AGING CHANGES IN THE OPTICAL ELEMENTS OF THE EYE

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(Paper JBO-062R received Dec. 5, 1995)

ABSTRACT

The cornea and the lens are the optical elements of the eye, responsible for correctly focusing light rays on the retina. As the eye ages, both the cornea and the lens undergo structural changes, some of which affect function. This review deals with such age-related changes and any functional manifestations arising from them.

Keywords cornea; eye lens; aging; optics.

1 INTRODUCTION

Aging is not a disease. Certainly there is a depletion of some functional capacities and a retardation of reflexes and reactions, but this only becomes an impairment when the ability to cope with daily tasks is compromised.

It is sometimes difficult to distinguish between pathological and physiological changes in the eye. For example, when does an age-related change in the transmittance properties of the eye lens warrant the label ''cataract''? With the appearance of deep coloration? with the loss of one line on a visual acuity chart? It is easier to say that most visually impairing processes are simply an extension of the (normal) aging process which, for reasons that may be based on genetic and/or environmental influences, varies in rate among individuals.

This review is concerned with the effect of age on the two optical elements of the eye: the cornea and the lens, and will limit to passing reference extended or accelerated effects of aging that cause sight loss.

The cornea and the lens are responsible for transmitting and focusing light onto the retina, which contains photoreceptor cells and from which signals relaying visual information are sent to higher centers in the brain. In order to transmit light, the optical elements need to be transparent; in order to focus, they need to be curved and have an appropriate index of refraction. Most of the refracting power (about two-thirds of the total) is provided by the cornea, the clear curved ''window'' at the front of the eye and hence the first element that incoming light rays strike. Its curvature and the relatively high difference in refractive indices between the corneal index of 1.375[1] and that of air converge the light entering the eye.

The lens provides the other one-third of the ocular power and, in addition, is solely responsible for adjusting the focus of the eye to cope with the changing vergence demands i.e., to enable the eye to see clearly over a range of distances. The ability of the lens to alter the ocular focusing power is called *accommodation* and the steady age-related decrease in this function, referred to as *presbyopia* (discussed in detail later), is one of the most characteristic signs of aging.

2 SENESCENT CHANGES OF THE CORNEA

The thickness of the central cornea (as measured in Europeans) is between 0.50 and 0.57 mm, with a slightly thicker periphery (reviewed in Ref. 2). It can be broadly separated into three sections: the epithelium on the outer surface of the cornea; the stroma, which composes 90% of the corneal thickness; and the endothelium, which lines the posterior cornea and is therefore the innermost layer. Two other very fine layers of the cornea are also often identified separately: Bowman's membrane, which lies between the epithelium and the stroma, and Descemet's membrane, which lies between the stroma and the endothelium.

2.1 THE EPITHELIUM

The main role of the corneal epithelium is to protect the rest of the cornea by acting as a barrier to water, larger molecules, and toxic substances. It also serves as a support for the tear film, which is composed of oily, aqueous, and mucous layers and serves to moisten and protect the cornea as well as to provide a smooth surface for traversing light rays.

The function of the epithelium as an effective barrier has been assessed from measurements of its permeability. Two studies that looked at epithelial

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permeability to fluorescein, using slit-lamp fluorophotometry, reported an increase with age.[3–4] However de Kruijf et al.[5] did not find any significant correlation between epithelial permeability and age. Since the data points in all three studies were scattered, perhaps the difference may be explained by the age ranges used: in the first two studies there was an adequate representation of data from eyes aged between 60 and 80 years while in de Kruijf et al.'s study[5] there were only 3 (out of 46 eyes) over the age of 50, the oldest being 67 years.

The epithelial cells possess the ability to regenerate and hence to repair any superficial injuries to the cornea. The repair process can take only days if the basement membrane is intact, since this provides adhesion of epithelium to Bowman's membrane (as first observed in rabbit eyes).[6] From an electron microscopic study, it was found that the basement membrane thickness with age; after the second decade this thickening is the result of membrane reduplication promoted by either cell death or basement membrane deterioration.[7]

2.2 THE STROMA

The stroma consists mainly of collagen and connective tissue. A decrease in the spaces between collagen fibrils and some fiber degeneration resulting in collagen-free areas was noted in the corneal stroma from an 80-year-old donor eye compared with observations made in two younger eyes (aged 16 and 46 years).[8] A more extensive study that looked at structure in corneas ranging from birth to age 90, using synchrotron x-ray diffraction, confirmed this and also found an increase in the cross-sectional area of the corneal collagen fibers with age.[9]

