FULL PAPER *Anatomy*

# Microvascular Anatomy of the Pig Eye: Scanning Electron Microscopy of Vascular Corrosion Casts

Hiroyoshi NINOMIYA[1)] and Tomo INOMATA[1)]

[1)]*Department of Laboratory Animal Science, Azabu University, 1–17–71 Fuchinobe、Sagamihara 229–8501, Japan*

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ABSTRACT. The microvasculature of the eye of 10 pigs was investigated using scanning electron micrographs of corrosion casts. The ciliary body, iris and bulbar conjunctiva were supplied by the iridociliary ring artery via the long posterior ciliary artery. Capillaries of the ciliary process were of large diameter (23.2–27.5 $\mu$m) with an irregular bore, forming a thoroughfare channel draining blood in the ciliary arterioles into the pars plana venous vessels. Arterioles and venules in the iris exhibited a zigzag or spiral features. The third palpebra was supplied by the anterior ciliary artery. The capillary bed of the third palpebra was dense and was formed by many rows of fine hair-pin loops. Capillaries in the bulbar conjunctiva formed a sparse network disposing approximately parallel to the epithelium and formed a well-developed venous plexus, draining into the vortex veins. Retinal arterioles formed a slender and long course to capillaries. Retinal capillaries were extremely thin (3.0–4.0 $\mu$m in diameter). The choroid was supplied by the short posterior ciliary arteries. Choroidal arterioles exhibited a thick and short course to the choriocapillaris. The choriocapillaris was flat and sinusoid-like (8.9–13.9 $\mu$m in diameter), forming a dense sheet-like network. Blood from the choroid emptied into the episcleral vein via the vortex vein. Blood from the retina was drained by the posterior ciliary veins. The functional significance of this vascular architecture was discussed.
KEY WORDS: corrosion casts, eye, microvasculature, SEM, swine.

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The porcine eye is a preferable animal model for investigation regarding ophthalmology [20]. Various characteristics of porcine retinal vessels, which are similar or dissimilar to the human retinal vasculature, are reported [20, 21]. Additionally, the postmortem pig eye has been widely used for teaching medical or veterinary students and for training ophthalmology residents. Simoens *et al.* reported the vasculature of the porcine retina using the corrosion cast technique and histology [21]. Morrison *et al.*, who studied the ciliary body microvasculature of 8 mammalian species, reported marked interspecies variation in vasculature among the mammalian eyes, and stressed that species differences should be considered in the interpretation of the mechanism of blood flow under normal as well as pathological conditions of the eye [12]. A detailed knowledge of the microvascular system and drainage of the eye may lead to an understanding of the possible role of the vascular system in disease of the eye and contribute to the foundation for teaching ophthalmology.

Various physiological monitoring techniques involving fluorescein angiography and/or infrared indocyanin green angiography have been employed for the study of the ocular circulation. However, these techniques lack the resolution at the microscopic level to show which part of the blood vessels plays a more important role in the ocular circulation. To answer the question we have employed the scanning electron microscopy (SEM) study of microcorrosion casts. This technique provides a three-dimensional view of the vascular bed along the entire thickness of the eye. Simoens *et al.* described the vasculature of the porcine retina with scanning electron micrographs, but did not show the vasculature of the anterior segment of the eye [21]. The purpose of the study reported here was to re-evaluate the description by Simoens *et al.* and to document the microvascular architecture of the choroid and the anterior segments of the porcine eye that they did not trace or view.

