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TRANSIENT EFFECTS OF HISTAMINE ON THE CAPILLARY FILTRATION COEFFICIENT

By

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ABSTRACT

TRANSIENT EFFECTS OF HISTAMINE ON THE CAPILLARY FILTRATION COEFFICIENT

By

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There have been many reports in the literature on the effect of local intraarterial histamine on the capillary filtration coefficient (CFC). CFC has been reported to increase during infusion of this agent but the reported magnitude of increase is widely variable. To assess if this reported variability was due, at least in part, to some time dependent effect on CFC, CFC was measured in isolated, denervated canine forelimb, hindpaw and gracilis muscle at timed intervals during local intraarterial histamine infused at two different doses (4 and 12 ug base/min per 100 ml/min blood flow). Propranolol (3 mg/kg) was administered to inhibit possible catecholamine mediated inhibition of histamine induced increases in CFC.

At a given dose, the increase in CFC was greatest after 10 minutes of drug infusion and returned to control values by the 25th minute of histamine. These data indicate that the effect of histamine to increase CFC is highly transient.

The relative contributions of increases in surface area and/or permeability to increases in CFC was assessed by maximally dilating the vasculatures of the three tissues with nitroprusside (increasing surface area to a maximum). Any further increase in CFC produced by combined nitroprusside-histamine infusion would then be due to

increased permeability. Both doses of histamine, when infused concomitantly with nitroprusside, produced further increases in CFC relative to CFC obtained during infusion of nitroprusside alone. Although the time course for the transient increase in CFC was similar at both doses of histamine in the three tissues, the magnitude of increase was less at the low dose. It was concluded that histamine transiently increases permeability to fluid in all three tissues and that the high dose produced greater increases in permeability than the low dose.

An equation was derived to estimate the ratio of the number of gaps which form between venular endothelial cells to the number of small pores. It was concluded that less than three percent of small pores need increase in radius to form large pores or gaps with radii ranging from 195 to 1000 angstroms to explain the increases in CFC demonstrated in the hindpaw and gracilis muscle.

DEDICATION

To my wife Mary and son David who showed much patience and understanding. Without their love and support, this thesis would never have been possible.

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INTRODUCTION

Since the discovery of the biological activity of histamine (beta-imidazolylethylamine) in 1910 (11) many types of evidence have accumulated implicating a role for histamine in a variety of physiologic and pathologic processes such as microcirculatory regulation, central nervous system function, tissue growth and repair, gastric secretion and inflammation (13). Of primary interest to this study are those actions of histamine relevant to pathological conditions characterized by abnormal transvascular fluid fluxes.

During the acute inflammatory response, the determinants of fluid transfer across the microvascular wall are markedly altered and produce drastic alterations in transmicrovascular fluid flux. The principal vascular events associated with inflammation include vasodilation, increased vascular permeability and emigration of leukocytes. A variety of evidence has accumulated implicating a role for histamine as a mediator of these events in the inflammatory process (13,132). The supportive documentation includes: (1) the effects of exogenously administered histamine on the vasculature mimic those seen in inflammation, (2) histamine is released in several types of inflammation, (3) anti-histamines are anti-inflammatory.

Local intra-arterial histamine increases fluid filtration and extravascular fluid volume. This effect is attributable to a rise in the transmural hydrostatic pressure gradient, a fall in the transmural colloid osmotic pressure gradient, and an increase in both

microvascular surface area available for exchange and microvascular permeability to filtered fluid (52). Although little controversy prevails among investigators concerning these mechanisms for histamine induced increases in fluid flux, disagreement does exist with regard to the time course of the increase in permeability associated with histamine administration.

Histamine causes an increase in permeability presumably due to a direct action on the microvascular membrane resulting in the formation of gaps between endothelial cells of the postcapillary venules (20,91-94,157). The gaps are widest after 5 to 10 minutes and subsequently close after 15 to 30 minutes suggesting that histamine acts to transiently increase fluid and protein flux from the vascular to interstitial compartment (20,42,91,92,101,102,134,136). However, Renkin and coworkers (19,74,121,122) have presented evidence for a sustained action of histamine on fluid and protein transport based on analysis of lymph flux data.

In addition, there have been many reports in the literature on the effect local intraarterial histamine on the capillary filtration coefficient but the magnitude of increase is widely variable (9,34,39, 43,64,78,97,126,128). It was felt that histamine may have some time dependent effect on the capillary filtration coefficient and that this might explain, at least in part, the variation.

Thus, a primary objective of this investigation was to evaluate the duration of the effect of histamine to increase the capillary filtration coefficient. A second objective was to determine the relative contributions of increases in surface area and permeability to increases in CFC.

The first several sections of the literature review contain material relevant to the anatomical basis of microvascular permeability, microvascular exchange processes and the physical factors regulating fluid exchange. Subsequent sections present background information on the effect of histamine on the determinants of fluid filtration with a particular emphasis on mechanisms and duration of the microcirculatory effects of histamine. Because this dissertation focuses on the effect of histamine on the capillary filtration coefficient in tissues consisting mainly of skin and skeletal muscle, the literature review will pertain to the effect of histamine in such tissues. Where appropriate, however, the effect of histamine on other tissues will be discussed.

LITERATURE REVIEW

I. Anatomical basis of microvascular exchange

The exchange of materials across the microcirculation is thought to occur across the capillaries and immediate postcapillary venules. These vessels consist of a single layer of flattened squamous epithelium (commonly referred to as endothelium) and its supporting basement membrane. A thin adventitia composed chiefly of connective tissue fibers and a discontinuous layer of connective tissue cells surrounds these layers. The basement membrane is an acellular structure consisting of a mucopolysaccharide matrix within which a fine filamentous network of collagen and reticulin fibers are embedded (90). The basement membrane does not represent an impermeable barrier; rather, it can be likened to a chain link fence which allows free exchange of fluid and solute with radii less than 55 angstroms (22,136). In addition, pericapillary cells are associated with the capillaries. These include fibroblasts, histiocytes and pericytes (90). Pericytes have a contractile function and may be involved in regulating microvascular permeability (2,96,124).

Types of endothelium

Three types of capillary endothelium have been described (90). Continuous capillaries represent the most common type of capillary and are found in muscle, skin, the central nervous system, lung and mesentery. The endothelial cells form a continuous layer and

surrounded by an uninterrupted basement membrane. The plasma membranes of adjacent endothelial cells are closely opposed and in places form discrete junctional complexes. The presence of these junctional complexes was purported to present a formidable barrier to the diffusion and ultrafiltration of solutes and water (106). However, recent work has suggested that the intercellular junctions are perforated by channels with radii ranging from 35 to 80 angstroms (16,76,118,120,156).

Fenestrated capillaries are found in organs where there is much fluid transport such as the kidney, small intestine and glands. The fenestrations appear to be pores of about 400 to 800 angstroms in diameter which may be covered by a diaphragm or may actually be open channels across the endothelium. The diaphragm, when present, represents thinned regions of the endothelium containing no cytoplasm. The endothelium of this type of capillary is surrounded by a continuous basement membrane which probably represents the important barrier to solute movements (90).

The capillaries of the liver, spleen and bone marrow are lined by discontinuous endothelium. That is, there are large gaps up to a 1000 angstroms in diameter between the endothelial cells. The basement membrane is also discontinuous or may be absent. Capillaries of this type offer little restriction to the movement of fluid and solute (90).

The immediate postcapillary venules are similar in structure to the capillaries being composed of a single layer of endothelial cells and surrounded by a basement membrane. They can be distinguished from capillaries by their wider diameter (8 to 30 microns) (2,124).

Although the postcapillary venules are morphologically distinct from capillaries, common usage of the term "capillary" often includes these vessels owing to the similarity in their structures. As will be discussed below, exchange of fluid and solute is thought to occur across both the capillary and postcapillary venular endothelium. Thus the terms "microvascular" and "transmicrovascular" exchange are more appropriate than are "capillary" and "transcapillary" exchange. However, the more widely used latter terms are less cumbersome and will be used synonymously in this dissertation.

Pore theory of capillary permeability

The work of Pappenheimer and coworkers (110) and Renkin (116) has provided the foundation for the pore theory of microvascular permeability. According to this theory, water and small hydrophilic substances cross the endothelium through water filled channels or pores under the driving force of hydrostatic and osmotic pressure gradients. This theory has unified many experimental observations and provides the physical basis for molecular sieving.

The mechanism of molecular sieving has been described by Grotte (55) and Arturson (5) and their coworkers who studied the exchange of dextran fractions of graded sizes. Their work indicated that the concentration of macromolecules in lymph was a function of its size and led to the conclusion that the microvascular endothelium contains two sets of pores, the "small" and "large" pore (leak) systems.

The small pore system consists of numerous pores of approximately 35 to 80 angstroms in radius which allow nearly complete protein sieving (16,108,118,120,147). The large pore system provides the main path for exchange of plasma proteins and consists of

relatively infrequent pores with estimated radii of 120 to 300 angstroms (5,55,147). Thus, depending on the large pore in question, partial or no protein sieving is allowed. The large pores are presumed to be present in the venular side of the microcirculation and this coupled with the fact that the area of the postcapillary venules is even greater than that of the capillaries may account for the higher permeability of the venules (4,65,66,82,133).

Early estimates of the ratio of small to large pores ranged between 10,000 to 1 (5) to 34,000 to 1 (55). Thus, it has been presumed that these large pores are quantitatively more important in explaining macromolecular transport whereas the small pores accounted for fluid and small hydrophilic solute exchange. More recent estimates of pore radii, the ratio of the numbers and areas of small to large pores and the fraction of the hydraulic conductivity through the large and small pores in various vascular beds is presented in Table 1. From the data presented in this table, it is apparent that the large pores may also contribute substantially to convective fluid exchange. Indeed, increases in the numbers of large pores during certain interventions probably accounts for the bulk of increased fluid exchange associated with such states (20,91-94,128).

In addition to these extracellular pores, the plasma membrane of the endothelial cell is perforated by very small pores with radii ranging from 4 to 10 angstroms (112). According to Curry (26) and Michel (100) and their coworkers, these exclusive channels for water associated with the endothelial cells account for approximately 10 percent of the total hydraulic conductivity and thus comprise an important path for water transfer.

Table 1. Predicted small and large pore radii (R small and R large respectively), fraction of hydraulic conductance through small (F small) and large (F large) pores, and ratios of small to large pore areas and numbers in several capillary beds. F other refers to the fraction of hydraulic conductance not accounted for by small and large pores. * This value probably represents dilution effects caused by superfusion of muscle. (Modified from reference 147, hindpaw data obtained from reference 148).

Organ	Specie	R small (A)	R large (A)	F small	F large	F Other	Ratio of small to large pore areas	Ratio of small to large pore numbers
Paw	Dog	47	195	0.820	0.130	0.050	114:1	2064:1
Lung	Dog	80	200	0.800	0.160	0.040	31:1	195:1
Skeletal muscle	Rat	67	220	0.650	0.018	0.330*	361:1	3610:1
Small intestine	Cat	46	200	0.900	0.050	0.050	340:1	6400:1
Liver	Cat	90	330	0.200	0.800	--	3.4:1	46:1

Morphologic correlates of the small and large pore systems

The identification of the morphologic correlates of these two pore systems is currently an area of intensive investigation and debate. It has been postulated that the endothelial junction is the site of the small pore (76,156). Other investigators conclude that the small pores are not located at the endothelial junction but rather at vesicular channels formed by confluence of chains of vesicles (107,137). The large pores may be represented by the micropinocytotic vesicles whose inner diameter averages 250 angstroms, a value in close agreement with most large pore estimates (136,151). In addition, patent transendothelial chains of vesicles free of size limiting structures (strictures and diaphragms) would possess an internal radius of 250 angstroms and thus could serve as large pores (137). A third possibility would be large (200 to 400 angstroms) interendothelial gaps. This possibility is commonly criticized on the grounds that such large gaps should be readily apparent upon electron micrographic examination of the microcirculation yet they have not been identified. However, the relative paucity of these large pores would make electron micrographic isolation difficult (69).

II. Exchange Processes

The exchange of materials across the microvascular walls is thought to occur by three distinct processes: (1) diffusion, (2) vesicular transport (micropinocytosis, cytopemphis) and (3) convection (bulk flow) (58).

Diffusion

The diffusive process represents the most fundamental mechanism by which almost all small solute exchange between the vascular and extravascular compartments occurs. The importance of diffusion as a primary mechanism for macromolecular exchange is controversial however. Numerous investigators contend that macromolecular exchange occurs primarily by the diffusive process (113,117,123) while others hold that it occurs primarily by bulk flow (63,85,126,127,129,147).

Diffusion results as a consequence of the random kinetic motion of individual molecules or ions. The rate of diffusion is dependent on several factors including the permeability of the microvascular wall and the nature of the diffusing substance and its solvent, the area of the microvascular wall available for exchange and the blood-interstitial fluid concentration gradient for the substance. The interrelation among these factors is expressed in equation form as Fick's Law of Diffusion:

$$ds/dt = DA(dc/dx)$$

where:

ds/dt = quantity of substance moved per unit time,

D = free diffusion coefficient of the diffusing substance, proportional to the inverse of the square root of the molecular weight of the substance,

A = area of the microvascular membrane available for diffusion,

dc/dx = concentration gradient

Fick's Law can also be expressed as:

$$ds/dt = PS(\Delta c)$$

where:

P = microvascular permeability of the diffusing substance,

S = area of the microvascular membrane available for diffusion,

Δc = concentration of the substance in the microvasculature minus the concentration of the substance outside the microvasculature.

For small molecules, such as water, ions, urea and glucose, diffusion is free and rapid resulting in little transmicrovascular concentration gradient. However, as the size of lipid insoluble molecules approaches that of the pores, the diffusion of these substances becomes progressively more restricted. Lipid soluble molecules, such as oxygen, carbon dioxide and anesthetic gases, pass freely through the plasma membranes of the microvascular endothelium. Consequently, these molecules pass with great rapidity between the plasma and interstitial fluid.

Vesicular transport

Vesicular transport (micropinocytosis, cytopemphis) has been suggested by several investigators (15,19,74,107,121,122,136,137,151) as a mechanism for the transport of large lipid insoluble molecules across the microvascular wall. According to the theory of vesicular transport, vesicles form as invaginations of the plasma membrane and are pinched off to form small cytoplasmic vesicles which diffuse to the opposing cell surface and discharge their contents (15). It should be emphasized that all components of the plasma and interstitial fluid except those that are too large to enter the

vesicles may be exchanged by this mechanism. However, owing to the approximate equal concentrations of small solutes in the plasma and interstitial fluid, vesicular transport is quantitatively important only for the plasma proteins (117). In addition, the population density of vesicles increases from the arteriolar to venular end of the microcirculation and thus may account, at least in part, for the observed higher permeability of the venular end of the microcirculation (151).

It should be pointed out that very recent evidence strongly suggests that vesicular transport is unlikely to occur (17,47). The organization of vesicular profiles in rat heart (17) and frog mesenteric (47) capillaries was reinvestigated in these studies. From examination of random thin sections, 50 percent of the vesicles appeared free in the cytoplasm with the rest opening to the surfaces of the endothelial cells. This result was in accord with many previous observations (15,107,136,151). However, three dimensional reconstruction of ultrathin sections revealed that all vesicles were actually parts of the endothelial cell membrane as caveolae or more complex invaginations and not free in the cytoplasm. These results imply that transendothelial vesicular transport is unlikely to occur.

Convection

In 1896, E. H. Starling presented the hypothesis that filtration of fluid out of the capillaries is opposed by the increasing colloid osmotic pressure of the blood, finally resulting in absorption of fluid at the venular end of the capillaries. He also described for the first time, the basic physical forces governing the movement of fluid across the microvascular walls (141).

According to Starling's classic hypothesis, the capillary hydrostatic pressure (Pc) and colloid osmotic pressure of the tissue proteins (πt) determines filtration into the tissues and the tissue hydrostatic pressure (Pt) and colloid osmotic pressure of the proteins in the capillary (πc) determines absorption from the tissues. The balance of fluid between the vascular and extravascular compartments is a result of the interrelation between these hydrostatic and osmotic forces. Landis (80) later verified Starling's hypothesis with measurements made in single capillaries in frog mesentery.

Staub (142), in a recent review, indicates that the first mathematical formulation of the original Starling hypothesis was presented by Iverson and Johansen (70) in 1929 as

$$Pc - Pt = \pi c - \pi t$$

This expression was later modified to include the permeability characteristics of the blood-tissue interface so that the volume flux across the microvasculature can be described by (77,109):

$$F = k[Pc - Pt - \sigma(\pi c - \pi t)] \qquad (1)$$

where k represents the capillary filtration coefficient and σ represents Staverman's osmotic reflection coefficient. The quantity in the brackets is termed the net filtration pressure. Filtration occurs when F is positive and absorption when F is negative.

Capillary hydrostatic pressure is the principle force acting to move fluid out of the blood into the extravascular compartment. It represents the force applied to the capillary wall by the kinetic impact of fluid molecules in plasma divided by the area of contact. It is directly dependent on capillary blood volume and compliance.

Numerous studies indicate that the capillaries are quite rigid (8,105,138). That is, changes in transmural pressure appear to have little effect of capillary diameter. This might be expected because tension in the wall of capillaries is very small as result of their small radius (18). In addition, they are surrounded by a basement membrane and are embedded in the surrounding tissue gel which limits the movement of the wall (48,105). Consequently, capillary blood volume is the primary factor determining capillary pressure.

