# A PHYSIOLOGICAL MODEL TO MEASURE OPTICAL AND BIOPHYSICAL CHANGES DURING AVIAN ACCOMMODATION

by

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# A NOVEL *IN SITU* PHYSIOLOGICAL MODEL TO MEASURE OPTICAL AND BIOPHYSICAL CHANGES DURING AVIAN ACCOMMODATION


## ABSTRACT

A model was developed to directly measure optical and biophysical changes to the intact crystalline lens during ciliary nerve-induced accommodation. Lenticular optics during accommodation was analysed as a function of chicken age and in ametropic chicken eyes. Biophysical changes to the anterior segment of accommodating ametropic chicken eyes were assessed using the ultrasound biomicroscope. Resting state lenticular focal lengths increased as a function of age, presumably in association with growth of the eye. The amount of lenticular accommodation decreased as a function of age. The optical quality in lenses from hatchlings was poor, regardless of accommodative state, suggesting that the lens was not fully developed. In general, spherical aberration was greater with accommodation for all age groups. Lenticular focal lengths were shorter and accommodation-associated changes in focal length were smaller for form-deprived myopic eyes compared to their controls. Induction of hyperopia with +15 D spectacle lenses resulted in attenuated, but opposite effects, with lenticular focal lengths longer and accommodative changes slightly greater for treated eyes than for their controls. Lenticular spherical aberration increased with accommodation in both form-deprived and lens-treated birds, but induction of ametropia had no effect on lenticular spherical aberration in general. Accommodation was associated with a decrease in anterior chamber depth and a bulging of the lens. Changes related to induction of myopia were subtle, while changes to hyperopic eyes were often undetectable, limited by the level of resolution of the biomicroscope.

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This work is dedicated to family:

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my mom, the ballast of a family of heads-in-the-clouds Ph.D. (+1 candidate) holders and the only one with the ability to get the electric can opener working, and finally,

my sister, Audrey - Dr. (to be) Choh#3 - Hurry it up, will ya?

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## LIST OF ABBREVIATIONS/ACRONYMS

| | |
|---|---|
| ANOVA | analysis of variance |
| BVFL | back vertex focal length |
| D | dioptre(s) |
| Hz | hertz |
| mA | milliampere(s) |
| mHz | megahertz |
| mm | millimetre(s) |
| s.d. | standard deviation |
| s.e.m. | standard error of the mean |
| TS | Tyrode's solution |
| UBM | ultrasound biomicroscope |
| v/v | volume/volume |
| w/v | weight/volume |
| × | times, by |

# I. INTRODUCTION

## 1.1 A physiological model to measure optical changes during accommodation

The avian eye differs both anatomically and physiologically from human and other mammalian eyes. For example, in birds, the iris and ciliary muscles are striated. However, like mammals, ciliary muscles are innervated by postganglionic ciliary nerves, which, themselves, receive input from the parasympathetic oculomotor (III) nerve at the ciliary ganglion (Martin and Pilar, 1963). Most avian eyes undergo accommodation through direct manipulation of the lens, due, in part, to various evolutionary structural differences. The ciliary processes are much larger and the diameter of the lens is augmented by the presence of a ring of columnar epithelial cells at the equatorial periphery, called the annular pad. Lenses are soft and malleable, and the corneo-scleral sulcus, which exists as a consequence of the scleral ossicles, permits a greater range of movement. Together, these structures make it possible for contraction of the ciliary muscle to directly squeeze the lens, resulting in changes to lenticular surface curvatures and an increase in refractive power.

Although it has been well-established that the crystalline lens plays a major role in vertebrate accommodation, imparting some, if not all of the accommodative power to the eye depending on species, its optical properties during accommodation have been difficult to assess, partly because the lens is located within the eye. Some investigators have examined the lens during accommodation using whole-field electrical stimulation (Glasser *et al.*, 1995; Sivak *et al.*, 1985; Sivak *et al.*, 1986b), pharmacological agents (Glasser and Howland, 1995) and a zonule-stretching apparatus (Glasser and Campbell, 1998). But, as these artificial *in vitro* techniques involved detachment of the ciliary nerve, accommodation was elicited by means of a mechanism other than that which occurs *in vivo*. Moreover, recent evidence

shows that the chicken lens contains actin and myosin in addition to other contractile proteins, indicating that the crystalline lens may not play the passive role during accommodation to which it has been ascribed (Bassnett *et al.*, 1999). In addition, evidence exists showing that receptors for acetylcholine, a neurotransmitter that elicits contraction of skeletal muscle at neuromuscular junctions, are present in the lens (Thomas *et al.*, 1998). Although it is probable that acetylcholine is not involved in an active contraction of the lens, the potential for pharmacological or whole-field electrical stimulation to directly affect the lens in an unknown manner renders these methods inappropriate. In a study by Glasser *et al.* (Glasser *et al.*, 1995), optical properties of the lens *in vivo* were examined during electrical stimulation of the Edinger-Westphal nucleus, the part of the brain that signals accommodation. Retinoscopy and keratometry were used to measure changes to the total power of the eye and to corneal curvatures, respectively, and therefore, lenticular optics was not directly measured but was instead, inferred. Moreover, because the lens was enclosed by the rest of the eye, changes to optical quality of the lens during accommodation could not be measured.

This study was therefore undertaken to develop a physiological accommodation model with which optical changes to the intact chicken crystalline lens can be measured directly and concomitantly with accommodation that has been induced via a natural *in vivo* pathway, *i.e.*, by electrical stimulation of the ciliary nerve. Controversy exists over the aetiology of presbyopia, the decline in accommodation that is associated with age, with a body of evidence showing that compromise to one, some or all of the individual accommodative components may play a role. The *in situ* accommodation model, described herein, was therefore used to assess the effect of age on the functional optics and spherical aberration of the crystalline lens.

Chickens are the ideal animal model with which to test optical properties of the lens during accommodation since they possess a direct accommodation mechanism, as mentioned above. They mature rapidly and are precocial animals, using their eyes the day of hatching. In addition, although they are the predominant animal with which to test the effects of induced ametropia on growth and refractive development of the eye, accommodation-associated characteristics of the lens, and to a lesser extent, of the rest of the eye, is lacking (see below).

## 1.2 Experimentally-induced ametropia

In normal young animals, growth of the eye is modulated to ensure that the image focal plane coincides with the retina. This process, called emmetropisation, is the underlying basis of an extensive body of work which shows that the development of refractive errors may be influenced by specific environmental visual cues. Ametropias (myopia and hyperopia) have been experimentally induced in a variety of animals, including, but not limited to, chickens (Irving *et al.*, 1992; Schaeffel *et al.*, 1988), tree shrews (Norton *et al.*, 1999; Siegwart and Norton, 1993) and monkeys (Hung *et al.*, 1995). Evidence exists which shows that regulation of growth of the eye is at the level of the retina (Troilo *et al.*, 1987), and furthermore, that the retina is able to discriminate between the different, specific visual cues. Thus, myopia, usually manifested as an increased axial length of the globe, is induced by form-deprivation of the eye or by imposition of a hyperopic defocus using negative (convex) spectacle lenses, while hyperopia, manifested by shorter axial lengths and choroidal thickening, is induced by exposure to constant light or to a myopic defocus by application

of positive (concave) lenses (Irving *et al.*, 1992; Schaeffel *et al.*, 1988; Wildsoet and Wallman, 1995).

Controversy exists over the role that accommodation may play in mediating emmetropisation. Studies showing that optic nerve-sectioned eyes elongate to become more myopic in response to form-deprivation (Troilo *et al.*, 1987) and positive lenses (Wildsoet and Wallman, 1995) indicate that control of emmetropisation is at the level of the retina and that connection to the brain is not necessary for emmetropisation to occur. In contrast, several studies support the idea that accommodation may be a driving force for growth of the eye to a myopic refractive state. In humans, near-work, which includes reading, writing or any other task requiring accommodation, has been associated with the development of myopia, and population studies indicate a high prevalence of myopia in students from some Asian countries, which are known to have exacting educational standards (Lin *et al.*, 1999; Saw *et al.*, 2000; Wu *et al.*, 2001). While there is support for the idea that myopia may be genetically inheritable, the Barrow, Alaska study showed that school-attending grandchildren of nomadic Inuits tended to be more myopic than their ancestors, who tended to be hyperopic (Young *et al.*, 1969), suggesting that environmental visual factors can influence growth of the eye. Moreover, it has been suggested that in chicks, accommodation may be the mechanism by which the retina detects the type of defocus and mediates refractive development, where eyes imposed with a diverging negative lens accommodate more and become myopic, while those imposed with a positive lens accommodate less and become hyperopic (Schaeffel *et al.*, 1988). Observations that chicks imposed with different spectacle lenses accommodated to become functionally emmetropic lend support to this idea (Irving *et al.*, 1992; Schaeffel *et al.*, 1988; Wildsoet and Wallman, 1995). Furthermore, findings by

Wildsoet and Wallman (Wildsoet and Wallman, 1995) that optic nerve-sectioned eyes did not fully compensate for negative spectacle lenses (hyperopic defocus) suggest that the brain, of which the accommodative apparatus is part, may be required to detect, and therefore regulate, hyperopic blur.

As mentioned above, the crystalline lens is a primary contributor to accommodation, but its role in experimentally-induced ametropias remains unclear. In fact, the effect on the lens itself remains somewhat controversial, with most investigations showing little or no effect in lenticular weight, focal length, or axial thickness. However, recent work by Priolo and colleagues (Priolo *et al.*, 2000) indicates that the optics of lenses from form-deprived eyes and eyes treated with +10 D spectacle lenses are degraded, showing that the crystalline lens, too, is an intraocular structure which may be affected by experimentally-induced ametropias. Given the potential importance of accommodation in experimentally-induced ametropias and of the lens in accommodation, this study was undertaken to determine whether experimentally-induced ametropias have an effect on lenticular accommodative function or on lenticular spherical aberration.

### 1.3 Ultrasound biomicroscopy

While the model described herein was developed to assess optical properties of the crystalline lens during accommodation, it should be noted that the biophysical characteristics of the lens and of other intraocular structures in the chicken eye during accommodation have also been continually difficult to assess. Either measurements are indirect in nature, and therefore can include known and unknown confounding effects, or measurements are made directly, but at the cost of altering other structures within the eye.

Ultrasound biomicroscopy is a relatively recent technology that has been developed to measure anatomical structures in a non-invasive manner. Like (B-scan) ultrasonography, two-dimensional images of the structure under investigation are displayed, but the resolution of the ultrasound biomicroscope (UBM) is much higher. In addition to the advantage of being able to directly measure structures within the eye without having to disrupt any surrounding tissues, collection of data using the UBM is based on sound wave echoes rather than on optics, which in the latter case, can give rise to confounding effects because of optically refractive components in the eye. For example, studies on the optics of the ageing human lens using Scheimpflug photography often involves correction for distortions from the camera and/or both corneal surfaces (Dubbelman *et al.*, 2001; Koretz and Cook, 2001). Although use of the ultrasound biomicroscope may also require corrections to account for the densities of the various ocular structures, adjustments are simpler and less mathematically taxing. Moreover, the UBM is capable of collecting data in real-time, a distinct advantage when attempting to measure changes during accommodation. Given these advantages, this study was undertaken to determine the biophysical characteristics of lenses from myopic and hyperopic eyes, and to quantify changes to these lenses during accommodation using the ultrasound biomicroscope.

## II. METHODS

### 2.1 Electrophysiological apparatus

Silver wires (AM Systems Inc. 7825) were sanded with fine sand paper to remove oxidised silver coating, then immersed in fresh 100% bleach. After 15 minutes, the wires were removed from the chloride-plating solution and rinsed in deionised water (10 minutes). Silver wire was placed in the lumen of Tygon® tubing (S-50-HL Class VI) that was attached at the distal end to a plastic syringe (3cc Becton-Dickinson 9585). The distal end of the silver wire was passed through a hole in the wall of the tubing, just proximal to the syringe and soldered to the positive pole of a stimulating wire. The hole was sealed using epoxy. A second silver wire was wrapped around the outside of the Tygon tubing and electrode tip, with the end proximal to the syringe soldered to the ground or negative pole of stimulating wire.

Suction electrode tips were made by gently heating some Tygon tubing (AAQ02133) and slowly pulling the ends so that the middle, heated portion of the tubing was attenuated. Tubing was allowed to cool, then cut at the smallest diameter. As ciliary ganglia vary in size, depending on the age of the chicken, several plastic tips were made and each was trimmed until ciliary ganglia from chickens of various ages could be suctioned snugly into the tips. Plastic tips were connected to the suction electrode tubing via a small plastic connector (Cole-Parmer Instrument Co. 06359-07).

Positive and negative poles of the stimulation wires were attached to their respective posts of a Photo-Optic Stimulation Isolation Unit (Grass PSIU6), itself attached to an S43 Grass stimulator.

## 2.2 Experimental Procedures

### *2.2.1 Birds*

White leghorn chickens (*Gallus domesticus*) were obtained the day of hatching and were sacrificed the same day (day 0), after 7 days, 14 days or 6 weeks. Chicks not immediately used were reared in stainless steel chicken brooders for a maximum of 14 days. After 2 weeks, birds to be kept for 6 weeks were moved to a room with wood shavings flooring. All chicks were fed chick starter and water *ad libitum*. Fluorescent lighting in the room was set to an artificial diurnal (14 h light/10 h dark) schedule. One- and two-year old chickens were obtained from the Poultry Research Centre at the University of Guelph.

Refractive errors in both eyes of hatchling chicks to be used for studying the effects of experimentally-induced ametropias on accommodation were measured using streak retinoscopy. These chicks were then unilaterally fitted with a velcro ring and translucent goggles or +15 D goggles, to induce form-deprived myopia and hyperopia, respectively. Ungoggled, contralateral eyes served as controls for the goggling procedure. Both eyes were again refracted 7 days later, prior to sacrifice.

### *2.2.2 Dissection*

Chickens were sacrificed by decapitation and heads were bissected sagitally. Dissections were carried out with eyes submerged in oxygenated (95% $O_2$:5% $CO_2$) Tyrode's solution (TS: 134 mM NaCl, 3 mM KCl, $NaHCO_3$, 1 mM $MgCl_2$, 3 mM $CaCl_2$). For each eye, the optic nerve was cut to expose the underlying ciliary nerve and ganglion, and the ciliary nerve and ganglion carefully extricated from surrounding tissue before enucleation of the eye. Incisions were made at the posterior of the globe to ensure easy access of oxygenated solution (TS) to the lens. Eyes to be measured using the ultrasound biomicros-

crope were left intact. All eyes were left in oxygenated Tyrode's solution until they were to be scanned.

*2.2.3 Optical measurements of the lens during accommodation*

Prior to scanning, the posterior portion of each globe was removed except for a wedge containing the ciliary nerves and ganglion. Eyes were pinned to a Sylgard® (Dow Corning 184) washer using minutiae pins or fine needles. The Sylgard washer, with pinned eye, was placed into a silicon base mould which formed the bottom of a chamber. The chamber was completed by fitting the base mould with a rectangularly-shaped glass tube, with a second, smaller open-ended tube attached to one of the glass piece walls. The suction electrode with various diameters of Tygon tubing tips to allow for a tight fit with the ciliary ganglion, was passed through the open-ended tube and the ciliary ganglion was suctioned into the pipette tip. The rest of the open-ended tube was filled with petroleum jelly to act as a temporary plug. The chamber was filled with 8% (v/v) fetal bovine serum in Tyrode's solution in order to visualise the refracted beams and to neutralise the optical effects of the cornea.

Lenses were scanned using a redesigned scanning laser monitor (Sivak *et al.*, 1986a). In brief, a low power helium-neon laser beam was passed up through a small circular window at the bottom of the scanner, at various motor-controlled x,y coordinates from the centre. The chamber, consisting of the mould and rectangular glass piece, and containing the eye, was placed in a slot above the laser and beams were captured by digital cameras. Prior to scanning, the optical axis of the lens (slope of beam vertical, or equal to 0) was determined by ScanTox® (v. 1.4.48), a computer program also responsible for controlling the position of the laser and for calculations of back vertex focal lengths. Eyes were scanned at various

eccentricities from the optical axis and back vertex focal lengths were recorded and stored on the computer. The back vertex for each lens was pre-determined from a camera image. eccentricities from the optical axis, the line that passes through the centre of the lens.

For each eye, lenses were scanned before stimulation, with stimulation, then finally in a post-stimulation relaxed state, and the data collected represented, respectively, the resting, accommodating and recovering states of the eye. Stimulus pulses were typically 0.3 ms at 30 Hz, with current held between 0.1 to 0.15 mA for eyes from young chickens, or 10× this current for one and two year-old chickens. Measurements were made for maximal irideal contractions, as assessed by eye prior to scanning. For scans during accommodation, the eye was stimulated prior to toggling the computer program to capture the beam image at each eccentricity while for scans in a non-accommodating state, images were captured without stopping the step-motor. Step sizes were selected to ensure that the number of beams passing through the eye was relatively consistent, regardless of age (or size) of the chicken. Step sizes were 0.10 mm, 0.13 mm, 0.15 mm, 0.24 mm for 0 day, 7 day, 14 day and 6 week old chickens respectively, and 0.29 mm for both one and two year old chickens (Table 1). Step sizes for lenses from 7 day old ametropic eyes and their controls were kept at 0.13 mm. During collection of the data, the three most central rays were omitted to avoid spurious variability associated with sutures, areas of disruption where the lens fibres meet at the anterior and posterior poles (Bantseev *et al.*, 1999; Kuszak *et al.*, 1994; Sivak *et al.*, 1994).

*2.2.4 Biophysical measurements of the anterior segment during accommodation*

Intact eyes were placed into the bevel of a cube-like Sylgard® washer with the cornea facing up. The eye and washer were placed into chamber consisting of a silicone mould base

fitted with a rectangularly-shaped glass tube that had a small, open-ended tube attached to one of the walls. The handmade suction electrode was passed through the smaller tube of the glass piece and through a hole located at the side of the Sygard washer that opened up into the space evacuated by the bevel. The rest of the small tube was filled with petroleum jelly to act as a temporary plug. The ciliary nerve was suctioned into the electrode tip and the eye was submersed in Tyrode's solution.

