Electron microscopy of the human corneal endothelium with reference to transport mechanisms

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The corneal endothelium of four presumably normal human eyes, removed at operation, was studied with the electron microscope. Thorotrast had been injected into the anterior chamber a few minutes prior to the enucleation. The Thorotrast particles reached the anterior end of the intercellular space within 3 minutes and had entered Descemet's membrane within 6.5 minutes after injection into the anterior chamber. The pathway of the particles is discussed. The fine structure of the human corneal endothelium is described in detail, and the findings are schematized in a diagram (text, Fig. 30).

Many investigators have studied the cornea with the aid of the electron microscope; however, few have concerned themselves with the details of the fine structure of the normal human corneal endothelium.1-9 In addition, Kaye and associates10-11 have reported a series of interesting experiments on the uptake and transport of colloidal particles by the rabbit corneal endothelium.

Recently, we had an opportunity to study the presumably normal corneal endothelium of several human eyes, into the anterior chamber of which Thorotrast had been injected very shortly prior to enucleation, which was required because of malignant tumors. The purpose of this study was, first, to investigate the transport of Thorotrast particles by the human corneal endothelium in vivo, in the hope that this might be related to the mechanism by which corneal nutrition is obtained from the aqueous humor; and, second, to obtain some information about the fine structure of the normal human corneal endothelium or, more strictly, of the functioning endothelium.

Materials and methods

Four human eyes, enucleated because of malignant tumors of the orbit, choroid, or lid and palpebral conjunctiva, were studied. The corneas of the eyes were clinically normal. Prior to enucleation, 0.1 to 0.15 ml. Thorotrast (Testagar and Company, Detroit, Mich., containing 24 to 26 per cent thorium dioxide) was injected into the anterior chamber of each eye immediately after removing the same volume of aqueous humor.

The corneal tissues were fixed with osmium tetroxide immediately after enucleation, and embedded in Epon, following the same procedures as reported before.12 The time from the moment of Thorotrast injection to that of the fixation and the age of the patients are as follows: 3 minutes (age 69); 5 minutes (age 5); 6.5 minutes (age 47); and 7 minutes (age 6).
The corneal tissues from both the central and the peripheral (limbal) area were cut into thin cross sections with an LKB-Ulrotome. The sections were treated with 2 per cent uranyl acetate, lead, or both (double staining), and then studied with a Siemens Elmiskop I. The high magnification pictures shown in this paper were taken at an original magnification of ×30,000.

Observations

The fine structure of the human corneal endothelium. The corneal endothelium consists of single-layered cells, 4 to 6 μ thick, with their thickest part in the center of each cell where a more or less flattened nucleus is located (Fig. 1).

The posterior cell membrane facing the anterior chamber forms a rather flat surface7 (Fig. 1), except for an occasional invagination (Fig. 22, arrow) or infolding (Fig. 23). The anterior cell membrane attached to Descemet's membrane also shows a rather flat section, particularly in the central area, although slight ripplelike indentations are sometimes observed (Fig. 1). These indentations are seen more often and are more marked in the peripheral area (Figs. 5 and 10), where cytoplasmic processes often penetrate some distance into Descemet's membrane (Fig. 4, p). This occurs particularly when the cell is unusually thin. This general appearance may be related to the formation of Hassall-Henle bodies.2-5, 14 Certain regions of the anterior cell membrane appear to be denser than in other parts and are accompanied by densification of a small band-shaped zone of adjacent cytoplasm (Figs. 10, e and 15, e); usually there is a narrow, slightly less dense area interposed between the dense cytoplasm and the cell membrane (Fig. 15, e). No noticeable thickening of the cell membrane, as seen by others,5, 7, 10 was observed in our specimens in this region. The lateral cell membrane, combined with that of the adjacent cell, forms an intercellular border, which is seen as a paired membrane structure following a circuitous course (Figs. 1 and 10).7, 9, 15 The tortuosity seems to be greater between limbal than between central cells. The posterior end of the intercellular border forms a terminal bar (Figs. 1 and 17).5, 7, 10, 11, 16 This structure is characterized by an extreme densification of the adjacent cytoplasm which is the lateral end of the "terminal web," and, in general, by the narrow low density gap of about 100 Å (Fig. 17, tb).10, 11 Within the gap, an intermediate thin, dense line is seen in high magnification when treated with uranyl acetate plus lead (double staining) (Fig. 19, tb). This thin line sometimes appears as a row of dots. The posterior end of the terminal bar is often recessed slightly from the general level of the cell surface which forms an obtuse angled curve toward the terminal bar. The intercellular space, other than at the terminal bar, generally is of low density, about 200 Å in width, the boundaries of which are quite parallel (Fig. 10, e). No wide or cavernous intercellular spaces were seen in fresh, well-fixed material. The intercellular space often appears to be slightly narrowed at its anterior end (Fig. 26). A junctional complex, which is believed to be a desmosome but appears to be poorly developed, is also sometimes observed clef1 by a rather wide gap 250 Å in width and accompanied by a densification of the adjoining cytoplasm (Fig. 16, ds). A thin, dense intermediate line is found within such a desmosomal cleft, which appears to be slightly denser than the other intercellular space. Apart from the terminal bar and desmosomes, regional narrow intercellular spaces about 100 Å in width, without noticeable densification of the adjacent cytoplasm, are sometimes seen in the intercellular border (Fig. 10, d). With high magnification, a thin intermediate line, or dotted line, as observed in the terminal bar, is also seen in this narrow area (Fig. 14). In the lateral cell membrane, which composes the intercellular border, a triple-layered structure about 80 Å in total thickness, i.e., two dense layers 25 Å each, interposed with a lighter 30 Å layer, which appears to be a unit membrane,15 was seen frequently with high magnification (Fig. 13), except at the terminal bar and the nar-

