Further observations on the fine structure of the cornea

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A summary of the fine structure of presumably normal cornea is presented and is augmented by details not previously published. Certain differences noted between epithelial and endothelial cells are discussed, in view of their possible bearing on the function of these layers. Corneal tissue obtained from patients afflicted with keratoconus, cornea guttata, or Wilson's disease is compared with normal cornea, and a scarred rabbit cornea is compared with its normal counterpart. The widespread alterations in fine structure are described and discussed.

Since the fine structure of the normal central and peripheral human cornea has been discussed recently, only a brief review of this subject and a few additional observations will be presented here; most of the time will be spent in describing changes observed in corneas which had been altered pathologically or experimentally. Two corneas with keratoconus have been examined, one cornea guttata, a second Kayser-Fleischer ring, and a scarred rabbit cornea.

The methods of preparation and observation of the tissue were the same as those described previously.

Central cornea

Epithelium. In most electron micrographs of corneal epithelium, a gradual decrease in the density of the cytoplasm from the basal to the squamous cells has been observed; occasionally in human epithelium, however, some of the outermost squamous cells show such a great diminution in density that they are in startling contrast with adjacent cells (Fig. 1). In these pale cells, the filaments are virtually the only visible component of the cytoplasm and, consequently, their quantity and distribution can be clearly discerned.

The pale cell in Fig. 1 seems to be disintegrating; only fragments of the cell membrane, particularly in the region of the desmosomes, can be made out. The swirl- ing bundles of filaments resemble those encircling perinuclear aggregates of cytoplasmic inclusions.

By contrast, the darker cells have intact cell membranes and large numbers of finger-shaped processes. Their cytoplasmic filaments are visible but are less prominent than those in the pale cells. There is no evidence, however, that the filaments are more numerous in the lighter cells; in other words, there is no evidence that the
cytoplasmic filaments are produced from degenerating nuclei or cytoplasmic inclusions. In width, they fall within the same range wherever it has been possible to measure them: less than 100 Å, and frequently around 50 Å.

Beneath the epithelium, and attached to it by desmosomes, is the basement membrane of the epithelium. In transverse sections of human cornea, the basement membrane varies in thickness, and is thickest where the desmosomes of the basal cells are attached to it (Fig. 2). The most common range found has been about 200 to 300 Å, which is about half the measured thickness of basement membranes in rabbit corneas (Fig. 3). Occasional thinning, splitting, or apparent discontinuities have been observed in human basement membranes, but not in those of other species. For example, the rabbit membrane in Fig. 3 appears to be a robust and continuous structure. From its inner surface, fine filaments can be traced into the stroma, where similar filaments can be seen between the collagen fibrils. Fig. 2 shows clearly the double dark plaques of the epithelial portions of the desmosomes as well as the intradesmosomal material between the plaques and the basement membrane; this material is sometimes obviously filamentous, but it appears only moderately so in these illustrations.

Oblique sections through the basement membrane contain basal cell desmosomes arranged in clusters (Fig. 4). One pattern consists of six dark plaques forming a hexagon, with a seventh placed centrally. Sections of striated or laminated structures have been observed, particularly in the peripheral cornea and in tangentially or obliquely sectioned desmosomes, but the relationship of these to the structures described by Odland has not been determined.

**Stroma.** Under the basement membrane of the epithelium and firmly attached to it is the stroma, the structural elementary unit of which is the collagen fibril. In the cornea the collagen fibrils are thin, relatively uniform in diameter, and, in well-fixed normal tissue, closely packed. Directly beneath the epithelium there is, in certain species, a region (Bowman's layer) in which the collagen fibrils are randomly oriented (Fig. 5). In the rest of the stroma they are aggregated into ribbon-like bundles, which are the microscopic fibers of the stroma. The greatest precision is achieved in the regions in which the fibrils in one layer are oriented at right angles to those in adjacent layers. In other regions the bundles branch and anastomose in a manner which must increase greatly the resistance of the stroma to being split (Fig. 6).