Capillary blood volume is regulated by mean arterial blood pressure (Pa), venous pressure (Pv), and the pre- and postcapillary resistances (Ra and Rv respectively). These factors are related by the following equation originally derived by Pappenheimer and Soto-Rivera (109):

$$Pc = \frac{Pa(Rv/Ra) + Pv}{1 + (Rv/Ra)} \qquad (2)$$

In this formulation, Pc is attributable to a single point, with all hemodynamic resistance either upstream (Ra) or downstream (Rv) from this point. However, there is no one anatomical site which could be described as the location of Pc under all conditions. In fact, Pc is not the same in all capillaries within an organ. For example, Pc in one capillary may favor filtration whereas in an adjacent capillary, Pc may be lower and favor absorption.

In any event, it is evident from equation 2, that an elevation in mean arterial pressure, venous pressure or venous resistance, or a reduction in arterial resistance results in an increase in capillary hydrostatic pressure, all other variables remaining constant. However, it is unusual for any physiologic or pharmacologic

intervention to affect only one of the variables. In many cases one stimulus will affect more than one of the variables in such a way as to produce opposing influences on capillary hydrostatic pressure. For example, if mean arterial pressure is lowered, this would tend to reduce capillary hydrostatic pressure. However, arterial resistance frequently decreases in response to a decrease in mean arterial pressure (autoregulation) so that the net effect on capillary hydrostatic pressure may be minimized.

Tissue pressure or the hydrostatic pressure in interstitial fluid is analagous to capillary hydrostatic pressure in that it represents the force applied to the wall of the capillary by the impact of fluid molecules in the interstitium divided by the area of contact. The classical view is that interstitial fluid is slightly positive and therefore opposes filtration. However, recent information indicates that tissue pressure is slightly negative in a variety of tissues (38,57,59,115,155). However, the magnitude as well as the sign of tissue pressure is the subject of considerable controversy (14,59) and further investigation is needed to resolve this problem.

Microvascular colloid osmotic pressure or oncotic pressure is the pressure due to dissolved protein in plasma and averages about 28 mm Hg in man. The total osmotic pressure of plasma is the pressure due to the presence of all the dissolved components of plasma and averages about 5500 mm Hg when measured relative to water across a semipermeable membrane. Even though the oncotic pressure represents only a small fraction of the total osmotic pressure, it is the former which is of prime importance in determining the movement of fluid across the microvascular wall. To understand why this is so, it is

important to remember that the plasma proteins are the only components of plasma which do not readily gain access into the extravascular compartment.

Therefore, the concentration of protein in the plasma is approximately four times that in the interstitial fluid whereas there is little transcapillary concentration gradient for the electrolytes which determine the bulk of the total osmotic pressure in the plasma and interstitial fluid (58).

The principal plasma proteins are albumin with an average molecular weight of 69000 daltons, globulins, 140000 daltons, and fibrinogen, 400000 daltons. Therefore one gram of albumin contains twice the number of molecules contained in one gram of globulins and six times the number of molecules contained in one gram of fibrinogen. Furthermore, the concentration of albumin is twice that of globulins while fibrinogen has a relatively negligible concentration. Thus nearly 75 percent (22 mm Hg) of the colloid osmotic pressure of plasma is due to the presence of albumin. The colloid osmotic pressure of plasma is almost 50 percent greater than that exerted by the plasma proteins alone. This results from the Donnan effect. That is, the proteins in plasma are negatively charged molecules and as such attract a large number of positively charged ions, mainly sodium. These sodium ions increase the number of osmotically active particles in plasma and therefore the osmotic pressure. Even more important is the fact that this so-called Donnan effect becomes progressively more pronounced as protein concentration is increased (58).

Interstitial colloid osmotic pressure is analogous to the oncotic pressure of plasma; ie, it is determined by the concentration of dissolved protein in the interstitial fluid. However, the sieving characteristics of the microvascular barrier restrict the movement of protein into the interstitium so that only small quantities of protein leak into the extravascular compartment. This results in a lower number of osmotically active particles in the interstitial fluid relative to plasma resulting in a colloid osmotic pressure of 5 mm Hg. Wiederhielm (153,154) and Comper and Laurent (24) point out that the oncotic pressure of the interstitial space may be much higher than this due to the presence of hyaluronic acid in the tissue. In addition, the tangled matrix-like structure of the interstitium acts to exclude large molecules from portions of the tissue (24). If the size of the spaces between the matrix fibers were on the order of capillary small pore sizes, as has been suggested for rat mesentery (41), molecular sieving would occur (119). Thus, the gel like nature of the interstitium may increase the effective concentration of protein in the extravascular space.

Most investigators studying the regulation of transmicrovascular fluid movement have emphasized the importance of intracapillary forces. From the foregoing discussion, it is apparent that interstitial hydrostatic and colloid osmotic forces may be of considerable importance in maintaining fluid balance.

The osmotic reflection coefficient represents a descriptive term introduced by Staverman in 1951 (143) to indicate the fraction of solute molecules which approach the pores of a semipermeable membrane and are reflected back. It is defined as the ratio of the observed

osmotic pressure to that predicted by Van't Hoff's law. It is equal to one if the membrane is impermeable to solute and equal to zero if the membrane is freely permeable.

The capillary filtration coefficient (k, CFC) represents the transmicrovascular hydrodynamic conductivity which, in turn, is a product of the microvascular surface area available for exchange (Am) and microvascular permeability to filtered fluid (Lp or hydraulic conductivity) (40,83). The filtration coefficient can be represented in equation form as:

$$CFC = (Am)(kt)/(n)(\Delta x) = (Am)(Lp)$$

where:

Am = area of the membrane available for filtration,

kt = filtration constant,

n = viscosity of the ultrafiltrate,

Δx = path (pore) length.

Because n and Δx are relatively constant, they are included in the hydraulic conductivity (Lp) or permeability term of the filtration coefficient (83). From this equation it is apparent that the filtration coefficient is influenced by several factors. These include the number of open capillaries, pore radius, number, and length, and the viscosity of the filtrate.

Wiederhielm (154) has demonstrated that the filtration coefficient at the arterial end of the capillary is only one sixth that of the venous end. Therefore, determinations of the capillary filtration coefficient represent a weighted average of CFC along the

capillary. The higher filtration coefficient at the venular end is attributable to the greater surface area and permeability of the venular end of the microcirculation, as pointed out above.

Because this dissertation focuses on the effect of histamine on the capillary filtration coefficient, it is appropriate to consider problems associated with its determination.

In most studies, the microvascular filtration coefficient is determined by gravimetric (109) or volumetric (98) techniques. With these techniques, the tissue under investigation is placed on a weighing device or in a plethysmograph. Arterial and venous pressures are adjusted such that the organ is isogravimetric (isovolumetric); that is, the Starling forces are in equilibrium and no net transvascular flux of fluid occurs. Venous pressure is suddenly elevated. The ensuing increased filtration rate is composed of two phases: an initial rapid increase in tissue weight attributable to vascular volume changes and a slower component attributable to filtration of fluid from the vascular to the interstitial compartment; a result of increased microvascular pressure and consequent disturbance of the Starling equilibrium (98,109). The filtration coefficient is determined by dividing the rate of weight or volume gain by the change in microvascular pressure and is expressed in ml fluid gained per minute per mm Hg transmicrovascular pressure gradient per 100 grams of tissue.

From the method of determination, it is apparent that the gravimetric (volumetric) techniques suffer from three fundamental problems. First, it is difficult to assess the endpoint of the vascular distension phase. That is, the magnitude of the elevated

filtration rate induced by increasing venous pressure may be obscured by intravascular volume changes associated with stress relaxation (visco-elastic creep or delayed compliance) of the veins (32,44,71,99). In order to distinguish between changes in vascular volume and transcapillary fluid movement, investigators have simultaneously measured tissue volume changes gravimetrically or volumetrically and vascular volume by indicators such as Cr^{51} labelled red cells (9,32). Results from these studies indicate that the slow component of weight or volume gain is solely attributable to transcapillary fluid filtration. Menninger and Baker (99) have criticized these results on the grounds that the labelled red cells are not accessible to all parts of the vasculature. That is, red cells do not enter many capillaries due to plasma skimming or because various segments of the capillary bed are closed due to precapillary constriction. These investigators suggest that a more appropriate approach to this problem would be to devise a method to independently assess transcapillary filtration. By comparing the volume of fluid filtered from plasma with such a method with changes in total tissue volume (gravimetric or volumetric technique), it would be possible to determine if the slow component was entirely due to transcapillary fluid transfer. Fluid transfer in splenectomized dogs was assessed by measuring changes in hematocrit since such changes reflect blood-tissue fluid transfer. By either method, however, it has been shown that the bulk of the vascular volume shift is complete within 30 to 60 seconds (9,32,99).

A second criticism of the gravimetric technique for determination of the filtration coefficient is that a continual readjustment of the Starling forces occurs as a result of fluid filtration (40,125). The movement of fluid from the blood to the tissues increases capillary oncotic pressure and tissue volume. The increase in tissue fluid volume increases tissue pressure and decreases interstitial oncotic pressure. The net effect of these readjustments is that net filtration pressure and thus filtration rate is reduced leading to a considerable underestimate of CFC. Such readjustment should be a slow process in tissues such as skin and muscle where interstitial compliance is high and tissue oncotic pressure low (7) or if CFC is low (125). Indeed, Pappenheimer and Soto-Rivera (109) have shown that fluid filtration induced by step increases in venous pressure is constant for at least forty minutes in isolated cat and dog hindlimbs. In tissues characterized by low compliance or high oncotic pressure or high filtration coefficient, this problem can be obviated by use of the zero time extrapolation technique of Drake and coworkers (37).

Third, since the rise in microvascular hydrostatic pressure in filtration coefficient determinations is accomplished by elevating venous pressure, an assumption must be made about what fraction of the increase in venous pressure is transmitted to the microvascular bed (40). A knowledge of the pre- to postcapillary resistance ratio is necessary if a quantitative estimate of this correction factor is to be made. In most cases, investigators either assume a constant pre- to postcapillary resistance ratio in the vicinity of 0.75 or do not introduce this correction. However, this does not appear to represent

a major problem because the filtration coefficient is relatively insensitive to this parameter (23). In addition, a recent report has proposed an approach which does not require knowledge of this ratio (78a).

Friedman (45) and Johns and Rothe (71) have raised the objection that increased venous pressure causes significant protein leakage into the tissues resulting in an enhanced filtration rate and consequently an overestimate of CFC. However, Richardson and coworkers (125) have calculated that upon elevation of venous pressure, the increased protein flux across the microvascular wall accounts for only 0.1 to 3 percent of the total volume flux. Also, Landis (84) detected very little protein loss from single capillaries when venous pressure was elevated as much as 60 mm Hg. Therefore, enhanced protein leakage does not represent a significant source of error in the determination of the capillary filtration coefficient.

Another problem, inherent in some tissues and not others, involves the fact that the elevation of venous pressure during CFC determinations elicits a venous-arteriolar reflex whereby precapillary resistance increases thereby altering the pre- to postcapillary resistance ratio and decreasing the number of perfused capillaries and attendant reductions in CFC (40,125). This effect is most pronounced at higher venous pressure elevations (72,103). However, in most studies the extent of error is minimized because the induced rise in venous pressure is standardized and quite small (40). In some tissues, a venous-arteriolar reflex is not present and CFC is independent of the level of venous pressure elevation (34,109).

It has been suggested that the induced increase in venous pressure for estimation of the filtration coefficient is transmitted not only to open capillaries but to capillaries closed by "precapillary sphincters" as well (86,126). Because there is no evidence that the microvasculature is closed at the venous end, these capillaries fill retrogradely. However, Aukland and Nicolayson (7) have calculated that filtration in these closed capillaries would be complete within one minute. Thus, any error which might be introduced by this mechanism would occur during the vascular volume shift and would not influence CFC determinations because the rate of filtration is not determined during this time.

Another objection to the gravimetric (volumetric) determinations of CFC is that the induced rise in venous pressure might stretch the pores in the microvascular walls (111,135) and thereby increase the permeability to filtered fluid leading to an overestimate of CFC. However, much work has indicated that large increases in capillary pressure do not alter diameter of capillaries and postcapillary venules (8,105,138) or the radii of the pores (78,88).

Finally, it should be emphasized that changes in the capillary filtration coefficient can, by definition, be due to either a change in microvascular surface area or permeability or both. Because of this, it is difficult to relate changes in the filtration coefficient induced by physiologic and pharmacologic interventions to changes in permeability or surface area alone.

There are several approaches to gaining information about changes in capillary permeability independent of changes in surface area. First, CFC can be determined when the vasculature is maximally

vasodilated, which should give a maximum surface area so that any further increase in CFC can be attributed to a change in permeability (9,64,126,128,140). Another approach to independent determinations of microvascular permeability is to measure concentrations of macromolecules in lymph draining the organ in question. It is assumed that if no change in relative concentrations of macromolecules of different sizes occurs, no change in permeability has occured. The problem with this approach is that except in pathological conditions the transendothelial route for macromolecular transport is probably not identical to that for the ultrafiltration of plasma. The most elegant approach to the independent analysis of permeability and surface area is that provided by Pappenheimer and coworkers (83,108,110). By measurement of both the capillary filtration coefficient and diffusional exchange of small solutes across the microvascular membrane, equivalent pore size can be determined.

III. Effect of histamine on microvascular fluid exchange

During the acute inflammatory response, the determinants of fluid transfer across the microvascular wall are markedly altered and produce drastic changes in transmicrovascular fluid flux. The principal vascular events associated with inflammation include vasodilation, increased vascular permeability and the emigration of leukocytes. Histamine is one of several compounds that have been proposed as mediators of the vascular events associated with

inflammation because its actions on the vasculature mimic those seen in inflammation, it is released in several types of inflammation and because antihistamines are anti-inflammatory (13,132).

Histamine's ability to increase permeability was initially reported by Dale and Laidlaw (27) and Sollman and Pilcher (139). However, the first detailed description of its ability to cause vasodilation, hypotension and increase vascular permeability came from Dale and Richards (28). This was followed by the work of Lewis and his coworkers on the "triple response" and the role of histamine in inflammation. Lewis (87) observed that stroking of the skin of the human forearm with a blunt edge produced a characteristic triad of signs: an initial vasodilation of the microcirculatory vessels along the line of the stroke followed by a dilation of the neighboring vessels and finally, wheal formation along the line of the stroke. A similar triple response was evoked by electrical, mechanical, chemical and thermal stimuli as well as by introduction of histamine into the skin. Lewis concluded that the vascular events associated with these inflammatory stimuli were mediated by histamine itself or a closely related factor which he designated "H substance".

Since the initial studies by Dale and Lewis and coworkers, the actions of histamine on the circulation have been extensively studied and debated.

Several types of evidence indicate that histamine increases fluid filtration and extravascular fluid volume. In many vascular beds, local intra-arterial administration of histamine over a wide range of doses (3-64 ug/min) greatly increases net transvascular fluid flux resulting in increased weight, volume and circumference of the tissues

in question (9,30,52,61,78,126,128). Medium to large doses (12-64 ug base/min) result in edema evident upon visual examination (52). The increase in weight, volume and circumference can only be partially attributed to increased vascular volume subsequent to arteriolar vasodilation since these changes exceed those seen with maximal vasodilation (52). Furthermore, studies in which simultaneous determinations of vascular volume and fluid filtration were made indicate that following the initial increase in vascular volume associated with vasodilation no subsequent changes in vascular volume occur. In contrast, fluid filtration as indicated by increasing tissue weight or volume continued (9,32). Histamine increases the rate of lymph flow in canine forelimb (51,62,79), hindpaw (19,74,121,122) and intestine (104) indicating that filtration is elevated and that increased tissue volume does not result from decreased lymph outflow.

The increased rate of net fluid filtration associated with locally administered histamine is attributable to a rise in the transmural hydrostatic gradient, a fall in the transmural colloid osmotic pressure gradient and an increase in both the microvascular surface area available for exchange and permeability to filtered fluid.

Direct intra-arterial administration of histamine reduces resistance and increases flow in a variety of tissues including forelimb (52,60-62,78a,79), hindlimb (31,35), skeletal muscle (9,78) and adipose tissue (43). Measurement of segmental pressures and resistances indicate that histamine's effect is primarily on small vessels in contrast to large arteries and large veins (30,52,60,61).

Presumably most of the effect is on arterioles because the magnitude of the resistance fall is quite large. Microcirculatory studies confirm this (2,3). The vasodilator effect of histamine is at least partly a result of formation of prostaglandins because the vasodilation caused by histamine is reduced in the presence of cyclo-oxygenase inhibitors (149). Prostaglandins either do not increase microvascular permeability (29,49) or increase it very weakly compared to histamine (46,145,150). Thus, it appears that prostaglandins do not mediate this effect of histamine.

The marked filtration which results from intra-arterial histamine administration suggests a rise in capillary pressure again presumably due to a fall in precapillary resistance. Indeed, direct measurements of capillary hydrostatic pressure in human skin demonstrate a rise in capillary pressure during histamine (81). In other organs a rise in capillary hydrostatic pressure can be inferred from an elevation in small vein pressure which must represent a minimum for capillary pressure (30,52,60,61,130). Judging from these results capillary hydrostatic pressure may increase as much as 20 to 30 mm Hg. The rise in small vein pressure appears to be primarily a result of increased flow rather than increased venous resistance (52). The evidence for this is that infusion of histamine at a relatively low dose (5 ug base/min) into canine forelimbs perfused at constant pressure results in large increases in small vein pressures (from approximately 13 mm Hg at control to 18 mm Hg during histamine) and limb weight (approximately 25 g in 30 minutes). When histamine at this dose was infused into forelimbs perfused at constant flow, small vein pressure was not changed relative to control and the increase in

limb weight was greatly attenuated (approximately 7 grams in 25 minutes) (52). Presumably the gain in limb weight in the constant flow study was attenuated because the rise in capillary pressure was prevented by such a preparation. In addition, calculations of postcapillary resistance in muscle suggest a decrease not an increase in this variable (31,34). These data are consistent with microcirculatory data indicating that although the precapillary vessels are more sensitive to histamine than are the postcapillary vessels, both dilate (2,3).

