Intraocular Pressure-Dependent and -Independent Phases of Growth of the Embryonic Chick Eye and Cornea

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The pattern and relative rates of diametric growth of the avian eye and cornea are described throughout embryonic development. The effect of reduced intraocular pressure on eye and corneal diametric growth also was investigated. Between embryonic day 4 (E4) and 1 day posthatching, the eye undergoes two distinct phases of linear growth. The first phase (E4-10) is very rapid (1.193 mm/day). The second phase, after E10, is significantly slower (0.346 mm/day). By contrast, over the same developmental period, the cornea undergoes three distinct and sequential phases of linear growth. The second phase of corneal growth (E7-10) is the most rapid (0.429 mm/day) and separates two periods of slow growth (0.211 mm/day during E4-7 and 0.128 mm/day after E10). After the sustained release of intraocular pressure by intubation on E4, growth of both the eye and cornea is reduced significantly. Operated eyes grow at a rate of 0.356 mm/day (E4-10) and 0.155 mm/day (E10-16). Intubation reduces corneal growth to a single phase of 0.125 mm/day (E7-16). Thus, from E4-10 both the eye and cornea possess intrinsic growth potentials that are elevated significantly by intraocular pressure. After E10, the rate of growth of both the eye and the cornea is independent of intraocular pressure. Because both control and intubated eyes change their growth rate on E10, this transition also is independent of intraocular pressure. This contrasts with the cornea which, after intubation, shows no detectable variation in growth rate. Correlation of eye with corneal growth demonstrates an exponential relationship in the presence of intraocular pressure and an almost linear relationship after intubation. Invest Ophthalmol Vis Sci 32:2483-2491, 1991

Eye development commences with the outgrowth of a vesicle from the lateral walls of the diencephalon that, on encountering the lateral head ectoderm, undergoes complex invagination to form the optic cup. The establishment of the optic cup subdivides the neuroectoderm into an inner prospective neural retina and outer prospective retinal pigmented epithelium and is associated temporally and causally with lens induction from the overlying ectoderm. The lens resides within the tips of the optic cup and stimulates the overlying ectoderm to synthesize and assemble the primary corneal stroma which provides the framework onto which neural crest-derived cells migrate to establish the corneal endothelium. After its formation, the corneal endothelium secretes hyaluronate into the primary stroma which then is invaded by a second population of neural crest-derived cells that differentiate as corneal fibroblasts and synthesize and assemble the secondary corneal stroma.

During avian embryogenesis, the major components and overall structure of the eye, including the cornea, are established during the first 7 days of in ovo development. The newly formed eye primordium grows rapidly to become the most prominent cranial structure during the first half of avian development. The enormous increase in size of the developing eye primarily reflects an increase in size of the vitreous chamber to accommodate elevated synthesis, and thus an increasing volume, of vitreous humor. Concomitant with the enlargement of the vitreous chamber, the lens and cornea also increase in size, resulting in overall coordinated eye growth. The initial phase of rapid ocular growth probably serves three important roles fundamental to ultimate eye function. First, it permits the maturation of the neural retina and early establishment of the central pathways of the visual system. Second, it establishes a framework on which the appropriate intrinsic and extrinsic musculature together with their peripheral innervation can be established. Third, the eye achieves an appropriate size and position, relative to other cranial
structures, before a potential restriction of growth imposed by intramembranous ossification of the skull, particularly the orbit.16

The development and biochemistry of the avian eye were described in detail, and because of their obvious significance to the mature visual system, the cornea, lens, and neural retina received considerable attention. Although it constitutes a major component of their development, growth of neither the eye nor the cornea were analyzed comprehensively. It has long been recognized that growth of the embryonic chick eye is related to sustained intraocular pressure.17 The release of intraocular pressure during the early stages of eye morphogenesis causes the scleral cartilage to be substantially thicker17 and the neural retina, but not the pigmented epithelium, to be highly folded.18 Although cell differentiation apparently is unaffected, the atypical morphology of these tissues indicates that tension forces are instrumental in their normal development.

The most dramatic effect of intubation on embryonic day 4 (E4) is a severe reduction of eye growth, a largely reversible effect that is not an artifact of surgical manipulation.18 A similar reduction of corneal growth after intubation shows that this is associated with overall eye growth and thus indirectly related to intraocular pressure.19 We extended these previous studies, restricted to the eye and cornea during the early stages of development, and we describe a comprehensive analysis of the avian eye and corneal growth both in the presence (E4 to 1 day posthatching) and absence (E4–16) of intraocular pressure.

