MICROVASCULAR PERMEABILITY: EFFECTS OF PROSTAGLANDIN E_1

By

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ABSTRACT

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A wide variety of experimental evidence indicates that prostaglandins are indirectly involved in the inflammatory process. This evidence includes the fact that prostaglandins of the "E" series can be recovered from a sundry of inflammatory exudates, and that non-steroidal, anti-inflammatory agents (cyclo-oxygenase inhibitors) can attenuate all forms of inflammation. However, discrepancy prevails concerning the direct microvascular permeability inducing effect of exogenous PGE_1 or PGE_2 when administered into a tissue or organ.

To study the direct effects of exogenous PGE_1 on vascular resistance and microvascular permeability, the canine forelimb technique was employed. The sixty minute, local intra-arterial infusion of PGE_1 (2.0 to 16.0 µg/min) into forelimbs perfused either naturally or at constant brachial artery inflow produced profound vasodilation and increases in lymph total protein concentration, yet only minute dose independent increases in lymph flow rate. The increase in lymph flow rate was similar in magnitude regardless of the method of forelimb perfusion, i.e., natural or constant brachial artery inflow. PGE_1 (16.0 or 32.0 µg/min) also generated marked elevations in forelimb

weight due to edema formation. The weight increases were substantially greater under natural inflow conditions.

Skin small vein pressures were markedly elevated in the forelimbs perfused at natural inflow, although they failed to change relative to control in the limbs perfused at constant inflow. This suggests an increase in capillary hydrostatic pressure with natural inflow and no increase with constant inflow. Interestingly, local, intra-arterial infusion rates between 2.0 to 16.0 μg/min yielded significant increases in mean systemic arterial blood pressure. In contrast, increasing the local intra-arterial infusion rate to 32.0 μg/min caused sustained reductions in mean systemic arterial blood pressure. All infusion rates studied produced a significant increase in hematocrit.

Following pre-treatment with indomethacin (5 mg/kg) for one hour, the local, intra-arterial infusion of PGE_1 (16.0 μg/min) produced vasodilation and increases in lymph total protein concentration similar to the values obtained in the absence of indomethacin. There were no significant changes in lymph flow rate observed in the absence of indomethacin, but dramatic increases in lymph flow rate were obtained under both natural and constant brachial artery inflow conditions following pre-treatment with indomethacin.

Therefore, these data suggest that PGE_1 may well function as an inflammatory mediator in the dog, yet its role may be different and its mechanism of action considerably more complex than the putative mediators of inflammation (i.e., histamine and bradykinin).

DEDICATION

To Linda and my parents

their love and understanding have lit my way
to accomplishment and joy

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LIST OF SYMBOLS AND ABBREVIATIONS

NF = naturally perfused forelimb

CF = forelimb perfused at constant inflow

cm = centimeter

mm = millimeter

μm = micrometer

gms = grams

mgm = milligram

μg = microgram

ml = milliliter

min = minute

Å = Angstrom

mm Hg = millimeters of mercury pressure

k = Capillary Filtration Coefficient (CFC)

INTRODUCTION

The prostaglandins are a group of metabolic products from membrane unsaturated fatty acids which possess diversified physiological and pathological roles. Since their discovery in 1935 by Euler (28,29), there has accumulated a variety of evidence implicating these autocoids in the inflammatory process (118). The supportive documentation includes that: 1) exogenously administered prostaglandins, primarily of the "E" series, increase microvascular permeability in several species; 2) prostaglandins are released and can be recovered from several different types of inflammatory exudates; 3) non-steroidal, anti-inflammatory drugs (i.e., indomethacin, aspirin, naproxen) inhibit the biosynthesis of prostaglandins, thus attenuating inflammation. Although no controversy prevails among investigators concerning prostaglandin release in inflammation and the ability of non-steroidal anti-inflammatory drugs to inhibit prostaglandin biosynthesis, disagreement does exist with regard to the extent that exogenously administered PGE_1 or PGE_2 can augment microvascular permeability.

In the rat, mouse, hamster and guinea pig, PGE_1 causes an increase in permeability due to a direct action on the microvascular membrane resulting in the formation of gaps between endothelial cells in the post-capillary venules (24,59,113,114). This appears to be similar to the mechanism by which histamine and bradykinin increase microvascular peremeability. In contrast, however, others argued that PGE_1 has no permeability inducing effects (52,120).

Even though most reports show that PGE_1 can cause edema in the rodent, the concentrations required are much higher than those recovered from inflammatory exudates. This observation has led to the hypothesis that prostaglandin generation contributes to the edema of inflammation by potentiating or increasing the vascular leakage induced by other putative mediators, including histamine and bradykinin (113,126).

In the few permeability studies reported for dogs, PGE_1 failed to increase microvascular permeability to macromolecules (25,41,58). However, in these experiments doses of PGE_1 were small and the duration of infusions was short. Also, a possible interference by other prostaglandins or eicosanoids produced in response to the exogenous PGE_1 could have antagonized the effect of PGE_1 on the microvascular membrane. Consequently, these factors may have contributed to the failure to obtain positive results.

With the preponderance of supportive evidence in the rat, mouse, hamster and guinea pig, and the valid criticisms for the canine studies being considered, a systematic examination of the effects of PGE_1 on fluid filtration and macromolecular efflux is warranted. Therefore, the present study is an attempt to systematically characterize the permeability properties of one hour, local infusions of PGE_1 into the canine forelimb over a dose range of 2.0 µg/min. to 32.0 µg/min. Several experiments will test systemic indomethacin pretreatment prior to the onset of the local PGE_1 infusion. This was designed to suppress endogenous prostaglandin synthesis and release which might interfere with the authentic microvascular effects of exogenous PGE_1.

SURVEY OF THE LITERATURE

The microcirculation is the end organ of the cardiovascular system and performs two vital functions. The primary one is cell nutrition and the second is body fluid balance. In general, this system operates by delivering blood to tissues and organs commensurate with their metabolic demands, and by regulating the exchange of fluid between the intravascular and extravascular compartments.

To understand these processes better, investigators have attempted to define the anatomical features of capillary and lymphatic vessels, and to experimentally manipulate the distribution of plasma across the vessel walls. As better microscopes became available, additional potential pathways for solute and/or solvent movement were postulated. The term permeability was introduced to explain the end result of solute and solvent distribution across capillaries within the limits of the experimental conditions. However, such studies do not directly explain the relationship between the known microstructure and the steady-state values of body fluid distribution. This review of the literature will deal first with the current status of the anatomical features of capillary and lymphatic vessels, and then with some of the analytical processes of plasma and interstitial fluid dynamics that are pertinent to this dissertation.

Microcirculation implies blood flow through small vessels at the level of the capillary. The term capillary was first introduced in

1661 by Marcello Malpighi in the frog lung (73). Malpighi demonstrated that these "porosities in the flesh" were long, thin-walled tubes, whereby blood flows from artery to vein. Even though centuries have elapsed since Malpighi's initial observations, a persistent diversity of opinions still exists regarding the descriptions of the patterns of distribution and structure of the microcirculatory system.

The capillary vessel of most mammalian tissues and organs consists of a single layer of flattened endothelial cells bound together within an acellular basal lamina. The materials comprising the basal lamina (mainly mucopolysaccharides) are presumably secreted by these cells. Substances may penetrate the endothelium by passing directly through the cells, that is, through two thicknesses of cell membrane and a layer of cytoplasm, or by penetration without entering the cell. This latter route occurs by extracellular channels or through junctions between cells which are referred to as pores (94).

The work of Pappenheimer (90), Renkin (93) and Solomon (110) provides the foundation for the pore theory. According to this theory, hydrophilic materials traverse the endothelial barrier through water-filled channels or pores, under the driving force of hydrostatic pressure and concentration gradients. This concept unifies many experimental observations and provides the physical basis for the phenomenon of molecular sieving, or the restriction of molecules whose diameter is larger than the pore.

The mechanism of molecular sieving has been characterized by Grotte (43) and Arturson (4), who studied the exchange of dextran fractions of different molecular sizes. Their work leads to the conclusion that there must be a "large pore system" and a "small pore system." The diameter of the large pores ranges from 250 Å to 700 Å. These pores

provide the main pathway for the permeation of plasma proteins and other macromolecules (43). Large pores are presumed to be present mostly in the venous portion of the microcirculation and to account for the higher permeability of the venular segment to proteins. Using a similar experimental approach, Landis and Pappenheimer (67) concluded that the small pores occur as cylinders or slits with a radial range of 45 Å to 90 Å. The so-called "large pore system" appears to be only in a 1:34,000 ratio to the "small pore system" (53).

The pore theory implies only that the capillary wall acts as if it had pores. Electron microscopists have sought evidence for such pathways in view of the postulated pore diameters. Electron micrographs have revealed that the ultrastructure of capillaries is not the same in all parts of the circulation (77). Three different types of capillary walls have been identified based on the number of pores in the endothelia: they are termed continuous (non-fenestrated), discontinuous, and fenestrated. Table I summarizes the various capillary types in tissues and organs.

In analogy with larger blood vessels, the capillary wall of the continuous type consists of three layers or tunics (86,88). The complete inner tunic is formed by the endothelial cells, which are markedly attenuated--0.20 to 0.40 μM thick over all portions except in the nuclear regions. A second, also complete, middle tunic is formed by the basement membrane, which envelopes scattered pericytes. Pericytes are precursors of smooth muscle cells, and very little is known about their role within the general functional scheme of the vascular wall. The outer layer consists of scattered bundles of collagen and elastic fibers, and of single connective tissue cells. Detailed accounts of

Table I. Ultrastructure of the capillary wall in various tissues and organs (130)

Tissue	Endothelium
All endocrine glands	fenestrated
Kidney:	fenestrated
Glomerulus	fenestrated
Tubules	fenestrated
Medulla	fenestrated
CNS blood-brain barrier	continuous
Muscle:	continuous
Skeletal	continuous
Cardiac	continuous
Smooth	continuous
Lung	continuous
Sinusoids of:	
Liver	discontinuous
Spleen	discontinuous
Bone marrow	discontinuous
Adipose tissue	continuous
Connective tissue	continuous
Intestinal mucosa	fenestrated
Dermis	continuous & fenestrated

of the ultrastructure of continuous endothelium are given in the literature (17,97,106).

The most characteristic feature of continuous endothelial cells is the presence of varying numbers of micro-pinocytotic vesicles, 700 Å in diameter (87). Most of these vesicles appear as invaginations of the plasma membrane on either surface of the cell and therefore are designated as plasmalemmal vesicles. A large number of closed vesicles not in contrast with the plasma membrane also can be found in the endothelial cell cytoplasm.

The margins of adjacent capillary endothelial cells overlap or interdigitate (88). Generally, the intercellular space is obliterated within its third tunic (luminal side) by an area of close approximation or an apparent fusion of the outer leaflets of the adjacent plasma membranes (87,88). Differentiations of the structure of the junctions have been reported in consecutive segments of the microvasculature (87).

Simionescu *et al.* (104,105) studied the distribution after intravascular injection of three tracer materials of various effective radii (10 Å-20 Å) within the endothelium of rat skeletal muscle capillaries. All three tracers were excluded from the narrow junctional regions, but did appear within cytoplasmic vesicles. Within 30 seconds of administration, the vesicles on the luminal (internal) surfaces of the endothelial cells were labeled with the tracer. At 60 seconds the labels were distributed uniformly in external and internal surface vesicles. Passage across the cell was concluded to occur entirely via the vesicles, either singly or through openings formed by chains of vesicles. In other studies utilizing colloidal carbon (radius: <150 Å), penetration of the endothelium did not occur (23). After injury,

however, it appeared to penetrate through widened intercellular clefts between adjacent endothelial cells in post-capillary venules.

The structural identity of a small pore system (40-45 Å) is a matter of dispute. Based on the experiments of Karnovsky (62), small pores were represented in skeletal muscle capillary endothelium. These findings have not been confirmed, although Simionescu *et al.* (105) have presented evidence indicating that the small pores are patent transendothelial channels built up by the vesicles in muscle capillaries. In post-capillary venules, on the other hand, junctions between adjacent endothelial cells did occur (102).

The existence of the small membrane-lined vesicles containing water-soluble tracers inside the endothelial cells supports the concept of vesicular transport proposed by Bruns and Palade (17). According to this theory, the vesicles first form invaginations on the luminal side of the cell membrane, develop a neck, and then are pinched off, to presumably drift across the cytosol and empty their contents at the opposite cell membrane. A basic drawback of the vesicular theory of transport is its inability to account for the observed rate of fluid exchange in tissues and for molecular sieving. Although it may participate in normal fluid and macromolecular transport in altered fluid-flux states, such as inflammation where loss of fluid and protein from the vascular compartment is greatly augmented, this mechanism as a normal regulator is improbable.

The endothelial cells of fenestrated capillaries display numerous intracellular openings (200-250 Å) which may be either opened or closed by a very delicate plasma membrane diaphragm (34). These occur primarily in the renal glomeruli, glands and intestinal mucosa. Tracer electron micrograph studies of capillaries in rat and mouse intestinal

mucosa showed concentrations of dextrans and glycogens outside a large fraction of the fenestrae (103). These tracers also were present in cytoplasmic vesicles; however, transit was considerably slower than through the fenestrae. The fenestrae, although usually closed by a diaphragm, are not only permeable to large proteins such as ferritin but also to large amounts of bulk fluid including solutes (127). This high protein permeability may be important in post-capillary venules since this would directly influence the reabsorptive capacity of the blood vessels.

Discontinuous capillaries possess endothelial cells with obvious intercellular gaps. This type of capillary is adapted for the transmural exchange of not only macromolecules but even blood cells. Therefore, a blood transfusion can be administered via a cannula inserted into the bone marrow. Liver, spleen and bone marrow are characterized by this type of capillary.

The exchange of materials between blood and interstitial fluid involves diffusive and convective processes that occur at the level of the microvascular membrane. Reciprocity between these two fluid compartments separated by the membrane takes place primarily by the diffusive process, which is determined by concentration gradients, and the physical properties of the microvascular membrane. The convective process, which results in the continuous flow of fluid through the microvascular membrane, is a consequence of the interaction between hydrostatic and osmotic forces. Fluid movement across the enormous surface area presented by the microcirculation is essential for the maintenance of osmotic equilibrium in the tissue environment, the facilitation of the transport of large molecules through the interstitium, and the regulation of blood volume.

In the tissue milieu, the movement of fluid is maintained by the convective transfer of blood through the capillary network and the radial transfer of blood ultra-filtrate through the tissue by a diffusive process. These two flows, namely the convective (intravascular) and the diffusive (extravascular), have a precise relationship to each other which is summarized by the concepts involved in the Starling hypothesis of fluid exchange (111,67). The Starling Equation for the radial movement of fluid between the blood and extravascular compartment is expressed as

$$Jv = k \left| (P_c + \pi_i) - (P_i + \pi p) \right|,$$

where:

Jv = rate of fluid movement,

k = capillary filtration coefficient,

P_c = capillary hydrostatic pressure,

π_i = tissue colloid osmotic pressure,

P_i = tissue hydrostatic pressure, and

π_p = plasma colloid osmotic pressure.

If the rate of fluid movement (Jv) is positive, net fluid filtration occurs; if this value is negative, net fluid reabsorption ensues.

According to this theory, the balance of fluid between the two compartments is a result of the exchange of fluid arising from the interaction of hydrostatic and oncotic pressures, mediated by the permeability characteristics of the blood-tissue interface. Classically, net filtration occurs at the arterial end of the capillary and net absorption at the venular end. This occurs because the transmural hydrostatic pressure gradient exceeds the transmural oncotic pressure

gradient at the arteriolar end, whereas the reverse is the premise at the venular end of the capillary.

The primary measurements necessary to examine the validity of the Starling hypothesis were first carried out by Landis in 1927 using micromanipulation techniques (66,67). These studies were performed on single capillaries in the frog mesentery. The results from these experiments showed a direct linear relation between capillary hydrostatic pressure and fluid movement across the microvascular membrane (Figure I). When the capillary hydrostatic pressure exceeded 12 cm H_2O, fluid passed from the vasculature to the interstitium. At pressures below 10 cm H_2O, fluid was withdrawn from the interstitium and into the capillary. At pressures between 10 and 12 cm H_2O, little or no movement of fluid occurred. This was the result of the capillary hydrostatic pressure being balanced by the osmotic pressure of the plasma proteins.

In addition to supporting the basic tenets of Starling's hypothesis, these data also provided a measure of the permeability of the microvascular membrane to isotonic fluid. The permeability of the microvascular wall to isotonic fluid or the hydrodynamic conductivity of the capillary wall is called the capillary filtration coefficient (k or CFC). This proportionality constant was computed by Landis from the slope of a straight line drawn through the data points (Figure I).

Experimentally, the capillary filtration coefficient is measured in ml/min/mm Hg/100 gms tissue, by recording the increase in weight of an organ or limb that is caused by a known increase in capillary hydrostatic pressure (34). The coefficient varies from tissue to tissue with an average whole body value for man of .0061 ml/min/mm Hg/100 gms

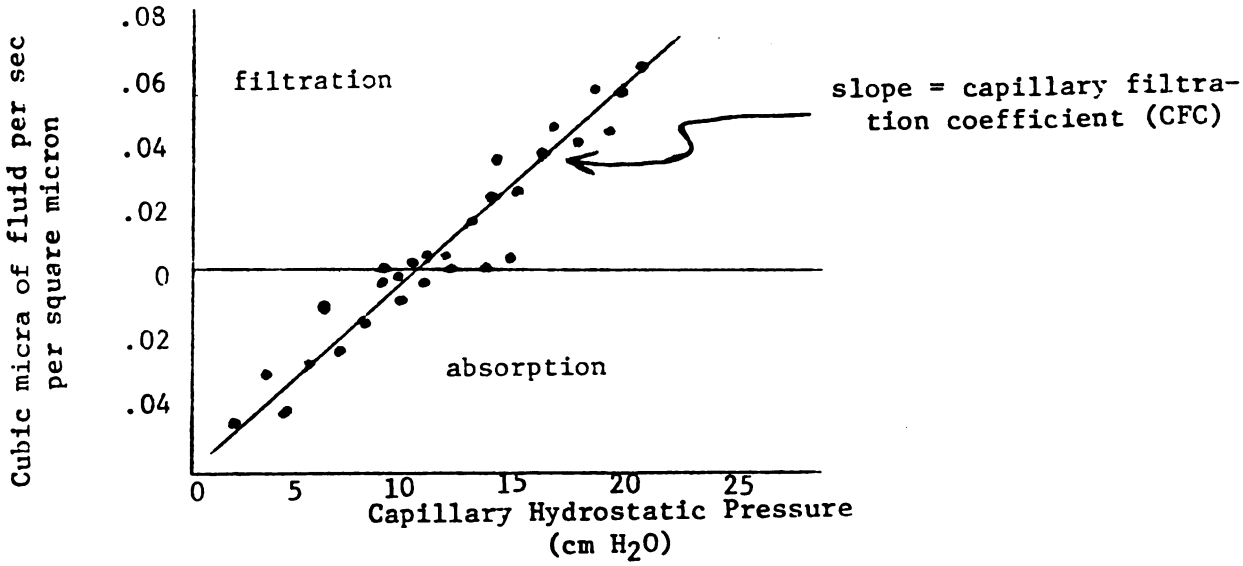


Figure I. Linear relation between capillary hydrostatic pressure and fluid movement across the microvascular membrane (67).

tissue (67). Under experimental circumstances the capillary filtration coefficient is used as an index of capillary permeability, which includes surface area available for diffusion.

