

Different Filling Patterns of the Choriocapillaris in Fluorescein and Indocyanine Green Angiography in Primate Eyes Under Elevated Intraocular Pressure

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PURPOSE. To investigate the hemodynamics of the choriocapillaris in primate eyes under elevated intraocular pressure.

METHODS. Indocyanine green angiography (ICGA) and fluorescein angiography (FA) were performed in two monkeys after elevation of the intraocular pressure (IOP) to 35 and 60 mm Hg.

RESULTS. ICGA and FA showed no perfusion delays in the choriocapillaris at 35 mm Hg. The slow dye filling at 60 mm Hg made it possible to observe the early phase angiography sequence. During ICGA, C-shaped precapillary arterioles multiplied in numbers and emerged in the posterior pole, forming the outer rim of the numerous mosaics or lobules. Dye subsequently infiltrated into the lobules. During FA, after numerous hyperfluorescent spots emerged in the posterior pole, each of the spots enlarged and became numerous mosaics with a dark outer rim. When the FA and ICGA images were superimposed, the C-shaped arterioles observed during the ICGA were shown to correspond to the outer dark rim of the mosaics seen during the FA. The earliest dye emergence was delayed during FA compared to ICGA, with 13 seconds elapsing in monkey 1 and 4 seconds in monkey 2. The horizontal diameter of the optic disc contained three to four mosaics in both monkeys.

CONCLUSIONS. The choriocapillaris lobules appear to be supplied from peripheral arterioles, not central. After blood perfuses the lobules, it drains into the venules at the center. Mosaic or lobular patterns during FA may reflect extravasated fluorescein from the fenestrated choriocapillaris.

Keywords: choriocapillaris, central venule theory, angiography

The posterior choroid is supplied by the short posterior ciliary arteries (SPCAs) that arise from the peripapillary area. SPCAs pierce the sclera and enter the choroid. Their branches feed the choriocapillaris, which is characterized by a continuous intercommunicating layer of wide lumen capillaries. The choriocapillaris plays an important role in the metabolism of the outer retina by supplying nutrients and removing waste products. The structure of the choriocapillaris in the posterior pole is known to have a lobular pattern.¹⁻⁴ In general, it is believed that the choriocapillaris hemodynamics involve arterioles at the center of the lobules that are responsible for feeding the lobules, after which the blood drains into the peripheral venules that are located in the outer rim of the lobules.¹⁻⁴ However, it is still disputed as to whether the outer rim of these lobules consists of venules or arterioles.⁵

Since the choriocapillaris has fenestrations,⁶ low molecular weight substances are able to freely permeate the area. Although 80% of sodium fluorescein (NaF) is bound to albumin in the blood,⁷ the unbound NaF freely penetrates the fenestration of the choriocapillaris. In fluorescein angiography (FA), the unbound NaF that readily leaks from the choriocapillaris is observed as background fluorescence during the early phase. Since the peak excitation (465–490 nm) and

the peak emission (520–530 nm) are blocked by the RPE, the choroidal vessels are not seen during FA. In contrast, since the excitation light and fluorescence of ICGA are at near infrared, they are not blocked by the RPE and thus, ICGA is more suitable for investigating the choroidal circulation. Another advantage is that 98% of the ICG is bound to plasma proteins in the blood.⁸ Furthermore, since the ICG does not easily penetrate the walls of the choriocapillaris, this allows better visualization of the blood flow in the choroidal vessels. Although intensity of the fluorescence is much weaker than that for FA, this can be overcome by using a more highly sensitive detector such as a video camera or a scanning laser ophthalmoscope (SLO) instead of a film-based conventional fundus camera. Heidelberg retinal angiography (HRA; Heidelberg, Inc., Heidelberg, Germany) uses an SLO that employs a higher power laser, higher sensitivity detector, and smaller confocal diaphragm, which results in a higher resolution. HRA is able to provide high-contrast images, as it performs real-time digital imaging processing during the examination and improves the image quality through the use of an averaging process.

