

Autogenous Veins as Arterial Substitutes: *

A Study of Their Histologic Fate with Special Attention to Endothelium

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THE FIRST SUCCESSFUL experiments in which autogenous vein grafts were interposed between the cut ends of arteries were performed by Carrel and Guthrie.^{1, 2} Since the reports of this work, several studies have been performed to determine the histologic fate of autogenous vein grafts used to replace segments of arteries. In 1949, the reported studies were reviewed by Schloss and Shumacker.¹⁷ These studies, which were concerned with the fate of grafts ranging in age from eight to 801 days, were summarized as showing that autogenous vein grafts persist as viable structures but undergo certain histologic changes consisting chiefly of fibrous reinforcement. Most investigators were of the opinion that the histologic changes that occurred resulted from the effects of arterial pressure upon the graft; however, there was considerable difference of opinion regarding the exact nature and degree of the histologic changes. Similar findings have been reported since 1949 in studies^{4, 12, 16, 18} in which autogenous vein grafts were examined from six months to two years and two months after operation. However, when grafts four or more years old have been examined, the findings have been quite different. In the latter studies,^{6, 11, 15} autogenous vein grafts have been described as thinned-out fibrous tissue tubes;

moreover, arteriosclerotic changes have been described in one graft recovered from a dog nine years after operation.¹⁰

Previous studies concerned with the histologic fate of autogenous vein grafts have been based upon gross and microscopic examinations of the grafts. Routine histologic sections have been used for the microscopic examinations. With this method, a detailed examination of the endothelial lining of the grafts has not been possible, since the endothelium is visualized only as a series of thinly flattened cells. In order to determine the true nature of the cells, endothelium has to be examined in *en face* preparations.^{3, 9}

This paper describes an experimental study in dogs which has been performed to further clarify the histologic fate of autogenous vein grafts used to replace segments of arteries. Special attention has been given to the morphology of autogenous vein graft endothelium which was examined in *en face* preparations.

Methods

The principles of laboratory animal care as promulgated by the National Society for Medical Research were observed.

Twenty-six mongrel dogs of both sexes weighing from 12.7 to 29.5 Kg. were used for the study. The dogs were anesthetized with sodium pentobarbital (25 mg./Kg.)

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administered intravenously. A segment of the left external jugular vein was excised to provide an autogenous vein graft. The vein graft was handled only with the operator's fingers and was kept moist with normal saline solution. The abdominal aorta was exposed through a left flank incision and mobilized from the level of the left renal artery to the trifurcation. A composite graft was then constructed by anastomosing a 3 cm. segment of the autogenous vein graft to a 3 cm. segment of a crimped, knitted Dacron graft. A composite graft was used in the study in order to determine if autogenous vein graft endothelium could later be demonstrated growing into the Dacron graft. It was reasoned that a demonstration of such growth would prove that the autogenous vein graft endothelium had retained its viability. After construction of the composite graft, a 6 cm. segment of abdominal aorta was excised. The free end of the Dacron graft was anastomosed to the proximal aorta and the free end of the vein graft anastomosed to the distal aorta. All anastomoses were done with continuous 5-0 arterial silk sutures. The vein graft was positioned so that venous valves did not interfere with aortic blood flow. The completed operative procedure is shown in Figure 1.

All dogs survived the operative procedure and received procaine penicillin 600,000 units and streptomycin 0.5 Gm. intramuscularly daily for one week. The dogs were later sacrificed with intravenous sodium pentobarbital. Three dogs were sacrificed at each of the following periods postoperatively: one week, two weeks, one month, one and one half months, two months, three months, three and one half months, and four months. Two dogs were sacrificed six months postoperatively. Ten minutes prior to sacrifice, the dogs were given heparin (1.5 mg./Kg.) intravenously to prevent clotting of blood on the endothelial surfaces of the vessels. After sacrifice, the composite graft of autogenous vein

