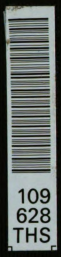
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The Inter-Relationship Between Prostaglandin E_1,
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and Macromolecular Permeability in the Canine Forelimb

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Arthur Neil Gorman

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M.S. degree in Physiology

George J. Gregor
Major professor

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THE INTER-RELATIONSHIP BETWEEN PROSTAGLANDIN E_1, VARIOUS VASOACTIVE SUBSTANCES, AND MACROMOLECULAR PERMEABILITY IN THE CANINE FORELIMB

By

Arthur Neil Gorman

A THESIS

Submitted to
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ABSTRACT

THE INTER-RELATIONSHIP BETWEEN PROSTAGLANDIN E_1, VARIOUS VASOACTIVE SUBSTANCES, AND MACROMOLECULAR PERMEABILITY IN THE CANINE FORELIMB

By

Arthur Neil Gorman

The effects of PGE_1, by itself and in combination with other vasoactive agents, on hemodynamics, lymph protein concentration, and weight, were examined in canine forelimbs perfused at constant inflow. PGE_1 (16 μg/min i.a.) produced profound vasodilation and caused marked increases in lymph protein concentration but failed to significantly increase lymph flow rate. Forelimb weights significantly increased owing to edema formation. Under similar conditions, this same dose of PGE_1 infused with isoproterenol (3 μg/min), vasopressin (0.8 Pressor Units/min), angiotensin II (2 μg/min), or methylprednisolone (15 μg/min) failed to increase lymph protein concentration relative to control. Serotonin (7 μg base/min i.a.) infused concomitantly with PGE_1 (16 μg/min i.a.) produced increases in lymph protein concentration and limb weights similar to those achieved with PGE_1 alone. The antagonism of the PGE_1-induced increase in protein efflux by isoproterenol, vasopressin, angiotensin II, and methylprednisolone was independent of changes in blood flow, vascular pressures, or perfused surface area. Thus, like

histamine and bradykinin, PGE_1 produces increases in microvascular permeability which can ve antagonized by other vasoactive agents.

To my lovely wife, Anita,

and to my wonderful parents;

their love has lit my way to accomplishment and joy.

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LIST OF SYMBOLS AND ABBREVIATIONS

mmHg	=	Millimeters of mercury pressure.
M.W.	=	Molecular weight.
gm %	=	Grams per cent.
Å	=	Angstrom units.
µm	=	Micrometer.
µg	=	Microgram
ng	=	Nanogram
ml	=	Milliliter
min	=	Minute
i.v.	=	Drug infusion into the vena cava.
i.a.	=	Drug infusion intra-arterially into the forelimb.
CF	=	Forelimb perfused at constant inflow.

INTRODUCTION

Although the issue has been under considerable debate, there is a great deal of evidence to indicate that prostaglandins, especially PGE_1, are intimately involved in the inflammatory process (102). Numerous studies (11,12,19, 20,47,74) conducted in rats, and guinea pigs have demonstrated that PGE_1 increases vascular permeability to macromolecules. These studies have been conducted using the vital dye technique which has a number of methodological drawbacks which may have contributed to the unusual variability of response to PGE_1 found by some investigators (20).

Using the more reliable hamster cheek pouch preparation, Svensjo (95) and Joyner et al. (45) have shown that PGE_1 increases the leakage of FITC-dextran from postcapillary venules by forming interendothelial cell venular gaps. However, they found PGE_1 to be considerably less potent than histamine or bradykinin in producing FITC-dextran leakage sites. They and others (57,107,108) have also reported that PGE_1 potentiates the direct actions of histamine and bradykinin on the microvascular membrane.

To date, only a few studies have been conducted in the dog. Greenberg and Sparks (27), Daugherty (13), and Joyner (43) have all failed to show that PGE_1 causes an

increase in microvascular permeability to macromolecules. However, these studies either did not measure protein fluxes directly, or did not study the effects of PGE_1 over a wide dose range. Recent work from our laboratory (58) has shown that a wide dose range of PGE_1 causes increases in lymph protein concentration and forelimb weights when infused locally intra-arterially into canine forelimbs perfused either naturally or at constant inflow. Lymph flow rate did not increase as expected. Following pretreatment with indomethacin, PGE_1 produced increases in lymph protein concentration similar to those produced in the absence of indomethacin, but now produced marked increases in lymph flow rate under both natural and constant inflow conditions.

Based on these data in the dog and similar findings in other species, it appears that PGE_1 is likely to have a role in inflammation and increases vascular permeability in a manner similar to that of histamine and bradykinin. Work from this laboratory (29,59,65) and others (98) has demonstrated that catecholamines possess the unique ability to antagonize the protein efflux produced by histamine and bradykinin owing to a direct action on the microvascular membrane which counteracts that of histamine and bradykinin. This antagonism is independent of changes in blood flow, microvascular pressure and perfused surface area (29,51,65). It is now known that other agents possess the ability to antagonize or potentiate the protein efflux produced by these agents (29,59,78). In this study, the effects of

catecholamines and a variety of other natural and synthetic agents were studied to determine if they antagonize or potentiate the direct actions of PGE_1 on the microvascular membrane.

SURVEY OF THE LITERATURE

The vascular system carries nutrients, hormones, electrolytes, gases, and macromolecules required by the cells throughout the body and it takes up the products of their metabolism for excretion. The actual exchange between the blood and tissues takes place in the capillaries and venules and can occur by filtration, diffusion or micropinocytosis.

Fluid filtration is governed by physical forces and can be expressed by the following equation derived by Starling (91).

$$F = k(Pc - Pi - \pi p + \pi i),$$

where

F = the rate of fluid movement across the capillary wall;

k = capillary filtration coefficient. This is a measure of the permeability of the microvascular wall to isotonic fluid. It is determined by the product of capillary permeability and surface area available for diffusion;

Pc = capillary hydrostatic pressure;

Pi = interstitial hydrostatic pressure;

πp = plasma colloid osmotic pressure;

πi = interstitial colloid osmotic pressure.

When the equation is positive, filtration of fluid occurs, and when it is negative, fluid reabsorption occurs.

Capillary hydrostatic pressure is determined by capillary blood volume and capillary compliance. Clough et al. (8) has reported changes of only 0.1 μm in the diameter of capillaries in the cat mesentery during systole. This very low compliance is probably due to the incompressible nature of the surrounding gel matrix (21). Since there is little change in compliance, Pc is determined primarily by capillary blood volume which is in turn determined by several physical factors affecting both inflow and outflow. These factors are related by the equation:

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

where

$\bar{P}c$ = mean capillary hydrostatic pressure;

$\bar{P}a$ = mean arterial pressure;

Pv = venous or outflow pressure;

Rv = venous resistance to outflow;

Ra = arterial resistance to inflow.

An increase in arterial pressure, venous pressure, or venous resistance will increase $\bar{P}c$. An increase in arterial resistance will lower $\bar{P}c$. Vascular resistances are related to vessel caliber which is determined by active changes due to vascular smooth muscle activity, passive changes (due to changes in transmural pressure), and blood viscosity.

Interstitial fluid hydrostatic pressure is analagous to capillary hydrostatic pressure but is that pressure found in the interstitial spaces. It had been generally accepted that Pi was slightly positive (55) and would oppose fluid

filtration. Guyton (32), however, has suggested that interstitial fluid pressure is subatmospheric, thus enhancing fluid filtration. This issue remains to be resolved.

Plasma colloid osmotic pressure is the pressure resulting from the concentration of dissolved protein in the blood. This oncotic pressure is estimated to be about 25 mmHg out of a total plasma osmotic pressure of 6,000 mmHg (3) but is of great importance for, unlike the electrolytes which contribute to the total osmotic pressure, plasma proteins do not diffuse readily into the interstitial spaces and are largely confined to the intravascular space.

Plasma proteins are primarily a mixture of albumin (M.W. 69,000), globulins (M.W. 140,000), and fibrinogen (M.W. 400,000). Approximately 65% of plasma colloid osmotic pressure is attributable to albumin and only 15% to globulins as the albumin molecule is only one-half the size of the golbulin molecule and is present in higher concentrations. These plasma proteins exert an oncotic pressure of about 19 mmHg. In addition, cations which bind to the negatively charged protein ions exert another 6 mmHg pressure.

Similarly, interstitial colloid osmotic pressure is determined by the protein concentration of the interstitial fluid. Average total protein concentration of interstitial fluid is about 3 gm % and the colloid osmotic pressure about 10 mmHg (106). Protein concentrations and colloid osmotic pressures vary from one tissue to another. In skin and skeletal muscle the average protein concentration has been

found to be 2.0 gm % yielding an oncotic pressure of about 5 mmHg. In the liver, where discontinuous sinusoids allow for even greater filtration, protein concentrations of greater than 3.3 gm % and oncotic pressures of 16 mmHg have been reported. Discontinuous sinusoids which allow proteins and other large molecules to freely pass through large gaps, are also found in the bone marrow and the spleen.

Two other types of capillaries have been distinguished in studies using electron microscopy. Continuous capillaries are found in smooth, skeletal, and cardiac muscle, as well as in connective tissue and the central nervous system (4). They are characterized by an uninterrupted endothelium and intercellular gaps 40 Å wide. These intercellular junctions may correspond to the "small pore" system postulated by Landis and Pappenheimer (55). These pores represent only 0.1 - 0.2 per cent of the total capillary surface area. Peroxidase, a protein tracer of relatively low molecular weight (40,000), has been shown to pass rapidly through these clefts while ferriten (M.W. 500,000) does not (49). Cerebral capillaries are impermeable to both peroxidase and ferriten reflecting the barrier function of the cerebral capillary wall (blood-brain barrier). Fenestrated capillaries, found in the renal glomeruli, endocrine glands, and the intestinal mucosa, have circular pores 1 μm in diameter which penetrate the endothelium. These pores are usually covered by a very thin diaphragm and appear to be regularly spaced approximately 1300 Å apart.

Tissue colloid osmotic pressure can be measured by different techniques. Measurements using implantable devices such as perforated capsules that theoretically equilibrate with the interstitial fluid may be inaccurate because of the possibility of contamination by plasma, or that the sampled fluid may not contain all osmotically active particles. A more common method, lymph fluid analysis, makes the assumption that the lymph accurately reflects the interstitial fluid contents. This method has been challenged, for changes in lymph concentration could occur as the lymph flows centrally from the terminal lymphatics to the larger lymph vessels. Lymph analysis may also not reflect protein concentration gradients that exist in the interstitium. Using dextran molecules of known molecular weight and size, Renkin and Garlick (80) found that concentrations were equal in lymph and interstitial fluid thus precluding the possibility of protein concentration gradients existing in the interstitium. Studies conducted by Garlick and Renkin (22) and Mayerson *et al*. (66) have shown that exchange only occurs at lymph nodes and not in the lymphatic trunks. Thus, if lymph is sampled before it reaches a node it should be a true reflection of what is at the terminal lymphatic vessel.

