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COLLATERAL CIRCULATION IN THE HIND LIMB OF DOG AS INFLUENCED BY VASOACTIVE COMPOUNDS

Iowa State University PH.D. 1981

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Collateral circulation in the hind limb of dog as influenced by vasoactive compounds

by

Bashir Ahmad Sheikh

A Dissertation Submitted to the
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LIST OF ABBREVIATIONS

ACH	Acetylcholine
ADP	Adenosine-diphosphate
ATP	Adenosine-triphosphate
AA	Arachidonic acid
CO	Cardiac output
CGA	Caudal gluteal artery
CNS	Central nervous system
C	Control
c.s. area	Crosssectional area
c-AMP	Cyclic adenosine monophosphate
DCIA	Deep circumflex iliac artery
DFA	Deep femoral artery
DCFA	Distal caudal femoral artery
ETEA	Eicosatetraenoic acid
EMF	Electromagnetic flowmeter
EIA	External iliac artery
Q_c	Flow across collateral bed
Q_p	Flow across peripheral bed
Q_s	Flow through stenosis
GMP	Gaunosine-monophosphate
G	Group
15HPAA	15-hydroperoxy arachidonic acid
HETE	12-hydroxyeicosatetraenoic acid

IMZ	Imidazole
INDO	Indomethacin
id	Internal diameter
IIA	Internal iliac artery
ia	Intraarterial
iv	Intravenous
METH	Methysergide
MCFA	Middle caudal femoral artery
MAO	Monoamine oxidase
od	Outside diameter
PCPA	Parachlorophenylalanine
$\Delta P, \Delta Ps$	Pressure gradient, Pressure gradients
PG,PGs	Prostaglandin, Prostaglandins
PGG_2, PGH_2	Prostaglandin endoperoxides
PGI_2	Prostacyclin
PCFA	Proximal caudal femoral artery
RH	Reactive hyperemia
rpm	Revolutions per minute
R or RZP	Reserpine
R_s	Resistance through stenosis
R_c	Resistance across collateral bed
R_p	Resistance across peripheral bed
SA	Saphenous artery

SRT	Serotonin
TX	Thromboxane
TXs	Thromboxanes

INTRODUCTION

The arterial circulation and its mechanism of control are remarkably well-adapted for the preservation of the entire organism. When blood flow to a vascular region is reduced by an arterial narrowing or occlusion, a series of pre-existing alternate channels assumes the nutritive role of the parent artery. These alternate channels, called collateral vessels, are preformed anastamosing branches of either functioning or nonfunctioning small arteries (Bellman et al., 1959; Friedenberg and Perez, 1965). In addition, neogenic collateral vessels may develop over a period of time under subacute and chronic conditions (Eriksson, 1970; Conrad et al., 1971).

Collateral channels can be classified into stem, midzone and reentrant vessels (Longland, 1953). Stem vessels, which originate proximal to a stenosis, supply blood to a second set called midzone vessels, which are composed of myriads of tiny channels normally invisible by arteriography (Winblad et al., 1959). These vessels usually have the greatest resistance, yet are important as they can enlarge with time and direct blood into distal reentrant vessels. Reentrant vessels carry retrograde blood flow during an arterial occlusion and join the parent vessel at some point distal to occlusion. The basic function of these

vessels is to carry blood in sufficient quantities to maintain the metabolic requirements (circulating equilibrium) of the part they serve. The extent to which the distal ischemic bed is preserved depends upon the capabilities of the collateral arteries, the site and rapidity of an arterial occlusion, the size, distribution, and vascular tone of the preformed collateral channels, and the metabolic demands of the tissue distal to the occlusion.

Despite recent advances in the knowledge of peripheral circulation, hemodynamic changes associated with collateral vessel function, during health and disease, have not been thoroughly characterized. This is chiefly due to overly simplistic approaches that have been used to study collateral vessel recruitment and function. Some of the interpretations thus evolved are as follows:

Maximum collateral blood circulation results from a mechanical occlusion (Eckstein et al., 1941; Winblad et al., 1959).

Occlusion associated with intravascular thrombosis depresses the collateral vessel recruitment (Schaub et al., 1976).

No significant improvement in blood flow occurs with therapeutic intervention following a mechanical obstruction except during strenuous exercise (Thulesius, 1963, 1972; Khudaiberdyerv and Kulikov, 1970).

Resistance across a stenosis is dynamic and is influenced by peripheral and collateral hemodynamics (Roth et al., 1976; Walinsky et al., 1979).

Intravascular obstruction is not purely a mechanical phenomenon and is always associated with endothelial cell damage (Turrito and Baumgartner, 1976) and ischemia (Kramer and Folts, 1973) which release a wide variety of vasoactive substances (Imhoff, 1961). These substances are believed to regulate the delivery of blood to the tissues (Afonso et al., 1974; Schaub et al., 1977a) often resulting in the necrosis of the part they serve (Bulle, 1957; Schaub et al., 1977b). SRT is one substance being released during arterial occlusion (Bloor et al., 1973; Sullenberger et al., 1959; Kordenat and Kezdi, 1979). Prostanoids, another family of vasoactive agents also are believed to be involved during obstructive episodes (Alexandar et al., 1975; Ogletree and Lefer, 1978; Folts and Beck, 1979).

A wide variety of factors are involved in determination of the flow reserve of a vascular bed. The term "flow reserve" refers to maximum flow rate during oxygen deficit following complete occlusion. The present study was designed to identify and analyze the numerous interacting factors that affect the flow reserve of the vascular bed, and also to characterize collateral hemodynamics during arterial

stenosis. More specifically, the purposes were as follows:

i) To determine if critical or complete stenosis of a vessel is associated with platelet aggregation and release of vasoactive substances.

ii) To determine whether the inhibition of these substances by antagonists may improve the collateral blood flow.

REVIEW OF LITERATURE

The existence of collateral circulation in an organism has been suggested in many different ways since the early second century. Antyllus (150), a great Greek surgeon, was first to report that the ligation of a vessel did not necessarily result in loss of the part it served. Fifteen centuries later, Lower (1669) found that the vessels which carry blood to the heart were linked together by anastamosing ends which were later observed by Haller (1757) in his preparations. However, the real stimulus to the study of collateral hemodynamics came following the remarkable experimental investigations of Hunter, 1785 (Owen, 1861, cited in John and Warren (1961)), who observed a continued growth of the stag's antler, following ligation of the main nutrient artery, with prodigious development of vascular channels, that bypassed the occluded artery with time. From his observations, he finally concluded that "the vessels go, where they are needed".

The impetus for recruitment and enlargement of collateral circulation has been attributed to a wide variety of factors. Some investigators believe that physical factors, principally the ΔPs and the flow velocities, served as prime stimuli for the opening of preexisting collateral channels (Dornhorst and Schaffer, 1951; Conrad et al., 1971; Giron et al., 1971; Barnes, 1980), whereas others proposed that blockade

of sympathetic nerves potentiated the collateral circulation (Nothnagel, 1889; Thies, 1933; Deterling et al., 1947; Donald and Fergusson, 1970; Kiss et al., 1970). Still others suggested that the interaction and release of vasoactive compounds from the obstructed vessel and the region distal to it play a role in the regulation of collateral blood flow (Imhoff, 1961).

A wide variety of factors are involved in the recruitment of collateral vessels. The studies of different investigators will be cited in the section with an emphasis on the stimulus for collateral vessel recruitment and the role of various metabolites on collateral circulation. The effect of stenosis, role of prostaglandins and serotonin in the regulation of collateral vessel functioning will be reviewed in subsequent sections.

Collateral Circulation

In their studies on collateral flow, Lewis (1940); Holeman (1949); Winblad et al. (1959); John and Warren (1961); and Leibow (1963) stated their belief that the recruitment of the collateral circulation following an inflow occlusion was due to interaction between increased ΔPs, increased flow velocities, vascular tone and accumulation of the metabolites in tissues distal to occlusion. Coffman (1966), however, contended that a variable response of

collateral recruitment was due to vasomotor tone and the reactivity of the collateral vessels to different vaso-active procedures.

Rosenthal and Guyton (1968) observed the rapid and major recruitment of collateral vessels during the first few seconds of an occlusion. This was followed by slow and prolonged changes over a period of time. The ensuing distal ischemia associated with hypotension caused vasodilatation of the collateral bed and suggested a possible regulatory role of ischemia in the modulation of collateral blood flow. In contrast, Thulesius (1963) could not find significant change in the collateral blood flow during the first few seconds following an occlusion, but he did notice improvement over the next 2-3 minutes. In their studies, peripheral vaso-dilatation always preceded the collateral recruitment (Fleish, 1935; Hilton, 1959) which significantly improved only during strenuous exercise (Khudaiberdyerv and Kulikov, 1970; Thulesius, 1972; Abramson, 1980). Similar findings were shown in the studies of Rutherford and Valenta (1971) who, following an exercise, observed a marked improvement in the muscle blood flow despite the presence of an arterial occlusion.

During their experiments to ascertain the determinants of blood flow in a region, Mohrman and Feigl (1978) reported that the rate of blood flow through a region was based on the

interaction between the alpha adrenergic constrictor mechanism and the metabolism of the vascular bed involved. However, Ribeiro et al. (1979) suggested that the oxygen induced increase in collateral blood to the ischemic myocardium was due to the protective and regulatory role of oxygen in the redistribution of blood flow in the region. The exact mechanism by which oxygen induced the increase in collateral blood flow could not be determined in these studies.

Dornhorst and Schaffer (1951) concluded that an arterial occlusion was associated with a significant decrease in the resistance of the associated collateral vascular bed. The decrease was metabolically based and was the consequence of the interaction among the increased ΔPs, the vasospasm and the vascular tone of the region. Vasospasm was also observed by Awdeh (1978) in the principal nutrient vessel following an inflow occlusion. He believed this spasm was of vascular origin and was caused by the mechanical stimulation of the occlusion.

Olsson et al. (1970) measured a three-fold increase in adenosine content of the blood in the ischemic tissue distal to obstruction and speculated that adenosine, a breakdown metabolite of ATP, played an important role in the recruitment of collateral blood flow. An intimate relationship between distal tissue hypoxia and peripheral vascular

adenosine concentration was also reported by Berne et al. (1971) during their studies on the role of adenosine in peripheral vasodilatation. Berne, however, in his follow-up studies in 1980, suggested that the adenosine released in response to an inflow occlusion was chiefly responsible for the collateral vascular recruitment. He observed that the adenosine level was closely correlated with the oxygen tension of the ischemic region. He believed that the adenosine-mediated maintenance of collateral blood flow was due to increased adenyl-cyclase activity resulting from increased adenosine contents in the tissue. Adenyl-cyclase increased c-AMP, which either reduced the cell permeability for calcium or blocked the calcium uptake by vascular smooth muscle cell.

Edholm et al. (1951) compared blood flow in obstructed and unobstructed arteries of canine hind limbs. They found a significant increase in the blood flow to the obstructed and distally hypotensive hind limb, as compared to the unobstructed one. They postulated that this was due to a contralateral steal effect. Brkic and Laszt (1973), on the contrary, reported a significantly improved blood flow in the contralateral unobstructed hind limb of rats as compared to ipsilateral obstructed limbs. The increase in blood flow to the contralateral unobstructed side can be attributed to the release

of vasodilatory agents and their subsequent transport to the contralateral limb, where they caused vasodilatation. A decrease in blood flow to the unexercised hind limb, as compared to the exercised limb was observed by Kitamura et al. (1980). They believed this decrease was due to reflex vasoconstriction in the unexercised limb, resulting from peripheral chemoreceptor stimulation. The accumulated metabolites, including PCO_2, increased hydrogen ion concentration and lactate, were responsible for peripheral chemoreceptor reflex activity.

Stenosis

Mechanical stenosis of an arterial segment often is used to simulate an atherosclerotic obstruction in the experimental research laboratory. Partial stenosis, as opposed to complete obstruction, has a complex influence on the blood flow through and beyond the narrowed segment and significantly alters the collateral and peripheral vascular hemodynamics. Since graded levels of stenosis of the principal nutrient vessel were produced in these studies, some of the significant effects of experimentally produced graded stenoses on collateral and peripheral vasculature will be discussed.

Gregg and Patterson (1980) reported that stenotic ΔPs ranging between 6-9 mm Hg were required to recruit collateral blood flow. Pressure gradients of this magnitude were obtained

when the lumen diameter was reduced to 50 percent of its original diameter, a point at which flow through the stenosed artery was significantly reduced. Folts et al. (1973), however, found no significant reduction in blood flow through a stenotic segment with 10 mm Hg ΔP and 52 percent reduction in cross-sectional area. They did find a decrease in flow, however, when a lumen cross-sectional area was reduced by 78 percent. This stenosis level was critical as it was associated with intravascular obstruction, endothelial cell damage, higher ΔP and lack of reactive hyperemic response following an occlusion. Similar findings were reported in the studies of Santamore et al. (1980), who showed that intravascular stenosis was associated with vasospasm, which enhanced the severity of the stenosis. They believed that the vasospasm was internally originated (potentiated) due to mechanical stimulation of the nerve endings in the vessel wall.

Beckman et al. (1979) observed an inverse relationship between reactive hyperemic blood flow rate and peripheral vascular resistance which did not change noticeably until the stenosis c.s. area was reduced by 80 percent. There was significant decrease in blood flow at this level of stenosis and the release of stenosis caused a reactive hyperemic response. Similar findings were reported by

Kubicka et al. (1979) in an extracorporeal circulation. They observed that 98 percent reduction in c.s. area of the artery was the critical point where blood flow dropped to 25 percent of its resting value. The flow, however, in their studies started to decrease when the lumen c.s. area was reduced by 75 percent. Sequential obstruction of potential collateral channels in these experiments did not significantly alter the blood flow distal to the stenosis.

Walinsky et al. (1979) defied the concept of fixed stenosis on the basis of his findings, which showed that the stenosis resistance was dynamic in nature and was influenced by a variety of factors. These included platelet aggregation, collateralization, turbulence and release of vasoactive metabolites. These factors decreased R_p, increased ΔP_s potentiated narrowing of the lumen by passive reverse stress-relaxation mechanism and augmented the hemodynamic severity of the stenosis. Earlier, Roth et al. (1976), had proposed that the normal blood supply to the peripheral bed, even in the presence of moderately severe stenosis, was due to the interaction between collateral and peripheral vascular resistances. They had further proposed that during distal vasodilatation resulting from increased metabolic activity following an exercise, the distal pressure decreases and ΔP increases. This causes already existing moderate severe

stenosis in the system to become functionally more severe, which not only affects vascular collateralization but flow through the stenosis as well. Similar observations by Carew et al. (1968) were attributed to peripheral vasodilatation and decreased intraluminal pressures distal to stenosis which lead to passive narrowing of the vessel lumen.

May et al. (1963) observed an inverse relationship between the blood flow and ΔP with alterations in degree and severity of stenoses. There was direct correlation between the ΔP across the stenosis and its length, which however, did not change the hemodynamics in less than subcritical range. In the critical range, a 4 times increase in length of stenosis was associated with 25 percent greater reduction in flow through the stenosis. The findings of Feldman et al. (1979) showed that the critical level of a stenosis could be reached at smaller decreases in c.s. area, with increase in stenosis length. A 75 percent stenosis over 15 mm length was as critical as a 96 percent stenosis over 1 mm length. Several stenoses in series in these studies produced an additive effect. Similar responses with stenoses in series also were reported by Gould and Lipscomb (1974) and Young (1979). Gould and Lipscomb (1974), in addition, noticed that stenosis greater than 85 percent, despite being associated with decreased

blood flow and flow reserve, did not change the resting blood flow distal to stenosis. Gould (1978), during his review of the functional aspects of a critical stenosis, suggested that percent decrease in c.s. area as a measure of stenosis severity was an unrealistic and oversimplified approach. This opinion was based on the observations that stenosis geometry and not the percent reduction in c.s. area alone altered pressures and flows. He mentioned that stenosis length, absolute diameter, divergence angles, eccentricity, turbulence and flow separation produce cumulative effects on the resistance, ΔP and flow reserve capacity in the region.

Young and Tsai (1973) in in-vitro studies reported that flow separation resulting from stenosis significantly influenced the pressure and flow characteristics, which showed a nonlinear relationship at critical level of stenosis. They believed that the ΔPs across the stenosis were dependent upon stenosis geometry, velocity of blood flow, inertial effects and viscosity of blood.

Young (1979), however, reviewed the effects of stenosis and stenosis geometry on collateral and peripheral vascular bed hemodynamics and proposed that the performance of a stenosis be described as a function of peripheral and collateral conductances and not simply by percent reduction in c.s. area. These observations were based on his own findings and findings of other researchers who had suggested that stenosis geometry

did not significantly influence vascular hemodynamics, except at moderate levels of stenosis. However, when peripheral resistance attains a minimal value, any increase in stenosis severity not only will decrease the blood flow further but will also influence the flow reserve in a significant manner. He suggested that stenosis resistance was not a fixed but a dynamic quantity which was influenced by collateral and peripheral vascular resistances. Similarly, Schwartz et al. (1980) reported that experimental or clinical stenosis was not a lesion with constant resistance. If it was, flow through a stenosis at worst would remain constant even if the oxygen demands of the distal ischemic bed increased markedly. But this is not usually the case, because dilatation of the peripheral arteriolar bed results in decreased arterial pressure beyond stenosis, which might cause a passive decrease in arterial caliber and intensify the severity of the stenosis. In previous studies (1979), they had shown that distal vasodilatation was associated with paradoxical decrease in coronary blood flow, a concomitant increase in ΔP and an increase in stenosis resistance to a level five times that of the unoccluded vessel. It was believed that these effects could have occurred due to release of vasoactive metabolites distally in the peripheral bed following transient coronary artery occlusions. The vasoactive substances intensively dilate the distal vascular

bed and potentiate the hemodynamic severity of the stenosis.

Prostaglandins

PGs are synthesized by virtually every tissue in the body (Christ and Vandorp, 1972). Their release can be stimulated by various means including hormones (bradykinin and angiotensin II), endogenous or exogenous neurotransmitters, tissue damage and decreased oxygen tension (Needleman et al., 1975a, 1975b, Needleman, 1976; Gunther and Cannon, 1980). Since there is no evidence that PGs can be stored, their release implies denovobiosynthesis (Piper and Vane, 1971). Little, if any, PGs can be detected in arterial blood because of efficient pulmonary destruction. Thus, PGs are considered as local hormones that are synthesized in response to different stimuli at or near their site of action.

PGs are formed by the enzymatic oxidation of polyunsaturated fatty acids (AA) i.e., 8, 11, 14 ETEA (Di homo-γ-linolenic acid) and 5, 8, 11, 14 ETEA which form PGs of 1 and 2 series, respectively. In the body, polyunsaturated fatty acids are not available in the free form but are bound to phospholipids and triglycerides. Therefore, PG synthesis can only follow the activation of lipases (phospholipase A_2 and possibly triglyceride lipase) which release bound fatty acids in free form. This free precursor of PGs (AA) is acted upon by cyclo-oxygenase with the incorporation of oxygen and results in the formation of PGG_2 and PGH_2.

Aspirin and aspirin like drugs inhibit the biosynthesis of PGs by inactivating cyclo-oxygenase. AA also is converted by lipooxygenase in platelets to a noncyclized product HETE.

Despite their own biological activity (vasoconstriction and platelet aggregation) PGG_2 and PGH_2 are very unstable intermediates and are very quickly (4-6 min.) converted to PGE, PGD and PGF series by isomerases and to TXA_2 by TXA_2 synthetase or by PG, 9 cyclase to PGI_2, a potent platelet deaggregating agent. The generation of these PGs in different body tissues is dependent upon the type of stimuli and production of different synthetic enzymes. Enzymes require endoperoxide as a substrate and lead to the formation of different types of PGs (Needleman and Kaley, 1978). Since different PGs produce different effects (complementary and antagonistic), the ultimate response of an organ or a region to PG biosynthesis often represents the pathophysiologic condition of the vascular bed involved.

The biosynthesis of PGs can be inhibited in vitro or in vivo by therapeutic doses of INDO (Flower, 1974). The dose level for inhibition of PG synthesis varies from species to species. For example, the guinea pig, as compared to the dog, requires 10-30 times more INDO concentration to effectively counter PG biosynthesis. In situations like this, very high doses of antagonists might be influencing other enzyme systems

in the body (Hamberg, 1972; Flower et al., 1972).

The primary focus of this section is to review currently available evidence regarding the endogenous synthesis of PGs in different vascular regions and their regulatory role in the maintenance of tissue viability by modulating local vascular blood flow.

Ali and McDonald (1977) observed that platelet incubation with AA caused biosynthesis of PGs within 2 minutes. The PGs synthesized were PGE_2, $PGF_{2\alpha}$, PGD_2 (stable forms) and PGG_2, PGH_2 and TxA_2 (unstable forms). The PG biosynthesis was effectively inhibited by INDO pretreatment in lower concentrations.

After studying the variable effects of PGs, Marmo et al. (1980) reported the direct, modulatory and reflex effects of PGI_2, 6-Keto $PGF_{1\alpha}$, $PGF_{2\alpha}$ and 15(S)-15 methyl $PGF_{2\alpha}$ upon cardiovascular and pulmonary systems. PGI_2 produced a transient hypotension, venous vasoconstriction and reflex sinus tachycardia with marked reduction in baroreceptorial carotid sinus reactivity. $PGF_{2\alpha}$ caused hypertension, tachycardia, arterial and venous vasoconstriction, increased CO, cardiac stimulation, increased sympathetic tone and changes in the excitability of barosensitive zone of the carotid sinus. $PGF_{2\alpha}$ also increased respiration by peripheral bronchconstricter effect and was also associated with stimulation of respiratory center via chemoreceptors.

Messina et al. (1976) investigated the possible regulatory role of PGs in local circulation and proposed that PGs produced both vasopressor and depressor activity in different vascular beds. This activity varied from species to species. PGs of E and A series were always associated with weaker vasodilatory response, whereas other PGs produced variable effects. They suggested that despite these variable effects, PGs play a homeostatically significant role in controlling vasoreactivity of vascular regions. They are essential in the regulation of vascular tone and resistance and act independently without interaction with other vasoactive agents like serotonin, histamine and bradykinin. During similar studies, Dusting et al. (1977), noticed that PGE_2 and TXA_2 opposed PGI_2 in the same vascular bed. PGE_2, TXA_2 and PGH_2 produced transient vasoconstriction, whereas PGI_2 and AA completely relaxed isolated coronary artery strips. INDO and 15-HPAA increased the resting vascular tone and abolished AA induced relaxation of arterial strips. The inhibition of AA induced vasorelaxation by 15-HPAA suggested that AA caused vasodilatation via PGI_2 formation.

Variable effects of PGs were also shown by Rose et al. (1974). AA caused hypotension, increased platelet aggregation and produced inconsistent effects on myocardial contractility. Aspirin completely abolished AA induced responses. PGE_2 and $PGF_{2\alpha}$, on the contrary, produced an immediate hypotension

and increased myocardial contractility, which remained unaffected by aspirin treatment. They suggested that AA induced responses were mediated by PGs (PGE_2 and $PGF_{2\alpha}$) or their intermediates (PGG_2, PGH_2). Similarly, in in-vitro studies on isolated coronary artery preparations, Kulkarni et al. (1976) observed that AA caused vasodilatation, whereas PGE_2 and $PGF_{2\alpha}$ produced vasoconstriction. PGE_1 on the other hand, caused vasodilatation which was potentiated by INDO. INDO along with aspirin and meclofenamate inhibited AA induced vasodilatory responses suggesting that its actions were mediated by PGs. They postulated that PGE_1, PGE_2 and $PGF_{2\alpha}$ acted by inducing changes in vascular smooth muscle cyclic nucleotides (Schultz et al., 1973) and that PGE_2 and $PGF_{2\alpha}$ induced vasoconstriction was due to increased c-GMP. PGE_1 related vasodilatation may have been due to increased c-AMP concentration (Flores and Sharp, 1972). Hatano et al. (1980), while studying PG effects in different vascular tissues, showed that $PGF_{2\alpha}$ was the most potent vasoconstrictive agent both in coronary and mesenteric arteries and PGI_2 was the most vasodilatory agent. PGE_1 and PGE_2 produced feeble contractile activity in mesenteric arteries. The variable effects of differing PGs could be attributed to the presence of two different types of receptors which would have mediated the contraction and dilatation produced by PGs.

