VEIN CRAFT INJURY DURING IN-SITU FEMORODISTAL BYPASS PROCEDURES.

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A thesis submitted for the degree of Doctor of Medicine (MD) to the University of Leicester 1992.
To Patricia Ann Healey
Statement of originality:

The work described in this thesis was performed in the laboratories of the Clinical Sciences Building at Leicester Royal Infirmary whilst I was Clinical Research Fellow and then Lecturer in Surgery in the Academic Department of Surgery.

The work is entirely my own except for the parts stated below which were conducted with the assistance of the following:

Design and construction of the organ bath:
Miss PAC Watt.

Processing of samples for scanning electron microscopy:
Mrs K Allen.

Histological assessment:
Dr S Muller and Dr C Bouch.

RD Sayers  Feb 1981
Autologous saphenous vein remains the conduit of choice for femorodistal bypass grafts to the below knee popliteal artery, tibioperoneal trunk and single calf vessels. The vein is used non-reversed (in-situ) and a valvulotome is passed along the vein to render the valves incompetent. During the first post-operative year 20-30% of these grafts develop intrinsic lesions which may lead to graft failure. These lesions may be isolated graft stenoses or longer diffuse areas of intimal hyperplasia. The aetiology of these intrinsic graft lesions remains unknown. However it has been that the key pathophysiological process in intimal hyperplasia is the migration of smooth muscle cells from the media of the vein into the intima where they proliferate and cause thickening.

Recently it has been suggested that intrinsic graft lesions in reversed vein grafts used for coronary artery bypass surgery may be due to injury to the vein during preparation for bypass grafting. However the preparation of in-situ femorodistal vein grafts differs from that of reversed grafts. Therefore a study has been performed to investigate whether damage to the vein occurs during preparation for in-situ femorodistal bypass grafting and to identify specific factors that may lead to injury.

An organ chamber system was designed to allow endothelial and smooth muscle cell injury to be studied in saphenous vein segments obtained from patients undergoing bypass surgery. Smooth muscle cell function was assessed by the contractile response of the vein to noradrenaline and endothelial function was assessed by the release of endothelium-derived relaxing factor (EDRF).

The results show that significant endothelial and smooth muscle cell injury occurs with the in-situ technique of vein grafting when compared to controls (p<0.05). This injury occurs during surgical preparation of the vein for use as an in-situ conduit. However, the use of x-ray contrast medium for intra-operative arteriography does not cause additional endothelial or smooth muscle cell injury. Intra-operative vasodilators such as papaverine and iloprost do not damage the endothelium or smooth muscle cells although papaverine does have a prolonged effect on smooth muscle cell function.
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This thesis would not have been possible without help from the following persons who I would like to thank:

Professor PRF Bell for his enthusiastic support and guidance. Dr H Thurston, Reader in Medicine for closely supervising this work and Miss PAC Watt for helping me learn the organ bath technique.

My thanks also to Mrs Karen Allen for processing samples for scanning electron microscopy, Dr Salli Muller and Dr Clive Bouch for help with histological assessment, George McTurk and Steve Cronier for scanning electron microscopy, and Dr Carol Jagger for statistical advice.

I am also indebted to the vascular surgeons at Leicester Royal Infirmary (especially Professor PRF Bell, Mr JD Beard, Mr JS Budd and Mr NJM London) and the cardiothoracic surgeons at Groby Road Hospital (especially Mr TJ Spyt, Mr RK Firmin, Mr J Bailey, Mr J Leverment and Mr A Soznowski) for providing vein samples.
Publications Arising From This Thesis

1. Valvulotome induced smooth muscle injury in femorodistal vein grafts.
   Sayers RD, Watt PAC, Bell PRF, Thurston H

   Sayers RD, Watt PAC, Muller S, Bell PRF, Thurston H

3. Endothelial cell injury secondary to surgical preparation of reversed and in-situ saphenous vein bypass grafts.
   Sayers RD, Watt PAC, Muller S, Bell PRF, Thurston H

Presentations Arising From This Thesis

   Sayers RD, Watt PAC, Bell PRF, Thurston H
   Presented to the Tripartite Meeting of British, Canadian and Dutch Vascular Surgical Societies.
   London November 1990.

2. Functional endothelial damage in reversed and non-reversed (in-situ) vein grafts.
   Sayers RD, Watt PAC, Bell PRF, Thurston H
   Poster Presentation to the Association of Surgeons of Great Britain and Ireland.

   Sayers RD, Watt PAC, Bell PRF, Thurston H
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SECTION ONE

INTRODUCTION
"The concept of using this vein in-situ and thus providing a conduit lined with normal endothelium is particularly attractive in these longer, low flow bypasses. The provision of a viable and hence non-thrombogenic surface is clearly its most important attribute."

AM Karmody and RP Leather 1984
CHAPTER ONE

PERIPHERAL VASCULAR DISEASE.

Introduction

Cardiovascular disease has become the single largest cause of death in the western world with myocardial infarction and cerebrovascular accidents being responsible for most of the fatal events (Report of the Working Group on Arteriosclerosis 1981). The primary cause of cardiovascular disease is atherosclerosis - a disease of arteries that leads to their progressive narrowing and eventual occlusion (Marchand 1904). Atherosclerosis may affect any artery but usually involves the coronary and cerebral circulations, and the aorta and lower limb vessels. The clinical effects of atherosclerosis result from distal ischaemia or infarction and give rise to ischaemic heart disease, cerebrovascular and peripheral vascular disease.

Peripheral vascular disease (PVD) is caused by atherosclerosis of the abdominal aorta and arteries of the upper and lower limbs. Although it is not usually a direct cause of mortality, lower limb ischaemia represents a major cause of morbidity. In addition there is a strong association between peripheral vascular disease and ischaemic heart disease, which results in the premature death of many of these patients (Fowkes 1988).
Pathology

The basic pathological lesion of atherosclerosis is the fibrous plaque. This is a raised yellow-white focal lesion that lies within the intima of the arterial wall and consists of a core of lipids (mainly cholesterol) that is covered by a fibrous cap. These plaques become more numerous as the disease progresses, and in severe cases may cover the entire intimal surface of an artery. As the plaques increase in size, they project into the lumen of the artery and cause progressive narrowing and eventual occlusion. Fibrous plaques often occur at arterial bifurcations which has led to the suggestion that haemodynamic factors, such as turbulent blood flow and low shear stresses, are important factors in their development (Mitchell 1965).

The distribution of plaques within the arterial system is often characteristic with the coronary, carotid and cerebral vessels being particularly affected. The disease also commonly affects the infrarenal segment of the abdominal aorta and the lower limb vessels.

In addition to gradual enlargement, fibrous plaques may undergo a number of complications which include distal embolisation and plaque rupture. Rupture is a particularly serious complication which is usually associated with haemorrhage within the plaque. This may produce a sudden increase in plaque size that can lead to acute occlusion of an already narrowed artery (Davies 1985).

Pathogenesis

There are two major events that occur in the pathogenesis of atherosclerosis which lead to the formation of fibrous plaques on the
luminal surfaces of arteries. Initially smooth muscle cells from the media of the arterial wall migrate into the intima where they proliferate and deposit extracellular connective tissue substances such as collagen and elastin. This is followed by the accumulation of intra and extracellular lipid which appears to be derived from the plasma (Woolf 1982). Unfortunately it is not possible to adequately explain the origins and progression of these two events with a single theory of pathogenesis, and historically, two theories have dominated our understanding.

In 1844 Carl Von Rokitansky introduced the "thrombogenic theory" which suggested that atherosclerosis was due to "excessive deposition of an inner vascular membrane". He thought that a constituent of blood was deposited on the luminal surface of the arterial wall during the formation and growth of fibrous plaques. This deposition occurred at sites of endothelial injury and was composed mainly of platelets, leucocytes and fibrin. The lipid in the plaques was derived from the subsequent breakdown of platelets and leucocytes.

However in 1853 Rudolf Virchow proposed that the material that Rokitansky had described was subendothelial in position and could not be derived from any surface deposition. Virchow maintained that atherosclerosis was caused by an invading stream of plasma constituents including lipids which crossed the luminal surface by a process of "imbibition" and resulted in local thickenings.

Response to injury theory

The "response to injury" theory was introduced by Ross and Glomset in 1976 in their extensive review of the pathogenesis of atherosclerosis. This theory proposes that "injury" to the endothelium
is the crucial initiating event that leads to the development of intimal smooth muscle proliferation (Ross 1976, Ross 1986).

It has been demonstrated that cigarette smoking, hypertension, diabetes and hypercholesterolaemia can cause damage to endothelial cells, and this damage is often more severe at arterial branch points where turbulent flow occurs. Monocytes can then adhere to these areas of endothelial damage and migrate to a sub-endothelial position where they are able to transform into tissue macrophages and accumulate lipid. In addition, platelets can adhere to sub-endothelial collagen in areas where endothelial cell loss has occurred. All of these cell types either contain, or can synthesize and release, chemoattractants and growth factors which are capable of stimulating smooth muscle cells to migrate from the media into the intima where they can proliferate.

Peripheral Vascular Disease

Peripheral vascular disease of the lower limb is caused by atherosclerotic narrowing or occlusion of the distal abdominal aorta and its lower limb branches which results in chronic ischaemia of the leg.

Initially, atherosclerotic narrowing of an artery produces minimal haemodynamic changes and is usually asymptomatic. It has been shown that an artery can become stenosed to a point where its luminal diameter is reduced by approximately 50% and its luminal area by 80% before there is significant reduction in flow or distal pressure (Mann 1938, Berguer 1974). However, if further narrowing occurs, there is a marked fall in both flow and distal pressure, and this degree of narrowing is referred to as the "critical stenosis". Once the critical stenosis is reached the arterial system is unable to deliver a sufficient volume of
blood to the peripheral tissues and the patient experiences ischaemic pain.

PVD usually presents clinically in one of three ways - intermittent claudication, ischaemic rest pain or critical limb ischaemia. In addition many patients experience a combination of these three problems.

Intermittent claudication

Intermittent claudication is usually the first symptom of arterial insufficiency and refers to ischaemic pain which occurs only during exercise when the oxygen demands of the muscles cannot be met. Exercise induces vasodilation of the distal vascular bed which exaggerates the fall in distal pressure. Unfortunately flow cannot be increased through a stenosed artery to compensate for the fall in pressure.

Intermittent claudication usually affects the calves because the superficial femoral artery which supplies blood to the muscles of the calf is commonly affected. However, if the aortoiliac segment is affected, this may produce pain in the buttocks or thighs, and occasionally the Leriche syndrome of buttock pain and impotence in men may occur if the internal iliac arteries which supply the pelvis are affected.

Ischaemic rest pain

If the luminal diameter of the artery is decreased even further, the arterial system will fail to satisfy the demands of the peripheral tissues at resting flow, the pain will become continuous, and is referred to as "ischaemic rest pain". Rest pain initially occurs in the
foot because this is the most distal part of the limb. It often occurs at night when cardiac output falls and may be partially relieved by positioning the limb with the foot dependent in order to allow gravity to aid perfusion.

Rest pain is often accompanied by tissue necrosis which manifests itself as ischaemic ulceration and gangrene. Tissue necrosis often follows a trivial injury because the healing process is impaired by ischaemia.

However many patients with intermittent claudication and mild rest pain experience a gradual improvement of their symptoms particularly if they stop smoking. This is due to the development of a collateral circulation which compensates for the relative ischaemia. If complete occlusion of a major vessel has occurred the limb is entirely dependent on collateral flow. However development of a collateral circulation takes time, and during its development the ischaemia may be severe enough to cause tissue necrosis. There may also be a dramatic worsening of ischaemia if the collateral circulation becomes blocked.

Critical limb ischaemia

Critical limb ischaemia is defined as "ischaemia which endangers the limb or part of the limb" (Bell 1982, Wolfe 1986). These patients usually have a combination of rest pain and tissue necrosis and unless urgent revascularisation is performed, amputation of a non-viable limb becomes inevitable.

The most widely accepted definition of critical limb ischaemia is that of the European Working Group (Dormandy 1989a) who stress the importance of grouping diabetic and non-diabetic patients separately.
They define chronic critical limb ischaemia by the following two criteria:

1. Persistently recurring rest pain requiring regular analgesia for more than two weeks and/or ulceration or gangrene of the foot and toes.

2. An ankle systolic blood pressure of less than 50 mm Hg.

This precise definition of critical limb ischaemia is important because it allows identification of those patients requiring urgent treatment. In addition various treatment options can be evaluated in a defined group of patients with severe peripheral vascular disease. Unfortunately, in the past, the reported results of both surgical and non-surgical treatment options for peripheral vascular disease have often grouped together patients with differing severities of disease. This has led to false evaluations of the various treatment options available for these patients.

Incidence and prevalence of peripheral vascular disease

In the Framingham study (Kannel 1985), which has followed normal individuals in the general population for the past 26 years, the incidence of claudication was found to be 0.2% in men aged 45-55 years and 0.5% in men aged 55-65 years. In the same population, the incidences of ischaemic cerebrovascular events and ischaemic heart disease were about half and four-times that of claudication, respectively. A higher incidence of claudication was reported in the smaller Basle study (Widmer 1964), partly because of more careful patient assessment and the additional use of non-invasive investigations.
The prevalence of arterial disease causing intermittent claudication in men aged less than 50 years is approximately 1.0 to 1.5% and rises to 5% in men over the age of 50 years (Dormandy 1989b). However this is almost certainly an underestimation of the true prevalence because most of the major studies have been concerned solely with symptomatic patients with claudication (Reunanen 1982).

The natural history of claudication is for symptoms to stabilise in 75% of patients soon after onset, and to improve in some patients as collaterals develop. However in 25% of patients the symptoms deteriorate significantly and they progress to severe claudication or rest pain and tissue necrosis. The overall outlook for patients presenting with claudication is poor (Dormandy 1989b) and their mortality rates at 5, 10 and 15 years are 30%, 50% and 70% respectively (figure 1.1). This is due to the strong association between arterial disease of the lower limb and similar disease in the coronary and carotid vessels which accounts for the fact that 80% of patients with peripheral vascular disease die from myocardial or cerebral infarction (Fowkes 1988).

Risk factors

The major risk factors for the development of peripheral vascular disease are smoking, hypertension and diabetes (Kannel 1976, Hughson 1978, Fowkes 1988). It is interesting that the Framingham study suggested that the risk factors for peripheral vascular disease may be slightly different to those for cardiovascular disease (Gordon 1972), and the controversy regarding the importance of lipids continues. The Framingham study suggested that hyperlipidaemia was a positive risk factor for cardiovascular disease and peripheral vascular disease. However Hughson's study from Oxford, on the prevalence and risk factors
Figure 1.1
Graph showing the fate of claudicants compared to the general population (after Dormandy 1989).
for claudication, found no association between claudication and hypercholesterolaemia but confirmed the association between claudication and both smoking and hypertension. On the other hand, Greenhalgh has reported that hypertriglyceridaemia, a controversial risk factor for ischaemic heart disease, is a positive risk factor for intermittent claudication (Greenhalgh 1971).

**Treatment**

The treatment options available for peripheral vascular disease include conservative measures and invasive procedures. "Stop smoking and keep walking" was the excellent advice given by Housley in a leading article in the British Medical Journal (Housley 1988). Stopping smoking slows the progression of the disease and allows a collateral circulation to develop (Juergens 1960, Quick 1982). Regular exercise increases the distance that the patient is able to walk before the onset of claudication, probably by dilating collateral vessels, although exercise also reduces blood viscosity (Ernst 1987).

The incidence of amputation in patients who develop claudication is known to be low and ranges between 1.6-1.8% depending on the availability of reconstructive arterial surgery (Kannel 1985, Widmer 1964). Therefore most patients with stable claudication may be reassured that they will never require amputation particularly if they stop smoking.

There are a large number of drugs, all of which are vasodilators to some degree, that have been promoted for the treatment of claudication. However there is no convincing evidence that any of these agents are effective. Many of the trials have been uncontrolled, and
even the controlled series are flawed because of the strong placebo effect of any medication and the spontaneous improvement that may occur as a collateral circulation develops (Housley 1988).

The invasive treatment of peripheral vascular disease involves revascularisation of the ischaemic limb. This is essential for patients with critical limb ischaemia, rest pain or tissue necrosis and should be undertaken with a certain degree of urgency. In addition, revascularisation should also be offered to patients with severe claudication if the disease significantly interferes with their lifestyle. However it must be remembered that these invasive procedure are not without risk and the benefits in terms of improving quality of life must outweigh the risks to both life and limb in each individual case.

Two major invasive treatment options are available - percutaneous transluminal angioplasty and reconstructive arterial surgery.

Percutaneous transluminal angioplasty

The technique of percutaneous transluminal angioplasty (PTA) was introduced in 1964 by Dotter and Judkins, and involves balloon dilation of isolated arterial stenoses or short occlusions. It is usually performed under local anaesthetic and in most cases the artery to be dilated is entered via the ipsilateral femoral artery, puncturing in an antegrade or retrograde fashion depending on the location of the lesion. Alternatively the contralateral femoral artery may be punctured and a lesion close to the inguinal ligament approached via the cross-over method. Normally the obstruction is first crossed with a guide wire before introducing the balloon, and dilation is then performed under fluoroscopic control over a period of 10-30 seconds. This leads to
fracturing of atherosclerotic plaques (Block 1985) and the resulting necrotic debris is removed by macrophages over the following weeks (Becker 1989).

The main indications for PTA include iliac, femoral and popliteal stenoses as well as short (<10cms) occlusions of the femoropopliteal segment. As technical expertise has increased, longer occlusions are being successfully treated and these include iliac occlusions and more distal lesions in the calf vessels. In addition, perioperative angioplasty is being used to dilate iliac stenoses prior to infrainguinal bypass or to improve outflow in calf vessels.

