Vein to Artery Grafts

A Quantitative Study of Revascularization by Vasa Vasorum and its Relationship to Intimal Hyperplasia

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Iliolumbar vein to iliac artery grafts were placed in 40 rats by microsurgical technique. Groups of animals were perfused with fixative at eight intervals between one and 20 weeks after operation, and sections of the graft and control arteries (the opposite iliacs) were analyzed microscopically. The revascularization of the graft by capillaries commenced within the first postoperative week. The level of vascularity (capillaries per cross-sectional mm²) increased during the first four weeks, maintained a constant level and declined after week 16. The grafts of the 17–20 week group were substantially less vascular than the earlier groups. Intimal thickening commenced at three to four weeks after operation, *i.e.* during the period of increasing graft vascularity. The mean intimal proportion of the graft was 14% at four to five weeks and at 17–20 weeks was 35% of the cross-sectional area of the graft wall. However, the actual thickness of the intima did not increase significantly with time, rather the whole graft wall tended to become thinner. At 17–20 weeks grafts which showed a high degree of intimal thickening had significantly fewer capillaries within their walls. Quantitative evidence is presented to suggest that the continued growth of the graft intima may not be supported by a similar increase in the number of vasa vasorum. Therefore, it is suggested that the reduced level of vascularity in grafts with hyperplastic intimae may form an ischemic basis for degenerative changes which are known to take place in some long-term grafts.

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SINCE ITS WIDESPREAD INTRODUCTION 30 years ago, the technique of grafting a segment of autologous vein has become the method of choice for the replacement of diseased peripheral and coronary arteries. Despite the diagnostic, surgical and technological advances which have increased the effectiveness of the technique, some grafts become occluded by the structural changes which occur subsequent to insertion. The most important of these is the proliferation of the "intima" (also called the neointima of hyperplastic intima), which commences within the first month after operation and can lead to graft occlusion within the first year.[4] Atherosclerosis is the other major structural degeneration which may cause failure in long-term grafts. It has been estimated that one-third of grafts will develop serious defects which would threaten long-term patency and, while half of these are preventable, "Atherosclerosis and intimal thickening are not remediable and will continue to be a major cause of loss of graft function."[12]

The pathogenesis of intimal hyperplasia is undetermined but it has been suggested that three factors—surgical trauma, altered arterial hemodynamics and mural ischemia may be involved.[2,4,5,12] What role does mural ischemia play in this process? In fact, how are vein grafts vascularized and what relationship does this have to the development of intimal hyperplasia?

There is some evidence from Indian ink injection studies that the circulation is re-established in the vasa vasorum of canine vein autocirculation is re-established in the vasa vasorum of canine vein autografts within 72 hours after insertion.[14] The origin of vasa vasorum in grafts has been investigated by wrapping grafts in impervious materials. Such studies have shown that the vasa vasorum arise from both the surrounding tissues and from the sites of anastomoses.[3,7] Decron® prostheses modify graft revascularization: grafts wrapped in loose (permeable) mesh for six months were more vascular than those in tightly-woven (impermeable) mesh or those grafts left un-

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supported. Moreover, there was more subendothelial proliferation in unsupported grafts.[5]

While it has been established that vein grafts do become revascularized, it is not known how the vascularity of the graft compares with that of a normal artery or if there is a relationship between the revascularization process and the development of intimal hyperplasia. The following research project was designed to provide quantitative data in order to answer these questions.

Materials and Methods

Grafting Procedure

In 40 young adult male rats, anesthetized with Pentobarbitone (40 mg/kg body weight), 5 mm segments of iliolumbar veins were dissected out and soaked in heparinized saline for approximately ten minutes. The left common iliac arteries were mobilized and cut between two Scovell-Lewis vascular clamps. The divided ends were irrigated with heparinized saline and the vein graft was then sutured end to end to the artery using 10.0 nylon (Ethicon) microsutures (Fig. 1). The diameter of the graft was approximately 1 mm and the graft was ischemic for 60 minutes. Upon removal of the clamps, blood flow was immediate and hemostasis was achieved in three to five minutes.