Materials and Methods

All procedures on embryos used in this study conformed to the ARVO Resolution on the Use of Animals in Research. The embryos were derived from White Leghorn (Gallus domesticus) eggs obtained locally. The eggs were incubated in a forced-draft incubator at a temperature of 38 ± 0.5°C. The embryos were aged by comparison with the normal developmental tables of Hamburger and Hamilton.20

Measurement of Eye and Corneal Growth

The embryos were dissected free of extraembryonic membranes in cold calcium- and magnesium-free Tyrode’s solution, pH 7.4. After their transfer to fresh cold calcium- and magnesium-free Tyrode’s solution, the intact eyeball was removed and oriented to be positioned with the anterior segment facing upward. Eye growth was determined from the average equatorial diameter of a minimum of three eyeballs at each of the developmental ages examined. The retinal pigmented epithelium provided a distinct boundary to the eyeball and enabled consistent measurements to be made. Using the choroid fissure as the ventral reference point, both the dorsoventral and nasotemporal diameters of the eyes were measured on a calibrated Wild dissecting microscope (Wild Heerbrugg, Herrbrugg, Switzerland). These two axes of measurement, which were never significantly different from one another, provided an average eye diameter directly proportional to overall eye size.18

Corneal diameter was used as an index of overall corneal growth. For each of the developmental ages examined, average corneal size was determined from at least three eyes. Each of the corneas was derived from previously measured eyeballs, enabling direct correlation between overall eye and corneal growth to be made. After the measurement of eyeball diameter, the cornea was dissected free of adherent tissues and flat mounted. Using the distinct limbal region as the corneal boundary, the average diameter of a cornea was determined from two measurements made at right angles to one another. Because in situ orientation of corneas cannot be determined after their isolation, these axes of measurement necessarily were random. Initial measurements showed that isolated corneas are circular structures at all stages of their development.

Reduction of Intraocular Pressure

A circular window, approximately 1.5-cm diameter, was cut into the shell of all eggs on incubation day 4, corresponding to E3. Approximately 50 μl of sterile Hanks’ buffered saline, pH 7.4, containing penicillin and streptomycin (Flow, Irvine, Scotland) was injected into each egg and the window sealed with adhesive tape. On the following day (E4), the adhesive tape was removed, and the embryos underwent in ovo microsurgery under fiberoptic illumination. Access to the right eye, naturally facing upward during this period of development, was generated by tearing the overlying extraembryonic membranes with watchmaker’s forceps. Using fine iridectomy scissors, a small incision was made into the dorsal aspect of the eyeball at a point midway between the edge of the cornea and the equator of the eye. A glass tube was inserted through this incision to establish continuous release of intraocular pressure by drainage from the vitreous chamber. The glass tubes used in this study were hand pulled from capillary tubing and measured under a calibrated microscope. The pieces of glass tubing inserted into the eyes were selected to measure approximately 3.0-mm long, 0.268 ± 0.025-mm internal diameter, and 0.412 ± 0.037-mm external diameter. Approximately 50 μl of sterile Hanks’ buffered saline, pH 7.4, containing antibiotics was injected into the egg and the window resealed with...
adhesive tape. After in ovo surgery, the eggs were returned to the incubator to permit continued development. After appropriate periods of postoperative incubation, the embryos were killed, and the diameters of intubated eyes and corneas were determined as described.

Results

Commencing on E4 and extending until 1 day posthatching, the diametric size of right and left eyes and right and left corneas was determined at 2-day intervals. The data presented in Figure 1 demonstrate that over this developmental period both the eye and the cornea grew rapidly and extensively. At each of the embryonic ages examined, there was no significant difference in size between right and left eyes (Fig. 1A). A parallel analysis also showed that there was no significant difference in size between right and left corneas (Fig. 1B).

The Effect of Intubation on Eye Growth

Glass tubing was inserted into the dorsal aspect of the right eye on E4 to prevent the accumulation of intraocular pressure. Figure 2 shows an E8 embryo, killed 4 days after in ovo surgery. In addition to demonstrating the tube inserted into the right eye, the operated eye was significantly smaller than its fellow control (left) eye. This difference was accentuated further on E12, by which time the control (left) eye was substantially larger than the operated right eye (Fig. 3). As previously described, insertion of a glass tube

Fig. 1. Commencing on E4, average equatorial diameter of right and left eyes (a) and average limbal diameter of right and left corneas (b) were determined at 2-day intervals until 1 day post-hatching. Columns are plotted to the average of the data and the solid caps represent the positive standard deviation. The data demonstrate that over this period both the eye and cornea increase their size dramatically. At each embryonic age there is no statistical difference in size between right and left eye or right and left cornea. L = left (diagonal shading); R = right (horizontal shading). Numbers on the horizontal axis refer to embryonic age in days.

