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YELLOWING OF THE HUMAN LENS: NUCLEAR AND CORTICAL CONTRIBUTIONS

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Abstract - The geometry of the cortex and nucleus of excised human lenses was determined by photography and dissection. Samples of the cortex and nucleus were measured spectro-photometrically to allow determination of light loss through the whole lens and the light loss per unit pathlength. It was concluded that the reduction of short wavelength light transmission with increasing age is due to two processes; first, the nuclear pathlength remains constant but there is increased pigment deposition; second, the pigment concentration does not change in the cortex but the pathlength increases.

Key Words - Lens Yellowing Aging Transparency Lens thickness

INTRODUCTION

In a previous paper (Mellerio, 1971) possible causes for the increase in "yellowing" of the' crystalline lens with advancing age were considered. Weale (1963a) had shown lenses became bigger and yellower with approaching senility and the 1971 study attempted to determine whether the rise in yellowing was due to the deposition of more absorbing (or scattering) pigment or to an increase in lens thickness, or to some combination of these two possibilities.

To decide which alternative applied, the light loss, expressed as photometric density per unit pathlength, was determined for a number of lenses and plotted against age. There was no obvious change in this value and it was concluded that the increase in lens thickness was responsible for increasing lenticular yellowing. Implicit in this conclusion is the assumption that the pigment (or scattering molecule) is uniformly distributed in the lens, or at least within the central area corresponding to a pupillary window of radius 4 mm which was the area that was measured.

However, that the lens is not homogeneous has been known since the invention of the slit lamp (Weale, 1963a) and is very obvious when examining a living lens with such an instrument (Brown, 1973a; Sigelman *et al,* 1974). The conclusions of the 1971 paper, based as they were on homogeneity, must be suspect. They can be valid in one sense only: overall, the lens behaves optically as if its absorption or scatter per unit pathlength is constant with age and increasing pathlength is the reason for reduced transmission. This must be a simplification for it is now apparent that pigment and scattering centres are not evenly distributed so that age changes must be more complex than were originally described. More recent studies confirm this complexity, e.g. Hockwin *et aL* (1984), Sigelman *et al.* (1974). Further, Zeimer and Noth (1984) have questioned the validity of Mellerio's 1971 suggestion that the large variation in light loss per unit pathlength from one lens to the next precludes any certainty in choosing an explanation for yellowing.

This paper describes how the spectral photometric density of the lens nucleus and cortex were separately determined, together with the pathlengths of each component, and how this information can be used to "reconstruct" a whole lens which behaves, fortuitously, as those reported in 1971.

METHODS

Human lenses taken within 8 hr of death were used either at once or after over night storage in a moist saline atmosphere at 4°C (Siew *et al.*, 1981). Storage produced no optical changes visible to the naked eye. Each lens was examined carefully and was rejected if not perfectly clear and free from opacities. This selection severely reduced the number of lenses that could be used. Any attached pigment or vitreous was removed and surplus fluid was blotted off with filter paper. The posterior lens surface was placed horizontally on a 3 mm diameter collar that could be rotated about its vertical axis. A series of photographs were taken of the lens so that its dimensions might be measured. The anterior profile was photographed from the side at four different rotational positions of the lens. It was turned over and the posterior profile photographed four times in a similar way. Each profile was traced from the enlarged film, averaged, and joined for the smoothest equatorial fit with the opposite profile to generate a profile of the complete lens. From this, the averaged thickness of the lens, here referred to as the pathlength, x_T, at any distance from the polar axis, here referred to as radius R, could be determined. The estimated errors of measurement were for x_T , ± 0.07 mm and for R, ± 0. 1 mm. Suspension of the lens in air on its collar would be expected to distort its shape by circumferential sag which would increase the apparent thickness of the reconstructed lens. The extent of this shape distortion was examined by tracing as much as possible of the profile from the upper surface round the edge of the lens and onto the lower surface. If this was done for the lens in both orientations, the overlapping profiles matched within the estimated errors given above.

