Pergamon

0042-6989(94)E0049-Q

Vision Res. Vol. 35, No. 1, pp. 37–50, 1995

0042-6989/95 $7.00 + 0.00

Moving the Retina: Choroidal Modulation of Refractive State

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Received 24 February 1993; in revised form 12 July 1993; in final form 17 February 1994

The chick eye is able to change its refractive state by as much as 7 D by pushing the retina forward or pulling it back; this is effected by changes in the thickness of the choroid, the vascular tissue behind the retina and pigment epithelium. Chick eyes first made myopic by wearing diffusers and then permitted unrestricted vision developed choroids several times thicker than normal within days, thereby speeding recovery from deprivation myopia. Choroidal expansion does not occur when visual cues are reduced by dim illumination during the period of unrestricted vision. Furthermore, in chick eyes presented with myopic or hyperopic defocus by means of spectacle lenses, the choroid expands or thins, respectively, in compensation for the specific defocus imposed. Consequently, when the lenses are removed, the eye finds its refractive error suddenly of opposite sign, and the choroidal thickness again compensates by changing in the opposite direction. If a local region of the eye is made myopic by a partial diffuser and then given unrestricted vision, the choroid expands only in the myopic region. Although the mechanism of choroidal expansion is unknown, it might involve either a increased routing of aqueous humor into the uveoscleral outflow or osmotically generated water movement into the choroid. The latter is compatible with the increased choroidal proteoglycan synthesis either when eyes wear positive lenses or after diffuser removal.

Accommodation Chicken Choroid Myopia Refractive error

INTRODUCTION

Like most other optical devices, eyes are generally thought to focus by lens adjustments that optically move the image plane. During ocular accommodation, most vertebrates move the image plane by rapidly adjusting the optical power of the eye, for example by increasing the curvature of the lens for near objects. Variants of this mechanism are found in fish, which displace the lens, and in birds, which alter the curvature of the cornea as well as the lens (Sivak, 1980; Schaeffel & Howland, 1987; Troilo & Wallman, 1987). A second, slower, way that vertebrates bring images into focus on the retina is by adjusting the growth of the eye as a whole so that its length becomes appropriate for the resting optical power of the eye (emmetropization) (Van Alphen, 1961, 1986; Schaeffel & Howland, 1988a; Troilo & Wallman, 1991). The strongest evidence for this emmetropization process is that, in the chick, the eye grows in compensation for defocus produced by spectacle lenses (Schaeffel, Glasser & Howland, 1988; Irving, Sivak & Callender, 1992). In this paper, we present evidence for a third focusing mechanism—intermediate in speed—in which the retina is moved forward and back by changes in the thickness of the choroid.

The choroid in chickens, as in other vertebrates, consists of two parts: the choriocapillaris, a network of fenestrated capillaries just behind the retinal pigment epithelium, and the main portion of the choroid, which contains numerous larger blood vessels, and, at least in birds, large lacunae. These structures are supported by an intervascular suspensory system comprised of extracellular matrix, smooth muscle fibers, fibroblasts and pigmented cells (Meriney & Pilar, 1987). The choroid supplies the outer retina with oxygen and nutrients and also functions as a heat sink (Bill, 1985). It is under the control of the autonomic nervous system, and is innervated from many divergent sources, including the oculomotor, trigeminal and facial nerves, as well as the ciliary, superior cervical and pterygopalatine ganglia (Bill, 1985). In addition, a plethora of putative transmitters have been localized to these terminals, including acetylcholine, VIP, substance P

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(Reiner, 1987) and somatostatin (Epstein, Davis, Gelman, Lamb & Dahl, 1988). The functional significance of this diverse pattern of innervation is unknown.

We observe changes in choroidal thickness in two experimental situations that present eyes with out-of-focus images: (i) eyes made myopic by prior visual deprivation; and (ii) eyes made functionally either myopic or hyperopic by spectacle lenses. In presenting these results, we will argue that modulation of choroidal thickness is a response to optical defocus, the choroid becoming thicker with myopic defocus (image in front of retina) and thinner with hyperopic defocus (image behind retina).

METHODS

Animals

White Leghorn chickens were hatched in our laboratory from eggs obtained from a commercial supplier (Truslow Farms, Chestertown, Md). They were raised in heated brooders on a 14:10 hr light:dark cycle.

Deprivation myopia experiments

To produce myopia by visual deprivation, we covered one eye of newly hatched chicks with a white, translucent plastic diffuser attached to the surrounding feathers (Wallman, Ledoux & Friedman, 1978). The fellow untreated eye served as a control in these and other experiments reported in this paper. The refractive error and the axial dimensions of all eyes were measured by low-frequency ultrasound either 10 ($n = 5$) or 32 ($n = 11$) days later, when the diffusers were removed, and then twice a week thereafter. In a related experiment, the diffusers were removed at 2 weeks of age, and the chicks ($n = 10$) were then put in a dim (0.05 lx) diurnal environment to assess the effect of reduced visual cues on choroidal thickness.

To produce eyes with myopia confined to half of the retina, another group of chicks ($n = 7$) was raised from hatching with one eye covered by a diffuser that permitted unrestricted vision only to the nasal half of the retina. At 2 weeks of age the diffusers were removed, the birds given 2 weeks of unrestricted visual experience, and local changes in choroidal thickness were characterized as described below.

Spectacle lens experiments

At 4 days of age, chicks had one eye covered by a custom-made panoramic spectacle lens (Conforma Contact Lenses, Norfolk, Va), with a 7 mm internal radius of curvature providing a 70–90 deg undistorted field of view. Lenses of either −15, −6, 0, +6 or +15 D* were used (6–7 chicks for each power). Each lens was mounted in an annulus of Velcro attached to a mating piece of Velcro cemented to the chicks' feathers by collodion. The lenses were kept quite clean by keeping birds on raised floors, sieving food to remove small particles, and cleaning the lenses approximately every 3 hr from about 10 a.m. to 9 p.m. After 4.5 days, refraction and ultrasound measurements were made.

*The front of the lens was about 5 mm from the cornea. As a result, the effective power of the lenses at the cornea would be −14, −5.8, +6.2, +16 D; because these differ so little from the optical power of the lenses and because we did not measure the distance from the lens to the cornea in all birds, we have retained the use of optical power in the text.

Measurement of refractive error

Birds were anesthetized with a mixture of chloral hydrate and sodium pentobarbital. Cycloplegia was obtained by 1 drop/min for 5–10 min of 10 mg/ml vecuronium bromide (Norcuron, Organon, West Orange, N.J.) and benzalkonium chloride (0.26 mg/ml) in saline. Refractive error was measured with a Hartinger refractometer (Jena Optik), as the median of 4–6 pairs (at orthogonal meridians) of measurements per eye, with the eye realigned with respect to the instrument after every other pair of measurements (Wallman & Adams, 1987).

Demonstration of choroidal thickness changes

We used six methods to illustrate the phenomenon of choroidal expansion; two of these methods (hemisected frozen eyes and low frequency A-scan ultrasound) were also used to quantify choroidal expansion in specific experiments:

(a) Hemisected frozen eyes. Immediately after an overdose of sodium pentobarbital anesthesia, the eyes were removed and mounted with optic axes approximately horizontal in Cryomatrix (Shandon, Pittsburgh, Pa) on the stage of a freezing microtome. Sections were taken until the vicinity of the optic axis was reached, as shown by the lens having its greatest thickness; at this point the eye was photographed from above.

(b) Histological sections. After eyes were fixed in 2% glutaraldehyde/2% paraformaldehyde in cacodylate buffer, 1 mm diameter punches were made through the eye wall near the posterior pole. These were imbedded in plastic and sectioned at 1 μm. Because we did not use this technique or the following one to make measurements, we did not attempt to assess the shrinkage during fixation or dehydration.