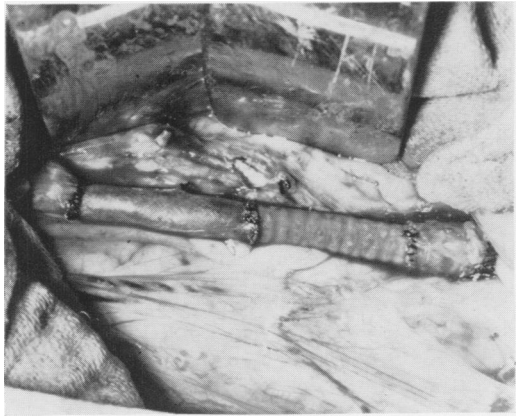


FIG. 1. Completed operative procedure.

and Dacron was removed in continuity with a short segment of the adjacent proximal and distal aorta. A segment of the right external jugular vein was removed from several dogs to provide a control vein specimen. Care was taken to avoid stretching or pulling the specimens since the endothelium of a vessel is easily damaged by such maneuvers.

Häutchen preparations, designed to delineate a single layer of endothelial cells for *en face* examination, were made from the lining of the autogenous vein and Dacron grafts and the segments of adjacent proximal and distal aorta. The procedure used for making the Häutchen preparations was a modification of the procedures described by Poole¹⁴ and Lautsch⁸; it will be described in detail in a forthcoming publication.

Routine histologic sections of the autogenous vein grafts and the control vein specimens were made and stained with 1) hematoxylin and eosin, 2) Masson's trichrome stain for connective tissue, 3) Rinehart-Abul-Haj stain for acid mucopolysaccharides (ground substance) and 4) Verhoeff's elastic stain.

Results

Specimens from 24 of the 26 dogs operated upon were satisfactory for study. The specimens from two dogs, followed for

