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the vitreous cortex from these sites were cut with scissors, fixed overnight in a solution of 1% paraformaldehyde and 1.25% glutaraldehyde in 0.1-mol/L cacodylate buffer (pH 7.4), and postfixed in 0.1-mol/L cacodylate buffer (pH 7.4) for 1 hour at 4°C. Specimens were dehydrated in a graded acetone series, followed by propylene oxide, and then embedded in epoxy resin (Epon)-araldite. Thick sections (1 μ) were stained with toluidine blue, and suitable areas were chosen for study. Blocks were trimmed, thin sectioned, stained with 2% uranyl acetate-lead citrate, and photographed with the use of a transmission electron microscope (Philips 200).

For scanning electron microscopy, dissected portions of the posterior vitreous were mounted onto filter paper, with the vitreous cortex facing up (away from the

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filter paper). Specimens on filter paper were fixed overnight in 1% paraformaldehyde and 1.25% glutaraldehyde in 0.1-mol/L cacodylate buffer (pH 7.4), and critical point dried in carbon dioxide. The tissue then was dehydrated in a graded alcohol series. Specimens and filter paper were mounted together on aluminum stub mounts and coated with gold. Photographs were taken with a scanning electron microscope (AMR 1000A).

RESULTS

By dark-field slit microscopy, the vitreous cortex in all eyes had a dense appearance that scattered light more intensely than did the inner portions of the vitreous (Fig 1). In all eyes from individuals aged 21 years or older (n=44; 37 eyes [84%] were from individuals aged 41 years or older), the posterior vitreous had two "holes" in the vitreous cortex (Fig 1). The prepapillary hole (a true hole in situ) measured approximately 1.0 to 1.25 mm in diameter, and the premacular hole was about four times larger. Ultrastructural studies demonstrated that the retina removed from the vitreous was intact in the eyes that had this microscopic appearance. The ILL had an irregular posterior aspect and a smooth anterior surface with no vitreous fibrils (Fig 2). The vitreous in these specimens had an outer layer of densely packed collagen fibrils (Fig 3) and no retinal elements.

In six (40%) of the 15 eyes from individuals aged 20 years or younger (four eyes [66.7%] were from individuals aged younger than 10 years), the appearance of the peripheral vitreous was similar to that of the older individuals, but the appearance of the posterior vitreous was entirely different. Figure 4 demonstrates the macroscopic structure observed in the vitreous of a 9-month-old female infant. Although the prepapillary hole is clearly seen, there appears to be no hole in the premacular vitreous cortex (Fig 4, top left and top right). The vitreous cortex in the posterior pole of this eye had more intense light-scattering properties than did the equatorial vitreous cortex (Fig 4, bottom left and bottom right). Scanning electron microscopy of the posterior vitreous in this eye demonstrated a membranous structure adherent to the vitreous cortex in the posterior pole (Fig 5, left). Transmission electron microscopy identified this structure as the ILL of the retina with vesicles of membranous material present on the posterior aspect (Fig 5, right).

