0014-4754/93/040277-05$1.50 + 0.20

Choroidal and iris angioarchitecture of the newt: a scanning electron-microscopic study of vascular corrosion casts

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Received 2 October 1992; accepted 18 December 1992

Abstract. The corrosion cast technique provided for the first time an excellent three-dimensional visualization of the vascular pattern of the choroid and iris in the newt eye. The results show the presence of a single arterial afference to the choroidal and iris capillaries: the ophthalmic artery is the origin of both ciliary arteries and the long posterior ciliary artery. Slightly behind the equatorial circumference of the eyeball the venous drainage consists of a single vessel on the dorsal side and two distinct vessels on the ventral one. It receives blood from both iris and choroid. The surface of the plastic endocasts shows some details of fine luminal structures of the endothelial cells. Shallow depressions may be regarded as imprints of endothelial cell nuclei, and they are distinctly different for arteries and capillaries. The angioarchitecture of the newt eye differs from that of brain in that hairpin-shaped capillary loops are not observed at all.
Key words. Vascular casts; scanning electron microscopy; choriocapillaries; iris; *Triturus cristatus carnifex*.

The angioarchitecture of each organ, i.e. the spatial distribution of its vessels (arteries, capillaries and veins), is a characteristic closely related to its histological structure and function. The vascular pattern has been studied in many tissues and organs using conventional methods based mainly on India-ink injection and tissue clearing[1-7]. These methods suffer from the disadvantage of being unable to show directly the three-dimensional distribution of vessels and the details of the system. The main problems in light microscopical analysis of injected specimens are tracing distinct vessels over long distances, and obtaining three-dimensional images of microvessels by reconstruction of serial sections.

The study of organ angioarchitecture remained difficult until the early 1970s, when Murakami[8] introduced for the first time the vascular corrosion cast technique. The injection-replication-SEM technique is a simple method which combines the high resolving power and the great depth of field of SEM with the ability of low viscosity resins to fill even the smallest capillaries. It has greatly improved our knowledge of the microangioarchitecture in normal and pathological tissues and organs and in developing structures[9].

Several SEM studies of vascular corrosion casts of vertebrate eyes have been reported in the literature[9], but at present the data concerning amphibian eyes are scarce and are related only to anurans[10-12]. There are no SEM researches on eye angioarchitecture in urodeles. In a previous study on the newt we limited our interest to cerebral vessels[13,14].

The aim of the present study is to provide new and detailed information concerning the angioarchitectural pattern of the eye in the newt and discuss it on the basis of what is known regarding anurans.

Materials and methods

Ten adult newts (*Triturus cristatus carnifex*) of both sexes were anesthetized in 1% ethyl m-aminobenzoate (Sigma). Using a Gilson Minipulse 3 peristaltic pump (constant flow of 3 ml/min), they were perfused first with 30 ml of Holtfreter's solution containing heparin (1500 IU/ml), and then with 50 ml of 3% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). Next, 7 ml of a freshly prepared low-viscosity methyl methacrylate ($\eta = 2.5-3$ cS)[15] was injected at a constant rate, at room temperature, through a short cannula inserted in the conus arteriosus. The base resin consisted of 5 ml methyl methacrylate monomer (K & K Laboratories) with 25 ppm hydroquinone (Fluka) in which 1% w/v of 2,4 dichlorobenzoyl peroxide (K & K Laboratories) was dissolved. This solution was prepolymerized with UV light to a viscosity of 2.5–3 cS using a Philips TL 40 W/12 ultraviolet-B fluorescent tube; 2.2 ml of hydroxypropyl methacrylate (K & K Laboratories), 0.07 g of benzoyl peroxide (Aldrich) and 0.11 ml of n-N-dimethyl aniline (Fluka) were added immediately before utilization.

An hour after the resin injection the specimens were placed in a water bath at 60 °C overnight, to accelerate and complete the process of polymerization. The heads were then removed and placed in 30% KOH for maceration. Skull bone decalcification was obtained by immersion in 2.5% HCl. Finally, the casts were washed in 5% trichloroacetic acid solution to remove any products of saponification still adhering[10], and then washed several times in distilled water. The clean replicas were frozen at −20 °C and the ice blocks freeze-dried to prevent vessel collapse[9]. After drying, the eye casts were dissected, mounted on stubs, gold-coated in an Edward

A150 sputter coater and examined in a Philips 515 scanning electron microscope at an accelerating voltage of 15 kV.

