10:29:04 OCA PAD AMENDMENT - PROJECT HEADER INFORMATION 05/24/93

Active

Project #: E-25-X25 Cost share #: Rev #: 2
Center # : 10/24-6-R7514-0A0 Center shr #: OCA file #:
Work type : RES
Contract#: AGMT DTD 920612 Mod #: 1 Document : AGR
Prime #: Contract entity: GTRC

Subprojects ? : N CFDA: N/A
Main project #: PE #: N/A

Project unit: MECH ENGR Unit code: 02.010.126
Project director(s):
KU D N MECH ENGR (404)894-6827

Sponsor/division names: AMERICAN HEART ASSOC /
Sponsor/division codes: 500 / 011

Award period: 920701 to 940630 (performance) 940801 (reports)

Sponsor amount	New this change	Total to date
Contract value	33,000.00	66,000.00
Funded	33,000.00	66,000.00
Cost sharing amount		0.00

Does subcontracting plan apply ?: N

Title: HEMODYNAMICS IN ANASTOMOTIC GRAFT FAILURE

PROJECT ADMINISTRATION DATA

OCA contact: Kathleen R. Ehlinger 894-4820

Sponsor technical contact

ARTHUR J. MERRILL, JR., M.D.
(404)952-1316

AMERICAN HEART ASSOCIATION
GEORGIA AFFILIATE
1685 TERRELL MILL ROAD
MARIETTA, GA 30067-8320

Sponsor issuing office

LINDA CERASA
(404)952-1316

AMERICAN HEART ASSOCIATION
GEORGIA AFFILIATE
1685 TERRELL MILL ROAD
MARIETTA, GA 30067-8320
FAX (404) 952-2208

Security class (U,C,S,TS) : U ONR resident rep. is ACO (Y/N): N
Defense priority rating : NONE NONE supplemental sheet
Equipment title vests with: Sponsor GIT X

Administrative comments -
ISSUED TO ADD $33,000 AND EXTEND TERMINATION DATE FROM 6/30/93 TO 6/30/94.

SR671

GEORGIA INSTITUTE OF TECHNOLOGY
OFFICE OF CONTRACT ADMINISTRATION

NOTICE OF PROJECT CLOSEOUT

Closeout Notice Date 10/28/94

Project No. E-25-X25 Center No. 10/24-6-R7514-0A0

Project Director KU D N School/Lab MECH ENGR

Sponsor AMERICAN HEART ASSOC/

Contract/Grant No. AGMT DTD 920612 Contract Entity GTRC

Prime Contract No.

Title HEMODYNAMICS IN ANASTOMOTIC GRAFT FAILURE

Effective Completion Date 940630 (Performance) 940801 (Reports)

Closeout Actions Required:	Y/N	Date Submitted
Final Invoice or Copy of Final Invoice	Y	
Final Report of Inventions and/or Subcontracts	Y	
Government Property Inventory & Related Certificate	N	
Classified Material Certificate	N	
Release and Assignment	N	
Other	N	

Comments

Subproject Under Main Project No.

Continues Project No.

Distribution Required:

Project Director	Y
Administrative Network Representative	Y
GTRI Accounting/Grants and Contracts	Y
Procurement/Supply Services	Y
Research Property Managment	Y
Research Security Services	N
Reports Coordinator (OCA)	Y
GTRC	Y
Project File	Y
Other	N
	N

NOTE: Final Patent Questionnaire sent to PDPI.

0

Georgia Tech

THE GEORGE W. WOODRUFF SCHOOL OF
MECHANICAL ENGINEERING

David N. Ku, MD, PhD
Associate Professor
Woodruff Faculty Fellow

Georgia Institute of Technology
Atlanta, Georgia 30332-0405
USA

October 20, 1994

Carl Lane, MD
Chairman, Research Committee
American Heart Association
Georgia Affiliate
1685 Terrell Mill Road
P. O. Box 6997
Marietta, GA 30065-6997

Dear Dr. Lane:

Enclosed please find the final report form for our Grant-in-Aid of the project entitled "Hemodynamic flow and turbulence in anastomotic graft failure by occlusive intimal hyperplasia". This report covers the period from 7-1-93 through 6-30-94. The Grant-in-Aid was most helpful in our understanding of the relationship between hemodynamics and graft intimal hyperplasia. Our results suggest that hemodynamics may be one of the most important factors in the development of graft occlusions.

Thank you very much for your support. If I can be of further information, please do not hesitate to call me.

Sincerely,

David N. Ku, MD, PhD
Associate Professor of Surgery
and Mechanical Engineering

DNK:mjk
Enclosure

Telephone: (404) 894-6827 david.ku@me.gatech.edu Fax: (404) 894-2291

An Equal Education and Employment Opportunity Institution

A Unit of the University System of Georgia

AHA Final Report for Grant-in-Aid
PI: David N. Ku, MD, PhD

Hemodynamic Flow And Turbulence In Anastomotic Graft Failure By Occlusive Intimal Hyperplasia

This project was designed to assess the effects of low shear stress and turbulence hemodynamics on vascular graft intimal hyperplasia.

Specific Aims included determining the rate of stenosis progression in a canine model, stimulating occlusion by changes in flow, and evaluating the effects of turbulence in an arterial-arterial graft. We were able to accomplish these goals as well as perform additional experiments.

The experimental design was to alter the hemodynamics in vivo and measure the changes in intimal thickening from this change.

The hemodynamic conditions were altered by varying graft diameter and changing flow rates through grafts in a dog and a pig model.

The results indicate that:

a) low shear stress stimulates intimal thickening in vivo,
b) turbulence can also stimulate intimal thickening but the separate effects from low shear stress are not significant,
c) occlusion is independent of the early thrombotic events,
d) occlusion is highly dependent on the long term shear stress through a graft,
e) the time history of stenosis progression indicates an advanced stage IV which is less proliferation and more synthesis of extracellular matrix,
f) flow reduction can greatly accelerate intimal hyperplasia to occlusive levels,
g) this hemodynamic stimulus for occlusion is operative in both dog and pig animal models, and

h) anti-inflammatory steroids at a dose of 0.125 mg/kg Dexamethasone is not effective in reducing flow induced intimal hyperplasia in pigs even though it has been reported to be effective in rats.

We conclude that:

a) hemodynamics is one of the critical factors leading to long-term graft occlusion by intimal hyperplasia,

b) long-term graft occlusion is not as strongly dependent on the initial thrombotic events,

c) the role of turbulence in arterial-arterial graft intimal hyperplasia is minimal,

d) occlusions may be preventable by the use of optimal graft diamters, and

e) future studies should focus on the cellular mechanisms by which low shear stress activates occlusion and extracellular matrix synthesis.

We have submitted two manuscripts for publication and have two additional ones in preparation. The preprints of two manuscripts are enclosed. One NIH R01 application for continuation of this project has been approved and is pending funding.

MS#971-OA

Revise 9-6-94

LOW SHEAR STRESS PROMOTES INTIMAL HYPERPLASIA IN A DOSE-RESPONSE MANNER

Tarek A. Salam, MD FRCS

Alan B. Lumsden, MD

William D. Suggs, MD

David N. Ku, MD PhD.

Section of General Vascular Surgery

Emory University, Atlanta, GA

and

George W. Woodruff School of Mechanical Engineering

Georgia Institute of Technology

Atlanta, GA

Running Title: Low Shear Stress and Intimal Hyperplasia

Key Words: Intimal hyperplasia, Grafts, Shear Stress, Taper, Flow, Orientation

Correspondence to: David N. Ku, MD, PhD
George W. Woodruff School of Mechanical Engineering
Georgia Institute of Technology
Atlanta, Georgia 30332-0405
Tel: (404) 894-6827
Fax: (404) 894-2291

Revision: 4-5-94

ABSTRACT

Purpose: This study was designed to evaluate the effects of wall shear stress and direction of graft taper on anastomotic neointimal hyperplasia rate in end-to-side polytetrafluoroethylene (PTFE) grafts.

Methods: Twenty 4 - 7 mm tapered PTFE grafts were inserted in the femoral and carotid locations in an end-to-side fashion bilaterally in five mongrel dogs to closely simulate the most common clinical configuration. Because shear stress is inversely proportional to the diameter to the third power, shear stress will be much greater at the 4 mm end in comparison to the 7 mm end of the graft. The grafts were harvested after 16 weeks using a pressure perfusion technique. Mean thickness of anastomotic neointimal hyperplasia and mid-graft pseudointima was measured by computer image analysis.

Results: At the time of implantation, the mean shear stress was 18 dynes/cm^2 at the smaller 4 mm end versus 3.4 dynes/cm^2 at the 7 mm end of the tapered grafts ($p < 0.0001$). Flow was significantly higher for the carotid grafts over the femoral grafts, thus 4 different shear stresses of 1.8, 5.0, 9.5, and 27 dynes/cm^2 could be differentiated ($p < 0.001$). Seventeen of twenty grafts were patent at the time of harvest. Thickening was concentric at each anastamosis. Mean anastomotic neointimal thickness was significantly greater at the 7 mm end compared to the 4 mm end of these grafts (0.29 mm vs. 0.18 mm, $p<0.05$), and was also significantly greater for all femoral grafts when compared to the carotid grafts (0.29 mm vs. 0.19 mm, $p<0.001$). The amount of intimal thickening was statistically related to the inverse of the local mean shear rate in a dose response manner yielding a correlation coefficient of 0.96. The thickness of anastomotic neointima or mid-graft pseudointima did not differ significantly when the direction of graft taper was reversed in the femoral or carotid locations.

Conclusion: This data shows that the development of *in vivo* anastomotic neointimal thickness was primarily determined by low wall shear stress even with a variety of flow conditions and graft orientations.

INTRODUCTION

Polytetrafluoroethylene (PTFE) grafts are among the most commonly used vascular grafts in arterial reconstructive procedures (1-5). However, late failure of these grafts from neointimal hyperplasia is a major problem confronting the vascular surgeon. Although the underlying etiology of neointimal hyperplasia has not been fully elucidated, a large number of theories have been proposed to explain this process on the basis of various hemodynamic factors (6-12). Among these factors, wall shear stress is hypothesized to play an important role in the regulation of production of neointimal hyperplasia.

Morinaga et al. have suggested that the variation in wall shear stress during a cardiac cycle is the essential hemodynamic factor related to intimal hyperplasia in arterially transplanted autogenous veins in the canine model (9). The regulatory role for shear stress as well as tangential stress in the modification of the vein graft structure transplanted into the arterial circulation has been also suggested by Zwolak et al (7). Binns et al. evaluated the role of shear stress in the healing of straight PTFE grafts of various diameters (3,6,and 8mm) and described an inverse relationship between the amount of mean shear stress and the associated anastomotic neointima and pseudointima (6). Fillinger et al. (15) studied the effect of tapered grafts were compared with straight grafts for a high flow/high shear Arterio-Venous fistula loop in the canine model (15). They observed that intimal-medial thickening was decreased in the 4 to 7 mm tapered grafts where volume of vibration was lower, suggesting that turbulence may enhance thickening. In a non-human primate model where blood flow was increased by creating an iliac arteriovenous fistula, Zarins et al. have demonstrated that arteries tend to adapt in size to maintain a specific wall shear stress under conditions of increased blood flow (14). Similar results have been reported in the canine model by Kamiya and Togawa (15). Furthermore, wall shear stress has been suggested to play a major role in the pathogenesis of atherosclerosis (16-25).

One approach to defining the relationship between hemodynamics and hemodynamics and intimal hyperplasia is to study the detailed variations in shear stress at an anastomosis (41-42). The shear stress variations at the anastomotic site can be directly compared to morphologic measurements of intimal thickening. However, there are practical limitations to this approach: a) the spatial separation between locations of high and low shear stress is quite small; b) the wall shear stress at a specific site is highly dependent on the exact local geometry and can vary from one surgical anastomosis to the next; and c) the morphologic reconstruction of intimal thickening is difficult to achieve with the required degree of spatial resolution. Definitive conclusions using this approach may be difficult to achieve. Further, previous studies of the role of hemodynamics in graft intimal hyperplasia have varied both shear stress and volume flow simultaneously. Thus, the effects of wall shear stress as an independent factor have not been demonstrated in a clear, quantitative manner for intimal hyperplasia.

