ACCOMMODATION, REFRACTIVE ERROR AND EYE GROWTH IN CHICKENS

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Abstract—We raised chickens with defocusing lenses of differing powers in front of their eyes. For this purpose, small hoods made from soft, thin leather were carefully fitted to their heads. Lenses were attached to the hoods by velcro fasteners and could be easily removed for cleaning. The powers of the lenses were such that their optical effects could be compensated for by accommodation. It was verified by infrared (IR) photoretinoscopy that the chickens could keep their retinal images in focus. Wearing a lens resulted in a consistent shift of the non cycloplegic refractive state (measured without the lens) which was in the direction to compensate for the lens. We used a sensitive technique (precision = ± 50 μm as estimated from the variability of repeated measurements) to measure the posterior nodal distance (PND) in excised eyes of birds grown with lenses. The PND, in turn, was used to compare eyes treated with different lenses. It was found that the PND was increased in eyes which were treated with negative lenses compared to those treated with positive lenses. This effect occurs independently in both eyes and it is not due to changes in corneal curvature. We discuss our result in terms of a closed-loop feedback system for the regulation of eye growth.

INTRODUCTION

Considerable interest has been shown in the growth of vertebrate eyes, because the tuning of the optical components to the length of the eye is so accurate. Indeed, the optical resolution of the emmetropic human eye is limited only by diffraction for pupils of 2.4 mm and smaller (Campbell and Gubisch, 1966).

However, the normal tuning of optical components to each other can be disturbed during development, as ametropia often occurs in human eyes. For a long time it was not known whether the optical tuning during eye growth was determined solely by genetic factors or whether, in addition, the growth of the eye was also influenced by visual experience (Duke-Elder, 1970). Recent research has demonstrated that visual deprivation in experimental animals during early postnatal development can result in optical defocus (Wallman et al., 1978; Raviola and Wiesel, 1985; Hayes et al., 1986; Hodos and Kuenzel, 1984). Also for humans some evidence has accumulated that visual deprivation or visual field loss during infancy may result in myopia (Rabin et al., 1981; Hoyt et al., 1981; O'Leary and Millodot, 1979). However the results seem not to be as consistent as those from animal models (von Noorden and Lewis, 1987).

It may be concluded that visual experience is necessary for the eye to grow in a proper way. However, it has not yet been shown that any feedback loops are acting to adjust the position of the retinal plane to the image plane. Visual deprivation has been achieved by lid suturing (Wiesel and Raviola, 1977 (monkeys); Yinon et al., 1980 (chickens); corneal opacification (Wiesel and Raviola 1979 (monkeys)) and, most frequently, by fixing small plastic shells in front of the eyes which may serve as total or partial occluders (Wallman et al., 1978; Hodos and Kuenzel, 1984; Hayes et al., 1986; Pickett-Seltner et al., 1987 (chickens)). In terms of a feedback system (the presence of which we assume for our discussion) such devices provide "open loop" conditions because the image quality cannot be improved by any regulatory growth processes in the eye. The finding, that enormous amounts of myopia may develop [mean values of about 30D in young chickens treated with total occluders (Wallman and Adams, 1986; Gottlieb et al., 1988)] and the strong increase in variability of the refractive state in different individuals are both in accord-

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 ance with the expected consequences of an “open loop” system. To provide “closed-loop” conditions to the developing eye the optical quality of the retinal image should be restorable by compensatory growth processes.

We used weak ophthalmic lenses (range +4 to −8D) to defocus chicken eyes during the first 30 days of life. Optical blur resulting from the lenses is different from the blur resulting from occluders in at least two ways. Firstly, the image quality may be improved immediately by accommodation. [The chickens’ accommodative range (about 20D) is far greater than that of man and produced by a powerful apparatus which includes a corneal and a lenticular component (Schaeffel et al., 1987; Troilo and Wallman, 1987).] Secondly, growth processes may correct for the defocus provided by the lens. However it should be noted that the image quality attained with the lens may not be as good as without the lens due to peripheral distortions, decentering or dust accumulated on the lenses. As stated above, blur of the retinal image which cannot be corrected for by accommodation may also result in increased growth of the vitreous chamber, and therefore effects similar to those occurring in occluded eyes may be superimposed on the growth pattern. To keep these as small as possible it is necessary to assure that with the lenses the chickens are able to keep their retinal images in focus. The power of the lenses should be matched to their accommodative ability. For example, Smith et al. (1980) simulated anisometropia in kittens using strong defocusing lenses (range −10 to −16D) and Crewther et al. (1986) treated monkeys monocularly with lenses of +6, −6 and −9D. In these cases, subsequent changes in refractive development cannot be the result of changes in accommodation, firstly because the untreated eye in both studies probably guided the accommodative tonus (accommodation is tightly coupled in both eyes) and secondly because with some lenses the animal’s accommodative power was insufficient to refocus the retinal image.

