Articles

Ocular microcirculation

Scanning electron microscopic study

*Jose Miguel Risco and Waykin Nopanitaya**

A simple vascular casting technique utilizing a low-viscosity plastic was used to study the three-dimensional ocular microcirculation of the cat eye. Vascular arrangements in different anatomic areas of the eye, i.e., iris, ciliary body, retina, optic nerve, and choroid, were clearly elucidated by scanning electron microscopy. This modified technique is described in detail, and scanning electron micrographs are presented to illustrate the reliable results.

Key words: ocular vessels, vascular cast, scanning electron microscopy

The microcirculation of the eye has been a subject of investigation for many years, and different techniques have been employed to study it. These techniques include standard histologic sections, flat preparation of digested tissue, injections of india ink, and plastic corrosion casting.

In this report a new technique utilizing an injection-corrosion method for studying ocular blood vessels is described in detail. This technique is similar to those previously reported in studies of the ocular circulation[1] but was modified by the addition of compounds which reduced the viscosity of the injected plastic medium to allow uniform filling of capillaries. In addition, this report presents the results of the application of the aforementioned technique to show the fine structure of several anatomic areas of the cat eye in heretofore unavailable detail. A brief interpretation and scanning electron micrographs of the iris, ciliary body, retinal vessels, optic nerve, and choroid are presented to illustrate the advantages of the technique.

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Materials and methods

Healthy adult domestic cats weighing 2 kg were used in this study. The animals were anesthetized with intravenous pentobarbital sodium (15 mg/kg) and fixed in a supine position. Both common carotid arteries and jugular veins were exposed after the animals were given a subcutaneous injection of 3 ml of lidocaine. The common carotid arteries proximal to the heart were ligated with 3-0 silk, and each was cannulated by a 22-gauge plastic cannula. Each cannula was secured to its artery with a 3-0 silk ligature. When the arterial cannulation was completed, the jugular veins were incised. Arterial perfusion was then begun with saline containing heparin (10 units/ml) at a gravity flow rate of 25 ml/min until the effluent of the open jugular veins became clear. As soon as the saline perfusion was completed (approximately 20 min), 20 ml of freshly mixed plastic mixture[2] was introduced simultaneously into both carotid arteries at a flow rate of about 10 ml/min. The plastic mixture having the most satisfactory low viscosity (20 poises) and suitable hardness consisted of 33 ml of Batson No. 17 corrosion compound (Polysciences, Inc., Paul Valley Industrial Park, Warrington, Pa.) (25 ml monomer base solution, 7.5 ml catalyst, 0.5 ml promotor) and 12 ml of Sevriton

