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LIGHT ABSORPTION AND SCATTER IN THE HUMAN LENS

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THE human crystalline lens grows bigger and yellower with increasing age (WEALE, 1963). SAID and WEALE (1959) were able to quantify the "yellowing" process in living human lenses in terms of photometric density, which increased towards the shorter wavelength end of the spectrum owing, they thought, to increased pigmentation and light scattering. There are at least three ways in which this could come about-either more absorbing (or scattering) pigment is deposited in the lens thus increasing its concentration, or the lens becomes thicker increasing the optical pathlength, or some combination of these two processes is established.

In order to decide if one of these alternatives applies, the light loss, expressed as photometric density per unit pathlength, was determined in twenty excised human lenses.

METHODS

A. *Experimental*

Human lenses were taken within 8 hr of death and used either at once, or stored overnight in a moist saline atmosphere at 4'. This storage produced no observable optical changes. Each lens was examined carefully and was rejected if not perfectly clear and free from opacities. This criterion severely reduced the number of lenses that could be used. Any attached pigment or vitreous was carefully removed and the lens was then put into a snugly-fitting collar in a small cell previously filled with saline which had been boiled and cooled to remove dissolved gases (Fig. 1). A cover slip was placed on top of the collar in such a way that no air bubbles were trapped. Excess saline was mopped up with filter paper.

The apparatus is shown in Fig. 1. The colour filters were by BaIzers Aktiengesellschaft, Liechtenstein, and when calibrated were found to have half-bandwidths of around 10 nm and peak transmissions at 404, 447, 506, 568 and 620 nm. The 404 nm filter had a secondary peak at 590 nm which was visually more important than the short wavelength peak. However, when the spectral sensitivity of the recording film (I1ford, FP4) and the spectral emission of the tungsten lamp were allowed for, the effective energy at long wavelengths was insignificant. The filters covered only the visible spectrum, for this is where the visual effects of yellowing are of importance.

A calibrated density step wedge was placed in the position of the lens and photographed in coloured light; the wedge was then replaced by the lens cell and another photograph taken. This procedure was repeated for each colour filter.

The lens fibres of the cortex were easily seen and hindered measurement of photometric density across the lenses. To remove the fibre outlines, the lenses were spun about their polar axes during photography. For this purpose the circular lens cell fitted into the centre of a large ball race driven by a pulley belt and electric motor.

In the first experiments of the series, the lenses were spun at 1000 rev/min. This speed was reduced later to 300 rev/min because at the higher rate the centrifugal stresses were of the same order as the elastic limit of the lens substance (FISHER, 1971) and it was thought that this might affect the optical properties of the tissue. The density measurements have revealed no systematic differences between fast and slow spun lenses, so the results have been considered together.

The photometric density of each lens was determined in four meridians with a Wooster Scanning Micro-densitometer Mk. III (Crystal Structures Ltd., Cambridge). The photographs of the standard wedge were used to calibrate the traces which were subsequently smoothed and averaged. The estimated measuring error was ±0.02 log units.

To calculate light loss per unit pathlength the lens thickness must be known. This was determined in the following way. The lens was balanced in air upon the end of a specially shaped spindle of a vertically mounted motor and excess moisture was removed with blotting paper. Whilst the lens was spinning (at 300 or 1000 rev/min) several photographs were taken of the profile using an electronic flash as light source. The lens was turned over and the profile of its other surface photographed. The profiles of each surface were traced from the enlarged film, averaged, and joined together for the smoothest fit at the equator to obtain the complete profile. From this profile the thickness, x, of the lens was determined for selected points of its radius, R. The estimated errors of measurement were for x, ±0.07 mm. and for R, ±0.1 mm.

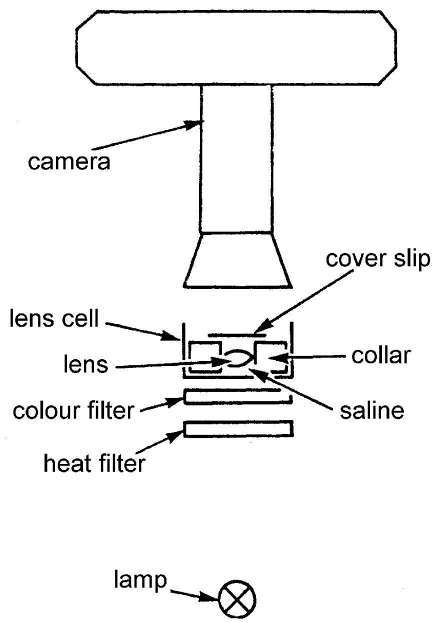

FIG. 1. Diagram of apparatus. The lens cell is full to the cover slip with saline, and a piece of 2 mm heat absorbing glass acts as a heat filter.

The changes in lens shape between spinning at 300 and 1000 rev/min must be less than the probable measurement error for no systematic differences could be detected between the high and low rotations. The difference between spinning in air and saline must also be small, and in any case the maximum change in lens thickness that can be produced in a young lens before exceeding the elastic limit is never more than 4 or 5 per cent (FISHER, 1971).

B. *Theory*

To observe lens yellowing, changes in the shorter wavelengths are compared with the changes in the longer wavelength part of the spectrum for the following reason. Figure 2 shows averaged density traces D_{404} and D_{620} for violet and red light respectively for a 28-year-oId lens. The density in red light is lower than that in violet light, but it increases towards the lens periphery. This increase is due to "vignetting" caused by the residual optical power of the lens in saline. It is impossible to neutralize the lens completely as its refractive index is not constant throughout its extent. This vignetting is not apparent in the trace for violet light because it is masked by the much greater density of the lens. If the red trace is substracted from the violet, the density difference, ΔD, is obtained and the effect of the vignetting is removed. The magnitude of the vignetting is expected to vary with wavelength, but the change from red to violet will be small and is ignored.

Reference to Fig. 3 may be made to justify the choice of 404 nm and 620 nm as the wavelengths used to calculate ΔD. This figure shows the photometric density at the five wavelengths already mentioned for groups of old and young Ienses as defined in the figure caption. Also plotted is the difference between these two groups. This difference increases with decreasing wavelength showing that the greatest changes occur between the red and violet light.

The density difference may be represented by the equation below which assumes the validity of Beer's and Lambert's laws.

$$\Delta D = D_{404} - D_{620} = \frac{c \cdot x}{2.3}(b_{404} - b_{620}) \qquad (1)$$

where ΔD is the density difference; D_{404} and D_{620}, the photometric density at 404 and 620 nm respectively; c, the concentration of a hypothetical absorbing pigment; *x*, the pathlength traversed by the light (thickness of lens); and β_{404} and β_{620} the molar absorption coefficients at the two wavelengths.

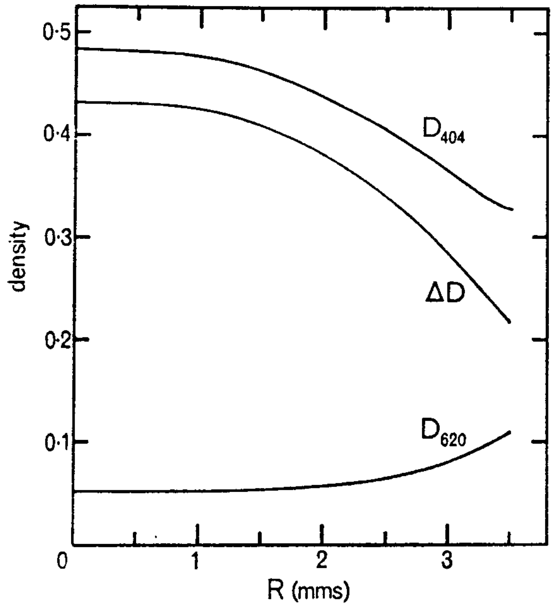

FIG. 2. Averaged photometric density traces plotted against lens radius, ***R***, from a 28-year-old lens. The upper trace, D_{404}, is for violet light (404 nm) and the lower, D_{620}, for red light (620 nm). ΔD shows the difference between D_{404} and D_{620}.

In equation (1) transmission losses have been considered wholly in terms of absorption. Reflection and scatter have been ignored although they are included in the density measurements. Reflection losses at the salinellens interfaces are small because their refractive indices are not too dissimilar, and reflections from the top and bottom of the lens cell will have been cancelled out by similar reflections from the top and bottom of the standard density wedge used to calibrate the films. Scatter losses require further consideration.

The only type of scattering likely to be of importance in the normal lens is Rayleigh scattering where the size of the scattering centres is small compared with the wavelength (λ) of the light. In this case the quantity of light scattered is proportional to λ^{-4}.

