Fine structure of retinal vessels in man and the macaque monkey

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The fine structure of the capillaries, arterioles, and venules of the human and monkey retina are described. The capillaries consist of endothelial tubes without fenestrations and a basement membrane in which pericytes are embedded. The structure of endothelial cells indicates that they are very active. Some difference in composition of the basement membrane material on the outer and inner surface of the pericytes may exist. The outer layers were vacuolated in the adult but not in young human or monkey material. This may represent a change due to age. The cytoplasmic organelles of the pericytes are concentrated on the outer (glial) side. Fibrillar material was found in their cytoplasm somewhat similar to that in smooth muscle. The endothelial cells in the monkey contained unusual cytoplasmic organelles with a highly organized and complicated internal structure. These are related to other cytoplasmic membrane structures.

In recent years, the interest of many investigators has been focused on the anatomy and histology of the retinal capillaries and larger vessels in normal and pathologic conditions, especially diabetic retinopathy. However, the ultrastructure of normal retinal vessels has not been extensively studied to date. Some descriptions of them were reported by Maeda1 and Bloodworth2 in the human, and by Kissen and Bloodworth3 in the rat.

The present paper is a report on the fine structure of retinal vessels of young and adult humans, which will be compared with that of the macaque monkey. This new data will be correlated with that reported by Maeda and Kissen and Bloodworth.

Materials and methods

Four human eyes, obtained surgically,* and the eyes of four macaque monkeys were used in this study. Two human eyes of white females, 4 and 7 years of age, were removed because of orbital rhabdomyosarcoma. The other two were from a 48- and a 42-year-old white male and female, respectively, which were removed because of small malignant melanomas of the posterior choroid. In each case, the retina appeared to be normal by light microscopy. The uveal tumors were not near the areas from which the retinas for this study were taken.

Immediately after enucleation, all of the eyes were cut into anterior and posterior parts near the ora serrata and were placed in a large volume of fixative. The posterior part of the eyes was

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then cut into narrow strips containing both central and peripheral retina. The retina and choroid were stripped without separation from the sclera, and fixation was continued in a fresh solution. These procedures were carried out in a buffered osmium tetroxide with 45 mg. per milliliter of sucrose at 0° C. Fixation time was 2 hours. After dehydration in ethanol, the tissues were embedded in either Araldite or Epon, according to standard procedures. The ultrathin sections were cut with a Porter-Blum microtome, mounted on carbon-coated grids, and stained with lead.4 Electron micrographs were taken with Siemens Elmiskop I at original magnifications of 5,000 to 16,000.

For light microscopy, thick sections (about 1 µm) were made from the same blocks and stained with either toluidine blue or by the periodic acid-Schiff reaction of Hotchkiss.

Results

Capillaries. These descriptions are based on the observations of capillaries found in the nerve fiber layer, inner and outer boundaries of inner nuclear, and in both the central and peripheral retinas of the human, and in the macaque monkey. The diameters of these capillaries ranged from about 3.5 to 6 µm. The retinal capillaries of both human and monkey of all ages consist of three layers, endothelial cells, a discontinuous single layer of pericytes, and a thick basement membrane.

The endothelial lining of a capillary wall, in cross section, consists, in most instances, of one or two cells. Fig. 1 shows a cross section of a capillary which passes through the nucleus of the endothelial cell. The endothelial cell body where the nucleus lies is thick, and projects into the capillary lumen, the cell body elsewhere consists of thin cytoplasmic extensions which completely encircle the capillary lumen. At the endothelial cell boundary, where the endothelial cells make contact with each other, a tight union is formed by means of a special structure which will be described below.

The oval nuclei of the endothelial cells extend lengthwise with the capillaries, and their contour appears irregular in cross sections. The chromatin substance of the nuclei is rather evenly distributed throughout the karyoplasm, with slight aggregations along the nuclear membrane.