While investigating clinical implications of PGI_2, Szczeklik et al. (1980) suggested that atherosclerosis was associated with disruption of PGI_2 in human lower extremities. PGI_2 infused intra-arterially or intravenously (5-10 ng/Kg/72 hrs) improved blood flow and relieved all the symptoms associated with atherosclerosis including pain and ischemic ulcers. The improvement caused by PGI_2 infusion was due to deaggregation of platelets in capillaries or due to proliferation of capillaries in the ischemic areas. Similarly, Ubatuba et al. (1979) reported that intravenous pretreatment with PGI_2 inhibited thrombus formation in carotid vessels with damaged endothelium. They suggested that PGI_2-induced inhibition of thrombus formation was due to platelet deaggregation and increased c-AMP concentration.

Moncada et al. (1976) postulated that the controlling factor in thrombus formation was the balance between TXA_2 and PGI_2 biosynthesis. TXA_2 and PGI_2 originate from common precursor endoperoxide (PGG_2 or PGH_2). These substances possess their own vasoconstrictive properties (Hamberg et al., 1975) and are responsible for platelet aggregation and ensuing release reaction (Hamberg et al., 1974). INDO inhibited PG biosynthesis by impairing transformation of PGG_2 or PGH_2 to TXA_2 or PGI_2 and caused decrease in blood flow (Needleman et al., 1977a). This then may not only enhance

platelet aggregation and deposition but eventually may lead to thrombus formation in a blood vessel.

Gunther and Cannon (1980) showed that temporary coronary occlusion was associated with increased PG synthesis in the ischemic areas of the myocardium. While investigating the assumed modification of angiotensin II activity by PGs, they noticed that administration of angiotensin II caused a marked increase in PG biosynthesis in isolated heart preparation. The response, however, was not pronounced in in-vivo coronary vasculature. Angiotensin II also potentiated adrenergic activity and cardiac ionotropism by increasing catecholamine secretion. Pretreatment with INDO led to significant decrease in PG biosynthesis and increased vascular resistance suggesting that cardiac PG synthesis may have been regulating the coronary vasoconstrictor effects of angiotensin II.

Folts et al. (1973) reported that 78 percent intravascular stenosis significantly decreased blood pressure and flow across the stenosis and abolished reactive hyperemic response. The achievement of critical stenosis at a lower level of reduction in c.s. area was believed to be due to intravascular obstruction which caused vasospasm and altered the ΔP and blood flow across the stenosis. While investigating the possible cause for myocardial infarction, they (1976) observed that 60-80 percent fixed stenoses were

associated with reductions in blood pressure and flow which returned cyclically back to control values following elimination of obstruction by physical poking or INDO treatment. On histologic examination, an amorphous mass of platelet aggregates was found in the narrowed lumen of the vessel. Platelet aggregation in the stenosed segment was due to platelet damage and activation by ADP. Later, Folts et al. (1977) investigated the possible interaction of collateral and peripheral hemodynamics during vasospasm and subsequent cyclic reductions in pressure and flows during 60-80 percent fixed stenoses. Vasospasm in these studies was associated with distal bed ischemia (manifested by S-T segment depression) and platelet aggregation. Platelet aggregation disappeared following pinching or administration of aspirin. They suggested that collateral and peripheral hemodynamics were not involved in platelet deaggregation, which must have been due to some other mechanisms involved and/or due to increased ΔP across the stenosis. Folts and Beck during subsequent studies (1979), showed a regular and spontaneous deaggregation of blood platelets in coronary vessels with 70 percent stenosis. Cyclical reductions of pressure and flows lasted 10-15 minutes. These events were potentiated by epinephrine and abolished by ibuprofen (a PG inhibitor). They suggested a possible role of epinephrine and PGs in the process of myocardial infarction. Vasodilator substances like

dipyridamole, nitroglycerine and papaverin inhibited the cyclic reductions of pressure and flow.

Folts and Rowe (1980), however, observed the disappearance of these oscillations with aspirin, ibuprofen, sulfapyrazone treatments and mechanical intervention (physical poking or pinching). By these results, they substantiated the previous findings that platelet aggregates were responsible for reductions in pressure and flow in coronary, carotid and femoral arteries. They proposed that the resulting vasospasm and platelet aggregation following an arterial occlusion was due to release of vasoactive PGs and not due to mechanical stimulation of the nerve endings in the arterial wall. In contrast, Uchida et al. (1980) suggested that the cyclic reductions of blood pressure and flow in the partially constricted coronary arteries were the consequence of vascular spasm and not platelet aggregation or thrombus formation. These suggestions were based on their studies with angiography and microscopy. During angiographic studies, they observed the downstream narrowing, accompanying a coronary stenosis, to be of uniform, smooth and symmetrical diameter which was uncharacteristic of platelet aggregation or thrombus formation. On microscopic examination they found no evidence of platelet aggregation. The disappearance of vasospasm with vasodilatory drugs like dipyridamole,

nitroglycerin and papaverin reinforced their previous observations in these studies. The researchers suspected that vasospasm was responsible for the cyclic reductions in pressure and flow. The vasospasm, they further suggested, was of vascular origin and was eliminated by nonspecific vasodilatory substances. During their preceding studies (1977a, 1977b, 1978a, 1978b), however, they had shown that reductions in pressure and flow in a partially stenosed coronary artery were abolished by aspirin, phenylbutazone, benzydamine, cu-chlorophylline and PGI_2. The reductions on the other hand were potentiated by epinephrine, PGE_2, lack of glutathione and 15-HPAA with inconsistent presence of platelet aggregation in the stenosed vessel. During these studies, Uchida et al. (1978b) had suspected the possible role of PGs, platelet aggregates or vasospasm in cyclic reductions of pressure and flow following partial occlusion.

While supporting the findings of Folts et al. (1977), Capurro et al. (1979) observed that cyclic reductions in coronary blood flow were due to platelet aggregates and PG biosynthesis. Aspirin induced improvement in flow was due to direct effect of aspirin on platelet aggregation and not to PG biosynthesis. Helenski et al. (1980), however, showed that thrombosis induced vasospasm was due to platelet activation, and simultaneous release of SRT and TXA_2, which not only directly affected the thrombosed artery but may have caused

vasospasm in the collateral vessels as well. Pretreatment with INDO relieved the vasospasm and improved Q_c. They suggested that INDO acted principally by inhibiting the biosynthesis of TXA_2 rather than of all other PGs and that SRT in some way was related to TXA_2 release. Release relationship between TXA_2 and SRT also was suggested by O'Donnel and Fiedel (1979).

Capurro et al. (1980) revealed that aspirin not only caused platelet aggregation in coronary arteries but also abolished AA induced vasodilatation in these vessels. Platelet aggregation potentiated by low doses of aspirin was inhibited at higher doses. PGI_2 induced vasodilatation was unaffected by aspirin. This was because aspirin inhibited the biosynthesis of PGs at the cyclo-oxygenase level, hence exogenous PGI_2 response was unaffected, whereas AA induced vasodilatory response was abolished. They suggested that AA induced vasodilatation was mediated by PGs, possibly PGI_2. During subsequent studies Capurro et al. (1981) found that aspirin pretreated animals responded more effectively to exogenous PGI_2 than those that were not treated. PGI_2 significantly improved blood flow to the myocardium following aspirin pretreatment. The response to adenosine, nitroglycerin and nitroprusside compounds, however, did not change with aspirin pretreatment. They postulated that improvement in blood flow following aspirin pretreatment could have been due

to its inhibitory effect on biosynthesis of PGs. These inhibited PGs could have included those with direct vasoconstriction effect and also those that might mediate their vasoconstrictive effects by release of catecholamines.

Jugdutt et al. (1979), while determining the role of PGs in myocardial infarction, observed that INDO potentiated infarction following coronary artery occlusion. This was due either to reduction in PGE_2 mediated increase in coronary blood flow or to unopposed vasoconstrictive effects of PGG_2, PGH_2 and TXS. They suggested, however, that the exact mechanism of INDO action was not clear and was yet to be elucidated. Similar findings were reported by O'Beaty and Donald (1979) in the canine hind limb. They contended that PGs were responsible for maintaining hind limb blood flow following inflow occlusion during rest and exercising states. The inhibition of PGs by INDO and meclofenamate increased vascular resistance and decreased blood flow under resting conditions. This decrease in blood flow was due to a nonspecific vasoconstrictive action of the inhibitory agent on the vasculature following an inflow occlusion. Alexandar et al. (1975), however, suggested that INDO induced decrease in RH at all levels of occlusion was due to inhibition of PGE_1. They believed that PGE_1 was the prime regulatory factor of blood flow during myocardial ischemia (Alexandar et al., 1973). The ischemia

of very short duration (less than a minute) may not have been associated with PGs release. Therefore, blood flow under these very acute situations must be regulated by substances other than PGs. PGs, however, do control blood flow regulation during longer durations of arterial occlusions (Needleman and Kaley, 1978).

Serneri et al. (1980) investigated the effects of ischemia produced by 3 minutes of arterial occlusion and 15 minutes of venous stasis, on the production of PGI_2. They found that PGI_2 was produced during the first minute of RH and declined subsequently with repeated episodes of intermittent ischemia. Despite similar observations during venous stasis, PGI_2 generation was three times less than arterial occlusion. Similar findings were reported by McGiff and Itskovitz (1973) on kidneys and Block et al. (1975) and Moncada et al. (1977a) in rabbit hearts. They believed that local blood flow changes due to arterial occlusion induce PGI_2 release which maintains blood supply to the ischemic region. INDO in these studies inhibited the synthesis of PGI_2. PGs played a regulatory role in the maintenance of blood flow, following an arterial occlusion, in the preparations of Owen et al. (1975). Decreased blood flow observed in these preparations following INDO treatment could have been due to: i) inhibition of normal PG biosynthesis, ii) inhibition of enhanced PG biosynthesis, or

iii) nonspecific vasoconstrictor effect of INDO on vascular smooth muscle. INDO action directly on platelet aggregation and indirectly in the modulation of neural tone was also speculated in these studies to be a possible cause of decreased blood flow in the region.

Rosenblum et al. (1980) reported that the cyclooxygenase inhibitors, aspirin and INDO enhanced platelet aggregation in mesenteric vessels. Mesenteric vessels normally produce PGI_2, which is a platelet deaggregating agent. Aspirin and INDO induced platelet aggregation was possibly due to inhibition of PGI_2 synthesis, which might have been regulating the microvasculature reactivity and tissue viability. Contrary to those studies, Glenn and Horan (1981) observed an adenosine mediated increase in coronary blood flow in ischemic myocardium following INDO treatment. They suggested that INDO potentiated increased blood flow may have been due to inhibition of vasoconstrictor PGs which were predominantly released during an inflow occlusion.

Flower (1974), while investigating the mechanism of INDO action, found that it acts in the early stages of PG synthetic pathway in a competitive irreversible fashion. INDO exerts its effect by combining slowly with the active site and decreasing the catalytic activity of the enzyme in a time dependent manner. The long lasting effects (24-48 hrs) following oral administration are due to its conversion to

active metabolite.

Moncada et al. (1977b) reported that imidazole inhibited the enzymic conversion of PGG_2 or PGH_2 to TXA_2 by platelet microsomes without affecting PGI_2 biosynthesis. Imidazole induced TXA_2 inhibition was complete and selective in nature (Needleman et al., 1977b). The studies of Busse and Seuter (1969), however, revealed incomplete inhibition of TXA_2 following methyl imidazole treatment of aggregated platelets. PGE_1 and PGI_2, on the other hand, not only deaggregated the platelets but also blocked TXA_2 and melonaldehyde production by elevating intracellular c-AMP concentration. Despite their role in platelet aggregation adenosine, ATP and AMP did not affect TXA_2 or melonaldehyde production. AA administration in their studies caused rabbit mortality, which was effectively blocked by INDO, aspirin and methyl imidazole treatment. On the basis of these observations, they suggested that pharmacological inhibition of platelet function could not be achieved by a uniform mechanism controlled by the AA pathway.

Serotonin

Coronary artery disease, complete occlusion and critical stenoses are associated with platelet aggregation (Haerem, 1972; Folts et al., 1973; Coe and Salzman, 1976; Uchida

and Murao, 1979). Interaction between platelets and damaged endothelium may lead to thrombus formation (Danese and Haimov, 1971; Horie et al., 1978). The aggregated platelets and thrombi formed, in addition to ischemic tissue, release a variety of vasoactive substances including SRT (Bulle, 1957; Gladkova and Vasiliev, 1971; Kordenat and Kezdi, 1979). SRT has been implicated in producing variable effects in different vascular beds, depending upon the number and type of receptors present and existing tone of the vascular bed (Dalessio, 1972).

Page and McCubbin (1953) examined the amphibaric action of SRT on vascular smooth muscle. When the neural tone was normal or increased, SRT behaved as a vasodilator. When the vascular tone was low, it acted as a powerful vasoconstrictor agent. Similarly, Haddy (1960) observed that SRT actively constricted larger blood vessels and dilated smaller ones. It lowered the systemic blood pressure in hypertensive animals and increased blood pressure in hypotensive animals. Fillion et al. (1971) reported cardiac stimulation in response to SRT administration in addition to the responses observed by Haddy (1960).

Bock et al. (1957) and Glover et al. (1958) noticed an increase in forearm and muscle blood flow with simultaneous decrease in skin blood flow following SRT administration. While comparing SRT and epinephrine effects on isolated

cerebral arteries, Toda and Fugita (1973) found that SRT induced contractions were more pronounced than epinephrine. The response was of reverse nature in mesenteric vessels. The researchers thought this response was due to transition in SRT receptor distribution from higher to lower vessels. The findings of Urthaler et al. (1980), however, showed significant vasoconstrictive effect of SRT on renal and mesenteric vessels. The SRT effects were not pronounced on coronary, carotid and femoral vessels. No change in forearm blood flow, following continuous intraarterial or intravenous infusion of SRT was observed by Daugherty et al. (1968). They speculated that the route of administration played no role in SRT actions. Zucker and Cornish (1980), in their studies, observed hypotension and bradycardia followed by delayed tachycardia and hypertension, following a single bolus injection of SRT. They suggested that SRT administered intraventrically invoked a hypertensive coronary chemoreflex.

Daicoff et al. (1968) and Swedenborj (1971) found that experimentally induced stenosis was associated with SRT release, which caused pulmonary vasoconstriction. Pretreatment with methysergide blocked the SRT induced vasoconstriction. They believed that methysergide acted peripherally and locally with a nonperceptible effect on CNS.

Lewis and Reichert (1926) reported that femoral artery thrombosis was associated with severe pain and ulceration of

the heel, which led to an asymptotic condition. Surgical removal of thrombus not only restored blood flow through the stenosis but also across the collateral vascular bed. During similar studies, Imhoff (1961) observed that the acute ligation of feline distal aorta produced only transient abnormalities, whereas ligation associated with blood clot caused paralysis, loss of femoral pulse and cold extremities. Aortograms obtained during Imhoff's studies showed the presence of collateral blood flow following an acute ligation which was significantly reduced in clot associated ligated animals. He believed that release of some vasoactive agents from the blood clot depressed the collateral blood flow. Sullenberger et al. (1959) found a severe depression of blood flow in the microvasculature of the ischemic tissue distal to occlusion. They attributed this depression in flow to the release of SRT from venous thrombosis. The release of SRT with strong vasoconstrictive properties following coronary artery ligation was also reported by Gladkova and Vasiliev (1971).

Schaub et al. (1976) investigated the role of thrombosis in the regulation of collateral blood flow in the feline hind limb. They showed that thrombotic limbs did not carry substantial quantities of collateral blood flow which they suspected was due to ADP and SRT release. SRT in addition, increased capillary permeability, caused endothelial cell

damage (Majno et al., 1967) and damaged collateral vessels rendering them nonfunctional. During follow-up studies, Schaub and his associates (1977b), compared the endogenously released SRT actions with those that were exogenously administered. Exogenous SRT (6 µg) in a closed aortic segment caused decrease in hind limb blood flow, which was abolished in cinencerin hydrochloride pretreated animals. Similar results were obtained when animals were pretreated with RZP and/or PCPA. These drugs decreased SRT levels and exhibited recovery in collateral blood flow. These observations suggested the possible role of SRT during thrombosis in the regulation of collateral blood flow (Schaub et al., 1977b). During identical studies in the coronary arteries, Kordenat and Kezdi (1979) improved collateral blood flow with METH in thrombus associated coronary myocardium.

Bell et al. (1967), Zarvas et al. (1971), Welch et al. (1973) and Reuse-Blom (1976) showed that endogenously released SRT following an acute ligation was responsible for a vascular spasm that lasted several hours and caused severe reduction in blood supply to the ischemic tissue. Similar vasospasm characterized by a pronounced vasoconstriction was also reported by Hellstrom (1979). It potentiated the myocardial ischemia, caused endothelial cell damage, produced vascular stasis and augmented thrombus formation in already sclerotic vessels. He suspected these responses were due to

release of vasoactive substances from the ischemic tissue.

METH is a potent SRT antagonist and has been frequently used to block SRT in various body tissues (Halpern et al., 1960; Owen et al., 1971). It competes for SRT uptake at the receptor site (Curran et al., 1967).

While investigating the METH actions in different vascular regions, Saxena (1974) observed a selective vasoconstriction in the carotid artery vascular bed following METH treatment. Femoral, superior mesenteric, renal and vertebral artery vascular beds were, however, not affected even in larger doses. This was due to quantitative variation in both type, population and sensitivity of receptors subserving METH induced peripheral vasoconstriction (Dalessio, 1972; Saxena, 1972). In contrast, Graham (1964) reported that METH was associated with arterial spasm in the hind limb, cramps in the lower legs and swelling of the feet in human beings. Similarly, Dalessio et al. (1961) and Curran et al. (1965) noticed a bilateral lower extremity vascular spasm following oral doses of METH. Saxena (1972), however, suggested that the net effect of METH in a vascular bed depended upon its pre-existing vascular tone. It causes vasoconstriction in a vascular bed with low vasomotor tone and vice versa.

Koe and Weissman (1966) investigated the effects of PCPA, a SRT blocking agent, and reported that PCPA was the selective

depletor of SRT in the brain as well as peripheral nerves and did not affect catecholamines, MAO and tryptophan decarboxylase in the body tissues. Bloom and Giarman (1968) described PCPA as specific inhibitor of tryptophan hydroxylase, a rate limiting enzymes in the synthesis of SRT. The longer duration of PCPA induced SRT depletion was due to the conversion of PCPA to an active metabolite, PCPA pyruvic acid, which caused irreversible conversion of tryptophan hydroxylase.

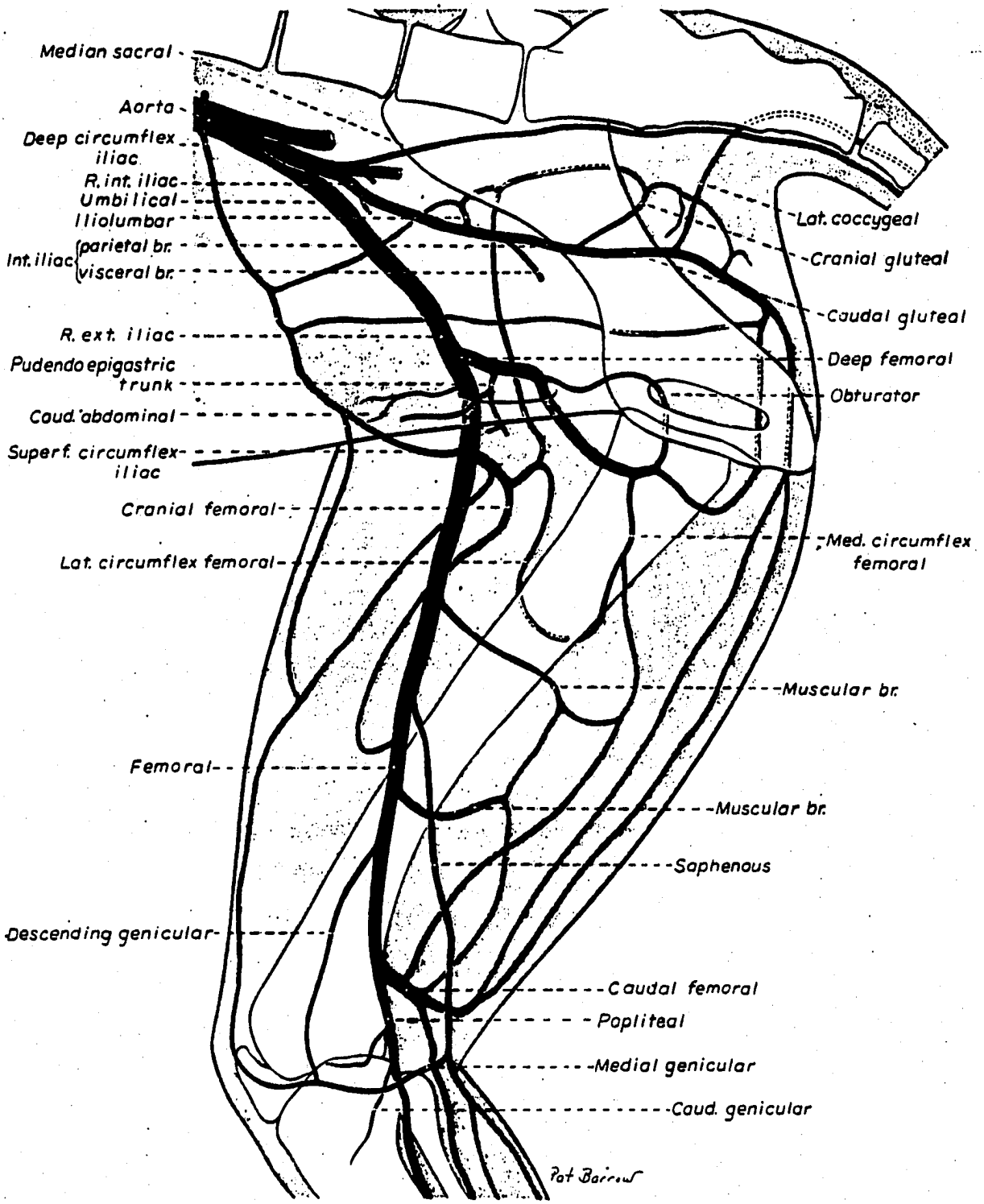
RZP, another depletor of SRT from platelets and body tissues, was studied by Carlsson et al. (1957). They suggested that the exact mechanism of RZP induced SRT depletion was not known. They believed RZP acted by simple displacement of SRT at the receptor site and destroyed the binding capability of the receptor for SRT by denaturing the storage site. They proposed a possible reserpine effect in the inhibition of the transport system and the storage of the granules.

MATERIAL AND METHODS

Experimental Model

The canine hindleg was selected to serve as a regional blood flow model of a main vessel-collateral arterial system. The arteries of the hindleg are easily accessible for instrumentation. A mechanical stenosis was used to simulate an obstruction to flow as might be found in the femoral arterial thrombosis. By this means, a model of arterial obstructive disease is developed. The DFA, the PCFA, the CGA and the SA including the muscular and the DCIA branches are the chief inputs of collateral blood supply to a peripheral bed, the hindleg below the stifle joint. The DCFA, another collateral artery, emerges from the femoral artery in the distal thigh region. It gives off branches to the tissues both above and below the stifle joint (Fig. 1). In the resting state, the ΔP is such that it normally carries blood from the femoral artery in a reverse direction (centrifugal) into the thigh region (collateral bed). Blood flow supplied into the thigh via DCFA (henceforth referred to as nutritional blood flow) is to meet nutritional requirements of the caudal portions of the thigh musculature and hamstring muscle group distal to the stifle joint. During an obstruction, when flow in the femoral artery is substantially reduced, the ΔP reverses and the DCFA receive blood from the descending branches of the cranially located thigh arteries and provides collateral

Figure 1. The chief arteries and some of the potential collateral vessels in the hind limb of the dog (reproduced from Anatomy of the Dog by Miller et al., 1979)


Median sacral
Aorta
Deep circumflex iliac
R. int. iliac
Umbilical
Iliolumbar
Int. iliac
parietal br.
visceral br.
R. ext. iliac
Pudendoepigastric trunk
Caud. abdominal
Superf. circumflex iliac
Cranial femoral
Lat. circumflex femoral
Femoral
Descending genicular
Lat. coccygeal
Cranial gluteal
Caudal gluteal
Deep femoral
Obturator
Med. circumflex femoral
Muscular br.
Muscular br.
Saphenous
Caudal femoral
Popliteal
Medial genicular
Caud. genicular
Pat Barrow

blood flow to the peripheral bed (henceforth referred to as collateral blood flow). The direction of collateral blood flow in DCFA is centripetal with respect to the femoral artery. The volume of flow carried by the DCFA has not been studied. In this study, therefore, an attempt was made to quantify the volume of DCFA flow under normal and obstructed flow conditions.

