Comparative effects of prostacyclin and acetylcholine upon collateral circulation to the canine lower hind limb during thrombosis

by

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ACh	Acetylcholine
A-V	Arterial-venous
cm	Centimeters
cpm	Counts per minute
DPC	Driving pressure collateral bed
DPP	Driving pressure peripheral bed
DPS	Driving pressure stenosis area
EMF	Electromagnetic blood flow
FA	Femoral artery
FV	Femoral vein
gm	Gram
Hg	Mercury
id	Inside diameter
IV	Intravenous
kg	Kilogram
LAD	Left anterior descending coronary artery
LV	Left ventricle
min	Minute
ml	Milliliter
mm	Millimeters
P	Blood pressure
ΔP	Blood pressure differential

P_1	Mean arterial blood pressure in the FA proximal to the stenosis (driving pressure for flow through the stenosis)
P_2	Mean arterial blood pressure in the FA distal to the stenosis
P_3	Mean arterial blood pressure in a branch at the level of the DCFA (potential collateral blood flow)
PA	Mean arterial blood pressure
PCFA	Proximal caudal femoral artery
PCFV	Proximal caudal femoral vein
p-CPA	Para-chlorophenylalanine
PG	Prostaglandin
PGE2	Prostaglandin E-2
PGH2	Prostaglandin H-2
PGI2	Prostaglandin I-2 (prostacyclin)
PV	Mean venous blood pressure at the FV (venous blood pressure)
Q	Blood flow
QC	Collateral blood flow
QP	Peripheral blood flow in the ipsilateral leg
QPC	Peripheral blood flow in the contralateral leg
QS	Stenosis blood flow
R	Resistance
RBS	Reference blood samples
RC	Collateral resistance
RP	Peripheral resistance
RM	Radioactive microspheres
RMT	Radioactive microsphere technique

RS	Stenosis resistance
SA	Saphenous artery
SV	Saphenous vein
Th	Thrombosis
Th + ACh	Thrombosis with acetylcholine
Th + PGI2	Thrombosis with prostacyclin
TXA2	Thromboxane A2
0-S	0 percent stenosis
100-S	100 percent stenosis
^{o}C	Degrees centigrade
%	Percent
"	Inches
μ	Micron
x	Magnification

INTRODUCTION

Collateral vessel function has not been precisely defined due to incomplete evaluation of the hemodynamic changes which take place during collateral vessel recruitment and function. Many factors influence collateral hemodynamics during arterial stenosis and thrombosis. In this study, an attempt will be made to measure some of these parameters and to determine if the vasodilators acetylcholine (ACh) and prostacyclin (PGI2) may improve collateral circulation during arterial thrombosis.

In many tissues, the arterial vasculature is designed to maintain nutrient supply during arterial occlusion. During occlusions, a network of preformed anastomosing arterial branches called collateral vessels supply nutritive blood flow to areas previously fed by occluded parent arteries.

Collateral vessels have been classified as stem, midzone, and reentrant vessels, depending on their location relative to an occlusion (Longland, 1953). The most proximal elements, stem vessels, supply blood to the midzone vessels, which are composed of a multitude of channels normally invisible in arteriographs (Winblad et al., 1959). Although midzone vessels have a high resistance, they can enlarge to allow blood flow into the distal elements, reentrant vessels.

During an arterial occlusion, reentrant vessels may conduct retrograde blood flow into the parent vessel distal to the occlusion, thus supplying sufficient blood to the tissues distal to the occlusion to keep

them viable. The degree to which the viability of the distal ischemic bed is maintained is a function of the extent of collateralization, how rapidly an occlusion develops, vascular tone, the location of arterial occlusion relative to the distribution and size of the collateral vessels, and the metabolic rate of the ischemic bed tissue.

Occlusion of an artery is not a simple mechanical event. It causes tissue ischemia and endothelial cell damage, which result in the release of vasoactive substances (Imhoff, 1961). Two such substances known to be released during thrombotic occlusion, serotonin (Kordenat and Kezdi, 1979) and thromboxane A-2 (TXA2) (Schaub et al., 1982), regulate blood flow to distal tissues and may cause tissue death following an occlusive incident (Schaub et al., 1977a, 1977b).

Previous studies of collateral blood flow (CBF) have generated several observations about their functions. It was observed that the resistance of a stenosis is dynamic and is interdependent upon peripheral and collateral bed hemodynamics (Roth et al., 1976; Wallinsky et al., 1979). Mechanical occlusion (stenosis) produced the greatest stimulus for CBF (Winblad et al., 1979), while the presence of an intravascular arterial thrombosis inhibits development of CBF (Schaub et al., 1976, 1977a, 1977b). Strenuous exercise is the only documented therapeutic method to improve CBF during a mechanical occlusion (Thulesius, 1963; Khudaiberdyerv and Kulikov, 1970).

This study was designed to monitor and analyze hemodynamic variables which interact to influence collateral vessels; to compare stenosis with and without the effects of thrombosis at the same vascular site; and to

determine the effects of ACh and PGI2 on CBF. By utilizing the hind limb of the dog as a model, a more comprehensive experimental design has been incorporated in this study than in previous experimental models.

REVIEW OF LITERATURE

Vascular disease in which nutritional blood flow to a body region has been compromised is a common problem in medicine today. Arterial thrombosis has been found to be one of the acute manifestations of a vascular disease which reduce or interrupt normal blood flow to a region.

In order to better understand the mechanisms involved in promoting survival of an organ or tissue during thrombotic insult, the study of collateral blood flow (QC) is warranted, and further investigation may provide valuable knowledge.

Stenotic Factors Which Affect Normal Blood Flow

Artificial stenoses have been employed in major nutrient arteries (arteries supplying nutritional blood flow to tissues) by many investigators in order to study impairment of circulatory function (Mann et al., 1938; Folts et al., 1974; Kubicka et al., 1979). During these experiments, the cross-sectional area of a vessel was reduced to study the relationship between lumen reduction or length of the stenotic segment and the degree of inhibition of blood flow.

In the carotid artery of the dog, the cross-sectional area (the area of a section at right angles to the lumen of the vessel) of the lumen was reduced 50% by insertion of an 8 mm long bakelite tube with an appropriate internal diameter without modifying blood flow through the artery. A 90% lumen reduction depressed blood flow by 50% (Mann et al., 1938). Other investigators found that significant flow reduction through the

vessel by insertion of 2 mm long plastic cylinders of varying internal diameters within a coronary artery did not occur until 78% reduction of luminal cross-sectional area was achieved (Folts et al., 1974). Kubicka et al. (1979) first noticed flow decreased with 75% reduction of lumen cross-sectional area. A 98% cross-sectional area reduction dropped vessel blood flow to 25% of its original value. Kubicka inserted a double limb extracorporeal shunt into the right common iliac artery of five dogs. One limb of the shunt maintained blood flow to the leg between maneuvers. The second limb of the shunt was constructed to allow placement of 1 cm long inserts, from 4 mm to 0.5 mm in internal diameter, in order to produce repeatable, progressive arterial stenoses. Flow proximal to the shunt was measured by the electromagnetic blood flow (EMF) technique following each progressive stenosis. Measurements were made with the circulation intact, then repeated following ligation of major collateral arteries such as the median sacral, internal iliac, and profunda femoris. Reduction of the number of potential collateral vessels did not affect the maximum blood flow observed by progressive arterial stenosis.

Percent decrease in cross-sectional area was an incomplete measure of the degree of a stenosis as found by Gould (1978). He stated that stenosis geometry modified resistance, pressure differential (ΔP), and flow reserve capacity of a vascular bed. Flow reserve refers to maximum flow rate which is achievable during oxygen deficit following a period of complete occlusion. Young and Tsai (1973) in their experiments found that stenosis geometry, in addition to flow turbulence and separation,

determined pressure drops across stenoses. Roth et al. (1976) and Young (1979) described factors other than stenosis geometry which modify vascular hemodynamics in critical stenoses. They stated that stenosis resistance (RS) was not fixed but rather dynamic in nature. When the pressure gradient was held constant, a change in RS was primarily determined by collateral circulation and peripheral resistance (RP). Gould et al. (1975) also showed that the resistance of a fixed stenosis could be variable; a decrease in RS increased flow through the stenosis ($R=\frac{\Delta P}{Q}$).

Stenosis length is another factor influencing blood flow. A four-fold increase in length of a critical stenosis produced a 25% reduction in flow (May et al., 1963). A stenosis is "critical" when a small decrease in lumen size is associated with appreciable reduction in blood flow. There was no effect of stenosis length upon blood flow during subcritical periods. Feldman et al. (1978) found that smaller decreases in cross-sectional area were necessary to reach critical stenosis when stenosis length was increased. In addition, it was found that stenoses in series produced additive effects. These findings were supported by results of Gould and Lipscomb (1974) and Young (1979).

It appears that RS is not static and could increase when the distal vascular bed dilated due to release of vasoactive substances (Wallinsky et al., 1979). In a stenosis less than 100%, RS through the section upstream from a peripheral bed was a function of the sum of the resistances of the two elements when collateral flow (QC) was absent (please refer to Figure 2), $QP = \frac{PA-PV}{RS+RP}$. Kreuzer and Schenk (1973), along with Schwartz et al. (1980), found that at constant driving pressure distal vasodilation

reduced RP, thus increasing QS and elevating RS. In a later study, Schwartz et al. (1980) observed that a lowering of coronary pressure proximal to the stenotic segment increased RS due to passive narrowing of the vessel from elastic recoil. Investigations by Santamore et al. (1980) and Wallinsky et al. (1979) agreed with Schwartz's findings that proximal vasoconstriction greatly increased stenosis severity. Such a passive narrowing could occur physiologically in or due to a tissue ischemia, thus depressing arterial pressure there and perhaps promoting vasodilation distal to the stenosis.

While ΔP and Q are the primary determinants of RS, both RC and RP also can be effective modulators of RS. All of the hemodynamic parameters are variable and are interdependent (RS on QS and ΔP; RS on RC and RP). Over the course of a long-term experiment, all variables are dependent upon anesthesia, surgery, physiological deterioration, and systemic buildup of extrinsic vasoactive agents.

Cardiovascular Occlusive Disease and Thrombosis

Prolonged or repeated occlusion of the arterial inflow to a tissue bed may endanger tissue function and viability. Impaired function is seen most frequently in tissues experiencing mechanical limitations of blood flow. These limitations can be due to compression, to thromboembolism, or to arterial occlusive disease. Dysfunction occurs when tissue oxygen demand exceeds oxygen supply as in peripheral arterial occlusive

disease, which annually debilitates many patients and results in prolonged and extensive morbidity and may result in loss of life.

Vasodilator drugs have been used in pharmacotherapy of peripheral arterial occlusive disease. Their efficacy in restoring circulation to ischemic tissue beds has been limited. Adrenergic blocking agents have been successfully used in the treatment of occlusive peripheral vascular disease; however, they possess several untoward effects which limit their usefulness.

Beneficial peripheral effects of alpha receptor blocking agents, e.g., phenoxybenzamine and methysergide maleate, may be overshadowed by systemic effects leading to reflex tachycardia and electrocardiogram modification. They promote systemic hypotension and may produce peripheral vascular insufficiency (Bergersen and Goth, 1976); therefore, the use of these agents is questionable.

The ergot alkaloids, mild alpha blockers, have been contraindicated in treatment of peripheral occlusive vascular disease due to powerful vasodilatory actions unrelated to their antiadrenergic properties (Gilman et al., 1980). The flow distribution to the already malperfused, ischemic region(s) could be compromised by active dilation of the blood vessels elsewhere.

Pharmacologic agents suitable for modulating the distribution of blood flow between prestenotic/thrombotic areas and the collateral-dependent areas ideally should not produce untoward effects on the systemic circulation, blood pressure regulation, and cardiac function.

Experimental Arterial Thrombosis

Experimental thrombosis models have been studied in order to more fully comprehend the nature of vasoactive substances released during cardiovascular occlusive diseases. Kingsley et al. (1967) stated that injury produced from roughening the intima of the arterial wall is a factor in the promotion of thrombus formation. Sheppard and French (1971) inserted and rotated a roughened metal probe within the aorta of several rabbits. Within 45 minutes after removal of the probe, platelet aggregation was observed at the injury site.

Many investigators employed electrical stimulation to disrupt the intimal lining of arterial vessels and encourage thrombosis (Duval et al., 1970; Piton et al., 1978; Romson et al., 1980; Sedlark et al., 1980).

Gianturco et al. (1975) induced a gradually forming arterial thrombotic occlusion in man. Their technique was recommended in a clinical trial but was not performed experimentally. They employed a stainless steel coil and Dacron tuft which induced a thrombus plug to form.

Szarnicki et al. (1981) employed this same device for transcatheter embolization of major systemic pulmonary arterial collateral vessels in a human patient for correction of pulmonary atresia. In the Szarnicki study, a stainless steel wire coil with a Dacron tuft attached to its inside diameter was integrated into a "loading cartridge" arrangement.

The loading cartridge was advanced through an angiographic catheter precisely placed fluoroscopically and released from the catheter at a previously determined site. After the coil was extruded from the catheter tip, it assumed a helical shape with the Dacron tuft enmeshed in the wire coil. Proper sizing of the coil was determined by the diameter of the vessel to be embolized. The risk of distal embolization was minimized with the correct coil size. Rapid thrombus formation occurred and a plug formed at the precise placement site. Angiograms were taken to document complete vessel occlusion.

Kordenat et al. (1972) and Kordenat and Kezdi (1979) produced gradual coronary arterial thrombosis in a canine model by insertion of a helical copper coil within the lumen of a coronary vessel. This technique, with several modifications, was our method of choice (please refer to the surgical procedure section).

The thrombotic occlusion model used by Kordenat to produce a slowly-forming thrombus consisted of precisely placing a helically-shaped coil of copper wire (thrombogenic) into a selected coronary arterial branch. The animals were anesthetized lightly with sodium pentobarbital, and a concentric, double lumen catheter was utilized to insert the coil into the artery. In this technique, the tip of the inner catheter, upon which the wire coil had been emplaced, was inserted into the left anterior descending coronary artery (LAD). The wire coil was directed into the LAD under fluoroscopic (image intensifier) visualization. By pulling the inner catheter back while holding the outer catheter stationary, the end of the outer catheter pushed against the wire coil and it was released

into the LAD. The copper wire coil, of sufficient size to become fixed in the LAD, was approximately 1.5 to 2.0 mm in overall diameter and 5 to 7 mm long. The occluding thrombus began to form immediately and was complete 15 to 20 minutes after the wire coil was inserted. Coronary blood flow via the LAD became successively depressed. Complete occlusion was confirmed electrocardiographically (S-T segment configuration changes) and angiographically. The gradually occluding intracoronary thrombus produced experimental infarction. This thrombosis model simulated coronary thrombosis without pre-existing arteriosclerosis in man (Kordenat and Kezdi, 1979).

Van Aken et al. (1980) and Barnes (1981) proposed that collagen may induce intravascular platelet aggregation and thrombosis. Van Aken inverted a flap of the aortic wall into the lumen of rats to expose blood to collagen and thus produce thrombosis. In a similar fashion, Constantine et al. (1972) inverted a segment of a side branch of the carotid artery in dogs to produce thrombosis.

Collateral Blood Flow

The first report of the existence of CF was in 150 by Antyllus, a Greek surgeon who observed that vessel ligation did not always result in damage to the perfused area (John and Warren, 1961). Lower in 1669 and Haller in 1757 documented the presence of anastamosing blood vessels in the heart (John and Warren, 1961). Continued growth of a stag's antler after main nutrient artery ligation in a study by Hunter, cited in John

and Warren (1961), focused interest upon and promoted further research into collateral vessel development.

The mechanisms for recruitment of QC and the impetus for recruitment of collateral vessels still are not clearly defined. Previous studies have shown controversial results.

Dornhorst and Sharpey-Schafer (1951) measured a significant decrease in collateral bed vascular resistance associated with arterial occlusion by clamping the femoral artery in the dog. The decrease was thought to be the result of depressed sympathetic vascular tone and increased pressure gradients resulting from the occlusion, which may have initiated the release of vasodilator substances. Their findings were supported by Imhoff (1961). He stated that the simple occlusion of the distal feline aorta (without thrombus formation) resulted in the release of vasoactive agents from the blood vessel and tissues distal to the occlusion, the release of these substances being associated with the initiation of CF. Berne (1970) concluded that adenosine and other vasoactive substances may have been responsible for peripheral vasodilation distal to the occlusion. A three-fold increase in blood adenosine levels was detected from tissues distal to an occlusion site (Olsson, 1970). Proposals that adenosine in ischemic tissue regions promotes collateral vessel vasodilation were supported in a later study by Berne (1980).

Coffman (1966) found genesis of QC was dependent upon predominant vasomotor tone and the existing state of vascular reactivity. Studies by Theis (1933) and Donald and Ferguson (1970) supported Coffman's conclusions with the discovery that sympathectomy enhanced collateral blood

flow, but experiments conducted by Rutherford and Valenta (1971) and Allwood (1962) found that sympathectomy did not improve QC.

Some have stated that following occlusion, increased pressure differentials and flow velocities were the predominant stimuli for collateralization (Longland, 1953; Winblad et al., 1959; John and Warren, 1961; Leibow, 1963; Giron et al., 1971; Barnes, 1980).

One of the most notable observations with respect to recruitment of CF was that strenuous exercise markedly improved post-occlusion CF (Khudaiberdyerv and Kulikov, 1970; Rutherford and Valenta, 1971; Thulesius, 1963; Abramson, 1980; Fedor et al., 1980). After exercise training, patients with occlusive disease exhibited improved circulation to affected extremities over that of the pre-exercise period.

Earlier, the benefits of exercise upon QC in the human subject had been subject to doubt. Allwood (1962) found that during exercise of the calf muscles in the subjects with occlusive vascular disease, CF to the foot and calf was diminished. Closure of small blood vessels due to redistribution of blood flow during exercise was suggested as the reason. When blood is directed away from a region of the body, driving pressure in the peripheral blood vessels may decrease. This decreased intraluminal pressure changes the tension exerted on the arterial wall by the smooth muscle in the wall and may allow the radius of small vessels to decrease. As hypotension progresses, a vessel's caliber could decrease progressively until it reaches critical closing pressure, at which

time the lumen completely closes. It is more probable that decreased vascular resistance elsewhere is the reason that the dependent vascular bed is deprived of blood flow.

Thrombus Inhibition of Collateral Blood Flow

Imhoff (1961) and Schaub et al. (1976, 1977a, 1977b) noticed only transient inhibition of pre-existing collateral vessels during simple ligation of the feline distal aorta. Ligation of the terminal aorta in Imhoff's study produced transient hind limb reflex abnormalities, but aortic occlusion by an experimentally induced blood clot resulted in hind limb paralysis, rear extremity hypothermia, and lack of a femoral pulse.

