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Anterior Corneal Dystrophy of American Dutch Belted Rabbits: Biomicroscopic and Histopathologic Findings

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Abstract. Spontaneously occurring anterior corneal opacities were present in related, juvenile American Dutch belted rabbits. Slit lamp biomicroscopy revealed focal opacities of epithelium, basement membrane, and subepithelial corneal stroma. Lesions were characterized histologically by thin and disorganized surface epithelium, thickened and intensely staining epithelial basement membrane, fimbriated and irregular basement membrane-stromal juncture, and disorganized subepithelial stroma. Biomicroscopic and histopathologic features of anterior corneal dystrophy of American Dutch belted rabbits appear similar to those of human anterior corneal dystrophies.

Reports of specific, primary anterior corneal dystrophies in laboratory or domestic animal species are infrequent. Corneal dystrophy characterized by thickened, elevated epithelium interspersed with areas of abnormally thin epithelium has been described in New Zealand white rabbits.[25] Basal epithelial cells and the epithelial basement membrane were normal in light microscopic studies of affected corneas. A familial superficial corneal dystrophy, exacerbated by tear film deficiency, was recently described in the Shetland sheepdog.[9] Lesions were central, bilateral, and circular or irregular and occurred in adult dogs. Corneal erosions occurred in one-third of 29 affected dogs. Transient superficial corneal opacities of neonatal Thoroughbred horses have been recently reported.[22] These lesions were self-limiting and unassociated with systemic disease; an environmental or infectious etiology was proposed. Anterior corneal dystrophy with primary involvement of the corneal epithelial basement membrane, either as an induced or spontaneously occurring lesion, has not previously been reported in animals.

Spontaneously occurring corneal opacities in animals are usually either related to systemic abnormalities and, therefore, are not true corneal dystrophies or affect primarily the corneal stroma. The rabbit is susceptible to both systemic and ocular manifestations of high dietary cholesterol.[17,32] Ocular lesions in affected rabbits included focal or diffuse white areas of lipid deposition within the perilimbal cornea. Circumferential corneal stromal lipid deposition has been described in rabbits fed high cholesterol diets,[5] and this model has been proposed as a useful one for studying arcus senilis.[5,32] Diffuse bilateral white crystalline opacities caused by an all-milk diet have been reported in a wild rabbit.[13]

Corneal opacities of animals associated with systemic disease include corneal lipidosis related to thyroid dysfunction and hyperlipoproteinemia in dogs,[6,14,30] mucopolysaccharidoses-related corneal opacities of cats,[15,21,24] immunologically-mediated corneal opacities in homozygous rhino mice,[37] and drug-induced opacities of rats administered narcotic analgesics.[28] Beagle, Siberian husky, Airedale, and collie dogs develop primary lipid keratopathies involving the corneal stroma.[8,10,11,23,27,29,31,35] These canine lipid keratopathies appear to be true dystrophies which histologically and biomicroscopically resemble central corneal crystalline dystrophy of man.[10,31]

A number of bilateral hereditary primary anterior corneal dystrophies are recognized in man. Multiple abnormalities of the epithelium, epithelial basement membrane, and subepithelial stroma characterize these familial dystrophies.[1,20,33,36] The most common forms of human anterior corneal dystrophy are the epithelial basement membrane dystrophies (map-dot-finger print dystrophy, Cogan's microcystic dystrophy) in which opacities are related to thickened, multilaminar basement membrane occurring intraepithelially and in the normal basement membrane location.[4,7,34] Clinically in human patients, primary dystrophic disorders of the corneal epithelium, the epithelial basement membrane, or the underlying corneal stroma may result in marked corneal opacification and the development of painful recurrent corneal erosions.[3,12]

In this study, young related American Dutch belted

rabbits with spontaneously occurring superficial corneal opacities were examined periodically with a slit lamp biomicroscope for 6 months, and lesions were photographed and studied histologically.

Materials and Methods

Five related 6-month-old American Dutch belted rabbits, three males and two females, were obtained from a private contract laboratory which had acquired the animals for ocular toxicity studies. The animals were rejected from toxicology trials when focal superficial corneal opacities were seen in three animals (two males and one female) during routine screening procedures. Following transfer to a university research facility, the rabbits were housed for 6 months in standard metal hutches and maintained on a commercial vegetarian rabbit chow diet (Purina Rabbit Chow, St. Louis, MO) supplemented with green leafy vegetables.