The increased transmural hydrostatic pressure gradient will wane with time owing to increased tissue volume and consequent increased tissue pressure. However, compliance measurements in skin (155), subcutaneous tissue (57,59) and muscle (38,115) indicate that the interstitium of these tissues is highly compliant and can accomodate increases in tissue volume up to twenty percent with very little change in interstitial fluid pressure. In fact, Pappenheimer and Soto-Rivera (109) have demonstrated that filtration rates following step increases in venous pressure are constant for forty minutes in the hindlimbs of cats and dogs indicating that a readjustment of the Starling forces has not occurred.

Much evidence has accumulated in recent years indicating that in addition to increasing the transmural hydrostatic pressure gradient, histamine also acts to increase fluid filtration by decreasing the transmural colloid osmotic pressure gradient. The reduction in the oncotic gradient arises from an increased interstitial oncotic pressure owing to an increased rate of leakage of protein from the vascular compartment into the interstitial space.

Much indirect evidence supports the concept that tissue colloid osmotic pressure is increased following histamine administration. Majno and coworkers (91-94) have provided electron micrographic evidence that histamine injected subcutaneously over a dose range of one to 28 ug increases the deposition of colloidal carbon particles on the basement membrane of postcapillary venules. Studies utilizing intravital microscopy of the hamster cheek pouch microcirculation indicate that superfusion of the pouch with a solution containing 10^{-5} molar histamine greatly increased the leakage of fluorescein labelled dextran from the postcapillary venules (146). It has also been demontrated that histamine markedly increases the rate of transport of radioactive and other dye labelled protein (6,12,97,101,134).

Another approach which suggests that the transmural colloid osmotic pressure gradient decreases with histamine administration is to measure the concentration of proteins (19,51,62,74,78a,79,121, 122,130) or exogenously administered dextrans (19,74,121,122,146) in the lymph during histamine administration. Results from such studies demonstrate a marked elevation in the concentration of these macromolecules in lymph relative to control. At higher doses of histamine (16-64 ug base/min), the concentration of macromolecules in lymph approaches that of plasma again indicating histamine may cause a marked reduction in the transmural colloid osmotic pressure gradient (19,62,74,121,122,130).

Histamine when infused into isolated canine forelimbs over a dose range of 6.8 to 68 ug/min produces a dose dependent fall in isogravimetric capillary pressure (Pci) from 10.7 mm Hg at control to 7.7 mm Hg at the highest dose (36). McNamee and Grodins (97) reported

a much larger fall in Pci (18.6 to 4.5 mm Hg) during perfusion of dog gracilis muscle with blood containing 3.3 to 5 ug histamine/ml blood. Since Pci represents the net sum of all forces opposing filtration (i.e., $Pci = Pt + \sigma(\pi c - \pi t)$), these results suggest a large fall in the oncotic pressure gradient or a decrease in the reflection coefficient or both.

Finally, histamine can increase tissue volume under conditions when capillary pressure is unchanged and only slightly exceeds plasma colloid osmotic pressure. In dog forelimbs perfused at constant pressure, infusion of high doses of histamine (60 ug base/min), increases limb weight by approximately 75 grams in 30 minutes (52). In a similar preparation perfused at constant flow, limb weight increased approximately 68 grams in 30 minutes (52). As discussed previously, administration of histamine leads to an increase in capillary pressure of 20 to 30 mm Hg during constant pressure perfusion. However, capillary pressure during constant flow perfusion is unchanged during histamine yet the weight gained by the forelimb in both preparations was similar. Furthermore, the forelimbs continued to gain weight when perfused at pressures of approximately 20 to 25 mm Hg, a value less than plasma colloid osmotic pressure (52). These data clearly indicate that histamine, at least at high doses, can increase fluid filtration relatively independent of changes in capillary pressure by decreasing the transmural colloid osmotic pressure gradient.

The increased protein efflux and interstitial fluid colloid osmotic pressure is attributable to a direct action of histamine on

the microvascular membrane to increase permeability (1,19,20,36,51,52, 62,74,91-94,97,121,122,157). Electron micrographic evidence suggests that histamine acts to induce the formation of venular interendothelial gaps (20,91-94,157) thereby increasing the number of large pores.

These morphologic observations are supported by physiologic data which demonstrate that histamine decreases the sieving characterstics of the blood-tissue interface such that the concentrations of all the plasma proteins or exogenously administered dextrans in the interstitial fluid increase (19,51,62,74,78a,79,121,122,130,146). In addition, the osmotic reflection coefficient for plasma proteins is decreased from a control of 0.9 to 1 to approximately 0.4 to 0.6 during histamine (36,97,104).

The mechanism of this increase in microvascular permeability is uncertain. It has been suggested that histamine induces venular interendothelial gap formation (20,91-94,157) by causing contraction of actomyosin-like fibrils within the venular endothelial cells resulting in their rounding up thereby effectively increasing the radius of the intercellular cleft to form large pores or "leaks" (93-94). Both the size and the number of these "leaks" increase with histamine in a dose dependent fashion (56). There is no evidence that histamine affects small pore radii. In fact, Diana and coworkers (34) have shown that effective small pore radii are unaffected by histamine.

Renkin and coworkers (19,74,121,122) have challenged the concept of increased pore size as an explanation of histamine's action to reduce the selectivity of the blood-lymph barrier. In their studies,

histamine acts to increase lymph flow and macromolecular concentration and macromolecular transport. Further, they demonstrated that increasing venous pressure during the administration of histamine does not alter the rate of macromolecular transport. Since macromolecule tranport via pores is thought to be sensitive to pressure whereas the vesicular transport of large solute is not, they suggested that histamine increases both the rate of vesicular transport across the microvascular endothelium and doubles the size of the vesicles. This concept is supported by the studies of Alksne (1) who showed that following the application of histamine, colloidal mercuric sulfide particles were present in vesicles whereas they were not present in the control state suggesting that vesicle radius had increased. However, the more recent electron micrographic studies of Casley-Smith and Window (20) demonstrate that neither vesicle diameter nor number increase during histamine. Although the number of vacuoles within the endothelium increased with histamine, their role in the transport of material is uncertain (20). Also protein transport is augmented in the dog forelimb if venous pressure is elevated during histamine infusion (51). This data is more compatible with the concept that histamine acts to form large pores or leaks rather than increasing the rate of vesicular transport. In addition, very recent evidence suggests that vesicular transport is unlikely to occur (17,47). Clearly, more studies are needed to resolve this controversy.

Intra-arterial histamine increases the capillary filtration coefficient in most (9,34,39,43,64,78,97,126,128) but not all (68) tissues. This implies that an increase in the microvascular surface area available for filtration and/or an increase in microvascular

permeability to filtered fluid occurred. Further, measurement of CFC provides a direct measure of the rate of fluid transfer across the microvascular walls per unit transcapillary pressure difference. From the foregoing discussion, it is apparent that both the rise in transmural hydrostatic pressure gradient and the fall in the transmural colloid osmotic pressure gradient associated with histamine administration contribute to a substantially elevated net filtration pressure and consequently increases the filtration rate. The rise in microvascular surface area and/or permeability implied by increased CFC augments the effect of elevated net filtration pressure on fluid filtration.

In almost all tissues, histamine increases the capillary filtration coefficient. However, the reported magnitude of increase in CFC is widely variable. For example, Kjellmer and Odelram (78) reported a six-fold increase in CFC of cat gastrocnemius muscle during infusion of 27 ug/min histamine. Fredholm and coworkers (43) reported an average 2 fold increase in CFC in canine subcutaneous adipose tissue during infusion of 75 ug/min/100g histamine. Diana and coworkers (34) noted a two-fold increase in CFC during infusion of 20 to 60 ug/min histamine into isolated dog hindlimbs. Rippe, Grega and coworkers (126,128) measured a three fold increase during infusion of histamine at 30 to 60 ug/ml perfusate into maximally dilated rat hindquarters. McNamee and Grodins (97), using isolated dog gracilis muscle, reported a 36 fold increase in CFC when 3.3-5ug histamine/ml was added to the perfusate. Baker (9) utilized a similar preparation and measured a 7.5 fold increase when histamine was infused at 5 ug/kg/min. Flynn and Owen (39) measured CFC during infusion of 3

ug/kg/min histamine into isolated skinned cat hindlimbs and found CFC increased by two fold. Finally, Haraldsson and coworkers (64) reported a 3 fold increase in CFC during perfusion of maximally dilated pancreatic glands in juvenile pigs with histamine at 50 uM.

Histamine is a vasodilator; therefore, increases in CFC are almost certainly attributable at least in part to increases in microvascular surface area. Because histamine increases CFC in vascular beds already maximally vasodilated with papaverine (9,64,126,128), it seems likely that a significant fraction of the increase in CFC caused by histamine results from an increase in capillary permeability. However, there is no evidence that small pore radius is increased by histamine (34). Rather, it appears the increase in permeability is mediated by an increased number of large pores (20,64,91-94,126,128).

The difference in reported magnitudes of change in CFC may be attributed to differences in species, tissues, dosages and/or time of measurement after the onset of histamine administration. Surprisingly little attention has been paid to possible transient effects of histamine on transvascular fluid exchange and the results of such studies are inconclusive although the bulk of the evidence favors the view that histamine induces transient rather than sustained increases in fluid and protein transport.

Micrographic studies demonstrate that histamine administered by subcutaneous injection (91,92,94) or by topical application (1,20,93) results in a transient widening of venular interendothelial clefts, increasing the number of large pores. One study also suggests that

extremely high doses of histamine cause clefts to open between capillary endothelial cells (114). The gaps are widest after 5 to 10 minutes and subsequently close after 15 to 30 minutes (20,42,91,92, 101,102,134,146).

Studies using intravital fluorescence microscopy also indicate that histamine transiently increases the permeabiity of venular endothelium. Leakage of fluorescein labelled albumin was increased following superfusion of cat and rat mesentery with histamine at 0.1 to 100 ug/ml superfusate, an effect which largely abated after 30 minutes (42). Similarly, increased leakage of fluorescein labelled dextrans was reported in the hamster cheek pouch during superfusion with histamine at 10^{-5} molar for four minutes. Again leakage ceased after 30 minutes (146).

These microscopic studies suggest that the action of histamine to increase permeability is highly transient lasting at most 30 minutes. These studies can be criticized on the grounds that they were made following single applications of histamine. Histamine administered in such a fashion is not likely to remain after 30 minutes owing to the ease with which it can diffuse through tissues and its rapid rate of metabolism which may explain the transient duration of the effect of histamine in these studies.

Indeed, Renkin and coworkers (19,74,121,122) have presented evidence that repeated subcutaneous injections of histamine into dog hindpaws increased lymph flow, protein concentration, and protein transport (flow times concentration). These effects of histamine lasted as long as histamine continued to be administered (up to four hours) suggesting histamine acted in a sustained fashion. However,

these investigators point out that their data were difficult to interpret owing to the long half time for washout of fluid and protein from the interstitium.

These results were criticized by Grega, et al (51) who showed that infusion of histamine at 4 ug base/min into dog forelimbs produced large increases in lymph flow, protein concentration and protein transport (flow times concentration). These increases reached a maximum within 20 to 30 minutes after the onset of histamine and waned over the remainder of the experiment.

More recently, fluorescence microscopy of the mesenteric circulation demonstrates that during continuous superfusion with histamine, the extravasation of fluorescein labelled albumin at the venules ceases after 20 to 30 minutes with the exception of a few localized regions. This result suggest that a large transient increase in permeability occurs followed by a smaller sustained increase (42).

Finally fluorescein labelled dextran has been used to examine the duration of the increase in permeability during local intra-arterial infusion of histamine (16 ug/min) into dog forelimbs (146). The labelled dextran was infused intravenously either during the control period or at selected intervals (0, 30 and 60 minutes) after the onset of histamine infusion. During the control period, concentrations of dextran increased in lymph and decreased in plasma resulting in a lymph to plasma protein concentration ratio of 0.12 after 30 minutes. In experiments where dextran was infused at the start of histamine infusion, the increase in the concentration of dextran in the lymph was much larger although the fall in plasma concentration was the same

as in the control period prior to histamine. The resulting lymph to plasma concentration ratio was 0.55 thirty minutes after the onset of histamine. However, if the dextran was infused 30 or 60 minutes after the start of histamine, no such increase was noted. The ratio of lymph to plasma dextran concentration was virtually identical in the groups given labelled dextran during the control period or 30 or 60 minutes after the onset of histamine indicating the permeability was the same in those groups. These investigators also demonstrated that the gaps formed at the venular interendothelial junctions of the hamster cheek pouch microcirculation were closed by the thirtieth minute after the start of histamine thus providing both physiologic and morphologic evidence of a transient increase in permeability.

STATEMENT OF OBJECTIVES

The technique to determine CFC proposed by Pappenheimer and Soto-Rivera (109) requires isogravimetric states for its determination. This method worked well under normal conditions. However, histamine infusion causes large amounts of fluid to escape to the extravascular space. In previous studies, blood flow to the organ under investigation was reduced to an ischemic state so that the organ remained isogravimetric. Thus, the first objective of this investigation was to devise a method to determine the effect of histamine on CFC without an isogravimetric state, i.e., without the possible complicating effects of ischemia.

The second objective of this research was to evaluate the duration of the effect of histamine to increase the capillary filtration coefficient (CFC). This was accomplished by determining the filtration coefficient at timed intervals during the local intra-arterial administration of histamine at two doses in three different tissues: isolated canine forelimb, hindpaw and gracilis muscle.

A third objective of this research was to determine the relative contributions of increases in the surface area available for exchange and permeability to filtered fluid to increases in CFC. This was accomplished by maximally vasodilating (increasing surface area to a maximum) the vascular beds of isolated canine forelimb, hindpaw and gracilis muscle. Any further increase in CFC induced by concomitant

infusion of histamine should then be due to increased permeability.

I believe these investigations are of considerable significance because the duration of the effect of histamine on fluid movement is controversial. Furthermore, there has been no quantitative assessment of the duration of the effect of histamine to increase fluid movement. In addition, the relative contributions of increases in permeability and surface area to increases in CFC was assessed thus providing an important first step in delineating the mechanism whereby histamine transiently increases CFC. Finally, a new method for calculation of the capillary filtration coefficient which does not require isogravimetric states is presented.

METHODS

I. General

One hundred fifteen mongrel dogs of either sex weighing 22.1 ± 0.4 kg were used in these investigations. All dogs were anesthetized with sodium pentobarbital (25 mg/kg intravenously, Butler, Inc., Columbus, OH), intubated with a cuffed endotracheal tube and placed on postive pressure respiration (Model 613, Harvard Apparatus Company, Millis, MS). Tidal volume and respiratory rate were adjusted to maintain blood gases and pH within the normal range. The right jugular vein and carotid artery were isolated and cannulated for infusion of drugs (heparin, propranolol, and supplemental doses of anesthetic) and measurement of systemic arterial pressure respectively.

II. Isogravimetric forelimb studies

The skin of the left forelimb was circumferentially divided about 2 to 4 centimeters above the elbow. The left brachial artery and vein and cephalic vein were isolated in preparation for cannulation. The muscles and remaining connective tissue were severed with electrocautery. Special care was taken to ensure minimal bleeding from the cut surface of the muscles. The humerous was cut and the cut ends packed with bone wax. Following these surgical preparations, approximately 30 minutes were allowed to elapse to allow for hemostasis. Immediately prior to the administration of heparin (10000 USP units, intravenously, Liquaemin Sodium, Organon, Inc., West

Orange, NJ), the forelimb nerves were severed. The brachial artery was temporarily occluded (1 to 2 minutes) and the brachial and cephalic veins were cannulated with 15 cm lengths of PE 320 tubing. Venous outflows were combined and directed via a Y-tube through a 1/4 inch fine adjustment needle valve (T Valve Stopcock, Metering Type, Ace Glass Corp., Vineland, NJ) to a reservoir. Blood from this reservoir was returned to the animal via a cannulated femoral vein using a pump (Holter Roller Pump, Model RE161, Extracorporeal Medical Specialties, Inc) interposed in this circuit. The needle valve permitted the precise venous pressure manipulations that were necessary for the determination of the capillary filtration coefficient. The left femoral artery was cannulated for withdrawal of arterial blood which was passed through a pump (Masterflex Roller Pump, Model 7564-00, Cole Parmer, Chicago, IL). This pump maintained flow constant through a cannula placed distal to a ligated portion of the right brachial artery.

III. Isogravimetric hindpaw studies

The skin overlying the right tibia was circumferentially divided 2 to 4 centimeters above the tarsus. The anterior tibial artery and the lateral saphenous vein were isolated in preparation for cannulation. The remaining connective tissue was severed with electrocautery. The tibia was cut and the cut ends packed with bone wax. Following these surgical preparations, approximately thirty minutes were allowed to elapse to allow for hemostasis. Following the administration of heparin (10000 USP units intravenously), the anterior tibial artery was temporarily occluded (1 minute) and the lateral cephalic vein was cannulated with a 15 cm length of PE 240 or

280 tubing depending on the size of the vein. Venous outflow from this cannula was directed through a fine 1/4 inch needle valve to a reservoir. Blood was returned to the animal via a cannulated femoral vein using a pump interposed in this circuit. The left femoral artery was cannulated for withdrawal of arterial blood which was passed through a pump. This pump maintained flow constant through a cannula placed distal to a ligated portion of the right anterior tibial artery.

