Cyclocryotherapy (CCT) is a frequently used cyclo-destructive modality in patients with glaucoma when other surgical procedures have failed.1-6 Newer trans-scleral laser (e.g., Nd:YAG and diode) methods for cyclodestruction have been used in the hope of reducing serious ocular complications associated with CCT, including phthisis bulbi and anterior segment ischemia. Transscleral Nd:YAG laser cyclophotocoagulation (TSNYC) is an appealing alternative to CCT because of the ability to titrate the amount of laser energy delivered to the eye. However, although both of these modalities effectively lower intraocular pressure (IOP), the superiority of one surgical technique has not been proved.

Cyclocryotherapy (CCT) is thought to reduce aqueous humor formation7-11 and has been shown to alter ciliary body blood flow12 in experimental animals, whereas TSNYC has been associated with decreased aqueous production13,14 and increased uveoscleral outflow.15 Both procedures create similar ocular inflammatory changes in rabbits.14 It is difficult to equate the effects of these two cyclodestructive procedures from these studies because different animal species...
(rabbits, cats, and monkeys), surgical protocols, and various postoperative study intervals were used. We recently described a cat model of IOP reduction after noncontact TSNYC.\textsuperscript{10} The cat has the advantage of having an anterior segment that more closely resembles the human than does the rabbit. The purpose of the current investigation was to use the cat model to compare the IOP-lowering effects of CCT and noncontact TSNYC, as well as to characterize the effects of these procedures on aqueous humor dynamics, ocular blood flow, and the blood–aqueous barrier.

**METHODS**

**Cyclodestructive Surgery**

One eye of 29 adult pigmented cats (\textit{Felix domestica}), each weighing 3 to 4 kg, was chosen by computer randomization to receive CCT or noncontact TSNYC. The fellow control eye did not have surgery and served as an internal control. Treated cats were randomized into a 3- or 12-week study period. All procedures were performed in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and with the approval of the Animal Care and Use Committee at Northwestern University. Results are reported as the mean ± SEM. Statistical comparisons used analysis of variance and nonparametric methods to compare results within an individual (Wilcoxon analysis) or between (Kruskal–Wallis analysis) treatment conditions. \( P < 0.05 \) was considered significant.

Cats were anesthetized with a mixture of ketamine hydrochloride (25 mg/kg) and xylazine (1 mg/kg) intramuscularly for both cyclodestructive procedures. Cyclocryotherapy was performed using a 3 mm cryoprobe and a nitrous oxide-cooled, cryo-ophthalmic unit (Frigitonics, Shelton, CT). According to the protocol by Higginbotham et al,\textsuperscript{11} the anterior edge of the cryoprobe was placed 2 mm behind the cornea, and 12 applications, equally spaced over 360°, were delivered for 60 seconds at \(-80^\circ \text{C}\). Noncontact TSNYC was performed with the laser beam defocused maximally (3.6 mm) beneath the scleral surface, as described by Rosenberg et al.\textsuperscript{16} Using the Lasag Microruptor II (Lasag, Thun, Switzerland) in the free-running, thermal mode, 7 to 9 J of energy was delivered (80 applications, in multiple rows as necessary) beginning 3 mm posterior to the corneal limbus.

A sub-Tenon’s injection of dexamethasone phosphate (12 mg) and topical atropine sulfate 1% were administered immediately after surgery. Topical prednisolone acetate 1% delivered four times daily and atropine 1% delivered two times daily were instilled for 2 weeks after surgery or until active ocular inflammation resolved. The intensity of ocular inflammation was graded from slit lamp examination on 0 to 3+ scale (for anterior chamber cells/high-power field: 0 = no cells, 1+ = <10 cells, 2+ 10 to 40 cells, 3+ = cell clumps; for flare (Tyndall effect), 0 = no flare, 1+ = mild, 2+ = moderate, 3+ = severe; for conjunctival reaction, 0 = no injection, 1+ = mild injection, 2+ = moderate injection and chemosis, 3+ = severe injection and prolapsing chemosis). Contralateral control eyes received no medications. Surgery was repeated using the same operative parameters (12 applications of CCT or 80 applications of TSNYC) if IOP was not reduced by at least 20% 2 weeks after surgery. Retreatment was performed to achieve a persistent IOP reduction for at least 3 weeks before aqueous humor dynamic studies and euthanasia.

