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MICROVASCULAR PATTERN OF THE RETINA IN THE JAPANESE MONKEY (*Macaca fuscata fuscata*)

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Abstract

The microvascular architecture of the retina in the Japanese monkey was revealed using scanning electron microscopy of microvascular corrosion casts.

At the optic nerve disc, the central retinal artery radiated to four primary retinal arterioles: the superior and inferior temporal arterioles and superior and inferior nasal arterioles. A dense retinal capillary network showed regional differences in density. The capillary networks observed were divided in four parts: the optic nerve disc, macula, equator, and ora serrata. At the optic nerve disc, the network was a multi-laminar structure containing the radial peripapillary capillaries in parallel with the nerve fibers at the innermost layer. At the macula, the retinal capillaries formed a ring around the central fovea which is an avascular area. At the equatorial part, the network was observed to be double-layered: a superficial capillary network located in the nerve fiber layer and a deep capillary network located at the external nuclear lamina. The former showed a 30-50 μm wide capillary-free zone (CFZ) around arterioles. The latter was undulated in smaller capillaries without the CFZ. At the ora serrata, the network changed to a single layer with coarse ovoid meshes, and the CFZ developed to 50-70 μm in width around arterioles. Hence, the microvasculature of the retina in the Japanese monkey showed regional variations correlating with the fine structure of the retinal layers, e.g., the multi-laminar capillary network for the thick nerve fiber layer at the optic nerve disc part, and the central avascular fovea for the absence of any structure inside the external nuclear lamina. Capillary-free zone occurred since the retinal capillaries were located in the same level as the arterioles.

Key Words: Microvasculature, retina, corrosion casting, Japanese monkey.

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Introduction

The fine vasculature of the retina and the capillary-free zone (CFZ) were first described by His in 1880. Michaelson (1954) demonstrated that the architecture of the adult human retina consisted of the superficial (primary) vasculature within the nerve fiber layer and an anastomosing deeper (secondary) capillary network within the inner nuclear layer, respectively, using India ink injection method. Michaelson (1954) reported that the secondary network ends before it reaches the ora serrata, additionally the third layer was found as the radial peripapillary capillaries (RPC) near the optic nerve head. Three-dimensional approach to elicit the capillary network of the retina was made by Kuwabara and Cogan (1960) utilizing the trypsin-digestion method. Similarly, Shimizu (1971) made microangiographical observations of the retinal capillary architecture. Recently, Ujiie (1976) and Shimizu and Ujiie (1978) examined the retinal microvasculature of the monkey employing plastic microcorrosion casts, and discussed the RPC and CFZ. Seki (1987) also studied the retinal vasculature in the rat using a similar approach. Snodderly *et al.* (1992) investigated neural-vascular relationships in the central retina of macaque monkey and stated that vascularities in the primate retina as seen in the central nervous system showed local variations by reflecting local variant metabolism in addition to the physical elements of the retina. We demonstrate three-dimensional characteristics of the retinal microvascular architecture on corrosion casts, capable to observe from both the vitreous and choroid sides by scanning electron microscopy (SEM), and furthermore evaluate that regional blood flow in the retina may be determined by the regional variations in vascularity.

Materials and Methods

Ten adult Japanese monkeys (*Macaca fuscata fuscata*) supplied by Co-operative Research Fund of the Primate Institute, Kyoto University, Japan, were used for this study. These animals were anesthetized with

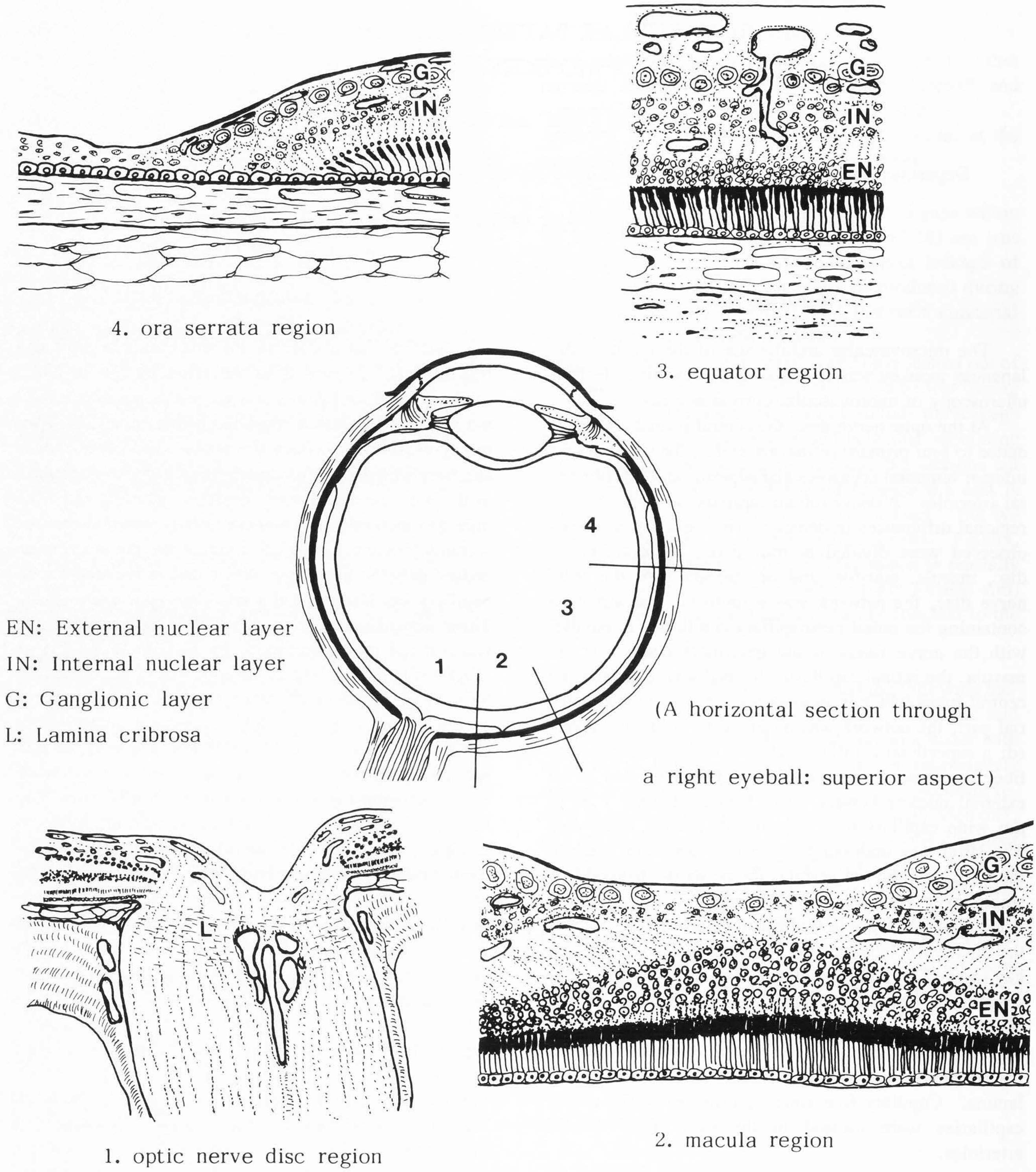


Figure 1. Histological illustrations of four regions of retina. **1.** optic nerve region; multilayered capillary network; **2.** macula region, avascular fovea; **3.** equator region, typically double-layered capillary network; and **4.** ora serrata region, a single network.