Figure 6 demonstrates the macroscopic appearance of the posterior vit-

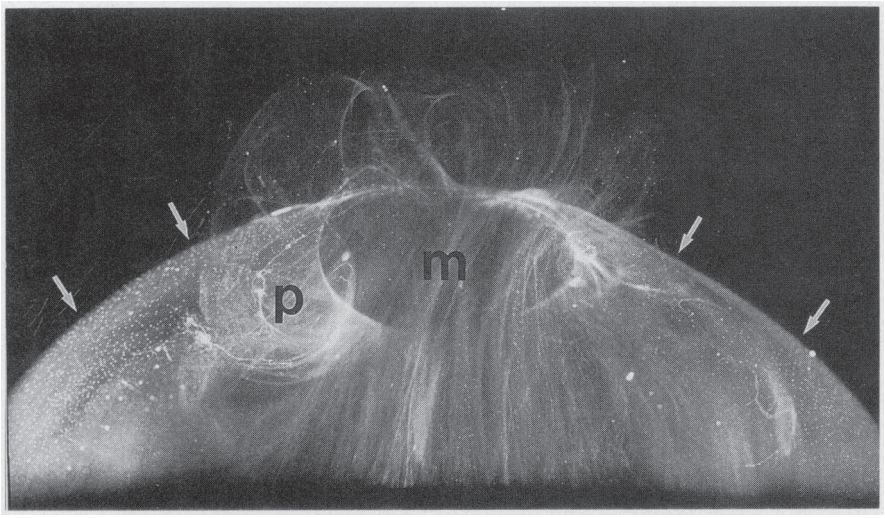
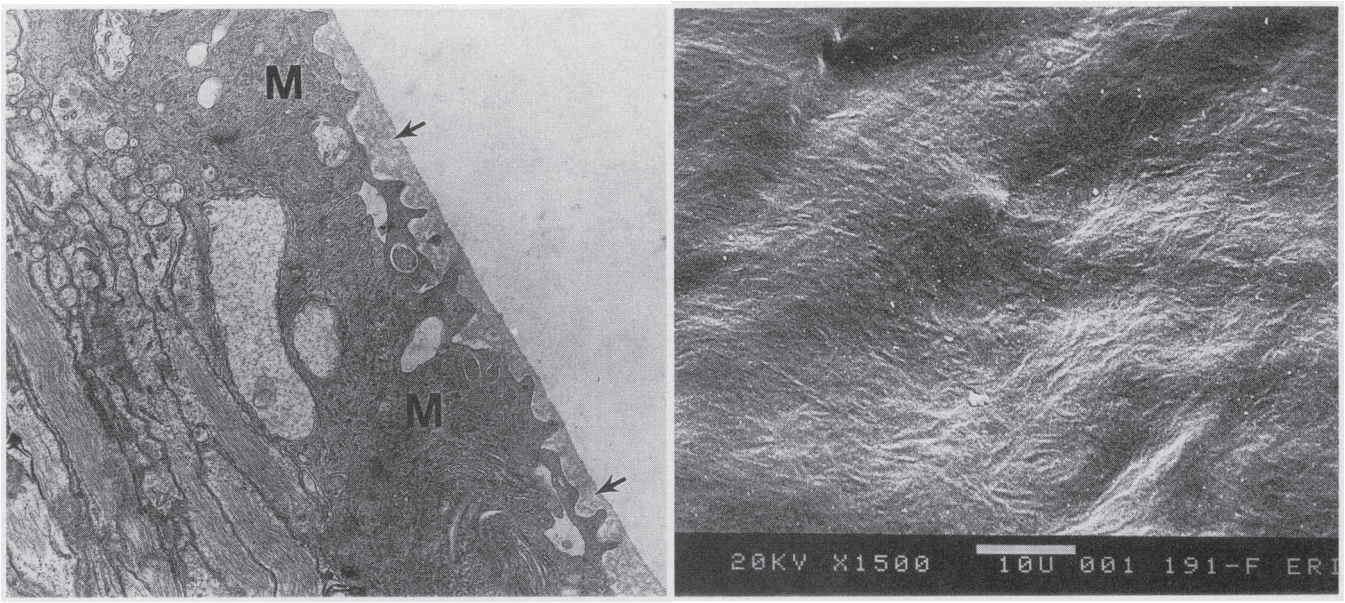


Fig 1.—Posterior vitreous in a 59-year-old man. The cortex (arrows) surrounds the entire vitreous. The premacular hole (m) is four times larger than the prepapillary hole (p) (original magnification approximately ×9).

Fig 2.—Ultrastructure of the inner retina after dissection away from the vitreous. Left, Müller's cells (M) insert into the posterior aspect of the internal limiting lamina (arrows). The anterior surface is smooth, whereas the posterior aspect follows the contours of the inner retinal cells (original magnification ×20 800). Right, Scanning electron microscopy of the anterior aspect of the internal limiting lamina, demonstrating the smooth surface and absence of vitreous collagen fibrils (original magnification ×2250).



