The histological and ultrastructural pathology of congenital hereditary corneal dystrophy: A case report

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This case report describes the pathological changes found in the markedly clouded and thickened corneal button from a child with bilateral congenital hereditary corneal dystrophy. When surveyed by light and electron microscopy, three types of abnormalities were evident: (1) superficial changes including small subepithelial bullae, interruptions of the thickened epithelial basement membrane, anchoring fibrils associated with the central corneal basement membrane, and thickness variations in Bowman’s layer; (2) stromal alterations, including disorganization of the stromal lamellae and enlargement of the collagen fibrils to almost twice normal diameter; and (3) a uniformly thinned Descemet's membrane with ultrastructural anomalies suggestive of a functionally defective endothelium. Although the superficial changes are for the most part nonspecific, the increased collagen fibril diameter may constitute a congenital stromal defect of diagnostic significance which is shown here for the first time. The alterations of Descemet's membrane and the indications of endothelial dysfunction are interpreted to implicate a congenital form of endothelial dystrophy as a possible factor in the pathogenesis of congenital hereditary corneal dystrophy.

According to the Franceschetti classification of heredofamilial corneal degenerations, three subgroups may be distinguished among those dystrophies that primarily involve the corneal parenchyma: (1) Classical forms, which are noncongenital and progressive, include: granular (Groenouw’s Type I), lattice (Haab-Dimmer), and macular (Groenouw’s Type II) dystrophies; (2) congenital forms, which are more or less stationary; and (3) diverse forms, including crystalline and fatty degenerations.

The histopathology of the classical forms has been the subject of numerous reports, the Jones and Zimmerman paper being especially notable. More recently, electron microscopic investigations have demonstrated unique ultrastructural alterations of the stroma in each of the three classical types. The congenital dystrophies, however, have not aroused such interest. Indeed, the English literature apparently includes only three histopathological reports of congenital hereditary corneal dystrophy (abbreviated hereafter as CHCD), while the ultrastructural pathology of this condition has remained altogether unexplored.

We have recently had the opportunity to study by light and electron microscopy a corneal button from one of Maumenee's
CHCD patients (Case 6 in reference 23). The purpose of this case report is, therefore, to illustrate the specific lesions of CHCD, to attempt a correlation of its clinical, histological, and ultrastructural features; and to reassess the advisability of classifying CHCD as a parenchymatous type of corneal dystrophy.

Case history

P. B. H. (JHH 123 25 76), a Caucasian boy, was 2 years and 9 months of age when first examined at the Wilner Institute on May 11, 1960 (date of birth: August 22, 1957).

Family history. The patient’s parents were alive and well. All members of the patient’s family represented in the pedigree pattern (Fig. 1) were negative for ocular disease. The patient’s mother had two sons by previous marriage who were in their late teens and had normal corneas. The patient’s one full brother, aged 5 years, also had normal eyes.

Physical examination. Extensive diagnostic work-up and repeated neurological, surgical, and pediatric consultations revealed no non-ocular abnormalities. Serologic tests for syphilis were negative. No abnormal mucopolysaccharides were present in the urine.

Present illness. The patient was the product of a full-term pregnancy and weighed 7 pounds 8 ounces at birth. Though his corneas were thought to be clear at birth, by about 2 months of age his parents had noticed bilateral clouding. Neither discharge nor redness or inflammation of the eyes was ever noted.

Eye examination at age 3 months revealed a bilateral corneal haze, primarily confined to the central cornea. Corneal diameters and filtration angles were normal. The iris had a peculiar slate-gray color, and no iris vessels were evident. The pupils were small and reacted well. The fundus could not be visualized clearly. At this time, as well as on four subsequent occasions within the following 10 months, examination under general anesthesia revealed intraocular pressures of approximately 6/5.5 (15 mm. Hg Schiötz).

By the end of the first year, the initially centralized corneal haze had increased to a dense grayish-blue infiltration throughout the whole cornea so that not even the iris was clearly visible. Corneal vascularization was never apparent. The patient’s general functional status at that time was considerably better than his ocular situation might seem to indicate; his general postnatal development with regard to sitting, walking, and talking had been normal.

Ocular examinations. On May 11, 1960, the eyes appeared straight and free from noticeable nystagmus and inflammation. The patient was able to follow light fairly well at a distance of 6 feet in a dark room and to follow hand movements at about 1½ feet. Extraocular movements were normal in all cardinal directions. Corneal sensitivity was bilaterally normal and equal. Corneal diameter measured approximately 11 mm. in the horizontal meridian. The corneas were almost completely opaque; they were like ground glass in appearance and almost as white as sclera (Fig. 2). On slit-lamp examination the corneal epithelium did not show any bullous changes; instead a pigskin-like roughening was evident in the stippled distortion of the light reflex (Fig. 2). The stroma was diffusely clouded and appeared to be almost three times normal thickness in all
areas from the limbus to the apex. No blood vessels were seen in the peripheral corneal stroma. The opacity of the cornea was so dense that the deeper layers of the cornea could not be seen clearly. However, the anterior chamber appeared of normal depth, and the pupil on transillumination appeared round and, also, it responded to light.

