Further electron microscopic study of hereditary corneal edema

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This report presents further observation on the fine structure of hereditary corneal edema. The anterior and middle stroma showed almost normal collagen lamellar formation. In the posterior stroma irregularity of interfibrillary distance was noted. Cross-sections of collagen fibrils varied in diameter. Two Descemet's membranes were found, the second one contained unusual banded materials. Most parts of the second Descemet's membrane were covered with thickened multiformed packed smaller collagen fibrils, and a fibroblast-like cell layer was present on the face of the anterior chamber. These observations support our earlier study which suggested that the morphologic changes occurred during fetal development.

Key words: heredity, corneal dystrophy, corneal edema, collagen fibrils, Descemet's membrane, endothelium, histopathology, electron microscopy.

Hereditary corneal edema, one of the congenital corneal dystrophies, is thought to be a dominant transmission. Electron microscopic study of our primary report differentiated this condition from a congenital hereditary corneal dystrophy described by Maumenee and Kenyon. Through electron microscopic studies of a corneal button from another patient afflicted with keratoplasty, this study provides additional information concerning the spectrum of morphologic changes in hereditary corneal edema.

Case history

We first examined J. E., a 25-year-old Caucasian woman, in June, 1969. At this time she gave a history of poor vision since birth. This had been diagnosed originally as congenital glaucoma, and a trephine operation had been performed in her right eye. Following surgery she had received topical medication to control her intraocular pressure, but had been off of this treatment for many years without consequent pressure elevation. At age 17 she began wearing a contact lens in her left eye. Her family history was negative, and her physical examination was within normal limits.

Ocular examination. The patient's best corrected visual acuity was counting fingers at 10 feet in her right eye and 20/80 to 20/100 in her left eye. In her right eye, refraction revealed a manifest correction of approximately 6 diopters, but this failed to improve her visual acuity. She had approximately 20 prism diopters of intermittent exotropia with 6 diopters of left hypertropia. Slit-lamp examination revealed fairly symmetrical corneas. The anterior stroma
and epithelium were regular, and the deeper two thirds of stroma had a fine, ground-glass haze. Although no guttata were seen, the corneas were thickened, and the endothelial pattern was markedly irregular. The anterior chambers were deep. There were iris cysts at the pupillary margin in both eyes. The lens appeared grossly clear. Indirect ophthalmoscopy showed normal discs and vessels in both eyes. Her intraocular pressure was 16 mm. Hg in each eye.

In September, 1969, a 7.5 mm. penetrating keratoplasty was done in the right eye. The graft remained clear, however, the patient's vision was poor due to amblyopia. In September, 1970, a 7.5 mm. penetrating corneal graft was done in the left eye (Fig. 1).

Material and methods

The corneal button was obtained at the time of operation (Sept. 15, 1970). It was fixed in 4 per cent glutaraldehyde with phosphate buffer at room temperature for 30 minutes and then sectioned into smaller pieces. These were postfixed with 1 per cent osmium tetroxide in the same buffer at 4° C. for 90 minutes, dehydrated through a series of ethyl alcohol solutions, and embedded in Epon.

Thick sections were stained with toluidine blue and studied simultaneously with the light microscope for orientation in electron microscopy.

The embedded tissue was cut with a Porter-Blum microtome and stained with uranyl acetate and lead citrate. Electron micrographs were taken with a Zeiss 9S-2.

Results

Because of scraping at operation, epithelial cell layers were absent. Bowman's membrane was present in all specimens and showed normal thickness.

Anterior and middle layers of collagen lamellae ran parallel to each other, and their arrangement was almost normal. However, in the posterior stroma, some collagen formation showed irregular alignment. (Fig. 2). At higher magnification, the arrangement of collagen fibrils which make up the individual lamellar bundles showed irregularity of the interfibrillary distance. An increase in the interfibrillary distance (collagen-free spaces) was seen, especially in the posterior stroma. A cross section of fibrils varied from 200 to 400 A in diameter. In a longitudinal section a periodicity of about 650 to 700 A was measured (Fig. 3). Granular substances were seen between the lamellae or adjacent to keratocytes (Fig. 4). Most keratocytes were normal, but some showed degeneration in the posterior stroma (Fig. 5).