Schaub similarly studied the responses of feline hind limb collateral circulation in two models of aortic occlusion: simple ligation and thrombus formation. For the simple ligation model, the caudal aorta was permanently ligated approximately 5 mm distal to the origin of the caudal mesenteric artery. The sixth lumbar arteries and the left deep circumflex iliac artery were ligated. Ligatures were secured around the right deep circumflex iliac artery and the aorta at the level of the trifurcation at the external iliac arteries. After evacuation of the blood from the aorta, the ligatures were tightened. The blood was evacuated by flushing the aorta with 37°C saline solution or by digital evacuation prior to tightening the ligatures.

Hemodynamic responses following simple ligation then were compared with those following thrombotic occlusion of the caudal feline aorta

(Schaub et al., 1976, 1977a, 1977b). In the thrombus occlusion model, experimental occlusion of the aorta by thrombosis was produced by temporarily ligating the aorta approximately 5 mm distal to the origin of the caudal mesenteric artery. The right and left deep circumflex iliac arteries were acutely ligated as were the sixth lumbar arteries. The aorta was ligated at the trifurcation after positioning a 16 gauge needle within it. The temporary ligation was removed allowing blood to enter the region, then the aorta was occluded again. Ten to 20 units of 100 μ/ml of thromboplastin were injected into the trapped blood, causing a clot in a 1.5 cm segment of aorta. The needle then was removed.

Postsurgically, all ligation cats treated in Schaub's studies initially showed depression of reflexes, demonstrated by ataxic gait and hind limb muscular weakness. After three days, however, all reflexes improved and only slight gait impairment was noticed. Aortograms indicated substantial blood flow adjacent to the area of aortic ligation, and the fifth and seventh lumbar vessels appeared dilated compared to the anterior lumbar vessels.

Cats subjected to aortic thrombosis had severely depressed or absent reflexes after recovery from anesthesia. Three days after occlusion, these subjects exhibited no improvement in neurological reflexes, and aortograms showed a lack of collateral vessel development.

Thus, occlusion by blood clot appeared to have impaired caudal blood flow to greater degree than did simple ligation and resulted in hind limb paralysis. Schaub et al. (1977a, 1977b) proposed that while the thrombus did not provide any greater mechanical impairment to blood flow than did

the simple ligation model, the release of vasoconstrictor substances by the thrombus was the cause of the more extensive impairment of collateral flow.

The release of a vasoconstrictor substance from aggregated platelets was suggested in an earlier study by Zucker (1947), who observed that vasoconstriction occurred not only in injured, platelet-containing blood vessels, but also in adjacent vessels. This adjacent vasoconstriction did not appear to be due to trauma or reflex, thus implicating vasoactive agents released by the platelets.

Schaub et al. (1977a, 1977b) suggested that serotonin was the vasoactive agent released by aggregating platelets or vessel endothelium which inhibited collateral blood flow by inducing vasoconstriction. Serotonin may reduce QC by its direct vasoconstrictive action on vascular smooth muscle. He reported that treatment with reserpine or para-chlorophenylalanine (p-CPA), both serotonin antagonists, improved collateral circulation to the feline hind limbs following clot formation in the descending aorta. Later Schaub et al. (1982) added that it was more probable that recirculation of platelet-derived thromboxane-A2 (TXA2) provided the impetus to depress QC. (Please refer to the section entitled "Prostaglandins and the Prostacyclin-TXA2 Interactions".) Perhaps other agents besides serotonin and TXA2 may mediate post thrombotic inhibition of CF.

The exact mechanisms of action for the vasoactive agents affecting CF have not been defined; vasoconstriction, platelet aggregation, or endothelial cell damage have been postulated (Schaub et al., 1977a, 1977b).

To date, the most definitive method for evaluating improvement of CF by therapeutic agents appears to be an arterial thrombosis model (Schaub et al., 1977a, 1977b).

Prostaglandins and the Prostacyclin-TXA2 Interactions

In this section, the role of prostaglandins and other autocoids in regulating local blood flow will be discussed. The prostaglandins, by their various actions, have been established as physiological mediators of blood flow regulation. A number of unstable but highly active intermediates have been isolated and characterized (please refer to Figure 1 for a diagram of the sequence of events leading to the release of vasoactive endoperoxides which regulate local blood flow).

PGA1, PGE1, and PGB1 were found to have vasodilatory effects in man and experimental animals (Goodman and Gilman, 1975). Dusting et al. (1978) investigated the vascular actions of arachidonic acid and its metabolites in perfused femoral beds in the dog, PGI2, PGE2, PGH2, and arachidonic acid induced vasodilation. PGI2 had an acute vasodilatory effect over five minutes in duration with a prolonged recovery period. The femoral bed was more sensitive to PGE2, and this dilator was 2-15x more potent than PGI2. PGH2 was of equal potency to PGI2, but its duration of action was shorter.

TXA2 is formed in the platelets and causes vasoconstriction (Bunting et al., 1976; Needleman et al., 1976) and platelet aggregation (Harker

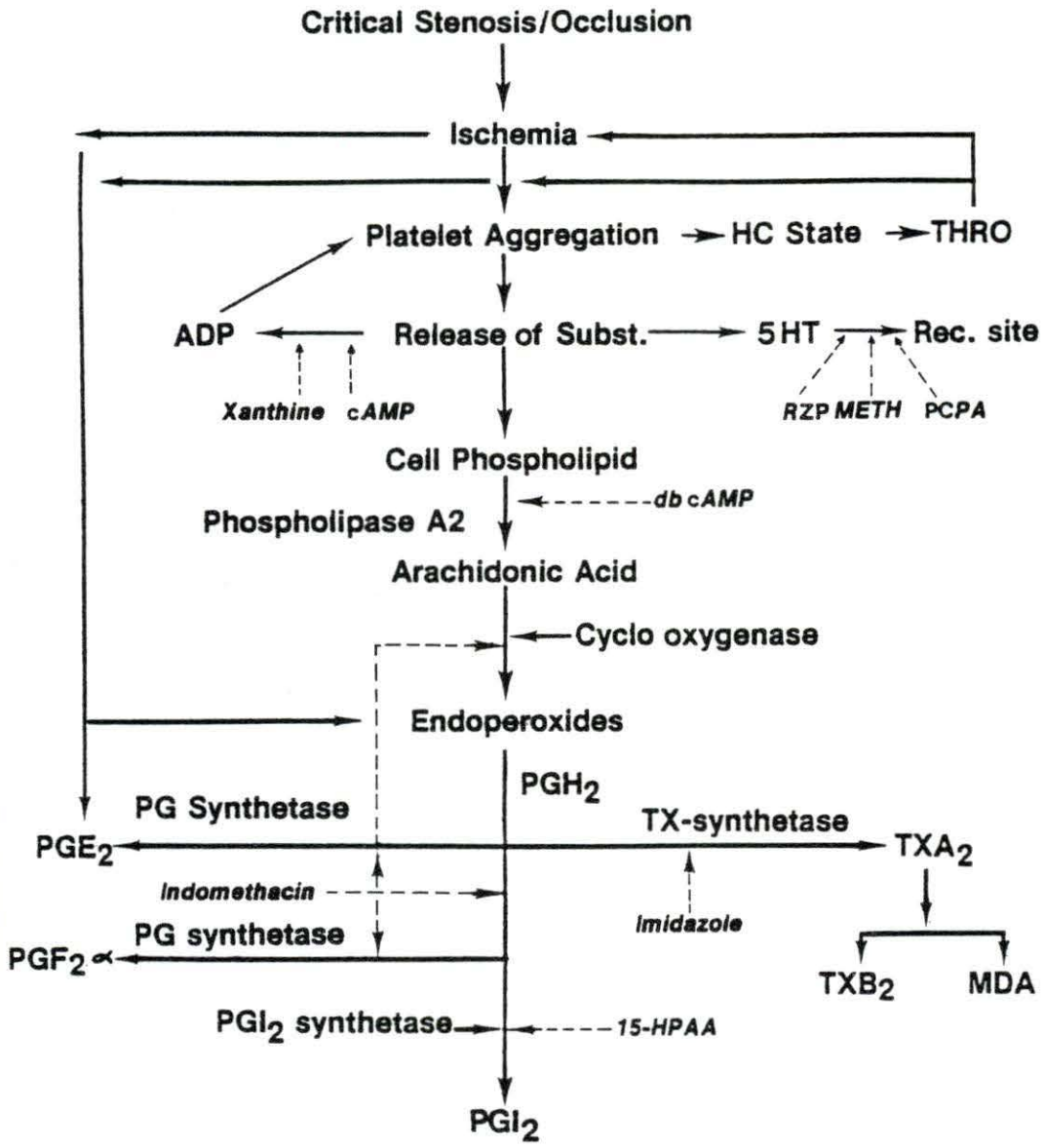


Figure 1. Sequence of events which lead to the endogenous release of various vasoactive substances (reproduced from Ph.D. dissertation by Sheikh, 1981)

and Slighter, 1972; Moncada and Vane, 1977). Dusting et al. (1978) observed vasoconstrictor effects from PGF2-α, TXA2, and the endoperoxide analog UH6619. PGF2-α was a weak vasoconstrictor. TXA2 was more effective in the mesenteric than in the femoral vascular bed, although its action lasted less than two minutes. Studies by Hammarstrom and Falardeau (1977) and Hamberg et al. (1975) suggest that TXA2 stimulates the release of serotonin, a powerful vasoconstrictor of vascular smooth muscle, from blood platelets.

PGI2 is formed in arterial and venous walls and acts to promote vasodilation while inhibiting platelet aggregation (Gryglewski et al., 1976; Bunting et al., 1976; Tateson, 1977; Moncada and Vane, 1978). Moncada and Vane hypothesized that endoperoxides released by the platelets were converted to PGI2 in the microsomal fraction of the intimal cells.

The PGI2-TXA2 Interaction

Two of the most prominent hormones in modulating vessel caliber and blood clotting are PGI2 and TXA2. These prostaglandins are synthesized endogenously in the arterial and venous intima and within blood platelets (Goodman and Gilman, 1975). Though TXA2 and PGI2 both are formed from arachidonic acid via the endoperoxidase system, they have opposite actions (Hammarstrom and Falardeau, 1977; Gorman, 1979). Their reciprocal actions provide a mechanism for regulation of local blood flow within the body.

Numerous experiments suggest that control of thrombogenesis is due to a balance between blood levels of PGI2 and TXA2 (Gryglewski et al., 1976; Moncada and Vane, 1978; Pace-Asciak, 1977; Dusting et al., 1978). Investigations by Miller et al. (1977) showed that TXA2 acted on platelets by depressing cAMP levels. Tateson et al. (1977) and Moncada and Vane (1978) observed that PGI2 promoted increased cAMP levels in platelets. Gorman (1979) further delineated the mechanism behind the PGI2-TXA2 interaction. He stated that TXA2 inhibits adenylate cyclase which depresses cAMP levels and promotes calcium release from platelets, causing their aggregation. Conversely, PGI2 activates the adenylate cyclase system, stimulating high levels of cAMP and sequestering calcium within the platelets, thus preventing aggregation. It appears that TXA2 and PGI2 have opposing effects on platelet cAMP levels, giving a controlled balance system in the regulation of vascular haemostasis (Moncada, 1980).

Selective inhibitors of TXA2 or PGI2 have been used to elucidate the degree and direction of control exhibited by these two hormones on platelet behavior and vasomotor tone. Inhibition of PGI2 resulted in increased platelet aggregation, due to predominant TXA2 effects. Inhibition of cyclooxygenase by indomethacin or meclofinamate promoted the vasoconstrictive effects induced by TXA2 (Dusting et al., 1978). These agents may prevent endogenous prostacyclin synthesis in the vasculature, thus enhancing TXA2-mediated vasoconstriction by reducing the counteracting vasodilation by endogenous PGI2.

During thrombosis, release of TXA2 by platelets has been shown to occur, resulting in vasoconstriction and platelet aggregation (Moncada

and Vane, 1978). Intra-arterial injection of prostacylin may counteract the constrictor effects of TXA2, may inhibit platelet aggregation, and may promote collateral perfusion to a thrombotically occluded vascular bed (Moncada and Vane, 1977). Antagonism of TXA2 formation led to a prolonged bleeding time and promoted vasodilation due to the prevailing actions of PGI2 (Nijkamp et al., 1977; Moncada, 1980).

PGI2, a circulating hormone that is not degraded significantly in the lungs as are many other prostaglandins, has a half-life of 30-40 minutes (Dusting et al., 1978). In this study, we attempted to use PGI2 to promote collateral blood flow during thrombosis.

Acetylcholine (ACh)

ACh was chosen as the second vasodilatory agonist in this project because it is a well-known, powerful vasodilator and has a very brief duration of action (5-7 minutes), an advantage when several agents are to be infused consecutively.

In the canine femoral artery, the response to ACh was reached within 20 to 50 seconds after the beginning, and lasted for 5 to 7 minutes after the cessation, of a continuous infusion (Mons et al., 1970). The dilation response was independent of retrograde propagation of nervous or myogenic impulses along the vascular wall, which implied local control was exerted. ACh causes vasodilation by stimulating muscarinic receptors on those vascular beds having postglanglionic parasympathetic effector cells.

MATERIALS AND METHODS

Experimental Design

A collateral system of vessels supplies blood to the ischemic lower hind leg peripheral bed during partial or complete occlusion of the FA. Figure 2 depicts circulation of the canine lower hind limb. Arterial thrombosis has been shown to inhibit collateral blood flow development more than simple ligation (Schaub et al., 1976). It has been proposed that a thrombosis may modulate the level of ischemia in a distal tissue by releasing either adenosine (Berne, 1970), prostacyclin (Moncada, 1980), serotonin (Schaub et al., 1977a, 1977b), or TXA2 (Schaub et al., 1982).

In this study, acute stenosis and thrombosis of the FA were compared in their abilities to affect collateral blood flow. An attempt was made to improve collateral blood flow during thrombosis by infusion of the vasodilators ACh and PGI2. ACh, a well-known vasodilator, was chosen due to its short duration of action. PGI2 was utilized for its ability to promote collateral blood flow by inhibiting the vasoconstrictor effects of TXA2 and preventing platelet aggregation in a vascular bed downstream of a thrombosed artery (Moncada and Vane, 1978). The mechanisms of action of ACh and PGI2 are different; each was discussed in the Review of Literature.

While four animals provided the data reported in this study, an additional six were utilized in pilot trials to develop surgical procedures

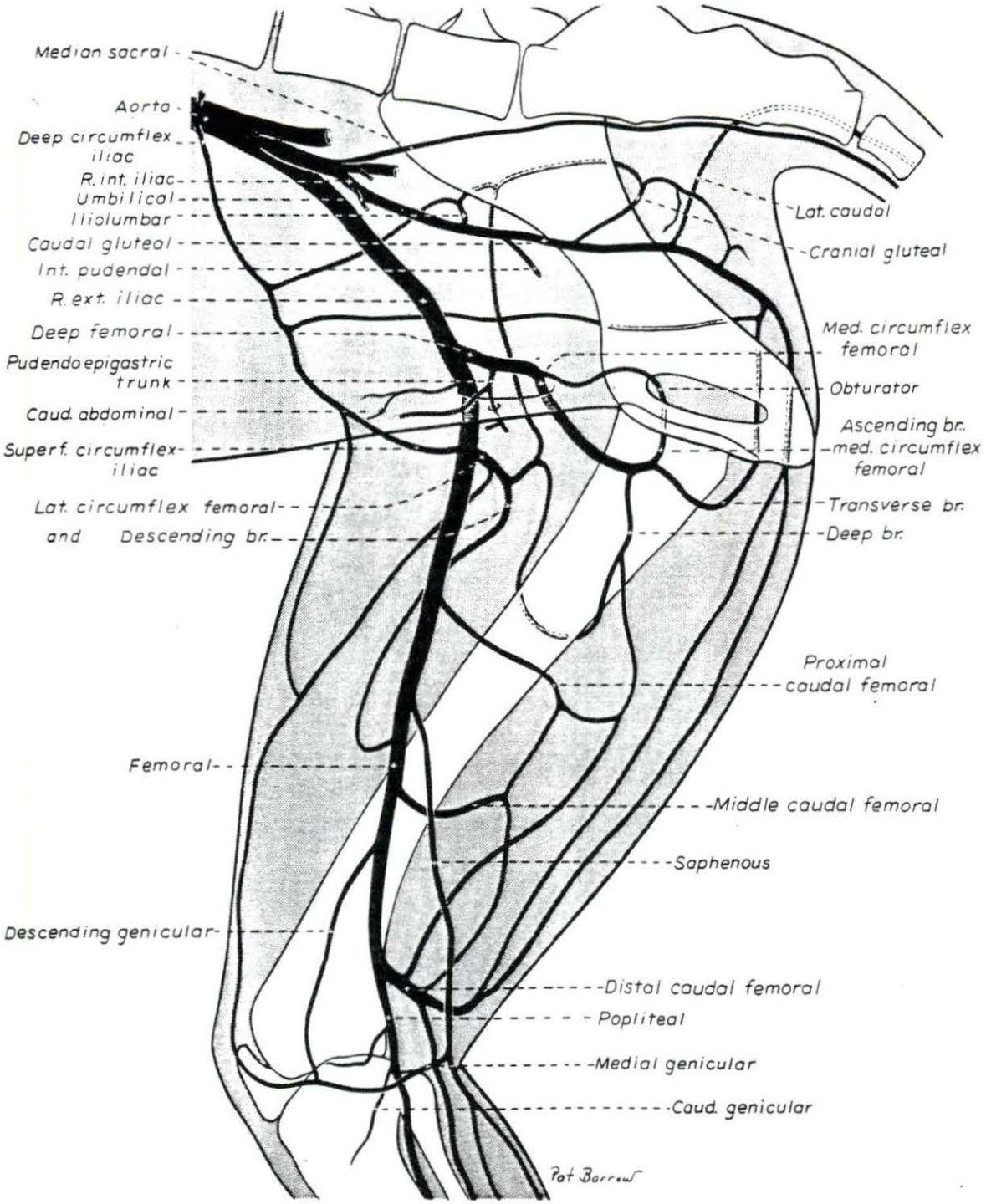


Figure 2. Diagram of the major arteries and some of the potential collateral vessels in the canine hind limb (reproduced from Anatomy of the Dog by Miller et al., 1977)

and to calibrate the measurement systems, and three for perfecting the sequence of administration of RM.

