Paracellular route of aqueous outflow in the trabecular meshwork and canal of Schlemm

A freeze-fracture study of the endothelial junctions in the sclerocorneal angle of the macaque monkey eye

Giuseppina Raviola and Elio Raviola

The intercellular junctions of the endothelial cells of the trabecular meshwork and canal of Schlemm were examined with the electron microscope in the macaque monkey eye by both thin-sectioned specimens and the freeze-fracturing technique. The endothelial cells that line the beams of the meshwork are joined by gap junctions and short, isolated strands of tight junction; zonulae occludentes are absent. Thus aqueous humor can freely traverse the patent endothelial clefts of the trabecular meshwork. The endothelial cells of the canal of Schlemm are joined by zonulae occludentes and a small number of minute gap junctions. In 57% of their length, the tight junctions consist of one or two strands; the strands are rarely more than four. They remain preferentially associated with the E-face of the membrane, run parallel to one another, and only exceptionally branch or anastomose. Thus they are provided with free endings and do not form a bidimensional network. As a result of this organization, the zonula occludens is traversed by meandering channels of extracellular space or slit pores, which connect the open endothelial clefts on the luminal and tissue fronts of the junction. The frequency of slit pores is 0.134 per micrometer of zonula occludens. They occupy 0.07% of the intercellular boundary and 0.0015% of the area of the endothelium. Estimates of the fluid conductance of the zonulae occludentes indicate that the intercellular clefts of the endothelium of Schlemm's canal filter but a small fraction of the amount of aqueous humor that leaves the anterior chamber through the conventional route.

Key words: intercellular junctions, trabecular meshwork, Schlemm's canal, macaque monkey, aqueous outflow

In its outflow pathway at the sclerocorneal angle, the aqueous humor percolates through the trabecular meshwork, gains access to the juxtaocular connective tissue, and finally penetrates the wall of Schlemm's canal. To understand the mechanism of aqueous outflow, it is important to estimate the contribution of the paracellular and transcellular routes in the movement of water and solutes across the endothelia that line the spaces of the trabecular meshwork and the lumen of Schlemm's canal. A precise knowledge of the structure of the specialized interendothelial junctions is therefore essential. Previous studies on this subject reported that the endothelial cells of the trabecular meshwork are connected...
by zonulae occludentes and adhaerentes, "attachment bodies," and adhering and gap junctions. Tight junctions between the endothelial cells of Schlemm's canal were repeatedly described, however, noted that in human eyes some of the endothelial clefts were patent throughout their length, and Shabo et al. suggested that the endothelial tight junctions of the canal were macular rather than zonular in shape. All these studies were based on the analysis of thin-sectioned specimens, which provide inadequate information on the extent and complexity of zonulae occludentes. We have therefore applied the freeze-fracturing technique to the study of the primate sclerocorneal angle and have elucidated the nature and organization of the specialized endothelial junctions in the trabecular meshwork and Schlemm's canal.

Materials and methods

Animals. Male and female, adult macaque monkeys were used for this study (two Macaca mulatta, one Macaca arctoides, and one Macaca fascicularis).

Conventional electron microscopy. With the animals under pentobarbital anesthesia, the eyes were enucleated and cut at the equator, and the anterior segment was immediately immersed in 2% formaldehyde, 2.5% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.3, containing 0.2% CaCl₂. The lens was gently removed, and the specimens were fixed for 2 hr at room temperature. After an overnight wash with cacodylate buffer at 4° C, the anterior segment was radially cut into wedges and postfixed for 2 hr at 4° C in 1% osmium tetroxide, 1.5% potassium ferrocyanide in distilled water, dehydrated, and embedded in Epon-Araldite. Thin sections were stained with uranyl acetate and lead citrate. Micrographs were taken with either an RCA-3G or a JEOL 100B electron microscope.

Freeze-fracturing. After aldehyde fixation, wedges of anterior segment were coated with agar and sectioned at 200 μm with a Smith-Farquhar tissue chopper. The plane of sectioning intersected the canal of Schlemm at a very oblique angle, so that its lumen was easily seen with the dissecting microscope. The region of the trabecular meshwork and Schlemm's canal was trimmed free of the surrounding tissues. Furthermore, to facilitate the identification of the endothelium of the canal in freeze-fracture replicas, small fragments of iris epithelium rich in melanosomes were detached from the posterior surface of the iris and delicately inserted into the lumen of the canal with the help of a 36-gauge needle. The tissue sections were equilibrated with 20% glycerol in cacodylate buffer at pH 7.4 for at least 30 min and subsequently sandwiched between two gold specimen carriers coated with a thin layer of 25% polyvinyl alcohol (Gelvatol 20-30; Monsanto Co., St. Louis, Mo.) in 25% glycerol. The specimens were rapidly frozen in the liquid phase of partially solidified Freon 22 (monochlorodifluoromethane), cooled with liquid nitrogen, mounted in a double replica device and fractured, and replicated in a Balzers apparatus (Balzers High Vacuum Corp., Santa Ana, Calif.) fitted with an electron beam gun for platinum shadowing and a quartz crystal monitor for standardizing the thickness of the replicas. The temperature of the stage was —110° C. The replicas were cleaned in methanol and bleach (5% to 6% sodium hypochlorite containing 10% to 15% potassium hydroxide), washed in several changes of distilled water, and mounted on Gilder grids (Marivac Services, Halifax, Nova Scotia). Replicas were examined either with a JEOL 100B or an AEI 6B electron microscope. The illustrations of the freeze-fracture replicas are presented with the shadow direction approximately from bottom to top.