sodium pentobarbital (40 mg/kg, body weight). Common carotid arteries were cannulated (metal cannula, 2.0 mm in diameter) and both vertebral arteries were ligated at the level of the 6th cervical vertebra. Thereafter, the carotid system was washed with 80 ml heparinized (5,000 IU/L) isotonic sodium chloride solution at 40°C until the efflux of the internal jugular vein became clear. On both sides, 199 ml oligomer of the methyl methacrylate with 2 grams benzoyl peroxide as a catalyst and 2 ml N,N-dimethylaniline as an accelerator (Ohta *et al.*, 1990) were injected through the common carotid arteries after rinsing the vascular bed (viscosity: 11 centipoise, injection pressure: 100-120 mm Hg). Injected specimens were left at room temperature for 2 hours. The eyeballs were removed under a dissecting microscope and incubated in a water bath at 60°C overnight, and macerated in 5% KOH solution at 40°C for one day with renewing the medium twice. They were rinsed with 5% formic acid for 30 minutes, washed with several changes of distilled water, frozen in the latter, and freeze-dried. The corrosion casts were mounted on specimen stubs, sputter-coated with gold, and examined under a scanning electron microscope (JSM T-300, JEOL) operated at an accelerating voltage of 5 kV.

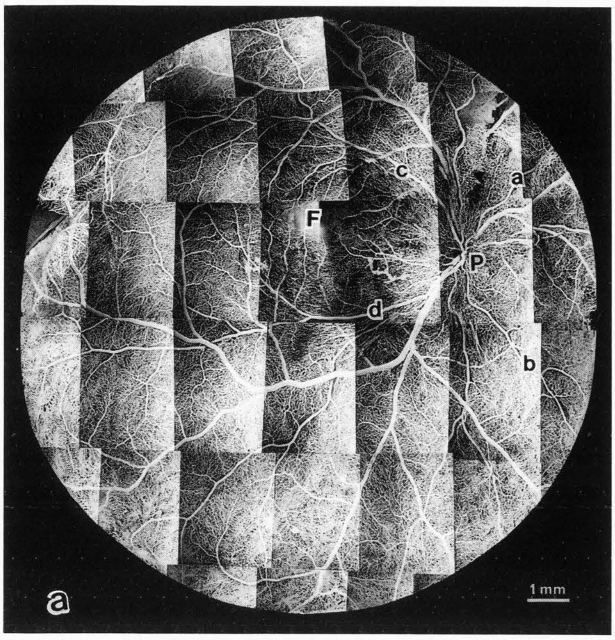
Results

To better describe the results, the retina of the Japanese monkey was divided into four parts: the optic nerve disc, macula, equator, and ora serrata (Fig. 1).

The central retinal artery penetrated the center of the scleral part of the lamina cribrosa toward the optic nerve disc, where it divided into four equal-sized arterioles as its primary branches: the superior and inferior nasal retinal arterioles, and the superior and inferior temporal arterioles (Fig. 2a). Each of these arterioles radiated with the respective satellite venule in the nerve fiber layer. The superior and inferior retinal temporal arterioles gave rise to the superior and inferior macular arterioles, respectively (Fig. 2a). These primary arterioles (80-100 μm in diameter) ran straight, and venules (100-150 μm in diameter) meandered slightly apart from arterioles. Capillary networks expanding between these arterioles and venules were basically composed of two layers: a superficial (vitreous side) network in the nerve fiber layer (Fig. 2a) and a deep (choroid side) network in the internal and external nerve plexiform layers (Fig. 2b).

Optic nerve disc

Radial peripapillary capillaries (RPC) showed a characteristic appearance in the thick nerve fiber layer of the optic disc (Fig. 2a). The RPC of the Japanese monkey, appearing as a C-pattern against the macula, diverged from the superficial capillary network and were



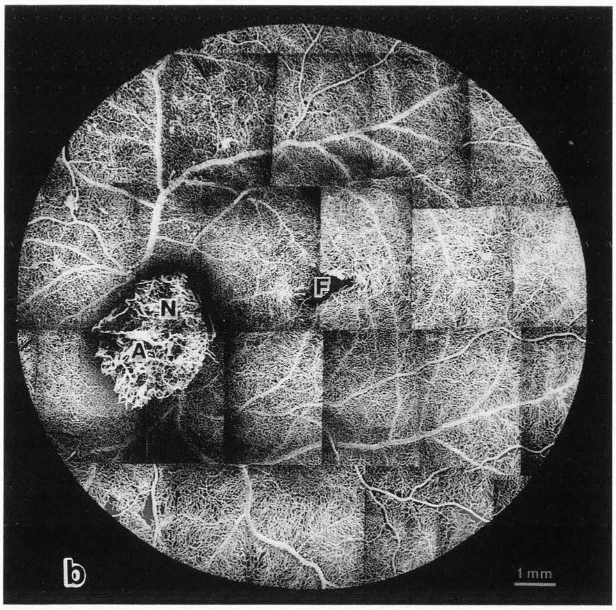


Figure 2a. Anterior (vitreous) view of a corrosion cast of the whole retinal vasculature. At the optic nerve disc (P), the central retinal artery divides into four primary arterioles; superior nasal retinal (a), inferior nasal retinal (b), superior temporal retinal (c), and inferior temporal retinal (d) arterioles. F: Central fovea. **2b**. Posterior (choroid) view of the same specimen. Fine capillary network expands on the choroid side of the retina. F: Central fovea, N: Optic nerve, A: Central retinal artery.