Each animal was subjected to five treatment periods. The treatments, in order of initiation, were 100% stenosis (100-S), 0% stenosis (0-S) or control, thrombosis (Th), thrombosis with ACh infusion (Th+ACh), and thrombosis with PGI2 infusion (Th+PGI2).

The order in which the experimental flow states were induced could not be randomized for several reasons. The 100-S regimen preceded the 0-S (control) period because it in itself established a flowmeter zero reading for QS during the control period. During 100-S, flow was considered to be free from the effects of vasoactive agents. Both 100-S and 0-S states had to be implemented before inducing the third experimental state, Th, since this state was irreversible once the thrombus was formed. After thrombosis was verified both visually and by reduction of QS to essentially zero and after the Th circulatory parameters were measured, collected, and stored, ACh infusion was started. When the response to ACh infusion stabilized, the Th+ACh period was identified and data were recorded.

Since the duration of action of ACh is short and transitory, approximately 5-7 minutes after the cessation of a continuous infusion (Mons et al., 1970), the evaluation of the second agent, PGI2, was possible if a recovery period of approximately 10 min- utes was instituted. It was infused as the final step in the experimental cycle (Th+PGI2) because of its relatively long duration of action, approximately 30 to 40 minutes (Dusting et al., 1978).

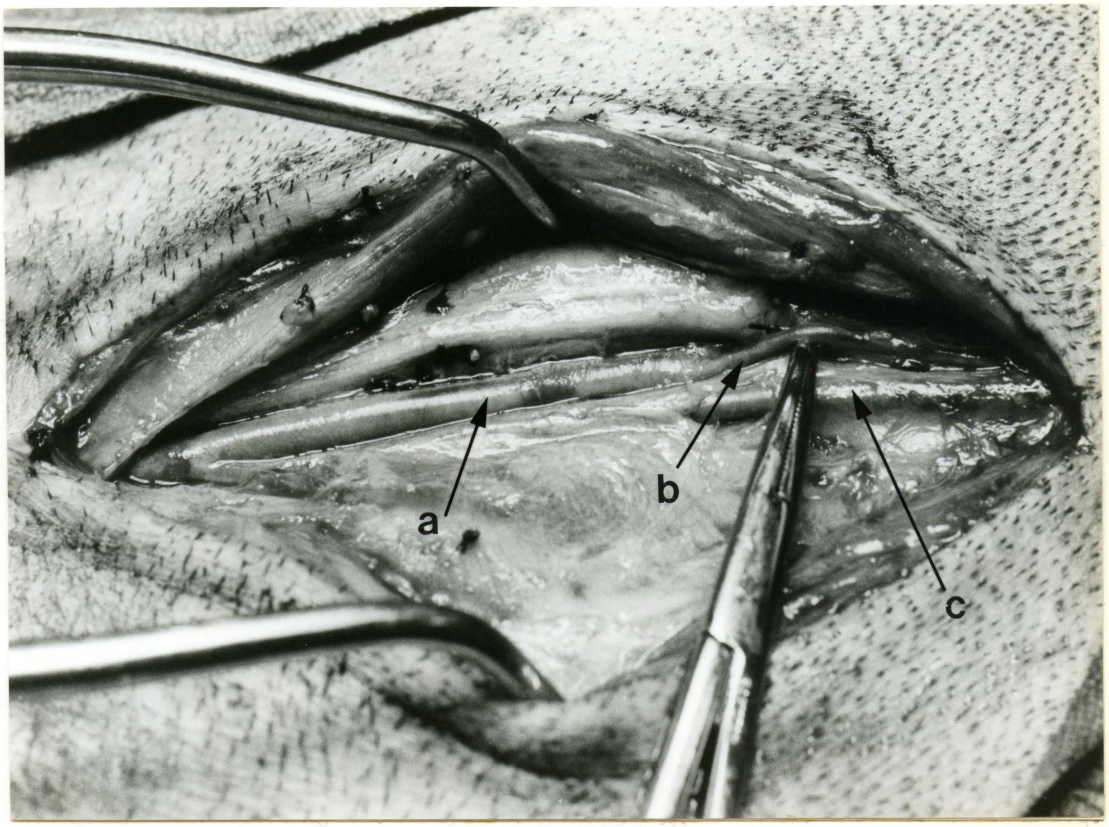
Pressures and flows were measured during each experimental period. A list of formulae used to calculate the experimental parameters is provided in the Appendix. Radioactive microspheres (RM), 15μ in diameter, were injected during each circulatory state in order to determine peripheral and collateral blood flow to the lower hind limbs, arteriovenous shunting, and total kidney blood flow. Tissue samples were collected for subsequent analysis for determination of peripheral blood flow by the radioactive microsphere technique (RMT) at the conclusion of each experiment.

Protocol for Implementing the Experimental Design

To induce the 100-S state, a vascular (bulldog) clamp was placed on the left FA approximately 4-6 cm above the origin of the saphenous artery (SA) and approximately 2 cm downstream from the EMF probe, between the sites for measurement of P_1 and P_2 (Plates 1 and 2). Blood flow and pressures were allowed to equilibrate, and EMF blood flow and arterial and venous pressures were recorded. Immediately after sampling, ^{46}Sc-labelled RM (15μ) were injected into the left ventricle (LV). After reference blood samples were withdrawn from the aortic and external thoracic arterial cannulae (according to Heymann et al., 1977), the vascular clamp was removed and the hind leg circulation was allowed to equilibrate.

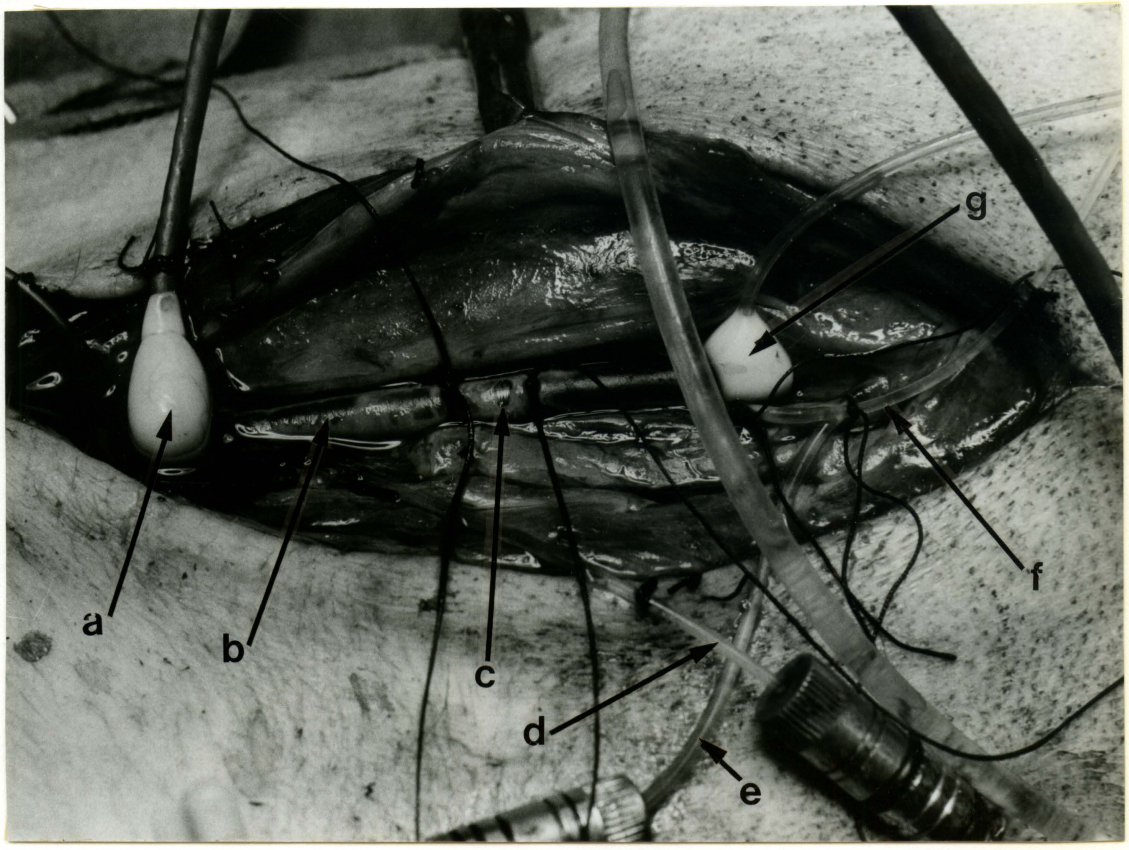
For the 0-S determination (control level), ^{118}Sn-labelled RM (15μ) were injected into the LV immediately after EMF flow and blood pressures

Plate 1. The surgical incision for isolation of the left femoral artery and saphenous artery



a) Femoral Artery
b) Saphenous Artery
c) Femoral Vein

Plate 2. The EMF probe and the vascular occluder in place on the femoral artery with the copper coil device emplaced within the femoral artery



a) EMF Probe
b) Femoral Artery
c) Thrombogenic Copper Coil Device
d) P1
e) P3
f) P2
g) Pneumatic Vascular Occluder

were measured. After recording O-S flow parameters, the FA was double clamped and a transverse incision was made in the left FA at the previously described stenosis site. To induce thrombosis, a polyvinyl tube 15 mm in length and 4 to 5 mm in diameter, heat-shrunk over a thrombus-inciting copper coil, then was inserted into the vessel lumen. The thrombogenic device was constructed by tightly coiling a thin copper wire, .005 mm in diameter, around a mandrel 2.0 to 3.0 mm in diameter. After this was done, a length of heat shrink polyvinyl tubing was secured around the copper coil. This thrombogenic device was trimmed to 15 mm in length and then removed from the mandrel. Thus, the copper wire coil was firmly attached inside the leak-proof polyvinyl tube, and, once implanted in the FA, it would be exposed to flowing blood (Figure 3). The copper wire initiated blood clotting and thrombus formation within the lumen of the thrombogenic device (Kordenat et al., 1972; Kordenat and Kezdi, 1979). Ligatures were installed around the vessel and tube above and below the incision site in order to prevent hemorrhage, and then the occlusions were released, thus allowing blood to flow through the coil. Within 30 to 60 minutes after insertion of the copper coil, a complete thrombotic occlusion of the FA (verified visually and by EMF measurements) had resulted (the Th state). After the thrombus had formed, pressures and zero flow were recorded and ^{85}Sr-labelled RM (15μ) were injected.

Immediately after these measurements were completed, ACh was infused (Sigma Chemical Co., St. Louis, Mo.; 0.123 ml/min, 500 mg/ml) in the aorta. After the response to the ACh had equilibrated, circulatory

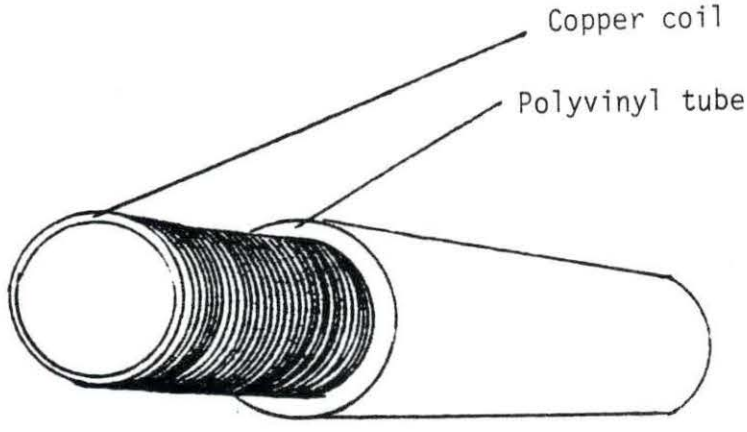


Figure 3. Exploded view of the copper coil device implanted within the left femoral artery to induce thrombosis

parameters were recorded (Th+Ach), then ^{95}Nb-labelled RM (15μ) were injected. After all measurements were completed, the ACh infusion was discontinued and circulatory parameters were allowed to equilibrate.

Finally, the sodium prostacyclin infusion (Burroughs Wellcome Co., Research Triangle Park, NC) was started and maintained at a rate of 0.494 ml/min (4,000 μg/ml) until hemodynamic parameters stabilized, then ^{141}Ce microspheres (15μ) were injected. These were recorded once this state (Th+PGI2) was established.

In order to standardize experimental procedure, the same isotope was used for each stage of the experiment in all subjects, hopefully imposing equal hemodynamic effect.

Pressure and Flow Relationships

Blood pressures were measured at the following locations:

PA: Mean arterial blood pressure in the distal abdominal aorta, above the origin of the common iliac arteries and the several smaller arteries which supply driving pressure to the hind limb collateral vascular bed.

P_1: Mean arterial blood pressure in the FA proximal to the stenosis (driving pressure for flow through the stenosis).

P_2: Mean arterial blood pressure in the FA distal to the stenosis.

P_3: Mean arterial blood pressure measured through a catheter directed downstream in the saphenous artery. This variable estimates

the driving pressure for the peripheral bed during Th, Th+ACh, and Th+PGI2 treatment periods.

PV: Mean venous blood pressure in the femoral vein (venous blood pressure).

Equations and Diagrams

The following equations were used to calculate vascular resistance at the femoral artery stenosis site (R_S) and in the collateral (R_C) and peripheral (R_p) vascular beds.

$$R_S = \frac{P_1 - P_2}{Q_S}$$

$$R_p = \frac{P_2 - PV}{Q_p}$$

$$R_C = \frac{P_a - P_3}{Q_C}$$

Dimensions of these variables are:

P (Pressure) = mm Hg

Q (Flow) = $ml \cdot min^{-1}$

R (Resistance) = $mm\ Hg \cdot min \cdot ml^{-1}$

A schematic representation of the experimental model with sites of pressure and flow measurement is shown in Figure 4.

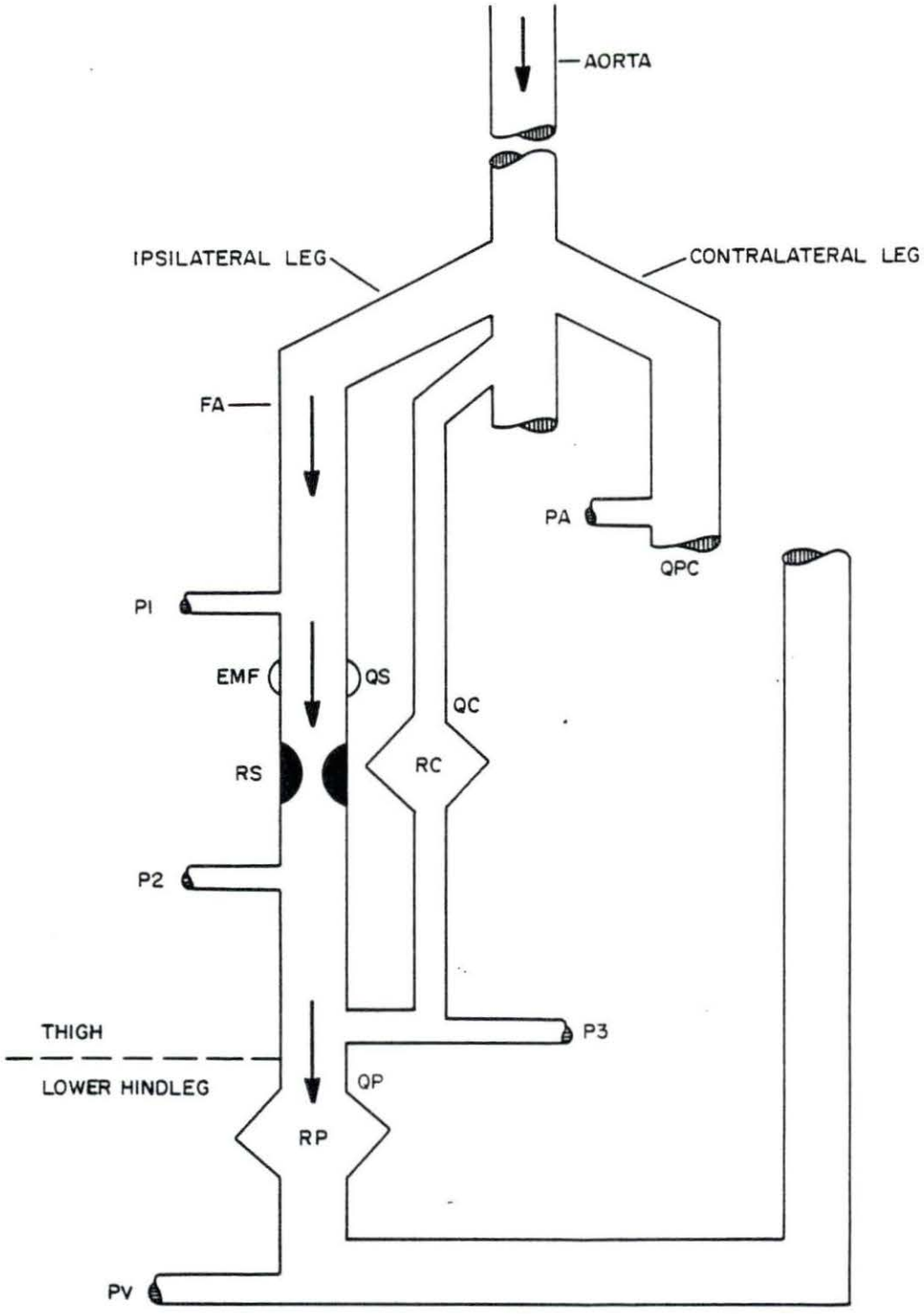


Figure 4. Schematic representation of the canine hind limb with various resistive vascular components and sites of pressure and flow measurements

Measurements

Blood flow measurement

Peripheral blood flow (QP) is a measure of the volume of blood returning from the hind leg distal to the proximal caudal femoral vein. The RMT was used to measure QP; this technique will be explained later in this section.

Collateral blood flow (QC) was calculated to be the difference between QP and QS, QC = QP - QS. When the FA was occluded by mechanical means or by complete thrombus formation, then QC = QP.

EMF blood flow measurement

Blood flow through the FA (QS) was measured by a 3 mm noncannulating EMF transducer (Model 4, In Vivo Metric Systems, Healdsburg, CA) with an electromagnetic flow measuring system (Biotronix Model BL 610, Chicago, IL). Please refer to Plate 2 for a photograph of the EMF probe in place.

Before beginning each experiment, the "cal" mode output of the EMF module, a reference voltage of 0.8 volt approximately equivalent to 150 ml/min., was utilized to adjust recorder sensitivity so that subsequently recorded blood flow experimental data fell within the recording range of the polygraph.