## MATERIALS AND METHODS

Ten pigs of a Yorkshire breed (12–15 kg in weight, young, male) were used for this study, they were anesthetized by barbiturates and the carotid artery was opened to bleed. The jugular vein was opened, a catheter was introduced in the carotid artery and then physiological saline solution warmed to 37°C was perfused via this catheter with a syringe under manual pressure. Perfusion was continued until the saline solution emerging from the jugular vein was completely cleared of blood. The orbital blood vessels were manually injected with approximately 30 m*l* of a combination methylmethacrylate monomer and Mercox (Dainippon Ink & Chemical Co., Ltd., Tokyo, Japan) (ratio in volume = 7:3). The injection pressure was 100 to 120 mmHg. After polymerization, the eye was macerated for 2–3 days by repeated baths in a 20 % NaOH solution at 50°C, and samples were then rinsed in distilled water. Under a dissecting microscope, each part of the eye was isolated, mounted on an aluminum stub, sputtered with gold in an ion- coater (IB-3, Eiko Engineering, Co., Ltd., Ibaraki, Japan) and examined under a scanning electron microscope (ABT-32, Topcon Co., Ltd., Tokyo, Japan). Animals were treated in accordance with Azabu University Animal Care and Use Guidelines. The animal facility at Azabu University is accredited by the Office for Protection from Research Risks (OPRR) (#A5393-01), U.S.A.

## RESULTS

The eyeball of the pig received most of its blood supply from the long posterior ciliary artery and short posterior ciliary artery and the chorioretinal artery. The topographical situation of main vessels is summarized in Fig. 1.

*Vasculature of the anterior part of the eyeball*: The ciliary process was supplied by the iridociliary ring artery, which originated from the long posterior ciliary arteries. The iridociliary artery traveled about halfway between the iris and the lateral border of the eyeball, where they gave rise to the ciliary process arterioles and capillary beds (Figs. 1 and 2). Microvessels in the ciliary process formed radially arranged closely packed plates of vessels (Fig. 3). Microvessels in the ciliary process consisted of the marginal capillaries, the intraprocess capillaries and the collecting venules (Fig. 4). The marginal capillaries exhibited an irregular bore with alternating expansions and were somewhat conglomerating and large in diameter (23.2–27.5 $\mu$m). They connected the ciliary process arterioles with the collecting venules directly, forming a 'thoroughfare channel'. The intraprocess capillaries were fed by the ciliary process arterioles and the marginal capillaries. Some of these capillaries connected the ciliary process adjacent to each other (Fig. 4, arrows). These capillaries drained into the collecting venules and exhibited a less undulation and a smaller diameter (7.2–11.6 $\mu$m). The ciliary collecting venules emptied into the pars plana venules (Figs. 1 and 3, double arrows). They then ran posteriorly, forming a well developed venous plexus which freely anastomosed with those of the anterior border of the choroidal veins. The pars plana venules then drained into the vortex veins via the choroidal veins (Fig. 3, arrow).

The blood supply of the iris arose primarily from the iridociliary ring artery which was augmented by the anterior ciliary arteries. Branches of this circular artery extended toward the pupil to supply the radially oriented capillary net of the iris. They were characterized by a zigzag or spiral feature, and formed a two-dimensional vascular net (67.3–77.7 $\mu$m in diameter) in the iris (Figs. 2 and 5). The venules also followed an undulating course and emptied into the vortex veins via the pars plana venules. Capillaries beneath the iridal epithelium formed a fine meshwork around the pupil and covered the iridal vessels (Fig. 5).

The third palpebra and bulbar conjunctiva were supplied by the anterior ciliary artery and partially by the long posterior ciliary artery. The capillaries in the palpebra formed a compact capillary bed with hair-pin loops (Fig. 6). The capillaries in the bulbar conjunctiva formed one layered relatively coarse capillary bed, which were approximately parallel and close to the basement membrane of the epithelium. Venules draining the palpebral and conjunctival capillaries were disposed deeper in the stroma and form a well developed venous plexus underneath the capillary network (Fig. 7). Then they were drained by the vortex veins.