Perfusions

The animals were divided into eight groups of five according to the age of the graft at the time of perfusion: one, two, three, four to five, six to eight, nine to 12, 13–16 and 17–20 weeks after operation. Perfusions were done via the left ventricle using 50 ml of heparinized saline followed by 200 ml of Karnovsky's fixative. The graft and control (right common iliac) arteries were dissected out and trimmed into blocks which were postfixed in 1% O_sO_4, dehydrated in ascending grades of alcohol and embedded in epon-araldite.

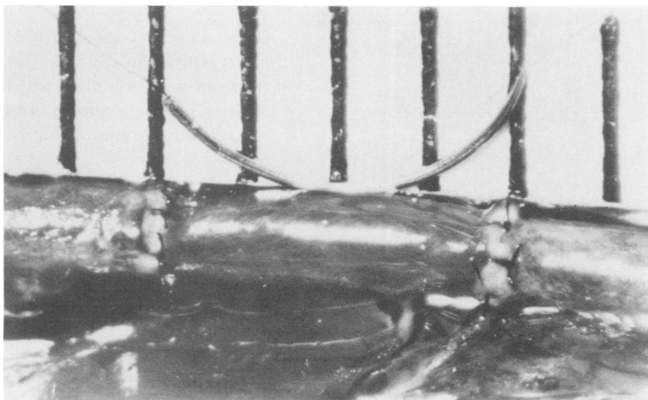

FIG. 1. The vein graft at the time of insertion. The scale is in mm and shows a BV3 (130 μm) needle with a 10-0 (22 μm) Ethilon suture.

TABLE 1. *Vein Graft Revascularization Compared with the Vascularity of Control Arteries (Fig. 1)*

Groups in Weeks Post-grafting	Graft Vascularity Caps./mm²·x̄ ± SEM n = 5 per group	Control Artery Vascularity Caps./mm²·x̄ ± SEM n = 5 per group
1	37 ± 17	47 ± 25
2	71 ± 24	61 ± 35
3	62 ± 12	25 ± 11
4–5	104 ± 17	23 ± 10
6–8	98 ± 16	49 ± 16
9–12	100 ± 8	25 ± 8
13–16	75 ± 12	10 ± 4
17–20	45 ± 17	47 ± 15
1 Way Anova	$p < 0.05$ F = 2.516 d.f. 32, 7 LSD (0.05) = 46	$p > 0.05$ NS F = 0.92 d.f. 32, 7 LSD (0.05) = 52

Analysis

The analysis was performed using 1 μm transverse sections stained with 1% Toluidine Blue in 1% Borax. The sections were examined at 480× magnification and the vasa vasorum were classified as capillaries, arterioles, and venules according to their size and histologic structure. Drawings of the sections were made using a Photolucider and the positions of the vasa vasorum were plotted accurately in relation to the various layers of the vessels, in particular to the intima of the graft. From these drawings, measurements of the areas of tissues of the sections and the width of the graft intimae were made using a "Kontron" Image Analyser. The numbers of vasa vasorum, capillaries, arterioles and venules per mm² of the graft and control sections were calculated and analyzed.

Results

General Observations

After operation, blood was seen to flow freely through the grafts (the wall, 50 μm thick, was almost transparent). A strong positive femoral pulse confirmed the success of the procedure. Three of the 40 grafts were excluded from the study because they were inflamed at the time of removal. An additional three grafts were done.

Quantitative data are represented graphically in Tables 1 and 2 and Figures 2 and 3. Photographs of cross-sections through a normal artery, the graft at one, four and 20 weeks after insertion are shown in Figures 4, 5, 6 and 7, respectively.

Revascularization

Capillaries appeared in the loose connective tissue surrounding the graft within one week after insertion

TABLE 2. *Thickness of Vein Grafts and Their Intimae Compared with Normal Control Arteries*