Results

The corrosion casts provide excellent three-dimensional visualization of the vascular pattern of the newt eye.

At the posterior pole, choriocapillaries are supplied with oxygenated blood coming from the ophthalmic artery (figs 1, 2). Before reaching this point, it divides into two ciliary arteries (nasal ciliary artery and temporal ciliary artery), which perforate the sclera just dorsally to the outlet of the optic nerve (fig. 2). Up to this point both ciliary arteries run tightly parallel, then, after penetrating the sclera, they diverge and go further in a horizontal plane, following – to a certain extent – the external surface of the choroidal capillary network. Each ciliary artery emits some secondary branches reaching the network below. The proximal branches (fig. 3), usually two per side, dorsal and ventral, are bigger and longer and join the choroidal vessels with a few very short twigs. The bigger branches originate sideways and obliquely leave the ciliary artery, forming an acute angle open in the temporal or nasal direction, according to which ciliary artery is examined. In contrast, the branches emerging from the distal segment of each ciliary artery are shorter and narrower, and originate from the lower part, forming a right angle and connecting perpendicularly to underlying capillaries without further twigs (fig. 3).

At the posterior pole, choriocapillaries form a vascular ring surrounding the outlet of the optic nerve from the ocular globe. This vascular ring and the choriocapillaries of the territory dorsal to it are supplied with blood from twigs emerging from a big branch of the nasal ciliary artery (figs 1, 2). This branch is one of the long proximal ramifications mentioned above.

In the choroid the vessels are essentially represented by capillaries which show a mainly uniform calibre. They are interconnected by anastomoses and form a monolayered, close network with fine meshes, disposed in an almost circular or elliptical shape (fig. 4).

A fine network is present in the choroid at the posterior pole of the ocular bulb and extends around from here. As the fore choroidal margin is approached, the appearance of the choriocapillary network is modified. It becomes less dense, because the meshes are larger, more elliptical and clearly elongated in shape (fig. 5). At this level choroidal vessels, arranged in extended meshes and anastomised at an acute angle, flow into vessels with slightly increasing diameter from which the ocular venous system originates. Slightly behind the equatorial circumference of the eyeball is the venous drainage system of the choroid. Ventrally, this system consists of venous stems of a nasal and temporal branch of the inferior ocular vein (fig. 5). In the dorsal region it consists of two separate veins (nasal superior ocular vein and temporal superior ocular vein) (fig. 6).

In the vascular endocast the fore zone of the eyeball appears scarcely convex. It is supplied by the long posterior ciliary artery coming from the ophthalmic artery at the posterior pole of the eye. It is caudally directed, passes over the choroidal network without connections with choriocapillaries (fig. 7), reaches the fore hemisphere from the temporal side, and here vascularises the iris (fig. 8). On reaching the iris, the long posterior ciliary artery bifurcates and continues as a big arterial circle circumscribing the pupil. The arterial circle of the iris branches into a few radially-oriented vessels which run through the iris as far as its periphery and give out twigs, giving rise to a mono-layered network with wide polygonal meshes. At the periphery these vessels connect with the choroidal venous system, which is slightly posterior to the equator of the eyeball. The surface of the plastic endocasts shows some details of the fine luminal structures of the endothelial cells. The ophthalmic artery, ciliary arteries and their branches exhibit evident shallow, clearly elliptical impressions (fig. 3). The long axes of these depressions parallel the vessel length. Choriocapillaries rarely show ovoidal impressions which are clearly shallower than those of other vessels (fig. 4).