The actual calibration of the EMF system was accomplished just prior to the termination of the experiment. The EMF transducer was calibrated in situ on the FA over a range of flow rates from 10 to 250 ml/min. The setup consisted of inserting a cannula into the FA distal to the EMF

probe. Blood was pumped by the dog's heart into a graduated cylinder by timed collection. Flow rate was controlled by a screw clamp on the tubing. The regression equation to relate flow and the polygraph and computer voltage outputs was developed from 4 to 5 flow rate measurements for each EMF transducer across the flow range to perform the calibrations.

Blood pressure measurement

Arterial blood pressures in the hind limbs were detected by Statham pressure transducers (Model P23Dc, Statham Co., Hato Rey, Puerto Rico). Venous pressure was monitored with a Statham Model PR-23-ID-300 pressure transducer. Each pressure transducer strain gauge diaphragm was mounted at the hydrostatic level of the hind legs. Pressure calibration values from 0 to 200 cm of water pressure were obtained from a water manometer.

Arterial pressure channel sensitivities were adjusted so that 200 cm of water pressure (147 mm Hg) produced +1.3 volt output. Venous channels had a +1.3 volt output for 50 cm of water pressure (37 mm Hg). The calibration procedure included a computer program which converted cm of water pressure into mm Hg pressure. Calibration factors were obtained from a sequential set of pressures applied to all four arterial pressure transducers. The arterial pressure transducers exhibited linear response to pressures greater than 50 mm Hg. Very few arterial pressure values in the data were less than 50 mm Hg.

A separate pressure calibration factor for the low range (less than 50 mm Hg) was determined for the venous transducer. Each pressure

transducer was calibrated by sampling four to five readings across the pressure range, and in the range of voltages received by the computer, the digitized values were converted to pressures (mm Hg) using the equation for a straight line ($y = mx + b$).

To prevent blood clotting in the pressure, infusion, or withdrawal cannulae, a 0.9% saline perfusion system was utilized. It consisted of a Model 1201, Harvard Apparatus Co. peristaltic pump (Harvard Apparatus Co., Millis, MA). The pump forced saline through a manifold system into all indwelling catheters when they were not being used for pressure measurements or infusions. The system was connected through a four-way stopcock to each catheter junction.

Data Processing

Pressure and flow information was recorded on a multi-channel polygraph (Grass Model 7, Grass Instrument Co., Quincy, MA). Each pressure and flow channel of the polygraph was electrically zeroed and balanced at the onset of each experiment in order to accurately set baselines for pressure and flow measurements. Flow and pressure information available at the output of the polygraph amplifiers was digitized through a custom-built analog-to-digital converter and transmitted into a PET 2032 professional microcomputer (Commodore Business Machines (CBM) Inc., Santa Clara, CA). Digitized data were stored on 5 1/4" floppy discs by utilizing a CBM Model 8050, Dual Disc Drive. A 5-second period of pressure and

flow information was sampled at 20 samples per second during each experimental treatment.

At the completion of the in vivo portion of each trial, the subject was euthanized with sodium pentobarbital (Sleep-Away, 1 ml/5 lb body weight, I.V., Fort Dodge Laboratories, Fort Dodge, IA), and the RM containing tissues of the lower hind limbs (muscle, bone, skin and paw), and the lungs and kidneys were isolated and saved for tissue perfusion determinations.

The Radioactive Microsphere Technique (RMT)

In the RMT, a known number RM of 15μ were injected into the LV of the heart for each step in the experiment. Uniform mixing of the RM with blood took place in the LV, and the mixture then was transported via the systemic arteries to all parts of the body, including the hind limb tissues of interest and the lungs and the kidneys.

Total lower limb peripheral blood flow (QP) was calculated from the sum of the individual tissue perfusion rates for muscle, bone, skin and paw. The RMT enabled one to detect not only any change in the rate of blood flow to each tissue type, but also in QP and QC to the lower hind limb, at each stage during the experiment.

Arterio-venous (A-V) shunting of the RM was calculated by determining RM entrapment in the lungs according to the model cited in Wagner et al., 1969). The fraction of shunted blood can be obtained from the following formula:

$S = \frac{Q_1}{Q}$, where

S = fraction of blood flow passing through shunts

Q_1 = the quantity (counts per minute) of RM in the lungs

Q = the counts per minute of the total injected dose

Q_1 represents microspheres which passed through or around systemic capillary beds and which reached the lungs by way of the venous system.

Kidney blood flow rates were calculated in order to verify that our measurements compared favorably with the normal values published by Nathanson and Jackson (1975) and Buckberg et al. (1971).

In each ampule, radionuclide-labelled 15μ microspheres were suspended in 10% Dextran to which a few drops of 5% Tween 80 (polyoxyethylene 80 sorbitan monooleate) were added by the manufacturer to prevent microsphere aggregation (Heymann et al., 1977). Five nuclides utilized in this study were: scandium (^{46}Sc), tin (^{113}Sn), strontium (^{85}Sr), niobium (^{95}Nb), and cerium (^{141}Ce). New England Nuclear Company (Boston, MA) supplied the ^{113}Sn containing microspheres, and 3M Company (St. Paul, MN) supplied the other four.

Prior to experimentation, microspheres were systematically examined for aberrant or aggregated RM. Aliquots were examined under a microscope at 100x. A drop of RM was precisely diluted with water; the mixture was placed on a slide. An optical measuring grid was used to determine average microsphere size. All samples were found to be within the acceptable range (15μ $\pm$ 5μ) specified by the manufacturers.

In order to calculate the correct volume of RM suspension to be injected for each nuclide, the amplitudes of the energy spectrum of each isotope were determined on a Model 1282 Compugamma Multichannel Pulse Height Analyser (LKB Wallac, Gaithersburg, MD). After the spectra were determined, the specific spectral regions for counting each isotope were established. The number of counts per minute per microsphere were determined by transferring 10-50 RM onto microscope slide coverslips by streak application. The number of RM on each coverslip was counted under a microscope. Each coverslip then was fragmented and placed within a 12 x 75 mm polyethylene culture tube (Number 14-956-10, Fischer Scientific Co., Pittsburgh, PA), positioned in the counter and measured for radioactivity.

Immediately before injecting RM into the subject, the 5 ml vial containing the RM was mixed vigorously for 10 minutes in a mixer (Model 1290, Lab Line Instruments Co., Melrose Park, IL) to ensure uniform suspension of the RM (Heymann et al., 1977).

Reference blood samples (RBS) were employed to check for uniformity of RM distribution throughout the circulatory system. RBS collected from two widely separated withdrawal sites from the animal should contain nearly equal numbers ($\pm$5%) of microspheres if adequate mixing and dispersal of the RM had occurred. The RBS were withdrawn from two sites, the external thoracic artery and the right FA starting 30 seconds prior to the onset of RM infusion and ending two minutes post infusion. Blood was simultaneously withdrawn at 2.50 ml/min into separate 10 ml glass syringes by a dual infusion/withdrawal pump (Model 946, Harvard Apparatus

Co., Inc., Millis, MA). The collected RBS were transferred to 12 x 75 mm polyethylene culture vials and counted for radioactivity.

Withdrawing a measurable number of RM at a known, constant flow rate also provided a standard for calculating the flow rates of the selected tissues and organs. The calculation of flow using this reference technique was performed by comparing the number of RM in tissue with the number of RM collected in the RBS for each RM injection according to the following formula:

$$Q = \frac{Q_{ar} x I_t}{I_{ar}}, \text{ where}$$

Q = unknown organ flow in ml/min

Q_{ar} = withdrawal rate for arterial reference sample in ml/min

I_t = radioactivity in tissue or organ sample counter per minute (cpm)

I_{ar} = radioactivity in arterial reference sample (cpm)

Animals

Four mongrel dogs approximately two years of age were obtained from Laboratory Animal Resources, Iowa State University. All subjects were in excellent health and ranged in weight from 25 to 30 kilograms.

Surgical Procedure

All subjects were anesthetized with 5% sodium pentobarbital (1 ml/kg body weight; Fort Dodge Laboratories, Fort Dodge, IA) via injection into the cephalic vein. Proper depth of anesthesia was maintained by additional injections as needed. An endotracheal tube was inserted to maintain an open airway. The animals were placed in dorsal recumbancy on a warm water heating pad (Model K 20/64N series, Hamilton Aquamatic K Module, Hamilton Industries, Cincinnati, OH) to maintain body temperature. Lactated Ringers solution with 5% sodium bicarbonate were constantly administered at a rate of 3.5 ml/min I.V. The electrocardiogram was monitored with a Sanborn 780-6A Viso Scope (Hewlett-Packard, Corvallis, OR).

The surgical sites were shaved. A cranio-caudal incision was made in the left mid-cervical region along the jugular furrow. The carotid sheath was identified and opened, exposing the left carotid artery for cannulation. An incision was made in the carotid artery, and a cardiac catheter was inserted and advanced towards the heart. The tip of this size 7 French catheter was bent by heating the tip to facilitate guiding it into the left ventricle. The catheter, attached to a recording pressure transducer, was advanced until a typical left ventricular pressure waveform was observed, indicating that the catheter tip had passed through the aortic valve into the left ventricle. The exposed portion of the catheter then was secured to the carotid artery with a ligature.

Through it aortic pressure was detected with a Statham Model 23Dc pressure transducer and displayed on the Viso Scope, and RM could be injected through it into the left ventricle (LV) later in the experiment.

A skin incision 6 to 8 cm in length was made cranio-caudally midway between the point of the left shoulder and the manubrium sterni. After retracting the fascia of the thoracic inlet, the external thoracic artery was exposed. After applying vascular occluders, an incision was made and a cannula was inserted retrograde for approximately 3 to 4 cm. Through this cannula, RBS could be withdrawn.

An initial skin incision was made extending proximodistally over the middle 2/3 of the left medial femoral region. A fascial incision was made along the caudal border of the sartorius muscle, parallel to the course of, and exposing, the FA and vein (see Plate 1). After these vessels were exposed, pressure cannulae were inserted retrograde into the origins of the left proximal caudal femoral artery (PCFA) and the SA for measurement of blood pressure in the FA above and below the sites intended for stenosis and thrombosis. Each cannula tip was advanced proximally almost flush with the FA lumen and secured with ligatures. Lower hind leg arterial driving pressure, P_3 (distal to the FA stenosis) was monitored by inserting a second pressure cannula through the incision in the SA in an antegrade direction away from the parent vessel.

Proximal from the origin of the PCFA, the fascia surrounding the FA was dissected free and a noncannulating EMF probe (Model 4, In Vivo Metric Systems, Healdsburg, CA) was inserted around the FA to measure blood flow (QS) at the stenosis/thrombosis site. A pneumatic vascular

occluder (Model OC4, In Vivo Metric Systems, Healdsburg, CA) was similarly emplaced on the FA 2 to 3 cm above the origin of the SA and approximately 2 cm downstream from the EMF probe. It was used repeatedly to establish zero blood flow (baseline setting) in the FA. Before initiating the experimental treatment, the hyperemic response elicited by occluding the FA was allowed to disappear. During the 100-S interval of the experiment, the FA was clamped midway between the flowmeter and vascular occluder to produce a complete stenosis.

To initiate a thrombosis, a vascular incision was made at this same site to insert a thrombogenic device into the left FA (the thrombogenic procedure was discussed earlier in this section).

A similar skin incision was made on the medial surface of the contralateral (right) thigh. The right femoral vein (FV) was isolated between the proximal caudal femoral vein (PCFV) and the saphenous vein (SV), and a cannula was advanced distally to monitor lower hind limb venous blood pressure and to obtain blood for radioimmunoassay (RIA) procedures. At approximately the same level as for the venous cannulation, a double lumen cannula was inserted into the FA and was advanced proximally until its tip resided within the descending aorta at a point just below the renal arteries. One lumen of the cannula was used for infusion of PGI2 or ACh. The second lumen was used to monitor pressure in the descending aorta (PA).

A hollow polyvinyl tube 15 mm in length and 2-3 mm inside diameter containing a tightly wound copper coil was inserted into the left FA to initiate a thrombosis (Plate 2).

Tissue Assay

Immediately after the completion of each in vivo experiment, the animals were euthanized and appropriate RM-containing tissues were collected and frozen at -5°C for later processing and analysis. Both hind limbs were removed below the stifle. Only those hind limb muscles below the stifle joint were taken; thigh muscles were carefully excluded. Lower hind limb tissues were divided into four portions, muscle, bone, skin and paw, and frozen for later perfusion analyses. Whole lungs and kidneys were also removed for assessment of RM shunting and renal blood flow, respectively.

All tissues were weighed (wet), desiccated in a drying oven (Model 31543 GCA, Precision Scientific, Chicago, IL), and reweighed (dry) on a torsion balance (Model Sc 15, Welch Scientific Co., Chicago, IL). While only wet weights were used for calculations of tissue perfusion, desiccated weights were used to ensure that tissue dehydration occurred which was adequate to avoid spattering during ashing. Each sample was ashed to reduce sample size at 325°C in a muffle furnace (Model F11625, Thermolyne Corporation, Dubuque, IA) fitted with a temperature control unit to maintain a constant temperature (Model 292P, Barber-Colman Co., Rockford, IL). Ashing was continued until samples were reduced to white ash, indicating that all combustible and volatile elements were removed. The RM remained in the residue. After ashing, each specimen was very carefully transferred to labelled 12 x 75 mm polyethylene culture tubes within a vented, paper-lined hood, approved for use with radioactive materials.

Samples were crushed and ground to a fine consistency. A clear plexiglass box 2 feet wide by 1.5 feet in height and depth, open in the front for access, was utilized during transfer to prevent aerosolized ash from contaminating the hood.

Tubes were transferred to the multichannel gamma counter and samples were measured for radioactivity. Raw counts were analyzed on the microcomputer according to the procedure of Heymann et al. (1977).

Gamma counter results were entered into the micrcomputer keyboard to calculate individual tissue perfusion and total QPC. Also, signal averaging of five second sampling intervals of digitized dynamic EMF flow and pressure waveforms was done to obtain mean values. Peripheral and collateral bed and stenosis hemodynamic parameters were calculated by utilizing mean values of flow, pressures, and radioactive microsphere perfusion data (please refer to the Appendix for a list of formulae). Previously stated formulae were utilized by the microcomputer to process the experimental hemodynamic data stored on disc and to arrive at the final parameter results.

RESULTS AND DISCUSSION

The objective of this investigation was to study the effects of thrombosis and stenosis upon the hind limb collateral and peripheral circulations and to determine the effects of ACh or PGI2 upon these same vascular channels during thrombosis.

Pressures and flows were measured to establish hemodynamic relationships in the ipsilateral leg (the leg in which the stenosis was applied). Contralateral leg peripheral flow (QPC) was determined for comparison purposes. The values determined are presented in Tables 1 through 17 and Figures 5 through 14. In this set of figures (5-14), the points representing the individual data points for each treatment are the important features and that the lines connecting the points do not reflect actual values (during transition from one state to the next).

Hind Limb Hemodynamics

The data from this experiment were not pooled, but rather, the individual animal responses are discussed separately for three reasons. First, the low number of animals in this study (n=4) combined with the wide range of subject responses indicated that examination of individual responses would most accurately reflect the results. Results were analyzed by use of one-way analysis of variance (Snedecor and Cochran, 1976). We did not find evidence that, during thrombosis, either ACh or PGI2 significantly changed QC or QP at the five percent confidence level ($p < 0.05$). Second, the nature of this investigation, with its highly

Table 1. Vascular hemodynamics in the hind limbs of dog 10 in each treatment period

Parameter[a]	Treatment				
	100%	0%	Th	Th+ACh	Th+PGI2
QPC	34.55	74.79	51.66	49.53	49.26
QP	12.16	11.35	2.07	1.27	5.15
RP	6.23	9.46	40.46	84.72	15.52
DPP	75.85	107.43	83.77	107.59	79.93
QC	1.11	-13.60	11.19	- 9.90	- 1.57
RC	50.40	- 3.72	6.84	- 4.68	-48.23
DPC	59.93	50.53	76.55	46.37	71.03
QS	11.05	24.95	- 9.12	11.17	6.72
RS	5.43	- 0.04	- 5.69	9.55	4.98
DPS	60.02	- 0.94	51.96	106.70	33.53

Definitions of parameters:

QPC	Peripheral blood blow in the contralateral leg
QP	Peripheral blood flow in the ipsilateral leg
RP	Peripheral resistance
DPP	Driving pressure peripheral bed
QC	Collateral blood flow
RC	Collateral resistance
DPC	Driving pressure collateral bed
QS	Stenosis blood flow
RS	Stenosis resistance
DPS	Driving pressure stenosis area

[a]Flows expressed in ml/min, resistances expressed in mm Hg/min/ml, pressures expressed in mm Hg.

Table 2. Hemodynamic parameters (blood pressures) versus experimental condition for dog 10

Hemodynamic parameter[a]	Stenosis		100% thrombosis-treatment		
	100%	0%	None	ACh	PGI2
P1	112.34	139.02	159.60	204.91	148.54
P2	52.32	139.96	107.74	98.21	115.01
P3	78.30	108.71	85.86	109.75	81.02
PA	134.24	159.25	162.41	156.12	152.06
PV	2.45	1.28	2.09	2.15	1.09

Definitions of parameters:

P1 Mean arterial blood pressure in the FA proximal to the stenosis (driving pressure for flow through stenosis)

P2 Mean arterial blood pressure in the FA distal to the stenosis

P3 Mean arterial blood pressure in a branch at the level of the DCFA (potential collateral blood flow)

PA Mean arterial blood pressure

PV Mean venous blood pressure at the FV (venous blood pressure)

[a]Pressures expressed in mm Hg.

Table 3. Vascular hemodynamics in the hind limbs of dog 11 in each treatment period

Parameter[a]	Treatment				
	100%	0%	Th	Th+ACh	Th+PGI2
QPC	17.66	138.18	16.14	71.09	20.09
QP	13.02	14.85	22.64	13.54	8.38
RP	3.58	1.71	2.79	3.53	4.17
DPP	46.73	25.41	63.27	47.83	34.97
QC	24.45	-86.41	33.55	25.46	14.58
RC	2.54	- 0.96	1.77	4.67	5.36
DPC	62.15	82.64	59.23	72.11	78.22
QS	-11.43	101.27	-10.91	-11.92	- 6.20
RS	-10.26	- 0.06	- 7.20	- 8.59	- 5.47
DPS	117.27	- 5.25	78.47	102.38	33.87

Definitions of parameters:

QPC	Peripheral blood flow in the contralateral leg
QP	Peripheral blood flow in the ipsilateral leg
RP	Peripheral resistance
DPP	Driving pressure peripheral bed
QC	Collateral blood flow
RC	Collateral resistance
DPC	Driving pressure collateral bed
QS	Stenosis blood flow
RS	Stenosis resistance
DPS	Driving pressure stenosis area

[a]Flows expressed in ml/min, resistances expressed in mm Hg/min/ml, pressures expressed in mm Hg.