After the whole lens had been photographed, it was dissected by discission of the capsule around the circumference and removal of the cortex by gentle teasing with two fine sable hair brushes. This process, of course, destroyed the integrity of the cortex. The material was carefully placed in a small phial until the optical measures were made (see below). The nucleus was left intact; it was transferred to the collar for photography of the anterior and posterior profiles in the same way that the whole lens was photographed. The result of this was a reconstructed nucleus which could provide at any radius, R, a nuclear pathlength, x_N. By subtraction of x_N from x_T the pathlength of the cortex, x_C could be found. The lens nuclei appeared fairly uniformly yellow by visual inspection: each was homogenised with a blunt metal spatula and placed in a phial until optical measurements were made. The older the lens, the harder it was to mash the nucleus. The division of the lens into two components and the forced homogeneity of each before photometric density measurements were made is a great simplification of the real situation. The division by brushes might be thought somewhat arbitrary, but consistent results were obtainable with practice, as is demonstrated by the similarity of the nuclear profiles in those cases where the two lenses of a donor were available.

The photometric densities of the cortex and nucleus sample were measured in special micro-cuvettes with a Shimadzu type MPS 50L spectrophotometer. Each micro-cuvette was formed from a brass plate 1.05 mm thick in which a 3.67 mm hole was drilled. Two small glass cover slips formed the optical wall, one at the front and one at the back of the hole in the brass plate. The small chamber for the sample could be filled without any air bubbles, with saline as a control, or the lens samples. The nuclear material of the older lenses was very stiff and it was difficult to fill the micro-cuvette accurately such that the cover slips were smooth and flat on the brass plate. With care, the pathlength of the nucleus sample was never more than 10% greater than the thickness of the brass plate. The micro-cuvette had a volume of 44 µl and was placed in the sample carrier of the spectrophotometer as close to the photomultiplier as possible to reduce the effects of light loss due to scatter. The sensitivity of the instrument was reduced by the use of the small diameter sample cell; the gain was increased but noise limited the performance of the measurements by restricting the short wavelength performance at high photometric densities. The estimated error in these measurements ± 0.02 log units. Both the cortex and nuclear material showed increased scatter after mixing and inserting into the microcuvette, the former more markedly than the latter. After a short period the samples had cleared, as judged by visual inspection. The slowest clearing took some 10-15min: photometric density measurements were taken when the samples had cleared.

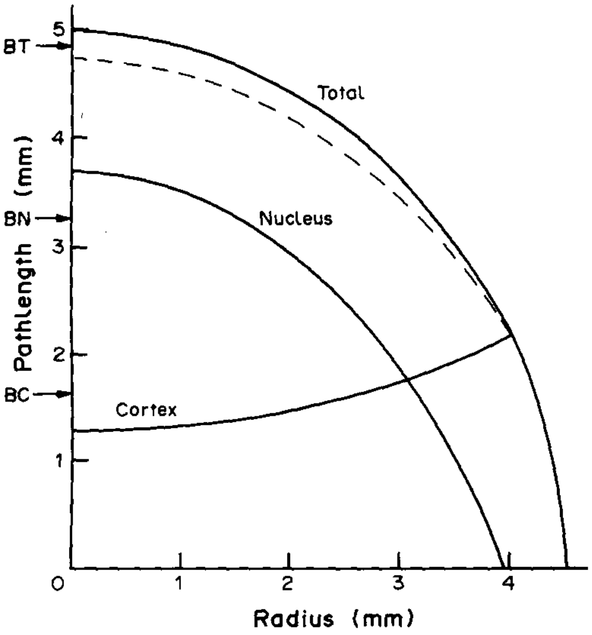

Figure 1
The mean pathlengths for the whole lens, the nucleus and the cortex at different values of lens radius, *R*, derived by averaging data from the 12 lenses in this study. The mean lens age is 56 years. The dashed line is derived from similar data for the series of lenses of mean age 37 years in Mellerio (1971). The arrows labelled BT, BN & BC show the pathlengths at *R* = 0 for the total lens, the nucleus and the cortex of the 45 year lens accommodated to 4D from Brown (1973a)

