Vision Research 129 (2016) 61–68

Contents lists available at ScienceDirect

Vision Research

journal homepage: www.elsevier.com/locate/visres

Off-axis astigmatism in the isolated chicken crystalline lens

Felix Maier [a], Siegfried Wahl [b], Frank Schaeffel [a,*]

[a] Section of Neurobiology of the Eye, Ophthalmic Research Institute, Elfriede-Aulhorn-Straße 5-7, 72076 Tübingen, Germany
[b] ZEISS Vision Science Lab, Ophthalmic Research Institute, Elfriede-Aulhorn-Straße 5-7, 72076 Tübingen, Germany

ARTICLE INFO

Article history:
Received 6 June 2016
Received in revised form 26 October 2016
Accepted 26 October 2016
Available online 3 November 2016

Keywords:
Astigmatism
Crystalline lens
Chicken
Refractive index
Ray tracing
Peripheral visual field

ABSTRACT

The chicken eye was previously found to have little off-axis astigmatism which is not explained by its special corneal shape but rather by the optical properties of the crystalline lens. To learn more about lens design, we studied off-axis astigmatism in the chicken lens *in situ* and compared it to a glass lens of similar power but with homogenous refractive index. After euthanasia, enucleated eye balls were cut in the equatorial plane right behind the scleral ossicles. The anterior segment was placed in a water-filled chamber. Several thin laser beams were projected in two perpendicular meridians through the lens under various eccentricities and the focal lengths were determined. Off-axis astigmatism across the horizontal visual field was determined as the differences in power in the two meridians. The same procedure was used for the glass lens. On-axis, the chicken crystalline lens had slightly more power in the vertical than in the horizontal meridian (−2.8 ± 0.7 D (SEM)). Astigmatism flipped sign and increased with eccentricity to reach +6.1 ± 2.1 D (SEM) at 33.5 deg off-axis, as expected from off-axis astigmatism. Even though this value appears high, it was still 2.5 times lower than in the glass lens. A ZEMAX model of a lens with a homogeneous index and with surface profiles taken of the natural chicken lens revealed even higher levels of off-axis astigmatism. Obviously, the natural chicken lens displays much less off-axis astigmatism than a glass lens with similar power. Since its shape does not explain the low off-axis astigmatism, it must be due to a refined internal refractive index structure.

1. Introduction

In an emmetropic vertebrate eye, peripheral refractive errors are low, indicating that the image shell is matched to the shape of the retina (human: Jaeken & Artal, 2012; Millodot, 1981; Seidemann, Schaeffel, Guirao, Lopez-Gil, & Artal, 2002, monkey: Hung, Ramamirtham, Huang, Qiao-Grider, & Smith, 2008). Still, inter-individual variability of refractive errors is higher in the periphery than in the fovea (human: Jaeken & Artal, 2012; Tabernero et al., 2012, monkey: Franz-Odendaal, 2008). While on-axis astigmatism declines sharply during early childhood (Howland, Atkinson, Braddick, & French, 1978; Mohindra, Held, Gwiazda, & Brill, 1978), considerable amounts of off-axis astigmatism are not corrected during emmetropization, at least in primate eyes (Gustafsson, Terenius, Buchheister, & Unsbo, 2001; Williams, Artal, Navarro, McMahon, & Brainard, 1996). Since peripheral astigmatism persists during development, questions arise as to whether (1) its correction is not possible with the designs that are available in natural visual systems (2) it is not worthwhile to correct it because neural visual acuity is too poor in the periphery to gain anything, or whether (3) it may, in fact, have a physiological function, like a role in emmetropization. Howland, at the 13th International Myopia Conference 2010, wrote “it is possible that the magnitude and sign of the off-axis astigmatism is estimated by the peripheral primate retina and is used to control emmetropizing growth of the eye”. Charman (2011) suggested that “... emmetropization may be guided by imagery in the peripheral retina, perhaps making use of oblique astigmatism.” However, eyes of chickens display little off-axis astigmatism (Maier, Howland, Ohlendorf, Wahl, & Schaeffel, 2015). Their emmetropization is nevertheless fast and accurate (Wallman, Adams, & Trachtman, 1981; Wallman & Winawer, 2004). Apparently, off-axis astigmatism is at least not necessary for emmetropization in chickens.

The lack of peripheral astigmatism in the chicken eye was not explained by a special corneal shape since it was found that the chicken cornea represents a scaled version of the human cornea (Maier et al., 2015). Therefore, it must be due to the design of the crystalline lens. As in humans, the lens of the chicken is flat in shape and is assumed to exhibit a gradient refractive index (GRIN) (Schaeffel & Howland, 1988). However, it has also been proposed that birds and reptiles have no GRIN at all because fiber cell compaction is lacking during development (Augusteyn, 2014a,

---

* Corresponding author.
E-mail address: frank.schaeffel@uni-tuebingen.de (F. Schaeffel).

http://dx.doi.org/10.1016/j.visres.2016.10.005
0042-6989/

2014b). The exact profile of the GRIN is not known for chickens. In the present study, we investigated the off-axis optics in isolated chicken lenses to learn more about their optical design.

2. Methods

2.1. Animals

Thirteen one-day old male chickens were obtained from a local hatchery (chicken farm Weiss, Kirchberg, Germany). They were kept in groups of five in large cages under a 12 h light/dark cycle, at a constant room temperature of 30° Celsius during the first 7 days and 28° Celsius thereafter. Food and water were available *ad libitum*. The treatment of the chickens was approved by the Commission for Animal Welfare of the Medical Faculty of the University of Tuebingen and in agreement with the ARVO statement for the use of Animals in Ophthalmic and Vision Research. Measurements were done on 24 chicken lenses (Table 1).