A transducer (50 MHz) of the biomicroscope was lowered from above the chamber until it was in the Tyrode's solution above the cornea. Ultrasound biomicrographs of the anterior segment of the eye, from the front surface of the cornea to the posterior pole of the lens were collected at a medium resolution (5 × 5 mm of visible ocular tissue) for eyes at rest and during stimulation (30 Hz, 0.1 - 1.5 mA). Images were collected at high resolution (2.5 × 2.5 mm) for front lenticular surfaces.

## 2.3 Analysis of the data

### *2.3.1 Back vertex focal lengths*

Data consisting of beam position, back vertex focal length and beam intensity were transferred to a spreadsheet program for analysis. For all eyes, the paraxial beams on either side of the optical axis and along the optical axis were omitted since the central regions of the all lenses are optically compromised by the presence of sutures, areas of disruption where the lens fibres meet at the anterior and posterior poles. Optical scans at the sutures result in focal lengths that are either unpredictably too short or too long, and are therefore inappropriate for inclusion. Given that accommodation is often associated with constriction of the

pupil and may therefore result in a lower number of points scanned across the lens, means were adjusted to match aperture sizes observed during accommodation.

*2.3.2 Ultrasound biomicrographs*

Ultrasound biomicrographs were exported as 256 × 256 pixel .pcx files or grabbed from a video tape recording, then transferred to a computer for analysis. Medium resolution images were used to measure lenticular thickness and anterior chamber depth. Corneal thicknesses were also assessed as a measure of the amount of user-error. All three measurements were made along the same axis. Front lenticular surface curvatures were traced 5× on high resolution images. The resulting x,y coordinates were used to determine the best-fitting parabolic function $y=Ax^2+Bx+C$, from which the A-coefficient, which determines steepness of a parabola was used to represent front lenticular surface curvatures.

## 2.4 Statistical tests

To examine the effects of age and accommodation, two-way repeated measures ANOVA (analysis of variance) tests at two-tailed $\alpha$ levels of 0.05 were used, with age as the independent, between-subjects factor and accommodative state as the repeated, dependent, within-subjects factor. Greenhouse-Geisser and Huynh-Feldt epsilon estimates were used to detect within-subjects differences and interaction (Levine, 1991). Comparisons of the means as a function of age were analysed using one-way analysis of variance (ANOVA), followed by the honestly-significant difference (HSD-)Tukey test. Changes associated with accommodation were assessed using one-way repeated measures ANOVA, followed by paired t-tests with a Bonferroni correction to account for multiple testing.

To examine the effects of induced ametropias and accommodation, two-way repeated measures ANOVA (analysis of variance) tests at two-tailed $\alpha$ levels of were used, with refractive error as the independent, between subjects factor and accommodative state as the repeated, dependent within-subjects factor. Greenhouse-Geisser or Huyhn-Feldt epsilon values were again used for detection of within-subjects differences or interaction, where appropriate (Levine, 1991). Comparisons between refractive errors and their respective controls were analysed using t-tests, while changes associated with accommodation were assessed using one-way repeated measures ANOVA, followed by paired t-tests with a Bonferroni correction to account for multiple testing. For all tests, if the data was not normally distributed, the tests on ranks (nonparametric) were used.

## III. RESULTS

### 3.1 Effects of age

*3.1.1 Lenticular accommodation*

Although eyes were scanned at consistent step sizes (See Methods; also Table 1), within each age group, eyes showed a range of irideal aperture sizes, inherently, as well as associated with accommodation, which made variations in the number of eccentric points scanned across the lens difficult to control for (Table 1). Comparison of beam number ranges shows that the range differences for younger chickens were at least double those of the 1- and 2-year old chickens (Table 1, compare range of 12 to 18 for hatchlings to range of 12 to 14 for one- and two-year olds). Nevertheless, the mean number of eccentric points scanned across the lens was more or less consistent with age, although this was not tested for. For relaxed eyes, both prior to and after stimulation, the mean number of beams passing through the pupil ranged from 13 to 16, with slightly lower means (13 and 14) for eyes from

**Table 1** - Step sizes from the optical centre of the lens, and the number (mean: range) of beams entering the pupil for each state of accommodation as a function of age

| Age | Step size (mm) | Mean and range of the number of beams entering the pupil for each state of accommodation | | |
|---|---|---|---|---|
| | | pre | during | post |
| 0 d (n=18) | 0.1 | 16: 12 to 18 | 11: 8 to 14 | 15: 12 to 18 |
| 7 d (n=17) | 0.13 | 16: 13 to 18 | 13: 10 to 17 | 16: 13 to 19 |
| 14 d (n=23) | 0.15 | 15: 13 to 18 | 13: 10 to 17 | 15: 14 to 18 |
| 6 w (n=22) | 0.24 | 13: 11 to 15 | 12: 8 to 14 | 13: 11 to 15 |
| 1 y (n=9) | 0.29 | 13: 12 to 14 | 13: 11 to 14 | 13: 12 to 14 |
| 2 y (n=12) | 0.29 | 14: 12 to 14 | 13: 12 to 14 | 14: 12 to 14 |

chickens 6 weeks and older (Table 1). During accommodation, the mean number of eccentric points were closer, ranging from 11 to 13. No trend was observed, since the smallest means (11 and 12) occurred for eyes from hatchlings and 6 week old chickens, respectively.

Without corrections for pupil size, the back vertex focal lengths (BVFLs) for lenses from hatchling chicks prior to stimulation averaged to 19.76 ± 0.47 (s.e.m.) mm (Table 2). The mean BVFL decreased with accommodation, averaging to 15.05 ± 0.51 mm, then increased to 19.43 ± 0.47 mm in the post-stimulated state. For 7 day old chickens, the average BVFL for lenses were slightly longer, beginning at 20.28 ± 0.29 mm in the pre-stimulus state, decreasing to 17.02 ± 0.37 mm during accommodation, then increasing up to

**Table 2** - Mean back vertex focal length and focal length range for each accommodative state as a function of age.

| Age | Mean focal lengths ± s.e.m. (mm) for each state of accommodation and focal length ranges (mm) in parenthesis | | |
|---|---|---|---|
| | pre | during | post |
| 0 d (n=18) | 19.76 ± 0.47 (12.57 to 29.98) | 15.05 ± 0.51 (7.73 to 27.87) | 19.43 ± 0.47 (12.84 to 27.25) |
| 7 d (n=17) | 20.28 ± 0.29 (11.57 to 27.38) | 17.02 ± 0.37 (12.37 to 23.48) | 20.09 ± 0.28 (13.06 to 27.15) |
| 14 d (n=23) | 23.27 ± 0.35 (14.50 to 35.83) | 19.35 ± 0.35 (11.93 to 28.01) | 22.84 ± 0.33 (14.79 to 35.35) |
| 6 w (n=22) | 29.15 ± 0.37 (17.79 to 47.06) | 25.55 ± 0.44 (19.86 to 40.18) | 28.94 ± 0.36 (19.72 to 47.02) |
| 1 y (n=9) | 30.22 ± 0.29 (23.62 to 46.12) | 28.89 ± 0.39 (23.12 to 44.86) | 29.93 ± 0.27 (23.65 to 45.57) |
| 2 y (n=12) | 29.96 ± 0.51 (23.63 to 47.51) | 28.58 ± 0.47 (22.87 to 48.72) | 29.72 ± 0.50 (23.33 to 50.65) |

20.09 ± 0.28 mm during the post-stimulus state. For 14 day old chickens, mean BVFLs were 23.27 ± 0.35 mm, 19.35 ± 0.35 mm and 22.84 ● 0.33 mm, for eyes in the pre-stimulus, stimulated, and post-stimulus states respectively. BVFLs for 6 week old chickens were much longer, with averages of 29.15 ± 0.37 mm, 25.55 ± 0.44 mm and 28.94 ± 0.36 mm for pre-stimulus, stimulated and post-stimulus states, respectively. For eyes from one year old chickens, mean BVFLs were 30.22 ± 0.29 for eyes prior to stimulation, 28.89 ± 0.39 mm during accommodation, and 29.93 ± 0.27 mm post-stimulus. For two year old chickens, the BVFLs were slightly shorter, with means of 29.96 ± 0.51 mm, 28.58 ± 0.47 mm and 29.72 ± 0.50 mm, for pre-stimulus, stimulated and post-stimulus states, respectively.

All mean lenticular back vertex focal lengths were adjusted for a constant aperture size prior to comparison (Fig. 1). A two-way repeated measures ANOVA revealed differences in the mean back vertex focal length as a function of both age ($p=0.000$), and accommodation ($p=0.000$). Significant interaction was also detected between the two factors ($p=0.000$) suggesting that lenticular focal lengths at the different accommodative states varied depending on the age of the chicken. Use of a one-way ANOVA revealed differences in mean pre-stimulus back vertex focal lengths as a function of chicken age ($p=0.000$). Specifically, there was an increase in the mean focal length at 14 days, with each of the means at 0 and 7 days significantly shorter than that at 14 days (Fig. 1). A second increase in mean focal length occurred at 6 weeks, with means for 6 week-, one year- and two year old chickens all significantly greater than those for chickens at 0, 7 and 14 days ($p<0.05$; HSD-Tukey test). No differences were detected between means for 0 and 7 day old chickens, or between means for 6 week, one- and two year old chickens. Although focal lengths for two year old chickens were slightly shorter than those for one year old chickens (Fig. 1;

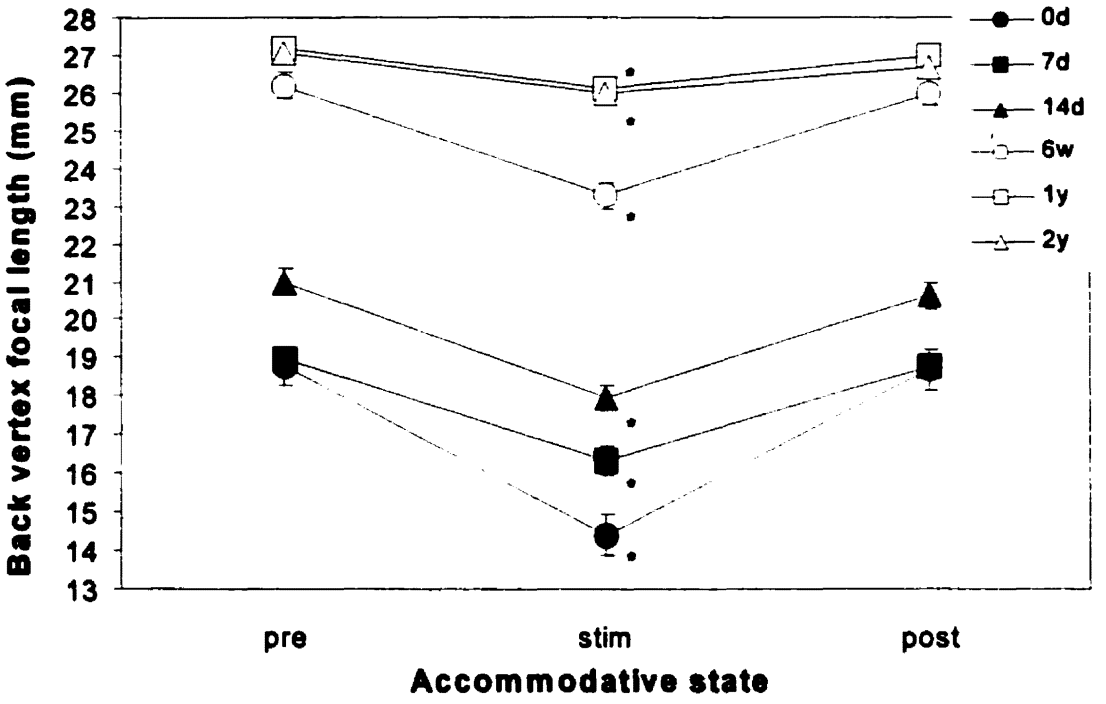



**Figure 1** - Mean back vertex focal lengths (± s.e.m.), adjusted for constant aperture size, for lenses from chickens aged 0 days (filled circle), 7 days (filled square), 14 days (filled triangle), 6 weeks (open circle), 1 year (open square), and 2 years (open triangle) old, for each accommodative state. Some error bars are covered by points on the graph. Within each age group, means denoted by asterisks were significantly shorter than those not marked ($p<0.05$; one-way repeated measures ANOVA with Bonferroni multiple comparison test). Note age-associated increases in mean back vertex focal lengths. For other comparisons, please see text.

compare values 29.96 ± 0.51 mm versus 30.22 ■ 0.29 mm, respectively), this difference was not significant. Together the results verify the assumption that the resting state focal length of the lens increases with age, presumably in association with normal axial growth or elongation of the eye (Priolo *et al.*, 1999).

For all age groups, mean lenticular focal lengths varied as a function of accommodation ($p=0.000$ for all groups except at one- and two years which were at $p=0.001$; one-way repeated measures ANOVA) (Fig. 1), with focal lengths during stimulation significantly shorter than those during the pre-stimulus and post-stimulus states ($p<0.05$; Bonferroni

multiple comparison test), indicating that stimulation of the ciliary nerve was able to induce a lenticular accommodative response. Although a hysteresis effect, shown by a difference between mean pre- and post-stimulus focal lengths, was observed in all age groups this lagging effect was only significant ($p<0.008$) in 2 year old chickens (Fig. 1).

Prior to assessment of lenticular accommodative function, all lenticular back vertex focal lengths were converted to dioptres (assuming thin lens in water, $n_w=1.33$). Changes during accommodation were quantified by subtracting dioptric values for the pre-stimulated state from those for the stimulated state. Analysis of the accommodative amplitudes as a function of chicken age revealed an age-associated reduction in the mean amount of accommodation (Fig. 2), an indication that chickens become presbyopic. Specifically,

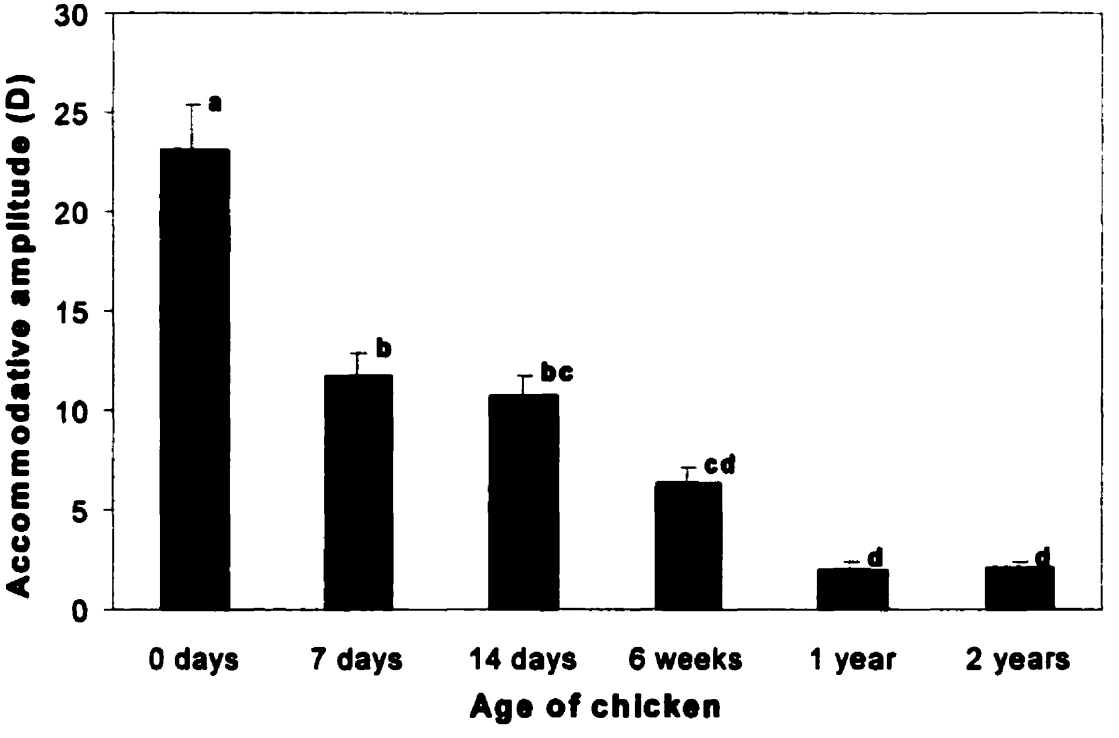



**Figure 2** - Mean change in accommodation (± s.e.m.), from pre-stimulus to stimulated state, as a function of age. Means denoted by the same letters (a,b,c,d) are statistically similar ($p>0.05$; one-way ANOVA with HSD-Tukey test). Note reduction in the amount of accommodation concomitant with increasing age, with significant reductions occurring at 7 days, and again at 1 year.

lenticular accommodation in hatchlings was significantly greater than for all other age groups, and means for 7 and 14 day old chickens were significantly greater than for 1 and 2 year old chickens ($p=0.000$; one-way ANOVA with HSD-Tukey test). The mean lenticular accommodative amplitude for 6 week old chickens, at an intermediate level between that for 14 day old chickens and those for 1 and 2 year old chickens, was not significantly different from either group. Accommodative amplitudes observed during recovery, calculated by subtraction of dioptric values for the post-stimulated state from those for stimulated state, showed exactly the same trend ($p=0.000$; one-way ANOVA with HSD-Tukey test; data not shown).