Fig. 2. The effect of intubation on E4 on the morphology of the E8 chick embryo head. A glass tube (arrowheads) inserted into the dorsal quadrant of the right eye causes it to be significantly smaller than the unoperated left eye. Despite the reduction in eye growth, intubation does not appear to disrupt or delay the development of surrounding craniofacial structures. Scale bar = 2.0 mm.
into the eye on E4 causes rupture of the neural retina in addition to a reduction of its growth. In contrast to the control eye, the E12 operated eye was covered partially by thickened eyelids, and the upper beak was crossed characteristically toward the operated side (Fig. 3).

During embryonic development, control (left) eyes underwent two distinct phases of growth (Fig. 4A). The first phase of eye growth extended from E4-10 and was both linear and very rapid. During this 6-day period, the eye grew in diameter from an average of 1.070 mm on E4 to an average of 7.983 mm on E10, an increase of approximately 750%. The second phase of eye growth was shown after E10 and, although only data up to and including E16 is presented in the figure, extended until at least 1 day posthatching. Although the second phase of eye growth also was linear, it was much slower, and over the 6-day period (E10-16), the average eye diameter increased to 9.910 mm, an increase in size of approximately 125%.

Eye growth was reduced significantly and permanently after intubation on E4 (Fig. 4A). By E10, the average diameter of intubated eyes (3.434 mm) had increased by only 320%. Between E10 and E16, intubated eyes continued to grow slowly and increased in diameter by only 180%. The data presented in the figure suggest that, despite a severe reduction in size, intubated eyes showed two phases of growth with a reduction in rate occurring on E10. Both the initial rapid and subsequent slow phases of intubated eye growth tended toward linearity although there was fluctuation in growth rate between 24 and 48 hr after the release of intraocular pressure.

An overall description of the pattern of diametric growth of the embryonic chick eye was extended to a detailed analysis of the relative rates of the two phases of control and intubated eye growth (Figs. 4B-C). From the data presented in Figure 4A, both control and intubated eyes showed a sharp change in diametric growth rate on E10. The relative rate of the first phase of eye growth (E4-10) was determined by normalizing the mean size of both E4 control and intubated eyes to unity. Values obtained from E5, E6, E7, E8, E9, and E10 eyes were then corrected to the normalized E4 eye diameter. Similarly, the relative rate of the second phase of eye growth, commencing on and extending beyond E10, was determined by normalizing the mean diametric size of both E10 control and intubated eyes to unity. Values obtained from E12, E14, and E16 control and intubated eyes, in addition to E18, E20, and 1 day posthatching control eyes, then were corrected to the normalized E10 eye diameter. The resultant data demonstrated that control eyes increased in diameter at a rate of 1.193 mm/
day from E4-10 (Fig. 4Bi) and 0.346 mm/day from E10 to 1 day posthatching (Fig. 4Bii). After intubation (E4-10), the eye diameter increased at a rate of 0.356 mm/day (Fig. 4Ci), and this decreased to 0.155 mm/day from E10-16 (Fig. 4Cii).

Control Versus Intubated Corneal Growth

Analysis of control corneas showed a sigmoidal increase in diameter from E4-16, with changes in diametric growth rate occurring on E7 and E10 (Fig. 5Ai). From E4-7, average corneal diameter increased by approximately 230% from 0.455-1.059 mm. By E10, the average corneal diameter was 2.320 mm, representing an increase in size of almost 220% since E7. The average diameter of the E16 cornea was 3.147 mm, representing an increase in size of 135% since E10 and 690% since E4. Thus, the measurement of corneal diameter demonstrated three phases of diametric growth of the developing cornea. The first phase (E4-7) and the second phase (E7-10) were both relatively rapid. The third phase, demonstrated after E10 and continuing uniformly until 1 day posthatching, was considerably slower.

After intubation on E4, corneal growth was reduced significantly and permanently (Fig. 5Aii). On E6, although the average diameter of corneas from intubated eyes increased, it was significantly less than control. From E6-7, the average diameter of corneas from intubated eyes decreased and remained relatively unchanged on E8 (Fig. 5Aii). After E8 and until E16, the maximum age we examined, the average diameter of corneas from intubated eyes increased gradually and linearly. Over the period from E4-16, the average diameter of corneas from intubated eyes increased by approximately 380%. However, corneas from E16 intubated eyes were approximately 55% smaller than controls.

