CHANGES IN CORNEAL CURVATURE DURING ACCOMMODATION IN CHICKS

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Abstract—Evidence is presented for an active corneal component of accommodation in chicks. Using anesthetized chicks, consistent increases in corneal curvature were observed during accommodation produced either by electrical stimulation of the Edinger-Westphal nucleus or by topical application of 0.4% nicotine sulfate to the cornea. Electrical stimulation produced a mean accommodative amplitude of 9.2 diopters (D) and a change in corneal power of 3.9 D whereas nicotine treatment elicited 8.1 D of accommodation and 6.1 D of change in corneal power. The change in corneal power increased proportionately with accommodative amplitude up to about 10 D of total accommodation where the corneal power change appeared to level off at approximately 6 D. This limit to corneal curvature change implies that it plays a proportionately greater role in the lower range of accommodation.

Accommodation Chickens (Gallus gallus domesticus) Cornea Edinger-Westphal nucleus

INTRODUCTION

Differences in ocular morphology suggest that birds and mammals may have different accommodative mechanisms. In birds, a subdivision of the avian ciliary muscle's longitudinal bundle, Crampton's muscle, inserts at the corneo-scleral border and, when contracted, may increase corneal curvature, thereby increasing refractive power (Slonaker, 1918; Walls, 1942; Meyer, 1977; but see Suburo and Marcantoni, 1983). Substantial changes in corneal curvature have been found by some researchers (Beer, 1892/93; Gundlach et al., 1945; Rosenthal, 1981) but not by others (Steinbach and Money, 1973; Levy and Sivak, 1980; Sivak et al., 1986). This study presents results showing that corneal changes are part of the accommodative response of chicks.

To control for methodological artifacts we used two in vivo methods to produce accommodation: (a) electrical stimulation of the Edinger-Westphal nucleus (EW) and (b) topical application of 0.4% nicotine sulfate to the cornea. The EW (also known in birds as the accessory oculomotor nucleus) is the only known source of the pre-ganglionic, parasympathetic neurons which innervate the ciliary ganglion (Narayanan and Narayanan, 1976; Reiner et al., 1983). The post-ganglionic ciliary neurons innervate the intraocular musculature and stimulate both accommodative and pupillary changes. Thus, electrical stimulation of the EW produces accommodation through the normal neural pathway and does not interfere with the eye directly.

METHODS

Twenty 4-week-old White Leghorn chickens (Gallus gallus domesticus) were used. All ocular measurements were made before and during induced accommodation and took place while the birds were anesthetized with urethane (2 g/kg i.p.), an anesthetic that almost entirely eliminates spontaneous eye movements (Burns and Wallman, 1981). During both sets of measurements the eyelids were held open with lid-retractors that produced no apparent deformation of the cornea.

Total accommodative change was defined as the difference in refractive error measured with a Hartinger refractometer (Aus Jena Coincidence Refractometer) before and during induced accommodation. Because of the small pupils of chicks (about 3 mm in diameter at rest and 2 mm when accommodated), the refractometer was adapted with a supplemental lens (about +11 D) to reduce the width of the light beam. To calibrate the refractometer we used two methods. In one method we used a
“mechanical” eye, which was simply a plano-convex lens of known power mounted parallel to a screen on a movable platform. By placing the screen at different distances from the lens, different degrees of “ametropia” were produced. Because the accuracy of this technique is limited by lens aberrations, we also refracted a single lens reflex camera focussed either at infinity, to simulate emmetropia, or at various other distances, to simulate varying amounts of myopia. To simulate hyperopic refractive errors, we simply moved a screen alone to varying distances from the refractometer. At a distance of 1 m, hyperopia of +1 D is simulated, at 1/2 m, +2 D of hyperopia is produced and so on. The data from these 3 methods of simulating refractive errors were fitted with a third order polynomial which served as a calibration curve.

Corneal curvature (measured as the radius of curvature) was assessed with a keratometer (Topcon OM-3) and with a photographic method described below. To adapt the keratometer for the highly curved corneas of chicks, a supplemental lens (+8 D) was attached in front of the keratometer along its optic axis. The keratometer was calibrated by measuring the curvatures of steel balls of known diameter. Approximately 4.0 mm² of cornea was sampled using the keratometer.

Both the refractometer and the keratometer were aligned with the pupillary axis, which, in chicks, closely approximates the optic axis (unpublished observations based on Purkinje-images). To align the refractometer, a circular fluorescent lamp was placed concentric with the refractometer’s optical axis. With the refractometer 10–15 cm from the eye, the reflected image of the circular lamp was clearly visible and could be placed coaxial with the pupil by adjusting the position of the chick’s head. Following alignment, the refractometer was translated along its optic axis toward the eye to the proper measurement distance, the circular lamp was turned off, and the refractive error was measured. We aligned the keratometer with a similar procedure. By disengaging the instrument’s image doubling prisms, only a single reflection of the keratometer’s circular mire is left, which can be concentrically aligned with the pupil.

