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Clinical features of the retinopathy, globe enlarged (*rge*) chick phenotype

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Abstract

The purpose of the study reported here was to characterize the clinical aspects of the autosomal recessive retinopathy, globe enlarged (*rge*) phenotype in chicks (*Gallus gallus*). *Rge*/rge, *rge*/+ and +/+ chicks were studied from hatch to 336 days of age by general clinical examination, post-mortem examination, vision testing with an optokinetic device, ophthalmoscopy, biomicroscopy, tonometry, central corneal pachymetry, a-mode ultrasonography, infrared photoretinoscopy and photokeratometry. Additionally, preliminary electroretinographic and histopathologic investigations were performed. There is a variable degree of vision loss in *rge*/*rge* chicks at 1 day of age with further chicks losing vision over the next few weeks until all chicks become functionally blind by 30 days of age (although some optokinetic responses remain in some of the *rge*/*rge* chicks). Over the first few weeks of life *rge*/*rge* chicks develop thicker corneas with a larger radius, hyperopia, shallower anterior chambers and enlarged globes both radially and axially, compared to controls. A preliminary ERG study showed that 1 day old *rge*/*rge* chicks have an elevated response threshold, a lower amplitude a-wave with a markedly shallow leading slope, a lack of both oscillatory responses and c-waves and, at brighter flashes, an increased b-wave amplitude. Light microscopy revealed no gross retinal abnormalities in young chicks to account for the blindness. A thinning of all retinal layers developed in parallel with globe enlargement. The *rge* defect is a unique progressive retinal dystrophy that results in a severe visual deficit, abnormal electroretinographic waveforms, and secondary globe enlargement.

Keywords: Electroretinogram; Autosomal recessive; Retinopathy; Slow retinal degeneration; Globe enlargement; *Gallus gallus*

1. Introduction

Hereditary blindness has been recorded in a number of different strains of chicken. The retinal degeneration (*rd*) chicken has been characterized and has proven to be a model for Leber Congenital Amaurosis in humans (Semple-Rowland, Lee, VanHooser, Palczewski, & Baehr, 1998). The *rd* chicken phenotype is an autosomal recessive, early-onset retinal dystrophy and is due to a *null* mutation in the photoreceptor guanylate cyclase gene (*GC1*), a gene that is mutated in some Leber Congenital Amaurosis patients (Semple-Rowland et al., 1998). The *rd*/*rd* chicks are blind from hatch and have a severe early-onset retinal degeneration with non-recordable ERGs (Ulshafer & Allen, 1985; Ulshafer, Allen, Dawson, & Wolf, 1984). Other forms of retinal dystrophy in chickens include retinal dysplasia and degeneration (*rdd*) and blindness with enlarged globe (*beg*). *Rdd* chicks are visually impaired at an early age and become totally blind. As the name suggests, the retina is dysplastic and then degenerates. Morphologically the *rdd* phenotype is recognizable by abnormalities in both the retinal pigment epithelium and the neural retina (Randall et al., 1983). The *beg* chicks are blind at hatch, have vestibular problems and develop globe enlargement (Pollock, Wilson, Randall, & Clayton, 1982).

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Chicks have been used in studies of the development of ametropias because alterations in globe dimensions are readily induced by alterations in the retinal image, including vision deprivation, defocus (Beresford, Crewther, Kiely, & Crewther, 2001) and constant light exposure (Li, Howland, & Troilo, 2000). Globe enlargement is also a feature of some retinal dystrophies such as *beg* and also the retinopathy, globe enlarged (*rge*) defect described in this study.

The *rge*/*rge* chick has an autosomal recessive mutation, identified as causing blindness and globe enlargement in some birds in flocks of commercial layer chickens in the UK (Curtis, Baker, Curtis, & Johnston, 1987; Curtis, Baker, Curtis, & Johnston, 1988). A flock of *rge*/*rge* birds was initially established by one of the authors (RC). A retinal origin for the blindness was suspected on the basis of preliminary electroretinographic investigations (Curtis et al., 1988). The name retinopathy, globe enlargement (*rge*) was selected to reflect the characteristics of the disorder.

A flock of *rge* chickens was established at Roslin Institute (Edinburgh) by crossing a single *rge*/+ bird from Curtis' original flock with a white leghorn. Subsequently a second flock was established at Michigan State University from hatching eggs imported from the Roslin Institute. This paper reports the initial clinical characterization of the *rge* phenotype.

2. Method

Breeding was performed to produce homozygous affected (*rge*/*rge*), heterozygous carrier (*rge*/+) and homozygous normal (+/+) chicks. Birds were group housed under 12 h light/dark cycles and fed a commercial chicken diet (Home Fresh Poultry Feed, Kent Feeds, Inc. Muscatine, IA).

All procedures using chicks were conducted in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and approved by the Institutional Animal Use Committee.

2.1. General examination, observation of behavior, ophthalmic examination and necropsy examination

21 birds, of which 9 (6 males and 3 females) were *rge*/*rge* and 12 were *rge*/+ (4 males and 8 females) were examined at hatch, then at 7, 14, 21, 28, 42, 49, 70, 77, 84, 98, 280, 308 and 336 days of age. A general physical examination was performed at the time intervals listed. The ability of the birds to locate and peck at food particles on the floor was evaluated. An ophthalmic examination was performed using a transilluminator, hand-held slit lamp and indirect ophthalmoscope. Comparative necropsies were performed on *rge*/*rge* and *rge*/+ and +/+ birds at hatch, 2 and 6 months of age. Tissue samples from the CNS, liver, muscle and heart were collected from 4 *rge*/*rge* and 4 *rge*/+ chicks and submitted for histopathology.

2.2. Vision testing with an optokinetic device

Visual acuity was assessed by the use of an optokinetic device. This consisted of a drum with a diameter of 38 cm, which could be lined with different sets of alternating black and white vertical stripes, and could be rotated, in alternating directions around the bird at a velocity of 48 degrees/second. The widths of black and white stripes stimulus used in this study were 4.5, 7, 13 and 25 mm with a spatial frequency of 0.37, 0.23, 0.13 and 0.06 cycles per degree, respectively. The head of the test bird was approximately at 19 cm from the stripes. Vision was assessed under both dim and bright light, 0.22 and 24.5 cd/m^2 respectively, as measured by a photometer (Research Radiometer IL 1700 with SED033 silicon light detector, International Light, Inc. Newburyport, MA). Birds were tested at 1, 14, 30, 60, 150 and 270 days of age. The response of the chick was observed and classified as positive response (head and neck turned to follow rotating stripes), partial response (head sometimes followed the rotation of the stripes but not every time) or negative response (the head never turned to follow the rotating stripes).