Capillary hydrostatic pressure (Pc) is directly dependent upon capillary blood volume and compliance. Clough *et al.* (22) have indicated that capillaries are quite rigid. They report a change of only 0.1 μm in radius in capillaries of the cat mesentery during systole. This rigidity results from the environment circumjacent to the capillaries. The basement membrane and gel matrix surrounding these microvessels give the capillaries little, if any, compliance (36). Since compliance is relatively constant in capillaries, changes in capillary blood volume are the primary factor in determining Pc. Capillary blood volume is influenced by systemic arterial pressure, venous pressure and the pre- and post-capillary resistances. The interrelationship of these factors is expressed in the following equation of Pappenheimer and Soto-Rivera (67):

$$\bar{P}c = (Pa - Pv)\frac{Rv}{Ra + Rv} + Pv,$$

where:

Pa = systemic arterial pressure,

Pv = venous pressure,

Ra = arterial resistance (pre-capillary), and

Rv = venous resistance (post-capillary).

An increase in Pa or Pv will increase Pc. Increasing Rv will raise Pc, whereas increasing Ra will lower Pc. Vessel resistances are inversely related to vessel caliber. This caliber is determined mainly by active changes in vascular smooth muscle activity and passively by changes in effective transmural pressure. Effective transmural pressure is the

pressure in the interstitial fluid environment of the capillary subtracted from the intraluminal pressure in the capillary. Changes in blood viscosity also affect resistance to blood flow. An increase in the viscosity of the blood will increase the resistance to flow. Blood viscosity is proportional to the hematocrit and the dissolved materials in the plasma.

The hydrostatic pressure of the interstitial spaces is determined by tissue compliance and interstitial fluid volume. Classically, it is accepted that this pressure is positive and will therefore oppose fluid filtration out of the capillaries. While this is the case for many tissues (kidney, brain, bone marrow, abdominal viscera), recent studies have suggested that it is sub-atmospheric in subcutaneous tissue (44). Guyton, using implanted perforated spheres in a variety of tissues (44), has concluded that this pressure is sub-atmospheric (-7 mm Hg). This conclusion, however, is under much criticism and further investigation is needed to resolve the question.

Plasma colloid osmotic pressure (oncotic pressure) is the pressure due to the concentration of dissolved proteins in the blood. Under normal conditions, the total osmotic pressure of plasma is about 6,000 mm Hg, with the oncotic pressure contributing about 25 mm Hg (12). This oncotic pressure is responsible for vascular fluid retention. It is achieved because the plasma proteins are largely confined to the intravascular space and therefore create an active tonicity within the vasculature. The electrolytes which constitute the bulk of the remaining total osmotic pressure pass freely through the capillary membrane, creating no tonicity between the two body fluid compartments.

Albumin, globulin and fibrinogen comprise the major plasma proteins. Albumin, which is found in the greatest abundance, has an

average molecular weight of 69,000 and a concentration of 4.6 gms %. Globulin has an average molecular weight of about 140,000 with a concentration of 2.5 gms %. Fibrinogen is the largest of the plasma proteins, having a molecular weight of about 400,000, but is found in a concentration of only 0.3 gms %. Of the total oncotic pressure, 19 mm Hg are attributable purely to the proteins, and the remaining 6 mm Hg are due to the cations which bind to the proteins by electronegative forces. This phenomenon is known as the "Donnan Effect." Since albumin is in the greatest concentration of the three proteins, it constitutes the largest fraction (70%) of the total oncotic pressure (12).

Interstitial fluid colloid osmotic pressure is contingent on the protein concentration of the interstitial fluid. The concentration of the interstitial protein is not uniform; it varies from 0.4 gms % to 3.3 gms %, depending on the tissue (67). In skin and skeletal muscle, the average interstitial protein concentration is about 2.0 gms %, which yields an oncotic pressure of about 5 mm Hg. More recent findings suggest that the total protein concentration of interstitial fluid is about 3.0 gms % and the colloid osmotic pressure about 10 mm Hg (125). In the liver, where capillary protein permeability is high, large amounts of plasma proteins cross the microvascular membrane, producing an interstitial fluid oncotic pressure of about 16 mm Hg or more. This corresponds to a minimal protein concentration of about 3.3 gms %.

Discussion of absolute values for interstitial fluid colloid osmotic pressure is under considerable debate. Measurements using implantable devices such as perforated capsules that theoretically equilibrate with interstitial fluid may be inaccurate because of the

possibility of contamination by plasma, or that the fluid sampled may not contain all the osmotically active substances. The most common method for estimating interstitial oncotic pressure is lymph fluid analysis. This method makes the assumption that lymph is a true reflection of the interstitial fluid contents. Critics of this view argue that changes could occur in lymph composition as it flows from terminal lymphatics upward to larger vessels due to gradients of protein concentration within the interstitial spaces. Renkin and Garlick (96) have shown that dextran molecules of known molecular weight and size are in equal concentration between lymph and interstitial fluid when injected into a tissue. This observation allowed them to conclude that there is no significant protein concentration gradient beyond the capillaries. Garlick and Renkin (37) have performed studies showing that exchange occurs only at lymph nodes and not in the lymphatic trunks. If lymph is sampled before it reaches a node, it should be a true reflection of what is at the terminal lymphatic vessel.

The terminal vessels of the lymphatics consist of a widely distributed closed-end network of highly permeable lymph capillaries that are similar to blood capillaries. In general, there are no tight junctions between endothelial cells, but they possess fine filaments that secure them to the surrounding connective tissue. With muscular contraction, these filaments may distort the lymphatic vessel such that spaces will open between adjacent endothelial cells, allowing the entrance of proteins, macromolecules and cells present in the interstitial fluid. The terminal lymphatics drain into larger vessels that ultimately enter the right and left subclavian veins.

Valves exist throughout all lymphatic channels. When a lymph vessel is compressed by pressure, lymph in the channel is squeezed in both directions. Since the valves are only unidirectional to flow, the fluid will be transported from the terminal lymphatics in the central direction past the valve. Factors that can compress the lymphatics and evoke the movement of lymph are: muscle contraction, arterial pulsations, passive movements of body parts and compression of body tissues from the outside. During exercise, lymph flow can increase substantially, about 15 times normal. When either the volume of interstitial fluid exceeds the drainage capacity of the lymphatics or the lymphatic vessels become blocked, interstitial fluid accumulates and gives rise to edema. The accumulation of fluid occurs primarily in the more compliant tissues (i.e., subcutaneous tissue).

The most fundamental mechanism by which substances are relocated between the vascular and interstitial compartments is by the diffusive process. Diffusion is a distributive process which is dependent on the thermal motion of molecules; therefore, the greater the concentration difference along an axis, the greater the rate of diffusion. This mode of exchange can be described by the Fick Law of Diffusion, which states that the quantity of substance moved per unit time is equal to the free diffusion coefficient of the molecule, the concentration gradient, and the area of the capillary membrane. These factors are related in the following equation:

$$ds/dt = D \cdot A \cdot dc/dt$$

where:

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

D = free diffusion coefficient for a molecule (this value is inversely proportional to the square root of the molecular weight),

A = area of the capillary membrane,

dc/dt = concentration gradient.

The site of diffusion of a molecule depends on whether the substance is water-soluble or lipid-soluble. Water-soluble substances pass through small pores in the endothelial cell. For small molecules such as water, ions, and urea, diffusion is free and rapid. However, for lipid-insoluble molcules of increasing size, diffusion becomes progressively more restricted, such that molecules above a molecular weight of 60,000 are almost completely impermeable to the capillary endothelium. Lipid-soluble molecules such as CO_2, O_2 and anesthetic gases pass freely through the intact cell membrane. Small, lipid-soluble molecules pass with great ease and rapidity between capillary and interstitium.

Micropinocytosis (cytopemphysis) is a slow transport mechanism that is probably responsible for the movement of large lipid-insoluble molecules between plasma and interstitial fluid. The process has been described previously in this survey of the literature. It appears that there should be little doubt that such a mechanism is involved in the normal or physiologic transport of certain large macromolecules across the microvascular membrane, based on numerous data and observations of this phenomenon by several well respected medical scientists (19,62, 95,104,105,127). However, the importance of this mechanism in pathologic conditions, such as inflammation, is questionable.

This dissertation focuses on the second of two vascular changes associated with acute inflammation: 1) hemodynamic adjustment and 2) alterations in vascular permeability. The increased vascular permeability with the escape of plasma proteins and fluid is known as exudation. It accounts for an increase in the volume of interstitial fluid (edema) and tissue swelling at the site of injury. The increased permeability first affects venules but rapidly extends to capillaries (98). The mechanisms leading to this increase in permeability in inflammation are still incompletely understood.

Several classes of mediators affecting permeability of blood vessels have been described, and most appear to have their effect on post-capillary venules. The mechanism by which permeability agents accomplish their biological effects is not known. The suggestion that permeability changes are due to increases in hydrostatic pressure in the capillaries, caused by contraction of venules, has not been substantiated. It has recently been suggested that permeability-inducing agents cause contraction of periendothelial cells (pericytes), which surround capillaries. Since these cells seem to be structurally attached to the endothelial cells, their contraction could result in pulling apart endothelial cells at their junctional zones, allowing the escape of fluids and solutes. Electron microscopic evidence of contractile-like agents in pericytes has been reported (107). However, this theory of changes in permeability awaits further study. Classical permeability inducing agents are histamine, bradykinin, SRS-A (slow reacting substance of anaphylaxis) and several basic peptides released from neutrophils (98,107). The prostaglandins are biologically active compounds whose role in the inflammatory process and the induction of permeability is currently under a great deal of speculative investigation.

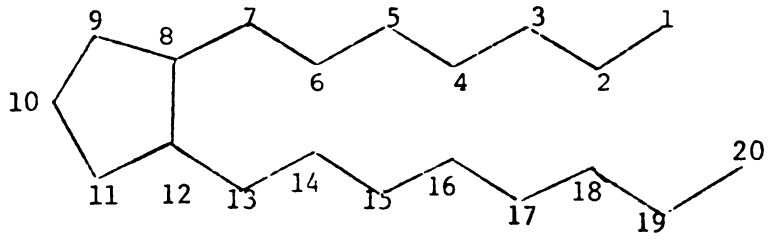
The prostaglandins are a group of endogenous, unsaturated fatty acids to which are attributed a diversified range of physiological activity. The first descriptive report of an effect ascribed to the prostaglandins was in 1930, when two American gynecologists, Drs. Raphael Kurzrok and Charles Lieb, observed that uterine muscle strips would relax or contract when exposed to human semen (65). In an attempt to procure a treatment for human sterility, these investigators recognized that uteri obtained from women who had completed successful pregnancies responded with relaxation when 1 cc of semen was applied. However, the uteri from women who had histories of complete or extended periods of sterility contracted with the addition of human seminal fluid. The significance of these findings not only indicated that the active constituent in the human semen was dependent upon the endocrine status of the uterus, but that this new substance possessed differential biological activity.

Although Kurzrok and Lieb made these preliminary observations, the credit for the discovery and isolation of prostaglandins belongs to the world-renowned Swedish medical scientist, Ulf S. von Euler. In 1935, it was Euler who clearly established that the active principle in human semen and extracts from sheep vesicular glands was a lipid-soluble acid which he called "prostaglandin" (28,29,30). The following excerpt is a brief account from the records of Euler regarding the identification of prostaglandins:

> Starting with the findings that the vesicular gland of the sheep was a rich source of the active compound, we found it only natural to seek more information about its chemical nature. The observation that it could be extracted with lipid solvents from an acid solution but was freely water soluble in an alkaline medium not only suggested that it was of acidic character, but also provide a simple means of purification. It thus appeared to have the properties

> of a fatty acid, which was somewhat surprising since very little was known about biologically active substances of this kind, and certainly nothing of their activity in the body.
>
> Through the good offices of Hugo Theorell in the chemistry department of the Karolinska Institute, who had just developed an apparatus for preparative electrophoresis allowing separation of various fractions, an active extract was separated. The results showed as expected, that the active principle migrated toward the anode.
>
> Since it appeared that the active compound was a new and previously unknown chemical, it was thought that it should receive a name of its own. Owing to its occurrence in extracts of the prostate and vesicular gland, it was called prostaglandin. (31)

For the next two and one-half decades, the investigation into this area was limited because of inadequate experimental techniques. The impetus for further biological research occurred in 1960, when Bergström and Sjövall isolated and chemically characterized the first pure samples of prostaglandin (10,11). They initially isolated two prostaglandin compounds which behaved differently, based on their partition between ether and an aqueous phosphate buffer. The compound soluble in ether was called prostaglandin E or PGE (11), and the compound soluble in the phosphate buffer was called prostaglandin F or PGF (10). Preceding the identification of PGE and PGF, Bergström and his associates were able to differentiate and isolate 13 structurally different compounds which were all derivatives of the parent-skeleton compound, prostanoic acid (9) (see Figure II). All prostaglandins contain 20 carbon atoms, a cyclopentane ring, two aliphatic side chains and a terminal hydroxy group. With the recent discoveries of many other prostaglandins and prostaglandin-like compounds, E. J. Corey has suggested that the term "eicosanoids" be used to describe all of the 20-carbon derivatives, whereas "prostanoids" refers only to those with a prostanoic acid skeleton (81).



All prostanoids contain 20 carbon atoms, a cyclopentane ring, two aliphatic side chains and a C-terminal hydroxy group.

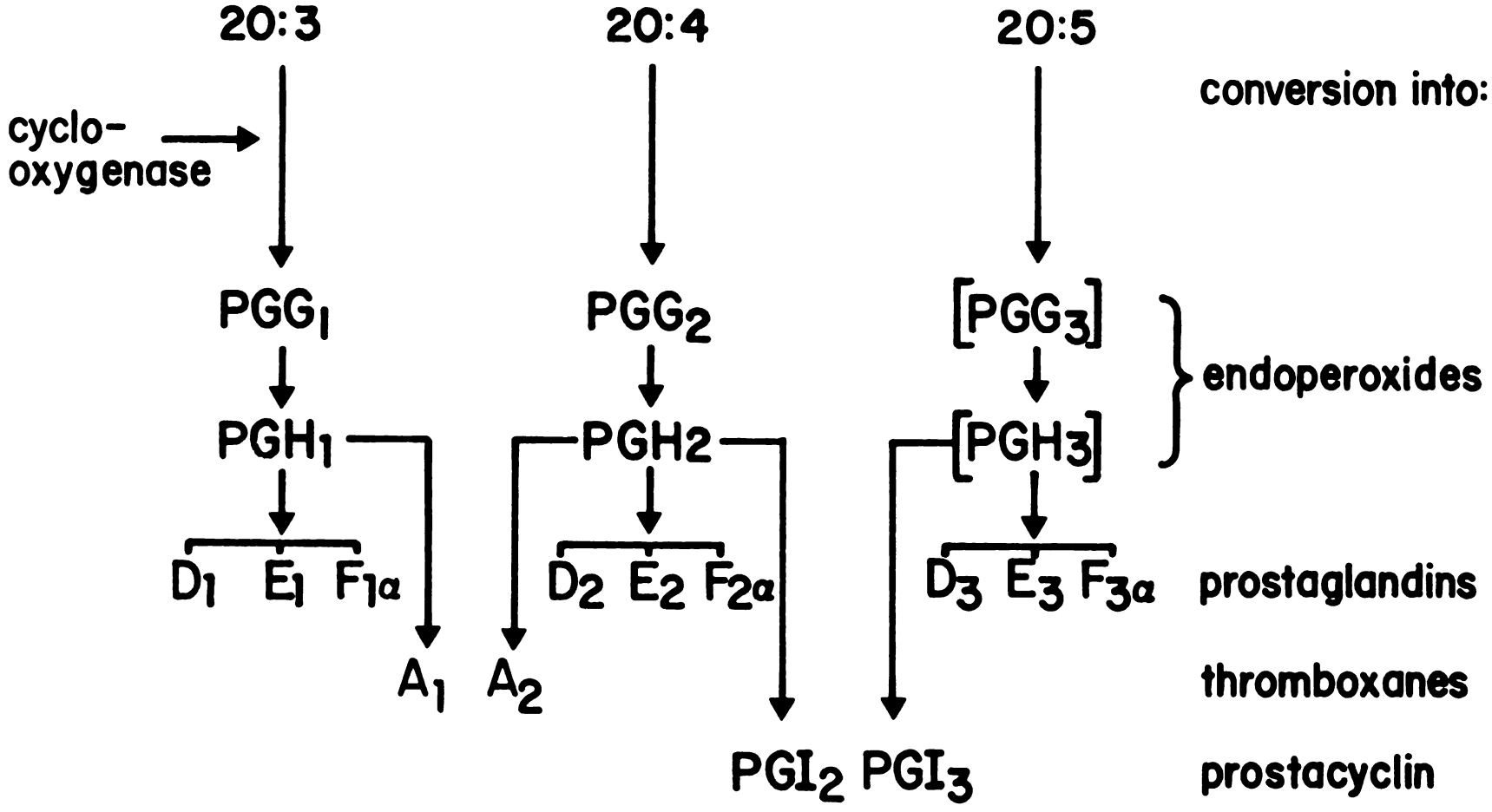
Figure II. Structure of prostanoic acid.

Physiologically, prostaglandins are synthesized from 20-carbon polyunsaturated fatty acids containing three, four or five double bonds. These fatty acids are present mainly in the phospholipid portion of the mammalian cell membrane, although serum triglycerides and cholesterol are also sources (79,53). Furthermore, prostaglandins have been identified in birds, reptiles, amphibians, fish and various members of the plant kingdom (53).

The principal fatty acid precursor of prostaglandins in man is eicosatetraenoic acid, which is commonly referred to as arachidonic acid (see Figure III). This compound contains four double bonds and generates those eicosanoids with two double bonds in their aliphatic side chains (i.e., PGE_2, $PGF_{2\alpha}$, PGD_2, PGI_2, TXA_2). Di-homo-γ-linolenic acid, which possesses three double bonds, yields eicosanoids that contain one double bond (i.e., PGE_1, $PGF_{1\alpha}$, PGD_1, TXA_1). Eicosanoids with one double bond are synthesized only in trace amounts in most mammalian tissues. Although it was originally assumed that these substances were of paramount importance in physiological control systems, recent advances have demonstrated that their significance is less than the eicosanoids derived from arachidonic acid (79). Eicosapentaenoic acid, the harbinger substance for the eicosanoids with three double bonds, incorporates five double bonds in its structure. The physiologic relevance of these eicosanoids is ambiguous and considerable investigation is necessary to elucidate their significance (79). The situation regarding the biosynthesis of the eicosanoids as of 1979 is summarized in Figure III.

