Retinal vascular patterns

VII. Acellular change

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The trypsin digestion technique has revealed a preferential loss of endothelial cells (in conditions other than diabetes) and eventually a total acellularity of many retinal capillaries. The surprise is that this may occur without recognized functional abnormality or without appreciable histologic change in the rest of the retina. Perhaps the retina has a large reserve capacity normally or perhaps the choroidal circulation compensates for loss of the retinal circulation. In any case the retina shows little reparative response to these capillary changes. Unlike what one expects in most other tissues, there is no formation of fibrous scar tissue, no infiltration with histiocytes, and no neovascularization. Instead, Müller's cells fill up the tissue defect and their processes insinuate into what had been the capillary lumina.

Previous papers have described the characteristic patterns and cytology of the retinal vessels, as revealed by the trypsin digestion technique, in normal and pathologic conditions. Electron microscopic observations have supplemented these descriptions. The importance of the mural cells has been especially emphasized. These cells appear to be differentiated pericytes encased within the wall of the retinal capillary and have an especial vulnerability in diabetes. Only casual reference has been made to the endothelial cells.

Loss or attenuation of the endothelial cells from the capillaries is a common finding, perhaps the most common finding, in various pathologic conditions and in otherwise normal eyes. Widespread loss of cellularity is a constant occurrence in the peripheral capillaries of elderly people. It is often preliminary to loss of the mural cells with the result that a totally acellular and fibrous plexus finally remains. The absorption of these acellular vessels is either extremely slow or nonexistent so that the remaining scaffolding preserves permanently the architectural pattern of the original blood vessels.

In the present paper we propose to demonstrate the early changes in the endothelial cells and the later changes in the acellular vessels as seen in flat preparations of the retinal vessels and in electron micrographs. We wish further to point out a unique reaction of Müller's cells of the retina accompanying loss of cells from the retinal vessels but a surprising lack of correlation between acellularity of retinal capillaries and histologic abnormalities in the retinal tissue.
Materials and methods

Retinal vessels from approximately 500 eyes were isolated by the trypsin digestion technique which we have previously described. About one-half of the eyes were obtained postmortem from persons not known to have ophthalmic disease and one-half were obtained surgically with a variety of ocular disorders.

Electron micrographic studies were carried out on the normal eyes of four elderly and three young persons, on three eyes with absolute glaucoma, and on two eyes with injury.

Correlative observations were made on twenty cats' eyes which had been damaged experimentally. The experiments included systemic poisoning by iodoacetic acid, mechanical trauma, ischemia, and photocoagulation.

Results

Normal retinal capillaries show a remarkably uniform caliber of their lumina and constant distribution of cells in their walls. The ellipsoidal pale nuclei of the endothelial cells are regularly spaced as are also the round, dark nuclei of the mural cells (Fig. 1B). In the developing stage and during the first decade of life the nuclei of both endothelial and mural cells are relatively abundant and frequently appear to overlap one another (Fig. 1A). With increasing age the nuclei in the capillary wall become less numerous per unit length and eventually the ratio of the endothelial cells to the mural cells declines progressively. Although the endothelial nucleus becomes somewhat elongated with age (Fig. 1C), the shape and stainability of the individual nuclei remain relatively constant.

The fine structure of normal retinal capillaries is characterized in practically all species by: (1) a thick basement membrane which forms the outer limits of the capillary and at the same time separates the endothelial and mural cells; (2) a thick endothelial lining of the relatively small lumen; and (3) the presence of mural cells.

