Summary: We used pliethysmograph techniques to measure or calculate the tissue and capillary forces and flows (capillary pressure, tissue and plasma oncotic pressure, transcapillary pressure drop, lymph flow, and interstitial pressure) in a dog hindpaw preparation in situ at three different venous pressures (PV). Since lymph was flowing from the preparation, the isovolumetric state represented an isofiltration state rather than the conventional isovolumetric or isogravimetric state. At an isofiltration capillary pressure of 12.8 mm Hg, lymph oncotic pressure averaged 3.8 mm Hg, plasma oncotic pressure averaged 20.9 mm Hg, and tissue pressure averaged -4.7 mm Hg (PV normal). The imbalance in transcapillary forces averaged 0.5 mm Hg and represented the lymph flow contribution (lymph flow/filtration coefficient) to maintenance of the normal capillary filtration pressure. As isofiltration capillary pressure increased to 14.5 mm Hg, lymph oncotic pressure increased by 1.7 mm Hg, lymph plasma oncotic pressure fell by 2.2 mm Hg, and the transcapillary pressure drop increased to 6.6 mm Hg (PV -5 mm Hg). At higher venous pressures the tissue pressure fell to -10 mm Hg, lymph oncotic pressure fell to 0.5 mm Hg, and the transcapillary pressure drop increased to 6.3 mm Hg (PV -10 mm Hg). At moderate levels of PV elevation, the transcapillary pressure drop and increased tissue pressure provided 80% of the counterbalancing tissue force, each contributing approximately 40%. At higher venous pressures, the only tissue force that opposed filtration was an increase in tissue pressure.
ics of lymphatic drainage. 10–14 The current concept envisions the existence of a slight imbalance of transcapillary hydrostatic and oncotic forces. This imbalance of forces provides the driving pressure for the continuous, albeit slow, leakage of fluid from the capillary into the interstitium. To maintain the normal steady state, the egress of fluid from the capillary is balanced by an equally sluggish outflow of fluid from the interstitium via the lymphatic system. In addition to providing a pathway for removal of interstitial volume, the lymphatics also serve to maintain the transcapillary oncotic gradient required for the establishment of normal transcapillary fluid balance. The lymphatic vessels maintain this gradient by transporting proteins out of the interstitium at a rate equal to the transcapillary protein flux.

The increased awareness of the functional interactions between capillary, interstitial, and lymphatic forces provided the impetus for studies focusing on regulation of transcapillary fluid movement in edema. In 1932 Krogh et al. 15 suggested that, following an increase in venous pressure or a decrease in plasma oncotic pressure, compensatory changes in interstitial and lymphatic forces provide a "margin of safety" against development of edema. Some recent studies 16–18 suggest an important role for interstitial fluid pressure elevations in preventing gross accumulation of interstitial fluid following an increase in capillary pressure. Other studies 19–21 support the concept that a reduction in interstitial oncotic pressure is the major safety factor against edema formation. Finally, some investigators 22 consider alterations in lymphatic flow as the major component of the "margin of safety." Yet, it is probable that all of these mechanisms contribute to some extent in every tissue.

Furthermore, the relative contribution of each compensatory mechanism probably varies from tissue to tissue, since interstitial compliance, extent and activity of lymphatic vessels, and normal interstitial oncotic pressure can be expected to vary from organ to organ. In addition, within a given tissue, the importance of the individual components of the "margin of safety" may vary with the magnitude of capillary pressure.

The purpose of the present study was twofold: (1) to quantify the interaction of capillary, interstitial, and lymphatic forces in the dog hindpaw under normal conditions, and (2) to determine the contribution of interstitial and lymphatic compensatory mechanisms to the prevention of gross accumulation of fluid in the dog hindpaw at different levels of capillary pressure. To achieve these goals, we determined simultaneously isovolumetric capillary pressure, interstitial fluid pressure (modified capsule technique), lymphatic flow, and lymph oncotic pressure in the canine hindpaw in situ. Increases in capillary pressure were induced by graded elevations of venous outflow pressure.