The stromal cells in human cornea appear to lie within the lamellae rather than between them, although a cell may be so near the edge of a layer that the distinction may be meaningless. Wherever it has been possible to identify and trace processes of adjacent cells, plasma membranes have always been observed between the contiguous ends; the processes appear to hold hands but not to fuse with each other.

Widths of most collagen fibrils of stroma, measured in twelve different species, fell between about 200 and 300 Å. The repeating period was found to be between 500 and 600 Å, which is the usual range for collagen fibrils in sections of plastic-embedded tissues.

**Descemet's membrane and endothelium.** At the junction of Descemet's membrane and stroma, collagen fibrils of the stroma are embedded in Descemet's membrane (Fig. 7). Below this is the organized portion of the membrane, and under this, in human cornea, is the portion which does not normally show any regularity of structure.

The innermost layer of the cornea is the endothelium, a sheet of flat cells with sinuous lateral cell membranes and numerous mitochondria. These cells do have desmosomes, but, except for those of the terminal bar type near the anterior chamber, these are very small and few in number.
Figs. 6-8. For legends see page 206.
Fig. 1. Tangential section through squamous cells of human cornea, showing portions of a pale cell and two cells of normal density. The dark cells have intact membranes and many interdigitating processes (ip). The pale cell has only fragments of membrane but shows clearly the copious filamentous component of the cytoplasm. Bundles of the filaments surround cytoplasmic inclusions. (×21,650.)

Fig. 2. Transverse section through the basement membrane (bm) of human corneal epithelium (ep) showing a number of basal cell desmosomes (d), and a few fibrils of Bowman's layer of the stroma (str). (×43,750.)

Fig. 3. A similar section through rabbit cornea. Intradesmosomal filaments can be seen between the dense regions of the cell membrane and the basement membrane. Fine filaments (f) extend from protuberances of the basement membrane into the stroma. (×43,650.)

Fig. 4. Oblique section through the same region in human cornea, cutting through many basal desmosomes. The dense substance is arranged in clusters of varied sizes and shapes, and occasionally forms an almost perfect face-centered hexagon (arrow). (×27,650.)

Fig. 5. Transverse section through human cornea showing the basement membrane (bm) of the epithelium, Bowman's layer (Bl) of unoriented collagen fibrils, and the transition between Bowman's layer and the oriented fibrils forming the lamellae of the stroma (str). (×16,550.)

Fig. 6. Human corneal stroma showing several lamellae (fibers), two of them branching. The collagen fibrils appear as dots when cut in cross section, as short segments when sectioned obliquely, and as fibrils when they lie in the plane of the section. (×26,900.)

Fig. 7. Human Descemet's membrane (Dm) and a portion of an endothelial cell (en) with nucleus (n). Only the portion of the membrane nearest the stroma (str) shows periodic structure. The arrow points to the irregular transitional zone between membrane and stroma. (×17,450.)

Fig. 8. Peripheral human cornea showing the contorted inner surface of the epithelium (ep) and its basement membrane (bm), desmosomes (d), the haphazardly oriented but loosely packed fibrils of the outer stroma in the region occupied by Bowman's layer in the central cornea, and the edge of a capillary (c), with a portion of an endothelial cell (en) and its laminated basement membrane (bm). (×16,800.)

Fig. 9. Deeper in the stroma of the peripheral cornea, the collagen fibrils are oriented into layers but vary considerably in width. (×16,050.)

Fig. 10. A Hassall-Henle body protrudes into the endothelium (en). It is penetrated by a system of tunnels in which can be found granules (g), processes of endothelial cells (p), smooth fibrils (sf), and fusiform long-spacing fibers (ls). Long-spacing fibers also form a discontinuous layer between the organized and the amorphous portions of Descemet's membrane. (×9,650.)