Early changes in the endothelial cells. As the earliest pathological change, endothelial cells become irregular in shape, nuclear stainability, and distribution (Fig. 2). This irregularity is seen either throughout the whole vasculature or in small lo-

Fig. 1. Vessel preparations of normal human retinas. (P.A.S.-hematoxylin stain, x256.) A, Highly cellular vessels of 8-year-old boy. B, A 40-year-old man; endothelial cells (e) and mural cells (m) are regularly spaced. C, An 80-year-old man; only two endothelial nuclei are seen in this field whereas mural cells are numerous. Capillaries are somewhat smaller.
ocalized areas. The reason for these changes is not always clear but often acute or subacute pathological conditions, such as sudden elevation of the intraocular pressure or injury, is found to be the possible cause. Although the destructive change is not apparent at this stage, acellularity seems to follow it.

The next noticeable, nonspecific change in the capillary wall is the disappearance of the endothelial nucleus (Fig. 1C). Depending on conditions of the retina and age, the sparseness of the endothelial nuclei varies considerably. And the severity of the vascular change is not necessarily correlated with histologic abnormalities in the rest of the retina. It is not uncommon to find total absence of endothelial nuclei in a large area of the vasculature of a histologically normal retina.

Capillaries which have lost endothelial nuclei appear to maintain their original caliber as long as mural cells are intact. Although red blood cells are not frequently found in capillaries which have lost their endothelium, electron microscopic study suggests that some of these capillaries may still be patent. Cross sections of retinal capillaries in instances where vessel preparations of the adjacent area have revealed extensive endothelial acellularity show that many are patent and have the normal endothelial lining (Fig. 3). The fact that the chances of hitting the nucleus of the endothelial cell in the ultrathin sections of this retina are extremely small, whereas the demonstration of the mural nucleus is quite frequent, may well indicate that these cross sections are of "endothelial acellular" capillaries.

The cytoplasmic lining of these capillaries presumably belongs to some surviving endothelial cells from distant portions of the same vessel, or from the adjacent capillary. The fine structure of these cells is not so different from the normal condition, except that the cytoplasm is a little thinner and the basement membrane belonging to the endothelial cell shows a relatively high incidence of vacuole formation, whereas the part which surrounds the main body of the mural cell usually avoids severe vacuolization (Fig. 4).

Endothelial and, occasionally, mural

![Fig. 2. Vessel preparation from the retina of a glaucoma patient. Endothelial cells are irregular in shape and distribution. Mural cells are regularly distributed. (P.A.S.-hematoxylin stain. x250.)](http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/932955/)
Fig. 3. Electron micrograph of a retinal capillary from a young patient with ocular injury. The vessel preparation of this retina (right) shows extensive endothelial necularity. The electron micrograph (left) probably corresponds to an area similar to that indicated by the line crossing a mural cell. The capillary is probably patent. The mural cell (Muc) is normal. Endothelial lining (E) may be somewhat thinner.

cells in the capillaries which are showing a decrease in the number of their endothelial nuclei, often show lipofuscin granules in their paranuclear areas. These masses of the lipofuscin are found in flat preparations as well as by electron microscopy (Fig. 5). They are P.A.S. positive and sudanophilic and easily distinguishable from melanin or hematogeneous pigments. Aside from senescence, lipofuscin granules are most commonly seen in the vessels of chronically pathological eyes. They were especially conspicuous in retinal capillaries of a few cases of chronic mild venous occlusion.

Another type of electron-dense material is seen within the vacuoles of the basement membrane. These are also of lipidic nature and composed of fine amorphous granules.

Small nodulelike accumulations of mitochondria in the endothelial and mural cells of this pathological stage may be more than an incidental finding, though the microorganelles appear to be within normal limits. This is also clearly observed in the vessels prepared from retinas which have been incubated in a nitro blue-tetrazolium solution containing succinic acid as the substrate, and followed by the trypsin digestion (Fig. 6). The blue-stained, nodulelike material corresponds to the site of the accumulation of the mitochondria observed electron microscopically.

Retinal tissue which is undergoing vas-
cular changes often shows a conspicuous increase of membranous structure around the vessels. This structure seems to have originated when the cell membranes of Müller's cell processes became closer together, as a result of the disappearance of some neuronal cells. Basement membrane often extends into these membranes. Fine fibrous material clinging around the vessels in the pathological cases, as is seen in flat preparations, corresponds to the above fibrous material (Fig. 7).