Following physical examination, a detailed ocular examination was performed on each animal with a portable slit lamp biomicroscope (Kowa SL-2, Keeler Instruments, McHenry, IL) and a binocular indirect ophthalmoscope (AO MK IV, Reichert Scientific, Buffalo, NY). Fluorescein stain (Fluor-I-Strip, Ayerst, New York, NY) was applied to evaluate the integrity of the corneal epithelium. The animals were examined at 2-week intervals for 6 months. Lesions were photographed with a photo slit lamp (FS-2, Nikon, Garden City, NY). As part of the initial screening procedure, blood was collected from each of the five rabbits for quantitation of serum fats. Following an overnight fast, blood was drawn from the marginal ear vein of each rabbit, allowed to clot for 20 minutes, and centrifuged. Serum was harvested and analyzed for triglycerides and total lipids.

At the conclusion of the observation period, the animals were sacrificed, and the eyes were enucleated and fixed in Karnofsky fixative (4.0% paraformaldehyde, 2.5% glutaraldehyde, 0.2 M phosphate bufffer pH 7.2). After fixation, each cornea was removed at the limbus with corneal scissors and sectioned linearly into 2 mm strips with a platinum razor blade. Linear strips were imbedded into glycol methacrylate (JB-4, Polysciences, Park Warrington, PA), sectioned at 3 μm and stained with toluidine blue and periodic acid-Schiff (PAS) stain. Jones hematoxylin and eosin (HE) stain was used for basement membrane differentiation. Stained sections of both affected and unaffected corneas were studied by standard light microscopy and phase contrast microscopy.

Near the end of the study, an on-site inspection of the facilities and husbandry practices of the commercial vendor was conducted to explore the possibility of an environmental etiology. At that time an additional 34 related rabbits were examined with a portable slit lamp biomicroscope at the rabbitry to determine the incidence of corneal lesions in breeding animals and to investigate the possible heritable nature of the disease.

Results

On initial ocular screening with a focal light source three (two males and one female) of the five rabbits studied had varying degrees of corneal opacification. A more detailed ocular examination using a portable slit lamp biomicroscope confirmed bilateral opacities in one male and one female and uniocular opacities in one male (Fig. 1). Biomicroscopically, the affected eyes were characterized by focal linear, curvilinear or plaque-like epithelial and subepithelial opacities of the central or paracentral cornea. Three of the five affected corneas had solitary localized lesions while each of two affected eyes had two distinct areas of opacification (Fig. 1). Opacities, which were superficial in each instance, varied in optical density from a fine granular stippling to more opaque areas of coarse granular opacities to easily seen dense plaques (Figs. 2–4).

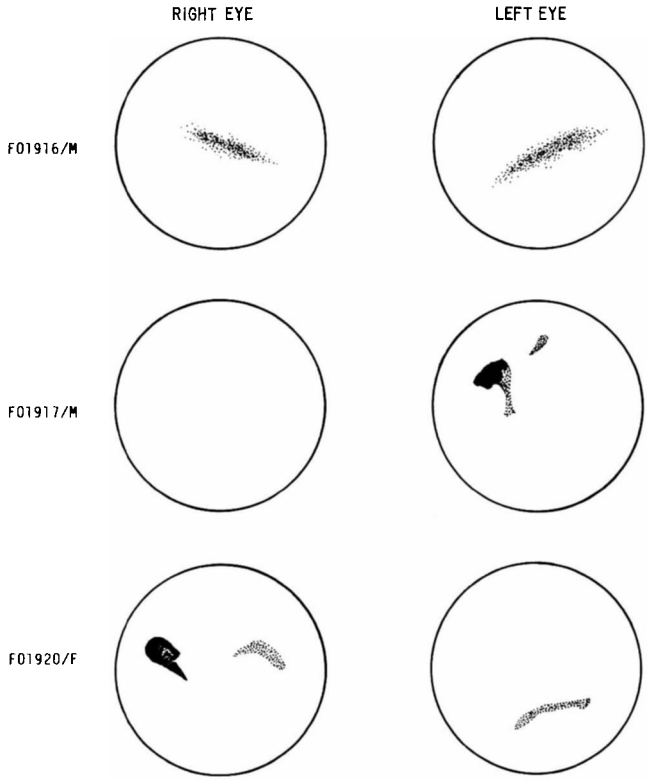

Fig. 1. Location, optical density and distribution of corneal lesions of three affected American Dutch belted rabbits.

Of the 34 animals (30 females and 4 males) examined at the rabbitry, nine females and one male had lesions similar to those in the three previously examined affected rabbits. Six of the nine affected females were sired by the affected male. Corneal opacities were the only ocular lesions noted. General physical examination of all animals examined was unremarkable, and no external evidence of systemic disease was apparent.