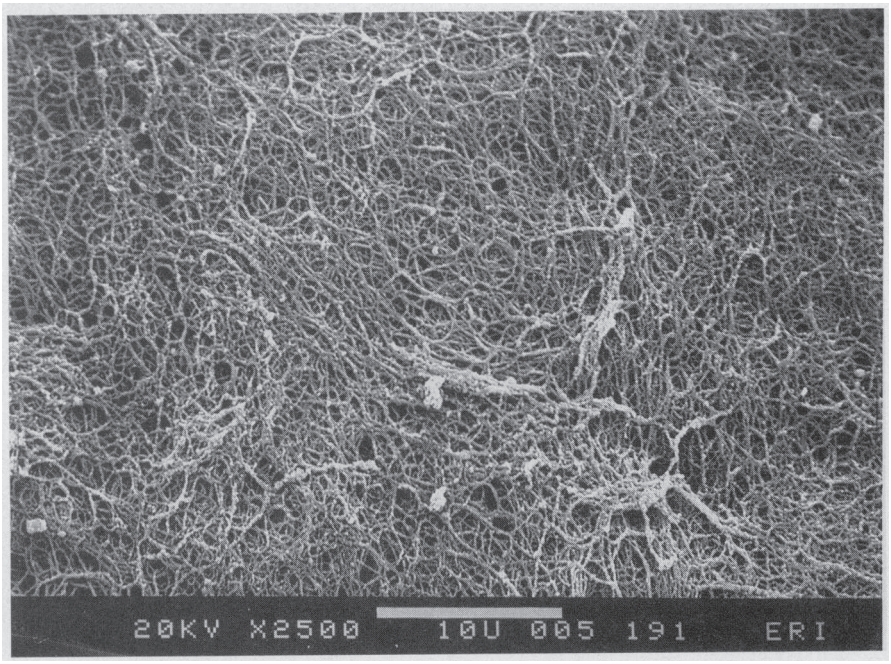
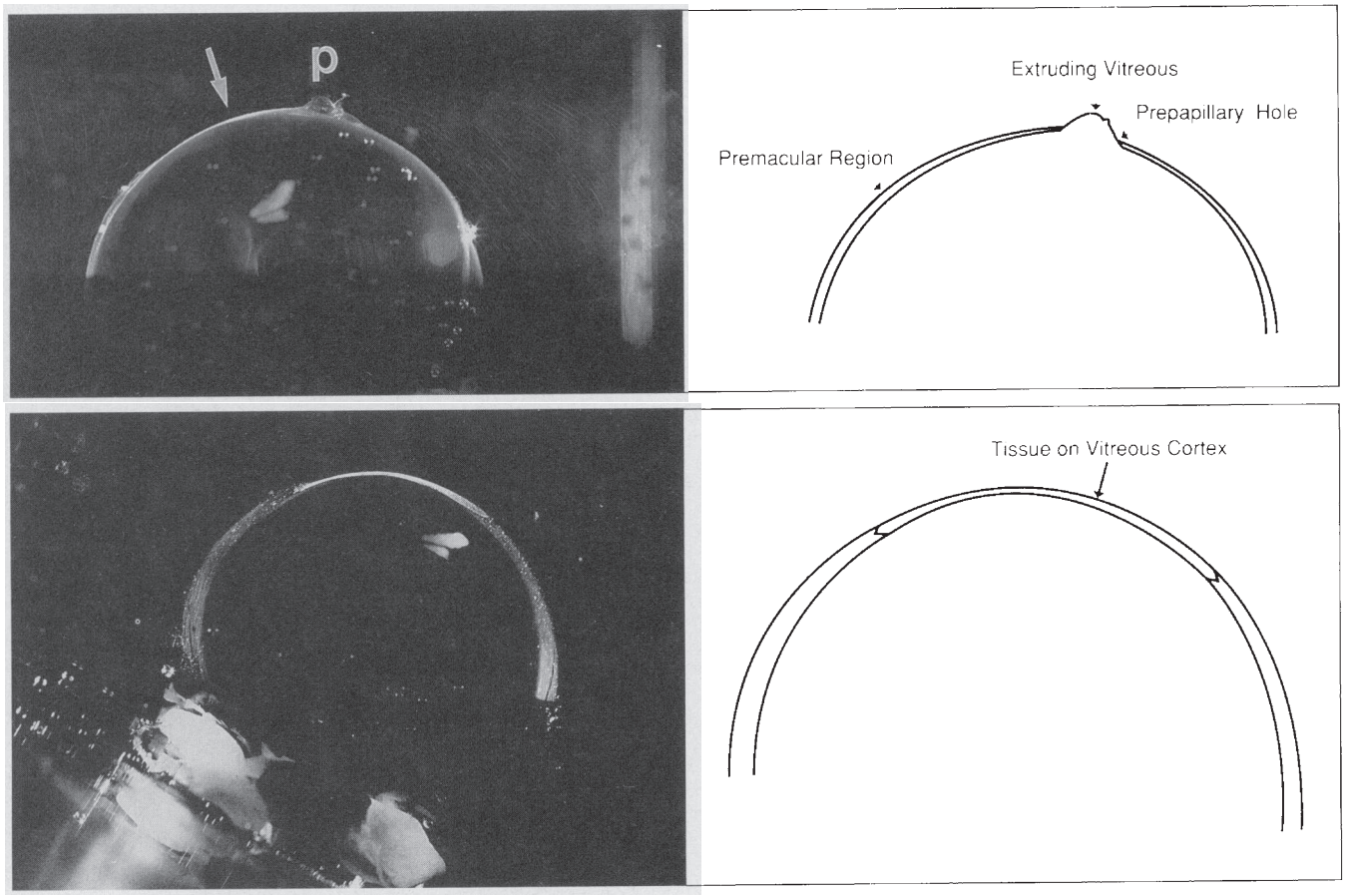