Fig. 11. Interposed between the layers (Dm) into which Descemet's membrane splits at the limbus are bundles of stroma-type collagen fibrils (c) and of much thinner fibrils (tf) which show the collagen macroperiod when suitably oriented, but which appear as amorphous masses when sectioned at right angles to their length. (×21,550.)
Figs. 9-11. For legends see opposite page.
Peripheral cornea

Near the limbus, the smooth inner surface of the epithelium becomes highly convoluted as the basal cells send a profusion of basement membrane-covered processes into the stroma. Bowman's layer loses its identity as it changes gradually into a layer of loosely packed fibrils and filaments, and capillaries can be found very close to the epithelium (Fig. 8). The outer layer of stroma looks as if it had been hastily put together, with collagen fibrils of assorted diameters (from well under 100 A to over 700 A) loosely assembled into irregular bundles (Fig. 9).

The Hassall-Henle bodies, or warts, of Descemet's membrane protrude into the endothelium, displacing all but thin layers of cytoplasm (Fig. 10). Within each wart is a branching and anastomosing system of tunnels, which may contain granules, fibrils, or endothelial cell processes. The long-spacing fibril which is seen in and around the warts resembles that which occurs in the trabecular region; it has a period similar to that of the organized portion of Descemet's membrane, but it forms spindle-shaped aggregates and has denser and wider dark bands and thicker connecting filaments. Smooth fibrils which resemble the connecting filaments are also found, often in association with the long-spacing fibers.

In this region, too, Descemet's membrane begins to undergo the subdivision which finally transforms it into a series of basement membranes for the cells of the trabecular region. The layers are separated by stroma-type collagen fibrils and fibrils with diameters of only about 75 A, but with the repeating period of collagen, as shown in Fig. 11. In the transverse or oblique sections, bundles of these fine fibrils may appear as amorphous masses if the individual fibrils are not resolved.

Keratoconus

Epithelium and basement membrane. In the two corneas with keratoconus which were examined, portions of the epithelium were indistinguishable from normal epithelium. At the other extreme, regions were found in which the layer had diminished to a thickness of about 15 μ and in which most of the cells looked distinctly abnormal (Fig. 12). In such areas the innermost cells are flattened and may be less dense than are the squamous cells. No obvious alterations were found in the appearance of the desmosomes and the interdigitating cell processes, or in the width of the intercellular space, except near the outer surface where enlarged spaces are seen even in normal epithelium. Mitochondria and other cytoplasmic inclusions were either more numerous or stood out more clearly in these cells than in normal corneal epithelium.

Like the epithelium, the basement membrane in keratoconus is also diversified in appearance. In transverse sections it may be thick or thin, continuous or discontinuous. Where large holes occur, processes of epithelial cells may protrude through them into the stroma (Fig. 13) and spread over varying distances under the basement membrane.

Fig. 12. Entire thickness of a thin region of the epithelium and a portion of Bowman's layer in a cornea from a patient with keratoconus. The cells are atypical in shape and vary widely in density. Mitochondria (m) can be seen in both light and dark cells. The cytoplasm of the dark cells looks as if it had been compressed, or condensed. (x10,250.)

Fig. 13. Inner edge of the corneal epithelium from another region of the same specimen. Processes (p) of epithelial cells extend into the stroma (str) through holes in the basement membrane (bm). (x23,000.)
Figs. 12 and 13. For legends see opposite page.
Figs. 14 and 16. For legends see opposite page.
Fig. 15. Section of paraffin-embedded corneal cornea, showing an invasion of the epithelium (ep) by the stroma (str). Bowman’s layer looks normal on either side of the interruption. (x475.)

Fig. 14. A large nerve in the epithelium of a cornea affected by keratoconus is oriented parallel with the basement membrane (bm) and consists of a bundle of unmyelinated fibers. Mitochondria (m) are numerous and well defined. (x17,700.)