If *a* and *s* represent the fraction of light incident on the lens lost by absorption and scatter respectively, the transmission is:

$$T = 1 - a - s$$

Now for Rayleigh scattering, $s = B.\lambda^{-4}$ where *B* is a constant, so

$$T = 1 - a - B \cdot l^{-4} \quad (2)$$

If lens transmission is plotted against λ^{-4} some indication of the importance of scatter loss may be obtained.

Conclusions about pigment concentration and distribution are not affected by scattering losses provided it is remembered that the term "concentration" refers to what may be a mixture of an absorbing pigment and a scattering substance, although it is possible that one molecule is responsible for both. With the method of measurement employed here, it is not possible to decide how much of the light loss is due to scattering and how much due to absorption.

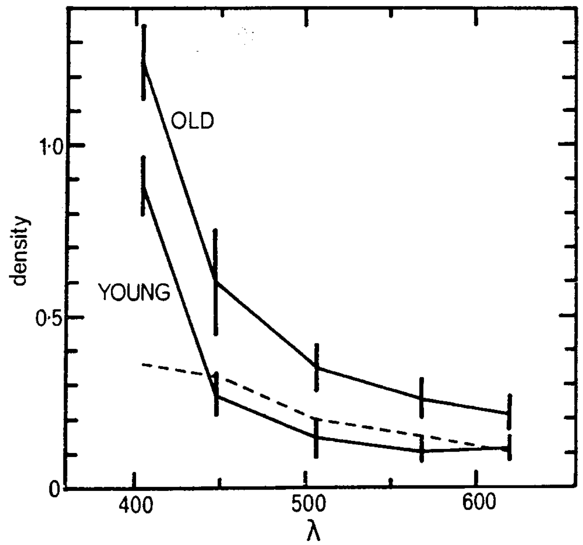

FIG. 3. Photometric density at the lens pole (radius, *R*=0) plotted against wavelength, λ (nm). The data are from two groups of lenses. The upper OLD points had ages 46, 49, 59 and 66 years. The lower YOUNG points had ages 19, 22, 22, 215, 31 and 32 years. The vertical bars represent ±1 S.D. The dashed line plots the difference between the OLD and YOUNG data.

RESULTS

Figure 3 shows the photometric density at the centre (*R* = 0) of 10 lenses for the five different wavelengths. The lenses were split into two groups as explained in the figure caption, young below 32 years and old over 46 years. The curves resemble those of SAID and WEALE (1959), except that they are about ⅓ of a log unit higher in violet light. Also plotted is the difference between the two curves which shows that the increase in density with age is progressively greater at shorter wavelengths.

In Fig. 4 the old and the young lens data are again employed: this time the transmission, *T*, is plotted against λ^{-4}. The young data points lie in a curve, but the old points approximate to a straight line which extrapolates to the *T* axis at 0.71.

The production of a mean pathlength versus radius plot by avergaing data from all the lenses to yield the curve in Fig. 5 was arbitrary, and can be justified only on grounds of convenience for later consideration, because both the lens shape and size change when it grows older. The thickness of the lens, x, at its pole (R = 0) increases, and the ratio of equatorial diameter to polar thickness decreases with age (Fig. 6). At first sight this might be interpreted as the lens' becoming more spherical, but this is not so, the shape changes being complex with the major portion confined to a conical bulging of the posterior surface (FISHER, 1971).

The photometric density difference was calculated at different points across each lens and plotted against the optical pathlength at those points. Figure 7 shows two typical plots, the straight lines being least squares fits. All the lenses produced rectilinear plots and, except in one case, the points were never further from the fitted line than their estimated errors of measurement.

The gradient of the fitted lines, $\Delta D/x$, is the density difference or light loss per unit pathlength and in Fig. 8 is shown plotted against age. Even including the solitary point at 54 years, there is no significant correlation of $\Delta D/x$ with age.

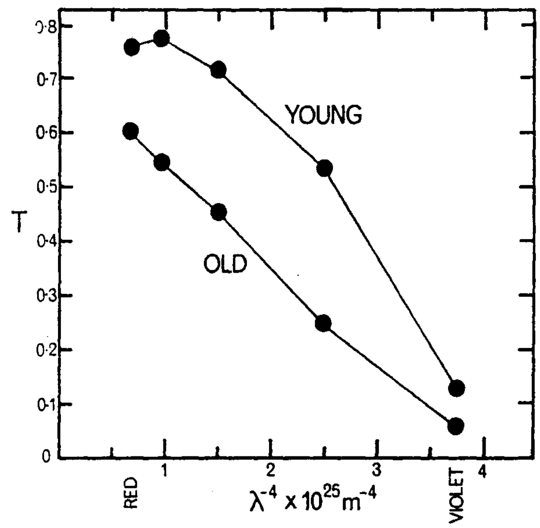

FIG. 4. Transmission, *T*, plotted against $\lambda^{-4} \times 10^{25}$ m^{-4} for the two groups of lenses described in Fig. 3.

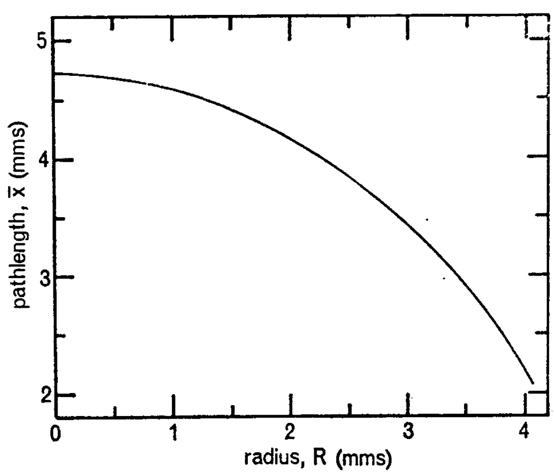

FIG. 5. Mean pathlength, $\overline{x}$, plotted against lens radius, *R*. The mean value was obtained by averaging data from the 20 lenses in this study, but as the lens changes shape with age certain caveats must be placed on this curve (see text). Mean age of lenses; 37.2 years.

DISCUSSION

(a) *Absorption and scatter*

As pointed out in a previous section, it is not possible to analyse the transmission losses into those due to absorption and those due to scatter, although Fig. 4 suggests that scattering is more important for old than for young lenses. This observation is supported by the work of WOLF and GARDINER (1965) who showed that lenticular back scatter of white light increased by more than an order of magnitude between the ages of 20 and 80. Examination of BOETTNER's and WOLTER's (1962) transmission and scatter data for the blue end of the visible spectrum reveals a similar trend.

The analysis could be assisted by an experimental determination of the angular distribution of the fraction of light scattered or by calculation of this fraction using the constant *B* in equation (2).

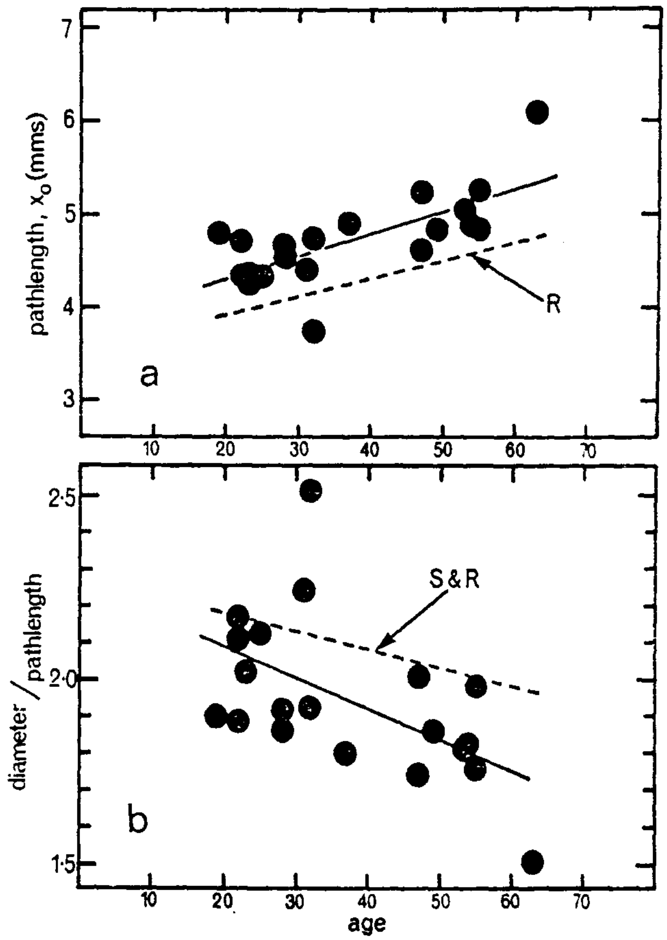

FIG. 6. (a) Pathlength, x_0, at the lens pole (R = 0) plotted against age for 20 lenses. The straight line is fitted by a least squares method; the increase is significant (r = 0.71, P = 0.001). The dashed line, *R,* was fitted through the data of RAEDER (1922) for 48 emmetropic lenses and is shown for comparison. (b) The ratio of lens diameter to polar pathlength, plotted against age. The fitted line shows a significant decrease (r = -0.55, P = 0.02). For comparison the dashed line, *S* and *R,* is the lens diameter data of SMITH (1883) divided by the thickness data of RAEDER (1922). Raeder made his measurements in living eyes but Smith examined excised lenses so his figures are smaller than for living lenses. This disparity is greatest at the younger ages thus reducing the slope of the *S* and *R* line in the figure.

