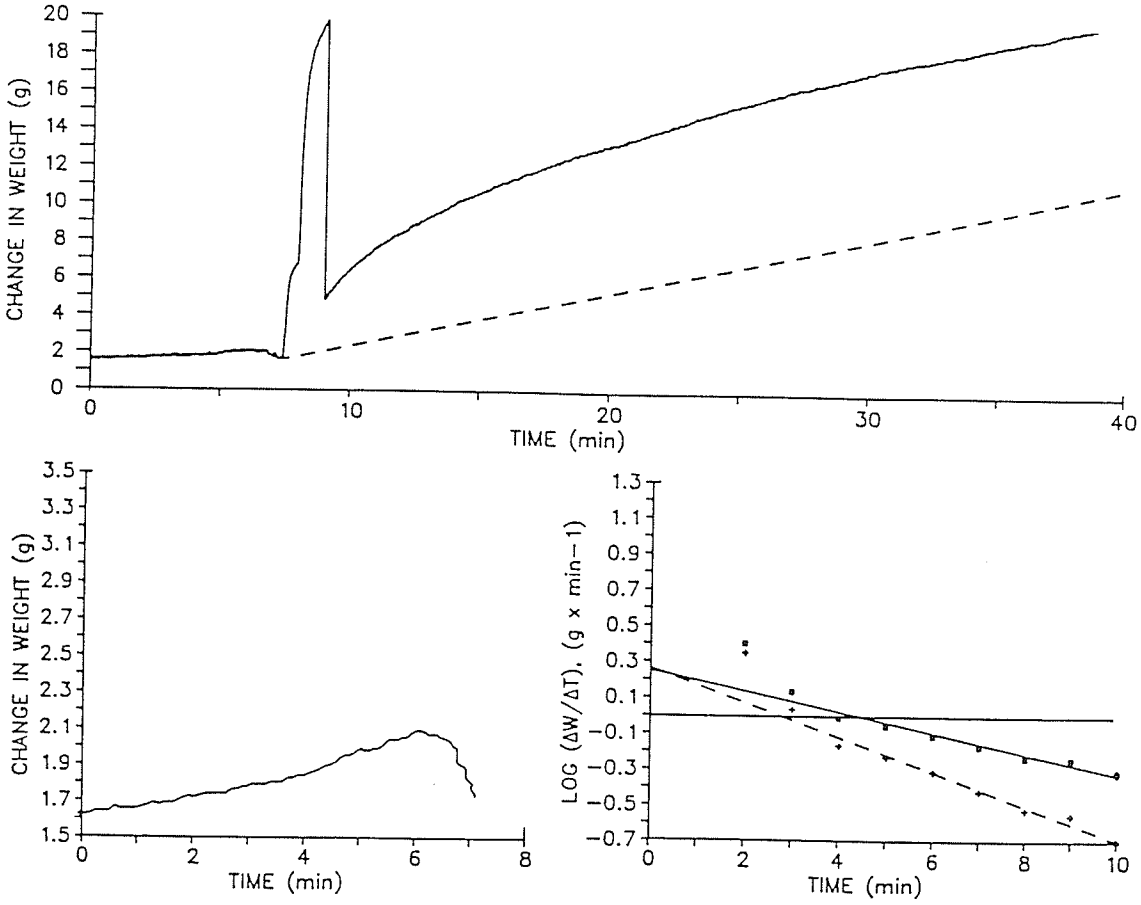
present from the time the change in P_c was introduced (Figure 10, top, dashed line). Figure 10, bottom right, represents determinations of K_f using the weight transient technique. The solid line represents a least-squares best fit obtained between 3 and 10 minutes using the logarithm of the rate of total lobar weight gain. As constant weight gain may reflect fluid exchange across structures other than the microvascular membrane, it was subtracted from the total weight change. The dashed line represents an extrapolate of the logarithm of weight gain after rate of constant weight gain was subtracted from the total weight change. There was no difference in the extrapolated values. Similar results were obtained in other experiments in group 3 where changes in P_c > 7 cmH_2O were applied. The average K_f obtained using the extrapolation method in group 3 lobes was 0.0069 ± 0.0029 $ml \cdot min^{-1} \cdot cmH_2O^{-1} \cdot g$ dry wt^{-1} (0.227 ± 0.069 $ml \cdot min^{-1} \cdot mmHg^{-1} \cdot 100g^{-1}$), about three times higher than the constant rate of weight gain determinations reported in Table 1 (page 37) but similar to values of K_f reported by Drake et. al. (1978) and Rippe et. al. (1985). In the preparation used for the experiments in Part I, rates of constant weight gain did not seem to significantly affect determinations of K_f using weight transient techniques over the range of P_c tested.