*3.1.2 Lenticular spherical aberration*

In lenses from hatchling chicks, spherical aberration (SA) varied non-monotonically between positive and negative (under-corrected and over-corrected, respectively), with an overall negative spherical aberration predominating (Fig. 3A). This pattern of spherical aberration was similar for all physiological states, with differences at each eccentricity between non-stimulus and stimulus focal length powers relatively consistent (Fig. 3A), suggesting that poor optical quality was inherent to the lens. These results were taken to indicate that the lens is not fully developed at this age. In contrast, lenses from all other age groups showed clearly negative, monotonic spherical aberrations for all physiological states (Figs. 3B,C, 4A-C), indicating improvement of lenticular optical quality from the hatchling stage (compare Fig. 3A to rest of Fig. 3B,C and all of 4).

To account for differences in aperture size (See Table 1), varying degrees of monotonic behaviour of some but not all lenses (Figs. 3 and 4), and the omission of the back vertex focal length at the optical centre (See Figs. 3 and 4; also Methods), the A-coefficient

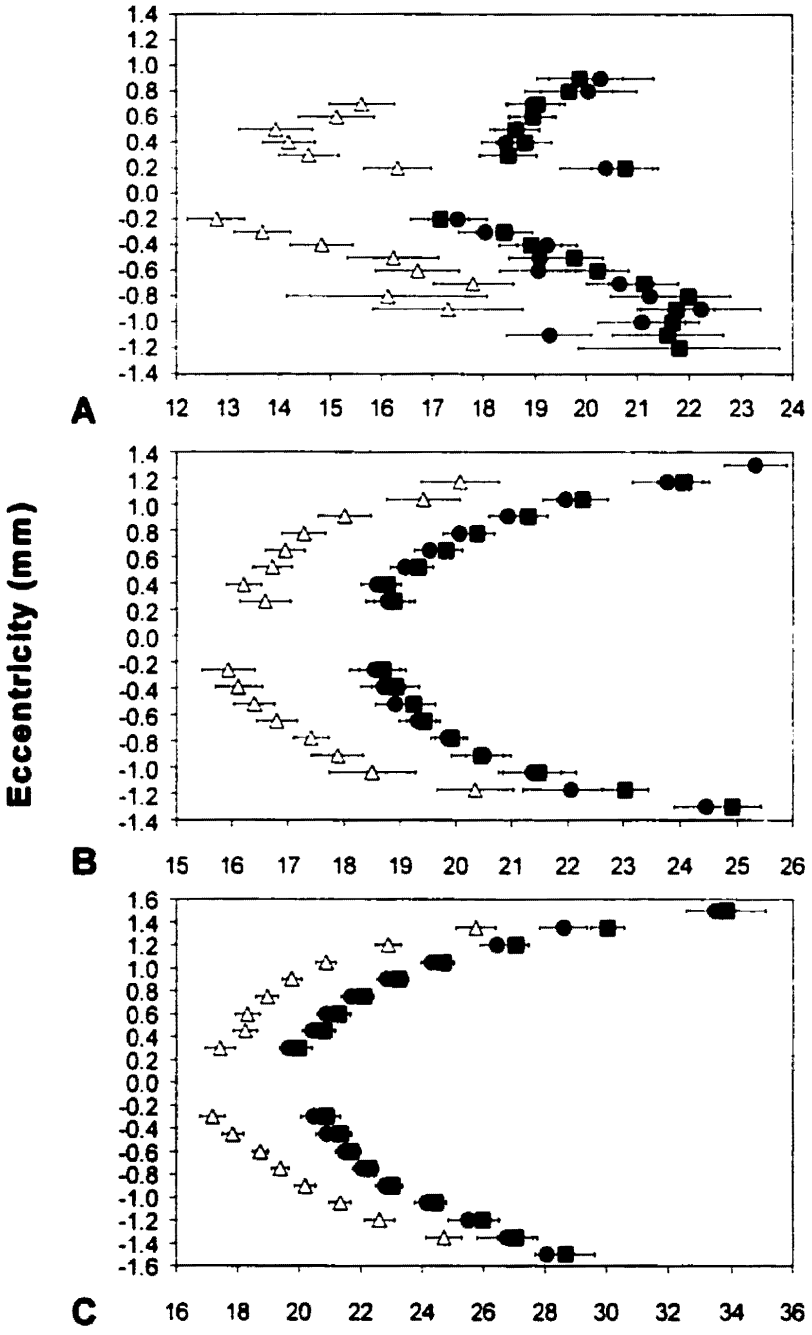



**Figure 3** - Mean back vertex focal lengths (± s.e.m.) of lenses from young chickens aged (**A**) 0 days, (**B**) 7 days, and (**C**) 14 days, plotted as a function of eccentricity. Each data point represents a mean of a minimum of 3 values measured at that eccentricity. Lenses were optically scanned prior to stimulation (square), during stimulation (triangle) and after stimulation (circle). Note that for all accommodative states, hatchling lenses show non-monotonic spherical aberrations (**A**) while spherical aberrations are monotonic and clearly negative in lenses of 7 and 14 day old chickens (**B** and **C**, respectively).

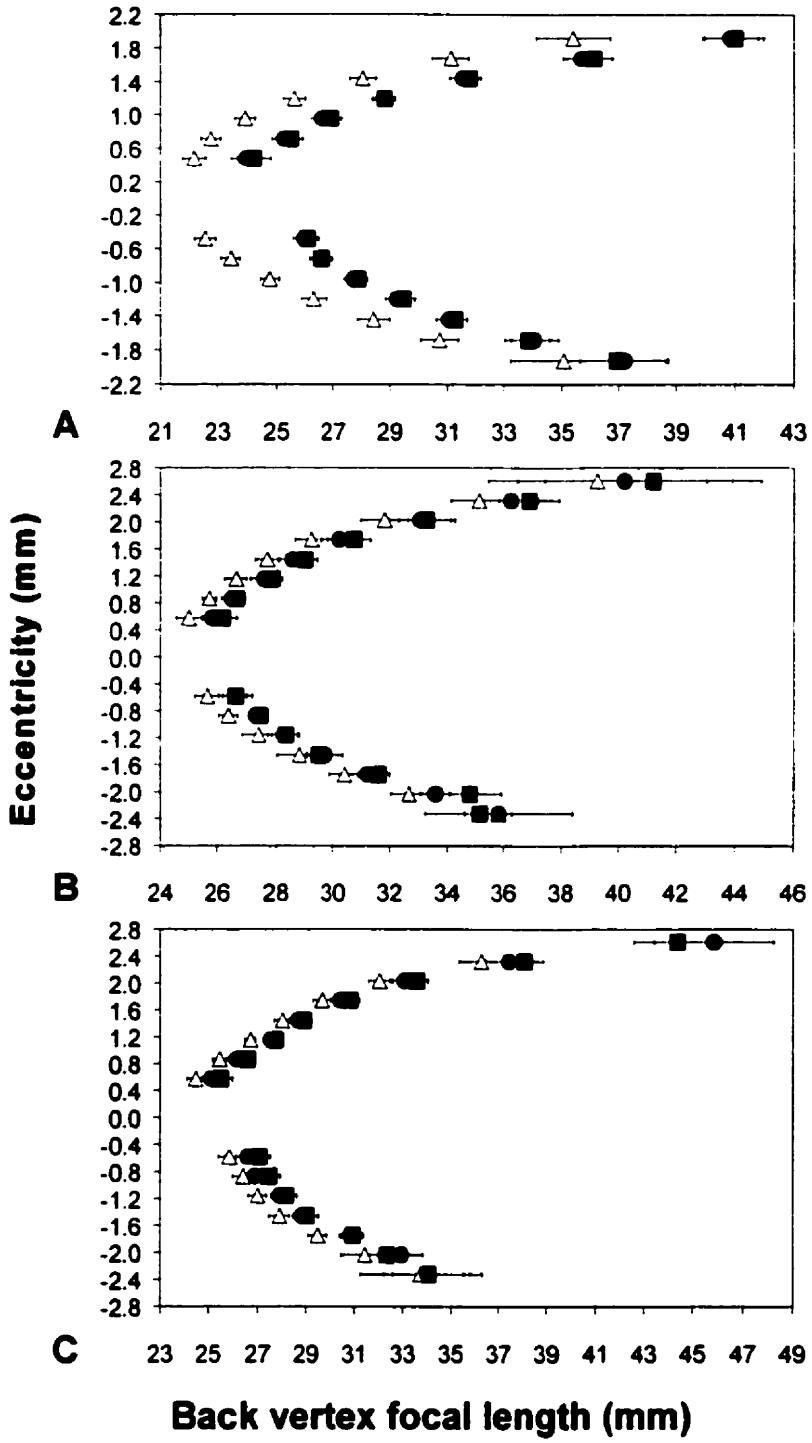



**Figure 4** - Mean back vertex focal lengths (± s.e.m.) of lenses from older chickens aged (**A**) 6 weeks, (**B**) 1 year, and (**C**) 2 years, plotted as a function of eccentricity. Each data point represents a mean of a minimum of 3 values measured at that eccentricity. Lenses were optically scanned prior to stimulation (square), during stimulation (triangle) and after stimulation (circle). Note reduced accommodative responses in 1- and 2-year old chickens (**B** and **C**, respectively).

of the parabolic function $y=Ax^2+Bx+C$ best-fitting each scan in dioptres (thin lens in water, $n_w=1.33$) was used to quantify lenticular spherical aberration (SA). Steeper parabolas, representing scans with greater spherical aberration, show higher A-coefficient values (Fig. 5). Use of a two-way repeated measures ANOVA on mean lenticular SA amounts revealed effects of both age ($p=0.000$) and accommodation ($p=0.024$), as well as interaction between the two effects ($p=0.001$) indicating that SA amounts at the various accommodative levels were dependent on the age of the chicken. Mean spherical aberration in lenses from eyes at rest decreased (or improved) as a function of age, with SA for lenses from one- and two-year

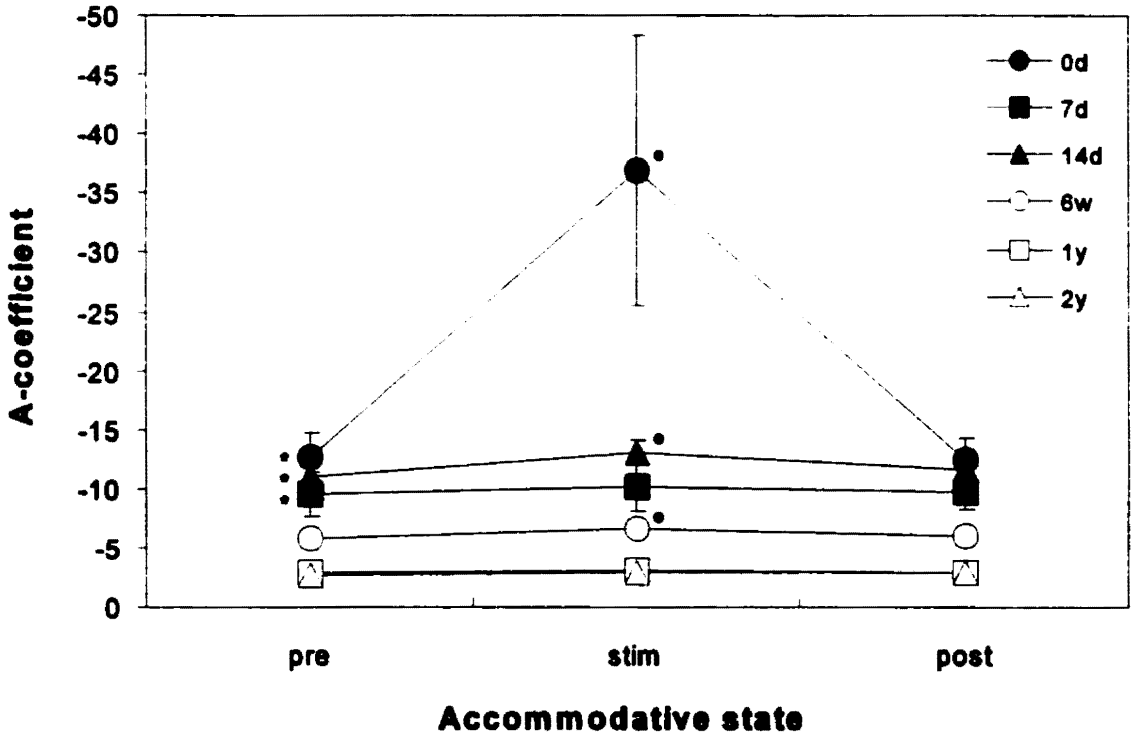



**Figure 5** - Mean parabolic A-coeffient value (± s.e.m.) representing spherical aberrations for lenses from chickens aged 0 days (filled circle), 7 days (filled square), 14 days (filled triangle), 6 weeks (open circle), 1 year (open square), and 2 years (open triangle) old. Some error bars are covered by points on the graph. Means denoted by asterisks were significantly greater than those of the same accommodative state from 1- and 2-year old chickens ($p<0.05$; one-way ANOVA with HSD-Tukey). Within each age group, means denoted by dots were significantly greater than those for the pre-stimulus state ($p<0.05$; one-way repeated measures ANOVA with Bonferroni t-tests). For other comparisons, please see text.

old chickens lower than for those from hatchling, 7 day and 14 day old chicks ($p<0.05$; one-way ANOVA with HSD-Tukey test). No differences were detected between means for 7 day and 6 week old chickens, or between means for 6 week, one- and two-year old chickens. SA amounts in recovering (post-stimulus) lenses showed the same age-associated changes and similarities ($p<0.05$; one-way ANOVA with HSD-Tukey test). SA for stimulated lenses from hatchling eyes were significantly greater than those for all other age groups ($p<0.05$; one-way ANOVA with HSD-Tukey test).

Lenticular SA amounts for lenses from stimulated eyes were higher than those from pre-stimulus eyes in all age groups, but only signficantly so in lenses from hatchling, 14 day and 6 week old chickens. In addition, differences were detected between stimulated and post-stimulus lenses in hatchling and 6 week old chickens. No trend was detectable for accommodation-associated differences in SA.

Given that a highly non-monotonic spherical aberration can be an indication of poor optical quality, the degree of non-monotonicity for each scan, defined herein as the variation from the expected back vertex focal length defined by the best-fitting parabola, was calculated as the deviation, or mean sum of squares, from its best-fitting parabola. To account for disparity in aperture size, non-monotonicity was calculated for the same number of points (6), representing about 61% of the pupil diameter of a stimulated eye for all age groups (Fig. 6). Use of a two-way repeated measures ANOVA revealed that non-monotonic deviation was affected by both age ($p=0.000$) and accommodation ($p=0.007$), with significant interaction between the two factors ($p=0.000$), indicating that the amounts of non-monotonic deviation at the different accommodative levels were dependent on the age of the chicken. Specifically, for all accommodative states, non-monotonic deviations in hatchling lenses

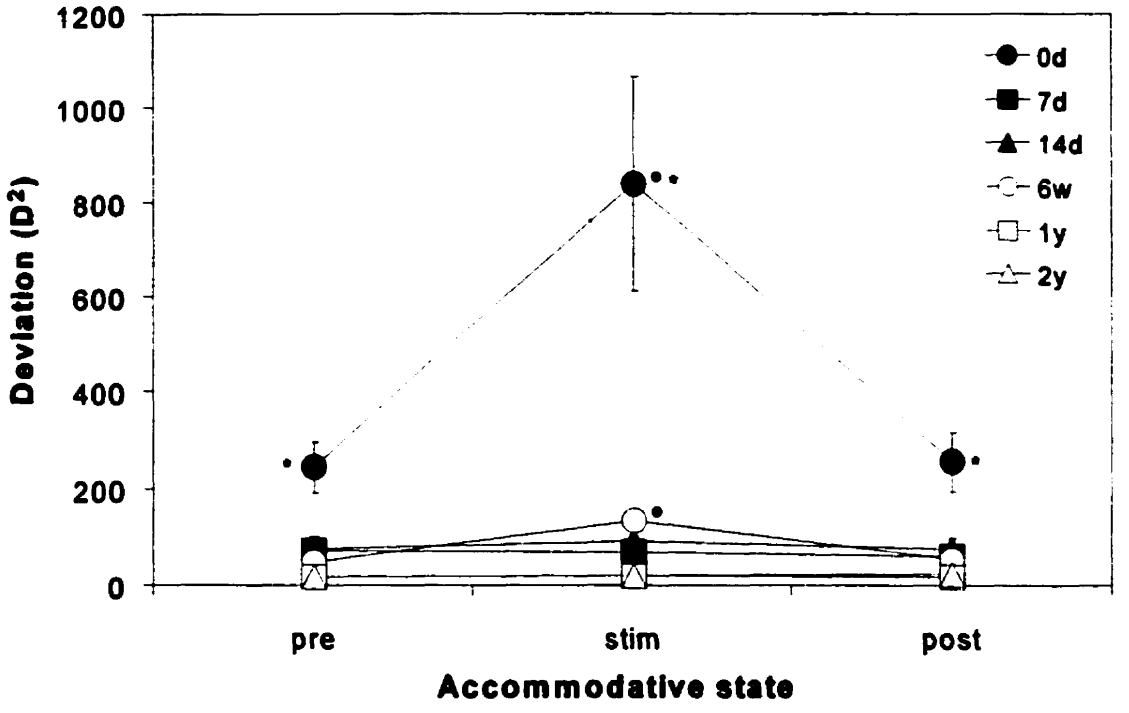



**Figure 6** - Mean deviation or non-monotonicity (± s.e.m.) for lenses from chickens aged 0 days (filled circle), 7 days (filled square), 14 days (filled triangle), 6 weeks (open circle), 1 year (open square), and 2 years (white triangle) old. Some error bars are covered by points on the graph. Asterisks denote significantly greater deviations compared to all other age groups ($p<0.05$; one-way ANOVAs with HSD-Tukey tests). Within each age group, means denoted by dots were significantly greater than those for the pre-stimulus state ($p<0.05$; one-way repeated measures ANOVA with Bonferroni t-tests). For other comparisons, please see text.

were signficantly greater than for all other age groups ($p<0.05$; one-way ANOVAs with HSD-Tukey tests), an indication that lenticular optical quality in these hatchlings was inherently poor. Analysis of the degree of non-monotonicity as a function of accommodation revealed an increase in hatchlings and 6 week old chickens ($p<0.05$; one-way repeated measures ANOVA with Bonferroni multiple comparison tests), an indication that accommodation was associated with worsening optical quality in only some age groups. Again, no trend was observed for accommodation-associated changes in deviation.

