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Normal development of refractive state and ocular component dimensions in the marmoset (*Callithrix jacchus*)

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Abstract

Refractive state and ocular dimensions were studied longitudinally in nine normal marmosets. Animals were anaesthetised and examined (with some exceptions) at 4, 6, 7, 8, 10, 15, 24 and 39 weeks of age. Cycloplegic retinoscopy showed that hyperopia early in life rapidly diminished. Refraction corrected for the artefact of retinoscopy stabilised by 8 weeks of age, but at a slightly myopic value, rather than at emmetropia. The ocular components continued to change throughout the period studied. Corneal radius, measured by photokeratometry, increased slightly during development. Anterior segment depth and vitreous chamber depth (VCD), measured by A-scan ultrasonography, increased throughout development while lens thickness initially increased and then decreased. Data from the eyes of these normal animals were compared with that from the contralateral eyes of animals which received short periods of monocular deprivation early in life (Troilo, D., & Judge S.J. (1993). Ocular development and visual deprivation myopia in the common marmoset (*Callithrix jacchus jacchus*). *Vision Research*, 33, 1311–24); eyes which viewed through no lens or a plano lens (Graham, B. & Judge, S.J. (1999)). The effects of spectacle wear in infancy on eye growth and refractive error in the marmoset (*Callithrix jacchus*). *Vision Research*, 39, 189–206), and eyes of normal animals in another colony. There were no significant differences between the first two groups and the normal animals in our colony while age-matched animals from the other colony were slightly but significantly less myopic than our animals.

Keywords: Eye growth; Primate; Refractive development

1. Introduction

1.1. The purpose of the study

This study started out as a control for the experiments, described in Graham and Judge (1999), in which we raised marmosets wearing spectacle lenses between the ages of 4 and 8 weeks. We wished to have control data, obtained in a longitudinal study with measurements made at the same ages as in the animals which wore lenses. We also had a number of auxiliary interests, the first of which was to test the assumption made earlier (Troilo & Judge, 1993) that the contralateral eye of monocularly lid-sutured animals grew normally. Secondly, we became interested in a number of more general issues such as what factors affect the inter-individual variation in ocular size early in life, and other questions about the pattern of growth in the normal primate eye.

1.2. The time-course of refractive development and post-natal eye growth in humans and other primates

As in other species, the primate eye is generally not emmetropic at birth, but hyperopic, with the degree of hyperopia varying between individuals. These neonatal refractive errors are usually reduced as the animal develops. This trend towards emmetropia has been described in humans (Mohindra & Held, 1981; Ehrlich, Atkinson, Braddick, Bobier & Durden, 1995; Saunders, Woodhouse & Westall, 1995), macaques (Kiely, Crewther, Nathan, Brennan, Efron & Madigan, 1987; Raviola & Wiesel, 1990), and in marmosets (Troilo & Judge, 1993).

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Human postnatal eye growth is considered to have two phases: a rapid infantile one, lasting 2 or 3 years, during which axial length increases by 5 mm (approximately 25%), and a slower juvenile one, lasting 10 or more years, in which axial length increases by a further 1 mm (5%) (Sorsby, Benjamin & Sheridan, 1961; Sorsby & Leary, 1970; Larsen, 1971a,b,c,d; Curtin, 1985). In humans the main interest has been in slow juvenile growth, rather than infantile growth, and although there are cross-sectional measurements on neonates and children from the age of 3 years onwards, there seems to be very little data, especially longitudinal data, on the infantile period (Sorsby & Leary, 1970; Wood, Mutti & Zadnik, 1996). Indeed there is a general shortage of longitudinal data, and the first truly comprehensive longitudinal survey which measures all the optical components in school-aged children is still underway, with only initial cross-sectional results published (Zadnik, Mutti, Friedman & Adams, 1993).

The most nearly comparable human study to ours would appear to be that of Larsen (1971a,b,c,d) but this was a cross-sectional study on children hospitalised for non-ophthalmological reasons, rather than a longitudinal study of normal children.

1.3. Macaques and chimpanzees

The development of refraction and/or its ocular components has been studied in chimpanzees (Young, Leary & Farrer, 1971); *Macaca mulatta*: (De Rousseau & Bito, 1981; Tigges, Tigges, Fernandes, Eggers & Gammon, 1990; Lambert, Fernandes, Drews-Botsch & Tigges, 1996); and *Macaca fascicularis* (Kiely, Crewther, Nathan, Brennan, Efron & Madigan, 1987). Much of these data are cross-sectional, and we are not aware of a systematic longitudinal study before ours.

1.4. Subjects and housing

The common marmoset (*Callithrix jacchus*) is a small, highly social, arboreal primate which inhabits the primary and secondary Amazonian and Atlantic coastal forests in the north-east of Brazil (Stevenson & Rylands, 1988). Marmosets are territorial animals, living in extended groups of three to eight animals or more. Females usually give birth to precocial, dizygotic twins, with the litter weight, excluding the placenta, being 15–25% of that of the mother's normal body weight, (Kleiman, 1977). Youngsters are weaned at around 60 days of age and reach adult body weight at around 8–12 months (Hearn, 1987). Sexual maturity is reached at 18–24 months. Marmosets are diurnal, with their daily activity pattern being such that the animals spend time at all levels of the rain forest. Whilst they tend to feed in lower levels of the forest they spend some time each day sunbathing on the top of the canopy. Social interaction within groups is common, taking the form of grooming and play. Vocal communication is used both within and between groups of animals.