Since the arachidonic acid cascade is of greater importance in mammalian tissues than the other two biosynthetic pathways, this

FIGURE III



Biosynthesis of prostaglandins from dihomo-γ-linolenic acid (C20:3), arachidonic acid (C20.4) and eicosopentaenoic acid (C20:5). (53)

treatise will concentrate on the significance of these compounds. Reference to other pathways will be made when necessary.

Arachidonic acid is obtained directly from the diet or by anabolic desaturation and chain elongation from dietary linolenic acid. It is transported in blood, largely bound to albumin, and is incorporated as a structural component of phospholipids in cell membranes and other subcellular structures of all tissues in the body (81).

It is commonly accepted that the majority of free arachidonic acid, as well as the other precursor substances (i.e., di-homo-γ-linolenic acid and eicosapentaenoic acid) are liberated from the membrane phospholipids by enzymatic cleavage. The enzyme or group of enzymes responsible are called acylhydrolases, which include mainly the phospholipases. Evidence remains nebulous as to the specificity of these enzymes for a particular precursor (68).

The specific acylhydrolases associated with prostaglandin synthesis are activated by a number of different physical and chemical stimuli (Table II). The accepted reaction sequence for prostaglandin synthesis is: stimulus ⟶ acylhydrolase activation ⟶ release of fatty acid precursor from various lipid sources ⟶ eicosanoid biosynthesis and release.

The acylhydrolase(s) is the rate-limiting enzyme in the prostaglandin synthetic pathway (121). This assumption is based on the fact that the activity of the other eicosanoid synthesizing enzymes in intact tissues are limited by the amount of available arachidonic acid. In addition, arachidonic acid can be provided from endogenous fatty acids by the administration of exogenous acylhydrolase (121).

Table II. Stimulants of acylhydrolase (121)

Stimulant	Tissue
Amines:	
catecholamines	spleen, stomach, brain, fat cells
serotonin	stomach, brain, fat cells
acetylcholine	skin, intestine, adrenals
Hormones:	
TSH	thyroid gland
ACTH	adrenal glands
LH	uterus
glucagon	liver
Peptides:	
bradykinin	lung, skin
angiotensin	spleen
Miscellaneous:	
collagen	platelets
thrombin	platelets
dust particles	lung, spleen
nerve stimulation	brain, kidney, lung, fat cells
anaphylaxis	lung, spleen
inflammation	skin
distention	lung, uterus, stomach
distilled H_2O	intestine

Based on the substantial number of diverse acylhydrolase stimulants (Table II), a perplexing question arises as to the mechanism or mechanisms by which acylhydrolases can be activated to augment arachidonic acid release. Currently, there are three proposed mechanisms which may account for the activation process (121). The first mechanism was forwarded from the observation that in the pancreas, phospholipase A_2 is stored as a zymogen granule and is activated by tryptic cleavage. Consequently, a stimulus would convert trypsinogen to trypsin and this would cleave phospholipase A_2 from its zymogen storage granule. This type of action is irreversible and, in most cases, not suitable for physiological control of prostanoid biosynthesis, which must depend on reversible activation mechanisms. However, in the platelet only, this could provide a mechanism of action since platelets may undergo irreversible damage subsequent to their activation (49). A second proposed mechanism involves a reversible activation achieved by changes in the concentration of free intracellular Ca^{++}. Some acylhydrolases of plasma membranes are Ca^{++} dependent. The optimal Ca^{++} concentration for maximal acylhydrolase activity of liver plasma membranes is 5 μM. This is considerably higher than the normal intracellular concentration of free Ca^{++}. Consequently, an induced increase of Ca^{++} would activate an acylhydrolase and enhance the cleaving activity. Supporting this theory is the fact that a variety of prostaglandin stimulants facilitate Ca^{++} release into the cell. These stimuli include thrombin, acetylcholine and the catecholamines. The third mechanism has been proposed by Verger and his co-workers (119). They suggest that the activity of acylhydrolases, particularly phospholipases, depends to a large extent on the structural arrangement of the phospholipid molecules in the cell membrane. When the normal lipid

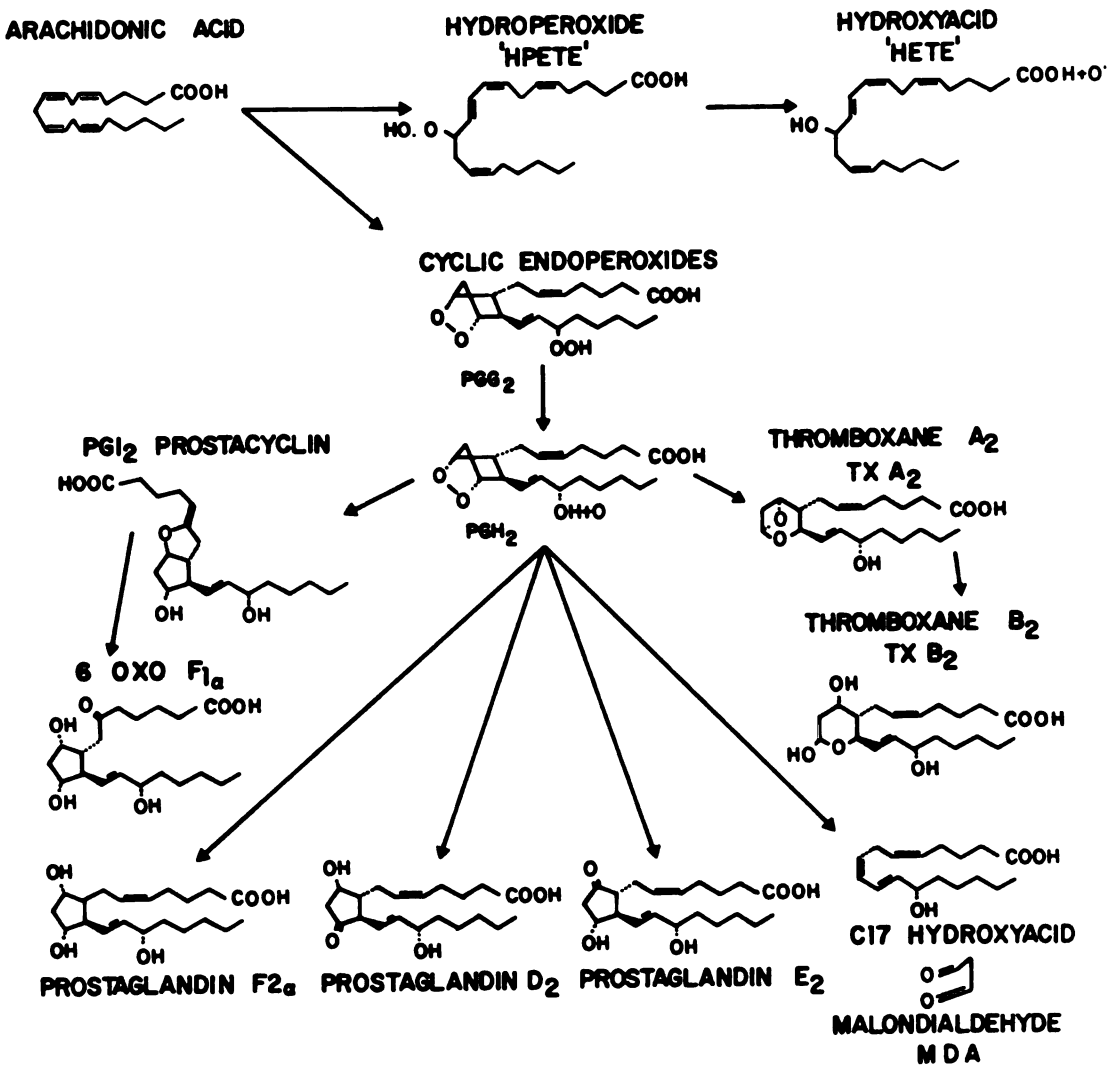
bi-layer structure is altered as in perturbation of the cell, cleavage of arachidonic acid from phospholipids will ensue.

Acylhydrolases are found in various organelles in the cell, including mitochondria, lysosomes, microsomes and the plasma membrane (38). Since acylhydrolases have several sub-cellular locations, it is assumed that its stimulation and activity are modulated by more than one mechanism depending on the state of the particular tissue or cells. It has also been noted that mepacrine is a specific inhibitor of acylhydrolase, and thereby prevents the release of free arachidonic acid (49).

Once released from the fatty acid pools, arachidonic acid is metabolized by two types of enzyme systems. One is a series of lipoxygenases that peroxidize arachidonic acid to form unstable hydroperoxides. The hydroperoxides are then rapidly reduced enzymatically by glutathione peroxidase to inactive hydroxyacids (Figure IV) (81). Different lipoxygenases have been described in different tissues depending on what carbon atom the peroxidation occurs (68). For example, the lipoxygenases in the lung peroxidize arachidonic acid at carbon atoms 11 and 12, whereas white cell lipoxygenase peroxidizes at carbon 5.

The other enzyme system has a cyclo-oxygenase that forms the prostaglandin endoperoxide PGG_2 (Figure IV). This is converted to another endoperoxide, PGH_2, by the action of a peroxidase enzyme (85). The half-lives of both endoperoxides are about five minutes at 37°C (78). The classical prostaglandins, PGE_2, $PGF_{2\alpha}$ and PGD_2, are formed directly from PGE_2 either by the action of an isomerase or by a non-enzymatic reduction which will yield $PGF_{2\alpha}$. PGD_2 is formed from PGH_2 in the presence of 11-keto-isomerase, which is a cytoplasmic (soluble) enzyme (68). PGD_2 is a major prostanoid identified in brain (1) and

FIGURE IV



METABOLIC PATHWAYS OF ARACHIDONIC ACID (81)

platelets (2). Although PGD_2 has been regarded as biologically inactive, it does inhibit platelet aggregation (68). Endoperoxide-ε-isomerase is a membrane bound enzyme which converts PGH_2 to PGE_2 (68).

Although $PGF_{2\alpha}$ can be formed directly from PGH_2 by a non-enzymatic reduction, speculation exists for another route of formation. PGE_2-9-keto-reductase, which reversibly converts PGE_2 to $PGF_{2\alpha}$, is a cytoplasmic enzyme that has been isolated from vascular tissue, kidney, lung, brain, heart, spleen, liver and blood cells (112,69). This enzyme requires NADP as a cofactor and may function as the major synthetic route for the formation of $PGF_{2\alpha}$ (69). Consequently, $PGF_{2\alpha}$ generation may not be directly from PGH_2, but rather through PGE_2 acting as an intermediate. It is interesting to speculate on the importance of this enzyme in regulating the diverse physiological processes involving these two classes of prostaglandins, yet no definitive evidence is available to elucidate this issue.

The prostaglandin endoperoxides are also transformed enzymatically into two other unstable products, prostacyclin (PGI_2) and thromboxane A_2 (TXA_2) (47,78,82). The half-lives of these compounds are three minutes and 30 seconds, respectively.

The cascade of arachidonic acid metabolism that leads to the formation of the cyclic endoperoxide PGG_2 is catalyzed by the enzyme complex known as cyclo-oxygenase. The enzyme catalyzes the reaction which incorporates molecular oxygen into the polyunsaturated fatty acid, arachidonate. The cyclo-oxygenase complex is composed of several sub-units and has a molecular weight of about 70,000 daltons (68). The activity of the enzyme is completely dependent upon adequate heme availability. Since competition for heme by various other proteins

exists within the cell, this could provide a means of regulating prostaglandin biosynthesis (68).

In 1971, Vane and others discovered that non-steroidal, anti-inflammatory agents, notably aspirin and indomethacin, inhibited prostaglandin biosynthesis (33,117). Since this initial discovery, much evidence has emerged to indicate that the antipyretic, analgesic and anti-inflammatory actions of these drugs are mainly mediated via inhibition of prostaglandin biosynthesis at the cyclo-oxygenase step (80). Because aspirin and indomethacin prevent the biosynthesis of all known eicosanoids, they have been used extensively as experimental tools to clarify whether prostaglandins are involved in various physiological or pathological events.

In 1975, Hamberg and his associates discovered a new group of biologically active compounds, thromboxanes (47). Since these substances occurred in the thrombocytes (platelets) and contained an oxane ring in their structure, the word thromboxane was proposed. TXA_2 is derived from PGH_2 (Figure IV) and stimulates platelet aggregation. It has also been shown that TXA_2 is identical with rabbit aorta contracting substance (RCS) originally described by Piper and Vane (91). TXA_2 generation is the major product of prostaglandin metabolism in platelets; however, other tissues possess thromboxane synthetase and can therefore produce TXA_2. This list includes iris, umbilical artery, placenta, leukocytes and lung fibroblasts (81). TXA_2 produces vasoconstriction and contraction of the trachea (85).

TXA_2 is potentially capable of causing myocardial infarction. Shimamoto and his co-workers (100) demonstrated that in rabbits intracoronary artery injection of TXA_2 could cause massive platelet aggregation capable of producing myocardial necrosis and typical

electrocardiogram changes associated with infarction. Pre-treatment with phthalazinol, which inhibits TXA_2, prevented the myocardial infarction induced by TXA_2 (101). Other TXA_2 inhibitors (specifically thromboxane synthetase inhibitors) include N0164, L-8027, dipyridamole and imidazole (68).

The platelet pro-aggregatory phenomenon is by far the most important effect of TXA_2, which reduces platelet adenylate cyclase activity, and leads to an increase in platelet cyclic AMP (79). Therefore, Vane (79) has proposed that cyclic AMP exerts a regulatory function on platelets. It is known that increasing levels of cyclic AMP will render platelets less aggregable. Consequently, when TXA_2 decreases cyclic AMP, platelets aggregate.

The counterpart eicosanoid of TXA_2 is the platelet anti-aggregatory compound, PGI_2. PGI_2 was discovered by the Wellcome group from England in 1976 (78,82). In that laboratory, Dr. Moncada and his associates were studying the vasoconstriction induced by TXA_2 in porcine aortas. When arachidonic acid, PGE_2, $PGF_{2\alpha}$ or PGD_2 was added to porcine microsome preparations, no prostaglandin-like activity was detectable by smooth muscle bioassay. However, when PGG_2 or PGH_2 was incubated in the same system, a substance called PGX was found which relaxed blood vessels and inhibited platelet aggregation (78). The structure of PGX was quickly solved by the joint efforts of the Upjohn group in the United States and the Wellcome group in England, and the name prostacyclin (PGI_2) was adopted (55).

Prostacyclin inhibits platelet aggregation by stimulation of adenylate cyclase (79). This effect leads to an increase in platelet cyclic AMP, thereby rendering the platelets less aggregable. Since TXA_2 reduces adenylate cyclase activity, Moncada and Vane (79) have

proposed a theory that cyclic AMP exerts a regulatory function on platelets, which depends on a balance between TXA_2 and PGI_2.

Prostacyclin is the major known product of arachidonic acid metabolism in all vascular tissues studied (79). A recent discovery of interest is that prostacyclin can be released into the pulmonary vein by the lungs and can therefore be present in arterial blood. However, unlike the traditional prostaglandins (E_2, $F_{2\alpha}$ and D_2), prostacyclin is not degraded to an inactive metabolite by the lung. This results from the absence of an active uptake process for prostacyclin by pulmonary capillary endothelial cells (122). Consequently, it has been proposed that prostacyclin is a circulating hormone which continually activates platelet adenylate cyclase causing platelets to be less aggregable. This hypothesis suggests a homeostatic system in which platelet aggregation is controlled *in vivo* by a prostacyclin mechanism that involves both circulating and locally produced prostacyclin (79).

Prostacyclin, thromboxane A_2 and the classical prostaglandins are considered to be major metabolites of the cyclic endoperoxide, PGH_2. However, under some conditions this endoperoxide may break down into a 17-carbon hydroxy acid (HHT) and malondialdehyde (MDA) (Figure IV) (81). The reaction occurs non-enzymatically by spontaneous degradation of PGH_2. It is thought that the biosynthetic pathways of TXA_2 and HHT formation are somehow linked since thromboxane synthetase also catalyzes HHT formation (81).

The rapid disappearance of physiologically active prostaglandins is catalyzed by intracellular enzymes (68). Tritium-labeled PGE_2 and $PGF_{2\alpha}$, administered intravenously to human subjects, are rapidly converted to the corresponding 15-keto-13,14 dihydro metabolites by the

enzymes prostaglandin dehydrogenase and Δ^{13} reductase (99). Initially, prostaglandin dehydrogenase catalyzes the oxidation of the 15-hydroxyl group to form a conjugated ketone at this position. This is usually followed by a reduction of the 13-14-trans double bond, catalyzed by Δ^{13} reductase. The resulting dihydro-keto derivative is the major prostaglandin metabolite in the peripheral circulation. Subsequent oxidations will follow yielding the mono- and dicarboxylic acids, which are predominant metabolites in the urine (68). Spontaneous hydrolytic cleavages of TXA_2 and PGI_2, the major degradative reaction sequences for these eicosanoids, yield the major metabolic by-products TXB_2 and 6-keto-$PGF_{1\alpha}$, respectively.

Although many regard prostaglandins as freely diffusible agents, current available evidence shows that some cell membranes represent a barrier to the passive diffusion of prostaglandins. Experiments have shown that cellular uptake in many tissues is an active or carrier-mediated process (16). Interference with the mediated transport by drugs, such as non-steroidal anti-inflammatory agents (indomethacin, naproxen, phenylbutazone), will increase the circulating levels of prostaglandins (15). Such active or carrier-mediated processes have been shown to exist in the brain (15), eye (15), lung (14), vagina (15), skin (70), kidney (13), and adipose tissue (68). Thus, active transport or carrier-mediated transport of eicosanoids across the cell membrane can be prerequisite to their degradation.

The first experimental evidence for a prostaglandin receptor was reported in 1972 by Kuehl and Humes (64). These investigators demonstrated a receptor for PGE_1 on the cell membranes of rat adipocytes. Using a binding assay technique, specific binding of tritiated PGE_1 was revealed in a particulate fraction of isolated rat adipocytes.

They also observed that PGE_2 would bind to the receptor with approximately the same affinity as PGE_1, while the binding capacity of A- and F-type prostaglandins to this receptor was insubstantial. Reports of a high-affinity PGE receptor have subsequently appeared for stomach, thyroid gland, uterus, liver, corpus luteum, kidney (99), skin (71), and the vasculature (56). These receptors which mediate the various physiologic responses initiated by E-type prostaglandins are located on the cellular plasma membranes (99). High affinity receptors for $PGF_{2\alpha}$ have also been demonstrated in cell membranes of corpus luteum, uterus and oviduct (99). Although it appears likely that receptors for the various eicosanoids exist in virtually all tissues, these receptor relationships for the different cells must be learned. Also, it is not yet understood the extent to which prostaglandins affect cells other than the ones catalyzing their biosynthesis (68).

The importance of prostaglandins in the inflammatory response has been implicated by three lines of research: 1) prostaglandins of the E series have been shown to have potent pro-inflammatory effects; 2) they are released in several different types of inflammation; 3) non-steroidal anti-inflammatory drugs (aspirin and indomethacin) inhibit the biosynthesis of prostaglandins.

