OBSERVATIONS ON ARTERIAL HOMOGRAFTS WITH SPECIAL REFERENCE TO PATHOLOGIC CHANGES

A Preliminary Report

WILLIAM A. HAWK, M.D.,
Department of Pathology

and

ALFRED W. HUMPHRIES, M.D.
Department of Vascular Surgery

ARTERIAL HOMOGRAFTS offer a bright promise to those patients who have advanced arterial disease of the major peripheral arteries and the distal aorta. In the present series, a diseased segment of a vessel has been replaced by a homograft in 264 patients, the majority of whom since have shown great clinical improvement. In nine patients in whom the homografts failed either during the immediately postoperative period or during the ensuing 18 months, the homografts were recovered for critical analysis at reoperation or at necropsy. The findings on these studies are presented in this report.

Materials and Methods

Nine homografts were available for study; five of these were recovered from 4 to 8 weeks and four from 9 to 18 months after the primary operation. Changes observed in the homografts in the early group were similar, and one was chosen for this report as representative for the preparation of photomicrographs and for comparison with the older segments.

All of the recovered homografts were processed in the same way. Blocks were taken from the central portion of the homografts and anastomotic lines, usually after fixation of the main portions of the specimen in Zenker's solution. The sections were stained with hematoxylin-eosin-methylene blue, Verhoeff's stain counterstained by the Van Gieson's method, Masson's trichrome stain, and Gomori's elastic tissue stain. Frozen sections were made on formalin-fixed segments and were stained with Sudan IV for lipids.

An unused freeze-dried femoral homograft was reconstituted as though for operative substitution and then was studied according to the procedures used for the collected material. The unused segment was essentially a fibroelastic tubular structure devoid of intima and lined by the internal elastic lamella (Fig. 1). There was a variable amount of adventitious tissue present. The media was composed of smooth muscle and fibrous tissue in the usual proportions.

Fig. 1 (Unused, reconstituted, femoral homograft segment). Fibroelastic segment showing pyknotic smooth muscle nuclei. Hematoxylin-cosin-methylene blue; X95.

Nuclei of the smooth muscle were hyperchromatic, flattened, and fusiform; the nuclear detail was obscured. The adventitious zone was well defined and was of variable thickness because it had been stripped in processing. It consisted of interlacing collagen bundles, some of which appeared to be basophilic. The adventitious arterioles were well preserved; their smooth muscle cells appeared to be similar to those of the media. Sections stained with the Verhoeff's method showed many parallel and anastomosing elastic bundles in the media and few in the adventitia (Fig. 2). The internal elastic lamella was especially prominent and represented the inner surface of the homograft segment. Masson's trichrome stain revealed abundant collagen in the adventitia and media. Moreover, the Masson's stain clearly demonstrated vacuolated phagocytes in an old atheroma that in the hematoxylin-eosin-methylene blue preparations were observed as poorly outlined cells with unstained nuclei. No lipid was demonstrable in the vascular wall in sections prepared with Sudan IV.

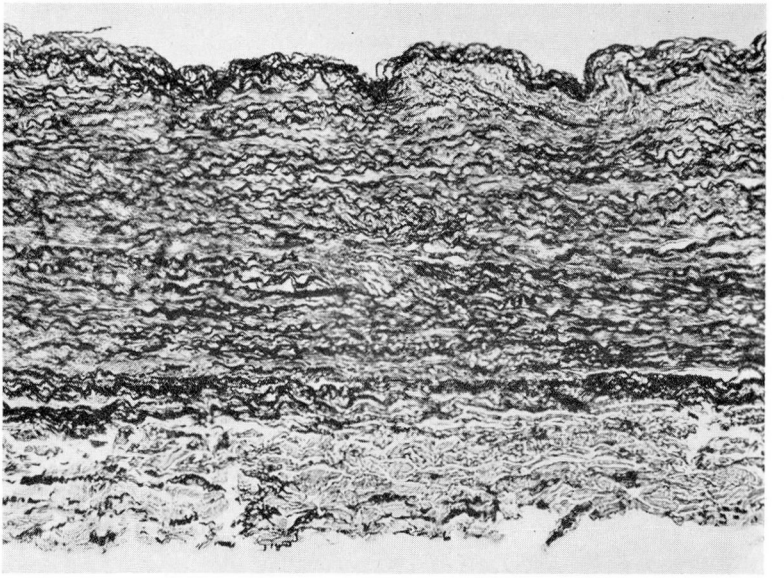

Fig. 2 (Unused, reconstituted, femoral homograft segment). Showing abundant elastic fibers in the media, and the internal elastic lamella as the lining surface. Verhoeff's stain; X140.

