DETERMINATION OF THE NATURE AND MECHANISM OF REVASCULARIZATION OF ISCHEMIC LIMBS VIA THE VENOUS ROUTE

Richard Baffour B.Sc. (Ghana) M.Sc. (McGill)

Department of Experimental Surgery

McGill University

Montreal, Quebec

Canada

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of Doctor of Philosophy

December 1987

TABLE OF CONTENTS

CHAPTER III

MATERIALS AND METHODS

CHAPTER IV

RESULTS

CHAPTER V

DISCUSSION

ABSTRACT

At present, the only effective treatment for atherosclerotic arterial obstruction is surgical revascularization. Unfortunately, in some patients, this is unsuccessful due to the extent of the disease; most of these patients require amputation of the limb because of severe pain or gangrene.

By expanding on the experimental work of others, we were able to successfully save the limbs of experimental animals (canine) with obstructed arteries by restoring circulation via the use of the venous system (which is not affected by atherosclerosis). Subsequently, we have also been able to save the limbs of a small group of patients using a similar procedure. Angiograms in the clinical and experimental setting revealed reversed flow in the venous bed with new vessel formation around and distal to the arteriovenous reversal. In the present study, we investigated whether or not the reversed venous flow provided nutrient perfusion to the hind limb, the role of severe ischemia in the development of the network of vessels appearing around and distal to the arteriovenous reversal and whether these vessel are newly formed. In addition, we also examined the role of lipid angiogenic factor and controlled exercise, as stimulants in the development of this network of vessels.

We have demonstrated that the reversed venous flow provides nutritive perfusion to the limb as measured by transcutaneous oxygen. In addition, we have shown that severe ischemia plays a

major role in neovascularization of the canine hind limb in the presence of arteriovenous reversal, as determined angiographically. We have also demonstrated that there is increased capillary growth in the presence of severe ischemia following arteriovenous reversal and that this capillary neoformation can be enhanced by *in vivo* administration of lipid angiogenic factor or controlled exercise. Finally, we have demonstrated increased tritiated thymidine uptake by endothelial cell nuclei in hind limbs with arteriovenous reversal, suggesting that there is new endothelial formation.

RESUME

A l'heure actuelle, le seul traitement efficace de l'obstruction artérielle due à l'athérosclerose est la revascularisation chirurgicale. Malheureusement, chez certains dont la maladie est trop étendue, cette technique est vouée à l'échec; la plupart de ces malades devront donc subir l'amputation d'un membre pour remédier à leur douleur intense ou à la gangrène.

En s'inspirant des travaux expérimentaux d'autres chercheurs, nous avons été en mesure de sauver les membres d'animaux (chiens) dont les artères étaient obstruées en rétablissant la circulation par le biais du système veineux (qui n'est pas atteint par l'athérosclérose). Par la suite, nous avons été en mesure de sauver les membres d'un petit groupe de malades en nous servant d'une technique similaire. Les angiogrammes effectués tant dans le milieu clinique que dans le milieu expérimental ont révélé une inversion de la circulation dans le lit veineux avec formation de nouveaux vaisseaux autour du lieu où l'inversion avait été réalisée et dans la partie distale par rapport à l'inversion. Dans l'étude dont il est fait état, nous avons tenté de préciser si la circulation veineuse inversée permettait la perfusion de nutriments aux membres postérieurs, nous avons également tenté de déterminer le rôle de l'ischémie grave dans le développement du réseau de vaisseaux qui apparaissent autour du

du lieu d'inversion artérioveineux et dans la région qui lui est distale et si ces vaisseaux sont de formation récente. En outre, nous avons étudié le rôle du facteur angiogénique lipoidique dans la stimulation du développement de ce réseau de vaisseaux.

Nous avons démontré que l'inversion de la circulation veineuse permet la perfusion nutritive du membre, tel qu'on a pu le mesurer par le taux d'oxygène transcutané. De plus, nous avons démontré qu'une ischémie grave joue un rôle prépondérant dans la néovascularisation du membre postérieur du chien en présence d'une inversion artérioveineuse, démontrée a l'angiographie. Nous avons également fait la preuve qu'il existe une croissance capillaire accrue en présence d'une ischémie grave qui fait suite à une inversion artérioveineuse et que cette nouvelle formation de capillaires peut être favorisée par l'administration in vivo du facteur angiogénique lipoidique ou par un exercice suivi. Enfin, nous avons démontré qu'il existe une captation accrue de thymidine tritiée par les noyaux des cellules endothéliales des membres postérieurs sur lesquels on a pratiqué une inversion artérioveineuse; cette observation donne à penser qu'il y a formation d'un nouvel endothélium.

ACKNOWLEDGEMENTS

I am deeply indebted to my supervisor, Dr. J.F. Symes for his unlimited help, patience and constructive criticism throughout this investigation and in the preparation of this manuscript.

I am also indebted to Dr. A. Graham, Dr. A. Sniderman, all the other staff of the Cardiovascular Research Unit (1984-1987) and Cardiovascular and Thoracic Surgery (Royal Victoria Hospital) for their superb help.

My sincere thanks goes to Dr. A. Common (Department of Radiology, Royal Victoria Hospital) for angiography. Also to Dr. B. Kopriwa (Department of Anatomy, McGill University) for processing of tissue specimens for autoradiography.

Lipid angiogenic factor (Feline CM Fr) was kindly provided by Dr. A.L. Griffith (Biochemistry Research Laboratories, Department of Biobehavioral Sciences, Boston University, Boston, Mass).

I would like to thank Maria Grant for typing the manuscript.

The present work was supported by funds made available to Dr. J.F. Symes by the Medical Research Council of Canada.

LIST OF ABBREVIATIONS.

AMP	Adenosine monophosphate
ATP	Adenosine triphosphate
AVA	Arteriovenous anastomosis
AVR	Arteriovenous reversal
Cap/mm^2	Capillaries per square millimeter
C	Celsius (degree centigrade)
Ci	Curie
Cm	Centimeter
DNA	Deoxyribonucleic acid
g	Gram
HDL	High density lipoproteins
^{3}H-Tdr	Tritiated-Thymidine
Kg	Kilogram
L	Liter
LDL	Low density lipoproteins
uCi	Microcurie
M	Molar or moles per litre
mg	Milligram
ml	Milliliter
mmHg	Millimeter of mercury pressure
mo	Month
0^o	Degree (360th part of the circumference of a circle)
pH	-- Log H^+ concentration
PTFE	Polytetrafluoroethylene (Teflon)
SEM	Standard error of the mean
$Tcp0_2$	Transcutaneous partial pressure of oxygen
wt	Weight

LIST OF FIGURES

Figure 10a: The Treadmill: Constructed in our Laboratory.

Figure 10b: Dog Running on the Treadmill.

Figure 10c: Same Dog Running on the Treadmill.

Figure 11: AVR: Day 10 Study. Representative angiogram from studies under Materials and Methods sections D, F, G, and H.

Figure 12: AVR: Day 22 Study. Representative angiogram from studies under Materials and Methods sections D, F, G, and H.

Figure 13: Transcutaneous pO_2.

Figure 14: Representative angiogram from a Group 1 animal.

Figure 15: Representative angiogram from a Group 2 animal.

Figure 16: Representative angiogram from a Group 3 animal.

Figure 17a: Representative angiogram from a Group 4 animal demonstrating sequential filling of the intense vascular network and reversed venous flow to the foot.

Figure 17b: Representative angiogram from a Group 4 animal demonstrating sequential filling of the intense vascular network, reversed venous flow to the foot and subsequent venous emptying of the limb.

Figure 17c: Representative angiogram from a Group 4 animal demonstrating the intense vascular network and venous emptying of the limb.

Figure 18: Leg muscle sections histochemically stained for alkaline phosphatase activity.

Dark spots represent capillaries.

A = Nonoperated Control Hind Limb (Contralateral muscle).

B = Operated Hind Limb (muscle).

Figure 19: Capillary density in muscles of canine hind limb.

Figure 20: Tritiated-Thymidine Incorporation.

Figure 21: Endothelial cells of small blood vessels indicating DNA synthesis. Two endothelial cell nuclei (arrows) are heavily labelled. A single pulse of tritiated-thymidine was given intravenously three hours before the animal was sacrificed.

Figure 22: AVR: Day 10: Capillary Density: (effect of controlled exercise: Treadmill).

Figure 23: AVR: Day 10: Capillary Density (effect of lipid angiogenic factor).

LIST OF TABLES

CHAPTER I

INTRODUCTION

A. PERIPHERAL VASCULAR DISEASE

I. General Introduction:

The primary role of the circulatory system is the transport of oxygen, nutrients, by-products of metabolism, heat and other constituents of blood to and from all tissues of the body. The energy for this important function is generated by the heart which pumps blood through a series of distributing vessels.

In accordance with Jean Poiseuille's law (1842), the flow of blood is controlled by the arterial blood pressure, resistance to the flow by the blood vessels and blood viscosity. Furthermore, the resistance of a vessel is inversely proportional to the fourth power of its radius. Thus, the radius of a vessel is of paramount importance in the circulatory system. In the presence of arterial lumenal narrowing, which is usually due to atherosclerosis, the efficient transport of blood to the peripheral capillary bed is impeded (1 - 4). With mild obstruction, characteristic symptoms become evident only after brief exercise, such as walking or climbing stairs. In contrast, more severe disease leads to symptoms even at rest and eventually necrosis of tissue.

II. Intermittent Claudication:

The term claudication, which is derived from Latin, means "limping". Intermittent claudication is a well characterized symptom of peripheral vascular disease and is almost always

atherosclerotic in origin. However, there are other rare causes, such as cystic adventitial disease of the popliteal artery and popliteal entrapment syndrome (5, 6).

The general complaints of these patients are muscle pain, cramp, fatigue and/or severe weakness with exercise, which are relieved by rest. The arterial blood flow may be adequate to maintain resting tissue requirement but, with exercise, the flow may not be increased enough to deliver the necessary oxygen to maintain muscle function. The location of intermittent claudication is determined by the level of atherosclerotic stenosis. Generally, symptoms felt in the lower back, buttocks or thigh are associated with distal aortic and common iliac disease. In contrast, calf and foot symptoms are caused by occlusion of the superficial femoral artery, while popliteal artery disease may lead to foot pain or numbness on walking. The etiology of the pain is unclear, although it is believed to be caused by muscular hypoxia and accumulation of metabolites. Metabolites such as lactic acid, phosphorus compounds, ammonia, phosphoric acid and potassium have all been suggested as the cause of the pain (7 - 9).

Intermittent claudication is relatively benign (10, 11). Forty per cent of patients improve, 40% remain unchanged and only 20% need surgical treatment (12). Furthermore, it has been reported that only 5.8% of intermittent claudication patients require amputation during a mean follow-up period of 2.5 years (13).

III. Ischemic Rest Pain:

With further progression of vascular disease, more arterial and collateral vessels may become occluded. Consequently, arterial flow becomes inadequate even at rest, causing more ischemia and pain (14). In addition to the reduction of total arterial blood flow, most of these patients have significant structural changes in the capillary vascular bed and it has been suggested that these changes may be the immediate cause of necrosis (15).

Pain at rest means the arterial flow to the extremity is so limited that even the small nutrient requirements of the skin is compromised and this signifies the start of severe peripheral vascular disease (16, 17). Ischemic rest pain is unrelenting and severe. It is usually worse at night when the patient is in bed, since in the horizontal position, the beneficial effects of gravity in carrying blood distally are lost (14). Thus the patient has to hang the foot over the side of the bed or spend the night in a chair in order to relieve the pain. However, this posture may lead to edema which in turn leads to further reduction of flow because of increased extracellular pressure. At this stage, another type of pain due to ischemic neuropathy may occur (14).

IV. Ulceration and Gangrene:

Ulceration and gangrene are the end stage of peripheral vascular disease of the extremities. It is well established that

the stereotype of chronic leg ischemia is gradual development of claudication which becomes worse with shortening of walking distance. This is followed by continuous pain at rest and eventually, deterioration in skin nutrition with ulceration and frank gangrene (15, 16, 18). Usually, ulcers occur on the toes or heel but may occur anywhere on the foot or lower leg. Similarly, gangrenous changes occur in the toes, heels and lateral aspects of the foot or proximal parts of the leg as a result of trauma (such as incisions, burns or heat) or by drug-induced vasoconstriction (19, 20).

B. THE ETIOLOGY AND PATHOGENESIS OF ATHEROSCLEROSIS

I. The Lesions of Atherosclerosis:

Despite recent advances in atherosclerotic research, the cause and pathogenesis of atherosclerosis remain unsolved. However, there is a general agreement that there are three different types of atherosclerotic lesions: fatty streak, fibrous plaque and complicated lesion.

Atherosclerosis is a disease process that starts early in life. The fatty streak begins in the intima and may cover about 10% to 30% of the intimal area of the aortic surface by the age 10 or 30 years, respectively (21). The lesion first becomes apparent as fatty dots or streaks on the intima of large arteries, localized primarily at proximal and lateral branch orifices and the shoulders of bifurcations. Eventually, the lesion spreads to smaller arteries. Histologically, the lesion

shows an intima thickened by the collection of lipid-laden foam cells, which may be either smooth muscle cells or macrophage type foam cells. Usually, this lesion causes little or no obstruction and no clinical symptoms. The fact that fatty streaks are present in the aorta of virtually every child by the age of 10 years has led to the suggestion that juvenile fatty streaks are the precursors of the large fibrous plaques of developed atherosclerosis (22). However, this suggestion has been challenged by several studies using topography, geographic pathology and chemistry, and also by preliminary studies using cellular markers (23 - 25). For instance, the ubiquity of fatty streaks in populations with a low incidence of clinical manifestations of atherosclerosis suggests that the lesion progression does not develop beyond the fatty streak stage (26).

In contrast, the primary lesion of chronic arterial occlusive disease is the fibrous plaque, which has a different distribution pattern as compared to the fatty streak (27). The fibrous plaque first appears in the abdominal aorta after the age of 20 and increases progressively with age (21). This lesion protrudes into the lumen of the artery and shows a central lipid pool of atheromatous gruel and cholesterol crystals surrounded by foam cells. Some of these foam cells may be monocytes (macrophages) and others are smooth muscle cells (28). The interstitium between the cells is expanded by the accumulation of elastic fibres, collagen, proteoglycans and lipid droplets (27, 29). The fibrous plaques are most frequent and severe in the arteries of the heart, brain and legs, where they may increase in

size, enough to obstruct the flow of blood. It is well established that progressive and degenerative changes in the fibrous plaque from adulthood to middle age may predispose individuals to cardiovascular disease later in life (30).

The final step that leads to clinically significant reduction in the flow of blood usually is thrombosis over a fibrous plaque. This lesion, which is referred to as a complicated lesion, is believed to be a fibrous plaque that has been modified by hemorrhage, calcification, cell necrosis and mural thrombosis.

In an attempt to explain the cause and pathogenesis of atherosclerosis, several hypotheses have been suggested. These hypotheses must take all the above lesions into account as well as explain the effects of the associated risk factors on the incidence and clinical events of atherosclerosis. Present hypotheses include the lipid hypothesis, the response-to-injury hypothesis, the monoclonal hypothesis and the clonal-senescence hypothesis of atherogenesis (21, 31 - 36).

II. The Risk Factors of Atherosclerosis:

Risk factors have been defined as habits and abnormalities which lead to an increased susceptibility to atherosclerosis (37). A risk factor may be a causative agent, a secondary manifestation of an underlying metabolic abnormality or an early symptom of atherosclerosis (26). Such factors are based on associations uncovered in epidemiological studies of both humans

and experimental animals. For instance, it has been observed that people moving from a geographic area of low incidence to an area of high incidence gradually tend to show the higher rates of atherosclerosis observed in such areas. Such studies demonstrate the importance of environmental factors in atherosclerosis.

The major established risk factors are age, hypertension, cigarette smoking, diabetes mellitus, and hypercholesterolemia. It should be mentioned that when the first four of these factors are not accompanied by elevated plasma cholesterol, these factors may not lead to premature atherosclerosis. This has been demonstrated by epidemiological studies in Japan (26).

a) Age:

It is well established that age has the strongest and most consistent correlation with atherosclerosis. Atherosclerotic plaques appear in the aorta in the first decade of life, in the coronary arteries in the second, and in the cerebral and peripheral arteries in the third (38). Several studies involving the use of multiple regression models in the analysis of clinical and autopsy data have demonstrated significant positive correlation between age and atherosclerosis (39, 40).

b) Hypertension:

Epidemiological studies of both humans and experimental animals have indicated that an elevated blood pressure

accelerates atherogenesis and increases the incidence of cardiovascular disease (26, 37, 41 - 43). Hypertension is a strong risk factor for stroke and to a lesser extent for peripheral vascular disease. Studies by Robertson and Strong have demonstrated that hypertensive patients are strongly associated with an increased degree of atherosclerosis than non-hypertensive patients(44). This difference has been shown to be significant at all ages, in both sexes and for both the aorta and coronary arteries (44). These findings have been substantiated by more recent investigations involving the use of multivariate linear regression analysis (40, 45).

c) Cigarette Smoking:

Cigarette consumption remains an important risk indicator for cardiovascular disease, particularly in elderly individuals (46 - 48). Susceptibility to ischemic heart disease and peripheral vascular disease and their complications is enhanced by cigarette smoking. The associated risks tend to increase in proportion to the number of cigarettes smoked (49). Several studies have demonstrated a strong positive correlation between cigarette consumption and the extent and character of cardiovascular disease (47, 49). However, the exact mechanism involved is by no means clear. Various investigators have postulated that nicotine, toxic agents, carbon monoxide, increased concentration of carboxyhemoglobin and/or decreasing oxygen supply may cause an increased risk of atherosclerosis in the smoker, although none of these factors have been definitely proven as being the culprit (50). For instance, the

administration of high doses of nicotine to experimental animal produces calcification and necrosis of the arterial media, a condition that is similar to Monckeberg's arteriosclerosis (50). However, such studies have still not conclusively proven that nicotine leads to atherosclerosis.

d) Diabetes Mellitus:

Diabetes mellitus increases the incidence and severity of peripheral vascular disease. This association has been demonstrated in a large variety of studies. Various groups have shown that peripheral atherosclerosis is up to 11 times more frequent in diabetics and that occlusive arterial disease develops about 10 years earlier, as compared to control groups (26, 44). Loss of limb occurs 5 times more frequently in the diabetic with symptomatic occlusive disease than in similarly affected, non-diabetic peers (26). Also, gangrene of the extremities due to atherosclerosis is about 40 times more frequent in a diabetic over the age of 50 than in a non-diabetic (26). Finally, diabetic groups have more fibrous plaque and calcification in the coronary arteries, as well as more stenosis, than non-diabetic groups (44).

Despite extensive research in the area of diabetes mellitus and its association with atherosclerosis, the exact mechanisms by which this disease enhances the development and severity of atherosclerosis remain unknown. Some experimental studies have demonstrated that hyperglycemia associated with insulin deficiency as in diabetes produced by alloxan or partial

pancreatectomy is not, per se, atherogenic (51, 52). Rabbits and rats rendered insulin-deficient with alloxan, developed less atherosclerosis than expected when fed a high cholesterol diet. However, this atherosclerosis prevention effect has been reversed by giving such experimental animals insulin (53). Furthermore, insulin has been shown to affect the metabolism of the arterial wall. Studies by Mahler have demonstrated that when insulin is given to alloxanized rats, there is a decrease in the production of endothelial lipase (54). In a similar study, Stout demonstrated that an intravenous injection of insulin into alloxanized rats led to a much greater incorporation of carbon-14-labelled glucose or acetate into the aortic lipid than when no insulin was given (55, 56). Insulin does not appear to accelerate the uptake of cholesterol by the aortic wall (57). However, it has been found to inhibit the regression of the lesions in the aorta that occurs when cholesterol-fed chicks are transferred to a normal diet (58).

e) Hypercholesterolemia:

Hypercholesterolemia has been associated with an increase in atherosclerosis, a conclusion established in several epidemiological studies (59 - 61). It has been demonstrated that an individual's risk of developing atherosclerosis increases exponentially at high levels of serum cholesterol (60). Studies involving prolonged diet-induced hypercholesterolemia in several species including non-human primates, have shown that such animals have a greater risk of developing atherosclerosis and its major complications (62 - 64).

Finally, a report from the Framingham study indicates that the important components of total serum cholesterol in relation to atherosclerosis are low density lipoproteins (LDL) and high density lipoproteins (HDL) (61). According to this report, high levels of LDL and/or low levels of HDL, are associated with atherosclerosis.

III. Hemodynamic Factors in Atherogenesis:

Atherosclerosis has been shown to occur at certain favoured sites, an observation that has led to the suggestion that local hemodynamic factors, such as shear stress, may be associated with atherogenesis. The favoured sites in question are branch vessels arising from the aorta, bifurcations, arterial bends, superficial femoral artery in the Hunter's canal and the common femoral artery under the inguinal ligament. Several hypotheses have been proposed to explain the effects of hemodynamic forces exerted upon the arterial wall. These include the pressure-related hypothesis, the wall-shear hypothesis, the turbulence-related hypothesis and the flow separation hypothesis (65 - 72).

The pressure-related hypothesis, proposed by Texon suggests low intraluminal and high extravascular pressures during a portion of the cardiac circle lead to a suction effect on the endothelial surface (65,66). Over time, the endothelium is believed to become damaged, eventually leading to atherosclerotic lesions. However, since negative transluminal pressure is unlikely during the cardiac circle, this hypothesis does not appear to be valid (73).

The wall-shear stress hypotheses have been put forward independently by Fry and Caro (67, 69). Fry has suggested that acutely elevating the shear stress forces on the endothelial surface to approximately 380 dynes/cm^2, could result in increased permeability and alterations in the endothelial architecture (67, 74, 75). These changes in turn could lead to local injury and erosion in the favoured sites resulting in lipoprotein accumulation and eventually atherosclerotic lesions. Thus, elevated shear stress may lead to atherogenesis. In contrast, Caro and others believe that there is an inverse relationship between shear stress and atherogenesis (69). These investigators have suggested that low stress areas of the vascular tree favour a lessened egress of lipid components, particularly cholesterol, from the vessel wall. Therefore, the development of atherosclerotic lesions in such regions may be favoured. Conversely, high stress regions would have less chance of developing atherosclerosis (69).

Turbulence has also been suggested as a causative factor in atherogenesis (70, 71, 76 - 78). It is believed that turbulence causes platelets and other blood components to stick together, eventually leading to the formation of thrombi or elevated plaques in the intima. Furthermore, turbulence is believed to cause endothelial injury, which alone can result in atherosclerotic lesions. However, there is no conclusive evidence to support the suggestions that turbulence plays a role in atherogenesis.

The flow separation hypothesis was proposed by Fox and Hugh (72). According to this hypothesis, flow separation at arterial branches and along curved segments results in areas of stasis. Hence, there is a stagnation of blood in such sites, leading to the accumulation of platelets, fibrin and lipoprotein, and eventually to atherosclerotic lesions. This phenomenon is believed to be the major hemodynamic force associated with atherogenesis.

Summary:

In spite of the various hypotheses, we still have no conclusive proof as to the precise role that hemodynamic forces play in atherogenesis. Extensive additional studies are required before definitive conclusions can be made.