TABLE 6. Summary of K_f and Pcrit from several studies.

Study	K_{f1}	K_{f2}	Pcrit cmH_2O	Pcrit mmHg
		Excised lobes		
Group 1	0.0022	0.085	9.53	7.01
	±0.0003	±0.013	±1.18	±0.87
Group 2*	0.0021	0.1027	12.2	9.0
	±0.0006	±0.021	±2.41	±1.76
Morriss et.al.(1980)		0.30	2.3	1.7
		±0.16	±4.9	±3.6
Ehrhart et.al.(1984)		0.07	6.7	4.9
		±0.029	±3.0	±2.2
		Intact lobes		
Morriss et.al.(1980)		0.09	27.3	20.1
		±0.03	±6.4	±4.7
Mink et.al.(1985)		0.086	22.3	16.4
		±0.058	±6.4	±6.2
		Intact animals		
Guyton and Lindsey (1959)		0.07	34	25

Values are means ± SD. K_{f1}, $ml \cdot min^{-1} \cdot cmH_2O^{-1} \cdot g$ dry wt^{-1}; K_{f2}, $ml \cdot min^{-1} \cdot mmHg^{-1} \cdot 100\ g^{-1}$; * Group 2 lobes before albumin.

FIGURE 10. Effect of constant weight gain on weight transient determinations of K_f. Top panel, lobar weight tracing at baseline and following a step increase in P_c from 12 to 22 cmH_2O. Dashed line illustrates the effect of constant weight gain if present from the time the pressure change is introduced. Bottom left panel, initial 8 minutes of the same lobar weight tracing using high sensitivity weight measurements. Bottom right panel, K_f determined by extrapolation before (solid line) and after (dashed line) rate of constant weight gain is subtracted from the total lobar weight change.



To shift Pcrit to higher values, 100 ml of 25% human serum albumin was added to each lobe in group 2 after initial rates of weight gain were determined at three levels of P_c. In this way, the zero filtration intercept would not be outside of the range of P_c tested, maintaining Zone III conditions. In six experiments, after the administration of albumin, P_c was lowered in steps to gradually decrease the rate of constant weight gain. To obtain a direct measurement of Pcrit, this process could be continued until a P_c was reached where weight gain stopped. However, in isolated lobe preparations, where lymphatic drainage has been interrupted, no net filtration occurs during isogravimetric conditions. However, intravascular protein could diffuse into the interstitium inducing a new imbalance of Starling's forces in favor of filtration such that an isogravimetric state would not be maintained. To avoid this potentially confounding phenomenon, P_c was reduced to produce very small rates of constant weight gain and Pcrit was obtained by extrapolation, although from rates of weight gain close to zero. Errors as the result of extrapolation would be minimized. However, at small rates of filtration, it is possible for protein diffusion still to influence the force imbalance across the microvascular membrane. If so, one would expect small rates of constant weight gain not

to satisfy a linear relationship with P_c. This was never the case. However, because of this possibility, in six other experiments identified with an asterisk in Table 2 (page 40), P_c was lowered to induce fluid reabsorption. In these experiments, Pcrit represented the zero rate of weight gain intercept of a least-squares linear regression obtained using positive and negative rates of weight change. There was no difference in the Pcrit obtained in these two subgroups (unpaired Student's t test).

Lunde and Waaler (1969) reported rates of weight loss and gain when changes in II were introduced. At left atrial pressures of 15 cmH_2O their preparations lost weight. When plasma colloid osmotic pressure (II_p) was lowered by 30%, weight gain ensued. Further changes from high to low and back to high II_p perfusates had the same effect. Presumably, decreases and increases in II_p moved the balance of forces above and below Pcrit, respectively. Constant rates of weight loss have also been observed by other investigators. Uter et. al. (1966) related constant rate of weight change to P_c in isolated lobe and lung preparations when the airways were filled with air, Ringer, or colloid solutions. The relationships reported in their study were very similar to the experiments reported herein. When P_c was lowered below a critical level, rates of constant weight loss proportional to P_c

followed a linear relationship. In a subsequent study, where the perfusate's II was increased, they demonstrated a modest increase in Pcrit and also a change in the slope relating the rate of constant weight change to the difference between P_c and II (Uter et. al., 1967). However, when Guyton and Lindsey (1959) decreased II, they failed to detect any change in K_f.