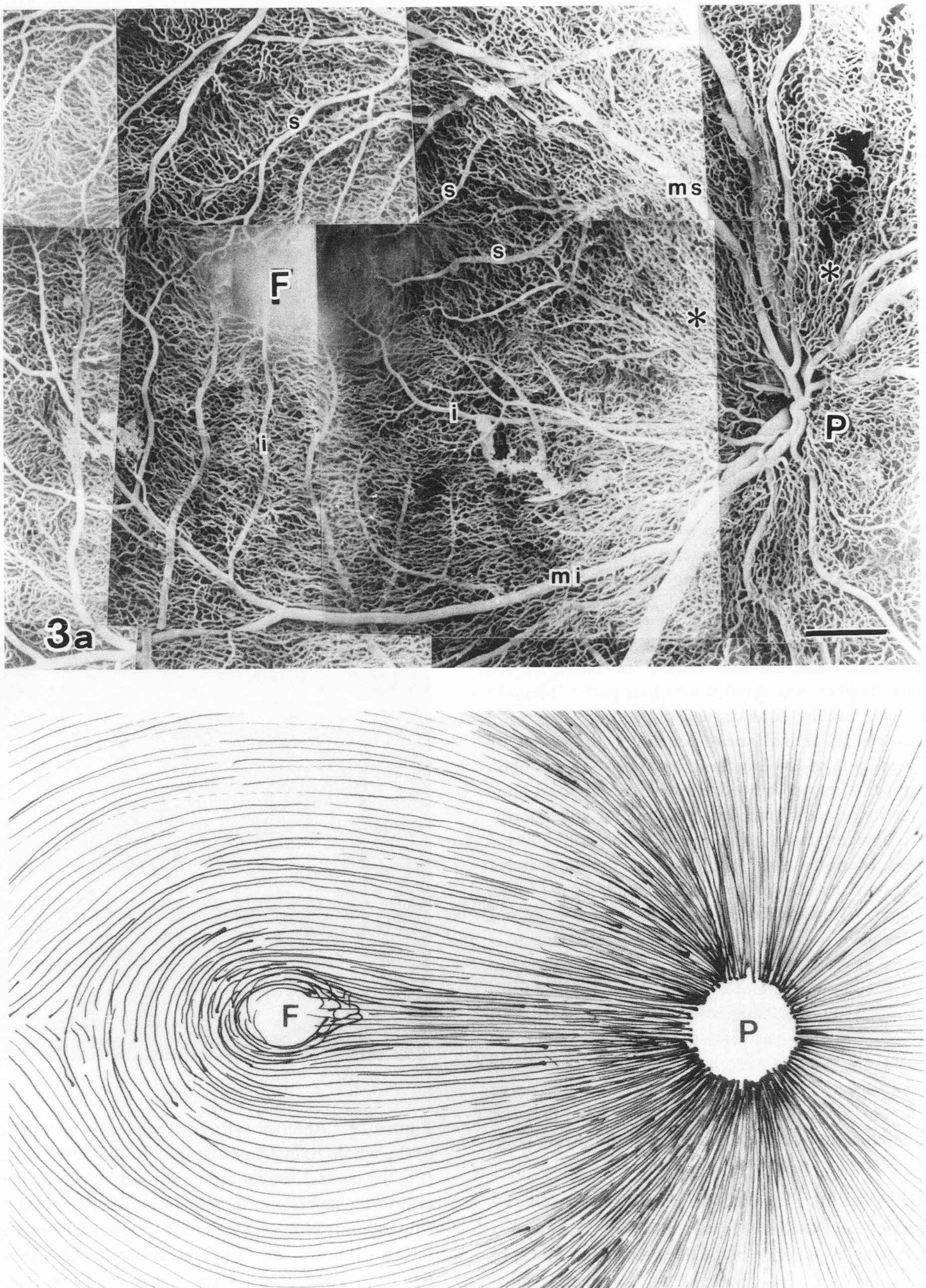

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Figure 3a (on the facing page at left). A close-up of the optical nerve disc (P) and macula areas. **3b**. Drawing of the nerve fibers between these areas. The superior (ms) and inferior (mi) macular arterioles diverged from the superior (s) and inferior (i) temporal retinal arterioles, respectively. The RPC (*) figures C facing to the macula like the pattern of the nerve fiber. The central fovea (F) is observed as an avascular area. Bar = 100 μm.

located superficial to the retinal arterioles and venules (Figs. 3a and 3b). They passed in an arcade for about 200 μm in the nerve fiber layer, becoming multilayered near the optic disc (Fig. 4). The density of the RPC was always in accordance with the density and passage of the nerve fibers between the macula and disc (Figs. 3a and 3b). The optic nerve head was divided into three lamellar regions from anterior to posterior: prelaminar (superficial layer and prelaminar region; Hayreh, 1974), laminar, and retrolaminar regions (Fig. 5a). In the prelaminar region, the capillaries (7-8 μm in diameter) were connected with peripapillary retinal vasculature and the vascular pattern of this region resembled the peripapillary network (Fig. 5b). Capillaries (5-6 μm in diameter) in the laminar region interconnected between the prelaminar and retrolaminar regions and passed straight along the optic nerve through the lamina cribrosa (Fig. 5b). The retrolaminar capillaries (7-8 μm in diameter) formed the network and were derived from tributaries of the pial vessel network which was contributed by branches of the short posterior ciliary arterioles, and centrifugal branches of the central retinal artery supplying the optic nerve (Figs. 5a and 5c). The arterial circle of Zinn was not observed around the optic nerve, but capillary ring at the anterior part of the retrolaminar region was formed by pial capillary network which did not have direct connection to the choroid vasculature (Fig. 5c).

Macula

In this region, the vascular architecture faded toward the central fovea since the nerve fiber layer disappeared up to the internal nerve plexiform layer in the central fovea (Fig. 3a). Around the macula, the superior and inferior macular arterioles gave rise to capillaries, meshworks of which concentrically surrounded the fovea. Also, the superficial capillary layer (on the vitreous side) gradually disappeared close to the fovea. Accordingly, only the deep capillary layer (on the choroid side) surrounded the fovea and formed a capillary ring surrounding an avascular area (Fig. 6a). Blood from the ring drained into four radiating venules (Fig. 6b).

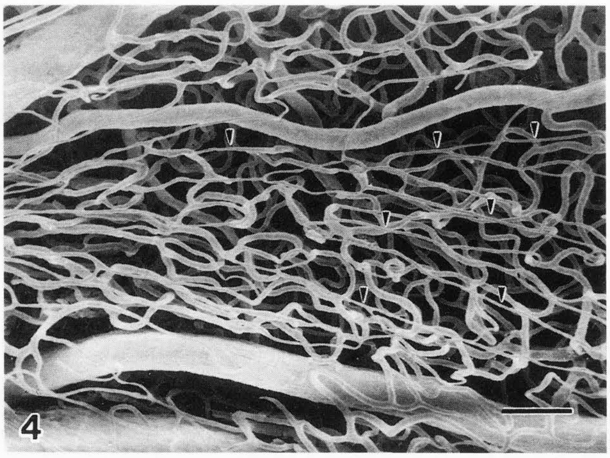


Figure 4. A close-up of the optic nerve disc area. The RPC (arrowhead) arches for about 200 μm distance from and along the passage of the nerve fibers. Bar = 50 μm.