### 3.2 Effects of experimentally-induced ametropias

*3.2.1 Lenticular accommodation*

Form-deprivation resulted in induction of myopia, an observation that is in keeping with other reports. Refractive errors in form-deprived eyes (n=31) ranged from -4.75 to -24.50 D and averaged -13.71 ± 0.97 D (s.e.m.), while the contralateral (control) ungoggled eyes (n=31) were hyperopic, with refractive errors ranging from +1.75 to +6.75 D and averaging +3.87 ± 0.22 D. As expected, axial lengths, as measured by A-scan ultrasonography, for form-deprived eyes were longer, at a mean length of 8.96 ± 0.11 mm, compared to those for control eyes, which averaged 8.28 ± 0.06 mm. Eyes imposed with +15 D lenses became hyperopic, ranging from +6.25 to +19.00 D and averaging to +14.36 ± 0.40 D, while refractive errors for their contralateral, ungoggled eyes ranged from +1.75 to +6.00 D and averaged to +3.48 ± 0.15 D. Axial lengths of defocus-imposed eyes were shorter, at 7.97 ± 0.05 mm, compared to their controls, at 8.48 ± 0.04 mm.

Although all eyes were optically scanned at 0.13 mm intervals, the number of eccentric points across the lens varied as a result of differences in pupil aperture sizes (Table 3). Changes in pupil size arose due to natural or inherent pupil size variation, or due to accommodation-associated pupillary constriction. For both eyes of form-deprived birds, the range of the number of beams passing through the pupil were slightly smaller for pre-stimulus eyes than for post-stimulus eyes (compare pre-stimulus ranges of 14 to 19 for control and 14 to 17 for treated eyes to 12 to 19 and 12 to 17 for post-stimulus eyes, respectively). Not surprisingly, the mean number of beams passing through lenses from control and treated stimulated eyes was smaller than for their respective unaccommodating counterparts. The range of beam numbers across myopic lenses were also slightly smaller

**Table 3** - The number of beams (mean: range) entering the pupil for form-deprived myopic (translucent goggle-imposed) and hyperopic (+15 D goggle-imposed) chickens for each state of accommodation.

| | Translucent goggle | | +15 D goggle | |
|---|---|---|---|---|
| | control | treated | control | treated |
| pre | 16: 14 to 19 | 15: 14 to 17 | 16: 13 to 17 | 16: 13 to 18 |
| stim | 13: 10 to 17 | 12: 10 to 15 | 12: 10 to 15 | 12: 10 to 15 |
| post | 15: 12 to 19 | 15: 12 to 17 | 15: 13 to 17 | 15: 13 to 17 |

for all accommodative states (10 to 15) compared to their controls (10 to 17), although means were still relatively consistent (Table 3). For lens-imposed birds, the range of eccentric beam numbers were more consistent, with ranges of 13 to 17 beams passing through all non-accommodating eyes, except for pre-stimulus control eyes at a range of 13 to 18. For both eyes, the mean number of beams passing through pre-stimulus lenses were slightly higher than those for post-stimulus lenses, with means of 16 compared to 15 beams, respectively. For lenses from stimulated treated and control eyes, the range and mean number of eccentric points were consistent, and again, smaller compared to lenses from non-accommodating eyes (Table 3). Overall, means and ranges for the number of beams passing through the lens were relatively consistent regardless of refractive error, although this was not tested for.

Without corrections for aperture size, mean back vertex focal lengths (BVFLs) for lenses from myopic eyes were 19.95 ± 0.21 mm (s.e.m.) prior to stimulation, 16.11 ± 0.24 mm during stimulation and 19.45 ± 0.18 mm in the post-stimulated state (Table 4). Mean BVFLs for lenses from the form-deprived control eyes were longer, at 21.07 ● 0.18 mm,

**Table 4** - Mean back vertex focal lengths ± s.e.m. (mm) and focal length range (mm) in parenthesis for form-deprived myopic (translucent goggle-imposed) and hyperopic (+15 D goggle-imposed) chickens for each state of accommodation.

| Accom-modative state | Translucent goggle | | +15 D goggle | |
|---|---|---|---|---|
| | control | treated | control | treated |
| pre | 21.07 ± 0.18 (16.61 to 32.84) | 19.95 ± 0.21 (12.75 to 26.12) | 20.49 ± 0.18 (15.77 to 32.04) | 21.28 ± 0.22 (16.18 to 33.50) |
| stim | 16.37 ± 0.25 (11.60 to 25.03) | 16.11 ± 0.24 (8.35 to 21.27) | 15.71 ± 0.17 (11.06 to 21.18) | 15.94 ± 0.18 (12.38 to 24.26) |
| post | 20.59 ± 0.19 (16.10 to 33.15) | 19.45 ± 0.18 (13.23 to 25.45) | 20.05 ± 0.18 (15.94 to 29.34) | 20.74 ± 0.22 (16.00 to 29.17) |

16.37 ± 0.25 mm and 20.59 ± 0.19 mm, for pre-stimulus, stimulated and post-stimulus states, respectively.

Prior to comparisons, mean back vertex focal lengths were adjusted to a constant irideal aperture size (Fig. 7). For form-deprived birds, use of a two-way repeated ANOVA revealed differences as a function of accommodative state ($p=0.000$) and refractive error ($p=0.008$). Significant interaction was also detected ($p=0.017$) indicating that the back vertex focal lengths at the various accommodative states were dependent on the refractive error of the eye. For both eye types, mean lenticular focal lengths for stimulated eyes were shorter than for those not stimulated, an indication that, as expected, stimulation of the ciliary nerve was able to induce a lenticular accommodative response ($p<0.0001$ for both eyes; one-way repeated measures ANOVA on ranks with Dunn's method for multiple comparison). The hysteresis effects observed for post-stimulus scans compared to baseline scans were also significant for both groups ($p<0.05$ for both eyes; Dunn's method for multiple comparison).

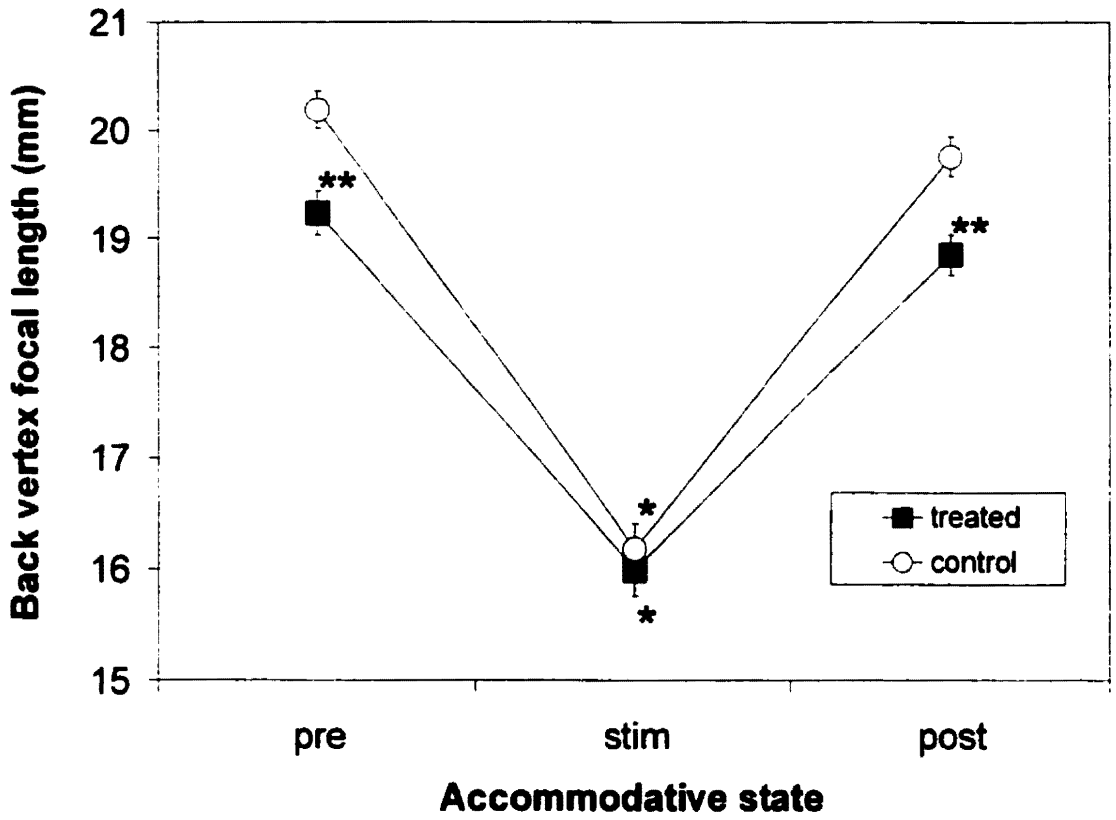



**Figure 7** - Mean back vertex focal lengths (±s.e.m.) adjusted for constant aperture size for lenses from form-deprived eyes (filled squares) and from their controls (open circles) at each accommodative state. For both eye types, focal lengths denoted by asterisks were shorter than for those not marked ($p<0.05$; Dunn's multiple comparison test on ranks). Means denoted by double asterisks were significantly shorter than those for control eyes at the same accommodative state ($p<0.05$; HSD-Tukey test).

Mean resting or baseline focal lengths for myopic eyes were significantly shorter than for their controls ($p=0.0008$), with differences in mean length at about 1 mm (Fig. 7), or in power at about 3 D (assuming thin lens in water, $n_w=1.33$; data not shown). Mean lenticular focal lengths for post-stimulus myopic eyes were also shorter than for their controls ($p=0.0009$), again showing differences in mean length and power of about 1 mm and 3 D, respectively. Together the results demonstrate that the crystalline lens is affected by visual or environmental cues and grows independently of a predefined genetic program.

The observation that mean lenticular focal lengths for stimulated treated eyes were similar to those for their controls (p=0.5722), together with the finding that focal lengths for lenses from myopic eyes are inherently shorter (Fig. 7), suggests that the accommodative apparatus was also affected by induction of myopia. For myopic eyes, both the mean accommodative amplitude, calculated as the difference between focal lengths for pre-stimulus and stimulus states, and mean amount of recovery from accommodation, calculated as the difference between focal lengths for the stimulated and post-stimulus states, were significantly reduced compared to their controls (Fig 8; p=0.019 and p=0.018, respectively). Conversion of accommodative and recovery amplitudes to dioptres resulted in dilution of the sensitivity of the statistical test, and myopia-associated differences in accommodative

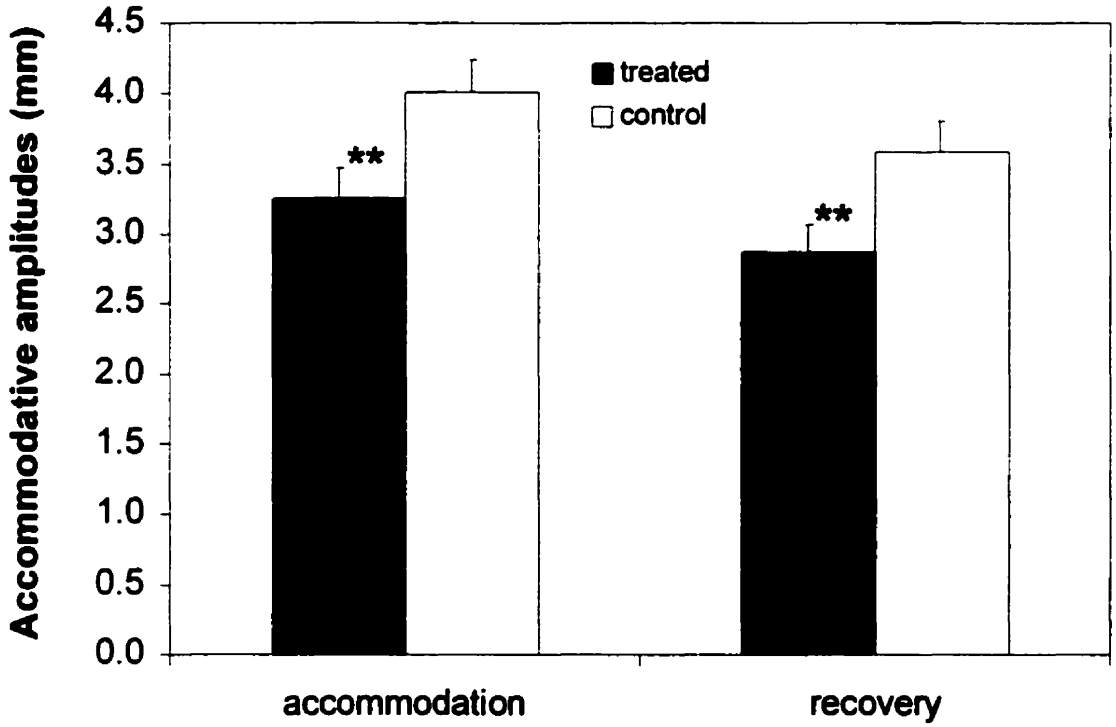



**Figure 8** - Mean accommodative and recovery amplitudes (±s.e.m.) for lenses from myopic eyes (filled bars) and their controls (open bars). For each accommodative change in amplitude, means denoted by double asterisks were significantly reduced compared to those not marked (p<0.05; paired t-test).

amplitudes became attenuated (p=0.141 and p=0.128, respectively; data not shown).

Prior to adjustments for aperture size, BVFLs for lenses from +15 D lens-treated eyes were 20.32 ± 0.20 mm, 15.80 ± 0.18 mm and 19.97 ± 0.20 mm for the pre-stimulus, stimulated and post-stimulated states, respectively (Table 4). Mean BVFLs for lenses from hyperopic control eyes were slightly shorter, an opposite trend to that observed for form-deprived chickens, with focal lengths at 19.77 ± 0.17 mm, 15.61 ± 0.17 mm and 19.43 ± 0.17 mm for the pre-stimulus, stimulated and post-stimulus states, respectively.

Mean BVFLs were also adjusted for a constant aperture size prior to comparisons (Fig. 9). Use of a two-way repeated measures ANOVA on lenticular focal lengths revealed a very strong accommodation effect (p=0.000) but more modest effects of refractive state (p=0.077) and interaction (p=0.065). In keeping with results for form-deprived chicks, mean BVFLs for stimulated lenses from both treated and control eyes were significantly shorter than for those during the pre- and post-stimulus states, and again, the hysteresis effects observed in both eye types, between lenses in the pre- and post-stimulus states, were also significant ($p<0.0001$ for both eye types; one-way repeated measures ANOVA on ranks with Dunn's multiple comparison tests).

Induction of hyperopia had opposite and more moderate effects than those for induction of myopia, with differences in mean lenticular focal lengths between treated and control eyes at rest, both prior to and following stimulation, at about 0.5 mm, or 1.75 D in power. However, use of the sign test clearly indicated that for these eyes, lenticular focal lengths for treated eyes were longer than for their controls (p=0.0046 and p=0.0039, respectively), which indicates that hyperopia is associated with specific changes to the lens. The findings that no differences were detected in lenticular focal lengths between treated and

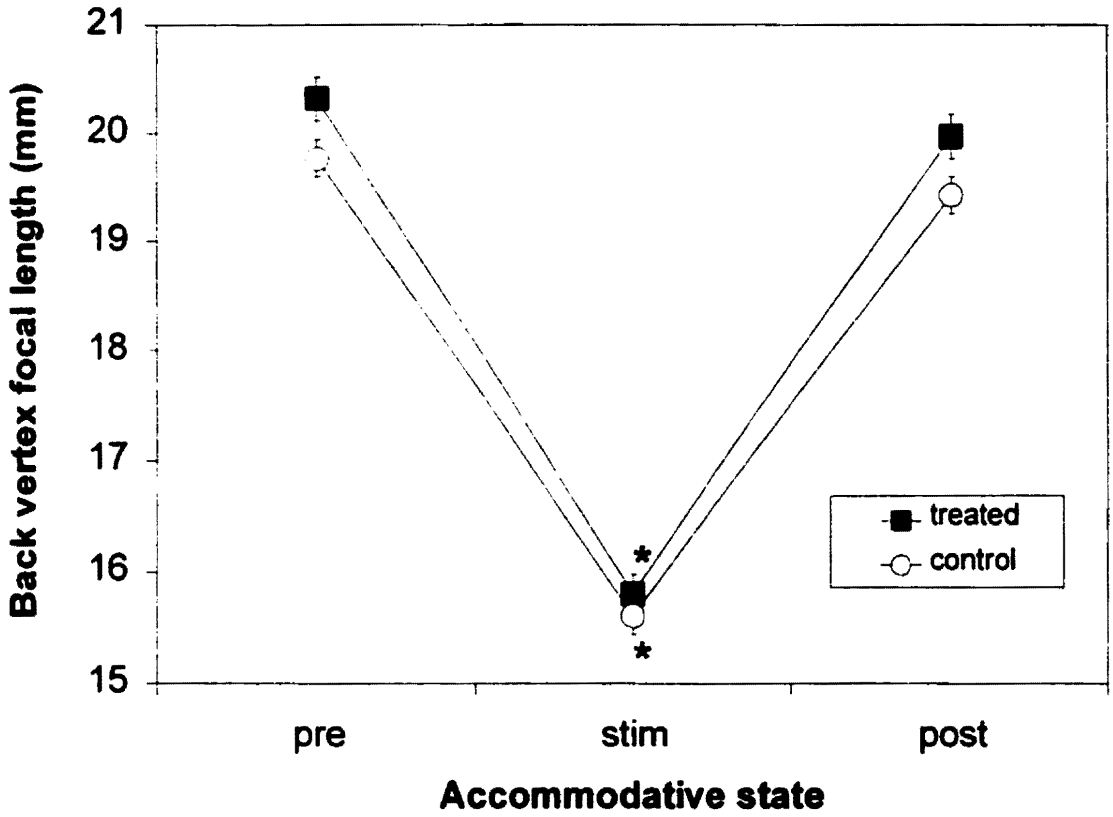



**Figure 9** - Mean back vertex focal lengths (±s.e.m.) adjusted for constant aperture size for lenses from +15 D lens-treated eyes (filled squares) and from their controls (open circles) at each accommodative state. For both eye types, focal lengths denoted by asterisks were longer than for those not marked ($p<0.05$; Dunn's multiple comparison test on ranks). For the resting accommodative states, focal lengths for treated eyes were longer than those for control eyes ($p<0.05$; sign test).

control eyes ($p=0.7488$; sign test) but that means for treated eyes were inherently longer suggests that induction of hyperopic refractive error also affects the accommodative apparatus. In contrast to results for form-deprived chickens, accommodative and recovery amplitudes were greater for treated eyes than for their controls (Fig. 10), although these associations were not very strong ($p=0.090$ and $p=0.100$, respectively).