Fig. 16. Section through the apex of an eruption of the corneal stroma into the epithelium (ep) in keratoconus. An epithelial cell process (p) protrudes through a hole in the basement membrane (bm). It is not known whether the cell (c) is of epithelial or stromal origin. (x16,650.)

Fig. 17. In the deeper layers of the corneal stroma in keratoconus, the collagen fibrils are more irregularly spaced than in normal cornea, and the lamellae are often thin and folded. (x14,700.)

Fig. 18. A portion of one type of cell seen in the corneal stroma in keratoconus, with its nucleus (n), mitochondria (m), rough endoplasmic reticulum (er), and abundant cytoplasmic filaments. Typical collagen fibrils (c) surround the cell. (x29,850.)

Fig. 19. Another type of cell in the corneal stroma in keratoconus is in intimate association with large masses of material (m), in the surface of which collagen fibrils may be embedded (arrows). (x23,400.)

Fig. 20. A third type of cell in the stroma in keratoconus has an irregular profile with many blunt processes. Its cytoplasm is packed with filaments (f). There are similar filaments which are clearly extracellular (fo) and others which appear to be partly inside and partly outside, or possibly on the surface (fio). Finer, curled (or twisted) filaments can be seen both within the cell and between the collagen fibrils outside the cell. (x52,850.)

Fig. 21. Descemet’s membrane (Dm) in these corneas with keratoconus looked normal, as did the endothelium in some regions. In this section the mitochondria (m) appear to be somewhat more numerous and better defined than is usual in normal endothelium. (x12,950.)
Figs. 17-19. For legends see page 211.
Figs. 20 and 21. For legends see page 211.
An unusually good specimen of a fairly large nerve was found in the epithelium of one of the corneas with keratoconus (Fig. 14). It contains many mitochondria and appears to be unmyelinated.

An even more striking phenomenon in keratoconus than the downgrowth of the epithelium is the upgrowth of the stroma (Figs. 15 and 16). The undulating patterns observed in these regions suggest that sheets of parallel collagen fibrils had pushed their way up through Bowman's layer and into the epithelium. Cells with abundant cytoplasmic inclusions have been observed near the apex of most of these eruptions of the stroma. Between the eruptions, Bowman's layer appears quite normal.

Stroma. Compared with normal corneal stroma (Fig. 6), that of keratoconus showed considerable disorganization. In many of the regions examined, the collagen fibrils were noticeably irregularly spaced, and the layers they formed were often thin and meandering as demonstrated in Fig. 17. In other regions, particularly near Descemet's membrane, the stroma seemed to be unaltered.

The stromal cells were unlike those observed in the central cornea. One type (or one stage) found is shown in Fig. 18. In the cytoplasm of this cell mitochondria, endoplasmic reticulum, and many fine filaments can be seen. Another type (Fig. 19) has few, if any, cytoplasmic filaments, but is closely associated with closely packed masses of material. Some of these masses appear to be completely enclosed by the cell, some are only partially encircled by cytoplasmic arms, and some simply lie in depressions of the cell and are exposed along their outer surfaces. Collagen fibrils are embedded near the surface of the masses which, in those areas, are similar in appearance to the zone of transition between Descemet's membrane and the stroma. Finally, there is a cell (Fig. 20) which is associated with filaments—filaments which are definitely inside the cell, filaments which are clearly outside the cell, and filaments which appear to be partly inside and partly outside. (As pointed out by Godman and Porter, caution should be exercised in drawing conclusions from such appearances; the depth of focus of the electron microscope and the difficulty in obtaining sufficiently large numbers of serial sections multiply the pitfalls for the unwary.)

Descemet's membrane and endothelium. In one cornea with keratoconus the two layers, Descemet's membrane and endothelium, showed no obvious changes, except for a possible increase in the number of mitochondria in the endothelial cells (Fig. 21). In the other specimen (Fig. 22) there were, in some regions, large intercellular vacuoles. No intracellular vacuoles of this magnitude have been observed in well-preserved endothelium, either normal or pathologically altered.

