Hereditary corneal dystrophy in the Manx cat: a preliminary report

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A progressive, apparently inherited corneal dystrophy is described in an inbred line of Manx cats. Initial changes in the cornea are seen at four months of age and characterized by anterior stromal edema. Progressive worsening of the condition produces severe bullous keratopathy with eventual breakdown of both epithelium and stroma. Light microscopic and ultrastructural studies in the advanced disease state revealed marked edema of the corneal stroma, disintegration of collagen material, and the formation of epithelial bullae. Ultrastructural evidence shows a normal endothelium to be present. The pathogenesis of this corneal dystrophy is not clear and further studies are underway.

The corneal dystrophies have been defined as hereditary primary alterations or degenerations of the cornea that affect either the limiting membranes and/or the stroma, occur bilaterally, and begin usually at an early age; are not accompanied by an inflammatory phenomenon or by vascularization; may be slowly progressive or stationary and, in general, are not associated with metabolic disorders or other systemic anomalies.1, 2, 3

In general, an attempt has been made to separate the numerous corneal dystrophies that exist on the basis of their location affecting the epithelium, endothelium, Descemet's membrane, or stroma. Additionally, the morphological, biomicroscopic, and hereditary aspects of the various corneal dystrophies have been used to classify them.

Many and varied types of corneal dystrophies have been described in man. Well defined, heredofamilial, degenerative corneal dystrophies have not been frequently reported in animals.4

This report describes a heredofamilial corneal dystrophy in a family of Manx cats.

In 1972, three Manx cats were presented for examination to the Ophthalmology Section, New York State Veterinary College. The owner has been raising Manx cats for approximately 15 years, utilizing an inbred strain that produced Manx cats that were both tailless and tailed. For the past five years, at least three generations of Manx cats had ocular problems. At approximately four months of age, the affected cats developed a bilateral "haziness" to the cornea which appeared most dense centrally. The cloudiness of the cornea progressed and became more severe so that by two years of
Fig. 1. Pedigree of line of inbred Manx cats with hereditary corneal dystrophy. The numbers of animals available for study are small and the exact mode of inheritance cannot be determined from data presently available.

age there was marked corneal edema. The owner reported that two members of this family of cats had enucleations secondary to what was described as "corneal perforations" occurring at two to three years of age.

Materials and methods

Cats which in previous matings had produced offspring with corneal edema were obtained and bred (see pedigree, Fig. 1).

Serial ocular examinations of breeding animals and progeny were performed every two months. Ocular examination of the progeny began at six weeks of age. Ocular examinations were performed utilizing the Nikon biomicroscope and Fison Indirect Ophthalmoscope. Serial photographs of corneal lesions were obtained with the Donaldson stereo-camera.

Shazam (cat No. 1) was euthanized and ocular tissues submitted for histologic and ultrastructural examination. One eye was fixed in Zenker's-Acetic acid solution, processed, and sectioned according to previously described techniques. The tissues were stained with hematoxylin and eosin, Masson's trichrome, periodic acid-Schiff, Von Giesson's collagen, alcian blue, and colloidal iron.

For electron microscopy, the anterior segment of the left eye was isolated by a transverse cut at the equator with a razor blade. The anterior segment was immediately immersed in cold 2.5 per cent glutaraldehyde in 0.0645 M cacodylate buffer, and the lens was removed by means of a zonulotomy performed under direct magnification. After 1.5 hours of fixation in glutaraldehyde, the tissues were washed in cacodylate buffer with sucrose (45 mg. per milliliter), postfixed in cold 2 per cent osmium tetroxide, dehydrated through ascending concentrations of ethanol, and embedded in an epoxy resin (Epon 812). Sections for light and electron microscopy were cut from the plastic blocks and stained with azure II-methylene blue and uranyl acetate-lead citrate, respectively.

Results

Experimental matings. Two matings were obtained involving an affected two-year-old male (cat No. 4) and a four-year-old, apparent carrier, normal-eyed female (cat No. 3). The first mating produced only one female kitten (No. 5) which developed corneal edema at age four months. By 18 months of age, the corneal edema had progressed markedly and severe bullous keratopathy was present. The second mating produced three kittens. One female kitten died at birth. One male kitten was very weak and could not rise from a "crawling" position to support its body weight; it was euthanized at four weeks of age. The third kitten, a female (No. 6), survived and was affected with corneal edema at four months of age.