Fig 3.—Scanning electron microscopy of the vitreous cortex after removal of the retina (same eye as Fig 2, right). Densely packed collagen fibrils are seen with no internal limiting lamina components. The compactness of the collagen fibrils is probably exaggerated by dehydration during processing (original magnification × 3750).

reous in a 14-year-old boy. The prepapillary hole in the vitreous cortex can be clearly identified, but there is no hole in the premacular region. The posterior pole appears to be covered by a "cap" of tissue that surrounds the prepapillary hole. Within this tissue, there are "imprints" of the fovea and branching linear patterns arising from the prepapillary hole (the former site of the optic disc) that follow the course of retinal blood vessels. The peripheral vitreous has the same appearance as the vitreous cortex of older individuals shown in Fig 1. Scanning electron microscopy demonstrated that the vitreous in this specimen contained a membrane adherent to the posterior vitreous cortex. The posterior surface of this cap of membranous tissue was covered by numerous vesicular structures (Fig 7). Transmission electron microscopy identified this tissue as the ILL of the retina (Fig 8). The vesicles on the posterior surface of this tissue were identified as the inner portions (or inner dense cytoplasm) of Müller's cells of the retina with "attachment plaques" present at the points of inser-

Fig 4.—Macroscopic structure of posterior vitreous in a 9-month-old female infant. Top left, The prepapillary hole (p) in the vitreous cortex is seen, but no hole is present in the premacular region (arrow) (original magnification approximately × 7). Top right, Cartoon companion of Fig 4, top left. Bottom left, Special illumination demonstrates the more intense light-scattering properties of the tissue on the premacular vitreous cortex (original magnification approximately × 7). Bottom right, Cartoon companion of Fig 4, bottom left.



tion into the ILL (Fig 8). Transmission electron microscopy of the retina in this region demonstrated marked disruption of the plasma membranes of all inner retinal cells with cytoplasmic organelles dispersed into the preretinal space.

COMMENT

This study demonstrates topographic and age-related variations in the strength of human vitreoretinal adhesion. Although the technique of specimen preparation did involve the creation of a ("surgical") plane of dissection at the equator, when the retina was peeled posteriorly, the adhesive forces between the ILL of the retina and the posterior vitreous cortex were the sole determinants (without artifactual influence) of the level of cleavage. In all eyes from individuals aged 21 years or older (the majority of whom were aged older than 41 years), adhesion between the ILL of the retina and the vitreous cortex in the posterior pole was weak, and the retina separated cleanly from the vitreous between the ILL and the posterior vitreous cortex. However, in 40% of eyes from individuals aged 20 years or younger, adhesion between the ILL and the posterior vitreous cortex was stronger than Müller's cell itself. Consequently, the inner portions of Müller's cells tore away from the retina and were found adherent to the ILL–vitreous cortex complex. This topographic difference is consistent with clinical observations by Schachat and Sommer[14] who suggested that stronger vitreoretinal adhesion at the macula accounted for a variety of vitreomaculopathies associated with PVD. Moreover, these results also demonstrate that this strong