Increased concentrations of prostaglandins, especially of the E series, have been recovered from inflammatory exudates in man (40,92), dog (3,57), and rat (128,129). Greaves *et al.* (40), using a skin perfusion technique *in vivo,* studied the prostaglandin activity of inflamed skin in patients with allergic contact eczema. Perfusates from 35 patients contained a mixture of E and F prostaglandins. Concentrations of PGE_1 recovered from inflamed tissues averaged 1.0 ng PGE_1/ml of perfusate. Control levels averaged 0.2 ng PGE_1/ml of

perfusate. The approximate distribution of total activity of PGE_1 was: E_1 12%, E_2 12%, $F_{1\alpha}$ 24%, and $F_{2\alpha}$ 49%. This is suggestive that prostaglandins participate in the pathogenesis of inflammation in man. In a similar study using human subjects, Plummer *et al.* (92) showed that an exudate from inflamed skin displayed augmented prostaglandin activity compared with exudates from contralateral non-inflamed skin. PGE_2 and PGE_1 activity increased 70% and $PGF_{2\alpha}$ increased 73% as compared to control. Inflammation was produced by application of Trafuril cream to normal abdominal skin. Normal human plasma levels of prostaglandins as measured by radioimmunoassay are: PGE_1 2.6 pg/ml, PGE_2 4.5 pg/ml and $PGF_{2\alpha}$ 12 pg/ml (26).

Although prostaglandins have been extracted from various inflammatory exudates and inflammatory diseases involving acute or chronic inflammation of the joints, skin and eyes can be controlled though not cured by cyclo-oxygenase inhibitors, there is no direct evidence for prostaglandins as putative mediators of inflammation. The application of a prostaglandin directly to a tissue causing edema remains a controversial conclusion. Considerable debate exists among investigators concerning the relative capacity to which PGE_1 and/or PGE_2 are involved in the transcapillary movement of fluid and plasma proteins. Possible reasons for this uncertainty include experimental techniques, species variability, PGE dose and routes of administration, and magnification of conclusions deduced from the data.

Greenberg and Sparks (41) studied the effects of PGE_1 on the vascular resistance, capacitance and capillary filtration coefficient in the isolated canine hind limb by measuring total outflow and pressure of the popliteal vein and changes in plethysmographic recordings in an enclosed hind limb. They found that the intra-arterial administration

of 0.001 to 1.0 μg/min of PGE_1 caused a decrease in resistance, an increase in the capillary filtration coefficient and an increase in the vascular capacity of the isolated hind limb. Constant inflow studies and experiments on isolated vascular smooth muscle strips indicated that PGE_1 caused relaxation, whereas $PGF_{2\alpha}$ caused contraction. They suggested that the increased capillary filtration coefficient during PGE_1 infusion is due to a decrease in pre-capillary sphincter tone, since 1) there was no net filtration associated with the increased capillary filtration coefficient and 2) the relative changes in resistance and the capillary filtration coefficient were similar in magnitude to those observed during exercise when there is no increase in capillary permeability. More recent work in the hind limb of the dog by Joyner (58) presents evidence that the subcutaneous injection of PGE_1 (0.01 to 100 μg/15 min) causes net fluid filtration but does not increase permeability, as ascertained from the protein concentration of collected lymph. However, Joyner proposed that PGE_1 enhanced transcapillary fluid movement, as demonstrated by an enhanced lymph flow, primarily by its vasodilatory properties. The vasodilation resulted in a decrease in the pre- to post-capillary resistance ratio resulting in net fluid filtration. In contrast, Daugherty (25) studied the effects of PGE_1 on skin and skeletal muscle vascular beds in the canine forelimb and observed little effect on filtration. In these studies, PGE_1 (2.0 to 10 μg/min) infused into the brachial artery produced large increases in both skin and muscle blood flows and decreases in skin and muscle vascular resistances. The small effect on filtration, as measured by forelimb weight, was postulated to be due to a proportional dilation of the pre- and post-capillary vascular segments. The prostaglandin infusion period was only three to five

minutes, which could have accounted for no observable changes in capillary filtration and/or permeability.

In general, it has been demonstrated that the systemic (i.v.) administration of PGE_1 decreases total peripheral resistance and systemic arterial pressure in rats (50,123), guinea pigs (83), dogs (75,84), and man (18). The cardiovascular effects of PGE_1 are markedly affected by the route of its administration. Bergström *et al.* (8) showed that the depressor effect of intravenously injected PGE_1 is markedly smaller than that of intra-arterially injected PGE_1 in anesthetized dogs. These studies indicate that PGE_1 is effectively inactivated by a single circulation through the lungs.

Other investigators employing the vital dye technique in the rat and guinea pig have presented conflicting data concerning the effect of PGE_1 on capillary fluid and protein movement. The vital dye technique involves the injection of Evans Blue into the systemic circulation of an animal, and then an estimation of the amount of dye leaking at the administration site of the prostaglandin. The injection site for the prostaglandin is usually skin. The dye is assumed to bind to circulating plasma proteins, and will therefore be transported with the escaping protein. Microvascular permeability changes are quantitated by either measuring the diameter of the dye-leakage in the area of application, or more precisely by recovering the extravasated dye by extraction from the injected tissue, and measuring the concentration of the dye spectrophotometrically. Another approach using the vital dye technique involves the intravenous injection of radioactively labeled albumin and the subsequent measurement of the radioactivity of extravasated protein at the site of prostaglandin injection.

Utilizing the vital dye technique in the rat and guinea pig, several investigators have shown that PGE_1 responds variably and unpredictably with respect to vascular permeability changes (24,35,126). PGE_1 had been observed to increase vascular permeability to various degrees (24,35), while other studies showed no apparent change in microvascular permeability (126,51). It is difficult to attempt to explain the reasons for these differences; however, the vital dye method has three apparent disadvantages which may have contributed: 1) The concentrations of PGE_1 at the testing site cannot be well controlled. Depending upon the rate of penetration and diffusion of PGE_1 in the tissue, concentration differences could have occurred. 2) The extent to which the dye binds to the plasma protein is uncertain and variable. 3) Finally, the effect of PGE_1 would be instantaneous and not a dynamic change in vascular permeability. This last effect is caused by the rapid metabolization of the autocoid by degradative enzymes located in the tissue.

Although criticisms exist for the vital dye procedure, the preponderance of the data utilizing this technique show increases in vascular permeability in response to PGE_1 in the rat and guinea pig (20,24,35,89,118). Kaley (61) noted increases in permeability, especially in venules, while Pangides (89) observed a direct relationship between the dose of PGE_1 used and the average diameter of the lesion. Another group of investigators has proposed that prostaglandins (E_1 and E_2) contribute to the edema of inflammation by potentiating or increasing the vascular leakage induced by other mediators such as histamine and bradykinin (33,70,126). This potentiation can be inhibited by indomethacin pre-treatment (33).

The hamster cheek pouch preparation has been employed to study permeability changes in the microcirculation. In this procedure, FITC fluorescein-labeled dextran (M.W. = 145,000) is administered intravenously. Then a portion of the hamster cheek pouch is dissected and viewed microscopically. Leakage sites are counted after topical application of various agents by areas of fluorescence. This technique is of more value than the vital dye technique, in that it allows for the dynamic study of macrmolecular leakage *in vivo*.

Using the more reliable hamster cheek pouch preparation, Joyner *et al.* (59) and Svensjo (113) have shown that PGE_1, in doses ranging from 1 to 100 ng, increases the leakage of FITC-dextran from postcapillary venules. These investigators do not believe that the augmented macromolecular leakage occurs as a result of vesicular transport. They suggest that interendothelial gaps form as adjacent endothelial cells "round up" in response to the prostaglandin or other permeability inducing agent in a manner similar to that of histamine or bradykinin.

STATEMENT OF THE PROBLEM

A wide variety of experimental evidence demonstrates that the prostaglandins, especially PGE_1, are intimately involved in the inflammatory process (53,118). PGE_1 causes an increase in microvascular permeability to macromolecules owing to a direct action on the microvascular membrane which results in the formation of large venular gaps similar to that produced by bradykinin and histamine (59,113,114). Some evidence suggests that the direct macromolecular permeability increasing action of PGE_1 is considerably less potent than that produced by histamine or bradykinin (113,118). This lesser direct effect is apparently offset by the unique ability of PGE_1 to potentiate the actions of histamine and bradykinin on the microvascular membrane (33,70,113,127). These actions of PGE_1 on the microvascular membrane, coupled with the elevated concentration of prostaglandins in inflammatory exudates (3,40,92,128,129), suggests an important role for these autocoids in inflammation. This hypothesis is further supported by the observation that pre-treatment with indomethacin or aspirin reduces the intensity of inflammatory responses (5,20,92).

The evidence discussed above implicating a role for the prostaglandins in inflammation is primarily from studies in rats, hamsters and guinea pigs. The few studies performed in the dog (25,41,58) provide no conclusive evidence that PGE_1 causes an increase in microvascular permeability to macromolecules, suggesting the possibility of

species variability. If true species variability exists, the relevance of conclusions based on studies in rats, hamsters, and guinea pigs to inflammatory processes in humans is questionable. In the dog studies, the doses of PGE_1 employed were small and the duration of the infusions were usually short. These factors could have contributed to the lack of definitive results. Because of the overwhelming evidence supporting a role for prostaglandins in the rodent studies, and the grounds for valid criticism of the canine studies, a systematic study of the effects of PGE_1 on fluid filtration and macromolecular efflux is clearly warranted. These observations prompted the present study to characterize the effects of one hour infusions with a dose range of PGE_1 from 2.0 to 32.0 μg/min, i.a., on fluid filtration and macromolecular efflux in the canine forelimb. Also, several experiments will test the effect of systemic indomethacin prior to the onset of a local PGE_1 infusion on fluid filtration and macromolecular efflux.

It is the interest of this study to correlate these pharmacological data with the morphological data already available from the Swedish investigators.

METHODS

These experiments were conducted on 51 mongrel dogs of either sex weighing approximately 30 kilograms (range: 20-40 kg). Anesthesia was induced with 30 mg/kg of sodium pentobarbital and was supplemented periodically throughout the duration of the experiment as required. A Harvard respiratory pump was used to ventilate each animal with room air at a frequency of 10 cycles per minute and a tidal volume that was adjusted according to the animal's weight.

The virtually intact canine forelimb perfused at either constant brachial artery or natural inflow was employed as the test organ to collect subcutaneous lymph and measure lymph total protein concentration. The surgical process necessary to prepare the forelimb consisted of electrocautery to make small incisions superficial to the axillary portion of the brachial artery, the cephalic vein (5 cm distal to the elbow), the second superficial dorsal metacarpal vein of the right forelimb and, in the constant brachial artery inflow preparations, an incision over the right femoral triangle. Additionally, an incision was made in the neck lateral to the trachea, for isolation and cannulation of the left common carotid artery and jugular vein.

Ensuing the neck vessel cannulations, a side branch of the brachial artery, a skin lymph vessel in the region of the cephalic vein and the dorsal paw vein were isolated. Following the intravenous administration of 10,000 U.S.P. units of heparin via the jugular vein

catheter, the small blood vessels were cannulated in an upstream direction with polyethylene tubing, thereby allowing for the measurement of brachial artery perfusion pressure and skin small vein pressure. The brachial artery side branch was cannulated with PE-50 tubing inserted to the point of bifurcation of this vessel from the major artery. PE-60 tubing was used to cannulate the skin small vein. The cannulated small vessel acts as an extension of the catheter and, therefore, the pressure measured is that which is present in the vessel or vessels to which the cannulated vessel unites. This pressure is a true lateral pressure only if the cannulated vessel is patent, as verified by the ability to freely withdraw blood from and to flush physiologic saline into the vessel. The presence of the catheter does not measurably alter the pressure in the arterial or venous system because, in the canine forelimb, the cannulated vessel is a negligible fraction of the total cross-sectional area of the arterial and venous vascular bed, and there are abundant artery to artery and vein to vein anastomoses (25,42,45). In the natural inflow experiments the cannulated side branch of the brachial artery was used for the local infusion of PGE_1.

The lymphatic vessel was cannulated in an upstream direction with polyethylene tubing for lymph collection. These lymph vessels in the cephalic vein area below the elbow drain forelimb skin and paw (76). Two or three were usually tied centrally and one of them was cannulated distally with a beveled 10 cm length of PE-10 tubing. The walls of these vessels were quite substantial, necessitating a needle puncture (22 gauge) prior to cannulation.

In the experiments employing a constantly perfused brachial artery forelimb, the brachial artery was isolated, tied and transected

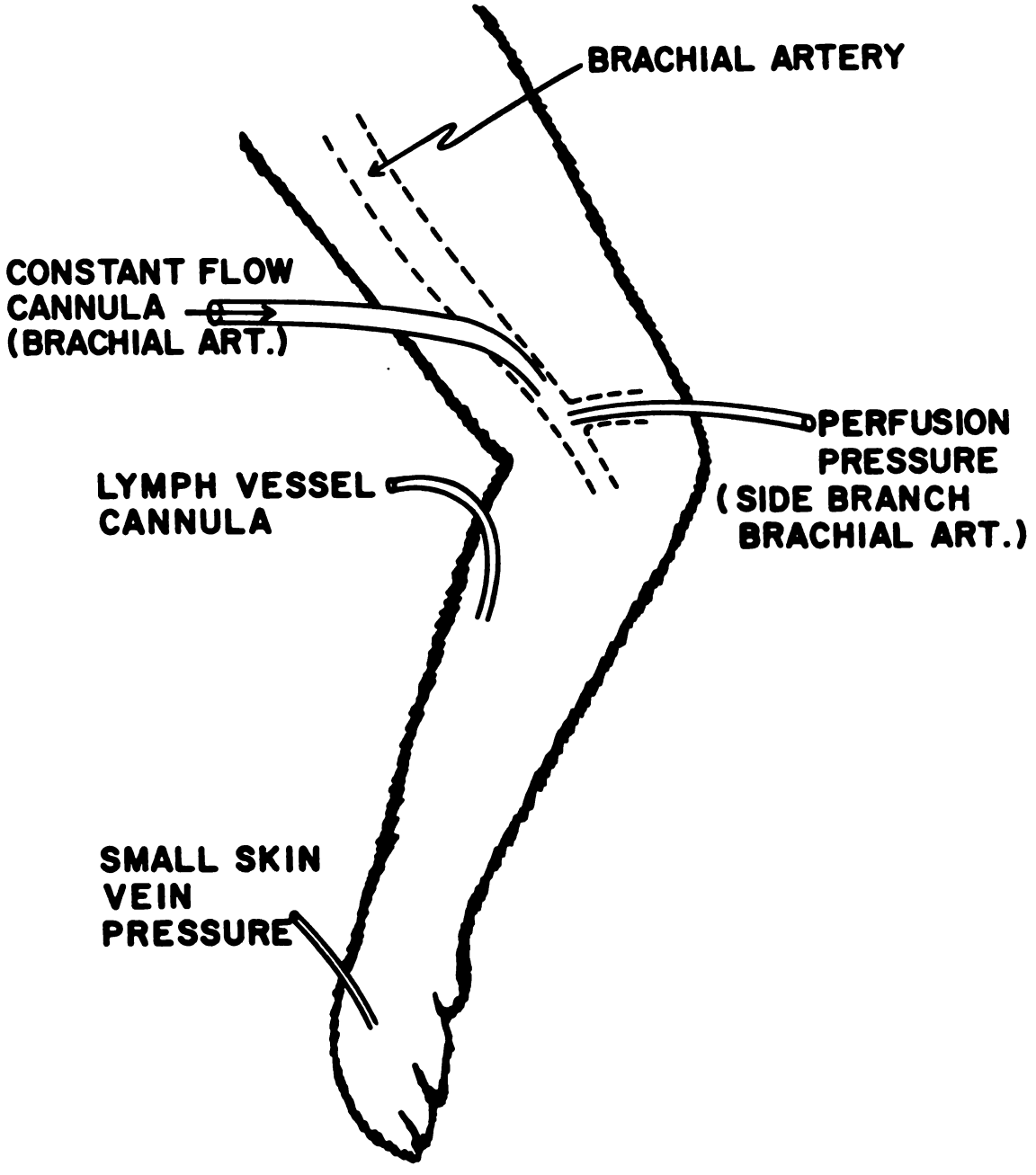
about 5 cm proximal to the previously cannulated side branch. Blood that was obtained from a cannulated femoral artery was pumped at a controlled flow into the transected brachial artery. A Sigmamotor pump (Model T68H, Sigmamotor Inc., Middleport, NY) was used to keep inflow constant at a value which produced a perfusion pressure similar to systemic pressure (see Figure V for summary).

Lymph was collected in 0.5 ml graduated cylinders constructed from plastic pipettes. Forelimb skin small vein pressure, brachial artery perfusion pressure (constant flow only), systemic blood pressure, and skin lymph flow rate were continuously monitored and recorded at 10 minute intervals. All pressures were measured with Statham pressure transducers (Model P23Gb, Statham Instruments, Inc., Oxnard, CA), connected to a direct writing oscillograph (Model 7754A, Hewlett-Packard Co., Palo Alto, CA).

After one 10 minute control period, followed by two consecutive 10 minute vehicle-control periods, the local intra-arterial administration of PGE_1 was begun. In the constant inflow preparations, PGE_1 introduction was accomplished by infusing directly into the circuit behind the Sigmamotor pump to insure adequate mixing of the drug. In the natural inflow studies, PGE_1 was infused directly into a side branch of the brachial artery. All concentrations of PGE_1 were administered at a delivery rate of 0.2 ml/min with a Harvard Apparatus infusion/withdrawal pump.

Arterial blood samples (5 ml aliquots) for ascertaining hematocrit and plasma protein concentrations were drawn from the cannula measuring systemic blood pressure. The blood samples were taken 10 minutes prior to the infusion of PGE_1, and at 30 minute intervals throughout the duration of the experiment. The protein concentrations

FIGURE V



Schematic diagram of the canine forelimb technique.

of the lymph and plasma samples were analyzed by the modified Biuret reaction (7). The samples were read spectrophotometrically in grams percent with an ACCU-STAT Blood Chemistry Analyzer (Clay Adams, Model 2000) which had been calibrated with samples of known protein concentration.

Modifications to this standard protocol were instituted in 15 of the 57 total experiments. In 12 studies 5 mg/kg of indomethacin was given as pretreatment. After 60 minutes this was followed by 16 μg/min of PGE_1 infused into the brachial artery of the forelimb for 60 minutes. The values for lymph flow rate and lymph protein transport were compared to those values obtained with 16.0 μg/min of PGE_1 alone using a group t-test. The indomethacin was given over a five minute interval into the jugular vein. In another series, the angiotensin I to angiotensin II converting enzyme blocker, SQ 14225 (captopril), was administered intravenously 20 minutes prior to, and throughout, the 60 minute duration of the local infusion of PGE_1. These experiments were performed on constant inflow preparations using three animals.