2.3. Tonometry, central corneal pachymetry and eye dimension measurements

Central corneal thickness (CCT), intraocular pressure (IOP), and axial globe length were measured following application of a topical anesthetic (proparacaine hydrochloride 0.5% ophthalmic solution USP—Alcon Laboratories, Forth Worth, Texas). In each case three readings were taken and averaged for each eye of each bird. These measurements were repeated on the same 21 birds, of which 9 (6 males and 3 females) were *rge*/*rge* and 12 were *rge*/+ (4 males and 8 females).

CCT was measured using an ultrasonic pachymeter (Pachette™, Ultrasonic Pachymeter Model DGH 500, Exton, PA) with the speed of sound in the cornea pre-set at 1640m/sec. The axial globe length was measured by A-mode ultrasonography (Humphrey—A/B Scan System 835—Dublin, California).

IOP was measured by applanation tonometry using a Tonopen (Mentor; Norwell MA). Additional, more detailed A-mode ultrasonographic measurements of the eyeball, including lens thickness, anterior and vitreous chamber depth were performed in another group of 16 *rge*/*rge* and 17 *rge*/+ chicks at 13 days of age (6 *rge*/*rge* and 5 *rge*/+), 33 days of age (7 *rge*/*rge* and 9 *rge*/+) and 92 days of age (3 *rge*/*rge* and 3 *rge*/+), using an ophthalmic A-mode ultrasonic biometer (Echorule—Phakosystems Inc. Downsville, Ontario, Canada). The

weight of the enucleated globe trimmed of extraocular tissues was also recorded in this group of birds.

The radial globe diameter (dorsal equator to ventral equator) of both eyes was measured using Castroviejo calipers immediately following enucleation of 15 *rge/rge* and 15 *rge/+* terminally anesthetized birds at 13, 33, 92, 200 and 270 days of age.

2.4. Refraction and measurement of corneal curvature

Infrared photoretinoscopy (Howland & Schaeffel, 1989; Schaeffel, Howland, & Farkas, 1986) and photokeratometry (Schaeffel & Howland, 1987) were performed on 16 *rge/rge* and 17 *rge/+* chicks at 13 days of age (6 *rge/rge* and 5 *rge/+*), 33 days of age (7 *rge/rge* and 9 *rge/+*) and 92 days of age (3 *rge/rge* and 3 *rge/+*). The infrared photoretinoscopy technique was calibrated against conventional retinoscopy in chickens and agrees with the results of the latter within a fraction of a diopter, the difference being due to the difference in wavelengths employed and the chromatic aberration of the chick eye (Howland, personal communication, 2003).

2.5. Electroretinography

A scotopic white light intensity series ERG was recorded from the left eye of 6 *rge/rge* chicks and 5 +/+ chicks within 12 h of hatch using a Utas 3000 Electrophysiology unit (LKC Inc, Gaithersburg, MD). The pupils were dilated by application of topical 1% vecuronium bromide (ESI Lederle, Philadelphia, PA), the chicks were anesthetized with isofluorane delivered in oxygen, and then dark adapted for 20 min. Stay sutures were placed in the conjunctiva to position the globe in primary gaze and a bipolar Burian Allen ERG corneal contact lens (Hansen Ophthalmic Inc. Solon, IA) positioned. Light stimuli were delivered via a Ganzfeld. The following flash intensities were used: −2.4, −2, −1.42, −1.194, −0.79, −0.39, −0.002, 0.394, 0.85, 1.4, 2.3 and 2.8 cdS/m^{-2}. All responses were recorded with the bandpass set at 1–3000 Hz and computer averaged.

2.6. Histopathology

Immediately following euthanasia bilateral enucleation was performed. The vitreous chamber was injected with 4% paraformaldehyde in 0.1 M sodium cacodylate buffer through the *pars plana* and the globe plunged into the same fixative at 4 °C. After 30 min the anterior segment including lens and vitreous was removed and the eye cup immersed in the same fixative overnight at 4 °C. Following dehydration through graded ethanol, collected tissues were embedded in a low viscosity glycol methacrylate plastic media, (Immuno-Bed. Electron Microscopy Sciences, Fort Washington, PA), and sectioned in 3 μm thick slices through the optic nerve in the superior–inferior plane of the retina. Tissue sections were then collected, mounted on glass slides and stained with toluidine blue or hematoxylin/eosin and analyzed.

2.7. Statistical analyses

Statistical analyses of the clinical measurements and results were performed by repeated measures analysis of variance (ANOVA), one-way ANOVA (radial globe diameter) and *t*-test (when comparing one time-point in two groups). Data from the vision testing using the optokinetic device was analyzed using an ordinal model for multinomial data. ERG means for amplitude and latency were averaged for each light intensity analyzed using logistic regression. The tests were all run in two different statistical analysis softwares (StatView and SAS 2001—version 8.2. SAS Institute Inc., Cary, NC). If any statistically significant difference was found the data were further analyzed using post hoc comparisons with Fisher's or Tukey-Kramer tests. Data were deemed significant when $P < 0.05$.

3. Results

A summary of the statistically significant differences in ocular dimensions, refraction and IOP of *rge/rge* chicks compared to controls is shown in Fig. 1. There was no significant difference between *rge/+* and wild type birds (+/+) for any of the measurements (data not shown).

3.1. Physical examination and post-mortem examinations

Rge/rge chicks appeared less active than *rge/+* and +/+ chicks immediately after hatch, although this was not quantified. The *rge/rge* birds sporadically exhibited a characteristic slight lateral nodding movement of the head and some "pecking at the air" behavior starting at 2 weeks of age. This characteristic behavior continued for the duration of the study. Affected and control birds responded similarly to sound stimuli produced by hand clapping.

No gross lesions nor any non-ocular abnormalities were observed in *rge/rge* birds on necropsy or light microscopy histopathologic examination of CNS, liver, muscle and heart (data not shown).

