Fine structure of the choriocapillaris and retinal capillaries

M. H. Bernstein and M. J. Hollenberg

The vascular requirements of the retina are met by two distinct morphologic capillary types. Of these, the choriocapillaris presents a highly polarized appearance with an attenuated and fenestrated endothelium. These specializations are lacking in the second type, namely, those vessels derived from the central artery of the retina. The functional roles of these capillaries would appear to be in accord with their morphologic characteristics. Injection studies with ferritin and studies of prolonged ingestion of silver nitrate in drinking water suggest that the choriocapillaris provides a major fraction of the fluid and nutrients moving into the retina.

The vascular needs of the retina are supplied from two sources, the central retinal artery and the choriocapillaris. The accessibility of the former to funduscopic examination has led to overemphasis of the importance of the retinal capillaries, overshadowing that of the choriocapillaris. This electron microscopic study presents findings which suggest that the choriocapillaris is more active as an exchange site than the deeper retinal vessels.

Electron microscopic analysis of capillary structure has led to several attempts at morphological classification. The relative scarcity of electron microscopic information at the functional level has largely precluded integration of morphological and functional concepts. As a first step toward the reconciliation of form and function, a simplified morphological classification of capillaries for this study is proposed, namely, that capillaries fall into two classes on the basis of endothelial morphology: (1) those with fenestrations, therefore with discontinuous cytoplasm, and (2) those with continuous cytoplasm, without fenestrations.

Fenestrations are found in the presence of a pronounced attenuation of cytoplasm. The amount of endothelial cytoplasm is reduced to a minimum; and in the fenestrae, the cell membranes are reduced to a single layer. The attenuated capillary wall forms one portion of a surface involved in massive fluid movement. This endothelial configuration presumably facilitates the movement of fluids and dissolved solutes of low molecular weight. Such attenuation exists in a functional context and is rarely, if ever, uniformly distributed, but rather exhibits a marked polarity. Capillaries are the exchange levels of a fluid transport system, and endothelial fenestration is a direct reflection of the existence of a preferred exchange direction. This polarization aspect of capillary fenestration has probably received less attention than

From the Department of Anatomy, Wayne State University School of Medicine, Detroit, Mich.

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it warrants. This is in part at least because
the most intensively studied site for this
specialization has been in the glomerular
capillaries of the kidney.\(^6\) In this site, the
polarization of the endothelial fenestration
is masked by the geometrical complexities
of the relationships of the capillaries and
the enveloping epithelium of the renal cap-
sule. There can be no question of the
existence of a preferred direction of ex-
change and a corresponding functional po-
larization in this system.

There is a further significance in our
simplified morphologic classification. Ves-
cicle formation, the morphological compo-
nent of pinocytosis, is a more prominent
feature of continuous endothelia. Since ex-
change is the primary function of capil-
laries, it would follow that fenestrae and
pinocytic vesicles are different manifesta-
tions of a similar activity. They may repres-
ent divergent adaptations to satisfy dif-
fering aspects of the underlying physiologi-
cal requirement. It should also be noted
that pinocytosis or vesicle formation gen-
ernally fails to exhibit any marked polariza-
tion.

The capillary beds supplying the retina
fall into two distinct classes on a morpho-
logical basis. The choriocapillaris exhibits
a pronounced polarity. The surface adja-
cent to the pigmented epithelium of the
retina is markedly attenuated and is abun-
dantly fenestrated.\(^6\) The endothelial nuclei
and the surrounding cytoplasm are gen-
erally found on the choroidal side. The

Fig. 1. Choriocapillaris from Rhesus (Macaca mulata) monkey. Extracellular connective tissue
space of the choroid is at the top. The endothelium is fenestrated where it abuts Bruch's mem-
brane. The choroidal side of the vessel is not fenestrated and is composed of two overlapping
endothelial processes. Bruch's membrane is a composite basement membrane with endothelial
and epithelial components. The main mass of Bruch's membrane is a discontinuous middle
layer which frequently appears to have a fibrillar component. The basal surface of the pig-
mented epithelium has a gentle undulant pattern. (x34,130.)
retinal capillaries, on the other hand, show no fenestrae and no orientation or polarization. They are characterized by a multi-layered pattern of continuous endothelial cytoplasm and basement membrane and the presence of pericytes or Rouget cells. Functional observations with electron-opaque markers have been used to further delineate the differences between choriocapillaris and retinal capillaries. Most of these observations have been made on ground squirrels. The morphological details are essentially the same for all animals that have been examined, including monkey, cat, and rabbit.

Morphological observations

Choriocapillaris. The choriocapillaris is an extensive, anastomosing capillary system derived from the choroidal vessels and lies completely within Bruch's membrane. The choriocapillaris vessels have a large lumen and relatively thin walls. On the choroidal side, we find overlapping endothelial processes, nuclei, and a richly vesiculated cytoplasm. On the opposite or retinal side, the dominant feature is the extensive attenuation of the endothelium and the numerous fenestrae.

