Late Failure in Vein Grafts:

Mediating Factors in Subendothelial Fibromuscular Hyperplasia

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Late failure of autologous vein grafts often results from excessive subendothelial fibromuscular hyperplasia. Varying factors have been implicated in this process, but the exact etiology remains unclear. In this study, three groups of animals were studied which had sections of common carotid artery replaced with autologous vein grafts. Group I had simple replacement, while Groups II and III had grafts supported by either tightly woven or loose mesh dacron prostheses. Thrombosis occurred only in the unsupported grafts. Unsupported grafts also had more subendothelial proliferation when compared to the loose mesh group. The combined thickness of the intima-media correlated inversely with the number of vasa present within the vein graft wall, *i.e.* significantly more vascularization was found in the mesh supported group when compared to the other groups. Grafts supported with the tightly woven prosthesis were relatively impervious to the ingrowth of vasa vasora. The data suggest that persistent distension of the graft wall by intra-arterial pressure influences the degree of subendothelial fibromuscular hyperplasia and may be interrelated to revascularization of the graft wall *per se*.

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LATE FAILURE OF AUTOLOGOUS vein grafts represents a persistent and significant problem.[2,3,10,11] Such failure, occurring more than six months postoperatively, usually results from subendothelial fibromuscular proliferation which compromises the graft lumen.[5,10,15,16] The mechanisms responsible for this proliferative response are poorly understood, although the available evidence indicates that devascularization of the vein segment,[5,18] overdistension of the vein prior to implantation,[14] decreased blood flow through the graft,[8] and ischemia of the graft wall[5] are involved in this phenomenon.

The purpose of this investigation was: 1) to determine if controlled dilatation of the vein graft, by means of a supportive prosthesis, alters subendothelial fibromuscular proliferation, and 2) to evaluate the effect of inhibition of vasa vasora ingrowth into the wall of the vein graft.

Materials and Methods

Eleven healthy mongrel dogs of either sex with an average weight of 22 kg were studied. The external jugular veins and common carotid arteries were exposed and mobilized through two lateral neck incisions. Venous dissection was completed with care to avoid unnecessary injury to the vessel wall. The excised vein segment was flushed gently against zero resistance with heparinized normal saline and placed in this solution at room temperature until the common carotid arteries were prepared for grafting (less than 15 min).

A 6 cm length of the common carotid artery was removed and replaced with the reversed vein segment in an end to end anastomosis. The time from removal of the vein to restoration of circulation through the graft was approximately 30 min.

The outside diameter of the proximal carotid artery and mid-region of the graft was measured 30 min. after restoration of blood flow. The true inside diameter was calculated by subtracting twice the thickness of the artery or vein wall from the outside diameter measurements.

The control group consisted of seven dogs which

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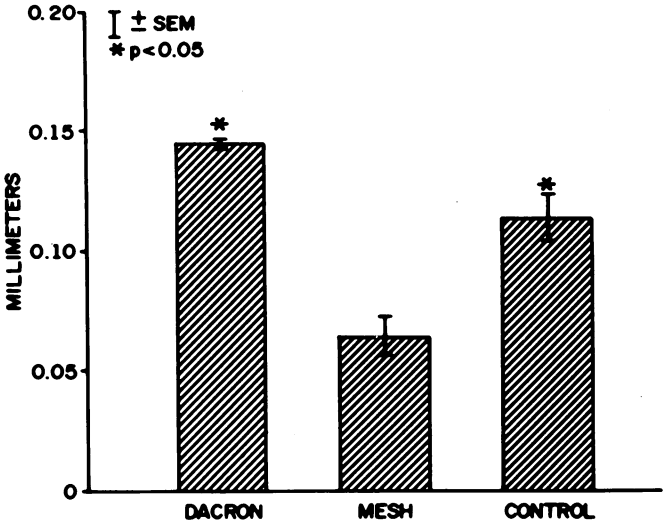


FIG. 1. Comparison of subendothelial wall thickness.

received a total of 14 unsupported grafts. The remaining four animals received eight grafts supported by a dacron mesh prosthesis (mesh group) on the right side and by a woven dacron prosthesis (woven group) on the left side.

