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A NEW APPROACH TO THE PREVENTION OF

ANASTOMOTIC NEOINTIMAL HYPERPLASIA

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ABSTRACT

The standard approach to the surgical management of occlusive atherosclerotic disease of the femoral, popliteal and tibial arteries is by the use of bypass grafting. One of the major problems influencing the long-term patency of infrainguinal bypass grafts is the development of distal anastomotic neointimal hyperplasia, which in many cases causes the failure of the graft, with associated thrombosis and a resultant significant morbidity and mortality. The need for an effective means of limiting the development of anastomotic neointimal hyperplasia forms the basis of this thesis.

Endovascular stents were developed for use in native arteries after balloon angioplasty to overcome vessel elastic recoil as well as to rescue immediate angioplasty failures and intimal dissections. The work presented in this thesis was performed in order to investigate the effect on the site and development of anastomotic neointimal hyperplasia of placing such a device across the distal anastomosis of an animal model of an arterial bypass graft. A canine model of an aorto-bi-iliac bypass graft was developed and two separate distal anastomotic patterns investigated in separate experiments, namely the end-to-end and the end-to-side configurations. The early and late effects of stenting the distal anastomoses were examined for both models.

In the following Sections, an overview of anastomotic neointimal hyperplasia is presented. After outlining basic histological and pathophysiological considerations, a review of a variety of operative and pharmacological approaches to anastomotic neointimal hyperplasia that have been described in the literature is presented. There follows a general review of the use of endovascular stents prior to a description of my own experimental work on endovascular anastomotic stenting and the results obtained. These results are then analysed with particular focus on possible future refinements and modifications of the experimental model as well as the clinical applicability of anastomotic stenting.

The work presented in this thesis represents a novel approach to the problem of anastomotic neointimal hyperplasia in lower limb arterial bypass grafts. It is hoped that some of the lessons learned in performing this study can be applied to future developments in this field.

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This work was funded in part by a grant from the Schneider Stent Company of St Paul, Minnesota who also provided the stents utilised in the experiments and I am deeply grateful to them, especially to their medical director, Dr Sheila Douglas. The grafts were donated by W.L. Gore Inc. of Flagstaff, Arizona and I wish to thank Mr Charles Smith, their Mid-West Marketing Director for his help.

DECLARATION

The work described in this thesis is completely original and all of the operative procedures were performed by me, assisted by Dr J.J. Hoballah, Assistant Professor of Vascular Surgery, University of Iowa.

DEDICATION

This thesis is dedicated to my wife, Clare, and to my parents, with my deepest gratitude for all of their love and support.

SECTION I

Introduction

By definition, intimal hyperplasia is an abnormal accumulation of cells and extracellular matrix in the tunica intima of an artery or vein. The increase in cell numbers is due to a proliferation and migration of smooth muscle cells from the underlying tunica media. Through a series of intracellular and extracellular reactions, some or all of these smooth muscle cells acquire the ability to secrete collagen, elastin and other extracellular matrix proteins.

In vascular surgical practice, intimal hyperplasia can develop at any location where operative or endovascular intervention has resulted in an alteration in the normal architecture of a vessel.[90,236] The extent of the intimal hyperplastic response cannot be predicted but all lesions have in common similar basic histological features. Intimal hyperplasia may be found after various vascular interventions such as surgical endarterectomy[37,56], transluminal balloon angioplasty[174,186], as a late complication of catheter embolectomy[31,235], or at the distal anastomosis of a peripheral arterial bypass graft.[97,188,268,271] The lesion may be insignificant both in terms of the degree of stenosis and the lack of symptoms in certain cases, but in others, may be a major cause of morbidity.[9,12,32]

In modern vascular surgical practice, the mainstay of the treatment of limb-threatening, occlusive atherosclerotic disease affecting the arteries of the lower extremity is by the use of bypass grafting. The use of a conduit to bypass the affected segment(s) of vessel, aims to restore the distal circulation and salvage the limb.

The best long-term patency rates for such grafts, particularly when they are anastomosed distally to arteries below the knee joint, have been reported with the use of the patient's own long saphenous vein used either *in situ* or in reversed orientation. The reported secondary patency at five years for such bypass grafts is up to 85%.[98,281]

However, in some patients it is not possible to use this vein as a conduit, either because of its prior use as a bypass graft, or because of previous vein stripping or because of its poor quality or small diameter. Under such circumstances, an alternative graft material is required. Some authors have described favourable results with other sources of autogenous vein.[48] On the other hand, various alternative prosthetic graft materials have been developed over the years. The prosthetic graft material in commonest use for infrainguinal bypass grafts in clinical practice today is polytetrafluoroethylene (PTFE). A considerable literature has accumulated on the patency rates for PTFE lower limb bypass grafts. For grafts where the distal anastomosis is placed at the above-knee popliteal artery level, the patency rates are comparable to those for autogenous vein.[219,281] However, for PTFE grafts to the below-knee popliteal artery and to the tibial arteries, the patency is significantly inferior to that for vein bypasses.[23,217] From the literature, the cause of approximately 25% of PTFE graft failures is distal anastomotic neointimal hyperplasia. The lesion develops as an accumulation of tissue at the anastomosis that extends into the proximal graft for a few millimeters which angiographically, appears as a concentric filling defect (Figure 1). As the lesion progresses, the degree of stenosis at the distal anastomosis increases until the flow through the bypass is sufficiently low as to lead to thrombosis occurring within the graft.

In the following chapters, I shall review the morphology, pathophysiology and the current theories as to the aetiology of anastomotic neointimal hyperplasia (A.N.H.). In addition the significance of A.N.H. to the practice of clinical vascular surgery will be outlined. Current concepts as to the various approaches to ameliorate anastomotic neointimal hyperplasia, both pharmacologically and surgically, will be presented. Experimental models of neointimal hyperplasia from the literature will be appraised and thereafter, I shall present a laboratory model that I personally developed and describe a novel approach to the prevention of anastomotic neointimal hyperplasia which I have investigated.

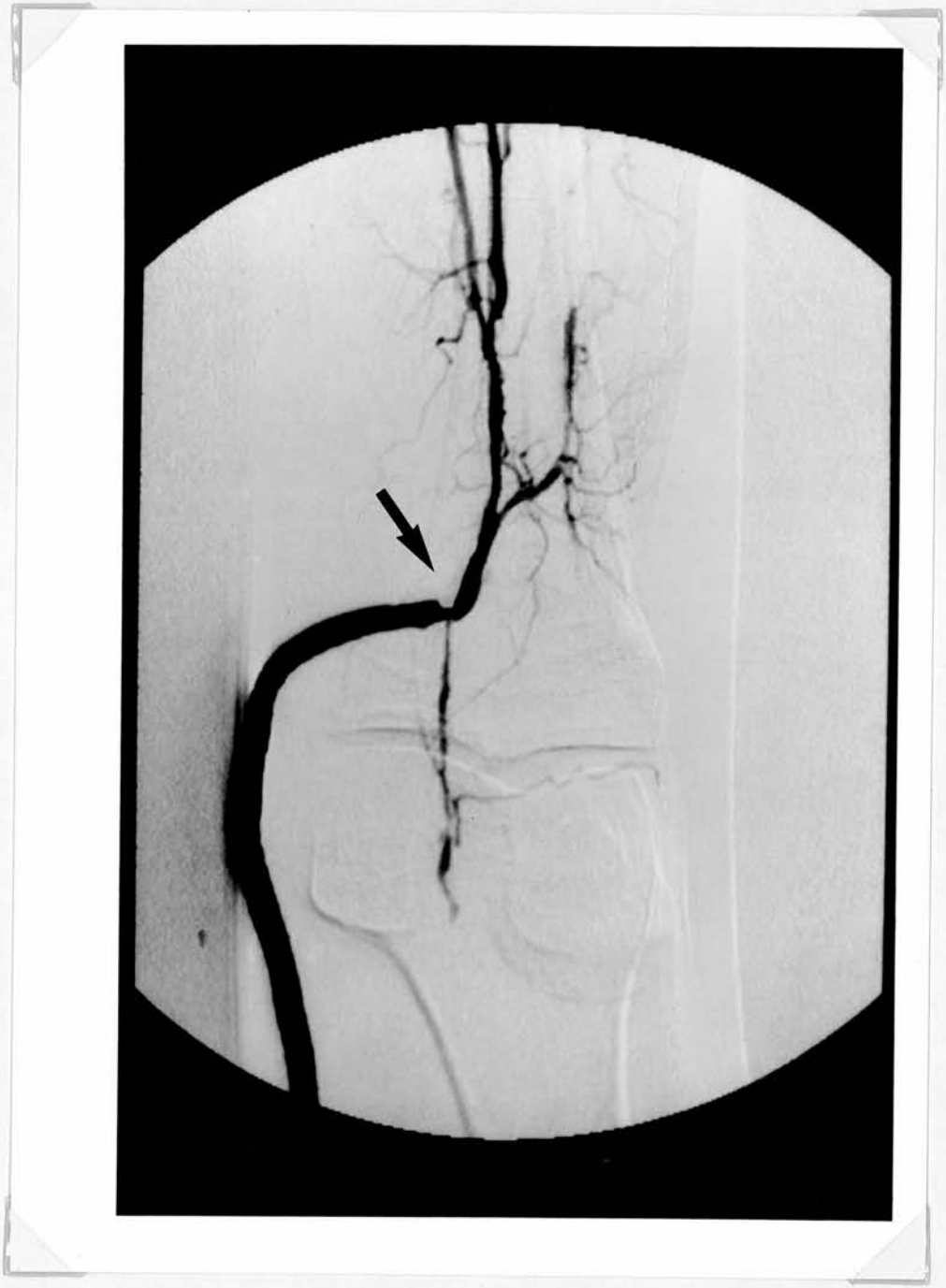

Figure 1: This angiogram shows a stenotic defect (arrow) due to neointimal hyperplasia at the distal anastomosis of a PTFE graft to the below-knee popliteal artery.

Historical Review

The formation of neointimal hyperplasia following operative procedures on blood vessels is not a new concept. In the early years of this century, the pioneers of modern vascular surgery encountered this lesion as a factor that frustrated their early attempts at vascular anastomosis. Alexis Carrel and co-workers were truly visionary in their laboratory experiments.[110] In the paper entitled "Anastomosis of Blood Vessels by the Patching Method and Transplantation of the Kidney"[43], Carrel described clearly the development of anastomotic intimal hyperplasia: "...within a few days of the operation, the stitches placed in making the anastomosis became covered in a glistening substance similar in appearance to the normal endothelium. In some cases, there was a ribbon-like deposit of fibrin around the line of the anastomosis. Irregular shaped clots sometimes occurred at points of defective union of the intimas...".

In another contemporary publication[42], Carrel described at length various methods he had utilised in a canine model for the "uniterminal" and "biterminal" anastomosis of veins and arteries. He was aware that poor surgical technique could lead to the deposition of blood factors that might have a detrimental effect on patency. Particular attention was paid to the anastomosis of vessels of different calibre: "...in order to approximate the opening of the vein to that of the artery, the wall of the former must be folded or puckered. On account of this venous folding, and of the thickness of the arterial wall, it is difficult to obtain an accurate approximation of the endothelium of the two vessels....in the bottom of these foldings, and in the interendothelial spaces, fibrin may be deposited and form the nucleus of an occluding thrombus...".

Carrel also attempted to construct an arterial bypass conduit from a tube fashioned from peritoneum, again in a canine model. Not surprisingly, his efforts were thwarted by graft thrombosis: "...in the third case, there was no infection, but thrombosis of the vessel had occurred. At each end of the peritoneal segment was found a fibrous clot, completely obliterating the lumen at the anastomosis...".

Thus it can be seen that since the earliest attempts at vascular reconstruction, surgeons have been aware of the potential for the development of neointimal hyperplasia at the site of anastomoses as well as at the sites of other varieties of vascular intervention. Since Carrel and Guthrie's time, the evolution of new vascular grafts and other innovations such as endovascular therapy have all been limited by the development of some degree of neointimal hyperplasia.

For the purposes of this thesis, subsequent comments will be restricted to the development of anastomotic neointimal hyperplasia in peripheral arterial bypass grafts.

Histology and Morphology

The lesion of anastomotic intimal hyperplasia is just as Carrel described it in 1906. Macroscopically, it appears as a plug of grey, white, or yellow tissue usually located just distal to the arterial side of an anastomosis and extends up onto the graft surface for 1-2cm.[35,83] The result is usually a circumferential stenosis. The lesion is less frequently found at the proximal as compared to the distal anastomosis of a peripheral arterial bypass graft.[166] A great deal of work has been done over the years to characterise and quantify at a microscopic level the cell types involved in this complex lesion.[22,25,91,132]

Sottiurai obtained tissue at the time of reoperation or amputation from 11 vein bypasses, 4 bovine heterografts, 7 dacron prostheses and 27 polytetrafluoroethylene grafts that had failed and were excised en bloc.[251] These samples were examined with light microscopy, scanning and transmission electron microscopy. He was able to characterise the lesions and also he compared the morphological differences seen in the various conduits. In addition, he was able to obtain additional information regarding the natural history of arterialization of vein grafts.

All vein grafts examined were lined with endothelial cells positioned in an orderly pattern, with the cellular long axis orientated parallel to the direction of blood flow. Underlying the apparently "normal" endothelial cell layer were smooth muscle cells and a subendothelial matrix consisting of abundant mucopolysaccharides and moderate amounts of collagen and elastin. Degenerative changes were also seen in a proportion of the smooth muscle cells as was the transformation of smooth muscle cells to myofibroblasts, which had the combined features of fibroblasts and myoblasts. Myofilaments were scanty but there was abundant rough endoplasmic reticulum seen confirming the secretory role that these cells possess in this situation.

Bovine heterografts exhibited somewhat different features from the vein bypasses when inspected microscopically. Endothelial coverage was limited to 2 to 4cm from the arterial anastomosis and in isolated areas adjacent to the anastomosis. Thickening of the bovine

heterograft near the artery was accompanied by subendothelial collagenous fibroplasia admixed with cellular debris.

The dacron grafts studied by Sottiurai had complete luminal lining with the longitudinal axes of fibroblastoid cells parallel to the direction of blood flow. These fibroblasts contained abundant endoplasmic reticulum within the cytosol and deep to them were a quantity of poorly organised elastin, collagen and ground substance. Orderly distribution of fibroblasts alternating with fibrous connective tissue elements formed a laminated arrangement throughout the thickness of the "pseudointima".

Unlike all of the other grafts analysed in this study by Sottiurai, polytetrafluoroethylene (P.T.F.E.) grafts lacked any sort of cell coverage at all. Rather, a gelatinous layer of haematogenous debris, including platelets, red blood cells, fibrin, white blood cells and plasma protein was seen to be adherent to the luminal surface of the P.T.F.E. graft. This occurred as a compact lining of the graft. Neointimal hyperplasia, manifested by subintimal fibroplasia, was found exclusively at the heel and toe of the anastomosis and in the floor of the native artery. The microscopic changes seen with the different failed graft conduits were similar, but the amount of neointimal hyperplasia seen in vein grafts was less than in the others. Indeed, only 6 of the 11 vein bypasses studied were found to have neointimal hyperplasia, whereas in all of the other conduits, A.N.H. was prominent and all but occluding the lumen. Sotturiai described the presence of smooth muscle cells with structure and function transformed to that of fibroblastoid appearance as the "sine qua non" of anastomotic neointimal hyperplasia. The dominant pathological features encountered in the intimal hyperplasia in each type of arterial substitute studied were myocyte degeneration, myofibroblast proliferation, and fibrous matrix accumulation. A laminated distribution of myocytes alternating with fibrous connective tissue in a concentric pattern characterized the sequential order of the pathomorphogenesis of intimal hyperplasia. It was concluded that in response to the physical stress caused by turbulent flow and mismatched properties of the graft and artery, myocytes undergo morphological degeneration and transformation. The

former eventuates cell death, while the latter leads to massive extracellular connective tissue production resulting in fibroplasia of the intima.

In subsequent studies, using animal models of vascular anastomoses, of both end-to-end and end-to-side configurations, Sottiurai et al made further observations on the cellular structure of the lesion of anastomotic intimal hyperplasia.[252,254] They found no A.N.H. in the anastomoses of the end-to-end configuration. In the end-to-side models, however, there were striking morphological differences between the A.N.H. seen at the toe and at the heel of the anastomosis. The A.N.H. at the toe was mostly a fibrocollagenous matrix with a scanty admixture of slender myofibroblasts. The latter accounted for less than 20 per cent of the volume of the anastomotic neointimal hyperplasia. By contrast, the A.N.H. encountered at the heel had a greater cellular component, with myofibroblasts accounting for 40 per cent of the lesion. The extracellular matrix at both the heel and the toe was predominantly collagenous with fragmented elastic tissue interspersed. The collagen bundles were both parallel to and oblique to the longitudinal orientation of the cellular components. The reasons for these observed differences between heel and toe A.N.H. are not known but it may be that the morphological variations reflect different regional blood flow patterns. Alternatively, they might simply represent different stages in the development of A.N.H., or indeed that A.N.H. may be the result of a remodelling process influenced by haemodynamic factors.

In a later study[250], Sottiurai et al made some other interesting observations on the cellular events involved in the evolution of A.N.H. Again, utilising electron microscopy, they observed that leucocytes appeared to adhere to the gap junctions between endothelial cells. Platelets were then seen to traverse these gap junctions (in the endothelium) at the heel and at the toe of the anastomosis. Vascular endothelial cells have a specific anatomical orientation, with the long axes of the cells orientated parallel to the direction of blood flow. The particular anatomical configuration that the distal process overlaps the adjacent proximal cell border is referred to as the "shingle effect". This arrangement allows stretching of the endothelial cell lining, for example during the cardiac cycle, without exposure of the underlying subendothelial matrix to cells and other elements of the circulating blood. The

pre-existing "shingle effect" is predisposed to injury by turbulent flow at the heel and toe. Endothelial cell "lifting" permits blood borne elements (platelets, white cells, fibrin, complememt, etc) to enter the subendothelial space without disrupting the endothelium. Via this route, these cells and substances are thought to gain access to the subintimal space and thereby are able to stimulate the proliferation and transformation of smooth muscle cells leading to the development of A.N.H.

In a series of papers reporting their experience with 4mm polytetrafluoroethylene (P.T.F.E.) grafts inserted into the iliac or carotid circulations of male baboons[59,60,62], Clowes et al furthered the understanding of the sequence of events leading to the development of P.T.F.E. graft-arterial anastomotic neointimal hyperplasia. They showed that:

(i) the endothelium over the graft arises from the neighbouring arterial endothelium and grows as a continuous surface monolayer towards the centre of the graft conduit.

(ii) smooth muscle cells also derived from the adjacent artery migrate and proliferate in association with the endothelial growing edge and form the bulk of the thickness of the neointima.

(iii) the endothelial growing edge appeared to be just ahead of the underlying smooth muscle cell layer.

(iv) the process is progressive and both endothelium and smooth muscle cells located discretely over the anastomosis continue to proliferate despite complete endothelial coverage. This process is decidedly different from the hyperplastic lesion seen in models of arterial injury in which smooth muscle cell proliferation ceases after the overlying endothelial layer is restored. As a result of this finding, the authors deduced that since normal endothelial cells only proliferate when they are separated from each other, continued endothelial cell proliferation must imply continued endothelial injury, albeit without frank denudation.

(v) intimal cross-sectional area in the region of the anastomosis is always greater than over the adjacent graft.

(vi) there was no evidence that fibroblasts underwent transformation into smooth muscle cells.

In a subsequent report of the late (6-12 month) findings in the same animal model[60], they found that the process of endothelial and smooth muscle cell growth continued although at a slower rate. Short grafts (7.1+/-0.2cm) in their study were found to achieve complete coverage of neointima whereas longer grafts (8.4+/-0.3cm) did not, implying that there was a critical length of graft above which, even in this "chronic" model, complete neointimal coverage would never be achieved. Intimal cross-sectional area was greatest at the anastomoses and at late times was principally due to an increase in connective tissue, the smooth muscle cell mass remained constant after 3 months.

Clowes et al[62] wondered if it would be possible to enhance the endothelialisation of the middle portion of the bypasses (furthest from the growing edge of neointima) by increasing the porosity of the graft material used, allowing the ingrowth of capillary tufts (which might act as a source of endothelial cells) and thereby reduce the thrombogenicity of the conduit. Again, they placed 4mm diameter P.T.F.E. grafts into the aorto-iliac circulation of baboons. The graft pore size was 60 micrometres, compared to 30 micrometres used in their prior studies. They found that intimal thickening, like endothelial and smooth muscle cell proliferation, was distributed evenly along each graft and was not confined to the anastomotic regions as was the case with 30 micrometre P.T.F.E. grafts. Furthermore, in the 60 micrometre grafts, capillaries derived from the granulation tissue surrounding the graft were seen to penetrate the wall and provide multiple sources of endothelium at the luminal surface of the graft. These capillaries were at most 100-500 micrometres apart and therefore endothelial coverage of the luminal surface by outgrowth was extremely rapid and appeared to be complete by 14 days.

These exciting results provoked the question as to whether these findings could be reproduced in human subjects. Kohler et al[149] reported on a clinical study aimed at determining if spontaneous endothelialisation of P.T.F.E. grafts can be achieved in human subjects. They constructed a graft composed of two equally long pieces of P.T.F.E., one having a pore size of 30 micrometers and the other 60 micrometers. In a study of ten such above-knee PTFE femoro-popliteal bypass grafts placed in eight patients with occlusive

peripheral vascular disease, spontaneous endothelialisation of the conduit did not occur. In comparing 30 micrometre pore size to 60 micrometres, capillary ingrowth was seen in the grafts of higher porosity, but it rarely extended more than half the distance from the outside of the graft lumen and thus could not stimulate the development of an endothelial lining.

A number of investigators have established different animal models in order to study the evolution of anastomotic neointimal hyperplasia. LoGerfo et al placed 6mm dacron femoro-femoral bypass grafts in dogs.[166] The proximal and distal anastomoses were of the end-to-side configuration, as in the standard bypass graft performed in humans for atherosclerotic occlusive disease. In half of the animals, the outflow from the distal anastomosis was bidirectional (achieved by ligating the recipient artery proximal to the profunda femoris artery take-off) whereas in the other half, the outflow was unidirectional (the femoral artery was ligated distal to the profunda femoris artery guaranteeing that all flow was directed down the femoral artery only). Grafts that remained patent were harvested at intervals of two to 196 days so that changes with time could be observed. Specimens were inspected histologically and it was found that midsaggital sections from both the proximal and the distal anastomoses exhibited an ingrowth of cells from the host artery. The pannus of growth extended 1-2cm into the graft prior to becoming a neointima of compact fibrin. In no instance was the centre portion of any graft lined with cells. In all cases, the distal anastomoses exhibited statistically greater amount of A.N.H. than its corresponding proximal anastomosis and this was irrespective of outflow type. Anastomotic hyperplasia was found to be progressive at both anastomoses in both groups. Electron microscopy of the pannus growth demonstrated a surface of polygonal shaped cells, absent of adherent blood cells. This lining was seen to be contiguous with the arterial endothelium across the suture line and was presumably derived from the host vessel endothelium. In contrast, the lining of the central portion of the grafts consisted solely of compact fibrin. Transition occurred abruptly between the pannus of cellular growth and the fibrinous lining. Logerfo et al speculated that the propensity for hyperplasia to occur downstream suggested that interactions between blood and the graft material might stimulate the hyperplastic response. As the grafts in their study were longer

than the short interposition grafts favoured by other investigators, it seemed likely that prolonged contact of blood with the graft might give rise to greater stimulation of the hyperplastic response at the distal anastomosis.

In a subsequent study from the same laboratory, Cantelmo et al reported on their findings when they compared dacron and P.T.F.E. grafts inserted into the canine carotid circulation.[41] In addition, they assessed the development of neointimal hyperplasia in both an end-to-end and an end-to-side model of proximal and distal anastomoses. For both groups (dacron and P.T.F.E.) there was a significantly greater amount of neointimal hyperplasia at the distal anastomosis compared to the proximal anastomosis ($p<0.001$-dacron; $p<0.005$-P.T.F.E.). There was a significantly greater response in the dacron prostheses at the proximal anastomosis compared to P.T.F.E. ($p<0.05$). However, unlike Sottiurai et al, they were unable to document a quantitative difference in anastomotic hyperplasia between end-to-end and end-to-side anastomoses. As a result of the findings of this study, the authors reiterated their belief that neointimal hyperplasia is greater at downstream anastomoses because after blood enters the prosthetic graft, it is activated by contact with the prosthetic surface (acute) or the acellular pseudointima (chronic) which leads to the release of thrombogenic substances. The first cells that are capable of responding to these activated circulating blood-borne elements are found in the region of the distal anastomosis, since the graft is lined by an acellular, fibrinous neointima. They concluded from this study that as downstream anastomotic hyperplasia was common to both types of prosthetic graft material investigated, that this may be the final common pathway of failure for other or possibly all prosthetic arterial grafts.

Further observations on the nature of the pannus growth of anastomotic hyperplasia were reported by Kuwano et al.[157] They placed 6mm P.T.F.E. interposition grafts into the iliac circulation of dogs and studied the harvested specimens at serial time intervals. They found two differing types of pannus growth. The so-called "smooth" type of pannus extending from the anastomotic artery into the graft, as described by others was seen in this model. Also, in a third of the grafts, a second "nodular" pannus lesion was identified. The authors

postulated that this might represent organisation of thrombus and they wondered if thrombus deposition was the starting point for the evolution of A.N.H.

Watase et al analysed the ultrastructure of what they described as "pseudointimal" hyperplasia in P.T.F.E. grafts inserted into the arterial and venous systems of albino rabbits.[286] From their observations, they concluded that intimal hyperplasia develops more rapidly in the venous system than in the arterial system, but that the mechanism of its pathogenesis is essentially the same, namely: early thrombosis, phagocytosis of the thrombus, appearance of fibroblasts, growth of endothelial-like cells, appearance of smooth muscle cells and pseudointimal thickening and proliferation of myofibroblasts producing collagen fibrils. Further, they proposed a number of possible sources of the endothelial-like cells which appeared near the anastomotic region. The most likely source, in their estimation, was direct migration from the host artery. However, they also suggested implantation of circulating multi-potential cells or ingrowth of scattered endothelial cells from the margins of the mouths of new vessels which penetrate the interstices of the graft. As we have seen from the work of Kohler et al[149], the latter two theories appear to have been refuted in human studies. These apparently contradictory findings from different authors may be partly explained upon the basis that a variety of animal models and species have been used. Other contributing factors may also have been the graft material used, the diameter and length of graft and the vessels into which it was placed and finally the use of adjuvant drugs such as antiplatelet agents and anticoagulants. The final comment to make at this stage pertaining to observations made with animal models of vascular grafting is that it is not possible to extrapolate with certainty results in an animal model to the findings in diseased human vessels.

Mitogens, Cellular Events and Messengers

Having outlined the morphological characteristics of the lesion of anastomotic neointimal hyperplasia, it is important to consider the means by which these changes occur. Clearly, a process as complex as this is the result of the interaction of a multitude of factors.
First, let us consider the various cell types involved in the lesion of intimal hyperplasia.

Endothelial Cells.

Williams[293] stated that as well as traumatic injury to endothelium being a stimulus to the development of intimal hyperplasia, metabolic injury, such as diabetic hyperglycemia can also stimulate a cellular intimal response.
Studies with porcine aortic wall cells in culture by Koo and Gotlieb[151] demonstrated that:
(i) the increased rate of migration and turnover of smooth muscle cells in response to injury is dependent upon the presence of endothelial cells.
(ii) the smooth muscle cell proliferation is maximal when the endothelial cells are proliferating.
(iii) medial proliferation of smooth muscle cells is not an essential prerequisite to intimal migraton as the smooth muscle cells can proliferate within the intima.
The significant role of the intact endothelial cell layer with respect to intimal hyperplasia in vein grafts has been demonstrated by a number of authors.[70,167,178,228] From these studies it can be hypothesised that the endothelial cells of the native artery adjacent to a prosthetic graft anastomosis must play a similarly significant role.
In their baboon prosthetic bypass graft model, Clowes et al showed that smooth muscle cells underlying an area of endothelial cell regeneration exhibited an active proliferation rate, while the smooth muscle cells in the native artery remained quiescent.[59] Also, the endothelial

monolayers that form over the proliferating smooth muscle cells exhibit an elevated cell turnover rate compared to endothelial cells in the native artery surface.

Using a P.T.F.E. interposition bypass graft placed in the rabbit carotid arterial system, Mason et al demonstrated that smooth muscle cell proliferation was greater at the distal than at the proximal anastomosis.[181] However, this difference did not appear to be related to the degree of endothelial loss or regrowth. A further possible link between endothelial cell function and the development of neointimal hyperplasia was suggested by Sharefkin et al.[243] Endothelin-1 is a 21 amino acid peptide secreted by endothelial cells which has vasoconstrictor action and mitogenic activity for vascular smooth muscle cells. Its mitogenic activity is synergistic with that of platelet-derived growth factor (P.D.G.F.). They hypothesized that endothelial-cell derived endothelin-1 might contribute to the development of intimal hyperplasia at the sites of the reendothelialisation of vascular grafts. Using a cell culture model subjected to varying degrees of laminar shear stress (shear stress is a function of volume flow rate/vessel diameter^3), they showed that the release of endothelin-1 from human endothelial cells (from umbilical vein) was inversely proportional to the level of shear stress. In other words, low shear stress, shown by others to be a stimulus of intimal hyperplasia, may achieve this via the enhanced secretion of endothelin-1 from endothelial cells. However, this factor can only be one of the culprits, as, in 1974, in electron microscopic studies of experimentally-induced Neointimal hyperplastic lesions, Imparato et al showed that these lesions occurred in both high flow and low flow areas.[130] Indeed, in the high flow models, hyperplastic lesions were seen as early as 2 days after the creation of an anastomosis between the renal artery and the vena cava in a canine model.

Endothelial cells are known sources of a number of growth factors[242] as well as "relaxing" factors.[150] Fox and DiCorleto[106] showed that the growth-promoting activities of cultured endothelial cells could be increased four times at the extremes of metabolic acidosis and alkalosis, another form of chemical injury..

Another important aspect of endothelial cell function is the anti-thrombotic nature of these cells. Quiescent endothelial cells actively secrete prostacyclin (PGI2) and produce an

anionically charged surface glycocalyx rich in heparan sulphate and chondroiton sulphate. Endothelial cells also cull platelet activators from the local environment by binding and inactivating thrombin via membrane-bound antithrombin III or thrombomodulin. PGI2 is a potent vasodilator and platelet inhibitor and its release is provoked by vascular trauma. In a rabbit aortic model, Korchi et al[152] showed that animals treated with a prostacyclin analogue (TRK-100) given subcutaneously had less intimal hyperplasia at the anastomosis than did hyperlipidemic rabbits, suggesting an inhibitory role for PGI_2. Sumpio and Banes showed that cyclic stretching did not alter basal endothelial cell production of PGI_2 but it increased PGI_2 synthetic capacity in a time-dependent manner.[264]

Smooth Muscle Cells

Medial smooth muscle cells begin to migrate into the intima within the first 24 hours of vascular injury.[26,52] Their proliferation can occur in either the media prior to migration or once they are located in the intima.[229] There are numerous factors driving these changes, including mitogen secretion by the various cellular elements present as well as the smooth muscle cells themselves. In a series of experiments with the adult rat carotid artery injury model, Bowen-Pope et al's findings suggested that production of platelet-derived growth factor (P.D.G.F.) by smooth muscle cells might be activated as a response to injury and might supplement the P.D.G.F. derived from attached, degranulated platelets and from activated endothelial cells, thereby contributing to the extensive and prolonged proliferation of smooth muscle cells in the intima of the injured vessel.[30]

Sterpetti et al[258] showed that smooth muscle cells from bovine thoracic aortas secreted increasing amounts of P.D.G.F. as the shear stress increased in their *in vitro* flow system model. This study suggested one means by which haemodynamic factors could induce cellular changes and mitogen release. Cyclical stretching related to pulsatile blood flow as well as the abnormal flow dynamics of vascular anastomoses (and other reconstructions as

well) are known to stimulate smooth muscle cell migration and proliferation by themselves.[5,54,61,88,162,253,265]

In early work with the rat carotid artery injury model, Clowes et al[57] showed that luminal narrowing was maximal after 2 weeks and less marked at 12 weeks. This was found to be due to the fact that the early luminal narrowing was mostly caused by vasoconstriction secondary to smooth muscle cell contraction, whereas the late narrowing was due to intimal thickening only. However, the total volume of smooth muscle cells remained more or less constant at both time intervals, indicating that continued intimal thickening at late time points is due to synthesis and accumulation of connective tissue and extracellular matrix, without further increases in smooth muscle cell numbers. Chidi et al[54] demonstrated experimentally that the source of subintimal collagen after arterial injury was transformed, media-derived smooth muscle cells and that there was no evidence from their study that endothelium contributed any of the collagenous extracellular matrix in ther intimal hyperplastic lesion. Chan et al[49] showed that the smooth muscle cells derived from stenotic lesions at the site of arterial balloon angioplasty progress to the third passage in cell culture whereas those derived from atherosclerotic plaques do not. Furthermore, the former are far less sensitive to the inhibitory effects of heparin than are normal or atherosclerotic plaque smooth muscle cells. Further studies by Birinyi et al, on tissue obtained from human anastomotic hyperplastic lesions recovered at the time of revisional surgery, showed the cells to be of vascular smooth muscle cell origin, to have the potential to stimulate smooth muscle cell growth, to contain mR.N.A. that encodes for P.D.G.F.-A and for P.D.G.F. receptors.[27] This "autocrine" effect might explain why platelet-inhibiting drugs, such as aspirin, have failed to have an impact on the development of anastomotic intimal hyperplasia. Further evidence for this hypothesis is provided by the work of Golden et al[112] who suggested that the continued proliferation of smooth muscle cells in high porosity grafts in baboons (as noted by Clowes et al) that have complete endothelial coverage implies an alternative source of growth factors than platelets. However, Sottiurai et al have provided convincing electron microscope

evidence that even an apparently intact endothelium can permit the passage of cells due to the loss of the normal so-called "shingle" effect, especially at the anastomotic level.[250,254]

Platelets

Because of their relatively small size as compared to the other cellular elements of the blood, platelets are marginated and thus pushed into contact with the vessel wall. Flow profiles are parabolic in large and medium-sized arteries and the flow is therefore slower along the walls than in the central lumen. Platelets are in contact with the wall "seeking" a breach in the endothelium or exposed subendothelial matrix.

Platelet adherence, aggregation and degranulation are the earliest events to occur after endothelial injury or restoration of blood flow through a bypass graft.[225] Importantly, a number of studies have shown that platelet adherence to the prosthetic material is not confined to the early period following graft placement.[3] Stratton et al used indium-111 labelled platelets to study patients with dacron aorto-femoral bypasses.[260] These grafts had been in place for between 9 and 120 months. Compared to normal control subjects (young adults with no graft in place) platelet deposition was consistently present and the difference between the 2 groups of subjects was highly significant ($p<0.001$). The authors concluded that their results implied that there was incomplete endothelial coverage of the graft flow surface. Also, there must be some sort of equilibrium between deposition and dissolution otherwise thrombosis would occur in every case.

Seeger et al sought to determine the differences between platelet accumulation on two different grafts (dacron and P.T.F.E.)[238] Using a canine carotid interposition graft (dacron on one side, P.T.F.E. on the other) indium-111 labelled platelet deposition was evaluated at 1, 7 and 21 days after graft implantation. Platelet accumulation decreased on all grafts between 1 and 7 days ($p<0.05$). However, platelet accumulation then increased on the dacron grafts between 7 and 21 days to levels greater than on day 1. P.T.F.E., on the other

hand, had a minimal increase after day 7 and this was well below the value obtained on day 1. The dacron grafts developed a thick, collagen-rich pseudointima whereas P.T.F.E. had a thin, incomplete pseudointima. The dacron grafts had inferior patency to that of the P.T.F.E. grafts (78% vs 89%). These findings may be explained by the findings of Sottiurai et al[251], in that although dacron achieves complete coverage with pseudointima, it is composed of fibroblastoid cells and collagen organised in a lamellated form and this is far from the non-thrombogenic nature of intact, quiescent vascular endothelium.

Further studies examining platelet-graft interactions were performed by Ito et al.[133] In a dacron carotid to aorta graft tunnelled subcutaneously in a canine model, they showed that these long grafts resulted in prolonged thrombocytopenia, a transient decrease in arachidonic acid (AA), ADP, and collagen-stimulated aggregation and a persistent decrease in ATP release to ADP and collagen stimulation after transit through the graft. This suggested that persistent platelet activation occurs even in grafts with a mature pseudointima. As anastomotic hyperplasia is greater at downstream anastomoses, Ito and collaegues postulated that blood interaction with the graft material leads to the release of mitogenic substances which have their effect on the first normal vascular cells they contact on exiting the conduit, i.e. at the distal anastomosis. Madras et al added credance to this theory by studying the thrombogenicity of blood after passage through various vascular grafts.[179] Samples of blood that had passed through a graft showed a marked predisposition to thrombus formation with no change in clotting time. Dacron grafts were found to preactivate blood more than P.T.F.E. grafts, which showed a lesser degree of activation, more like that of autogenous vein.

White Blood Cells

The role of the white blood cell is not as well defined as that of the other cell types involved in the genesis of intimal hyperplasia. A proportion of circulating leucocytes are marginated or adherent to the arterial endothelium. Electron microscope studies have shown both monocytes and lymphocytes adherent to and even penetrating the endothelium. Further, the standard balloon catheter-induced arterial injury model causes leucoytes to adhere to the de-endothelialised surfaces.[52,114] Studies have shown that smooth muscle cells and macrophages secrete substances that are chemotactic for leucocytes. Another agent that is chemotactic for monocytes is P.D.G.F. Monocyte derived macrophages not only have scavenger functions at the site of vascular injury, but also have the capacity to secrete effector proteins, growth factors and mitogens, implying a role in the healing process.[115] The white cells thus sequestered then secrete growth factors that stimulate the proliferation and migration of smooth muscle cells. Neutrophil polymorphonuclear leucocytes may play a destructive role.[289] Exposed to complement, immune complexes and endotoxins, neutrophils induce endothelial damage through oxygen-free radicals. Neutrophil activation may lead to the detachment of the endothelial cells that are marginally adherent to the site of injury causing a persisting exposure of the subendothelial matrix. Studies of arterial balloon catheter injury have demonstrated an inhibitory effect on the development of intimal hyperplasia by administering corticosteroids.[51,52,66] This may be due to the effects that these agents have on neutrophils which include decreasing the adhesiveness of the cells, decreasing the chemotactic response of the neutrophils to various agents present at the site of arterial injury as well as the inhibition of neutrophil degranulation.

A further stimulus to platelet aggregation within the graft may be under the control of the complement system. Adherence of leucoytes to the irregular graft pseudointima, with the subsequent release of both aggregation factors and mitogenic substances, is at least partly caused by complement released in response to contact with the graft's polymer surface. In a series of in vitro experiments, Coleman et al compared the effects on complement activation

of various graft materials alone and in combination with suture materials using a radioimmunoassay for the measurement of complement C5a levels.[68] Polypropylene and P.T.F.E suture material caused significant C5a activation whereas novafil (polybutester) did not. Both dacron and P.T.F.E. graft material caused significant activation. The addition of suture materials to the P.T.F.E. graft did not increase the C5a levels above the P.T.F.E. graft material alone. However, dacron together with polypropylene or novafil suture (but not P.T.F.E.) elevated C5a levels significantly. Since the patterns of C5a activation in vitro paralleled those of platelet activation in vivo by the various materials tested, Coleman et al concluded from their study that complement activation may be implicated in the pathogenesis of neointimal hyperplasia by activation of platelets.

The Role of Mitogens and Growth Factors

The early critical events in the development of anastomotic neointimal hyperplasia are endothelial injury or denudement and immediate platelet adherence. Even if the endothelial cells are intact, as is the case at an anatomosis months after the placement of a bypass graft, the so-called "shingle" effect seen in normal endothelial cell homeostasis as described by Sottiurai appears to be disrupted.[254] Whether endothelial cell injury leads to cell death or transformation into mitogen-secreting cells is not certain.(A mitogen is a substance that induces blast transformation, D.N.A. and R.N.A. synthesis).

In any event, within minutes of injury, platelets and leucocytes adhere to the exposed subendothelial collagen. Platelets aggregate and release vasoactive and prothrombotic substances in addition to growth factors such as platelet derived growth factor (P.D.G.F.) and transforming growth factor-B (T.G.F.-B).[34,35] Monocytes migrate into the subendothelial layer, differentiate into tissue macrophages and release growth factors.[115] Lymphocytes within the thrombus generate cytokines such as interleukin 1 that are mitogenic for smooth muscle cells and fibroblasts. Studies have shown that the interleukin 1 induces P.D.G.F. gene expression in the smooth muscle cells themselves.[223] This has led a number of workers to describe a so-called "autocrine" system: the smooth muscle cells have been shown to produce the very growth factors (secreted by other cells that are also present) that stimulate their own growth.[34,194,293] Clearly, under normal conditions of arterial wall "quiescence", these events and functions are not apparent. Endothelial cells have also been shown to be a source of mitogens under certain circumstances[293], and thus the cellular events associated with the pathogenesis of neointimal hyperplasia clearly consist of an extremely complex network of interrelated factors.[137]

Growth factors are polypeptides and are the primary extracellular regulators of cellular proliferation and growth. Signal transduction is initiated by binding of the growth factor to its specific cell surface receptor. This leads to the activation of intracellular messengers (eg cAMP, calcium) and thereby to cellular proliferation. Growth factors can exert this effect

through receptors on the secretory cell itself (autocrine activity), on cells nearby (paracrine activity) or at a remote site (endocrine activity). Platelet-derived growth factor and transforming growth factors A and B are examples of growth factors that can exert autocrine activity. This may be one pathway by which endothelial and smooth muscle cells escape normal regulatory control and become autonomous leading to the proliferative lesion of intimal hyperplasia. There are a number of growth factors that are known to have effects on the cells of the arterial wall and these include platelet-derived growth factor (P.D.G.F.), fibroblast growth factor, epidermal growth factor and transforming growth factors A and B (Table 1).

Growth Factor	Macrophages	Monocytes	Platelets	Endothelial cells	Smooth Muscle Cells
P.D.G.F.	+	+	+	+	+
T.G.F.-B	+	+	+	+	+
F.G.F.	+	+	-	+	+
E.G.F.	+	-	+	-	-
T.G.F.-A	+	+	-	-	-

Table 1: **Growth factors implicated in intimal hyperplasia and their cells of origin.**
(Adapted From: Bull DA, Hunter GC, Putnam CW. Growth Factors and the Arterial Wall. Perspectives in Vascular Surgery 1991;2:88-103.[34])

Platelet-Derived Growth Factor (P.D.G.F.) is a cationic glycoprotein with a molecular weight of 31,000. As can be seen from Table 1, under certain conditions, P.D.G.F. is secreted by a variety of cell types other than platelets including macrophages, monocytes, smooth muscle cells and endothelial cells. Both rapidly-growing and quiescent endothelial cells secrete P.D.G.F in culture, but the rate of release can be varied by certain conditions. Both tumour promoters and endotoxin can stimulate the release twofold. This seems to be related to the injurious effects of these agents on the endothelial cells. P.D.G.F. has an A and a B polypeptide chain. The 2 chains are encoded by genes on different chromosomes

and each chain can be produced independent of the other. Therefore, P.D.G.F. may be secreted as either P.D.G.F.-AA, or AB, or BB. Its half-life in the circulation is approximately two minutes. As well as stimulating the proliferation of vascular smooth muscle cells acting synergistically with other growth factors, a phenomenon described by Grotendorst[116], P.D.G.F. is also mitogenic and chemotactic for fibroblasts and stimulates the migration of monocytes and macrophages.[34,194] This chemoattractant function occurs before D.N.A. synthesis. Chemotaxis occurs at a concentration of P.D.G.F. lower than that required for mitogenesis. The migration of smooth muscle cells in response to P.D.G.F. requires both R.N.A. and protein synthesis. However, in vitro studies have shown P.D.G.F. to inhibit endothelial cell migration. As this a key event in the formation of anastomotic neointimal hyperplasia, with the endothelial growing edge "ahead" of the smooth muscle cell component, by implication, other mitogens must be involved. The B chain of P.D.G.F. is produced under the control of the c-*sis* oncogene found on chromosome 22. In response to P.D.G.F. secreted by platelets, macrophages, etc., the c-sis oncogene is activated and this leads to further secretion of P.D.G.F. and increased expression of the P.D.G.F. receptor on the smooth muscle cell membrane. This suggests a mechanism by which smooth muscle cells could become autonomous: once secreted, P.D.G.F. may then act on its producer cell by binding to the receptors on the cell surface causing further proliferation and migration.[30,162,163,258]

In a study comparing endothelial cell-seeded P.T.F.E. to non-seeded grafts in a canine thoraco-abdominal aortic graft model, Kaufman et al showed that P.D.G.F. production was greater in the seeded grafts and did not decrease with time in either group.[138] Furthermore, the amount of P.D.G.F. production was maximal at the distal anastomosis of the grafts (Table 2).

	4 Weeks		12-20 Weeks		52+Weeks
	Seeded	Control	Seeded	Control	Seeded
Proximal Anastomosis					
A-200	18+/-12	43+/-14	17+/-15	11+/-6	1+/-1
A-100	41+/-14	44+/-9	34+/-12	24+/-11	6+/-5
g-100	193+/-37	145+/-34	113+/-47	110+/-20	292+/-79
G-200	102+/-19	76+/-22	68+/-25	104+/-24	362+/-83
Midgraft	13+/-7	13+/-4	18+/-10	76+/-25*	36+/-17*
Distal Anastomosis					
G-200	**43+/-11~**	101+/-39	61+/-43	**33+/-16~**	212+/-82*
G-100	**83+/-24~**	147+/-45	109+/-57	**29+/-12~**	289+/-105*
A-100	155+/-48	93+/-25	88+/-67	120+/-54	80+/-43
A-200	86+/-46	53+/-24	56+/-50	92+/-47	44+/-31

Table 2: **Intimal thickness of canine arterial and graft segments**
* $p<0.05$ compared to 4 weeks ~ $p<0.001$ compared to proximal anastomosis
(From Kaufman BR, DeLuca DJ, Folsom DL, et al J Vasc Surg 1992;15:806-16.[138])

Fibroblast Growth Factor (F.G.F.) is a potent smooth muscle cell mitogen as well as a stimulator of endothelial cell proliferation and migration. F.G.F. is secreted by capillary endothelial cells, monocytes and smooth muscle cells. There are two forms of F.G.F.: a basic and an acidic form. Both are 154 amino acid single chain polypeptides with a molecular weight of 15,000. They have a high affinity for heparin. Basic F.G.F. is important in maintaining the integrity of the endothelium of the vessel wall. Its role as a mitogen for endothelial cells is therefore to induce the restoration of an intact, non-thrombogenic endothelial layer after injury.[34] Under normal circumstances, F.G.F. exists mostly as an intracellular protein. It seems likely that cell injury or death leads to its extracellular release where it acts as an autocrine/paracrine repair factor. In the quiescent state, F.G.F. is found in the extracellular matrix of endothelial cells bound to heparan sulphate. This union not only prevents its breakdown but also augments its mitogenic

capability. When arterial injury occurs, this F.G.F. becomes activated by the release of heparanases from platelets, macrophages and T lymphocytes.[52]

Epidermal Growth Factor (E.G.F.) is a single chain polypeptide of 53 amino acids with a molecular weight of 6,000. It is released by platelets and macrophages. It is mitogenic for fibroblasts and smooth muscle cells and acts synergistically with other growth factors. Little is known about its in vivo functions since most of the published work regarding this substance to date has been in vitro.[34]

Transforming Growth Factors (T.G.F.) are so called as they have the ability to promote the growth of kidney fibroblasts independent of a matrix.[255] T.G.F.-A is similar in structure and function to E.G.F and promotes the migration and proliferation of endothelial cells. It is produced by monocytes. T.G.F.-B is secreted by platelets, monocytes and macrophages and is a chemoattractant for fibroblasts, monocytes and macrophages. T.G.F.-B stimulates smooth muscle cell proliferation but inhibits endothelial cell proliferation and migration. This inhibitory effect on endothelial repair implies an effort to prolong the exposure and stimulation of underlying smooth muscle cells. T.G.F.-B is found bound in the extracellular matrix (to chondroitin sulphate) and its contrary effects on endothelial cells and smooth muscle cells may be an important factor in the pathogenesis of neointimal hyperplasia. Further evidence for the role of TGF in the genesis of neointimal hyperplasia came from a study reported by Mc Caffrey et al who showed that fucoidan, a heparin-like, non-anticoagulant ligand of TGF inhibited smooth muscle cell migration and proliferation in vitro.[175]

Thrombin and other components of the coagulation cascade are known to stimulate growth factor release from platelets and vascular smooth muscle cells as well as having a mitogenic effect on fibroblasts.[111] After endothelial injury, the circulating blood is exposed to sudendothelial elements and platelet aggregation and thrombus formation occur immediately. Bowen-Pope et al[30] have shown that release of P.D.G.F. by cultured human and bovine endothelial cells can be stimulated by physiological concentrations of thrombin. Thus thrombin and other factors may play a role in the subsequent chain of events.

Growth factors	Endothelial cells	Smooth muscle cells	Fibroblasts
PDGF	+	+	+
TGF-B	-	+	+
FGF	+	+	+
EGF	+	+	+
TGF-A	+	Nil	Nil

Table 3: **Growth factors causing cell proliferation and their target cells.**
+ = mitogen; - = inhibitor
(Adapted from Bull DA, Hunter GC, Putnam CW. Growth Factors and the Arterial Wall. *Perspectives in Vascular Surgery* 1991;2:88-103.[34])

Returning to the concept of endothelial injury, within hours of such an insult to the vessel's integrity, endothelial cells migrate to cover the denuded area. The distance between the margins of the denuded area determines the extent of endothelial migration and proliferation. As shown by Clowes et al[57], in a rat model the process of re-endothelialisation ceases after 6 weeks whether or not complete coverage is achieved. Basic F.G.F. is abundant in association with actively replicating endothelial cells, indicating the significant role of this substance in the restoration of endothelial coverage. Simultaneously, smooth muscle cells from the tunica media proliferate and migrate through gaps in the internal elastic lamina into the tunica intima. This process is limited in experimental models of arterial injury, but is continuous at graft-to-artery anastomoses. The release of the growth factors such as the ones described

above results in the stimulation and proliferation of the cells of the vessel wall. In the familiar sequence of events that follows arterial injury, once repair is achieved, these events cease and vessel wall homeostasis returns. However, this orderly process of repair may become dysfunctional and lead to anastomotic neointimal hyperplasia. The continuing presence of growth factors in the vicinity of the injury (or anastomosis) leads to the selection of a clone of autonomous smooth muscle cells, with no inhibition of growth, resulting in persisting cell proliferation and extracellular matrix deposition. As observed by Clowes et al[61], the intimal hyperplastic plaque requires blood supply to support its evolution. Stimulation of neovascularisation is a function of F.G.F. which is secreted by endothelial cells, macrophages and platelets. The discovery of the relationship of these events to expression of the c-*sis* oncogene in platelets has led some authors to make the comparison between the intimal hyperplastic response and some of the events seen in growing tumours.[34,255]

Growth factors	Endothelial cells	Smooth muscle cells	Fibroblasts	Monocytes	Macrophages
PDGF	+	+	+	+	+
TGF-B	-	+	+	+	+
FGF	+	+	+	Nil	Nil
EGF	+	Nil	Nil	Nil	Nil
TGF-A	+	Nil	Nil	Nil	Nil

Table 4: **Growth factors stimulating or inhibiting cell migration and their target cells.**
+ = stimulation of migration; - = inhibition
(Adapted from Bull DA, Hunter GC, Putnam CW. Growth Factors and the Arterial Wall. *Perspectives in Vascular Surgery* 1991;**2**:88-103.[34])

AETIOLOGY OF ANASTOMOTIC NEOINTIMAL HYPERPLASIA

From the above review of the structural and cellular events that are associated with the development of anastomotic neointimal hyperplasia, the aetiology of the lesion would appear to be multifactorial. Mechanical factors and abnormal flow patterns related to the bypass material itself and the anastomotic geometry are among the likely aetiological factors. Understanding these mechanisms more precisely is important for a number of reasons. The development of improved prosthetic graft materials and the evolution of operative approaches aimed at overcoming mechanical causes of A.N.H. and the ability to "manipulate" pharmacologically the lesion of intimal hyperplasia are dependent upon a profound understanding of the importance of the various factors and events leading to its formation.

Basic Physiological Considerations

Compliance

A primary function of the aorta and its major and minor branches is to convert the highly pulsatile output of the heart to a more uniform steady flow of blood in the arteriolar and capillary circulation.[182] This function requires that peripheral vascular input impedance (related to arterial resistance, fluid inertia and arterial compliance [capacitance /distensibility]) is matched to the output impedance of the left side of the heart. Thus arterial compliance is a key factor in blood flow in the major arteries. Strictly speaking, compliance is defined as the fractional change in volume per unit change in pressure [C=(DV/V)/DP]. In cylinders of fixed length, as in the blood vessels, this equation simplifies to twice the fractional change in diameter per unit change in pressure: C=2(DD/DP)/D.

The term impedance is used to describe opposition to pulsatile flow, just as resistance describes opposition to steady flow.[263] The impedance of a vessel is a factor of fluid inertia, distal resistance, diameter and compliance of the conduit itself. Since at a given time, resistance and inertia of fluid are relatively constant, differences in flow between conduits are most directly related to diameter changes and/or changes in compliance. When there is a sudden change in compliance and or geometry (branching or stenosis or enlargement), an impedance mismatch is said to occur, with some loss of energy. This loss is independent of any energy being absorbed by viscoelastic walls of the vessel. A graft that is equal in diameter and compliance to the host artery in theory transmits a pulse wave without alteration; compliance mismatch and diameter changes cause a change in the pulse wave velocity, shape and amplitude. The effect is bimodal with increased pulse amplitude at the proximal and decreased pulse amplitudes at the distal end of the non-compliant conduit. The effect of this change for a non-compliant vessel is an increase in the amplitude of the pressure wave, which results in local increased wall tensile stress. Compliance mismatch refers to the difference in compliances between two conduits that are anastomosed to each other and is a relative statement of the degree of resultant wall tensile stresses placed on both conduits.[183] It should be borne in mind that diameter mismatch is equally problematic since impedance is also dependent on the diameter of the conduit. Sudden changes in diameter entail conversion of flow energies to wall shear energies. Impedance is due to fluid inertia and compliance, as we have seen. It is also used to characterise the efficiency with which pulsatile energy is transmitted to the periphery.

Early attempts to manufacture prosthetic graft materials focussed largely on the thrombogenicity of the graft surface without taking mechanical properties into account. However, it became apparent that the distensibility of a bypass graft is often less than that of the host artery and that this property can change during the period of implantation. Most of the conduits used currently as arterial bypass grafts are significantly less compliant than a normal artery. This has been suggested as a possible cause of the unsatisfactory patency rates obtained with prosthetic as well as with biological grafts. The proposed mechanism by

which this occurs is that an increased impedance, associated with the graft's reduced compliance, produces a decrease in blood flow through the graft and renders it more likely to occlusion from thrombosis. However, the compliance of the graft *per se* is unlikely to have a significant effect on the total impedance of a major arterial branch, and mean flow in a relatively short graft should not be affected. An example of this phenomenon is a theoretical 2 fold decrease in arterial compliance between the iliac and popliteal arteries, but this can be expected to produce a 10% decrease in flow only. It is the abrupt change in compliance at the proximal and distal anastomoses that renders compliance in vascular grafts an important factor. Such a change can reduce flow in the graft and increase platelet deposition near the anastomosis as well as generating structural stresses that might affect the formation of neointimal hyperplasia. Thus compliance may influence the deposition of thrombus-related blood elements, or the attachment of endothelial cells as well as healing of the external surface.

Wall tensile stresses are a function of the elastic modulus and are directly dependent on wall thickness and on luminal diameter. In theory, there would not be any wall stress to a luminally-matched, end-to-end anastomosis of similar tissues. However, any other variance, including such factors as end-to-side anastomosis, a marked change in luminal diameter, a change in elastic modulus or compliance of each component would result in significant wall stress.