(c) Sections 1 mm thick of the posterior eye wall from fixed eyes. After fixation in 2% paraformaldehyde/1.25% glutaraldehyde in 0.1 M phosphate buffer, sections of the posterior eye wall were cut freehand with a razor blade and photographed under dark-field microscopy.

(d) High-frequency ultrasound images of the posterior eye wall (B-scan). Anesthetized chickens were positioned with the optic axis of one eye vertical, and a latex waterbath was placed over the eye. A 50 MHz ultrasound transducer traversed the pupil, driven by a two-axis stepping-motor positioner. The echoes were digitized at 100 MHz and images of these echoes were generated with a video printer.

(e) Low-frequency A-scan ultrasound. This method was used for all *in vivo* monitoring of axial dimensions and choroidal thickness unless described otherwise. In

anesthetized birds, a 7.5 MHz ultrasound transducer was placed along the optic axis of the eye via a gel-coupled water-filled standoff, and conventional A-scan ultrasound traces were digitized at 20 MHz by a digital storage oscilloscope and subsequently analyzed to obtain axial ocular dimensions. Four sweeps comprising two independent alignments of the probe with the eye were averaged. We used a sound velocity of 1.6078 mm/μsec for the lens and a sound velocity of 1.534 mm/μsec for the other media (Wallman & Adams, 1987). Because we saw no echo from the retina–choroid interface, we measured the thickness of the retina and choroid combined. From this measurement, one can infer the approximate thickness of the choroid alone by subtracting an estimate of retinal thickness [0.25 mm, according to Barrington (1990)]. As a measure of the repeatability of the measurements we used the standard deviations obtained from repeated measures of the same birds; these were approx. 57 μm. We treat the differences in the thickness of the "choroid + retina" as arising from the choroid alone because the changes in retinal thickness (thinning during deprivation and returning to normal with recovery) are quite small [22 μm in 2-week-old chicks (Barrington, 1990)].

(f) High-frequency ultrasound measurement of the axial spacing of ocular components (A-scan). From several adjacent scan lines that made up the B-scan image described in (d), the analytic signal magnitude (Gammell, 1981) was computed and plotted, yielding an A-scan trace of high resolution.

Characterization of local choroidal thickness changes

Eyes were frozen and hemisected as described in (a) above, and on the resulting photographs two outlines—of the retinal pigment epithelium and of the inner scleral margin—were traced on a digitizing tablet. The spacing between these outlines represents choroidal thickness. To align normal eyes to form averages of their outlines, we took advantage of the facts that (a) the central 100 deg of the back of the eye approximates an arc, the center of curvature of which lies near the axis of symmetry of the outline of the eye and (b) the largest diameter of the chick eye is the equatorial diameter. For the non-deprived eye of each bird, we used one algorithm to find the center of curvature of the posterior pole, and another algorithm to find the equatorial diameter of the eye (Wallman, Gottlieb, Rajaram & Fugate-Wentzek, 1987). We then formed a coordinate system with the x-axis parallel to the equatorial diameter and the origin at the center of curvature of the posterior globe. We take the liberty of referring to the y-axis of this coordinate system as the "optic axis". This procedure could not be used with the partially deprived eyes because the asymmetry of the contours would result in spurious equatorial axes. For these eyes we aligned, by eye, the anterior half of the contour of the partially deprived eye with a superimposed, left–right-reversed image of the contour traced from the normal fellow eye, and transferred to it the coordinate frame of the normal eye (determined as described above).

Measurement of choroidal and scleral proteoglycan synthesis

To measure the incorporation of sulfate into proteoglycans, 6 mm diameter punches of choroid from approximately the central part of the eye were pinned onto Sylgard-lined petri dishes in the defined medium N2 (Bottenstein & Sato, 1979), which was labeled with $Na_2{}^{35}SO_4$. Scleral punches were also labeled in N2. The choroids or scleras were incubated for 18–24 hr at 37°C, and were then digested in proteinase K (Sigma) at 60°C overnight. The glycosaminoglycans were precipitated with cetylpyridinium chloride, filtered and scintillation counted (methods in Rada, Thoft & Hassel, 1991).

Electron microscopy of choroidal smooth muscle

One mm tissue punches of the posterior eye wall, fixed in 2% glutaraldehyde/2% paraformaldehyde in cacodylate buffer, were embedded in Lowicryl K4M, sectioned at 100 nm and collected on grids. Tissue was incubated with primary antibody against smooth muscle actin (Sigma) at 1:1000 and then with gold-conjugated secondary antibody, before staining with uranyl acetate.

RESULTS

Choroidal changes in eyes made myopic by previous deprivation

Eyes wearing diffusers developed substantially elongated vitreous chambers and perhaps slightly thinner choroids. As a consequence, when the diffuser is first removed, the retina experiences substantial myopic blur, because the retina is now behind the eye's plane of focus. Subsequently, the choroid thickened over the next week (young birds) or month (older birds), pushing the retina forward toward the plane of focus and thereby substantially correcting the myopia caused by the previous visual deprivation.

We have documented this choroidal thickening using three histological techniques differing in whether the tissue was fixed or embedded (Fig. 1, left column). In frozen, unfixed, hemisected eyes [Fig. 1(a)] an increase in choroidal thickness is observed, as evidenced by the increased separation between the retinal and scleral boundaries in the formerly deprived eyes. Photomicrographs of plastic-embedded sections of eyes [Fig. 1(b)] provide greater detail about this change in thickness; the expansion mostly involves the outer choroidal region adjacent to the sclera, which shows enlarged lacunae (L) and greatly increased cross-sectional area. In fixed eyes that were neither frozen nor embedded [Fig. 1(c)], choroidal thickening is also evident, and, again, expansion of the outer region of the choroid appears to underlie the choroidal thickness changes. That this phenomenon is apparent using all three histological techniques indicates that it is unlikely to be an artifact associated with shrinkage or swelling during tissue processing.

We also documented choroidal thickening in intact living eyes using high frequency B-scan ultrasound

[Fig. 1(d)], low frequency A-scan ultrasound [Fig. 1(e)] and high frequency A-scan ultrasound [Fig. 1(f)]. The distance between retinal and scleral peaks is increased in eyes that had been previously deprived. That this choroidal thickening can be seen both in the living animal as well as in preserved material confirms that it represents a real biological response of this tissue.

Longitudinal studies using ultrasound show the time–course of the choroidal thickening (Figs 2 and 3). In the younger birds, the peak of the choroidal expansion occurred within 7 days after the diffusers were