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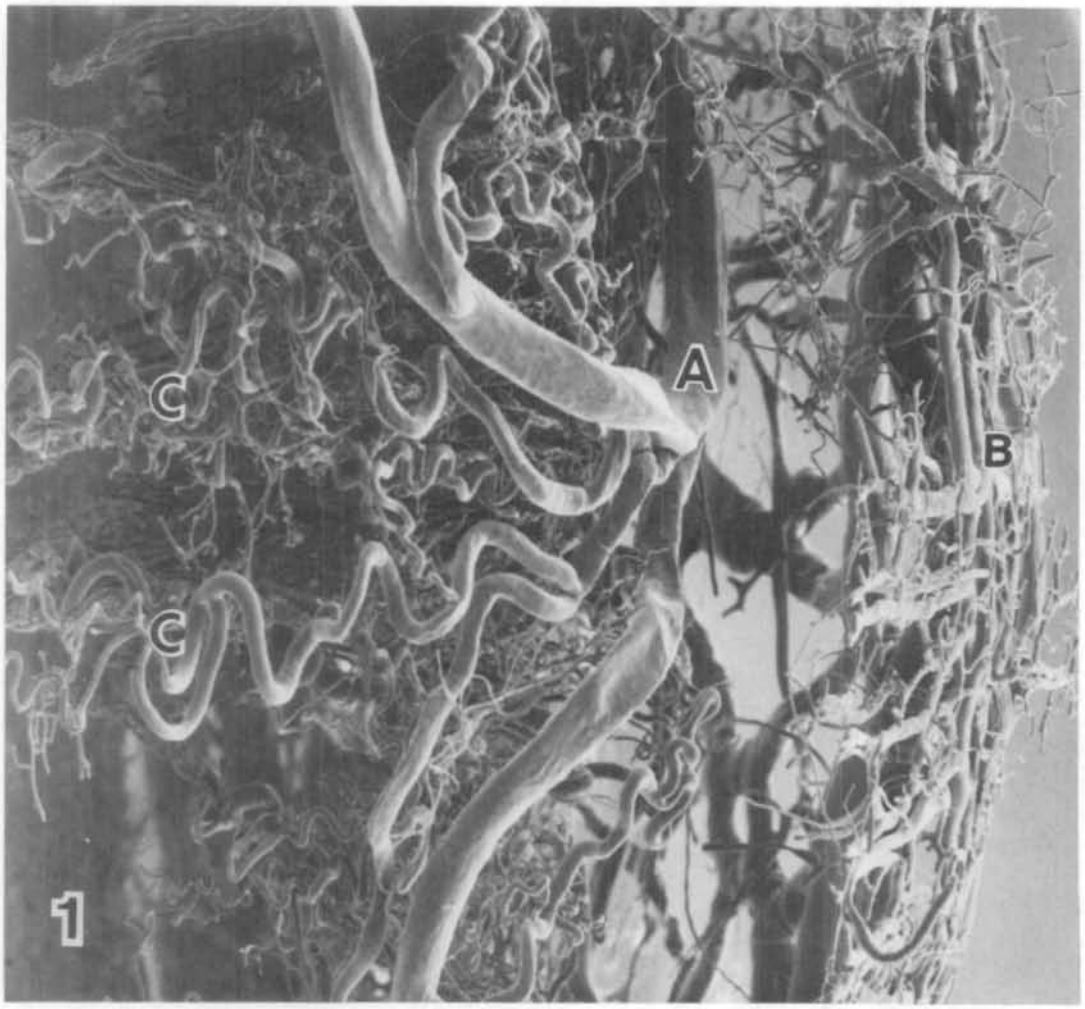

Fig. 1. A portion of the anterior segment microcirculation. The long posterior ciliary artery *(A)* bifurcates to form the major arterial circle of the iris. The intrascleral plexus or circle of Hovius *(B)* and the ciliary process *(C)* are shown. (×45.)

(Amalgamated Dental Trade Distributor, Ltd., London, England, commercially available in any dental suppliers in U.S.). The plastic-injected eyes were left in situ at room temperature for 30 min, enucleated, and immersed in warm (80° C) water for 4 hr to allow the plastic to harden. The ocular tissues were digested in 40% KOH at 60° C for 4 days. The KOH was changed twice daily. The casts were washed in several changes of distilled water and then air-dried in a dust-free container at room temperature. The dried vascular casts were carefully dissected and were mounted on scanning electron microscope (SEM) stubs with conductive adhesive media. Samples were lightly coated (10 nm) with gold-palladium and examined with an ETEC U2 Autoscan scanning electron microscope operating at an accelerating voltage of 20 kV.

Results

The ocular vessel casts obtained by the procedure described allowed excellent three-dimensional visualization of the microcirculation of the cat eye. Five different areas of each ocular cast were observed: iris, ciliary body, retina, optic nerve, and choroid.