On February 14, 1967, the patient's ocular status was essentially unchanged. He had been attending a school for the blind and apparently had been doing fairly well. Visual acuity was reduced in the right eye to counting fingers at a distance of 8 inches and in the left eye to hand motions at a one foot distance without any glasses. There was good light perception and projection and good colored-light perception in all quadrants. Extraocular movements were full with gross nystagmoid movements but no rapid nystagmus. Tensions were approximately 6/5.5 (15 mm. Hg Schiotz). Slit-lamp examination showed corneal opacities located predominantly in the posterior two thirds of the stroma. The epithelium was unchanged with respect to its roughened appearance. The anterior chamber could not be evaluated because of corneal opacity. The ERC was normal.

On February 17, 1967, a 6 mm. penetrating corneal graft was performed on the right eye. During the operative procedure, the anterior chamber seemed quite shallow and could be entered only with extreme difficulty. The surgeon's impression was that the iris might be adherent to the posterior corneal surface; hence, it could not be judged with certainty whether the trephine had penetrated the entire thickness of the cornea or whether the endothelium and perhaps some of Descemet's membrane had dissected free of the stroma and lay against the iris.

This graft remained crystal clear postoperatively for 4 weeks. Under the slit lamp, the details of the iris and an anterior chamber of normal phakic depth could be seen. During the fifth postoperative week an early homograft reaction occurred, and the graft became clouded. Systemic prednisone and topical dexamethasone (Decadron) caused the graft to clear so that the iris was again visible. Six weeks after the operation, the patient was discharged on systemic and topical dexamethasone. During the third postoperative month, the graft remained clear and smooth. At that time the patient could count fingers at a distance of 3 feet. There was no epithelial bedewing and only slight stromal haziness. The anterior chamber was of normal depth and clear by slit lamp. An excellent red reflex was obtainable, but the patient would not hold still to permit detailed fundoscopic examination. On May 16, 1967 (12 weeks postoperatively), a second homograft reaction occurred. With the slit lamp, corneal vascularization could be seen up to the margin of the transplant in almost all areas, though no neovascularization was apparent in the transplant itself. The graft became definitely edematous in both epithelium and stroma but did not approach the thickness of the patient's extremely thickened left cornea. The epithelial edema would not clear appreciably even with 50 per cent glycerine. Increased local and
systemic steroids accomplished no remission of the bullous keratopathy and were, therefore, discontinued after one month. The patient is under consideration for future regrafting.

Material and methods

The excised corneal button was halved immediately following careful surgical removal and was placed in appropriate fixatives. One of the corneal halves was fixed in formalin, embedded in paraffin, and sectioned in the conventional manner for light microscopy. The other specimen was fixed for electron microscopy in cold 2 per cent osmium tetroxide (buffered to pH 7.8 with M/14 veronal acetate solution containing 1 per cent calcium chloride and 40 mg. per milliliter sucrose). After about 20 minutes in the cold, fixation was continued for one hour at room temperature. The fixed tissue was dehydrated in a graded ethanol-water series and embedded in Araldite epoxy resin. Thin sections were cut on a Cambridge-Huxley microtome, mounted on uncoated copper grids, doubly stained with uranyl acetate and lead citrate (for 10 minutes each), and examined with an RCA EMU-3F microscope at 50 kv.

For purposes of comparison, the normal human cornea from an enucleated specimen with malignant posterior melanoma was prepared for electron microscopy according to the previously described procedure.

Results

Light microscopy. When the histologic CHCD specimen (Fig. 3A) is directly compared with a normal human cornea (Fig. 3B), the marked thickening of the pathological cornea is obvious. Whereas

Fig. 3A. Survey light micrograph of the CHCD corneal button in histologic section. Compare stromal thickness of CHCD specimen to that of the similarly prepared normal corneal specimen (Fig. 3B). (Hematoxylin and eosin stain; ×60.)

Fig. 3B. Survey light micrograph of a normal human cornea in histologic section. Separation of the epithelium and Bowman's layer from the stroma, spaces between stromal lamellae, breaks in Descemet's membrane, and absence of endothelium are fixation and handling artifacts. (Hematoxylin and eosin stain; ×60.)
the normal range of corneal thickness is 0.46 to 0.67 mm.34 the CHCD cornea has swollen to almost three times normal thickness (measuring ca. 1.63 mm. in Fig. 3A). Stromal thickening is clearly responsible for virtually all of this increase.

At higher magnifications other anomalies become apparent. As shown in Fig. 4, the corneal epithelium varies considerably in thickness, from a rather thin 20 μ (and about five cell layers) to a relatively normal 50 μ (and about nine cell layers). Especially in the thinned areas, the basal cells are distorted, suggesting regeneration. Many cells in the basal layer are edematous. Small subepithelial bullae (Fig. 5) are a frequent occurrence between the basal cells and Bowman's layer. Bowman's layer appears thickened in some areas (up to 25 μ thick in Fig. 4) but absent in others (extreme right of Fig. 4). The leveling tendency of the corneal epithelium is

Fig. 4. Light micrograph illustrating the superficial alterations in the CHCD cornea. The corneal epithelium varies in thickness, and the basal epithelial cells show hydropic swelling (circled). Bowman's layer is highly irregular, with extremes of thickening and thinning (opposed arrows) occurring in close proximity. Numerous vesicular water clefts (*) are present in the superficial stroma. (Hematoxylin and eosin stain; ×400.)