Results

Trabecular meshwork

Thin sections. In addition to the usual complement of cytoplasmic organelles, the flattened endothelial cells of the beams of the trabecular meshwork contained lipid droplets, up to 0.5 μm in diameter, which were often associated with dense inclusions, probably melanosomes (Fig. 1). At the cell surface, a cilium was frequently seen protruding in a channel bounded by the plasma membrane. Smooth and coated invaginations of the plasmalemma were present in varying numbers at the cell surface and vesicles; both coated and smooth were scattered throughout the cytoplasm. Rows of two or more plasmalemmal (pinocytotic, micropinocytotic) vesicles fusing with one another and continuous with the cell membrane occurred commonly (Fig. 2). The fibroblasts of the juxtacanalicular connective tissue were star-shaped and thus possessed very irregular outlines. They typically contained flattened cisterns of the rough endoplasmic reticulum...
Fig. 1. *Macaca mulatta*. Trabecular meshwork. Tangential section through an endothelial cell that lines a trabecular beam. In addition to the usual complement of organelles, the cytoplasm contains lipid droplets (arrows) intimately associated with dense bodies resembling melanosomes. Dense glycogen particles are scattered throughout the cytoplasm. (×29,500.)

Fig. 2. *Macaca mulatta*. Tangential section through the periphery of an endothelial cell of the meshwork. The cytoplasm contains bundles of filaments (Fl) and a number of plasmalemmal vesicles, either isolated or fusing with one another (arrowheads). (×31,000.)

Fig. 3. *Macaca mulatta*. The fibroblasts of the juxtacanalicular connective tissue are characterized by flattened cisterns of the rough endoplasmic reticulum containing a dense lamina bisected by a lucent space. (×27,000)
Fig. 4. Macaca mulatta. Gap junction between endothelial cells of the trabecular meshwork as seen at high magnification (large arrow). Inset, Endothelial cell of a beam sends an attenuated process across an intertrabecular space (ITS) to make a gap junction (Gj) with an endothelial cell of a neighboring beam. In places, the plasma membranes of adjacent endothelial cells approach each other focally and their cytoplasmic aspect is decorated by a feltwork of fine filaments (arrowheads). Cv, Coated vesicles. (×100,000; inset ×21,000.)
enclosing a dense lamina bisected by a lucent space (Fig. 3 and ref. 19).

The endothelial cells of the beams and the fibroblasts of the juxtacanalicular connective tissue were joined by two kinds of specialized junctions. In places, the plasma membranes of adjacent cells were focally fused and their cytoplasmic aspect was decorated by a feltwork of fine filaments (Fig. 4). These minute surface specializations resembled zonulae occludentes in their organization, but their location along the interendothelial cleft was variable. Furthermore the fact that most clefts were patent throughout their length suggested that these regions of fusion were not zonular in shape. The second type of junctional specialization was represented by gap junctions. These were infrequent, varied considerably in size, and were characterized by a straight or gently curving course (Fig. 4).

Freeze-fracturing. In freeze-fracture replicas of the angle, the beams can be cross-fractured; in this instance, the cytoplasm of their endothelium and their connective tissue core were readily identified. On the other hand, when the fracture plane ran parallel to the long axis of the beams, it exposed large expanses of the plasma membrane of their endothelial cells (Fig. 5). As usual, intramembrane particles were more numerous on the inner leaflet or P-face of the plasma membrane on both fronts of the endothelial cells. Two sorts of specializations were present at the cell surface: openings of plasmalemmal vesicles and intercellular junctions. The number of stomata of plasmalemmal vesicles was quite variable in different cells and in different regions of the surface of the same cell. They formed irregular clusters or were aligned in rows which ran parallel to the long axis of the cell. They had a diameter of about 6 nm and resembled valvate papillae on the P-face and shallow volcanoes on the outer membrane leaflet or E-face (Fig. 6).