Equator

Typically, double-layered capillary networks extended between the macula and equatorial parts (Figs. 7a and 7b). Capillaries diverging from each arteriole in regular intervals and at right angles to the parent vessel repeatedly branched and anastomosed with adjacent capillaries, to form the superficial capillary network (Fig. 7a). These capillaries were usually large in diameter (6-7 μm) and formed large meshes (about 150 μm) of capillary networks in the nerve fiber layer (Fig. 7a). The deep capillary network was made of fine capillaries with slight tortuosity and did not extend in a plane but undulated in small mesh (about 100 μm) of the capillary network in the internal and external nerve plexiform layers (Fig. 7b). The two capillary networks communicated with each other. Blood from the deep network drained into the venules in the nerve fiber layer. A capillary-free zone (CFZ), of 30-50 μm in width was observed around arteriolar branches, but no CFZ was seen around venules (Figs. 8a and 8b).

Ora serrata

Close to the ora serrata, the internal and external nerve plexiform layers adhered to each other, and the boundary between the nerve cell and fiber layers gradually disappeared. According to these changes, the double-layered capillary networks adhered to form a single network (Figs. 9a and 9b). Capillaries (7 μm in diameter) of this network diverged from the arterial branches at 700-900 μm intervals, and branched repeatedly to form a capillary network (Fig. 9a). Its meshworks were coarse (200-300 μm), spreading in a plane with a wide CFZ (50-70 μm in width) around arterial vessels, but no

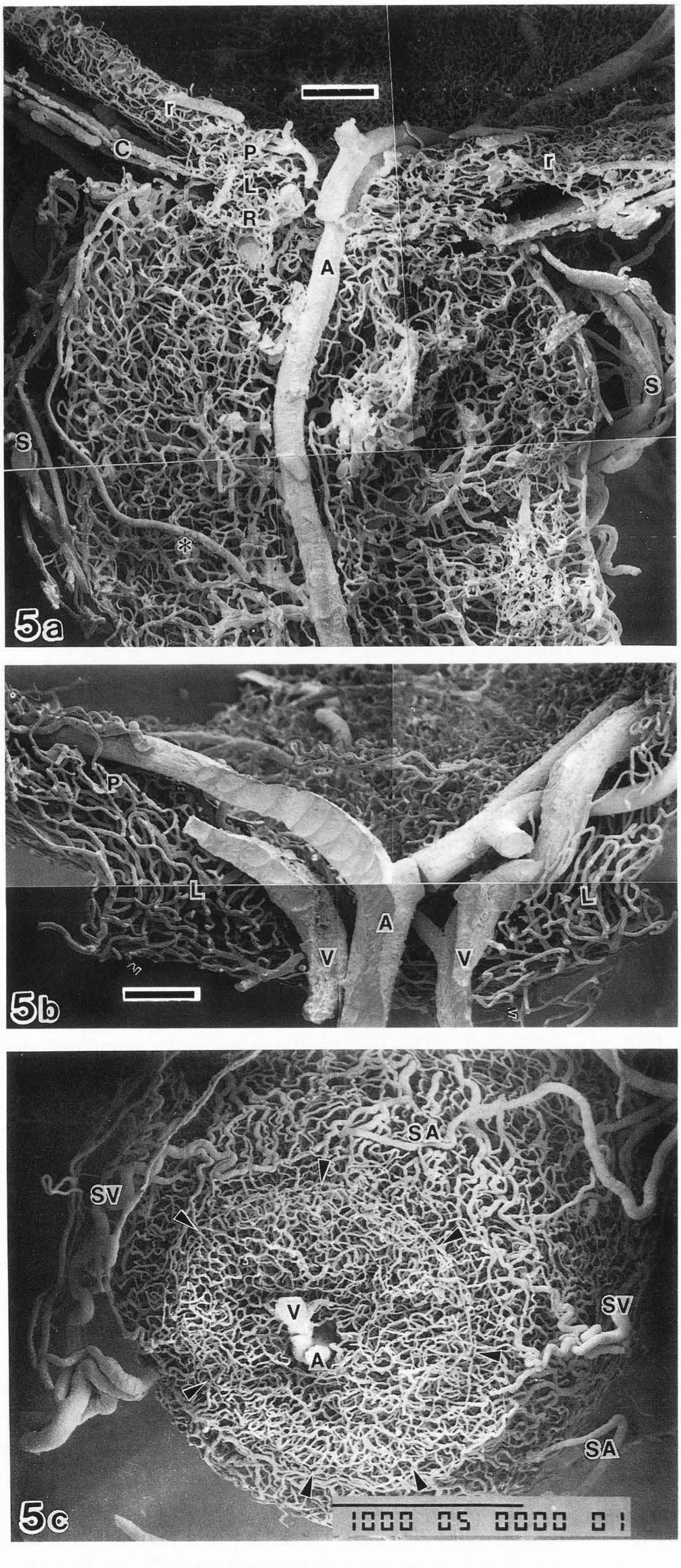
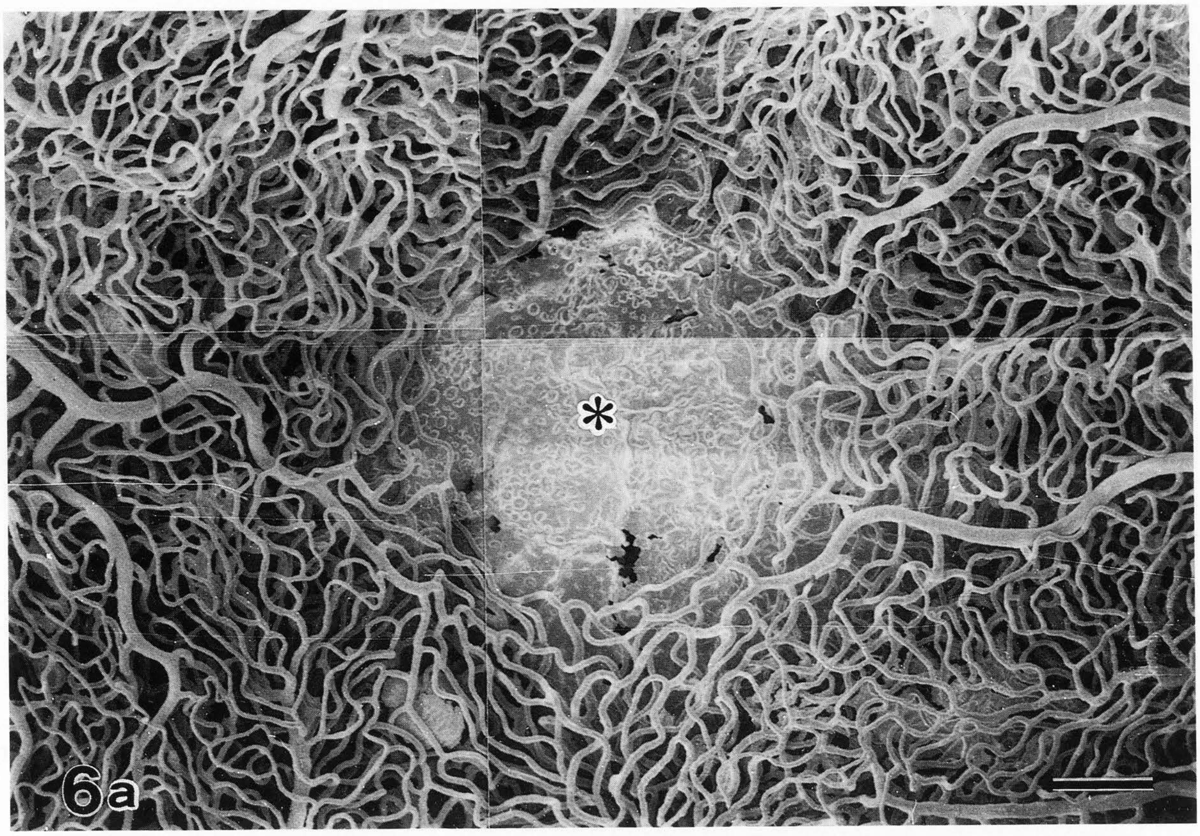