Fig. 22. This section of endothelium in keratoconus contains a number of large intercellular vacuoles (v), some of which contain cloudlike precipitates. Large terminal bars (d) and a strong attachment of the endothelium to Descemet's membrane (dense regions along the cell membrane may be desmosomal in function) apparently prevent the vacuoles from breaking through into the anterior chamber or into Descemet's membrane. Note again the many mitochondria (m). (×16,150.)

Fig. 23. This anvil-shaped wart of cornea guttata shows the usual long-spacing component of Descemet's membranes (f↓); the spindle-shaped long-spacing fibers (f↓); bundles of fine fibrils with the fundamental collagen period (f↓); and thin, smooth fibrils both inside and on the surface of the wart (f↓) which is outlined by a thin line and a few aggregates of a dark material (dm). (×11,500.)

Fig. 24. This wart is covered by and completely buried in the thin smooth fibrils. The dark material (dm) here occurs in clumps. (×22,950.)
Figs. 22-24. For legends see opposite page.
Cornea guttata

**Descemet's membrane.** The outstanding feature of the single specimen of cornea guttata which was examined was a thickened Descemet's membrane which showed organized structure throughout its entire thickness, including the protuberances characteristic of this dystrophy, the warts. In the wart shown in Fig. 23, for example, a number of fibrous species previously met elsewhere can be seen: (1) the typical long-spacing component of Descemet's membrane; (2) spindle-shaped aggregates of "heavy duty" long-spacing fibrils; (3) the thin fibrils with the repeating period of collagen; and (4) smooth fibrils, often closely associated or even possibly continuous with the cross-striated types, or forming a layer on the inner surface of Descemet's membrane. In other regions, one or another of these components may be present in greater proportion.

The wart in Fig. 23 represents the anvil-shaped type, flat on its surface and somewhat undercut on its sides, which are covered with a layer of fine fibrils. Fig. 24 shows one side of a wart which is similar except for the fact that the depression between it and the adjacent wart has been completely filled by the fine fibrils. Both Descemet's membrane and the warts are outlined by a dense, partly fibrous, partly granular substance; this forms large masses in some regions, but occurs only as an irregular dark line (Fig. 23) or a few granules in others.

When the long-spacing material is properly oriented in the section (Fig. 25), it appears as hexagonal figures which are as beautiful and precise as those observed in tangential sections of beef Descemet's membrane.14

In the spindle-shaped long-spacing fiber of the normal peripheral cornea, a row of granules was occasionally seen midway between adjacent dark bands. Here, in Descemet's membrane of cornea guttata, a further elaboration of this type of fiber has been found (Fig. 26). The repeating unit, bounded by two wide dark bands, is polarized and consists of a wide dark band, a light band of almost the same width, a pair of narrow dark bands separated by a narrow light band, another narrow light band, and a single narrow dark band which may or may not be separated from the next wide dark band by a narrow light band (there is a possibility that the single narrow dark band is one of a pair, the other of which has fused with the wide dark band). In a single fiber all the component fibrils are oriented in the same direction. Two fibers may join laterally in a head-to-tail position, however, with their wide dark bands in phase but with their subbands apparently shifting from one position to another relative to the wide bands.

No typical endothelium was seen in any of the sections examined, and only one flattened cell was found where the endothelium should have been. It was positioned between the layer of loose filaments and the dense material directly adjacent to it elsewhere (Fig. 27).

**Fig. 25.** The hexagonal figures characteristic of tangentially sectioned, organized Descemet's membrane can be seen in the warts of cornea guttata. The arrow points to a complete hexagon. Multiple or unusually thick connecting filaments are prevalent in these warts. (×33,750.)