Clinically observable age-related changes in the stroma are an increased turbidity[10] and an increase in stromal density (reviewed in Ref. 11). Dustlike opacities can sometimes be seen in the central posterior stroma of older eyes (over 50). These are referred to as cornea farinata and they rarely have any detrimental effect on visual function. It has been suggested that they may be linked to the degradation of corneal collagen[8] or to changes in the distance between collagen fibers and the appearance of collagen-free spaces.[12]

2.3 THE ENDOTHELIUM

The most important senescent change in the cornea, from the clinical perspective, is endothelial degeneration or distrophy. The pattern of arrangement of young endothelial cells resembles a honeycomb. This pattern becomes more and more irregular with age as cell density decreases and cell size increases.[10,13–24] Mean cell loss calculated from 103 eyes of older individuals was estimated to be around 2% over a 2-year period.[22] In addition, the cell shapes become increasingly more variable (pleomorphism).[13–14,16–17,25] Laing et al.[16] reported, from *in vivo* studies, that the mean cell area doubles between the ages of 20 and 80 years but because of the age-related increase in pleomorphism and polymegathism (variation in cell size), the value for mean area in very old tissue may not be representative.

It is believed that the increase in endothelial cell size is an act of compensation for a decrease in number: the remaining cells extend to cover spaces left by adjacent cells which have died. The endothelium can cope with a measure of cell loss and subsequent extension of remaining cells. Two common signs of senescence which indicate that these processes are occurring are (1) the formation of corneal guttata: excrescences on Descemet's membrane that appear to make pitlike markings in the endothelium; and (2) loss of cell outline and the appearance of shrivelling.

The endothelium is responsible for regulating the fluid balance of the cornea in order to maintain the hydration of the stroma at a level of about 78% water and thus to retain transparency. The control of hydration of the cornea by the endothelium operates on two levels: as an active pump and as a passive fluid barrier: fluid leaks through into the stroma and is removed by the metabolic pump. When the number of cells that have degenerated exceeds the number needed for proper endothelial function, there is an impairment of the barrier function and aqueous humor seeps into the cornea, resulting in a disruption of the structural order and a subsequent increase in turbidity and light scatter. It is not certain whether there is a gradual increase in permeability of the corneal endothelium with age. One report (a conference abstract) claims that there is an increase of 24% in endothelial permeability to fluorescein over an age range from 5 to 79 years[26] while Bourne et al.[27] reported no such change. From another study of 26 pairs of donor corneas, ranging from 11 to 91 years, the density of pump sites was found to be constant with age but to increase in corneas that showed early signs of the degenerative changes normally associated with a greater permeability.[23]

The presence of edema or recovery from hydration are more reliable indicators of endothelial function than cell density values. The rates and amounts of recovery from hypoxic stress, which lead to corneal edema, induced by contact lens wear and eye closure, have been used to assess endothelial function.[28–30] The rate of recovery from induced stress was found to decrease with age. An age-related shift from aerobic to anaerobic metabolism, which would result in a lower tolerance to conditions of stress, has been shown in a study on 89 human donor corneas ranging in age from 1 to 79 years.[24]

2.4 PHYSICAL FEATURES

The temperature of the central cornea was found to decrease with age, by about 2 deg over an age

range between 1 and 80 years.[31] The reasons for this, and any functional significance it may have, are not clear at present. The tactile sensitivity of the cornea also decreases with age,[32–34] which suggests that the change in sensitivity is caused by an age-related decrease in the numbers of nerve fibers and pain receptors.[2]

From biomicroscopic studies, it was found that the cornea thins with age by about 0.0045 mm per decade.[35] A previous study[36] reported a greater rate of corneal thinning of 0.0007 mm/year from a sample of 839 Greenland Eskimos of age range 7 to 89 years. Alsbirk also observed that in adults the rate of thinning was significantly higher for males than for females[36] and that males who worked indoors had thicker corneas than the average general population.[37] It was noted that the women spent most of their time indoors, prompting the suggestion that corneal thinning may not be solely the effect of senescence but that environmental factors may also play a role.[37] Familial resemblance was also found, implying a genetic influence. Comparison of the data for Eskimos with those from 98 Danes showed a significant ethnic difference, but only for males.[36]

Corneal thinning with age has not been a universal finding. Olsen[38] did not find any significant difference in corneal thickness between a younger (20 to 40 years) and an older (60 to 80 years) group of subjects. The discrepancy between Olsen and Ehlers[35] and Olsen[38] is hard to explain given that similar techniques and approximately the same subject numbers were used.

