Electron microscopic studies on Fuchs' combined dystrophy
II. Anterior portion of the cornea

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Anterior portions of the central corneas of Fuchs' combined dystrophy were studied with the electron microscope, with the use of the same corneal buttons as in Part I of this study, in which changes of the posterior portions were reported. The epithelium showed various grades of cellular edema, mostly in the basal layer. Some of the edematous cells appeared to burst and coalesce to form subepithelial bullae. Possibly as a result, together with proliferation of epithelial cells, marked undulations of the epithelial base and "epithelial quasi islands" with fissures were formed. Intercellular spaces with desmosomes were usually closed and tight junctions of the surface layer could be seen as in the controls. Alterations of the subepithelial region paralleled or followed the epithelial changes. Cells appeared in this region at an early stage. Serial sections showed these to come from the anterior stroma. Later, active fibroblasts and collagen fibrils increased, forming large subepithelial masses of connective tissue. These filled all spaces between the irregular epithelial base mentioned above and Bowman's layer, including the fissures separating the epithelial quasi islands. At a later stage fibroblasts became inactive and fewer and the collagen fibrils became thicker. Alterations of Bowman's layer and stroma were less marked, although some changes were seen in the stroma as compared with normal controls. All this indicates that the changes in the anterior cornea in Fuchs' dystrophy are secondary to the involvement of the posterior portions which were discussed in Part I, and that the subepithelial alterations are to form a subepithelial connective tissue. Development of anterior bullous changes may be largely dependent upon the balance between the barrier function of the posterior banded region of Descemet's membrane and dysfunction of the altered endothelium.

Key words: Fuchs' dystrophy, cornea, dystrophy, epithelium, bullous keratopathy, electron microscopy, ultrastructure, pathology, Bowman's layer, stroma.

Although Fuchs' combined dystrophy was originally recognized by its epithelial involvement,1 its primary locus is now believed to be the posterior portion of the central cornea (discussed in Part I of this study2). Electron microscopy of this region revealed various morphologic changes of the endothelium and Descemet's membrane, and fine structural analysis led us to a new hypothesis concerning the mechanism of alterations of these tissues.3

There are some histologic reports on the anterior corneal portions of Fuchs' combined dystrophy,1, 5-14 but ultrastructural studies on these regions are very few.15 In
this paper (Part II) we have attempted to elucidate: (1) detailed fine structural changes of the anterior portions of the cornea, (2) whether the anterior changes are really secondary to the posterior involvement, (3) the sequence of the changes, and (4) what relations may exist between the alterations of the anterior portions and those of the posterior.

Materials and methods

The materials, methods, and cases used are the same as for Part I. Corneal buttons obtained by perforating keratoplasty of seven cases of clinically typical Fuch's combined dystrophy were fixed with 3 to 4 per cent glutaraldehyde followed by 1 per cent OsO₄, embedded in Araldite, and studied with the electron microscope.

Observations

Case 1. Epithelial change was not marked, except that posterior portions of the basal cells were often edematous. The epithelial basement membrane was normal. However, groups of flattened cells were interposed between the epithelium and Bowman's layer (Fig. 4); the spaces for these cells were formed by partial elevation of the epithelial base. Many of these cells appeared to be fibroblasts, but some resembled Schwann cells with unmyelinated nerve fibers (Fig. 5). The cells were usually surrounded by a dense sheathlike area, similar to basement membrane, but containing thin fibrils (about 100 A diameter). Between the sheath area and the epithelial basement membrane there was usually a narrow layer of collagen fibrils (150 to 200 A in diameter) (Fig. 5). Bowman's layer was almost entirely normal, but a rare condition was found in serial sections wherein a row of cells appeared to cross the Bowman's layer from the anterior stroma to the subepithelial region (Fig. 6). The stroma did not show striking changes. However, when compared with normal control corneas, it was noted in places that (1) the collagen lamellae were wavier, (2) interfibrillar spaces were wider (Fig. 19), and (3) regions composed of fine granular or filamentous material were more marked within collagen lamellae and adjacent to keratocytes (Fig. 20).

