The Mosaic Pattern of the Inner Surface of Vertebrate Retina

Taeko Masutani-Noda and Eichi Yamada

Department of Anatomy (Prof. E. Yamada), University of Tokyo Faculty of Medicine, Tokyo, Japan

Received September 29, 1982

Summary. Retinas of various vertebrates (bullfrog, crow, bull, pig and human) were treated with HCl and collagenase for removal of the basement membrane. The exposed basal surface of Müller cells was observed under a scanning electron microscope. In the nerve fiber layer, the Müller cells divide into small basal processes which extend towards the basal surface, where they terminate with a mosaic pattern. This pattern varies somewhat from species to species and from region to region of the retina.

Müller cells extend radially through the entire thickness of the retina from the outer to the inner limiting layer. Morphology of Müller cells has been studied by many researchers (Cajal, 1893; Polyak, 1941; Wolter, 1961; Pedler, 1961, 1963; Hogan and Feeney, 1963; Hogan et al., 1971; Villegas, 1964; and others). These studies described that, in the nerve fiber layer, Müller cells terminate in a trumpet shape, fanning out at the inner retinal boundary. However, the exact mode of the terminations of the Müller cell at the basal surface of the retina has yet to be clarified. We removed the basement membrane using the HCl-collagenase digestion method (Evan et al., 1976). This procedure enabled us to observe the exposed basal surface of Müller cells directly under the scanning electron microscope (SEM). The mosaic pattern of the endings of the Müller cells thus revealed will be demonstrated in this paper.

MATERIALS AND METHODS

Retinas of the bullfrog (Rana catesbiana), crow (Corvus levaillantii), bull, pig and human were used. The eyes were enucleated and dissected. All materials except those of the human were fixed with 2.5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4. The human retina was obtained from a cadaver which was fixed by perfusion with formaldehyde and ethanol. The retina was cut into small pieces. To remove the basement membranes, the HCl-collagenase method (Evan et al., 1976) was used. The tissues were rinsed several times in 0.1 M phosphate buffer and placed in 5.5 N HCl for 60 min at 60°C. After HCl digestion, the tissues were rinsed again several times in 0.1 M phosphate buffer, then placed in a collagenase solution (Worthington, Type II) at a concentration of 200 units/ml of 0.1 M phosphate buffer for 3 hrs at 37°C. After rinsing several times with 0.1 M phosphate buffer, the tissues were postfixed in 1.3%
OsO₄ in 0.1 M phosphate buffer for 2 hrs, dehydrated with a series of acetone and dried by the CO₂ critical point method. The specimens were coated with gold-palladium and observed in a Hitachi HFS-2S scanning electron microscope. For transmission electron microscopy (TEM), the retinas were fixed with 2.5% glutaraldehyde in 0.1 M phosphate buffer followed by 1% OsO₄ in 0.1 M cacodylate buffer. The tissues were then embedded in Epon 812, sectioned on a Porter-Blum MT-1 ultra microtome and thin sections were observed in a Hitachi H-700 electron microscope after staining with uranyl acetate and lead citrate.

**OBSERVATIONS**

Figure 1 shows an overview of the vertically fractured retina at the equatorial region...

---

Fig. 1. Overview of fractured pig retina. NF nerve fiber layer, GC ganglion cell layer, IP inner plexiform layer, IG inner granular layer, OP outer plexiform layer, OG outer granular layer, OL outer limiting layer, RC layer of rods and cones. × 550

Fig. 2. Higher magnification of the nerve fiber layer of the pig retina. × 3,500

Fig. 3. Mosaic pattern of the inner retinal surface of the crow. Peripheral region near the ora serrata. In the equatorial region, the elements forming the mosaic pattern are smaller but similar in shape. × 8,500

Fig. 4. Tangential section through the inner surface of the crow retina. Profiles of foot processes are evident. The basement membrane is seen in the upper right corner. × 18,000

Fig. 5. Vertical section of the inner area of the crow retina. Many foot processes of Müller cells extend through the nerve fiber (NF) layer towards the inner retinal surface and terminate there. B basement membrane, V vitreous body. × 18,000
Fig. 3-5. Legends on the opposite page.
of the pig eyeball. At the bottom of this figure, many rods and a few cones form the rod and cone layer. The outer limiting layer is formed by the attachment of apical processes of the Müller cells, which extend microvilli among the bases of the rods and cones, called the fiber basket. The inner limiting layer is composed of basal endings of Müller cells which are covered by a basement membrane. Figure 2 shows an inner area of the same retina at higher magnification and also shows the basal foot processes of Müller cells which radiate towards the inner surface of the retina. They issue sheet-like, lateral extensions which interdigitate with each other and also surround the space where the bundle of nerve fiber is located. The basal ends of the foot processes are flat and cover the inner surface of the retina throughout.