The basal surface of the pigment epithelium of the retina faces the attenuated surface of the choriocapillaris and shows marked indications of transport activities, although there are broad variations in various species that have been examined. Species variations range from gentle undulations in the monkey retina (Fig. 1) to the deep and extensive infoldings found in the ground squirrel (Fig. 2). Unlike the attenuated endothelial cytoplasm, all of the...

Fig. 2. Choriocapillaris of ground squirrel retina. The fenestrated endothelium is seen apposed to Bruch's membrane. The basal surface of the pigmented epithelium is marked by extensive infoldings. This tissue sample was fixed one hour after the injection of 250 mg. per 100 Cm. Thorotrast. Particles of thorium dioxide are visible in the lumen of the vessel. (×24,270.)
epithelia appear to be involved in pinocytic vesicle formation.

**Bruch's membrane.** Interposed between the cellular layers of the retina and choroid is a compound basement membrane identified as Bruch's membrane. There are thin, dense amorphous basement membrane components present adjacent to the epithelial and endothelial surfaces (Figs. 1 and 2). Between these margins there is a variable intermediate layer which contains a discontinuous lamina densa, with a fibrillar component intermingling with the amorphous material. These structural elements are best seen in tangential sections (Fig. 3). Bruch's membrane may range in total thickness from 2,000 to 5,000 Å.

**Retinal capillaries.** The capillaries derived from the central artery of the retina are normally distributed in two fairly distinct layers. These are generally designated as the superficial and deep capillary beds. They have a continuous endothelium and no fenestrae (Fig. 4). The endothelial cells abut to form junctions, but there are also frequent overlappings of endothelial processes. Peripheral overlapping processes are generally separated by layers of basement membrane material. The wall structure of these capillaries is further complicated by the presence of pericytes along the capillaries. These latter cells are also enclosed by layers of basement membrane material. The basement membrane here is sharply different from that described for Bruch's membrane. First of all, this basement membrane is a unitary structure of uniform electron density. The membrane splits to enclose overlapping endothelial segments (Fig. 7) as well.

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**Fig. 3.** Electron micrograph of a tangential section through a portion of Bruch's membrane. There is a discontinuous fibrillar meshwork embedded in the amorphous ground substance of the basement membrane. (×68,600.)
as the pericytes. The result is that the normal cross-sectional profile of these capillaries shows a basement membrane encircling the endothelium with additional layers of basement membrane material commonly visible enclosing either tabs of endothelial cytoplasm from adjacent cells or portions of the pericytes (Figs. 4 and 7).

Consistent with the fact that the substance of the retina represents a peripheral extension of the central nervous system, the capillaries derived from the central artery of the retina are covered almost entirely by glial cell processes (Fig. 4).

Movement of electron-opaque material

**Thorium dioxide.** To elucidate the functional characteristics of the distinctive types of capillaries supplying the retina, studies with electron-opaque markers have been initiated. Two markers have been introduced by intravascular injection: thorium dioxide in particles of 90 to 150 Å (Thorotrast) and ferritin. Injected Thorotrast (250 mg. per 100 Gm.) was visible in the lumen of all vessels examined. Fig. 2 shows thorium dioxide particles in the choriocapillaris one hour after injection. Thorium dioxide was never observed outside the blood vessels.

**Ferritin.** Intravascular injections of dialyzed or cadmium-free ferritin have been followed at various times after injection. For periods up to two hours there is a continuing movement of ferritin through the choriocapillaris and the retinal capillaries.

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Fig. 4. Cross section of a retinal capillary. The endothelium is continuous and relatively thick. Pinocytic vesicles may be seen at the luminal surface; a larger number of such vesicles may be observed at the outer endothelial surface. Two overlapping endothelial processes are present in this section. The uniform, homogeneous basement membrane is continuous around all the endothelial cells seen. The capillary is separated from the surrounding neuroepithelium by glial cell processes. (27,200.)
This movement can be observed as early as 15 minutes after injection and apparently continues at a steady rate primarily determined by the local concentrations of ferritin present in the vessels. Histochemically, ferritin is present in all capillaries with a slight indication of higher concentration in the choriocapillaris. This histochemical observation is qualitatively supported by electron microscopic data.

The ferritin appeared to diffuse readily out of the capillary lumen into the substance of the basement membrane. From the choriocapillaris, the primary diffusion barrier would appear to be the middle or intermediate zone of Bruch’s membrane. Comparison of observations at thirty minutes (Fig. 5) and at two hours (Fig. 6) shows a progressive accumulation of the marker in the basement membrane material. Occasional ferritin particles can be seen in the extracellular tissue spaces on the choroidal side of the choriocapillaris. The retinal capillaries also show some movement of ferritin through the endothelium (Fig. 7). There is considerably less migration through the retinal capillaries than through the choriocapillaris.