An alternative approach would be to create specific hemodynamic conditions *in vivo* where the spatial locations of high and low shear stress are further apart so that spatial resolution is no longer a significant problem. The relationship between wall shear stress and intimal thickening can then be studied more easily without the problems of misregistration and resolution for histologic sectioning. Creation of more uniform hemodynamic conditions would also reduce the relative importance of anatomic variation inherent in studying several anastomoses.

This study was designed to evaluate the relative effects of wall shear stress and direction of graft taper on the development of *in vivo* anastomotic neointimal hyperplasia and mid-graft pseudointima in tapered arterial-arterial PTFE grafts, independent of total flow rate. By the use of a tapered graft, two different shear stresses can be achieved for any single flow rate. By comparing direction of taper, the relative influence of graft orientation on vascular healing can be evaluated as well. The normal clinical geometry of an end-to-side graft anastomosis is preserved. By controlling diameter, a much larger variation in shear stress can be achieved and these variations in shear stress can be spatially resolved for statistical comparison with intimal thickening.

METHODS

A range of shear stress levels at each anastomosis of arterial-arterial grafts was achieved by using tapered grafts. Standard PTFE grafts were inserted in an end-to-side manner to best simulate clinical anatomies.

Five adult male mongrel dogs weighing 20-28 kg each were used in this study. Dogs were selected because their vascular response to injury has been well studied previously (6,8,22,23,26). All animal care was performed in accordance with the "Principles of Laboratory Animal Care" and the "Guidelines for the Care and Use of Laboratory Animals" (NIH Publication No. 80-23, revised 1985). Animals were pretreated with acepromazine (0.1mg/kg) and atropine HCl (0.04mg/kg) administered intramuscularly, and anesthesia was induced with thiopental sodium (10-20mg/kg intravenously). The animals were endotracheally intubated and maintained with 1% to 2.5% isoflurane.

Under sterile conditions, the common femoral and the common carotid arteries were exposed bilaterally. After systemic heparin anticoagulation (100 IU/kg), 4 to 7 mm tapered polytetrafluoroethylene (PTFE) vascular grafts (Gore-Tex®, W. L. Gore & Associates, Inc., Flagstaff, AZ) were cut to a length of 8 cm and implanted bilaterally in the carotid and femoral locations. The grafts were placed in a paired fashion with the direction of graft taper reversed on one side for each anatomical location (Figure 1). End-to-side anastomoses were performed and the intervening segment of native artery was ligated with 0 silk between the anastomoses near the "heel" of each anastomosis to eliminate a blind "cul-de-sac" (Figure 2). The arteriotomy length at each anastomosis was approximately twice the graft diameter at that site. An end-to-side anastomosis was selected as this is more commonly performed clinically than the end-to-end anastomosis. The anastomoses were performed using running CV-7 PTFE suture (Gore-Tex®, W.L. Gore & Associates, Inc., Flagstaff, AZ) with aid of loupe magnification. All wounds were closed in layers using 4-0 polyglactin sutures (Vicryl, Ethicon, Inc.). Cephonicid (10mg/kg) was given intravenously 1 hour before and 6 hours after surgery, followed by cephalexin (500mg) orally for 3 days for antibiotic prophylaxis. During recovery all grafts were checked weekly for patency by direct palpation and Doppler ultrasound.

An end-to-side anastomosis produces shear stress variations over space and time within the hood of the graft. The mean shear stress can be approximated to first order by the Poiseuille formula for straight tubes. The wall shear stress is most dependent on the local diameter of the tubes. The end-to-side geometry only modulates the shear stress around this mean level. The wall shear stress will be different for the same total flow rate or viscosity if one compares the two ends of the tapered graft. After establishing hemostasis, the blood flow rate was measured at all four sites by use of transit time ultrasonography (T201 Ultrasonic Blood Flowmeter; Transonic Systems Inc.).

The average wall shear stress (τ_w) in dyne/square centimeter was calculated as: $\tau_w=32\mu Q/\pi D^3$ where μ is the viscosity of blood (assumed to be 0.035 Poise), Q is the mean flow rate (milliliters per second), and D is the diameter (centimeters) (14). This steady flow shear stress estimate has been shown to be appropriate for the time-averaged mean wall shear stress for pulsatile flow signals (14, 44). This formula for steady flow in a straight tube provides a reasonable approximation for the spatially and time-averaged wall shear stress in the hood of the graft where the thickness measurements were taken (41, 42).

Tissue preparation and morphometry: After 16 weeks, the grafts were harvested and the animals sacrificed as follows. The animals were anesthetized and the grafts were exposed. Patency or occlusion of the grafts was documented prior to harvest using a 5MHz Doppler ultrasound probe. A sternotomy was performed and Ringer's solution was infused at 80 mm Hg pressure through a wide-bore needle into the left ventricle while the animal was synchronously exsanguinated via a cannula placed in the right atrium. Once the blood was cleared from the circulatory system, the arteries and the implanted grafts were perfusion-fixed *in situ* for 30 minutes at 120 mmHg pressure using 5% Glutaraldehyde. Grafts were then harvested with 2 centimeters segments of attached proximal and distal artery and fixed in 5% glutaraldehyde. The animals euthanised with Buthanasia (0.2 ml/kg).

The grafts were embedded in paraffin, sectioned perpendicular to the vessel long axis at each anastomosis as well as in the mid-graft area at the specific locations shown in Figure 3. The sections were then stained with Hematoxylin and Eosin. Morphometric measurements of the anastomotic intimal hyperplasia and mid-graft pseudointima was performed by computer image analysis software (Optimas,

Bioscan, Inc., Edmonds, WA) on a magnified image relayed from a microscope-mounted video camera to a digitizing pad and video monitor (Thomas Optical, Columbus, GA). Thickness was determined at 8 points around the circumference and spatially averaged to yield a mean thickness.

Statistical Analysis: Statistical analysis was performed on a IBM PS/2 computer with use of GraphPAD InStat statistical software. Results were expressed as the mean ± standard error of the mean. The grafts in different locations and taper orientation were compared with respect to (i) anastomotic intimal hyperplasia, and (ii) pseudointimal thickness at the mid-graft section using the paired Student's *t* test. Chi-square analysis was used to determine significant differences in graft patency rates. Results were considered significant if $p < 0.05$.

RESULTS

Volumetric flow rates for the 20 grafts at the time of implantation in the carotid and femoral locations are shown in Table I. The calculated mean shear stress was 18 dyne/cm^2 at the smaller 4 mm end versus 3.4 dyne/cm^2 at the 7 mm end. Flow was consistently 2-3 times greater through the grafts in the carotid location, thus four levels of shear could be distinguished at the ends of each graft. The mean wall shear stress for the femoral grafts was 9 ± 1 dyne/cm^2 at the 4mm end and 2 ± 0.3 dyne/cm^2 at the 7mm end ($p < 0.001$). For the carotid grafts, the corresponding values were significantly higher than the femoral grafts, averaging 27 ± 2 dyne/cm^2 and 5 ± 0.4 dyne/cm^2 at the 4mm and the 7mm ends, respectively ($p < 0.0001$).

At the time of harvest after 16 weeks, a total of 17 of 20 grafts were patent and available for analysis. Patency of the grafts was as follows: 4 to 7 mm taper carotid grafts, 5 of 5; 7 to 4 mm taper carotid grafts, 4 of 5; 4 to 7 mm taper femoral grafts, 3 of 5; 7 to 4 mm taper femoral grafts, 5 of 5 (Table II). No statistically significant difference in patency could be detected between any of the various subgroups.

Anastomotic neointimal thickness was measured at each anastomosis (locations A, B, D, and E, Figure 3) and was defined as the thickness of all material between the endothelial lined flow surface and

the subjacent graft. The histologic appearance of the neointima was that of organized spindle-shaped cells near the lumen with a progression toward hypocellular disorganization toward the graft. The transition from the artery to the pannus in-growth was smooth and regular, without obvious distortion of the lumen circularity. The neointima was generally concentric without favoring the hood, toe, or heel of the anastomosis. The thickening of the graft was relatively uniform. There was slightly less thickening along the normal artery wall (not statistically significant). It is unknown whether the artery wall in the anastomotic section exhibited any adaptation or compensation for luminal stenosis such as with normal arteries.

Representative histologic sections of anastomotic neointima are shown in Figure 4. Figure 4(a,b) illustrates the ingrowth of neointimal cells from the media over the PTFE material. Figure 4(c,d) illustrates high power views of the neointima at two ends of a femoral graft. At the lumenal surface, the dense myocytes were aligned in a circumferential direction. Deeper in the tissue, away from the lumen, the cells were more randomly oriented with a substantial amount of extracellular material. The cells have penetrated into the interstices of the graft.

The mean anastomotic neointimal thickness for all grafts in the femoral location was 0.29 ± 0.02mm which was significantly greater compared to the corresponding neointimal thickness in the carotid grafts which measured 0.19 ± 0.02mm, $p < 0.01$ (Table III). Average anastomotic intima was also thicker at the wide (7mm) ends of all grafts (0.29 ± 0.03mm) compared to their narrow (4mm) ends (0.18 ± 0.03mm, $p < 0.05$ (Table IV)). Figure 4(c,d) contrasts the neointimal thickness at the 7mm end with the much thinner neointima at the 4mm end from the same femoral graft. Less overall neointimal thickening was seen for the carotid grafts which experienced greater overall levels of shear stress (Figure 5).

The inverse relationship between the amount of shear stress and the associated neointimal thickness in this model is shown graphically in Figure 6. The relationship can be expressed mathematically as:

$$\text{Intimal Thickness} = 0.15 \times (1/\text{wall shear stress}) + C.$$

Linear regression analysis between intimal thickness and inverse wall shear yielded a very strong

correlation coefficient of 0.956. C is a constant which would likely depend on the implantation age of the graft.

No significant difference in mean anastomotic neointimal thickness was found when grafts tapering from the 7mm end proximally to the 4mm end distally were compared to grafts with reversed direction of taper (4mm end proximally and 7mm end distally) (Table V).

The mid-graft pseudointimal lining (Section C) was primarily composed of friable thrombotic debris with no endothelial cells present, in contrast to the cellular neointimal growth at the anastomoses. The fibrin accumulation of pseudointima is illustrated in Figure 7. Although the lower shear stress femoral grafts were also associated with a thicker pseudointima formation compared to the carotid grafts, this difference could not be shown to be statistically significant with this number of animals ($p = 0.30$) (Table III). Thickness of the mid-graft pseudointima was also not significantly different when comparing the direction of graft taper (0.24 ± 0.07 versus 0.29 ± 0.03 mm, Table V).

DISCUSSION

These findings clearly support the hypothesis that low wall shear stress influences the thickness of *in vivo* anastomotic neointimal hyperplasia, independent of total flow rate. Normal arteries can experience widely ranging pulsatile flow rates that may or may not be typified by a single measurement in an anesthetized animal. Likewise, the exact amount of shear stress at anastomoses is complex and likely varies over a wide physiologic range in vivo. In the present study, the narrow end (4 mm) of the tapered grafts consistently experienced a ~~much~~ more than 5X higher shear stress compared to the wide end (7 mm) at all flow rates, since wall shear stress is related inversely to the cube of the radius. Thus, a difference in shear stress was isolated as the experimental variable even in the face of physiologically fluctuating flow rates. In this study, overall low shear stress was the primary factor influencing intimal thickening in comparison with the effects of distal location anastomosis or direction of taper since those factors were not found to be significant in this study. Similarly, neointimal thickness variations were found to be independent of the endothelial cell coverage, which was consistent over the entire surface of all graft anastamoses. While not

measured directly, turbulence was expected to be small or absent in these arterial-arterial grafts. Likewise, no differences in pulse and mean pressure were observed.

