content could partly be due to loss by proteolysis, and the evidence presented may suggest proteolysis due to increased enzyme activities in cataract lenses, with the exception of leucine aminopeptidase. This has also been shown in previous studies for human cataract lenses. However, rates of hydrolysis for arylamide derivatives for human cataract lenses far exceeded the rates in normal human lenses. It is entirely possible there may be a species-specific leucine aminopeptidase in the Cataract Resistant mouse lenses, Emory Mouse lenses, and human lenses. Another possible explanation for the low leucine aminopeptidase activities in the Emory Mouse cataract homogenates may be an age related inactivation or degradation of the enzyme because of structural alteration which may be responsible for the markedly diminished activity observed when compared to human cataract lenses.

A comparison of relative substrate specificities on Arg-Arg-2-NNap and Boc-Arg-Pro-2-NNap derivatives revealed additional apparent similarities between the human and mouse lens extracts.

Key words: cataract, proteases, mouse lens, Emory Mouse

From the Department of Biochemistry, Medical University of South Carolina, Charleston, South Carolina; and Emory University, Laboratory for Ophthalmic Research, Atlanta, Georgia. Supported by Grants from the National Eye Institute, EY-00429 and EY-00260, and in part by a Department Grant to Emory University from Research to Prevent Blindness, Inc. Submitted for publication: September 13, 1984. Reprint requests: A. A. Swanson, PhD, Department of Biochemistry, Medical University of South Carolina, 171 Ashley Avenue, Charleston, SC 29425.

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Cataract Induction in Rabbits with the Nd-YAG Laser

Glen Cangelosi, Marguerite B. McDonald, and Keith S. Morgan

The study of occlusion amblyopia and its therapy has involved animal models of stimulus deprivation achieved by various means, none of which closely simulates human congenital cataract. The authors used the Nd-YAG laser as a means of inducing cataracts in rabbit eyes. Twenty rabbit eyes were treated at various frequency and power settings. High energy YAG laser pulses of 10 mJ produced cataracts that at first resembled large bubbles. Over several days, these bubbles coalesced into smaller opacities. With multiple treatments (usually 150–200 pulses), the authors could create a cataract of a specific size and position. Six months later, 100% of these laser-treated rabbit eyes showed persistent cataracts that resembled human congenital cataracts. It appears that the YAG laser can provide a reproducible and reliable method of inducing specific types of cataracts in animal eyes without damaging other ocular structures.

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To assess the efficacy of various forms of surgical treatment and methods of aphakic correction in preventing or reversing amblyopia, it would be useful to have an animal model that closely simulates human congenital cataract. Various methods of inducing cataracts in animals have been tried in the past. These models have included lid closure, translucent plastic contact lenses, cycloplegics, sutured nictitating membranes, or "needling"-induced traumatic cataracts. However, all have demonstrated drawbacks that limit their effectiveness in mimicking human congenital cataract and the development of amblyopia.

This study was undertaken to demonstrate that the Nd-YAG laser could be used as a reliable tool in
producing a noninvasive means of inducing cataracts in the rabbit model. We are presently attempting to extend this procedure to the primate animal model and to evaluate the ability of the resulting lesions to mimic the human visual defect caused by congenital or acquired cataract.

Materials and Methods. Animal care and treatment in this investigation were in compliance with the ARVO Resolution on the Use of Animals in Research. Short acting mydriatics (tropicamide 1%, phenylephrine 2.5%) were instilled in both eyes of 10 albino rabbits (body weight, 5-10 kg). Photographic documentation of the clarity of the lenses was obtained. A topical anesthetic (proparacaine hydrochloride) was instilled in the eyes. For laser treatment, the rabbits were secured behind the Nd-YAG-100 model slit lamp (American Medical Optics; Irvine, CA) by means of restrictive wrappings, as well as an assistant’s firm grip.

The parameters of treatment were defined in terms of millijoules (mJ) per pulse, number of pulses, and focus of the laser. Various numbers of pulses (20-80) and power settings (10-15 mJ) were used in the initial session to create intralenticular lesions. All laser pulses were directed within the lens nucleus or the posterior capsule, depending on the type of cataract desired. Additional laser sessions were required to determine the number of lesions needed to obtain clinically significant cataracts. Photographs were taken immediately following treatment, and 1 day, 1 wk, 1 mo, 3 mo, and 6 mo later.