If compensatory changes in growth patterns in the eye are expected as a result of the optical defocus provided by the lenses they may be very small compared to the changes seen in eyes which were deprived of form vision. This can be illustrated by the following consideration. The total refractive power of the dioptric system in a chicken eye of 10.5 mm axial length (dioptric equivalent of the anterior focal length) is about 150D. If a lens of 4 D is added, the change in total refractive power is only a few percent. If, in the very first approximation, it is assumed that only the plane of the retina is shifted during compensatory growth (as it would occur for ametropia completely axial in origin), the new position would differ by only some 200 μm. This is much smaller than the differences in axial length observed in occluder experiments (Gottlieb et al., 1988; Hodos and Kuenzel, 1984; Hayes et al., 1986).

In eyes with axial myopia image magnification and posterior nodal distance (PND) are increased compared to emmetropic controls (Duke-Elder, 1970). In the present paper we restricted our observations mainly to comparisons of the PND’s from eyes treated with different lenses and developed a very sensitive technique to record the PND in excised eyes.

MATERIALS AND METHODS

Animals

One-day-old chicks were obtained from local breeding colonies (Cornell K-Strain). The birds were kept under 14/10 hr light dark cycle. Food and water were available ad libitum. Additionally, data from five 26-day-old chickens (White Leghorn) originating from City College, NYC (Prof. Wallman) were used.

Hoods and lenses

Because chickens create a dusty environment, their lenses will be quickly turned into diffusers due to the layer of dust adhering to them if they are not regularly cleaned. Therefore they cannot be rigidly attached to the head as has been done for occluders in other studies. Hence we employed hoods made from soft, thin leather which were carefully fitted to their heads. The hoods could be tightened by their velcro fasteners and could be easily removed. This happened each night when the chicks remained in the dark. Five different sizes of hoods were necessary from day 7 to day 35 (Fig. 1). The chickens raised with hoods but without lenses did not differ significantly in body weight from those without hoods. The wearing of lenses, however, resulted in a slight decrease in body weight (about 10%). Lenses were cut from the center of ophthalmic lenses and had a diameter of 16 mm. They were attached to the hoods by velcro fasteners on their rims and were thereby easily removable for cleaning and centering which was done every 1–2 hr during the day. During clean-
Fig. 1. Chickens wearing the leather hoods. Small lenses (cut from the center of ophthalmic lenses) were attached to the hoods with velcro fasteners and were easily removable. (a) Chicks wearing lenses for 2 days (age: 11 days). (b) Chickens at day 30 of age. Note the chicken with occluders attached to the hood which allow only frontal vision.
Fig. 2. Infrared photoretinoscopy in ametropic chicken eyes. A computer program based on a video frame grabber (see appendix) allows portions of multiple frames to be recorded from a restricted area in the video image. Simultaneously the IR LED's in the photoretinoscope are flashed sequentially. Thus refractions at several eccentricities can be obtained in about 500 msec. **Leftmost column:** the refractive state (+0.25D) with the lens (+4D, first trace) is similar to that one seen in an untreated control chicken (not shown). **Second column:** after removal of the lens a hyperopic refraction (about +3D) is observed. Note the fundus reflexes in the top of the pupil. **Rightmost column:** myopic chicken eye (which has been treated with an occluder, refraction about −6D). Note the fundus reflexes in the bottom of the pupil. Figure indicates eccentricities (mm).
ing, lenses were off for about 10 sec. In this manner the amount of optical blur resulting from dust on the lens could be kept to a minimum. We could always obtain clear photoretinoscopic reflexes when chickens were refracted through the lenses and it is clear that the lens treatment is not comparable to a treatment with goggles which deprive the birds of form vision (Hodos and Kuenzel, 1984). In additional experiments, occluders made from black plastic foil were used. A vertical slit was cut in the frontal segment of the occluder [Fig. 1(b)] to allow for restricted vision in the frontal visual field (about 10–20 deg width, 10 deg lateral from the rostro-caudal axis). It has been shown that eye growth is almost linear during the first 50 days of life (Schaeffel et al., 1986; Hayes et al., 1986) and that the susceptibility to the development of ametropia is high over this period (Wallman and Adams, 1986). Because the hoods with lenses weighed approx. 3.4 g or approx. 10% of their hatching body weight, it constituted too great a load for the newly hatched chick. Accordingly we allowed the chickens to gain some weight during the first week of life before we started with the experiments.