Discussion

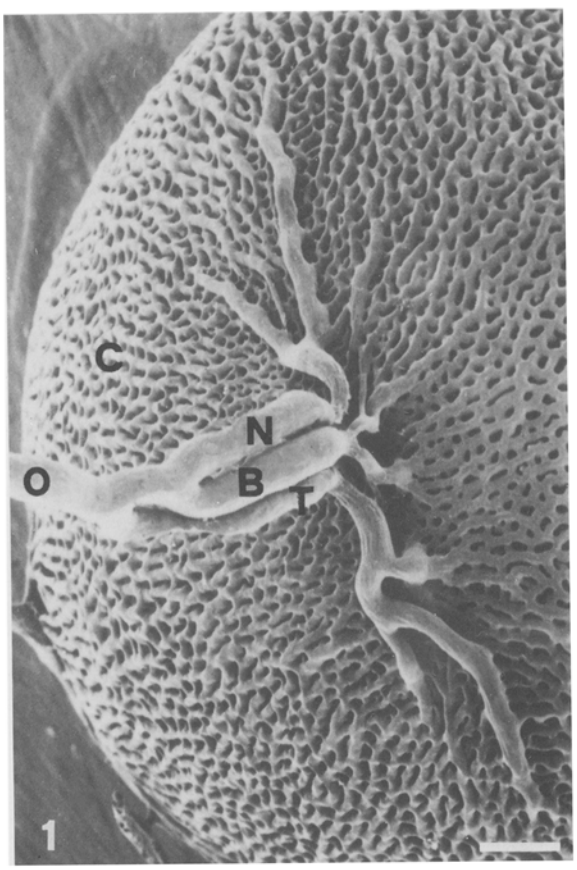
An accurate explanation of the results of many physiological studies needs knowledge not only of the structure and physiology of blood-tissue interfaces, but also of the microvascular angioarchitecture. The high resolving power of the SEM-corrosion cast method introduced by Murakami[8,16] and Nowell and Lohse[17] has provided more accurate data on the three-dimensional arrangement of vessels in tissues and organs. Unlike conventional light system methods, this SEM technique allows differentiation between arteries and veins and especially, for the first time, supplies a three-dimensional image of the whole vascular bed, so that the entire course of single blood vessels can be traced over long distances and anastomotic relations can be observed in detail.

Figure 1. Choroidal vascular cast of the right eyeball viewed from the dorsal side. C, choriocapillaries; O, ophthalmic artery; N, nasal ciliary artery; T, temporal ciliary artery; B, big proximal branch of the nasal ciliary artery (bar: 0.2 mm).

Figure 2. The same as in figure 1, viewed from the ventral side. A vascular ring surrounds the outlet of the optic nerve (arrowhead) from the eyeball (bar: 0.2 mm).

Figure 3. Elongated imprints of endothelial cell nuclei (arrows) are evident on ciliary arteries and their branches. Big arrowheads, proximal branches; thin arrowheads, distal branches (bar: 0.1 mm).

