The Role of the Iris in Chick Accommodation

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PURPOSE. Peripheral defocus, higher-order aberrations, and accommodation interact with pupil size to influence retinal image quality and possibly eye growth. Iridectomy (ID) provides a fixed, enlarged pupil. Results from in vitro studies suggest that ID may reduce or eliminate accommodation in the chicken. This paper further investigates the effects of ID on chicken accommodation, eye growth, and refractive development.

METHODS. Refraction, biometry, and corneal curvature were measured, before, and after topical instillation of nicotine in 43 White-Leghorn chickens that had undergone monocular ID. Intraocular pressure (IOP) was measured, and eyes were imaged with anterior segment optical coherence tomography (OCT) during accommodation. In vitro preparations were used to examine accommodation responses in a lens-scanning instrument.

RESULTS. Iridectomy induced small but significant decreases in anterior and vitreous chamber depths and an increase in lens thickness (LT). IOP was similar in iridectomized and control eyes from 1 week on. In vivo, nicotine induced similar accommodative changes in iridectomized and control eyes. OCT images revealed a forward displacement of the iris during accommodation in control eyes. Iridectomized and control eyes showed similar increases in LT. In vitro, iridectomized eyes showed minimal nicotine-induced accommodation.

CONCLUSIONS. Refraction and eye growth were minimally affected by ID in chickens, implying that emmetropization was unaffected and supporting the use of ID as a tool in emmetropization and myopia studies. The greatly attenuated accommodative responses in vitro for iridectomized eyes suggest a role of biomechanical factors in the chick. IOP was unaffected by the surgery, implying that the iris musculature is not essential for maintaining aqueous outflow pathways. (*Invest Ophthalmol Vis Sci.* 2011;52:4710–4716) DOI:10.1167/iov.10.6819

Interest in manipulating optical aberrations and peripheral refractive errors as possible treatments for controlling myopia is driving ongoing research involving specially designed multifocal lenses in animal models for myopia. The present study used the chick, the most commonly used model, to investigate the ocular effects of iridectomy (ID), which induces a fixed dilated pupil. We have used ID in some of our studies to avoid the confounding effects of variations in pupil size,

which are known to influence the magnitude of higher-order aberrations and peripheral refractive errors, and thus retinal image quality.^{1–3} However, the effect of this surgery on ocular accommodation and intraocular pressure (IOP) in the chick remain unresolved, although changes in either or both of these parameters may introduce other confounding factors for the above lens studies.

The role of accommodation in the development of myopia and emmetropization is not fully understood. While the apparent association between excessive near work and myopia opened the possibility that excessive accommodation might be responsible, it has not proven to be a factor in form deprivation myopia induced with translucent goggles in chicks; histologic studies of ciliary body morphology show no evidence of change,⁴ and ciliary nerve section to eliminate accommodation does not prevent form deprivation myopia.^{5,6} Results from studies using negative lenses to induce myopia lead to a similar conclusion; neither Edinger-Westphal lesioning⁷ nor ciliary nerve section,⁶ both methods of eliminating accommodation, prevent lens (defocus)-induced eye growth changes. Nonetheless, in a binocular lens experiment in which one eye also underwent ciliary nerve section, eyes showed similar rates of ocular growth, even though the unlesioned eye would have been required to accommodate almost continuously for the animal to see clearly.⁶ This observation and another in which ciliary nerve section was found to alter the response to competing defocus stimuli⁸ leave open the question of whether accommodation plays a role in emmetropization.

The possibility that raised IOP may underlie the increased ocular enlargement seen in myopia also remains unresolved. In three studies of young children, higher than normal IOP has been reported for myopic eyes, but in one of these studies, the differences were observed only after the children became myopic.^{9–11} Results from the only two studies to address this question in chicks were also inconclusive. IOP was observed to initially decrease in eyes wearing either negative or positive lenses, although the faster-growing eyes showed a later relative increase in IOP.¹² However, in a related study,¹³ topical timolol, an IOP-lowering drug, failed to inhibit lens-induced myopic growth in young chicks.