Hemodynamic Measurements

Blood flow measurement

Morphological and tracerdye studies of the dog have shown that the femoral artery supplies blood to the hind leg and the femoral vein returns all the peripheral blood from the hind leg distal to the proximal caudal femoral vein. During partial or complete femoral artery occlusion, blood is supplied to the ischemic lower hind leg peripheral bed by the collateral vessels in the thigh region. The flow through the femoral artery (Q_s) and its corresponding vein, the femoral (Q_p) was measured by two 3 mm id. cannulating EMF transducers (Biotronix, BLG G10 Electromagnetic flowmeter, Biotronix Lab. Inc., Riversprings, Md). To determine the volume of flow accurately, these flow transducers were calibrated by the method of Young et al. (1975) at the end of each experiment (see Appendix A). Collateral blood flow was estimated as the difference between Q_p and Q_s.

$$Q_c = Q_p - Q_s.$$

Blood pressure measurement

Blood pressures were measured at 5 different locations in the hind limb by Statham Model P23Ac transducers (Statham Medical Instruments, Inc., Hato Rey, Puerto Rico) (Lambert and Wood, 1947). All of the transducers were mounted at the same elevation as the hind leg. They were calibrated with known pressures before the start of the experiment (see Appendix A). Site selection for each pressure measurement was based on its potential role in collateral and peripheral hemodynamics. The pressures measured were as follows:

Pa: Mean arterial blood pressure at the level of abdominal aorta, very close to the origin of common iliac artery and just above the region where several arteries branch and contribute to the hind limb collateral vascular bed (collateral circulation driving pressure).

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

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

P_3: Mean arterial blood pressure in a branch at the DCFA level (potential collateral blood pressure).

PV: Mean arterial blood pressure at the femoral vein upstream from the flow transducer (venous blood pressure).

The following equations were used to calculate the R_s, R_c, and R_p.

$$R_s = \frac{P_1 - P_2}{Q_s}$$

$$R_c = \frac{P_a - P_3}{Q_c}$$

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

Dimensions of these variables are:

P (Pressure) = mm Hg

Q (Flow) = $ml.sec^{-1}$

R (Resistance = $mm\ Hg.sec.ml^{-1}$

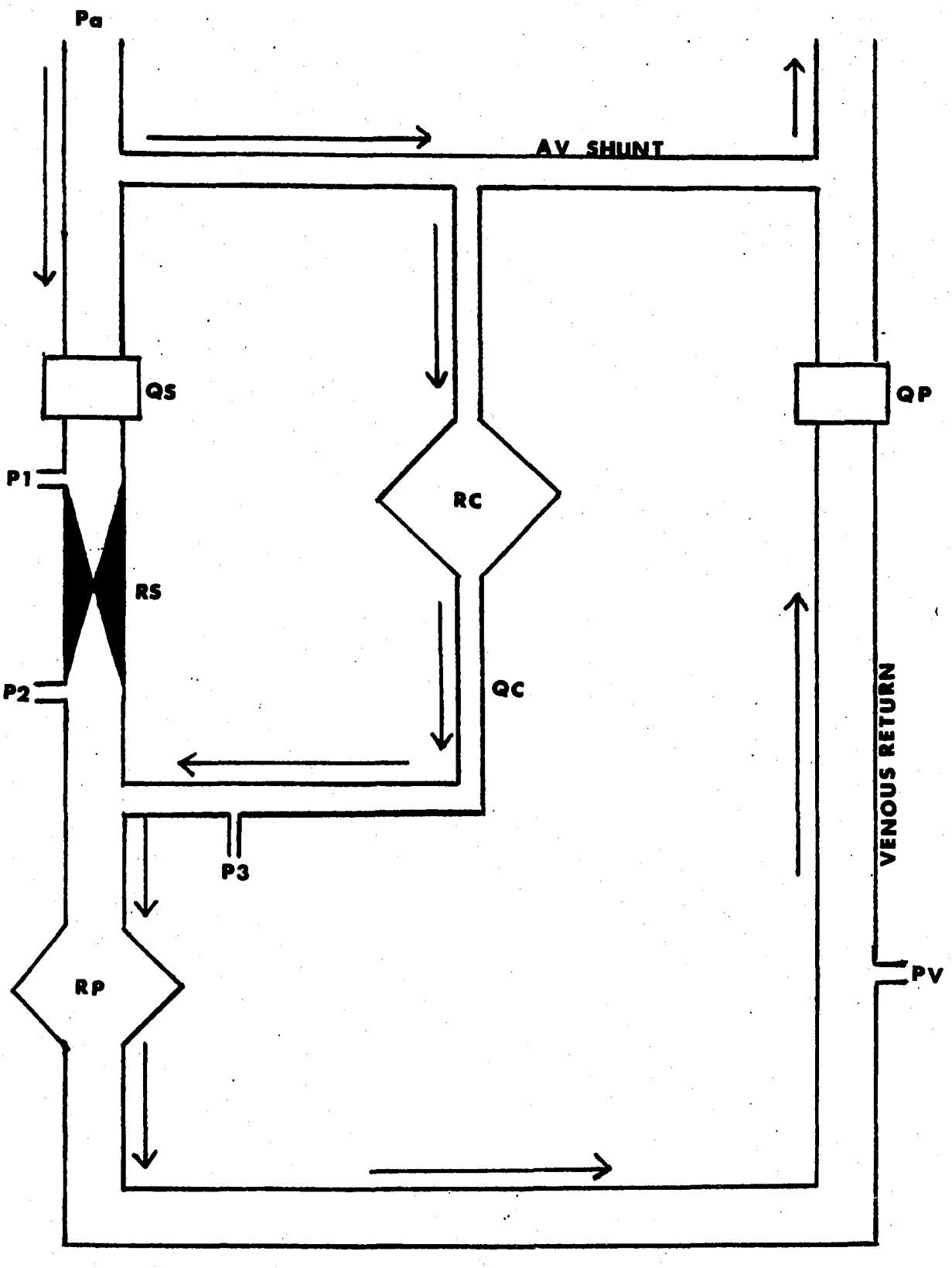
The schematic representation of the experimental model showing the distinctive sites of pressure and flow measurements is shown in Fig. 2.

Preparation of the Experimental Animal

Anesthesia

The experiments were performed on 28 mongrel dogs of both sexes weighing 22 to 36 Kg. The animals were lightly anesthetized with thiamyl sodium (Surital, Parke-Davis and Co., Detroit, MI.) 30 mg/Kg iv, and were cannulated with an endotracheal tube to facilitate proper ventilation. During the

Figure 2. Schematic representation of hind limb of the dog with various resistive vascular components and sites of pressure and flow measurements


Pa
AV SHUNT
QS
QP
P1
RC
RS
P2
QC
VENOUS RETURN
P3
RP
PV

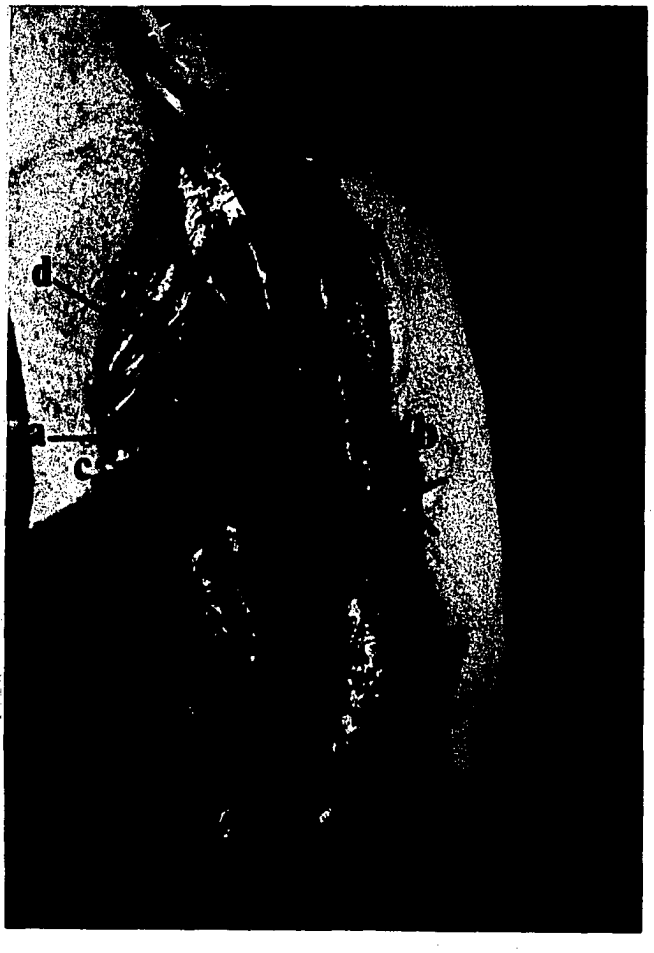
course of the experiment, the animals were maintained in the surgical plane of anesthesia with α-chloralose (Sigma, St. Louis, MO.) 25 mg/Kg iv, as and when required. Body temperature was maintained constant with heating pads and was monitored on YSI telethermometer (Yellowsprings Instrument Co., Yellow Springs, Ohio). The EKG was monitored with a Corbin-Farnsworth LIFE GUARD device (Gould Inc., Instrument Systems Division, Cleveland, Ohio). A constant infusion (3.5 ml/Min iv) of dextrose 5 percent in bicarbonated lactate Ringer solution was given to maintain fluid balance during the experimental period.

Surgical procedure

To expose the femoral artery for the emplacement of an instrumented bypass device, an incision was made between MCFA and DCFA on the medial aspect of the left thigh. The sartorius and semimembraneous muscles were dissected at their insertions and the medial fabella, located adjacent to the condyle of the femur, was removed. The femoral artery and the corresponding vein were exposed and isolated in the region between the MCFA and the DCFA (Plate 1).

A second incision was made at the PCFA level in the contralateral limb and the PCFA was isolated. Cautery was used during the surgical procedure to minimize hemorrhage from the exposed tissues following administration of heparin.

Plate 1. The surgical incision on the medial aspect of the thigh between MCFA and DCFA.



a - Femoral vein
b - Femoral artery
c - DCFA
d - MCFA

At this point, the animal was given heparin (3 mg/Kg iv). Heparin at half this initial dose was repeated at hourly intervals in order to avoid clotting problems during hemodynamic measurements.

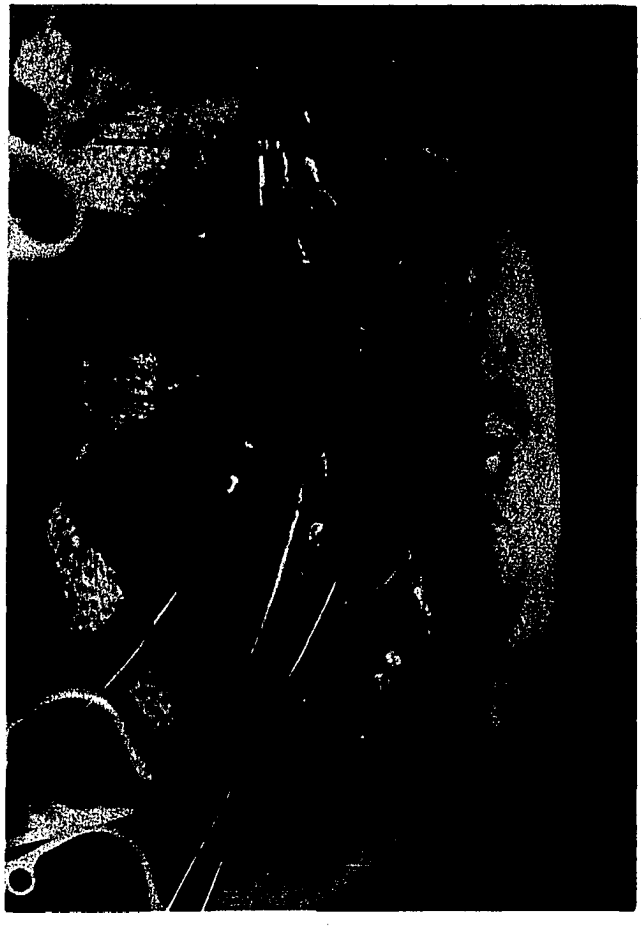
Cannulation

A branch of the DCFA was cannulated to record P_3. This procedure was followed by cannulation of the contralateral PCFA to record Pa. The cannula in PCFA was advanced into the abdominal aorta to a point very close to the origin of the common iliac artery. At this stage, the femoral arterial and venous segments were cannulated (Plate 2) with a specially designed extracorporeal perfusion apparatus consisting of the two bypass circuits, one for the femoral artery and one for the corresponding femoral vein (Plate 3). The decision to use extracorporeal bypass systems was made: i) to avoid the loss of potential collateral vessels for measuring blood pressures and ii) to avoid the artifacts due to closeness of the hemodynamic measuring devices which would have made estimation of pressures and flows difficult.

Arterial Bypass System

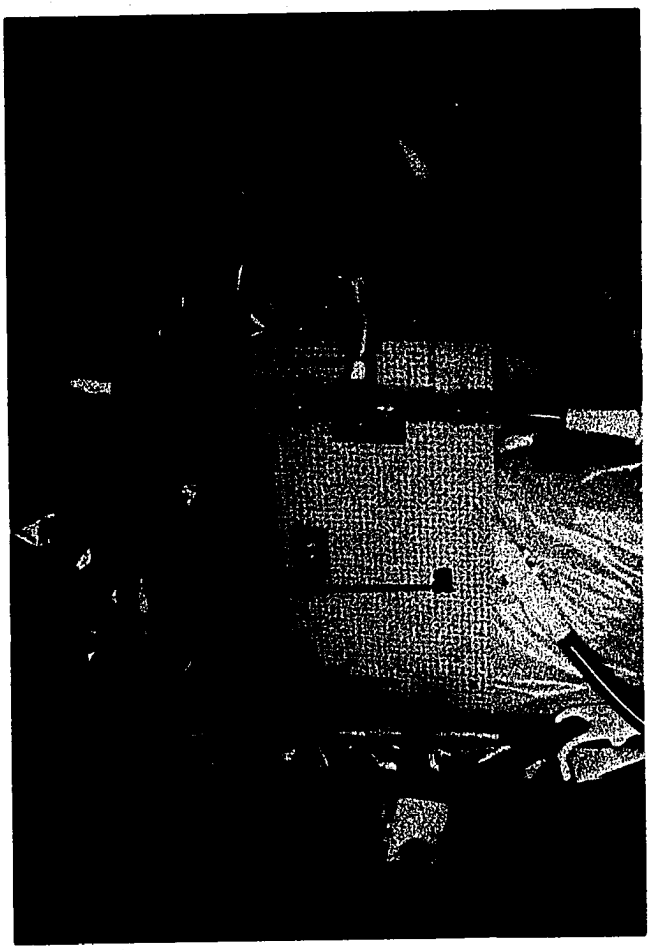
The arterial bypass system was composed of two pressure taps proximal (P_1) and distal (P_2) to a stenotic segment (R_s),

Plate 2. Femoral artery and femoral vein cannulated with extracorporeal bypass circuits.



a - Femoral artery
b - Femoral vein
c - Infusion tap

Plate 3. Extracorporeal bypasses for artery (a) and vein (b) are shown



a 3 mm id EMF transducer (Q_s) with large diameter (greater than femoral artery diameter, medical grade silicone rubber tubing at both ends which led to the cannulas.

Venous Bypass System

The venous bypass included a pressure tap (P_v) and KCl coated silver reference electrode proximal to EMF transducer (Q_p) and a platinum hydrogen electrode distal to the flow probe with medical grade silicone rubber tubing at both ends. The total length of the tubing including connectors and EMF transducers was 76 cms in each bypass.

Flow and Resistances in the Bypass Circuits

To determine the flow resistive effects of these bypasses, an experiment was performed. The fluid dynamics of each extracorporeal bypass was examined under a constant pressure head and varying flow conditions. The data thus obtained are presented in Appendix B (Tables B1-B3). According to these data, flow in 1-2.5 ml/sec range was laminar. This flow rate did not significantly change the fluid dynamics in the bypass. However, when flow rate increased beyond 3 ml/sec, turbulence occurred and resistance increased to 1.99 and 1.04 mm Hg·sec·ml^{-1} in the arterial and venous bypass, respectively (Appendix B, Fig. B1). The bypass circuit flow resistance at 0%

stenosis thus was almost insignificant compared to that measured with a critical stenosis applied (Appendix B, Fig. B2). In the arterial segment, the higher initial resistance at the lowest flow rate could not be explained. It may have been due to a small pressure differential across the arterial segment which may not have been accurately detected by the pressure transducers. On the basis of these observations it was concluded that bypass systems did not significantly affect the in vivo hemodynamics.

The pressure taps P_1, P_2 and P_v in the extracorporeal bypasses, along with catheters from the P_a and P_3 were then connected to their corresponding transducers. All the transducers were mounted at the same elevation. Standard cardiac catheters (id = 1.42 mm and od = 2.33 mm) were used for all pressure measurements except P_3, for which a polyethylene catheter P.E. 50 (id = 0.76 mm, od = 1.22 mm) was used.

Instrumentation

Pressures and flows were recorded on a Grass model 7 polygraph (Grass Instrument Co., Quincy, Mass.). Outputs from the polygraph were simultaneously being recorded as a raw (analog) data on a 7 channel instrumentation tape recorder/reproducer (Honeywell model 5600, Honeywell Test

Instruments Division, Denver, CO.) and Laboratory Digital Computer (Pdp 8e, Digital Equipment Corporation, Maryland, Mass.) for instantaneous analysis of the analog data (analog to digital conversion). The digitized signal was stored on storage oscilloscope monitor over 12 sec periods and included 256 sampling points. Signals that lasted for a minimum of 4 seconds were instantaneously analyzed and averaged each time for digital output. The analyzed signals included 6-10 pressure and flow waveforms (approximately 80-85 sampling points) depending upon the heart rate of the animal. This was done in an effort to get representative and reliable data (complete set-up of data acquisition equipment is schematically shown in Fig. 3).

Experimental Design

Endothelial cell damage, platelet aggregation and distal ischemia are all associated with partial or complete arterial occlusions. They trigger a biochemical sequence of events which eventually leads to the release of vasoactive substances including PGs, TXs and SRT (Fig. 4). In this study an attempt was made to identify and characterize the regulatory role of these compounds by observing the influence of their respective antagonists at 6 levels of progressive stenosis (0, 60, 80, 90, 95 and 100 percent reduction in flow c.s. area

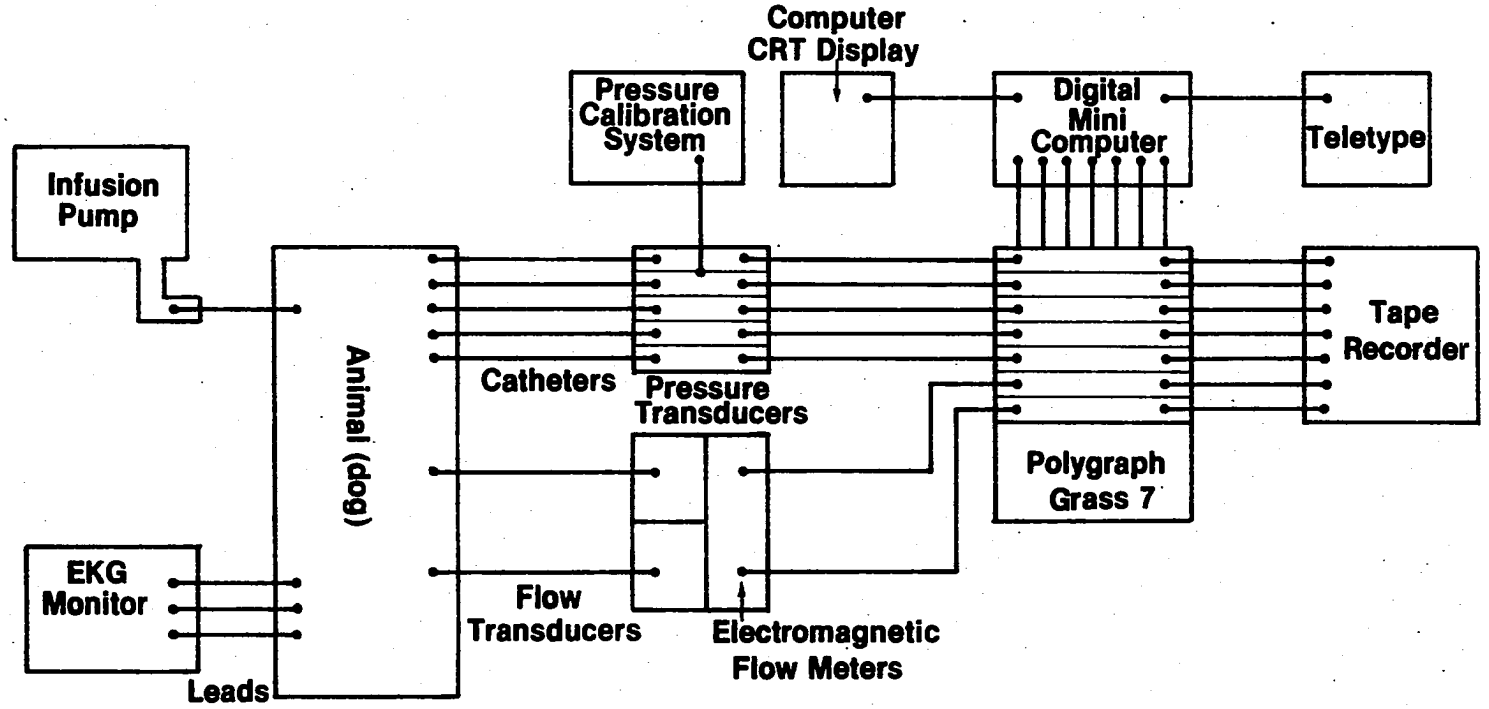


Figure 3. Schematic representation of data acquisition equipment used during pressure and flow measurements

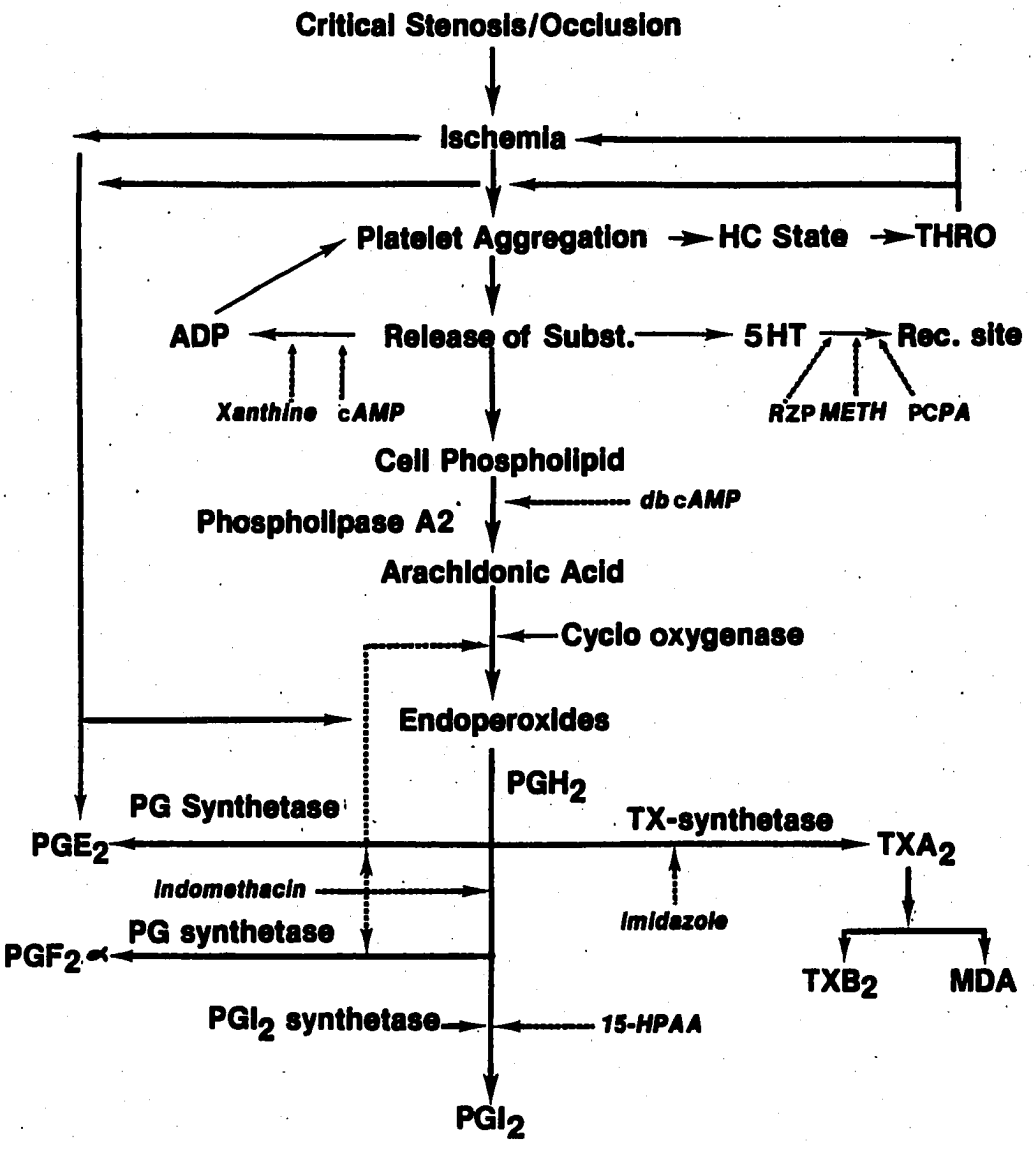


Fig. 4. Sequence of events which lead to the endogenous release of various vasoactive substances (dotted lines indicate various blocking agents).

in the femoral artery). SRT was antagonized at the receptor site with METH (Curran et al., 1967; Saxena, 1974) or RZP (Carlsson et al., 1957; Shore et al., 1955; Shore, 1962) and at the neuronal level by PCPA and RZP (Koe and Weissman, 1966; Bloom and Giarman, 1968).

