SPECULAR MICROSCOPY OF THE CORNEAL ENDOTHELIUM IN DOGFISH SHARK (*Squalus acanthias*), GOLDFISH (*Carassius auratus*), AND BULLFROG (*Rana catesbeiana*)

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In 1920 Vogt first described the endothelial appearance of the posterior human corneal surface by using the specular reflection of the biomicroscope (Graefes Arch Clin Exp Ophthalmol 101:123, 1920). In 1968 Maurice utilized specular reflection and devised the first laboratory specular microscope that could be used to study excised living corneas (Experientia 24:1094, 1968). With certain modifications the laboratory model was used to view the endothelial surface *in vivo*, thus providing a unique view of the monolayer without interrupting its morphology or function.

The purpose of this study was to use the clinical specular microscope to document the appearance of the corneal endothelium *in vivo* in fish and the bullfrog. This study represents the first report on the specular microscopic appearance of the corneal endothelium in the dogfish shark, goldfish, and bullfrog.

Endothelial specular micrographs were taken with the Keeler Konan wide-field specular microscope using a x40 magnification contact dipping cone gently placed on the cornea. The cornea was anesthetized with topical 0.5% proparacaine in the pithed dogfish shark handheld bullfrog. Goldfish were anesthetized with MS-222. Tri-X black and white film was processed and enlarged to 200x. A calibration (0.01 mm) micrometer was photographed with the specular microscope for fixed-frame cell density measurements. Whenever possible, computer-assisted morphological analysis was performed using software developed by Inaba et al (Invest Ophthalmol Vis Sci 25 suppl:240, 1984) and described by Schultz et al (Am J Ophthalmol 98:401, 1984). The morphological appearance for the various species is shown in Figures 1-3.

When initially viewed with the specular microscope, the dogfish shark endothelium contained 2200 cells/mm^2 and showed a very irregular morphological appearance quite different from the regular polygonal pattern observed in normal mammalian species (Fig. 1A). The endothelium looked similar to the "reversal pattern" of Chandler's syndrome, a disease state in man where the endothelium has epithelial-like characteristics (Hirst, Am J Ophthalmol 89:11, 1980; Invest Ophthalmol Vis Sci 64:603, 1983). After several minutes of viewing, the endothelial monolayer lost its "reversal pattern" and appeared to swell (Fig. 1B). The endothelial cell monolayer began to disrupt and the cells could be seen sloughing from their basement membrane matrix (Fig. 1C).

The goldfish corneal endothelium had a cell density of 500 cells/mm^2 and displayed a nonpolygonal endothelium with rounded borders like those of a jigsaw puzzle (Fig. 2). No cells were lost during applanation.

By comparison, the bullfrog corneal endothelium had a polygonal cell monolayer similar to that observed in mammals (Fig. 3). Because of its polygonal pattern, the bullfrog specular micrograph was subjected to computer-assisted morphometry. The mean cell area was 1823 um^2 with a cell

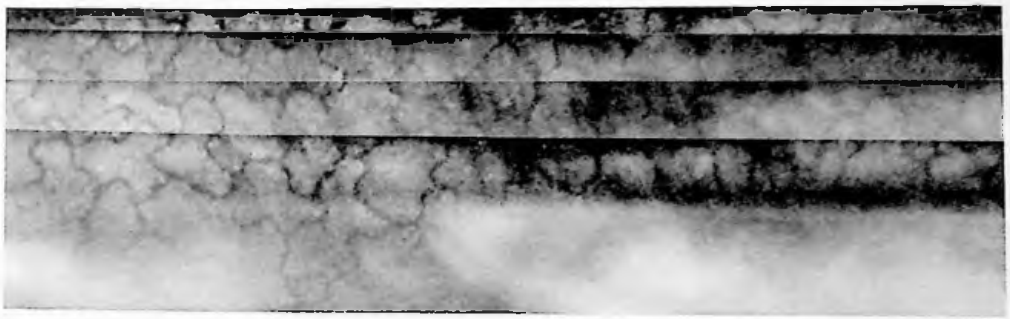

Figure 2. Specular Photomicrograph of the Endothelium of a Goldfish. The endothelium is nonpolygonal and contains 500 cells/mm^2. (x200 magnification)