**Noninvasive Experiments**

**Intraocular Pressure.** Using a pneumatonometer (Model 30R; Digilab, Cambridge, MA), IOP was measured in both eyes of awake cats with topical 0.5% proparacaine hydrochloride. The average of three pressure measurements was recorded. Baseline IOP was measured at least three times weekly for a minimum of 2 weeks before surgery. Pressure was measured 1 to 3 days after surgery, every 3 to 4 days for the first 2 weeks, and at least weekly thereafter until the animal was killed.

**Aqueous Humor Flow Rate.** Aqueous humor flow was calculated using the Goldmann equation, \( F = (\text{IOP} - P_v)c, \) where \( F \) is the rate of aqueous flow (\( \mu l/\text{minute} \)), \( P_v \) is episcleral venous pressure (mm Hg), and \( c \) is outflow facility (\( \mu l/\text{minute} < \text{pd} > \text{mm Hg} \)). Outflow facility was determined (2-minute tonogram with a 10 g weight) using an electronic tonometer (Digilab). Episcleral venous pressure was measured with a 3 mm planapating head attached to a force-displacement transducer mounted on a Haag–Streit biomicroscope.\textsuperscript{17}

**Fluorophotometry.** Anterior chamber fluorophotometry was performed to study the blood–aqueous barrier to fluorescein entry using a slit lamp–mounted fluorophotometer.\textsuperscript{18} An intravenous injection of 0.3 ml of 10% sodium fluorescein was administered, and anterior chamber fluorescein concentration was measured 5 minutes after injection and then every 10 minutes for 50 minutes.\textsuperscript{19} The calibrated fluorophotometer was linear between fluorescein concentrations of 10\(^{-4}\) and 10\(^{-7}\) mg/ml.

**Invasive Experiments**

**Ocular Blood Flow.** Ocular blood flow was estimated using 15-\( \mu \)m\textsuperscript{85}Sr-labeled microspheres (\( N = 15 \)).\textsuperscript{20} Under intramuscular anesthesia with the animal ventilated (Harvard Respirator; Harvard Apparatus, Dover, MA) through a tracheostomy, a baseline blood sample from a cannulated femoral artery was obtained. A tho-
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Results

Inflammation

All treated eyes demonstrated early postoperative 1+ to 2+ conjunctival reaction and anterior chamber inflammation. The severity and duration of ocular inflammation was similar among eyes undergoing CCT and TSNYC. Two eyes in each 12-week treatment group developed severe 3+ inflammation and corneal epithelial defects after surgery that resolved after 5 to 7 days under a conjunctival flap. By 3 weeks after surgery, topical antiinflammatory medications were discontinued in every animal because anterior chamber cells were no longer present. Mild anterior chamber flare persisted in all treated eyes. No rubecosis iridis, lenticular opacities, or synechiae were noted. Corneal neovascularization not exceeding 1.5 mm in treated eyes was common.

Intraocular Pressure

Cyclocryotherapy. Pretreatment IOP was similar (N = 8) between treated and control eyes in the 3-week study period (24.3 ± 3.6 mm Hg in treated and 24.6 ± 3.1 mm Hg in control eyes). Intraocular pressure was reduced significantly (P < 0.001) in all treated eyes (17.9 ± 1.8 mm Hg) at the end of the 3-week study period (Table 1). One eye required retreatment 1 week after surgery. Another eye had an IOP spike (>10 mm Hg) on the first postoperative day before IOP reduction.