Hoods (initially without lenses) were applied first at day 7. The chickens did not accept the hoods well during the first two hours as they stopped foraging. However the hoods were perfectly well tolerated later on, and all of the chickens foraged and gained weight. Also, no cases of ocular inflammation were observed. Lenses were first attached to the hoods at day 9. We used two different experimental groups which allowed us to evaluate inter-individual variability: the parameter most important for our measurements was the posterior nodal distance in the eye (see below) which is not only dependent on our manipulations but also on individual variability of eye size, age and body weight. Variability resulting from these factors is eliminated if differently treated eyes within one individual are compared. For the first experimental group, 10 chickens were raised with lenses of different optical power, but in all animals the same lens was used in both eyes. In a second experiment, the left eye received a positive lens and the right eye a negative lens (4 chickens) and vice versa (4 chickens). If two equally strong lenses were used the chicks accepted them immediately. Lenses of differing power initially induced a turning reaction towards the side of the more negative lens which disappeared after a few hours. Additionally, we raised 5 chickens treated with binocular occluders and 4 untreated control chickens for comparison.

**Refractions**

All refractions presented in this paper were done non cycloplegically. During an experimental session the focus of every eye was observed for two minutes and the most hyperopic reading was recorded. Measuring the refractive state in the uncyclopleged eye results in increased variability, because the amount of tonic accommodation may vary from day to day. However from frequent repetition (daily refraction of the animals) and the variability observed (see Fig. 4) we conclude that the differences described between eyes treated with lenses of different sign are significant. Non-cycloplegic refraction is advantageous for our experiments in that the natural accommodation tonus is conserved, a factor which might be important for normal eye growth. We used IR-photoretinoscopy to refract the birds as described in a previous paper (Schaeffel et al., 1986). Since then, the technique has been improved in several ways (Schaeffel et al., 1987). Additionally, a computer program (see Appendix) was used which allowed the multiple recording of video frames while IR LED's of increasing eccentricity were flashed simultaneously. With this method, several refractions from different eccentric light sources could be obtained from one eye within about 500 msec (Fig. 2). This improved the accuracy of the refraction.

**Calibration of IR photoretinoscopy vs streak retinoscopy**

IR photoretinoscopy was checked against conventional streak retinoscopy by refracting two chickens (noncycloplegically) twenty times using both techniques. A white light source was attached to the IR photoretinoscope (which otherwise produces no visible light) in order to mimic the retinoscope, because it was thought that the white light from the streak retinoscope could have attracted the chickens' attention. The differences seen in refractive state with the two techniques were not significant. In a second experiment 5 chickens (age 30 days) in which accommodation was paralyzed by ablation of the Edinger Westphal nucleus by D. Troilo (City College, NYC) both eyes were refracted using streak retinoscopy by the authors and
D. Troilo. Subsequently, IR photoretinography was performed. The correlation between the results of the two techniques was very high (IR vs streak: \( y = 0.88x - 0.187, r = 0.952, P < 0.0001 \)). Except in one case, the refractions did not differ by more than 1D. [A more hyperopic reading might be expected for infrared light due to the longitudinal chromatic aberration in the eye (Mandelman and Sivak, 1983). A possible explanation for the lack of this effect will be given below.]

**Measurement of posterior nodal distance, axial length and corneal refractive power**

Chickens were sacrificed with an overdose of ether anaesthetic between day 28 and 35. The eyes were excised in phosphate buffer (pH 7.4, 320 mosmol) to prevent osmotic pressures and mechanical stress and carefully freed from extraocular tissue. Alternately, the left or the right eye was measured first. An excised chicken eye provides excellent transscleral images (Rochon-Duvigneaud, 1922; Vakkur et al., 1963) due to its rather stable shape (scleral ossicles), its comparatively translucent fundus (Schaeffel et al., 1986) and good optical quality even after enucleation [Fig. 3(a)].

Four bright light guide apertures arranged in a square were positioned in front of the eye at a distance of about 640 \( \mu \)m (visual angle subtended about 16 deg). The light sources were imaged on the retina and, for an emmetropic eye, the diameter of the dots seen transsclerally from the backside of the globe was smaller than 0.3 mm. By inserting different ophthalmic lenses in front of the eye it could be seen that (a) the focus of the spots could not be improved and (b) there was no detectable increase of blur if lenses weaker than \( \pm 2 \)D were added. Thus the minimal diameter of the dots as seen transsclerally is determined by scattering of light in the tissue layers of the fundus and not by optical defocus. Eyes of known refractive error could be well refocused by appropriate lenses [Fig. 3(b), for a myopic eye of 7D]. Additionally, several eyes were refracted after enucleation. No differences greater than 1D were observed between the living and the enucleated eye. The observations suggest that the optical parameters which are important for our subsequent measurements are reasonably conserved in the excised eye. The posterior nodal distance (PND) was calculated from the distances of the light spots (without any correcting lenses) on the retina by a simple ray equation