2.5 CORNEAL SHAPE AND REFRACTIVE INDEX

The shape of the cornea is, in general, not perfectly spherical but exhibits some toricity, referred to more commonly as astigmatism. It is more curved in the vertical than in the horizontal meridian in young eyes, but this reverses with age. Such changes are best investigated in longitudinal studies, which avoid the scatter in data that arises from individual variations. A study conducted by Lyle[39] on 462 eyes over a mean period of 24 years showed that in most subjects (over 70%), age-related changes in corneal shape are small (0.25 D or less). Up to age 30 there is a slight increase in the curvature of the vertical meridian whereas over the age of 30 this trend reverses. Interestingly, Morgan[40] found this trend only in females as they aged from 13 to 33 years.

Other studies have measured astigmatism in individuals from various age groups. Phillips[41] found that the incidence of eyes with a greater curvature in the vertical meridian decreases over an age range of 10 to 80 years. Kiely, Smith, and Carney[42] measured and compared the radii of corneal curvature of subjects from four different age groups: 16 to 20 years, 21 to 40 years, 41 to 60 years, and 60 to 60 years. They showed that the radius of curvature decreased (the curvature steepened) in all meridians measured (horizontal, vertical, and the two diagonals), with the smallest change observed in the vertical meridian. Similar findings were reported in a more recent study,[43] indicating that the age-related changes in corneal astigmatism result from a relative steepening of the horizontal meridian rather than a flattening of the vertical meridian. This confirms the earlier study by Reading,[44] who cited Marin-Amat's explanation for the age-related changes in corneal shape. Marin-Amat[45] stated that in the very young, who engage in no close visual work, the major pressure on the cornea comes from the lids and hence it is squeezed in the vertical direction. In adults the demands on convergence are greater with the increased amount of near work and the pressure effects on the horizontal meridian arise from the extraocular muscles involved in convergence. Hayashi et al.[43] suggested that the variability of external factors, such as lid pressure and reduced action of the extraocular muscles, was too large to explain the alterations in corneal curvature and that structural changes, which occur with age, are more likely to be responsible. The refractive index of the cornea, the other parameter of power, was not found to alter with age.[46]

2.6 CORNEAL TRANSMITTANCE

The cornea transmits light from 300 to 2500 nm with no significant age-related differences.[47] The lack of an age dependence on light transmittance through the cornea has been confirmed by Beems and van Best[48] using donor eyes, and by van den Berg and Tan,[49] using *in vivo* data of Tan (referred to by van den Berg and Tan[49]). However, Lerman's findings[50] do not concur with those mentioned above, indicating variations with age in corneal light transmittance. Insufficient information on method and samples in the latter study renders it difficult to explain why these results differ from all the others.

3 REFRACTIVE STATE OF THE EYE

Hirsch[51] found a decrease in the proportion of emmetropes (those who do not require spectacle correction for distance vision) after the age of 45 and concomitant increases in the proportions of both myopes (short-sighted individuals) and hypermetropes (long-sighted individuals), the latter showing the greater increase. This supports previous studies (reviewed in Hirsch[51]). From a clinical study of patient records, an increased tendency toward myopia, up until about the age of 20, was observed and thereafter the refractive state was seen to shift gradually with age toward hypermetropia,[52] confirming a previous study.[53] However, the results of Saunders' study,[52] as correctly noted by Saunders, were not from a ran-

domly selected group of subjects but from an ''optically biased'' one, since all subjects involved had come to seek some refractive correction, and therefore could be taken as representative of the population. The age-related changes in the corneal shape (discussed above) result in a similar trend in the astigmatism of the eye.[52–55]

4 SENESCENT CHANGES OF THE LENS

The human lens is a biconvex spheroid with the posterior surface being the more curved. For a more detailed discussion of lens dimensions, the reader is referred to Refs. 2 and 56. The lens is located behind the iris and is contained within a thin semielastic capsule. Fine zonular fibers are attached to the capsule around the lens equator and these link the lens to the ciliary muscle. The lens alters its accommodative state, and hence the power of the eye, by changing its shape: it becomes more curved as the demand for refractive power increases with near visual tasks and it flattens for distance vision. This change in shape is initiated by the ciliary muscle, which moves back for distant vision, pulling the zonules taut and hence causing a stretching and flattening of the lens. For near vision, the muscle moves forward, releasing zonular tension and allowing the lens to assume the appropriate rounder shape.