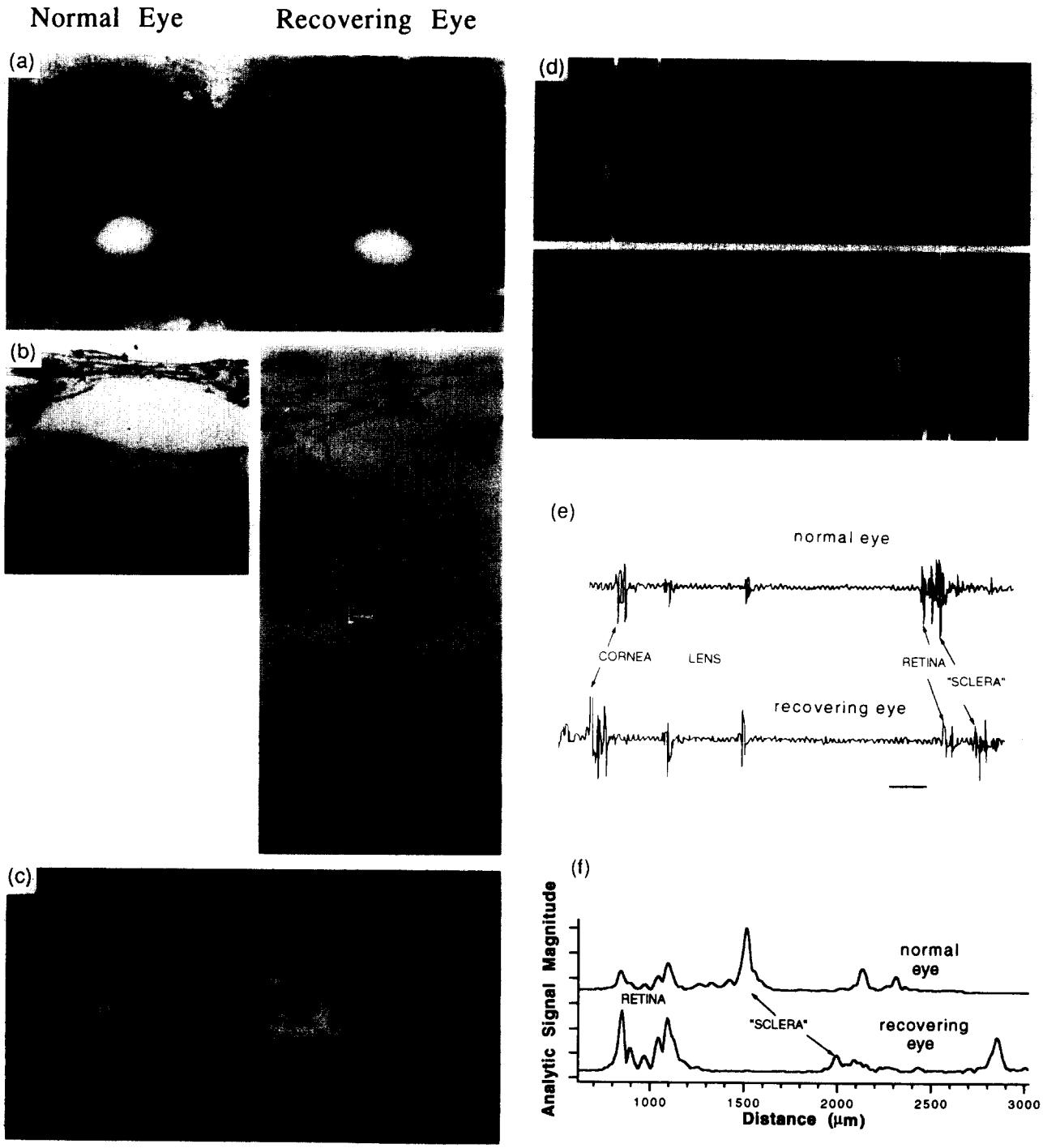

FIGURE 1. Choroidal expansion in eyes recovering from myopia induced by prior form-deprivation. In (a)–(c), recovering eyes are on the right, untreated fellow eyes are on the left. (a) Unfixed hemisected eyes. Arrowheads indicate choroidal boundaries. Scale bar, 2 mm. (b) Plastic-embedded sections at the posterior pole of eyes. Sclera begins just above pictures. L, lacuna; P, pigment cell; PE, retinal pigment epithelium; arrowhead indicates choriocapillaris. (c) One-mm-thick sections of the posterior eye wall. Ch, choroid, delimited by arrows; L, lacuna; R, retina. (d) High-frequency B-scan ultrasound image. R, retina; S; sclera; echo to left of retina is posterior lens surface. (e) Low-frequency A-scan ultrasound trace, representative of those used for measurements of thickness of "choroid + retina" in subsequent figures. Front and back lens peaks straddle the "lens" label. Scale bar, 2 μsec. (f) High-frequency A-scan ultrasound trace, in which the analytic signal magnitude (Gammell, 1981) is plotted against distance.

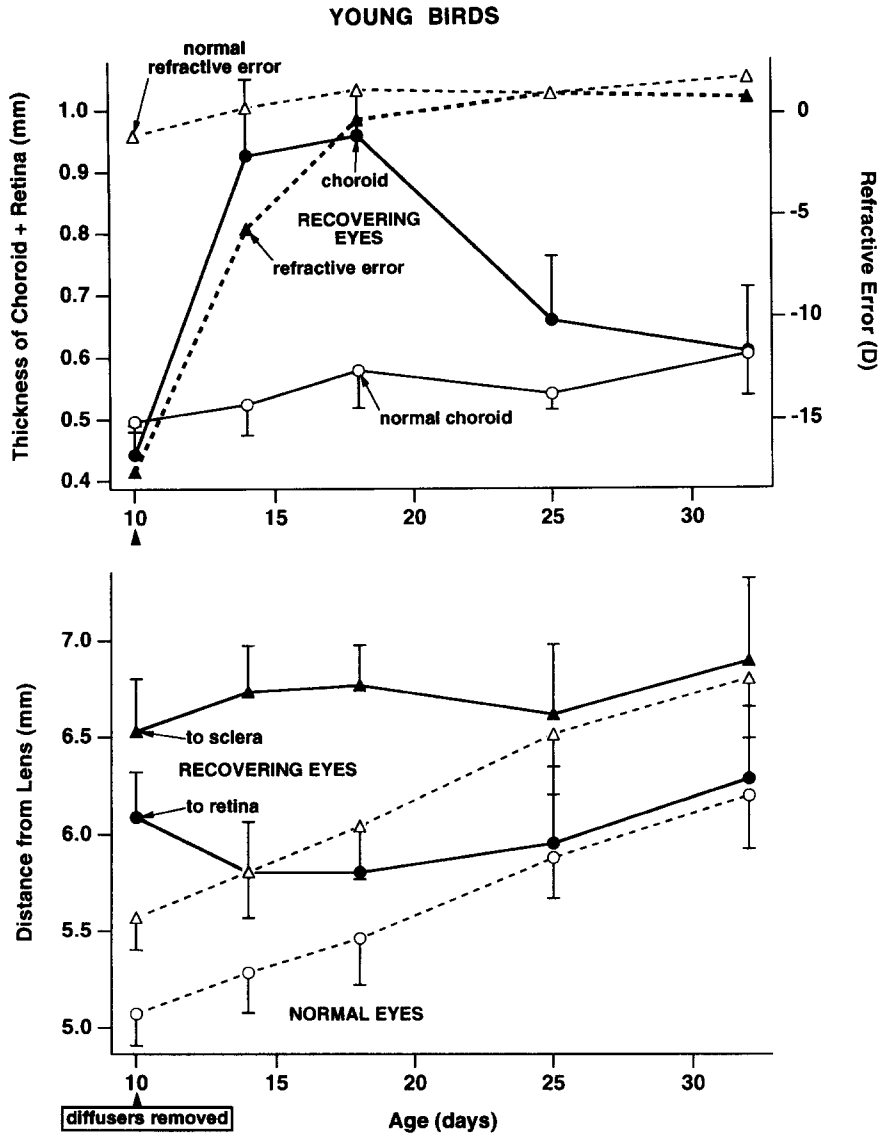

FIGURE 2. Relation of changes in thickness of "choroid + retina" to changes in refractive error (a), and associated changes in distance from lens to retina and to sclera (b) following removal of ocular diffusers at 10 days. The thickness of "choroid + retina" in the upper panel is equal to the distance from lens to sclera minus that to retina in (b); all thickness measurements were by low-frequency ultrasound. (a) shows that the early course of the recovery from myopia closely parallels the expansion of the choroid; (b) shows that this occurs because the retina is pushed forward toward the image plane. As the eye becomes normal in refraction and length, the choroid returns to normal thickness. Solid symbols are previously deprived, recovering eyes; open symbols are fellow control eyes. In (b), triangles represent distance to sclera; circles, distance to retina. Each data point is the mean of five eyes; error bars are standard deviations. Arrows on the x-axes of both panels indicate when the diffusers were removed.

removed, with no overlap between peak choroidal thickness of recovering and normal (fellow control) eyes, that is, the thinnest "choroid + retina" among the recovering eyes (0.77 mm) was thicker than the thickest among control eyes (0.61 mm). The choroidal thickness changes approximately three-fold, increasing to 0.7 mm, assuming the retina maintains a constant thickness of 0.25 mm. The change in choroidal thickness with age is statistically significant [one-way ANOVA for repeated measures of the differences between normal and recovering eyes of individual birds compared across time;* $F(4,12) = 6.05$, $P < 0.01$]; *post hoc* tests confirmed the statistical significance of the increase in choroidal thickness after 4 and 8 days of recovery (age: 14 and 18 days) compared to that at the time the diffusers were removed (Tukey's test, $P < 0.01$).