Table 4. Hemodynamic parameters (blood pressures) versus experimental condition for dog 11

Hemodynamic parameter[a]	Stenosis		100% thrombosis-treatment		
	100%	0%	None	ACh	PGI2
P1	116.00	108.98	118.18	115.08	118.28
P2	- 1.27	114.23	39.71	12.70	84.41
P3	51.76	34.57	68.24	51.79	39.71
PA	113.92	117.21	127.48	128.90	117.93
PV	5.03	9.15	4.97	3.96	4.73

Definitions of parameters:

P1 Mean arterial blood pressure in the FA proximal to the stenosis (driving pressure for flow through stenosis)

P2 Mean arterial blood pressure in the FA distal to the stenosis

P3 Mean arterial blood pressure in a branch at the level of the DCFA (potential collateral blood flow)

PA Mean arterial blood pressure

PV Mean venous blood pressure at the FV (venous blood pressure)

[a]Pressures expressed in mm Hg.

Table 5. Vascular hemodynamics in the hind limbs of dog 12 in each treatment period

Parameter[a]	Treatment				
	100%	0%	Th	Th+ACh	Th+PGI2
QPC	22.49	23.33	12.18	5.63	11.12
QP	26.57	21.53	30.74	22.08	15.98
RP	2.80	3.09	2.50	3.23	4.52
DPP	74.48	66.55	77.11	71.41	72.36
QC	25.53	-15.63	30.24	20.26	15.62
RC	1.20	- 3.34	1.60	2.29	3.71
DPC	30.66	52.18	48.25	46.35	57.93
QS	1.04	37.16	0.50	1.82	0.36
RS	15.79	0.39	81.18	13.62	46.13
DPS	16.43	14.68	40.59	24.79	16.60

Definitions of parameters:

QPC	Peripheral blood flow in the contralateral leg
QP	Peripheral blood flow in the ipsilateral leg
RP	Peripheral resistance
DPP	Driving pressure peripheral bed
QC	Collateral blood flow
RC	Collateral resistance
DPC	Driving pressure collateral bed
QS	Stenosis blood flow
RS	Stenosis resistance
DPS	Driving pressure stenosis area

[a]Flows expressed in ml/min, resistances expressed in mm Hg/min/ml, pressures expressed in mm Hg.

Table 6. Hemodynamic parameters (blood pressures) versus experimental condition for dog 12

Hemodynamic parameter[a]	Stenosis		100% thrombisos-treatment		
	100%	0%	None	ACh	PGI2
P1	107.66	105.74	125.01	117.91	122.64
P2	91.23	91.05	84.42	93.11	106.03
P3	80.18	72.77	82.24	76.17	78.46
PA	110.85	124.96	130.49	122.52	136.40
PV	5.70	6.22	5.13	4.75	6.10

Definitions of parameters:

P1 Mean arterial blood pressure in the FA proximal to the stenosis (driving pressure for flow through stenosis)

P2 Mean arterial blood pressure in the FA distal to the stenosis

P3 Mean arterial blood pressure in a branch at the level of the DCFA (potential collateral blood flow)

PA Mean arterial blood pressure

PV Mean venous blood pressure at the FV (venous blood pressure)

[a]Pressures expressed in mm Hg.

Table 7. Vascular hemodynamics in the hind limbs of dog 13 in each treatment period

Parameter[a]	Treatment				
	100%	0%	Th	Th+ACh	Th+PGI2
QPC	22.75	28.43	17.40	153.26	62.22
QP	13.50	36.25	10.40	13.50	7.33
RP	3.41	2.36	7.00	3.64	7.03
DPP	46.17	85.71	72.90	49.18	51.56
QC	12.10	-52.69	-80.85	12.14	11.22
RC	8.01	- 0.75	- 0.65	3.47	7.67
DPC	73.54	39.55	52.69	42.07	86.05
QS	1.40	88.94	91.25	1.36	- 3.89
RS	28.86	0.25	0.01	12.33	-14.65
DPS	40.41	22.37	1.18	16.78	56.97

Definitions of parameters:

QPC	Peripheral blood flow in the contralateral leg
QP	Peripheral blood flow in the ipsilateral leg
RP	Peripheral resistance
DPP	Driving pressure peripheral bed
QC	Collateral blood flow
RC	Collateral resistance
DPC	Driving pressure collateral bed
QS	Stenosis blood flow
RS	Stenosis resistance
DPS	Driving pressure stenosis area

[a]Flows expressed in ml/min, resistances expressed in mm Hg/min/ml, pressures expressed in mm Hg.

Table 8. Hemodynamic parameters (blood pressures) versus experimental condition for dog 13

Hemodynamic parameter[a]	Stenosis		100% thrombosis-treatment		
	100%	0%	None	ACh	PGI2
P1	132.56	132.94	133.00	143.25	134.83
P2	115.78	110.57	131.81	107.53	77.85
P3	55.04	96.42	85.74	12.48	61.95
PA	103.74	135.97	138.44	142.42	148.00
PV	12.48	10.70	12.84	9.88	10.39

Definitions of parameters:

P1 Mean arterial blood pressure in the FA proximal to the stenosis (driving pressure for flow through stenosis)

P2 Mean arterial blood pressure in the FA distal to the stenosis

P3 Mean arterial blood pressure in a branch at the level of the DCFA (potential collateral blood flow)

PA Mean arterial blood pressure

PV Mean venous blood pressure at the FV (venous blood pressure)

[a]Blood pressures expressed as mm Hg.

Table 9. Kidney blood flow (ml/min) in each treatment period

	Treatment				
Subject	100%	0%	Th	Th+ACh	Th+PGI2
10	458.0	237.0	291.0	183.0	469.0
11	347.0	210.0	316.0	251.0	424.0
12	395.0	454.0	366.0	344.0	453.0
13	372.0	421.0	349.0	391.0	455.0

Table 10. Muscle blood flow (ml/min) to the ipsilateral, left, leg in each treatment period

	Treatment				
Subject	100%	0%	Th	Th+ACh	Th+PGI2
10	1.69	3.35	0.43	0.27	0.96
11	1.76	1.93	3.48	2.77	1.31
12	7.10	5.56	8.55	5.06	----[a]
13	2.31	8.44	1.48	3.89	1.27

[a]Incomplete data.

Table 11. Muscle blood flow (ml/min) to the contralateral, right, leg in each treatment period

	Treatment				
Subject	100%	0%	Th	Th+ACh	Th+PGI2
10	12.79	21.03	22.83	20.57	5.75
11	1.68	1.74	2.96	23.09	2.53
12	6.69	7.29	3.79	1.15	1.08
13	4.73	7.06	4.89	55.50	6.23

Table 12. Skin blood flow (ml/min) to the contralateral, right, leg in each treatment period

	Treatment				
Subject	100%	0%	Th	Th+ACh	Th+PGI2
10	4.38	23.00	----[a]	2.62	6.01
11	3.68	2.98	3.60	4.03	7.78
12	4.21	3.08	1.68	1.65	3.97
13	4.68	4.89	2.88	10.85	9.17

[a]Incomplete data.

Table 13. Skin blood flow (ml/min) to the ipsilateral, left, leg in each treatment period

	Treatment				
Subject	100%	0%	Th	Th+ACh	Th+PGI2
10	0.96	0.40	0.27	0.23	0.51
11	2.42	1.55	1.33	0.96	0.70
12	7.39	4.17	3.11	3.61	2.93
13	1.23	2.01	0.67	0.70	0.41

Table 14. Bone blood flow (ml/min) to the contralateral, right, leg in each treatment period

	Treatment				
Subject	100%	0%	Th	Th+ACh	Th+PGI2
10	0.65	1.12	0.54	0.26	10.16
11	0.89	0.83	1.31	0.78	9.16
12	2.48	3.04	1.55	1.41	2.30
13	2.60	2.43	0.95	3.83	15.09

Table 15. Bone blood flow (ml/min) to the ipsilateral, left, leg in each treatment period

	Treatment				
Subject	100%	0%	Th	Th+ACh	Th+PGI2
10	2.72	2.29	0.55	0.30	1.33
11	2.77	2.60	5.15	2.54	2.14
12	2.79	3.60	3.08	3.57	5.50
13	2.29	4.58	2.28	2.43	1.34

Table 16. Paw blood flow (ml/min) to the ipsilateral, left, leg in each treatment period

	Treatment				
Subject	100%	0%	Th	Th+ACh	Th+PGI2
10	2.11	0.36	0.14	0.08	0.52
11	1.19	1.87	2.17	0.89	0.64
12	1.44	1.33	2.89	2.46	5.12
13	1.71	3.96	1.51	0.85	1.01

Table 17. Paw blood flow (ml/min) to the contralateral, right, leg in each treatment period

	Treatment				
Subject	100%	0%	Th	Th+ACh	Th+PGI2
10	0.23	0.19	0.12	0.11	11.42
11	3.48	51.15	1.65	4.60	----[a]
12	0.89	0.91	0.46	0.42	2.28
13	1.58	2.17	1.22	1.94	7.12

[a]Incomplete data.

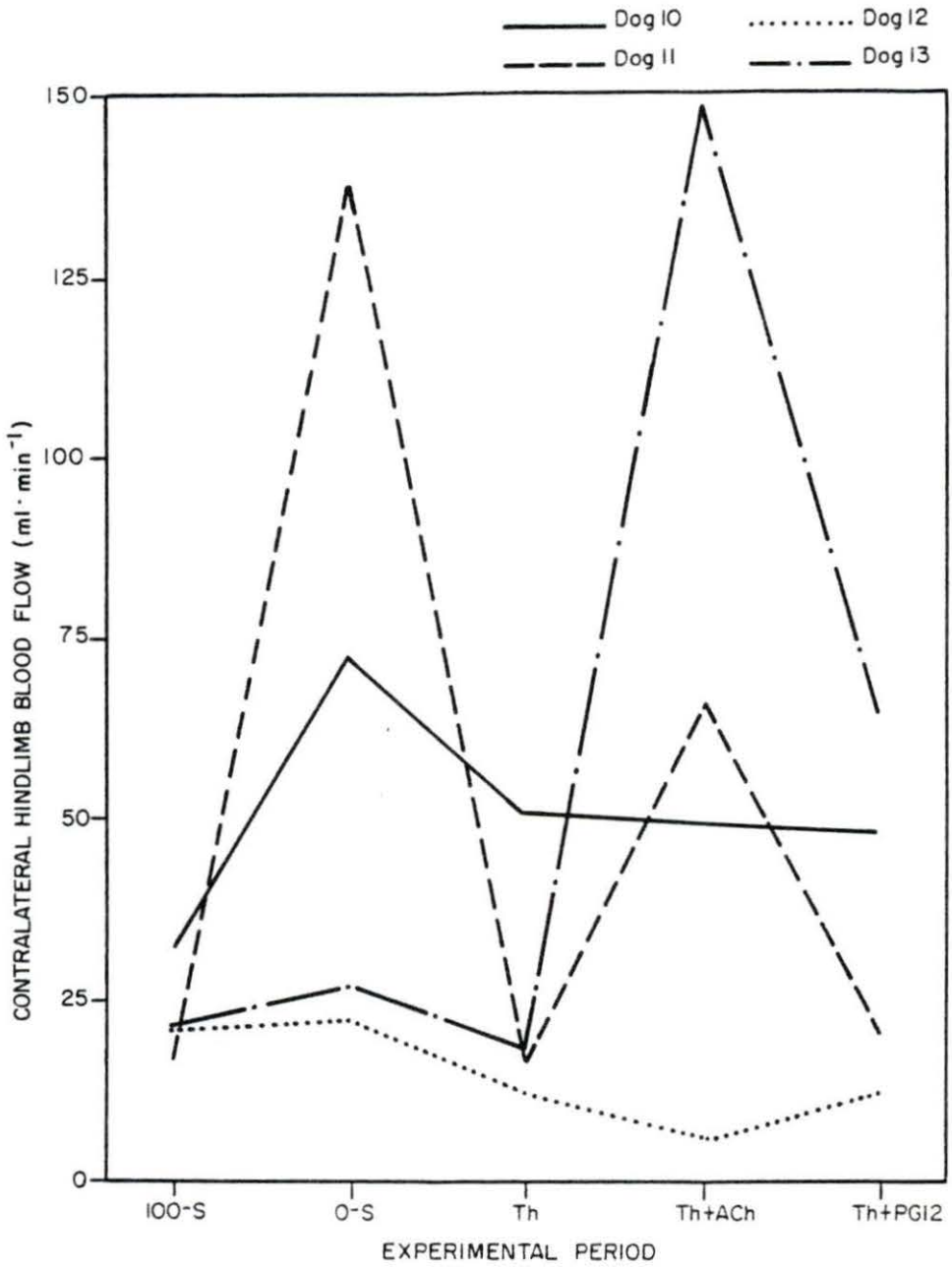


Figure 5. Peripheral blood flow in response to the experimental treatments in the contralateral, right, hind limb

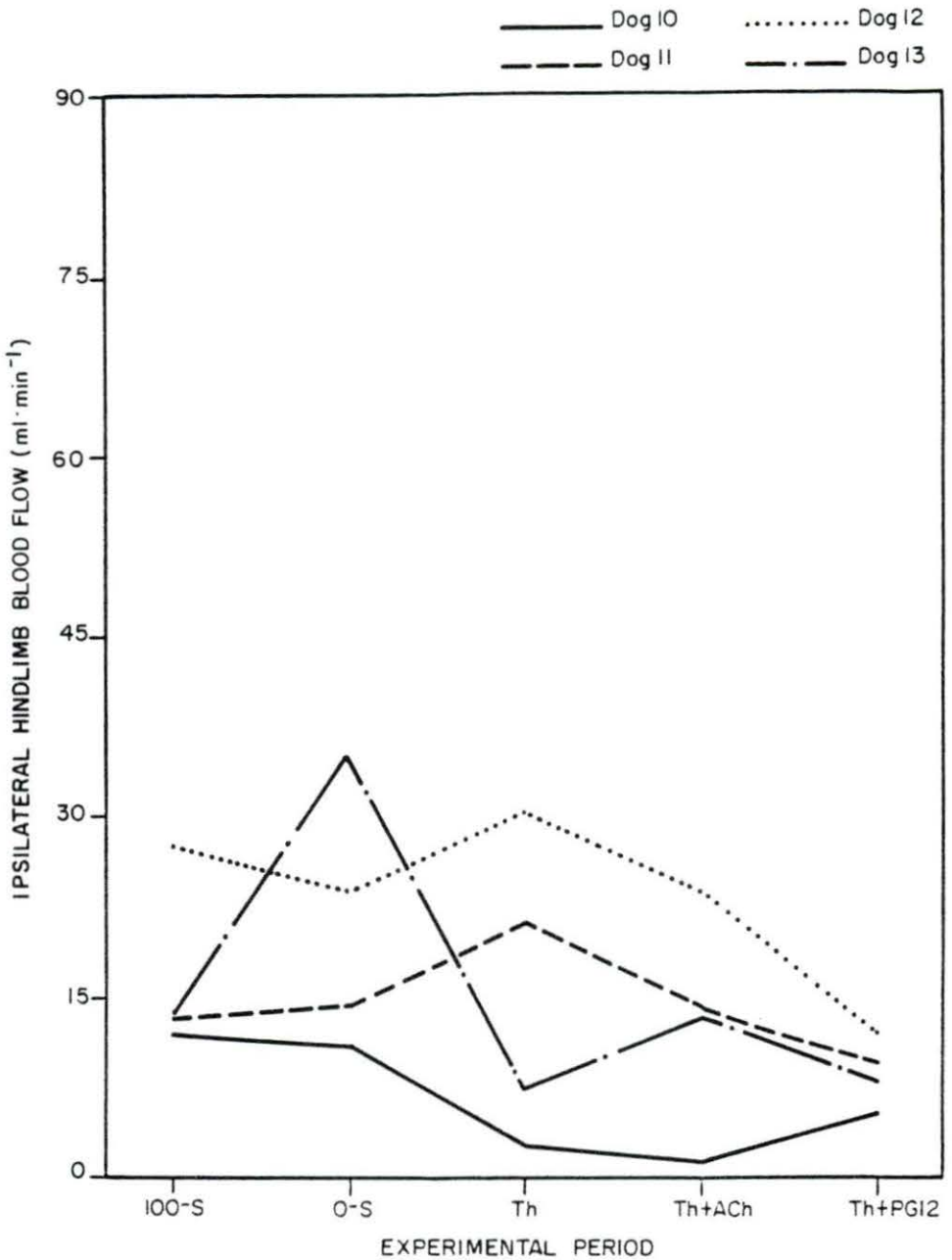


Figure 6. Peripheral blood flow in response to the experimental treatments in the ipsilateral, left, hind limb

