In Vitro Changes in Back Vertex Distance of Chick and Pigeon Lenses: Species Differences and the Effects of Aging

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We have used a scanning laser technique to measure *in vitro* changes in back vertex distance of chick and pigeon lenses. Enucleated eyes were dissected, leaving the lens naturally suspended by the ciliary body and intraocular muscles. Ray tracing techniques were used to measure the resting back vertex distance of the lenses by passing a laser beam through the lens and scanning it across the pupillary aperture. The pupil diameter was measured videographically. The measurements were repeated while the intraocular muscles were pharmacologically stimulated with increasing concentrations of either nicotine or carbachol. Drug stimulation caused changes in pupil diameter and changes in the back vertex distance of the lenses. These experiments were conducted on the eyes of young chicks, young pigeons, and on the eyes of three pigeons older than 10 yr. The lenses from the eyes of the old pigeons had the greatest resting back vertex distance, followed by those of the young pigeons and finally those of the young chicks. Lenses from the eyes of young chicks and young pigeons underwent similar drug-induced changes in back vertex distance, but the lenses from old pigeon eyes showed an almost complete absence of such changes. Further, we demonstrated that, just as in the chick eye, lenticular changes in pigeon eyes are due to a contraction of the iris muscle. This is evident because after the iris has been removed the lens undergoes no change in back vertex distance during stimulation. We conclude with a discussion of the lenticular accommodative ability of the pigeon eye with reference to the recently reported accommodative mechanism of the chick eye and a comparison of chick and pigeon iris morphology.

Lenticular accommodation   Scanning laser   Spherical aberration   Nicotine   Carbachol   Presbyopia

INTRODUCTION

The mechanisms and extent of lenticular accommodation in birds has been the subject of extensive investigation since Philip Crampton (1813) first described the ciliary muscles of the avian eye. It has since been reported that birds exhibit a wide range of accommodative mechanisms and abilities (Cramer, 1853; Müller, 1857; Beer, 1893; Gundlach, Chard & Skahen, 1945; Goodge, 1960; Walls, 1967; Levy & Sivak, 1980; Schaeffel & Howland, 1987; Glasser, Troilo & Howland, 1994; Glasser, Murphy, Troilo & Howland, 1995). Few studies, however, have systematically described the accommodative range of different bird species or made direct comparisons.

Chicks and pigeons are among the most commonly used species in studies of avian vision, yet their accom-

modative abilities remain unclear. Gundlach et al. (1945) measured a maximum of 19 D of drug-induced accommodation in pigeons but made no attempt to identify the corneal and lenticular contribution. A more recent study showed that chicks have roughly 7 D of lenticular and 8 D of corneal accommodation, and that pigeons have only about 9 D of accommodation, almost all of which is corneal in origin (Schaeffel & Howland, 1987). Other studies on accommodation in either chicks or pigeons have both supported and challenged these values. Edinger–Westphal (EW) stimulation and topical application of nicotine sulfate in chicks produced about 6 and 9 D of accommodation respectively (Troilo & Wallman, 1987). We have previously measured up to 25 D of EW-stimulated accommodation in chicks, which included up to 9 D of corneal accommodation (Glasser et al., 1994, 1995). EW stimulation in pigeons has been used to produce up to 7 D of accommodation (Erichsen, Hodos & Kurkjian, 1993).

Among all the visual requirements of birds, feeding presents perhaps the greatest accommodative demand. For example, aquatic birds such as the cormorant have a strong accommodative range to overcome the loss

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in optical power that the eye undergoes while the birds feed under water (Hess, 1909). On the other hand, those birds which do not have visually demanding feeding behaviors frequently have little or no accommodation. Owls, for example, which are known to be able to capture their prey in the dark using auditory cues alone (Payne, 1971), have been shown to generally have a limited range of accommodation (Murphy & Howland, 1983; Howland, Howland, Schmid & Pettigrew, 1991). This might suggest that granivorous and insectivorous birds, which feed by pecking at objects on the ground, have similar accommodative abilities. However, chicks and pigeons, which have similar feeding habits and similar eye morphology, are thought to have very different accommodative abilities. Chicks have been shown to accommodate up to about 25 D using both the cornea and the lens (Glasser et al., 1994, 1995), but previous studies show pigeons to have only about 7–9 D of accommodation (Schaeffel & Howland, 1987; Erichsen et al., 1993).

Little is known about the effects of aging on accommodation in birds. An age-related reduction in accommodative range has been reported in only one study on chicks (Sivak, Hildebrand, Lebert, Myshak & Ryall, 1986). In pigeons, however, there is only the suggestion of mild presbyopic effects with aging (Hodos, Miller & Fite, 1991). Here we have used an *in vitro* technique to make a direct comparison of lenticular accommodation in chick and pigeon eyes and to show presbyopic changes in aged pigeon eyes.

Electrical stimulation has been used to induce accommodation in · excised bird eyes while measuring the changes in focal length of the lens (Sivak, Hildebrand & Lebert, 1985; Sivak et al., 1986). Because the iris and ciliary muscles of bird eyes are predominantly striated and contain nicotinic receptors, contractions of the iris muscle and accommodation can be induced by the application of nicotine (Pilar & Vaughan, 1969; Pilar, Nunez, McLennan & Meriney, 1987; Troilo & Wallman, 1987). We have previously used electrical and nicotine stimulation to induce accommodation in excised chick lenses while measuring changes in back vertex distance with a scanning laser device (Glasser et al., 1995). In that study, we measured stronger changes in back vertex distance with nicotine sulfate stimulation than with electrical stimulation (Glasser et al., 1995). Here we have used drug stimulation over a range of increasing concentrations to ensure that the strongest possible responses are induced and to compare the sensitivity of chick and pigeon irises over the entire range. Because the chick iris is known to contain smooth muscle fibers (Nishida & Sears, 1970; Scapolo, Pierone, Filogamo & Veggetti, 1988), and because it has been shown to have a non-nicotinic contractile response (Pilar et al., 1987), we have also used the acetylcholine analog, carbamylcholine chloride (carbachol), to stimulate chick eyes. We wished to test if carbachol could cause an increase in lens power without causing pupillary constriction.

The ability to induce and measure changes in back vertex distance in excised eyes has provided a powerful tool for understanding avian accommodation. We have used it here to show that pigeon lenses undergo changes in back vertex distance similar to chick lenses. We have also used this technique to demonstrate that lenses from the eyes of three pigeons, each more than 10 yr old, do not undergo significant changes in back vertex distance with pharmacological stimulation. This provides evidence for presbyopic-like changes in pigeon eyes with aging. A preliminary report of these results has appeared elsewhere (Glasser & Howland, 1994).

MATERIALS AND METHODS

Subjects

Eight 4-week old Cornell K-Strain White Leghorn chicks, one 3-month old, two 6-month old, and three > 10-yr old (16, 11, and 10 yr old) racing pigeons were used. Chicks were housed in 12/12 hr light/dark cycle from day 1 with *ad libitum* food and water. Pigeons were obtained from local racing pigeon owners and were housed in 12/12 hr light/dark cycle with *ad libitum* food and water until used.

Ocular dissections

All birds were euthanized with an overdose of inhalant ether. The eyes were immediately enucleated and maintained in oxygenated Tyrode's solution (Pilar & Tuttle, 1982). The corneo-scleral border of the eye was glued (cyanoacrylate, Krazy Glue) to the edge of a bevelled hole in a 2 × 2 cm square, $\frac{1}{8}$-in. thick plexiglas plate. The cornea protruded through the hole, and the posterior segment of the eye remained behind the plate. The eye could easily be dissected by clamping the plate in a dissecting dish filled with Tyrode's solution.

The cornea was removed by first making an incision with a scalpel blade and then cutting circumferentially around the cornea at the corneo-scleral border using iridectomy scissors. The posterior segment of the globe (the sclera, retina, choroid, and vitreous) was removed by making a circumferential cut through the sclera at the equator of the eye. Any remaining vitreous gel was removed from the posterior ciliary body and lens by gently lifting it out with fine forceps. The remaining eye preparation, with the lens suspended by the iris and ciliary body, remained attached to the plexiglas plate. Neither the lens nor any of the intraocular muscles were affected by the dissection. Contractions of the iris remained viable for up to 2 hr under ideal conditions.