Two Descemet's membranes were found in this case (Fig. 6). The first Descemet's membrane was gently undulated and thinned. Its thickness was about 2.3 μ and banded materials with a periodicity of about 1,000 to 1,100 A were seen throughout the whole layer. The nonbanded homogeneous layer was completely absent. The spaces between the two Descemet's membranes consisted of numerous multiformed collagen fibrils. The duplicated Descemet's membrane was thicker than the first one and its thickness was about 4 μ. No distinct border existed on the endothelial side. Large areas of the second Descemet's membrane were covered with fibrous regions, but some parts were covered with the endothelium. Two different banded materials were seen in the second Descemet's membrane and border areas (Figs. 7 and 8). One was a spindle-shaped long-spacing banded material with 1,000 to 1,100 A periodicity. The other has a periodicity of about 500 A. Their banded materials were distributed at random and transform to each other in some areas (Fig 9). The region between the second
Figs. 2 and 3. (Fig. 2) Formation of collagen fibrils showing disorganized alignment. (Original magnification ×29,000.) (Fig. 3) Cross section of collagen fibrils. Diameter of fibrils varies from 200 to 400 Å. Interfibrillar distances are irregular. (Original magnification ×87,000.)
Figs. 4 and 5. (Fig. 4) Granular substances (h) showing between the lamellae or adjacent keratocytes (K). (cf) Collagen fibrils. (Original magnification ×29,000.) (Fig. 5) Degenerated keratocyte. Mitochondria (m) are swollen with pale matrix. (N) Nucleus. (Original magnification ×14,000.)

Descemet's membrane and endothelium showed a mixture of collagen fibrils and bundles of these fibrils with 500 Å banded materials. At higher magnification, a longitudinal section of the latter banded materials was composed of bundles of about 200 Å collagen fibril materials (Fig. 10). The parts of the second Descemet's membrane which was covered with endothelial cells consisted of groups of 100 Å fibrous materials with no periodicity and homogeneous substances (Fig. 11).

Most endothelial cells were absent and replaced by a single layer of fibroblast-like cells. Each cell contained a long-oval nucleus with condensed chromatin, several round or oval type mitochondria, few rough endoplasmic reticulum, a well-developed Golgi complex, and scattered filaments. Several vesicles opened to the anterior chamber (Figs. 12 and 13). Only one specimen was observed to have endothelial cells and few lymphocytes (Fig. 14). They were located on the second Descemet's membrane. Endothelial cells had many microvilli on the face of the anterior chamber, and each contained an oval-shaped nucleus, few mitochondria, and distended endoplasmic reticulum. Some of them showed filaments throughout the cytoplasm. Lymphocytes were located between endothelial cells.

Discussion

Marked corneal edema was seen in our first reported case of hereditary corneal edema.1 This present case had less corneal edema, and ultrastructural stromal exami-
Fig. 6. Two Descemet's membranes of hereditary corneal edema. The first (original) membrane (DM-1) is thinned and consists of banded materials. No distinct border of the second (duplicated) membrane (DM-2) exists on the endothelium side. (St) Stroma; (cf) collagen fibrils. (Original magnification ×5,400.)

nation showed almost normal collagen formation. No large collagen-free spaces were found, however, in a careful examination of cross sections, the irregularity of the interfibrillar distance was noted, especially in the posterior stroma. Cross sections of collagen fibrils measured 200 to 400 A in diameter. The bigger collagen fibrils seem to have resulted from altered collagen fibrillogenesis during fetal development rather than from excessive hydration as suggested by Kenyon and Mau-menee.3

Another stromal finding was granular substances between lamellae or adjacent to keratocytes. Such substances were observed in swollen corneas4,5 and Fuchs' dystrophy.6