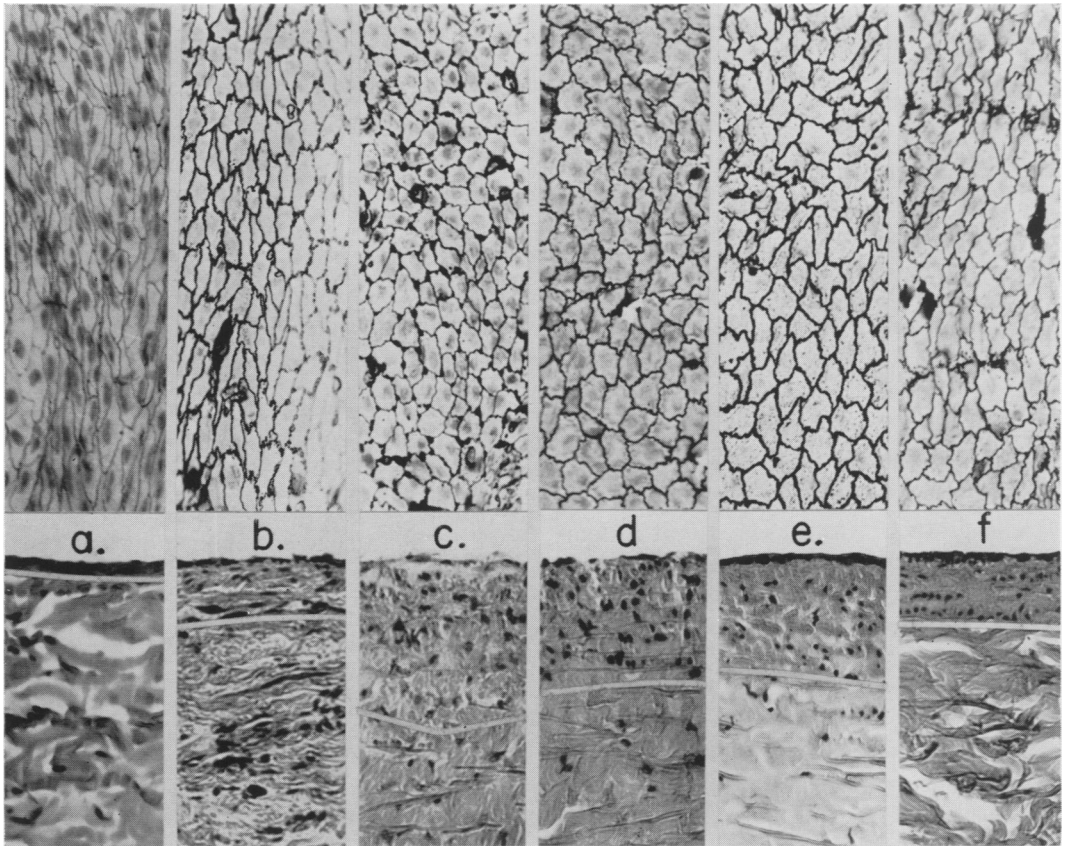


FIG. 2. The results of Häutchen preparations and routine sections (H & E). A line drawn in each routine section represents the border between the media and adventitia. ($\times 160$.) a: Normal control; b: 1 week postoperative; c: 2 weeks postoperative; d: 1 month postoperative; e: $3\frac{1}{2}$ months postoperative; f: 6 months postoperative.

one and one half and two months, respectively, were unsatisfactory for study because the grafts were occluded by an organized thrombus. Although Häutchen preparations from the one and two week postoperative groups were satisfactory for study, these preparations were found to contain less endothelial cells and more fibrous tissue cells than preparations from the other groups.

All vein grafts which were satisfactory for study were slightly dilated at the time of sacrifice. In eight dogs, a hematoma was present around the Dacron grafts alone. The lumen of the grafts was not compromised by the hematoma, but there was considerable fibrous tissue reaction around

both the Dacron and vein grafts. In the other dogs, in which there was no hematoma around the Dacron grafts, there was only minimal fibrous tissue reaction around the grafts.

In Häutchen preparations of control vein specimens, normal endothelium appeared to be composed of single nucleated, polygonal-shaped cells which were bordered with delicate argyrophilic material. The size of the cells was generally uniform and the long axis of the cells was parallel to the long axis of the vein (Fig. 2a).

One Week Postoperative. The endothelium of the autogenous vein grafts, as visualized in Häutchen preparations, appeared only slightly altered (Fig. 2b). The

endothelial cells were generally larger than normal and the argyrophilic borders were wrinkled and denser. Endothelium was present at each end of the Dacron graft in all preparations. The endothelium at the distal end of the Dacron graft was contiguous with the vein graft endothelium, while the endothelium at the proximal end of the Dacron graft was contiguous with the aortic endothelium. The area of endothelium at the proximal end of the Dacron graft was larger than that present at the distal end. The two areas of endothelium at each end of the Dacron graft were separated by a fibrin clot which lined the other inner surfaces of the graft. A few collections of large *mononuclear cells* with silver-stained borders were present in this clot (Fig. 3). These *mononuclear cells* were larger than ordinary mononuclear cells and had vesicular nuclei; occasionally, they were seen as islands of cells which had the same mosaic features displayed by endothelial cells. The *mononuclear cells* were believed to represent young endothelial cells developing from a source other than the endothelium present at each end of the Dacron graft.

Routine sections of the vein grafts showed a very slight increase in wall thick-

ness (Fig. 2b). The cellular elements of the vein grafts were well preserved. Mucopolysaccharides were slightly increased in the media and moderately increased in the adventitia.

Two Weeks Postoperative. A hematoma was present around the Dacron graft in one dog in this group; however, there was no difference between the histologic findings in this dog and the other two dogs in this group.

The Häutchen preparation findings in this group were similar to those described for the one week postoperative group (Fig. 2c). However, in preparations from the two week postoperative group, a slightly larger area of endothelium was present at each end of the Dacron graft; also more collections of large *mononuclear cells* were present in the fibrin clot and these showed definite features of isolated endothelium.

