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Structure of the vitread face of the monkey optic disc (*Macacca mulatta*)

SEM on frozen resin-cracked optic nerveheads supplemented by TEM and immunohistochemistry

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Abstract. The authors applied frozen resin cracking after hexamethyldisilazane (HMDS) desiccation on the monkey optic disc region. The cracked face through the central part of the optic disc showed that the inner limiting membrane of the retina continued into the limiting membrane of Elschnig, and this, in turn, continued into the central meniscus of Kuhnt. At the disc margin the membrane was about 70 nm in thickness, due to a large fibrillar component. Elschnig's membrane was about 50 nm in thickness and was composed of both fibrils and flocculent material. The membrane covering the central meniscus of Kuhnt was about 20 nm in thickness. The number of fibrils here was very low, and the membrane consisted of flocculent material. The positive immunohistochemical stainings for GFA and vimentin of Elschnig's membrane and Kuhnt's meniscus were noteworthy. The positive staining disappeared when the membrane continued into the inner limiting membrane of the retina, supporting the different structural composition.

Introduction

The vitreoretinal border region of the human optic disc was first described in 1879 by Kuhnt [9] and later in 1901 by Elschnig [3]. Both stated that the internal limiting membrane, although thin, covers the optic disc except at the site of the hyaloid artery.

Wolter [11] found that the inner limiting membrane of the human retina was part of the Müller cell fibers. Consequently, the only area of the retina without Müller's radial fibers, i.e., the optic disc area, lacks an inner limiting membrane.

Anderson et al. [2] stated that the rather abrupt thinning of the basement membrane accounts for the light-microscopical impression that the inner limiting membrane of the retina ends at the disc margin.

Since we have optimized the frozen resin-cracking technique [6], which provides valuable information on the structural elements of cracked tissue, we decided to apply this technique to monkey retinas in order to visualize the structural composition of the basement membrane and the vitreoretinal border region at the optic disc.

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Materials and methods

Twelve monkey eyes (*Macacca mulatta*) were immersion-fixed in 4% phosphate-buffered neutral formaldehyde at room temperature for at least 24 h and washed in buffer. The fixed eyes were transsected, and discs of the optic disc region with retina, adjacent choroid and sclera were trephined out, using a corneal trephine 4 mm in diameter. The specimens were frozen resin-cracked in accordance with our previously published method [6]. Briefly, the discs were dehydrated in graded ethanol and embedded in Epon 812 without catalyst, frozen, and cracked into halves with a cutting edge and a hammer. The resin was removed by using propylene oxide. The specimens were desiccated for 5 min in hexamethyldisilazane and then air dried. They were glued to metal stubs with silver paste and coated with 50 nm gold palladium before SEM. All specimens were examined in a JSM-35 scanning electron microscope at 15 kV at tilting angles of 0–60. Agfapan 100 professional (ASA 100/Din 21) and positive copies enlarged three times were used for photographic recording.

For control purposes, two formalin-fixed specimens from the optic disc region were processed directly using standard procedures for transmission electron microscopy (TEM). The disc region was cut at four different levels (Fig. 1), and each level was examined separately.

Two specimens were processed and stained according to routine paraffin technique for light microscopy. Representative sections were examined for GFA, NSE, S-100 protein, and vimentin using rabbit immunoglobulins against the appropriate proteins, visualized by the PAP method.

