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Macular pucker

II. Ultrastructure*

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Abstract. Twenty symptom-producing epimacular membranes removed during vitreous surgery were examined by light microscopy, scanning and transmission electron microscopy. These membranes contain cells of glial and pigment epithelial origin, but one also finds myofibroblasts and fibroblasts which cannot be identified morphologically as to their origin. The membranes can be classified into two types. Membranes in one group are composed of numerous alternating layers of collagen and cells and some internal limiting lamina. The second type of membrane is generally composed of a single layer of cells with large sheets of internal limiting lamina from the retinal surface and little if any collagen. Both types of membranes have cells on the retinal side of the removed internal limiting lamina, presumably derived from neurosensory retina.

Zusammenfassung. 20 symptomatische epimakuläre Membranen wurden mikrochirurgisch entfernt und histologisch, elektronenmikroskopisch und mit dem Rasterelektronenmikroskop untersucht. Die Membranen enthalten Zellen gliösen und pigmentepithelialen Ursprungs sowie Fibroblasten und Myofibroblasten. Die Membranen können in zwei Gruppen eingeteilt werden. Die erste Gruppe enthält abwechselnd Schichten von Kollagen und Zellen sowie Lamina limitans interna. Die zweite Gruppe besteht aus einer Lage von Zellen ohne Kollagen aber mit riesigen Stücken der Lamina limitans interna. In beiden Gruppen findet man Zellen auf der retinalen Seite der Lamina limitans interna, die wahrscheinlich von der Retina selbst stammen.

Introduction

Macular epiretinal membranes have been referred to by a variety of names, among them macular pucker, surface wrinkling retinopathy, and premacular fibrosis (Gass 1977; Roth and Foos 1971; Bellhorn et al. 1975). These names have been determined primarily by either clinical or pathologic studies. With the advent of vitreous surgery and a special biopsy system (Hickingbotham et al. 1981) it is possible to peel these membranes maintaining in vivo orientation and study their microscopic characteristics compared to their clinical appearance. This study of 20 surgically removed epimacular membranes was designed to describe the ultrastructural detail of these membranes by scanning and transmission electron microscopy. This can be correlated to their clinical appearance. We suggest reasons why puckering occurs preferably in the macula and point out that removal of internal limiting lamina is very frequent and seemingly well tolerated.

Materials and methods

Twenty macular epiretinal membranes were removed during vitreous surgery using special biopsy forceps (Hickingbotham et al. 1981), which maintains orientation of the membrane after removal from the eye. Immediately after removal from the eye, the membrane still held in the forceps, was washed for 2 min in 0.1 M cacodylate buffer with 5% sucrose (pH 7.4) to remove the vitrectomy infusion fluid. The membrane was then placed in 2% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) for 45 min, removed from the forceps and mounted on a specimen stub, which retained orientation of the membrane throughout the remainder of specimen preparation (Hickingbotham et al. 1981). The membrane was postfixed in 2% osmium tetroxide in 0.1 M cacodylate buffer for 1 h, washed in buffer, stained en bloc with 3.5% uranyl acetate for 1 h, and dehydrated in a graded ethanol series. In the final dehydration step, one-half of each specimen was cut away and placed in a flat embedding mold and infiltrated with plastic embedding medium. Following polymerization 0.5–1.0 μm sections were cut and stained with toluidine blue and basic fuchsin for light microscopy, and 60-nm sections stained with uranyl acetate and lead citrate were examined by transmission electron microscopy.

The remaining half of the specimen was critical-point dried from carbon dioxide, sputter coated with gold-palladium, and examined by scanning electron microscopy in the backscatter and secondary electron imaging modes.

Two membranes were serially sectioned and studied by light microscopy to histologically confirm their general topography after observation by scaning electron microscopy.

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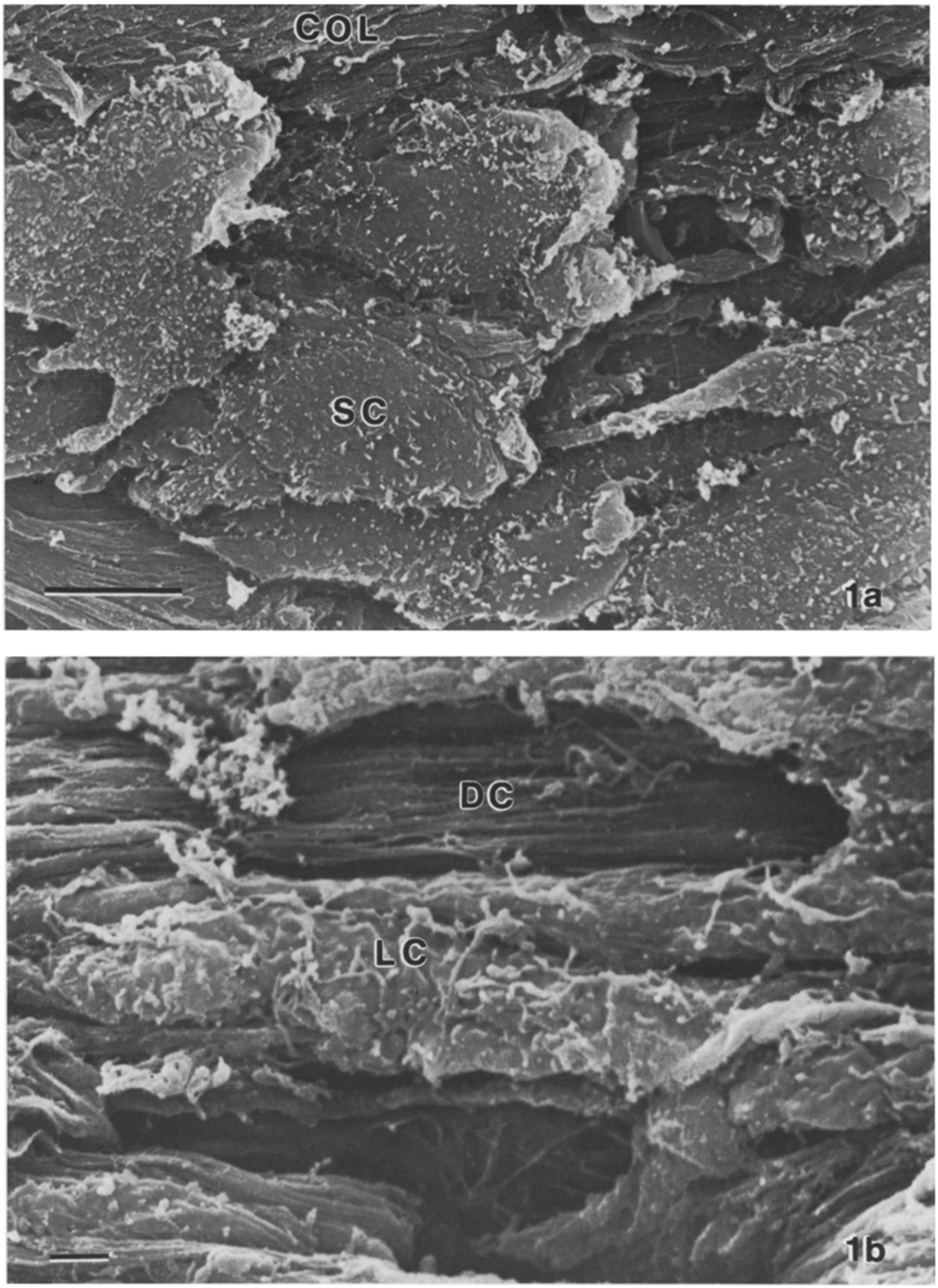