In captivity, marmosets will breed readily and will pair-bond for long periods. Birth of triplets is common and of quadruplets rare, though without human intervention (removing young to give supplementary food) only two of the young will usually survive beyond the first few weeks of life. Marmosets have a gestation period of approximately 144 days. Post-partum mating occurs, with the time between litters being 154–157 days. The behaviour of young marmosets changes much over the first 2 months of life. During the first 2 weeks of life they are constantly being carried by one or other of the older family members. Throughout this time they spend much of their time sleeping or clinging with their faces in the fur of the adult carrying them. They take a more active interest in their surroundings over the following few weeks, though they still spend nearly all of their time being carried. During this period they attend to parts of their home cage and the individuals in it, but do not spend time looking outside the home cage. At this time most objects of interest are within a few centimetres of the youngsters. In our colony the maximum distance to which they attend at this age is around half a metre—a limit set by the size of the cages in use at the time. From the age of 4 weeks they begin to spend time off the parents or older juveniles, exploring the home cage and looking across the room at other family groups. This is the time when they begin to feed themselves, though they still take their mother's milk. Again the minimum distance they attend to is just a few centimetres but the maximum will be a few metres.

1.5. Marmoset vision and accommodation

Marmoset peak foveal cone density (190667 ± 15658 S.D. cones mm^{-2}) is similar to that of humans and macaques, and the marmoset has higher cone densities in the periphery (Troilo, Howland & Judge, 1993; Wilder, Grünert, Lee, & Martin, 1996). Based on this figure and a schematic eye for the marmoset, the Nyquist limit on visual acuity at the fovea was estimated to be 30 cpd—a value compatible with the behavioural data reported by Ordy & Samorajski (1968).

In a similar fashion to other New World monkeys, marmosets exhibit polymorphism of the cone pigments (Travis, Bowmaker & Mollon, 1988). While male monkeys are all dichromatic, females may be either dichromatic or trichromatic. Behavioural experiments have shown that the pattern of inheritance of these features is usually consistent with a model in which a medium-

to long-wave pigment is expressed at a single polymorphic locus on the X-chromosome (Tovee, Bowmaker & Mollon, 1992).

Using infrared photorefraction, Troilo, Howland & Judge (1993) reported amplitudes of accommodation in six marmosets, aged between 0.5 and 11.4 years, of more than 15 D. Younger animals were not measured.

2. Methods

2.1. Our data set

We studied nine normal animals longitudinally until the age of 273 days (39 weeks). A much larger data set was available of measurements at 4 weeks of age, the usual age at which our lens-rearing experiments started. We also made cross-sectional measurements of refraction, but not vitreous chamber depth (VCD), in 20 adult animals in a second colony in which the animals had been raised since birth in room-sized enclosures rather than cages, to see if these animals were as myopic as our own adult animals.

2.2. Oxford animals

Measurements were made as systematically as practicable on nine animals that never wore lenses and on whom the only experimental procedure was the repeated anaesthesia needed to make the optometric measurements. These nine normal animals included four sets of twins and one singleton from five different family groups. Two of the four pairs were males, while the remaining five animals were females. Animals were measured, with a few exceptions at 4, 6, 7, 8, 10, 15, 24 and 39 weeks (28, 42, 49, 56, 70, 105, 168 and 273 days) of age, by which time growth was almost complete. The exceptions were that the second, third and fourth animals were not measured at 4 weeks; the 5th and 6th animals were not measured at 6 weeks; the 5th, 6th, 7th and 8th animals were not measured at 7 weeks, and the 5th and 6th animals were not measured at 8 weeks.

For reasons beyond our control, it was necessary to house the marmosets in three different animal rooms at different times. All rooms were illuminated on a 12:12h day:night cycle. Prior to early September 1994, the illumination incident on the cage fronts was approximately 175 lx, with the illumination within the cages ranging from 10 to 88 lx. After this time the illumination on the cage fronts was approximately 350 lx, with a range of 88 to 200 lx within the cages. Within the remainder of the room the illumination ranged from 88 to 700 lx. The temperature was held constant at 24 ± 1.0°C. The internal dimensions of the cages were 0.80 m high, by 0.61 m wide, by 0.57 m deep. Each cage had a nest box measuring $0.16 \times 0.24 \times 0.18$ m, with a wooden floor. The cage floors and the cage fronts were metal grids while the roof and other three sides of each cage were solid. Horizontal wooden perches were present in every cage and whenever possible, other wooden branches were placed in each cage. Animals not only used the wooden perches and branches for climbing but also for scent marking and adults were often seen gouging wood with their teeth.

Animals were given a varied diet, consisting mainly of egg sandwiches (sliced wholemeal bread with mashed boiled egg and milk) which were given 5 days a week, and Mazuri Primate Diet (expanded pellets, 20 g adult^{-1} day^{-1}, Special Diet Services, UK). A standard multivitamin supplement (Vitracell ZM, Univet, UK) was added to each sandwich. On 1 day each week vitamin D_3 (10^3 iu adult^{-1}) was provided. On 2 days each week animals were given coarsely chopped fresh fruit instead of egg sandwiches. In addition, a forage mix consisting of dried fruit, cereal and seeds was scattered in the cage daily. Occasionally, food was presented in a novel manner, such as fruit suspended from pieces of string. Water was available ad libitum, from bottles attached to the cage front. For the first 8 weeks following birth, cow's milk was also made available.