3.2. Vision testing

From hatch *rge/rge* chicks were less efficient than *rge/+* and +/+ chicks at pecking food particles placed on the floor, spending an average of 3 attempts before actually picking up the particle. By 30 days of age the *rge/rge* chicks did not attempt to peck at food particles placed

	rge Phenotype	Result and SD for *rge/rge*	Result and SD for *rge/+*
Corneal Radius	Increased at 33 days of age****	4.68 ± 0.17 mm	3.90 ± 0.19 mm
Central Corneal Thickness	Increased at 42 days of age**	258.75 ± 10.50 μm	243.83 ± 3.55 μm
Axial Globe Length	Increased at 92 days of age****	14.41 ± 0.16 mm	12.55 ± 0.97 mm
Radial Globe Diameter	Increased at 33 days of age**	1.62 ± 0.96 cm	1.38 ± 0.12 cm
Anterior Chamber Depth	Decreased at 13 days of age***	0.858 ± 0.68 mm	0.980 ± 0.42 mm
Vitreous Chamber Depth	Increased at 33 days of age***	7.56 ± 0.32 mm	6.58 ± 0.34 mm
Eyeball Weight	Increased at 33 days of age***	1.42 ± 0.10 g	1.14 ± 0.90 g
Refraction	Hyperopia (higher at 33 days of age)***	12.00 ± 2.30 D	4.44 ± 1.60 D
Intraocular Pressure	Decreased at 49 days of age**	17.60 ± 1.26 mmHg	20.25 ± 1.57 mmHg

Fig. 1. Summary of the main clinical features observed in chicks presenting the *rge* phenotype: $^{**}P < 0.05$, $^{***}P < 0.001$, $^{****}P < 0.0001$.

on the floor. Once the *rge/rge* chicks were familiar with their environment they were able to consistently locate feed and water containers. From approximately 30 days of age *rge/rge* chicks failed to show an "escape response" to the visual stimulus produced by the approach of a person, making the *rge/rge* chicks easier to catch than the *rge/+* and +/+ chicks. Visual menace response was absent in the *rge/rge* chicks by about 30 days of age but present in all the age-matched *rge/+* and +/+ chicks.

An assessment of visual acuity using the optokinetic device showed that the percentage of *rge/rge* chicks showing a response (partial or complete) to the rotating stripes decreased with age (Fig. 2). At each time-point, and for each width of stripe, the percentage of chicks showing any response was lower under the dimmer room lighting. Although at 150 days of age 60% of *rge/rge* chicks showed some response to the widest stripe under bright lighting conditions (Fig. 2) they were not able to peck at food particles, had an absent menace response and "escape response".

The correlation of reduction in response to stripes of smaller widths, with increasing age and with lower light levels in comparison to *rge/+* or +/+ chicks was significant ($P < 0.001$).

3.3. Ophthalmic examination and globe biometry findings

The pupillary light reflex became increasingly sluggish in the *rge/rge* chicks, although it did not disappear completely during the time of the study. Funduscopy revealed that about half the *rge/rge* chicks, at approximately 42–49 days of age, had a number of white to gray linear fundus lesions typically extending from the pecten to the periphery (Fig. 3A). Additionally, from the same age, most of the *rge/rge* fundi appeared to have reduced pigmentation and the pecten appeared to be smaller in size.

Globe enlargement developed in the *rge/rge* chicks (Fig. 3B, 4 and 5) resulting in a characteristic exposure of sclera at the medial canthus region bilaterally. The area exposed increased with age. Clinical examination showed that the cornea was flatter and the anterior chamber shallower in the older *rge/rge* chicks compared to *rge/+* and +/+. In the older birds the anterior surface of the lens was almost in contact with the posterior corneal surface (Fig. 3C). *Rge/rge* birds commonly developed anterior subcapsular and cortical cataracts by 4–6 months of age.

Morphometric measurements of the globe confirmed the clinical impression of a progressive globe enlargement both in the radial globe diameter (Figs. 3B and 4, as measured on enucleated globes) and in the axial direction (Fig. 5, a-mode ultrasound). The mean radial diameter of the globe was significantly greater than *rge/+* and +/+ by 33 days of age ($P < 0.05$) (Fig. 4) and the axial globe length by 92 days of age ($P < 0.0001$) (Fig. 5). Globe mass increased in parallel with globe enlargement and by 33 days of age *rge/rge* globes were significantly heavier than *rge/+* ($P < 0.001$). To investigate the possibility that globe enlargement was secondary to an increase in IOP tonometry was performed. There was no significant difference in IOP between *rge/rge*, *rge/+* and +/+ chicks until 49 days of age, at which time the IOP of *rge/rge* chicks was lower than *rge/+* and +/+ ($P < 0.05$) (Fig. 1) and remained lower for the duration of the study (data not shown).

A detailed ultrasound study of axial globe measurements showed that the anterior chamber of *rge/rge* chicks was significantly shallower than that of *rge/+* from the first time point measured (13 days of age, $P < 0.05$) (Fig. 1) and continued to become shallower with age ($P < 0.001$), confirming the slit-lamp observations. The increase in axial globe length in *rge/rge* chicks was due primarily to an increase in the vitreal chamber depth, which was significantly different from *rge/+* by 33