At the time of the on-site visit it was discovered that the vendor was striving to establish a specific-pathogen-free (SPF) colony and had maintained a "closed" colony with no "out crosses" for several generations. Therefore, the colony consisted of highly inbred ani-

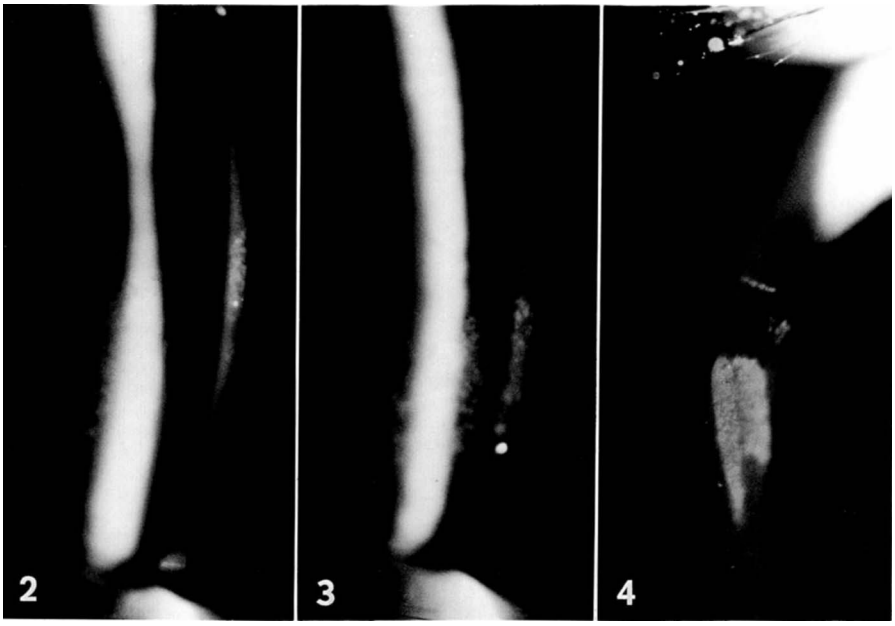

Fig. 2. Slit lamp photograph of left superior central cornea of affected male rabbit (F01916). Note fine granular stippling of anterior cornea.

Fig. 3. Slit lamp photograph of left inferior central cornea of affected male rabbit (F01916). Superficial opacities are more coarsely granular than opacities seen in Fig. 2.

Fig. 4. Slit lamp photograph of right cornea of affected female rabbit (F01920). Plaque-like opacity of superficial paracentral cornea is delineated by the slit beam. Note distinct demarcation between affected and nonaffected cornea.

mals. The housing provided was spacious, clean and well-ventilated. No enviromental irritants or husbandry practices were identified which might have contributed to the occurrence of the corneal lesions. These observations combined with the finding that six of nine affected females were sired by one affected male, suggested a familial disease.

In the affected rabbits mean values for serum triglycerides (111 mg/dl) and total lipids (263 mg/dl) were less than the corresponding mean values for the unaffected rabbits (triglycerides 161 mg/dl; serum lipids 330 mg/dl). However, numbers of animals in each group were not sufficient to state a significant difference.

Histologically, lesions were similar but varied in severity. Periodic acid-Schiff and Jones hematoxylin and eosin (HE) stains demonstrated irregularly thickened epithelial basement membrane throughout affected areas. Junctional areas between the abnormal epithelial basement membrane and the superficial corneal stroma appeared fimbriated and irregular (Fig. 5). The most prominent histopathologic changes were noted in areas where dense plaque-like lesions were seen biomicroscopically. In the most severely affected areas (i.e., most optically dense), the basement membrane was accentuated with Jones HE stain and appeared markedly thickened and irregular (Fig. 6). Differential interference contrast illumination also enhanced visualization of thickened basement membrane and adjacent abnormal anterior corneal stroma which had disorganized collagen lamellae (Fig. 7). Epithelium was frequently thin and disorganized in affected areas, and epithelial basal cell nuclei stained intensely (Fig. 7). Detachment of the basal epithelial cells from the basement membrane was occasionally noted within affected foci.

Discussion

Spontaneously occurring ocular disease of laboratory rabbits is of interest to the biomedical community because the rabbit is commonly used in toxicologic studies to assess the degree of ocular irritation induced by topically administered test substances. It is important to identify and define spontaneously developing ocular surface diseases which could result in misinterpretation of ocular findings following exposure to investigative materials.