A number of new therapeutic options has also been introduced including laser angioplasty, percutaneous atherectomy and embolectomy, and the implantation of endovascular prostheses (stents). The role of these new techniques together with the precise indications and outcome of conventional angioplasty remains to be demonstrated from the ongoing clinical trials.

The major complication of PTA appears to be a re-stenosis rate of around 40% (Johnston 1982) depending on the site of the lesion. The use of anti-platelet drugs and other techniques such as endothelial cell seeding in preventing re-stenosis is currently being evaluated. However in most vascular centres, PTA has become established as the first-line treatment of stenoses or occlusions in lower limb vessels.

Reconstructive vascular surgery

Modern vascular surgery became possible following the successful introduction of a number of important developments including arteriography, heparin, blood transfusion and antibiotics. The two basic
techniques for restoring blood flow to a limb whose main artery is affected by atherosclerosis are endarterectomy and arterial bypass.

Endarterectomy involves removal of atherosclerotic disease from an artery by dissecting in the plane of cleavage between the diseased intima and the underlying normal arterial wall. This technique is most successful when the artery is large (e.g. the aortoiliac segment), the occlusion or stenosis is short, and the diseased intima splits easily from the normal arterial wall. Its use in the lower limb has almost ceased but it remains a very effective method of treating carotid artery disease.

Arterial bypass surgery involves suturing a replacement conduit (vein or prosthetic material) to the patent artery above and below the diseased segment. In the lower limb it has become an established surgical treatment of symptomatic occlusive arterial disease.
CHAPTER TWO

INFRAinguinal VEIN BYPASS GRAFTS.

Introduction

Infrainguinal bypass surgery is an effective method of revascularising an ischaemic limb which has a stenosis or occlusion of the femoropopliteal segment. If the disease is confined to the superficial femoral artery, a femoropopliteal bypass from the common femoral artery in the groin to the popliteal artery above the knee is used. Unfortunately the disease often extends below the knee into the distal popliteal artery and its branches, and in this situation a femorodistal bypass to the below knee popliteal segment, tibioperoneal trunk or a single calf vessel is required. A wide variety of conduits, including autologous saphenous vein and prosthetic materials have been used as grafts for these bypass procedures with varying degrees of success. The indications for operation, factors affecting outcome, complications and results of surgery in terms of mortality rates, graft patency rates and limb salvage are now emerging from long term follow up studies. In this chapter the femoropopliteal operation will be considered briefly and then femorodistal procedures will be discussed in detail.
Femoropopliteal bypass

The first above knee femoropopliteal bypass was performed by Kunlin in 1949. He used autologous saphenous vein as a conduit and reversed it so that the valves would not obstruct the flow of blood (Kunlin 1949, Kunlin 1951). This procedure became a very popular operation for treating symptomatic occlusive disease of the superficial femoral artery. Unfortunately, over-enthusiasm for the operation led to inappropriate surgery on many patients with mild stable claudication who would have been better treated conservatively. Post-operative failure of the graft occurred in many cases and often produced a critically ischaemic limb that required amputation. Nowadays femoropopliteal bypass is reserved for patients with severe ischaemia and in this carefully selected group it remains a very effective operation.

Femorodistal bypass

Femorodistal bypass allows revascularisation of an ischaemic limb in patients with a superficial femoral artery occlusion which extends into the popliteal trifurcation and beyond. The distal end of the graft may be anastomosed to the below knee popliteal segment, tibioperoneal trunk or a single calf vessel.

The results of these distal procedures vary considerably but in general terms the more distal the site of graft insertion, the shorter the graft survival time (Harris 1983, Denton 1983). The initial attempts at femorodistal procedures produced such poor results that many surgeons advocated primary amputation in patients with femorodistal disease rather than attempted limb salvage (Stoney 1978, Thompson 1980). Over the last few years, surgical techniques have improved, and have resulted
in progressively more distal reconstructions being successfully performed (Evans 1970).

However these distal procedures are only appropriate in patients with critical ischaemia, tissue necrosis or severe rest pain in whom the benefits of successful revascularisation outweigh the risks of the operation.

Choice of conduit

There is considerable controversy regarding the best conduit for infrainguinal bypass procedures. It has been suggested that saphenous vein should always be used because the patency rates are superior to prosthetic alternatives (Grimley 1979, Bergan 1982, Tilanus 1985, Michaels 1989). However other studies have failed to show a significant advantage for vein compared to prosthetic grafts in the above knee situation (Rosen 1986, Yashar 1981). This has led to the recommendation that a prosthetic graft should be used for an above knee femoropopliteal bypass and the saphenous vein preserved in case a femorodistal bypass procedure becomes necessary at a later date (Haimov 1979, Quinones-Baldrich 1984, Sterpetti 1985). In the femorodistal situation autologous long saphenous vein has been shown to be superior when compared to prosthetic alternatives. It has emerged as the conduit of choice and represents the "gold standard" against which all other grafts are compared.

The suitability of the vein may be assessed pre-operatively by Duplex scanning and its position marked on the skin to facilitate placement of the incision at operation. Sometimes the long saphenous vein may be unsuitable or unavailable for use (Szilagyi 1979) because of poor calibre, phlebitic change, or prior harvesting for coronary artery
bypass grafting. In this situation, it may be possible to use the saphenous vein from the opposite leg, or vein from the arm, but occasionally prosthetic grafts such as polytetrafluoroethylene (PTFE) (Veith 1978), Dacron (Stephen 1977) and glutaraldehyde-tanned human umbilical vein (HUV) (Dardik 1979, Dardik 1980) have to be used. Although the results of prosthetic grafts for femorodistal bypass are inferior to those for vein, the incorporation of a vein cuff at the distal anastomosis may improve patency (Miller 1984).

The in-situ technique of vein grafting

In the above knee situation the saphenous vein is used reversed so that the valves do not obstruct the flow of blood. However if this technique is used in the distal situation there is a size discrepancy between artery and vein graft particularly at the distal anastomosis. In addition reversing the vein results in loss of natural taper which may be haemodynamically significant, and there is a period of mural ischaemia caused by disruption of the vasa vasorum as the vein is removed.

The in-situ method of vein grafting was introduced to overcome these problems. This technique uses the saphenous vein non-reversed (in-situ) to allow a better size match between artery and vein graft at each anastomosis and the valves are then rendered incompetent by passing a valvulotome along the vein.

The first attempt at in-situ grafting was made by Charles Rob during a femoropopliteal bypass in 1959. He used the blunt end of a varicose vein stripper to destroy the venous valves, and although he achieved arterial flow down the graft the bypass failed shortly afterwards. At about the same time Karl Victor Hall in Oslo started to
devise better methods of valve disruption. Initially he surgically excised each valve leaflet through a transverse venotomy in the valve sinus which, although time consuming, resulted in the first reported successful series of in-situ grafts (Hall 1962). Following this Hall introduced a valve stripper which could be passed from the distal end of the vein and would engage and tear each valve cusp when withdrawn (Hall 1978). The subsequent introduction of better and more reliable valvulotomes has led to renewed interest in the in-situ method and it has now become the technique of choice for femorodistal bypass in many specialised vascular centres. The in-situ technique also allows the saphenous vein to be used as the bypass conduit in over 90% of femorodistal reconstructions because fewer small calibre veins are excluded (Beard 1989a).

There have been very few trials to compare the in-situ and reversed techniques of vein grafting. The study by Harris showed little difference in patency rates for the two methods, but the study only involved femoropopliteal grafts both above and below the knee and not distal reconstructions to single calf vessels (Harris 1987).

Patient selection

A femorodistal bypass procedure should only be performed in patients with critical limb ischaemia, tissue necrosis or rest pain. Recent evidence suggests that a policy of reconstruction in these patients is an economically more effective alternative to primary amputation (Cheshire 1991).

A femoropopliteal procedure has similar indications although some surgeons would also offer it to patients with severe claudication. In all cases the benefits of successful revascularisation in terms of limb
salvage, relief of rest pain and improved quality of life must outweigh the risks of the operation.

Pre-operative assessment

A careful pre-operative assessment must be made in any patient being considered for infrainguinal bypass surgery with special attention paid to both cardiorespiratory and cerebrovascular function. These patients are usually elderly with widespread vascular disease, and aggressive medical treatment of any concurrent disease is essential to optimise the patients condition before surgery.

In addition, factors which will affect future mobility should be assessed. Patients with severe limb contractures or immobility secondary to dementia may be better served by primary amputation.

The two most important pre-requisites prior to performing infrainguinal bypass surgery are the demonstration of adequate inflow of blood to the femoral artery in the groin and the identification of a patent vessel on which to site the distal anastomosis.

Traditionally, the pre-operative arteriogram has been the most widely used investigation to assess both of these important factors and it is usually adequate for an above knee femoropopliteal procedure. If there is any doubt about the adequacy of the inflow, percutaneous intra-arterial pressure measurements may be performed before and after vasodilators such as papaverine (Quinn 1975). A significant pressure drop indicates a stenosis in the aortoiliac segment which should be corrected by balloon angioplasty or reconstructive surgery prior to, or at the same time as infrainguinal bypass grafting.

Unfortunately if the patient requires a femorodistal bypass procedure, conventional pre-operative arteriography may not always
adequately demonstrate the distal calf vessels especially in the presence of severe multisegment disease or poor cardiac function (Beard 1988a). Intra-arterial digital subtraction angiography (DSA) may provide better resolution of the distal vessels, especially when combined with reactive hyperaemia (Scott 1989), but the technique is not widely available. Alternatively, auscultation at the ankle with a Doppler probe is often able to demonstrate patent calf vessels which may not be revealed by conventional arteriography or DSA, but again it is dependent on adequate perfusion pressure.

The introduction of a new technique known as pulse-generated run-off (PGR) has overcome many of these problems (Beard 1988a). PGR is performed by placing a pulsatile cuff around the patient's calf which generates blood flow in patent calf vessels as it rapidly inflates and deflates. The PGR technique allows the identification of patent calf vessels not detected by arteriography or ankle Doppler and can also demonstrate whether a calf vessel is in continuity with the pedal arch, which is an important determinant of femorodistal graft patency and allows the best vessel to be chosen for the distal anastomosis (Simms 1988). It has been suggested that the combination of intra-arterial DSA and PGR is the best method of selecting suitable patients for femorodistal bypass (Scott 1989).

The policy in Leicester

It is the policy of the academic department of surgery in Leicester to always attempt revascularisation, if possible, in patients with critical limb ischaemia, tissue necrosis, rest pain or severe claudication. All patients are assessed by conventional pre-operative arteriography and ankle Doppler pressure measurements. In the above knee
situation a prosthetic graft is always used and the vein saved for a possible future distal vascular procedure.

Those patients who require femorodistal bypass procedures are further assessed by PGR to identify patent calf vessels. The suitability of the long saphenous vein is determined by Duplex scanning and its position marked on the skin. The in-situ technique of vein grafting is the method used during the operation.

The in-situ vein femorodistal bypass operation

The operation of femorodistal bypass may be performed under general or regional anaesthesia with the patient lying supine. The lower abdomen, groin and affected limb are exposed, the skin prepared with clear antiseptic solution, and the foot is placed in a transparent bag to allow inspection during the procedure.

The groin is dissected through a slightly oblique incision to expose the common femoral, superficial femoral and profunda femoris arteries which should be controlled with slings. The oblique incision permits better exposure of the proximal long saphenous vein and the saphenofemoral junction.

Following this, the distal vessels below the knee are explored. The distal popliteal artery and tibioperoneal trunk may be exposed via a medial below knee incision. A medial mid-calf incision is used to approach the posterior tibial artery, and the peroneal artery can also be exposed with this approach by incising the interosseous membrane. If the pre-operative assessment showed the anterior tibial artery to be the best distal vessel, it may be exposed via a lateral incision at mid-calf level.
Intra-operative assessment of "run-off"

It has been established that the most important factor which decides the immediate success of a femorodistal bypass grafts is the peripheral resistance in the vessel onto which the graft is anastomosed i.e. the outflow of blood into the foot (Parvin 1985). Although it is not possible to measure this "run-off" pre-operatively, it can be measured intra-operatively and thus predict the likely success of the graft.

Therefore before proceeding with the vein bypass, the run-off of the vessel chosen for the distal anastomosis should be checked. This may be assessed either by performing an intra-operative arteriogram or measuring the resistance to blood flow.

An operative arteriogram (figure 2.1) may be performed using 20 mls. of contrast medium injected directly into the artery via a 23 G (blue) butterfly needle (Patel 1988). This will confirm the patency of the distal vessels but gives little indication of their functional capacity. A more physiological test is the measurement of resistance to blood flow. The peripheral resistance may be determined by inserting a cannula down the artery via a small arteriotomy. Then 50-100 mls. of heparinised blood is infused at a constant flow rate whilst measuring the pressure (Parvin 1985). Resistance is determined from the formula:

\[
\text{Resistance (PRU)} = \frac{\text{Pressure (mmHg)}}{\text{Flow (mls/min)}}
\]

(PRU = peripheral resistance unit)
Figure 2.1

Intra-operative pre-reconstruction distal arteriogram.
A resistance of greater than 2 peripheral resistance units (PRUs) is inevitably associated with subsequent graft failure and another site for the anastomosis should be found or the procedure abandoned (Parvin 1985). If poor run-off was anticipated and consent obtained then a primary amputation should be performed.

In-situ vein bypass

Exposure of the long saphenous vein along its entire length allows identification and ligation of all the tributaries, and reduces the risk of vein wall damage by blind passage of the valvulotome (Beard 1989a). It should also avoid the problem of missed tributaries which may form arteriovenous fistulas. However a popular alternative is to pass the valvulotome blind, and cut down at the site of each valve where a tributary is usually to be found (Gannon 1986). The proximal end of the vein is detached from the saphenofemoral junction with a cuff of femoral vein and the defect oversewn with 6/0 prolene. The common femoral, superficial femoral and profunda femoris arteries are clamped and a longitudinal arteriotomy made in the common femoral artery. A limited endarterectomy may be necessary if the femoral vessels are severely diseased. The proximal anastomosis is performed with continuous 5/0 prolene. The clamps on the femoral artery are then removed and arterial blood allowed to flow to the first valve. The distal end of the vein is divided and a 2.5mm valvulotome is then passed from the distal end along the vein and pulled back through each valve in turn. Blood will flow down the vein graft as each valve is rendered incompetent and finally emerge from the distal end of the vein. The distal anastomosis is then performed with 6/0 prolene using loupe magnification.
Completion assessment

Once a femoropopliteal or femorodistal vein bypass has been completed, it is important to check for errors. A pulse in the graft does not guarantee flow and foot pulses are rarely immediately palpable. A completion arteriogram can be simply performed via a cannula inserted into a side branch or by direct puncture of the vein graft. The graft is occluded proximally and 20mls of contrast infused down the graft with the film cassette centred on the distal anastomosis (figure 2.2). The arteriogram will pick up most technical errors such as kinking, intimal flaps at the distal anastomosis and the presence of persistent side branches or valves.

The commonly accepted alternative to completion arteriography is measurement of graft blood flow although the use of vascular endoscopy is increasing. Electromagnetic flowmeters are expensive and require careful calibration, but the recently developed Doppler flowmeter is simple to use (Beard 1988a&b). Measurement of graft pressure as well as flow permits calculation of the peripheral resistance. Injection of a vasodilator such as papaverine (15mg) down the graft increases the sensitivity of the test and a resistance of < 1PRU is satisfactory for a femorodistal bypass (Beard 1989a).

Intra-operative vasodilators

Recent attention has focused on adjuvant therapy to improve femorodistal graft patency if the intra-operative peripheral resistance is high. Iloprost, a stable prostacyclin analogue, is a powerful
Figure 2.2

Intra-operative completion arteriogram after in-situ vein femorodistal bypass graft.
vasodilator and platelet inhibitor which has been used both intra-
operatively and post-operatively to increase graft flow in an attempt to

Results of in-situ femorodistal vein grafts

The success of infrainguinal reconstructions varies according to
the severity of the disease, the expertise of the surgeons and the type
of graft used (Bell 1985). It is difficult to compare the results of
in-situ vein grafts because many of the reported series are uncontrolled
with poorly documented patients, the follow-up time and the site of the
distal anastomosis varies, and there is lack of clear distinction
between those patients operated on for claudication and those with rest
pain, tissue necrosis or critical ischaemia. In addition some series
quote primary patency rates whereas others use secondary patency rates
which include grafts that have been successfully revised. However, in
general terms, the more distal the reconstruction and therefore the
longer the graft, the higher the chances of failure.

The best reported series of femorodistal reconstructions are from
Leather in the United States with a 5 year secondary patency rate of 78%
for below-knee popliteal and 62-79% for more distal bypasses, depending
on the vessel used (Leather 1988). More typical figures in this country
are a 3 year primary patency of 45% (Budd 1990) and secondary patency of
57% (Beard 1989).

The most important factors determining early graft patency appear
to be the level of the distal anastomosis, the run-off, and the calibre
and quality of the long saphenous vein (Beard 1989, Budd 1990). For
anastomoses to calf arteries, continuity with the pedal arch appears to
be an important factor (Simms 1988).
Post-operative problems

Post-operative failure of infrainguinal vein grafts may be divided into immediate, early and late causes (Wolfe 1987a). Immediate failure occurs within the first post-operative month, early failure occurs between 1 month and 1 year and late failure occurs after the first post-operative year.

Immediate failure

Immediate failure is almost entirely due to errors of technique or judgement at the time of surgery (Whitemore 1980). This may be an imperfect anastomosis because of narrowing or an intimal flap, or failing to select correctly the site of the distal anastomosis. Most of these problems should be avoidable with the use of routine intra-operative arteriography or resistance measurements (Parvin 1985).

The graft should be closely monitored during the first 24 hours as this is when most occlusions will occur. The subcutaneous position of the graft makes it easily palpable under the thigh wound and a Doppler probe will confirm the presence or absence of flow if there is any doubt. If the graft occludes, exploration and thrombectomy is usually worthwhile but it may be difficult to determine the cause of occlusion once it has occurred.