Case Reports

Case 1 (Eight-week graft). A diabetic, 56-year-old woman was admitted to the hospital because of intermittent claudication of the right calf after one block of normal walking; the condition had been present for one year without progression. In the right leg, there was a 2 plus femoral pulse with no pulsations below that level. Pulses in the left leg were essentially normal. The oscillometric measurements on the right were as follows: thigh ½ unit at 100 mm. Hg, below the knee ½, and above the ankle ½ unit. At operation a superficial femoral freeze-dried homograft was placed from the level of the bifurcation of the femoral artery to the popliteal space. The homograft was a primary failure. A second attempt at grafting was made two months later, at which time the first homograft was obtained for study. At the second operation, a segment of the right superficial femoral artery was replaced by a 14.5-cm. aortic homograft with end-to-end anastomoses.

The first aortic homograft was thickened, covered by dark-red, shaggy, fibrous tissue, and had the external diameter of a normal femoral artery. Because of technical difficulty, the homograft was removed in short segments. The lumen was completely occluded by a light-red thrombus that was adherent to the wall. The anastomotic lines were intact and the minute wedge-shaped depressions at these sites were filled with fibrin-like material. The intimal surface adjoining the clot was light ocher, smooth, and without intimal plaques, ulcerations, or focal zones of calcification. The proximal portion of the homograft had been plicated to reduce the size of the lumen, and five black-silk sutures were present.

Histologically, the graft appeared to be similar to the unused segment. The tunica intima was well defined but in all sections was devoid of any endothelial covering, and

appeared as a network of interlacing and occasionally nodulated fibers having a looser arrangement than those of the underlying tunica media. Elastic tissue stains showed moderately abundant elastic fibers, but these were not so dense as those in the underlying media. Masson's trichrome stain revealed only scant amounts of collagen in the tunica intima. This portion of the tunica intima corresponded to the internal elastic lamella of the muscular arteries. The elastic tissue content of the media appeared of normal amount; both the intima and the media were acellular at this eight-week stage.

About the periphery of the homograft there was an irregular but moderately thick cuff of granulation tissue that was heavily infiltrated by lymphocytes and plasma cells and showed a foreign-body reaction with many giant cells (Fig. 3). Numerous fibroblasts

Fig. 3 (Case 1—Eight-week-old homograft). Section showing dense foreign-body and inflammatory reaction in the adventitious zone. Hematoxylin-eosin-methylene blue; X30.

were present and they partially penetrated the superficial outer layers of the segment and formed fibrous tissue bundles with few or no elastic fibers. The inflammatory reaction was sharply delimited from the media, and the fibroblastic penetration apparently was limited to the scanty adventitia remaining on the vascular segment. Where the aortic segment had been plicated to reduce the size of the lumen, fibroblasts aggregated along the surgically cut surface and extended inward for a short distance parallel to the medial fibers (Fig. 4).

Case 2 (Nine-month graft). A nondiabetic, 53-year-old man was admitted to the hospital because of intermittent claudication of the left calf at two blocks of normal walking; it had been present for three months with no progression. The pulses were normal in the right leg. In the left leg, he had a 3 plus femoral pulse and no pulsations

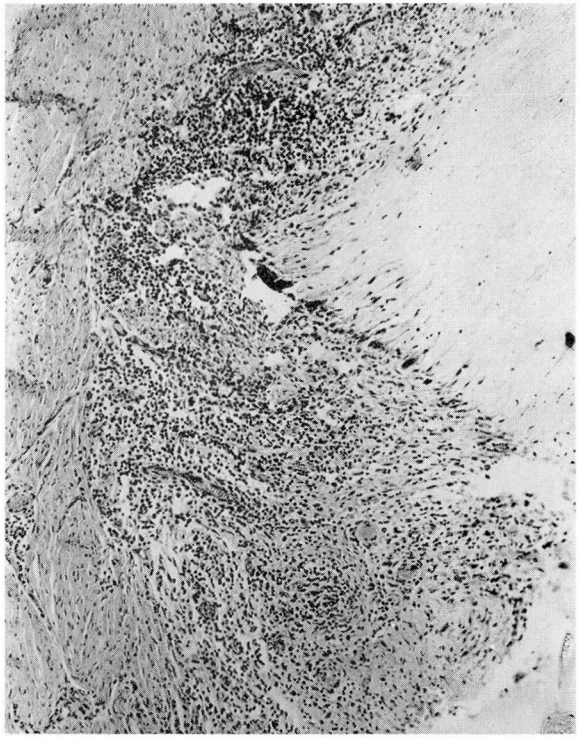

Fig. 4 (Case 1—Eight-week-old homograft). Free margin of plicated homograft (upper right part of photograph) showing granulation tissue, marginal foreign-body reaction, and fibroblasts penetrating parallel to medial fibers. Hematoxylin-eosin-methylene blue; X80.

below that level. The oscillometric measurements in the right leg were normal; in the left leg they were as follows: thigh ¾ unit at 100 mm. Hg, below the knee ½, above the ankle ½ unit. On December 28, 1954, a superficial femoral artery was replaced with a freeze-dried, plicated, aortic homograft in Hunter's canal, resulting in full restitution of pulses. Nine months postoperatively he developed an acute aneurysm of the homograft with rupture. The ruptured homograft was successfully replaced and was obtained for study.