Figure 5 (at left). **a.** A horizontal section through a cast of the optic nerve head. The central retinal artery (A) gives off the centrifugal branch (*) supplying the optic nerve and pia matter, and no branches at the optic nerve head. The optic nerve head is divided into three laminar regions: prelaminar (P), laminar (L) and retrolaminar (R) regions. C. Choroid vasculature, r: Peripapillary retinal vasculature, S: Short posterior ciliary artery. Bar = 200 μm. **5b**. A close-up of the prelaminar and laminar regions, separating from the retrolaminar region. The prelaminar capillaries (P) resemble the peripapillary retinal network. Capillaries in the laminar region (L) pass straight through the lamina cribrosa. A: Central retinal artery, V: Central retinal vein. Bar = 300 μm. **5c**. Anterior (vitreous) view of the retrolaminar region. Capillary ring (arrowheads) at the anterior part of this region is formed by pial capillary network. A: Central retinal artery, SA: Short posterior ciliary artery, SV: Short posterior ciliary vein, V: Central retinal vein. Bar = 1000 μm (1 mm).

Figure 6 (on the facing page at right). An avascular area in the macula area viewed from vitreous (**6a**) and choroid (**6b**) sides. The vascular architecture around the macula fades toward the central fovea superficial capillary. Only the deep capillary network forms a capillary ring surrounding the avascular area (*). Blood from the ring drains into four radiating venules (V). Bars = 50 μm.