Groups in Weeks Post-grafting	Graft Width (mm)		Intimal Proportion (%)	Control Artery (mm)
	Total	Intima		Total Width
4–5				
x̄	0.2440	0.0448	13.97	0.0896
SEM	0.0119	0.0216	4.33	0.0078
6–8				
x̄	0.1656	0.0547	33.77	0.1057
SEM	0.0132	0.0034	3.63	0.0141
9–12				
x̄	0.1767	0.0332	16.21*	0.0805
SEM	0.0464	0.0169	9.09	0.0100
13–16				
x̄	0.1563	0.0473	29.11	0.0996
SEM	0.0256	0.0119	4.01	0.0120
17–20				
x̄	0.1423	0.0536	35.04	0.1019
SEM	0.0190	0.0142	6.11	0.0079
1 way Anova	$p > 0.05$ NS $F = 2.24$ d.f. 20, 4 LSD (0.05) = 0.0779	$p > 0.05$ NS $F = 0.034$ d.f. 20, 4 LSD (0.05) = 0.4389	$p < 0.05$ $F = 2.91$ d.f. 20, 4 LSD (0.05) = 17	$p > 0.05$ NS $F = 0.93$ d.f. 20, 4 LSD (0.05) = 0.0314
2 way Anova between the total widths of the control arteries and the grafts—$p < 0.05$, $F = 16.011$, d.f. 4, 1, LSD (0.05) = 0.563				

n = 5 per group.

* One graft in this group was almost devoid of an intima.

(Fig. 5). By three weeks, the connective tissue had thickened and consolidated, with the original graft tissue remaining as small, isolated patches of necrotic tissue. Capillaries had increased in number and some had differentiated into arterioles and venules. The numbers of arterioles and venules constituted a small proportion of the total number of vasa and they did not differ significantly throughout the study period. The vast majority of vasa vasorum were capillaries and these, being the nutrient vessels, were used in the comparative quantitative analysis.

The numbers of capillaries per mm² of the groups of grafts at the various time periods ranged from a mean of 37 at one week to 104 at four to five weeks where

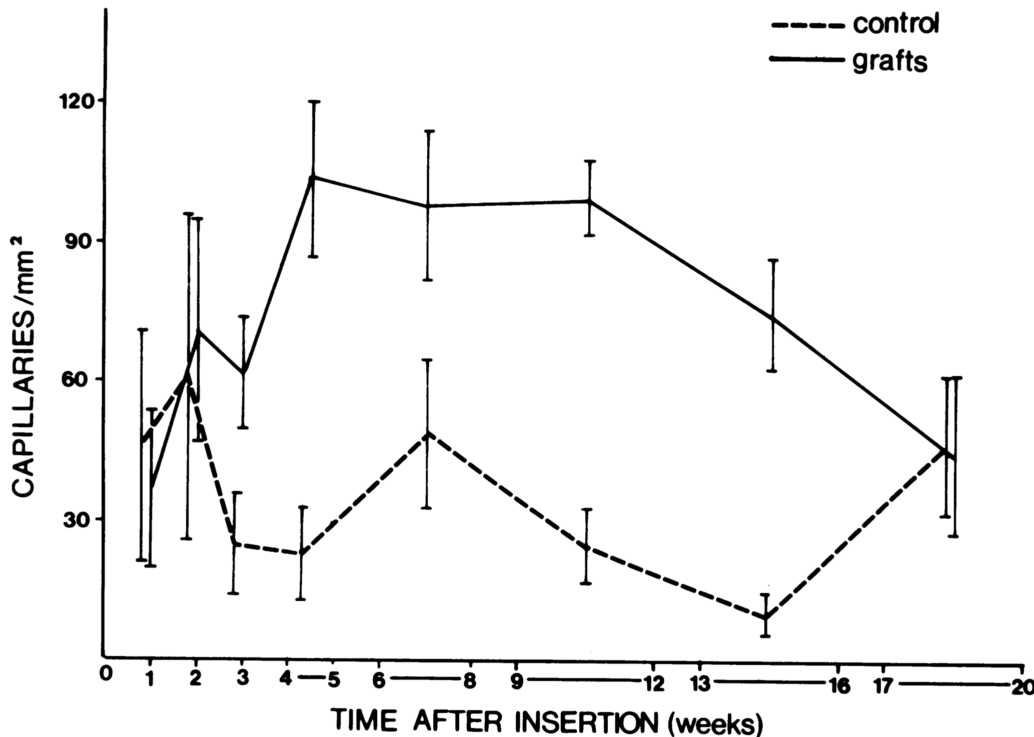

FIG. 2. The vascularity of vein grafts and control arteries showing mean ± SEM values for the experimental and control groups (Table 1).