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The line shown in each figure represents 1 μ unless otherwise indicated.

Fig. 1. Low magnification view of Descemet's endothelium (E) in the central area of a normal human cornea. Numerous mitochondria are seen in the cytoplasm. The terminal web (tw) is almost free from cell organelles. The anterior cell membrane bordering Descemet's membrane (D) is rather flat. The intercellular border (ic) has a tortuous course. Thorotrast particles injected into the anterior chamber (A) are seen on the free surface; tb, terminal bar; n, nucleus.

Fig. 2. Mitochondria (m) with cristae running parallel to their long axis, seen in the endothelial cytoplasm.

Fig. 3. Mitochondria (m), in which the cristae appear to be arranged concentrically or spirally, found in the endothelial cytoplasm. Endoplasmic reticulum (er) is also seen.

Fig. 4. Endothelial cells from the limbal area. Note the enormously long mitochondrion (m) containing longitudinal cristae among numerous other mitochondria in the cytoplasm. A cytoplasmic process (p) protrudes into Descemet's membrane (D); endoplasmic reticulum (er).

Fig. 5. Endothelial cell from the limbal area showing numerous mitochondria (m) in the cytoplasm, except in the terminal web (tw) area where only vesicles containing Thorotrast particles (v) are seen. A centriole (c) is seen accompanied by well-developed Golgi complex (g). Endoplasmic reticulum (er) is also seen. Injected Thorotrast particles are located on the free surface facing the anterior chamber (A). The anterior cell membrane adjacent Descemet's membrane (D) appears as a wavy line.

Fig. 6. The centrioles (c) located near the terminal web (tw) are surrounded by a network of filamentous components which are similar to that constituting the terminal web. Thorotrast particles are seen on the free surface. g, Golgi complex; A, anterior chamber.

Fig. 7. Section of a probable "myelin figure" found occasionally in the cytoplasm. This structure consists of concentrically arranged, multilayered membranes in the periphery and a less dense center, and is surrounded by a single limiting membrane. In this particular figure, there is a dense body in the less dense central area.

Fig. 8. A "multivesicular body" seen in the cytoplasm.

Fig. 9. A high magnification of another "myelin figure." The apparently single limiting membrane seen in the lower magnification is shown as a triple-layered structure (arrows) similar to a unit membrane. The lucent area (a) may be an artifact.

Fig. 10. Intercellular border between endothelial cells in the limbal area. The intercellular border shows a complicated tortuosity. In the intercellular space (c), which generally is about 200 Å in width, a narrow region (d), about 100 Å wide, is seen occasionally. The anterior cell membrane (a) is somewhat wavy and occasionally contains an apparently denser region (e). In the cytoplasm of this particular picture, endoplasmic reticulum (er) and free RNP particles (r) are abundant. The terminal web (tw) is clearly shown. Thorotrast particles are seen on the free surface, some attached directly to the surface and the others in clumps some distance apart from it. D, Descemet's membrane; A, anterior chamber. Regions marked by a, b, c, d, and e, or similar areas, are shown at high magnification in Figs. 11, 12, 13, 14, and 15, respectively.

Fig. 11. Triple-layered structure (arrows) seen in the anterior cell membrane (a). D, Descemet's membrane.