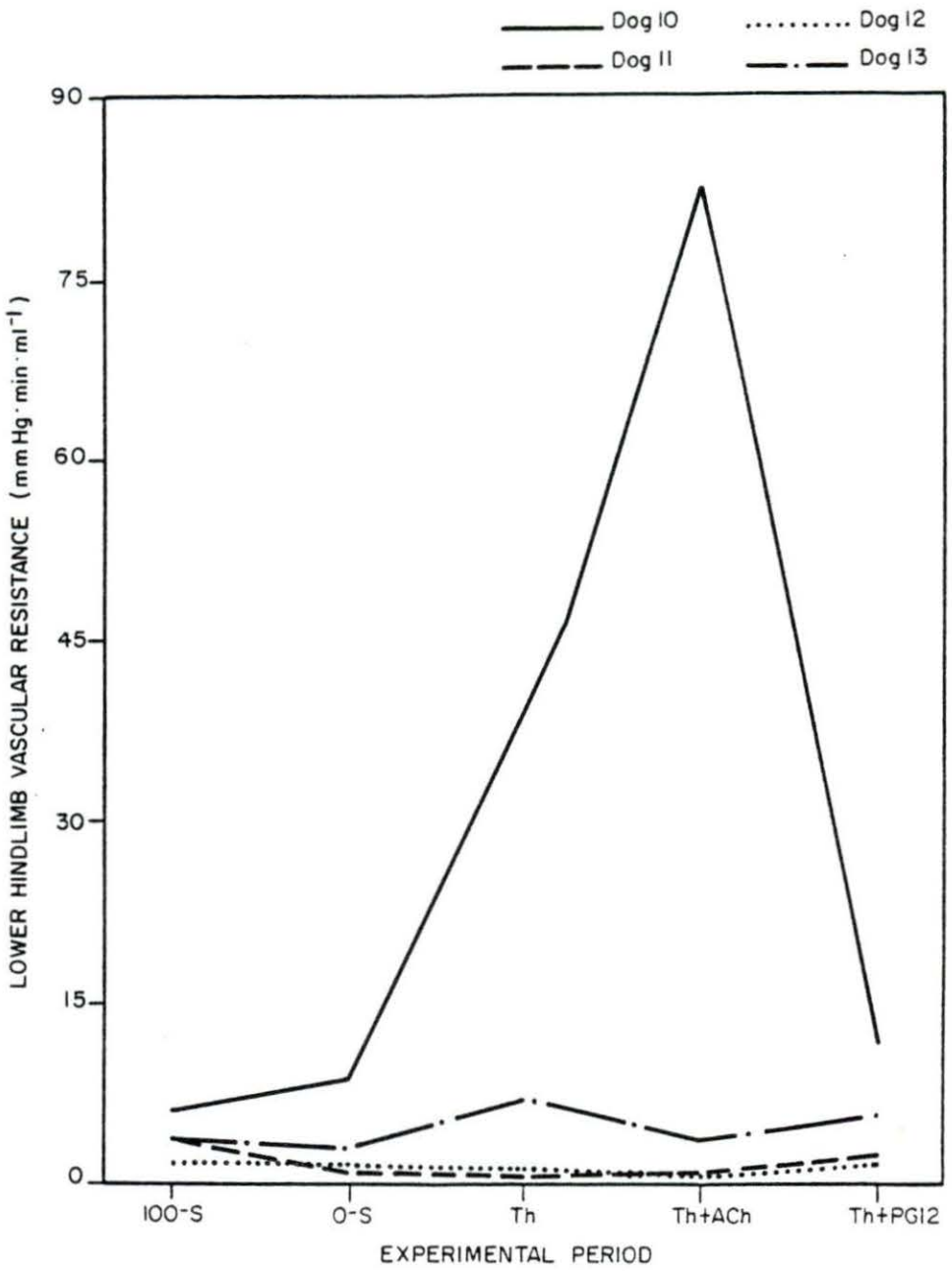


Figure 7. Peripheral vascular resistance in response to the experimental treatments in the ipsilateral, left, hind limb

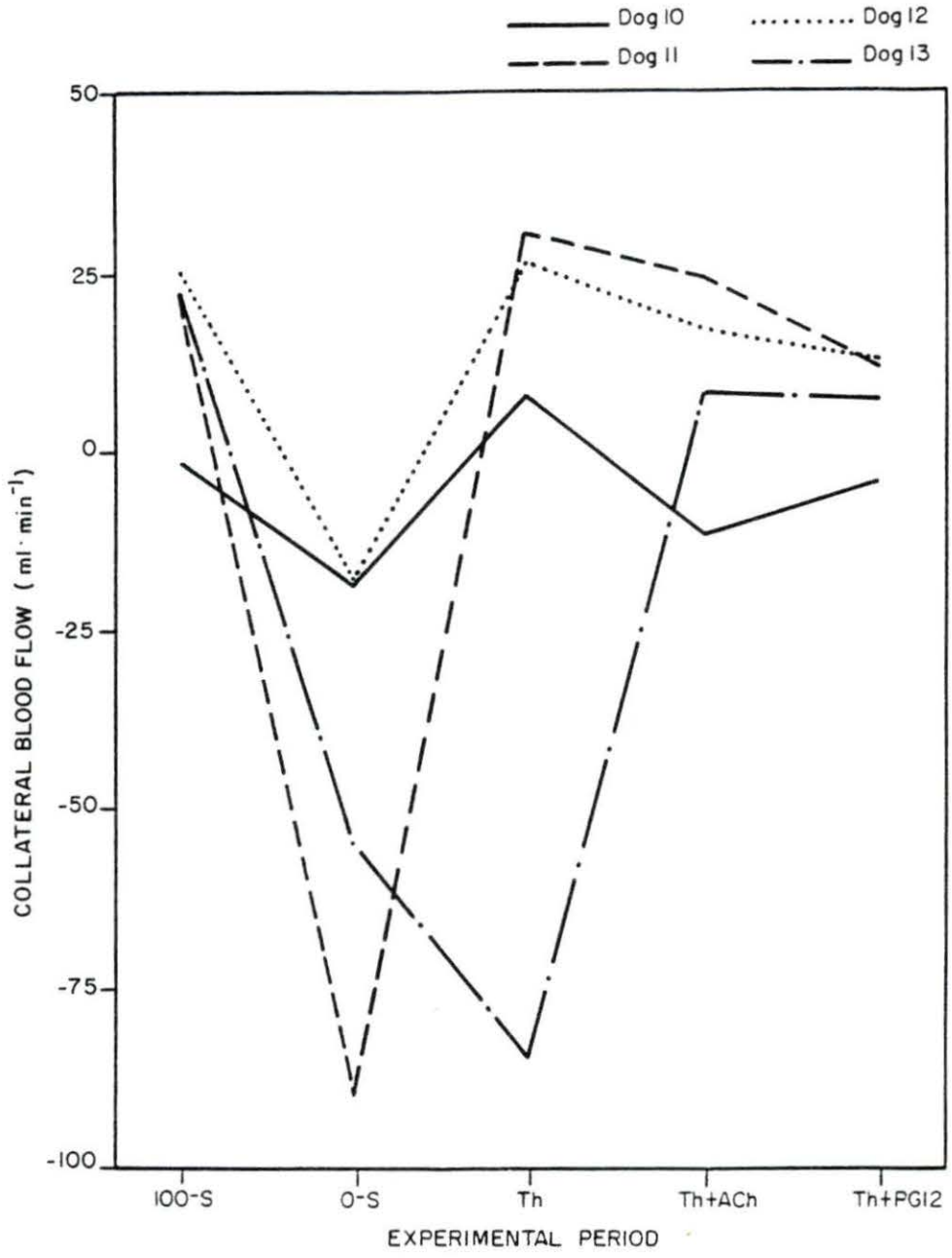


Figure 8. Collateral blood flow in response to the experimental treatments in the ipsilateral, left, hind limb

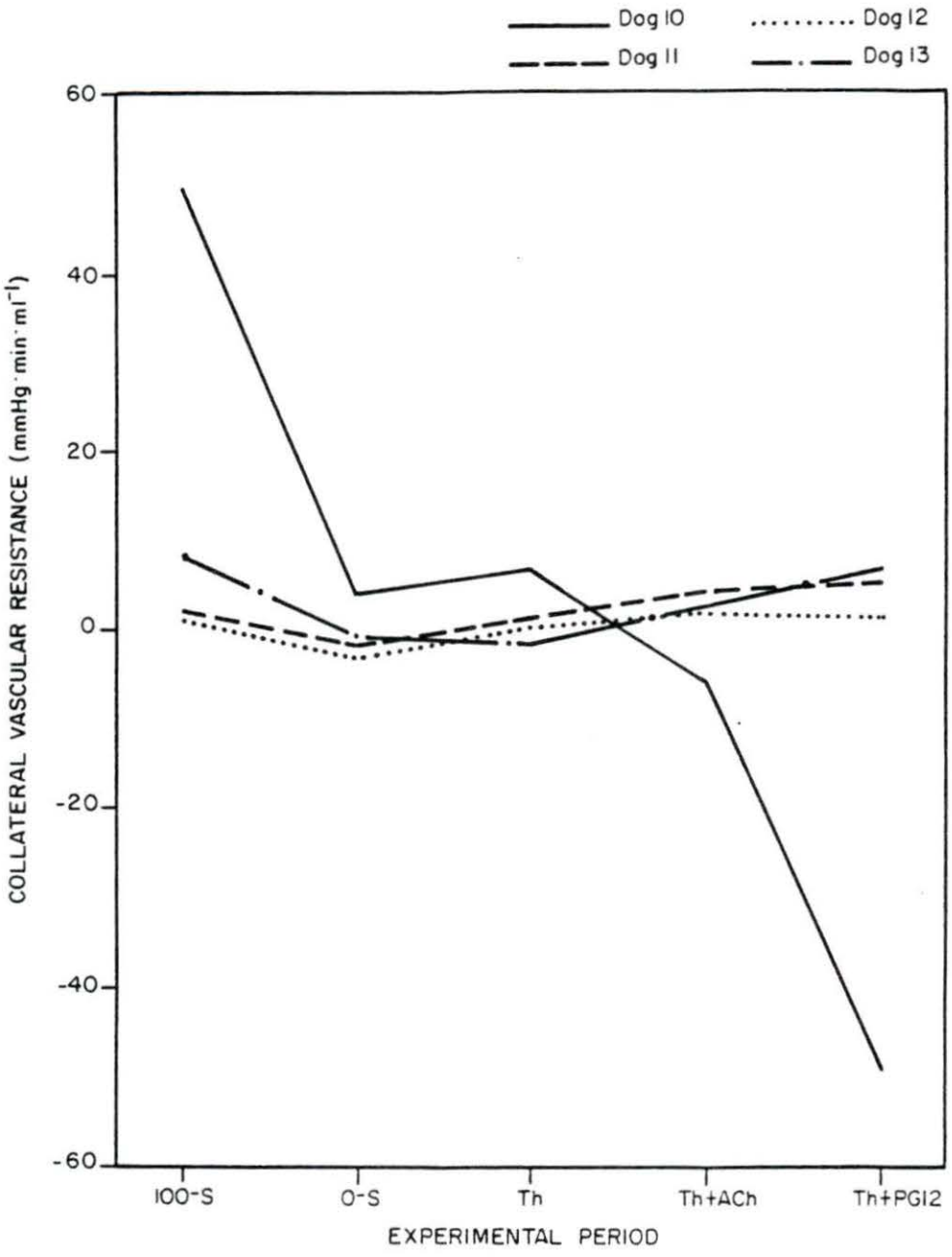


Figure 9. Collateral vascular resistance in response to the experimental treatments in the ipsilateral, left, hind limb

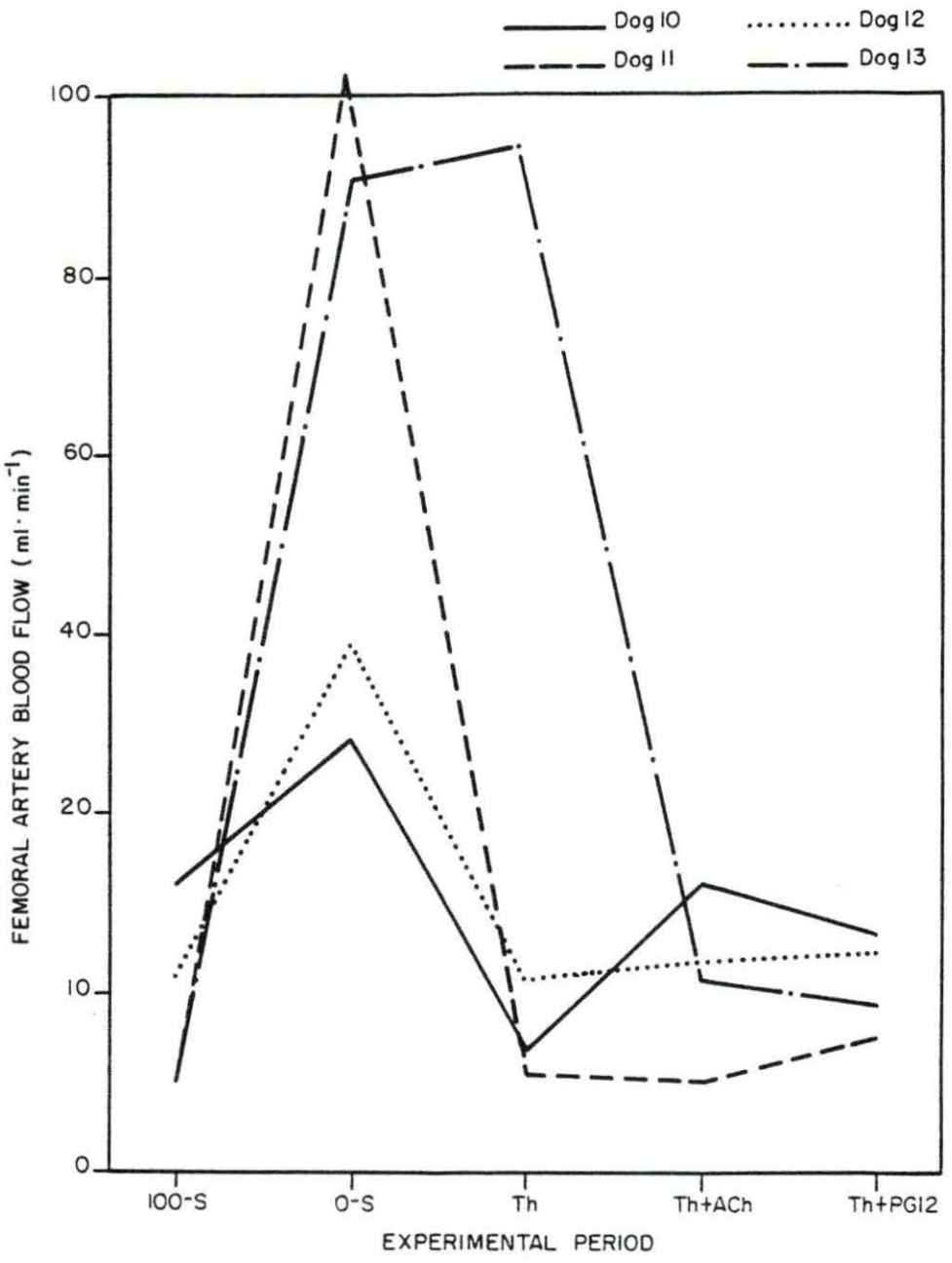


Figure 10. Stenosis blood flow in response to the experimental treatments in the ipsilateral, left, hind limb

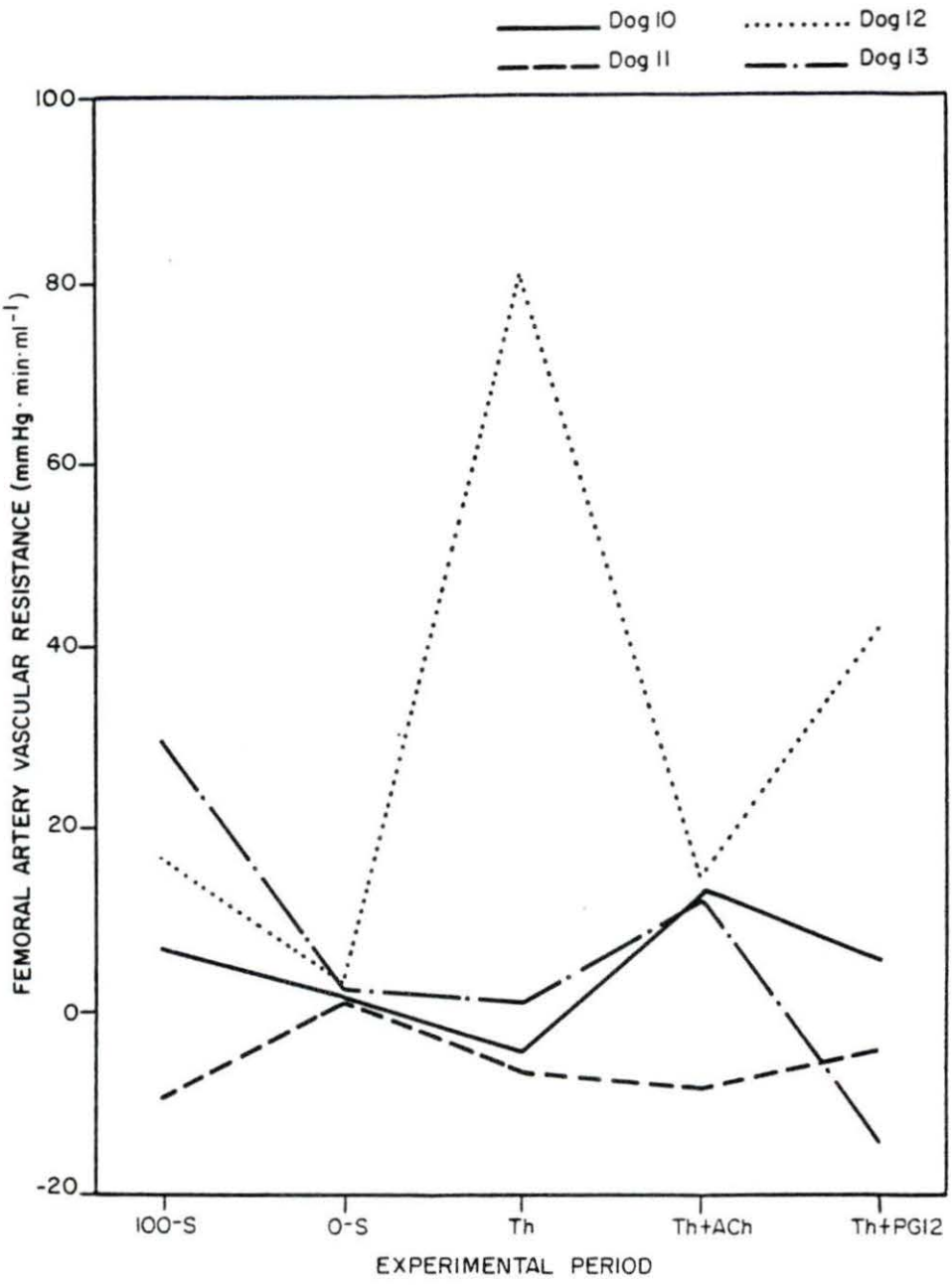


Figure 11. Stenosis vascular resistance in response to the experimental treatments in the ipsilateral, left, hind limb