The dacron mesh prosthesis was wrapped around the grafts after the anastomoses were completed. The free edges were sutured together longitudinally, constricting the diameter of the graft uniformly by 10%. The same length of carotid artery was removed on the contralateral side, and the proximal anastomosis was performed as previously described. The distal end of the vein segment was occluded temporarily with a vascular clamp and the diameter of the graft measured. A woven prosthesis of proper size was selected to assure consistent reduction of the diameter of the graft by 10%. The vein segment was threaded through the prosthesis and the distal anastomosis completed. Stay sutures were placed through both ends of the prosthesis and the adventitia of the proximal and distal artery.

Six months following this preparation the animals were restudied and the brachiocephalic artery cannulated through a standard thoracotomy incision. The grafts were flushed *in situ* with normal saline at a pressure of 120 mm Hg followed by a 2.5% glutaraldehyde–formaldehyde fixing solution in 0.1 M phosphate buffer at a similar pressure. They were removed and placed in fresh fixative for an additional 24 hours followed by a thorough washing in phosphate buffer.

Longitudinally oriented tissue sections for light microscopic evaluation were dissected in transverse sections from five regions of the arteriovenous graft: proximal carotid artery, proximal, middle and distal graft, and distal carotid artery. Sections were selected from those regions which appeared thickest on gross examination. Tissue samples were placed in 10% neutral buffered formalin and routinely processed for light microscopy. Microscopic sections were stained with hematoxylin and eosin, Verhoeff von Gieson and hematoxylin-phloxine-saffron stains.

Specimens for electron microscopic evaluation were selected from areas adjacent to those areas selected for light microscopy. Thin sections, 400 to 600 Å in thickness were mounted on copper grids and stained with saturated uranyl acetate and lead citrate solutions.

The thickness of the subendothelial region was measured using a grid type reticule of known diameters in the ocular of a light microscope. Micrometer graduation intervals were determined for objectives used in this study. The subendothelial layer of the graft was defined to include the tunica intima and media. Three measurements of the thickness of this layer were made from each of the hematoxylin-phloxine-saffron stained sections. The same grid was used to count the number of vasa vasora in the graft wall underlying the sites where the subendothelial layer was measured. Vasa vasora were counted only if they were present within the grid at a standard magnification (240×). Transverse sections of the graft wall were used to determine the orientation of vasa vasora in various regions of the graft.

Results

A total of 22 arteriovenous grafts were inserted in the common carotid arteries of 11 dogs. One graft from a control group animal hemorrhaged on the fourth postoperative day and was removed. Thrombosis was present in three grafts from the control group at the time of sacrifice. Grafts from the woven and mesh groups did not have thrombosis at the time of necropsy.

The ratio of carotid artery diameter to graft diameter in the control group at the time of surgery was 1:2.5 which increased to 1:3.4 at the time of necropsy ($p < 0.05$). Changes in ratio were not observed in grafts from the woven and mesh groups.

The intimal surface appeared qualitatively similar in grafts from all groups. Anastomotic suture lines were covered by a dense, slightly raised layer of opaque tissue which quickly tapered off as it extended into the intimal surfaces of the adjacent artery and graft. A few small irregular opaque intimal thickenings were observed in all grafts but did not exceed 0.5 cm^2. Atherosclerotic plaques of the grafts were not observed.

The average subendothelial thickening (Fig. 1) in control group grafts was 0.11 mm ± 0.01 (mean ± SEM) and in woven group grafts, 0.14 mm ± 0.01 (p N.S.). The thickness of the combined intima-media of mesh group grafts was 0.06 mm ± 0.01 which

represents a statistically significant difference when compared to either control group ($p < 0.05$) or woven group grafts ($p < 0.05$).

Microscopically, in all groups, the subendothelial layer was composed primarily of smooth muscle cells, and fibrous connective tissue. Smooth muscle cells of the most superficial layer, comparable to the tunica intima, were oriented parallel to the long axis of the graft. The deeper layer of smooth muscle cells, comparable to the tunica media, was oriented in either a circular or spiral fashion relative to the long axis of the graft.