Short strands, identical to those typical of zonulae occludentes, were occasionally present within the membrane of the endothelial cells (Fig. 7). They were either isolated or associated with the periphery of gap junctions; their length was quite variable. They had a straight or undulating course, and sat on creases of the P-face of the plasma membrane. They were complemented by grooves on the E-face, but they occasionally fragmented upon fracturing and thus they appeared unequally partitioned between the two membrane leaflets. When the fracture plane shifted from the interior of one membrane into the adjacent one, P-face strands and E-face grooves were found in perfect register across a narrowing of the intercellular space; thus the intramembrane strands corresponded to linear regions of membrane fusion between endothelial cells. It must be stressed that these isolated, short tight junction strands were set far apart from one another and never gave rise to a zonula occludens; they clearly corresponded to the minute surface specializations seen in thin-sectioned specimens.

The gap junctions between endothelial cells varied both in shape and dimensions: they consisted of as few as 10 particles or reached up to 1 μm in diameter (Fig. 8). They were distributed at random on the cell surface, often isolated but occasionally in groups of two or more. The distribution of the junctional particles was rarely homogeneous. As a rule, they were loosely packed and enclosed islands of smooth membrane matrix. However, large junctions occasionally consisted of linear domains of hexagonally packed particles that regularly alternated with aisles of smooth membrane matrix.

Schlemm’s canal

Thin sections. The endothelium of the canal consists of flattened cells, their nuclei bulging into the lumen. Their cytoplasmic organelles were unremarkable except for occasional crystalline inclusions within the cisterns of the rough endoplasmic reticulum (Fig. 9, inset A); similar structures have been described in a variety of ocular blood vessels in both adult and prenatal monkeys. Uncoated invaginations of the plasmalemma occurred in variable numbers on both the luminal and abluminal fronts of the endothelium (Fig. 9, inset B), whereas in the cytoplasm plasmalemmal vesicles were infrequent. In places, coated vesicles were also found. At the cell margins, the plasma mem-
Fig. 5. _Macaca mulatta_. Representative fracture through a beam of the trabecular meshwork. In the lower left corner the fracture plane has crossed an intertrabecular space (ITS). Subsequently, it has exposed the E-face of the luminal plasmalemma of an endothelial cell (EJ), its cytoplasm (CytJ), and the P-face of its basal plasmalemma (PJ). After traversing the connective tissue core of the beam (BC), it has cut across the cytoplasm of a second endothelial cell (Cyt2) and has finally exposed the P-face of its luminal membrane (P2). The core of the beam consists of collagen fibrils sandwiched between the thick basal laminae of the investing endothelial cells (Bl1 and Bl2). (x25,000.)
brane usually followed a gently undulating course; less frequently, digitations or lamellae originating from the periphery of one cell interlocked with similar processes of the neighboring cell. The intercellular clefts had variable widths, but in one or more regions of their course, the adjoining plasma membranes approached each other focally and appeared decorated on their cytoplasmic aspect by a feltwork of fine filaments. When these regions of close surface approximation between endothelial cells were favorably oriented with respect to the plane of sectioning, the adjacent membranes appeared to fuse and thus obliterated the intercellular space (Fig. 9). Interendothelial clefts patent throughout their length were rarely seen. Gap junctions between endothelial cells were present but infrequent (Fig. 9, inset B).

Freeze-fracturing. In replicas of freeze-fractured specimens, the endothelium of the canal of Schlemm could be positively identified from the surrounding tissues because of the presence in the vessel lumen of the small fragment of iridial epithelium, rich in spherical melanosomes, which had been introduced before freezing. Since fracturing with the double replica device exposed broad expanses of the wall of the canal, areas up to 3600 \( \mu m^2 \) of endothelium could be analyzed in montages of electron micrographs. The present description concerns the inner wall of the canal.

The endothelial cells were very elongate elements with sinuous boundaries and interdigitated with one another through short, blunt protrusions (Fig. 10). The plasma membrane on both surfaces of the endothelial cells revealed a variable number of stomata of plasmalemmal vesicles, which appeared as shallow volcanoes on the E-face and vallate papillae on the P-face. (\( \times 52,500 \).)

**Fig. 6.** *Macaca mulatta.* Trabecular meshwork. In an attenuated portion of an endothelial cell the fracture process has exposed the E-face of the membrane on one surface of the cell and the P-face of the membrane on the other surface. In between is the cross-fractured cytoplasm (Cyt). Stomata of plasmalemmal vesicles (arrowheads) are present on both fronts of the cell; they appear as shallow volcanoes on the E-face and vallate papillae on the P-face. (\( \times 52,500 \).)
cell surface studded with vesicles was found next to another that was practically smooth (Fig. 10). No attempts of quantitation were made because studies on nerve endings have clearly shown that chemical fixation profoundly distorts the temporal parameters of vesicle interaction with the cell surface. 18