Broadly speaking and from a functional perspective, only two senile changes occur in the lens: a loss of accommodative capacity, presbyopia, and an increased impedance to the transmission of light which, when it reaches a stage at which vision is impaired, can be referred to as cataract.

4.1 PRESBYOPIA

Presbyopia is a universal condition: every individual who reaches their sixth decade of life will have experienced its manifestations in the form of difficulties with focusing on close objects. Though the signs become evident in the late middle years of the human lifespan, the process is a gradual one that starts in early infancy. It is not until the near point recedes beyond an arm's length that the individual becomes aware of symptoms: blurred vision, eye strain and, not uncommonly, headaches. The explanations for presbyopia have been largely based on perceived changes in rheological properties, i.e., changes in elasticity of the lens and its capsule. (The capsule is believed to play a role in accommodation by exerting a moulding force on the lens.)

''Sclerosis'' or hardening, resulting from dehydration of the tissue, was long considered to be at least partly responsible for the loss of lenticular elasticity (reviewed in Refs. 57–58). While there is evidence that the lens becomes less deformable with age, this does not account for the entire loss of accommodative capacity.[59] Moreover, significant water losses with age have not been found beyond prenatal and early postnatal life.[60] This confirms biochemical studies that reported observing no changes in protein/water proportions in the lens as it ages.[61–62] A slight decrease in the inner regions of the lens (approximately 5% of total water from between the ages of 3 and 77 years) which was reported by Lahm, Lee, and Bettelheim,[63] was determined from lens sections and hence the finding may, at least partly, reflect age-related differences in susceptibility to evaporation given that there is less protein-bound water in older lenses.[63]

Increased adhesion between the fiber cells of the lens[60] and/or binding of certain structural proteins to cell membranes[64–65] are the more likely explanations for the age-related increase in lenticular rigidity. Fisher also found that Young's modulus of elasticity for the capsule decreases with age,[66] indicating that the capsule becomes more flaccid. A loss of contractile force of the ciliary muscle, once thought to contribute to presbyopia, seems unlikely to be a significant factor.[57–58]

Although it is superficially simple to understand, there is as yet no adequate explanation for presbyopia and this may be because, despite the fact that it progresses with age, presbyopia may not be exclusively a consequence of senescence.[67] It has been found that presbyopia does not fit with the aging trends of other human biological and related functions: its rate is approximately twice as fast.[68–69] A new hypothesis of presbyopia, based on this observation, has been proposed: that presbyopia is a multifactorial process resulting from the combined effects of growth and senescence.[67] Pierscionek and Weale[67] have suggested that a particular signal in lens cell differentiation causes changes, over time, in the geometry of the lens and its attachments. This hypothesis takes into account not only the observations of comparative aging trends[68] but also the experimental findings regarding lens shape[70–72] and changes in the position of the zonular fibers[73] with age. It supports an earlier suggestion[74] that the continual growth of the lens is likely to be a cause of the decrease in accommodative amplitude with age.

Although presbyopia is a universal condition, and it is accepted that in general all accommodative capacity is lost by the end of the seventh decade, the rate of loss varies. The variation is greatest when one makes a global comparison. Individuals who live in countries that are closer to the equator develop presbyopia earlier in life than those living further from the equator. In addition, people who live at high altitudes show a slower rate of development of presbyopia than those in lower regions. The predominant factors contributing to the rate of lenticular aging are most likely to be the amount of ultraviolet radiation and the ambient temperature.[75–78] However, it should not be overlooked that, since the determination of accommodative amplitude is subjective and global studies use a number of practitioners and a patient rather than a

subject database, prescribing habits of the eye clinician as well as factors that influence patient self-selection must be considered in the analysis of results.[79]