Early failure

For many years it was thought that the poor results of infrainguinal bypass procedures compared to aortoiliac reconstructions
were due to progression of proximal or distal disease and it was therefore considered that intervention to alter outcome was not possible. However, in 1973 Szilagyi suggested that the development of intrinsic lesions in vein grafts led to narrowing of the lumen, poor blood flow and subsequent thrombosis (Szilagyi 1973). Despite this evidence, and similar findings by other authors (Whitemore 1980, Campbell 1981), it has only been relatively recently that interest in the concept of vein graft lesions has developed and much of this has come from the ability to non-invasively assess the graft post-operatively.

It is now recognised that up to 30% of infrainguinal vein grafts will develop stenoses in the period 1-12 months after surgery (Szilagyi 1973, Moody 1989, Grigg 1988a&b) and it is during this crucial period that up to 77% of failures are known to occur (Wolfe 1987a&b).

Late failure

Late failure occurs after the first post-operative year and is probably related to progression of disease in the arteries proximal or distal to the graft (DePalma 1979). Control of risk factors, particularly smoking, may slow its progression.

Vein graft surveillance

Following the demonstration that intrinsic lesions occur in vein grafts, attention has focused on post-operative monitoring and follow-up to detect these lesions and correct them before graft failure occurs. Intrinsic graft lesions may be treated by percutaneous balloon...
angioplasty, patch angioplasty, a short vein bypass or excision of the lesion and insertion of a new segment of vein. This approach has been shown to be worthwhile because revision of a functioning vein graft produces better results than either graft thrombectomy or replacing a failed graft (Berkowitz 1981). Hence the success rate of graft thrombectomy is only 50% for grafts performed for rest pain or critical ischaemia and the subsequent amputation rate is approximately 50–80% (Wolfe 1987a&b).

Therefore a number of different vascular centres have introduced graft surveillance programmes and are evaluating different methods of detecting vein graft lesions. One of the simplest tests is measurement of the ratio between the resting Doppler ankle pressure and the resting Doppler brachial pressure. This ratio is known as the ankle/brachial pressure index (ABPI) and it falls if an obstruction to the flow develops (Taylor 1977). However some authors have found this to be inaccurate since a fall in the ABPI of up to 0.15 lies within the limitations of the test (Ouriel 1982). However this problem may be partly overcome by the addition of a standard treadmill exercise test which increases the sensitivity of the test and reveals some stenoses that would otherwise have remained undetected (Laing 1980, Cohen 1986). Despite this, some authors suggest that less than 25% of angiographic stenoses are detected by ABPIs even if exercise testing is added to improve sensitivity (Grigg 1988a&b, Wolfe 1987b, Moody 1990).

The other popular screening method at present is Duplex scanning (Bandyke 1987, Bartlett 1988, Brennan 1991). Duplex scanning allows any graft identified by ABPI as being "at risk" to be further assessed. If a stenosis is detected (figure 2.3), it is confirmed by conventional arteriography before being treated by PTA (figure 2.4). Such a policy of
Figure 2.3

Colour Duplex scan of an in-situ vein graft stenosis.
Figure 2.4
Arteriogram of vein graft stenosis (arrow) before, during and after dilation with percutaneous transluminal angioplasty.
intervention before graft failure has been shown to improve long term
graft patency by up to 20% (Moody 1990, Bandyke 1987, Bartlett 1988).

However Duplex scanning has its limitations and it is often
difficult to adequately visualise the graft below the knee. Therefore
assessment at this level may have to consist of blind Doppler sampling
in the absence of a clear image. This problem may be overcome in the
future when colour-coded Duplex scanners become more widely available
(Polak 1990).

A precise assessment of any vein graft may be obtained with
standard arteriography but this is invasive. The advent of intravenous
digital subtraction angiography (IVDSA) has provided a relatively non-
invasive assessment with minimal morbidity which can be preformed on an
out-patient basis (Wolfe 1987a&b). The role of IVDSA is currently being
evaluated, but it is not widely available at present.
INTRODUCTION

Early failure of infrainguinal vein bypass grafts appears to be due to the development of intrinsic lesions within the graft. Two main types of lesions have been identified:

1. Intimal hyperplasia
2. Vein graft stenoses

Intimal hyperplasia is a diffuse process that may involve both the graft and the anastomosis (Szilagyi 1973) whereas vein graft stenoses are usually isolated lesions confined to the graft (Moody 1989). Both lesions cause luminal narrowing which leads to poor blood flow and eventual thrombosis.

The aetiology of these two lesions is unknown and it is uncertain whether they represent similar or different pathological processes. Intimal hyperplasia appears to be due to the accumulation of smooth muscle cells in the intima of the graft which causes thickening (Spray 1976, Brody 1972, McGeachie 1981). However, the histological nature of vein graft stenoses is not well documented although the few
that have been examined in Leicester have shown dense intimal fibrosis (unpublished data).

In addition the overall relationship between these two lesions is confusing. Most of the clinical reports in the literature concentrate on the importance of intimal hyperplasia particularly in reversed coronary artery bypass grafts whilst the recently introduced vein graft surveillance programmes for infrainguinal grafts have focused on vein graft stenoses.

_Intrinsic lesions in vein grafts_

The concept of intrinsic lesions in infrainguinal vein grafts was introduced in a classic paper by Szilagyi in 1973. He studied 377 reversed femoropopliteal vein grafts of which 316 were to the above-knee, and 61 were to the below knee popliteal artery. The fate of these grafts was followed over a 10 year period by angiography which was performed in the immediate post-operative period and then at 1-3 yearly intervals.

Post-operative angiograms were performed on 220 of the above-knee grafts and 40 of the below-knee grafts. These angiograms revealed that 85 intrinsic lesions had developed in these 260 grafts (33%) and that 6 different types of lesions were identified (table 1).

Intimal thickening and atherosclerosis were diffuse lesions often involving the whole length of the graft, whereas fibrous stenoses, suture stenoses and fibrotic valve cusps were usually localised.

The earliest lesions seen were suture stenoses (mean time of onset 9 months). Fibrotic valve cusps, intimal thickening and fibrous stenoses occurred in the intermediate period (14, 16 and 19 months respectively),
<table>
<thead>
<tr>
<th>Type of lesion</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>(n=260)</td>
<td></td>
</tr>
<tr>
<td>Intimal thickening</td>
<td>21 (8%)</td>
</tr>
<tr>
<td>Atherosclerosis</td>
<td>20 (8%)</td>
</tr>
<tr>
<td>Fibrotic valve cusp</td>
<td>15 (6%)</td>
</tr>
<tr>
<td>Fibrous stenosis</td>
<td>11 (4%)</td>
</tr>
<tr>
<td>Aneurysmal dilation</td>
<td>10 (4%)</td>
</tr>
<tr>
<td>Suture stenosis</td>
<td>8 (3%)</td>
</tr>
</tbody>
</table>

**Table 1**

and aneurysmal dilation and atherosclerosis occurred late (28 and 45 months respectively). All of these lesions progressed with time although there was variation in the rate of progression.

Fibrous stenoses were often located close to the anastomoses and it was suggested that these were caused by traumatic application of vascular occlusion clamps during the operation. Suture stenoses were found closely related to areas where a side-branch had been suture-ligated. They were thought to have been caused by placing the suture too close to the vein wall which resulted in luminal narrowing as it was tightened.

Histological examination was performed on grafts which were removed from patients who had died or had undergone revisional graft surgery. Twenty one samples of vein were obtained of which 17 were from above knee grafts and 4 were from below knee grafts. Eight of these samples consisted of complete grafts obtained at post-mortem and 13 consisted of segments of grafts removed at operation. The average duration of implantation of these grafts was 3 years.

Histological examination of the grafts with intimal thickening showed an overgrowth of the subendothelial smooth muscle fibres and fibrin layering on the intimal surface.

In addition, fibrous stenoses, suture stenoses and fibrotic valve cusps were found to be localised lesions with the rest of the graft being normal. In contrast, intimal thickening, atherosclerosis and aneurysmal dilation tended to be diffuse and affected long segments of the graft.
Saphenous vein histology

In order to understand the pathophysiological changes that occur in intrinsic vein graft lesions it is necessary to review the normal histological structure of the saphenous vein.

All veins contain 3 layers: the intima, media and adventitia. The intima lies adjacent to the lumen and consists of a thin monolayer of endothelial cells lying on a basement membrane of collagen. The media consists of layers of smooth muscle cells together with collagen and elastin fibres. The adventitia is the outermost layer and consists mainly of collagen bundles with bands of elastin fibres.

The saphenous vein has some unique features because of its anatomical location in the leg which creates haemodynamic stresses that are unusual for a vein. It is therefore relatively thick-walled with more smooth muscle layers and elastin than would normally be found in a vein of comparable size (Szilagyi 1973, Cheanvechai 1976).

Preparation of veins for bypass grafting

In the first week after grafting all veins show a set of characteristic histological changes which are thought to occur during removal, preparation and anastomosis of the vein to the artery and its subsequent exposure to a high pressure environment. The surgical preparation of vein grafts is different depending on whether the vein is to be used reversed or in-situ. If the vein is to be reversed it is initially exposed along its entire length, the side branches are ligated and the vein is then distended to identify leaks before removal from the surrounding tissues (figure 3.1). All of these manoeuvres involves
Figure 3.1

Preparation of a reversed saphenous vein bypass graft showing distension with saline to identify leaks.
handling the vein and in addition the vasa vasorum are disrupted which leads to ischaemia of the vein wall.

The most important aspect of the preparation of in-situ grafts is the destruction of the venous valves which would otherwise obstruct the flow of blood. This is achieved by the passage of a valvulotome (figures 3.2 and 3.3) along the vein from the distal end. As this instrument is pulled back it cuts each valve cusp which allows arterial blood to flow along the vein. In addition the side branches must be identified and ligated to prevent the development of arteriovenous fistulae which would divert the flow of arterial blood into the venous system. It is not essential to expose and mobilise the saphenous vein to perform these two manoeuvres and many surgeons prefer to pass the valvulotome blindly along the vein and cut down at the site of each valve where a side branch is usually located. However this may lead to vein injury (Gannon 1986) and others prefer to expose the vein completely which allows the valvulotome to be passed under direct vision and all side branches to identified (Beard 1989a).

Histological changes after grafting

The histological changes that occur in the first week after grafting have been mainly studied in reversed vein grafts. The most striking feature is the loss of a large proportion of the endothelial cell layer and elevation of the remaining endothelium by oedema and leucocyte infiltration (Brody 1972). The subsequent exposure of the underlying connective tissue allows deposition of fibrin, platelets and inflammatory cells (Stanley 1982, Bulkley 1977, Fuster 1979). These inflammatory cells can also infiltrate into the media and adventitia
Figure 3.2

A Hall 2.5mm valvulotome.
Figure 3.3

Intra-operative photograph showing the use of a valvulotome.
(Ramos 1976), and may even result in necrosis of smooth muscle cells

Similar histological changes of endothelial loss and smooth muscle
damage also occur in the adjacent artery from the use of vascular
occlusion clamps and the insertion of sutures at the anastomosis

The extent of this endothelial and smooth muscle cell damage is
highly variable and can range from minimal injury to necrosis and cell

Cellular regeneration

Following these changes of inflammation, mural thrombosis, and
cell death, there is a period of regeneration of cells and connective
tissue over the next few weeks. The regeneration of endothelial cells is
initially quite rapid and is then followed by a slower period of growth
(Chidi 1977). In addition, the speed of re-endothelialisation may depend
on the extent of damage which has occurred during surgery. Experimental
studies have shown that a 1cm. long piece of vein will be re-
endothelialised within 1 week (Dirrenberger 1978) and that 30-90% of the
surface of a 3-5cms. long vein graft will be re-endothelialised at 6
weeks (Ramos 1976).

The origin of the regenerating endothelial cells is debatable and
they may arise from the surviving graft tissue, the adjacent artery and
the surrounding connective tissue bed. Whilst some studies have shown
that they originate from the existing endothelium (Fonkalsrud 1978),
others have shown that when the graft is entirely denuded of endothelium
regeneration occurs from the host artery (Dilley 1983).
The smooth muscle cell changes of oedema, necrosis and infiltration by inflammatory cells persist for about two weeks after grafting (Brody 1972). Following this, fibroblasts and collagen fibres are found in increasing numbers in the media of vein grafts during the next four weeks (Spray 1976, Ramos 1976, Brody 1972, Bergeur 1980, Barboriak 1978).

The adventitia of the graft becomes incorporated into the surrounding connective tissues and it quickly becomes difficult to distinguish between the two (Bergeur 1980). The vasa vasorum are re-established within one week after grafting (Wyatt 1964, McGeachie 1981) by ingrowth of vasa vasorum from the adjacent artery and connective tissue (Brook 1974, McCune 1952).

The re-innervation of the vein graft by adrenergic nerves which also grow in from the adjacent artery and connective tissues (Meagher 1984, Waris 1984) is a longer process than re-vascularisation and does not become fully established until 2-4 weeks after the operation (Meagher 1984).

**Intimal hyperplasia**

Intimal hyperplasia was first described in 1910 by Carrel during his studies on different techniques for anastomosis of blood vessels. He noted that "within a few days after the operation, the stitches placed in making the anastomosis became covered with a glistening substance similar in appearance to the normal endothelium".

It is likely that what Carrel described at the site of anastomoses is the normal response of injured endothelium. However it is the abnormal continued proliferation and overgrowth of smooth muscle cells in response to endothelial injury that is the lesion that represents
intimal hyperplasia (DeWeese 1982). Since this original description, intimal hyperplasia has been demonstrated in arteries after peripheral and carotid endarterectomy, in vein grafts in the coronary and peripheral circulations and in prosthetic grafts.

**Intimal hyperplasia in arteries**

In 1964 Szilagyi reviewed 67 patients who had undergone aortoiliac endarterectomy for occlusive arterial disease. He found that an atheromatous-like lesion had recurred in 41% of the iliac vessels and in 15% of the aortas. Similarly in 1976 intimal hyperplasia after carotid endarterectomy was reported in a study of 29 patients who had developed recurrent stenoses between 5 months and 13 years after operation. In 9 of these patients intimal fibrosis was seen within the first post-operative year (Stoney 1976). At re-operation a pale homogenous layer with a smooth shiny surface was noted at the previous endarterectomy site and histological examination of these lesions showed fibrocytes scattered loosely in the mucopolysaccharide ground substance. In another study of 32 patients with recurrent stenoses after carotid endarterectomy, it was also noted that the recurrent lesions were located at the same site as the original endarterectomy site (Clagett 1986). A more recent review of 116 carotid endarterectomies found that 47 out of 103 cases of recurrent stenoses were caused by intimal hyperplasia (Bartlett 1987).

**Intimal hyperplasia in vein grafts**

In 1971 the first case of late occlusion of aortocoronary saphenous vein bypass grafts was reported in a patient who died 114 days...
post-operatively of cardiac failure. Despite early post-operative angiographic evidence of graft patency, post-mortem examination showed marked intimal fibrosis of the vein grafts (Grondin 1971). In a larger review of 97 aortocoronary saphenous vein grafts examined at post-mortem, intimal fibrous plaques consisting of cellular connective tissue were found in 14 out of 55 patients who had died within the first post-operative year (Bulkley 1977). In addition intimal hyperplasia in saphenous vein grafts has been reported as occurring as early as 4 weeks after coronary artery bypass surgery (Batayias 1977).

The classic description of intimal hyperplasia in infrainguinal bypass grafts as opposed to coronary artery bypass grafts was by Szilagyi and has been described earlier.

Pathological features of intimal hyperplasia in vein grafts

Intimal hyperplasia in vein grafts is a chronic structural change caused by the formation of a thickened fibrocellular layer between the endothelium and the internal elastic lamina (Dilley 1988). This process develops in all mature vein grafts and is regarded by many as an unavoidable response of the vein to grafting (Fuchs 1978, Walton 1985). The crucial event in the development of intimal hyperplasia is the accumulation of smooth muscle cells in the intima of the vein graft which proliferate and cause thickening. This process has been reported as early as 2-4 weeks after grafting (Spray 1976, Brody 1972, McGachie 1981). The origin of these smooth muscle cells is unclear although it seems likely that they are medial smooth muscle cells which migrate into the intima. It has been also been suggested that smooth muscle cells may already be present in the intima of a donor vein (Szilagyi 1973, Spray 1976, Cheanvechai 1976, Lawrie 1976), and that
these cells proliferate after grafting. However myointimal cells are not present in all veins used for grafting but all vein grafts subsequently develop intimal hyperplasia. Others authors have suggested that cells from the adjacent artery or connective tissue contribute to intimal hyperplasia in the graft (Spaeet 1975) in a similar manner to that seen in the neointima that develops in prosthetic grafts (Madras 1981, Poole 1958, Ghidei 1968, Clowes 1985). Another possible contribution is the deposition and organisation of thrombotic material from the circulating blood on the intimal surface of the vein graft (Szilagyi 1973, Unni 1974, Hutchins 1980, Bulkley 1977, Jones 1973).

Vein graft stenoses

The first reported case of a vein graft stenosis was by Breslau in 1965. He described a stenosis in the mid-portion of a reversed femoropopliteal vein graft 16 months after implantation that appeared to be related to a fibrotic valve. In 1971 Downs reported 8 cases of localised stenoses in vein grafts that he also attributed to fibrotic valves. Once again the classic description of vein graft stenoses was by Szilagyi in 1973. However in more recent times the concept of these lesions being fibrosed valve leaflets has been challenged, and in particular Bosher in 1979 suggested that vein graft stenoses occurred separate from the sites of the venous valves and that the pathogenesis was related to local wall trauma.

