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## STRUCTURE AND PHYSIOLOGY OF THE EYE LENS

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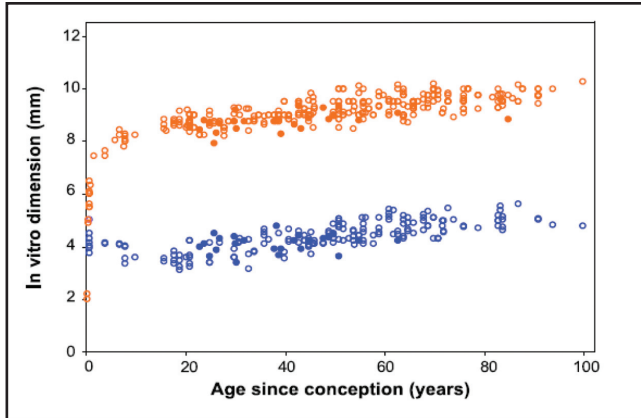
The eye lens functions as an optical element as well as a biological monitor of growth, aging, and of pathologies, both ocular and systemic. Its functional properties, provision of refractive power, alteration of that power with accommodation, and the maintenance of transparency are determined by the structural features and their organization. This chapter discusses lens structure and physiology, how this may be related to function, and changes that occur with age.

### LENS GROWTH

The human eye lens is a biconvex structure with an anterior surface that is less curved than its posterior counterpart. The newborn human lens has an equatorial diameter of approximately 6 mm and a sagittal thickness of 4 mm.<sup>1</sup> Lens growth continues throughout life, albeit at a reduced rate in adult years, to an equatorial diameter between 9.5 and 10 mm and a sagittal thickness of approximately 5.5 mm,<sup>1</sup> as shown in Figure 7-1. Lens biometric parameters, however, must be put into the context of whether they are measured *in vivo* or *in vitro*. In the former, the lens is under tension with equatorial and sagittal dimensions depending on accommodative state, whereas in the latter (*in vitro*), the lens is thought to assume its most relaxed state. It remains uncertain as to what exact state of accommodation this

corresponds. The curvature of the lens increases with age, as would be expected given that the lens continues to grow well after the growth of the eyeball has ceased, thereby preventing expansion in the equatorial direction. It should be noted that studies of lens shape that are conducted on lenses removed from the eye cannot give sufficient information about age-related changes because the *in vivo* shape depends also on the ciliary muscle force and on zonular tension.

The geometry of the lens and its relation to the zonule and ciliary muscle alters with age and was observed by Farnsworth and Shyne<sup>2</sup> as an anterior “shift” of the zonule with age. The zonular insertions clearly cannot detach and then reattach themselves at a more anteriorly placed point on the capsule, thus this apparent shift must be caused by the growth mode. The differentiated lens fiber cells originate from epithelial cells that line the anterior capsule, and it has been hypothesized that the epithelial cells migrate a given distance toward the equator before differentiating.<sup>3</sup> This would gradually shift the point at which fiber cell differentiation starts—more anteriorly—altering the geometry of the lens and associated tissue and leading to an apparent anterior shift of the zonule, making it more difficult to alter the shape of the lens with age.<sup>3</sup> The capsule under the anterior zonular insertions has been found to be a region of local thinning with the maximum thickness of the capsule occurring between these insertions and the anterior pole (ie, the midperiphery).<sup>4</sup> The capsule increases in thickness with age



**Figure 7-1.** Dimensions (mm) of in vitro human lens equatorial diameters (orange) and sagittal thicknesses (blue) plotted against age since conception (years). (Reprinted from *Exp Eye Research*, vol 90(6), Augusteyn RC, On the growth and internal structure of the human lens, pp 643-654, Copyright 2010, with permission from Elsevier.)

at the pole; and some increase in thickness, followed by a plateauing around the sixth to seventh decade, is found in the midperiphery.<sup>4</sup> The capsule is thinnest at the posterior pole and this, together with the capsular thickness at the equator, does not alter with age.<sup>4</sup>

An intriguing aspect of lenticular growth and physiology are features seen when looking into a live eye using a clinical biomicroscope. These features appear to be lamellar demarcations of certain points during growth and have been termed the *zones of discontinuity*. Although the zones must scatter or reflect light (otherwise they would not be visible to the clinician), they do not affect vision, and their function or significance remains unclear, as does the contribution they may make to accommodation. They do not appear to be caused by local changes in the refractive index gradient. Fiber optic sensing, which permits measurement of refractive index at any point in the lens, did not show any evidence of small peaks or dips in the refractive index gradient.<sup>5</sup> These zones have thus far been reported only in the human lens, which is an accommodating lens, and their structural nature requires further investigation.

