
The effects of transcorneal freezing on protein content of aqueous humor and intraocular temperature at the posterior surface of the cornea, the angle, the iris, and the ciliary processes were determined in rabbits and cats. Normal aqueous protein concentration was 40 ± 2 mg/dl in rabbits and 43 ± 4 mg/dl in cats. In rabbits, total aqueous protein content reached its highest level (2790 ± 302 mg/dl) 3 hr after transcorneal freezing, decreased by 50% after 4 hr, and was not significantly different from normal after 7 days. In cats, total aqueous protein content also reached its highest level (1610 ± 290 mg/dl) 3 hr after corneal freezing. Fluctuations occurred thereafter, but protein content was not significantly different from normal after 7 days. The temperature at the corneal endothelium always decreased to below 0° C with a 10 to 25 sec application of the cryoprobe to the cornea in rabbit and cat. Intraocular temperature did not decrease below 24° C at the angle or ciliary processes during application of the cryoprobe to the cornea in rabbit and cat. Intraocular temperature did not decrease below 24° C at the angle or ciliary processes during application of the cryoprobe for up to 25 sec, whereas the temperature at the pupillary margin of the iris sometimes decreased to near 0° C with a 15 to 25 sec application.

Transcorneal freezing has frequently been used as a noninvasive method for destroying portions of the corneal endothelium in studies of endothelial regeneration and wound healing. In rabbit, extensive cellular division occurs at the margin of the wound, and damaged cells are completely replaced by new cells. In contrast, little cellular division occurs in cat corneas, and the new endothelial layer is composed of enlarged, flattened cells that migrate from the margin of the wound onto the area of Descemet’s membrane that had been denuded of cells. The regenerated endothelium in cat appears to be similar to stressed endothelium in the human and has been proposed as a model for evaluating intraocular solutions, drugs, or devices that might have an adverse effect on stressed endothelial cells. Various forms of ocular injury (e.g., paracentesis) are known to produce a breakdown of the blood-aqueous barrier as reflected by an increase in protein concentration in the aqueous humor. The purpose of this study was to determine if transcorneal freezing causes a breakdown of the blood-aqueous barrier of rabbits and cats and to measure to what extent intraocular temperatures are lowered during freezing. A difference in response of the blood-aqueous barrier might explain the difference in regenerative capacity of the corneal endothelium in rabbit and cat.

Methods

Transcorneal freezing. Brass cryoprobes were machined to fit the radius of corneal curvature with diameters suitable for freezing approximately 50% of the corneal endothelium (8 mm for rabbits and 9 mm for cats). New Zealand white rabbits (2.6 to 4.3 kg) and cats (2.4 to 5.9 kg) were given an intramuscular injection of ketamine hydrochloride (10 to 15 mg/kg), and a topical anesthetic (benzoxinate hydrochloride, 0.4%) was applied to the corneas prior to injury. Rabbit eyes were properted to facilitate probe placement, whereas a speculum was used in cat eyes. The probes were cooled in liquid nitrogen (−196° C) and placed on the central corneal surface for 5, 10, 15, 20, or 25 sec.

Intraocular temperature. Ketamine hydrochloride, 30 to 50 mg/kg, and sodium pentobarbital, 10 to 30 mg/kg, were administered intramuscularly in separate syringes to provide anesthesia. Nasal congestion, when it occurred, was relieved by 0.2 mg of atropine sulfate intramuscularly.

Following propertosis of the eye in rabbits, the anterior chamber was entered at the limbus with a No. 64 Beaver blade. In cats, a lid speculum was inserted and the nictitating membrane excised before the anterior chamber was entered. A copper constantan (Type T) thermocouple (Honeywell Co.) was inserted through the wound. A mattress suture (5-0 proline or silk) was placed across the wound and tied to reform the anterior chamber. The thermocouple tip was then positioned at the posterior surface of the cornea, the pupillary margin of the iris, the angle, and the periphery of the posterior chamber (as close to the ciliary processes as possible without direct visualization). Bleeding did not occur in any eye. Control values for temperature were obtained, and the cryoprobe was then applied to the cornea for 5, 10, 15, 20, or 25 sec while temperature changes were monitored. Nine rabbit and nine cat eyes were used for the temperature measurements.

Protein content in aqueous humor. Rabbit and cat corneas were frozen for 15 sec with an 8 or 9 mm diameter probe cooled in liquid nitrogen. Each eye was paracentised once at specified times.
(5 and 15 min, 1, 2, 3, 4, 6, 12, and 36 hr, and 3 and 7 days) following transcorneal freezing. Aqueous humor from frozen and control eyes was assayed for protein content. To facilitate aqueous humor withdrawal in rabbits, retrobulbar lidocaine (1%) injections were made 5 min prior to paracentesis. Retrobulbar injections alone had no effect on protein content.

**Results**

**Intraocular temperature.** Application of the freezing probe to rabbit cornea for 10, 15, 20, or 25 sec reduced the temperature at the endothelium to mean values of −27°C or lower (Fig. 1). The temperature at the pupillary margin of the iris decreased significantly with 10, 15, 20, or 25 sec of application but never went below freezing. Temperature at the angle and near the ciliary processes never decreased below 24°C, even with 25 sec application of the cryoprobe.