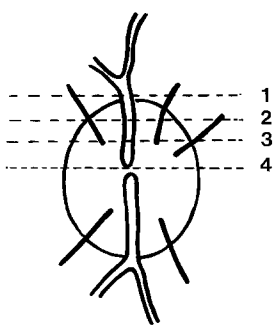


Fig. 1. Sketch of levels examined through the disc area

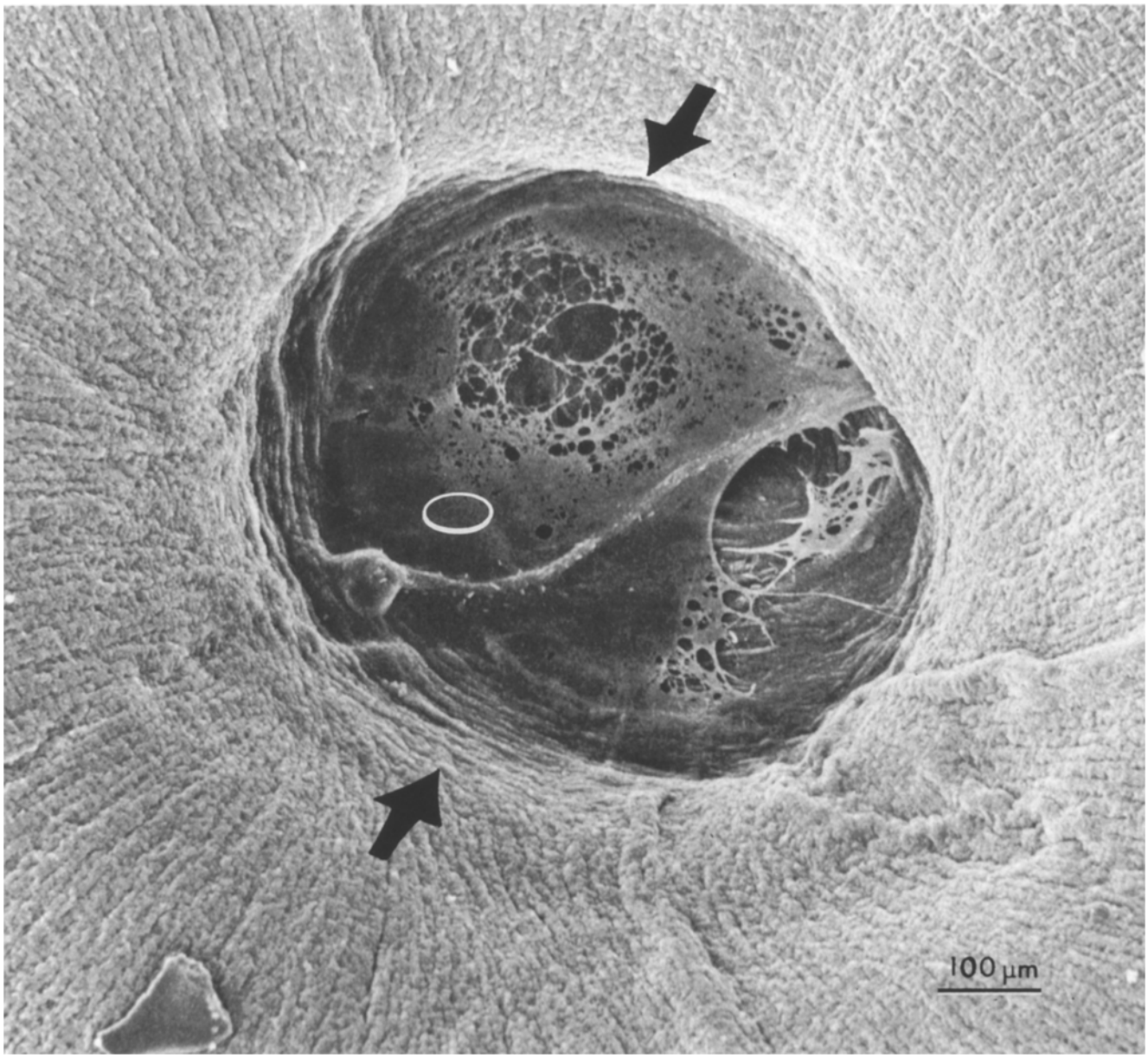


Fig. 2. SEM survey of monkey disc. *Arrows* at disc margin. *White ring* on inner limiting membrane of Elschnig (lab. no. 801b/86, SEM 1195, ×162). Bar = 100 µm

Results

The technique employed revealed the inner limiting membrane of the retina to be a homogeneous, wavy membrane. The disc margin was clearly seen (Fig. 2). Details of the inner limiting membrane of Elschnig showed a gradual thinning (Fig. 3A), and defects were seen, through which the plasma membrane of glial cells appeared (Fig. 3B). The cracked face through the central part of the optic disc showed that the inner limiting membrane of the retina continued into the inner limiting membrane of Elschnig and this, in turn, continued into the central meniscus of Kuhnt (Fig. 4).

