breakdown was occurring in the intact calcium-induced cataract one would certainly expect accompanying changes in Na\(^+\) or K\(^+\).

Since little if any change in hydration or cation levels was observed, it is postulated that in the earliest stage of opacification, scattering centers might result from calcium-protein interactions both in the membranes and the cytosol. This hypothesis is presently being pursued in a study involving histologic examination to determine whether localized disorder of fiber cells might contribute to light scattering in this cataract.

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From the Institute of Biological Sciences, Oakland University, Rochester, Mich. The study was supported by Research Grants EY-03681, EY-00483, and EY-00484 from the National Eye Institute of the National Institutes of Health and is part of the Cooperative Cataract Research Group activity. Submitted for publication Aug. 10, 1981. Reprint requests: Kenneth R. Hightower, Institute of Biological Sciences, Oakland University, Rochester, Mich. 48063.

**Key words:** cataract, calcium, lens, rabbit, proteins

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**Decrease in canine corneal endothelial cell density and increase in corneal thickness as functions of age. ROBERT M. GWIN, IRVING LERNER, J. KAY WARREN, AND GLENWOOD GUM.**

Fifty-nine normal dogs, ranging in age from 6 weeks to 132 months were examined with contact specular microscopy to determine the relationship of age to corneal endothelial cell density, morphology, and corneal thickness. Canine corneal endothelial cells appear quite similar to those of other species studied, including man. The hexagonally shaped canine endothelial cells tend to enlarge with age, with the population in young animals averaging around 2500 cells/mm\(^2\) and the number of cells in older dogs being frequently below 2100 cells/mm\(^2\). A significant increase in corneal thickness was observed with age. Healthy canine corneal endothelial cells appear to maintain a functional monolayer by enlargement and migration and represent a reasonable model for future endothelial cell study. **(INVEST OPHTHALMOL VIS SCI 22: 267-271, 1982.)**

With the popularity of advanced surgical techniques such as phacoemulsification, intraocular lens implantation, and corneal transplantation\(^1\)\(^\text{-}^3\) and the development of the clinical specular microscope, trauma to corneal endothelial cells by these procedures has been well documented.

Several studies show that normal human endothelial cell populations decrease with age, resulting in a significantly larger size of individual cells.\(^4\)\(^\text{-}^5\) These data indicate that human endothelial cells maintain a functional monolayer by the process of enlargement and migration, rather than by active mitosis. Endothelial cell regenerative activity appears to vary in animal species. In the rabbit, mitotic activity of endothelial cells is extensive after endothelial cell loss.\(^6\) In contrast to man, the number of rabbit corneal endothelial cells increases with age.\(^7\) It has been shown, however, that the cat and primate have limited capacities to actively divide and respond to injury in a manner more similar to man.\(^8\)\(^,^9\) The regenerative capacity of canine corneal endothelial cells is unknown.

The purpose of this paper is to describe the normal aging changes of canine corneal endothelial cells with regards to cell density, morphology, and corneal thickness.

**Materials and methods.** Dogs used in this study were either normal beagles and schnauzers obtained from dog colonies at the University of Florida or were dogs of various breeds and ages
Fig. 1. Specular microscopic views of normal canine corneal endothelium. A, Four-month-old schnauzer (3248 cells/mm²). B, Ten-month-old schnauzer (2814 cells/mm²). C, Six-year-old German shepherd (1995/mm²). D, Eleven-year-old mixed breed (1855/mm²).
examined immediately prior to cataract surgery. The majority of these dogs were either poodles or cocker spaniels. The ages of dogs within each breed varied greatly.

All dog eyes were examined and photographed (Carl Zeiss photo slitlamp; Carl Zeiss, Inc., Oberkochen, West Germany) prior to specular microscopy. All eyes were free of inflammation or other discernible ocular disease and had normal intraocular pressure. Corneal diameters differed minimally in the breeds examined; however, dogs less than 3 months of age tended to have corneal diameters 2 to 3 mm smaller than did adult dogs. Contact specular microscopy and photography were performed with a Bio-Optics contact LSM-2000B specular corneal endothelial microscope (Bio-Optics, Arlington, Mass.) under heavy tranquilization. Calculations of endothelial cell density per square millimeter was standardized by using a counting reticle in the photography system. In counting endothelial cells, 10 squares were counted in at least three separate photographs of each region. These counts were then averaged to arrive at a single count. Cells were counted in both the central and peripheral cornea. Central corneal readings were made from a central corneal region equidistant with the limbus in the horizontal and vertical planes. Peripheral readings were taken in the lateral and superior regions of the cornea 2 to 3 mm from the limbus. Corneal thickness was also measured with a digital pachometer within the specular microscope.