At reoperation a large aneurysmal dilatation of the femoral aortic homograft was found in Hunter's canal. The aneurysm measured 12.0 cm. in length and was flattened on its undersurface and protruded anteriorly in a rounded domelike fashion. It was surrounded by a large hematoma that was readily evacuated. The external surface was covered with abundant, thick, shaggy, densely adherent, fibrous tissue. Superiorly, a 4.5-cm. segment of the patient's vessel entered the aneurysm and the anastomotic site appeared to be well united and smooth. The patient's distal arterial segment was identified and the suture line at the anastomosis was found to be intact. On dissection the proximal arterial wall of the patient was 3.0 mm. in thickness and showed an extensive atheromatous process with numerous irregular yellowish raised plaques. The arterial segment was slightly constricted, where it anastomosed with the homograft. The aneurysm itself was filled with dark-red, extremely adherent, clotted blood, and yellowish pultaceous material.

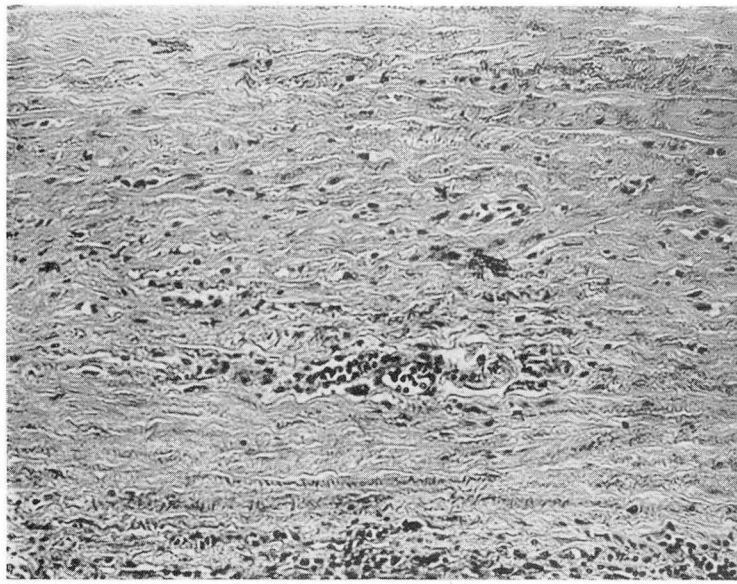

Fig. 5 (Case 2—Nine-month-old homograft). Blood-filled capillary in the outer portion of the media (lower part of photograph). Hematoxylin-eosin-methylene blue; X170.

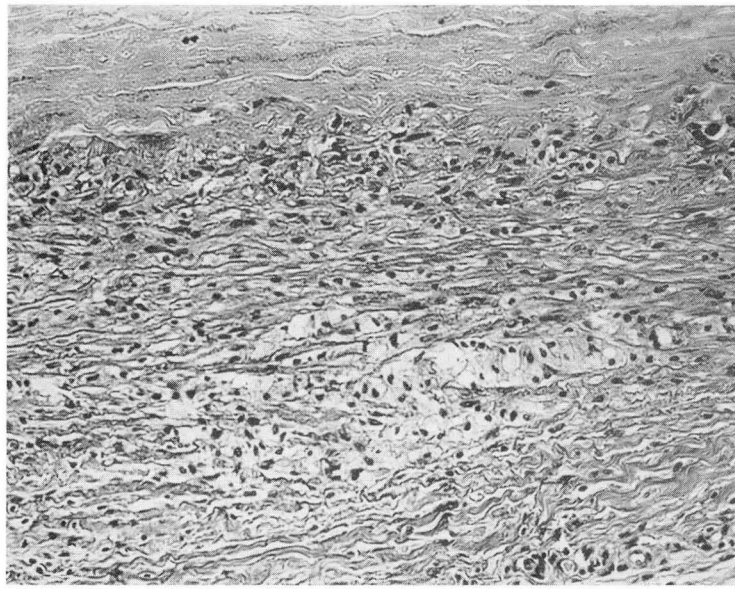

Fig. 6 (Case 2—Nine-month-old homograft). Collection of lipophages with scant lymphocytic infiltrate in the media. Hematoxylin-eosin-methylene blue; X170.