Recently there has been renewed interest in vein graft stenoses. This is due to the increasing interest in in-situ femorodistal vein bypass techniques and the development of vein graft surveillance programmes which allow follow-up of these grafts (Grigg 1988a&b, Wolfe 1987a&b, Moody 1989, Moody 1990, Berkowitz 1981). The incidence of
vein graft stenoses has been reported to be between 11-33% (Berkowitz 1981, Sladen 1981, Whitney 1976). The histology of these lesions remains unclear mainly because most have been treated by balloon angioplasty, patch angioplasty or a short reversed vein bypass graft. The stenosis has therefore not been excised and subjected to histological examination. However there have been a few reports that claim that they are similar to the intimal and smooth muscle hyperplasia as described earlier (Szilagyi 1973, Bulkley 1977, Lawrie 1976). This has not been our experience in Leicester and the few that we have examined histologically have shown dense intimal fibrosis (unpublished data)(figure 3.4).

**Aetiology of intrinsic vein graft lesions**

In 1973 Szilagyi not only documented the occurrence of intrinsic vein graft lesions but also speculated about their aetiology, and suggested that injury to the vein during surgical preparation might be a causative factor. He emphasised the importance of avoiding traumatic handling of the vein and minimising the warm ischaemia period during which the vein is lifted from the surrounding tissue and deprived of its blood supply. He proposed that blood should be allowed to flow through the vein for as long as possible while the tributaries are dissected and ligated and that the vein should be immediately placed in a cold solution of isotonic glucose after removal. In addition he stressed the importance of close post-operative monitoring and surveillance of vein grafts preferably by angiography.

He went on to suggest that these techniques of careful handling of the vein at operation would allow intrinsic lesions to be partly avoided, and graft surveillance would allow lesions to be identified and
Figure 3.4

Histology of an in-situ vein graft stenosis showing intimal thickening and dense intimal fibrosis.
corrected before graft failure occurred. While Szilagyi's original
time of vein wall damage is still accepted, the ways in which this
damage occurs has been challenged. In particular there is doubt
regarding the relevance of surgical clamps, side-branch ligation and
fibrosis of valve cusps.

A recent study by Moody of 74 femoropopliteal vein grafts involved
intra-operative marking of the sites of side branches, valves and
location of clamps with radio-opaque surgical clips placed in the
surrounding fat (Moody 1991). During the follow-up period 22 stenoses
were identified in 18 grafts by Duplex scanning. Only one coincided with
a previously marked area and this was the site of a valve. In the 34
in-situ grafts in this study, 10 residual valve cusps were identified by
Duplex in 7 grafts, but there was no evidence of turbulent flow at these
points. It was concluded that there is no correlation between valve
sites, side-branches, clamp sites or residual valve cusps and the
development of vein graft stenoses. It was also suggested that
endothelial injury during surgical preparation of the vein may be a more
likely cause of subsequent vein graft stenoses.

This concept of endothelial injury causing the subsequent
development of vein graft lesions is not new. Infact Ross who originally
proposed the "injury hypothesis" theory of atherosclerosis believes that
intimal hyperplasia may be an early lesion on the pathway to the
formation of an atherosclerotic plaque (Ross 1976).

Recent attention has also focused on the relationship between
smooth muscle cell proliferation and endothelial injury. This concept
has been investigated by Angelini who has developed an organ culture
system for human saphenous vein (Angelini 1991). Using this technique,
the basis of intimal proliferation can be studied in segments of vein
which remain viable in tissue culture. Angelini documented smooth muscle
proliferation and intimal thickening in veins after 14 days in culture. In addition, veins in which the endothelium was removed showed reduced intimal thickening whereas veins that had been fully prepared for use as reversed coronary artery bypass grafts produced marked intimal thickening. It was suggested that surgical preparation of reversed vein grafts caused not only endothelial injury, but smooth muscle injury as well, which was followed by smooth muscle cell proliferation and intimal hyperplasia.

Thus it would seem that further studies are required to define the histological nature of vein graft stenoses and the role of various aetiological factors in the subsequent development of intrinsic vein graft lesions. In particular the importance of endothelial and smooth muscle cell injury needs to be determined.
CHAPTER FOUR

ENDOTHELUM AND VASCULAR TONE.

Introduction

Vascular endothelium is a structurally simple but functionally complex tissue. Although only one cell thick it constitutes a dynamic interface between the blood and the rest of the body. Until relatively recently vascular endothelium was thought to act merely as a semi-permeable, non-thrombogenic lining of blood vessels. However over the last few years it has been realised that the endothelial cell is far more important than was previously considered. It is now known that these cells have several important functions which include regulation of coagulation, regulation of the inflammatory and immune responses, and regulation of vascular tone (table 2).

The regulation of vascular tone is one of the most important roles of vascular endothelium and involves interactions with several different families of vasoactive agents (figure 4.1). These include the renin-angiotensin-aldosterone system, the kallikrein-kinin system, prostaglandins, prostacyclin and endothelium-derived relaxing factor (EDRF).
Table 2

Functions of endothelial cells.
Figure 4.1

Interactions between the renin-angiotensin-aldosterone, kallikrein-kinin and prostaglandin systems.
The renin-angiotensin-aldosterone system

Renin is a glycoprotein which is released by the juxtaglomerular cells of the kidney and acts on the plasma α2 globulin angiotensinogen to produce a decapeptide angiotensin I. Angiotensin I is converted to its active form, angiotensin II by the action of the endothelial-derived angiotensin converting enzyme (ACE). Angiotensin II, an octapeptide, has several important actions. It acts on vascular smooth muscle to produce marked vasoconstriction, it directly suppresses renin release and it stimulates aldosterone secretion. Aldosterone is produced by the adrenal cortex and acts mainly to increase sodium re-uptake in the kidney which in turn leads to an increase in the extracellular fluid volume by osmoregulation.

Renin release is stimulated by a fall in blood pressure or extracellular volume. The subsequent production of angiotensin II increases total peripheral resistance by its vasoconstrictive actions and therefore restores the blood pressure towards normal.

The kallikrein-kinin system

Kallikreins are proteolytic enzymes which act on a number of circulating globulins called kininogens to produce several vasodilator peptides of which the most important is bradykinin. Bradykinin is released in response to local tissue injury and is an important mediator of local tissue reactions. Bradykinin causes dilation of blood vessels both directly and by release of additional mediators such as vasodilator prostaglandins (PGI₂, PGE₂) and EDHF. Bradykinin is inactivated by several enzymes but most notably ACE.
Prostaglandins

Prostaglandins are a group of vasoactive eicosanoids which are produced by vascular endothelial cells and act on vascular smooth muscle. They are formed from membrane phospholipids by the action of phospholipases which release arachidonic acid. The two most important prostaglandins are PGE$_2$ and PGI$_2$ (prostacyclin), both of which cause relaxation of vascular smooth muscle. The release of PGE$_2$ and PGI$_2$ is also stimulated by bradykinin.

Prostacyclin

In 1976 Vane and his colleagues discovered that endothelial cells produce a substance that they called prostacyclin (Moncada 1975). Prostacyclin is a bicyclic eicosanoid which is formed from arachidonic acid by the cyclic endoperoxide prostaglandin synthetase. Arachidonic acid, the precursor of prostacyclin, is produced from endothelial phospholipids by phospholipase A$_2$. This release is stimulated by a number of factors including stress exerted on the cell membrane, thrombin, platelet-derived growth factor and adenine nucleotides. Prostacyclin is released on both the mural and luminal sides of the endothelial cell and its half-life in the bloodstream is less than 1-2 minutes. The main actions of prostacyclin are vasodilation and inhibition of platelet aggregation, and these effects are mediated by the intracellular formation of cyclic adenosine monophosphate (c-AMP)(Higgs 1983, Vane 1987).
Endothelium-derived relaxing factor (EDRF)

In 1980 Furchgott and Zawadzki discovered by accident that the vascular relaxation induced by acetylcholine on rabbit aorta was dependent on the presence of endothelium and that this effect was mediated by a substance released from endothelial cells, which was later known as EDRF (Furchgott 1980). They demonstrated that removal of the endothelium (by rubbing) prevented the relaxing action of acetylcholine. This discovery of an endothelial-derived relaxing factor finally gave a satisfactory explanation to the apparent paradox of the divergent in vivo and in vitro responses to acetylcholine and histamine. Whilst both agents act as vasodilators in vivo they may cause contraction of arteries in organ baths because most of the endothelial cells are removed during cutting and preparation of the vessel.

Endothelium-dependent relaxation has subsequently been demonstrated in many vascular preparations including arteries, veins and microvessels. It occurs in response to a variety of substances such as acetylcholine, adenine nucleotides, thrombin, substance P, histamine, and the calcium ionophore A23187. In addition other stimuli such as hypoxia, increased flow and electrical stimulation also cause endothelial-dependent relaxation of vascular tissues in vitro. However some compounds such as the nitrovasodilators (sodium nitroprusside, glyceryl trinitrate) atrial naturetic factor, β-adrenergic agonists and prostacyclin cause endothelium-independent relaxation (Furchgott 1984, Griffith 1984, Busse 1985, Moncada 1986).

Several ingenious approaches have been used to establish that EDRF is released from endothelial cells. These have involved demonstrating that a biologically active substance is transferred from a donor to a
detector tissue. One system consisted of a "sandwich" arrangement of two rabbit aortic strips placed intima to intima. The EDRF donor had intact endothelium and the detector was denuded of endothelium (Furchgott 1984). Acetylcholine was shown to stimulate EDRF release from the donor and to cause relaxation of the pre-contracted detector tissue. Other approaches have included a perfusion system in which a donor rabbit aorta was placed in series with a detector (endothelium removed) ring of vascular tissue. Stimulation of the donor with acetylcholine again caused relaxation of the detector (Griffith 1984, Rubany 1985).

These techniques have established that EDRF is a short-lived substance with a half-life of only a few seconds in oxygenated physiological salt solutions (Griffith 1984, Cocks 1985). It is released under basal conditions and after stimulation with substances such as acetylcholine (Griffith 1984, Rubany 1985, Martin 1985). The effects of EDRF are mediated by stimulation of the soluble guanylate cyclase with a consequent elevation of intracellular cyclic-GMP levels (Rapoport 1983). Thus EDRF, like prostacyclin, is dependent on an intracellular second messenger system.

In addition to vasodilation, EDRF has been shown to inhibit platelet aggregation (Azuma 1986, Furlong 1987, Radomski 1987a), and to cause disaggregation of already aggregated platelets. These actions of EDRF on platelets may be synergistic with those of prostacyclin which performs similar functions (Radomski 1987b).

EDRF and nitric oxide

The original suggestion that EDRF was prostacyclin was ruled out by demonstrating that the vasodilator response was not blocked by the cyclo-oxygenase inhibitor indomethacin (Furchgott 1987). Other early
suggestions included a product of the arachidonic acid lipoxygenase (Singer 1983, Forstermann 1984) or of the cytochrome P-450 system (Pinto 1986).

Then in 1988 Furchgott suggested that EDRF may be nitric oxide (NO) (Furchgott 1988) and at the same time Ignarro came to the same conclusion that EDRF may be NO or a closely related species (Ignarro 1988). It has since been established that nitric oxide is synthesized from the amino acid L-arginine by an enzyme known as the NO synthase (Palmer 1988).

The evidence that supports these suggestions comes from demonstrations of the similarities between EDRF and NO. Thus it has been shown that EDRF and NO release from vascular endothelium can be detected by the same chemical means which involves measuring the chemiluminescent product of their reaction with ozone (Downes 1976). In addition, the biological actions of EDRF and NO on vascular strips (Palmer 1987, Hutchinson 1987) and platelets (Radomski 1987a) have also shown that the two compounds are indistinguishable (Moncada 1988b). Both EDRF and NO cause relaxation of vascular strips that declines at the same rate during passage down a bioassay cascade (Palmer 1987) and both EDRF and NO act on vascular smooth muscle (Rapoport 1983) and platelets (Mellion 1981) through the stimulation of the soluble guanylate cyclase and which leads to elevation of cyclic-GMP levels.

The importance of EDRF

The implications of the existence of EDRF mediated vasodilation are numerous. It is regarded by many as the endogenous nitrovasodilator (Moncada 1988a) and is responsible for the vasodilator tone which exists throughout the cardiovascular system. This EDRF-dependent tone allows
flow in the system to be locally regulated. In addition EDRF may play an important protective role in preventing thrombosis. If platelets should start to aggregate in a vessel, their release of products such as ADP and serotonin would lead to EDRF-mediated vasodilation and the consequent increase in flow would tend to flush away any developing thrombus (figure 4.2).

The loss of EDRF-mediated tone may be important in certain cardiovascular diseases. Atherosclerosis is associated with localised impairment of the release or effects of EDRF and thus increases the risks of vasoconstriction and thrombosis (Frieman 1986, Ludmer 1986, Cohen 1983). A reduction in the release of EDRF has been demonstrated in atherosclerotic human coronary arteries (Forstermann 1986) and the response of the coronary circulation to acetylcholine is decreased in patients with coronary artery disease (Drexler 1989). This may be an important mechanism in coronary artery vasospasm where functional endothelial injury is present in the absence of positive angiographic findings. There is also evidence that a deficiency in EDRF-mediated vasodilation may be present in essential hypertension (Linder 1990, Panza 1990) and if generalised might contribute to increased blood pressure (Rees 1989, Vallance 1989a).

EDRF release from veins

It has been established that EDRF is also released from the endothelium of human veins (Collier 1990, Vallance 1989b) but this release is less than that seen in arteries (Thom 1987, Luscher 1988). It has been shown that the maximum degree of acetylcholine induced endothelium-dependent vasodilation in superficial veins is only 30% of that seen in arteries (Luscher 1988). This is contrast to the larger
Figure 4.2
The effects of loss or injury to endothelium on EDRF regulation of vascular tone.
endothelium-dependent response produced by bradykinin and the
endothelium-independent responses of glyceryl trinitrate both of which
are said to produce full vasodilation (Collier 1972, Collier 1978).
These responses may reflect differences in the ability of the vessel to
synthesize or release EDRF or differences in acetylcholine receptors on
endothelial and smooth muscle cells in arteries and veins.

Functional endothelial injury

In the "endothelial injury" theory of atherosclerosis, Ross
discusses the problems of defining endothelial injury (Ross 1986).
Whilst severe injury cause loss of endothelial cells which is easily
detectable by histological techniques, it is also likely that less
severe injury causes functional endothelial cell injury without cell
loss. This non-denuding type of injury will be reflected in the ability
of endothelial cells to produce substance such as prostacyclin and EDRF.
Therefore the release of these two substances may be used as markers of
endothelial injury and also give a quantitative assessment of its
severity.

This concept has been applied to determine endothelial injury
during preparation of reversed saphenous coronary artery bypass vein
grafts. A comprehensive study of functional injury caused by preparation
of these veins has been made by Angelini and his colleagues. Initially
they studied metabolites involved in cellular energy production as
markers of tissue injury (Angelini 1985). It is known that both hypoxia
and ischaemia are associated with a fall in the concentrations of ATP
(Levitsky 1975) with a consequent rise in the production of degradation
products such as AMP, adenosine, inosine and hypoxanthine. They found
that surgical preparation of the vein which included stripping of the
adventitial layer, side branch ligation and distension with blood and then storage in the patient's own heparinised blood at room temperature were all associated with metabolic damage. In particular uncontrolled distension of the vein was very traumatic. They also looked specifically at endothelial function in terms of prostacyclin and EDRF release. Prostacyclin production was significantly reduced in reversed veins prepared for bypass grafting when compared to controls (Angelini 1987a).

EDRF release was studied using a bioassay cascade system in which a human saphenous vein sample was used as an EDRF donor and an endothelium-denuded porcine coronary artery ring was used as the detector (Angelini 1989). They compared veins that had undergone minimal surgical handling with veins that had been fully prepared for reversed bypass grafting. There was a significant reduction in EDRF release from surgically prepared veins compared to controls and that this impaired release of EDRF was also associated with some loss of endothelial cell coverage. On the basis of their studies they have stressed the importance of handling veins carefully during surgery so as to leave the vein with a metabolically intact endothelium (LoGerfo 1981, Angelini 1987b, Gottlab 1977). These findings of reduced EDRF production after preparation of reversed vein grafts have been supported by the work of others (Lawrie 1990).

However it must be stressed that these findings apply to reversed veins used for coronary artery bypass grafting and as yet there have not been any major human studies of cellular damage associated with the in-situ technique of femorodistal bypass grafting.
CHAPTER FIVE

AIMS, MATERIALS AND METHODS.

Introduction

Femorodistal bypass has become an established method of treatment of lower limb ischaemia in patients with a superficial femoral artery occlusion that extends into the popliteal artery and its branches. The saphenous vein has emerged as the "gold standard" conduit for these distal reconstructions and the in-situ technique of vein grafting has solved many of the technical problems associated with difficult anastomoses onto small vessels. However the problems of intrinsic vein graft lesions remain, and they represent the major cause of failure in the first post-operative year. Evidence is now emerging from studies of reversed vein grafts that surgical injury during preparation of the vein may be important in the development of these lesions. Experimental studies have shown that both structural and more recently functional injury occurs to both the endothelial and smooth muscle layers.

However surgical injury during preparation of in-situ vein grafts has not been extensively investigated. The preparation of in-situ vein grafts differs from that for reversed vein grafts and there are several intra-operative manoeuvres which are specific to femorodistal bypass procedures. These include the warm ischaemic period following
mobilisation of the vein, the use of a valvulotome to destroy the valves, the use of X-ray contrast media for intra-operative arteriography and the use of intra-operative vasodilators such as papaverine and iloprost.

It is important to establish the effects of these procedures on the endothelium and smooth muscle in order to provide a basis for future work on the aetiology of in-situ vein graft lesions.