Grafts from control and woven groups were characterized by a thickened subendothelial layer which showed both smooth muscle cell hyperplasia and hypertrophy with varying amounts of collagen (Figs. 2 and 3). Grafts from the mesh group contained a relatively thinner subendothelial layer which characteristically did not show the degree of smooth muscle cell hyperplasia or hypertrophy present in the other groups (Fig. 4).

The number of vasa vasora present in the graft wall

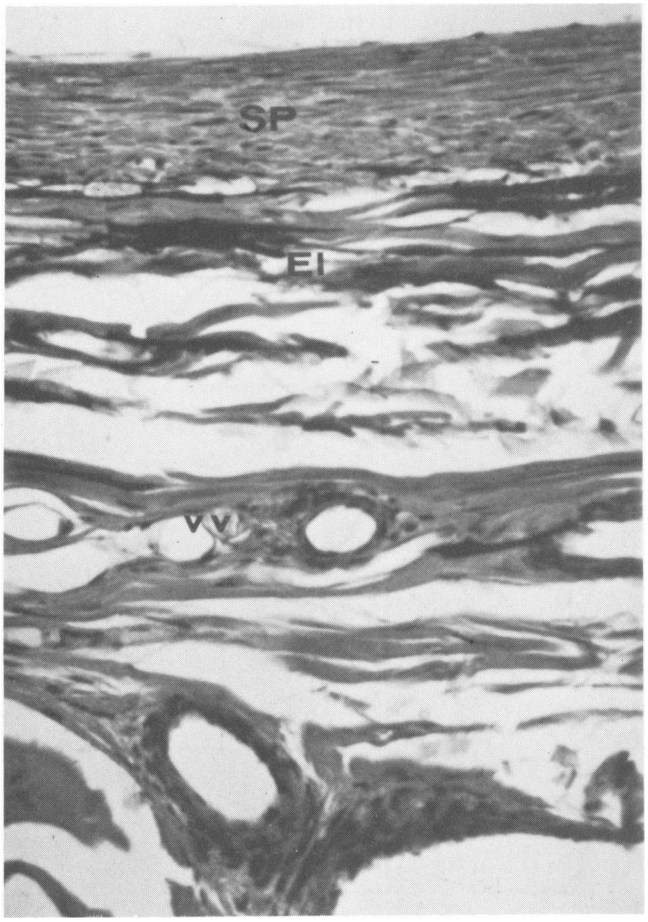


FIG. 2. Section from a control graft showing thickened subendothelial layer (SP), small bands of elastic fibers (El) and vasa vasora (vv). (Verhoeff von Gieson stain, 240×).