Baseline IOP was similar (n = 6) between treated (24.5 ± 1.8 mm Hg) and control (23.2 ± 1.8 mm Hg) eyes in the 12-week study period. Intraocular pressure was reduced significantly (P < 0.01) in all treated eyes (15.5 ± 1.2 mm Hg) at the end of the study interval (Table 1). However, all eyes required one retreatment to maintain IOP reduction.

Transscleral Nd:YAG Laser. Before treatment, IOP was similar (N = 8) between treated and control eyes in the 3-week study period (20.8 ± 3.1 and 20.9 ± 3.0 mm Hg, respectively). Intraocular pressure was reduced significantly (P < 0.001) in all treated eyes (15.6 ± 4.0 mm Hg) at the end of the study period (Table 1). Two eyes required retreatment 1 week after surgery.

Pretreatment IOP was similar (N = 7) between treated (20.4 ± 4.1) and control (21.4 ± 4.0) eyes in the 12-week study period. Intraocular pressure was reduced significantly (P < 0.001) in all treated eyes (16.1 ± 2.0 mm Hg) at the end of the study interval (Table 1). Three eyes required retreatment—one eye required one retreatment, another eye required two, and the third eye required three retreatments. There was no difference in the magnitude of IOP reduction between eyes receiving a single treatment compared to eyes requiring multiple treatments. One eye had an IOP spike (>10 mm Hg) on the second day before IOP lowering. The decrease in IOP was similar between eyes treated with CCT and TSNYC both 3 and 12 weeks after surgery.

Aqueous Humor Dynamics

Aqueous Humor Flow Rate. Tonographic outflow facility (~0.25 μl/minute<sub>pd</sub> mm Hg) and episcleral venous pressure (~8 mm Hg) were not altered by either cyclodestructive technique. Both techniques proved difficult in the cat, and calculated aqueous
flow (μl/minute) was determined only in animals with satisfactory measurements on both eyes at the given study time interval. Calculated flow was lower in the treated than in the control eye 3 and 12 weeks after either cyclodestructive procedure.

**Cyclocryotherapy.** In 3-week animals (N = 3), aqueous flow was not statistically different in the treated (2.5 ± 0.2) and control (4.0 ± 1.8) eyes. In 12-week animals (N = 5), flow was lower (P < 0.04) in the treated (1.6 ± 0.8) than in the control (5.2 ± 1.9) eyes.

**Transscleral Nd:YAG Laser.** Three weeks after treatment, aqueous humor flow was lower in the treated (1.7 ± 1.7) than in the control (5.1 ± 4.3) eyes. However, because of the small number of animals with satisfactory tonograms (N = 3) and the large variance in results, this difference was not significant (P > 0.11). Twelve weeks after TSNYC, flow was significantly (P < 0.04; N = 5) lower in the treated (3.5 ± 1.9) than in the control (5.9 ± 2.3) eyes.

**Aqueous Humor Protein**

**Cyclocryotherapy.** Aqueous humor protein concentration was higher in all CCT-treated eyes than in contralateral control eyes. In 3-week animals, protein concentration in treated eyes was 138.5 ± 119.0 mg/dl compared to control eyes 45.8 ± 9.9 mg/dl (P < 0.01; N = 6). In 12-week animals, protein concentration in treated eyes was 326.7 ± 44.6 mg/dl, and in control eyes it was 47.7 ± 11.7 mg/dl (P < 0.03; N = 6).

**Transscleral Nd:YAG Laser.** Aqueous humor protein concentration also was elevated in all TSNYC-treated eyes. In 3-week animals, protein concentration in treated eyes was 182.1 ± 54.8 mg/dl compared to 56.1 ± 20.7 mg/dl in control eyes (P < 0.02; N = 7). In 12-week animals, the concentration in treated eyes was 266.7 ± 154.6 mg/dl compared to control eyes 46.3 ± 12.1 mg/dl (P < 0.02; N = 3). The percent increase in aqueous protein comparing the treated to the control eye was similar for CCT- and TSNYC-treated animals both 3 and 12 weeks after surgery.