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PND = \frac{D^*A'}{A}
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were \( (D) \) is the distance of the plane of the light guide arrangement to the eye, \( (A) \) the diagonal distance of the light guide apertures in that plane and \( (A') \) the diagonal distance of the transscleral light spots. The chicken eye is rather flat and the error resulting from the treatment of the retina as a plane is not detectable for the angles subtended by the light guides smaller than 20 deg. The PNDs increased slightly with time due to mechanical deformation in the excised globe but the changes did not exceed 1% during the first 5 min after enucleation. As the measurements were completed within 3 min, this error has not been considered in subsequent calculations. Quick data collection was made possible by a computer program which detected the position of the centers of the light spots in a highly magnified video image (17 \( \mu \)m per pixel, total frame size \( 480 \times 512 \) pixel) taken from backside of the globe. We used a video camera equipped with a 105 mm, f/1.8 Nikkor lens and several extension tubes. As the magnification is a very critical parameter in measuring distances, the camera lens was set to a fixed focus and the camera was moved to achieve sharp focus. The computer program also calculated the PND (see Appendix) from the distances between the two pairs of dots. A correction for different magnification in the vertical and the horizontal meridian had to be included (ratio of horizontal to vertical magnification = 0.842). The holder containing the eye was fixed on a graduated rotatable table with gradations accurate to 1 min of arc. With this arrangement the PNDs were recorded for every 10 deg across the visual field. Using a second video camera positioned from above, the outer dimensions of the eye could be measured simultaneously from a video image magnified to 38.5 \( \mu \)m per pixel. Measurements of axial length were not as exact as those from transscleral images as the influence of the parallax was important. The variability observed while turning the eye along its optical axis was \( \pm 0.15 \) mm. As the cornea provides the major refractive surface in the eye, corneal refractive power in the alert, living bird was determined shortly before dissection by IR photokeratometry (Schaeffel and Howland, 1987. The distances of the first Purkinje reflexes created by 8 IR LED's arranged in a circle were evaluated). A computer program similar to that used for the
Fig. 3. Transscleral images as seen from the backside of the excised eye. The four small light dots are the images of four bright light guide apertures positioned in front of the eye (diagonal visual angle about 16 deg, distance from eye to light guide array about 640 mm). (a) Photograph of an emmetropic eye. Note the correct focus of the image as indicated by the small size of the imaged dots. (b) In a myopic chicken eye (refraction about -6D) the transscleral image appears to be out of focus with +2D lens, but it can be refocused by inserting lenses in front of the eye. The power of the correcting lens ranges from +2D (strongest defocus) to -8D, applied in steps of two diopters (photographs from video images). (c) Infrared light (provided by IR LED's, about 890 nm) apparently cannot be focused as well as white light. Because the defocus is not correctable by additional lenses (left: no lens, right: -6D) it is concluded that the scattering of infrared light in the tissue layers of the fundus is greater than that of white light.
transscleral images detected the position of the 8 reflex dots in the video image of the cornea and calculated corneal radius of curvature (see Appendix). Each eye was measured at least four times. Standard deviations for the corneal refractive power did not exceed ±1D. The low variability of the measurement ruled out the possibility of spontaneous accommodation which, in turn, would affect corneal radius of curvature. We also measured corneal radius of curvature in some freshly enucleated eyes. No significant changes were observed between the living and enucleated eyes.

RESULTS

Development of the noncycloplegic refractive state

The refractive state could be easily observed in chickens while they were wearing lenses. It was found that all chickens were able to keep their retinal images in focus because no myopic or hyperopic photoretinoscopic reflexes (see Schaeffel et al., 1986) could be seen in the pupil through the lenses. Lenses stronger than +4D, however, were not accepted, as the chickens which wore them closed their eyes for most of the time. It was striking that even +4D lenses did not produce myopic refractions in the evening of the first day of wearing lenses, indicating that the chickens could relax their accommodation to compensate for the lenses.

We made the following observations of the development of the refractive state (measured with the lenses removed).

(1) Regarding positive lenses. All eyes treated with positive lenses became consistently hyperopic. The shift in resting refractive state could be first detected after about 5 days of treatment. The developmental course and the scattering of the values from the daily refractions can be seen in Fig. 4. The hyperopic defocus did not increase further after 10 days and did not exceed +4D.

(2) Regarding negative lenses. None of the chickens became as strongly myopic as one would expect from the power of the negative lenses. Instead, the chickens appeared to be focused at the plane of the refracting device and the observer. This would be equivalent to a myopic defocus relative to infinity of about −1.5 D (i.e. the reciprocal of the distance of the photoretinoscope which was about 0.7 m). It must be noted that for our photoretinoscope positioned at 0.7 m, the thresholds for detection of a reflex were −2.2 and −0.8 D, respectively relative to infinity. Therefore, when no reflex was seen the data were plotted at the center of this interval at −1.5 D (Fig. 4). Myopia stronger than −2.2 D was seen only occasionally at about 20 days of age and disappeared later on. However, the apparent asymmetry in the development of ametropia in both directions (hyperopic and myopic) decreases if a moderate retinoscopic artefact of approx. 1 D for small eyes (Glickstein and Millodot, 1970) is taken into account.