Once aligned, spherical equivalents for both refractive error and corneal curvature were computed by averaging the two orthogonal measurements taken on the principal meridians. The data from refractometry and keratometry were the average of 6–8 repeated measures of the spherical equivalent refractive error or corneal curvature. The instruments were realigned after every second or third measurement. Since each datum was a mean, the standard error of the mean provides an estimate of the precision of our techniques. The average standard error determined for refractometry was about ±0.3 D while that for keratometry was within ±0.02 mm (about 1 D). Our precision of keratometry is similar to those reported in several studies reviewed by Ludlam, Wittenberg and Rosenthal (1965).

Besides keratometry, corneal curvature was measured by photographing the first Purkinje-images of three fiber optic light guides arranged in an equilateral triangle centered on the camera’s optic axis. The area of cornea sampled by this technique was approximately 0.3 mm². The device was easily aligned with the eye’s optic axis by moving the chick’s head until the reflected triangles arising from the first, third and fourth Purkinje-images were concentric (Fig. 1). The following calibration technique was repeated before each eye was measured. With the camera’s lens-to-film distance fixed, a ruler was photographed to determine the magnification; then several steel balls were individually placed at the camera’s anterior focal point and the images of the three light sources were photographed to permit construction of a calibration curve. The chick’s eye was then

![Fig. 1. Representation of the view through the Purkinje-image camera when all three sets of Purkinje-images form equilateral triangles which are concentrically aligned with the eye’s optic axis. In reality, all three sets of images are not in focus simultaneously. The large, outer images are the third Purkinje-images which arise from the corneal surface. The middle images are the first Purkinje-images reflected from the anterior lens surface. The small, inverted inner images represent the fourth Purkinje-images reflected from the posterior lens surface.](attachment://purkinje_image_diagram.png)
aligned as discussed above and the distance of the eye to the camera was adjusted by moving the bird toward or away from the camera until the first Purkinje-images were brought into sharp focus. The photographic images were later projected onto a digitizing tablet to determine the diameter of the circle drawn from the Purkinje-images. This technique yields a single estimate of corneal curvature by determining the diameter of a circle with a circumference which passes through each light's first Purkinje-image. The standard errors of repeated measurements of photographs of the first Purkinje-images for individual eyes were, like keratometry, within ±0.02 mm. Since the Purkinje-image camera's depth of field (about ±1 mm) was larger than that of the keratometer (< ±0.25 mm), this could produce errors of approximately ±0.05 mm (±1.5 D) (see Bennett and Francis, 1962).

Edinger–Westphal stimulation

Monopolar electrodes (Rhodes SNEX-300: tips 0.25 mm long and 0.1 mm in diameter) were stereotaxically placed into the EW and their position adjusted to produce accommodative responses to electrical stimulation. Typically, large accommodative changes were accompanied by brisk pupillary effects (either dilations or contractions), iris bulge, and noticeable changes in light reflected from both corneal and lenticular surfaces. If vertical eye movements were produced, the electrode was moved dorsally since the oculomotor nucleus lies ventral to the EW; if torsional eye movements were produced the electrode was moved anteriorly. When accommodative responses were observed, the electrode was fixed in place with skull screws and dental acrylic. The bird was then removed from the stereotaxic instrument for testing. Measurements during electrically induced accommodation were paired with, and recorded immediately after, a measurement of the eye at rest. Electrical stimulation (20–30 μA, 110 Hz, 0.5 msec pulse duration) was produced using a Grass SD-9 stimulator and CCU-1-A constant current isolation unit. At the end of each experiment, marking lesions were made in order to verify electrode placement. The birds were then sacrificed, perfused with Heidenhain's solution and their brains sectioned at 50 μm and stained with neutral red for histological inspection. We used only those birds in which stimulation sites were verified to have been in the EW.

Nicotine treatment

Because avian ciliary muscle is striated and contains nicotinic receptors, application of nicotine sulfate to ciliary muscle produces accommodation. Two drops of 0.4% nicotine sulfate were applied to the cornea 3 min before measurements were made of the refractive state of the eye and of the curvature of the cornea (technique of Rosenthal, 1981). Typically, we observed the maximum effect of the drug 4–5 min following application. All effects began to drop off sharply at 10–12 min and varying degrees of corneal clouding were often observed at different points along the drug's time course. Because of the brief action of the drug and its tendency to cause corneal clouding, all accommodation measurements were completed within ten minutes of application. Unlike measurements made during EW stimulation, those taken during nicotine-induced accommodation were not paired with the resting state measurements. All of the resting state measurements for a given eye were recorded together before the application of nicotine. The short time-course of nicotine-induced effects necessitated measuring corneal changes only by keratometry (7 chicks, 13 eyes) whereas the corneal changes of the EW-stimulated chicks (13 chicks, 13 eyes) were measured by both corneal measurement techniques. In order to randomize the order of the measurement procedures with respect to the temporal effects of nicotine, 6 of the 13 nicotine-treated eyes were measured by refraction first, and 7 by keratometry first.