C. THROMBOSIS AND PERIPHERAL VASCULAR DISEASE

Thrombosis is the central event in the evolution of vascular disease and may lead to ischemic necrosis, a finding more characteristic of arterial than venous lesions. Thrombosis contributes to plaque formation, progression of atherosclerosis and occasionally embolization to smaller, more distal arteries. The mechanisms involved in intravascular thrombosis consist of complex and intricate dynamic interactions between the vascular wall and the components of the flowing blood. These include injury to normal or already damaged vessel walls, platelet adhesion and aggregation and activation of the clotting mechanism (79, 80). Briefly, platelets tend to deposit on exposed collagen of damaged endothelial surfaces or ulcerated atherosclerotic

plaques. This is followed by the release of platelet intracytoplasmic calcium, which then causes the contraction of the platelet and the release of adenosine diphosphate and thromboxane A2 (81 - 84). These compounds in turn cause platelet aggregation. During the complex processes of platelet adhesion and aggregation, the clotting mechanism may be activated, leading to the formation of thrombin and fibrin. The fibrin causes stability and fixation of the thrombus (85, 86).

The symptoms and signs of arterial thrombus depend upon the size and location of the artery occluded, and the speed of the development of the thrombosis. A partial thrombotic obstruction of the vascular lumen may not cause any immediate clinical symptoms. Such a thrombus usually occurs over an ulcerated atherosclerotic plaque and may eventually accelerate atherosclerosis. In contrast, a complete sudden arterial thrombosis may present as an emergency with an acutely ischemic limb. Under such conditions, the limb will be cold and pale. In addition, there may be pain, paresthesia, and motor paralysis, eventually leading to necrosis of limb. Finally, thrombus can dislodge and embolize into a distal artery, worsening symptoms of claudication and pain or causing sudden acute occlusion.

D. NEOVASCULARIZATION IN PERIPHERAL VASCULAR DISEASE

Neovascularization is the sequence of events associated with capillary formation. This process is of paramount importance in a variety of biological processes including collateral circulation, wound healing, tumor growth and embryonic development (87). The molecular events that cause neovascularization remain unclear. However, several mechanisms have been proposed to explain this process. It has been suggested that a mechanical or biochemical stimulus can induce new vessel growth.

In terms of a mechanical stimulus, it is well established that significant hyperemia develops in response to an arteriovenous fistula (88, 89). This is believed to occur primarily as a result of an increased velocity of blood flow (90, 91). It has been shown that an increase in the flow rate of blood causes shear stress of the vessel wall and this leads to passive dilatation and enlargement of existing collaterals by active growth (90, 91).

With regard to a biochemical stimulus, a second event, entirely different from pre-existing vessel enlargement has been proposed as the mechanism for neovascularization (92). This second event may occur later or at least not as quickly as pre-existing vessel enlargement (92). Capillary neoformation, has previously been ascribed to such substances as a transferable factor promoting vascular growth, tumor angiogenic factor, human follicular fluid, angiogenesis factor from human myocardial

infarct, lipid angiogenic factor and angiogenin (87, 92 - 96). In an ischemic environment, these biochemical factors may be released thereby producing neovascularization (97). According to Folkman and associates, capillary neoformation occurs in stages (98). First, in the presence of a biochemical stimulus, endothelial cells degrade the vascular basement membrane and protrude through the wall of the vessel. This is followed by a directional locomotion of the endothelial cells from the parent vessel to form a capillary sprout. Next, a lumen is formed in the sprout and endothelial proliferation takes place within the sprout whereas endothelial locomotion occurs at the tip of the sprout. The tip of one sprout joins with another to form a capillary loop through which blood begins to flow. Eventually, new sprouts develop from each loop to form an intense capillary network. As a final step, a new basement membrane is formed which later incorporates microvascular pericytes into it (98). All the above stages are believed to be caused either directly or indirectly by angiogenic factors (98).

Neovascularization induced by angiogenic factors is an area of active research. It is generally believed that learning more about the physiological mechanisms involved in neovascularization may lead to the development of more effective therapies for peripheral ischemic disease or myocardial infarction.

E. THE TREATMENT OF PERIPHERAL VASCULAR DISEASE

I. Medical Therapy:

In spite of the fact that medical treatment is palliative, not curative, it still remains of vital importance, since the underlying cause of atherosclerosis persists even after surgery.

a) Measures Designed to Influence Peripheral Vascular Disease:

These are general measures that may help patients with peripheral vascular disease. Such measures include weight loss since excess body weight reduces the claudication distance of patients. Patients should be advised to keep their feet dry and clean and that trauma and extremes of temperature are to be avoided. Furthermore, patients should be advised to use correctly fitting shoes, to do daily inspections of their feet for indications of infection, and never go barefoot. Also, patients should be encouraged to have their nails and callouses attended to professionally.

b) Drug Therapy:

Drugs have generally been ineffective for the treatment of peripheral vascular disease. Several vasodilator drugs, such as nylidrin, isosuprine and tolazoline, increase blood flow to animal as well as normal human extremities (99 - 104). Clinical trials with these agents have been unsuccessful. In spite of

several claims, especially by pharmaceutical companies, there is no evidence that any vasodilator drug is effective in the treatment of peripheral vascular disease.

In contrast, pentoxifylline, a methylxanthine derivative, has been used by several investigators for oral treatment of intermittent claudication. Various randomized, double-blind trials have shown that the pentoxifylline-treated patients increased their walking distance compared to patients treated with placebo (105 - 110). Pentoxifylline appears to improve capillary blood flow by increasing red blood cell flexibility thereby enchancing the passage of these cells through the vascular bed (111). This effect on red blood cells is believed to be due to increased cyclic adenosine monophosphate (AMP) and adenosine triphosphate (ATP) concentrations resulting from phosphodiesterase inhibition (111). In addition, pentoxifylline is believed to decrease the concentration of fibrinogen in blood, preventing aggregation of erythrocytes and platelets, thereby reducing blood viscosity (112).

Fibrinolytic agents, on the other hand, are effective treatment for the acute manifestation of peripheral vascular disease. Streptokinase or urokinase accelerate the generation of plasmin which lyses thrombi in veins, pulmonary and coronary arteries as well as peripheral arteries (113). It may be used as a prelude to surgical intervention or as a treatment in patients who are unsuitable candidates for surgical procedures (113, 114).

Generally, it appears that thrombolytic therapy is much less successful in patients with chronic occlusive disease compared to more recent occlusions (115).

II. Surgical Treatment:

a) Direct Arterial Reconstruction:

At present, the only effective means of relieving pain and improving the nutrition in the extremities of patients with peripheral vascular disease is by direct arterial revascularization. Surgery is effective palliation and may accomplish limb salvage, but unfortunately, it has no impact on the underlying cause of the disease. It should also be mentioned that not every patient with peripheral vascular disease should have surgical intervention, since the majority of patients may improve with appropriate medical management and an additional number may stabilize with time. Generally, the indications for surgery are ischemic rest pain, numbness, ulceration, gangrene or interference with the ability to earn a living.

There are several revascularization techniques in the armamentarium of the vascular surgeon for the patient with peripheral vascular disease who requires surgery. These techniques include endarterectomy, angioplasty, aorto-bifemoral bypass, extra-anatomical axillo-femoral or femoral-femoral bypass grafting, and saphenous vein bypass. However, in spite of all these procedures, there are numerous on-going studies by several investigators for the development of effective and lasting arterial revascularization to salvage extremities that would otherwise be lost by amputation.

Aorto-iliac disease:

The procedure of choice for patients with an ischemic limb due to diffuse aorto-iliac occlusive disease is an aorto-bi-femoral bypass using a synthetic graft. The operative mortality rate of this procedure is between 3% - 9% and the long term graft patency rate is between 70% - 97% at 5 years (116 - 119). Some studies have reported a graft patency rate of about 75% at 10 years (120). Such long term patency has been suggested to be due improved graft material, which may provide a more biocompatible pseudointima and secondly, to the present practice of extending the distal anastomosis down onto the profunda femoral artery, thereby ensuring a good out-flow tract (119 - 123).

The major problems associated with this operation are graft infection, progression of atherosclerosis in the distal vessels, which may result in poor run-off, pseudoaneurysms and aorto-enteric fistula formation. In recent years, the incidence of graft infection has been reduced with peri-operative antibiotics and with the use of improved bioprosthetic grafts that allow early tissue incorporation (120, 124 - 127). The other two problems, pseudoaneurysm and aorto-enteric fistula formation, are believed to be reduced by the use of an end-to-end proximal anastomosis. This type of anastomosis has been suggested to improve hemodynamics and to ensure good closure of the retroperitoneal tissue over the proximal suture line (120, 128, 129).

Another revascularization technique used to bypass aortoiliac disease is the use of a graft that connects an axillary artery to both femoral arteries. This operation, which has an operative mortality of 2%, is appropriate for high-risk patients and those with aortoiliac graft occlusion or infection (130, 131). The most frequent complication of an axillo-femoral-femoral graft is thrombosis, which may occur in up to 40% of these patients within one year. The long term patency rate of the graft is about 76% at 5 years (130).

Femoro-popliteal and Distal Disease:

In patients with femoro-popliteal disease, the operation of choice is to bypass the obstruction, frequently with a reversed, autogenous, saphenous vein graft. The patency rate of this operation is between 58% to 72% at five years and 15-40% at eight to ten years (117, 132 - 135). The operative mortality is 2% - 7% and 5 year mortality is about 48% (129). However, the long term results of this operation have been inconsistent and variable. It has been suggested that the outcome depends on many variables, including age, the state of health of the patient, the length of the graft, the size and quality of the vein graft, peripheral vessel run-off and the presence of diabetes mellitus (136). However, there is no general consensus among surgeons as to whether or not the graft patency rates of this operation correlate with the above mentioned variables (133).

Finally, many ischemic extremities have been salvaged by distal grafting, to the lower calf vessels beyond the popliteal trifurcation (121, 126 - 128, 137). In such operations, as above, the reversed saphenous vein is commonly used. The patency rate of this graft is 73% initially, with a progressive decrease to about 25% at 8 - 11 years.

b) In-Situ Saphenous Vein Arterial Bypass:

The first successful use of the in-situ saphenous vein bypass was reported by Hall in 1962 (138). However, poor results led to the abandonment of this technique. In 1972, the technique was reintroduced with a great deal of success by Leather and associates (139). At present, many surgeons consider this the operation of choice for femoral-popliteal, femoral-tibial or femoral-peroneal bypass to revascularize ischemic limbs.

Essentially, this technique involves leaving the saphenous vein in its native bed, its valves rendered incompetent with a valve cutter. The advantages of this operation are: (1) improved hemodynamics, since the large end of the vein is anastomosed to the large femoral artery, and the narrow end of the vein is used at the smaller distal anastomosis (140, 141), (2) reduced endothelial damage since most of the vein is not mobilized, leaving the vasa vasorum intact to supply endothelial nutrition (142 - 147). These advantages seem to relate to superior results, with a patency rate following distal bypass of 71% at five years (148). In spite of these advantages, the in-situ technique has some inherent problems which conventional

reversed saphenous vein grafts do not. One of these is the development of communication between the saphenous vein and the deep venous system (149). These arteriovenous fistulae may be present at the time of surgery but may also develop post-operatively if previously competent valves break down and allow arterial flow to enter the low resistance deep venous system. These fistulae can steal enough flow so that distal arterial flow is severely compromised. In the patient with poor distal run-off distal graft thrombosis may occur (150, 151).

c) Sympathectomy:

There is a growing consensus that lumbar sympathectomy may be a suitable procedure for some properly selected patients. Generally, interruption of the sympathetic chain, either by surgical section or by injection of phenol, produces vasodilation, which leads to increased limb flow (152, 153). However, the proportion of this increase that goes to the skin is far greater than going to the underlying muscle. Sympathectomy may increase skin blood flow, thereby relieving ischemic rest pain and enhancing the healing of small ulcers or small areas of gangrene (154). However, this operation does not have any beneficial effect in the treatment of intermittent claudication.

Nevertheless, lumbar sympathectomy still remains a controversial operation since its introduction by Adson and Brown in 1924 (155, 156). There are conflicting results in the literature about its role in the treatment of peripheral vascular disease. For instance, three different groups, headed by

Haimovici, Szilagyi and Kim, have reported the efficacy of lumbar sympathectomy in the treatment of patients with severe ischemic limbs not amenable to direct revascularization (157 - 159). In another study, Lee demonstrated that a majority of patients with digital gangrene who were poor candidates for direct arterial revascularization, sympathectomy was effective in the salvage of limbs and toes (160). Berardi and Siroospour demonstrated that the beneficial effects of sympathectomy were decreased by factors such as rest pain, ulceration and gangrene (161).

On the other hand, Fulton and Blakely have been unsuccessful in using sympathectomy to relieve rest pain, salvage gangrenous limbs or even delay amputation in some patients (162). Bergan and Trippel reported that in their experience, ischemic limbs actually worsened, judged angiographically, following sympathectomy (163). Such conflicting results may be due to the fact that an heterogenous population of patients were involved in all the various studies mentioned above.

d) Amputation:

Amputation of the lower limb still remains part of the armamentarium of the vascular surgeon despite advances in the treatment of peripheral vascular disease. Generally, amputations become necessary when a limb is not amenable to direct revascularization due to diffuse distal blood vessel disease, the presence of intractable rest pain, ischemic necrosis of tissue or ulceration and gangrene that do not respond to treatment.

The main objectives of lower extremity amputation are to achieve primary healing of the amputation site, relief of pain, restoration of ambulatory ability and preservation of limb length. Hence, these factors must be taken into consideration when a surgeon is determining the level of amputation. In recent years, there has been a definite trend toward below-knee amputation, compared to the safer approach at the above-knee level. There is renewed interest in improving the patient's rehabilitation and quality of life by performing below-knee amputation, even though such an operation has some associated risks. Below-knee amputation has led to an increase in the number of patients who ambulate using prosthetic devices (164, 165).

The mortality rate for amputation has ranged from 20% to 40% (166 - 169), but, more recent series report decreased mortality rates, some as low as zero (17). Late mortality rates range from 25% to 50% at 2 years and 50% to 75% in 5 years (17). It should be pointed out that most of these patients tend to die from other cardiovascular problems, such as coronary artery or cerebral vascular disease.

CHAPTER II

A. HISTORICAL BACKGROUND OF ARTERIOVENOUS REVASCULARIZATION

Advanced ischemia of an extremity due to diffuse distal arterial disease is frequently not amenable to standard, currently available revascularization techniques, including bypass grafting, endarterectomy or sympathectomy. Consequently, some patients with extensive disease require amputation of the limb because of severe pain and/or gangrene.

The fact that the venous system is not affected by even the most severe atherosclerotic process has led several investigators over the years to explore the possibility of revascularizing ischemia tissue by the creation of an arteriovenous fistula. For the most part these attempts have been unsuccessful due to venous congestion or inadequate tissue perfusion (170).

Two forms of arteriovenous revascularization must be distinguished. Arteriovenous fistula consists of creating a communication between the arterial and venous system to allow flow of blood in the normal direction from distal to proximal in the vein. Arteriovenous reversal implies an arteriovenous anastomosis with ligation of the proximal venous limb such that flow is forced distally in a retrograde fashion through the venous system (the arterial pressure combined with surgical ablation causing incompetence of the venous valves).

Arteriovenous reversal, perhaps the most radical revascularization concept, is also the oldest, with the first experimental attempt taking place in 1881 and the first clinical attempt by a Spanish surgeon, San Martin y Satrustequi in 1902 (171).

Occasional clinical success resulted in several trials prior to 1920, but ultimately the poor results reported from careful clinical studies of the centrally located arteriovenous reversal procedure, such as those of Szilagyi and associates in 1951, led to the abandonment of this technique (172). More recently, experimental studies by several groups have demonstrated more favourable results in arteriovenous reversal (173 - 179).

Experimental studies by Root and Cruz demonstrated that immediate end-to-end arteriovenous reversal led to massive tissue edema (173). They utilized a canine model in which all branches of the external iliac, ipsilateral internal iliac and distal arteries to one limb were ligated. In addition, a side-to-side fistula was created at the popliteal artery level and the distal artery and proximal vein were immediately ligated. All the animals developed massive edema with loss of limb function 24 hours post-operatively. Furthermore, 40% of these dogs had to be sacrificed as a result of muscle necrosis and even though the survivors regained some use of their limbs, the fistulas eventually thrombosed (173). In another study, Matolo and associates demonstrated revascularization of turkey (obligatory

bipeds) lower extremities utilizing an immediate end-to-side arteriovenous reversal (174). In their study as well, all the animals developed massive edema and 60% lost their limbs within 3 months from venous congestion and ischemia.

This massive edema, usually associated with immediate arteriovenous reversal, was reduced by staged retrograde perfusion by Hierton in 1961 (175). In a clinical study, Hierton initiated staged retrograde venous perfusion for discrepancy in length of lower extremities. Formation of an arteriovenous fistula, later followed by ligation of the femoral vein proximal to the fistula, created a true arteriovenous reversal and avoided excessive edema (175).

A similar approach was taken by Bernstein's group who created an experimental animal model of severe limb ischemia and were able to demonstrate limb salvage without excessive edema formation (176 - 179). In one of these studies, an ischemic canine hind limb preparation was created by the ligation of the femoral artery and its branches in one leg. In addition, the terminal aorta and its pelvic branches as well as the ipsilateral 6th lumbar artery and the ipsilateral deep circumflex iliac artery were all ligated and divided. In the control group, there was uniform irreversible ischemia which eventually caused gangrene (179). In a second group, an immediate arteriovenous reversal anastomosis was created at the popliteal level in addition to the ligation of several pelvic vessels, as previously described in the control group. This preparation caused massive

edema and poor wound healing. Only one-third of these animals survived with a patent arteriovenous anastomosis; none of these survivors had normal limb function (179). Finally, in a third group of animals, a staged arteriovenous reversal anastomosis was performed. An end-to-side arteriovenous fistula was initially created at the popliteal level. Again, there was complete ipsilateral devascularization as performed in the first two groups. Ligation of the proximal popliteal vein was carried out days later. This staged arteriovenous reversal led to 100 per cent limb survival and the animals developed only mild edema. Furthermore, doppler ultrasonography and angiography provided objective evidence of excellent tissue perfusion in these limbs (179).

We felt that this concept of staged arteriovenous reversal was important to enable the limb to be functional as well as viable. We used a canine ischemic hind limb preparation to investigate the physiologic consequences of staged arteriovenous reversal. In our laboratory, we developed an animal model in which severe irreversible ischemia was created in one limb which was then revascularized by a staged arteriovenous reversal procedure at the popliteal level (Fig. 1). We were indeed successful in salvaging nine out of ten limbs (180). More important, studies using radioactive microspheres provided new insight into the mechanism by which nutrient tissue flow evolves as a result of arteriovenous revascularization. Microspheres of 15um in diameter were used, allowing them to be trapped at the capillary level. The data suggested an initial decreased flow in

all muscle groups of the arterovenous reversal leg to approximately 25 per cent of that in the control leg ($p < 0.005$). However, after 10 days, flow had increased in the arteriovenous reversal leg to levels significantly higher than on Day 1. In the lateral thigh, for example, the flow with the fistula open had increased from 26 to 55 per cent of that in the control leg. Interestingly, with the fistula closed, flow was not significantly different from that seen when the fistula was open (55 vs 49%). This would signify a high degree of flow via collateral vessels formed in response to the arteriovenous fistula. Similarly, there was a significant increase in blood flow to the distal leg (gastrocnemius and foot) in the arteriovenous reversal leg compared to the baseline flow when the fistula was open (e.g. foot 88 vs 25%). However, occlusion of the fistula resulted in a significant drop in flow (e.g. foot 88 to 45%) indicating that the major source of nutrient flow to these areas was via the reversed venous route (180). Clearly, reversal of venous flow does occur and can result in perfusion of tissue in the limb distal to the fistula. Of greater importance, however, is the formation of an intense network of new vessels in the region of the fistula which then appears to extend distally with time, as demonstrated by the post-operative angiogram in Figure 2. We were able to demonstrate this second mechanism arteriographically and quantitate its contribution to nutrient flow using radioactive microspheres.

At the same time this experimental study was completed, a patient presented to our Vascular Service who had severe unremitting rest pain without tissue necrosis and numerous attempts of conventional revascularization had been unsuccessful because of inadequate run-off. This patient adamantly refused amputation and consented to undergo the experimental procedure as it was outlined to him. He was carefully informed that the operation was indeed experimental, and that if unsuccessful, amputation would ultimately be necessary. The operative procedure itself was uncomplicated and there was no problem with excessive limb edema or cardiac failure secondary to the arteriovenous fistula. The proximal venous limb was left open in order to maximize flow through the anastomosis. A prosthetic graft extending from the femoral artery to the peroneal vein was implanted and blunt instrumentation used to attempt breakdown of venous valves distal to the anastomosis (Fig. 3). The ischemic symptoms in the foot remained unchanged for approximately 10 days and then gradually ameliorated such that by the end of about six weeks the patient no longer complained of significant rest pain. Arteriographic evaluations carried out at 4 months, at seven months and again at 12 months demonstrated extensive perfusion of the distal limb (Fig. 4). Doppler studies showed normal perfusion at rest and no significant drop in ankle pressure with exercise (181).

As a result of this preliminary success, we felt justified in setting up a clinical protocol in order to further evaluate this procedure and determine its role in the management of severe

limb ischemia. In this protocol, patients presenting to our vascular surgical service with severe rest pain with or without early dry gangrene, in whom complete evaluation, including arteriography with the use of distal delayed filming and hyperemia, fails to demonstrate adequate outflow for conventional revascularization procedures (as determined by two vascular surgeons) were offered arteriovenous revascularization. In fact, the majority of these patients have already undergone previous distal bypass which has failed as a result of inadequate outflow. Once the procedure and the investigations we wish to carry out to monitor its progress are explained to the patient and the appropriate consent form signed, the patient undergoes a femoral artery to tibial vein bypass with a distal anastomosis located in the mid-calf region. PTFE grafts have been routinely utilized as the saphenous vein is generally not available. Prior to the creation of the distal anastomosis, distal valves in the vein are rendered incompetent using instruments suitable for in-situ saphenous vein grafting. These patients are subsequently followed at regular intervals using doppler studies, arteriography and clinical evaluation.

To date, 12 patients with intractable (narcotic dependent) rest pain have been entered into the protocol. Among these 12 patients there was one operative death (8.3%) resulting from a massive myocardial infarction on the 12th post-operative day. In four patients, there was early graft occlusions (two due to inadequate inflow, two due to inadequate tibial vein). Two patients with patent grafts required amputation at two weeks and

four months because of progressive necrosis of the forefoot while a third with a patent graft had a Symes amputation that healed. In all, four limbs were salvaged (37.7%) during an average follow-up of 44 months.

Angiograms performed at four months or later demonstrate extensive vascularization of the limbs with reversed venous flow to the ankle region. The mean values for the five limbs with patent grafts are as follows:

	Pre-Op.	1 week	4 mos	12 mos	24 mos	36 mos
Ankle/ brachial index	0.25	0.29	0.53	0.62	0.65	0.82
Ankle Waveform (mm)	2.6	19	39	45	50	42

Our initial clinical experience demonstrates that limbs can be revascularized by the arteriovenous route. There is no question of dramatic improvement, documented objectively, in the patients in whom the procedure has succeeded. However, many important questions remain unanswered. For example, one great limitation in the clinical setting is the time required for improvement which varied from two to eight weeks when the procedure succeeded. Clearly, if the procedure is to have wider applicability, we must find a means to accelerate the revascularization process.