*3.2.2 Lenticular spherical aberration*

An advantage to use of the physiological accommodation model presented here is the ability to directly assess the optical quality of the lens. All optical scans showed negative,

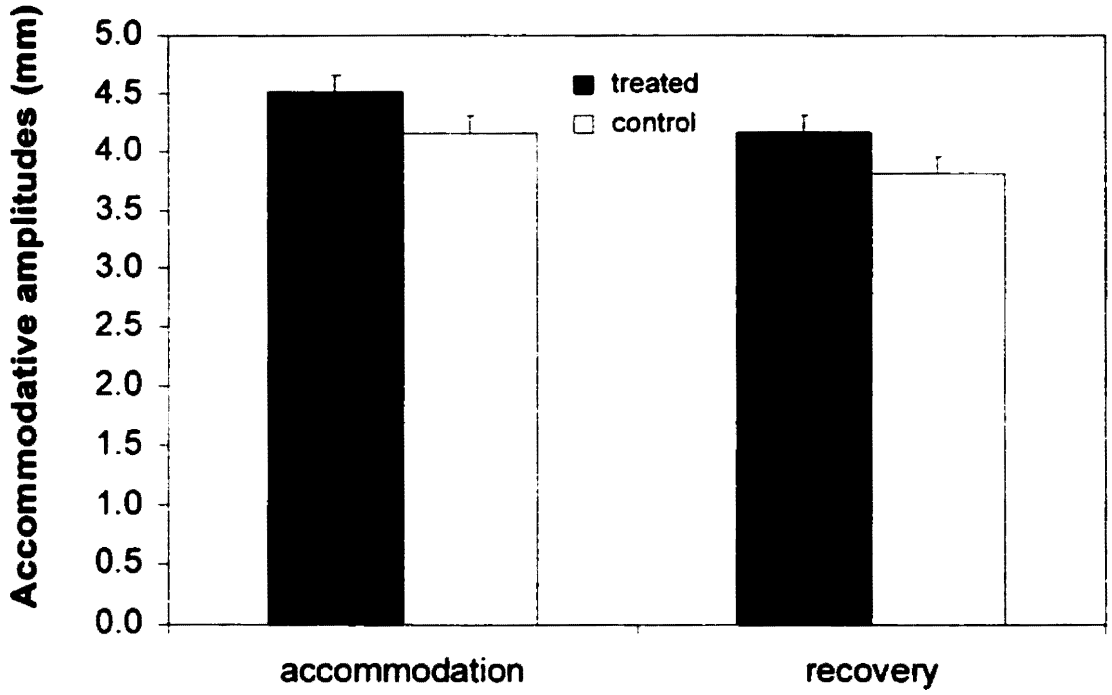



**Figure 10** - Mean accommodative and recovery amplitudes (±s.e.m.) for lenses from +15 D lens-treated eyes (filled bars) and their controls (open bars). Means for treated eyes were slightly greater than for their controls. Please see text for details.

monotonic spherical aberration (SA), regardless of refractive error or accommodative state (Fig. 11). Spherical aberration was quantified as the A-coefficient of the parabolic function $y=Ax^2+Bx+C$ that best-fit each scan in dioptres (assuming thin lens in water, $n_w=1.33$). Steeper parabolas, representing scans with greater spherical aberration, show higher A-coefficient values (Figs. 12 and 13). Two-way repeated measures ANOVAs revealed that for both form-deprived and hyperopic chickens, refractive error had no effect on the amount of lenticular spherical aberration ($p=0.922$ and $p=0.856$, respectively) (Figs. 12 and 13). In myopic birds, lenticular spherical aberration (SA) were similar in pre- and post-stimulus eyes, but increased with stimulation, regardless of refractive error ($p=0.000$ and $p=0.0018$ for control and treated eyes, respectively; one-way repeated measures ANOVAs on ranks with Dunn's multiple comparison tests) (Fig. 12). Eyes for hyperopic birds were similar;

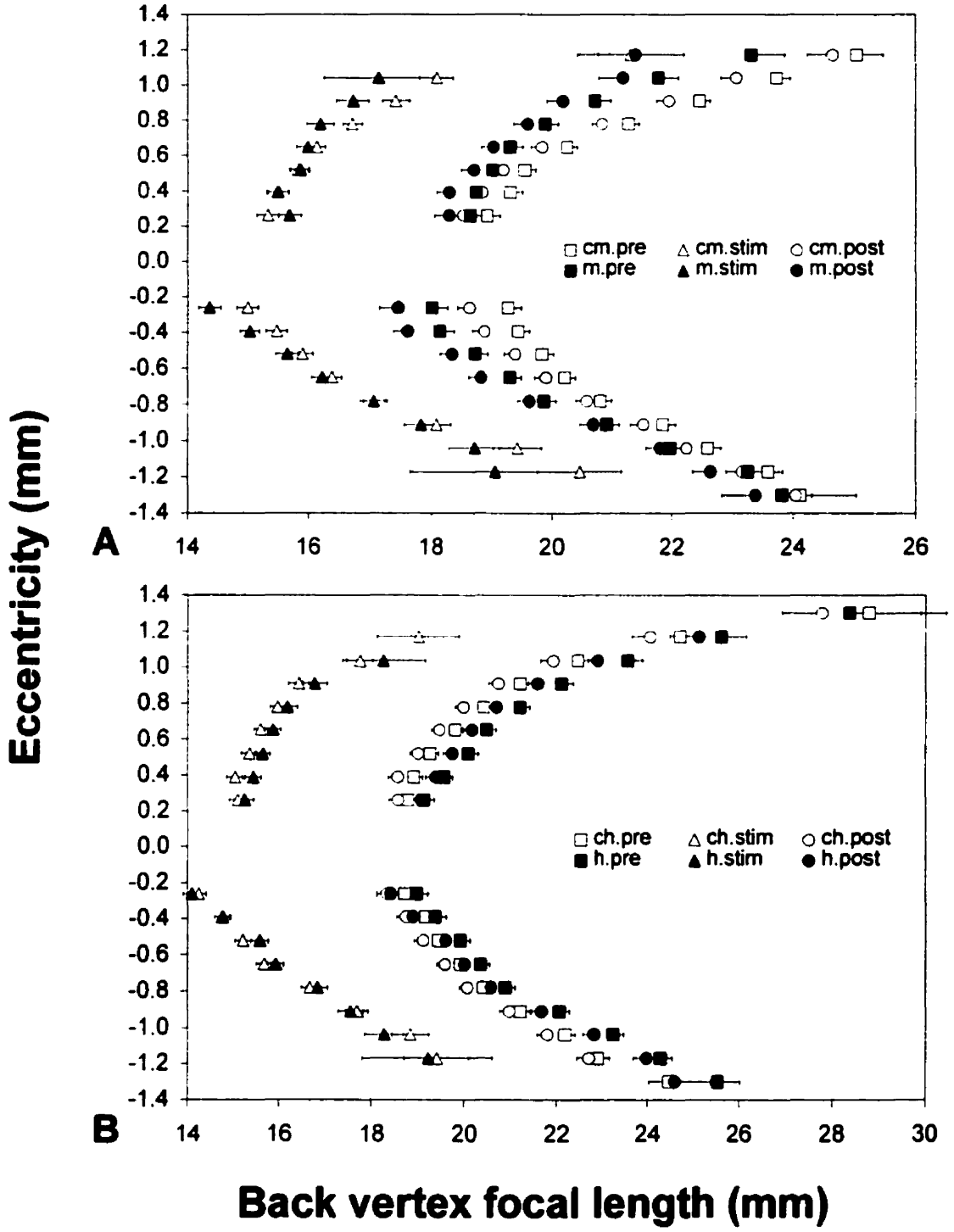



**Figure 11** - Mean back vertex focal lengths (±s.e.m.) of lenses from **(A)** form deprived myopic and **(B)** +15 D lens-treated hyperopic chickens, plotted as a function of eccentricity. Each data point represents a mean of a minimum of 3 values measure at that eccentricity. Lenses from treated (filled) and control (empty) eyes were optically scanned prior to stimulation (squares), during stimulation (triangles) and after stimulation (circle). Note that for all accommodative states, spherical aberrations are monotonic and clearly negative.

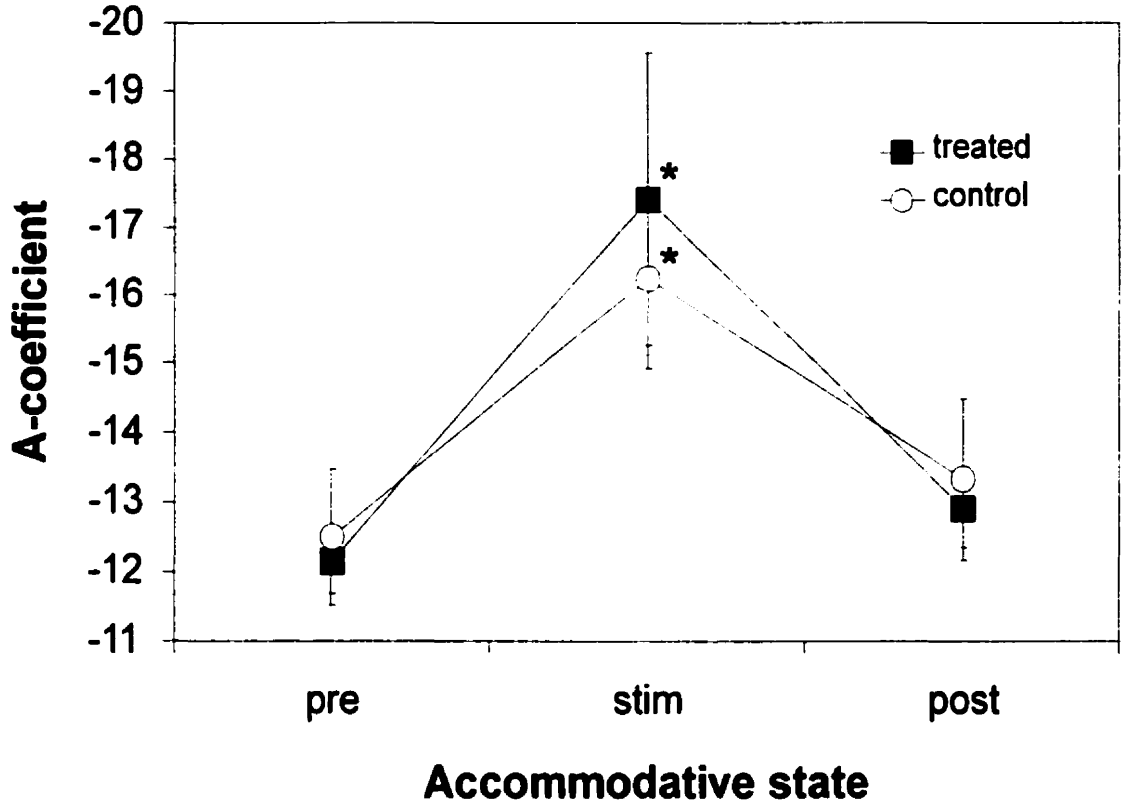



**Figure 12** - Mean parabolic A-coefficient value (±s.e.m.) representing spherical aberrations for lenses from form-deprived eyes (filled squares) and their controls (empty circles). For both eye types, means denoted by asterisks were significantly greater than those not marked ($p<0.05$; Dunn's multiple comparison test).

spherical aberration was the same for pre- and post-stimulus eyes, but significantly increased as a function of accommodation regardless of treatment ($p=0.000$ for both eye types; one-way repeated measures ANOVAs on ranks with Dunn's multiple comparison tests) (Fig. 13). Analysis of the amount of non-monotonicity, measured as the deviation from the best fitting-parabola for each scan, also revealed no effect of refractive error (data not shown).

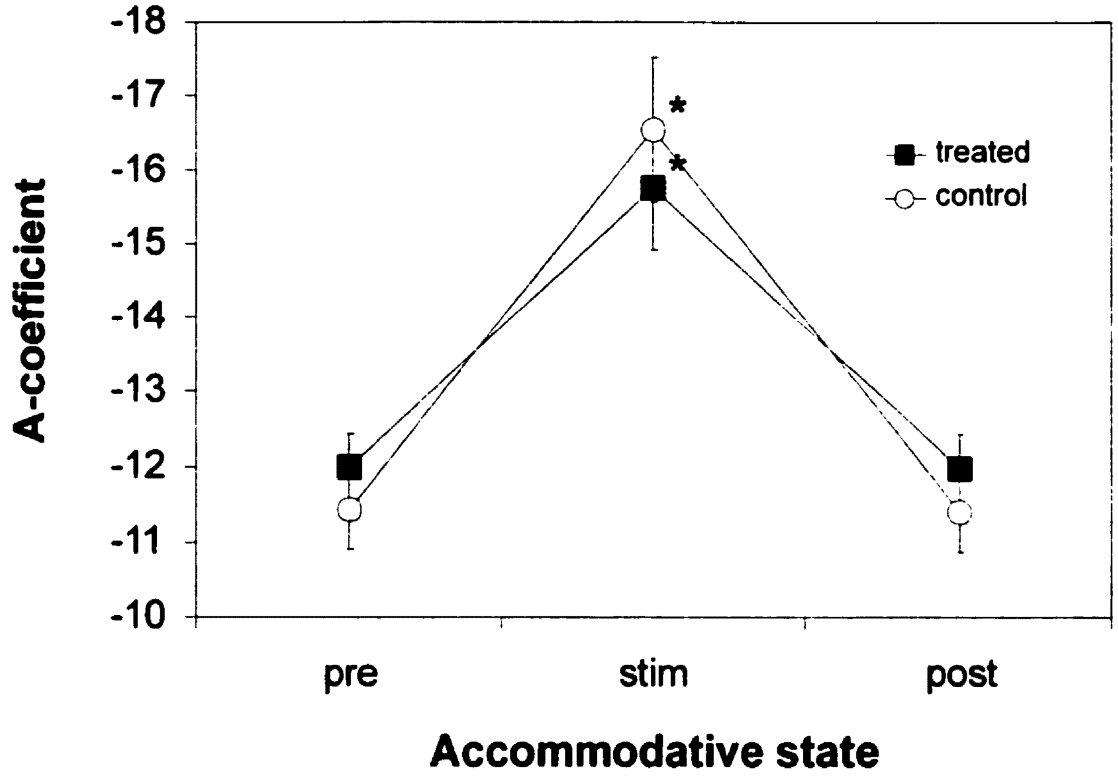



**Figure 13** - Mean parabolic A-coefficient value (±s.e.m.) representing spherical aberrations for lenses from +15 D lens-treated eyes (filled squares) and their controls (empty circles). For both eye types, means denoted by asterisks were significantly greater than those not marked ($p<0.05$; Dunn's multiple comparison test).

### 3.3 Anterior segment measurements during accommodation

In keeping with other reports, form-deprivation resulted in induction of myopia. Refractive errors for form-deprived eyes (n=12) ranged from -9.25 to -21.50 D and averaged -13.46 ± 1.13 D (s.e.m.), while the contralateral (control) ungoggled eyes (n=12) were hyperopic, with refractive errors ranging from +2.25 to +4.50 D and averaging +3.44 ● 0.17 D. As expected, axial lengths, as measured by A-scan ultrasonography, for form-deprived eyes were longer, at a mean length of 9.38 ± 0.11 mm, compared to those for control eyes, which averaged 8.64 ± 0.10 mm. Eyes treated with +15 D defocus lenses became hyperopic (n=13), by amounts ranging from +8.50 to +19.00 D and averaging to +14.15 ● 0.79 D, while

refractive errors for their contralateral, ungoggled eyes (n=13) ranged from +3.00 to +5.75 D and averaged to +4.02 ± 0.23 D. Axial lengths of defocus-imposed eyes were shorter, at 8.10 ± 0.08 mm, compared to their controls, at 8.64 ± 0.09 mm.

Use of the ultrasound biomicroscope resulted in images for which intraocular structures were clearly distinguishable, including, at medium resolution, both corneal surfaces, the iris, and both lenticular surfaces, although posterior lenticular curvatures were harder to detect (Fig. 14). For all eyes, regardless of refractive error, stimulation of the ciliary nerve resulted in constriction of the pupil apertures and forward and backward movement of the front and back lenticular surfaces respectively (Fig. 15A,B). For 18 of 44 eyes analysed, stimulation was associated with a backward movement of the central regions of the cornea, which was taken to indicate the occurrence of corneal accommodation (Fig. 15B).




**Figure 14** - Representative ultrasound biomicrograph of a chicken eye for which ocular structures such as corneal surfaces (C), iris (I) and both anterior (AL) and posterior (PL) surfaces of the lens are clear defined. Bar = 0.5 mm.