Routine sections of the vein grafts showed a considerable increase in media thickness due to muscular hypertrophy and edema (Fig. 2c). There was a slight increase in adventitial fibrous tissue. Mucopolysaccharides were considerably increased in the intima and media and moderately increased in the adventitia.

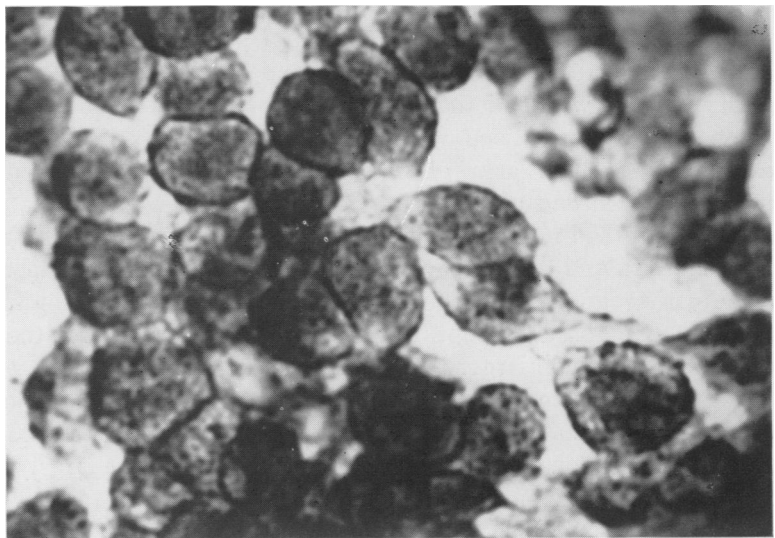


FIG. 3. Large "mononuclear cells." Häutchen preparation $\times 650$.

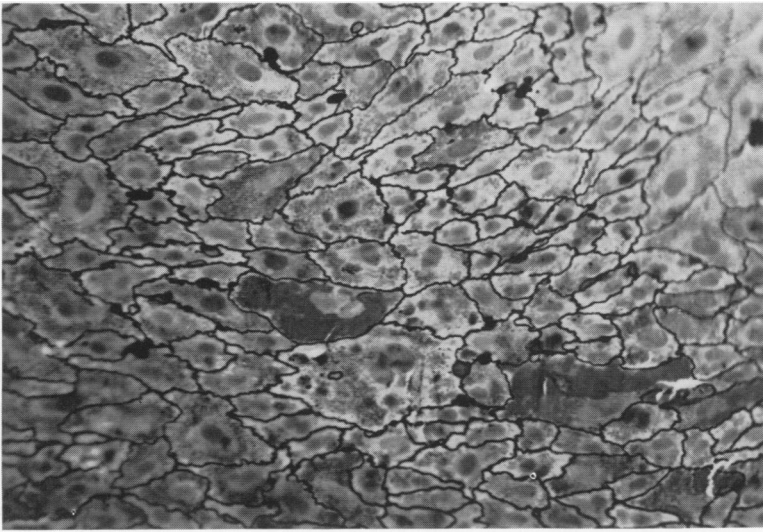


FIG. 4. Endothelium lining the Dacron graft. Note extreme irregularity of individual cells and multinucleation. Häutchen preparation $\times 175$.

Elastic fibers in the media appeared separated.

One Month Postoperative. The endothelium of the vein grafts appeared intact and regular in Häutchen preparations (Fig. 2d).

A hematoma was present around the Dacron graft in two dogs in this group. In one dog, the hematoma covered the entire external surface of the Dacron graft. The Dacron graft was lined with fibrin clot except for a small area of endothelium present at each end of the graft. In the other dog, the hematoma covered all but the posterior external surface of the Dacron graft. The posterior inner surface of the graft was lined with endothelium; the other inner surfaces were lined with fibrin clot except for a small area of endothelium present at each end of the graft. One dog in this group had no hematoma around the Dacron graft, and the graft was completely lined with endothelium. The endothelium lining the Dacron graft appeared irregular; the cells were much larger than those seen in the vein grafts and many contained multiple nuclei (Fig. 4).