The long posterior ciliary artery reached the iris, bifurcated, and continued as a major arterial circle (Fig. 1). The two sites of bifurcation were located 180° apart. Branches

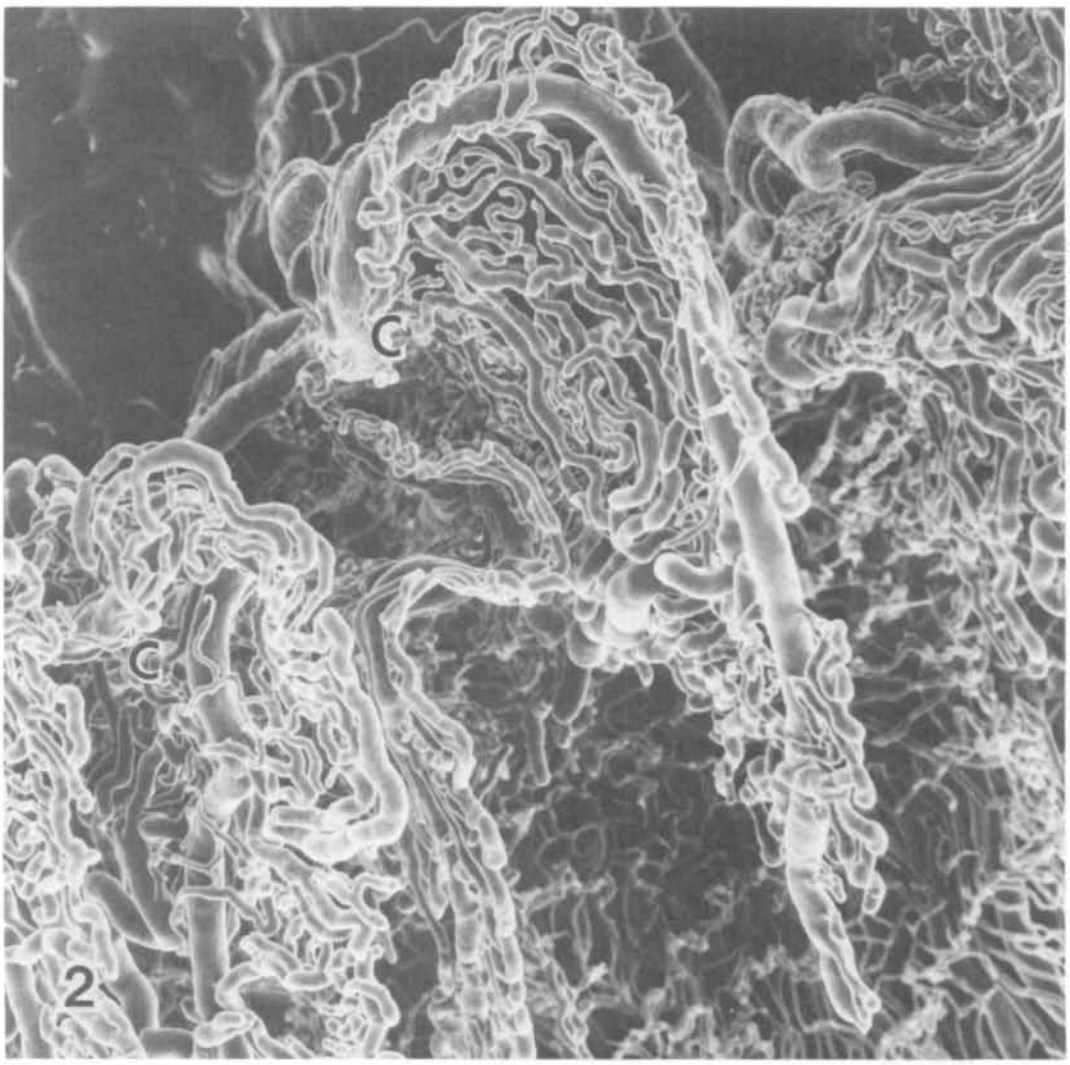

Fig. 2. The ciliary processes (*C*). A major arterial vessel branches into small capillaries. (×100.)

from the long posterior ciliary artery supplied the ciliary body. The major arterial circle of the iris branched tortuously as it entered the iris. The intrascleral plexus or circle of Hovius consisted of microvessels forming a complex vascular network around and posterior to the limbus.