Scanning laser measurements

The eye preparation, still attached to the plexiglas plate, was transferred to the scanning laser apparatus described previously (Glasser et al., 1995). The eye preparation was settled horizontally with the iris facing upward in a glass chamber filled with Tyrode's solution (Fig. 1). A video camera, attached to a frame grabber board in a personal computer (PC), was mounted above the eye preparation so the iridial aperture could be viewed on the monitor. The camera magnification was

calibrated, and the pupil diameter was measured on the PC by drawing a circle to fit the pupil. The scanning laser apparatus, also under PC control, was used to measure the back vertex distance of the lens (Glasser *et al.*, 1995). The PC was programmed to first locate, compute, and record the slope and intercept of a laser beam (helium–neon, 633 nm) passing undeviated through the optic axis of the lens. The PC then moved the laser to the edge of the pupil and scanned it across the pupil. During the scan, at a frequency of roughly 2 Hz, a frame was grabbed, the laser beam was located in the frame, and the slope and intercept of the laser beam passing through the lens was computed and stored to disk.

To determine the back vertex distance of the lens, a second computer program calculated the intersection point of each laser beam with the optic axis. All inter-section points were averaged to produce a mean focal point. Since laser beams with small angles of deviation from the optic axis often intersected the optic axis unusually close to or far from the lens, the mean could be arbitrarily distorted in one direction or another. Therefore, the mean focal point was *not* used to calculate the back vertex distance. Instead, the point used was that of best image focus, i.e. the point where the distance of separation of all laser beams that cross the optic axis (i.e. the "waist" of the rays) is at a minimum. This point
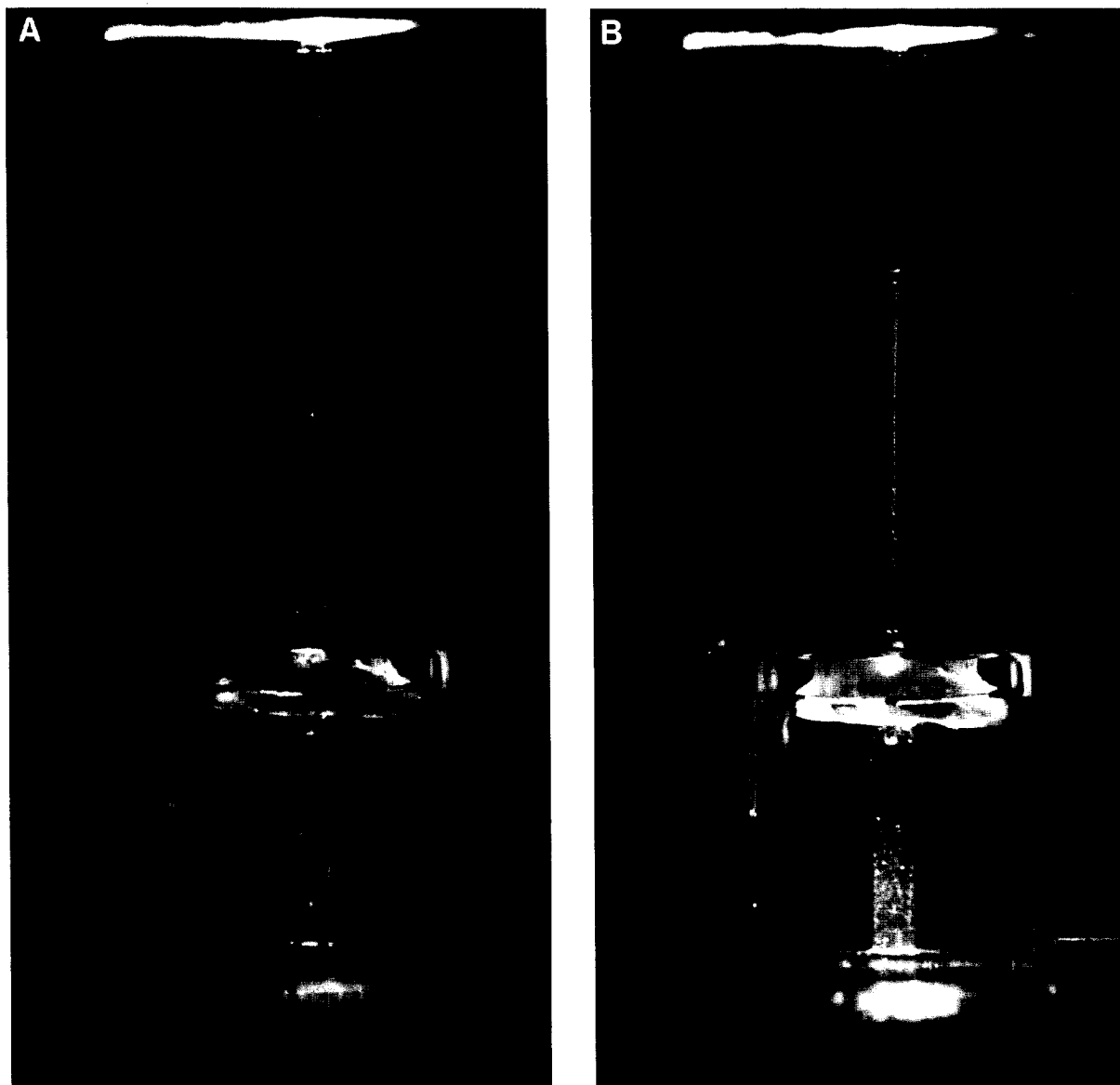

FIGURE 1. Long exposure photographs of the glass chamber of the laser scanning apparatus housing the eye preparation while relaxed (A) and while stimulated (B). The eye preparation is mounted horizontally in the glass chamber. The parallel laser beams pass up through the base of the glass chamber filled with Tyrode's solution, pass through the lens, and are refracted and drawn to a focal point above the lens. To obtain these photographs, a long exposure photograph was taken lasting the entire duration of a single laser scan across the pupillary aperture. The 35 mm camera was placed in front of the glass chamber to provide the same view that is usually seen by the video camera. (A) Note that almost all the rays that pass up through the base of the lens also pass through the dilated pupillary aperture. (B) Note that in the stimulated state, the constricted pupil reduces the number of rays passing up through the lens to just the paraxial rays and that the focal point is moved visibly closer to the lens. The glass chamber is 2.9 cm wide.

was located by scanning along the optic axis from a given distance before the mean focal point to a given distance after the mean focal point. At each point along the optic axis, the two most peripheral laser beams were found, and the distance between them was computed. The shortest distance of separation over the considered range of the optic axis provided a measure of the best focus of all the laser beams. The distance between this point and the posterior surface of the lens was then used to represent the back vertex distance. This method proved more reliable than using the mean intersection point of all laser beams with the optic axis. Further, the method we used represents the plane of minimum point spread.

Drug treatments

An outlet tube was connected to the base of the glass chamber of the scanning laser apparatus via an electronic valve. This mechanism allowed solutions of one drug concentration to be quickly drained from the chamber and replaced with another of higher concentration. The pupillary aperture and the back vertex distance were measured for nicotine sulfate (Sigma) or carbachol (Sigma) concentrations of 0, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5}, 10^{-4} and 10^{-3} g/ml. After the pupil diameter and back vertex distance were measured at each concentration (requiring about 3 min), the working solution was drained and the next higher concentration of oxygenated solution was poured into the glass chamber. The chick irises were observed to respond to the drugs immediately, so measurements were begun as soon as the solutions were replaced. The pigeon irises, however, showed a somewhat delayed reaction. Therefore the measurements were delayed for 2 min until the drug had time to act. Once measurements were completed for the entire drug concentration series (roughly 30 min), the first eye preparation was removed, the chamber was rinsed thoroughly with fresh Tyrode's solution, and the experiment was repeated on the second eye preparation.