RESULTS

A consistent increase in corneal curvature (i.e. a decrease in the radius of curvature) was

<table>
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<tr>
<th>Table 1. Corneal radius of curvature measurements for electrically-stimulated and nicotine-treated chicks (means in mm ± SD)</th>
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<tbody>
<tr>
<td><strong>EW-stimulated chicks</strong></td>
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<tr>
<td>Keratometry (n = 13)</td>
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<tr>
<td>Before stimulation: 3.63 (0.11)</td>
</tr>
<tr>
<td>During stimulation: 3.49 (0.13)</td>
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<tr>
<td>Difference: -0.13 (0.06)</td>
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<tr>
<td>Purkinje Images (n = 12)</td>
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<td>Before stimulation: 3.58 (0.10)</td>
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<tr>
<td>During stimulation: 3.48 (0.12)</td>
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<tr>
<td>Difference: -0.10 (0.07)</td>
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<tr>
<td><strong>Nicotine-treated chicks</strong></td>
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<tr>
<td>Keratometry (n = 13)</td>
</tr>
<tr>
<td>Before stimulation: 3.71 (0.11)</td>
</tr>
<tr>
<td>During stimulation: 3.49 (0.11)</td>
</tr>
<tr>
<td>Difference: -0.22 (0.08)</td>
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observed during accommodation for all the animals tested (Fig. 2). The change in corneal curvature from the resting to accommodated state (summarized in Table 1) was significant in all cases (paired t-tests: nicotine-treated [keratometry], t (12) = −9.93, P < 0.0001; EW-stimulated [keratometry], t (12) = −8.38, P < 0.0001; EW-stimulated [Purkinje-image photography], t (11) = −5.38, P < 0.0001). There was no statistically significant difference in the corneal curvature measurements obtained using keratometry or Purkinje-image photography on the same EW-stimulated eyes [paired t-test, t (23) = 1.67, P = 0.11].

In order to determine corneal power changes produced by the increase in curvature, the corneal radius of curvature was converted to refracting power using the general ray-tracing formula for a single refractive surface

$$F_c = \frac{n_c - 1}{r_c}$$

where:

- $F_c$ = refracting power of the anterior surface of the cornea.
- $n_c$ = refractive index of chicken cornea = 1.362 (Sivak et al., 1978).
- $r_c$ = anterior corneal surface radius of curvature (in meters).

Figure 3 shows the relationship between corneal power change and the amplitude of accommodation for individual birds undergoing either

![Fig. 2. Change in corneal radius of curvature measured by keratometry and Purkinje-image photography during accommodation. A reduction in radius of curvature is equivalent to an increase in curvature. Top: ranked change in corneal radius of curvature for nicotine-treated eyes measured by keratometry. Middle: ranked change in corneal radius of curvature during Edinger–Westphal stimulation measured by keratometry. Bottom: Change in corneal radius of curvature measured by Purkinje-image photography and aligned with the same eyes shown in the middle panel.](image)
Corneal accommodation in chicks

Fig. 3. Scatter plot showing corneal power change versus the amplitude of accommodation for Edinger–Westphal stimulation (solid symbols) and nicotine treatment (open symbols). Corneal power change was calculated from keratometric readings. For some nicotine-treated eyes, keratometry was the first measurement made (triangles); for others, refractometry was first.

Discusstion

The results of this study strongly argue for an active corneal component of accommodation in chicks. Using either EW stimulation or nicotine treatment and either measurement technique, increases in corneal curvature are observed throughout the range of accommodative responses produced (up to 19 D). The mean amplitude of accommodation for the nicotine-treated eyes was significantly greater than for the EW-stimulated chicks (15.1 D [SD = 2.7] vs 9.2 D [SD = 4.7]; two sample t-test, t (24) = -3.92, P < 0.001). The mean change in corneal power for nicotine-treated eyes was also greater than in EW-stimulated birds (6.1 D [SD = 2.3] vs 3.9 D [SD = 1.7]; two sample t-test, t (24) = -2.92, P < 0.01).