The endothelial cells of Schlemm’s canal were joined by zonulae occludentes and a small number of minute gap junctions usually associated with tight junction strands (Fig. 10, inset). The zonulae occludentes consisted of one to eight superimposed strands (Figs. 11 to 15), although in 57% of the junctional length the number of strands was reduced to one or two (variations in the number of strands along the zonula occludens are reported in Table I). The strands remained preferentially associated with the outer membrane leaflet (Fig. 11) and left complementary grooves on the inner leaflet (Figs. 12 and 13). During the fracture process, however, fragments of the intramembrane strands were frequently avulsed from the E-face and appeared associated with the inner leaflet (Fig. 12). As a result, particles and short strands were intermittently inserted along the grooves of the P-face and short grooves irregularly alternated with the strands of the E-face. The P-face grooves sat on top of shallow creases of the inner leaflet of the plasmalemma, whereas the E-face strands lay at

Fig. 7. Macaca mulatta. Trabecular meshwork. The endothelial cell membrane contains tight junction strands (Tj). These fragment upon fracturing and appear irregularly partitioned between P- and E-fracture faces. They may be solitary or associated in groups, but they never give rise to a zonula occludens. Their course is usually undulating; they neither branch nor anastomose and are thus characterized by free endings. Frequently, they are associated with gap junctions (Gj). The fragments of the strands sit on linear elevations of the P-face or lie at the bottom of shallow depression of the E-face. Furthermore, when the fracture plane shifts from the interior of one membrane into the bilayer of the adjacent one, the strands are consistently in register with a narrowing of the intercellular cleft (arrowhead). This finding indicates that the strands correspond to linear regions of fusion between the membranes of the adjacent cells. (∗=49,500.)
Fig. 8. *Macaca arctoides*. Gap junctions between endothelial cells of the trabecular meshwork. On the P-face the particles are loosely packed and enclose islands of smooth membrane matrix. Note the fragments of the outer leaflet of the adjoining junctional membrane (asterisks) containing complementary arrays of pits. Stomata of plasmalemmal vesicles are indicated by arrowheads. (×56,000.)
Fig. 9. Macaca mulatta. Schlemm's canal. The intercellular space between neighboring endothelial cells is variable in width. In places the adjoining membranes approach each other focally and fuse (arrows). At the site of fusion a small transparent body is occasionally seen spanning the thickness of both junctional membranes. These are decorated on their inner aspect by a tenuous mat of filamentous material. Inset A, Crystalline inclusion within a cistern of the endoplasmic reticulum in an endothelial cell. Inset B, Small gap junction between endothelial cells of Schlemm's canal (Gj). On the left is a plasmalemmal vesicle continuous with the cell surface (arrowhead). (×149,000; inset A ×55,000; inset B ×130,000.)
Fig. 10. *Macaca mulatta*. Elongate shape of the endothelial cells of Schlemm’s canal. The micrograph is crossed diagonally by a zonula occludens (arrowheads). Note that the surface of the two cells on the lower right is studded with openings of plasmalemmal vesicles whereas the cell on the upper left possesses very few stomata. Inset, small gap junction between endothelial cells of Schlemm’s canal (arrowheads). (×20,500; inset ×166,000.)
the bottom of shallow valleys of the outer leaflet. When the fracture plane shifted from the interior of one membrane to the other junctional membrane, strands and grooves were seen in perfect register with one another across a narrowing of the intercellular space (Fig. 13). It is thus clear that the intramembrane strands correspond to the sites of membrane fusion seen in thin sections of the canal endothelium.

The most unusual feature of this zonula occludens was the arrangement of its junctional strands: they were commonly parallel to one another and had a straight or slightly undulating course; rarely, they bent at an angle and only exceptionally did they branch or anastomose. Even when the number of strands was very high (Fig. 14), they did not form a bidimensional network and thus possessed free endings or spurs. As a result of this organization, continuous, narrow, corridors of smooth membrane matrix traversed the entire thickness of the zonula occludens in a zigzagging course through the labyrinth of junctional strands (Fig. 15). Their number was low because the junctional strands were usually very long.

These corridors of smooth membrane matrix represent interruptions in the zonula occludens, i.e., regions of the interface between adjacent endothelial cells in which a continuous channel meanders throughout the junction and thus connects the patent intercellular cleft on the tissue front of the zonula occludens with the patent cleft on its luminal front (Fig. 16). These labyrinthine channels will be referred to as slit pores in the rest of this paper.

Comparison with thin-sectioned specimens showed that slit pores in cross section had the shape of a biconvex lens, intervening between two adjacent sites of membrane fusion. Their maximal width (Fig. 16) was 10 nm. Their mean length or length of the interruption in the zonula occludens (Fig. 16) was measured in freeze-fracture replicas as the transverse dimension of the corridors of smooth membrane matrix at their narrowest point and it amounted to 63 nm. Their mean depth or path length through the zonula occludens (Fig. 16, arrow) was 1.5 μm. At the narrowest point of their course, their mean cross-sectional area was 440 nm².