This latter approach is probably impossible because it depends on the refractive index which is not constant, and on the number, which is unknown, of scattering dipoles in the light path. It is also unlikely that absorption does not change with λ; it appears to do so in the case of the young lenses (Fig. 4) where it would be responsible for the increasingly negative slope of their plot.

(b) *Distribution of lens pigment*

If the light loss is due to only one lens pigment and it is assumed that scatter and absorption losses are equivalent, then equation (1) may be written:

$$\frac{\Delta D}{x} = \frac{c \cdot \Delta b}{2.3} \tag{3}$$

where $\Delta\beta = (\beta_{404} - \beta_{620})$ showing that $\Delta D/x$ is proportional to pigment concentration, the molar absorption coefficients being constants. If two or more pigments are present the equation is

$$\frac{\Delta D}{x} = \frac{1}{2.3}[c_1 \cdot \Delta b_1 + c_2 \cdot \Delta b_2 \ldots.] \tag{4}$$

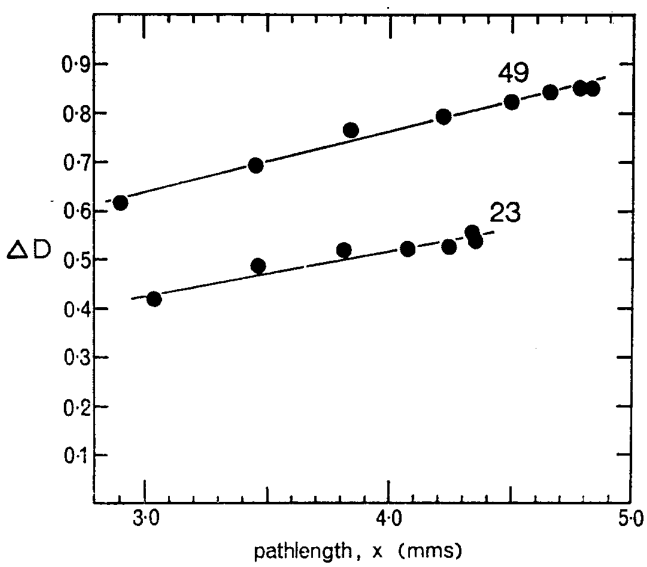

FIG. 7. Density difference, ΔD, plotted against pathlength, x, for a 49 and a 23 year lens. The lines are least squares fits. Estimated error of measurement in ΔD is ± 0.03 log units.

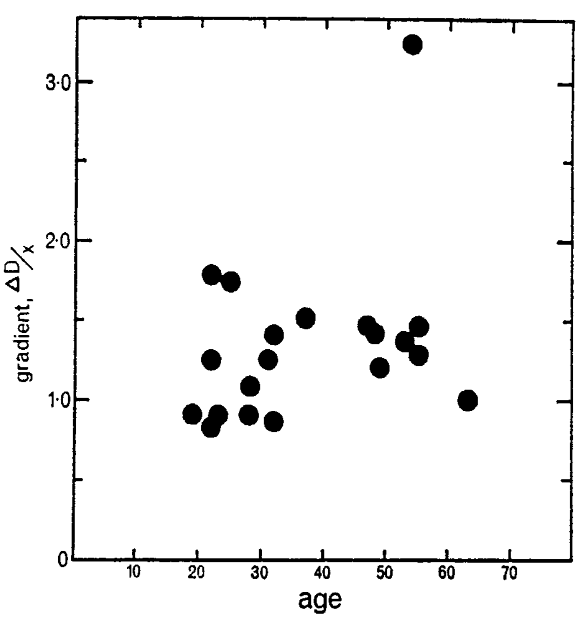

FIG. 8. Gradient, $\Delta D/x$, of the fitted lines (see Fig. 7) for the 20 lenses plotted against age. There is no significant change with age.

where the suffixes indicate difrerent pigments. If $\Delta\beta_1 = \Delta\beta_2$ . . . it would in effect be the same as having only one pigment present.

The rectilinear nature of the plots of ΔD vs. x (Fig. 7) shows that c in equation (3) or the sum of the terms in brackets in equation (4) are constant across the lens. Therefore, if only one pigment is present its concentration is uniform throughout the central 7-8 mm of the lenses that were examined. With more than one pigment, as equation (4) shows, their individual concentrations could vary but their sum cannot. It would be reasonable to conclude, though, that these concentrations are constant or else the distributions would have to be complementary to produce relationships like those in Fig. 7.

Figure 8 shows that $\Delta D/x$ does not change with age, and this leads to the conclusion that the distribution of lenticular pigment remains unchanged between 20 and 60 years.

(c) Senile yellowing

Whether one thinks in terms of pigment concentration or of light loss per unit pathlength, the constancy of $\Delta D/x$ with age denies pigment build-up as a cause of senile yellowing. Although $\Delta D/x$ does not change, there are obviously changes in lenticular pigment. COOPER and ROBSON (1969) have shown that extracted lens pigment changes its wavelength of maximum absorption during the fourth decade of life, and in Fig. 4 the difference between the young and old lenses suggests changes in the importance of the fractions a and s in determining lens transmission. Whatever changes occur they must be complex and complementary for there is no overall change in light loss per unit pathlength.

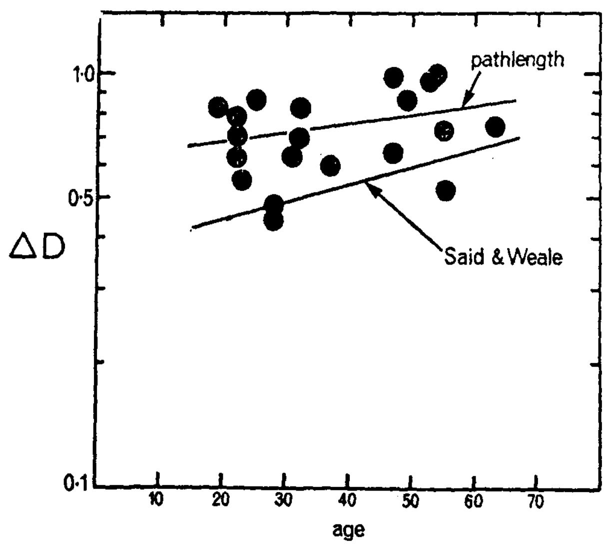

FIG. 9. Density difference, ΔD, at the lens pole (R = 0) plotted against age. The value of ΔD was obtained from the fitted lines of the ΔD vs. x plots (Fig. 7) at the maximum value of x which corresponded with R = 0. Note the ordinate has a logarithmic scale. The line labelled "Pathlength" is taken from Fig. 6a and is vertically placed in an arbitrary position so that it passes amongst the data points. The "Said and Weale" line is taken from their 1959 density data and has been adjusted to the same wavelengths and mean lenticular pathiengths as used in the present study (see text).

If $\Delta D/x$ does not increase with age (Fig. 8) the alternative of increasing pathlength must be considered to see if it is responsible for reduced senile transmission. Figure 6a demonstrates the significant increase of polar thickness with aging, and Fig. 9 shows the density difference, ΔD, plotted on a logarithmic scale against age. The scatter of points is very large, (a difficulty also encountered by BOETTNER and WOLTER, 1962) but the fitted line from Fig. 6a is shown arbitrarily placed amongst the points. That this line will pass amongst the points indicates that increasing thickness could explain the reduced transmission shown here and elsewhere (SAID and WEALE, 1959). Also in Fig. 9 for comparison is a line taken from the data of Said and Weale and adjusted to the wavelengths and mean lens pathlengths of the present study. (Said and Weale measured lens transmission with a large pupil which had an effective pathlength smaller than the polar pathlength, see below.) If the line was slightly higher it would pass through the points satisfactorily; the difference could well be a matter of technique or selection of material.