In contrast to the EW stimulations, there is no apparent relationship between the magnitude of corneal power change and the amplitude of accommodation produced with nicotine (Fig. 3, open symbols) probably because of a ceiling effect. The nicotine always elicited amplitudes of accommodation above that at which maximal curvature changes are produced by EW stimulation.) The greater scatter of the nicotine data may have several explanations. Variability resulting from the drug’s brief action is suggested by the relatively greater corneal effects observed when keratometric readings are made prior to refractometry (open triangles) compared to when refractometry is performed first (open squares). Since the results of EW stimulation suggest that accommodation greater than 10 D is associated with maximal corneal change, and since nicotine always produced at least this level of accommodation, individual differences in maximal corneal change could produce some scatter of the data. Furthermore, variability in drop size and retention of the drop on the corneal surface, as well as measurement difficulties because of corneal clouding, may also contribute to the scatter of the nicotine data points. Finally, the presence of corneal clouding suggests that nicotine may have direct effects on the cornea possibly contributing to curvature changes. The mean nicotine-induced corneal power change of 6 D is, nevertheless, consistent with the ceiling effect suggested by EW stimulation.

In general, nicotine produces larger effects than EW stimulation. Perhaps the ciliary muscle is able to contract more powerfully than it does when normally stimulated by the nervous system. Alternatively, electrode placement or the stimulation parameters used may not have been maximally effective in driving all accom-
accommodation-producing neurons. This is suggested by the greater variability in accommodative amplitude produced by EW stimulation than by nicotine treatment (SD = 4.7 and 2.7 respectively) although the two techniques produce comparable maximum responses. Despite the greater variability, EW stimulation has the advantage of providing a more reliable method for studying accommodation because, unlike nicotine, it is easy to control the accommodative responses, there is no critical temporal element, and it does not involve manipulation of the eye directly.

Corneal curvature changes during accommodation in various bird species have been found by others. Beer (1892/93) also reported increases in corneal curvatures in electrically stimulated excised eyes of pigeons, chickens, ducks, hawks, and owls; the largest corneal effects being observed in the predatory species. Corneal curvature changes were measured by the movements of pins inserted into the cornea or by changes in Purkinje-images. Gundlach et al. (1945) reported instances of a pigeon spontaneously showing 17 D of corneal accommodation and of 4-5 D of corneal change being produced by nicotine applied to the cornea. Unfortunately, Gundlach et al. did not perform systematic experiments and the refractive changes of the eyes were not measured.

In contrast, Steinbach and Money (1973) reported no corneal curvature changes in the Great Horned Owl on the basis of the movements of light reflected from a mirror attached to the cornea. Accommodation was assumed to occur when a mouse was moved toward the subject, but was not directly measured. In addition, in several species of diving ducks, no corneal curvature changes were seen during high levels of nicotine-induced accommodation (Levy and Sivak, 1980), although Crampton's muscle is typically reduced in waterfowl, and a corneal accommodation mechanism would be of little use for vision underwater because of the nearly equal refractive indices of water and cornea.

Unlike Gundlach et al. (1945), Levy and Sivak (1980) could find no corneal curvature changes during 5 D of nicotine-induced accommodation. In preliminary experiments we observed no consistent corneal effects in several 1-2 month old pigeons (Columba livia) although only 2 D of accommodation was elicited with the same nicotine treatment used on the chicks. The higher amplitude of accommodation produced by Levy and Sivak is probably due to their use of a more concentrated nicotine sulfate solution (2% vs 0.4%). These results do not rule out the possibility that a corneal component of accommodation exists in the pigeon, which could not be measured because of the small amplitudes of accommodation in both studies.

There has also been some disagreement regarding the existence of corneal accommodation in chickens. Rosenthal (1981), using Purkinje-image photography, reported corneal curvature changes amounting to about 8 D of corneal power change during 21 D of nicotine-induced accommodation in chickens. Sivak et al. (1986) however, found no corneal effects in the electrically stimulated eyes of chickens in vitro. They measured the change in ocular focal length by projecting parallel laser beams along the optic axis emerging through a window cut in the back of the globe. The change in focal length of the eye was not significantly different when the cornea was immersed in water than when it was exposed directly to air. If corneal accommodation had occurred, the change in focal length would have been less when the cornea was in water. One possible reconciliation of our results with those of Sivak et al. is that the normal intraocular pressure, which would be eliminated by opening the eye, may be important for the expression of accommodation-related corneal curvature changes. Coleman (1970) proposed a mechanism for human accommodation in which hydrostatic forces are an integral component. Suburo and Marcantoni (1983) have suggested, based on anatomical evidence, that a similar mechanism exists in chicks.

Regardless of the mechanism of accommodation in chicks, the results presented here indicate that corneal change plays an integral role. The maximum change in corneal curvature produces a change in corneal refractive power of about 6 D and typically occurs at levels of accommodation greater than 10 D. Since we observed up to 19 D of accommodation in our chicks, this implies that at amplitudes of accommodation greater than 10 D, lenticular changes become progressively more important than corneal changes. In related work, using Purkinje-image photography and A-scan ultrasonography, we have observed large lenticular changes during accommodation and have found that the amplitude of both the corneal curvature changes and the total amplitude of accommodation decline with age (Troilo and Wallman, 1985).
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REFERENCES