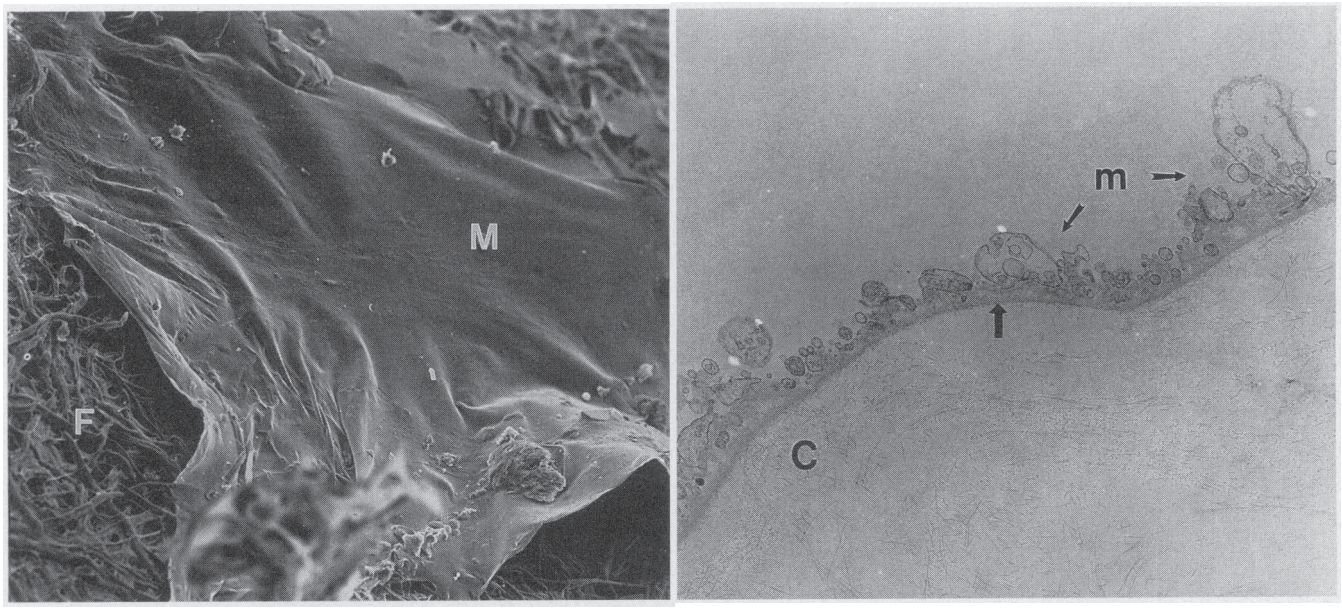
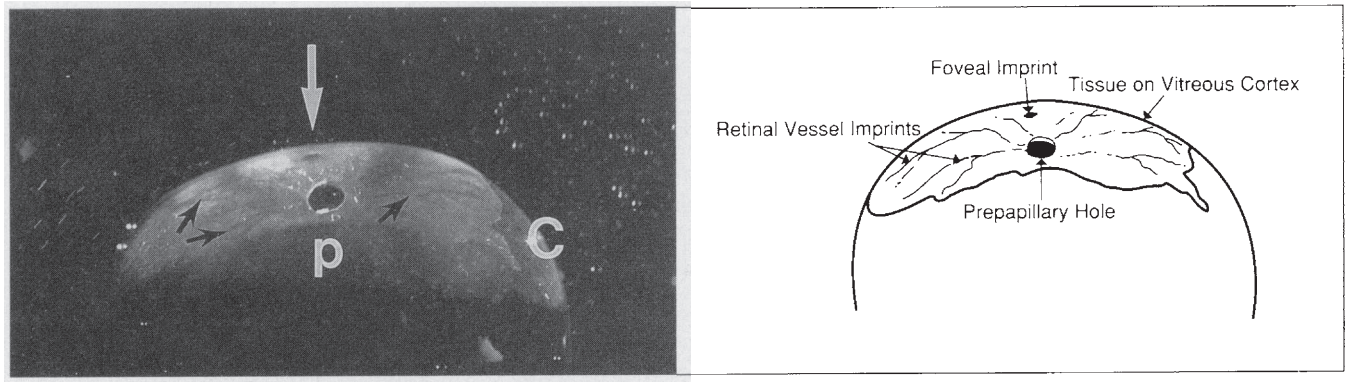


Fig 5.—Ultrastructural appearance of the posterior vitreous cortex shown in Fig 4. Left, Scanning electron microscopy demonstrates a membranelike structure (M) that was adherent to the premacular region of the vitreous. The coarse fibers of the filter paper (F) are seen beneath the membrane (original magnification ×600). Right, Transmission electron microscopy demonstrates that this structure consists of the internal limiting lamina (thick arrow) bordered by collagen fibrils (c) on its anterior aspect and membranous vesicles of inner retinal origin (m, thin arrows) on its posterior aspect (original magnification ×21 300).