Figure 4. Very shallow imprints of endothelial cell nuclei with irregularly ovoidal shape (arrowheads) are present on choriocapillaries (bar: 0.05 mm).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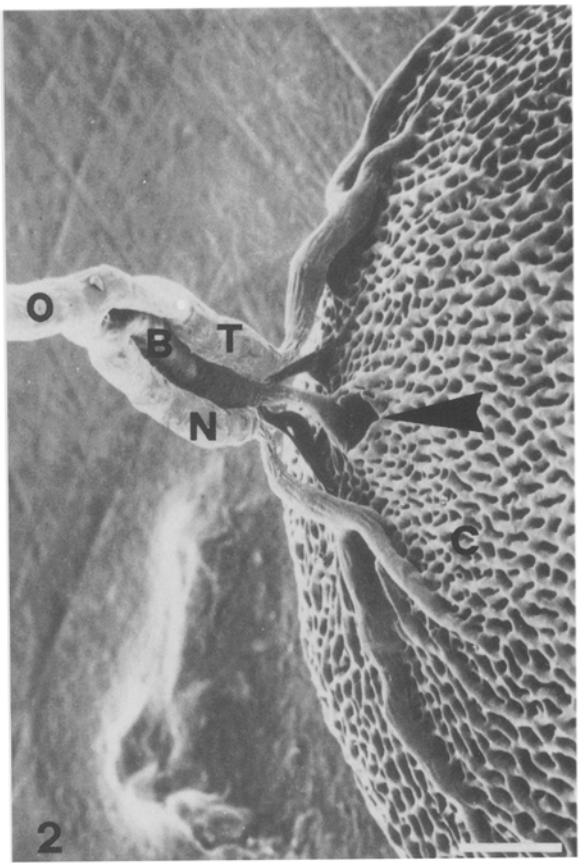
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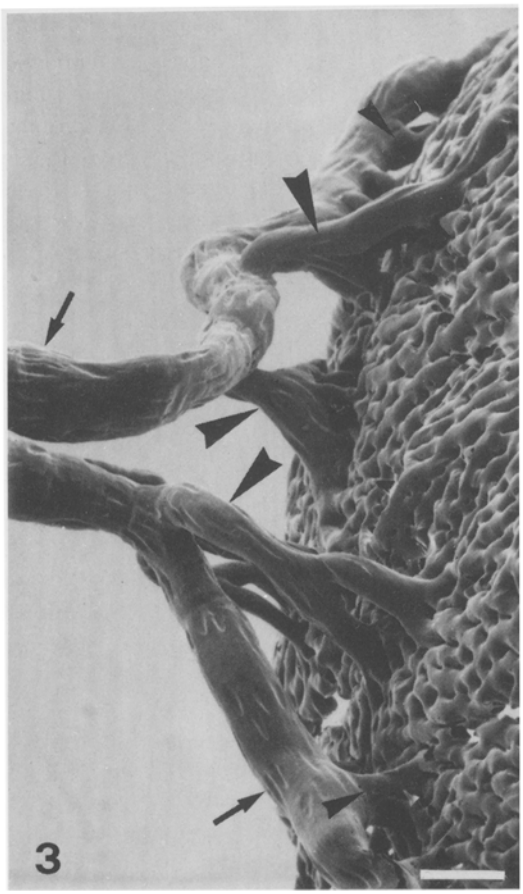
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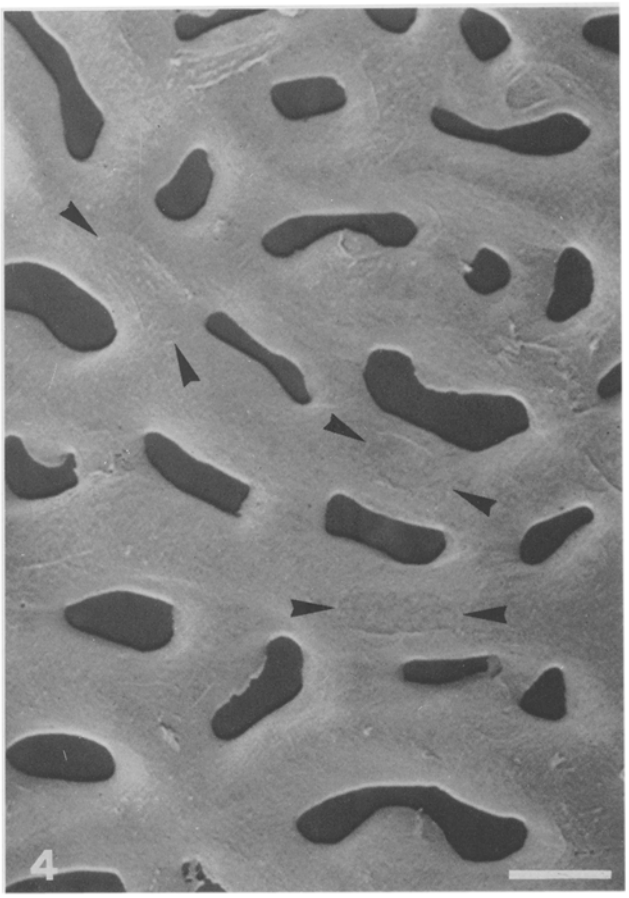
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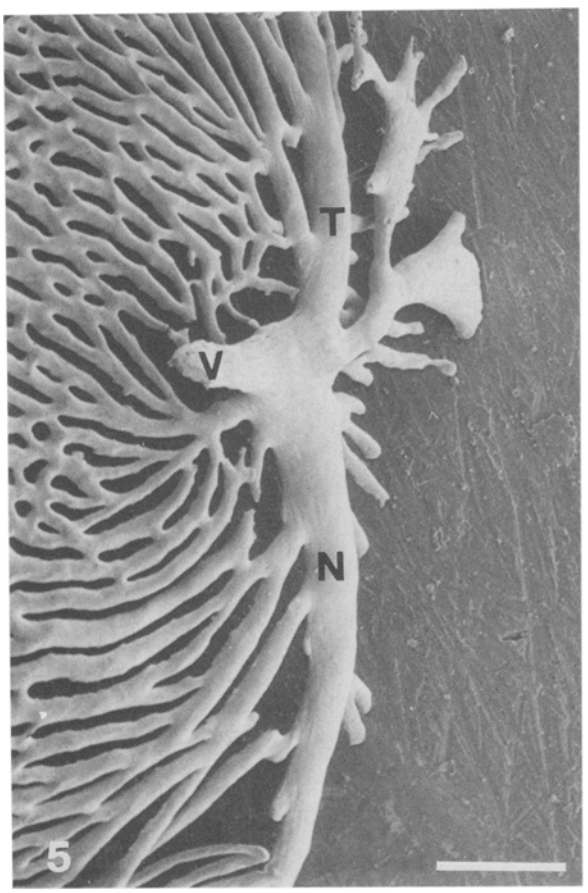
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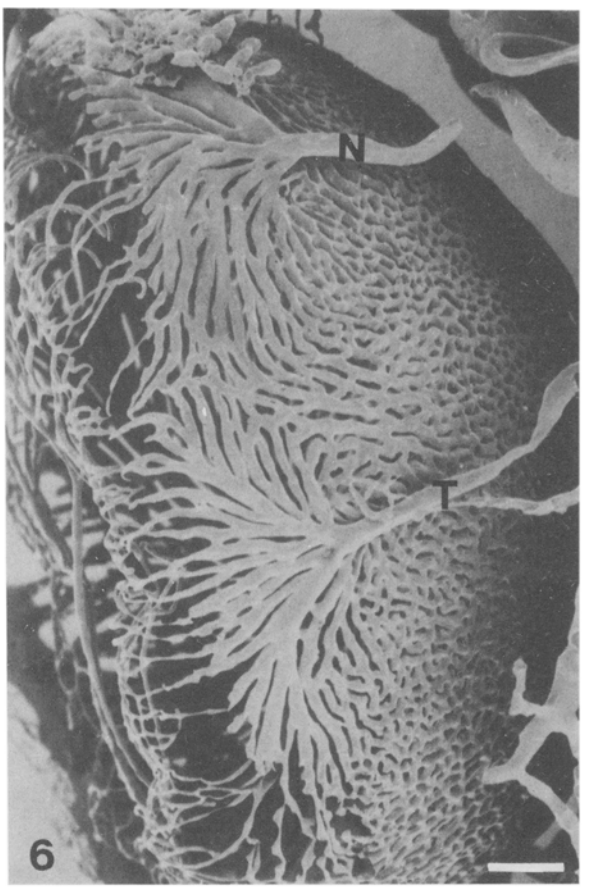