Fig. 1. a Scanning electron micrograph of the vitreal surface of a multilayered membrane. Foci of stellate cells (*SC*) in scale-like arrangement with numerous microvilli, seen on a layer of loosely packed collagen (*COL*). Bar = 10 μm. **b** Through voids in the layer of loosely packed collagen (*LC*), a third more consistently oriented layer of densely packed collagen is seen (*DC*). Bar = 1 μm (same specimen as Fig. 1 a)

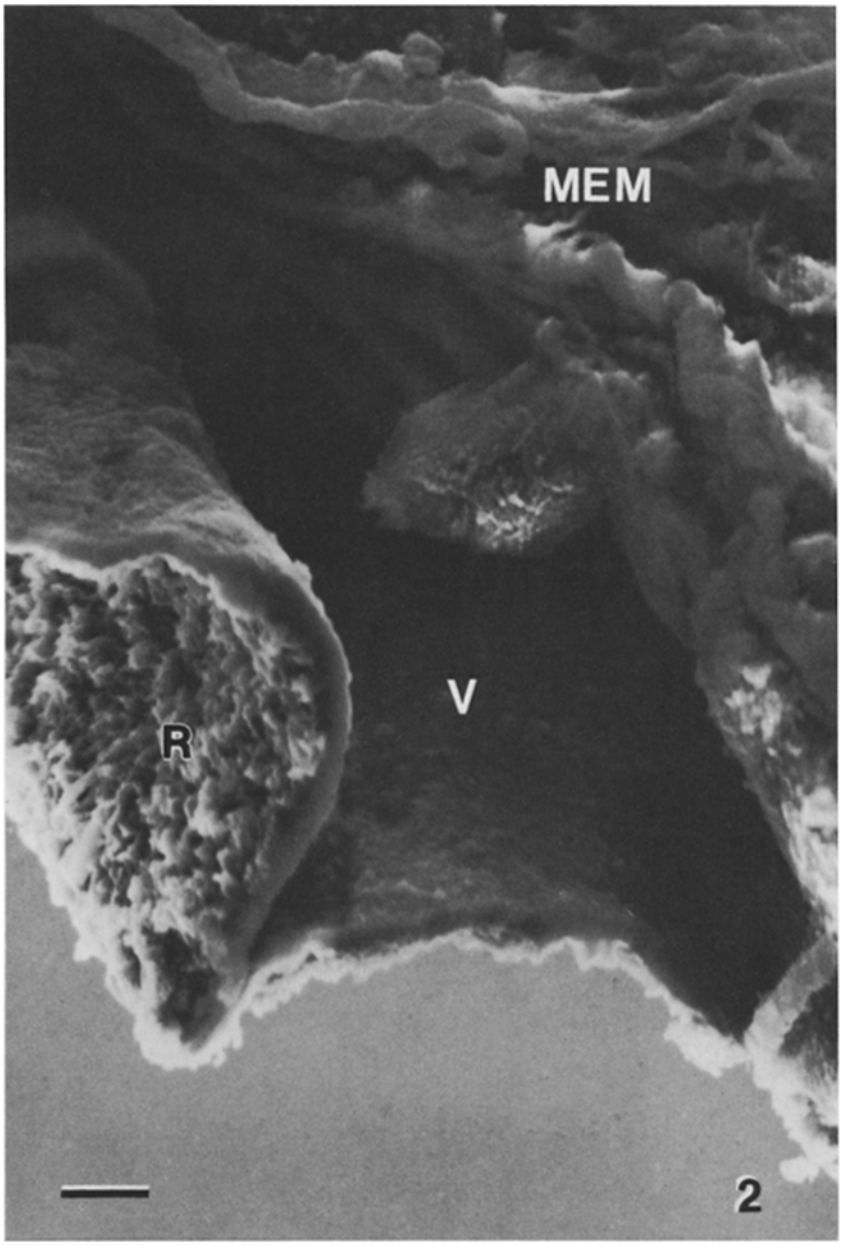


Fig. 2. Scanning electron micrograph, with multilayered membrane (*MEM*) on the smooth vitreal side (*V*) of internal limiting lamina of the retina. The irregular retinal surface (*R*) of the internal limiting lamina faces away from the membrane. Bar = 10 μm

Results

Each of the 20 epimacular membranes obtained by biopsy was placed in one of two classifications according to their histological and ultrastructural appearance. Cross-sectioned membranes which revealed several distinct layers of cells and collagen were placed in one group, and membranes showing only a single layer of cells on internal limiting lamina (ILL) comprised the second group.

Multilayered membranes

Scanning electron microscopy (SEM) of the anterior (vitreal) surface of multilayered membranes showed foci of stellate cells which overlapped each other in a scale-like arrangement (Fig. 1 a). These cells had numerous microvilli on the anterior surface. Between the cells, the next layer of the membrane could be seen. This consisted of loosely packed collagen with no particular directional arrangement (Fig. 1 b). Through voids in this loose collagen layer, a third layer formed by densely packed bundles of collagen fibers was seen. Visualization of deeper layers of the membrane from the vitreal side was not possible. When the specimens were turned over and viewed form the posterior (retinal) side, pieces of basal lamina attached to a layer of fine, randomly arranged collagen fibrils, or to cells on the posterior surface of the membrane were found. The basal lamina material was between 500 and 1,000 Å thick and had a smooth surface facing the membrane, and an irregular surface facing away from the membrane (Fig. 2). In the areas with no basal lamina the cells on the posterior surface of the membrane were fusiform in shape with long cell processes which often intermeshed with the fine collagen fibers (Fig. 3).

When multilayered membranes were viewed by transmission electron microscopy (TEM) the lamina on the posterior surface of the membranes was found to be consistent