**Methods**

**ANIMAL PREPARATION**

Twelve mongrel dogs were anesthetized with sodium pentobarbital (30 mg/kg, iv). Body temperature was maintained at 37 ± 1°C with heat lamps. The hindpaw was isolated in situ in the following manner: The femoral artery, anterior tibial artery, lateral saphenous vein and two lymphatics coursing along the saphenous vein were isolated. Three to five other lymph vessels seen during the isolation procedure were cut and opened to the air for free drainage. All surgical incisions were made 5 to 7 cm above the ankle region. Following surgery, the dog was heparinized (5 mg/kg, iv) and the vessels were cannulated. The paw was perfused with autologous blood via an extracorporeal circuit that connected the anterior tibial artery to the femoral artery. Anterior tibial blood flow and pressure were monitored with an electromagnetic flowmeter (BL-310) and a P23Ac transducer, respectively. The lateral saphenous vein was catheterized and connected to a venous reservoir. The venous pressure was monitored with a P23Bc pressure transducer. The two lymphatics were cannulated with polyethylene (PE) 90 tubing. After completion of all cannulations, the leg was clamped tightly above the level of the incisions and cannulations (Fig. 1).

**MEASUREMENT OF INTERSTITIAL FLUID PRESSURE**

Four to six weeks prior to the experiment a small, porous, polyethylene capsule similar to that described by Ott et al. 19 was implanted in the subcutaneous tissue of the dorsal hindpaw. The capsules were constructed of porous PE, the pores averaging 35 μm in diameter (Bolab). The PE material was cut into a cylinder measuring 4 mm in diameter and 10 mm in length. A hole was drilled through the center of the cylinder along its long axis. A length of PE tubing (inner diameter, 1.5 mm) was perforated at one end. The perforated end was inserted through the hole in the PE cylinder. The PE cylinder and tubing were fused by heat at one end and the melted end of the capsule was flattened and smoothed. This procedure provided a firm attachment of the PE 190 catheter to the miniature capsule. The perforations in the PE tubing provided communication between the capillary pores and the lumen of the catheter. Vinyl cement was used to seal the joint between the catheter and capsule on the other end of the cylinder. The capsule-catheter system was filled with sterile saline, the free end of the catheter was
sealed with heat, and the miniature pressure-measuring device was implanted beneath the skin. After implantation, the dogs were injected with penicillin for 5 days and fed a protein-rich diet for 1 month. On the day of the experiment the catheter was exteriorized and connected to a P23Gb low displacement transducer. The zero reference point was the hydrostatic level of the implanted porous capsule.

An important assumption in our study is that the pressure measured from the chronically implanted capsule is a true measure of interstitial fluid pressure in the hindpaw. A recent criticism of the technique is that the fibrous connective tissue which lines the capsule may be impermeable or nearly impermeable to plasma proteins and, consequently, the capsule may reflect the sum of interstitial hydrostatic and oncotic forces. However, direct assessment of capsule permeability suggests that under physiological conditions protein oncotic pressure gradients have little effect on the pressure measured in implanted capsules. Furthermore, the fibrous lining associated with the porous PE type of capsule is much thinner and apparently more permeable than the lining which grows into the larger hollow capsules. Therefore, we feel that the assumption of equality of capsule and interstitial fluid pressures is valid. More detailed discussions of interstitial fluid pressure measurement are presented elsewhere.

**DETERMINATION OF TISSUE VOLUME**

Monitoring of tissue volume is essential to determine capillary pressure, capillary filtration coefficient, and changes in interstitial fluid volume. To determine tissue volume the hindpaw was placed in a Lucite plethysmograph (Fig. 1). The space between the paw and the opening in the plethysmograph was sealed with plasticized hydrocarbon gel (Plastibase, Squibb). Care was taken not to compress blood and lymphatic vessels. Consequently, blood flow and lymph flow were not altered by the procedure. The sealed plethysmograph was connected to a shallow Lucite reservoir with wide diameter by a short length of Tygon tubing. The reservoir was suspended from the lower lug of a precalibrated force-displacement transducer (Grass FT 10C). The plethysmograph-reservoir system then was filled with Tyrode's solution at room temperature. Evaporation of fluid from the reservoir was prevented by covering the wide opening with aluminum foil. A beam-type counterbalancing system was attached to the upper lug of the force-displacement transducer to provide a sensitive measurement of volume changes. The reservoir was placed at the level of the plethysmograph to avoid excessive back pressure on the tissue. Changes in tissue volume caused movement of fluid into or out of the reservoir; thus, tissue volume changes were translated into weight changes. The wide diameter of the reservoir served to minimize increases in back pressure when large volumes of fluid were translocated from the plethysmograph into the reservoir.

**DETERMINATION OF LYMPH FLOW AND ONCOTIC PRESSURES OF PLASMA AND LYMPH**

The rate of lymph flow was calculated from the velocity of movement of the meniscus within the PE 90 tubing connected to the lymph vessels. Velocity was measured with a ruler and magnifying comparator. The flows measured represent free flow in the anesthetized dog in the absence of artificial massaging or pumping.