Fig. 5. Light micrograph of the epithelium and superficial stroma of the CHCD cornea. Edema fluid between the basal epithelial cells and Bowman's layer has produced a small subepithelial bulla (*). (Hematoxylin and eosin stain; ×400.)
clearly demonstrated in this case, as the epithelial thickness varies inversely with the irregularities of Bowman's layer, to maintain a smooth surface contour.

With such pronounced stromal swelling, the lamellar organization of the stroma is lost, giving the stroma a diffusely amorphous, macerated appearance (Figs. 3A and 6). As a consequence of extreme edema and lamellar dissociation, the stromal cells have become scattered and seemingly reduced in number (compare Figs. 3A and 3B). In addition, vesicular water clefts of varying size (30 to 80 μ in length in Figs. 4 to 8) separate the indistinct lamellae, especially in the anterior and posterior stromal zones (Figs. 3A and 7). These pathological processes pervade the entire cornea.

Descemet's membrane, which normally thickens with advancing age from about 6 μ in the newborn infant to as much as 20 μ in the adult,2 is only about 3.0 μ thick in the CHCD specimen (Fig. 8). It is, however, remarkably uniform in thickness and is reasonably adherent to the posterior stromal surface. Hence, the surgeon's impression notwithstanding (see Case history), the histological character of Descemet's membrane offers no suggestion of its having been damaged or partially dissected during surgery.

No traces of endothelial cells could be found anywhere on the specimen. Colloidal iron, PAS, and Masson stains disclosed neither hyaline degeneration of the stromal lamellae nor other abnormalities.

**Electron microscopy.** The demonstrable abnormality of the histologic material is also evident at the ultrastructural level. In particular, distinct changes are notable in the epithelium and basement membrane, in Bowman's layer and the stroma, and in Descemet's membrane and the endothelium.

**Epithelium and basement membrane.** The general architecture of the epithelium in CHCD closely resembles that of the normal human cornea as described by Jakus13,14 and by Teng.15 That is, as depicted in Figs. 9 and 10, three main types of cells that occur in layers are present:

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**Fig. 6.** Light micrograph of the intermediate stroma of the CHCD cornea. Diffuse edema has caused the normally compact stromal lamellae to dissociate into finely macerated fibrils. (Masson stain; ×400.)
Fig. 7. Light micrograph of the deep stroma of the CHCD cornea. Vesicular water clefts of various sizes (*) are particularly numerous in the posterior stromal layers. (PAS stain; x200.)

Fig. 8. Light micrograph showing alterations deep in the CHCD cornea. Water clefts and disorganization of the lamellae are especially prominent. The PAS-positive Descemet's membrane (arrows) is thin but uniform, and (in the complete absence of endothelium) it borders directly on the anterior chamber (AC). (PAS stain; x400.)

(1) the columnar basal cells (B Ep in Fig. 10) form a continuous layer closely applied to Bowman's layer; (2) polygonal cells (I Ep in Fig. 9) compose the intermediate layer, 1 to 2 cells thick; and (3) a superficial layer, 5 to 6 cells thick, consists of flattened cells (S Ep in Fig. 9).

For the most part, the usual cytoplasmic organelles are found in the expected distribution for a nonkeratinizing epithelium, with the most common cytoplasmic component being the ubiquitous feltwork of fine tonofilaments (Figs. 11 and 12) of ca. 50 A diameter and indeterminate length,
Fig. 9. Low-power electron micrograph of the corneal epithelium in CHCD. The superficial epithelial layer (S Ep) consists of large squamous cells, whereas cells of the intermediate layer (I Ep) are polygonal in shape. Both cell types have single nuclei (N). Occasional intracellular vacuoles (*) and a few mitochondria (M) are evident in the superficial cells. In the intermediate cells, however, large numbers of mitochondria form sizable perinuclear aggregates. The convoluted cell membranes (CM) are studded with numerous desmosomes (circled). The flattened cell (Sec?), with particularly electron-dense cytoplasm, may represent a secretory or "dark" cell (found only rarely by Teng). The continuous process of disintegration and shedding of the more superficial cells is also apparent; the outermost cell (Di), bordering the corneal free surface (FS), consists of only necrotic debris and is devoid of identifiable fine structure, and the cell immediately beneath (Dj) shows pyknotic nuclear degeneration and loss of cytoplasmic organelles. (×7,500.)
Fig. 10. Low-power electron micrograph of the epithelial-stromal junction in CHCD. An entire basal epithelial cell (B Ep) is shown in cross section. This cell is essentially columnar, although, (as is typical of epithelial irregularities in CHCD) its configuration is skewed. The single nucleus (N), and the numerous mitochondria (M) that are arranged as a perinuclear cap, are prominent intracellular features. The extensively interdigitated lateral cell membranes (CM) are difficult to visualize at this magnification; hence, the cell border is more clearly demarcated by the electron-dense desmosomes (circled) which, occurring peripherally along the lateral cell membranes, serve as specialized sites for intercellular attachment. At the basal end of the cell, a vacuole-like area (*) is possibly evidence of the hydropic swelling of the basal cells, which is also evident in the corresponding light micrographs of this area (see Fig. 4). The cell membrane (BCM) covering the basal surface of the basal cells possesses innumerable hemidesmosomal attachment sites, which are barely distinguishable at this magnification. Immediately subjacent to the basal cell membrane lies the basement membrane (BM) of this epithelium, which is also best viewed at higher magnification (see Fig. 12). This basement membrane, in turn, rests upon Bowman's layer of the stroma (Str) which consists of collagen fibrils with no apparent organizational pattern. (x8,500.)
Fig. 11. This electron micrograph represents a higher power view of the basal perinuclear zone of a basal epithelial cell in CHCD. The intracellular cytoplasmic components are more clearly evident as: a large nucleus (N) contained in a double-membraned nuclear envelope; numerous mitochondrial profiles (M) showing minimally developed internal fine structure; rare cisternae of the rough-surfaced endoplasmic reticulum (ER); aggregations of ribonucleoprotein particles (R); and an extensive feltwork of fine tonofilaments (TF). (×27,000.)