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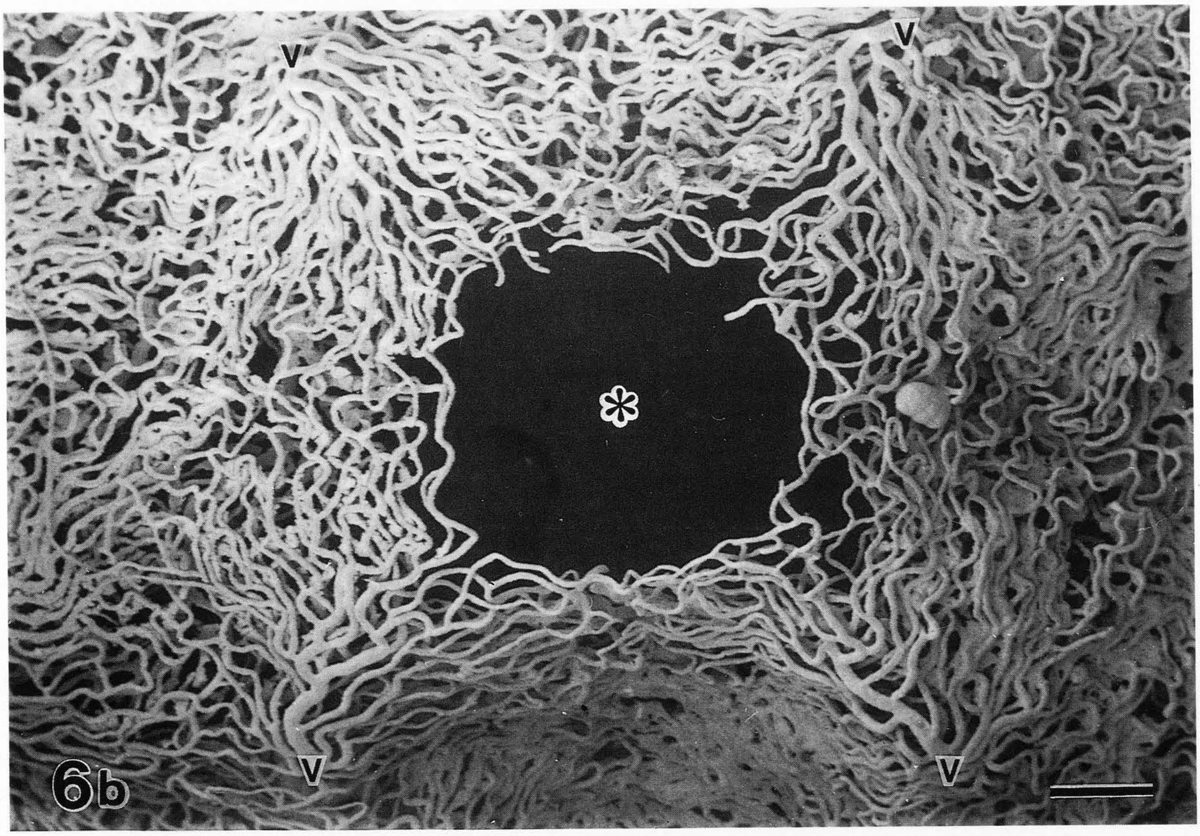
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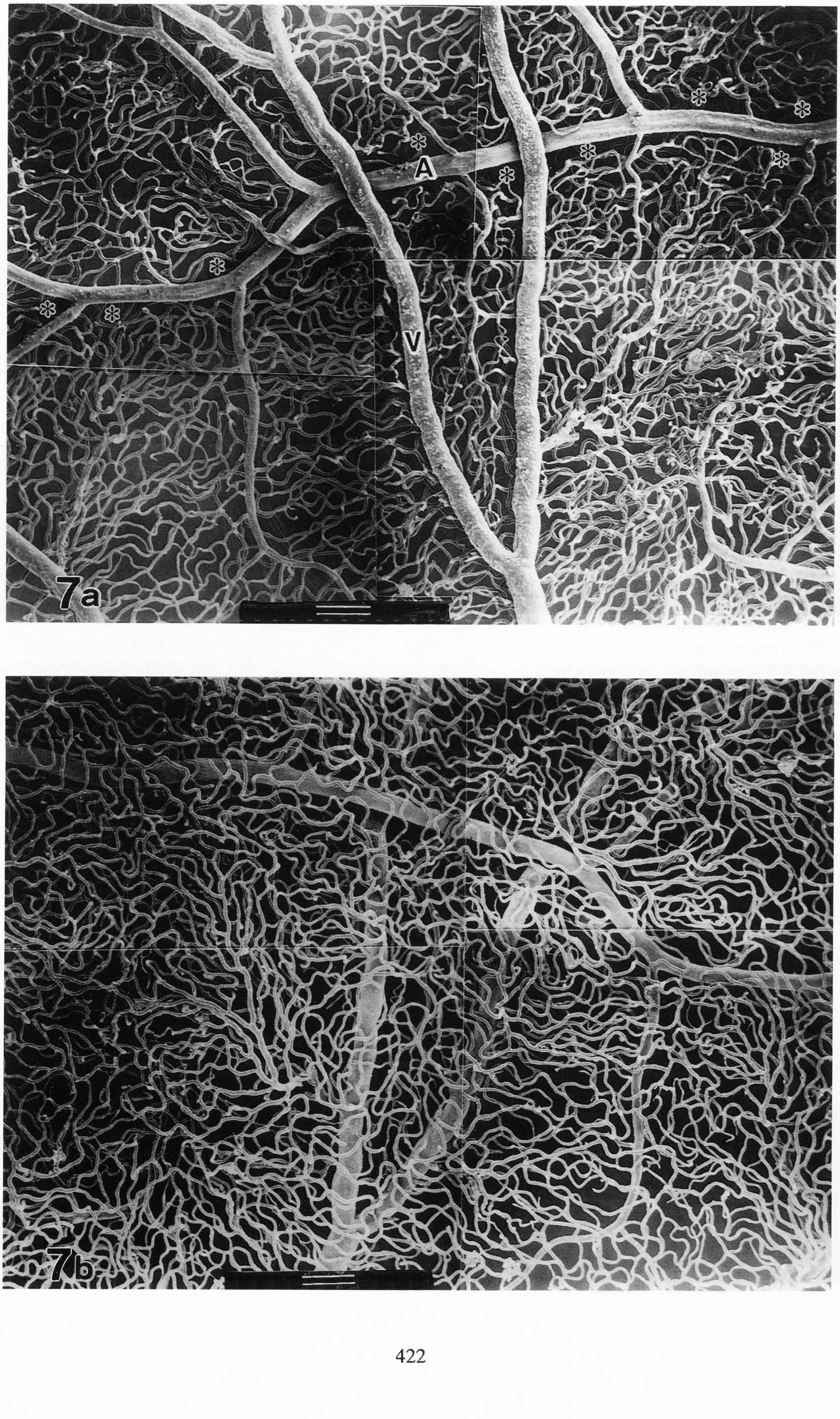
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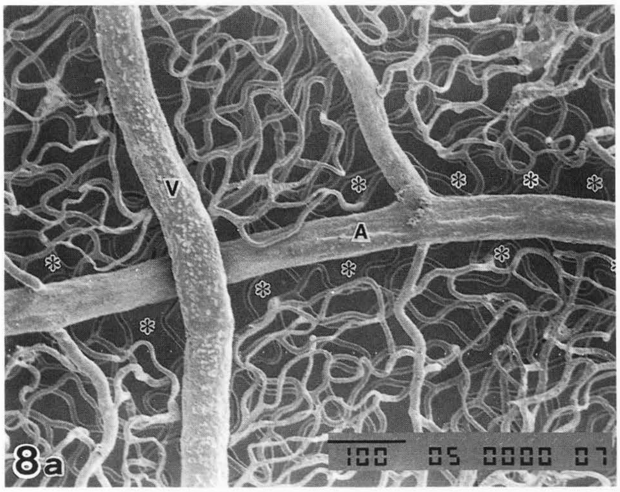

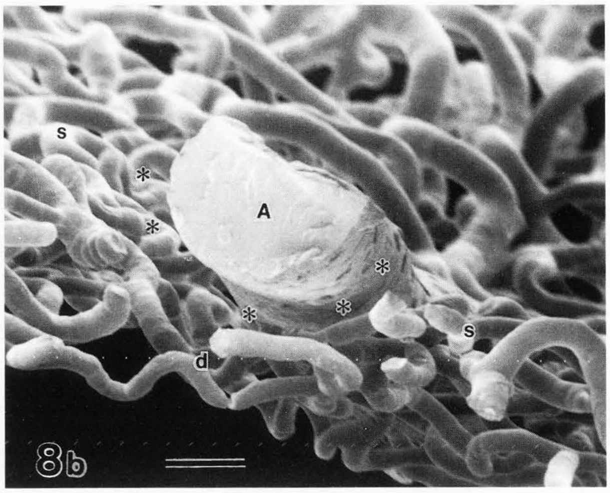

Figure 7 (on the facing page at left). Double-layered capillary networks viewed from vitreous (**7a**) and choroid (**7b**) sides. Superficial capillaries form large meshworks and the deep capillary network is undulated in small meshwork. *: Capillary free zone. Bars = 100 μm.

Figure 8a. A close-up of CFZ in Fig. 7a. Bar = 100 μm. **8b**. Cross-section around the arteriole. Bar = 30 μm. A CFZ (*) is observed around the arteriole (A), but there is no CFZ around the venule (V). s: Superficial capillary network; d: Deep capillary network.

CFZ was present around venous vessels (Figs. 9a and 9b). Blood from the capillaries drained into venules passing on the choroid side (Fig. 9b). Meshworks around the ora serrata were more coarse (300-500 μm) and elongated in the meridian direction, with capillary loops along the ora serrata (Fig. 10).