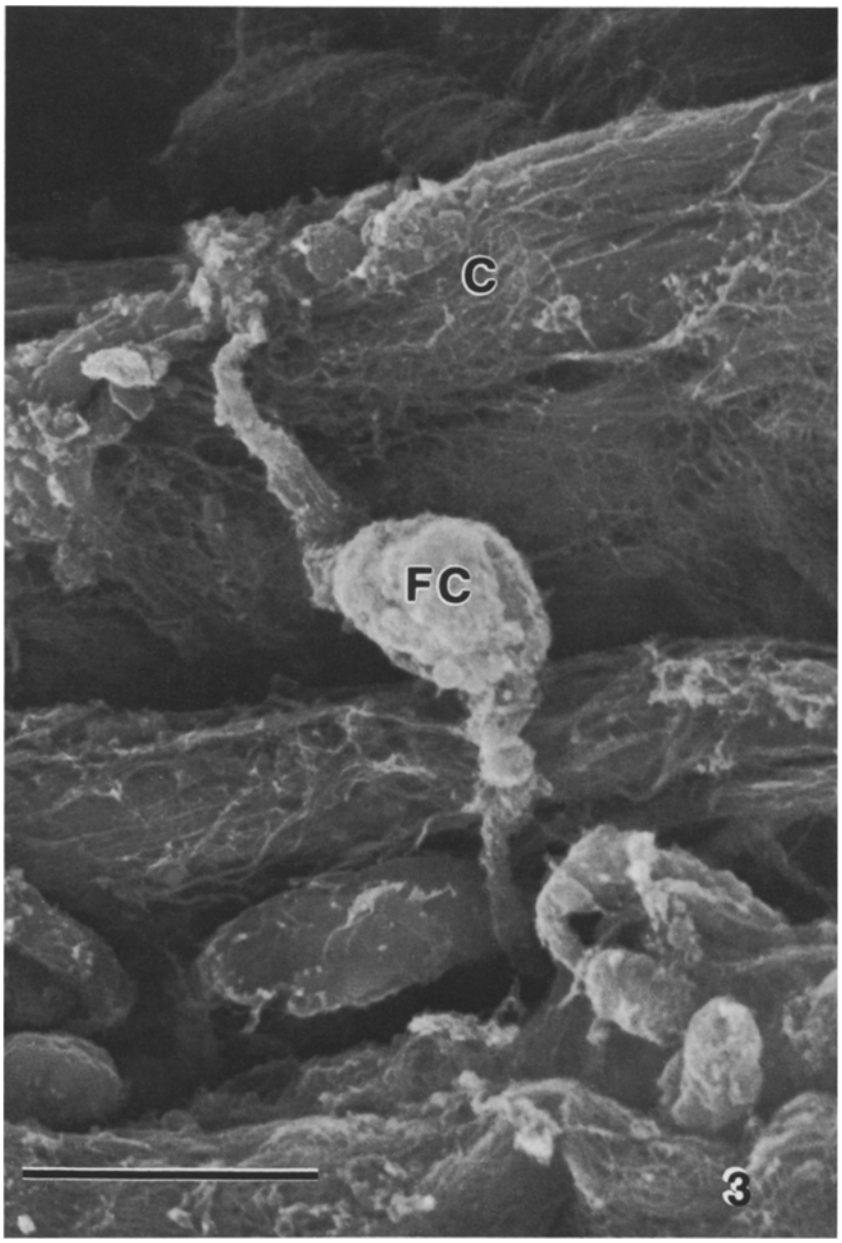
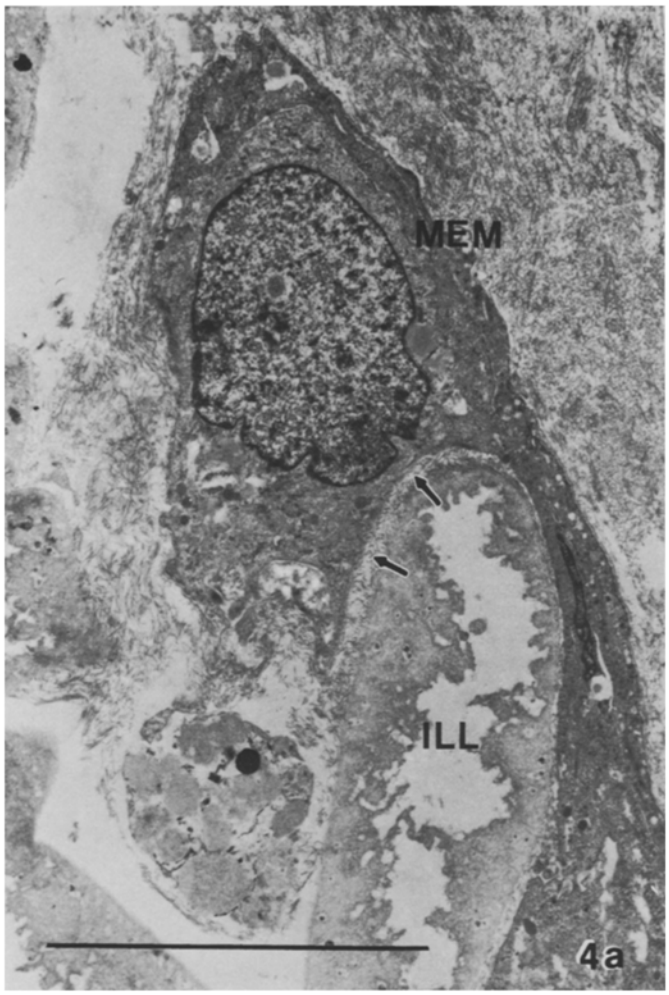


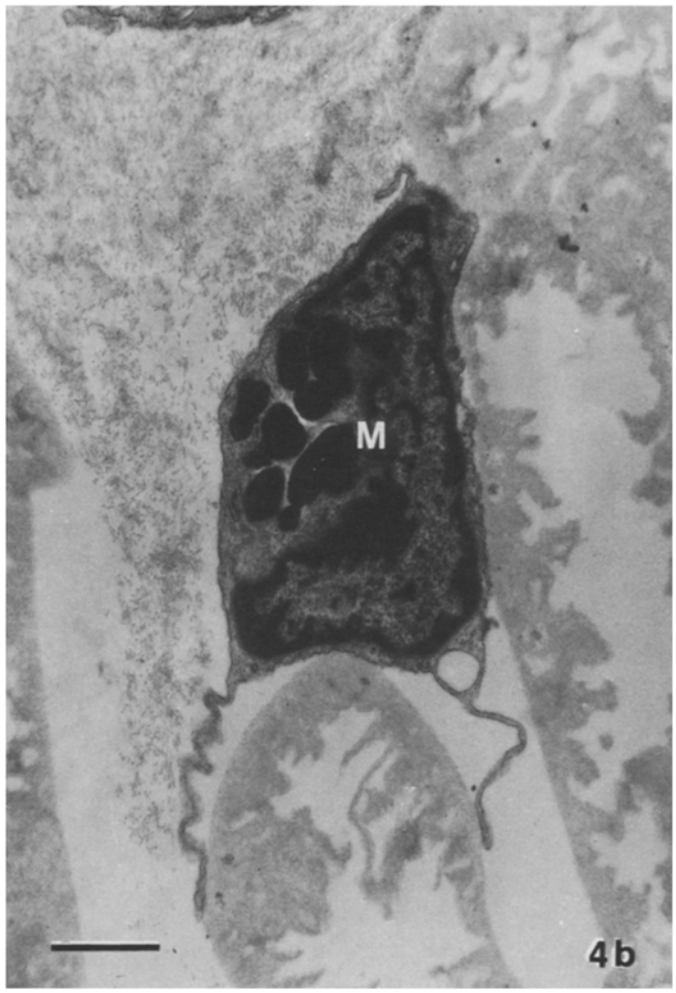
Fig. 3. Posterior surface of multilayered membrane showing fusiform-shaped cells (*FC*) intermeshing with fine collagen fibers (*C*) in areas where basal lamina is missing. Bar = 10 μm

with the internal limiting lamina (ILL) of the retina. The ILL had a smooth surface which faced the membrane, and an irregular surface which faced away from the membrane (Fig. 4a, b). Fine collagen fibers (~20-nm diameter) seen between the posterior surface of the membrane and the ILL had a 59-nm periodicity and appeared to fuse into the internal limiting lamina. Denser, more organized collagen bundles found in the deeper layers of the membranes were banded (59-nm periodicity) and had diameters ranging from 15 to 80 nm (Fig. 4a).