which fill most of the ground substance of these cells. Only two seemingly incidental departures from the usual situation deserve additional comment. (1) Unusually large numbers of small irregularly shaped mitochondria (60 to 80 per cell cross section) are found in the basal perinuclear zones of the basal and intermediate cells (Figs. 10 and 11), whereas, in the normal corneal epithelium of both rabbit17 and human,13 occasionally mitochondria have been seen. (2) Most cells of all three types contain several cytoplasmic vacuoles of varying sizes (Figs. 9 and 10). These vacuoles appear mainly empty and are especially pronounced in the cells of the basal layer, where they probably constitute the ultrastructural counterpart of the histologically demonstrable hydropic swelling of these cells (Fig. 4).

Numerous well-developed desmosomes of the typical macular variety (described by Farquhar and Palade3 in various epithelial and found by Jakus10 in the corneas of several species) are found along the complex interdigitations of the cell margins at all levels. The multitude of these attachment sites, and their especially fine preservation in this specimen, frequently facilitate the morphological comparison of many desmosomes (each oriented in a slightly different plane of section) within a single viewing field (as in Fig. 13).

The basement membrane of this epithelium forms a continuous electron-dense layer (the lamina densa)34 along the in-
Fig. 12. The epithelial-stromal junction in CHCD is shown at higher magnification in this electron micrograph (cf. Fig. 10). The tonofilaments (TF) of the basal epithelial cell (B Ep) extend down to the cell border and may, in fact, function in the adhesion of the epithelium to Bowman's layer of the stroma (Str). A more certain role in the attachment process is played by the hemidesmosomes (HD), appearing as individual electron-dense plaques along the basal cell membrane (BCM). The basal cell membrane (BCM) is separated from the epithelial basement membrane (BM) by a 500 Å electron-translucent space (the lamina lucida). The underlying basement membrane (the lamina densa) appears as a continuous band of amorphous material, of intermediate electron density, which varies between 750 and 900 Å in thickness. Banded collagenous anchoring fibrils (AF) arise from the surface of Bowman's layer and insert into the posterior aspect of the basement membrane. The random arrangement of collagen fibrils in Bowman's layer is also evident. (x34,000.)
Fig. 13. For legend see opposite page.
extend perpendicularly toward the basement membrane, arborizing into finer filaments as they anchor in the membrane's posterior aspect. First described extensively by Palade and Farquhar, who aptly termed them anchoring fibrils, these fibrils have also been found beneath the basement membranes of several epidermal and mucosal tissues, in which they are thought to anchor the basement membrane to the underlying collagenous tissue. Similar fibrils have been observed by McTigue and Fine in association with the basement membrane of normal human peripheral cornea. They have not, however, been previously described in the human central cornea. The banded character of these fibrils is demonstrated in Fig. 12, while Fig. 14, B illustrates their arborization and (as occasionally seen) their regular spacing along the basement membrane.

Both Jakus and Kayes and Holmberg have noticed occasional thinning, splitting, and apparent discontinuities in human corneal basement membranes. In the CHCD cornea, however, basement membrane discontinuities assume a much greater order of magnitude, for here, total absences of basement membrane can occur for intervals of many microns and can be clearly related to the presence of the small subepithelial bullae discernible in histological sections (Fig. 5). This phenomenon is surveyed in Fig. 14, A and is detailed in the higher magnifications of this same area, Fig. 14, B and C. At the left of Fig. 14, A (enlarged in B), the basement membrane is continuous and normal in appearance and is attached above and below by frequent hemidesmosomes and anchoring fibrils, respectively. In the middle area of Fig. 14, A (enlarged in C), the sudden cessation of both the basement membrane and its attendant hemidesmosomes is apparent, as the intact plasma membrane of the basal cells and the surface of Bowman's layer extend confluenly for a short distance, but then separate at the margin of the microbulla. Of additional significance are the pockets of basement membrane-like material found bordering the basal epithelial surface. Although only two of these pockets are visible at the periphery of the bulla shown in Fig. 14, A, similar collections of basement membrane material occur all along infoldings of the basal cell plasma membrane within the central areas of the microbullae. Along the directly underlying surface of Bowman's layer, by contrast, only rarefied amorphous debris that bears no resemblance to basement membrane is evident.

Bowman's layer and the stroma. The normal ultrastructural appearance of Bowman's layer is that of a fairly uniform band ranging between 8 and 14 μ in thickness and consisting of randomly oriented collagenous fibrils packed into a dense feltwork that is not sharply demarcated from the more highly organized underlying stroma.