B. PURPOSE OF THE PRESENT STUDY

As discussed earlier, conventional revascularization techniques for advanced lower limb ischemia may fail in the presence of diffuse distal arterial disease. We have shown both experimentally and clinically that severely ischemic limbs may be salvaged by infrapopliteal reversal of venous flow (180, 181). In the experimental study, Graham and colleagues demonstrated both angiographically and with microsphere injections that the arteriovenous fistula was associated with a marked increase in collateralization and neovascularization (180). Clinically, Symes and colleagues have demonstrated the efficacy of femorotibial arteriovenous revascularization for the salvage of an otherwise irreversibly ischemic limb (181). In the patients' who underwent this procedure, extensive vascularization was also seen to evolve over time in the region of the fistula. These new vessels seem to be responsible for eventually increasing the circulation to the limb.

This study was undertaken to further our understanding of the neovascularization process which ensues when an arteriovenous reversal procedure is utilized to revascularize a severely ischemic limb. We hope that learning more about the physiological factors involved in this procedure may lead to the development of a more effective therapy for the salvage of many previously irreversibly ischemic limbs.

CHAPTER III

MATERIALS AND METHODS

All chemicals used were reagent grade and were purchased from commercial suppliers. Tritiated-thymidine (6.7 Ci/mole) was obtained from New England Nuclear and was measured in a Beckman Liquid Scintillation Systems, LS 1800.

A. THE DEVELOPMENT OF ISCHEMIC CANINE HIND LIMB MODEL

As indicated in the previous section on arteriovenous revascularization, we felt that the concept of staged arteriovenous reversal was important to enable the limb to be functional as well as viable. In order to investigate the physiological consequences of staged arteriovenous reversal, Graham and colleagues developed a severe ischemic hind limb model in our laboratory (180).

It is well established that dogs have extensive collaterals. For example, ligation of the superficial artery alone does not cause ischemia. Consequently, the most difficult part in the development of this model was to ensure a severely ischemic hind limb preparation. In the initial studies, three groups of mongrel dogs with different levels of unilateral ischemic hind limbs were created, by trial and error. These groups were achieved by the ligation of different collateral vessels of the pelvis and hind limb.

In group I, all the branches of the femoral artery as well as the deep femoral artery of the left hind limb were ligated. Then, an arteriovenous anastomosis (AVA) was performed at popliteal level in an end-to-side fashion. The distal end of the divided popliteal artery was ligated. A silk suture was then placed loosely around the popliteal vein proximal to the AVA and its ends were left in the subcutaneous tissue. To determine the baseline flow (i.e. severity of ischemia) in different muscle groups, radioactive microspheres (niobium-95) of diameter 15um were injected with the AVA temporarily occluded. On post-operative Day 5, the animals were reanaesthetized and the loosely placed silk suture was tied, producing arteriovenous reversal (AVR). The follow-up studies were carried out on post-operative Day 10. Once again, the dogs were reanesthetized and two further microsphere flow studies were performed using cerium-141 and chromium-51. Microspheres were injected with the AVR open and repeated with the AVR occluded. The dogs were sacrificed and the flow to paired muscle groups in the left and right limbs was calculated by the reference sample technique (182). Niobium-95 reflected baseline ischemic flow, while the cerium-141 and chromium-51 determined the total limb flow with the AVR open and the collateral flow when the AVR was occluded. In addition to the microsphere flow studies, ischemia was also assessed by microscopic examination. The data from these assessments indicated that the above surgical technique did not cause ischemia.

Consequently, a second group of animals with more collaterals ligated was investigated. In group (II), ischemia was prepared as described for group I, but in addition, the terminal aorta, the bilateral internal iliac arteries and the sacral artery were all ligated. Once again, an AVA was performed as described for group I and the baseline ischemic flow was assessed by niobium-95. On post-operative Day 5, the AVA was converted to AVR and on Day 10, the follow-up studies were performed as previously described. As in group I, the surgical technique used in group II also failed to produce severe ischemia.

Thus, a third group of dogs with additional collaterals ligated was investigated. In group III, the dogs were prepared in a manner similar to group II. In addition, the ipsilateral deep circumflex artery as well as the umbilical arteries were all ligated. An AVA was created in the usual fashion, which was later converted to AVR. Ischemia was assessed as described for groups I and II, on Day 1 and Day 10. In group III, the surgical technique caused severe ischemia as judged by microspheres and microscopic studies. If the AVA was ligated on Day 1, the limbs became totally necrotic over a period of 24-48 hours with the average time being 36 hours. Pathologic examination revealed gross and microscopic changes of acute ischemic necrosis. This finding suggested that in group III, the operated limbs were initially perfused only by the AVA and later (on post-operative Day 5) by the AVR. This third group became our severely ischemic hind limb model, which is discussed in more detail (with illustrations) below.

B. ANIMAL MODEL

Mongrel dogs weighing from 20-25 kgs were anaesthetized with sodium pentobarbital (25 mg/kg) and received pre-operative antibiotics. Through a longitudinal incision in the left groin region all branches of the femoral artery were ligated (Fig. 5b). A second incision in the left pelvic region was then used to ligate the distal aorta and all pelvic branches which potentially supply collaterals to the left limb. These include the terminal aorta, both internal iliac arteries, the sacral artery, the left deep circumflex artery, the 6th and 7th lumbar arteries and the left deep femoral artery (Fig 5a). After heparinizing the animal, an end-to-side anastomosis was carried out between the proximal end of the divided popliteal artery and the popliteal vein (Fig 5c). A silk suture was placed loosely around a proximal venous limb for subsequent ligation 5 days later (Fig.1). The incisions were then closed and the animals returned to their cages.

C. ANGIOGRAPHY

Serial conventional angiograms were performed by standard techniques on all animals on Day 10, Day 22 or Day 28 to demonstrate the extent of vascularization of the limb. The left common carotid artery was exposed through a ventral incision in the neck and the distal end of the artery was ligated. Using the Seldinger technique a 7 French multihole catheter was introduced into the exposed artery and advanced to a position about 3 cm proximal to the aortic bifurcation. A test injection of contrast agent (5-10 ml of MD-76, diatrizoate meglumine and sodium) was

observed fluoroscopically prior to filming. A total of 30 ml of MD-76 was injected at the rate of 10 ml per second with serial filming over 10 seconds on a puck changer. This was followed by semi-selective angiography in which the catheter was advanced to the external iliac artery of the operated hind limb. Fifteen milliliters of contrast agent at a rate of 5.0 ml per second was used at this site.

D. ASSESSMENT OF TISSUE PERFUSION TRANSCUTANEOUS OXIMETRY

Transcutaneous oximetry is a non-invasive and continuous method for monitoring oxygen tension, shown to be useful for the detection and assessment of peripheral vascular disease (183 - 186). Several investigators have demonstrated that transcutaneous pO_2 is a good indicator of tissue perfusion and correlates well with different grades of ischemia (184 - 188). We felt that transcutaneous oximetry would be useful in the assessment of tissue perfusion of the hind limb in our experimental animals.

In these experiments, two groups of animals (12 in each group) were used; final follow-up studies were done on post-operative day 10 or 22. Transcutaneous oximetry (Cutaneous pO_2, Monitor 820, Kontron Medical) was used to assess tissue perfusion of the limbs on day 1, 10 and 22. Each animal's right leg served as its own control. The machine was calibrated according to the specifications of the manufacturer and the heating temperature of the electrode was set at 44°C. The site to be investigated was shaved and cleaned with alcohol swabs, after

which the electrode was applied using contact gel, and secured in place by adhesive tape. The electrode was allowed to equilibrate before transcutaneous pO_2 was recorded.

E. ISCHEMIA AS A DETERMINANT OF NEOVASCULARIZATION FOLLOWING ARTERIOVENOUS REVERSAL: AN ANGIOGRAPHIC STUDY

This study was undertaken to evaluate the role of ischemia as a stimulus to neovascularization. This is an initial step in defining the exact mechanisms by which arteriovenous reversal increases limb nutrient flow. Our hypothesis was that severe tissue ischemia is necessary in order to provide the setting for neovascularization in response to the presence of an AVR.

Four groups of animals, each consisting of three mongrel dogs (20 - 25 kg), were used for these experiments. The dogs were fasted overnight and anaesthetized with intravenous sodium pentobarbital (Somnotol), 25 mg/kg body weight. Ventilation through an endotracheal tube was provided by a Bird Mark 8 respirator. All dogs received pre-operative antibiotics and were maintained on this medication for four days.

I. Group I: Non-Ischemic:

A longitudinal incision was made in the medial thigh of the left leg. Following the administration of 2000 units of heparin, a side-to-side arteriovenous anastomosis was then performed at the popliteal level without ligation of the distal artery, as shown diagramatically in (Figure 6). The incision was closed and

the animals returned to their cages. After four weeks, the dogs were reanaesthetized and angiograms were obtained. This group served as the control since the arterial inflow was not interrupted.

II. Group II: Distal Ischemia:

An anastomosis was carried out as before except that the distal artery was ligated. Thus, a functional end-to-side arteriovenous anastomosis (AVA) was created, (Figure 7). The pelvic vessels, deep femoral and all branches of the superficial femoral were left intact so as to determine their contribution to limb vascularity. A silk suture was placed loosely around the popliteal vein proximal to the AVA and its ends were left in the subcutaneous tissues as the incision was closed.

The dogs were returned to their cages and on post-op day 5, the loosely placed silk suture was tied, producing arteriovenous reversal (AVR), (Figure 7). Follow-up angiograms were performed after four weeks.

III. Group III: Partial Ischemia:

Pre-operatively, the dogs were treated as described for the previous two groups. An arteriovenous anastomosis was carried out as described for Group II (Figure 7). In addition, all branches of the superficial femoral artery were ligated, leaving only the deep femoral artery intact. Furthermore, a retroperitoneal approach was used to expose the terminal aorta

and its branches. The terminal aorta, both internal iliac arteries, the sacral artery, left deep circumflex artery, and the left 6th and 7th lumbar arteries were all ligated (Figure 8). The incisions were then closed and the animals returned to their cages. On post-op Day 5, the AVA was converted to AVR. After four weeks, follow-up angiograms were obtained.

IV. Group IV: Severe Ischemia:

An arteriovenous anastomosis was performed as previously described for Groups II and III. In addition, all possible sources of collateral flow to the left limb including the deep femoral artery were ligated (Figure 9). Thus, the left limb of these animals was initially perfused only by the AVA and later (on post-op Day 5) by the AVR. Again, angiograms were obtained after four weeks.

E. QUANTIFICATION OF NEOVASCULARITY BY CAPILLARY DENSITY DETERMINATION

The presence of alkaline phosphatase activity in the walls of blood vessels was discovered independently by Gomori and by Takamatsu (189 - 191). These investigators developed the first histochemical technique for the demonstration of alkaline phosphatase in the endothelium of capillaries and of small blood vessels.

More recently, these techniques have been used to determine capillary density (cap/mm^2) in experimental animals which have

been subjected to various stimuli, such as nerve stimulation and exercise (192 - 195). These studies have demonstrated capillary growth and an absolute increase in capillary number in both skeletal and cardiac muscles. Furthermore, it has been shown that this increase can be distinguished from the dilatation of pre-existing capillaries.

The experiments under this section were performed to investigate the feasibility of using capillary density measurements to precisely quantify the neovascularization process in the hind limbs of our experimental animals. Two groups of animals, each consisting of 6 mongrel dogs, were used for this set of experiments. The groups differed in terms of the post-operative day (10 or 22) on which the final follow-up studies were undertaken. A modified method of Romanul was utilized in this study (196). Briefly, cannulas were placed in the abdominal aorta and inferior vena cava of the dogs on post-operative days 10 or 22. The animal was sacrificed and the hind limbs perfused with 3L of warm ($37^{o}C$) 0.2% sodium nitrite in normal saline followed by 2L of normal saline and 2L of filtered azo dye alkaline phosphatase incubating medium (20 mg sodium - naphthyl phosphate and 20 mg 0-dianisidine, in 0.1M stock "Tris" buffer (pH10); final pH of mixture, 9.5). After 1 hour, the vascular tree was rinsed with 2L of normal saline and tissue samples were removed from every muscle in the hind limbs. These samples were then frozen in isopentane (pre-cooled by dry ice) and sectioned at a thickness of 10 microns using a cryostat. The sections were

counter-stained in Mayer's Haemalum and mounted in glycerine jelly as described by the histochemical technique of Pearse (197). Finally, capillary density (cap/mm^2) was determined microscopically.

G. DETERMINATION OF NEW VESSEL FORMATION USING TRITIATED-THYMIDINE UPTAKE AND AUTORADIOGRAPHY

Tritiated-Thymidine uptake is an established and reliable method for measuring cell proliferation (198). Several studies have demonstrated that neovascularization is associated with proliferation of vascular endothelial cells and that there is a positive correlation between proliferation and the uptake of tritiated-thymidine (199, 200). In some of these studies, the presence of tritiated-thymidine labelled endothelial nuclei was confirmed by autoradiography (201).

Our previous studies have demonstrated angiographically that the arteriovenous fistula was associated with an intense network of vessels in the region of and distal to the fistula. We hypothetized that it was possible to use tritiated-thymidine uptake to determine if angiographically visualized vessels are simply previously dormant channels which are opened up or newly proliferating vessels. Once again two groups of animals (6 in each group) were used in these experiments. The animals were sacrificed on post-operative day 10 or 22 for the follow-up studies. Tritiated-thymidine (^{3}H-TdR) was administered intravenously as a single pulse (0.5 uCi/g of body weight) on the

tenth or on the twenty-second post-operative day. The ^{3}H-TdR was allowed to circulate for 3 hours and then cannulas were placed in the abdominal aorta and inferior vena cava (3 cm proximal to the bifurcation). The animal was sacrificed and the hind limbs perfused with 1.5L of heparinized saline to remove as much free radioactivity as possible.

I. Tissue Radioactivity Determination:

Three samples from every muscle in both hind limbs were taken. Aliquots of muscle (wet tissue weight, approximately 500 mg) were transferred to glass counting vials. Two millilitres of 0.5 M protosol (tissue solubilizer) were added to each sample vial. The vials were then placed in a 55^{o}C water bath until tissue dissolution occurred. The digests were decolorized by the addition of 20% benzoyl peroxide in toluene followed by an additional 30 minutes of incubation.

After cooling to room temperature, 0.5 ml aliquots of the digests were transferred to counting vials and 10 mls of scintillation solution (econofluor) were added.

Radioactivity was measured using a liquid scintillation counter (Beckman Beta Spectrometer Systems, LS 1800), and results were expressed as microcuries per gram of wet tissue.

II. Autoradiography:

Representative tissue samples were fixed in 10% buffered formalin, embedded in paraffin and cut at a thickness of 4 microns. The sections were then deparaffinized and autoradiographs prepared by standard techniques (202). Briefly, the slides were exposed for 14 days at 4°C, developed and then fixed with hematoxylin. The presence and location, in relation to blood vessels, of tritiated-thymidine labelled endothelial nuclei was confirmed microscopically.

H. STIMULATION OF NEOVASCULARIZATION

In order to determine whether or not certain interventions might stimulate the neovascularization process both to progress further distally and to occur more rapidly, three groups of animals were randomized as discussed below.

Group I: Control:

The arteriovenous revascularization procedure was carried out as above, without attempt being made to enhance the process of neovascularization. Tissue perfusion was determined by transcutaneous pO_2 and neovascularization was assessed by capillary density determination (as described in Materials and Methods, Sections D and F, respectively) on the tenth or twenty-second post-operative day.

I. Group II: Muscle Stimulation:

In this group, the animals were trained to run on a moving belt exercise machine (treadmill) (Fig 10 a,b,c). The slope of the treadmill was 20^{o} and the speed was 10 km/hr. The arteriovenous revascularization procedure was performed after the animals had become well accustomed to running on the treadmill. From the third post-operative day, the animals were subjected to 3 daily periods (30 min. each) of controlled exercise (running) on the treadmill. Finally, neovascularization was assessed as described for Group I (control).

II. Group III: Lipid Angiogenic Factor:

During the first decade of this century, various investigators demonstrated that the omentum can be effective in supplying blood to ischemic areas (203 - 205). Subsequent studies have shown that the pedicled omentum is capable of providing collateral circulation to the ischemic heart (206, 207).

More recently, Goldsmith and colleagues have showed that revascularization occurred at an omental-cerebral and omental-spinal cord interface, resulting in an extensive new source of blood for the brain and spinal cord, despite the absence of ischemia in these organs (208 - 213). Subsequent work by Goldsmith's group led to the discovery of a potent angiogenic factor in the lipid fraction obtained from the omentum after extraction in a chloroform-methanol solvent mixture (95).

Furthermore, they have demonstrated that administration of this omental lipid fraction can cause increased vascular perfusion, as assessed by in vivo nuclear imaging techniques using tagged erythrocytes labelled with technetium - 99m (214).

We investigated the feasibility of using this lipid angiogenic factor to accelerate the neovascularization process resulting from our surgical technique. Each operated dog received a 5 ml daily injection of lipid angiogenic factor, for a period of 10 days. The injection was given intramuscularly in alternating hind limbs. Thus, each dog received a total of 50 ml over the ten day period. Finally, tissue perfusion and neovascularization was assessed as previously described.

CHAPTER IV

RESULTS

Clinical assessment of the animals in the various groups demonstrated the limbs to be functional with mild edema, which subsided within an average of six days. Furthermore, no gross evidence of tissue necrosis was seen.

A. Angiography:

Representative Angiograms from Studies under Materials and Methods Sections D, F, G and H:

The angiograms were satisfactory and consistent in all the various groups of animals. The angiograms performed on the tenth post-operative day revealed perfusion of the operative limbs by the arteriovenous fistula and reversed flow in the major veins to the mid-calf. Contrast opacified an intense vascular network at the level of the fistula, with subsequent filling of deep veins and drainage from the pelvis (Fig. 11).

Similarly, the angiograms performed on the 22nd post-operative day demonstrated more extensive perfusion of the operated limbs via the arteriovenous fistula. The angiograms revealed reversed venous flow distally to the foot, compared to Day 10. These angiograms showed more numerous and larger vessels spreading out from the region of the fistula, extending medially and laterally into the thigh as well as distally to the mid-calf. Finally, the deep veins fill from this dense network and return blood to the pelvis (Fig. 12).

B. Assessment of Tissue Perfusion:

Transcutaneous Oximetry:

The experiments in this section monitored tissue oxygenation following arteriovenous reversal. In these animals no attempt was made to enhance neovascularization. These experiments were performed as described under Materials and Methods (Section D).

The results represent an average of the 24 dogs studied. On Day 1, the values for transcutaneous pO_2 at the thigh (57 $\pm$ 5 mmHg) and calf (12 $\pm$ 2 mmHg) were significantly lower ($p < 0.01$) in the operated limbs compared to control limbs (77 $\pm$ 4 mmHg and 80 $\pm$ 5 mmHg, respectively). At Day 10, the transcutaneous pO_2 of the thigh (67 $\pm$ 6 mmHg) and calf (47 $\pm$ 1 mmHg), still remained significantly lower. On Day 22, transcutaneous pO_2 of both the thigh and calf had increased in the operated limb to level equal to their control counterparts (79 $\pm$ 2 mmHg vs 81 $\pm$ 2 mmHg and 78 $\pm$ 2 mmHg vs 76 $\pm$ 2 mmHg, respectively) (Table 1, Fig. 13).

C. The Role of Ischemia as a Stimulus in Neovascularization:

Satisfactory angiograms were obtained in all twelve animals. Consistent angiographic findings were evident in each group.

Group I: Non-Ischemia:

This group was designed to investigate the role of the arteriovenous fistula itself in neovascularization. The angiograms showed the development of arterial and venous collateral channels at the level of the fistula (Fig. 14). Furthermore, there was a high density of contrast medium in the main arteries as well as collaterals; contrast passed rapidly through the accompanying veins and venous collaterals.

Group II: Distal Ischemia:

The angiograms of this group showed less dilatation and enlargement of collaterals at the level of the fistula (Fig. 15). However, there was good flow of contrast medium through the arteriovenous reversal and retrograde into the distal venous system. In addition, the angiograms indicated that there was dilatation of the vein distal to the fistula. The deep femoral artery provided collaterals to the distal popliteal and tibial vessels.

Group III: Partial Ischemia:

As in Group II, the operated limbs were perfused by the deep femoral artery and the arteriovenous fistula. The angiograms indicated dilatation and enlargement of the native vessels as

well as distention of the vein distal to the fistula (Fig. 16). There was rapid retrograde flow in the veins to the level of the ankle and flow of contrast through some branches of the main vein, distal to the fistula.

Group IV: Severe Ischemia:

In this group, the operated limbs were perfused by the arteriovenous fistula, and after day 5, by the arterovenous reversal only. The most striking angiographic finding was neovascularization. These angiograms showed intense formation of knobbly vessels which were not seen in any of the previous three groups (Fig. 17a). Furthermore, with delayed exposures, contrast medium flowed from the main artery, through the arteriovenous reversal to the main distal vein, then filled an intense vascular network which spread out from the anastomotic area, extending medially and laterally into the thigh and distally to the mid-calf (Fig. 17 b, c). Finally, the deep femoral veins drain from this network, returning blood to the pelvis (Fig. 17c).

D. Quantification of Neovascularity by Capillary Density Determination:

These experiments were performed to determine new capillary formation. The experiments were carried out as described under Materials and Methods (section F).

Cross-sections of leg muscles stained for alkaline phosphatase activity permitted a detailed study of capillaries. The capillaries appeared in cross section as dark spots when stained for alkaline phosphatase which indicated endothelial cells. It should be mentioned that the nuclei of endothelial cells were not stained. As shown in Fig. 18, the section of muscle from the operated hind limb indicated higher capillary density compared to the contralateral muscle from the unoperated control hind limb. In addition, the distribution of the capillaries was homogenous, especially in the operated hind limb.

The capillary density count was determined microscopically from cross sections of muscles stained for alkaline phosphatase activity, as previously described. Examples of such sections are shown in Fig. 18. The results represent an average of 12 dogs (two experimental groups, each consisting of six dogs). The muscles from the operated hind limbs showed a substantial increase in capillary density as compared to the contralateral normal muscle (Fig. 18). In the medial thigh, after ten days, the capillary density in the operated hind limb was significantly greater than that in the control (876 ± 38 vs 598 ± 42 cap/mm^2, $p = 0.002$). Similar results were obtained in the muscles from

the foot (823 ± 52 vs 627 ± 34 cap/mm^2, p = 0.0007) (Table 2, Fig. 19). After 22 days, more capillaries per square millimeter were found in the operated hind limb. Once again, in the medial thigh, the values were 987 ± 56 vs 618 ± 30 cap/mm^2 (p = 0.0007) for the operated and control hind limbs, respectively. Similarly, in the calf, the values were 1011 ± 46 vs 613 ± 24 cap/mm^2 (p = 0.0004) (Table 3, Fig. 19). The differences between the two operated hind limbs at day 10 and day 22 were not statistically significant.