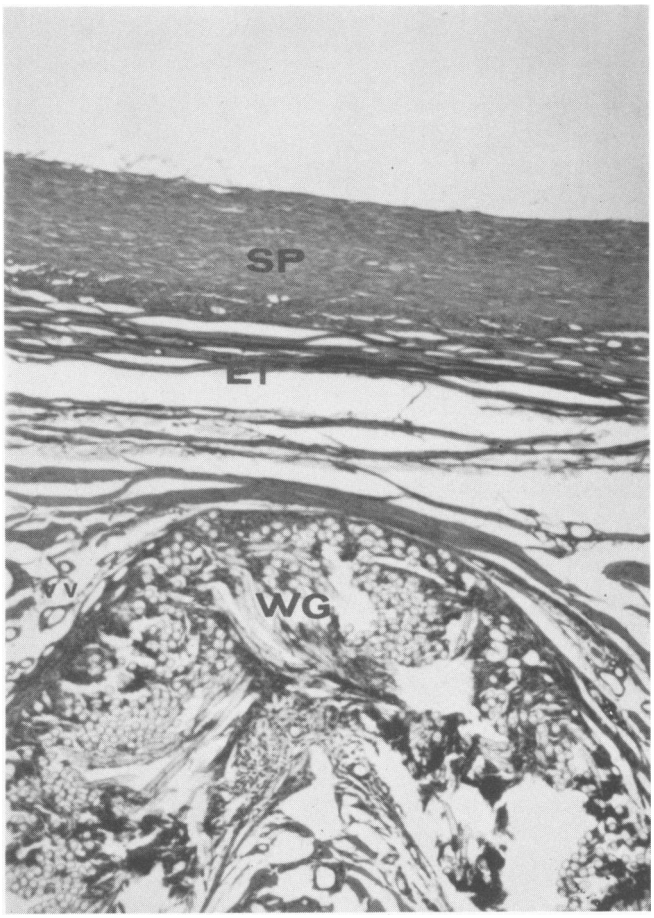


FIG. 3. Woven group graft showing subendothelial proliferation (SP). The prosthesis is clearly shown (WG). Small elastic fibers (El), and vasa vasora (vv) are present. (Verhoeff von Gieson stain, 240×).

was determined on sections taken parallel to the long axis of the vessel. Control grafts contained an average of 3.94 ± 0.2 (mean ± SEM) vasa vasora per field examined and the woven group 3.76 ± 0.3. These groups were statistically similar. Vasa vasora were more numerous in the mesh group 6.24 ± 0.3 and differed significantly from both control group ($p < 0.05$) and woven group ($p < 0.05$) grafts (Fig. 5).

Transverse sections from the distal ends of the woven grafts contained more vasa vasora in their walls than were present in the mid-region of the graft. The majority of these vasa were oriented parallel to the longitudinal axis of the graft.

Control and woven grafts were characterized ultrastructurally by the presence of a continuous, intact endothelium. Endothelial cell basement membranes were inconsistently thickened when compared to those of the mesh group. The subendothelial layer was composed of well differentiated hypertrophic smooth muscle cells. The interstitium contained collagen, proteoglycans and occasional elastic fibers (Figs. 6 and 7).

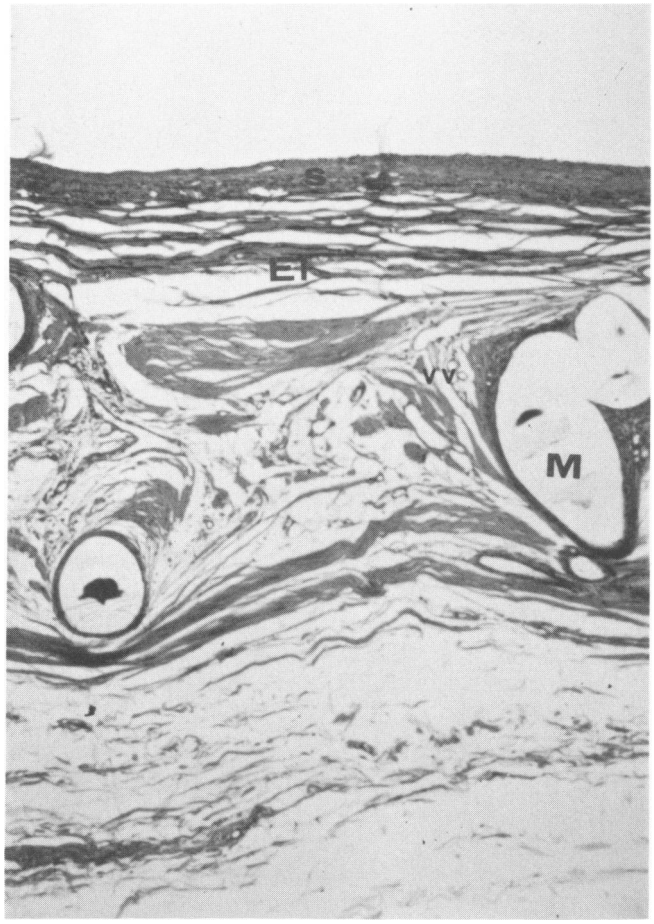


FIG. 4. Section from a mesh group with very little subendothelial thickening(s). Elastic fibers are present (El) and the prosthesis is clearly shown (M). Vasa vasora (vv) are also indicated. (Verhoeff von Gieson stain, 240×).