A difference in thickness between Elschnig's membrane (midperiphery) and Kuhnt's meniscus (center of disc) was observed. The thickness in the center of the disc (Fig. 1, level 4; Fig. 5A) of the vitreopapillary border region was about the same as that of the plasma membrane of glial cells, i.e., 20 nm. The number of fibrils was very low, and the membrane consisted of a flocculent material (Fig. 5B, D, E).

At the midperiphery of the disc, the membrane was about 50 nm in thickness and followed closely the irregularities of the underlying glial cell surfaces. It was composed of both fibrils and the flocculent material (Fig. 5C, F, G). The membrane at level 1 was about 70 nm in thickness due to a large fibrillar component and smoothed out the irregularities in the surfaces of the underlying cells (Fig. 6).

The immunohistochemical staining of the membrane for S-100 and NSE was negative, whereas staining for GFA and vimentin was positive in glial sheaths of the optic nerve, connective tissue of the cribriform layer (vimentin), and the cells of the central meniscus of Kuhnt. The fact that

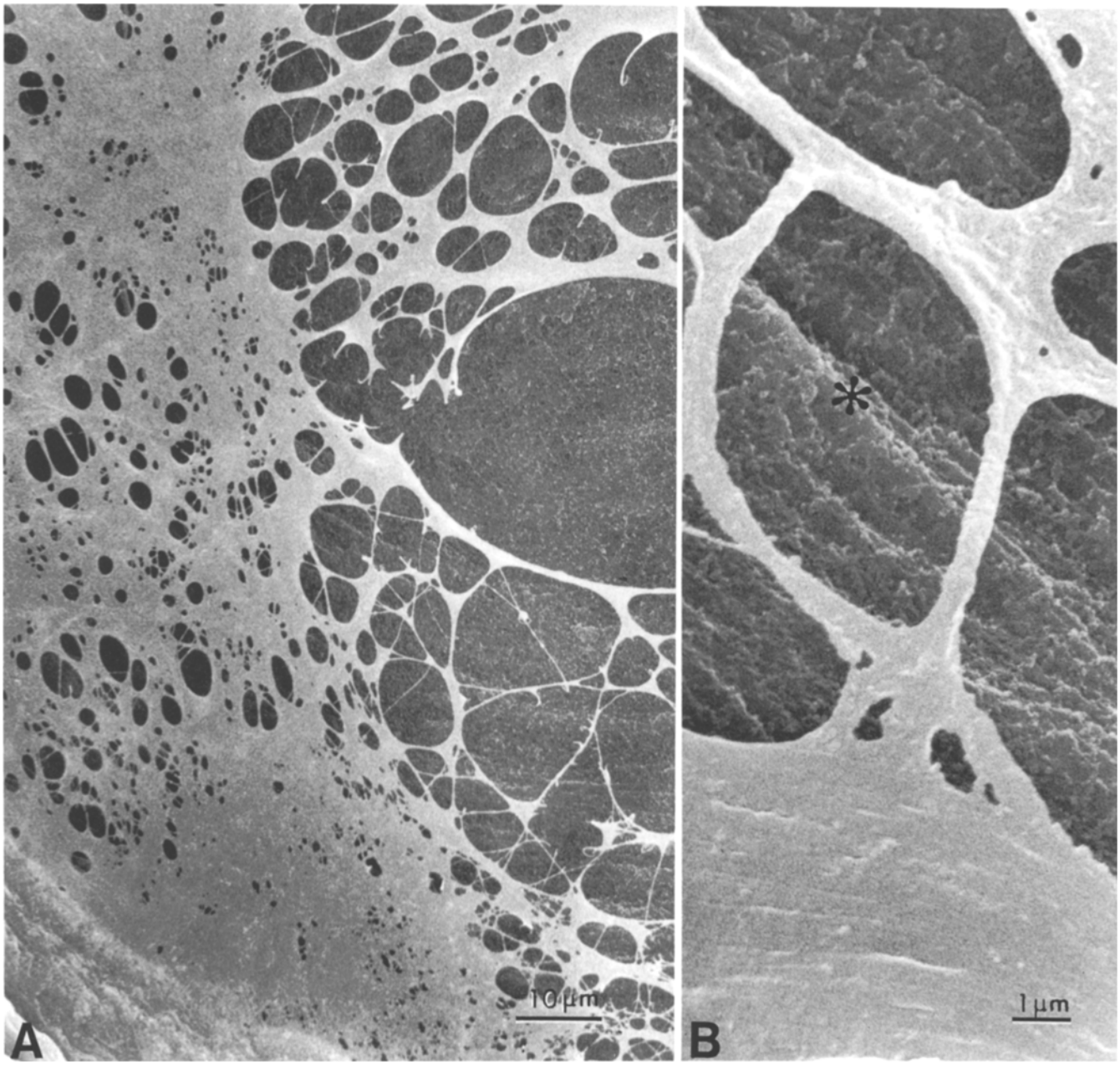


Fig. 3. A Details of inner limiting membrane of Elschnig. The gradual thinning of the membrane is seen (lab. no. 801a/86, SEM 1195, ×1320). Bar = 10 µm. **B** View of glial cells on plasma membrane (*asterisk*) seen through defects in fibrillary layer of the membrane (lab. no. 801a/86, SEM 1195, ×9000). Bar = 1 µm

GFA and vimentin of Elschnig's membrane stained positive was noteworthy, but the positive staining disappeared when the membrane continued into the inner limiting membrane of the retina.

Discussion

We found earlier that the inner limiting membrane of the retina consisted of structural elements situated between the plasma membrane of Müller cells and the vitreous. We were able to distinguish a dense fibrillar meshwork close to the plasma membrane and a loose net of fibrils reaching from the denser parts into the vitreous proper [7].

By using the methods presented, we have now been able to follow the vitreopapillary border region from the inner limiting membrane of retina through four levels of the nervehead. The study confirmed the fast thinning-out of the border region at the optic disc, as previously described by Anderson et al. [2], Gärtner [5], Anderson [1], Hogan [8], and Roth and Foos [10], but we found no thickening of this border membrane, as stated by Kuhnt [9] and Elschnig [3], at the central meniscus of Kuhnt. The inner limiting membrane did not end at the margin of the normal optic disc either, as stated by Wolter [4]. Our findings seemed to support the statements by Anderson et al. [1, 2]: that the abrupt thinning of the basement membrane probably