At the conclusion of all experiments the animals were sacrificed. The experimental (right) and control (left) forelimbs were then severed approximately two centimeters proximal to the humeral condyle for measurement of forelimb weights. The brachialis, biceps and triceps muscles were carefully dissected down to their tendons of insertions on the radial and ulnar tuberosities. Diligent care was assumed to insure that the limbs were always severed at the same points on the humeral condyle. The forelimbs were then exsanguinated and weighed. Limb weights were compared (experimental vs. control) using a paired t-test. Previous work by our group, in the same preparation,

produced no significant changes in limb weights. Therefore, neither the ethanol-saline vehicle nor the trauma produced by the surgery were responsible for the significant increases in limb weights seen in dogs with marked increases in lymph total protein concentration. Moreover, our group has shown that when lymph total protein concentration did not significantly increase, the experimental and contralateral control limb weights were similar. Consequently, this method appears to be valid for ascertaining changes in limb weights.

All other data were statistically analyzed by the Analysis of Variance (Randomized Complete Block Design) and the means were compared to control by the Least Significant Difference Test (10).

PGE_1 was procured from The Upjohn Company, Kalamazoo, Michigan, compliments of Dr. John E. Pike (U-10136, Lot No. 12874-JHK-102C and 11894-VOV-77). Stock solutions were prepared by dissolving 10 mg of PGE_1 into 10 ml of absolute ethanol. Appropriate dilutions were made using normal saline as the solvent. Infusion rates of PGE_1 used in these experiments were: 2 µg/min, 4 µg/min, 16 µg/min, and 32 µg/min.

Indomethacin solutions (Sigma Chemical Co., I 7378; (1- p-chlorobenzoyl -5-methoxy indole-3-acetic acid) were prepared by dissolving an appropriate amount of indomethacin, based on the animal's weight, into 100 ml of 1N saline containing 90 mg of $NaHCO_3$. Dissolution of the indomethacin took approximately two hours of continual stirring. The $NaHCO_3$ raised the pH of the 1N saline to 8.2-8.4, which aided in the dissolution of the indomethacin and augmented its half-life to about three hours at room temperature.

Captopril was obtained from the Squibb Laboratories, New Brunswick, New Jersey. It was prepared for infusion by dissolving it

it in normal saline to an appropriate concentration for an infusion at a rate of 14 µg/kg/min.

RESULTS

The data in this section are presented in Tables III through VII. Measurements of vascular pressures and collection of lymph samples were made over 10 minute periods. Arterial blood samples were taken every 30 minutes. In Tables III through VI, three control readings followed by six experimental readings were acquired. The control readings were of two types. The -20 minute control reading was measured following a 10 minute period where no drug or vehicle was infused. The -10 and 0 minute readings were taken during the brachial artery infusion of a vehicle solution (ethanol-saline). At 0 minutes the vehicle infusion was terminated and replaced with the intra-brachial artery infusion of PGE_1 for 60 minutes.

In Table VII, the format is essentially the same as in Tables III through VI, except that at -60 minutes a "solution and/or drug free" control reading was taken followed by the intravenous injection of indomethacin. The -10 and 0 minute readings were acquired during the vehicle infusion period.

Table III

In the natural and constant brachial artery inflow forelimb preparations, PGE_1 (2.0 µg/min, i.a.) moderately increased systemic arterial blood pressure. Perfusion pressure markedly decreased in the constant inflow experiments, while skin small vein pressure minimally

increased. Skin small vein pressure rose substantially in the naturally perfused forelimbs.

Lymph total protein concentration and lymph protein transport increased in both forelimb preparations, yet lymph flow rate changed minimally. Plasma protein concentrations remained unaltered relative to control values, while the hematocrit ratios increased.

Table IV

Four micrograms per minute of PGE_1 infused into the brachial artery of constantly perfused forelimbs for 60 minutes augmented systemic arterial blood pressure to the same extent as 2.0 µg/min of PGE_1 (Table III). Perfusion pressure markedly decreased, while skin small vein pressure remained unchanged. Lymph total protein concentration and lymph protein transport increased with only a very slight enhancement of lymph flow rate. These lymph parameters are almost identical to those obtained at an infusion rate of 2.0 µg/min of PGE_1 (Table III). Plasma protein concentration remained unaltered, while the hematocrit ratio increased relative to control values.

Table V

At 16.0 µg/min, PGE_1 did not significantly alter systemic arterial blood pressure in either the natural or constant brachial artery inflow forelimb preparations. In the constant flow studies, perfusion pressure decreased markedly as skin small vein pressure remained unchanged. Skin small vein pressure increased in the naturally perfused forelimbs.

In both preparations, lymph total protein concentration and lymph protein transport increased relative to control levels, yet lymph flow rate increased only slightly. The total lymph protein

concentration in the constant inflow experiments increased by almost 2.0 gms %, which is greater than the values obtained with 2.0 or 4.0 µg/min of PGE_1 (≃1.2 gms %). Hematocrit ratios increased in both preparations, while the plasma protein concentration remained unchanged in the constantly perfused forelimbs. Interestingly, the plasma protein concentration slightly increased relative to control in the naturally perfused forelimbs.

Forelimb weight measurements were obtained only from naturally perfused forelimbs. These forelimbs significantly increased in weight by 55 grams.

Table VI

The local, brachial artery infusion of PGE_1 (32.0 µg/min) moderately decreased systemic arterial blood pressure in naturally perfused forelimbs and slightly lowered this pressure in the constant inflow forelimbs. Skin small vein pressure did not change in the constant inflow experiments, while it increased in the natural flow studies. Perfusion pressure was greatly reduced in the constant brachial artery inflow preparations.

The total protein concentrations of the lymph rose with both experimental procedures; however, the increment was somewhat lower than the levels achieved at an infusion rate of 16.0 µg/min of PGE_1 (Table V). In the naturally perfused forelimbs, lymph flow rate increased relative to control, which is different from the lymph flow rates obtained with the other three infusion rates (Tables III, IV and V). At constant inflow, however, lymph flow rate increased minimally, which is similar to the results obtained with the other infusion rates. Lymph protein transport increased in both forelimb procedures.

Plasma protein concentration increased in both preparations, whereas at other infusion rates plasma protein concentration levels remained relatively unchanged (Tables III, IV and V). Hematocrit ratios also increased in both forelimb procedures.

In the naturally perfused forelimbs, weights were significantly augmented by an increment of 50 grams and in the constant inflow forelimbs, weights increased significantly by 26 grams.

Table VII

The systemic administration of 5 mg/kg of indomethacin failed to alter significantly the control values of the measured parameters in both naturally and constantly perfused forelimbs. Following this pre-treatment with indomethacin, the local, intra-arterial infusion of 16.0 µg/min of PGE_1 for 60 minutes did alter the vascular lymph and plasma parameters quantified.

In the constant brachial artery inflow studies, systemic arterial blood pressure increased significantly (≈10 mm Hg), while little change was observed at natural inflow. These results are somewhat similar to those obtained with the infusion of 16.0 µg/min of PGE_1 alone (Table V). Perfusion pressure markedly decreased, being almost identical to the results shown in Table V. Skin small vein pressure increased at natural inflow but did not change at constant inflow.

Lymph total protein concentration increased in both forelimb preparations. Interestingly, lymph flow rates were statistically different from those obtained when PGE_1 was infused into the brachial artery without prior systemic pre-treatment with indomethacin (Table V). The flow rates of the lymph in both preparations increased markedly, with a larger increase observed at natural inflow. As a result, lymph protein

transports increased significantly from those values obtained at 16 μg/min of PGE_1 alone.

Hematocrit ratios increased significantly and plasma protein concentrations failed to change in both natural and constant inflow forelimbs. Forelimb weights also significantly increased by 64 grams in the natural flow forelimbs and by 14 grams in the constant flow forelimbs.

Captopril Studies

In three constantly perfused brachial artery forelimbs, 15 μg/kg of captopril (SQ 14225) was administered intravenously 20 minutes prior to and throughout the 60 minute duration of the intra-brachial artery infusion of 16.0 μg/min of PGE_1. No significant change from control was observed in any of the vascular lymph or plasma parameters measured.

Table III. Effects of locally infused PGE_1 (2.0 µg/min, I.A.) into the forelimb for 60 minutes on lymph flow, lymph protein transport, protein concentration of lymph and plasma, vascular pressures and hematocrit (n=6) (NF = natural flow, CF = constant flow)

		Control	Vehicle Control		PGE_1 Infusion Period					
Time (minutes)		-20	-10	0	10	20	30	40	50	60
Systemic Arterial Blood Pressure (mm Hg)	NF	118	119	120	127	129	132†	131†	130†	129
	CF	131	134	137	150†	156*	158*	159*	157*	158*
Perfusion Pressure (mm Hg)	CF	121	124	125	60*	62*	64*	65*	65*	65*
Skin Small Vein Pressure (mm Hg)	NF	12	11	11	17*	18*	17*	17*	18*	17*
	CF	11	11	11	12	13*	13*	14*	14*	13*
Lymph Flow Rate (ml/10 min)	NF	.02	.01	.01	.02	.04	.05	.04	.07†	.07†
	CF	.01	.01	.01	.02†	.03*	.03*	.03*	.02†	.02†
Lymph Total Protein (grams %)	NF	2.4	2.5	2.5	2.7	2.8	3.1*	3.2*	3.4*	3.2*
	CF	2.0	2.1	2.2	2.6†	2.7	2.8*	2.8*	2.7†	2.8*
Lymph Protein Transport (mg/10 min)	NF	.42	.33	.34	.65	1.15	1.40	1.24	2.45†	2.32†
	CF	.20	.24	.27	.48	.74*	.82*	.69†	.59†	.48
Plasma Protein (grams %)	NF			5.7			5.6			5.6
	CF			5.8			6.0			6.0
Hematocrit	NF			38			41*			42*
	CF			38			43*			44*

*$p \leq 0.01$ relative to zero time.

†$p \leq 0.05$ relative to zero time.

Table IV. Effects of locally infused PGE_1 (4.0 µg/min, I.A.) into the forelimb for 60 minutes on lymph flow, lymph protein transport, protein concentration of lymph and plasma, vascular pressures and hematocrit (n=6) (CF = constant flow)

Time (minutes)		Control -20	Vehicle Control -10	Vehicle Control 0	PGE_1 Infusion Period 10	20	30	40	50	60
Systemic Arterial Blood Pressure (mm Hg)	CF	124	124	127	138†	141†	138†	141†	141†	142†
Perfusion Pressure (mm Hg)	CF	120	118	118	56*	58*	60*	62*	64*	69*
Skin Small Vein Pressure (mm Hg)	CF	11	11	11	11	12	12	12	12	12
Lymph Flow Rate (ml/10 min)	CF	.02	.02	.01	.03	.05*	.04*	.03	.03	.02
Lymph Total Protein (grams %)	CF	2.4	2.4	2.4	2.7	3.0*	3.4*	3.5*	3.4*	3.5*
Lymph Protein Transport (mg/10 min)	CF	.33	.32	.30	.87	1.51†	1.56*	1.19†	1.04	.70
Plasma Protein (grams %)	CF			6.0			6.1			6.0
Hematocrit	CF			37			41*			42*

*$p \leq 0.01$ relative to zero time.

†$p \leq 0.05$ relative to zero time.

Table V. Effects of locally infused PGE_1 (16.0 µg/min, I.A.) into the forelimb for 60 minutes on lymph flow, lymph protein transport, protein concentration of lymph and plasma, vascular pressures, forelimb weight and hematocrit (n=6) (NF = natural flow, CF = constant flow)

		Control	Vehicle Control		PGE_1 Infusion Period					
Time (minutes)		-20	-10	0	10	20	30	40	50	60
Systemic Arterial Blood Pressure (mm Hg)	NF		121	122	130	127	128	128	129	130
	CF	117	116	115	116	114	118	121	121	122
Perfusion Pressure (mm Hg)	CF	106	104	107	52*	55*	56*	57*	59*	64*
Skin Small Vein Pressure (mm Hg)	NF		10	9	19*	18*	18*	18*	18*	17*
	CF	13	13	13	13	13	14	14	14	14
Lymph Flow Rate (ml/10 min)	NF		.02	.02	.04	.08	.06	.06	.08	.08
	CF	.01	.01	.01	.05*	.05*	.06*	.05*	.04†	.05*
Lymph Total Protein (grams %)	NF		1.9	2.0	2.1	2.3	2.8*	2.8*	2.7*	2.7*
	CF	2.0	1.9	1.9	2.3	3.1*	3.7*	3.7*	3.5*	3.5*
Lymph Protein Transport (mg/10 min)	NF		.32	.36	.78	1.71*	1.40†	1.22†	1.29†	1.55*
	CF	.25	.22	.23	.92	1.80*	2.30*	2.16*	1.53*	1.74*
Plasma Protein (grams %)	NF			5.3			5.6			5.7*
	CF			5.6			5.8			5.9
Hematocrit	NF			40			45*			45*
	CF			37			43*			44*

Table V (continued)

		Control	Experimental	Difference
Forelimb Weight (grams; n=5)	NF	703	759	55†

*$p \leq 0.01$ relative to zero time.

†$p \leq 0.05$ relative to zero time.

Table VI. Effects of locally infused PGE_1 (32.0 µg/min, I.A.) into the forelimb for 60 minutes on lymph flow, lymph protein transport, protein concentration of lymph and plasma, vascular pressures, forelimb weight and hematocrit (n=6) (NF = natural flow, CF = constant flow)

Time (minutes)		Control	Vehicle Control		PGE_1 Infusion Period					
		-20	-10	0	10	20	30	40	50	60
Systemic Arterial Blood Pressure (mm Hg)	NF		121	120	106*	104*	107*	107*	107*	108*
	CF	116	116	113	105	108	108	109	111	112
Perfusion Pressure (mm Hg)	CF	105	104	103	47*	47*	49*	51*	51*	52*
Skin Small Vein Pressure (mm Hg)	NF		12	12	20*	20*	19*	19*	20*	19*
	CF	12	13	12	12	12	12	13	13	13
Lymph Flow Rate (ml/10 min)	NF		.02	.02	.08†	.13*	.16*	.15*	.15*	.16*
	CF	.02	.02	.02	.02	.03	.04†	.03	.03	.02
Lymph Total Protein (grams %)	NF		2.2	2.2	2.6	2.8†	3.1*	3.1*	3.3*	3.1*
	CF	2.4	2.4	2.3	2.6†	2.6†	2.7*	2.6†	2.7*	2.7*
Lymph Protein Transport (mg/10 min)	NF		.38	.36	2.08	3.88*	5.22*	5.40*	5.10*	5.10*
	CF	.35	.39	.37	.44	.73†	.87*	.72†	.71†	.57
Plasma Protein (grams %)	NF			5.9			6.3*			6.4*
	CF			5.7			6.2*			6.2*
Hematocrit	NF			37			42†			43*
	CF			38			44*			44*

Table VI (continued)

		Control	Experimental	Difference
Forelimb Weight (grams; n=6)	NF	704	754	50*
	CF	746	771	26†

*$p \leq 0.01$ relative to zero time.

†$p \leq 0.05$ relative to zero time.

Table VII. Effects of indomethacin (5 mg/kg) injected intravenously followed by PGE_1 (16.0 µg/min, I.A.) infused locally into the forelimb for 60 minutes on lymph flow, lymph protein transport, protein concentration of lymph and plasma, vascular pressures, forelimb weight and hematocrit (n=6) (NF = natural flow, CF = constant flow)

		Indo. Injection	Vehicle Control		PGE_1 Infusion Period					
Time (minutes)		-60	-10	0	10	20	30	40	50	60
Systemic Arterial Blood Pressure (mm Hg)	NF	118	120	119	121	118	117	114	115	113
	CF	132	133	133	142†	145*	145*	144†	143†	142
Perfusion Pressure (mm Hg)	CF	123	131	132	58*	59*	59*	60*	60*	61*
Skin Small Vein Pressure (mm Hg)	NF	13	12	12	26*	26*	25*	25*	25*	24*
	CF	11	10	10	11	11	11	11	11	11
Lymph Flow Rate (ml/10 min)	NF	.03	.04	.04	.20*Ω	.30*Ω	.32*Ω	.29*Ω	.29*ω	.27*ω
	CF	.01	.02	.02	.08*	.12*ω	.10*	.09*	.07ω	.07ω
Lymph Total Protein (grams %)	NF	2.0	2.1	2.2	2.7†	3.3*	3.1*	2.9*	3.0*	3.0*
	CF	2.6	2.9	2.9	3.2	3.5*	4.0*	4.0*	4.0*	4.0*
Lymph Protein Transport (mg/10 min)	NF	.54	.68	.79	4.66*Ω	9.48*Ω	9.77*Ω	7.86*Ω	8.35*Ω	7.69*Ω
	CF	.40	.42	.49	2.42Ω	4.24*ω	4.48*Ω	3.78*ω	2.96†ω	2.81†
Plasma Protein (grams %)	NF	6.0		5.9			6.0			6.0
	CF	5.8		5.8			6.0			6.0

Table VII (continued)

Time (minutes)		Indo. Injection -60	Vehicle Control -10	Vehicle Control 0	PGE_1 Infusion Period 10	20	30	40	50	60
Hematocrit	NF	33		34			38*			38*
	CF	37		37			43*			43*
		Control			Experimental				Difference	
Forelimb Weight (grams; n=6)	NF	733			798				64*	
	CF	695			709				14†	

*$p \leq 0.01$ relative to zero time.

†$p \leq 0.05$ relative to zero time.

Ω$p \leq 0.01$ relative to the values obtained with 16.0 µg PGE_1/min alone (Table V).

ω$p \leq 0.05$ relative to the values obtained with 16.0 µg PGE_1/min alone (Table V).

DISCUSSION

The role of "E" series prostaglandins in the inflammatory process has evoked substantial controversy for several years (35,58, 70,118,120,117,126). Although these prostanoids have been recovered from various inflammatory exudates, and inflammation can be attenuated by cyclo-oxygenase inhibitors (i.e., PG synthetase inhibitors), variable results have been obtained concerning the ability of PGE_1 or PGE_2 to mimic the edema of inflammation when given exogenously into a tissue. The data in the present study demonstrate increases in forelimb weight in the dog following local, intra-brachial artery infusions of PGE_1 for 60 minutes. This provides evidence for edema formation subsequent to an increase in net fluid filtration. The rise in lymph total protein concentration results from an increase in microvascular permeability to macromolecules. The precise mechanism by which the augmented microvascular permeability occurs cannot be ascertained from these experiments. However, work carried out by Swedish laboratories (59,113,114) suggests that it probably results from a direct action of PGE_1 on the microvascular membrane causing the formation of large venular gaps or pores. The heightened skin small vein pressure observed in the naturally perfused forelimbs suggests an increase in capillary hydrostatic pressure, since skin small vein pressure represents a minimum for capillary hydrostatic pressure (45,74). These observations imply that the mechanism of edema formation is by a

decrease in the transmural oncotic pressure gradient and an increase in the transmural capillary hydrostatic pressure gradient. In the constant brachial artery inflow preparations, the edema was attributable primarily to a decrease in the transmural oncotic pressure gradient, since skin small vein pressure failed to change from control in these forelimbs.

PGE_1 produces only increases in microvascular permeability in doses exceeding those needed to produce vasodilation. Theoretically, this is physiologically important, since it permits a means for "E" series prostaglandins to function in the local regulation of blood flow without increasing microvascular permeability to macromolecules (125). It would be unprofitable if these agents simultaneously increased net fluid filtration.