The mechanism of accommodation in the avian eye varies from that of mammals in a number of important ways, and there are also differences between avian species. While mammals exhibit only lenticular accommodation, mediated by smooth muscle bearing muscarinic receptors, birds have both corneal and lenticular accommodation, mediated by skeletal muscle bearing nicotinic receptors.¹⁴ In the former case, only the lens surfaces undergo steepening; in the latter case, the cornea also steepens, contributing to the net increase in ocular refracting power. Young chicks can accommodate over 25 D through lenticular and corneal mechanisms.¹⁵

Among avian species, the iris musculature plays a variable role in accommodation. Evidence for iris-mediated lenticular accommodation in birds was first offered in 1853, when Cramer¹⁶ observed that accommodative lens movements were absent in iridectomized pigeons. In some diving birds, the anterior lens surface protrudes through the pupil of the iris

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during accommodation, providing convincing evidence for an accommodative role of the iris in these species. In the chick, Glasser et al.^{17,18} attributed accommodation-related changes in the curvature of the chick lens to the contraction of peripheral and oblique iris muscle fibers, based on anatomic observations and in vitro measurements of accommodation in response to electrical stimulation. In the latter studies,¹⁸ ID was found to eliminate lenticular accommodation, leading to their conclusion that the iris was necessary for accommodation in the chick. As a mechanism for accommodation, they proposed that contracting muscle fibers at the peripheral edge (root) of the iris applied force through the ciliary processes to the equatorial surface of the lens, thereby deforming it.

In the study reported here, the effects of ID on biometric and refractive parameters, nicotine-induced accommodation, and IOP were evaluated in vivo, and its effects on nicotine-induced accommodation were also evaluated using in vitro preparations from some of the same eyes. Some of these data have been reported in abstract form (Ostrin LA, Liu Y, Mok Yee J, Choh V, Wildsoet CF. *IOVS* 2010; 51:ARVO E-Abstract 1719).

METHODS

This study into the ocular effects of ID used 43 White Leghorn chickens, which were housed in a 12-hour light/dark cycle with food and water available ad libitum. Details of the number and age of birds used in each experiment in this study are summarized in Table 1. In brief, all chickens underwent monocular ID surgery, whose ocular effects were subsequently studied in terms of refractive development, ocular growth, IOP, and nicotine-induced accommodation. Both in vivo and in vitro studies of accommodation were undertaken. Methods were approved by the UC Berkeley Animal Care and Use Committee and conformed to the ARVO statement for the Use of Animals in Ophthalmic and Vision Research.

Monocular ID was performed under gaseous anesthesia (2% isoflurane in oxygen) on chicks aged between 8 and 32 days. A small (1.5 mm) temporal limbal incision was made with a sharp-tip keratome, the iris grasped at its base with microfine, flat-tipped forceps through the incision and 180° from the incision, gently detached from its root in one motion, taking care not to contact the anterior lens surface, and then drawn out through the incision. The anterior chamber was then irrigated with sterile PBS to remove any tissue debris and blood. The limbal incision was self-sealing. Immediately after the surgery and for the next 4 days, dexamethasone and vigamox were applied topically. The contralateral fellow eyes were not treated in any way and served as controls in all experiments.

In vivo measurements, which comprised streak retinoscopy, high-frequency A-scan ultrasonography, and corneal topography, were car-

ried out 5 days after surgery. Chicks were positioned on a custom-made stand with beak bar for both retinoscopy and high-frequency A-scan ultrasonography and were also anesthetized for the latter (1.5% isoflurane in oxygen). For corneal topography measurements, birds were hand held for alignment purposes. The youngest group of four 16-day-old birds did not undergo corneal measurements. Room lights were dimmed to a minimal level to control for accommodation. Both iridec-tomized eyes (IDEs) and fellow control eyes (CEs) of each bird were measured before and at timed intervals after the topical instillation of two drops of 0.4% nicotine solution. An additional drop was instilled 30 seconds later if no accommodative response or miosis (in CEs) was observed. The latter choice reflects the presence of nicotinic rather than muscarinic receptors on the intraocular muscles of the avian eye, which comprises skeletal rather than smooth muscle.¹⁹ Baseline values provided indices of the effects of the ID surgery alone, and the nicotine-induced changes provided indices of accommodation-related changes.