Each ciliary process contained a major arterial vessel which randomly branched to form a capillary network (Fig. 2). Endothelial mural impressions were evident in the large arterial vessel and were arranged parallel to the longitudinal axis of the vessel (Fig. 3). The pars plana vessels were closely associated with the ciliary process and ran parallel one another in an anteroposterior direction.

The major retinal arteries entered the eye at the periphery of the optic disc. Minute branches of retinal capillaries were sharply angulated away from the main vessel. Small retinal capillaries divided by right-angle branching and were distributed in two layers within the retina (Fig. 4). Annular endothelial cell impressions were always present at the branching sites (Fig. 5).

The optic nerve was quite striking when

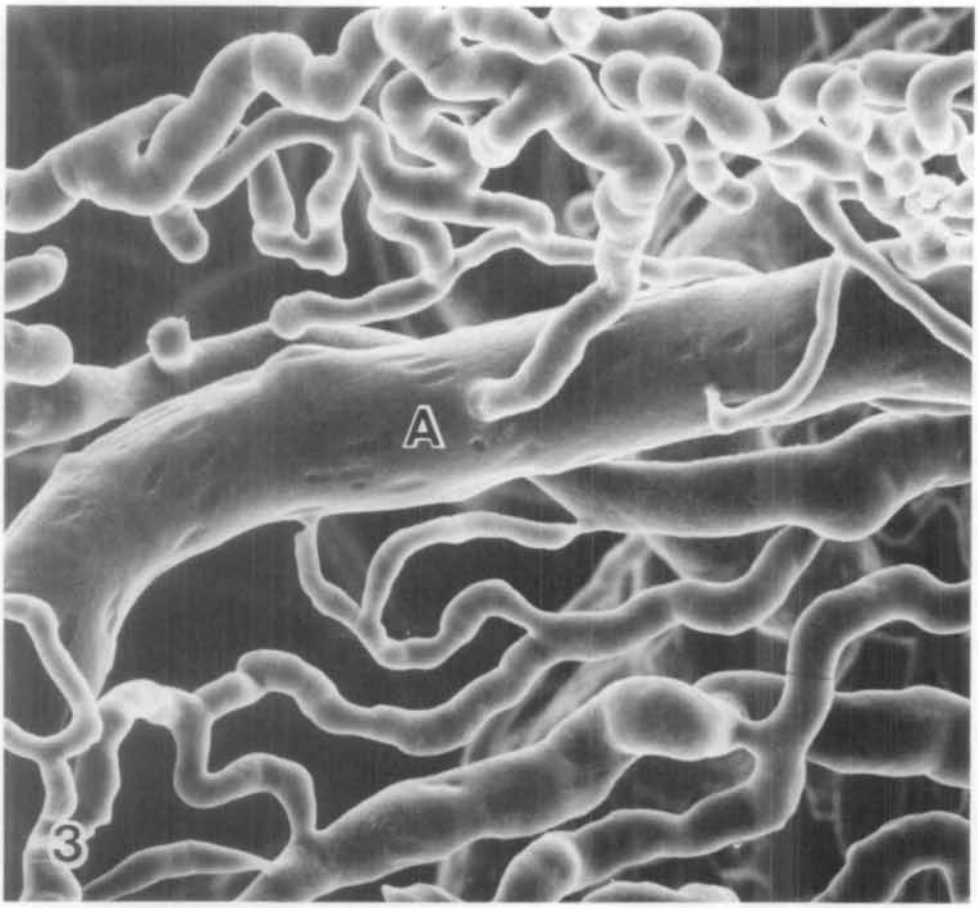

Fig. 3. A major arterial vessel *(A)* of the ciliary process at higher magnification. Note endothelial nuclear impressions on the artery. (×400.)