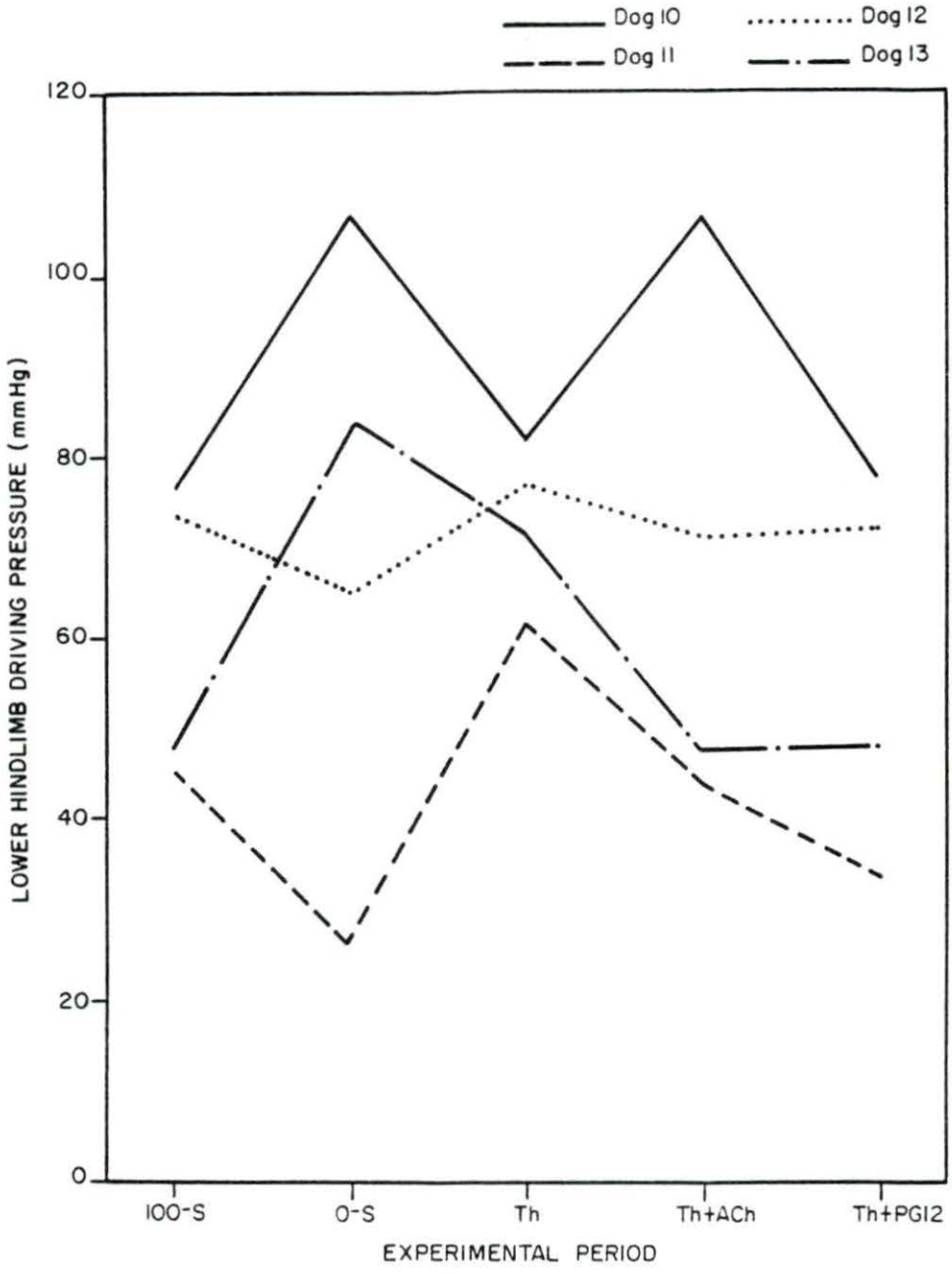


Figure 12. Peripheral driving pressure in response to the experimental treatments in the ipsilateral, left, hind limb

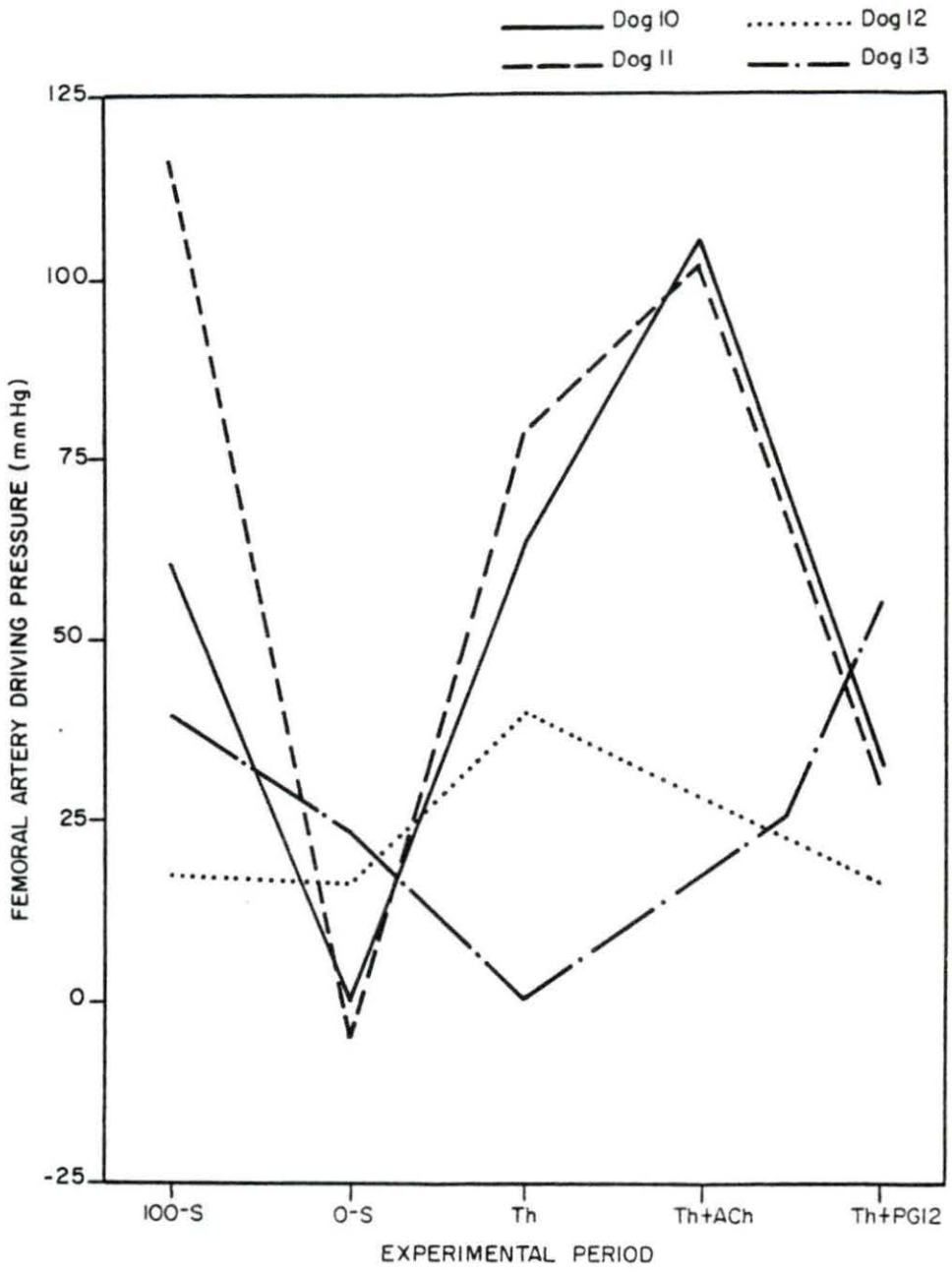


Figure 13. Stenosis driving pressure in response to the experimental treatments in the ipsilateral, left, hind limb

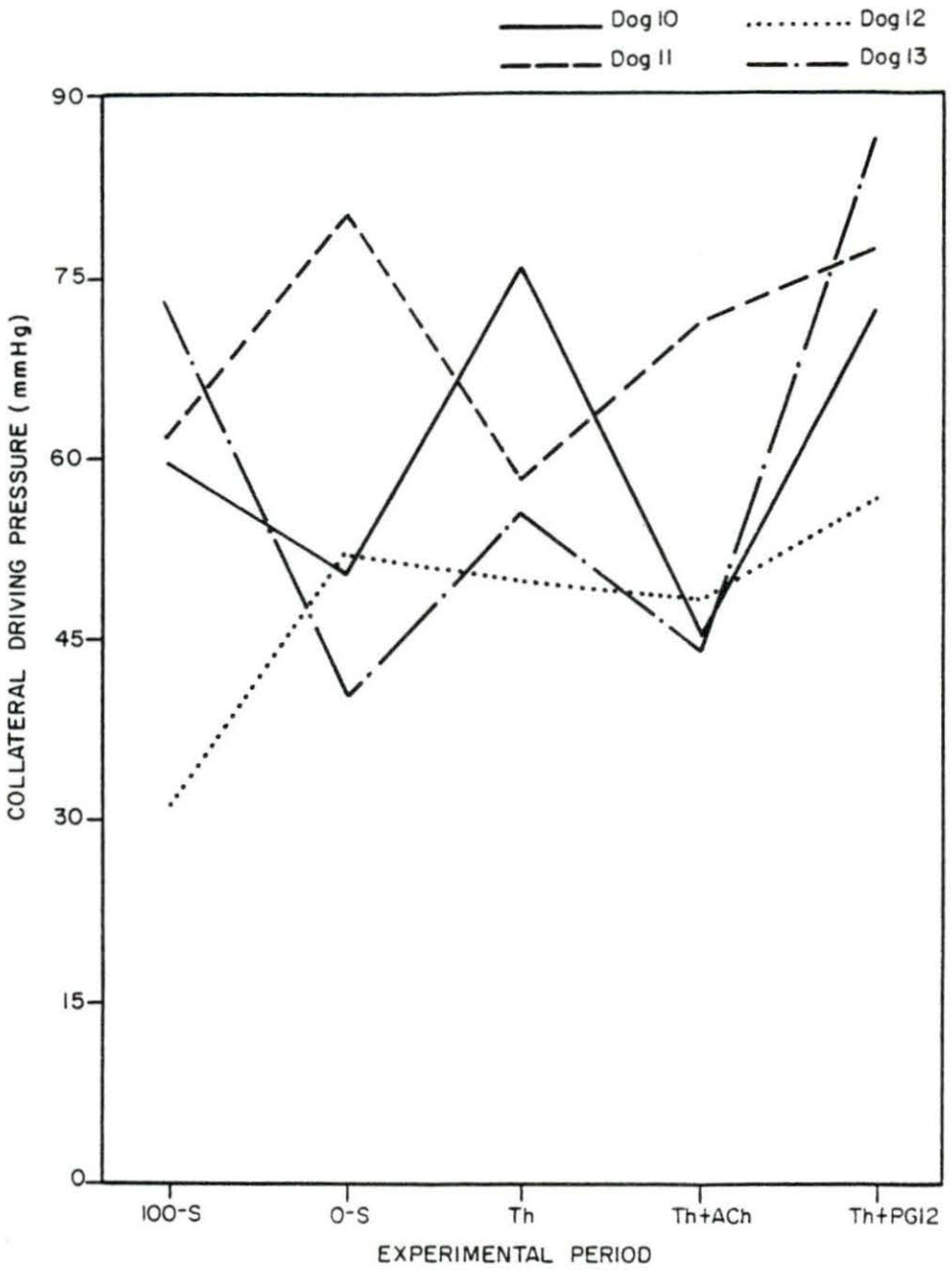


Figure 14. Collateral driving pressure in response to the experimental treatments in the ipsilateral, left, hind limb

invasive experimental design, created considerable "physiologic reactance" (Fry, 1960), treatment responses in the experimental animal which would not be consistent with those present in an intact, unoperated subject, which contributed to the variability of responses. Schwartz et al. (1961) reported that dissection of the canine femoral artery stimulates vasoconstriction of the distal vasculature. They stated that the sympathetic pathway for peripheral vasoconstriction is associated with vascular trauma. Third, two different types of hemodynamic responses to thrombosis were observed in this study. These contributed to the nonuniformity of the data and may be ascribed to the existence of two populations of dogs, arachidonic acid sensitive and arachidonic acid insensitive (suggested by Allen and Clark, 1983).

Stenosis

During the stenosis experimental period (100-S), a vascular clamp was applied to the FA at the previously described site (please refer to the Materials and Methods section) to produce 100% stenosis. After measurements were taken, the clamp was removed, and measurements of the normal (control) state were taken after the circulation had equilibrated. In this way, 100% stenosis was compared with the nonoccluded state.

In the ipsilateral leg, in dogs 10 and 12, QP was greater and RP was less during 100-S than 0-S; however, the differences were small (Tables 1 and 5, Figures 6 and 7).

QP was slightly less during 100-S in dog 11 (Table 3) and markedly so in dog 13 (Table 7) than 0-S. RP was elevated during 100-S in both animals' ipsilateral legs.

Peripheral blood flow in the contralateral leg (QPC) was consistently depressed by stenosis of the ipsilateral leg in all four subjects (Tables 1, 3, 5 and 7, Figure 5). This may have been due to the release of vasoconstrictor metabolites.

Subjects 10 and 12 responded differently than expected to 100-S compared to 0-S in that QP was less after release of the occlusion on the FA than during 100-S. This reaction could not only be due to changes in vasomotor tone of the peripheral vessels over the course of these two experimental stages, but also to trauma from the experimentally-induced arterial occlusion. Thus, clotting of the red blood cells within the peripheral bed of the ipsilateral leg in these subjects may have induced the drop in blood flow and RP increase after release of the stenosis.

QC reversed (Figure 8) and RC declined (Figure 9) after release of the stenosis during the 0-S period. These findings are consistent with the belief that during occlusion of the FA in the mid-thigh region, flow in collateral channels reversed so that the lower hind limb could receive blood from the thigh collateral bed via the DCFA and other channels rather than the thigh musculature receiving distal femoral artery blood as appears frequently to be the normal case.

Release of the stenosis resulted in a decline in RS in all animals (Figure 11) including dog 11 which had a slightly negative resistance value due to a negative pressure differential across the stenosis site,

which may be the result of a drift in the blood pressure baseline calibration.

The decrease in QS due to stenosis, as compared with the 0-S period, indicates that occlusion of the FA interrupted flow through the stenosis region (Figure 10). However, had we measured complete occlusion of the FA during 100-S, QS should have been zero and RS should have been infinite in value ($RS=\frac{\Delta P}{Q_S}$). The computer averaged values for QS did not equal zero nor infinity for RS during 100-S due to slight inaccuracies in the digital averaging technique.

In dog 11, the RS data were negative in value indicating a reverse pressure gradient through the stenosis site. P2 was greater than P1 during 0-S (Table 4). The return of QS during 0-S to a large positive value in part supports the contention that there was experimental error in measurement of the pressure gradient across the stenosis.

Thrombosis

After 0-S measurements were complete, the thrombogenic device was inserted within the FA at the stenosis site. In two out of four subjects, QPC and QP decreased during thrombosis, and in three of four animals, RP was elevated compared to the 0-S data.

In dogs 11 and 12, the response to thrombosis was such that QP and peripheral perfusion pressure (DPP) in the ipsilateral hind limb was not depressed by thrombosis as occurred in the other two subjects (Tables 4 and 6, Figure 12). QP and DPP were greater during thrombosis in dogs 11

and 12, and RP was depressed less during thrombosis than during stenosis. During the control period, RP was greater in dog 12 than during thrombosis, and RP in dog 11 was only slightly greater during thrombosis than the control value. Subjects 10 and 13 showed a marked decline in QP and elevation of RP compared to stenosis. The level of collateralization in dogs 11 and 12 may have been greater than in dogs 10 and 13 to account for the difference in response to thrombosis. In both dogs 11 and 12, RP was less during thrombosis than during stenosis. An elevated QP in both subjects which exceeded the increase in DPP during thrombosis accounts for the lower RP value during thrombosis compared to stenosis. During the control period (O-S), RP was greater in dog 12 than during thrombosis (Th). This was due to the relatively large QP value in these two subjects.

In dogs 11 and 12, it was possible that occlusion of the FA with a bulldog clamp may have induced vasospasm in the distal peripheral bed which could account for the greater decline in flow during 100-S than during thrombosis. Thrombosis failed to inhibit QP in dogs 11 and 12; in fact, DPP was elevated during thrombosis in both subjects. This may have been due to a lack of vascular reactivity. This lack of response to thrombosis in the peripheral bed may have been due to the length of time the subject was under anesthesia, systemic acidotic conditions, or local ischemic metabolites, which compromised vascular responses and resulted in vasodilation, possibly irreversible in nature. Had the vascular smooth muscle remained responsive along with the receptors and extrinsic innervation, the peripheral bed may have vasoconstricted in response to

thrombosis.

Another possibility is that vasodilator agents may have been predominant in dogs 11 and 12 but not in dogs 10 and 13 so that, despite the presence of a thrombus, dilation occurred.

In dog 10, it appears from the QPC data that vascular response to thrombosis was less than durring 100-S in the contralateral leg. Peripheral perfusion to the contralateral leg was greater during all periods of thrombosis, compared to 100 percent stenosis. This observation suggests that vasodilation due to hypoxia and accumulation of metabolic byproducts may have occurred in the peripheral bed rendering it insensitive to vasoconstrictive metabolites released during thrombosis in this subject.

The predominant effects of thrombosis were decreased QP and QPC (observed in three out of four cases for QPC and two of four cases for QP), and elevated RP. In dogs 10 and 13 in which QP was inhibited by thrombosis, these results imply that vasoactive substances such as serotonin or TXA2, released by the thrombus, have vasoconstrictive properties. RP with thrombosis was from 2-6x greater than RP during 100-S alone.

QC and RC were greater during thrombosis than during 100-S in three out of four subjects for QC and two of four subjects for RC. Thrombosis appeared to be an equal or greater stimulus than stenosis to initiation of collateral blood flow. Elevated resistance in the collateral bed may have resulted from the release of vasoconstrictor substances from the thrombus which would have impeded maximal collateral perfusion to the occluded area. However, in all four subjects, PA was elevated to a degree that resulted in increased collateral driving pressure (DPC) values

(Figure 14). Lack of a negative response to thrombosis was apparent in dogs 11 and 12; in fact, QS was greater than control (0-5) or stenosis (100-2) during the Th period. This may indicate that the ability of the peripheral circulation to responsd to vasoconstrictive substances released during thrombosis may have been compromised. It is more probable that dogs 11 and 12 were arachidonic acid insensitive animals, compared to dogs 10 and 13.

In dog 13, the collateral bed failed to respond to thrombosis, but there was an incomplete occlusion of the FA at the thrombus site. Incomplete thrombosis was indicated by larger QS and smaller RS values compared to the 0-S values. Completely occluding thromboses did form in dogs 10 and 12, as confirmed by elevated resistance and almost nonexistent flow across the stenosis/thrombosis segment of the FA, which we considered equal to zero. In dog 11, a negative QS value (which should be considered equal to zero) was recorded which accounts for the negative RS value and is not consistent with the DPS value. The reason for these slight inaccuracies is due to the digital averaging scheme utilized for the computer. Measurement error and signal averaging of the blood pressure over a nonwhole number of cardiac cycles may have caused errors in the data.