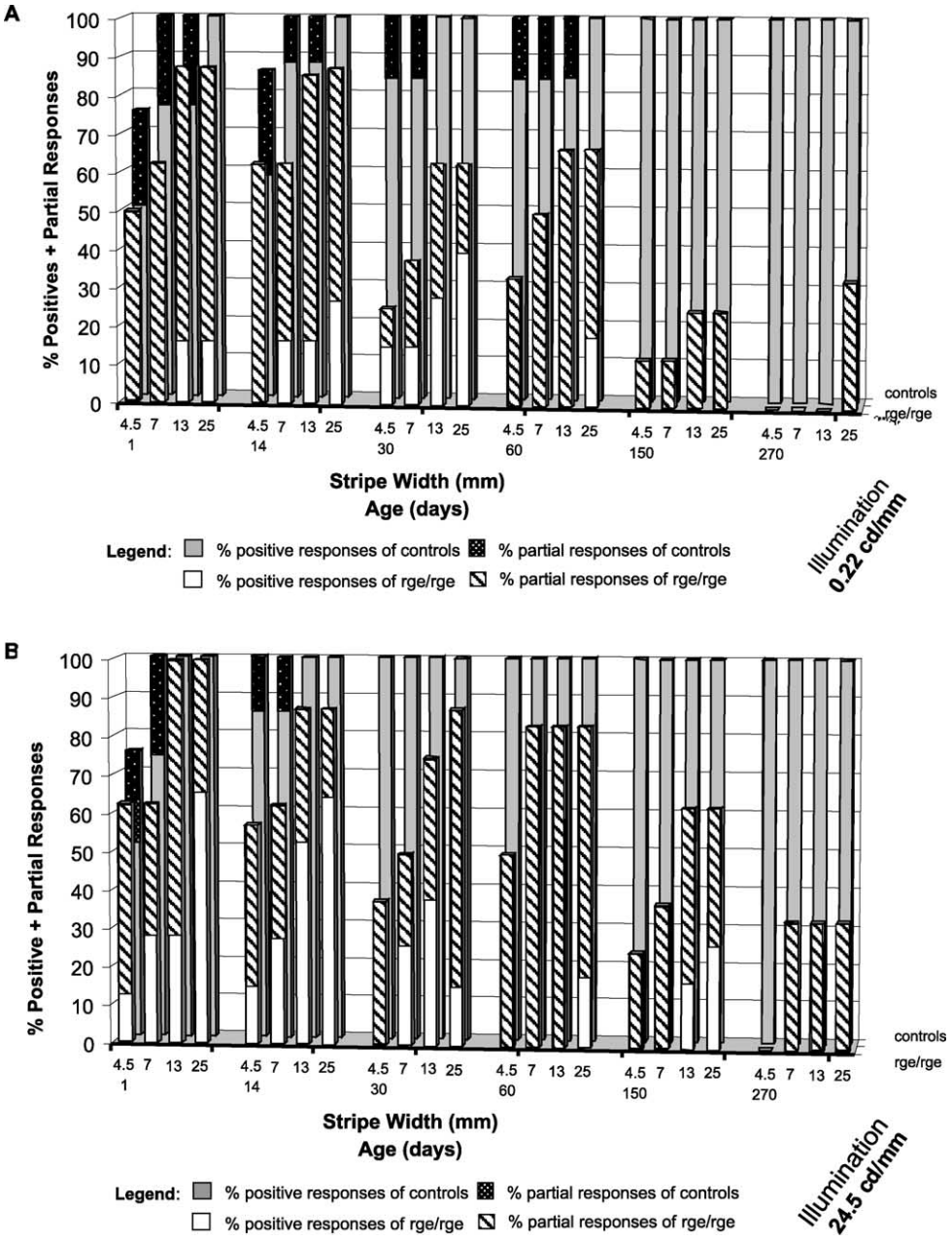

Fig. 2. Comparison of the percentages of control (*rge*/+, +/+) and *rge*/*rge* birds that responded positively or partially to the optokinetic device, under two different light intensities, (A) 0.22 cd/m^2 and (B) 24.5 cd/m^2. 1 day of age $n = 4$ controls and 8 *rge*/*rge*; 14 days of age $n = 7$ controls and 8 *rge*/*rge*; 30 days of age $n = 6$ controls and 8 *rge*/*rge*; 60 days of age $n = 6$ controls and 6 *rge*/*rge*; 150 days of age $n = 6$ controls and 8 *rge*/*rge*; 270 days of age $n = 2$ controls and 3 *rge*/*rge*.

days of age ($P < 0.001$) (Fig. 1). The axial thickness of the lens was not significantly different from *rge*/+ and +/+ at any time-points measured ($P = 0.34$). Pachymetry reveal that there was a significant increase in CCT in *rge*/*rge* chicks compared to *rge*/+ and +/+ by 42 days of age ($P < 0.05$) (Figs. 1 and 6), this statistical difference was maintained for the duration of the study (Fig. 6).

Corneal curvature (radius) was measured by infrared photokeratometry to investigate the change in radius detected by ophthalmoscopic and biomicroscopic examination. The mean corneal curvature (corneal radius) of *rge*/*rge* and *rge*/+ chicks was not significantly different at 13 days of age (3.4 ± 0.1 and 3.4 ± 0.08 mm, respectively, $P = 0.79$), but by 33 days of age the cornea of *rge*/*rge* chicks was had flattened resulting in a significantly greater corneal radius than that of the *rge*/+ chicks (4.68 ± 0.17 and 3.9 ± 0.19 mm, respectively, $P < 0.0001$). By 92 days of age there was further flattening of the cornea in the *rge*/*rge* chicks (corneal radius in *rge*/*rge* 6.8 ± 0.4, and in *rge*/+ 4.8 ± 0.2 mm, $P = 0.0015$).

Infrared photoretinoscopy was performed to investigate the effects of the abnormal globe morphology of

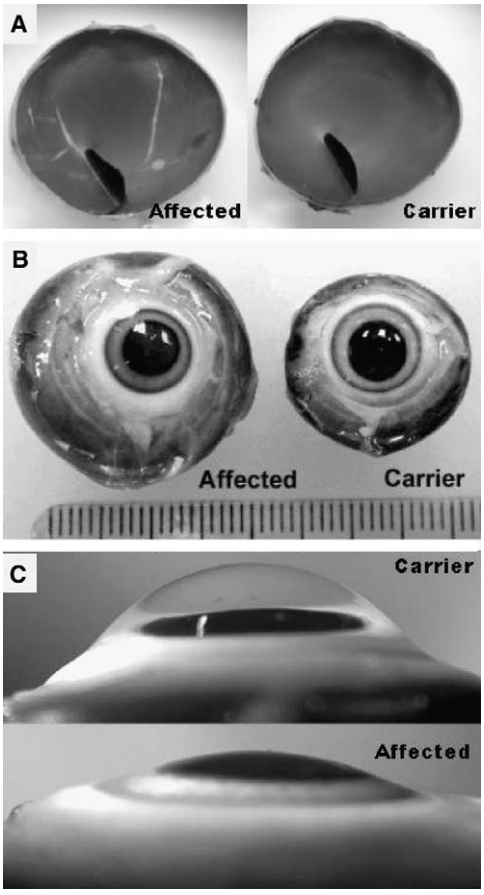

Fig. 3. (A) Macroscopic appearance of the eyecups of an affected (*rge/rge*) and a carrier (*rge/+*) chick at 45 days post-hatch. Note the presence of white linear lesions in the *rge/rge* retina (arrows). Histology of this lesion can be seen in the Fig. 10. (B) Macroscopic appearance of the globes of an affected (*rge/rge*) and a carrier (*rge/+*) chick at 180 days post-hatch. Note the difference in radial diameter of the globes. (C) Comparison of the anterior segment of an affected (*rge/rge*) and a carrier (*rge/+*) chick at 180 days post-hatch. Note the flatter cornea and very shallow anterior chamber of the affected eye.