The increase in slope of constant rate of weight gain to P_c relationships observed in the present study could reflect an increase in microvascular permeability associated with the administration of albumin. Because outdated human serum albumin was used initially, this seemed a distinct possibility. However, in the last three experiments, fresh albumin solutions were used and similar changes in the relationship were observed. This finding is contrary to observations made by other investigators who report small increases in K_f when protein concentration is decreased (Huxley and Curry, 1985). No explanation could be derived from the data. If this phenomenon was indeed secondary to microvascular permeability changes, it was not of sufficient magnitude to prevent increases in Pcrit in association with increased II.

The estimates of the membrane drag reflection coefficient (0.57 ± 0.10 and 0.66 ± 0.08, Table 3, page 41) obtained in these experiments are higher than those

reported by Rippe et. al. (1985), where σ was found to be 0.48 using a weight transient approach in isolated dog lobes, and also higher than estimates reported by Parker et. al. (1981) in anesthetized dogs (σ_d = 0.50) using analysis of lung lymph. The calculations of σ_d shown in Table 3 could be too high if the change in II_t associated with the administration of albumin was overestimated. However, experiments with prolonged left atrial pressure elevations in sheep have shown that changes in II_t calculated from lymphatic samples may be underestimated unless enough time is allowed to establish a true steady state of lymph protein concentration with filtration (Parker et. al., 1985). If this is also the case in dogs, estimates reported by Parker et. al. (1981) may have been too low.

The calculations of K_f and σ_d obtained in this study suggest that the integrity of the microvascular membrane is not significantly affected by the isolation procedure. The control group demonstrates that this preparation remains stable over several hours and that the rate of edema formation tends to accelerate slightly only when the volume of fluid accumulated exceeds 30-40% of initial lobar weight. The fact that these preparations gain weight continuously at low pressures may been attributed to increased microvascular permeability but this could also

be explained by considering that Pcrit is lower than the range of P_c used in most gravimetric studies. In isolated lobe preparations, low Pcrit may be related to lymphatic and bronchial vessel obstruction without injury to the microvascular membrane. In this preparation, constant rates of weight gain did not significantly influence determinations of K_f using the weight transient method.

II. LASER COLORIMETRY

Since it is difficult to separate vascular from extravascular volume changes in weight tracings obtained from lung and lobar preparations, the time course of vascular volume expansion following an increase in P_c remains controversial. Although it is common practice to consider vascular volume changes to have been completed three to five minutes following the hydrostatic pressure change, several studies indicate that this may not be a valid assumption. Lunde and Waaler (1969) conducted experiments in which they used the emitted radioactivity of ^{51}Cr-labelled red cells as an independent measurement of vascular volume. Changes in pulmonary radioactivity were followed continuously with a scintillation counter pointed at the isolated rabbit lung preparation. When small changes in hydrostatic pressure were introduced, weight and radioactivity signal changes were

indistinguishable. This was attributed to a lack of sensitivity of the technique. With pressure changes of approximately 15 mmHg, the rate in weight gain after 3 minutes exceeded the rate of change in pulmonary radioactivity, which decreased with time, but did not completely reach a plateau during the period of observation (10 minutes). The difference was interpreted as filtration. Similar findings were reported by Lee and Lee (1979) also using ^{51}Cr-labelled red cells. Under Zone II conditions, elevation of P_c produced rapid increases in weight and radioactivity followed by a slower gain. The blood volume change was estimated to represent 40-70% of the total weight change. Since weight and tissue radioactivity remained higher than the original values when P_c was return to baseline, they suggested that the increased radioactivity could be consistent with recruitment of microvessels. Greenway et. al. (1970) performed similar experiments in isolated, in situ, cat liver preparations. Following changes in P_c, radioactivity increased for about 30 minutes. When vascular volume was subtracted from total hepatic volume changes, the rate of filtration appeared constant. Chang et. al. (1979), using multiple indicators, calculated the changes in vascular, extravascular and cellular spaces following hydrostatic pressure changes. Their results were similar to the

results reported herein: lobes always gained weight, the rate of weight gain was proportional to the microvascular hydrostatic pressure, no fluid reabsorption was detected and vascular volume changes persisted for as long as 15 minutes following microvascular hydrostatic pressure increases of 10 mmHg.