## STRUCTURAL PROTEINS: THE LENS CRYSTALLINS

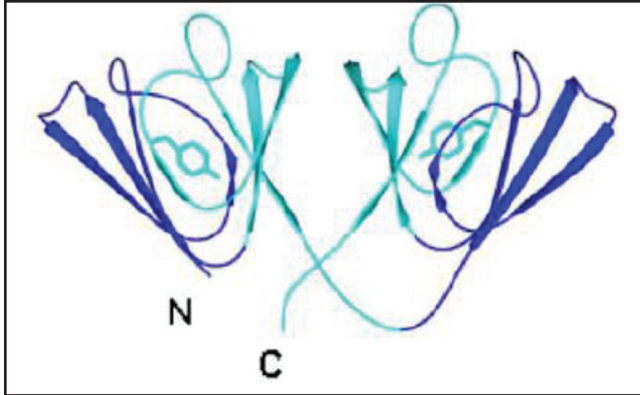
The major structural entities of the lens are the crystallin proteins, which comprise approximately 35% of the wet weight of the human lens and were classified for vertebrates in 1893 by Morner<sup>6</sup> into 3 major

classes:  $\alpha$ -,  $\beta$ - and  $\gamma$ -crystallins, based on physico-chemical properties. The most common differential feature is molecular weight or size, with  $\alpha$ -crystallin being the largest protein class and  $\gamma$ -crystallin being the smallest. Subsequently, structural similarities between the  $\beta$ - and  $\gamma$ -crystallins have led to these protein classes being considered part of a larger “superfamily.” A recent classification of the major crystallins that are expressed in the mammalian lens divides them into 16 subgroups— $\alpha$ -crystallins:  $\alpha A$  and  $\alpha B$ ;  $\beta$ -crystallins:  $\beta B1$ ,  $\beta B2$ ,  $\beta B3$ ,  $\beta A1/A3$ ,  $\beta A2$ ,  $\beta A4$ ; and  $\gamma$ -crystallins:  $\gamma A$ ,  $\gamma B$ ,  $\gamma C$ ,  $\gamma$ ,  $\gamma D$ ,  $\gamma E$ ,  $\gamma F$ ,  $\gamma S$ .<sup>7</sup>

The most abundant of the crystallin classes in the human lens are the  $\alpha$ -crystallins. This class of proteins has been shown to possess heat shock properties and has a chaperone function,<sup>8</sup> protecting the lens when it is exposed to conditions of stress such as temperature elevation, exposure to oxidation, or heavy metals. This protective role is thought to be exercised by  $\alpha$ -crystallins binding with sections of the smaller protein classes, which may have started to unfold when exposed to stress factors, thereby preventing the formation of aggregates<sup>8</sup> that would scatter light and manifest as opacities. Whether chaperone activity in response to laboratory-induced stresses applied to nonhuman cells (to many of which the lens in the living human eye would never be exposed) can truly be replicated in the aging human lens cannot be said with certainty.

It has been suggested that  $\alpha$ -crystallins have a role in inhibiting apoptosis (cell death), in lens cell development and regulation of the cell cycle, and in differentiation.<sup>9</sup>  $\alpha B$ -crystallin has also been found in other tissues of the body, is induced in situations of oxidative stress,<sup>9</sup> and is associated with a number of neurological and muscular disorders; but, surprisingly, few associations have been made between  $\alpha B$ -crystallin and lens aging conditions in humans.<sup>6</sup>

Far less is known about the functions of  $\beta$ - and  $\gamma$ -crystallins than about possible functions of  $\alpha$ -crystallin. The  $\beta$ - and  $\gamma$ -crystallins share similarities in secondary structure (antiparallel  $\beta$  sheets), which resembles the motif found on ancient Greek vases and is referred to as the “Greek key” (Figure 7-2).<sup>7</sup> The functional significance of a Greek key motif in these proteins, however, is not clear. It has been suggested that this structural form assists with packing of the proteins to minimize light scatter, and it has also been shown that a number of proteins with this secondary structure bind  $Ca^{2+}$  ions.<sup>5</sup> Because  $Ca^{2+}$  has been linked with cataract formation, this property may be important in maintaining optical integrity.<sup>6</sup>



**Figure 7-2.** Monomeric  $\gamma$ -crystallin showing the Greek key motif. N and C indicate the amino (NH<sub>2</sub>) and carboxyl ends (COOH), respectively. (Reprinted from *Prog Biophys Mol Bioly*, vol 86(3), Bloemendal H, de Jong W, Jaenicke R, Lubsen NH, Slingsby C, Tardieu A, Ageing and vision: structure, stability and function of lens crystallins, pp 407-485, Copyright 2004, with permission from Elsevier.)