(3) Regarding the correlation between lens powers and amount of refractive error. While positive lenses produced clear hyperopic refractions of about 2–3D and negative lenses myopic refractions of about −1.5D there was no correlation between the strength of positive or negative lens and the degree of hyperopia and myopia, respectively (Fig. 5). However the regression between refractive state and the power of the applied lens was highly significant if calculated from all the lenses used (y = 0.369x + 0.809, d.f. = 20, T = 10.0, P < 0.0001, for both eyes of one individual treated with the same lens, and y = 0.385x + 0.856, d.f. = 14, T = 5.2, P < 0.001 for both eyes treated with different lenses, t-test). Apparently, the two eyes of one individual did not seem to be coupled in the development of the refractive state, because essentially the same result was seen independently of whether the fellow eye was treated equally or differently.

(4) Regarding the occlusion experiment. In agreement with results from other workers (Gotlieb et al., 1988; Hodos et al., 1985; Pickett-Seltzer et al., 1987), myopia usually developed rapidly in eyes which were restricted to frontal vision (Fig. 6). Accommodation was apparently lost in strongly myopic chickens which did not change their focus in response to a target which we presented. This was in contrast to lens wearing chickens which always displayed a very active accommodation. Myopia did not develop in all chickens wearing occluders. Two of them remained almost emmetropic with no obvious decline in accommodative ability.

Posterior nodal distance (PND)

To measure the PND in an eye across the visual field, the eye has to be aligned in a defined plane. The nasal pole in the excised eye can be easily found from the eccentric position of the pupil in the cornea (it is displaced towards the
heal). Angles given below were measured against the "axis of symmetry" in the eye (the perpendicular bisector of the equatorial diameter of the globe, as estimated from outside) while the eye was turned in the horizontal plane. The orientation of that axis was estimated by eye to an assumed accuracy of ± 2 deg. The axis subtends an angle of about 7 deg to the pupilary axis which is oriented more towards the nasal pole. The quality of the transscleral images (composed of four dots) remained surprisingly good while the eye was turned in the horizontal plane. Up to at least −60 deg (temporal retina) and 40 deg (nasal retina) there was no obvious defocus or increase in dot size. However the brightness of the dots declined in the peripheral retina due to the decreasing cross sectional area of the pupil. Some distortion could be seen in the very peripheral field as the magnification became bigger in the vertical than in the horizontal meridian (data not shown). In all eyes we observed a clear decrease in image magnification in the nasal retina [Fig. 9(a–c)].

We also tried to evaluate longitudinal chromatic aberration in the eye by replacing the image generating light guides in front of the eye by IR LED's. Surprisingly, we didn't measure an increase in PND for infrared light (890 nm) as would be expected from longitudinal chromatic aberration. Instead, the distance between the centers of the dots viewed transsclerally was even slightly decreased (corresponding to a decrease in the posterior nodal distance equivalent to half a diopter). Also the diameter of each dot

![Fig. 4(a)](image-url)
was clearly increased [Fig. 3(c)] and they could not be refocused by lenses placed in front of the eye. Thus it appears that white light and infrared light are differently scattered and reflected in the fundus, and that the reflecting plane for infrared light is less accurately defined.

Relation of the posterior nodal distance to axial length and refractive state

A series of experiments was performed in collaboration with David Troilo (City College, NYC) to test whether the PND can be used as an anatomical correlate for changes in refractive state. In 10 chicken eyes (the chickens belonging to another experimental group, age 26 days), axial length (ax) was measured by ultrasonography as described Wallman and Adams (1987) and by the technique described in the present paper (digitized video image). Ultrasonography gave a shorter axial length (by 0.24 mm of the mean) which can be expected because in the video image the external dimensions of the eye are measured. Both measurements were highly correlated (video image vs ultrasound: \( y = 1.35x - 3.29, \ r = 0.922, \ P < 0.001 \)). A high correlation was also ob-
served for the PND and the axial length as measured by either technique (PND vs ax (ultrasound): $y = 0.7x - 0.443$, $r = 0.938$, $P < 0.001$, and PND vs ax (video image): $y = 0.584x + 0.544$, $r = 0.943$, $P < 0.001$). Finally, axial length and PND were plotted vs the refractive error (as measured with the accommodation paralyzed). The correlations observed were significant as well [ax (ultrasound) vs refraction: $y = -8.54x + 88.4$, $r = 0.773$, $T = 3.45$, $P < 0.01$, ax (video image) vs refraction: $y = -6.8x + 72.5$, $r = 0.902$, $T = 5.9$, $P < 0.001$, and PND vs refraction: $y = -12.34x + 83.94$, $r = 0.803$, $T = 3.81$, $P < 0.01$]. It has to be kept in mind that inter-individual variability contributes to the measurement variability and that the correlations probably would be increased if both eyes in a single animal were compared.