**Fluorophotometry**

**Cyclocryotherapy.** Peak aqueous fluorescein levels occurred 15 minutes after intravenous dye injection in the treated and control eyes. In both 3- and 12-week animals, anterior chamber fluorescein concentration (ng/ml) was higher in CCT-treated eyes. Three weeks after surgery, fluorescein concentration in treated eyes was 9.8 ± 4.0 compared to 5.0 ± 3.1 in the control eyes (P < 0.01; N = 5). Anterior chamber fluorescein concentration was also significantly higher (P < 0.04; N = 5) in treated (5.6 ± 1.2) than in control eyes (2.4 ± 1.0) 12 weeks after CCT. The treated-to-control eye ratio of fluorescein concentration 3 weeks after treatment was +2.5 ± 1.6 (range +1.4 to +6.3), which was similar to the magnitude of the fluorescein increase in the 12-week animals (+2.9 ± 1.8; range +1.2 to +5.7).

**Transscleral Nd:YAG Laser.** Anterior chamber fluorescein concentration was higher in TSNYC-treated eyes at the end of both study periods. In 3-week animals (N = 8), fluorescein concentration in treated eyes (10.2 ± 7.0) was significantly (P < 0.01) greater than in control eyes (5.3 ± 4.9). Fluorescein levels were also higher (P < 0.02) in the 12-week animals (N = 7); treated eyes 3.4 ± 2.0 and control eyes 1.0 ± 0.4. The treated-to-control eye fluorescein ratios were similar between the 3-week (+2.8 ± 2.8; range +1.1 to +9.4) and 12-week (+3.7 ± 3.0; range +1.9 to +10.1) animals. There was no significant difference in the 3- and 12-week ratios comparing CCT and TSNYC treatment.

**Ciliary Body Blood Flow**

**Cyclocryotherapy.** No difference in ciliary body blood flow (ml/minute·100 g tissue) was detected between treated and control eyes at the conclusion of either the 3- or the 12-week study interval. The ratio of ciliary body blood flow in treated compared to control eyes at the end of the 3-week study period (N = 4) was 1.1 ± 0.4 (range, 0.9 to 1.6), and at the end of
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FIGURE 1. Cat ciliary body 12 weeks after cyclocryotherapy (CCT). Nonpigmented epithelium (NPE) and pigmented epithelium (PE) are disorganized, and there is diffuse pigment dispersion. Ciliary epithelium is absent at the ciliary body base (arrows). Clusters of PE and NPE cells are present within ciliary body stroma and processes. Cystic structures are present.

Transscleral Nd:YAG Laser. Ciliary body blood flow was not affected by TSNYC at the end of either study period. The ratio of ciliary body blood flow in treated compared to control eyes at 3 weeks (N = 4) was 0.7 ± 0.2 (range, 0.4 to 0.9), and at 12 weeks (N = 4) it was 1.3 ± 0.4 (range, 1.0 to 1.9).

Pathology

Cyclotherapy. The ciliary body in treated eyes appeared flattened and irregularly shaped when examined grossly. An irregular, whitish membrane covered its surface. Light microscopy demonstrated that most of the ciliary processes had markedly disorganized nonpigmented (NPE) and pigmented (PE) ciliary epithelium (Fig. 1). The greatest amount of ciliary body damage occurred at the base of the processes. The epithelium was often absent here. Many processes appeared normal toward their apices so that areas of both normal and abnormal epithelium were present within the same ciliary process (Fig. 2). There was pigment dispersion throughout the ciliary body. Intercellular junctional complexes could still be identified. Mitochondria and rough endoplasmic reticulum were increased in number. Cysts were lined by NPE with normal junctional membrane specializations. Intracellular membrane profiles were present. The glycocalyx was normal.

Transscleral Nd:YAG Laser. The ciliary body in treated eyes was blunted, and a whitish membrane

FIGURE 2. Normal ciliary processes (large arrow) adjacent to abnormal processes (small arrow). Pigment dispersion and ciliary epithelial destruction are apparent.

pigmentation, and the filtration angle appeared normal.