Grafts from the mesh group had thin and occasionally discontinuous endothelial cell basement membranes with rare fibroblasts present in the subendothelial layer. Smooth muscle cells were not hypertrophic in these grafts (Fig. 7). The major difference between the control and woven grafts and the mesh grafts was in the thickness of the smooth muscle cell layers, two to seven cells thick in the mesh group and ten to 40 cells thick in the control and woven groups.

Discussion

Subendothelial fibromuscular proliferation in arteriovenous grafts is a phenomenon which occurs as early as four weeks after implantation and which may result in obliteration of the graft. Various factors have been suggested to explain this proliferation. Ramos, et al.,[12] have demonstrated a variable degree of subendothelial proliferation in arteriovenous grafts subjected to overdistension (600 mm Hg) prior to implantation into the arterial system. They also found disruptive changes in these grafts which preceded this proliferation suggesting that overdistension is related to a thicker graft wall. Abbott, et al.[1] found similar changes with loss of elasticity in graft walls subjected to distension at lower pressure (300 mm Hg) prior to implantation. Several studies have documented marked *in vivo* increases in the diameter of vein grafts used as either arterial replacements[4,14] or bypasses.[7]

Wrapping the grafts with a loose, supportive prosthesis (mesh group) in the present study, reduced the diameter of the graft uniformly by 10% when compared to the nonsupported grafts (control group). Our findings suggest that there are beneficial effects of reducing graft dilatation with respect to changes which result in early graft failure, *i.e.* endothelial alteration with subsequent thrombosis and/or aneurysmal dilatation and rupture. There is also an apparent beneficial effect on later changes which occur in the graft wall, *i.e.* mesh group grafts had combined intimal–medial thickness which was substantially less than that of the control group. This was associated with a substantial increase in the number of vasa vasora per microscopic field when compared to the control group. In addition, the newly developed vasa vasora penetrated the dacron mesh prosthesis. This finding suggested that revascularization of the graft may influence the extent and severity of subendothelial fibromuscular proliferation.

The second experimental group (woven group) was designated to evaluate the combined effects of reducing graft dilatation and inhibiting vasa vasora penetra-

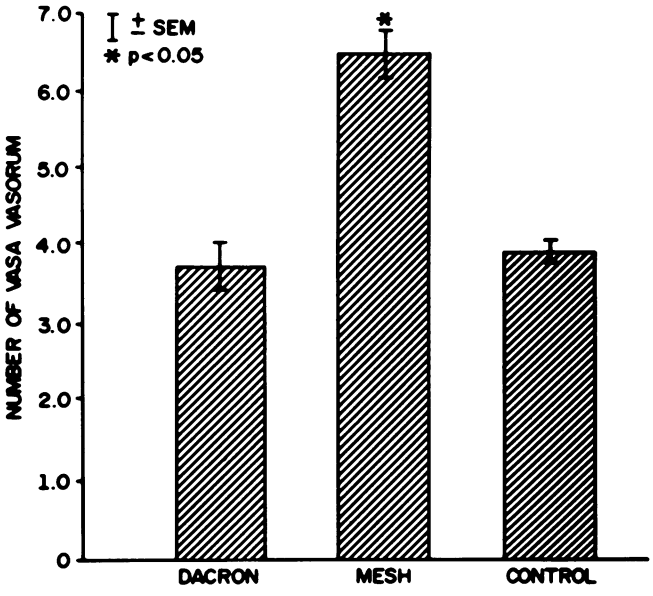
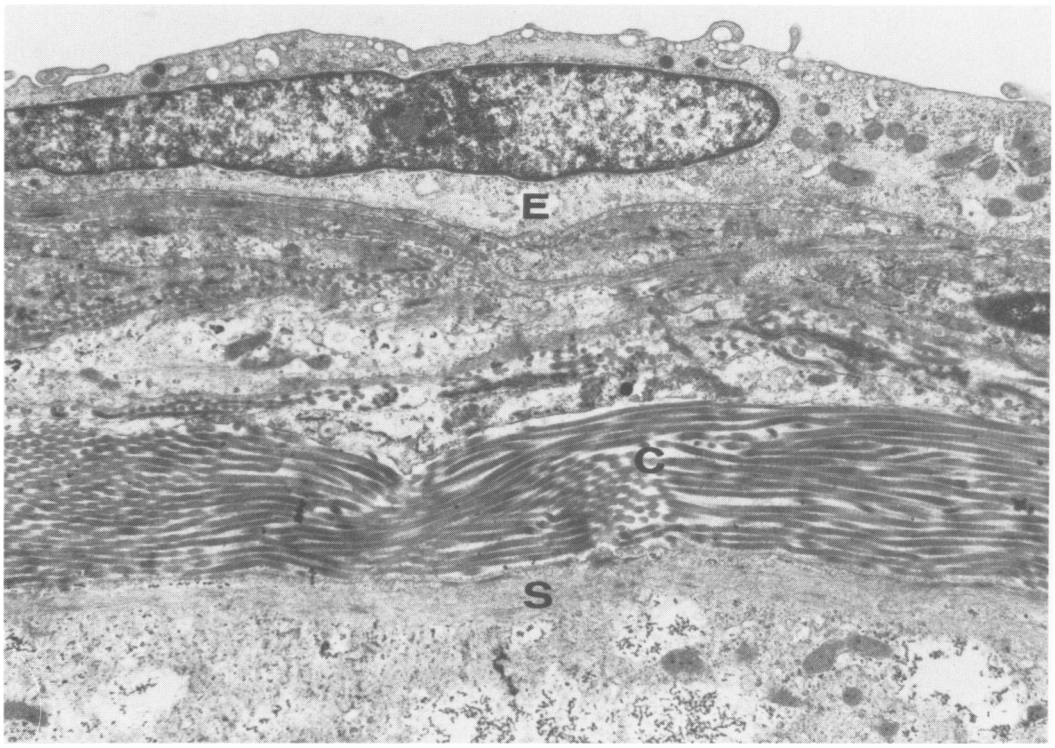