Case 2. Various changes were found in the epithelium and subepithelial area of the central cornea where typical bullous alterations were seen clinically. The thickness of the epithelial layer varied due to large infoldings or undulations of the epithelial base (Fig. 11); the spaces between the epithelial base and Bowman's layer were filled with subepithelial connective tissue described below. Some regions of the epithelium appeared as if one to several epithelial islands were piled under the superficial epithelial layer, with each island separated by a continuation of the subepithelial connective tissue (Fig. 13). However, narrow cytoplasmic bridges were often seen connecting those epithelial quasi islands with each other and with the superficial epithelial layer (Fig. 14). Various grades of cellular edema were observed, mainly at the basal layer of this altered epithelium. In general, the edematous epithelial cells were characterized by decreased electron density of the cell bodies, including the nuclei (Fig. 7); some such cells had accumulations of glycogen-like particles in the cytoplasm (Fig. 8). Finally, some of the edematous cells appear to have burst or emptied themselves leaving a little cell debris in the periphery. Coalescence of such emptied cells sometimes formed a large vacant space between the epithelium and Bowman's layer; this was possibly a subepithelial bulla (Fig. 9). Apparently normal, epithelial basement membrane was usually seen at the epithelial base, including the circumference of the quasi islands, but it was occasionally missing, particularly beneath the edematous cells. In wide areas of the central cornea a subepithelial layer of varying thickness filled the spaces between the irregularly undulating epithelial base and the almost normal Bowman's layer (Figs. 7, 10 to 13). The subepithelial layer consisted of many cells (mostly active fibroblasts) (Figs. 10 and 12), abundant collagen fibrils (about 200 A in diameter),
some dense areas composed of fine filaments and thin fibrils (about 100 Å), and areas of empty spaces (Fig. 12). Stromal changes were similar to those of Case 1, but the keratocytes often appeared fatter than normal, with more developed rough-surfaced endoplasmic reticulum (RSR) (Fig. 18).

**Case 3.** The epithelium showed undulations of its base and occasional epithelial quasi islands as in Case 2, but changes of the cellular fine structure were much less marked. A broad subepithelial connective tissue of irregular thickness (up to 20 μ) was formed; this was similar in Case 2, but the number of cells here (mostly inactive fibroblasts) was fewer and the main elements were collagen fibrils (150 to 200 Å diameter) and dense filamentous areas (Fig. 16). These findings suggested a quiescent stage of elapsed bullous keratopathy. Bowman's layer and stroma were similar to those in Case 1.

**Case 4.** A very massive (up to 350 μ thick) subepithelial connective tissue layer was found in the central cornea. The epithelium covering this region was thinner than normal (about 30 μ; 4 to 5 layers) and showed various edematous changes at the basal layer, as seen in Case 2. The subepithelial connective tissue consisted of thick collagen fibrils (about 300 Å in diameter), some thin fibrils (about 100 Å in diameter), and a very few inactive fibroblasts. Arrangement of these elements was loose with wide interfibrillar spaces (Fig. 17). An artificial separation between this and Bowman's layer was seen. These findings suggest a late stage of subepithelial connective tissue formation, the epithelial changes possibly representing a recurrent bullous alteration due to uncompensated conditions of this cornea. The structure of the Bowman's layer and stroma was similar to that in Case 2, but widening of the interfibrillar spaces was more marked.

**Case 5.** The epithelium showed occasional formation of quasi islands with narrow epithelial fissures as in Cases 2 and 3, and the basal layer showed some edematous changes. However, the thickness of the epithelial layer was even and within normal limits (45 to 50 μ). Relatively thin fibrils (about 150 Å in diameter) filled the epithelial fissures. A thin layer of the same fibrils was also seen under the apparently normal epithelial basement membrane. Frequently, still deeper, there was a layer of dense filamentous material similar to basement membrane. The combination of these subepithelial elements gave an impression of apparently thickened epithelial basement membrane with more fibrillar elements. This subepithelial area with a total thickness of less than 1μ could be interpreted as a minimal grade of subepithelial connective tissue formation; connective tissue cells were seldom in this layer. Bowman's layer and stroma were similar to those in Case 2.

**Case 6.** A local inflammatory change was seen in the central cornea. The lesion spread from the epithelium to the anterior stroma, with the latter most widely involved. Cells found in this area were neutrophils, some lymphocytes, degenerated cells with pyknotic nuclei, and many macrophages. As such inflammatory changes are believed to be a secondary complication of this disease, their detailed descriptions are omitted. Otherwise, the changes of the anterior cornea appeared similar to those of Case 5.