As in our first report, the most characteristic findings were in the posterior portion. Two Descemet's membranes were found, each thinner than that of a normal membrane. A normal Descemet's membrane consists of two regions, with the one closer to the anterior chamber having a homogeneous structure. The region closer to the stroma shows regularly arranged fibril bundles with a banding of about 1,000 to 1,100 A.7,8 The first Descemet's membrane lacked the homogeneous structural region and demonstrated banded material which is the same as described by Jakus.7,8 The second Descemet's membrane also lacked the homogeneous structural region and had two different banded materials. One had a spindle-shaped long-spacing banded material which had the same periodicity as the first Descemet's membrane. The other banded material had a periodicity of 500 A and was composed of bundles of the
Figs. 7 and 8. (Fig. 7) Second Descemet’s membrane containing two different banded materials. One is spindle-shaped long-spacing banded material (large arrows), the other is 500 Å banded material (small arrows). (Original magnification ×30,000.) (Fig. 8) Border region of the second Descemet’s membrane consists of 200 Å collagen fibrils (cf), 500 Å banded materials (small arrows), and high density homogeneous substances (d). Long-spacing banded material (large arrows). (Original magnification ×43,000.)
smaller collagen fibrils. Spindle-shaped long-spacing banded materials are seen in the normal peripheral Descemet's membrane showing a Hassal-Henle body,\(^7\)\(^8\) new regenerative Descemet's membrane,\(^9\) and pathologic Descemet's membrane.\(^6\)\(^8\)\(^9\) Descemet's membrane of Fuchs' dystrophy is characterized by a greatly increased thickness and consists of three different banded regions\(^11\) or five different banded regions.\(^12\) Two different banded materials which are closest to the endothelium in Fuchs' dystrophy are similar to that of the second Descemet's membrane. Occasionally, 500 Å banded materials can be seen to transform to long-spacing banded materials at the border region as described in Fuchs' dystrophy. Both banded materials seem to be composed of 200 Å collagen fibrils as shown in Figs. 8 and 9. Such fibrillar materials may be presumed to be produced from fibroblast-like cells of posterior layer. In order to explain the appearance of unusual banded materials in Fuchs' dystrophy, Iwamoto and associates\(^12\) suggested that the anterior banded and nonbanded regions probably represent the remnant of normal Descemet's membrane, and the pathologic changes are added posteriorly. The abnormal endothelium produces not only the basement membrane-like material, but also collagen fibrils.

The posterior surface of Descemet's membrane in congenital hereditary corneal dystrophy is directly exposed to the anterior chamber.\(^3\) In our first case of hereditary corneal edema, the normal endothelium of the posterior corneal layer had been replaced with several fibroblast-like cell layers.\(^4\) Recently, such fibroblast-like cells have been reported in the endothelium of Fuchs' dystrophy and the endothe-
Figs. 10 and 11. (Fig. 10) Fibrous region between the second Descemet's membrane and the posterior cell layer. 500 Å banded materials also consist of 200 Å collagen fibrils. (Original magnification ×40,000.) (Fig. 11) The second Descemet's membrane (DM-2) near the abnormal endothelium (En) showing groups of 100 Å fibrous materials with no periodicity (large arrows) and homogeneous substances. Coated vesicles (small arrows). (Original magnification ×38,000.)

Lium associated with retrocorneal membranes in failed penetrating keratoplasties. When associated with corneal grafts, the cells of the wound and the retrocorneal membrane are of host origin. Maruyama and co-workers showed that in alkaline corneal burns the retrocorneal membranes were made of fibroblast-like cells; and suggested this was overgrowth of the stimulated endothelium. In the present case, the normal endothelium was also replaced by fibroblast-like cells. Their morphology was slightly different from typical fibroblasts because they possessed fewer rough endoplasmic reticulum. These cells probably arise from the regenerative endothelial cells and may have a different function.

The abnormal endothelial cells were located on the second Descemet's membrane and had many microvilli on the face of the anterior chamber. Their cytoplasm contained few mitochondria, distended endoplasmic reticulum, and filaments. Occasionally, intracytoplasmic filaments can be seen in the normal endothelial cell, tissue cultured endothelial cell, and regenerative endothelial cell, but those in our present case were more abundant.

Several lymphocytes were located between the endothelial cells, and may have caused the formation of the abnormal posterior cells. The endothelium which produced the second Descemet's membrane could have been damaged by uveitis, the subsequent infiltration of lymphocytes causing severe endothelial damage. The large parts of endothelium may again be replaced by the regenerative endothelial
Figs. 12 and 13. (Fig. 12) Fibroblast-like cell has several mitochondria (m), well-developed Golgi complex (G), few endoplasmic reticulum, and cytoplasmic filaments (f). Many vesicles (small arrows) open to the anterior chamber (AC). (cf) Collagen fibrils. (Original magnification x14,000.) (Fig. 13) The second Descemet's membrane (DM-2) is covered with the abnormal endothelial cells (En) with invaded lymphocyte (Ly). (AC) Anterior chamber. (Original magnification x6,300.)

Fig. 14. The abnormal endothelial cell showing few mitochondria, distended endoplasmic reticulum, and filaments. (DM-2) Second Descemet's membrane; (G) Golgi complex; (N) nucleus. (Original magnification x13,000.)
cells, and, finally, these cells may alter to fibroblast-like cells. The remaining endothelial cells may also change pathologically.

Thus, hereditary corneal edema specimens suggest that during fetal development an unknown determinant, possibly hereditary, causes altered collagen fibrillogenesis and endothelial damage.

We wish to acknowledge the valuable technical assistance provided by Mr. John G. Valenti.

REFERENCES