E. Determination of New Vessel Formation:

Tritiated-Thymidine Uptake:

The experiments were performed as described in Materials and Methods (Section G). Average results from 12 dogs are again presented (two experimental groups, each consisting of six dogs). As shown in Table 4 and Fig. 20, a progressive and significant increase in tritiated thymidine uptake by endothelial cells in the thigh and calf regions was demonstrated by Day 10 in the operated hind limbs as compared to the control. The mean values in the medial thigh were .30 ± .01 vs .25 ± .01 uCi/g of muscle wet weight ($p < 0.005$) and in the calf .31 ± .01 vs .27 ± .01 uCi/g ($p < 0.05$. A further increase, particularly in the calf, was seen at Day 22 (.36 ± .01 vs .27 ± .01 uCi/g ($p < 0.005$) (Table 4, Fig. 20).

Autoradiography revealed extensive labelling of endothelial cell nuclei in the operated hind limb which was more than 95%

of the total labelled nuclei (Fig. 21). In contrast, less than 5% of the total labelled nuclei in the control hind limb were endothelial cell nuclei.

STIMULATION OF NEOVASCULARIZATION:

This series of experiments was carried out to determine whether the rate of neovascularization in our ischemic limb preparation can be enhanced by either controlled exercise (running on a treadmill) or the use of lipid angiogenic factor. Once again, the experiments were performed as described under Materials and Methods (Section H).

F. Effect of Muscle Stimulation by Exercise:

a) Transcutaneous Oximetry:

The results are an average of four dogs. After controlled exercise, transcutaneous pO_2 measurements increased an average of 16% in the operated limbs compared to operated hind limbs without exercise (Table 5). In the controlled exercise animals, the thigh of the operated hind limbs showed an increase of 6% whereas that of the calf was 26%. The average increase of the control or nonoperated hind limbs of the excised animals was only 10%.

b) Capillary Density:

The results showed moderate increase in capillary density as a result of controlled exercise. In the operated hind limbs, there was a 20% increase in capillary density in the animals that underwent controlled exercise, compared to the operated hind limbs without exercise (Table 6, Fig. 22). Similarly, there was

24% increase in the foot of the operated and exercise animals (Table 6, Fig. 22). The data also showed an average increase of 12% in the unoperated hind limbs of the exercised animals.

G. Effect of Lipid Angiogenic Factor:

Note that both hind limbs of the treated animals received equal amounts of lipid angiogenic factor. Two dogs received this treatment.

a) Transcutaneous Oximetry:

The mean values of transcutaneous pO_2 showed an increase of 27% in the operated hind limb of the treated animals compared to an untreated but operated hind limbs, after ten days (Table 7). In the thigh, the increase was 15% and that in the calf was 38%. However, there was a slight increase of 6% in the unoperated hind limb of the treated animals when compared to its counterparts of the untreated animals.

b) Capillary Density:

The data are shown in Table 8 and Fig. 23. It can be seen that there was a tremendous increase in capillary density as a result of the lipid angiogenic factor treatment. This increase was apparent even after 10 days of treatment. In the medial thigh, there was a 61% increase in capillary density in the operated hind limbs of the treated animals, compared to the untreated operated hind limbs. Similarly, there was 66% increase

in the foot of the operated and treated animals. The results also indicated that there was an average increase of 13% in capillary density in the unoperated hind limbs of the treated animals.

CHAPTER V

DISCUSSION

A. Angiography:

We used angiography to demonstrate the extent of vascularization of the limb in our experimental animals. The angiograms performed on the AVR dogs revealed an intense network of vessels in the region of the arteriovenous fistula. This massive network of vessels increases over time and is much greater at day 22 than day ten. This suggests that the formation of this network does not result from an increase in venous congestion, since a more immediate effect would be expected. However, this network of vessels seen angiographically is merely an anatomic rather than a functional assessment. In the present study, there is no doubt that the increase in the density of vessels leads to improved tissue perfusion of an ischemic hind limb and that these limbs are viable clinically with increased distal oxygenation (confirmed by transcutaneous pO_2). This network of vessels may be similar to collateral vessels developing around a chronic vascular occlusion in an ischemic limb, allowing patients to maintain or actually improve the perfusion of the limb.

B. Transcutaneous Oximetry:

As noted earlier, transcutaneous oximetry is a non-invasive, continuous, quantitative method for monitoring oxygen tension in the skin. A number of investigators have demonstrated the usefulness of this technique in the assessment of peripheral vascular disease (183 - 186). In addition, transcutaneous pO_2 is a good indicator of tissue perfusion and correlates well with

different grades of ischemic (184 - 188). In one study, Ohgi and associates used transcutaneous oximetry to evaluate the ischemia in patients with peripheral vascular disease (184). These investigators demonstrated that the mean pretibial transcutaneous oxygen tension values correlate well with the grade of Fontaine's classification of clinical severity of ischemic legs. There was no insufficiency of the pretibial skin circulation, as measured by transcutaneous oxygen tension values in Grade 1. However, in Grades 2, 3 and 4, there were significant decreases of pretibial skin transcutaneous oxygen tension (184). Hauser and Shoemaker used a transcutaneous oxygen tension regional index to assess tissue perfusion in peripheral vascular disease (187). They defined this index as the ratio of extremity to chest transcutaneous oxygen tension. This index avoids the effects of changes in systemic oxygen delivery upon local transcutaneous oxygen tension values (187). This index was unchanged by exercise in normal subjects whereas patients with intermittent claudication showed a large decrease when exercised until symptomatic. In addition, these investigators successfully used this transcutaneous regional perfusion index to quantify limb ischemia and evaluate clinical progression of peripheral vascular disease (187).

In our investigations, we felt that mild edema of the operated limbs might limit the reliability of transcutaneous oximetry resulting in values that are artifically low. However, this was not a significant problem, since the edema usually

subsided within one week. In this study, transcutaneous oximetry demonstrated a steady and significant improvement in the operated hind limbs, over time. There was progressive increase in transcutaneous oxygen tension values at the thigh and calf levels, between the tenth and twenty-second post-operative days. In fact by the twenty-second post-operative day, there was no difference between the values of the control and operated hind limbs. The rise in transcutaneous oxygen tension paralleled the progressive increase in the vessels as revealed angiographically. The results obtained in our studies are unique. We have demonstrated extremely low tissue perfusion after our surgical procedure. By the twenty-second post-operative day, the operated hind limb transcutaneous oxygen tension values were as good as those of control. To our knowledge, no one outside our laboratory has previously demonstrated this finding.

C. The Role of Ischemia as a Stimulus in Neovascularization:

This study examined the impact of an arteriovenous fistula on vascular formation as judged angiographically. The outcome, however, depends on the nutritive blood supply to the limb. In the absence of ischemia, new vessels become evident but these are centered about the fistula and almost certainly serve as secondary conduits to allow increased venous return to the heart. Their formation is not dependent on ischemia and they are not likely to be nutrient vessels. Creation of an arteriovenous reversal (Groups 2-4) results in retrograde venous flow but in the absence of ischemia little or no neovascularization occurs.

With severe ischemia (Group 4), however, a unique angiographic picture is seen. In our previous study, using microspheres, this neovascular network was shown to be involved in tissue nutrition (180). Morphologically, these vessels are easily distinguished from the normal arterial-to-arterial collaterals seen around chronic arterial occlusive lesions. Furthermore, in the present study, ischemia was shown to be a major determinant of this neovascularization process following arteriovenous reversal.

Significant hyperemia develops in response to an arteriovenous fistula (88, 89). This is believed to occur principally as a result of increased velocity of blood flow (90, 91). An increase in the rate of flow causes shear stress of the vessel wall and this leads to passive dilatation and enlargement of existing collaterals by active growth (90, 91). In contrast, a major factor to neovascularization may be an unidentified biochemical stimulus. Angiogenesis has previously been ascribed to such things as a transferable factor promoting vascular growth, tumor angiogenic factor, angiogenesis factor from human myocardial infarcts, lipid angiogenic factor, and angiogenin (92-96). In an ischemic environment, these biochemical factors may be released and are somehow capable of producing neovascularization (97).

Our data from Group I animals supports the generally established fact than an arteriovenous fistula leads to the development of collaterals. In comparison, the results of Groups II and III show less collateral development. However, we see

enlargement of alternate blood vessels which may be caused by the different levels of ischemia associated with these two groups. Finally, in the severely ischemic limbs in Group IV, the results demonstrated an intense neovascularization at the level of the arteriovenous fistula as well as extending distally. This finding correlates with other published studies on the role of ischemia in neovascularization (91, 97, 215, 216). Schaper, in 1971, created myocardial ischemia by implanting ameroid constrictors around the circumflex branch of the left coronary artery of mongrel dogs. This procedure initiated vascular growth, demonstrated by the incorporation of tritiated thymidine by endothelial cells (91). Furthermore, various ophthalmological studies have shown a direct association between retinal ischemia and neovascularization (97, 215, 216).

D. Neovascularity by Capillary Density Determination:

This study assessed capillary formation in response to staged arteriovenous reversal. The major finding is a consistent and substantial increase in capillary density as a result of our surgical procedure.

As earlier noted in the Materials and Methods section, capillary density of the muscle of our experimental animals was determined by staining for alkaline phosphatase activity, indicating endothelial cells. Our primary concern was to quantify the total number of capillaries per cross-sectional area of muscle fibers. A major problem with this technique might have been muscle atrophy, which would increase capillary density due

to a decrease in the cross-sectional area of the fibers. Fortunately, this was not the case since there was no evidence of muscle shrinkage or atrophy in any of the operated hind limbs. Consequently, any increase in capillary density in the operated hind limb would imply capillary neoformation.

In our investigations, capillary density was significantly increased in the operated hind limb muscles by day ten and increased further by 22 days, compared with the control values. This suggests progressive increases in the total number of capillaries as a result of the growth of new capillaries. This observation agrees with previous investigators who demonstrated capillary growth as well as an absolute increase in capillary number in both skeletal and cardiac muscle, as a response to various stimuli (for example, nerve stimulation and exercise) (192 - 195, 217). Myrhage and Hudlicka demonstrated capillary growth in chronically stimulated skeletal muscle by intravital microscopy and histochemical techniques (193). In their studies, animal tenuissimus and extensor hallucis proprius muscles were stimulated electrically at 10 Hertz, eight hours a day for one to two weeks. These stimulations caused increased capillary density in the stimulated muscles but not in control or sham operated muscles. In addition, they actually observed capillary neoformation, as sprouts budding from the wall of existing capillaries by an intravital microscope connected to a closed-circuit TV system (193).

The molecular events that cause capillary neoformation are unclear. It has been suggested that hemodynamic (mechanical) and/or angiogenic factors may be involved in capillary neoformation. Based upon available evidence, this process procedes in the following manner. First, there is an immediate response, wherein pre-existing capillaries enlarge by vasodilatation and cell division in the presence of the appropriate stimuli (92). Capillary growth and sprouting follows later. This second event represents capillary neoformation.

In our experimental set-up, it seems that the increase in capillary density was due to capillary neoformation, since there was an absolute increase in the total number of capillaries per square millimeter.

E. Tritiated-Thymidine and Autoradiography:

This study examined the effects of reversed blood flow on vascular proliferation in ischemic canine hind limbs. Our investigations indicate increasing uptake of tritiated thymidine by the vascular endothelial cells, confirmed by autoradiography. Thus, there is evidence of vascular proliferation as a result of our surgical procedure.

Normal adult vascular endothelium has an extremely slow turnover rate, rarely dividing, so mitoses are infrequently encountered. Tritiated thymidine is taken up by replicating DNA

in endothelial cells. Neovascularization is associated with proliferation of vascular endothelial cells; there is a positive correlation between proliferation and the uptake of tritiated thymidine (199 - 201). Folkman and associates have previously demonstrated with autoradiography that tritiated thymidine was incorporated into regenerating endothelial cells (201). In their study, tumor cells or tumor angiogenic factor was injected into the subcutaneous tissue of rats. This was followed by the injection of tritiated thymidine as single pulse, at different intervals and tissues were examined by autoradiography and electron microscopy. This revealed tritiated thymidine labelling in the endothelial cells as early as six hours in the rats that received tumor cells; there was more synthesis of DNA as well as evidence of capillary neoformation within 48 hours. Similarly, there was evidence of capillary neoformation and endothelial cell DNA synthesis after 48 hours in the rats that were injected with tumor angiogenic factor (201).

A major limitation of tritiated thymidine uptake is its inability to differentiate between new endothelial and expansion of existing endothelial cells. The proliferation of endothelium results in the widening of pre-existing capillaries and/or capillary neoformation (92). A number of studies have demonstrated a close relationship between cellular proliferation and capillary neoformation (218 - 220). Mandache and associates have demonstrated proliferation of heart tissues in various forms of experimental cardiac hypertrophy (218). These workers, produced cardiac hypertrophy in rats by swimming exercise or by the creation of aortic stenosis. Then, at different intervals,

tritiated thymidine was administered intraperitoneally and the hearts were removed and examined by electron microscope autoradiography. This revealed labelled endothelial cells in the capillaries of the hearts from exercised rats. In contrast, there was no evidence of labelled cells in the hearts of rats with aortic stenosis and only two labelled endothelial cells were found in the heart of normal rat (218).

In our studies we believe that tritiated thymidine uptake was due to capillary neoformation since the tritiated thymidine was given as a single pulse on the tenth or twenty-second post-operative day. Any opening of pre-existing capillaries should have taken place at the time of tritiated thymidine administration.

F. Stimulation of Neovascularization:

Controlled Exercise (Treadmill):

We examined the effect of controlled exercise (running on a treadmill) as a stimulus to neovascularization in our experimental animals. The results demonstrate that controlled exercise can moderately enhance tissue perfusion and cause neovascularization to occur more rapidly and more distally.

By the tenth post-operative day, the transcutaneous oxygen tension level of the operated hind limb of the exercised animals was increased by an average of 16% when compared to operated hind limbs of unexercised animals. Similarly, the capillary density was increased (18%) in the operated hind limb of exercised animals. However, there was also a moderate increase in capillary density (12%) in the unoperated hind limbs of the exercised animals. These observations are in agreement with previous findings that neovascularization is stimulated by exercise (217, 221, 222). In one study, Anverse and associates used morphometric techniques to study the effect of exercise on the capillary vasculature of the rat heart (217). These investigators demonstrated that moderate treadmill running by rats produced an increase in the numerical density, luminal surface and total length of capillaries in the myocardium (217).

It is believed that both hemodynamic and angiogenic factors may be responsible for neovascularization in exercised muscle (195). The hemodynamic stimulus is believed to be the increased capillary blood flow which occurs during exercise. The angiogenic stimulus may be the various metabolites such as adenosine diphosphate and lactic acid, which are released from muscles during exercise; these have been shown to cause neovascularization (223, 224).

G. Stimulation of Neovascularization:

Lipid Angiogenic Factor:

In this preliminary study, we investigated the role of lipid angiogenic factor as a stimulant to neovascularization. Our data suggests that lipid angiogenic factor can enhance tissue perfusion and neovascularization in our ischemic hind limb preparation after arteriovenous reversal. Goldsmith and his group have studied the effect of omental lipid fraction injected into the corneas of rabbits resulting in rapid angiogenesis, determined microscopically. Subsequently, they showed that omental lipid fraction causes increased vascular perfusion to the area of a standardized wound in the limb of cats (214). This standard wound was, created by removing a segment of the femoral artery and ligating its divided ends. This was then followed by the intramuscular injection of the omental lipid fraction into the wound or at a distant site (214). This administration of the omental lipid fraction caused increased vascular perfusion as assessed by *in vivo* nuclear imaging techniques using tagged erythrocytes labelled with technetium-99 m (214). The angiogenic factor (s) present in the omental lipid fraction is yet to be identified and purified to chemical homogeneity. It is generally believed that these angiogenic molecules may be prostaglandins (E_1 and E_2 series); a number of studies have shown that such prostaglandins are angiogenic (225 - 227). Benezra studied the ability of prostaglandins (E_1, E_2, D_2, A_1 and Fa_1), fibroblast or epidural growth factors and synthetic chemoattractants to induce

the proliferation of new blood vessels in the rabbit cornea (225). Prostaglandin E_1 was shown to have the strongest ocular neovascularization effect, whereas prostaglandin E_2 had a weaker effect. In contrast, prostaglandins D_2, A_1, Fa_1 as well as growth factors and synthetic chemoattractants had no effect (225).

Our results indicate the operated hind limbs showed a rapid increase in nutritive tissue perfusion when measured by transcutaneous oxygen tension. This increased tissue perfusion is probably the result of an increased and rapid development of new capillaries. These findings are very encouraging, since they indicate the potential of using in vivo administration of an angiogenic factor to enhance neovascularization in ischemic limbs. To our knowledge, there is no published report that has demonstrated augmentation of tissue perfusion as well as capillary neoformation, quantitatively, in the presence of limb ischemia, by lipid angiogenic factor. Although these preliminary results are very suggestive, further study is planned to confirm these findings.

CHAPTER VI

SUMMARY

In the present study, we have investigated the physiological mechanisms by which new vessels develop following arteriovenous reversal. The major findings from these investigations are as follows:

1. Nutrient flow is provided to the ischemic hind limb by arteriovenous reversal as indicated by increasing values of transcutaneous oxygen.

2. Severe ischemia acts as a stimulant to neovascularization in the canine hind limb, following arteriovenous reversal, as determined angiographically.

3. There is increased capillary growth in the presence of severe ischemia and arteriovenous reversals as shown by capillary density values. This capillary neoformation may be enhanced to progress further distally and more rapidly by either intramuscular administration of lipid angiogenic factor or controlled exercise (treadmill). The enhancement caused by lipid angiogenic factor appears to be greater than that of controlled exercise (57 vs 18%).

4. As a result of our surgical procedure, there is a new endothelial cell formation as indicated by increasing tritiated thymidine uptake.

The slow but progressive increase in the cutaneous oxygen levels parallels the increase in tritiated thymidine uptake and in capillary density. Additionally, the development of a neovascular network around and distal to the arteriovenous reversal seen angiographically also paralells these studies. Clearly, it is this capillary network which ultimately perfuses the limb rather than simply reversed venous flow which for the most part is ultimately shunted back to the venous system. This data distinguishes the success of our experience from the failures of others. Finally, we hope that the knowledge from our studies on new blood vessel formation can be used to develop more effective therapies for the salvage of ischemic limbs, especially when conventional revascularization techniques are not feasible.

CHAPTER VII

CLAIMS TO ORIGINALITY

In addition to all the points raised in the summary, the author lays claim to the following observations: In the presence of severe ischemia, arteriovenous reversal provides nutritive tissue perfusion which results from the development of an intense network of new vessels in response to the procedure. The development of these new vessels can be enhanced by in vivo administration of lipid angiogenic factor or controlled exercise. These observations are viewed by the author as being original contributions to scientific knowledge.

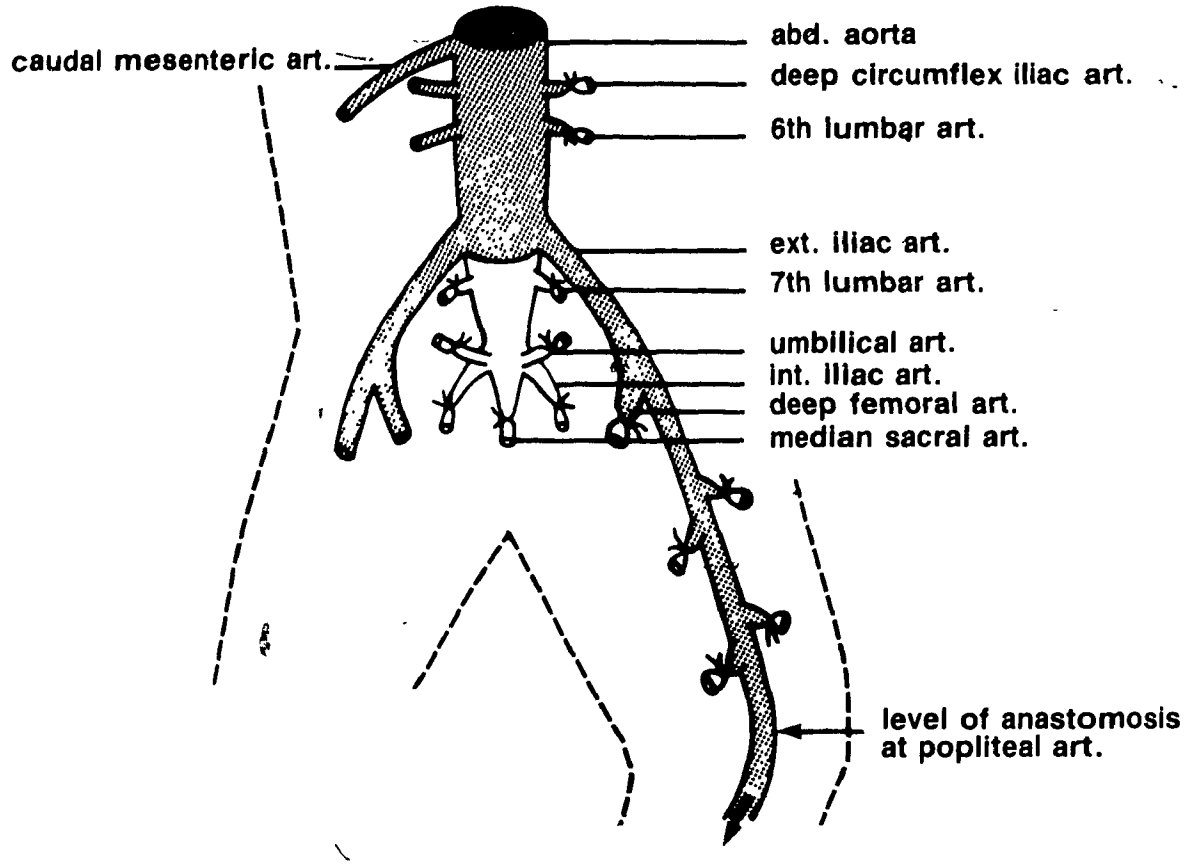
Portions of these studies described herein have appeared in the following original articles:

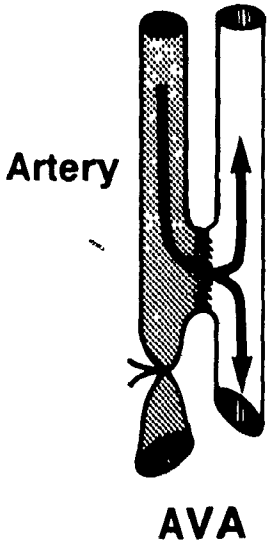
1. Graham AM, Baffour R, Burdon T, Sniderman A, Symes JF: Demonstration of neovascularization in response to arteriovenous reversal in ischemic canine limb. Surg Forum XXXVI: 458, 1986.

2. Baffour R, Danylewick R, Burdon T, Sniderman A, Common A, Graham AM, Symes JF: An angiographic study of ischemia as a determinant of neovascularization in arteriovenous reversal. Surgery, Gynecology and Obstetrics 166:28-32, 1988.