The quantitative, non-linear relationship between neointimal thickening and wall shear stress shown in Figure 6 is strikingly similar to that shown by Ku et al (24) for early atherosclerotic intimal thickening at the carotid bifurcation. This relationship between intimal thickening and low wall shear stress has also been described for the abdominal aorta (39). Low shear stress conditions have been demonstrated to induce smooth muscle cell proliferation and increase intimal area and thickness in similar calibre PTFE grafts (6,26,27). Increased levels of shear stress from 26 to 78 dynes/cm^2 appears to reduce the total smooth muscle cell area for more porous PTFE grafts in baboons (28). Clinical experience with PTFE grafts for peripheral occlusive disease clearly indicates that low flow and low shear portend poor graft longevity. The results for balloon injury models are more mixed (29). This widely consistent relationship pattern between low shear and intimal thickening *in vivo* demonstrates that normal artery adaptation and the early healing of vascular graft anastomoses is directly influenced by local wall shear stress conditions. These results primarily apply to the early responses of intimal thickening, typical of normal adaptive healing in arterial grafts. It remains to be seen whether low shear stresses can induce pathologic occlusive disease as well (25).

The measurements of intimal thickening in this study permitted direct comparison with the average wall shear stress expected at the graft anastamoses. The hemodynamic variation in wall shear stress at the hood of the anastomosis has been quantified using laser Doppler anemometry and computational fluid dynamics (41, 42). The wall shear stress within the anastomoses is slightly lower (~50%) than the Poiseuille theoretical values. However, the level of the spatially and time-averaged wall shear stress is dominated by the local diameter. The diameter changes in this study caused variations of more than 500% in wall shear stress. Attempts to measure differences in thickness at 1 mm intervals along the hood of the graft were not statistically resolvable. Our conclusions are based, instead, on measurements taken approximately 3 cm apart with no problems in spatial resolution or statistical difference.

Our canine model of intimal hyperplasia thickening demonstrates an orderly smooth muscle cell prolific response with small amounts of extracellular matrix proteins. The lesions did not proceed to full occlusion. It is not known whether this degree of intimal thickening is merely an adaptive process or whether this is the precursor to occlusive intimal hyperplasia (25, 43).

In this study we have quantitatively demonstrated a direct link between level of shear stress and intimal thickening in a large animal model of graft intimal hyperplasia. The strong statistical correlation suggests that hemodynamics can account for approximately 80% of the intimal thickening. However, this study does not rule out the contribution of other factors such as compliance mismatch, hypertension, or other mechanical factors. These other factors were not tested in this model. Nor does this study test the importance of growth factors and thrombosis. No conclusions are made as to the mechanism of how and why shear stress produces intimal thickening.

The mechanism by which shear stress influences the development of neointimal hyperplasia is not well understood. The pannus in-growth is likely to be composed of predominantly smooth muscle cells (30). However, the fluid-wall shear stress interface is primarily experienced by the endothelial cell. One mechanism for neointimal thickening may be that the endothelial cells sense the local wall shear and regulate the amount of underlying smooth muscle cell growth. Clearly, endothelial cells can change their physiological behavior and morphologic appearance in response to wall shear stress (31). Shear-sensitive endothelial cell mechanoreceptors have been postulated as regulators of the adaptation process seen in arteries in response to increased shear stress (32). Such receptors, however, have not been identified. An "endothelial-derived relaxation factor" described by Furchgott (33) and an "endothelial-derived constriction factor" described by Langille and O'Donnell (34) are other mediators that may also play a role in the adaptive response of the arterial wall to alterations in shear stress. A separate humoral factor acting on the media in response to alterations in shear stress has been suggested (15). The role of surface characteristics were not studied in this model (35-36).

Reckter and Gordon have used PCNA techniques to show that SMC proliferation can be high in the anastomotic intimal hyperplasia areas of PTFE grafts in an arterio-venous fistula (AVF) arrangement

(27). Although these mechanisms have been found to be operative in a variety of systems ranging from the adaptive response of native vessels to AVF grafts (14) to highly porous PTFE grafts (37), some or all of them may also regulate the thickness of neointimal hyperplasia in response to alterations in shear stress for the clinically-used prosthetic grafts used in this study. The fact that most of these mediators are endothelium-derived or endothelium-dependent may explain the localization of neointimal hyperplasia in these grafts to the juxta-anastomotic region. In contrast to native vessels, the commonly used human prosthetic grafts are rarely, if ever, fully-lined with endothelium except for a few millimeters adjacent to the anastomosis (30).

This study demonstrates that shear stress is a primary determinant of intimal thickness which overrides factors such as taper direction or distal location of anastomosis. We did not detect a significant difference in patency at four months, in anastomotic intimal hyperplasia thickness, or mid-graft pseudointimal thickness when the direction of graft taper was reversed. The lack of effect of the direction of graft taper on the amount of neointimal hyperplasia in tapered grafts in our study is consistent with that of Byer et al. (38) who compared straight 6 mm PTFE grafts and 4 to 7 mm tapered PTFE grafts in the aorta-iliac position in a canine model. They noted no significant difference in anastomotic intimal hyperplasia between the two graft configurations, although the 4 to 7 mm reverse tapered grafts were associated with a higher absolute patency rate. A tapered graft study by Fillinger et al. utilized higher flow rates from an A-V fistula graft which yielded much higher shear stresses, approximately ten times greater than used here (13). Turbulent effects, which were dominant in their model, were not important in our study. The insignificance of taper direction is indirectly supported by recent studies showing that in-situ saphenous vein grafts are essentially equivalent in failure rate to the reversed saphenous vein grafts (reversed direction of taper) in the femoro-popliteal position. For more distal bypasses, other factors such as size mismatch at the anastomosis come into play.

The graph shown in Figure 7 suggests that grafts with smaller diameters may be better. However, this interpretation would not be correct. Instead the graph should be interpreted to suggest that larger-diameter grafts are worse. Other studies which indicate that severe thrombosis can occur for high levels

of shear stress (40). Thus, very low shear stresses can induce a greater amount of intimal thickening but high shear stresses with a very small diameter graft would be more prone to occlusion by thrombosis. In combination, these two pieces of information would suggest that there is an optimum shear stress or optimum diameter for graft longevity in which intimal thickening is slow and thrombosis is not present (6).

Such a shear stress optimum appears to exist for arteries (14). In normal arteries, the *in vivo* shear stress appears to be determined by the flow rate and diameter. Several studies have shown that the diameter can change for arteries and will adapt to short-term and long-term, chronic changes in shear stress. Grafts are different in that they are not biologic organs and cannot adapt over time. Therefore, the intimal thickening may cause occlusion faster than with arteries which can adapt into intimal thickening by increasing their diameter (25).

In conclusion, this study demonstrates that lower shear stress produces a substantially thicker amount of anastomotic neointimal hyperplasia in a quantitative, dose-response manner, independent of pulsatile total flow volume. Graft taper orientation does not significantly affect the thickness of anastomotic neointima or mid-graft pseudointima in PTFE arterial-arterial grafts. In comparison with the dominant mean shear levels at the anastomoses, small local variations in shear from surgical geometry appear to be minor. These results apply to *in vivo* PTFE grafts composed of the pore-size and material configuration used most commonly in clinical vascular surgery today. Since mean wall shear is primarily determined by the graft diameter, selection of an optimal size graft for typical flow rates may increase the long term patency of prosthetic grafts (6). Additional future studies should focus on the stimulation of intimal thickening by hemodynamic measures with the objective of distinguishing the occlusive response.

REFERENCES

1. Imparato AM, Bracco A, Kim GE, Zeff R. Intimal and neointimal fibrous proliferation causing failure of arterial reconstructions. Surgery 1972;72:1007-17.
2. Cacciatore R, Inderbitzi R, Stirnemann P. Five years experience with infra-inguinal arterial reconstruction: a comparison of venous with PTFE bypass. VASA 1992;21:171-6.
3. Whittemore AD. Autogenous saphenous vein versus PTFE bypass for above-knee femoropopliteal reconstruction. J Vasc Surg 1992;15:895-7.
4. McCollum C, Kenchington G, Alexander C, Franks PJ, Greenhalgh RM. PTFE or human umbilical vein for femoro-popliteal bypass: a multi-center trial. Eur J Vasc Surg 1991;5:435-43.
5. Johnson WC, Squires JW. Axillo-femoral (PTFE) and infrainguinal revascularization (PTFE and umbilical vein). Vascular Registry of the New England Society for Vascular Surgery. J Cardiovasc Surg 1991;32:344-9.
6. Binns RL, Ku DN, Stewart MT, Ansley JP, Coyle KA. Optimal graft diameter: Effect of wall shear stress on vascular healing. J Vasc Surg 1989;10:326-37.
7. Zwolak RM, Adams MC, Clowes AW. Kinetics of vein graft hyperplasia: Association with tangential stress. J Vasc Surg 1987;5:126-36.
8. Bassiouny HS, White S, Glagov S, Choi E, Giddens DP, Zarins CK. Anastomotic intimal hyperplasia: Mechanical injury or flow induced. J Vasc Surg 1992;15:708-17.
9. Morinaga K, Okadome K, Kuroki M, Miyazaki T, Muto Y, Inokuchi K. Effect of wall shear stress on intimal thickening of arterially transplanted autogenous veins in dogs. J Vasc Surg 1985;2:430-3.
10. Abbott WM, Megerman J, Hasson JE, L'Italien G, Warnock DF. Effect of compliance mismatch on vascular graft patency. J Vasc Surg 1987;5:376-82.
11. Fillinger MF, Reinitz ER, Schwartz RA, Resetarits DE, Paskanik AM, Bredenberg CE. Beneficial effects of banding on venous intimal-medial hyperplasia in arteriovenous loop grafts. Am J Surg 1989;158:87-94.
12. Kuroki M, Okadome K, Inokuchi K, Sugimachi K. Intimal hyperplasia: The permeation of serum-

derived substance into the arterial autovein graft under abnormal blood flow. Jpn J Surg 1988;18:300-7.

13. Fillinger MF, Reinitz ER, Schwartz RA et al. Graft geometry and venous intimal-medial hyperplasia in arteriovenous loop grafts. J Vasc Surg 1990; 11:556-66.
14. Zarins CK, Zatina MA, Giddens DP, Ku DN, Glagov S. Shear stress regulation of artery lumen diameter in experimental atherogenesis. J Vasc Surg 1987;5:413-20.
15. Kamiya A, Togawa T. Adaptive regulation of wall shear stress to flow change in the canine carotid artery. Am J Physiol 1980; 239:H14-21.
16. Ku DN, Zarins CK, Giddens DP, Glagov S. Separated and secondary flow and early atherosclerosis in the human carotid bifurcation. In: Role of Blood Flow in Atherogenesis, eds Yoshida Y et al, New York: Springer-Verlag, 1988: 73-79.
17. Caro CG, Fitz-Gerald JM, Schroter RC. Atheroma and arterial wall shear: Observation, correlation and proposal of a shear-dependent mass transfer mechanism for atherogenesis. Proc R Soc Lond (Biol) 1971;117:109-59.
18. Zarins CK, Giddens DP, Bharadvaj BK, Sottiurai VS, Mabon RF, Glagov S. Carotid bifurcation atherosclerosis: Quantitative correlation of plaque localization with flow velocity profiles and wall shear stress. Circ Res 1983;53:502-14.
19. Zarins CK, Bomberger RA, Glagov S. Local effects of stenoses: Increased flow velocity inhibits atherogenesis. Circulation 1981;64(Suppl 2):221-7.
20. Butterfield AB, Miller CW, Lumb WV, McLeod FD, Nelson AW, Histand MB. Inverse effect of chronically elevated blood flow on atherogenesis in miniature swine. Atherosclerosis 1977;26:215-24.
21. Towne JB, Quinn K, Salles-Cunha S, Bernhard VM, Clowry LJ. Effect of increased arterial blood flow on localization and progression of atherosclerosis. Arch Surg 1982;117:1469-74.
22. Sako Y. Effects of turbulent flow and hypertension on experimental atherosclerosis. JAMA 1962;179:36.
23. Flaherty JT, Ferrans VJ, Pierce JE, Carew TE, Fry DL. Localizing factors in experimental

atherosclerosis. In: Likoff W, et al. eds Atherosclerosis and coronary heart disease. New York: Grune & Stratton, Inc. 1972:40-83.