From the examination of 160 μm of zonula occludens, we estimated the frequency of slit pores as 0.134/μm of zonula occludens. Their total length (or length of open endothelial junction) represents 0.87% of the intercellular boundary between endothelial cells. From the examination of 3600 μm² of endothelium, we estimated that slit pores occupy 0.0015% of the area of the endothelium.

Table I gives the details of the measurements done on the zonulae occludentes of the endothelium of the Schlemm canal. The high dispersion of the measurements of slit pore length and depth is due to the fact that a small number of pores had a length of 120 to 250 nm (about 10%) or a depth of 0.2 to 0.3 μm (about 10%).

Discussion

Trabecular meshwork. Freeze-fracturing demonstrates that the endothelial cells lining the spaces of the trabecular meshwork are connected by gap junctions and short strands of tight junction, either isolated or associated with the periphery of gap junctions. We can thus rule out the presence of zonulae occludentes. This finding explains why blood-borne ultrastructural tracers, such as horseradish peroxidase, promptly penetrate the anterior chamber when the flow in Schlemm’s canal is reversed. Furthermore, it suggests that aqueous humor can freely move along the patent interendothelial clefts from the intertrabecular spaces to the core of the beams and juxtanacanalicular connective tissue, thus gaining access to the abluminal front of the endothelium of the canal of Schlemm.

In addition to this paracellular route, the presence of plasmalemmal vesicles in the endothelial cells suggests the possibility that fluid can also cross the endothelium by vesicular transport. Finally, it has been claimed that large discontinuities exist in the endothelial lining of the trabecular spaces, and these would, of course, represent the major pathway for fluid movement toward Schlemm’s canal. It must be noted, however, that these
Fig. 11. *Macaca mulatta*. Boundary region between adjacent endothelial cells. On the lower right, fracturing has exposed the P-face of the membrane of a lamellar process of an endothelial cell (Pf₂), and after crossing the cytoplasm (Cyt) it has exposed the E-face of the membrane on the other surface of the process (Ef₁). On the left, the fracture plane has shifted into the interior of the membrane of an adjoining cell and exposed its P-face (Pf₁). Two zonulae occludentes (Zo₁ and Zo₂) are visible at the interfaces between the lamellar process and the adjacent endothelial cells. Their strands remain preferentially associated with the E-face (Zo₂), follow an undulating course, and terminate freely without branching or anastomosing. They lie at the bottom of shallow valleys on the E-face and leave complementary grooves on the P-face. The grooves are not visible at this magnification, but they sit on linear elevations of the inner leaflet (Zo₁). (×58,000.)
Table I. Intercellular boundary, tight junction strands, and slit pores in the endothelium of Schlemm’s canal

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
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<tbody>
<tr>
<td>Length of intercellular boundary/cm² of endothelium</td>
<td>2.5 x 10³ cm (a)</td>
</tr>
<tr>
<td>Number of strands in zonulae occludentes</td>
<td>1 strand, 14%</td>
</tr>
<tr>
<td></td>
<td>2 strands, 43%</td>
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<tr>
<td></td>
<td>3 strands, 18%</td>
</tr>
<tr>
<td></td>
<td>4 strands, 18%</td>
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<tr>
<td></td>
<td>5 or more strands, 7%</td>
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<tr>
<td>Mean length of slit pores/cm²</td>
<td>6.3 x 10⁻⁶ cm (±5.3, S.D., 29 measurements) (b)</td>
</tr>
<tr>
<td>Maximal width of slit pores/cm²</td>
<td>10⁻⁶ cm (c)</td>
</tr>
<tr>
<td>Mean cross-sectional area of slit pores/cm²</td>
<td>4.4 x 10⁻¹² cm² (d)</td>
</tr>
<tr>
<td>Mean depth of slit pores/cm²</td>
<td>1.5 x 10⁻⁴ cm (±0.79, S.D., 29 measurements) (e)</td>
</tr>
<tr>
<td>Number of slit pores/cm of intercellular boundary</td>
<td>1.34 x 10⁻² (f)</td>
</tr>
<tr>
<td>Number of slit pores/cm² of endothelium</td>
<td>3.35 x 10⁻⁶ (g)</td>
</tr>
<tr>
<td>Length of open junction/cm of intercellular boundary</td>
<td>8.4 x 10⁻³ cm</td>
</tr>
<tr>
<td>Length of open junction/cm² of endothelium</td>
<td>21 cm</td>
</tr>
<tr>
<td>Area of slit pores/cm² of endothelium</td>
<td>1.47 x 10⁻⁴ cm²</td>
</tr>
<tr>
<td>Area of Schlemm’s canal endothelium in Macaca mulatta</td>
<td>0.27 cm² (i)</td>
</tr>
<tr>
<td>Total area of slit pores in Schlemm’s canal endothelium</td>
<td>4 x 10⁻⁶ cm³</td>
</tr>
</tbody>
</table>