Fig 6.—Posterior vitreous in a 14-year-old boy. Left, The prepapillary hole (p) is surrounded by tissue with light-scattering characteristics different from those of the vitreous cortex (c). This tissue contains what appears to be the "imprints" of the fovea (white arrow) and the retinal vessels (black arrows) (original magnification approximately ×9). Right, Cartoon companion of Fig 6, left.



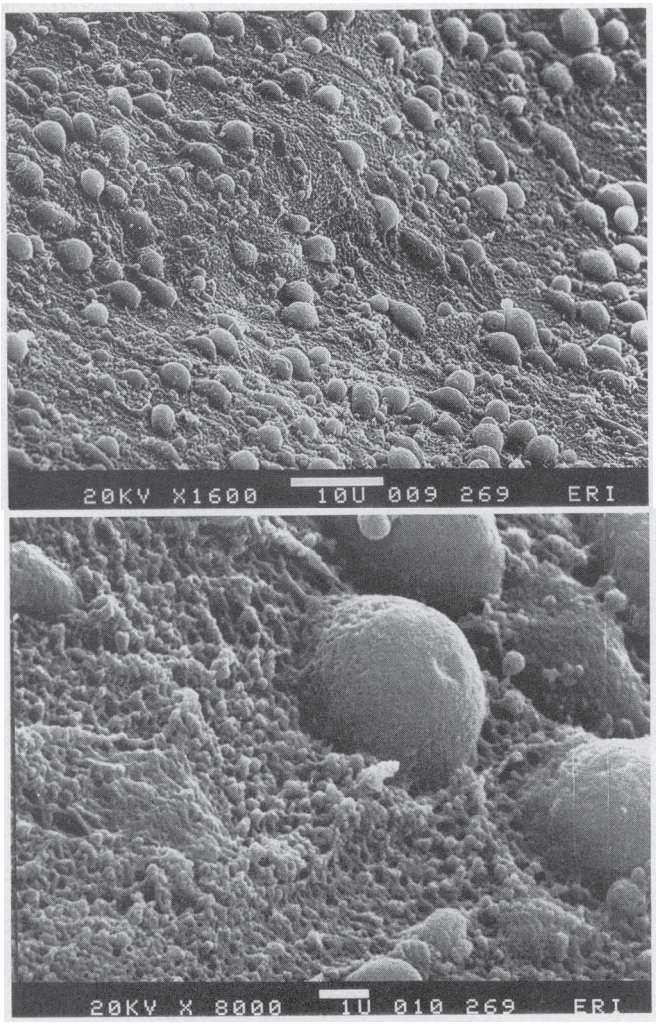


Fig 7.—Scanning electron microscopy demonstrates that the posterior surface of the tissue shown in Fig 6 contains numerous vesicular structures. Top, Low original magnification (×2400). Bottom, High original magnification (×12 000).