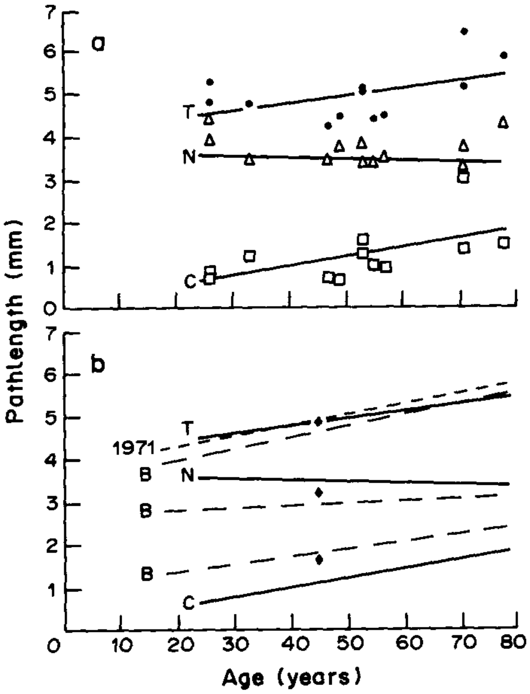

Figure 2
(a) the pathlength at *R* = 0 for the total lens (filled circles), the nucleus (empty triangles) and the cortex (empty square) plotted against age. The solid lines are regression lines. (b) The regression lines as in (a) plus the regression line for the total lens from Mellerio (1971), dashed and marked 1971. Also shown are the three regression lines from Brown (1973); the three diamonds are points for the 45-year old lens accommodated to 4D from Brown (1973a).

RESULTS

Twelve lenses from ten individuals who ranged from 26 to 78 years old were used: the mean age was 51.6 years. Figures 1 to 3 are concerned with lens geometry or physical dimensions.

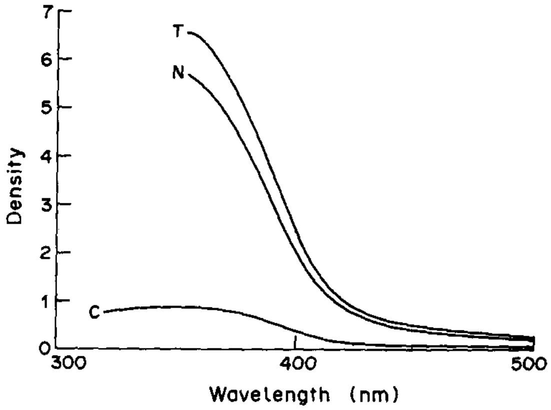

Figure 3
The mean spectral density of the whole lens, T, the nucleus, N, and the cortex, C, reconstructed from the 12 lenses in this study; the mean age of the lens is 51.6 years. The losses above 500 nm were small and have not been plotted

Figure 1 shows the pathlengths for the cortex, nucleus and total lens for the mean 52-year-old lens derived by averaging the pathlengths for each lens. Also shown for comparison is the total pathlength from the series of 20 lenses in the 1971 paper (Mellerio, 1971). The mean age of the 1971 series was 37.2 years. Figure 2(a) shows the pathlengths for the cortex, nucleus and total lens at the lens pole, $R = 0$, plotted against age. Least squares regression lines were fitted to the data and the equations and correlations are shown here:

$x_T = +\,0.017\ \text{age} + 4.09;$

$r = +\,0.47;\ P = 0.2$

$x_N = -\,0.004\ \text{age} + 3.70;$

$r = +\,0.20;\ P = 0.5$

$x_C = +\,0.022\ \text{age} + 0.16;$

$r = +\,0.56;\ P = 0.05.$

Figure 2(b) shows these lines again together with the total lens pathlength regression line from 1971:

$x_T = +\,0.024\ \text{age} + 3.83;$

$r = +\,0.71;\ P = 0.01.$

The 1971 data and the present data are not significantly different ($P = 0.01$) and represent samples from the same statistical population. The regression lines from Brown (1973b) are also shown for comparison.

From the values of photometric density of each sample and the pathlengths of each lens, the densities of the nucleus, cortex and whole lens were calculated for each lens on the assumption that Beer's and Lambert's laws apply. From these "reconstructed" values, the density of the mean cortex, nucleus and lens shown in Fig. 1 was derived and the spectral density is shown in Fig. 3. Of great interest is the age variation of density of the reconstructed lenses: Fig. 4 shows the density at a wavelength of 400 nm at R= 0 plotted against age. Also shown is the density at

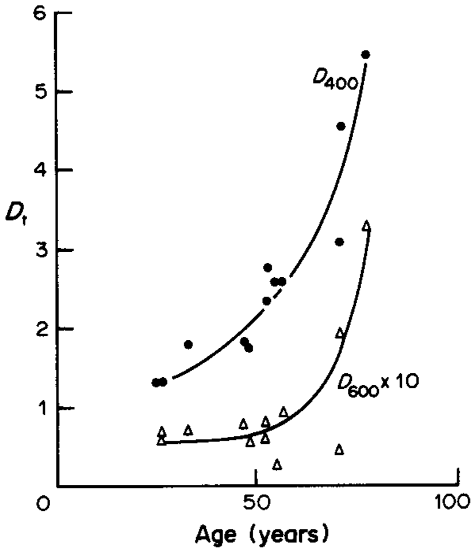

Figure 4
The light loss for the whole reconstructed lens as density D_T, plotted against age at R = 0 for a wavelength of 400 and 600 nm. Note that the D_{600} plot is on a ten times scale. the lines suggest by-eye trends.