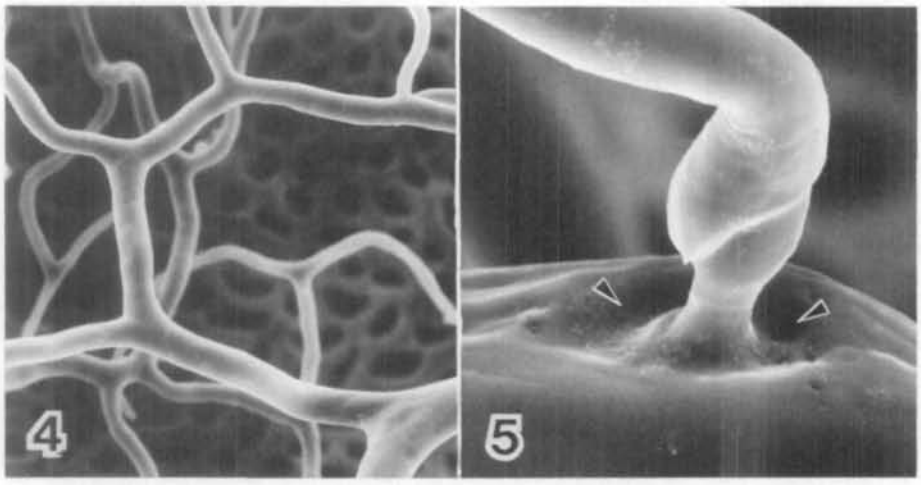

Fig. 4. Retinal capillaries dividing by right angle branching. Two layers of vessels are shown. The background is occupied by the choriocapillaris. (×400.)

Fig. 5. Annular impressions *(arrowheads)* in the site of retinal artery branching (×1,800.)