Traversing the length of the homograft was a continuous black-silk suture that had previously plicated the homograft to correct the size of the lumen. The wall of the aneurysm ranged from 0.5 to 1.0 cm. in thickness and was firm and rubbery; the distal opening was completely occluded by an atherosclerotic process.

In none of the histologic preparations could a lining endothelium be found in the aortic graft. The lumen of the aneurysm contained abundant old fibrin clot that was not organized but, in a few areas, the clot was invaded by fibroblasts. In many zones the inner two thirds of the media was acellular; the outer one third contained evenly distributed but sparse numbers of fibrocytes. An occasional blood-filled capillary was present in the outer portion of the media (Fig. 5). Numerous collections of lipophages were present, some just beneath the intimal tunic but most in the central and outer portions of the media (Fig. 6). In several sections, the media was thinned to approximately one half of its average thickness, and in one section a thin portion contained especially numerous foci of well-preserved lipophages. Verhoeff's stain showed abundant elastic tissue fibers in the relatively unaffected portions, but where the segment was thinned, the elastic fibers were condensed and sparse in number (Fig. 7). It appeared that the collections of lipophages produced the disruption in continuity of elastic tissue, maximally affecting the areas of greatest thinning. Masson's trichrome stain showed abundant collagen in the media in many areas, but the collagen fibers were spread apart and displaced by lipophage infiltration. In one thinned area, there was a pronounced reduction in collagen, particularly in the outer one half of the media. The adventitia of the homograft was comprised largely of dense collagen with a focal scanty lymphocytic infiltrate.

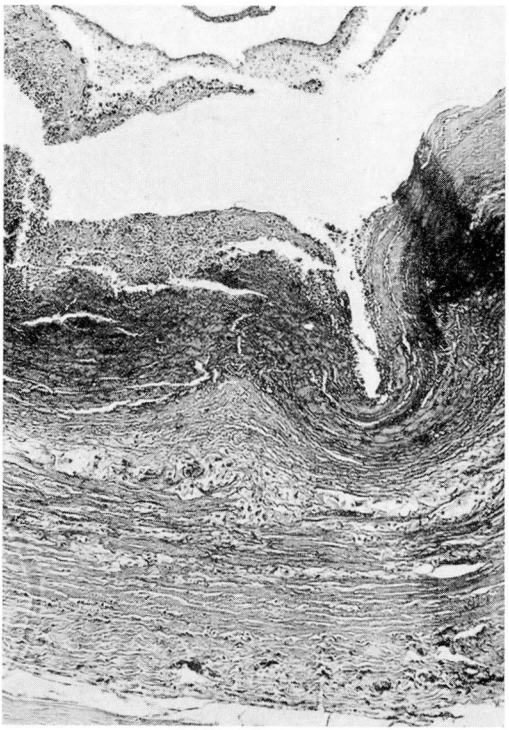

Fig. 7 (Case 2—Nine-month-old homograft). Section from aneurysmally dilated homograft showing sparse elastic fibers in broad outer zone of media, marked lipophage infiltration, and reduction in thickness of the media. Verhoeff's stain; X80.

Case 3 (Eleven-month graft). A nondiabetic, 59-year-old man was admitted to the hospital because of chest pain and intermittent claudication of the left buttock and left thigh which occurred after one-half block of normal walking; this condition had been present for six years and was becoming worse. The pulses on admission were as follows: right iliac 3 plus, right femoral 3 plus, right popliteal 3 plus, right dorsal pedal 0, right posterior tibial 2 plus. On the left side there were no pulsations below the aorta. Preoperative oscillometric measurements were as follows: on the right side—thigh ¾ unit at 100 mm. Hg, below the knee 2, above the ankle 1¾ units; on the left side—thigh ¼ unit at 100 mm. Hg, below the knee ½, above the ankle 0. A freeze-dried homograft was substituted for the aortic bifurcation including the left femoral bifurcation. Eleven months later the homograft suddenly ruptured because of erosion by sharp calcific points on the patient's left common iliac artery where it was cut at the time of the original homograft. An emergency operation was performed and another homograft was installed between the right common iliac portion of the original graft and the right femoral artery. The patient developed an infected retroperitoneal hematoma and died six days postoperatively.