Over the week during which the choroid reached maximum thickness in the younger birds [Fig. 2(a)], the degree of myopia diminished. Because the time-course of the increased choroidal thickness parallels the recovery from myopic refractive error in the previously deprived eyes, the choroid appears to contribute

*We have used differences between treated and fellow control eyes in this and other analyses reported in this paper to reduce variability among individual animals and so to improve the sensitivity of the analyses.

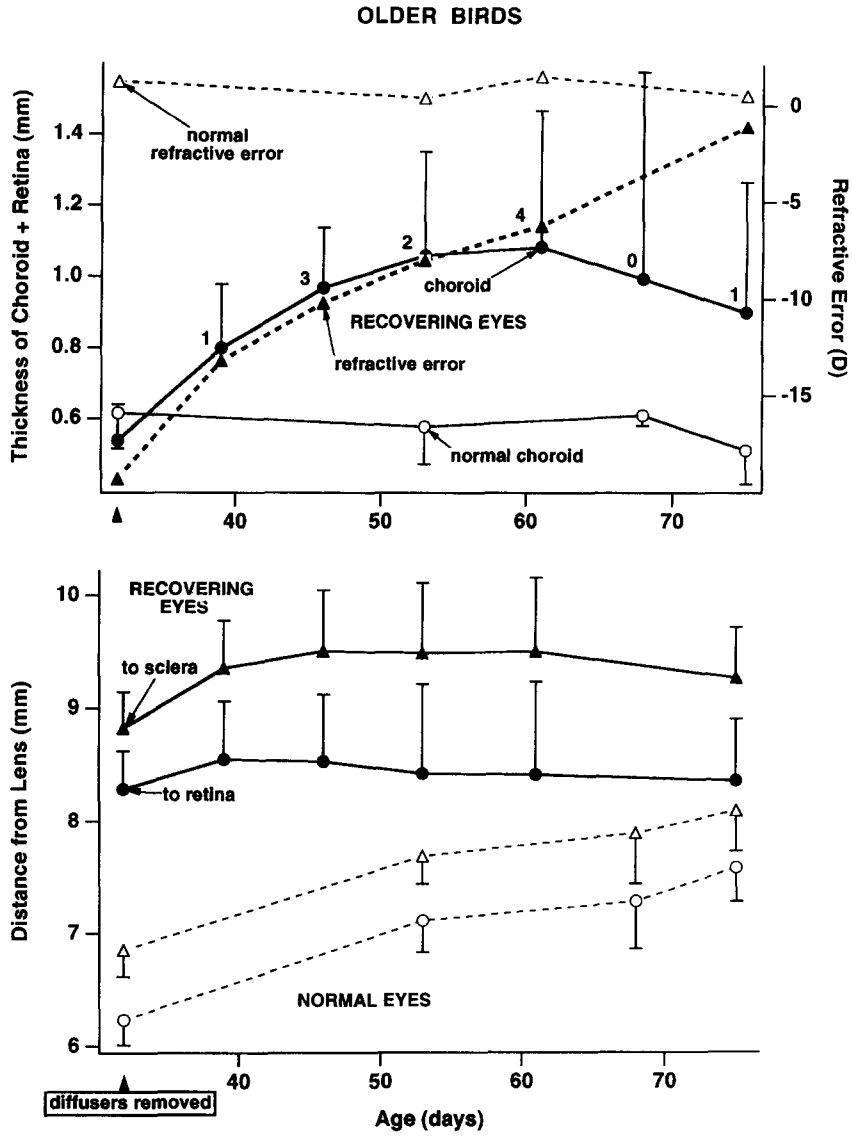

FIGURE 3. Same as Fig. 2, but for older birds wearing diffusers from hatching until 32 days. Note that in these birds the eye has enlarged too much for the maximal choroidal expansion (at 54–62 days) to result in emmetropia (a) and to regain its normal dimensions (b) during the experiment; perhaps because complete recovery does not occur, the choroid remains expanded. Each data point is the mean of 11 eyes; error bars are standard deviations. The increasing standard deviations with age in the older birds reflect the fact that choroidal thickness of individual eyes peaked at different ages (the digit above each point shows the number of eyes peaking in choroidal thickness at that age).

substantially to the recovery. When we plot separately the distances from the lens to the retina and to the sclera against age, beginning when the diffusers were removed, we find that the expanding choroid pushes the retina forward, closer to the lens [Fig. 2(b)] causing recovery from myopia.

To calculate the effect of choroidal thickening on refractive error, we used a procedure similar to that of Troilo and Judge (1993). We first computed the total optical power of the recovering eyes at each age as

$$P = n_v/(0.85 \times \text{axial length}) - \text{R.E.}$$

in which [0.85 × axial length (by ultrasound)] would be the estimated focal length of an emmetropic chicken eye (Wallman & Adams, 1987) if the optics were in air; dividing 1 by this figure yields the optical power in diopters; multiplying by n_v (the refractive index of the vitreous humor, 1.336) takes account of the different speed of light in vitreous; subtracting the refractive error (R.E.) compensates for the eyes not being emmetropic. Next we used a variant of this equation to estimate what the refractive error of the eye would be if its axial length was longer by the amount of the choroidal expansion (the difference between the thickness of the choroid in the recovering and the fellow untreated eye, Δchor), i.e. R.E. without choroidal change $= n_v/[0.85 \times (\text{axial length} + \Delta\text{chor})] - P$. Finally, we subtracted from this predicted refractive error, the actual refractive error for each bird at each age to show how much of the refractive error is attributable to the change in choroidal thickness.

As Table 1 shows, in the younger birds, the change in choroidal thickness makes the refractive error slightly more (0.8 D) myopic than the −17.6 D measured at the

TABLE 1. Estimates of the refractive effect on recovering eyes of measured differences in choroidal thickness

Days of deprivation	Days of recovery	Measured refractive error (D)	Choroidal thickness difference (expl eye – fellow eye) (mm)	Predicted effect of choroidal thickness difference (D)
10	0	−17.6	−0.05	−0.8
	4	−5.6	0.40	5.4
	8	−0.2	0.38	4.9
	15	1.0	0.03	0.2
	22	0.8	0.01	0.01
32	0	−19.3	−0.08	−0.6
	21	−8.0	0.43	3.0
	43	−1.1	0.39	2.6

end of the period of deprivation (the choroid thins slightly), and 5.4 D less myopic than measured 4 days after vision was restored; thus the choroidal expansion accounts for approximately half of the 12 D recovery over the latter period. After 15 days of recovery, however, the choroid of the recovering eye is no thicker than that of the fellow eye and hence no longer influences the refractive recovery.