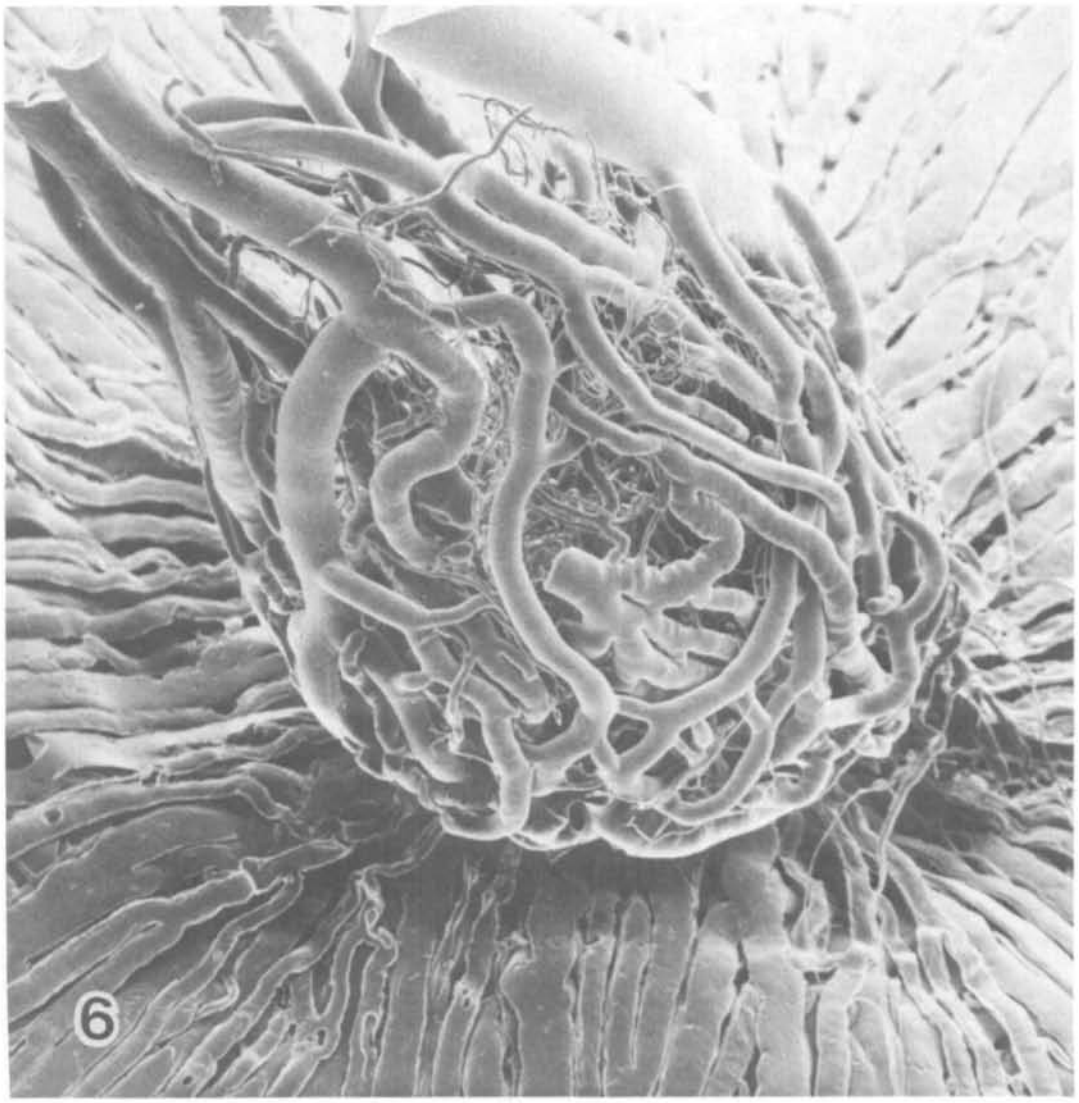

Fig. 6. Microvessels of the optic nerve as seen posteriorly. Large cilioretinal vessels surround the optic nerve. The large choroidal vessels are radially arranged around the optic nerve. (×45.)

viewed posteriorly. Large cilioretinal arteries surrounded the optic nerve. Large choroidal arteries were seen branching from these large vessels in a radial pattern (Fig. 6). No central artery was observed. The choriocapillaris in the posterior pole consisted of a single-layered network of fine channels arranged in a lobular pattern (Fig. 7). Each lobule consisted of a centrally located vessel from which capillaries radiated. When the choroid was studied from a semilateral view at the edge of the specimen, the arteriovenous system could be observed. The choriocapillaris network appears to be supplied and drained by vessels oriented perpendicular to the capillaries layer and connected to larger choroidal arteries and veins (Fig. 8). The lumen of the choriocapillaris measured 8 to 15 μm.

Discussion

The injection-corrosion technique used in this study is simple, rapid, and reliable. A one-step mixing of the casting medium sim-