The choroidal vasculature is composed of three layers that include the choriocapillaris, Sattler layer, and Haller layer. The



vascular lumens are largest in the Haller layer. When using ICGA, the overlapped fluorescence from the outer choroidal vessels can make it difficult to observe the choriocapillaris. Thus, in order to observe the hemodynamics of the choriocapillaris, it is necessary to perform early phase angiography prior to the dye filling in the outer choroidal vessels. Another problem that can be an issue is the rapid blood flow in the choriocapillaris. The choroidal blood flow is pulsatile and its velocity is 20-fold faster than that found in the retina.⁹ Thus, the sequence of the dye filling in the choriocapillaris is difficult to obtain. Hayreh was able to overcome these obstacles by using the ocular hypertension model in primates.¹

The purpose of the current study was to further investigate the choriocapillaris hemodynamics using Hayreh's model, with the ICGA and FA evaluated by HRA. The use of both ICG and NaF dyes provide different images of the same flow through the same structures, and allows important conclusions. The most important of these is that the choriocapillaris lobule is supplied from peripheral arterioles, not central, as many have thought.

METHODS

Animals

This study was conducted in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. The study was approved by Gunma University Animal Care and Experimentation Committee. Two healthy monkeys (*Macaca fuscata*) were used in the study. Monkey 1 was a 6.72 kg 11-year-old female and monkey 2 was a 3.19 kg 3-year-old female. Before all examinations, the monkeys were anesthetized by an intramuscular injection of a mixture of ketamine hydrochloride (10 mg/kg) and xylazine hydrochloride (2 mg/kg). Topical ocular surface anesthesia (0.4% oxybuprocaine hydrochloride) was instilled to reduce discomfort. Topical 1% tropicamide and 2.5% phenylephrine hydrochloride were instilled for mydriasis during the angiography. Before angiography, we confirmed normal fundi in both eyes of the two monkeys by ophthalmoscope.

Angiography

Angiography was performed in the left eye of each animal. The ocular hypertension model was created using the Constellation Vision System (Alcon Surgical, Fort Worth, TX, USA). After the conjunctival sac was sterilized with 5% povidone iodine, a 25 G trocar was inserted into the vitreous cavity through the pars plana at 1.5 mm posterior to the limbus. The trocar was then connected to an infusion line with artificial aqueous humor. This intraocular pressure (IOP) control system automatically maintained the adjusted IOP in real time to ± 2 mm Hg. Angiography was first performed by HRA without infusion (measured IOP: 8 mm Hg). Second and third angiography were performed at the infusion pressures of either 35 or 60 mm Hg. The examination was performed once a day, with experimental intervals of more than 1 week. The SLO of the HRA used blue laser excitation light (488 nm) for FA and a diode laser (785 nm) for ICGA. A barrier filter with a cutoff at 500 nm was used for the FA, while an 810-nm block excitation was used during the ICGA. The camera angle was set at 30°, which included the optic disc and the macula. Video images were obtained using the simultaneous mode. ICGA and FA were performed separately. During the first step of the ICGA procedure, 1 mL of a 10 mg/mL ICG solution was

intravenously injected via the antecubital vein. Then 10 minutes later, 1.0 mL of 10% fluorescein sodium was injected. SLO acquires 30 frames per second during a 30-second period of the early phase.

RESULTS

There was no delay of the dye filling in the choriocapillaris during ICGA and FA at an infusion pressure of 35 mm Hg compared to the same procedures without any artificial infusion pressure. Because of fast dye filling in the choriocapillaris, it was not possible to identify the sequences of the early phase dye filling during the ICGA and FA at an infusion pressure of 35 mm Hg.