2.3. Optometric measurements

Cycloplegia was induced 30 min before each measurement session using 1–2 drops of 1% cyclopentolate hydrochloride (Smith and Nephew). For all measurements, marmosets were anaesthetised with a mixture of 0.9% alphaxalone and 0.3% alphadolone acetate (Saffan, Pittman-Moore, UK, 0.09 ml 100 g^{-1} i.m. (weight less than 100 g) or 0.14 ml 100 g^{-1} i.m. (weight over 100 g)). During measurements animals were kept in a prone position with the head stabilised with padded restraints on either side. The eyelids were retracted with a speculum, to obtain good visualisation of the front of the globe. If necessary the cornea was prevented from drying by the instillation of 0.3% Hypromellose eye drops (Schering-Plough).

2.3.1. Refractometry

Refractive state was measured by streak retinoscopy using hand-held trial lenses. Refractive corrections were measured (in diopters) along both the horizontal and vertical meridia of each eye. The spherical equivalent correction was calculated as the mean of two measures of horizontal refraction and two measures of vertical refraction, with the standard clinical convention of negative refractions for myopic errors and positive refractions for hyperopic errors. The cylindrical correction was calculated by subtracting the mean horizontal correction from the mean vertical correction. All refractive error data are corrected for working distance (ap-

proximately 2/3 m) but not for the artefact of retinoscopy (Glickstein & Millodot, 1970) unless stated. The small eyes of marmosets do yield a considerable artifact of retinoscopy: +6 D in neonates (7 mm long eyes), reduced to +2.6 D in adult animals (11 mm long eyes).

We only made measurements in the principal meridia, assuming that, as in humans, the common axes of astigmatism are near the horizontal and vertical meridia.

While cycloplegia is effective at relaxing accommodation and eliminating the need for fixation by the animal, it also produces a dilated pupil. This widens the zone in which the reflex can be seen and increases the uncertainty of the point of reversal. In such a dilated pupil the reflex is not identical across the whole of the pupil. In some cases 'scissoring' occurs, in which case different portions of the streak of light may be seen to move in opposite directions to one another. We always neutralised the reflex in the very centre of the pupil.

2.3.2. Keratometry

The radius of corneal curvature was measured with a photokeratometer similar in design to that of Howland & Sayles (1985). This recorded the first Purkinje images of three fibre optic light sources mounted in an equilateral triangle centred on the camera lens (micro-Nikkor 55 mm f2.8) axis and at right angles to it. With the parameters used, the sample was from an area of the cornea approximately 1 mm^2 and this was assumed to be spherical.

The photokeratometer was aligned with the eye's optic axis with the help of eight alignment LED diodes in the plane of the fibre optic sources. By using the split-prism view-finder of the camera, the distance between the sources and the images of the alignment diodes could be held constant to 1%. Because this distance was held constant, the separation of the images of the fibre optic sources recorded on the camera film was directly proportional to the radius of curvature of the cornea. The constant of proportionality was determined (to within a standard error of $\pm 0.2\%$) by calibrating the photokeratometer with ball-bearings whose radii of curvature had been measured with a micrometer. To ensure that measurements of the images on the film negatives were made as accurately as possible a video image analyser (Seescan, Cambridge) calibrated with a microscope graticule slide was used.

2.3.3. Ultrasonography

Anterior segment depth (distance from the front of the cornea to the front of the lens), lens thickness and VCD were measured along the eye's pupillary axis, using A-scan ultrasonography (Teknar A-scan III with a 7.7 MHz transducer). A standoff was attached to the probe tip in order to position the focus of the probe in the eye and allow easy, hand-held application. Because the thickness of the cornea (~ 0.35 mm) is not much greater the wavelength of the ultrasound fundamental frequency, $\lambda \sim 0.2$ mm[1], the thickness of the cornea could not always be resolved.

As well as positioning the focus of the transducer within the eye, the stand-off was also used to narrow down the diameter of the probe tip so that the probe could be easily applied between the lid retractors in very young animals. The stand-off was filled with lubricant gel (Johnson and Johnson), and the end covered with a thin plastic membrane. Ultrasound transmission gel (Parker) was then used to interface this with the eye. Four measures were taken from each eye and averaged. Velocities of ultrasound in the different parts of the eye were taken from the values for the human eye given by Coleman, Lizzi & Jack (1977). Because resolution of the anterior and posterior surfaces of the cornea was not always possible, a weighted average velocity (1550 ms^{-1}) was calculated for the anterior segment (cornea plus anterior chamber depth) (Troilo & Judge, 1993).

Ideal traces show clear, steeply rising peaks for the retina, the two lens surfaces, and the cornea. The lens peaks should be of similar thickness and size, and should just not be saturated at the top of the display. There is a tendency for the peak representing the front of the cornea to be somewhat smaller than the lens peaks. We judged as satisfactory ultrasound traces in which peaks heights (relative to a saturation level of 100%) met the following criteria: cornea greater than 50%, lens greater than 75% and vitreo-retinal-interface greater than 85%, and such traces were certainly very consistent (standard deviation over 100 repeated measures in the same session = 0.04 mm). A theoretical analysis (Judge, in preparation) suggests that the reason why this is so is that the 15 mm stand-off requires the probe to be very accurately aligned in order for the reflections from all four surfaces (but particularly from the posterior lens surface) to reach the transducer: small errors of alignment result in one or other reflection missing the transducer.

2.4. Recovery from anaesthetic

Saffan is the anaesthetic most strongly recommended for use with marmosets. Full recovery from anaesthesia took 1–2 h after the last dose of anaesthetic. Prior to their return to the home cage, marmosets were closely observed to check for signs of stress or for indications that their condition was not stable enough for their return. All animals, in particular those of less than weaning age, were closely observed on their return to the home cage.

[1] $\lambda = v/f$, with $v \sim 1500$ ms^{-1} and $f = 7.7$ MHz.