The lymph vascular system forms a "drainage system" as lymph is conducted from the lymphatic capillaries through successively larger vessels that ultimately empty into the venous system. The lymph is propelled centrally through one-way valves that only permit unidirectional flow. When

a lymph vessel is distended with fluid, contractile elements or smooth muscle cells, if present in the vessel wall, contract (31). In addition to the pumping caused by the intrinsic contraction of the vessel walls, any external factor that compresses the lymph vessel can also contribute to the movement of lymph fluid. Such factors are muscle contraction, passive movements of parts of the body, arterial pulsations, and compression of the body tissues from the outside. Though usually ignored, a variety of vasoactive agents have the ability to contract or relax lymphatic vessels in vivo and in vitro.

Diffusion is the most important means by which substances are transferred between the plasma and interstitium. This process results from the random thermal movement of water molecules and dissoved particles. Hence, the greater the concentration gradient across the membrane, the greater the rate of diffusion. Fick's Law describes the process of diffusion:

$$\frac{ds}{dt} = D \cdot A \cdot \frac{dc}{dx},$$

where

$\frac{ds}{dt}$ = the amount of substance moved per unit time;

D = the diffusion coefficient for a particular molecule. (This value is inversely proportional to the square root of the molecular weight of the particle.);

A = the cross-sectional area of the capillary membrane;

$\frac{dc}{dx}$ = the concentration gradient across the capillary membrane.

Thus, the amount of substance which diffuses per unit time is equal to the product of the diffusion coefficient, the area of the capillary membrane, and the concentration gradient. Small molecules such as water and urea diffuse rapidly through capillary pores. Lipid-insoluble substances must also diffuse through these pores but due to the larger size of these molecules their diffusion is more restricted. With increasing size diffusion becomes much more difficult. Molecules with molecular weights of 60,000 or greater are almost completely impermeable. Substances which are lipid-soluble such as CO_2 and O_2 can diffuse directly through the lipid membrane of the cell. Since these substances can diffuse across the entire capillary membrane, their rates of diffusion are several hundred times the rates of most lipid-insoluble molecules.

Micropinocytosis (cytopempsis) is a relatively slow transport process that may be responsible for the movement of large lipid-insoluble molecules between plasma and interstitial fluid. This process takes place via vesicles which invaginate from the plasma-membrane and migrate across the cytoplasm from one surface of the endothelial cell to another where the contents are released. The actual passage of the vesicle across the cell may be passive, but the structural reorganization of the membrane during invagination and exocytosis is regarded as an active, energy-consuming

process. Although such a mechanism is known to exist, its quantitative importance in capillary and venular exchange is under considerable debate.

It has been repeatedly suggested that the vesicles are of major importance for transport of large molecules (5,81,87) and may contribute to a "large pore" system (81) which permit plasma proteins, hormones and antibodies to pass freely into the interstitium. It has been hypothesized by Renkin's group (5,44,79) that this mode of transport may play a primary role in changes in vascular permeability associated with inflammation. Increasing the permeability of the microvascular membrane will result in the escape of plasma protein from the vasculature to the interstitial fluid which will raise the oncotic pressure of the interstitium. The increased interstitial fluid oncotic pressure will enhance fluid movement out of the vasculature. Using microperoxidase tracer in mice, Simionescu et al. (88) found that leakage of macromolecules is primarily restricted to venules 8 - 16 μm in diameter. Svensjo et al. (98) reported similar findings using the hamster cheek pouch preparation. This procedure can be used to study changes in permeability in the microvasculature. FITC fluorescein-labeled dextran (M.W. 145,000) is administered intravenously and a portion of the cheek pouch is dissected and viewed microscopically (96). Leakage sites are counted, after topical application of various agents, as areas of fluorescence appear. The tissues can then be collected and examined via electron

microscopy. Histamine and bradykinin, two of the most important edemogenic and vasoactive agents in the body, produced a dose-related increase in the number of leakage sites in venules 9 - 16 μm in diameter (99). No leakage sites were detected in the arterioles or the capillaries. However, Svensjo *et al*. and other investigators (82) do not believe that the increased macromolecular leakage occurs as a result of increased vesicular transfer. They propose that interendothelial gaps form as adjacent endothelial cells "round up" in response to these vasoactive agents (36). These gaps are believed to be formed by active contraction, presumably as a result of shortening of actinomyosin-like filaments within these cells (63). Numerous morphological studies have demonstrated these gaps in tissues exposed to histamine (6,60,61,62) and bradykinin (30,33,86). The gaps formed were from 0.08 μm to 1.4 μm in width, large enough to easily allow passage of macromolecules.

In contrast, there is no direct evidence to support the claim of Carter, Joyner, and Renkin (5) that inflammatory mediators increase vesicular transfer. Morphological studies have been unable to substantiate this claim.. Since cytopempsis must be somewhat of an active process (81), it would be expected that interference with cellular metabolism would curtail vesicular transport. However, micropinocytosis has proven to be relatively resistant to oxygen lack and/or to inhibitors of cellular metabolism (92). Active processes are temperature sensitive and yet it has been

demonstrated (82) that marked cooling of the rat hindquarters failed to alter the increased clearance of macromolecules in response to elevations in venous pressure. Jennings and Florey (42) observed no change in vesicular uptake of ferriten during cooling, whereas Rippe and Grega (82) have shown that cooling markedly reduced the increase in macromolecular efflux produced by histamine in rats. Indeed, this effect was immediately reversible by rewarming the preparation to 37° C. Thus, at present, there is no conclusive evidence to support the hypothesis that cytopempsis plays a significant role in transcapillary movement. On the other hand, a significant amount of morphological data is available to attest to the formation of venular gaps as a means by which protein efflux can occur during inflammation.

Inflammation is characterized by vasodilation, increased vascular permeability, pain, and migration of leukocytes into the inflamed area (56). The high concentration of plasma proteins in inflammatory exudates as compared with normal extravascular fluid (90) makes it certain that whatever other changes are present, an increased permeability of the vessel wall to protein is an essential feature of inflammation.

Histamine and bradykinin have been implicated in playing a major role in inflammation. It has been shown that local administration of histamine (1 - 64 μg base/min) or bradykinin (0.8 - 10 μg/min) into the canine forelimb significantly increases net fluid filtration in skin and

skeletal muscle (28,35,52,59,65) resulting in significant increases in the weight and volume of the perfused limb. The increase in net fluid filtration is due to both an increase in microvascular pressure resulting from the vasodilation produced by these autocoids, as well as a decrease in the transmural oncotic pressure gradient. The decrease in the transmural oncotic pressure gradient is due to an enhanced rate of protein efflux into the interstitium (36) subsequent to an increase in vascular permeability to macromolecules.

The relative contributions of these pressure-dependent and pressure-independent effects are dose related. Low doses of histamine (e.g. 5 μg base/min) and bradykinin (e.g. 0.8 μg/min) infused into the naturally perfused forelimb produce increases in lymph flow, small skin vein pressure (a minimun for capillary hydrostatic pressure), and limb weight (28,52). These same doses infused into forelimbs perfused at constant inflow still increase lymph flow greatly but small skin vein pressures are not altered and the weight gain is largely attenuated (28,59). Thus, under conditions where microvascular pressure is held constant, the edema is considerably reduced.

High doses of histamine (e.g. 64 μg base/min) and bradykinin (e.g. 10 μg/min) increase blood flows and skin small vein pressures to the same levels as do the lower doses, yet the edema is three to four times greater with the higher doses. Under constant inflow conditions the same

large increases in limb weight are seen despite the fact that skin small vein pressures, and inferentially, capillary hydrostatic pressures , probably remain at control levels. Thus, with the higher doses of histamine and bradykinin, the pressure-independent effects of these autocoids are dominant in producing edema (28,52), whereas at the lower doses, the pressure-dependent increases in capillary hydrostatic pressure are important in the genesis of the edema formation.

Other agents have also been found to contribute to the inflammatory response. Among them are the prostaglandins. Prostaglandins are potent vasoactive agents having a wide range of actions and are synthesized from 20 - carbon polyunsaturated fatty acids. The effects of prostaglandins were first noted by Kurzrok and Lieb (54) in 1930 when they observed that uterine muscle strips would relax or contract when exposed to human semen. A few years later von Euler (103) reported that human and sheep seminal fluid extracts had potent stimulatory actions on smooth muscle and lowered arterial blood pressure in experimental animals. He demonstrated that the biological activity of the seminal fluid was associated with a lipid-soluble acid which he called prostaglandin. During the 1950's and early 1960's pioneering work by Bergstrom and Samuelsson (2) found that at least four major groups of prostaglandins existed; i.e., the E, F, D, and A groups. Two other groups, the thromboxanes (TXA_2) (37) and prostacyclins (PGI_2) (71) have recently been isolated.

The main precursor for these prostaglandins is arachidonic acid (5,8,11,14, eicosatrienoic acid) which has four double bonds and gives rise to prostaglandins with two double bonds. The arachidonic acid found in the cell membrane is derived from the diet, either from elongation and desaturation of the essential fatty acid linolenic acid found in vegetables, or from the arachidonic acid content of meats. Even very slight chemical or mechanical stimuli can activate the enzyme phospholipase A_2 (72) which releases arachidonic acid from membrane phospholipids. The enzyme complex known as cyclo-oxygenase or prostaglandin synthetase, which seems to be present in the membranes of all cells, converts the arachidonic acid into the unstable cyclic endoperoxide PGG_2. This substance is rapidly converted to yet another unstable cyclic endoperoxide, PGH_2. At this point the biochemical pathways diverge. Depending on the tissue in question different prostaglandins will be synthesized, probably due to the differential activity of certain enzymes in their respective tissues. PGE_2, a vasodilator and bronchodilator is formed from PGH_2, as is $PGF_{2\alpha}$ which is a venoconstrictor and bronchoconstrictor. It has been pointed out that there is a certain symmetry between the various prostaglandins. The PGE and PGF compounds have been shown to have opposing actions in most tissues (41). It has recently been shown in monkeys, chickens, and pigeons (94) that PGE_2 can be converted to $PGF_{2\alpha}$ by the enzyme PGE_2 9-Keto Reductase. It has been postulated that $PGF_{2\alpha}$ may be converted to PGE_2 as well, but as yet no evidence exists to support this theory.

Prostacyclin (PGI_2) is formed from PGH_2 in the walls of arteries and veins. PGI_2, which has a half-life of only two to three minutes (72), increases platelet adenyl cyclase activity. This results in increased cyclic-AMP levels and inhibition of platelet aggregation. PGI_2 prevents platelet aggregation in vivo and in vitro at concentrations of 1 ng/ml. Prostacyclin is also a vasodilator and is broken down rapidly into 6-oxo-$PGF_1\alpha$ which has only weak anti-aggregatory activity.

The symmetry of the system is preserved by thromboxane A_2 (TXA_2), another product of PGH_2. TXA_2 is found in the platelets and constricts blood vessels (37). It is metabolized to the inactive compound, thromboxane B_2, in approximately 30 seconds. TXA_2 decreases adenyl cyclase activity which decreases cyclic-AMP levels and promotes platelet aggregation. Thus PGI_2 and TXA_2 are in opposition to one another. It has been postulated by Moncada and Vane (72) that the delicate balance of these two prostaglandins is essential for proper homeostasis and that prostacyclin may one day be used in a clinical setting to prevent thrombosis.