Spherical aberration measurements

The avian lens has been shown to exhibit negative spherical aberration (Sivak, Ryall, Weerheim & Campbell, 1989). We, therefore, analyzed our back vertex distance measurements to get some idea of the spherical aberration of the lenses. When the eye was

unstimulated and the pupil was dilated, 20–25 laser beams passed through the pupil, but when the eye was maximally stimulated and the pupil constricted, only about 10 laser beams passed through the pupil. These 10 central laser beams that represent the paraxial rays intersect the optic axis closer to the lens than do the more eccentric laser beams due to the negative spherical aberration of the avian lenses. With that in mind, we first computed the mean back vertex distance of the lenses using all the laser beams passing through the pupil (to give the *total back vertex distance*) and again using only the paraxial laser beams (to give the *paraxial back vertex distance*). For the dilated pupil, the difference between these two distances represents a measure of the negative spherical aberration of the lens.

Data presentation

For each eye, the pupil measurements and scanning laser measurements were made three times for each drug concentration. The mean pupil diameter, mean total back vertex distance, and mean paraxial back vertex distance were then calculated from the three measurements and expressed in mm. For each group of animals, the means obtained for each eye at each drug concentration were averaged and expressed as a mean and a SE. To compare the results among the three animal groups tested, the resting (unstimulated) mean for each group was normalized to 100%, and subsequent drug concentrations were then expressed as a percentage change from the resting state.

Specific experiments

Pupil diameter and lens back vertex distance were measured in the eyes of five 4-week old chicks, two 6-month old pigeons, and three >10-yr old pigeons before and after stimulation with increasing concentrations of nicotine. Similarly, pupil diameter and lens back vertex distance were measured in the eyes of three 4-week old chicks before and after stimulation with increasing concentrations of carbachol. It has previously been shown that lenticular accommodation in chicks is mediated by the iris (Glasser et al., 1995). To demonstrate that this is also true in pigeons, we measured the back vertex distance in two iridectomized pigeon eyes that were stimulated using increasing concentrations of nicotine. First, with the iris intact, the back vertex

FIGURE 2 (facing page). Computer analysis of the laser scanning data of a chick lens (A) relaxed and (B) stimulated with 10^{-5} g/ml nicotine. The computer program reads in the slopes and intercepts of the laser beams passing through the lens (towards the left of the diagram), reconstructs them (horizontally), then calculates the mean focal point and the best convergence point of all the rays. From this information, the computer calculates the total back vertex distance and the paraxial back vertex distance (for details see text). The vertical dotted lines represent the horizontal scale in mm. The magnification in the vertical axis has been increased 10-fold to improve the resolution of the diagrams. (A) In the unstimulated condition, 22 rays pass through the pupil to give a total back vertex distance of 33.50 mm and a paraxial back vertex distance of 26.50 mm. The solid dots are positioned at the x, y coordinates corresponding to the horizontal position where each ray intersects the optic axis and the vertical position where the ray exits the lens. The curve described by the dots demonstrates the spherical aberration of the lens with the paraxial rays intersecting the optic axis closer to the lens than the more eccentric rays. (B) When the eye is stimulated, only 17 beams pass through the constricted pupil, and the focal length of the lens is reduced to give a total back vertex distance of 22.50 mm and a paraxial back vertex distance of 25.17 mm. Note the disruption of the negative spherical aberration of the lens during stimulation.
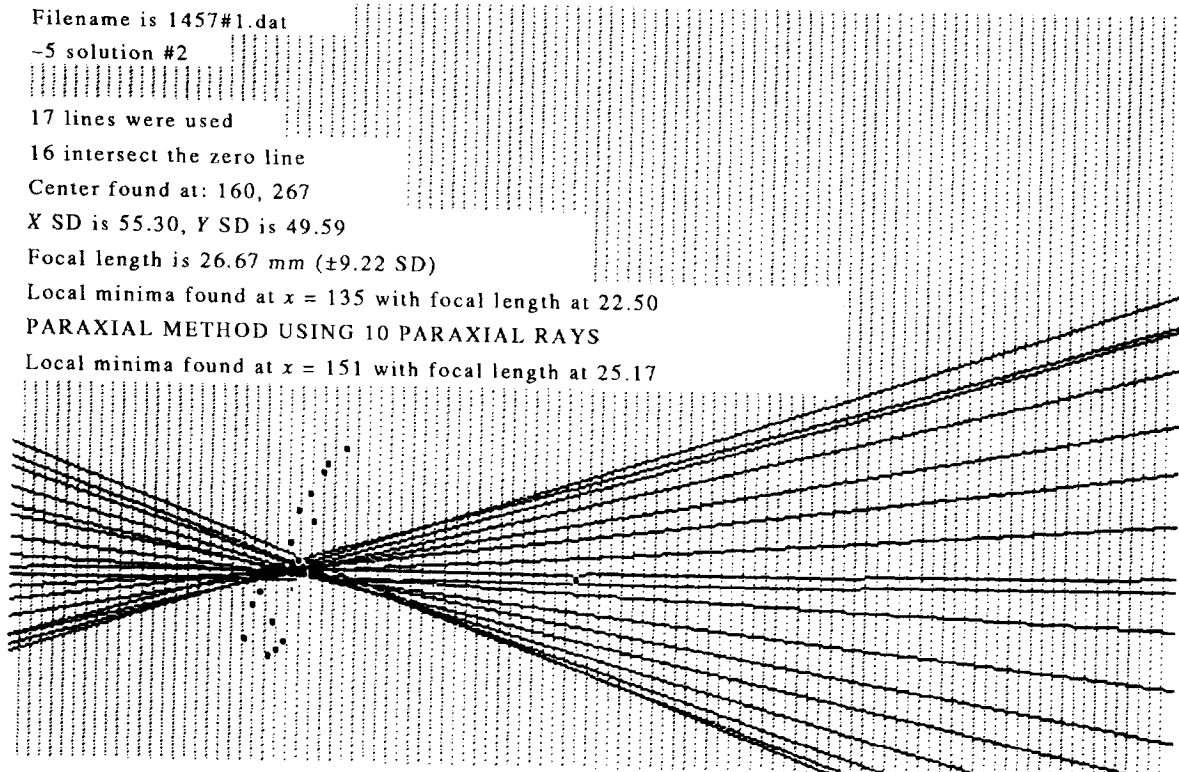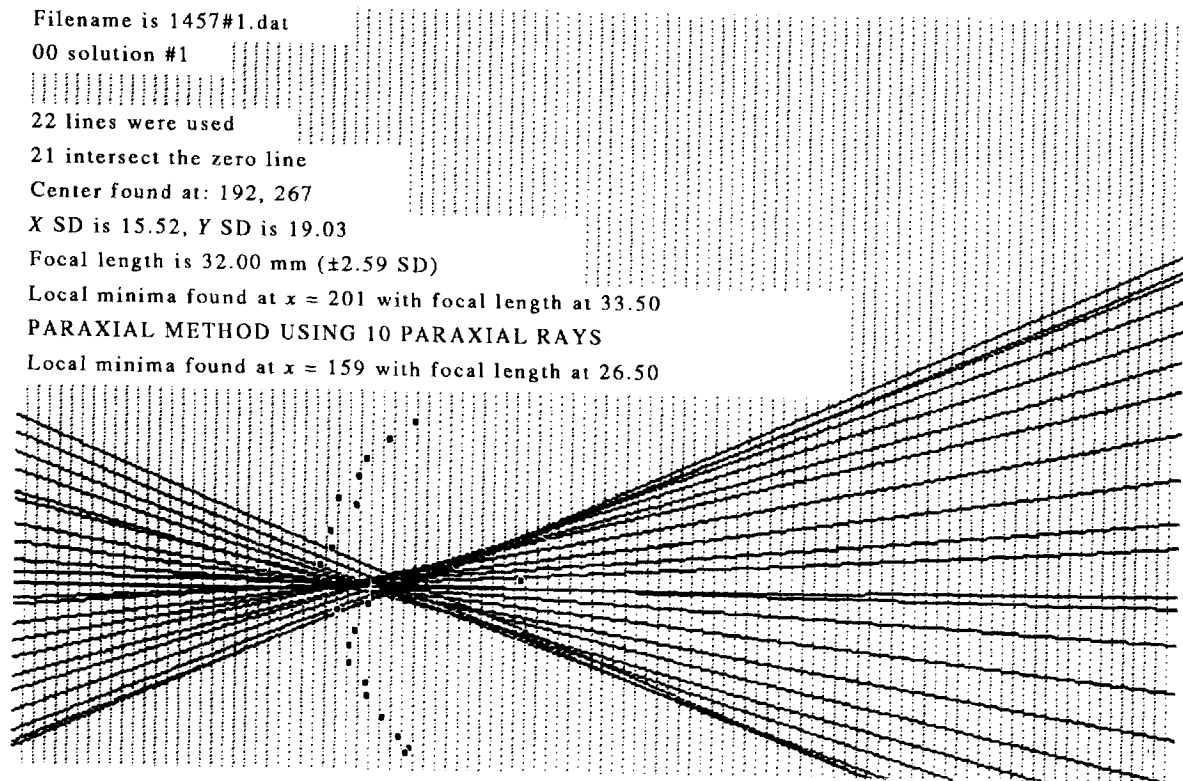
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00 solution #1

