Chronic Vascular Changes in the Walls of Experimental Berry Aneurysms of the Aortic Bifurcation in Rabbits

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SUMMARY Experimental berry aneurysms were fashioned from an autogenous venous transplant by microvascular surgery at the aortic bifurcation of rabbits maintained on a stock diet. Structural changes in the aneurysms and in the host aorta were studied for periods up to 3 years 9 months postoperatively. Phlebocoelexsclerosis developed in the aneurysms and progressed to severe fibrosis, calcification, ossification, mural thrombosis and lipid deposition resembling human atherosclerosis, with eventual loss of the media. The host aorta exhibited pronounced intimal thickening at the bifurcation. The experiments demonstrated the importance of hemodynamic stress in the accelerated production of the degenerative changes in the walls of berry aneurysms.

CONTROVERSY continues over the etiology of berry aneurysms of the cerebral arteries, partly because of difficulties in understanding the structure of the sac wall.1,3 Since berry aneurysms are rare in animals other than man,4 experimental aneurysms were fashioned by microvascular surgery at the rabbit aortic bifurcation to study structural changes in their walls when subjected to chronic hemodynamic stress peculiar to this type of aneurysmal dilatation.

Materials and Methods

Anesthesia, induced in 21 young stock rabbits (2 to 3 kg in weight) by intravenous sodium pentobarbital, was sustained by open ether. A segment of the external jugular vein, previously mobilized from the neck and free of valves, was anastomosed aseptically in the crotch (apical angle2) of the aortic bifurcation, and adjacent medial aspect of the common iliac arteries to form an end-to-end anastomosis in relation to the aorta. The free end of the vein was then ligated. The size of the sac could be varied and, by means of 2 such ligatures, a small, blind, blood-filled venous sac was left in situ in 12 rabbits. Further details of this technique have been published.4 The iliolumbar arteries arising from the lateral aspect of the common iliac arteries near the foramen were ligated routinely.

The rabbits were fed on a stock pellet diet with water ad libitum and were killed by intravenous sodium pentobarbital at intervals varying from 1 day to 3 years 9 months postoperatively. The abdominal aorta with the aneurysm was removed but, in some rabbits, the aorta was perfused with fixative for a few minutes prior to removal. All tissues were fixed in 10% buffered formalin. Step serial blocks were cut transversely through the lower abdominal aorta and aneurysm in 6 rabbits, and tissues were embedded in paraffin or gelatin. In the remaining 19 rabbits, segments of the abdominal aorta and the ventral wall of the aneurysm were embedded in paraffin wax or gelatin. The aortic bifurcations with the residual aneurysms were paraffin-embedded for serial sectioning, the forks being so oriented that they were cut longitudinally to provide Y-shaped sections, with the aneurysm in the acute angle of the Y. Paraffin sections were stained with hematoxylin and eosin, Verhoeff's elastic tissue stain and eosin, Mallory's phosphotungstic acid hematoxylin (PTAH) and toluidine blue. Frozen gelatin-embedded sections were stained with Feitrot 3B and Harris' hematoxylin. Serial sections were mounted 3 to 5 per serially-numbered slide. Approximately 80% of paraffin sections and all frozen gelatin sections were stained.

Results

The experimental aneurysms were remarkably similar to naturally-occurring berry aneurysms of man (fig. 1). They became spherical and enlarged postoperatively, frequently exceeding 1 cm in diameter at sacrifice. Too large for the pelvis, they bulged ventrally, being aligned eccentrically to the aortic axis, a configuration not infrequent in man.3 Perianeurysmal fibrosis made dissection from perivascular fat difficult. The small blind segment of vein containing blood was not easily identifiable, except for the sutures and white fibrous tissue adherent at the fundus. Siderotic pigmentation was not evident.

The inner surface of the sacs was shiny and smooth, except for remnants of recent blood clot adhering to the wall. Two sacs contained mural thrombus, and in one aneurysm (3 years 9 months postoperatively) there was a yellowish-white zone resembling an atheromatous lesion, the lipid being confirmed histologically (fig. 2).