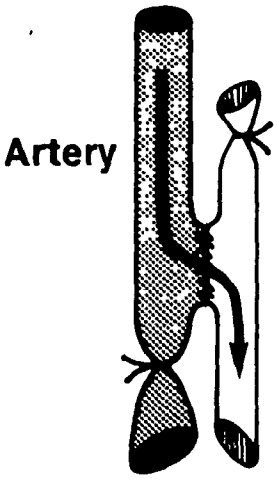
Figure 1

CANINE ISCHEMIC LIMB PREPARATION





AVA



AVR

Artériovenous anastomosis (AVA) which is subsequently converted to an arteriovenous reversal (AVR)

Figure 2

Representative angiogram of ischemic limb, demonstrating intense new vessel formation around and distal to anastomosis (arrow).

Figure 3

Intraoperative arteriogram demonstrates patent polytetrafluoroethylene (PTFE) graft to peroneal vein anastomosis and reversed flow in vein distally.

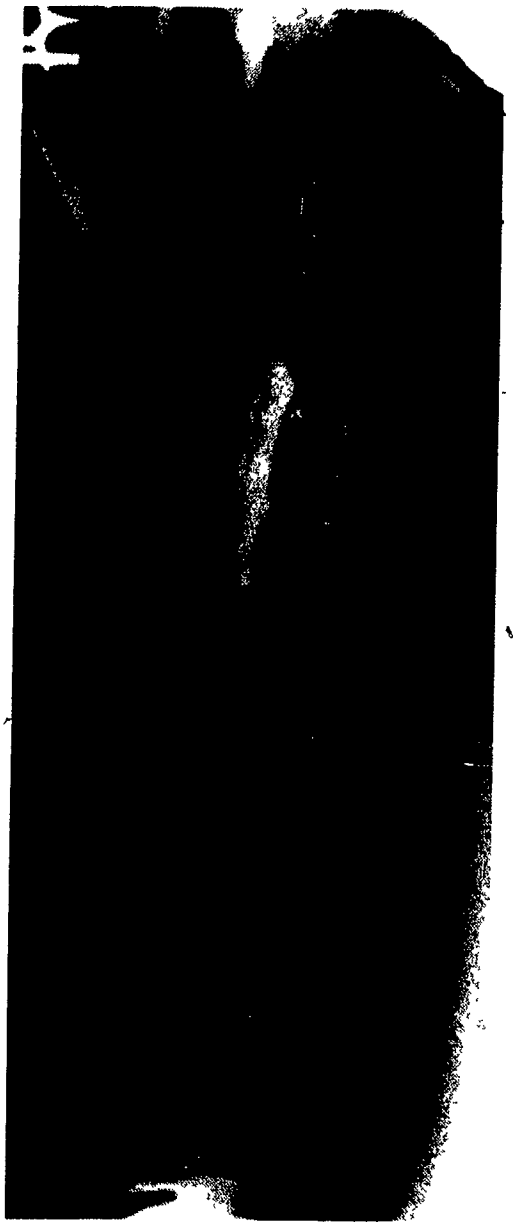
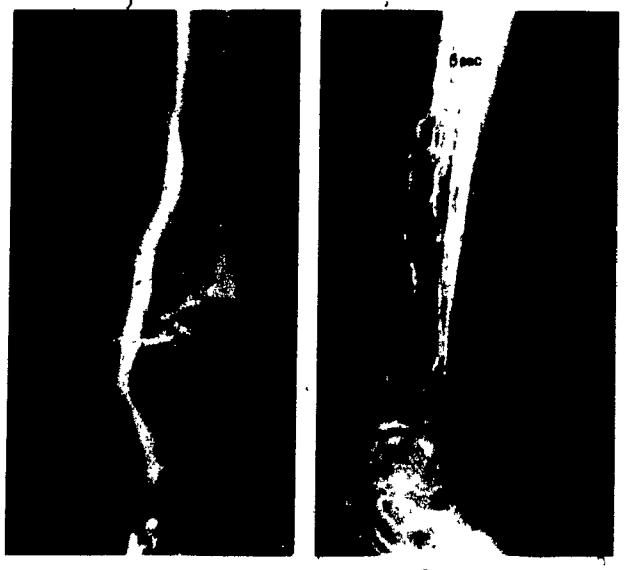


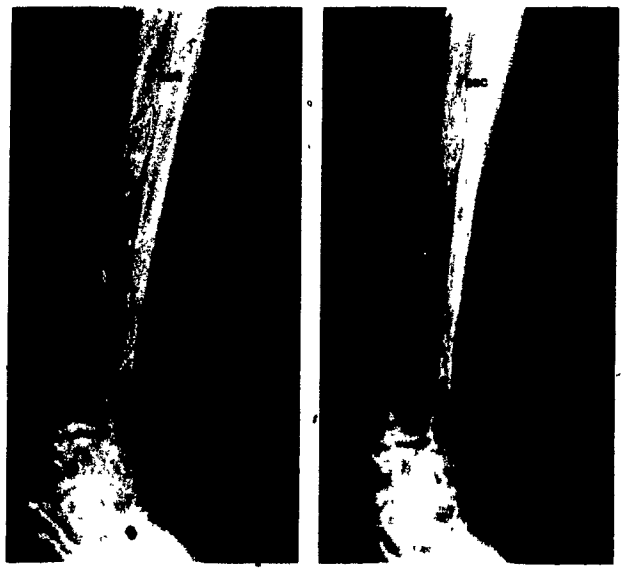
Figure 4

Arteriogram 4 months postoperatively:

(a) 2.5 seconds, showing patent PTFE graft and rapid filling of popliteal vein,
(b) 5 seconds,
(c) 6 seconds, showing extensive neovascularization extending to foot,
(d) 7 seconds, showing venous return from foot.



4a 4b



4c 4d

Figure 5a

Illustration of pelvis vessels ligated.

Figure 5b

Illustration of branches of the femoral artery ligated.

Figure 5c

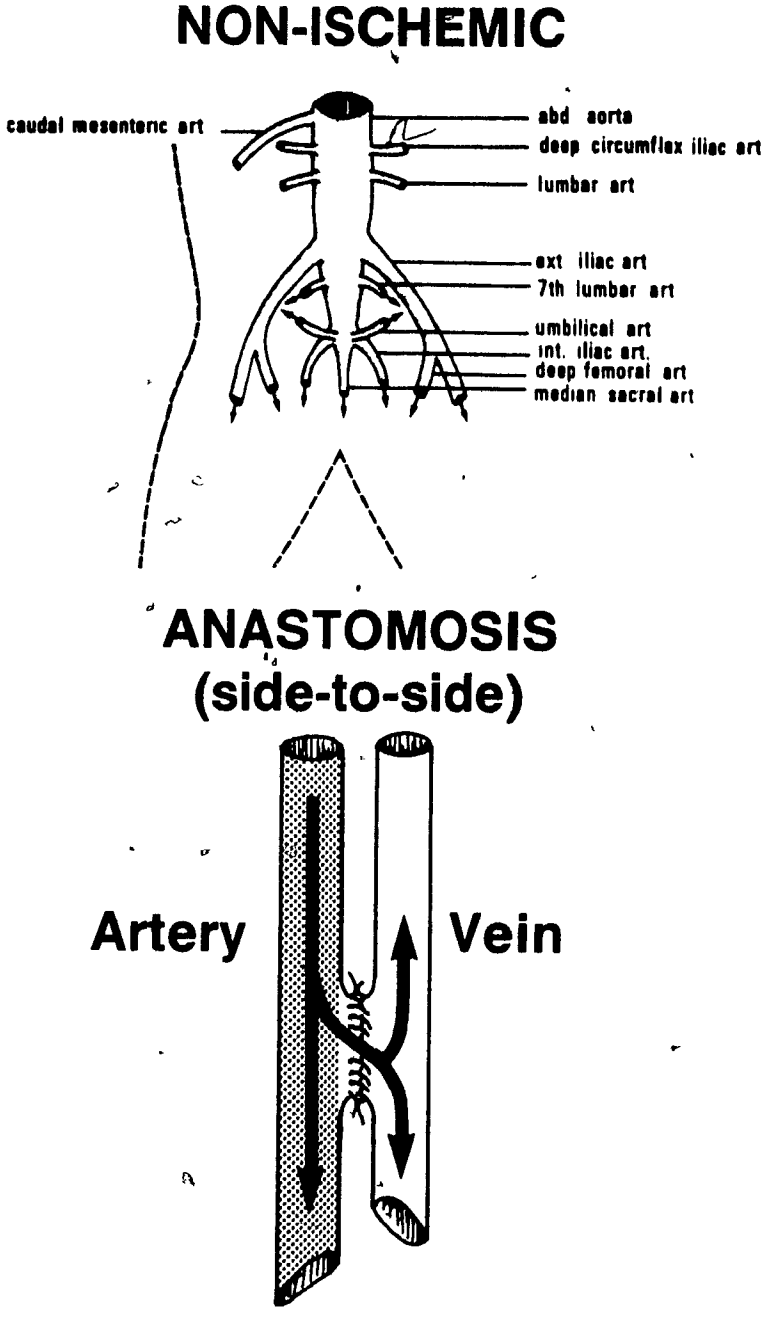
Arteriovenous anastomosis and a silk suture placed loosely around the proximal vein.

5a

5b

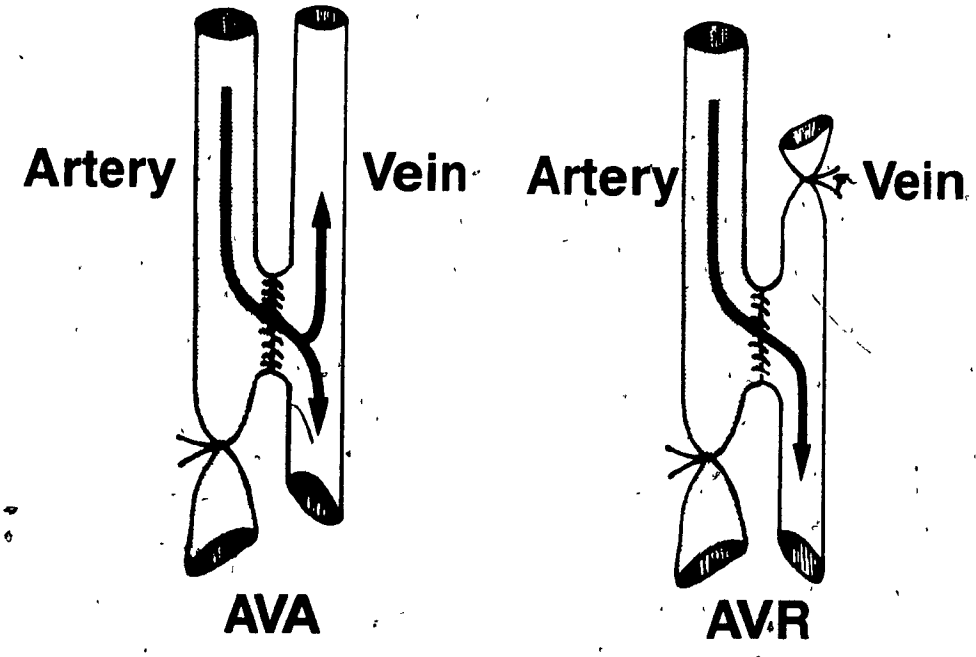
5c

Figure 6



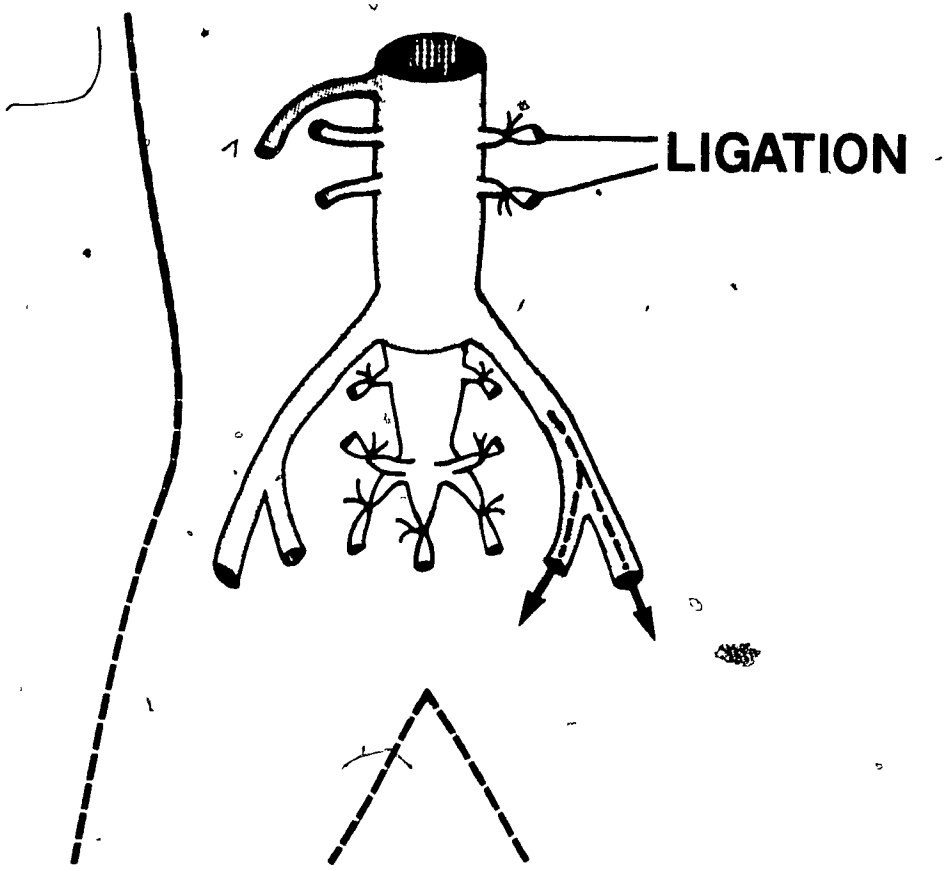
Diagramatic illustration of the operative procedure in Group 1.

Figure 7



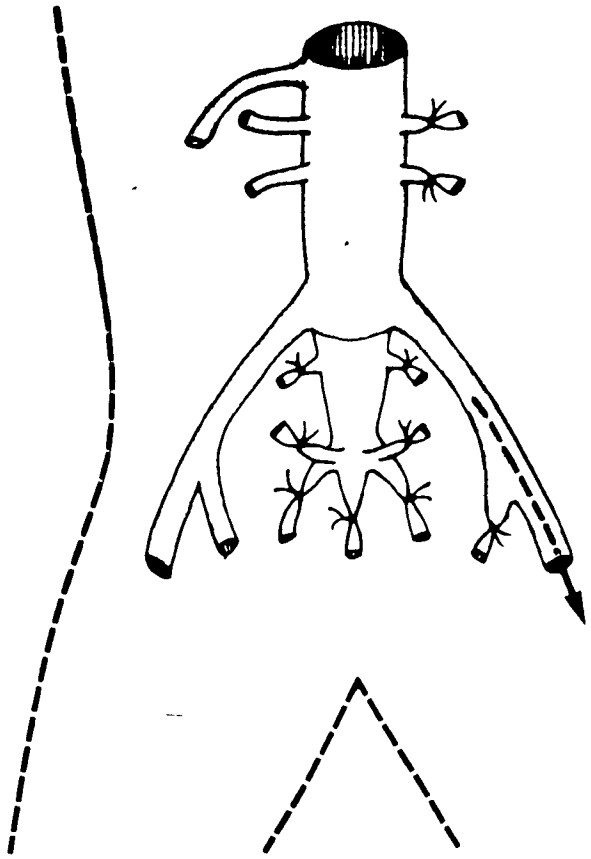
Diagramatic illustration of the popliteal anastomosis in Groups 2-4.

Figure 8



Diagramatic illustration of vessels ligated in Group 3.

Figure 9



Diagramatic illustration of vessels ligated in Group 4.

Figure 10a

The treadmill:

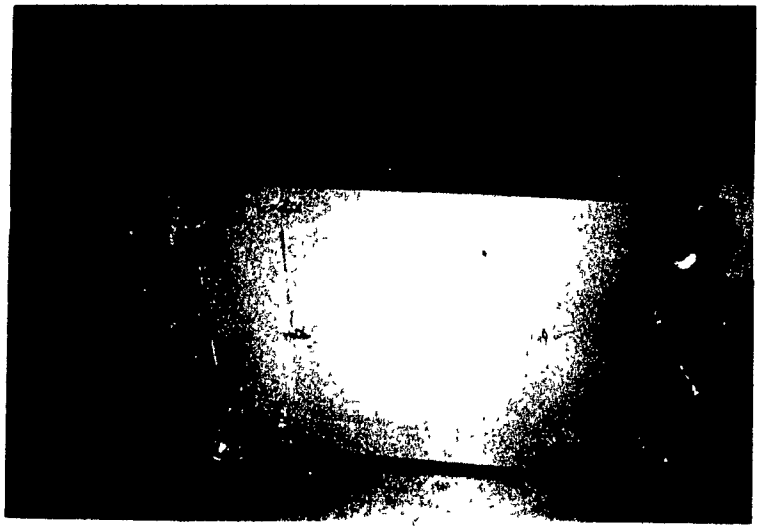
constructed in our laboratory.

Figure 10b

Dog running on the treadmill.

Figure 10c

Same dog running on the treadmill.



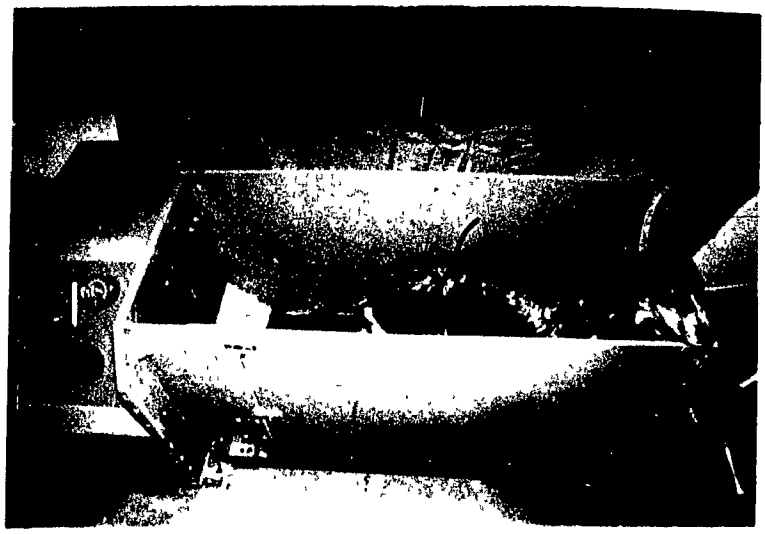


Figure 11

AVR: DAY 10 STUDY

Representative angiogram from studies under materials and methods sections D,F,G and H. Angiogram shows early formation of new vessels around the arteriovenous anastomosis (heavy arrow) and reversal of the flow in the venous system (light arrow).

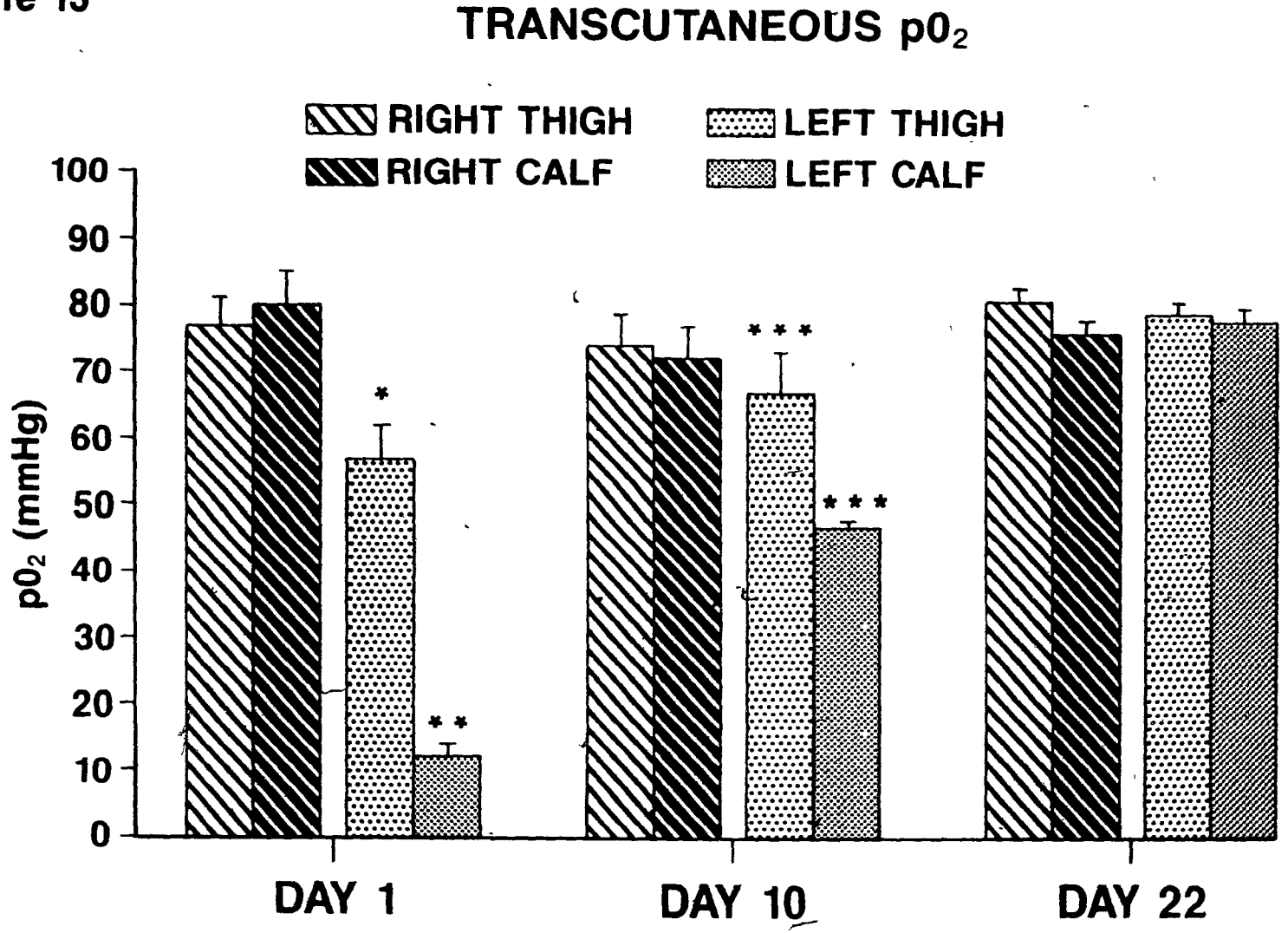
Figure 12

AVR: DAY 22 STUDY

Representative angiogram from studies under materials and methods sections D,F,G and H.

Angiogram demonstrates intense new vessel formation around and distal to anastomosis (arrow).

Figure 13



RIGHT LEG (control)

LEFT LEG (operated on)

* $p < .01$

** $p < .005$

*** $p < .05$

Figure 14

Representative angiogram from a Group 1 animal.


1

Figure 15

Representative angiogram from a Group 2 animal.


R

Figure 16

Representative angiogram from a Group 3 animal.

Figure 17a

Representative angiogram from a Group 4 animal demonstrating sequential filling of the intense vascular network and reversed venous flow to the foot.


