Corneal Opacity Associated with Eye Disease in Hatchery-Reared Lake Trout*† (33667)

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(Introduced by H. M. Klitgaard)

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It has previously been reported (1, 2) that lake trout raised in a hatchery environment develop cataracts, corneal opacities and lesions which can progress to corneal perforation, extrusion of the lens and blindness. Further studies on the causative factors and the prevention of this eye disease by Steucke *et al.* (3) have shown that trout raised under total cover from daylight had a low incidence of disease; whereas, trout raised without any protective cover showed a high incidence of eye disease. They have also concluded that dietary factors had no influence on the incidence of eye disease. The measurement of tissue water, sodium, and potassium of normal and pathological lake trout corneas (4, 5) has shown an increase in the total water content of the diseased cornea with a concomitant decrease in total corneal sodium and potassium. Associated with this drop in total corneal sodium and potassium is the maintenance of corneal impermeability to both sodium and water until the time of total corneal perforation when sodium and water permeability increase.

Grossly, the diseased cornea is opaque and shows either enlargement of the entire corneal diameter or keratoconus. In a light microscopic histological study, Hoffert and Fromm (2) reported that there is no inflammatory response (i.e., lymphatic infiltration) or vascular infiltration within the cornea. It was the purpose of the present study to describe the ultrastructural pathology of the corneal lesion.

Material and Methods. Lake trout (*Salve linus namaycush*) used in these studies were provided by the Michigan Conservation Department from their hatcheries at Grayling and Marquette, Michigan. Corneas were excised from 3 normal and 15 diseased eyes, fixed in 3% glutaraldehyde in 0.2 *M* sodium cacodylate buffer containing a trace of $CaCl_2$ for at least 8 hr, and rinsed in 0.2 *M* sodium cacodylate containing sucrose and $CaCl_2$. Small "pie-shaped" wedges were then cut from the center of the cornea and post-fixed in 2% osmium tetroxide in sodium cacodylate buffer for 4 hr. These wedges were dehydrated in alcohol and propylene oxide and embedded in Epon 812. Sections were cut on a Porter-Blum microtome, stained in uranyl acelate and lead citrate and examined in an RCA-EMU3G electron microscope.

Observations and Discussion. Direct comparison of plastic embedded light sections of normal and diseased corneas reveals significant changes in the corneal stroma while the corneal epithelium appears normal. These alterations include an overall increase in stromal thickness, loss of parallel arrangement of collagen lamellae and increases in the size of individual keratocytes. Changes in the endothelium include vacuolization of the cytoplasm, pyknosis of the nuclei and general distortion of cell shape.

Thickness measurements of the various corneal layers with an ocular micrometer showed the greatest increase in the stroma with a corresponding drop in the ratio of epithelial thickness to stromal thickness. The thickness of the corneal epithelium of the normal lake trout was 0.12 mm [$N = 6$] and of the stroma was 0.11 mm [6] with an