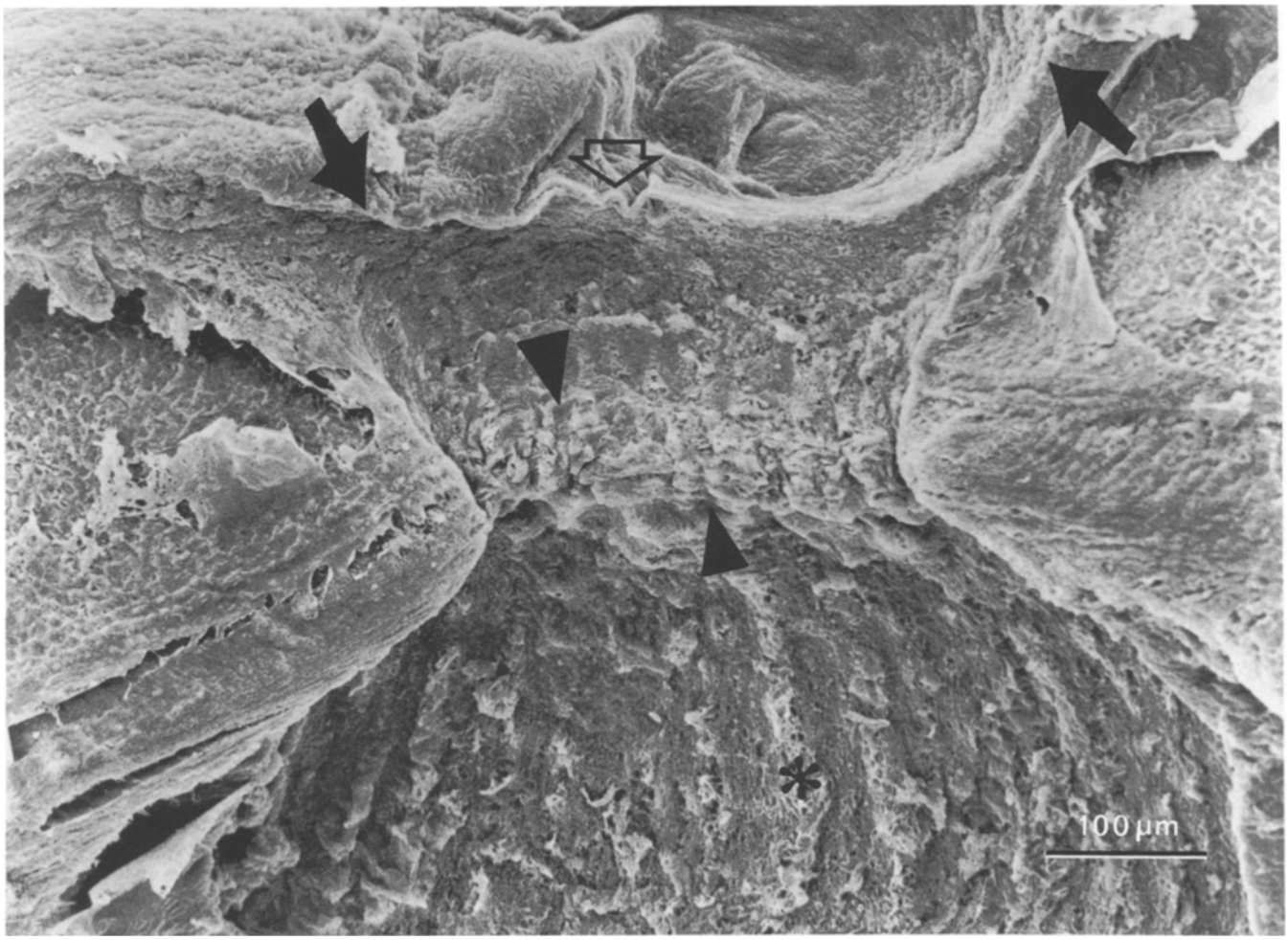


Fig. 4. Cracked face through central part of disc. *Open arrow* on inner limiting membrane of Elschnig. *Arrows* on disc margin. *Arrowheads* on cribriform layer of disc. *Asterisk* on optic nerve (lab. no. 800b/86, SEM 1194, ×224). Bar = 100 µm

accounts for the impression gained in light microscopy that the inner limiting membrane of the retina ends at the disc margin.