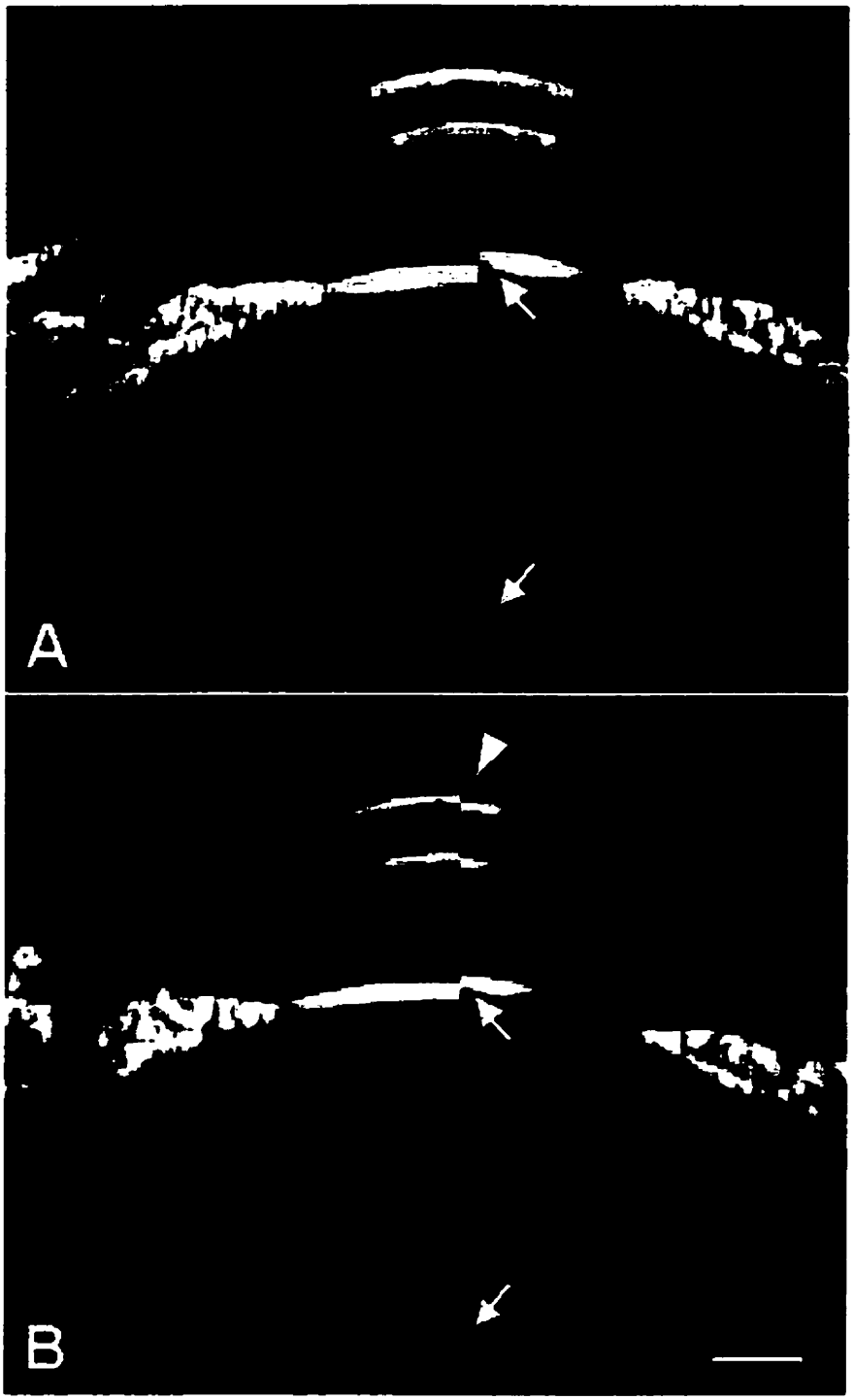



**Figure 15** - (**A**) Cut-away superimposed ultrasound biomicrographs of chick eyes at rest (left) and undergoing accommodation (right). Note that both front and back lenticular surfaces move during accommodation (arrows) while the cornea does not. (**B**) A subset of eyes also showed corneal accommodation (arrowhead) which involved backward movement of the cornea. Bar = 0.5 mm.

Form-deprivation myopia

Mean anterior chamber depths decreased concomitantly with accommodation in both treated and control eyes of form-deprived birds (p=0.000; two-way repeated measures ANOVA), averaging to about a 2 to 3 pixel, or 0.05 to 0.06 mm, difference respectively (Table 5). These changes were usually related to forward movement of the anterior surface of the lens, and in cases when corneal accommodation was present, to backward movement of the cornea (Figs. 15A,B). Although its effect was smaller, refractive error was also associated with changes in anterior chamber depth (p=0.056; two-way repeated measures ANOVA), with the average depth for myopic eyes at rest deeper than for their controls (Fig. 16A, Table 5) by about 8 pixels, or 0.16 mm. In addition, in 8 of 9 pairs of eyes, anterior

**Table 5** - Means ± s.e.m. in mm for measurements of various ocular components for form-deprived eyes and their controls at rest and during accommodation. Means ● s.e.m. in pixels are in parenthesis.

| Ocular component | control | | treated | |
|---|---|---|---|---|
| | at rest | accommodating | at rest | accommodating |
| anterior chamber depth (n=9) | 0.81 ± 0.02 (41.3 ● 0.9) | 0.75 ± 0.02 (38.2 ± 1.1) | 0.97 ± 0.03 (49.5 ± 1.8) | 0.92 ± 0.04 (47.0 ± 2.1) |
| lenticular thickness (n=12) | 1.82 ± 0.01 (93.3 ± 0.7) | 1.93 ± 0.02 (98.8 ± 1.0) | 1.86 ± 0.02 (95.3 ± 0.8) | 1.95 ± 0.02 (99.6 ± 1.0) |
| front lenticular curvature (n=12) | 0.17 ± 0.01 | 0.24 ± 0.02 | 0.18 ± 0.01 | 0.23 ± 0.01 |
| corneal thickness (n=9) | 0.263 ± 0.006 (13.5 ± 0.3) | 0.264 ± 0.006 (13.5 ± 0.3) | 0.251 ± 0.006 (12.8 ± 0.3) | 0.255 ± 0.005 (13.1 ± 0.3) |

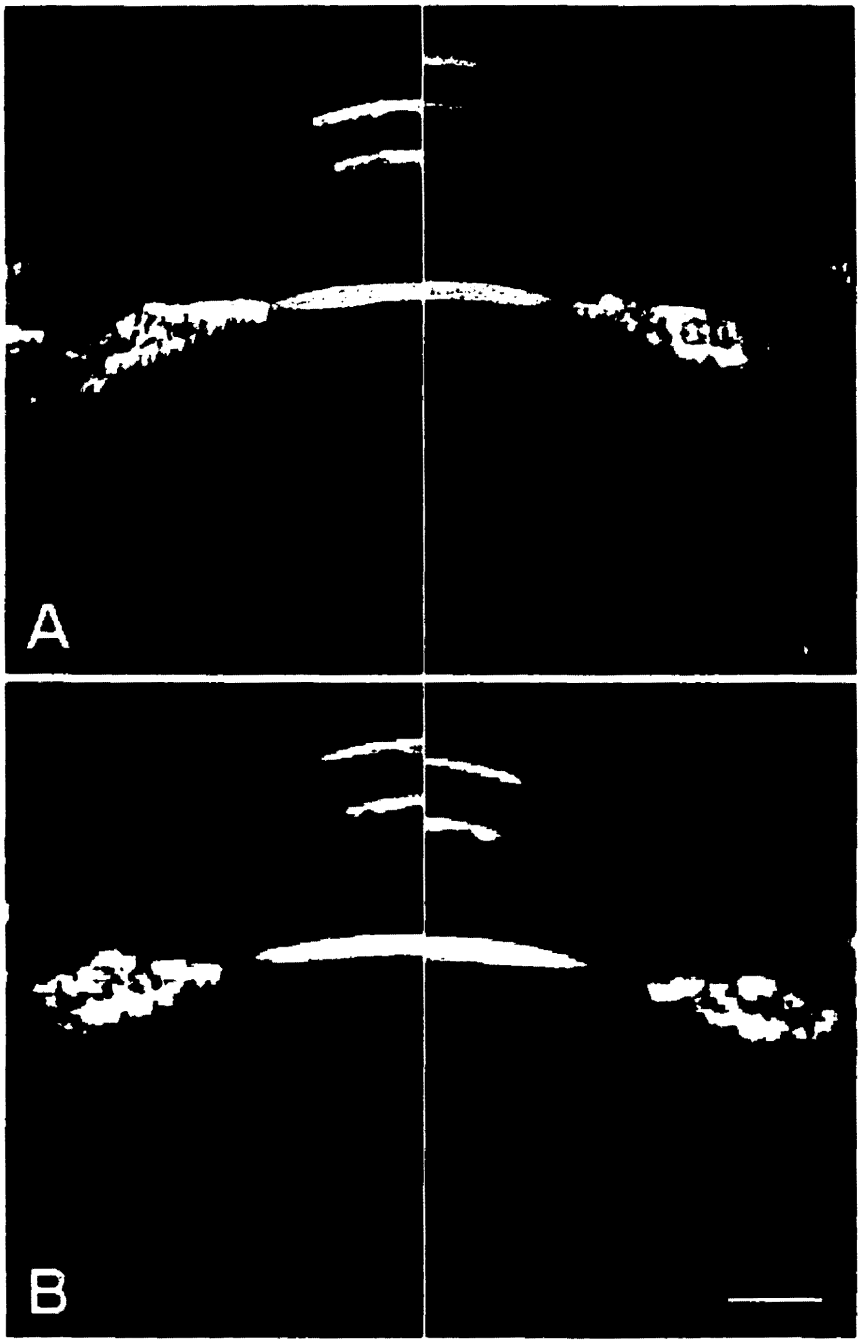



**Figure 16** - Micrographs of treated (right) and control (left) eyes from (**A**) form-deprived and (**B**) +15 D lens-treated chickens. Micrographs are aligned at the posterior poles of the lens. Note differences in anterior chamber depth of between treated and control eyes for both form-deprived and +15 D lens-treated chickens. Bar = 0.5 mm.

chambers were deeper for form-deprived eyes compared to their controls, in both the resting and accommodative states ($p=0.0391$ for both accommodative states; sign test) (Fig. 17).

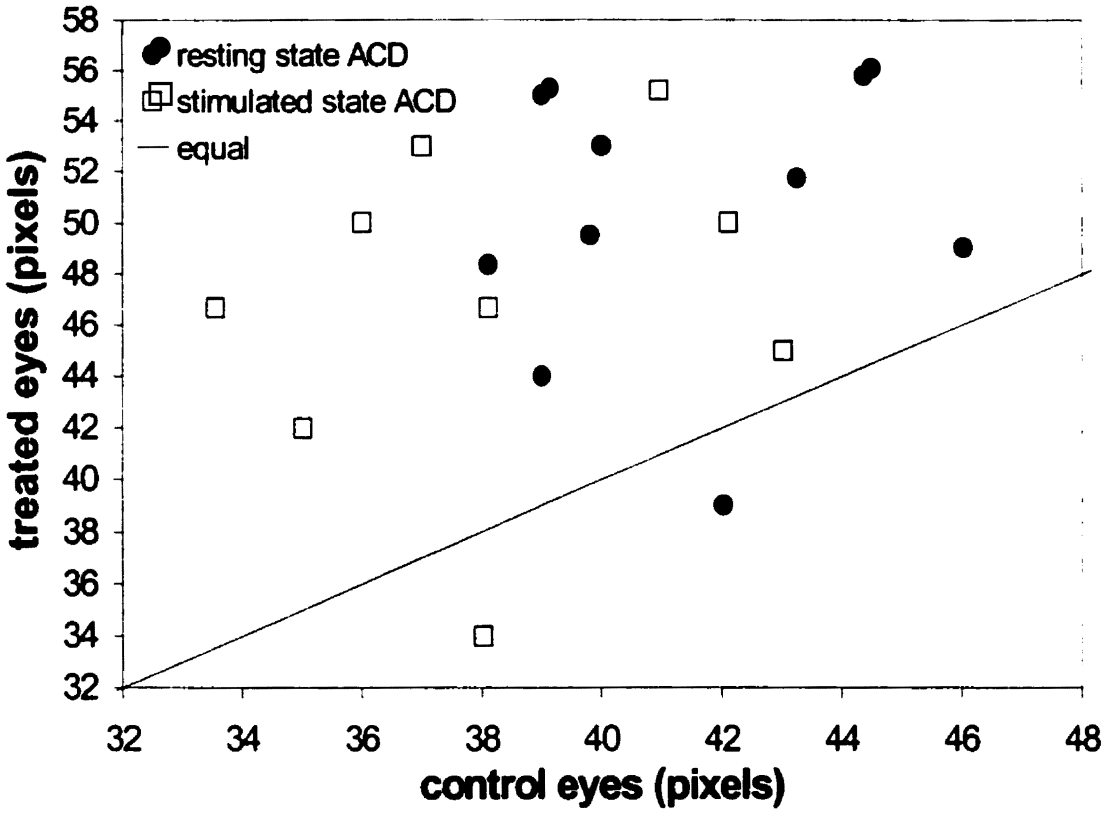



**Figure 17** - Resting state (filled circles) and stimulated state (empty squares) anterior chamber depths for treated eyes plotted against their controls for form-deprived chickens. Note the majority of plots are to the left of, or above the "equal" line (8 or 9 pairs), indicating that in general, chambers for treated eyes were longer than their controls (p=0.0391; sign test).

Taken together, the results suggest that anterior chamber depths were greater for form-deprived eyes.

Analysis of the mean lenticular thickness revealed an effect of accommodation (p=0.000), but not of refractive error (p=0.252; two-way repeated measures ANOVA). Accommodation was associated with a robust increase in lenticular thickness, with differences of about 4 and 6 pixels, or 0.09 and 0.11 mm, for treated and control eyes respectively (Table 5). Although refractive error effects were not very strong (see above), for all pairs of eyes analysed (n=12), lenticular thicknesses for resting form-deprived eyes

were greater than those for their controls (p=0.0005; sign test) (Fig. 18). However, it should be noted that the mean difference between the control and treated eyes was about 2 pixels, or 0.04 mm, which is just above the minimum detectable level of resolution.

Analysis of the front surface curvatures revealed no effect of refractive error (p=0.949) but an increased steepening with accommodation (p=0.000; two-way repeated measures ANOVA). Unlike measurements for lenticular thicknesses and anterior chamber depths, no other trends were observed.

Differences in mean corneal thicknesses were all less than 1 pixel, both as a function of refractive error and accommodation (Table 5), indicating that while there was slight user-

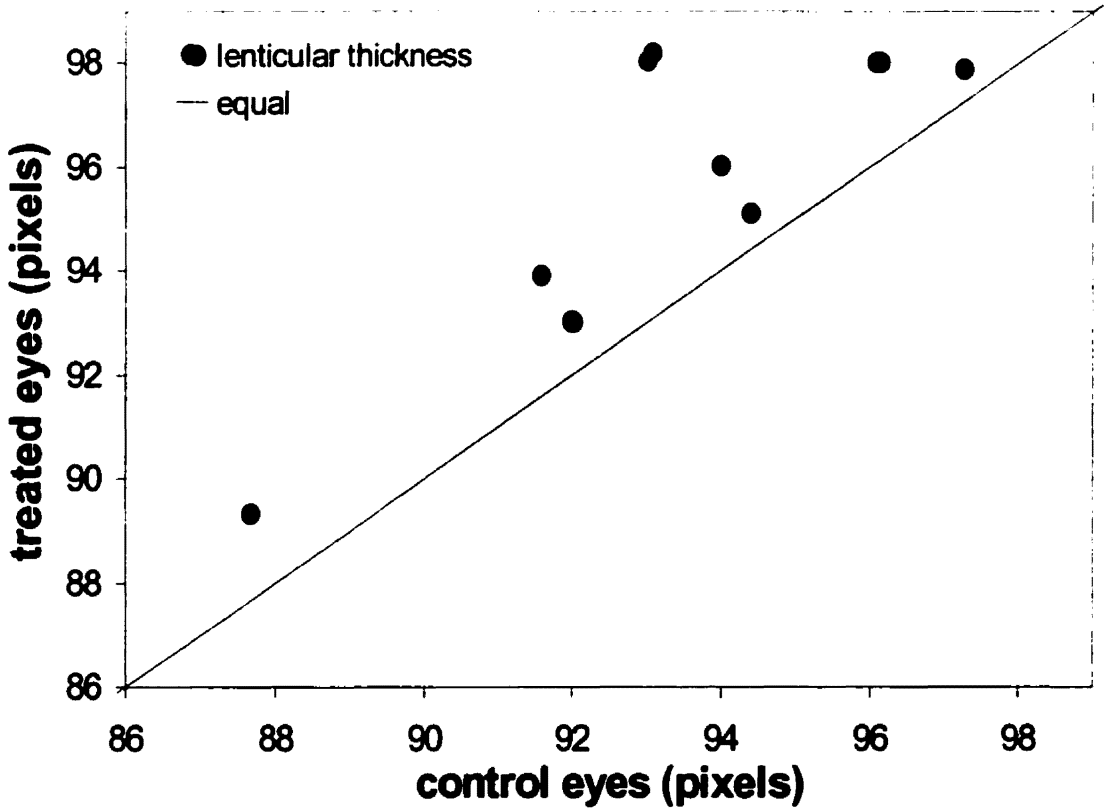



**Figure 18** - Resting lenticular thicknesses (filled circles) for treated eyes plotted against their controls for form-deprived chickens. Note that all plots are to the left of, or above the "equal" line (n=12), indicating that lenses from treated eyes were thicker than their controls for all pairs (p=0.0005; sign test).

error variability, their amounts were below the resolution detectable and were therefore considered negligible.