600 nm magnified 10 times for clarity. The choice of 400 and 600 nm is arbitrary, but these values conveniently span most of the visible spectrum.

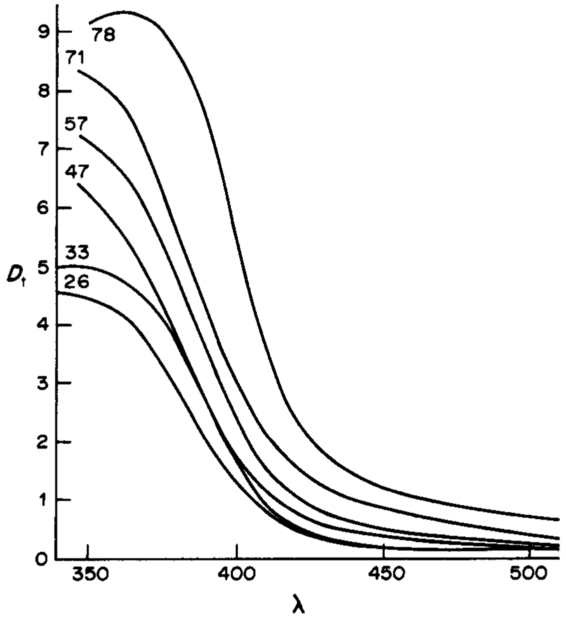

Figure 5
The light loss, as density D_T, of reconstructed lenses at $R = 0$ plotted against wavelength for six lenses of the ages shown.

Figure 5 shows the light loss as density of the whole "reconstructed" lens plotted against wavelength for six lenses of different ages chosen so as to span the age range of the material. Only the shorter wavelengths, where the densities are highest and the age changes most, are plotted.

In this paper the light loss in the samples was measured as the density which is defined as the logarithm to the base 10 of the reciprocal of the transmission. This loss could be due to absorption and/or scatter and it would be of interest to determine the magnitude of each component. Although the Shimadzu MPS 50L, a double beam instrument, was used in such a way as to minimise light losses due to scatter and the effects of fluorescence, some of the measured values may reflect these mechanisms (Weale, 1985). This study did not specifically measure scattered light and is principally concerned with where in the lens light loss occurs rather than in its detailed causative mechanisms. Thus it is not possible to estimate the magnitude of the scattering losses.

Figure 6 shows the total light loss, as density, per unit pathlength (in this case 1 mm) for the homogenised nucleus and cortex plotted against age for a wavelength of 400 nm. The lines are least squares regression lines

$D_N/x = +0.016\text{ age} - 0.205;$

$r = +0.917; P < 0.001$

$D_C/x = -0.277;$

$r = -0.133; P > 0.5.$

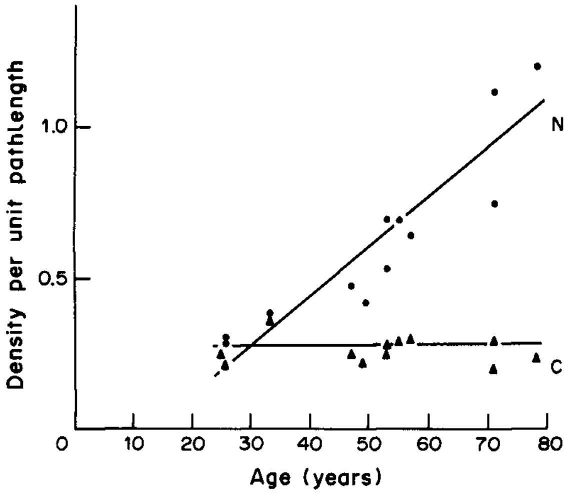

Figure 6
The light loss, as density, per unit pathlength (D/x) for the nucleus (circles) and for the cortex (triangles) at a wavelength of 400 nm, plotted against age.