*Measured on 3.6 x 10⁻⁵ cm² of replica of endothelium montages of electron micrographs taken at an original magnification of 3000x.
*Measured on 1.6 x 10⁻⁵ cm² of zonulae occludens in montages of electron micrographs taken at an original magnification of 20,000x.
*Minimal transverse dimension of the labyrinthine corridors of smooth membrane matrix which cross the entire zonula occludens.
*Measured in thin-sectioned specimens.
*Measured with a planimeter in micrographs of thin-sectioned specimens enlarged 10⁶ times.
*Counted on 1.6 x 10⁻⁵ cm² of zonulae occludens.
*Calculated from the equation $\pi D_c P_s$, where $D_c$ is the mean diameter of the cornea (1.07 cm) and $P_s$ is the perimeter of Schlemm's canal (0.08 cm, measured at the light microscope in a meridian, tissue-chopper section of the sclerocorneal angle).

Discontinuities may be generated during the procedure of specimen preparation for microscopy.

Schlemm’s canal. Traditionally, the canal of Schlemm has been regarded as a vessel sui generis, for it shares with lymphatic vessels a discontinuous basal lamina, but it resembles venules in its wide lumen and continuous endothelium. An additional physiologically important property emerges from our findings: the endothelial junctions of Schlemm’s canal are simple, but essentially continuous zonulae occludentes. In this respect Schlemm’s canal is remarkably similar to the blood capillaries of the omentum, mesentery, diaphragm,21 and lung22 and differs from muscle or visceral venules in which tight junctions are absent and the interendothelial clefts are patent throughout their length.21,23 Plasmalemmal vesicles in the endothelium of the canal are present in moderate numbers, and transendothelial channels formed by chains of fusing vesicles24 are absent. In spite of the fact that we have examined in our freeze-fracture replicas large expanses of canal endothelium (3600 µm²), we have not observed openings of giant vacuoles on the luminal front of the endothelium. This finding, however, does not rule out the existence of giant vacuoles, for these seem to appear in large numbers only when the pressure in the anterior chamber exceeds the pressure in the lumen of Schlemm’s canal.24 Therefore we will limit our discussion to the functional implications of the
Figs. 12 to 15. *Macaca mulatta*. Variations in the complexity of the zonulae occludentes between endothelial cells of Schlemm’s canal.

Fig. 12. Fracture process has exposed the P-face of the junctional membrane. The zonula occludens is represented by a single strand (arrow), but most of it was avulsed during fracturing and left a tenuous, complementary groove (arrowheads) on top of a shallow elevation of the inner leaflet. (×89,000.)

Finding that the lining of Schlemm’s canal shares occluding endothelial junctions and a complement of plasmalemmal vesicles with continuous blood capillaries of muscle and viscera.

Physiologists have postulated that the high permeability of the capillary wall is due to the presence of two hypothetical sets of water-filled pores of different size and frequency. Small pores are envisioned either as cylindrical channels up to 12 nm in diameter or as 8 nm slits, with a density of 10 to 15 units/μm² of endothelial surface. Large pores or leaks should have a diameter of 50 to 70 nm and very small density, approximately one per 20 μm² of endothelial surface. Small pores account to a large extent for the transport of water and small lipid-insoluble molecules, whereas the large pore system accounts for the transendothelial transport of molecules larger than serum albumin. Uncertainties still exist as to the morphological correlate of the small pore system in continuous (nonfenestrated) capillaries. There is now general agreement that plasmalemmal vesicles, which are thought to shuttle back and forth between the luminal and tissue fronts of the endothelium, cannot account for fluid movement across the capillary wall. In fact, they transport bloodborne tracers at a rate that is much slower than that postulated by physiologists for the small pore system. It was suggested that in muscle capillaries the interendothelial clefts are occasionally patent throughout their length and thus represent the site for movement of water and small molecules across the capillary wall. However, more recent studies with the freeze-fracturing technique have shown that the endothelial clefts are sealed by simple but continuous zonulae occludentes. Alternative pathways for fluid movement across the capillary endothelium in muscle are channels...
formed by chains of fused vesicles,
but it is unclear whether these structures are present in adequate numbers in the capillaries of other organs.