24. Ku DN, Giddens DP, Zarins CK, Glagov S. Pulsatile flow and atherosclerosis in the human carotid bifurcation: Positive correlation between plaque location and low and oscillating shear stress. Arteriosclerosis 1985;5:293-302.
25. Glagov S, Giddens DP, Bassiouny H, White S, Zarins CK. Hemodynamic effects and tissue reactions at grafts to vein anastomosis for vascular access. In: Vascular Access for Hemodynanics - II, ed. Sommer BG, Henry ML, Precept Press, Inc., 1991; 3-20.
26. Kraiss, LW, Kirkman TR, Kohler TR, Zierler B, Clowes AW. Shear stress regulates smooth muscle proliferation and neointimal thickening in porous polytetrafluoroethylene grafts. Arteriosclerosis and Thrombosis 1991; 11:6 1844-52.
27. Rekhter M, Nicholls S, Ferguson M, Gordon D. Cell proliferation in human arteriovenous fistulas used for hemodialysis. Arteriosclerosis and Thrombosis 1993; 13:4 609-617.
28. Kohler TR, Kirkman TR, Kraiss LW, Zierler BK, Clowes AW. Increased blood flow inhibits neointimal hyperplasia in endothelialized vascular grafts. Circulation Research 1991; 69:6 1557-65.
29. Kohler TR, Jawien A. Flow affects development of intimal hyperplasia after arterial injury in rats. Arteriosclerosis and Thrombosis 1992; 12:8 963-71.
30. Sottiurai VS, Yao JST, Batson RC, Sue SL, Jones R, Nakamura YA. Distal anastomotic intimal hyperplasia: Histopathologic character and biogenesis. Ann Vasc Surg 1989;3:26-33.
31. Nerem RM. Vascular fluid mechanics, the arterial wall, and atherosclerosis. J Biomech Engng 1992; 114:274-82.
32. Rodbard S. Negative feedback mechanisms in the architecture and function of the connective and cardiovascular tissues. Perspect Biol Med 1970;13:507-27.
33. Furchgott RF. Role of endothelium in responses of vascular smooth muscle. Circulation Res 1983;53:557-73.
34. Langille BL, O'Donnell F. Reductions in arterial diameter produced by chronic decreases in blood

flow are endothelium- dependent. Science 1986;231:405-7.

35. Mathisen SR, Wu D, Sauvage LR, Usui Y, Walker MW. An experimental study of eight current arterial prostheses. J Vasc Surg 1986;4:33-41.

36. Greisler HP. In: New Biologic and Synthetic Vascular Prosthesis, Austin: R. G. Landes Co, 1991.

37. Clowes AW, Kirkman TR, Reidy MA. Mechanisms of arterial graft healing; A rapid transmural capillary ingrowth provides a source of intimal endothelium and smooth muscle in porous PTFE prostheses. American Journal Physiology May 1986; 220-30.

38. Byer A, Moss CM, Keys RC, Hobson RW, Padberg F, Campion T. Reversed tapered vs. straight PTFE (Gore-Tex) grafts in dogs. J Cardiovasc Surg 1988;29:470-5.

39. Moore JE, Xu C, Glagov G, Zarins CK, Ku DN. Fluid wall shear stress measurements in a model of the human abdominal aorta: Oscillitory behavior and relationship to atherosclerosis, in submission.

40. Hanson SR, Markou C., Lindahl AK, Ku DN, Scarborough RM, Phillips DR, Gorecki M, Pinet A, Garfinkel LI, Kelley A, Harker CA. "Inhibition of in vivo thrombosis formstrain in an arterial stenosis model." Abstract XIVth Congress International Society on Thrombosis and Hemostosis, 1993.

41. Steinman DA, Vinh B, Ethier CR, Ojha M, Cobbold RSC, Johnston KW. A numerical stimulation of flow in a two-dimensional end-to-side anastomosis model. J Biomech Engng 1993, 115: 112-118.

42. Loth F. Velocity and wall shear measurements inside a vascular graft model under steady and pulsatile flow conditions. PhD Thesis, Georgia Institute of Technology, Atlanta, GA, 1993.

43. Bassiouny HS, White S, Glagov S, Choi E, Giddens DP, Zarins CK. Anastomotic intimal hyperplasia: Mechanical injury or flow induced. J Vasc Surg 1992; 15:708-17.

44. He X, Ku DN. Unsteady entrance flow development in a straight tube. J Biomech Engng, 1994: in press.

FIGURE LEGENDS

Figure 1. Diagramatic representation of the study design and placement of tapered grafts in dogs. Note the reversal of direction of graft taper on the right side.

Figure 2. Diagrammatic representation of the end-to-side anastomoses. The intervening segment of native artery is ligated close to each anastomosis.

Figure 3. Pattern of graft sectioning for morphometric analysis. Sections A,B: proximal anastomosis; section C: mid-graft section; sections D,E: distal anastomosis.

Figure 4.

(a),(b): Low power microscopic sections through the anastomoses at the 4mm and 7mm ends of a tapered graft, respectively. Note the difference in neointimal thickness between the two ends. L:lumen; N:neointima.

(c),(d): High power sections of the anastomotic neointima in a femoral graft. This endothelium-lined layer is formed of highly organized spindle-shaped cells.

Figure 5. High power sections of the anastomotic neointima in a tapered carotid graft for the (a) 4 mm end and (b) 7 mm end. Note the thinner neointima at the 4 mm end which experiences higher shear stress. Likewise, the 7 mm carotid neointima is thinner than the 7 mm femoral neointima (Figure 4d).

Figure 6. Graph of anastomotic neointimal thickness (mean $\pm$ SE) in relation to shear stress at the anastomoses of tapered grafts. F:femoral grafts; C:carotid grafts; 4:4mm end; 7:7mm end.

Figure 7. High power section of the mid-graft pseudointima. This layer is composed mainly of friable thrombotic debris with no endothelial lining.

Table I. Flow rates and shear stress for the 4mm and 7mm ends of the tapered grafts in the carotid and femoral location

	Flow rate (ml/min)	*Shear stress (dyne/cm²)*	
		4mm end	*7mm end*
Carotid grafts	$290 \pm 23^{*}$	$27 \pm 2^{*}$	$5 \pm 0.4^{*+}$
Femoral grafts	102 ± 41	9 ± 1	$2 \pm 0.3^{+}$

$^{*}p < 0.0001$ compared with the corresponding end of the femoral grafts.
$^{+}p < 0.001$ comparing the 4 mm end to the 7 mm end.

Table II. Graft patency by type and location.

Graft type	*Direction of graft taper*		*All grafts*
	4 to 7mm	*7 to 4mm*	
Carotid grafts	5/5	4/5	9/10
Femoral graft	3/5	5/5	8/10
Total	8/10	9/10	17/20

Table III. Thickness of anastomotic neointima and mid-graft pseudointima for carotid and femoral tapered grafts.

	Anastomotic Neointima(mm)	*Mid-Graft Pseudointima(mm)*
Carotid grafts	$0.19 \pm 0.02^{*}$	$0.21 \pm 0.04^{+}$
Femoral grafts	0.29 ± 0.02	0.31 ± 0.06

$^{*}p < 0.01$ compared with the femoral grafts.
$^{+}p = 0.30$ (NS) compared with the femoral grafts.

Table IV. Thickness of anastomotic neointima at the narrow (4mm) and the wide (7mm) end indicating differences between the carotid and femoral positions for the tapered grafts.

Graft end	*Carotid grafts*	*Femoral grafts*	*All grafts*
Narrow end (4mm)	0.13 ± 0.02 *+	0.22 ± 0.04 *	0.18 ± 0.03*
Wide end (7mm)	0.26 ± 0.04 +	0.36 ± 0.03	0.29 ± 0.03

*$p < 0.05$ compared with the 7mm end of the grafts.
+$p < 0.05$ compared with femoral graft ends of the same dimensions.

Table V. Thickness of anastomotic neointima and mid-graft pseudointima for 7 to 4mm taper vs. 4 to 7mm taper grafts.

Direction of Graft taper	*Anastomotic Neointima (mm)*			*Mid-Graft Pseudointima (mm)*
	7mm end	*4mm end*	*Mean*	
7 to 4 mm	0.32±0.04+	0.13±0.02+	0.24±0.0	0.24 ± 0.07+
4 to 7 mm (Reversed)	0.26±0.04	0.21±0.04	0.23±0.03	0.29±0.03

+p = Not significant compared with the reversed grafts.