All materials used currently as arterial bypass grafts have compliances that differ from the native artery. In other words, there is an inevitable compliance mismatch present when any of these conduits are placed. This may be made more marked with tissue reaction and fibrosis that occurs in the time after implantation although studies by Walden and Lye on both autogenous vein and prosthetic materials (dacron and PTFE) failed to confirm this experimentally. In contradiction to these findings, Kinley et al stated that prosthetic grafts become more "rigid" with time whereas host artery distensibility remains constant.

Abbott has studied extensively the comparative compliances of various conduits, both after implantation in human subjects and in experimental animals. Abbott pointed out that blood

vessels become less distensible at higher pressure and therefore, the compliance of an artery is greater at diastolic pressure than it is at systolic pressure.[1] To avoid ambiguities, he recommended that compliance values be reported with the pressure range over which they are measured.

Table 5 illustrates the range of compliance measured in a variety of conduits as published in the literature.

Conduit	Compliance (radial changes/mm Hg 10^{-2})
Human	
Artery Normal	9.1% +/- 1.6%
Diseased	5.9% +/- 1.5%
Vein Early	4.4% +/- 1.8%
Long-term	3.4% +/- 0.36%
Umbilical Vein	3.7% +/- 0.5%
Dacron	1.9% +/- 0.3%
PTFE 4mm-Early	1.4% +/- 0.6%
Long-term	1.03% +/- 0.39%
6mm-Early	1.2% +/- 0.3%
Long-term	1.6% +/- 0.2%
Canine	
Artery	8.7% =/- 3.1%
Vein Early	4.09% +/- 1.65%
Late	2.2% +/- 0.16%

Table 5: **The compliance of various conduits when placed into both the human and canine circulation.**
(Adapted from Bunt, TJ. Neointimal Hyperplasia. Chapter in Iatrogenic Vascular Injury: A Discourse on Surgical Technique.[35])

There is a considerable body of evidence in the literature to support the theory that compliance mismatch is an aetiological factor in the development of anastomotic intimal hyperplasia. Baird et al[11] hypothesized that a difference between the circumferential compliance of a small diameter vascular prosthesis and its host artery is detrimental to graft patency. In a canine model in which a ligated segment of femoral artery was bypassed with a

10cm graft of either autogenous vein, dacron or PTFE, Kidson et al found a direct correlation between the compliance of the conduit and patency. This was true for grafts harvested at both two weeks and three months and the differences were statistically significant[141,143].

Duration	Vein	Dacron	PTFE
TWO WEEKS	83	64	32
THREE MONTHS	76	29	14

Table 6: **Differential patency of conduits implanted into a canine model at 2 weeks and 2 months** (Kidson et al[141]).

None of the grafts studied showed a significant change in compliance after implantation. The vein grafts in this study maintained their compliance despite a doubling of wall thickness in 2 weeks without concomitant reduction in internal diameter.

Walden et al[285] similarly demonstrated a significant correlation between the compliance and the patency rates for a variety of arterial grafts after their implantation into the human circulation at the femoro-popliteal level (Figure 2).

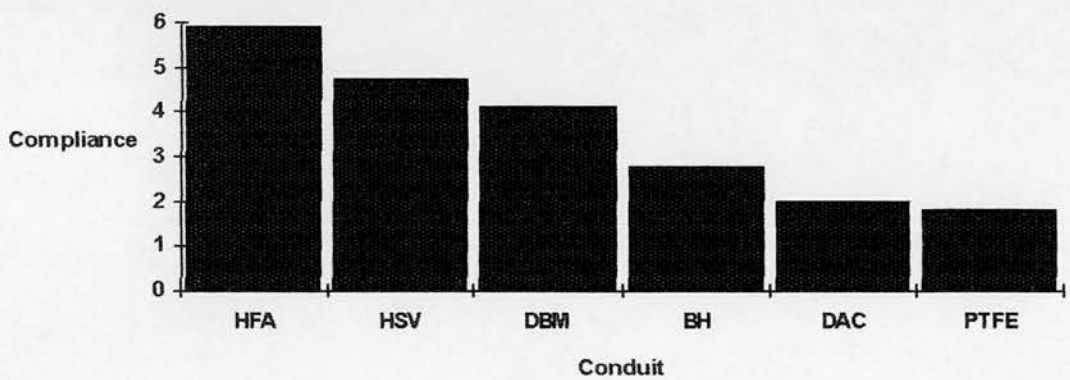

Figure 2: **Compliance (per cent radial change per mmHg x 10^{-2}) of various conduits.**
HFA: Human Femoral Artery; HSV: Human Saphenous Vein
DBM: Gluteraldehyde-treated Umbilical Vein Grafts
BH: Bovine Heterografts; DAC: Dacron; PTFE: Polytetrafluoroethylene
(Adapted from Walden et al, Arch Surg 1980;115:1166-1169.[285])

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It should be borne in mind that compliance mismatch was only one factor considered responsible for the difference in patency rates between the various conduits assessed in this study. The inherent thrombogenicity varies between graft materials and flow rates and patterns are dictated by the graft materials as well as the inflow and outflow arteries in the individual cases.

Conduit	% Patent (1 year)	% Patent 2 (years)
Host Artery	-	-
Saphenous vein	88	84
Umbilical vein	83	80
Bovine heterograft	65	59
Dacron	65	42
PTFE	60	42

Table 7: **Patency of the various conduits placed at femoro-popliteal level in humans** (Walden et al[285]).

Abbott et al[1] designed a model to investigate compliance mismatch as the sole variable in a canine model of femoral artery bypass. Segments of the experimental animals' own carotid arteries were either: i)filled with 0.025% buffered gluteraldehyde (pH 7.4) at 100mm Hg and immersed in normal saline solution externally for 30 minutes (compliant graft), or: ii)similarly filled but instead immersed in 10% gluteraldehyde for 1 hour (stiff graft). The former was 100% and the latter 40% as compliant as the native artery. Thus the luminal surfaces of both grafts were exposed to identical concentrations of gluteraldehyde. These grafts were then implanted into the femoral circulation with both anastomoses being fashioned in an end-to-end configuration. At the time of implantation, compliance measurements were made and the "stiff" grafts were found to be associated with greater anastomotic compliance mismatch than were the "compliant" group. The "compliant" grafts in this model were significantly more compliant than the "stiff" grafts, but the

physicochemical surface properties of the lumen did not differ between the two. At harvest, 86% of compliant grafts remained patent compared to 43% of stiff grafts ($p<0.05$). The authors concluded from this study that even when flow surfaces are identical, compliance mismatch is significantly deleterious to bypass graft patency.

Hong-De Wu et al[84] have recently reported work which seems to contradict these earlier findings described by Abbott et al. They attempted to study the "direct and sole effect" of compliance mismatch on the development of anastomotic intimal hyperplasia in a canine model. They used dogs of apparently equally low thrombogenic potential (by performing screening tests of platelet aggregation) and performed carotid bypasses using 6cm long, 5mm diameter externally-reinforced dacron grafts. Each animal had an interposition bypass with an end-to-end anastomosis on one side. A model of an end-to-side anastomosis was established on the contralateral carotid artery, by placing an "on-lay" graft with this anastomotic configuration at either end and ligating the native artery mid-way between the two anastomoses. Control, iso-compliant grafts of excised 4cm portions of carotid artery were interposed into the femoral arteries. Specimens were harvested at 6 months and one year. There were only two occluded graft limbs, both of these were due to spontaneous thrombosis not related to the development of anastomotic neointimal hyperplasia. Follow-up angiography failed to demonstrate any progressive narrowing of the non-compliant anastomoses. Quantitative analysis failed to show any significant difference in anastomostic neointimal thickness between the compliant and non-compliant grafts at either time interval. The main criticism of this particular study is that the flow surfaces of the bypasses were very different and also there are differences between the haemodynamics of the carotid and femoral circulations which may have influenced the development of neointimal hyperplasia. Nevertheless, studies such as this reiterate the complexity of the pathogenesis of anastomotic neointimal hyperplasia and show that compliance mismatch is certainly not the only aetiological factor.

Okuhn et al[202] have also performed laboratory animal experiments which have cast some doubt on the significance of the role of compliance mismatch in the development of

anastomotic neointimal hyperplasia. They placed plastic, non-compliant tubes around one iliac artery in a canine using the other undissected side as control. Through femoral artery PTFE "sidearm" grafts, they measured end-diastolic vessel diameter and pulse pressure. Measurements were repeated at 3 and 6 months, after which the animals were sacrificed and the arteries examined histologically. The banded segments showed a significant and sustained compliance mismatch between the adjacent artery on the same side and the contralateral, untreated artery. In spite of this, the intima of the banded vessels was almost indistinguishable from that of the contralateral control side. This was in marked contrast to the anastomoses of the PTFE "sidearms" which all showed marked, near-occlusive anastomotic neointimal hyperplasia. The authors concluded from this complicated experiment that compliance mismatch could not be implicated in the neointimal hyperplastic response as there was no difference between the banded and control arteries. However, this study did not address the issue of disruption of vascular endothelium (injury theory) as an important initial event in the pathological process. One could speculate that had they combined a balloon catheter endothelial injury (a standard experimental methodology for the induction of intimal hyperplasia) to the banding of the vessel, their results might well have been very different.

Hasson et al[122] demostrated that in a canine model of femoral artery end-to-end anastomosis, pulsed ultrasound could be used to obtain longitudinal profiles of vessel diameter and compliance near the anastomoses. They found that although the arterial diameter decreases monotonically to a minimum level at an anastomosis, arterial compliance first increases by approximately 50% before decreasing to 60% of the control value. They coined the term "para-anastomotic hypercompliance zone" (PHZ) and found that this region lay 3.6mm from the anastomosis. The PHZ also occurred in the artery adjacent to compliant or stiff grafts and they hypothesized that it may be due to transmitted effects of the suture line on the arterial wall. As such, this zone doubles the compliance mismatch anticipated at an anastomosis even when an artery is anastomosed to an apparently isocompliant graft.

Finally, they suggested that such a PHZ could be the link between anastomotic neointimal hyperplasia and the mechanical events that act at a vascular anastomosis.

Suture Technique

Various different sutures and suturing techniques are used in clinical practice for vascular reconstructions. Most often, a continuous or interrupted suture is utilized. Klein et al[145] hypothesized that these two techniques might have differing effects on anastomotic compliance and thereby influence the development of anastomotic neointimal hyperplasia and consequently, graft patency. They used a canine femoral artery model in which compliance measurements were made for the normal artery and then after it had been divided and re-anastomosed with either a continuous or an interrupted 6/0 polypropylene suture. Using an electromagnetic rheoangiometric loop probe, they measured static and dynamic compliance and compared the data for each suture technique. They found that dynamic diameter compliance was significantly decreased at the continuous sutured anastomoses compared to the proximal and distal native artery ($p<0.01$). For the interrupted anastomoses, the same applied for the anastomosis compared to the proximal artery ($p<0.01$), but not to the distal artery. The interrupted suture anastomoses were significantly more compliant than their continuous counterparts ($p<0.05$). This study seemed to show that the interrupted anastomosis was significantly more compliant than one sewn with a continuous suture technique. However, as this study was only designed to show the immediate differences at operation, no comment could be made about the effect these observations had in the long-term.

Continuing their work on the paranastomotic hypercompliance zone (PHZ), Hasson et al investigated further the effects of suture technique.[123] Again they utilized the "Echo-tracker" ultrasonic probe device to measure compliance at and around vascular anastomoses. Using a canine femoral artery model of end-to-end anastomosis, they compared the effects of

interrupted to continuous suturing (with 6/0 polypropylene). There was a difference in the incidence of the para-anastomotic hypercompliance zone. Eighteen (86%) of the continuous sutured anastomoses exhibited this property, while it was only seen in eight (50%) of interrupted anastomoses ($p<0.03$). In some cases, the PHZ was seen proximal and distal to the anastomoses, in others only proximal or distal and in others not at all. This finding was irrespective of the suture technique used ($p>0.6$). However, PHZ was present in 30/42 (71%) of sides proximal or distal to 21 continuous suture lines but in only 12/32 (38%) sides of 16 interrupted anastomoses ($p<0.01$). The site of peak compliance (when PHZ did occur) was centred 3.8 +/- 1.2mm from the anastomosis, similar to the findings in their earlier work. Again, the location of PHZ was unrelated to the suture technique. Both compliance and diameter measured at the anastomosis were significantly lower when continuous sutures were utilised. However, the amount of increase in compliance at the PHZ could not be related to changes in diameter, compliance or indeed to suture technique. The findings from this important study essentially echoed those of Klein et al[145]. The authors concluded that even when the compliance of a prosthetic graft exactly matches that of the native artery, a compliance mismatch can arise because of a suture line effect. In such circumstances, the results of their experiments imply that an interrupted suture technique would be preferrable. Continuous sutured anastomoses are associated with decreased diameter and compliance. As such, such an anastomosis is less able to expand with each pulse and therefore has to transmit longitudinal stress to the adjacent arterial wall which is relatively fixed by the sutures. This might explain the greater incidence of PHZ in continuous compared to interrupted anastomoses. The work on para-anastomotic hypercompliance by Hasson et al showed that PHZ is not a constant finding and even when present, it does not seem to be necessarily related to suture technique or to compliance or diameter changes. Therefore, other mechanical and dynamic factors must be acting, either together with or as well as compliance mismatch, to bring about the cellular events that lead to neointimal hyperplasia.

Graft Diameter and Length

Herring et al performed experiments with interposition dacron or PTFE grafts in the canine carotid and femoral arteries in order to establish a relationship between the diameter of the conduit, the length of the conduit and long-term patency.[126] They found that for 4mm internal diameter dacron grafts, 2/3 grafts measuring 20mm in length were patent at one month, but when the length was increased to 40mm or more, not one of eight remained open. For 4mm internal diameter PTFE grafts, 80% of conduits 40mm long remained patent. However, for grafts of 60mm in length, none remained patent. When 4mm diameter, 40mm long grafts of either material were compared, no dacron bypass stayed open whereas 80% of the PTFE grafts did. For 3mm diameter grafts, the length had to be even shorter to ensure the grafts remained patent. Small differences in length made a vast difference in patency, for example, 60% of 4mm long grafts were patent at one month compared to only 14% of 8mm long bypasses. The conclusion from this experimental work was that the patency of small arterial prostheses in a canine model is a function of their length, radius to the fourth power and the graft material itself. The so-called critical length for a prosthesis (Lc) becomes infinite when the diameter of the graft is between 5 and 6mm. Madras et al[180] stated that diameter matching was at least as important as the matching of elastic properties of host artery and graft material for the long-term patency of small arterial bypass grafts. The ratio of graft diameter to artery is a more significant determinant of impedance (resistance to pulsatile flow) matching both for blood flow and for pulse wave. Impedance is proportional to the vessel diameter to the power 2.5 but only to the square root of the elasticity. Diameter-matched anastomoses allow the blood flow and pulse wave to traverse the anastomotic region with minimal energy dissipation, thereby avoiding physical stress to the adjacent arterial wall. As has been shown by many authors, stress at anastomoses related to pulse waves during the cardiac cycle (cyclic stress) is a stimulus of smooth muscle cell proliferation and migration.

The standard end-to-side vascular anastomosis has a so-called "cobra-head" configuration. Madras et al[180], and other authors[35] have applied the law of Laplace to this arrangement. The "cobra-head" enlarges the diameter of the artery at the anastomotic level. As (wall) tension=pressure x radius of curvature, a two to three-fold increase in wall tension may be the result in an end-to-side anastomosis at a small diameter artery. This is far greater than the stress resulting from simple compliance or impedance mismatch.

Kinley et al showed that prosthetic vascular grafts are essentially non-distensible, whereas host arterial diameter can vary by up to 10% during the cardiac cycle.[144] They confirmed in both in vitro and in vivo studies that when the ratio of graft-to-host artery radius (diameter) was approximately 1.4, the resultant shear stress was the minimum possible and therefore the most desirable. Interestingly, when such a graft:artery radius ratio was utilized in a canine aortic interposition graft model using a 4/0 "Ethiflex" suture, the shear stresses measured were about one-thirtieth of the tensile breaking stress of the suture material.

A link between the work of Hasson et al[122,123] and that of Madras[180] and Kinley[144] amongst others was provided by Chandran et al[47] who used a finite element simulation of an end-to-end graft artery anastomosis to evaluate the distribution of para-anastomotic compliance and stresses. They found that a region of increased compliance was present 4mm from the anastomosis on the arterial side. In addition a region of increased stress was located 0.5mm from the anastomosis on the graft side. Also, increasing the diameter of the graft compared to the host artery resulted in a larger increase in the compliance of the artery in the anastomotic region, thereby exaggerating the para-anastomotic hypercompliance zone.

Anastomotic Geometry, Flow-Related Phenomena & Shear Stress

Sottiurai et al reported in several publications on their experimental findings from which they concluded that the geometry of the end-to-side anastomotic configuration was a significant factor in the development of intimal hyperplasia.[250,254] In a canine model of autologous artery end-to-end and end-to-side anastomoses, they found that with the former configuration, intimal hyperplasia did not occur. With the end-to-side model, however, the cellular and matrix components occurred in differing proportions at certain locations at or near the anastomosis. Intimal hyperplasia at the toe was mostly fibrocollagenous with only a few, slender myofibroblasts (comprising less than 20% of the lesion). At the heel, however, there was a far greater cellular component (40% of the intimal hyperplasia). Since similar changes were not seen in their model of end-to-end anastomosis, they concluded that the different geometry of the end-to-side configuration must give rise to non-laminar blood flow and flow separation leading to endothelial injury, with subsequent repeated exposure of subintimal smooth muscle cells to blood borne mitogenic substances with the resultant cellular proliferation and migration and associated matrix secretion.

Bassiouny et al[17] investigated the sites of occurrence of neointimal hyperplasia in the distal (end-to-side) anastomoses of canine ilio-femoral bypass grafts with reversed autogenous vein on one side and 6mm thin-walled PTFE on the other. Anastomotic geometry was standardized with a hood length-to-vessel diameter ratio of 4:1 and a maximum hood width of 6 mm at the centre of the anastomosis. Histological analysis revealed two distinct regions for the occurrence of intimal hyperplasia: one at the suture line and a second discrete location in the floor of the native artery at anastomotic level. However, in this study, there were no histological differences between the two locations, with the relative amounts of cells and extracellular matrix being the same for the lesions in both graft and floor of native artery. Maximal neointimal thickness along the suture line was significantly greater in the PTFE grafts than in the vein bypass grafts ($p<0.05$). The same did not apply to the neointimal hyperplasia located in the floor of the host artery. The vessels and grafts from some of the

experimental animals were harvested and silicone casts made of the anastomoses, so that in vitro pulsatile flow visualisation studies could be performed using "Amberlite" particles and a special computerized apparatus for producing flow velocity waveforms and monitoring flow with electromagnetic flowmeters. There was no difference between the inflow or outflow volumetric flow rates between vein and PTFE grafts. An end-to-side anastomosis produced luminal cross-sectional enlargement at the graft-artery suture line and an adverse pressure gradient. This geometrical transition led to secondary flow patterns which varied at different points in the cardiac cycle. In early systole, the flow entering the anastomosis was laminar and particles were skewed towards the graft hood. Flow stagnated on the arterial floor and at this point there was oscillation in a proximal and distal direction. Secondary flow patterns were seen at the suture line and lateral walls of the anastomosis. The video studies showed marked turbulence here as velocity increased at peak systole. Flow separation was also seen along the lateral walls where particle residence time was prolonged, clearance of particles was prolonged and particles were seen to accumulate. Intimal thickening was not seen along the hood of the graft where flow was laminar and high shear and particle residence times were short. On the other hand, arterial floor neointimal thickening occurred in a location of stagnation where low shear was seen. Separation, low shear and long particle residence time were also seen at the heel and lateral anastomotic wall.

The concept that the geometry of the end-to-side anastomotic configuration significantly influences the patterns of flow has been investigated extensively. Fluid flowing in a tube has a central mainstream flow that is bounded by a slower-moving layer near the tube's wall. This so-called "boundary layer" is attached to the wall by the interaction of friction and the viscous forces of the fluid itself. When the diameter of the tube changes abruptly (as at an anastomosis) or if the direction of flow changes direction suddenly, the fluid flows against a local pressure gradient. The slower-moving fluid near the wall has less momentum than the mainstream and stops or even changes direction. Therefore a zone of fluid disturbance or flow separation develops between the mainstream fluid and the wall. LoGerfo et al studied this so-called "boundary layer separation" in an in vitro model of end-to-side anastomosis

using 8mm diameter dacron graft material and plastic tubing.[168] They noted that fluid energy losses across the anastomoses were insignificant and that flow disturbances seemed to be the important mechanical problems seen. The anastomosis resulted in a ring of slow moving fluid that cut across the main flow stream. Fractionation of flow was secondary to adverse local pressure gradients that were formed and augmented during diastole. At clinically-relevant Reynold's numbers (Re=VDr/n, where V is velocity of flow, D is diameter of the vessel, r is fluid density and n is fluid viscosity) LoGerfo found that flow was unstable but not turbulent. Within the flow separation area, wall shear stress was low making this an area where fluid in contact with the wall moves slowly. In this model, a large proportion of the side branch flow (the graft) was derived from the boundary separation layer. Thus it was postulated that the blood entering the graft might have a higher potential for carrying activated platelets that have been displaced retrograde and circumferentially and derived from the slower moving outer shell of the main limb flowstream.

Further work from the same laboratory by Crawshaw et al[76] looked at flow disturbances at distal end-to-side anastomoses, again in an in vitro model. In this experiment, they analysed fluid flow patterns utilizing dye boluses and still and cine photography. Anastomotic angles of 15^{o} and 45^{o} were assessed. In addition they described the "proximal outflow segment" as being the anastomotic artery analogue proximal to the anastomosis and the "distal outflow segment" as the artery distal to this. At an inlet angle of 15^{o} with the proximal outflow segment occluded, there was no flow disturbance. This arrangement is close to representing an end-to-end anastomosis. This was the case as the proximal outflow segment was opened until the flow through it exceeded 10% of the mainstream flow. Above this, flow separation occurred. When the inlet angle was 45^{o}, a zone of separation was observed just distal to the anastomosis even with the proximal outflow segment occluded. This involved the near-wall flow in the proximal part of the distal outflow segment adjacent to the toe of the anastomosis. Flow was in fact reversed in this region. When this reverse flow reached the anastomosis, it rejoined the outer mainstream and passed back into the centre stream. When the proximal outflow segment was then opened, flow into it exceeded 40% of main flow before the

separation extended proximally into the hood of the anastomosis. Dye injected distally travelled retrograde across the toe into the hood and thence into the proximal segment. Near-wall velocity measurements decreased as the percentage of inlet flow exiting up the proximal outflow segment increased. Such low velocities allow increased contact of activated platelets and other circulating blood cells with the vessel wall, in vivo. One conclusion that might be drawn from this work is that it is disadvantageous to have a patent outflow artery proximal to the site selected for anastomosis. However, the patency of this vessel, even for a short distance proximal to the anastomosis not only provides retrograde tissue perfusion but also increases the flow in a graft that may well be placed into a restricted run-off with significant outflow resistance. The other conclusion from these in vitro studies is that flow disturbance is minimal with a low inlet angle.

LoGerfo et al attempted to confirm these in vitro findings in a canine model of end-to-side distal anastomosis.[166] They placed 6mm dacron femoro-femoral bypasses in order to bypass a ligated femoral artery. They manipulated the anatomy in order to compare the effect on anastomotic neointimal hyperplasia of the patency of the proximal outflow segment. Unidirectional outflow from the graft was ensured by ligating the recipient femoral system below the profunda femoris artery (occluded proximal outflow segment), whereas bidirectional flow was guaranteed by ligating it proximal to the profunda femoris (patent proximal outflow segment). Animals were sacrificed at intervals up to 6 months after implantation. The downstream anastomosis showed a significantly greater hyperplastic response than the proximal anastomosis in both unidirectional and bidirectional models (both $p<0.001$). Furthermore, the intimal hyperplasia so measured was progressive over time for both modes of outflow ($p<0.01$). There was no significant increase in the extent of downstream hyperplasia in the model that had greater flow separation, ie the bidirectional flow anastomosis. This finding appeared to contradict the previously-described in vitro experiments from the same laboratory. The authors concluded that in such an animal model, it may be that the downstream location is a significantly greater factor than boundary layer separation in the genesis of anastomotic neointimal hyperplasia. Nevertheless, the specific

location of the hyperplasia coincided with the areas of flow separation identified in their prior in vitro models. They also suggested that the propensity for the hyperplasia to occur at the downstream anastomosis implied that interactions between blood factors and the graft material may stimulate the hyperplastic response.

Kohler et al investigated the effects of increased flow on the development of anastomotic neointimal hyperplasia in a baboon model of end-to-side anastomosis.[146] They placed 5-7cm long, 4mm diameter PTFE aorto-iliac bypass grafts and on one side created a distal arterio-venous fistula between the superficial femoral artery and vein. The other side acted as a control. The mean flow across the grafts with a distal fistula was 785 +/- 101ml/min compared to a figure of 230 +/- 35ml/min for the control grafts ($p<0.001$). The peak systolic velocity, time-averaged velocity and average shear were all similarly, significantly increased on the fistula side compared to controls. The presence of a fistula did not change the pattern of endothelial coverage but did cause a marked reduction in the cross-sectional area of the neointima compared to control grafts at two weeks, two and three months ($p<0.01$). They also looked at the neointimal response to the reversal of such fistulae and found that fistula closure caused increased neointimal thickening. Fistulae were also created at 2 months in some animals on the previous control side. At 3 months, these grafts had slightly less neointima but the difference between them and controls was not significant. Studies of the relative cell:matrix ratio in the neointima analysed showed that the decreased graft neointimal thickening on the side of the fistula appeared to be due to reduction in both the smooth muscle cell and matrix content, since the volume fractions of smooth muscle cells and matrix did not change. This was explained by a reduction in smooth muscle cell number without a change in the amount of matrix each cell produces. The neointima remained sensitive to flow changes at late times, since ligation of fistulae lead to a significant increase in neointimal thickness and although not statistically significant, the creation of a late fistula lead to a decrease in neointimal build-up in control grafts. Kohler et al postulated that at a cellular level these events were likely to be mediated by the endothelium through differential production of mitogens. Certainly the work of authors such as Hsieh et al[129] indicated a link

between shear stress and the level of mRNA coding for platelet-derived growth factor by vascular endothelial cells.

Okadome et al correlated prospectively the flow waveform of PTFE above-knee femoro-popliteal bypass grafts to long-term patency.[201] Type "0" waveforms (a steep acceleration in systole and a steep deceleration in diastole with reverse flow in late diastole - as in a normal femoral artery) and type "I" waveforms (steep acceleration and deceleration, but no diastolic flow reversal) were associated with superior patency rates compared to grafts with type "II" waveform (deceleration characterized by slow deceleration in mid-diastole): 94% at 1 and 2 years and 79% at 3 years for type "0" and type "I", compared to 74% at 1 year, 66% at 2 years and 49% at 3 years for grafts with type "II" flow waveform ($p<0.05$). Flow waveform analysis was proposed as a method for predicting which conduits would be at risk of developing anastomotic neointimal hyperplastic lesions and thereby forecasting the long-term patency of such bypasses. The authors recommended performing a composite PTFE/vein bypass in situations where the run-off gave rise to type "II" waveform in the grafts.

The same authors performed laboratory animal experiments using a canine iliac artery 6mm diameter, 3cm long PTFE interposition graft model.[200] They simulated a poor run-off situation by ligating the femoral artery just distal to the profunda femoris artery take-off (low-flow, low shear) and compared the distal anastomotic neointimal hyperplasia that developed to that on the contralateral side which had an identical graft placed, but with a normal run-off. In a simultaneous experiment, a model of low-flow but near-normal wall shear stress was created by having an arterio-venous fistula proximal to the iliac interposition graft on one side, the contralateral side acted as the control graft again. The results of this experiment showed that in the low flow, low shear model (ligated distal femoral artery) the distal anastomotic neointimal hyperplasia that developed was significantly greater than on the control side, at both one and three months after graft placement ($p<0.01$). Furthermore, there was not a significant increase in the neointimal hyperplastic response at the distal anastomosis of the grafts with a proximal arterio-venous fistula (low flow, near-normal

shear). Okadome et al concluded that the canine poor run-off model had a propensity to develop neointimal hyperplasia at the distal anastomosis. They warned that the same might apply to PTFE lower extremity bypasses in patients with limited run-off.

Keynton et al studied the effects of anastomotic angle and flow rate on the flow patterns and shear stresses acting on the native artery at a distal anastomosis in an in vitro model.[140] End-to-side anastomoses were fashioned from "Plexiglass" with various anastomotic angles of 30°, 45° and 60° being studied separately. Flow visualisation studies were performed using a blood analogue solution containing silicone carbide particles. For all 3 anastomotic angles and a variety of Reynold's numbers, the flowstream entering the host artery had a velocity profile that was skewed towards the outer wall (floor of anastomotic artery). The impingement of the flowstream on the outer wall also resulted in circumferential motion and a point of flow stagnation was seen at the anastomosis. Flow separation was seen to occur in a pattern similar to that reported in the model of end-to-side anastomosis of Crawshaw et al, with fluid flowing retrograde into the proximal outflow segment. When anastomotic angles were compared, the amount of circumferential motion was greatest at 60° and least at 30°. When the effect of angle was assessed for shear, it was found that the normalized axial shear rates were greatest in the 45° specimen. The authors concluded that the 60° angle would be unfavourable for implantation of a bypass at femoral level since under comparable flow conditions, an extra separation zone was seen at the anastomosis itself. The low shear rates within this region correlate with the intimal hyperplasia observed clinically.[17] It would appear from this study that an anastomotic angle of 45° is the best option as the other angles tested proved disadvantageous in terms of shear and additional separation zones.

Ojha et al used rigid in vitro "Plexiglass" models of end-to-side anastomoses with an anastomotic angle of 45° in an attempt to identify the haemodynamic factors involved in the pathogenesis of distal anastomotic neointimal hyperplasia.[198] They showed that the flow field seen in a model of an anastomosis differs markedly from that seen in a gradually curved blood vessel. When their test fluid was circulated through the model with pulsatile flow, they demonstrated a region of flow separation immediately distal to the toe of the junction along

the near wall. At the same time they observed that the proximal host artery contained a zone of slow-moving recirculation. Low wall shear stress was seen at the near wall just distal to the anastomosis, in the proximal host vessel where flow was virtually stagnant and in the floor of the host artery where entry flow is split into forward and retrograde flows. This lattermost area was a location of a complex mixture of both high and low shear which in itself may account for the preponderance of the neointimal hyperplastic process for this area. Low shear has been implicated as a major aetiological factor by other authors, but Ojha et al noted that this entity was present in the proximal host artery and yet this area is not subject to significant neointimal hyperplasia, implying that shear stress is not the sole haemodynamic factor involved. The limitations of in vitro models are again exemplified by this study as patency of the proximal outflow segment and vessel-graft compliance mismatch have not been taken into account and undoubtedly, in vivo, both play an important role.

In a more recent study[199], the same authors have studied the influence of anastomotic angle on wall shear stress in an in vitro model of end-to-side anastomosis, subjected to pulsatile flow. Anastomotic angles from 20 degrees to 60 degrees were assessed. They found that for all angles, low shear stress was present at the heel of the anastomosis and in the bed of the native artery opposite the heel, possibly due to occlusion of the proximal end of the host vessel. At the toe, flow separation increased as the anastomotic angle increased. In the floor of the native artery opposite the toe of the anastomosis, increasing the anastomotic angle was associated with an increase in wall shear stress. Increasing the anastomotic angle also led to greater fluctuation of cycle to cycle wall shear in the toe region of the host vessel. The authors concluded from this study that the lowest achievable anastomotic angle was associated with the least flow separation at the toe and the least fluctuation in shear in the floor of the native artery. Both of these effects might, they suggested, be associated with less anastomotic neointimal hyperplasia. They suggested that the excellent patency rates achieved by Taylor et al[270] using vein patches on the toe of tibial PTFE grafts might owe their success to the effect they have on the anastomotic angle and consequently on wall shear stress.

Kohler et al performed experiments with autogenous jugular vein grafts interposed into the rabbit carotid circulation.[147] They wrapped the grafts with PTFE, either "tightly" or "loosely". They found that at graft harvest, the "tight" wrap grafts (low wall stress, determined by the ratio of luminal radius to wall thickness) had significantly less wall area and they concluded that high wall stress was a significant factor in the development of wall thickening in vein grafts. Other laboratory work by Zwolak et al studying the kinetics of experimental vein grafts concluded that there was a direct correlation between vein graft hyperplasia and tangential stress.[304]

Zarins et al stated that artery luminal diameter in an experimental model of atherosclerosis was regulated by shear stress.[302] They created an arteriovenous fistula in the iliac circulation of cynomolgous monkeys which were then fed a diet rich in cholesterol. After six months blood flow and flow velocity were both significantly greater in iliac arteries that had anarterio-venous fistula compared to controls. However, calculated wall shear stress was no different between the two sides because of a twofold increase in luminal diameter on the fistula side. On the treated side there was a twofold increase in media cross-sectional area, but no change in media thickness or total wall thickness. Tangential wall tension (calculated using LaPlace's Law, T=Pr) and wall stress (from Hagen Poiseuille formula, which relates wall stress to 4 times the viscosity of blood times blood flow divided by psi times the third power of the vessel radius) were two times greater on the fistula side compared to controls. The authors concluded that increased flow results in arterial dilatation and normalization of wall shear stress and that the latter might regulate lumen diameter. Increased flow did not enhance plaque deposition or vessel wall thickness under these conditions.

Cyclic strain

Pevec et al[212] established an in vitro model of an end-to-side anastomosis with a 6mm thin-walled PTFE graft material anastomosed to "Penrose" latex rubber tubing in a fashion similar

to that used by Chandran et al.[47] This rubber tubing has similar compliance in vitro to the human femoral artery. This model was subjected to pulse waves of physiological pressure, rate and contour in order to establish areas of the end-to-side anastomosis where abnormal strain patterns exist. They found that within the relatively non-compliant graft, circumferential strain (change in circumference/baseline circumference) was relatively constant until the heel of the anastomosis was reached. At this point, the circumferential strain increased markedly, reaching a maximum (three times baseline) one third and one half of the way between heel and toe, returning to near baseline levels just distal to the toe. The strain across the suture line was shown to increase to a similar degree at the same location. The regions of increased strain seen in this in vitro model correspond to the sites of formation of anastomotic neointimal hyperplasia. In addition, this study has important implications for the development of anastomotic pseudoaneurysms.

Several authors have correlated cyclic stretch and strain with increased protein and collagen synthesis by vascular smooth muscle cells in vitro[89,266] and these findings have been extrapoltaed to the relationship between stress and strain and patency of bypass grafts in vivo.[124] Sumpio et al examined the effect of repetitive stretching on smooth muscle cell collagen production, using porcine aortic medial smooth muscle cells in cell culture.[265] They found that not only was collagen synthesis stimulated by mechanical deformation but so too was the synthesis of non collagen protein.

Sottiurai et al also showed an in vitro relationship between cyclic stretch and altered smooth muscle cell morphology, in particular the amount and configuration of the secretory rough endoplasmic reticulum.[253] Also, smooth muscle cells subjected to stretching became elongated with their long axis orientated in the direction of stretch.

One clinical correlate of these findings is the arterio-venous fistula for haemodialysis access in which intimal hyperplasia is often seen to occur within the venous component distal to the anastomosis.[134] This vessel is subjected to pulsatile arterial blood flow for the first time when the fistula is created and theoretically, cyclic stretch and strain must be at least partly

responsible for the development of anastomotic intimal hyperplasia in this setting. Clearly, increased flow, diameter and compliance mismatch are other factors involved.

Summary

Having reviewed the principal mechanical events acting at the anastomotic level, it is important to attempt to correlate these to the cellular events outlined in the previous section.[244]

At the time of creation of a vascular anastomosis, endothelial injury secondary to vessel handling, clamping and resultant ischemia is added to by the presence of a suture line which may compromise healing and subsequent endothelial cell function. Furthermore, the cells of the arterial tunica media are also damaged and respond by proliferating and adopting a secretory role.

With the anastomosis formed and flow through the graft established, the cellular components of the blood come into contact with the thrombogenic surface of the conduit. On reaching the distal anastomosis, they are activated and capable of secreting a variety of mitogenic substances that can stimulate the migration and proliferation of medial smooth muscle cells. The formation of boundary layer separation zones that further stimulate platelet activation as do areas of low and high wall shear are other contributory factors. Mismatch of compliance, which can lead to the modulation of the amplitude of transmitted pulse waves and cyclical stretch of the native arterial wall at the anastomotic level lead to chronic injury of the endothelial cells which in turn leads to loss of the "shingle effect" and further platelet and cellular deposition, with mitogen release as described above. These factors also stimulate the so-called "autocrine" mechanism of smooth muscle cell proliferation and migration that has been shown to occur independent of other sources of stimulation.

The geometrical configuration of the end-to-side anastomosis leads to a number of mechanical and haemodynamic events that can lead to the stimulation of platelets and other

cells and thus to mitogen release and the development of neointimal hyperplasia. The lesion tends to be far more significant at the distal rather than the proximal end-to-side anastomosis. One explanation for this is that proximally, the cellular components within the flowing blood have not yet been activated by contact with the graft's luminal surface. It can be conjectured that within the boundary separation layer that must occur at the proximal anastomosis, platelets and the other circulating cells are activated and stimulated to secrete mitogens which act on the endothelial cells and subintimal cells at the distal anastomosis. Also, for tubular grafts, the conformational stress is probably greater at the smaller diameter distal artery which is opened up to a greater extent by the arteriotomy than is the wider proximal artery.

SECTION II

Anastomotic Neointimal Hyperplasia

Clinical Correlation: Prosthetic Bypass Grafts

It has been reported by many authors in clinical series that clinical judgement and technical excellence apart, after the progression of atherosclerosis in native inflow and outflow arteries, the major cause of the failure of infrainguinal prosthetic bypass grafts is anastomotic neointimal hyperplasia.[192,218,271,283] Often, ANH and progression of atherosclerosis occur in combination and under such circumstances it is often not possible to implicate one or the other alone. Undoubtedly, ANH is also encountered in association with autogenous and umbilical vein[38] bypass conduits, but for the former in particular, the pathophysiology and haemodynamics are considerably different from the prosthetic bypasses.[86,87] Because the experimental work presented later in this thesis involves the use of polytetrafluoroethylene (PTFE) grafts in a canine model, I shall focus on the clinical aspects of ANH as a cause of failure of prosthetic bypass grafts with particular emphasis on PTFE.

One of the unavoidable problems with clinical series in respect to the incidence of ANH is that more often than not, there is no histological proof that this is indeed the cause of graft failure. Instead, one has to rely on the angiographic appearances of a stenotic lesion at the distal anastomosis of the graft as being diagnostic of ANH.[192] On the other hand, angiographic proof of clear disease progression in the run-off vessels distal to the graft with no evidence of a filling defect at anastomotic level is the best way of discriminating disease progression alone as the cause of graft occlusion.

It has been shown clearly in prospective randomized trials that autogenous greater saphenous vein is the conduit of choice for infrainguinal arterial bypass to the below-knee popliteal artery and to the tibial and peroneal arteries.[23,281] The problem arises when this conduit is not available for use as happens 20-30% of the time, either because of its poor quality, small

diameter, or because of its prior use for bypass, either coronary or infrainguinal. In these circumstances, many authors advocate the use of autogenous vein from other sources such as the lesser saphenous vein and arm veins[32,48], claiming that these conduits offer comparable patency rates to greater saphenous vein and superior long-term patency rates to any of the prosthetic conduits currently avaliable. Even so, in some such limb salvage situations, it is necessary to make the choice between the use of a prosthetic graft to the below-knee popliteal or a tibial artery and primary amputation.[275]

Veith et al were of the opinion that in this situation a distal bypass utilising PTFE was the better option for the patient.[281] In their experience, PTFE bypass grafts to the infrapopliteal arteries yielded a primary patency rate of 12% at 4 years, significantly less than that for autogenous saphenous vein, which was 49% at 4 years ($p<0.001$). For randomized grafts placed to the above-knee popliteal artery, the 4 year primary patency rate for autogenous saphenous vein bypasses was 61% compared to 38% for PTFE ($p>0.25$). At the below-knee popliteal artery level, there was a significant benefit from the use of ASV with 4 year primary patency rate of 76% against a primary patency rate of 54% for PTFE ($p<0.05$). When the data for randomized and obligatory PTFE grafts to the popliteal artery were compared the former gave rise to significantly better primary patency rates than the latter: 47% vs. 29% at 4 years ($p<0.025$). One of the conclusions to arise from this study was that the use of PTFE for limb salvage can be justified on the basis that in a high-risk patient group, life expectancy may not exceed the likely primary patency of the graft. Clearly, one of the faults of many reported retrospective or non-randomized studies is that often the use of obligatory PTFE grafts in unfavourable circumstances has a significant negative influence on patency rates and graft performance.[23] As well as giving clear evidence for the superior patency rates for autogenous vein bypasses in all infrainguinal locations, but especially in grafts to the below-knee popliteal and tibial arteries, Veith et al deduced some interesting points relating to the failure of vein and PTFE grafts.[283,284] Failures occurring in the early months after bypass placement are thought to be due to technical factors, those that occur between 2 and 18 months are mostly as a result of anastomotic neointimal hyperplasia and graft failure beyond

18 months after operation is largely attributable to the progression of atherosclerosis. These conclusions can be contrasted to those of Whittemore et al who stated that for autogenous vein infrainguinal bypass grafts, failure within the first 30 days of placement was due to technical mishap; those occurring within the first year were the result of vein graft stenosis and those that occur after 12 months are usually due to progression of distal disease. In the large prospective randomized series reported by Veith et al, with a follow-up of 5 years, autogenous saphenous vein and PTFE grafts to the popliteal artery failed with more or less equal frequency for the first 18 months, but after this the latter failed far more frequently. The authors hypothesized that PTFE grafts to the popliteal level may, in some way, promote the progression of distal atherosclerosis.[281] If this is so, the implications as far as re-operation and secondary or tertiary bypass are concerned are great, as potential targets for subsequent bypass may thus be compromised. In any event, the patency rates for the PTFE grafts in the prospective series of Bergan et al[23] and the later series of Veith et al are not dissimilar to those published by other authors, although a variety of reporting methods and a mixture of retrospective and prospective studies make comparison of such series difficult (see infrainguinal PTFE graft meta-analysis table).

What is the incidence of anastomotic neointimal hyperplasia occurring in PTFE infrainguinal arterial bypass grafts? O'Donnell et al reported their experience in the management of failed PTFE femoro-popliteal and tibial grafts.[192] Intimal hyperplasia was the sole cause of 19% of graft failures, as common as progression of disease in the inflow vessels. However, progression of atherosclerosis in the outflow tract accounted for 64% of failures. They were able to document accurately the occurrence of these entities with respect to time. In the period 1-6 months after graft implantation, worsening of distal disease was responsible for the failure of 7 grafts compared to 4 graft occlusions caused by anastomotic neointimal hyperplasia. In the 7-12 month period, progressive distal atherosclerosis was again more prevalent. Between 13 and 24 months both showed an equal incidence and after 2 years, failure was almost exclusively due to run-off vessel disease progression. The association of PTFE grafts to the popliteal and infra-popliteal arteries with an apparent exaggerated

progression of atherosclerosis caused O'Donnell et al to question whether the angiographic appearances within the popliteal artery were in fact neointimal hyperplasia within the floor of the native artery rather than atheroma itself. However, in spite of this, such changes were inevitably associated with progression of atheromatous changes in far distant tibial and peroneal arteries. O'Donnell speculated, as has LoGerfo[165], that activation of blood cells, such as platelets and macrophages by contact with the thrombogenic graft surface leads to their activation and to the subsequent release of mitogenic substances at points downstream where the atherosclerotic process is already underway. This hypothesis was of course speculative but would certainly explain the findings of both O'Donnell's study and that of Veith et al. It also serves to highlight the grey area of overlap between the neointimal hyperplastic response and atherosclerosis. Another point to arise from this important study is the fact that this group used antiplatelet agents post-operatively as a matter of routine. This fact may have had some bearing on the incidence of anastomotic neointimal hyperplasia observed in this study, making it somewhat lower than that reported by other authors.

Ascer et al reported their experience utilizing an aggressive policy of reoperation for PTFE bypass failure and stressed the importance of distal outflow site and operative technique in determining the successful outcome of such interventions.[9] In a series of 724 PTFE bypasses performed for critical ischemia over a 6 year period, 165 (23%) failed. This series included infrainguinal bypass grafts as well as non-anatomic grafts in the form of axillo-femoral and femoro-femoral grafts. The commonest identifiable cause of PTFE late graft failure was progression of distal disease in 37% of cases. Neointimal hyperplasia at the distal anastomosis was seen in 25% of infrainguinal grafts that failed but in only 10% of the extra-anatomic graft failures, suggesting that anastomotic neointimal hyperplasia is more of a problem when PTFE is anastomosed to smaller diameter outflow arteries. Of note in this series, no obvious cause for graft occlusion was found in 33% of cases.

Taylor et al performed a prospective audit over a 6 year period of a series of 159 PTFE infrainguinal bypass grafts.[271] Sixty-two per cent of grafts were to infrageniculate arteries, 28% being to tibial or peroneal arteries. More than half of the patients had single vessel run-

off or an isolated popliteal segment. Thirty-seven (23%) grafts failed during follow-up. Progression of distal disease was the cause of failure in 22% of cases and anastomotic neointimal hyperplasia resulted in 19% of failures. Taylor also encountered a considerable number of grafts (19%) in which the cause of bypass occlusion was never identified. As far as neointimal hyperplasia is concerned, Taylor et al stated that its rate of onset and severity were highly variable and appeared to bear no relationship to the above-knee or below-knee location of the graft, to the state of the run-off arteries and to the presence of such risk factors as diabetes and smoking. As a result of their experience with this series of patients, Taylor et al have subsequently developed an anastomotic vein patch that has had a significant impact on the development of anastomotic neointimal hyperplasia with associated improved patency rates.

Quinones-Baldrich et al reviewed the UCLA experience with failed primary PTFE infrainguinal bypass grafts.[217,218] During a 10 year follow-up period, 111 of 322 (34%) grafts failed. In almost every case, a cause of failure was firmly established. In 47 of these 111 (42%) cases, progression of distal disease was the cause of graft thrombosis. This was associated with neointimal hyperplasia at the distal anastomosis in 25/47 instances (57%). In addition, isolated anastomotic neointimal hyperplasia was seen in 7 further instances. All of these grafts failed within 12 months of implantation. This report did not encounter the progression of distal disease associated with PTFE grafts as noted by both Veith and O'Donnell. Quinones-Baldrich et al were adamant that failure of a PTFE infrainguinal bypass graft due to anastomotic neointimal hyperplasia and/or progression of distal disease is best treated with the replacement of the conduit with an entirely new autogenous vein bypass wherever possible, and that secondary reconstruction with PTFE should be reserved for those patients with a complete lack of useable autogenous vein.

The results of secondary revascularisation with autogenous vein are very impressive. Edwards et al reported a series of 103 secondary vein bypass grafts with 5 year primary and secondary patencies of 57% and 71% respectively.[98] These results are especially impressive

compared to those for prosthetic grafts as 67 of the 103 bypass grafts were to infrapopliteal arteries.

There is clearly a limit to what can be done to restrict the progression of distal atherosclerosis after the placement of an infrainguinal bypass graft. O'Donnell et al felt that one way of influencing such events was to avoid the use of PTFE since, in their experience, there seemed to be a circumstancial deterioration in distal disease in the years after bypass placement which did not seem to occur with autogenous vein grafts. Much work has been focussed on risk factors and the relative contributions of smoking, hypertension, diabetes and hyperlipidaemia. Nevertheless, it seems from the above review of the literature on failed and failing infrainguinal PTFE arterial bypass grafts that the cause of failure in up to 25% of cases is anastomtoic neointimal hyperplasia. The poor results of reoperation and the effect on limb salvage have made the limitation or prevention of this problem the subject of much investigation over the years. In the series of 111 PTFE graft failures reported by Quinones-Baldrich et al, the poor results of graft salvage are exemplified.[218] Patients treated with thrombectomy +/- vein patch had a primary patency and limb salvage for this secondary procedure of 40% and 45% respectively at 30 months. For cases treated by graft extension with autogenous vein, the figures were 32% and 43%; for those treated by graft extension with PTFE, primary patency was 30% and limb salvage 43% at 30 months. These figures are clearly inferior to the primary patency rates for patients treated with an entirely new autogenous vein bypass graft in whom the primary patency of these new conduits was 55% with a limb salvage rate of 72% at 30 months. Ascer et al also reported poor patency after the salvage of thrombosed PTFE grafts.[9] For graft thrombosis due to anastomotic neointimal hyperplasia (25% of infrainguinal graft failures and 10% of non-anatomic grafts), their operative approach consisted of dissection of the distal anastomosis, a longitudinal incision on the hood of the graft directly over the anastomosis, proximal graft thrombectomy followed by vein patch angioplasty. They stressed the importance of not attempting to excise the hyperplastic lesion although they did recommend carefully extracting visible thrombus. Using this approach and distal extension for graft failure due to disease progression, the

secondary patency for above-knee femoro-popliteal grafts was 24% at 36 months. For grafts to the below-knee popliteal artery the 36 month patency was 12% and for tibial grafts 10%. As a result of this experience, as was the case in the report published by Quinones-Baldrich et al, Ascer and co-workers recommended the placement of a totally new bypass for a below-knee PTFE graft thrombosis based on their superior results with graft replacement over graft salvage at this level. They placed 27 new bypasses, 12 PTFE, 9 vein and 6 composite PTFE/vein. These new conduits had a primary patency at 24 months of 48% for grafts to the popliteal level and 39% for tibial grafts. Thus it can be seen from this reported experience that salvage of grafts after failure does not give durable results.

Further information on the incidence of anastomotic neointimal hyperplasia as a cause of infrainguinal PTFE bypass graft thrombosis has been gleaned from the outcome of thrombolytic therapy as a means of treating occluded grafts. At the University of Iowa, 22 such PTFE bypasses have been treated with direct intra-arterial urokinase thrombolysis.[71] Seventeen grafts underwent "successful" lysis in that either complete clot lysis occurred or adequate lysis resulted such that the underlying cause of graft occlusion was revealed. In one case, the run-off status was so poor that it was decided to replace the PTFE graft with an entirely new autogenous vein graft. Nine grafts (41%) had anastomotic stenoses due to neointimal hyperplasia treated with either percutaneous transluminal balloon angioplasty or surgical revision. A further patient had a popliteal artery stenosis treated by balloon angioplasty. In six (35%) cases where lysis was successful, no cause for graft occlusion could be ascertained. Life table analysis of all PTFE infrainguinal grafts subjected to an attempt at thrombolysis (intent to treat) showed a secondary patency at 15 months of 30%. Balloon angioplasty of anastomotic stenoses did not give rise to sustained patency with only three of eight grafts remaining patent for longer than 15 months. The two grafts (both to the above-knee popliteal artery) treated by vein patch angioplasty of the distal anastomosis after successful thrombolysis had revealed distal anastomotic neointimal hyperplasia, remained patent at 17 and 28 months respectively. Based upon this experience, we recommended that

the optimal treatment of a thrombosed infrainguinal PTFE graft was its replacement with an entirely new autogenous vein bypass, if possible.

Valji et al reported a similar experience with the thrombolytic treatment of 15 prosthetic grafts using pulsed spray urokinase.[278] Five grafts (33%) required intervention (either endovascular or surgical) to treat anastomotic neointimal hyperplastic lesions of the distal anastomosis. Obviously the data from series of thrombosed grafts treated with thrombolytic therapy do not give a strictly accurate idea of the incidence of ANH as the grafts that are not successfully reopened using this technique are often not included in the analysis, thereby exaggerating the incidence of the condition.[29] Nevertheless, it is clear from these reports that ANH is a significant cause of medium-term graft occlusion.

Thus if the development of graft occlusion secondary to anastomotic hyperplasia can be prevented, many patients will be spared these therapeutic dilemmas and compromises. Veith et al have now recommended a policy of utilizing autogenous vein for primary infrainguinal bypass wherever possible.[281,284] However, some authors have stated a preference for the use of PTFE for primary bypass, especially to the above-knee popliteal artery in order to preserve vein for secondary bypass.[217,219]

In some circumstances, as alluded to above, one's choice of conduit is limited to a prosthetic graft. In the case of secondary bypass, this is more often the case than in primary grafts, as the ipsilateral long saphenous vein may have been utilized as a bypass conduit previously. Because of the poor patency rates of PTFE bypass grafts to the infrageniculate arteries, some authors have questioned the place of bypass with prosthetic material altogether. On the other hand, Yang et al have reported favourably on the use of PTFE for secondary femoro-popliteal bypass, especially in patients with inadequate available autogenous vein.[299] They reviewed their experience with 73 secondary infrainguinal bypass grafts performed with PTFE, 64% of which were to the below-knee popliteal artery. The primary patency of these grafts at 4 years was 38% but with the use of a rigorous surveillance protocol, the secondary patency was 55% and limb salvage was 74%. These data would seem to support the use of such conduits under certain circumstances, but also as 48 reinterventions were required to

maintain or restore patency, this series shows that PTFE grafts require aggressive follow-up to preserve patency and that, although atherosclerosis is the main patient-related factor in failure, anastomotic hyperplasia is undoubtedly the main graft-related factor.

Another area of vascular surgical practice in which anastomotic neointimal hyperplasia is a continuing cause of failure of a considerable number of grafts is in the field of vascular access for haemodialysis. Jenkins et al reported on the medium-term follow-up of a series of 80 arterio-venous shunts performed on 56 dialysis patients.[134] There were 40 PTFE shunts and 40 autogenous vein grafts. Twenty-six (65%) of the PTFE shunts developed a stenosis in the run-off vein adjacent to the distal anastomosis extending into the vein for a variable length and these lesions were confirmed histologically to be secondary to intimal hyperplasia. Run-off stenoses were not observed in vein shunts although the vein grafts themselves developed intrinsic stenoses in eight cases (20%). The authors noted that one sign of the presence of outflow stenosis included increased pressure in the dialysis line and this was an indication for angiography. Intervention to correct such stenotic lesions prior to shunt thrombosis resulted in improved patency. The authors suggested that the incidence of ANH encountered in the PTFE shunts in their series leant further weight to the compliance mismatch theory for the pathogenesis of the lesion.

Palder et al reported on a series of 324 arterio-venous conduits performed on 256 patients with end-stage renal disease requiring haemodialysis.[205] There were 154 Cimino fistulae and 163 PTFE grafts and 7 miscellaneous shunts. Interestingly, the cumulative 48 month patency rates for the PTFE shunts (60%) were superior to those of the autogenous grafts (44%) but this was explained by the large number of 30 day Cimino failures, most of which were due to the use of an inadequate vein. Excluding these grafts, the 48 month patencies were in fact similar for both groups. These authors found superior patency for PTFE forearm loops compared to more proximal grafts, which was different from the findings of Jenkins et al. However, they did state that most patients requiring a proximal shunt had previously undergone a number of more distal fistulae. In this series, 80 of the 163 PTFE grafts (49%) had a complication, 91% of which were due to thrombosis. Forty-four per cent

of thromboses occurred with no obvious underlying cause and 87% of these responded successfully to simple thrombectomy. Recurrent thrombosis occurred in 56% of all occlusions and the commonest identifiable cause was venous outflow stenosis secondary to intimal hyperplasia immediately proximal to the graft-venous anastomosis.

A number of difficulties are encountered when comparing the many reported series of infrainguinal revascularisation procedures using polytetrafluoroethylene (PTFE) including the variation in reporting methodology, the fact that some series are prospective and randomised whereas others are simply a retrospective review of all bypass grafts performed using PTFE. Also, inclusion and exclusion criteria vary between authors, starkly illustrated by the data from the report by Veith et al[281] in which obligatory PTFE bypass grafts fare worse than randomised conduits. Nevertheless, since its introduction into clinical practice in the 1970's, a vast clinical experience has been gained worldwide with PTFE in the infrainguinal position and I have performed the following meta-analysis in order to compare the outcome of bypass with PTFE as regards medium-term graft patency. Where deducible, the incidence of anastomotic neointimal hyperplasia as a cause of bypass failure has been recorded, although such information was volunteered by a minority of authors.