2.5. Animals from a second colony

The refraction of 20 animals of varying ages (range 40–601 weeks) was measured on day 1. Animals were maintained in two family groups in separate rooms. Within each room cages were 6 m long by 4 m wide by 3 m high. Cages were made of timber with 4 cm wire mesh making up walls and the ceiling. The stone floor of the cage was covered with a deep layer of sawdust litter. Branches, dowel perches, tables and 4 cm gauge netting was provided to increase use of space within the cages. Humidity, temperature and lighting levels were similar to those in the Oxford colony. In the second colony, animals were fed twice each day, with New World primate diet being mixed with roughly chopped fresh fruit, boiled eggs and potatoes. Water was available ad libitum.

Prior to the measurement of refraction in these animals, cycloplegia was induced with cyclopentolate. Animals were returned to their cages and then recaptured for measurements to be made. Animals were not anesthetised. Streak retinoscopy with a retinoscopy-rack of lenses (Clement Clarke) was used to determine refractive state in both the horizontal and vertical meridia of each eye. During measurements the animals were held by an experienced technician with whom they were well acquainted. The data took 5–10 min per animal to collect.

2.6. Data analysis

Data were stored on a personal computer (Dan 486) and analysed using the SPSS for Windows (version 6.0) statistical software package. Graphs were created on a Sun workstation using the Graph View program (Cherwell Scientific).

3. Results

There were no significant differences between the two eyes in refractive errors or the sizes of any of the ocular components, at any age, in the group of nine normal animals (one factor analysis of variance on the difference between the two eyes: spherical refraction $P = 0.85$, cylindrical refraction $P = 0.17$, corneal power $P = 0.24$, anterior segment depth $P = 0.82$, lens thickness $P = 0.75$, VCD $P = 0.84$). For this reason and for simplicity, graphs show data from just the right eyes of these animals.

3.1. Refraction

The eyes of infant animals grow so as to eliminate the hyperopia characteristic of neonates. Fig. 1 shows spherical and cylindrical refractive error as a function of age. Data are corrected for working distance but not for the artefact of retinoscopy. As the filled symbols representing the group of nine normal animals clearly shows, there is a significant decrease in the spherical refractive error with age. There is a small but significant amount of 'with the rule' astigmatism[2] (mean 0.45 D ± 0.07 S.E.M., t-test of $\mu = 0$, $P < 0.001$). This does

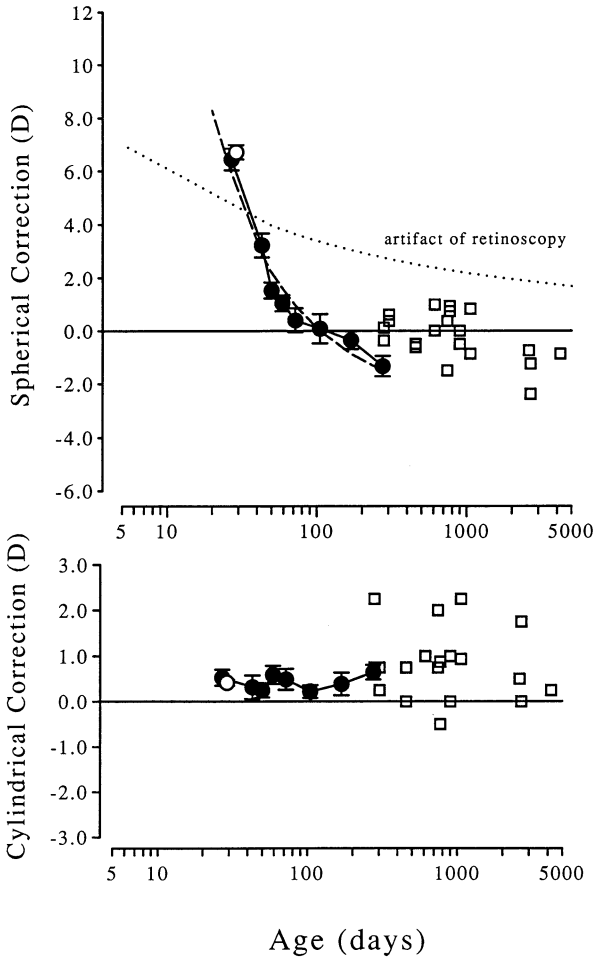

Fig. 1. Normal development of refractive state in the marmoset. Solid circles show mean ± S.E. of refraction of right eyes of nine marmosets. The hollow circle represents mean refraction of one eye of 57 other animals from which data was collected at 4 weeks of age. Hollow squares represent individual refractions of the right eyes of adult animals from a second colony. The upper panel shows the spherical correction, determined by averaging refractions along the horizontal and vertical meridians. The artefact of retinoscopy, represented by the dotted line, was calculated from the regression line of axial length versus log(age) assuming a retinal thickness to the photoreceptor outer segments of 215 μm (Troilo, Howland & Judge, 1993). The dashed line shows the power function least squares fit of the data from the group of nine normal animals. The lower panel shows the cylindrical correction (horizontal-vertical refraction) of the same eyes shown in the upper panel. A number of the adult animals from the second colony are noticeably more astigmatic than other animals from either colony.

[2] Astigmatism in which the meridian with least curvature is horizontal.

not change significantly with age (one-factor ANOVA, $P = 0.684$).