Acting in parallel with this choroidal mechanism is another recovery mechanism, known from previous studies in chicks and tree shrews (Wallman & Adams, 1987; Norton, 1990; Sivak, Barrie, Callender, Doughty, Seltner & West, 1990; Troilo & Wallman, 1991), in which a decreased rate of ocular elongation and decreased scleral growth (Nickla, Gottlieb, Christensen, Peña, Teakle, Haspel & Wallman, 1992; Rada, McFarland, Cornuet & Hassell, 1992), together with continued growth (and flattening) of the cornea and perhaps the lens, increases the focal length of the eye's optics until the physical length and focal length become matched (Wallman & Adams, 1987). To separate the consequences of the recovery mechanism associated with decreased ocular elongation (i.e. decreased scleral growth) and that associated with the choroidal thickening just described, consider Fig. 2(b). The expanding choroid initially results in the vitreous chamber (as delimited by the retina) being reduced (Fig. 2, recovering eye, "to retina" curve), although the eye continues to elongate for a few days, as shown by the increasing distance to the sclera (recovering eye, "to sclera" curve). Later, this ocular elongation slows, ceasing by 18 days of age, although the focal length of the optics continues to increase (data not shown). By this time, the refraction has become nearly normal and the choroid then returns to normal thickness, so that between 25 and 32 days of age the eye regains its normal length, refraction, and choroidal thickness (Fig. 2).

In the birds deprived for 32 days, both the choroidal expansion and the change in refractive error are slower, continuing for a month after the diffusers are removed. Furthermore, these eyes are more variable in the rate of choroidal expansion; the numbers above each data point in Fig. 3(a) show the number of treated eyes that peaked at each age. As fellow control eyes were not measured

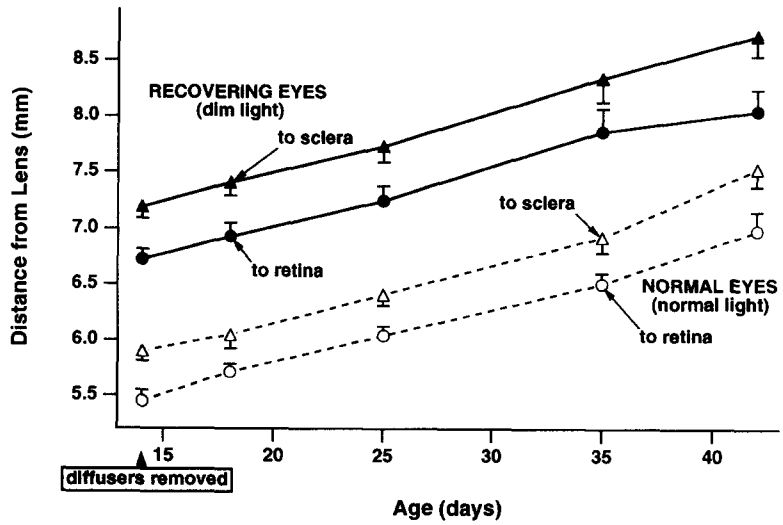

FIGURE 4. Growth of the vitreous chamber as a function of age in birds put in dim (0.05 lx) illumination when their diffusers were removed at 2 weeks of age. Both "recovering" myopic eyes (in dim light) and untreated control eyes (in normal light) continue to elongate, whether measured at the retina or sclera. In contrast to the results of recovering eyes in normal illumination (Figs 2 and 3), in dim illumination the thickness of the "choroid + retina" does not increase. Error bars are SEs. Conventions are the same as in Figs 2 and 3.

at all time points, statistical analysis of the data was restricted to those time-points for which there were complete data sets. Because of this limitation and the temporal variability in choroidal expansion just noted, age-related changes in choroidal thickness are only marginally significant as analyzed by one-way ANOVA for repeated measures [differences between recovering and fellow normal eyes of individual birds compared across time, $F(2,8) = 4.17$, $P = 0.058$]. *Post hoc* tests confirmed the statistical significance of the increase in choroidal thickness between 0 and 21 days after diffuser removal (age 32 and 53 days; Tukey's test; $P < 0.05$) while the difference between 0 and 43 days after diffuser removal (age 32 and 68 days) just failed to reach significance (Tukey's test; $P > 0.05$). Nonetheless, the presence of choroidal expansion in these birds is as consistent as in the younger birds in that in every bird the thickness of the "choroid + retina" is greater in the recovering eye than in the fellow eye (mean is 77% greater after 21 days of recovery).

In the older birds ocular elongation at the time of removal of the diffusers is so great that the eyes do not recover completely during the measurement period and hence the choroid stays expanded [Fig. 3(a)]; this choroidal expansion reduces the myopia by 3 D (Table 1). These results imply that the choroid returns to its normal thickness only when the eye has nearly recovered from myopia. In this case, choroidal expansion did not push the retina forward but merely served to compensate for the continued ocular growth ["to sclera" curve, Fig. 3(a)].

Dim visual environments prevent choroidal expansion in myopic eyes

If chicks made myopic by wearing a diffuser over one eye are put into very dim light (0.05 lx) at the time the diffusers are removed, the extent of recovery from the myopia is greatly reduced (Gottlieb, Marran, Xu, Nickla & Wallman, 1991). This is presumably because the visual cues to defocus are attenuated and the depth of focus is increased by the low acuity. Under these conditions, the eyes show no choroidal expansion (cf. Fig. 4 to Figs 2 and 3) and remain myopic (mean refractive error at the end of the recovery period is -12.87 D compared to $+1.04$ D for birds reared in normal light levels). Thus, a one-way ANOVA for repeated measures of differences between recovering and fellow untreated eyes of individual birds in dim light revealed no significant effect of time. The lack of a compensatory response in choroidal thickness in this visually "reduced" environment lends further support to the argument that the choroidal expansion we see under normal illumination in previously deprived eyes is a response to visual cues and not a secondary, non-specific effect of having previously worn diffusers.

Local myopia causes local choroidal expansion

In chicks made myopic in only half of the eye by having previously worn diffusers that covered half of the visual field, the eyes develop expanded choroids only in the previously deprived myopic segment (Fig. 5). Surprisingly, the three-fold choroidal expansion seems comparable in magnitude to that seen if the entire eye was myopic [0.4 mm expansion in locally myopic (Fig. 5, bottom) vs 0.5 mm expansion in globally myopic (Fig. 2)], although precise comparisons are not possible because the ages of the birds differed, and thus, by measuring the locally myopic group at 1 week of recovery, we may not have captured the peak of choroidal expansion.

To test whether the local choroidal expansion was statistically significant we assessed the degree of