Figure 3 illustrates en-face view of the inner surface of the crow retina, which is uncovered by HCl-collagenase treatment. The true surface of the retina thus is exposed by removal of the basement membrane. The picture clearly demonstrates the arrangement of foot processes in a mosaic pattern. Each component of the mosaic represents each foot process, and is oval or oblong in shape. These findings are also supported by the thin sectioning technique.

Figure 4 is a TEM image of a tangential section through the inner surface of the crow retina and shows a mosaic pattern formed by the foot processes. It is obvious that the profiles of the foot processes are not uniform but vary in shape and size.

Fig. 6. Vertical section of the inner area of the human retina. The macular region. The basement membrane (B) is rather thick in this region. The basal surface of the foot processes is irregular and shows small protrusions. ×12,000

Fig. 7. Inner surface of the human retina under the SEM. Equatorial region. ×4,600
Fig. 8-10. Inner surface of the bovine retina under the SEM. Fig. 8. Equatorial region. $\times 6,400$. Fig. 9. Peripheral region near the ora serrata. $\times 2,100$. Fig. 10. Optic disc region. $\times 6,400$
Figure 5 is a TEM view of a vertical section of the same material showing longitudinal profiles of Müller cell foot processes. These processes terminate at the inner limiting layer through the nerve fiber layer. The basement membrane covers the basal surface of these processes. Fine fibrils of the vitreous body scatter in various directions forming a thick layer next to the basement membrane. In both figures, no special attachment structure is observed between the foot processes. These findings clearly indicate that each element of the mosaic pattern as seen in the SEM corresponds to each foot process of the Müller cells.

As mentioned earlier, the basal surfaces of the foot processes of Müller cells are usually smooth and flat. In the human retina, the basal surfaces often show small irregular protrusions. These protrusions are demonstrated in TEM views of a vertical section through the inner limiting layer (Fig. 6) as well as in en-face SEM views of the layer after treatment with the HCl-collagenase technique (Fig. 7). The outer surface of the basement membrane is irregular and follows the contours, including the protrusions, of the basal surfaces of the foot processes, while the inner surface is rather smooth and flat (Fig. 6). En-face SEM view (Fig. 7) also shows that most of the profiles of the foot processes are oblong. However, the profiles of the foot processes vary according to the regions of the retina and also to the animal species.

Figures 8-10 illustrate the inner retinal surface of the three different regions in the bovine eye after removal of the basement membrane by the HCl-collagenase technique.

Fig. 11 and 12. Inner surface of the frog retina under the SEM. Fig. 11. Equatorial region. A mosaic pattern likened to a jig-saw puzzle is seen. ×5,700. Fig. 12. Peripheral region near the ora serrata. The pieces of mosaic are smaller with few processes. ×5,700
At the equatorial region (Fig. 8), the inner retinal surface shows a typical mosaic pattern somewhat like a flag-stone pavement. The elements of the mosaic are roughly oval, polygonal or oblong in shape, and the size also varies. At the peripheral region near the ora serrata (Fig. 9), their shapes become quite slender and the overall pattern appears rather vortical. Gaps between the foot processes are probably due to artificial separation during preparation of the specimen. At the optic disk region, a different pattern is demonstrated as illustrated in Figure 10. Here, the foot processes issue sheet-like lateral extensions in a lamellar fashion, which interdigitate with those from neighboring foot processes.

Another example of the regional differences in the frog retina is shown in Figures 11 and 12. At the equatorial region (Fig. 11), each element of the mosaic pattern shows highly irregular shapes like pieces of a jigsaw puzzle, and tightly interlocks with each other. At the peripheral region near the ora serrata (Fig. 12), Müller cells decrease in height and become more cylindrical. In the inner retinal surface of this region, the pieces of the mosaic are smaller and have few processes.