Data from animals in the second colony were averaged as all of these animals were adult. Mean refractive error for these animals was approximately zero before the artefact of retinoscopy was taken into account (R eye, -0.23 D ± 0.20 S.E.M.; L eye, -0.32 D ± 0.16 S.E.M.), therefore the animals are really myopic. Again these animals showed a significant degree of 'with the rule' astigmatism (R eye, mean 0.83 D ± 0.17 S.E.M., t-test of $\mu = 0$, $P < 0.001$). There were no significant differences between refractions of animals of different sex (one-factor ANOVA, $P = 0.549$) or of different parents (one-factor ANOVA, $P = 0.181$). There was a significant difference between the spherical refractions of the nine Oxford animals at their final measurement age and four animals of comparable age in the second colony (one-factor ANOVA, $P < 0.05$), with the Oxford animals being significantly more myopic than animals in the second colony. The actual difference in refraction between the groups is approximately one diopter. However, there was no significant difference between the cylinder corrections of the same animals (one-factor ANOVA, $P = 0.25$).

3.2. Ocular components

Fig. 2 shows the mean corneal power, anterior chamber depth, and lens thickness of the nine normal (Oxford) animals as a function of age, and Fig. 3 shows mean VCD and axial length as a function of age. There are significant changes in all of the ocular components with time (one-factor ANOVA, corneal power $P < 0.05$, all other components $P < 0.0001$).

The main trend in corneal power is a decrease with age, i.e. the cornea flattens (Fig. 2(a) and Table 1). Lens thickness initially increases but then decreases after 10 weeks of age (Fig. 2(b) and Table 1). Anterior segment depth, VCD and axial length all increase with age (Fig. 2(b), Fig. 3(a) (b), Table 1). The major contribution to the increase in axial length (AL) is from the increase in VCD. There is a significant increase in the VCD/AL ratio with increasing age (from 0.58 to 0.64: one-factor ANOVA, $P < 0.0001$). There is also a significant increase in the proportion of the axial length represented by the anterior chamber depth (0.14–0.16: one-factor ANOVA, $P < 0.01$) and a significant decrease in the proportion of axial length represented by the lens thickness (0.27–0.19: one-factor ANOVA, $P < 0.0001$) (Table 1, Fig. 4).

The unfilled symbols in Figs. 2 and 3 show the mean values for a much larger group of animals that were also normal at 4 weeks of age—the animals described in Graham and Judge (1999) to which lenses were fitted at that age. These values are not significantly different from the mean values at the same age for the group of normal animals we studied longitudinally (represented by the filled circles). Even the visually conspicuous difference in corneal power shown in Fig. 2a is not significant at the $P = 0.05$ level because of the large S.E. and small n (we were not able to measure corneal curvature in all nine animals at that age).

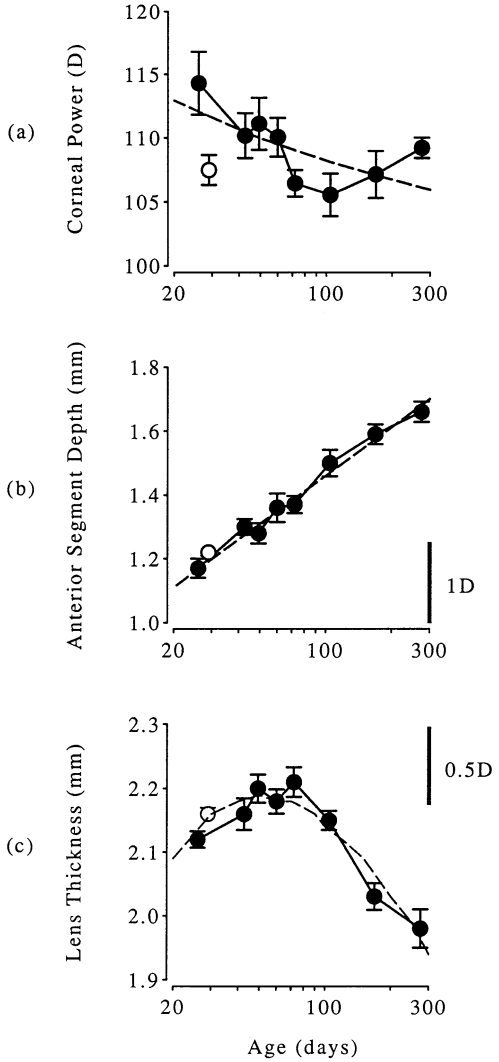

Fig. 2. Development of (a) corneal power; (b) anterior segment depth and (c) lens thickness. In the upper panel the dashed line shows the power function least-squares fit of the data from the group of seven normal animals. The dashed line in panel (b) shows the regression of anterior segment depth on log(age). In the lower panel the dashed line shows the data fitted with a cubic function. Scale bars are based on the data of Troilo & Judge (1993) and it can be seen from these bars in this figure and Fig. 3 that changes in refraction are brought about mostly by increases in the depth of the vitreous chamber. Error bars represent $\pm$S.E. See Table 1 for regression equations.

3.3. Comparisons with fellow eyes of lid-sutured or lens-wearing eyes

3.3.1. Vitreous chamber depth

The VCD of these normal eyes was compared with that in eyes from two other groups: eyes which wore

either no lens or a plano lens early in life (see Graham & Judge, 1999, for details of lens-wearing) ('pseudo-normal' eyes); and fellow eyes of lid-sutured eyes (Troilo & Judge, 1993). We fitted linear regressions of VCD to the $\log_{10}$ of age in days. There were no significant differences between the three groups of eyes with respect to either the gradients of the regression lines or the constant terms (unpaired t-tests) (Table 2).