Within the first few days postoperatively, there was histological evidence of inflammation with a thin layer of thrombus lining the sacs. By 19 days, this was largely resolved. The wall exhibited intimal thickening with the wall thickness in places being equal to that of the iliac arteries. In aneurysms from 1 to 6 months, there was medial thickening, predominantly at the lateral sides of the sac, and only remnants of elastic laminae were present in the media and adven-
Intimal thickening was fibromuscular (fig. 3) with little elastic tissue proliferation. Residual elastic laminae were less wavy than usual and considerably fragmented. Near the fundus, the media had disappeared and the intima was usually thinner and more fibrotic than laterally. In places, the intima contained atypical, loosely arranged and stellate muscle cells, and exhibited diffuse metachromasia. Strands of collagen extended into the perianeurysmal adipose tissue, at times incorporating groups of fat cells into the adventitia. Vasa vasorum invaded the outer wall, occasionally extending into the intima when intimal proliferation was pronounced.

In older aneurysms (up to 3 years 9 months postoperatively) the wall varied in thickness, but was thicker than the inferior vena cava. The wall exhibited fibromuscular proliferation similar to that of arterial intimal proliferation, but with less fibrillary elastica and less thickening. Elsewhere the wall was often fibrotic, thin (fig. 4) and devoid of media. Medial remnants and fragmented adventitial elastic laminae were found more readily at the lateral sides of the pouch but, for the most part, the media in old sacs was fibrotic. The intima, when cellular, exhibited metachromasia, but staining was less pronounced in the media and in the host arteries. Metachromasia was
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FIBROTIC WALL OF ANEURYSM MORE THAN 3 YEARS POSTOPERATIVELY. THERES VARIATION IN THICKNESS OF WALL AND THE MEDIA IS ABSENT. FIBROSIS HAS EXTENDED INTO NEIGHBORING ADIPOSE TISSUE INCORPORATING SOME INTO OUTER PART OF WALL. NOTE ABSENCE OF ELASTIC TISSUE. VERHoeff'S ELASTIC TISSUE STAIN. X 65.

In 14 aneurysms older than one month, there was mural or intramural fibrin in the wall, or overt mural thrombosis in 7, but thrombus was present in 5 of the 7 aneurysms older than 3 years. There was fibrin in an atheromatous deposit in one aneurysm (fig. 5), and intramural microhemorrhages in 2 sacs. In one aneurysm containing macroscopic thrombus was a mural tear with fibrin infiltration of the wall nearby. In only one long-term aneurysm was there thrombus in the neighboring host aorta.

Calcific stippling was present in several aneurysms and calcified plaques or bone formation in 5. The fundus was prone to calcification.

Minimal quantities of lipid were seen in some recently produced aneurysms and were related to surgical trauma and thrombosis. Lipid deposits were observed in 5 of 11 aneurysms older than one year postoperatively. Diffuse extracellular lipid deposition (fig. 6), in addition to zones containing cholesterol crystals, were observed in the limited frozen section material, and multiple foci of lipid deposits containing numerous cholesterol clefts and a few lipophages were found in 2 aneurysms of more than 3½ years' duration (figs. 2 and 7).

Intimal proliferation at the aortic fork was thicker and more extensive than that following simple arteriotomy and contained multiple, discontinuous elastic laminae in the intima at the facial and dorsal aspects of the bifurcation. The thickening was more pronounced on the medial aspect of the common iliac arteries, where the internal elastic lamina usually terminated near the entrance to the sac (fig. 1). Intimal proliferation on the lateral aspect of the common iliac arteries (fig. 1) resembled lateral pads in cerebral arteries of man and of other animals. The lateral angle thickening was more prominent than, and independent of, intimal proliferation related to the origin of the ligated iliolumbar arteries, and displayed a remarkable similarity to that of non-lipid-contain-

faint or absent in relatively acellular and hyaline fibrotic zones of old aneurysms. Perianeurysmal fibrosis varied and did not appear to be progressive.

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ing intimal thickening in man (fig. 8). No lipid was demonstrable in paraffin sections of these arterial changes.