In the cytoplasm, Golgi apparatus and centrioles are encountered in the vicinity of the nucleus. The mitochondria in the endothelial cells are relatively small in size and number. Tubular or vesicular components, some endoplasmic reticulum with ribonucleoprotein particles, and free dense particles are scattered throughout the cytoplasm. In addition, some very fine filamentous structures are found. A number of pinocytic vesicles, or caveolae, are recognizable along both the lumen and basal cell borders (Fig. 2). The free surface of endothelial cells facing the capillary lumen is generally irregular, especially near the junction with adjacent endothelial cells. At this point, the cell membrane projects in a flangelike process into the lumen. In Fig. 1 a pair of such flangelike processes can be seen on the margins of both adjoining cells (1).

A specialization for attachment at the cell at this point can be found in endothelial cells of retinal capillaries, Figs. 1, 4c, and 6. There is an increase in electron density of the cell membranes of adjacent endothelial cells forming a terminal bar at their region of contact. The dense portions of the cell membranes are continuous from the free to the basal surface facing the basement membrane.

The endothelial cells of retinal capillaries in the macaque monkey show the same structural characteristics as those of the human retina, as far as the above descriptions are concerned. The only exception is a peculiar (crystallike) body in the cytoplasm (Fig. 3). These bodies were seen only in monkey retinal endothelial cells, but were found in all individuals examined. They were roughly ovoidal, ranging from 0.2 by 0.5 µm to 0.4 by 1.2 µm in diameter, and had no distinct limiting membrane, but were connected with the surrounding vesicular components at their periphery (Figs. 4a–4d). Occasionally they were in contact with the endothelial cell membrane. A regular internal structure is recog-
All electron micrographs were taken from material embedded in Araldite except Fig. 4a, which was embedded in Epon 812; and all were stained with the highly alkaline solution of lead salt according to Karnovsky's method. The scale in each micrograph represents 1 micron.

b, Rosenthal body; Bm, basement membrane; C, centriole; cf, collagen fibril; E, erythrocyte; En, endothelial cell; EN, nucleus of endothelial cell; G, Golgi apparatus; GC, ganglion cell; gl, glial cell; m, mitochondria; MN, nucleus of muscle cell; Nl, nucleolus; n, nerve fiber; P, pericyte; PN, nucleus of pericyte; V, vacuole in basement membrane; v, pinocytic vesicle.

**Fig. 1.** A cross section of a retinal capillary in the inner nuclear layer. The capillary wall is composed of two endothelial cells with no fenestrations or pores. Half of the capillary wall is covered by a thick endothelial cell containing an irregularly shaped nucleus with a prominent nucleolus. The other half is covered with a thin cytoplasmic extension of an endothelial cell. At the endothelial cell boundary, a dense cell membrane of the adjacent endothelial cells form a terminal bar, and a pair of flangedike processes can be seen on the margins of adjoining cells (1). A small mitochondrion, endoplasmic reticulum, and free ribonucleoprotein particles are scattered throughout the cytoplasm. The outside of the capillary is surrounded by the cell processes of the pericytes and separated from the neural tissue by a thick basement membrane. (Young human \( \times 27,000 \).)
Figs. 2 and 3. For legends see opposite page.
Figs. 4a-4d. Electron micrographs of the peculiar bodies at higher magnification, illustrating several patterns of their internal structures. (Monkey ×70,000.)

Fig. 2. An electron micrograph showing the relationship between an endothelial cell and a pericyte of a retinal capillary. To the left of the figure (•••), the processes of both endothelial cell and pericyte indent the cytoplasm of the other and are closely attached to each other without a thick interposing basement membrane. On the right (•••), they are in contact with a smooth surface. The pinocytic vesicles can be seen along both luminal and basal cell membranes of the endothelial cell. However, in the pericyte, they are found only on the neural side; the capillary side is occupied by homogeneous material. (Young human ×37,000.)

Fig. 3. A portion of a capillary in the nerve fiber layer of the macaque monkey retina. The capillary consists of endothelial cells, pericytes, and homogeneous basement membrane. The endothelial cell contains a peculiar body (b) which has a regular internal structure. (Macaque monkey ×20,000.)
Fig. 5. A part of a capillary in the inner nuclear layer of adult human retina. The basement membrane is split into layers with the interposition of the processes of the pericytes. The basement membrane between the endothelial cells and the pericytes is composed of homogeneous material and is relatively uniform in thickness. In contrast the basement membrane facing the glial cells contains vacuoles of various sizes and varies considerably in thickness. Small aggregations of dense material can be seen in the vacuoles. (Adult human >28,000.)