Fig. 12. Triple-layered structure (arrow) seen in the posterior cell membrane (b).

Fig. 13. Triple-layered structure (arrows), as in a unit membrane, seen in each of the lateral cell membranes which constitute the intercellular border limiting the intercellular space (c).

Fig. 14. The intermediate line within the narrow region (d) of the intercellular border is seen clearly at portions shown by arrows.

Fig. 15. The apparently dense area (c) of the anterior cell membrane in high magnification. The cell membrane of this area also appears to have a trilaminar structure, which seems to be
slightly narrower than that of the other part (see Fig. 11). This area is accompanied by a densification of the adjacent cytoplasmic area, which is separated from the membrane by a slightly less dense intermediate area.

Fig. 16. A poorly developed desmosome (ds); c, intercellular border.

Fig. 17. The terminal web (tw) consists of a complicated, fine network of filamentous components, the density of which is extremely intensified near the terminal bar (tb), which contains a narrow gap, about 100 Å in width. Thorotrast particles are seen on the free surface and in the intercellular space above, but not in the terminal bar.

Fig. 18. This particular area of the terminal web (tw) shows a somewhat tubular rather than fibrillar pattern.

Fig. 19. A terminal bar (tb) showing an intermediate thin, dense line in the less dense central area. This structure may be equivalent to a zonula occludens (Farquhar and Palade).

Fig. 20. A portion of the terminal bar area, which is seen occasionally. The area, zo, appears to be the equivalent of the zonula occludens; and the area, za, farther from the cell surface, seems to be similar to a "fascia adhaerens."

Fig. 21. A narrow area containing filamentous components (f) seen frequently in the cytoplasm adjacent to the Descemet's membrane (D).

Fig. 22. Posterior, central region of an endothelial cell, facing the anterior chamber. Numerous Thorotrast particles are seen on the free surface. Vesicles containing Thorotrast particles (o) are found in a series from the free surface to the deeper cytoplasmic area, piercing the terminal web (tw). An invagination (arrow) of the free surface is seen.

Fig. 23. An infolding of the posterior cell membrane is shown. The terminal web (tw) follows the curve of the infolding.

Fig. 24. An endothelium fixed 6.5 minutes after the injection of Thorotrast into the anterior chamber. The particles are abundant on the free surface facing the anterior chamber (A). No particles are seen in the terminal bar (tb); instead, there are two vesicles containing particles in the lower cytoplasmic area. Many particles are found throughout the intercellular space other than the terminal bar (tb). Some particles are seen within Descemet's membrane (arrow).

Fig. 25. A case fixed 3 minutes after the Thorotrast injection. The particles (arrows) are seen almost reaching the anterior end of the intercellular space, but not within Descemet's membrane (D).

Fig. 26. A case 7 minutes after the Thorotrast injection. Some particles (arrows) are seen within the Descemet's membrane (D), as well as in the intercellular space, which appears to be slightly narrowed at the anterior end.

Fig. 27. The same case as in Fig. 26. Many particles (arrows) are seen in Descemet's membrane (D), as well as in the intercellular space which is cut obliquely.

Fig. 28. Many Thorotrast particles are seen on the free surface. Vesicles containing particles (1 and 2) are seen in the cytoplasm near the terminal bar. One vesicle containing particles (3) is connected with the intercellular space. Similar vesicles (o) associated with the intercellular space are seen in other areas. The particles are found in the intercellular space (4 and 5), other than the terminal bar region. These suggest, but do not prove, that the particles are carried from the anterior chamber (A) to the intercellular space above the terminal bar by the vesicles (1, 2, and 3) and then through the intercellular space (3, 4, and 5). The terminal bar (tb) is cut tangentially.

Fig. 29. In this picture the particles are seen throughout the intercellular space. The usual narrow (100 Å) gap is not seen at the cell interface adjacent to the anterior chamber in this section. Instead, the intercellular space appears to open into the anterior chamber and to contain Thorotrast particles which could have entered it from the anterior chamber. Some cytoplasmic density adjacent to this area, reminiscent of that seen associated with terminal bars, is observed. Some particle-containing vesicles associated with the intercellular space are also seen (o). A, anterior chamber.
Fig. 30. The fine structure of the human corneal endothelium described in this paper is schematized in this diagram. Terminal web, TW; nucleus, N; endoplasmic reticulum, ER; free RNP particles, R; Golgi complex, G; centriole, C; desmosome, D; Descemet's membrane, DM.