At necropsy the thoracic and upper abdominal aorta showed severe arteriosclerosis. An infected hematoma extended from beneath the inguinal ligament on the left to the posterior portion of the pancreas and surrounded the psoas muscle, the sciatic nerve, the left kidney, and the adrenal. A recent aortic homograft segment, 20.0 cm. in length, was anastomosed to the right portion of the aortic bifurcation and to the right femoral artery distal to the femoral bifurcation. The proximal portion of the left homograft was ligated near the bifurcation. A 10-cm. segment of the old homograft extended from the region of the bifurcation down into the hematoma in the left inguinal region. Dissection of the vessels showed intact suture lines for the recent right femoral aortic homograft and a recently formed reddish-brown friable thrombus partially occluding the central portion of the old homograft. In several areas in the new segment there were yellowish, subintimal plaques of gritty consistence which were slightly elevated above the lining surface. The old homograft segment showed a roughened surface and had a tendency to separate into two layers. The distal portion was severely autolyzed and was lost in the hematoma; it was presumed to have sloughed off. The calcified segment of the left common iliac artery had been removed at the previous emergency operation.

Section of the 11-month-old homograft showed perivascular fibroblastic proliferation in an area that contained much hemosiderin pigment and notable infiltration with neutrophils, lymphocytes, and plasma cells. Numerous focal zones of autolysis were present. This autolytic process principally involved the adventitia, and the media singularly was spared and was present as an acellular necrotic segment without endothelial lining. Elastic tissue stains revealed abundant elastic fibers in parallel arrangement and in approximately the same amount as in a comparable unused aortic segment from the patient. Masson's trichrome stain reflected an amount of collagen similar to that in an unused aortic segment.

The recent homograft segment was identical to the unused segment except for the presence of old atheromas that were completely acellular but contained focal deposits of calcium. This segment was not included as part of this study.

Case 4 (One-year and one-and-one-half-year grafts). A nondiabetic, 47-year-old woman was admitted to the hospital because of claudication in the back and in both hips after three blocks of normal walking; the condition had been present for four years and was worsening. The preoperative pulses were normal on the right side; on the left side they were as follows: 2 plus femoral, 2 plus popliteal, and 2 plus posterior tibial. The preoperative oscillometric measurements were: on the right—below the knee 2½ units at

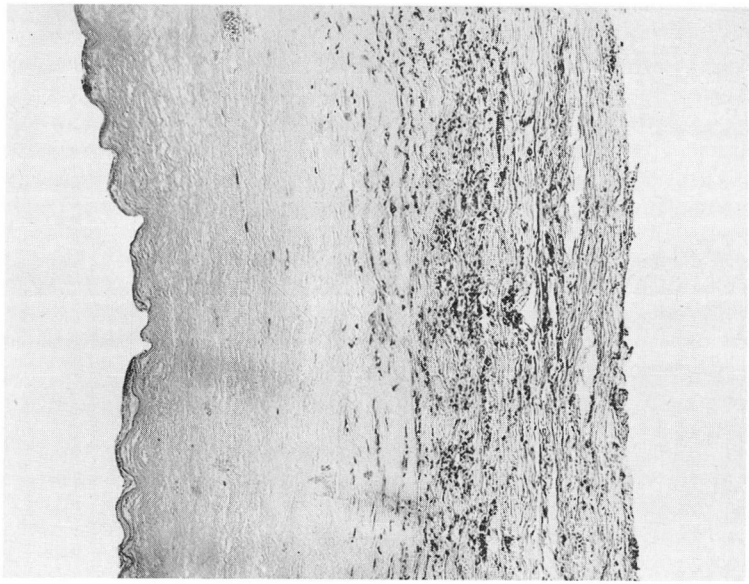

Fig. 9 (Case 4—One-year-old homograft). Extent of fibrous tissue infiltration of the media. The majority of the nucleated cells are fibrocytes. Hematoxylin-eosin-methylene blue; X80.

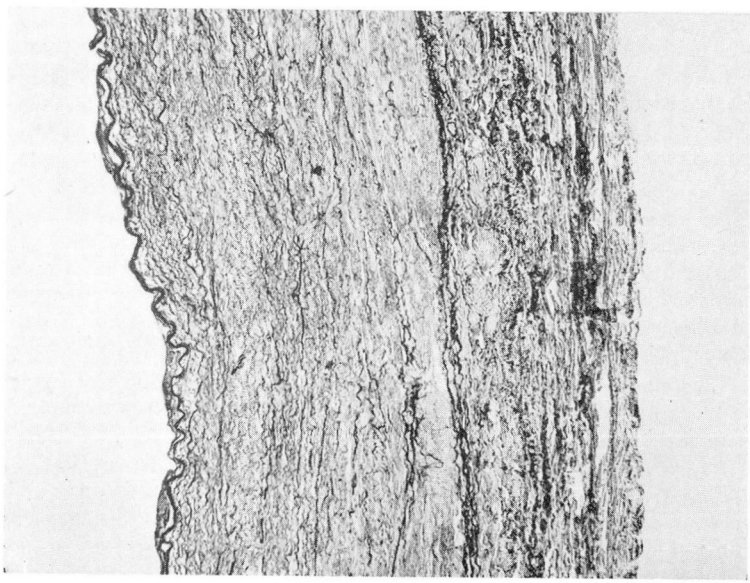

Fig. 8 (Case 4—One-year-old homograft). Showing the internal elastic lamella as the lining surface. Verhoeff's-Van Gieson's stains; X85.