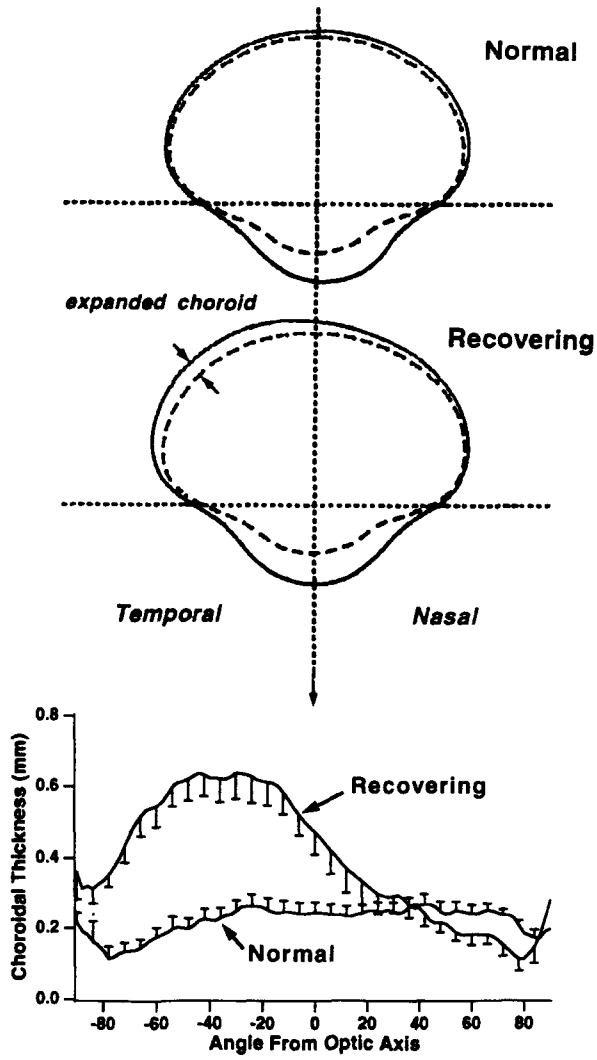

FIGURE 5. Averaged digitized tracings of photographs from above of hemisected eyes [as in Fig. 1(a)] in which the temporal retina of one set of eyes (recovering) had been deprived of form-vision by partial diffusers for 2 weeks from hatching, leaving the deprived half of the eye myopic. The diffusers were then removed and the birds allowed to recover for 2 weeks. The inner contour of the sclera (solid line) and the retinal pigment epithelium (dashed line) delimit the choroid. The choroidal thickening (arrows) is limited to the myopic half of the eye. The graph (lower panel) shows the thickness of the choroid as a function of the angle from the axis ("optic axis") that in normal eyes is the axis of symmetry. SEs are shown as downward error bars for the recovering eyes and as upward error bars for the fellow control eyes ($n = 7$ pairs of eyes).

asymmetry of each eye and compared the locally myopic and normal eyes. To do this, we measured for each eye, at 2 deg intervals, the distances both to the sclera and to the retinal pigment epithelium from the origin of the coordinate system described in the Methods. We then divided each measurement on the temporal (previously deprived) side of the eye by the corresponding measurement on the nasal (non-deprived) side. Ratios >1 indicate that the temporal side is longer than the nasal side. After 1 week of recovery, the mean ratio for the measurements to the sclera was significantly larger (1.08) than the mean ratio for the retina (1.03; significantly different by t-test, $P < 0.05$, $n = 7$), indicating that the retina had been pushed forward into a more symmetric shape by the local expansion of the choroid. The local choroidal expansion presumably contributes to the very rapid recovery from partial deprivation myopia observed by Wallman and Adams (1987), and strengthens our hypothesis that the choroidal changes in thickness are in response to image defocus.

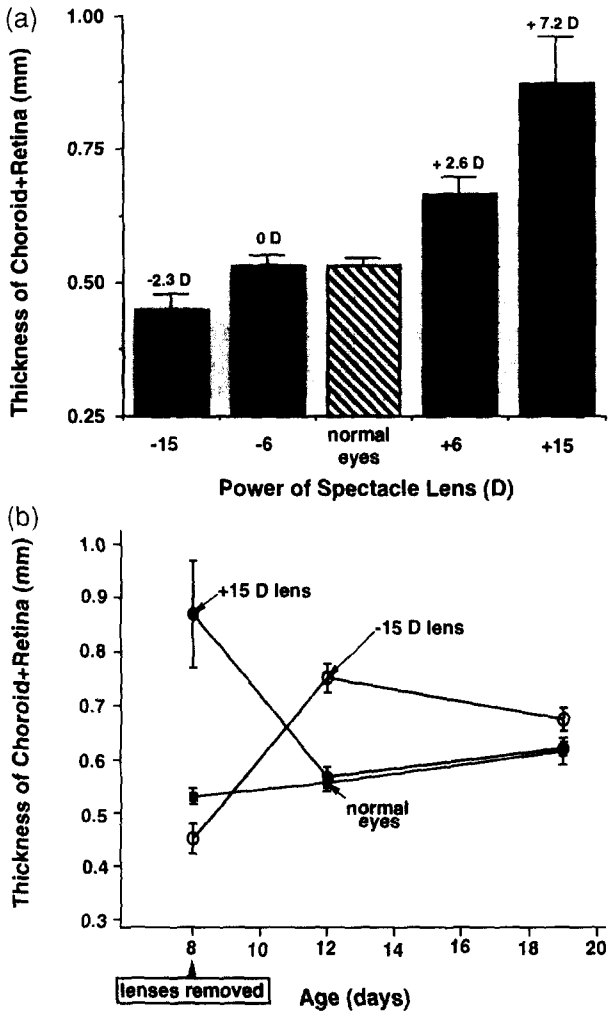

FIGURE 6. Effect of 4.5 days of monocular spectacle lens wear beginning at 3 days of age on the thickness of "choroid + retina", assessed by A-scan ultrasonography in anesthetized eyes under cycloplegia. (a) Thickness of "choroid + retina", plotted as a function of lens power ($n = 6$–7 in all cases), is shown to be related to the optical power of the lens worn. The middle bar shows all the untreated fellow eyes ($n = 23$). The numbers above the bars are estimates of the amount of refractive error attributable to the difference in choroidal thickness between the lens-treated and fellow control eyes. (b) Effect of removal of lenses on subsequent thickness of "choroid + retina". The eyes previously made functionally myopic by wearing plus lenses are hyperopic when the lenses are removed; their choroids now become thinner. Those previously with minus lenses are myopic without the lenses; their choroids become thicker. All error bars are SEs.

Eyes made myopic or hyperopic with spectacle lenses show compensatory changes in choroidal thickness

The three results presented up to this point suggest that the choroidal expansion occurring when the diffusers are removed is a response to myopic blur. To test this hypothesis more directly, we used positive spectacle lenses to produce myopic defocus in normal eyes (by adding to the optical power of the eye, these lenses cause images to be focused in front of the retina, as occurs in myopia); in addition we used negative spectacle lenses to produce equivalent amounts of hyperopic defocus (images focused behind the retina). In eyes with myopic defocus, the choroid expands within days, pushing the retina forward, thereby partially correcting the imposed myopia. Conversely, in eyes with hyperopic defocus the choroid thins, pulling the retina back toward the sclera, again partially correcting the imposed refractive error. We analyzed these data in three ways: First, analysis of variance showed that the lens power accounts for a significant proportion of the variance in choroidal thickness [one-way ANOVA comparing lens-treated eyes: $F(4,46) = 19$, $P < 0.0001$]. Second, correlating the choroidal thickness with the optical power of the spectacle lenses showed a strong relationship ($r = 0.80$, $P < 0.001$, d.f. $= 23$). Finally, to determine whether the lens-treated eyes in the positive-lens and negative-lens groups were each different from their fellow untreated eyes, we tested the interocular difference in choroidal thickness and found it to be significantly different from zero in each group (positive lens birds, $n = 11$, mean difference $= 236\ \mu$m, $t = 3.57$, $P = 0.005$; negative lens birds, $n = 13$, mean difference $= -56\ \mu$m. $t = 2.17$, $P = 0.05$).

Using the same algorithms used above to estimate refractive change attributable to choroidal change, we find that for eyes with +15 D lenses, the choroidal expansion reduces the imposed myopia by 7.2 D; in contrast, for eyes with −15 D lenses, the choroidal thinning reduces the imposed hyperopia by 2.3 D [Fig. 6(a)]. (These estimates are offered with the caveat that if the spectacle lenses changed the retinal thickness, this would confound our estimates.) Positive lenses induce larger thickness changes than negative lenses, presumably because the choroid can expand much more than it can thin.