Venous plasma and lymph samples were collected to determine plasma and lymph oncotic pressures, respectively. Oncotic pressures were determined with a Prather-type osmometer fitted with a UM-10 membrane (Millipore).

In our present study, we assume equality of lymph and interstitial oncotic pressures. Although this assumption is implicit in most studies of transcapillary exchange, the existence of a protein-concentrating mechanism in lymph vessels recently has been proposed. However, several studies indicate that in subcutaneous and other tissues the concentrating ability of the lymphatics is weak or nonexistent. Taylor et al. showed that capsule fluid and lymph in the hindpaw were characterized by identical protein levels. In addition, Rusili and Arfors, using more appropriate sample interstitial fluid, found no significant difference between hindpaw tissue fluid and lymph with respect to plasma proteins. Furthermore, Nicolaysen and Staub demonstrated equality of interstitial and lymph albumin activity by direct microscopic analysis of Evans blue albumin fluorescence in lung tissue. Thus, although we cannot completely exclude the possibility of lymphatic concentrating ability, the available experimental data do not lend support to the concept.

**DETERMINATION OF CAPILLARY PRESSURE**

Through the use of the whole organ approach, one can obtain an estimate of the effective hydrostatic pressure within the exchange vessels. Although this pressure is known as the isovolumetric capillary pressure, it probably represents a space average pressure for capillary and venular compartments.

Mean capillary hydrostatic pressure was determined by step-flow and zero-flow techniques. Briefly, the step-flow techniques consist of progressively elevating venous pressure and maintaining the isovolumetric state by counter-balancing decreases in arterial pressure. Venous pressure was elevated by raising the venous outflow reservoir. Tibial artery pressure was lowered by graded compression of the extracorporeal tubing connecting the anterior tibial and femoral arteries. As the arterial and venous pressures were altered in steps, the blood flow also was reduced in stepwise...

*We have assumed that lymphatic fluid represents tissue fluids relative to transcapillary fluid flux. This assumption has been argued since the early studies of Drinker (Yoffey and Courtier, Taylor et al., Ruszyynski et al., Rusili and Arfors, Cailey-Smith, and Guyton et al.), with some investigators stating that lymph proteins reflect a good cross section of tissue fluid from the area drained by the lymphatic, and others stating that lymph bears no correspondence to tissue fluid. Certainly one cannot argue that lymph draining an organ is representative of tissue fluid surrounding each capillary; but, since we measure an average functional capillary pressure, tissue pressure, etc., then lymph fluid must reflect some average tissue fluid in a steady state. The possibility certainly exists that lymph may be concentrated as it courses through the lymphatic system; however, there is no experimental evidence that supports this hypothesis if lymph fluid is compared to "tissue fluid" in the same preparation and the lymph is sampled before the lymphatic vessels pass through nodal structures. In all experiments reported in the literature, lymphatic proteins are comparable to "tissue fluid samples." This does not mean that the question is settled, but only demonstrates that our techniques are not adequate at the present time to answer this very important and baffling question: Does lymph truly reflect tissue fluids?"
fashion because the arteriovenous pressure difference was diminished. By extrapolation of the venous pressure-flow relationship to zero blood flow, the isogravimetric capillary pressure was obtained. An alternative and simpler approach is to occlude the arterial inflow to the organ and subsequently elevate the venous pressure to maintain the isovolumetric state. The venous pressure required to achieve the isovolumetric condition is defined as the zero-flow isogravimetric capillary pressure. Since changes in total tissue volume reflect not only changes in interstitial fluid volume but also alterations in blood volume, maintenance of a constant total volume does not necessarily indicate absence of alteration in capillary filtration. Therefore, we modified these methods of determining capillary pressure by achieving and maintaining an "isofiltration" state, namely, by adjusting venous pressure to keep the slope of the slow component of tissue volume change at zero for at least 3 minutes. In general, the isofiltration values were 2-3 mm Hg higher than isovolumetric measurement.

In some experiments, capillary pressure was obtained by both step-flow and zero-flow isofiltration techniques. The values obtained were not significantly different. Therefore, zero-flow determinations were used in most cases, especially at the higher venous pressures.

**DETERMINATION OF CAPILLARY FILTRATION COEFFICIENT**

When venous pressure is elevated, the increase in tissue volume consists of an initial rapid phase followed by a longer lasting slow phase. The rapid phase reflects pooling of blood within the blood vessels and the slow component is due to accumulation of interstitial fluid produced by acceleration of transcapillary filtration. We plotted the rate of tissue volume change as a function of time (first 10 minutes of volume change) on semilogarithmic paper and extrapolated the slow component to zero time to obtain the initial rate of transcapillary filtration. The capillary filtration coefficient was calculated as the quotient of initial transcapillary fluid flux and the change in capillary pressure. The difference between the isovolumetric capillary pressures before and after the venous pressure elevation was used as the change in capillary pressure.