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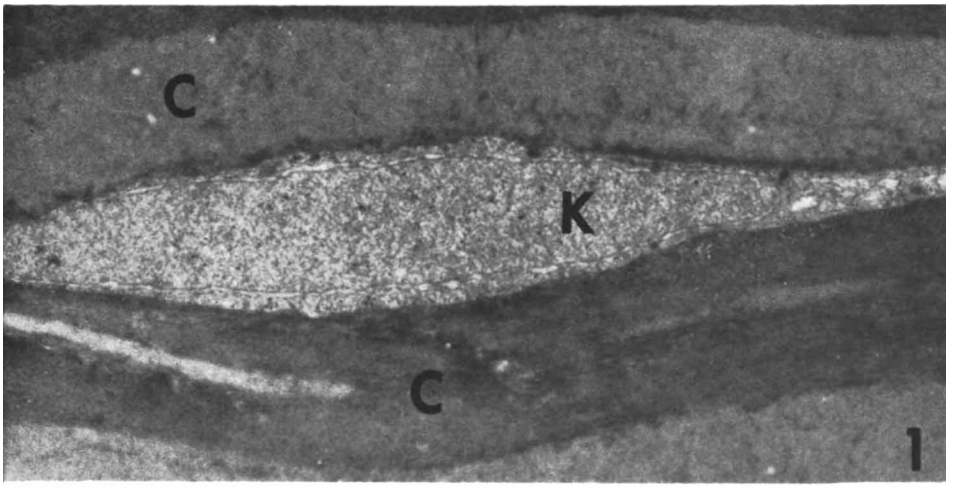
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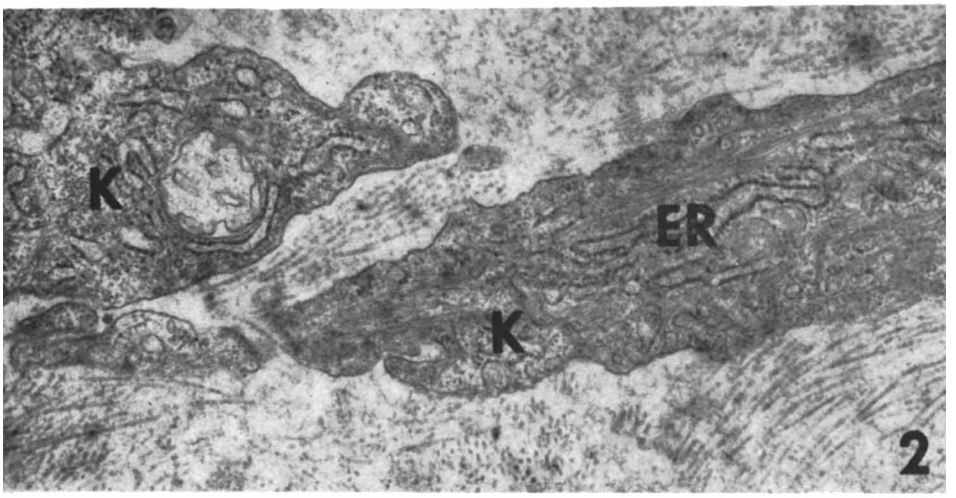
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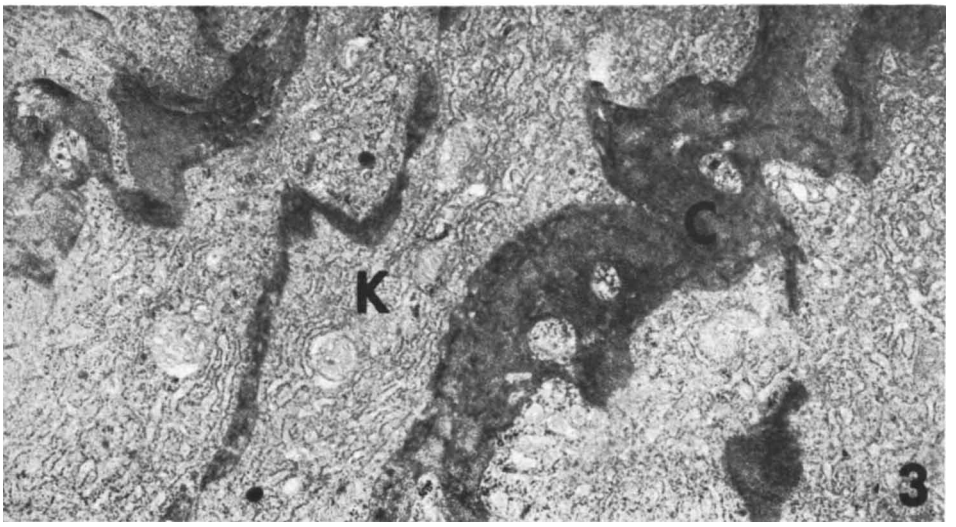
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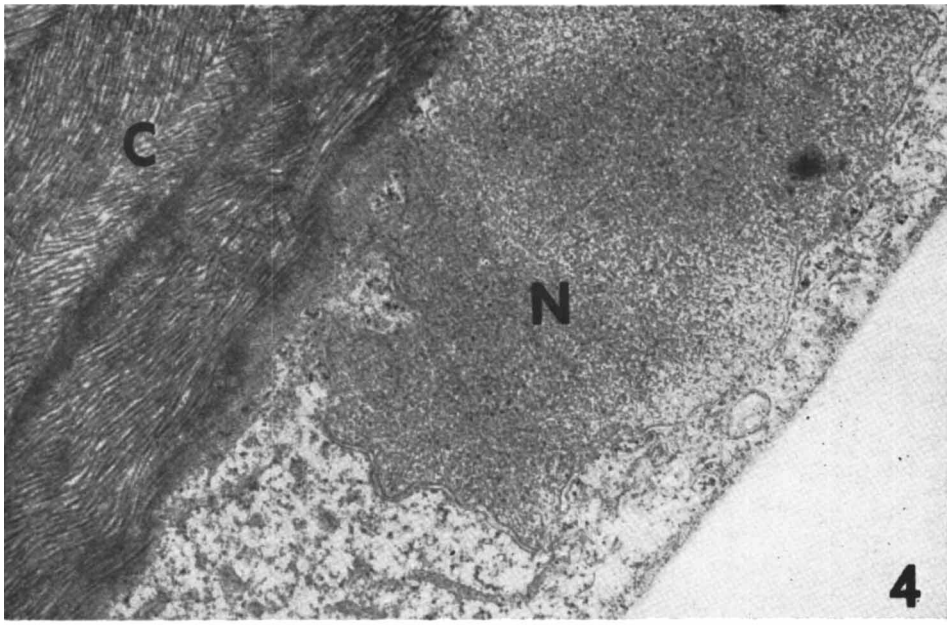
FIG. 1. An electron micrograph illustrating normal lake trout corneal stroma: A keratocyte (K) lies between two collagen lamellae (C); ×12,500.