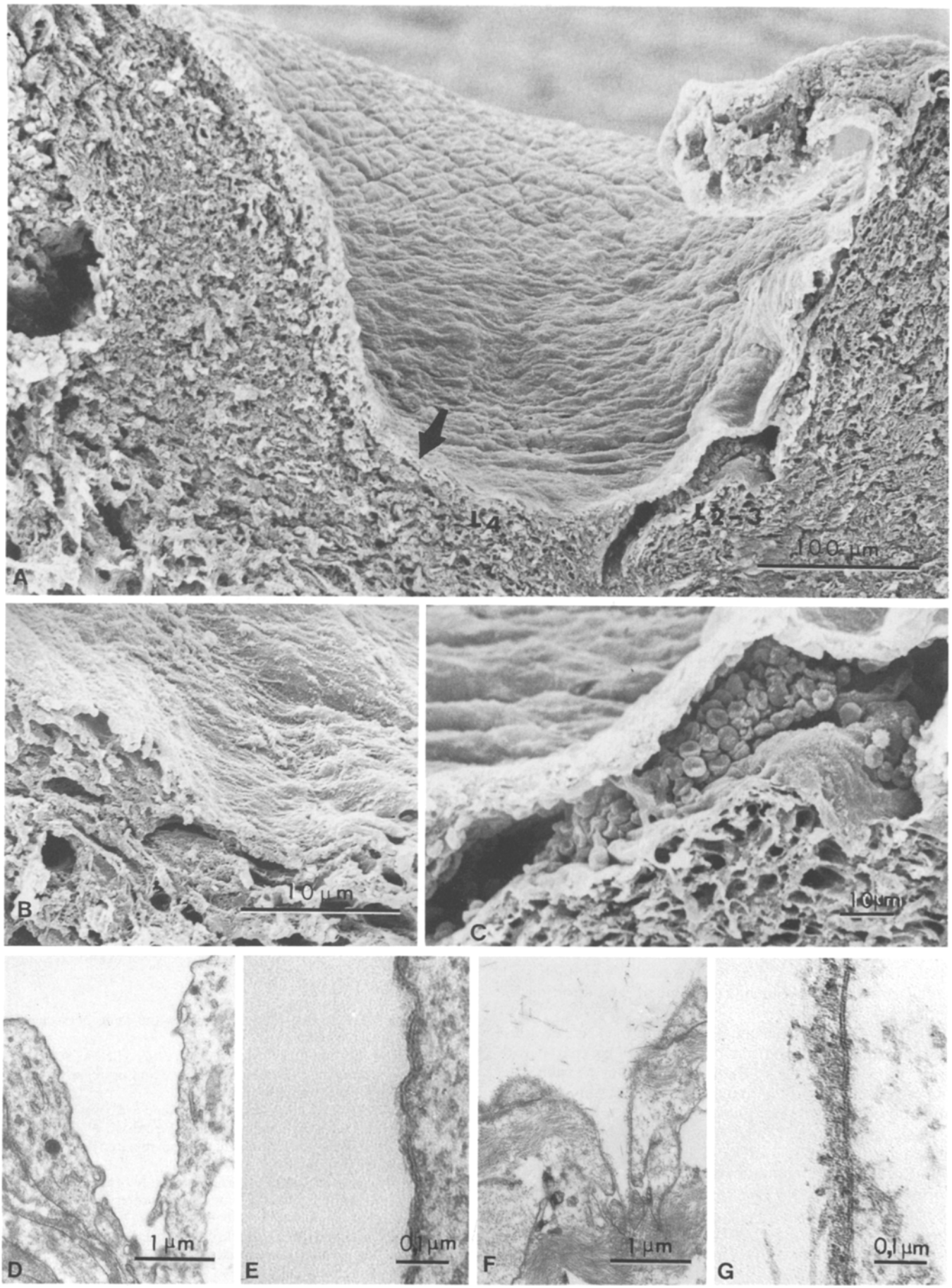
The inner limiting membrane of Elschnig was about 50 nm in thickness, and the part covering the central meniscus of Kuhnt was about 20 nm, which is in agreement with the findings of Hogan et al. [8], although we were not able to verify his finding of a less-dense zone between the membrane and the cell membrane. This thinning-out of the vitreopapillary border region may indicate the area in which sheaths of the fetal hyaloid artery fuse with the papillary face, supplementing this with mesodermal material, as indicated by the positive reaction for vimentin. Another explanation may be that the basement membrane is thin because the underlying cells are astrocytes, which would account for the positive GFA reaction.