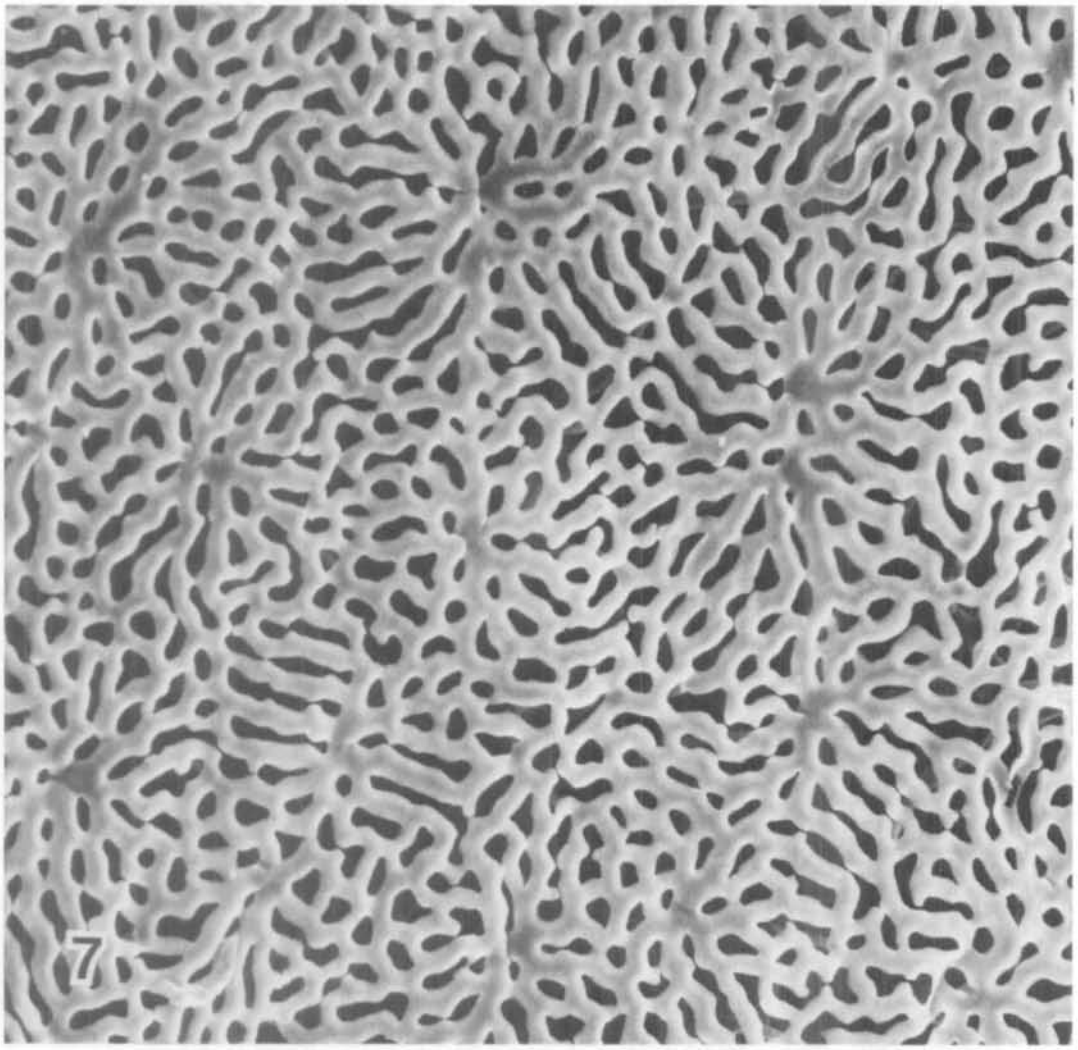

Fig. 7. The choriocapillaris at the posterior pole. A lobular arrangement of capillaries is shown. (×240.)

plifies the procedure. This plastic mixture has a low viscosity (20 poises) which provides uniform filling of microvessels. In addition, the vascular casts made from this medium are stable under heavy electron bonbardment at the high accelerating voltages (20 kV) which are required for high-resolution work. Most SEM studies using corrosion casts, especially the recently published work by Shimizu and Ujiie,[1] employed another type of plastic which can withstand electron irradiation at considerably low accelerating voltages (5 kV). Such low voltages prohibit detailed observation and high resolution by SEM. In our study, high resolution and excellent details of the casts' surfaces were achieved, e.g., the mural endothelial nuclear impressions on the surfaces of vascular casts were detailed enough to allow the differentiation of arteries from veins.[1, 3] The endothelial nuclear impressions of arteries were always oriented along the longitudinal axis of each artery, whereas the venous endothelial impressions were randomly distributed without a characteristic orientation. The annular arrangement of endothelial impressions at the site of arterial branching of the retina vessels probably represents the arteriolar sphincter.[4]