22 lines were used

21 intersect the zero line

Center found at: 192, 267

X SD is 15.52, Y SD is 19.03

Focal length is 32.00 mm (±2.59 SD)

Local minima found at $x = 201$ with focal length at 33.50

PARAXIAL METHOD USING 10 PARAXIAL RAYS

Local minima found at $x = 159$ with focal length at 26.50

Filename is 1457#1.dat

−5 solution #2

17 lines were used

16 intersect the zero line

Center found at: 160, 267

X SD is 55.30, Y SD is 49.59

Focal length is 26.67 mm (±9.22 SD)

Local minima found at $x = 135$ with focal length at 22.50

PARAXIAL METHOD USING 10 PARAXIAL RAYS

Local minima found at $x = 151$ with focal length at 25.17

FIGURE 2. *Caption opposite.*

distance was measured in the unstimulated eyes. The irises were then dissected from the eyes and the back vertex distance was again measured during nicotine stimulation for the range of increasing concentrations. The irises were removed by holding the pupillary edge with fine forceps while using fine iridectomy scissors to cut circumferentially around the peripheral ciliary margin of the iris. This procedure completely removed the iris without affecting any of the other intraocular tissues.

Histology

In order to examine and compare the accommodative apparatus of chick and pigeon eyes, histology was done on 10 eyes of 4-week old chicks euthanized for the purpose and on the same four eyes of the 6-month old pigeons used for the scanning laser measurements. Chicks were euthanized with an overdose of inhalant ether, the eyes were enucleated, the corneas and posterior segments removed as previously described, and the anterior segment preparation placed in chilled 4% glutaraldehyde fixative overnight. Due to the relative scarcity of pigeons, it was necessary to do histology on the same pigeon eyes that had been used for the scanning laser measurements. After the scanning laser measurements were completed, the eye preparations were carefully removed from the plexiglas plate, washed for 5–10 min in fresh Tyrode's solution, and then placed in chilled 4% glutaraldehyde fixative overnight. All pigeon and chick tissues were then decalcified by soaking in 10% EDTA for 3 days, dehydrated to 95% ethanol, embedded in methacrylate (Polaron), sectioned at 10 μm, and stained with basic fuchsin/methylene blue.

RESULTS

In vitro changes in back vertex distance

As demonstrated previously on the eyes of waterfowl (Sivak et al., 1985), our results show that it is possible to induce significant changes in focal length of chick and pigeon lenses in vitro. As illustrated in Fig. 2, there is a distinct decrease in the back vertex distance of the lenses with drug stimulation and a reduction in the number of laser beams passing through the constricted pupil. The presence and extent of the negative spherical aberration is immediately evident in the unstimulated lenses, but it is usually disrupted during stimulation.

Nicotine stimulation of young chick eyes

As shown in Fig. 3(A, B), the resting total back vertex distance (paraxial plus eccentric laser beams) of the 10 4-week old chick lenses was 30.27 mm. In the unstimulated condition (0 g/ml nicotine), a 5.27 mm negative spherical aberration is seen by comparing the paraxial back vertex distance measurements and the total back vertex distance measurements. Chick eyes had a resting pupil diameter of 3.08 mm. At 10^{-8} and 10^{-7} g/ml, the total back vertex distance, paraxial back vertex distance, and pupil diameter all showed a tendency to increase from the rest condition, although

the change was not significant. Chick eyes responded with a decrease in pupil diameter and a decrease in back vertex distance at between 10^{-7} and 10^{-6} g/ml of nicotine with a maximal response at 10^{-5} g/ml. This change resulted in a 13.88% (paired t-test: $t = 4.33$, $P < 0.05$) decrease in paraxial back vertex distance, a 27.16% (paired t-test: $t = 7.68$, $P < 0.05$) decrease in total back vertex distance, and a 38.64% (paired t-test: $t = 6.77$, $P < 0.05$) decrease in pupil diameter from the rest state.
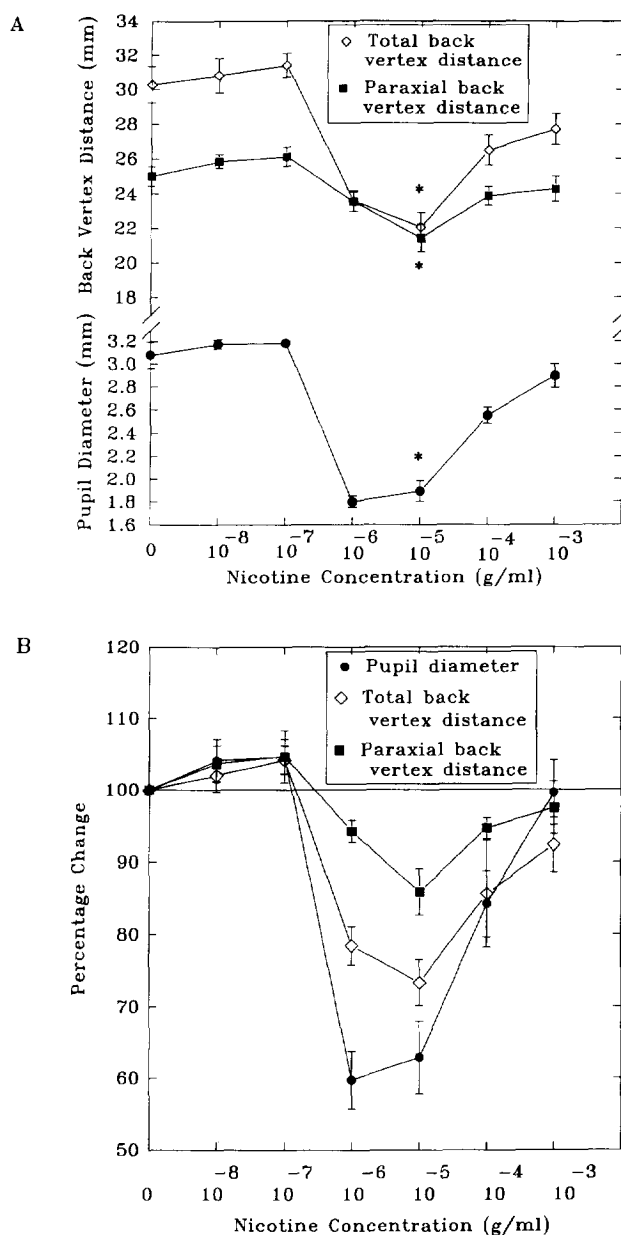

FIGURE 3. (A) Back vertex distance and pupil diameter measurements of 10 young chick lenses stimulated with increasing concentrations of nicotine and (B) the same data plotted as a percentage of the unstimulated values (0 g/ml nicotine). The data show that, with respect to the unstimulated condition, all three measures initially increase at the lower concentrations of nicotine stimulation, followed by a decrease at between 10^{-7} and 10^{-6} g/ml. At higher nicotine concentrations, the three measures begin to return to their pre-stimulated state as the iris muscle begins to relax at supramaximal doses of nicotine. (*Significance from the unstimulated state, paired t-test. Total back vertex distance, $t = 7.68$, $P < 0.05$; paraxial back vertex distance, $t = 4.33$, $P < 0.05$; pupil diameter, $t = 6.77$, $P < 0.05$.)