DISCUSSION

Geometric considerations

The division of the lens into nucleus and cortex by teasing with brushes is a somewhat arbitrary process but it yields consistent results which compare with those from *in vivo* studies in the literature as shown in Fig. 1. Brown (1973a, b), using a slit lamp technique, showed in one report (1973a) that his oldest subject (45 years), when accommodated at 4 D, had a cortex thickness of 1.63 mm and a nucleus thickness of 3.21 mm at the lens pole ($R = 0$). These points are shown on Figs 1 and 2 for comparison with the mean lens of the present study which had an age of 51.6 years. The total pathlength of Brown's lens was 4.84 mm which compares well with 4.98 mm of the older, fully accommodated, and therefore fatter, lens in Fig. 1. However, the data shown by Brown in his presentation to the Ciba Foundation in 1973 (Brown, 1973b) tell a more complete story: in a series of 52 eyes, he showed thickness values that must be considered more reliable but which nevertheless show a spread of values at any age of the order of ± 0.3 to 0.4 mm. The whole lens data in Fig 2 also show a considerable spread but it may be concluded that there is substantial agreement between the present study and that of Brown (1973b) for the whole lens. In Brown's 1973b series, the accommodative state of the eyes was not specified but if they were unaccommodated, this would be a factor that would go some way to explain why his values are smaller for the younger lenses than those in the present study. Residual differences are small and probably due to limitations of sampling. Brown's data show only a very small increase in nuclear thickness in agreement with the findings of this study. The increases in cortex thickness on aging are responsible for practically the whole lenticular change. Although Brown's whole lens data (1973a, b) can be considered to be in reasonable agreement with those of this study, the values for the nucleus and cortex are different. That there are differences is not unexpected because such very different techniques were employed for separation of the lens components. A process which in effect unpeels the lens substance from the outside and which stops at the first signs of resistance to the brushes may not correspond exactly with the optical definition of the limits of the nucleus.

Light loss and aging

As is well known from slit lamp examination, the yellowing and scattering in a lens is not uniform throughout its thickness (Lerman, 1983; Hockwin *et al.,* 1984). A study of lenticular light loss should not therefore assume lenticular homogeneity, as has often been the case and which was so in the investigation of Mellerio (1971). Division into nucleus and cortex is simplistic but is an attempt to use easily demonstrated morphological differences to help in understanding optical changes. Figure 3 clearly shows nuclear and cortical differences. Comparisons using this figure for the averaged data (mean age 52 years) are preferred to the perhaps more appealing option of comparison of data for a younger lens more nearly like that of the typical "standard" 25-year-old observers usually employed in laboratory experiments for two reasons. First, it is not until the fifth decade of life that lenticular density takes on significant values at middle and longer wavelengths. Second, variations between individuals can be quite large and using averaged results offers some protection from accidental and unknowing use of atypical data.

Other authors (see, for example Weale, 1973b) show that above 440 nm the photometric density is low at any age. The densities from Fig. 3 are similar to those of Zigman *et al* (1976, about 0.9 for 52 years at 440 nm) and Cooper and Robson (1969, 0.5 for an old lens at 440 nm), and with those of Said and Weale (1959, 0.43 for an extrapolated age of 52 years at 440 nm). At shorter wavelengths, and below 400 nm, discrepancies become larger and the data from the present study (e.g. 2.5 for 52 years and 400 nm) generally follow those of Cooper and

Robson (1969, 2.0 for 63 years and 400 nm) and the extrapolation of Zigman *et aL* (1976, between 1.5 and 2.0 for lenses 50-70 years at 400 nm). There are problems with the data of Ziginan *et al.* because their densities at long wavelengths are of the order of half a log unit instead of the expected 0.05. Said's and Weale's (1969) data suggest a low extrapolated figure in the region of 0.65 at 400 nm for a 50-year-old lens whereas Fig. 3 shows a density of about 2.5 which is higher also than the figure of 1.4 for the "old" lens population Mellerio (1971). Weale (1985) shows a density of about 1.1 log units at 50 years and 400 nm for *in vivo* measurements and a spread of values rising from about 0.3 log units in young lenses to well over 1 log unit by 70 years. That the Said and Weale figures are low, as are others in the literature (e.g. Coren and Girgus, 1972, using a psychophysical method but tied to Said's and Weale's data) may arise because of the methods of measurement adopted. Density measures on excised lenses are higher at shorter wavelengths and have the advantage of being more direct than *in vivo* techniques. However, they suffer from the usual criticisms of all *in vitro* techniques, but even so their mutual agreement is not great. Small sample sizes may be another factor but even in large samples, as Coren and Giraus (1972) show with 256 subjects, the scatter cache one third of a log unit or more. It is unfortunate that with the most interesting older lenses and wavelengths below 400 nm, the variation of values within and between studies reaches such high values. The reasons for this probably lie mainly with true inter-lens variations. Selection criteria for including a lens in a study vary from laboratory to laboratory and this must influence the outcome of the measurements. The role of light scatter and fluorescence, too, may or may not, be allowed for in the measurements of light loss by calculation or by spectrophotometric configuration. It is likely that the selection of clear lenses for the present paper, which limits the investigation to the study of photometrically young but chronologically old lenses (Lerman, 1983), may be another factor responsible for the differences between the investigations.