It has been suggested that the changing zonular force in the act of accommodation may stimulate cell division in the anterior epithelium of the lens.[80] This would increase the growth rate of the lens, making it harder and harder to deform. Support for this hypothesis comes from a study which showed that drugs capable of altering zonular force also cause a change in the rate of cell proliferation in the lens.[81] Several other factors have been shown to influence the rate of development of presbyopia: trauma, diseases, drugs, malnutrition, and refractive error.[82]

4.2 THE ''LENS PARADOX'' AND ITS SOLUTION

Until very recently, age-related changes in the optical parameters of the lens were considered to be somewhat perplexing. The curvatures of the lens have been shown to increase with age by *in vivo*[70–71] and *in vitro*[72] studies. This should result in greater refractive power and the onset of myopia (short-sightedness). In fact, the opposite occurs: the eye loses its ability to focus on near objects while its refractive state remains unaltered for distance vision. This inconsistency between observation and expectation was termed the *lens paradox*. However, the focusing or refractive power of any lens depends not only on its shape but also on its refractive index, which is directly related to the density of the lens material. In the case of the eye lens, the refractive index is not uniform but smoothly varying, increasing in magnitude toward the center. In the nuclear (central) region of the lens, the index magnitude is almost constant, the greatest variations occurring in the cortex (periphery). The paradox was further compounded by the notion that the lens has a varying refractive index because of its unique growth mode in which new cells are synthesized on the lens surface with no concomitant loss of existing tissue. This cellular ''overlay,'' which is thought to compress the inner layers of the lens, and in this way to increase the tissue density, would also be expected to cause an age-related increase in refractive index since the lens continues to grow throughout life. Amazingly, this unsubstantiated notion, the origin of which is obscure, was accepted by some lens researchers unquestioningly. Its weaknesses have since been pointed out.[83–84] Returning to the paradox, an age-related increase in the index gradient would accentuate the tendency toward myopia.

A suggestion was made that the lenticular refractive index may decrease with age (to compensate for the increase in curvature and therefore prevent the ''myopization'' of the eye).[85] Although a slight increase in nuclear water content with age has been reported,[86] the data points are scattered and the significance of the rise is based on lenses that are post-presbyopic; in the age range up to 50 years, the data show no increase in water content. Moreover, a change in protein/water proportions with age has not been shown in previous studies (as discussed above) and indeed no measurable decrease in refractive index with age has been found (reviewed in Ref. 11).[83,87–88] This is supported by protein densitometric data.[89] The fundamental point, which some lens researchers have failed to recognize, is that an overall increase or decrease in nuclear refractive index would not solve the lens paradox, for the nuclear refractive index is almost constant, and therefore very little refraction takes place within it. The bending of light, the extent of which determines the refractive power, depends on a *change* in refractive index, as Snell's law tells us.

An answer to the paradox was provided by Pierscionek,[88] who recognized that only a change in the gradient of refractive index, which occurs in the cortex, can sufficiently compensate for the increase in curvature. She proposed a synchrony between growth of the lens and the way in which the refractive index is distributed, so that the distant refractive state of the eye remained unaltered in spite of continued tissue accretion and increases in lens curvature. Calculations using a lens model[90] and experimental estimations from clinical data[91] support Pierscionek's hypothesis, which may finally lay the ''paradox'' to rest.

4.3 LENTICULAR TRANSMITTANCE

The functional loss incurred by the presbyopic process can be considered as nothing more than an inconvenience compared with the impairments that can result when changes in lenticular transparency impede the transmission of light. Lens transmittance ranges from ultraviolet to 1900 nm[47] but there is a strong age dependence in transmission of both ultraviolet and visible wavelengths.[47,92] The age-related factors that attenuate light transmission are absorption and light scatter. However, a certain amount of attenuation is tolerated without any detriment to vision. It should also be added that even when an opacity is visible in the lens by clinical (biomicroscopic) examination, it does not necessarily imply a visual degradation (reviewed in Ref. 93). The relationship between visual performance and lens appearance will be discussed later.