Table 8: META-ANALYSIS OF INFRAINGUINAL PTFE BYPASS GRAFTS

Author/ year (ref #)	No of grafts	Diameter	Outflow artery	30 day patency	12 month patency	24 month patency	36 month patency	% failed due to ANH
Burnham/ 1978 (36)	47	NS	BKPop29 Tib18	91	72	NS	NS	NS
Clyne/1979 (64)	20	NS	Pop18 Tibial 2	86	50	NS	NS	NS
Echave/1979 (97)	250	Tapered 6.5-4.5mm	Popliteal	NS	74	56	NS	2.5
Edwards/1979 (99)	29	6/6-4.5mm	Tibial	63	21	NS	NS	NS
Haimov/1979 (119)	184	NS	AKPop(59) BKPop(125)	NS	77 71	59 56	NS	NS
Veith/1979 (283)	175	6mm	Popliteal	94	$1^{O}70/\ 2^{O}80$	$1^{O}65/\ 2^{O}75$	$1^{O}65/\ 2^{O}75$	25
Evans/1980 (102)	98	8/6/4mm	AK Pop (73) BK Pop (25) Isolated seg	95 88 100	69 66 91	64 60 91	57 60 91	NS
Gupta/1980 (118)	175	6mm	Popliteal	$1^{O}90/2^{O}$ 97	$1^{O}68/2^{O}80$	$1^{O}65/2^{O}77$	$1^{O}62/2^{O}77$	25
Hobson/1980 (128)	36	NS	Pop 15 Tibial 21	84	65	65	NS	NS
Sladen/1980 (248)	105	6mm	AK Pop (14) BK Pop (74) Tibial (11)	NS 85 64	86 54 9	64 42	NS 30	NS
Veith/1980 (282)	148	6mm	AK Pop (82) BK Pop (66)	96 97	83 87	79 86	79 86	NS
Weisel/1980 (288)	67	6mm	AK Pop (24) BK Pop (26) Tibial (17)	95 82 50	75 58 18	50 35 18	50 35 18	NS
Hallett/1981 (120)	100	6/6-4.5mm	AKPop (28) At knee (10) BK Pop (54) Tibial (8)	95 90 90 50	88 68 60 25	80 22 47 25	80 NS 47 25	NS
Kidson/1981 (142)	44	6mm	AK Pop (27) BK Pop (17)	92 76	68 38	55 25	NS	NS
Simone/1981 (247)	65	8/6/ 6-4.5mm	Popliteal	83	63	51	51	NS
Yeager/1981 (300)	63	6mm	Pop (33) Tibial (30)	95 86	70 20	60 20	55 8	NS
Bergan/1982 (23)	137	6mm	AKPop (33) BKPop (46) Tibial (58)	97 97 82	83 72 48	77 68 25	72 60 20	NS
Brewster/ 1983 (32)	208	NS	AK Pop (75) BKPop (133)	95 90	83 52	71 43	63 39	NS
Christenson/ 1983 (55)	281	6mm	AKPop(153) BKPop(74) Tibial(54)	95 79 78	85 56 56	82 53 54	80 50 52	NS

Cranley/1983 (75)	89	NS	Pop(73) Tibial(13)	78 69	57 33	44 31	41 31	NS
McAuley/ 1983 (173)	127	NS	AK Pop (90) BK Pop (37)	85 73	68 55	62 50	47 50	NS
Quinones-Baldrich/1984 (216)	63	6mm	Popliteal AK47BK16	96	81	79	79	NS
Charlesworth 1985 (50)	134	NS	Pop (128) Tibial (16)	89	53	47	41	NS
Sterpetti/1985 (32)	90	NS	AKPop	NS	78 82	74 78	62 68	28
Williams/ 1985 (292)	180	6mm	Popliteal	70	40	29	20	NS
Veith/1986 (281)	269	6mm (pop) 6-4.5mm (infrapop)	AK Pop(91) BK Pop (80) Tibial (98)	98 97 96	85 88 48	70 82 33	60 55 30	NS
Taylor/1987 (271)	159	NS	AK Pop (48) BK Pop (66) Tibial (45)	97 97	90 86	NS	85 60	20
Flinn/1988 (105)	75	NS	Tib/peroneal	NS	57	45	37	NS
Quinones-Baldrich/1988 (219)	146	6mm	AKPop(101) BKPop(45)	$1^{0}98$ $1^{0}96$	$1^{0}85$ $1^{0}80$	$1^{0}80$ $1^{0}60$	$1^{0}74$ $1^{0}49$	NS
Whittemore/ 1989 (291)	215	NS	AKPop(118) BKPop(76) Tibial(21)	NS	70 53 25	60 38 12	40 32 12	NS
Quinones-Baldrich 1992	322	6mm	AK Pop 219 BKPop 75 Tibial 28	$1^{0}97$ $1^{0}95$ $1^{0}70$	$1^{0}85$ $1^{0}77$ $1^{0}45$	$1^{0}77$ $1^{0}78$ $1^{0}42$	$1^{0}70$ $1^{0}70$ $1^{0}23$	29
Taylor/1992 (270)	256	6mm	Pop 173 Tibial 83		90 74	85 66	82 58	NS
John/1993 (135)	113	6mm	AKPop	90	73	60	46	NS

(NS: Not stated)

This meta-analysis represents a selection of reported series of infrainguinal bypass with PTFE from the time of the introduction of this conduit into clinical vascular surgical practice to the present day. While it is difficult to compare individual series with others for the reasons stated previously, several points appear to arise form this analysis. For bypass grafts to the above-knee popliteal artery, the long-term patency of PTFE is comparable to that of

autogenous saphenous vein. However, for infrageniculate grafts, the patency rates are inferior to those reported in the literature for vein. The exception is the series of Taylor et al[270] in which the results of PTFE bypass with the Taylor patch are exceptionally good even to the tibial arteries. Aside from this, from the late 1970s to the present day, there has not really been an appreciable improvement in the patency rates achieved with PTFE. Also, in those reports where it is quantified, the incidence of anastomotic neointimal hyperplasia as a cause of bypass graft thrombosis seems to be fairly standard at between 20% and 30%. It is interesting to note that, whilst appearing to confer some haemodynamic advantage, the tapered PTFE graft has not been universally adopted and indeed has itself been associated with ANH.

Secondary Revascularistaion Procedures with PTFE: Literature Review

There are fewer data reported in the literature relating to the use of PTFE for secondary infrainguinal arterial reconstruction. Such reports as there are give a very favourable impression of the use of PTFE in this setting. It may be that others with a less promising experience have not reported their findings in the vascular surgical literature.

Author/Year (ref #)	No. Grafts	Diameter	Outflow artery	30 day patency	12 month patency	24 month patency	36 month patency
Bartlett/1987 (16)	247 (PTFE) 21 (vein)	NS	Pop Tibial	2^{O} 97	2^{O} 80	2^{O} 65	2^{O} 55
Dennis/1988 (82)	26	NS	BK Pop 2 Tibial 24	70	45	30	30
Yang/1991 (299)	73	6mm	AK Pop (26) BK Pop (47)	NS	1^{O} 62 2^{O} 72	1^{O} 44 2^{O} 58	1^{O} 41 2^{O} 58

(NS: Not stated)

Table 9: Secondary Infrainguinal Bypass with Polytetrafluoroethylene

TECHNIQUES AIMED AT MINIMISING THE DEVELOPMENT OF ANASTOMOTIC NEOINTIMAL HYPERPLASIA.

From the above review, it is clear that the development of ANH in prosthetic infrainguinal bypass grafts is due to several associated mechanical factors acting in particular at the distal anastomosis of these conduits. Because of this multifactorial aetiology, a single practical solution to the problem has so far proved elusive. A number of authors have described surgical and pharmacological approaches in an attempt to ameliorate the problem of anastomotic neointimal hyperplasia. In the following section, the published data will be reviewed relating to such techniques as anastomotic venous collars and patches, the use of tapered and composite grafts and the seeding of grafts with vascular endothelial cells. A brief review of published work on the pharmacological control of anastomotic neointimal hyperplasia will also be presented.

1) Anastomotic Vein Patches/Collars.

The use of a cuff or patch of vein at the distal anastomosis of a PTFE bypass graft to a distal popliteal or a tibial artery is not a new concept. A number of modifications on this theme has been reported over the past decade.

It was Siegman[245], writing in 1979, who first described the technique of vein cuff interposition at the distal anastomosis of a vein bypass to the infrapopliteal arteries. He also recommended the technique of vein interposition cuff at the anastomosis of prosthetic graft to autogenous vein in a composite lower extremity bypass, in order to make such a graft more haemodynamically favourable.

However, much of the credit for the introduction of the use of venous interposition collars goes to Miller who reported his group's experience of their anastomotic technique in

1984.[185] They reported the use of a vein collar at the distal anastomosis of 114 infrainguinal PTFE bypass grafts: 85 to the popliteal level and 29 to the infrapopliteal arteries. Over a median follow-up of 8 months, the patency of the femoropopliteal grafts was 77/85 (90%) and that of the femoro-tibial grafts was 21/29 (72%). Miller recommended the use of either proximal or distal long saphenous vein or a segment of a thigh branch of the long saphenous vein. The vein used should be 2 to 3mm in diameter and the length of vein should be between two and three times the length of the arteriotomy (Figure 3). Prior to adopting this technique, in order to facilitate the anastomosis of stiff prosthetic grafts to friable, diseased arteries, Miller had placed an eccentric vein patch at the distal arteriotomy in order to open the arteriotomy up to make suture placement easier, in a similar way to that described by Bernhard. By utilizing the vein collar described initially by Siegman, it was possible to place all sutures accurately, and thereby appose artery and vein intima precisely. In addition, the anastomosis of the prosthetic graft was made far easier and there was less risk of traumatizing a diseased artery. Finally, Miller et al reported that thrombectomy of such a graft was far easier than that in a conduit in which the PTFE graft was anastomosed directly to the small diameter run-off artery.

Encouraged by the results of Miller et al, other authors have had a similarly favourable experience with the use of the distal anastomotic vein cuff. In a series of 41 PTFE grafts to the below-knee popliteal (11 cases) or tibial and pedal arteries (30 cases) all placed for critical ischaemia in patients with no available autogenous vein, Tyrrell et al noted a 47% secondary patency rate at 13.9 months.[275] The limb salvage rate was 70% in this series. They felt that not only did the vein collar facilitate the actual performance of the distal anastomosis, but that it made for a longer anastomosis. In addition, they felt that the vein cuff acted as a transit zone between the relatively stiff, non-compliant graft and the host artery, thereby averting a sudden, large change in compliance between the two conduits. This may be due to the fact that the longitudinal compliance of a vein is greater than its transverse compliance, indicating that the orientation of the vein in the collar may be important. Certainly, Tyrrell et al felt that

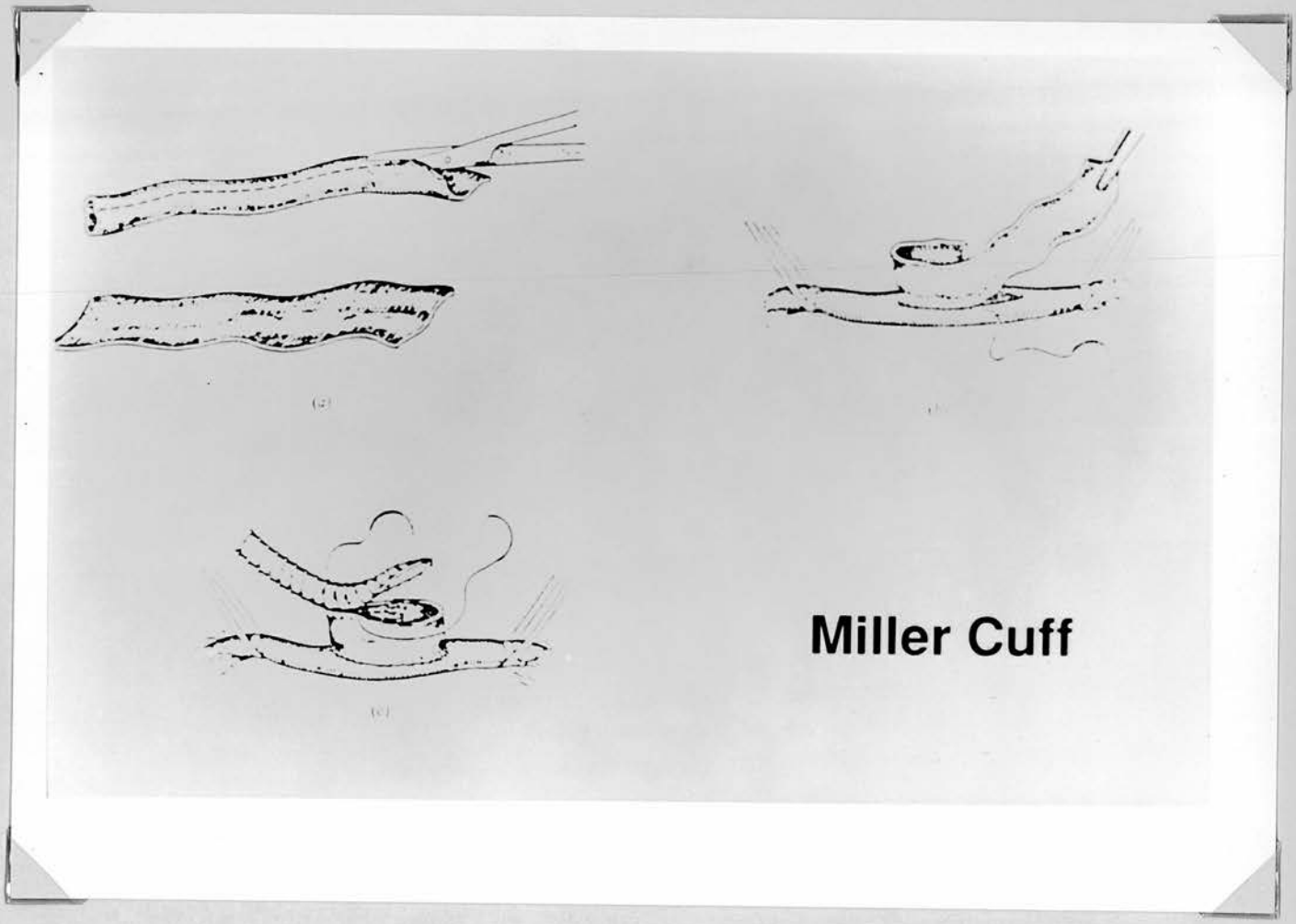


Figure 3: **The Miller Cuff.**
A segment of vein is opened longitudinally, after which it is sewn to the arteriotomy as shown, forming a collar to which the distal end of the PTFE graft is anastomosed

important. Certainly, Tyrrell et al felt that in the situation of the threatened limb with no available autogenous vein, the use of PTFE

grafts with distal anastomotic vein collars gave rise to patency rates and limb salvage that indicate that this approach was preferable to primary amputation.

Taylor et al described another variation of the distal anastomotic vein patch which they had used for infrageniculate lower extremity bypass grafts with impressive early patency rates.[270] They felt that the Miller cuff inevitably was associated with a great deal of turbulence as well as a high anastomotic angle. The Taylor patch modification of distal anastomotic vein patching consists of making a long (3 to 4cm) arteriotomy in the host artery. A U-shaped slit is made in the underside of the graft which is angled minimally so as to ensure that the graft is as near parallel to the artery as is possible. The graft is then sutured in place and the hood is incised in line with the arteriotomy for a length 2cm proximal to the heel level. A vein patch is then sutured in place over this diamond-shaped defect, the distal sutures being interrupted. Care is taken not to narrow the patch at the level of the PTFE-artery interface (Figure 4). Taylor et al reported the results of a series of 256 femoro-popliteal and femoro-tibial grafts performed utilising the Taylor patch technique at the distal anastomosis.[270] In addition, as a matter of routine, they placed a 3 cm long patch of autogenous vein onto the hood of the proximal anastomosis. The 1, 3 and 5 year primary patency rates for the femoro-popliteal grafts in this series were 91%, 81% and 71% respectively. The comparable primary patency rates for the tibial grafts were 74%, 58% and 54%. The authors observed a 14% incidence of anastomotic neointimal hyperplasia as a cause of graft failure in this series, compared to a figure of 25% in a series of PTFE grafts performed at their institution previously, without the use of a distal anastomotic vein patches. Their prior experience with vein patch angioplasty and extension vein graft as a means of correcting ANH after graft failure had been that these techniques were associated with no further ANH. Taylor et al felt that the vein patch might reduce compliance mismatch but also suggested that the venous endothelium might be responsible for the release of fibrinolytic and anti-platelet substances.

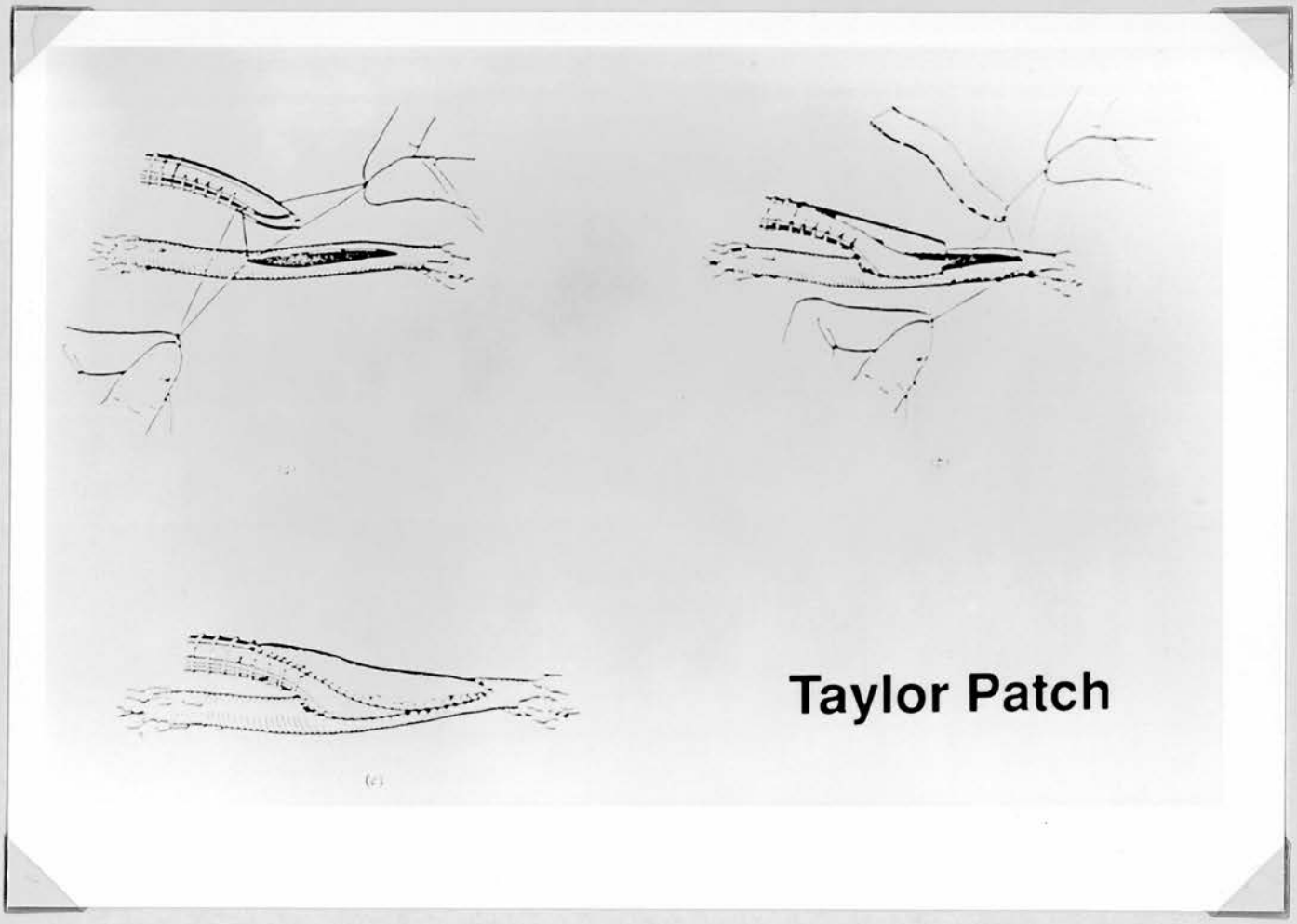


Figure 4: **The Taylor Patch.**
A V-shape is cut from the toe of the distal PTFE, after which the heel of the graft is sewn in place as usual. A segment of autogenous vein is then sewn in place forming a long, boot-shaped distal anastomosis.

A further modification of this approach to construction of the distal anastomosis of PTFE grafts to the infrageniculate arteries was described by Tyrrell and Wolfe.[276] They felt that a major disadvantage of the Taylor patch was that the heel of the anastomosis to the host artery consisted of the PTFE graft material itself and they felt that this might lead to the continued development of ANH at this location, although Taylor's later report failed to confirm this. Also, they reiterated Taylor's reservations regarding turbulence within the Miller cuff. By designing a spiral "boot" of vein they managed to include the advantage of the Miller cuff of not having any PTFE in direct anastomosis with the host artery whilst maintaining the apparent haemodynamic advantages of the Taylor patch. As yet there have been no clinical data published to support its use in preference to the techniques favoured by Miller and Taylor.

The Miller cuff has also been utilized in an attempt to enhance the patency rates of umbilical vein bypass grafts to the crural arteries. Buth et al reported a series of 12 such bypass grafts.[38] At a mean follow-up of 9 months (range: 3-18 months), two of the 12 conduits (17%) had occluded. Because of this favourable early experience, they have adopted this approach for every umbilical vein bypass to the infrageniculate vessels.

Another variation on the theme of the incorporation of a portion of autogenous venous tissue at the distal anastomosis of prosthetic grafts was reported by Batson and colleagues, who utilised the so-called "Linton" vein patch.[18] They placed a vein patch into the arteriotomy at the site selected for the distal anastomosis. This patch was twice the length of the intended PTFE anastomosis (Figure 5). The patch was then incised longitudinally in its mid-portion and the distal anastomosis performed. In a series of 68 PTFE grafts to the distal popliteal artery (48), the tibio-peroneal trunk (12) and to tibial arteries (8), the 6, 12, 24 and 36 month cumulative patency rates were 87%, 74%, 65% and 65% respectively. Interestingly, of the late graft failures assessed, the commonest cause of graft thrombosis was neointimal hyperplasia at the distal anastomosis.

In the same paper, Batson et al reported on the ultrastructural analysis of 27 thrombosed PTFE grafts (performed without vein patch) that had been analysed in their laboratory.[18]

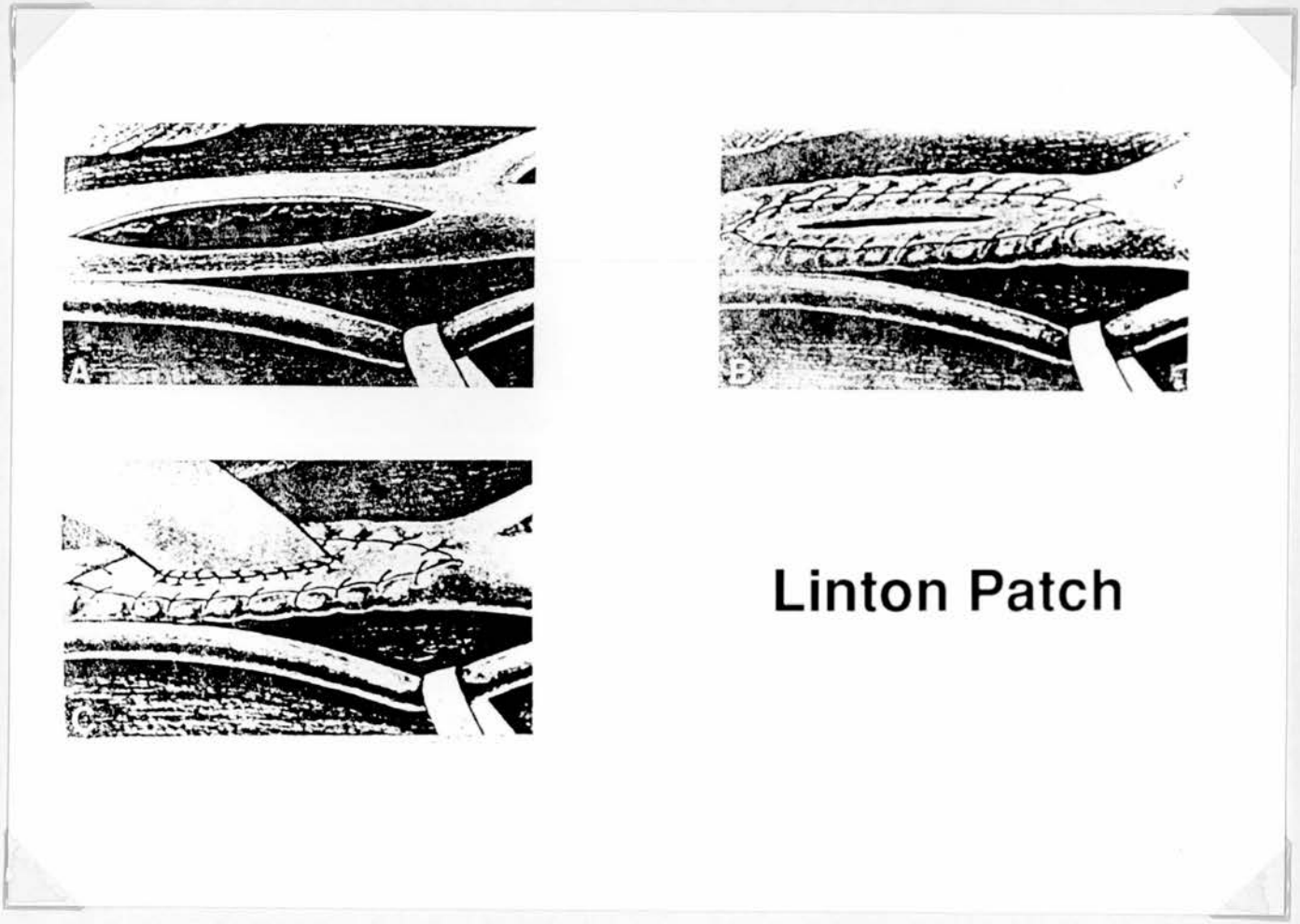


Figure 5: **Linton Patch.**
A vein patch is sewn to the distal arteriotomy and is then incised longitudinally. The PTFE is then anastomosed to this vein patch in standard end-to-side fashion.

ANH was found in every case and it was this finding that had led them to institute a policy of placing a distal anastomotic (Linton) vein patch in every case of infrageniculate PTFE graft. The authors were of the opinion that A.N.H. was due to compliance mismatch and that a vein patch not only overcame this problem but had the advantage of not being subject to kinking as seen in composite PTFE-vein grafts that have the venous component crossing the knee joint.

Laboratory Studies with Anastomotic Vein Cuffs

The clinical experience with vein collars and patches has stimulated workers to investigate these adjuvant techniques in the laboratory in order to shed some light on how the apparently superior patency rates reported can be attributed to the presence of the autogenous vein collar or patch.

Sottiurai et al performed PTFE grafts with or without distal anastomtic vein patches in a canine model of an ilio-femoral bypass.[250] They found that a vein patch was still associated with the formation of distal anastomotic neointimal hyperplasia, but to a significantly lesser degree than non-patched anastomoses. Also patency and Doppler waveform analysis was more favourable for patched compared to non-patched anastomoses.

Suggs et al placed 4mm PTFE grafts in the carotid circulation of dogs. One side had a vein cuff constructed at both the proximal and distal anastomoses and the other side acted as a control.[262] All dogs were treated with aspirin for one week prior to the operative procedure and afterwards until the time of sacrifice. They found that the most severe ANH was found at the toe of the anastomosis and in the proximal host artery in relation to both proximal and distal anastomoses of the non-cuffed sides, especially in the distal anastomoses. Immunocytochemical and histological studies showed that there was a proliferation of smooth muscle cells related to the ANH in non-cuffed specimens and that this was absent from the vein cuffed anastomoses. Four of the eleven non-cuffed grafts remained patent whereas 7 of

the 11 cuffed grafts were patent at sacrifice. As well as suggesting a role for the vein cuff in the inhibition of ANH, the authors felt that their study reiterated the observation that haemodynamic factors promote ANH at stereotyped sites just proximal or distal to graft-artery anastomoses. One caveat that Suggs et al stressed was the individual thrombogenicity of the subjects studied that may have had a significant effect on graft patency itself, quite apart from any effect of ANH. However, in spite of this fact, which can be the case in human subjects as well, there was no doubt that the incidence of ANH was decreased significantly by the presence of an anastomotic vein cuff. The authors postulated that this finding could be due to a haemodynamic effect of the cuff, which may disperse kinetic energy more widely than occurs when the graft is anastomosed directly to the artery. They wondered if this led to less injury to the arterial wall thereby reducing the cellular reponses to the unfavourable haemodynamics. Suggs et al also suggested that the venous endothelial cells themselves may be responsible for the secretion of substances that inhibit platelet adherence and mitogenesis, a hypothesis favoured by Taylor amongst other authors.
Further experimental work was performed by Beard et al who were specifically interested in the haemodynamics of the vein interposition cuff.[19] They used cadaveric internal mammary arteries and anastomosed 6mm PTFE grafts in end-to-side fashion either incorporating a vein cuff or not (controls). The in vitro model consisted of an hydraulic system containing whole human citrated blood with a packed cell volume of 45 per cent. Flow through each anastomosis was measured over a range of arterial pressures using an electromagnetic flowmeter. For arteries with an internal diameter of less than 2mm, flow was significantly higher in vein cuff anastomoses compared to the non-cuffed controls ($p<0.001$). This difference increased as mean arterial pressure increased above 40mm Hg. The same findings were observed in arteries of diameter greater than 2mm, although in this group the difference only just reached statistical significance ($p=0.05$). Similarly, resistance across the vein cuffed anastomoses decreased significantly as mean arterial pressure increased, this was not the case in control anastomoses. The authors concluded that these results might explain the promising patency rates of infrainguinal PTFE grafts performed with a distal anastomotic "Miller" vein

cuff. In the arteries of diameter less than 2mm, they suggested that the cuff had the effect of allowing the anastomosis to distend as the perfusion pressure increased, thereby reducing the anastomotic resistance to flow. The fact that this did not seem so vital in vessels with a diameter greater than 2mm implied that anastomotic dimensions were of less importance for arteries of this size.

Further in vitro experiments on the possible mechanism of action of the anastomotic vein cuff by Tyrrell and co-workers showed that autogenous vein stretches significantly more longitudinally than transversely and that intact human saphenous vein is anisotropic that is that it has the property of being more "compliant" longitudinally than transversely.[274,277] This is also the case for host artery and therefore the best "match" between vein and artery is obtained if their longitudinal axes are aligned at the anastomsois. The authors concluded that orientating the vein collar or patch's longitudinal dimension with that of the recipient artery takes maximal advantage of the elastic properties of the venous wall. This group showed that the vein collar did not act as an elastic "reservoir" which stores blood in peak systole and thus allows for flow in diastole, as had been suggested by others. However, they demonstrated from casts taken of direct PTFE-artery anastomoses that even in what was thought to be a technically "perfect" anastomosis, with pulsatile blood flow there is protrusion of the PTFE into the arterial lumen, giving rise to what they described as "oval distortion". This distortion was significantly less in anastomoses constructed with either a Miller cuff or a Taylor patch. In their view, this oval distortion effectively constituted a significant stenosis at the anastomotic artery level as well as probably influencing the distribution of shear stress at the anastomosis.[274]

2) Composite Prosthetic/Vein Grafts

When adequate vein is not available for an all autogenous bypass, another approach that has been used in an attempt to improve the patency rates for prosthetic infrainguinal grafts to below the knee joint level is the composite bypass. The philosophy behind such an approach is that prosthetic grafts crossing the knee do not seem to perform as well as autogenous vein bypasses. The prosthetic material is used for the thigh portion of the graft to which is anastomosed a portion of available autogenous vein (either long or short saphenous or arm vein). This latter component crosses the knee and is anastomosed to either the popliteal artery below the knee joint or to a tibial artery. This approach seems to be reasonable in theory, but the results of reported clinical experience have not been particularly favourable and the patency rates do not approach those of all autogenous bypass grafts.

One of the earliest reports of the use of composite grafts was published in 1961 by Dale et al.[77] In the laboratory, initially, they performed composite grafts in a canine model. They found that the patency of such conduits was best in the high flow, aortic location. Of the 5 experimental grafts placed at this level, only one failed. However, at the femoral artery level, 10 of 11 experimental grafts failed. In spite of these equivocal laboratory results, they decided to utilise the composite graft in a series of 16 patients with ischaemic lower extremities who lacked adequate autogenous vein for use as a bypass graft. They used a teflon prosthetic graft which was anastomosed to the autogenous vein component in an end-to-end fashion using silk sutures. No attempt was made to "fish mouth" or otherwise taper this end-to-end anastomosis and therefore there was an abrupt change in diameter at the prosthetic graft-to-vein anastomosis. This may account for the fact that of the 16 grafts placed, 13 thrombosed within 7 months and the longest recorded patency was 12 months. This early experience, and the disappointing results, may partly be explained by the nature of the stiff prosthetic material utilised and the apparently haemodynamically-compromised method of anastomosing the vein to the prosthetic graft.

In 1973, Linton and Wirthlin reported a series of 40 femoro-popliteal composite dacron-autogenous vein bypass grafts.[164] The authors were clearly of the opinion that having the venous component traversing the knee joint increased graft patency. Furthermore, they developed a spatulated prosthetic graft to vein anastomosis which gave rise to a much more streamlined conduit. In addition, they used dacron grafts rather than the early teflon prosthetic material used in the study of Dale et al. They monitored this series of bypasses for 5 years and reported a cumulative patency rate of 67.9% at 1 year, 59.9% at 2 years and 53% at 3 years. These patency rates were lower than those for a simultaneous series of all autogenous vein bypasses from the same institution, but were considerably better than the patency of prosthetic grafts alone or for the technique of endarterectomy and vein patching. Unfortunately, there is no indication in this particular study as to the incidence of distal anastomotic problems, in particular A.N.H.

Lord et al took the concept of composite prosthetic-vein infrainguinal bypasses a step further.[172] Their initial experience using the end-to-end prosthetic to vein anastomotic technique, reported by Dale, had been equally poor. Their modification of the composite bypass evolved when they found themselves in the situation of an attempted vein bypass in which the distal portion of vein did not function properly. Therefore they joined the proximal prosthetic component of the graft to the distal venous component in end to side fashion before ligating the venous component proximal to this prosthetic-vein anastomosis. It was then possible to anastomose the vein to the distal popliteal artery. They went on to use this technique in 23 further bypasses for limb salvage to either the distal popliteal artery or to the tibial arteries. The follow-up in this series was only 6 months and the cumulative patency rate of 46.4% appears poor until it is appreciated that in over half of these patients the run off artery was either an isolated popliteal segment or a single tibial vessel only. Because of these results, the authors were keen to stress their firm belief in the long saphenous vein as the conduit of choice and the feeling that this composite bypass technique should be reserved for limb salvage patients with no available autogenous vein.

Burnham et al were particularly condemning of the technique of composite PTFE-vein grafts to the tibial artery level.[36] In a series of 47 PTFE grafts to either the distal popliteal or tibial arteries, they placed 6 composite grafts. Of these, 3 were fashioned according to the end-to-end anastomotic technique of Linton and Wirthlin[164], only one graft remained patent during follow-up. In the other 3 cases, they adopted the sequential technique favoured by Friedmann and colleagues[107], in which the prosthetic component is anastomsoed to the popliteal artery above the knee and the venous portion of the graft is taken from this point to the distal anastomotic level. All 3 such grafts failed. Based upon this limited experience with the composite graft technique, Burnham et al dismissed it from their clinical practice.

In 1981, more promising results with composite infrainguinal bypass grafts were published by Snyder et al.[249] In a non-randomised study, they compared the patency rates for grafts in 68 patients undergoing composite PTFE-autogenous vein bypass to a group of 89 patients undergoing bypass with PTFE grafts alone. In all but five cases, the autogenous tissue for the composite graft was saphenous vein but in the remaining five, four cases used the cephalic vein and in one, endarterectomised superficial femoral artery. The groups studied were evenly matched for risk factors and also for the indications for operation, the bulk of which were for limb salvage. Also the patency of the run-off vessels was comparable in both groups. The distal anastomosis was to the infrageniculate level in 82% of the composite grafts but in only 60% of the PTFE-alone group. The prosthetic-to-vein anastomosis was performed in the "fluted" manner reported by Linton and Wirthlin.[164] These authors made no special effort to avoid crossing the knee joint with prosthetic grafts. Using life table analysis, the cumulative patency of the composite grafts at all levels was 70% at one year, 63% at two years and 58% at three years compared to figures of 60%, 47% and 25% respectively for the PTFE-alone group. The authors performed a meta-analysis of the published data on vein graft patency and compared this with the patency data of their composite grafts. Certainly, as far as 12 to 18 months follow-up, the two patency curves were very similar, but in such a small series, beyond this time interval, the standard error was greater than 10% and therefore no accurate conclusions could be reached about the long-

term patency rates. Even so, this report by Synder and colleagues on PTFE-autogenous vein composite bypass grafting was the first to really give any evidence in support of the use of this technique.

The following year, La Salle et al reviewed their group's experience with composite bypass grafts to the distal popliteal artery.[161] In this retrospective review, the prosthetic material used for the composite graft was dacron in 29 cases and PTFE in 10 cases. In the group of grafts that comprised prosthetic material only, there were 47 PTFE grafts and 32 dacron grafts. Using life table analysis, for the composite grafts, the one, two and three year cumulative patency rates were 54%, 50% and 42% respectively. The figures for the prosthetic grafts alone were 51%, 40% and 35% respectively. These authors felt that whilst in theory, having autogenous tissue anastomosed to the run-off artery was preferable, the main problem with composite grafting appeared to be related to the prosthetic graft-to-vein anastomosis. Not only did the authors feel that this was a technically-demanding anastomosis to perform, but they were also of the opinion that this was a site for the formation of neointimal hyperplasia related to compliance and mechanical mismatch between the prosthetic graft and the autogenous vein component. Because their results with composite bypass grafting were not significantly different from those using a prosthetic graft alone to the below-knee popliteal artery level, the authors stated that they had abandoned completely the composite graft.

More recently in 1987, Britton and Leveson have been encouraged by their experience with composite bypass grafting to the anterior tibial artery in the treatment of the threatened lower extremity, where adequate autogenous saphenous vein was not available.[33] In a series of 25 such bypasses using 6mm PTFE anastomosed to non-reversed saphenous vein, they experienced a 12 month cumulative patency rate of 65% which was associated with a limb salvage rate of 72%. They were of the opinion that other series' poor results were due to the fact that the saphenous vein component had been reversed and therefore at the prosthetic graft to vein anastomosis, there had been considerable diameter discrepancy leading to problems at this level. They felt that by using the vein in a non-reversed orientation, the

larger diameter of the vein was anastomosed to the prosthetic graft and the lesser diameter was anastomosed to the native tibial vessel. They thought that this orientation conferred a significant haemodynamic advantage on the conduit.

As mentioned earlier, another variation on the theme of the composite prosthetic-vein bypass graft is the so-called "sequential" graft, a technique initially described by DeLaurentis and Friedmann in 1970.[81] In a later publication, Friedmann reported their long-term follow-up data using this sequential graft.[107] Twenty-eight grafts were placed using the composite technique described above. The distal anastomosis was to the popliteal artery in 15, to the anterior tibial artery in 9, to the peroneal artery in two and to the posterior tibial artery in two cases. Over a follow-up period that ranged from 4-60 months, the cumulative graft patency was 71%. Grafts to the popliteal artery had an overall failure rate of 27%, whilst those to anterior tibial artery had an overall failure rate of 33%. These results were comparable to those experienced by the same authors for femoro-popliteal bypass grafting with autogenous vein and for this reason, the authors recommended this technique as an option when adequate saphenous vein was not available for infrainguinal bypass.

From this brief review of the literature on composite prosthetic-vein infrainguinal bypasses, it can be seen that although one or two authors have had reasonable results, on the whole, the patency rates of such conduits have been inferior to those obtained with autogenous vein and it is for this reason, presumably, that this method has not been globally accepted as an alternative bypass technique when adequate vein is not available.

3) Endothelial Seeding

In the previous two sections, I have described surgical approaches developed in an attempt to improve the results of infrainguinal bypass grafts using prosthetic conduits, on the assumption that the cause of the failure of these bypass grafts is due to mechanical and haemodynamic factors either at the distal anastomosis or due to kinking of the prosthetic material as it crosses the knee joint. Another approach aimed at improving the patency of prosthetic grafts has been focussed on the cellular events taking place both at the distal anastomosis and within the conduit itself. As described earlier, the only portions of PTFE grafts to achieve luminal endothelial cell coverage are the regions of the anastomoses through an ingrowth of endothelial cells from the native artery associated with an underlying mass of proliferating smooth muscle cells. This ultimately leads to the development of anastomotic neointimal hyperplasia. The remainder of the luminal surface of the bypass becomes lined with a layer of organised fibrin and thrombus, but never becomes covered by endothelial cells.
The theory behind the large amount of work that has been done in the field of endothelial seeding is that were it possible to have complete endothelial coverage of the endoluminal surface of such a prosthetic bypass, the thrombogenicity of the graft would be reduced and therefore patency potentially enhanced. Also, full endothelialisation might decrease the stimulation of medial smooth muscle cells by mitogenic substances derived from circulating blood cells and platelets. Early experimental work by Herring et al[125], in which dacron grafts seeded with autologous vein endothelial cells were compared to control grafts, showed that when explanted, the seeded grafts had a progressive increase in thrombus-free surface and had significantly thinner "inner capsule". Graham et al inserted seeded grafts into the thoraco-abdominal aorta of dogs and at 4 weeks found that 80% of the seeded grafts' surface was endothelialised compared to only 10% of the sham-seeded controls.[290] Again in a canine model in 1982, Stanley et al placed bilateral ilio-femoral dacron grafts with one side being seeded with autogenous vein endothelial cells.[256] In addition the animals received oral aspirin and dipyridamole. All of the grafts were patent whilst the animals were administered

the medications, but when they were stopped, the seeded grafts were found to have a significantly greater patency rate of 73% compared to 27% in the control group. At graft harvest, the seeded grafts were found to be completely endothelialised between 2 and 4 weeks from the time of the original operation. The implication of such studies is that a combination of endothelialisation of the graft together with the administration of antiplatelet medications decreases graft thrombogenicity and enhances graft patency. Much work has been done on the technical aspects of endothelial cell seeding, particularly in relation to endothelial cell adherence, as one of the problems encountered by many authors has been that cell detachment occurs with the restoration of blood flow to the graft.[139,239] Various authors have experimented with fibronectin and fibrin polymers as possible "adhesion agents".[290]

Unfortunately, thus far, the promising laboratory results in animal models of endothelial cell seeding have not been replicated in the human trials that have been published. For example, Herring et al seeded dacron grafts with endothelial cells and placed them in the femoro-popliteal, axillo-femoral and femoro-femoral locations.[290] However there was no difference in patency between seeded and control grafts. It was interesting to note that in patients who continued to smoke after the bypass operation, the early patency of seeded grafts was actually poorer than that seen in the controls. Further trials by Walker et al and by Herring et al have similarly failed to demonstrate any benefit of endothelial seeding on the long-term patency of grafts.[290]

4) Distal Arterio-Venous Fistula

A number of authors have reported the successful implementation of the technique of an adjuvant arterio-venous fistula during the construction of a prosthetic bypass to the tibial arteries.[78,79,121] This technique owes its success to the resultant high flow through the graft that prevents sluggish blood flow and consequent thrombosis due to limited run-off, rather any direct effect on the development of anastomotic neointimal hyperplasia. However, it has been suggested by some authors that by inducing such abnormal flow within the bypass graft, it may be that the end result is to induce the development of ANH which is clearly counterproductive. Suffice to say that, as with some of the other techniques descibed above aimed at improving the patency rates of tibial arterial prosthetic graft reconstructions, the distal arterio-venous fistula has yet to gain universal acceptance.

5) Pharmacological Approach to Neointimal Hyperplasia.

As well as the above operative and physiological interventions as an attempt at limiting the development of anastomotic neointimal hyperplasia, much work has been focussed on the use of drugs in this field. Many workers have shown both experimentally and clinically the beneficial effect of a large variety of pharmacological agents. Several different classes of drugs have been studied and shown to be effective in this role.[83] These include the antiplatelet agents, anti-inflammatory agents, immunosuppressive drugs, drugs normally used for the treatment of hypertension, anticoagulant medications and various agents that are in fact, lipid metabolites. The fact that such a wide variety of agents has been studied tends to confirm that the lesion of anastomotic neointimal hyperplasia is complex and its aetiology is probably multifactoral. Furthermore, there is apparent controversy in the literature on the pharmacological manipulation of ANH as, often the results of studies on the same agent from different centres appear to contradict each other. One concludes that we are still some way from finding the ideal pharmacological prophylaxis against anastomotic neointimal hyperplasia.

Antiplatelet Agents

The antiplatelet agents that have been studied extensively are aspirin and dipyridamole.[298,301] These agents act by inhibiting the synthesis of prostaglandin in platelets by blocking the arachadonic acid pathway at different levels. Aspirin irreversibly acetylates platelet cyclo-oxygenase, whereas dipyridamole increases the intracellular cyclic adenosine monophospate (cAMP) level, thereby inhibiting the precursors of thromboxane. The result of these effects is that both drugs prevent platelet adherence and aggregation.[58,160]

Experimental work on the action of antiplatelet agents with regard to neointimal hyperplasia was performed by Friedman et al[108], who reported a reduced level of neointimal hyperplasia following aortic balloon catheter injury in thrombocytopaenic rabbits. However, subsequent laboratory and clinical reports from various different authors have been contradictory regarding the role of aspirin in this setting. In particular, some workers have demonstrated a positive effect of aspirin with regard to platelet adherence to prosthetic grafts, whereas other studies have shown that the antiplatelet agents decrease subsequent platelet aggregation and platelet thrombus formation, but have no effect on the initial platelet deposition.[176,177,214] With regard to the patency of bypass grafts, again positive effects in the laboratory have not always been borne out clinically. For example, in the study of Kohler et al[148], the effect of aspirin and dipyridamole on the patency of lower extremity bypass grafts was observed in a prospective, double-blinded, randomised trial. The authors concluded that aspirin and dipyridamole administered post-operatively in their study did not improve the overall patency rates of either vein or PTFE intrainguinal bypass grafts. On the other hand, Clyne et al reported a significantly beneficial effect in clinical trials using dacron or PTFE femoro-popliteal grafts when a short course of aspirin and dipyrimadole was administered post-operatively.[65] Cheseboro et al found that the use of post-operative aspirin and dipyrimadole in combination after aorto-coronary vein bypass improved late patency rates significantly.[53] Quinones-Baldrich et al showed that aspirin increased significantly the patency of an end-to-side iliac anastomosis, but had no significant effect on neointimal hyperplasia.[220]

The recently published reports of the Antiplatelet Trialist Group[6,7] indicated that in the fields of coronary artery bypass grafts, peripheral vein and prosthetic bypass grafts, as well as after endovascular interventions, such as percutaneous transluminal balloon angioplasty, there appeared to a be a positive benefit upon long term vessel and graft patency with the use of antiplatelet agents, in particular aspirin, post-operatively. It seems that when the antiplatelet agents are commenced immediately after the procedure, there is a far greater beneficial effect on patency than if there is delay between the procedure and the commencement of such treatment. It seems, however, from the mixed reports summarised above from the laboratory

and clinical fields, that the effect on patency of the antiplatelet agents is probably not so much related to the effect on anastomotic neointimal hyperplasia per se as to the prevention of early graft or vessel thrombosis. Clearly more work is necessary in this field experimenting with new agents and combinations of agents.

Anticoagulants

The anticoagulant used routinely in vascular surgical practice to prevent intra-operative thrombosis is heparin. Heparin is a naturally-occuring polymer containing chains of sulphonated mucopolysaccharides.[58] It is concentrated in mast cells and it is found in several tissues and cell types, including vascular endothelial cells. The mode of action of heparin is to inhibit the plasma-bound proteolytic clotting cascades. Certain sizes of heparin polymers combine with anti-thrombin III to enhance the enzymatic action of this agent. As well as possessing anticoagulant properties, heparin has been shown to be antimitogenic, having the capacity to inhibit smooth muscle cell migration and proliferation both in vivo and in vitro.[52,63,191] The precise mechanism of this antimitogenic action is not known, although endothelial cells have been shown to secrete a heparin-like substance and so it may be that heparin can inhibit smooth muscle cell proliferation which seems to occur in the normal "resting" vessel wall.[45] Certainly, the effect of heparin on the inhibition of arterial smooth muscle cells occurs at a dose below that required to achieve anticoagulation and indeed certain fractions of heparin with non-anticoagulant properties still maintain this anti-proliferative activity.[95] Needless to say, as with many of the other agents that have been proved beneficial with regard to anastomotic neointimal hyperplasia, studies have been published demonstrating the opposite finding, including work by Cambria et al[40], with experimental vein grafts, in which heparin failed to suppress cellular proliferation and intimal hyperplasia in autogenous interposition vein grafts placed into the rat aorta.

More recently, a similar action to conventional heparin has been demonstrated experimentally with the low molecular weight heparins.[39,295] In the clinical studies published to date, however, no significant long-term benefit on graft or vessel patency has been demonstrated. One of the problems with the intermittent injection of heparin, which was stressed by Clowes and Reidy[58], is that repeated injections of larger doses of heparin can produce transient anticoagulation. The authors observed that if repeated cycles of anticoagulation and coagulation occur because of these intermittent heparin injections, thrombosis can develop in some situations. They concluded that any clinical strategy which would result in a beneficial effect of heparin would need to result in a steady-state low level of anticoagulation.

If the aim of using systemic heparinisation during and immediately after the placement of an arterial bypass graft is to prevent immediate thrombosis, it seems logical to assume that the use of long-term anti-coagulatant medication might positively influence the rate of late graft thrombosis.[153] Warfarin is the oral anti-coagulant used in standard clinical practice. This drug is a competitive inhibitor of vitamin K and suppresses the hepatic synthesis of the four vitamin K-dependent clotting factors II, VII, IX and X. Although anticoagulants are used by many practitioners post-operatively, especially in patients with poor run-off and in secondary and tertiary bypass grafts, there is little evidence in the literature to support this practice.[28,92,279] The risks of haemorrhagic side-effects, some of which are potentially life-threatening, as well as the cost of monitoring anti-coagulant control are but two factors which question the use of warfarin when the beneficial effects on graft patency are not proved.

However, there have been some prospective studies performed to assess the effect of long-term anti-coagulation on the late patency of infra-inguinal bypass grafts. In 1988, Kretschmer et al published the results of a survival study of 119 patients who were treated with warfarin after the placement of an autogenous saphenous vein femoro-popliteal bypass grafts.[154] The treated group had a significantly greater five year survival than controls. There were 11 graft occlusions in the treated group compared to 17 in the control group. In a subsequent publication on the same group of patients and grafts, Kretschmer et al reported

a significantly greater primary patency rate, limb salvage rate and patient survival in the group treated with warfarin compared to controls.[155]

As is the case with the antiplatelet agents, other published data on the use of anticoagulation in the post-operative period after bypass placement come from the field of coronary artery bypass grafting. In a randomised trial, Pfisterer et al[213] studied prospectively the relative effects of antiplatelet and anticoagulant treatment on the early and late thrombosis rate of aorto-coronary vein bypass grafts. There was no significant difference in the patency rates of the two treated groups although both were superior to the patency rates of the control (placebo) group. The incidence of haemorrhagic complications was greater in the warfarin group. The authors concluded that there appeared to be no advantage of the use of warfarin over aspirin on the late patency of coronary artery bypass grafts.

Clearly, more prospective studies are required before any decisive conclusions can be reached about the routine use of oral anticoagulants after peripheral artery bypass grafting. It would appear that any benefit on patency is via the inhibition of coagulation rather than than on any direct influence on the events leading to the development of anastomotic neointimal hyperplasia.

Antihypertensive Agents

A considerable amount of attention has been focussed upon the possible role of these drugs in the prevention of anastomotic neointimal hyperplasia. Angiotensin converting enzyme (ACE) inhibitors, calcium channel blockers and alpha-1-adrenergic antagonists have all been reported to be effective in suppressing anastomotic neointimal hyperplasia.

Angiotensin converting enzyme inhibitors prevent the conversion of angiotensin I into its active derivative angiotensin II. Angiotensin II is a very potent vasoconstrictor and is thought to work principally through its effect on the smooth muscle cells in the arterial tunica media. Angiotensin II may also interact with platelet activation via a specific binding site.

Experimental models of both arterial balloon catheter injury[215] and experimental vein grafts in animals[193] have shown a significantly beneficial effect with regard to anastomotic neointimal hyperplasia of the use of ACE inhibitors. In the former experiment, it was with the use of silazapril and in the vein graft experiment it was with captopril. However, there are no published results from any human studies as yet.

A further study using another drug saralasin which is an angiotensin II receptor antagonist was reported by Xian-Mang and colleagues.[209] In a balloon catheter aortic endothelial denudation model in rats, they demonstrated that this drug was able to interfere with the proliferative response to vascular injury. As a result, they concluded that their work provided strong support for the hypothesis that angiotensin II does indeed induce smooth muscle cell proliferation after aortic injury in the rat.

Another group of antihypertensive agents that have been studied is the calcium channel blockers, in particular nifedipine. These drugs act by interrupting the normal function of membrane calcium channels, thereby reducing the availabilty of calcium for various intracellular functions. Calcium has been implicated in a number of the cellular events leading to the development of anastomotic neointimal hyperplasia, including platelet activation, the release of platelet-derived growth factor, the proliferation of medial smooth muscle cells and the subsequent ability of these cells to produce extracellular matrix. Various experimental data exist as to the positive effects of calcium channel blocking drugs on ANH. Weigand et al placed interposition vein grafts in a canine model and observed the effects on animals that recieved nifedipine compared to controls.[287] They proved that nifedipine was associated with significantly less anastomotic neointimal hyperplasia in the vein grafts than was seen in untreated, control dogs. Similar positive effects have been demonstrated with the drug verapamil, another calcium channel antagonist, on animal arterial injury models and experimental vein bypasses.[100]

The other group of antihypertensive agents that has shown promise in the laboratory in terms of reducing anastomotic neointimal hyperplasia is the alpha-adrenergic receptor antagonists. These receptors are found on vascular smooth muscle cells and when stimulated give rise to

profound vasoconstriction. Using the alpha-1-antagonist doxazosin, Vashisht et al demonstrated a long-term reduction in intimal hyperplasia when using this agent in a rabbit aortic endothelial denudation model.[280] Other work from the same laboratory by O'Malley et al[195], using prazosin, showed a similar beneficial effect.

Although these drugs have shown marked promise in the laboratory, little information is available from their use in the clinical field to support any obvious benefit with the prophylaxis against anastomotic neointimal hyperplasia. As with many of the drugs that have been shown experimentally to inhibit the hyperplastic response, the dosage required to produce these effects in humans can often give rise to unacceptable side-effects.

Anti-inflammatory agents

The exact role of the white blood cell, and in particular the neutrophil, has yet to be clearly elucidated as far as anastomotic neointimal hyperplasia is concerned.[52,58] After arterial endothelial injury, there is adherence of white blood cells to the exposed subintima as well as a localised inflammatory response with the release of agents such as chemoattractants and cytokines. White blood cells are able to respond to and to secrete mitogenic substances such as macrophage-derived growth factor and fibroblast-derived growth factor.[67] Both of these substances have a direct effect on the stimulation to migration and proliferation of the medial smooth muscle cells which are exposed to the circulating blood at the time of arterial injury. With these cellular events in mind, some workers have hypothesised that there may be a role for the use of some of the currently used anti-inflammatory agents, including the corticosteroids, in the prophylaxis against ANH.

Chervu et al documented that animals treated with corticosteroids developed significantly less intimal hyperplasia than non-treated controls in a balloon catheter endothelial injury model in the carotid artery of the rabbit.[51] Subsequent work from the same group using dexamethasone proved that the suppression of intimal hyperplasia using this agent was dose-

depedent.[66] Furthermore, they proved that above the dose required to achieve this effect in the rabbit model, which was 0.05mg per kg, no further improvement was noted.