The synthesis of PGs and TXs were inhibited at the cyclo-oxygenase level by INDO (Flower, 1974; Moncada et al., 1976; Rosenblum et al., 1980). TXA_2 was specifically blocked at the thromboxane synthetase level by IMZ (Hamberg et al., 1975; Moncada et al., 1977b).

The 28 animals were divided into 6 groups. Each group received either an agonist or antagonist drug or both following measurements of hemodynamic variables under resting, untreated conditions. G1, G3, and G4, each received ACH and SRT as agonists, whereas G2 received only SRT as agonist. G2 to G6, were all subjected to various antagonist treatments, i.e., METH, PCPA, R + PCPA, INDO and IMZ, respectively.

Pressures and flows were measured at 6 graded levels of stenosis during the C, ACH, METH, INDO and IMZ treatments in various groups (Table 1), whereas during SRT, PCPA and R+PCPA the pressures and flows were measured at 0, 95,and 100 percent stenosis levels.

All the 8 animals in G1 were subjected to DCFA occlusion to test: i) the hypothesis that this vessel serves as the

Table 1. Experimental design showing groups, treatments, and number of animals in each treatment

Groups	Group 1			Group 2			Group 3			Group 4			Group 5		Group 6	
Treatments[a]	C	ACH	SRT	C	METH	SRT	PCPA	ACH	SRT	R+PCPA	ACH	SRT	C	INDO	C	IMZ
	8[b]	8	6[c]													
				4	4	4										
							4	4	4							
										4	4	4				
													4	4		
															4	4

[a] ACH - (1-2 g/Kg/Min ia)
SRT - (15-30 µg/Kg/Min ia)
METH - (100 µg/Kg iv 30 minutes prior to measurements)
PCPA - (300 mg/Kg orally every 72 hrs prior to experiment)
R or RZP - (5 mg/Kg orally 8-10 days before the experiment)
INDO - (20 mg/Kg orally 1-2 hrs prior to measurements)
IMZ - (10 µg/Kg iv 1/2-1 hr prior to measurements).

[b] Numbers in treatment column represent the number of animals subjected to a treatment.

[c] Variation in the number of animals subjected to each treatment within a given group was either due to fewer animals treated or to rejection of data deemed unreliable because it was obtained late in a long experimental period.

principal collateral recipient vessel (Plate 4), and ii) to quantify the nutritional blood flow supplied by this vessel to the thigh musculature. The DCFA receives nutritional blood flow from the femoral artery under normal flow conditions. ACH was administered in 5 of these animals and blood flow was measured before and after DCFA occusion at 0, 95 and 100 percent stenosis levels.

Blood samples were obtained for analysis of SRT concentration at 0, 95 and 100 percent stenoses from animals in G1, G2, G3 and G4. SRT concentration was determined by the spectro-photo-fluorometric assay procedure of Maickel et al. (1968). The principal steps in this procedure originally were adapted by Bogdanski et al. (1956) and were subsequently modified by Waalkes (1959). An assay kit (Regis Chemical Co., Morton Grove, Illinois) based on the Maickel et al. (1968) method was used for SRT analysis (the stepwise analytical procedure for SRT determination is shown in Appendix C).

Data Analysis

All of the data obtained were tabulated. Statistical analysis included nested split, split plot experimental design using a model incorporating a multivariate two way classification with unequal number of observations and paired student "t" test (Snedecor and Cochran, 1976).

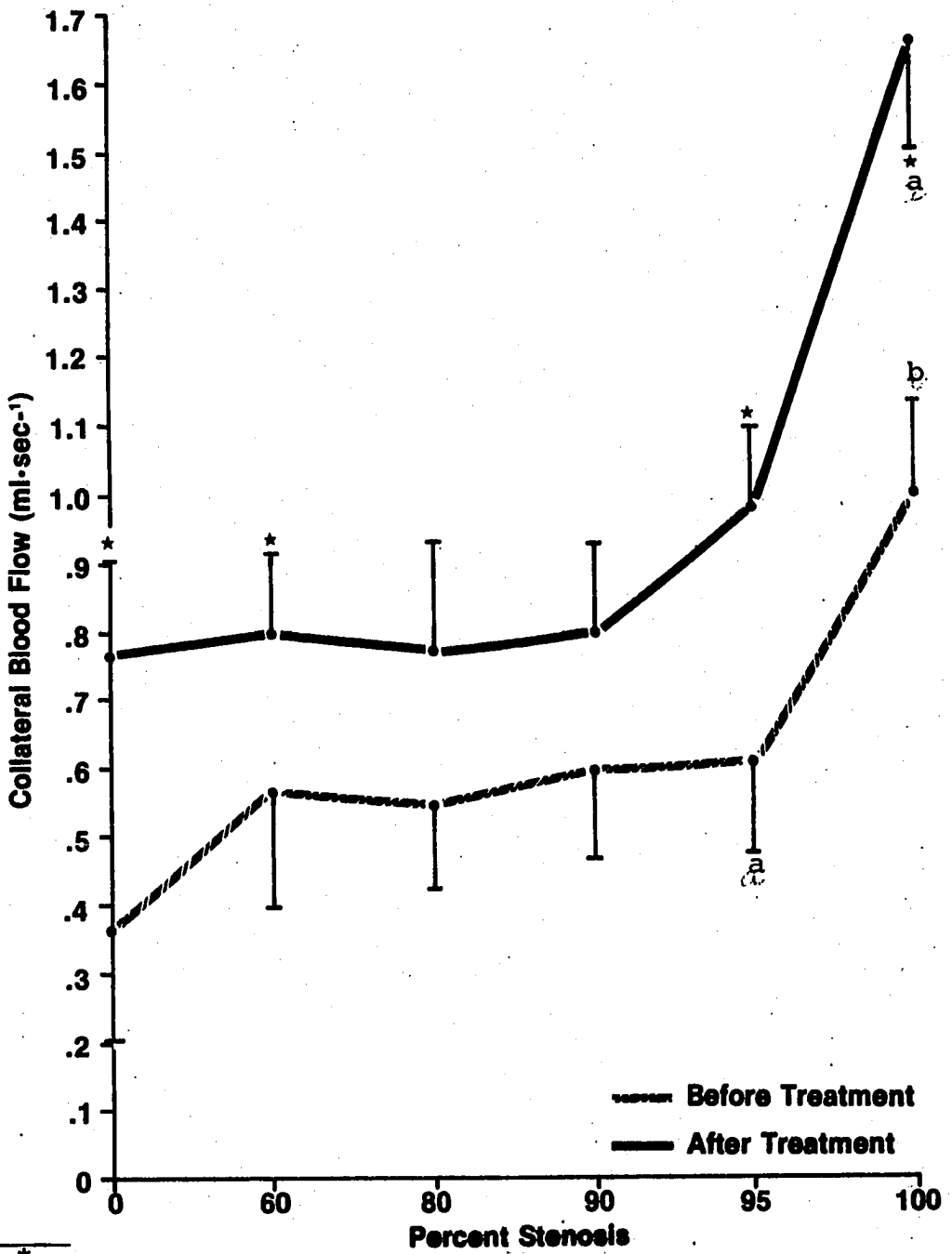
Plate 4. Femoral artery (a) and corresponding vein (b) are isolated with DCFA (c) and MCFA (d)

RESULTS AND DISCUSSION

The objective of the present investigation was to determine the influence of endogenously released vasoactive substances on collateral and peripheral hemodynamics during graded arterial stenoses. Pressures and measured flows were utilized to calculate the hemodynamics in the region. The values thus determined are presented in Fig. 5 to Fig. 22 and Table 2 through Table 9.

Q_c (Fig. 5) in resting, untreated animals did not change significantly until a 95 percent decrease in c.s. area was reached. R_c (Fig. 6) and R_p (Fig. 7), however, remained unchanged during this period, suggesting that maximum vasodilatation of both vascular beds had occurred before the experiment was started. R_s (Fig. 8) increased with each grade of stenosis but attained significance only at 95 percent decrease in c.s. area. Contrary to this, a 90 percent stenosis caused a significant increase in R_s under elevated flow conditions (Fig. 8). R_p (Fig. 7) dropped markedly at all levels of stenosis, whereas Q_c (Fig. 5) and R_c (Fig. 6) remained unchanged throughout the experiment.

The above observations are identical to those reported by Roth et al. (1976) and Walinsky et al. (1979), who suggested a limited role of collateralization during distal vasodilatation, despite decrease in blood flow through the



*P<.05 between treatments in a group.

[a]P<.05 within treatment in a group.

[b]P<.05 within treatment in a group.

Figure 5. Collateral blood flow during graded levels of femoral artery stenosis before and after acetylcholine infusion in the peripheral vascular bed

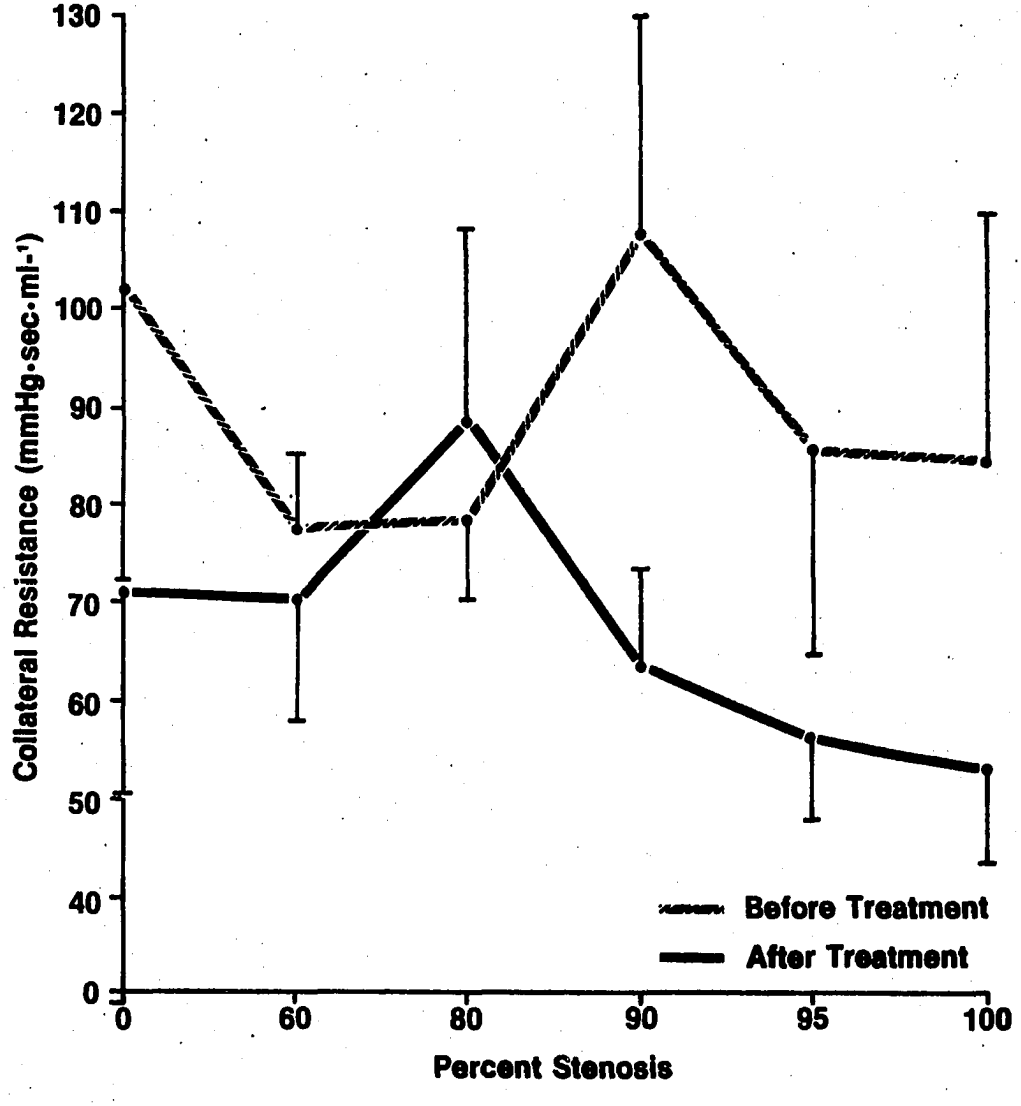
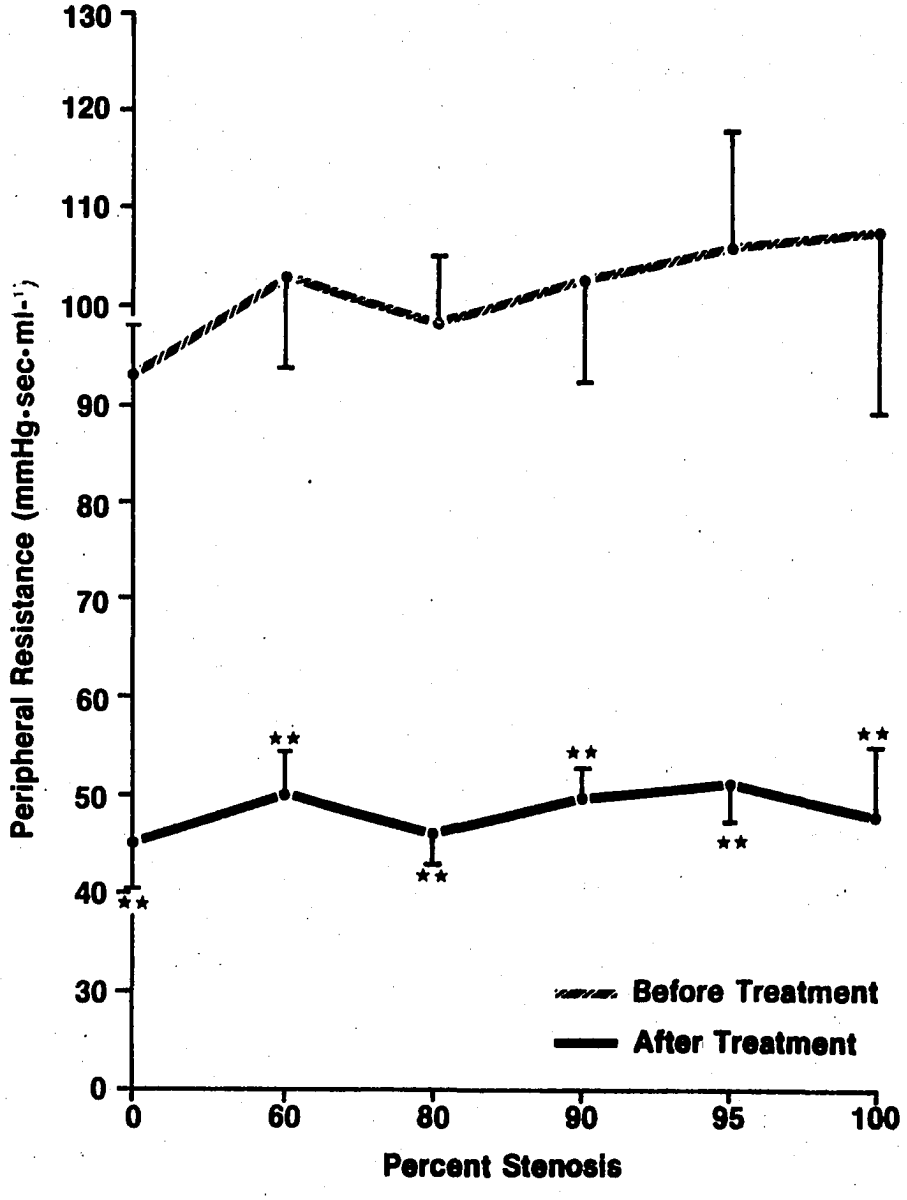
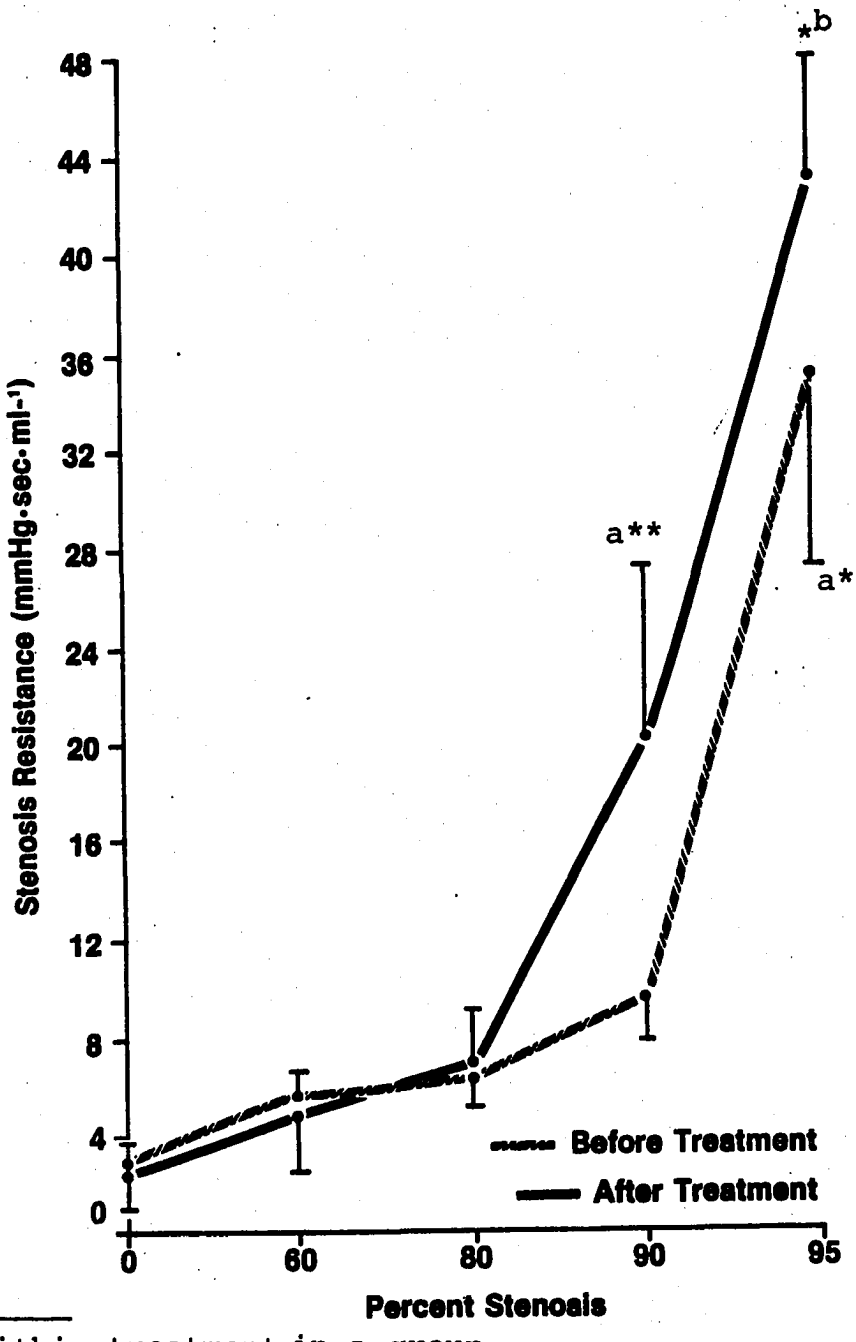


Figure 6. Collateral vascular resistance during graded levels of femoral artery stenosis before and after acetylcholine infusion in the peripheral vascular bed



**P<.01 between the treatments in a group.

Figure 7. Peripheral vascular resistance during graded levels of femoral artery stenosis before and after acetylcholine infusion in the peripheral vascular bed



[a]P<.05 within treatment in a group.

[b]P<.01 within treatment in a group.

*P<.05 between treatments in a group.

**P<.05 between treatments in a group.

Figure 8. Femoral artery resistance during graded levels of stenosis before and after acetylcholine infusion in the peripheral vascular bed

stenosis due to vasoplasticity. Distal vasodilatation caused by drug infusion, ischemia or hypoxia is associated with a decrease in pressure and resistance distal to stenosis with simultaneous increase in R_s. This increase leads to potentiation of the hemodynamic severity of the stenosed segment (Young et al., 1977; Schwartz et al., 1979, 1980). The decrease in Q_s during distal vasodilatation is believed to be due to passive narrowing, which occurs immediately downstream from the stenosis. Distal dilatation causes increase in ΔP across the stenosis by lowering distal pressure. The lateral pressure at the site of the stenosis must be the same as the lateral pressure in the artery distal to the stenosis. The decrease in distal pressure, therefore, will cause the pressure in the stenosed segment also to decrease. This leads to the passive narrowing of the vessel lumen, which potentiates the R_s with simultaneous decrease in Q_s (Carew et al., 1968). The exact role of collateral hemodynamics during distal vasodilatation is not clearly understood. One explanation of the unchanged R_c observed in these studies could be that they were functioning at their maximum capacity during the resting untreated state, and no significant improvement in Q_c would have occurred except during strenuous exercise (Khudaiberdyerv and Kulikov, 1971; Thulesius, 1972).