It has been theorized that the lens retains transparency for a greater part of its life because of the structural organization between the constituent proteins and water.[94–95] Benedek[95] reasoned that as long as proteins were densely packed, short-range order was sufficient for unhindered light transmission. Experimental evidence in support of Benedek's proposal has been found in calf [96] and in human lenses.[97]

4.3.1 Light Scatter

In the clinic, the lens is observed with a slit-lamp (biomicroscope) . Information about the appearance of the lens and any opacities it may contain is conveyed to the observer's (examiner's) eye via backscattered rays. Biomicroscopic studies have shown an increase in image density (an increase in backscattered light and hence a decrease in transmittance) with age.[98–104] The rate of age-related increase in backscatter is slight up to the age of 40 and rapid thereafter.[101,104–105] While slit-lamp studies are usually conducted using a white light source, increased light scatter with age, using a longer wavelength source (700 nm), has also been reported.[106–108]

A relationship between light scatter and the constituent proteins of the lens was reported by Spector, Li, and Sigelman[109] who compared the backscatter seen in the functional eye with the amount of high molecular weight protein (HMWP) extracted in the soluble fraction. They found that in the nuclear region of the lens, the rates of increase in backscattered light and in the HMWP fraction were similar.

It has been suggested that as the lens ages there is a change in the refractive index difference between proteins and their environment, and that the resultant fluctuations (which while occurring over small regions and therefore not having a measurable effect on the overall index profile) are responsible for the increase in light scatter.[110] Evidence of a synergetic process, in which conformational changes to lens proteins cause a release of water previously bound to the protein aggregates, has been described.[63,110]

Although the transmittance of the lens decreases with age, lens transparency, determined by image-forming capacity, does not. This was investigated by Weale[111] who found no age-related changes (age range 32 to 89 years) but a lower transparency of female lenses compared with their male counterparts. Other studies which claim changes in transparency with age[101,104,107] have based their conclusions on measurements of backscattered light and hence have really referred to transmittance. In view of Weale's findings,[111] it would be worthwhile to take heed of the difference, albeit subtle, between transmittance (determined on the basis of backscattered light) and transparency (based on image quality).

4.3.2 Relation of Light Scatter to Visual Acuity

The image of the lens seen clinically does not correlate well with the patient's acuity and hence cannot be used to predict visual function. Changes in scatter from the anterior eye (lens and cornea) with age were compared with age-related changes in visual acuity and were not found to concur.[98] This was supported by de Waard et al.[112] who showed that the relationship between forward scattered light (light that passes through to the retina) and backscattered light has considerable individual variation and hence the amount of light transmitted to the retina cannot be estimated from the backscatter seen in the slit-lamp. It has been noted that the amount of intraocular[113] or backscattered light[114] is dependent to some extent on the pigmentation of the eye and hence any measurements of scattered or stray light need to take this factor into account.

Bettelheim and Ali[115] recognized the need to formulate a relationship between forward and backscattered light. They measured the angular variation in light scatter from *in vitro* lenses from 23 normal eyes aged between 3 and 76 years using a white (tungsten) source, and two wavelength components from a mercury source: the blue line (435.8 nm) and the green line (546.1 nm). Acceptable correlations between forward and backscattered light were found for the blue and green sources but not for the white light source, the most commonly used source in routine slit-lamp examination.

A gross association between visual acuity and the relative extinction of blue light compared with white light has been observed[116]: in cases where the extinction ratio dropped below 0.5, acuity was below the nominated normal level (6/12 or 20/40).

It is important to realize that the backscattered light consists of both diffusely scattered and reflected components; the former may be detrimental to vision, the latter is not. Hence, if a correlation is to be sought, between an image of the lens seen through a slit-lamp and the visual acuity of the patient or subject, it is only the proportion of diffusely scattered light that is relevant. A simple way of separating the two components of backscatter is with linear polarizers: one to polarize the incident beam and the other, with its transmission axis at 90 deg to the axis of the first polarizer, in front of the eye piece. From basic optics, the reflected light, which remains polarized, will be blocked by the crossed polarizer (analyzer) and only the diffusely scattered light will reach the observer. This method of polarizing light biomicroscopy (PLB) has been shown to yield very interesting qualitative results[117–118] and was employed recently in a quantitative study which showed that using PLB, the images of the lens are correlated with visual acuity, while with standard biomicroscopy there is no correlation.[119]

4.3.3 Absorbance

The lens yellows with age and the coloration continues to deepen. This renders the lens an ever more effective absorbance filter for short wavelength radiation and it has been shown that the wavelength of maximum absorption decreases with age.[120] Said and Weale[92] showed that the optical density of the lens increases with age, particularly for the short wavelength end of the visible spectrum. The accumulation of a single pigment to explain the yellowing of the lens could only be sup-

ported by the data up to the age of 25. Beyond this, either accumulation of other pigments with different density spectra or an increase in scatter could explain the results.[92]

The largest proportion of yellow pigment is contained within a particular (insoluble) protein fraction that was found to be related to the absorbance of the lens.[121] It is worth noting that the state of extracted proteins should not be considered as indicative of their state *in vivo*. Hence the insoluble proteins should not be regarded as being insoluble within the lens but rather as the fraction that has an increased vulnerability to insolubilization (most likely through aging) when removed from the functional tissue.