(d) Visual importance of lens shape

WEALE (1961, 1968) considered what effects the variable pathlength of the lens had upon various aspects of vision. For his calculations he assumed the absorbing or scattering pigment was uniformly distributed across the lens, and this study confirms his assumption. Although the only lens profile available to him was unusually thin, the magnitude of the effect is not greatly altered if calculated from the pathlength data of Fig. 5.

The modification of the Stiles-Crawford effect of the first kind, SCE 1, by the lenticular shape, as demonstrated in WEALE'S (1961) paper, has been recalculated here using the mean pathlength data of Fig. 5. If T_c and T_p refer to lens transmission at the centre and periphery respectively, then the SCE 1 that would be measured if the lens was flat or absent (the retinal SCE 1) is:

$$h'=h\,\frac{T_c}{T_p} \tag{5}$$

where η is the ratio of the light intensity of the central to peripheral beams when matched for equal brightness, it being assumed that the position of maximum luminous efficiency ($\eta = 1$) is at the pupil centre and that this corresponds with the lens pole. Figure 10 shows plots of $1/\eta$ against λ as taken from WALRAVEN and BOUNIAN (1960) using the results of STILES (1939). Also shown is the corrected ratio, $1/\eta'$using the pathlength data of this study, and of Weale's paper. Below about 500 nm the retinal SCE 1 is seen to be greater than would be expected from measurement of the central and peripheral beams outside the eye.

The variation of lenticular pathlength would not be expected to modify the SCE 2 (change in hue of the peripheral beam) as it is usually measured with monochromatic light. With non-monochromatic sources the lens effect might have to be considered with SCE 2.

Another way of considering the effects of variable lenticular pathlength is to use the effective pupillary area, A_e, in calculating light flux through the pupil. A_e, is defined as that pupillary area which would admit as much light into an eye with a disk-shaped lens of thickness everywhere equal to its polar pathlength x_o, as is admitted by a pupil of true area A_o, which for convenience of calculation may be regarded as having a lens of integrated or effective pathlength x_e This effective pathlength varies with pupil size as shown in Fig. 11a.

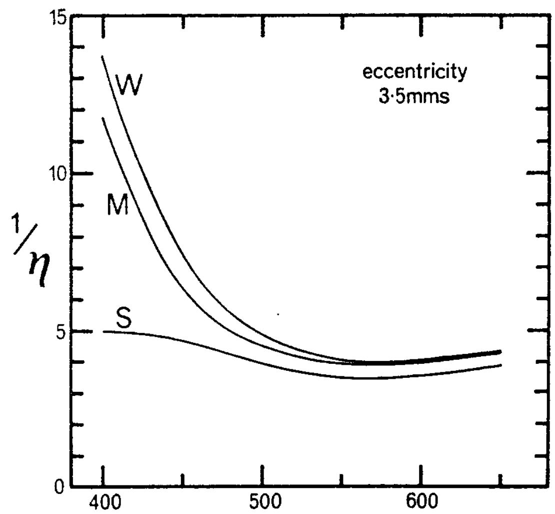

FIG. 10. Reciprocal of the relative luminous efficiency, η, plotted against wavelength, λ nm, for a point of entry for the peripheral beam 3.5 mm from the point of maximal efficiency. Curve *S* is STILES (1939) curve from WALRAVEN and BOUMAN (1960); *W* is this curve corrected for lenticular pathlength variations using the pathlength data available to WEALE (1961); *M* is calculated using the data of Fig. 5.

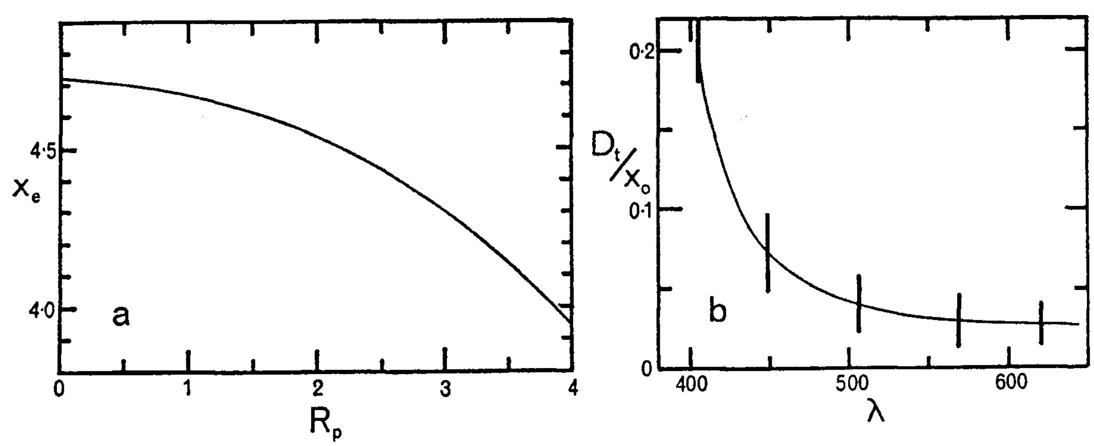

FIG. 11. (a) Effective, or integrated lenticular pathlength, x_e mm, plotted against real pupil radius, R_p mm. (b) Photometric density per unit pathlength, D_t/x_o, at the lens pole plotted against wavelength, λ nm, for 10 lenses of all ages. The vertical bars represent ± 1 S.D.

This figure is based on the mean pathlengths in Fig. 5, so the value for A_e given below is to some extent age dependent. (The pupil radius in Fig. 11a is the real radius and is not corrected for corneal magnification. Figure 12 may be used with real or apparent dimensions.)

$$\log A_e = \log A_o + \frac{m}{2.3}(x_o - x_e) \tag{6}$$

and

$$\frac{m}{2.3}=\frac{D_t}{x_o} \tag{7}$$

where μ is the absorption coefficient and D_t, the photometric density.

Figure 11 b shows average values of D_t/x_o at the lens pole (R = 0) plotted against λ for a group of 10 lenses. Figure 12 shows A_e plotted against pupil radius, R_p for four different wavelengths. A_e is only much larger than the true area at very short wavelengths and with pupil diameters greater than, say, 5 mm. As A_e varies with λ, it follows that the spectral distribution of a light may be modified by alterations in pupil size.

CRAWFORD (1949) measured the scotopic visibility curve in 50 people with natural pupils. If the mean diameter of the pupil was 7 mm, the changes in the scotopic curve for viewing through a pupil of 2 mm may be calculated. This would represent an extreme case of a laboratory experiment for naturally the pupil would never be so small in scotopic conditions, even in old people.

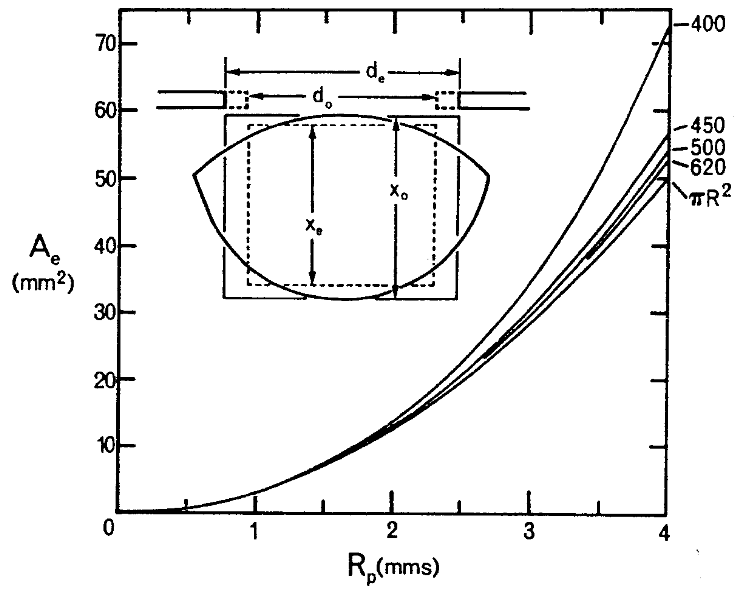

FIG. 12. Effective pupillary area, A_e, plotted against pupil radius, R_p. The curves are labelled on the right with their respective wavelengths; the lowest curve, πR^2, shows true pupillary area. Real or apparent pupillary dimensions may be used. Inset: a lens behind a pupil of area $A_o = \pi d_o^2/4$ may be replaced by the disc-shaped lens of thickness x. which is shown by the dashed line. Alternatively, if the lens is replaced by the disc-shaped lens of thickness x_o shown in solid line, for equal light flux a pupil of larger area, $A_e = \pi d_e^2/4$, is required.