Further work from the laboratory, aimed at delineating more clearly the mode of action of glucocorticoids with regard to neointimal hyperplasia published by Longenecker, showed that the glucocorticoid hormones have direct and pronounced growth-inhibiting effects on aortic smooth muscle cells in culture, but less significant effects on endothelial cells.[170,171] The authors suggested that glucocorticoids inhibit smooth muscle proliferation by decreasing the sensitivity of medial smooth muscle cells to mitogenic stimulation by high density lipo-proteins, presumably derived from white blood cells.

A clinical study of the effect of methylprednisolone on restenosis after coronary artery balloon angioplasty reported by Pepine et al[211] did not show any benefit in the steroid-treated group. However, the methodology involved and the agent used in this particular study may have been responsible, since methylprednisolone is a short-acting steroid and the patients were only given one dose intravenously before angioplasty, with no further pharmacological treatment thereafter. It may well be that the corticosteroids are the most promising agents in terms of drug prophylaxis against anastomotic neointimal hyperplasia. However, it can be predicted that once again the systemic side-effects of these agents, in doses necessary to suppress ANH, may prove to be unacceptable.

It has been suggested that other immunosuppressive agents such as azathioprine and cyclosporine may inhibit the neointimal hyperplastic response by a similar immunosuppressive effect principally aimed at white blood cell function.[67] However experimental reports are limited in this field at present.

Lipid metabolites

In recent years, much attention has been focussed on the effect of fish oil derivatives in regard to lowering the incidence of atherosclerotic heart disease.[187] From studies in

eskimos[13], who have extremely low plasma levels of low-density lipoprotein (LDL) and high levels of the Omega-3 polyunsaturated fatty acid, eicosapentaenoic acid (EPA), a substance found in high quantities in fish oil, an association between the serum lipids and the low incidence of atherosclerotic disease has been made. EPA competes directly with arachadonic acid in the prostaglandin synthesis pathway.[159] EPA is converted in this pathway to thromboxane A3, which has no effect on platelet function, compared to thromboxane A2, the normal by-product of arachadonic acid metabolism which is a strong promotor of platelet aggregation. Thus far, several animal studies have shown promising results using EPA in an attempt to suppress neointimal hyperplasia.[85,103]

Ohara et al placed PTFE grafts in the rabbit aorta in a model of hypercholesterolaemia.[197] The results showed that EPA reduced anastomotic neointimal hyperplasia in expanded PTFE grafts in the hypocholesterolaemic animal model. Landymore et al placed external jugular vein interposition grafts into the femoral arteries of adult mongrel dogs that had been fed a high cholesterol diet.[159] The control group were compared to a group given a diet also containing EPA to demonstrate a significant inhibition of neointimal hyerplasia in this hypocholesterolaemic model in the group treated with EPA compared to controls. The authors concluded that EPA inhibits platelet-mediated neointimal hyperplasia and suggested that cod-liver oil could be used clinically to influence the development of neointimal hyperplasia in vein grafts. These promising animal experimental studies will need further investigation in human clinical trials before any long-term role for fish oil products in the inhibition of anastomotic neointimal hyperplasia in infrainguinal arterial bypass grafts can be deduced.

Other Therapeutic Modalities

Recently, other potential non-surgical methods of limiting the development of anastomotic neointimal hyperplasia have been described after successful experimental work in the animal laboratory. Eton et al reported their experience with a rabbit carotid artery intimal injury model treated with photofrin (an agent preferentially adsorbed by smooth muscle cells within intimal hyperplastic lesions) together with local continuous wave tunable dye laser.[101] They showed that such treatment resulted in significantly less intimal hyperplasia than was seen in controls. Such novel approaches suggest yet further possible means of combating neointimal hyperplasia. As yet no human trials of this therapeutic modality have been reported.

Genetic researchers have also offered an alternative approach to the problem of restenosis after balloon angioplasty which could have important implications for the treatment of anastomotic neointimal hyperplasia as well.[14] They performed balloon angioplasty on the femoral arteries of pigs. The treated segments of vessel were then seeded with a human respiratory virus which acted as a carrier of the gene which encodes for thymidine kinase. This enzyme does not harm the vascular smooth muscle cells directly, but makes them susceptible to the drug ganciclovir. This agent is phosphorylated by the thymidine kinase causing it to insinuate itself into the DNA of the dividing cells and thus halt cell replication. In the porcine model, animals so-treated had a 50% to 90% reduction in arterial intimal thickening compared to untreated controls.

Both of these techniques offer exciting prospects for the non-surgical approach to the prevention of neointimal hyperplasia, but obviously, more work is required before human trials can be performed, not least to guarantee that no long-term adverse native vessel damage, such as aneurysm formation, results from such treatments.

Pharmacological Control of Neointimal Hyperplasia: Summary

From this review of the literature on the pharmacological inhibition of anastomotic neointimal hyperplasia, it can be seen that whilst some agents have shown marked promise in the laboratory, this has not been borne out in clinical practice. Furthermore, often for every positive report from one author a contradictory report comes from another laboratory. These two observations serve to illustrate the complexity of the pathogenesis of anastomotic neointimal hyperplasia. Clearly more work is required in the field of the pharmacological approach to the control of this lesion before the ideal agent is arrived at. The recent Anti-Platelet Trialist Group's report, however, would seem to imply a beneficial effect of the administration of low dose aspirin per and post-operatively on vessel and graft patency after all vascular interventions and certainly anti-platelet agents are the mainstay of drug therapy in clinical practice at present.

SECTION III

ENDOVASCULAR STENTS

Introduction

The concept of the use of an endovascular tubular device to effect vascular repair is not a new one. In 1894, Abbe, a surgeon from New York, experimented with the canine femoral artery and feline aorta in an attempt to devise a means of reapproximating severed arteries. After first dividing the vessels, Abbe used a glass tube shaped like an hour glass, placing the thicker ends within the proximal and distal end of the severed artery and then tightening the artery over the glass tube at either end with a circumferential silk tie. The prosthesis within the canine femoral arteries thrombosed in every case, but the device placed in the feline aorta remained patent.[109]

Several investigators attempted to improve upon Abbe's technique, using various different devices made from such diverse materials as magnesium and ivory. In 1901, Peyr used a magnesium tube to reunite the femoral vein after excision of a groin tumour. Although the patient died of unrelated complications on the third post-operative day, at autopsy the prosthesis was found to be patent.[109] These early attempts at the use of prosthetic devices to unite divided arteries were succeeded by the development of suture techniques with which we are now familiar.

In more modern times, the use of endovascular prostheses in the form of stents was introduced in the 1960's by Dotter. He popularised the technique of transluminal catheter dilatation of arterial atherosclerotic stenoses. However, this procedure can be complicated by secondary luminal thrombosis and Dotter believed that the deployment of a post-angioplasty, endoluminal device might prevent this occurring.[93] Initially, in a canine model, he used polyethylene, polyamide and teflon tubular prostheses but in every case, the stented vessels

thrombosed. After this initial experience he then experimented with coil-spring devices, again in a canine model, and followed these animlas for a number of years with serial angiograms. He was able to demonstrate the continued patency of these devices up to two and a half years after their deployment in the canine femoral artery.[93] The early experience of Dotter and co-workers illustrated many of the fundamental problems that have presented themselves to the subsequent designers of endovascular stents in subsequent years.

With the materials currently available it is probably impossible to manufacture the "ideal" stent. Schatz[232], and more recently Becker[21], have written extensively on what they believe to be the ideal characteristics of a metallic endovascular stent. As the primary use of stents is as a means of preventing restenosis after balloon angioplasty and to rescue immediate angioplasty failures or angioplasty-induced intimal dissections, the stents should be deployable using the same technology and techniques as are used for percutaneous transluminal balloon angioplasty. Because vessels of different lengths and diameters require treatment, the stents should be marketed in appropriately corresponding dimensions. Often, vessels of a very small diameter have to be negotiated to reach the target lesion(s) and so the smaller the diameter of the catheter loading system is, the better. Equally the higher the expansion ratio of the stent, namely the ratio of its unexpanded to its expanded state, the better. A stent should be radiopaque, so that it can be visualised on fluoroscopic and radiological imaging technology and the catheter on which it is mounted should be easily visualised with the same imaging facilities. The stent should be flexible, especially in the longitudinal plane, but should also be rigid enough to oppose vessel wall elastic recoil. One of the most important characteristics required is that the stent material should be of low thrombogenicity and be inert. With such an array of requirements, it is not surprising that a number of different stent designs have been developed over the past two decades.

Underlying the various different models that have been produced are two basic means of stent deployment, namely the balloon-expandable stent and the self-expanding stent.

Balloon-Expandable Stents

The best known version of this particular design is that popularised by Palmaz.[206] This stent comprises a single piece of stainless steel tubing bearing staggered rows of rectangular slots etched into the wall. The stent is mounted and crimped onto an angioplasty balloon and delivered to the target within a protective sheath. When the target site for balloon angioplasty is reached, the sheath is withdrawn, the stent expanded and deployed by inflation of the balloon. When a satisfactory result is visualised radiologically, the balloon is deflated and removed. When the stent is expanded, the rectangular slots enlarge and thus diamond shaped openings are created. When expanded fully, the Palmaz stent is alleged to have only 10% of its exposed surface area comprised of the metallic elements. This stent is relatively rigid and inflexible and is able to withstand elastic vessel wall recoil. It is this design of stent that has undergone the most scrutiny in human clinical trials to date.

The Strecker stent is more flexible than the Palmaz stent.[21] Rather than stainless steel, it is made of tantalum interwoven wire and this metal is more radiopaque than steel. The method of deployment is very similar to that of the Palmaz stent but the proximal and distal ends of the Strecker stent have a thin silicone sleeve that acts as a retaining device.

A third type of balloon-expandable stent is the "book binder" or "Gianturco-Roubin" stent.[21] This is made from stainless steel and particularly designed for use in small vessels. It is made of surgical suture wire and the wire is wrapped cylindrically with bends adopting alternating U and inverted U configurations every 360 degree turn. This is the most flexible of the balloon-expandable stents, but because of its configuration, it has a low expansion ratio and therefore it is not suitable for larger peripheral vessels.

Self-expanding Stents

There are two basic types of self-expanding stent that have been developed and tested. One is a stent that adopts a set configuration which is triggered by thermal memory when exposed to body temperature. The other is a spring mechanism triggered by unloading the device from a constraining delivery catheter.

Stents with thermal memory are made of an alloy of titanium and nickel called "Nitinol". This alloy has a unique characteristic which is that it has a thermally-triggered shape memory. Therefore it can be loaded onto a catheter in an elongated state, but when exposed to body temperature it regains a functional configuration in the shape of a spring coil. It is possible to alter the temperature at which this material changes its configuration by varying the proportions of titanium and nickel contained in the alloy. A number of investigators, including Dotter[94], Cragg[72,73] and Sutton[267] have reported successful deployment with long-term patency of these devices in canine models.

The best-known spring-loaded, self-expanding stent is the "Wallstent" endoprosthesis.[231] These stents are manufactured in a range of diameters from 2.5 to 15mm when fully expanded. They comprise 16 to 20 filaments of surgical grade stainless steel woven into a tubular flexible self-expanding braid configuration. The filaments of the smaller stents are 0.075 to 0.1mm in diameter. The crossing points of each filament are free to pivot and therefore the stent is flexible in the longitudinal direction. The smaller stents are delivered on a 5 French-gauge catheter and the larger ones are mounted on a 7 French-gauge catheter. When mounted on the delivery catheter, the stent is stretched to its lowest profile and is contained within a constraining, rolling membrane. To deploy the Wallstent, it is first positioned at the target site. The mounting catheter has a side port which can be injected with either radiopaque contrast or fluid which will lubricate the constraining membrane. By protracting the metallic insert, the constraining membrane is retracted and as it rolls back the stent is deployed. The stent expands to be contained within the diameter of the containing vessel.

The "Gianturco" Z-stent is made from tempered stainless steel and is also self-expanding.[96] It is mounted on a delivery catheter and after deployment the non-tapered outer catheter is withdrawn. Again, in this design, an inner pushing catheter allows deployment without the position of the stent changing.[21]

Stent Incorporation

The process of stent incorporation into the vessel wall forms a fairly uniform pattern, irrespective of the stent type used. Fibrin deposition and minor thrombus formation between the stent filaments is followed by full endothelial coverage after as little as 2-3 weeks. This process is dependent upon good inflow and outflow so that excessive thrombus is not deposited, which if significant, can result in stent occlusion. Krupski et al reported their laboratory experience with the Wallstent in a baboon model of carotid endarterectomy.[156] They found that the deployment of a stent at such sites of arterial disruption gave rise to significantly less platelet adherence and early thrombus formation. Many authors have reported that the neointima that develops in association with deployed stents actually stabilises with the establishment of endothelial coverage such that at six months from the time of stent implantation, the neointimal thickness is fairly predictable between 50 and 400 micrometers.[15,24,230] Proponents of the Palmaz stent claim that the minimal late neointimal thickness is related to the fact that these stents only have 10% of their surface area comprised of the metallic component, whereas the self-expanding stents, such as the Wallstent, when fully expanded have up to 20% of their surface area comprised of the metallic filaments.[21] Others have argued that the flexibility of the self-expanding stents is responsible for an unstable growth surface on the endoluminal aspect of the stent which stimulates smooth muscle cell proliferation within the underlying arterial media and thus, gives rise to a greater degree of neointimal thickness.[232] These would appear to be reasonable arguments in favour of using the more rigid Palmaz type of balloon-expandable

stent. However, these devices are clearly harder to manoeuvre around tortuous vessels and therefore deployment can be technically more demanding.[207,296] Furthermore stent deployment with this device is reportedly associated with a higher incidence of stent migration and embolisation than is seen with the self-expanding devices. Also, in inexperienced hands, it is easy to overexpand the stent and thereby damage the recipient vessel. Thus it can be seen that although the previously-listed characteristics of the ideal stent are the ultimate goal, none of the devices that are currently available for clinical practice do not fulfill every criterion.

Clinical Results of Endovascular Stenting

Balloon-expandable stents

Endovascular stents have now been used extensively both in the coronary and peripheral circulations and there are reasonable follow-up data available in order that the late outcome of the stenting of balloon angioplasty sites in the coronary, renal, iliac and peripheral arteries can be assessed and compared to either angioplasty alone or other conventional surgical means of intervention.

In 1988, Palmaz et al reported the initial experience with the Palmaz balloon-expandable stent in the treatment of 15 patients with symptomatic iliac artery stenosis.[207] The immediate results in this initial patient group were excellent with all patients having a substantial clinical and haemodynamic improvement after stent placement. The trans-stenotic pressure gradient after stent deployment changed from a predilatation mean of: 32.3 +/- 16.7 mmHg to 3.1 +/- 4.2 mmHg. The ankle-arm indices before the procedure were: 0.68 +/- 0.22 and afterwards: 0.96 +/- 0.24. In this early publication, follow-up was only to a maximum of 12 months, but all patients seemed to have maintained a good clinical result at that time. Eleven subjects underwent angiography at 6 months and in every case, a thin layer consistent with neointimal tissue was seen between the opaque metal mesh of the stent and the vessel lumen. This layer averaged 0.8mm in thickness and appeared to be uniformly distributed within the luminal surface of the stent. Of note, where the authors had deployed tandem stents, there did not appear to be any significant increase in neointimal thickness compared to vessels in which only one stent had been placed.

Rees and colleagues[221], from the same group, reported on the use of percutaneous balloon angioplasty combined with Palmaz stenting in the treatment of iliac artery occlusions in a group of 12 patients. Using either a guidewire or a hot tip laser, the occlusions were successfully traversed after which balloon angioplasty and Palmaz stent deployment was

utilised. In all but one patient, a mean increase in ankle brachial index of 0.15 was observed and after deployment of the stent the trans-stenotic pressure gradients decreased to less than 5 mmHg, with a mean of 0.58 mmHg. Again, in this study, the follow up was short with a range of 1-14 months and a median of 6 months. However the results in this short follow-up period appeared good with patients both haemodynamically and symptomatically stable. Subsequently, Palmaz et al reported on the multicentre study group's first 171 iliac artery balloon angioplasty procedures which were followed by stenting.[208] This report gave further encouraging results, with 89% of patients showing clinical benefit over a median follow-up of 6 months, ranging between 1 and 24 months. The interesting point to note in this report of a larger experience was that stent deployment was associated with a procedure-related complication rate of 9.7%. Most of the complications were related to either groin haematomata or distal embolisation of thrombus or atherosclerotic debris. Also, at the autopsy of one patient who died from metastatic carcinoma, important information was obtained regarding the mode of stent incorporation. It seemed from assessing this patient's stented iliac artery, that Palmaz balloon-expandable stents are incorporated into the arterial wall through a process of neointimal tissue formation followed by endothelial coverage of this surface. There was some residual thrombus in this autopsy specimen which was inspected 2 months after stent placement. This would indicate that the incorporation of stents in humans takes place over a longer period of time than in experimental animals. Whilst obviously documenting encouraging results, these reports from Palmaz's group lacked any long-term follow-up.

In 1992, Richter et al published the long term results of a prospective, randomised trial in which balloon angioplasty alone of iliac artery stenoses and occlusions was compared to combined balloon angioplasty and stenting.[222] This represented the first randomised study of this technique. One hundred and eighty-five patients were studied, 93 treated with angioplasty and stenting and 92 with balloon angioplasty alone. The mean post-procedure trans-stenotic pressure gradient for the stented group was 1.8 mmHg compared to 6.7 mmHg for the post-balloon angioplasty alone group. This was highly significant ($p<0.0001$).

Complications were far fewer in the stented group in this study, partly because there were 4 dissections leading to thrombosis in the balloon angioplasty alone randomised group. Looking at the long-term results, for the stented group there appeared to be a so-called "restenosis plateau" seen after 6 months where the degree of luminal narrowing stabilised at about 15% through to 24 months and beyond, whereas in the balloon angioplasty-alone group, there was a gradual increase in the mean percentage of luminal narrowing from about 12% immediately post-procedure, to 22% at 12 months and almost 30% at 24 months. Only 2% of the stented patients had required additional therapy by 36 months, compared to 28% of the balloon angioplasty-alone group.

The study of Richter et al illustrates one of the problems encountered with percutaneous transluminal balloon angioplasty alone, namely angioplasty-induced dissection.[222] Becker et al reported on a series of 12 iliac angioplasties which were complicated by severe intimal dissection.[20] In every case, Palmaz stents were deployed either at the same time or at a subsequent, separate procedure in an attempt to rescue these dissections. An average of three stents were employed for each vessel so-treated. All 12 arteries showed marked improvement angiographically. In eight of the 12 vessels followed-up with angiography at one year, all were patent, with clear neointimal formation, but in only one case was the lumen obviously stenosed. The authors concluded that balloon angioplasty induced intimal dissection could be treated satisfactorily by the deployment of multiple Palmaz stents.

More information on the nature of incorporation into arteries of the Palmaz stent in humans has been published by Bergeron and colleagues[24], who inspected 15 such stents with the angioscope. The stents had been deployed in the iliac, femoral and popliteal arteries. The mean time from stenting to angioscopic inspection was six months, with a range from two to 12 months. In all cases complete stent endothelialisation was observed. This appeared as a uniform smooth white layer with a thickness that appeared proportional to the duration of placement. The authors noted that endothelialisation occurred faster in the femoral and popliteal arteries than in the iliac vessels where partial exposure of the stent struts was noted in some of the 12 month subjects. They also noticed that the proximal end of the stent was

always the last area to become covered with endothelium. They could not distinguish angioscopically the neoendothelium on the stented lumen from the normal arterial endothelium and indeed, they had to confirm their location angiographically in order to arrive at this conclusion. They commented that the maximal thickness of neointima, which they measured at 250 micrometers, was reached by eight weeks and in fact the neointimal thickness appeared to decrease thereafter. They recommended the use of antiplatelet therapy until full endothelialisation had occurred after which this therapy could be discontinued safely There is now an extensive reported experience of the use of the Palmaz balloon-expandable stent in the coronary circulation. This stent has been used in this area both after balloon angioplasty and to salvage inadvertent coronary artery dissection at the time of balloon angioplasty. Schatz et al reported on their early experience in 1991.[233] Delivery of the device after coronary angioplasty was successful in 94% of cases. By three months, 92% of stented patients remained asymptomatic. It was interesting to note that in the two groups of patients compared in this study, those receiving aspirin and dipyridamole were compared to those receiving aspirin, dipyridamole and warfarin. The former group sustained significantly more peri-procedure complications, more subacute arterial closures and more myocardial infarctions. Fischmann et al[104] reported longer follow-up of the United States multicentre trial of Palmaz stent placement after coronary artery balloon angioplasty. Eighty-one per cent of patients treated remained event-free at one year. Patients who had been treated for a recurrent coronary artery stenosis had a less favourable outcome than those being treated for a de novo stenosis. Carrozza et al[44] described a similar experience and found 88% event-free survival at six months and 70% survival after three years, in a group of 220 patients treated with coronary artery Palmaz stent deployment after balloon angioplasty.
Herrmann and colleagues were less optimistic about the longer-term results of the use of stenting to rescue failed percutaneous transluminal coronary artery balloon angioplasties.[127] Although they experienced good initial success rates, subsequent complications related to subacute thrombosis occurred in almost 20% of patients. As such, they concluded that patients who are treated should be monitored very closely and anticoagulated fully. It is

presumed that the relatively high thrombosis rate experienced by most authors with coronary stenting is related in part to the small diameter of the vessel after angioplasty and stenting. Wong and Schatz[296] deduced that there were certain settings in which coronary artery stenting was contraindicated including patients with diffuse disease, poor inflow or outflow, a lesion length greater than 15mm, tortuosity of the proximal vessel and a vessel diameter of less than 2.5mm. Also, in their experience, this therapeutic approach was best avoided in patients who could not tolerate anticoagulation, antiplatelet therapy or who had recently sustained a myocardial infarction. Clearly with such limitations upon the use of stenting in the coronary arteries, a certain amount of caution should be exercised in this field.

Self-expanding stents

A considerable amount of experimental work in the animal laboratory setting preceded the clinical use of self-expanding stents. Duprat et al inserted multiple self-expanding stainless steel stents into arteries of various calibres in a canine model.[96] The stents tested ranged in unconstrained diameter from 3mm to 5mm. Follow-up ranged from 4 to 13 weeks. They found that one month after insertion, the self-expanding stents placed in small arteries were totally covered by the proliferation of neointima. Larger stents placed within the canine aorta were only partially covered with neointima. The authors described a so-called stent:artery ratio (SAR) which was computed using the diameter of the stent when fully expanded and the angiographic diameter of the artery being stented. They found that when the SAR was greater than 1.2, excessive eccentric proliferation of the neointima was observed. As the SAR increased, the expansile force of the stent against the vessel wall increased and this seemed to stimulate excessive neointimal proliferation. The authors postulated that this may result in vascular luminal narrowing. The results showed that in small arteries, the selection of an appropriately-sized stent was a major determinant of subsequent vessel patency. With an SAR of 1 to 1.2, the vessel lumen is maintained and the stent wires become covered with

a thin layer of neointima. If, however, the SAR is greater than 1.2, spasm and thrombosis possibly caused by excessive trauma to the vessel wall may occur as well as excessive proliferation of the neointima. Both of these factors can compromise the vessel lumen over a longer period of time, that is, more than six months. The authors found that stents equal in diameter to the recipient artery were large enough to prevent stent migration, while stent diameters greater than 1.2 times the diameter of the recipient artery were found histologically to "overstretch" the arterial wall.

Further experimental work with the self-expanding stainless steel stent was reported by Rousseau and colleagues[224] who inserted this prosthesis in 28 animals and assessed the performance of the stent from the point of view of thrombogenicity, the tendency to migrate, the "implantation zone" and stent incorporation into the vascular wall. No antiplatelet or anticoagulation therapy was given during the study. Angiographic and histological analysis showed that the prosthesis had a very low thrombogenicity when it was well adapted to the native vessel diameter and that it was incorporated fully into the vessel wall by a neointima by 3 weeks from the time of implantation. No stent migration occurred and branch vessel flow was preserved, even in those vessels in which the ostia were traversed by the prosthesis. These authors noted thrombotic complications when the stent traversed an area of rapid change in vessel diameter, when the end of the prosthesis was engaged in a side branch of a main vessel and when the ratio of unconstrained to implanted diameter was greater than 1.5. This important study by Rousseau et al[224] also described in detail the histological analysis of the stented vessels at various time periods during their follow-up. In the first few hours after implantation, a thin fibrin and platelet layer had formed over the prosthesis with evidence of polymorphonuclear leucocytes localised at the prosthetic filaments. Occasionally, they noticed thrombus adherent to the filaments, but in too small a quantity to influence the vessel's calibre. At 1 week, microscopically, a thin, translucent membrane covered the luminal surface of the stent. At 3 weeks, the stent was seen to be tightly adherent to the internal elastic lamina without actually damaging this structure. The thickened neointima was rich in fibroelastic cells and contained a few haemosiderin-laden macrophages. At this point,

the endoluminal surface was completely covered by endothelial cells. Specimens harvested at six months demonstrated identical findings to those seen 3 weeks. In particular, there was no sign of progression of the thickness of the neointima, which was found to average 250 micrometers in vessels of diameter 5mm. Stents crossing the ostia of branch arterial vessels were studied carefully. Histologically, all branches were patent, with the metal filaments enclosed individually by neointima and without significant reduction of vascular lumen.

The thrombogencitiy of endovascular stents is an important factor in their successful deployment.[203] This particular aspect was studied in a porcine model by Parsson et al.[210] They created an artificial stenosis in the external iliac artery and vein of the pig using catgut ligature. A month later, the iatrogenic stenosis was treated with balloon dilatation and the deployment of a "Wallstent". Stent sizes were selected in such a way that the ratio of stent to vessel did not exceed 1.5. Throughout the study, arteriography demonstrated that all side branches within the stented area continued to be patent as did the main arteries. On the venous side, however, one month after placement of the catgut ligature, three iliac veins demonstrated moderate to severe stenosis and in three animals occlusion of the iliac vein with collateral flow was noted at venography. In these animals a collateral was identified and dilated and stented. All venous stents distended the stenosis completely and no technical problems were encountered. Platelet labelling studies demonstrated an increase of activity over time in the arterial stents but a decrease in activity on the venous side. Complete endothelialisation was noted by 3 weeks and the authors related this to the size of the Wallstent filaments (0.09mm diameter). They postulated that longer endothelialisation times reported for other stent types was due to the larger filament size in these devices. They found that smaller diameter stents developed proportionately greater amounts of neointimal hyperplasia. Platelet adherence was less in stents placed in the venous circulation compared to those in the arterial circulation.

Clinical experience with the self-expanding "Wallstent" has been limited to Europe, since these devices are not licenced for human use in the United States as yet. Zollikofer et al reported a series of 41 patients treated with balloon angioplasty followed by Wallstent

placement for stenotic or occlusive lesions of the iliac, femoral and popliteal arteries.[303] In the iliac artery group, after stent placement, 96% of the vessels were patent at a mean follow-up of 16 months (range 6-30 months). However, in the femoro-popliteal artery group, of 11 patients followed up, only six had patent stented segments at a mean of 20 months (range 7-26 months). The authors concluded that the use of self-expanding stents was an excellent adjunct to balloon angioplasty for complex iliac artery lesions, but that they had grave reservations about the applicability of this technology to the femoro-popliteal segment.

Long and colleagues performed a prospective study of 49 consecutive patients with 53 iliac artery lesions.[169] All were treated with percutaneous transluminal balloon angioplasty and the insertion of a self-expanding stent in 47 cases and a balloon-expandable stent in 6 cases. The primary patency of these iliac arteries after stenting was 94% at 12 months, 66% at 24 months and 44% at 36 months. The secondary patency, after further intervention, was 96% at 12 months, 96% at 24 months and 88% at 36 months. However, it should be noted that in both primary and secondary patency figures the standard error exceeded 10% after only 24 months. The authors noted that neointimal hyperplastic lesions occurred in seven cases (13.5%) and in a further 6% of cases, restenosis occurred at the end of the stent because of incomplete coverage of the original lesion.

The self-expanding stent has also been used in other locations. Goy and colleagues reported on a series of 56 patients treated for coronary artery stenosis with balloon angioplasty followed by Wallstent deployment.[113] Occlusion of the stent was documented in eight patients, the earliest occurring 30 minutes after angioplasty and the latest eight months after implantation. Coronary artery bypass grafting was required in four patients, eight patients suffered myocardial infarction in the territory of the stented vessel, seven patients died within 19 months of implantation and 10% sustained restenosis within the stent itself. At a mean late follow-up a mean of 34 months (range: 24-43 months), 51% of patients were asymptomatic and a total of 78% were in a better functional class than that documented prior to stent implantation. The authors concluded that the implantation of the self-expanding intracoronary stent appeared to be a therapeutic option, but that stent occlusion was an

significant limiting factor and that again small vessel size appeared to be a major problem with the use of the Wallstent. Serruys et al reported similar experience with the Wallstent used in the coronary circulation, 24% of stents inserted occluded and of these 4/5 were in the first 14 days of implantation.[240] Significant restenosis occurred in over a third of stents. These authors were pessimistic about the long term outcome of coronary artery stenting.[241]

Another area of clinical practice which was alluded to earlier is the development of haemodynamically significant stenoses due to neointimal hyperplasia in the venous outflow component of haemodialysis fistulae. Several authors have described their experience using the self-expanding endovascular prosthesis in the treatment of such lesions. Antonucci et al used the "Wallstent" to treat 10 venous stenoses in 7 patients undergoing chronic haemodialysis by way of either a Cimino fistula, a PTFE loop or an arterio-venous xenograft.[8] Four of the 10 venous stenotic lesions were central in either the brachio-cephalic or subclavian veins, the others were more peripherally located in close relation to the fistula itself. In every case, a balloon angioplasty was performed prior to stent deployment, in part to "test the nature of the lesion". In all patients, stenting was immediately successful, one patient sustained an occlusion due to poor compliance with anticoagulation, but this was successfully recanalised percutaneously. All patients were able to recommence haemodialysis immediately after stent placement. Three of the 10 lesions treated in this manner required further intervention within three to nine months and again, these were all achieved percutaneously. The authors were of the opinion that the venous stenosis occurring in arterio-venous fistulae was due to a combination of neointimal hyperplasia and perivenous fibrosis. Because of this, conventional balloon angioplasty tended to have a very poor success rate, with a 12 month patency rate after intervention documented at 45%. They felt that the self-expanding stent might overcome some of the elastic recoil encountered in these fibrotic lesions after conventional balloon angioplasty.

Similar conclusions were reached by Gunther and colleagues who treated a group of patients with occluded PTFE arterio-venous shunts and Cimino fistulae with the surgical removal of clot followed by balloon angioplasty and stent placement.[117] However, their follow-up was

only for a matter of a few months and therefore conclusions about long-term patency could not be reached from their study. Nevertheless, they were of the opinion that stenting certainly had a role to play in the salvage of these fistulae in patients on long term haemodialysis.

Summary

This review has demonstrated that the use of endovascular stents has so far been limited in clinical practice to adjunctive therapy after percutaneous transluminal balloon angioplasty in the treatment of arterial stenoses or as a means of rescuing angioplasty failures or dissections. In the larger calibre, iliac arteries, the results have been reasonably promising in the studies published to date. However, when stents are utilised in smaller calibre vessels such as the superficial femoral, popliteal, renal and coronary arteries, restenosis and thrombosis either subacutely or in the medium-term seem to be a significant problem, with reocclusion occurring in a significant proportion of stented arteries.[226,294] Clearly, in some situations, the absence of inflow and outflow disease is essential, as is careful case selection. The use of stents in lengthy, stenotic lesions in diffusely-diseased vessels does not give as durable results as in isolated, short, proximal stenoses. Further studies with longer follow-up will obviously be required before the use of stenting is accepted as standard practice after balloon angioplasty and as an alternative to standard arterial bypass procedures.

SECTION IV

Endovascular Stenting and the Salvage of the Failing Bypass Graft

Published Data

As well as the description of their use in the salvage of arterio-venous haemodialysis fistulae, there are some anecdotal reports in the literature regarding the use of stents for the salvage of bypass grafts, principally in the coronary circulation.

Stratienko and colleagues used a combination of urokinase infusions followed by Palmaz stent deployment to salvage five occluded saphenous vein coronary artery bypass grafts.[259] A total of 13 stents were used. Angiographic follow-up was available at an average of five months after recanalisation: three of five grafts were widely patent, the remaining two exhibiting greater than 50% luminal stenosis. These restenoses correlated with the clinical recurrence of symptoms. These results were quite encouraging, but were not gained without complications. Four patients suffered groin haematomata and one patient a significant retroperitoneal haematoma.

Strumpf et al[261] used Palmaz stents to treat 26 saphenous vein coronary artery bypass graft stenoses in patients with recurrent angina after coronary bypass grafting. Ninety-two per cent of the stents were implanted without incident. Stent embolisation occurred in two patients and 20% of patients had vascular complications including haematoma, pseudoaneurysm and bleeding from the femoral arterial access site. At a mean follow-up of five months, 14 patients were available for repeat arteriography. Two of these patients developed recurrent ischaemia ascribed to their venous graft from instant restenosis. The clinical recurrence rate was 15%. The authors were cautiously optimistic about the use of stenting for vein graft stenosis, but clearly their follow-up was very limited.

The "Wallstent" has also been used in the field of coronary artery bypass stenosis. De Scheerder et al reported the use of the Wallstent in 91 degenerated vein grafts from a population that was at high surgical risk primarily because of unfavourable coronary artery anatomy.[234] There was an acute stent thrombosis rate of 11% and a myocardial infarction rate of 4.6%. Forty-six patients had an angiogram performed between 3 and 6 months after treatment and the restenosis rate was quite high at 35%.

There are one or two anecdotal reports in the peripheral vascular literature of the use of endovascular stents for vein bypass salvage and for the treatment of neointimal hyperplastic lesions after recanalisation of prosthetic grafts with thrombolytic therapy.[80]

Neville et al have recently described work from their animal laboratory looking at the effects of stenting vein grafts in a sheep model.[190] The theory behind their work was that vein bypasses are prone to develop fibrointimal stenotic lesions. Often the use of balloon angioplasty alone to treat such stenoses does not give satisfactory results because of elastic recoil of the conduit after dilatation. The authors showed that it was feasible to place stents in vein grafts six weeks after they had been placed, with acceptable patency rates up to six months after stent depolyment.

To date there have been no reports in the literature of the use of endovascular stenting as a specific means of prophylaxis against the development of anastomotic neointimal hyperplasia.

ENDOVASCULAR ANASTOMOTIC STENTING AS A MEANS OF LIMITING THE DEVELOPMENT OF ANASTOMOTIC NEOINTIMAL HYPERPLASIA

Hypothesis

In the preceding chapters, I have reviewed the extent of the problem that anastomotic neointimal hyperplasia (ANH) poses in the field of peripheral vascular surgery. For the purposes of this thesis, I have restricted my comments to the incidence of this pathological process in prosthetic infrainguinal bypass grafts and more specifically, to those bypasses fashioned from polytetrafluoroethylene (PTFE). Whilst the various prophylactic interventions aimed at minimising ANH that have been described (namely the use of vein patches and collars, the composite prosthetic-vein bypass and the seeding of grafts with endothelial cells) have shown some promise, both in laboratory experimental models and in some clinical reports, none of these techniques have gained universal acceptance into vascular surgical practice.

From the above brief outline of vascular stents, it can be seen that these devices were developed not only to treat immediate angioplasty failures but also to prevent the recurrence of arterial stenosis due to the development of neointimal hyperplasia after balloon angioplasty. The results so far have been mixed. In the larger arteries of the iliac circulation, the published results for late vessel patency after combining balloon angioplasty with stenting do seem to be superior to those for angioplasty alone. In the smaller calibre, more distal femoral and popliteal vessels, the patency rates have not been as promising. Case selection is important, since with angioplasty alone or combined with stenting, certain lesions are doomed to early restenosis after endovascular therapy, particularly long stenoses and occlusions, those associated with diffuse disease of the vessel and in situations of suboptimal inflow or outflow. These latter statements are also applicable to the use of stents in the coronary circulation. Arterio-venous fistulae for haemodialysis often fail because of neointimal hyperplasia of the

venous outflow that tends to occur a few centimetres distal to the anastomosis. Clinical reports of the use of angioplasty combined with endovascular stent placement have shown some promise. In addition, stents have been used to salvage both coronary artery bypass graft stenoses and anecdotally, vein grafts in the peripheral circulation. No reports exist of the use of stenting as a possible prophylaxis against the development of ANH.

The hypothesis behind the experimental work described in the coming chapters was that, in a canine model of a PTFE graft-to-artery anastomosis, the deployment of a flexible metallic endovascular stent (the "Wallstent") across the distal anastomosis of such a bypass might somehow influence the mechanical events at the anastomosis and thereby modify the site, rate and degree of development of ANH.

Previous Studies on Endovascular Anastomotic Stenting.

The Vascular Surgery Section of the University of Iowa has previously investigated the effects of endovascular anastomotic stenting in a canine model.[297] In this pilot study, an end-to-end arterial anastomosis was performed in a canine model at the level of the femoral arteries. Twelve adult mongrel dogs were studied. Each animal had both femoral arteries below the inguinal ligament surgically exposed. The vessels were divided transversely and then re-anastomosed with a continuous 6/0 polypropylene suture. One anastomosis was selected at random for stenting (using the "Wallstent") so that each animal acted as its own control. Dogs were sacrificed at 3 weekly intervals, 3 dogs at each time. After fixation, the stents were actually removed from the vessels using electron microscopy forceps under the dissecting microscope. Sections were taken at representative levels of stented and control arteries and stained with elastochrome stain. Computer digitization was performed and the luminal, intimal and medial areas were calculated. Ten of the 12 dogs were available for statistical analysis. The stent filaments were incorporated into the vessel wall by 3 weeks and were covered by a thickened neointima lined by a layer of cells that appeared to be vascular

endothelium by light microscopy. The stented areas showed a moderate neointimal hyperplastic response along the entire length of the stented vessel. Similar changes were seen to a lesser extent in sections adjacent to the stent and at the non-stented anastomoses. The area of the lumen at the stented anastomoses was significantly greater than that at the control anastomoses at all time intervals ($p<0.0001$). However, the intimal area at the stented anastomoses was significantly greater than at the non-stented anastomoses ($p<0.0001$). When different sections were compared within the stented arteries, luminal area at the anastomoses was significantly greater than at sections 0.5cm or more remote from the stent at all time intervals ($p<0.02$). Also, the intimal area at the anastomoses was significantly greater than at sections 0.2cm or more away from the stent ($p<0.0003$). This study also demonstrated that there were no differences in the arterial media when comparing stented to non-stented arteries, no matter what time interval of the study was analysed. From these results, it would seem that although the stents invoked a significant neointimal hyperplastic response, the effect of this accumulation on the luminal area was offset by the apparently "dilating" effect of the stent. The stents appeared to be well incorporated into the vessel wall and were endothelialised by three weeks. Of particular interest was that the distending effect of the stent at the anastomotic level seemed to overcome the luminal narrowing caused by the continuous anastomsotic suture which was seen in the non-stented controls.

The next logical step in the investigation of endovascular anastomotic stenting was to observe what the effect this technique would have on the neointimal hyperplastic response in an animal model of a graft-to-artery vascular anastomosis.

Study Design

Adult conditioned mongrel dogs of either sex weighing 20-25 kilogrammes were used for these experiments. The animals were maintained in accordance with the guidelines in "Principles of Laboratory Animal Care" and "Guide for the Care and Use of Laboratory Animals" (National Institute of Health Publication Number 80-23, revised 1985).

The canine model was selected partly because of its prior successful use in the pilot study of arterial stenting. Also, the ISCVS/SVS Committee on Standards for assessing new graft materials in experimental animals[2] recommended the dog as the experimental animal of choice and although we planned to use an accepted prosthetic graft material, that is, polytetrafluoroethylene (PTFE), the technique of graft-arterial endovascular anastomotic stenting had not been reported previously.

We decided to establish two separate experiments in order to assess the two commonly used distal anastomotic configurations: namely, the end-to-end and the end-to-side configurations. Much of the study design was identical for both experimental models, but the technical details and anatomy of each operative procedure and the analysis of the results will be considered separately.

Based upon the results of previously reported laboratory and clinical work with stents, we divided each group of animals into two separate sub-groups according to the planned interval between graft implantation and graft harvest. Previous studies, including the work previously reported from our own laboratory, have shown that full stent endothelial coverage is complete in the canine model three weeks after stent deployment. Furthermore, by two to three months, the neointimal hyperplasia associated with the stents seems to become stable and non-progressive. For these reasons, we chose to sacrifice half of each group of dogs at four weeks (the so-called "early" group) and the remaining half at 12 weeks (the "late" group).

After consultation with our colleagues in the Department of Biological Statistics at the University of Iowa, it was decided that the minimum number of dogs necessary for any

statistically significant conclusions to be reached from these experiments required the allocation of 3 dogs to each time interval in both models. Thus a total of 12 animals were used in the entire study.

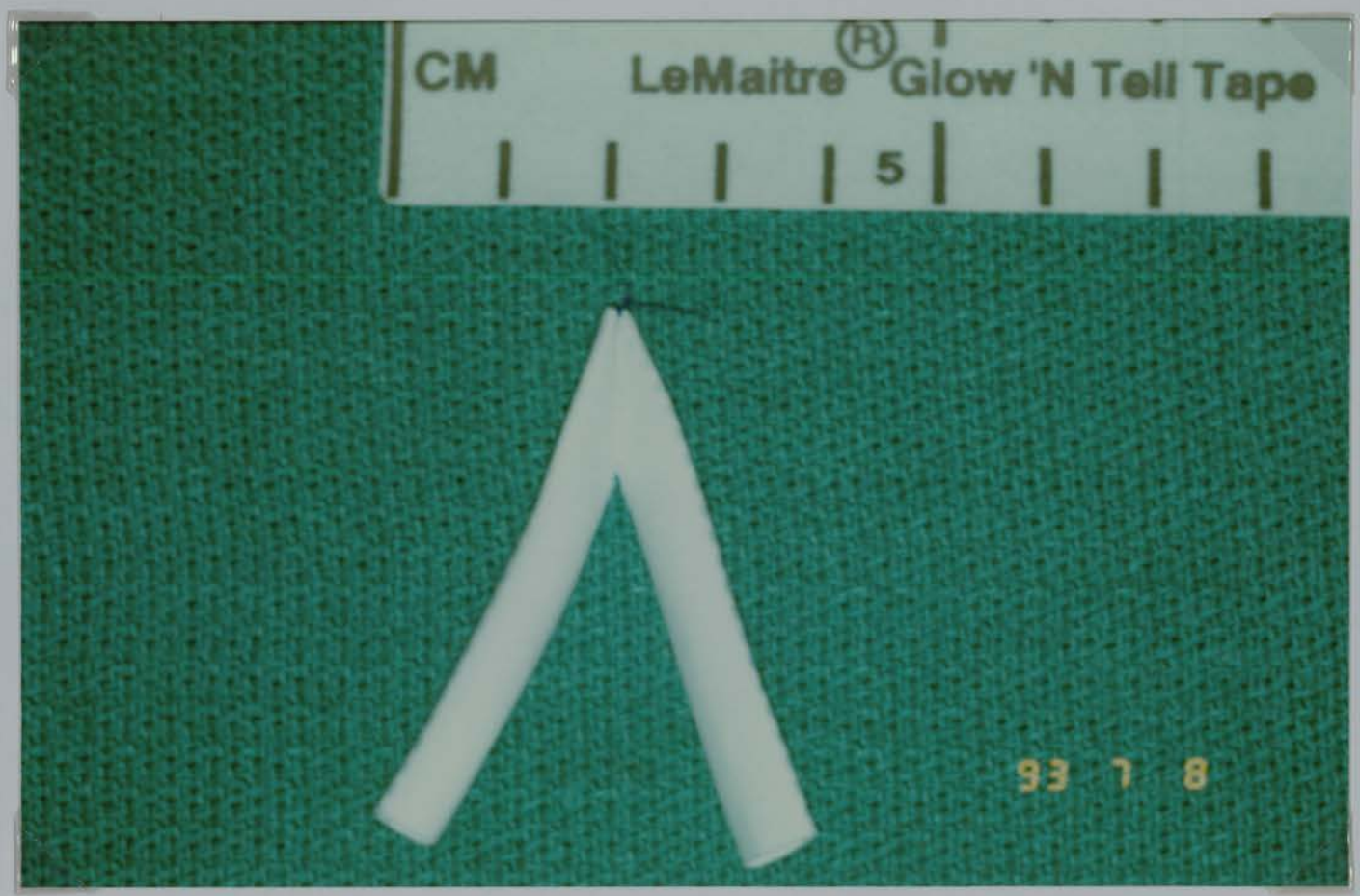


Figure 6: A hand-sewn PTFE bifurcated graft as used in every experimental animal.

Materials and Methods

Grafts

In both models, a bifurcated graft was placed in the aorto-iliac segment of the canine arterial circulation. The grafts were composed of two lengths of 6mm external diameter, thin-walled expanded polytetrafluoroethylene (PTFE, "Goretex", W.L. Gore, Flagstaff, Arizona) with a pore diameter of 30 micrometres, which were anastomosed proximally using a continous 6/0 polypropylene suture (Ethicon). Thus, a bifurcated graft was created with each limb of the graft measuring 6cm in length. All grafts used in both models were hand-sewn by me and were as identical to each other as was possible (Figure 6). Details of the distal graft limb configuration will be described in detail in the sections relating to each of the distal anastomotic models.

Stents

For this experiment, the stent selected for use was the "Wallstent" (Schneider Stent Corporation, St Paul, MN). As described above, these devices are made from approximately 16 fine stainless steel interwoven filaments, each filament having a diameter of 0.09mm. These self-expanding stents are manufactured upon a 7 French guage delivery catheter. The stent is held in its unexpanded state within a constraining membrane. The delivery catheter has a side arm half way along its length, through which saline or other fluid can be injected to lubricate the membrane in preparation for deployment. The proximal end of the device has a metal plunger that is advanced up the catheter at the time of stent release. This leads to the retraction of the membrane and the expansion of the stent (Figure 7). The inherent design of this delivery system allows spontaneous expansion of

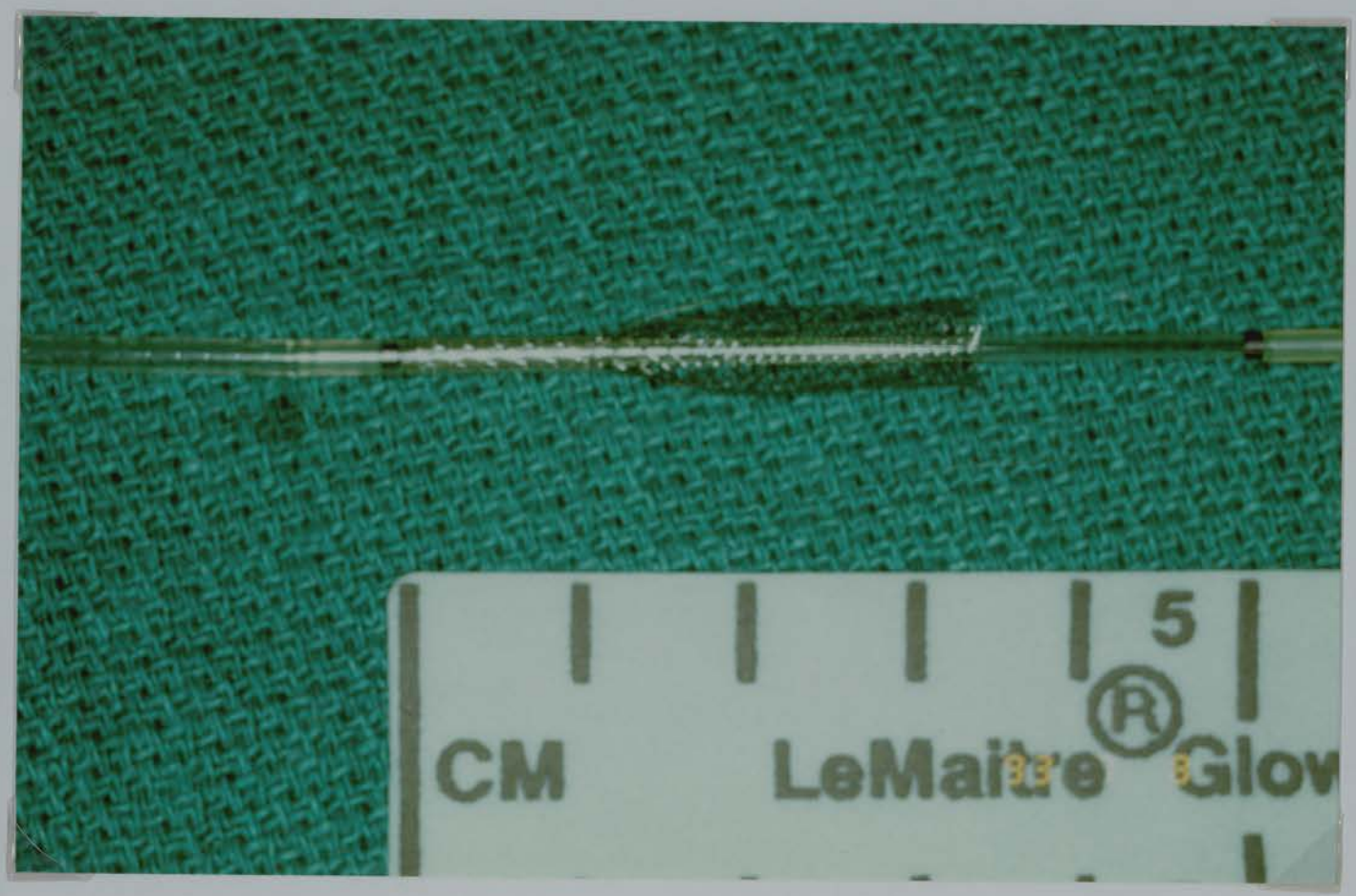


Figure 7: At deployment, the constraining membrane retracts allowing the Wallstent to expand fully.

the stent without the need for balloon expansion. As we wanted to avoid any form of inadvertent "angioplasty" or over-distension of the recipient vessel, the self-expanding Wallstent was the most suitable device for this experiment. Also, as it can flex in any direction without loss of luminal diameter, the Wallstent was the ideal choice for stent placement across an end-to-side anastomosis (Figure 8).

Anastomotic Sutures

For the graft construction, a 6/0 polypropylene suture was used. In all cases, the distal anastomoses were sewn using a continuous 6/0 polypropylene suture. We adopted a parachute technique and used 2.5X loupe magnification at all times. The proximal aortic anastomosis in both models was of the "onlay", end-to-side configuration and was sewn with a continuous 5/0 polypropylene suture in every case.

Anaesthesia

All animals were fasted for 12 hours prior to the operative procedure. An 18g intravenous cannula was inserted into a superficial forelimb vein. The dogs were pre-anaesthetised with pentobarbitol sodium at a dose of 15mg/kg. They then underwent endotracheal intubation and anaesthesia was maintained with a mixture of 1% halothane and room air administered via a Harvard respirator. No muscle relaxants were utilised.

Circulating volume was maintained with intravenous isotonic saline. In most cases, 500-1000 mls were administered during the operative procedure.

At the end of the procedure, 100% oxygen was administered and once the animals had a gag reflex and were rousable, the endotracheal tube was removed. Post-operative

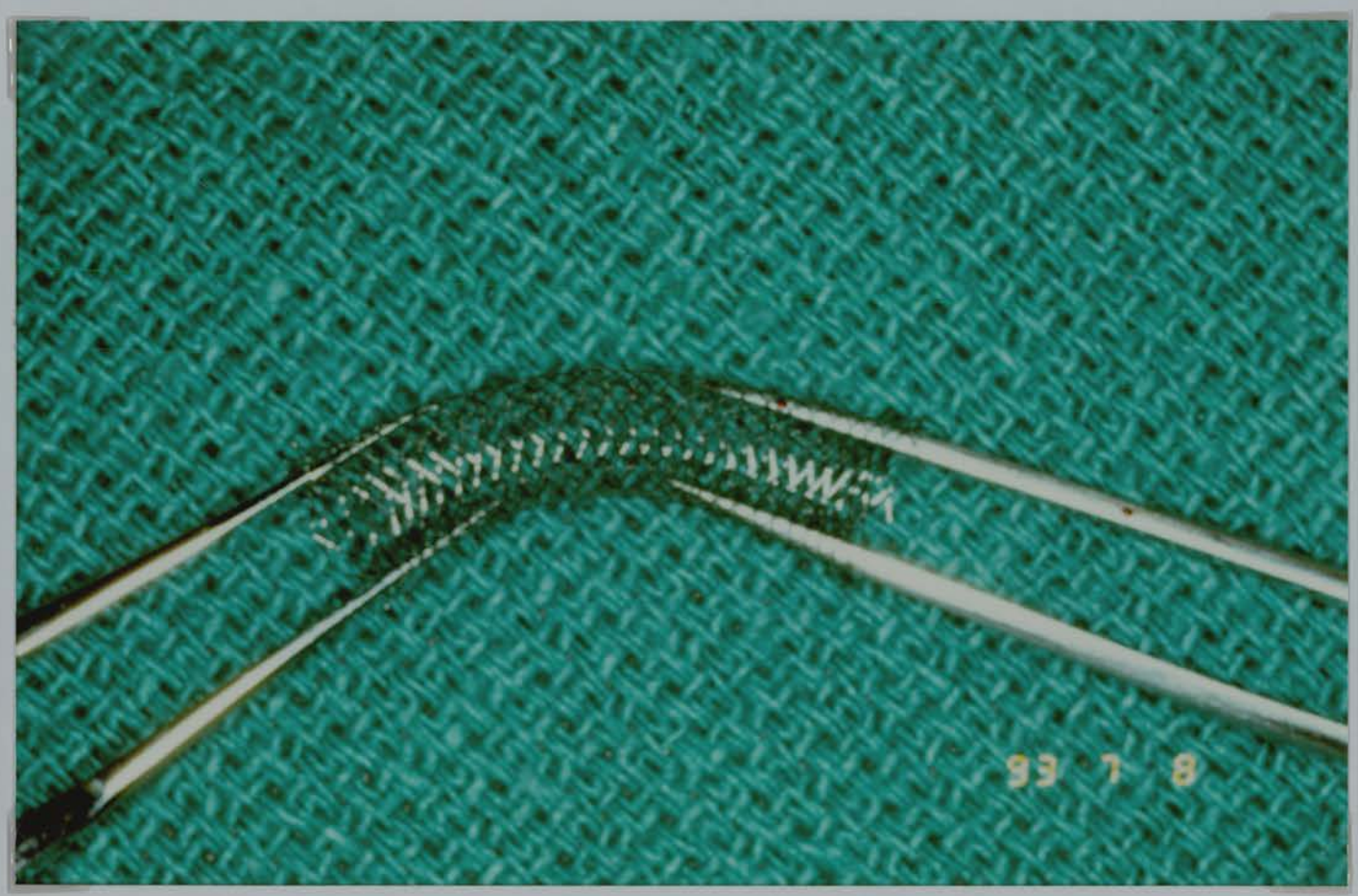


Figure 8: The Wallstent is flexible in all directions without loss of luminal diameter.

monitoring consisted of hourly pulse and respiration recording until the dog was fully ambulant. This usually took between 3 and 5 hours.

Other Drugs Administered

Antibiotic prophylaxis was given at the induction of anaesthesia and consisted of 3mls of a solution of penicillin benzathine 150,000 units/ml, procaine penicillin 150,000 units/ml and sodium formaldehyde 1.75mg/ml given by intramuscular injection.

Five minutes before the application of vascular clamps, intravenous heparin was administered at a dose of 50 units/kg. Heparinisation was not reversed.

Intra-operative angiography was performed with Conray 280 contast medium. A total of 20-30 mls of contrast were administered to each animal.

Heparinised isotonic saline was used to flush graft limbs during the operative procedure. The solution was made up by adding 1000 units of sodium heparin to 500 mls of isotonic saline.

Post-operatively, no anticoagulant or antiplatelet therapy was administered.

Operative Procedure

Exposure of Vessels

The method of vessel exposure and dissection was identical for both the end-to-end and end-to-side models.