The  $\beta$ -crystallins can be separated into the acidic ( $\beta$ A1/A3,  $\beta$ A2,  $\beta$ A4) and basic ( $\beta$ B1,  $\beta$ B2,  $\beta$ B3) groups.<sup>7</sup> In the mammalian lens,  $\beta$ -crystallins are expressed in fiber cells, and in the human lens this expression increases throughout life.<sup>6</sup> Species variations in the types of  $\beta$ -crystallins are found in the lens, and it has been suggested that this may be linked to lens water content,<sup>7</sup> hence having a functional correlation with the refractive index.

The  $\gamma$ -crystallins, like the  $\beta$ -crystallins, are specific to lens fiber cells and are the last of the crystallins to be synthesized during the differentiation of epithelial cells to fiber cells.<sup>7</sup> In the human lens, this is the least abundant protein class, with  $\gamma$ C and  $\gamma$ D-crystallins constituting 81% of this class of proteins in the embryonic human lens, and by the age of 10 years, only  $\gamma$ D are detectable.<sup>6</sup> Although  $\gamma$ -crystallins are low in abundance, they have the highest specific refractive increment (a measure of how much the refractive index increases with an increase in concentration) of all the protein classes.<sup>10</sup> Lenses with higher water content, hence a greater capacity to accommodate, have a relatively lower content of  $\gamma$ -crystallins; the human lens has little  $\gamma$ -crystallin beyond the nuclear region.

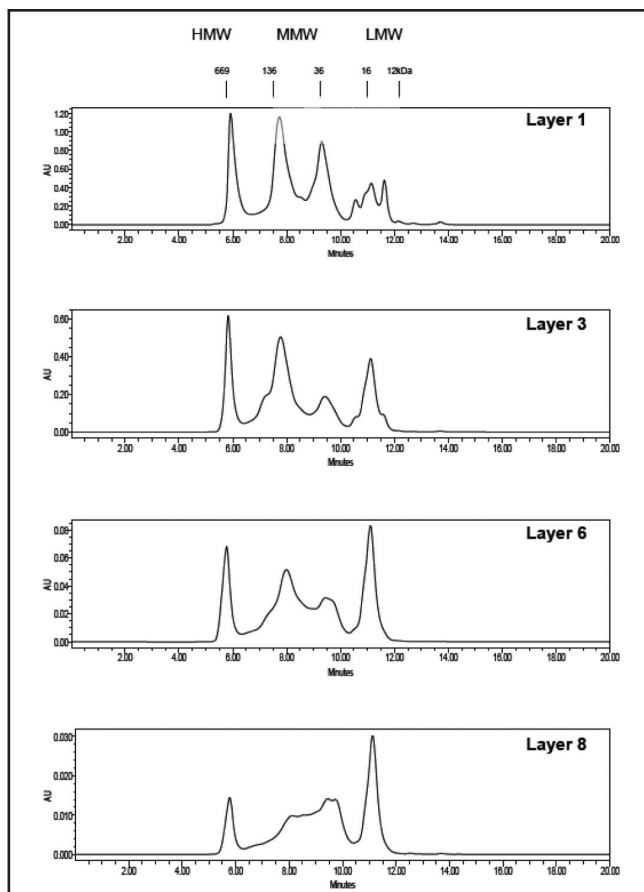
## CRYSTALLIN DISTRIBUTIONS AND FUNCTIONAL CORRELATES

The crystallin proteins, and their concentrations and individual distribution patterns, are linked to the

optical parameters. The crystallin protein distributions and concentrations determine the magnitude of the refractive index at localized points and the form of the index gradient across the lens. The more fundamental and far more complex structure/function link of the crystallin proteins is related to the maintenance of transparency with age and with the internal displacements that occur when the lens accommodates.