IV. Isogravimetric gracilis muscle studies

The skin overlying the right gracilis muscle was sectioned along the length of the muscle. The gracilis muscle was freed from the surrounding connective tissue by blunt dissection. The gracilis artery and vein were isolated and the obdurator nerve was sectioned with electrocautery. Special care was taken to ensure that all collateral vessels were ligated before sectioning to ensure minimal hemorrhage. These vessels were sectioned between double ligatures. Isolation of the muscle from the origin at the pubis and insertion on the tibia was accomplished by means of tight ligatures. After these surgical procedures were complete, approximately 30 minutes were allowed to elapse to allow for hemostasis. Following administration of heparin (10000 USP units intravenously), the left femoral artery was cannulated for withdrawal of arterial blood which was passed through a pump. This pump maintained flow constant through a cannula placed distal to a ligated portion of the right femoral artery confluent with the right gracilis artery. Outflow from the gracilis vein was directed, via a cannula placed in a ligated portion of the femoral vein confluent with the right gracilis vein, through a 1/4

inch fine adjustment needle valve to a reservoir. Blood from this reservoir was returned to the animal via the cannulated left femoral vein using a pump interposed in this circuit.

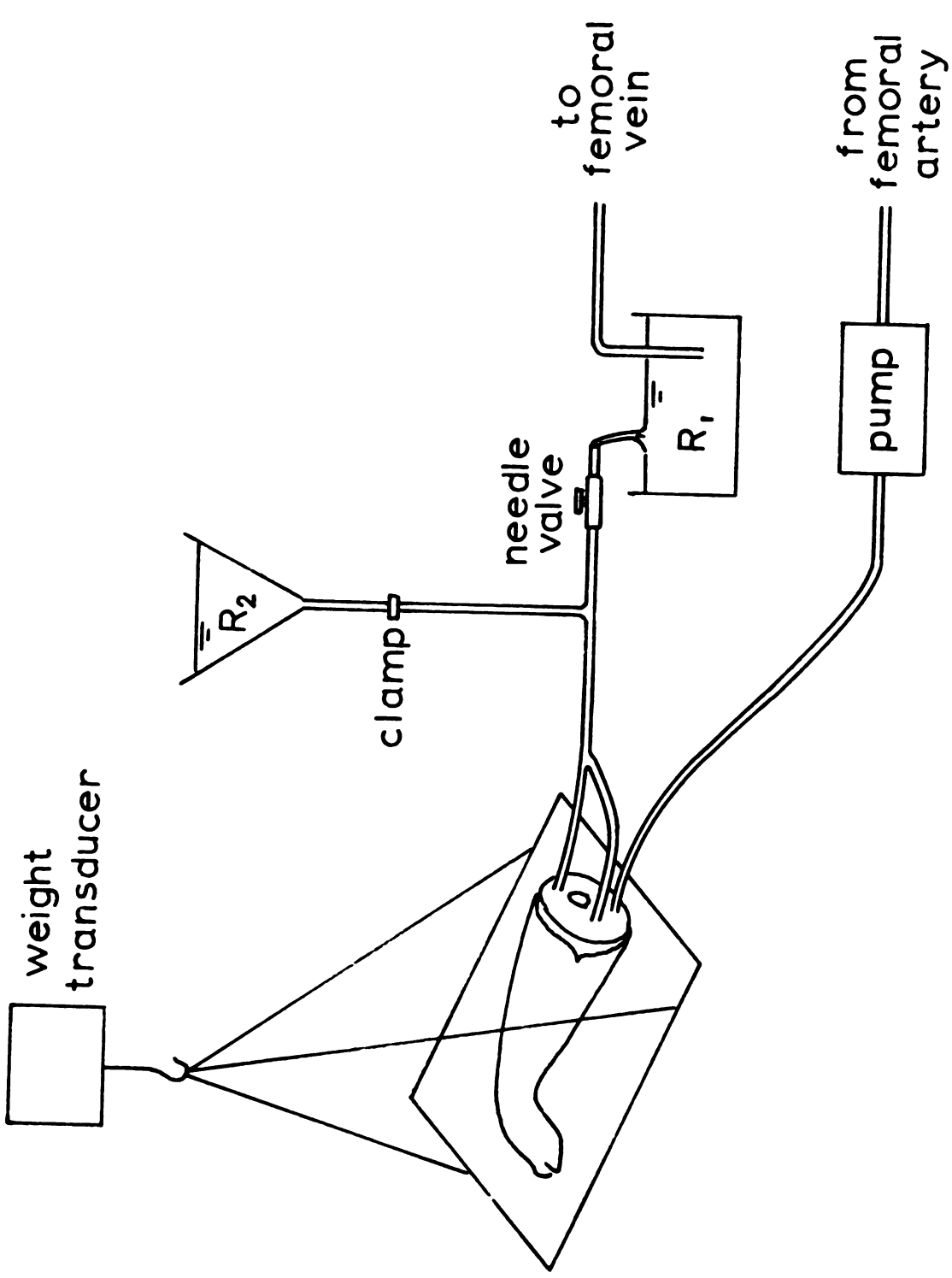
V. Pressure and limb weight recording

After all cannulas were positioned, the organ under investigation was placed on a wire mesh grid attached to a sensitive strain gauge (Unimeasure/80, Unimeasure, Inc., Pasadena, CA). In the forelimb studies the sensitivity of the gauge was adjusted so that placement of a 5 gram weight on the grid produced a pen deflection of 20 to 25 mm on the recording paper. In the gracilis muscle and hindpaw studies, the sensitivity of the gauge was adjusted so that placement of a 5 gram weight on the grid resulted in a pen deflection of 50 to 65 mm on the recording paper. Blood flow in the control period was adjusted so that the organ remained isogravimetric and flow was maintained at this level throughout the experimental protocol. The organ under investigation was coated with an inert silicone spray and covered with cellophane to prevent drying. A diagram of the experimental preparation is depicted in Figure 1.

Arterial, perfusion and venous pressures were recorded with low-volume displacement pressure transducers (Statham, model P23Gb, Gould Medical Products, Statham Industries, Oxnard, CA) and continuous recordings of pressures and limb weight were made with a direct writing oscillograph (Grass model 5D polygraph, Grass Instruments Co., Quincy, MA).

In all experiments, because of the recent finding (54,126) that the catecholamines antagonize histamine induced increases in fluid and protein fluxes via interaction with the beta-receptors, propranolol (3

Figure 1. Schematic of the experimental preparation to study the effects of histamine on the capillary filtration coefficient in the isolated dog forelimb. A similar preparation was used for the isolated hindpaw and gracilis muscle studies.


weight transducer
clamp
R_2
needle valve
R_1
to femoral vein
from femoral artery
pump

mg/kg body weight, Sigma Chemical Co., St. Louis, MO) was administered just after suspension of the limb from the strain gauge. In 12 to 14 animals from each group, propranolol was administered after an initial CFC determination in order to assess the effect of this agent on CFC. Adequacy of beta-blockade was periodically tested by challenge with a 2 microgram bolus injection of isoproterenol (Isuprel Hydrochloride, Breon Laboratories, Inc., New York, NY). Blockade was considered adequate when no decrease in perfusion pressure was noted.

VI. Determination of isogravimetric capillary pressure (Pci)

Isogravimetric capillary pressure was determined by the method of Pappenheimer and Soto-Rivera (109). Briefly, arterial perfusion pressure was reduced and venous pressure increased to give four or five isogravimetric states. Isogravimetric venous pressure was plotted as a function of the corresponding flow rate. The y intercept of such a plot yields Pci while the slope yields postcapillary resistance (see Figure 2).

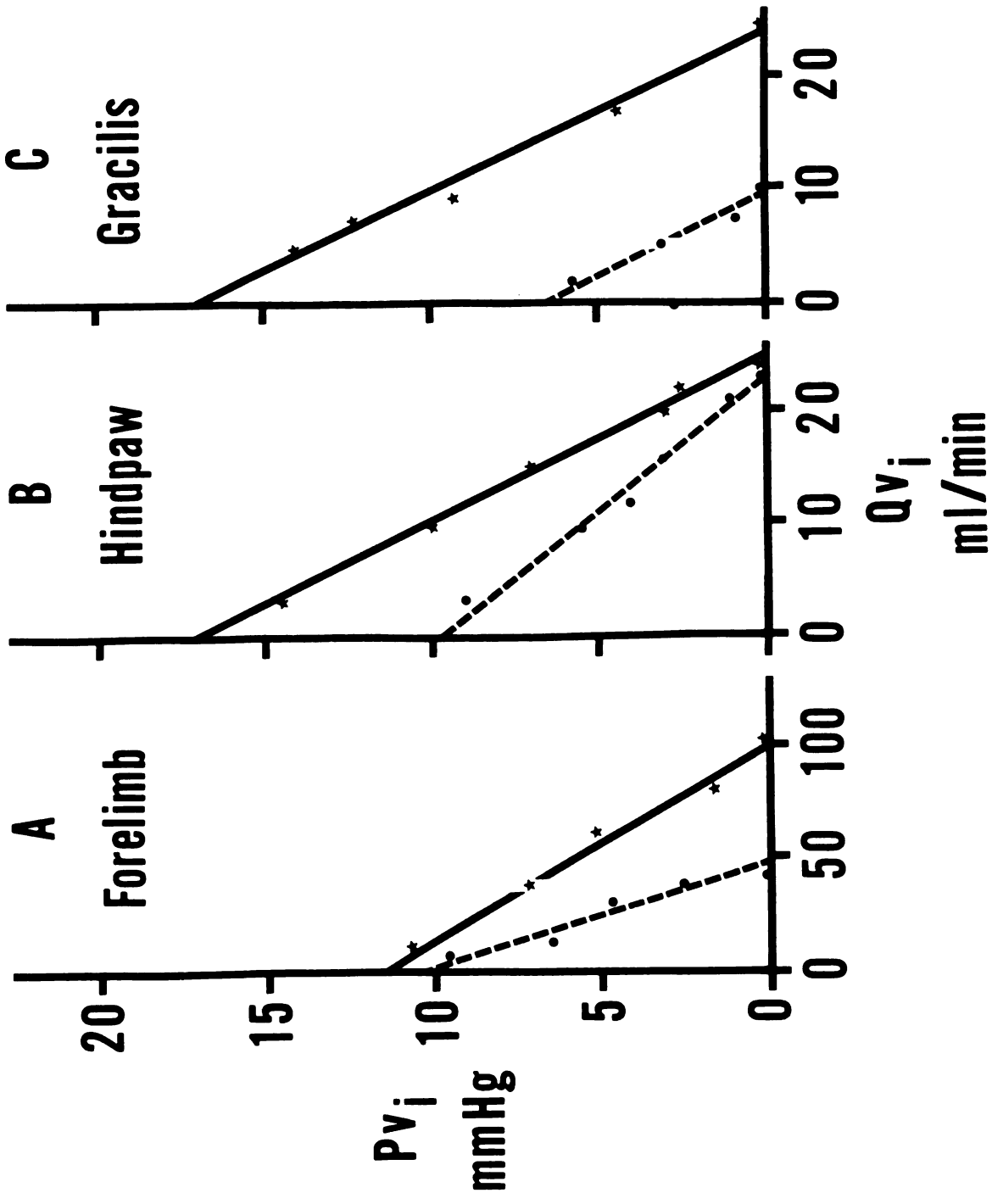
VII. Determination of CFC

The capillary filtration coefficient was determined by a modification of the method of Pappenheimer and Soto-Rivera (109). After measuring an initial venous pressure (Pv_1) and filtration rate (F_1, g/min) venous pressure was suddenly elevated 5 to 15 mm Hg and following the initial vascular transient, venous pressure (Pv_2) and filtration rate (F_2) were recorded. CFC was then calculated according to the formula:

$$CFC = \frac{F_2 - F_1}{Pv_2 - Pv_1} \qquad (3)$$

The filtrate was assumed to have unit density; thus CFC was expressed

Figure 2. Relation between isogravimetric venous pressure (Pv_i) and flow (Qv_i) depicting (1) linearity of Pv_i over a wide range of blood flows and (2) a comparison between control and histamine isogravimetric data in the same forelimb (Figure 2A), hindpaw (Figure 2B), and gracilis muscle (Figure 2C).


A
Forelimb
B
Hindpaw
C
Gracilis
20
15
10
5
0
Pvi
mmHg
0
50
100
0
10
20
0
10
20
Qvi
ml/min

as milliliters per minute per millimeter Hg per 100 grams. A derivation of this equation is given under Treatment of Data.

VIII. Treatment of Data

The original method of Pappenheimer and Soto-Rivera requires isogravimetric states for the determination of CFC (109). This method worked well under normal conditions. However, histamine infusion causes large amounts of fluid to escape to the extravascular space. In previous studies, blood flow to the organ under investigation was reduced to an ischemic state so that the organ remained isogravimetric. We wished to determine the effect of histamine on CFC without an isogravimetric state, i.e., without the possible detrimental effects of ischemia complicating the analysis. The theoretical basis is as follows. Using the Starling equations and from the Poiseuille equation,

$$F = CFC(Pc - Pci) \tag{4}$$

$$Pci = Pt + \sigma(\pi c - \pi t) \tag{5}$$

$$Pc = Pv + RvQv \tag{6}$$

where

F = rate of fluid movement across the microvasculature,

(+) = filtration,

(-) = reabsorption,

Pc, Pt = capillary and tissue hydrostatic pressures,

πc, πt = capillary and tissue colloid osmotic pressures,

σ = osmotic reflection coefficient,

Rv = venous resistance,

Qv = venous flow rate,

Pv = venous pressure

we obtained the equation (Equation 3) we used to estimate CFC.

After recording an initial venous pressure (Pv_1) and filtration rate (F_1), venous pressure is elevated (Pv_2). The ensuing increased filtration rate (F_2) is composed of two phases: an initial, rapid increase in tissue weight attributable to vascular volume changes and a slower component attributable to filtration (98,109) (see Figure 3). The filtration rate (F_2) and venous pressure (Pv_2) are measured during this slow component. Because this elevation of venous pressure increased capillary pressure, the two filtration states can be described by equation 4:

$$F_1 = CFC(Pc_1 - Pci) \tag{7}$$

$$F_2 = CFC(Pc_2 - Pci) \tag{8}$$

We assumed that conditions in and around the microvascular wall are not altered to any significant degree between the two filtration states, such that CFC and Pci are not greatly influenced. This assumption is probably valid since only 45 to 60 seconds elapsed between the first and second filtration state determinations. For each CFC determination, F_1, Pv_1, F_2, Pv_2 are obtained experimentally. Thus equation 7 can be subtracted from equation 8 to obtain:

$$F_2 - F_1 = CFC(Pc_2 - Pc_1) \tag{9}$$

Capillary pressures associated with the two states can be calculated from equation 6:

$$Pc_1 = Pv_1 + Rv_1Qv_1 \tag{10}$$

$$Pc_2 = Pv_2 + Rv_1Qv_1 \tag{11}$$

Our experiments show (Figure 2) venous resistance is constant over a

Figure 3. Diagram of tissue weight and venous pressure during capillary filtration coefficient determinations.