A $> 10^{-5}$ g/ml nicotine concentration became supramaximal and caused the intraocular muscles to die and the pupil diameter and back vertex distance to return towards their rest condition.

Nicotine stimulation of young pigeon eyes

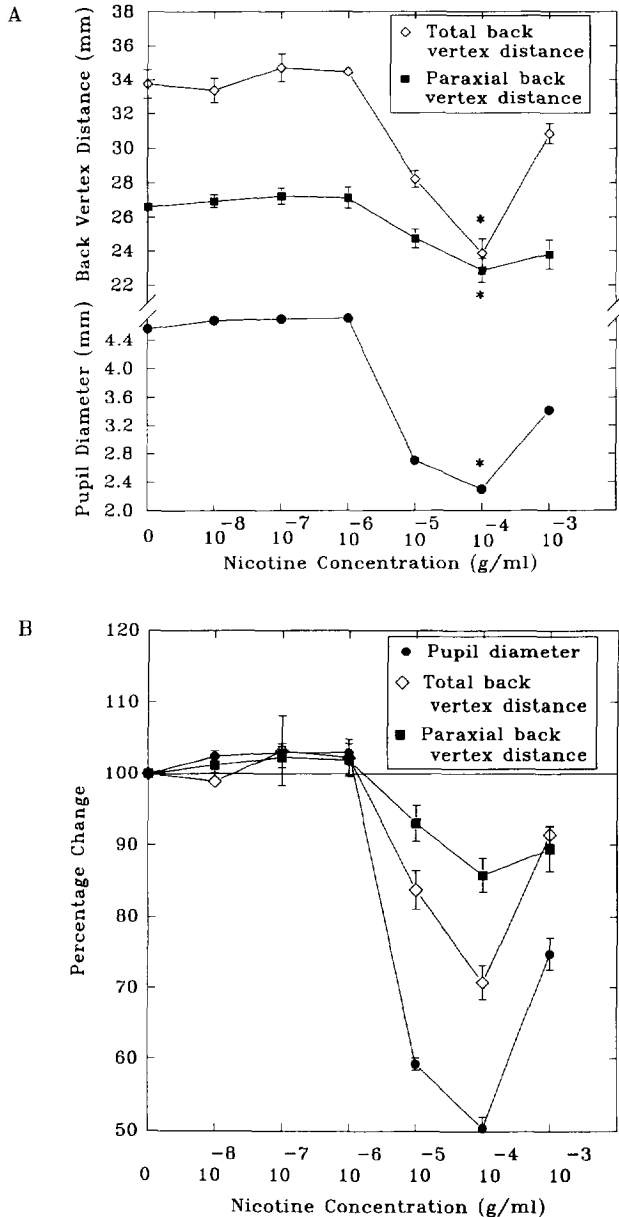As shown in Fig. 4(A, B), the total resting back vertex distance of 33.75 mm for the four 6-month old pigeon

FIGURE 4. (A) Back vertex distance and pupil diameter measurements of four young pigeon lenses stimulated with increasing concentrations of nicotine and (B) the same data plotted as a percentage of the unstimulated values (0 g/ml nicotine). Unlike in the young chick eyes, there is no effect of nicotine stimulation at the lower concentrations. All measures show a decrease at between 10^{-6} and 10^{-5} g/ml, reflecting the reduced sensitivity of the pigeon irises to nicotine stimulation. At higher nicotine concentrations, the three measures begin to return to their pre-stimulated state as the iris muscle begins to relax at supramaximal doses of nicotine. (*Significance from unstimulated state, paired t-test. Total back vertex distance, $t = 10.67$, $P < 0.05$; paraxial back vertex distance, $t = 6.21$, $P < 0.05$; pupil diameter, $t = 22.46$, $P < 0.05$.)

lenses was greater than that of the chick lenses. Similarly, the resting pupil diameters for the pigeon eyes (4.56 mm) was greater than that of the chicks. In the unstimulated condition (0 g/ml nicotine), the pigeon lenses showed a greater degree of negative spherical aberration (7.14 mm difference between paraxial and total back vertex distance) than the chick lenses. Pigeon eyes responded at higher concentrations of nicotine (10^{-6}–10^{-5} g/ml) than the chick eyes with a maximal response at 10^{-4} g/ml. A 14.09% (paired t-test: $t = 6.21$, $P < 0.05$) change in paraxial back vertex distance and 29.24% (paired t-test: $t = 10.67$, $P < 0.05$) change in total back vertex distance of the pigeon eyes were similar to those of the chicks. The pigeon pupil diameter underwent a greater constriction [49.56% (paired t-test: $t = 22.46$, $P < 0.05$)] than that of the chicks. At $> 10^{-4}$ g/ml of nicotine, the pigeon intraocular muscles began to die.

Nicotine stimulation of > 10-yr old pigeon eyes

As shown in Fig. 5(A, B), the six > 10-yr old pigeon lenses had a greater resting back vertex distance (38.44 mm) than the lenses of the 6-month old pigeons. The resting pupil diameters were similar for the two groups. As with the 6-month old pigeon eyes, the > 10-yr old pigeon eyes did not show any change in back vertex distance or pupil diameter at the lower concentrations of nicotine. In the unstimulated condition, the > 10-yr old pigeon lenses had a similar degree of negative spherical aberration (7.62 mm) to the 6-month old pigeon lenses. As with the 6-month old pigeon eyes, the > 10-yr old pigeon eyes were stimulated by nicotine concentrations of between 10^{-6} and 10^{-7} g/ml. The two most striking results of the > 10-yr old pigeon eyes are the absence of a significant change in paraxial back vertex distance with increasing nicotine concentration (paired t-test: $t = 1.69$, $P = 0.151$) and the reduced change in total back vertex distance as compared to the 6-month old pigeons [13.24% (paired t-test: $t = 4.73$, $P < 0.05$)]. The > 10-yr old pigeon eyes also showed a significantly reduced pupillary constriction [36.45% (paired t-test: $t = 9.95$, $P < 0.05$)] compared to the 6-month old pigeon eyes.

Carbachol stimulation of chick eyes

As shown in Fig. 6(A, B) for a group of six 4-week old chick lenses, the resting total back vertex distance (31.53 mm) and paraxial back vertex distance (23.79 mm) were similar to the first group of chick lenses. The degree of negative spherical aberration for this group of lenses (7.73 mm) was, however, closer to the two groups of pigeon lenses than the first group of chick lenses. The unstimulated pupil diameter was also greater than in the initial group of chick eyes (3.44 mm). Carbachol stimulation initially resulted in a 15.99% (paired t-test: $t = -12.57$, $P < 0.05$) pupillary dilation at 10^{-6} g/ml with no change in back vertex distance. At between 10^{-5} and 10^{-6} g/ml of carbachol, pupil diameter and back vertex distance began to decrease. A concentration of 10^{-4} g/ml of carbachol had the strongest constrictive effect on the iris, resulting in a 24.42% (paired t-test: $t = 9.43$, $P < 0.05$) decrease in

paraxial back vertex distance, a 30.57% (paired t-test: $t = 15.51$, $P < 0.05$) decrease in total back vertex distance, and a 22.38% (paired t-test: $t = 19.49$, $P < 0.05$) pupillary constriction.