In monkey 1, pulsatile dye filling was observed during the ICGA and FA at an infusion pressure of 60 mm Hg. During the ICGA, a network of faint fluorescence emerged in the posterior pole at 18 seconds after the dye injection via the antecubital vein (Fig. 1A). Fluorescence intensity increased at 20 seconds. The observed network consisted of C-shaped branches of the arterioles which formed the outer rims of the numerous dark mosaics or lobules (Fig. 1B). Dye extravasated from the rims and then infiltrated into the dark lobules at 21 seconds (Fig. 1C). The repeated pulsatile dye filling led to the angiogram of the choriocapillaris becoming homogeneous with a loss of the mosaic pattern observed until 27 seconds (Fig. 1D). Subsequently, increased background fluorescence from the outer choroidal vessels obscured the angiogram of the choriocapillaris. During the FA, numerous faint fluorescent spots emerged in the posterior pole at 31 seconds after the dye injection via the antecubital vein (Fig. 1E). At 33 seconds, each of the fluorescein spots expanded (Fig. 1F) and then formed numerous mosaics or lobules throughout the posterior pole at 34 seconds (Fig. 1G). Each lobule had a dark rim in its periphery (Fig. 1G). The lobular hyperfluorescence then fused together with the dark rim disappearing until it became homogeneous background fluorescence at 39 seconds (Fig. 1H). A triangular nonperfusion island was seen at the temporal area that was adjacent to the optic disc throughout all of the phases of the ICGA and FA procedures (Figs. 1A-H). The angiograms for the FA at 34 seconds and for the ICGA at 21 seconds in the posterior pole were superimposed (Fig. 2). In order to distinguish the angiograms, yellow was used for the FA (Fig. 2A) while green was used for the ICGA (Fig. 2B). When the FA and ICGA images were superimposed using a photo editing program (Photoshop version 13; Adobe Systems, Inc.), C-shaped branches of the arterioles in the ICGA corresponded to the dark rim around the hyperfluorescent mosaics seen in the FA (Fig. 2C).

When the infusion pressure was set at 60 mm Hg in monkey 2, the ICGA showed a star-shaped hyperfluorescence that multiplied in numbers and then emerged in the posterior pole at 16 seconds after the dye injection via the antecubital vein (Fig. 3A). The star shapes connected with each other, forming an incomplete network at 17 seconds (Fig. 3B). As a result, the observed network surrounded the dark mosaics. Subsequently, the dye filled into the dark mosaics in some of the areas at 20 seconds (Fig. 3C).

During the FA, the observed hyperfluorescent spots emerged in the posterior pole at 20 seconds (Fig. 3D). These numerous spots enlarged at 22 seconds (Fig. 3E) and developed numerous mosaics or lobules with dark outer rims at 24 seconds (Fig. 3F).

The horizontal diameter of the optic disc contained approximately 3 to 4 mosaics in both monkeys (Figs. 1, 3).