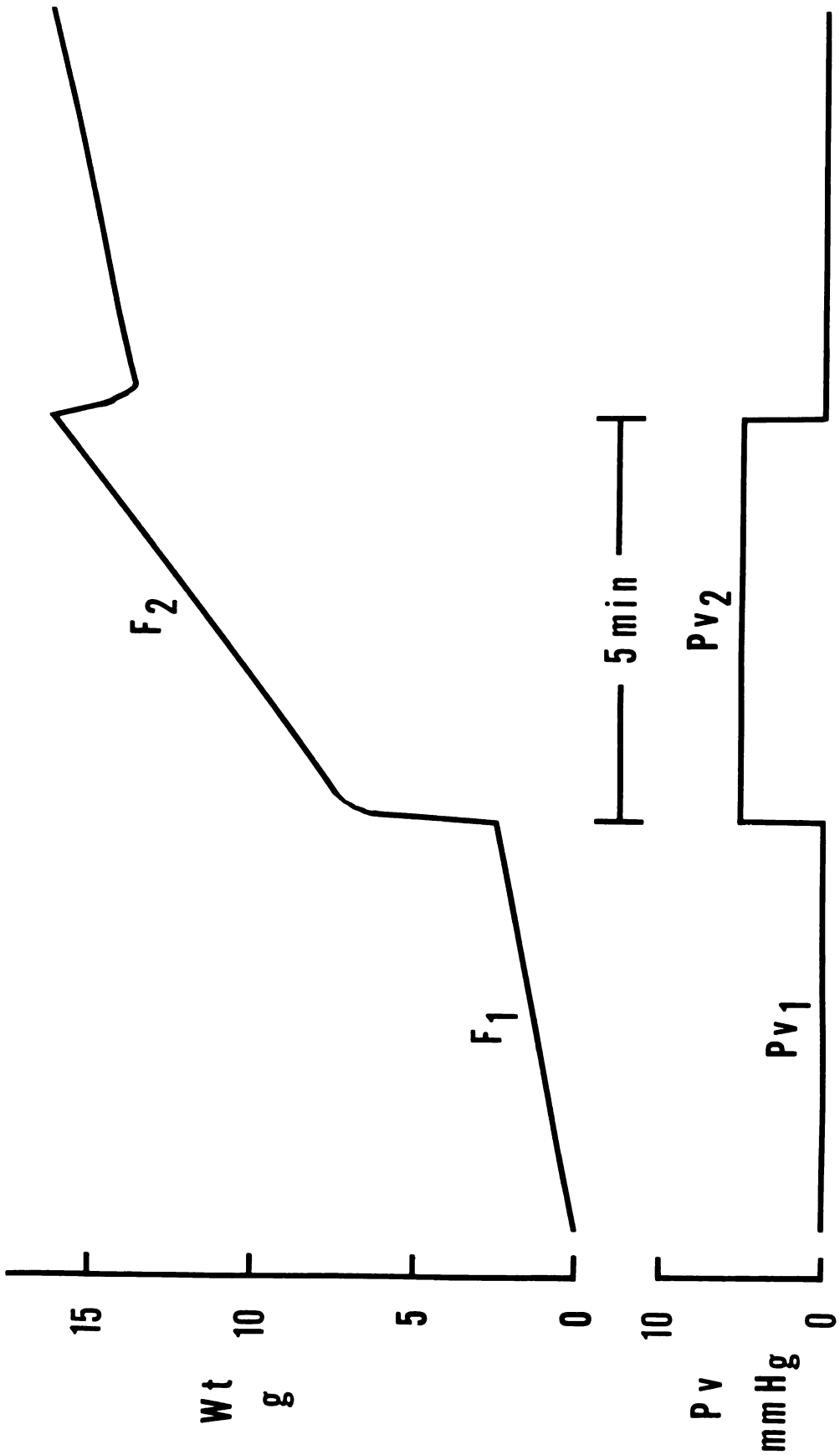
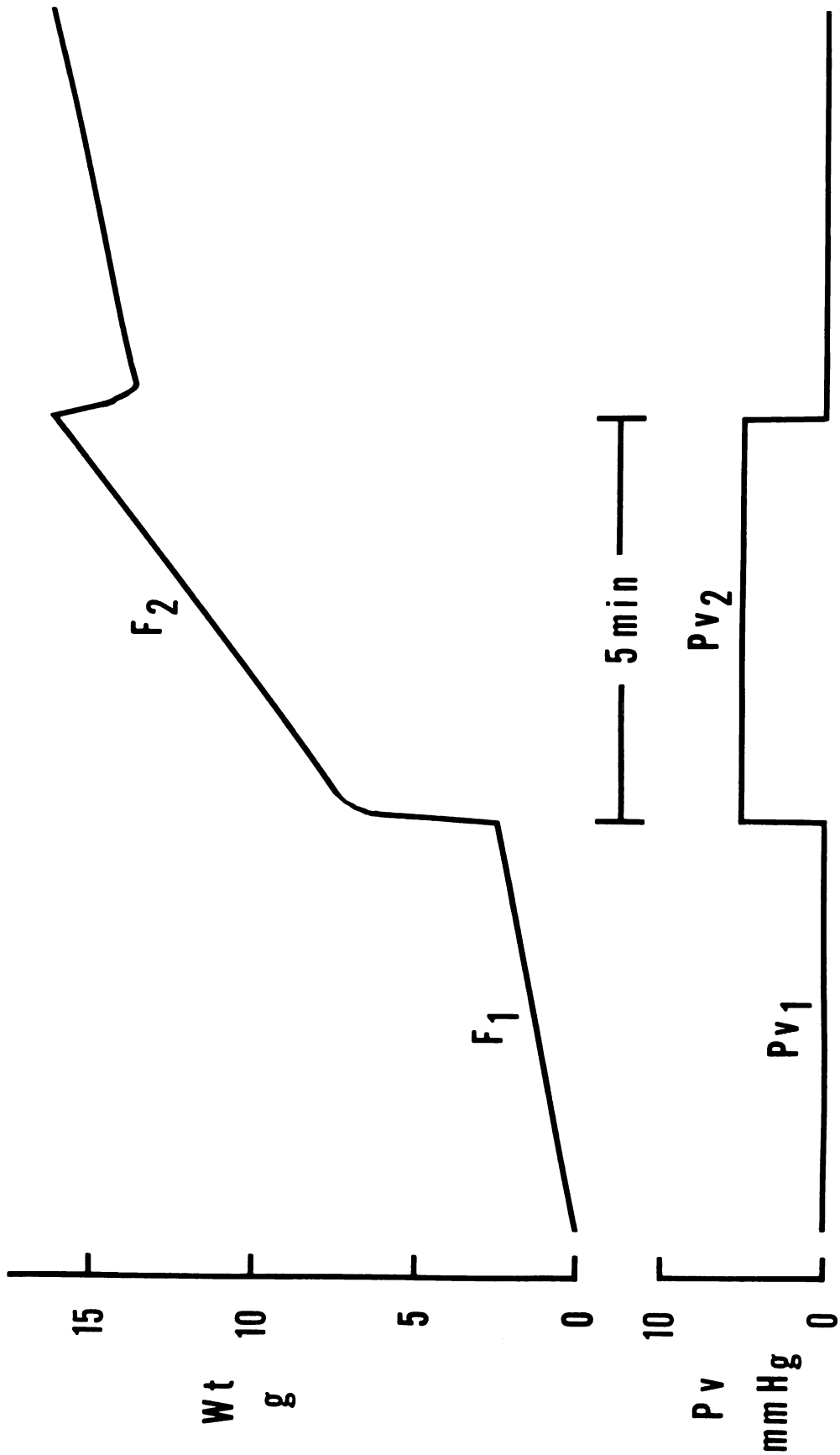

15
10
5
0
Wt
g
F2
F1
5 min
Pv2
Pv1
10
0
Pv
mmHg

Figure 3. Diagram of tissue weight and venous pressure during capillary filtration coefficient determinations.


15
10
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Wt
g
F2
F1
5 min
10
0
Pv
mmHg
Pv2
Pv1

wide range of venous pressures; thus Rv_1 and Rv_2 can be regarded as equal. In addition, our experiments show that 100 percent of the increase in venous pressure is transmitted back to the arterial side of the circulation. Qv_2 was less than Qv_1 owing to the increased rate of fluid filtration brought about by the increased capillary pressure. However, the filtration rate F is much less than Qv and Qv_1 and Qv_2 never differed by more than 5 percent. Thus Qv_1 and Qv_2 are approximately equal. Therefore, equation 10 can be subtracted from equation 11 to obtain:

$$Pc_2 - Pc_1 = Pv_2 - Pv_1 \qquad (12)$$

Substituting $(Pv_2 - Pv_1)$ for $(Pc_2 - Pc_1)$ in equation 9 and rearranging we obtained equation 3.

IX. Experimental Protocols

Series 1 (forelimb), 2 (hindpaw), 3 (gracilis muscle). Effect of saline and time on CFC, mean arterial blood pressure (Pa), and perfusion pressure (Pp).

Propranolol (3mg/kg) was administered intravenously. After beta-blockade was complete as judged by no response to isoproterenol, a control CFC determination was made. An infusion of saline (0.123 ml/min) was inititiated and maintained throughout the remaining protocol. CFC was determined after the 5th, 10th, 15th, 20th, 25th, 45th, 65th and 85th minutes of saline infusion. Beta-blockade was periodically tested by challenge with isoproterenol.

Series 4 (forelimb), 5 (hindpaw), 6 (gracilis muscle). Effect of nitroprusside over time on CFC, Pa, and Pp.

Propranolol (3 mg/kg) was administered intravenously. After beta-blockade was complete, a control CFC determination was made. An

infusion of sodium nitroprusside (30 to 75 ug/min, Sigma Chemical Co., St. Louis, MO) was then inititiated and maintained throughout the remaining protocol. The dose of nitroprusside was adjusted to produce a maximal fall in perfusion pressure. CFC was determined after pressures and weight had stabilized (approximately 5 minutes). Next, an infusion of saline (0.123 ml/min) was begun and CFC determined after the 5th, 10th, 15th, 20th, 25th, 45th, 65th and 85th minute of saline and nitroprusside. The following experiments were conducted to determine if maximal vasodilation was equivalent to maximal recruitment. After the last CFC determination, blood flow to the forelimb (series 4) and gracilis muscle (series 6) was stopped for 10 minutes. Following this period of complete ischemia, flow was returned to its initial value and after 1.5 minutes CFC was determined. After the last CFC determination in the hindpaw series (series 5), an infrared heat lamp was directed at the hindpaw and the temperature of the hindpaw was elevated to 44 degrees centrigrade. Hindpaw temperature was measured via a thermocouple (Yellow Springs) placed subcutaneously. After maintaining the temperature of the hindpaw at 44 degrees for 5 minutes, CFC was determined.

Series 7 (forelimb), 8 (hindpaw), 9 (gracilis muscle). Effect of the low dose of histamine over time on CFC, Pa, and Pp.

These series were similar to series 1, 2 and 3 except histamine (4 ug base/min per 100 ml/min blood flow, Sigma Chemical Co., St. Louis, MO) was infused instead of saline.

Series 10 (forelimb), 11 (hindpaw), 12 (gracilis muscle). Effect of the high dose of histamine over time on CFC, Pa, and Pp.

These series were similar in protocol to series 1, 2 and 3 except histamine (12 ug base/min per 100 ml/min blood flow) was infused instead of saline.

Series 13 (forelimb), 14 (hindpaw), 15 (gracilis muscle). Effect of nitroprusside and nitroprusside plus histamine (low dose) on CFC, Pa, and Pp.

These series were similar to series 4, 5 and 6 except histamine (4ug base/min per 100 ml/min blood flow) was infused instead of saline. CFC was determined after the 5th, 10th, 15th, 20th, 25th, 45th, 65th and 85th minute of histamine infusion.

Series 16 (forelimb), 17 (hindpaw), 18 (gracilis muscle). Effect of nitroprusside and nitroprusside plus histamine (high dose) on CFC, Pa, and Pp.

These series were similar to series 13, 14 and 15 except histamine was infused at 12 ug base/min per 100 ml/min blood flow.

At the end of the experiments, the tissue under investigation was ensanguinated and weighed. The gain in weight (fluid) accrued during the experimental period was assessed from the calibrated limb weight tracing from the polygraph. This value was subtracted from the final tissue weight to yield the initial tissue weight. CFC was expressed in terms of initial tissue weight.

X. Statistical Analysis

Statistical analysis was performed using a 2-way analysis of variance (ANOVA) and means were compared by the Duncan's test. An unpaired Students t-test was used to compare the means between groups.

A p value of less than 0.05 was considered significant. Least square linear regression analysis was used to best fit a line to the isogravimetric capillary pressure data (144).

RESULTS

Tables 2-4 show the effect of histamine (12 ug base/min per 100 ml/min blood flow) on the capillary filtration coefficient (CFC), mean arterial blood pressure (Pa) and perfusion pressure (Pp) in the forelimb (Table 2), hindpaw (Table 3) and gracilis muscle (Table 4). In the forelimb (Table 2), CFC increased from a control of 0.014 $\pm$ 0.002 to 0.036 $\pm$ 0.006 and 0.036 $\pm$ 0.007 ml/min/mm Hg/100 g after the 5th and 10th minute of histamine infusion. Subsequent CFC estimations were not different from control. The Pp fell from a control of 121 $\pm$ 8 mm Hg to 60 $\pm$ 8 mm Hg after five minutes of histamine infusion and remained depressed at this level throughout the remaining protocol. Intraarterial histamine also produced a sustained fall in mean arterial blood pressure.

In the hindpaw (Table 3), CFC averaged 0.0119 ml/min/mm Hg/100g during the control period. It was significantly elevated after the 5th, 10th, 15th and 20th minute of histamine but was not significantly different from control at later times. Local intraarterial histamine also produced sustained decreases in Pa and Pp.

In the gracilis muscle (Table 4), CFC averaged 0.0086 ml/min/mm Hg/100g during the control period. Histamine infusion produced marked increases in CFC estimated after the 5th, 10th, 15th and 20th minute of histamine. Subsequent CFC measurements were not different from control. The perfusion pressure fell from a control of 105 $\pm$ 9 mm Hg

to 42 mm Hg after five minutes of histamine infusion and remained depressed at this level for the remaining protocol. Systemic blood pressure was unaffected by histamine in these experiments.

Table 2 (Forelimb). Effect of histamine (12 ug base/min per 100 ml/min blood flow) on the capillary filtration coefficient (CFC), mean arterial blood pressure (Pa) and perfusion pressure (Pp) measured at timed intervals. Values represent mean ± standard error, n = 6, * = significantly different from control at $p < 0.05$, average forelimb blood flow = 13.3 ± 0.5 ml/min/100g, average forelimb weight = 633.0 ± 22.0 grams.

		Histamine infusion (min)							
	Control	5	10	15	20	25	45	65	85
CFC ml/min / mm Hg/100g	0.014 ±0.002	0.036* ±0.006	0.036* ±0.007	0.024 ±0.003	0.022 ±0.004	0.018 ±0.002	0.018 ±0.004	0.015 ±0.002	0.013 ±0.001
Pa (mm Hg)	113 ±6	105* ±10	101* ±8	99* ±8	99* ±8	99* ±8	99* ±8	102* ±7	102* ±7
Pp (mm Hg)	121 ±8	60* ±8	60* ±7	62* ±8	62* ±6	61* ±8	66* ±8	64* ±6	66* ±6

Table 3 (Hindpaw). Effect of histamine (12 ug base/min per 100 ml/minute blood flow) on the capillary filtration coefficient (CFC), mean arterial blood pressure (Pa) and perfusion pressure (Pp) measured over timed intervals. Values represent mean ± standard error, n = 6, * = significantly different from control at $p < 0.05$, average hindpaw blood flow = 13.3 ± 1.3 ml/min/100 g, average hindpaw weight = 240.5 ± 32.5 grams.

			Histamine infusion (min)							
		Control	5	10	15	20	25	45	65	85
CFC	ml/min mm Hg/100g	0.0119 ±0.0027	0.0280* ±0.0057	0.0319* ±0.0064	0.0266* ±0.0042	0.0253* ±0.0067	0.0143 ±0.0019	0.0141 ±0.0061	0.0191 ±0.0060	0.0117 ±0.0030
Pa	(mm Hg)	111 ±16	101* ±20	98* ±11	95* ±12	93* ±13	90* ±13	88* ±11	88* ±7	81* ±6
Pp	(mm Hg)	84 ±8	68* ±5	71* ±7	70* ±6	69* ±6	73* ±8	69* ±6	67* ±6	74* ±9

Table 4 (Gracilis). Effect of histamine (12 ug base/min per 100 ml/min blood flow) on the capillary filtration coefficient (CFC), mean arterial blood pressure (Pa) and perfusion pressure (Pp) measured at timed intervals. Values represent mean ± standard error, n = 9, * = significantly different from control at $p < 0.05$, average gracilis blood flow = 9.0 ± 1.1 ml/min/100g, average gracilis weight = 63.4 ± 5.3 grams.

		Histamine infusion (min)							
	Saline infusion (min)								
	Control	-5	5	10	15	20	25	45	65	85
CFC ml/min / mm Hg/100g	0.0086 ±0.0011	0.0080 ±0.0009	0.0680* ±0.0146	0.0602* ±0.0103	0.0550* ±0.0111	0.0340* ±0.0080	0.0225 ±0.0057	0.0120 ±0.0011	0.0122 ±0.0012	0.0119 ±0.0013
Pa (mm Hg)	117 ±7	117 ±6	111 ±7	111 ±7	109 ±7	108 ±7	109 ±7	109 ±7	106 ±7	111 ±7
Pp (mm Hg)	105 ±9	105 ±8	42* ±4	46* ±5	46* ±4	47* ±4	46* ±4	46* ±4	46* ±4	46* ±4

Tables 5-7 show the effect of nitroprusside and nitroprusside plus histamine on CFC, Pa and Pp in the three tissues. In the forelimb (Table 5), CFC averaged 0.013 $\pm$ 0.004 ml/min/mm Hg/100g during the control period. During the infusion of nitroprusside alone CFC averaged 0.021 $\pm$ 0.002 ml/min/mm Hg/100g, a value not statistically different from the control value. When histamine infusion was superimposed on the nitroprusside infusion, CFC increased to 0.036 $\pm$ 0.003, 0.033 $\pm$ 0.008 and 0.031 $\pm$ 0.007 after the 5th, 10th and 15th minute. CFC averaged 0.0110 $\pm$ 0.0018 ml/min/mm Hg/100g during the control period in the hindpaw (Table 6). During the infusion of nitroprusside alone, CFC averaged 0.0145 $\pm$ 0.0017 ml/min/mm Hg/100g, a value not statistically different from the control value. In the gracilis muscle (Table 7), CFC averaged 0.0095 $\pm$ 0.0012 and 0.0141 $\pm$ 0.0023 ml/min/mm Hg/100g during control and nitroprusside alone infusion, respectively. When histamine was infused concomitantly with nitroprusside, CFC was significantly increased after the 5th, 10th and 15th minute. In all three series (Tables 5-7), nitroprusside produced a marked fall in perfusion pressure. The concomitant infusion of histamine with nitroprusside produced no further decrease in perfusion pressure. Mean arterial blood pressure was also significantly reduced by nitroprusside in the forelimb and hindpaw series (Tables 5 and 6) but not in the gracilis series (Table 7). Concomitant infusion of histamine with nitroprusside produced no further reduction in Pa in the forelimb and hindpaw but significantly reduced Pa relative to control in the gracilis.

Comparison of CFC measurements tabulated in Tables 2 and 5 (forelimb), 3 and 6 (hindpaw), and 4 and 7 (gracilis) show that CFC

measurements obtained during infusion of the high dose of histamine alone were not statistically different from CFC measurements obtained during combined nitroprusside-histamine infusions. This result suggests that nitroprusside did not affect histamine induced increases in CFC.

Table 5 (Forelimb). Effect of nitroprusside and nitroprusside plus histamine (12 ug base/min per 100 ml/min blood flow) on the capillary filtration coefficient (CFC), mean arterial blood pressure (Pa) and perfusion pressure (Pp) measured at timed intervals. Values represent mean ± standard error, n = 6, * = significantly different from control at $p < 0.05$, average forelimb blood flow = 13.8 ml/min/100g, average forelimb weight = 594.5 ± 43 grams.