Hyperopia

Analysis of mean anterior chamber depths in +15D lens-treated chicks revealed an effect of accommodation (p=0.000) but no effect of refractive error (p=0.502). As for myopic birds, anterior chamber depths decreased with stimulation, showing similar differences, of about 3 pixels, or 0.05 mm, between accommodative states for both control and treated eyes. However, in contrast to measurements for eyes from form-deprived birds, anterior chamber depths in resting +15 D lens-treated eyes were smaller than for their controls (Fig. 16B) in 10 of 13 pairs (p=0.0386; sign test) (Fig. 19), by a mean difference of close to 2 pixels, or 0.04 mm (Table 6). These differences, much smaller in magnitude to those exhibited by form-deprived eyes, are just above the resolvable limit of the UBM (see above; Table 5).

Use of a two-way repeated measures ANOVA on mean lenticular thickness showed that there was an effect of accommodation (p=0.000) and a more modest effect of refractive error (p=0.160). Changes in lenticular thickness increased with accommodation for both treated and control eyes, with differences of about 5 pixels, or 0.10 mm for both eyes, which was similar in magnitude to the accommodation-associated increase observed in myopic birds. Induction of hyperopia had more varied effects on lenticular thickness than those for form-deprivation (Fig. 20). For eyes at rest, lenses from treated eyes were thinner for 6 pairs, the same for 4 pairs, and thicker for 3 pairs (p=0.5078; sign test). The group comprising the greatest number of eyes was that which showed treated lenses were thinner. Differences in lenticular thickness were greater in general for this group (compare differences, or distances

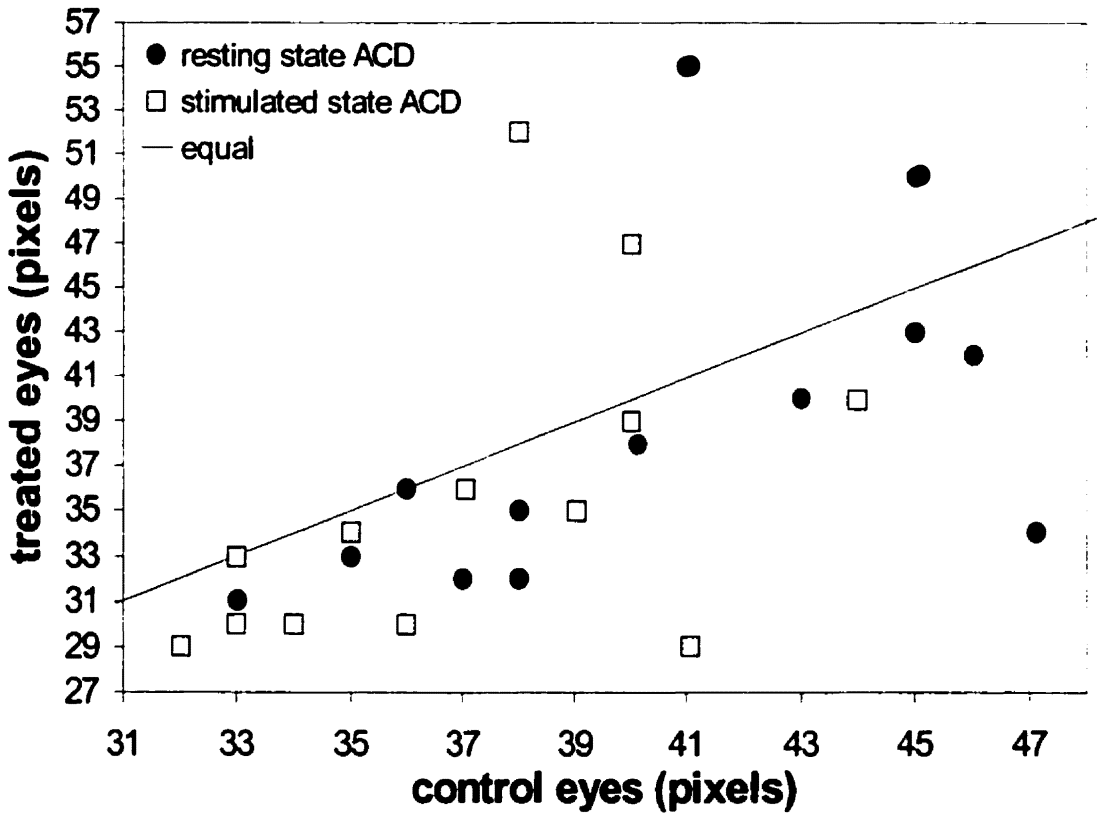



**Figure 19** - Resting state (filled circles) and stimulated state (empty squares) anterior chamber depths for treated eyes plotted against their controls for +15 D lens-treated chickens. Note the majority of plots are to the right of, or below the "equal" line (10 or 13 pairs), indicating that in general, chambers for treated eyes were shorter than their controls (p=0.0386; sign test).

from equal or trend line, in Fig. 20 for groups above and below the line), however, the combination of differences with the other two groups led to an attenuation of lenticular thickness differences, resulting in a mean change of slightly less than 1 pixel, which is below the minimum detectable change resolvable by the UBM (Table 6).

As with myopic birds, analysis of mean front lenticular surfaces revealed no change in steepness with refractive error (p=0.884) but increasing steepness with accommodation (p=0.000). No other trends were observed.

**Table 6** - Means ± s.e.m. in mm for measurements of various ocular components for +15 D lens-treated eyes and their controls at rest and during accommodation. Means ± s.e.m. in pixels are in parenthesis.

| Ocular component | control | | treated | |
|---|---|---|---|---|
| | at rest | accommodating | at rest | accommodating |
| anterior chamber depth (n=13) | 0.79 ± 0.02 (40.3 ± 1.3) | 0.72 ± 0.02 (37.1 ± 1.0) | 0.75 ± 0.04 (38.6 ± 2.0) | 0.70 ± 0.04 (35.7 ± 2.0) |
| lenticular thickness (n=13) | 1.82 ± 0.01 (93.2 ± 0.5) | 1.92 ± 0.01 (98.4 ± 0.7) | 1.80 ± 0.01 (92.5 ± 0.5) | 1.90 ± 0.01 (97.1 ± 0.5) |
| front lenticular curvature | 0.162 ± 0.003 | 0.247 ± 0.007 | 0.160 ± 0.003 | 0.247 ± 0.009 |
| corneal thickness (n=13) | 0.269 ± 0.007 (13.8 ± 0.4) | 0.271 ± 0.008 (13.9 ± 0.4) | 0.278 ± 0.004 (14.2 ± 0.2) | 0.275 ± 0.005 (14.1 ± 0.3) |

For +15 D lens-treated chickens, differences in the mean corneal thicknesses were also less than 1 pixel, both as a function of refractive error and accommodation, and were therefore considered to be negligible or non-existent.

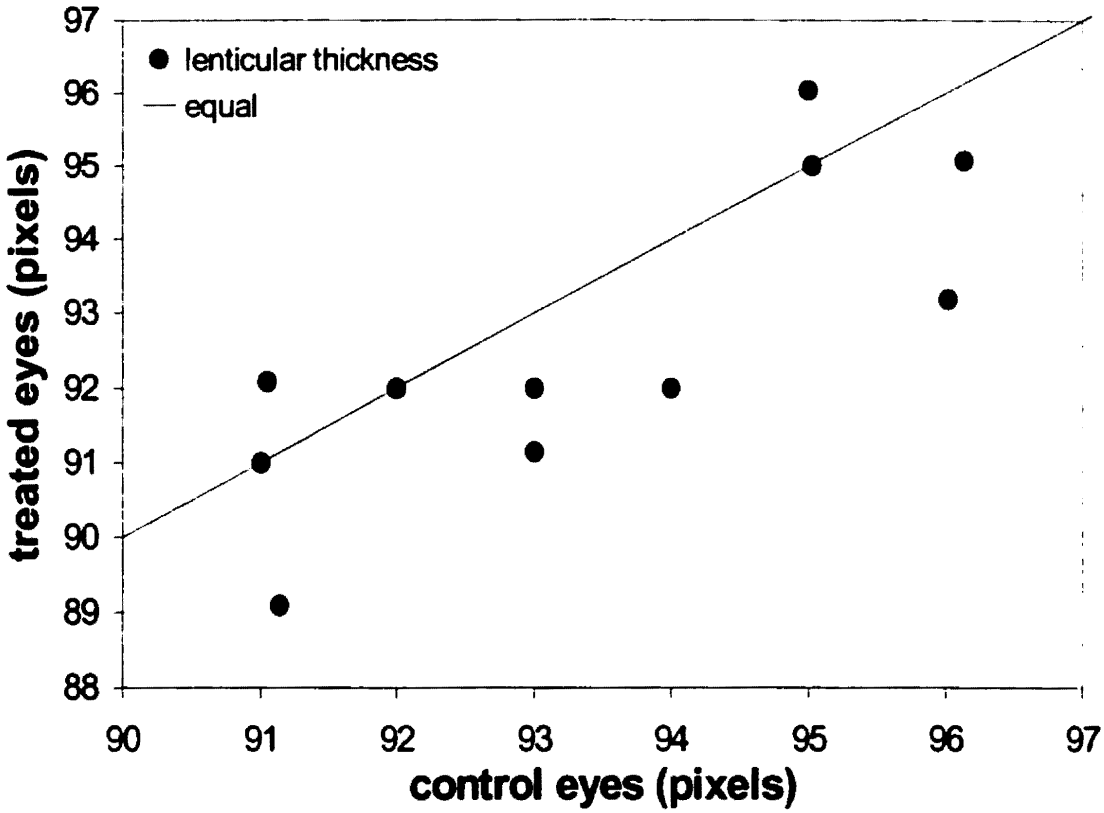



**Figure 20** - Resting lenticular thicknesses (filled circles) for treated eyes plotted against their controls for +15 D lens-treated chickens. Location of plots were more varied (p=0.5078; sign test). Note that more plots lie to the right of, or below the "equal" line (n=6) than to the left, and of these plots, differences were greater (farther from the "equal" line).

## IV. DISCUSSION

### 4.1 Effects of age on lenticular accommodation and spherical aberration

This is the first physiological study to directly examine optical properties of the lens during accommodation that has been induced in a manner approximating the *in vivo* condition. Given that all intraocular structures remained in their natural anatomical configurations and that accommodation was induced for all age groups, *i.e.*, stimulation of the ciliary nerve resulted in shorter focal lengths (Fig. 2), using a method that results in accommodation *in vivo*, the results presented herein were taken to represent functional optics as they would be in the intact eye.

This is the first study to show an adverse effect of age on lenticular accommodative function in chickens, with reduction in lenticular accommodation associated concomitantly with increasing age, a characteristic of presbyopia. Although age-matching of chickens to humans has not been analysed, it must be noted that chickens are precocial birds, opening and using their eyes the day of hatching. Hens usually begin laying eggs by the end of 5 months, an indication that one- and two year old chickens may be comparable to middle-aged humans. As chickens older than 2 years were not available, whether even older chickens would show further reduction in lenticular accommodation ability or a lack of accommodative response altogether, remains unknown.

As with humans, it is also difficult to determine the aetiology of presbyopia in chickens. Changes in the accommodative amplitude may be due to biophysical changes to the lens, or weakening of the ciliary muscle or both. It must be noted that hardening of the lens, whether due to changes in thickness or an increase in lenticular protein concentrations, would have an effect on the ciliary muscle, with requirements of greater ciliary muscle

function. As reported here, lenticular focal lengths for two year old chickens were slightly shorter, although not significantly, than those for one year old chickens, which raises an intriguing possibility that with more samples or with even older chickens, lenticular focal lengths would shorten with increasing age. Physiologically, this is possible if growth of the lens is accompanied by an increased protein accumulation, causing an increase in refractive index and/or thickening of the lens. If this were to occur, it would be an indication that the refractive index of the lens had increased, and that the ciliary muscle could have been affected because of the increased effort required to squeeze a less flexible or thicker lens.

The changes in accommodative amplitude, presented here, correlate well with results of Glasser *et al.* (Glasser *et al.*, 1995) which showed lenticular accommodative changes of about 10 D for 4 week old birds. Estimates of the change in accommodation for 4 week old chickens in the work presented here would fall somewhere between about 6 to 10 D (estimate from Fig. 2). Differences in dioptric values may be accounted for by variations in chicken strain and environments in which the chickens were raised. In their study, Glasser and colleagues (Glasser *et al.*, 1995) suggested that the 10 D change in accommodation they observed was probably not the true extent of lenticular accommodation in the chick, because of backward movement of the lens, mediated by loss of intraocular pressure (IOP) and removal of the vitreous, and because they had previously measured greater accommodative amplitudes using 0.011% nicotine stimulation. It remains unknown whether the arguments expressed by Glasser and colleagues hold true for the work reported here. The degree of backward movement of the lens, if any, was not assessed. Thus, it is possible that backward movement of the lens occurred in the experiments described here, caused by removal of the back of the globe. On the other hand, there were several differences in the study reported

here that may have helped to alleviate loss of IOP. Vitreous was not removed, and the eye was placed near the bottom of the chamber. Hence, some intraocular pressure may have been recovered by the volume of Tyrode's saline weighing down upon the vitreous and through it, the lens. In addition, eyes from hatchlings underwent corneal accommodation (data not shown), which is an IOP-dependent process that requires 15-20 mmHg in the eye (Glasser *et al.*, 1994). Together, these observations suggest that for hatchling eyes at least, the minimum IOP criterion was met, and that intraocular pressure loss was attenuated. Finally, it should be noted that back vertex focal lengths were measured for the maximum irideal contraction inducible by a physiological paradigm, and that the great amount of accommodation observed pharmacologically may be an extremely artificial circumstance; although the lens is capable of generating the amount of accommodation observed, this accommodative amplitude would not be observed naturally, or *in vivo*.

As expected, resting lenticular focal lengths of young chickens increased as a function of chicken age (Fig. 1), presumably in association with axial elongation of the eye. However, these focal lengths, as reported here, are slightly longer than those reported by Priolo and colleagues (Priolo *et al.*, 1999), who examined optical properties of excised chickens lenses *in vitro* as a function of age. This difference is probably attributable to the isolation of the lens in the previous study, where disruption of the anatomical structures supporting the lens *in vivo* causes changes to the shape of the lens. "Rounding up" of the lens once it has been free of its supporting anatomy has been previously shown by Glasser and colleagues (Glasser *et al.*, 1995). In addition, a more recent and sensitive version of the scanning laser monitor was used in the study reported here, which may have contributed to the differences observed

between the two studies. However, regardless of the differences, the pattern for lenticular focal length distribution as a function of age between the two studies are similar.

The finding that the hysteresis effect was significant only in 2 year old chickens must be interpreted cautiously. It could be an indication that the lenticular function is detrimentally affected in older chickens. However, as post-stimulus focal lengths were collected to ensure that stimulation of the ciliary had no optical or physiologically deleterious effects, the recovery time between the end of collecting data for stimulated eyes to the beginning of collection of post-stimulus focal lengths was not controlled for. Presumably, longer recovery times would result in smaller differences between pre- and post-stimulus focal lengths.

An advantage to the use of the physiological *in vivo* accommodation model described herein, is its usefulness in directly measuring the effects of age and accommodation on lenticular spherical aberration. As reported here, after 7 days, lenticular spherical aberration became monotonic (Figs. 3, 4 and 6) and negative, an observation that is in keeping with other reports. It should be noted that high amounts of negative spherical aberration may not necessarily result in poor vision, since the amount and type of spherical aberrations at the cornea currently remain unknown. However, while it is possible that the effects of the cornea may act to counter the negative spherical aberration observed in the lens, it is less likely that the erratic nature and clearly high amount of non-monotonicity exhibited by hatchling lenses, regardless of accommodative state (Figs. 3 and 6), can be compensated for by the cornea. Taken together, the results indicate that the lens was not fully developed at this age.

It remains unclear whether the high degree of spherical aberration observed in some stimulated lenses arose because of changes to the shape of the lens or because of changes to the refractive index of the lens. Given that focal lengths were measured for "distant" objects (collimated light), it might be expected that accommodation would be associated with degradation of optical quality. That the greatest increase in SA and degree of non-monotonicity was observed in hatchlings might be related to the observation that hatchlings also showed the greatest accommodative ability. While it must be noted that an age-associated trend was not observed for SA and non-monotonicity, for all age groups, SA in stimulated lenses were greater than for their unstimulated counterparts, although not significantly so, in all age groups.

Accommodation in some birds also includes a corneal component. In chickens and pigeons, changes to the cornea can account for up to half of the total amount of accommodation (Schaeffel and Howland, 1987), whereas, in hooded mergansers, lenticular accommodation plays a dominant role (Sivak *et al.*, 1985), especially when these diving ducks are in water, and power from the cornea is neutralised. It must be noted that although the corneal contribution was not measured in this study, the cornea may play a significant role in chicken vision during accommodation. Whether its effect on spherical aberration, if it exists, is synergistic with the lens during accommodation, working to improve optical quality, or deleterious is not known.

### 4.2 Effect of experimentally-induced ametropias on lenticular accommodation and spherical aberration

This is the first study to examine the effects of experimentally-induced ametropias on lenticular accommodative function and on lenticular optical quality in chickens for which accommodation has been induced in a mechanism similar to that which exists in nature, *i.e.*, via electrical signals at the ciliary nerve. As for studies examining age effects, intraocular structures remained in their natural anatomical configurations and stimulation of the ciliary nerve resulted in accommodation, as measured by shorter focal lengths (Figs. 7 and 9), indicating that the results measured were representative of the functional optics of intact eyes.

This is the first study to show that in chickens, lenticular back vertex focal lengths are shorter for myopic eyes than for their controls, suggesting that the crystalline lens, too, is affected by experimentally-induced myopia, and is in fact, a contributor to the resulting myopia (Fig. 7). Presumably, the increase in power of the lens reported here reflects a change either in the lenticular refractive index or in the shape the lens, or both. It may be speculated that changes to the shape of the lens could arise from an increased growth of fibre cells, or alternatively, from an increased basal ciliary muscle. It remains unclear which of these mechanisms are applicable to the work presented here.