*Vasculature of the posterior part of the eyeball*: The blood supply to the laminar optic nerve was derived from

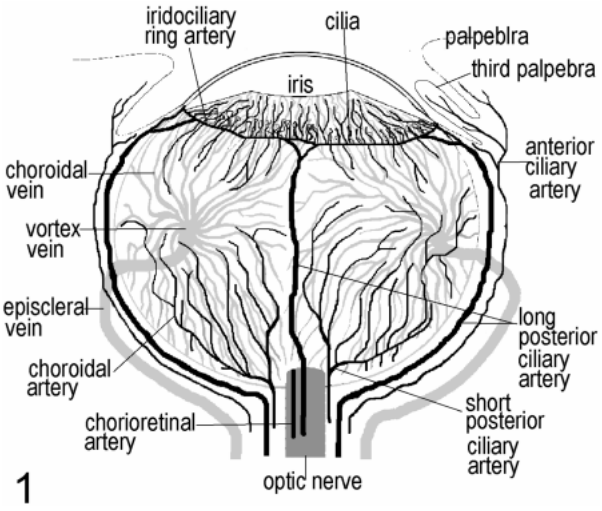


Fig. 1. Diagrammatic representation of bulbar vessels of the swine eye.

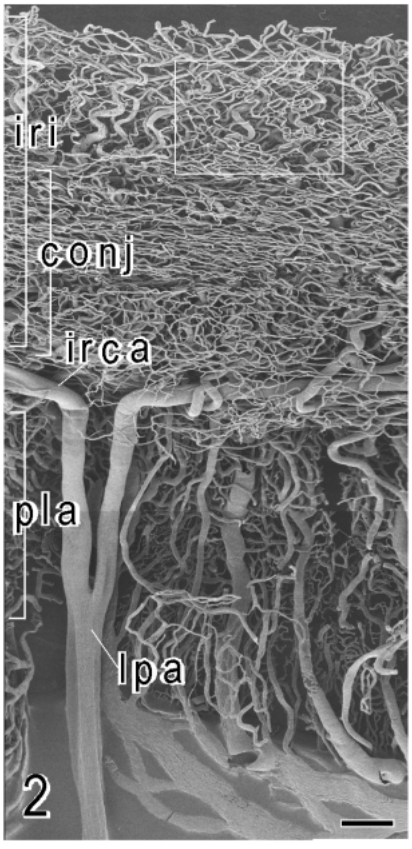


Fig. 2. Montage of scanning electron micrograph of the iris (iri). Viewed from outside the eye. conj: bulbar conjunctiva, irca: iridociliary ring artery, lpa: long posterior ciliary artery, pla: pars plana. Bar=285 $\mu$m.

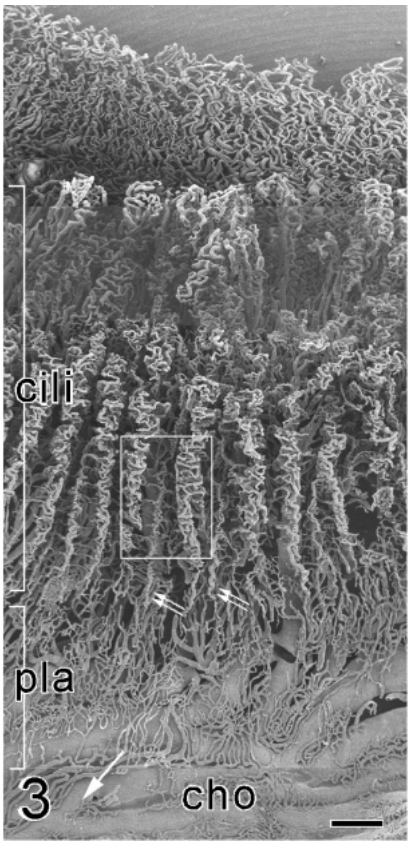


Fig. 3. Montage of scanning electron micrograph of vasculature of the ciliary process. Note the radially arranged capillaries and collecting venules (double arrows) merging into the venules in the pars plana (pla). Viewed from inside the eye. The vortex vein (arrow) is seen through the choriocapillaris (cho). cili: cilia. Ba =285 $\mu$m.