**DETERMINATION OF INTERSTITIAL VOLUME INCREASES AND INTERSTITIAL COMPLIANCE**

Since the slow component of tissue volume change represents an increase in interstitial fluid volume the magnitude of the slow component was used as a measure of the change in interstitial volume induced by elevation of venous pressure. The instantaneous interstitial volume change was plotted against the prevailing interstitial fluid pressure and interstitial compliance was calculated from the slope of this curve.

**CALCULATION OF VASCULAR RESISTANCES**

Total vascular resistance (R_T) was calculated from the hindpaw blood flow (F_A), arterial pressure (P_A), and saphenous vein pressure (P_V), or

\[ R_T = \frac{P_A - P_V}{F_A}. \]

Precapillary resistance (R_A) was calculated from paw flow, arterial pressure, and isovolumetric capillary pressure (P_C), or

\[ R_A = \frac{P_A - P_C}{F_A}. \]

Finally, postcapillary resistance (R_V) was calculated from

\[ R_V = \frac{P_C - P_V}{F_A}. \]

In the calculation of R_A and R_V, it is assumed that total blood flow is equivalent to capillary flow. If there were large arteriovenous shunt flows in our preparation, then the resistance calculations could be in error. Since neither pre- nor postcapillary resistance changed significantly with increasing venous pressures (Fig. 2), then shunt flow must not have been a problem in our preparation; either the shunt flow remained constant with increasing venous pressure or was an insignificant portion of total blood flow.

**CALCULATION OF PRESSURE DROP ACROSS THE CAPILLARY WALL**

The net transcapillary filtration pressure, or pressure drop (ΔP), was calculated from isovolumetric capillary pressure (P_C), interstitial fluid pressure (P_i)}, plasma oncotic pressure (π_P)}, and lymph oncotic pressure (π_L), or

\[ ΔP = P_C + π_L - π_P - P_i}. \]

**EXPERIMENTAL PROTOCOL**

During the isolation and preparation period, a slight rise in interstitial fluid pressure always was observed, presumably due to a slight diminution of precapillary resistance. After the experimental set-up was complete, venous pressure was set at 3 ± 1 (mean ± SD) mm Hg and arterial pressure was lowered from 118 ± 5 to 100 ± 4 mm Hg. The reduction of arterial pressure was required to return interstitial pressure to the level present before the operative procedures. Throughout the experiments, systemic arterial pressure was maintained at approximately 100 mm Hg. After the tissue reached an isovolumetric state, control determinations of capillary pressure, interstitial fluid pressure, lymph and plasma protein osmotic pressures, and lymph flow were obtained. Venous pressure then was elevated to 20 mm Hg and maintained at this level until a new isovolumetric state

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1 When calculating the transcapillary pressure drop (ΔP) it was assumed that the reflection coefficient of the plasma proteins (π_P) was equal to 1. The correction equation should state that: \[ ΔP = P_C + P_L - π_P - P_i}. \] If π_P < 1 a variety of ΔP values can be calculated. If we had collected all the lymph flow in this preparation, then π_L could be evaluated. Unfortunately, we did not collect all the lymph draining the paw preparation, but we can at least approximate π_L assuming that bulk flow across the capillary is much greater than the diffusional term. Assuming that the final lymphatic protein concentration is similar to capillary filtrate (at the minimal lymph protein concentration which was observed at P_L = 35), then a sieving coefficient of 0.025 can be calculated which yields a of 0.975. Obviously, this calculation is an oversimplification of the real system since the diffusional term is neglected, but we feel that it indicates a π for plasma proteins very close to unity in our preparation. Furthermore, many investigators assume that π = 1 and describe protein leak across capillaries as being solely due to either diffusion or vesicular transport, since the kinetics of lymphatic protein flux appear to be diffusional with π = 1 (no bulk flow component). The problem of assessing a cannot be settled with our present data, even when all forces except total lymph flow are known, but it appears from this and other data that a is very close to 1 in the hindpaw.
was attained; at this time the above determinations were repeated and the capillary filtration coefficient was calculated. The procedure was repeated after a new isovolumetric state was achieved following elevation of venous pressure to 35 mm Hg.

After the experiment, the contralateral paw was cut and removed from the hindlimb. After dissection, total tissue, bone, skin, and muscle weights were determined. The paw consisted of 45% bone, 45% skin, and 10% muscle. Variables normalized to tissue weight are expressed in terms of 100 g of soft tissue, i.e., skin and skeletal muscle.