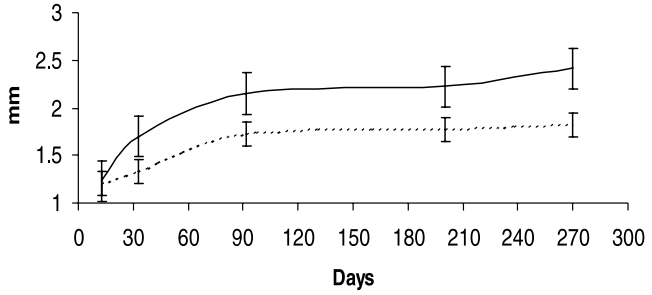

Fig. 4. Radial globe diameter of *rge/rge* (uninterrupted line) and control (dashed line) eyes with respective standard error bars on each time-point evaluated. Note that the mean radial globe diameter of the *rge/rge* eyes became significantly different (greater) than the controls at 33 days of age (this is before the axial length was significantly greater). 13 days of age ($n = 3$ *rge/rge* and 4 *rge/+*), 33 days of age ($n = 4$ *rge/rge* and 4 *rge/+*), 92 days of age ($n = 3$ *rge/rge* and 2 *rge/+*), 200 ($n = 3$ *rge/rge* and 2 *rge/+*) and 270 days of age ($n = 2$ *rge/rge* and 3 *rge/+*).

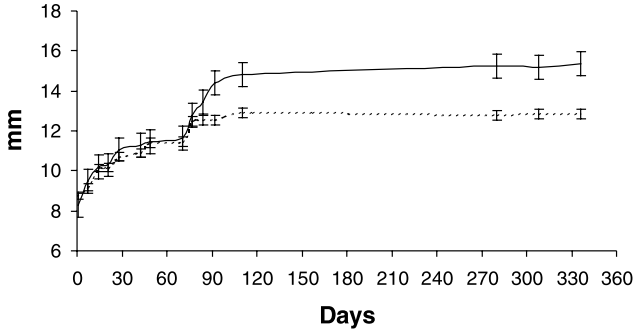

Fig. 5. Mean axial globe length of *rge/rge* (uninterrupted line) and control (dashed line) eyes with respective standard error bars on each time-point evaluated. Note that the mean axial globe length of the *rge/rge* eyes became significantly greater than the controls at 92 days of age. $n = 21$ birds, 9 were *rge/rge* and 12 were *rge/+*. Examined at 1, 7, 14, 21, 28, 42, 49, 70, 77, 84, 92, 98, 280, 308, 336 days of age.

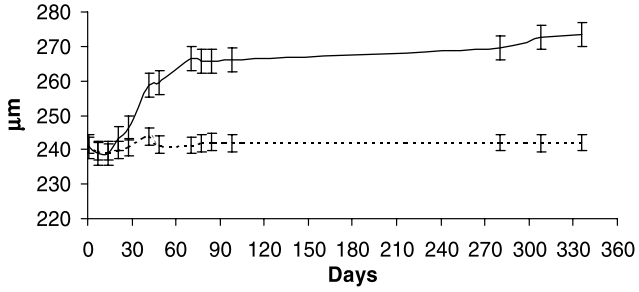

Fig. 6. Mean CCT of *rge/rge* (uninterrupted line) and control (dashed line) eyes with respective standard error bars on each time-point evaluated. Note that the mean CCT of the *rge/rge* eyes became significantly different (thicker) than the controls at 42 days of age. $n = 21$ birds, 9 were *rge/rge* and 12 were *rge/+*. Examined at 1, 7, 14, 21, 28, 42, 49, 70, 77, 84, 98, 280, 308, 336 days of age.

rge/rge chicks on refraction. The *rge/rge* and *rge/+* chicks had a similar degree of hyperopia at 13 days of age (10.8 ± 2.5 and 10.4 ± 1.7 diopters, respectively), by 33 days of age the degree of hyperopia of *rge/+* chicks had decreased to 4.4 ± 1.6 diopters ($P < 0.001$) while that of *rge/rge* chicks remained significantly greater at 12.0 ± 2.3 diopters ($P < 0.001$). Measurements taken at 92 days of age showed that the degree of hyperopia of *rge/+* chicks was 4.3 ± 0.57, while that of *rge/rge* chicks was 9.8 ± 7.4 diopters.

3.4. Electroretinography

The mean dark adapted a-wave and b-wave thresholds (threshold considered to be a response greater than 3 μV) of the *rge/rge* chicks at 12 h of age was elevated by approximately 1.4 log units (0.0 log cdS/m^2) compared to the +/+ chicks (−1.4 log cdS/m^2), the amplitude of the a-wave response to all flash intensities was decreased and the latency increased ($P < 0.001$). Furthermore, the steepness of the leading slope of the a-wave was de-