100 mm. Hg, above the ankle ¾ unit; on the left—below the knee ½, above the ankle ½ unit at 100 mm. Hg. A freeze-dried homograft was substituted for the aortic bifurcation, resulting in full restitution of normal pulses and oscillometric measurements. The patient remained well for seven months, when she again developed intermittent claudication. This condition was treated symptomatically. Six months later she was found to have a segmental occlusion of the left common iliac artery; the diseased segment of artery was removed and a bypass homograft connecting the right common iliac artery to the left common iliac artery was installed. Part of the first homograft was removed during that operation. Her condition remained unchanged for six months, by which time all pulses on the left lower extremity had disappeared. At a third operation, it was found that she had developed a secondary block at the femoral bifurcation with occlusion of the superficial femoral artery at that point. We believe that this represented diminution in exit flow which brought about an occlusion of the left half of the first homograft. The right side of the bifurcation had remained open. Accordingly, the bypass homograft was removed and a new prosthesis was substituted which included the femoral bifurcation.

At the second operation, one year after the initial grafting procedure, a segment of artery, 4.0 cm. in length and 0.5 cm. in diameter, was obtained. One end of the vessel showed an attached femoral graft of slightly larger caliber, the lumen of which was completely occluded. The artery itself was completely occluded 1.0 cm. from the site of the homograft. The anastomotic line appeared to be well healed and bound externally by dense collagen; the internal defect between the homograft and the patient's vessel was filled with fibrous tissue. Grossly, no intima appeared to cover the anastomosis.

Histologically, the patient's artery was occluded by an organizing thrombus; no endothelial lining could be discerned within the homograft. The defect between the donor segment and the patient's vessel was filled with fibrous tissue.

Multiple sections from the central portion of the graft showed essentially a tubular segment lined by the internal elastic lamella (Fig. 8). The inner portion of the media was acellular, but the outer one third to one half contained a few fibrocytes (Fig. 9). The distribution of the fibrocytes was sparse in many areas and more dense in others, particularly toward the adventitia. In focal areas, there was disruption of the media by a collection of irregular spaces, some of which contained the outlines of poorly preserved lipophages with foamy cytoplasm, but with no nuclei visible (Fig. 10). Masson's trichrome stain readily revealed these cells, stained the nuclei, and demonstrated the abundant collagen in the media and the adventitia. Verhoeff's stain showed the internal elastic lamella lining the vessel, but indicated a reduction in the content of elastic fibers in the media and the adventitia. Gomori's stain, however, revealed the elastic tissue fibers and they did not appear reduced in amount (Fig. 11). Sudan IV preparations showed that throughout the wall lipid droplets were focally dense, particularly beneath the internal elastic lamella.

The surgical specimen removed at the time of the third operation, one and one-half years after transplantation, was an aortic bifurcation homograft with the proximal aortic anastomotic line and an 0.8-cm. segment of the patient's aorta. A 2.0-cm. length of the right common iliac artery and a 3.5-cm. length of the left common iliac artery were part of the homograft. A 1.8-cm. length of femoral homograft was anastomosed end-to-side to the anterior portion of the right common iliac segment and was passed toward the left as part of the bypass. This was a portion of the one-year-old graft described above which had been ligated and left in the patient. The opened specimen showed complete thrombosis of the right iliac segment and of the more recent bypass. The left iliac portion showed considerable roughening of the lining surface by a focal mural thrombus. The aortic anastomosis was well united externally by dense fibrous tissue, and internally there

Fig. 10 (Case 4—One-year-old homograft). Section showing irregular atheromatous spaces in the media containing cells (lipophages) mostly with unstained nuclei; only a few pyknotic forms evident. Hematoxylin-cosin-methylene blue; X95.

was a slight wedge-shaped depression which appeared filled with fibrous tissue. The right iliac anastomosis was available as a second specimen and this showed a similar type of union. The left iliac anastomosis unfortunately could not be obtained.

Sections from the aortic anastomosis showed good union between the donor segment and the patient's vessel. Dense collagen filled the triangular defect at the junction of aorta and homograft, and a dense adventitia abundant in collagen also was present. No endothelium could be seen. The patient's own vessel showed numerous atheromas. Similar changes were noted at the other anastomoses and in none could endothelium be identified.