4

Figure 17b

Representative angiogram from a Group 4 animal demonstrating sequential filling of the intense vascular network, reversed venous flow to the foot and subsequent venous emptying of the limb.

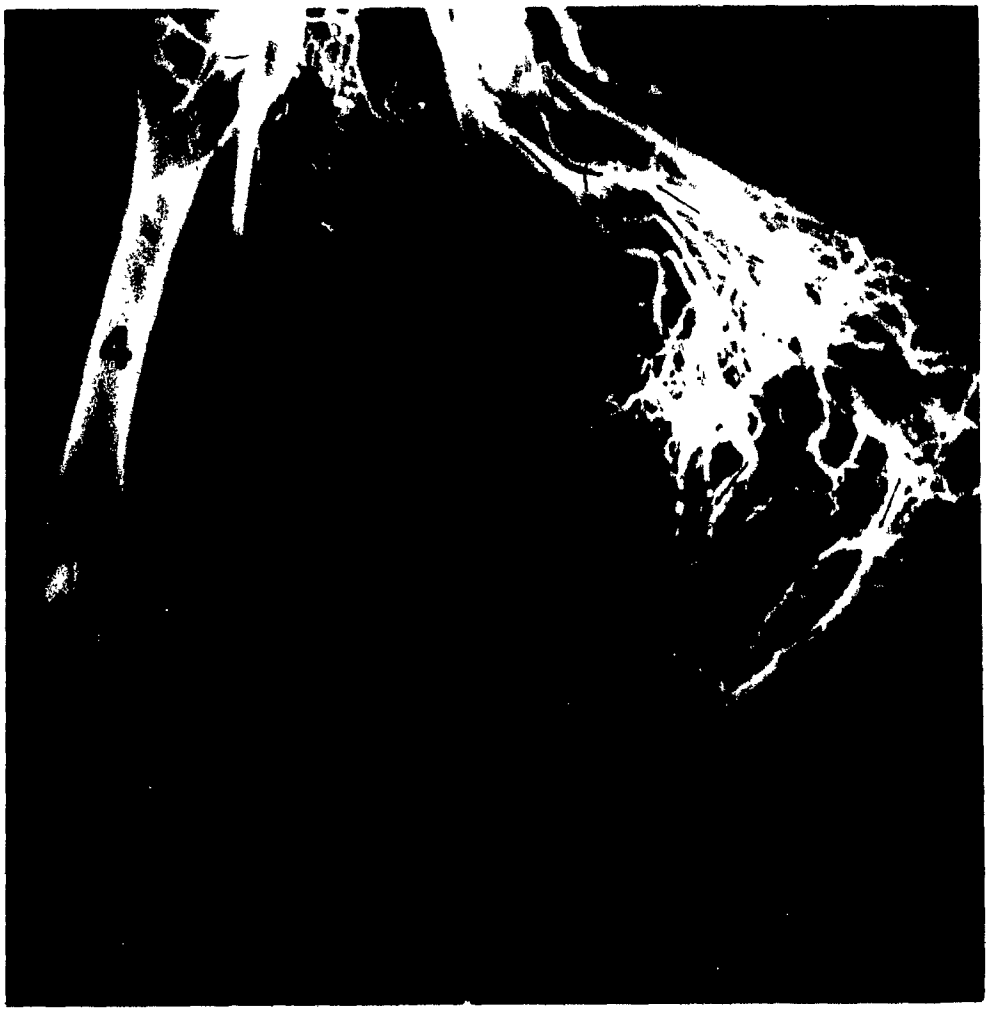


Figure 17c

Representative angiogram from a Group 4 animal demonstrating the intense vascular network and venous emptying of the limb.

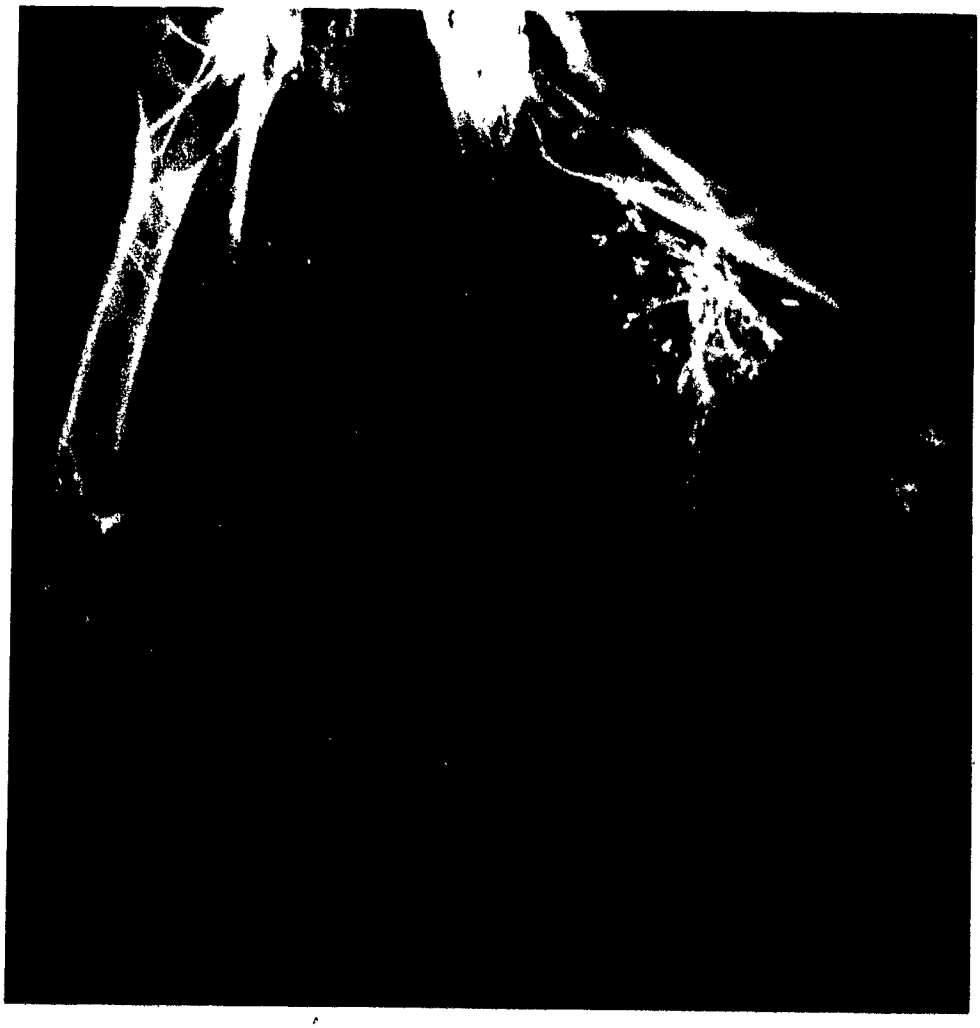


Figure 18

Leg muscle sections histochemically stained for alkaline phosphatase activity.

Dark spots represent capillaries.

A = Nonoperated control hind limb (contralateral muscle) (×250)

B = Operated hind limb (muscle) (×250)

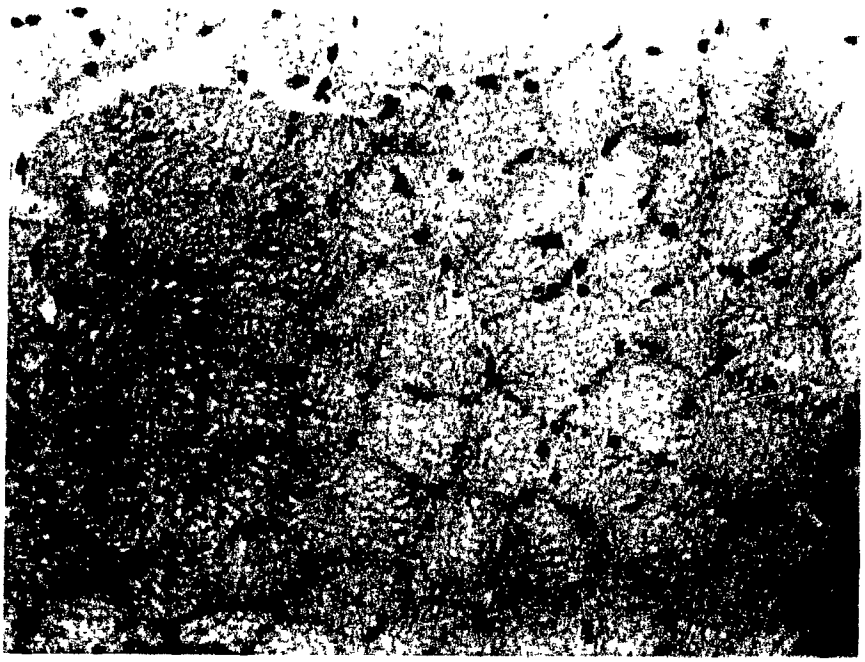


Figure 19

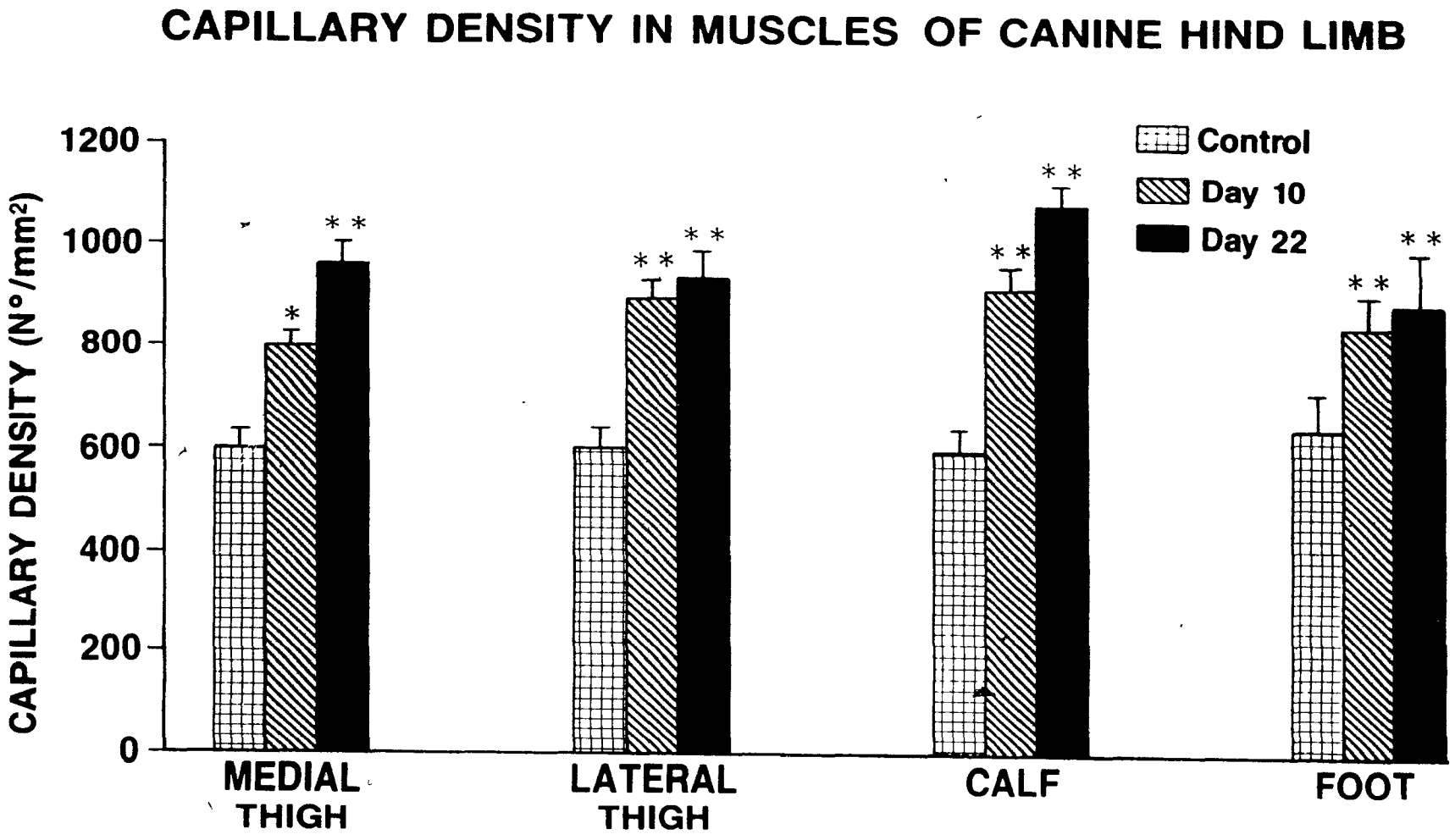

CAPILLARY DENSITY IN MUSCLES OF CANINE HIND LIMB
CAPILLARY DENSITY (N°/mm²)
1200
1000
800
600
400
200
0
Control
Day 10
Day 22
* *
*
MEDIAL THIGH
LATERAL THIGH
CALF
FOOT
Values are in means ± SEM
Student's (paired t-test)
* p = 0.02 vs control
* * p ≤ 0.001 vs control

Figure 20

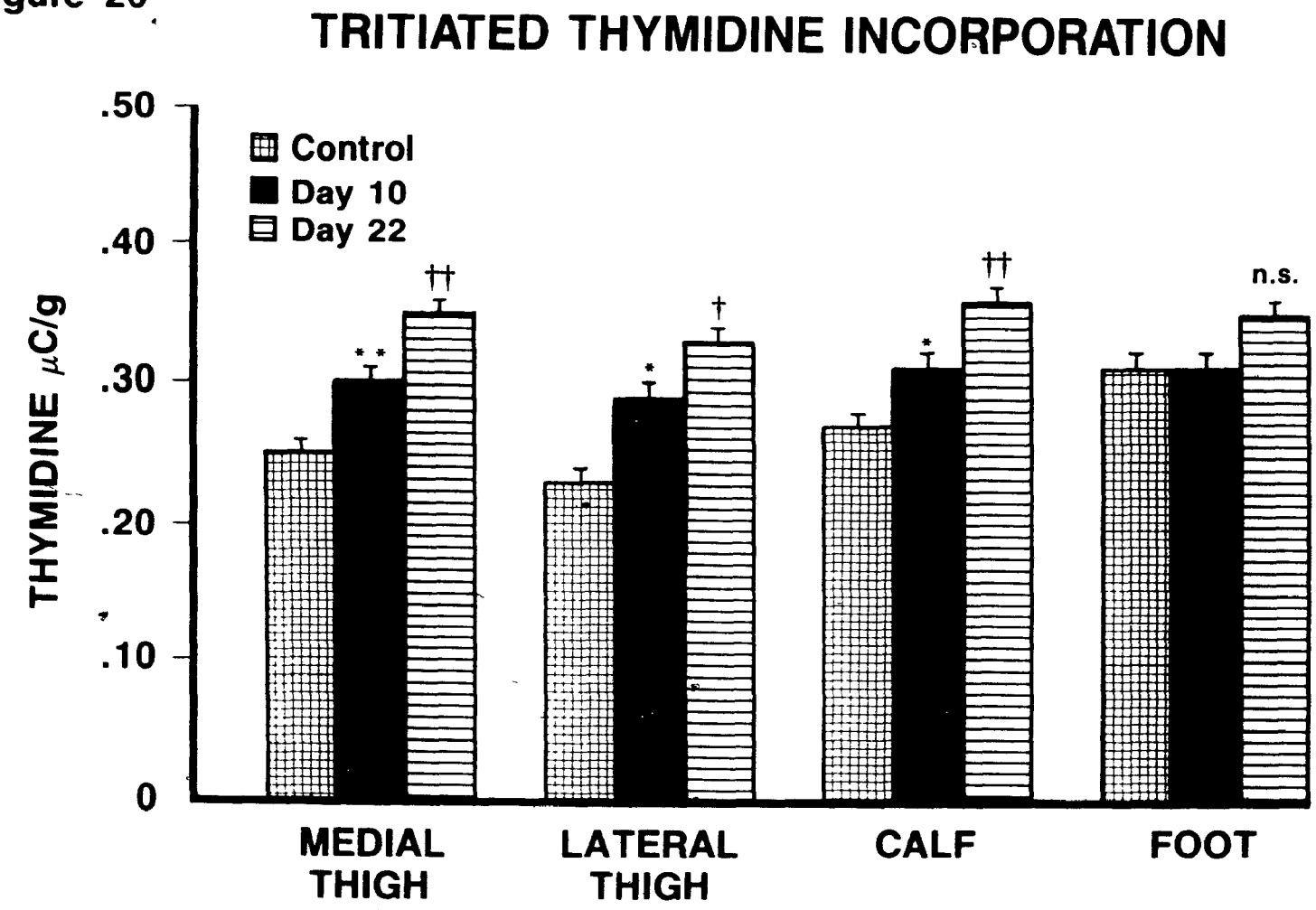

TRITIATED THYMIDINE INCORPORATION
THYMIDINE μC/g
.50
.40
.30
.20
.10
0
Control
Day 10
Day 22
**
††
*
†
*
††
n.s.
MEDIAL THIGH
LATERAL THIGH
CALF
FOOT
* p<0.05
** p<0.005
vs control
† p<0.05
†† p<0.005
vs day 10 values
n.s. = not significant

Figure 21

Endothelial cells of small blood vessels indicating DNA synthesis. Two endothelial cell nuclei (arrows) are heavily labelled. A single pulse of tritiated thymidine was given intravenously three hours before the animal was sacrificed.

Figure 22

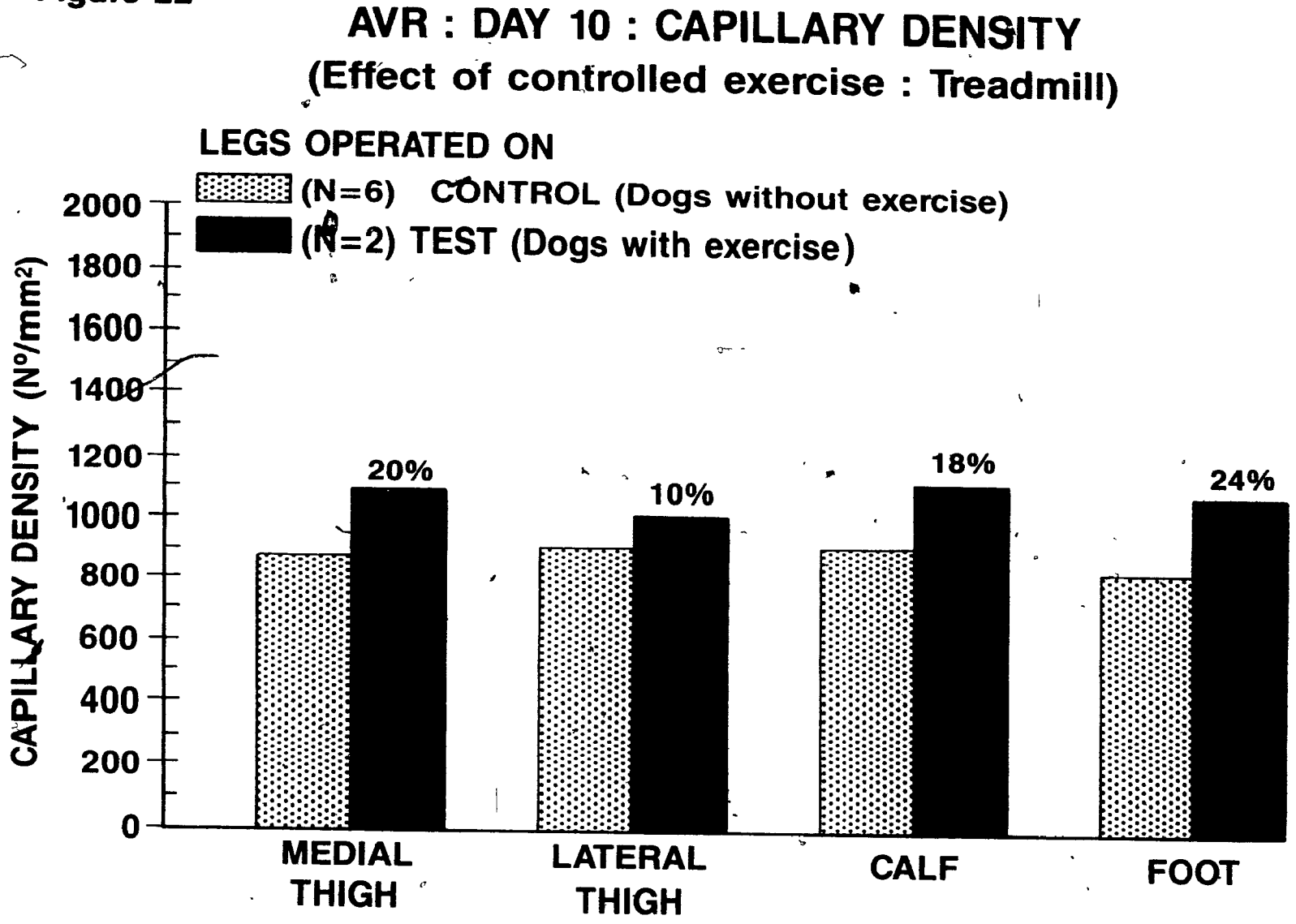

AVR : DAY 10 : CAPILLARY DENSITY
(Effect of controlled exercise : Treadmill)
LEGS OPERATED ON
(N=6) CONTROL (Dogs without exercise)
(N=2) TEST (Dogs with exercise)
CAPILLARY DENSITY (N°/mm²)
2000
1800
1600
1400
1200
1000
800
600
400
200
0
20%
10%
18%
24%
MEDIAL THIGH
LATERAL THIGH
CALF
FOOT

Figure 23

AVR: DAY 10:CAPILLARY DENSITY
(effect of lipid angiogenic factor)