Figs. 4c and 4d. For legend see preceding page.
Fig. 6. A part of a retinal capillary and surrounding neural tissue in the nerve fiber layer. The endothelial cells overlap each other at the cell boundary where a terminal bar is continuous with the basement membrane. Portions of two ganglion cells can be seen in the micrograph. They are separated from the retinal capillary by a very thin layer of glial cells cytoplasm. (Young human 21,500.)
Fig. 7. A part of a small arteriole found in the nerve fiber layer. Muscle coat of vessel consists of four layers of smooth muscle cells which show characteristic elongated nucleus, a small amount of cytoplasmic organelles situated near the end of the nucleus, and fine myofilaments. Intercellular spaces of the arteriole are filled with basement membrane material and many collagen fibrils can be seen on the glial side in the adventitia. (Young human x27,000.)
Fig. 8. A part of a vein in the nerve fiber layer. In the endothelial cell, Golgi apparatus and centriole are found in the vicinity of the nucleus. A few layers of smooth muscle cells coat the endothelial cells. A thick layer of collagen fibrils covers the outside of the vein. (Adult human \( \times22,000 \).)
Retinal vessels

shape may be starlike, for they occasionally appear as fragments of cytoplasm in a section (Figs. 1, 2, 3, 5, and 6). These cells can be easily distinguished from endothelial cells by their fine structure. The cytoplasm of pericytes contains relatively small amounts of the ordinary organelles, such as mitochondria and vesicular components, but those seen are similar in structure to those of endothelial cells. However, the distribution of the organelles is peculiar, in that they are mainly found in the portion of the cell adjacent to the neural tissue. The rest of the pericyte cytoplasm is filled with less dense materials which, in some instances, form very fine filamentous structures, so that this region somewhat resembles smooth muscle cells (Figs. 2, 5, and 6).

The nucleus of the pericyte is oval, or flat, in cross section, and is similar to the nucleus of endothelial cells. In the nuclear region, typical Golgi apparatus, centrioles, mitochondria, and endoplasmic reticulum can be seen.

The pericytes are almost entirely covered by a dense basement membrane. In some small areas, however, pericyte and endothelial cells are so closely attached that no basement membrane material is apparent between them. In some instances, close apposition of two cells is characterized by Singer-like processes of the cytoplasm of either the pericyte or endothelial cells, which indent or penetrate into the cytoplasm of the other, forming a most intimate contact (Fig. 2). Pinocytic vesicles are also found in pericytes along the cell membrane next to the neural tissue, very rarely adjacent to the capillary (Fig. 2). No significant difference in the structure of pericytes, or in their relation to endothelial cells, was discovered in the young and adult human or monkey.

The basement membrane of retinal capillaries has been reported to be homogeneous in structure in the rat, but occasionally unlined vacuolar structures, or holes, were found in this membrane around human retinal capillaries. The basement membrane of monkey retinal capillaries, however, is similar to that of rats, and is composed of a homogeneous, dense material, in which are embedded portions of the cell bodies of the pericytes (Fig. 3). Some difference in the basement membrane of young and adult human retinal capillaries was found in the present study.

The basement membrane is usually separated by pericytes into an inner layer situated between endothelium and pericyte, and an outer layer facing the glial cells. Both layers of the basement membrane are similar in density, structure, and thickness (about 0.1 μ) in the young human material. In the adult, however, these two layers vary considerably in structure and thickness (Fig. 5). The outer layer contains vacuoles, or holes, of various sizes, so that it has a “Swiss cheese” appearance, as described by Bloodworth. It varies from 0.1 to 1.4 μ in thickness. In general, small aggregations of a dense material can be seen in such vacuoles (Fig. 5). It is noteworthy that vacuolization is not seen in the inner layer of the basement membrane.