**Posterior Nodal Distances for various treatments**

**Chickens wearing lenses.** The PND's (measured at 0 deg) from the first experimental group (identical lenses on both eyes) are shown in Fig. 8. PNDs were significantly bigger ($P < 0.025$, one-tailed t-test) in chickens which had worn negative lenses as compared to those which had worn positive lenses. There was some difference in the mean body weight in that chickens wearing plus lenses were heavier (183 ± 17 g) compared to those wearing minus lenses (155 ± 32 g). However it was not significant (d.f. = 10, $T = 1.79$, $P < 0.1$). Probably the difference in the PND between chickens wearing positive and negative lenses would have been larger if the chickens of the two groups had more similar body weights. Finally, the axial lengths from both groups were found to be not significantly different ($10.46 ± 0.29$ mm, negative lenses, vs $10.39 ± 0.29$ mm, positive lenses, d.f. = 18, $T = 0.52$, NS).

For the second experimental group, in comparing the PND's from the right eye (treated with a negative lens) and the left eye (treated with a positive lens) it can be seen that in all but one individual the PND was affected in a consistent way [Fig. 9(a)]. The reciprocal experiment gave similar results [Fig. 9(b)]. As it has already been found for the first experimental group (identical lenses in both eyes) the defocusing lens seemed to act more efficiently in the temporal retina. For comparison, data of the four control chickens (untreated) are shown in Fig. 9(c). Here, the differences in the PNDs from both eyes do not exceed 0.15 mm which must be considered as the maximal natural variability between both eyes. In all chickens, irrespective of their treatment, it can be seen that the PND is longer in the temporal than in the nasal retina.

The interocular difference in PND (at 0 deg) is plotted vs the difference in applied lens power (always the lens power of the left eye minus the lens power of the right eye) in Fig. 10(a). The regression ($y = 0.024x - 0.0093$) is significant (d.f. = 6, $T = 3.43$, $P < 0.01$, one-tailed t-test). The level of significance increases for the temporal retina (-20 deg, Fig. 10(b), $y = 0.035x - 0.001$, d.f. = 6, $T = 4.60$, $P < 0.005$, one-tailed t-test). In contrast to the first experimental group, differences in axial length were
Fig. 6. Development of the refractive state in three chickens treated with binocular occluders which allowed only frontal vision. Note the rapid development of myopia (more than 1D a day) as compared to Fig. 4.

weakly, but significantly, correlated to the differences in lens power ($-0.325 \pm 0.28$ mm, left eye with plus lens, vs $0.063 \pm 0.16$, right eye with plus lens, $d.f. = 6$, $T = -2.40$, $P < 0.05$, one-tailed $t$-test).

*Chickens wearing occluders.* The PND in chickens wearing partial occluders which became myopic (3 out of 5) was strongly increased (up to 1 mm at day 30). The result is in accordance with the large body of data already available for chickens treated with occluders (Wallman and Adams, 1986; Gottlieb et al., 1987; Hayes et al., 1986; Pickett-Seltner et al., 1987).

As yet, we have not examined the relationship between refractive state and PND in these animals in a systematic way.

**Corneal refractive power**

The cornea is the major refractive surface in the eye, and thus influences the refractive state very significantly. Corneal refractive power is, as are other optical parameters in the eye, a function of eye size and therefore body weight, age and individual variability. Therefore it is not easily compared in different animals from the first experimental group. The data become more
A-- Chicks treated with 

Fig. 7. Corneal refractive power vs axial length of the eye for chickens which wore the same power lens in both eyes. For this group it is difficult to compare the absolute values of corneal refractive powers of chickens which wore positive as opposed to negative lenses, because absolute eye size varied among individuals. Therefore corneal refractive power is plotted vs axial length in an effort to remove the influence of body size. However, the slopes are not significantly different and we conclude that there is no consistent effect of the lenses on corneal curvature. See also Table 1.

Table 1.

useful if an internal parameter is included to characterize absolute eye size. Figure 7 shows a plot of corneal refractive power (anterior surface only) vs axial length. The slopes are not different for chickens with positive and negative lenses which suggests that corneal refractive power has not changed consistently in response to the action of the applied lens. A direct comparison becomes more useful if different lenses are used in one animal (second experimental group). In four out of eight animals interocular differences in corneal refractive power were significant. However the sign of the differences was completely random here, and the same was observed in chickens wearing identical lenses in front of both eyes (Table 1).