Serotonin

Since its discovery in the blood serum by Rapport and his associates (1948), SRT has occupied a unique place as an important autacoid. Its role as a modulator of blood flow, following an arterial insult, has been investigated over the past 25 years (Bulle, 1957; Daicoff et al., 1968; Gladkova and Vasiliev, 1971; Schaub et al., 1976; Kordenat and Kezdi, 1979). SRT has variable actions in body tissues. It causes vasoconstriction of large arteries and vasodilatation of smaller ones in low doses (Goodman and Gilman, 1975). Amphibaric actions of SRT can also be observed in various vascular beds depending upon their vascular tone (Page and McCubbin, 1953).

To determine its actions, SRT was administered ia in the hind limb peripheral vascular bed of 6 dogs during arterial stenosis at 0, 95 and 100 percent levels (Table 2). Despite a slight improvement in peripheral blood flow, Q_c, R_c, R_p and R_s did not change significantly, suggesting no SRT effect on hind limb vasculature. Daugherty et al. (1968) also found nonsignificant variation in canine forelimb hemodynamics following ia or iv administration of SRT. The vasoconstriction attributed to SRT in the feline hind limb preparations, following thrombus associated occlusions, could have been due to interaction of SRT with PGs (Schaub et al.,

Table 2. Vascular hemodynamics in the hind limb of the dog before and after serotonin treatment

Level of stenosis	Hemodynamic variables (n=6)							
	QC		RC		RP		RS	
	C	SRT	C	SRT	C	SRT	C	SRT
0	.43±.15	.48±.19	135.7±53	231.9±103	132.4±53	121.3±28.0	2.78±1.2	4.08±2.4
95	.54±.16	.48±.22	413.38±317	218±247	132.75±27	135.24±25.8	26.97±16.0[a]	21.8±10.6[a]
100	1.03±.27[a]	.82±.17[a]	118.28±44	111.66±30	151.9±36	123.1±23.82	3899±518[b]	3750±484[b]

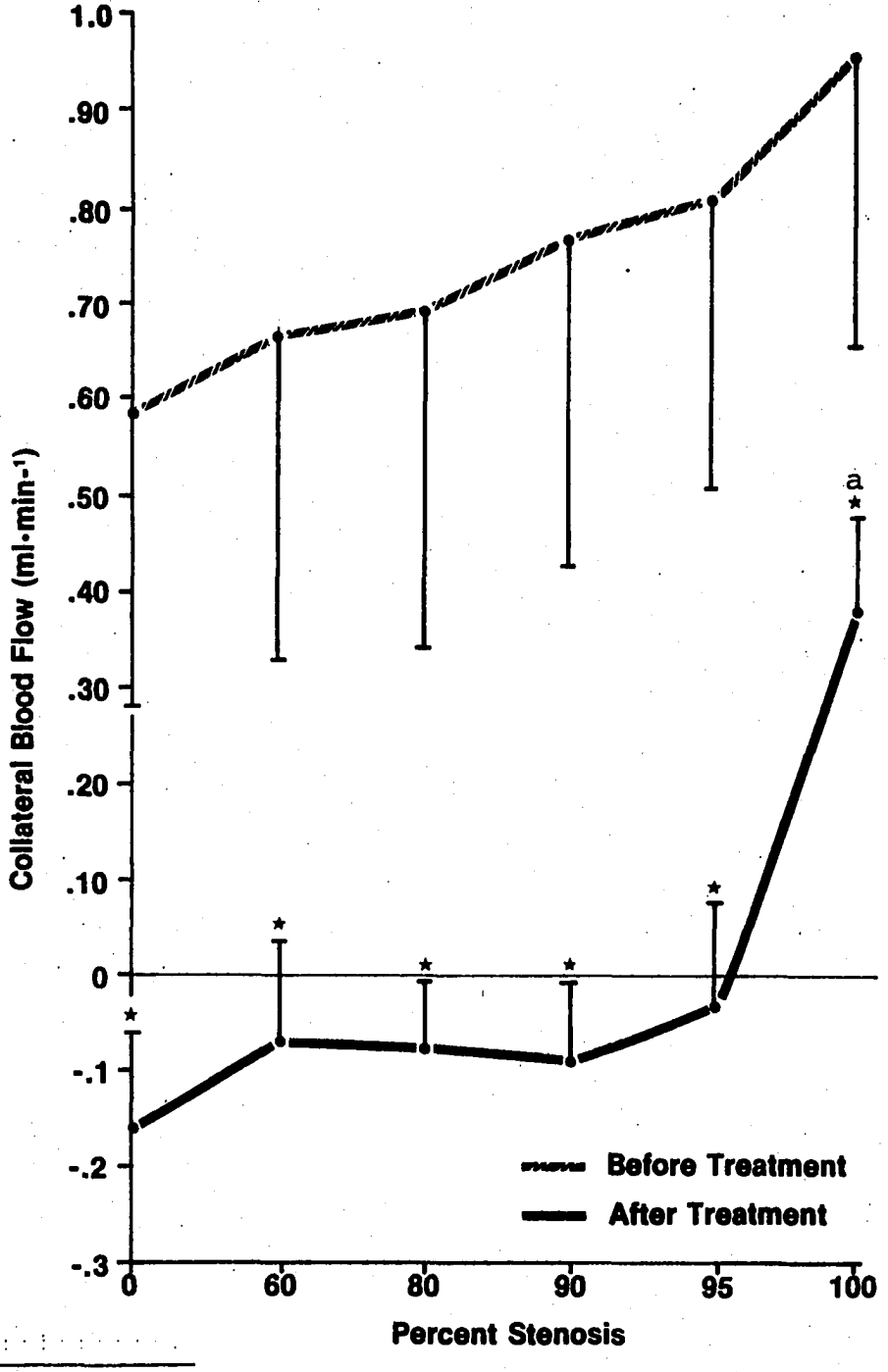
[a] $P<.05$ within treatment in a group.

[b] $P<.05$ within treatment in a group.

1976). Its effects might have been mediated via TXA_2 released from the aggregated platelets (O'Donnel and Fiedel, 1979). The resulting changes in the biochemical environment of the hind limb vasculature due to thrombus formation might also have potentiated the SRT effect reported in their studies (Rosenthal, 1972). Variation in the species of experimental animals may also be a causal factor of the SRT effect.

Methysergide

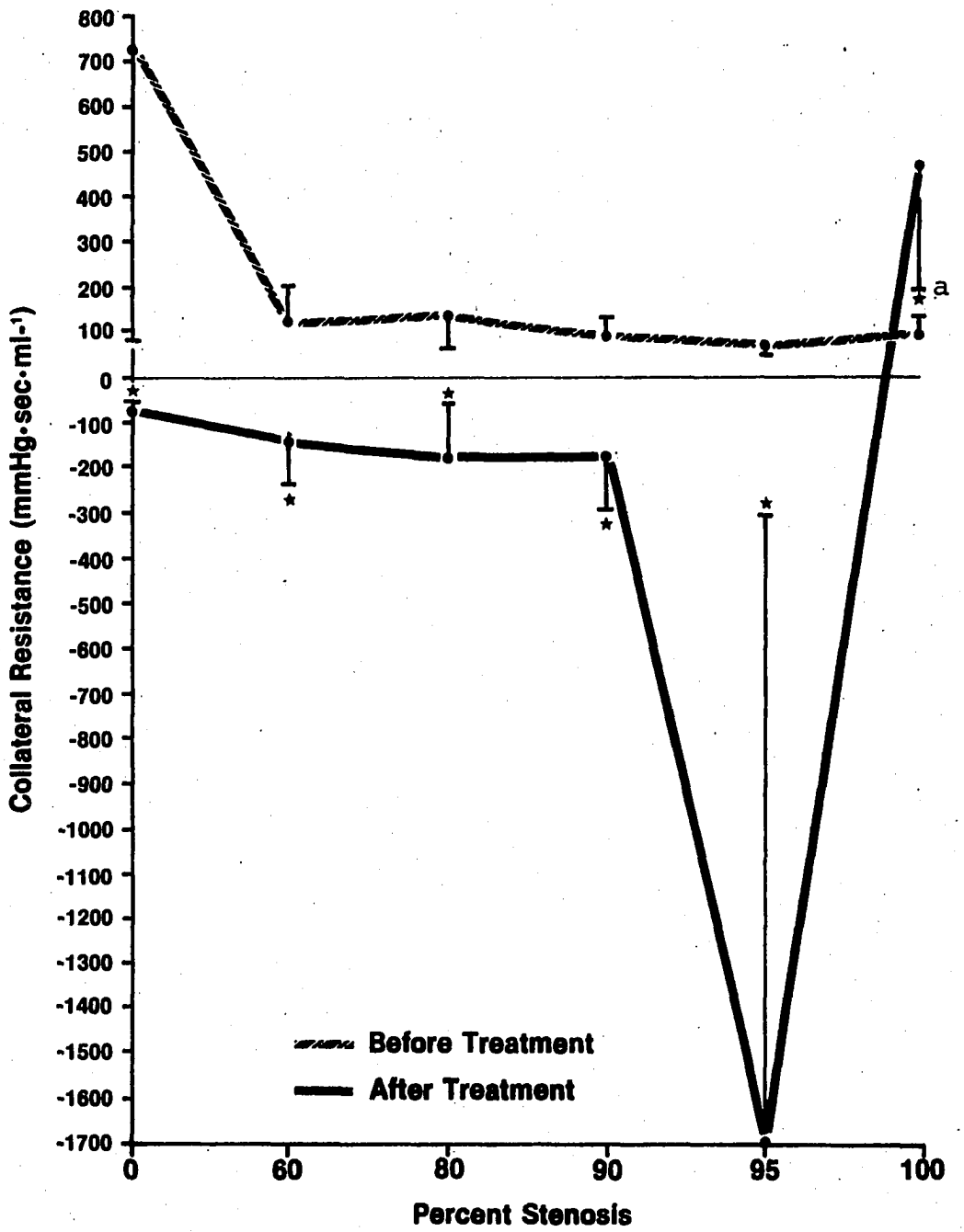
To ascertain if SRT was being endogenously released during the process of occlusion, antagonists were used in the present study to determine SRT effects. METH, which competitively inhibits SRT at its receptor site in vascular beds, was one of the inhibitors administered in 4 animals. The results obtained before methysergide treatment showed no significant change in Q_c (Fig. 9), R_c (Fig. 10), R_p (Fig. 11), and Q_p (Fig. 12), whereas R_s changed significantly at 95 percent decrease in c.s. area (Fig. 13). A profound change in collateral and peripheral hemodynamics occurred following METH treatment. Q_c (Fig. 9) stopped flowing through the collateral bed and flow did not occur until 95 percent stenosis level was attained. During this period, the blood was being supplied to the affected collateral bed from the



*P<.05 between the treatments in a group.

aP<.05 within treatment in a group.

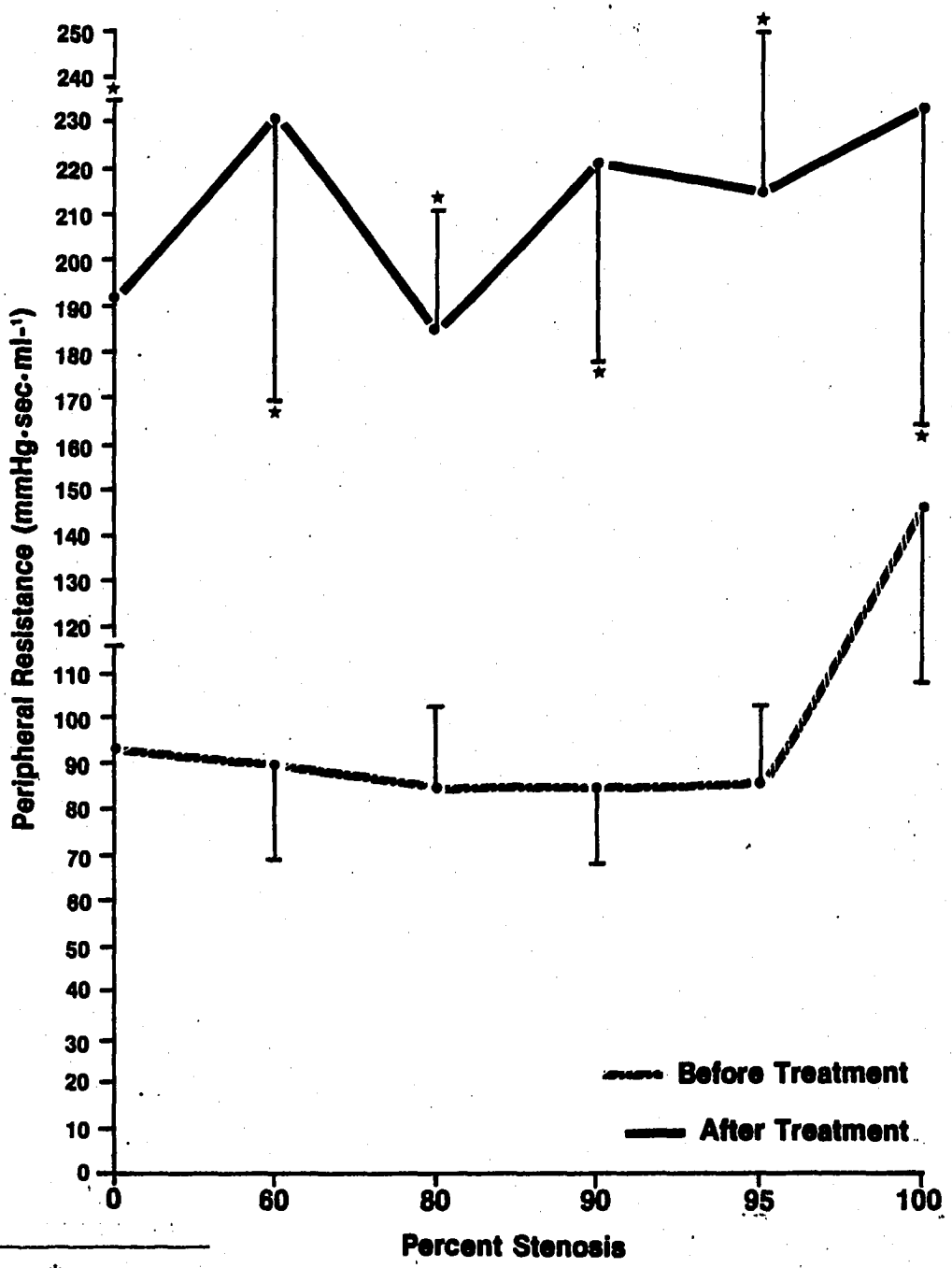
Figure 9. Collateral blood flow during graded levels of femoral artery stenosis before and after methysergide treatment



*$P < .05$ between the treatments in a group.

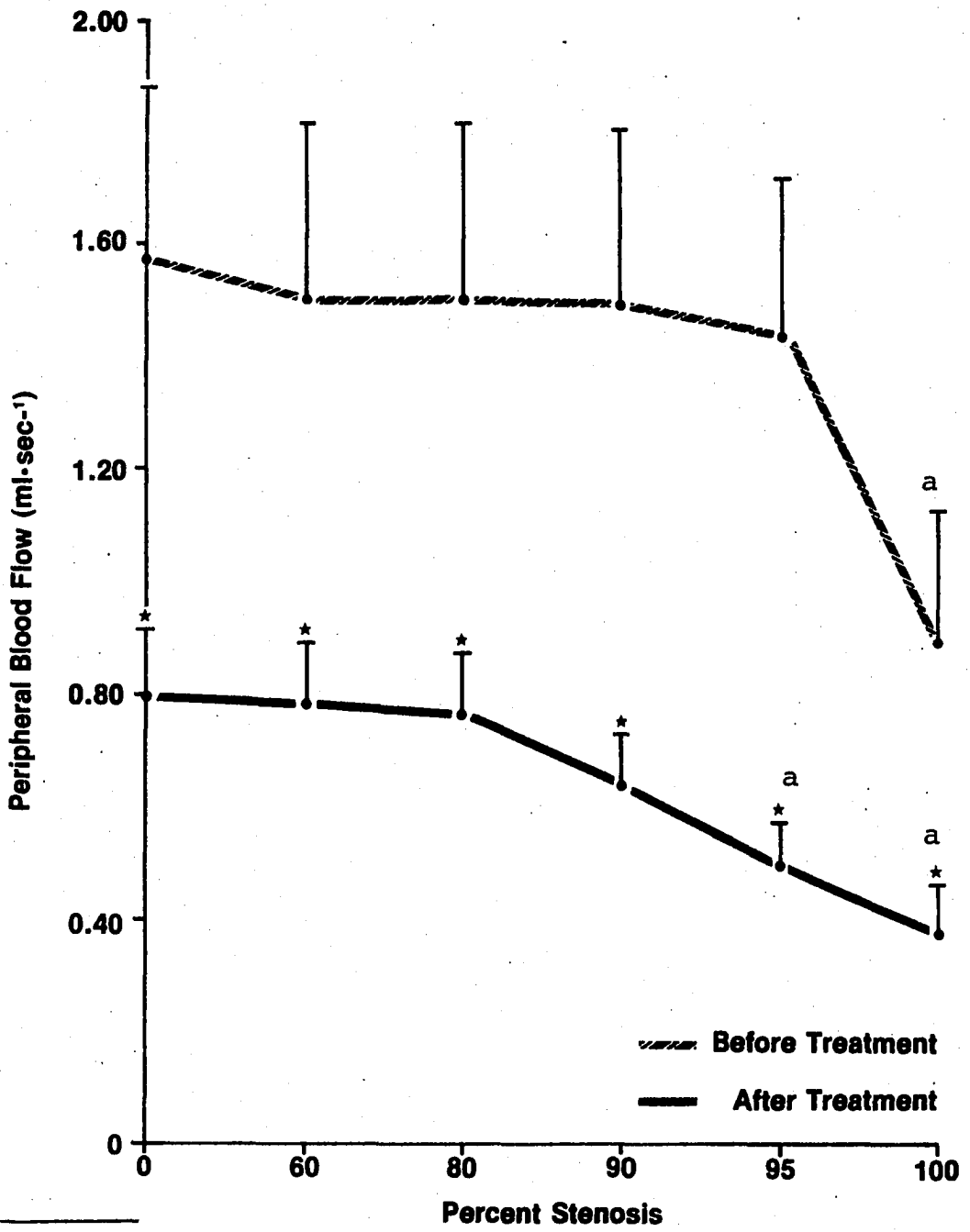
[a]$P < .05$ within treatment in a group.

Figure 10. Collateral vascular resistance during graded levels of femoral artery stenosis before and after methysergide treatment



*P<.05 between the treatments in a group.

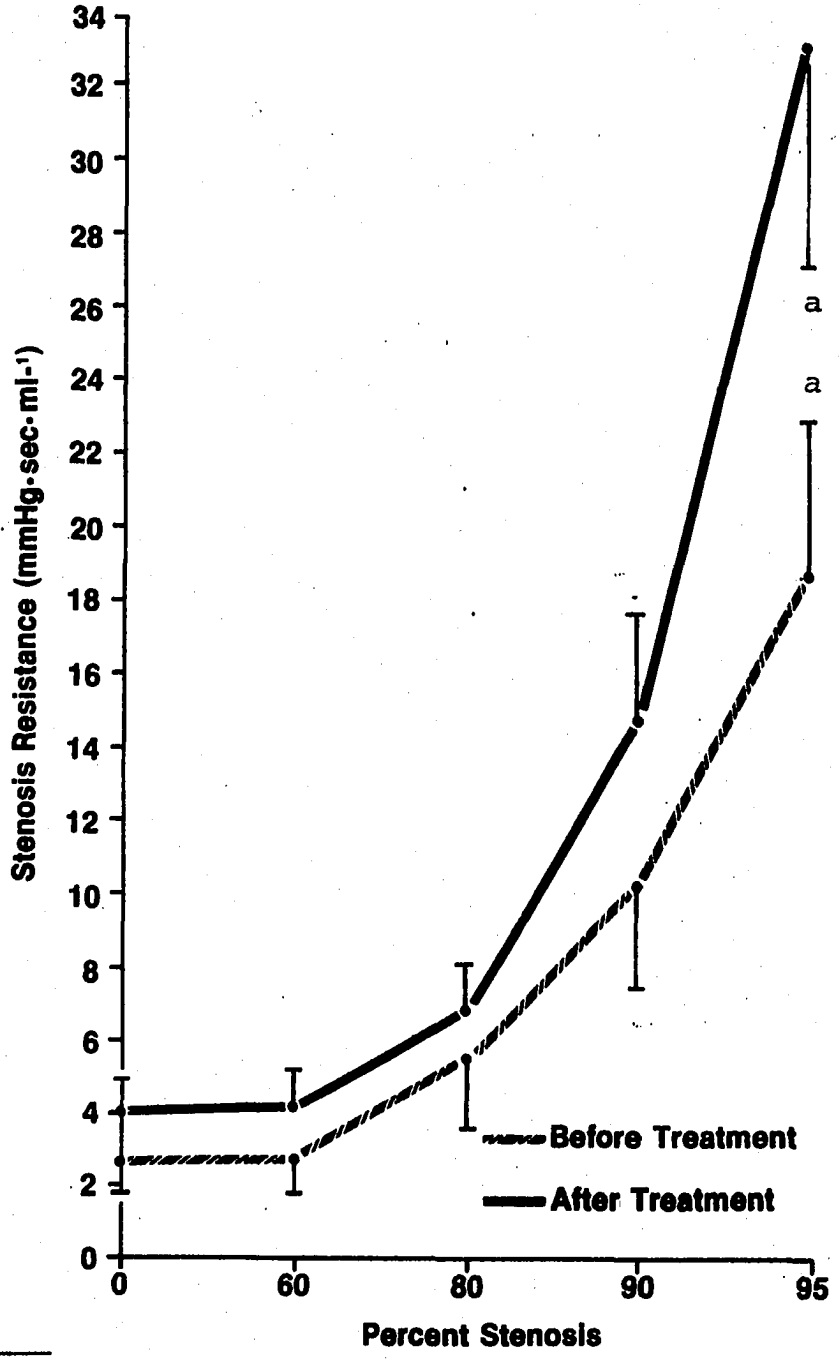
Figure 11. Peripheral vascular resistance during graded levels of femoral artery stenosis before and after methysergide treatment



*P < .05 between the treatments in a group.

[a]P < .05 within treatment in a group.

Figure 12. Peripheral blood flow during graded levels of femoral artery stenosis before and after methysergide treatment



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[a] $P < .05$ within treatment in a group.

[b] $P < .05$ within treatment in a group.

Figure 13. Femoral artery resistance during graded levels of stenosis before and after methysergide treatment

partially obstructed femoral artery. Q_c, however, started flowing through the collateral bed when the femoral artery was completely occluded. R_c (Fig. 10) remained undefined (negative value) until 95 percent stenosis, because of no flow situation in the collateral bed. It was significantly higher at 100 percent stenosis when compared to pretreated values. The peak negative value of R_c, with a large standard error of the mean, was due to least negative value of Q_c (-.01) in one animal. This increased R_c to a very large negative value was still nonsignificant as compared to lower levels of stenoses. R_p (Fig. 11) increased 2-3 times from pretreated values following METH treatment. It remained higher and did not change significantly during graded stenoses. Q_p (Fig. 12) dropped significantly following METH treatment and further decreased at 95 and 100 percent stenosis levels. R_s (Fig. 13) following METH did not change from those of pretreated values due to low flow rates through the stenosis before, and after, treatment.

The decrease in Q_c and Q_p with concomitant increase in R_c and R_p observed in these studies may have been due to selective localized vasoconstriction produced by METH. Systemic arterial blood pressures were not affected during the experimental period. CO may have been the other factor to be influenced by METH, but it has been shown that even large doses of METH do not affect CO (Saxena, 1974). Similar

findings were reported by Graham (1964) in the femoral vascular bed and in the mesenteric and distal aortic regions by Saxena (1974). Ureles and Rob (1964) found an acute ischemia of the left foot, which was associated with the obstruction of the popliteal and dorsal pedis arteries as a result of METH induced arterial spasm.