The ratio of the light flux for large to small pupils is given by the ratio of the large to small effective pupil areas. This ratio at four wavelengths is compared with that in red light (620 nm) and Table 1 shows by what factor the scotopic visibility curve should be multiplied to correct for viewing through a 2 mm pupil. As the table shows, the correction is small except at short wavelengths. These corrections lead to a spectral shift of the peak of the curve towards longer wavelengths, but it is small, probably no more than 1 nm.

TABLE 1

λ (nm)	factor
400	0.865
450	0.945
500	0.985
550	0.995
620	1.000

Factor to modify scotopic visibility curve for 2 mm pupil viewing.

Acknowledgements I should like to thank Mr. R. F. FISHER for help with this study, and Mrs. B. PETTET for technical assistance.

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Abstract-The light transmission of freshly excised normal human lenses has been measured at five visible wavelengths. The lens profile has also been recorded and used to calculate light loss per unit pathlength of lens. This loss, although made up of absorption and scatter the contributions of which cannot be quantified, may be regarded as due to a hypothetical absorbing pigment. The loss is directly proportional to pigment concentration and remains constant throughout the central 7-8 mm of the lens and between the ages of 20 and 60. Because of this constancy, senile lenticular yellowing is attributed to increasing lens thickness. The visual importance of this light loss in the lens is briefly considered.

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A calibrated density step wedge was placed in the position of the lens and photographed in coloured light; the wedge was then replaced by the lens cell and another photograph taken. This procedure was repeated for each colour filter.

The lens fibres of the cortex were easily seen and hindered measurement of photometric density across the lenses. To remove the fibre outlines, the lenses were spun about their polar axes during photography. For this purpose the circular lens cell fitted into the centre of a large ball race driven by a pulley belt and electric motor.

In the first experiments of the series, the lenses were spun at 1000 rev/min. This speed was reduced later to 300 rev/min because at the higher rate the centrifugal stresses were of the same order as the elastic limit of the lens substance (FISHER, 1971) and it was thought that this might affect the optical properties of the tissue. The density measurements have revealed no systematic differences between fast and slow spun lenses, so the results have been considered together.

The photometric density of each lens was determined in four meridians with a Wooster Scanning Micro-densitometer Mk. III (Crystal Structures Ltd., Cambridge). The photographs of the standard wedge were used to calibrate the traces which were subsequently smoothed and averaged. The estimated measuring error was ±0.02 log units.

To calculate light loss per unit pathlength the lens thickness must be known. This was determined in the following way. The lens was balanced in air upon the end of a specially shaped spindle of a vertically mounted motor and excess moisture was removed with blotting paper. Whilst the lens was spinning (at 300 or 1000 rev/min) several photographs were taken of the profile using an electronic flash as light source. The lens was turned over and the profile of its other surface photographed. The profiles of each surface were traced from the enlarged film, averaged, and joined together for the smoothest fit at the equator to obtain the complete profile. From this profile the thickness, x, of the lens was determined for selected points of its radius, R. The estimated errors of measurement were for x, ±0.07 mm. and for R, ±0.1 mm.

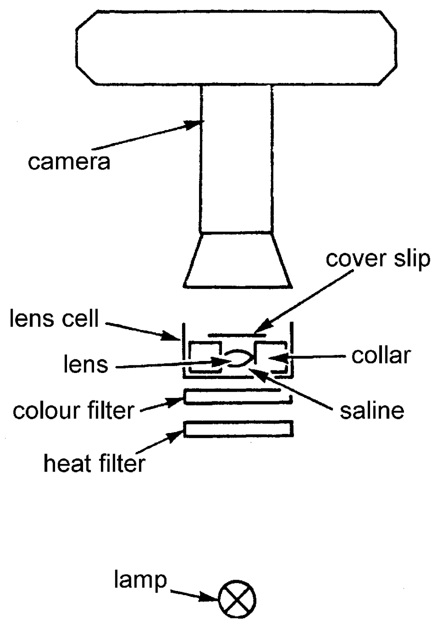

FIG. 1. Diagram of apparatus. The lens cell is full to the cover slip with saline, and a piece of 2 mm heat absorbing glass acts as a heat filter.

The changes in lens shape between spinning at 300 and 1000 rev/min must be less than the probable measurement error for no systematic differences could be detected between the high and low rotations. The difference between spinning in air and saline must also be small, and in any case the maximum change in lens thickness that can be produced in a young lens before exceeding the elastic limit is never more than 4 or 5 per cent (FISHER, 1971).

B. *Theory*

To observe lens yellowing, changes in the shorter wavelengths are compared with the changes in the longer wavelength part of the spectrum for the following reason. Figure 2 shows averaged density traces D_{404} and D_{620} for violet and red light respectively for a 28-year-oId lens. The density in red light is lower than that in violet light, but it increases towards the lens periphery. This increase is due to "vignetting" caused by the residual optical power of the lens in saline. It is impossible to neutralize the lens completely as its refractive index is not constant throughout its extent. This vignetting is not apparent in the trace for violet light because it is masked by the much greater density of the lens. If the red trace is substracted from the violet, the density difference, ΔD, is obtained and the effect of the vignetting is removed. The magnitude of the vignetting is expected to vary with wavelength, but the change from red to violet will be small and is ignored.

Reference to Fig. 3 may be made to justify the choice of 404 nm and 620 nm as the wavelengths used to calculate ΔD. This figure shows the photometric density at the five wavelengths already mentioned for groups of old and young Ienses as defined in the figure caption. Also plotted is the difference between these two groups. This difference increases with decreasing wavelength showing that the greatest changes occur between the red and violet light.

The density difference may be represented by the equation below which assumes the validity of Beer's and Lambert's laws.

$$\Delta D = D_{404} - D_{620} = \frac{c \cdot x}{2.3}(b_{404} - b_{620}) \qquad (1)$$

where ΔD is the density difference; D_{404} and D_{620}, the photometric density at 404 and 620 nm respectively; c, the concentration of a hypothetical absorbing pigment; *x*, the pathlength traversed by the light (thickness of lens); and β_{404} and β_{620} the molar absorption coefficients at the two wavelengths.

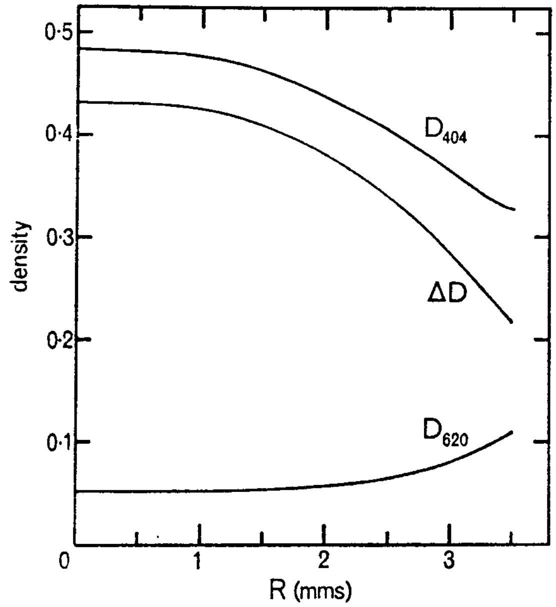

FIG. 2. Averaged photometric density traces plotted against lens radius, ***R***, from a 28-year-old lens. The upper trace, D_{404}, is for violet light (404 nm) and the lower, D_{620}, for red light (620 nm). ΔD shows the difference between D_{404} and D_{620}.

In equation (1) transmission losses have been considered wholly in terms of absorption. Reflection and scatter have been ignored although they are included in the density measurements. Reflection losses at the salinellens interfaces are small because their refractive indices are not too dissimilar, and reflections from the top and bottom of the lens cell will have been cancelled out by similar reflections from the top and bottom of the standard density wedge used to calibrate the films. Scatter losses require further consideration.

The only type of scattering likely to be of importance in the normal lens is Rayleigh scattering where the size of the scattering centres is small compared with the wavelength (λ) of the light. In this case the quantity of light scattered is proportional to λ^{-4}.