			Histamine infusion (min)							
			Nitroprusside infusion (min)							
		Control	-5	5	10	15	20	25	45	65	85
CFC	ml/min mm Hg/100g	0.013 ±0.004	0.021 ±0.002	0.036* ±0.003	0.033* ±0.008	0.031* ±0.007	0.026 ±0.003	0.024 ±0.003	0.022 ±0.002	0.017 ±0.003	0.022 ±0.003
Pa	(mm Hg)	133 ±5	118* ±13	116* ±14	114* ±13	113* ±13	111* ±13	112* ±13	113* ±12	112* ±11	113* ±12
Pp	(mm Hg)	105 ±19	55* ±11	55* ±11	54* ±11	54* ±11	54* ±11	55* ±11	56* ±11	56* ±12	56* ±13

Table 6 (Hindpaw). Effect of nitroprusside and nitroprusside plus histamine (12 ug base/min per 100 ml/min blood flow) on the capillary filtration coefficient (CFC), mean arterial blood pressure (Pa) and perfusion pressure (Pp) measured over timed intervals. Values represent mean ± standard error, n = 6, * = significantly different from control at $p < 0.05$, average hindpaw blood flow = 14.1 ± 2.1 ml/min/100 g, average hindpaw weight = 237.0 ± 28.0 grams.

			Histamine infusion (min)							
			Nitroprusside infusion (min)							
		Control	-5	5	10	15	20	25	45	65	85
CFC	ml/min	0.0110	0.0145	0.0298*	0.0268*	0.0217*	0.0211*	0.0151	0.0156	0.0161	0.0163
	mm Hg/100g	±0.0018	±0.0017	±0.0072	±0.0040	±0.0014	±0.0022	±0.0013	±0.0011	±0.0015	±0.0010
Pa	(mm Hg)	133	117*	114*	114*	113*	111*	112*	108*	112*	113*
		±5	±8	±9	±10	±9	±10	±10	±10	±9	±9
Pp	(mm Hg)	86	53*	49*	50*	49*	50*	51*	50*	50*	50*
		±5	±1	±1	±1	±1	±1	±1	±1	±1	±1

Table 7 (Gracilis). Effect of nitroprusside and nitroprusside plus histamine (12 ug/min per 100ml/min blood flow) on the capillary filtration coefficient (CFC), mean arterial blood pressure (Pa) and perfusion pressure (Pp) measured at timed intervals. Values represent mean ± standard error, n = 8, * = significantly different from control at $p < 0.05$, average gracilis blood flow = 13.8 ± 1.8 ml/min/100g, average gracilis weight = 66.0 ± 7.0 grams.

		Histamine infusion (min)							
		Nitroprusside infusion (min)							
	Control	-5	5	10	15	20	25	45	65	85
CFC ml/min / mm Hg/100g	0.0095 ±0.0012	0.0141 ±0.0023	0.0706* ±0.0175	0.0586* ±0.0110	0.0446* ±0.0116	0.0506* ±0.0116	0.0222 ±0.0052	0.0153 ±0.0016	0.0151 ±0.0018	0.0151 ±0.0021
Pa (mm Hg)	114 ±9	94 ±9	73* ±11	64* ±13	61* ±13	59* ±12	64* ±11	64* ±11	64* ±11	66* ±11
Pp (mm Hg)	135 ±10	47* ±4	52* ±3	51* ±5	55* ±6	56* ±7	53* ±5	55* ±5	53* ±5	55* ±5

The effect of the low dose of histamine (4ug base/min per 100 ml/min blood flow) on CFC, Pa and Pp in the three tissues is shown in Tables 8-10. Histamine infusion produced increases in CFC after the 5th and 10th minute in the forelimb series (Table 8), after the 5th, 10th and 15th minute in the hindpaw series (Table 9) and after the 5th, 10th, 15th, 20th and 25th minute in the gracilis muscle series (Table 10). In the forelimb series (Table 8), Pa fell from a control of 103 $\pm$ 16 mm Hg to 88 mm Hg after the 5th minute of histamine and remained at this level throughout the remaining protocol. Pa was not significantly different from control except after the 45th and 65th minute of histamine in hindpaw series (Table 9) while Pa was unaffected by histamine in the gracilis muscle series (Table 10). Histamine infusion at this dose produced significant sustained reductions in Pp relative to control in all three tissues.

Table 8 (Forelimb). Effect of histamine (4 ug base/min per 100 ml/min blood flow) on the capillary filtration coefficient (CFC), mean arterial blood pressure (Pa) and perfusion pressure (Pp) measured at timed intervals. Values represent mean ± standard error, n = 6, * = significantly different from control at $p < 0.05$, average forelimb blood flow = 11.0 ± 1.1 ml/min/100 g, average forelimb weight = 582 ± 65 grams.

		Histamine infusion (min)							
	Control	5	10	15	20	25	45	65	85
CFC ml/min / mm Hg/100g	0.007 ±0.002	0.024* ±0.005	0.023* ±0.007	0.016 ±0.005	0.016 ±0.004	0.016 ±0.004	0.011 ±0.004	0.012 ±0.002	0.011 ±0.003
Pa (mm Hg)	103 ±16	88* ±15	86* ±13	84* ±12	82* ±12	78* ±11	67* ±6	55* ±8	55* ±9
Pp (mm Hg)	92 ±5	63* ±3	61* ±2	60* ±2	61* ±2	60* ±2	68* ±2	71* ±2	78* ±2

Table 9 (Hindpaw). Effect of histamine (4 ug base/min per 100 ml/min blood flow) on the capillary filtration coefficient (CFC), mean arterial blood pressure (Pa) and perfusion pressure (Pp) measured over timed intervals. Values represent mean ± standard error, n = 9, * = significantly different from control at $p < 0.05$, average hindpaw blood flow = 15.6 ± 2.1 ml/min/100 g, average hindpaw weight = 230.5 ± 23.0 grams.

		Histamine infusion (min)							
	Control	5	10	15	20	25	45	65	85
CFC ml/min / mm Hg/100g	0.0096 ±0.0019	0.0140* ±0.0017	0.0171* ±0.0019	0.0145* ±0.0016	0.0129 ±0.0010	0.0114 ±0.0008	0.0113 ±0.0006	0.0112 ±0.0013	0.0110 ±0.0015
Pa (mm Hg)	103 ±5	103 ±6	102 ±5	99 ±5	97 ±6	96 ±6	90* ±6	89* ±6	93 ±5
Pp (mm Hg)	98 ±8	83* ±7	83* ±7	81* ±8	81* ±8	81* ±7	82* ±8	83* ±9	80* ±10

Table 10 (Gracilis). Effect of histamine (4 ug base/min per 100 ml/min blood flow) on the capillary filtration coefficient (CFC), mean arterial blood pressure (Pa) and perfusion pressure (Pp) measured at timed intervals. Values represent mean ± standard error, n = 10, * = significantly different from control at $p < 0.05$, average gracilis blood flow = 11.7 ± 1.9 ml/min/100 g, average gracilis weight = 78.0 ± 2.9 grams.

		Histamine infusion (min)							
	Control	5	10	15	20	25	45	65	85
CFC mm/min / mm Hg/100g	0.0082 ±0.0018	0.0135* ±0.0029	0.0132* ±0.0025	0.0142* ±0.0029	0.0133* ±0.0032	0.0139* ±0.0034	0.0110 ±0.0026	0.0098 ±0.0021	0.0085 ±0.0013
Pa (mm Hg)	101 ±6	100 ±5	100 ±5	98 ±4	98 ±5	98 ±5	94 ±4	95 ±6	94 ±6
Pp (mm Hg)	108 ±5	82* ±4	82* ±4	84* ±4	84* ±4	86* ±3	90* ±4	97* ±4	99* ±5

Tables 11-13 show the effect of nitroprusside alone and nitroprusside plus histamine (4 ug base/min per 100 ml/min blood flow) on CFC, Pa and Pp in the three tissues. In all three series (Tables 11-13), nitroprusside infusion produced a slight but not statistically significant increase in CFC. Concomitant infusion of histamine with nitroprusside increased CFC after the 5th, 10th, 15th, 20th and 25th minute of histamine in the forelimb series (Table 11), after the 5th and 10th minute in the hindpaw (Table 12) and gracilis muscle series (Table 13). In all three series, nitroprusside infusion significantly reduced both Pa and Pp from their control levels. When histamine infusion was superimposed on nitroprusside infusion, no further reduction in Pa and Pp resulted.

Comparison of the estimates of CFC tabulated in Tables 8 and 11 (forelimb), 9 and 12 (hindpaw), and 10 and 13 (gracilis muscle) showed there was no difference between CFC measurements obtained at any time between the groups which received histamine alone or histamine and nitroprusside.

While the time course of the increase in CFC induced by the low and high doses of histamine were similar, the magnitudes of increase were always less at the lower dose. Also, the time course of the rate of weight gain was similar to the transient changes in CFC. That is, rate of weight gain was markedly elevated during the first 10 to 20 minutes of histamine and began to decrease over the remaining protocol, finally returning to near isogravimetric levels by the 45th minute of histamine infusion.

Table 11 (Forelimb). Effect of nitroprusside and nitroprusside plus histamine (4 ug base/min per 100 ml/min blood flow) on the capillary filtration coefficient (CFC), mean arterial blood pressure (Pa) and perfusion pressure (Pp) measured at timed intervals. Values represent mean ± standard error, n = 6, * = significantly different from control at $p < 0.05$, average forelimb blood flow = 14.8 ± 1.0 ml/min/100 g, average forelimb weight = 510.5 ± 69.0 grams.

		Histamine infusion (min)								
		Nitroprusside infusion (min)								
	Control	-5	5	10	15	20	25	45	65	85
CFC ml/min / mm Hg/100g	0.009 ±0.001	0.014 ±0.003	0.017* ±0.001	0.018* ±0.002	0.017* ±0.001	0.018* ±0.001	0.016 ±0.001	0.014 ±0.002	0.014 ±0.003	0.014 ±0.002
Pa (mm Hg)	105 ±7	75* ±8	77* ±8	78* ±9	77* ±10	81* ±9	80* ±8	80* ±7	83* ±7	87* ±7
Pp (mm Hg)	107 ±11	60* ±5	63* ±8	60* ±7	59* ±6	63* ±5	61* ±6	61* ±6	59* ±5	60* ±5

Table 12 (Hindpaw). Effect of nitroprusside and nitroprusside plus histamine (4 ug base/min per 100 ml/min blood flow) on the capillary filtration coefficient (CFC), mean arterial blood pressure (Pa) and perfusion pressure (Pp) measured at timed intervals. Values represent mean ± standard error, n = 6, * = significantly different from control at $p < 0.05$, average hindpaw blood flow = 18.3 ± 2.0 ml/min/100 g, average hindpaw weight = 217.0 ± 24.0 grams.

			Histamine infusion (min)							
			Nitroprusside infusion (min)							
		Control	-5	5	10	15	20	25	45	65	85
CFC	ml/min mm Hg/100g	0.0117 ±0.0022	0.0173 ±0.0030	0.0237* ±0.0027	0.0219* ±0.0027	0.0177 ±0.0021	0.0180 ±0.0030	0.0166 ±0.0021	0.0171 ±0.0011	0.0171 ±0.0022	0.0169 ±0.0022
Pa	(mm Hg)	131 ±8	119* ±12	115* ±11	113* ±12	116* ±11	115* ±11	117* ±10	119* ±8	122* ±7	121* ±7
Pp	(mm Hg)	98 ±9	58* ±4	57* ±5	55* ±5	55* ±4	54* ±5	55* ±4	59* ±4	60* ±4	60* ±4

Table 13 (Gracilis). Effect of nitroprusside and nitroprusside plus histamine (4 ug base/min per 100 ml/min blood flow) on the capillary filtration coefficient (CFC), mean arterial blood pressure (Pa) and perfusion pressure (Pp) measured at timed intervals. Values represent mean ± standard error, n = 7, average gracilis blood flow = 11.5 ± 2.7 ml/min/100 g, average gracilis weight = 79.1 ± 7.1 grams.

		Histamine infusion (min)							
	Nitroprusside infusion (min)								
	Control	-5	5	10	15	20	25	45	65	85
CFC ml/min / mm Hg/100g	0.0086 ±0.0026	0.0120 ±0.0027	0.0175* ±0.0049	0.0181* ±0.0037	0.0136 ±0.0044	0.0137 ±0.0036	0.0154 ±0.0038	0.0155 ±0.0039	0.0125 ±0.0028	0.0135 ±0.0040
Pa (mm Hg)	130 ±8	126 ±8	111* ±7	105* ±8	102* ±8	102* ±8	102* ±8	101* ±8	104* ±7	103* ±6
Pp (mm Hg)	136 ±7	56* ±6	54* ±6	56* ±7	60* ±6	55* ±5	64* ±4	61* ±4	61* ±5	60* ±6

Tables 14-16 show the effect of local intraarterial nitroprusside infusion on CFC, Pa and Pp in the three tissues. After the 5th minute of nitroprusside infusion, CFC was increased by 60%, 70% and 55% relative to the control period in the forelimb (Table 14), hindpaw (Table 15) and gracilis (Table 16), respectively and remained at this level throughout the remaining protocol. Nitroprusside infusion also produced sustained decreases in both Pa and Pp.

The data depicted in Figure 4 show that 10 minutes of complete ischemia resulted in no change in CFC relative to CFC obtained when the vasculature of the forelimb and gracilis muscle was maximally dilated with nitroprusside suggesting that these vascular beds were maximally recruited. In addition, no reactive dilation was noted in any experiment following the ischemic period, again suggesting that the organs were maximally dilated and recruited. The data depicted in Figure 5A show that CFC was increased when the temperature of the hindpaw was elevated to 44 degrees centrigrade. The results depicted in Figure 5B suggest that this effect is due to a decrease in viscosity of the ultrafiltrate rather than to an increase in the number of perfused capillaries because CFC increased in a direct relation to the decrease in viscosity. The mean ratio of CFC measured at $44^{o}C$ to that measured at $34^{o}C$ was 1.31 ± 0.55 which was not statistically different from the ratio of viscosity of 0.9 percent saline at $34^{o}C$ to 0.9 percent saline at $44^{o}C$ (1.21).

Table 14 (Forelimb). Effect of nitroprusside on the capillary filtration coefficient (CFC), mean arterial blood pressure (Pa) and perfusion pressure (Pp) measured at timed intervals. Values represent mean ± standard error, n = 6, * = significantly different from control at $p < 0.05$, average forelimb blood flow = 13.6 ± 1.1 ml/min/100 g, average forelimb weight = 505.5 ± 28.0 grams.

			Saline infusion (min) / Nitroprusside infusion (min)							
	Control	-5	5	10	15	20	25	45	65	85
CFC ml/min / mm Hg/100g	0.013 ±0.001	0.019* ±0.003	0.018* ±0.002	0.018* ±0.002	0.018* ±0.002	0.018* ±0.003	0.019* ±0.004	0.017* ±0.002	0.018* ±0.003	0.018* ±0.003
Pa (mm Hg)	133 ±9	103* ±18	100* ±16	98* ±16	100* ±12	99* ±11	95* ±11	92* ±10	98* ±9	98* ±9
Pp (mm Hg)	119 ±13	60* ±5	58* ±4	60* ±5	60* ±5	58* ±3	59* ±4	60* ±4	60* ±6	58* ±7

Table 15 (Hindpaw). Effect of nitroprusside on the capillary filtration coefficient (CFC), mean arterial blood pressure (Pa) and perfusion pressure (Pp) measured over timed intervals. Values represent mean ± standard error, n = 5, * = significantly different from control at $p < 0.05$, average hindpaw blood flow = 19.1 ± 8.8 ml/min/100 g, average hindpaw weight = 223.0 ± 21.5 grams.

			Saline infusion (min) / Nitroprusside infusion (min)							
	Control	-5	5	10	15	20	25	45	65	85
CFC ml/min / mm Hg/100	0.0090 ±0.0007	0.0164* ±0.0020	0.0175* ±0.0037	0.0168* ±0.0025	0.0182* ±0.0029	0.0167* ±0.0028	0.0172* ±0.0031	0.0180* ±0.0032	0.0169* ±0.0027	0.0174* ±0.0027
Pa (mm Hg)	135 ±12	114* ±9	112* ±10	113* ±10	114* ±10	113* ±10	115* ±10	115* ±10	119* ±9	120* ±7
Pp (mm Hg)	104 ±19	51* ±6	51* ±6	53* ±7	51* ±7	52* ±7	53* ±7	55* ±7	54* ±7	52* ±7

Table 16 (Gracilis). Effect of nitroprusside on the capillary filtration coefficient (CFC), mean arterial blood pressure (Pa) and perfusion pressure (Pp) measured at timed intervals. Values represent mean ± standard error, n = 6, * = significantly different from control at $p < 0.05$, average gracilis blood flow = 11.6 ± 2.2 ml/min/100 g, average gracilis weight = 88.0 ± 8.0 grams.

			Saline infusion (min) / Nitroprusside infusion (min)							
	Control	-5	5	10	15	20	25	45	65	85
CFC ml/min / mm Hg/100g	0.0107 ±0.0032	0.0163* ±0.0035	0.0177* ±0.0032	0.0175* ±0.0044	0.0166* ±0.0021	0.0179* ±0.0048	0.0163* ±0.0038	0.0170* ±0.0039	0.0148* ±0.0045	0.0163* ±0.0010
Pa (mm Hg)	119 ±9	110* ±9	103* ±5	102* ±5	101* ±6	100* ±6	101* ±5	102* ±4	102* ±6	109* ±6
Pp (mm Hg)	131 ±6	50* ±7	52* ±6	54* ±5	53* ±6	54* ±6	56* ±5	58* ±4	58* ±5	60* ±5

Figure 4. Paired determinations of capillary filtration coefficient (CFC) obtained during maximal vasodilation with nitroprusside (NP) and following 10 minutes of complete ischemia in forelimb (Figure 4A) and gracilis muscle (Figure 4B). Average CFC for these interventions were not statistically different in either tissue.