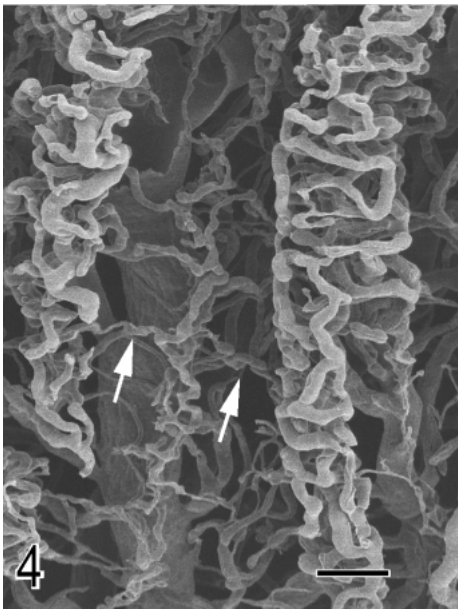


Fig. 4. Enlargement of area outlined in Fig. 3 showing thick marginal capillaries of the ciliary process. Arrows show interprocess vascular connections.Bar=100 $\mu$m.

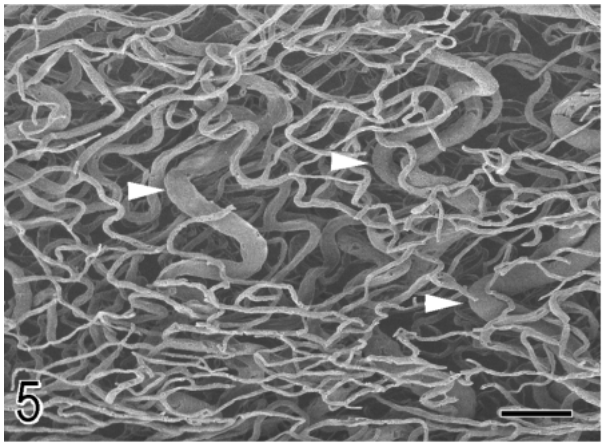


Fig. 5. Enlargement of area outlined in Fig. 2 showing the zigzagging iridal arteries (arrow heads) and fine capillary network covering the arteries. Bar=100 $\mu$m.

the chorioretinal artery and longitudinal pial vessels. Connections between some optic nerve capillaries and choriocapillaris were not found in the porcine eye. The venules from the optic nerve drained into the posterior ciliary vein.

The retinal vasculature of the pig has been described in detail by Simoens *et al.* [24], we have little to add. The blood supply of the retina arose from the chorioretinal artery. This artery divided into four or five and enters the retina at the periphery of the optic disc. In the retina the retinal arteries pursued a wavy course to the base of the ciliary body. The major branches, which had a luminal diameter of up to 60.5–65.6 $\mu$m, ramified into smaller arterioles by means of side-branches at mostly right angles to the parent artery. The precapillary arterioles (6.7–14.5 $\mu$m in diameter) took a relatively long course and then expanded into capillaries. At their branching site retinal precapillary arterioles showed luminal constrictions, known as "intra-arterial cushions", which might control blood flow at the branching sites (Fig. 8, arrow). Periarteriolar capillary-free zones were not avascular but exhibited sparse capillaries in the porcine eye (Fig. 8). The retinal capillaries were quite thin (3.0–4.0 $\mu$m in diameter) and formed a thin layer of capillary net in the superficial region (vitreous side) of the retina. They drained into the postcapillary venules, which extended into a venous network just under the retinal capillary network. The venules then went up to drain into larger veins, which merged into the posterior ciliary vein via the retinal vein near the optic disc.

The short posterior ciliary arteries formed branches as the

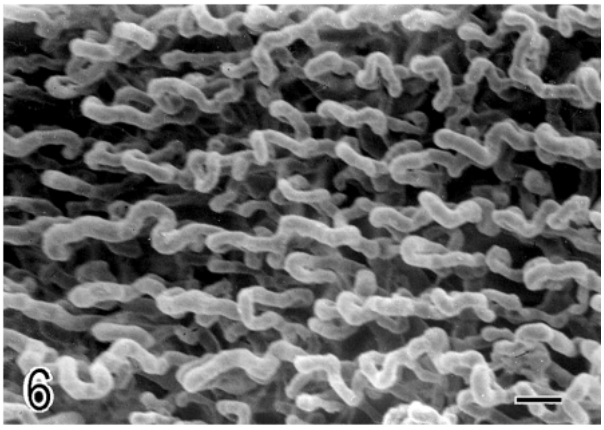


Fig. 6. Capillaries in the third palpebra. Note the dense capillary bed with hair-pin loops just under the epithelium. Bar=3.3 $\mu$m.