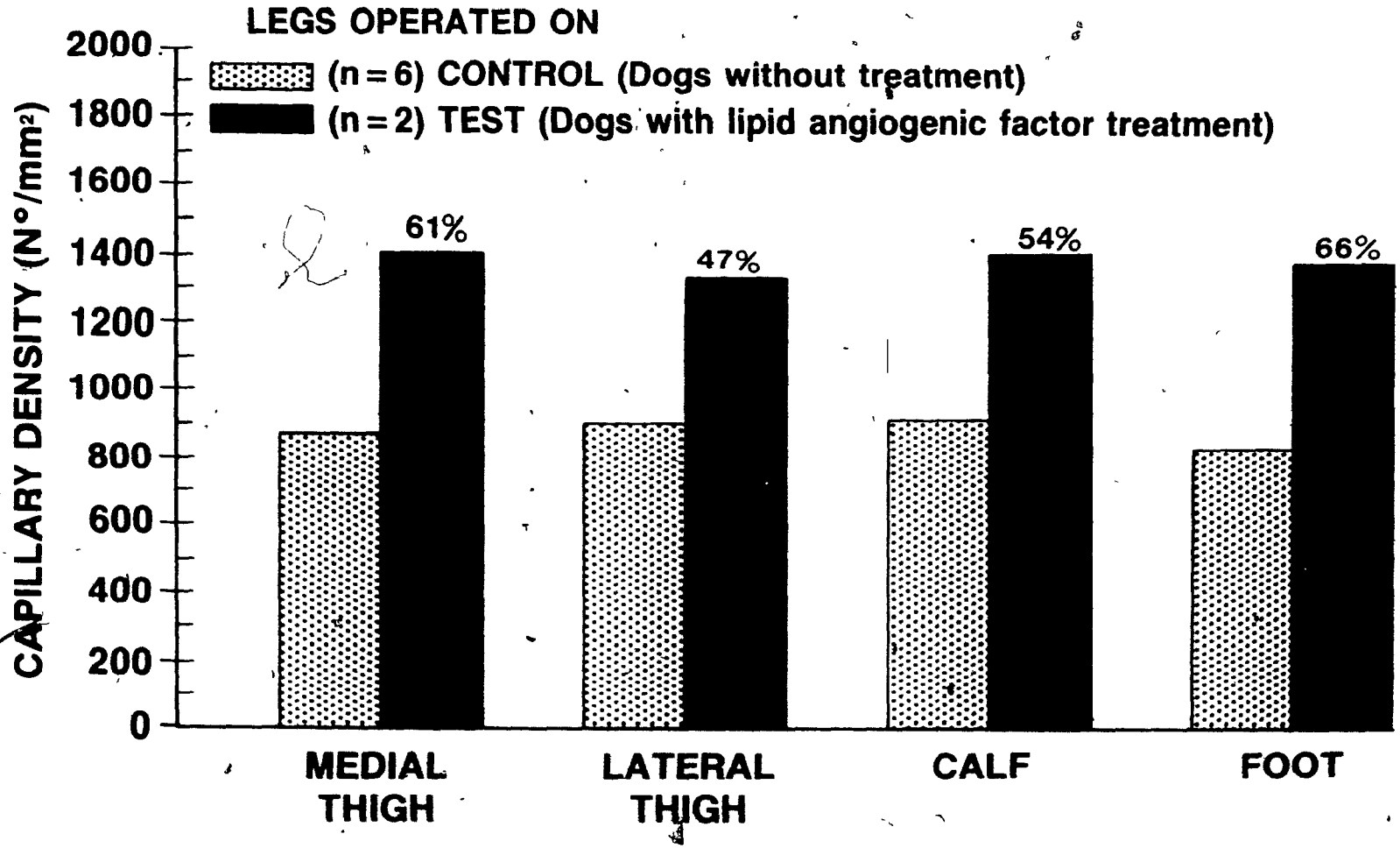


Table 1

TRANSCUTANEOUS pO_2 (mmHg)

	DAY 1		DAY 10		DAY 22	
	R	L	R	L	R	L
THIGH	77 ± 4	57 ± 5*	74 ± 5	67 ± 6***	81 ± 2	79 ± 2
CALF	80 ± 5	12 ± 2**	72 ± 5	47 ± 1***	76 ± 2	78 ± 2

(paired-t-test)

MEAN ± SEM (N=24)

R = RIGHT LEG (control) L = LEFT LEG (operated on)

* $p < .01$

** $p < .005$

*** $p < .05$

Table 2

ARTERIOVENOUS REVERSAL : DAY 10 STUDY

CAPILLARY DENSITY IN MUSCLES OF CANINE HIND LIMB

	RIGHT LEG (control) (capillaries/mm^2)	LEFT LEG (operated on) (capillaries/mm^2)
MEDIAL THIGH	598 ± 42	876 ± 38*
LATERAL THIGH	588 ± 41	905 ± 48**
CALF	596 ± 38	910 ± 52†
FOOT	627 ± 34	823 ± 52‡

Values are means ± SEM (N=6) Student's (paired t-test)

* p = 0.002
** p = 0.0002
† p = 0.0004
‡ p = 0.0007

Table 3

ARTERIOVENOUS REVERSAL : DAY 22 STUDY

CAPILLARY DENSITY IN MUSCLES OF CANINE HIND LIMB

	RIGHT LEG (control) (capillaries/mm^2)	LEFT LEG (operated on) (capillaries/mm^2)
MEDIAL THIGH	618 ± 30	987 ± 56*
LATERAL THIGH	612 ± 21	936 ± 61**
CALF	613 ± 24	1011 ± 46†
FOOT	626 ± 34	893 ± 44‡

Values are means ± SEM (N=6) Student's (paired t-test)

* p = 0.0007

** p = 0.0014

† p = 0.0004

‡ p = 0.0002

Table 4

THYMIDINE INCORPORATION

Tritiated-Thymidine incorporation (μCi/g wet wt)

	CONTROL	DAY 10	DAY 22
MEDIAL THIGH	.25 ± .01	.30 ± .01**	.35 ± .01‡
LATERAL THIGH	.23 ± .01	.29 ± .01*	.33 ± .01†
CALF	.27 ± .01	.31 ± .01*	.36 ± .01‡
FOOT	.31 ± .01	.31 ± .01 (NS)	.35 ± .00 (NS)

Values expressed as mean ± SEM (N=12) Student's t-test

* $p < 0.05$

** $p < 0.005$, vs control

† $p < 0.05$

‡ $p < 0.005$, vs day 10 values

NS = not significant

Table 5

AVR : DAY 10 STUDY

EFFECT OF CONTROLLED EXERCISE (treadmill)

	TRANSCUTANEOUS pO_2 (mmHg)			
	DOGS WITHOUT EXERCISE (N=12)		DOGS WITH EXERCISE (N=4)	
	RIGHT LEG	LEFT LEG (operated on)	RIGHT LEG	LEFT LEG (operated on)
THIGH	74	67	81	71
CALF	72	47	80	59
AVERAGE INCREASE	(10%)		(16%)	

Table 6

AVR : DAY 10 STUDY

EFFECT OF CONTROLLED EXERCISE (treadmill)

	CAPILLARY DENSITY (Cap/mm^2)			
	DOGS WITHOUT EXERCISE (N=6)		DOGS WITH EXERCISE (N=2)	
	RIGHT LEG	LEFT LEG (operated on)	RIGHT LEG	LEFT LEG (operated on)
MEDIAL THIGH	598	876	699	1090
LATERAL THIGH	588	905	679	1008
CALF	596	910	658	1113
FOOT	627	823	660	1083
AVERAGE INCREASE		(12%)		(18%)

Table 7

AVR: DAY 10 STUDY

EFFECT OF LIPID ANGIOGENIC FACTOR TREATMENT

	TRANSCUTANEOUS pO_2 (mmHg)			
	UNTREATED CONTROL DOGS (N=12)		DOGS TREATED WITH ANGIOGENIC FACTOR (N=2)	
	RIGHT LEG	LEFT LEG (operated on)	RIGHT LEG	LEFT LEG (operated on)
THIGH	74	67	77	77
CALF	72	47	78	65
AVERAGE INCREASE		(6%)		(27%)

Table 8

AVR : DAY 10 STUDY

EFFECT OF LIPID ANGIOGENIC FACTOR TREATMENT

	CAPILLARY DENSITY (Cap/mm^2)			
	UNTREATED CONTROL DOGS (N=6)		DOGS TREATED WITH LIPID ANGIOGENIC FACTOR (N=2)	
	RIGHT LEG	LEFT LEG (operated on)	RIGHT LEG	LEFT LEG (operated on)
MEDIAL THIGH	598	876	679	1409
LATERAL THIGH	588	905	668	1334
CALF	596	910	673	1402
FOOT	627	823	681	1369
AVERAGE INCREASE			(13%)	(57%)

REFERENCES

1. Byar D, Fiddian RV, Quereau M et al: The fallacy of applying Poiseuille Equation to Segmental Arterial Stenosis. Am Heart J 70:216, 1965.

2. Fiddian RV, Byar D, Edwards EA: Factors Affecting Flow through a Stenosed Vessel. Arch Surg 88:83, 1964.

3. Kindt GW, Youmans JR: The Effect of Stricture Length on Critical Arterial Stenosis. Surg Gynecol Obstet 128:729, 1969.

4. May AG, DeWeese JA, Rob CA: Hemodynamic Effects of Arterial Stenosis. Surgery 53:513, 1963.

5. Haid SP, Conn J Jr, Bergan JJ: Cystic Adventitial Disease of the Popliteal Artery. Arch Surg 101:765, 1970.

6. Darling RC, Buckley CJ, Abbott WM et al: Intermittent Claudication in Young Athletes: Popliteal Artery Entrapment Syndrome. J. Trauma 14:543, 1974.

7. Kissin M: The Production of Pain in Exercising Skeletal Muscle During Induced Anoxemia. J Clin Invest 13:37, 1934.

8. Katz LN, Linder E, Landt H: On the Nature of the Substance (s) Producing Pain in Contracting Skeletal Muscle: Its Bearing on the Problems of Angina Pectoris and Intermittent Claudication. J Clin Invest 14:807, 1935.

9. Harpuder K, Stein ID: Studies on the Nature of Pain Arising from an Ischemic Limb. II Biochemical Studies. Am Heart J 25:438, 1943.

10. Richards RL, Forest H: The Treatment of Peripheral Vascular Disease. Practitioner 215:431, 1975.

11. Verstraete M: Current Therapy for Intermittent Claudication. Drugs 24:240, 1982.

12. Editorial: Intermittent Claudication: Brit Med J 1(6019): 1165, 1976.

13. Imparato AM, Kim GE, Davidson T et al: Intermittent Claudication: Its Natural Course. J. Cardiovasc Surg 17(1):99, 1976.

14. DeWolfe VG: Assessment of the Circulation in Occlusive Arterial Disease of the lower Extremities. Mod Concepts Cardiovasc Dis 45(1):91, 1976.

15. Editorial: The Skin Microcirculation and Pathogenesis of Ischemic Necrosis and Gangrene. Scand J Clin Lab Invest 37:473, 1977.

16. Larsen OA, Lassen NA: Medical Treatment of Occlusive Arterial Disease of the Legs. Walking Exercise and Medically Induced Hypertension. Angiologica 6:288, 1969.

17. Coffman JD: Intermittent Claudication and Rest Pain: Physiologic Concepts and Therapeutic Approaches. Prog Cardiovasc Disease 22(1):53, 1979.

18. McGrath MA, Graham AR, Hill DA, et al: The Natural History of Chronic Leg Ischemia. World J Surg 7:314, 1983.

19. Alexander CS, Sako Y, Mikulic E: Pedal Gangrene Associated with the use of Dopamine. N Engl J Med 293(12):591, 1975.

20. Greene SI, Smith JW: Letter: Dopamine Gangrene. N Engl J Med 294 (2):114, 1976.

21. Woolf M: Factors that may Affect the Extent and Severity of Atherosclerosis: In Pathology of Atherosclerosis. Butterworths and Co (Publishers) Ltd. London, Boston, Toronto, p 153-185, 1982.

22. Smith EB: Molecular Interactions in Human Atherosclerotic Plaques. Am J Path 86(3): 665, 1977.

23. Tejada C, Strong JP, Montenegro MR et al: Distribution of Coronary and aortic Atherosclerosis by Geographic Location, Race and Sex. Lab Invest 18:509, 1968.

24. Smith EB: The Relationship Between Plasma and Tissue Lipids in Human Atherosclerosis. Adv Lipid Res 12: 1, 1974.

25. Pearson TA, Wang A, Solez K, Hepinstall RH: Clonal Characteristics of Fibrous Plaques and Fatty Streaks from Human Aortas. Am J Pathol 81:379, 1975.

26. Arteriosclerosis: Report of the Working Group on Arteriosclerosis of the National Heart, Lung and Blood Institute. NIH Publication No. 81-2034, p 5-9, 1981.

27. Ross R and Glomset JA: The Pathogenesis of Atherosclerosis. N Engl J Med 295:369, 420, 1976.

28. Geer JC, McGill HC Jr., Strong JP: The Fine Structure of Human Atherosclerotic Lesions. Am J Pathol 38:263, 1961.

29. Wolinsky H: Mesenchymal Response of Blood Vessel Wall. Cir Res 32:543, 1973.

30. Bierman EL, Ross R: Aging and Atherosclerosis. Atherosclerosis Rev 2:79, 1977.

31. Goldstein JL, Brown MS: The Low-Density Lipoprotein Pathway and its Relation to Atherosclerosis. Ann Rev Biochem 46:897, 1977.

32. Fredickson DS, Goldstein JL, Brown MS: The Familial Hyperlipoproteinemias. The Metabolic Basis of Inherited Disease. 4th Edition by JB Stanbury, JB Wyngaarden, DS Fredickson. New York, McGraw-Hill, 1978.

33. Zilversmit DB: Atherogenesis: a Postprandial Phenomenon. Circulation 60 (3) 473, 1979.

34. Brewer HB Jr., Schaefer EJ, Osborne JC et al: High Density Lipoproteins; an Overview. Report of the HDL Methodology Workshop. March 1979, Edited by K Lippel. Bethesda, National Institute of Health (NIH Publication No. 779-1661) p 29-41, 1979.

35. Benditt EP, Benditt JM: Evidence for a Monoclonoal Origin of Human Atherosclerotic Plaque. Proc Natl Acad Sci (USA) 70: 1753, 1973.

36. Martin GM, Spraque CA: Symposium on In Vitro Studies Related to Atherogenesis: Life Histories of Hyperplastoid Cell Lines from Aorta and Skin. Exp Mol Pathol 19:125, 1973.

37. Stamler J, Berkson DM, Lindberg HA: Risk Factors, Their Role in the Etiology and Pathogenesis of Atherosclerotic Diseases. In the Pathogenesis of Atherosclerosis. Edited by R.S. Wissler and J.G. Geer. Baltimore, Williams and Wilkins, p 41-119, 1972.

38. Robertson WB: Some Factors Influencing the Development of Atherosclerosis. A Survey in Jamaica, West Indies. Journal of Atherosclerosis Research 2:79, 1962.

39. Draper NR, Smith H: Applied Regression Analysis. p 167-168, 1966 New York.

40. Rhoads GG, Blackwelder WC, Stemmermann GN et al: Coronary Risk Factors and Autopsy Findings in Japanese-American Men. Lab Invest 38:304, 1978.

41. Kannel WB, Schwartz MD, McNamara PM: Blood Pressure and Risk of Coronary Heart Disease. The Framingham Study. Diseases of the Chest 56:43, 1969.

42. Kannel WB, Wolf PA, Verter J et al: Epidemiological Assessment of the Role of Blood Pressure in Stroke. JAMA 214:301, 1979.

43. Paul O: Risks of Mild Hypertension: A Ten Year Report. Br Heart J 33: Supplement: 116, 1971.

44. Robertson WB, Strong JP: Atherosclerosis in Persons with Hypertension and Diabetes Mellitus. Lab Invest 18:538, 1968.

45. Solberg LA, Hjerman J, Helgeland A, et al: Association Between Risk Factors and Atherosclerosis Lesions Based on Autopsy findings in the Oslo Study: A Preliminary Report. In: Atherosclerosis IV. Edited by G. Schettler, Y. Goto, Y. Hata and G. Klose, Berlin: Springer-Verlag p 88-102, 1977.

46. Paffenbarger RS Jr, Notkin J, Krueger DE et al: Chronic Disease in Former College Students II. Methods of Study and Observations on Mortality from Coronary Heart Disease. Amer J Public Health 56:926, 1966.

47. Hammond EC, Garfinkel L: Coronary Heart Disease, Stroke and Aortic Aneurysm. Factors in Etiology. Arch Environ Health 19:167, 1969.

48. Dyer A, Stamler J, Shekelle RB et al: Hypertension in Elderly. Med Clin North Am 61:513, 1977.

49. Strong JP, Richards ML, McGill HC Jr., et al: On the Association of Cigarette Smoking with Coronary and Aortic Atherosclerosis. J Atherosclerosis Res 10:303, 1969.

50. Gotto AM Jr.: Diagnosis and Management of Risk Factors for Atherosclerosis. In: Atherosclerosis. A Scope Publication. The Upjohn Company, Kalamazoo, Michigan p 9-35, 1977.

51. Duff GL, McMillan GC: The Effect of Alloxan Diabetes on Experimental Cholesterol Athersclerosis in the Rabbit. J Exp Med 89:611, 1949.

52. Chute AL, Orr JL, O'Brien MJ et al: Vascular Lesions in Alloxan Diabetic Rats. Archieves of Path 52: 105, 1951.

53. Duff GL, Brechin JH, Kinkelstein WE: The Effect of Alloxan Diabetes in Experimental Cholesterol Atherosclerosis in the Rabbit. J Exp Med 100:371, 1954.

54. Mahler R: Diabetes and Arterial Lipids. Quarterely Journal of Medicine 34:488, 1965.

55. Stout RW: Insulin-Stimulated Lipogenesis in Arterial tissue in Relation to Diabetes and Atheroma. Lancet 2:702, 1968.

56. Stout RW: Insulin Stimulation of Cholesterol Synthesis in Arterial Tissue. Lancet 2:467, 1969.

57. Christensen S, Jensen J: Uptake of Labelled Cholesterol from Plasma by Aortic Intima-Media in Control and Insulin Injected Rabbits. J Atherosclersis Res 5:258, 1966.

58. Stamler J, Pick R, Katz LM: Effect of Insulin in the Induction and Regression of Atherosclerosis in the Chick. Cir Res 8:572, 1969.

59. Keys A: Seven Countries, A Multivariate Analysis of Death and Coronary Heart Disease. Harvard University Press. Cambridge, Mass, 1980.

60. Cornfield J: Joint Dependence of Risk of Coronary Heart Disease on Serum Cholesterol and Systolic Blood Pressure: A Discriminant Function Analysis. Fed Proc 21(4)pt 2:58, 1962.

61. Gordon T, Castelli WP, Hjortland MC, et al: Predicting Coronary Heart Disease in Middle-Aged and Older Persons. The Framington Study. JAMA 238(6):497, 1977.

62. Stamler J: Population Studies. In: Nutrition, Lipids and Coronary Heart Disease. A Global View. Edited by Levy R, Rifkind B, Dennis B, Ernst N, New York, Raven Press , 1979.

63. Taylor CB, Patton DE, Cox DE: Atherosclerosis in Rhesus Monkeys. VI. Fatal Myocardial Infarction in a Monkey Fed Fat and Cholesterol. Arch Pathol 76:404, 1963.

64. Armstrong ML, Warner ED: Morphology and Distribution of Diet-Induced Atherosclerosis in Rhesus Monkeys. Arch Pathol 92:395, 1971.

65. Texon M: A Hemodynamic Concept of Atherosclerosis with Particular Reference to Coronary Occlusion. Arch Intern Med 99:418, 1957.

66. Texon M, Imparato AM, Helpern M: The Role of Vascular Dynamics in the Development of Atherosclerosis. JAMA 194:1226, 1965.

67. Fry DL: Acute Vascular Endothelial Changes Associated with Increased Blood Velocity Gradients. Cir Res 22:165, 1968.

68. Fry DL: Certain Chemorheologic Considerations Regarding the Blood Vascular Interface with Particular Reference to Coronary Artery Disease. Circulation 39 and 40 (Suppl 4): 38, 1969.

69. Caro CG, Fitz-Gerald JM, Schroter RC: Atheroma and Arterial Wall Shear. Observation, Correlation and Proposal of a Shear Dependent Mass Transfer Mechanism for Atherosclerosis. Proc Roy Soc Lond 177:109, 1971.

70. Mitchell JRA, Schwartz CJ: Arterial Disease. Blackwell Scientific Publishers Ltd. Oxford, 1965.

71. Wesolowski SA, Fries CC, Sabini AM et al: The Significance of Turbulence in the Hemic Systems and in the Distribution of the Atherosclerosis Lesion. Surgery 57:155, 1965.

72. Fox JA, Hugh AE: Localization of Atheroma: A Theory Based on Boundary Layer Separation. Brit Heart J 28:388, 1966.

73. Oka S: A Theoretical Approach to the Effect of Shear Stress on the Development of Atheroma. Thrombosis Res 8(suppl II):305, 1976.

74. Zweifach BW: Structural Basis of Permeability and Other Functions of Blood Capillaries. In: Cold Spring Harbor Symposium on Quantitative Biology, Long Island Biological Association Cold Spring Harbor, Long Island, p 216, 1940.

75. Fry DL: Certain Histological and Chemical Responses of the Vascular Interface to Acutely Induced Mechanical Stress in the Aorta of the Dog. Cir Res 24:93, 1969.

76. Sako Y: Effects of Turbulent Flow and Hypertension on Experimental Atherosclerotic. J Amer Med Ass 179:36, 1962.

77. Scarfstein H, Gutstein WH, Lewis L: Changes in Boundary Layer Flow in Model Systems. Implications for Initiation of Endothelial Injury. Cir Res 13:580, 1963.

78. Rodbard S, Johnson AC: Deposition of Flow Borne Materials in Vessel Wall. Cir Res II:664, 1962.

79. Mustard JF, Murphy EA, Rowsell HC et al: Factors Influencing Thrombus Formation In Vivo. Am J Med 33:621, 1962.

80. Mustard JF, Packham MA, Kinlough-Rathbone RL: Mechanisms in thrombosis. In: Haemostasis and Thrombosis. (Eds Bloom A.L. and Thomas D.P.). Churchill Livingstone, Edinburgh, London, Melbourne and New York, p 503-526, 1981.

81. Fuster V, Chesebro JH: Antithrombotic Therapy: A Role of Platelet-Inhibitor Drugs. I. Current Concepts of thrombogenesis: Role of Platelets (First of Three Parts). Mayo Clin Proc 56(2):102, 1981.

82. Zucker MB, Nachmias VT: Platelet Activation. Arteriosclerosis 5(1):2, 1985.

83. Sixma JJ, Wester J: The Hemostatic Plug. Semin Hematol 14:265, 1977.

84. Gerrard JM, White JG: Prostaglandins and Thromboxanes "Middlemen" Modulating Platelet Function in Hemostasis and Thrombosis. Prog Hemost Thromb 4:87, 1978.

85. Groves HM, Kinlough-Rathbone RL, Richardson M et al: Platelet Interaction with Damaged Rabbit Aorta. Lab Invest 40:194, 1979.

86. Groves HM, Kinlough-Rathbone RL, Richardson M, et al: Thrombin Generation and Fibrin Formation Following Injury to Rabbit Neointima: Studies of Vessel Wall Reactivity and Platelet Survival. Lab Invest 46:605, 1982.

87. Frederick JL, Shimanuki T, Dizerega GS: Initiation of Angiogenesis by Human Follicular Fluid. Science 224:389, 1984.

88. Holman E: Problems in the Dynamics of Blood Flow: Conditions Controlling Collateral Circulation in the Presence of an Arteriovenous Fistula Following the Ligation of an Artery. Surg 26:880, 1949.

89. John HT, Warren R: The Stimulus to Collateral Circulation. Surgery 49:14, 1961.

90. Schaper W: Tangential Wall Stress as a Molding Force in the Development of Collateral Vessels in the Canine Heart. Experimenta (Basel) 23:595, 1967.

91. Schaper W: Brabander De M, Lewi P: DNA Synthesis and Mitoses in Coronary Collateral Vessels of the dog. Cir Res 28:671, 1971.

92. Kumar S, West D, Shahabuddin S et al: Angiogenesis Factor from Human Myocardial Infarcts. Lancet Aug 13:2 (8346):364, 1983.

93. Cuttino JT Jr., Bartrum RJ Jr., Hollenberg NK, et al: Collateral Vessel Formation: Isolation of a Transferrable Factor Promoting a Vascular Response. Basic Res Cardiol 70:568, 1975.