The inner limiting membrane of Elschnig consisted of fibrils and of a flocculent material and did not contain the spider-cell nuclei found by Elschnig [3]. This discrepancy may perhaps be ascribed to differences between monkey and man, or to the fact that Elschnig, using light microscopy, was unable to distinguish between the limiting membrane itself and the underlying cellular layer.

The central meniscus of Kuhnt consisted of a thin flocculent material covering the plasma membrane of glial cells. It was of even thickness and followed the irregularities of the glial surface. This is in contrast to the thick membrane over the retina, which fills in the irregularities of the glial surface and presents a smooth face towards the vitreous, as also stated by Anderson et al. [2], Anderson [1], and Roth and Foos [10].

Defects can be seen in the basement membrane at the

Fig. 5A–G. Lab. no. 802b/86, SEM 1196. **A** Cracked face through central part of disc. *Arrow* on inner limiting membrane of Elschnig. Levels 4 (L4 = center of disc) and 2,3 (L2,3 = midperiphery) are shown in **B** and **C** (×300). Bar = 100 µm. **B, C** Levels 4 and 2, 3, as indicated in **A**. Note the difference in thickness in the center and at the midperiphery (**B** ×3000; C, ×1000). Bars = 10 µm. **D, E** TEM of level 4 (center). Partial lack of fibrillar components is seen in **D** (×15000). Bar = 1 µm. Here the membrane consists of thin flocculent material on the plasma membrane of glial cells, visible only in high magnification (**E** ×99000). Bar = 0.1 µm. **F, G** TEM of level 2, 3 (midperiphery). In **F**, the inner limiting membrane of Elschnig is thin, but continuous and follows the irregularities of the underlying glial cell surfaces (**F** ×15000). Bar = 1 µm. In high magnification, the membrane is seen to be composed of fibrils and of flocculent material (**F** ×99000). Bar = 0.1 µm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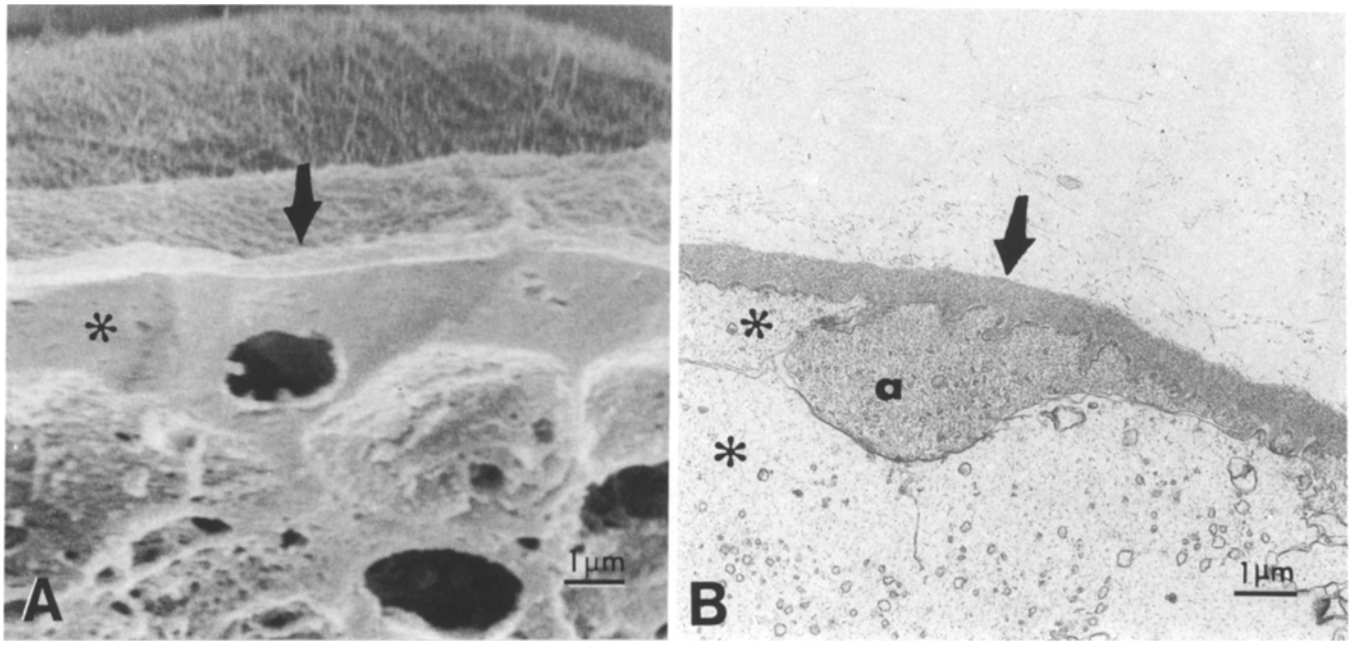