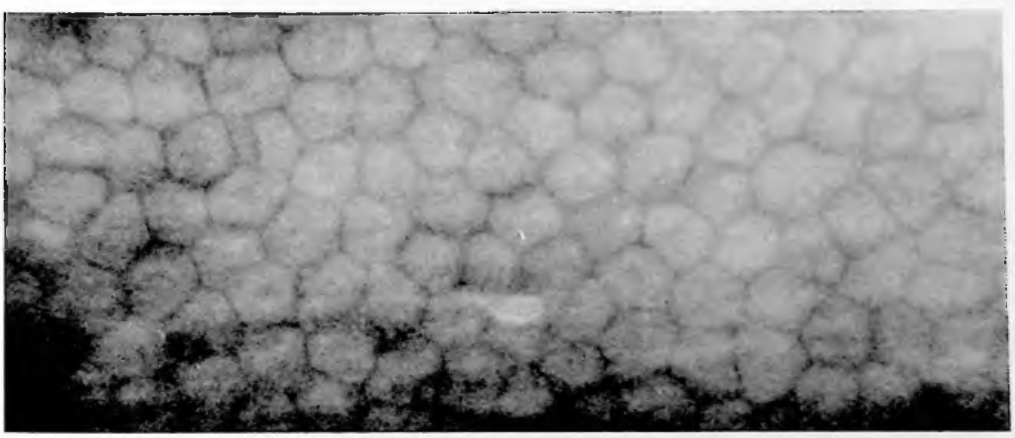

Figure 3. Specular Photomicrograph of the Endothelium of a Bullfrog. The endothelium is polygonal and contains 550 cells/mm^2. (x200 magnification)

density of 550 cells/mm^2. Only minimal polymegethism was present with a coefficient of variation of the mean cell area of 0.16. The monolayer showed significant pleomorphism with 50% of the cell population comprised of hexagonal-shaped cells in contrast to the 60-70% hexagonal cells seen in man (Yee, Curr Eye Res 4:671, 1985).

Recently, several studies have suggested that the morphologic appearance of human endothelium may reflect the endothelial functional status (Shaw et al, Ann Ophthalmol 11:885, 1978; Rao et al, Ophthalmology 91:1135, 1984). These investigators suggest that a nonuniform and/or an irregularly arranged monolayer of cells may represent a physiologic or structurally compromised endothelium. When one considers the minimal role of the endothelium in maintaining corneal transparency in the elasmobranch and teleost, one might expect that their endothelium would lack a uniform, regular polygonal pattern as observed in the dogfish shark and goldfish. In contrast, the bullfrog endothelium demonstrates a relatively uniform polygonal endothelial pattern that is very similar to what has been documented in a variety of mammalian species, which aids both structurally and functionally in maintaining corneal transparency.

The findings obtained in this study show that the corneal endothelium of the dogfish shark, goldfish, and bullfrog can be viewed in vivo with the clinical specular microscope. These studies also suggest a correlation exists between endothelial morphology and its functional capacity. (This research was supported in part by NIH grants R01 EY00933 and P30 ES01985.)

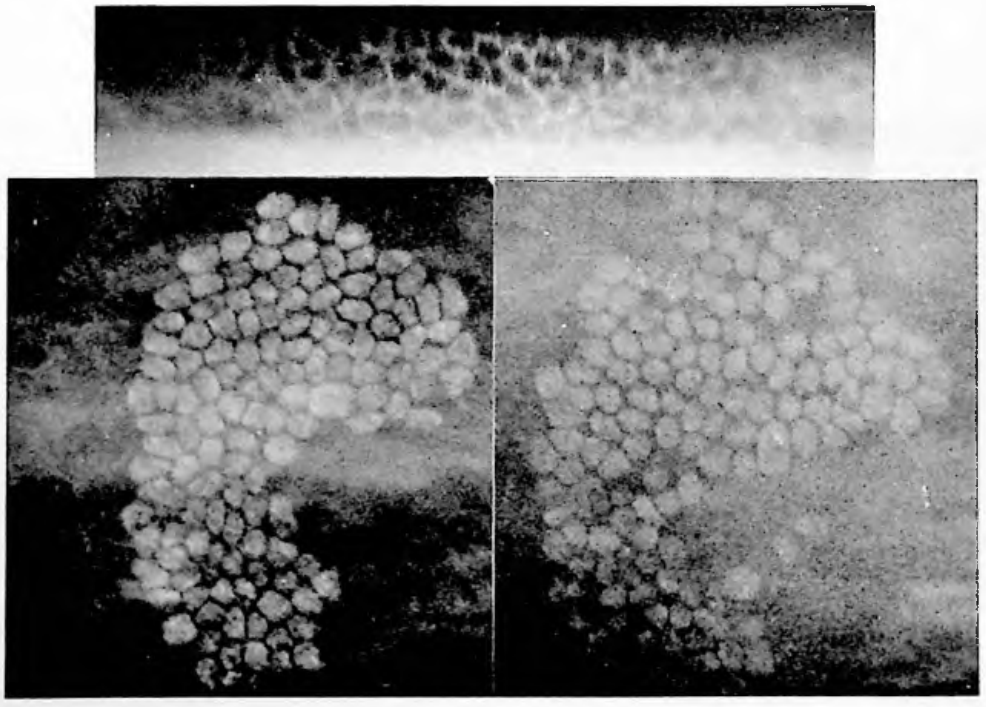

Figure 1. Specular Photomicrographs of the Endothelium of the Dogfish Shark. A. Endothelium of cornea with an intact epithelium, a normal cell density of 2200 cells/mm^2. B. Endothelium of cornea with damaged epithelium but with the endothelial cells intact. C. With applanation of the corneal endothelium by the specular microscope, the cells begin to break and slough from the cornea. All photos have a x200 magnification.