The increase in microvascular permeability yielded by PGE_1 is considerably less than that produced by bradykinin and/or histamine (72,74). The average maximal increase in lymph total protein concentration produced by PGE_1 is about 1.5 grams percent (Table V). Large doses of histamine or bradykinin increase the total protein concentration of the lymph to values approaching that in plasma (≈5.5 gms %) (72,74).

The inability of others to demonstrate an effect of PGE_1 on macromolecular permeability in the dog is probably related to the doses studied (25,41,58). These investigators performed the majority of their experiments at infusion rates below 2.0 μg/min. In addition, the short duration of the infusion and the failure to directly measure the efflux of protein or macromolecular tracer substances may account for their conclusions. However, the data presented in this

dissertation using levels of PGE_1 above 2.0 µg/min are consistent with the data from many other laboratories using rats, mice and hamsters (24,35,113).

PGE_1 affects lymph flow rate differently from bradykinin and histamine (46,72,74). At infusion rates between 2.0 and 16.0 µg/min, PGE_1 produced very small dose independent increases in lymph flow rate. These lymph flow rates were similar in magnitude under both natural and constant brachial artery inflow conditions. In contrast, 2 µg histamine base/min produces an increase in the protein concentration of the lymph similar to that elicited by PGE_1, yet produces extremely large increases in lymph flow rate (46). The lymph flow rates induced by histamine were substantially greater in the naturally perfused forelimbs than those perfused at constant brachial artery inflow. Following pre-treatment with indomethacin, PGE_1 (16.0 µg/min, i.a.) yielded an increase in the total protein concentration of the lymph similar to that produced without indomethacin, but produced very dramatic increases in lymph flow rate (Table VII). These elevated lymph flow rates were statistically greater from the flow rates obtained at 16.0 µg/min of PGE_1 alone. Moreover, these flow rates were proportionally greater in forelimbs naturally perfused than in those perfused at constant brachial artery inflow.

It is interesting to speculate the reasons why indomethacin pretreatment enhanced the rate of lymph flow produced by PGE_1. Since indomethacin inhibits cyclo-oxygenase, it is tenable to conclude that in the absence of indomethacin one or more eicosanoids produced subsequent to the cyclo-oxygenase step (Figures III and IV) could be liberated in response to exogenous PGE_1 and actively constrict prenodal lymphatic vessels, thereby impeding drainage. It has long been

known that the topical application of various vasoactive agents can affect lymphatic contractility (108,116). The mechanism is believed to occur by contraction of smooth muscle cells, since the anatomical structure of lymphatic vessels includes, in addition to an external adventitia and an internal endothelium, a tunica media made of smooth muscle cells arranged in several layers (116). This PGE_1 induced lymphatic contraction might occur via the action of certain enzymes in the prostaglandin biosynthetic cascade beyond the cyclo-oxygenase step, which would increase the relative amounts of various eicosanoids in the body.

For example, stimulation of 9-keto-reductase by PGE_1 would result in enhanced levels of $PGF_{2\alpha}$. Also, this enzyme could convert the exogenous PGE_1 to $PGF_{1\alpha}$. $PGF_{2\alpha}$ and $PGF_{1\alpha}$ are vasoconstrictors (27,31) and are antagonists of PGE_1 induced permeability in the rat (24). It is possible that either of these prostaglandins could be actively constricting pre-nodal lymphatics. Thus, not only would indomethacin prevent endogenous $PGF_{2\alpha}$ formation by inhibiting cyclo-oxygenase, it would also prevent $PGF_{1\alpha}$, since it also inhibits 9-keto-reductase (80). PGE_1 could likewise stimulate thromboxane synthetase in the platelet causing excessive release of TXA_2. TXA_2, like $PGF_{2\alpha}$ or $PGF_{1\alpha}$, is a vasoconstrictor (79,81) and could possibly cause active constriction of lymphatic vessels.

The theory that exogenous prostaglandin introduced into a tissue to cause an imbalance of the prostaglandin synthetic cascade, thereby increasing the relative amounts of other eicosanoids, is not a novel idea. Chahl and Chahl (20) have shown that part of the action of PGE_1 in inflammation is produced indirectly by releasor stimulation of the synthesis of other prostaglandins and their precursors. Their

experiments show that in rats pre-treated with indomethacin the area of swelling on the skin induced by PGE_1 is significantly reduced from the amount of swelling produced by PGE_1 alone. Conceivably, in the indomethacin pre-treated rats, drainage was not impeded, resulting in an attenuated area of edema.

When cyclo-oxygenase is blocked, this terminates the competition for substrate (i.e., arachidonic acid, di-homo-γ-linolenic acid and eicosopentaenoic acid) by cyclo-oxygenase and the lipoxygenases (Figure IV), allowing lipoxygenases to act freely and yield larger amounts of hydroperoxides and hydroxy-acids. Indomethacin could therefore be indirectly stimulating the production of these latter substances, which might participate in the opening of lymphatic vessels.

Another possible explanation for these data would be that normal circulating levels of TXA_2, $PGF_{1\alpha}$ and $PGF_{2\alpha}$ antagonize the permeability effects of exogenous PGE_1 (24) and may also exert a normal tonic effect on the lymphatics. Administration of indomethacin would prevent further generation of $PGF_{2\alpha}$ and $PGF_{1\alpha}$ by the vascular endothelium (51) and TXA_2 by the platelet (79,81), thereby reducing their circulating levels. Consequently, the tonic effects on the lymphatics would be removed along with any microvascular antagonizing effects. Thus, after one hour, the local infusion of PGE_1 would effectively increase lymph flow rate concomitant with the increased total protein concentration of the lymph.

It should be noted that at 32.0 μg/min, PGE_1 produced very pronounced increases in lymph flow rate (Table VI), yet the increase in lymph total protein concentration was no greater than that produced at 16.0 μg/min (Table V). Perhaps at this dose the direct action of PGE_1 largely counteracts the effects of the presumed endogenously released

lymphatic constrictor substance, the existing prostaglandin microvascular antagonist, or a lymphatic, tonically active substance.

The local intra-arterial infusions of PGE_1 at 2.0 and 4.0 µg/min produced significant increases in mean systemic arterial blood pressure (Tables III and IV). The elevated mean arterial pressure was somewhat less at 16.0 µg/min of PGE_1 (Table V). Since the augmented blood pressure is not attributable to the ethanol-saline vehicle (39), it must be ascribed to an action of PGE_1. It is well known that PGE_1 exerts an effect on the central cardiovascular centers which causes an elevation in arterial blood pressure (51). This could explain the observed hypertensive effects of the lower PGE_1 doses. When the PGE_1 infusion rate was increased to 32.0 µg/min, mean systemic arterial blood pressure decreased. It is possible that at this high dose, the central pressor effect may be overwhelmed by the peripheral depressor effect. This is supported by the fact that Kadowitz *et al.* (60) have shown that PGE_1 decreases the release of norepinephrine from adrenergic neurons via stimulation of presynaptic receptors.

Captopril (SQ 14225) inhibits the converting enzyme which transforms Angiotensin I to Angiotensin II. This converting enzyme, which is richly abundant in the lung, is also believed to catalyze the conversion of bradykinin to inactive fragments. In a single passage of bradykinin through the pulmonary circulation, the lungs destroy up to 90% of the active peptide. It has been suggested that the permeability inducing effects by PGE_1 or PGE_2 in the microcirculatory bed is mediated in part via bradykinin (52). This hypothesis is currently unsettled and requires additional experimentation. In this study, three dogs were treated with 15 µg/kg/min of captopril 20 minutes prior to and throughout the 60 minute local, brachial artery infusion of PGE_1 (16

μg/min). If the microcirculatory effects of PGE_1 are mediated in part by bradykinin, then a more dramatic effect on permeability should be observed when bradykinin inactivation is prevented. However, no significant change from control was observed in any of the vascular lymph or plasma parameters quantified, indicating that the effects of PGE_1 are probably not mediated by bradykinin.

Examining the data generated from the 51 dogs used in these studies, there is more variability in the lymph parameters than that which is observed for either bradykinin or histamine (72,74). This unusual variability of response has also been noted by Freeman and West (35) in their studies of inflammation on rats. The reason for this variation is not immediately clear, although it could be related to the release of an endogenous substance by PGE_1 (PG and/or catecholamine). Additional experimentation is needed to resolve this point.

In summary, the local intra-arterial infusions of PGE_1 (2.0 to 16.0 μg/min, i.a.) for 60 minutes into the canine forelimb perfused either naturally or at constant inflow produced marked vasodilation and increases in lymph total protein concentration, yet only minute dose independent increases in lymph flow rate. The increase in lymph flow rate was similar in magnitude with either the natural or constant inflow preparation. PGE_1 (16.0 or 32.0 μg/min, i.a.) also produced increases in forelimb weight due to edema formation. The weight increases were substantially greater under natural inflow conditions. Following pre-treatment with indomethacin, PGE_1 produced vasodilation and increases in lymph total protein concentration similar to that in the absence of indomethacin and also produced significant increases in the flow rate of lymph. Therefore, these data suggest that PGE_1 may well function as an inflammatory mediator in the dog. However, its

role is probably different and its mechanism of action more complex than the putative mediators of inflammation (i.e., histamine and bradykinin).

APPENDICES

APPENDICES

This appendix lists, in the form of tables, all the individual observations for the experiments performed in this study. Also listed are the means, standard error of the mean and the statistical significance.

Table A-III. Effects of locally infused PGE_1 (2.0 μg/min, I.A.) into the forelimb for 60 minutes on lymph flow, lymph protein transport, protein concentration of lymph and plasma, vascular pressures and hematocrit (n=6) (natural flow)

	Control	Vehicle Control		PGE_1 Infusion Period					
Time (minutes)	-20	-10	0	10	20	30	40	50	60
Systemic Arterial Blood Pressure (mm Hg)	125	130	130	130	150	155	145	140	140
	130	130	130	130	120	120	120	120	110
	90	85	85	112	125	125	130	130	130
	125	125	130	132	130	135	135	135	137
	110	110	112	125	120	120	122	120	120
	130	135	135	130	127	135	135	137	137
means	118	119	120	127	129	132†	131†	130†	129
standard error	±6	±8	±8	±3	±5	±5	±4	±4	±5
Skin Small Vein Pressure (mm Hg)	12	9	9	20	23	20	21	20	18
	11	11	11	16	16	16	16	18	16
	15	12	12	19	20	20	19	19	19
	12	12	12	18	17	17	17	17	17
	10	10	10	14	15	15	13	15	15
	13	13	14	17	16	16	16	16	16
means	12	11	11	17*	18*	17*	17*	18*	17*
standard error	±1	±1	±1	±1	±1	±1	±1	±1	±1

Table A-III (continued)

Time (minutes)	Control	Vehicle Control		PGE_1 Infusion Period					
	-20	-10	0	10	20	30	40	50	60
Lymph Flow Rate (ml/10 min)	0.02	0.02	0.02	0.04	0.03	0.04	0.03	0.03	0.05
	0.01	0.01	0.01	0.01	0.01	0.02	0.01	0.25	0.25
	0.01	0.01	0.01	0.01	0.06	0.06	0.06	0.05	0.05
	0.02	0.02	0.02	0.04	0.03	0.05	0.03	0.02	0.02
	0.03	0.01	0.01	0.02	0.07	0.06	0.04	0.04	0.02
	0.02	0.01	0.01	0.02	0.04	0.04	0.05	0.03	0.01
means	0.02	0.01	0.01	0.02	0.04	0.05	0.04	0.07†	0.07†
standard error	±.00	±.00	±.00	±.01	±.01	±.01	±.01	±.04	±.04
Lymph Total Protein (grams %)	2.6	2.9	3.0	3.5	3.2	3.6	3.5	3.5	2.8
	2.4	2.3	2.4	2.3	2.3	2.4	2.5	3.5	3.6
	2.7	2.6	2.7	3.2	3.6	3.7	3.8	4.2	4.3
	2.1	2.0	2.1	2.4	2.6	2.7	2.7	2.5	2.4
	1.9	2.0	2.0	2.1	2.1	2.4	2.9	2.9	2.7
	2.6	2.9	2.8	2.7	3.2	3.7	3.8	3.7	3.8
means	2.4	2.5	2.5	2.7	2.8	3.1*	3.2*	3.4*	3.2*
standard error	±.1	±.2	±.2	±.2	±.2	±.3	±.2	±.3	±.3

Table A-III (continued)

Time (minutes)	Control -20	Vehicle Control -10	0	PGE$_1$ Infusion Period 10	20	30	40	50	60
Lymph Protein Transport (mg/10 min)	0.52	0.58	0.60	1.40	0.96	1.44	1.05	1.05	1.40
	0.24	0.23	0.24	0.23	0.23	0.48	0.25	8.75	9.00
	0.27	0.26	0.27	0.32	2.16	2.22	2.28	2.10	2.15
	0.42	0.40	0.42	0.96	0.78	1.35	0.81	0.50	0.48
	0.57	0.20	0.20	0.42	1.47	1.44	1.16	1.16	0.54
	0.52	0.29	0.28	0.54	1.28	1.48	1.90	1.11	0.36
means	0.42	0.33	0.34	0.65	1.15	1.40	1.24	2.45†	2.32†
standard error	±.06	±.06	±.06	±.18	±.27	±.23	±.30	±1.28	±1.36
Plasma Protein (grams %)			5.5			5.6			5.7
			5.9			4.8			5.0
			5.5			5.5			5.5
			5.4			5.6			5.5
			6.0			6.1			6.1
			5.9			6.0			5.7
means			5.7			5.6			5.6
standard error			±.1			±.2			±.2

Table A-III (continued)

Time (minutes)	Control -20	Vehicle Control -10	0	PGE_1 Infusion Period 10	20	30	40	50	60
Hematocrit			40			43			44
			41			44			44
			38			43			43
			32			38			38
			34			36			37
			40			42			43
		means	38			41*			42*
		standard error	±2			±1			±1

*$p \leq 0.01$ relative to zero time.

†$p \leq 0.05$ relative to zero time.

Table B-III. Effects of locally infused PGE_1 (2.0 µg/min, I.A.) into the forelimb for 60 minutes on lymph flow, lymph protein transport, protein concentration of lymph and plasma, vascular pressures and hematocrit (n=6) (constant flow)

		Control	Vehicle Control		PGE_1 Infusion Period					
Time (minutes)		-20	-10	0	10	20	30	40	50	60
Systemic Arterial Blood Pressure (mm Hg)		120	120	125	135	145	150	155	150	155
		140	155	160	155	155	155	150	150	150
		125	125	130	140	155	165	165	160	160
		125	127	128	150	160	160	162	160	162
		135	137	140	170	170	170	170	170	170
		135	140	145	147	150	150	150	150	150
	means	131	134	137	150*	156*	158*	159*	157*	158*
Perfusion Pressure (mm Hg)		115	120	125	45	45	50	50	50	55
		120	145	150	80	80	80	80	80	80
		115	115	120	60	65	70	70	70	65
		105	105	105	57	60	60	62	60	62
		132	130	132	62	67	67	65	65	65
		137	130	120	55	55	57	60	62	65
	means	121	124	125	60*	62*	64*	65*	65*	65*

Table B-III (continued)

Time (minutes)		Control -20	Vehicle Control -10	0	PGE_1 Infusion Period 10	20	30	40	50	60
Skin Small Vein Pressure (mm Hg)		10	10	10	10	11	13	13	13	13
		15	15	15	16	17	15	16	17	15
		11	11	11	12	12	13	13	12	12
		12	13	13	16	16	17	18	18	18
		9	9	9	10	10	11	11	11	11
		9	9	9	10	10	10	10	10	10
	means	11	11	11	12	13*	13*	14*	14*	13*
Lymph Flow Rate (ml/10 min)		0.01	0.01	0.02	0.02	0.05	0.02	0.02	0.03	0.04
		0.01	0.02	0.02	0.03	0.03	0.02	0.02	0.02	0.02
		0.01	0.01	0.01	0.03	0.03	0.03	0.03	0.02	0.02
		0.01	0.01	0.01	0.01	0.01	0.02	0.02	0.01	0.01
		0.01	0.01	0.01	0.02	0.02	0.02	0.03	0.03	0.01
		0.01	0.01	0.01	0.01	0.03	0.05	0.03	0.02	0.01
	means	0.01	0.01	0.01	0.02*	0.03*	0.03*	0.03*	0.02*	0.02*

Table B-III (continued)

Time (minutes)		Control -20	Vehicle Control -10	Vehicle Control 0	PGE_1 Infusion Period 10	20	30	40	50	60
Lymph Total Protein (grams %)		1.3	1.4	1.5	1.5	2.1	2.5	2.7	2.7	2.7
		1.8	1.8	1.8	2.4	2.4	2.3	2.2	2.0	1.9
		1.9	2.1	2.1	2.2	2.1	2.2	2.0	2.2	1.9
		1.7	1.8	1.9	2.4	2.9	2.3	2.3	2.3	2.6
		2.1	2.4	2.4	3.0	2.9	2.8	2.7	2.6	2.5
		3.4	3.4	3.4	3.8	3.8	4.6	4.8	4.4	5.0
	means	2.0	2.1	2.2	2.6†	2.7†	2.8*	2.8*	2.7†	2.8*
Lymph Protein Transport (mg/10 min)		0.13	0.14	0.30	0.30	1.05	0.50	0.54	0.81	1.08
		0.18	0.36	0.36	0.72	0.72	0.46	0.44	0.40	0.38
		0.19	0.21	0.21	0.66	0.63	0.66	0.60	0.44	0.38
		0.17	0.18	0.19	0.24	0.29	0.46	0.46	0.23	0.26
		0.21	0.24	0.24	0.60	0.58	0.56	0.81	0.78	0.25
		0.34	0.34	0.34	0.38	1.14	2.30	1.29	0.88	0.50
	means	0.20	0.24	0.27	0.48	0.74*	0.82*	0.69†	0.59†	0.48

Table B-III (continued)

Time (minutes)	Control -20	Vehicle Control -10	0	PGE$_1$ Infusion Period 10	20	30	40	50	60
Plasma Protein (grams %)			4.7			4.6			4.6
			5.6			5.4			5.8
			5.8			5.9			5.8
			6.4			6.9			6.7
			6.0			6.4			6.2
			6.4			6.6			6.8
		means	5.8			6.0			6.0
Hematocrit			33			37			38
			44			48			48
			43			50			52
			32			35			37
			38			44			43
			40			42			43
		means	38			43*			44*

*$p \leq 0.01$ relative to zero time.

†$p \leq 0.05$ relative to zero time.