Results in the cat were similar. The temperature at the posterior surface of the cornea was consistently below freezing with 10, 15, 20, or 25 sec applications (Fig. 2). The temperature of the iris decreased significantly during application of the cryoprobe but was seldom below freezing. Temperature at the angle and near the ciliary body was minimally affected by application of the cryoprobe.

**Protein concentration.** The average protein concentration in normal aqueous humor of rabbit was 40 ± 2 mg/dl. Within 5 min after transcorneal freezing, aqueous humor protein content had doubled (90 ± 11 mg/dl); this was significantly different (p < 0.01) from the normal protein content (Fig. 3). Fifteen minutes after freezing, aqueous humor protein increased to 1448 ± 111 mg/dl, and at 3 hr the protein content was at its highest level (2790 ± 302 mg/dl). By the fourth hour, aqueous humor protein content decreased almost 50% (1480 ± 308 mg/dl) from peak values. After 12 hr, aqueous humor protein content decreased gradually until, at 7 days, it was not significantly different from normal.

The average protein concentration in normal
aqueous humor of cat was $43 \pm 4$ mg/dl. Total aqueous protein content was not significantly different than control content 5 min after transcorneal freezing (Fig. 4). After 15 min, total protein content had increased significantly ($p < 0.01$) to $265 \pm 69$ mg/dl. Aqueous humor protein was at its highest level after 3 hr ($1610 \pm 290$ mg/dl). In the following hours, protein content fluctuated from $670 \pm 192$ mg/dl after 4 hr to $1325 \pm 153$ mg/dl after 6 hr to $198 \pm 44$ mg/dl after 12 hr. At 7 days after freezing, total protein content ($127 \pm 43$ mg/dl) was not significantly different from normal.

Discussion. This study has shown that transcorneal freezing causes an increase in the protein content of aqueous humor in both rabbit and cat. This is in agreement with previous studies in rabbits involving other forms of ocular trauma. Protein content, however, increased to a greater extent in rabbit than in cat (particularly at the early times following injury). These results provide a possible explanation for the lack of cellular division.
seen in regenerating cat endothelium. Growth factors have recently been shown to stimulate cellular division in corneal endothelial cells in tissue culture.\(^9\) Perhaps insufficient growth factor leaks into injured cat eyes to stimulate mitosis in the endothelial cells.

The greater increase in protein in rabbit eyes could be due to differences in (1) sensitivities of rabbit and cat eyes to ocular trauma, (2) types or levels of individual proteins, (3) depth or volume of the anterior chamber, or (4) outflow facility. The volume of the aqueous humor in cat is three times that of rabbit, and the outflow facility in cat (1.56 \(\mu\)l/min/mm Hg) is about six times higher than in rabbit (0.21 to 0.34 \(\mu\)l/min/mm Hg).\(^9\) The fluctuations in protein concentration in cat between 4 and 36 hr could be due to high outflow facility, late tissue damage, late alterations in vascular or ciliary process permeability, or temporary blockage of the angle by sloughing endothelial cells. In both species, protein concentration returned to normal by 1 week, indicating that the permeability of the blood-aqueous barrier is only temporarily affected after corneal freezing.

Our purposes in measuring changes in intraocular temperature during transcorneal freezing were to determine if intraocular temperature is lowered sufficiently to explain the breakdown of the blood-aqueous barrier or cause damage or death in tissues other than the cornea. Intraocular temperatures at the angle and ciliary processes never fell below 24° C. In contrast, the temperature of the iris sometimes decreased to near 0° C in both rabbit and cat. It is possible that tissue damage and/or prostaglandin release occurs in the iris at low temperatures, resulting in altered permeability in the iris or ciliary processes.\(^4\)

From the Departments of Ophthalmology and Physiology, The Medical College of Wisconsin, Milwaukee, and Research Service, Veterans Administration Center, Wood, (Milwaukee), Wis. This investigation was supported in part by NEI Research Grant EY-04196 and Ophthalmic Research Center Grant EY-01931, medical research funds from the Veterans Administration, and an unrestricted grant from Research to Prevent Blindness, Inc. Submitted for publication April 19, 1978. Reprint requests: Dr. D. L. Van Horn, Research Service/151, Veterans Administration Center, Wood, (Milwaukee), Wis. 53193.

Key words: aqueous humor, blood-aqueous barrier, cornea, corneal endothelium, corneal injury

REFERENCES


Etiology of corneal sensitivity changes accompanying contact lens wear. KENNETH A. POLSE.

Corneal touch threshold was monitored while corneal edema was experimentally induced by exposing the cornea to either an oxygen-free environment or hypotonic saline. No change in sensitivity occurred during these conditions. Contact lens wearers who were fully adapted to their lenses and did not develop corneal edema during wear showed an increase of 96% in corneal touch threshold. Refitting these subjects with an experimental contact lens which caused a 6% increase in corneal thickness did not further alter the corneal sensitivity. The decrease in corneal sensitivity accompanying contact lens wear is independent of corneal edema and is likely a result of sensory adaptation to a mechanical stimulant.

It is well known that a marked decrease in the sensitivity of cornea and lid margin occurs during and after adaptation to contact lenses.\(^5\) Clinicians have observed that contact lens wear can increase the corneal touch threshold to high

0146-0404/78/121202+05$00.50/0 © 1978 Assoc. for Res. in Vis. and Ophthal., Inc.