Several types of cells were found in the mutilayered membranes (Table 1). Cells containing abundant intracytoplasmic filaments closely resembling glial cells (Fig. 4c), as well as cells containing the peripherally located fibrils and dense bodies usually associated with myofibroblasts were seen (Fig. 4d). Cells containing numerous desmosomes, junctional complexes and non-membrane-bound pigment common to pigment epithelial cells were found (Fig. 4e). Other cells containing membrane-bound pigment and having long cytoplasmic processes consistent with macrophages were present (Fig. 4b). Nearly all of the cells within the membrane were surrounded by collagen fibers which were directionally aligned. The collagen surrounding the myofibroblasts appeared to be more organized than the collagen surrounding non-contractile cells and frequently lay coaxially to the myofibroblast.

Commonly, well-developed cell junctions and desmosomes were identified as the attachment mechanism between cells in the multilayered epiretinal membrane (Figs. 4e, 5). Where the cells were directly adherent to the internal limiting lamina, attachment plaques similar to those described by Foos (1972) and Bellhorn et al. (1975) were found. An additional type of attachment site was also found, which appeared as a dense peak fusing with the





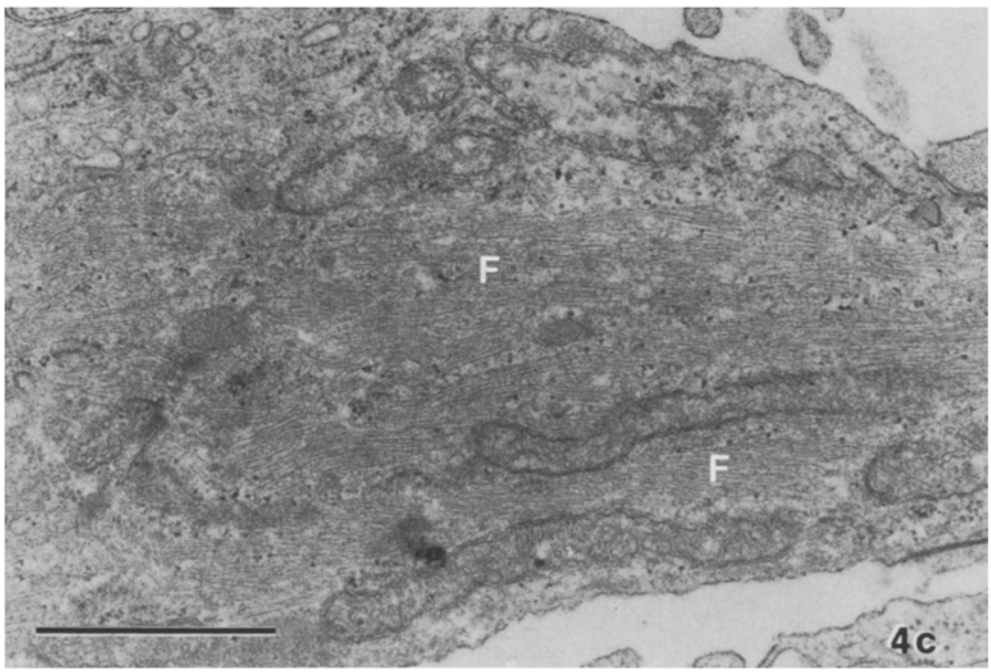
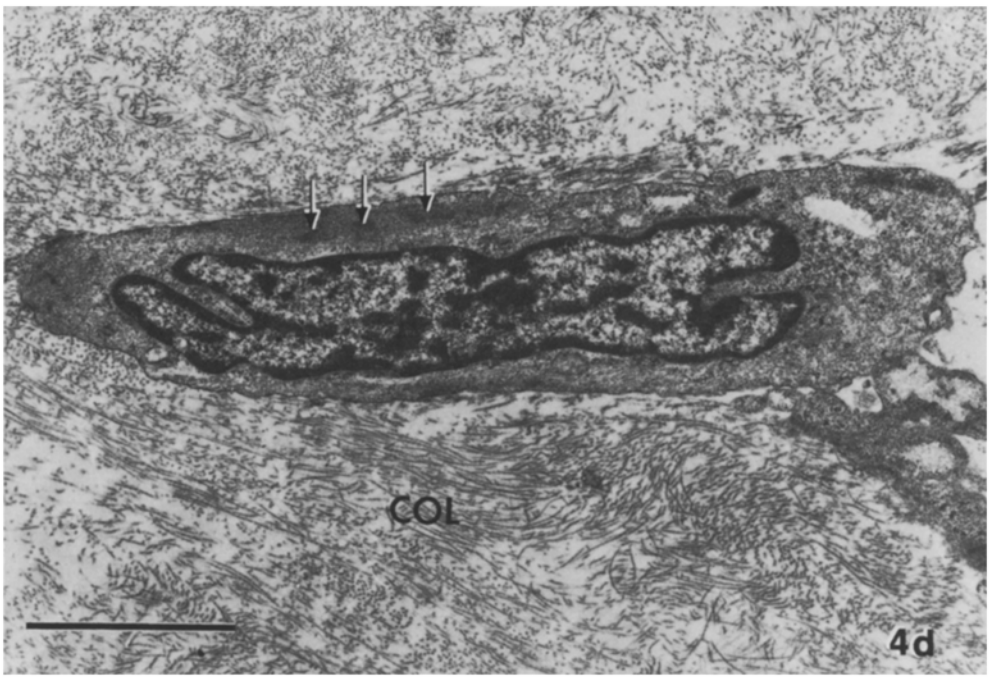


Fig. 4a–e. Transmission electron micrograph of various cell types found in multilayered membranes. **a** Multilayered membrane (*MEM*) with thin layer of collagen (*arrows*) between internal limiting lamina (*ILL*) and the cell. Bar = 10 μm. **b** Macrophage (*M*) on surface of the internal limiting lamina. Bar = 1 μm. **c** Cell containing many intracytoplasmic filaments (*F*) closely resembles glial cell. Bar = 1 μm. **d** Cell containing fibrils and dense bodies (*arrows*) resembles myofibroblast, surrounded by aligned collagen (*COL*). Bar = 1 μm. **e** Cell containing non-membrane-bound pigment and cell junctions suggests pigment epithelial cell origin. Bar = 1 μm