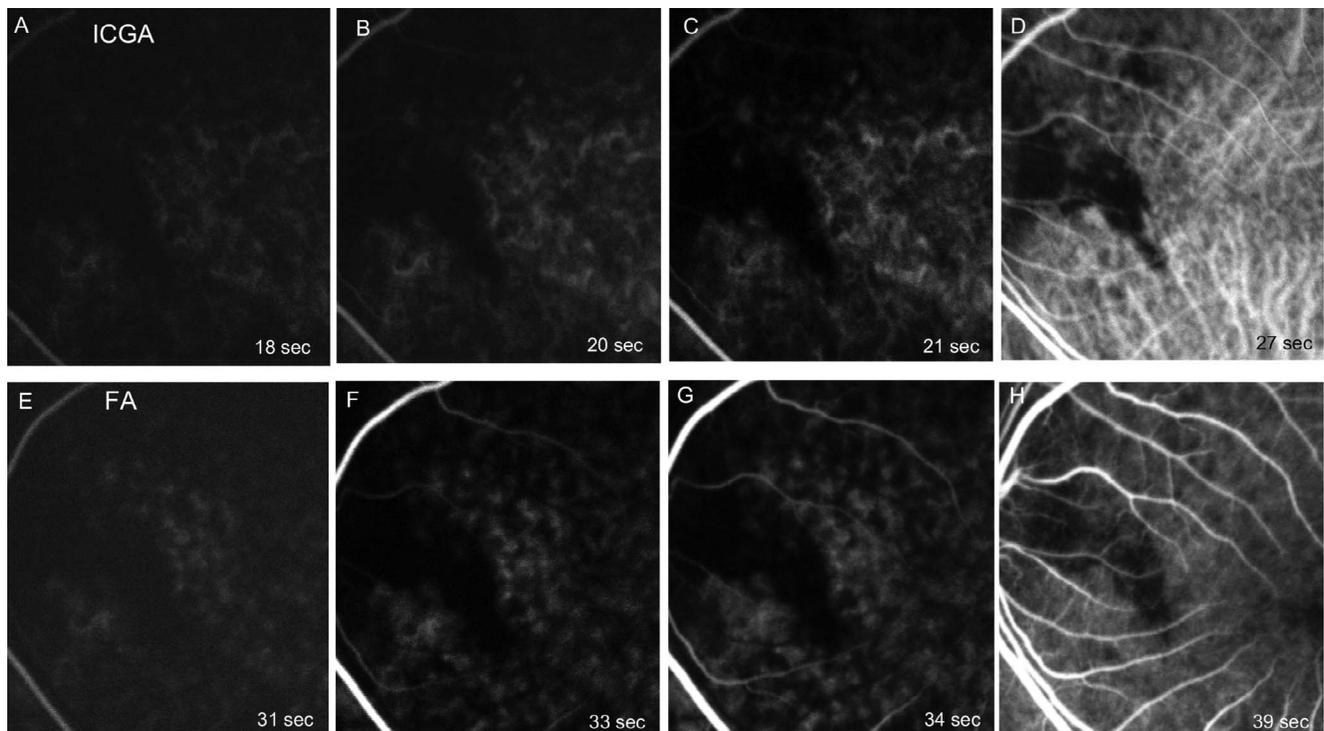


FIGURE 1. ICGA (*upper row*) and FA (*lower row*) in monkey 1 at a 60 mm Hg infusion pressure. (A) Network of faint fluorescence emerged in the posterior pole at 18 seconds after dye injection. (B) Increased fluorescence intensity was observed at 20 seconds. The network consisted of C-shaped branches of arterioles, which formed the outer rims of the numerous dark mosaics or lobules. (C) Dye extravasated from the rims and infiltrated into the dark lobules at 21 seconds. (D) Angiogram of the choriocapillaris became homogeneous with a loss of the mosaic pattern until 27 seconds. Increased background fluorescence from the outer choroidal vessels obscured the angiogram of the choriocapillaris. (E) During the FA, numerous faint fluorescent spots emerged in the posterior pole at 31 seconds. (F) Each of the fluorescein spots expanded at 33 seconds. (G) Hyperfluorescent spots formed numerous mosaics or lobules throughout the posterior pole at 34 seconds. Each lobule had a dark rim in its periphery. (H) Lobular hyperfluorescence fused together and upon disappearance of its dark rim, there was homogeneous background fluorescence until 39 seconds. A triangular nonperfusion island was seen at the temporal area that was adjacent to the optic disc throughout all phases of the ICGA and FA (A-H). Horizontal diameter of the optic disc contained about 3 to 4 mosaics.

DISCUSSION

We observed different dye-filling patterns of the choriocapillaris during the ICGA and FA procedures in the two monkeys with an intraocular pressure that was elevated to 60 mm Hg. Through the use of ICGA, we were able to observe the choroidal angiography without blockage effect by RPE. This was a great advantage as compared to Hayreh's experiment. During the ICGA, C-shaped branches of the arterioles

multiplied in numbers and then emerged in the posterior pole at 18 seconds after the dye injection (Fig. 1A). These precapillary arterioles formed a network that corresponded to the outer rims of the numerous dark mosaics or lobules at 20 seconds (Fig. 1B). ICG dye then infiltrated into the lobules at 21 seconds (Fig. 1C). Thereafter, the lobular pattern gradually became homogeneous and was then obscured by the increased fluorescence from the outer choroidal vessels until 27 seconds (Fig. 1D). An inversed pattern in the dye filling of