Nicotine stimulation of iridectomized pigeon eyes

To demonstrate that the iris is involved in changing the focal length of the pigeon lens, we stimulated two eyes of a 3-month old pigeon with increasing
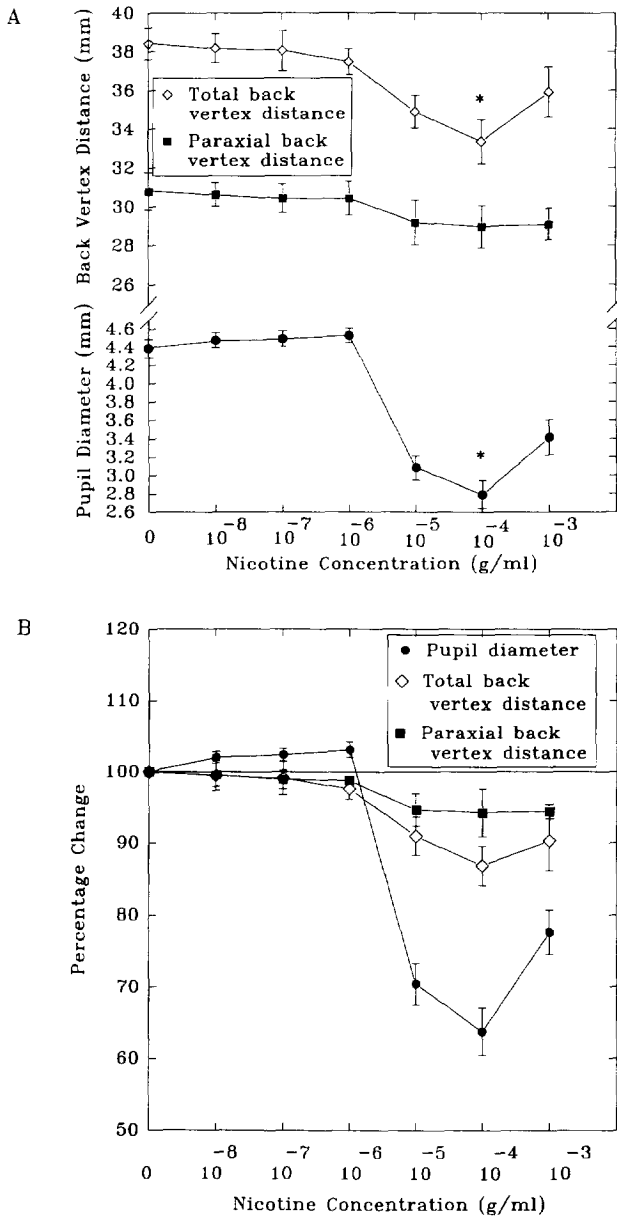

FIGURE 5. (A) Back vertex distance and pupil diameter measurements of six >10-yr old pigeon lenses stimulated with increasing concentrations of nicotine and (B) the same data plotted as a percentage of the unstimulated values (0 g/ml nicotine). As with the young pigeons, there is little change in the three measures at lower drug concentrations, although there is a very slight increase in pupil diameter. There is a decrease in pupil diameter and total back vertex distance at between 10^{-6} and 10^{-5} g/ml, but no significant decrease in paraxial back vertex distance. Although there is a relatively strong pupillary constriction, it is noticeably less than in the young pigeons. The iris is still relatively sensitive to the increasing nicotine concentrations, but the change in back vertex distance is reduced as compared to the young pigeons, representing presbyopia. As before, there is a tendency towards the pre-stimulated state as the iris muscle begins to relax at supramaximal doses of nicotine. (*Significance from rest state, paired t-test. Total back vertex distance, $t = 4.37$, $P < 0.05$; paraxial back vertex distance, *not significant* at 10^{-4} g/ml, $t = 1.69$, $P = 0.15$; pupil diameter, $t = 9.95$, $P < 0.05$.)
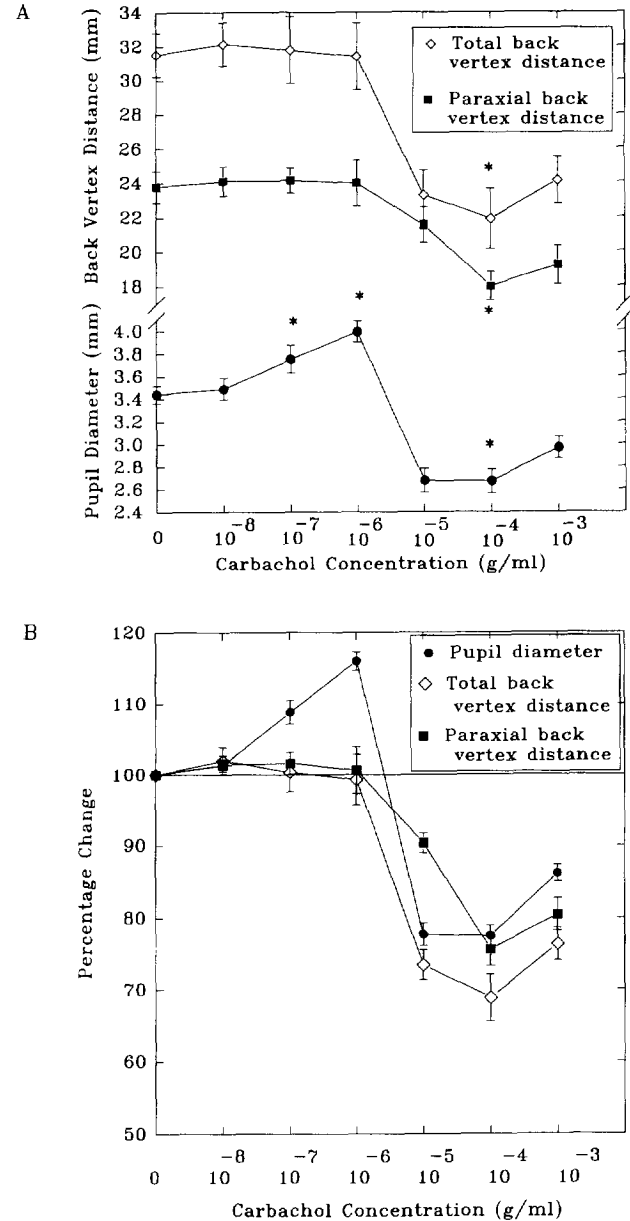
FIGURE 6. (A) Back vertex distance and pupil diameter measurements of six young chick lenses stimulated with increasing concentrations of carbachol and (B) the same data plotted as a percentage of the unstimulated values (0 g/ml nicotine). The data show that, with respect to the unstimulated condition, the pupil diameter increases at the lower carbachol concentrations without an associated increase in back vertex distance. This pupillary dilation at low carbachol concentrations probably reflects the muscarinic stimulation of smooth dilator fibers in the chick iris primarily at the pupillary margin of the iris. At between 10^{-6} and 10^{-5} g/ml carbachol, there is decrease in the back vertex distance of the lenses, which is stronger in magnitude than the change measured from nicotine stimulation. At higher carbachol concentrations the three measures begin to return to their pre-stimulated state as the iris muscle begins to relax at supramaximal doses. (*Significance from rest state, paired t-test. Total back vertex distance, $t = 15.51$, $P < 0.05$; paraxial back vertex distance, $t = 9.43$, $P < 0.05$; pupil diameter at 10^{-7} g/ml, $t = -5.15$, $P < 0.05$; at 10^{-6} g/ml, $t = -12.57$, $P < 0.05$; at 10^{-4} g/ml, $t = 19.47$, $P < 0.05$.)

concentrations of nicotine after the irises had been dissected from the eye preparation. The results presented in Fig. 7 show that there is no change in back vertex distance of the lenses with increasing nicotine concentration after the irises have been removed. The initial resting total and paraxial back vertex distance measurements with the iris intact show the back vertex distance of the 3-month pigeon lenses is considerably less than that of the 6-month or >10-yr old pigeon lenses. It is also evident that the lenses from the 3-month old pigeon eyes (with and without the irises) show almost negligible negative spherical aberration, quite unlike the lenses from older pigeons. At the first two measurements (iris intact, unstimulated and iris removed, unstimulated) approx. 2 mm of spherical aberration is evident; however, for all the subsequent measurement points, no spherical aberration can be detected.