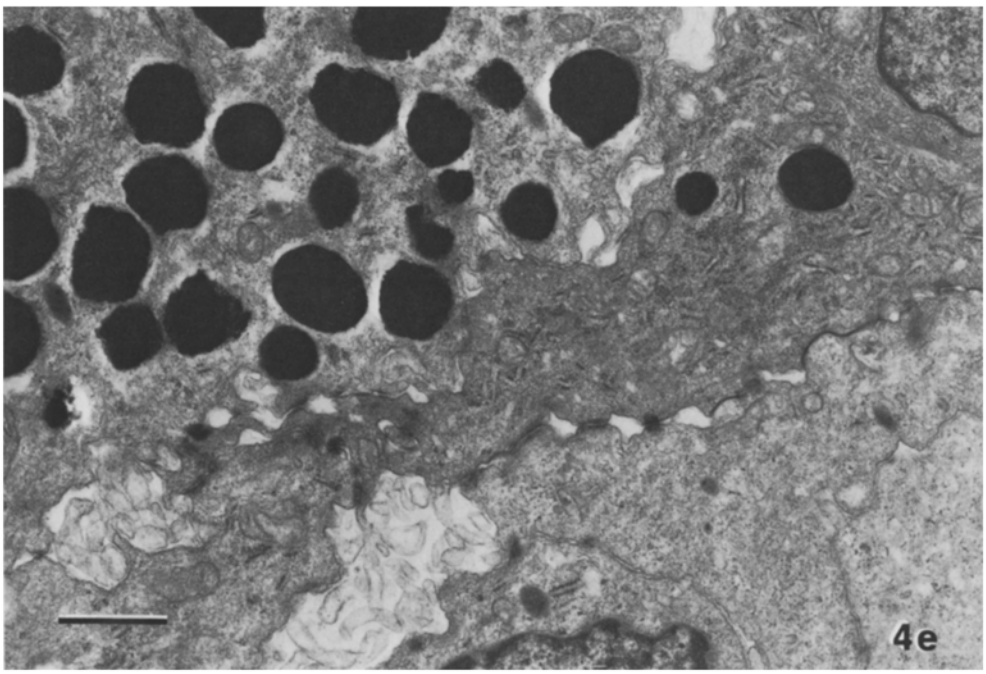


Fig. 4d, e

ILL (Fig. 6). No attachment plaques were seen when the previously described thin layer of collagen was present between the ILL and the membrane.

Single cell layer membranes

SEM of single cell layer membranes showed large sheets of basal lamina with foci of cells on its anterior surface (Fig. 7). Very little collagen was found in these membranes, thus the cells were attached directly to the basal lamina (Fig. 8). TEM confirmed that the basal lamina material seen in these membranes was internal limiting lamina of the retina. The types of cells found in single-layer membranes were primarily fibroblast-like. Cells of glial origin were seen more frequently in single cell layer membranes than in multiple layered membranes. Pigmented cells were rarely seen (Table 1). Attachment plaques like those described for multilayered membranes were much more numerous in the single-layered membranes.

In both the single and multilayered membranes, cells were seen on the retinal surface of the internal limiting lamina of the peeled surgical speciemens. These cells conformed precisely to the irregular surface of the internal limiting lamina and had numerous intracellular filaments and mitochondria indicating glial origin (Fig. 9).

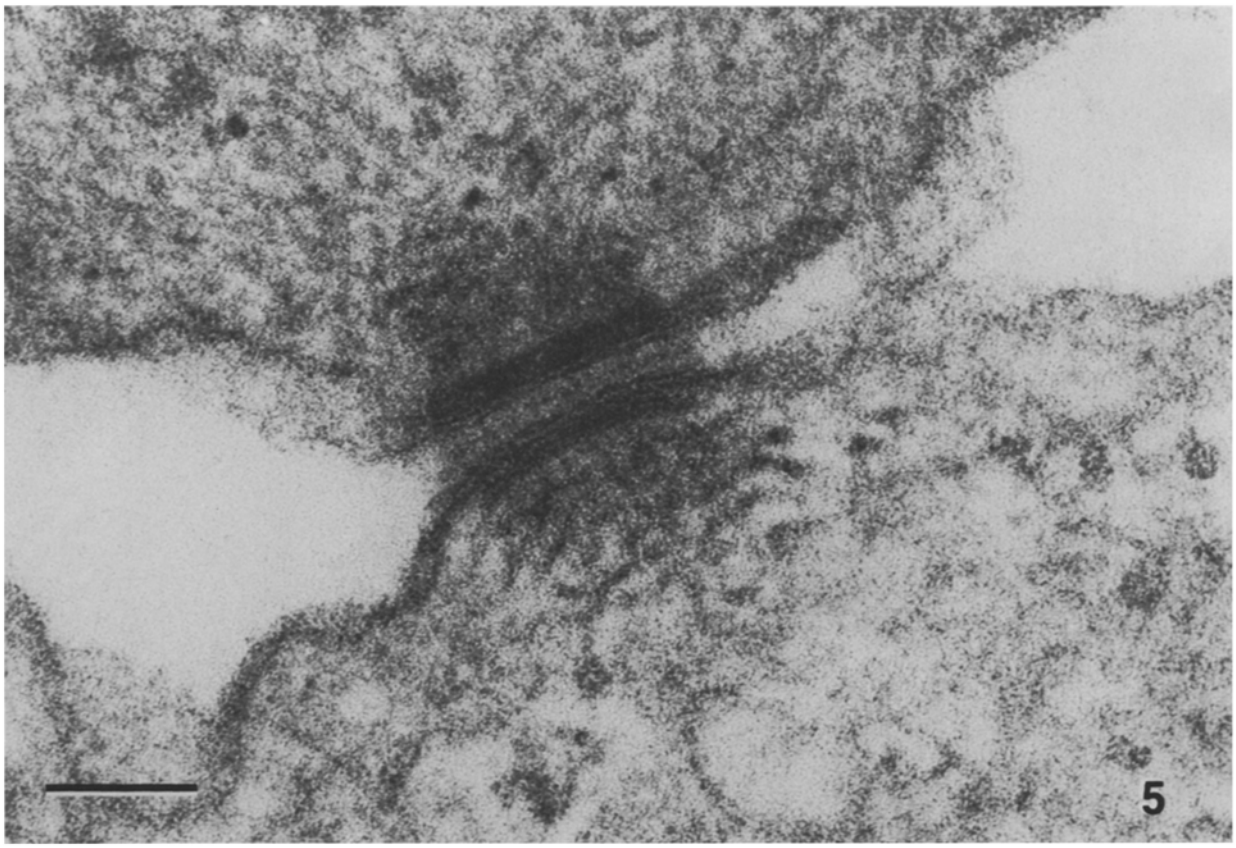


Fig. 5. Desmosome, frequently found between cells in both multi- and single-layer membranes. Bar = 0.1 μm

