Retinal arteriolar annuli

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Arterial annuli occur at the junction of side-arm branches of retinal arteries in a number of species, and we have studied them in the owl monkey, cat, dog, rat, and pig. They were initially described as being PAS-positive, but they can also be demonstrated with the Masson's, elastic–Van Gieson's, and Gridley's quadruple stains. In most instances the annuli were hypercellular, and some of the cells had small dark nuclei which appeared to be different from those of surrounding cells. There is no evidence that annuli contain muscular or elastic tissue components; most likely they are composed of basement membrane or immature collagen. Man and rhesus monkey do not have arteriolar annuli, nor do they show hypercellularity at sites of arterial side-arms.

Kuwabara and Cogan were the first to point out that PAS-positive "annuli" occurred at the sites of side-arm branching of retinal arterioles in the cat and rat, but not in other species which they studied. They felt that the annuli, which they considered to be unassociated with increased cellularity, might play a role in retinal circulation, possibly having some hydrostatic function. Their observation was confirmed and extended by subsequent authors using similar retinal digest preparations stained with PAS, and Friedman and associates noted annuli in vivo in cats. Apparently, such structures are not specific for intraretinal vessels, as Agarwal and co-workers have found similar ones in the preretinal hyaloid vessels of the frog, Rana tigrina, and the teleostean fish, Macrones seenghali. These latter authors prefer the term "annular valves" to annuli, and suggest that they play a role in circulatory regulation. In this paper, the microscopic appearance and stain reactions of annuli in a number of species are described, and the possible significance of these structures is discussed.

Materials and methods

Species studied. Retinal vessels of mature man, rhesus monkey (age unknown), owl monkey (Aotus trivirgatus, age unknown), mature cat, mature dog, mature rat, and immature pig were studied.

Digest technique. Formalin-fixed retinas were divided into several parts and digested in pepsin and trypsin. The digested specimens were then mounted on slides and stained.

Stains. Digest preparations were stained with periodic acid–Schiff reagent (PAS) and hematoxylin, Masson's trichrome, elastic van Gieson (EVG), or Gridley's quadruple stain. Stained and unstained digests were also examined by polarized light.

Results

Annuli were seen at side-arm branchings of arteries in the owl monkey, cat, dog, rat,
and pig (Fig. 1), but were absent in man and rhesus monkey (Fig. 2). They occurred at the majority of side-arm branching sites of main stem arteries in the cat and rat, and were also visible at many secondary right-angle branches of the arterial tree, particularly in the cat (Fig. 3). Fewer or less prominent annuli were seen in the dog and pig; in the owl monkey, they were noted mainly at secondary branches. With the PAS stain, the annuli appeared red to purple, depending in part on the over-all intensity of the staining. The annuli stained a bright blue with Masson's, pink with EVG, and an intense green with the Gridley stain (Table 1). Birefringence was not noted at sites of annuli in either stained or unstained preparations. In most specimens, the closer most annuli were to the disc, the more intensely they stained. This was not invariable, however, and an occasional side-arm branch at the posterior pole had no obvious annulus. An increase in PAS or other stain reaction was seen rarely at arterial bifurcations, but never at venous branchings. Twisted vessels caused an increase in the intensity of color at various vessel sites, mimicking the staining of annuli, and due to superimposition of wall structures.

When seen from the side, the annuli had a somewhat conical appearance (Fig. 4). The base which contained most of the increased staining material lay mainly within the wall of the larger vessel, and only small liplike protrusions extended up the wall of the side-arm branch. The annuli stained most intensely at their center and their color faded gradually into the surrounding tissue; often there was more coloration of the annulus on the side nearest the disc and it sometimes appeared to taper. When
Fig. 2. Absence of annuli at primary (black arrow), and secondary (open arrow) side-arm branches of a retinal artery in a human. (Pas and hematoxylin; ×510.)

seen on end, annuli appeared round or oval and completely encircled the lumen of the side-arm branch (Fig. 5). In the cat, the diameter of an annulus was usually about twice that of the arteriolar side-arm branch (range 1.4 to 3.0 times), and similar measurements were obtained in the rat. In other species, there was a lower annulus-arteriolar ratio.

Perhaps the most interesting finding was that the vast majority of annuli, regardless of species, were cellular. This was most obvious in the cat and rat and least apparent in the owl monkey. There was an abundance of cells within the area of the annulus, especially the portion located in the major vessel, but occasionally extending to involve the side-arm as well. A number of the cells appeared distinctly different from the other cells in the neighboring portion of the vessel wall; they had small, dense, dark nuclei (Figs. 6A and 6B). They were noted particularly in lightly stained PAS-hematoxylin preparations and those stained with Masson's trichrome. In human and rhesus monkey, species without obvious annuli, there was no increase in cellularity at side-arm junctions.

In no species, including dog, were there visible constrictions of the side-arm.
branches at the site of annuli; pseudoconstrictions were noted where vessels were twisted.

Discussion

It has been suggested that arteriolar annuli may somehow regulate blood flow in the microvascular bed of the retina.\(^1\)\(^,\)\(^6\) Impetus for thinking that local regulatory mechanisms are present in the terminal portion of the bed came from Thuránszky,\(^10\) who studied the retinal circulation of the cat using water objectives placed within the open eye of the anesthetized animal, as well as quick-frozen specimens. His conclusions were that retinal capillaries had a low concentration of erythrocytes and that only about one third of them contained erythrocytes at any one time. Furthermore, he published pictures of "constrictions" at arteriolar branch points. Friedman and co-workers\(^8\) utilized a sclerouveal window and high magnification to study the retinal circulation in the living cat. They found, contrary to Thuránszky, that most if not all of the retinal capillaries were open to and contained red blood cells. Though they visualized arteriolar annuli, they did not see them acting as sphincters, nor did they visualize any pref-
**Fig. 4.** Side view of an annulus in a cat showing the conical appearance and hypercellularity. (Masson's; ×2,000.)