Histology

The detailed functional anatomy of the chick iris has been described previously (Glasser *et al.*, 1995). Here we limit our histological observations to a comparison between the chick and pigeon iris. As seen in Fig. 8, the iridial stroma of the chick iris is more dense than that of the pigeon iris. There are substantially fewer circumferential muscle fibers (seen in cross section) in the pigeon iris than in the chick iris. These fibers are uniformly scattered throughout the thickness of the pigeon iris, whereas in the chick iris they predominate in the anterior

iridial stroma. The chick iris has the greatest density of fibers approximately midway across the iris, and towards the root of the iris there remains a relatively dense distribution of muscle fibers. In the pigeon iris, however, there is a relatively uniform distribution of circumferential muscle fibers between the pupillary margin and the iris root with few fibers towards the iris root. The pigeon iris is more vascularized than the chick iris with the greatest concentration of blood vessels on the anterior surface of the iris. The cross-sectional diameter of the largest blood vessels in the pigeon iris are often three to four times greater than those of the chick iris.

In the chick the attachment of the ciliary processes to the lens is located beneath the ciliary region of the iris. In the pigeon, however, this attachment is peripheral to the ciliary region of the iris and is associated more with the ciliary body than with the iris. In the pigeon the ciliary processes attach to the lens at the apical circumferential edge of the lens, whereas in the chick they are more distinctly on the anterior surface of the lens. In both the chick and pigeon eye, the ciliary processes are firmly attached to the lens. The chick lens is distinctively thicker and more spherical than the pigeon lens.

DISCUSSION

These experiments show that it is possible to induce considerable changes in back vertex distance of young chick and pigeon lenses *in vitro*. It should be kept in mind that the magnitude of the changes in back vertex distance are specific to the wavelength of the laser beam used. It seems likely, however, that these changes represent the real lenticular accommodative process of the avian eye. The ability to induce such changes *in vitro* provides a powerful tool to study and compare the lenticular accommodative process of different bird species. In support of the role of the iris in lenticular accommodation, we see first that changes in back vertex distance are always accompanied by changes in pupil diameter with pharmacological stimulation. Second, for both nicotine and carbachol stimulation, the maximal change in back vertex distance of the lenses always occurs at the same drug concentration that induces the maximal pupillary constriction. Third, removal of the pigeon iris prevents any change in the back vertex distance of the lens.

It is interesting to compare the responses of the chick and pigeon eyes. At least one previous report has suggested that the accommodative amplitude differs greatly between chicks and pigeons and that pigeons lack lenticular accommodation (Schaeffel & Howland, 1987). The results presented here provide empirical, albeit *in vitro*, evidence against both of these ideas. Our results also indicate that the two species show differential sensitivities to nicotine concentrations. We observed that the responses of the pigeon irises to appropriate drug concentrations were somewhat slower than responses of the chick irises. The possibility exists, therefore, that a direct comparison of chick and pigeon eyes is inappropriate due to the species differences. However,
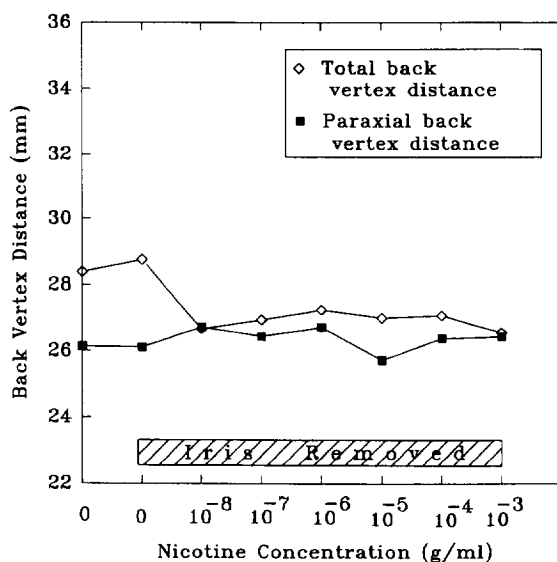
FIGURE 7. The results of an experiment demonstrating that the iris mediates lenticular accommodation in the pigeon eye. The total back vertex distance and the paraxial back vertex distance were first measured in two young pigeons' eyes with the iris intact and unstimulated (0 g/ml nicotine). The iris was then dissected out, and the back vertex distance was measured while exposing the iridectomized eyes to increasing concentrations of nicotine. Once the iris had been removed, there was no change in back vertex distance as a function of nicotine stimulation. This demonstrates that the iris is responsible for changing the curvature of the lens in the pigeon eye just as in the chick eye (Glasser *et al.*, 1995). Error bars are not shown because the measurements are from two eyes only, and pupil diameters were not measured because the irises had been removed.
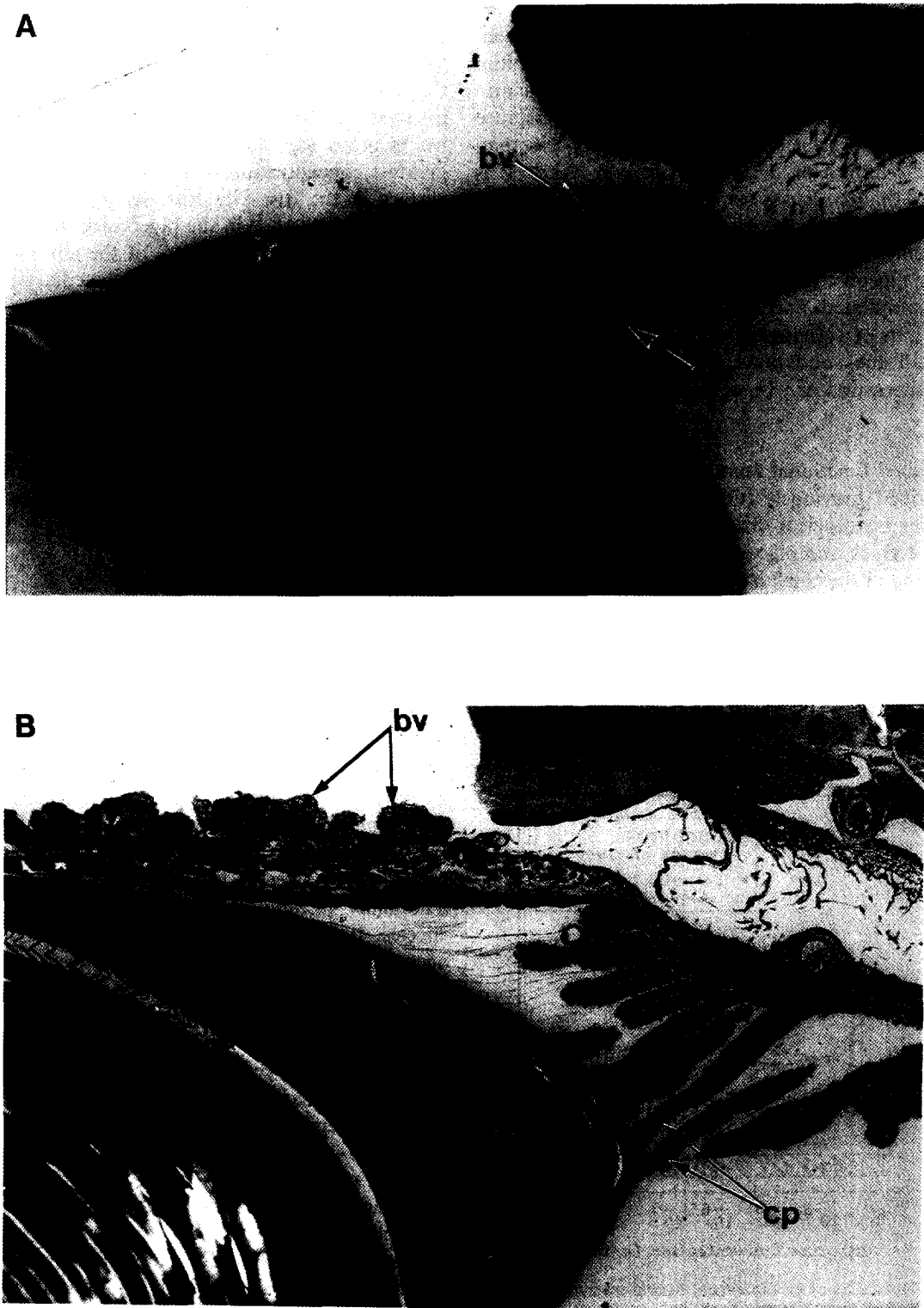
FIGURE 8. Histological sections of the chick (A) and pigeon (B) iris and lens. In both eyes, only the peripheral edge of the cornea (co) remains after the central cornea was removed to facilitate fixation. The stroma of the chick iris (ir) is considerably more dense than that of the pigeon. In the pigeon iris there is a relatively even but diffuse distribution of circumferential muscle fibers, whereas in the chick iris there is a considerably greater abundance, particularly in the anterior two-thirds of the iris. The pigeon iris has abundant large diameter blood vessels (bv) on the anterior surface of the iris, but the chick iris has far fewer blood vessels, and they are smaller in diameter. The peripheral ciliary margin of the chick iris is densely packed with circumferential muscle fibers, whereas the stroma of this region of the pigeon iris is particularly diffuse. The ciliary processes (cp) of the chick iris are tucked beneath (posterior to) the ciliary region of the iris, whereas in the pigeon, they are associated with the ciliary body beyond (lateral to) the iris. In the chick the ciliary processes attach to the anterior surface of the lens (le), but in the pigeon they are attached to the peripheral apex of the lens. The chick lens is thicker and more spherical than the pigeon lens, which is quite flattened in appearance.