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Fig. 13. In the CHCD cornea, a high-power electron micrograph of the border between superficial epithelial cells shows the varied appearances of desmosomes (numbered) viewed in different planes of section. That these intercellular attachment sites are specializations of the cells' plasma membranes (open arrows) is clearly evidenced by the continuity of the plasma membrane along the length of the desmosome. Thus, in transverse section (1), the electron-dense parallel plaques are most clearly defined and appear to share an additional parallel midline component. In slightly oblique section (2), the midline component is still present but is perpendicularly intersected by regularly spaced pairs of fine filaments which bridge the intercellular space. In still more oblique planes (3), the midline component disappears altogether, but the bridging filaments remain and appear to be continuous with the dense bundles of tonofilaments which converge toward the plaque areas from the adjacent cytoplasm. As the tangential plane is approached (4), the continuity of the tonofilaments across the intercellular space is more clearly suggested. And in completely tangential section (5), the macular character of the desmosome becomes apparent, as each plaque is seen as an electron-dense circular or oval area of 150 to 300 μ diameter. (×42,500.)
Fig. 14. For legend see opposite page.
According to Jakus, these fibrils, when embedded in epoxy resins, measure between 160 and 240 Å in diameter and occasionally display the longitudinal periodicity of 500 to 600 Å usual for collagen.

In the CHCD specimen, Bowman's layer was noted in histologic section to have gross undulations in its normally smooth contours and marked irregularities of thickness. These same abnormalities are also visible by electron microscopy, but there is no readily apparent correlation between these irregularities and the occurrence of other superficial alterations such as subepithelial bullae. The area shown in Fig. 14, A, for example, though directly adjacent to a subepithelial bulla, has retained an entirely normal configuration; here, Bowman's layer consists of disoriented collagen fibrils of uniform diameter (ca. 240 Å) and could be followed in the uncropped electron micrograph to a depth of at least 8 µ.

The ultrastructural architecture of the normal human corneal stroma has been thoroughly described by Jakus and by others and need not be extensively restated here. Indeed, in Fig. 17, a specimen of central stroma from a normal human cornea shows all the features relevant to this study:

1. The collagen fibrils constituting the bulk of the stroma are fine cylindrical fibrils of uniform diameter, which lie in ordered parallel alignment to form ribbon-like lamellae. The lamellae, in turn, are orthogonally oriented and constitute the stromal fibrils seen by light microscopy. The lamellar interfaces are smooth, and generally course parallel to the surface of the cornea.

2. Cross-sectional measurements of several hundred fibrils of this normal specimen gave a mean fibril-diameter range of 240 to 260 Å. Longitudinal periodicity was occasionally measurable at ca. 550 Å (cf. Jakus' normal values of 200 to 300 Å diameter and 500 to 600 Å repeating period for stromal collagen fibrils of 12 species).

Several counts of collagen fibril density gave a mean value of 504 fibril cross sections per square micron with a standard deviation of 19.0.

3. The stromal cells or keratocytes appear to lie within lamellae. They are spindle-shaped and have numerous attenuated processes, an example of which is shown in Fig. 17.

Considering the gross thickening of the CHCD stroma and the abnormal diffuseness of its normally compact fibrous structure by light microscopy, one might also expect the changes in stromal fine structure to be especially profound. Yet in low-power electron micrographs, few purely

Fig. 14. A, Between the basal epithelial layer (B Ep) and Bowman's layer of the stroma (Str) the edge of a bulla (B) is shown in this survey electron micrograph of the CHCD cornea. (Bracketing arrows indicate the areas shown at higher magnification in Fig. 14, B and C.) At the extreme right of this figure, infoldings of the basal cell membrane (open arrows) appear to contain basement membrane-like material. The underlying surface of Bowman's layer seems relatively free of cellular debris. (x8,000.) B, This electron micrograph reproduces at higher magnification the left (bracketed) portion of Fig. 14, A. Over most of the area shown, the epithelial-stromal junction is apparently normal, with an intact basal-cell membrane (BCM) and the usual frequency of hemidesmosomes (HD). Also, the basement membrane (BM) is generally uniform and continuous. In one area, the regularly spaced anchoring fibrils (AF) clearly arborize into finer filaments near their point of entry into the basement membrane. As the edge of a small bulla is approached, however, the picture abruptly changes (*); although the basal-cell membrane remains mainly intact, the basement membrane and its attendant hemidesmosomes completely cease to exist. (x24,000.) C, This electron micrograph is an enlargement of the middle (bracketed) portion of Fig. 14, A. Here the basal cell membrane (BCM), in the absence of hemidesmosomes and basement membrane, is difficult to visualize. In those areas where it can be followed, however, it seems to be intact but irregular. Since no intervening basement membrane is present, the basal cell membrane lies directly against Bowman's layer. (x30,000.)
ultrastructural abnormalities are obvious. In many parts of the middle stroma (Fig. 15), the regular organization of the collagen fibrils into well-defined lamellae is admirably maintained, and the keratocyte perikarya and cell processes are also intact and apparently normal. Only some generalized increase in stromal density is perhaps evident. At this same level of resolution, however, other areas (Fig. 16) show obvious disturbances of stromal architecture: (1) Compressions and rarefactions of large numbers of stromal fibrils have destroyed the highly organized uniformity of fibril packing; and (2) loss of parallel fibril alignment, and undulations of fibril bundles, obscure the usual lamellar pattern. These changes are most likely the result of both focal (i.e., water clefts) and diffuse stromal edema and correspond directly with the changes previously noted by light microscopy. It is interesting to note that similar nonhomogeneous swelling—which causes not only dispersion but also apparent aggregation of the collagen fibrils, has been observed by Langham (personal communication) in experimentally edematous rabbit corneas.