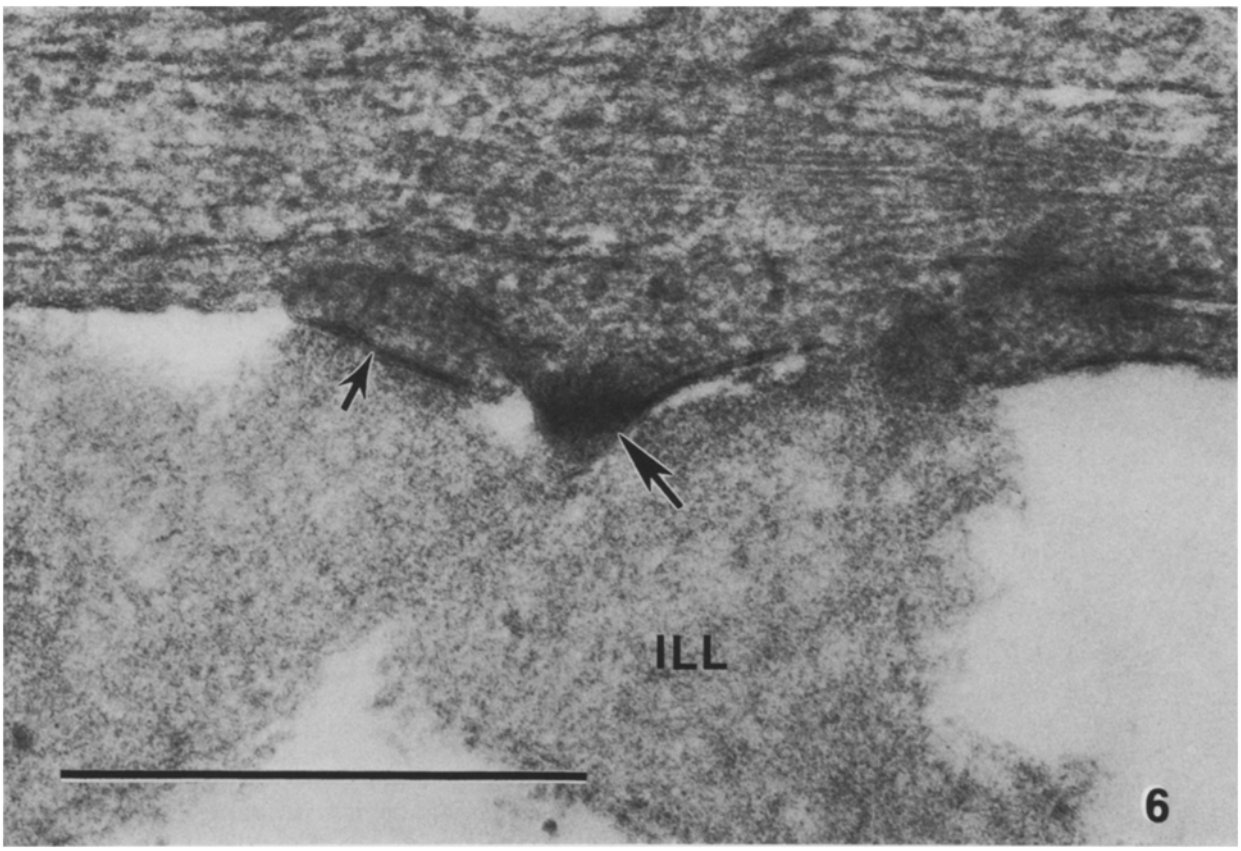


Fig. 6. Attachment sites to internal limiting lamina (*ILL*) found in epimacular membranes. *Small arrow* indicates plaques as described by Foos (1972). *Large arrow* indicates a second type of plaque consisting of a dense peak which fuses with the ILL. Bar = 0.5 μm

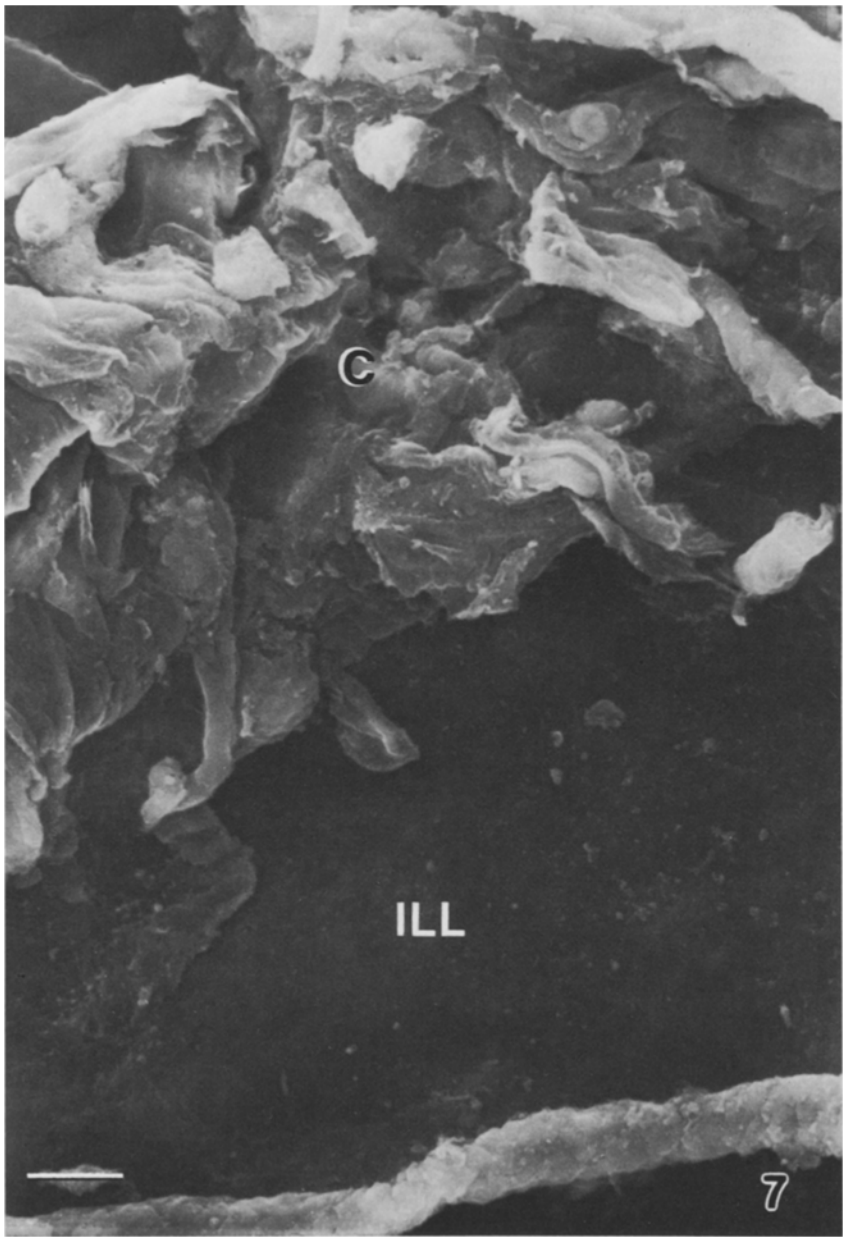


Fig. 7. Scanning electron micrograph of single-layer membrane showing large sheet of internal limiting lamina (*ILL*) with focal areas of cells (*C*) on the smooth vitreal surface. Bar = 10 μm

Discussion

Gass (1977) has subdivided macular epiretinal membranes into three clinical groups: clear, crinkled, and opalescent. Using histological specimens from enucleated eyes, Foos has divided the retinal membranes into a simple retinal surface wrinkling membrane, and a complex type of membrane with more cellular and collagenous material (Roth and Foos 1971; Foos 1974; Foos 1977a).