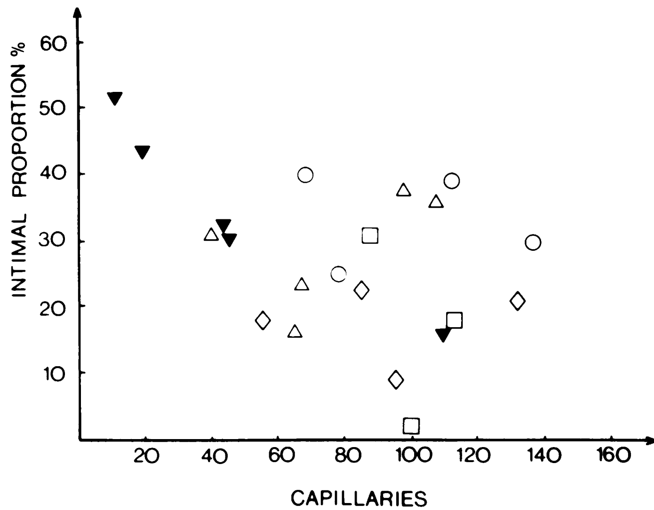

FIG. 3. A scatter diagram of the number of capillaries per mm² in vein grafts correlated with the intimal percentage (a measure of intimal hyperplasia) from four to 20 weeks after insertion. Symbols: ◇ = 4–5 wk; ○ = 6–8 wk; □ = 9–12 wk; △ = 13–16 wk; ▼ = 17–20 wk. There is a significant ($p < 0.05$) inverse correlation ($R = -0.9334$) in the 17–20 wk group (▼). Thus grafts with the greatest degree of intimal hyperplasia are relatively less vascular than other grafts.

they remained relatively constant until 12 weeks after operation, after which time there was a decline in vascularity (Table 1, Fig. 2). Between three and 16 weeks after insertion the grafts remained significantly more vascular ($p < 0.05$) than the corresponding normal iliac arteries in animals from the same group. The comparison between graft vascularity and arterial vascularity was made in animals from the same age/treatment group to overcome the possible changes that may have occurred due to aging. However, there was no significant difference between the vascularity of the control arteries with time, *i.e.*, there was no measurable aging effect. By contrast, there was a significant difference ($p < 0.05$) between the vascularity of the groups of grafts with time. By 17–20 weeks after operation the vascularity of the grafts and that of the control arteries was almost the same.

Intimal Hyperplasia

An "intimal" layer formed on the inner (lumenal) surface of the original elastic lamina of the vein. This lamina was always obvious as a fine line approximately 1 μm thick. The intima itself consisted of a layer of endothelial cells with an underlying layer of smooth muscle cells (Figs. 6 and 7). Intimal hyperplasia was seen in one graft by three weeks after operation, and was present in all but one graft of the four to five week group. In this group a mean of 14% of the cross-sectional area of the grafts was made up by the intima. In the 17–20 week old grafts the mean was 35% of the cross sectional area made up by the intima (Table 2). The development of the intima was faster in some animals than in others and this accounts for the large standard errors associated with these data from the various groups of grafts. For example, intimal hyperplasia was minimal in one animal in the 9–12 week group and this contributed to the reduced mean intimal thickness in this group.

In reality, the actual thickness of the intima did not vary significantly ($p > 0.05$) throughout the various study groups from four to five to 17–20 weeks after operation. However, the intimal percentages of these same groups of grafts did differ significantly ($p < 0.05$). In effect this was not a useful index of intimal hyper-