The outside of the capillary basement membrane is covered by cytoplasmic extensions of glial cells. Fig. 6 illustrates a relationship between the capillary and retinal elements. The very thin cytoplasm of the glial cells separates a ganglion cell from the capillary. When the basement membrane is homogeneous, the surface membrane of the glial cells facing it is rather smooth, whereas a glial surface facing a vacuolized basement membrane shows an irregular contour as shown in Figs. 5 and 6. As observed in light microscopy, the glial cells cover the capillaries and insulate them from the nerve cells. Some have their nuclei situated in the vicinity of the capillary. At least two types of glial cells surrounding the capillaries can be distinguished from each other by their nuclei. Those of one are small and dense, whereas those of the other are large and light in appearance. There is not sufficient evidence to distinguish these cells as special
perivascular glial cells on the basis of their internal morphology from retinal glia proper.

**Arterioles and venules.** Several sizes of arteries and veins were observed in the nerve fiber layer of human and macaque monkey retinas. Except where noted below, no morphological differences between the retinal vessels of these species could be recognized. The arterial vessels are distinguished from venules by the number of muscle layers around them. Venules, even large ones, had only a few layers of muscle, the arterioles many more.

The endothelial cells are similar in fine structure to those of the capillaries. The peculiar, highly organized bodies in the endothelial cells of monkey capillaries were also found, although less frequently, in arterial and venular endothelial cells of the monkey retina, but never in the human material.

In the arterial vessels, the pericytes are replaced by several layers of smooth muscle cells. The number of muscle layers varies from a few to more than ten, depending upon the size of the vessels. The fine structure of smooth muscle cells resembles that of muscular arteries elsewhere, in having characteristic flat nuclei, few cytoplasmic organelles, and homogeneous materials in which very fine filamentous structures can be seen (Fig. 7). Vesicles or caveolae are found along muscle cell membranes, not only on the vessel side, but on the glial side also. The spaces between the muscle cells were filled with dense basement membrane material, and some collagen fibrils were found in this material near the glial cells (Fig. 7).

The muscle coats of venules consist only of a single or double layer of muscle cells, even in large-sized veins found near the papilla (Fig. 8). The fine structure of these muscle cells is very similar to that of the pericytes. Collagen fibrils are also seen in the outer layers of the basement membrane of these venules, and tend to increase in amount in the larger vessels. In those areas, usually near the papilla, where an artery is accompanied by a vein, their adventitia fuse without interposition of retinal tissues.

No vacuolization of the basement membrane could be seen around the arterioles or venules of various sizes, in either the human or monkey retinas. No recognizable structural difference in these vessels, with respect to age or species, was found.

**Discussion**

In the adult and young human and macaque monkey eyes, the fine structure of the retinal vessels was observed in the nerve fiber and inner and outer zone of the inner nuclear layer. In each case there is no significant difference in fine structure attributable to the location of the vessels within the retina. This is in agreement with the report on retinal vessels of the rat.

The retinal capillaries consist of endothelial cells, pericytes, and a basement membrane, and are very similar to those found in the nervous system elsewhere. The resemblance is quite reasonable, because they serve tissue developed in a very similar manner from neural ectoderm. The endothelial cells in human retinal capillaries cover the capillary wall completely, having no fenestrations or pores, and in this respect are similar to those found in muscle, electric organ, central nervous system, and lung.

A few microvilli, or thin flanges, project from the cell surface toward the capillary lumen, especially at the margin of the endothelial cells. Such structures have been reported on the endothelial cells of capillaries of the choroid rete mirabile in the fish eye and in the cerebral cortex of rats. In the former, large vacuoles were also found near the endothelial junction. In the present study, however, no such vacuoles are found; therefore, it is uncertain that the flange region here has the same behavior with respect to pinocytosis, as proposed by Fawcett and Wittenberg in the rete of the fish eye.