Clinical appearance. When presented for initial clinical examination, Shazam (cat No. 1), the three-year-old castrated male, the source of the histologic study reported, had severe bilateral corneal edema. Biomorphic examination revealed the corneal edema to be most marked centrally and involved the anterior half of the corneal stroma and the corneal epithelium. The central aspect of the corneal epithelium contained large areas of epithelial bullae. The peripheral third of the cornea contained slight stromal and epithelial edema, however, no epithelial bullae were observed. Biomicroscopic ex-
Fig. 2. Clinical development of hereditary corneal edema in the Manx cat. A, very mild stage of corneal edema characterized by central corneal epithelial edema. This stage is first clinically detectable at four to five months of age. B, a slight increase in corneal edema at seven to nine months of age. There is yet no severe bullous keratopathy although there is a more pronounced anterior stromal edema. C and D, more advanced corneal edema in a cat 1.5 years of age. At this time bullous lesions in the corneal epithelium are noted and the corneal stroma is markedly thickened when examined biomicroscopically. E, very severe stage of corneal edema at 2.5 years of age. A marked bullous keratopathy is present. Bullae, which occasionally break down at this stage of the disease, are not seen in this photograph. F, severe deep interstitial keratitis and endophthalmitis that developed subsequent to breakdown of epithelial bullae and secondary infection with *Pseudomonas aeruginosa*. The eye was enucleated.
amination of the corneal endothelium could not be effectively performed. Further examination of the anterior ocular segment revealed no blepharospasm, no scleral or conjunctival vascularization, and a normal anterior chamber and iris. Fundus examination was performed and the retina and optic nerve head appeared normal. Accurate intraocular pressure recordings could not be obtained because of the severe bullous degeneration of the corneal epithelium. The remaining physical examination performed revealed no remarkable abnormalities.

Sheba (cat No. 3) was an intact, female Manx cat. Biomicroscopic examination of the anterior ocular segment revealed no abnormalities. Fundus examination was normal. The remaining physical examination revealed no detectable abnormalities.

Hobo (cat No. 4) was a 14-month-old intact Manx cat. Ocular examination revealed bilateral corneal edema most marked in the central aspect of the cornea. Biomicroscopic examination revealed stromal edema involving the anterior one-half of the stroma. Centrally, there were epithelial bullae. The bullae were smaller than those seen in the three-year-old castrated male. The peripheral 1/3 of the cornea appeared clear. Again, there was no blepharospasm or increased vascularization of the anterior ocular segment. Fundus examination was normal.

Cats No. 5 and No. 6 are both females and developed corneal edema at approximately four months of age. By one year of age, very severe corneal edema with bullous keratopathy had developed in cat No. 5. Periodic breakdown of corneal epithelial bullae produced pain, blepharospasm, and photophobia.

Light microscopy. Light microscopic sections of the globe from three different areas were obtained and examined. These areas included: (1) temporal cornea; (2) cen-
Fig. 4. A, Full-thickness section of central cornea from cat with severe hereditary corneal edema. There is severe cystic degeneration of the epithelium. There is marked stromal edema with separation of corneal lamellae. There are several, large pale staining areas in the anterior central stroma. The endothelium is artifactually damaged. Zenkers-Acetic acid fixed, Von Gieson's collagen stained. Photomicrograph, ×25. B, Higher magnification of 3, A, ×40.

central cornea; and (3) nasal cornea. The central cornea was marked by increased thickness in the anterioposterior dimension being three times greater than normal. In the central cornea the superficial epithelial cells were very flattened and squamous in appearance. Often these cells covered an underlying cystic area. In several areas there were irregularities of the epithelial surface resulting from rupture of underlying interepithelial cysts. The basal epithelial cells stained poorly, were swollen, and exhibited spongiosis. Numerous squamous appearing epithelial cells on top of the basal cells were irregular in size and stained poorly. There were numerous interepithelial cystic spaces. Most of the intercellular spaces appeared clear, however, some contained flocculent material resembling proteinaceous elements found within edema fluid (Fig. 3).

Severe changes were present in the cen-
Fig. 5. Central corneal epithelium showing intercellular edema of the basal epithelium. Cellular debris (thin arrow) is present in the edematous areas. The intercellular space is enlarged and there are discontinuities in the basement membrane (blunt arrow). Uranyl acetate and lead citrate stain, ×19,000.