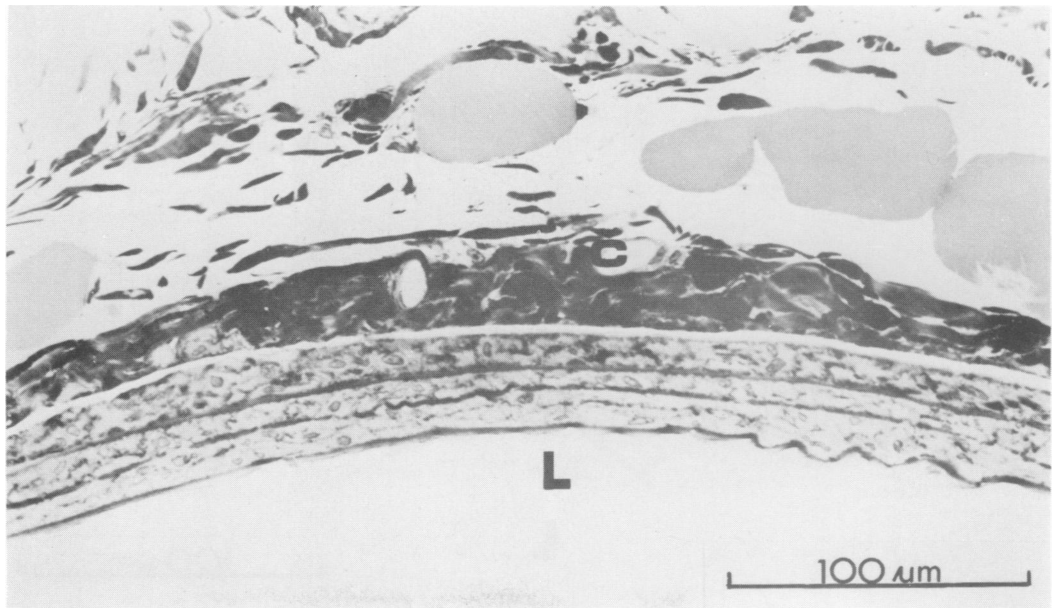

FIG. 4. A cross-section of the normal iliac artery of the rat showing 2 vasa vasoral capillaries (C) in the adventitia. L = Lumen.

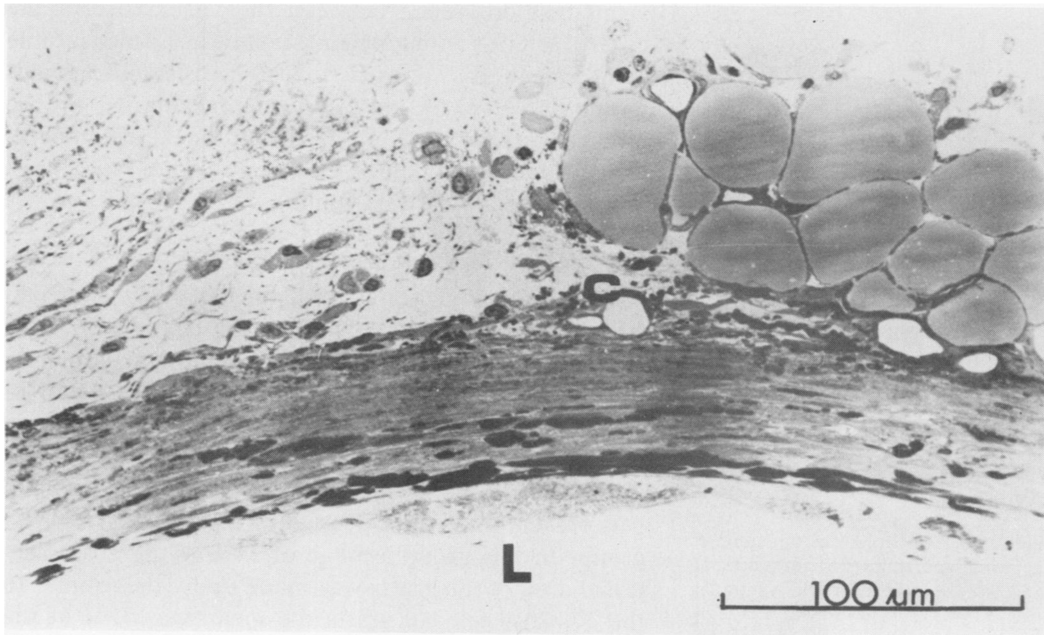

FIG. 5. A cross-section of a vein graft one week after insertion showing capillaries (C) in the outer layer. L = Lumen.

plasia because the factor which accounted for this observation was the actual reduction in thickness of the whole graft wall progressively with time from four to five to 17–20 weeks. While this was not significant ($p > 0.05$) the trend can be seen in Table 2. There was no significant change in the thickness of the control arteries over the study period. However, the grafts were significantly thicker ($p < 0.05$) than the control arteries. The development and structure of the intimae of similar vein grafts have been described previously.[8]

The scatter diagrams for the data pairs, intimal proportion and number of capillaries for the groups of grafts four to five, six to eight, nine to 12, and 13–16 weeks after insertion (Fig. 3) showed a tendency toward an inverse relationship, but were not statistically significant ($p > 0.05$). However, the same data pairs for the 17–20 week group were statistically significant ($p < 0.05$) and showed a strong inverse relationship. That is, grafts with thicker intimae had relatively fewer capillaries in their walls.