94. Folkman J, Merler E, Abernathy C, Gretchen W: Isolation of a Tumor Factor Responsible for Angiogenesis. J Exp Med 133:275, 1971.

95. Goldsmith HS, Griffith AL, Kupferman A, Catsimpoolas N: Lipid Angiogenic Factor from Omentum. JAMA 252:2034, 1984.

96. Fett JW, Strydom DJ, Lobb RR et al: Isolation Characterization of Angiogenin, an Angiogenic Protein from Human Carcinoma Cells. Biochemistry 24:5480, 1985.

97. Federman JL, Brown GC, Felberg NT, Felton SM: Experimental Occular Angiogenesis. Am J Ophthalmol 89:231, 1980.

98. Folkman J: Toward an Understanding of Angiogenesis: Search and Discovery. Perspectives in Biol & Med 29:1, 1985.

99. Coffman JD: Vasodilator Drugs in Peripheral Vascular Disease (Letter) N. Engl J Med 301 (3):159, 1979.

100. Stallworth JM, Lee WH, Belisle C et al: A Clinical and Laboratory Comparison of the Effects of Commonly used Vasodilator Drugs on Arterial Disease. Am Surg 24:700, 1958.

101. Gillespie JA: An Evaluation of Vasodilator Drugs in Occlusive Vascular Disease by Measurement. Angiology 17:280, 1966.

102. Strandness DE Jr.: Ineffectivness of Isoxsuprine on Intermittent Claudication. JAMA 213:86, 1970.

103. Thomas M, Campbell H, Heard G: The Effect of Vasodilator Drugs on Skin blood Flow in Peripheral Vascular Occlusion. Br J Surg 55:588, 1968.

104. Zetterquist S: Muscle and Skin Clearance of Antipyrene from Exercising Ischemic Legs Before and After Vasodilating Trials. Acta Med Scand 183:487, 1968.

105. Angelkort B: Influence of Pentoxifylline (Trental 400) on Microcirculation Post-Stenotic Blood Pressure and Walking Capacity in Patients with Occlusive Arterial Disease. IRCS Med Sci 5:370, 1977.

106. Bollinger A, Frei C: Double Blind Study of Pentoxifylline Against Placebo in Patients with Intermittent Claudication. Pharmatherapeutica 1:557, 1977.

107. Schubotz R: Double Blind Trial of Pentoxifylline in Diabetes with Peripheral Vascular Disorders. Pharmatherapeutica 1:172, 1976.

108. Porter JM, Cutler BS, Lee BY et al: Pentoxifylline Efficacy in the Treatment of Claudication. Multicenter Controlled Double-Blind Trial with Objective Assessment of Chronic Occlusive Arterial Disease Patients. Am Heart J 104:66, 1982.

109. Roekaerts F, Deelers L: Trental 400 in the Treatment of Intermittent Claudication: Results of Long-Term, Placebo-Controlled Administration. Angiology 35(7):396, 1984.

110. Strano A, Davi G, Avellone G, et al: Double-Blind, Crossover Study of the Clinical Efficacy and the Hemorheological Effects of Pentoyifylline in the Patients with Occlusive Arterial Disease of the Lower Limbs. Angiology 35(7):459, 1984.

111. Muller R: Pentoxifylline. A Biochemical Profile. J Med 10:307, 1979.

112. Aviado DM, Porter JM: Pentoxifylline: A New Drug for the Treatment of Intermittent Claudication. Mechanism of Action, Pharmacokinetics, Clinical Efficacy and Adverse Effects. Pharmacotherapy 4(6):297, 1984.

113. Marder VJ, Bell WR: Fibrinolytic Therapy. In: Hemostasis and Thrombosis. Basis Principles and Clinical Practice (eds) RW Colman, J Hirsh, VJ Marder and EW Salzam. Lippincott, Philadelphia p 1037-1057, 1983.

114. Verstraete M: Biochemical and Clinical Aspects of Thrombolysis. Semin Hematol 15:35, 1978.

115. Verstraete M, Vermylen J, Donati MB: The Effect of Streptokinase Infusion on Chronic Arterial Occlusions and Stenosis. Ann Intern Med 74:377, 1971.

116. Mulcare RJ, Royster TS, Lynn RA et al: Long-Term Results of Operative Therapy for Aortoiliac Disease. Arch Surg 113:601, 1978.

117. Hansteen V, Lorensten E, Sivertssen E: °Long-Term Follow-up of Patients with Peripheral Arterial Obliterations Treated with Arterial Surgery. Acta Chir Scand 141:725, 1975.

118. Malone JM, Moore WS, Goldstone J: The Natural History of Bilateral Femoral Bypass Grafts for Ischemia of the Lower Extremities. Arch Surg 110:1300, 1975.

119. Malone JM, Moore WS, Goldstone J: Life Expectancy Following Aortofemoral Arterial Grafting. Surgery 81:551, 1977.

120. Brewter DC, Darling RC: Optimal Methods of Aortoiliac Reconstruction. Surgery 84:739, 1978.

121. Yates SG, Barros D'Sa AA, Berger K et al: The Preclotting of Porous Arterial Prosthesis. Ann Surg 188:611, 1978.

122. Moore WS, Chvapil M, Seiffert G, Keown K: Development of an Infection Resistant Vascular Prosthesis. Arch Surg 116:1403, 1981.

123. Morris GC Jr, Edwards W, Colley DA et al: Surgical Importance of Profunda Femoris Artery. Arch Surg 82:32, 1961.

124. Szilagyi DE, Smith RF, Elliot P et al: Infection in Arterial Reconstruction with Synthetic Grafts. Ann Surg 176:321, 1972.

125. Lindenaver SM, Fry WJ, Schaub G et al: The Use of Antibiotics in the Prevention of Vascular Graft Infections. Surgery 62:487, 1967.

126. Kaiser AB, Clayson KR, Mulherin JL Jr, et al: Antibiotic Prophylaxis in Vascular Surgery. Ann Surg 188:283, 1978.

127. Sauvage LR, Berger K, Wood SJ et al: An External Velour Surface for Porous Arterial Prosthesis. Surgery 70:940, 1971.

128. Knox GW: Peripheral Vascular Anastomotic Aneurysms. Ann Surg 183:120, 1976.

129. Storey RJ, Albo EJ, Wylie EJ: False Aneurysm Occurring After Arterial Grafting Operations. Am J Surg 110:153, 1965.

130. Johnson WC, LoGerfo FW, Vollman RW et al: Is Axillo-Bilateral Femoral Graft an Effective Substitute for Aortic-bilateral Iliac/Femoral Graft? An Analysis of Ten Years Experience. Ann Surg 186:123, 1977.

131. Oblath RW, Green RM, DeWeese JA et al: Extra-Anatomic Bypass of the Adominal Aorta. Management of Post-Operative Thrombosis. Ann Surg 187: 647, 1978.

132. Reiche FA: Criteria for Evaluation of New Arterial Prosthesis by Comparing Vein with Dacron Femoropopliteal Bypass. Surg Gynecol Obstet 146:714, 1978.

133. Miller VM: Femoropopliteal Bypass Graft Patency. An Analysis of 156 Cases. Ann Surg 180:35, 1974.

134. LoGerfo FW, Corson JD, Mannick JA: Improved Results with Femoropopliteal Vein Grafts for Limb Salvage. Arch Surg 112:567, 1977.

135. DeWeese JA, Rob CG: Autogenous Venous Grafts Ten Years Later. Surgery 82:755, 1977.

136. Clowes AW, Whittemore AD, Couch NP: Vein Graft Failure After Femoropopliteal Reconstruction. Ethiology and Management. Circulation 62 (Suppl III):15, 1989.

137. Whittemore AD, Clowes AW, Tilney NL et al: Iliofemoral Reconstruction for Aortoiliac Occlusive Disease. Circulation 62:235, 1980.

138. Hall KV: The Great Saphenous Vein Used "In-Situ" as an Arterial Shunt After Extripation of the Vein Valves. Surgery 51:492, 1962.

139. Leather RP, Shah DM, Karmody AM: Infrapopliteal Arterial Bypass for Limb Salvage: Increased Patency and Utilization of the Saphenous Vein used In-Situ. Surgery 190:1000, 1981.

140. Hall KV, Rostad H: In Situ Vein Bypass in the Treatment of Femoropopliteal Atherosclerotic Disease: A Ten Year Study. Am J Surg 136:158, 1978.

141. Klimach O, Chapman BLW, Underwood CJ et al: An Investigation into How the Geometry of an End-to-Side Anastomosis Affects its Function. Br J Surg 71:43, 1984.

142. Brody WR, Kosek JC, Angell WW: Changes in Vein Grafts Following Aorto-Coronary Bypass Induced by Pressure and Ischemia. J Thorac Cardiovasc Surg 64:847, 1972.

143. Sottiurai V, Fry WJ, Stanley JC: Ultrastructural Characteristic of Experimental Arterial Medial Fibrodysplasia Induced by Vasa Vasorum Occlusion. J Surg Res 24:169, 1978.

144. McGeachie J, Campbell P, Prendergast F: Vein to Artery Grafts: A Quantitative Study of Revascularization by Vasa Vasorum and its Relationship to Intimal Hyperplasia. Ann Surg 194:100, 1981.

145. Boncheck LI: Prevention of Endothelial Damage During Preparation of Saphenous Veins for Bypass Grafting. J Thorac Cardiovasc Surg 79:911, 1980.

146. Bush HL, McCabe ME, Nabseth DC: Functional Injury of Vein Graft Endothelium. Role of Hypothermia and Distension. Arch Surg 119:770, 1984.

147. Cambria RP, Megerman J, Abbot WM: Endothelial Preservation in Reversed and In-Situ Autogenous Vein Grafts. Ann Surg 2023:50, 1985.

148. Leather RP, Karmody AM: In-Situ Saphenous Vein Arterial Bypass for the Treatment of Limb Ischemia. Adv Surg 19:175, 1986.

149. May AG, DeWeese JA, Rob CG: Arterialized In Situ Saphenous Vein. Arch Surg 91:743, 1965.

150. Galland RB, Young AE, Jamieson CW: In Situ Vein Bypass: A Modified Technique. Ann R Coll Surg Engl 63:186, 1981.

151. Samuels PB, Plested WG, Haberfelde GC et al: In Situ Saphenous Vein Arterial Bypass: A Study of Anatomy Pertinent to its Use In Situ as a Bypass Graft with a Description of a New Venous Valvulotone. Am Surg 34:122, 1968.

152. Collins GJ Jr, Rich NM, Andersen CA et al: Acute Hemodynamic Effects of Lumbar Sympathectomy. Am J Surg 136:714, 1978.

153. Hoffman DC, Jepson RP: Muscle Blood Flow and Sympathectomy. J Surg Res 14:151, 1973.

154. Postlethwaite JC: Lumbar Sympathectomy. A Retrospective Study of 142 Operations on 100 Patients. Brit J Surg 60:878, 1973.

155. Adson AW, Brown GE: The Treatment of Raynaud's Disease by Lumbar Ramisection and Ganglionectomy and Perivascular Sympathectomy of the Common Iliac. JAMA 84:1908, 1924.

156. Adson AW, Brown GE: The Treatment of Raynaud's Disease by Resection of the Upper Thoracic and Lumbar Sympathetic Ganglia and Trunks. Surg Gynecol Obstet 48:577, 1929.

157. Haimovici H, Steinman C, Karson JH: Evaluation of Lumbar Sympathectomy: Advanced Occlusive Arterial Disease. Arch Surg 89:1089, 1964.

158. Szilagyi DE, Smith RF, Scerpella JR et al: Lumbar Sympathectomy: Current Role in the Treatment of Arteriosclerotic Occlusive Disease. Arch Surg 95:753, 1967.

159. Kim GE, Ibrahim IM, Imparato A: Lumbar Sympathectomy in End Stage Arterial Occlusive Disease. Ann Surg 183:157, 1976.

160. Lee BY, Madden JL, Thoden WR, McCann WJ: Lumbar Sympathectomy for the Gangrene. Long-Term Follow-up. Am J Surg 145:398, 1983.

161. Berardi RS, Siroospour D: Lower Sympathectomy in the Treatment of Peripheral Vascular Occlusive Disease. Ten Year Study. Am J Surg 130:309, 1975.

162. Fulton RL, Blakeley WA: Lumbar Sympathectomy: A Procedure of Questionable Value in the Treatment of Arteriosclerosis Obliterans of the Legs. Am J Surg 116:735, 1968.

163. Bergan JJ, Trippel OH: Arteriograms in Ischemic Limbs Worsened After Lumbar Sympathectomy. Arch Surg 85:643, 1962.

164. Kihn RB, Warren R, Beebe RW: The "Geriatric" Amputee. Ann Surg 176:305, 1972.

165. Wray CH, Still JM Jr, Moretz WH: Present Management of Amputations for Peripheral Vascular Disease. Ann Surg 38:87, 1972.

166. Eidemiller LR, Awe WC, Peterson CG: Amputation of the Ischemic Extremity. Am Surg 34:491, 1968.

167. Lim RC, Blaisdell FW, Hall AD et al: Below-Knee Amputation for Ischemic Gangrene. Surg Gynecol Obstet 125:493, 1967.

168. Otteman MG, Stahlgren LH: Evaluation of Factors Which Influence Mortality and Morbidity Following Major Lower Extremity Amputations for Arteriosclerosis. Surg Gynecol Obstet 120:1217, 1965.

169. Silverstein MJ, Kadish L: Study of Amputations of the Lower Extremity. Surg Gynecol Obstet 137:579, 1973.

170. Bernheim BM: Arteriovenous Anastomosis Reversal of Circulation as a Prevention of Gangrene in the Extremities. Ann Surg 5:195, 1912.

171. San Martin Y Satrustequi A: Chirurgie de l'Appareil Circulatoire: l'Anastomose Arterio-Veineuse. Sem Med p 395, 1902.

172. Szilagyi D, Jay GD, Munnell ED: Femoral Arterioevenous Anastomosis in the Treatment of Occlusive Arterial Disease. Arch Surg 63:435, 1951.

173. Root HD, Cruz AB Jr.: Effects of an Arteriovenous Fistula on the Devascularized Limb. J Amer Med Assoc 191 (8):109, 1965.

174. Matolo NM, Cohen SE, Wolfman EF Jr.: Use of an Arteriovenous Fistula for Treatment of Severely Ischemic Extremity: Experimental Evaluation. Ann Surg 184:622, 1976.

175. Hiertonn T: Arteriovenous Fistula for Discrepancy in Length of Lower Extremities. Acta Orthop Scand 31:25, 1961.

176. Gausewitz S, Gerard DF, Dilley RB, Bernstein EF: Arteriovenous Anastomosis (AVA) and Arteriovenous Fistula (AVF) in Revascularizing the Ischemic Canine Hind Limb. Surg Forum 29:211, 1978.

177. Gerard DF, Dilley RB, Bernstein EF: Acute Physiologic Effects of Arteriovenous Anastomosis in Devascularized Canine Hind Limb. Surg Forum 28:206, 1977.

178. Gerard DF, Gausewitz S, Dilley RB, Bernstein EF: Acte Physiologic Effects of Arteriovenous Anastomosis and Fistula in Revascularizing the Ischemic Canine Hind Limb. Surgery 89:485, 1981.

179. Johansen K, Bernstein EF: Revascularization of the Ischemic Canine Hind Limb by Arteriovenous Reversal. Ann Surg 190 (2):243, 1979.

180. Graham AM, Sniderman A, Jothy S, Homan J, Symes JF: Staged Reversal of Venous Flow for Revascularization of the Severely Ischemic Limb. J Surg Res 35:11, 1983.

181. Symes JF, Graham AM, Stein L, Sniderman A: Salvage of a Severely Ischemic Limb by Arteriovenous Revascularization: A Case Report. Canadian J Surg 27(3):274, 1984.

182. Heyman MA, Payne BD, Offman J, Rudolph AM: Blood Flow Measurements with Radionuclide Labelled Particles. Prog Cardiovasc. Dis 20 (1):55, 1977.

183. Huch A, Huch R: Transcutaneous Noninvasive Monitoring of pO_2. Hosp Pract II (6):43, 1976.

184. Ohgi S, Ito K, Mori T: Quantitative Evaluation of Skin Circulation in Ischemic Legs by Transcutaneous measurement of Oxygen Tension. Angiology 32:833, 1981.

185. White RA, Nolan L, Harley D et al: Noninvasive Evaluation of Peripheral Vascular Disease Using Transcutaneous Oxygen Tension. Am J Surg 144, 68, 1982.

186. Franzeck UK, Talke P, Bernstein EF et al: Transcutaneous pO_2 Measurements in Health and Peripheral Artery Occlusive Disease. Surgery 91(2):156, 1982.

187. Hauser CJ, Shoemaker WC: Use of Transcutaneous pO_2 Regional Index to Quantify Tissue Perfusion in Peripheral Vascular Disease. Ann Surg 197(3):337, 1983.

188. Ohgi S, Ito k, Hara H, Mori T: Continuous Measurement of Transcutaneous Oxygen Tension on Stress Test in Claudicants and Normals. Angiology 37:27, 1986.

189. Gomori G: Microtechnical Demonstration of Phosphatase in Tissue Sections. Proc Soc Exp Biol Med 42:23, 1939.

190. Gomori G: The Distribution of Phosphatase in Normal Organs and Tissues. J Cell and Comp Physiol 17:71, 1941.

191. Takamatsu H: Histologische und biochemische studien uber die phosphatase. I. Histochemische untersuchungsmethodik der phosphatase und deren verteilung in verschiedenen organen und geweben. Trans Soc Path Japan 29:492, 1939.

192. Cotter M, Hudlicka O, Vrbova G: Growth of Capillaries During Long-Term Activity in Skeletal Muscle. Bibl Anat (11):395, 1973.

193. Myrhage R, Hudlicka O: Capillary Growth in Chronically Stimulated Adult Skeletal Muscle as Studied by Intravital Microscopy and Histological Methods in Rabbits and Rats. Microvasc Res 16(1):73, 1978.

194. Wright AJA, Hudlicka O: Capillary Growth and Changes in Heart Performance Induced by Chronic Bradycardial Pacing in Rabbits. Cir Res 49:469, 1981.

195. Hudlicka O, Dodd L, Renkin EM, Gray SD: Early Changes in Feber Profile and Capillary Density in Long-Term Stimulated Muscles. Am J Physiol 243(4):H528, 1982.

196. Romanul FCA, Bannister RG: Localized Areas of High Alkaline Phosphatase Activity in the Terminal Arterial Trees. J Cell Biology 15:73, 1962.

197. Pearse AGE: Histochemistry. London J and A. Churchill Ltd. 3rd Edition, p 713, 1968.

198. Cleaver JE: Thymidine Metabolism and Cell Kinetics. North-Holland Research Monographs. Frontiers of Biology vol. 6, Amesterdam, North-Holland Publishing Co. 1967.

199. Folkman J, Haudenschild C: Angiogenesis in vitro. Nature 288(5791):551, 1980.

200. Glaser BM, D'Amore PA, Michels RB et al: The Demonstration of Angiogenic Activity from Occular Tissues. Preliminary Report. Ophthalmology 87(5):440, 1980.

201. Cavallo T, Sade R, Folkman J et al: Tumor Angiogenesis. Rapid Induction of Endothelial Mitoses Demonstration by Autoradiography J Cell Biol 54:408, 1972.

202. Baserga R, Malamud D: Light Microscopic Autoradiography. In: Autoradiography: Techniques and Application, Chapter 2, New York, Harper and Row 1969.

203. Derenzi E, Boeri G: Isolated Spleen Tissue Revascularized by the Greater Omentum. Berl Klin Woch 40:773, 1903.

204. Morison R: Remarks on Some Functions of the Omentum. Br Med J 1:76, 1906.

205. Wilkie DPS: Some Function and Surgical Use of the Omentum. Br Med J 2:1102, 1911.

206. O'Shaughnessy L: An Experimental Method for Providing a Collateral Circulation to the Heart. Br JSurg 23:665, 1936.

207. Knock FE: Cardio-Omentopexy and Implantation of Multiple Loops for Revascularization of the Heart. Surg Forum 9:230, 1958.

208. Goldsmith HS: Omental Transposition for Peripheral Vascular Insufficiency. Rev Surg 24:379, 1967.

209. Goldsmith HS, Chen WF, Duckett SW: Brain Vascularization by Intact Omentum. Arch Surg 106:659, 1973.

210. Goldsmith HS, Duckett SW, Chen WF: Spinal Cord Vascularization by Intact Omentum. Am J Surg 129:263, 1975.

211. Idem: Prevention of Cerebral Infarction in the Dog by Intact Omentum. Am J Surg 130:317, 1975.

212. Idem: Prevention of Cerebral Infarction in the Monkey by Omental Transposition to the Brain. Stroke 9:224, 1978.

213. Goldsmith HS: Salvage of End-Stage Ischemic Extremities by Intact Omentum. Surgery 88:732, 1980.

214. Goldsmith HS, Griffith AL, Catsimpoolas N: Increased Vascular Perfusion After Administration of an Omental Lipid Fraction. Surg Gynecol Obstet 162:579, 1986.

215. Patz A, Eastman A, Higginbotham DH et al: Oxygen Studies in Retrolental Fibroplasia: The Production of the Microscopic Changes of Retrolental Fibroplasia in Experimental Animals. Am J Ophthal 36:1511, 1953.

216. Ashton N: Pathological Basis of Retrolental Fibroplasia. Br J Ophthalmol 7:385, 1954.

217. Anverse P, Ricci R, Olivetti G: Effects of Exercise on Capillary Vasculature of the Rat Heart. Circulation. 75 (Suppl 1):1, 1987.

218. Mandache E, Unge G, Appelgren LE, et al: The Proliferative Activity of the Heart Tissues in Various Forms of Experimental Cardiac Hypertrophy Studied by Electron Microscope Autoradiography. Virchows Archiv Abt B Zellpath 12:112, 1973.

219. Mandache E, Unge G, Ljungquist A: Myocardial Blood Capillary Reaction in Various Forms of Cardiac Hypertrophy. An Electron Microscopical Investigation in the Rat. Virchows Arch Abt B Zellpath 11:97, 1972.

220. Buchner T, Wagner H, Junge-Hulsing G, Schmitt G: Zur proliferation der zellen in Granulationsgewebe. Arzneim Forsch 18:170, 1968.

221. Dawson CA, Horvath SM: Swimming in Small Laboratory Animals. Med Sci Sports 2:51, 1970.

222. Hudlicka O: Effect of Training on Macro and Microcirculatory Changes in Exercise. Exercise and Sport Sci Reviews. 5:181, 1977.

223. Frazer RA, Ellis EM, Stalker AL: Experimental Angiogenesis in the Chorio-Allantoic Membrane.

224. Imre G: Studies on the Mechanism of Retinal neovascularization. Br J Ophthalmol 48:75, 1962.

225. Ben Ezra D: Neovasculogenic Ability of Prostaglandins, Growth Factors, and Synthetic Chemoattractants. Am J Ophthalmol 86:455, 1978.

226. Ziche M, Jones J, Gullino PM: Role of Prostaglandin E_1 and Copper in Angiogenesis. J Natl Cancer Inst 69:475, 1982.

227. Form DM, Auerbach R: PGE_2 and Angiogenesis. Proc Exp Biol Med 172:214, 1983.