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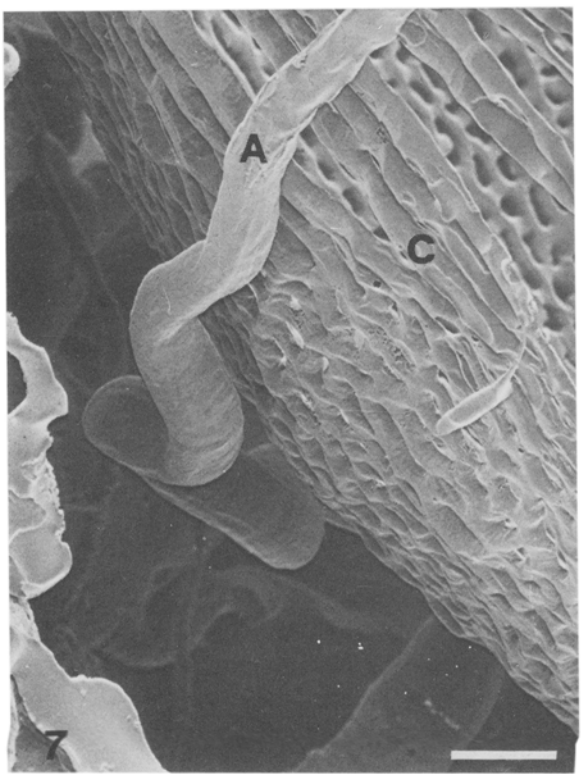
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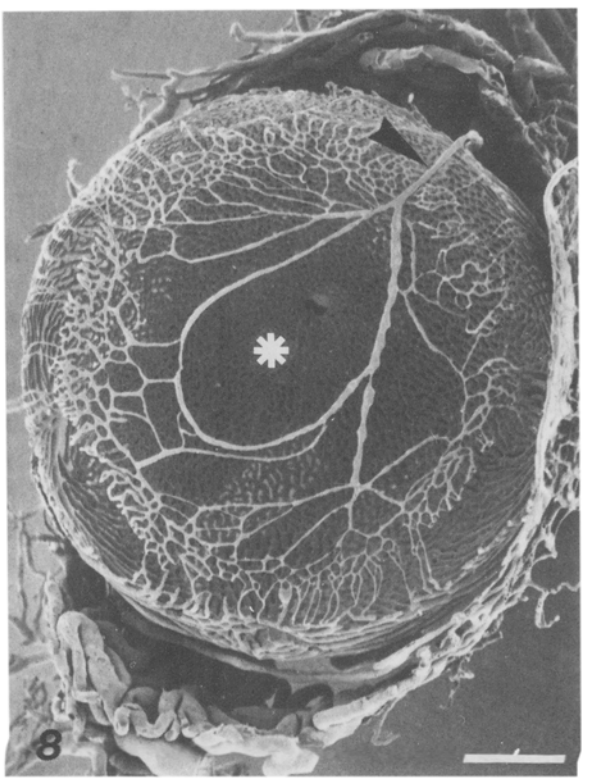
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In our SEM study on *Triturus* we have demonstrated the presence of a single arterial afference (ophthalmic artery) to choroidal capillaries. In *Rana*, Miodoński and Bär[10] and Miodoński et al.[12] reported the existence of an additional arterial supply for the choriocapillaries. It concerns especially the area surrounding the outlet of the optic nerve, i.e. the optic artery.