**Results**

**Endothelial cell density.** One hundred forty-seven cellular density readings from 59 dogs ranging in age from 6 weeks to 132 months were evaluated. Most dogs under 1 year of age had endothelial cell counts over 2600 cells/mm² (Fig. 1, A and B). These cells had a configuration identical to those observed in other species, with a constant hexagonal shape and uniform size.

Between 1 and 9 years of age the majority of dog corneas had lower endothelial cell densities, ranging from 2300 to 2500 cells/mm². Hexagonal cellular shape and uniform size remained constant, with pleomorphism rarely seen (Fig. 1, C).

Measurements of cell densities in six dogs over 120 months of age revealed decreased cell density (ranging from 1900 to 2100 cells/mm²). In these cases there was definite pleomorphism of cells; however, the corneas remained clear, suggesting persistence of a functional monolayer (Fig. 1, D).
A statistical analysis of variance of endothelial cell density in normal dogs revealed a significant decrease in cellular density with age ($p < 0.0006$). Regression analysis of this data revealed a mathematical model with a negative slope (Fig. 2).

Evaluation of central and peripheral endothelial cell densities in all eyes revealed mean density counts of 2335 cells/mm$^2$ (S.E. ± 103) centrally and 2310 cells/mm$^2$ (S.E. ± 80) peripherally. With t test analysis, no significant difference was observed ($p > 0.785$).

Additionally, no significant difference was found between left and right eye cellular densities ($p > 0.629$).

**Corneal thickness.** Evaluation of corneal thickness in the same group of dogs (123 readings) disclosed a significant gradual increase with age ($p < 0.001$). Although the majority of dogs under 2 years of age had corneal thickness ranging from 0.50 to 0.60 mm, dogs over 2 years of age consistently had thicker corneas ranging from 0.60 to 0.90 mm in thickness. Regression analysis of these data demonstrated a mathematical model with a positive slope (Fig. 3).

A significant increase ($p < 0.001$) in the mean corneal thickness was found between the central (0.61 mm, S.E. ± 0.009) and peripheral (0.67 mm, S.E. ± 0.010) corneal readings.

**Discussion.** These preliminary investigations into the physical and regenerative properties of canine endothelial cells provide new insights of clinical and research significance. There is a need for superior animal models in the study of naturally occurring, spontaneous, and inherited disease of the corneal endothelium. Unlike the cat or monkey, the dog has been shown to have both primary corneal endothelial cell dystrophy$^{10}$ and chronic open-angle glaucoma.$^{11}$ These naturally occurring disease processes make the dog potentially more valuable than other species heretofore described.

The numbers of dog endothelial cells per square millimeter are quite similar to those found in the human cornea. Most dog corneal endothelium demonstrated cells of relatively uniform size (homomegethism). However, an increased degree of pleomorphism was seen with age, and some corneas definitely had obviously variable-sized endothelial cells (polymegethism). In an aging response similar to man, the number of cells per square millimeter decreases significantly in the normal aging canine corneas. The extremely high

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**Fig. 3.** Relationship of corneal thickness to age in the normal dog. Each point represents a single reading from either the central or peripheral cornea of each cornea examined.
cell counts in very young puppies provides further indications of the ability of these cells to enlarge and retain a functional monolayer in the growing animal. Concomitant to increased individual endothelial cell size in the aging dog, corneal thickening is observed. Although a cause-and-effect relationship between these phenomena has not been proved, the importance of endothelial cell integrity in normal stromal hydration is well known. Other factors such as variation in collagen fibril numbers or structural arrangement and thickness of Descemet’s membrane may play a significant role in corneal thickness.

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Key words: corneal endothelium, dog, specular microscopy, endothelial regeneration, animal model, aging

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