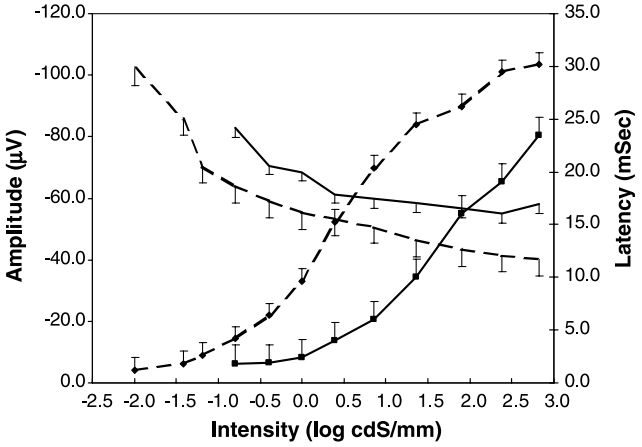

Fig. 7. Mean a-wave amplitude and latency, with respective standard error bars, 12 h after hatch, using an intensity series of flashes in a dark-adapted *rge/rge* and control +/+ birds. The black solid line represents the mean a-wave amplitude curve of the *rge/rge* birds. The black dashed line represents the a-wave amplitude curve of +/+ controls. The gray solid line represents the a-wave latency curve of the *rge/rge* birds. Gray dashed lines represent the a-wave latency curve from +/+ controls. Note the increased threshold, and difference in amplitude with increasing intensities between the *rge/rge* and control birds ($n = 7$ +/+ and 7 *rge/rge* birds).

creased compared to +/+ chicks. After a delayed threshold the b-wave response of *rge/rge* chicks increased with increasing flash intensity to a greater extent than the +/+ chicks (Figs. 7–9) and as the flash intensity increased above 1.4 log cdS/m^2 the b-wave amplitude of the *rge/rge* chicks was greater that that of the +/+ chicks ($P < 0.0001$). Furthermore the b-wave latency, which

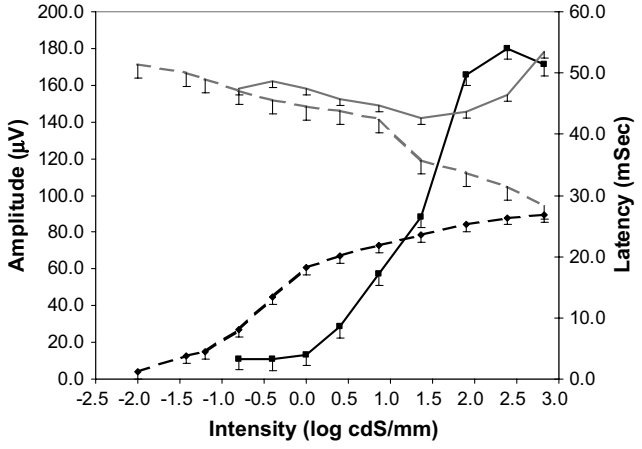

Fig. 8. Mean b-wave amplitude and latency, with respective standard error bars, 12 h after hatch, using an intensity series of flashes in a dark-adapted *rge/rge* and control +/+ birds. The black dashed line with markings represents the mean b-wave amplitude curve of the *rge/rge* birds. The black solid line represents the b-wave amplitude curve of +/+ controls. The gray solid line represents the b-wave latency curve of the *rge/rge* birds. The gray dashed line represents the b-wave latency curve from +/+ controls. Note that with higher light intensities (1.4, 1.9 and 2.4 log cdS/m^2) b-wave amplitude is higher in *rge/rge* than controls ($n = 7$ +/+ and 7 *rge/rge* birds).

following b-wave threshold was similar to controls and initially decreased with increasing flash intensity, started to increase with increasing light intensities. The ERGs recorded from *rge/rge* chicks had a lack of both oscillatory potentials and c-waves (Fig. 9B).

3.5. Histopathology (Fig. 10)

Examination of the retina in day-old *rge/rge* chicks by light microscopy (not shown) revealed grossly normal retinal morphology. The *rge/rge* chicks developed a slowly, progressive thinning of all retinal layers, but this was not obvious until the chicks were over 2 months of age (not shown) and worsened with age. After this age photoreceptor inner and outer segments became swollen and outer segments shortened. The outer nuclear layer thinned with increasing age and the remaining cell bodies became swollen. The outer plexiform layer became more severely thinned than the other retinal layers. There was a reduction in cell numbers in the inner nuclear layer and some of the remaining cells were swollen. Thinning of the inner plexiform layer and loss of ganglion cells was also apparent in older birds. The nerve fiber layer was the best preserved of all retinal layers and had a similar thickness as the controls in comparable retinal areas at all ages examined. Histological examination of the linear gray retinal lesions that had been observed ophthalmoscopically in *rge/rge* chicks revealed that the area consists of a focal loss of retinal pigment epithelium, with reduced thickness of the overlying inner and outer nuclear layer and accumulation of disorganized eosinophilic material where the photoreceptor outer segments originally were.

4. Discussion

This preliminary study confirms that *rge/rge* chicks suffer from a unique autosomal recessive retinal dystrophy phenotypically different from previously described chick retinal dystrophies such as *rd*, *rdd* and *beg* (Pollock et al., 1982; Randall et al., 1983; Semple-Rowland et al., 1998; Ulshafer & Allen, 1985; Ulshafer et al., 1984). The vision of *rge/rge* chicks deteriorates over the first few weeks after hatch and is worse under lower lighting conditions. They become unable to peck at small crumbs of food and lose vision related responses such as menace and escape responses progressively during the first few weeks of age. Some *rge/rge* birds have no visual responses as assessed by the optokinetic device at one day after hatch, whereas others retain some optokinetic responses for several weeks. However, even those that retain some optokinetic responses did not have sufficient vision to allow them to peck at food particles beyond about 30 days of age.