A tonometer (TonoLab; iCare, Espoo, Finland) was used to measure IOP in both eyes of individual chickens, 1 day before the surgery, as well as 1 hour, 24 hours, and 4 days postoperatively, and thereafter weekly for 4 weeks. The tonometer provided the average of the most similar four out of six IOP readings. Each data point represented the average of two such measurements per eye. A topical anesthetic was not necessary for these tonometer IOP measurements. The chickens were gently restrained but not anesthetized to avoid the IOP-altering effects of general anesthesia with ketamine^{20,21} or isoflurane.²²

In vivo imaging of the anterior ocular segments of some of the birds was undertaken using a noncontact optical coherence tomography (OCT) system (Visante, Zeiss, Dublin, CA), 1 week after their ID surgery. For imaging, birds were anesthetized with a combination of 15 mg/kg ketamine and 3 mg/kg xylazine, administered as an intramuscular injection. The birds were placed on the custom-built stand with beak bar to facilitate ocular alignment for imaging. Each eye was imaged both before and at 1 minute intervals over about 5 minutes after the topical instillation of two drops of 0.4% nicotine solution; the iridec-tomized eye was measured first, and then the bird was repositioned for imaging of the fellow eye. Custom software and calipers built into the instrument were used to measure anterior chamber depth and axial lens thickness (LT) from captured images, from which nicotine-induced changes were subsequently derived.

In vitro imaging of the anterior segments used a scanning laser system specifically designed for this purpose (ScanTox v. 2.0.154; XTOX Scientific, Napean, Ontario).²³ In brief, the instrument includes a chamber for mounting the anterior ocular segment, including the lens and attached ciliary processes and muscle, through which a low-power helium-neon laser beam is directed to pass axially through the chamber and crystalline lens of the mounted explant. A computer drives a stepper motor linked to the laser and to a video camera, which is used to capture images of the laser's path through the chamber. For measurement, the lens is initially centered by adjusting its location (*x* and *y* coordinates), until the laser beam passes through the lens without bending; this position corresponds to the optical axis of the lens. The position of the laser beam is then moved, in a stepwise manner under computer control, away from the optic axis along the diameter of the lens to the limit imposed by the pupil boundary. The step size was set to allow approximately 20 to 25 beams to pass through the pupil (0.1–0.16 mm step size, depending on the age of the chicken and size of pupil in the case of CEs). In this way, a set of beam traces is generated, from which the back vertex distance (BVD) of the lens is calculated. A computer program determines the point of intersection of the laser beam with the optical axis for all rays, and values for all rays are averaged to determine the BVD. Pupil size is derived from the number of beams passing through the lens and step-size setting. In vitro testing was carried out within 1 week of in vivo testing in all cases. Chicks were euthanatized by CO₂ asphyxiation, both eyes immediately enucleated and then submerged in oxygenated Tyrode's solution. To isolate the intact anterior segment for measurement, eyes were bisected at the equator, posterior to the ciliary processes. For

TABLE 1. Number and Age of Birds at Time of Measurements for Each Experiment

Group	Experiment	Group Size	Age (Days)
I	In vivo ocular development and accommodation	4	16
		15	22
		5	33
		5	45
		7	54
	In vitro accommodation (lens scanning)	4	18
		3	42
		5	19–22
II	In vivo OCT imaging	5	19–22
	In vivo IOP measurements	7	14–42

Monocular iridectomies were performed at least 5 days before measurements. IOP, intraocular pressure; OCT, optical coherence tomography.