In Schlemm’s canal, transendothelial channels are absent and plasmalemmal vesicles are much less numerous than in muscle capillaries; it is therefore unlikely that these structures transport significant amounts of fluid through the endothelial cells. An alternative pathway is represented by the intercellular boundary, for the presence of zonulae occludentes between endothelial cells by no means excludes fluid movement along the paracellular route. Even if we accept the interpretation that each tight junction strand corresponds to a single fibril shared by the adjoining membranes,
which brings adjacent cells into intimate contact and forms a permeability barrier, physiological studies show that these intercellular seals are permeable to ion fluxes. A careful analysis of the recent literature indicates that both the structure and complexity of the specialized junctions between endothelial cells vary in different segments of the vascular tree and in different organs. These differences in morphology include variations in the complexity of the network formed by the tight junction strands, the presence of interruptions, and the tendency of the strands to remain preferentially associated with the inner or the outer leaflet of the plasma membrane. Few unbranching, parallel strands are present in the capillaries of the rat omentum and mesentery, whereas a more elaborate network occurs in the en-
Fig. 14. Occasionally, for a short distance, the tight junctions between endothelial cells consist of numerous strands. The strands, however, have free endings and do not branch or anastomose. (×55,000.)

dothelium of large vessels such as the mesenteric and renal veins. A common feature of the zonulae occludentes in the endothelium of these vessels is the tendency of the intramembrane strands to fragment upon fracturing and to remain preferentially associated with the outer leaflet of the plasma membrane. On the other hand, in organs such as the brain the endothelial cells are connected by junctions that match in complexity those of other epithelia. A property of these junctions is that their strands remain associated with the inner leaflet of the plasma membrane. Venules of muscle and visera are unique, for strands are absent and the endothelial clefts are open throughout their length. Clearly, variations in the structure of endothelial junctions correlate with differences in the hydraulic conductivity (Lp) of the vessel wall. Penetrated capillaries have the highest Lp, and among continuous vessels, those provided with simple zonulae occludentes have an Lp of intermediate values. For these vessels, physiologists have postulated that as little as 2% up to as much as 30% of the total Lp might be ascribed to the cell pathway, but in all cases the intercellular pathway appears to be of substantial importance. At the other extreme, the capillaries of the brain, which are provided with complex zonulae occludentes, have a very low Lp, close to that of single, isolated animal cells. In these vessels, all water transport is probably accounted for by the cell membrane pathway. Thus zonulae occludentes seem to limit, rather than prevent, passage of water and ions between cells, and a wide range of tightness exists in different endothelia, as has been shown to be the case for epithelial tight junctions in general.

The zonulae occludentes of Schlemm's canal consist of a small number of parallel strands, which only exceptionally branch or anastomose; thus they do not form a network as in the case of the tight junctions of other epithelia. As a result the zonulae occludentes
Fig. 15. In places, continuous corridors of smooth membrane matrix are found meandering throughout the labyrinth of the tight junction strands (arrows). These corridors represent regions in which the intercellular cleft is patent throughout its length from the luminal to the tissue fronts of the endothelium. (×89,000.)

Fig. 16. Slit pores are labyrinthine channels that traverse the entire thickness of the zonula occludens and thus connect the tissue and luminal fronts of the endothelium of the canal of Schlemm.
are traversed by tortuous, narrow channels or slit pores, which correspond to regions in which the intercellular cleft is patent throughout its length. The number of these slit pores (\(3.35 \times 10^6/cm^2\) of endothelium) is 3 orders of magnitude smaller than the number of openings postulated for the small pore system in muscle capillaries (\(10^9/cm^2\)). Furthermore, they occupy a smaller fraction of the endothelial surface (0.0015%) than the small pore system (0.1% for a pore depth or path length of \(10^{-4}cm^3\)). The fluid conductance (\(Q\)) of the slit pores of Schlemm's canal can be estimated by applying Poiseuille's law of fluid flow between parallel plates:

\[
Q = \frac{\Delta PA}{3 \eta d} \left(\frac{w}{2}\right)^2
\]

where \(\Delta P\) = pressure difference across the endothelium; \(d\) = depth or path length of slit pores; \(A\) = area of slit pores; \(w\) = width of slit pores; and \(\eta\) = viscosity of the filtrate. Substitution in equation 1 of \(\Delta P = 5\) mm Hg or \(6.65 \times 10^3\) dyne/cm\(^2\); \(\eta = 0.007\) poise; and the values for \(d\), \(A\), and \(w\) given in Table I yield \(Q = 2.1 \times 10^{-9}cm^3/sec\) or \(1.26 \times 10^{-4}fu/min\). This quantity represents a very small fraction of the total amount of aqueous humor that leaves the anterior chamber (about 1.6 \(\mu l/min\) in *Macaca fascicularis*). It is therefore unlikely that the equivalent of the small pore system in Schlemm's canal is exclusively represented by the slit pores.

It must be stressed that equation 1 neglects the influence of the finite length of the slit pores and it may therefore overestimate their conductance by a factor of 2.7. In addition we have overestimated the pressure drop across the canal endothelium by neglecting the resistance of the trabecular meshwork. On the other hand, we may underestimate the width of the slit pores, since we did not take into account the shrinkage caused by dehydration and embedding. We may also have underestimated the number of slit pores, for only those with a relatively small depth can be followed throughout their course in the freeze-fracture replicas. Very deep pores, however, have high resistance, and their contribution to the flow across the canal endothelium is probably negligible.