Dihomo-gamma-linolenic acid (8,11,14 eicosatrienoic acid) contains three double bonds and gives rise to the 1-series of prostaglandins, i.e. those prostaglandins containing just a single double bond. The biochemical pathways involved here are similar to those of the 2-series discussed above. Cyclo-oxygenase converts dihomo-gamma-linolenic acid into the cyclic endoperoxide PGG_1 which rapidly converts to

PGH_1. PGH_1 gives rise to PGE_1 and $PGF_{1\alpha}$. The relationship between these two prostaglandins is similar to that of their 2-series counterparts.

The 1-series prostaglandins are not as prevalent as the 2-series prostaglandins and their physiologic importance is largely unknown. This is not to say that they have no role. PGE_1 has been shown to have high anti-aggregatory activity (77) due to its ability to increase cyclic-AMP levels(25), and can inhibit aggregation of human platelets (53) at a dose of 1 μg/ml. If the precursor dihomo-gamma-linolenic acid is administered, significant amounts of PGE_1 are synthesized in sheep vesicular gland (84) and the microsomes of bovine vesicular gland (112). Dihomo-gamma-linolenic acid competes with arachidonic acid for the cyclo-oxygenase of human platelets (111) and thus may effect the production of the 2-series prostaglandins.

In 1971 Vane (101) proposed that the anti-inflammatory action of aspirin arises from the inhibition of prostaglandin synthesis. This view has been supported by reports that aspirin can inhibit prostaglandin synthesis in human platelets (89) and semen (9). It is now known that low concentrations of aspirin-like drugs inhibit cyclo-oxygenase (72), the first enzyme in the arachidonic acid cascade, and thus inhibit the formation of the endoperoxides and all their subsequent products. Aspirin-like drugs have analgesic, antipyretic and anti-inflammatory properties, thus their ability to inhibit prostaglandin synthesis fits well with the belief

that prostaglandins are involved in the inflammatory process.

There is a great deal of evidence to implicate prostaglandins in inflammatory processes. Increased concentrations of prostaglandins, mainly PGE's, have been recovered from inflammatory exudates in man (14, 26, 76), dog (1), and rat (109). Anaphalaxis (75), tissue ischemia (16, 67), mechanical stimulation (15, 23), chemical inflammatory agents (110), and scalding injuries (1) all release prostaglandins, especially PGE's. PGE_1 induces migration of leukocytes (48) in concentrations of 1 μg/ml or greater. In man, 100 mg of PGE_2 causes pain when injected subdermally (10). PGE_1 also sensitizes pain receptors for subsequent infusions of histamine or bradykinin (17); i.e. concentrations of these autocoids which did not ellicit pain in untreated skin were very painful after treatment with PGE_1. In cats and rabbits (70) PGE_1 induces a dose-dependent pyresis with doses up to 10 μg. Thus, prostaglandins, especially PGE_1, reproduce many of the cardinal signs of inflammation.

PGE_1 can also affect net fluid filtration. Weiner and Kaley (104) have shown that in the rat mesocecum preparation in vivo, topical applications of PGE_1 in doses of 1 to 10 μg/ml, produces a dilation of metarterioles, precapillary sphincters and venules concomitant with an augmentation of capillary blood flow leading to increased fluid filtration. However considerable debate exists among investigators concerning the relative capacity to which PGE_1 is involved in the movement of fluid and macromolecules across

the microvascular membrane. The different experimental techniques employed, different PGE_1 doses and routes of administration, species variability as well as other factors, may have contributed to the conflicting data found in the literature.

Many investigators have employed the vital dye technique which involves the injection of a vital dye (e.g. Evans Blue) into the systemic circulation of the experimental animal and the subsequent measurement of the amount of dye leakage at the site of administration of the prostaglandin The injection site is usually skin. The dye is assumed to bind to circulating plasma proteins and will therefore be transported with filtered proteins. Changes in microvascular permeability 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 tissue and measuring the concentration of the dye spectrophotometrically. A modification of the method involves the intravenous injection of readio-actively labelled albumin and the subsequent measurement of the radioactivity of the extravasated protein at the site of prostaglandin injection. However, the vital dye technique has three major drawbacks. 1) The extent to which the dye binds to the plasma protein is variable and uncertain. 2) The concentrations of PGE_1 at the site of leakage are not well controlled. Depending on the rate of penetration and diffusion of PGE_1 in the tissue,

concentration differences could occur. 3) The injected PGE_1 would only have short-lived effects owing to the rapid metabolism of the agent by degradation enzymes located in the tissue. Thus it is not surprising that there is a diversity of opinion among investigators utilizing this technique.

In 1963, Horton, using the vital dye procedure, reported that PGE_1 increased permeability in guinea pig skin (40). Since that time, other investigators (11,12,19,20,47, 74) have also shown increases in vascular permeability in response to PGE_1 in both rat and guinea pig. Kaley (47) noted increases in permeability, especially in venules, while Panagides and Tolman (74) observed a direct relationship between the dose of PGE_1 used and the average diameter of the lesion. Another group of investigators utilizing the vital dye technique, have reported that while PGE_1 itself does not alter vascular permeability, it potentiates the effects of bradykinin and histamine (17,57,100,107,108). This potentiation can be inhibited by indomethacin pretreatment (7).

Using the more reliable hamster cheek pouch preparation (as described above), Svensjo (95) and Joyner, Svensjo and Arfors (45) have shown that PGE_1, in doses ranging from 1 to 100 ng, increases the leakage of FITC-dextran from postcapillary venules in a manner similar to that of histamine and bradykinin; i.e. via formation of interendothelial venular gaps. The number of leakage sites

was linearly related to the amount of PGE_1 administered, but PGE_1 was found to be at least ten times less effective than bradykinin in producing FITC-dextran leakage sites. PGE_1 also potentiated the response to bradykinin (95). Some investigators (108) have suggested that PGE_1 only increases protein efflux due to its ability to increase blood flow. However, when terbutaline, a β-agonist, was administered together with PGE_1, arteriolar blood flow was increased to levels even greater than those achieved with PGE_1 alone, while the number of leakage sites decreased. Panagides and Tolman (74) also noted that isoproterenol, a β-agonist, inhibited the PGE_1 evoked increase in permeability, although it too increases blood flow. Thus it appears that the increases in permeability attributable to PGE_1 are not due solely to increases in blood flow. The antagonism of the increased macromolecular permeability by β-agonist has been postulated to be due to the ability of the β-agonists to relax the contractile elements of the venular endothelial cells, and thus close the interendothelial gaps (97).

The evidence discussed above suggesting a role for PGE_1 in inflammation has been from studies conducted in rats, guinea pigs, and hamsters. So far, the few studies conducted in dogs have not provided substantial evidence to support this contention. Rosenthale (83) injected PGE_1 into the dog knee joint and it became inflamed. Greenberg and Sparks (27) studied the effects of PGE_1 on the vascular resistance, capacitance and capillary filtration coefficient

in the isolated canine hindlimb by measuring the total venous outflow of the popliteal vein and the changes in pleythysomographic recordings in an enclosed hindlimb. They found that the intravenous administration of 0.01 to 10 μg/min of PGE_1 increases blood flow and vascular volume which is evidence for an increase in vascular capacitance. PGE_1 also increased the capillary filtration coefficient, indicating either an increase in capillary permeability or an increase in capillary surface area due to increased relaxation of precapillary sphincters. They postulated that the predominant effect of PGE_1 infusion was a decreased precapillary sphincter tone (or increased relaxation), since there was no net filtration associated with the increased capillary filtration coefficient, and 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. Daugherty (13) studied the effects of PGE_1 on skin and skeletal muscle vascular beds in the dog forelimb and observed little effect on filtration. In these studies, PGE_1 (2 to 10 μg/min) infused into the brachial artery produced large increases in both skin and muscle blood flows whereas total vascular resistance in skin and muscle decreased. However, 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 above studies did not measure protein fluxes directly nor did they study the effects of a wide dose range of PGE_1. It has been shown (18) that large amounts of PGE_1 can be inactivated in a single passage through the lungs and liver (39). Thus the doses used in the above studies may have been insufficient. The duration of the infusions were also very short.

Recent work by Joyner (43) afforded a direct measurement of protein fluxes in the hindlimb of the dog. Subcutaneous injections of 0.01 μg of PGE_1 failed to show increases in vascular permeability as ascertained from the protein concentration of collected lymph. Joyner proposed that PGE_1 enhanced transcapillary fluid movement, as evidenced by an increased lymph flow, primarily by its vasodilatory properties. But Joyner himself raised the issue as to the accuracy of the study. Lymph samples were collected at 30 minute intervals and, if the increase in permeability was of a short duration, as was likely with the low doses employed, the permeability effects could have been masked. Moreover, in the same study, when the vital dye technique was used in the dog, PGE_1 (10-20 μg administered intradermally) produced bluing in 71% of the trials.

Recently, a systematic study of the effects of a wide dose range of PGE_1 on fluid filtration and macromolecular efflux was conducted in our laboratory (58). Sixty minute local intra-arterial infusions of PGE_1 (2 to 16 μg/min) into canine forelimbs perfused either naturally or at constant

inflow produced profound vasodilation and increases in lymph total protein concentration, but only slight dose-independent increases in lymph flow. PGE_1 (16 or 32 μg/min i.a.) also produced marked increases in forelimb weight owing to edema formation. Following pretreatment with indomethacin (5 mg/kg, i.v.), PGE_1 produced vasodilation and increases in lymph total protein concentration similar to those produced in the absence of indomethacin, but now produced marked increases in lymph flow rate under both natural and constant inflow conditions.

Based on these data in the dog and similar findings in other species, it appears that PGE_1 is likely to have a role in inflammation and increases vascular permeability in a manner similar to that of histamine and bradykinin. Work from this laboratory (29,59,65) and others (98) has demonstrated that catecholamines possess the unique ability to antagonize the protein efflux produced by histamine and bradykinin owing to a direct action on the microvascular membrane which counteracts that of histamine and bradykinin. This antagonism is independent of changes in blood flow, microvascular pressure and perfused surface area (29,51,65). It is now known that other agents possess the ability to antagonize or potentiate the protein efflux produced by these agents (29,59,78). In this study, the effects of catecholamines and a variety of other natural and synthetic agents were studied to determine if they antagonize or potentiate the direct actions of PGE_1 on the microvascular membrane.

METHODS

Thirty-five mongrel dogs of either sex, having an average weight of 27 kilograms (range: 17-45 kg), were anesthesized with sodium pentobarbital (30 mg/kg) and ventilated with room air using a Harvard respiratory pump.

In these studies, the intact canine forelimb perfused at constant inflow was used to collect skin lymph and measure lymph protein concentration. The surgical procedure consisted of using an electrocautery to make small incisions superficial to the brachial artery, cephalic vein (5 cm below the elbow), and second superficial dorsal metacarpal vein in the right forelimb. A side branch of the brachial artery, a lymph vessel, and a vein, respectively, were isolated. After administering 10,000 U.S.P. units of heparin intravenously, these vessels were cannulated in an upstream direction with polyethylene tubing for monitoring brachial artery perfusion pressure, lymph collection, and small skin vein pressure, respectively. The side branch of the brachial artery was cannulated with PE-50 tubing while the small vein in the paw was cannulated with PE-60 tubing. The cannulated small vessel acts as an extension of the catheter, and thus the catheter measures pressure in the vessels to which the cannulated vessel connects (30, 33, 34, 86), which is

representative of all small venous vessels in the skin of the paw.