If ***a*** and ***s*** represent the fraction of light incident on the lens lost by absorption and scatter respectively, the transmission is:

$$T = 1 - a - s$$

Now for Rayleigh scattering, $s = B.\lambda^{-4}$ where ***B*** is a constant, so

$$T = 1 - a - B \cdot l^{-4} \quad (2)$$

If lens transmission is plotted against λ^{-4} some indication of the importance of scatter loss may be obtained.

Conclusions about pigment concentration and distribution are not affected by scattering losses provided it is remembered that the term "concentration" refers to what may be a mixture of an absorbing pigment and a scattering substance, although it is possible that one molecule is responsible for both. With the method of measurement employed here, it is not possible to decide how much of the light loss is due to scattering and how much due to absorption.

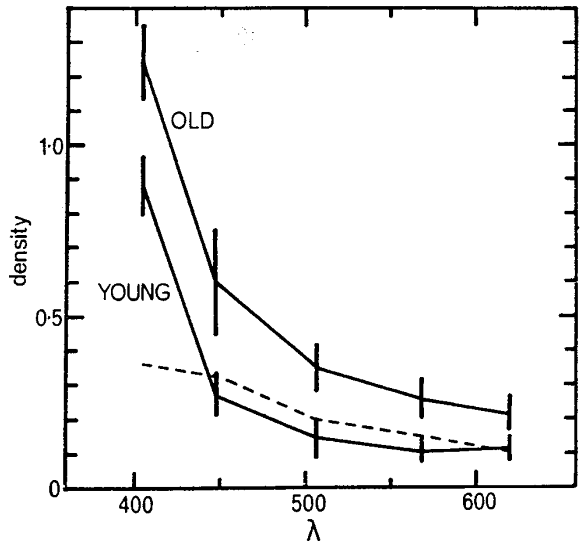

FIG. 3. Photometric density at the lens pole (radius, R=0) plotted against wavelength, λ (nm). The data are from two groups of lenses. The upper OLD points had ages 46, 49, 59 and 66 years. The lower YOUNG points had ages 19, 22, 22, 215, 31 and 32 years. The vertical bars represent ±1 S.D. The dashed line plots the difference between the OLD and YOUNG data.

RESULTS

Figure 3 shows the photometric density at the centre (R = 0) of 10 lenses for the five different wavelengths. The lenses were split into two groups as explained in the figure caption, young below 32 years and old over 46 years. The curves resemble those of SAID and WEALE (1959), except that they are about ⅓ of a log unit higher in violet light. Also plotted is the difference between the two curves which shows that the increase in density with age is progressively greater at shorter wavelengths.

In Fig. 4 the old and the young lens data are again employed: this time the transmission, T, is plotted against λ^{-4}. The young data points lie in a curve, but the old points approximate to a straight line which extrapolates to the T axis at 0.71.

The production of a mean pathlength versus radius plot by avergaing data from all the lenses to yield the curve in Fig. 5 was arbitrary, and can be justified only on grounds of convenience for later consideration, because both the lens shape and size change when it grows older. The thickness of the lens, x, at its pole (R = 0) increases, and the ratio of equatorial diameter to polar thickness decreases with age (Fig. 6). At first sight this might be interpreted as the lens' becoming more spherical, but this is not so, the shape changes being complex with the major portion confined to a conical bulging of the posterior surface (FISHER, 1971).

The photometric density difference was calculated at different points across each lens and plotted against the optical pathlength at those points. Figure 7 shows two typical plots, the straight lines being least squares fits. All the lenses produced rectilinear plots and, except in one case, the points were never further from the fitted line than their estimated errors of measurement.

The gradient of the fitted lines, $\Delta D/x$, is the density difference or light loss per unit pathlength and in Fig. 8 is shown plotted against age. Even including the solitary point at 54 years, there is no significant correlation of $\Delta D/x$ with age.

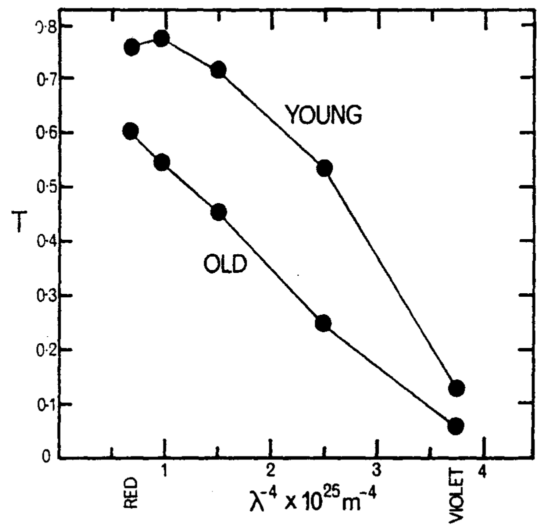

FIG. 4. Transmission, *T*, plotted against $\lambda^{-4} \times 10^{25}$ m^{-4} for the two groups of lenses described in Fig. 3.

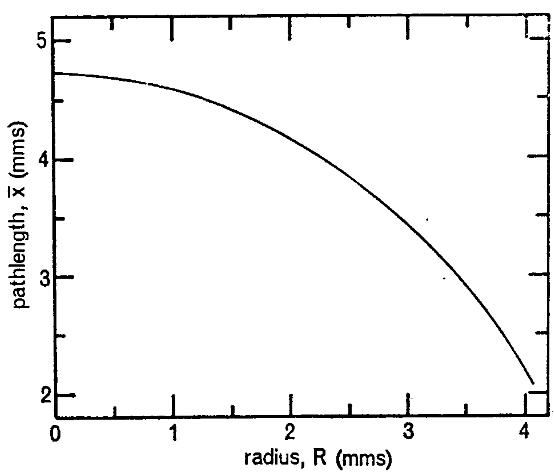

FIG. 5. Mean pathlength, $\bar{x}$, plotted against lens radius, *R*. The mean value was obtained by averaging data from the 20 lenses in this study, but as the lens changes shape with age certain caveats must be placed on this curve (see text). Mean age of lenses; 37.2 years.

DISCUSSION

(a) *Absorption and scatter*

As pointed out in a previous section, it is not possible to analyse the transmission losses into those due to absorption and those due to scatter, although Fig. 4 suggests that scattering is more important for old than for young lenses. This observation is supported by the work of WOLF and GARDINER (1965) who showed that lenticular back scatter of white light increased by more than an order of magnitude between the ages of 20 and 80. Examination of BOETTNER's and WOLTER's (1962) transmission and scatter data for the blue end of the visible spectrum reveals a similar trend.

The analysis could be assisted by an experimental determination of the angular distribution of the fraction of light scattered or by calculation of this fraction using the constant *B* in equation (2).

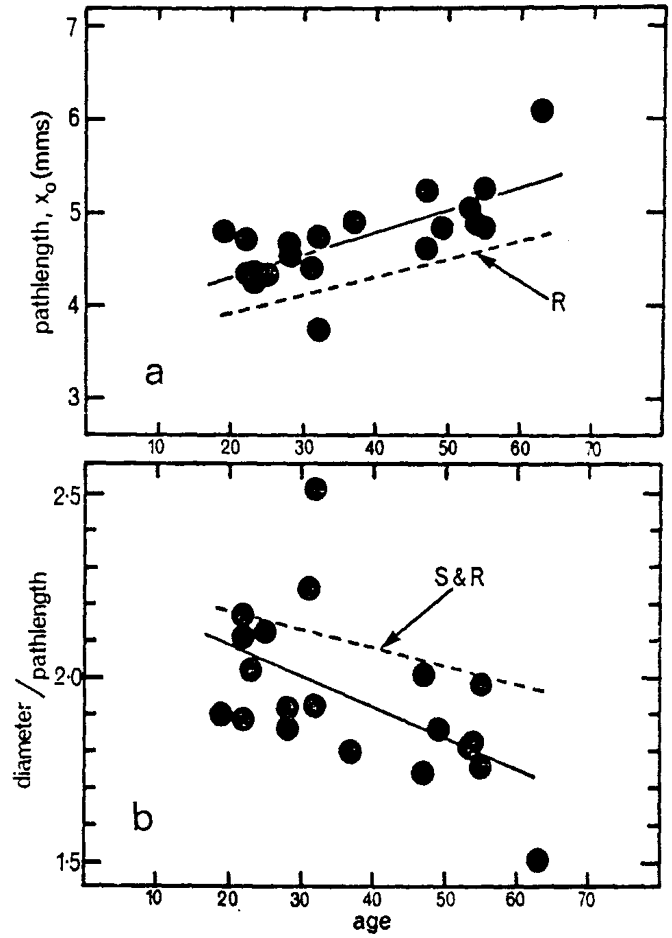

FIG. 6. (a) Pathlength, x_0, at the lens pole (R = 0) plotted against age for 20 lenses. The straight line is fitted by a least squares method; the increase is significant (r = 0.71, P = 0.001). The dashed line, *R,* was fitted through the data of RAEDER (1922) for 48 emmetropic lenses and is shown for comparison. (b) The ratio of lens diameter to polar pathlength, plotted against age. The fitted line shows a significant decrease (r = -0.55, P = 0.02). For comparison the dashed line, *S* and *R,* is the lens diameter data of SMITH (1883) divided by the thickness data of RAEDER (1922). Raeder made his measurements in living eyes but Smith examined excised lenses so his figures are smaller than for living lenses. This disparity is greatest at the younger ages thus reducing the slope of the *S* and *R* line in the figure.