Discussion

Microvascular architecture of the retina has been examined by different methods, such as the India ink injection, trypsin digestion, fluorescent microradiography, microangiography, and serial histological reconstruction. However, it has been difficult to make three-dimensional observations on both the vitreous and the choroid sides or separating each other in one specimen. Microvascular corrosion casting is most helpful to three-dimensionally examine the retinal vascular architecture at both the vitreous (internal) and choroid (external) sides of the retina, so that regional variations of the retinal capillary network in the both layers were well demonstrated in this paper.

Optic nerve disc

Since the existence of the radial peripapillary capillaries (RPC) was indicated by Michaelson (1954), differences on RPC between animal species have been investigated. Henkind (1967) made a comprehensive survey of past studies on the RPC and designated its differential diagnosis. However, the RPC may not be a spherical vasculature, as reported by Ujiie (1976) and Shimizu and Ujiie (1978), but a kind of satellite blood vessels of the nerve fiber. Nerve fibers of the optic nerve disc part become thicker close to the optic disc, so that capillary networks in it become multilayered proportionally. Morphological variations between animal species are determined by passages and density of the nerve fibers. Beside, the passage is remarkably influenced by the presence and form of the optic nerve disc.

On the vasculature of the optic-nerve head, there have been many discrepancy in investigators' opinions on correlations between the scleral vasculature and circulation in the optic nerve and between the peripapillary retinal vasculature around the disc and the capillary network of the optic-nerve head (**human**: Awai, 1985; Francois and Neetens, 1965; Fryczkowski *et al.*, 1984; Olver *et al.*, 1990; Zhao and Li, 1987; **rabbit**: Prince *et al.*, 1960; Prince and McConnell, 1964; Ruskell, 1964; Sugiyama *et al.*, 1992; **rat**: Pannarale *et al.*, 1991; Seki, 1987; Yoshimoto *et al.*, 1980; **cat**: Risco and Nopanitaya, 1980; Wong and Macri, 1964). As described by Anderson (1970), Anderson and Braverman (1976), Ernest and Potts (1968), Weiter and Ernest (1974), Lieberman *et al.* (1976), and Wybar (1956), an arterial circle of Zinn is neither observed in the primate, nor ramifications to the optic nerve from the scleral vasculature as shown by Araki (1976) and Risco *et al.* (1981). Correlations between the capillary networks

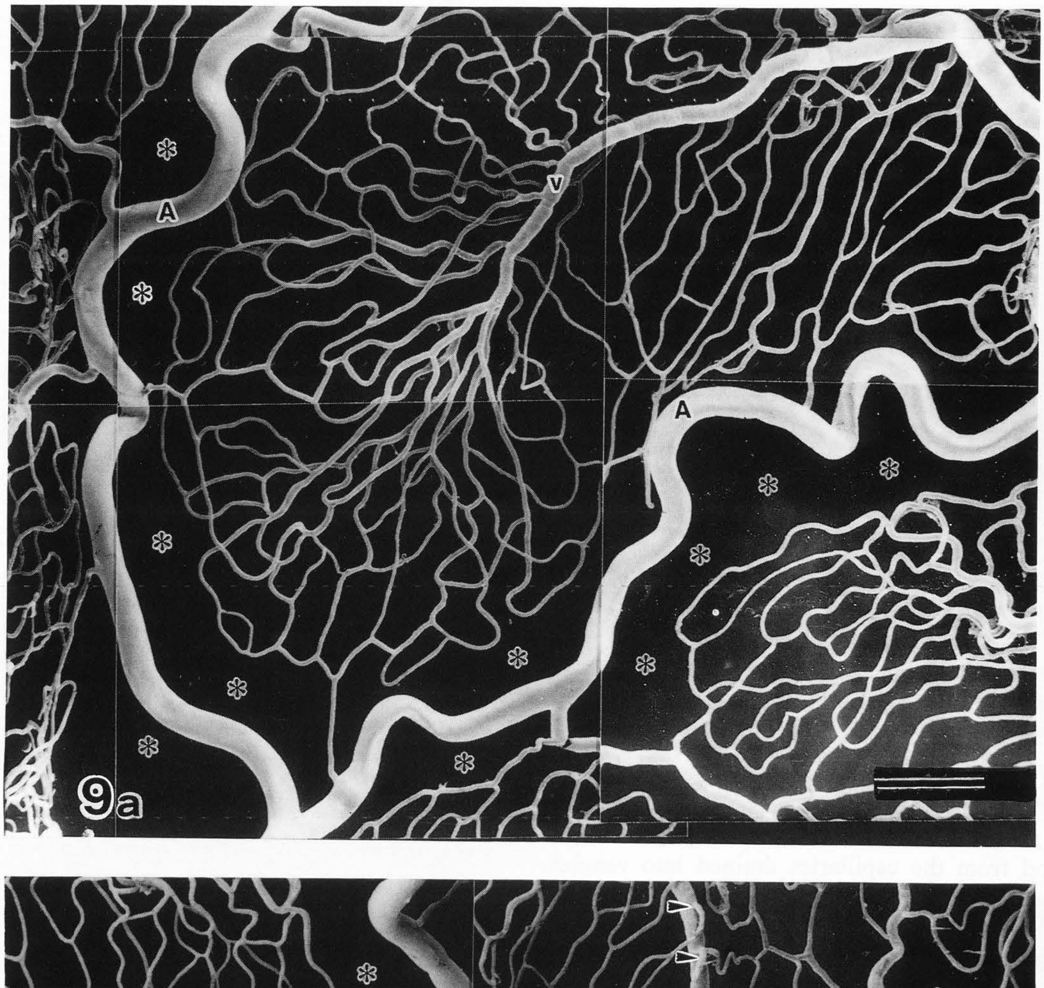
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Figure 10 (at right). Scanning electron micrograph of the ora serrata part. Meshwork around the ora serrata is longitudinally elongated in the meridian direction, with capillary loops along the ora serrata (arrowheads). Bar = 80 μm.