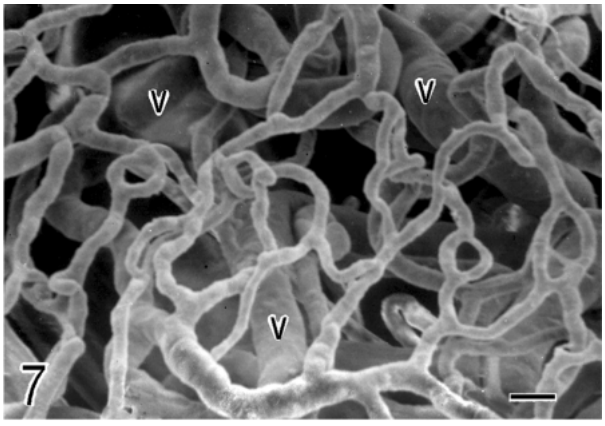


Fig. 7. Capillaries in the bulbar conjunctiva. Note the relatively sparse capillary network and the well developed venous network (v). Bar=33.3 $\mu$m.

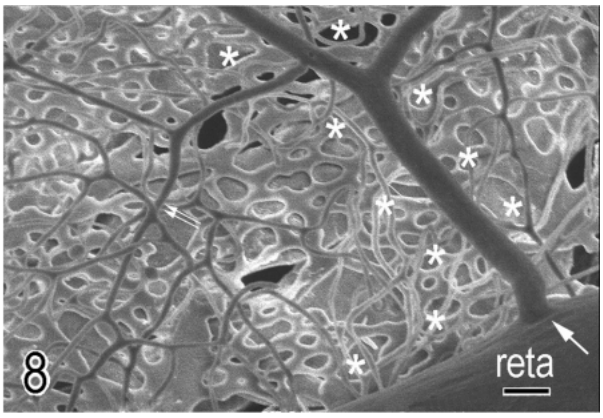


Fig. 8. A retinal artery (reta) with thin and long precapillary arterioles (double arrow). An intra-arterial cushion is clearly seen (arrow). The periarteriolar capillary-free zone is not avascular but with a rather sparse network (asterisks). Background is the choriocapillaris. Bar=100 $\mu$m.

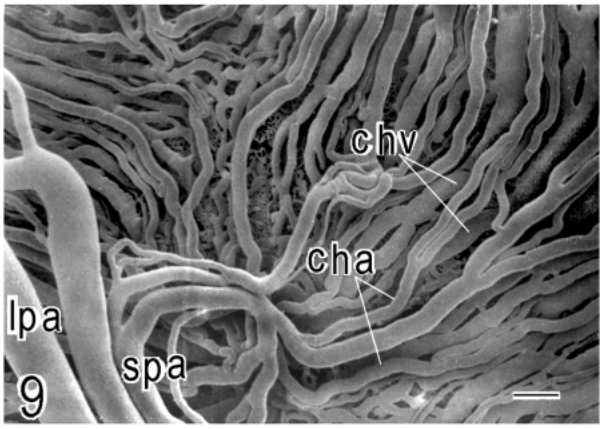


Fig. 9. Choroidal vasculature (scleral view) showing choroidal arteries (cha) branching from the short posterior ciliary artery (spa). Choroidal veins (chv) running parallel to the arteries are seen. lpa: long posterior ciliary artery. Bar=277 $\mu$m.