The abdominal aorta and iliac arteries were exposed through a midline incision. The small intestine was packed away in the right upper quadrant and the sigmoid colon retracted laterally with a self-retaining retractor.

Using a caliper, the diameter of the iliac arteries was measured prior to any surgical handling, so that the diameter of stent utilised would correspond to the normal physiological diameter of the iliac artery. The vessels were then dissected in preparation for grafting.

The canine aorta ends in a trifurcation: there are two external iliac arteries and a common internal iliac artery. The external iliac artery is approximately 10 cm in length and divides into a profunda femoris and superficial femoral branch at the level of the inguinal ligament. The internal iliac artery divides in the pelvis into paired hypogastric arteries and a median sacral artery. The aorta itself has paired lumbar branches as in the human and the arteria intestinalis caudalis (inferior mesenteric artery) originates from its anterior surface 2-3 cm proximal to the aortic trifurcation.

Both external iliac arteries were dissected from their origin to a point just distal to the profunda/superficial femoral bifurcation so that control of the profunda and superficial femoral arteries could be achieved with number 1 silk ties used as vessel slings. The distal aorta was then dissected and the lowermost lumbar arteries were divided between 2/0 silk ligatures. The inferior mesenteric artery was dissected free and carefully preserved. The internal iliac artery was left undisturbed.

With complete vascular control, systemic, intravenous heparin was administered at a dose of 50 units/kg body weight. Five minutes after this, the aorta was clamped using a "side-biting"

clamp, placed from below, the jaws being directed under the external iliac arteries and enclosing the internal iliac artery origin. Microvascular, atraumatic "Yasergill" clamps were applied to the distal-most aspect of the external iliac arteries, allowing access to the entire length of this vessel on both sides. Next, the side for stenting was selected by drawing labelled cards from an envelope.

End-to-end Model

The side chosen for stenting had the distal anastomosis sewn first. The distal graft limbs for the end-to-end model were left cut transversely with no taper or fluting. The external iliac artery was then doubly-ligated at its origin with number 1 silk ties. When this vessel was divided, it retracted distally but it was elected routinely to excise a portion of this vessel such that the graft could be anastomosed without any significant graft kinking. Care was taken not to excise an excessive length of artery to ensure that the anastomosis was not under excessive stretch or strain. The length of vessel excised was approximately 3 cm in every case. Handling the arteries led to an inevitable degree of spasm and for this model it was decided to routinely open longitudinally the anterior surface of the distal portion of the external iliac artery for a length of 3 mm. The anastomosis was then sewn using a continuous 6/0 polypropylene suture with a parachute technique under 2.5X loupe magnification. When the anastomosis was complete, the sutures were left loose initially to allow deployment of the stent under direct vision.

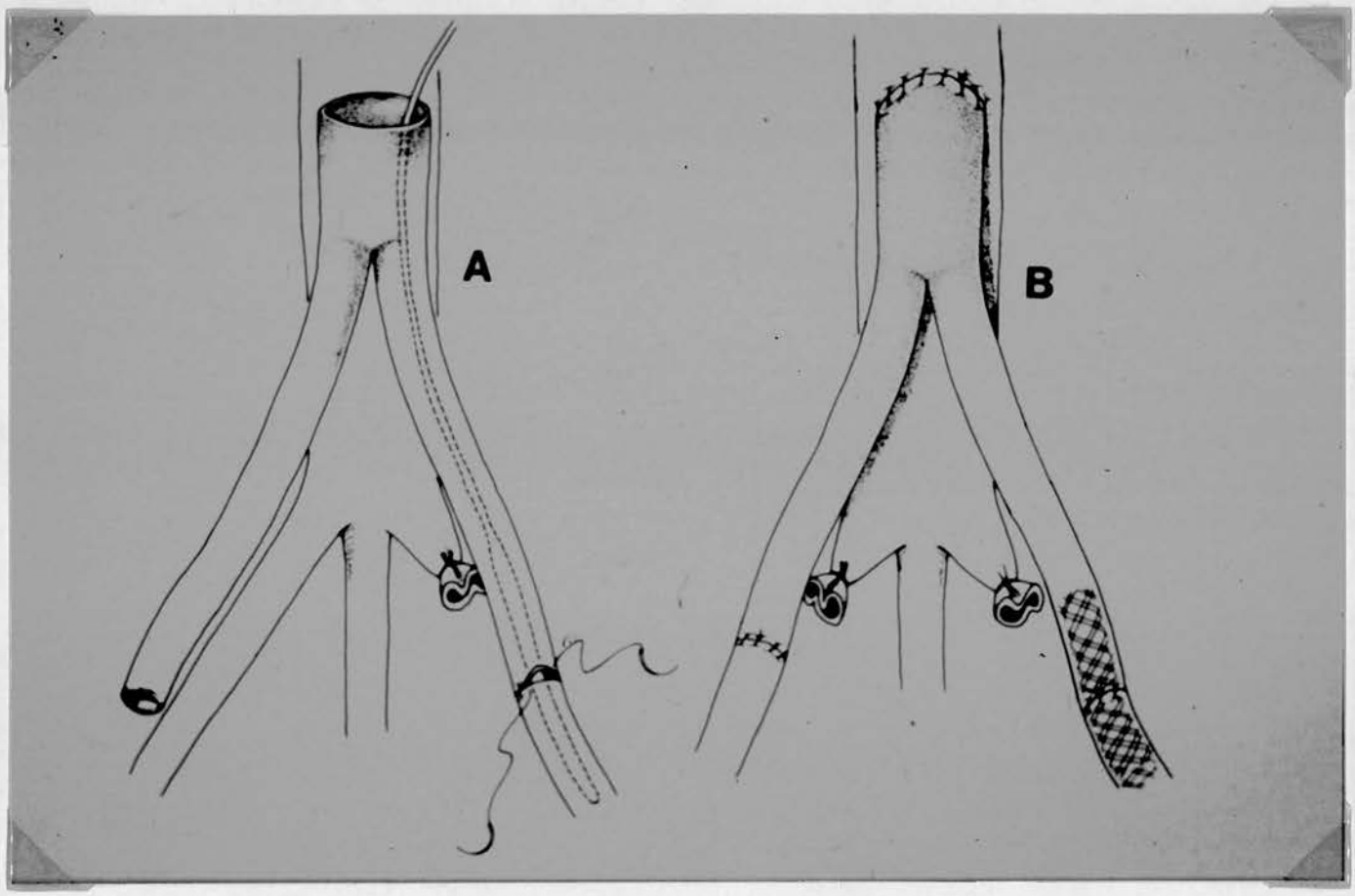


Figure 9a: **End-to-end model.**
The stent delivery device is seen placed across the anastomosis selected for stenting.

Figure 9b: Diagrammatic representation of the completed graft showing the stent deployed evenly across one distal anastomosis.

Stent Deployment

In all cases, after vessel diameter measurement prior to surgical handling, a stent of unconstrained internal diameter of 5 mm was utilised. Prior to stent deployment, normal saline was injected via the side port of the delivery device in order to lubricate the constraining membrane thoroughly. When adequate saline had been injected, fluid was seen to emerge from the tiny holes at the distal tip of the catheter. The device was introduced through the proximal end of the bifurcation graft and positioned equally across the anastomosis (Figure 9a). While one operator maintained control of the stent's position (by visualising the device through the untied anastomotic suture line) the other advanced the metal plunger, thereby retracting the membrane and allowing the stent to expand. When fully expanded, 1 cm of the stent lay within the native artery and 1 cm lay in the distal graft (Figure 9b). The delivery device was then simply removed via the proximal graft. The graft limb and the clamped segment of external iliac artery were then irrigated with heparinised saline solution and the anastomotic suture pulled tight and tied down. The clamps were left on with the segment isolated from the circulation while the other anastomoses were fashioned.

Attention was then turned to the opposite iliac anastomosis. This was performed in identical fashion, using a continuous 6/0 polypropylene suture with a parachute technique and again under 2.5x loupe magnification. Prior to tying the anastomotic suture, this segment was also flushed out with heparinised saline.

Finally, the proximal, aortic anastomosis was fashioned. A 1.5cm longitudinal arteriotomy was performed just to the right of the midline on the distal aorta, carefully avoiding the inferior mesenteric artery. The anastomosis was performed with a continuous 5/0 polypropylene suture. Before tying the suture and prior to the restoration of distal blood flow, both graft limbs and the proximal internal iliac artery were clamped and the aorta flushed to guarantee that there was no thrombus present. Then with the aortic anastomosis

Figure 10: **End-to-end Model.**
Operative photograph showing both distal anastomoses.

secured, the distal clamps were released one at a time and flow restored to the lower extremities.(Figure 10)

End-to-side model

As in the end-to-end model, the side selected at random for stenting was sewn first. Both distal graft limbs were cut to a standardised angle of 45 degrees. The external iliac artery was clamped proximally at its origin and distally at its bifurcation using microvascular, atraumatic Yasergill clamps. In the mid-portion of the external iliac artery, at a point where the graft would lie without either kinking or excess tension, a 10 mm longitudinal arteriotomy was performed. Again, using a continuous 6/0 polypropylene suture, the anastomosis was fashioned under 2.5x loupe magnification with a parachute technique. When the anastomosis was completed, the suture was left loose in readiness for stent placement.

Stent Deployment

Once again, after lubrication of the constraining membrane with isotonic saline, the stent delivery device was introduced via the proximal end of the bifurcation graft and positioned across the anastomosis (Figure 11a). The stent was deployed under direct vision through the loose anastomotic suture, such that 1 cm lay within the native artery and 1 cm lay within the PTFE graft (Figure 11b). After the stent delivery catheter had been carefully withdrawn from the graft, the stented graft limb was flushed out, via the proximal graft, with heparinised saline. At this point, the anastomotic suture was tightened and tied down securely. The clamps were left applied so that this segment of graft and artery was isolated from the circulation and remained completely free of blood whilst the other anastomoses were completed.

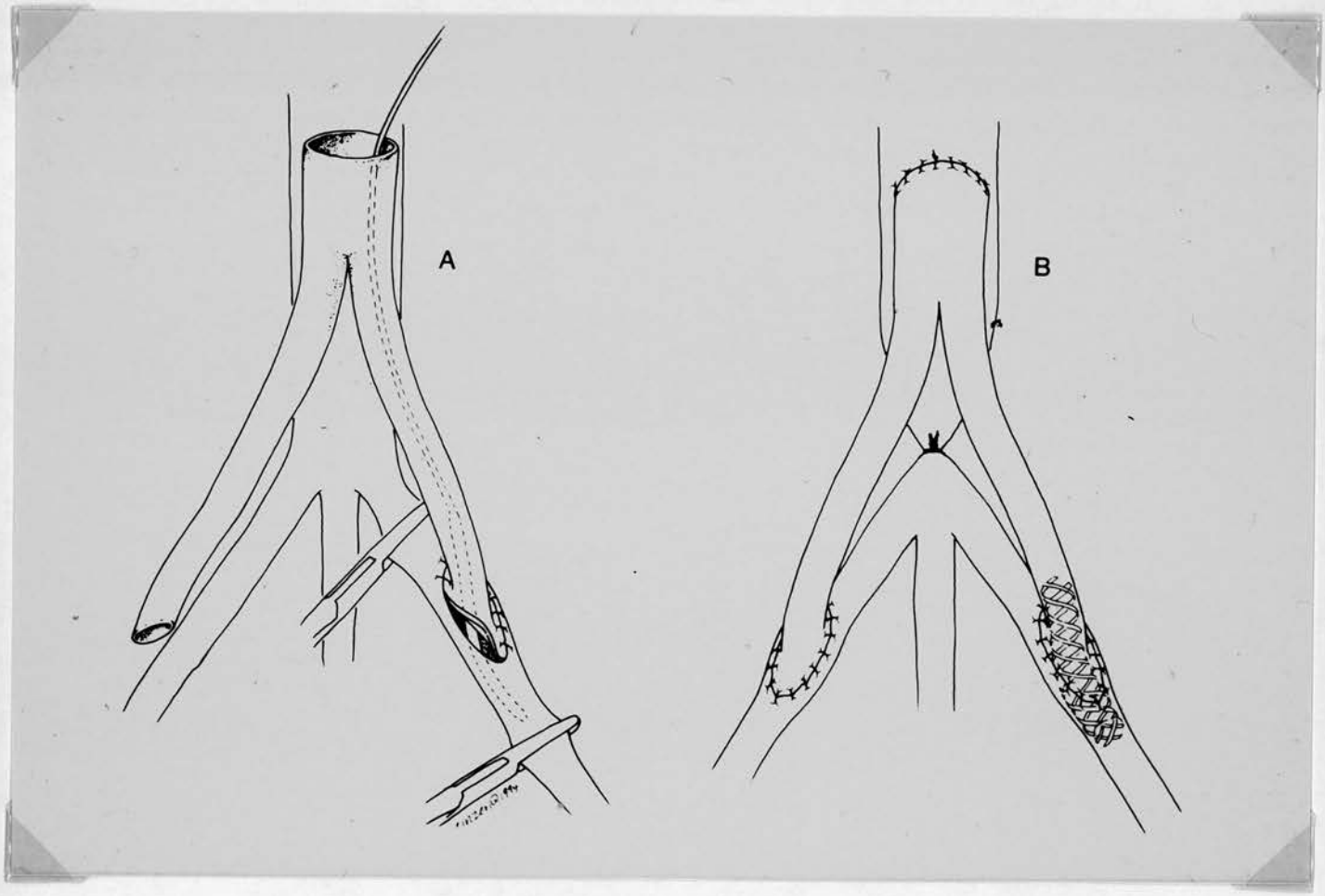


Figure 11a: **End-to-side Model**
The stent delivery device was positioned accurately across the anastomosis under direct vision.

Figure 11b: Diagram shows completed graft.

Next, the opposite distal anastomosis was performed in identical fashion. The iliac arteriotomy, which was of a standard length for all anastomoses was performed at an identical level on the contralateral external iliac artery. Prior to tightening and tying the suture, again the graft limb and iliac segment were thoroughly flushed out with heparinised saline.

Finally, the aortic anastomosis was fashioned. In the end-to-side model, we wanted to maintain retrograde perfusion from the distal anastomoses, since in clinical practice, this is one of the main advantages of this anastomotic configuration. The canine anatomy leant itself to this end in that by leaving the internal iliac artery intact and by ligating the aorta just distal to the aortic anastomosis, retrograde flow from both distal anastomoses could perfuse the internal iliac system. We were especially interested to observe the effects of such flow on the stented graft limbs in terms of endothelialisation of the stent where it crossed the proximal outflow artery. Therefore, prior to performing the aortic anastomosis, the distal-most portion of the aorta was slung with two number one silk ligatures in preparation for its ligation after the anastomosis had been completed.

The aortic anastomosis was then performed in an identical manner to that for the end-to-end model, although it was sited a little more proximally on the aorta to allow ligation distally, without compromising the internal iliac artery origin and its direct communication with both external iliac arteries. The inferior mesenteric artery was again preserved by locating the aortic anastomosis slightly to the right of the midline. The aortic anastomosis was sewn with a continuous 5/0 polypropylene suture and before tying the suture, it was thoroughly flushed out and then flushed with heparinised saline to guarantee that there was no thrombus present prior to releasing the clamps and restoring the distal circulation. The distal aorta was ligated with the previously sited silk ligatures immediately prior to release of the clamps (Figure 12).

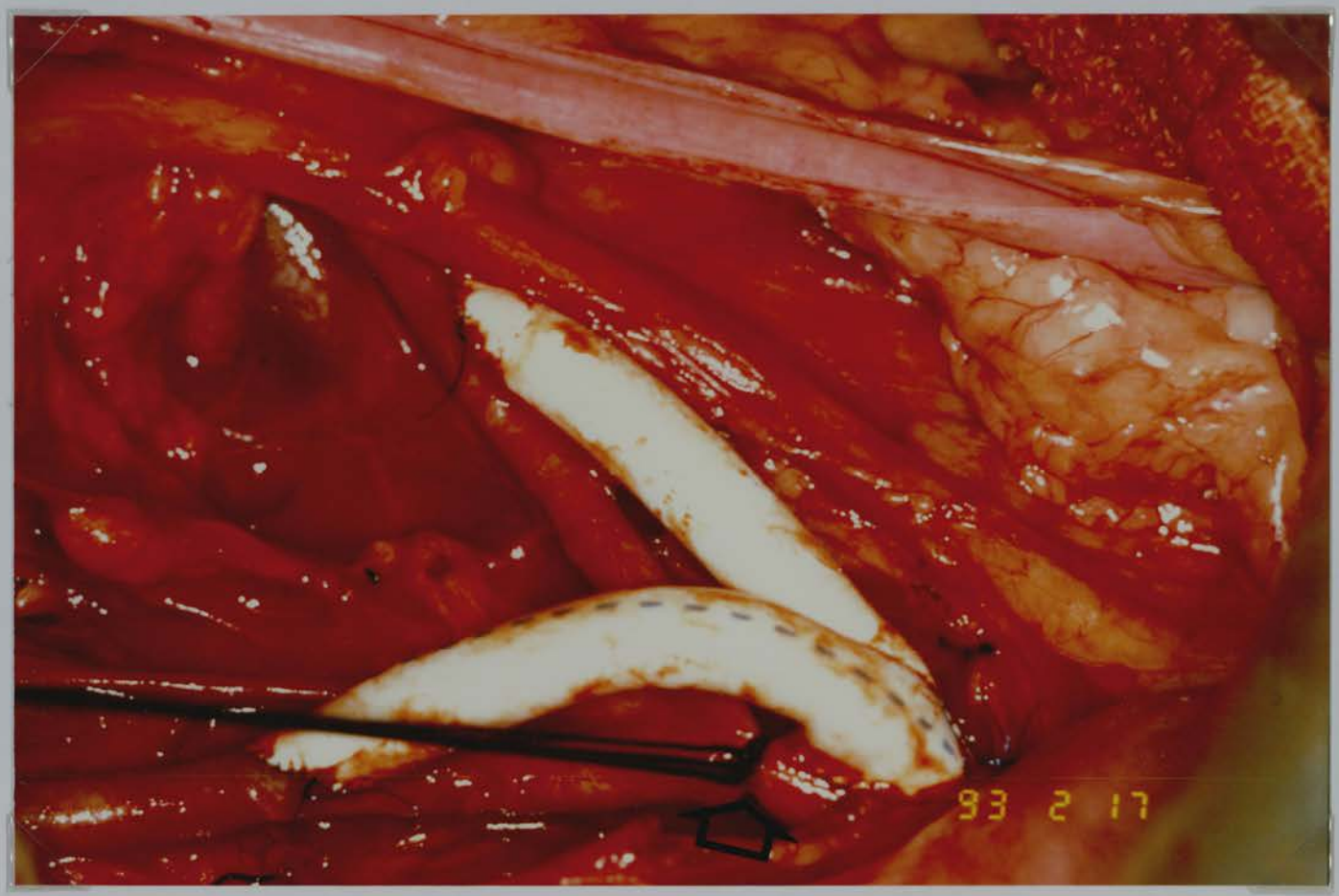


Figure 12: **End-to-side Model.**
Operative photograph showing the completed graft. The aorta has been ligated distal to the proximal anastomosis of the graft (arrow).

Completion Angiography

After being flushed with heparinised saline, a 20 G plastic intravenous cannula attached to a 60cm length of extension tubing was inserted into the proximal graft hood in preparation for completion angiography. A plain abdominal radiograph of the abdomen was taken to confirm the location and side of placement of the stent. Under fluoroscopic control, and without graft inflow occlusion, 10mls of Conray 280 contrast was injected into the graft and patency of the bypass and run-off arteries confirmed. Thereafter, a further 10-20 mls of contrast were injected and a hard-copy angiogram was obtained of the aorta, graft and femoral and more distal arteries. When a satisfactory image was obtained, the cannula was flushed with heparinised saline and then removed and the graft puncture closed with a simple stitch of 6/0 polypropylene.

Wound Closure

After guaranteeing haemostasis, the posterior peritoneum was closed over the graft with a continuous 3/0 vicryl suture. The small intestine was replaced in anatomical orientation, as was the sigmoid colon, and the greater omentum was draped over the gut to act as a barrier between the wound and the intestine in standard fashion.

The abdomen was closed en masse using continuous 0 vicryl. The panniculus carnosus, a discrete layer in the canine, was closed with continuous 3/0 vicryl. The skin was closed with subcuticular 3/0 vicryl.

Post-operative Care

At the completion of the operative procedure, the dogs were administered 100% oxygen via the endotracheal tube until they had a gag reflex and a flexion response to tactile stimuli. At this point, under close observation, the endotracheal tube was removed and the animals were transferred from the operating room to a recovery area. Here, they were monitored until they were able to stand on all four limbs without support.

For the first 24 hours after the surgery, they were observed in the Med Labs kennels where during working hours they were cared for by a qualified veterinary nurse. Out of hours, they were monitored by myself.

Without exception, by the first post-operative day, all animals were on normal diet, were fully mobile and were fit for transfer to the kennels at the Oakdale Facility. At this location they received care from licensed dog handlers and were monitored by myself on a regular basis.

Monitoring Graft Patency

Graft patency was monitored as follows:

1) **Femoral pulse palpation**

   Daily for the first week, then weekly until the time of sacrifice.

2) **Transcutaneous continuous wave Doppler**

   Daily for the first week, weekly thereafter.

3) **Mid-term angiography.**

Mid-term Angiography

For animals in the "early group", the mid-term angiograms were performed two weeks after graft insertion, whereas for those in the "late" group, the mid-term angiograms were performed at six weeks from the time of the original operative procedure. The dogs were transferred back to the University Med Labs facility the day before this procedure and fasted for 12 hours.

A forearm vein was cannulated and the dogs were anaesthetised with intravenous sodium pentothal, at a dose of 15mg/kg. No other drugs were administered during this procedure. The animals were allowed to breathe room air.

Under sterile conditions, the left common carotid artery was exposed through a short longitudinal left cervical incision. After gaining adequate control of this vessel and slinging it with two number 1 silk ligatures, a 5 mm longitudinal arteriotomy was performed and a standard angio-catheter was introduced and passed into the vessel. Under fluoroscopic guidance, the catheter was passed into the aortic arch and then steered into the descending

thoracic aorta and thence, into the proximal abdominal aorta. A test injection of 10mls of Conray 280 was performed and fluoroscopically, the patency of the graft and run-off arteries was confirmed. A second injection of 10-20 mls of contrast was then performed and a hard-copy angiogram was obtained. Also, a plain radiograph was taken to document stent location and to help with the recording of stent migration, if this had occurred. The catheter was flushed with heparinised saline and then removed. The common carotid artery was then ligated above and below the level of the arteriotomy with the two number 1 silk ligatures that had been placed earlier. Haemostasis was ensured after which the wound was closed with subcuticular 3/0 vicryl to the skin. The animals generally recovered fully from this procedure within two to three hours and were simply observed for the rest of that day before being transferred back to the kennels the following day and monitoring was continued as described above.

Sacrifice and Specimen Retrieval

At the appointed time, (either four or 12 weeks after graft placement) the dogs were returned to the laboratory operating room for sacrifice and graft harvest. Intravenous sodium pentothal (15mg/kg) was administered via a forelimb vein cannula. No other anaesthetic agents were required. The animals breathed room air.

The abdomen was entered through the previous midline incision.

The retroperitoneum was dissected and the distal aorta, iliac arteries and the graft were exposed, with care not to handle the graft directly, if at all possible. At this stage it was noted that while the dissection was quite straightforward on the control side, on the stented side, the surrounding tissues were found to be adherent to the stented region with numerous lymph nodes visible within the adherent fibrous tissue. Because of this, on the stented side no clearly discernible dissection plane existed between the graft and surrounding tissues and therefore, extra care had to tbe taken to guarantee that the vessel wall and graft were not

inadvertently damaged. When this region had been dissected free, including circumferential dissection of the distal aorta, which required the ligation of the lower three lumbar arteries bilaterally, the aorta was exposed proximally as far as the diaphragm. The distal external iliac arteries were also dissected free as was the internal iliac artery, guaranteeing complete control of the arterial tree at this level.

Via a proximal aortic puncture with a 20G cannula and extension tubing (as before) a further arteriogram was obtained to confirm graft and run-off vessel patency, using 20 mls of Conray 280 contrast.

At this stage the dogs were humanely euthenised using a lethal intravenous injection of 20 mls of concentrated potassium chloride solution.

Next, the vessels and graft were prepared for pressure-perfusion fixation.

The supra-coeliac aorta was cross clamped and divided. The proximal aortic stump was ligated and the distal stump was cannulated with an intravenous giving set, which was tied securely into place, guaranteeing a water-tight seal. At first, the aorta, graft and run-off arteries were thoroughly flushed with isotonic saline to ensure that all blood had been cleared from the system. Then, the saline was replaced with the fixative fluid. This consisted of 2% gluteraldehyde in 0.1 molar solution of sodium cacodylate buffer at a pH of 7.2. When 20-30 mls of this solution had been rapidly infused, one superficial femoral artery, both profunda femoris arteries and the internal iliac artery (2 cm from its origin) were all ligated with number 1 silk. Thus, one superficial femoral artery was left open and a further 10 mls of fixative fluid were injected to make sure that the system was clear of any other fluid or blood. At this stage the remaining superficial femoral artery was ligated. The injection apparatus was connected, by way of a "three-way tap" to a manometer. A further volume of the buffered 2% gluteraldehyde solution was injected until pressure of 90 mm Hg was reached. Then the "three-way tap" was closed off allowing the specimen to be fixed at a pressure of 90 mm Hg. Care was taken to make certain that no leaks existed in the system and the pressure was re-checked every 15 minutes throughout the pressure-perfusion fixation period.

After a one hour period of in situ pressure-perfusion fixation, the ligated arteries were divided distally and the specimen removed from the abdomen, the cannula still being in situ at this stage. The entire specimen was then immersed completely in a glass vat containing the same cacodylate-buffered 2% gluteraldehyde solution. At this stage, without suddenly decompressing the fixed graft, the cannula was removed from the proximal aorta and the graft and artery block was left for a further 24 hours of immersion fixation in a refridgerator at a temperature of 4 degrees centigrade.

Sections

In every case, transverse sections were taken at identical levels from both the stented graft limb and the non-stented, control graft limb. The reference point was the anastomotic suture line in all cases, so that as close to identical levels were sectioned from each side. We used an electron microscopy microtome to cut the sections. These devices, which are extremely sharp, were able to transect the stent, which was embedded in the fixed specimens to such a degree, that no significant disruption of the vessel architecture occurred during sectioning.

End-to-end Configuration

A total of five transverse sections was taken from each side of every animal in this experimental group. Bearing in mind that the stents were 2 cm in length when deployed, the sections were taken at 1 cm intervals starting 2 cm distal to the anastomosis (Section 1), then 1 cm distal to the anastomotic suture line, a level that corresponded to the distal margin of the stent/native artery interface (Section 2), a section from the graft-artery anastomotic level, that is from the middle of the stent (Section 3), a further section 1 cm proximal to the anastomosis, at the proximal stent/graft interface (Section 4) and a fifth section from the graft

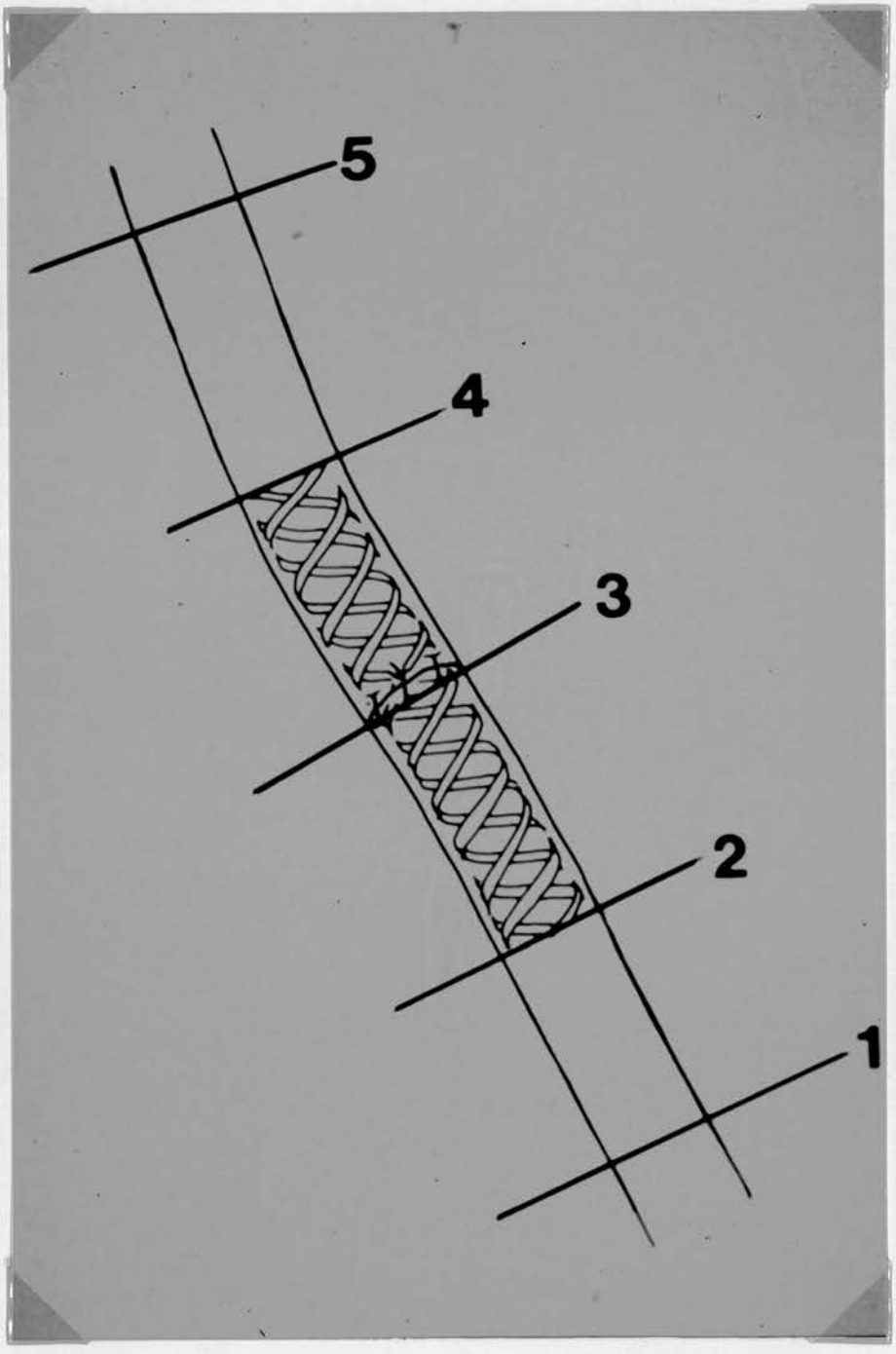


Figure 13: **End-to-end Model.** Sections taken from each graft limb.

1 cm proximal to the proximal margin of the stent, or 2 cm proximal to the anastomosis (Section 5) (Figure 13). Each section was given a five digit identification number to help with data processing later. The ten sections from each dog were mounted in a separate storage box on individual electron microscopy mounts to aid with subsequent imaging and analysis.

End-to-side Configuration

Six sections were taken from each graft limb in every animal in this group. Section 1 was from the run-off artery, 2 cm distal to the anastomotic suture line, 1 cm distal to the stent. Section 2 was taken from the run-off artery at its interface with the distal margin of the stent or a corresponding level on the control side. Section 3 was a section through the graft-artery anastomosis itself. Section 4 was taken from the proximal stent/graft interface level, 1 cm proximal to the anastomotic suture line. Section 5 was taken 1 cm more proximal into the graft, that is 2 cm proximal to the suture line. Finally, Section 6 was taken from the run-off artery 1 cm proximal to the distal anastomosis, an area of interest in this study, was retrograde flow was maintained in this model of end-to-side distal anastomosis (Figure 14). Thus, each animal had twelve sections taken. Again these were individually numbered and stored in a separate container for each dog on electron microscopy mounts.

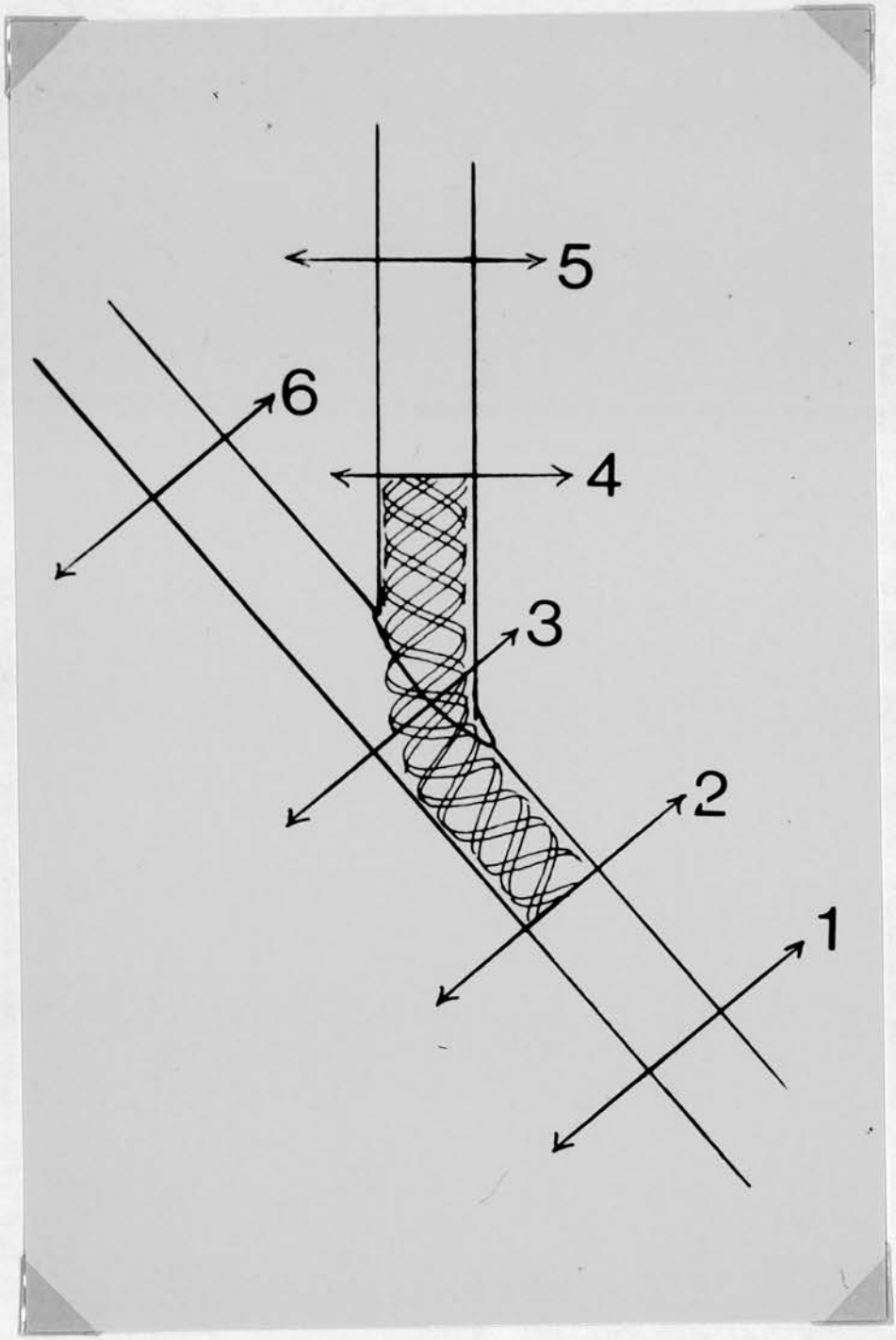


Figure 14: **End-to-side Model.** Sections taken from each graft limb.

Preparation of Sections for Analysis

The methodology used for the preparation and analysis of sections obtained from both the end-to-end and the end-to-side anastomotic configurations was the same, although the sections taken differed slightly between the two experiments.

We wished to glean as much information on the intimal and luminal changes associated with anastomotic stenting. At the same time, if possible, we wanted to study the morphology of the graft and artery on both the stented and the non-stented (control) sides. It was decided not to use light microscopy, as had been the case in the previous pilot study. This decision was partly due to concern over intimal disruption during stent filament extraction, and the very laborious and time-consuming nature of this particular procedure. However, we also wished to examine the sections taken with scanning electron microscopy to see if we could learn more about the cellular events and the nature of luminal coverage in the stented grafts. Therefore, with the help of colleagues in the Central Electron Microscopy Research Facility at the University of Iowa, the sectioned arteries and grafts were prepared for analysis as follows.

After pressure-perfusion fixation for one hour and immersion fixation for 24 hours as described above, specimens were rinsed in 0.1 M sodium cacodylate buffer three times, twenty minutes for each rinse and then osmicated (1% OsO4 in 0.1 M sodium cacodylate pH 7.2 for one hour). Three buffer rinses were then performed before stepwise dehydration in ethanol. Once the ethanol concentration was 100%, it was replaced with hexamethyldisilizane and allowed to air dry. Once dried, specimens were mounted on Cambridge style stubs and sputter coated in an Emitech K-550 with gold/palladium. The specimens were imaged on an Hitachi S-4000 scanning electron microscope.

Analysis of Sections.

From each of the standard sections taken from every graft limb, the mean luminal area and the mean intimal thickness were calculated.

Computer images of each section were created using a MegaPlus camera (Eastman Kodak Company, San Diego, California). Using a programme called "grabber", the computer images were transferred from the camera onto a Macintosh Workstation (Apple Inc., Cupertino, California). The digital images were then transferred to an Indigo Workstation for analysis. Objects in the digital images were traced using "Mtrace" software on a Silicone graphics Indigo workstation (Silicon Graphics Inc., Mountain View, California). "Mtrace" was developed by Randall J. Frank at the University of Iowa Image Analysis facility. Using a computer mouse, this programme allows the user to define the edge of contours or so-called regions of interest (ROI) thereby outlining objects in the image. This programme was ideal for tracing the lumen of each section. When this tracing was completed, the computer froze the tracing on the screen in green, while I traced the next outline, the outer aspect of the graft neointima/artery intima. The boundary between the intima and the vessel media or the graft material was very clearly seen in all cases. This tracing meant that between the two tracings, the intima of the section was contained. The intima was rarely of uniform thickness. Where it appeared to be extremely thin, it was possible to "zoom" in on the area of interest up to a magnification of 500% and thus it was possible to trace the outlines extremely accurately.

For sections of the native artery (Sections 1 and 2 in both models, and Section 6 in the end-to-side model), a third outline was traced on the outer aspect of the tunica media, but in some cases, it was not possible to distinguish with certainty the boundary of the media with the adventitia.

The "mtrace" programme is able to measure the area inside each contour traced and this was used to measure the residual luminal area of every section that was examined. Similarly, it was possible to measure the area of the tunica media for the sections that involved a portion of native artery. In order to measure accurately the mean intimal thickness for each section, I

utilised another computer software package that was at my disposal. This second programme, "Wall thickness", also developed by Randall J. Frank, was designed to radially sample two concentric contours (traced as described above) and measure the difference in radius at each angle. This difference was defined as the wall thickness at that particular angle. Subtracting radius 1 from radius 2 gave the intimal thickness at each radian. A mean intimal thickness was thus calculated for each section by measuring the wall thickness at each radian (giving 360 values for each section) and calculating the mean value. This methodology ensured that the lack of true uniformity of intimal thickness around the circumference of each section did not skew the intimal thickness data.

All data obtained from the Image analysis facility were then down-loaded onto a Macintosh computer and stored as database files using the programme "Microsoft Excel" 4.0. Separate directories were created for the end-to-end and end-to-side models and these were subdivided into files for early and late specimens.

Statistical Analysis

The intimal thickness and luminal area data were analyzed using univariate repeated measures of analysis of variance (ANOVA). This analysis involved three factors: a "between animal" factor, GROUP (early vs. late), and two "within animal" factors, STENT (stent vs. no stent) and SECTION. Comparisons of interest were examined using one degree of freedom mean contrasts and then tested with the t-test statistic. The standard errors used in computing the t-test statistic for these contrasts were estimated from the between and within animal mean square errors. A separate analysis was performed for the data from the end-to-end and end-to-side models. The standard error estimates were based on the pooled estimate of variability obtained from the analysis of variance.

RESULTS

1) Morbidity

There were no significant intra-operative or immediate post-operative complications.

There were no wound-related complications.

There appeared to be no adverse reactions to the intra-operative angiograms. In addition, there were no complications related to the mid-term angiograms.

There was no evidence of graft infection, either clinically or at the time of animal sacrifice and graft harvest.

One animal, which had been allocated to the "Early" group of the end-to-side model, died at between two and three weeks from causes unrelated to the operation or graft (there was a dog fight at the kennel). An autopsy was performed and the graft was found to be intact and on opening the aorta and graft, both graft limbs were patent. This animal was obviously omitted from any subsequent analysis.

2) Patency

a) End-to-end Model

One dog in the "early" group had a patent graft on the mid-term angiogram and clinically, prior to harvest, both limbs of the graft were patent. However, at the time of sacrifice, both graft limbs were found to be occluded. It was decided to open this specimen. On doing so, it was apparent to the naked eye that on the stented side, there was an accumulation of neointimal hyperplasia at the proximal stent-graft interface. On the control side a similar lesion was found located at the anastomotic suture line between the graft and the native

artery. In one animal in the "late" group, the control side was occluded at the time of sacrifice and again neointimal hyperplasia was seen at the suture line extending proximally into the graft for a few millimetres. As the grafts had been patent on mid-term angiograms and by femoral pulse palpation and insonation right up to the week prior to sacrifice, it was assumed that the occlusions had occurred shortly before sacrifice. However, as noted on the pre-sacrifice angiograms, the canine clearly has the capacity to establish excellent collateral flow by way of the profunda femoris and internal iliac arteries. This makes clinical and even Doppler assessment of patency difficult. Thus it can only be hypothesised that these occlusions occurred between the mid-term angiogram and sacrifice.

	Patent	**Occluded**
Control	4	2
Stent	5	1

Table 10: Patency of Graft Limbs in the End-to-end Distal Anastomotic Model

From the above table, it can be seen that the overall graft limb patency rate was 75% (9/12) and that there was no significant difference between control and stented graft limbs. The occluded graft limbs were excluded from statistical analysis.

b) End-to-side Model

The angiograms performed before the dogs were sacrificed showed that two graft limbs had occluded between the time of the mid-term angiogram and sacrifice. These occlusions

occurred in one stented and one control graft limb in different animals both in the "late" group. In both cases, the run-off artery was patent from the level of the distal anastomosis distally. This was presumably because retrograde flow from the contralateral distal anastomosis was able to traverse the internal iliac artery origin at the aortic trifurcation and thereby perfuse the vessels on the side of the graft limb occlusion. The two occluded graft limbs were excluded from statistical analysis.

	Patent	Occluded
Control	4	1
Stent	4	1

Table 11: Patency of Graft Limbs in the End-to-side Distal Anastomotic Model

The overall graft limb patency was 80% (8/10) and again there was no difference between the controls and stented sides.

3) Electron Microscopy

From the tissue that remained after sections were taken for the morphometric analysis, longitudinal sections were cut and prepared for inspection with the scanning electron microscope. These studies showed that in all cases, by four weeks after implantation ("early" group) the luminal surface of all stents in both the end-to-end and the end-to-side groups was completely covered with a confluent monolayer of cells orientated with their long axis parallel to the direction of blood flow (Figure 15,16).

Figure 15: Scanning Electron Micrograph (x50) shows complete coverage of the luminal surface of the stent.

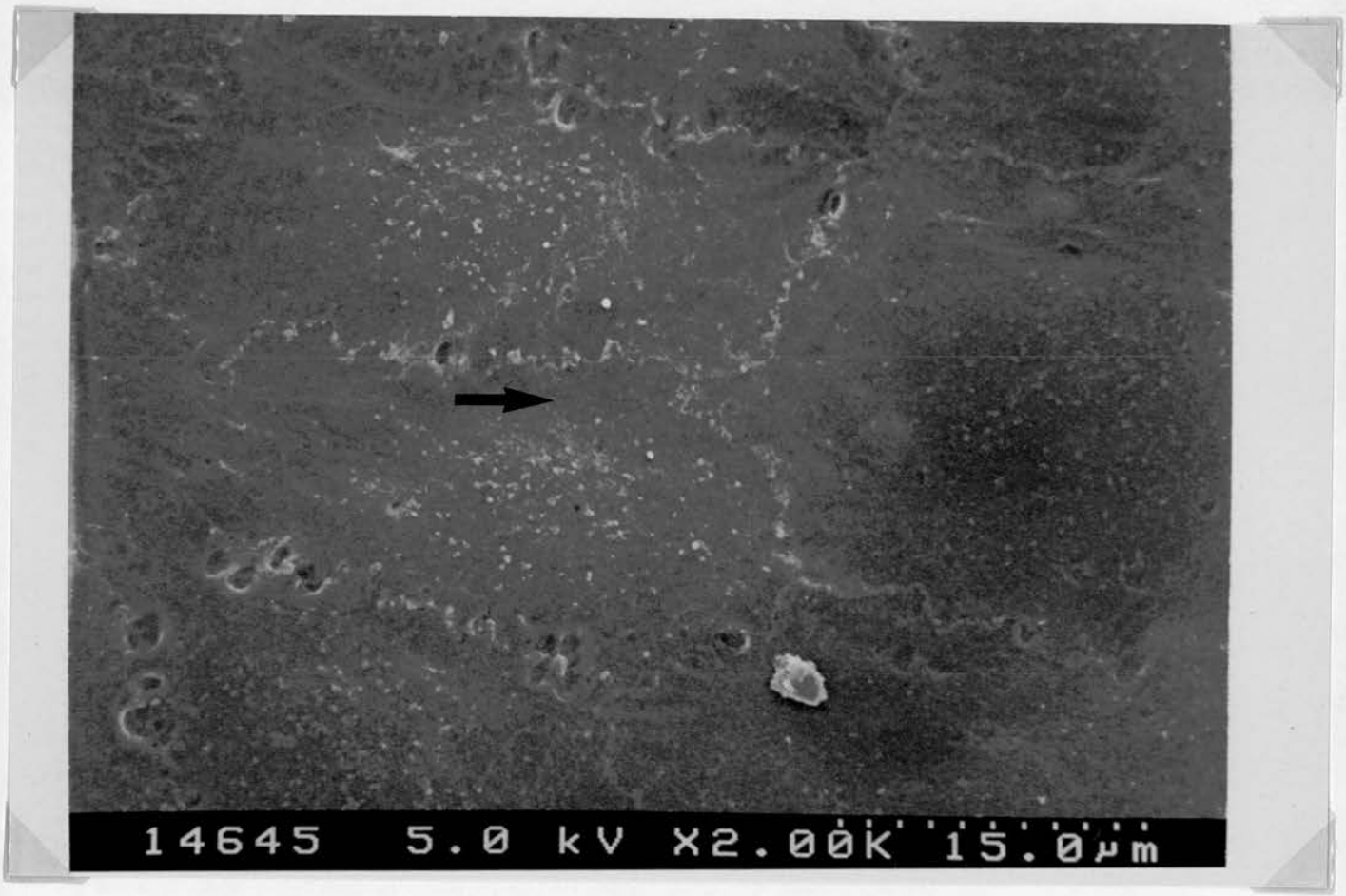


Figure 16: Scanning Electron Micrograph (x2.00K) shows the luminal surface of the stent to be covered by a layer of contiguous cells with their longitudinal axis parallel to the direction of blood flow (arrow).

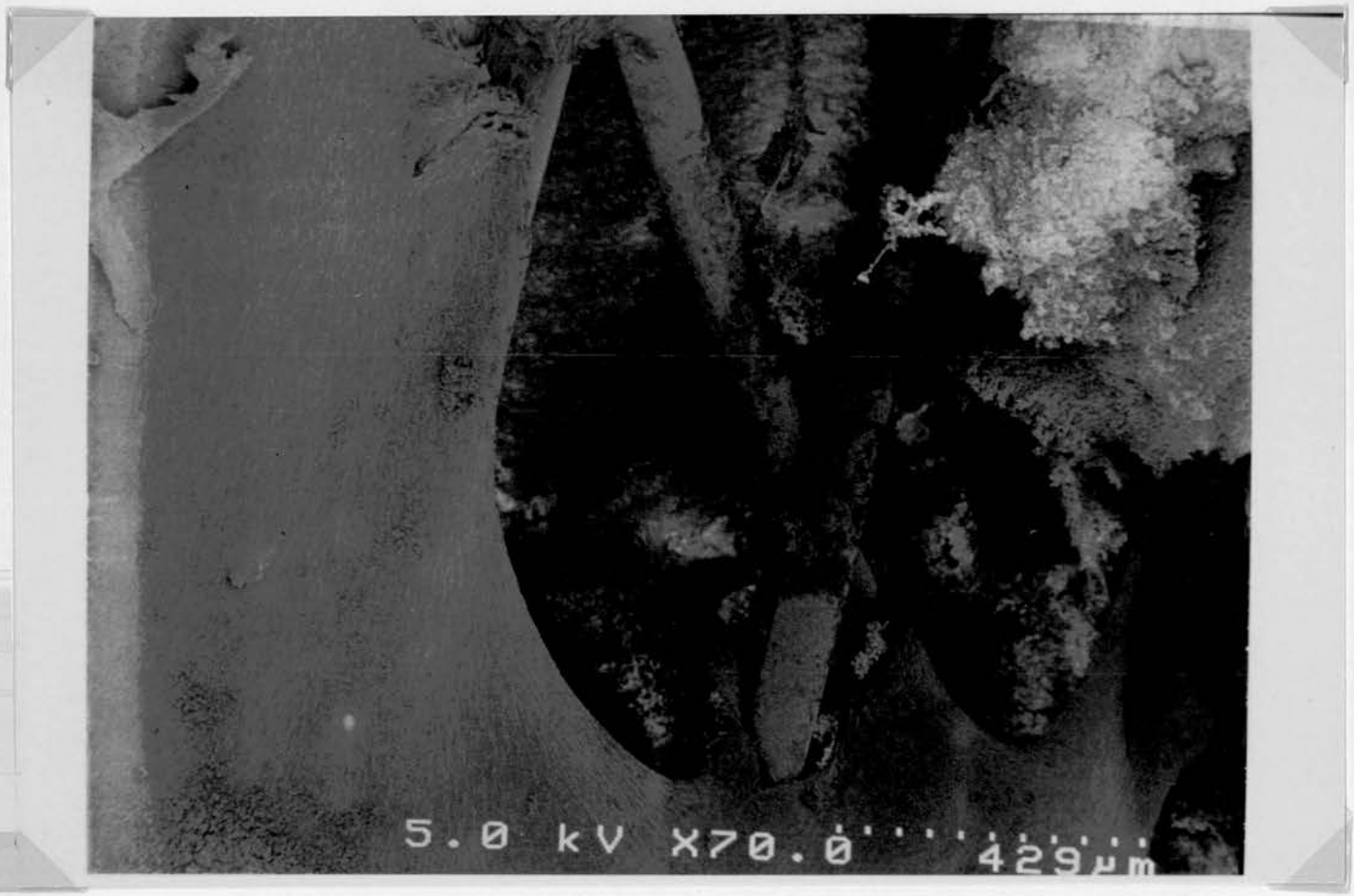


Figure 17: Scanning Electron Micrograph (x70) shows no cellular coverage of the stent filaments where the stent crossed the outflow artery at the distal end-to-side anastomosis.

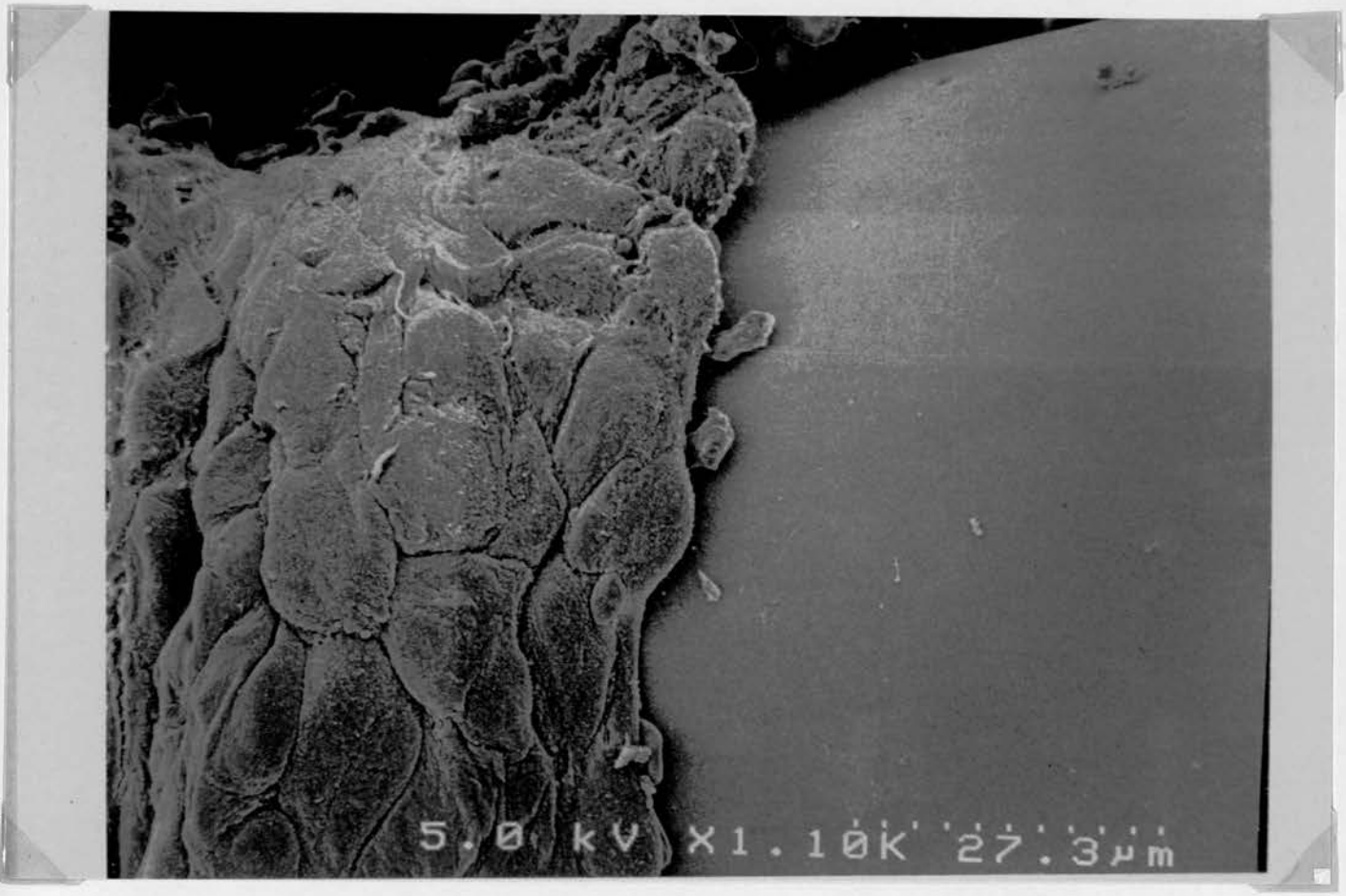


Figure 18: Scanning Electron Micrograph (x1.10 K) shows an abrupt end to the cellular coverage where the stent filaments crossed the outflow artery in the end-to-side model.

In the end-to-side model, we were especially interested in the area where the stent crossed the outflow artery at the distal anastomotic level. Without exception, at this level, the stent was not covered by the confluent cellular monolayer and the stainless steel stent filaments were seen exposed (Figure 17). Further micrographs at higher power showed very clearly the edge of the cell layer as a discrete zone of demarcation, presumably indicating that the endothelial cells did not encroach across blood flowing through the vessel at this level (Figure 18).

As cell marker studies were not performed, it is not possible to state categorically the nature of the cells that formed the endoluminal coverage of all stents. However, based upon a high index of suspicion, as well as the high power electron microscope appearances of these cells as well as data from other workers' studies with the Wallstent, one can state with reasonable certaintity that the cells seen in both models were vascular endothelial cells. If this is the case, it seems that by stenting a graft-artery anastomosis, complete endothelial coverage of the conduit can be achieved.