**Results**

**VASCULAR AND HEMODYNAMIC RESPONSES TO VENOUS PRESSURE ELEVATION**

The effects of venous pressure elevation on vascular resistance, blood flow, capillary pressure, and capillary filtration coefficient are summarized in Figure 2. At a normal venous pressure of 5 mm Hg, the blood flow was 23.5 ± 3.5 ml/min per 100 g, which is within the normal range of 20–30 ml/min per 100 g obtained by others. In the normal state, total vascular resistance is 4.0 ± 7 mm Hg/ml/min per 100 g. The precapillary resistance accounts for more than 90% of the total resistance to blood flow. Thus, because of the high ratio of precapillary to postcapillary resistance, capillary pressure in the hindpaw is less than 13 mm Hg under control conditions.

When venous pressure was elevated above 5 mm Hg, total resistance was not altered significantly, but postcapillary resistance fell and precapillary resistance increased slightly; consequently, the ratio of pre- to postcapillary resistance doubled when venous pressure was elevated to 35 mm Hg. In the face of a diminishing arteriovenous pressure difference and constant total vascular resistance, hindpaw blood flow fell as venous pressure increased. When the venous pressure was elevated from 5 to 20 mm Hg, 80% of the venous pressure change was transmitted to the capillary level. With further elevation of venous pressure to 35 mm Hg, approximately 87% of the increase in venous pressure was reflected to the capillary level. The capillary filtration coefficient averaged 0.028 ml/min per mm Hg per 100 g and was independent of venous pressure.

**EFFECTS OF INCREASED CAPILLARY PRESSURE ON INTERSTITIAL AND LYMPHATIC FORCES**

The effects on interstitial and lymphatic forces of changes in capillary pressure induced by venous pressure elevations are shown in Figure 3. At the “resting” capillary pressure of 12.8 ± 1.2 mm Hg, the interstitial pressure was -4.7 ± 0.8 mm Hg, the plasma oncotic pressure averaged 20.9 ± 2.2 mm Hg, and the lymph oncotic pressure was 3.8 ± 0.5 mm Hg. Thus, the transcapillary filtration pressure or net imbalance of forces across the capillary was very small, averaging 0.5 ± 0.3 mm Hg. Although the numerical value for the filtration pressure at a capillary pressure of 12.8 mm Hg is significantly different from zero (P < 0.05), it should be pointed out that the sum of the experimental errors inherent in determination of the

![Figure 2](http://example.com/figure2.png)

**Figure 2** Effects of venous pressure on capillary filtration coefficient, precapillary (R_A) and postcapillary (R_V) resistance, ratio of pre- to postcapillary resistance, blood flow, and isofiltration capillary pressure in canine hindpaw. Bars represent ± 1 SD; N = 12.

![Figure 3](http://example.com/figure3.png)

**Figure 3** Effect of increased capillary pressure on lymph flow (times control), transcapillary pressure drop, lymph and plasma oncotic pressure, and capsular pressure (interstitial fluid pressure) in canine hindpaw. Bars represent ± 1 SD; N = 12.
Four individual forces probably is larger than 0.5 mm Hg. Consequently, although a small transcapillary pressure drop must exist to account for the filtrate appearing in the lymphatics, it is not possible to determine its absolute value by summing the transcapillary forces. An alternative approach to calculation of the pressure drop across the capillary wall is to divide total lymph flow in the steady state by the prevailing capillary filtration coefficient. Unfortunately, we were not able to collect all of the lymph originating from the hindpaw in the present experiments, hence this approach was not possible.

Following elevation of venous pressure from 5 to 20 mm Hg, a new isofiltration state was reached in 30-50 minutes. In the face of an increase of 12 mm Hg in capillary hydrostatic pressure, interstitial fluid pressure rose by 4.6 mm Hg, lymph oncotic pressure fell by 2.2 mm Hg, and the transcapillary pressure drop increased to 5.6 mm Hg. No significant change in plasma oncotic pressure was observed. The increase in transcapillary filtration rate caused an accumulation of interstitial fluid volume of 1.7 ± 0.4 ml/100 g and lymph flow increased 10-fold from its control value of 8.6 ± 1.4 × 10⁻⁴ ml/min. A further increase of venous pressure to 35 mm Hg disrupted the prevailing isovolumetric state, but a new steady state was achieved 120-160 minutes later. With the consequent elevation of capillary pressure by another 13.1 mm Hg, interstitial fluid pressure rose to +10 mm Hg, lymph oncotic pressure fell to 0.5 mm Hg, and the transcapillary filtration pressure rose to 6.3 mm Hg. Interstitial fluid volume increased to a value 17.5 ml/100 g above the control level, and edema of the hindpaw was observable. Lymph flow increased to 12.8 times the control value. The plasma oncotic pressure was unchanged.