The mean interocular difference in corneal refractive power was $2.56 \pm 1.41 D$ (absolute values) for all the chickens wearing lenses. This is significantly higher than the difference in corneal refractive powers seen in our control chickens wearing no lenses at all ($0.65 \pm 0.17 D$, d.f. = 20, $T = 5.56, P < 0.001$). It has to be noted that the treatment with a lens results in an increased variability in corneal refractive power but the sign of the change is not related to the sign of the lens. We therefore believe that growth processes are responsible for the increased variability in corneal refractive power rather than different amounts of tonic (corneal) accommodation.

DISCUSSION

We have applied defocusing lenses to chicken eyes during early postnatal development. The treatment resulted in consistent shifts in non-cycloplegic refractive state. These shifts were always in the direction which compensated for the defocus provided by the lens. In the excised eyes the PND was found to be bigger in eyes treated with negative lenses than in those treated with positive lenses. These facts alone are not sufficient to show that the changes in the PND by itself were responsible for the differences measured in the refractive state. To justify this conclusion it has to be shown that the optical power of the lens was invariant. However, because it has been observed that the PND is closely related to the refractive state (see "results"), the following considerations may be justified.

Relation between changes in PNDs and refractive state

In a first order approximation we may assume that the ametropia resulting from the application of lenses is completely axial in origin. As the PND is equivalent to the anterior focal...
Fig. 9. Posterior nodal distance (PND) as measured across the horizontal visual field in the excised eyes (second experimental group). (a) The right eye has been treated with a negative lens and the left eye with a positive lens (lens power encircled). Note that the PND is increased in eyes with a negative lens (except in chick 3C, which had worn the weakest lens). Also it can be seen that the difference between the two eyes is stronger in the temporal retina than in the nasal retina. (b) The reciprocal experiment gives the same results (left eye with the negative lens and right eye with the positive lens). (c) Untreated control chickens.
length of the eye, small changes in the position of the retina (expressed as $\Delta\text{PND}$) may be directly converted into a shift in refractive state. The absolute amount of defocus expected is given by

$$\text{defocus (D)} = 1000/\text{PND} - 1000/(\text{PND} - \Delta \text{PND})$$

were PND is given in (mm).

Data necessary for the estimation can be taken from Fig. 8 and Fig. 9. It can be seen that, for the first experimental group, eyes treated with positive lenses would then be more hyperopic by 4.2D compared to those treated with negative lenses (at 0 deg). In fact, the mean change in the measured refractive state was 3.6D. By comparison, the mean difference in the power of the positive and the negative lenses used in the experiment was 10D.

To evaluate the second experimental group, the shift in refractive state calculated from the change in the PND ($y$) has been compared with the difference in refractive power of the lenses ($x$) applied before. The regression lines were $y = 0.55x + 0.12$, d.f. = 6, $T = 3.55$, $P < 0.01$ (for 0 deg) and $y = 0.77x + 0.76$, d.f. = 6, $T = 4.79$, $P < 0.005$, one-tailed t-test (for -20 deg, temporal retina). From the slopes it can be seen that the “efficiency” of the compensatory growth (as calculated from the PND’s) could reach 77%. Again, the means of the calculated differences in refractive state were similar to the means of the measured differences in refractive state (5.2D vs 4.3D, respectively).

Regarding the efficiency of the compensation for lenses

The changes in non cycloplegic refractive state were not as strong as it might have been expected from the power of the lenses which were worn if the compensation were 100% efficient. The slope is 0.38 if refractive state is plotted vs the applied lens power (see Fig. 5). Because the changes in the PND can well explain the change in refractive state, we believe that compensatory eye growth occurred but that it was in fact incomplete. A possible reason could be the reduced retinal image quality with the lens.

Effects of the lenses on the PND were bigger in the temporal than in the nasal retina [Fig. 9(a, b)]. This implies that the differences in refractive state were bigger there as well. Unfortunately, we did not collect the data for the
off-axis refractions in a consistent way because of the difficulties in aligning the axis of the eye in the alert bird for the refraction.

From Fig. 9(c) it can be seen that also in untreated chickens the PND across the horizontal visual field is not symmetrical with respect to the "optical axis" of the eye. On the other hand, there is no consistent asymmetry in the shape of the posterior part of the globe in untreated chickens (Wallman et al., 1987). However the lens in the eye appears to be tilted towards the nasal pole by a few degrees (Hayes et al., 1986, and personal observation). Nonparaxial ray tracing with different models of gradient index lenses showed that this could account for the longer PND in the temporal retina (unpublished observation). If so, then in order to keep the refractive state constant across the visual field the lens must have a focal length which must be variable with changing line of sight.