3.3.2. Refraction

It turned out to be simpler to compare the development of refraction between groups of animals if absolute refraction was considered, i.e. if corrections were made for the retinoscopic artefact. The artefact of retinoscopy was calculated using the method of Troilo, Howland & Judge (1993), but substituting VCD measures for AL because we wished to include data where axial length was not available. The artefact of retinoscopy, A, is given by:

$$A = \frac{n_{vit}RT}{1.48 \cdot VCD(1.61 \cdot VCD + RT)}$$

where the refractive index of vitreous humour n_{vit} is 1.336; retinal thickness to the middle of the photoreceptor outer segments RT is 215 μm; and the coefficients 1.48 and 1.61 arise from substituting the estimate VCD which is equal to 0.62 AL in the equation given by Troilo, Howland & Judge (1993).

Examining the development of absolute refraction it was observed that this is close to linear with age prior to 8 weeks of age. In order to test whether the data from Troilo & Judge (1993) and that reported here were significantly different from one another, linear regressions were fitted to data from the nine normal animals, the pseudo-normal animals and to data between 20 and 60 days of age from the animals of Troilo & Judge (Fig.

Table 1
Linear regressions for data in Figs 2 and 3

Regression	R
Anterior segment depth = $0.46+0.50 \cdot \log_{10}$ (d)	0.88
Vitreous chamber depth = $1.90+2.04 \cdot \log_{10}$ (d)	0.95
Axial length = $4.82+2.36 \log_{10} \cdot$(d)	0.96
Anterior segment depth = $0.20 \cdot AL-0.45$	0.87
Vitreous chamber depth = $0.86 \cdot AL-2.27$	0.99

Age (d) in days, corneal power in diopters and other variables in millimetres.
All regressions significant at $P<0.0001$.

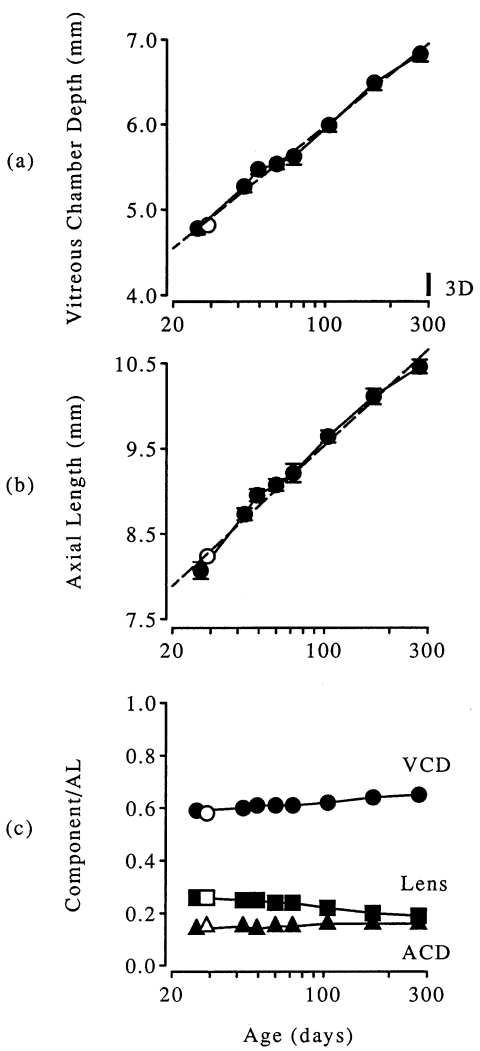

Fig. 3. Development of (a) vitreous chamber depth and (b) axial length for the same animals represented in previous Figures. The lower panel (c) shows the ratios of vitreous chamber depth (VCD, circles), lens thickness (LENS, squares) and anterior segment depth (ACD, triangles) to axial length (AL). Dashed lines in panels (a) and (b) represent the regressions of vitreous chamber depth and axial length against log(age) respectively. See Table 1 for regression equations.

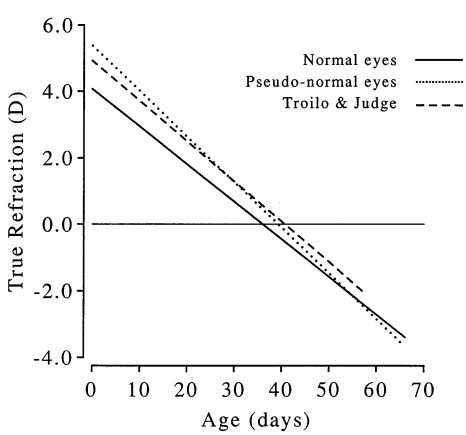

Fig. 4. The time-course of 'emmetropization' does not differ between various groups of normal eyes. Plot of linear regression of refraction corrected for the artefact of retinoscopy (true or absolute refraction) on age early in life before refraction has asymptoted. See Table 1 for further details.

Table 2
Parameters of linear regression of VCD on $\log_{10}$ of age in days, for right eyes of nine normal animals, pseudo-normal eyes of lens-wearing animals, and fellow eyes of those lid-sutured by Troilo & Judge (1993)

Data set	*n*	Gradient	Constant (*D*)
Normal	9	2.036 ± 0.082	1.899 ± 0.158
Pseudo-normal	24	1.907 ± 0.034	2.037 ± 0.064
Troilo & Judge	23	1.906 ± 0.063	1.999 ± 0.168

Mean $\pm$ S.E.

2). Unpaired *t*-tests showed there were no significant differences between the three groups in either slope or intercept (Table 3).

As the absolute refraction does not change significantly after 70 days of age the mean refraction of the data at this point and afterwards was taken to be the value for the asymptote in absolute refraction. Substituting this value in the regression equations in Table 3 then gave the age at which refraction stabilised.