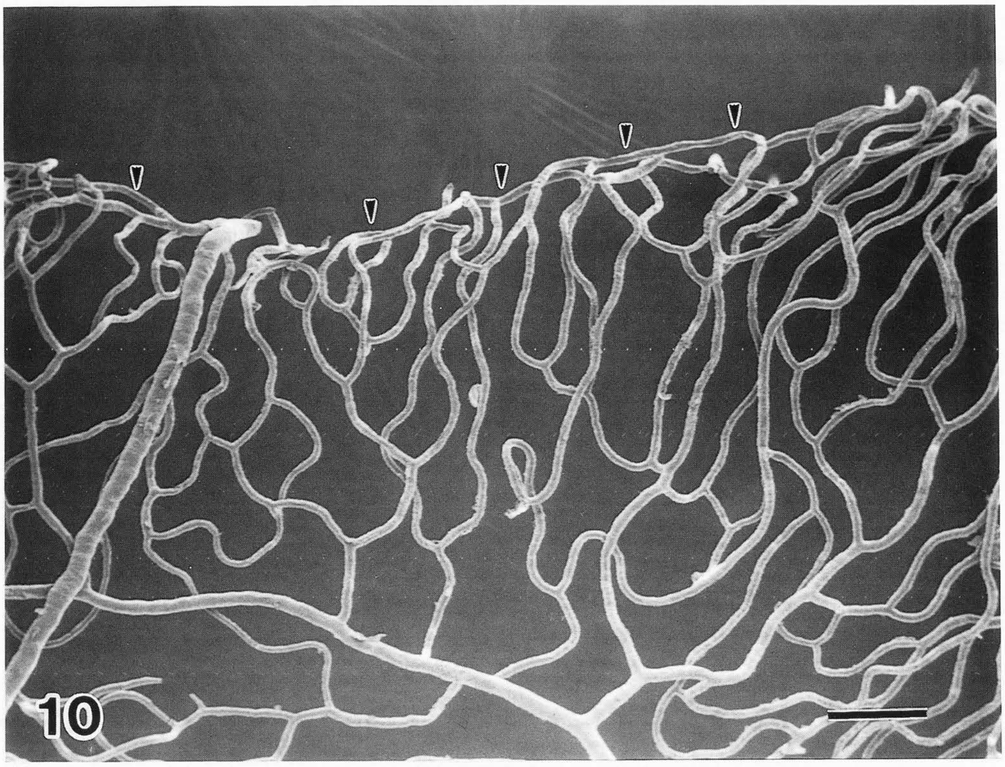

Figure 9 (on the facing page at left). A single capillary network of the same specimen (as shown in Fig. 8) viewed from vitreous (**9a**) and choroid (**9b**) sides. A single capillary network is formed by the superficial capillary network according to a disappearance of the deep network. Its meshwork is coarse, spreading in a plane with a wide CFZ (*) around arterial vessels (A), but CFZ does not occur around venous vessels (V). Blood from the capillaries drains into venules (V) passing in the choroid side (arrowheads). Bars = 100 μm.

around the disc and the network of the optic-nerve head were observed as a communication with each other by Lieberman *et al.* (1976) and Ojima (1977), although this was denied by Ernest and Potts (1968) and Araki (1976). In this paper, an apparent demarcation was recognized between the capillary network around the disc and the capillary of the optic nerve in the Japanese monkey by demonstrating straight capillaries in the laminar region of the optic nerve head, connecting with each other through the lamina cribrosa. Also, a capillary ring at the anterior part of the retrolaminar region is observed in this study. This ring is formed by pial capillary network which has no direct connection to the choroid vasculature, but both the pial network and choroid vasculature are derived from the short posterior ciliary arteries.

Macula

In this region, cell layers have disappeared from the superficial side, and the vascular architecture has also disappeared in the direction of an avascular part. According to these changes, a capillary ring surrounding the central fovea is constructed by capillaries of the deep layer without the presence of the CFZ. These capillaries appear in a concentric circular arrangement which is quite different from meshworks in other parts.

Equator

Since the CFZ was reported by His (1880), it has been understood that capillaries do not surround each arteriolar branch. However, Toussaint and Kuwabara (1961) affirmed their presence without supplying any evidence. Ujiie (1976) observed the retinal capillary architecture beneath the CFZ by microcorrosion casts. We have discussed the formation of the CFZ around the arteriole and its disappearance around the venule. Capillaries diverging from the arteriole at equal intervals always form a superficial capillary network and show an avascular boundary belt from arterioles in the same level as their network, namely, on the vitreous side. In contrast, the venule always receives blood from the deep side, namely, the choroid side. The superficial capillary network is distributed in areas around the venule. Viewed in terms of venular branches from the vitreous side, the CFZ may not appear around the venule. When the arterioles and venules were scanned from both the vitreous and choroid sides, this difference was evident.

Ora serrata

Cell layers decrease in number and disappear toward anterior direction from the rod and cone lamina (choroid side) to the nerve fiber layer (vitreous side), reversing the order in the macular part. Linked with this change, the vascular architecture of the deep network begins to disappear and the original double layers change to a single layer in the shape of a coarse meshwork. In the original, single-layered capillary network in the ora serrata part, the CFZ is obviously observed as a wider zone. The capillary network in the optic nerve region of the retina does not communicate with that of the ciliary body part, and capillary loops are directed toward the margin of the ora serrata in a regular form.

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Discussion with Reviewers

S. Aharinejad: You describe four segments of the retina with different vascular patterns, some being more densely vascularized, the other less. What is the reason for this difference? Can the metabolism of different areas cause different vascular density?
Authors: Yes. Regional variations in vascularity and vascular patterns reflect the regional variations in metabolism, rather than thickness of the retina.

S. Aharinejad: Have you measured the thickness of the retina in different areas you investigated?
Authors: Retinal thicknesses ranging from the nerve fiber layer to the light receptor cells are about 500 μm near the optic nerve disc, 300-400 μm at the equator region, and 100 μm near the ora serrata.

S. Aharinejad: Is there a difference between capillary diameters in the optic nerve disc and optic nerve fiber?
Authors: Capillary diameters in the optic nerve disc and optic nerve fiber are: 7-8 μm in the peripapillary retinal region, 7-8 μm in the prelaminar region, 5-6 μm in the laminar region, 7-8 μm in the retrolaminar region and 8-10 μm in the optic nerve. Capillaries in the laminar region are the narrowest, therefore, an ischemic ophthalmic disease easily occurs in this region.

J. Morrison: Can you comment from your observations on the artery of origin of capillaries in the lamina cribrosa portion of the optic nerve head as opposed to the nerve fiber layer region?
Authors: Capillaries in the lamina cribrosa portion of the optic nerve head interconnect between the retrolaminar region continuing with the optic nerve vasculature and the prelaminar region with peripapillary retinal vasculature. Therefore, their arterial origins are the central retinal artery and the short posterior ciliary arteries.

J. Morrison: Do you find any evidence for choroidal vascular supply to the optic nerve head?
Authors: The optic nerve head vasculature does not have direct connections with the choroid vasculature, but both vasculatures are derived from the short posterior ciliary arteries.