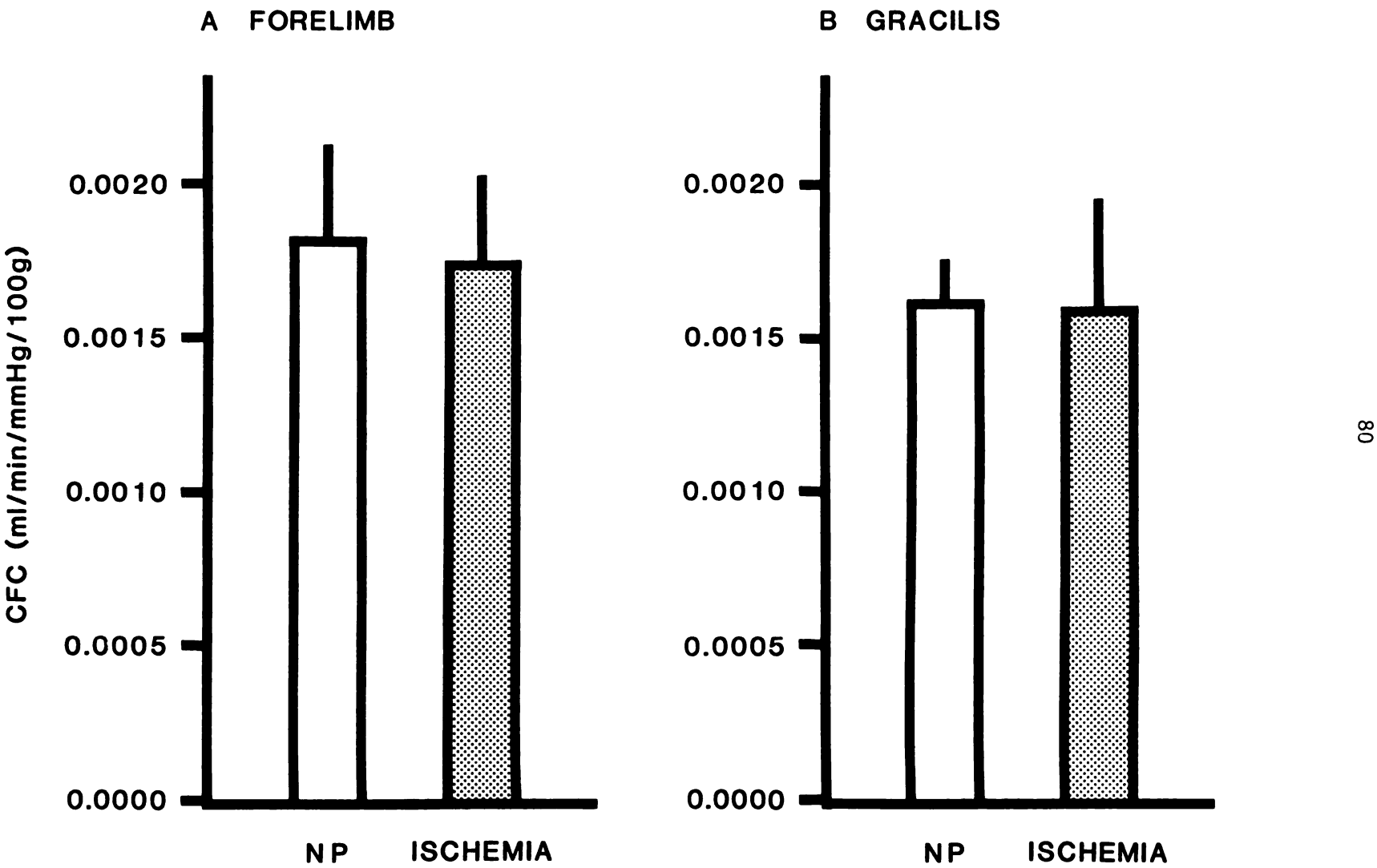
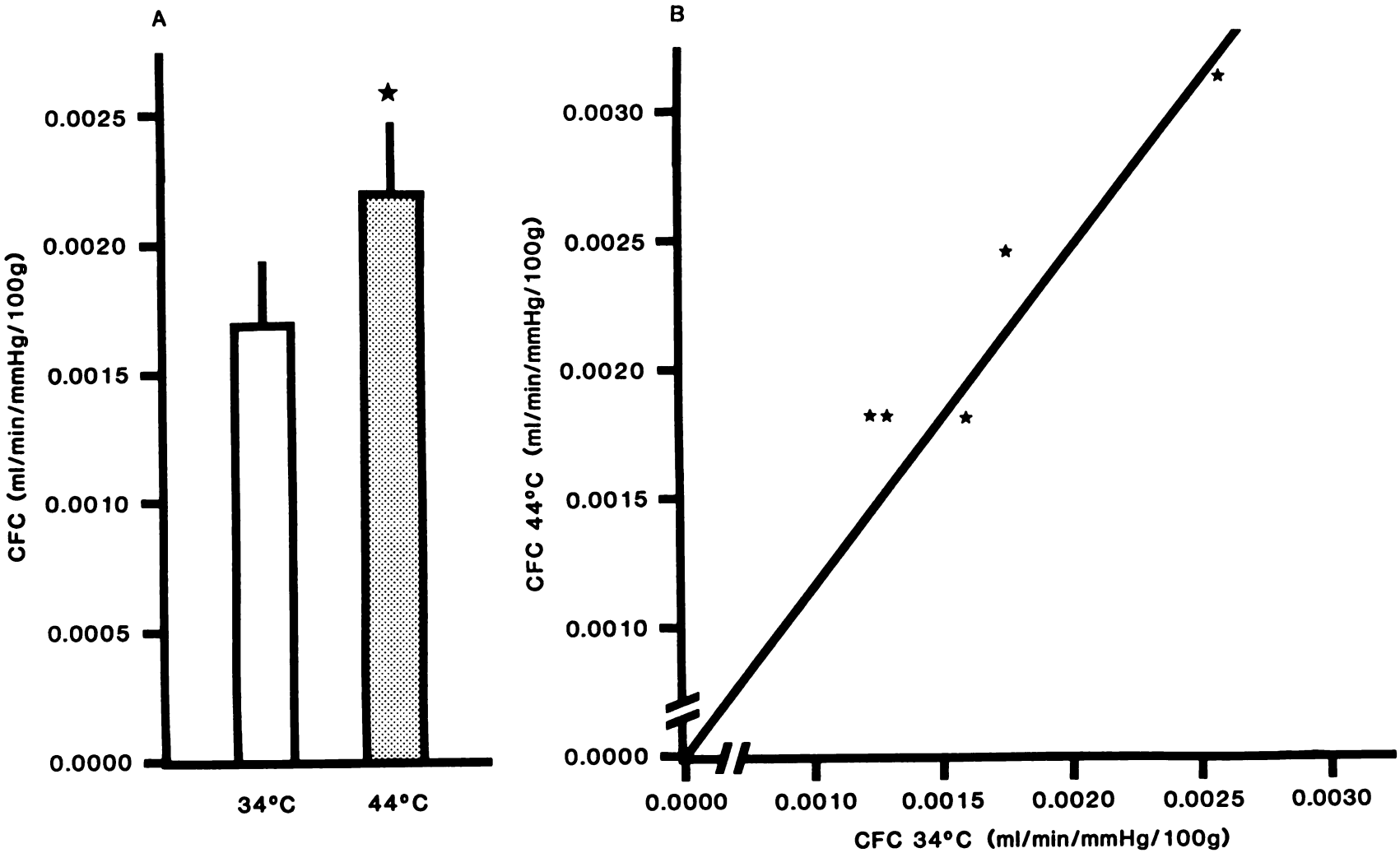

A FORELIMB
B GRACILIS
CFC (ml/min/mmHg/100g)
0.0020
0.0015
0.0010
0.0005
0.0000
NP
ISCHEMIA

Figure 5. A. Paired determinations of capillary filtration coefficient (CFC) in the maximally dilated hindpaw measured at 34 and 44°C (n = 5). Average CFC determined at 44°C was significantly different from CFC obtained at 34°C (* = significantly different at $p < 0.05$). B. Paired determinations of CFC measured at 34 and 44°C. Slope of line drawn through origin is equal to 1.21, the ratio of viscosity of 0.9 percent saline at 34°C to viscosity of 0.9 percent saline at 44°C.


A
0.0025
0.0020
0.0015
0.0010
0.0005
0.0000
CFC (ml/min/mmHg/100g)
34°C
44°C
B
0.0030
0.0025
0.0020
0.0015
0.0010
0.0000
CFC 44°C (ml/min/mmHg/100g)
0.0000
0.0010
0.0015
0.0020
0.0025
0.0030
CFC 34°C (ml/min/mmHg/100g)

It is evident from Tables 17-19 that infusion of the saline vehicle had no effect on CFC, Pa or Pp in the three tissues. In addition, repeated periodic challenge with isoproterenol of the beta blockade produced by the initial propranolol infusion produced no fall in perfusion pressure indicating that beta blockade remained complete throughout the experimental period in all three preparations.

The data depicted in Figure 6 shows that propranolol was without effect on CFC. On the average, propranolol reduced Pa from 136 $\pm$ 3 to 121 $\pm$ 5 mm Hg in the forelimb, 146 $\pm$ 5 to 134 $\pm$ 6 mm Hg in the hindpaw, and 136 $\pm$ 4 to 122 $\pm$ 5 mm Hg in the gracilis but had no effect on perfusion pressure in all three tissues.

Table 17 (Forelimb). Effect of saline (0.123 ml/min) infusion on the capillary filtration coefficient (CFC), mean arterial blood pressure (Pa) and perfusion pressure (Pp) measured at timed intervals. Values represent mean ± standard error, n = 5, average forelimb blood flow = 14.0 ml/min/100 g, average forelimb weight = 660.0 ± 72.0 grams.

	Saline infusion (min)						
	Control	5	15	25	45	65	85
CFC ml/min / mm Hg/100g	0.010 ±0.002	0.010 ±0.002	0.010 ±0.003	0.008 ±0.002	0.009 ±0.001	0.010 ±0.001	0.009 ±0.001
Pa (mm Hg)	116 ±11	119 ±13	118 ±14	120 ±14	122 ±18	121 ±18	121 ±25
Pp (mm Hg)	126 ±17	121 ±18	125 ±18	125 ±18	130 ±22	144 ±24	150 ±30

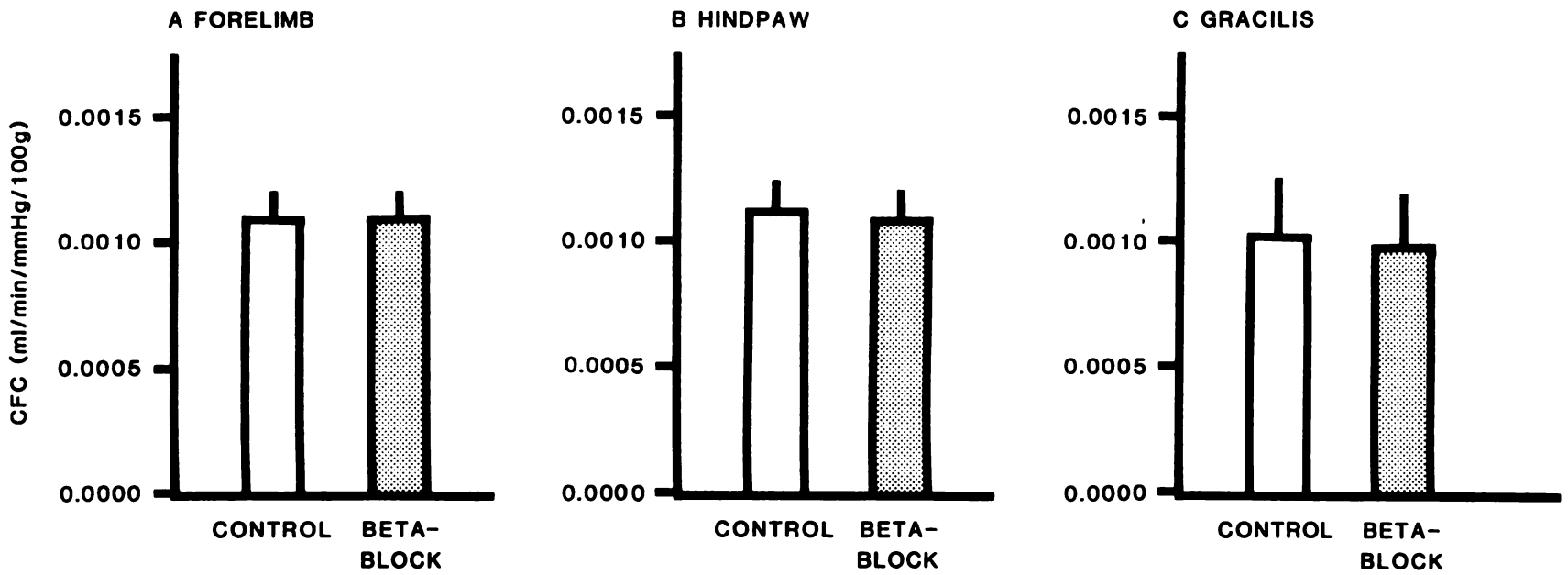
Table 18 (Hindpaw). Effect of saline (0.123 ml/min) infusion on the capillary filtration coefficient (CFC), mean arterial blood pressure (Pa) and perfusion pressure measured at timed intervals. Values represent mean ± standard error, n = 4, average hindpaw blood flow = 17.8 ± 1.8 ml/min/100 g, average hindpaw weight = 212.0 ± 21.8 grams.

		Saline infusion (min)							
	Control	5	10	15	20	25	45	65	85
CFC ml/min / mm Hg/100g	0.0110 ±0.0024	0.0107 ±0.0016	0.0133 ±0.0013	0.0113 ±0.0023	0.0130 ±0.0027	0.0119 ±0.0015	0.0108 ±0.0004	0.0109 ±0.0010	0.0105 ±0.0025
Pa (mm Hg)	120 ±8	123 ±7	125 ±6	124 ±7	124 ±7	123 ±8	124 ±7	122 ±8	122 ±9
Pp (mm Hg)	98 ±9	104 ±8	102 ±11	103 ±11	106 ±12	107 ±12	110 ±12	111 ±18	115 ±25

Table 19 (Gracilis). Effect of saline (0.123 ml/min) infusion on the capillary filtration coefficient (CFC), mean arterial blood pressure (Pa) and perfusion pressure (Pp) measured over timed intervals. Values represent mean ± standard error, n = 4, mean gracilis blood flow = 9.4 ± 2.5 ml/min/100 g, mean gracilis weight = 60.5 ± 10.0 grams.

			Saline infusion (min)							
		Control	5	10	15	20	25	45	65	85
CFC	ml/min mm Hg/100g	0.0095 ±0.0034	0.0098 ±0.0013	0.0079 ±0.0004	0.0126 ±0.0063	0.0084 ±0.0013	0.0091 ±0.0021	0.0085 ±0.0032	0.0097 ±0.0017	0.0096 ±0.0011
Pa	(mm Hg)	124 ±4	125 ±2	126 ±2	126 ±3	126 ±3	128 ±4	128 ±4	127 ±4	128 ±7
Pp	(mm Hg)	119 ±11	122 ±10	124 ±8	126 ±8	126 ±10	127 ±10	134 ±14	141 ±18	148 ±24

Figure 6. Comparison of average capillary filtration coefficient (CFC) measured during control and following beta-blockade with propranolol (3 mg/kg) (BETA-BLOCK) in forelimb (Figure 6A), hindpaw (Figure 6B), and gracilis muscle (Figure 6C).


A FORELIMB
B HINDPAW
C GRACILIS
CFC (ml/min/mmHg/100g)
0.0015
0.0010
0.0005
0.0000
CONTROL
BETA-BLOCK

DISCUSSION

The data presented in Tables 2-4 show the effect of the high dose of histamine (12 ug base/min per 100 ml/min blood flow) to increase CFC is transient, lasting at most 25 minutes. CFC increased 2.6 fold relative to control in the forelimb, 2.7 fold in the hindpaw and 7.9 fold in the gracilis during the first 10 minutes of drug infusion. CFC measurements obtained after this time in the forelimb, after the 20th minute in the hindpaw and after the 25th minute in the gracilis muscle were not significantly different from control.

Local intraarterial infusion of the low dose of histamine (4 ug base/min per 100 ml/min blood flow) also produced a transient increase in CFC of similar time course but of lesser magnitude (Tables 8-10). CFC increased 2 fold relative to control in the forelimb, 1.8 fold in the hindpaw and 1.6 fold in the gracilis muscle during the first 10 minutes of histamine infusion. This apparent dose dependent effect of histamine is in accord with the studies of Flynn and Owen (39) who showed a dose dependent increase in CFC in skinned cat hindlimbs when histamine was infused over a range of 0.031 to 3.1 ug/kg hindlimb/min.

Comparison of CFC estimated during infusion of histamine at the high dose in forelimb, hindpaw and gracilis muscle suggests that the microvasculature of the gracilis muscle responds to a greater degree (i.e., CFC increased to a greater magnitude). However, CFC determinations in the forelimb and hindpaw actually represent underestimations because CFC cannot be determined by gravimetric

techniques in tissues such as bone since the interstitial space cannot accomodate increased volume (40). The forelimb consists of 43 percent bone, 37 percent muscle and 20 percent skin (75) whereas the hindpaw consists of 45 percent bone, 10 percent muscle and 45 percent skin (21). Using these percentages, values from Tables 2, 3, 8 and 9 for control CFC and CFC determined after 10 minutes of histamine were normalized to soft tissue weight and are presented in Table 20.