choroidal arteries, which ran toward the anterior eye segments and supply the entire choroid. All choroidal arteries to the choriocapillaris were relatively flattened and ran in parallel arrays so as to interdigitate with choroidal veins draining this region (Fig. 9). The arteries ramified two or three times into precapillary arterioles. The choroidal precapillary arterioles were large in diameter (19.2–22.2 $\mu$m) and were characterized by a very short course to the choriocapillaris (Fig. 10), which was contrary to the arterioles in the retina. The capillaries of the choroid were somewhat flattened, sinusoid-like with a luminal diameter of 8.9–13.9 $\mu$m, and formed a very dense, freely anastomosing single layer capillary bed in the choroid (Fig. 11). As the choroidal capillary bed continued anteriorly along the inner wall of the eyeball, it became increasingly less dense and finally joins with vessels of the ciliary body at the ora serrata. Intra-arterial cushions were observed frequently in the precapillary arterioles in the choroid as well as in the retina. With the corresponding arterioles, the venules gathering the choriocapillaris also showed a very short course. These venules then became larger venules with a diameter of about 40 $\mu$m. The choroidal veins coursed anteriorly, ran parallel to arteries and followed the curvature of the eyeball in the choroid layer, then coalesced into the vortex. In some cases, small tributaries of the posterior ciliary vein drained a limited region of the choroid around the optic nerve.

*Venous drainage of the eyeball*: The venous blood from the entire choroid and the anterior segments of the eyeball drained into the vortex veins. There were four vortex veins, located obliquely on the dorsal, ventral, nasal, and temporal side near the equator between the horizontal and ventrical meridians of the eyeball. The vortex veins were broad and significantly flattened. They emptied into the episcleral veins following the curvature of the eyeball in the orbital fossa. The posterior ciliary vein of the pig was a small vein, unlike rats, draining blood only from the retina.

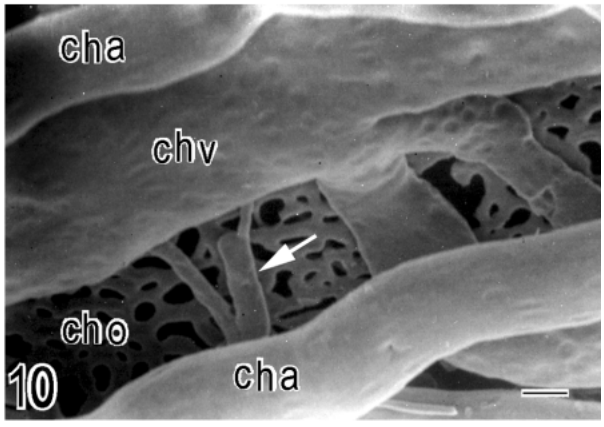


Fig. 10. Choroidal arteries and veins. Viewed from scleral side. Note the thick and short precapillary arterioles (arrow). Compare to the retinal arterioles in Fig. 8. cha: choroidal artery, chv: choroidal vein, cho: choriocapillaris. Bar=33.3 $\mu$m.

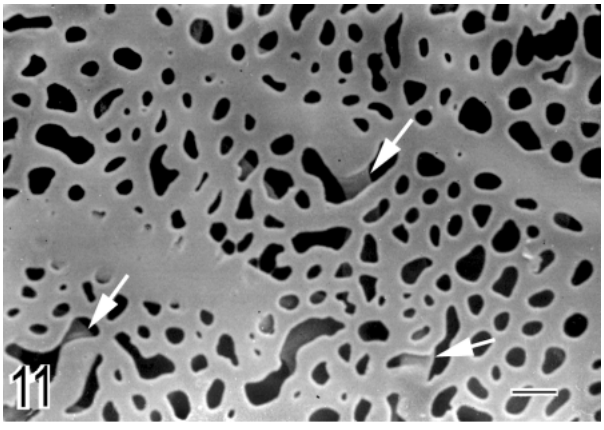


Fig. 11. Choriocapillaris viewed from inside the eye. Retinal vessels are removed. Arrows show precapillary arterioles. Bar=33.3 $\mu$m.