Results. The preliminary trials of high energy Nd-YAG pulses produced large bubbles in the lens nucleus during the procedure (Fig. 1); by retroillumination, the central lens was seen to be irregularly refractile (Fig. 2). One week later, the areas of the large bubbles were reduced to smaller, dense opacities (Fig. 3). Repeated applications of pulses (150-200) performed during two or three sessions were needed to induce cataracts of various positions and sizes. More sessions appeared to be necessary to produce larger and more dense cataracts, although no quantitative relationship was established. The time interval between sessions varied from 1 to 7 days, and had no apparent effect on the results of the laser treatment.

Higher energy pulses (15 mJ) yielded larger bubbles but not substantially larger areas of opacity than lower energy pulses (10 mJ). Higher energy pulses also increased the incidence of damage (rupture) to either the anterior or posterior lens capsule. Lower energy pulses (<10 mJ) resulted in lesions without significant “bubble” formation, and required numerous additional pulses to produce a cataract comparable to those created with higher energy pulses.

Six months following treatment, 100% of the laser-treated rabbit eyes showed persistent cataracts, stable in both configuration and density and comparable to human congenital cataracts (Fig. 4).

Discussion. The finding by Wiesel and Hubel that...
a brief period of monocular stimulus deprivation has considerable impact on the developing feline visual system has prompted much interest in the use of animal models for the study of amblyopia, and the timing of the critical period has been determined in kittens and in nonhuman primates. Although the critical period for humans has not yet been established, there is psychophysical as well as clinical evidence to indicate that the visual nervous system is extremely plastic until approximately 4–5 yr of age, during which time unilateral congenital cataract can be devastating to normal binocular visual development.

Numerous attempts have been made to induce lesions that simulate cataracts in young animals, in order to further the study of amblyopia; however, all of the previous methods for producing experimental amblyopia have some disadvantages. Translucent contact lenses may fall out or be removed by the animal, and such lenses can induce corneal epithelial defects or corneal ulcers if not fitted properly. Wiesel and Hubel used this technique to study the effects of visual deprivation in cats, and found it to be “not wholly satisfactory” as a result of lens-induced recurrent conjunctivitis. Suturing nictitating membranes may produce chronic irritation and the possibility of slippage or “recession” of the coverage. Cyclopegics have been used to create amblyopia, but do not affect distance visual acuity, and young mammals also show an uncanny resistance to “de-focusing” mechanisms. Von Noorden et al. used tarsorrhaphies to create amblyopia in monkeys, noting “vigorous effects of the mother animal to open the lids by licking the wound.” Needling-induced cataracts are subject to the risk of iatrogenic infection, uveitis, and glaucoma.

The laser method of inducing cataract described here confers on the investigator a relatively fine control of the configuration, as well as the timing, of the lesion. There may be some concern that the quantity of energy delivered to the eye could damage either the corneal endothelium or the retina; however, studies performed in animals have demonstrated no evidence of morphologic abnormalities in the endothelium of rabbit eyes that have undergone YAG laser surgery (capsulotomies). Also, Q-switched YAG
Lasers have been used successfully to cut vitreous membranes as close as 4 mm from the retina without damage, as determined by fluorescein angiography. The risk of retinal complications during laser lens surgery is minimized by the reduction in radiant energy as the diverging laser beam passes through the vitreous to the retina. The question of undesirable side effects of YAG laser treatment, such as transient or permanent increase in intraocular pressure, was not addressed in this study, as it was felt that intraocular pressure measurements in rabbit eyes would not necessarily be predictive of results in primate eyes. This work in rabbits was a very preliminary study to determine if lens opacities could be produced. Further work in nonhuman primates will include more extensive ocular examinations, including pre- and post-treatment intraocular pressure measurements.

Our laser technique can be used to create an isolated cataract with a large or small opacity, which opens the way for investigation into the role that the cataract itself plays in stimulus deprivation amblyopia. It should be possible to utilize this technique to study the amblyogenic effect of different size cataracts induced at various developmental stages in the nonhuman primate, as well as the effect of cataract removal followed by optical rehabilitation. The clinical implications of such studies would be of some importance, because there are conflicting opinions but little data concerning the efficacy of various forms of optical correction in children with congenital or traumatic cataracts.

**Key words:** cataract, rabbit, Nd-YAG laser, animal model, amblyopia

From the Lions Eye Research Laboratories, LSU Eye Center, Louisiana State University Medical Center School of Medicine, New Orleans, Louisiana. Supported in part by PHS grants EY-02580 and EY-02377 from the National Eye Institute, National Institutes of Health, Bethesda, Maryland. Submitted for publication: October 12, 1984. Reprint requests: Keith S. Morgan, MD, LSU Eye Center, 136 South Roman Street, New Orleans, LA 70112.

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