No difference in the fine structure of the capillary endothelial cells was found.
in the young and older (42 and 48 years) retinas, nor was any difference distinguished in these cells between the human and monkey retinas, except the peculiar geometrically patterned body in the monkey endothelial cells. The number of these bodies varied in individual animals and were more frequent in the central than in the peripheral retina. The highly regular internal structure of the inclusions suggest that the size of its components is on the order of macromolecules. Examples of highly organized crystalline-like structure have been reported in several cell types; e.g., as Reinke’s crystal in the interstitial cell of human testis; lipoprotein granules in the cortical collecting tubules of mouse kidney; fibrillar structures in spermatid nuclei in certain insects; yolk platelets in amphibian oocytes; hemosiderin deposits in some cell types; and intranuclear crystalline aggregates of virus in the cell infected with a virus. In addition, a local differentiation of endoplasmic reticulum, the myeloid body of the pigment epithelium, has been called a membrane crystal. In the pigment epithelium of the bat retina, a peculiar lamellar structure was reported by Yamada. The internal structures of the peculiar body reported in the endothelial cell of monkey retinal vessels varies slightly in each section, giving the appearance of an hexagonal honeycomb meshwork, or sievelike structure. Such variation of the patterns might be caused by the directions of sectioning, as well as by complexity of its construction. Another characteristic of this body is its relation to the vesicular or tubular components in the cytoplasm, which can be seen at its periphery. Since this structure has continuity with cytoplasmic membranes and complexity, it can hardly be considered as an aggregation of particles, such as hemosiderin or virus. It may be more reasonable to consider that the peculiar body in the endothelial cell of monkey retinal capillary is a kind of membrane structure, and belongs to the same category as the lamellar body in the pigment epithelium of the bat eye, or as the myeloid bodies in the pigment epithelium of lower vertebrates. The complexity and size of the component units have prevented a three-dimensional reconstruction of its internal structure based on the several patterns found in sections. The nature of this peculiar structure is still unknown, but attempts to investigate it histochemically are in progress. Since this body was found in the retinal tissue, and shows some similarity to the myeloid bodies in the pigment epithelium, it is tempting to consider the possibility that it is photosensitive and could play some role in the contraction of retinal vessels.

The pericytes of the retinal capillary are cells surrounding the outside of the endothelium. They are flat, polymorphic cells with branching processes as described by Wolter in the human retina treated with trypsin digestion and silver stains. The electron microscopical observation on the pericytes in human and monkey retina agrees well with Wolter’s observations of pericytes. In sections the pericytes appear in most cases as fragments or processes of cytoplasm, and the nuclei were rarely seen. Practically the whole surface of each pericyte is covered by basement membrane material and is separated from both the endothelial and glial cells. At some points, however, the opposed membranes of the endothelial cells and pericytes are in contact without interposition of basement membrane material. Such close appositions of cell membranes were also found in the smooth muscles in several tissues, and were assumed to be attachment devices of adjacent cells. Such devices may serve a similar function between the endothelium and the pericytes. That part of the pericyte adjacent to the capillary was quite homogeneous, because the cytoplasmic organelles were compressed into the glial side of the cell. The number of pinocytic vesicles found along the cell membrane suggests further that the pericytes are active in the transfer of substances. The basement membrane of the
retinal capillaries is thick, and fills the space between endothelium and neural tissue, enclosing the pericytes. That of 4- and 7-year-old humans and of monkey capillaries was composed almost invariably of homogeneous material, and contained no collagen fibrils, although fine collagen fibrils were observed in the wall of the capillaries of larger caliber near the glial cells. In contrast, the basement membrane of the adult human retina is found always to contain vacuoles. Such vacuoles were never seen in the basement membrane of the monkey, and seldom in that of the young human. Similar vacuolizations of basement membrane were reported\(^1\) to occur in the retinal capillaries of the normal adult human. It is uncertain whether these vacuoles are an artifact of preparation; however, none were seen in the rat or monkey, and only rarely in young humans. Furthermore, the vacuoles were found only at the glial side of the basement membrane. These facts suggest that the basement membrane of the adult human capillary facing the glial cell consists of different components than those in the young human and in animals, which of course may also be young. Therefore, the vacuolization of the capillary basement membrane may have some relationship to age, or possibly to early degenerative changes. It is significant that no perivascular space between endothelium and neural tissue as described by light microscopy is demonstrable in the electron micrographs.

The arterioles and venules in the retinal nerve fiber layer were structurally similar in each specimen. The number of smooth muscle layers of the arterial vessels was greater in proportion to the vessels' caliber, whereas no such increase in the number of muscle layers with vessel diameter was seen in the venules. Many collagen fibrils were found in the adventitia of both arteries and venules. The neural elements of the retina were completely separated by basement membrane, or adventitia, from the vascular components.

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REFERENCES