A final consideration concerns the slit pores that have considerable length (>120 nm). Their frequency is small (about 10%), but we do not know whether their shape in vivo is the same as in fixed specimens. If we assume that in vivo they are cylindrical, then their mean radius would be 61.2 nm and their total area in Schlemm's canal would be \(1.1 \times 10^{-5}cm^2\). From the equation

\[
Q = \frac{\Delta PAr^2}{8\eta d}
\]

which defines fluid flow through cylindrical pores, their conductance would be \(1.5 \times 10^{-3}fu/min\), a value still very small when compared to the total aqueous outflow.

The fact that slit pores in the endothelium are tortuous and few in number explains why the frequency of intercellular clefts open throughout their length in thin-sectioned specimens is exceedingly low. Shabo et al. noted these patent clefts, but our freeze-fracture findings do not support their conclusion that tight junctions in Schlemm's canal are macular rather than zonular in shape. We did not investigate the problem whether, in the canal endothelium, there is a discrepancy between number of strands in freeze-fracture replicas and points of membrane fusion in thin sections, as seems to be the case for the zonulae occludentes of the capillaries of rat omentum and mesentery. It seems unlikely, however, that some of the intramembrane strands may not correspond to lines of fusion between adjoining plasmalemmas, since in all instances in which the fracture plane shifts from the interior of one junctional membrane into the bilayer of the adjacent one, the strands are consistently in register with a constriction of the intercellular cleft.

Fluid can also filter across the strands of the zonula occludens. Because no data are available on the conductance of the tight junction strands, one can grossly estimate the hydraulic conductivity of the endothelial junctions of the canal of Schlemm by com-
comparison with the vessels of an organ in which
the number and radius of the pores (i.e.,
length of intercellular boundary per square
centimeter of endothelium and structure of
endothelial zonulae occludentes) are similar
and the hydraulic conductivity is known.
Such an organ is the lung. In the endothe-
lium of the canal of Schlemm and in lung
capillaries, the number of strands of the
tight junctions is approximately the same.39
Furthermore, in both tissues interruptions or
slit pores are occasionally present. Because
the length of intercellular boundary per unit
of endothelial surface is also very similar
(2.5 × 10^7cm/cm² in Schlemm's canal and
2.2 in the rabbit lung, calculated from ref.
40), we can assume that the canal wall has the
same filtration constant as lung capillaries
(Lp = 3.4 × 10^{-11}cm²/sec • dyne•cm). If we
apply Darcy's equation,26 which describes
the viscous flow of fluids through inert pores
of fibrous materials, we can estimate the
quantity of aqueous humor that may cross the
walls of the canal along the intercellular
boundaries (Qf):

\[ Q_f = \frac{K A_m \Delta P}{\eta \Delta x} \]  

where K = specific filtration constant of the
porous material or membrane; Am = area of
membrane; \( \Delta P \) = pressure across the mem-
brane; \( \Delta x \) = path length through the mem-
brane (approximately 0.3μm); and \( \eta \) = vis-
cosity of filtrate. Substitution in equation 3 of
\( K = 3.4 \times 10^{-11} \text{cm}^3/\text{sec} \); \( A_m = 0.27 \text{cm}^2 \)
(area of Schlemm’s canal endothelium); and
\( \Delta P = 5 \text{ mm Hg or } 6.65 \times 10^4 \text{ dyne/cm}^2 \) (dif-
ference in hydrostatic pressure across the
endothelium of the canal37) yields: \( Q_f = 6.1 \times 10^{-6} \text{cm}^3/\text{sec} \) or approximately \( 3.7 \times 10^{-8} \mu\text{l/min} \).

Again, this quantity represents a very
small fraction of the total amount of aqueous
humor that leaves the anterior chamber
through the conventional route. Thus it
seems unlikely that the intercellular spaces in
the endothelium of the canal of Schlemm
play a major role in aqueous outflow from the
anterior chamber. The contribution to aque-
ous transport across the canal endothelium of
dissipative mechanisms such as diffusion
along the cell membrane pathway and vesicu-
lar transport remains to be established.

In summary, the interendothelial clefts
of the trabecular meshwork are open and
permit free movement of aqueous humor
from the anterior chamber to the wall of
Schlemm’s canal. The endothelial cells of the
canal of Schlemm are joined by simple
zonulae occludentes with a small number of
interruptions or slit pores. These junctions
are probably leaky to water and small solutes.
However, the quantity of fluid that leaves the
interior of the eye through the intercellular
clefts of Schlemm’s canal seems to represent
a very small fraction of the total aqueous
outflow through the conventional route.

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