At higher magnification, however, an additional level of stromal alteration becomes strikingly obvious. In Figs. 18 to 20, the stromal fibrils appear greatly thickened
Fig. 16. In contrast to Fig. 15, this low-power electron micrograph of the central stroma shows the extremes of inhomogeneous swelling and lamellar disorganization that occur in the CHCD cornea. Here, the normally even lamellar contour (cf. Fig. 17) has been broken; irregularities of fibril spacing are extensive, and a gross separation of the lamellae (*) is present. Several keratocyte processes (K) are seen. (×8,500.)

in the CHCD specimen. Comparison of the CHCD stroma in Fig. 18 with the normal stroma in Fig. 17 at the same magnification provides a most graphic contrast, as the CHCD fibrils are shown to be almost twice normal diameter (470 to 490 Å versus 240 to 260 Å). In addition, some of the fibrils have a diameter of approximately 720 Å. And although the fibrils have for the most part kept their parallel alignment, the disturbance of the regular packing pattern is also obvious. Even in areas where the lamellar arrangement is relatively undisturbed and where fibril orientation is still regular (as in Figs. 19 and 20), the same increase in fibril size is apparent. Moreover, the mean collagen fibril density for the CHCD cornea is calculated to be 294 fibril cross sections per square micron with a standard deviation of 20.3, a highly significant departure from the normal (ₙ = 504; S.D. = 19.0). Despite these marked changes, the longitudinal fibril periodicity remains at about 600 Å, and the keratocytes look entirely normal (Fig. 20).

Descemet's membrane and the endothelium. In the normal cornea, Descemet's membrane is thought to represent the hyperplastic basement membrane of the corneal endothelium." 13 Hence, the endothelial-cell membrane is usually found in direct apposition to the posterior aspect of Descemet's membrane. Kayes and Holmberg14 have found Descemet's membrane to consist of "... two zones, with the one closest to the anterior chamber having few randomly arranged fibers in a moderately electron-dense matrix. The zone closest to the stroma shows regularly arranged fiber bundles with a banding of about 900 to 1,200 Å. No distinct border exists between these zones or between Descemet's mem-
brane and the stroma. Stromal fibers and both banded and nonbanded material are found interspersed.\textsuperscript{19}

Given this background, the typical appearance of Descemet's membrane in the CHCD specimen (Fig. 21) is indeed abnormal. As was predictable from the histological observations (Fig. 8), this layer is considerably thinned (approximately 3.5 \( \mu \) in Fig. 21; cf. 6 to 20 \( \mu \) normal histological range). In addition, however, electron micrographs show that: (1) the non-banded homogeneous layer is completely absent, so that all of Descemet's membrane would seem to consist of banded material (with ca. 1,100 \( \AA \) periodicity); (2) the posterior surface is covered by a thin (approximately 0.3 \( \mu \)) layer of randomly oriented collagen fibrils; and (3) even if the now absent endothelium were inadvertently removed at surgery, there are no gross surface irregularities that might additionally indicate the partial dissection of Descemet's membrane.

Discussion

These findings will be interpreted in the same order as the presentation of the ultrastructural observations.

\textit{Epithelium and basement membrane.}

Concerning the epithelial changes, it is advisable to heed Jones and Zimmerman,\textsuperscript{14} who in their histopathological differentiation of the classical corneal dystrophies, caution, "the alterations in the superficial cornea—variations in thickness of the epithelium, eosinophilic subepithelial plaques, fragmentation and dehiscences in Bowman's membrane—were often so striking that attention was sometimes diverted from..."
So it is with CHCD, where similar superficial changes would seem to be equally nonspecific.

Specific superficial change is, however, perhaps evidenced in the central CHCD cornea by the frequent appearance of the diffusely thickened epithelial basement membrane and its associated anchoring fibrils that are normally characteristic only of human peripheral cornea. Since the adhesion of the corneal epithelium is known to be strongest at the periphery, these patchy limbuslike alterations might well account for the paradoxically minimal bullous change in the epithelium of the dystrophic specimen despite the presence of extreme stromal edema. In addition, these same alterations, in conjunction with the microscopically evident subepithelial microbullae, would also explain the clinically apparent pigskin-like roughening of the CHCD corneal surface. Francois similarly implies that slight granularity of the CHCD epithelium is related to epithelial edema.

At the ultrastructural level, the triad of subepithelial microbullae, interruptions of the basement membrane, and pockets of basement membrane at the basal cell interface are probably related causally. In a later report these findings will be more fully discussed in comparison with the electron microscopic appearance of other bullous keratopathies.

**Bowman's layer and stroma.** The observed irregularities of Bowman's layer are
Fig. 19. An electron micrograph of the central stroma of the CHCD cornea. As in Fig. 15, neither the ordered fibril array nor the pattern of lamellar organization has been destroyed (cf. Fig. 18), even though marked enlargement of fibrils has occurred. (x36,000.)

probably of little or no significance. Indeed, Kaufman and Clower have described several cases in which irregularities of Bowman's layer were nonprogressive and virtually asymptomatic.