Calculations of transvascular flux using the colorimetric approach suggest that a significant proportion of the weight change after the first three minutes (Figure 7, page 47) are the result of slow vascular volume changes. It is generally accepted that the slow exponential weight gain reflects readjustment of Starling's forces across the microvascular membrane with filtration (Drake et. al., 1978; Taylor and Parker, 1984). However, the constant rates of filtration observed colorimetrically suggest that slow vascular volume changes are predominantly responsible for the slow exponential shape of the lobar weight time course. Slow stress relaxation of pulmonary vessels following elevation of hydrostatic pressure, as proposed by Sarnoff et. al. (1952), would explain these findings. If tissue pressures do, in fact, change with filtration, fluid reabsorption should occur once microvascular hydrostatic pressure is returned to baseline. Reabsorption was not detected with colorimetric measurements of fluid exchange even though

slow exponential weight decreases were observed, again suggesting the possibility of slow vascular volume changes. The measurements appeared to be correct in that calculations of filtered volumes using the on-line colorimetric device correlated well with measurements obtained with the microhematocrit technique (Table 4, page 49). Also, given enough time (about 20 minutes), rates of filtration calculated from changes in weight and colorimetric estimates of filtration became closer (Figure 8, upper panel).

Maron et. al. (1987) have found circuit hemolysis to significantly influence calculations of filtered volumes using their hematocrit-protein technique. Therefore, they proposed a correction procedure based on measuring free hemoglobin in the perfusate. Measurements of free hemoglobin conducted during the colorimetric experiments proved very variable and unreliable. Nevertheless, since absorption of free hemoglobin was negligible at a wavelength of 815 nm, hemolysis did not appear to interfere significantly with measurements of transvascular flux using laser colorimetry. Also, using the cyanomethemoglobin technique, the degree of hemolysis in these experiments was determined to be very small. However, colorimetric and microhematocrit calculations may have underestimated fluid exchange by about 0.8 $ml \cdot hr^{-1}$

(Hancock et. al., in press). Although hemolysis of a significant degree would underestimate edema accumulation and perhaps explain differences between weight changes and filtered volumes following P_c increases, it would not account for the failure to detect fluid reabsorption from colorimetric or microhematocrit determinations of filtered volumes, where it should have resulted in filtered volume overestimations.

As previously described, the microvascular filtration coefficient (K_f) is frequently estimated using the weight transient technique, where the slow exponential rate of weight change is extrapolated to the time P_c is changed (Drake et. al., 1978; Taylor and Parker, 1984; Unruh et. al., 1984). To the extent it is influenced by slow vascular changes, this method will overestimate K_f. Alternatively, K_f may be determined by relating rates of constant filtration to the change in P_c. As shown in Table 5 (page 51), K_f obtained in this way (K_{f2}) as well as K_f obtained using least-squares linear regressions through colorimetric measurements of transvascular fluid flux (K_{f3}) were four-fold lower than K_f obtained using the extrapolation technique (K_{f1}). These results support the measurements of K_f reported by Drake and Gabel (1981). There was good agreement between determinations of K_f using colorimetric and constant weight gain estimates of

filtration. In Table 5, K_f was also measured by relating the change in constant Q_f obtained colorimetrically to the first change in P_c (K_{f4}). These determinations of K_f are in close agreement with K_f calculations reported by Ehrhart et. al. (1984) and with values reported in Part I using the slope of the relationship between rates of constant weight gain and P_c in isolated excised canine lobes.

The results suggest that, following changes in vascular hydrostatic pressure, vascular volume changes continue over a much longer period than is generally accepted. The constant rate of filtration observed suggests that fluid filtration is greatly influenced by an accessible, compliant compartment. The rate of constant weight gain would be determined by the Starling force imbalance at the microvascular membrane, the hydrostatic pressure in the compliant compartment and the resistance encountered by the fluid in its course through membranes and tissues. Over the range of microvascular pressures tested, transvascular forces acting across the microvascular membrane never reached equilibrium and, as such, a state of constant filtration proportional to pulmonary capillary hydrostatic pressure persisted. Prolonged vascular volume changes, perhaps due to stress relaxation of pulmonary vessels in response to an increase

in capillary pressure, appear to be responsible for the exponential time course of lobar weight change. Rates of constant filtration will, therefore, be a closer measure of transvascular flux than the initial weight time course.

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