Treating the lens as a colored filter, its density was calculated from color matches made by 265 subjects ranging in age from 8 to 79 years.[122] The results showed an increase in lens density with age, with little variation in the absorption per unit path length, suggesting that density increases as a function of increase in lens thickness. A similar conclusion was reached from a study on excised human lenses.[123] In the latter study, it was assumed that there is only one pigment in the lens and that scatter and absorption losses were equivalent. However, Zeimer and Noth,[124] using scanning photometry to measure transmittance of the nuclear part of the *in vivo* lens, made the important observation that the amount of light lost per millimeter increases with lens age. This implicates a change in tissue properties as partly responsible for the changes in transmittance factors.

Using a psychophysical method,[125] it was found that absorption by the lens did not change much with age until the seventh decade, after which a threefold increase in the lens density index was observed. It should be noted that in this study subjects with cataractous lenses were included in the analysis because the authors considered cataract to be an extension of the aging process. Since the cataracts were all from eyes aged over 60, the effect of cataracts may have contributed to the large increase in lens density index after this age and therefore masked any strictly age-related trends.

However, similar changes in absorption characteristics of the human lens after the age of 60 have been shown in other studies. Pokorny, Smith, and Lutze[126] analyzed the literature concerning the spectral density of the lens and its rate of change with age and found that the constant rate of increase in lens density with age between the ages of 20 and 60 more than triples thereafter. They divided the spectral density function into two components, one affected by age and the other a residual component, and found that their analysis supported the suggestion made by Tan (referred to by Pokorny et al.[126]), that two active factors are responsible for the spectral density function of the lens: one attributable to growth and the other to aging. The latter factor is, according to Tan, related to the increasing coloration of the lens with age and may reflect the increase in one of the fluorogens (the one associated with lens yellowing) reported by Lerman and Borkman.[127]

4.3.4 Fluorescence

Biochemical studies on lens proteins have shown an age-related increase in blue fluorescence associated with the insoluble protein fraction,[128–129] which also rises with age. Lerman and Borkman[127] found two fluorescent compounds in the lens nucleus. The age-related increase in one of these, a blue fluorescing species (excitation: 340 to 360 nm, emission: 415 to 435 nm), was correlated with the increase in yellowing while the other, activated by longer wavelengths (excitation: 415 to 435 nm, emission: 500 to 520 nm), and possibly a secondary product of the first species, was related to the brown color seen in old lenses. A later study supported these findings.[130]

A number of other fluorescent species have been found[131] and age-related increases in blue-green fluorescence from *in vivo* and *in vitro* studies have been reported.[132–138] An increase in absorption[139] and fluorescence[140–141] of light at the far end of the visible spectrum (orange, red) was found in older lenses and in lenses with nuclear coloration.

Shifts in fluorescence intensity with age toward longer wavelengths,[137,142–143] suggest that the concentrations of fluorescent species within the lens change or that there may be a conversion of one fluorescent species to another.[143] In the case of fluorescence originating from proteins (tryptophan fluorescence), changes in the composition and conformation of the latter may be the cause of the shift to a longer emission wavelength.[137] Suarez, Oronsky, and Koch[97] showed that changes in structural organization occur after the age of 55 years concomitant with an increase in the fluorescence and the urea-insoluble protein fraction which, the authors suggest, may be indicative of cross-links formed between proteins and sugars.

Age-related changes to the cornea and lens are not sight threatening and at most cause a depletion in functional capacity which can be compensated for, i.e., presbyopia. However, the rate of aging varies and if it is excessively rapid, detrimental changes can occur. Efficient functioning of the ocular elements of the eye in later life depends on the rate of age-related change and this in turn depends greatly on environmental exposures as well as on the nutritional and behavioral habits in younger years.

Acknowledgment

The author is supported by the National Health and Medical Research Council of Australia (N.H.M.R.C.) and wishes to acknowledge financial assistance from Essilor International.

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