TABLE 2. Biometric, Refractive Error, and Corneal Power Data for Iridectomized and Fellow Control Eyes of 36 Chicks, Measured 5–7 Days Postsurgery, Both before and after Instillation of Topical 0.4% Nicotine (mean $\pm$ SE)

Parameter	Eye	Baseline (No Nicotine)		After Topical Nicotine	
		Δ (IDE – CE)		Δ (IDE – CE)	
Anterior chamber depth, mm	IDE	1.63 $\pm$ 0.30	–0.09 $\pm$ 0.09*	1.50 $\pm$ 0.33	–0.03 $\pm$ 0.11*
	CE	1.72 $\pm$ 0.27	($P < 0.001$)	1.53 $\pm$ 0.29	($P < 0.05$)
Lens thickness, mm	IDE	2.62 $\pm$ 0.26	+0.06 $\pm$ 0.06*	2.85 $\pm$ 0.23	+0.02 $\pm$ 0.10
	CE	2.56 $\pm$ 0.26	($P < 0.001$)	2.83 $\pm$ 0.26	($P = 0.09$)
Vitreous chamber depth, mm	IDE	5.81 $\pm$ 0.69	–0.12 $\pm$ 0.18*	5.75 $\pm$ 0.70	–0.12 $\pm$ 0.20*
	CE	5.93 $\pm$ 0.62	($P < 0.001$)	5.88 $\pm$ 0.60	($P < 0.001$)
Retinal thickness, mm	IDE	0.24 $\pm$ 0.02	0.001 $\pm$ 0.01	0.24 $\pm$ 0.02	–0.01 $\pm$ 0.01*
	CE	0.24 $\pm$ 0.01	($P = 0.53$)	0.25 $\pm$ 0.02	($P < 0.001$)
Choroidal thickness, mm	IDE	0.32 $\pm$ 0.17	0.004 $\pm$ 0.07	0.31 $\pm$ 0.16	+0.03 $\pm$ 0.07*
	CE	0.32 $\pm$ 0.16	($P = 0.71$)	0.28 $\pm$ 0.16	($P < 0.01$)
Corneal power, D	IDE	93.64 $\pm$ 11.33	+0.28 $\pm$ 1.18	100.10 $\pm$ 15.53	+0.08 $\pm$ 2.39
	CE	93.33 $\pm$ 11.38	($P = 0.16$)	100.01 $\pm$ 14.67	($P = 0.84$)
Refractive error, D	IDE	+1.77 $\pm$ 0.77	–0.09 $\pm$ 0.93	–15.22 $\pm$ 8.40	–0.09 $\pm$ 1.90
	CE	+1.87 $\pm$ 0.64	($P = 0.57$)	–15.13 $\pm$ 7.33	($P = 0.78$)

Maximum (peak) values are shown. P derived from two-tailed paired t -tests; *significant result. CE, control eye; IDE, iridectomized eye.

imaging, the anterior segment was first placed cornea down on a Sylgard washer and the sclera pinned in place, before transferring the preparation to the silicon base mold that formed the bottom of the scanning laser chamber; finally the rectangular glass tube forming the sides of the chamber was placed in position and the chamber filled with 20 mL oxygenated Tyrode's solution with 5% fetal bovine serum added to allow visualization of the laser beam passing through the chamber. In all cases, preparations from IDEs were tested before preparation from CEs, which were maintained in Tyrode's solution infused with oxygen until measured. In this way, it was possible to rule out tissue degradation as an explanation for any observed reduction in accommodation in preparations from IDEs.

RESULTS

Effects of ID on Refractive Development, Ocular Growth, and IOP

Ocular measurements for the iridectomized eyes were averaged across all ages and compared with equivalent data for fellow CEs using the two-tailed paired t -test. Although the differences were small, iridectomized eyes had significantly shorter anterior chambers (-0.09 mm, $P < 0.001$) and vitreous chambers (-0.12 mm, $P < 0.001$) and thicker lenses ($+0.06$ mm, $P < 0.001$) compared with fellow eyes (Table 2). However, these interocular differences in axial dimensions were not reflected in significant differences in refractive error (-0.10 ± 0.98 D, $P = 0.57$). Interocular differences in corneal power also did not reach statistical significance at any age ($+0.31 \pm 1.25$ D, $P = 0.16$).