**COMPLIANCE OF THE HINDPAW INTERSTITIUM**

The compliance of the hindpaw interstitium and its dependence on interstitial fluid pressure was quantified by plotting the change in interstitial fluid volume against the instantaneous interstitial pressure. The slope of the volume-pressure relationship reflects the interstitial compliance. As shown in Figure 4A, the compliance of hindpaw interstitium is low (0.36 ml/mm Hg per 100 g) in the negative range of interstitial pressure and increases 5-fold in the positive pressure range. Although the compliance at negative interstitial pressures is similar to data from previous measurements on the whole hindlimb, the hindpaw interstitium remains rather stiff in the positive pressure range when compared to the behavior of the hindlimb as a whole.

The homeostatic significance of interstitial compliance is better appreciated when interstitial fluid pressure is plotted as a function of interstitial fluid volume (Figure 4B). Assuming a normal interstitial fluid volume of 17 ml/100 g of hindpaw soft tissue, only a 2% increase in interstitial fluid volume is required to elevate capillary pressure by 1 mm Hg in the negative pressure region. Following an increase in capillary pressure, the low distensibility of the hindpaw interstitium allows a large counterbalancing rise in interstitial fluid pressure with only small accumulation of interstitial fluid. The efficacy of interstitial fluid pressure in counteracting changes in capillary pressure is significantly diminished in the positive pressure range, since a 9% increase in interstitial volume must occur to elevate interstitial pressure by 1 mm Hg.

**Discussion**

Several compensatory mechanisms potentially are available in any given tissue to prevent the development of gross edema following elevation of venous pressure. As illustrated in Figure 5, these compensatory reactions can be subdivided conveniently into myogenic, interstitial, and lymphatic components of the "margin of safety." The major purpose of the present study was to determine the extent of participation of individual safety mechanisms in providing protection against edema formation in the canine hindpaw when venous pressure is elevated.

**MYOGENIC COMPENSATIONS**

In several tissues of the body, the arterioles and precapillary sphincters constrict when local transmural pressure increases. This intrinsic myogenic vasoconstriction can be elicited by an increase in venous pressure. The myogenic modulation of precapillary resistance and capillary exchange capacity can be viewed as a local mechanism for "autoregulation of transcapillary filtration." Thus, arteriolar constriction serves to prevent dramatic alterations of capillary pressure following elevation of venous pressure. In addition, myogenic reduction of the number of open capillaries increases hydraulic resistance to transcapillary fluid movement. Hence, a rise in capillary pressure can be offset by a compensatory reduction of the capillary filtration coefficient and, consequently, transcapillary fluid flux is maintained near the control level. When present and operating in concert, these myogenic compensations can provide a high degree of protection against the edema-inducing effects of short-term venous pressure elevations.

Our experimental results suggest the absence of signifi-
One possible reason for this discrepancy may lie in the son to the 25-fold increase observed for the entire hindlimb. Pressure measurements were obtained from capsules implanted in subcutaneous tissue, but weight changes of the whole hindlimb were used to calculate interstitial volume changes. Since a large fraction of the whole limb consists of muscle, use of this technique will yield valid results only if the compliances of subcutaneous tissue and muscle interstitium are identical. On the other hand, the physical characteristics of the paw interstitium may actually differ from those of other subcutaneous regions of the hindlimb. If so, the absence of a sharp, marked increase in compliance in the positive pressure region may represent a long-term adaptation of the interstitium to existing hydrostatic influences in dependent areas.

Another potential interstitial safety mechanism is the reduction of interstitial oncotic pressure caused by dilution of proteins following accumulation of fluid in the interstitial spaces. The results of the present study suggest that the reduction of interstitial oncotic pressure following venous pressure elevation is not the result of simple dilution. For example, the 10% increase in interstitial volume at a venous pressure of 20 mm Hg could account for only a 10% reduction of oncotic pressure, yet the interstitial oncotic activity fell to less than 50% of the control level. At a venous pressure of 35 mm Hg, the 100% increase of interstitial volume could explain a reduction of oncotic pressure to 1/5 of control, but the measured oncotic activity was nearly 1/4 of the control level. As discussed below, lymphatic washout of interstitial proteins is the most probable mechanism responsible for this disproportionate reduction of oncotic pressure.