It is significant that vasa vasorum were never found within the intimal layer and were only seen in the outer layer of dense fibrous tissue (often referred to as the

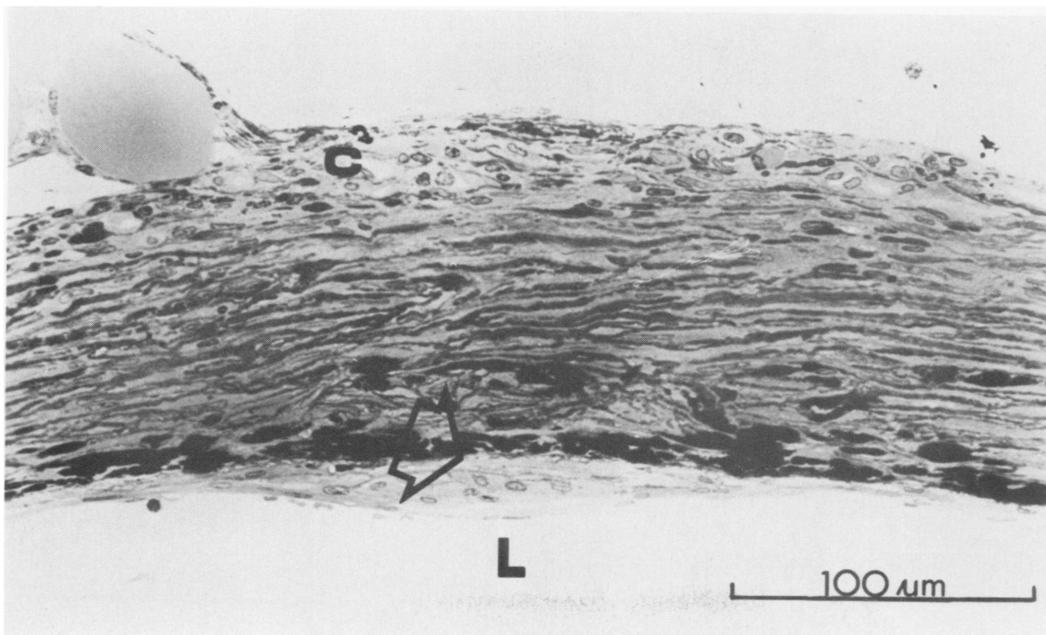

FIG. 6. A cross-section of a vein graft four weeks after insertion. Intimal hyperplasia has commenced on the lumenal (L) side of the arrow, which marks the elastic lamina of the vein.