IOP data for IDEs and fellow CEs collected from a group of seven birds tracked over a 4-week period after the ID surgery are shown in Figure 1. IDEs exhibited an acute lowering in IOP, which returned to normal within the week after the surgery. Mean IOPs for IDEs were 19.9 ± 2.70 mm Hg before surgery but had dropped to 15.1 ± 2.6 and 16.1 ± 2.62 mm Hg, 1 and 24 hours after the surgery. The latter values were significantly lower than those of CEs, which recorded similar IOPs before and after the surgery (19.9 ± 3.62 mm Hg, $P = 0.93$; 20.4 ± 1.4 mm Hg, $P < 0.0001$; and 24.4 ± 2.0 mm Hg, $P < 0.0001$, at baseline and 1 and 24 hours postsurgery, respectively). This difference in IOP between IDEs and CEs had disappeared by 1 week after surgery.

In Vivo Nicotine-Induced Ocular Changes

Topical nicotine induced similar changes in vivo in both IDEs and CEs (Fig. 2A), reaching a peak approximately 2 minutes after the instillation of the last drop, with bigger variation observed in IDEs in terms of the onset of peak accommodation and the minimal dose required to elicit an accommodative response. Thus both eyes showed significant reductions in anterior and vitreous chamber depth (ACD, VCD) and increases in LT (Fig. 2B) after nicotine instillation. Peak values for ACD and LT for the two eyes were not significantly different between eyes (Table 2). Nonetheless, the nicotine-induced reduction in VCD was significantly greater for IDEs compared with CEs (-0.13 mm, $P < 0.001$). CEs showed a slight increase in retinal thickness (RT, $+0.005 \pm 0.009$ mm, $P < 0.05$) and decrease in choroidal thickness (CT, -0.04 ± 0.06 mm, $P = 0.05$) after instillation of nicotine, and while these changes are small, they introduced significant interocular differences at peak accommodation (RT, -0.01 mm, $P < 0.001$; CT, $+0.03$

FIGURE 1. Difference in mean intraocular pressure (IOP) ($\pm$ SE) from between the IDEs and CEs in seven chickens, measured before monocular ID, and at 1 and 24 hours postoperatively, and then at 1-week intervals for 4 weeks. *Significant decreases in ID compared with CEs ($P < 0.05$).

FIGURE 2. (A) Nicotine-induced total accommodation and corneal accommodation for IDEs and CEs with age. (B) Nicotine-induced changes in ACD, VC, and LT in the IDEs and CEs with age.

mm, $P < 0.01$). IDEs underwent a small significant decrease in retinal thickness (-0.002 ± 0.005 mm, $P < 0.05$) but no change in CT (-0.008 ± 0.04 mm, $P = 0.29$).

Nicotine-induced corneal steepening and myopic shifts in refractive error were observed at all ages in both IDEs and CEs. Changes in cornea and refractive error were not significantly different between IDEs and CEs (Tables 2 and 3; $P = 0.65$ and 0.64 , respectively). For both IDEs and CEs, the magnitude of these changes decreased with age (Table 3), and on average, corneal accommodation accounted for $37.4 \pm 6.4\%$ of the total

FIGURE 3. Anterior segment OCT images captured from the CE (A, B) and IDE (C, D) of one chick. (A) and (C) show the eyes in their unaccommodated state, whereas (B) and (D) show accommodating eyes, after the topical instillation of 0.4% nicotine.

accommodation-related change in refraction, although its contribution to accommodation was seen to decrease significantly with age only in IDEs ($F_{3,28} = 8.54$, $P < 0.0005$).