The many other organelles in the cytoplasm are mitochondria. Each of the numbered upper circles in Descemet's membrane shows in higher magnification the corresponding encircled area of the lower figure.

1. The posterior cell membrane.
2. The terminal bar, mostly a "zonula occludens." 3. Narrow region of intercellular space, about 100 Å wide. 4. General intercellular space, ca. 200 Å in width. 5. The apparently dense area of the anterior cell membrane.

Row areas. A similar substructure suggestive of the unit membrane was also sometimes found in the anterior (Fig. 11) and the posterior cell membrane (Fig. 12).

The cytoplasm contains a large number of mitochondria (Figs. 1 to 5), many of which have cristae running parallel to their long axis (Fig. 2, m), and some with these arranged concentrically (Fig. 3, m), as has been observed in animal corneas. It is conceivable that these are cross sections of the former. In others, the more usual crista pattern is seen. The mitochondrial shape varies; one as long as 6 μ with longitudinal cristae was observed (Fig. 4, m). Rough-surfaced endoplasmic reticulum (ER) (Figs. 3, 4, 5, and 10, er), smooth-surfaced ER of vesicular type, well-developed Golgi apparatus (Figs. 5, g and 6, g), free RNP particles (Fig. 10, r), and centrioles (Figs. 5, c and 6, c) are also commonly found. A rounded structure characterized by thin, concentrically arranged, multilayered membranes in
its periphery and a less dense central area was sometimes observed (Figs. 7 and 9). This structure is encircled by a single membrane (Fig. 7) which, in high magnification, shows a trilaminar structure about 80 A in total thickness (Fig. 9, arrow) as does a unit membrane. A multivesicular body, as seen in the other tissues, was found in the cytoplasm, although seldom (Fig. 8). The cytoplasm in a zone 0.2 to 0.5 μ in width, adjacent to the anterior chamber, has a peculiar structure which generally appears to consist of a complicated, fine meshwork of filamentous components (Fig. 17, tw), or occasionally of fine tubules (Fig. 18, tw). This zone is almost completely free from cell organelles (Figs. 1, 10, and 17, tw). In the specimen studied, the only organelles occasionally seen in this area are vesicles containing injected Thorotrast particles (Figs. 5 and 22), and sometimes centrioles. This zone is tentatively called the "terminal web" (see Discussion). The centrioles, frequently accompanied by a Golgi apparatus, are almost always located near the terminal web (Figs. 5, c and 6, c). Filamentous components similar to those forming the terminal web were sometimes seen around the centrioles (Fig. 6). One end of a centriole appeared to be embedded in the terminal web, as mentioned. A similar but less well-developed zone of filamentous components is sometimes observed in the cytoplasm near Descemet's membrane (Fig. 21, f) and, occasionally, also adjacent to the intercellular border and around the nucleus. The observations of the fine structure of the corneal endothelium described above are summarized in Fig. 30.

**Transport of Thorotrast particles across the human corneal endothelium.** The following observations apply to each case studied. An appreciable amount of Thorotrast particles was found on the endothelial surface (Figs. 1, 10, 22, and 24) of the entire cornea, some of which were attached directly to the cell surface while others were seen as clumps some distance from the cell surface (Fig. 10); the latter fact may suggest the existence of some coating material. Vesicles containing the particles were seen in the cytoplasm, located mostly in the posterior half of the cell body and most frequently very near the anterior chamber. They were often encountered in the posterior central region of the cell very far from the lateral cell membrane. Fig. 22 suggests that these vesicles result from pinocytosis, and also shows that they can pass through the terminal web, which is otherwise almost free from cell organelles. A cross section of a partial infolding of the posterior surface might assume an apparent vesicular shape, but this could be distinguished from a genuine vesicle because of the terminal web which follows the curve of the cell membrane, as is shown in Fig. 23. Genuine vesicles containing Thorotrast particles were also seen near the lateral cell membrane, some of which (Figs. 28, v and 29, v) appeared to be connected with the intercellular space in which Thorotrast particles were located (Figs. 17, 24 to 29). The terminal bars with about 100 A gaps and the narrow regions of the intercellular space were always devoid of the particles. Occasionally, however, the intercellular region corresponding to the terminal bar appeared to be filled with the particles (Fig. 29). It is possible that this is an artifact rather than a condition which is an occasional normal situation. Fig. 28 suggests that the particles are carried by vesicles in the cytoplasm from the anterior chamber to the intercellular space anterior to the terminal bar. Thorotrast particles passed very quickly from the anterior chamber through the endothelium. In both cases when Thorotrast had been in the anterior chamber for 3 and 5 minutes, respectively, before fixation, the particles reached the anterior end of the intercellular space, but were not seen in the Descemet's membrane (Fig. 25). In the 6.5 minute and the 7 minute cases, the particles were seen within the Descemet's membrane, having passed through the intercellular space (Figs. 24, 26, 27).
Discussion