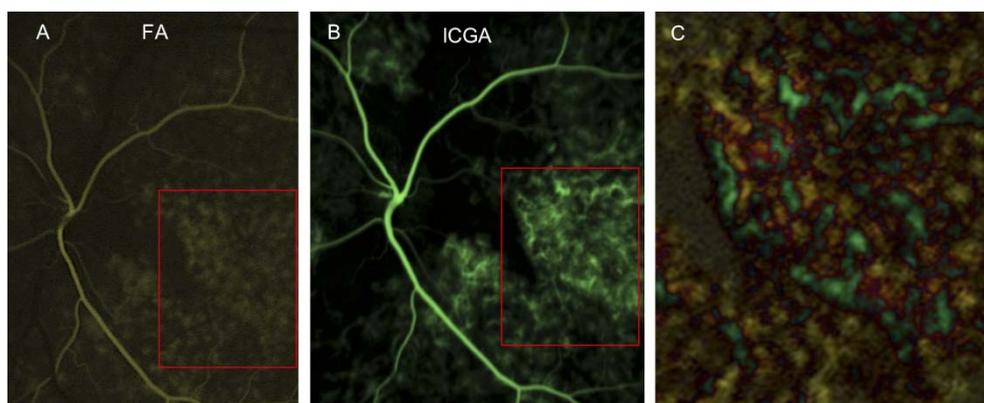


FIGURE 2. (A) FA (*yellow color*) at 34 seconds in monkey 1. (B) ICGA (*green color*) at 21 seconds in monkey 1. (C) Areas within the *red-lined squares* in (A, B) were superimposed using Photoshop. The C-shaped branches of the arterioles observed during the ICGA corresponded to the dark rim around the hyperfluorescent mosaics seen during the FA.

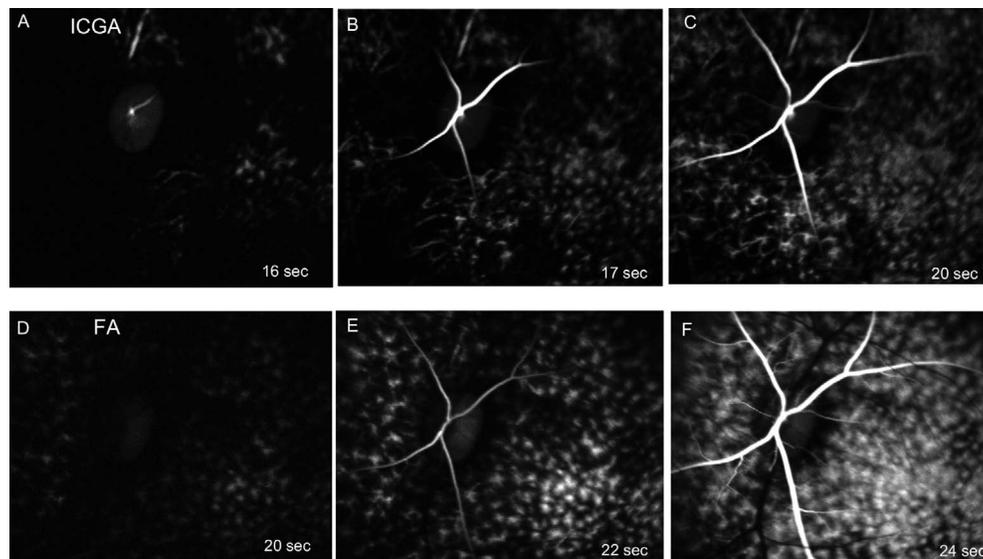


FIGURE 3. ICGA (*upper row*) and FA (*lower row*) in monkey 2 at a 60 mm Hg infusion pressure. (A) The star-shaped hyperfluorescence multiplied in numbers and emerged in the posterior pole at 16 seconds after the dye injection via the antecubital vein. (B) Star shapes connected to each other and formed an incomplete network at 17 seconds. This network surrounded the dark mosaics. (C) In some areas, the dye filled into the dark mosaics at 20 seconds. (D) Hyperfluorescent spots emerged in the posterior pole at 20 seconds. (E) The numerous spots enlarged at 22 seconds. (F) At 24 seconds the spots developed numerous mosaics or lobules with the dark outer rims. Horizontal diameter of the optic disc contained about three to four mosaics.

the choriocapillaris was observed during the FA. Faint fluorescent spots emerged in the posterior pole at 31 seconds (Fig. 1E). These enlarged to become numerous hyperfluorescent spots (Fig. 1F) at 33 seconds and then developed numerous lobules or mosaics with a dark outer rim at 34 seconds (Fig. 1G). Subsequently, the lobular hyperfluorescence fused together and after the loss of the dark rim, it became homogeneous with the background fluorescence until 39 seconds (Fig. 1H). When the ICGA and FA were superimposed, the results showed that the C-shaped precapillary arterioles observed in the ICGA corresponded to the dark outer rim seen in the FA (Fig. 2). Similar dye-filling patterns for the ICGA and FA were observed in monkey 2 (Fig. 3).