**Case 7.** The epithelium appeared normal except for some changes in the nuclei, which in the epithelial cells at the basal layer showed marked clumping of the chromatin substance within the central region, while those in the middle layer contained a striking tubular formation (Figs. 1 and 2). The epithelial basement membrane showed apparent thickening up to 2.5 μ (average 0.5 to 1.0 μ). This area was composed of dense filamentous material similar to basement membrane, but contained occasional vague configurations of collagen fibrils and a few connective tissue cells. Bowman's layer seemed intact, and the stromal change was similar to that in Case 1.
Fig. 1. An epithelial cell nucleus at the middle layer of epithelium (Case 7), showing flower-like tubular formations (t). Line represents one micron, unless otherwise indicated. (Original magnification ×8,400.)

Fig. 2. Higher power view of one tubular formation (t) in Fig 1. It appears to consist of bundles of hollow tubular structure about 100 Å in diameter with hexagonal cross section. These bundles show a honeycomb pattern at cross section (arrow), and a laminated figure at longitudinal section; in the latter, thin periodic lines seem to cross the laminae. (Original magnification ×100,000.)

Fig. 3. An early change of the anterior portion of the cornea (Case 7) showing apparently thickened epithelial basement membrane (bm). ep, Epithelium; B, Bowman's layer; c, connective tissue cell. (Original magnification ×22,000.)
Fig. 4. An early stage of changes in the anterior portion of the cornea (Case 1). Slightly edematous alterations (e) can be seen at basal portions of basal epithelial cells (ep), and groups of cells (c) appear in the subepithelial region. Bowman's layer (B) is intact. (Original magnification ×3,400.)

Fig. 5. One of the cells in the subepithelial region (Case 1). This (c) appears to be a Schwann cell with a nerve axon (n). Other cells were identified as fibroblasts. Note that a sheath-like area (s) with thin fibrils surrounds the cells. ep, Epithelium; b, subepithelial basement membrane; B, Bowman’s layer. (Original magnification ×25,000.)

Fig. 6. A rare area found by serial sectioning (Case 1) where rows of cells appear to cross Bowman’s layer (B) from anterior stroma to subepithelial region. ep, Epithelium. (Original magnification ×3,750.)
Fig. 7. Edema of basal cells (e) in the epithelium (Case 2). s, Subepithelial connective tissue; B, Bowman's layer. (×2,200.)

Fig. 8. Accumulation of glycogen particles (g) seen in edematous basal epithelial cells. (×18,500.)

Fig. 9. Subepithelial bulla (Case 2). Probably edematous basal cells have burst leaving cell fragments (e) in the periphery and the emptied spaces coalesced to form the bulla (b). ep, Epithelium; B, Bowman's layer. (×18,500.)
Fig. 10. Many cells (c) in subepithelial connective tissue (sc) (Case 2). ep, Epithelium. (x6,400.)

Fig. 11. A large infolding of subepithelial connective tissue (sc) (Case 2). ep, Epithelium; e, edematous basal cells; B, Bowman's layer. (x970.)

Fig. 12. Subepithelial connective tissue with an active fibroblast (f) (Case 2). The connective tissue is composed mainly of collagen fibrils (c); many empty spots (e) may represent inter-fibrillar edema. ep, Epithelium; B, Bowman's layer. (x6,000.)
Fig. 13. Epithelium of bullous keratopathy (Case 2). The epithelium is separated into several layers by large fissures (s), as if epithelial quasi-islands were formed underneath a superficial epithelial layer (ep). The fissures or interspaces (s) are filled with a continuation of subepithelial connective tissue (sc). B, Bowman's layer. Note that intercellular spaces with desmosomes are all closed. (×2,500.)
Fig. 14. Cytoplasmic bridge (b) which connects epithelial quasi islands (qi), such as shown in Fig. 13 (Case 2). s, Epithelial fissure, which is a continuation of subepithelial connective tissue. (Original magnification x7,250.)

Fig. 15. Epithelial surface, showing a tight junction (arrow) at the apical portion of an intercellular space (Case 3). m, Micropla; d, desmosome. (Original magnification x148,500.)

Fig. 16. A thick subepithelial connective tissue layer at a late stage of epithelial involvement (Case 3). It consists of many collagen fibrils (c) (about 200 A in diameter), dense filamentous material (d), and a few inactive fibroblasts (f). (Original magnification x20,000.)

Fig. 17. A part of an enormously thick subepithelial connective tissue layer (Case 4) at a stage presumably later than that of Fig. 16. It consists mainly of thick collagen fibrils (c) (about 300 A in diameter) and some thin fibrils (t) with wide interfibrillar spaces. (Original magnification x16,800.)
Fig. 18. Stromal keratocyte (k) seemingly activated with more cell organelles than usual and with thick cytoplasm (Case 2). (x23,000.)