Similar to the trends evident in the A-scan ultrasonography data, OCT anterior segment imaging revealed IDEs to have significantly shorter ACDs compared with CEs (0.10 ± 0.06 mm, $P < 0.05$) and thicker lenses (Fig. 3), although the latter difference did not reach statistical significance (0.022 ± 0.04 mm, $P = 0.33$). Likewise, after the instillation of nicotine, ACD was reduced and LT increased in all eyes (Table 4), although here the recorded change in LT was significantly smaller in IDEs compared with CEs (-0.11 mm, $P < 0.005$).

In contrast to our findings in vivo, nicotine-induced changes were limited to anterior segments prepared from CEs in vitro. Representative traces for IDEs and CEs of a 6-week-old chick are shown in Figure 4. In this example, 15 minutes after exposure to 100 μ M nicotine solution, BVD had changed from 24.6 to 11.0 mm and pupil size from 3.78 to 1.6 mm in the CE preparation (Fig. 4A). The scans also revealed a change in spherical aberration from negative before exposure to nicotine to positive after exposure. The width of the equivalent scans from the IDE (Fig. 4B) was wider (4.14 mm), limited only by the lens perimeter in the absence of the iris. The iridectomized preparation also showed exaggerated negative spherical aber-

TABLE 3. Peak Ocular and Corneal Accommodation Induced In Vivo by Topical Nicotine Instilled into Both Iridectomized and Control Eyes of Chickens Aged between 22 and 54 Days

Age (Days)	Eye	Ocular (Total) Accommodation (D)	Corneal Accommodation (D)	Corneal Contribution (%)
22 ($n = 15$)	IDE	25.3 ± 1.4	10.7 ± 1.7	42 ± 7
	CE	>24	10.1 ± 2.5	<42
33 ($n = 5$)	IDE	12.3 ± 2.3	5.4 ± 1.8	43 ± 9
	CE	13.0 ± 3.2	5.2 ± 1.7	41 ± 12
45 ($n = 5$)	IDE	8.1 ± 0.8	2.6 ± 1.9	31 ± 23
	CE	10.9 ± 1.9	3.8 ± 1.9	33 ± 13
54 ($n = 7$)	IDE	5.6 ± 2.6	1.4 ± 1.0	16 ± 12
	CE	6.7 ± 2.5	2.6 ± 0.8	38 ± 7

Accommodation calculated as change after nicotine instillation, and corneal contribution represents corneal accommodation as percentage of ocular accommodation. CE, control eye; IDE, iridectomized eye.

TABLE 4. Nicotine-Induced Changes in Anterior Chamber Depth and Lens Thickness of Iridectomized and Control Eyes, Measured In Vivo by OCT Anterior Segment Imaging ($n = 5$) and High-Frequency A-Scan Ultrasonography ($n = 4$) in 17 Day-Old Chicks (t -Tests)

Eye	Δ ACD (mm)		Δ LT (mm)	
	OCT	US	OCT	US
IDE	-0.14 ± 0.08	-0.12 ± 0.06	$+0.18 \pm 0.03$	$+0.19 \pm 0.06$
CE	-0.19 ± 0.05	-0.22 ± 0.07	$+0.29 \pm 0.05$	$+0.27 \pm 0.04$
IDE - CE	$+0.05$ ($P = 0.33$)	$+0.1^*$ ($P < 0.05$)	-0.11^* ($P < 0.005$)	-0.08^* ($P < 0.05$)

ACD, anterior chamber depth; CE, control eye; IDE, iridectomized eye; LT, lens thickness; OCT, optical coherence tomography; US, ultrasonography.

ration. However, nicotine had little to no effect on BVD or spherical aberration of the iridectomized preparation.

Of seven pairs of eyes previously shown to respond with accommodation to nicotine in vivo, only preparations from CEs showed nicotine-induced accommodative changes in vitro. In vivo, the IDEs of four 18 day-old chicks exhibited greater than 25 D of accommodation and those of three 6 week-old chicks, 7.6 ± 0.5 D of accommodation, in response to topical nicotine, similar to the responses of CEs (>25 D and 9.25 ± 1.9 D, respectively). In contrast, iridectomized prepa-

rations showed minimal decrease in BVD in response to nicotine in vitro ($6.2 \pm 5\%$ compared with $41.5 \pm 20\%$ for fellow eyes; Fig. 5).