Selective vasoconstriction following methysergide seen in these studies is difficult to explain. It may have been due to its direct action on the affected vascular bed or by the sensitization of the vascular tissue to the actions of the catecholamines (Dalessio, 1962; Saxena, 1972). However, the net effort of METH in a particular vascular bed is believed to be dependent upon pre-existing vascular tone (Saxena, 1974) despite its own feeble vasoconstrictive properties (Curran et al., 1967). Vasoconstriction may be potentiated in the presence of ischemia or hypoxia. In the present study, R_c and R_p had attained a minimum value during the course of the experiment so that the collateral and peripheral beds were maximally dilated. The vasomotor tone under these conditions must also have attained its minimum value, which during METH treatment was reversed, and produced severe hemodynamic alterations in the hind limb vascular bed.

The improvement in blood flow observed by Kordenat and Kezdi (1979) in thrombotically occluded coronary vessels

during METH treatment has no bearing on these studies. Occlusion in their studies was associated with thrombus and platelet aggregation, which must have released significant quantities of SRT, produced vasoconstriction, and potentiated the vasomotor tone of the vasculature. Under these conditions, METH would have blocked the SRT induced vasoconstriction, hence the net result would be vasodilatation and improvement in blood flow. Their studies also were in the coronary vascular bed, which possesses different vasoactive properties than hind limb vasculature.

SRT following METH treatment did increase the collateral blood flow, which, however, was significantly lower than it was in pretreated animals even at severe stenosis levels (Table 3) resulting in higher R_c values. R_p did not change from pretreated values. The increase in Q_c during SRT infusion can be attributed to its partial effectiveness at higher dose levels. Despite Q_c increase following METH treatment with SRT, it was still lower than in pretreated values. This may have been due to residual vasoconstrictive effect of METH in the canine hind limb vascular bed (Curran et al., 1967).

Table 3. The vascular hemodynamics in the hind limb of the dog in methysergide pretreated animals following serotonin treatment

Level of stenosis	Hemodynamic variables (n=4)							
	Q_c (ml.sec^{-1})		R_c (mm Hg·sec·ml^{-1})		R_p (mm Hg·sec·ml^{-1})		R_s (mm Hg.sec.ml^{-1})	
	CMT[1]	SRT	CMT	SRT	CMT	SRT	CMT	SRT
0	.58±.35	.17±.08	724±647	245.6±633	90.32±23	120.33±21.0	2.7±.57	3.52±1.0
95	.80±.31	.44±.02*	70.7±26	339.32±146*	85.65±18	95.0±15	18.53±5.22	34.9±5.9*[a]
100	0.96±.33	.98±.23[a]	99.72±38	103.82±22	135.66±50	60.24±12	6382±2221[a]	7329±735*[a]

[1] CMT, control animals in methysergide group.

* P<.05 between treatments in a group.

[a] P<.05 within treatment in a group.

Parachlorophenylalanine

PCPA is the most potent and selective depletor of SRT in brain and nervous tissue (Koe and Weissman, 1966). It inhibits SRT biosynthesis by inactivating tryptophan hydroxylase, a rate limiting step in the synthesis of SRT (Bloom and Giarman, 1968). PCPA in this study was used to block the SRT at the neuronal level, to ascertain if the suggested vasoconstriction by SRT (Sullenberger et al., 1959) was mediated via neurotransmission.

Animals (n=4) subjected to PCPA treatment showed a marked improvement in Q_c at 95 and 100 percent stenosis levels, with concomitant decrease in R_c (Table 4). These changes, however, were not significantly different from those observed under resting, untreated conditions. R_p remained unaffected during the course of the experiment and dropped to low values following ACH treatment. This decrease was due to distal vasodilatation caused by ACH infusion in the hind leg peripheral bed (Schwartz et al., 1979). These results reinforce previous observations that SRT is not the factor involved in the regulation of the blood flow.

Table 4. Vascular hemodynamics in the hind limb of the dog following PCPA and ACH treatment

Level of stenosis	Hemodynamic variables							
	Q_c (ml.sec^{-1})		R_c (mm Hg.sec.ml^{-1})		R_p (mm Hg.sec.ml^{-1})		R_s (mm. Hg.sec.ml^{-1})	
	PCPA	ACH	PCPA	ACH	PCPA	ACH	PCPA	ACH
0	.25±.1	.19±.1	440.5±182	252.8±97	126.4±16	60.3±11*	.87±.18	1.01±0.71
95	.43±.15[a]	.57±.1[a]	129.8±60[a]	112.13±40	131.73±24.5	80.52±15*	28.95±2.6[a]	29.92±50[a]
100	.81±.19[b]	1.51±.13[b]**	56.49±15.8[a]	46.61±8.4	134.6.16±50	50.87±7.2*	3851±427[b]	6813±491[b]

*P<.05 between treatments in a group.

**P<.01 between treatments in a group.

[a]P<.05 within treatment in a group.

[b]P<.05 within treatment in a group.

Reserpine

RZP is an SRT antagonist. It releases all bound SRT from brain, platelets and body tissues. It inactivates or denatures the storage area at the receptor site and interferes with its transport mechanism (Carlsson et al., 1957; Goodman and Gilman, 1975).

Four animals were administered RZP and PCPA to eliminate SRT effects in the body. No significant change in collateral and peripheral hemodynamics was observed in these animals following R+PCPA treatment. A change did occur, however, when the animals were subjected to an elevated flow condition by continuous infusion of ACH (Table 5). The response with ACH was highly pronounced. Q_c increased markedly with simultaneous drop in R_c at 95 and 100 percent stenosis levels. R_s, though changed before and after ACH infusion, but the change was significant only at 95 and 100 percent stenosis levels.

R_p was lower in PCPA treated animals following SRT infusion. This was chiefly due to increased Q_p, since ΔPs across the peripheral bed were not affected (Table 6). Q_c increased greatly at 100 percent stenosis in SRT administered animals. The marked increase in Q_c, without simultaneous decrease in R_c before and after SRT infusion is difficult to explain. It may have been due to increased ΔPs

Table 5. Vascular hemodynamics in the hind limb of the dog after R+PCPA and ACH treatment

Level of stenosis	Hemodynamic variables							
	Q_c (ml.sec^{-1})		R_c (mm Hg.sec.ml^{-1})		R_p (mm Hg.sec.ml^{-1})		R_s (mm Hg.sec.ml^{-1})	
	R+PCPA	ACH	R+PCPA	ACH	R+PCPA	ACH	R+PCPA	ACH
0	.41±.26	.85±.32	131.62±55	82.78±54	116.19±20	43.38±4.2**	1.86±0.6	0.7±.19
95	.48±.16	.99±.18**	125.5±32	48±15*	140.0±17	50.67±6.7**	38.11±15[a]	32.5±13[a]
100	.57±.08	1.39±.33*	79.9±15	45.7±9*	186.6±42	63.0±11*	4768±1470[b]	4465±900[b]

*P<.05 between treatments in a group.

**P<.01 between treatments in a group.

[a]P<.05 within treatment in a group.

[b]P<.05 within treatment in a group.

Table 6. Vascular hemodynamics in the hind limb of the dog after PCPA and SRT treatments

Level of stenosis	Hemodynamic variables							
	Q_c (ml.sec^{-1})		R_c (mm Hg.sec.ml^{-1})		R_p (mm Hg.sec.ml^{-1})		R_s (mm Hg.sec.ml^{-1})	
	PCPA	SRT	PCPA	SRT	PCPA	SRT	PCPA	SRT
0	.25±.12	.3±.1	450.5±182	279.6±127	126.4±16.8	60.14±11*	1.12±.7	1.44±.5
95	.43±.15[a]	.57±.1[a]	129.77±60[a]	112.13±40	131.73±24	80.55±15*	28.95±2.6[a]	29.92±5.0[a]
100	.81±.19[b]	1.51±.12[b]**	56.5±15	44.1±9.8	151.64±40	50.87± 7*	3856±425[b]	6836±467[b]

*P<.05 between treatments in a group.

**P<.01 between treatments in a group.

[a]P<.05 within treatment in a group.

[b]P<.05 within treatment in a group.

across the collateral bed at severe stenosis levels.

The response obtained after SRT administration in R+PCPA treated animals (Table 7) was similar to that obtained in PCPA treated animals. Q_c was higher at all stenosis levels and R_c did not change significantly, perhaps because of the larger doses of SRT (30 μg/Kg/Min) infused in the present group of animals. The larger SRT doses may have overridden the PCPA and R+PCPA effect and, in some way, may have influenced the collateral as well as peripheral hemodynamics.

To determine if SRT was one of the vasoactive agents being released during the process of occlusions, blood samples were collected at 0, 95 and 100 percent decrease in the lumen c.s. area in resting untreated, METH, PCPA and R+PCPA treated groups of animals (Table 8). No significant differences in SRT contents were found to exist in any of the groups before SRT infusion, although the concentration was lower in PCPA and R+PCPA treated animals. SRT levels, however, were 3 times higher at all levels of stenosis following SRT infusion in these groups.

The lack of variation in SRT concentration at varying levels of stenosis in different groups of animals before SRT administration is a result that conflicts with the findings of Sullenberger et al. (1959); Schaub et al. (1977a) and Kordenat and Kezdi (1979). They observed that significant quantities of SRT were released and suggested that intravascular

Table 7. Vascular hemodynamics in the hind limb of the dog after R+PCPA and SRT treatments

Level of stenosis	Hemodynamic variables							
	Q_c (ml.sec^{-1})		R_c (mm Hg.sec.ml^{-1})		R_p (mm Hg.sec.ml^{-1})		R_s (mm Hg.sec.ml^{-1})	
	R+PCPA	SRT	R+PCPA	SRT	R+PCPA	SRT	R+PCPA	SRT
0	.44±.23	.85±.32	218.37±122	82.78±54	116.19±20.7	40.38±4.2*	1.86±.6	0.77±.19
95	.48±.16	.99±.18*	125.4±31.9[a]	47.97±15[a]	140.95±17	50.17±6**	26.8±4.0[a]	32.96±5.5[a]
100	.54±.08	1.39±.33*	80±15[b]	45.7±9[b]	186±41.9	62.9±11*	3576±584[b]	4611±708[b]

*P<.05 between treatments in a group.

**P<.01 between treatments in a group.

[a]P<.05 within treatment in a group.

[b]P<.05 within treatment in a group.

Table 8. The serotonin contents in the canine blood during graded stenoses following treatments with various agonists and antagonists

Group number	Treatment	Serotonin contents (µg/ml) 0% Stenosis	95% Stenosis	100% Stenosis
G1	C	.62 ± .06	.56 ± .07	.51 ± .05
	SRT	2.03 ± .3*	1.91 ± .4*	1.94 ± .45*
G2	C	.63 ± .1	.50 ± .08	.58 ± .08
	METH	.65 ± .11	.6 ± .09	.53 ± .1
	SRT	1.25 ± .3*	1.02 ± .3*	1.05 ± .35*
G3	PCPA	.38 ± .1	.4 ± .1	.37 ± .1
	ACH	.5 ± .2	.53 ± .07	.48 ± .06
	SRT	1.41 ± .14*	1.34 ± .2*	1.48 ± .2*
G4	R+PCPA	.37 ± .04	.39 ± .03	.41 ± .04
	ACH	.49 ± .09	.51 ± .05	.43 ± .03
	SRT	1.49 ± .3*	1.32 ± .3*	1.48 ± .3*

*$P<.05$ between treatments within a group.

thrombosis, with its attendant cell damage and platelet aggregation, may have been the cause. The differences in vascular beds and experimental animal species in their studies, is another probable contributing factor for the variation in these results.

Indomethacin

PGs are local hormones and are released by almost every tissue in the body, including the blood vessel wall. Stimuli for release include mechanical damage, surgical trauma, endothelial cell damage, and decreased oxygen tension. PGI_2 is constantly released from the vessel wall due to platelet and vessel wall interaction. The PGI_2 synthetase is located in the endothelial cells of the vessel wall in an inactive form. The moving platelets in circulating blood, when they come into contact with the vessel wall, activate PGI_2 synthetase and result in PGI_2 biosynthesis. The PGI_2 does not allow deposition of platelets to the vessel wall under normal flow conditions (Owen et al., 1975; Dusting et al., 1977; Moncada et al., 1977a, 1978). During endothelial cell damage, disruption in the synthesis of PGI_2 occurs and causes platelet deposition, platelet aggregation, thrombus formation and TXA_2 release (Horie et al., 1978; Moncada et al., 1976).

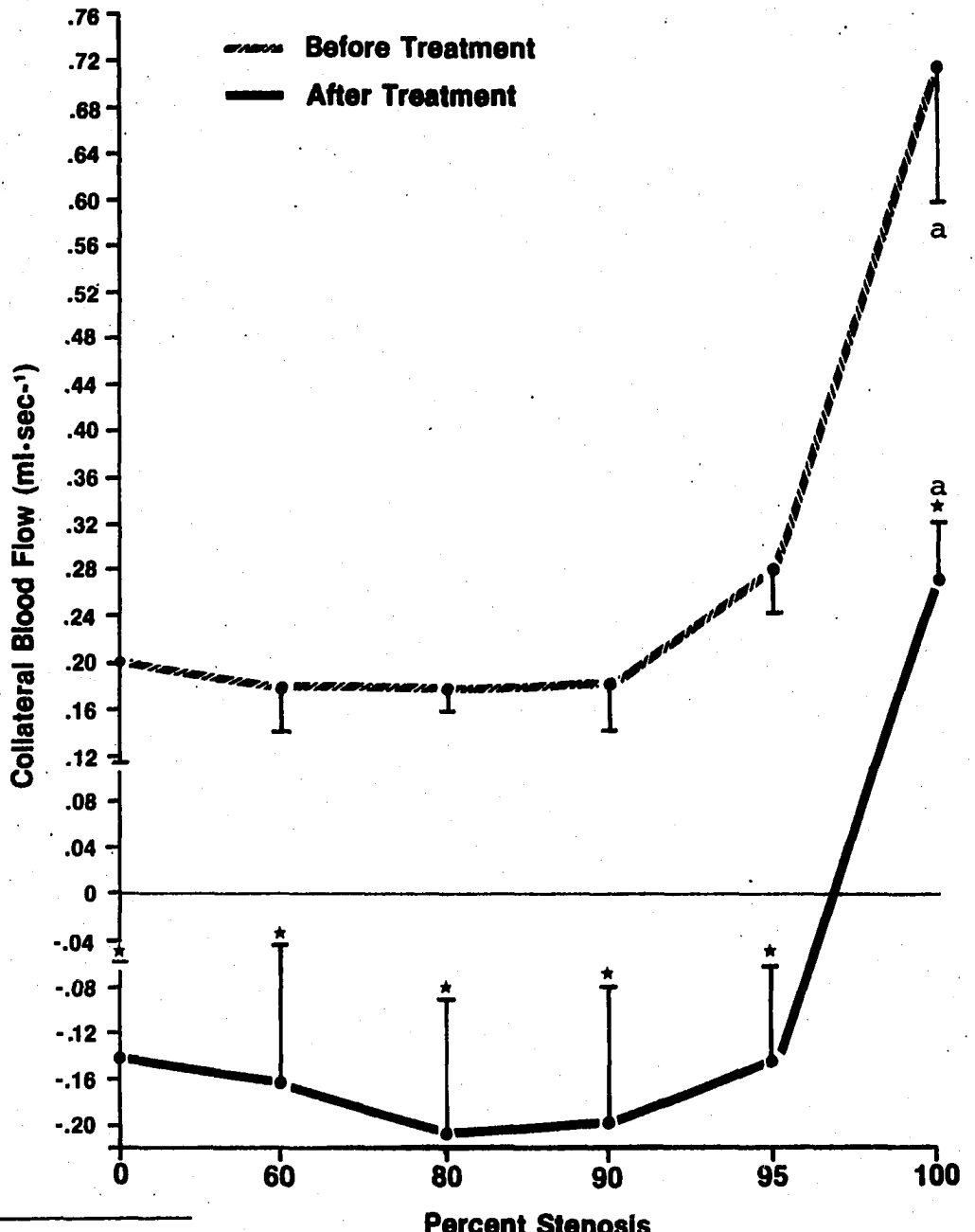
Within a wide range of perfusion pressures and arterial oxygen concentrations, hind limb vascular blood flow adjusts automatically to meet the metabolic demands of the tissues in the region. The adjustment is a result of endogenous release of vasoactive PGs and other substances. The role of PGs in the regulation of blood flow is well-documented

(Alexandar et al., 1975; Needleman and Kaley, 1978). PGI_2 is the most predominantly released substance during arterial occlusion (Moncada et al., 1977a; Serneri et al., 1980). However, during endothelial cell damage and thrombus formation, TXA_2 is the predominant vasoactive compound (Ellis et al., 1976; Moncada et al., 1976).

To ascertain the role of these vasoactive PGs in the regulation of blood flow during an arterial occlusion, and to identify the vasoactive PGs, INDO was used to antagonize the endogenously released PGs. INDO inhibits PG biosynthesis during the initial stages of biosynthetic pathway at the cyclo-oxygenase level (Flower, 1974).

Animals (n=4) treated with INDO showed paradoxical changes from pretreatment levels in collateral and peripheral hemodynamics. Q_c ceased during partial occlusions (Fig. 14). At this point, blood to the thigh region was being supplied from the partially obstructed femoral vessel. This redistribution of flow through the stenosis caused a severe decrease in Q_p (Fig. 17) with concomitant increases in R_p (Fig. 16). Q_c started flowing through the collateral bed at 100 percent stenosis and was still significantly lower than it was at the pretreated level. R_c (Fig. 15) was significantly higher at this point. R_s (Fig. 18), however, did not change in pre- and post-treated animals due to insignificant variations in Q_s between the treatments.

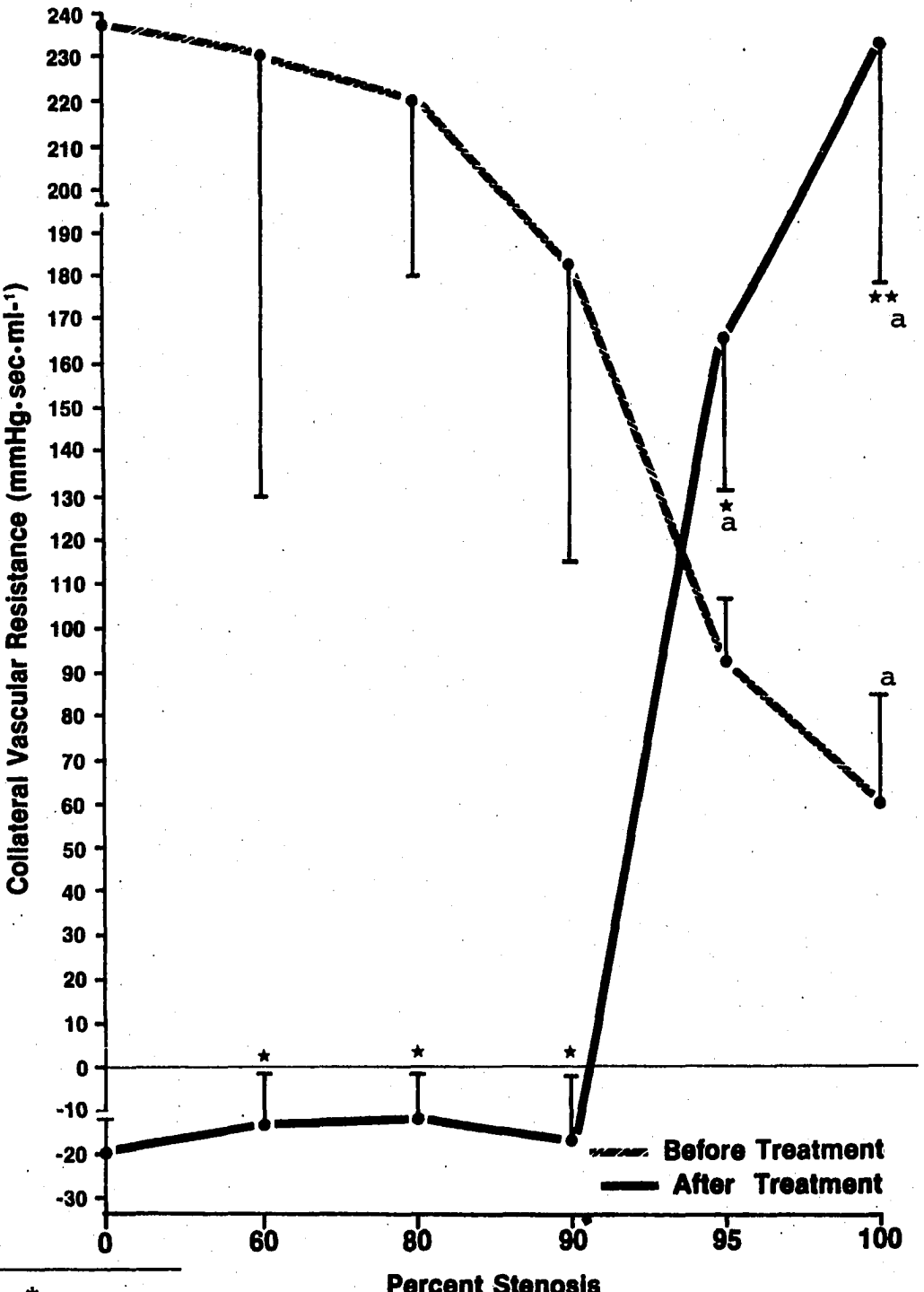
Figure 14. Collateral blood flow during graded levels of femoral artery stenosis before and after indomethacin treatment



*P<.05 between the treatments in a group.

[a]P<.05 within treatment in a group.

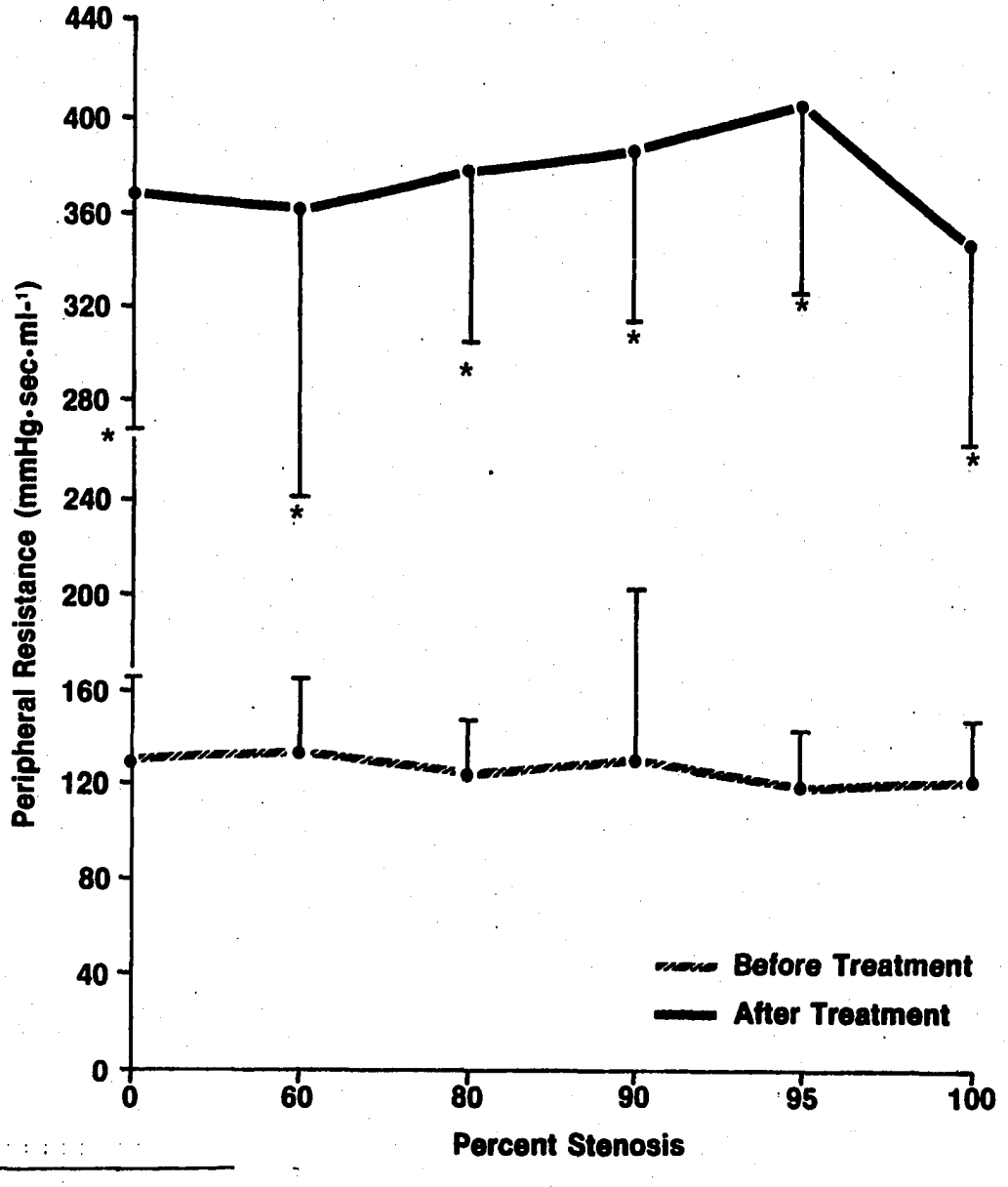
Figure 15. Collateral vascular resistance during graded levels of femoral artery stenosis before and after indomethacin treatment



*P<.05 between the treatments in a group.