FIG. 2. In the early stages of the disease, the cytoplasmic processes of the keratocytes (K) develop an extensive endoplasmic reticulum (ER) and increase in size; ×19,000.

FIG. 3. Enlarged keratocytes (K) in a more advanced stage of the lesion displace the normal parallel alignment of collagen lamellae (C); ×12,500.



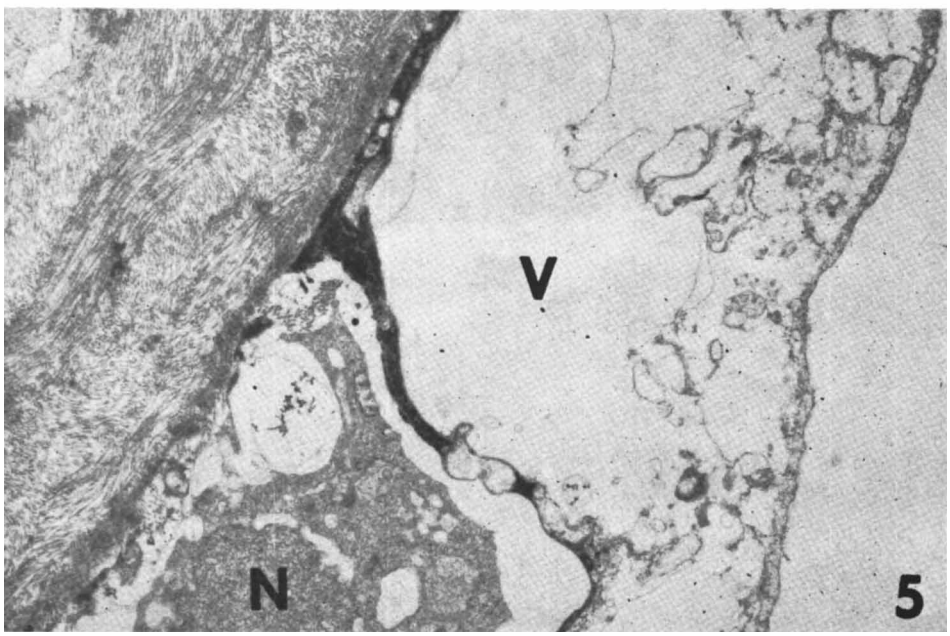


FIG. 4. Corneal endothelial cell of the normal lake trout with a large nucleus (N) and adjacent collagen lamellae; ×20,000.

FIG. 5. Degenerating endothelial cell of the diseased cornea with cytoplasmic vacuolization (V) and a pyknotic nucleus (N); ×14,000.

epithelial/stroma ratio of 1.17. The diseased cornea had an epithelial thickness of 0.10 mm (6) and the stroma was 0.16 mm (6) with an epithelial/stroma ratio of 0.66. Electron microscopic examination of diseased corneas also revealed no changes in the epithelium. The fine structure was similar to previously reported studies (6) of normal trout corneal ultrastructure; however, there were marked differences in the stroma and endothelium.

The ultrastructural appearance of the collagen lamellar pattern of the normal corneal stroma can be observed in Fig. 1. A keratocyte lies between two adjacent collagen lamellae. During the progression of the disease, keratocytes appear to enlarge and develop an extensive endoplasmic reticulum (Fig. 2). The enlarged keratocytes in a more advanced stage of the lesion displace the normal parallel alignment of the collagen (Fig. 3).

Figure 4 illustrates the fine structure of the endothelium of the normal lake trout cornea. The endothelial cells have fewer cytoplasmic organelles than observed in mammalian corneas (7). Descemet's membrane is not found. In the course of the disease, the endothelial cells become distorted in shape due to vacuolization which occurs within the cytoplasm (Fig. 5).

The pathological changes which we have observed involve both the corneal endothelium and stroma with alterations in both cellular and noncellular components. It is significant that morphological changes are not observed in the epithelium. Since the epithelium is normal in the diseased corneas, the observed changes in stroma and endothelium can be related to the observed changes in corneal transparency.

An interesting correlate to this disease is the ability of mammalian corneal keratocytes to undergo transformation into fibroblasts during corneal wound repair (8) or mild increase in number below the sloping edge of a corneal ulcer in Mooren's ulcer (9). Associated with these keratocyte transformations are alterations in the optical transparency of the cornea. However, the stromal cells in corneal wound repair or Mooren's ulcer do not enlarge or develop to the extent that has been observed in this lake trout corneal lesion.

Summary. Lake trout raised in a hatchery environment develop corneal lesions and opacities with a gross configuration similar to that observed in human keratoconus. Ultrastructural examination of this lesion shows that in the early stages of disease, the endothelial cells become distorted in shape and vacuolization occurs within the cytoplasm. As the corneal changes progress, the keratocytes enlarge and develop extensive endoplasmic reticulum, increasing the stromal thickness and displacing the parallel alignment of the collagen lamellae. There are no observable changes in epithelial thickness or morphology.

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