3.3.2.1. Refraction stabilises at a myopic value rather than emmetropia. Unlike in the chick (Wallman, Adams & Trachtman, 1981), marmoset eye refraction overshoots emmetropia. In the normal animals in the Oxford colony, refraction became significantly myopic by 10 weeks of age, when the estimated artefact of retinoscopy was taken into account, and the asymptotic value of refraction was -3.4 D.

The control eyes of the monocular-deprived animals studied by Troilo & Judge (1993) also became myopic at about the same age, though the asymptotic value of refraction was significantly less myopic: -2.0 D. Furthermore, we found that the mean value of refraction was also myopic in the (adult) animals in a second colony, measured by the same highly experienced retinoscopist (BG). Moreover, a previous cross-sectional study of normal adult animals (Troilo & Judge 1993) found a mean myopia of -3.1 D (artefact corrected).

3.3.2.2. Factors affecting initial value of VCD and refraction. In other experiments, described in Graham and Judge (1999), we found that there were sometimes

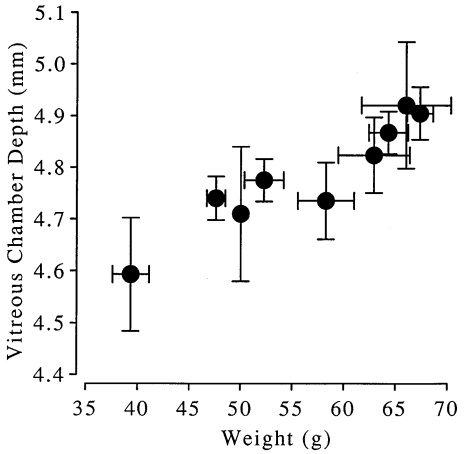

Fig. 5. Weight and vitreous chamber depth at 4 weeks of age co-vary ($R = 0.68$). Symbols show mean $\pm$ S.E. of weight and VCD of animals with common parents. MANOVA shows parentage has a significant effect on weight, but not VCD.

significant differences between groups of animals in the mean initial values of VCD and refraction. We therefore investigated possible factors influencing these variables, namely the parentage of the animals and the sex of the animals. We also looked at whether there was evidence of a secular variation in initial VCD and refraction which could conceivably have been associated with changes in colony size or location (the colony had to be moved to a different room twice).

Because we routinely began our lens-wearing experiments at the age of 4 weeks, and all animals were measured before experiments started, we had a large number (64) of normal animals of this age available for analysis: 37 were males and 27 females.

Parentage just failed to have a significant effect on the size of the vitreous chamber (MANOVA, $P = 0.079$), with the effects of parentage accounting for 19% of the total variance. There was a highly significant effect of parentage on weight of animals at 4 weeks of age (MANOVA, $P < 0.001$, with parentage accounting for 61% of the total variance. Weight was significantly correlated with VCD ($R = 0.68$) (Fig. 5). There were no significant differences between males and females in VCD, refraction or weight. We looked for the possibility of a secular trend in VCD of offspring, by comput-

Table 3
Linear regression of refraction on age in days, for right eyes of nine normal animals, pseudo-normal eyes of lens-wearing animals, and fellow eyes of those lid-sutured by Troilo & Judge (1993)

Data set	*n*	Gradient	Constant (*D*)	Asymptote (*D*)	Age (days)
Normal	9	-0.113 ± 0.019	4.1 ± 0.9	-3.4	66
Pseudo-normal	24	-0.137 ± 0.011	5.4 ± 0.6	-3.5	65
Troilo & Judge	23	-0.121 ± 0.025	4.9 ± 1.2	-2.0	57

Mean $\pm$ S.E.
Units of gradient were dioptres$\cdot$day^{-1}.

ing the average values for animals born in each 4 month period between April 1992 and April 1996. No such trend was found (one-factor ANOVA, $P = 0.27$). The 4 month period was chosen to ensure that no parents had more than one set of offspring in each period.

4. Discussion

4.1. Comparisons with previous data on lid-sutured animals

The unoperated eyes of the monocularly lid-sutured animals studied by Troilo & Judge (1993) grew in a way that did not differ significantly from the growth of the eyes of the normal animals studied here.

This is a useful finding because there was always the possibility that these eyes might not have grown normally.

It is particularly noteworthy that the refraction of the normal animals did not stabilize at emmetropia, but at a small degree of myopia. The same was true of Troilo & Judge's animals and it is good to have been able to reject the possibility that this feature of their data was some consequence of the fellow eyes having been lid-sutured early in life.

4.2. Features of marmoset ocular and refractive development

4.2.1. Corneal power decreases with age

Although our keratometry measurements are not as precise as we would wish, it is clear that the general trend is for corneal power to decrease over the period studied, as was suggested by the earlier study of Troilo & Judge (1993). Both Sorsby, Benjamin & Sheridan (1961) and Friedman, Mutti & Zadnik (1996) have reported a small decrease in corneal power in humans, about 0.3 D, between the ages of 8 and 15 years in the more recent study. Kiely, Crewther, Nathan, Brennan, Efron & Madigan (1987) also found a decrease with age in corneal power in juvenile macaques.

4.2.2. Anterior segment depth increases with age

We found that anterior segment depth increases with age, as it does in humans and macaques (Sorsby, Benjamin & Sheridan, 1961; Larsen, 1971a; Kiely, Crewther, Nathan, Brennan, Efron & Madigan, 1987).

4.2.3. Lens thickness increases to a maximum at the age of 10 weeks and then decreases

We found that lens thickness increased until the third month of life and then decreased. Although this tendency can, in retrospect, be seen in the earlier data of Troilo & Judge (1993) they did not remark on it.