Fig. 6A, B. SEM and TEM of the membrane at level 1 (*arrows*). Note the thickness of the membrane due to the large fibrillar component. Müller cells (*asterisks*) underlie a large part of the membrane with a possible astrocyte (*a*) in between. The thick membrane fills all irregularities in the surfaces of the underlying cells (lab. no. 804/86, SEM 1052, ×7800). Bars = 1 µm

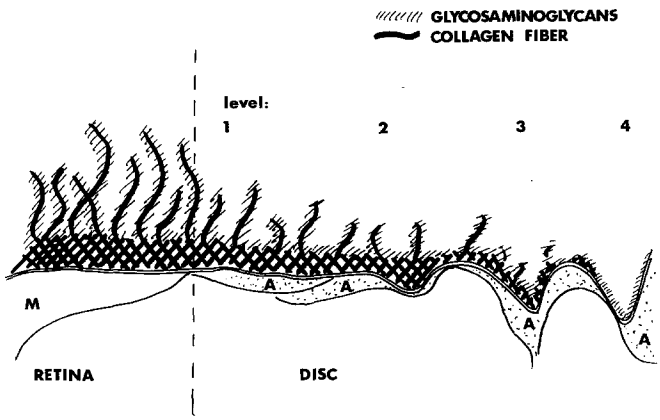

Fig. 7. Sketch of vitreous-disc border region based on the present study. **A** = astroglia, **M** = Müller cell

optic disc that are probably due to shrinkage artifacts such as the observed detachment of the membrane covering the optic disc.

The very thin membrane of Elschnig may act as a less efficient anatomical and functional barrier compared with the limiting membrane of the retina. This may explain the clinical observation that retinal-epiretinal vessels are most often observed at the nervehead.

This less efficient barrier function may be further supported by the findings of Roth and Foos [10] and Foos and Roth [4] that approximately 50% of 504 enucleated normal human autopsy eyes had an epipapillary membrane. It was composed of flat glial cell processes protruding through gaps in Elschnig's membrane.

The observations found in the present study may be expressed as sketched in Fig. 7. In short, at the disc margin the inner limiting membrane of Elschnig continues uninterruptedly and peripherally into the inner limiting membrane of the retina, and is composed of three main layers: the plasma cell membrane of Müller cells, a thick basal membrane, loose collagen fibrils intermingling with the vitreous, covered by glycosaminoglycans (GAG).

Approaching the center of the disc, the Müller cell plasma membrane is replaced by the membrane of astroglia. The thick basal membrane is thinned out and is lost centrally. The fibrils are reduced in number and length, and centrally only GAG remain on the plasma membrane surface.

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