Material used in this study was obtained by biopsy from eyes in which epiretinal membrane formation lead to reduced vision. These membranes were divided into two groups based on their ultrastructural appearance. The first type was a multilayered membrane containing a variety of cells and large amounts of collagen with varying amounts of internal limiting lamina of the retina. The second type of membrane consisted of a single layer of cells and little or no collagen, but large sheets of internal limiting lamina of the retina were present.

Based on our histologic and ultrastructural observations we are inclined to agree with Foos' classificaton (Roth and Foos 1971; Foos 1974; Foos 1977a). Our clinical observations, published in a separate paper, also favor two basic types of membranes (Trese et al. 1983).

When the two types of membranes were compared, two main differences were noted: (1) the amount of collagen and (2) the amount of internal limiting lamina removed with it.

It is interesting to speculate why one type of membrane contains large amounts of collagen and the other very little. One possible explanation is that the collagen present in the membrane might be vitreal collagen incorporated into the layers of the membrane during its formation. It has been shown that following posterior vitreous detachment, cortical vitreal collagen remains on the internal limiting lamina of the retina (Foos 1974). It may be possible that the cells forming the membrane would then grow on or into this vitreous collagen, and therefore the amount of

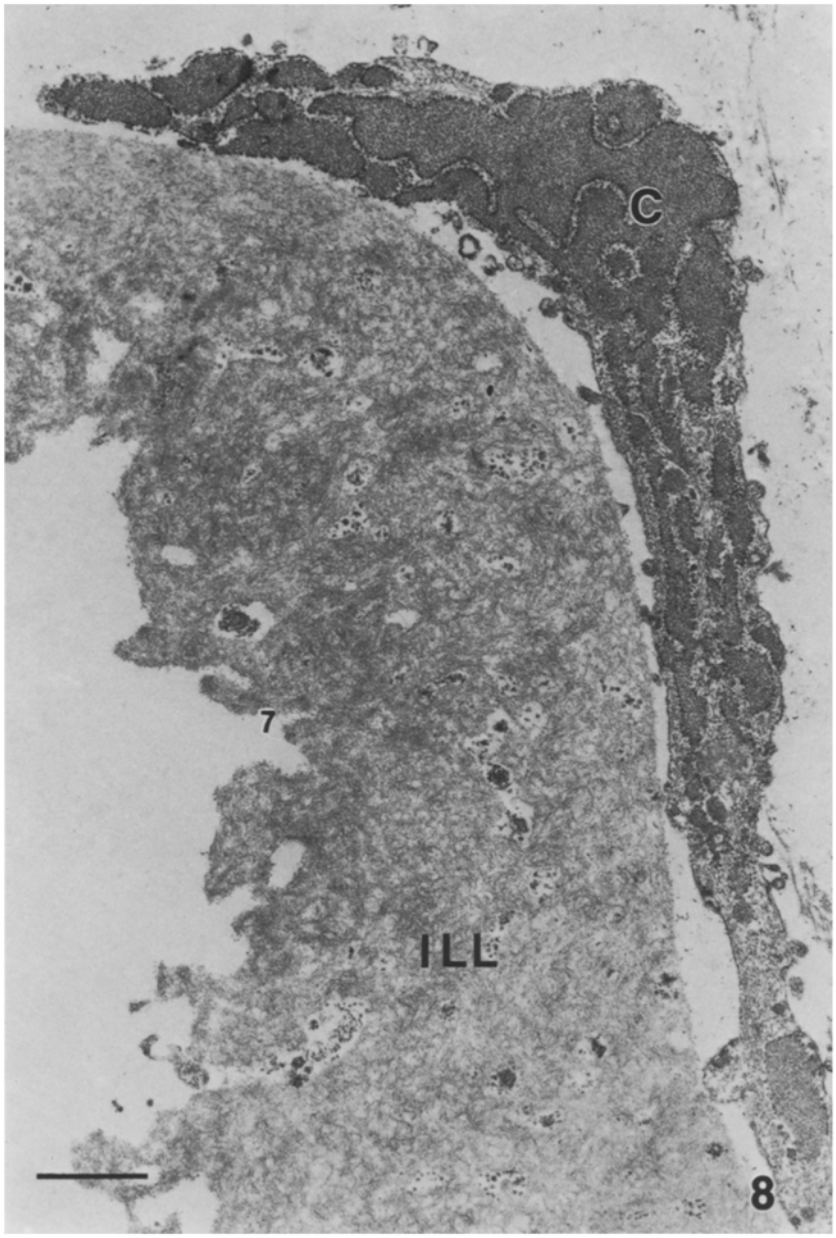


Fig. 8. Transmission electron micrograph of fibroblast-like cell (*C*) on surface of internal limiting lamina (*ILL*) with practically no collagen interposed. Bar = 10 μm

collagen in an epiretinal membrane would depend solely on the amount of remaining cortical collagen present on the ILL at the time of membrane formation. We do not believe that this is an important factor, because the amount of collagen found in some of these membranes is much greater than one would expect from simple collapse of vitreous. More importantly, the fibers are directionally aligned (Figs. 1b, 4d) in a way we would not anticipate after vitreous collapse.

Another more likely explanation for the large amounts of collagen in some membranes is that the collagen is produced by the cells in the membrane. This would not exclude the presence of vitreal collagen in initial membrane formation, but it would explain the progressive formation of cell and collagen layers as the membrane grows thicker. We do not feel that the measurement of collagen fiber diameter is a definitive means of differentiating between collagen that is native to the vitreous body and collagen that may be produced by proliferating cells in our specimens. Vitreous collagen typically ranges between 10 and 25 nm in diameter, and occasionally shows banding at 12–25 nm. Interstitial collagen, such as that which may be formed by proliferating cells, commonly has a 64-nm periodicity, and in our specimens the diameters ranged from 15 to 80 nm. This overlap in diameter prevents us from consistently distinguishing vitreal collagen from interstitial collagen by morphologic means. However, the smaller-diameter fibers, where overlap occurs, were rarely found between the cell layers.

The difficulties in gathering accurate historical information as to the length of time a membrane has been present within the eye makes it impossible to determine whether the age of the membrane relates to the amount of collagen present. However, the amount of collagen relates well to the clinical appearance of the membrane (Trese et al. 1983) (Table 1).