Table 1
Number of chicken lenses measured at different ages.

Age [days]	Chickens	Eyes
10	2	3
16	2	3
21	2	4
27	1	2
28	2	4
29	2	4
43	2	4

2.2. Preparation

Chickens were sacrificed by an overdose of diethyl ether inhalation and decapitated. A short line was drawn with a tissue marker on the sclera from the inner canthus towards the estimated pupil center. A second line was drawn in the superior quadrant of the sclera, perpendicular to the first mark. Subsequently, the eye lids were removed, the eye ball gently pulled out of the orbit, the optic nerve cut and the eye ball completely removed from the orbit. While the fellow eye was removed, the initially dissected eye was stored in ice-chilled ringer solution. One eye was randomly selected for the first measurements. The eye ball was aligned with its optical axis in the horizontal plane, kept in place with a pair of tweezers and then cut in half with a razor plate just behind the scleral ossicles. Due to the rigidity of the scleral ossicles, no visible mechanical distortion occurred in the anterior eye segment. The posterior half of the eye cup was discarded. The anterior segment was placed on a circular conical hole (see Fig. 1C), the cornea facing down. Special care was taken to align the pupil center with the center of the hole and to achieve a symmetrical protrusion of the cornea on the bottom side of the holder. Furthermore, the eye segment was aligned so that the horizontal line drawn with the tissue marker matched the plane in which the laser beams were projected from different angles of eccentricity. All these adjustments were done by the operator as judged by eye but the estimated angular alignment errors were less than 3 deg.

2.3. Experimental setup

The conical circular hole of the holder had a diameter of 15 mm diameter on the upper and 10 mm on the lower side. It was drilled in a polyacrylic plate of 3 mm thickness (Fig. 1C). The angle of the slope of the wall of the hole was 50.2 deg, relative to the plane of the plate, and matched about the slope of the limbus of the eye. No rotationally asymmetric forces could act on the tissue to induce astigmatism. The anterior eye cup rested on the limbus, not the cornea, so there was no contact of the cornea with the holder, no matter the age. The holder carrying the anterior eye segment was then transferred in a polyacrylic box filled with *Aqua bidest.* Several drops of Eosin Y, fluorescing at 530 nm, were added to the water to make the laser beams visible. The box was attached to a horizontal metal rod and height and lateral position could be controlled by an adjustable stage. The laser diode (15.0 mW, "Fixed Focus Green Laser Diode Module", 532 nm, Edmund Optics, Karlsruhe, Germany) could be rotated around the box by a metal lever to vary the angle of incidence of the laser beams (Fig. 1A and B). Nine thin parallel beams with a diameter of 0.2 mm and an inter-beam distance of 0.3 mm were generated using a beam widener (5X Beam Expander 532 nm, Edmund Optics, Karlsruhe, Germany) and multiple apertures (custom made).

Crystalline lenses were measured under thirteen different angles, ranging from +40.2 deg to −40.2 deg in steps of 6.7 deg. The optical axis of the eye (zero angle of eccentricity) was estimated by the operator by centering the pupil in the hole of the holder. Up to eight laser beams entered the pupil but, in smaller eyes, their number could decline down to 4 or 5. For more oblique angles of incidence, the number of rays passing through the pupil declined symmetrically on both sides of the visual field until only 2–3 rays contributed in the far periphery due to the narrowing cross-sectional area of the pupil. In these cases, the point of best focus was localized by determining the thinnest common beam diameter generated by all three rays. Laser beams were clearly visible and the focal points could be determined for various angles of incidence (Fig. 1A). A RGB CCD-Camera (DFK 23UP031, USB3.0 Color Camera, The Imaging Source, Bremen, Germany) imaged the path of the laser beams. Images were superimposed in one single image (Fig. 1B) using Adobe Photoshop CS6 (Adobe Systems Incorporated, San Jose, CA, USA) to visualize the focal points. The focal lengths were determined under the assumption that the position of the principle plane (denoted in Fig. 1B as a black asterisk) did not vary with angle of incidence. But even if the position of the principle plane would have varied with angle of incidence, its position should have remained the same for rays entering in two perpendicular planes, as long as rotational symmetry of the lens can be assumed. Therefore, the dioptric values of the astigmatism were assumed to be correct.

2.4. Data analysis

Astigmatism was determined at 13 different angular positions over the horizontal visual field by comparing the positions of the focal points of sets of laser beams entering either in the horizontal or the vertical meridian. The differences in focal lengths were converted into diopters, where f_1 was the focal length in the horizontal meridian and f_2 in the vertical meridian:

$$astigmatism\ [D] = \frac{1000}{f_1[mm]} - \frac{1000}{f_2[mm]}$$

Since off-axis astigmatism causes an increase in dioptric power for rays entering in the horizontal meridian, it was assumed that relative shorter focal lengths in the horizontal meridian would be due to off-axis astigmatism. Astigmatism measured at the corresponding angular positions in both eyes of an animal was averaged. To verify the validity of the measurements, an artificial 100 D glass lens with similar power and a homogenous refractive index (EdmundOptics #63535, double-convex, uncoated, N-SF5, n = 1.6727) was also measured. The artificial lens was double-convex with symmetrical spherical surfaces on both sides with a radius of curvature of 12.71 mm, a center thickness of 3.5 mm, an edge thickness of 1.45 mm and a diameter of 10 mm. The shape of the glass lens was