DISCUSSION

The near normal ocular dimensions and lack of difference in refractive errors of IDEs implies that emmetropization was not disrupted by surgical ID. The IDEs are compared with the CEs. While the refraction and biometric data were not measured for the two eyes before ID, young chicks show minimal natural anisometropia, and so no systematic interocular differences would be expected.^{24–26} Indeed, the lack of change in refractive error implies compensation for the small ID-related decrease of ACD and increase of LT. The latter changes also suggest that the iris exerts a small, albeit significant restraining influence on the unaccommodated lens in the chicken eye. The decrease in VCD in IDEs may reflect expansion of the lens into the vitreous chamber. These ID-related changes in axial dimensions in the chicken are similar to those that have been reported with total ID in rhesus monkeys.²⁷

In vivo, nicotine-stimulated accommodation was similar in IDEs and fellow CEs as measured with streak retinoscopy, A-scan ultrasonography and corneal topography, although decreased accommodation in IDEs is implied by ACD and LT derived from OCT images. However, differences in protocols more likely account for these differences in results. For OCT imaging, only one drop of 0.4% nicotine was instilled in IDEs, to avoid systemic interactions with drugs used for anesthesia

FIGURE 4. BVD plotted against pupil location for the CE (A) and IDE (B) of one chick before and after exposure to 100 μ M nicotine solution from an in vitro lens-scanning experiment.

FIGURE 5. Mean change in BVD after exposure to 100 μ M nicotine, plotted against time, for in vitro preparations from control (open symbols) and ID (closed symbols) eyes.

(ketamine and xylazine). In contrast, multiple drops of nicotine were used in other measurements, after observing the greater variation in the minimum effective dose required to elicit an accommodative response and the onset of peak accommodation in IDEs. We offer a pharmacokinetic explanation for the latter difference, specifically, that the pigment of the iris serves as a depot for sustained release of nicotine, which will drain more quickly from the anterior chamber of the IDEs that lack this depot than from CEs.

We found a temporary decrease in IOP after ID, which lasted approximately 4–5 days postsurgery. There are several possible explanations for why the IOP initially dropped. The reduction in IOP could have been due to leakage of aqueous through the surgical wound or to mild intraocular inflammation secondary to the surgery.^{28,29} It is possible that the removal of the iris in IDEs also reduced the mechanical resistance to aqueous outflow, although this effect would likely have been more enduring.

Consistent with previous *in vitro* lens-scanning studies in chick and pigeon, anterior segment preparations of IDEs showed little to no accommodation in response to nicotine, despite IDEs showing near normal accommodative responses *in vivo*, and preparations from fellow CE retaining accommodative responses *in vitro*. Note that changes in BVD reported here represent changes in lens power, because immersion of the cornea in the Tyrode's solution neutralizes its refracting power. Changes in the biomechanical properties of the anterior ocular structures involved in accommodation, after removal of the posterior vitreous chamber, seem the only plausible explanation for the lack of accommodation *in vitro* after ID. While we found no significant differences in IOP measured *in vivo* between CE and IDEs, 1 week and on postoperatively, around the time *in vitro* studies were undertaken, it is possible that *in vivo*, aqueous flow through the anterior chamber provides a mechanical restraining influence on the lens in IDEs, complemented by the action of the iris only in the control (intact) eye, and which remains as an influence *in vitro* for the latter only.