Regarding the poor focusing of transcleral images in infrared light

Scattering of light in the tissue layers of the fundus is increased for longer wavelengths. It might be concluded that retinoscopic techniques are less accurate in infrared light. However this is not necessarily true: The intensity profile of the photoretinoscopic reflex in the pupil is determined by a superposition of the contributions from different layers in the fundus. It is assumed that the retina remains about equally thick during development and that the layers do not change their individual reflectivity in a consistent fashion. Based on these assumptions the technique is dependable if once calibrated in a natural eye (see "Methods").

Peripheral and central regulation of eye growth. It has been shown by Trollo et al. (1987) that increased growth of the vitreous chamber and myopia can be induced by ocular occlusion even after the eye has been disconnected from the CNS (by cutting the optic nerve). In contrast, the eye remains small and becomes hyperopic if it has not been occluded after the optic nerve cut. Trollo et al. (1987) suggest the presence of an internal mechanism in the eye for regulating its growth, which depends on the "retinal activity". (Deprived eyes are assumed to have low "retinal activity".) Additionally, a "central mechanism" is believed to act to control eye growth. Based on their model, our effects should be related to the "central mechanism". We have shown in a previous paper (Schaefel et al., 1986) that accommodation is completely independent in both eyes. This is also necessary for the chicken to keep both eyes in focus for two different lenses. The accommodative tonus has been frequently suspected (for example McKanna and Casagrande, 1981; Hodos and Kuenzel, 1984; Yinon, 1984) to be a factor in the process of tuning the retinal plane to the image plane. The effects described in our paper are consistent with this idea. The changes observed in the PND could correspond to a change in refractive state which can approach about 77% of that one expected from the applied lenses. The results are also consistent with the idea of a "closed-loop" system which includes the accommodative tonus as a parameter. However it can not be ruled out that local mechanisms in the retina which have been shown to control eye growth in chickens (Wallman et al., 1987) are sensitive enough to detect such small amounts of defocus as the ones provided by the lenses. Then, however, accommodation would not be necessary at all to tune eye growth. It also remains to be explained how the local differences in the PNDs (compare the temporal with the nasal retina, Fig. 9) can be produced by a central accommodative feedback loop.

Currently there are no models available to describe how the accommodative tonus might influence eye growth in chickens. One possible mechanism could include changes in intraocular pressure which result from changes in accommodative tonus. Future experiments have to show whether or not the intra-ocular pressure changes during accommodation and whether changes in the mechanical properties of the scleral coat can result from presumed accommodative changes in pressure. Finally it has to be kept in mind that accommodation mechanisms are quite different in birds and primates. But understanding the process of "emmetropization" in vertebrates generally.

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APPENDIX

The programs described are designed to be used in conjunction with the Oculus-200 real time video digitizer board (Coreco, Canada). The “frame grabber” allows one to store a frame in an array of 480 × 512 points (pixels) in the 256K of on-board RAM memory for later analysis. The image is digitized to an accuracy of 7 bits per pixel which provides 127 gray levels.

Program 1. Calculation of the PND from the Video Image of the Transcortical Light Spots

The stored frame is displayed on an auxiliary monitor. The user adjusts a box on the monitor screen from the keyboard so as to create an active window within which the image analysis is conducted. The program begins a search of the light spots. When a spot is found the coordinates of the position are sent to a routine to locate the center of the spot which is subsequently marked on the image. Coordinates and radius are stored in an array. The search routine is called again. When another spot is found its coordinates are compared with the coordinates of the previous spot to assure that it is a new one. The search continues until four light spots are found or the bottom of the active window is reached. The diagonal distances between the four spots are calculated in pixels. The program also asks for parameters such as optical magnification (pixel/mm), threshold for distinguishing a spot from background luminance and the external variables necessary to calculate the PND from the light guide arrangement.
**Program 2**

Determination of the corneal radius of curvature from the position of 8 Purkinje reflexes on the video image of the cornea.

In contrast to above, the search routine continues until 8 spots have been found within the active window. The spots are now sorted to assure that the correct (diagonal) distances between them are calculated. The end positions following sorting are:

1
2 3
4 5
6 7
8

The distances 1–8, 2–7, 3–6, 4–5 are then computed from the previously recorded center coordinates of the eight spots. Subsequently corneal radius of curvature is calculated as described elsewhere (Schaeffel and Howland, 1987).

**Program 3**

Multiple grabbing of video frames.

The program operates in conjunction with a photoretinoscope of six eccentricities. It is designed to grab six frames synchronized with the flashing sequence of the IR LED's (which are positioned at different eccentricities from the optical axis of the video camera). As in the programs described above, an active window on the video monitor has to be defined by the user. The smaller the window the faster is the image recorded. The window size must be kept small enough to ensure that the final six images can be displayed on the video monitor simultaneously. Once the window size has been adjusted around the relevant part of the image, six buffers of this size are defined in the memory. These will be used to store the six recorded images until they can be displayed. The timing of the LED's by the electronic circuit driving the photoretinoscope must be adjusted to match the grabbing frequency.