The relative rates of control corneal growth was determined over the developmental periods E4–7, E7–10, and E10 to 1 day posthatching. For the first period of growth, the average diameter of E4 corneas was normalized to unity and corneal size on E5, E6, and E7 corrected to this starting value. For the second phase of growth, the average diameter of the E7 cornea was normalized to unity and corneal size on E8, E9, and E10 corrected to this starting value. Similarly, for the third phase of growth, the average diameter of the E10 cornea was normalized to unity and corneal size on E12, E14, E16, E18, E20, and 1 day posthatching corrected to this value. For corneas from intubated eyes, the relative rate of growth was determined over the complete developmental period from E4–16 by normalizing the E4 value to unity and correcting corneal diameter on E5, E6, E7, E8, E9, E10, E12, E14, and E16 to this initial value. These data show that control corneal diameter increased at a rate of 0.211 mm/day from E4-7 (Fig. 5Bi), 0.429 mm/day from E7-10 (Fig. 5Bii), and 0.128 mm/day from E10 to 1 day posthatching (Fig. 5Biii). From E4-16, cor-
Corneas from intubated eyes increased in diameter at a constant rate of 0.125 mm/day (Fig. 5c).

**The Relationship Between Eye and Corneal Growth in Control and Intubated Eyes**

The average diameters of eyes and corneas from either control or intubated eyes were arranged in order of increasing size regardless of embryonic age. Eye diameter then was plotted against corneal diameter for either control or intubated eyes. For the developmental period from E4 to 1 day posthatching, the increase in average diameter of control eyes and their corneas showed an extremely close and exponential association (Fig. 6A). Thus, although both structures grew rapidly and necessarily in conjunction with one another, the eye increased in size at a greater rate than the cornea. Over the developmental period from E4-16, correlation of the average diameter of intubated eyes and their corneas was more linear (Fig. 6B). By contrast with the disproportionate growth of the control eye and cornea, these data therefore demonstrated that, in the absence of intraocular pressure, corneal growth was more directly proportional to overall eye growth.

**Discussion**

Together with the developing brain, the eye serves as a template on which the major processes associated with facial morphogenesis are established.21,22 In addition to the obvious importance of a precisely developed visual system, eye development and growth thus constitutes a significant and substantial component of craniofacial development. The sustained release of intraocular pressure by intubation severely reduces growth of the embryonic chick eye17 and cornea19 without disrupting their histogenesis.18 More recently, a major interest in growth of the hatching chick eye arose from the demonstration of its ability to grow into focus and thereby compensate for experimentally induced changes in focal length.23 However, growth of the embryonic chick eye and cornea were not analyzed in detail. Employing diameter as an index of overall size,18,19 we described a comprehensive analysis of growth of the embryonic chick eye and cornea in both the presence and absence of intraocular pressure.

During the initial stages of in ovo development, the avian embryo transiently is oriented with its right eye naturally facing upward. Although unlikely, we considered the possibility that during these early stages the right eye and cornea could be growing at different rates than the left eye and cornea. Our data demonstrated that there was no significant difference in size between either right and left eyes or right and left corneas at any of the developmental stages examined. Thus, any difference in eye or corneal size resulting from subsequent surgical manipulation cannot be an artifact of growth differences intrinsic to the operated eye.
Between E4 and 1 day posthatching, control eye size increased dramatically in two sequential phases of linear growth. The first phase, before E10, was very rapid; the second phase, after E10, was considerably slower. The elimination of intraocular pressure by intubation on E4 substantially reduced both phases of eye growth. Nevertheless, intubated eyes continued to grow, and like control eyes, this growth occurred in two sequential phases which both tended toward linearity. The reduction of eye growth after intubation was most pronounced from E4-10, and consequently this phase primarily was dependent on sustained intraocular pressure. Intubation also revealed that, during this period, the eye was capable of a slow rate of growth in the absence of intraocular pressure. It remains to be determined whether the rate of eye growth normally achieved from E4-10 was the combination of intraocular pressure independent and dependent elements.