The lymph vessels in the area of the cephalic vein below the elbow drain forelimb skin and paw (36,69). Two or three were usually tied centrally and one of them was cannulated distally with a 10 cm length of PE-10 tubing which had been beveled at the cannulating end. The walls of these vessels were quite substantial requiring that they be punctured with a 22 gauge needle prior to cannulation.

The brachial artery was then isolated, tied off and transected about 5 cm proximal to the side branch which had been cannulated. Blood was obtained from a cannula inserted into the femoral artery and pumped at a controlled flow into the transected brachial artery. A Sigmamotor pumb (Model T68H, Sigmamotor Inc., Middleport, N.Y.) was used to keep inflow constant at a value which produced a perfusion pressure similar to aortic pressure. Aortic pressure was monitored via a cannula in the left carotid artery.

All pressures were monitored with Statham pressure transducers (Model P23Gb, Statham Instruments, Inc., Oxnard, California), connected to a direct writing oscillograph (Model 7754A, Hewlett-Packard Co., Palo Alto, California).

Lymph was collected at 10 minute intervals in miniature 0.3 ml graduated cylinders, constructed from plastic pipettes. Forelimb small skin vein pressure, aortic pressure, and brachial artery perfusion pressure were continuously monitored and recorded at the end of each 10 minute period.

After two consecutive control periods local (intra-arterial) administration of PGE_1 and/or other drugs was begun by infusing directly into the circuit behind the Sigmamotor pumb. Arterial blood samples (5 ml) for measuring hematocrit and plasma proteins were drawn from the cannula monitoring aortic pressure. Samples were taken five minutes before the local infusions of drugs began, as well as at 30 minute intervals throughout the experiment. The protein concentration of the lymph and plasma samples were analyzed by the modified Biuret reaction (51). The samples were read spectrophotmetrically in grams percent with an ACCU-STAT Blood Chemistry Analyzer (Clay Adams, Model 2000) which had been calibrated with samples known protein concentrations. Lymph protein transport was calculated as follows: Lymph Protein Transport = (10)(Lymph Flow Rate)(Lymph Protein Concentration).

PGE_1 was obtained from the Upjohn Company, Kalamazoo, Michigan (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 up using normal saline as the solvent, and the PGE_1 was then infused into the animal at a rate of 16 μg/min for 60 minutes.

The following drugs were also used in this study and prepared in normal saline: 1) isoproterenol hydrochloride, 3 μg/min infused for 60 minutes, Winthrop Laboratories; 2) vasopressin, 0.8 Pressor Units/min infused for 60 minutes, Calbiochem Co., Inc.; 3) angiotensin II, 2 μg/min infused for 60 minutes, Sigma Chemical Co.; 4) methylprednisolone

(Solu-Medrol), 15 μg/min infused for 60 minutes, Upjohn Co.; 5) serotonin creatinine sulfate, 7 μg base/min infused for 60 minutes, Sigma Chemical Co.. All drugs were administered intra-arterially at a delivery rate of 0.2 ml/min with a Harvard infusion/withdrawal pump. Control infusions of ethanol alone, in the same preparation, produced no significant changes in any of the measured parameters.

At the conclusion of the experiment the animals were sacrificed. The right and left forelimbs were then severed approximately 2 cm above the humeral condyle, and the brachialis, biceps, and triceps muscles were carefully dissected down to their tendons of insertions on the ulnar and radial tuberosities. Great care was taken to insure that the limbs were always severed at the same points on the humeral condyle. The limbs were then exsanguinated and weighed. Limb weights (experimental vs. contralateral control) were compared by using a paired t test. Control infusions of ethanol alone, in the same preparation, produced no significant changes in limb weights, thus neither the ethanol vehicle nor the trauma produced by surgery were responsible for the significant increases in limb weights seen in dogs with significantly increased lymph total protein concentrations. Moreover, when lymph total protein concentration did not significantly increase, the experimental and contralateral control limb weights were always very similar. Thus, this method is a valid means whereby changes in limb weights may be detected. All other data were statistically analyzed by Analysis of Variance (Randomized Complete Block Design) and the means compared to control by the Least Significant Difference Test (93).

RESULTS

Table 1

In limbs perfused at constant inflow, intra-arterially infused PGE_1 (16 μg/min) produced a moderate increase in lymph total protein concentration ($p<.01$). Lymph flow rate increased slightly but not significantly ($p>.05$) by the 20 minute sample period and then waned. There was a tendency for a slight but insignificant ($p>.05$) increase in lymph protein transport. Limb weights were significantly greater ($p<.01$) in the experimental limbs when compared to the contralateral control limbs. Plasma protein concentrations were not changed while the hematocrits were significantly increased ($p<.01$). Systemic arterial pressure increased significantly ($p<.01$) and remained elevated throughout the infusion period. Perfusion pressure decreased markedly ($p<.01$) and small skin vein pressure increased significantly but minimally ($p<.01$) toward the end of the infusion period.

Table 2

Table 2 shows the effects of isoproterenol (3 μg/min, i.a.) infused concomitantly with PGE_1 (16 μg/min, i.a.) into limbs perfused at constant inflow. Lymph flow rate, lymph protein concentration and lymph protein transport were unchanged. Systemic arterial pressure and small skin vein

pressure were not significantly altered. Perfusion pressure was markedly decreased ($p<.01$) for the duration of the infusion period. Plasma protein concentrations were not changed while the hematocrits were significantly increased ($p<.01$).

Table 3

No changes in lymph flow rate or lymph protein transport were seen when vasopressin (0.8 Pressor Units/min, i.a.) was infused concurrently with PGE_1 (16 μg/min, i.a.) in forelimbs perfused at constant inflow. Lymph total protein concentration minimally decreased ($p<.01$) and then returned to control levels by the end of the infusion period. The weights of the experimental and control limbs were not significantly different. Systemic arterial pressure was significantly increased ($p<.01$) at the start of the infusion period and then waned, returning to control levels. Perfusion pressure and small skin vein pressure were unchanged. Plasma protein concentrations and hematocrits were not significantly different from control.

Table 4

In limbs perfused at constant inflow, angiotensin II (2 μg/min, i.a.) in combination with PGE_1 (16 μg/min, i.a.) did not change lymph flow rate, lymph total protein concentration or lymph protein transport. The weights of the perfused limbs were moderately greater than those of the contralateral control limbs ($p<.05$). Systemic arterial pressure increased at the onset of the infusion period and gradually waned. Perfusion pressures significantly decreased ($p<.01$) and small skin vein pressure remained unchanged. Plasma

protein concentrations were not changed while the hematocrits were significantly increased ($p<.01$).

Table 5

No changes in lymph flow rate, lymph total protein concentration or lymph protein transport were seen when methylprednisolone (15 μg/min, i.a., CF) was infused concurrently with PGE_1 (16 μg/min, i.a., CF). The weights of the experimental and control limbs were not significantly different. Systemic arterial pressure increased ($p<.01$) while perfusion pressure decreased markedly ($p<.01$). Small skin vein pressure remained unchanged. Plasma protein concentrations were not changed while the hematocrits increased significantly ($p<.01$).

Table 6

Table 6 shows the effects of serotonin (7 μg base/min, i.a.) infused concomitantly with PGE_1 (16 μg/min, i.a.) into a limb perfused at constant inflow. Lymph flow rate increased slightly but not significantly ($p>.05$) by the 20 minute sample period and then waned. Lymph protein concentration increased significantly and remained elevated throughout the infusion period. There was a tendency for a slight but insignificant ($p>.05$) increase in lymph protein transport. The weights of the perfused limbs were markedly greater than those of the contralateral control limbs. Systemic arterial pressure decreased minimally but not significantly while perfusion pressure was moderately decreased. Small skin vein pressure increased moderately. Plasma protein

concentrations were unchanged while the hematocrits increased significantly.

Thus, PGE_1, and concomitant infusions of serotonin and PGE_1, produced significant increases in both lymph protein concentration and limb weight. Of the other four agents, only angiotensin II, by producing a moderate increase in limb weight, significantly altered either lymph total protein concentration or limb weight. Generally, systemic arterial pressure and hematocrit were significantly increased. Lymph flow rate, lymph protein transport, and plasma protein concentration remained unchanged relative to control in all groups.

Table 1. Effects of PGE_1 (16 μg/min i.a., CF) on lymph flow and protein transport, lymph and plasma protein concentrations, vascular pressures, hematocrit, and limb weights. (n=7)

	Control		Infusion Period					
	-10	0	10	20	30	40	50	60
Systemic Arterial Pressure (mmHg)	124	124	139*	139*	141*	141*	141*	141*
Perfusion Pressure (mmHg)	109	110	55*	56*	56*	55*	56*	57*
Skin Small Vein Pressure (mmHg)	11	12	12	13	13	13x	13x	13x
Lymph Flow Rate (ml/10 min)	.01	.02	.04	.07	.06	.03	.02	.02
Lymph Total Protein (gms %)	2.6	2.8	2.7	3.1	3.4*	3.3x	3.4*	3.2x
Lymph Protein Transport (mg/10 min)	.38	.44	1.31	2.82	2.53	1.04	.85	.84
Plasma Protein (gms %)		5.5			5.7			5.7
Hematocrit		39			44*			45*

	Control	Experimental	Difference
Limb Weight after 60 min infusion (gms)	665.3 ±28.9	687.9 ±29.8	22.6** ±3.7

*=P < .01 relative to zero time
**=P < .01 relative to contralateral control limb weight
x=P < .05 relative to zero time

Table 2. Effects of PGE_1 (16 μg/min i.a., CF) and isoproterenol (3 μg/min i.a., CF) on lymph flow and protein transport, lymph and plasma protein concentrations, vascular pressures, and hematocrit. (n=5)

	Control		Infusion Period					
	-10	0	10	20	30	40	50	60
Systemic Arterial Pressure (mmHg)	133	132	127	133	136	140	140	140
Perfusion Pressure (mmHg)	114	117	58*	65*	65*	67*	66*	66*
Skin Small Vein Pressure (mmHg)	15	17	12	11	11	11	12	12
Lymph Flow Rate (ml/10 min)	.03	.02	.04	.03	.02	.02	.02	.01
Lymph Total Protein (gm %)	2.4	2.5	2.5	2.4	2.4	2.3	2.4	2.4
Lymph Protein Transport (mg/10 min)	.69	.52	.85	.63	.50	.49	.44	.29
Plasma Protein (gm %)		6.0			6.0			6.1
Hematocrit		41			47*			49*

*=P < .01 relative to zero time

x=P < .05 relative to zero time

Table 3. Effects of PGE_1 (16 μg/min i.a., CF) and vasopressin (0.8 Pressor Units/min i.a., CF) on lymph flow and protein transport, lymph and plasma protein concentrations, vascular pressures, hematocrit, and limb weights. (n=5)