The central portions of the iliac homografts were not significantly different from the one-year-old segments. No evidence of endothelium was noted, and the internal elastic lamella lined the structure. The collagen and elastic tissue content of the media closely resembled that of the previously described one-year-old segment. The adventitia was present as an enveloping fibrous lamina of variable thickness, which contained focal lymphocytic infiltration and focal zones of fibroplasia. Sudan IV stains revealed lipid droplets in the vascular wall, which were focally aggregated particularly beneath the elastic lamella and in the adventitia.

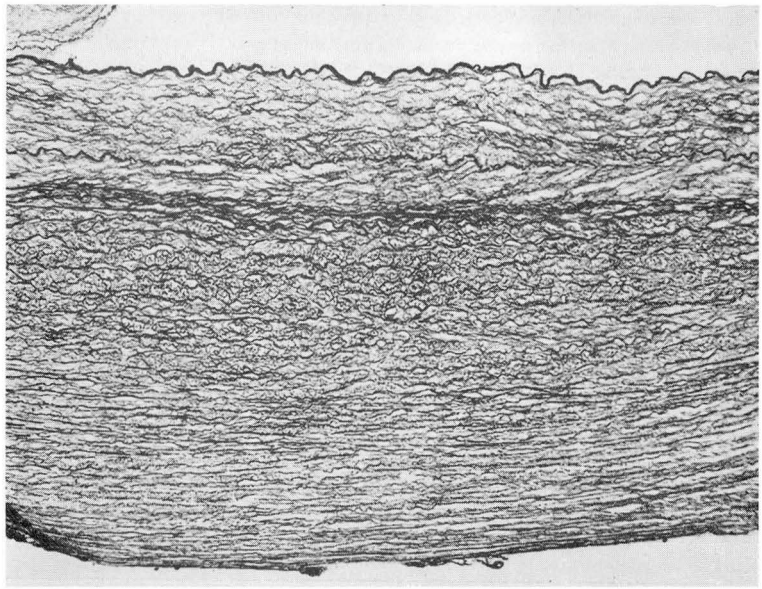

Fig. 11 (Case 4—One-and-one-half-year-old homograft). The Gomori elastic tissue stain shows the internal elastic lamella as the lining surface. There appears to be no significant reduction in elastic fiber content of the media. X95.

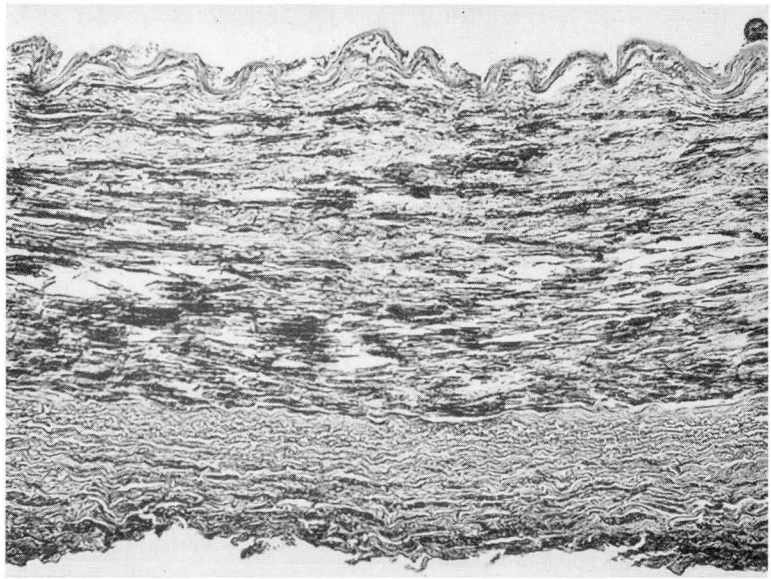

Fig. 12 (Case 4—One-year-old homograft). Showing fragmentation of the media and numerous irregular spaces. Masson's trichrome stain; X140.

The sections from the one-year-old bypass homograft showed no adventitious contribution from the patient. The internal elastic lamella was the lining membrane, but in small foci was covered by fibrin clot that distally occluded the lumen. The media had a peculiar appearance in the sections stained with hematoxylin-eosin-methylene blue, in that the fibromuscular tissue was fragmented and numerous irregular spaces were present (Fig. 12). The adventitia appeared as necrotic, irregularly interrupted bundles of collagen. The Masson's trichrome stain showed fragmentation of the collagen of the media and a marked diminution in collagen content. The tissue that failed to take the fast green stain appeared as short, rectangular, fragmented, coarse fibers. Verhoeff's stain showed an intact internal elastic lamella and a marked reduction in elastic tissue in the media, but well-retained elastic tissue in the adventitia. The elastic fibers that remained in the media appeared condensed and, in most foci, were discontinuous and of short, irregular contour.