Routine sections of the vein grafts showed a moderate increase in media thickness due to muscular hypertrophy and

edema (Fig. 2d). There was a slight increase in adventitial fibrous tissue. Mucopolysaccharides were increased in the media and adventitia.

One and a Half Months Postoperative. A hematoma was present around the Dacron graft in one dog in this group. The Häutchen preparation from this dog was unsatisfactory. The Häutchen preparation findings in the other dog in this group with no hematoma around the Dacron graft were the same as those described for the two weeks postoperative group.

Routine sections of the vein grafts showed an increase in media thickness due to muscular hypertrophy and edema. There was a moderate increase in adventitial fibrous tissue. Mucopolysaccharides were slightly increased in the intima.

Two Months Postoperative. A hematoma was present around the Dacron graft in both dogs in this group. The Häutchen preparation findings were the same as those described for the two weeks postoperative group.

Routine sections of the vein grafts showed an increase in media thickness due to muscular hypertrophy. Adventitial fibrous tissue was slightly increased. Mucopolysaccharides were slightly increased in

the media. Elastic fibers in the media and adventitia appeared separated.

Three Months Postoperative. The Häutchen preparation from one dog in this group was unsatisfactory. Häutchen preparations from the other two dogs in this group showed regular vein graft endothelium. The Dacron grafts were completely lined with endothelium.

The findings in routine sections of the vein grafts were the same as those described for the two months postoperative group.

Three and a Half Months Postoperative. The endothelium of the vein grafts appeared regular and intact in Häutchen preparations (Fig. 2e).

A hematoma was present around the Dacron graft in two dogs in this group. The Dacron grafts were lined with fibrin clot except for a small area of endothelium present at each end of the graft. One dog in this group had no hematoma around the Dacron graft, and the graft was completely lined with endothelium.

The findings in routine sections of the vein grafts were the same as those described for the two-month postoperative group (Fig. 2e).

Four Months Postoperative. The endothelium of the vein grafts appeared regular and intact in Häutchen preparations. The Dacron grafts were completely lined with endothelium.

Routine sections of the vein grafts showed an increase in media thickness due to muscular hypertrophy and an increase in fibrous tissue. Adventitial fibrous tissue was moderately increased and was collagenized. Mucopolysaccharides were slightly increased in the adventitia. Elastic fibers in the adventitia appeared separated.

Six Months Postoperative. The Häutchen preparation findings in this group were the same as those described for the four-month postoperative group (Fig. 2f).

Routine sections of the vein grafts showed denser fibrous tissue in the media

than was present in the four-month postoperative group. However, the wall thickness of the vein grafts was less than that of the grafts in the four months postoperative group (Fig. 2f). Mucopolysaccharides were slightly increased in the media and adventitia. Elastic fibers in the media appeared separated.

Results of the study may be summarized as follows:

1. The endothelium of the autogenous vein grafts, as visualized in Häutchen preparations, remained intact and appeared only slightly altered (Fig. 2). The endothelial cells were generally larger than normal and their silver-stained borders were wrinkled and denser.

2. The wall thickness of the autogenous vein grafts was increased; this resulted from an increase in fibrous tissue in both the media and adventitia and muscular hypertrophy in the media. The increase in wall thickness was more prominent at two weeks and one month postoperatively, and became less prominent as the age of the grafts increased (Fig. 2).