After the lenses are removed, the type of defocus the eyes experience is reversed: those eyes previously wearing positive lenses, having partially compensated for the induced myopia, now find themselves hyperopic. This produces a rapid thinning of the choroid, which partially corrects the hyperopia [Fig. 6(b)]. Conversely, those eyes

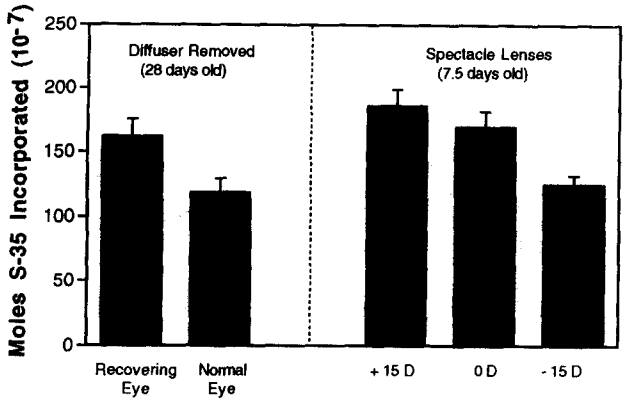

FIGURE 7. Incorporation of $^{35}SO_4$ into proteoglycans in 6 mm punches of posterior choroid. Left: choroids from recovering and normal eyes of chicks ($n = 7$) given 7 days of normal vision following 21 days of visual deprivation in one eye. Right: choroids from chicks wearing either positive ($n = 10$), negative ($n = 9$) or plano ($n = 4$) spectacle lenses for 4.5 days beginning at 3 days of age. Error bars are SEs.

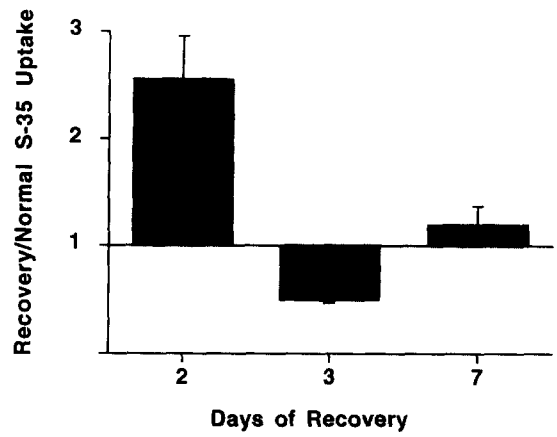

FIGURE 8. Incorporation of $^{35}SO_4$ into proteoglycans in 6 mm punches of posterior sclera at three intervals after diffusers were removed from eyes. Values plotted are means of the ratios of experimental and fellow control eyes. Note that incorporation decreases but only after 3 days. Error bars are SEs ($n = 12$ for 2 days, 20 for 3 days, 11 for 7 days).

previously with minus lenses now find themselves myopic and their choroids thicken. The choroidal thickening and thinning produced by positive and negative lenses respectively, together with the opposite changes after the lenses are removed, constitute the strongest evidence that the sign (myopic or hyperopic) and degree of defocus determine choroidal thickness.

Proteoglycan synthesis

Uptake of radioactive sulfate into proteoglycans is increased in thickened choroids and decreased in thinned choroids. The choroids from eyes recovering from deprivation myopia show significantly higher incorporation of sulfate than choroids from fellow normal eyes (Fig. 7 left; paired t-test comparing previously deprived and normal eyes, $P = 0.02$). Choroids from eyes wearing +15 D spectacle lenses show higher incorporation than those wearing 0 D lenses, while those wearing −15 D lenses show lower incorporation [Fig. 7 right; one-way ANOVA comparing the three lens treatment groups, $F(2,20) = 8.5$, $P < 0.01$; all three groups are significantly different from each other by Tukey's test, $P < 0.05$].* Modulation of choroidal thickness may be controlled at least partially by regulating the synthesis of these large, osmotically active extracellular matrix molecules.

*To confirm that the labeled sulfate is incoporated into proteoglycans, we sent labeled choroids from birds that had worn +15 and −15 D lenses to J. Rada of University of Pittsburgh for further analysis: chromatography using a sepharose CL6B column after extraction with 4 M guanidine indicated the presence of molecules of approximately the size of decorin and another larger proteoglycan. Similar results to those obtained by incorporation of labeled sulfate were obtained by incorporation of labeled glucosamine. Either method provides only an approximate estimate of net proteoglycan synthesis as we did not measure the rate of turnover of the choroidal proteoglycans during the incubation period; in the sclera, however, the turnover is quite low (data not shown). An additional complication of the sulfate-uptake method is that variations in the degree of sulfation of proteoglycans can influence the amount of incorporation measured.

We, like others, have found that changes in ocular length are associated with changes in scleral proteoglycan synthesis (Rada *et al.*, 1991; Nickla *et al.*, 1992). In eyes recovering from deprivation myopia, proteoglycan synthesis decreases compared to fellow normal eyes, but only after a lag of several days (Fig. 8); a one way ANOVA for repeated measures (treated eye − control eye differences compared) shows the time factor was significant [$F(2,40) = 24.3$, $P < 0.001$]; both day 3 and day 7 time points are significantly different from day 2 by Tukey's test ($P < 0.05$). Similar findings were reported by Rada *et al.* (1992). The biochemical change in the recovering sclera parallels that of the anatomical one; the reduction in ocular elongation occurs only after several days (Figs 2 and 3).

Electron microscopy

In preliminary experiments, we find that the choroid contains elongated, non-vascular smooth muscle that is immunoreactive for smooth muscle actin (Fig. 9). This confirms earlier reports of smooth muscle cells in the choroid (Walls, 1942; Meriney & Pilar, 1987).

DISCUSSION

We have presented six lines of evidence arguing that modulation of choroidal thickness is a response to optical defocus in which the choroid becomes thicker with myopic defocus (image in front of retina), thereby pushing the retina forward toward the image plane, and thinner with hyperopic defocus (image behind retina), thereby pulling the retina back, once again toward the image plane. These lines of evidence are: (1) when vision is restored after visual deprivation, the choroids of the myopic eyes rapidly increase several-fold in thickness, ameliorating the myopia; (2) as the myopia diminishes further because of a decreased rate of ocular elongation combined with continued growth of the cornea and lens, the choroid thins back to normal; in older eyes, which

FIGURE 9. Electron micrograph of choroidal cells labeled with antibodies to smooth muscle actin, showing long fibers not associated with blood vessels. M, muscle cell; F, fibroblast; C, collagen.

remain longer than normal and myopic, the choroid remains expanded; (3) if the previously deprived, myopic eyes are given vision under dim illumination, in which visual cues are attenuated, no choroidal expansion occurs and the eyes remain myopic; (4) eyes made locally myopic by local deprivation develop choroidal expansion only in the previously deprived region; (5) eyes made functionally myopic with positive spectacle lenses develop thickened choroids, while those made functionally hyperopic develop thinned choroids; and (6)

when the lenses are removed, and the sign of the refractive error is thus reversed, the thickened choroids now become thin, and the thin ones now expand.

Although there is some cause for skepticism about how accurately one can measure the real-life thickness of a blood-filled tissue like the choroid, we have confirmed the basic phenomenon of choroidal expansion by six methods (Fig. 1): hemisected frozen eyes, thick sections of the posterior wall of fixed eyes, histological sections of plastic-embedded tissue, high-frequency ultrasound imaging of the posterior eye wall (B-scan), and low- and high-frequency ultrasound measurement of the axial spacing of ocular components (A-scan). These methods are complementary: the ultrasound measurements, being made in live animals, best reflect the actual state of the choroid, but it is difficult to be certain which reflecting layer is responsible for which echo; the photographs of sections substantiate that the shifts in the ultrasound peaks are due to changes in choroidal thickness.

The existence of a choroidal focusing mechanism was hypothesized more than 50 yr ago by Gordon Walls (1942). Two papers since then have reported choroidal changes that we interpret as being the same phenomenon reported here, i.e. thickened choroids in the eyes of chicks made myopic and then permitted normal vision. However, the adaptive nature of the choroidal response was not then appreciated (Harrison & McGinnis, 1967; Hayes, Fitzke, Hodos & Holden, 1986).

Does the choroid play a role in control of eye growth?