Discussion

Berry aneurysms are rare in other animals, but have been reported in 3 chimpanzees. No satisfactory technique has been developed to induce them spontaneously in experimental animals but the present aneurysms are similar morphologically to berry aneurysms of man (fig. 1), although the wall is initially composed of a thin-walled vein. Many cerebral aneurysms in the early stages consist merely of endothelium and a thin layer of residual but attenuated adventitial tissue. The wall retains its ability to proliferate, to undergo repair and to develop premature atherosclerosis, although the time sequence of these changes is unknown. The present aneurysms are artificial but respond to hemodynamic stresses to which they are exposed, and probably constitute the most satisfactory model yet available. The loss of media from these aneurysmal walls indicates that there is no basis for arguing that the absence of media in human berry aneurysms is substantiation for medial defects (raphes) being a contributing factor in the etiology of berry aneurysms.

Veins, used as arterial transplants or in arteriovenous fistulae, do not become arterialized but undergo musculo-elastic intimal proliferation referred to as phlebosclerosis. When severe, it progresses to overt atherosclerosis at times associated with thrombosis and aneurysm formation. The relationship of phlebosclerosis to atherosclerosis is controversial, but the former appears to merge into the latter, with no sharp line of demarcation. The difference seems to be one of degree and both are accentuated by hypertension and hemodynamic parameters.

The aneurysmal walls did not become arterialized. Operative trauma is unlikely to have caused the changes, since control phlebotomy consistently produces negligible scarring, and spontaneous age changes in rabbit arteries and veins are minimal.
Mild changes in these aneurysms provided consistent comparisons with human phlebosclerosis and were similar to those of arterial by-pass grafts and arteriovenous fistulae. Like the latter, however, they closely resemble atherosclerosis when severe.

Veins normally exhibit little tendency to develop severe atherosclerosis but, when subjected to severe hemodynamic stress, irrespective of serum lipid levels, they develop atherosclerosis at an accelerated rate. The fibrosis, acellular hyaline changes, calcification, ossification, thrombosis and lipid deposits in these aneurysmal sacs are consistent with the observation that berry aneurysms in man develop atherosclerosis at an accelerated rate. It is likely that the true incidence of lipid deposition in the sac was higher, for only small samples of the wall were taken from most of the aneurysms for frozen section. The development of atherosclerotic lipid deposits in the absence of dietary supplements of cholesterol is indicative of the important role of hemodynamics in atherogenesis. The similarity of the degenerative changes in these sacs to those in the anastomosed veins of experimental arteriovenous fistulae is close, even though the hemodynamics differ in the 2 lesions. It would appear likely, therefore, that the degenerative changes in these lesions are a nonspecific response to hemodynamic stresses.

That the sequestered blood-filled segment of vein at the tip of the aneurysm failed to undergo atheromatous degeneration was expected because in man segments of arteries and veins sequestrated from the flowing blood by thromboembolic phenomena or sur-

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**Figure 6.** Fibrotic sac wall exhibiting diffuse fat infiltration (dark zone) beneath the intima (45 months postoperatively). Adipose fat cells below. Fettrot 3B and hematoxylin. × 80.

**Figure 7.** Thick fibrotic relatively acellular wall containing atheromatous plaque recognizable by the accumulation of cholesterol clefts (43 months postoperatively). Note abundant vasa vasorum in outer part of wall and absence of elastica. Verhoeff's elastic tissue stain. × 100.

**Figure 8.** Transverse section of common iliac artery immediately beyond entrance to experimental berry aneurysm 21 months postoperatively. Note extensive eccentric intimal thickening with medial thinning similar to that occurring in man. Verhoeff's elastic tissue stain. × 80.
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...gically, do not progress to atheroma as would be consistent with the thrombogenic theory of atherogenesis. There was no evidence that mural thrombus in the aneurysmal sac played a dominant role in the pathogenesis of the chronic changes occurring in aneurysmal walls.

Intimal thickenings at forks of cerebral arteries of human fetuses and neonates and of other animals occur over the crescentic flow divider and in the daughter branches (lateral pads) immediately beyond the lateral angles subtended by the parent stem and branches. Small iliolumbar branches arise from the lateral aspect of the common iliac arteries at or immediately beyond the aortic bifurcation, which may obviate flow separation in the iliac arteries, accounting for the absence of lateral pads at the rabbit aortic bifurcation. In these experiments, where iliolumbar branches were ligated, there was pronounced intimal thickening along the lateral aspect of the common iliac arteries, and this was more extensive than, and sometimes distinct from, that related to orifices of small iliolumbar branches.

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