3. The elastic fibers of the vein grafts appeared separated, but did not seem to be decreased in total amount.

4. Mucopolysaccharides (ground substance) were increased especially in grafts from the two-week postoperative group.

Discussion

The endothelium of the specimens in this study was examined in *en face* preparations. This type of preparation, termed a Häutchen preparation, was previously used by a number of investigators^{3, 5, 7, 8, 9} for detailed examinations of the endothelial lining of arteries and arterial plastic prostheses. In Häutchen preparations, it was possible to clearly visualize the structural details of the cells and the relationship of the cells to one another. The superiority of this method for detailed examination of endothelium was evident when Häutchen

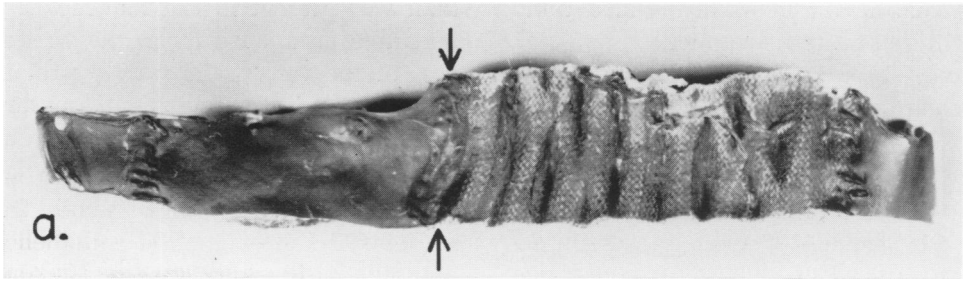


FIG. 5a. Two weeks postoperative. Composite graft and segments of host aorta opened longitudinally and exposed to silver nitrate. Note the tissue growth at the vein-Dacron graft junction indicated by arrows. ($\times 1.5$.)

preparations were compared with routine histologic sections.

In order to be able to detect abnormal changes in morphology, the endothelium of the autogenous vein grafts was compared with the endothelium of control vein specimens. In general, the endothelium of the autogenous vein grafts appeared only slightly altered. However, it was of interest that less satisfactory Häutchen preparations were obtained from grafts examined one and two weeks postoperatively; less endothelial cells and more subendothelial fibrous tissue cells were present in these preparations. This finding could be explained by either improper technic in making the preparations or an increased friability of the endothelial cells in these

specimens. The latter explanation was believed more likely, since the same technic was used for all preparations in the study. In addition, the preparations from the one and two week postoperative groups were the last to be made so that our experience with the technic was greatest at that time.

The endothelium of some of the autogenous vein grafts was proved viable by demonstrating gross and microscopic evidence of growth of endothelium from the vein graft into the adjacent Dacron graft (Fig. 5). The demonstration of such growth was limited to 1) the one and two week postoperative specimens, 2) the specimens older than two weeks in which a hematoma was present around the Dacron graft and 3) one specimen in the one and a half

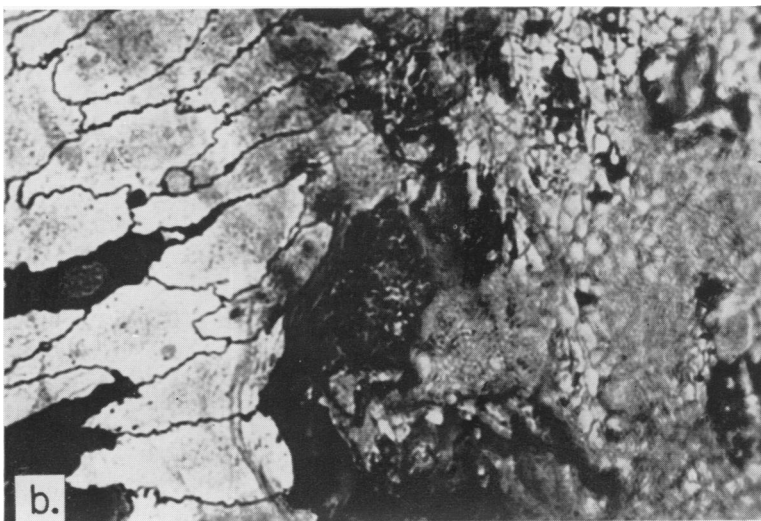


FIG. 5b. Two weeks postoperative. Häutchen preparation taken at the area indicated by arrows shows endothelium extending from the vein graft to the Dacron graft. ($\times 650$.)

month postoperative group. The Dacron graft in other specimens was completely lined with endothelium so that it was impossible to differentiate endothelial growth from the vein graft alone. In specimens in which endothelial growth from the vein graft was demonstrated, the extent of growth from the host aorta into the opposite end of the Dacron graft exceeded that from the vein graft. This finding can be interpreted as indicating that the growth potential of the autogenous vein grafts in this study was decreased unless perhaps there is a normal difference in growth potential between venous and aortic endothelium.