	Control		Infusion Period					
	-10	0	10	20	30	40	50	60
Systemic Arterial Pressure (mmHg)	118	122	142*	137x	131	127	122	121
Perfusion Pressure (mmHg)	103	109	99	98	112	114	112	109
Skin Small Vein Pressure (mmHg)	11	12	13	11	11	11	11	11
Lymph Flow Rate (ml/10 min)	.01	.02	.01	.01	.01	.01	.02	.02
Lymph Total Protein (gm %)	2.9	2.8	2.5	2.3*	2.5x	2.4	2.5	2.4
Lymph Protein Transport (mg/10 min)	.35	.40	.29	.31	.34	.28	.38	.43
Plasma Protein (gm %)		5.9			6.0			6.2
Hematocrit		40			42			43

	Control	Experimental	Difference
Limb Weight after 60 min infusion (gms)	693.1 ±50.0	693.2 ±52.0	00.1 ±3.1

*=P < .01 relative to zero time

x=P < .05 relative to zero time

Table 4. Effects of PGE_1 (16 μg/min i.a., CF) and angiotensin II (2 μg/min i.a., CF) on lymph flow and protein transport, lymph and plasma protein concentrations, vascular pressures, hematocrit, and limb weights. (n=7)

	Control		Infusion Period					
	-10	0	10	20	30	40	50	60
Systemic Arterial Pressure (mmHg)	134	135	156*	150*	145^{x}	144	141	140
Perfusion Pressure (mmHg)	122	124	78*	72*	72*	71*	71*	71*
Skin Small Vein Pressure (mmHg)	11	10	12	12	11	11	11	11
Lymph Flow Rate (ml/10 min)	.01	.01	.02	.03	.02	.02	.02	.02
Lymph Total Protein (gm %)	2.7	2.7	2.9	3.1	3.3	3.2	3.2	3.0
Lymph Protein Transport (mg/10 min)	.27	.27	.51	1.48	.88	.86	.78	.55
Plasma Protein (gm %)		6.0			6.2			6.0
Hematocrit		41			47*			46*

	Control	Experimental	Difference
Limb Weight after 60 min infusion (gms)	587.7 ±34.8	600.1 ±33.0	12.5^{xx} ±4.4

*=P < .01 relative to zero time

x=P < .05 relative to zero time

xx=P < .05 relative to contralateral control limb weight

Table 5. Effects of PGE_1 (16 μg/min i.a., CF) and methylprednisolone (15 μg/min i.a., CF) on lymph flow and protein transport, lymph and plasma protein concentrations, vascular pressures, hematocrit, and limb weights. (n=5)

	Control		Infusion Period					
	-10	0	10	20	30	40	50	60
Systemic Arterial Pressure (mmHg)	122	126	144*	143*	142*	140^x	139^x	138
Perfusion Pressure (mmHg)	117	118	50*	51*	52*	53*	53*	52*
Skin Small Vein Pressure (mmHg)	12	12	12	13	13	13	13	13
Lymph Flow Rate (ml/10 min)	.02	.02	.01	.02	.02	.01	.01	.01
Lymph Total Protein (gm %)	2.5	2.7	2.5	2.6	2.7	2.8	2.9	2.7
Lymph Protein Transport (mg/10 min)	.59	.60	.30	.38	.46	.37	.29	.39
Plasma Protein (gm %)		5.1			5.4			5.3
Hematocrit		37			44*			42^x

	Control	Experimental	Difference
Limb Weight after 60 min infusion (gms)	774.0 ±49.0	793.8 ±45.6	19.8 ±9.4

*=P < .01 relative to zero time x=P < .05 relative to zero time

Table 6. Effects of PGE_1 (16 μg/min i.a., CF) and serotonin (7 μg base/min i.a., CF) on lymph flow and protein transport, lymph and plasma protein concentrations, vascular pressures, hematocrit, and limb weights. (n=5)

	Control		Infusion Period					
	-10	0	10	20	30	40	50	60
Systemic Arterial Pressure (mmHg)	129	130	119	114	113	113	115	118
Perfusion Pressure (mmHg)	118	118	79*	81*	83*	84*	87*	88*
Skin Small Vein Pressure (mmHg)	13	13	14	15[x]	17*	17*	17*	17*
Lymph Flow Rate (ml/10 min)	.01	.02	.02	.06	.03	.03	.03	.04
Lymph Total Protein (gm %)	2.7	2.6	2.8	3.3[x]	3.7*	4.1*	3.8*	3.7*
Lymph Protein Transport (mg/10 min)	.27	.45	.58	2.03	1.36	1.10	1.29	1.34
Plasma Protein (gm %)		6.0			6.2			5.9
Hematocrit		39			42[x]			43*

	Control	Experimental	Difference
Limb Weight after 60 min infusion (gms)	592.8 ±37.6	628.3 ±36.5	35.5** ±5.9

*=P < .01 relative to zero time
x=P < .05 relative to zero time
**=P < .01 relative to contralateral control limb weight

DISCUSSION

In forelimbs perfused at constant inflow (Table 1), PGE_1 (16 μg/min i.a.) infused for 60 minutes significantly increased lymph total protein concentration and limb weight. Small skin vein pressure remained unchanged relative to control until min 40, by which time lymph protein concentration had significantly increased. Thus the edema produced by PGE_1 in a constantly pump-perfused forelimb is not attributable to an increased microvascular pressure, but to an increased net fluid filtration subsequent to a decrease in the transmural colloid osmotic pressure gradient owing to an increase in microvascular permeability to macromolecules.

This conclusion is supported by the work of Svensjo (95) and Joyner et al. (45). These investigators have shown that PGE_1 increases the leakage of FITC-dextran (M.W. 145,000) from postcapillary venules in the hamster cheek pouch preparation in a manner similar to that of histamine and bradykinin (99); i.e. via formation of interendothelial cell venular gaps. These gaps form in venules 9 to 16 μm in diameter, owing to the active contraction of endothelial cell filaments. Thus, PGE_1 increases macromolecular efflux by increasing vascular permeability in the venular section of the microvasculature.

It has recently been suggested by Williams and Peck (108) that PGE_1 has no direct effect on permeability but simply increases blood flow and thereby potentiates the leakage of macromolecules produced by histamine and bradykinin. However, in this study PGE_1 has been shown to increase protein efflux under constant inflow conditions which would not allow any increase in total blood flow.

When the β-agonist isoproterenol (3 μg/min i.a.) was infused concomitantly with PGE_1 (Table 2), it failed to increase lymph total protein concentration. Joyner et al. (45) has shown that terbutaline, a β-agonist, markedly reduces the macromolecular leakage evoked by PGE_1 in the hamster cheek pouch preparation. The edemogenic effects of histamine (29, 65) and bradykinin (59) are also antagonized by isoproterenol. Although isoproterenol markedly increases nutritional flow (82), the simultaneous infusion of isoproterenol with these autocoids intra-arterially into the canine forelimb prevents the marked increase in protein efflux usually produced by histamine and bradykinin (59,65). Norepinephrine also antagonizes the increase in microvascular permeability produced by these autocoids (29,59,65). This antagonism of the histamine and bradykinin protein efflux is completely independent of changes in blood flow, microvascular pressure, and perfused surface area (29,51,65). Instead, it reflects a direct action on the microvascular membrane which counteracts that produced by histamine and bradykinin. This conclusion is supported by morphological data which demonstrates that the marked increase

in venular leakage sites of FITC-dextran (M.W. 145,000) evoked by histamine and bradykinin is greatly reduced by the simultaneous application of catecholamines (98).

Similarly, the antagonism of the PGE_1-induced protein efflux by the β-agonists is completely independent of changes in blood flow, microvascular pressure, and perfused surface area. Both PGE_1 and isoproterenol are potent vasodilators which are expected to increase surface area and, under constant inflow conditions, fail to decrease skin small vein pressure and, inferentially, microvascular pressure. Thus, as with histamine and bradykinin, isoproterenol and terbutaline may physiologically antagonize the action of PGE_1, presumably by causing the relaxation of the actinomyosin-like filaments of the venular endothelial cells, thus closing the interendothelial gaps.

It is interesting to note that, like histamine and bradykinin, PGE_1 only produces increases in macromolecular permeability in doses exceeding those necessary to produce significant vasodilation. Subcutaneous injections of PGE_1 in doses as low as 10 ng/ml produce profound erythmea (26), and Daugherty (13) found that only 1-2 μg infused intra-arterially in the canine forelimb preparation produced maximal vasodilation. Thus, as with some of the other ubiquitous prostaglandins, PGE_1 may have a physiological function, such as local regulation of blood flow, in addition to its role as a mediator of inflammation.

The increase in macromolecular permeability produced by PGE_1 is considerably less than that produced by the other two autocoids. This has been noted by other investigators in the rat (102) and hamster (45). In the present study PGE_1 produced an average maximal increase in lymph total protein concentration of approximately 1 gm %, whereas high concentrations of histamine and bradykinin increase the total protein concentration of lymph to values approaching that of plasma (29,59). The more modest increases in vascular permeability attained with large doses of PGE_1 suggest that the inability of other investigators (13,27) to demonstrate an effect of PGE_1 on macromolecular permeability in the dog may be due to the dose studied. It has been shown that large amounts of PGE_1 can be inactivated in a single passage through the lungs and liver (18,39). In addition, these investigators failed to directly measure protein efflux, and therefore it is possible that the quantitatively smaller changes in fluid flux were not easily measurable with the techniques they employed. However, results from numerous other studies (11,12, 19,20,45,74,95) in rats, guinea pigs, and hamsters, are consistent with the findings of this study.

PGE_1 differs from histamine and bradykinin in yet another respect. Despite the increase in lymph total protein concentration, lymph flow rate (Table 1) does not significantly increase as it does with these other two autocoids (35,52, 64,65). Changes in lymph flow rate have traditionally been considered to reflect changes in transvascular fluid fluxes,

and increases in net fluid filtration generally produce roughly proportional increases in lymph flow rate. For instance, with low doses of bradykinin (0.8 μg base/min) infused locally intra-arterially into canine forelimbs perfused at constant inflow, a comparable increase in lymph total protein concentration (e.g. 1 gm %) produced a 10 to 15-fold increase in lymph flow rate (59). In the present study, only a 3 to 6-fold increase in lymph flow rate was observed (Table 1). Since drainage of the limb was somehow impeded it would be expected that for a comparable increase in lymph total protein concentration, the PGE_1 limbs would be more edematous than the low-dose bradykinin limbs. Indeed, the PGE_1 limbs were 60% heavier than the bradykinin limbs were, when comparable increases in lymph total protein concentration occurred (59).

Following pretreatment with indomethacin (5 mg/kg, i.v.), PGE_1 (16 μg/min) infused locally intra-arterially into canine forelimbs perfused at constant inflow (58) produced a significant increase in lymph total protein concentration similar to that produced in the absence of indomethacin, but under these same conditions produced a 10 to 15-fold increase in lymph flow rate as would be expected from the bradykinin data. Thus it appears that the PGE_1 causes the liberation of some endogenous substance which constricts the prenodal lymph vessels impeding drainage of the interstitium and compounding the edema. Since indomethacin blocks this constriction and restores normal lymph drainage, it is proposed that the endogenous agent involved is another prostaglandin. It is interesting to speculate as to the identity of this agent. Both

thromboxane A_2 and $PGF_{2\alpha}$ can constrict vessels, and $PGF_{2\alpha}$ has been shown to antagonize the increases in vascular permeability elicited by PGE_1 (12,74). This agent may also have contributed to the unusual variability of the data found in the literature. Additional experimentation is needed to resolve these issues.