The lesions of real diagnostic significance would then seem to be within the edematous stroma, where the marked enlargement of the collagen fibrils to almost twice normal diameter constitutes a distinctive alteration wholly unlike the stromal lesions of the classical corneal dystrophies. Further indication that CHCD represents a unique variant of stromal edema is again afforded by the experimental corneal-swelling studies of Langham and Cox (personal communication), who found that the swelling process in the rabbit cornea was confined mainly to the ground substance, affecting only the intercollagen spacing and leaving the fibril dimensions unchanged.

Since a future report will be devoted to the detailed comparison of the CHCD stromal lesions with other causes of corneal edema and opacification, the present discussion of these changes will therefore be limited to a few observations made by other workers which may shed some light on the nature of the fibril enlargement and the loss of corneal transparency.

Concerning the enlargement of the collagen fibrils, two related speculative possibilities are presently available: (A) collagen fibrils of initially normal size have swelled by becoming excessively hydrated, or (B) enlargement of the fibrils is due to a defect in fibrillogenesis.

There is some evidence to support the former possibility of excessive fibril hydration. Dr. Maurice Langham, who calculated the corneal water content of another of Maumenee's CHCD patients (Case 4 in reference 23), reported that in this specimen "a water content of 82.2 per cent
contrasts to the normal value of 74 to 75 per cent and is indicative of a markedly edematous cornea. It is true that the previously mentioned findings of Langham and Cox argue against the likelihood of fibril hydration in CHCD since the absorption of any aqueous excess is usually confined to the interfibrillar ground substance. Yet, there is additional presumptive evidence which leads us to consider the possibility of fibril hydration as not altogether untenable.

In particular, the normal difference in fibril size between the immense scleral fibrils (up to 2,000 Å in diameter) and the much smaller central corneal fibrils (250 to 300 Å in diameter) is well known, as are the qualitative and quantitative differences between sclera and central cornea regarding their mucopolysaccharide content. On the basis of these facts, Jakus has conjectured that since the collagen precipitated from extracts of cornea and sclera appear to be identical, then the mucopolysaccharides must be instrumental in determining the configuration which collagen assumes. By this same mechanism, a mixture of corneal and scleral mucopolysaccharide types at the limbus could account for the wide variation in fibril diameters (up to 700 Å) and the irregularities in fibril packing observed there. There is also more concrete evidence for the importance of mucopolysaccharides in
Fig. 21. An electron micrograph of Descemet's membrane of the CHCD cornea. Descemet's membrane lies in direct apposition to the posterior surface of the corneal stroma (Str). In the CHCD specimen, Descemet's membrane is thinned (to approximately 3.5 μ, in this figure). It consists of band material (arrows) having a periodicity of ~1,100 Å. Because the endothelium is entirely absent, the posterior surface of Descemet's membrane is directly exposed to the anterior chamber (AC), save for the thin covering of collagen fibrils (CF). (x22,500.)

determining the structural organization of the cornea. (1) Recent studies by Matthews indicate that it is the interaction of stromal collagen with large polymers of acid mucopolysaccharide that accounts for the regular alignment of the fibrils. (2) Moreover, Hedbys has proposed that the mucopolysaccharides of the interfibrillar ground substance are responsible for the swelling properties of the cornea. Thus, if it is the quality and quantity of the mucopolysaccharide ground substance surrounding each fibril which also regulates the degree of fibril hydration, we could speculate that an abnormal stromal mucopolysaccharide composition to be the cause of fibril enlargement by hydration in CHCD. And indeed, since it is the interfibrillar ground substance which normally obscures the details of fibril surface struc-
Congenital hereditary corneal dystrophy

In the central cornea (as in Fig. 17), the strikingly clear longitudinal periodicity of the CHCD stromal collagen (Fig. 20) is perhaps an indication that something is amiss among the mucopolysaccharides of the CHCD cornea.

The alternative possibility of defective early fibrillogenesis must also be considered. Jakus\textsuperscript{19, 13} has shown that a wound in the adult central cornea stimulates the deposition of collagen fibrils resembling those found in the adult peripheral cornea. These new fibrils are loosely packed and occur in a wide spectrum of widths, approaching twice the normal diameter. In the wider fibrils the characteristic periodicity of collagen is clearly visible. To Jakus, these findings suggest, "... both the scar and the limbus are more mature than the central cornea and that conditions in the adult central cornea are different from those which existed there during histogenesis of the stroma."\textsuperscript{9, 11} On this basis, therefore, one might conjecture that the similarities of collagen fibril size and organization occurring in CHCD central cornea, in normal limbal cornea, and in corneal scar tissue indicate a premature maturation of corneal development in CHCD (perhaps related to the availability of specific mucopolysaccharides, as was previously hypothesized), in which the stromal collagen synthetic mechanism fails to retain its embryonic potential and is thereby limited to the production of collagen fibrils of large diameter.

Further support for the possibility that the uniform collagen fibril enlargement of CHCD represents a congenital defect of fibrillogenesis is afforded by the yet unpublished studies of Zinn (personal communication) who has demonstrated that lensectomy in the 4 day chick embryo will result in the formation of corneal collagen fibrils that are from two to three times the normal diameters, the enlarged fibrils being of uniform dimension in any single eye. Finally, the previously noted occurrence of anchoring fibrils in the central CHCD cornea is also entirely consistent with this concept of partial limbalization of the embryonic cornea in CHCD.