The findings in routine sections of the autogenous vein grafts were in general agreement with those reported in other studies^{4, 12, 16, 17, 18} in which the grafts were examined from eight to 801 days after operation. In none of the grafts was there any evidence to suggest degeneration of the original cellular elements. The changes that occurred were believed to have represented those of adaptation of the vein graft to arterial blood pressure.

One of the interesting side features of this study was the demonstration of large *mononuclear cells* in Häutchen preparations of the Dacron grafts incompletely lined with endothelium (Fig. 3). These *mononuclear cells* were present in the fibrin clot which lined a part of the inner surface of the Dacron grafts. In the majority of instances, the cells were completely isolated from the endothelium growing into the Dacron grafts from the vein grafts and the aorta. The *mononuclear cells* were larger than ordinary mononuclear cells and had vesicular nuclei; the borders of the cells were silver-stained and the cells were arranged in the same mosaic patterns as endothelium. It was our belief that the *mononuclear cells* were young endothelial cells developing from a source other than the vein grafts or the aorta. Similar findings and conclusions have been reported by

Florey⁵ and Petry¹³ who studied the growth of endothelium into arterial plastic prostheses. The possible sources from which the *mononuclear cells* or young endothelium could develop include 1) multipotential mesenchymal cells which invade the graft from surrounding tissues, 2) elements of the blood and 3) endothelium in capillary buds which invade the graft.

With one exception, the Dacron grafts examined one month or more after operation were completely lined with endothelium if there was no hematoma around the external surface of the graft. The single exception was a specimen from the one and a half month postoperative group. In two dogs from the two week postoperative group, there was no hematoma around the Dacron graft and the grafts were incompletely lined with endothelium. It was therefore evident that complete endothelial coverage of the Dacron grafts in this study probably took place between two weeks to one month postoperatively when no hematoma formed around the grafts. The presence of a hematoma was believed to have slowed the rate of endothelial coverage primarily by interfering with the development of *mononuclear cells* or new endothelium from a source independent of the vein grafts or aorta.

Summary

A study has been performed in dogs to further clarify the histologic fate of autogenous vein grafts used to replace segments of arteries. Special attention has been given to the morphology of autogenous vein graft endothelium.

In 26 dogs, a 6 cm. segment of abdominal aorta was excised and replaced with a composite graft consisting of a 3 cm. segment of autogenous vein anastomosed to a 3 cm. segment of Dacron. The Dacron graft was interposed between the proximal aorta and the vein in order to determine if vein endothelium could be demonstrated growing into the Dacron. It was reasoned that a

demonstration of such growth would prove that autogenous vein graft endothelium had retained its viability. The dogs were followed for periods ranging from one week to six months before sacrifice. Häutchen preparations, designed to delineate a single layer of endothelium for *en face* examination, were made from the lining of the vein and Dacron grafts and adjacent segments of proximal and distal aorta. Routine histologic sections of the autogenous vein grafts were stained with H & E and stains to delineate muscle, fibrous tissue, elastic fibers, and ground substance.

Specimens from 24 dogs were satisfactory for study. Autogenous vein graft endothelium, as visualized in Häutchen preparations, remained intact in all specimens and appeared only slightly altered. Endothelial growth from the vein grafts into the Dacron was demonstrated in all specimens in which the Dacron graft was incompletely lined with endothelium. A study of routine histologic sections showed that other cellular elements of the vein grafts persisted without evidence of degeneration. There was, however, an increase in fibrous tissue and ground substance, muscular hypertrophy, and some separation of the elastic fibers.

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