Table 20. Comparison of CFC normalized to soft tissue weight obtained during the control period and after 10 minutes of histamine infusion in isolated canine forelimb, hindpaw and gracilis muscle.

	Soft tissue CFC (ml/min/mm Hg/100 g soft tissue)			
	Control	Histamine (high dose)	Control	Histamine (low dose)
Forelimb	0.025	0.063	0.023	0.040
Hindpaw	0.022	0.058	0.017	0.031
Gracilis	0.0086	0.0680	0.0082	0.0142

It is apparent that while control CFC is greater in the forelimb and hindpaw, the increase in CFC induced by histamine is remarkably similar at a given dose in all three tissues except for the gracilis at the low dose. The relatively higher control CFC in the forelimb and hindpaw is probably due to a higher microvascular surface area and/or permeability.

While these data clearly demonstrate a large transient effect of histamine on CFC, it seems unlikely that the transient effects alone can account for the large variation in reported CFC.

It has recently been demonstrated that the catecholamines antagonize the effect of histamine to increase the efflux of fluid and protein from the vascular to extravascular compartment (53,54,95,126). This antagonism appears to be mediated via interaction with the beta receptor because combined histamine-norepinephrine infusions into animals pretreated with propranolol increased fluid and protein efflux whereas animals which were not pretreated demonstrated no such increase. In addition, isoproterenol completely blocks histamine induced increases in CFC (126) and lymph flow and protein concentration (53,54). These observations provide an attractive hypothesis to explain the transient increase in CFC. That is, because local intraarterial histamine decreased arterial blood pressure (Tables 2-13), catecholamine levels may have been increased reflexly. Indeed, Robinson and Jochim (131) have shown that blood catecholamines increase during hypotension induced by histamine infusion. These increased catecholamine levels during histamine may then act to decrease the effect of histamine on the microvascular membrane. However, this possibility is unlikely because the forelimb, hindpaw and gracilis muscle vasculatures were beta blocked with propranolol.

Measurement of CFC provides a direct measure of transcapillary hydrodynamic conductivity which, in turn, is a product of the microvascular surface area available for exchange and microvascular permeability to filtered fluid. Therefore, the changes in CFC induced by histamine are due to changes in surface area or permeability or both.

The data presented in Tables 5-7 and 11-13 suggest that the contribution of changes in microvascular surface area may be less important than changes in microvascular permeability. In these experiments, forelimb, hindpaw and gracilis muscle vasculatures were maximally dilated with nitroprusside suggesting that surface area was at a maximum. That is, the dose of nitroprusside was adjusted to produce a maximal fall in perfusion pressure. Since this maximal fall in perfusion pressure was sustained throughout the infusion of nitroprusside (Tables 14-16) and blood flow was held constant, it follows that vascular resistance remained constant and hence surface area may also have remained constant. This line of reasoning suggests that the transient increase in CFC produced by concomitant infusion of histamine into vascular beds already maximally dilated with nitroprusside is due to a transient increase in permeability to filtered fluid.

Although this approach is attractive, it relies on the assumption that both histamine and nitroprusside produce proportionate changes in both vascular resistance and CFC, unless they influence vascular permeability. That is, it must be assumed that maximal vasodilation results in maximal recruitment of capillaries. However, CFC can change without any change in vascular resistance if vascular smooth muscle tone in precapillary resistance vessels can change independent of changes in tone of "precapillary sphincters". For example, stimulation of sympathetic nerves to the lower hindlimb vascular bed in cats produces an increase in vascular resistance and CFC (89). Because it would seem that an increase in resistance should result in no change or a decrease in CFC, it has been suggested that resistance

and CFC are independent variables and are controlled by different smooth muscle (89). Thus, in the present study, it was important to demonstrate that maximal vasodilation was equivalent to maximal recruitment of capillaries.

The results depicted in Figure 4 suggest that maximal vasodilation with nitroprusside is equivalent to maximal recruitment in the forelimb and hindpaw. In these experiments, CFC was determined during maximal vasodilation and after 10 minutes of complete ischemia, a maneuver known to maximally recruit capillaries (10) but not alter permeability (33). Filtration coefficients estimated during these maneuvers were identical suggesting that all exchange surface for filtration was available. To determine if the hindpaw vasculature was maximally recruited, CFC was determined in the maximally dilated hindpaw maintained at 34 or 44 degrees centrigrade. The increase in temperature would tend to make available previously unopened capillaries (50). It should be pointed out that single capillary studies indicate that microvascular permeability is unaffected by temperature (114) over this range. Heating the paw to 44 degrees resulted in a significantly elevated CFC relative to CFC obtained at 34 degrees. However, when the decrease in viscosity of the ultrafiltrate was accounted for (Figure 5), no difference in CFC at the two temperatures was evident, suggesting that surface area was at a maximum.

Thus assuming that nitroprusside produces no change in vascular permeability, these results tabulated in Tables 5-7 and 11-13 suggest that histamine at either dose produces a transient increase in permeability of the vasculatures of isolated canine forelimb, hindpaw

and gracilis muscle. This transient increase in permeability produced by continuous local intraarterial histamine could not have been due to a time dependent deterioration of the preparations because CFC was unchanged throughout the experimental period in control experiments (Tables 17-19). Miller and coworkers (102) have recently attempted to quantitate the effect of nitroprusside and histamine on vascular permeability. While low to moderate doses (10^{-7} to 10^{-5} M) of histamine or nitroprusside produced arteriolar dilation, only histamine produced significant leakage of fluorescein labelled albumin. Only at very high doses of nitroprusside (greater than 10^{-4} M) was protein leakage evident and even at these doses, the increase in leakage was very small (30 percent relative to control). Since blood concentrations of nitroprusside averaged only 2.8×10^{-6} to 1.2×10^{-5} M in these studies, it is unlikely that nitroprusside produced any significant increase in permeability. In addition, the relative increase in CFC induced by nitroprusside is similar to that seen with other vasodilators which do not increase permeability such as ATP or acetylcholine (78).

Comparison of the CFC data presented in Tables 5 and 11, 6 and 12, and 7 and 13 suggests that histamine may transiently increase the permeability of the microvascular wall in a dose dependent fashion. This conclusion is based upon the fact that infusion of the low dose of histamine into maximally dilated vascular beds produced increases in CFC which were significantly less than increases at the high dose. This result corroborates the findings of Baker (9) who showed that a

very low dose of histamine (5 ug/kg muscle/min) significantly increased CFC in maximally dilated gracilis muscle. Infusion of histamine at 60 ug/kg muscle/min produced further increases in CFC.

The capillary filtration coefficient is an operational term which does not indicate which parts of the microvascular barrier to fluid movement are involved. However, the time course of the increase in CFC is similar to that reported for the transient widening of venular interendothelial gaps induced by histamine. The gaps are widest (radius = 500 to 5000 angstroms) after 5 to 10 minutes and subsequently close after 15 to 30 minutes (20,42,91,92,101,102, 134,146). The data in the present study show that the magnitude of CFC is greatest after 10 minutes of histamine and returns towards control values over the subsequent 15 minutes (Tables 2-13). Taken together, these results suggest that histamine acts to transiently increase permeability by forming large pores or gaps in the microvascular wall.

It has recently been demonstrated that the luminal endothelial cell membrane contains receptors for histamine (67). These receptors are especially numerous in the venules and are preferentially located at interendothelial junctions which are rich in cytoplasmic filaments. This finding provides strong support for the concept that the opening of venular interendothelial junctions induced by histamine is due to contraction of filaments in the cytoplasm (93,94) and that histamine induced increases in CFC may be mediated by large pore formation in the venular side of the microcirculation.

Physiologic evidence supports the assertion of the electron microscopists that histamine acts to increase the permeability of the barrier to fluid movement by forming large pores (128). Histamine when infused into isolated maximally dilated rat hindquarters increases CFC 3 fold yet PS for Cr-EDTA increased very little (less than 10 percent). In addition, efflux of Cardio-Green labelled albumin was elevated indicating increased permeability to macromolecules. These data indicate that while histamine induces large increases in hydraulic conductivity and large molecular permeability, small molecular permeability increased only marginally. These observations were interpreted to support the concept that histamine acts to increase the number of large pores (128). The reason for this is because of their large radius, these large pores are very important for macromolecular exchange and also for hydraulic conductivity as this increases with the fourth power of the radius. However, as a result of their small cross-sectional area relative to that for the small pores, the large pores exert far less influence on the diffusional exchange of small hydrophilic molecules (128). For these reasons, small hydrophilic molecular permeability would be affected very little even if the number and/or size of the large pores increased markedly.

If it is accepted that these large interendothelial gaps do form in response to histamine, it should be possible to estimate the ratio of the number of these gaps to the number of small pores (N_G/N_S) in

the gracilis muscle and hindpaw vasculatures from the following equation (see Appendix for derivation):

$$\frac{N_G}{N_S} = \frac{[(CFC_{H+N}/CFC_N) - 1][(N_L/N_S)(R_L)^4 + (R_S)^4]}{(R_G)^4 - (R_S)^4}$$

where all values except N_G/N_S are known or obtainable experimentally. In this equation, N_G, N_L and N_S refer to numbers of gaps, large pores and small pores respectively; R_G, R_L and R_S refer to the radius of the gaps, large pores and small pores respectively; CFC_{H+N} and CFC_N refer to CFC obtained during histamine plus nitroprusside infusion and nitroprusside alone infusion respectively. From this equation, it is evident that when R_G = 1000 angstroms (45,91,92), R_L = 220 angstroms (Table 1), R_S = 67 angstroms (Table 1), N_L/N_S = 1/3610 (Table 1) and CFC_{H+N}/CFC_H = 4 (From gracilis data presented in Table 7) the ratio of the number of gaps to small pores (N_G/N_S) would be 1:16000. If R_G were 5000 angstroms (45,91,92), this ratio would be even smaller. Owing to the relative paucity of these gaps predicted by this equation, they would be difficult to isolate in electron micrographic studies yet they appear to be found with relative ease.

The answer to this apparent discrepancy may lie in the fact that the microvascular barrier to fluid filtration may actually be represented by a series of barriers of different hydraulic conductivities. That is, in control conditions, the microvascular endothelium may represent the principal resistance to fluid filtration. However, when histamine induces venular interendothelial gap formation, structures beyond the microvascular wall such as the basement membrane may provide the principal resistance. Support for this concept comes from the studies of Majno and coworkers (91,92) and

Alkasne (1) who demonstrated that in control conditions, colloidal carbon or mercuric sulfide particles do not penetrate interendothelial clefts. Under the influence of histamine, gaps formed between venular endothelial cells. These colloidal particles penetrated the gaps but most were retained at the basement membrane. These observations suggest that while the gaps may be 500 to 5000 angstroms in radius, their "equivalent" pore radius may be much less.

Because it is necessary to postulate the existence of large pores between the blood and lymph to explain the presence of protein in the interstitial space and lymph (147), it may be assumed that the radii of pores through the basement membrane or other structures (i.e., interstitial gel matrix) might be on the order of the large pore radii, i.e., 220 angstroms. Thus while the gaps may be 500 to 5000 angstroms in radius, their "equivalent" pore radius may be only 220 angstroms. If this is so, the equation above predicts the ratio of gaps to small pores to be 1:37.

Thus an increase in radius of approximately 0.0062 or 2.7 percent of the available small pores to form large pores or gaps with radii of 1000 or 220 angstroms respectively could account for the histamine (high dose) effects in gracilis muscle. Similar analysis of the effect of histamine at the low dose would predict an increase in radius of approximately 0.001 or 0.45 percent of the available small pores to form large pores or gaps with radii of 1000 or 220 angstroms respectively in gracilis muscle.

Similar analysis using hindpaw data presented in Table 1 (R_S = 47 angstroms, R_L = 195 angstroms, N_L/N_S = 1/114) and CFC data presented in Table 6 (CFC_{H+N}/CFC_N = 1.8) for the high dose of histamine predicts

N_G/N_S to be 1:71000 if R_G is 1000 angstroms and 1:103 if R_G is 195 angstroms. The low dose effects in the hindpaw suggest that N_G/N_S is 1:190,000 if R_G is 1000 angstroms and 1:138 if R_G is 195 angstroms. Therefore, an increase in radius of approximately 0.0014 or 0.97 percent of the available small pores to form large pores or gaps with radii of 1000 or 195 angstroms respectively could account for the high dose effects of histamine in the hindpaw. The low dose effects of histamine in the hindpaw can be accounted for by an increase in radius of approximately 0.00053 or 0.26 percent of the available small pores to form large pores with radii of 1000 or 195 angstroms respectively.

SUMMARY AND CONCLUSIONS

The duration of the effect of histamine to increase the capillary filtration coefficient (CFC) was evaluated at two different doses in three different tissues. In isolated canine forelimb, hindpaw, and gracilis muscle, local intraarterial histamine infusion transiently increased CFC. In all three tissues at either dose, the increase in CFC was greatest after 10 minutes of histamine and returned to control levels by the 25th minute of histamine infusion. However, the magnitude of increase in CFC was greater at the higher dose. When CFC was normalized to soft tissue weight, the magnitude of increase in CFC was similar in all three tissues at a given dose except for the gracilis at the low dose suggesting that histamine produced similar effects on surface area and/or permeability in the three tissues.

Measurement of CFC provides a direct measure of transcapillary hydrodynamic conductivity which, in turn, is a product of the microvascular fluid movement available for exchange and microvascular permeability to filtered fluid. Therefore, the transient increase in CFC induced by histamine was due to changes in surface area or permeability or both.

In order to determine the relative contributions of surface area and permeability to histamine induced increases in CFC, the vasculatures of these tissues were maximally dilated with nitroprusside, increasing surface area to a maximum. Any further increase in CFC induced by concomitant infusion of histamine with

nitroprusside should then be due to increased permeability. Both doses of histamine, when infused concomitantly with nitroprusside, produced further increases in CFC although the increase was less at the lower dose. It was concluded that histamine transiently increases permeability to fluid in all three tissues at either dose but that the higher dose produced greater increases in permeability.

This transient increase in permeability was probably not related to a beta antagonistic action of the catecholamines since the vasculatures of these tissues were beta-blocked with propranolol.

An equation was derived to estimate the ratio of the number of gaps which form during histamine to the number of small pores. It was concluded that only a small proportion (less than 3 percent) of small pores need increase in radius to form large pores or gaps to explain the increases in CFC demonstrated in the hindpaw and gracilis muscle.

APPENDIX

APPENDIX

Evidence has been reviewed by Pappenheimer (108) that fluid flow through membranes with porosities of greater than 20 angstroms in radius may be approximated by application of Poiseuille's law. By application of the double pore theory (55) and Poiseuille's law, fluid flow across the microvascular walls can be described by:

$$F = [(N_L\pi(R_L)^4/8ln) + (N_S\pi(R_S)^4/8ln)] \cdot [\Delta P] \qquad (13)$$

where N_L and N_S represent the numbers of large and small pores respectively, R_L and R_S represent the radii of large and small pores, l is pore path length, n is the viscosity of the filtrate, ΔP is the driving pressure (transmicrovascular hydrostatic pressure gradient minus transmicrovascular colloid osmotic pressure gradient). The quantity in brackets represents CFC. Thus in the control state, CFC (CFC_C) represents the sum of the conductivities of the large and small pore systems or:

$$CFC_C = [(N_L\pi(R_L)^4) + (N_S\pi(R_S)^4)]/8ln \qquad (14)$$

It has been demonstrated that histamine induces large gaps to form from small pores between venular endothelial cells (42,91-94) thus providing an additional path for fluid movement. Thus during histamine administration, CFC represents the sum of the conductivities of small and large pores and venular interendothelial gaps:

$$CFC_H = [(N_G\pi(R_G)^4) + (N_L\pi(R_L)^4) + ((N_S - N_G)\pi(R_S)^4)]/8ln \qquad (15)$$

where N_g and R_g represent the number and radii of the gaps respectively.

If CFC is obtained during maximal vasodilation (surface area at a maximum) with nitroprusside (CFC_N) and during combined histamine nitroprusside infusion (CFC_{H+N}), it is assumed that microvascular surface area is constant in both states. Assuming that path length is the same for large and small pores and for gaps, and that viscosity of the filtrate passing through the pores and gaps is the same, and that laminar flow occurs, Equation 15 can be divided by Equation 14 to obtain:

$$\frac{CFC_{H+N}}{CFC_N} = \frac{[N_G(R_G)^4] + [N_L(R_L)^4] + [(N_S - N_G)(R_S)^4]}{[N_L(R_L)^4] + [N_S(R_S)^4]} \quad (16)$$

Multiplying the right hand term of Equation 16 by $(1/N_S/1/N_S)$ obtains:

$$\frac{CFC_{H+N}}{CFC_H} = \frac{[(N_G/N_S)(R_G)^4] + [(N_L/N_S)(R_L)^4] + [(1 - (N_G/N_S))(R_S)^4]}{[(N_L/N_S)(R_L)^4] + (R_S)^4} \quad (17)$$

Rearrangement of Equation 17 yields:

$$\frac{N_G}{N_S} = \frac{[(CFC_{H+N}/CFC_N) - 1][(N_L/N_S)(R_L)^4 + (R_S)^4]}{(R_G)^4 - (R_S)^4} \quad (18)$$

With the exception of N_G/N_S, all values are known or obtainable experimentally. Therefore, estimation of the ratio of the number of gaps which form during histamine to the number of small pores (i.e., N_G/N_S) is possible.

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