Aims

The aims of the experimental work presented in this thesis are to investigate the degree of endothelial and smooth muscle damage that occurs after preparation of veins for in-situ femorodistal bypass surgery. In particular the following questions will be answered:

1. Does surgical handling and valvulotome use cause endothelial and smooth muscle cell damage to in-situ vein grafts.

2. Does the use of X-ray contrast medium (Niopam) damage the endothelium and smooth muscle cells.

3. Does the intra-operative use of vasodilators (papaverine and iloprost) damage the endothelial and smooth muscle cells.
Methods of studying endothelial and smooth muscle cells

There are a number of different techniques which allow investigation of endothelial and smooth muscle cell damage in vein grafts. These include both structural and functional assessment:

Investigation of structure

One of the simplest ways of investigating endothelial and smooth muscle cell structure is histological study. The saphenous vein can be fixed in formaldehyde, stained with Haematoxylin and Eosin (H&E) or Elastin Van Gieson (EVG), set in wax and thin sections cut with a microtome. When viewed under a light microscope both the endothelial cell lining and the smooth muscle cells of the media may be visualised. In addition special stains may be used to demonstrate the presence of endothelial cells and these include the monoclonal antibodies Q Bend 10, factor VIII and Von Willebrands factor.

More detailed assessment of structure may be obtained by scanning electron microscopy (SEM). This involves more complex preparation of the vein including initial fixation in glutaraldehyde, dehydration with increasing concentrations of alcohol, osmium staining, critical point drying and sputter-coating with gold.

Investigation of function

Endothelial function may be quantitatively assessed by the measurement of EDRF or prostacyclin production by endothelial cells. The
impaired production of either of these substances allows a quantatative assessment to be made of functional endothelial damage.

Endothelium-dependent relaxation and EDRF release may be measured using an organ bath system involving rings or strips of vascular tissue. In addition, if contraction studies are combined with these relaxation studies, then an assessment may also be made of the functional integrity of the underlying smooth muscle.

The organ chamber

An organ chamber apparatus was designed to allow contraction and relaxation studies to be performed on segments of saphenous vein (figure 5.1 and 5.2). This consisted of a glass organ bath (Palmer Bioscience Ltd, Kent, UK) which was filled with physiological salt solution (composition: NaCl 118mM; NaHCO₃ 25mM; KCl 4.5 mM; KH₂PO₄ 1mM; CaCl₂ 2.5mM; MgSO₄.7H₂O 1mM; glucose 6mM) from a reservoir. The temperature in the organ bath was constantly monitored and maintained at 37°C by allowing heated water to circulate through the outer glass jacket of the bath. In addition the physiological salt solution from the reservoir passed through a heating coil before entering the bath. The reservoir and organ bath were gassed with a 5% CO₂ / 95% O₂ mixture to achieve a pH of 7.45.

Collection of samples

Samples of saphenous vein were obtained from patients undergoing infrainguinal or coronary artery bypass surgery. In all cases consent was obtained from the patient and the studies performed had the approval
Figure 5.1

Photograph of the organ chamber apparatus.
Figure 5.2
Diagrammatic illustration of a vein mounted on a plate and suspended in a heated, gassed organ bath.
of the local ethical committee. The samples of vein were collected from the operating theatre and immediately placed in cold (4°C) calcium-free physiological salt solution. The samples were transported from theatre to the laboratory in a sealed container surrounded by crushed ice.

**Laboratory preparation of vein samples**

In the laboratory the sample of vein was placed in a dish containing cold (4°C) calcium-free physiological salt solution and viewed under a light microscope. Any excess fat was carefully dissected off the adventitia using micro-dissection scissors and forceps. Each end of the vein, which had usually been handled by the surgeon, was cut off to leave a segment of vein approximately 5mm. in length. This segment was then cut open along its long axis and opened out to from a rectangular strip. Extreme care was taken to avoid damaging the endothelial surface during this manoeuvre. A 6/0 suture was then passed through the mid-point of one of the long edges of the vein strip. The strip of vein was then fixed to a stainless steel plate, endothelial surface adjacent to the plate, by passing the opposite long edge of the vein over two sharpened pins on the plate. The plate and attached piece of vein were then immersed in the organ bath and the suture from the top edge was fixed to a pressure transducer (Palmer Bioscience Ltd, Kent, UK) which measured isometric tension. The pressure transducer was connected to a Washington MD2 two channel chart recorder (Palmer Bioscience Ltd, Kent UK) to allow a graphical record of force to be made.
Optimum passive tension

An equilibrium period of 1 hour was allowed to elapse during which the physiological salt solution (PSS) in the organ bath was changed 2-3 times. The passive tension on the vein was then set to 1 gramme by tightening a micrometer gauge to which the suture from the vein was attached. Following this the vein was then exposed to noradrenaline $10^{-7}$M (Sigma Chemicals Co. Ltd, Dorset, UK) and the force of contraction recorded. Then the vein was rinsed with PSS from the reservoir and the tension allowed to return to 1 gramme. After a period of 10 minutes the passive tension was increased to 2 grammes and the contraction to noradrenaline $10^{-7}$M recorded. This was repeated until the optimum passive tension that would elicit the maximum response to noradrenaline was found. This passive tension was usually between 1-3 grammes.

Contraction studies

After the passive tension had been set to give the best response to noradrenaline, a further equilibrium period of 1 hour was allowed to elapse during which the vein was rinsed several times by changing the PSS solution. A cumulative logarithmic dose contraction curve to noradrenaline ($10^{-8}$ to $10^{-5}$M) was then performed in the presence of cocaine ($10^{-6}$M) (Sigma Chemicals Co. Ltd, Dorset, UK). The cocaine was used to prevent neuronal re-uptake of noradrenaline so that the maximum response for each particular dose was produced. A typical dose contraction curve for noradrenaline is shown in figure 5.3.

In some cases the contractile response of the vein to high potassium (123 mM) PSS was determined. This was achieved by replacing the standard PSS in the organ bath with high potassium PSS which had
Figure 5.3
Diagrammatic illustration of a cumulative noradrenaline dose contraction curve.
previously been heated to 37°C in a water bath and gassed with a 95% 
O₂/5% CO₂ mixture. High potassium PSS causes contraction of vascular 
smooth muscle by a direct depolarising action on the cell membrane. 
This response is usually preserved despite extensive cellular damage and 
is therefore useful to check viability of severely damaged veins.

**Relaxation studies**

After completion of the noradrenaline curve the vein was rinsed 
with PSS and allowed to return to the previously set passive tension. 
After an interval of 20 minutes relaxation curves were performed and a 
similar interval of 20 minutes was allowed to elapse between curves. The 
relaxation curves were performed after a half-maximal contraction had 
been produced by exposure to a suitable dose of noradrenaline.

A variety of pharmacological substances were used including the 
endothelium-dependent agents acetylcholine (10⁻⁸ to 10⁻⁵M), bradykinin 
(10⁻⁹ to 10⁻⁶M), adenosine (10⁻⁸ to 10⁻⁵M), histamine (10⁻⁹ to 10⁻⁵M), 
and the endothelium-independent agent sodium nitroprusside 
(10⁻⁹ to 10⁻⁵M) (Sigma Chemicals Co. Ltd, Dorset, UK). 
A typical curve demonstrating endothelial-dependant relaxation produced 
by acetylcholine is shown in figure 5.4.

Following completion of the relaxation curves the vein was fixed 
under tension with either 2% buffered glutaraldehyde or formaldehyde 
(Sigma Chemicals Co. Ltd, Dorset, UK) and stored at 4°C prior to further 
processing for histological assessment by light or scanning electron 
microscopy (SEM).
Figure 5.4
Diagrammatic illustration of endothelium-dependent relaxation produced by acetylcholine after initial half-maximal contraction with noradrenaline.
Calculation of results

The pressure transducer which measured the force of contraction or relaxation was initially calibrated so that the deflection produced on the chart recorder by a given force was known. Then after each cumulative dose of noradrenaline the deflection was recorded and the force generated was calculated in grammes. The relaxation responses were calculated as percentages of the initial half-maximal contractions to noradrenaline.

Expression of data

The noradrenaline contractions have been expressed in graph form plotting force of contraction against log doses of noradrenaline, and the potassium contraction responses have been expressed as histograms. The relaxation responses have been expressed as graphs of percentage relaxation against dose. In all cases the mean ± the standard error of the mean has been used to show the variation in the responses.

Statistical analysis

The concentration of noradrenaline required to produce a half maximal contraction (ED_{50}) and the maximum response to noradrenaline for each of the various control groups was calculated. This data was then plotted as a histogram and both the maximum responses and ED_{50}s produced a skew distribution. The data was therefore regarded as not normally distributed and non-parametric statistical analysis performed.
The dose contraction curves to noradrenaline were analysed for differences in maximum contraction and ED_{50}s using the Mann-Whitney U-test for unpaired samples and the Wilcoxon matched-pairs test for paired samples. The maximum relaxation responses are reported and the data was analysed by calculating the areas under the relaxation curves produced for each vein sample. These areas were then compared using the Mann-Whitney or Wilcoxon tests. All tests were performed using the Unistat-III statistical package (Unistat Ltd, London, UK) and an Atari PC3 personal computer (Atari Corporation, Sunnyvale, USA).

The effects of transport temperature

The initial studies with the organ bath technique were performed in order to establish 2 facts:

1. Are the methods reliable and reproducible?
2. What is the optimum transport temperature for the vein?

Samples of saphenous vein were collected from 8 patients undergoing coronary artery bypass grafts. The veins were obtained by careful dissection from the lower calf region immediately after the start of the vein harvest procedure. Two segments of vein were obtained from each patient: one was placed in calcium-free PSS at room temperature (24°C) for transport to the laboratory and the other was placed in calcium-free PSS at 4°C and the container transported in an ice bucket containing crushed ice. In the laboratory each sample of vein was dissected and prepared in calcium-free PSS at either 4°C or 24°C. The samples of vein were then placed in the organ bath and allowed to
reach 37°C over the following hour. After the optimum passive tension had been set to noradrenaline the responses to the following agents were studied:

- Noradrenaline ($10^{-8}$ to $10^{-5}$M) with cocaine ($10^{-6}$M)
- High potassium (123 mM) PSS
- Acetylcholine ($10^{-8}$ to $10^{-5}$M)
- Acetylcholine ($10^{-8}$ to $10^{-5}$M) with Indomethacin ($10^{-5}$M)
- Sodium nitroprusside ($10^{-9}$ to $10^{-5}$M)

Relaxation responses to acetylcholine were studied in the presence of indomethacin (Sigma Chemicals Co. Ltd, Dorset, UK) in order to ascertain whether the venous endothelium was releasing contracting factors such as thromboxane. Any effects due to thromboxane are blocked by indomethacin which is a prostaglandin synthetase inhibitor. In this situation, the relaxation response to acetylcholine will be enhanced by the presence of indomethacin.

**Results**

The mean age of the patients in this study was 63 ± 2 years and their mean blood pressure was 108 ± 3 mmHg. These patients were not hypertensive nor diabetic.

The contraction response to high potassium (123 mM) PSS is shown in figure 5.5. The 24°C group of veins produced larger contractions than the 4°C group (9.81 ± 1.88g and 7.87 ± 1.72 g respectively). However, these differences did not reach statistical significance ($p>0.05$, Wilcoxon matched-pairs).
Figure 5.5

Contraction responses to high potassium (123 mM) PSS in veins at 4°C and 24°C.
The contractile responses to noradrenaline are shown in figure 5.5. The 24°C group produced larger maximum contractions than the 4°C group (10.27 ± 1.86g versus 8.31 ± 1.41g respectively) but again these differences were not significantly different (p>0.05, Wilcoxon matched-pairs).

Similarly the ED$_{50}$s for the 2 groups (table 3) were not significantly different (p>0.05, Wilcoxon matched-pairs).

<table>
<thead>
<tr>
<th>K$^+$ (g)</th>
<th>NA max (g)</th>
<th>ED$_{50}$ (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4°C</td>
<td>7.87 ± 1.72</td>
<td>8.31 ± 1.41</td>
</tr>
<tr>
<td>24°C</td>
<td>9.81 ± 1.88</td>
<td>10.27 ± 1.86</td>
</tr>
</tbody>
</table>

Table 3
Maximum contractions produced by high potassium (123mM) PSS (K$^+$) and noradrenaline (NA) and ED$_{50}$s for noradrenaline produced by veins at 4°C and 24°C.

The relaxation responses to acetylcholine, acetylcholine with indomethacin and sodium nitroprusside are shown in figures 5.7, 5.8 and 5.9. The veins at 4°C produced better relaxation responses than the 24°C group when exposed to acetylcholine alone and in the presence of indomethacin. In addition the relaxation response to acetylcholine was reduced by indomethacin. In the case of sodium nitroprusside the relaxation response was slightly better for the 24°C group. The maximum contractile response was produced by the 10$^{-6}$M dose of acetylcholine and the 10$^{-5}$M doses of acetylcholine with indomethacin and sodium.
nitroprusside (table 4). However none of these differences reached statistical significance (p>0.05, Wilcoxon matched-pairs).

<table>
<thead>
<tr>
<th></th>
<th>ACH (%)</th>
<th>ACH/Indo (%)</th>
<th>NAP (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4°C</td>
<td>51.13 ± 9.79</td>
<td>36.38 ± 11.98</td>
<td>95.88 ± 3.59</td>
</tr>
<tr>
<td>24°C</td>
<td>35.00 ± 9.5</td>
<td>32.38 ± 9.34</td>
<td>99.38 ± 0.63</td>
</tr>
</tbody>
</table>

Table 4:
Maximum relaxation responses produced by acetylcholine (ACH), acetylcholine with indomethacin (ACH/Indo) and sodium nitroprusside (NAP) in veins at 4°C and 24°C.
Figure 5.6
Cumulative dose contraction curve to noradrenaline in veins at 4°C and 24°C.
Figure 5.7

Endothelium-dependent relaxation with acetylcholine in veins at 4°C and 24°C.
Acetylcholine (M) + Indomethacin (10^{-5} M)

Figure 5.8

Endothelium-dependent relaxation with acetylcholine in the presence of indomethacin in veins at 4°C and 24°C.
Endothelium-independent relaxation with sodium nitroprusside in veins at 4°C and 24°C.
Discussion

These results show that it is possible to study contraction and relaxation responses of vein samples in the organ bath and that the results are reliable and reproducible. The contractile responses of the vein samples to agents such as noradrenaline and high potassium PSS allow a quantitative assessment to be made of the integrity of the smooth muscle cells. If smooth muscle damage is present, then the contractile responses to both of these agents will be impaired. As noradrenaline acts on the α-receptors of the smooth muscle cell, the contractile response to noradrenaline is a very sensitive indicator of cellular injury. On the other hand, high potassium PSS has a direct depolarizing effect on the smooth muscle cell membrane and is not dependent on the presence of an intact receptor. This response tends to be preserved until severe damage has occurred and it is therefore a useful method of detecting whether any viable smooth muscle remains if the response to noradrenaline is absent.

The organ bath system also allows the functional integrity of the endothelium to be assessed by relaxation responses to acetylcholine and other endothelium-dependent vasodilators. Acetylcholine acts on the muscarinic receptors on the endothelial cell surface and causes release of endothelial-derived relaxing factor (EDRF) which leads to relaxation of the underlying smooth muscle. Damage to the endothelium, which may result in both endothelial cell loss and functional impairment of the remaining endothelial cells will be reflected in an impaired relaxation response. If there is no detectable response to endothelium-dependent relaxing agents, it is important to ensure that the smooth muscle cells are still capable of relaxing. This may be determined by eliciting a relaxation response to sodium nitroprusside which is an endothelium-
independent vasodilator acting directly upon the smooth muscle cells and whose actions are not affected by the presence or absence of endothelium.

For these studies to assess the degree of damage caused to the vein by various intra-operative manoeuvres, it is obviously important not to cause any additional injury during preparation of the vein in the laboratory. Therefore it is important that the vein is removed from the patient with the minimum of surgical handling. Similarly, in the laboratory, meticulous surgical technique is required to prepare the sample and mount it on the stainless steel plate. Failure to do so will result in damage to the endothelial and smooth muscle layers. It is also important that the temperature and pH in the organ bath is maintained at physiologically normal level to provide a homeostatic environment.

The vein samples were transported from theatre to the laboratory in PSS which is a balanced physiological salt solution. There have been several studies of reversed veins which have looked at the effects of various media on endothelial preservation. Several of these media including Holmans (Angelini 1989), PSS (Lidbury 1989), MEM (Modified Eagles Medium) (Logerfo 1981), Hanks balanced salt solution (Abbott 1974) and Krebs-Ringer-bicarbonate-HEPES (Angelini 1987) do not have a deleterious effect on endothelial and smooth muscle cells when used as storage media for reversed vein grafts. However other storage solutions such as heparinised blood (Angelini 1989) and warm saline (Logerfo 1981) have been shown to lead to further injury to vein graft endothelium.

The initial experiments on veins in the organ bath which have been presented in this chapter were designed to study the effects of temperature during vein transport and its role in the preservation of a metabolically functioning endothelium and smooth muscle layer. The
results showed that the smooth muscle functions better at 24°C whereas endothelial function was better at 4°C. However none of the differences were statistically significant. Many other studies have looked at the effects of temperature on venous endothelium in reversed grafts and no overall consensus of opinion has been reached. Some authors have shown that storage of veins at 4°C in solutions such as isotonic saline, blood or St Thomas's Hospital cardioplegia solution lead to decreased endothelial function (Angelini 1987). Similarly Lawrie have shown that veins stored at 2-4°C had severe depression of EDRF production (Lawrie 1990). These findings are supported by others who have shown that the use of cold solutions such as MEM or saline are harmful to the endothelium (Logerfo 1981). On the other hand, Abbott has suggested that cold balanced salt solution leads to better endothelial preservation (Abbott 1974) and Gundry has shown that warm saline leads to massive endothelial cell loss whereas warm blood leads to only moderate endothelial cell injury and cold blood and saline fully preserved the endothelial layer (Gundry 1980).