Other substances which are released into the circulation during hemorrhage have been found to antagonize the protein efflux evoked by histamine and bradykinin (29,59,78). The simultaneous infusion of vasopressin (0.8 Pressor Units/min i.a.) with PGE_1 (Table 3) prevented the increase in lymph total protein concentration and limb weights, as it prevented the increase in vascular permeability induced by histamine and bradykinin. However, angiotensin II (2 μg/min i.a.), which does not block the edemogenic effect of bradykinin, does block the increase in lymph total protein concentration produced by PGE_1 (Table 4). Apparently the 2 μg/min dose of angiotensin II was near the effective threshold dose for antagonizing the PGE_1-induced protein efflux, as two of the seven dogs showed increases in lymph total protein concentration and limb weight. Hence, there was a slight but significant increase in limb weight for this group. However, lymph total protein concentration was not significantly increased indicating an inhibition of the protein efflux by angiotensin II.

Low doses of methylprednisolone (15 μg/min i.a.), a glucocorticoid, (Table 5) also block the edemogenic action

of PGE_1 although only a much larger dose (30 mg/kg i.v.) is effective in preventing the increases in macromolecular permeability elicited by histamine and bradykinin (29,78). Seeing that the more modest increases in vascular permeability elicited by PGE_1 were also more susceptible to inhibition by other vasoactive substances than histamine or bradykinin were, serotonin (7 μg base/min i.a.) was infused simultaneously with PGE_1. This dose did not block the increase in lymph total protein concentration or limb weight. Lymph flow increased slightly but not significantly, to levels similar to those achieved when PGE_1 was infused alone. A higher dose of serotonin (15 μg base/min i.a.) does prevent the edemogenic effects of histamine and bradykinin (29,78), and so it is likely that the dose of serotonin used in this study was simply insufficient to inhibit the actions of PGE_1. Further experimentation is needed to determine whether serotonin, in higher concentrations, in fact inhibits the edemogenic effects of PGE_1.

The antagonism of the PGE_1-induced protein efflux demonstrated in this study by a number of agents cannot be simply attributed to decreases in blood flow and/or perfused surface area. No changes in blood flow were possible as all experiments occurred under constant inflow conditions. Isoproterenol increases perfused surface area (82) and yet this catecholamine antagonized the edemogenic actions of PGE_1. Changes in vascular pressures were not responsible either. Methylprednisolone blocked the PGE_1 response even while perfusion pressure was extremely low, while serotonin did not

block the development of edema with perfusion pressures that were relatively high. Vasopressin antagonized the PGE_1 effect but produced no change in perfusion pressure. Skin small vein pressures and, inferentially microvascular pressures, were unchanged in all groups except for a small increase in the serotonin group due to this agent's ability to constrict skin small veins (24). Thus, it must be concluded that the antagonism of the PGE_1-induced protein efflux by isoproterenol, vasopressin, and low doses of methylprednisolone represents a direct action of these agents on the microvascular membrane. The antagonism by angiotensin II may be due to a direct action on the microvascular membrane, or may be due to an indirect effect owing to its ability to release catecholamines from the adrenal medulla and adrenergic terminals (24).

Local intra-arterial infusions of PGE_1 (16 μg/min), PGE_1 and vasopressin (0.8 Pressor Units/min), PGE_1 and angiotensin II (2 μg/min), and PGE_1 and methylprednisolone (15 μg/min) produced significant increases in systemic arterial pressure. Local intra-arterial infusions of PGE_1 and isoproterenol (3 μg/min) and PGE_1 and serotonin (7 μg base/min) produced no change in systemic arterial pressure. Under these conditions all groups showed significant increases in hematocrit except one. Vasopressin did not produce a significantly increased hematocrit. These results can best be explained if PGE_1 causes an increase in sympatho-adrenal activity. Others have shown that PGE_1 causes a reflex increase in systemic arterial pressure and heart rate (50,73)

which can be blocked by pretreatment with ganglionic blocking agents. PGE_1 does not have a direct action on the adrenal medulla (68) and studies have shown that PGE_1 inhibits the release of norepinephrine from adrenergic terminals (38,46,85,105). The pressor response does not occur in dogs with their adrenal glands excluded from the circulation (50). Thus it is suggested that the increases in systemic arterial pressures and hematocrits may be due to catecholamine release from the adrenal medulla. However, we have no data to suggest what the stimulus for the sympathoadrenal discharge might be as mean arterial pressure did not fall, and PGE_1 does not cause a release of adrenal catecholamines. Histamine produces a similar vasodilation, and yet no increase in systemic arterial pressure occurs although it causes a direct release of catecholamines. Perhaps large doses of PGE_1 have a direct stimulatory effect on central vasomotor neurons which produce an increase in sympathetic outflow. Further experimentation is needed to resolve this issue.

In general, the present study shows that PGE_1 in large doses increases macromolecular permeability in a manner similar to that of histamine and bradykinin. This data correlates well with morphological data available from Svensjo's laboratory. The increases in vascular permeability produced by PGE_1 are more modest than those produced by histamine and bradykinin, and are susceptible to antagonism by a greater number of other vasoactive agents. None of the

agents studied measurably potentiated the protein efflux produced by PGE_1. Lymph flow rate failed to increase significantly despite the increase in vascular permeability induced by PGE_1. Serotonin did not block the increased lymph total protein concentration produced by PGE_1, and it too failed to significantly increase lymph flow rate. Local intra-arterial infusions of PGE_1 (16 μg/min) produced significant increases in systemic arterial pressures and hematocrits, suggesting that PGE_1 causes an increase in sympatho-adrenal activity, perhaps via a direct action on central vasomotor neurons.

SUMMARY AND CONCLUSIONS

The canine forelimb perfused at a constant inflow was used to examine the effects of local intra-arterial infusions of PGE_1, and to determine if catecholamines and other vasoactive agents antagonized or potentiated the actions of PGE_1 on the microvascular membrane. The results indicate that large doses of PGE_1 (16 μg/min i.a.) produced profound vasodilation and increased macromolecular permeability by mechanisms similar to that of histamine and bradykinin, as evidenced by marked increases in lymph protein concentration and forelimb weight owing to edema formation. This data correlates well with morphological data from other laboratories. Lymph flow rate did not change relative to control.

The increases in lymph protein concentration and forelimb weight elicited by PGE_1 are more modest than those produced by histamine and bradykinin and are susceptible to inhibition by isoproterenol, vasopressin, angiotensin II, and methylprednisolone. The antagonism of the PGE_1-induced protein efflux by these agents is independent of changes in blood flow and vascular pressures, and may represent a direct action of these agents on the microvascular membrane.

In contrast to the other four agents, concomitant infusions of serotonin and PGE_1 produced increases in lymph

protein concentration, lymph flow rate, and forelimb weight similar to those achieved with PGE_1 alone. Thus serotonin failed to antagonize the PGE_1-induced protein efflux. None of the agents studies potentiated the protein efflux produced by PGE_1. Local intra-arterial infusion of PGE_1 produced significant increases in systemic arterial pressures and hematocrits, suggesting that PGE_1 causes an increase in sympatho-adrenal activity. In summary, these results indicate that PGE_1 may function as a mediator of inflammation in the dog, and that some natural or synthetic chemical agents can antagonize its effect on microvascular permeability.

APPENDICES

APPENDIX

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 statistical significance.

The data in the appendix tables corresponds to the mean values in Tables 1-6 as follows:

Table Number	Appendix Table Number
1	A1
2	A2
3	A3
4	A4
5	A5
6	A6

Table A1. Effects of PGE_1 (16 μg/min i.a., CF) on lymph flow and protein transport, lymph and plasma protein concentrations, vascular pressures, hematocrit, and limb weights. (n=7)

	Control		Infusion Period					
	-10	0	10	20	30	40	50	60
Systemic Arterial Pressure (mmHg)	125	130	160	165	165	165	165	165
	120	120	125	120	120	125	120	120
	140	142	160	155	155	145	140	140
	125	130	138	138	144	146	146	148
	135	135	158	158	160	160	158	155
	120	118	112	115	120	120	125	127
	118	118	122	115	110	116	120	120
means	124	124	139*	139*	141*	141*	141*	141*
± standard error	4	5	7	7	7	7	6	6
Perfusion Pressure (mmHg)	105	110	60	60	60	60	60	60
	100	95	52	52	52	52	55	52
	130	135	57	58	60	60	60	60
	125	135	45	48	52	55	55	58
	110	105	58	60	65	65	68	70
	105	102	52	52	55	55	55	57
	115	115	50	50	50	50	50	50
means	109	110	55*	56*	56*	55*	56*	57*
± standard error	5	7	2	2	2	3	2	2
Skin Small Vein Pressure (mmHg)	11	18	12	13	13	13	13	13
	12	12	14	15	15	15	16	16
	11	11	13	13	13	13	13	13
	11	12	14	15	15	16	15	16
	12	13	14	15	16	16	16	16
	10	10	11	12	12	12	12	12
	11	12	13	13	13	14	14	14
means	11	12	12	13	13	13[x]	13[x]	13[x]
± standard error	0	1	1	1	1	1	1	1

Table A1. Continued.

	Control		Infusion Period					
	-10	0	10	20	30	40	50	60
Lymph Flow Rate	.02	.04	.02	.02	.02	.02	.01	.01
(ml/10 min)	.01	.01	.01	.01	.01	.01	.01	.01
	.01	.01	.01	.01	.01	.01	.01	.01
	.02	.01	.02	.04	.06	.02	.01	.02
	.01	.01	.01	.01	.01	.01	.01	.01
	.01	.01	.01	.10	.16	.06	.04	.05
	.02	.02	.18	.28	.15	.06	.06	.06
means	.01	.02	.04	.07	.06	.03	.02	.02
± standard error	.00	.00	.03	.04	.03	.01	.01	.01
Lymph Total Protein	2.4	2.6	2.6	3.2	3.4	3.7	3.5	3.6
(gm %)	2.1	2.0	1.7	2.0	1.6	1.6	1.6	1.8
	3.5	3.5	3.4	4.0	4.5	4.2	4.1	3.8
	2.3	2.4	2.4	2.5	2.7	2.8	3.0	2.8
	2.4	2.6	2.0	2.1	2.2	2.1	2.5	2.4
	2.1	2.8	2.7	3.0	4.6	4.1	4.2	3.9
	3.5	3.5	4.0	5.1	4.8	4.6	4.7	4.2
means	2.6	2.8	2.7	3.1	3.4*	3.3[x]	3.4*	3.2[x]
± standard error	0.2	0.2	0.3	0.5	0.5	0.5	0.4	0.4
Lymph Protein Transport	.48	1.04	.52	.64	.68	.74	.35	.36
(mg/10 min)	.21	.20	.17	.20	.16	.16	.16	.18
	.35	.35	.34	.40	.45	.42	.41	.38
	.46	.24	.48	1.00	1.62	.56	.30	.28
	.24	.26	.20	.21	.22	.21	.25	.24
	.21	.28	.27	3.00	7.36	2.46	1.68	1.95
	.70	.70	7.20	14.28	7.20	2.76	2.82	2.52
means	.38	.44	1.31	2.82	2.53	1.04	.85	.84
± standard error	.07	.13	1.06	2.10	1.34	.45	.41	.39

Table A1. Continued.