This latter approach is probably impossible because it depends on the refractive index which is not constant, and on the number, which is unknown, of scattering dipoles in the light path. It is also unlikely that absorption does not change with λ; it appears to do so in the case of the young lenses (Fig. 4) where it would be responsible for the increasingly negative slope of their plot.

(b) *Distribution of lens pigment*

If the light loss is due to only one lens pigment and it is assumed that scatter and absorption losses are equivalent, then equation (1) may be written:

$$\frac{\Delta D}{x} = \frac{c \cdot \Delta b}{2.3} \tag{3}$$

where $\Delta\beta = (\beta_{404} - \beta_{620})$ showing that $\Delta D/x$ is proportional to pigment concentration, the molar absorption coefficients being constants. If two or more pigments are present the equation is

$$\frac{\Delta D}{x} = \frac{1}{2.3}[c_1 \cdot \Delta b_1 + c_2 \cdot \Delta b_2 \ldots.] \tag{4}$$

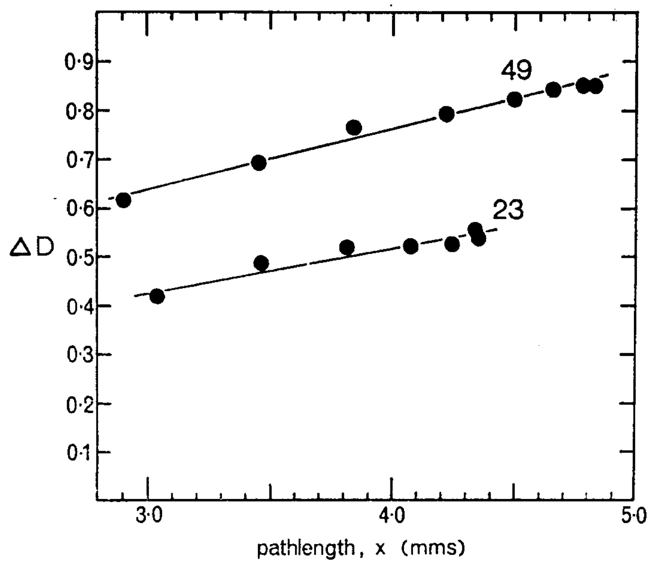

FIG. 7. Density difference, ΔD, plotted against pathlength, x, for a 49 and a 23 year lens. The lines are least squares fits. Estimated error of measurement in ΔD is ± 0.03 log units.

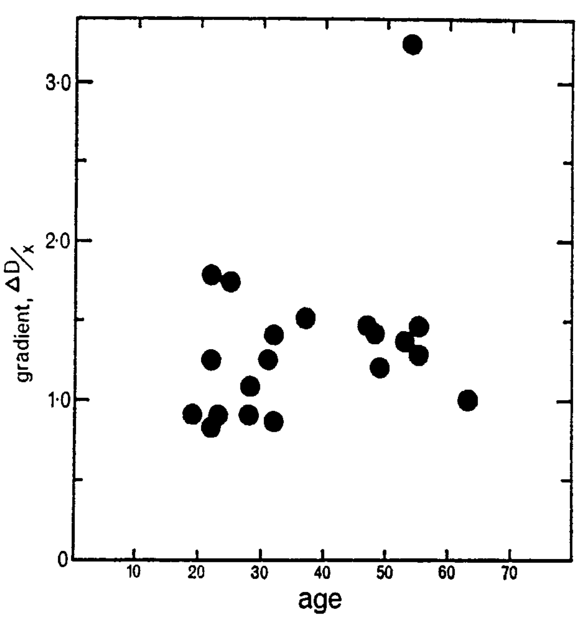

FIG. 8. Gradient, $\Delta D/x$, of the fitted lines (see Fig. 7) for the 20 lenses plotted against age. There is no significant change with age.

where the suffixes indicate difrerent pigments. If $\Delta\beta_1 = \Delta\beta_2$ . . . it would in effect be the same as having only one pigment present.

The rectilinear nature of the plots of ΔD vs. x (Fig. 7) shows that c in equation (3) or the sum of the terms in brackets in equation (4) are constant across the lens. Therefore, if only one pigment is present its concentration is uniform throughout the central 7-8 mm of the lenses that were examined. With more than one pigment, as equation (4) shows, their individual concentrations could vary but their sum cannot. It would be reasonable to conclude, though, that these concentrations are constant or else the distributions would have to be complementary to produce relationships like those in Fig. 7.

Figure 8 shows that $\Delta D/x$ does not change with age, and this leads to the conclusion that the distribution of lenticular pigment remains unchanged between 20 and 60 years.

(c) Senile yellowing

Whether one thinks in terms of pigment concentration or of light loss per unit pathlength, the constancy of $\Delta D/x$ with age denies pigment build-up as a cause of senile yellowing. Although $\Delta D/x$ does not change, there are obviously changes in lenticular pigment. COOPER and ROBSON (1969) have shown that extracted lens pigment changes its wavelength of maximum absorption during the fourth decade of life, and in Fig. 4 the difference between the young and old lenses suggests changes in the importance of the fractions a and s in determining lens transmission. Whatever changes occur they must be complex and complementary for there is no overall change in light loss per unit pathlength.

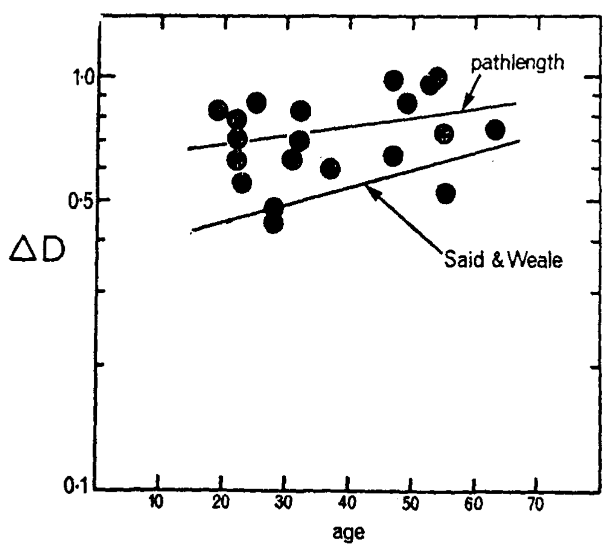

FIG. 9. Density difference, ΔD, at the lens pole (R = 0) plotted against age. The value of ΔD was obtained from the fitted lines of the ΔD vs. x plots (Fig. 7) at the maximum value of x which corresponded with R = 0. Note the ordinate has a logarithmic scale. The line labelled "Pathlength" is taken from Fig. 6a and is vertically placed in an arbitrary position so that it passes amongst the data points. The "Said and Weale" line is taken from their 1959 density data and has been adjusted to the same wavelengths and mean lenticular pathiengths as used in the present study (see text).

If $\Delta D/x$ does not increase with age (Fig. 8) the alternative of increasing pathlength must be considered to see if it is responsible for reduced senile transmission. Figure 6a demonstrates the significant increase of polar thickness with aging, and Fig. 9 shows the density difference, ΔD, plotted on a logarithmic scale against age. The scatter of points is very large, (a difficulty also encountered by BOETTNER and WOLTER, 1962) but the fitted line from Fig. 6a is shown arbitrarily placed amongst the points. That this line will pass amongst the points indicates that increasing thickness could explain the reduced transmission shown here and elsewhere (SAID and WEALE, 1959). Also in Fig. 9 for comparison is a line taken from the data of Said and Weale and adjusted to the wavelengths and mean lens pathlengths of the present study. (Said and Weale measured lens transmission with a large pupil which had an effective pathlength smaller than the polar pathlength, see below.) If the line was slightly higher it would pass through the points satisfactorily; the difference could well be a matter of technique or selection of material.