An understanding of how structural entities may relate to function and how this relationship is altered with age can be gleaned by following the growth mode of the lens and by considering interspecies comparisons. Because the lens grows by accrual of tissue on its surface, with no concomitant cell or protein losses, fractionation of the lens into layers from periphery to lens center separates the lens into tissue fractions laid down at different stages of lens development and life of the animal.<sup>11,12</sup> The classes of crystallin proteins and their relative proportions within a lens depend on the species. Even within lenses that contain similar types of crystallins, distribution patterns of proteins across lenses can vary greatly. An interspecies comparison in Figures 7-3 and 7-4 shows chromatographic separations of the protein classes on the basis of molecular weight from the outermost (peripheral) and the innermost (central) layers of a porcine lens (see Figure 7-3)<sup>11</sup> and a toad lens (see Figure 7-4).<sup>12</sup> In the porcine lens, the most recently synthesized fractions in layer 1 show that there is a single peak eluting high molecular weight (HMW) proteins and 2 peaks of middle molecular weight (MMW) proteins (which represent  $\alpha$  and  $\beta$ -crystallins, respectively). In layer 1 of the toad lens (see Figure 7-3),<sup>11</sup> there are 3 peaks in each of the HMW and MMW range, and there is a greater proportion of low molecular weight (LMW) proteins than in the porcine lens. From the outermost to innermost layers, the proportion of LMW proteins increases. Comparing the innermost layer (layer 8 in the porcine lens and layer 9 in the toad lens), the soluble protein in the toad lens center (see Figure 7-4)<sup>12</sup> is comprised exclusively of a LMW protein identified as  $\gamma$ -crystallin,<sup>12</sup> whereas in the porcine lens, significant amounts of the other protein classes are present (see Figure 7-3).<sup>11</sup>

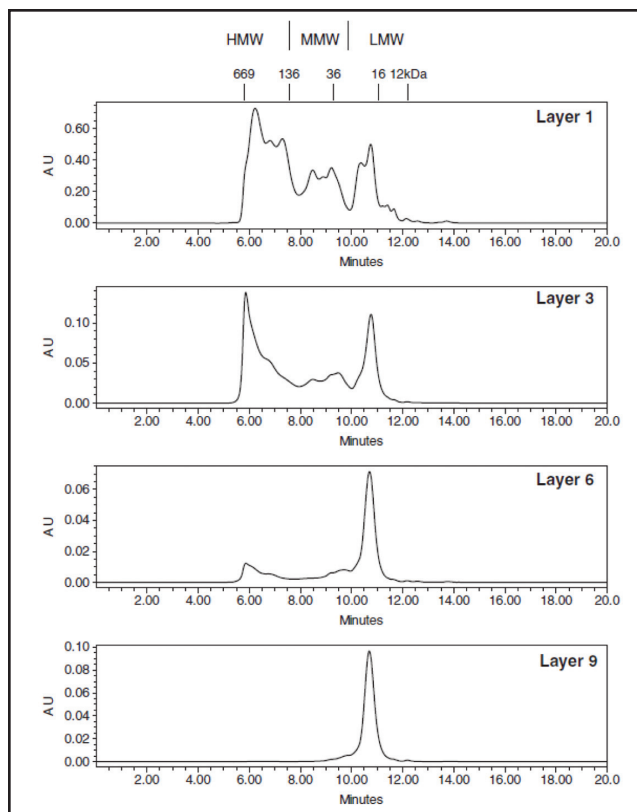
Despite such wide variations in protein distribution patterns, most lenses have a refractive index gradient shape that can be approximated to a second-order polynomial, with the exception of the human lens,<sup>5</sup> and all healthy lenses from any species are transparent. Hence, there are no particular sets of crystallin distribution patterns that are unique for the



**Figure 7-3.** Chromatographic profiles of protein distributions from layered sections of a porcine (*Sus scrofa domestica*) lens from the outermost (layer 1) to the innermost (layer 8) sections. The high, middle, and low molecular weight peaks (HMW, MMW, and LMW, respectively) show elution of the different crystallins. Molecular weight is given in kilodaltons (kDa). (Reprinted with permission from Keenan J, Orr DF, Pierscionek BK. Patterns of protein distribution in porcine eye lenses. *Mol Vis.* 2008;14(146-147):1245-1253.)

formation of the refractive index gradient or any specific crystallin proportions required to maintain transparency. There is, however, a link between lens hardness and  $\gamma$ -crystallin content, and this is significant with respect to accommodative function.