Morphologically, anterior corneal dystrophy of the American Dutch belted rabbit is unique among animals but strikingly similar to human corneal dystrophies biomicroscopically and histologically. Comparisons of histopathologic features of anterior corneal dystrophy of the Dutch belted rabbit with three specific forms of anterior corneal dystrophy of man are pre-

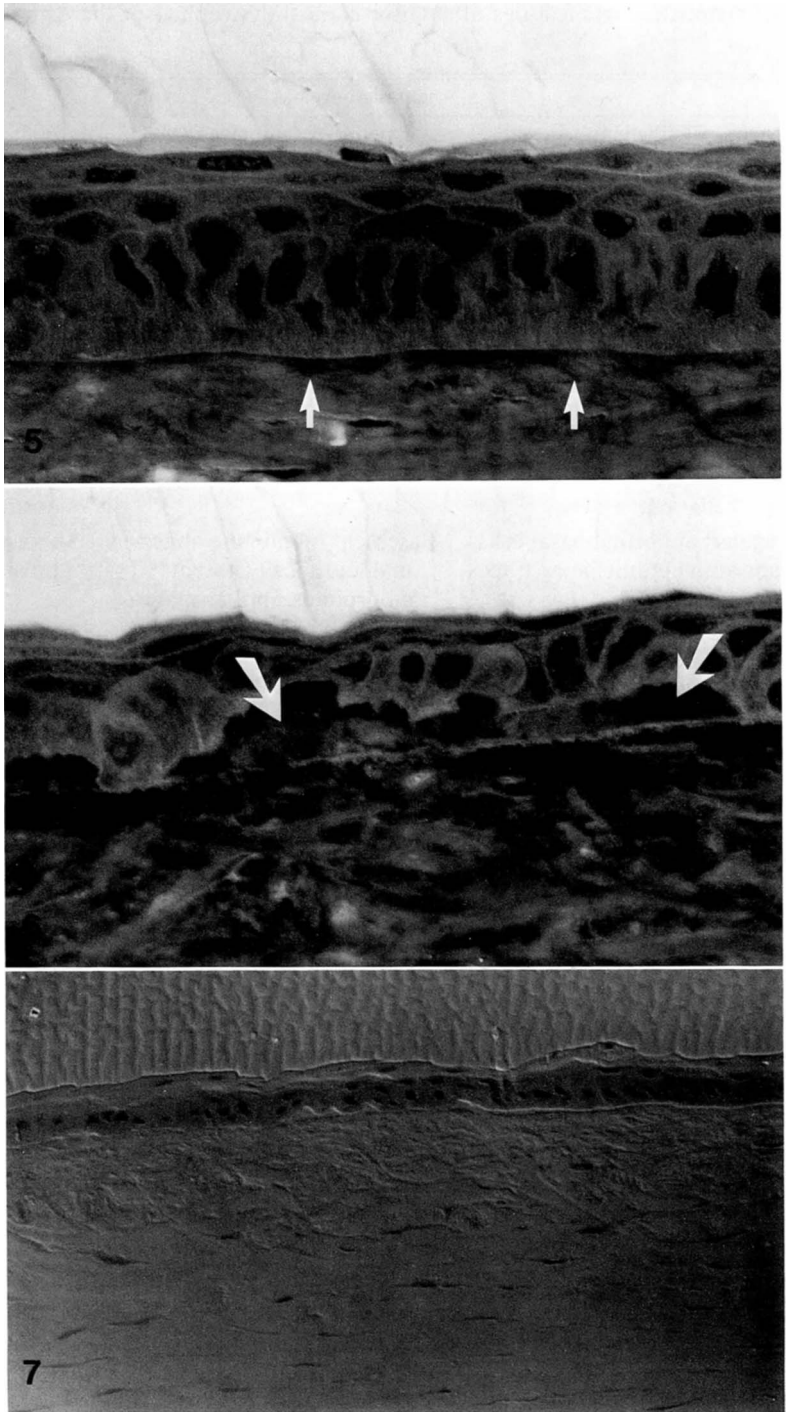

Fig. 5. Histologic section of anterior cornea of left eye of male rabbit (F01917). Note irregularly thickened basement membrane and fimbriated junction between abnormal basement membrane and anterior stroma (arrows). Jones HE.

Fig. 6. Histologic section of anterior cornea of left eye of female rabbit (F01920). Note markedly thickened abnormal basement membrane in area normally occupied by basal epithelial cells (arrows). Jones HE.