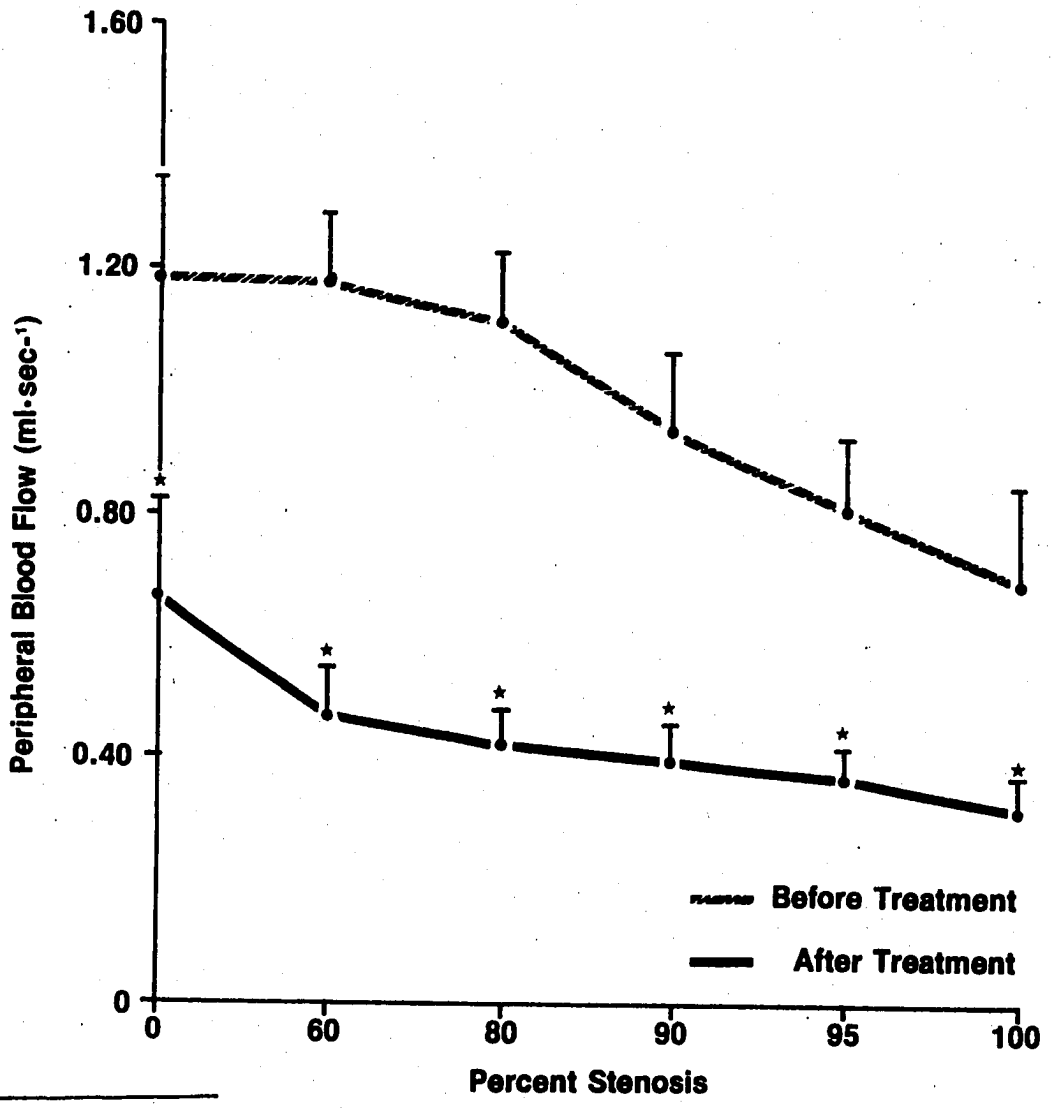
**P<.01 between the treatments in a group.

aP<.05 within treatment in a group.



*P<.05 between the treatments in a group.

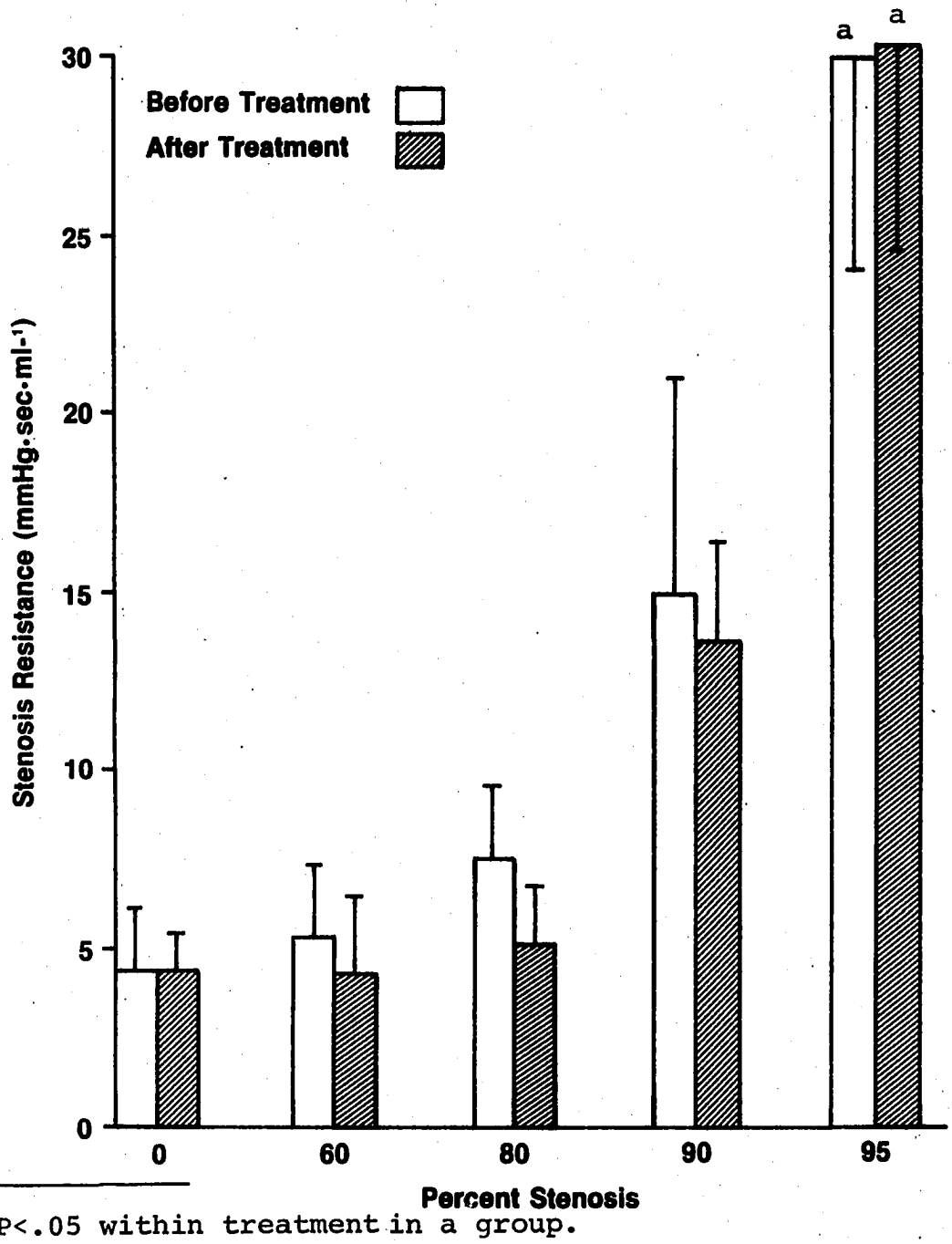
Figure 16. Peripheral vascular resistance during graded levels of femoral artery stenosis before and after indomethacin treatment



*P<.05 between the treatments in a group.

Figure 17. Peripheral blood flow during graded levels of femoral artery stenosis before and after indomethacin treatment

Figure 18. Femoral artery resistance during graded levels of stenosis before and after methysergide treatment



[a]P<.05 within treatment in a group.

These findings confirm the results of Afonso et al. (1974), Alexandar et al. (1973, 1975), and Owen et al. (1975), who reported that the regulatory role of hypoxia-induced PGs release was blunted by INDO and led to a marked decrease in reactive hyperemic response and PG biosynthesis. Hintz and Kaley (1977) observed that PG synthetase inhibition by INDO was associated with decreased peak vasodilatation and decreased hyperemic response (Kramer et al., 1976). Similar observations were made by O'Beaty and Donald (1979) and Rosenblum et al. (1980) during their studies on femoral and mesenteric vascular beds, respectively.

Although the exact mechanism of INDO action in a vascular bed is yet to be characterized, as an antagonist its inhibition of PG synthesis at the cyclo-oxygenase level is well documented. It inhibits PG biosynthesis by inactivating cyclooxygenase enzyme. Its direct vasoconstrictive effects via increased catecholamines and its role in modulation of vascular tone have also been suggested (O'Beaty and Donald, 1979).

In the present study, R_c and R_p were at their minimum value before, and attained 2-3 times higher value following, INDO treatment. Such a pronounced change could only have occurred by the inhibition of vasodilator PGs, chiefly PGI_2. This may have been released during experimental preparations and occlusions. However, the selective vasoconstrictor effect

of INDO, either direct or potentiated by catecholamines, cannot be ruled out.

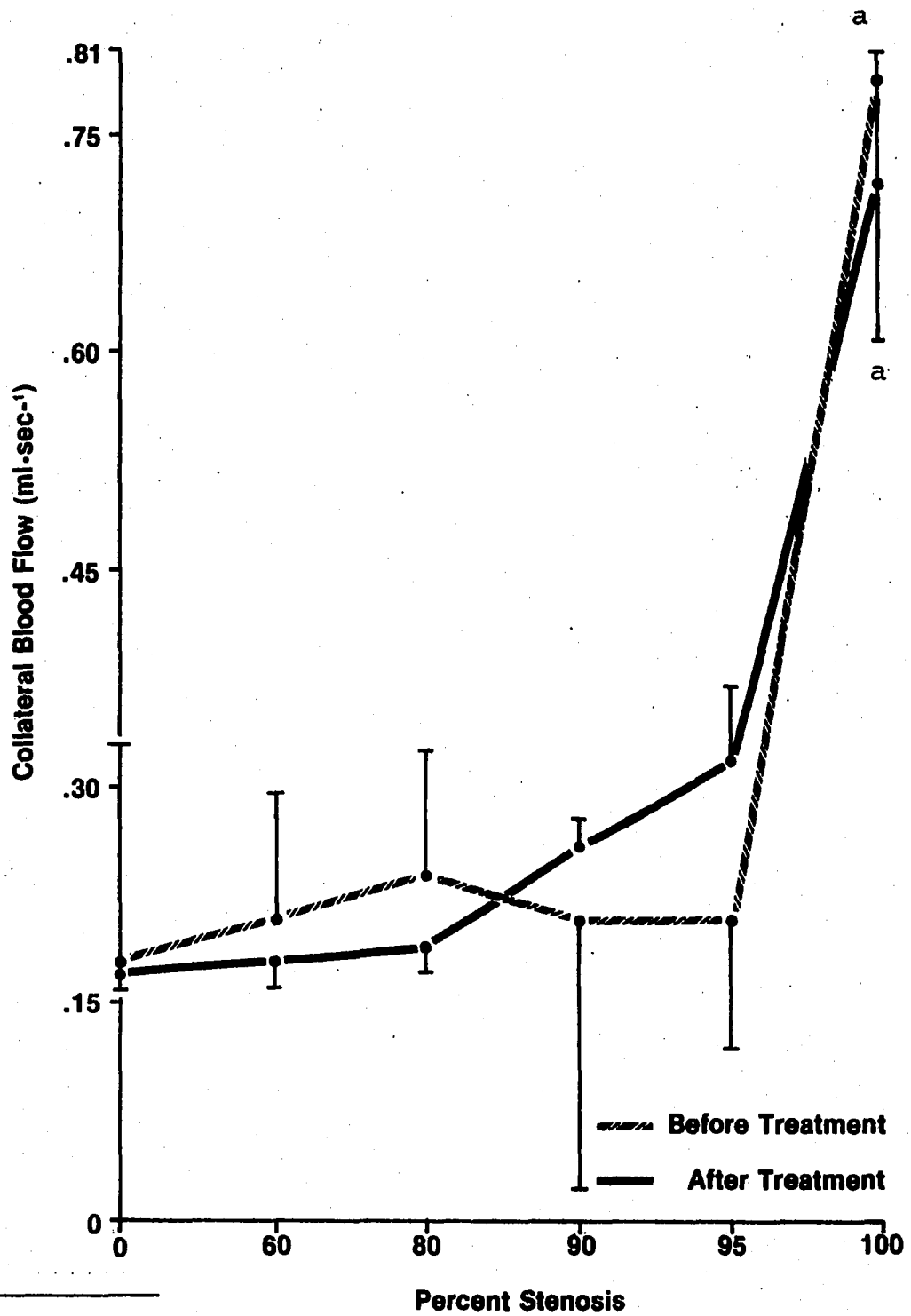
Imidazole

Critical stenosis has been associated with platelet aggregation and subsequent cyclical reductions in blood flow in femoral arteries (Folts and Rowe, 1980) and in coronary arteries (Folts et al., 1976; Cappuro et al., 1980; Uchida et al., 1980). Platelet activation and aggregation cause the release of vasoactive PGs, principally TXA_2, which causes vasoconstriction in the arterial bed (Ellis et al., 1976; Moncada et al., 1976).

IMZ, as a selective inhibitor of TXA_2, was administered to ascertain if critical stenosis was associated with platelet aggregation and TXA_2 release in these preparations. TXA_2 has been implicated in the inhibition of collateral recruitment during different vascular insults (Needleman et al., 1977b; Helenski et al., 1980). Imidazole acts by inactivating the TXA_2 synthetase and does not allow the conversion of PGG_2 or PGH_2 to TXA_2, whereas the synthesis of other PGs continues via their normal synthetic pathways (Moncada et al., 1977b).

IMZ treatment (n=4) did not produce a significant variation in vascular hemodynamics from the level of hemodynamics in pretreated animals. Q_c (Fig. 19) did not change

Figure 19. Collateral blood flow during graded levels of femoral artery stenosis before and after imidazole treatment



[a]P<.05 within treatment in a group.

significantly until a 100 percent decrease in the c.s. area was achieved. R_c (Fig. 20) and R_p (Fig. 21) remained at lower values during the experiment, suggesting a maximum vasodilatation at the beginning of the experiment. The variable responses at 90 percent stenosis in R_p were non-significantly different from other values because of a larger standard error of the mean. This response occurred because in one of the animals the peripheral bed was not maximally dilated and showed no Q_c and lower Q_p at 90 percent stenosis level. This lower Q_p value not only increased the R_p, but also produced a great variation within the group itself. The insignificant drop in R_c was due to increased pressure gradients across the collateral bed. R_s (Fig. 22) changed significantly at 90 percent stenosis, whereas at lower levels of stenosis the difference was insignificant.

Similar findings were reported by Uchida and Murao (1979) following IMZ treatment in coronary arteries. However, the findings of Helenski et al. (1980) revealed improved Q_c following INDO treatment. These findings have no bearing on the present study or on those of Uchida and Murao (1979). Subacute preparations and their association with thrombus predominantly would have released TXA_2 as a vasoactive PG in their studies. Due to absence of cyclical reductions of flow through, and pressure down stream from the

Figure 20. Collateral vascular resistance during graded levels of femoral artery stenosis before and after imidazole treatment

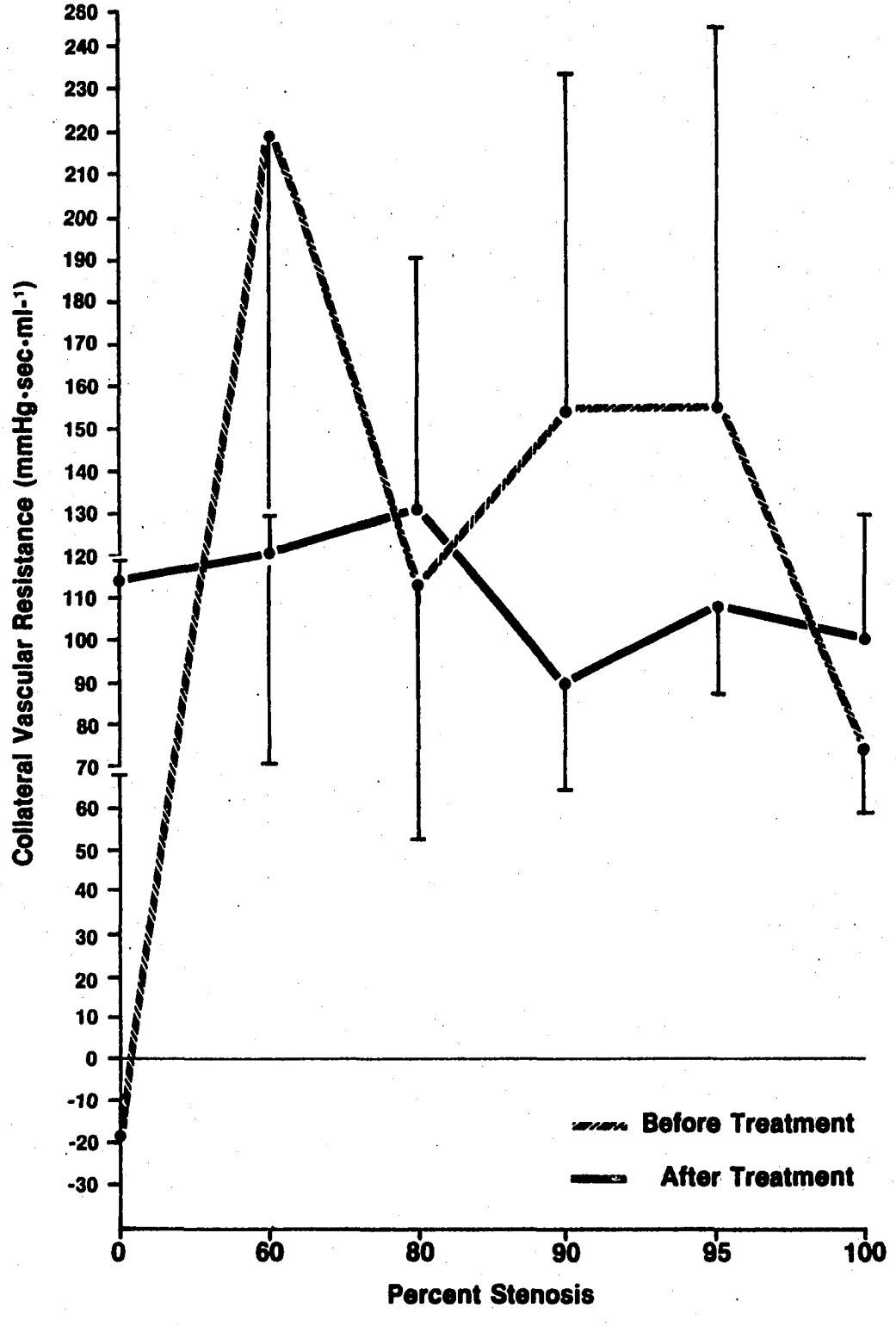

Collateral Vascular Resistance (mmHg·sec·ml⁻¹)
280
240
230
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
-20
-30
0
60
80
90
95
100
Percent Stenosis
Before Treatment
After Treatment

Figure 21. Peripheral vascular resistance during graded levels of femoral artery stenosis before and after imidazole treatment