The refractive index of the human lens increases across the cortex to a maximum between 1.39 and 1.41 (for wavelengths of 633 to 652 nm), which is constant throughout the nucleus.<sup>5</sup> Inconsistency exists in the literature regarding age-related changes in the nuclear refractive index. Increases and decreases with age in the water content of the nucleus have been reported.<sup>5</sup> Changes in water content will result in changes in refractive index. The discrepancies between findings are likely to reflect differences in tissue handling as well as in techniques used to



**Figure 7-4.** Chromatographic profiles of protein distributions from layered sections of a toad (*Bufo gargarizans*) lens from the outermost (layer 1) to the innermost (layer 9) sections. The high, middle and low molecular weight peaks (HMW, MMW, and LMW, respectively) show elution of the different crystallins. Molecular weight is given in kilodaltons (kDa). (Keenan J, Elia G, Dunn MJ, Orr DF, Pierscionek BK. Crystallin distribution patterns in concentric layers from toad eye lenses. *Proteomics.* 2009;9(23):5340-5349. Copyright John Wiley and Sons. Reproduced with permission.)

determine the refractive index and suggest that interpretations of findings need to be reviewed. The most recent studies fail to recognize well-established work in the biochemical literature in which proportions of protein and water were carefully measured, and no change in overall protein/water proportions in the adult lens with age was found.<sup>13</sup> If indeed there were any changes in nuclear refractive index with age, these would not have a significant effect on refraction because the nucleus, unlike the cortex, has no index gradient. Hence, they could not compensate for the curvature increases with age to prevent myopia. If there was no such compensatory mechanism, there would be no presbyopia. The older eyeball would have a progressively more curved lens, with no compensatory decrease in refractive index, which would add to the power of the eye. This would result in an increase

in myopia with age rather than the ubiquitous loss of functional capacity to focus on near objects that is experienced from the fifth decade of life.

## AGE-RELATED CHANGES

Aging of the lens leads to 2 clinical manifestations—the loss of accommodative capacity (presbyopia) and a decrease in transmittance, which, if coupled with excessive light attenuation in the form of either scatter or absorption that detrimentally affects vision, causes cataract. Because both presbyopia and cataract are age-related, it may be considered that the latter is an acceleration of the former and that the changes in lens function leading to presbyopia, if continued, may lead to cataract. If that were to be the case, and because little is known about the links between protein changes and accommodation, early senile cataract may be a structural model for presbyopia development. Cataract is a protein-folding disease, with chaperone function and heat-shock capacities decreasing with age.<sup>7,8</sup> It is notable, however, that in species where the center of the lens is almost entirely  $\gamma$ -crystallin and hence cannot avail of the chaperone protection of  $\alpha$ -crystallin, lenses can retain transparency even under the imposition of heat-induced stress.<sup>7</sup> Such lenses are also unable to accommodate as the tight packing of  $\gamma$ -crystallin renders the lens rigid. The human lens accommodates and needs to withstand external and internal stresses for a longer period than most animal lenses because the human lifespan is comparatively greater. Hence, it should be noted that for a number of human conditions and pathologies, especially those that are chronic or age-related, animal models can be of limited use. Genetic manipulations may have some relevance to genetic conditions, but with respect to the aging of the lens, the most common type of cataract is senile cataract and the ubiquitous manifestation of aging—presbyopia—is not represented accurately in any animal model. Aging of the lens is a conglomeration of exposure to lifestyle and environmental factors (to many of which no laboratory animal will ever be exposed) over decades, which is far longer than any laboratory animal could survive. Age-related changes in the human lens to the crystallin proteins and to their environment within the lens require more epidemiological data and investigation of the environmental and lifestyle factors.

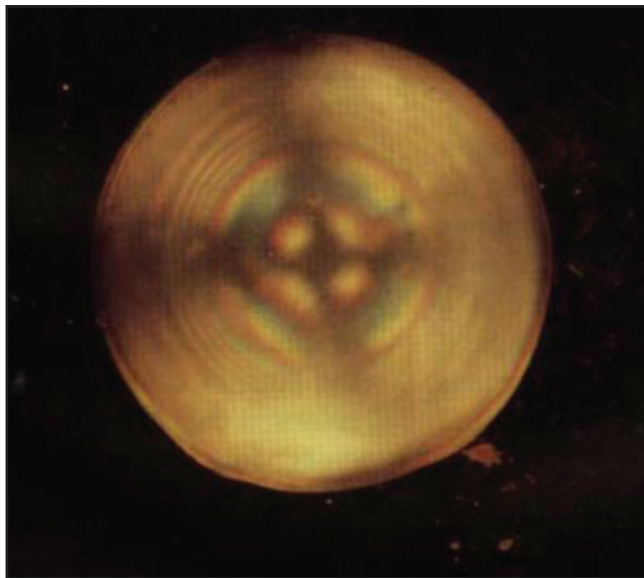
When crystallin proteins are extracted from young lenses, most are water-soluble. With age, an increasing