	Control		Infusion Period					
	-10	0	10	20	30	40	50	60
Plasma Protein (gm %)		5.3			6.1			4.9
		5.0			5.1			5.6
		6.6			6.8			6.9
		5.1			4.9			5.5
		5.8			6.1			6.0
		4.9			5.0			5.1
		5.8			5.9			5.8
± standard error of means		5.5			5.7			5.7
		0.2			0.2			0.2
Hematocrit		40			52			52
		37			38			42
		45			50			47
		38			45			47
		44			49			46
		40			43			43
		36			38			38
± standard error of means		39			44*			45*
		1			2			2

Table A1. Continued.

	Control	Experimental	Difference
Limb Weight after 60 min infusion (gms)	712.0	745.5	33.5
	741.5	757.0	15.5
	669.5	675.5	6.0
	594.0	616.5	22.5
	695.5	727.0	31.5
	701.0	730.0	29.0
	543.5	564.0	20.5
means	665.3	687.9	22.6**
± standard error	28.9	29.8	3.7

*=P < .01 relative to zero time
**=P < .01 relative to contralateral control limb weight
x=P < .05 relative to zero time

data expressed as mean ± standard error of the mean

Table A2. Effects of PGE_1 (16 μg/min i.a., CF) and isoproterenol (3 μg/min i.a., CF) on lymph flow and protein transport, lymph and plasma protein concentrations, vascular pressures, and hematocrit. (n=5)

	Control		Infusion Period					
	-10	0	10	20	30	40	50	60
Systemic Arterial Pressure (mmHg)	125	125	100	105	108	110	115	115
	110	110	117	120	125	130	126	128
	142	147	145	158	160	160	160	160
	130	130	140	142	150	160	160	155
	160	146	135	140	135	140	140	142
means	133	132	127	133	136	140	140	140
± standard error	8	7	8	9	9	9	9	8
Perfusion Pressure (mmHg)	125	125	50	50	50	55	55	55
	90	90	45	50	50	50	48	50
	120	130	60	60	60	60	60	60
	110	110	75	110	110	115	105	105
	125	130	58	55	56	56	60	60
means	114	117	58*	65*	65*	67*	66*	66*
± standard error	7	8	5	11	11	12	10	10
Skin Small Vein Pressure (mmHg)	12	12	11	11	11	12	13	13
	11	11	11	11	11	11	11	12
	7	10	8	9	9	9	9	9
	30	35	13	9	10	10	10	10
	14	15	15	15	15	15	15	15
means	15	17	12	11	11	11	12	12
± standard error	4	5	1	1	1	2	1	2

Table A2. Continued.

	Control		Infusion Period					
	-10	0	10	20	30	40	50	60
Lymph Flow Rate	.01	.01	.01	.01	.01	.01	.01	.01
(ml/10 min)	.12	.07	.12	.07	.05	.06	.04	.02
	.02	.01	.03	.02	.02	.01	.01	.01
	.01	.01	.01	.02	.01	.01	.01	.01
	.01	.02	.01	.01	.01	.01	.02	.01
means	.03	.02	.04	.03	.02	.02	.02	.01
± standard error	.02	.01	.02	.01	.01	.01	.01	.00
Lymph Total Protein	2.2	2.5	2.4	2.1	2.3	2.2	2.4	2.3
(gm %)	1.7	1.8	2.1	2.4	2.4	2.6	2.8	2.7
	3.6	3.7	3.5	3.3	3.2	2.8	2.9	2.9
	1.9	2.1	1.6	1.7	1.7	1.6	1.7	1.8
	2.7	2.5	2.7	2.7	2.5	2.4	2.0	2.2
means	2.4	2.5	2.5	2.4	2.4	2.3	2.4	2.4
± standard error	0.3	0.3	0.3	0.3	0.2	0.2	0.2	0.2
Lymph Protein Transport	0.22	0.25	0.24	0.21	0.23	0.22	0.24	0.23
(mg/10 min)	2.04	1.26	2.52	1.68	1.20	1.56	1.12	0.54
	0.72	0.37	1.05	0.66	0.64	0.28	0.29	0.29
	0.19	0.21	0.16	0.34	0.17	0.16	0.17	0.18
	0.27	0.50	0.27	0.27	0.25	0.24	0.40	0.22
means	0.69	0.52	0.85	0.63	0.50	0.49	0.44	0.29
± standard error	0.35	0.19	0.45	0.27	0.19	0.27	0.17	0.06

Table A2. Continued.

	Control		Infusion Period					
	-10	0	10	20	30	40	50	60
Plasma Protein (gm %)		5.8			6.1			6.0
		5.4			5.3			5.9
		6.4			6.4			6.5
		6.4			6.5			6.5
		5.8			5.8			5.6
means		6.0			6.0			6.1
± standard error		0.2			0.2			0.2
Hematocrit		40			50			50
		42			44			47
		44			44			50
		38			50			52
		42			48			47
means		41			47*			49*
± standard error		1			1			1

*=P < .01 relative to zero time x=P < .05 relative to zero time

data expressed as mean ± standard error of the mean

Table A3. Effects of PGE_1 (16 μg/min i.a., CF) and vaso pressin (0.8 Pressor Unites/min i.a., CF) on lymph flow and protein transport, lymph and plasma protein concentrations, vascular pressures, hematocrit, and limb weights. (n=5)

	Control		Infusion Period					
	-10	0	10	20	30	40	50	60
Systemic Arterial Pressure (mmHg)	80	100	105	95	100	100	98	98
	108	105	135	118	112	112	110	112
	125	130	125	135	135	135	132	130
	150	150	195	180	160	150	145	140
	125	125	150	155	150	140	125	125
means	118	122	142*	137x	131	127	122	121
± standard error	12	9	15	15	11	9	8	7
Perfusion Pressure (mmHg)	85	100	100	75	160	180	160	180
	110	115	75	63	57	55	55	55
	92	90	75	100	100	93	93	100
	120	120	122	125	125	130	130	100
	110	120	122	125	120	110	120	110
means	103	109	99	98	112	114	112	109
± standard error	6	6	11	13	17	21	18	20
Skin Small Vein Pressure (mmHg)	9	9	8	8	10	10	8	9
	6	6	7	7	7	7	7	7
	15	15	11	11	11	11	11	11
	8	9	12	11	11	11	10	9
	17	22	25	20	15	14	17	19
means	11	12	13	11	11	11	11	11
± standard error	2	3	3	2	1	1	2	2

Table A3. Continued.

	Control		Infusion Period					
	-10	0	10	20	30	40	50	60
Lymph Flow Rate	.02	.01	.01	.01	.01	.01	.02	.02
(ml/10 min)	.01	.01	.01	.01	.01	.01	.01	.01
	.01	.01	.01	.01	.01	.01	.01	.01
	.01	.04	.02	.03	.03	.02	.03	.04
	.01	.01	.01	.01	.01	.01	.01	.01
means	.01	.02	.01	.01	.01	.01	.02	.02
± standard error	.00	.01	.00	.00	.00	.00	.00	.01
Lymph Total Protein	2.8	2.8	2.6	2.0	2.2	1.9	2.3	2.2
(gm %)	3.2	2.8	2.4	2.1	2.4	2.5	2.4	2.3
	2.4	2.2	2.0	1.9	2.0	2.3	2.2	2.3
	2.0	2.0	1.9	2.1	2.2	2.3	2.2	2.4
	4.2	4.2	3.8	3.2	3.6	2.8	3.2	3.0
means	2.9	2.8	2.5	2.3*	2.5x	2.4	2.5	2.4
± standard error	0.4	0.4	0.3	0.2	0.3	0.1	0.2	0.1
Lymph Protein Transport	.56	.28	.26	.20	.22	.19	.46	.44
(mg/10 min)	.32	.28	.24	.21	.24	.25	.24	.23
	.24	.22	.20	.19	.20	.23	.22	.23
	.20	.80	.38	.63	.66	.46	.66	.96
	.42	.42	.38	.32	.36	.28	.32	.30
means	.35	.40	.29	.31	.34	.28	.38	.43
± standard error	.06	.11	.04	.08	.09	.05	.08	.14

Table A3. Continued.

	Control		Infusion Period					
	-10	0	10	20	30	40	50	60
Plasma Protein (gm %)		5.6			5.9			5.6
		5.4			5.5			5.8
		5.8			5.8			6.1
		5.4			5.7			5.8
		7.2			7.2			7.8
means		5.9			6.0			6.2
± standard error		0.3			0.3			0.4
Hematocrit		43			46			46
		33			36			39
		36			38			37
		45			43			44
		45			46			49
means		40			42			43
± standard error		2			2			2

	Control	Experimental	Difference
Limb Weight after 60 min infusion (gms)	868.5	873.5	5.0
	573.0	568.0	-5.0
	694.0	694.5	0.5
	624.5	616.0	-8.5
	705.5	714.0	8.5
means	693.1	693.2	0.1
± standard error	50.0	52.0	3.1

*=P < .01 relative to zero time x=P < .05 relative to zero time

data expressed as mean ± standard error of the mean

Table A4. Effects of PGE_1 (16 μg/min i.a., CF) and angiotensin II (2 μg/min i.a., CF) on lymph flow and protein transport, lymph and plasma protein concentrations, vascular pressures, hematocrit, and limb weights. (n=7)

	Control		Infusion Period					
	-10	0	10	20	30	40	50	60
Systemic Arterial Pressure (mmHg)	122	125	145	140	135	130	127	127
	150	150	155	150	142	140	138	132
	110	110	148	140	132	125	118	110
	156	157	168	155	150	152	150	150
	115	115	148	148	148	148	148	148
	138	138	150	145	145	150	150	150
	147	152	175	170	160	160	157	160
means	134	135	156*	150*	145x	144	141	140
± standard error	7	7	4	4	4	5	5	7
Perfusion Pressure (mmHg)	100	105	70	65	62	60	55	55
	150	150	80	70	70	68	65	68
	115	115	65	48	46	46	45	42
	150	155	125	125	120	125	130	133
	80	70	46	50	60	58	60	57
	130	130	60	60	60	60	60	60
	130	140	100	88	85	82	80	80
means	122	124	78*	72*	72*	71*	71*	71*
± standard error	10	11	10	10	9	10	11	11
Skin Small Vein Pressure (mmHg)	14	14	14	14	14	14	14	14
	11	11	11	11	12	11	11	11
	6	6	14	12	10	9	8	7
	11	11	14	14	13	12	13	12
	15	13	10	9	9	9	10	9
	8	8	10	10	10	10	10	10
	9	9	12	12	12	12	12	12
means	11	10	12	12	11	11	11	11
± standard error	1	1	1	1	1	1	1	1

Table A4. Continued.