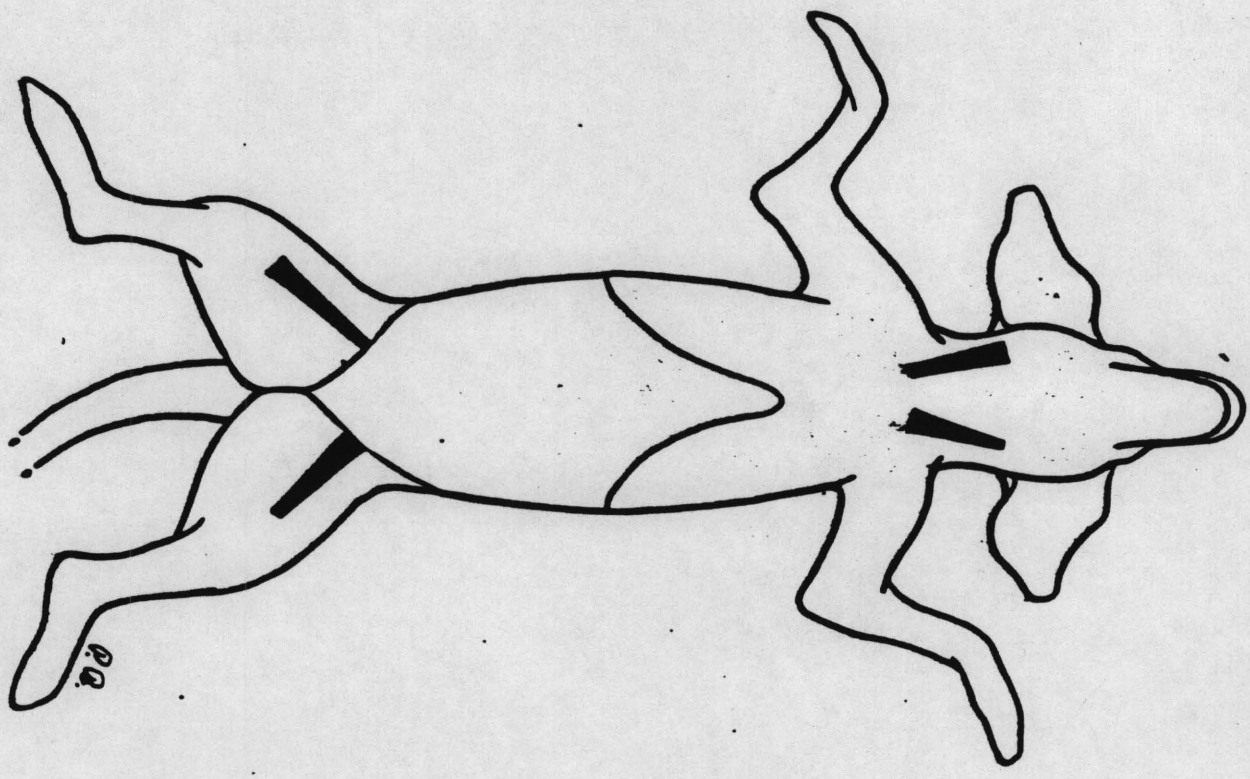


Figure 1

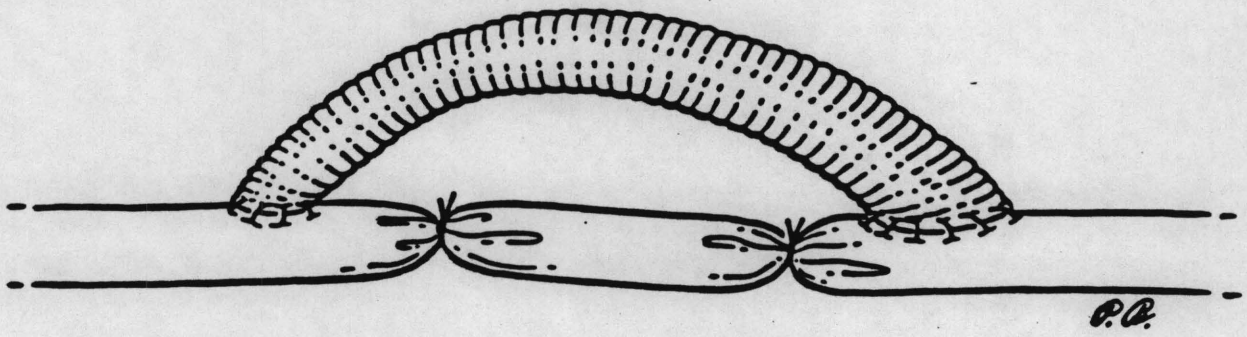


Figure 2

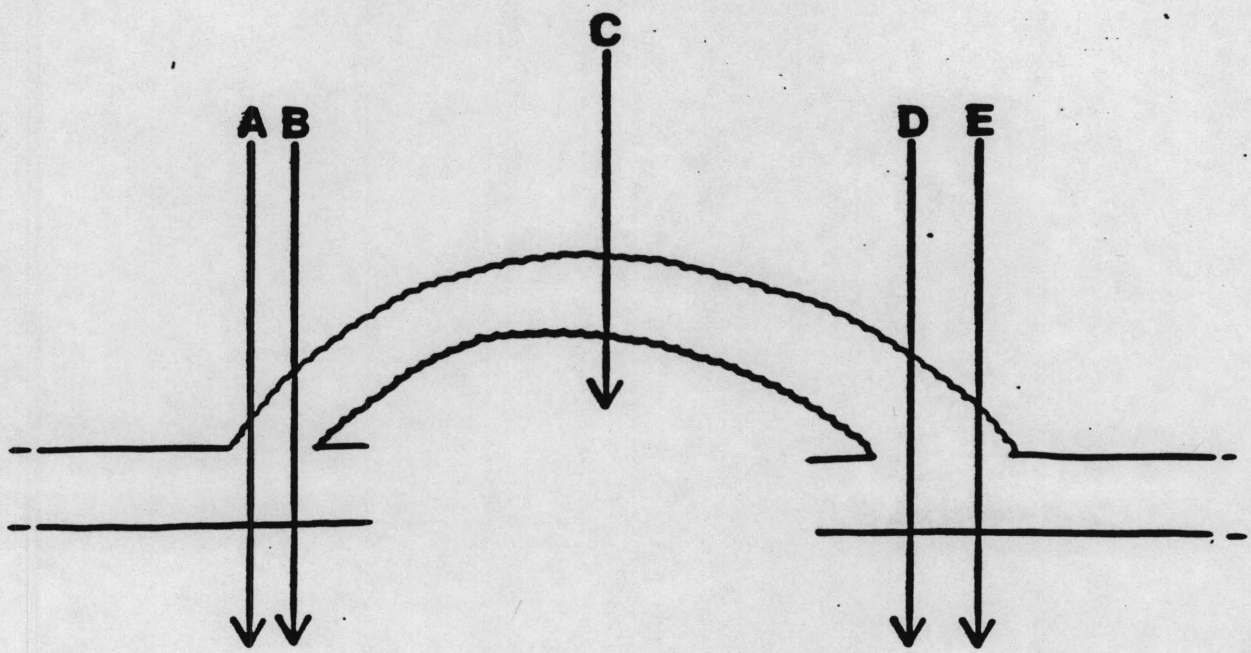
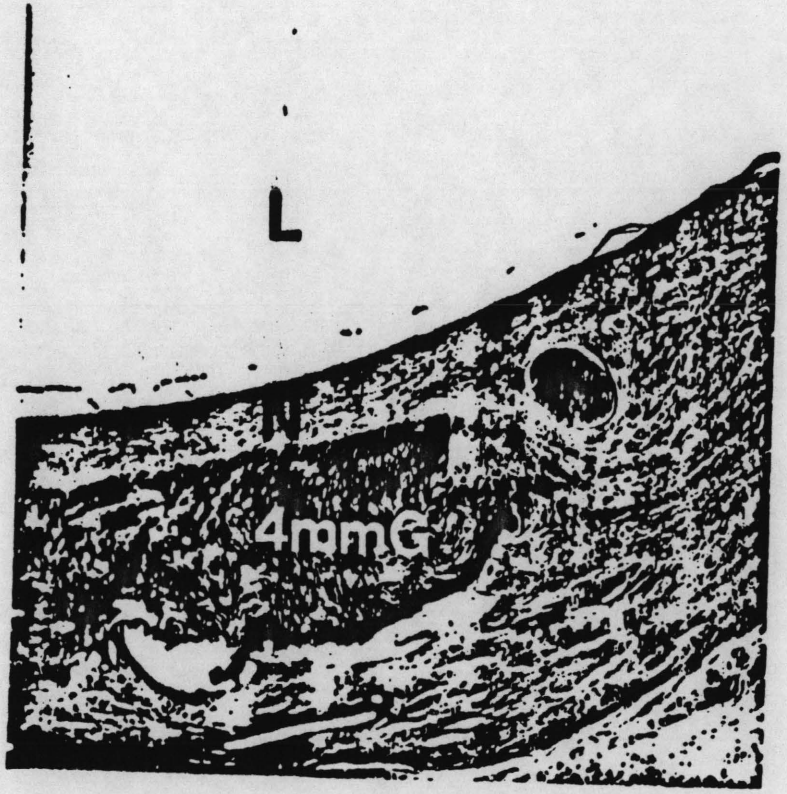
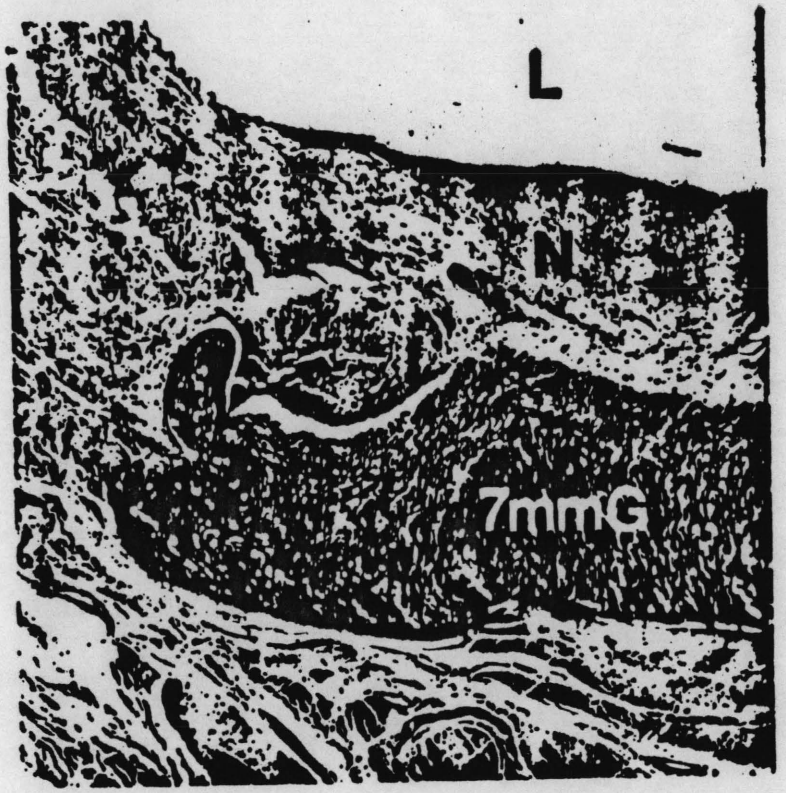


Figure 3

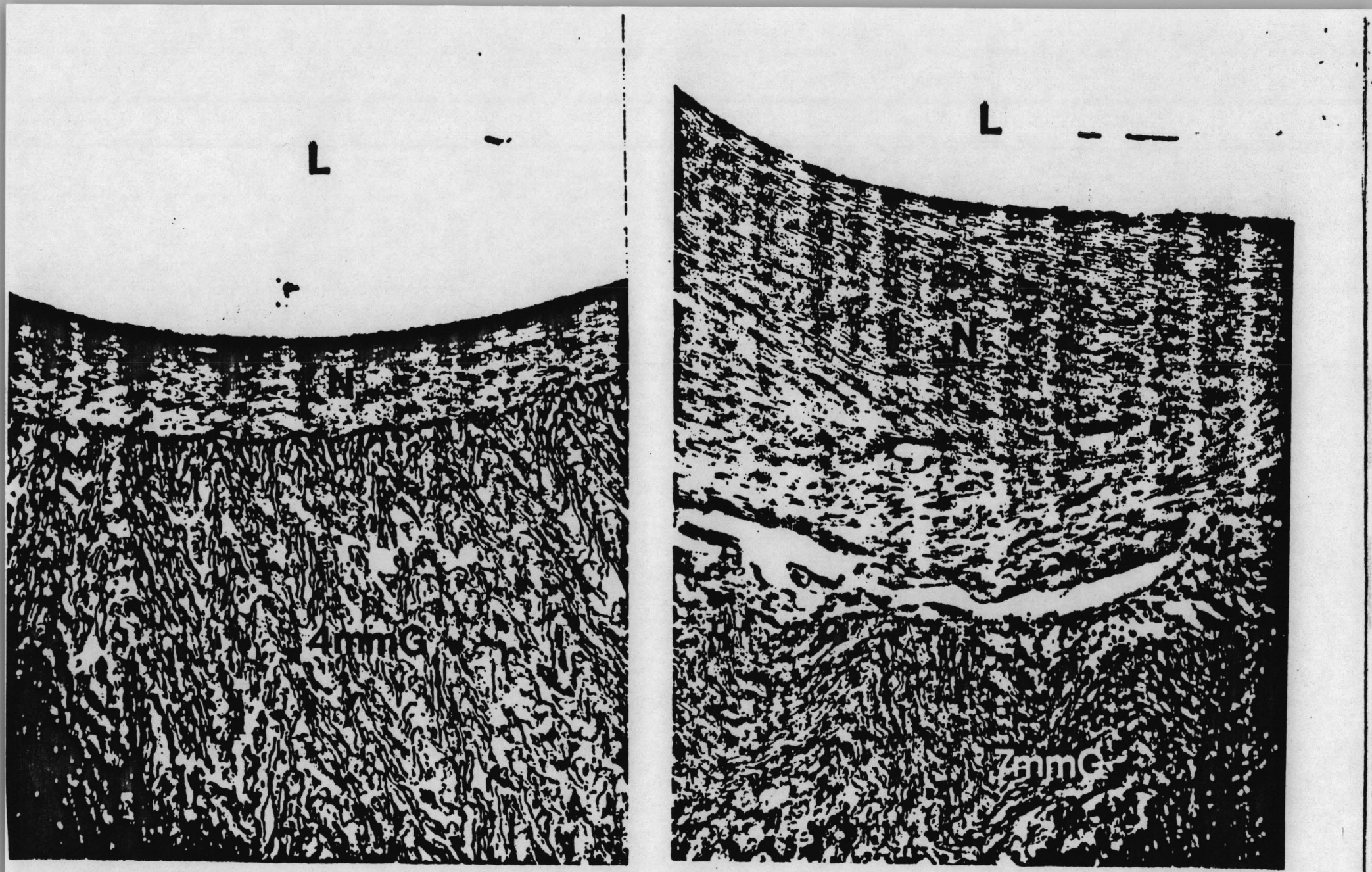


(a)



(b)