Comment

From the study of the present series the fate of homograft segments appears to be as follows. In part they become "vitalized" by fibrocytic infiltration of the remaining adventitia and of the outer layers of the media. From a practical point of view, the patient actually contributes a new adventitia that is moderately vascular; indeed, a few capillaries can be found in the outer layers of the media where there has been fibrous tissue infiltration. The elastic tissue of the media appears to be well retained. The internal elastic lamella or its equivalent in the aorta serves as the lining surface, and in none of our sections have we observed the ingrowth of endothelium from the patient's own vessel.

Creech, DeBakey, Cooley, and Halpert[1] noted that the intimal surface near the anastomotic line becomes covered by endothelium and the central portions by an amorphous fibrinoid substance. Barberio, Pate, Sawyer, and Hufnagel[2] also reported this endothelialization as occurring experimentally in animals, but they used only fresh aortic homografts. There is no doubt that such endothelial ingrowth does occur and a possible reason that we have not observed it, is that in all of the cases presented, thrombosis with occlusion of the homograft has been present. This cessation of blood flow probably suppresses proper nutrition to the intima and causes the disintegration of the endothelial ingrowth over the anastomoses.

Homografts are prone to develop arteriosclerosis, and this is logical since, as Creech and his group[1] point out, the homograft is subjected to the same internal environment as the patient's own vessels. Fisher and Fisher[3] were able to produce arteriosclerotic changes in homografts in experimental animals. Creech and associates[4] observed that when serum cholesterol levels in dogs were maintained at 1000 mg. per 100 ml., both the homograft and the dog's aorta developed atheromatous changes, and that lower levels of serum cholesterol produced changes in the homograft only. In Case 2 the arteriosclerotic process was manifested by numerous collections of lipophages within the media but without deposits of free cholesterol. These collections of lipophages disrupted the

continuity of the medial elastic fibers and collagen bundles, and in some areas appeared to occupy a considerable portion of the media. Infiltration by lipophages was most noticeable in those zones associated with the greatest thinning of the media. The aneurysmal dilatation of the homograft apparently is related to the infiltration by lipophages.

The durability of homograft segments would seem to be excellent. With one exception (Case 2), we have observed no failure of a homograft because of structural changes within the homograft itself. Probably this durability is due to the preservation of the elastic tissue component and to the presence of adventitia supplied by the patient. In some cases the elastic tissue undergoes alteration in staining reaction and Verhoeff's stain fails to reveal all of the elastic fibers that are readily visible by the Gomori method. There was no significant diminution of elastic fiber content in the one-year-old and in the one-and-one-half-year-old specimens. The collagen of the media was similarly well retained. This retention of structural integrity supports the view that homografts are good for many years and the experience of others is in close agreement.[1]

The peculiar changes observed in the one-year-old bypass homograft segment in Case 4 were not seen in any of our other material. The fragmentation of the media with condensation and reduction in elastic fiber content and diminution of collagen were interpreted as changes relating to nonfunction. This bypass segment was present in the patient for one year and was ligated at its distal end. The patient had not contributed a new adventitia as evidenced by the acellular and necrotic appearance of the adventitious zone. Evidently this homograft segment acted as a nonirritating foreign body and slowly underwent the degenerative changes observed.

Summary

Arterial freeze-dried homografts are essentially fibroelastic tubular "prostheses" of considerable durability. At least in the central portions of the homografts the lining surface appears to be the internal elastic lamella or its equivalent. The elastic tissue content of the media of homografts is well retained after one and one-half years though it undergoes changes indicated by altered staining properties. To a great extent the adventitia is supplied by the patient and, in some homografts, fibrous connective tissue may infiltrate the outer layers of the media. Endothelialization was not observed, and this lack in these cases might be the result of thrombosis within and near the homograft. Arteriosclerosis may develop in the media of homografts and in one case was sufficiently severe to permit formation of an aneurysm.

References

1. Creech, O., Jr.; DeBakey, M. E.; Cooley, D. A., and Halpert, B.: Structural alterations in human aortic homografts one to two and one-half years after transplantation. Surg., Gynec. & Obst. **103**: 147-154, Aug. 1956.

2. Barberio, J. R.; Pate, J. W.; Sawyer, P. N., and Hufnagel, C. A.: Some effects of cortisone on aortic grafts. Research Report, Project NM 007 081.10.04 Naval Medical Research Institute, National Naval Medical Center, Bethesda, Maryland.
3. Fisher, E. R., and Fisher, B.: Effect of induced arteriosclerosis on fresh and lyophilized aortic homografts in rabbit. Surgery **40**: 530-542, Sept. 1956.
4. Creech, O., Jr.; Jordan, G. L., Jr.; DeBakey, M. E.; Overton, R. C., and Halpert, B.: Effect of chronic hypercholesterolemia on canine aortic transplants. Surg., Gynec. & Obst. **101**: 607-614, Nov. 1956.