Pecten of the Chick Eye Demonstrated by Vascular Casts

By

N. MATSUNAGA and T. AMEMIYA

Department of Ophthalmology, Nagasaki University School of Medicine, Nagasaki, Japan.

—Received for Publication, April 18, 1990—

Key words: Pecten oculi, Methylmethacrylate, Vascular cast, KOH digestion, Capillary

Summary: Vascular casts demonstrated the capillary network and characteristic features of the inner surface of the capillaries in the chick pecten oculi. Pecten capillaries anastomosed freely with each other to form a complicated network of many layers of capillaries. The capillary diameter varied from capillary to capillary, and sometimes ampulla-like features were seen. Numerous pores in the casts of the capillaries corresponded to endothelial processes. Pits represented of nuclear imprints. These scanning electron microscopic findings in vascular casts were not incompatible with those seen by transmission electron microscopy.

It is well known that the pecten oculi of birds contains many capillaries with numerous basal and luminal membrane infoldings of the endothelial cells (Dieterich, 1973). Since capillary structures of the pecten oculi are similar to the brush border of the epithelial cells of the proximal convoluted tubules of the kidney, it has been assumed that there is active transport between the vitreous and the endothelium of the pecten capillary (Amemiya, 1982, 1983). We attempted to demonstrate stereoscopically the capillary network and the characteristic features of the inner surface of the capillaries in the pecten oculi.

Materials and Methods

14-day-old chicks were anesthetized with intraperitoneal pentobarbital sodium and their chests were opened by splitting the sternum. Warm Ringer solution (35°C) containing heparin sodium (500 units/100ml) and procaine hydrochloride (0.5gm/100ml) was perfused through a catheter inserted into the left ventricle. Blood was removed through an opening in the right atrium or the inferior vena cava. The perfusion pressure was about 100mmHg. Then 2% glutaraldehyde in 0.05M cacodylate buffer with 0.22M sucrose was perfused for about five minutes. Methylmethacrylate resin was injected through the same catheter. After two hours at room temperature, the eye of the chick was enucleated carefully and macerated in a bath of concentrated sodium hydroxide (20%), and then rinsed in distilled water. The vascular cast was dried at room temperature and mounted on stubs with silver paste,

then sputtercoated with gold for viewing in a scanning electron microscope by an Eicho ion coater.

Some specimens were digested with KOH: They were digested with 5N KOH for 20 minutes and fixed in 2% osmium tetroxide in veronal acetate buffer. After the materials had been dehydrated in a graded series of ethanol, they were dried by the critical point drying method and sputtercoated.

After the pecten filled with methylmethacrylate had been fixed in glutaraldehyde and postfixed in osmium tetroxide, it was dehydrated in a graded series of ethanol and embedded in Luveak 812. Ultrathin sections for electron microscopy were stained with uranyl acetate and lead citrate and examined by JEOL 1200 EX and JEOL 100B electron microscopes.

Results

Scanning electron microscopy showed the following findings:

1. The capillaries of the pecten were connected with each other and formed a vast network of many layers (Figs. 1, 2).
2. The caliber of the capillaries was not uniform. In some areas, they had beaded and ampulla-like appearances (Fig. 3).
3. At higher magnification, numerous pores and pits were seen on the surface of the cast. The former seemed to indicate cytoplasmic processes, and the latter impressions of nuclei of endothelial cells (Fig. 4).

4. Capillary networks resembling accordion pleats and anastomoses were clearly seen after digestion with 5N KOH (Fig. 5). The pecten capillaries contained double vessels filled with methylmethacrylate resin (Fig. 6).

Transmission electron microscopy showed the following findings:

1. The pecten capillaries had numerous basal and luminal membrane infoldings.
2. The capillaries were filled with methylmethacrylate resin, but the resin did not get into the spaces between the membrane infoldings (Fig. 7).

Discussion

The pecten of birds is composed of blood vessels and glial cells with pigmented granules. The capillaries of the pecten oculi have thin endothelial cell cytoplasm and numerous basal and luminal membrane infoldings. The structure and anastomoses of the pecten capillary network have not previously been demonstrated stereoscopically. Raviola (1967) injected a mixture of carbon and gelatine from a carotid artery to form vascular casts, while Seaman and Storm (1963) injected vinyl. But they could not make vascular casts of the pecten oculi. Since Murakami (1971) reported that methylmethacrylate is useful in the making of vascular casts, this method has been applied to the construction of all kinds of vascular casts. For example, the vascular structures of the ciliary body, choroid and retina have been clearly demonstrated (Tano and Chandler, 1981). Uziie (1976) showed pores and pits on the surface of vascular casts, the former representing microvilli and the latter nuclear imprints. Matsusaka (1984) made many kinds of vascular casts and described vasomotion captured by the cast. Hossler (1984) made methylmethacrylate vascular casts of avian eyes. He noted that two or three arteries to the pecten (*Aa. pectinis oculi*) arise from the ophthalmotemporal artery near the optic nerve run parallel to the base of the pecten at about the level of the choriocapillaries and send branches into the pecten, usually one or two for every pleat. However, he did not show the characteristic features of the inner surface or the running of the pecten capillaries. In this study, vascular casts of the pecten oculi of chick eyes showed numerous pores and pits and

a complicated network made up of layers of capillaries. The pecten capillaries were filled with methylmethacrylate. The scanning and transmission electron microscopic findings were not incompatible with each other. The ampulla-like part of the capillaries may play a role similar to that of venous valves in preventing transient stagnation and slow blood flows.