4) Angiography

Examples of the completion angiograms obtained for the end-to-end and the end-to-side models are shown in Figures 19 and 20. It can be seen that by uniplanar angiography, the stented anastomoses did appear to be more tapered and streamlined than the non-stented controls in both models of distal anastomotic configuration.

The quality of the mid-term, fluoroscopic angiograms was adequate to ensure graft and run-off artery patency but no reproducible data regarding vessel lumen and wall thickening could be obtained with the equipment available and images obtained.

The angiograms performed immediately prior to sacrifice were performed primarily to confirm patency. It was possible to document neointimal thickening since the residual lumen of the vessel as outlined by the contrast was seen to lie within the stent in some cases.

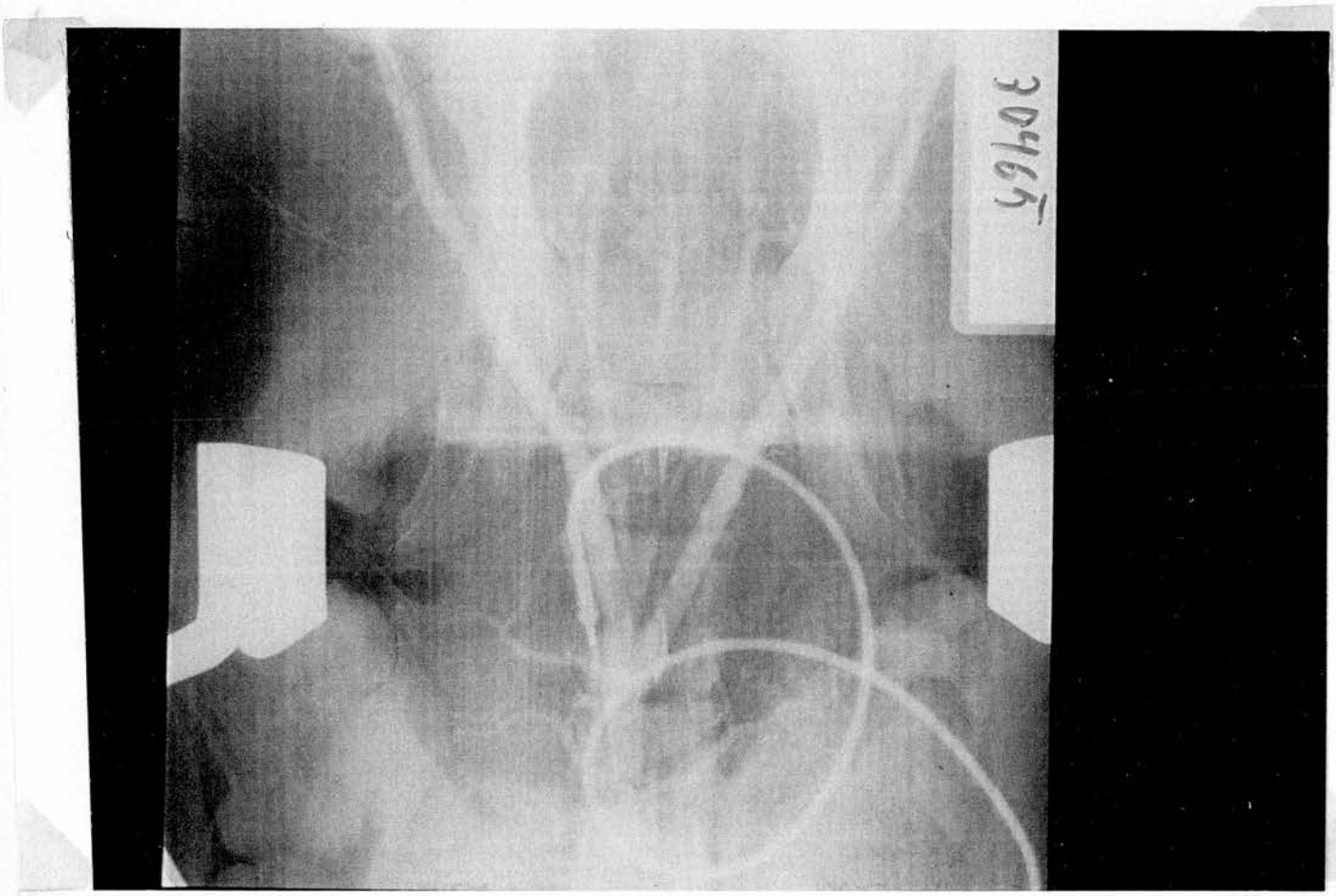


Figure 19: **End-to-end Model:** Completion Angiogram.

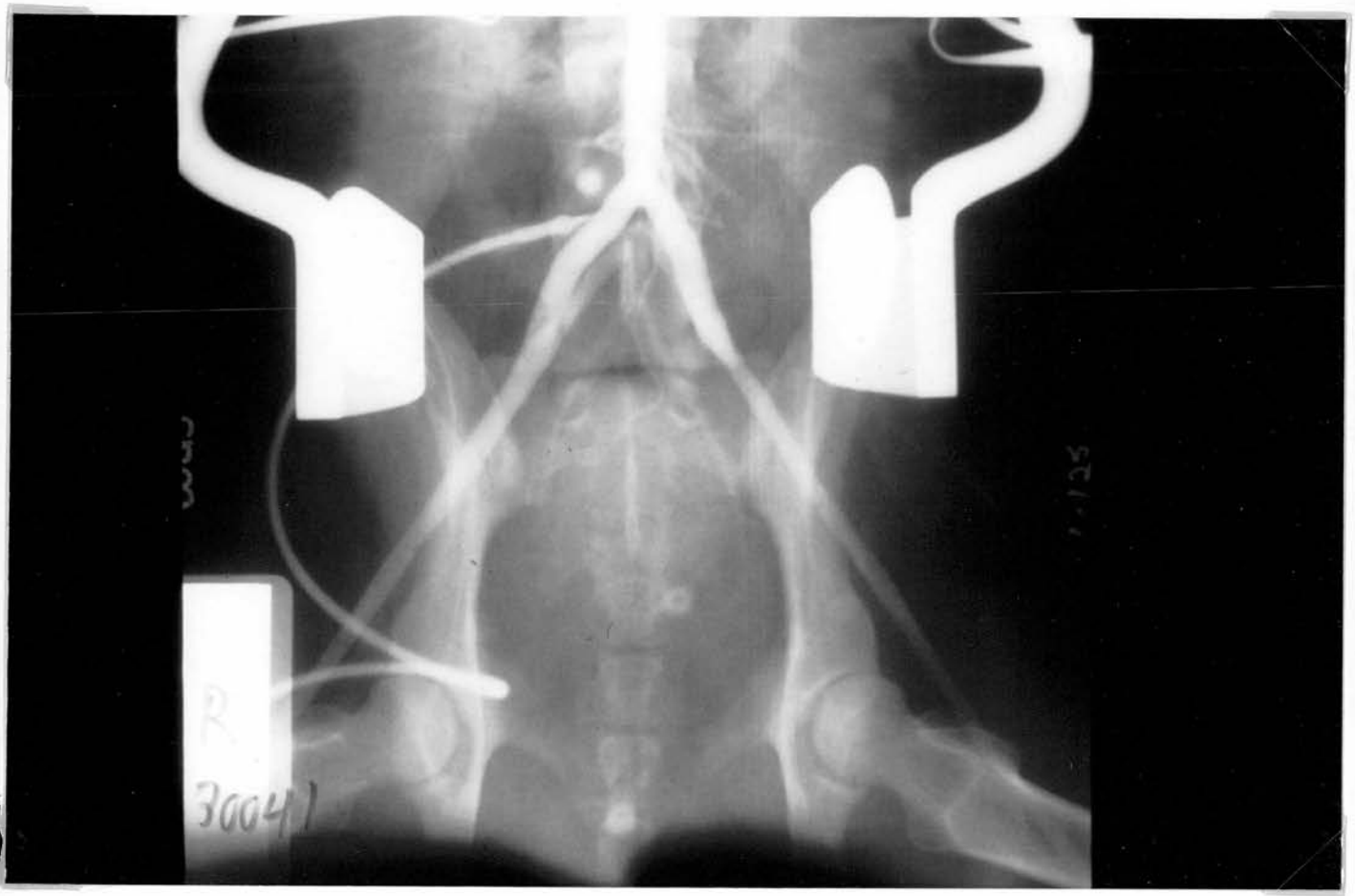


Figure 20: **End-to-side Model:** Completion Angiogram.

In both of the end-to-side model graft limbs that occluded, one stented and one control, the proximal outflow artery and the run-off artery distal to the anastomosis were patent and as mentioned above this can be seen angiographically to be due to flow from the contralateral graft limb (Figure 21). Thus the presence of a stent across an end-to-side anastomosis did not impair blood flow in either direction. In addition, as seen by s.e.m., this uni- or bi-directional flow was associated with an absence of cell coverage of the stent filaments at this level.

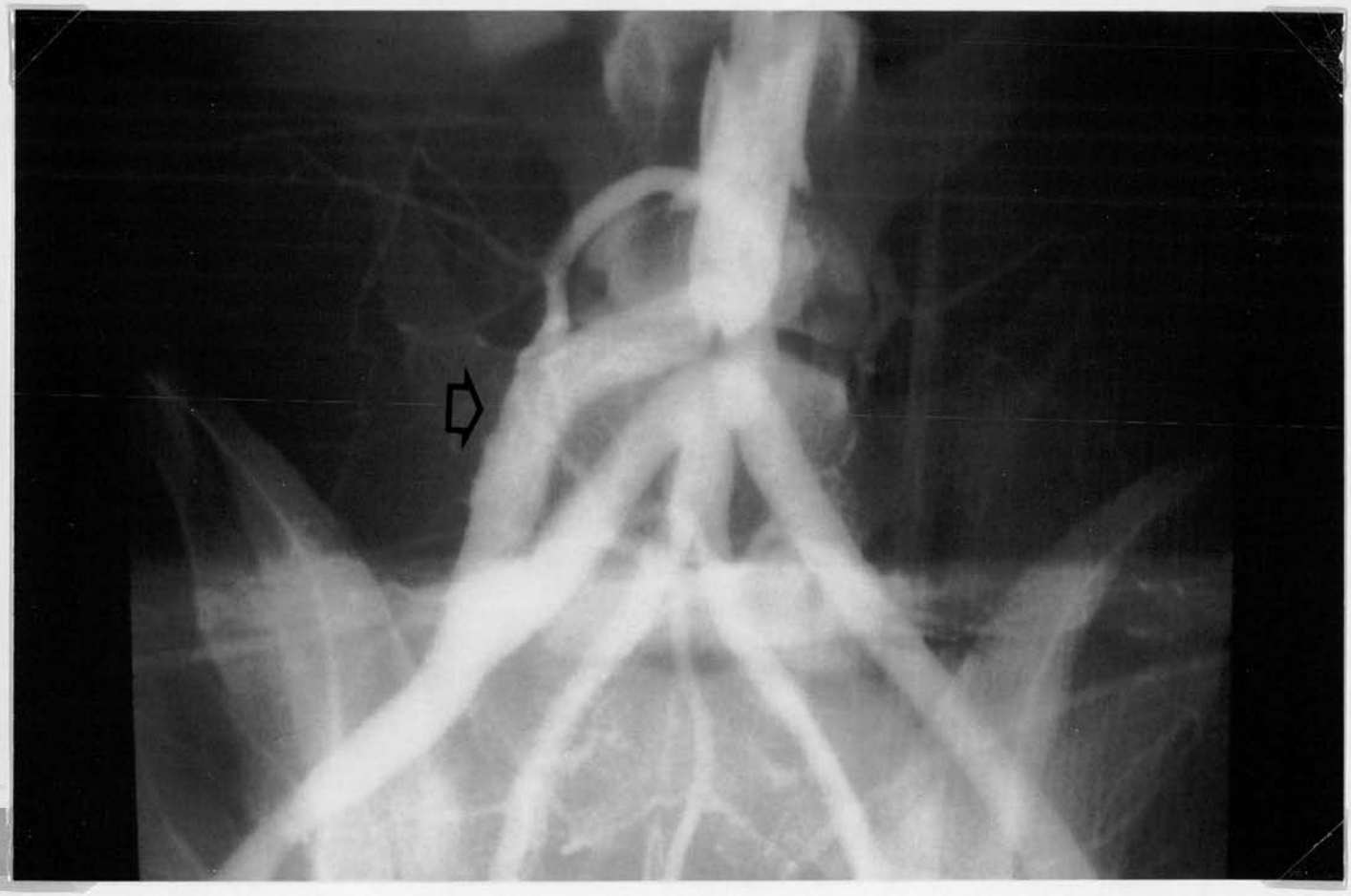


Figure 21: **End-to-side model. Sacrifice Angiogram.**
The stented graft limb is patent (arrow) but the control graft limb is occluded. Retrograde flow across the stented anastomosis has maintained flow in the run-off arteries on the control side.

5) Intimal Thickness Data (see Appendix I)

a) End-to-end Model

The statistical analysis demonstrated a significant three factor interaction between group (late or early), stent or no stent and Section (1 to 5).

There was no significant difference in the intimal thickness between the control and stented graft limbs at any level for the early group (Figure 22).

In the late group, however, the intimal thickness was significantly greater for the control side compared to the stented side at the anastomotic level (Section 3), ($p=0.0078$). The converse applied to Section 4 (proximal stent-graft interface) where the intimal thickness in the late group was significantly greater for the stented graft limbs compared to the non-stented controls ($p=0.0145$) (Figure 23).

When the control and stented sides were considered separately and intimal thickness compared between the early and late specimens in each group, a significantly greater intimal thickness was observed in the late control graft limbs at the anastomostic level compared to the early specimens ($p=0.0002$) (Figure 24). This was not seen in the stented grafts at the anastomotic level with no significant difference between early and late animals at this level. However, in Sections 4 and 5 (proximal stent-graft interface and proximal graft) the stented group had a significant increase in intimal thickness between 4 and 12 weeks ($p=0.006$ and $p=0.0008$ respectively) which was not seen at comparable levels in the control graft limbs.(Figure 25)

Figure 22

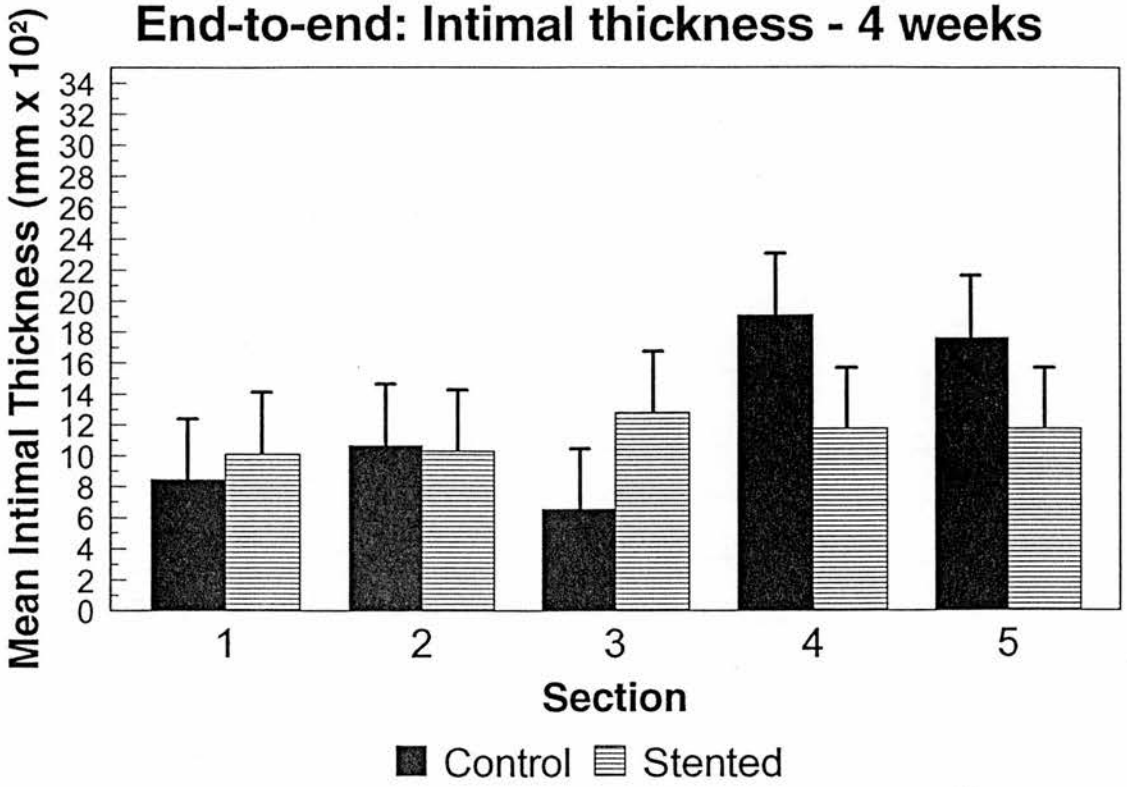
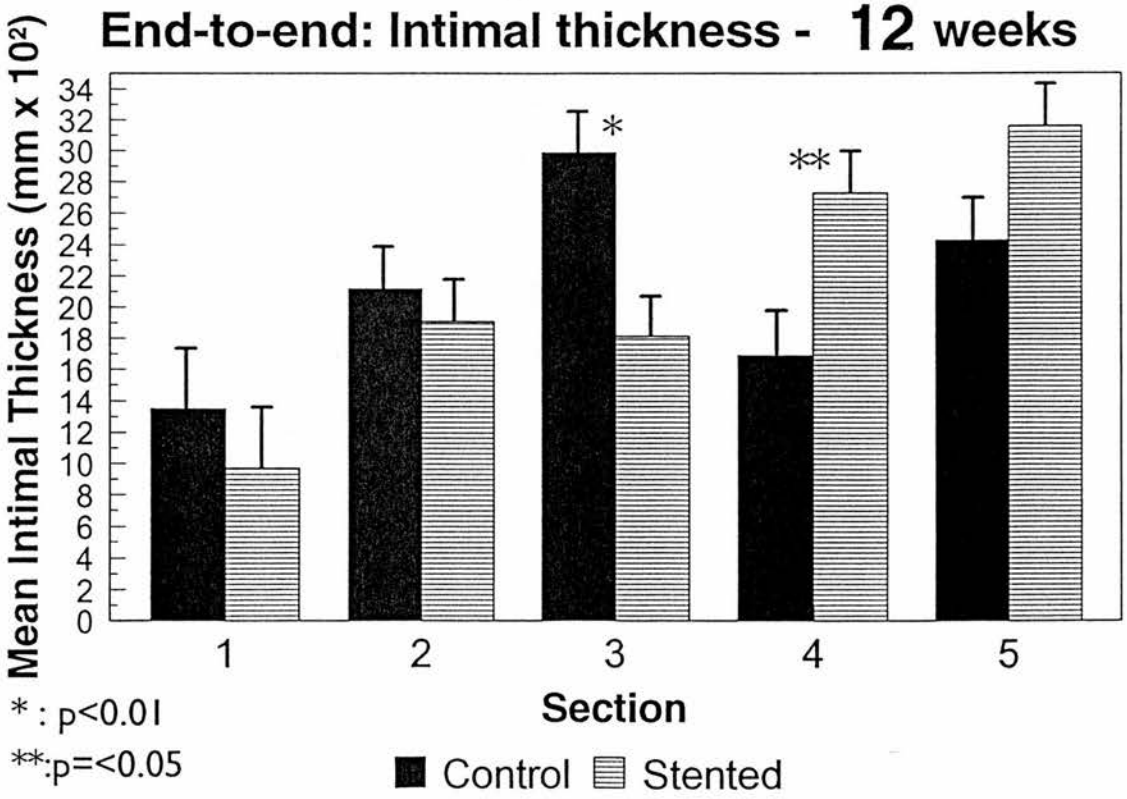


Figure 23



* : p<0.01

**:p=<0.05

Figure 24

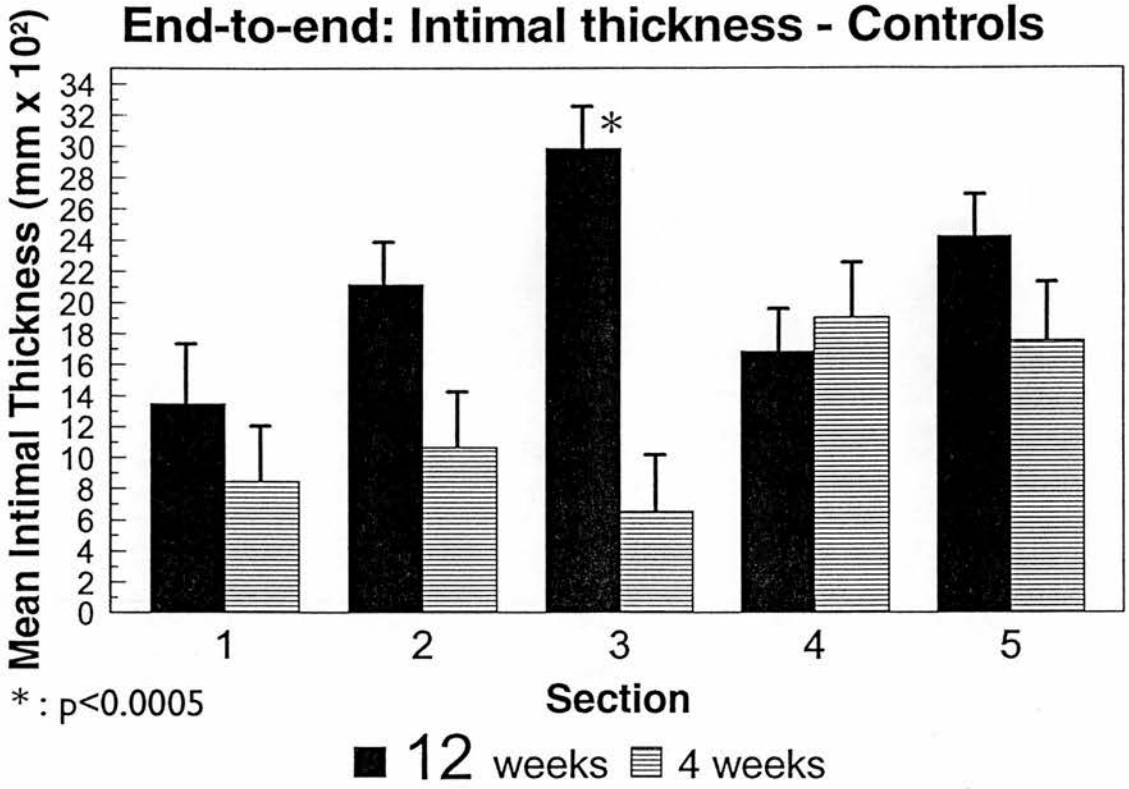
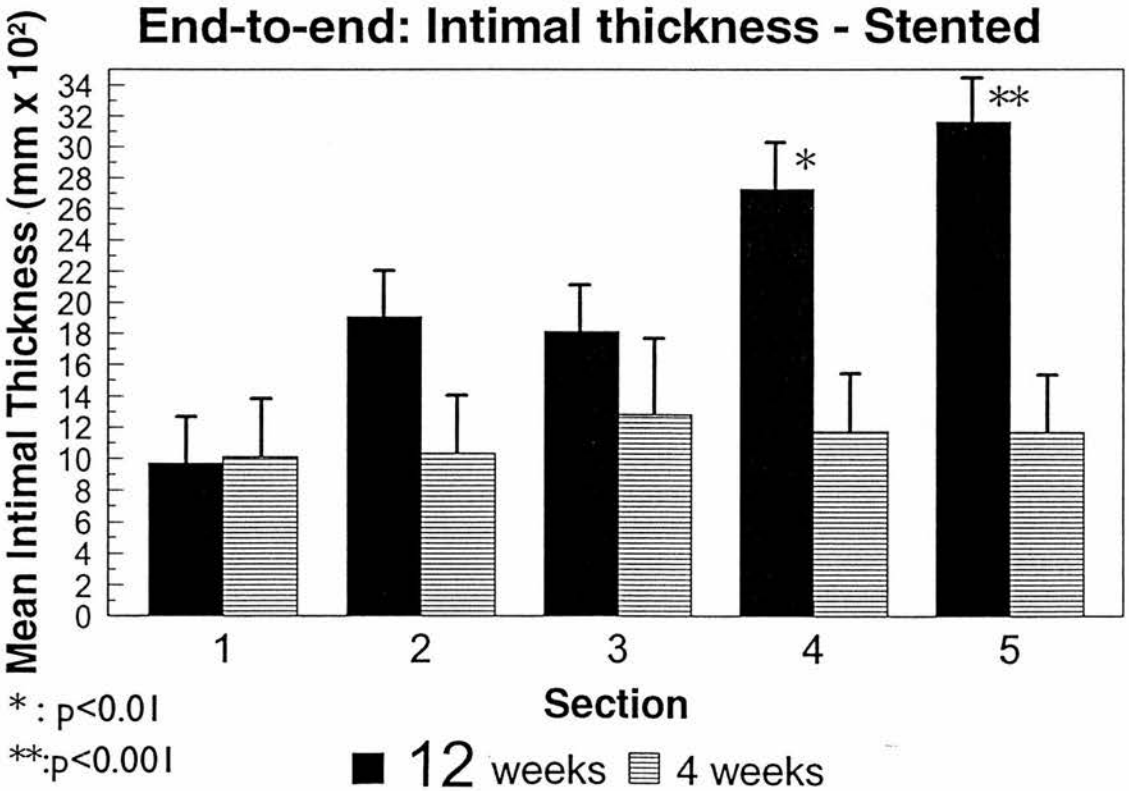


Figure 25



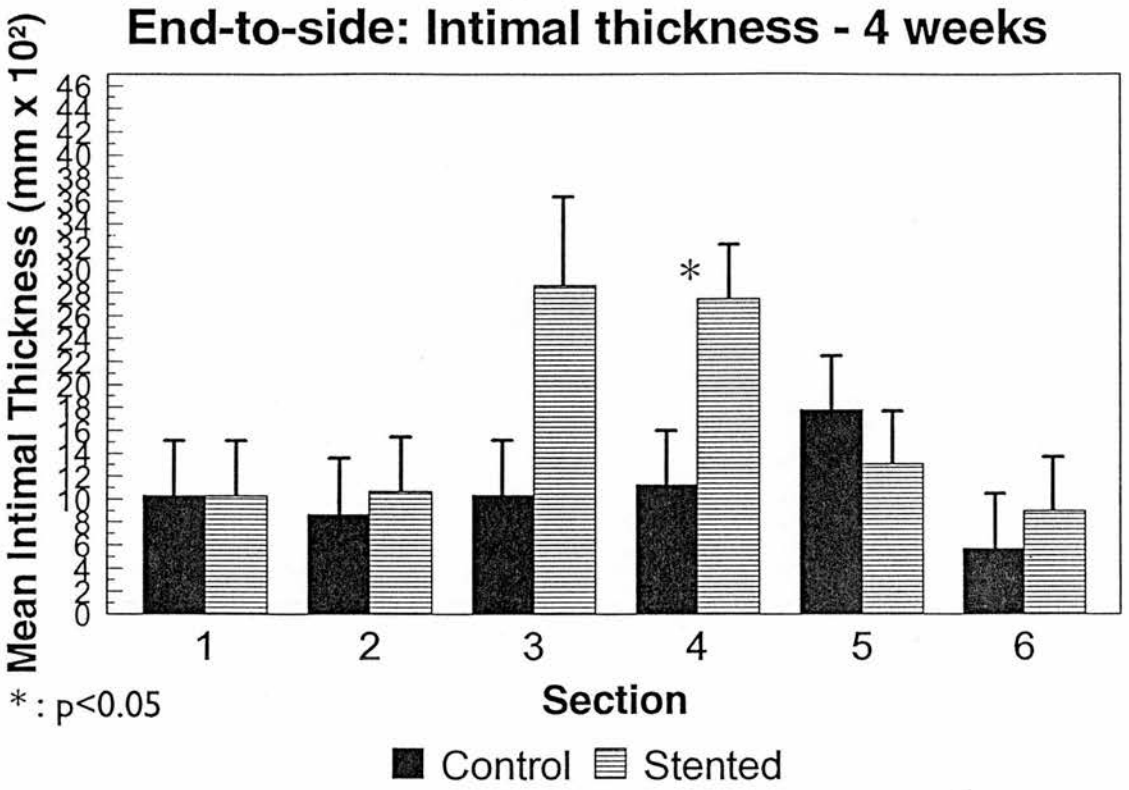
b) End-to-side Model

Again a significant three factor interaction was observed.

In the early group, the intimal thickness was significantly greater in stented graft limbs compared to controls at Section 4 (proximal stent-graft interface) ($p=0.0127$) (Figure 26). In the late group, the intimal thickness was greater in non-stented , controls at the anastomotic level, but this difference just failed to reach statistical significance ($p=0.0651$). At Sections 4 and 5 in the late grop, there was a significantly greater intimal thickness in stented sections compared to controls ($p=0.0458$ and $p=0.005$ respectively) (Figure 27).

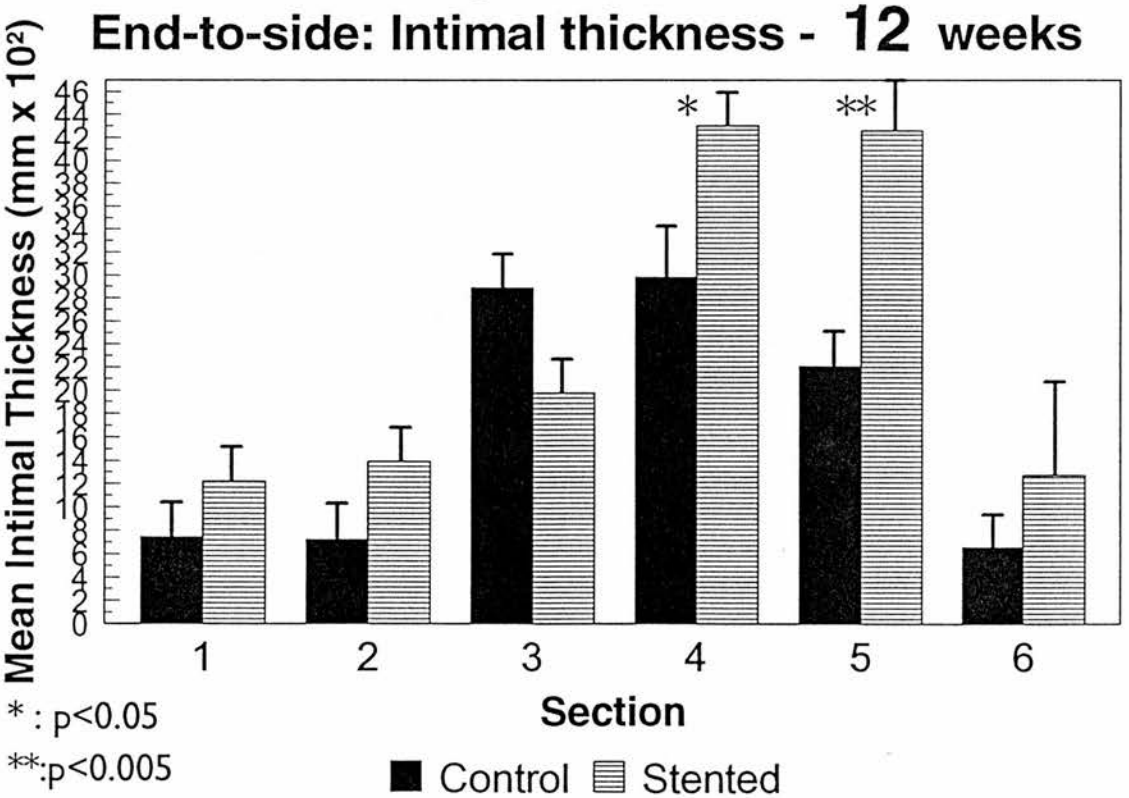
When controls and stented graft limbs were considered separately, and the early group compared to the late, the controls were seen to develop significantly greater intimal thickness at Sections 3 and 4 of the late group ($p=0.0068$ and $p=0.018$ respectively) (Figure 28). For the stented graft limbs, there was no significant increase in intimal thickness at the anastomotic level (Section 3) between early and late specimens ($p=0.3288$). However, there was a significant increase in the intimal thickness between four and 12 weeks in stented graft limbs at section 4 ($p=0.0207$) and Section 5 ($p=0.0004$) (Figure 29).

Figure 26



* : p<0.05

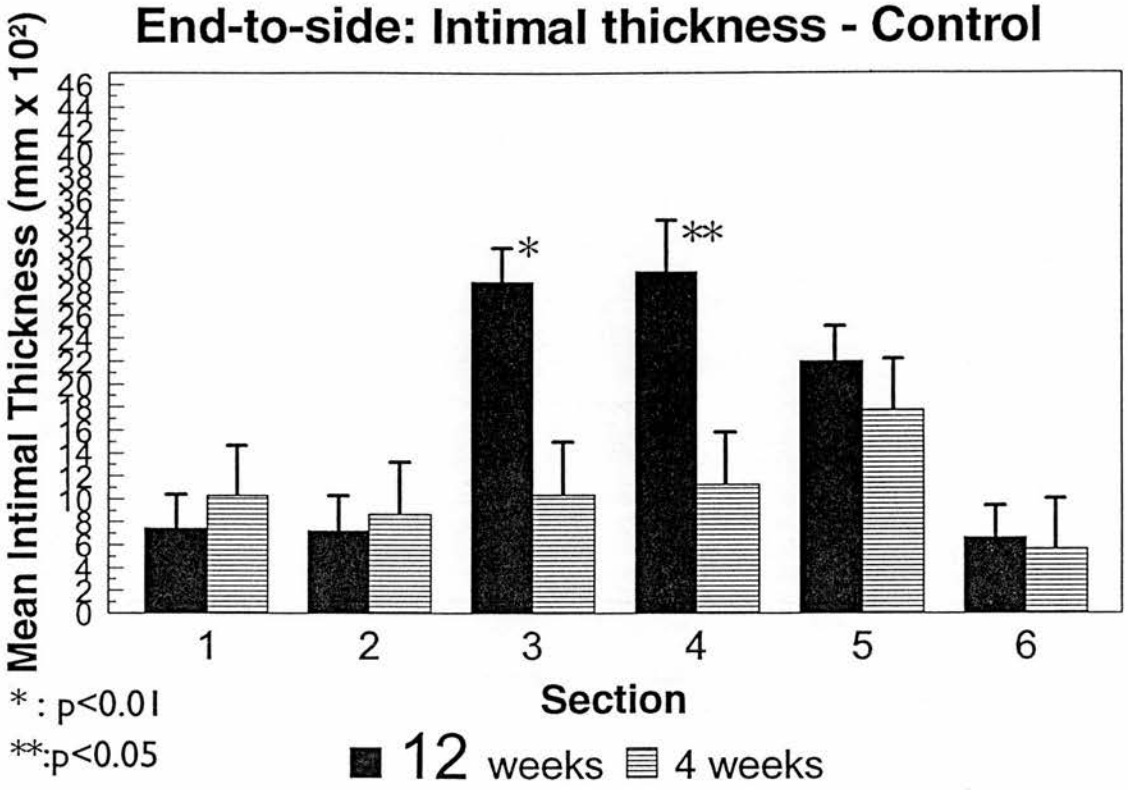
Figure 27



* : p<0.05

**:p<0.005

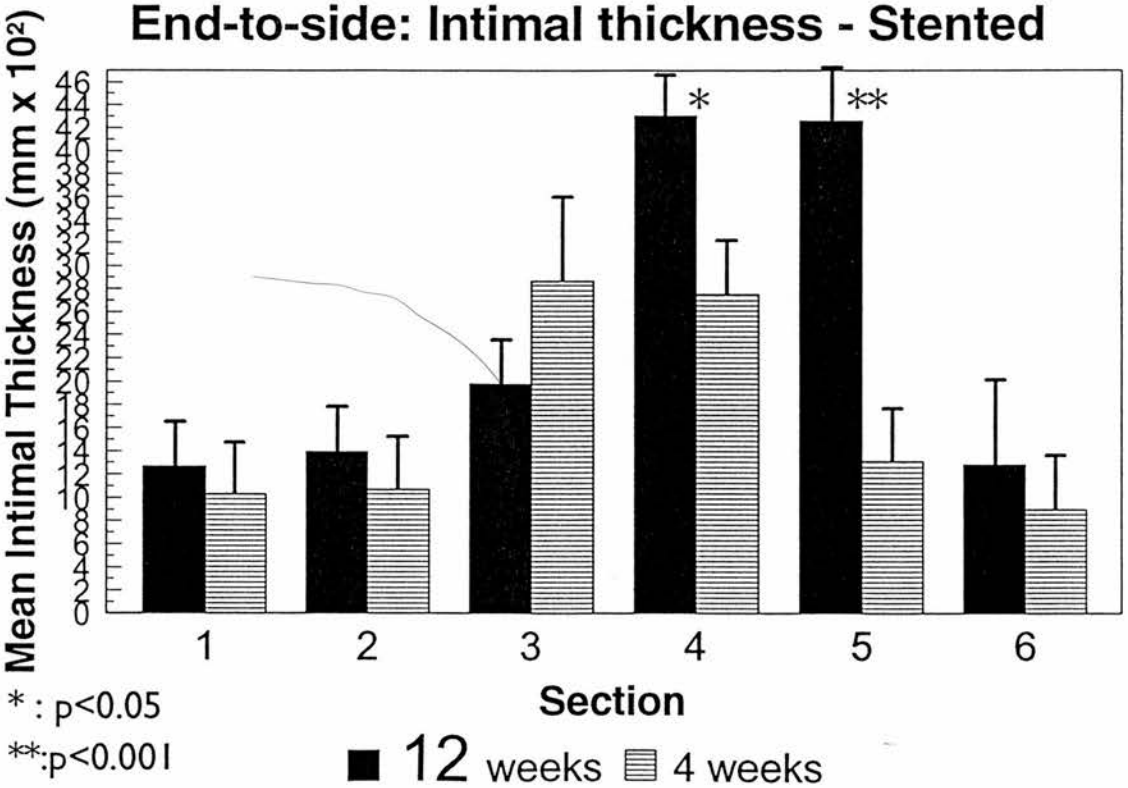
Figure 28



* : p<0.01

**:p<0.05

Figure 29



* : p<0.05

**:p<0.001

6) Luminal Area Data (see Appendix I)

a) End-to-end Model

There was no significant three and two factor interaction. There was no overall difference in luminal area between early and late and between stent and control.

There was no significant difference in mean luminal area between the stented and controls for both the early and the late groups at all sections (Figures 30 and 31).

When mean luminal area was analysed for controls and stented graft limbs separately, and early compared to late, only at Section 1 (run-off artery, 1 cm distal to the stent) of the control group was a significant difference in luminal area noted ($p=0.0351$), with the late luminal area being greater than the early (Figures 32 and 33).

Figure 30

End-to-end: luminal area - 4 weeks

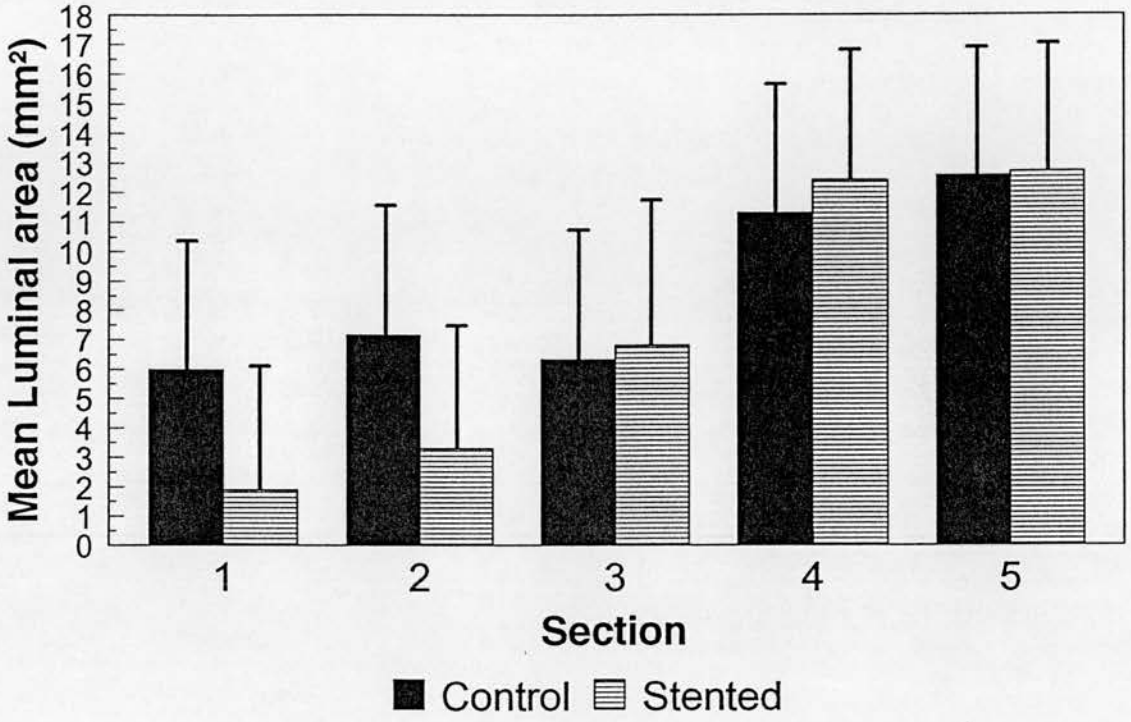


Figure 31

End-to-end: luminal area - 12 weeks

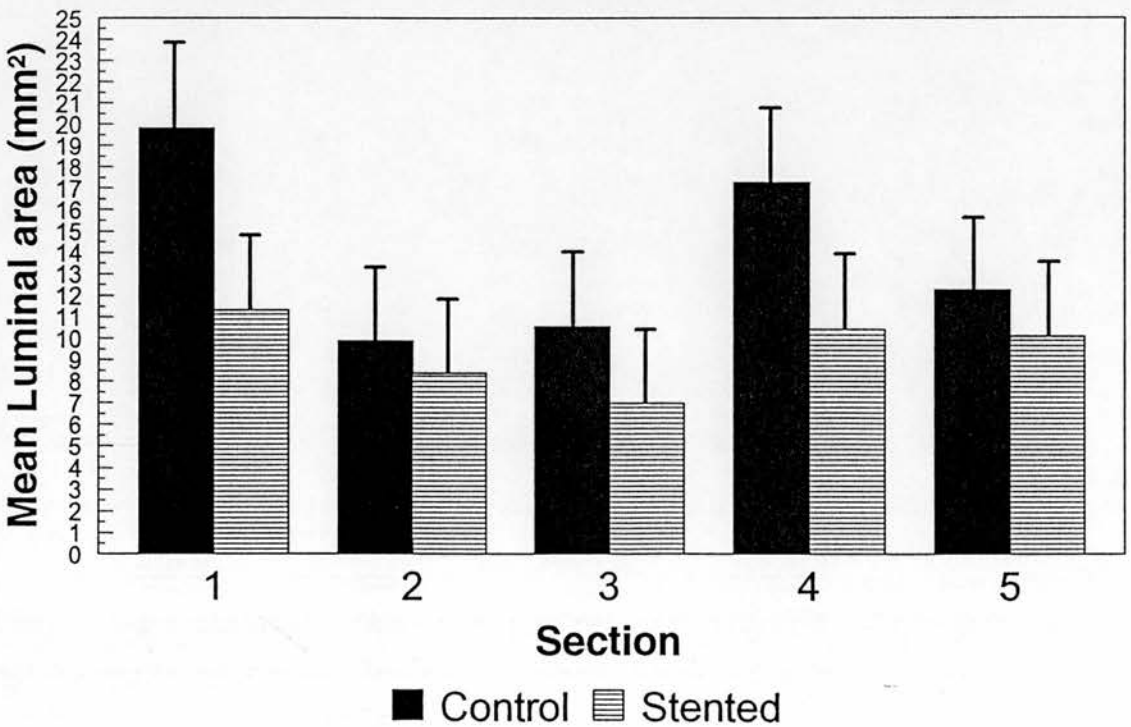


Figure 32

End-to-end: luminal area - Control group

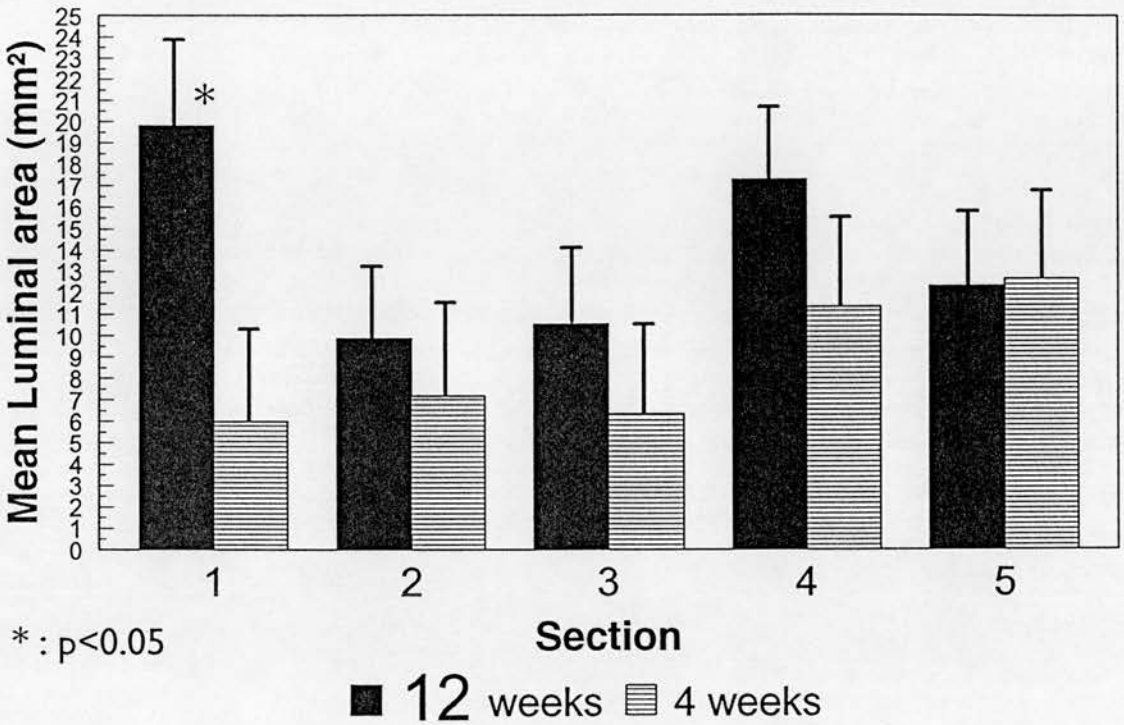
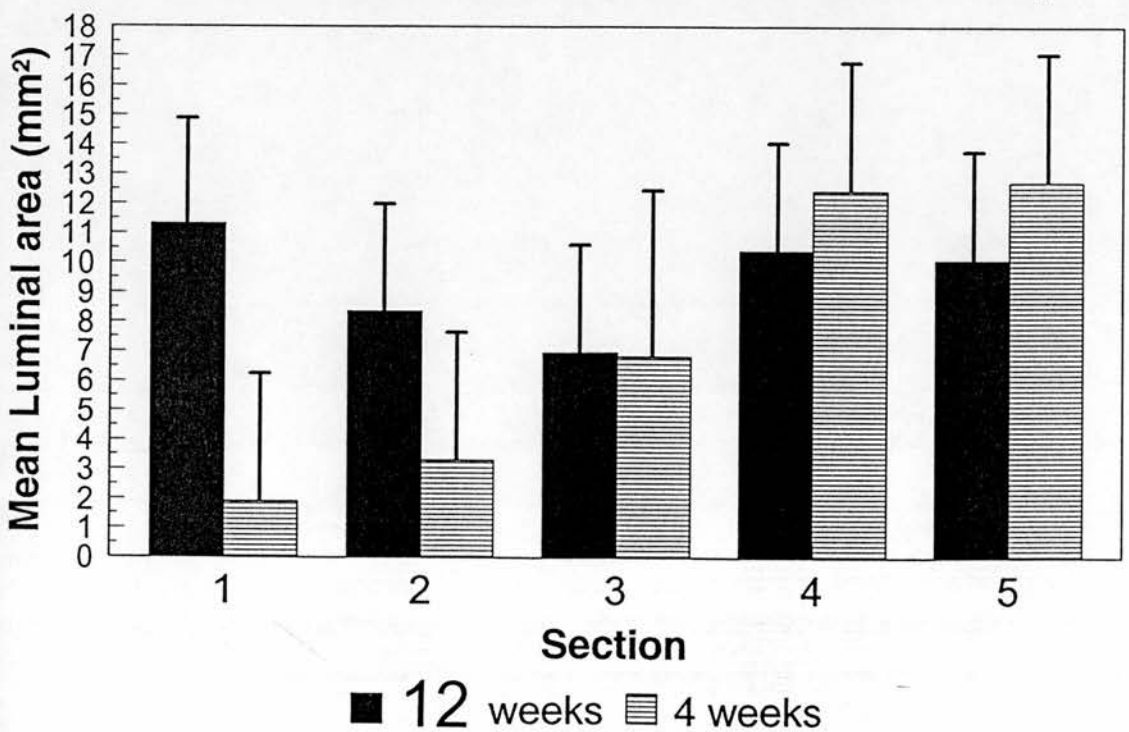


Figure 33

End-to-end: luminal area - Stented Group



b) End-to-side Model

There was no significant three and two factor interaction. There was an overall significant stent effect (when luminal area was compared across groups and sections).

There was no significant difference in luminal area between the control and stents graft limbs of the early group (Figure 34).

In the late group, there was a significantly greater luminal area in the controls at Section 2 (distal stent-artery interface, p=0.0005), Section 4 (proximal stent-graft interface, p=0.0130) and Section 6 (proximal outflow artery, 1 cm proximal to the anastomosis, p=0.0110) (Figure 35).

When luminal area was considered for the control and stented graft limbs separately and early compared to late for each group, there was no significant difference at any section for the stented graft limbs. However, for the nonstented controls, the luminal area was greater in the late group compared to the early at Section 1 (run-off artery at a level comparable to 1 cm distal to stent, or 2 cm distal to the anastomosis, p=0.0326), Section 2 (a level corresponding to the distal stent-artery interface, p=0.0261) and Section 6 (proximal outflow artery, 1 cm proximal to the anastomosis, p=0.0123) (Figures 36 and 37).

Figure 34

End-to-side: luminal area - 4 weeks

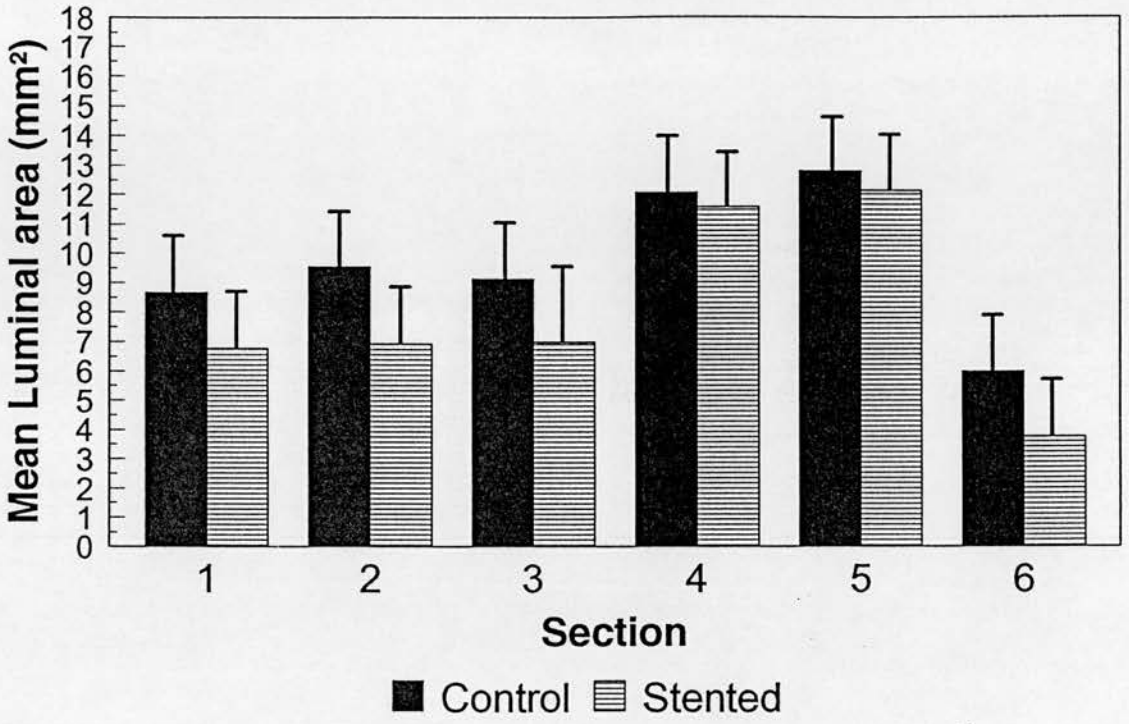
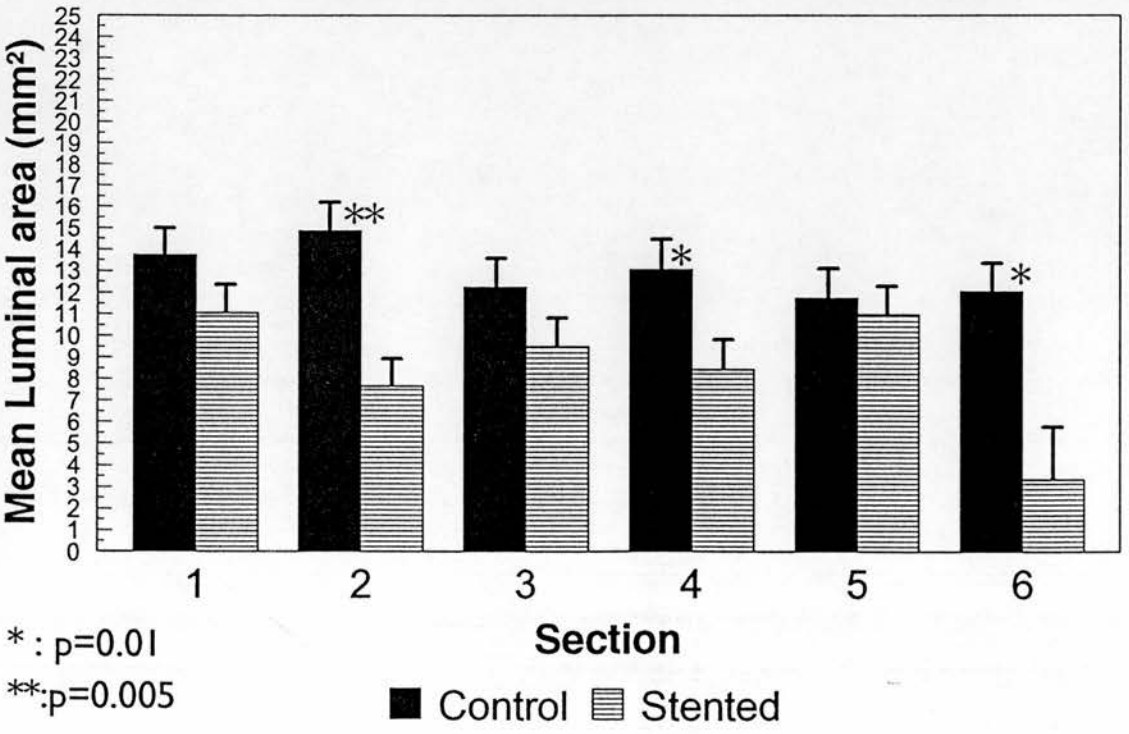


Figure 35

End-to-side: luminal area - 12 weeks

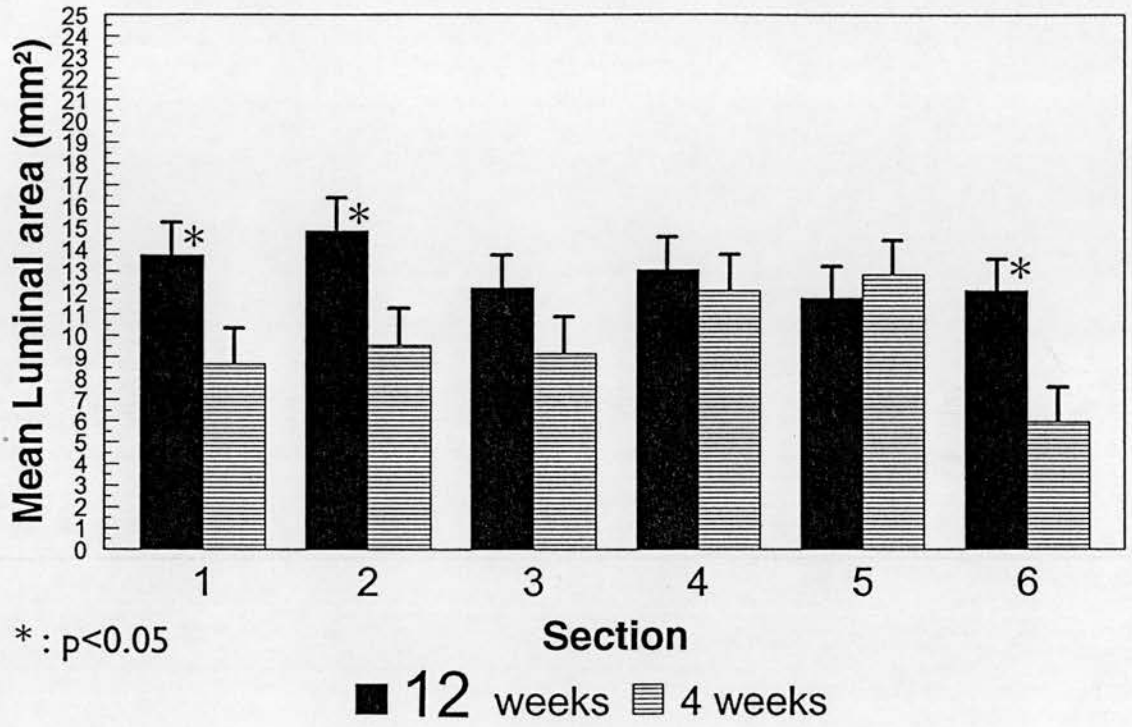


* : p=0.01

**:p=0.005

Figure 36

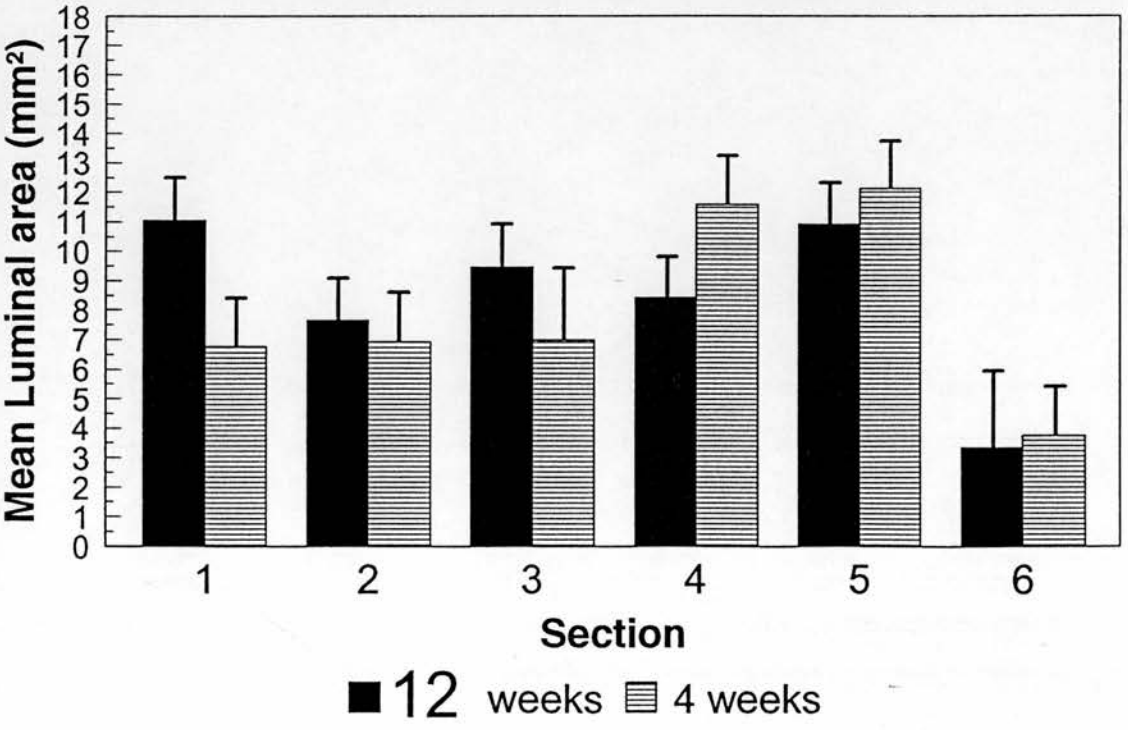
End-to-side: luminal area - Control group



* : p<0.05

figure 37

End-to-side: luminal area - Stented Group



7) Tunica Media Data

No statistically significant differences were observed in the comparison of the stent vs. no stent for both late and early groups in Sections 1 and 2. The same was observed when comparing early vs. late in the stented and nonstented sides. These results hold for both the end-to-end and the end-to-side data. Note that in interpreting these results the sample size of the study was very small and therefore the power of the test to detect certain defined differences was probably low.