In *Rana*[10,12] the optic artery, included in the vascular sheath of the optic nerve, flows in a capillary network around the entrance of the optic nerve into the eyeball. This three-dimensional capillary network, placed externally to the choriocapillaries and surrounding the distal part of the optic nerve, is absent in the newt. In *Triturus* (present work) and in *Rana*[10,12] the choriocapillaries are nourished by two ciliary arteries (nasal ciliary artery and temporal ciliary artery) which, owing to their origin and vascularization zone, can be compared to the mammalian short posterior ciliary arteries. In mammalian choriocapillaries the star-like angioarchitectonic pattern and the kind of nourishment from posterior short and long ciliary arteries[18-20] are related to a lobular and segmental circulation in the choroid. Such a situation is absent in *Triturus* as well as in *Rana*[10,12] which both show a very regular pattern, especially in the posterior pole of the eyeball.

Shallow depressions on our cast surface may be regarded as imprints of endothelial cell nuclei, and they are distinctly different for arteries and capillaries. According to Miodoński et al.[21] and Hodde et al.[22] the artery imprints are ovoidal shallow depressions showing their long axes parallel and oriented lengthwise in the vessel.

The nuclear imprints on choriocapillaries are little elliptical depressions. This situation recalls a similar condition found in brain hairpin-shaped capillary loops[13,14]. It is opportune to recall that different features of nuclear imprints on vascular casts may be related to the different structure of their walls as well as to different vascular resistance to the pressure of resin flow and other variables that determine the final quality of the casts[9,23-26].

The retina itself is avascular, and is fed indirectly by blood flowing in the vessels of the choroid, which is situated externally to it. According to a previous study on newt brain angioarchitecture[13,14], we can also find a bidimensional vascular network in meningeal sheaths. But, while in the newt brain it originates hairpin-shaped capillaries which penetrate and directly feed the brain parenchyma, which is fairly thick, in the eyeball the choroid does not give rise to vessels for the underlying nervous structure (the retina), since this is thin, and free diffusion of nutrients from the choriocapillary blood is adequate to maintain function.