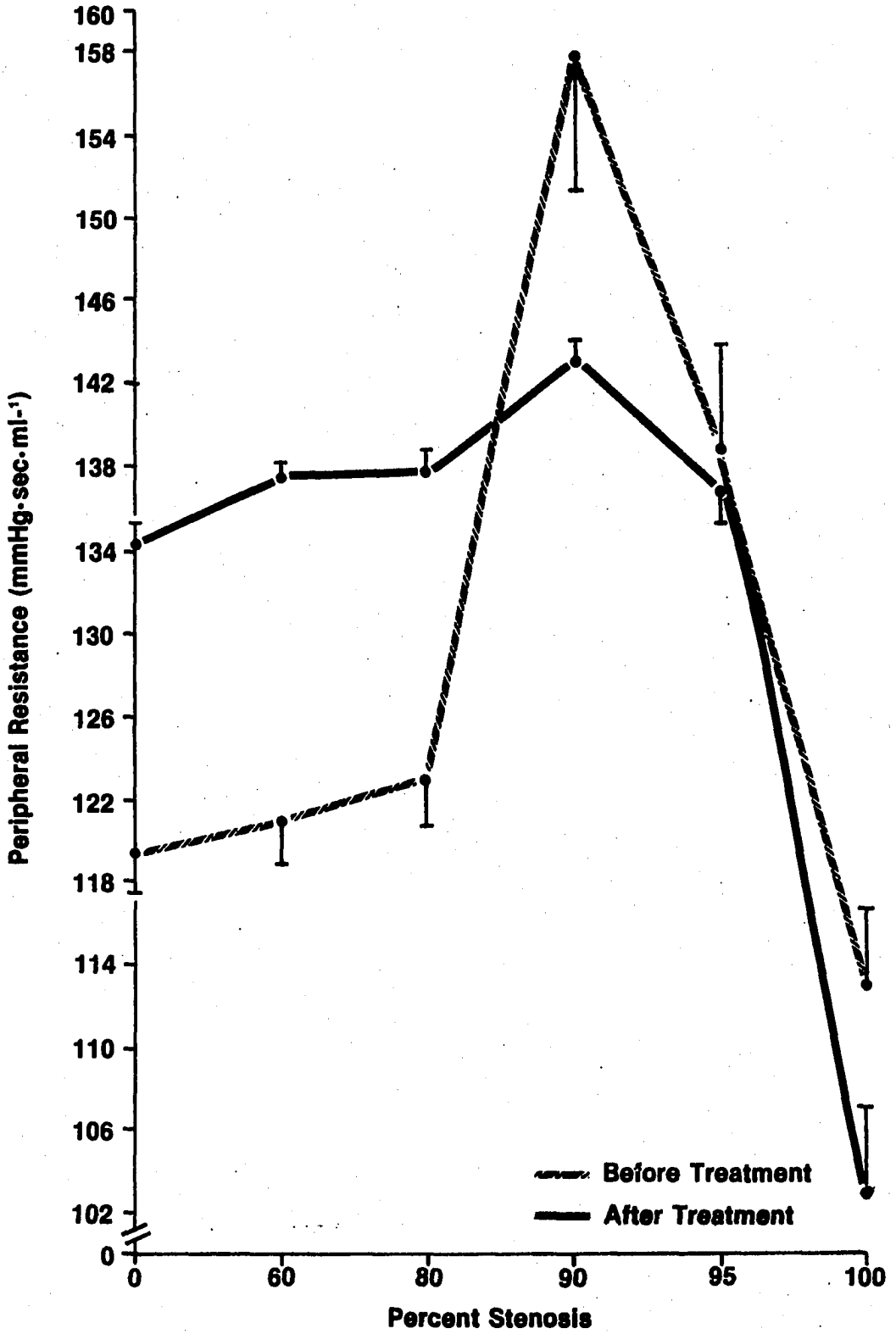
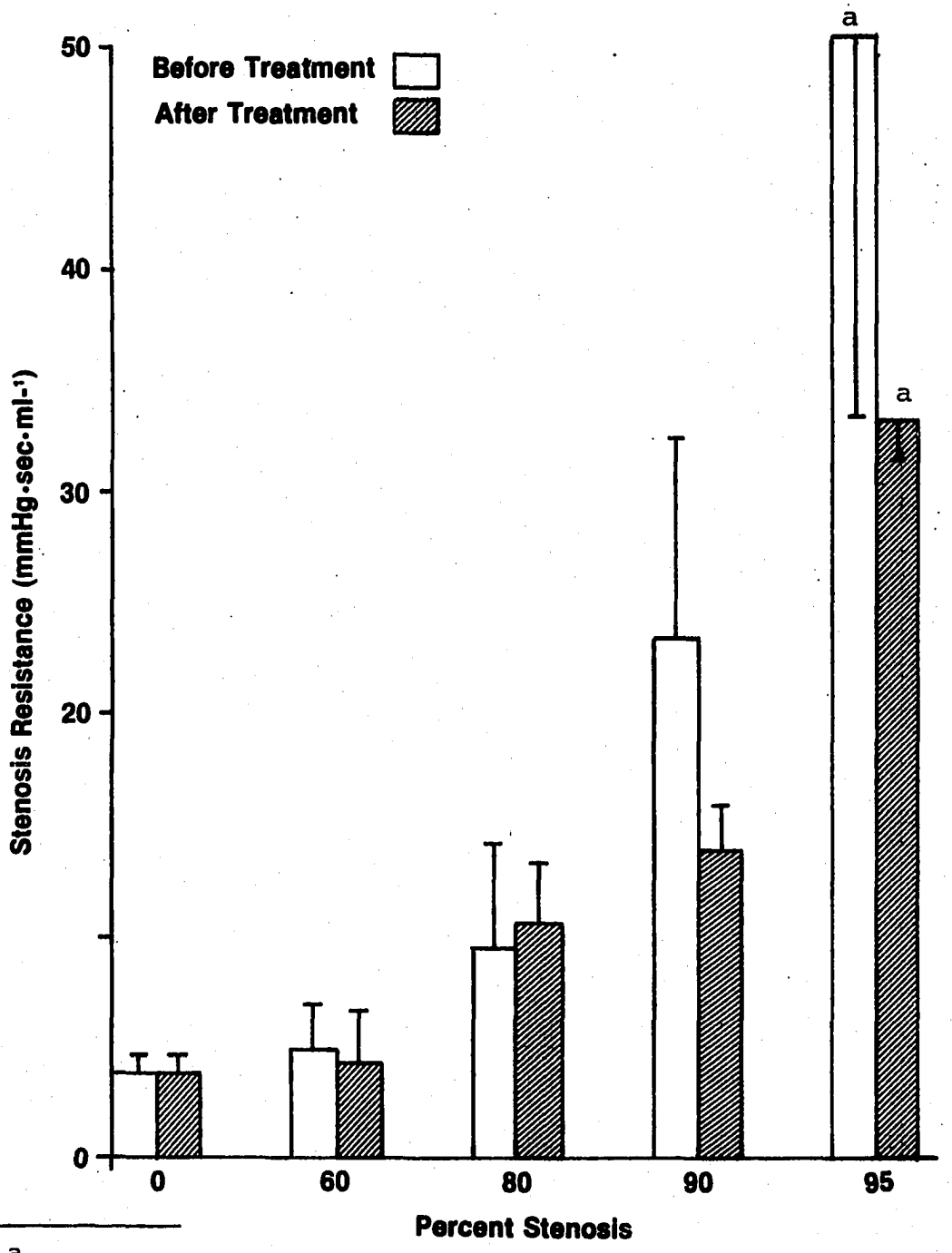

160
158
154
150
146
142
138
134
130
126
122
118
114
110
106
102
0
Peripheral Resistance (mmHg·sec·ml⁻¹)
0
60
80
90
95
100
Percent Stenosis
Before Treatment
After Treatment

Figure 22. Femoral artery resistance during graded levels of stenosis before and after imidazole treatment



[a]P<.05 within treatment in a group.

stenosis coupled with insignificant response to imidazole treatment, one may assume that platelet aggregates and TXA_2 were not involved in the regulation of blood flow in these studies.

DCFA Occlusion

It was hypothesized in this study that DCFA, by virtue of its extensive anastamosing connections with vessels both in ascending and descending directions, was an important collateral recipient artery. An attempt was made to quantify the blood flow in the DCFA both during resting and elevated flow conditions. Animals(n=8) with DCFA occlusion (Table 9) showed a marked decrease in blood flow during resting and elevated flow conditions. This, however, was not significantly different at a 95% decrease in lumen c.s. area. Q_c on the other hand remained unchanged during DCFA occlusion. These findings are in agreement with the findings of Kubicka et al. (1979), who observed that there was no significant variation in collateralization following the ligation of medial sacral, II, and DF arteries. In this study, therefore, the carrying capacity of the DCFA is quantitatively estimated, which despite a slight decrease, did not significantly change under elevated flow conditions.

Table 9. The blood flow through the femoral artery and collateral vascular bed during graded stenoses with and without DCFA occlusion under normal and elevated flow conditions

Treatment	Q_s (ml.sec^{-1})			Q_c (ml.sec^{-1})		
	0%	95%	100%	0%	95%	100%
Control						
WDO	1.12 ± .08	.61 ± .04[a]		.36 ± .02	.52 ± .2	.93 ± .2[a]
DO	.78 ± .06*	.63 ± .04		.19 ± .07	.26 ± .11	.64 ± .08[a]
ACH						
WDO	2.65 ± .37	1.17 ± .3		.2 ± .47	.66 ± .34	1.51 ± .36[a]
DO	1.68 ± .23**	.9 ± .13		.16 ± .07	.65 ± .15[a]	1.09 ± .05[a]

WDO = DCFA patent.

DO = DCFA occluded.

ACH = acetylcholine.

*P<.05 between treatments in a group.

**P<.05 between treatments in a group.

[a]P<.05 within treatment in a group.

SUMMARY AND CONCLUSIONS

The objective of the present investigation was to determine the influence of endogenously released vasoactive substances on the canine hind limb vasculature during graded arterial stenoses. The study was conducted on 28 mongrel dogs weighing between 22 to 36 Kg. Animals were divided into 6 groups (G1-G6) which received either agonist (Serotonin or Acetylcholine) or antagonist (Methysergide, Parachlorophenylalanine, Reserpine and Parachlorophenylalanine, Indomethacin or Imidazole) or both. The collateral and peripheral hemodynamics were measured during partial and complete occlusions of the femoral artery.

Resting, untreated animals (GI) did not show a significant variation in collateral and peripheral resistances following partial or complete occlusion of the femoral artery. Peripheral resistance dropped to less than 50 percent of resting level under elevated flow conditions. The collateral blood flow and resistance remained unchanged during this period. No serotonin effect was seen on hind limb vasculature. Methysergide treatment (GII) invoked a 2-3 fold increase in collateral and peripheral resistances, and collateral blood flow completely ceased during partial stenoses. During this period, nutritional blood flow was supplied to the collateral bed from the partially obstructed femoral vessel. This

redistribution of flow caused a severe drop in flow supplied to the peripheral bed distal to stenosis. The reduction was presumably due to the localized vasoconstrictive effects of methysergide in a maximally dilated vascular bed. The fact that collateral and peripheral hemodynamics were the same as in animals treated with either parachlorophenylalanine or reserpine and parachlorophenylalanine (G III and IV) suggests that serotonin was not involved in producing the vascular responses observed in these preparations.

Indomethacin treatment caused paradoxical changes in the hind limb vasculature with peripheral resistances increasing 2-3 fold. No blood flowed through the collateral vascular bed during partial occlusion of the femoral artery. The collateral vascular bed, however, received nutritional flow from the partially constricted femoral artery. Redistribution of flow, therefore, caused severe reductions in the rate of blood flow to the hind leg distal to occlusion and significantly increased peripheral resistance over the resistance of the untreated animals. High resistances and low blood flows observed during indomethacin treatment could be attributed to the treatment's inhibition of vasodilatory prostaglandins (principally PGI_2) that might have been released during graded stenoses. However, the nonspecific localized vasoconstrictor effects of indomethacin and its direct effect upon vascular tone in the region cannot be

ruled out.

Imidazole treatment produced no effect on the canine hind limb vasculature, probably due to lack of platelet aggregation and thromboxane A_2 release in these studies.

The distal caudal femoral artery (DCFA) carried 30 percent of the femoral artery blood flow under normal conditions. Collateral blood flow did not change significantly when DCFA was occluded. This study quantitated for the first time, the blood flow carried by the DCFA from the femoral artery. It is also shown that DCFA is not the only collateral recipient vessel present in the hind limb. Other vessels which either carry collateral blood flow and empty into patent DCFA, or drain distally, when occluded, may be present. The possibility that these vessels recruit additional collateral channels following DCFA occlusion cannot be ruled out.

The studies conclude that in these preparations serotonin or thromboxane A_2 were not involved in producing vascular responses and were not released during the process of occlusion. In contrast, vasoactive prostaglandins (principally PGI_2) were believed to be released and played the regulatory role in the maintenance of the blood flow to the hind limb vasculature. Their inhibition by indomethacin inhibited this regulatory response.

Before these results can be safely generalized, further

study is needed to characterize the underlying biochemical regulatory mechanisms. In particular, the effects of tissue and blood oxygen levels on prostaglandin biosynthesis and the interaction between prostaglandins, hormones and neurotransmitters need to be more clearly understood.

FUTURE RESEARCH

The present investigation has given some insights into the possible release of vasoactive regulators including the problems associated with it. Future studies, therefore, could be planned in different phases with an ultimate objective of developing a therapeutic agent to counter the obstructive obliterans disease in human beings as a replacement for surgical intervention.

Phased investigations could proceed as follows:

1. a) The present study could be redesigned (involving less invasiveness and more animals) and duplicated with a direct approach of assaying the release of vasoactive PGs, chiefly PGI_2.

   b) If PGI_2 assays reveal significantly higher levels following partial occlusions, 15-hydroperoxy-arachidonic acid could be used as the antagonist rather than indomethacin which is believed to have other effects, apart from PG biosynthesis inhibition.

   c) If possible, tissue oxygen tension could be measured in the affected region and correlated with blood flow and PGI_2 release.

2. Endeavors then could be made to successfully produce and reproduce an acute experimental arterial thrombosis, in an effort to simulate the obstructive obliterature disease in human beings. This phase could be reinforced with steps b and c in phase 1 except that TXA_2, SRT in addition to PGI_2 could be assayed and subsequently inhibited.
3. These studies should include the use of inhibitors in a search for a satisfactory therapeutic agent to overcome a thrombotic crisis without producing any side effect in the system.

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APPENDIX A

The polygraph was electrically zeroed and balanced at the beginning of each experiment. It was necessary to avoid the offsets in determination of the calibration factors and pressure and flow measurements. The calibrations were obtained from the Pdp8e Lab. digital computer. The calibration factor was determined in mm Hg pressure equivalent to 1 volt of electrical output for each transducer. To obtain reliable estimates of pressure calibration, a manometer filled up to 2000 mm H_2O was used. A computer program which converted mm H_2O pressure into mm Hg pressure was used for calibration. Calibration factors were obtained over a wide range of pressures applied simultaneously to all the five pressure transducers. All transducers showed a close linear response for pressures ranging between 1000 and 2000 mm H_2O. There was nonlinearity in response of transducers when pressures were dropped to 500 mm H_2O and less. The calibration factor obtained in the lower range was used for the one transducer which detected venous pressure. The calibration factors for the remaining four transducers were measured in systemic arterial blood pressure ranges (1900-2000 mm H_2O). In an effort to obtain error free and truly representative values, 4-5 close readings for each transducer were averaged. Before the data were obtained from

an experimental animal, using these calibration factors, known applied pressures were compared with those obtained from the transducers. If the two values fell within ±1 mm Hg pressure of each other, the calibration factors were considered accurate and reliable for data acquisition purposes.

EMF transducers were calibrated over a wide range of steady flow rates with a constant pressure head reservoir which contained blood from the experimental animal. The calibration set-up contained both EMF transducers (Q_s, Q_p) in extracorporeal circuits with a flow controlling device between the collecting point and the bypass system. Flow rate was measured by a timed collection in a graduated cylinder. Flow was varied by increasing or decreasing the resistance as and when required. The calibration factors were estimated at different flow rates. Four to five close readings for each flow probe were averaged. A known timed collection of blood flow was compared with that of EMF, using these calibration factors. If the results were within ± .05 ml/sec, the calibration factors were considered reliable and were used for data acquisition from the experimental animal.

APPENDIX B

Table B1. Fluid dynamics in the arterial and venous bypasses of the extracorporeal perfusion apparatus

Arterial bypass		Venous bypass	
Flow (ml/sec)	Resistance mm Hg·sec·ml^{-1}	Flow (ml/sec)	Resistance mm Hg·sec·ml^{-1}
0.62	0.64	0.95	0.25
1.73	1.20	2.33	0.75
3.57	1.99	3.33	1.04
3.64	2.08	3.73	1.08
4.65	2.48	3.87	1.09
5.22	2.73	5.00	1.39
5.80	2.96	6.28	1.62

Table B2. Fluid dynamics during graded stenoses in the stenotic arterial segment and total bypass circuit

Level of stenosis	Bypass				Arterial segment			
	P_1 (mm Hg)	P_2 (mm Hg)	Flow ml/sec.	Resistance mm Hg sec·ml^{-1}	P_1 (mm Hg)	P_2 (mm Hg)	Flow ml/sec.	Resistance mm Hg sec·ml^{-1}
0	39.29	12.42	6.84	3.93	35.38	29.59	7.15	0.81
60	39.63	12.55	6.86	3.95	35.62	29.80	7.19	0.81
80	41.51	9.33	6.30	5.11	38.25	23.84	6.57	2.19
90	44.91	1.10	5.22	8.39	42.91	11.08	5.17	6.16
95	53.34	6.84	1.62	28.70	52.68	10.68	1.59	24.43
100	54.12	3.55	0.14	361.21	55.39	6.06	0.24	205.54

Table B3. Flow rates and Reynolds numbers in bypass systems

Arterial bypass		Venous bypass	
Flow ml/sec	Reynolds #	Flow ml/sec	Reynolds #
0.64	450	0.95	669
1.62	704	2.33	1640
1.73	814	3.33	2344
3.57	2513	3.73	2626
3.64	2562	3.87	2724
4.65	3274	5.00	3520
5.22	3675	6.28	4421
5.80	4083		

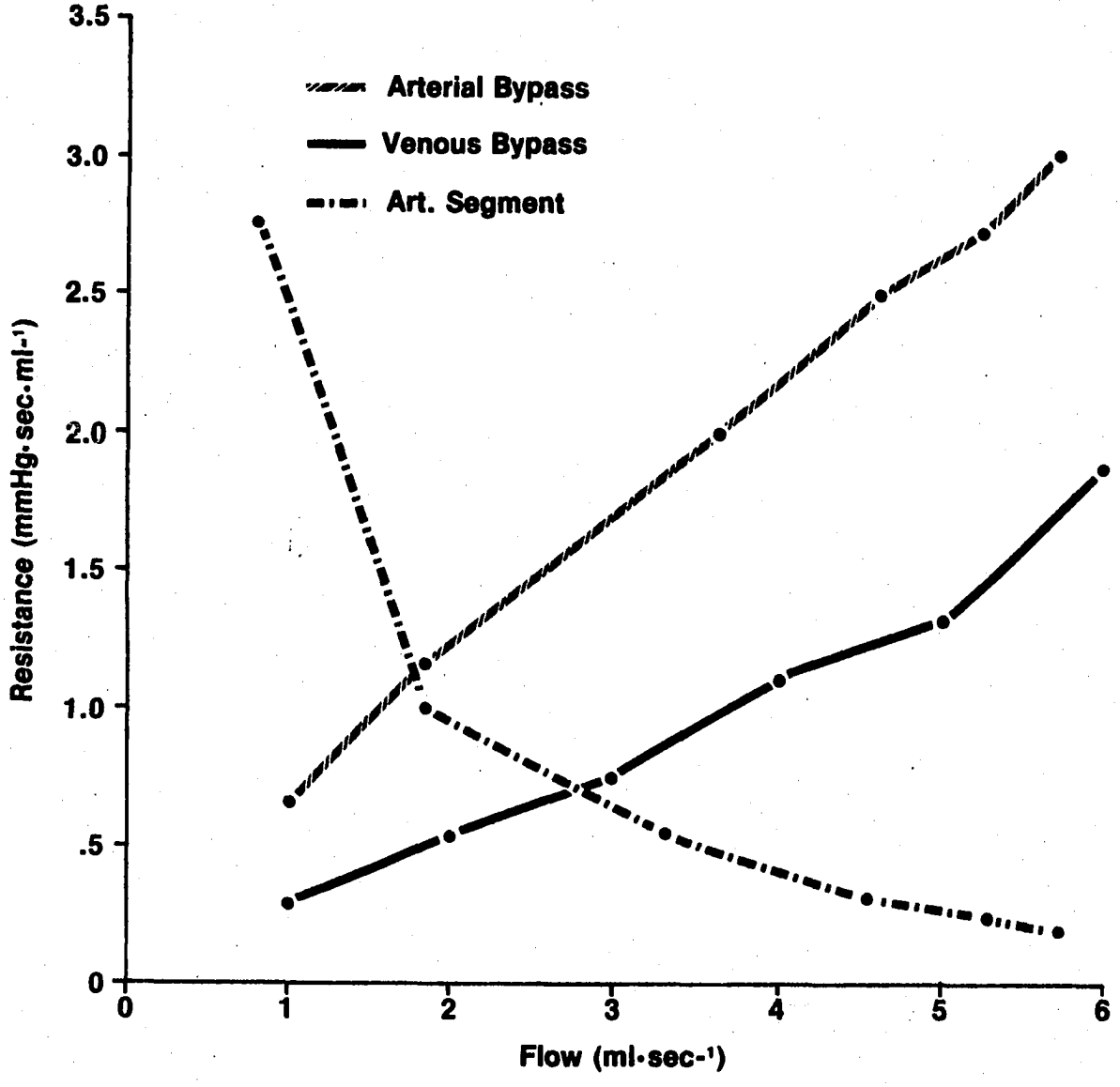

Figure B1. Resistance and flow relationship of arterial segment in the bypass, arterial bypass and venous bypass circuits

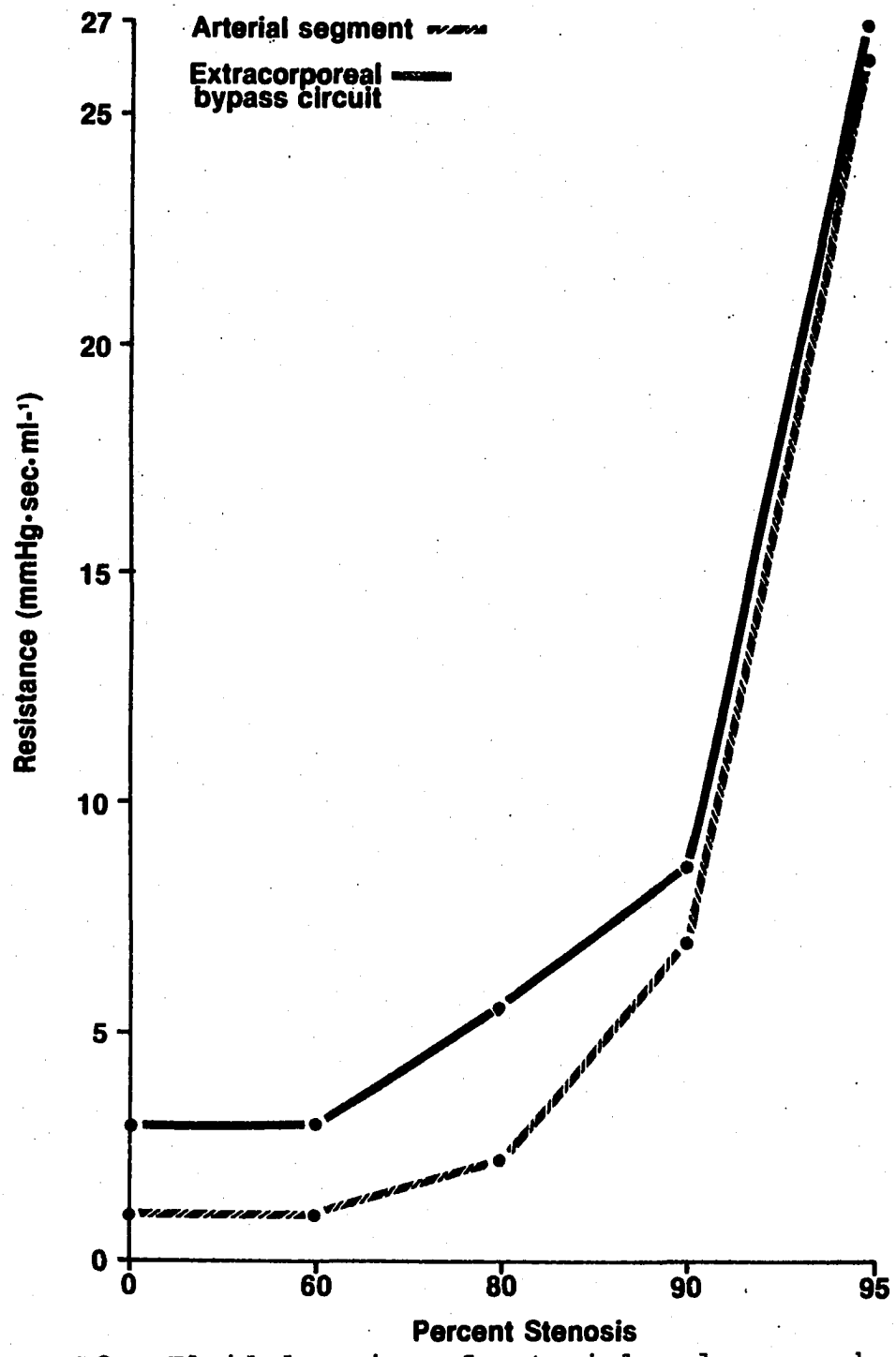

Figure B2. Fluid dynamics of arterial and venous bypass circuits during graded stenoses

APPENDIX C.

Serotonin analysis:

3 ml of whole blood was diluted with 5 ml of distilled water to hemolyze erythrocytes (henceforth it will be referred as a sample)

1 ml of 10 percent $ZnSO_4$ solution was then added and sample mixed

This was followed by addition of .5 ml 1N NaOH. The sample was mixed again

The sample was then centrifuged at 2500-3000 rpm for 10 minutes

The supernatant was harvested

1 ml of supernatant was added to a tube containing 10 ml of n-butanol

The sample was centrifuged for 5 minutes

The supernatant was isolated once again

2.5 ml of supernatant was shaken mechanically with 7.5 ml of n-heptane and .2 ml of .1 N HCl

The mixture was then centrifuged for 10 minutes

The butanol-heptane interphase was the aspirated and discarded

The remaining aliquot of extracted serotonin (.1-.15 ml) was then allowed to react with .6 ml of ortho-phaldialdehyde (OPT) (4 mg/100 ml 10N HCl) which resulted in a fluorescent derivative in boiling water both for 15 minutes

The fluorescence was read at 360 mμ activation spectrum and 470 mμ emission spectrum along with standard containing known concentration of serotonin after cooling in tap water

Serotonin concentration was then calculated by the following formula

$$\text{Serotonin } (\mu g/ml) = \frac{\text{Fluorescence of the sample}}{\text{Fluorescence of the standard}} \times \text{std. concn.} \times \text{dilution}$$