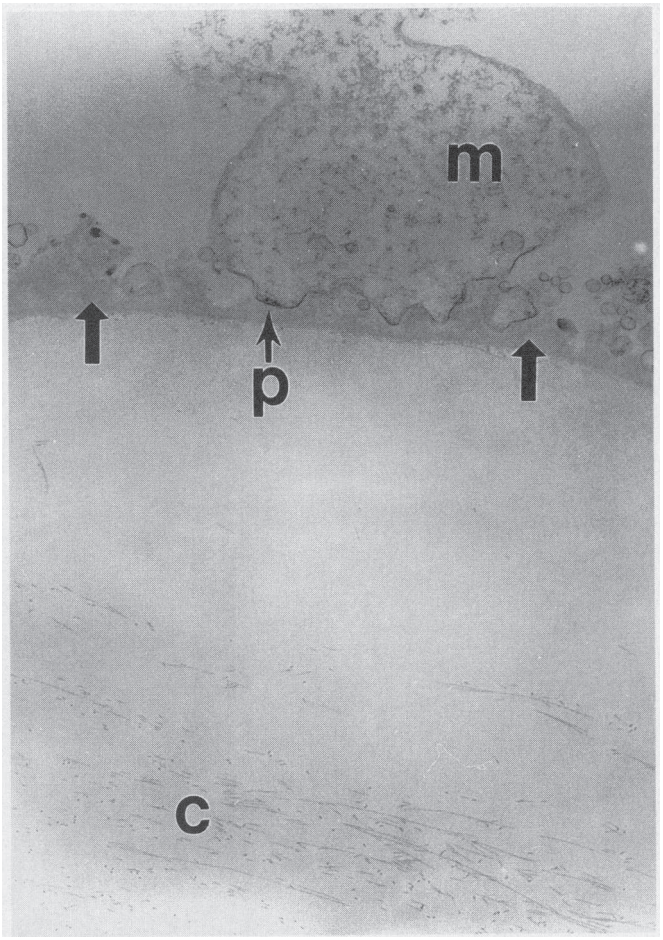


Fig 8.—Transmission electron microscopy of the posterior vitreous shown in Figs 6 and 7 demonstrates the internal limiting lamina (thick arrows), collagen fibrils (c) of the posterior vitreous cortex, and inner portion of a Müller's cell (m) inserting into the internal limiting lamina with attachment plaques (p) at this insertion (original magnification ×20 800). The space within the vitreous cortex is probably artifact, resulting from specimen preparation for electron microscopy.

adhesion is not focal, ie, limited to the macula, the optic disc, and along retinal blood vessels. Rather, the phenomenon is distributed throughout the posterior pole in a "sheetlike" configuration that encompasses the macula, as well as the peripapillary posterior pole.

The exact source of the adhesive forces between the vitreous and retina is not known. Early studies[15,16] reported the presence of vitreous collagen fibril insertion into the ILL. Other investigations[17] found fine fibrillar attachments between the vitreous and retina, but these were neither identified nor confirmed. Studies[18] of frozen, resin-cracked, and enzyme-digested monkey retina demonstrated a dense fibrillar meshwork close to the basement membrane of Müller's cells, whereas there was a loose network of fibrils that extended from this region into the vitreous. Other studies[19] have shown that the ILL is composed of type IV collagen associated with glycoproteins. Laminin, fibronectin, and proteoglycans have been identified in the human ILL.[20] The findings of the present study suggest that extracellular matrix components between the ILL and vitreous cortex are likely to be responsible for the sheetlike adhesion observed in these experiments.

Several studies[18,21-25] have shown that the thickness of the ILL of the retina increases with age. Kohno et al[21] found that in age-related thickening of the ILL, there is a bilaminar deposition of fibronectin and laminin in the ILL of the posterior pole but not of the equatorial fundus. It is possible that this thickening adversely affects the ability of Müller's cells to synthesize and maintain the components of the extracellular matrix at the ILL–vitreous cortex interface,[26-30] thus weakening vitreoretinal adhesion.[31,32] Such weakening leads to dehiscence at the ILL–vitreous cortex interface and allows liquid vitreous to dissect a plane between the vitreous cortex and retina.[1-3] This results in true PVD.[31] However, if the process of liquefaction occurs without adequate dehiscence between the vitreous cortex and the ILL, traction will be exerted at sites of persistent adhesion, and, as demonstrated in this study, retinal elements can tear away and remain adherent to the vitreous cortex. This may explain the severity of rhegmatogenous events in patients with high myopia and vitreous degenerations[31] where liquefaction is advanced but there is no vitreoretinal dehiscence.

Elucidating the nature of the adhesive forces at the vitreoretinal interface would contribute greatly to our understanding of PVD, the most common event in the life of the human vitreous. In particular, the composition of the extracellular matrix that bonds the vitreous cortex to the ILL of the retina should be investigated. Such information would improve our understanding of the conditions that result in retinal tears and detachment and macular holes.[28,31] Characterizing the mechanisms underlying vitreoretinal adhesion could also enable the development of means by which vitreoretinal adhesion could be enhanced or weakened, thereby inhibiting or facilitating PVD. This could be very useful since, depending on the clinical circumstances, it may be advantageous to be able to prevent or induce PVD.

Steven J. Brennan performed all technical aspects of electron microscopy.

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