An important question is why was there apparent reverse dye filling between the FA and ICGA? Leakage of NaF through fenestrated capillaries within the lobules certainly plays a part, producing a high concentration of dye within the lobules, which is then visualized on FA. However, another factor that strongly influences the relative appearance of the ICG and FA angiograms is the screening effect of the overlying RPE. These two factors together provide a more complete explanation. Thus, in the case of the ICG, the near IR excitation and emission wavelengths are minimally absorbed by RPE melanin. In the case of the FA, the blue excitation especially and also the emission centered around 520 nm are significantly absorbed by RPE melanin. As a result, a higher concentration of NaF than that of the ICG may be required for visualization on the FA than on the ICG of the same anatomic structure at a given time. Thus, at an early point when the arterioles at the outer rims are just seen on ICG, the fluorescein dye may not be visible. As time goes on, both dyes enter the capillaries of the lobule, but now, due to leakage, a much higher concentration of fluorescein is achieved than in the arteriole, and becomes visible. The ICG dye also is now visible, but leakage is not required. This is why the FA dye apparently first appears in the central lobules. This is also consistent with what was observed by Hayreh,

who did not have ICG angiography, and who therefore interpreted the pattern to mean that the lobule was filled centrally by a central arteriole. The ICG is more sensitive by virtue of not being screened by the RPE, and reveals the true state of affairs: that the feeding arterioles are at the periphery. It would follow logically that venous drainage would be central, but this was not explicitly demonstrated.

In our animals, the earliest dye filling into the lobules during FA was delayed 13 seconds as compared to the ICGA in monkey 1 and 4 seconds in monkey 2. Delayed dye filling during FA may be attributed to the time lag for the dye that leaks through the fenestration and then infiltrates beneath the RPE. Our results suggested that the C-shaped precapillary arterioles located at the outer rim of the lobules were responsible for supplying the choriocapillaris, with the blood then draining into the venules at the center of the lobules (Fig. 4).

Another point of interest is that of the apparent shapes of the peripheral arterioles themselves. In monkey 2, the precapillary arterioles usually appeared star-shaped, while they often appeared in a C-shaped pattern in monkey 1. These findings are not inconsistent, but have a unified explanation based on the interlocking configuration of the lobules themselves. In monkey 1, there usually was a brighter filling of approximately half of the rim, producing a C-shape. This suggests that there was filling from the arteriolar entry along an edge, with dye propagation around one rim. The star-shaped filling pattern in the second monkey indicates that the point of entry of the arteriole into the pattern of outer rims is at a vertex of these lobules. As a result, the dye travels into multiple edges of the patterns that share this vertex. More generally, the point of insertion of the arteriole and the connectivity of the lobular rims will determine the exact pattern of observed dye filling.

The early phase dye filling pattern during our FA was similar to that observed by Hayreh.¹ In both studies, fluorescence first appeared at the center of the lobules or segments, which was then followed by filling of the structures. Each of these lobules

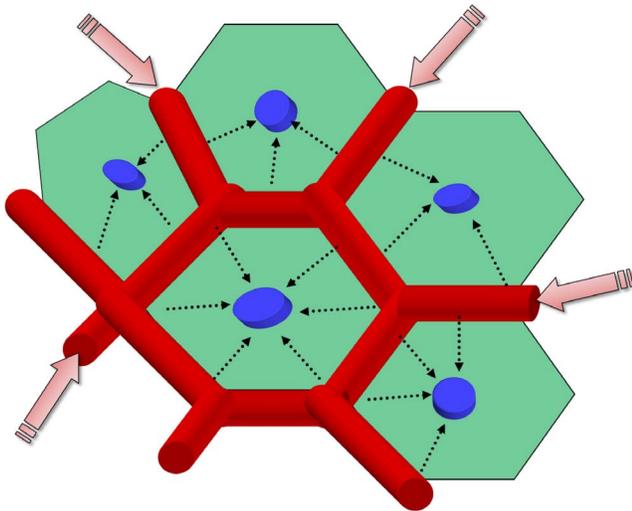


FIGURE 4. Central venule theory. Precapillary arterioles at the outer rim of the lobules supply the choriocapillaris, with the blood then presumably draining into venules at the center of the lobules.