Concerning the loss of corneal transparency, very little can be said which does not relate to the work of Maurice,\textsuperscript{24} who indicated that the transparency of the cornea depends upon its degree of hydration so that significantly increased hydration leads to swelling and loss of transparency. To explain this phenomenon he postulated that for a tissue to be transparent its fibrils must be parallel, equal in diameter, and have their axes disposed in a lattice. In conditions of excess hydration, disruption of the regular packing of the stromal collagen fibrils would result in excessive scattering of light rays and, hence, in a loss of transparency.

In addition, however, the increased diameter of CHCD fibrils must also be considered as a possible source of light scattering. In physical situations where light scattering by fibrils is assumed to occur by each fibril, independent of its neighbors, the degree of scattering is proportional to the square of the fibril radius. Maurice has proved that the corneal collagen fibrils do not scatter light energy independently. Yet even in his lattice theory of corneal transparency, the scattering of light by individual fibrils, as a function of their diameter, must still be a factor. Hence, in the stromal edema of CHCD, these enlarged fibrils might augment the light-scattering effect of lattice perturbation, to render the cornea opaque.

**Descemet's membrane and the endothelium.** The observation of Descemet's membrane in CHCD as a narrow, banded zone covered only by a thin layer of loose collagen fibrils and completely devoid of the usual nonbanded zone and endothelial cell layer, also deserves further attention. This seemingly unusual situation is, however, not without parallel.

1. Kayes and Holmberg\textsuperscript{29} have commented extensively on the thickened and irregular Descemet's membrane in Fuchs' dystrophy. In this condition, most of the increased thickness of Descemet's mem-
brane is represented by an increase in material having the periodicity of the banded bone. At the same time, the posterior, nonbanded zone becomes relatively thinned (often to extinction) with a loss of matrix so that many of the fibrous components not usually found in this part of the membrane become visible. These findings have led Kayes and Holmberg to hypothesize that fibrous elements are formed within the nonbanded region and subsequently are incorporated into the adjacent banded region.

2. Jakus, while examining a specimen with cornea guttata, similarly noted an extremely thickened Descemet's membrane which showed organized structure throughout its entire thickness and which was covered posteriorly by a layer of loose filaments and undescribed dense material. She interpreted this thickening to be the result of endothelial hyperactivity preceding its ultimate degeneration.

Despite the structural similarities of Descemet's membrane in both of these endothelial dystrophies and in CHCD, Descemet's membrane in Fuchs' dystrophy and in cornea guttata is markedly thickened whereas in CHCD it is considerably thinned. A possible explanation for this discrepancy is perhaps afforded by consideration of the embryogenesis of Descemet's membrane. In the neonatal rabbit (up to 8 days old), Descemet's membrane is thin but complete; its density is less than in the adult cornea, and remnants of stromal collagen fibrils are still evident within the mass of the membrane. In man, Descemet's membrane is first visible at the end of the sixth fetal month but does not attain a normal mature appearance until the last month of intrauterine life. Further histochemical studies indicate that the part of the membrane which is first laid down consists of banded material; the area of subsequent growth is the zone of nonbanded material.

If the histological and ultrastructural appearance of Descemet's membrane in the CHCD specimen is interpreted in accordance with the previously cited evidence from the literature, then one speculative interpretation of our observations can be hypothesized. Indeed, knowing that CHCD is a congenital condition, the observed abnormalities of Descemet's membrane suggest that, although a true endothelium must have been present during some period of embryonic development, this endothelium may have been functionally defective. In such a situation, if the dystrophic endothelium had ceased to elaborate more membrane material, the developmentally arrested Descemet's membrane would then be thin and completely banded. And as a second result of this endothelial malfunction, severe stromal edema could result, even in the presence of normal intraocular pressures.

In conclusion, it is perhaps also appropriate to comment on the classification of CHCD as a primary parenchymatous type of corneal dystrophy. In this regard Maumenee has already speculated that the possible mechanism for the congenitally abnormal corneal hydration of this condition might result either from abnormal embryonic development of the stroma itself or from a congenital form of endothelial dystrophy. With respect to the first possibility, we have already discussed the effects that mucopolysaccharide abnormalities and premature maturation might have on fibrillogenesis and corneal hydration. It is the second possibility, however, for which the preceding discussion now lends additional support. In all four of Maumenee's previous cases in which material was available for histologic examination, although indications of possible endothelial dystrophy were occasionally apparent, the endothelium was not well enough preserved to permit differentiation between the possibilities of dystrophy and damage. In the present case, however, it would seem that even if the observed absence of endothelium was due to loss in operative or postoperative handling rather than due to total endothelial degeneration, the electron microscopic appearance of
Descemet's membrane is highly suggestive that a functionally normal endothelium could not have been present beyond the embryonic period. Realizing, to be sure, the limitations of electron microscopic investigation of a single surgical specimen, it must be re-emphasized that these interpretations can at this point be only speculatively justified. Should, however, future studies of similar case material further substantiate this view, it would perhaps then be advisable to consider reclassification of congenital hereditary corneal dystrophy as a primary endothelial dystrophy so that cause (endothelial dysfunction) and effect (stromal edema) might no longer remain confused.

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REFERENCES

25a. McTigue, J. W., and Fine, B. S.: The base-