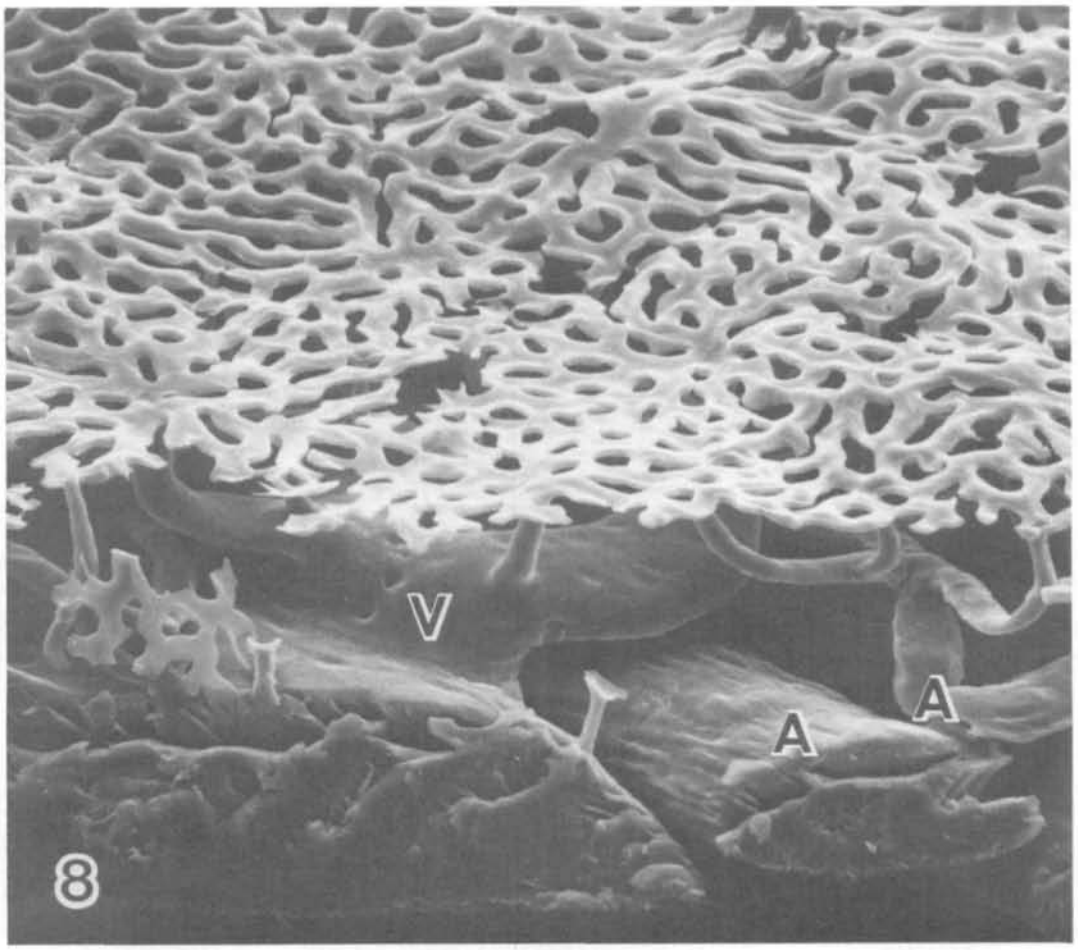

Fig. 8. The arteriovenous system of the choroidal circulation. Veins *(V)* are clearly differentiated from arteries *(A)* by the nuclear impression arrangement. (×300.)

Some findings merit further comment. In regard to the vessels of aqueous drainage, we have observed plastic filling of the intrascleral plexus most probably through the communications between the more anterior choroidal veins or the vortex veins and the circle of Hovius.[5] Similar findings using scanning electron microscopy of plastic luminal replicas have recently been demonstrated in the dog eye.[6] The lobular architecture of the choriocapillaris in the posterior pole was a constant and striking finding in our study. Similar vascular arrangement has been observed in humans[7–10] and in monkeys.[11, 12] This is in contrast to the rat, where there is not outstanding lobular distribution of the choriocapillaris vessels.[13]

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