**Fig. 5.** View of an annulus in a cat showing the oval appearance and hypercellularity. (Masson's; ×2,000.)
ential channels. Keith, Cunha-Vaz, and Shakib\textsuperscript{11} repeated Thuránszky's work and confirmed many of his findings, but they made no mention of annuli or sphincters. They felt that capillaries of the central retina continually appeared and disappeared, but that a few larger channels acted as preferential pathways between arteriole and venule and always remained open. At the present time, there is no general agreement about blood flow in the retinal capillary bed, nor is there sufficient

**Fig. 6A.** Side view of annuli in a rat (arrows) showing hypercellularity. (PAS and hematoxylin; \(\times800\).)

**Fig. 6B.** Schematic drawing of a primary side-arm branch annulus showing the morphology and cellular components.
Table I. Stain reactions of annuli compared with reaction of collagen, muscle, elastic tissue, and basement membrane

<table>
<thead>
<tr>
<th>Stain</th>
<th>Collagen</th>
<th>Muscle</th>
<th>Elastic tissue</th>
<th>Basement membrane</th>
<th>Annuli</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAS</td>
<td>Pink or red*</td>
<td>—</td>
<td>—</td>
<td>Rose to purple</td>
<td>Red to purple</td>
</tr>
<tr>
<td>Masson’s</td>
<td>Blue</td>
<td>Red</td>
<td>—</td>
<td>—</td>
<td>Blue</td>
</tr>
<tr>
<td>EVG†</td>
<td>Pink to red</td>
<td>Yellow</td>
<td>Blue-black</td>
<td>—</td>
<td>Pink</td>
</tr>
<tr>
<td>Gridley’s</td>
<td>Green</td>
<td>Red</td>
<td>Violet</td>
<td>—</td>
<td>Green</td>
</tr>
</tbody>
</table>

*Immature collagen.12
†Weigert’s resorcin-fuchsin counterstained with Van Gieson’s.

Table II. Species examined for arteriolar annuli by retinal digest techniques

<table>
<thead>
<tr>
<th>Species</th>
<th>Annuli</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Owl monkey</td>
<td>+</td>
<td>*</td>
</tr>
<tr>
<td>Cat</td>
<td>+</td>
<td>1, 2, 6</td>
</tr>
<tr>
<td>Dog</td>
<td>+</td>
<td>2, 7, 8</td>
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<tr>
<td>Rat</td>
<td>+</td>
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<tr>
<td>Pig</td>
<td>+</td>
<td>6</td>
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<tr>
<td>Macrones</td>
<td>+</td>
<td>4</td>
</tr>
<tr>
<td>Rana</td>
<td>+</td>
<td>3</td>
</tr>
<tr>
<td>Rabbit</td>
<td>— (+)</td>
<td>1, 2, 5</td>
</tr>
<tr>
<td>Mouse</td>
<td>— (+)</td>
<td>1, 6</td>
</tr>
<tr>
<td>Man</td>
<td>—</td>
<td>1, 7</td>
</tr>
<tr>
<td>Rhesus monkey</td>
<td>—</td>
<td>6</td>
</tr>
<tr>
<td>Calf</td>
<td>—</td>
<td>3, 6</td>
</tr>
<tr>
<td>Hamster</td>
<td>—</td>
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</tbody>
</table>

Abbreviations: —, No annuli; +, annuli noted.
*Present study.

The staining reactions suggest that the annuli are not composed of muscle or elastic tissue, but may represent basement membrane or immature collagen.12 The intense PAS stain probably indicates that the annuli have a high carbohydrate content. Immature or incompletely cross-linked collagen stains red with PAS and gives reactions similar to those obtained with the other stains. Unlike mature collagen, the immature form may possess rubber-like elasticity and is apparently related to basement membranes. In this regard it is interesting that the retinal arteries of the rat have well-defined annuli and prominent basement membranes. Indeed basement membranes are secreted by undifferentiated cells of developing retinal vessels.14 Whether the small darkly staining nuclei seen in many annuli belong to a different cell population than the endothelial cell or intramural pericyte is unknown, but they are unlikely to represent the so-called “cushion cells” described by Loewenstein15 in retinal precapillaries on both the arterial and the venous side of the circulation.

It is conceivable that annuli develop simply by the heaping up of basement membrane as the side-arm branch develops. If this were true, however, one might expect to find annuli in all species...
with retinal arterial side-arm branches. Yet man and rhesus monkey lack annuli although they have prominent side-arm arterioles, and interestingly there is no hypercellularity at such branches in either species. At present, we are studying the maturation of retinal vessels in species with annuli, and this may provide insight into when and how they develop.

It is impossible to do more than speculate about the role of annuli in retinal circulation. Since they apparently lack muscle or elastic tissue components, they probably do not actively contract; possibly they could swell under changing metabolic circumstances and constrict the lumen. It appears to us that their function may be to maintain the luminal opening of the side-arm branch and possibly prevent it from dilating excessively with pressure alterations. In none of the specimens we have examined, including dog, have we seen stenosis of the base of a side-arm arteriole, although such an appearance has been recorded by Mutlu and Leopold and Engerman and colleagues in the dog. Neither have we seen any obvious dilatation at the site of annuli.

At the present time, nothing is known either about the development of arteriolar annuli or about their function in health or disease. More information must be gathered about their ultrastructure and chemical composition before we assign them a role in retinal vascular regulation.

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REFERENCES