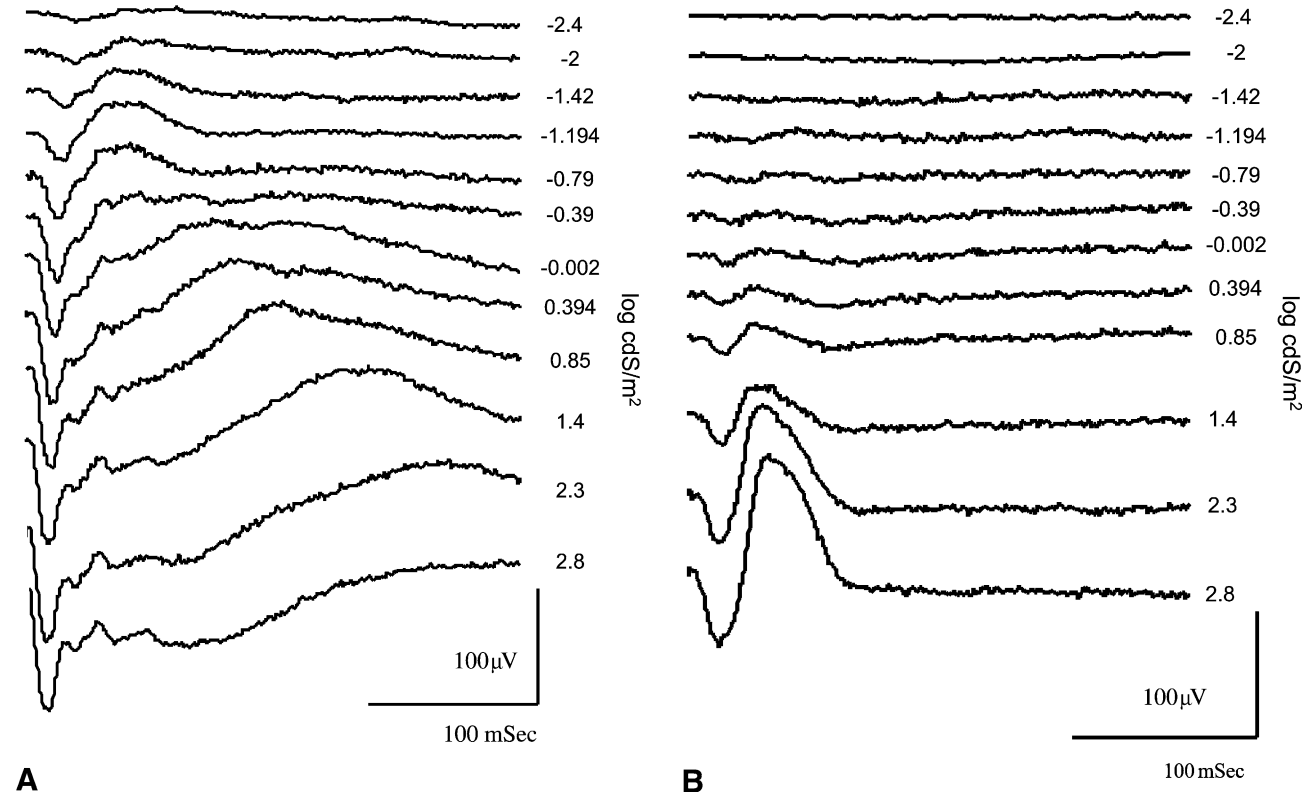

Fig. 9. (A) Representative ERG recordings from a control +/+ bird at hatch. Light intensities are indicated in the figure. Note the high a-wave amplitude, reduced latencies of a-wave and b-wave and presence of the c-wave with stronger flashes, compared to the ERG of Fig. 9 a (from a +/+ chick). Also note the low threshold for eliciting an ERG response (B) Representative ERG recordings from an *rge/rge* bird at hatch. Light intensities as for 9 A. Note the low a-wave amplitude, increased latencies of a-wave and b-wave and absence of a c-wave and oscillatory potentials with any flash intensity. Also note the high threshold for eliciting an ERG response.

Electroretinography performed within 12 h of hatching revealed that the *rge/rge* chicks have an increased a-wave threshold, and a reduced and abnormally shaped a-wave, suggesting abnormal photoreceptor function. The raised dark-adapted threshold of response correlates with the results of vision testing that showed that *rge/rge* chicks tend to have poorer vision under lower lighting levels. The marked reduction in the slope of the leading edge of the a-wave suggests reduced photoreceptor sensitivity. The b-wave threshold is also delayed. Interestingly, the b-wave in response to the brighter flashes is greater in amplitude and has a longer latency than that of the control chicks (*rge/+* and +/+). This difference in b-wave response in *rge/rge* chicks when compared to controls may be partly due to the reduced and slower a-wave response in the *rge/rge* chicks. The ERG of *rge/rge* chicks has a noticeable lack of oscillatory potentials and c-wave, a feature noted in the light-adapted ERG of normal chicks (Ookawa, 1971). When the ERG changes are considered in conjunction with the reduced scotopic vision it may indicate that there is a more severe abnormality in rod function than cone function. A more detailed ERG study to investigate rod and cone function and the deterioration in the ERG of the *rge/rge* chicks with age will be reported elsewhere.

The *rge/rge* chicks, in addition to vision loss and ERG abnormalities, develop marked changes in globe morphology. The dimensions of the globe of *rge/rge* chicks are not different from *rge/+* or +/+ chicks at 1 day of age but they subsequently develop a marked increase in globe size not associated with a change in IOP. A decrease in anterior chamber depth was the earliest morphological change detected. The increased globe size is primarily due to an increase in the vitreous chamber volume, initially, causing an in increase in radial globe diameter and then in axial globe length. A flattening of the cornea (increased corneal radius), and an increase in corneal thickness also developed in the *rge/rge* chicks.

The *rge/rge* chicks had a significantly greater degree of hyperopia than *rge/+* by 33 days of age. The refractive error of *rge/rge* and *rge/+* chicks was similar at 13 days of age (the first time point at which they were refracted), with both groups exhibiting hyperopia. Normal chicks have previously been shown to be hyperopic at hatch and with differential ocular growth they then tend towards emmetropia (Li & Howland, 1999). In this study the refraction of *rge/+* chicks examined changed towards emmetropia as expected while the globe of *rge/rge* chicks failed to undergo normal emmetropization. Although the mean degree of hyperopia of *rge/rge*