The increasing yellowing with age is demonstrated in Figs 4 and 5. There is little increase in light loss at longer wavelengths until the eighth decade, but at shorter wavelengths, there is an accelerating increase in loss. But which part of the lens is responsible and is this loss due to absorption or scatter? And does it rise because of an increase in concentration of absorber or scatter, or because of an increase in pathlength?

Reasons for increased yellowing

Inspection of Fig. 6 shows that there is no increasing light loss with age in the cortex but that there is an important rise in loss per unit pathlength in the nucleus. It is possible, therefore, to suggest reasons for the increase in lens yellowing as it grows older. The cortex gets thicker and the light loss increases only because of this thickness change, its light loss per unit pathlength remaining constant. In the nucleus, the pathlength remains constant but the loss per unit pathlength goes up thus raising the nuclear photometric density with age. Taken overall, these processes sum to give a yellowing lens. The way this summation occurs, fortuitously leads to the conclusion reached by Mellerio (1971) that yellowing is due to increased pathlength with age and not pigment accumulation. This is an important realisation because it means that the visual effects of the increased yellowing proposed in 1971 must still stand.

The objections of Zeimer and Noth (1984) regarding the large range of light loss per unit pathlength values reported by Mellerio (1971) are largely overcome in this study. The range of values is smaller and a different technique gives a surer but more complex set of reasons for the yellowing of the lens with age.

The increasing light loss in the nucleus may be due to rises in absorption brought about by pigment deposition, or by increased scattering from newly laid down scattering centres or by existing components that undergo conformational change that increase their scattering capability. That protein molecules can act as effective scattering centres is well known so the report by Fagerholm *et al.* (1 98 1) that the nuclear protein concentration does not increase with age might favour the suggestion that increases in scattering can not be responsible for nuclear yellowing. However, Siew *et al.* (1981) have shown that collapse of the nuclear network of proteins into smaller denser conglomerates on aging will increase scatter loss. The question is still an open one, but increasing nuclear scatter would not appear to be a major factor in aging.

Table 1 summarises the findings of this study by comparing the properties and changes in a 25 and a 70-year-old lens at a wavelength of 400 nm, chosen because it is close to the shortest that is of visual interest. The ratio of the total density of the cortex to that of the nucleus is approximately the same for both ages. This supports the conclusion that it is fortuitous that the light loss changes in both components sum to resemble changes throughout an assumed homogeneous one-component lens.

Finally, it is interesting to note the turbidity produced in the freshly homogenised samples, especially in the cortex, due presumably to disruption of local relations between water and protein aggregations (Maraini and Mangili, 1973). This may be of interest when examined in the light of the work on the reasons for cold cataracts, water imbalance and "water lakes" reported by Lerman (1983). The self-clearing of the samples reported here is also of interest, especially in view of the ideas of lenticular syneresis developed by Siew *et al.* (1981). Could this clearing be a form of "self-healing"? If so, it is certainly worth further investigation.

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	25 years				70 years			
	x (mm)	D_S (mm^{-1})	D	% total density	x (mm)	D_S (mm^{-1})	D	% total density
nucleus	3.5	0.25	0.88	0.81	3.5	1.00	3.5	0.89
cortex	0.8	0.25	0.20	0.19	1.8	0.25	0.4	0.11
total density			1.08				3.95	

Table 1. Summary of the properties of a 25- and a 70-year-old lens at a wavelength of 400 nm. x is pathlength at the lens pole; D_S is light loss (density) per unit pathlength, D is light loss (density) of nucleus or cortex.

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