Fig. 19. A stromal region with widened interfibrillar spaces (from noninflamed area of Case 6). (x40,600.)

Fig. 20. A stromal region with wide areas of finely granular or filamentous substance (f) (Case 1). k, Keratocyte. (x23,000.)
Discussion

The epithelium showed various grades of edematous changes in many cases, shown typically in Case 2. The edema, starting apparently at the basal portions of the basal cells (Case 1), involved mainly the basal layer of the epithelium, but sometimes the middle layer as well. The superficial layer was usually intact. Finally, some edematous cells seemed to burst and coalesce to form large subepithelial bullae (Case 2). The spaces thus formed may eventually be filled with subepithelial connective tissue which is discussed below; as a result large infoldings or undulations of the epithelial base may be produced (Fig. 11). Such a process, together with partial proliferation of the epithelial cells, may cause formation of epithelial quasi islands with fissures (Fig. 13). The intercellular spaces of the epithelial cells with numerous desmosomes were usually closed in all the cases we studied, and in the surface cells tight junctions were seen (Fig. 15) as often as in controls. Such tight junctions of the epithelium were noted in the normal epithelium by others.10-18 All these indicate that bullous changes of the epithelium started with intracellular edema of the basal cells and resulted from some influences, such as osmotic pressure change, which were brought from the posterior side. Tubular formation was found in the nuclei of epithelial cells at the middle layer of epithelium (Figs. 1 and 2). To our knowledge, such a structure has not been reported before. Although this was most prominent and typically seen in Case 7, a similar and less prominent structure was also visible in the nuclei of epithelial cells at the same layer of others cases, as well as in normal controls. Therefore, this tubular formation may not be specific to this disease.

Subepithelial alterations appeared to parallel or follow the epithelial involvement. Taking into consideration the histories of the patients, and the structural changes in their anterior corneas, it would seem that the subepithelial modification proceeded in the same order as in Cases 7 (?), 1, 2, 3, and 4. If this were true, the following sequence would be possible: The early change of this layer is either apparent thickening of the epithelial basement membrane (Case 7) or subepithelial cell deposits (Case 1). Which of these is really the earlier is not clear, but the lesser changes of the epithelium (except for the nuclei) in Case 7 suggest this to be the earlier stage. By serial sectioning, it was shown that the cells deposited in the subepithelial layer of Case 1 probably migrated from the anterior stroma, at least in part (Fig. 6) as presumed by McTigue.15 As the subepithelial change advanced, the number of active fibroblasts increased paralleling the greater amount of collagen fibrils (Case 2). At a later stage, fibroblasts became inactive and fewer and the main constituents were a large amount of collagen fibrils (about 200 Å in diameter) and dense filamentous materials (Case 3). At a further late stage, diameter of collagen fibrils increased (about 300 Å) with lesser amounts of dense materials and fewer cells, but with wider interfibrillar spaces (Case 4). In these series, the thickness of the subepithelial connective tissue also increased as the presumed stage proceeded, although this may not be a necessary process. However, all these indicate that the subepithelial changes are, in principle, the formation of subepithelial connective tissue which may act to repair the damaged epithelium and also as a barrier. This confirms earlier light microscopy,6,13 but no blood vessels were seen in the tissues studied.

Bowman's membrane was usually intact. Stromal changes were also less marked, but partial changes were seen as mentioned. When compared with normal controls they were characterized by (1) wavier collagen lamellae, (2) wider interfibrillar space, (3) presence of activated keratocytes, and (4) more areas of granular or filamentous material within collagen lamellae and around keratocytes.

In conclusion based on all of our data, there appears to be no doubt that the
changes in the anterior portion of the cornea (Part II) are secondary to those of the posterior portion (Part I). The most serious epithelial and subepithelial involvement was seen in Case 2, which possessed an incomplete posterior banded region within the thin Descemet's membrane. The fewest epithelial changes were found in Cases 1 and 7, in which a thick posterior banded region was formed within the thick Descemet's membranes. In Fuchs' combined dystrophy the posterior banded region of Descemet's membrane, including warts, may act as a barrier to prevent further permeation of the aqueous humor which may have passed through the altered endothelium. Development of the anterior bullous changes may be largely dependent upon the balance between the barrier function of the posterior banded region and dysfunction of the endothelium, as a similar assumption was made by Kayes and Holmberg.19

REFERENCES