**Fig. 26.** The spindle-shaped long-spacing fibers of Descemet's membrane in cornea guttata frequently have at least three, and sometimes four, thin dark bands within the macroperiod (arrows). This section also shows several bundles of thin fibrils with the collagen repeating period (f2) and scattered loose aggregates of smooth fibrils (f1). (×34,800.)

**Fig. 27.** Only one cell was found where the endothelium should have been. It consists of a nucleus (n) surrounded by a thin layer of cytoplasm and is interposed between the layer of filaments and the dark material (dm) directly adjacent to it elsewhere. (×17,200.)
Figs. 25-27. For legends see opposite page.
Epithelium. The epithelium in this specimen was very thin and consisted of only a few layers of flattened cells. In some regions these were greatly compressed by thick layers of filaments which separated the epithelium into two layers. The edge of one of these fibrous masses is seen in Fig. 28, outlined in part by basement membrane. Here the intact epithelium is about 20 μ thick, the fibrous layer about 7 μ, and the outer and inner epithelial sublayers about 10 μ and 3 μ respectively.

A similar fibrous layer, of variable thickness, was interposed between the epithelium and Bowman’s layer of the stroma. The two fibrous layers were generally similar in appearance, and in one region they were seen to be continuous. Their component filaments ranged in width between about 50 and 200 A and, when oriented into bundles, displayed the repeating period of collagen (Fig. 29).

In one area the subepithelial layer was augmented by several layers of collagen fibrils of varied but rather large diameter (up to about 400 A), separated by and also embedded in a filamentous material (Fig. 30). Bowman’s layer was immediately beneath this layer.

Stroma. In this single specimen of cornea guttata, Bowman’s layer of the stroma was easily recognized regardless of its position relative to the epithelium. Its collagen fibrils were of the expected diameters and were disposed in the usual haphazard fashion. The only difference noted (and this may have been due to the handling of the specimen and the elapsed time between removal from the eye and fixation rather than to the pathologic condition) was a somewhat greater separation between the fibrils than that found in other human corneas.

Too little of the stroma beneath Bowman’s layer was examined to permit any generalization to be made. That which was examined, however, appeared to be quite normal.

Kayser-Fleischer ring

Descemet’s membrane. The Kayser-Fleischer ring has been described as a ring of copper-containing granular deposits in Descemet’s membrane. A similar pattern has now been found in a second cornea. The ring begins just central to the zone containing the Hassall-Henle bodies and stops before Descemet’s membrane has been completely subdivided into thin layers. Fig. 31 shows a shallow wart which contains the granular deposits (the disposition of the granules into two or three layers was less marked in this cornea than in the previous one). The channels described earlier were undoubtedly the tunnels of the Hassall-Henle bodies, although Descemet’s membrane was quite flat.

Stroma. An interesting structure found in the stroma of this specimen is a long-spacing fiber with wide, dense bands spaced about 1,100 A apart, but with connecting filaments either so thin or of such low contrast that they are not resolved. These fibers are spindle shaped, are generally located near stromal cells, and may line up to form long, thin aggregates (Fig. 32).

Fig. 28. The thin epithelium of cornea guttata is separated into two layers and compressed by an invading fibrous layer (f), which is surrounded, at least in part, by basement membrane (bm). Nuclei (n), mitochondria (m), and desmosomes look normal. (×9,050.)

Fig. 29. When the filaments in the fibrous layer within the epithelium, or in the one between the basement membrane and Bowman’s layer, associate into bundles, they show the macroperiod of collagen (arrow). (×29,500.)

Fig. 30. In one region, the fibrous layer beneath the epithelium was augmented by a layer consisting of very fine filaments and collagen fibrils showing intraperiod banding (arrow). (×54,500.)
Corneal scar tissue

A small perforating cut was made in the cornea of a rabbit and allowed to heal. Two months later the eye was enucleated. After fixation with buffered osmium tetroxide, the scar was slightly darker than the rest of the cornea.