Central corneal stroma. The stromal lamellae were greatly separated and there was very poor staining of collagen. Keratocytes were present although their processes were reduced in size and the cells more rounded in appearance. A large area of poorly defined corneal lamellae was present in the central, outer one-half of the corneal stroma. The absence of normal collagen material could be demonstrated with Von Giesson's collagen stain. Neovascularization or inflammatory cells were not seen in any area of the corneal stroma. Descemet's membrane appeared to be normal in thickness in the central cornea and thicker than normal in the peripheral cornea. The endothelium had been artifactually detached in the light microscopic section (Fig. 4).

Electron microscopy. The central cornea of cat No. 1 showed the basal epithelial cells to be more oval in shape and frequently resembled corneal wing cells. Many mitochondria were swollen and in some the cristae were degenerate. The tonofibrils and ribosomes were normal. Necrotic epithelial cells were in a few loci.

The epithelial intercellular space was greatly enlarged and cytoplasmic projections were unusually evident. Sometimes membrane-bound structures of various density were in the intercellular space. Occasionally, the basal cells were separated from the basement membrane by a thin layer of epithelial cell processes and a space containing varying amounts of membranous and granular material. The space between basal epithelial cells and the basement membrane was enlarged. Filaments from hemidesmosomes to the basement membrane were absent in much of the area (Fig. 5).

In some areas, the anterior stromal layer was distinct as a zone of randomly oriented collagen fibers (Fig. 6). The space between collagen fibers was increased in foci in the anterior 1/3 of the stroma. The collagen fibers were randomly oriented, but were more widely spaced resulting in a less distinct anterior stromal layer (Fig. 7).
Occasionally, there were foci in which the collagen fibers were fragmented or frayed. There were areas of stromal rarefaction with very poorly staining collagen fibrils. Collagen fibrils seen in these areas were variable in size and an electron-dense granular material was interspersed between some of the collagen fibrils (Fig. 8). A few keratocytes had increased amounts of endoplasmic reticulum. Processes of other keratocytes were degenerate or contained areas of focal cytoplasmic degradation.

Long-spacing bundles or banded material were present in the posterior half of Descemet's membrane. The bundles occurred more frequently near the endothelium. Thin fibrils and accumulations of basement membrane-like material were near the long-spacing bundles. The endothelium was normal (Fig. 9).

Electron microscopic examination of the peripheral cornea showed marked intercellular epithelial edema, but absence of epithelial bullae. The epithelial basement membrane was absent in small focal areas underlying the basal epithelium. In these areas, epithelial hemidesmosomes were also absent.

The anterior stromal lamellae were normal, and the collagen fibrils throughout showed good spacing with little disorientation. In contrast, the posterior stroma had random diameter collagen fibrils, great disorganization, and abortive attempts at forming distinct lamellar planes. In some areas the collagen fibrils had very poor staining characteristics and in some areas appeared absent. A quite homogenous fibrillar matrix was found in these areas (Fig. 10).

Descemet's membrane in the peripheral cornea was abnormally thick and contained long spacing collagen bundles (Fig. 11).

One eye from cat No. 6 was enucleated at six months of age and prepared for electron microscopic examination as previously described. Very similar, although not nearly so advanced, changes as those of cat No. 1 were found in the corneal stroma. The endothelium was normal in all corneal tissue examined.

Discussion

Hereditary corneal edema as a manifestation of a corneal dystrophy has not been reported in the cat. Thus we have found...
Fig. 7. Area of stromal edema has resulted in loss of uniform spacing of collagen fibers. Uranyl acetate and lead citrate stain, ×24,000.

Fig. 8. Anterior stroma from central cornea shows collagen fibrils that are irregularly spaced. An electron-dense, amorphous matrix occupies areas where normal collagen fibrils should be (asterisk). Uranyl acetate and lead citrate stain, ×19,000.
isolated reports of corneal edema in cats occurring both unilaterally and bilaterally. Among the earliest reports was that of Knape who recorded corneal edema in a mixed-breed cat where the condition had been present for two months. His histologic report indicated that the cornea was two to five times thicker than normal with severe stromal edema. The endothelium in this case was absent over a large area of the cornea and Knape hypothesized that the loss of endothelium was associated with previous anterior uveitis.