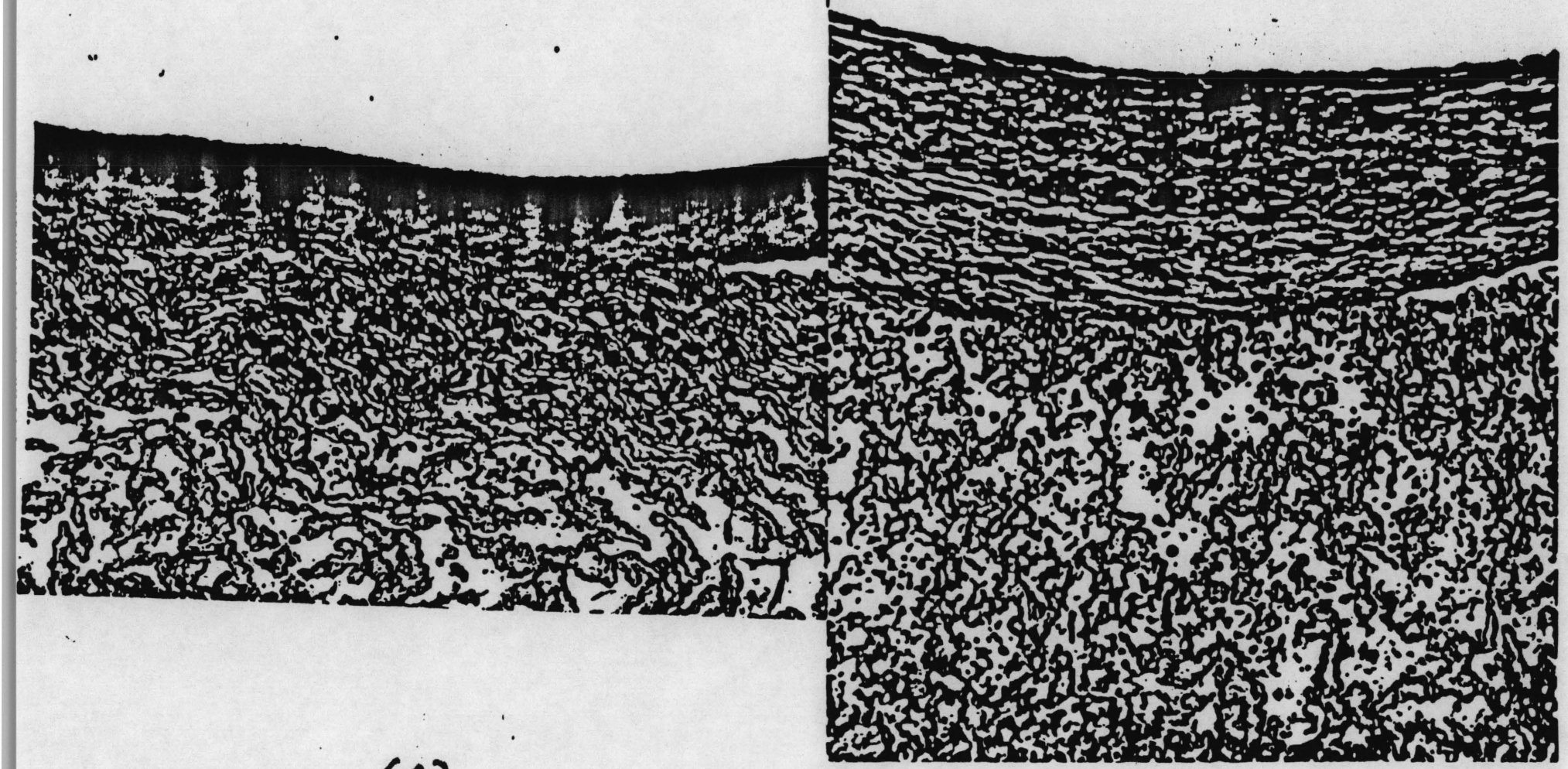
Figure 4



(c)

(d)

Figure 4



(a)

(b)

Figure 5

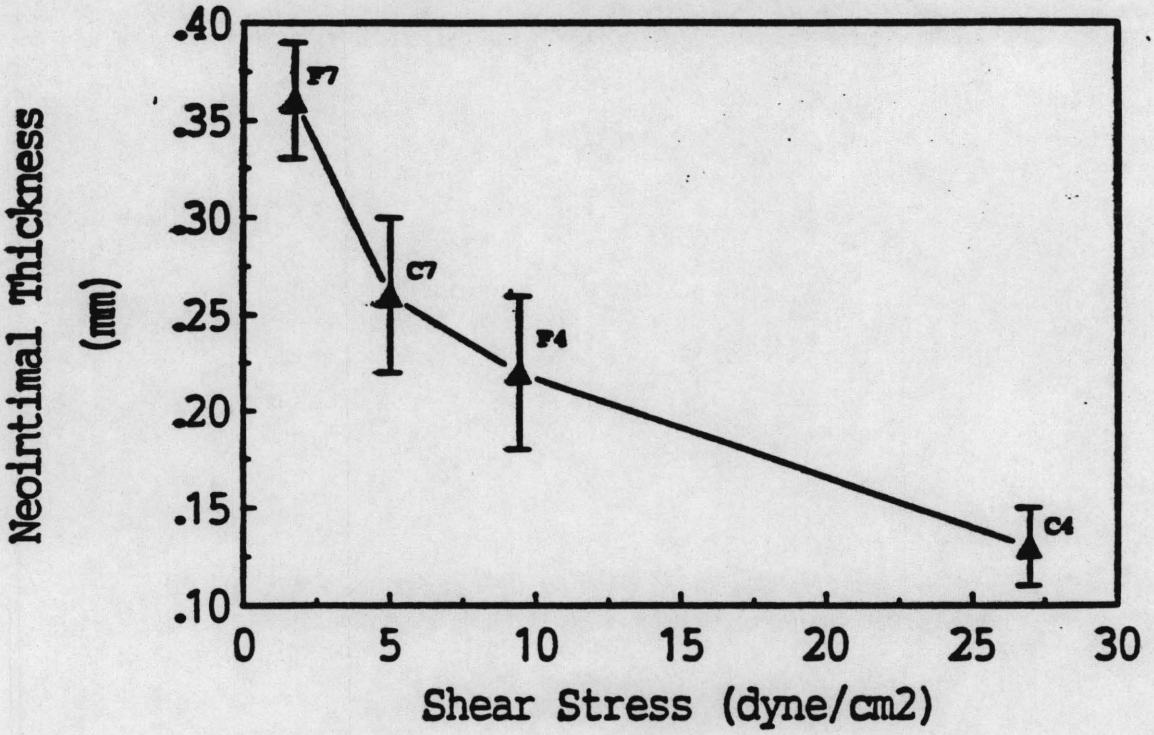

Neointimal Thickness
(mm)
.40
.35
.30
.25
.20
.15
.10
0
5
10
15
20
25
30
Shear Stress (dyne/cm2)
F7
C7
F4
C4

Figure 6

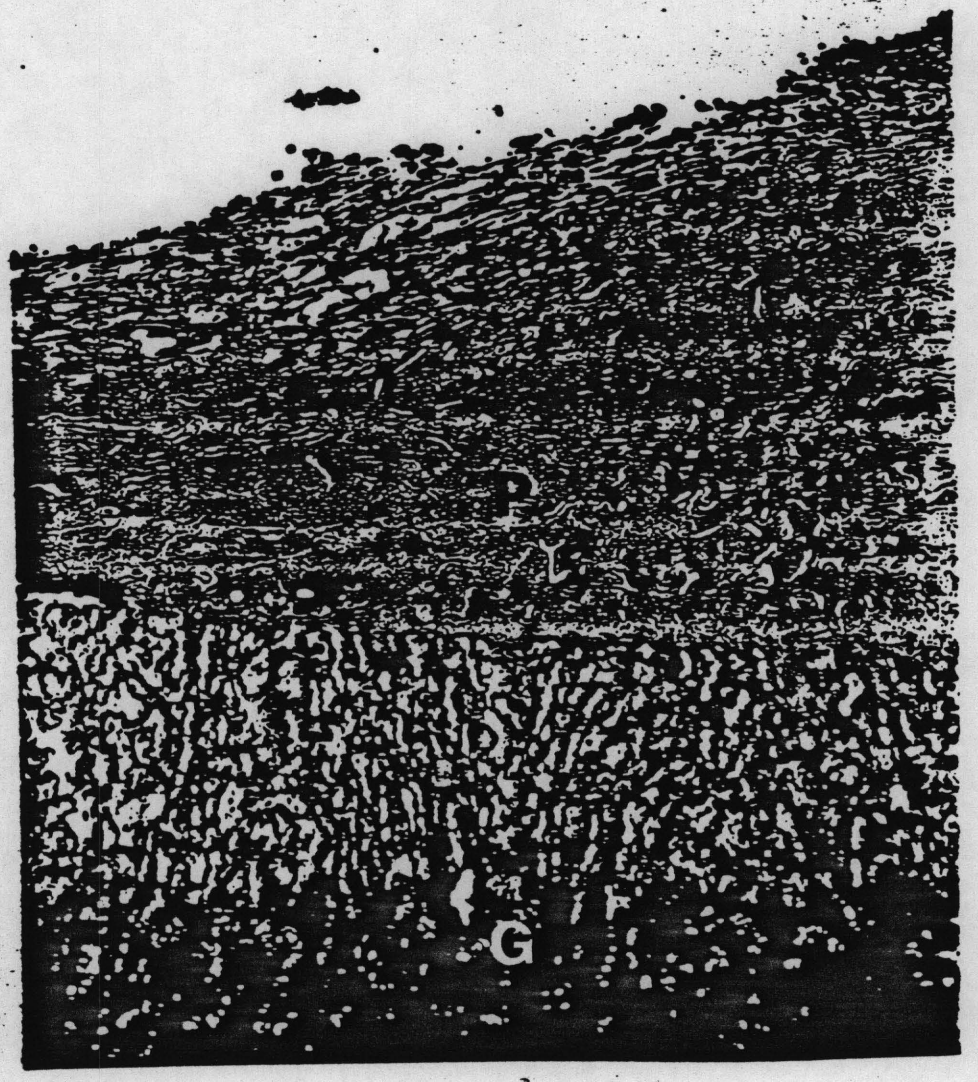


Figure 7

OCCLUSIVE INTIMAL HYPERPLASIA IS ACCELERATED BY HEMODYNAMIC MANIPULATIONS

Tarek A. Salam, MD FRCS

Alan B. Lumsden, MD

David N. Ku, MD PhD

Section of General Vascular Surgery

Emory University, Atlanta, GA

and

School of Mechanical Engineering

Georgia Institute of Technology, Atlanta, GA

Running Title: Occlusive Intimal Hyperplasia

Correspondence to: David N. Ku, MD PhD
Georgia Institute of Technology
School of Mechanical Engineering
Atlanta, GA 30332-0405
Phone: (404) 894-6827
Fax: (404) 894-2291

Acknowledgements: Appreciation is expressed to Carol Ames who performed the histological preparation.

8/30

ABSTRACT:

Occlusive intimal hyperplasia in artificial vascular grafts may be stimulated by a variety of biochemical and hemodynamic factors. This study was designed to evaluate the effect of reduction of blood flow rate and increased turbulence on the development of anastomotic neointimal hyperplasia in polytetrafluoroethylene (PTFE) grafts. After two weeks, flow was reduced by 50% on one side by banding the distal artery in 5 animals (Group I), and the mid-graft section in another 5 animals (group II). Grafts on the opposite served as controls. The grafts were harvested after 10 weeks and intimal thickness was measured by computer image analysis.

For the control grafts, 20 of 20 were patent at the time of harvest. In contrast, only 9 of 20 of the banded grafts were patent ($p < .01$). Graft occlusion in the study grafts occurred at a mean of 32 days after flow reduction. Histologically, a endothelium-lined thick hypocellular layer of intimal hyperplasia was present at the anastomoses of the banded grafts. In contrast, the anastomoses of the control grafts were lined by a thin, highly-organized cellular layer. The mean thickness of the intimal hyperplastic response at both the proximal and distal anastomoses of the banded grafts was significantly greater compared to the control grafts. This data shows that reduction of blood flow rate in PTFE grafts accelerates the production of anastomotic intimal hyperplasia with rapid progression to occlusion, independent of early thrombotic events.