even with the species differences, by using increasing drug concentrations, it is still possible to induce similar changes in back vertex distance in the young chick and young pigeon eye preparations.

We can only speculate as to why the pigeon iris shows a delayed response to nicotine stimulation. The difference in the vascularization of the chick and pigeon irises may suggest there is a difference in the permeability of the iridial stroma of these two species. It is equally possible that the affinity of the circumferential muscle fibers for nicotine sulfate may differ. It is beyond the scope of this study to explain this difference, but it is of obvious importance, especially when considering future studies or interpreting previous studies using pharmacologically induced accommodation *in vivo*.

In the > 10-yr old pigeons at maximal nicotine stimulation (10^{-4} g/ml), the total back vertex distance does not tend towards the paraxial back vertex distance as happens in the other groups of eyes. This is because the sub-maximal pupillary constriction in the > 10-yr old birds allows more than just the paraxial laser beams through the pupil, so that the total back vertex distance measurement includes some of the more eccentric laser beams. By contrast, in the younger bird eyes, under conditions of maximal pupillary constriction, the only laser beams passing through the pupil are the paraxial beams, so that the total back vertex distance measurement is identical to the paraxial back vertex distance measurement. Because, in these > 10-yr old pigeon eyes, there is no significant change in paraxial back vertex distance with nicotine stimulation, the change in total back vertex distance is entirely due to pupillary constriction on the negative spherically aberrated lenses. The absence of a change in paraxial back vertex distance represents a real deficit in the ability to change the focal power of the lens, i.e. a real lenticular presbyopia in the > 10-yr old pigeon eyes. However, it is possible that this lack of change in lens power might be due in part to a weaker iris muscle contraction (reflected by the reduced pupillary constriction). The reduced responsiveness of the older irises might be due to a desensitization through loss of receptors or to other age-related physiological changes.

The carbachol series was used to stimulate non-nicotinic smooth muscle fibers of the iris. The chick iris is known to contain smooth muscle fibers that aid in pupillary dilation (Nishida & Sears, 1970; Scapolo *et al.*, 1988), and has been shown to exhibit a non-nicotinically mediated contraction in young chicks (Pilar *et al.*, 1987). We wished to determine if: (1) we could stimulate an increase in lens power without an associated change in pupil diameter; or (2) if pupillary dilation caused an *increase* in back vertex distance of the chick lenses due to some kind of negative accommodation [as suggested by the low doses of nicotine (Fig. 3)]. The low doses of carbachol clearly show that a smooth muscle mediated pupillary *dilation* does occur without an associated change in lens power. At higher carbachol concentrations, the specific muscarinic effects are presumably overridden by stimulation of nicotinic receptors, produc-

ing results that are a little more difficult to interpret. Carbachol induced only a 22% pupillary constriction relative to the rest condition, whereas nicotine induced a 39% pupillary constriction. However, carbachol caused a greater change in both the total (31% for carbachol vs 27% for nicotine) and the paraxial (24% for carbachol vs 14% for nicotine) back vertex distances for the chick lenses. One possible interpretation of this result is that the reduced pupillary constriction is due to the muscarinic effect on the smooth dilator fibers in the chick iris, while the strong changes in back vertex distance are due to nicotinic stimulation of the more peripheral iris musculature. It is this peripheral iris musculature that has been shown to exert the major force in changing the focal length of the chick lens (Glasser *et al.*, 1995).

It has also been shown that lenticular accommodation in chicks is mediated primarily by the peripheral iris musculature squeezing the peripheral margin of the lens (Glasser *et al.*, 1995). We have demonstrated here that, if the iris is removed from the pigeon eye, there is no change in back vertex distance of the lens with nicotine stimulation. The iris can be removed without affecting the ciliary muscles, yet no change in back vertex distance occurs during nicotine stimulation. This provides evidence that, as in the chick eye, the iris causes the change in back vertex distance of the lens. It remains unclear, however, whether the precise action of the pigeon iris is identical to that of chicks. The anatomy of the chick and pigeon eyes differ enough to suggest that their modes of lenticular accommodation also differ. This work includes no systematic studies of mechanisms in the pigeon eye, so the following proposed differences in lenticular accommodation between the chick and pigeon eyes are based solely on the histological comparisons.

In the chick eye a contraction of the peripheral muscle fibers of the iris pushes directly on the ciliary processes (Glasser *et al.*, 1995). These substantive peripheral muscle fibers of the chick iris are all but absent in the pigeon iris. The pigeon iris may, therefore, be better suited to pull the peripheral edges of the lens together during pupillary constriction rather than actively squeezing the edge of the lens as in the chick eye. In the pigeon iris, as the circumferential muscle fibers contract, the iris constricts, pulling the ciliary body and associated ciliary processes axially. This action would draw the equatorial edges of the lens together, increase the curvature of the anterior and posterior lens surfaces, and thereby increase the focal power of the lens. When the iris relaxes, the lens would be returned to its rest position by the outward pull of the ciliary body and the pectinate ligament. Both of these elements are ideally suited to serve this function, as has been described in the chick eye (Glasser *et al.*, 1995).

Our characterization of negative spherical aberration of chick and pigeon lenses (namely a comparison between the total back vertex distance and the paraxial back vertex distance) is unsophisticated. Nevertheless, the degree of negative spherical aberration we measured in the lenses from 4-week old chicks is greater than one previously published report for chick lenses (Sivak *et al.*,

1989). Sivak *et al.* (1989) show data from a 15-day old chick lens with approx. 4.2 mm of negative spherical aberration. We have shown here that lenses from 3-month old pigeons show considerably less negative spherical aberration than the lenses of 6-month old pigeons. It is possible, therefore, that there is a developmental change in the spherical aberration of the avian lens. It is interesting to note that in spite of the histological differences in shape and thickness of the young chick and young pigeon lenses, they both have a similar degree of spherical aberration.

These experiments provide no indication of what role, if any, the negative spherical aberration of the lens plays in the intact eye. It is possible that the spherical aberration is corrected through complementary aberrations of the cornea to create a sharp image on the retina and prevent any change in focus during pupillary constriction. Alternatively, it is possible that the negative spherical aberrations of the lens remain uncorrected, compromising the image quality but allowing the focal power of the eye to be changed by simply constricting the pupil. The latter might be of particular value in aged pigeons' eyes, for example, where the focal length of the lens can no longer be changed through changes in lens curvature.

SUMMARY

Our experiments show that, contrary to previous reports, the lenses from young chick and young pigeon eyes do undergo similar changes in lens power, at least when subjected to drug-induced stimulation *in vitro*. The lenses from >10-yr old pigeon eyes, however, do not undergo significant changes in paraxial back vertex distance with drug stimulation. This provides evidence for presbyopic-like changes in the eyes of >10-yr old pigeons. If the pigeon iris is removed, the back vertex distance of the lens does not change with drug stimulation. So, in spite of their morphological differences, chick and pigeon irises both mediate lenticular accommodation, possibly through slightly different mechanisms.

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