Under the epithelium, which had not regained its original thickness, the basement membrane was still incomplete and, where it was present, it showed an even greater than normal variation in thickness. Interestingly, within the larger gaps of the basement membrane short segments can be seen, associated with and completing the basal desmosomes (Fig. 33).

The regenerating stroma resembles both embryonic stroma and that of adult peripheral cornea. The cells are numerous, large, and packed with rough endoplasmic reticulum and mitochondria (Figs. 33 and 34), while the collagen, particularly near the epithelium, occurs in a wide spectrum of widths, from filaments too thin to be measured accurately to fibrils about 500 Å wide. Deeper in the stroma the orientation into layers is better but the variation in width persists (Fig. 35). (Neither the range in widths nor the greatest widths, however, approach those observed in the sclera, where the range may encompass a greater than tenfold difference in fibril width.)

The regenerating Descemet's membrane consists of a layer about 2 μ thick adjacent to the endothelium and a series of parallel but much thinner and irregular layers in the stroma (Fig. 36). In the thick layer a number of small islands of long-spacing fibers can be seen.

Discussion

The cells of the epithelium and the endothelium of the cornea are distinctly different in their fine structure. The cytoplasmic filaments so prominent in the epithelial cells have been seen in the endothelium only near the limbus, and even here they were not present in abundance. In the epithelium they appear to fill every part of the cell not occupied by the nucleus or cytoplasmic inclusions. That they could constitute a structural framework, an intracellular skeleton, is suggested by the pale squamous cells; with little more than the cytoplasmic filaments, desmosomes, and fragments of cell membrane left, these cells manage to maintain their proper size and shape. It can be imagined that destruction of the remaining desmosomes would be the next step and that this would be followed by desquamation of the cell.

The endothelial cells also lack the profusion of finger-shaped processes and desmosomes of their ectodermal counterparts. In transverse sections, their lateral membranes proceed from stroma to anterior chamber in smooth, lazy waves. At their free surfaces, the cells are tacked together by large terminal bars, but other desmosomes between the cells are few in number and very small. Perhaps some of the dark,
Figs. 31-33. For legends see opposite page.
thickened regions of the cell membrane facing Descemet's membrane may be desmosomal in function (a possible involvement in vesicle formation of similar regions in the peripheral cornea has been discussed earlier1).

These differences in structure between epithelium and endothelium suggest differences in behavior. One such difference was described by Speakman,6 who found that, while the intact, well-preserved endothelium was extremely resistant to stains which stained the epithelium, vacuolated endothelium took up the same stains readily. The implication is that the formation of vacuoles (between rather than within the cells, as Speakman had concluded) so changes the permeability properties of the cell membrane that it permits passage of certain stains.

Of more general interest, however, is any clue which the fine structure might provide about the mechanism of fluid transfer by the epithelium and endothelium, particularly if the transfer takes place largely through the intercellular spaces, as Maurice8 has suggested. The many interdigitating processes of the epithelial cells must increase the length of the pathway between the basement membrane and the outer surface of the cornea, while the large number of desmosomes must limit the volume of the intercellular space by limiting the size of the vacuoles which can form between them. In the endothelium, on the other hand, the pathway between Descemet's membrane and the anterior chamber is much shorter because the actual distance between the two is much less, because there is only a single layer of cells, and because there are no small interdigitating processes or many desmosomes to introduce detours. Also, because of the few desmosomes and the absence of a cytoplasmic skeleton, the endothelial cells must be able to change shape rapidly and, by forming vacuoles, change tremendously the volume of the intercellular space. The sensitivity of these cells to their environment in vitro could be just an exaggerated form of their normal activity in regulating the flow of fluid into, or out of, the cornea.