INTRODUCTION

Intimal hyperplasia at the anastomotic ends of vascular grafts remains an important problem to the clinical treatment of symptomatic vascular diseases. Expanded polytetrafluoroethylene (PTFE) vascular grafts are among the most commonly used grafts for peripheral vascular disease as well as for vascular access for hemodialysis. The majority of these grafts fail due to progressive proliferation of smooth muscle cells at the edge of the graft eventually leading to total occlusion of the lumen (1). In the earliest period of implantation, thrombosis and platelet deposition occurs on the artificial surface of clinically used PTFE grafts (18). A second phase of the early healing process involves a migration of endothelial and smooth muscle cells from the host arteries. At a later point in time, the cellular neointima increases in bulk to produce full occlusion of the lumen. In humans, the mean time to occlusion is approximately two to four years. It is this latter occlusive process which is important clinically and is the main subject of this article.

Hypotheses for intimal hyperplasia include a number of putative agents including platelet activation (1-3), immunologic factors (4), and mitogenic factors (5). Pharmacologic agents to minimize or inhibit these processes at the vascular anastomosis, however, have been unsuccessful (6,7). There is considerable evidence that hemodynamic conditions at the anastomosis play a major role in the development of neointimal hyperplasia (8,9). Differences in circumferential compliance of the native vessel and graft material (10), low wall shear stress (11,12), and non-laminar flow (13), have all been proposed as factors contributing to the proliferative process.

Controversy remains as to the relative roles of these factors in the development of intimal hyperplasia. Part of the problem is that the human occlusive process takes years to develop and most animal models of intimal hyperplasia produce very thin amounts of tissue in-growth at the anastomotic sites. Typically, the animal forms of

intimal thickening are smooth muscle constructs which are better termed intimal fibromuscular hypertrophy which is histologically different from the disorganized intimal hyperplasia seen in human occlusive disease (16). These short term models of intimal hyperplasia put undue emphasis on the initial events of the vascular graft healing process and may not fully address the factors related to the occlusive process.

Our previous work has shown that anastomotic intimal thickening is directly related the levels of low wall shear stress in vascular grafts, independent of local flow rates ( ). This new study is designed to induce low flow, with and without turbulence, and to characterize the development of occlusive neointimal thickening which ultimately results in poor patency of the grafts. Several questions are addressed by this study: Is occlusive intimal thickening dependent on the early events related to thrombosis? Can hemodynamic conditions accelerate anastomotic graft intimal thickening into full occlusion? Our initial hypothesis is that the early events of graft healing are not as critical and that low wall shear stresses play a relatively important role in the development of occlusive disease.

EXPERIMENTAL DESIGN

The effects of low flow on the development of occlusive disease was studied in a canine model. The dog was chosen because PTFE grafts do not develop neointima throughout the graft, similar to humans, and we have a large experience with this model. End-to-side arterial-arterial grafts were placed in a standard fashion using clinically approved graft material to simulate directly typical human hemodynamic conditions. The protocol was designed to create changes in flow conditions well after platelets have adhered and after the graft has been covered by a pseudointima of fibrin. Thus, the role of changes in hemodynamic factors could be evaluated independent of the early effects of thrombosis, graft material surface, and initial migration of arterial cells into the anastomoses.

Control and study grafts were placed in the carotid and femoral positions in dogs in a paired manner. The right sided grafts served as controls and the left sided grafts served as the study grafts. Arterial grafts generally remain patent for more than 20 weeks in this dog model (20). Two animal groups were studied. Group I had grafts placed in the four positions in the normal fashion described below. After two weeks, the flow through the study grafts was reduced by 50% using a double ligature stenosis placed in the distal artery receiving the flow. Since all other parameters were equal, the 50% reduction in flow caused a 50% reduction in the wall shear stress. Thus, the Group I study grafts experienced low shear stress relative to the control grafts in the same dog.

A similar double ligature stenosis was placed on the Group II study grafts at a location 2 cm just upstream of the distal anastomosis. The distal anastomosis in these grafts experienced lower shear stress and also increased levels of turbulence downstream of the ligature. Group I and Group II grafts were distinguished by their exposure to turbulence in addition to lower shear stresses.

MATERIALS AND METHODS

Ten mongrel dogs of either sex weighing 20-28 kg. were used in this study. Dogs were selected because their vascular response to injury has been well studied previously (11-15). All animal care was performed in accordance with the "Principles of Laboratory Animal Care" and the "Guidelines for the care and use of Laboratory Animals" (NIH Publication No. 80-23, revised 1985). Animals were pretreated with acepromazine (0.1mg/kg) and atropine HCl (0.04mg/kg) administered intramuscularly, then anesthesia was induced with thiopental sodium (10-20mg/kg intravenously), the animals were endotracheally intubated and maintained with 1% to 2.5% isoflurane. Prophylactic cephalosporin (Monocid) 10mg/kg was given intravenously immediately prior to skin incision.

Under sterile conditions, the common femoral arteries and the common carotid arteries were exposed bilaterally. After systemic heparin anticoagulation (100 IU/kg), proximal and distal control of the arteries was obtained with vascular clamps. Six (6) mm PTFE grafts were implanted in an end-to-side fashion at all four sites. An end-to-side anastomosis was selected as this is clinically more commonly performed than the end-to-end anastomosis. The arteriotomy length at each anastomosis was approximately twice the graft diameter at that site. The anastomoses were performed using running CV-7 PTFE suture (W.L.Gore & Associates Inc.) with aid of loop magnification. Care was exercised to place corresponding anastomoses at the same level bilaterally. After completion of the anastomoses, the intervening native vessel was ligated with 0 silk between the anastomoses near the "heel" of each anastomosis to eliminate a blind "cul-de-sac", and flow was established through the grafts (figure 1a). After establishing hemostasis, all wounds were closed in layers using polyglactin 910 sutures (Vicryl, Ethicon, Inc.).

After two weeks, both carotid and femoral grafts were exposed bilaterally, and blood flow was measured at all four sites over the native artery just proximal to the proximal anastomosis by use of transit time ultrasonography (T201 Ultrasonic Blood Flowmeter; Transonic Systems Inc.). The grafts on one side were used for the study while those on the opposite side served as controls. The animals were divided into two groups of five each. In one group (Group I), an 0 silk ligature was placed around the native artery on the study side about one centimeter distal to the graft and gradually tightened until the flow rate recorded by the flowmeter was reduced by about 30%. A second 0 silk ligature was then placed about one-half of a centimeter distal to the first one and similarly tightened until the flow rate is reduced to about 50% of the initial value (figure 1b). This method of double ligature allows for more precise control of flow during placement of the stenosis and resulted in a persistent decrease in flow over time.

In the other group (Group II), ligatures were placed in a similar fashion around the central portion of the graft on the study side until similar reduction of flow rate was reached (Figure 1c). The placement of the stenosing ligature on the mid-graft section adds increased turbulence to the reduced flow effect on the distal anastomosis. In both groups, no intervention was done on the control side. Assignment to the study or control side was done at random. During recovery all grafts were checked weekly for patency with a directional Doppler at a site beyond the distal anastomosis.

Tissue preparation and morphometry

Eight weeks following the intervention described, the grafts were harvested and the animals sacrificed as follows. The animals were anesthetized as described herein and the grafts were exposed. Patency or occlusion of the grafts was documented prior to harvest using a Doppler ultrasound probe and flow rate was again measured just proximal to the proximal anastomosis. A sternotomy was performed and Ringer's solution was infused at 80 mmHg pressure through a wide bore needle into the left ventricle while the animal was synchronously exsanguinated via a cannula placed in the right atrium. Once the blood was cleared from the circulatory system, the arteries and the implanted grafts were perfusion fixed in situ for 30 minutes at 120 mmHg pressure using 5% glutaraldehyde. Grafts with 2 centimeters segments of attached proximal and distal artery were then harvested and fixed in 5% glutaraldehyde, and the animals euthanised with Buthanasia (0.2 ml/kg).

All grafts, regardless of patency, were submitted to histological examination and morphometric analysis. The grafts were embedded in paraffin and sectioned transversely at each anastomosis as well as in the mid-graft area and then stained with Hematoxylin and Eosin or VanGieson-Masson trichrome. Morphometric analysis of intimal thickness area the anastomotic intimal hyperplasia and mid-graft pseudointima was performed using a Thomas Optical measurement system with Optimas Bioscan

software. Additional morphologic measurements were made as to cell count per high powered field cell orientation, and extracellular matrix amount at different depts of the intimal hyperplasia.

Statistical Analysis

The study grafts were compared to their controls with respect to (i) patency rates, (ii) thickness of the anastomotic intimal hyperplasia at both the proximal and distal anastomoses. Statistical analysis was performed on a PS/2 IBM computer with use of Instat statistical software. The paired two-tailed Student t test was used to compare the study grafts to their controls. Chi-square analysis was used to determine significant differences in patency rates. Results were expressed as the mean $\pm$ standard error of the mean and differences were considered significant if $p < 0.05$.

RESULTS

A persistent reduction of flow rate by 50% of the initial value was achieved by banding of the study grafts of both Group I and Group II. The flow rates at the time of placement of the stenosis for the control and study grafts in both groups are shown in Table I. This flow reduction persisted over the study period as shown in Table II which lists the mean flow rates at harvest for the patent grafts in the study group and their controls. At the time of harvest 10 weeks after implantation, all of the 20 control grafts were patent while only 9 of 20 grafts with flow reduction were patent (6 in Group I and 3 in Group II) compared to their corresponding control grafts. This difference in patency was statistically significant for both Group I grafts ($p < 0.05$) and Group II grafts ($p < 0.01$) compared to controls (Figure 2). There was not a statistically significant difference between the patency rates of Group I and Group II.

Only two of the study grafts and none of the control grafts acutely thrombosed within the first three days after banding and were excluded from further analysis. The

remaining occluded grafts over a period of time ranging between two and six weeks (mean of 32 days).

Microscopic examination of the occluded grafts revealed a thick layer of intimal hyperplasia lined by a single layer of endothelium with near total occlusion of the lumen of the graft at the anastomoses (Figure 3). The intimal hyperplasia was not uniform in appearance. At the lumenal surface, an intact endothelial cell layer uniformly covered the smooth muscle cells. Near the surface of the lumen, the cells were highly oriented, spindle-shaped, and aligned in a circumferential manner and exhibited very little extracellular substance (see Figure 4a). In marked contrast, the deep portion of the intimal hyperplasia underneath this surface was highly disorganized, with a chaotic pattern of rounded cell shapes and (Figure 4b). Cell density was low due to a large amount of extracellular material. Histologic staining indicated that much of the extracellular material was collagen suggesting that these cells were in a synthetic mode instead of a proliferative mode.

The mean thickness of anastomotic intimal hyperplasia was significantly greater for the unoccluded study grafts in Group I and II compared to their corresponding control grafts (Table III). The increased thickness in the study grafts was significant at both the proximal and distal anastomoses. In general, the intimal thickness was greater at the proximal ends instead of the distal anastomoses, although this difference did not reach statistical significance. Although the mean thickness of anastomotic intimal hyperplasia was greater for the grafts in Group II when compared to the grafts in Group I both at the proximal and distal anastomoses, this difference, was not statistically significant.

The markedly cellular intimal hyperplasia noted at the anastomoses of the occluded grafts was also distinctly different histologically from the pseudointima within the mid-graft sections which consisted primarily of an amorphous acellular mass of thrombotic debris and fibrin with complete lack of endothelial cells (Figure 5).

DISCUSSION

The early thrombotic and cellular migration events related to normal graft healing were constant between the study and control grafts in this model of intimal hyperplasia. The fact that occlusion was strongly different between the control and study grafts indicates that these early events may play a relatively minor role in the occlusive process. Likewise, since the graft surface is typically covered by pseudointima after the first two weeks (18), the graft surface composition is unlikely to have been the major stimulus for graft occlusion.