Corneal dystrophies affecting man have been grouped into those affecting primarily the anterior limiting membrane of the cornea, those primarily affecting Descemet's membrane or the endothelium, or a combination of these forms.

Because the very early lesions of corneal dystrophy in the Manx cat present as edema of the anterior corneal stroma we very carefully reviewed the primary dystrophies of the corneal stroma that have been recognized in man. It should be emphasized that clinically the dystrophy seen in Manx cats begins as an anterior stromal edema progressing to marked epithelial edema. There are no definitive opacities located within the corneal stroma. The stromal edema that develops is most marked centrally and assumes a uniform, bilateral

Fig. 9. The endothelium from the central cornea appears normal. Bundles of long-spacing collagen (arrow) fibrils are located in the posterior zone of Descemet's membrane. Uranyl acetate and lead citrate stain, ×13,000.
appearance without any lattice or branch- ing formation. The corneal epithelium slowly becomes edematous, however, no deposits of material are seen to infiltrate the anterior stroma or epithelium. The signs, biomicroscopic appearance, and histologic description of the corneal dystrophy exhibited in the Manx cat could not be placed within any of the known anterior corneal dystrophies.

The systemic disorders of acid mucopolysaccharide metabolism in man have been extensively reported and classified into six well-defined syndromes on the basis of the genetic, clinical, and biochemical characteristics. There are definitive and progressive ocular lesions described in the mucopolysaccharidoses including corneal clouding, retinal pigmentary degeneration, and optic atrophy. The biochemical and ultrastructural pathology in the mucopolysaccharidoses has been well described. The corneal lesions in these disorders are characterized by alterations of corneal archi-
Corneal dystrophy in Manx cat

Volume 15
Number 1

25

tecture and the appearance of vacuole-distended cells and extracellular AMP. The corneal lesions appear to develop independently of lesions developing in surrounding tissues.1-12

Our study of hereditary corneal edema in the Manx cat indicates that the abnormality does not appear to be associated with abnormal acid mucopolysaccharide metabolism. There is no ultrastructural evidence of alterations in corneal stroma associated with the systemic mucopolysaccharidoses or macular corneal dystrophy. Additionally, a histochemically demonstrable increased amount of acid mucopolysaccharide could not be found in the cornea. Other systemic abnormalities that have been described for the acid mucopolysaccharidoses were not observed.

The ultrastructural findings in the advanced cases of corneal dystrophy in the Manx cat are very interesting. There is severe stromal edema and nonuniform separation of collagen fibrils with several large areas of collagen-free “lakes.” These changes have been associated with many types of chronic corneal edema.13, 14 The endothelium has been examined in a six-month-old kitten and in the cat reported in this study who was three years of age. The corneal endothelium in affected cats has been found to be normal after ultrastructural examination. Alterations of Descemet’s membrane were found and are described as long spacing collagen fibrils. The presence of these fibrils is not specific for corneal dystrophy in the Manx cat but have been associated with chronic corneal edema in a number of conditions in man.11 In Fuch’s dystrophy of man, the greatly thickened Descemet’s membrane could be subdivided into five different regions. One hypothesis which has been proposed is that the major alterations in Fuch’s dystrophy occur in the posterior aspect of Descemet’s membrane. The altered endothelial cells may undergo transformation into cells with morphology and function similar to fibroblasts and begin to produce basement membrane-like material and collagen fibrils. The combination of these two products forms a fibrillar region in Descemet’s membrane. Some of this fibrillar material disintegrates into thin fibrils and is transformed into long spacing collagen bundles. The degree to which disintegration of the collagen material is influenced by edema fluid or altered enzyme function is not accurately known.1, 8

We have described a progressive, hereditary corneal dystrophy that developed in an inbred line of Manx cats. There is marked edema of the corneal stroma and disintegration of collagen material. Progressive worsening of the condition produces severe bullous keratopathy with eventual breakdown of both epithelium and stroma. We have demonstrated the pathologic findings in the advanced stage of the condition. Ultrastructural evidence indicates a normal endothelium to be present. The pathogenesis of this hereditary dystrophy is not clear. Further studies are underway to more clearly define the pathogenesis of the problem in early affected animals.

REFERENCES