Destructive changes, such as karyolysis or karyorrhexis, are also seen especially in eyes with acute ischemia or following embolism. These changes seem to be followed by a localized or diffuse death of the capillary. In flat preparations they are seen as wrinkled acellular strands.

Except in the case of diabetic retinopathy, proliferation of endothelial cells is rare. Proliferation of the mural cell has not yet been observed.

Late stage of the acellular vessels. Eventually the damaged capillaries lose the nuclei of mural cells as well as of endothelial cells. Totally acellular capillaries are not patent, and are no longer functioning as circulatory channels. As retinal tissue seemingly has very little capacity for absorbing dead tissue, particularly the basement membrane substance, remnants of the capillaries remain at the original site, preserving the normal pattern of plexus for a long period of time. Damaged vessels in the nonspecific pathological condition appear to be simply abandoned (Fig. 8).
Fig. 5. Lipofuscin particles (Lf) seen in the endothelial cell of an elderly person. Lipidic granules (Lg) are also seen in the vacuole in the basement membrane. Vessel preparation of this retina (left) shows P.A.S.-positive granules in the capillary wall.

Fig. 6. Dehydrogenase activity in the retinal capillaries of a glaucoma patient. A, Succinic acid dehydrogenase activity. Large-sized granules are characteristically found in pathologic retinas. (×128.) B, DPNH diaphorase activity is demonstrated in the mural cell and in groups of mitochondrias (arrow). (×256.)
Fig. 7. Increased membranous structure about a retinal capillary of an elderly person. Basement membrane substance is being deposited in some membranes (arrow). Note the accumulation of mitochondrias in the mural cell. Vessel preparation from the same retina (below) shows membranous structures attached to the vessel (arrow). (P.A.S.-hematoxylin stain. ×256.)

paratory process or neovasculogenesis to replace the damaged vessels is either absent or extremely rare. The reparatory reaction of the surrounding retinal tissue is also minimal.

Depending upon the nature and degree of the damage, acellular strands are seen either as clusters, or as a total acellular vascular bed. Even in cases of sharply circumscribed retinal degeneration, damaged vessels stay at the original site without appreciable change in the surrounding vascular beds.

These damaged vessels are seen as P.A.S.-positive material in the histopathological specimen. Electron microscopically, they are demonstrated either as a collapsed structure of basement membrane surrounded by Müller’s cells, or as a structure which maintains the original caliber of the capillary but filled with prolongation of Müller’s cells (Figs. 8C, 9).
Fig. 8. A 57-year-old man with central artery occlusion of 4 weeks duration. A, All capillaries have lost cellularity, but still maintain their original vascular pattern. (P.A.S.-hematoxylin stain. ×184.) B, Histology shows good preservation of the outer half of the retina. The inner layers have lost cells. One large collapsed vessel is seen in the center. No capillary is recognizable. (Hematoxylin-eosin stain. × 184.) C, Electron micrograph of the nerve fiber layer of the same retina shows totally acellular capillary. The lumen (L) is filled with the extension of the cytoplasm of Müller's cell. The basement membrane is swollen and vacuolated.
Fig. 9. Capillary of the cat retina of which the large arteries were damaged by photocoagulation two months previously. Capillary lumen and the space of the mural cell cytoplasm (M) are completely filled with Müller's cell. Higher magnification (right) shows the identical structure in both outside and inside (L) of the capillary. The basement membrane preserves its original structure quite well. All experimentally damaged, and clinico-pathological cases show similar findings.

In either case, the basement membrane appears to be swollen or looser than normal, and shows occasional breaks. The cytoplasm of the Müller's cell which has invaded into the capillary lumen is identical to that which is in the surrounding retina. No collagenous tissue or fibrous thrombus is demonstrable in these lumina.

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