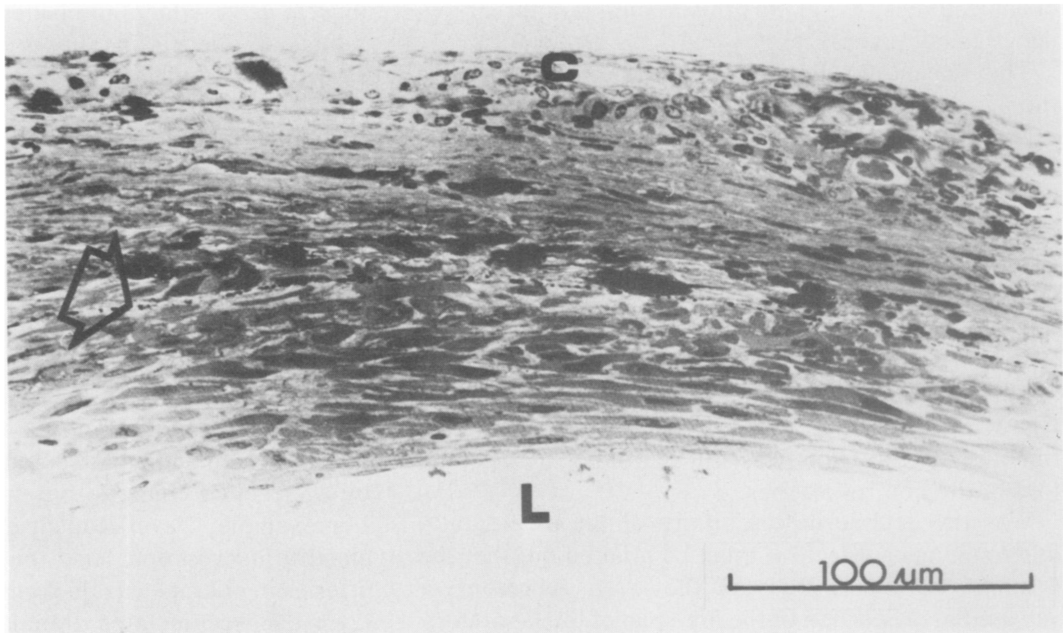

FIG. 7. A cross-section of a vein graft 20 weeks after insertion. Intimal hyperplasia has occurred on the inner lumenal (L) side of the elastic lamina of the original vein (arrow). A capillary (C) is present in the outer layer of the graft.

"media" of a vein graft) outside the thin elastic lamina of the original vein graft (Figs. 5–7). Furthermore, it is noteworthy that no small vessels were seen to pass from the lumen into the tissues of the graft.

Discussion

This study had two main objectives: first, to quantitate the revascularization process in vein grafts over the first 20 weeks after insertion and, secondly, to relate this vascularity to the intimal changes which occur in the grafts during this time. The results have shown that revascularization begins within one week of the insertion of the graft. Following an increase in vascularity in the first four weeks, there was a consistently high level of vascularity from four to 16 weeks which declined after the sixteenth after operation week —at which time the intima had been well established. Furthermore, there was an inverse correlation between the vascularity of the graft and the degree of intimal hyperplasia in the late (17–20 week) grafts. Thus grafts which had a high degree of intimal hyperplasia had comparatively few capillaries within their walls.

Inflammatory tissue surrounding the graft was probably the source of new vasa for the vein grafts. Due to the sampling techniques used (cross-sections from the centre of the graft) the origin of the vasa could not be determined directly—although some capillaries were seen to sprout from adjacent muscle, connective and inflammatory tissue. However, previous studies[3,7,14] provide evidence that vasa vasorum around grafts originate from both the ends of the host artery, in the region of the anastomoses, and from the surrounding inflammatory tissues. According to McCune et al.,[7] the latter source seems to be more important because when arterial grafts were wrapped in plastic, to prevent capillary ingrowth, the revascularization was minimal and it was stressed that "the transplants be surrounded by as much vascular tissue as possible in order to promote vascularization." However, when similar vein grafts were wrapped in Teflon® to prevent the ingrowth of capillaries from surrounding tissues, revascularization of the graft was actually "more marked in the wrapped grafts"—the vessels grew in from the anastomoses.[3] The difference between these two results, and others, was explained by Brook et al.[3] as being due to technical reasons; the more detailed histological identification of capillaries in their study and the longer period of investigation of grafts than had been used previously. Whichever source is the greater contributor of capillaries, the revascularization process is rapid and commences within the first week after graft insertion.

The quantitative analysis of vasa vasorum revealed that although a few small arterioles and venules had formed by one week after graft insertion, the numbers of these vessels did not increase significantly throughout the study period (1–20 weeks). The vast majority of vasa vasorum was made up of capillaries which did change in number. Thus these were considered to be the important "nutritive" vessels. Previous studies of the revascularization of blood vessel grafts have directly or indirectly associated the vasa vasorum with the nutrition of the graft. It is generally held that vasa vasorum are nutritive vessels and, while there is good circumstantial evidence, from the results of oc-

clusive studies, to support this assumption direct evidence is not given.[7,8,11]

The reason for the decline in the vascularity of the 17–20 week grafts is unclear. Possibly this was a result of the final resolution of a protracted inflammatory process. Although there was no evidence of inflammation at postoperative stages later than four to five weeks, the connective tissue component of the graft wall consolidated and tended to diminish in thickness progressively with time after grafting. This was only a trend and was not statistically significant. However, even though the vascularity of the grafts at 17–20 weeks approximated that of the normal control arteries, from the same animals, the mean total thickness of the grafts was still 1½ times thicker than that of the control arteries.