Transmission electron microscopy confirmed observations made from light microscopy specimens. Damaged areas of ciliary body were most prominent at the base of the processes, where the epithelium was usually obliterated. Both ciliary epithelial layers were extremely disorganized, multilayered, and misshapen (Fig. 3). Pigment dispersion was present throughout the ciliary body. Intercellular junctional complexes could still be identified. Mitochondria and rough endoplasmic reticulum were increased in number. Cysts were lined by NPE with normal junctional membrane specializations. Intracellular membrane profiles were present. The glycocalyx was normal.

FIGURE 3. Electron micrograph of cat ciliary body 12 weeks after cyclocryotherapy. Nonpigmented epithelium (NPE, large arrow) and pigmented epithelium (PE, small arrow) cells are disorganized and multilayered. Intercellular junctions (not shown) could be identified.
Areas of damaged PE and NPE appeared more discrete than in CCT eyes. In some sections, only the PE was damaged, whereas the greatest amount of ciliary body damage occurred at the base of the ciliary processes despite some processes appearing normal toward the apices. Electron microscopic findings were the same as those present in cyclocryotherapy eyes, except that areas of damaged pigmented epithelium and nonpigmented epithelium appeared more focal in TSNYC eyes.

covered the surface, although this was less prominent than that seen in CCT eyes. Histologically, either the processes showed no detectable changes or they had mild to severe tissue damage. As in CCT eyes, the greatest amount of ciliary body damage occurred at the base of the processes, and the tips were spared. In some sections, only the PE was damaged, whereas in others, NPE and PE were disrupted totally (Fig. 4). Areas of damaged PE and NPE appeared more discrete than in CCT eyes. Vascular engorgement within the ciliary processes was obvious. Clumping of PE was present in areas of normal ciliary epithelium. Pigment dispersion and cystic formations, while diffusely present, appeared to be in smaller amounts than those seen in CCT eyes, although this was difficult to quantify. Hemorrhage, inflammatory cells, and necrosis were absent. The anterior chamber angle was normal. Electron microscopic findings were the same as those described for CCT eyes.

DISCUSSION

Many investigations on the effects of cyclodestructive surgery have been performed in the rabbit.16,14,22-25 This laboratory animal has a number of weaknesses that limit interpretation of the effects of cyclodestructive surgery. It is difficult to localize the ciliary body during surgery without damaging adjacent structures because the rabbit anterior segment is crowded by a large lens, and the ciliary processes extend onto the iris. Moreover, the rabbit blood–aqueous barrier is exceedingly fragile.27 In contrast, the cat ciliary body is identified easily because it extends 4 mm posterior to the corneal limbus,28 and its blood–aqueous barrier is less labile than the rabbit. Although the cat eye differs from the human eye (e.g., the human ciliary body is 1.5 mm posterior to the corneal limbus25,26), CCT11 and TSNYC10 have been shown to lower IOP in this animal. The current study confirms that both of these cyclodestructive modalities can reduce IOP for periods as long as 12 weeks after surgery (Table 1).

Our results demonstrate a similar IOP lowering effect of CCT and TSNYC in the cat. The IOP reduction is not associated with alterations in measured tonographic outflow facility or episcleral venous pressure. Calculated aqueous humor flow is significantly reduced 12 weeks after both procedures. Although flow and IOP are reduced in all the 3-week animals, only a small number of cats at this interval (three in each group) had satisfactory tonograms on both treated and control eyes, limiting the statistical, but not the clinical, interpretation of this time interval. The high rate of aqueous flow in our control cat eyes, compared to a normal rate of $\sim2.5 \mu l/minute in human eyes, has been reported by other investigators.31 Although inflow reduction can account for the measured IOP decrease, we did not evaluate uveoscleral outflow, which accounts for $<$3% of aqueous outflow in the cat.31 Enhanced uveoscleral outflow and IOP reduction have been reported after contact TSNYC of the pars plana, but not the pars plicata, in the monkey.15 Because the cat ciliary body is more than twice as large as the human ciliary body, most, if not all, of the cyclodestructive treatment in our study was administered to the pars plicata. Although it is possible that some treatment reached posteriorly to the pars plana, where disruption of the PE occasionally could be identified, there was no histopathologic evidence of enlargement of the suprachoroidal space in any of our specimens.