**LYMPHATIC COMPENSATIONS**

Several investigators have observed an augmentation of lymph flow following elevation of venous pressure. Recently, Taylor et al. described a direct relationship between lymph flow and interstitial fluid pressure in the negative pressure region. However, at an interstitial pressure of 1–2 mm Hg, lymph flow tended to reach a maximum value and further elevation of interstitial pressure produced no further augmentation of lymph flow. A similar relationship was obtained in this study (Fig. 3). The direct relationship between lymph flow and interstitial fluid pressure is probably due to (1) acceleration of lymph formation consequent to an increase of the interstitium-to-terminal lymphatic pressure difference, and (2) an increase in the direct lymphatic compensations of the "margin of safety."
myogenic activity (i.e., frequency and force of contraction) of muscular lymphatics in response to stretch of their walls. The plateau of the lymph flow-interstitial pressure curve may represent the maximum pumping activity of the contractile lymph vessels under the conditions of our experiments.

On first thought, it is difficult to conceive of lymph formation vis-à-vis subatmospheric interstitial fluid pressure. Yet, several mechanisms have been proposed to explain this phenomenon. Guyton and Coleman envisioned pulsatile interstitial pressure as the driving force for lymph formation. In this scheme, interstitial fluid flows into the terminal lymphatic during the positive pressure phase of the interstitial pulsation, at which time interstitial pressure is higher than the assumed near-atmospheric pressure within the terminal lymph vessel. Allen has suggested that negative lymph pressure induced during the rebound phase of passive tissue motion may provide the gradient required to drive interstitial fluid into the terminal lymphatics. Indeed, Allen and others have recorded negative pressures in the larger lymph vessels, suggesting the possible existence of markedly negative pressure in the terminal ramifications of the lymphatic system. Another possibility is that the contraction of muscular lymphatics sets up the suction force required to remove fluid from the interstitium. In the latter scheme, recoil of the relaxing muscular lymphatic wall exerts tension on the lymph and thereby induces a negative lymph pressure. At the terminal lymphatic, lymph pressure would be more negative than interstitial fluid pressure. Although circumstantial evidence can be invoked to support each of the above mechanisms, the concepts must remain speculative until definitive measurements of local interstitial fluid pressure and terminal lymphatic pressure can be obtained simultaneously in the canine hindpaw.

The contribution of lymph flow augmentation to the "margin of safety" is critically dependent on the sensitivity of the lymphatics to changes in interstitial pressure or volume. In the hindpaw, the lymph propulsion system is highly sensitive to increases in pressure or volume, or in both, of the interstitium. For example, an increase of 4.6 mm Hg in interstitial fluid pressure, or a 10% rise in interstitial volume, elicits a 10-fold increase in the flow of lymph from the hindpaw. Thus, the efficacy of the lymphatic component of the "margin of safety" is amplified by the low compliance of the hindpaw interstitium.

The importance of lymph flow augmentation in prevention of edema probably can be understood best in terms of the transcapillary pressure drop maintained by the lymphatic pump in the steady state (Figure 6). Under normal conditions, lymph flow is sluggish and the transcapillary forces are not far removed from true equilibrium. However, with an increase of 12 mm Hg in capillary pressure, a 10-fold acceleration of lymph flow dissipates 5.6 mm Hg, or 43% of the capillary pressure rise. If lymph flow had remained constant, the elevation of interstitial fluid pressure and reduction of interstitial oncotic pressure would have been greater by a total of 5.6 mm Hg. Thus, in the absence of lymph flow augmentation, interstitial fluid volume would have increased much more to provide the higher interstitial pressure and lower oncotic activity. Consequently, the lymph vessels serve to dissipate at the capillary wall a fraction of the capillary pressure increase and thereby prevent transmission of the potential effects of this fraction of pressure elevation to the interstitial space.

As mentioned earlier, the acceleration of lymph flow serves to remove proteins, as well as water, from the interstitium. The washout of proteins by high rates of lymph flow is probably the major mechanism for reduction of interstitial oncotic pressure following elevation of capillary pressure in the hindpaw. Thus, interstitial oncotic activity is lowered by clearance of a large fraction of the interstitial protein pool, rather than by dilution of a constant quantity of protein consequent to fluid accumulation in the interstitial space.