The work presented here is also the first to show that myopia has an adverse effect on lenticular accommodation in chickens (Fig. 8). The results clearly indicate that much of the loss of lenticular accommodation may be attributed to the inherently shorter lenticular focal lengths exhibited by myopic lenses and the similar lenticular focal lengths for stimulated myopic and control eyes (Fig. 7). Together, these findings suggest that the

accommodative apparatus in myopic eyes is weaker, incapable of producing the same accommodative amplitudes as for control eyes. Although the weakened myopic accommodative response may be due to changes in the responses of one, some or all portions of the accommodative pathway, beginning at the ciliary ganglion and ending at the ciliary muscles, it is also possible that the amount of accommodation was limited by biophysical constraints, specifically, by thicker or harder lenses which, because they were less flexible, could not be squeezed further.

Conclusive associations for hyperopic birds were clearly harder to demonstrate, despite the increased number of birds sampled. However, the findings that longer lenticular focal lengths were associated with experimentally-induced hyperopia at a 92% confidence level ($p=0.077$) and that focal lengths for treated eyes were longer in 30 of 39 pairs (Fig. 10), strongly argue that experimentally-induced hyperopia should have a significant effect on the lens with further sampling. Moreover, hyperopic birds exhibit opposite trends to those for myopic birds, such as longer lenticular back vertex focal lengths ($p=0.0046$) and greater lenticular accommodative amplitudes for defocus-treated eyes compared to their controls (Fig. 9 and 10), suggesting that the lens and accommodative apparatus are not only affected by induction of hyperopia, but also, that they respond in a manner that is distinct from that for form-deprivation. Thus, overall, the results suggest that the lens is affected by, and responds distinctly to, specific visual cues.

While the trends for myopic and hyperopic birds were generally reciprocal, there were differences between the two groups which may account for the diminished responses observed in hyperopic birds. Refractive errors for both control groups were hyperopic, resulting in similar refractive error signs for, and smaller differences between, treated and

control eyes in hyperopic birds. Thus, the effect of refractive error may have been augmented in myopic birds but diminished in hyperopic birds, and these differences may have been sufficient to cause a diminished association between lenticular focal lengths and refractive error in hyperopic birds. The refractive errors for the control eyes reported here are in keeping with those of a study by Irving and colleagues, which showed that control eyes of chickens treated for 7 days with negative or positive lenses of various powers were hyperopic, regardless of sign or power of the spectacle lens (Irving *et al.*, 1992). However, in contrast, studies by Pickett-Seltner *et al.* (Pickett-Seltner *et al.*, 1988), Wildsoet and Wallman (Wildsoet and Wallman, 1995) and Priolo *et al.* (Priolo *et al.*, 2000), show that their control groups tended to be emmetropic, the same refractive error as the treated eye, or myopic, respectively. Presumably, the hyperopic refractive errors for both control groups reported here reflect the young age of the chicks and differences between studies may be related to variability in chickens. It is also possible errors were made during refractive error measurements, but, as the same method was used for all eyes and the mean refractive error for defocus-treated eyes were slightly less hyperopic than expected (+14.36 ± 0.40 D) this seems less likely.

While it remains unknown whether lenses from treated eyes of hyperopic birds exhibited longer focal lengths because their refractive indices were reduced or because they were flatter or thinner, or both, it must be noted that there are inherent limitations with either of these mechanisms. Unlike the rest of the eye, the lens grows throughout life, with "shells" or concentric layers of fibre cells continuously added to pre-existing layers of the lens. Under normal circumstances, fibre cells do not die or become phagocytosed; the lens contains all of its original cells, with the oldest cells compacted toward the centre of the lens.

Together, these cellular growth mechanisms present barriers for the lens to become hyperopic; as there is no additional mechanism to alleviate growth changes, unlike the eye itself which can rely on thickening of the choroid to further reduce retinal distance from the anterior of the eye, the lens can only become thinner by a decrease or cessation in the growth. Moreover, thinning of the lens cannot rely on compaction of fibre cells towards the centre of the lens since the lens would become more powerful and the eye more myopic by the subsequent increase in refractive index.

If changes to the crystalline lens are indeed cellular and not due to changes in resting ciliary muscle tone, it remains to be determined how changes to the crystalline lens are mediated. Given that the lens is enclosed within the eye, it is constantly exposed to, and cannot avoid any factors that may be released or upregulated by the retina in response to the imposed blurs. Changes to the lens may therefore be an epiphenomenon of the changes that are occurring globally in the eye. That the lens responds in a specific manner to distinct visual cues, contributing to the final refractive error of the eye rather than reducing its effect, may imply that the crystalline lens is genetically pre-programmed to respond to specific putative retinal factors, or that the lens itself is capable of distinguishing and up- or down-regulating its own growth changes. It currently remains unknown what these putative signals are, if they exist, and whether regulation involves up- or down-regulation of receptors in the lens or not.

Given that spectacle lenses were able to induce lenticular responses, and moreover, that these responses were diametrical to those for myopic birds, it may be speculated that imposition of a hyperopic defocus, by a negative or convex lens, would result in similar effects as for form-deprivation myopia. Although studies exist which show that the eye is

capable of discriminating between, and responds differently to, form-deprivation and lens-induced myopia (Bartmann *et al.*, 1994; Schaeffel *et al.*, 1995; Schaeffel *et al.*, 1994), the majority of studies indicate that the effects of the two treatments are similar (Irving *et al.*, 1992; Norton *et al.*, 1999; Troilo and Wallman, 1991; Wildsoet and Wallman, 1995). However, as this paradigm was untested, the effects of hyperopic defocus on lenticular accommodation remain unknown.

In a previous report, Priolo and colleagues (Priolo *et al.*, 2000) found that lenticular optical quality was degraded for myopic eyes but could not find any differences in lenticular focal length between myopic eyes and their controls, or between hyperopic eyes and their controls. In contrast, the results presented here clearly show that lenticular focal lengths are affected by form-deprivation and, albeit less strongly, by imposition of a myopic blur. It should be noted that in addition to the greater number of chickens tested here, there are several other differences between the study here and the one reported by Priolo *et al.* (Priolo *et al.*, 2000). In the previous study, focal lengths at the sutural regions were included in calculations of mean focal length and focal length variability. As briefly mentioned above (see Methods) sutural regions are associated with unpredictably variable focal lengths (Bantseev *et al.*, 1999; Kuszak *et al.*, 1994; Sivak *et al.*, 1994) which can result in masking of smaller effects. Focal lengths at these regions were omitted for the study here. In addition, a more recent and sensitive version of the scanning laser monitor was used in the study here, which may have allowed for detection of smaller differences in focal length compared to the original scanning laser monitor used in the previous study. Finally, Priolo and colleagues (Priolo *et al.*, 2000) excised lenses from the eye, whereas lenses remained *in situ* for the study reported here. It has been previously shown that excision of lenses causes

them to "round up" (Glasser *et al.*, 1995). Given the possibility that treatment-associated differences in focal length reported here may be attributable to a difference in accommodative tone of the eye, isolation of the lens from the zonules and the rest of the accommodative apparatus may have inadvertently caused neutralising effects. The combination of these experimental and analytical modifications may have acted together to cause the different results observed for this study and for those previously reported (Priolo *et al.*, 2000).

It must be noted that an unresolved issue concerning the accommodation model reported here involves the potential loss of power associated with loss of intraocular pressure (IOP) and backward movement of the lens during accommodation (Glasser *et al.*, 1995). As was the case for the age study, the degree of backward movement of the lens, if any, was not assessed. However, the same arguments hold; the vitreous was not removed, and eyes were placed at the bottom of the scanning chamber, which together may have helped alleviate IOP loss by combining the weights of vitreous and volume of the Tyrode's solution to push down on the lens. Again, it should be noted that great amounts of accommodation elicited by pharmacological agents (Glasser and Howland, 1995) do not necessarily reflect the accommodative amplitudes that would be achieved naturally or *in vivo*. Finally, in the study reported here, accommodation was clearly associated with very significant and obvious changes in back vertex focal length and spherical aberration, which may render the question of whether accommodation-associated changes were even greater, a moot point.

In a previous report, it was shown that induction of myopia was associated with degradation of optical quality (Priolo *et al.*, 2000), while in the study reported here, no differences in lenticular spherical aberrations were detected between myopic eyes and their controls (Figs. 12 and 13), nor in the degree of non-monotonicity between the two eye types

(data not shown). However, Priolo and colleagues (Priolo *et al.*, 2000) used focal length variability as a measure of optical quality, where high variability indicated poor optical quality, while the report here uses the steepness of the best-fitting parabola (see Fig. 11) to indicate high spherical aberration, and deviation from the best-fitting parabola to indicate non-monotonicity. The advantage to using parabola-based calculations lies in the ability to account for aberrations at the cornea. Several studies report that aberrations of the crystalline lens are eliminated by equal and opposite aberrations of the cornea, resulting in zero aberrations for the whole eye (Artal *et al.*, 2001; Sivak, 1982).

It remains unclear whether the cornea plays a role in neutralising or augmenting the spherical aberration observed for the lens both at rest and during accommodation in the study here. It must be noted that chickens also undergo corneal accommodation, which may have implications in the amount of aberrations of the whole eye. In order to eliminate the accommodation-associated increase in lenticular spherical aberration, corneal aberrations would also need to increase, assuming equal and opposite aberrations. It also remains unknown what effects ametropias have on corneal spherical aberration. In fact, it remains contentious whether corneal curvatures are even affected by induction of ametropia. Various form-deprivation studies indicate that the cornea steepens, flattens or curvatures stay the same, while Irving and colleagues observed corneal flattening with imposition of high positive lenses, and no changes for negative lenses (Irving *et al.*, 1992). Presumably steepening or flattening of the cornea, if it occurs, would have different effects on the magnitudes of spherical aberration, but whether these changes in the amount of putative positive spherical aberrations work in concert with the lens to eliminate spherical aberration of the whole eye is not known.

### 4.3 Ultrasound biomicroscopy of the anterior segment

This is the first study to examine the biophysical characteristics of the anterior segment of myopic and hyperopic eyes undergoing accommodation. As expected, accommodation in the chick eye was associated with thickening of the lens, a reduction in anterior chamber and a steepening of front lenticular surface curvature. The results showing that thickening of the lens was associated with movement of both lenticular surfaces during accommodation, with the anterior surface moving into the anterior chamber, and the posterior surface moving into the vitreous chamber, are consistent with the observations of Glasser and colleagues (Glasser *et al.*, 1995) who used slit-lamp illumination to observe changes to the lens during accommodation elicited by stimulation of the Edinger-Westphal nucleus. While it was not possible to measure the curvatures for posterior lenticular surfaces using the UBM (Figs. 14, 15 and 16), given that accommodation was associated with both steepening of the front lenticular surface and bulging of the lens into both anterior and vitreous chambers, it may be speculated that accommodation was also associated with an steepening of the posterior lenticular surface, as was previously reported (Glasser *et al.*, 1995).

In their study, Glasser *et al.* (Glasser *et al.*, 1995) reported that lenticular thickness increased by 0.2 mm with accommodation and that movement of the two lenticular surfaces were equal, with the amount of bulging into the anterior chamber equal to that into the vitreous chamber. Measurements for some of the eyes in the study here indicated a varied response amongst the eyes, with some showing equal amounts of change in anterior and posterior surface movement (n=16), and with others showing more anterior surface movement (n=7), and still others more posterior surface movement (n=17). While some of

these accommodation-associated changes were obviously greater at one surface than the other, the majority of changes were within 1 pixel of each other (30 of 40 eyes) and therefore these differences should be interpreted cautiously. However, it should be noted that for the 10 eyes that did show a clear difference in the amount of movement between front and back surfaces, for all 10, bulging at the posterior pole was greater than at the anterior pole (by differences ranging from 2 to 4 pixels). It remains enigmatic what factors were responsible for the increased movement of the posterior pole, whether, for example, the apparent increase in posterior surface movement represents a shift of the lens backwards. It has been previously reported that backward shifting of the lens is possible if there is loss of intraocular pressure (Glasser *et al.*, 1994), but given that these eyes were whole, a loss of intraocular pressure should not have occurred, although this was not tested for. Another possibility is that the intraocular pressure in these eyes were lower to begin with, although how such a mechanism would have occurred, also remains unclear.

The results of the study here indicate that the accommodation-associated increase in lenticular thickness was half the magnitude of that previously reported (Glasser *et al.*, 1995). While the method of induction of accommodation differed between the two studies, the resultant amounts of lenticular accommodation were similar; in the previous study, stimulation of the Edinger-Westphal nucleus elicited about 15 D of lenticular accommodation, while the method used here, via electrical stimulation of ciliary nerve, has been previously shown to generate approximately 15 to 19 D of lenticular accommodation (converted from BVFLs for ametropic eyes, section 3.2.1, assuming $n_w$=1.33; data not shown), suggesting that differences in lenticular thickness are not related to the method of induction, nor to the amount, of accommodation. Given that eyes in the study here were

from younger chickens, and that there is an age-related decrease in resting state lenticular power in chickens (Fig. 2), it may be speculated that the 0.01 mm change in thickness for the smaller, more powerful lenses of the study here is equivalent to the 0.02 mm change in thickness for the older, less powerful lenses of the previous study. Further differences may lie in breed of the chickens, or in the use of an A-scan ultrasound, rather than the UBM as reported here.

It remains unclear why corneal accommodation in the study here was associated with a backward shift, or translation, of the whole cornea into the anterior chamber and toward the crystalline lens (Fig. 15B). Because corneal translation was observed in 17 of 44 eyes, it was considered that this movement may be an artefact of the experimental procedure. However, observations of the scleral region and side of the eye clearly indicate that movement of the cornea was relative to the rest of the eye, nor was corneal movement associated with the greater movement of the posterior pole of the lens, observed in some of the eyes. Preliminary analysis reveals that there is a slight steepening in curvature for the central region of the cornea for the study here, but that this change is very slight and well below the differences observed between the inner and outer corneal surfaces themselves (data not shown). Observations of the ciliary region for the eyes here suggest that corneal accommodation originates near the limbus, with inward pulling or negative pressure radially, or centripetally, conducted along the plane of the cornea, much like the mechanism for corneal accommodation proposed by Glasser and colleagues (Glasser *et al.*, 1994). As data were primarily collected for the crystalline lens, clearer details describing the corneal mechanism are needed and further analysis of the ciliary region is required in order to elucidate the mechanism observed in the study here.

Changes as a function of refractive error were clearly more difficult to assess, partly because of the subtle changes associated with myopia and hyperopia. Like the previous study showing that a myopic refractive state had greater effects on lenticular focal length than a hyperopic refractive state (See section 3.2.1), the study here shows that form-deprivation had greater effects on anterior chamber depth and lenticular thickness, while changes to hyperopic lenses were less consistent (compare both proportion and spread of points for Figs. 18 and 19 for anterior chamber depths, and Figs. 18 and 12 for lenticular thicknesses). However, despite the lack of clear findings, results showing that anterior chambers tended to be deeper and lenses thicker in myopic birds compared to their controls, and that chambers were shallower and more lenses thinner for lens-treated hyperopic eyes compared to their controls, indicates that induction of myopia and hyperopia generally resulted in opposite effects.

The UBM is set for certain sound wave speeds that have been empirically derived for humans. It remains unclear whether the density of chicken structures are equivalent to humans but given that measurements were compared relative to each other, the actual velocity used for the UBM is largely irrelevent. It must be considered, too, that the effects of experimentally-induced ametropias on the refractive index of the lens remain unknown, which could affect any measurements or comparisons made on the UBM. An increase in refractive index would result in a greater density and therefore greater sound velocity; without lens velocity corrections, measurements for the myopic lenses would be underesti-mated (shorter) because echoes would be reflected back faster. In contrast, a decrease in refractive index of the lens would result in slower sound velocities, and measurements for hyperopic eyes would be over-estimated (longer) since echoes would be reflected back

slower. Given that differences were detectable between ametropic and control eyes without lens velocity corrections, refractive error-dependent velocity corrections may also be largely unnecessary. In addition, it must be noted that biochemical changes associated with experimentally-induced ametropias have been difficult to quantify (Pickett-Seltner *et al.*, 1988; Zaidi, 2001) which may suggest that if they exist, the changes are subtle and that changes to lens velocity values would also be nominal. It has been recently reported that lens velocity does not change with age (Dubbelman *et al.*, 2001), further suggesting that even if a refractive index change exists, it may not be detectable.

In summary, it was found that the UBM was a good tool for measuring changes during accommodation, and moreovoer, that it may be advantageous over other techniques due to its ability to capture real-time images in a non-invasive manner. However, it should be recognised that there are limits to the resolution of the structures in question. Thus, while measurement changes to various parameters were robust during accommodation, the more subtle changes associated with experimentally-induced ametropias were less clear. Measurements at a higher resolution may help to elucidate some of these uncertainties.

### 4.4 General conclusion

A model utilising ciliary nerve stimulation to measure accommodation-associated changes was developed; optical changes to the lens in ageing and ametropic chickens were examined in addition to biophysical changes to the anterior segment of the eye. It should be noted that because the protocol used in this study was established to model accommodative changes in the eye *in vivo*, its use is limited only by accessibility or visualisation of the intraocular structure(s) in question. While accommodation-associated optical and

biophysical changes were examined in the study here, it should be noted that morphological and (neuro)pharmacological changes to the lens during accommodation remain untested. In addition, other structures related to accommodation, such as the ciliary muscle and cornea, have been neglected. Moreover, synapses at the iris are functional in embryonic chicks by Stage 35 (Landmesser and Pilar, 1974; Pilar *et al.*, 1987), suggesting that synapses at the ciliary muscles are also functional at this time and that the accommodative apparatus is functional while the chick is still *in ovo*. This model may therefore be used to test optical, biophysical and/or morphological changes to the accommodative apparatus during development of the chick. Finally, while the model was developed here to test accommodation-associated changes in chickens, it should be noted the *in situ* model described here may be ported to test accommodative changes in other birds which show different accommodative behaviours, such as the kestrel or diving mergansers, or indeed in other animals in which acccommodation is stimulated by ciliary nerve stimulation.

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