A schematic representation of the fine structure of human Descemet's endothelium based on the study of many electron micrographs is shown in Fig. 30. The cytoplasmic area adjacent to the anterior chamber has a peculiar structure which we have called "terminal web" and have described. This region is similar to the "terminal web" described electron microscopically in the intestinal epithelium by Palay and Karlin. The terminal web, named by Sauer, was studied histochemically in many cells by Leblond and co-workers. In this paper, the term "terminal web" is used for that specific area of the human corneal endothelium, because of its similarity in the electron micrographs to the "terminal web" reported in the intestinal epithelium, because of its location in the cell and its close relationship to the terminal bar, as well as to the centrioles. This area may correspond to that reported as "a relatively thick osmic acid-positive layer" or as "the area of greater electron density."

A terminal bar was found at the posterior end of the intercellular border as seen by others in both rabbit and man, but, on rare occasions, a typical terminal bar was absent (Fig. 29). Recently, Farquhar and Palade proposed a new nomenclature distinguishing three different junctional complexes: (a) zonula occludens (tight junction), which is continuous around each cell; (b) zonula or fascia adhaerens (intermediary junction); and (c) macula adhaerens (desmosome), thereby dropping the term, "terminal bar." In our studies, the so-called terminal bar of the corneal endothelium seems to consist almost exclusively of a "zonula occludens" with associated cytoplasmic densification, as defined by Farquhar and Palade. A region reminiscent of the "fascia adhaerens" was seen rarely, immediately anterior to the zonula occludens (Fig. 20). In the intercellular border, there are narrow regions about 100 A in width where the adjacent cytoplasm is not more dense than elsewhere. This narrow region is similar to the terminal bar (zonula occludens) in its width and in the presence of an intermediate thin, dense line. The intermediate line seen in both the terminal bar and in such narrow regions probably represents the line of fusion of the outer leaflets of the unit membrane.

An unknown structure found in several cells was characterized by multilayered, concentrically arranged membranes with a less dense center. It somewhat resembles a cross section of a myelinated nerve fiber; although its nature is not clear, it may be a type of "myelin figure."

Thorotrast particles, injected into the anterior chamber, reached the anterior end of the intercellular space within 3 minutes and were found in the Descemet's membrane in 6.5 minutes. Their passage through the endothelium appears to be mainly intercellular, except in the terminal bar area. This confirms the findings of Kaye and associates in the rabbit, using Thorotrast in vitro and other markers in vivo, although in their experiments the Thorotrast particles were not seen in the Descemet's membrane until after 30 to 45 minutes of exposure in vitro, and none was found in the cytoplasm even after one hour of exposure in vivo. In addition, the wide dilation of the intercellular space found in their preparations was not seen in our material. They concluded, as a result of their studies of the in vitro rabbit preparations, that the Thorotrast particles were carried in vesicles around the terminal bar. In our observations, the zonula occludens were devoid of Thorotrast particles, and some of our electron micrographs showed Thorotrast-filled vesicles which could be interpreted as en route around the terminal bar (Figs. 24 and 28). These two facts are in agreement with the reports on rabbit material. Occasionally, however, particles were found within the terminal bar area (Fig. 29). The intercellular space in such areas was wider (200 A) than the usual terminal bar space (100 A). Such appearance could be artifactitious. However, the possibility should not be neglected that the terminal bars
may open momentarily, permitting the entrance of anterior chamber material into the intercellular spaces. This concept is entirely speculative, and is suggested by a few observations such as the one in Fig. 29. The narrow (100 Å) regions seen in the intercellular spaces other than the terminal bar may occupy a limited plaquelike area, thus permitting the particles to pass through the intercellular space around the “narrow” area. The latter may function as merely an attachment device.

The rapid movement of Thorotrast particles from the anterior chamber to the Descemet’s membrane through the endothelium, together with the conceivable vigorous activity of the endothelial cells with numerous mitochondria, suggests the possibility of active participation of the endothelium with respect to the transfer of material. The vesicles containing Thorotrast particles located in the posterior central region of the cell body far from the intercellular borders may be concerned with phagocytic activity rather than with transport.

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REFERENCES

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