Our study protocol eliminated events in the early post-operative period from study, concentrating on the latter events of hemodynamic changes from low flow, low shear, and turbulence. Low shear stress, or a change to a low shear stress condition after initial healing events, stimulates occlusive tissue expansion. The histologic appearance of the canine intimal hyperplasia is almost identical to descriptions of advanced human intimal hyperplasia (16). There is an intact endothelial cell surface which overlies a disorganized array of ovoid smooth muscle cells embedded in a large amounts of extracellular matrix material. The intimal hyperplasia is limited to approximately two centimeters of perianastomotic graft without coverage in the mid-portion. Our past studies of the natural history of canine intimal hyperplasia indicates that the growth can be sudden and episodic, not linear (11). Thin amounts of intimal thickening at early time points reveal a highly organized stroma of intimal fibromuscular hypertrophy and a paucity of extracellular matrix. Later stages of thick intima have a characteristic layer of highly organized smooth muscle cells near the lumenal surface but highly disorganized, hypocellular masses of extracellular matrix deep near the graft. The thrombosis occurring in the central area of pseudointimal hyperplasia appears to be non-contributory to the cellular processes at both anastomotic sites.

The results from this study are very similar to the clinical experience of PTFE grafts in humans. Grafting in the face of low flow situations with either poor inflow or high outflow resistance can lead to early occlusion (19). Intimal thickening has been shown to be directly related to low conditions with exponential rises in thickness seen when shear stresses are below 5 dynes/cm^2 (20).

Turbulence is an important hemodynamic factor which may or may not contribute significantly to the development of intimal hyperplasia. Fillinger et al studied the amount of turbulence in arterio-venous fistula (AVF) grafts in dogs and concluded that higher Reynolds numbers and turbulence volumes enhanced early intimal thickening (13). The studies cannot be directly compared, however, since the flow rates and turbulence levels through the AVF grafts were much greater than with arterial-arterial grafts and the amount of thickening in our study was far greater. However, the poor patency rates in our group of grafts exposed to turbulence is consistent with this conclusion, although the results were not statistically significant different from low flow alone.

Several studies exist as to the role of various agents on the healing of balloon injured arterial segments (eg 17, 18, 21). The thin amounts of cellular thickening of the normal arterial wall to these injuries may or may not be analogous to the occlusive intimal hyperplasia in our graft model.

Intimal hyperplasia should not be considered as scar tissue, but rather a viable biologic entity that is constantly undergoing remodeling under the influence of blood flow dynamics (14, 16, 18). The increased thickness of anastomotic intimal hyperplasia in response to reduction of blood flow rate shown in the present study, may represent an adaptive response of the vessel wall to meet the altered flow conditions (16, 20). Persistence of the low flow state at the anastomosis is likely to continue to stimulate an intimal proliferative response. In our canine model of graft occlusion, hemodynamic low wall shear stress overwhelmed all other variables and accelerated occlusion in a

short amount of time.

Endothelial cells and smooth muscle cells grown in culture have been shown to respond to low shear stress conditions (18, 22). The morphology of endothelial cells grown in low shear or static conditions are random in orientation without a preferred direction. As the intimal thickening progresses, one would expect the deeper layers to be more insulated from the shear effects of hemodynamics and experience a more static environment. PTFE grafts are essentially non-distensible under physiologic pressure conditions (18). Thus, the perigraft cells would be expected to experience the least amount of stress from pressure pulses and circumferential stretch as well. Abbot and Megerman have suggested that the low compliance of PTFE grafts is associated with higher rates of intimal hyperplasia (10). In our model, the deepest layers of cells in the perigraft region exhibited the most disorganization and extracellular matrix material. The appearance of the intimal hyperplasia in this region strongly suggests that growth of neointima in this region from the production of extracellular matrix is the dominant mechanism by which intimal hyperplasia becomes occlusive. Rodbard has hypothesized that the normal adaptive response of cells to static conditions is the production of large amounts of extracellular matrix production (23). It is possible that the lack of stretch in the PTFE graft removes any mechanical stimulus to the smooth muscle cells in this layer and transforms the cells into a synthetic mode with production of large amounts of extracellular matrix. The highly synthetic mode of these cells and the hypocellularity in this region would suggest that proliferation is not the major culprit, but that synthesis is. Future studies of the response of endothelial cells to low levels of wall shear stress and the response of underlying smooth muscle cells to endothelial cell products should provide important information on the cellular and molecular mechanisms producing the transformation of thin, organized healing into occlusive growth.

Our study lends support to the clinical significance of flow limiting stenosis in

vascular grafts used in lower extremity bypass procedure for atherosclerotic occlusive disease, and in vascular access for hemodialysis. The results emphasize the importance of serial evaluation of these grafts with measurement of flow rates to permit early detection of flow limiting stenoses. Prompt intervention prior to rapid acceleration of intimal hyperplasia to complete occlusion may then allow more graft salvage. The study also provides a model for the accelerated production of intimal hyperplasia within 10 weeks for possible future pharmacologic or further hemodynamic manipulations aimed at reducing of this proliferative process.

In conclusion, a new animal model of accelerated graft occlusion from hemodynamics is presented. The results indicate that low shear stress can stimulate occlusion, independent of early events related to thrombosis on the artificial PTFE graft surface. The intimal hyperplasia in this model matches the histological appearance of intimal hyperplasia in humans. Future studies of graft occlusion should focus on smooth muscle cell organization and synthesis instead of only cell proliferation.

Table I. Blood flow rate before and after banding at the time of placement of the stenosis.

	Flow rate (ml/min)	
	Before banding	After banding
Group I		
Control grafts	200±47	
Study grafts	190±28	90±15
Group II		
Control grafts	210±47	
Study grafts	230±33	120±18

Table II. Number of patent grafts and mean blood flow rate at time of harvest (10 weeks).

	No. of patent grafts at harvest	Flow rate (ml/min)
Group I		
Control grafts	10	120±25
Study grafts	6	90±6
Group II		
Control grafts	10	130±37
Study grafts	3	100±12

Table III. Mean neointimal thickness at the proximal and distal anastomoses of the patent study grafts compared to their controls. Thickness in occluded grafts would be 3 mm or 10 times greater.

	Anastomotic Neointimal Thickness (mm)			
	Group I grafts		Group II grafts	
	Proximal Anastomosis	Distal Anastamosis	Proximal Anastomosis	Distal Anastamosis
Study grafts	0.604±0.13	0.579±0.13	0.967±0.13	0.858±0.11
Control Grafts	0.340±0.05	0.246±0.02	0.367±0.06	0.281±0.02
p value <	0.04	0.02	0.002	0.001

REFERENCES

1. Ferns GA, Raines EW, Sprugel KH, Motani AS, Reidy MA, Ross R. Inhibition of neointimal smooth muscle accumulation after angioplasty by an antibody to PDGF. Science 1991;253:1129-32.
2. Hagen PO, Wang ZG, Mikat EM, Hachel DB. Antiplatelet therapy reduces aortic intimal hyperplasia distal to small diameter vascular prostheses (PTFE) in nonhuman primates. Ann Surg 1982;195:328-39.
3. LoGerfo FW, Quist WC, Nowak MD, et al. Downstream anastomotic hyperplasia. A mechanism of failure in Dacron arterial grafts. Ann Surg 1987;197:479-83.
4. Hoepp LM, DeWeese JA, Elbadawi A. Effect of steroids and immunosupression on anastomotic intimal hyperplasia in femoral arterial bypass grafts. Surg Forum 1976;27:215-6.
5. Barrett TB, Benditt EP. Sis (platelet-derived growth factor B chain) gene transcript levels are elevated in human atherosclerotic lesions compared to normal artery. Proc Natl Acad Sci USA 1987;84:1099-103.
6. Clowes AW, Karnovsky MJ. Failure of certain antiplatelet drugs to affect myointimal thickening following arterial endothelial injury in the rat. Lab Invest 1977;36:452-64.
7. McCready R, Price M, Kryscio R, Hyde G, Mattingly S, Griffen W. Failure of antiplatelet therapy with ibuprofen (Motrin) to prevent neoinitimal fibrous hyperplasia. J Vasc Surg 1985;2:205-13.
8. Berguer R, Higgins RF, Reddy DJ. Intimal hyperplasia. An experimental study. Arch Surg 1980;115:332-5.
9. Shin CS, Hatem JN, Abach IF. Effects of diminished distal blood flow on the morphologic changes in autogenous vein grafts. Surg Gynecol Obstet 1978;147:189-92.

10. Abbott WM, Megerman J, Hasson JE, L'Italien GJ, Warnock DF. Effects of compliance mismatch on vascular graft patency. J Vasc Surg 1987;5:376-82.
11. Salam TA, Lumsden AB, Suggs WD, Ku DN. Effect of wall shear stress and graft orientation on vascular healing of tapered grafts. Surg Forum 1992,43:322-3.
12. Binns RL, Ku DN, Stewart MT, Ansley JP, Coyle KA. Optimal graft diameter: Effect of wall shear stress on vascular healing. J Vasc Surg 1989;10:326-37.
13. Fillinger MF, Reinitz ER, Schwartz RA, Resetrits.DE, Paskanik DB, Bredenberg CE. Beneficial effects of banding on venous intimal-medial hyperplasia in arteriovenous loop grafts. Am J Surg 1989;158:87-94.
14. Sottuiurai VS, Yao JS, Batson RC, Sue SL, Jones R, Nakamura YA. Distal anastomotic intimal hyperplasia: Histopathologic charater and biogenesis. Ann Vasc Surg 1989;3:26-33.
15. Bassiouny HS, White S, Glagov S, Choi E, Giddens DP, Zarins CK. Anastomotic intimal hyperplasia: Mechanical injury or flow induced. J Vasc Surg 1992;15:708-17.
16. Glagov S, Giddens DP, Bassiouny HS, White S, Zarins CK. Hemodynamic effects and tissue reactions at graft to vein anastomosis for vascular access. In: Vascular Access for Hemodynanics - II, ed. Sommer BG, Henry ML, Precept Press, Inc., 1991; 3-20.
17. Mason RA, Hui J, Campbell R, Giron F. Effects of endothelial injury on smooth muscle cell proliferation. J Vasc Surg 1987;5:389-98.
18. Grieisler HP. New biologic and synthetic vascular prostheses. RG Landes Co., Austin, 1991.
19. Clinical low flow
20. Salam TA, Lumsden AE, Suggs ED, Ku DN. Low shear stress promotes intimal hyperplasia in a dose-response manner. In submission.
21. Kohler TR, Jawien A. Flow affects development of intimal hyperplasia after

arterial injury in rats. Arteriasclerosis and Thrombosis 1992; 12:8 963-71.

22. Nerem RM. Vascular fluid mechanics, the arterial wall, and atherosclerosis. J Biomech Engng 1992; 114:274-82.

23. Rodbard S. Negative feedback mechanisms in the architecture and function of the connective and cardiovascular tissues. Perspect Biol Med 1970;13:507-27.

FIGURE LEGENDS

Figure 1. End-to-side vascular graft placement in the carotid and femoral arteries in a canine model. (a) Graft configuration at onset of study. (b) Group I - Placement of a flow reducing stenosis on the native artery one centimeter downstream of the distal anastamosis. (c) Group II - Placement of a flow-reducing stenosis on the PTFE graft one centimeter upstream of the distal anastamosis.

Figure 2. Patency of grafts ten weeks after implantation and eight weeks after placement of flow reducing stenosis.

Figure 3. Neointimal hyperplasia in a highly stenotic graft subjected to flow reduction two weeks after implantation. Note the straited cells just beneath the lumen, and the disorganized, chaotic whirls of hypercellular thickening near the graft.

Figure 4a. High-magnification view of the striated layers near the blood-intimal surface of the occlusive intimal hyperplasia. Note the intact, thin, normal appearing endothelial cells at the lumenal surface.

Figure 4b. High-magnification view of the deep portion of occlusive intimal hyperplasia near the graft surface. Note the hypocellularity, large amount of extracellular matrix material, and lack of organized lamellae in this region.

Figure 5. High-magnification view of the pseudo-intimal hyperplasia from the mid-portion of the grafts. Note the absence of cellular elements and the amorphous appearance of fibrin and trapped erythrocytes.

Figure 6. Intimal hyperplasia at the anastomotic site of a graft which was totally occluded clinically. Note the similar morphology to the highly stenotic graft shown in Figure 2.