FIG. 5. Comparison of absolute numbers of vasa vasorum per 0.25 mm × 0.25 mm.



FIGS. 6 and 7. Electron photomicrographs of a woven group graft (Fig. 6) and a mesh group graft (Fig. 7). There is more collagen (c) in the woven group graft when compared to the mesh group graft. Collagen fibers are closer to the luminal surface of the mesh graft and deeper within the wall of the woven graft. Endothelial cell basement membranes (bm) in the mesh graft are thinned and discontinuous. Endothelial cells were intact (E) in both groups. Well-differentiated smooth muscle cells (s) can be seen in both groups (Figure 6, 28,000×, Figure 7, 22,500×).

tion on subendothelial fibromuscular proliferation. This was accomplished by using a very tightly woven, supportive prosthesis which reduced the diameter of the graft by 10% but, in addition, physically impeded the penetration of vasa into the graft wall. The woven group grafts, when compared to the mesh group, had considerably fewer vasa vasora per microscopic field. The majority of the vasa vasora in this group were

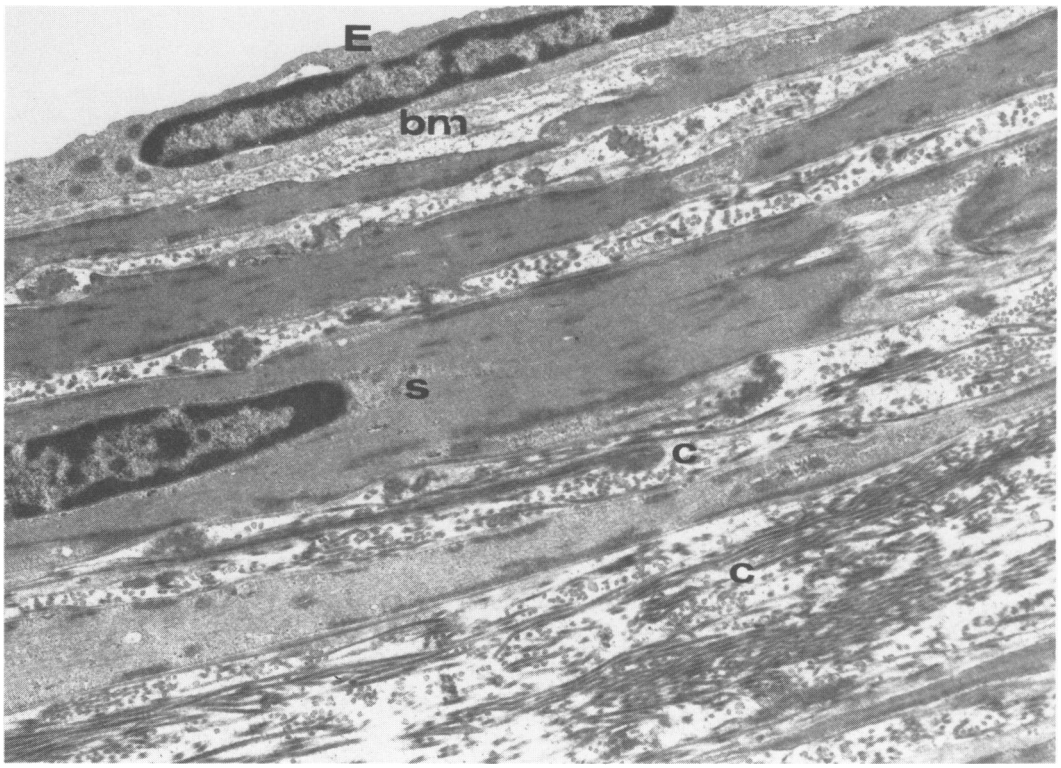


FIG. 7. See legend for Figures 6 and 7.

observed entering the graft from the two anastomotic sites. Relatively few new vessels penetrated the tightly woven prosthesis. Vasa vasora from the control group grafts penetrated the graft wall perpendicular to the long axis of the graft and only a few entered from the adjacent carotid artery.

Brody et al.[6] have examined the comparative effects of veins that were transected and reanastomosed *in situ*, devascularized *in situ* or transected and transplanted into the arterial system as bypass grafts. Veins transected and reanastomosed *in situ* showed preservation of normal vein architecture. Veins devascularized *in situ* had changes that were similar to those segments used as arteriovenous bypasses. Wyatt et al.[18] have studied revascularization of femoral vein bypass grafts in dogs, and concluded that this process can occur either by direct anastomosis of existing vasa vasora with vessels from the graft bed or by proliferation and invasion of vessels from the graft bed into the graft adventitia and media. Within two months after being placed in the arterial system, the pattern of vasa vasora in the graft was similar in distribution to venae vasora of control veins, suggesting that a direct anastomosis had occurred.