Thrombosis and ACh

After thrombosis of the FA, ACh was infused into the systemic circulation to determine its effects on the peripheral and collateral circulatory beds of the lower hind limb during thrombosis. Collateral flow was inhibited by ACh in dog 11 as QC declined 50% after ACh infusion and RC increased 200%.

Peripheral and collateral perfusion were decreased by ACh in three out of four subjects (dogs 10, 11 and 12). QPC, QP, and QC were depressed by ACh infusion during thrombosis. In these animals, it was possible that, in a systemic response to recirculating vasodilator agents, blood was shunted away from the hind limb circulation distal to the thrombosis site to other locations where ACh-modulated vasodilation also occurred. This "femoral steal" effect corresponds to observations of a "coronary steal" effect in the coronary circulation reported by Flameng et al. (1973) and Schaper et al. (1973). This "steal" effect may occur when administration of a vasodilator further compromises blood flow to an already malperfused tissue bed by shunting blood flow away from that region by decreasing peripheral resistance in other vascular tissue beds. Therefore, in dogs 10, 11 and 12, ACh infusion resulted in greater malperfusion of the lower hind limb during thrombosis. An alternative explanation for the observed decrease in QP and QC may be that the ACh acted to depress heart rate and contractility, thereby lowering cardiac output centrally and depressing hind limb perfusion peripherally.

This response was not evident in the other two subjects; dogs 11 and 13 had 6x and 11x increases in QPC, respectively, during ACh treatment. QP was also elevated and RP declined dog 13. It appears that in dog 13, peripheral perfusion to both ipsilateral and contralateral legs was improved by administration of ACh. Improvement in QP and QPC during ACh administration may have been due to lesser degrees of central cardiac or peripheral "steal" effects of ACh. This may be due to a degree of vasoresponsiveness of the hind limb beds in these subjects not present in dogs 10 and 12 and in the ipsilateral leg in dog 11.

The response of the collateral circulation to ACh during thrombosis in dog 13 was difficult to assess due to the lack of complete thrombosis. But since QS approached zero and RS values were consistent with values obtained during 100-S, it appears that during this experimental period (TH + ACh) the FA finally was completely occluded by thrombus.

Hind limb collateral perfusion during ACh administration in dog 10, characterized by negative values for QC and RC, was absent. That is, the thigh appeared to be receiving blood from the DCFA in quantity similar to that of the 0-S period. This observation is difficult to reconcile but may be a result of a discrepancy in the portion of the circulation model depicting the pressure gradient across the collateral bed.

Thrombosis and PGI2

PGI2 improved QPC during thrombosis as did ACh in dog 11 and 13. In dogs 10 and 12, the response to PGI2 and ACh was similar in that both vasodilator drugs inhibited QPC during thrombosis. However, this inhibition was negligible except in dog 12, in which ACh produced a 50% decline in QPC.

The reaction of the ipsilateral leg to PGI2 infusion was different from that in the contralateral leg. QP declined and RP was elevated in three out of four animals in response to PGI2, and in each, PGI2 promoted peripheral perfusion to a lesser degree than ACh. In dog 10, QP was elevated 4x during Th + PGI2 over Th + ACh. In the individual (dog 13) in which ACh was minimally effective in increasing QP, PGI2 depressed QP, and RP was increased. It appears that although the value for RP was similar to that of the Th period, the equality was due to the fact that both QP and DPP declined during PGI2 infusion.

Compared to the ACh treatment, QC declined and RC increased with PGI2 infusion during thrombosis in all four subjects. PGI2 and ACh were both ineffective in promoting collateral circulation in these animals. The "femoral steal" effect, previously mentioned in reference to ACh administration, may also have resulted from systemic effects of PGI2 on vascular beds. Both ACh and PGI2 thus may have shunted blood away from the thigh collateral bed during thrombosis and increased malperfusion of the tissues distal to the arterial thrombosis.

Negative values for QC and RC expressed for the Th + PGI2 period in dog 10 suggest negative collateral flow. However, for dog 11, a negative QS value seems invalid during O-S because the positive gradient discounts the possibility of negative QS. Therefore, the resultant negative RS values obtained during the other experimental periods suggest that QS may have been measured incorrectly.

Ranges of Tissue and Organ Perfusion Rates

Kidney blood flow

Total kidney blood flow measured by he RMT in the four dogs ranged between 183-469 ml/min (Table 9). This range compared favorably with RMT values reported by Nathanson and Jackson (1975). They found that blood flow to the kidneys ranged from 200-800 ml/min in the dog. The agreement of our data with their published information suggests that we had accurately measured blood flow with the RMT and that our dogs had reasonable cardiac outputs and renal blood flows.

Muscle blood flow

Lower hind limb muscle perfusion during O-S averaged 4.89 ml/min in the ipsilateral leg (Table 10) and 9.28 ml/min for the contralateral leg (Table 11). Kane and Grim (1969) reported an average muscle blood flow of 5.6 ml/min. Their studies were conducted on noninstrumented subjects using an isotope washout technique. In light of the increased physiologic reactance of our invasively instrumented subjects, our results compare to a reasonable degree. In our preparation, the ipsilateral leg

required extensive surgical intrusion. There was a lesser degree of invasiveness in the contralateral leg. Our subjects were not heparinized in order to permit thrombosis to develop in the FA. Therefore, due to the invasivity of the preparation, embolization at the micro and macro levels may have partially impeded perfusion to the ipsilateral limb. That is, surgical trauma may have stimulated vasoconstriction and distal muscle ischemia, thus inhibiting lower hind limb blood flow.

Skin blood flow

During O-S, skin perfusion rates ranged from 2.98-23.00 ml/min in the contralateral leg (Table 12) and 0.40-4.17 ml/min in the ipsilateral leg (Table 13). Skin blood flow rates were lower in the ipsilateral leg. Again, this may have been due to the high degree of surgical trauma to that leg. Kane and Grim (1969) stated that the average blood flow to the skin was 8.4 ml/min, which was comparable to what was measured in this study for the contralateral leg. The range of skin blood flow rates measured in this experiment is reasonable as skin blood flow rates may vary greatly with the physiological state of the animal and are affected by depth of anesthesia, ambient temperature, and release of vasoactive agents.

Bone blood flow

Contralateral leg blood flow rate to the bone tissue averaged 1.86 ml/min (Table 14) and 3.28 ml/min in the ipsilateral leg (Table 15) during O-S. Gross et al. (1981) stated that in the dog, bone blood flow

may vary from 2-30 ml/min. Our results fall within the range of previously reported data for bone blood flow. It appears that the ipsilateral leg had higher bone blood flow values than did the contralateral leg. The greater surgical trauma in the ipsilateral leg has been shown to depress lower hind limb blood flow in other tissues, however bone blood flow to the more invasively prepared ipsilateral leg was greater than the contralateral leg. The invasiveness of the preparation of the ipsilateral limb did not inhibit flow to the bone more than in the contralateral limb, as it had in other tissues. This may have been due to physiological reasons not readily discernible. However, the bone blood flow measurements were at the low end of the normal range, and this may be attributed to anesthesia, the long duration of the experiment, or the high degree of surgical invasiveness.

Paw blood flow

Blood flow to the paw during the control period (O-S) in the ipsilateral leg ranged from 0.36 to 3.96 ml/min (Table 16). Contralateral leg paw blood flow was calculated to be 0.19 to 2.17 ml/min during the control period in three out of four animals (Table 17). A value of 51.15 ml/min was determined for dog 11's paw blood flow. This value seems exceptionally high compared to the values recorded for the other subjects, and the reason for this outlying value is not readily apparent.

Femoral Shunting ("Steal")

Ischemia-induced intrinsic release of vasoactive substances may have augmented the shunting of blood away from already malperfused regions during vasodilator administration. The infusion catheter for vasodilator administration was positioned within the terminal aorta so as to expose the beds fed by the terminal branches of the aorta first to the infusates. Upon recirculation, the remainder of the circulatory beds of the body would be exposed to any intact agonists. It is possible recirculating ACh may have diminished cardiac output in some cases. PGI2 degradation is not complete in the lungs enabling it to reach other tissue beds. The thigh region may have been relatively more responsive than the lower hind limb to the vasodilators since it was subjected to less surgical trauma. Thus, it and other systemic nutritional beds could have been more responsive to vasodilator agents and more able to maintain perfusion rates. Blood flow may have been directed primarily to the thigh musculature's arterio-capillo-venous channels before reaching the lower leg.

The lower hind limb may have been made insensitive to the actions of agents such ACh or PGI2 due to the vasoconstrictive effects of serotonin and/or TXA2, which may override the actions of the vasodilators during thrombotic occlusion. Schaub et al. (1977a) found that serotonin was released during arterial thrombosis and postulated that TXA2 was also

associated with vasoconstriction which occurred during thrombosis. Non-ischemic areas responsive to the vasodilators could achieve greater decreases in vascular resistance and, thus, increased flow. A nonresponding, constricted, or thrombotically-occluded and maximally dilated, circulatory bed would maintain its relatively high resistance, with resultant shunting of blood to other dilated areas.

Percent Shunting of Radioactive Microspheres (RM)

Shunting of RM via arterio-venous channels which would pass the nominal 15μ spheres was determined by dividing the number of RM reaching the lungs by the total RM dose injected into the left ventricle. In our study, we found that from 1.2 to 4.8 percent of the RM dose was shunted into the lungs. Kaihara et al. (1968) reported 5 to 10 percent shunting into the lungs with 15μ RM in dogs. Our results show that excess shunting did not occur in this study, supporting the validity of the data for the parameters measured by the RMT.

Total Hind Limb Blood Flow

Blood flow to the lower hind limbs was calculated by summing the four tissue flow rates. Flow to the ipsilateral leg for dogs 10, 11, 12 and 13 during O-S were 24.95, 101.27, 37.16 and 88.94 ml/min, respectively; and for the contralateral leg, 74.79, 14.85, 23.33 and 28.43 ml/min, respectively. Coffman (1966) measured femoral artery inflow for the canine hind limb, and the results averaged 36.8 ml/min. Venous outflow was

determined by Kane and Grim (1969), and the results for two separate studies measured hind limb blood flow to be 45.8 ml/min and 74.2 ml/min.

Our values compare well with previously documented values.

SUMMARY AND CONCLUSIONS

The objectives of this research were to compare the capacity of acute stenosis and thrombosis of the FA to affect QC and to determine if QC could be enhanced by infusion of the vasodilators ACh and PGI2. In order to validate the experimental model for this investigation, pilot trials were initiated on nine dogs to establish several procedures new to this laboratory:

1. The RMT was successfully introduced for measurement of QP, kidney, and hind limb tissue blood flows.
2. Computer programs were implemented for data acquisition, storage, and calculation of parameters.
3. A copper coil device which induced an intra-arterial thrombus to gradually form was employed.

The actual experimentation was conducted on four mongrel dogs from 25 to 30 kg in weight. Each animal was subjected to five experimental treatments; they were, in sequence, 100-S, 0-S, Th, Th + ACh, and Th + PGI2. Hind limb collateral and peripheral hemodynamics were measured during all treatment periods.

After hemodynamic measurements were made during the initial treatment period (100-S), the stenosis was removed, and changes in peripheral, collateral, and stenosis hemodynamics were observed. QPC increased only slightly in dogs 12 and 13, but in dogs 10 and 11, QPC was markedly elevated after release of the stenosis (during 0-S). In the latter two subjects, stenosis of the ipsilateral leg greatly inhibited peripheral blood

flow in the contralateral limb. The inhibition of QPC could have been due to vasospasm in the contralateral leg in response to the vascular trauma of clamping the ipsilateral FA during 100-S (Schwartz et al., 1961).

QP was inhibited during 100-S in dog 13; again, this may have been caused by distal vasoconstriction associated with the vascular trauma of clamping the FA during 100-S. The increase in QP following the release of the stenosis in dog 13 may have been due to another factor, reactive hyperemia. The initial phase of the reactive hyperemia response is characterized by vasodilation and increased blood flow to a previously occluded site after release of the occlusion (stenosis) if the vascular smooth muscle can respond to the build-up of local catabolites. After the catabolites were flushed out and local oxygen levels restored, then a secondary vasoconstriction would result. However, this response was absent in the other three subjects, and this lack of response in these subjects is consistent with the small change in RP between the 100-S and 0-S periods. The viability of the vascular smooth muscle may have been compromised, accounting for the lack of response to stenosis in the peripheral bed in dogs 10, 11, and 12.

It is interesting to notice that the small increase in RP during 0-S may have prevented greater QP in dog 10 despite the concurrent increase in DPP. Dog 13 exhibited an increase in DPP and QP, and RP declined slightly during 0-S. Collateral blood flow and resistance declined during this period, although DPC values were inconsistent between animals.

There were two different responses to thrombosis. This may have been due to a lack of viability of the vascular smooth muscle in nonresponsive subjects, or more probably due to a lack of sensitivity of the vascular smooth muscle to release of arachidonic acid metabolites during thrombosis. According to Allen and Clark (1983), there may exist two discrete canine populations, arachidonic acid sensitive and arachidonic acid insensitive animals. This may account for the two different responses to thrombosis in our study. In subjects which QP and DPP were elevated, RP was unchanged and DPC declined (dogs 11 and 12). The second type of response to thrombosis in dogs 10 and 13 was characterized by depressed QP and DPP, a larger RP value, and elevated DPC. In subjects whose peripheral circulation was inhibited by thrombosis, more blood may have been directed towards the collateral bed in response to thrombosis in dogs 10 and 13, as shown by the larger DPC values. All subjects consistently had greater QC, with little change in RC, while QPC declined during thrombosis.

ACh appeared to improve QC in only one subject (dog 13), while it depressed QC slightly in the other three. The increased QC in dog 13 is not valid; the reason for the apparent increase from the Th to the Th + ACh period is not due to any ACh effect but is due to a lack of complete thrombotic occlusion of the FA during Th as evidenced by the large QS value. QS declined during the Th + ACh period, indicating that complete occlusion of the FA occurred at that time, an event which provided the stimulus for collateral blood flow which was absent in the Th period. ACh infusion inhibited QC in those three subjects in which a complete

thrombotic occlusion formed during the Th period. RC declined and RP increased due primarily to a decrease in DPC and elevation of DPP which suggests blood flow may have been directed away from the collateral bed due to a peripheral "steal" effect in which infusion of the vasodilator further compromised blood flow by shunting flow away from that region.

QPC was improved by ACh in two subjects (dogs 11 and 13), while a slight decline occurred in dogs 10 and 12. QP was also improved in dog 13, and RP declined slightly. PA was slightly elevated in dogs 11 and 13, whereas dogs 10 and 12 had slightly depressed PA after ACh infusion, which suggests that cardiac output may have been depressed by ACh in dogs 10 and 12 but not in dogs 11 and 13, accounting for their elevated QPC values. This may also be due to lesser peripheral "steal" or a degree of vasoresponsiveness not present in the contralateral hind limb beds of dogs 10 and 12.

PGI2 infusion was ineffective in improving QC over Th or Th + ACh levels in this trial, although DPC values were elevated over Th + ACh levels in all subjects and were greater than Th values in all subjects but dog 10. In subjects 10 and 13, it is interesting to note that both the Th and the Th + PGI2 treatment periods produced increases in QPC. This may indicate a greater sensitivity to arachidonic acid and its metabolites in these subjects than in dogs 11 and 12. However, QC was not improved in dogs 10 and 13, although the elevation of DPC with infusion of PGI2 was more pronounced in dogs 10 and 13 than in dogs 11 and 12. The slight increase in RC during PGI2 infusion (which occurred in all subjects except dog 10) may have prohibited elevation of QC.

Only dog 10 had greater QP and depressed RP and RC during PGI2 infusion than in previous Th and Th + ACh periods, despite a decline in DPP.

QPC values for dogs 11 and 13, which were elevated during ACh infusion, declined with infusion of PGI2. QPC in dog 13 remained greater during Th + PGI2 than during the Th period; however, PGI2 produced no significant difference in the other three subjects.

In conclusion, we were unable to demonstrate any significant difference with one-way analysis of variance ($p < .05$) in the four subjects between the peripheral and collateral effects of stenosis or thrombosis, and neither ACh nor PGI2 were significantly effective in promoting CF during thrombosis ($p < .05$).

There were problems in obtaining uniform response to our experimental treatments due to the high degree of surgical invasiveness of the animal preparation. The occurrence of vasospasm, spontaneous intravascular thrombosis, and release of vasoactive substances may have occurred due to manipulation at the surgical sites. The validity of data obtained from such an extensively instrumented animal model may be questioned in light of the divergent nature of some of the results.

The smaller number of subjects (n=4) and the high degree of individual variation in this study made interpretation of trends difficult. The existence of arachidonic acid sensitive and arachidonic acid insensitive subjects in this study could have resulted in the two different observed responses to thrombosis and could have further impeded interpretation of the ACh and PGI2 treatments.

Simplification of the model in this investigation into a less invasive preparation would be necessary to permit evaluation of more normal physiological responses. Future refinements could also include the use of EMF probes which are electronically zeroed to prevent baseline drift. Baseline drift made measurement of QS zero flow difficult and resulted in erroneous negative values for QS and RS in our experimental data.

Finally, our choice of using P3 as an estimate for collateral bed downstream pressure (and lower hind limb driving pressure) may not have been appropriate to measure the hemodynamic events occurring in the collateral bed. Nevertheless, P3 appeared to be the only accessible anatomic site for tapping into the common point between these two arterial beds. Further experiments would be warranted to define the optimum placement of a blood pressure cannula with respect to measurement of lower hind limb and collateral bed properties.

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APPENDIX: LIST OF FORMULAE

LIST OF FORMULAE

$$DPC = PA - P_3$$

$$DPP = P_1 - P_2$$

$$DPS = P_2 - PV$$

$$PA - PV = QP\left(RP + \frac{RS \cdot RC}{RS + RC}\right)$$

$$Q(RMT) = \frac{Qar \cdot It}{Iar}$$

Q = unknown organ flow in ml/min
Qar = flow of arterial reference sample in ml/min
It = amount of radioactivity or cpm in tissue or organ sample
Iar = amount of radioactivity or cpm in arterial reference sample

$$QP = QS + QC$$

$$QS = QP - QC$$

$$QC = QP - QS$$

$$RC = \frac{PA - P_3}{QC}$$

$$RP = \frac{P_2 - PV}{QP}$$

$$RS = \frac{P_1 - P_2}{QS}$$

$$S = \frac{Q_1}{Q}$$

S = fraction of blood flow passing through shunts
Q_1 = the quantity (cpm) of RM in the lungs
Q = the total injected RM dose

$$y = mx + b$$

y = y coordinate
m = slope of the line
x = x coordinate
b = y intercept