The functions which have been ascribed to the pecten oculi are: transport of nutrition to the retina, accommodation mechanism, stabilization of intraocular pressure, maintenance of temperature in the eye, screening of the retina from the sun's rays, and navigation (Duke-Elder, 1958). The complicated network of pecten capillaries, resembling a labyrinth, seems to increase the surface of blood vessels per unit area and to provide for a rapid and efficient exchange of materials between the vitreous and pecten capillaries.

References

- 1) Amemiya, T.: Electron histochemical study of alkaline phosphatase activity in the pecten oculi of the chick. *Graefes Arch. Clin. Exp. Ophthalmol.*, **219**: 11-14, 1982.
- 2) Amemiya, T.: Glycogen metabolism in the capillary endothelium. Electron histochemical study of glycogen synthetase and phosphorylase in the pecten capillary of the chick. *Acta Histochem.*, **73**: 93-96, 1983.
- 3) Dieterich, C. E. & Dieterich, H. J.: Fine observation of the pecten oculi capillaries of the chicken. *Cell Tissue Res.*, **146**: 473-489, 1973.
- 4) Duke-Elder, S.: *System of Ophthalmology*, Vol. 1. The Eye in Evolution. 410-417, Henry Kimpton, London, 1958.
- 5) Hossler F. E. & Olson, K. R.: Microcorrosion casting, scanning electron microscopy and stereology. *Am. J. Anat.*, **170**: 205-221, 1984.
- 6) Matsusaka, T. & Fukuda, M.: Ultrastructures suggesting the vasomotion on vascular casts. *Folia Ophthalmol. Jpn.*, **35**: 2078-2083, 1984. (In Japanese)
- 7) Murakami, T.: Application of the scanning electron microscope to the study of the fine distribution of the blood vessels. *Arch. Histol. Jap.*, **32**: 445-454, 1971.
- 8) Raviola, E. & Raviola, G.: A light and electron microscopic study of the pigeon eye. *Am. J. Anat.*, **120**: 427-462, 1967.
- 9) Seaman, A. R. & Storm, H.: A correlated light and electron microscope study on the domestic fowl (*Gallus domesticus*). *Exp. Eye Res.*, **2**: 163-172, 1963.
- 10) Tano, Y. & Chandler, D. B.: Vascular casts of experimental retinal neovascularization. *Am. J. Ophthalmol.*, **92**: 110-120, 1981.
- 11) Uziie, K.: Three-dimensional view of retinal capillary. *Acta Soc. Ophthalmol. Jap.*, **80**: 634-644, 1976. (In Japanese)

Explanation of Figures

Plate I

Fig. 1. Scanning electron micrograph of a vascular cast. A vast network of many layers is clearly shown.
(original magnification $\times 200$. Bar $100 \mu\text{m}$)

Fig. 2. Cast of a capillary network. This shows the labyrinth-like running of tortuous and winding capillaries.
(original magnification $\times 400$. Bar $10 \mu\text{m}$)

1

2

Plate II

- Fig. 3. The caliber of the capillaries is not uniform. They have beaded and ampulla like appearances.
(original magnification $\times 600$. Bar $10\ \mu\text{m}$)
- Fig. 4. Scanning electron micrograph of a cast showing numerous pores and pits on the surface.
(original magnification $\times 3,000$. Bar $10\ \mu\text{m}$)

3

4

Plate III

Fig. 5. Scanning electron micrograph of a pecten treated with KOH digestion. Pleats of the pecten and anastomoses of capillaries are seen.

(original magnification $\times 150$. Bar $100\ \mu\text{m}$)

Fig. 6. Scanning electron micrograph of a pecten treated with KOH. The pecten capillaries are filled with methylmethacrylate resin (*).

(original magnification $\times 2000$. Bar $10\ \mu\text{m}$)

5

6

7

Plate IV

Fig. 7. Transmission electron micrograph of a pecten injected with methymethacrylate resin. The pecten capillaries have numerous basal and luminal membrane infoldings. The capillaries are filled with methymethacrylate resin (*), but the resin does not get into the space between the membrane infoldings.
(original magnification $\times 5,000$. Bar $1\ \mu\text{m}$)