In a human cross-sectional study Larsen (1971b) reported a thinning of the crystalline lens by 0.5 mm between birth and the age of 13 years, with 60% of the effect occurring before the age of 3 years; and Zadnik, Mutti, Fusaro & Adams (1995) have confirmed in a longitudinal study of children between the ages of 6 and 13 years that the lens does indeed thin-by 0.2 mm. If one uses as a rough guide to equivalent ages in marmosets and humans, 1 month of marmoset growth as equivalent to 1 year of human growth, then the period in human life where one would want to look for the sort of initial lens thickening we have found in the marmoset would be in infants under 2 years of age. Larsen (1971b) showed that eyes of children aged 1–2 years had significantly thinner lenses than those of neonatal 1–4 days old, and so if there is early lens thickening in humans it would have to occur and revert in the first year of life, where Larsen made very few measurements.

We are not sure why lens thickness changes in this non-monotonic way. One obvious question about the decrease in lens thickness is whether it is due to active remodelling of the lens tissue or passive responses to external factors: one might, for example, imagine zonular tension increasing as the eye grows, and the increased tension flattening the lens.

4.2.4. Growth curves for VCD and axial length

Although it is not our purpose here to enter into a detailed discussion of the allometry of eye growth, we think it worthwhile to point out that growth of VCD and axial length of infant and juvenile eyes seems generally to be close to logarithmic with time, not only for marmosets (Troilo & Judge, 1993 and Fig. 3), but also for macaques (Fig. 1 of Tigges, Tigges, Fernandes, Eggers & Gammon, 1990; Table 1 of Lambert, Fernandes, Drews-Botsch & Tigges, 1996; axial length data only), and humans (Tables 1 and 2 of Larsen 1971c (VCD), and Table 4 of Zadnik, Mutti, Friedman & Adams, 1993 (VCD and AL)).

4.2.5. Cage myopia?

The finding that there is a significant difference in refraction between normal, age-matched, animals in the two colonies suggests a number of possibilities.

In a separate control experiment (Whatham and Judge, unpublished observations) we have shown that anaesthesia with Saffan has no effect on cyclopleged refraction: there is no difference in refraction of the same animals whether they are or are not anaesthetised.

It has long been known that macaques reared in restricted visual environments become somewhat myopic (Young, 1964), and while it has been suggested that this could be a deprivation effect (Wallman, 1993) the usual interpretation is that the myopia is a functional response to being raised in an environment where

no object is further than some finite distance. In the chick there is good evidence (Miles & Wallman, 1990) that animals raised in environments of low height develop myopia in the upper visual field and this must be a functional response.

The most likely interpretation of the greater myopia in the Oxford animals is therefore that this was a cage myopia, related to the much smaller cages in use at the time in Oxford compared with those of the second colony. The use of a much smaller mesh in the cage fronts of the Oxford cages (1.2×2.4 cm) compared with that of cages of the second colony (4.0×4.0 cm) may have contributed by discouraging our animals from making much use of the distant view potentially available but we presume that the main demand on quality of focus in the distance is set by the most distant target to which the animal can attempt to jump, and this would certainly have been much smaller (~ 0.5 m) in the Oxford cages in use at the time of this study than in the cages of the second colony which were an order of magnitude larger in linear dimension.

4.2.6. Reliability of retinoscopy

We assessed the reliability of our retinoscopic measurements in two ways. First by comparing retinoscopic measurements made on a small number of animals by one of two other experienced retinoscopists (David Troilo and Andrew Whatham). Second, we made some comparison measurements using a Hartinger refractometer. (Model 110, Zeiss Jena). Although this is not a technique we routinely use, it involves a smaller element of judgement than retinoscopy and so even in less practised hands should be equally valid. The differences between these control measurements and our standard ones were small.

We also considered possible causes of systematic errors in the retinoscopic measurements and their interpretation. One source of error would be spherical aberration combined with the use of an inappropriate pupil diameter, and to avoid this we always neutralised the central reflex.

It would be desirable to be able to directly check the veracity of the usual assumptions, which we followed, about the cause of the retinoscopic artefact. O'Leary & Millodot (1978) found that in humans two reflective layers contributed to the retinoscopic reflex: a specularly reflecting layer they presumed to be at the vitreo-retinal interface and a diffusely reflecting layer near the pigment epithelium. In young human eyes reflection from the vitreo-retinal interface predominates, while in older eyes reflection from the deeper layer predominates. It would be very useful to investigate whether reflection at the vitreo-retinal interface predominates in young marmosets. An indirect way of determining this would be to compare retinoscopy with a method of refraction that is not subject to these uncertainties, such as the electroretinographic technique of Fitzke, Hayes, Hodos & Holden (1985), or to carry out retinoscopy while animals perform a visual discrimination task that requires accurate accommodation.

4.2.7. Factors influencing eye size early in life

We have shown that there is an effect of parentage on weight at the onset of our experiments, but that there is not a significant effect of parentage on the initial value of vitreous chamber depth at the onset of our experiments.

This implies an incomplete correlation between VCD and body weight at the age of 4 weeks, and indeed the correlation is only moderate ($R = 0.68$, i.e. accounted for $R^2 = 0.46$ of the variance). It is of course possible that the two variables would correlate better at different ages of life; it is well-established that in humans the time-courses of ocular growth and increase in body weight are not very well correlated, and the same appears to be true in the marmoset (unpublished observations).

It is pleasing to see that there was no secular variation in the ocular size of the marmosets, because we had been concerned about whether a number of intermittent stress factors (loud noise from building renovations at one stage, expressions of hostility between animals in adjacent cages at another, and having to move the colony twice) might have affected growth and development.

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