**INTERACTION OF CAPILLARY, INTERSTITIAL, AND LYMPHATIC FORCES**

Another objective of the present study was to determine the relative contribution of the individual compensatory mechanisms to the regulation of transcapillary fluid balance at different levels of capillary pressure. As shown in Figure 7, the relative magnitude of individual force changes is dependent on the initial capillary pressure. When capillary pressure is elevated from 12.8 to 24.9 mm Hg, 39% of the total 12.1 mm Hg of compensation is due to elevation of interstitial fluid pressure. Dissipation of pressure at the capillary wall caused by augmentation of lymph flow provides another 43% of the total compensatory reaction.
The remaining 2.2 mm Hg (18%) is a result of the reduction of interstitial oncotic pressure produced mainly by lymphatic washout of the interstitial protein pool. The compensation profile is dramatically altered when capillary pressure is increased from 24.9 to 38 mm Hg, an increase similar in magnitude to the previous perturbation. At this initial capillary pressure, interstitial oncotic pressure already is very low (1.6 mm Hg) and lymph flow is near its maximum rate. Consequently, reduction of oncotic pressure and greater pressure dissipation by the lymphatics now can provide very little compensation when capillary pressure is further elevated. As pointed out above, in the absence of the lymphatic force dissipation mechanism, the total capillary pressure rise is transmitted to the interstitium. In the face of an inadequate interstitial oncotic reserve, this complete transmission of the capillary force increment to the interstitial spaces must necessarily be hydraulic in nature. Thus, a rise in interstitial fluid pressure is the major compensation (93%) at the higher capillary pressure. However, due to the increased compliance in the positive interstitial pressure region, the volume of fluid which must accumulate to (93%) at the higher capillary pressure. However, due to the increased compliance in the positive interstitial pressure region, the volume of fluid which must accumulate to moderate edema.

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Regional Refractoriness within the Ventricular Conduction System

An Evaluation of the "Gate" Hypothesis

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SUMMARY We studied the refractoriness of Purkinje fibers with the intent of localizing critical sites of block of premature impulses. To preserve the ventricular conducting system (VCS) nearly intact in vitro, we used a modification of the Elizari preparation. This was superfused with a physiologic salt solution. Action potential durations increased progressively from the His bundle to the distal Purkinje fibers along three pathways: (1) the main right bundle branch and moderator band; (2) the anterior border fibers of the left bundle and anterior false tendons; (3) the posterior border fibers of the left bundle and posterior false tendons. The action potential durations near the terminations of the false tendons were the longest ones found. The interior fibers of the left bundle branch had action potentials of shorter duration and greater variability than those of simultaneously activated fibers in the right bundle branch or the border fibers of the left bundle branch. Similarly, on the right side, the septal branches of the right bundle had action potentials of shorter duration than those of the moderator band. We also found that the fibers with short action potential durations provided the quickest pathways to septal myocardium. When extrastimuli were applied to the His bundle, block in a bundle branch always occurred in the proximal 1 or 2 cm of the main bundle branch. Experiments performed in vivo in which extrastimuli were delivered to the atrium or His bundle and recordings made from the terminations of false tendons and the distal ends of the main right bundle branch confirmed the finding that the critical sites of block were located in the proximal main bundle branches.

SOME TIME AGO, it was suggested tentatively by Hoffman and co-workers that peripheral Purkinje fibers might be especially prone to block conduction of premature impulses, i.e., that the longest refractory periods might reside in distal sites of the ventricular conduction system (VCS). Later, this suggestion was tested in vitro with certain segments of the bundle branches which included the false tendons. It was found that the durations of action potentials increased distally, attaining maxima near the terminations of the false tendons. The functional refractory periods of these segments of the bundle branches were imposed by the distal sites. By extrapolation, the hypothesis was formulated that there are uniform maxima of refractory periods at peripheral sites throughout the VCS which function collectively as a "gate" limiting the passage of premature impulses originating either within the VCS or within ventricular myocardium. These conclusions derive from data obtained from relatively small preparations of false tendons and adjoining islands of myocardium from the right or left ventricle. Recently, detailed studies of activation of the entire canine left bundle branch in vitro revealed complexities of function of the total structure which would not have been apparent in such dissected preparations. Consequently, it appeared desirable to reexamine the refractory properties of the VCS in vitro, using preparations that preserved the VCS more nearly intact. Under these conditions, the critical site of block of premature impulses was not distal but proximal in the main bundle branches. This finding was corroborated by studies performed in vivo.

METHODS

STUDIES IN VITRO

Twenty mongrel dogs were anesthetized with sodium pentobarbital (30 mg/kg, iv) and their hearts were removed through a right lateral thoracotomy incision. The VCS was isolated by a modification of the method described by Elizari et al. The His bundle was exposed by means of a single, strategically placed cut in the atrial septum aligned...
Interaction of capillary, interstitial, and lymphatic forces in the canine hindpaw.
H I Chen, H J Granger and A E Taylor

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