## DISCUSSION

Capillaries supplying the anterior margin of the ciliary process exhibited an irregular and thick bore and formed thoroughfare channels, draining blood from the ciliary arterioles into the pars plana venules, bypassing the ciliary processes entirely. This pattern of venous drainage is seen in other species [12]. The marginal capillaries may be responsible for the very high blood flow values compared to the choroid, kidney cortex and cardiac muscle [1] and blood flow velocities of the ciliary process [7]. This hemodynamics would be anticipated considering the ciliary process function secreting aqueous humor. The ciliary angioarchitecture with the separate plates of vessels arranged concentrically is presumably to facilitate unrestricted pupillary dilation and constriction in the iris.

Arterioles and venules of the iris were arranged radially and exhibited a uniform, round bore and zigzag or somewhat coiled feature, presumably to accommodate changes in the state of the pupil.

Capillaries in the third palpebra were characterized by a dense capillary bed with hair-pin loops just under the epithelium. Such subepithelial vascular organization may play a role in secreting aqueous humor, and exchanging nutrients and gasses between the cornea and the palpebral vessels across the palpebral epithelium when the lids are shut during sleep as suggested by Oduntan [15]. The bulbar conjunctiva consisted of one layered capillary network just under the epithelium and a rich venous plexus in the stroma. This microvascular organization may be responsible for the ready absorption of eye drops in the eye.

The retinal arteries gave rise to arterioles by means of side-branches, mostly at right angles. The retinal capillaries were extremely thin in diameter (3.0–4.0 $\mu$m), and red blood cells might barely pass through. The ridges at the origins of precapillary arterioles in the retinal and choroidal arteries represent intra-arterial cushions, which are sphincter-like thickenings of the intima, and play a significant role in regulating blood flow at the branching sites [4]. Our observation of these cushions in the retinal vessels is consistent with previous corrosion cast studies of ophthalmic arteries in rats [4, 14], pigs [21], monkeys [13] and humans [9]. The branching pattern with right angles, intra-arterial cushions and capillaries with extremely thin diameter may be responsible for causing plasma-skimming in the retinal capillary network [6, 11, 18].

The blood supply to the choroid was via short precapillary arterioles with comparatively large diameter (19.2–22.2 $\mu$m) and compactly arranged thick capillaries, whereas the blood supply to the retina was via long arterioles with narrow diameter (6.7–14.5 $\mu$m) and extremely thin capillaries forming a relatively sparse network. The anatomical differences between the two vessels may indicate a difference in the velocity of blood flowing to the choroid and retina, in which choroidal blood flows in relatively high values [5]. Indeed, on angiographic inspection, the filling pattern of fluorescein angiography in the eye was successively observed at the choroidal phase, which is characterized by the first filling of the choriocapillaris and then the retinal arterial phase, which in turn is characterized by a hyperfluorescence of the retinal arteries [5]. Again, the rich blood supply to the choriocapillaris with high oxygen pressure and a high vascular permeability would be anticipated considering its role in diffusing oxygen and nutrients through the choriocapillaris to nourish the retina. Such rich choroidal vasculature compensates for decreases in arterial blood pressure and thereby remains relatively stable within a physiological range of arterial blood pressure [19].

The venous drainage of the porcine eye resembled that of humans. The entire choroid and the anterior segment of the eyeball involving the iris, ciliary body and bulbar conjunctiva were drained only by vortex veins in the pig. In contrast, the rat eye has two drainage pathways; the entire choroid is drained by the posterior ciliary vein and the ante-

rior segments of the eyeball are drained by the vortex veins [2]. This is the only difference from a rat, in which the blood from the posterior half of the eyeball drains into the posterior ciliary veins. Rats have been exclusively used for the study of elevation of the intraocular pressure by cauterization of vortex veins. However, the studied rats showed no elevation of intraocular pressure [8, 10]. Presumably another venous drainage via the posterior ciliary vein compensates for the cauterized vortex veins to prevent the intraocular pressure from elevating. A pig has only one pathway for irrigation of blood from the choroid.

The circle of Zinn-Haller seen in human beings [17], which surrounds the optic nerve supplying the peripapillary choroid and the optic disc, was not observed in pigs as in rats [22], dogs [3] and monkeys [16].

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