Is the modulation of choroidal thickness involved in emmetropization—the growth of the eye toward emmetropia from myopia or hyperopia? One possibility is that the choroid itself mediates the scleral response. For example, if a thicker choroid provides a greater diffusional barrier to a stimulatory growth factor secreted by the retina or retinal pigment epithelium, or if it affords greater protection from stretching of the sclera by the intraocular pressure (Van Alphen, 1961, 1986), then scleral growth might decrease after the choroid becomes thicker in myopic eyes. Alternatively, the choroidal response may constitute another blur-reducing feedback circuit in parallel with the one that adjusts ocular elongation toward emmetropia (Schaeffel & Howland, 1991; Wallman, 1991), perhaps using the same visual cues. These two circuits would therefore also be in parallel with the accommodation feedback circuit, which acts to reduce blur as well.

Choroidal responses may also improve the dynamics of the emmetropization system by preventing the rapidly growing eye from overshooting emmetropia when correcting myopia or hyperopia. This overshoot could potentially occur because there seems to be a lag period between changes in defocus and changes in scleral growth rate, as shown by the fact that when normal vision is restored to previously deprived myopic eyes, the posterior sclera continues to grow faster than normal for several days, whether measured by changes in vitreous chamber depth at the sclera (Figs 2 and 3), or by incorporation of sulfate into scleral proteoglycans (Fig. 8; Rada *et al.*, 1992). In the case of hyperopic eyes, for example, continued growth during this lag period would cause them to grow past their appropriate eye length and become myopic. The rapid thinning of the choroid in these hyperopic eyes would result in emmetropia—and hence the initiation of decreased growth—before the eye reaches its appropriate length. This would, in effect, anticipate the lag period, and prevent any growth overshoot.

Age-dependence of choroidal response

We found that both the choroidal expansion after visual deprivation and the subsequent thinning as deprivation myopia declined were more rapid in younger eyes. Might choroidal modulation be limited to early postnatal life, as appropriate for a mechanism in the service of emmetropization? The more rapid expansion in younger animals suggests this is so; the slower choroidal thinning in older animals may be in compensation for a slower action of the scleral recovery mechanism in older animals. Specifically, because the scleral recovery mechanism can not directly make the eye less myopic (the eye presumably cannot shrink), but can only stop its elongation, the recovery results from the focal length of the eye's optics increasing as the cornea (and perhaps the lens) continues to flatten. Thus, the maximum rate of recovery by decreased scleral growth (i.e. with ocular elongation completely halted) depends mostly on the rate of corneal flattening. Because this declines with age (Wallman & Adams, 1987; Troilo & Wallman, 1991), so too would the rate of recovery attributable to the scleral mechanism. Thus, if the choroid returns to normal thickness only when the myopia is eliminated by the scleral mechanism, one would expect this thinning to occur more slowly, if at all, in the older animals. Myopic adult chickens can maintain thickened choroids for years (Harrison & McGinnis, 1967).

Mechanisms of choroidal expansion

The mechanism underlying the modulation of choroidal thickness is unknown. We put forward three possibilities. First, increases in thickness might be achieved by increasing the amount of highly charged proteoglycans in the choroidal extracellular matrix, thereby causing water to enter and the choroid to swell (Myers, Armstrong & Mow, 1984). This is supported by the finding that expanded choroids have a higher rate of sulfate incorporation into proteoglycans than do choroids of normal eyes (Fig. 7), although we do not yet know whether an increase in proteoglycan synthesis of this magnitude is sufficient to account for the increase in choroidal thickness.

Alternatively, a thicker choroid might be produced by an increase in the degree of fenestration of the capillaries of the choriocapillaris, permitting increased entry of large osmotically active molecules into the extracellular space. By controlling the concentration of such

molecules in the choroid, the retinal pigment epithelium, which is thought to regulate the number of pores in the adjacent choriocapillaris (Korte, Burns & Bellhorn, 1989), could determine choroidal thickness.

A third possibility is that modulation of choroidal thickness may involve changes in the amount of aqueous humor that leaves the eye by each of the two outflow pathways—the direct drainage into the Canal of Schlemm, and the indirect uveoscleral pathway. We have preliminary evidence that the lacunae of the choroid are connected both to the anterior chamber (we found horseradish peroxidase in the lacunae of the anterior choroid 4 hr after its injection into the anterior chamber) and to the vasculature (we frequently see blood cells in the lacunae post-mortem). Perhaps the eye can adjust the relative resistance of the two outflow pathways, thereby shunting varying amounts of fluid to the choroid.

Whatever the basic mechanism of modulation of choroidal thickness, a possible contributing factor is the non-vascular smooth muscle that straddles the chick choroid (Fig. 9). The degree of contraction of this smooth muscle could influence choroidal thickness. Thus, localized choroidal thickening, like that shown in Fig. 5, may reflect local differences in muscle tone. A similar suggestion was made by Walls (1942).

Conceivably, changes in choroidal expansion may also be related to changes in choroidal bloodflow. There is evidence in birds that choroidal bloodflow is controlled by the Edinger–Westphal nucleus (Fitzgerald, Vana & Reiner, 1990), the source of the preganglionic fibers to the ciliary ganglion, and that deprivation of form vision causes drastic reductions in choroidal bloodflow (Reiner, Fitzgerald & Hodos, 1991). However, in preliminary experiments, we find that ciliary ganglionectomy does not prevent choroidal thickening in eyes recovering from myopia.

Experimental and clinical implications

The evidence just presented that choroidal thickness depends on the refractive status of the eye forces the reexamination of the results of many studies that assumed, reasonably enough, either (i) that refractive status is a function only of ocular length and the focal length of the eye's optics, or (ii) that vitreous chamber length can only be modulated by changes in the length of the eye, and thus that ultrasound and caliper measurements are essentially equivalent measures of eye length. In particular, many studies of animal eyes showing either compensation for spectacle lenses, recovery from ametropias, or drug effects on eye growth should now be reexamined to separate the effects of choroidal and scleral changes.

More importantly, these results imply the existence of a choroidal compensatory mechanism that is sensitive to retinal image defocus. This mechanism appears to act locally within the eye. First, as shown here, when normal vision is restored to an eye made myopic in half of the eye, the choroidal expansion is restricted to that region. Second, we find that optic nerve section does not interfere with choroidal thickening, either during recovery from myopia induced by partial form-deprivation (Xu, 1992) or in the presence of ametropias induced by spectacles (Wildsoet & Wallman, 1992). Therefore, local visual cues can determine local choroidal thickness just as local deprivation cues determine local ocular elongation.

Do similar choroidal changes occur in humans? If so, several observations would require reinterpretation. For example, the clinical observation that optically correcting hyperopia leads to a small increase in measured hyperopia (Borish, 1970) might be due, at least in part, to a previously thinned choroid expanding once the ametropia is corrected. It is also claimed that giving spectacles to myopes aggravates their myopia (Duke-Elder & Abrams, 1970; Garner, 1983; Medina, 1987); choroidal changes in the opposite direction might be the basis for such an effect as well. Furthermore, studies in which prolonged close vision was interpreted as leading to increased tonus of accommodation could alternatively be explained by the choroid thinning in response to the functional hyperopia present during close viewing (the image being focused behind the retina). In humans, the magnitude of the refractive effects of choroidal changes would almost certainly be much smaller than in chicks because the larger eye size (and greater focal length) results in a proportionally smaller refractive effect of a given amount of choroidal expansion.

In conclusion, we have shown that the growing chick eye can change the position of the retina relative to the eye's plane of focus by modulating the thickness of the choroid, a response intermediate in speed between ocular accommodation and ocular elongation. It seems remarkable that even a local region of the retina can infer the sign of the optical defocus and use it to adjust choroidal thickness to bring images into focus. How general this phenomenon is across species and what its biophysical mechanism might be are as yet unknown.

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Acknowledgements—We are grateful to Dr Ronald Silverman of Cornell University School of Medicine for the high-frequency ultrasound measurements. This research was funded by NIH EY02727 to JW and NHMRC 880904 to CW.