Fig. 7. Histologic section of anterior cornea of left eye of female rabbit (F01920) viewed with differential interference contrast illumination. Corneal epithelial thickness is diminished to 2–3 cell layers, basal cell nuclei are small and stain intensely, the basement membrane is thickened and irregular, and associated superficial corneal stroma (analogous to Bowman's layer) is disorganized. Within the anterior stroma note relative acellarity and irregular arrangement of collagen lamellae. PAS.

Table 1. Comparison of histopathologic features of anterior corneal dystrophies of the American Dutch belted rabbit and man.[1,4,7,20,26,33,34,36]

Description	Anatomic Layers Affected/Lesions		
	Epithelium	Basement Membrane (BM)	Subepithelial Stroma
Dutch belted rabbit anterior corneal dystrophy	Thin; disorganized; basal cell nuclei small and intensely stained, focal detachment of basal epithelial cells from basement membrane	Thickened; intensely staining; irregular	Irregular junction with epithelial BM; relative acellularity; disorganized lamellae
Meesman's juvenile epithelial dystrophy	Epithelial vesicles; abnormal maturation of cells with intracellular "peculiar substance"	Thickened (250 μm)	Normal
Epithelial basement membrane dystrophy	Abnormal epithelial cells; epithelial microcysts; fibrillar epithelial material	Thickened (200–600 μm); intensely staining	Fibrillar subepithelial material; Bowman's layer and stroma normal
Reis Bucklers' dystrophy	Irregular; abnormal basal cells; abnormal epithelial architecture	Basement membrane abnormal and focally absent; amorphous fibrillar material found in place of BM	Abnormal Bowman's layer (fibrosis); fibrillar elements

sented in Table 1. Similar pathologic features include abnormalities of the epithelial cells, basement membrane, and subepithelial stroma.

Corneal opacities seen in this inbred strain of American Dutch belted rabbits appear to fulfill criteria of a true corneal dystrophy.[36] Corneal dystrophies occur spontaneously, are non-inflammatory, typically involve the central cornea, are often bilateral, and frequently occur symmetrically. They are unassociated with systemic disease, primarily involve a single corneal layer (although other layers may become secondarily affected), and are familial.[36] Corneal opacities in affected rabbits occur spontaneously, and the central or paracentral cornea is involved. Lesions are often bilateral but not always symmetrical. No clinical or histologic evidence of local inflammation is present, and the animals are free of systemic disease. The corneal epithelial basement membrane is consistently abnormal; however, abnormalities of the overlying epithelium and underlying stroma also occur.

Clinicians have been concerned with the renewal of tight epithelial-basement membrane adhesions following abrasion of the corneal epithelium.[12] Poor adhesions of the basal epithelial cells to the epithelial basement membrane may result in recurrent epithelial erosions of the corneal surface which are painful and disabling in human beings and animals. In human patients, the anterior corneal dystrophies are a primary cause of recurrent corneal epithelial erosions.[3,12,36]

The importance of normal basement membrane complexes to the maintenance of tight adhesions between the epithelial basal cells and the anterior stroma (analogous to Bowman's layer) has been investigated in laboratory animals.[17–19] Rabbit cornea was used to demonstrate that epithelial cells undergo rapid migration and mitosis (i.e., 4 days) to cover denuded basement membrane. Tight adhesions formed between epithelial cells and the original basement membrane within 7 days in normal rabbit cornea.[19] When the epithelium and basement membrane were removed from monkey corneas, 8 weeks were required for reformation of the new corneal epithelium-basement membrane complex with resulting firm attachment by anchoring filaments to the underlying stroma.[18] Precise contributions of corneal epithelium and Bowman's layer keratocytes to the formation and renewal of the corneal epithelial basement membrane merit further study.

Since rabbits in the present study with corneal epithelial basement membrane disease have abnormal corneal epithelium and superficial corneal stroma, these animals may be useful for investigating the basic interrelationships and sequential contributions of basal epithelial cells and adjacent stromal keratocytes to corneal epithelial basement membrane formation. Ultrastructurally corneal epithelial basement membrane consists of basal lamina collagen and a reticulum with ground substance and resembles other basement membranes.[16] It is believed that the basal lamina collagen is a product of the epithelial cells and that the reticular fibers, with their associated polysaccharide matrix, are products of connective tissue fibroblasts.[2]

Based on present findings, the term anterior corneal dystrophy of American Dutch belted rabbits is suggested as the most appropriate designation for this disorder. Researchers are alerted to this suspected familial disease and are encouraged to carefully screen all American Dutch belted rabbits prior to initiation of

biomedical studies relating to the eye. Ultrastructural and genetic studies are needed to further characterize this entity which is proposed as an animal model for the study of anterior corneal dystrophies of man.

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