Thus the results from all of these studies are confusing. It was therefore decided that because the endothelium functioned better at 4°C in the experimental results from the organ bath, all veins should be transported and prepared at 4°C.

Conclusions

The successful establishment of the organ bath technique allowed further studies to be made of the responses of normal saphenous vein and the effects of the various intra-operative manoeuvres during femorodistal bypass procedures on endothelial and smooth muscle cell integrity.
CHAPTER SIX

ORGAN CHAMBER STUDIES OF NORMAL AND VARICOSE SAPHENOUS VEINS.

Introduction

Varicose veins tend to become more common with increasing age. It is therefore not unusual for patients undergoing infrainguinal vein bypass procedures to have a degree of varicose disease of their long saphenous vein. If these varicosities are not severe, the vein may still be suitable for use as a bypass conduit, whereas severe disease usually excludes it from further use and alternative sources of vein or even prosthetic grafts may have to be found. Although the aetiology of varicose veins remains unknown it is probably multifactorial. Recently it has been suggested that an endothelial abnormality may contribute to their development (Thulesius 1988).

It was therefore considered important to study the contraction and relaxation responses of normal and varicose human saphenous veins to determine whether there are any differences between them. If there are differences, then this factor may influence the results produced by any further study of vein samples. In addition, since the samples of saphenous vein for this study were collected from the groin and thigh region of patients, it was possible to determine whether veins from
other areas of the leg (ie. distant from the calf region) produce similar types of contraction and relaxation responses to those produced by veins from the calf region.

Methods

The vein samples for this study were obtained from patients undergoing either varicose vein surgery or carotid endarterectomy with vein patching. Varicose vein surgery is a very common general surgical procedure which involves disconnection of the long saphenous vein from the femoral vein in the groin and ligation and division of all branches running into the saphenofemoral junction. Carotid endarterectomy is a complex vascular procedure performed on patients with symptomatic stenoses of the internal carotid artery in an attempt to reduce the risk of embolic stroke. This involves removal of the atherosclerotic plaque which develops at the carotid bifurcation and acts as the source of emboli to the brain. Following completion of the endarterectomy the carotid arteriotomy is closed with a vein patch in order to avoid narrowing the artery. In Leicester it is the policy to use the long saphenous vein from the upper thigh because it has been demonstrated that rupture of the carotid artery patch, a potentially fatal complication, is less likely when the saphenous vein from the thigh as opposed to the ankle is used (Riles 1990, Archie 1990).

Eleven samples of saphenous vein were obtained from 9 patients undergoing saphenofemoral disconnection and compared to 11 samples of saphenous vein obtained from 7 patients undergoing carotid endarterectomy. The veins from patients undergoing carotid endarterectomy were regarded as a control group. All samples were obtained from the groin and upper thigh region and were therefore
site-matched. In addition the varicose samples were all from patients who had a saphena varix in their groin. The veins were transported in calcium-free PSS at 4°C, and prepared and placed in the organ bath as described previously.

Following a 1 hour equilibrium period the optimum passive tension was set and after a further 1 hour period a dose response curve to noradrenaline ($10^{-6} \text{M}$ to $10^{-5} \text{M}$) was performed in the presence of cocaine ($10^{-6} \text{M}$). After rinsing the veins with PSS to wash out the noradrenaline, relaxation responses of half-maximally contracted veins were studied with acetylcholine ($10^{-8}$ to $10^{-5} \text{M}$) and sodium nitroprusside ($10^{-9}$ to $10^{-5} \text{M}$) at 20 minute intervals.

**Results**

The mean ages and blood pressures of patients from the 2 groups is shown in table 5. The mean blood pressure (BP) for each patient was calculated from the formula:

$$\text{Mean BP} = \text{Diastolic BP} + \frac{1}{3} (\text{Systolic BP} - \text{Diastolic BP})$$

These patients were not hypertensive nor diabetic.

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean age (years)</th>
<th>Mean BP (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>63 ± 4</td>
<td>107 ± 5</td>
</tr>
<tr>
<td>Varicose</td>
<td>48 ± 6</td>
<td>104 ± 5</td>
</tr>
</tbody>
</table>

Table 5 Mean age and mean blood pressures of patients from control and varicose groups.
The passive tension required to produce the maximum contractile response to noradrenaline was similar for the control (2.79 ± 0.34g) and the varicose group (3.03 ± 0.25g). The contractile responses to noradrenaline are shown in figure 6.1. The maximum contractile response of the control and varicose groups of veins were similar (9.73 ± 1.41 and 10.96 ± 1.77g respectively)(table 6) and there was no statistically significant difference between them (Mann-Whitney U-test). The ED₅₀ for the two groups were also not significantly different (control 0.29 ± 0.16µM, varicose 0.54 ± 0.31µM)(Mann-Whitney U-test)(table 6).

The endothelium-dependent relaxation produced by acetylcholine is shown in figure 6.2. The maximum relaxation response was produced by the 10⁻⁶M dose for the control group (48 ± 12%) and the 10⁻⁷M dose for the varicose group (23 ± 11%)(table 6). There was no significant difference in relaxation between the two groups (Mann-Whitney U-test). The endothelium-independent responses to sodium nitroprusside are shown in figure 6.3. The maximum response for the 2 groups was produced by the 10⁻⁵M dose and was 99 ± 1% for the control group and 100 ± 0% for the varicose group (table 6). These differences were also not significantly different (Mann-Whitney U-test).
Figure 6.1

Cumulative dose contraction curve to noradrenaline in control and varicose veins.
Figure 6.2

Endothelium-dependent relaxation with acetylcholine in control and varicose veins.
Figure 6.3

Endothelium-independent relaxation with sodium nitroprusside in control and varicose veins.
Table 5 Maximum contractions to noradrenaline (NA) and ED\textsubscript{50}s, maximum endothelium-dependent relaxation with acetylcholine (ACH) and maximum endothelium-independent relaxation with sodium nitroprusside (NAP) in control and varicose groups of veins.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Varicose</th>
</tr>
</thead>
<tbody>
<tr>
<td>NA Max (g)</td>
<td>9.73 ± 1.41</td>
<td>10.96 ± 1.77</td>
</tr>
<tr>
<td>NA ED\textsubscript{50} (\textmu M)</td>
<td>0.29 ± 0.16</td>
<td>0.54 ± 0.31</td>
</tr>
<tr>
<td>ACH Max (%)</td>
<td>40 ± 12</td>
<td>23 ± 11</td>
</tr>
<tr>
<td>NAP Max (%)</td>
<td>99 ± 1</td>
<td>100 ± 0</td>
</tr>
</tbody>
</table>

**Discussion**

The contractile responses with noradrenaline of the control and varicose groups to were very similar and there was no significant difference between the maximum response produced or the ED\textsubscript{50}s. The endothelium-dependent relaxation responses to acetylcholine were larger in the carotid group although no overall difference was detected when the data was analysed. Similarly the endothelium-independent relaxation responses to sodium nitroprusside were very similar and again there was no overall difference.

These results illustrate several important points. Firstly, samples of saphenous vein from other areas of the leg distant from the calf region produce similar contraction and relaxation responses. Secondly, there dose not appear to be a difference between varicose and
non-varicose saphenous vein in terms of their contraction and relaxation responses, despite previous suggestions to the contrary (Thulesius 1988).

Therefore if vein samples are obtained from patients with varicosities of their long saphenous system, this does not alter the results obtained and any differences between a control group and study group will be due to the variable parameter under investigation and not due to the fact that the vein is varicose.

Conclusions

Saphenous vein samples from the proximal and distal parts of the limb produce similar contraction and relaxation responses. These responses are also similar in varicose and non-varicose veins.
CHAPTER SEVEN

ENDOTHELIAL AND SMOOTH MUSCLE INJURY AFTER SURGICAL PREPARATION OF REVERSED AND IN-SITU SAPHENOUS VEIN BYPASS GRAFTS.

Introduction

The preparation of reversed vein grafts involves exposure and mobilisation of the vein, ligation of side-branches, stripping of the adventitia and distension to identify leaks. The effects of these manoeuvres on the endothelial and smooth muscle cells have been extensively studied in reversed coronary artery bypass grafts. There is a considerable loss of endothelial cells and the remaining ones are functionally impaired with reduced ability to produce prostacyclin and endothelium-derived relaxing factor (EDRF). In addition the function of the smooth muscle cells is impaired and this seems to be particularly related to uncontrolled distension which generates very high pressures within the vein. The recent evidence linking these effects with the subsequent development of intimal hyperplasia has led to renewed interest in the surgical techniques employed (Angelini 1991). The emphasis is now on careful handling of the vein with avoidance of over-distension by using pressure-regulating devices. In addition the task of removing the vein, which used to be delegated to the most junior member
of the surgical team, is often now performed by the most experienced surgeon.

In the lower limb the use of reversed vein grafts is now uncommon. For above knee reconstructions there is an increasing tendency to use prosthetic grafts and for distal procedures to below the knee, the in-situ technique is the most preferred. This technique has the advantage of better size-match between artery and vein graft at the anastomoses which facilitates ease of operation. In addition, it has been claimed that in-situ vein grafts have the additional advantage of an intact endothelial lining which leads to less thrombogenicity (Karmody 1984). However it seems surprising that use of a valvulotome is so atraumatic. Unfortunately there are very few studies in the literature that have investigated these claims. One study that compared endothelial preservation in reversed and in-situ vein grafts did report superior endothelial cell preservation for in-situ grafts in an animal model (Cambria 1985). However endothelial preservation has not been previously studied in human in-situ vein grafts.

It was therefore proposed to study endothelial and smooth muscle cell structure and function after surgical preparation of reversed and in-situ vein grafts.

Methods

The initial study was on 28 samples of saphenous vein which were obtained from 23 patients. These patients were undergoing either infrainguinal or coronary artery bypass grafting. These patients were not diabetic nor hypertensive and their mean ages and blood pressures are shown in table 7.
Three groups of veins were studied which had been surgically handled in different ways. The first group (control) were obtained after minimal surgical dissection using a no-touch technique (10 vessels, 8 patients). The second group (reversed) were samples taken after they had been fully prepared for reversed bypass grafting (9 vessels, 8 patients). This included full exposure and mobilisation of the vein, ligation of side-branches and uncontrolled distension with saline. The third group (in-situ) were obtained after preparation for in-situ vein grafting which involved exposure and mobilisation of the vein, ligation of side branches and the passage of a valvulotome (9 vessels, 7 patients). A 2.5mm Hall valvulotome was used for each in-situ graft and it was passed as atraumatically as possible. All samples of vein for each group were taken from the calf region to ensure that they were matched for site and size. Therefore, in the in-situ group, the vein was obtained from the distal end of the graft.

Table 7 Mean ages and mean blood pressures for patients from control, reversed and in-situ groups.
The veins were transported from theatre to the laboratory in cold (4°C) calcium-free physiological salt solution in a sealed container. In the laboratory the vein was carefully cleaned of fat and adventitia and a ring of vein 5mm in length was cut open to form a rectangular strip. All dissection was performed under a light microscope with the vein in calcium free physiological salt solution. One end of the vein was attached to the stainless steel plate and the other to the force transducer to measure isometric contraction. The stainless steel plate and vein were placed in the organ bath containing physiological salt solution at 37°C and gassed with a 5% CO2 / 95% O2 mixture to achieve a pH of 7.45.

After an equilibrium period of one hour each vein was stretched and the passive tension set to achieve a maximum contractile response to noradrenaline. A further equilibrium period of one hour was allowed to elapse before a cumulative dose contraction curve to noradrenaline (10^-8M to 10^-5M) was performed in the presence of cocaine 10^-6M. After several rinses with fresh physiological salt solution over a period of 20 minutes, each vein was sub-maximally contracted with noradrenaline before relaxation studies were performed using the following:

**Endothelium-dependent vasodilators:**

- Acetylcholine (10^-8M to 10^-5M)
- Bradykinin (10^-9M to 10^-6M)
- Adenosine (10^-8M to 10^-5M)
- Histamine (10^-9M to 10^-5M)

**Endothelium-independent vasodilator:**
Sodium nitroprusside (10^{-9} M to 10^{-5} M)

Finally the vessels were fixed with 2% buffered glutaraldehyde and processed for histology and scanning electron microscopy assessment.

**Results**

The resting tension required to produce the maximum contractile response to noradrenaline was the same for the control and reversed groups but reduced in the valvulotome group (2.50 ± 0.27g, 2.50 ± 0.27g and 1.5 ± 0.55g respectively).

The dose response curve to noradrenaline for the 3 groups is shown in figure 7.1. The control veins produced a maximum contraction of 9.44 ± 1.61g, which was reduced to 8.84 ± 1.38g in the reversed group and further reduced to 3.47 ± 1.75g in the in-situ group (table 8). There was a significant difference between the maximum contractile responses of the control and in-situ groups (p<0.05, Mann-Whitney U-test). However the difference in the maximum contractile response between the control and reversed groups was not significant (p>0.05, Mann-Whitney U-test).

In addition the concentration of noradrenaline required to induce a half maximal contraction (ED_{50}) was increased in the in-situ (0.87 ± 0.45pM) and reversed groups (0.20 ± 0.06pM) compared to controls (0.08 ± 0.02pM)(table 8). The difference in ED_{50}s between the control and in-situ groups was significant (p<0.05, Mann-Whitney U-test) but there was no significant difference between the control and reversed groups (p>0.05, Mann-Whitney U-test).
Figure 7.1

Cumulative dose contraction curve to noradrenaline in control, reversed and in-situ groups of veins.
Table 8 Optimum passive tension, maximum contraction to noradrenaline (NA) and ED$_{50}$ for control, reversed and in-situ groups of veins.

<table>
<thead>
<tr>
<th></th>
<th>Passive tension</th>
<th>Max NA</th>
<th>ED$_{50}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(g)</td>
<td>(g)</td>
<td>(µM)</td>
</tr>
<tr>
<td>Control</td>
<td>2.50 ± 0.27</td>
<td>9.44 ± 1.61</td>
<td>0.08 ± 0.02</td>
</tr>
<tr>
<td>Reversed</td>
<td>2.50 ± 0.27</td>
<td>8.84 ± 1.38</td>
<td>0.20 ± 0.06</td>
</tr>
<tr>
<td>In-situ</td>
<td>1.50 ± 0.55</td>
<td>3.47 ± 1.75</td>
<td>0.87 ± 0.45</td>
</tr>
</tbody>
</table>

It was found that the control and reversed groups of veins would produce repeated contractions of the same magnitude to noradrenaline, but the in-situ group of veins failed to re-contract to a second exposure. This prevented further study of relaxation responses in the in-situ group because this requires the vein to be sub-maximally contracted before exposure to relaxing agents.

However endothelium-dependent and independent relaxation responses were studied in the control and reversed groups. The relaxation responses produced by acetylcholine, bradykinin, adenosine, histamine and sodium nitroprusside are shown in figures 7.2-7.6. The maximum responses produced are shown in table 9. However there was no significant difference between the 2 groups in their responses to any of the relaxing agents used (p>0.05, Mann-Whitney U-test).
Figure 7.2

Endothelium-dependent relaxation responses with acetylcholine in control and reversed groups of veins.
Figure 7.3

Endothelium-dependent relaxation responses with bradykinin in control and reversed groups of veins.
Figure 7.4

Endothelium-dependent relaxation responses with adenosine in control and reversed groups of veins.
Figure 7.5

Endothelium-dependent relaxation responses with histamine in control and reversed groups of veins.
Figure 7.6

Endothelium-independent relaxation responses with sodium nitroprusside in control and reversed groups of veins.
Control | Reversed
--- | ---
ACH (%) | 10 ± 6 | 6 ± 5
BK (%) | 29 ± 9 | 17 ± 9
Aden (%) | 17 ± 8 | 4 ± 3
Hist (%) | -38 ± 8 | -62 ± 15
NAP (%) | 100 ± 0 | 100 ± 0

Table 9 Maximum endothelium-dependent relaxation responses to acetylcholine (ACH), bradykinin (BK), adenosine (aden) and histamine (hist) and maximum endothelium-independent relaxation response to sodium nitroprusside (NAP) for control and reversed groups of veins.

In order to allow relaxation studies to be performed in the in-situ group, a second series of veins which had been prepared for use as in-situ grafts was obtained from patients undergoing femorodistal bypass procedures (5 patients, 8 vessels). These patients were normotensive (mean blood pressure 106 ± 2 mmHg) and not diabetic with a mean age of 67 ± 4 years. The veins were collected, prepared and mounted in the organ bath as described previously.

The passive tension which would give the best contractile response was not determined for this group as this would mean repeated exposures to noradrenaline. Similarly a noradrenaline dose response was not performed. Instead, the passive tension was pre-set to the previously observed mean passive tension of 1.5g for the first in-situ group of veins. The veins were then sub-maximally contracted with a single bolus injection of noradrenaline and a cumulative relaxation curve performed with acetylcholine. Following this the response of the vein to high
potassium (123mM) PSS was determined. This was performed by replacing the PSS in the organ bath with PSS containing potassium which had previously been heated to 37°C in a water bath and gassed with a 5% CO₂ / 95% O₂ mixture to achieve a pH of 7.45. Potassium causes contraction of vessels by a direct depolarising effect on the smooth muscle cell membrane and this ability is the last response to be abolished in damaged vessels. This effect allows the viability of severely damaged, poorly responsive veins to be determined.

Eight vessels were studied which had been obtained from 5 patients (table 10). One vessel did not show any response to noradrenaline or high potassium (123mM) PSS. Of the remaining 7 vessels, responses to both a bolus of noradrenaline and potassium were elicited. The mean contraction to noradrenaline of the 7 vessels was 3.3 ± 1.14g, and the mean contraction to potassium of these vessels was 4.86 ± 1.37g. None of these vessels produced any endothelium-dependent relaxation responses to acetylcholine (figures 7.7 and 7.8).