The involvement of the endothelium in cornea guttata is generally acknowledged, although its absence or its degenerated appearance is the point usually stressed. The greatly altered fine structure of Descemet's membrane and the addition to it of the warts argue for a hyperactivity of the endothelium preceding its ultimate degeneration, as was suggested by Klien.9 The warts are reminiscent of the early stages of Descemet's membrane which were observed in chick embryos,4 in which the continuous membrane seems to be formed by the growth and eventual fusion of isolated islands of membrane. A similar sequence of wart growth and fusion, followed by the formation of more warts, might account for the discrepancy in the thickness of Descemet's membrane as reported by different observers. The membrane would presumably continue to increase in thickness until the endothelium degenerated.

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Fig. 34. In another region of the stroma of the same scar bundles of fine filaments (f) and collagen fibrils (c) of widely varying widths can be seen. The cell process, with its abundant endoplasmic reticulum (er), resembles those seen in chick embryonic corneas. (×20,800.)

Fig. 35. In the deeper stroma of the scar the collagen fibrils are better oriented into layers, but they still vary in diameter, as can be seen in those which appear in cross section. (×31,000.)

Fig. 36. Descemet's membrane has regenerated partially and consists of a layer about 2 μ thick (Dm) and a number of parallel, but much thinner, layers separated by stromal collagen. Small long-spacing fibers (arrow) can be seen in the thickest layer. (×27,250.)
An interesting point is that Descemet’s membrane in cornea guttata shows organized structure throughout its entire thickness. This could result either from a rearrangement of the normally amorphous portion of the membrane or by the formation of long-spacing elements which move into this region. The appearance of hexagonal figures in sections near the edge of a wart indicates that the sheets of interconnected filaments and nodes follow the contours of the wart. The thin fibrils with the collagen period, previously seen in the peripheral cornea, are the first fibrils with the fundamental, rather than the doubled, collagen repeating period which have been observed within Descemet’s membrane.

Other alterations observed in this specimen include an attenuation of the epithelium and the presence of fibrous layers between the cells and between the basement membrane and Bowman’s layer of the stroma. The tentative identification of these new filaments and fibrils as collagen is based on the appearance of the macroperiod of collagen in closely packed bundles of filaments and of the period, including subbands, in individual, thicker fibrils. The continuity between the two layers and the presence of basement membrane around the layer in the epithelium suggest that the fibrous layer was first deposited below the basement membrane and then pushed its way outward.

In keratoconus, the sequence of events which results in the invasion of the epithelium by stromal collagen and in the downgrowth of epithelium into the stroma is not known. The normal appearance of both the basement membrane and Bowman’s layer between the discontinuities suggests that these gaps could be consequences, rather than initiators, of abnormal activity in the epithelium and in the stroma.

Whereas the alterations in the basement membrane and in Bowman’s layer appear to be localized, those in the deeper stroma are widespread. The impression is gained that the stromal cells have all been mobilized and activated, and are trying to repair some sort of generalized damage; they appear to be producing fibrils and filaments, and masses of a Descemet’s membrane-like material. It can be imagined that in some regions they overcompensated and formed more than the needed amount of stromal collagen, which finally buckled and pushed up into the epithelium.

An even greater degree of activation of cells appears to be called for in the healing of a corneal wound. The stromal cells in the rabbit scar resemble, in fact, those in a chick embryonic cornea, being large in size and having a high concentration of rough endoplasmic reticulum. The new collagen fibrils, on the other hand, are more like those found in the adult peripheral cornea. They are more varied in width and more haphazardly disposed than embryonic stroma fibrils. The regenerating Descemet’s membrane is also similar to the multilayered membrane seen near the limbus of adult cornea.

The transparent central cornea can be thought of as the successful outcome of a precise programing of the cells charged with its production. Then, having completed their task, these cells appear to settle into a state of relative inactivity and gradually to forget the instructions they once received. Never entirely, however, for if called upon for repair duty, they respond enthusiastically with products at least similar to those they formerly made. The question confronting us is whether or not, by changing the environment of the cells or by any other means, we can instruct them to reproduce what they once did so well.

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REFERENCES

Figs. 34-36. For legends see opposite page.