DISCUSSION

General Comments

To date, there have been no reports in the literature of the use of endovascular anastomotic stenting as a possible means of limiting the development of distal anastomotic neointimal hyperplasia in infrainguinal bypass grafts. The only documented use of stents in the field of bypass grafting has been as a means of salvaging failing or failed grafts.[80] Most of this experience has been gained with coronary artery bypass grafts, the conduit in question being either autogenous saphenous vein[234,240,259] or internal mammary artery.[4] More recently, several authors have reported the successful use of stenting in the salvage of failed or failing arteriovenous haemodialysis access fistulae where the stent has been deployed after percutaneous transluminal balloon angioplasty of neointimal hyperplasia within the venous component of such a graft.[8,117]

As far as infrainguinal arterial bypass grafting is concerned, there is little debate that for grafts to the below-knee popliteal artery and to the tibial arteries, the conduit of choice is autogenous saphenous vein.[23,131,184,281] However, in cases where the ipsilateral saphenous vein is not usable or has been stripped and there is insufficient alternative autogenous vein available, as is often the case in secondary bypass or in patients with prior coronary artery bypass graft, an alternative conduit is utilised. Although the results for prosthetic grafts to the below-knee popliteal artery are reasonable, they are certainly significantly inferior to those for autogenous saphenous vein and those for other sources of autogenous vein, such as arm vein.[48] At the tibial level, the patency rates for prosthetic grafts are even less favourable with the best patency rates in reported series being about 40% at three years, although most authors have reported patency rates of around 25% at three years. Faced with these poor results, in a situation where a prosthetic graft is the only option for bypass, some authors have advocated primary amputation without attempting a limb

salvage bypass, the philosophy being that with patency rates as poor as these for prosthetic bypass, there seems little clinical or economic rationale behind persisting with apparently fruitless efforts at bypass.

Others have taken the opposite viewpoint, stating that since the results of bypass for limb salvage with autogenous vein are so promising, it surely must be possible to develop modifications of surgical technique in order to improve the patency rates for prosthetic grafts to infrageniculate arteries and by so doing, possibly emulate the patency and the limb salvage rates seen with autogenous vein.[270,282,284] Much work has been directed at developing different prosthetic materials. The graft material in commonest clinical use nowadays is polytetrafluoroethylene (PTFE), but numerous alternatives are being marketed and it seems that manufacturers are in major competition to produce the "ultimate" graft! Dacron, human umbilical vein and cryopreserved human saphenous vein are but three of the readily-available alternatives on the market in the United Kingdom today. Reports from different centres have documented favourable patency rates with all three of these conduits, although none can approach the late patency rates for vein in bypass grafts to the infrageniculate arteries. As the experimental work reported in this thesis utilised PTFE, I shall restrict comments to the use of this particular graft material. Even with modern graft technology, it can be seen from the meta-analysis of results for infrainguinal bypass grafting utilising PTFE that the results have remained significantly inferior to autogenous vein right up to the present day, indicating that adapting the conduit material alone is not sufficient to improve its performance.

As outlined in Section II, several different surgical approaches have been developed to attempt to limit the development of anastomotic neointimal hyperplasia. The theory behind the composite graft was that prosthetic graft material kinked when crossing the knee joint and that to avert this by anastomosing a segment of autogenous vein to the prosthetic material above the knee such that the autogenous tissue crossed the knee might influence patency in a positive way. It was hoped that an additional benefit of anastomosing autogenous tissue to the native artery might be a positive effect as far as the development of anastomotic neointimal hyperplasia was concerned. However, from my review of the literature on

composite bypasses, it can be seen that in most authors' experience, the hypothetical advantages of such a bypass configuration have not been borne out in clinical practice.[77,161,164,172] Indeed, some authors reported the finding of ANH at the prosthetic-to-vein anastomosis.[161]

The anastomotic vein cuff and its various "descendants", most notably the Taylor patch, were developed initially to make the anastomosis of prosthetic grafts to small arteries technically easier to perform.[185,245,274] Subsequent clinical experience has shown very promising results for the patency of infrageniculate PTFE grafts using a distal anastomotic vein patch and Taylor himself has reported a 71% patency rate for popliteal grafts and 54% for tibial grafts at 5 years.[270]

The exact way in which the short interposition vein collar or patch exerts these beneficial effects on patency is not clear. It may be that by averting the abrupt change in compliance between the prosthetic material and the native artery, the vein cuff contributes to reducing the hyperplastic response. There may also be a "seeding-like" effect due to the presence of endothelial cells from the vein patch or cuff at the anastomotic level. Alternatively, especially with the Taylor patch technique, in which a long arteriotomy is fashioned prior to placement of the vein patch, the resultant "streamlining" of the anastomosis together with marked reduction in the anastomotic angle may confer a haemodynamic advantage on patched anastomoses leading to less wall shear and flow disturbance, both of which have been implicated in the genesis of anastomotic neointimal hyperplasia.

There is now an extensive literature on the clinical outcome of the use of endovascular stents in both the coronary and peripheral circulations. These devices were first developed to rescue immediate percutaneous transluminal balloon angioplasty failures and intimal dissections and have been used subsequently as a means of reducing the incidence of restenosis after successful angioplasty. Also, they are used to counteract vessel wall elastic recoil after angioplasty as well as to "tack" down intimal flaps and tears. In the smaller calibre arteries, the results of endovascular stenting have been reasonable, although early thrombosis has been a problem in a significant number of cases. At the iliac artery level, the

results of endovascular stenting have been very promising and of note, compared to angioplasty alone, long-term vessel patency has been superior and the need for further intervention significantly less.[222] As outlined in the preceding overview of vascular stents, human and animal studies have demonstrated that these devices achieve full endothelial coverage and are incorporated into the recipient vessel after a relatively short period of time. In animal models, this has been shown to occur within three weeks of stent deployment, whilst in human studies, the interval to full endothelial coverage is somewhat longer at approximately three months. It is postulated that full endothelial coverage prevents adherence of circulating thrombogenic blood proteins and cells and so averts the resultant mitogenic stimulation of the underlying exposed medial smooth muscle cells with the potential for a subsequent hyperplastic response.

With these various observations in mind, it seemed possible that the placement of an endoluminal stent across the distal anastomosis between a prosthetic graft and native artery might influence the site and amount of development of anastomotic neointimal hyperplasia.

Choice of Experimental Model

The prosthetic graft material in commonest use in clinical practice for infrainguinal arterial bypass grafts in the United Kingdom today is polytetrafluoroethylene (PTFE) and therefore this was the graft material chosen for the model used in these experiments. From my own meta-analysis of the literature of infrainguinal PTFE bypass grafts, it was apparent that for PTFE grafts used to bypass arteries below the inguinal ligament, the diameter of conduit used most frequently was 6 mm and this was the graft diameter selected.

One of the criticisms levelled at other animal bypass graft models of anastomotic neointimal hyperplasia is that often a short interposition graft is placed in a large diameter artery with high rates of blood flow, such as the aorta. Thus, the extrapolation of conclusions drawn from such animal experiments to the human situation has to be viewed with caution. Other

authors have utilised a cross-femoral bypass in a canine model.[166] This model certainly utilised a longer conduit implanted into smaller diameter arteries, but the problem of controls arises with single grafts within the same animal. Furthermore, the work of Kinley et al[144] and of Clowes et al[60] on critical graft lengths and resultant thromboses indicated the need for our experiment to comprise a 6 mm PTFE graft of reasonable length although it is felt that for 6 mm diameter grafts, the critical length, above which thrombosis is inevitable, is far greater than for any conduit used in clinical practice. The graft limbs were all 6 cm in length. The report of the committee of the SVS/ISCVS on the assessment of new graft materials[2] recommended the use of the canine as an animal experimental model. For these reasons, for our experimental work, it was decided to place a bifurcated graft into the aorto-iliac circulation of a canine model. Nevertheless, we were aware that caution must be exercised when extrapolating to the setting of the human subject with advanced atherosclerotic occlusive disease, the results of any technique involving the study of the disease-free arteries of a healthy animal.

The number of animals studied in each sub-group was admittedly small. As there have been no previous reports of the outcome of graft-artery anastomotic stenting, we did not know what sort of patency rates and therefore meaningful data on anastomotic neointimal hyperplasia we could achieve. For the planned statistical analysis, the minimum number of dogs that could be allocated to each group was three.

The times chosen for graft retrieval were selected based upon the experience of other authors using the Wallstent in animal arteries. By four weeks ("early" group) complete endothelial coverage was achieved in every one of our specimens. Other workers had reported that the neointima within the Wallstent, when it is placed in the native arteries of the dog, is stable and non-progressive by ten weeks and therefore for our study of graft-to-artery anastomotic stenting, the so-called "late" group was sacrificed at 12 weeks for both anastomotic configurations.

Technical Considerations

The advantage of utilising the "Wallstent" for a study of anastomotic stenting was that these stents are allegedly radially compliant and longitudinally flexible. Thus it was possible to place a stent across an end-to-side anastomosis without distorting the geometry or reducing the lumen. We were anxious not to perform any form of angioplasty or arterial overdilatation whilst placing the stent and therefore the vessel's diameter was measured before any surgical handling took place. Reports from other authors have suggested that the placement of an excessively large stent can exaggerate the residual vessel lumen diameter and also have a negative effect on the vessel media, giving rise to medial atrophy and fibrosis.[46] As it happened, the size of dog used for these experiments (20-25 kg) had as a standard finding an external iliac artery diameter between five and six millimetres in every case, so that the size of stent selected was the same in every animal. The use of the more rigid and less pliable balloon-expandable Palmaz stent might possibly have resulted in a degree of angioplasty, as well as considerable distortion of the anastomosis, particularly in the end-to-side anastomotic configuration.

Stent placement under direct vision was an important component of our model and so sewing the stented anastomosis first and placing the stent via the proximal graft was the logical method of stent deployment. Previously, in the pilot work of Worsey et al[297], the stent had been introduced via a distal branch artery, but there was concern that so doing could traumatise the run-off vessels with a resultant adverse effect on stent performance and thus might falsely skew the results in favour of the control graft limbs. From a practical viewpoint, as well as requiring a further surgical incision in the animal's groin, the side branches of the canine superficial femoral artery could be easily traumatised by the introduction of a 7 French guage catheter.

There were no complications related to stent deployment, in particular there were no cases of stent migration. In clinical practice, this is a very real complication of the use of balloon-expandable stents, another reason for our choice of the "Wallstent". At either end of the

"Wallstent", the filaments are slightly splayed outwards. This helps fixation to the native vessel and certainly, we found that when released from its constraining membrane, the stent "clicked" open and was fixed firmly in place in every case. Given the results in both models with regard to the significantly greater intimal thickness in late specimens at the sections taken from the stent-graft interface (Section 4), it may be that this apparent "shelf" had an adverse effect on haemodynamic factors such that it precipitated an excessive accumulation of neointimal hyperplasia at that level.

Equally, it was clear that the mere presence of a stent across a graft-to-artery anastomosis has an immediate lumen-reducing effect. One problem of assessing the effect on luminal area using our methodology was highlighted in the section taken at the anastomotic level in the end-to-side model. Although the stent was flexible in all planes, there was an inevitable loss of some of the native vessel floor from the conduit's lumen. This negatively-skewing effect was at least partly overcome by having a standard anastomotic angle of 45 degrees, but even so it could not be eradicated completely. For these two reasons, the luminal area data are more difficult to interpret and changes in lumen were obviously not always due solely to the accumulation of neointimal thickening, but rather, may have been due in part to a limitation of the experimental model itself.

Another technical point worthy of mention is that of the operative findings at the time of the sacrifice laparotomy. Without exception, in both anastomotic configurations, the stented graft limb and artery in the vicinity of the anastomosis were involved in a thick mass of fibrous tissue. Dissection was difficult and extreme care had to be taken to avoid damaging the specimen, as no obvious plane between the graft and surrounding tissue was apparent. The control side, by contrast, had a thin pseudo-capsule around it and a clear dissection plane was easily developed between the graft and this surrounding tissue. These findings have some important implications. First, if similar events occur in human arteries after stenting, operating on these vessels after endovascular stenting may prove technically demanding.

In a study of the compliance changes in canine iliac arteries after endovascular stenting, published since these experiments were completed, Back et al[10] have also encountered an

intense periadventitial fibrous reaction around the stented vessels that was not seen in control arteries. They observed that vessel compliance was altered from the moment that a stent was deployed and that over time compliance decreased significantly. They suggested that the fibrous reaction they encountered might account for this finding. Furthermore, they postulated that such a mismatch in compliance might have haemodynamic and mechanical consequences. They argued that decreased compliance in the stented segment increased impedance to blood flow by increasing the loss of pulsatile energy in the vessel wall. This could lead to decreased distal perfusion, increased pressure wave reflection and thus increased pulsatile mechanical stresses at the interface between the non-compliant stented segment and the native artery. Although the methodology used by Back et al did not assess arterial compliance at fixed points, but rather only measured dynamic compliance over 2 to 3 cm segments of vessel, their suggestion is certainly interesting to contemplate in light of the findings from these studies of endovascular anastomotic stenting.

Graft Patency

As shown in the Results Section, the patency rates achieved in both experiments were similar, namely, 75% for the end-to-end model and 80% for the end-to-side model. There appeared to be no differences between stented and control graft limbs in this regard. Apart from intra-operative heparinisation and the injection of heparinised saline into graft limbs whilst clamps were applied, no other form of anticoagulants or antiplatelet agent was administered either during the operative procedure, or in the post-operative period.

No screening tests for hypercoagulability were performed on any of the animals. It is conceivable that there were some differences between the individual experimental animals in terms of coagulation. In particular, it has been reported that there are recognised differences between the arachidonic acid pathway in some animal platelets and in the human. It has been shown that platelet-rich plasma obtained from the majority of mongrel dogs fails to aggregate

when stirred with sodium arachidonate, despite the fact that the platelets form thromboxane A2.[136] However, platelet-rich plasma from a minority of dogs makes an equal quantity of thromboxane A2, but aggregates irreversibly when stirred with sodium arachidonate. It is possible that the animals in which graft limb occlusion occurred belonged to the minority in which such arachidonate sensitivity existed. Having said that, in one animal in the end-to-end group, both graft limbs were found to be occluded at the time of sacrifice, and when the specimen was opened, an obvious accumulation of neointimal hyperplasia was seen in both graft limbs. On the control side, the lesion had occurred at the level of the anastomotic suture line and extended back into the graft for a few millimetres, whereas on the stented side, the build up of neointimal hyperplasia was seen at the proximal stent-to-graft interface and extended proximally into the graft from this level. Thus an obvious cause for graft thrombosis was present, independent of any platelet or other coagulation problem, in the only animal to suffer bilateral graft limb occlusion. Given the patency rates achieved and the findings in those graft limbs that did occlude, it seems that our policy of ignoring the arachidonate sensitivity issue did not influence the outcome of the experiments significantly. To compare this approach to clinical practice, it is only the occasional patient who undergoes thorough coagulation screening prior to infrainguinal bypass. Such detailed studies of clotting tend to be limited to patients with a suspected or known hypercoagulable disorder or to those patients who present with a graft thrombosis in whom no obvious underlying graft, anastomotic or run-off vessel problem is found.

It is concluded that in a canine model of PTFE aorto-bi-iliac bypass, in which the distal anastomosis is formed in either the end-to-end or the end-to-side configuration, endovascular anastomotic stenting can be performed with acceptable patency rates up to 12 weeks after graft placement.

Electron Microscope Studies

In every animal in both models, complete cellular coverage of the stent's lumen was observed by 4 weeks. The cells formed a confluent monolayer on the luminal surface of all stents and had the appearances on scanning electron microscopy of vascular endothelial cells. However, as we did not perform any endothelial cell marker studies, this statement is speculative rather than categorical. Even so, other workers have demonstrated beyond doubt that the Wallstent becomes fully endothelialised by a similar time period after stent deployment within the arteries of dogs.

In the end-to-side model, the electron microscopy studies yielded some additional interesting findings. The luminal surface of the stents was again completely covered except where the stent crossed the outflow artery. This was a constant finding. Higher power views taken from the stent filaments at this level showed an obvious discrete "edge" to the cell monolayer. This would tend to imply that blood flowing across the filaments prevents any cell adherence from taking place. This observation has important implications for the practice of endovascular stenting in general as it seems from this experiment at least, that it is possible to place a stent across the ostium of a branch artery without causing occlusion of the vessel.

Analysis of Intimal Thickness and Luminal Area Data

End-to-end Model

This experimental work showed that at the anastomotic level, at 12 weeks, the stented graft limbs had significantly less intimal thickening compared to the non-stented, control graft limbs ($p=0.0078$). However, at the proximal stent-graft interface, the opposite applied, with the stented graft limbs developing significantly greater intimal thickness compared to controls ($p=0.0145$). At the anastomotic level, the intimal thickening appeared to stabilise between 4 and 12 week subjects on the stented side whereas, there was a significant increase in intimal thickness over time in the non-stented, controls at this level ($p=0.0002$). In the stented graft limbs, however, there was a significant increase in intimal thickness over time in the graft proximal to the stent which was not seen at corresponding levels in controls.

The assessment of luminal area failed to demonstrate any significant differences between the controls and stented graft limbs in either early or late groups. Over time, there was a significant increase in the luminal area ($p=0.0351$) of the run-off artery 2 cm distal to the anastomosis in non-stented controls that was not seen in native vessels distal to the stented graft limbs.

The data on intimal thickness would imply a significantly beneficial effect of the stent at the anastomotic level. Not only did late controls have significantly greater intimal thickness at this level, but also, the lesion appeared to be progressive over time in controls whereas, within the stented section, the intima appeared to stabilise over time. This would certainly correspond with prior studies of stenting in canine arteries, which have shown the neointima within the Wallstent to be a non-progressive lesion. The significantly greater intimal thickness at Section 4 (proximal stent-graft interface) and Section 5 (proximal graft) seen in the stented graft limbs at 12 weeks, suggests that whatever it is that induces the development of neointimal hyperplasia may have been relocated to this level, away from the anastomosis, by the placement of a stent across the suture line.

The luminal area data are a little less easy to interpret. At the anastomotic level, in animals sacrificed at 12 weeks, one might have expected a significantly greater luminal area in the stented graft limbs. There was significantly less intimal thickness (IT) at this level compared to non-stented controls and it might have been expected that the concentric decrease in luminal area that is present at a continuous suture line reported by other authors[123,145] would have been overcome by the fully-expanded stent. However, in these experiments, we utilised stents the diameter of which corresponded to the physiological diameter of the native artery in order to avoid any such angioplasty effect. Also, the work of Back et al[10] has shown that dynamic radial compliance is lost from these stents after their deployment within the canine iliac arteries after as short a time as one week. Thus it would seem that although the stent was associated with significantly less IT at the graft-artery anastomosic level, the physical presence of the stent within the conduit, combined with the effect of accurate stent:vessel diameter matching must have been sufficient to counteract any significant beneficial effect on the luminal area of lesser degree of accumulation of ANH.

The changes at Section 4 are also hard to interpret. There was a significantly greater IT at 12 weeks in the stented compared to the non-stented subjects and yet the effect on luminal area did not reach statistical significance. It is possible that this finding was to attributable to the relatively small number of subjects in each limb of the study. However, the statistical methodology utilised was very sensitive and should not have been adversely influenced by the number of subjects alone. The fact that increased intimal thickening was not associated with a corresponding decrease in lumen could be related to technical factors at the time of specimen retrieval. The specimens were pressure-perfusion fixed at physiological pressures (90 mm Hg). It can only be assumed that the PTFE proximal to the stent in stented graft limbs, and at the anastomotic suture line in control graft limbs did undergo a degree of expansion during the fixation process, enough to eradicate the direct effect of the intimal changes on the luminal area.

The other interesting finding in this model was of a significant increase in lumen of the run-off artery distal to the anastomosis seen in controls but not seen in stented graft limbs. This

was seen in the absence of any significant differences in intimal thickness and may reflect a compensatory dilatation of the artery distal to the constricting effect of the anastomotic suture line. It is possible that the stent had a sort of "streamlining" effect on the anastomosis and the run-off artery distal to it.

End-to-side Model

Because of the more complex haemodynamic environment associated with this anastomotic configuration, it was predicted that we would encounter an assortment of interesting effects due to the placement of a stent across such an anastomosis. We were not to be disappointed! That we experienced a patency rate of 75% was most encouraging, since, apart from any other considerations, this confirms the aorto-bi-iliac bypass as a serviceable model for the study of anastomotic neointimal hyperplasia in the canine.

At the anastomotic level, we again encountered greater intimal thickening in controls compared to stented graft limbs at 12 weeks, although this difference just failed to reach statistical significance ($p=0.0651$). However, the reverse again applied at the proximal stent-graft interface: the stented graft limbs developed significantly greater IT at both Sections 4 ($p=0.0458$) and Section 5 ($p=0.0050$). Interestingly, the difference was also seen to be statistically significant in Section 4 of the early group ($p=0.0127$).

As was the case with the end-to-end distal anastomotic configuration, the IT within the stented graft/artery segment did not show any significant progression between 4 and 12 weeks. In the non-stented controls, however, at the graft-artery anastomotic level (the level corresponding to mid-stent level) there was a significant increase in IT between 4 and 12 week subjects ($p=0.0068$). In both controls and stented graft limbs, at Section 4, there was a significant progression of IT between early and late subjects and this was also seen in Section 5 of the stented group.

The luminal area was significantly greater for 12 week controls at Section 2 ($p=0.0005$), Section 4 ($p=0.0130$) and Section 6 ($p=0.0110$). When the controls and stented graft limbs were considered separately and early luminal area compared to late, there was a significant increase in lumen over time for controls at Sections 1 ($p=0.0326$), Section 2 ($p=0.0261$) and Section 6 ($p=0.0123$). In other words, these changes were seen at all of the sectioned levels that involved native artery alone.

The stent conferred an advantage, albeit a non-significant one, on the development of neointimal hyperplasia at the graft-to-artery anastomotic level and the IT within the stented segment of graft and artery did not progress with time. However, again, significantly greater IT was seen proximal to the stent than at corresponding levels in the controls. These changes developed in the animals sacrificed at 4 weeks as well as in those at 12 weeks. What is more, the changes seen in Section 5 of the 12 week animals imply that the neointimal hyperplasia was progressing proximally into the graft with time. In other words, the area of the proximal stent-graft interface acted in a similar manner to the anastomotic region in the non-stented controls. The previously-mentioned "step" associated with the Wallstent's proximal (and distal) margin may have been responsible for some of the altered flow dynamics that could be responsible for the IT changes seen.

The luminal area changes encountered corresponded with the intimal thickness changes in that at the anastomosis, there was no significant difference between controls and stented graft limbs. At the proximal stent-graft interface, a significantly greater lumen was seen in 12 week controls compared to stented graft limbs and this corresponded to the significantly greater IT seen in the stented sides. Again, the IT and LA data did not tally in the proximal graft sections and once again, fixation artefact might have been responsible for this apparent inconsistency. The interesting point to arise from the luminal area data is the apparent compensatory dilatation seen in sections of the the native artery in controls distal to the anastomosis and in the outflow artery one centimetre proximal to the anastomosis. These changes were not seen in stented sections. Also the differences seen in the luminal area were in sections where no difference in IT existed between stented limbs and controls. It may be that the stent had an effect on shear forces and cyclical stretch at these levels, preventing such changes. It can only be speculated that in the controls, various vasoactive substances (such as endothelium-derived relaxing factor and prostacyclin, amongst others) were able to act unopposed allowing for a compensatory dilatation of the native artery. Some of these effects may have been due to the full endothelial cell coverage seen in the stented graft limbs, not seen in the controls.

Summary

This study of endovascular anastomotic stenting in a canine model of PTFE-to-artery bypass grafting has demonstrated that the self-expanding, flexible "Wallstent" can be placed across such anastomoses without undue influence on patency up to 12 weeks after stent deployment. This applies to a bypass where the distal anastomosis is of either the end-to-end or the end-to-side configuration.

In both of the models of distal anastomotic configuration, the stented graft limbs were associated with less intimal thickening (IT) at the graft-to-artery suture line than were the nonstented control graft limbs.

However, in both models, there was a significant accumulation of IT at the proximal stent-graft interface, a feature not seen at comparable levels in controls.

Intimal thickening within the 2 cm stented graft-artery segment stabilised after 4 weeks, whereas in controls, there was a significant increase in IT between 4 and 12 weeks.

The native arteries of the control sides appeared to undergo some form of compensatory dilatation, a feature not associated with anastomotic stenting.

Scanning electron microscopy studies showed full endoluminal cellular coverage by 4 weeks, most probably due to endothelialisation. In addition, the area of the stent that crossed the anastomotic artery in the end-to-side model did not gain any cellular coverage and flow was maintained both prograde and retrograde through the stent filaments in all cases.

Conclusions

What are the possible mechanisms by which the placement of a stent across a vascular anastomosis could give rise to these effects on the site of development of and the amount of neointimal hyperplasia? From the preceding overview of neointimal hyperplasia, it is clear that many factors influence the development of this lesion. It is reasonable to assume that the stent had some influence on some or all of these factors to some extent.

Let us consider some of the cellular events that occur after stent deployment. Immediately after the stent is deployed within a vessel, plasma proteins from the circulating blood, including fibrin, become adherent to the filaments of the stent. Cell adherence then occurs with platelet, white and red blood cell aggregation in this area resulting in cell and platelet-rich fibrin thrombus deposition. If there is an underlying exposed media, these aggregated cells can then start the mitogenic cascade that leads to smooth muscle cell migration, proliferation and transformation into a pool of collagenous matrix-secreting cells, thereby leading to the development of neointimal hyperplasia. Endothelial cell coverage of the luminal surface of the stent is complete after a few weeks in animal models and a few months in humans. It is presumed that complete endothelial coverage limits thrombus formation and has an inhibitory effect on cell adherence and the stimulation of the underlying medial cellular elements is blocked.

In the model of anastomotic stenting described in the above experiments, certain differences must exist from the events that follow angioplasty and stent deployment within a native artery alone. The proximal half of the stent was deployed onto the luminal surface of a PTFE graft. The mid-point of the stent lay at the graft-to-artery suture line and the distal half of the stent was sited within the native artery. Thus, the stent was in contact with three separate surfaces which might be expected to behave differently. All surfaces were exposed to circulating blood elements through the interstices of the stent filaments. At the anastomotic suture line, the underlying medial smooth muscle cells were inevitably exposed to circulating blood elements. However, as IT was significantly less in stented sections at this level at 12 weeks in

the end-to-end model and less, but not significantly so, in the end-to-side model, endothelial cell coverage may have had the effect of limiting the duration of and amount of smooth muscle cell stimulation that took place, by reducing the amount of cell adherence and mitogen secretion. In the non-stented,control PTFE-artery anastomoses, the endothelial cell covered neointima was seen to migrate proximally from the suture line into the graft for a length of several millimetres, as reported by others. In the stented portion of PTFE in these experiments, from the anastomotic suture line 1 cm proximally to the proximal margin of the stent, no such "plug" of neointima was seen, the IT being uniform from the level of the suture line to the proximal margin of the stent. As this area also developed full endoluminal cellular coverage by 4 weeks, it was assumed that this cell coverage prevented the development of a plug of neointima at this level and also limited the thickness of the neointima within the stented portion of graft material.

At the proximal stent-graft interface and proximally into the graft of stented graft limbs, we saw significantly greater neointimal hyperplasia than at comparable levels in controls, irrespective of the distal anastomotic configuration. This finding tends to imply that factors, of either a cellular or a mechanical nature, or both, had been translocated from the graft-to-artery suture line proximally to the stent-graft interface.

There is good evidence from the literature that cyclic stretching, as occurs during pulsatile blood flow, stimulates the proliferation of medial smooth muscle cells and also induces them to secrete extracellular matrix. It is possible that, at the anastomotic level, the presence of a relatively rigid, stainleess steel endovascular stent prevented such cyclical stretch and its resulting effects. The alleged radial compliance of the Wallstent has recently been shown to be lost very soon after stent deployment[10], further strengthening this argument as a possible mechanism for the significant reduction in IT seen in the stented graft limbs at the anastomotic level compared to non-stented controls.

As discussed earlier, the role of compliance mismatch in the aetiology of anastomotic neointimal hyperplasia has yet to be established conclusively. It has to be assumed that within the stent the compliance of the conduit was uniform. As such, any mismatch of

compliance which has been shown to be present at the graft-artery suture line by other workers, should have been negated. We observed a significant accumulation of IT at the proximal stent-graft interface and it could be hypothesised that any mismatch of compliance present at the graft-artery anastomosis had been relocated to this level. The fact that this finding was seen in both models of distal anastomotic configuration tends to lend credance to this argument. The work of Hasson et al[122,123] on para-anastomotic hypercompliance zones (PHZ) showed that the suture line at a vascular anastomosis was responsible for an increase in compliance just distal to the anastomosis with a return to normal vessel compliance distal to this point. The PHZ in their experimental model was always located within 3-4 mm of the anastomotic suture line. It is reasonable to assume that any such zone that existed in the model utilised in the experiments under consideration here would have been included within the stented section of artery. However, as the measurement of such haemodynamic parameters was not included in our project, this can only be a speculative hypothesis.

As mentioned above, the recent report by Back et al[10] on the measurement of compliance changes in the canine iliac artery after the deployment of the Wallstent may have contributed some useful information on the role of compliance mismatch in the development of anastomotic neointimal hyperplasia. The authors found that the stented sections of artery became less compliant from the moment of stent deployment. There was a clear difference in compliance between the stented sections of vessel and neighbouring non-stented sections. However, in their neointimal thickness data, there was no obvious accumulation of neointima at the stent-artery interface. This study may have provided evidence that compliance mismatch is not a significant factor in the genesis of neointimal hyperplasia. The obvious difference between the work of Back et al[10] and the experiments detailed in this thesis is that here we have considered the effects of stenting graft-artery anastomoses, whereas Back and colleagues placed stents within untreated native arteries. These authors clearly deployed stents that induced a degree of angioplasty as there was a significant difference in luminal area between stented and control arteries. Furthermore, they experienced evidence of

atrophy of the tunica media, a feature that was not apparent in the present work. This may have been due to the deployment of slightly over-sized stents in their study.

In common with the in vitro studies of Crawshaw et al[76], the recent work of Ojha and colleagues[199] showed that minimising the anastomotic angle in an end-to-side distal anastomosis led to least flow disturbance within the vicinity of the anastomosis and gave rise to an anastomisis least likely to develop neointimal hyperplasia. All grafts in our end-to-side model were cut to an angle of 45 degrees in an attempt to standardise the anastomotic angle. Although the Wallstent is flexible longitudinally, and can therefore traverse an end-to-side anastomosis without compromising the stent's lumen, it is conceivable that since its natural orientation is straight when fully deployed, that the stent, once located across the anastomosis tended to straighten the anastomotic angle to something less than the control, non-stented side. If this was so, it might explain the lesser degree of IT seen at the graft-artery anastomotic level in the end-to-side model. In the end-to-end model, where there were no such geometric considerations, it may be that any cyclical distortion of the anastomosis during the cardiac cycle may have been overcome by the presence of the stent.

The in vitro studies reported by Crawshaw et al[76] implied that patency of the proximal outflow segment of an end-to-side distal ananstomosis was an unfavourable factor for the flow patterns leading to the development of ANH. Subsequent animal studies from the same group with a canine femoro-femoral dacron bypass failed to show any significant difference in ANH at the distal anastomosis between dogs with a patent proximal outflow artery and those with the vessel occluded immediately proximal to the anastomosis.[166] In our experimental model of end-to-side anastomosis, the proximal outflow artery was left patent and in fact flow via this vessel went to either the internal iliac system or the opposite external iliac artery. There was no significant difference in IT within the proximal outflow artery itself but the nonstented native artery did undergo a compensatory dilatation which was also noted at the other native artery sections. All of the stented native arteries remained patent, even in the case where the stented graft limb occluded. Thus in the model used for these

experiments, the proximal outflow segment was patent in both the stented graft limbs and the nonstented controls.

The description by LoGerfo et al[168] of the boundary layer separation zone seen at arterial bifurcations and at vasular anastomoses may also be pertinent to the findings at the proximal stent-graft interface seen in both the end-to-end and end-to-side models. Because of the apparent "step" where the stent was fixed to the luminal surface of the graft, areas of flow reversal and of "eddy" flow may well have resulted within the boundary layer of flowing blood. Such areas of low shear, where circulating cells are in contact with the luminal surface of the conduit for longer periods than is the case with laminar flow are recognised locations for the development of ANH. In addition, it is at the proximal stent-graft interface that circulating blood cells and platelets, already stimulated by contact with the surface of the prosthetic graft material first came into contact with the edge of the fully endothelialised stented graft portion. It is possible that for these reasons (boundary layer changes, diameter change, edge of endothelialised neointima) significant quantities of ANH developed at the stent-graft interface and extended proximally into the graft from this level.

ENDOVASCULAR ANASTOMOTIC STENTING
FUTURE DEVELOPMENTS AND CLINICAL IMPLICATIONS

The experimental work reported in this thesis constitutes the first description of the use of endovascular stenting as a possible method of limiting the development of anastomotic neointimal hyperplasia. It is true to say that at the anastomotic level, the presence of a stent led to the development of significantly less neointimal hyperplasia, although, as we have seen, this was at the expense of an accumulation of neointima at the proximal stent-graft interface. It is interesting to speculate on future developments of experimental methodology that might reproduce these beneficial effects of stenting at the anastomotic level whilst overcoming the neointimal changes at the proximal stent-graft interface. This problem might conceivably be overcome by the use of larger diameter stents with an "intentional" angioplasty effect, stents of greater length or tandem stents. All of these approaches might make it possible to overcome the significantly greater intimal thickening at the stent-graft interface level encountered in the current series of experiments, by increasing the diameter of the conduit at this level.

Alternatively, other stent designs could be used in the same canine model of an aorto-iliac bypass graft. One could hypothesise that the use of a balloon-expandable Palmaz stent might effectively "angioplasty" the region of the proximal stent-graft interface. In addition, such stents, being non-flexible in a longitudinal direction might have the effect of reducing the anastomotic angle and so "straighten" an end-to-side anastomosis potentially making it more favourable haemodynamically.

The current experimental model could be repeated in order to study, in vivo, the effect of anastomotic stenting on flow within the stented graft limb and run-off artery compared to the control side and whether blood flow through a stented anastomosis changes with time. Much information could be gleaned about the role of compliance mismatch in the aetiology of anastomotic neointimal hyperplasia by measuring the compliance, in vivo, of such a model,

looking particularly at the graft-to-artery anastomosis on the nonstented, control side and comparing this region to the area of the stent-graft interface on the stented side. Compliance could be measured directly, as in the methodology adopted by Hasson et al[122] in their work on para-anastomotic hypercompliance zones or by utilising endovascular technology, as in the work of Back et al.[10]

The effects of different stent types and dimensions could also be assessed in a similar manner. The Wallstent was selected for use in these initial studies of anastomotic stenting as it is flexible in all directions. This was thought to make it the best choice of device to avoid distortion of the geometry of the anastomosis, especially in the case of the end-to-side configuration. It could be postulated that a more rigid, less flexible stent, such as the balloon-expandable Palmaz stent might actually serve to lessen the anastomotic angle by "straightening" the anastomosis and this could influence the site and development of anastomotic neointimal hyperplasia. We were meticulous in measuring vessel dimensions prior to handling so as to avoid selecting an excessively large stent diameter. The fact that we did not observe any of the medial atrophy described by others would tend to imply that no overdistension of the native arteries took place. However, this may also be why the positive effects of stenting on intimal thickening were not always so clearly reflected in significant luminal area changes. It may be that, at the expense of inducing thinning/atrophic changes in the tunica media, the use of an over-sized stent could have positive effects on neointimal thickness whilst also enhancing the residual luminal area.

Biodegradable stents could have a role in this field. Cong and colleagues[69] used a stent composed of a mixture of glyceride, diglyceride, triglyceride and zinc to facilitate the construction of microvascular anastomoses in a rabbit femoral artery model. Not only did stenting enhance the time taken to perform the anastomosis but also it improved endothelial apposition. Studies of the vessels after up to 70 days post-operatively failed to demonstrate any significant difference in the thrombosis rate between stented and non-stented anastomoses. The stent material dissolved and there was no evidence of any injurious effect of the device upon the arterial intima or media. Could this experimental work in the field of

microsurgery be translated to the current area of interest, namely large vessel anastomoses between prosthetic grafts and arteries? If so, it could be hypothesised that the stent could induce endothelial coverage of the anastomosis, after which it would be absorbed. The question here is whether the presence of the rigid stent structure is necessary to prevent late neointimal hyperplasia. Even so, this is another exciting area worthy of further investigation. Another approach altogether, which is as relevant to the field of arterial stenting after percutaneous balloon angioplasty as it is to endovascular anastomotic stenting is the possibility of using drug-impregnated stents. For example, antiplatelet agents could then act locally preventing platelet adherence and degranulation thereby limiting the amount of thrombus formation and also mitogen secretion. It is possible that such agents acting as a local "depot" could limit the extent of development of the neointimal hyperplastic lesion until full endothelialisation was achieved since after this time, it seems that the lesion becomes stable within stented arteries and as we have shown in these experiments, in the region of stented graft-to-artery anastomoses.

The ultimate question to ask about this experimental work is what is the potential role of anastomotic stenting in clinical vascular surgical practice. Anastomotic neointimal hyperplasia continues to be a significant cause of the late failure of prosthetic infrainguinal bypass grafts, accounting for over 20% of graft thromboses in most clinical series. Our experiments in a canine model have shown that it is possible to limit the development of neointimal hyperplasia at the anastomotic level by the placement of a stent across a distal graft-artery anastomosis.

It has also been demonstrated that where a stent crosses a patent artery with blood flowing through it, the device does not gain cellular coverage and the stent filaments remain exposed in the flowing bloodstream. Extrapolated to the clinical setting, this finding has important implications for the placement of stents across branch arteries, the point being that doing so does not inevitably lead to the occlusion of the branch artery in question. This has particular significance with respect to the endovascular grafting of abdominal aortic aneurysms, a technique that utilises endovascular stents to secure the proximal and distal ends of the

endovascular graft. Where important branch arteries, such as the renal arteries, are close to the level of the proximal stent deployment, it may be that to place the stent across the ostia of these vessels is safe. Further studies on this particular aspect of endovascular stenting are required to clarify this issue.

The placement of endovascular grafts to replace aneurysms[158] or occluded segments of native arteries, the so-called "endovascular bypass", has as a key component of the procedure, the fixation of the prosthetic graft proximally and in some cases distally, with endovascular stents.[74] The findings in our experiments of significant accumulations of neointimal hyperplasia at the proximal stent-graft interface may have particular relevance to this clinical setting. To extrapolate our results to the endovascular bypass procedure, it is possible that time will show that these grafts may fail because of the development of neointimal hyperplasia at either the proximal end, or the distal end, or both ends of the graft at the stent-graft interface.

A final technical point that has a great deal of relevance to the practice of clinical vascular surgery in the coming decades relates to our findings at reoperation at the time of graft harvest. As reported by Back et al amongst others, the stent appears to invoke a transmural reaction around the stented graft and vessel. In every case, a considerable amount of densely adherent fibrous tissue was encountered, which made dissection technically demanding. In addition, recognition of anatomical structures involved in this mass of fibrous tissue, for example the ureters, was made difficult owing to the bulk of the fibrotic tissue. Endovascular therapy, including stenting, has now become an established component of the treatment of occlusive and more recently aneurysmal vascular disease. With time, there will undoubtedly be a need to re-operate on the arteries that have been treated with endovascular stents. From our experience in the canine model at least, such operations may be rendered technically difficult by the presence of a stent and the fibrotic reaction it induces.

It is hoped that the positive results of this work on endovascular anastomotic stenting as a means of limiting the development of anastomotic neointimal hyperplasia in infrainguinal

bypass grafts will stimulate further research into what is an exciting new approach to a major complication of the surgical treatment of peripheral vascular disease.

APPENDIX I

Analysis of Lumen, Intima, and Media data
Comparing Stent vs. No stent and Early vs. Late

The data were analyzed using a univariate repeated measures analysis of variance. This analysis involved three factors: a between animal factor, GROUP (early vs. late), and two within animal factors, STENT (stent vs. no stent) and SECTION. Comparisons of interest were examined using one degree of freedom mean contrasts and then tested with the t-test statistic. The standard errors used in computing the t-test statistic for these contrasts were estimated from the between and within animal mean square errors.

A separate analysis was performed for the data from the end-to-end model and for the data from the end-to-side model. A summary of the unvariate analysis of variance, the pairwise comparisons (no stent vs. stent compared at each section for each group; early vs. late compared at each section for stent and no stent), and the mean and standard error estimates are presented in the following pages.

The standard error estimates were based on the pooled estimate of variability obtained from the analysis of variance.

Analysis of Luminal Area

End-to-Side Model

Test of main factor effects and interactions:

Factor	df	Mean Square	Denominator df	Denominator mean square	F	p-value
Group	1	38.502	3.18	17.489	2.20	0.2295
Stent	1	111.546	3.73	4.757	23.45	0.0100
Group/stent	1	21.872	3.68	4.765	4.59	0.1047
Section	5	22.597	16.71	4.942	4.57	0.0082
Group/section	5	13.376	16.67	4.944	2.71	0.0572
Stent/section	5	6.095	12	3.796	1.61	0.2320
Group/stent/section	5	3.100	12	3.796	0.82	0.5603

No significant three and two factor interaction. There was a significant overall stent effect (comparing luminal area averaged across groups and sections).

No Stent (NS) vs. Stent(S) for each group and section:

Group	Section	Difference (NS-S)	Standard Error	df	t	2-tailed p-value
L	1	2.695	1.633	14.59	1.65	0.1203
L	2	7.192	1.633	14.59	4.40	0.0005
L	3	2.748	1.633	14.59	1.68	0.1138
L	4	4.613	1.633	14.59	2.82	0.0130
L	5	0.766	1.633	14.59	0.47	0.6462
L	6	8.707	2.956	13.46	2.95	0.0110
E	1	1.896	2.001	14.59	0.95	0.3586
E	2	2.597	2.001	14.59	1.30	0.2144
E	3	2.140	2.925	14.06	0.73	0.4764
E	4	0.496	2.001	14.59	0.25	0.8075
E	5	0.658	2.001	14.59	0.33	0.7467
E	6	2.202	2.001	14.59	1.10	0.2888

The luminal area in the non-stented side was significantly greater compared to the stented side in Sections 2, 4 and 6 of the late group. No significant difference in luminal area was observed between the non-stented and the stented side in all sections of the early group.

Early (E) vs. Late (L) for stent and no stent at each section:

Stent/ No Stent	Section	Difference (L-E)	Standard Error	df	t	2-tailed p-value
NS	1	5.084	2.223	21.07	2.29	0.0326
NS	2	5.319	2.223	21.07	2.39	0.0261
NS	3	3.095	2.223	21.07	1.39	0.1784
NS	4	0.971	2.223	21.07	0.44	0.6667
NS	5	-1.090	2.223	21.07	-0.49	0.6291
NS	6	6.086	2.223	21.07	2.74	0.0123
S	1	4.286	2.223	21.07	1.93	0.0675
S	2	0.724	2.223	21.07	0.33	0.7477
S	3	2.458	3.082	23.98	0.80	0.4330
S	4	-3.149	2.223	21.07	-1.42	0.1713
S	5	-1.197	2.223	21.07	-0.54	0.5960
S	6	-0.419	3.319	22.48	-0.13	0.9007

A significantly greater mean luminal area was observed in the late group compared to the early group in Sections 1, 2, and 6 of the non-stented side. No significant difference in mean lumen area in the stented side was observed between the late and early group.

MEAN AND STANDARD ERROR ESTIMATES: End-to-side model: Luminal area

Group	Stent/No stent	Section	Mean	SE
L	NS	1	13.731	1.406
L	NS	2	14.839	1.406
L	NS	3	12.209	1.406
L	NS	4	13.028	1.406
L	NS	5	11.680	1.406
L	NS	6	12.018	1.406
L	S	1	11.036	1.406
L	S	2	7.647	1.406
L	S	3	9.461	1.406
L	S	4	8.414	1.406
L	S	5	10.915	1.406
L	S	6	3.311	2.837
E	NS	1	8.646	1.722
E	NS	2	9.519	1.722
E	NS	3	9.114	1.722
E	NS	4	12.056	1.722
E	NS	5	12.770	1.722
E	NS	6	5.932	1.722
E	S	1	6.750	1.722
E	S	2	6.922	1.722
E	S	3	6.974	2.742
E	S	4	11.563	1.722
E	S	5	12.112	1.722
E	S	6	3.730	1.722

Analysis of Luminal Area

End-to-End Model

Test of main factor effects and interactions:

Factor	df	Mean Square	Denominator df	Denominator mean square	F	p-value
Group	1	131.694	2.98	54.123	2.43	0.2172
Stent	1	90.139	3.03	133.659	0.67	0.4712
Group/stent	1	26.525	3.03	133.017	0.20	0.6852
Section	4	54.667	12.62	24.870	2.20	0.1282
Group/section	4	48.896	12.63	24.859	1.97	0.1609
Stent/section	4	8.395	10	11.566	0.73	0.5941
Group/stent/section	4	8.402	10	11.566	0.73	0.5937

No significant three and two factor interaction. There was no significant overall difference between early and late and between no stent and stent.

No Stent(NS) vs. Stent(S) for each group and section:

Group	Section	Difference (NS-S)	Standard Error	df	t	2-tailed p-value
L	1	8.480	5.651	6.33	1.50	0.1816
L	2	1.497	5.210	4.81	0.29	0.7858
L	3	3.583	5.210	4.81	0.69	0.5234
L	4	6.829	5.210	4.81	1.31	0.2490
L	5	2.170	5.210	4.81	0.42	0.6949
E	1	4.156	6.381	4.81	0.65	0.5447
E	2	3.880	6.381	4.81	0.61	0.5707
E	3	-0.485	7.428	7.76	-0.06	0.9496
E	4	-1.130	6.381	4.81	-0.18	0.8667
E	5	-0.146	6.381	4.81	-0.02	0.9826

No significant difference in mean luminal area between the no stent and the stent for both early and late groups in all the sections.

Early (E) vs. Late(L) for Stent and No Stent at each section:

Stent/ No Stent	Section	Difference (L-E)	Standard Error	df	t	2-tailed p-value
NS	1	13.812	5.998	15.96	2.30	0.0351
NS	2	2.676	5.582	12.86	0.48	0.6396
NS	3	4.205	5.582	12.86	0.75	0.4648
NS	4	5.916	5.582	12.86	1.06	0.3087
NS	5	-0.322	5.582	12.86	-0.06	0.9549
S	1	9.488	5.582	12.86	1.70	0.1132
S	2	5.059	5.582	12.86	0.91	0.3814
S	3	0.136	6.754	19.57	0.02	0.9841
S	4	-2.043	5.582	12.86	-0.37	0.7203
S	5	-2.639	5.582	12.86	-0.47	0.6443

A greater mean luminal area was observed in the late group compared to the earlier group in Section 1 of the non-stented side.

MEAN AND STANDARD ERROR ESTIMATES: End-to-end model: Luminal area

Group	Stent/No stent	Section	Mean	SE
L	NS	1	19.7885	4.157
L	NS	2	9.8393	3.530
L	NS	3	10.5253	3.530
L	NS	4	17.2157	3.530
L	NS	5	12.2517	3.530
L	S	1	11.3073	3.530
L	S	2	8.3420	3.530
L	S	3	6.9420	3.530
L	S	4	10.3853	3.530
L	S	5	10.0803	3.530
E	NS	1	5.9750	4.324
E	NS	2	7.1630	4.324
E	NS	3	6.3205	4.324
E	NS	4	11.2985	4.324
E	NS	5	12.5725	4.324
E	S	1	1.8195	4.324
E	S	2	3.2830	4.324
E	S	3	6.8069	5.758
E	S	4	12.4280	4.324
E	S	5	12.7190	4.324

Analysis of Intimal Thickness

End-to-end Model

Test of Main Factor effects and interactions:

Factor	df	Mean Square	Denominator df	Denominator mean square	F	p-value
Group	1	0.08297	2.88	0.00270	30.77	0.0129
Stent	1	0.00026	2.85	0.00343	0.08	0.8016
Group/stent	1	0.00032	2.85	0.00342	0.09	0.7803
Section	4	0.01460	12.42	0.00428	3.41	0.0426
Group/section	4	0.00458	12.40	0.00429	1.07	0.4130
Stent/section	4	0.00053	9	0.00121	0.44	0.7772
Group/stent/section	4	0.00977	9	0.00121	8.06	0.0048

There was a significant three factor interaction between group, stent and section.

No Stent (NS) vs. Stent (S) for each group and section:

Group	Section	Difference (NS-S)	Standard Error	df	t	2-tailed p-value
L	1	0.03784	0.04106	10.97	0.92	0.3766
L	2	0.02076	0.04106	10.97	0.51	0.6232
L	3	0.11740	0.03425	8.75	3.43	0.0078
L	4	-0.10402	0.03425	8.75	-3.04	0.0145
L	5	-0.07363	0.03425	8.75	-2.15	0.0609
E	1	-0.01696	0.04194	8.75	-0.40	0.6956
E	2	0.00318	0.04194	8.75	0.08	0.9413
E	3	-0.06270	0.05722	11.52	-1.10	0.2955
E	4	0.07370	0.04194	8.75	1.76	0.1137
E	5	0.05875	0.04194	8.75	1.40	0.1957

Mean intimal thickness was significantly greater in the stented compared to the non-stented side in Section 4 of the late group. The reverse was observed for Section 3 of the late group where mean intimal thickness was significantly greater for the non-stented side compared to the stented side.

Early (E) vs. Late (L) for Stent and No Stent at each section:

Stent/ No Stent	Section	Difference (L-E)	Standard Error	df	t	2-tailed p-value
NS	1	0.05038	0.05612	26.18	0.90	0.3775
NS	2	0.10501	0.05612	26.18	1.87	0.0725
NS	3	0.23327	0.05134	22.33	4.54	0.0002
NS	4	-0.02194	0.05134	22.33	-0.43	0.6732
NS	5	0.06723	0.05134	22.33	1.31	0.2037
S	1	-0.00442	0.05134	22.33	-0.09	0.9322
S	2	0.08743	0.05134	22.33	1.70	0.1024
S	3	0.05317	0.06443	25.36	0.83	0.4169
S	4	0.15578	0.05134	22.33	3.03	.00060
S	5	0.19961	0.05134	22.33	3.89	0.0008

Significantly greater intimal thickness in the non-stented side of the late group compared to the early group in Section 3. The same was observed for Sections 4 and 5 of the stented side.