Acknowledgments. This study was supported by grants from the Ministero della Pubblica Istruzione and the Ministero dell'Università e della Ricerca scientifica e tecnologica.

1 Herrick, C. J., J. comp. Neurol. *61* (1935) 297.
2 Roofe, P. G., J. comp. Neurol. *61* (1935) 257.
3 Michaelson, I. C., Retinal Circulation in Men and Animals. C. C. Thomas, Springfield 1954.
4 Bubis, J. J., and Luse, S. A., Z. Zellforsch. *62* (1964) 16.
5 Axelsson, C., Acta otolaryngol. *243* (1968) 5.
6 Rhodin, J. A. G., in: Histology, a Text and Atlas, p. 803. Ed. J. A. G. Rhodin. Oxford University Press, London, New York 1974.
7 Rhodin, J. A. G., in: Blood Vessels and Lymphatics in Organ System, p. 97. Eds D. I. Abramson and P. B. Dobrin. Academic Press, San Diego, London 1984.
8 Murakami, K. T., Arch. Histol. Jpn. *32* (1971) 445.
9 Lametschwandtner, A., Lametschwandtner, U., and Weiger, T., Scanning Microsc. *4* (1990) 889.
10 Miodoński, A. J., and Bär, T., Cell Tissue Res. *249* (1987) 101.
11 Miodoński, A. J., and Bär, T., Cell Tissue Res. *250* (1987) 465.
12 Miodoński, A. J., Kus, J., and Bär, T., in: Cell and Tissues, a Three-dimensional Approach by Modern Techniques in Microscopy, p. 527. Ed. P. M. Motta. Alan R. Liss, New York 1989.
13 Lazzari, M., Franceschini, V., and Ciani, F., in: Atti 53° Congresso Unione Zoologica Italiana, p. 399. Mucchi, Modena 1990.
14 Lazzari, M., Ciani, F., and Franceschini, V., J. Morph. *210* (1991) 239.
15 Gannon, B. J., Biomed. Res. *2* (1981) 227.
16 Murakami, T., Arch. Histol. Jpn. *38* (1975) 151.
17 Nowell, J. A., and Lohse, C. L., Scanning Microsc. *1* (1974) 267.
18 Risco, J. M., and Nopanitaya, W., Invest. Ophthal. Vis. Sci. *19* (1980) 5.
19 Kohler, T., and Leiser, R., Acta anat. *116* (1983) 55.
20 Alm, A., in: The Physiology and Pharmacology of the Microcirculation, vol. 1, p. 299. Ed. N. A. Mortillaro. Academic Press, New York 1983.
21 Miodoński, A. J., Hodde, C. K., and Bakker, C., Beitr. Elektronenmikr. Direktabb. Oberfl. *9* (1976) 435.
22 Hodde, K. C., Miodoński, A. J., Bakker, C., and Veltman, W. A. M., Scanning Microsc. *2* (1977) 477.
23 Weiger, T. A., Lametschwandtner, A., and Adam, H., Mikroskopie *39* (1982) 187.
24 Weiger, T., Lametschwandtner, A., and Stockmayer, P., Scanning Microsc. *1* (1986) 243.
25 Lametschwandtner, A., Lametschwandtner, U., and Weiger, T., Scanning Microsc. *2* (1984) 663.
26 Christofferson, R. H., and Nilsson, B. O., Philips Electron Optics Bull. *127* (1989) 43.

Figure 5. Detail of the vascular cast of the right eyeball showing nasal (N) and temporal branch (T) of inferior ocular vein (V). Choroidal vessels are arranged in elongated meshes (bar: 0.2 mm).
Figure 6. Dorsal view of the vascular cast of the left eyeball showing nasal superior ocular vein (N) and temporal superior ocular vein (T) (bar: 0.2 mm).
Figure 7. Detail of the long posterior ciliary artery (A) which passes over the choroidal network without connections with choriocapillaries (C) (bar: 0.1 mm).
Figure 8. Vascular cast of the left eyeball viewed from the anterior pole. The long posterior ciliary artery (arrowhead) originate a big arterial circle circumscribing the pupil (asterisk) (bar: 0.5 mm).