<table>
<thead>
<tr>
<th>Vessel No.</th>
<th>NA Dose (M)</th>
<th>Contraction NA (g)</th>
<th>Contraction K⁺ (g)</th>
<th>ACH (%) (10⁻⁸⁻¹⁰⁻⁵M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3x10⁻⁷</td>
<td>3.00</td>
<td>5.04</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>3x10⁻⁷</td>
<td>8.40</td>
<td>9.60</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>1x10⁻⁶</td>
<td>0.75</td>
<td>2.55</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>1x10⁻⁶</td>
<td>0.45</td>
<td>2.25</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>3x10⁻⁵</td>
<td>0.45</td>
<td>0.15</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>1x10⁻⁵</td>
<td>0.00</td>
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<td>1x10⁻⁷</td>
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</table>

Table 10 Maximum contractions to noradrenaline (NA) and high potassium PSS (K⁺), and endothelium-dependent relaxation responses with acetylcholine (ACH) for 2nd in-situ group of veins.
Figure 7.7

Contraction responses to high potassium (123mM) PSS in control and 2nd in-situ group of veins.
Endothelium-dependent relaxation responses with acetylcholine in control and 2nd in-situ group of veins.
Histological assessment

Seven samples of vein from the control, reversed and in-situ groups were prepared as paraffin sections (4µm) having previously been fixed at their respective passive tensions in the organ bath with 2% formaldehyde. These samples were stained with Haematoxylin and Eosin (H&E) and Elastin Van Gieson (EVG).

In addition endothelial cells were assessed with a novel monoclonal antibody to human endothelium, Q Bend 10 (Unipath Ltd, Bedford, UK), using the labelled Avidin Biotin immunoperoxidase technique. The primary antibody was used at a dilution of 1:50. The degree of endothelial cell loss and smooth muscle damage was assessed by a pathologist who had no prior knowledge of the group origin of the vein sample. A simple semi-quantitative scoring system was employed with the degree of endothelial damage assessed as follows (tables 11-13):

0 Negligible
1 Minimal/patchy cell loss
2 Severe/total cell loss

Smooth muscle damage was similarly documented as:

0 Normal
1 Minimal/slight necrosis
2 Severe necrosis

The intima of each vein was normal on naked-eye examination. Nearly all of the in-situ veins showed severe or complete endothelial
cell loss when assessed by light microscopy. Damage to the medial smooth muscle was generally either severe or moderate and "injury scores" were correspondingly high. The veins from the reversed group demonstrated less severe damage. Endothelial cell loss, although present, was patchy and similarly smooth muscle damage was either slight or absent. The control veins showed minimal endothelial cell and muscle damage and therefore produced low "injury scores" (figures 7.9-7.14).

<table>
<thead>
<tr>
<th>Control (n=7)</th>
<th>Reversed (n=7)</th>
<th>In-situ (n=7)</th>
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<td>0</td>
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<td>2</td>
<td>8</td>
<td>12</td>
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</table>

Table 11 Endothelial injury scores for control, reversed and in-situ groups of veins.

<table>
<thead>
<tr>
<th>Control (n=7)</th>
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<th>In-situ (n=7)</th>
</tr>
</thead>
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<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>5</td>
<td>9</td>
</tr>
</tbody>
</table>

Table 12 Smooth muscle injury scores for control, reversed and in-situ groups of veins.
Table 13 Combined endothelial and smooth muscle injury scores for control, reversed and in-situ groups of veins.

<table>
<thead>
<tr>
<th></th>
<th>Control (n=7)</th>
<th>Reversed (n=7)</th>
<th>In-situ (n=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1</td>
<td>4</td>
<td></td>
</tr>
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<td>1</td>
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<tr>
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</tr>
<tr>
<td>0</td>
<td>2</td>
<td>3</td>
<td></td>
</tr>
</tbody>
</table>

Scanning electron microscopy (SEM) assessment

Samples of vein from each of the three groups were also processed for scanning electron microscopy assessment after fixation with 2% glutaraldehyde at their respective passive tensions in the organ bath. These samples were washed in 0.1M cacodylate buffer (Sigma Chemicals Co. Ltd, Dorset, UK) for 1 hour and then dehydrated through graded ethanol (50% for 30 minutes, 70% for 60 minutes, 90% for 15 minutes and 100% for 15 minutes) and finally immersed in three changes of analar acetone. They were then dried using a Polaron E3000 carbon dioxide critical point dryer (Bio-Rad Microscience Ltd, Hemel Hempstead, UK). The specimens were mounted on copper stubs with colloidal silver and sputter coated with gold-palladium in a Polaron E5150 sputter coater (Bio-Rad Microscience Ltd, Hemel Hempstead, UK). The coated stubs were viewed and photographed on an ISI-DS 130 dual stage scanning electron microscope (International Scientific Instruments, Buxton, UK).
The vein samples from the control group showed preservation of endothelium (figures 7.15 and 7.16). The reversed group of veins showed some loss of endothelial cells but overall the coverage was good. In contrast, the in-situ veins showed severe damage to the endothelial layer and in some cases complete loss of endothelial cells with exposure of the underlying smooth muscle cells (figures 7.17 and 7.18).
Figure 7.9

Histological picture of a vein from the control group showing normal endothelial and smooth muscle cells (Stain H&E).
Figure 7.10

Histological picture of a vein from the reversed group showing patchy loss of endothelial cells (Stain H&E).
Figure 7.11

Histological picture of a vein from the in-situ group showing complete loss of endothelial cells (Stain H&E).
Figure 7.12

Histological picture of a vein from the control group stained with the endothelial marker Q Bend 10 showing presence of endothelial cells.
Figure 7.13

Histological picture of a vein from the reversed group stained with the endothelial marker Q Bend 10 showing patchy loss of endothelial cells.
Figure 7.14

Histological picture of a vein from the in-situ group stained with the endothelial marker Q Bend 10 showing complete loss of endothelial cells.
Figure 7.15

Scanning electron micrograph of a vein from the control group showing endothelial cells.
Figure 7.16

Scanning electron micrograph of a vein from the reversed group showing presence of endothelial cells (right) and loss of endothelial cells with exposure of the basement membrane and smooth muscle cells (left).
Figure 7.17

Scanning electron micrograph of a vein from the in-situ group showing complete loss of endothelial cells and exposure of the basement membrane and smooth muscle cells.
Discussion

These results show the degree of structural and functional damage that surgical preparation of reversed and in-situ vein grafts causes to the endothelial and smooth muscle cells. In the reversed group there was no significant difference in the endothelial function when compared to controls as measured by EDRF release. These findings do not support the findings of others who have reported impaired EDRF release after distension of reversed vein grafts (Angelini 1989). However histological studies did show patchy endothelial cell loss in this group compared to controls. The histological studies of the in-situ group showed severe or complete endothelial cell loss and the functional studies confirmed this finding in that EDRF release could not be detected.

Assessment of the smooth muscle function showed that the maximum contraction to noradrenaline was reduced in the reversed group and further reduced in the in-situ group compared to controls. However the maximum contractile responses between the control and reversed groups were not significant different and similarly there was no significant difference between the ED₅₀s of these groups. For the in-situ group both the maximum contractile response to noradrenaline and the ED₅₀s were significantly different compared to controls and in addition the in-situ veins failed to produce repeated contractions to noradrenaline.

Histological studies of smooth muscle in the reversed group showed normal or slightly damaged smooth muscle cells. However in the in-situ group the damage to the smooth muscle cells was moderate to severe with some veins showing smooth muscle cell necrosis.

The cause of endothelial and smooth muscle injury in the in-situ group is due to a combination of surgical handling of the vein during dissection, mobilisation and ligation of side-branches and the passage
of a valvulotome along its luminal surface. The vein samples in the
in-situ group were all obtained from the distal end of vein grafts and
it is possible that more severe injury occurs here due to the actual
insertion of the valvulotome and that the degree of damage documented by
these studies is not as severe in the proximal and mid-portions of the
vein graft. In addition although a 2.5mm Hall valvulotome was used in
each case, the diameter of the distal end of the vein was not measured.
However because vein is the conduit of choice for femorodistal bypass
procedures, attempts are made to use the saphenous vein at all costs in
preference to a prosthetic graft. This sometimes leads to a small
diameter (<2.5mm) vein being used which is often the same size or
smaller than the valvulotome, and in these cases severe damage would be
expected.

It is of interest that there does not seem to be any difference in
the incidence of intrinsic vein lesions in reversed and in-situ grafts.
However the only study that has compared them looked at vein grafts to
the below knee popliteal artery and not more distal procedures to single
calf vessels. In addition the precise relationship between injury and
subsequent development of vein graft lesions has not been defined. It is
possible that the mere presence of cellular damage and not its severity
may be the most important aetiological factor.

There are several ways in which the degree of damage seen in
in-situ grafts may be reduced. The smallest possible valvulotome should
always be used (a 2mm valvulotome is now commercially available) and it
should be passed as atraumatically as possible. Excessive handling of
the vein graft should be avoided, and in addition modification of the
operative technique should be considered. Complete exposure and
mobilisation of the vein is a method that was introduced by Beard and
his colleagues in Bristol (Beard 1989a). The alternative is to avoid
exposure of the vein and to cut down at each valve site where a side-
branch is always found. This would lead to less handling and exposure of
the vein with possibly less damage (Gannon 1986).

Conclusions

Preparation of veins for in-situ grafting causes significant
damage to the endothelium and smooth muscle cell layers. This is
probably caused by a combination of surgical handling during exposure
and mobilisation of the vein and ligation of side-branches, and the
passage of a valvulotome along its lumen.
CHAPTER EIGHT

THE EFFECTS OF INTRA-OPERATIVE ARTERIOGRAPHY AND X-RAY CONTRAST MEDIUM ON VEIN GRAFTS.

Introduction

There are several other intra-operative procedures performed during femorodistal bypass operations which might have a damaging effect on the endothelium and smooth muscle cells of the vein graft. These include the use of X-ray contrast media for intra-operative arteriography, and the use of intra-operative vasodilators such as papaverine and iloprost. The effects of Niopam (a non-ionic contrast medium), which is routinely used as a contrast medium, will be discussed in this chapter and the effects of vasodilators (papaverine and iloprost) will be discussed in chapter 9.

Intra-operative arteriography

After the distal anastomosis of a femorodistal bypass has been completed, it is important to check for technical errors. It is inadequate to rely upon the presence of a palpable pulse in the graft as this does not guarantee adequate flow. An intra-operative completion arteriogram is therefore usually performed by inserting a cannula into a
side-branch of the graft or a needle directly into the graft itself. The graft is then occluded proximally with a vascular clamp and 20mls. of contrast medium infused down the graft with a film cassette placed under the leg and centred on the distal anastomosis. The arteriogram will identify most technical errors such as kinking and twisting of the graft, the presence of persistent side-branches or valves and intimal flaps at the distal anastomosis.

It has been established in both animal experiments and clinical studies that the intravascular use of contrast media may lead to endothelial damage (Ausman 1964, Mersereau 1961). This has been implicated as a possible factor in side-effects such as thrombosis, vascular pain and anaphylaxis which may be seen after arteriography. However there have been few studies that have dealt with changes to venous endothelium and they have all been performed in animal models. They have suggested that the non-ionic contrast media cause less endothelial damage at comparable concentrations than the ionic agents (Morettin 1984).

It is the policy in Leicester to use Niopam (iopamidol) a non-ionic contrast medium for arteriography. It was therefore proposed to study whether this agent caused endothelial or smooth muscle injury to vein grafts.

Methods

Eight samples of saphenous vein were collected from patients undergoing coronary artery bypass grafting. The samples were obtained from the distal end of the vein at the level of the ankle immediately after exposure of the vein had commenced. The sample of vein was removed with the minimum of handling and immediately placed in cold (4°C)
calcium-free PSS for transport to the laboratory. Under the microscope, the vein was carefully cut open to form a strip and then the strip was cut in half to produce two samples of vein each 5mm in length. Each sample was placed in a petri dish with the endothelial surface uppermost. Two millilitres (2 ml) of Niopam was pipetted into the petri dish onto the endothelial surface of the vein sample. After 2 minutes the Niopam was removed and replaced with cold calcium-free PSS. The control sample was exposed to 2 ml of calcium-free PSS for 2 minutes. The samples of vein were then placed in the organ bath in the standard way.

Following the usual equilibrium period the optimum passive tension was set to give the maximum response to noradrenaline. Then the following pharmacological agents were used to study endothelial and smooth muscle function:

- Noradrenaline ($10^{-8}$ to $10^{-5}$M)
- High potassium (123mM) PSS
- Acetylcholine ($10^{-8}$ to $10^{-5}$M)
- Iloprost ($10^{-10}$ to $10^{-7}$M)
- Sodium nitroprusside ($10^{-9}$ to $10^{-5}$M)

Statistical analysis

The samples in this group were paired. Therefore the Wilcoxon matched-pairs test was used to determine differences between groups accepting $p<0.05$ as being significant.
Results

The mean age of patients in this study was 62 ± 3 years and their mean blood pressure was 106 ± 3 mmHg. These patients were not hypertensive nor diabetic.

The passive tension that would produce the maximum contractile response to noradrenaline was the same for the control and Niopam groups (2.50 ± 0.27g). The contractile responses to noradrenaline and high potassium (123mM) PSS are shown in figures 8.1 and 8.2. The mean response to potassium in the control group was 10.13 ± 1.44g compared to 10.07 ± 1.63g in the Niopam group (table 14). The maximum contractile response to noradrenaline was 10.14 ± 1.17g in the control group and 9.11 ± 1.60g in the Niopam group (table 14). There was no statistical significance between the responses to potassium or the maximum contractile responses to noradrenaline (Wilcoxon matched pairs, p>0.05).

It was not possible to calculate an ED$_{50}$ for the noradrenaline responses because the rate of change of force between the $10^{-8}$M and $10^{-7}$M doses was so rapid.

<table>
<thead>
<tr>
<th>$K^+$ (g)</th>
<th>Max NA (g)</th>
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<tr>
<td>Control</td>
<td>10.13 ± 1.44g</td>
</tr>
<tr>
<td>Niopam</td>
<td>10.07 ± 1.63g</td>
</tr>
</tbody>
</table>

Table 14 Maximum contraction responses to noradrenaline (NA) and high potassium (123mM) PSS ($K^+$) in control and Niopam groups of veins.
Figure 8.1

Cumulative dose contraction curve to noradrenaline in the control and Niopam groups of veins.
Figure 8.2

Maximum contraction responses to high potassium PSS in the control and Niopam groups of veins.
The relaxation responses to the endothelium-dependent agent acetylcholine and the endothelium-independent agents iloprost and sodium nitroprusside are shown in figures 8.3 to 8.5. The maximum relaxation responses are shown in table 15. There was no significant difference in relaxation between the control and Niopam groups with any of the relaxing agents used (Wilcoxon matched-pairs, p>0.05).

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Niopam</th>
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<tbody>
<tr>
<td>ACH (%)</td>
<td>59 ± 13</td>
<td>49 ± 15</td>
</tr>
<tr>
<td>ILO (%)</td>
<td>83 ± 5</td>
<td>56 ± 16</td>
</tr>
<tr>
<td>NAP (%)</td>
<td>94 ± 3</td>
<td>94 ± 4</td>
</tr>
</tbody>
</table>

Table 15 Maximum endothelium-dependent relaxation responses with acetylcholine (ACH) and maximum endothelium-independent relaxation responses with iloprost (ILO) and sodium nitroprusside (NAP) in control and Niopam groups of veins.

The vein samples were examined histologically and the appearances of the endothelial and smooth muscle cells assessed using the same scoring system as described previously. All samples showed minimal or patchy endothelial cell loss with normal smooth muscle cells and there was no differences between the 2 groups.
Figure 8.3

Endothelium-dependent relaxation responses with acetylcholine in the control and Niopam groups of veins.
Endothelium-independent relaxation responses with iloprost in the control and Niopam groups of veins.
Endothelium-independent relaxation responses with sodium nitroprusside in the control and Niopam groups of veins.
Discussion

Completion arteriography following femorodistal bypass procedures is usually performed with the proximal portion of the graft temporarily occluded by a vascular clamp. This clamp is released and flow restored in the graft immediately after the film has been taken. In this study, x-ray contrast medium (Niopam) was introduced directly onto the endothelial surface of the vein samples for 2 minutes which is a much longer and more direct exposure than that which occurs in vivo.

Despite this prolonged exposure, Niopam did not appear to cause any damage to the endothelium or smooth muscle cells of the vein samples. The contraction responses to noradrenaline and the endothelium-dependent and endothelium-independent relaxation responses were very similar for the control and Niopam groups. In addition, analysis of the data did not show any statistically significant differences between the 2 groups. These finding were supported by histological assessment of the veins which showed minimal endothelial injury in the control and Niopam groups.

Previous studies have suggested that ionic contrast media may cause endothelial injury (Morettin 1984). Niopam is a non-ionic agent and did not appear to have any effects on either the endothelium or smooth muscle cells of the vein.

Conclusions

Niopam does not appear to cause injury to the endothelium or smooth muscle cells of vein grafts. Therefore it would seem to be an
acceptable contrast agent for completion arteriography during femorodistal bypass.
CHAPTER NINE

THE EFFECTS OF INTRA-OPERATIVE VASODILATORS (PAPAVERINE AND ILOPROST) ON VEIN GRAFTS.

Introduction

This chapter looks at the use of intra-operative vasodilators during femorodistal bypass procedures. There are 2 commonly used agents—papaverine and iloprost.

Papaverine

Vasospasm is a common arterial response to low blood flow and surgical handling and results in a narrowed luminal diameter which can make vascular anastomoses technically difficult. In order to alleviate this problem many cardiothoracic centres advocate the use of the vasodilator drug papaverine during surgical preparation of vein grafts.

Vascular smooth muscle relaxation is mediated by the activation of the enzyme guanylate cyclase which causes an increase in intracellular levels of c-GMP. The enzyme phosphodiesterase regulates the levels of c-GMP within the cell by destroying the guanosine nucleotide (Vanhouette 1987). Papaverine acts by inhibiting phosphodiesterase
causing an increase in c-GMP which leads to relaxation of the smooth muscle in the vessel wall (Lee 1978).