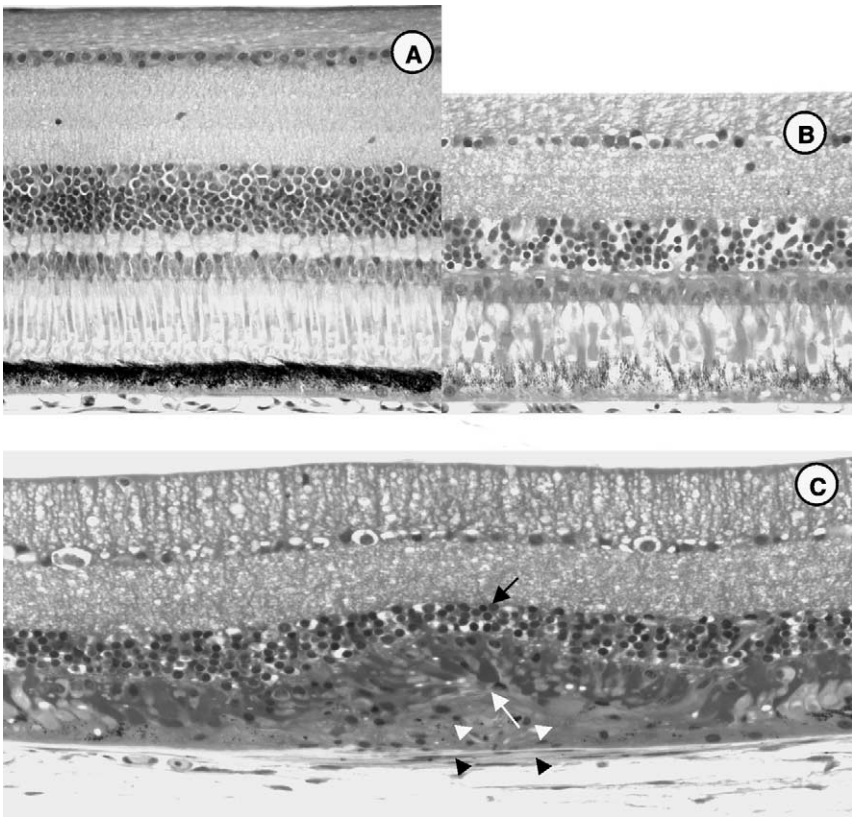

Fig. 10. Photomicrograph (400×) of the central retina of (A) an *rge*/+ bird and (B) and *rge*/*rge* bird, both are from 450 days old females. Euthanasia was performed during daytime, after two hours of dark adaptation, in both birds. Note the generalized thinning of all outer retinal layers and RPE atrophy with dispersed granules in the *rge*/*rge* bird. Additionally, ganglion and inner nuclear layer cell bodies appear edematous and less numerous in the *rge*/*rge* retinas with a loss of the architecture mainly affecting the outer nuclear layer and photoreceptor outer segments. (C) Photomicrograph (400×) of the linear gray retinal lesion in the central retina of an *rge*/*rge* bird at 45 days of age. Note the mild focal fibrosis at the level of the inner choroid (black arrowheads) and the absence of RPE (white arrowheads). There is an accumulation of eosinophilic material in the subretinal space (white arrow). The adjacent photoreceptor inner and outer segments are disorganization and there is thinning and displacement of the outer and inner nuclear layers (black arrow).

chicks decreases with age, the range of refractive errors within the *rge*/*rge* group appears to increase. The increase in vitreous chamber size and decrease in anterior chamber depth tend to reduce the degree of hyperopia. It is conceivable that these latter changes develop as a compensatory mechanism for the hyperopia. The early increase in the radial diameter of the globe may be responsible for the flattening of the cornea. The resulting defocus (hyperopia) will then induce a compensatory increase in axial length.

The mechanisms underlying environmentally induced alterations in globe growth in chicks have been investigated. Eye growth is controlled by levels of dopamine and melatonin, which have antagonistic effects (Schaeffel, Bartmann, Hagel, & Zrenner, 1995; Scher, Wankiewicz, Brown, & Fujieda, 2002). Melatonin is produced in the retina and is involved in dark-adaptation of the retina (Arushanian & Ovanesov, 1999; Scher et al., 2002) as well having a suppressive effect on dopamine (Schaeffel et al., 1995; Scher et al., 2002). Retinal melatonin levels are higher at night in chicks and other vertebrates (Hamm & Menaker, 1980; Skene, Vivien-Roels, & Pevet, 1991) and are decreased following light adaptation (Scher et al., 2002). They were also shown to be decreased in the constant light exposure chick (Li & Howland, 1999; Li et al., 2000; Zawilska & Wawrocka, 1993). The reduced antagonistic effects of melatonin on dopamine-induced scleral growth is believed to account for the increase in globe size in the constant light exposure chick. Further evidence to support this theory was provided by the finding that intramuscular injections of melatonin partially prevented ocular changes in chicks exposed to constant light (Li & Howland, 1999). Some of the abnormalities in the electroretinogram of *rge*/*rge* chicks are similar to those resulting from light-adaptation and the morphological changes in the globe are similar to those seen in constant light exposure. This may give a clue as to the underlying photoreceptor dysfunction. Measurement of retinal melatonin and dopamine levels in *rge*/*rge* chicks is planned to further investigate their role in the abnormal globe growth that is a feature of the disease.

Preliminary histological studies do not show any evidence of retinal dysplasia (as seen in the *rdd* chick)

(Randall et al., 1983) and morphological changes at the light microscope level develop slowly and rather late in the disease process (unlike the *rd* chick that suffers from a rapid loss of photoreceptors (Semple-Rowland et al., 1998; Ulshafer & Allen, 1985; Ulshafer et al., 1984). It would appear that vision loss in *rge/rge* chicks is not due primarily to photoreceptor cell death, but rather an abnormality in photoreceptor function. As vision deteriorates a thinning of all retinal layers is obvious and seemed to parallel globe enlargement. It is conceivable that the globe enlargement results in the same number of retinal cells spread over a greater retinal area and thus is responsible for at least some of the retinal thinning observed. Unfortunately there is a paucity of published investigations of alterations in retinal morphology in chicks with environmentally induced globe enlargement, so it is not clear whether the thinning of retinal layers that develops in the *rge/rge* chick can be simply explained by the alterations in globe size. Radiating white lesions were seen on the fundus of some *rge/rge* chicks. These lesions grossly appear to be similar to those previously described in induced myopia in chicks, which were likened to 'lacquer cracks' seen in highly myopic people (Hirata & Negi, 1998).

A more detailed description of the ultra-structural changes in the retina of *rge/rge* chicks will be reported in a future publication.

The *rge* phenotype is one of a progressive early onset vision loss associated with abnormal ERG responses, but not accounted for by photoreceptor loss. Gross alterations in globe morphology follow and are likely to be secondary to photoreceptor dysfunction. The recent mapping of the *rge* locus to chicken chromosome one (Morrice et al. personal communication, 2002) suggests a number of candidate genes to investigate and eventual identification of the causal gene mutation will make the *rge* chicken a valuable model for the understanding of retinal function and the mechanisms that control ocular growth, and may possibly show that it is a model for human retinal disease.

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