	Control		Infusion Period					
	-10	0	10	20	30	40	50	60
Lymph Flow Rate	.01	.01	.01	.01	.01	.01	.01	.01
(ml/10 min)	.01	.01	.01	.01	.01	.01	.01	.01
	.01	.01	.01	.01	.01	.01	.01	.02
	.01	.01	.05	.18	.09	.09	.08	.05
	.01	.01	.01	.01	.01	.01	.02	.01
	.01	.01	.01	.01	.01	.01	.01	.01
	.01	.01	.01	.01	.01	.01	.01	.01
means	.01	.01	.02	.03	.02	.02	.02	.02
± standard error	.00	.00	.01	.02	.01	.01	.01	.01
Lymph Total Protein	3.1	3.5	3.2	3.3	3.8	3.4	3.4	3.2
(gm %)	3.0	3.0	3.5	4.7	5.0	4.5	4.6	4.0
	3.1	3.1	2.8	3.1	3.2	3.0	2.8	2.6
	2.0	2.2	3.9	4.8	4.8	4.7	4.4	4.4
	1.5	1.5	1.4	1.4	1.5	1.5	1.6	1.5
	3.0	2.2	2.3	2.0	2.3	2.3	2.2	2.2
	3.1	3.1	3.1	2.7	2.8	3.1	3.1	2.8
means	2.7	2.7	2.9	3.1	3.3	3.2	3.2	3.0
± standard error	0.2	0.3	0.3	0.5	0.5	0.4	0.4	0.4
Lymph Protein Transport	.31	.35	.32	.33	.38	.34	.34	.32
(mg/10 min)	.30	.30	.35	.47	.50	.45	.46	.40
	.31	.31	.28	.31	.32	.30	.28	.26
	.20	.22	1.95	8.64	4.32	4.23	3.52	2.20
	.15	.15	.14	.14	.15	.15	.32	.15
	.30	.22	.23	.20	.23	.23	.22	.22
	.31	.31	.31	.27	.28	.31	.31	.28
means	.27	.27	.51	1.48	.88	.86	.78	.55
± standard error	.02	.03	.24	1.19	.57	.56	.46	.28

Table A4. Continued.

	Control		Infusion Period					
	-10	0	10	20	30	40	50	60
Plasma Protein (gm %)		6.4			6.8			6.6
		6.4			6.5			6.7
		5.4			5.4			5.5
		6.8			6.8			6.2
		5.4			5.8			5.7
		5.8			6.0			5.8
		5.8			5.8			5.7
means		6.0			6.2			6.0
± standard error		0.2			0.2			0.2
Hematocrit		46			50			50
		35			46			45
		42			47			47
		43			47			47
		40			46			45
		38			40			40
		45			51			49
means		41			47*			46*
± standard error		1			1			1

Table A4. Continued.

	Control	Experimental	Difference
Limb Weight after 60 min infusion (gms)	624.5	626.0	1.5
	504.0	528.5	24.5
	517.0	517.0	0.0
	473.0	496.0	23.0
	687.0	698.0	12.0
	707.5	709.0	1.5
	601.0	626.0	25.0
means	587.7	600.1	12.5^{xx}
$\pm$ standard error	34.8	33.0	4.4

*=P < .01 relative to zero time

x=P < .05 relative to zero time
xx=P < .05 relative to contralateral control limb weight

data expressed as mean $\pm$ standard error of the mean

Table A5. Effects of PGE_1 (16 μg/min i.a., CF) and methylprednisolone (15 μg/min i.a., CF) on lymph flow and protein transport, lymph and plasma protein concentrations, vascular pressures, hematocrit, and limb weights. (n=5)

	Control		Infusion Period					
	-10	0	10	20	30	40	50	60
Systemic Arterial Pressure (mmHg)	115	120	135	135	132	130	132	135
	132	130	145	137	144	145	145	144
	85	88	135	135	135	130	130	125
	152	155	147	150	150	147	150	142
	128	135	160	160	150	148	140	142
means	122	126	144*	143*	142*	140x	139x	138
± standard error	11	11	5	5	4	4	4	4
Perfusion Pressure (mmHg)	90	80	42	40	40	40	40	40
	122	120	52	55	56	58	58	58
	104	108	45	45	45	45	45	45
	130	138	50	55	55	55	60	55
	140	143	60	62	62	65	62	62
means	117	118	50*	51*	52*	53*	53*	52*
± standard error	9	11	3	4	4	5	4	4
Skin Small Vein Pressure (mmHg)	15	18	13	13	13	13	13	12
	10	9	14	13	14	14	14	14
	9	9	10	11	11	11	12	12
	9	9	10	11	11	11	11	11
	15	13	15	15	15	15	15	15
means	12	12	12	13	13	13	13	13
± standard error	1	2	1	1	1	1	1	1

Table A5. Continued.

	Control		Infusion Period					
	-10	0	10	20	30	40	50	60
Lymph Flow Rate	.02	.01	.01	.03	.02	.02	.01	.01
(ml/10 min)	.01	.01	.01	.01	.01	.01	.01	.01
	.01	.02	.01	.02	.03	.01	.01	.01
	.06	.06	.02	.01	.02	.02	.01	.02
	.01	.01	.01	.01	.01	.01	.01	.02
means	.02	.02	.01	.02	.02	.01	.01	.01
± standard error	.01	.01	.00	.00	.00	.00	.00	.00
Lymph Total Protein	1.5	1.5	1.2	1.4	1.7	2.1	2.5	2.1
(gm %)	2.6	3.0	3.0	2.8	2.8	2.8	2.8	3.0
	2.8	2.8	2.8	2.9	2.6	2.7	2.9	2.7
	2.6	2.8	2.5	2.7	2.6	2.6	2.9	2.4
	2.8	3.3	3.1	3.4	3.6	3.6	3.5	3.4
means	2.5	2.7	2.5	2.6	2.7	2.8	2.9	2.7
± standard error	0.2	0.3	0.3	0.3	0.3	0.2	0.2	0.2
Lymph Protein Transport	0.30	0.15	0.12	0.42	0.34	0.42	0.25	0.21
(mg/10 min)	0.52	0.30	0.30	0.28	0.28	0.28	0.28	0.30
	0.28	0.56	0.28	0.58	0.78	0.27	0.29	0.27
	1.56	1.68	0.50	0.27	0.52	0.52	0.29	0.48
	0.28	0.33	0.31	0.34	0.36	0.36	0.35	0.68
means	0.59	0.60	0.30	0.38	0.46	0.37	0.29	0.39
± standard error	0.25	0.28	0.06	0.06	0.09	0.05	0.02	0.09

Table A5. Continued.

	Control		Infusion Period					
	-10	0	10	20	30	40	50	60
Plasma Protein (gm %)		4.8			4.9			4.8
		5.5			5.6			5.5
		4.7			5.2			4.7
		5.0			5.1			5.1
		5.7			6.1			6.4
means		5.1			5.4			5.3
± standard error		0.2			0.2			0.3
Hematocrit		30			32			30
		38			48			50
		38			49			42
		37			44			42
		43			48			48
means		37			44*			42x
± standard error		2			3			3

	Control	Experimental	Difference
Limb Weight after 60 min infusion (gms)	683.5	739.5	56.0
	733.5	739.5	6.0
	735.5	742.5	7.0
	964.5	974.5	10.0
	753.0	773.0	20.0
means	774.0	793.8	19.8
± standard error	49.0	45.6	9.38

*=P < .01 relative to zero time x=P < .05 relative to zero time

data expressed as mean ± standard error of the mean

Table A6. Effects of PGE_1 (16 μg/min i.a., CF) and serotonin (7 μg base/min i.a., CF) on lymph flow and protein transport, lymph and plasma protein concentrations, vascular pressures, hematocrit, and limb weights. (n=5)

	Control		Infusion Period					
	-10	0	10	20	30	40	50	60
Systemic Arterial Pressure (mmHg)	132	135	142	140	142	140	140	148
	127	125	125	127	125	125	125	125
	125	125	100	95	95	100	102	108
	125	130	100	85	85	85	90	90
	135	135	130	122	120	115	120	120
means	129	130	119	114	113	113	115	118
± standard error	2	2	8	10	10	10	9	10
Perfusion Pressure (mmHg)	125	118	72	76	76	72	75	82
	127	125	125	127	125	125	125	125
	130	125	57	58	60	65	65	65
	100	105	55	55	60	60	65	65
	110	115	85	90	95	100	105	105
means	118	118	79*	81*	83*	84*	87*	88*
± standard error	6	4	13	13	12	12	12	12
Skin Small Vein Pressure (mmHg)	15	14	16	17	20	20	20	20
	12	12	12	13	14	14	14	15
	12	13	15	15	15	16	16	16
	11	10	9	10	11	11	12	11
	15	15	18	22	23	24	25	25
means	13	13	14	15x	17*	17*	17*	17*
± standard error	1	1	2	2	2	2	2	2

Table A6. Continued.

	Control		Infusion Period					
	-10	0	10	20	30	40	50	60
Lymph Flow Rate	.01	.01	.03	.09	.04	.03	.03	.04
(ml/10 min)	.01	.01	.01	.02	.02	.03	.01	.02
	.01	.01	.02	.14	.07	.04	.02	.02
	.01	.05	.03	.02	.03	.01	.09	.04
	.01	.01	.01	.02	.01	.02	.02	.08
means	.01	.02	.02	.06	.03	.03	.03	.04
± standard error	.00	.01	.00	.02	.01	.01	.01	.01
Lymph Total Protein	3.4	3.4	3.4	3.8	5.2	5.7	5.3	5.2
(gm %)	2.0	2.3	2.8	3.1	3.5	3.6	3.7	3.8
	2.5	2.3	2.4	3.5	4.0	4.3	4.1	3.8
	2.4	2.3	2.9	3.2	3.2	3.4	3.5	3.4
	3.1	2.8	2.7	2.9	2.8	3.3	2.6	2.2
means	2.7	2.6	2.8	3.3[x]	3.7*	4.1*	3.8*	3.7*
± standard error	0.3	0.2	0.2	0.2	0.4	0.4	0.4	0.5
Lymph Protein Transport	.34	.34	1.02	3.42	2.08	1.71	1.59	2.08
(mg/10 min)	.20	.23	.28	.62	.70	1.08	.37	.76
	.25	.23	.48	4.90	2.80	1.72	.82	.76
	.24	1.15	.87	.64	.96	.34	3.15	1.36
	.31	.28	.27	.58	.28	.66	.52	1.76
means	.27	.45	.58	2.03	1.36	1.10	1.29	1.34
± standard error	.03	.18	.15	.90	.47	.28	.51	.26

Table A6. Continued.

	Control		Infusion Period					
	-10	0	10	20	30	40	50	60
Plasma Protein (gm %)		7.2			7.5			7.2
		5.1			5.5			4.7
		5.3			5.2			5.4
		5.6			6.0			5.8
		7.0			6.8			6.4
means		6.0			6.2			5.9
± standard error		0.4			0.4			0.4
Hematocrit		35			37			40
		44			50			50
		41			44			45
		39			37			38
		38			42			42
means		39			42[x]			43*
± standard error		2			2			2

	Control	Experimental	Difference
Limb Weight after 60 min infusion (gms)	520.0	541.0	21.0
	715.0	736.5	21.5
	549.0	592.0	43.0
	645.5	690.5	45.0
	534.5	581.5	47.0
means	592.8	628.3	35.5**
± standard error	37.6	36.5	5.9

*=P < .01 relative to zero time x=P < .05 relative to zero time
**=P < .01 relative to contralateral control limb weight
data expressed as mean ± standard error of the mean

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