Based on results of similar *in vitro* studies involving IDEs, Glasser et al.¹⁷ attributed a role for the iris muscle in lenticular accommodation in the chick and pigeon. However, as we have shown in chicks, *in vivo* accommodation is minimally affected by ID, thus arguing against an active role for the iris in accommodation. Our OCT images of the anterior segment, captured during accommodation, also suggest that the iris is passively moved anteriorly, as the lens expands (Fig. 3B), rather than being actively involved in accommodation, as argued in previous studies.¹⁷

While our results suggest that the iris does not play an active role in lenticular accommodation in chicks, this finding does not rule out a role for the iris in accommodation in aquatic birds, which lack the benefit of corneal accommodation underwater. Consistent with species differences in the role of the iris in avian accommodation, it has been shown that some aquatic birds have enlarged iris musculature, which may better support a role in accommodation,³⁰ and some diving birds as well as penguins are reported to exhibit accommodation-induced anterior lenticonus,^{31–33} which has been attributed to the squeezing action of the peripheral iris sphincter on the lens. However, our OCT images from accommodating chicks show no evidence of anterior lenticonus.

West et al.³⁴ attribute lenticular accommodation in the chick to a molding influence on the anterior surface of the lens of the ciliary processes, resulting from contraction of the ciliary muscle. The possible role of intraocular pressure gradients is raised in another report³⁵ that proposes as a mechanism for accommodation in the chicken that contraction of the ciliary muscle stretches the choroidal coat and increases the pressure behind the lens. This model is similar to Coleman's theory of

accommodation in humans, which ties lens shape changes to a pressure gradient between the vitreous and aqueous compartments, generated by ciliary muscle contraction.³⁶ While these models are compatible with our *in vitro* results for IDEs, considered in isolation, they do not explain why CE retained accommodation *in vitro*. Instead, our results suggest any significant influence of pressure gradients may be limited to the anterior segment, perhaps generated through interactions between the iris and peripheral anterior lens surface.

Early literature is inconsistent on the existence of corneal accommodation in various avian species. Corneal accommodation was not present in pigeons or ducks during nicotine-induced accommodation,³¹ electrical stimulation in chickens,³⁷ or in behavioral studies in owls.³⁸ However, our results in chickens are consistent with results of more recent studies involving freely accommodating chickens³⁹ and nicotine-stimulated accommodation in chicks.⁴⁰ Corneal accommodation accounted for approximately 37% of overall accommodation in both CE and IDEs in our chickens, comparing favorably to equivalent figures around 40% reported by others.^{40,41} The magnitude of corneal accommodation decreased with age in both iridectomized and normal eyes, implying age-related changes in either or both corneal stiffness and ciliary muscle contractility in chicken eyes. It has been shown that corneal accommodation in chicks is mediated by the ciliary muscle.⁴¹ Given the relatively young age of the chickens in the present study, changes in corneal stiffness seem a more plausible explanation. Although the decrease of accommodative amplitude with age may also partly reflect corneal flattening during normal development, which reduces the dioptric influence of corneal steepening of similar magnitude, and hence its contribution to accommodation.

Total ID has been used to visualize the zonules, ciliary processes, and lens equator during accommodation in primates.^{42–44} In contrast to our findings that ID had no significant effect on nicotine-induced accommodation in the chicken, *in vivo*, total ID has been shown to cause a decrease in nicotine-induced accommodation in primates.²⁷ Similar to results reported here in chickens, the resting corneal curvatures were not significantly altered in iridectomized monkey eyes, while the anterior chambers were shallower. However, results of pharmacological studies in iridectomized monkey eyes were inconsistent; carbachol-induced accommodation was reduced by 40% in IDEs compared with contralateral CE,²⁷ whereas pilocarpine-induced accommodation was unaffected by ID. Nonetheless the investigators interpreted the decreased response to carbachol in iridectomized eyes as evidence for an iridial component to primate accommodation, limited to conditions involving extreme contraction of the pupil; they suggested that constriction of the iris sphincter pulled the ciliary muscle forward, thereby increasing accommodation. As in the present study of chickens, these authors also did not observe iridogenic anterior lenticonus during accommodation in their monkeys.

Overall, our results provide new insights into the mechanism of accommodation in the chicken and support the use of ID as a tool for enlarging the pupil in emmetropization and myopia studies using the chicken model.

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