Table B-IV. Effects of locally infused PGE_1 (4.0 µg/min, I.A.) into the forelimb for 60 minutes on lymph flow, lymph protein transport, protein concentration of lymph and plasma, vascular pressures and hematocrit (n=6) (constant flow)

	Control	Vehicle Control		PGE_1 Infusion Period					
Time (minutes)	-20	-10	0	10	20	30	40	50	60
Systemic Arterial Blood Pressure (mm Hg)	105	110	110	120	120	115	120	120	120
	140	135	135	140	162	150	155	157	160
	97	100	110	115	122	125	125	125	125
	140	135	140	145	140	135	135	135	135
	140	145	155	190	190	190	190	190	190
	120	120	120	120	110	110	120	120	122
means	124	124	127	138†	141†	138†	141†	141†	142†
standard error	±8	±7	±7	±11	±12	±12	±11	±11	±12
Perfusion Pressure (mm Hg)	100	100	100	57	57	57	60	60	62
	130	130	135	70	70	70	72	75	77
	95	95	95	50	55	55	55	57	57
	135	135	125	45	50	50	52	60	60
	140	140	140	55	55	60	60	65	90
	120	110	112	60	62	65	70	67	70
means	120	118	118	56*	58*	60*	62*	64*	69*
standard error	±8	±8	±8	±4	±3	±3	±3	±3	±5

Table B-IV. Effects of locally infused PGE_1 (4.0 µg/min, I.A.) into the forelimb for 60 minutes on lymph flow, lymph protein transport, protein concentration of lymph and plasma, vascular pressures and hematocrit (n=6) (constant flow)

	Control	Vehicle Control		PGE_1 Infusion Period					
Time (minutes)	-20	-10	0	10	20	30	40	50	60
Systemic Arterial Blood Pressure (mm Hg)	105	110	110	120	120	115	120	120	120
	140	135	135	140	162	150	155	157	160
	97	100	110	115	122	125	125	125	125
	140	135	140	145	140	135	135	135	135
	140	145	155	190	190	190	190	190	190
	120	120	120	120	110	110	120	120	122
means	124	124	127	138†	141†	138†	141†	141†	142†
standard error	±8	±7	±7	±11	±12	±12	±11	±11	±12
Perfusion Pressure (mm Hg)	100	100	100	57	57	57	60	60	62
	130	130	135	70	70	70	72	75	77
	95	95	95	50	55	55	55	57	57
	135	135	125	45	50	50	52	60	60
	140	140	140	55	55	60	60	65	90
	120	110	112	60	62	65	70	67	70
means	120	118	118	56*	58*	60*	62*	64*	69*
standard error	±8	±8	±8	±4	±3	±3	±3	±3	±5

Table B-IV (continued)

Time (minutes)	Control	Vehicle Control		PGE_1 Infusion Period					
	-20	-10	0	10	20	30	40	50	60
Skin Small Vein Pressure (mm Hg)	13	14	14	16	16	16	16	16	16
	12	12	12	11	11	12	12	11	11
	9	9	9	9	10	10	10	10	10
	16	15	15	16	17	17	17	17	16
	7	7	7	9	9	10	10	10	10
	9	9	9	7	7	7	8	8	8
means	11	11	11	11	12	12	12	12	12
standard error	±1	±1	±1	±2	±2	±2	±1	±1	±1
Lymph Flow Rate (ml/10 min)	0.01	0.01	0.01	0.08	0.12	0.12	0.05	0.05	0.04
	0.02	0.02	0.01	0.02	0.04	0.04	0.06	0.05	0.04
	0.01	0.01	0.01	0.02	0.02	0.02	0.02	0.02	0.02
	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
	0.01	0.01	0.02	0.03	0.05	0.04	0.04	0.03	0.02
	0.03	0.03	0.02	0.01	0.03	0.02	0.02	0.02	0.02
means	0.02	0.02	0.01	0.03	0.05*	0.04*	0.03	0.03	0.02
standard error	±0	±0	±0	±0.01	±0.02	±0.02	±0.01	±0.01	±0.01

Table B-IV (continued)

Time (minutes)	Control	Vehicle Control		PGE$_1$ Infusion Period					
	-20	-10	0	10	20	30	40	50	60
Lymph Total Protein (grams %)	3.3	3.3	3.3	3.9	4.4	4.5	4.4	4.4	4.3
	2.2	2.0	2.0	2.6	2.3	2.4	3.3	2.9	3.1
	2.6	2.6	2.6	3.0	3.6	4.1	4.0	3.9	3.8
	3.2	3.1	3.1	3.1	3.2	3.1	3.4	3.2	3.3
	1.8	1.8	1.7	1.6	2.4	3.3	3.2	3.3	3.3
	1.4	1.5	1.6	2.0	2.0	2.7	2.8	2.6	2.9
means	2.4	2.4	2.4	2.7	3.0*	3.4*	3.5*	3.4*	3.5*
standard error	±.3	±.3	±.3	±.3	±.4	±.3	±.2	±.3	±.2
Lymph Protein Transport (mg/10 min)	0.33	0.33	0.33	3.12	5.28	5.40	2.20	2.20	1.20
	0.44	0.40	0.20	0.52	0.92	0.96	1.98	1.45	1.20
	0.26	0.26	0.26	0.60	0.72	0.82	0.80	0.78	0.36
	0.32	0.31	0.31	0.31	0.32	0.31	0.34	0.32	0.35
	0.18	0.18	0.34	0.48	1.20	1.32	1.28	0.99	0.68
	0.42	0.45	0.32	0.20	0.60	0.54	0.56	0.52	0.26
means	0.33	0.32	0.30	0.87	1.51	1.56*	1.19	1.04	0.70
standard error	±0.04	±0.04	±0.02	±0.45	±0.76	±0.78	±0.31	±0.28	±0.21

Table B-IV (continued)

Time (minutes)	Control -20	Vehicle Control 10	Vehicle Control 0	PGE_1 Infusion Period 10	20	30	40	50	60
Plasma Protein (grams %)			6.2			6.1			5.6
			7.0			6.8			6.6
			5.2			5.3			5.3
			6.0			6.2			6.2
			5.0			5.4			5.5
			6.7			6.6			6.7
		means	6.0			6.1			6.0
		standard error	±.3			±.3			±.2
Hematocrit			32			37			37
			30			37			37
			39			43			43
			41			42			41
			38			45			49
			43			44			44
		means	37			41*			42*
		standard error	±2			±1			±2

*$p \leq 0.01$ relative to zero time.

†$p \leq 0.05$ relative to zero time.

Table A-V. Effects of locally infused PGE_1 (16.0 µg/min, I.A.) into the forelimb for 60 minutes on lymph flow, lymph protein transport, protein concentration of lymph and plasma, vascular pressures, forelimb weight and hematocrit (n=6) (natural flow)

	Control	Vehicle Control		PGE_1 Infusion Period					
Time (minutes)	-20	-10	0	10	20	30	40	50	60
Systemic Arterial Blood Pressure (mm Hg)		117	120	132	135	140	140	145	145
		115	117	117	107	100	95	95	95
		127	127	135	125	125	125	125	122
		112	115	118	120	125	130	132	135
		128	128	150	152	155	152	152	155
		125	125	127	125	125	125	125	125
	means	121	122	130	127	128	128	129	130
	standard error	±3	±2	±5	±6	±7	±8	±8	±9
Skin Small Vein Pressure (mm Hg)		7	7	17	17	18	18	18	18
		12	12	17	16	15	14	13	13
		9	8	19	18	18	17	16	15
		9	9	16	11	11	11	12	11
		14	12	30	30	30	30	30	30
		9	8	17	16	16	16	16	16
	means	10	9	19*	18*	18*	18*	18*	17*
	standard error	±1	±1	±2	±3	±3	±3	±3	±3

Table A-V (continued)

Time (minutes)	Control -20	Vehicle Control -10	Vehicle Control 0	PGE_1 Infusion Period 10	PGE_1 Infusion Period 20	PGE_1 Infusion Period 30	PGE_1 Infusion Period 40	PGE_1 Infusion Period 50	PGE_1 Infusion Period 60
Lymph Flow Rate (ml/10 min)		0.01	0.01	0.03	0.05	0.04	0.05	0.03	0.06
		0.01	0.01	0.05	0.10	0.04	0.03	0.03	0.02
		0.01	0.01	0.01	0.02	0.02	0.03	0.04	0.02
		0.04	0.05	0.04	0.03	0.03	0.03	0.05	0.03
		0.03	0.03	0.10	0.26	0.23	0.18	0.34	0.39
		0.01	0.01	0.01	0.02	0.02	0.02	0.02	0.02
	means	0.02	0.02	0.04	0.08	0.06	0.06	0.08	0.08
	standard error	±.01	±.01	±.01	±.04	±.03	±.03	±.05	±.04
Lymph Total Protein (grams %)		3.0	3.0	3.0	3.4	4.2	4.1	4.3	4.0
		2.1	2.1	2.7	2.8	4.0	4.2	4.0	3.8
		1.4	1.4	1.4	1.7	2.8	2.3	2.1	2.3
		1.8	1.8	1.8	1.7	2.0	2.2	2.0	2.1
		1.2	1.3	1.5	1.7	1.5	1.2	1.1	1.2
		2.1	2.1	2.2	2.4	2.4	2.5	2.7	3.0
	means	1.9	2.0	2.1	2.3	2.8*	2.8*	2.7*	2.7*
	standard error	±.3	±.3	±.3	±.3	±.4	±.5	±.5	±.4

Table A-V (continued)

Time (minutes)	Control -20	Vehicle Control -10	0	PGE_1 Infusion Period 10	20	30	40	50	60
Lymph Protein Transport (mg/10 min)		0.30	0.30	0.90	1.70	1.68	2.05	1.29	2.40
		0.21	0.21	1.35	2.80	1.60	1.26	1.20	0.76
		0.14	0.14	0.14	0.34	0.86	0.56	0.69	0.46
		0.72	0.90	0.72	0.51	0.60	0.66	0.10	0.42
		0.36	0.39	1.50	4.42	3.45	2.16	3.74	4.68
		0.21	0.21	0.22	0.48	0.48	0.50	0.54	0.60
	means	0.32	0.36	0.78	1.71*	1.40†	1.22†	1.29†	1.55*
	standard error	±.09	±.11	±.24	±.67	±.47	±.30	‡52	±.70
Plasma Protein (grams %)			5.4			5.8			6.0
			5.3			5.8			5.7
			4.6			4.9			4.7
			5.2			5.5			5.7
			5.7			6.1			6.4
			5.6			5.4			5.6
		means	5.3			5.6†			5.7*
		standard error	±.2			±.2			±.2

Table A-V (continued)

Time (minutes)	Control -20	Vehicle Control -10	0	PGE$_1$ Infusion Period 10	20	30	40	50	60
Hematocrit			40			48			48
			34			43			40
			42			44			45
			45			48			50
			46			50			48
			35			36			37
		means	40			45*			45*
		standard error	±2			±2			±2

	Control	Experimental	Difference
Forelimb Weight (grams; n=5)	672	711	39
	725	743	18
	630	648	18
	769	923	154
	721	768	47
means	703	759	55†
standard error	±24	±46	±25

*$p \leq 0.01$ relative to zero time.

†$p \leq 0.05$ relative to zero time.

Table B-V. Effects of locally infused PGE_1 (16.0 µg/min, I.A.) into the forelimb for 60 minutes on lymph flow, lymph protein transport, protein concentration of lymph and plasma, vascular pressures, forelimb weight and hematocrit (n=6) (constant flow)

	Control	Vehicle Control		PGE_1 Infusion Period					
Time (minutes)	-20	-10	0	10	20	30	40	50	60
Systemic Arterial	120	120	120	105	102	102	102	105	105
Blood Pressure	105	102	105	97	102	125	130	132	137
(mm Hg)	92	90	90	95	90	90	95	95	90
	142	142	145	165	165	170	175	170	175
	100	100	102	107	100	95	95	95	95
	140	140	125	125	125	125	130	130	130
means	117	116	115	116	114	118	121	121	131
standard error	±9	±9	±8	±11	±11	±12	±13	±12	±12
Perfusion Pressure	120	105	110	55	58	60	62	65	65
(mm Hg)	95	97	100	37	42	40	42	42	47
	95	92	92	50	52	52	55	52	55
	116	120	130	85	90	95	93	105	120
	95	95	95	35	37	37	37	37	35
	115	115	115	52	52	54	54	55	55
means	106	104	107	52*	55*	56*	57*	59*	64*
standard error	±5	±5	±6	±7	±8	±9	±8	±10	±11

Table B-V (continued)

Time (minutes)	Control	Vehicle Control		PGE_1 Infusion Period					
	-20	-10	0	10	20	30	40	50	60
Skin Small Vein Pressure (mm Hg)	10	11	12	12	13	13	15	15	16
	9	8	8	8	8	8	8	8	8
	11	11	11	12	12	12	12	11	12
	16	16	16	16	16	18	17	17	18
	14	14	13	15	14	14	14	14	18
	16	16	16	17	17	17	16	16	16
means	13	13	13	13	13	14	14	14	14
standard error	±1	±1	±1	±1	±1	±1	±1	±1	±1
Lymph Flow Rate (ml/10 min)	0.02	0.01	0.01	0.04	0.04	0.04	0.03	0.02	0.03
	0.01	0.01	0.01	0.01	0.01	0.03	0.01	0.01	0.01
	0.01	0.01	0.01	0.02	0.06	0.07	0.07	0.07	0.06
	0.01	0.01	0.01	0.01	0.01	0.02	0.01	0.01	0.01
	0.01	0.02	0.02	0.09	0.10	0.09	0.09	0.07	0.06
	0.02	0.01	0.01	0.10	0.10	0.10	0.11	0.05	0.05
means	0.01	0.01	0.01	0.05*	0.05*	0.06*	0.05*	0.04†	0.05*
standard error	±.00	±.00	±.00	±.02	±.02	±.01	±.02	±.01	±.02

Table B-V (continued)

Time (minutes)	Control	Vehicle Control		PGE_1 Infusion Period					
	-20	-10	0	10	20	30	40	50	60
Lymph Total Protein (grams %)	1.7	1.7	1.8	1.8	2.2	2.9	3.4	3.3	3.1
	2.1	1.6	1.6	2.9	2.6	3.5	2.8	2.5	2.7
	2.6	2.4	2.5	2.9	3.8	4.0	4.3	4.0	3.8
	2.1	2.0	2.2	2.3	2.6	3.2	3.1	3.0	3.1
	2.0	2.0	2.0	2.1	3.8	4.8	5.1	5.0	4.9
	1.5	1.7	1.5	1.8	3.3	3.8	3.4	3.3	3.1
means	2.0	1.9	1.9	2.3	3.1*	3.7*	3.7*	3.5*	3.5*
standard error	±.2	±.1	±.1	±.2	±.2	±.3	±.3	±.4	±.3
Lymph Protein Transport (mg/10 min)	0.34	0.17	0.18	0.72	0.88	1.16	1.02	0.66	0.93
	0.21	0.16	0.16	0.29	0.26	1.05	0.28	0.25	0.27
	0.26	0.24	0.25	0.58	2.28	2.80	3.01	2.80	2.28
	0.21	0.20	0.22	0.23	0.26	0.64	0.31	0.30	0.31
	0.20	0.40	0.40	1.89	3.80	4.32	4.59	3.50	2.94
	0.30	0.17	0.15	1.80	3.30	3.80	3.74	1.65	3.72
means	0.25	0.22	0.23	0.92	1.80*	2.30*	2.16*	1.53*	1.74*
standard error	±.02	±.04	±.04	±.30	±.63	±.64	±.76	±.56	±.59

Table B-V (continued)

Time (minutes)	Control -20	Vehicle Control -10	0	PGE$_1$ Infusion Period 10	20	30	40	50	60
Plasma Protein (grams %)			5.5			6.1			6.6
			4.9			5.2			5.1
			5.9			6.0			6.0
			5.9			5.9			6.0
			5.7			5.7			5.8
			5.8			5.9			6.1
		means	5.6			5.8			5.9
		standard error	±.2			±.1			±.2
Hematocrit			40			47			52
			36			40			41
			37			38			38
			37			44			45
			40			44			44
			34			42			43
		means	37			43*			44*
		standard error	±1			±2			±2

*$p \leq 0.01$ relative to zero time.

†$p \leq 0.05$ relative to zero time.

Table A-VI. Effects of locally infused PGE_1 (32.0 µg/min, I.A.) into the forelimb for 60 minutes on lymph flow, lymph protein transport, protein concentration of lymph and plasma, vascular pressures, forelimb weight and hematocrit (n=6) (natural flow)

	Control	Vehicle Control		PGE_1 Infusion Period					
Time (minutes)	-20	-10	0	10	20	30	40	50	60
Systemic Arterial Blood Pressure (mm Hg)		125	122	117	112	112	110	110	107
		122	122	95	85	90	87	87	90
		110	105	105	105	105	105	105	107
		120	120	90	92	95	95	95	97
		125	125	102	100	105	107	105	110
		125	125	125	130	137	137	137	135
	means	121	120	106*	104*	107*	107*	107*	108*
	standard error	±2	±3	±5	±6	±7	±7	±7	±6
Skin Small Vein Pressure (mm Hg)		14	14	19	19	19	19	19	18
		9	9	16	15	14	15	15	15
		11	10	16	16	16	16	17	17
		12	12	21	19	18	18	18	18
		12	12	20	19	18	18	18	17
		15	16	27	29	30	30	30	29
	means	12	12	20*	20*	19*	19*	20*	19*
	standard error	±1	±1	±2	±2	±2	±2	±2	±2

Table A-VI (continued)

Time (minutes)	Control -20	Vehicle Control -10	Vehicle Control 0	PGE_1 Infusion Period 10	20	30	40	50	60
Lymph Flow Rate (ml/10 min)		0.02	0.02	0.19	0.25	0.22	0.23	0.22	0.23
		0.01	0.01	0.05	0.07	0.03	0.03	0.06	0.03
		0.01	0.01	0.02	0.12	0.19	0.27	0.11	0.21
		0.03	0.02	0.02	0.08	0.16	0.16	0.20	0.19
		0.01	0.02	0.09	0.12	0.20	0.17	0.17	0.16
		0.02	0.02	0.12	0.13	0.15	0.16	0.16	0.16
	means	0.02	0.02	0.08†	0.13*	0.16*	0.15*	0.15*	0.16*
	standard error			±.03	±.03	±.03	±.04	±.02	±.03
Lymph Total Protein (grams %)		2.3	2.4	3.1	4.2	4.1	3.7	3.6	3.3
		2.1	2.1	2.6	2.5	2.5	2.9	3.1	2.9
		2.7	2.7	3.2	2.9	2.9	2.8	3.5	3.0
		3.0	2.8	2.9	2.7	4.0	4.1	4.4	4.2
		1.8	1.7	2.0	2.1	3.4	3.2	3.0	3.1
		1.4	1.5	1.9	2.2	1.9	2.0	1.9	1.8
	means	2.2	2.2	2.6	2.8†	3.1*	3.1*	3.3*	3.1*
	standard error	±.2	±.2	±.2	±.3	±.4	±.3	±.3	±.3

Table A-VI (continued)

Time (minutes)	Control -20	Vehicle Control -10	Vehicle Control 0	PGE_1 Infusion Period 10	20	30	40	50	60
Lymph Protein Transport (mg/10 min)		0.46	0.48	5.89	10.50	9.02	8.51	7.92	7.59
		0.21	0.21	1.30	1.75	0.75	0.87	1.86	0.87
		0.27	0.27	0.64	3.48	5.51	7.56	3.85	6.30
		0.90	0.56	0.58	2.16	6.40	6.56	8.80	7.98
		0.18	0.34	1.80	2.52	6.80	5.44	5.10	4.96
		0.28	0.30	2.28	2.86	2.85	3.20	3.04	2.88
	means	0.38	0.36	2.08	3.88*	5.22*	5.40*	5.10*	5.10*
	standard error	±.11	±.05	±.81	±1.35	±1.21	±1.17	±1.12	±1.14
Plasma Protein (grams %)			5.7			6.2			6.1
			6.0			6.1			6.1
			6.6			7.0			7.0
			6.0			6.5			6.7
			5.6			6.2			6.1
			5.2			6.0			6.2
	means		5.9			6.3*			6.4*
	standard error		±.2			±.2			±.2

Table A-VI (continued)

Time (minutes)	Control -20	Vehicle Control -10	0	PGE_1 Infusion Period 10	20	30	40	50	60
Hematocrit			36			39			39
			42			44			44
			36			40			40
			34			40			40
			40			44			45
			32			46			49
		means	37			42†			43*
		standard error	±2			±1			±2

	Control	Experimental	Difference
Forelimb Weight (grams; n=6)	754	840	86
	760	801	41
	533	575	42
	693	717	24
	731	787	56
	753	801	48
means	704	754	50*
standard error	±36	±39	±8

*$p \leq 0.01$ relative to zero time.