Before E10, the eye expands without restriction by surrounding tissues. Because it is the most prominent cranial structure, this phase of very rapid growth must facilitate the precise positioning of topographically associated tissues around the eye during the early stages of development. This period of growth coincides with the enhanced accumulation of vitreous fluid which, in turn, must be accommodated by additional growth of the neural retina and retinal pigmented epithelium together with the pericocular mesenchyme. The growth of each of these tissues was initiated by cell proliferation, but subsequently, the surface area of both the neural retina and retinal pigmented epithelium increased primarily by lateral spreading and flattening of these two cell sheets. However, because intubation causes the neural retina to be highly folded, the lateral expansion of this tissue was independent of eye size and did not promote eye growth.

The rate of growth of both control and intubated eyes decreased sharply on E10, and therefore, this transition must be independent of intraocular pressure. This transition in growth rate coincided temporally with the establishment of the scleral cartilage from the pericocular mesenchyme. Because the scleral cartilage encapsulates the eye, it may restrict growth indirectly by either suppressing the synthesis of vitreous humor or stimulating its rate of turnover. Preliminary studies indicated that intraocular pressure per se does not change during avian development, and consequently the term “pressure” may be misleading. Thus, alterations in vitreous humor volume must be accommodated directly by corresponding changes in overall eye size and vice versa. The relationship between scleral cartilage and eye growth was highlighted by the demonstration that experimentally induced alterations in eye dimension elicited corresponding changes in both the proliferative and biosynthetic activity of scleral chondrocytes.

After E10, eye growth was restricted potentially by both the scleral cartilage and the establishment of the bones of the orbit. Although the eye continues to grow, this is in conjunction and coordinated with a general increase in head size. We therefore concluded that eye growth after E10 was independent of intraocular pressure, and this was reinforced by the demonstration that, after intubation, the rate of eye growth from E4-10 was identical to the rate of growth of control eyes from E10 to 1 day posthatching. Intubated eyes reduced their rate of growth on E10 because of an overall perturbation of facial development around the operated eye (Fig. 3). This disruption must result in a much smaller orbit in addition to substantially thicker scleral cartilage. Thus, commencing on E10, eye growth was constrained extrinsically, and the
growth rate of intubated eyes was reduced additionally because they already were significantly smaller.

Growth of the eye closely correlated with a corresponding increase in diametric size of the cornea. Analysis of corneal size with respect to age revealed three phases of diametric growth from E4 to 1 day posthatching. Initial corneal growth (E4–7) was relatively rapid and increased further from E7–10. The subtle transition in growth rate exhibited around E7 was not an artifact; we made many measurements of E6, E7, and E8 corneas, and these reinforced our initial and unexpected observation. The supplementary elevation of growth rate from E7–10 reflected an increase in corneal diameter in addition to the establishment of corneal curvature that began on E8 and also was related to intraocular pressure. Thus, during this phase of its growth, the surface area of the cornea increased more rapidly than its circumference. We did not analyze corneal curvature in our study, but our measurements of flat-mounted corneas combine both parameters. After E10 corneal growth was reduced, and this effect persisted at a constant rate until at least 1 day posthatching. Intubation on E4 reduced subsequent corneal growth to a single slow phase. Thus, the transitions in corneal growth rate demonstrated on E7 and E10 were both a function of intraocular pressure. The rate of corneal growth in intubated eyes was identical to the slow phase of corneal growth normally demonstrated by control corneas after E10. We therefore concluded that corneal growth was dependent on intraocular pressure before E10 and independent of this after E10.

Throughout their development, control eye and corneal growth were correlated exponentially. The control eye grew extensively, and this, in turn, promoted the growth of the contiguous cornea. Because eye growth from E4–10 was dependent on intraocular pressure, intubation should result in a concomitant reduction in corneal growth, as we demonstrated and as has been shown elsewhere. Although our data showed that corneal growth from E4–10 was dependent on intraocular pressure, this dependency was indirect. Intraocular pressure promoted the growth of the eye which then facilitated corresponding corneal growth. This was shown by the linear correlation between eye and corneal size after intubation.

After surgery the rate of diametric growth of the cornea from E4–16 was identical to that of control corneas from E10 to 1 day posthatching. Thus, this rate of corneal growth was indirectly independent of intraocular pressure and related to overall growth of the eye which, under both conditions, was directly independent of intraocular pressure. From E10–16 the cornea acquires many of the physical properties characteristic of its normal function, most notably its transition from opacity to transparency, in addition to being extensively innervated. In a subsequent paper (Bee and Roche, manuscript in preparation), we demonstrate that intubation resulted in precocious development and innervation of the embryonic chick cornea. Thus, this novel system provided the unique opportunity to investigate the mechanisms by which the development and innervation of the cornea is controlled, and transparency is achieved.

Key words: eye, cornea, development, growth, pressure

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