Total ciliary body blood flow in the cat is not altered 3 or 12 weeks after CCT or TSNYC. Thus, damage to the vascular supply of the ciliary body, with subsequent reduction in aqueous production caused by ischemia, cannot account for reduced IOP in our animals. Green et al12 reported decreased ciliary body blood flow in albino rabbits 1 week after 180° CCT. The treated ciliary body was compared to the untreated portion within the same eye. Reduced blood flow was measured in the treated portion, whereas blood flow through the untreated portion was almost twice that of normal. The difference in these results from our study may reflect animal (rabbit versus cat) and postoperative intervals (1 week versus 3 or 12 weeks).

The normal blood–aqueous barrier limits entry of proteins into the aqueous humor. In our study, CCT
and TSNYC result in similar physiologic alterations in the barrier and histopathologic injury to the ciliary epithelium. Breakdown of the barrier results in increased protein and enhanced entry of intravenously injected fluorescein in the anterior chamber. Specialized intercellular junctions (zonulae occludens) are thought to be responsible for maintaining the integrity of the blood–aqueous barrier and are located between cells of the NPE. Marked pigment dispersion seen on microscopic examination after CCT and TSNYC represents rupture of cell membranes and consequent disruption of the blood–aqueous barrier. Persistent elevation of aqueous protein and an increased fluorescein entry into the aqueous humor signifies that blood–aqueous barrier function is not restored 12 weeks after surgery. These findings are consistent with Quigley, who found altered pigmented cells that lacked basement membranes and intercellular junctional complexes 1 to 3 months after CCT in monkeys. It also correlates with the presence of chronic flare after CCT and TSNYC. Higginbotham and coworkers have found the blood–aqueous barrier in cats remains highly permeable to fluorescein 6 weeks after CCT. Lincoff et al measured increased anterior chamber protein in rabbits 1 year after CCT.

Our study does not address the long-term (>3 months) time course after cyclodestructive surgery, particularly the histologic and functional alteration in eyes that “recover from” surgery. There is conflicting evidence in the literature that recovery of IOP may relate to repair of the altered cells or to the increased functional role (e.g., aqueous humor production) of the remaining ciliary epithelium. It has been noted, experimentally and clinically, that eyes undergoing a greater extent of CCT or TSNYC are less likely to require repeat treatment to control IOP.

The current study did not find differences in the cat between CCT and TSNYC in the effect on IOP, aqueous humor dynamics, blood–aqueous barrier, and ciliary body blood flow. The delivery of discrete applications of focused laser energy to the ciliary body does not appear to have an advantage over CCT. Our conclusions are restricted to the evidence obtained from healthy experimental cats, and this may not necessarily apply to patients with glaucoma. Evaluation of the effects of cyclodestructive surgery by monitoring IOP alone may not be sufficient. The histologic disruption of the ciliary epithelium is consistent with the observed reduction in aqueous humor formation by active secretory processes decreasing IOP and with the measured breakdown of the blood–aqueous barrier, which could increase the rate of water entry into the anterior chamber by nonsecretory processes (e.g., ultrafiltration). The ultimate clinical IOP response will depend on the balance of these two effects and on the underlying status of the glaucomatous damage to the outflow pathways. The similar physiologic and anatomic alterations after CCT and TSNYC in the cat suggest that both techniques may have similar clinical effects regarding IOP reduction. This can only be determined in randomized clinical trials.

Key Words
aqueous humor dynamics, blood–aqueous barrier, cat, ciliary body blood flow, cyclocryotherapy, transscleral Nd:YAG cyclodestruction

References


29. Hampton C, Shields MB. Transscleral neodymium:YAG cyclophotocoagulation: A histologic study of