Was the decline in vascularity of the older grafts due to an aging effect? It must be pointed out that the 6 month period of study of the grafts represents a substantial proportion of the life-span of the laboratory rat. While there is no evidence available on the quantitative decrease in vasa vasorum with age, qualitative degenerative changes in vasa vasorum of human aortae have been reported.[9] Several factors were suggested which may be responsible for these degenerative changes including: aging, hypertension, inflammation, infection and chemical factors (such as cholesterol and histamine levels). It is unlikely that aging affected the vascularity of these late grafts because there was no evidence of any of these factors in the grafts and, furthermore, there was no significant change in the vascularity of the control arteries with time.

The results of the present study show that there is a significant inverse correlation between the number of capillaries and the percentage of the graft made up by the intima in the 17–20 week grafts. Thus grafts with thicker intimae had comparatively fewer capillaries in their walls. These results are similar to those of Karayannacos et al.,[5] who also found an inverse correlation between the vascularity of canine vein grafts and the degree of "subendothelial proliferation" (intimal hyperplasia), six months after insertion. They also showed that grafts supported with a loose-mesh (permeable) dacron prosthesis were more vascular and had less subendothelial proliferation than those enclosed in a tightly-woven (impermeable) prosthesis. Presumably the tight mesh inhibited the revascularization of the graft tissues.

It is suggested that the arterial pulsation of the graft induces the muscular intima to develop. In some cases, as the intima thickens, the progressive decrease in blood supply available from the vasa vasoral capillaries stimulates a proliferative response in the smooth muscle cells, which intensifies the hyperplasia of the intima. Evidence in support of this hypothesis is shown by the marked proliferation of the intimae in aortae rendered ischemic by the obstruction of their vasa vasorum with thrombin gelatin.[9]

The other possible source of nutrition for the hyperplastic intimae of vein grafts is from the lumen. It is generally held that the avascular media of a muscular artery is supplied by nutrients from the lumen.[1,13] In fact, the "intima" of a vein graft is very similar structurally to the muscular media of an artery. However, as the graft thickens the deeper cells of the intima become more distant from the lumen—a situation which (as explained above) induces further hyperplasia. This phenomenon could possibly be aggravated by age related changes that may occur in the intima. For example, Laver-Rudich et al.[6] found that at the electron microscopic level the subendothelial layer of arteries in old rats was thicker than in young rats. This layer had accumulated granular and fibrillar material and cell debris. In the present study it was not possible to study the intimae of the grafts and control arteries with the electron microscope and, furthermore, there was no evidence of any increase in thickness of the control arteries with age. However, it must be emphasised that the hyperplastic and degenerative changes that take place in arteries, and vein grafts, are probably the result of a number of etiologic influences.

Thus intimal hyperplasia may be a self-perpetuating process which can actually lead to frank degeneration of the graft wall. For example, in the present study, signs of smooth muscle degeneration were evident amongst the intimal cells in the deepest layer of the intima of a 20-week-old graft. This particular graft had a very thick intima and only a small number of capillaries within its wall. The degenerating structure of its intima was attributed to the effects of mural ischemia—the degenerating cells in this thick graft received inadequate nutrition by being halfway between the lumen and the vasa vasorum, the diffusion distance from both sources being too great.

One major difference exists between the present study and that of Karayannacos et al.[5] In contrast to the present study, their observations were made at 6 months postoperatively when the vascular pattern of the graft and the intima are established. In the present study, the relationship between graft vascularity and intimal hyperplasia was explored during their period of development. During this time the grafts were significantly more vascular than the corresponding control arteries. Thus mural ischemia is not responsible for the genesis and early development of the intima.

Rather, evidence is presented to suggest that mural ischemia may be a factor which influences the severity of intimal hyperplasia in some vein grafts.

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