A second difference between the membranes was that various amounts of internal limiting lamina were present in these specimens. A review of the attachment mechanisms

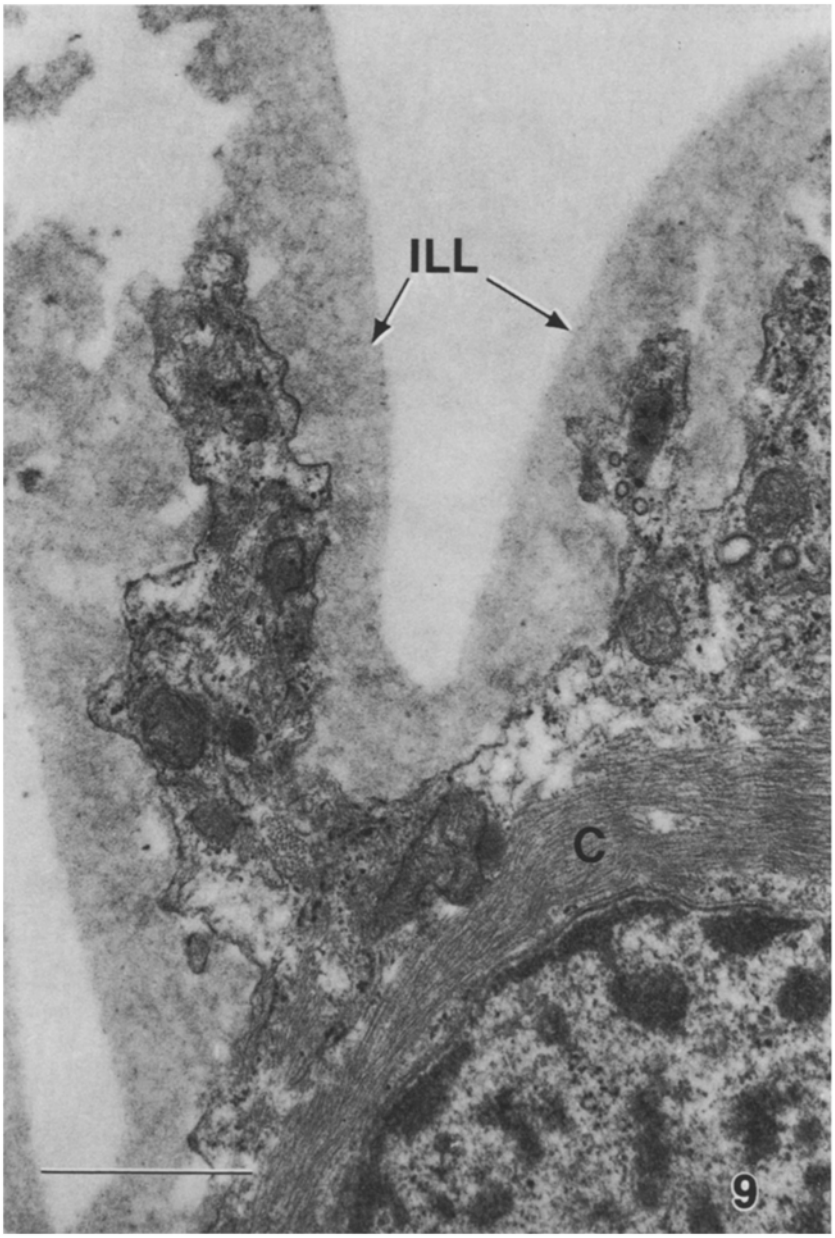


Fig. 9. Cell of neurosensory retina (*C*) closely conforming to the retinal side of the internal limiting lamina (*ILL*). Glial origin suggested by intracytoplasmic filaments. Bar = 1 μm

between the internal limiting lamina and the neurosensory retina may offer an explanation as to why internal limiting lamina can be peeled relatively easily from the retina. Foos (1972) and Bellhorn et al. (1975) observed that in contrast to the retinal periphery the posterior pole had fewer attachment plaques. These plaques are thought to play a role in the strength of the attachment between the internal limiting lamina and the cellular elements of the retina. Thus, Foos explains why dome-shaped sublaminar hemorrhages are located predominantly in the posterior pole.

In single-layered membranes, we found large numbers of attachment plaques between the cells of the membrane and the internal limiting lamina. Therefore removal will cause large areas of internal limiting lamina to separate from the posterior pole.

It is not difficult to explain why multilayered membranes bring smaller amounts of internal limiting lamina with them when peeled. These membranes have fewer attachment plaques because of collagen interposed between the membrane cells and the internal limiting lamina. Therefore the attachment of multilayered membranes to the ILL is weaker. Thus, peeling results in randomly spaced fragments of internal limiting lamina.

There has been concern that peeling of the internal limiting lamina may result in complications. Although Constable et al. (1981) have demonstrated that the internal limiting lamina of the retina is regenerated following trephine injury to dogs, it has not yet been shown whether or not the same is true in humans. Since we have observed major visual improvement and normal clinical appearance of retinas from which large pieces of internal limiting lamina were removed by surgical stripping (Trese et al. 1983), we suspect the ILL can regenerate. Although both types of epimacular membranes had cells of the neurosensory retina attached to the retinal side of the internal limiting lamina, this also did not appear to have a detrimental effect on retinal function (Trese et al. 1983).

The mechanism of epimacular membrane formation has

Table 1. Cell types and amount of collagen in each membrane as it relates to clinical appearance and the presence of retinal breaks (Ret. = retinal)

Case no.	Clinical appearance	Fibroblasts	Pigmented cells	Glial cells	Large amount of collagen	History of Ret. Break
1	Opaque	×	×		Yes	×
2	Opaque	×	×		Yes	×
3	Opaque	×			Yes	
4	Opaque	×	×		Yes	×
5	Opaque	×	×		Yes	×
6	Opaque	×	×	×	Yes	×
7	Opaque	×			Yes	
8	Opaque	×	×		Yes	×
9	Opaque	×		×	Yes	
10	Opaque	×	×		Yes	×
11	Opaque	×	×		Yes	×
12	Transparent	×		×	Small	
13	Transparent	×	×	×	None	×
14	Transparent	×		×	None	
15	Transparent	×		×	None	
16	Transparent	×			None	
17	Transparent	×		×	None	
18	Transparent	×			None	
19	Transparent	×			None	
20	Transparent	×			None	

previously been investigated (Foos 1977b; Gywat et al. 1978; Machemer and Laqua 1975; Laqua and Machemer 1975). Such membranes are formed when cells proliferate along the vitreal surface of the internal limiting lamina of the retina. The cells arrive on the internal limiting lamina perhaps by one or two mechanisms: (1) growing through a break in the internal limiting lamina of the retina, in which case the cells may be derived from the astroglial elements within the neurosensory retina, and (2) a mechanism involving a full thickness retinal break which need not be in the macula, through which retinal pigment epithelial cells migrate and proliferate along the retinal surface. These two mechanisms can occur in combination. Our data show that pigment epithelial cells were always present in eyes with a history of retinal breaks, and usually had complex multilayered membranes, a condition suggested by Kenyon and Michels (1977).

The mechanism of puckering in the macula can be attributed to contraction of the cells that have grown along the internal limiting lamina of the retina. We have identified cells with the ultrastructural characteristics of myofibroblasts in our specimens (Gabbiani et al. 1978), as have other investigators (Kampik et al. 1981). These cells probably constitute the main contractile element of epimacular membranes. The effect of contracting epiretinal membranes on the retina is also determined by the strength of the attachment of that membrane to the retinal surface, and the attachment between the internal limiting lamina and the neurosensory retina. Strong attachments may result in major retinal disorganization. Weaker attachments between the membrane and the internal limiting lamina may result in spontaneous peeling of the membrane. We suspect that detachment of the internal limiting lamina may occur as well if the attachment between the membrane and the ILL is stronger than the attachment between the ILL and the neurosensory retina.

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