DISCUSSION

To observe the genuine cell surface under the SEM, we must remove the basement membrane with which the cell is covered. EVAN et al. (1976) introduced a technique for removing this covering with HCl and collagenase, and successfully revealed the basal surface of the renal epitheliocytes. This technique has been widely applied to various tissues and cells. The inner retinal surface is also covered by a basement membrane, since, embryologically, this surface corresponds to the basal surface of the retina. The present study first demonstrates the inner surface of the retina under the SEM by removing this basement membrane by the HCl-collagenase method.

The internal surface showed a characteristic mosaic pattern formed by the basal surface of the foot processes from Müller cells. The Müller cell in the retina has been studied by many light microscopists. CAJAL (1893), for example, described in detail the shape of Müller cells in various vertebrates using Golgi’s silver impregnation technique. He showed that Müller cells of the teleost retina closely resembled those of amphibians and mammals. He also mentioned that the basal portion of the Müller cell in the mammalian retina was often bifurcated at the nerve fiber layer. However, TEM observation of sectioned materials clearly indicated that the basal portion of the Müller cell divides into many small branches. These branches radiate towards the inner surface of the retina through the nerve fiber layer and terminate as foot processes on the basement membrane.

The mosaic pattern of the inner retinal surface was previously observed by light microscopy using reduced silver precipitates (PEDLER, 1961). However, PEDLER interpreted this pattern as an artificial one produced by the removal of the vitreous body and by silver staining of the tissue, forming the mosaic pattern. He assumed that this mosaic pattern is concerned with the attachment of the vitreous body.

It is evident from our observation that this mosaic pattern is formed by the foot processes of the Müller cells, and we assume that the reduced silver precipitates may represent the contour of these processes.

On the other hand, WOLTER (1961) demonstrated mosaic pattern on a frozen section stained with silver carbonate. He suggested that only the basal endings of Müller cells were stained with this technique.
What is the functional significance of the mosaic pattern? As well known, the Müller cell is a large, modified glial cell which traverses the entire thickness of the retina. The cell plays an important role in both mechanical and metabolical support for the neuronal elements in the retina. Furthermore, the vertebrate retina is often devoid of vascularization. Thus, the numerous basal foot processes of Müller cells are probably related to the metabolic activity through the cell surface, since the surface area of the cell is extensively increased by these processes. This situation somewhat resembles that of the renal epitheliocyte. The differences in the mosaic pattern according to the retinal regions as well as to the animal species may reflect the differences in arrangement and the components of the diverse neuronal elements within the retina, although the fundamental morphology of the Müller cell is similar throughout the various vertebrates examined thus far. Exact relationships between the different mosaic patterns and functional meaning remains to be detected in further investigations.

Finally, from this preparation, we cannot tell how many foot processes or mosaic elements are derived from a single Müller cell. In other words, we cannot determine the area and extent of the inner retinal surface which is covered by a single Müller cell from the basal view of the cell.

REFERENCES

Cajal, S. R. y.: The vertebrate retina. (1893) In: (ed. by) R. W. Rodieck: The vertebrate retina. W. H. Freeman and Company, San Francisco, 1973 (p. 773–904).

Evan, A. P., W. G. Dail, D. Dail, D. Dammrose and C. Palmer: Scanning electron microscopy of cell surfaces following removal of extracellular material. Anat. Rec. 185: 433–446 (1976).

Hogan, M. J. and L. Feeney: The ultrastructure of the retinal vessels. III. Vascular-glial relationships. J. Ultrastr. Res. 9: 47–64 (1963).

Hogan, M. J., J. A. Alvarado and J. E. Weddell: Histology of the human eye. Saunders Co., Philadelphia, 1971 (p. 461–490).

Pedler, C.: The inner limiting membrane of the retina. Brit. J. Ophthal. 45, 423–438 (1961).

Polyak, S. L.: The retina. University of Chicago Press, Chicago, 1941 (p. 343–354).

Villegas, G. M.: Ultrastructure of the human retina. J. Anat. (Lond.) 98: 4: 501–513 (1964).

Wolter, J. R.: Silver carbonate techniques for the demonstration of ocular histology. In: (ed. by) G. K. Smelsers: The structure of the eye. Academic Press, New York, 1961 (p. 111–138).