MEAN AND STANDARD ERROR ESTIMATES:

End-to-end model: Intimal thickness

Group	Stent/No stent	Section	Mean	SE
L	NS	1	0.1345	0.03962
L	NS	2	0.2113	0.03962
L	NS	3	0.2983	0.03248
L	NS	4	0.1686	0.03248
L	NS	5	0.2425	0.03248
L	S	1	0.0966	0.03248
L	S	2	0.1905	0.03248
L	S	3	0.1809	0.03248
L	S	4	0.2726	0.03248
L	S	5	0.3161	0.03248
E	NS	1	0.0841	0.03975
E	NS	2	0.1062	0.03975
E	NS	3	0.0650	0.03975
E	NS	4	0.1905	0.03975
E	NS	5	0.1753	0.03975
E	S	1	0.1010	0.03975
E	S	2	0.1031	0.03975
E	S	3	0.1277	0.05559
E	S	4	0.1169	0.03975
E	S	5	0.1165	0.03975

Analysis of Intimal Thickness

End-to-Side Model

Test of main factor effects and interactions:

Factor	df	Mean Square	Denominator df	Denominator mean square	F	p-value
Group	1	0.05289	3.25	0.00772	3.25	0.0729
Stent	1	0.04530	3.82	0.00180	25.12	0.0084
Group/stent	1	0.00038	3.65	0.00178	0.22	0.6707
Section	5	0.05248	16.64	0.00544	9.65	0.0002
Group/section	5	0.01209	16.47	0.00546	2.21	0.1021
Stent/section	5	0.00393	10	0.00338	1.16	0.3906
Group/stent/section	5	0.01162	10	0.00338	3.44	0.0456

There was a significant three factor interaction between group, stent and section.

No stent (NS) vs. Stent (S) for each group and section:

Group	Section	Difference (NS-S)	Standard Error	df	t	2-tailed p-value
L	1	-0.04928	0.04485	11.19	-1.10	0.2950
L	2	-0.06653	0.04485	11.19	-1.48	0.1656
L	3	0.09173	0.04485	11.19	2.04	0.0651
L	4	-0.13234	0.05866	10.81	-2.26	0.0458
L	5	-0.20572	0.05866	10.81	-3.51	0.0050
L	6	-0.06168	0.08790	10.39	-0.70	0.4983
E	1	0.00035	0.05493	11.19	0.01	0.9950
E	2	-0.02026	0.05493	11.19	-0.37	0.7191
E	3	-0.18311	0.08407	10.62	-2.18	0.0529
E	4	-0.16281	0.05493	11.19	-2.96	0.0127
E	5	0.04692	0.05493	11.19	0.85	0.4109
E	6	-0.03342	0.05493	11.19	-0.61	0.5551

Mean intimal thickness is significantly greater in the stented compared to the non-stented graft limbs in Sections 4 and 5 of the late group and Section 4 of the early group. Mean intimal thickness at Section 3 of the late group is greater for non-stented compared to stented in the late group but just fails to reach statistical significance.

Early (E) vs. Late (L) for Stent and No Stent at each section:

Stent/ No Stent	Section	Difference (L-E)	Standard Error	df	t	2-tailed p-value
NS	1	-0.02611	0.06329	27.25	-0.41	0.6832
NS	2	-0.01422	0.06329	27.25	-0.22	0.8239
NS	3	0.18521	0.06329	27.25	2.93	0.0068
NS	4	0.18586	0.07372	28.07	2.51	0.0180
NS	5	0.04301	0.06329	27.25	0.68	0.5025
NS	6	0.00946	0.06329	27.25	0.15	0.8823
S	1	0.02351	0.06329	27.25	0.37	0.7132
S	2	0.03205	0.06329	27.25	0.51	0.6166
S	3	-0.08964	0.08975	21.89	-1.00	0.3288
S	4	0.15540	0.06329	27.25	2.46	0.0207
S	5	0.29564	0.07372	28.06	4.01	0.0004
S	6	0.03773	0.09859	19.31	0.38	0.7061

Significantly greater intimal thickness in the nonstented side of the late group compared to the early group in Sections 3 and 4. The same was observed for Sections 4 and 5 of the stented side.

MEAN AND STANDARD ERROR ESTIMATES:

End-to-side model: Intimal thickness

Group	Stent/No stent	Section	Mean	SE
L	NS	1	0.0739	0.03977
L	NS	2	0.0721	0.03977
L	NS	3	0.2886	0.03977
L	NS	4	0.2978	0.05501
L	NS	5	0.2202	0.03977
L	NS	6	0.0656	0.03977
L	S	1	0.1261	0.03977
L	S	2	0.1386	0.03977
L	S	3	0.1969	0.03977
L	S	4	0.4302	0.03977
L	S	5	0.4259	0.05501
L	S	6	0.1272	0.05501
E	NS	1	0.1030	0.08553
E	NS	2	0.0863	0.04904
E	NS	3	0.1034	0.04904
E	NS	4	0.1120	0.04904
E	NS	5	0.1772	0.04904
E	NS	6	0.0561	0.04904
E	S	1	0.1026	0.04904
E	S	2	0.1066	0.04904
E	S	3	0.2865	0.08039
E	S	4	0.2748	0.04904
E	S	5	0.1303	0.04904
E	S	6	0.0895	0.04904

Analysis of Tunica Media Data

No statistically significant differences were observed in the comparison of the stent vs. no stent for both late and early groups in Sections 1 and 2. The same was observed when comparing early vs. late in the stented and non-stented sides. These results hold for both the end-to-end and the end-to-side data. Note that in interpreting these results the sample size of the study was very small and therefore the power of the test to detect certain defined differences may have been low.

APPENDIX II

PRESENTATIONS BASED UPON THIS THESIS

1) **Endovascular Anastomotic Stenting in a Canine Model.**
R.T.A. Chalmers.

Presented at the University of Edinburgh School of Surgery Day, Royal College of Surgeons, Edinburgh, November, 1993.

2) **The Effects of Polytetrafluoroethylene Graft Anastomotic Stenting in a Canine Model.**
R.T.A Chalmers, J.J. Hoballah, W.J. Sharp, T.F. Kresowik, J.D. Corson.

Presented at the 22nd Annual Symposium of the Society for Clinical Vascular Surgery, Tucson, Arizona, March, 1994.

3) **The Effect of Endovascular Stenting on the Healing of a Polytetrafluoroethylene End-to-end Anastomosis in a Canine Model.**
R.T.A. Chalmers, J.J. Hoballah, W.J. Sharp, T.F. Kresowik, J.D. Corson.

Presented at the Annual Meeting of the Association of Surgeons of Great Britain and Ireland, Harrogate, England, April 22nd, 1994.

4) **The Effect of an Intraluminal Stent on Neointimal Hyperplasia at an End-to-side Polytetrafluoroethylene Graft Arterial Anastomosis.**
R.T.A. Chalmers.

Presented at the Joint Clinical and Scientific Meeting of the Royal College of Surgeons of Edinburgh and the Royal Society of Medicine, Edinburgh, May 26th, 1994.

5) **The Effect of Endovascular Anastomotic Stenting in a Canine Model.**
R.T.A. Chalmers.

Presented at the First Tripartite Meeting of the Vascular Surgical Society of Great Britain and Ireland, the British Society of Interventional Radiologists and the Angiology Forum, Stratford-upon-Avon, England, June 3rd, 1994.

APPENDIX III

PUBLICATIONS BASED ON THIS THESIS

1) **Chalmers R.T.A.**, Hoballah J.J., Sharp W.J., Kresowik T.F., Corson J.D.
THE EFFECT OF AN INTRALUMINAL STENT ON NEOINTIMAL HYPERPLASIA AT AN END-TO-SIDE POLYTETRAFLUOROETHYLENE GRAFT-ARTERIAL ANASTOMOSIS.
Am J Surg 1994;**168**:85-90.

2) **Chalmers R.T.A.**, Hoballah J.J., Sharp W.J., Kresowik T.F., Corson J.D.
THE EFFECT OF AN ENDOVASCULAR STENT ON THE HEALING OF A POLYTETRAFLUOROETHYLENE END-TO-END ANASTOMOSIS IN A CANINE MODEL.
Br J Surg 1994;**81**: 1443-1447.

3) **Chalmers R.T.A.**, Hoballah J.J., Sharp W.J., Kresowik T.F., Corson J.D.
CHANGES IN ARTERIAL WALL COMPLIANCE AFTER ENDOVASCULAR STENTING. Letter to the Editors.
J Vasc Surg 1995;**21**:543-544.

The Effect of an Intraluminal Stent on Neointimal Hyperplasia at an End-to-Side Polytetrafluoroethylene Graft Arterial Anastomosis

Roderick T.A. Chalmers, MB, ChB, FRCS (Ed), Jamal J. Hoballah, MD, William J. Sharp, MD, FACS, Timothy F. Kresowik, MD, FACS, John D. Corson, MB, ChB, FRCS(Eng), FACS, *Iowa City, Iowa*

BACKGROUND: Anastomotic neointimal hyperpla- plays a significant role in the late failure of frainguinal prosthetic arterial bypass grafts. revious work from our laboratory revealed at placing a stent across an end-to-end arterio- terial anastomosis resulted in an increase in luminal area as well as in the intimal thick- ss (IT) at the anastomotic level. This study s designed to evaluate the effects on neointi- l hyperplasia when a stent is placed across an l-to-side polytetrafluoroethylene (PTFE) graft erial anastomosis.

METHODS: A canine model of an end-to-side astomosis was developed using a 12 × 6 mm ytetrafluoroethylene aortobi-iliac graft. A self- anding stainless steel Wallstent was placed oss one randomly selected distal anastomosis ving the opposite side as a control. Dogs were rificed at 4 and 12 weeks. At sacrifice, the ft and intact anastomoses were pressure-per- on fixed with glutaraldehyde. Sections of h distal graft, anastomosis, and recipient ry were obtained for analysis. Computer im- s of each section were digitized to determine luminal area and the mean IT. The data e analyzed statistically using univariate re- ed measures of analysis of variance.

RESULTS: One animal died prior to early sacri- Eight of 10 graft limbs remained patent at ifice. Of the 2 limbs that occluded, one was ted and one was nonstented. At 4 weeks, ed graft limbs had significantly greater IT at proximal stent level (mean difference be- n control and stented sides 0.163 mm ± 4, $P = 0.01$). Stented and nonstented anas- ses had similar luminal area and IT at levels where sections were taken.

At 12 weeks, control limbs had significantly greater IT at the anastomotic level compared to the 4-week measurements (mean difference 12 weeks versus 4 weeks 0.185 mm ± 0.06, $P = 0.006$). In the stented limbs, IT at the anastomotic level had stabilized and was not significantly thicker than at 4 weeks. The control limbs had greater IT at the anastomotic level than the stented limbs (mean difference between controls and stented sides at 12 weeks 0.091 mm ± 0.044, $P = 0.06$).

At the proximal end of the stent, IT progressed significantly between the 4th and 12th weeks (mean difference 12 weeks versus 4 weeks 0.155 mm ± 0.06, $P = 0.02$). The IT at the proximal end of the stent at 12 weeks was significantly greater than the IT at a comparable level in the controls (mean difference stent versus control 0.132 mm ± 0.05, $P = 0.04$). The luminal area in the control limbs was significantly greater than in the stented anastomoses at levels corresponding to either end of the stent (mean difference at proximal end 4.163 mm^2 ± 1.633, $P = 0.01$; mean difference at distal end 7.192 mm^2 ± 1.633, $P = 0.0005$). However, there was no difference in luminal area at the anastomotic level.

CONCLUSION: We conclude that the presence of an intraluminal stent alters the siting and degree of anastomotic neointimal hyperplasia in a canine model of an end-to-side anastomosis resulting in translocation of the intimal hyperplastic response to the proximal graft stent interface in a magnitude similar to that which would normally be found at the anastomosis.

Anastomotic neointimal hyperplasia (ANH) is thought to be the major cause of late failure of prosthetic infrainguinal arterial bypass grafts.[1-3] A major goal of vascular research is to understand the etiology of ANH in order to develop means to control it. Undoubtedly multiple factors are involved in the evolution of ANH. Mechanical events at an end-to-side anastomosis are extremely complex, involving compliance and size mismatches,[4-6] high and low shear stresses,[7,8] and cyclical stretching.[9]

The hypothesis of this study was that placing a flexible metallic intraluminal stent across a prosthetic graft-to-artery end-to-side anastomosis might influence the me-

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orted in part by a grant from Schneider Stent Co., St. Paul, Min- grafts supplied by W. L. Gore and Associates, Inc., Flagstaff,

ited at the 22nd Annual Meeting of the Society for Clinical r Surgery, Tucson, Arizona, March 2–6, 1994.

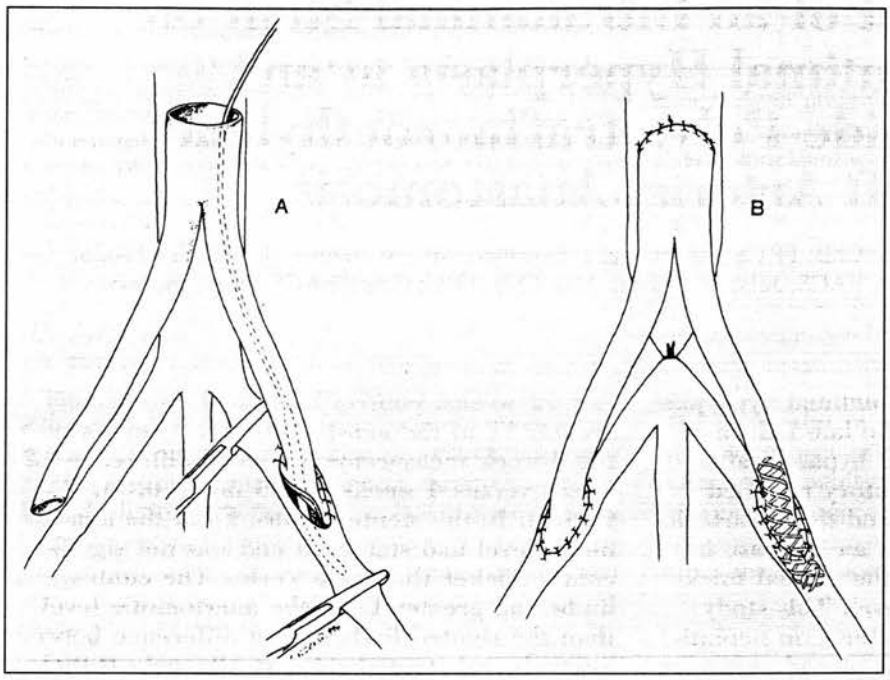

Figure 1. (A) The delivery system is shown being positioned under direct vision. **(B)** The deployed stent is seen crossing the completed left distal anastomosis.

chanical events at the anastomosis and hence modify the site and extent of ANH.

MATERIALS AND METHODS

Six conditioned male and female mongrel dogs weighing 20 to 25 kg were used. The animals were maintained in accordance with the guidelines in "Principles of Laboratory Animal Care" and "Guide for the Care and Use of Laboratory Animals" (National Institute of Health Publication Number 80-23, revised 1985). Each dog was pre-anesthetized with pentobarbital sodium (15 mg/kg), intubated, and maintained on 1% halothane admixed with room air and administered via a Harvard respirator. Intravenous isotonic saline was given as required to maintain circulating volume. Antibiotic prophylaxis consisting of 3 mL of a solution containing penicillin benzathine 150,000 U/mL, procaine penicillin 150,000 U/mL and sodium formaldehyde 1.75 mg/mL was given intramuscularly 30 minutes prior to skin incision. A bifurcated graft was created from two 6-cm lengths of thin-walled polytetrafluoroethylene (PTFE) 6 mm in diameter with a pore diameter of 30 μm (PTFE-Gortex, W.L. Gore and Associates, Inc., Flagstaff, Arizona), anastomosed with a continuous 6/0 polypropylene suture.

The abdominal aorta and iliac arteries were exposed through a midline abdominal incision. Before the iliac arteries were handled, their external diameter was measured with a caliper to identify the size of stent needed to correspond to the normal arterial diameter. The vessels were then isolated and prepared for anastomosis. Intravenous heparin was administered at a dose of 50 U/kg 5 minutes prior to arterial clamping. The side selected at random for stenting was sewn first. The distal limb of the graft was cut at a 45° angle. The length of the iliac arteriotomy was standardized to 10 mm. Using a parachute technique under 2.5× loupe magnification, the anastomosis to be stented was performed with a continuous 6/0 polypropylene suture technique. The suture line was left loose initially to allow accurate placement of the stent under direct vision.

The delivery device of a Wallstent (Schneider Stent Co., St. Paul, Minnesota) of unconstrained diameter equivalent to that of the native artery was introduced through the proximal end of the bifurcation graft and positioned equally across the anastomosis (**Figure 1A**). The stent is made of woven stainless steel and is manufactured within a constraining membrane which is mounted on a 7-Fr gauge catheter. The Wallstent is deployed by retracting the constraining membrane. It measures 2 cm in length when unconstrained. When expanded, 1 cm lay within the graft and 1 cm within the native artery. The anastomotic suture was then tightened and tied down. The opposite distal nonstented anastomosis was performed in identical fashion. The aortic anastomosis was of end-to-side, "onlay" configuration and was performed with continuous 5/0 polypropylene suture. Just before releasing the clamps and restoring the distal circulation, the aorta immediately distal to the proximal anastomosis was ligated with two heavy silk ligatures (**Figure 1B**) to ensure that all aortic flow entered the proximal end of the graft. Retrograde flow was preserved at the distal end-to-side anastomoses. We felt that this model was a close representation of the clinical situation of a graft bypassing an occluded artery.

Initial patency was assessed by intra-operative completion angiography via a proximal aortic needle puncture. No anticoagulants or antiplatelet agents were used following surgery. Postoperatively, graft patency was monitored by femoral pulse palpation and the use of a transcutaneous continuous wave Doppler examination performed daily for the first week and weekly until the time of sacrifice. In all cases at midterm, under a short general anesthetic with pentobarbital sodium, angiography of the bypass and runoff vessels was performed via a left carotid cutdown.

gs were separated into 2 groups: early (sacrificed at 4 ks) and late (sacrificed at 12 weeks). At the time of ifice, each animal was anesthetized with pentobarbital um. The aorta and graft were exposed through the pre- s incision and an angiogram was performed through oximal aortic needle puncture. The animal was then n a lethal dose of intravenous potassium chloride. The and vessels were flushed with 0.9% saline and then sure-perfusion fixed at 90 mm Hg with a 2% glu- dehyde in 0.1 molar cacodylate buffer for 1 hour. The els and graft were then explanted en bloc and im- ed in 2% glutaraldehyde for 24 hours.

ctions were taken at fixed levels from stented and non- ted sides (**Figure 2**) so that they could be compared ctly and all animals in the study could also be com- d. Computer images of all sections were created us- a Megaplus camera (Eastman Kodak, San Diego, fornia) controlled by a MacIntosh FX Workstation ple Inc., Cupertino, California). Images were traced g "mtrace" software on a Silicon Graphics Indigo kstation (Silicon Graphics Inc., Mountain View, Cali- a). "Mtrace", developed by the University of Iowa, /s the contours of images to be outlined, thereby mak- t possible to calculate areas, perimeters, and minor najor axes. A second computer program called "wall ness" was used to radially sample concentric con- and measure the difference in radius (ie, IT) at each . A mean IT for each section was calculated from data. Thus we were able to measure the luminal area -T of each section.

were analyzed using univariate repeated measures alysis of variance. This analysis involved three fac- between-animal factor (early versus late) and two -animal factors (stent versus no stent, and section section). Sites of interest were evaluated by com- the means with one degree of freedom and tested he *t*-test statistic.

JLTS

dog died postoperatively from causes not related to aft or operative procedure. The graft was not har- and this animal was exluded from statistical anal- here were no wound complications. The angiograms ned prior to sacrifice showed that 2 of the 10 graft vere occluded. Both were in the late group. One was and one nonstented. However, the outflow arteries atent proximally and distally in all 10 extremities angiographically.

ing electron microscopy studies of longitudinal taken of the stents showed that at 4 weeks the lu- urface of all stents was covered with a monolayer oriented with their long axes parallel to the direc- lood flow. However, without exception, where the ossed the outflow artery at the anastomotic level, ot covered by a similar layer of cells and the stain- l was exposed.

early sacrifice group, stented limbs had signifi- greater IT than did controls at the level corre- g to the proximal end of the stent (stented side m, controls 0.112 mm, difference 0.163 mm, $P =$ igure 2, Section 4). For all other sections in the early group, there was no significant difference in IT or luminal area between stented and control graft limbs.

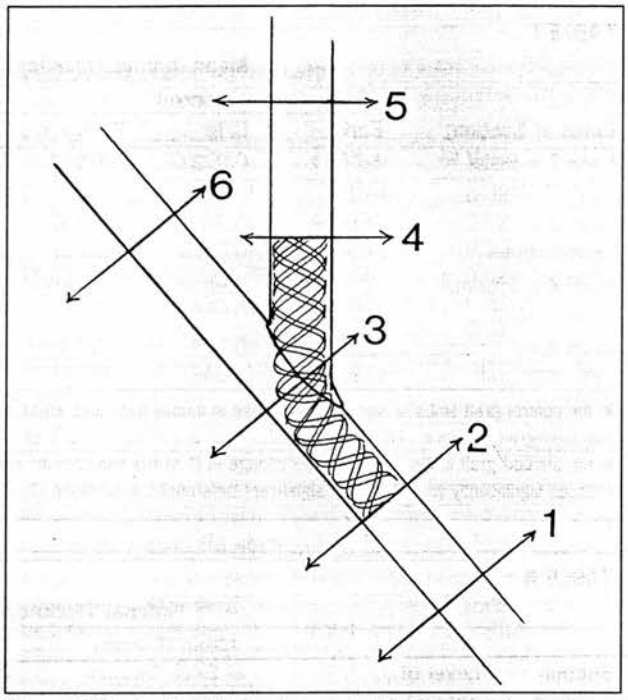

Figure 2. The levels of the sections submitted for analysis are demonstrated.

The control graft limbs in the late sacrifice group had significantly greater IT at the anastomotic level than had been seen in the early group (Figure 2, Section 3). This was not true of the stented graft limbs, whose IT at the anastomotic level was similar in the early and late groups. These findings imply that ANH at the anastomatic level progressed during the 8-week interval in the nonstented limbs, but stabilized in the stented limbs (**Table I**). At 12 weeks, control limbs had greater IT at the anastomotic level than stented limbs, but the difference just failed to reach statistical significance (**Table II**).

As in the early sacrifice group, stented limbs at 12 weeks had significantly greater IT than controls at the level corresponding to the proximal end of the stent. Furthermore, this difference had increased and extended to a more proximal level within the graft (Figure 2, Sections 4, 5) (Tables I, II).

Compared to the early sacrifice control group, the late sacrifice group had a luminal area significantly greater in the run-off artery and in the anastomotic artery at a level 1 cm proximal to the anastomosis (Figure 2, Sections 1, 2, and 6) (**Table III**). This response was not seen in the stented limbs, which had similar luminal areas at 4 and 12 weeks. Compared to stented graft limbs, the luminal area in the late group of controls (**Table IV**) was significantly greater at levels corresponding to the proximal and distal ends of the stent, as well as the outflow artery 1 cm proximal to the anastomosis (Figure 2, Sections 2, 4, and 6) (Table IV).

COMMENTS

Endovascular stents were developed to rescue immediate angioplasty failures and also to reduce the incidence of restenosis after percutaneous transluminal balloon angioplasty. To date, there have been no reports of the use of

TABLE I

Mean Intimal Thickness (mm), Early versus Late

	Control				Stent			
Level of Section	**Early**	**Late**	**Difference**	***P* value**	**Early**	**Late**	**Difference**	***P* value**
Level 2 = Distal end of stent	0.086 (0.049)	0.072 (0.039)	- 0.014 (0.06)	0.82	0.107 (0.049)	0.139 (0.039)	0.032 (0.06)	0.6
Level 3 = Anastomosis	0.1003 (0.049)	0.288 (0.039)	0.185 (0.06)	0.006	0.287 (0.080)	0.196 (0.039)	0.103 (0.049)	
Level 4 = Proximal end of stent	0.112 (0.049)	0.98 (0.0550)	0.186 (0.07)	$P = 0.01$	0.275 (0.049)	0.430 (0.039)	0.155 (0.06)	0.02
Level 5 = Proximal graft	0.220 (0.039)	0.177 (0.049)	0.043 (0.06)	0.50	0.425 (0.055)	0.130 (0.049)	0.295 (0.07)	0.004

In the control graft limbs, a significant increase in Intimal thickness areas was noted at the anastomotic level and at a level corresponding to the proximal stent-graft interface. No significant difference at sections 1, 2, 5 or 6.

In the stented graft limbs, there was no change in IT at the anastomotic level but at the proximal stent-graft interface, and in the proximal graft, IT increased significantly with time. No significant differences at sections, 1, 2, 3 or 6.

TABLE II

Intimal Thickness (IT) at 12 weeks

Section Number	**Level of Section**	**Mean IT (mm) at Each Section**		**Difference in Mean IT**	**Standard Error**	***t* Value**	**2-Tailed *P* Value**
		Control	**Stent**				
3	Anastomosis	0.288	0.196	0.092	0.044	2.04	0.06*
4	Proximal end of stent	0.298	0.430	-0.132	0.05	-2.26	0.04†
5	Proximal graft	0.220	0.426	-0.204	0.05	-3.51	0.005†

At the anastomotic level, IT was greater in controls but the difference was not significant. More proximally, in Sections 4 and 5, the IT in stented graft limbs was significantly greater than that seen in controls. There was no significant difference in IT between control and stented sections at levels 1, 2 or 6.

†Statistically significant.

nts as a means of limiting ANH in bypass grafts. In pre-
us work from our laboratory,[10] the effects of stenting on
end-to-end arterio-arterial anastomosis were studied. In
nonstented controls, there was a significant decrease in
cross-sectional luminal area at the anastomotic level.
wever, the stented anastomoses had a significantly
ater amount of IT at the anastomotic site. This model
vided us with preliminary data regarding the healing
racteristics of a stent at an anastomotic site. Since an
rately performed arterio-arterial anastomois is known
eal satisfactorily and does not present a clinical prob-
we would not suggest the use of a stent for this ap-
ation. The next logical step was to study a model of a
t artery anastomosis to further evaluate these findings.
H has long been recognized as a cause of the late fail-
f prosthetic infrainguinal arterial bypasses.[1-3] In pros-
bypass grafts, the characteristic lesion is a "plug" of
e composed of smooth muscle cells and extracellular
x in the floor of the native artery, around the suture
and extending backwards into the distal end of the
for several millimeters.[11,12]

etiology of ANH is probably multifactorial. A num-
f investigators have demonstrated experimentally, both
o and in vitro, that a combination of mechanical phe-
na act at an end-to-side anastomosis. Several authors
implicated a mismatch of compliance between the
etic graft material and the native artery at the anas-
ic level.[4,5,13] In a canine model of isocompliant arte-
d-to-end anastomoses, Hasson et al[14] utilized pulsed ultrasound to obtain longitudinal profiles of diameter and compliance. They demonstrated an area of "hypercompliance" just distal to the anastomosis in this model and suggested that it might be at least partly a consequence of transmitted effects of the suture line on the arterial wall.

Madras et al[6] stated that the standard end-to-side, "cobra-head" anastomosis inevitably leads to an increase in the diameter of the host artery at the anastomotic level. Applying the law of Laplace to this region he observed that a 200% to 300% increase in wall tension could arise from this increase in diameter, especially when a relatively large-diameter graft is anastomosed to a small artery. It is generally held that diameter-matched anastomoses allow the blood flow and pulse wave to traverse the anastomotic region with minimal energy dissipation, thereby limiting physical stress to the adjacent arterial wall.[15,16] These physical stresses are key events in the stimulation of the cells involved in the anastomotic hyperplastic process.[9,17] Sottiurai[18] has stressed the importance of the "non-biological" geometry of the end-to-side anastomosis in the genesis of ANH.

LoGerfo et al[19] performed cross-femoral Dacron bypass grafts in a series of dogs. They found that the downstream anastomosis developed a significantly greater amount of anastomotic hyperplasia compared to the upstream anastomosis. However, there was no significant increase in the amount of downstream hyperplasia with unidirectional or bidirectional outflow, made possible by ligating the femoral artery above or below the level of the profunda take-off.

The presence of a stent eliminated the compensatory increase in the lumen found in the nonstented counterparts possibly by altering shear forces and/or cyclical stretch at that level. The smaller luminal area observed at the proximal stent-graft interface reflects the greater IT caused by the stent at that level in the presence of a fixed graft diameter. The use of the Wallstent in this experiment caused an inevitable slight "step" at the stent-graft interface, which may be at least partly responsible for altered flow dynamics in this region. Possible future developments of this experimental model include the use of a tapered graft or different stent designs to overcome the observed negative stent effect.

Electron microscopic studies demonstrated that the stents were covered completely with a monocellular layer by 4 weeks. The only area not covered was at the inflow segment of the anastomotic artery. We assume this is related to the presence of prograde and retrograde flow through the interstices of the stent and has important implications with regard to the placement of stents across major branch arteries.

We conclude from this study of endovascular anastomotic stenting in a canine model of PTFE end-to-side arterial anastomosis that, while stenting reduces the accumulation of neointimal hyperplasia at anastomotic level, it is at the expense of negative changes within the graft at the graft-stent interface. These include a decrease in luminal area and an increase in IT. Also, the presence of a stent seems to prevent the native artery from undergoing a compensatory increase in luminal area distal to the anastomosis, seen in the nonstented group in this experiment.

Further studies are required to test the effects of different stent types, anastomotic dimensions, and configurations on ANH.

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DISCUSSION

Dr Salama: Is there difficulty having the stent not kink as you bring it into the common iliac?

Dr. Chalmers: The Wallstent is flexible in all directions.

itish Journal of Surgery 1994, 81, 1443–1447

Effect of an endovascular stent on healing of an end-to-end polytetrafluoroethylene–artery anastomosis in a canine model

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canine model of end-to-end anastomosis between the ac arteries and polytetrafluoroethylene grafts was veloped; a self-expanding Wallstent was placed across e anastomosis. The opposite limb acted as a control. imals were killed at 4 or 12 weeks. Sections were taken d the intimal thickness and luminal area calculated. At weeks intimal thickness was significantly greater at astomoses in control sections ($P=0{\cdot}007$), and at the erface between the proximal stent and graft in stented graft limbs ($P=0{\cdot}01$). Control graft limbs had significantly enhanced intimal thickness at the anastomotic level at 12 weeks compared with that at 4 weeks ($P=0{\cdot}0002$), while there was no such increase for the stented side. There was no significant difference in luminal area between control and stented graft limbs. Anastomotic neointimal hyperplasia in a canine graft–artery bypass model is modified by endovascular stenting.

clinical reports of lower-extremity polytetrafluoroethylene TFE) grafts, over 20 per cent of thromboses are caused by ıstomotic neointimal hyperplasia[1–3]. There are probably ny interacting factors responsible for the genesis of such perplasia; mismatch of both compliance[4,5] and diameter[6] ween the prosthetic graft material and the native artery, l cyclical stretching during the cardiac cycle[7,8] have been subject of much investigation. The hypothesis underlying present study was that, in an end-to-end anastomosis ween a PTFE graft and native artery, the presence of an lovascular stent might alter some or all of these hanical factors and so modify the development of stomotic neointimal hyperplasia. The results of endo- ular arterial graft anastomotic stenting in a canine model described.

terials and methods

ıdult mongrel dogs of each sex weighing between 20 and 25 kg used. Animals were cared for according to the guidelines in *ciples of Laboratory Care* and *Guide for the Care and Use of oratory Animals* (National Institutes of Health publication no. 23, revised 1985). Anaesthesia was induced with pentobarbital ım 15 mg/kg, after which animals were intubated and tained on 1 per cent halothane and room air administered via a ard respirator. Circulating volume was maintained with venous isotonic saline. Antibiotic prophylaxis at induction of sthesia was 3 ml of a solution of benzathine penicillin 00 units/ml, procaine penicillin 150 000 units/ml and sodium aldehyde 1·75 mg/ml given intramuscularly. Before operation a rcated graft was hand-sewn using 6/0 polypropylene to unite 5-cm lengths of 6-mm diameter thin-walled PTFE (GORE-TEX; Gore, Flagstaff, Arizona, USA) of pore width 30 μm.

e abdominal aorta and iliac arteries were exposed through a ne abdominal incision. The diameters of the iliac arteries were ıred with a caliper before vessel handling so that the diameter stent to be used corresponded to the normal diameter of the . The iliac arteries and distal aorta were then dissected in ration for grafting. Intravenous heparin 50 units/kg was ıistered 5 min before the arterial clamps were applied. The limb selected for stenting was chosen at random and mosed first. The external iliac artery was ligated at its origin o. 1 silk and a segment of the artery excised to ensure that the graft would not kink. The distal portion of the external iliac artery was spatulated with a 3-mm arteriotomy and, using a continuous 6/0 polypropylene suture with a parachute technique under 2·5× loupe magnification, the anastomosis was fashioned. The sutures were left loose initially to allow the stent to be positioned precisely under direct vision. The Wallstent (Schneider, St Paul, Minnesota, USA) is made from woven stainless steel and is mounted within a constraining membrane on a 7-Fr catheter. A stent of 5-mm unconstrained diameter was used in all cases. The device was introduced through the proximal end of the graft and deployed accurately across the anastomosis so that it lay equally within the graft and the artery (*Fig. 1a*). The anastomotic suture was then tightened and tied. The opposite iliac anastomosis was then performed in an identical fashion. Finally, the aortic anastomosis was sewn with a continuous 5/0 polypropylene suture. This anastomosis was of end-to-side 'onlay' configuration (*Fig. 1b*). Completion arteriography was performed via a proximal aortic needle puncture before wound closure to assess the technical result at both anastomoses.

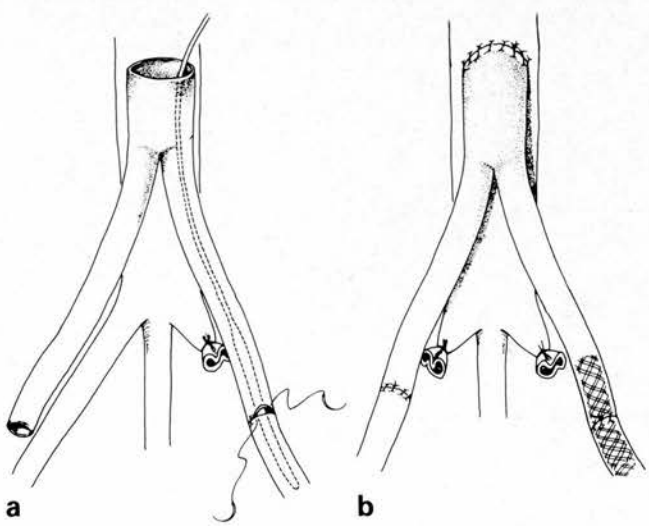

Fig. 1 **a** Diagrammatic representation of the stent placed via the proximal graft and deployed under direct vision. **b** The completed graft showing the aortic 'onlay' anastomosis and the external iliac artery ligated at its origin on both sides. The stent is deployed across the left distal anastomosis

accepted 30 May 1994

Graft patency was monitored in the postoperative period by femoral pulse palpation and transcutaneous continuous-wave Doppler ultrasonography, daily for the first week and once a week thereafter until the animals were killed. No anticoagulant or antiplatelet therapy was administered after the surgical procedure. The patency of the graft and run-off arteries was further monitored by arteriography at midterm in all animals. This procedure was performed via a carotid cut-down under brief general anaesthesia with intravenous pentobarbital sodium.

The dogs were separated into two groups for the purposes of analysis: those killed at 4 weeks and those at 12 weeks. The animals were anaesthetized with intravenous pentobarbital sodium before death. The graft and vessels were exposed via the previous midline abdominal incision. An arteriogram was obtained via a proximal aortic needle puncture to visualize both distal anastomoses. A lethal dose of intravenous potassium chloride was then administered. The graft and arteries were flushed with isotonic saline solution and then pressure-perfusion fixed with 2 per cent glutaraldehyde in cacodylate buffer at 90 mmHg for 1 h. The vessels and grafts were then explanted and fixed in 2 per cent glutaraldehyde for a further 24 h. Sections were taken at five fixed levels: (1) the run-off artery 1 cm distal to the stent; (2) the interface between the stent and the artery; (3) the anastomotic level; (4) the interface between the proximal stent and the graft; and (5) the graft 1 cm proximal to the stent (*Fig. 2*).

With a Megaplus camera (Eastman Kodak, San Diego, California, USA) computer images of all the sections were obtained and stored on a Macintosh FX workstation (Apple, Cupertino, California, USA). Images were traced with 'm-trace' software (Silicon Graphics, Mountain View, California, USA). This program allows the contours of images to be outlined, making it possible to calculate the area and perimeter. Another program (Wall Thickness; University of Iowa, Iowa City, Iowa, USA) enabled sample concentric contours to be determined radially. The difference in radius at 360° was measured and an average intimal thickness for each radian of each section obtained. Additional longitudinal and transverse sections were taken from the residual specimens and examined with scanning electron microscopy.

Data were analysed by univariate repeated-measures analysis of variance, involving a between-animal factor (4 *versus* 12 weeks) and two within-animal factors (stent *versus* no stent, section *versus* section). Pairwise comparisons were made for controls *versus* stent at each section in all animals. Animals of the 4- and 12-week groups were also compared at each section for differences between controls and stented graft limbs. Comparisons of interest were examined using one degree of freedom mean contrasts and tested with the *t* statistic. The standard errors used in computing the *t* statistic for these contrasts were estimated from the between- and within-animal mean square errors.

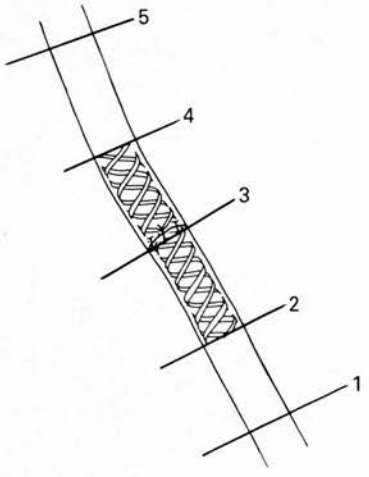

Fig. 2 Sections were taken at five fixed levels: 1, run-off artery 1 cm distal to the stent; 2, interface between the distal stent and the artery; 3, graft–artery anastomosis; 4, proximal stent and the graft; and 5, graft 1 cm proximal to the stent. Corresponding levels were sectioned on the unstented control side

Results

There were no deaths and no wound-related complicati
Three graft limb occlusions were found at death. One ani
killed at 4 weeks had occlusion of the control and sten
graft limbs and was excluded from statistical analy
Necropsy of this animal revealed an accumulation of n
intimal hyperplasia at the proximal stent–graft interface
the stented side, while a similar plug of neointi
hyperplasia was located at the suture line between the g
and artery on the control side. In one animal killed at
weeks the control side was occluded and a plug of neoint
was again seen at the suture line extending proximally i
the graft for a few millimetres. As the grafts had been pa
on arteriography at midterm and by femoral pulse palpat
up to 1 week before death, it was assumed that occlus
had occurred in the week before sacrifice. The midt
arteriograms did not give reliably quantifiable informa
on intimal thickening.

Scanning electron microscopy

Scanning electron microscopy showed that in all cases
4 weeks the luminal surface of the stent was covered wit
confluent monolayer of cells, orientated with the longitudi
axis parallel to the direction of blood flow (*Figs 3* and
Transverse sections showed the neointima to be composed
abundant fibrous material with a few cells interspersed.

Fig. 3 Scanning electron micrograph (× 2000 magnification) showing the luminal surface of the stented anastomosis covered confluent layer of cells with their longitudinal axes parallel to th direction of blood flow

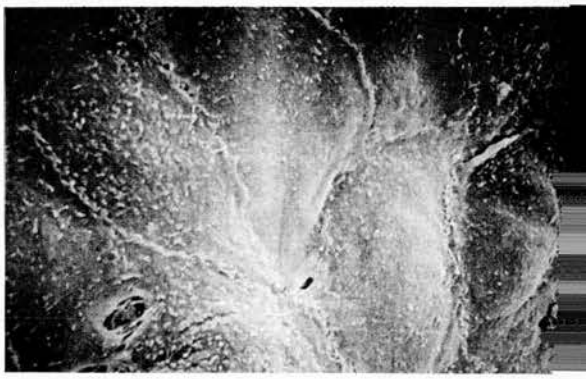

Fig. 4 Scanning electron micrograph at a higher power (× 35(showing a monolayer of cells covering the luminal surface of t stented graft

'able 1 Mean intimal thickness at 12 weeks

ection	Mean intimal thickness for all animals (mm)		Difference(s.e.) in mean intimal thickness (mm)	*t*	Two-tailed *P*
	Controls	Stented			
	0·134	0·097	0·037(0·041)	0·92	0·38
	0·211	0·190	0·021(0·041)	0·51	0·62
	0·298	0·181	0·117(0·034)	3·43	0·007
	0·169	0·273	−0·104(0·034)	−3·04	0·01
	0·242	0·316	−0·074(0·034)	−2·15	0·06

ıble 2 Mean intimal thickness in animals killed at 4 and 12 weeks

Section	Mean intimal thickness (mm)		Difference(s.e.) in mean intimal thickness (mm)	*t*	Two-tailed *P*
	12 weeks	4 weeks			
ɔntrol					
1	0·134	0·084	0·050(0·056)	0·90	0·38
2	0·211	0·106	0·105(0·051)	1·87	0·07
3	0·298	0·065	0·233(0·051)	4·54	0·0002
4	0·169	0·190	−0·021(0·051)	−0·43	0·67
5	0·242	0·175	0·067(0·051)	1·31	0·20
ɔnted					
1	0·097	0·101	−0·004(0·051)	−0·09	0·93
2	0·190	0·103	0·087(0·051)	1·70	0·10
3	0·181	0·128	0·053(0·064)	0·83	0·41
4	0·273	0·117	0·156(0·051)	3·03	0·006
5	0·316	0·116	0·200(0·051)	3·89	0·0008

le 3 Mean luminal area in animals killed at 4 and 12 weeks

ection	Mean luminal area (mm^2)		Difference(s.e.) in luminal area (mm^2)	*t*	Two-tailed *P*
	12 weeks	4 weeks			
trol					
	19·788	5·975	13·813(5·998)	2·30	0·035
	9·839	7·163	2·676(5·582)	0·48	0·64
	10·525	6·321	4·204(5·582)	0·75	0·46
	17·216	11·298	5·918(5·582)	1·06	0·31
	12·252	12·572	−0·320(5·582)	−0·06	0·95
ted					
	11·307	1·820	9·487(5·582)	1·70	0·11
	8·342	3·283	5·059(5·582)	0·91	0·38
	6·942	6·807	0·135(6·754)	0·02	0·98
	10·385	12·428	−2·043(5·582)	−0·37	0·72
	10·080	12·719	−2·639(5·582)	−0·47	0·64

nal thickness

weeks there were no significant differences in intimal ness between stented and control graft limbs at all s. At 12 weeks, however, there was significantly greater ness at the anastomotic level (section 3) when the mean ness in controls was compared with that in stented graft s (0·298 *versus* 0·181 mm, $P = 0·007$). At the interface een the proximal stent and the graft (section 4), intimal ness was greater in stented prostheses than in controls the difference was statistically significant (stented 1 0·273 mm *versus* control mean 0·169 mm, $P = 0·01$) *le 1*).

hen the differences in intimal thickness between animals nd 12 weeks were compared for control and for stented limbs, a significantly greater thickness was observed at nastomotic level (section 3) for controls at 12 weeks compared with that at 4 weeks (mean 0·298 mm *versus* 0·065 mm, $P = 0·0002$). At the anastomotic level there was no significant difference in intimal thickness between 4- and 12-week stented graft limbs. However, at the interface between the proximal stent and the graft, and 1 cm proximally into the prosthesis (sections 4 and 5) there was a significant increase in thickness for stented grafts between 4 and 12 weeks (*Table 2*).

Luminal area

There was no significant difference in mean luminal area between the control and stented graft limbs for both early and late groups in all sections. When the data for control and stented graft limbs were analysed separately, comparing luminal area at 4 and 12 weeks, the only significant

difference was for section 1 (the run-off artery 1 cm distal to the stent) of the control side ($P = 0{\cdot}035$) (*Table 3*).

Discussion

Stenting is associated with significantly less intimal thickening at the distal graft–artery suture line than in controls (each animal acted as its own control). However, the reverse applied at the interface between the proximal stent and the graft. The thickening within the stented graft limbs stabilized with no significant difference between animals killed at 4 and 12 weeks. However, for controls at corresponding levels this did not apply and intimal thickening increased significantly over time at the graft–artery anastomotic level. In the stented graft limbs there was also a significant increase in thickening over time in the graft proximal to the stent. Interestingly, none of these changes in intimal thickness correlated with a significant change in mean luminal area. The significantly greater lumen in the run-off artery of late controls may reflect compensatory dilatation of the artery distal to the constricting effect of the anastomotic suture line. This was not seen in the stented anastomoses and it is possible that the stent has a streamlining effect on the anastomosis.

In a parallel study of anastomotic stenting of a PTFE–artery anastomosis using an end-to-side configuration[9], a stent reduced the amount of thickening at the anastomotic level at the expense of a significantly greater amount at the interface between the proximal stent and the prosthesis. This finding was reproduced in the present end-to-end graft–artery model. It is noteworthy that the effect of anastomotic stenting on intimal thickening was similar in each model. The results of both experiments imply that stents may influence the mechanical events taking place at a vascular anastomotic suture line irrespective of whether the anastomosis is end to end or end to side.

Several investigators have reported that a combination of factors act at a graft–artery anastomosis to stimulate the cellular events that ultimately lead to the development of anastomotic neointimal hyperplasia. One theory is that a mismatch of compliance between the graft material and host artery may be responsible for the poor patency of prostheses anastomosed to small-diameter arteries. However, there is a lack of definitive evidence linking compliance mismatch alone to the development of such hyperplasia. Hasson *et al.*[10] performed arterial end-to-end anastomoses with a continuous suture in a canine femoral artery model, and measured the diameter and compliance at the anastomosis and its vicinity with pulsed ultrasound. They found that the arterial diameter decreased monotonically to a minimal level at the anastomosis. However, arterial compliance increased by about 50 per cent in the perianastomotic region before decreasing to 60 per cent of control values. The authors coined the term 'para-anastomotic hypercompliance zone' to describe this area of increased compliance, which was also observed when they implanted PTFE grafts into the canine carotid circulation. They hypothesized that the increased cyclical stretch associated with this zone could be a stimulus to smooth muscle cell proliferation and thus provide a link between compliance and anastomotic neointimal hyperplasia. Chandran *et al.*[11] described an *in vitro* finite-element analysis model of an end-to-end graft–artery anastomosis. They found that the para-anastomotic hypercompliance zone was larger with PTFE and Dacron (DuPont, Wilmington, Delaware, USA) than with vein. However, larger tensile stresses were present in the wall of the vein grafts compared with that in prostheses. Furthermore, increasing the diameter of the conduit compared with that o the host artery resulted in a significant increase in hype compliance on the arterial side. As the site of development o anastomotic neointimal hyperplasia is mainly at the dist anastomosis and into the distal few millimetres of th prosthetic graft, it is reasonable to assume that mechanic factors acting in this region, including compliance mismatc may be implicated in the pathogenesis of hyperplasia. W *et al.*[12] hold a contrary opinion. They placed externall supported Dacron grafts into the canine carotid circulatio end to end on one side and end to side on the other. Contr grafts consisted of autogenous carotid artery segmen implanted into the femoral circulation. There was n statistically significant difference in anastomotic neointim thickness in the compliant and non-compliant grafts ov time. The authors concluded that compliance mismat between grafts and arteries did not cause anastomot neointimal hyperplasia.

A number of authors, including Golden *et al.*[13] an LoGerfo[14], have hypothesized that activation of certai components of circulating blood, such as platelets an macrophages, by exposure to the foreign material of th proximal graft causes these to secrete mitogenic substance These mitogens can stimulate medial smooth muscle cells the distal anastomosis, leading to their migration a proliferation and the development of anastomotic neointim hyperplasia. Clowes *et al.*[15] proposed the theory of repeat endothelial injury at the distal anastomosis leading continuous stimulation of endothelial and smooth mus cells, both of which are also sources of mitogenic substanc In baboon carotid and iliac graft–artery models, th observed both endothelial and underlying smooth mus cells migrating from the host artery into the graft. With tim both cell types continued to proliferate, presumal stimulated by the repetitive injury of arterial pulsation.

One theoretical advantage of a stented anastomosis m be that the early endothelialization of the stent surf contributes to the stabilization of the anastomotic neointin hyperplasia compared with the progressive lesion comparable levels observed in the controls. Oth experimental studies[16] have shown that, in cell cultu cyclical stretching stimulates vascular smooth muscle cells alter their morphology and secrete collagen, further evide for the association between mechanical factors and development of hyperplasia. It is possible that, by damp this cyclical strain, the presence of the stent limits development of hyperplasia.

The evolving concept of endovascular bypass has, as a technical point, the fixation of the ends of the graft v stents. The present study has shown that it is feasible to pl endovascular stents of 5 mm diameter across vasc anastomoses without significant early graft thrombosis. ' introduction of an endovascular femoropopliteal by graft for occlusive disease, as described recently by Ci and Dake[17], requires the placement of an endovasc PTFE graft after percutaneous transluminal ball angioplasty of the stenotic superficial femoral ar segment. The conduit is secured with a proximal (an some cases with a distal) stent. This procedure has b performed on arteries of approximately the same diamet those in the canine model described; it is possible that it be complicated by anastomotic neointimal hyperplasi locations similar to those in the present experiment.

From the present study of endovascular anastom stenting it may be concluded that a stent can be placed ac a graft–artery anastomosis without significant early thrombosis. There is a statistically significant beneficial e

in limiting the development of anastomotic neointimal hyperplasia at the graft–artery suture line, but this is at the expense of greater intimal thickening in the graft, just proximal to the stent. Furthermore, a stent prevents the run-off artery undergoing dilatation for several centimetres distal to the anastomosis.

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Pulse-generated run-off *versus* dependent Doppler ultrasonography for assessment of calf vessel patency

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Pulse-generated run-off (PGR) is an established technique in the assessment of calf vessel patency. Dependent Doppler ultrasonography is proposed as a fast and simple alternative. Twenty-six limbs with severe ischaemia were evaluated by PGR, dependent Doppler examination and intra-arterial digital subtraction angiography (DSA). PGR was performed and scored as previously described. Dependent Doppler ultrasonography was performed after 5 min of foot dependency and scored as for PGR. Angiograms were scored by an independent radiologist, who awarded 2 for a vessel widely patent to the ankle, 1 for a diseased vessel crossing the ankle and 0 if no vessel was visualized. Of 78 calf vessels evaluated, 59 (76 per cent) appeared patent on PGR and dependent Doppler examination but only 33 (42 per cent) appeared patent to the ankle with intra-arterial DSA. There was very good agreemei between PGR and dependent Doppler for detection patent calf vessels ($\kappa = 0{\cdot}93$). Doppler signals were biphas in six calf vessels on dependency and in 22 vessels wi PGR. PGR and dependent Doppler ultrasonograph detected 26 vessels communicating with the pedal ar compared with seven detected angiographically. There w good agreement between PGR and dependent Dopp examination for diagnosis of the most suitable vessel f distal anastomosis ($\kappa = 0{\cdot}80$). The wide availability a simplicity of dependent Doppler ultrasonography me that no patient with a critically ischaemic limb should denied reconstructive surgery on the basis of angiograp findings alone.

Evaluation of calf vessel patency and communication with the vascular arch of the foot is essential before femorodistal bypass for determining surgical feasibility and for preoperative planning[1]. In severely ischaemic limbs standard angiographic techniques may fail to reveal distal run-off, despite the use of vasodilators or hyperaemia[2,3]. Intra-arterial digital subtraction angiography (DSA) has improved assessment of proximal calf vessels but visualization of distal calf vessels and of the pedal arch remains difficult[4]. Pulse-generated run-off (PGR) scoring gives a more functional picture of run-off status; it may predict calf vessel resistance and hence subsequent graft patency rates[5]. PGR requires time, an occlusive cuff and a pulse generator, which may not be available in all hospitals. In contrast, dependent Doppler ultrasonographic examination is easily performed on the ward and provides a simpler functional assessment of calf vessel status. This study prospectively compared dependent Doppler examination, intra-arterial DSA and PGR.

Patients and methods

Twenty-five patients, 19 men and six women of median age 64 years, undergoing evaluation of severe ischaemia in 26 limbs were studied. All had rest pain and ten had ischaemic tissue loss. Fifteen patients were diabetic, ten had cardiac symptoms and all were former smokers.

All patients underwent conventional retrograde transfemoral angiography supplemented with intra-arterial DSA imaging of calf vessels. To obtain optimal visualization of calf vessels the arterial catheter was withdrawn into the iliac vessels before DSA. A radiologist, blind to the non-invasive results, assessed the angiograms. A vessel widely patent to the ankle scored 2, a diseased vessel patent to the ankle scored 1 and an occluded vessel scored 0. In addition, each vessel seen to communicate with the pedal arch scored 1.

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PGR was performed with evaluation of the foot arch previously described[6].

Dependent Doppler examination was performed by a secc observer, unaware of the PGR results, after 5 min of f dependency with an 8-MHz pencil probe. The pedal arch v evaluated using the method of Roedersheimer *et al.*[7]. B dependent Doppler and PGR were scored 2 for a biphasic signa for a monophasic signal and 0 for an absent signal, givin maximum possible calf vessel score of 6. Each vessel communi ing with the foot arch scored 1, giving a maximum limb vessel sc of 9.

Statistical analysis

Differences in calf vessel score between PGR, dependent Dop and intra-arterial DSA were assessed by paired *t* test and the 95 cent confidence interval (c.i.) of the difference in scores. Diagn of patent calf vessels by PGR and dependent Doppler examina was compared by calculating a κ value as a measure of agreem as was selection of the most suitable vessel for distal anastomosi these two techniques[8]. The limits of agreement between PGR dependent Doppler calf vessel score were calculated as descr by Bland and Altman[9].

Results

Of 78 calf vessels studied, angiography diagnosed 33 per cent) as patent; PGR and dependent Doppler u sonography both diagnosed 59 (76 per cent) as patent shown in *Table 1* there was excellent agreement betv PGR and dependent Doppler in the diagnosis of patent vessels.

Fig. 1 shows the mean calf and limb vessel scores for I dependent Doppler and angiography. Dependent Do examination detected only six biphasic signals comp with 22 detected by PGR. Thus PGR had a signific greater calf vessel score than dependent Doppler ($P < 0$ 95 per cent c.i. of difference 0·26–1·04). The 95 per limits of agreement between PGR and dependent Do

at compliance mismatch is not a significant factor in the use of neointimal hyperplasia?

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Changes in arterial wall compliance after endovascular stenting

To the Editors:

We read with interest the article by Back et al. (J V
SURG 1994;19:905-11) on their experimental work
cerning compliance changes in a stented canine iliac a
We recently reported our findings in a canine model
aortobiiliac polytetrafluoroethylene bypass graft, in v
a Wallstent (Schneider Stent Co., St Paul, Minn.)
deployed across one distal anastomosis in an attem
control the development of anastomotic neointimal h
plasia.[1,2] In our experiments, we measured the a
diameter with a caliper before vessel handling to sel
appropriately sized stent, and we did not encount
atrophy of the tunica media experienced by Back et
their study, the diameters of the stented vessels were g
than those of the nonstented controls. This rais
question about whether oversizing of the sten
overdistension of the artery caused the medial atro

Finally, Back et al. reported finding a neointi
uniform thickness associated with the stents. The
observed a loss of compliance in the stented portion
vessel. Hence, at the interface of the normal artery a
stent, a mismatch of compliance existed. Because th
not encounter a significant accumulation of neoint
the stent-to-artery interface, is their work in fact ev

APPENDIX IV

AWARDS AND DISTINCTIONS

1) **The Effects of Polytetrafluoroethylene Graft Anastomotic Stenting in a Canine Model.**

Winner of the Peter B. Samuels Award of the Society for Clinical Vascular Surgery for the Best Vascular Research Paper, Arizona, USA, March, 1994.

2) **Endovascular Anastomotic Stenting in a Canine Model.**

Finalist in the Cheyne Medal Competition, University of Edinburgh School of Surgery Day, November, 1993.

3) **The Effect of an Intraluminal Stent on Neointimal Hyperplasia at an End-to-Side Polytetrafluoroethylene Graft-Arterial Anastomosis.**

Finalist for the Surgeon-in-Training Medal at the Joint Meeting of the Royal College of Surgeons of Edinburgh and the Royal Society of Medicine, Edinburgh, May, 1994.

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