†$p \leq 0.05$ relative to zero time.

Table B-VI. Effects of locally infused PGE_1 (32.0 µg/min, I.A.) into the forelimb for 60 minutes on lymph flow, lymph protein transport, protein concentration of lymph and plasma, vascular pressures, forelimb weight and hematocrit (n=6) (constant flow)

	Control	Vehicle Control		PGE_1 Infusion Period						
Time (minutes)	-20	-10	0	2	10	20	30	40	50	60
Systemic Arterial	102	102	105	60	90	85	85	87	90	92
Blood Pressure	90	92	92	50	102	120	120	120	117	120
(mm Hg)	115	115	117	125	127	130	132	132	132	132
	117	117	117	70	112	117	115	120	130	130
	125	125	100	55	75	75	77	77	80	80
	145	145	140	95	125	120	117	117	115	115
means	116	116	113	76*	105	108	108	109	111	112
standard error	±8	±8	±7	±12	±8	±9	±9	±9	±9	±9
Perfusion Pressure	97	97	100		37	37	40	40	42	42
(mm Hg)	85	85	85		42	47	50	50	50	52
	105	105	107		47	47	47	50	50	50
	105	105	107		55	57	60	65	70	70
	110	100	90		45	40	40	40	40	40
	130	130	130		55	55	55	60	55	55
means	105	104	103		47*	47*	49*	51*	51*	52*
standard error	±6	±6	±6		±3	±3	±3	±4	±4	±4

Table B-VI (continued)

Time (minutes)	Control	Vehicle Control		PGE_1 Infusion Period					
	-20	-10	0	2	10	20	30	40	50	60
Skin Small Vein Pressure (mm Hg)	16	16	16	11	12	12	12	13	13
	13	14	14	12	13	14	14	14	14
	13	13	12	15	15	15	15	15	16
	14	14	14	13	13	13	14	14	14
	11	11	10	11	11	11	12	12	13
	7	7	12	9	9	9	9	9	9
means	12	13	12	12	12	12	13	13	13
standard error	±1	±1	±1	±1	±1	±1	±1	±1	±1
Lymph Flow Rate (ml/10 min)	0.05	0.05	0.05	0.07	0.12	0.14	0.10	0.10	0.07
	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
	0.01	0.01	0.01	0.01	0.02	0.03	0.02	0.03	0.02
	0.01	0.01	0.02	0.01	0.03	0.03	0.02	0.01	0.01
	0.01	0.02	0.01	0.01	0.01	0.01	0.02	0.02	0.02
means	0.02	0.02	0.02	0.02	0.03	0.04†	0.03	0.03	0.02
standard error	±.01	±.01	±.01	±.01	±.02	±.02	±.01	±.01	±.01

Table B-VI (continued)

Time (minutes)	Control	Vehicle Control		PGE_1 Infusion Period					
	-20	-10	0	2	10	20	30	40	50	60
Skin Small Vein Pressure (mm Hg)	16	16	16		11	12	12	12	13	13
	13	14	14		12	13	14	14	14	14
	13	13	12		15	15	15	15	15	16
	14	14	14		13	13	13	14	14	14
	11	11	10		11	11	11	12	12	13
	7	7	12		9	9	9	9	9	9
means	12	13	12		12	12	12	13	13	13
standard error	±1	±1	±1		±1	±1	±1	±1	±1	±1
Lymph Flow Rate (ml/10 min)	0.05	0.05	0.05		0.07	0.12	0.14	0.10	0.10	0.07
	0.01	0.01	0.01		0.01	0.01	0.01	0.01	0.01	0.01
	0.01	0.01	0.01		0.01	0.01	0.01	0.01	0.01	0.01
	0.01	0.01	0.01		0.01	0.02	0.03	0.02	0.03	0.02
	0.01	0.01	0.02		0.01	0.03	0.03	0.02	0.01	0.01
	0.01	0.02	0.01		0.01	0.01	0.01	0.02	0.02	0.02
means	0.02	0.02	0.02		0.02	0.03	0.04†	0.03	0.03	0.02
standard error	±.01	±.01	±.01		±.01	±.02	±.02	±.01	±.01	±.01

Table B-VI (continued)

Time (minutes)	Control -20	Vehicle Control -10	Vehicle Control 0	PGE_1 Infusion Period 2	10	20	30	40	50	60
Lymph Total Protein (grams %)	1.7	1.7	1.6	2.0	1.9	1.9	2.1	2.0	2.1	
	3.4	3.4	3.4	3.6	3.5	3.4	3.0	3.5	3.4	
	2.0	2.0	2.0	2.0	2.0	2.1	2.3	2.3	2.1	
	2.4	2.4	2.4	2.4	2.8	2.6	2.5	2.7	2.6	
	2.1	2.1	2.0	2.2	2.3	2.9	2.8	2.7	2.7	
	2.5	2.5	2.6	3.6	3.2	3.5	3.1	3.0	3.0	
means	2.4	2.4	2.3	2.6†	2.6†	2.7*	2.6†	2.7*	2.7*	
standard error	±.2	±.2	±.3	±.3	±.3	±.3	±.2	±.2	±.2	
Lymph Protein Transport (mg/10 min)	0.85	0.85	0.80	1.40	2.28	2.66	2.10	2.00	1.47	
	0.34	0.34	0.34	0.36	0.35	0.34	0.30	0.35	0.34	
	0.20	0.20	0.20	0.20	0.20	0.21	0.23	0.23	0.21	
	0.24	0.24	0.24	0.24	0.56	0.78	0.50	0.81	0.52	
	0.21	0.21	0.40	0.22	0.69	0.87	0.56	0.27	0.27	
	0.25	0.50	0.26	0.36	0.32	0.35	0.62	0.60	0.60	
means	0.35	0.39	0.37	0.44	0.73†	0.87*	0.72†	0.71†	0.57	
standard error	±.10	±.10	±.09	±.19	±.32	±.37	±.28	±.27	±.19	

Table B-VI (continued)

Time (minutes)	Control -20	Vehicle Control -10	0	PGE_1 Infusion Period 2	10	20	30	40	50	60
Plasma Protein (grams %)			5.3				6.2			6.2
			6.2				6.8			7.0
			5.8				6.6			6.6
			5.0				5.4			5.6
			5.9				6.0			6.2
			6.0				6.2			5.8
		means	5.7				6.2*			6.2*
		standard error	±.2				±.2			±.2
Hematocrit			36				41			43
			39				47			49
			32				38			37
			40				43			44
			40				48			47
			38				45			43
		means	38				44*			44*
		standard error	±1				±2			±2

Table B-VI (continued)

	Control	Experimental	Difference
Forelimb Weight (grams; n=6)	760	779	19
	672	674	2
	1127	1200	73
	699	714	15
	762	806	44
	453	452	2
means	746	771	26†
standard error	±89	±99	±11

*$p \leq 0.01$ relative to zero time.

†$p \leq 0.05$ relative to zero time.

Table A-VII. Effects of indomethacin (5 mg/kg) injected intravenously followed by PGE_1 (16.0 µg/min, I.A.) infused locally into the forelimb for 60 minutes on lymph flow, lymph protein transport, protein concentration of lymph and plasma, vascular pressures, forelimb weight and hematocrit (n=6) (natural flow)

	Indo. Injection	Vehicle Control		PGE_1 Infusion Period					
Time (minutes)	-60	-10	0	10	20	30	40	50	60
Systemic Arterial Blood Pressure (mm Hg)	105	110	110	127	120	125	122	122	120
	120	117	120	100	107	105	105	105	110
	130	130	130	150	140	135	125	125	120
	125	135	135	142	139	130	125	120	115
	130	125	125	135	130	130	130	135	135
	100	105	95	70	70	75	75	80	80
means	118	120	119	121	118	117	114	115	113
standard error	±5	±5	±6	±12	±11	±9	±9	±8	±8
Skin Small Vein Pressure (mm Hg)	10	10	10	25	24	23	23	22	22
	14	14	14	27	27	28	27	29	29
	15	14	14	30	28	27	25	25	24
	14	12	12	30	29	28	27	28	27
	10	7	12	29	27	27	28	27	22
	13	12	11	14	18	19	20	20	20
means	13	12	12	26*	26*	25*	25*	25*	24*
standard error	±1	±1	±1	±3	±2	±2	±1	±1	±1

Table A-VII (continued)

Time (minutes)	Indo. Injection -60	Vehicle Control -10	Vehicle Control 0	PGE_1 Infusion Period 10	20	30	40	50	60
Lymph Flow Rate (ml/10 min)	0.02	0.01	0.02	0.01	0.20	0.20	0.14	0.15	0.10
	0.01	0.02	0.02	0.11	0.17	0.18	0.16	0.17	0.18
	0.06	0.08	0.07	0.25	0.48	0.47	0.45	0.45	0.33
	0.02	0.03	0.04	0.32	0.33	0.34	0.33	0.30	0.34
	0.01	0.01	0.02	0.11	0.31	0.44	0.33	0.38	0.36
	0.07	0.08	0.08	0.29	0.31	0.29	0.30	0.26	0.30
means	0.03	0.04	0.04	0.20*Ω	0.30*Ω	0.32*Ω	0.29*Ω	0.29*Ω	0.27*Ω
standard error	±.01	±.01	±.01	±.04	±.04	±.05	±.05	±.05	±.04
Lymph Total Protein (grams %)	1.7	2.1	2.1	2.8	4.4	4.0	3.9	3.8	3.8
	2.1	2.5	2.5	3.1	3.2	3.2	3.2	2.9	2.7
	1.9	1.8	2.1	2.5	2.9	2.9	2.3	2.9	2.7
	1.7	1.7	1.8	1.8	2.5	2.3	2.0	2.0	2.0
	3.5	3.5	3.5	4.1	4.0	3.6	3.4	3.6	3.8
	1.2	1.3	1.2	1.8	2.6	2.6	2.8	2.6	2.7
means	2.0	2.1	2.2	2.7†	3.3*	3.1*	2.9*	3.0*	3.0*
standard error	±.3	±.3	±.3	±.4	±.3	±.3	±.3	±.3	±.3

Table A-VII (continued)

Time (minutes)	Indo. Injection	Vehicle Control		PGE_1 Infusion Period					
	-60	-10	0	10	20	30	40	50	60
Lymph Protein Transport (mg/10 min)	0.34	0.21	0.42	2.80	8.80	8.00	5.46	5.70	3.80
	0.21	0.50	0.50	3.41	5.44	5.76	5.12	4.93	4.86
	1.14	1.44	1.47	6.25	13.93	13.63	10.35	13.05	8.91
	0.34	0.51	0.72	5.76	8.25	7.82	6.60	6.00	6.80
	0.35	0.35	0.70	4.51	12.40	15.84	11.22	13.68	13.68
	0.84	1.04	0.96	5.22	8.06	7.54	8.40	6.76	8.10
means	0.54	0.68	0.79	4.66*Ω	9.48*Ω	9.77*Ω	7.86*Ω	8.35*Ω	7.69*Ω
standard error	±.15	±.19	±.16	±.55	±1.27	±1.63	±1.04	±1.60	±1.43
Plasma Protein (grams %)	5.2		5.5			6.0			5.8
	5.9		5.8			5.8			5.6
	6.9		6.5			6.5			6.6
	5.2		5.2			5.3			5.7
	6.8		6.4			6.8			6.8
	6.0		5.7			5.3			5.6
means	6.0		5.9			6.0			6.0
standard error	±.3		±.2			±.3			±.2

Table A-VII (continued)

Time (minutes)	Indo. Injection -60	Vehicle Control -10	Vehicle Control 0	PGE_1 Infusion Period 10	20	30	40	50	60
Hematocrit	28		28			33			32
	22		22			24			24
	38		38			47			45
	38		38			46			45
	35		35			40			40
	38		40			39			40
means	33		34			38*			38*
standard error	±3		±3			±4			±4

	Control	Experimental	Difference
Forelimb Weight (grams; n=6)	706	762	56
	696	779	83
	799	872	73
	719	770	51
	693	729	36
	786	873	87
means	733	798	64*

*$p \leq 0.01$ relative to zero time.

†$p \leq 0.05$ relative to zero time.

Ω$p \leq 0.01$ relative to the values obtained with 16.0 g PGE_1/min alone (Table V).

Table B-VII. Effects of indomethacin (5 mg/kg) injected intravenously followed by PGE_1 (16.0 µg/min, I.A.) infused locally into the forelimb for 60 minutes on lymph flow, lymph protein transport, protein concentration of lymph and plasma, vascular pressures, forelimb weight and hematocrit (n=6) (constant flow)

	Indo. Injection	Vehicle Control		PGE_1 Infusion Period					
Time (minutes)	-60	-10	0	10	20	30	40	50	60
Systemic Arterial	105	105	105	115	120	125	125	122	122
Blood Pressure	140	155	155	150	150	145	140	145	140
(mm Hg)	150	150	150	152	155	155	155	150	150
	150	150	150	167	165	160	155	155	150
	112	120	120	120	130	135	135	135	135
	135	120	115	150	152	150	152	152	152
means	132	133	133	142†	145*	145*	144†	143†	142†
standard error	±8	±9	±9	±8	±7	±5	±5	±5	±5
Perfusion Pressure	100	100	102	55	52	55	52	52	55
(mm Hg)	120	120	120	50	50	50	55	50	50
	145	155	157	62	65	65	67	67	67
	140	150	155	75	70	65	62	65	65
	110	130	130	65	67	70	75	75	75
	125	130	125	40	47	50	50	50	52
means	123	131	132	58*	59*	59*	60*	60*	61*
standard error	7	8	9	5	4	4	4	4	4

Table B-VII (continued)

Time (minutes)	Indo. Injection -60	Vehicle Control -10	Vehicle Control 0	PGE_1 Infusion Period 10	20	30	40	50	60
Skin Small Vein Pressure (mm Hg)	12	14	14	14	15	15	15	15	15
	11	10	10	11	11	10	10	10	10
	12	10	10	11	11	12	12	12	12
	7	5	5	6	6	5	5	4	3
	9	7	7	7	7	7	8	8	8
	12	13	13	17	17	17	17	17	17
means	11	10	10	11	11	11	11	11	11
standard error	±1	±1	±1	±2	±2	±2	±2	±2	±2
Lymph Flow Rate (ml/10 min)	0.01	0.01	0.01	0.10	0.11	0.10	0.09	0.10	0.14
	0.01	0.01	0.01	0.18	0.29	0.20	0.18	0.11	0.08
	0.02	0.03	0.03	0.07	0.07	0.08	0.06	0.05	0.04
	0.01	0.02	0.02	0.05	0.10	0.09	0.04	0.04	0.04
	0.01	0.01	0.01	0.03	0.06	0.06	0.07	0.07	0.08
	0.02	0.01	0.02	0.02	0.03	0.09	0.07	0.06	0.05
means	0.01	0.02	0.02	0.08*	0.12*ω	0.10*	0.09*	0.07†ω	0.07†ω
standard error	±0	±0	±0	±.02	±.04	±.02	±.02	±.01	±.02

Table B-VII (continued)

Time (minutes)	Indo. Injection -60	Vehicle Control -10	Vehicle Control 0	PGE_1 Infusion Period 10	20	30	40	50	60
Lymph Total Protein (grams %)	1.8	2.0	2.0	2.2	2.3	3.0	3.1	3.2	3.0
	3.1	3.4	3.4	4.1	5.0	5.7	6.0	5.5	5.8
	2.1	2.4	2.5	2.5	2.9	3.3	3.2	3.4	3.0
	2.5	2.7	2.7	2.9	3.0	3.6	3.5	3.6	3.8
	2.8	3.2	3.2	3.2	3.4	3.6	3.8	4.0	4.0
	3.5	3.8	3.8	4.0	4.5	4.9	4.2	4.3	4.2
means	2.6	2.9	2.9	3.2	3.5*	4.0*	4.0*	4.0*	4.0*
standard error	±.3	±.3	±.3	±.3	±.4	±.4	±.4	±.3	±.4
Lymph Protein Transport (mg/10 min)	0.18	0.20	0.20	2.20	2.53	3.00	2.79	3.20	4.20
	0.31	0.34	0.34	7.38	14.50	11.40	10.80	6.05	4.64
	0.42	0.72	0.75	1.75	2.03	2.64	1.92	1.70	1.20
	0.50	0.54	0.54	1.45	3.00	3.24	1.40	1.44	1.52
	0.28	0.32	0.32	0.96	2.04	2.16	2.66	2.80	3.20
	0.70	0.38	0.76	0.80	1.35	4.41	3.09	2.58	2.10
means	0.40	0.42	0.49	2.42ω	4.24*Ω	4.48*ω	3.78*ω	2.96†ω	2.81†
standard error	±.07	±.08	±.09	±1.01	±2.06	±1.41	±1.43	±.68	±.58

Table B-VII (continued)

Time (minutes)	Indo. Injection	Vehicle Control		PGE_1 Infusion Period					
	-60	-10	0	10	20	30	40	50	60
Plasma Protein (grams %)	5.5		5.4			5.2			5.2
	6.7		6.8			7.0			7.0
	5.8		5.7			5.8			5.8
	5.4		5.8			6.1			6.0
	5.8		5.8			6.2			6.0
	5.7		5.5			5.8			6.0
means	5.8		5.8			6.0			6.0
standard error	±.2		±.2			±.2			±.2
Hematocrit	32		32			32			34
	28		28			33			34
	40		42			48			45
	41		41			50			49
	47		40			48			48
	41		39			49			48
means	37		37			43*			43*
standard error	±2		±2			±3			±3

Table B-VII (continued)

	Control	Experimental	Difference
Forelimb Weight (grams; n=6)	607	620	13
	948	989	41
	548	561	13
	589	592	3
	536	545	9
	943	945	2
means	695	709	14†
standard error	±79	±83	±6

*p ≤ 0.01 relative to zero time.

†p ≤ 0.05 relative to zero time.

Ωp ≤ 0.01 relative to the values obtained with 16.0 µg PGE_1/min alone (Table V).

ωp ≤ 0.05 relative to the values obtained with 16.0 µg PGE_1/min alone (Table V).

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