During in-situ femorodistal bypass procedures vasospasm does not seem to be a frequently encountered problem. However papaverine is still used as a vasodilator during completion assessment of the vein bypass. One of the commonly accepted alternatives to completion arteriography is measurement of graft blood flow using the Doppler flowmeter (Beard 1989b). In addition measurement of graft pressure by insertion of a needle into the graft allows calculation of the peripheral resistance. It is in this situation that papaverine is used to augment graft blood flow and thus increase the sensitivity of the test, with a resistance of <1PRU suggesting that the procedure is satisfactory.

Commercially available papaverine is supplied as papaverine hydrochloride and its pH ranges from 3.2 and 3.6 (Mills 1989). During femorodistal bypass it is used undiluted (15mg in 1ml) and injected as a bolus down the graft. It is possible that papaverine causes cellular injury to the vein graft and its effects were therefore studied using the organ bath technique.

Methods

Eight samples of saphenous vein were collected from patients undergoing coronary artery bypass grafting. The samples were obtained from the distal end of the vein at the level of the ankle immediately after exposure of the vein had commenced. The sample of vein was removed with the minimum of handling and immediately placed in cold (4°C) calcium-free PBS for transport to the laboratory. Under the microscope, the vein was carefully cut open to form a strip and then the strip was cut in half to produce two samples of vein each 5mm in length. Each
sample was placed in a petri dish with the endothelial surface uppermost. Two millilitres (2 ml) of papaverine hydrochloride was pipetted into the petri dish onto the endothelial surface of one vein sample. After 2 minutes the papaverine was removed and replaced with cold calcium-free PSS. The control sample was exposed to 2 ml of calcium-free PSS for 2 minutes. The samples of vein were then placed in the organ bath in the standard way.

Following the usual equilibrium period the optimum passive tension was set to give the maximum response to noradrenaline. Then the following pharmacological agents were used to study endothelial and smooth muscle function:

- High potassium (123mM) PSS
- Noradrenaline (10^{-8} to 10^{-5}M)
- Acetylcholine (10^{-8} to 10^{-5}M)
- Sodium nitroprusside (10^{-9} to 10^{-5}M)

Statistical analysis

The samples in this group were paired. Therefore the Wilcoxon matched-pairs test was used to determine differences between groups accepting $p<0.05$ as being significant.

Results

The mean age of patients in this study was 62 ± 1 years and their mean blood pressure was 99 ± 4 mmHg. These patients were not hypertensive nor diabetic. The passive tension that would produce the maximum contractile response to noradrenaline was identical for the
control group and the papaverine groups (3.0g). The responses to noradrenaline and potassium are shown in figures 9.1 and 9.2. There was a statistically significant difference in the response to potassium (control 8.90 ± 1.76g, papaverine 2.30 ± 1.35g)(Wilcoxon matched-pairs, p<0.05)(table 16). Similarly the maximum contractile response to noradrenaline was higher in the control group (9.11 ± 1.18g) than in the papaverine group (2.39 ± 0.88g)(table 16) and these differences were also significant (p<0.02, Wilcoxon matched-pairs). The mean ED50 for the noradrenaline curve of the control group (0.15 ± 0.03pM) was significantly less than that of the papaverine group (0.2 ± 0.06pM) (p<0.05, Wilcoxon matched-pairs)(table 16).

<table>
<thead>
<tr>
<th>K⁺ (g)</th>
<th>NA max (g)</th>
<th>ED50 (pM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control 8.90 ± 1.76</td>
<td>9.11 ± 1.18</td>
<td>0.15 ± 0.03</td>
</tr>
<tr>
<td>Papaverine 2.30 ± 1.35</td>
<td>2.39 ± 0.88</td>
<td>0.2 ± 0.06</td>
</tr>
</tbody>
</table>

Table 16 Contraction to high potassium (123mM) PSS (K⁺), maximum contraction to noradrenaline (NA) and ED50s in control and papaverine groups of veins.

Three of the 8 samples in the papaverine group did not produce any response to these two agents whereas their controls reacted to both agents. This meant that only 5 of the 8 veins were available for further studies of endothelial function. The maximum responses of the 2 groups to acetylcholine and sodium nitroprusside are shown in figures 9.3 and 9.4.
Figure 9.1

Cumulative dose contraction curve to noradrenaline in control and papaverine groups of veins.
Figure 9.2

Contraction responses to high potassium (123mM) PSS in control and papaverine groups of veins.
Figure 9.3

Endothelium-dependent relaxation with acetylcholine in control and papaverine groups of veins.
Endothelium-independent relaxation with sodium nitroprusside in control and papaverine groups of veins.
There was no significant difference in the relaxation responses in the 5 veins that were available for study (p>0.05, Wilcoxon matched-pairs) (table 17).

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Papaverine</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACH (%)</td>
<td>40 ± 9</td>
<td>19 ± 12</td>
</tr>
<tr>
<td>NAP (%)</td>
<td>85 ± 13</td>
<td>99 ± 1</td>
</tr>
</tbody>
</table>

Table 17 Maximum endothelium-dependent relaxation with acetylcholine (ACH) and maximum endothelium-independent relaxation with sodium nitroprusside (NAP) in control and papaverine groups of veins.

The vein samples were examined histologically and endothelial and smooth muscle cell appearances scored as described previously. The 3 samples from the papaverine group that did not contract with noradrenaline showed moderate endothelial cell injury and some degree of smooth muscle cell damage. The remaining veins in the papaverine group and all of the control veins showed minimal endothelial cell injury.
Iloprost

Iloprost is a stable prostacyclin (PGI$_2$) analogue that is a potent vasodilator and also inhibits platelet aggregation (Dormandy 1989). Its actions are not dependent upon the presence of an intact endothelium (Schroder 1987). It also acts in the presence of indomethacin, indicating that endogenous cyclo-oxygenase products are not required for vasodilation (Schroder 1987). Iloprost is thought to act by binding to specific vascular smooth muscle PGI$_2$ receptors (Schillinger 1980).

In femorodistal bypass procedures for critical limb ischaemia iloprost has been used to augment graft flow (Shearman 1990, Hickey 1991). It has been suggested that this effect occurs by lowering the high resistance to flow which exists in the microcirculation (Dormandy 1989) and which may be caused by vasospasm, endothelial cell swelling and luminal obstruction by neutrophils (Gidlof 1987).

Iloprost may be given as a bolus down the vein graft during intra-operative assessment of graft flow and/or as a post-operative intragraft infusion. The effects of iloprost on the endothelium and smooth muscle cells of the vein graft were therefore studied in order to determine whether it has any damaging effects on the endothelium and smooth muscle of vein grafts.

Methods

Eight samples of saphenous vein were collected from patients undergoing coronary artery bypass grafting. The samples were obtained from the distal end of the vein at the level of the ankle immediately after exposure of the vein had commenced. The sample of vein was removed with the minimum of handling and immediately placed in cold (4°C)
calcium-free PSS for transport to the laboratory. Under the microscope, the vein was carefully cut open to form a strip and then the strip was cut in half to produce two samples of vein each 5mm in length. Each sample was placed in a petri dish with the endothelial surface uppermost. Two millilitres (2 mls) of iloprost was pipetted into the petri dish onto the endothelial surface of one vein sample. After 2 minutes the iloprost was removed and replaced with cold calcium-free PSS. The control sample was exposed to 2 mls of calcium-free PSS for 2 minutes. The samples of vein were then placed in the organ bath in the standard way.

Following the usual equilibrium period the optimum passive tension was set to give the maximum response to noradrenaline. Then the following pharmacological agents were used to study endothelial and smooth muscle function:

- High potassium (123mM) PSS
- Noradrenaline (10^-8 to 10^-5M)
- Acetylcholine (10^-8 to 10^-5M)
- Sodium nitroprusside (10^-9 to 10^-5M)

**Statistical analysis**

The samples in this group were paired. Therefore the Wilcoxon matched-pairs test was used to determine differences between groups accepting p<0.05 as being significant.
Results

The mean age of patients in this study was 65 ± 3 years and their mean blood pressure was 99 ± 3 mmHg. These patients were not hypertensive nor diabetic. The passive tension that would produce the maximum contractile response to noradrenaline was 2.88 ± 0.15g for the control group and the 3.0 ± 0g for the iloprost group.

The responses of the control and iloprost groups to potassium are shown in figure 9.5. The iloprost group produced a larger contraction compared to control (control 7.54 ± 0.96g, iloprost 10.02 ± 1.31g) but there was no significant difference between the responses (p>0.05, Wilcoxon matched-pairs)(table 18). The contractile responses to noradrenaline are shown in figure 9.6. Again the iloprost group produced larger responses than the control group. Although there was no significant difference in the ED50s (control 0.1 ± 0.02pM, iloprost 0.07 ± 0.01pM)(p>0.05 Wilcoxon matched-pairs), there was a significant difference in the maximum responses to noradrenaline (control 7.62 ± 0.72g, iloprost 11.38 ± 0.90g)(p<0.05 Wilcoxon matched-pairs)(table 18).

The maximum responses produced by acetylcholine and sodium nitroprusside are shown in table 19. Analysis of the relaxation responses to acetylcholine and sodium nitroprusside (figure 9.7 and 9.8) showed that there was no significant differences between the control and iloprost groups (p>0.05 Wilcoxon matched-pairs).
Figure 9.5

Contraction responses to high potassium (123mM) PSS in control and iloprost groups of veins.
Figure 9.6

Cumulative dose contraction curves to noradrenaline in control and iloprost groups of veins.
Endothelium-dependent relaxation with acetylcholine in control and iloprost groups of veins.
Figure 9.8

Endothelium-independent relaxation with sodium nitroprusside in control and iloprost groups of veins.
Table 18 Contraction responses to high potassium (123mM) PSS (K⁺), maximum contractions to noradrenaline (NA) and ED₅₀ in control and iloprost groups of veins.

<table>
<thead>
<tr>
<th></th>
<th>K⁺ (g)</th>
<th>NA max (g)</th>
<th>ED₅₀ (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7.54 ± 0.96</td>
<td>7.62 ± 0.77</td>
<td>0.1 ± 0.02</td>
</tr>
<tr>
<td>Iloprost</td>
<td>10.02 ± 1.31</td>
<td>11.38 ± 0.90</td>
<td>0.07 ± 0.01</td>
</tr>
</tbody>
</table>

Table 19 Maximum endothelium-dependent relaxation with acetylcholine (ACH) and maximum endothelium-independent relaxation with sodium nitroprusside (NAP) in control and iloprost groups of veins.

<table>
<thead>
<tr>
<th></th>
<th>ACH (%)</th>
<th>NAP (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>35 ± 9</td>
<td>100 ± 0</td>
</tr>
<tr>
<td>Iloprost</td>
<td>22 ± 7</td>
<td>100 ± 0</td>
</tr>
</tbody>
</table>

Histological examination of the vein samples showed normal endothelium or minimal endothelial injury and there was no differences between the 2 groups.
Discussion

There have been few previous studies on the effects of papaverine on endothelial and smooth muscle cell function. One report from Sottiurai did study the effects of papaverine on smooth muscle cell morphology during preparation of reversed vein grafts. No difference was found in veins that had been distended with and without the presence of papaverine at a follow-up of between 1 and 12 months (Sottiurai 1985). However, this study did not investigate smooth muscle cell injury at the time of implantation and was only concerned with morphological changes. A similar study by LoGerfo suggested that papaverine preserved the endothelial morphology when used as a distending agent for reversed vein grafts (LoGerfo 1981).

The results produced in this chapter disagree with these findings. There was a statistically significant difference between the control and papaverine groups in their maximum contractile responses to potassium and noradrenaline. Similarly, the ED50s for the noradrenaline curves were significantly different. There are two possible explanations for this, either the acidic nature of papaverine leads to smooth muscle cell injury, or alternatively the impaired smooth muscle contraction with papaverine may be caused by complete inhibition of the contractile machinery. Whilst the latter effect would be expected to recover, the former would not. The endothelium-dependent responses to acetylcholine were impaired in the papaverine group when compared to controls but these differences did not reach significance. This may be a reflection of the fact that only 5 veins were available for relaxation studies to be performed because 3 veins did not produce any response to contracting agents. Thus, the small sample size may prevent statistical significance from being reached. Whilst it is possible to speculate that veins which
do not contract will not relax, it is not actually possible to
demonstrate this. However, histological examination of the veins did
show endothelial and smooth muscle cell injury in the 3 veins that did
not contract to noradrenaline.

The experiments performed with iloprost also produced interesting
findings. The iloprost group showed larger contractions to potassium and
noradrenaline when compared to controls. Although these results did not
reach significance with potassium, there was a significant difference in
the maximum response to noradrenaline. Thus iloprost appears to have the
opposite effect to papaverine on smooth muscle cell function. However,
the endothelium-dependent relaxation with iloprost was less than that
seen in the control group although the difference was not significant.

The results presented in this chapter show that papaverine appears
to have a prolonged effect on the smooth muscle cells of the vein
samples but it remains unclear whether this is a temporary effect on
smooth muscle cell function or permanent smooth muscle cell damage.
Iloprost, on the other hand, did not appear to have any effect on either
the endothelium or smooth muscle cells of the vein samples.

Conclusions

Papaverine appears to have a prolonged effect on smooth muscle
cells of vein grafts whereas iloprost does not appear to have any effect
on either the endothelium or smooth muscle cells.
SECTION THREE

DISCUSSION.
CHAPTER TEN

FINAL DISCUSSION, CONCLUSIONS AND FUTURE WORK.

Vascular surgery is a relatively young specialty which has progressed rapidly. Its role in the treatment of lower limb ischaemia needs to be constantly re-defined, particularly in the light of the continuing advances made in alternative techniques such as percutaneous transluminal angioplasty. However, femorodistal bypass procedures have become an established treatment option for lower limb ischaemia in carefully selected patients. The initial attempts at femorodistal bypass produced such poor results that many questioned the wisdom of such procedures and advocated amputation as the only realistic option. However the surgical techniques have improved and particularly the introduction of the in-situ method has led to femorodistal bypass becoming firmly established in many vascular centres. It is now realised that pre-operative assessment with techniques such as dependent Doppler ultrasound or PGR is vital to determine calf vessel patency, and that the use of intra-operative resistance measurement and arteriography can improve peri-operative decision making.

Unfortunately the problems associated with graft failure remain and the development of intrinsic vein graft lesions represent the major barrier to long term patency. Therefore it is important that attention should be focused not only on the detection and correction of these lesions but also on their aetiology and possible ways in which they
might be prevented. In order to do this, it is vital to investigate the changes that occur in vein grafts at a cellular level. There has already been a considerable amount of research into the development of vein graft lesions in reversed vein grafts and the recent work by Angelini has finally established a definite link between vein injury associated with the reversed technique and the subsequent development of intimal hyperplasia. However, there has been very little research into the in-situ technique of vein grafting and it has been assumed that the studies of reversed vein grafts apply equally to in-situ grafts.

The experimental work presented in this thesis attempts to redress this imbalance and specifically investigates the in-situ technique of femorodistal grafting. The development of the organ chamber has allowed the important cellular components of the vein (endothelium and smooth muscle) to be studied in vitro. A functional assessment of the degree of injury to these two important layers can be made, and these findings can be supported by evidence of structural damage that is provided by histological data. The results show that injury to both the endothelium and smooth muscle layers does occur during preparation of veins for in-situ bypass. The most important cause of this injury appears to be surgical handling of the vein during the operation which includes mobilisation, ligation of side-branches and passage of the valvulotome. This is reflected in functional impairment of the smooth muscle cells in their ability to respond to contracting agents and decreased production of EDRF caused by loss or injury to the endothelium. These findings are supported by the results of histology and scanning electron microscopy which shows that both endothelial cell loss and smooth muscle cell damage after preparation of veins for in-situ bypass. The other intra-operative manoeuvres that were studied (x-ray contrast medium, papaverine and iloprost) did not appear to cause significant additional
injury to the veins although papaverine did produce a long lasting
effect on smooth muscle cell function.

There are two important areas where future work is required. In
the clinical field, attempts must be made to reduce vein injury during
the operation by re-evaluating alternative technique of preparing in-
situ vein grafts. It may be possible to reduce damage to the endothelium
and smooth muscle cells by avoiding full mobilisation of the vein.
Although it has been suggested that this technique may lead to vein wall
perforation, there has never been a randomised trial to compare these
two methods.

In addition new designs of valvulotomes are constantly being
introduced which are designed to minimise endothelial trauma and each
one needs careful evaluation. The use of intra-operative angioscopy
would allow the luminal surface of the vein wall to be inspected and
provide an assessment of the effects of different types of valvulotomes
and different techniques of grafting.

The other major area where future research is needed is the
investigation of the relationship between the in-situ technique and the
development of vein graft lesions. The organ culture technique described
by Angelini could be adapted to provide an in vitro model of in-situ
vein grafts. This would allow the study of the relationship between
endothelial and smooth muscle cell injury and the subsequent changes in
the vein wall that lead to graft lesions. Once this has been
established, it should be possible to investigate ways in which
proliferation and migration of smooth muscle cells can be prevented with
new therapeutic regimes.
APPENDIX A

Composition of normal, calcium-free and high potassium (123mM) PSS.

<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>CaCl free</th>
<th>High potassium</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
<td>118</td>
<td>118</td>
<td>0.0</td>
</tr>
<tr>
<td>KCl</td>
<td>4.5</td>
<td>4.5</td>
<td>123</td>
</tr>
<tr>
<td>CaCl₂</td>
<td>2.5</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>MgSO₄·7H₂O</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>KH₂PO₄</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>NaHCO₃</td>
<td>25</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>Glucose</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
</tbody>
</table>

(Values are mmol/l)


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