**Silver nitrate.** In a different type of experiment, adult ground squirrels were placed on 0.25 per cent silver nitrate solution in lieu of drinking water. This regime was well tolerated by the animals for long periods of time. Minimal treatment periods of eight to nine weeks were required before there was any appreciable deposition of silver. This is similar to earlier observations on rat kidney. Silver deposits appeared in Bruch’s membrane and to a lesser degree on the choroidal side.
Fig. 6. Choriocapillaris of ground squirrel two hours after the injection of ferritin. Residual ferritin is visible in the lumen of the capillary and in increased amounts in all three components (endothelial, epithelial, and intermediate) of Bruch's membrane. Endothelial fenestrae, cut in a grazing section, are seen in the upper portion of the capillary. (×34,800.)

side of the choriocapillaris (Fig. 8). There was no deposition relating to the capillary beds derived from the central retinal artery. Indications are that much more extended treatment periods are required to achieve silver deposition in these sites.12 Comparison of Bruch's membrane and the basement membrane of a glomerular capillary after nine weeks of silver nitrate treatment (Fig. 9) shows a striking similarity. If the observed silver deposits are proportional to the fluid flux at a given site, this is a surprising and significant correspondence.

Discussion

The weight of the evidence presented here emphasizes the importance of the choriocapillaris in the microcirculatory economy of the retina. This is in sharp contrast to most reports of funduscopic observations in the ophthalmologic literature which relate exclusively to the central artery of the retina and its terminal branches.13

Our observations on ferritin show that some of this electron-opaque material moves through the endothelium both in the choriocapillaris and in the capillary beds of the retina. There are indications of a greater movement through the choriocapillaris and with a preferred direction of movement toward the retina. This would suggest a correspondingly greater role for the choriocapillaris in providing for the vascular demands of the retina. The significance of the choroidal circulation to the maintenance of normal retinal function is further emphasized in the human retina by the absence of the retinal capillaries in the area of the fovea, the site at which visual function is most critical.
Fig. 7. Retinal capillary of ground squirrel, thirty minutes after ferritin injection. Ferritin particles are present in the lumen of the capillary and adsorbed to the surface of the endothelium. Material adsorbed to the surface is taken into the cytoplasm by vesicle formation. (×49,500.)

Fig. 8. Ground squirrel choriocapillaris after nine weeks' ingestion of silver nitrate. Silver grains have been deposited in Bruch's membrane and to a lesser extent in the choroid. (×27,160.)
This study has not been directly concerned with transport mechanisms. The data, however, afford some insight into these mechanisms, on a morphological basis. Pinocytosis, at the level of fine structure has become an electron microscopic truism, and a phenomenon which we expect to find. And, indeed, pinocytosis is the principal observable transport phenomenon seen in the retinal capillaries. The data also show that transcapillary movement of these markers into the retina is probably greatest from the choriocapillaris, through the fenestrated endothelium. The paucity of pinocytic vesicle formation at this site is indicative that transport through such a fenestrated endothelium involves mechanisms in addition to vesicle formation.

The silver nitrate data indicate that the development of silver deposits in these circumstances is a relatively slow process. This is the only situation to date in which we have been able to demonstrate a functional difference between choriocapillaris and retinal capillaries. The slow onset of silver deposition and its localization indicate that we are dealing with a condition involving long-term fluid movement and a prolonged period for saturation of the tissue. As the initial filtration barrier, Bruch’s membrane apparently is the preferred site of nucleation and deposition. The absence of silver deposition in the comparable sites in the retinal capillary beds suggests that these areas pass lesser volumes of fluid. This would be in accord with the suggestion of Missotten that the capillaries of the central artery have a higher internal pressure and a greater flow rate than is true of the choriocapillaris. It would follow that the relative stasis in the chorio-
capillaris would permit a more massive fluid movement through the vessel walls. In this context, the more substantial movement of the relatively large molecule of ferritin through the choriocapillaris would also be a by-product of these pressure and flow relations.

Each of the electron-opaque markers used in this work has individual characteristics which contribute to its behavior. Clearly, one difference between thorium dioxide and ferritin is that the former is a biologically unacceptable foreign substance. Ferritin, on the other hand, is physiologically acceptable, even though it may be further characterized as a "foreign" protein. The implication is that endothelial plasma membrane acts as an initial barrier with acceptance and rejection capabilities. The results with silver nitrate suggest that this plasma membrane selectivity has an intrinsic size or molecular weight limit and that dissolved silver nitrate is below this limit. Silver deposition would appear to involve long-term saturation with nucleation occurring preferentially in dense filtration barriers in positions of greatest fluid movement. The similarity of results in kidney and retina would suggest that fluid movement at a given site in either a glomerular capillary or the choriocapillaris is of the same order of magnitude.

The blood-retinal barrier is generally considered to be an extension of the blood-brain barrier and, in accord with other observations, the primary barrier appears to be either the basement membrane material in Bruch's membrane or the layered basement membrane enveloping the capillaries of the retinal vessels. In the latter case, the primary barrier is further supported by glial cell processes encasing the capillary.

REFERENCES