amount of extracted crystallins is water-insoluble, and this has been thought to be indicative of increased aggregation in the living lens that eventually leads to cataract. It has been shown that even a very old lens—from which up to 80% of the proteins extracted were water-insoluble—retained its transparency.<sup>14</sup> It is most likely that older proteins, which may retain the structural features that support transparency within the lens, become more vulnerable to insolubilization when removed from their cellular environment. This may also provide some insight into their behavior within the lens, as well as the possibility that the unfolding of proteins and the bonds that form after translation could contribute to the increase in rigidity that leads to loss of accommodation.

## STRUCTURAL ORDER

Few investigations have linked the mechanical and the optical properties of the lens and none, to the author's knowledge, considers the effect of lens order on the function of accommodation. Order has been considered only with regard to the transparency; short-range spatial order is believed to be sufficient to prevent opacification.<sup>15</sup> Evidence for this assertion was reported by Delaye and Tardieu.<sup>16</sup> Recently, it has been proposed that the degree of spatial order required for transparency may vary across the lens and that it may be greater in the cortex than in the nucleus.<sup>17</sup> The reason for this is the considerable difference between the refractive index of the cortical cytoplasm and that of the fiber cell membranes. Without a higher degree of spatial order, such a difference in refractive index would result in light scatter. The relatively small difference between cytoplasmic and membrane indices in the nucleus indicate that the degree of spatial order does not have to be high to prevent scatter and opacification. However, it is interesting to note that when the lens is dehydrated, the protein arrangement that remains manifests what would appear to be a higher degree of structural order than encountered in the fresh (hydrated) lens (Figure 7-5).<sup>18</sup>

When a structure with a high degree of order is illuminated with linearly polarized light and is viewed through an analyzer (a polarizer with its axis at 90 degrees to that of the linearly polarized light), 4 arms of a dark cross and colored rings are seen within the structure. The arms (isogyres) and the colored rings (isochromatics), which become a set of dark/light fringes if monochromatic light is used as



**Figure 7-5.** Isochromatics (colored rings) in a dehydrated fish lens (Blue Eye Trevally, *Hyperoglyphe antarctica*). (Reprinted from *Exp Eye Res*, vol 59(1), Pierscionek BK, Isochromatics in eye lenses, pp 121-124, Copyright 1994, with permission from Elsevier.)

the source, are characteristic features seen in crystals, when the latter are viewed between crossed polarizers. The high degree of organization in crystals, with accurate spacing between the atoms, may, if the spacing varies in different planes, result in birefringence—the property of possessing 2 or sometimes 3 refractive index values that depend on the direction of light travel through the crystal and the spacing of atoms along that direction. Double imagery results by looking at an object through a birefringent crystal held in certain orientations.

Because birefringence produces characteristic patterns when viewed through crossed polarizers, the presence of isogyres and isochromatics in the optical elements of the eye has been interpreted as an indication of birefringence.<sup>19</sup> It has, however, been shown that isogyres can be produced in nonbirefringent structures<sup>20</sup> and that curvature alone can result in their formation.<sup>21,22</sup> The explanation provided is that the orientation of linearly polarized light twists as its direction is changed by refraction through or reflection from a highly curved structure, which produces the isogyre pattern.<sup>21,22</sup> Isochromatics, however, are linked with higher-ordered structures that do demonstrate birefringence. Why the dehydrated lens shows these patterns,<sup>18</sup> indicating a highly ordered protein backbone, remains unanswered. Knowledge of the

structural order and whether it is indeed long range will provide insight into how the layers of the lens may be displaced with accommodation and how this capacity may be lost with age.

## SUMMARY

Many aspects of the structure/function relationship of the eye lens remain unclear and leave a number of questions unanswered. The most fundamental of these questions is how an organization of 3 classes of proteins with complicated shapes and differing proportions across the tissue can produce a transparent structure that is maintained over decades and how the order and organization of proteins can alter with no adverse effects on optics when the lens accommodates. An understanding of the structural organization and the level of protein order and how these alter with age is required before the changes that occur with presbyopia can be fully comprehended.

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