exhibited a dark outer rim of hypofluorescence. Hayreh postulated that each segment was an independent unit with a central terminal arteriole responsible for supplies along with draining venules that were positioned around the periphery of the segment (Fig. 5).^{1,2} Torczynski and Tso³ reported finding a distinct lobular arrangement in the posterior pole in their flat preparations of the human choriocapillaris. They suggested that their findings indicated that the central arterioles were responsible for feeding the lobule, with the peripheral venules draining the blood. A later study by Yoneya and Tso,⁴ who examined corrosion vascular casts in postmortem eyes, confirmed these previous results.

In the 1980s, ICGA was introduced as a beneficial method that could be used in the ophthalmology field. Flower used a subtraction algorithm that subtracted a high speed image from the image immediately following and was able to demonstrate the sequence of dye filling in the choriocapillaris during ICGA in primates without artificially elevated IOP.¹⁰ The ICGA subtraction method showed that the choriocapillaris consisted of discrete lobular segments. The dye filling cycle in the choriocapillaris begins in the macular area and then progresses radially toward the periphery in a wavelike manner. Flower postulated that blood may flow from one lobule into or even through an adjacent one depending on the pressure gradient. However, these observations are not in agreement with the concept that each lobule functions as an independent circulation unit.

Using vascular casts and scanning electron microscopy techniques, Fryczkowski⁵ was able to distinguish arteries and veins based on the shape of the endothelial cell nuclear indentations in the choroidal vascular mold. Anatomically, this method showed that the lobular appearance of the choriocapillaris existed only in part of the posterior pole. In addition, Fryczkowski⁵ also found one or more collecting venules in the center of 86% of the anatomic lobules, while central feeding arterioles were observed in 14%. McLeod and Luty¹¹ used combined alkaline phosphatase histochemical staining with flat embedding in glycol methacrylate to study the choroidal vascular pattern in the choroid from postmortem eyes. They reported that the posterior region exhibited a lobular pattern with a centrally located venule along with arterioles located at the lobular periphery.

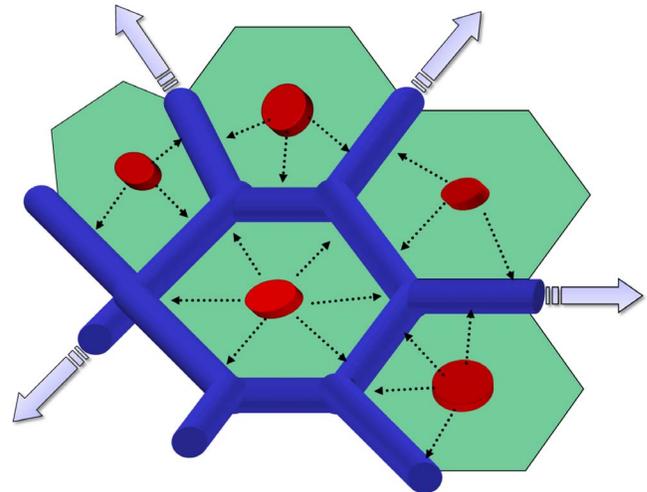


FIGURE 5. Central arteriole theory. The lobule is an independent unit of the circulation. The terminal arteriole at the center feeds the lobule with the blood then draining into the venules at the periphery.

Strength of the study: We have shown that the use of both ICG and NaF dyes provides different images of the same flow through the same structures, and allows important conclusions. The limitation is that only two animals were studied, hence the experiments should be repeated in other primates.

In conclusion, we appear to have answered a long-standing question about the vascular architecture of the choriocapillaris lobules, namely, that the arteriolar supply is from the edges, and that the lobules are organized in a tiled fashion with shared arteriolar edges. Thus, precapillary arterioles appear not to be terminal arterioles but instead form local networks by anastomosis. This should help our understanding of the perfusion of the outer retina in health and disease.

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