Loss of structural and/or functional endothelial integrity has been shown in several studies to result in increased permeability of the vessel wall to blood constituents. Certain of these constituents now have been implicated as stimuli for smooth muscle proliferation. Fisher-Dzoga et al.,[9] have noted smooth muscle cell proliferation *in vitro* when cultures were exposed to hyperlipemic serum. The stimulus for proliferation in this study was associated with low density lipoproteins. Ross[13] has reported a nondialyzable platelet fraction capable of stimulating smooth muscle proliferation *in vitro*. Wight[17] using the same *in vitro* model described increased glycosoaminoglycan and collagen deposition by proliferating smooth muscle cells when exposed to this same platelet fraction. Alterations in the endothelium of grafts may result over a period of time in increased permeability of the graft wall to substances which are capable of stimulating proliferation of smooth muscle cells.

The present study suggests that reduction of dilatation of arteriovenous grafts by use of a supportive prosthesis does have a beneficial effect on the graft with respect to early failure resulting from thrombosis and/or rupture. Furthermore, there is an indication of a definite relationship between the density of vasa vasora within the graft wall and the thickness of subendothelial fibromuscular layer.

A specific density of vasa vasora appears necessary to maintain functional and structural integrity within the graft media. Fewer vasa, as produced in the woven graft group, could result in relative ischemia and/or necrosis of smooth muscle cells followed by a proliferative response of remaining viable cells comparable to the effect noted in unsupported vein grafts.

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