(d) Visual importance of lens shape

WEALE (1961, 1968) considered what effects the variable pathlength of the lens had upon various aspects of vision. For his calculations he assumed the absorbing or scattering pigment was uniformly distributed across the lens, and this study confirms his assumption. Although the only lens profile available to him was unusually thin, the magnitude of the effect is not greatly altered if calculated from the pathlength data of Fig. 5.

The modification of the Stiles-Crawford effect of the first kind, SCE 1, by the lenticular shape, as demonstrated in WEALE'S (1961) paper, has been recalculated here using the mean pathlength data of Fig. 5. If T_c and T_p refer to lens transmission at the centre and periphery respectively, then the SCE 1 that would be measured if the lens was flat or absent (the retinal SCE 1) is:

$$h' = h\,\frac{T_c}{T_p} \tag{5}$$

where η is the ratio of the light intensity of the central to peripheral beams when matched for equal brightness, it being assumed that the position of maximum luminous efficiency (η = 1) is at the pupil centre and that this corresponds with the lens pole. Figure 10 shows plots of 1/η against λ as taken from WALRAVEN and BOUNIAN (1960) using the results of STILES (1939). Also shown is the corrected ratio, 1/η’using the pathlength data of this study, and of Weale's paper. Below about 500 nm the retinal SCE 1 is seen to be greater than would be expected from measurement of the central and peripheral beams outside the eye.

The variation of lenticular pathlength would not be expected to modify the SCE 2 (change in hue of the peripheral beam) as it is usually measured with monochromatic light. With non-monochromatic sources the lens effect might have to be considered with SCE 2.

Another way of considering the effects of variable lenticular pathlength is to use the effective pupillary area, A_e, in calculating light flux through the pupil. A_e, is defined as that pupillary area which would admit as much light into an eye with a disk-shaped lens of thickness everywhere equal to its polar pathlength x_o, as is admitted by a pupil of true area A_o, which for convenience of calculation may be regarded as having a lens of integrated or effective pathlength x_e This effective pathlength varies with pupil size as shown in Fig. 11a.

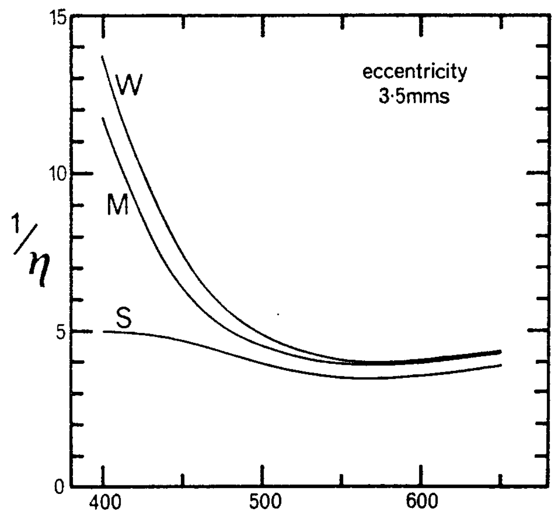

FIG. 10. Reciprocal of the relative luminous efficiency, η, plotted against wavelength, λ nm, for a point of entry for the peripheral beam 3.5 mm from the point of maximal efficiency. Curve *S* is STILES (1939) curve from WALRAVEN and BOUMAN (1960); *W* is this curve corrected for lenticular pathlength variations using the pathlength data available to WEALE (1961); *M* is calculated using the data of Fig. 5.

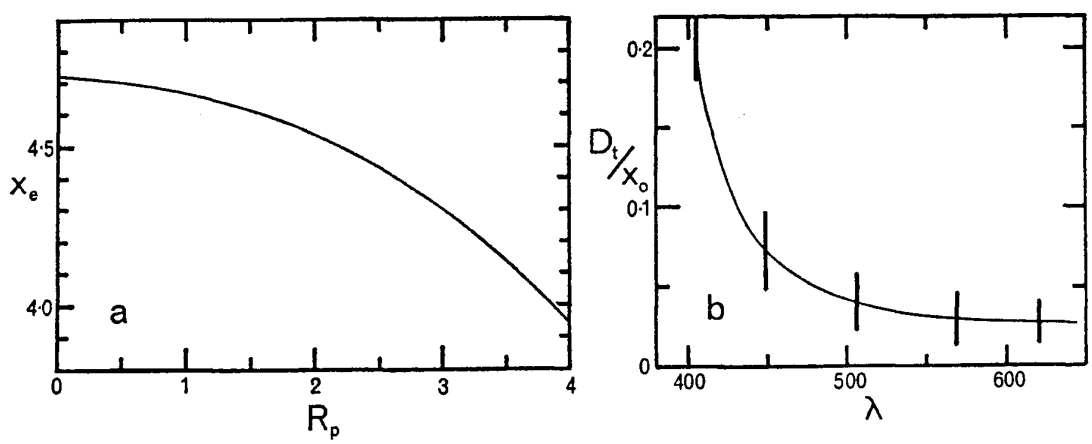

FIG. 11. (a) Effective, or integrated lenticular pathlength, x_e mm, plotted against real pupil radius, R_p mm. (b) Photometric density per unit pathlength, D_t/x_o, at the lens pole plotted against wavelength, λ nm, for 10 lenses of all ages. The vertical bars represent ± 1 S.D.

This figure is based on the mean pathlengths in Fig. 5, so the value for A_e given below is to some extent age dependent. (The pupil radius in Fig. 11a is the real radius and is not corrected for corneal magnification. Figure 12 may be used with real or apparent dimensions.)

$$\log A_e = \log A_o + \frac{m}{2.3}(x_o - x_e) \tag{6}$$

and

$$\frac{m}{2.3} = \frac{D_t}{x_o} \tag{7}$$

where μ is the absorption coefficient and D_t, the photometric density.

Figure 11 b shows average values of D_t/x_o at the lens pole ($R = 0$) plotted against λ for a group of 10 lenses. Figure 12 shows A_e plotted against pupil radius, R_p for four different wavelengths. A_e is only much larger than the true area at very short wavelengths and with pupil diameters greater than, say, 5 mm. As A_e varies with λ, it follows that the spectral distribution of a light may be modified by alterations in pupil size.

CRAWFORD (1949) measured the scotopic visibility curve in 50 people with natural pupils. If the mean diameter of the pupil was 7 mm, the changes in the scotopic curve for viewing through a pupil of 2 mm may be calculated. This would represent an extreme case of a laboratory experiment for naturally the pupil would never be so small in scotopic conditions, even in old people.

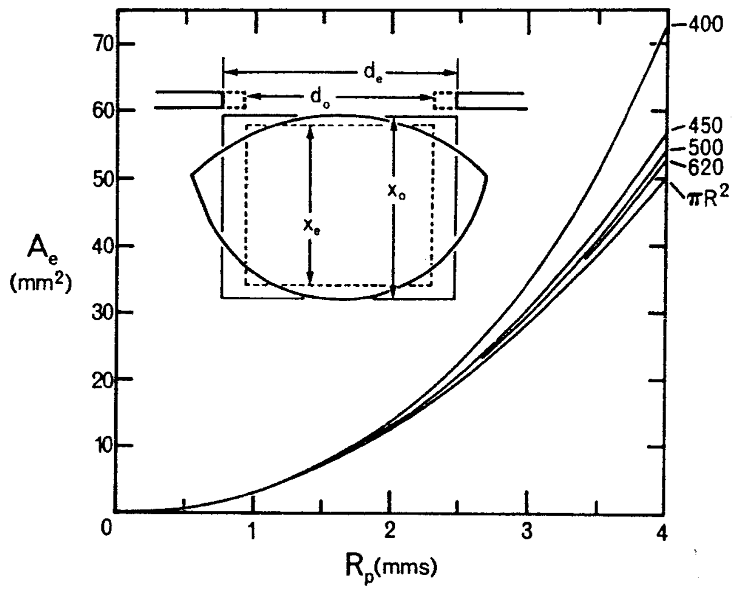

FIG. 12. Effective pupillary area, A_e, plotted against pupil radius, R_p. The curves are labelled on the right with their respective wavelengths; the lowest curve, πR^2, shows true pupilIary area. Real or apparent pupillary dimensions may be used. Inset: a lens behind a pupil of area $A_o = \pi d_o^2/4$ may be replaced by the disc-shaped lens of thickness x. which is shown by the dashed line. Alternatively, if the lens is replaced by the disc-shaped lens of thickness x_o shown in solid line, for equal light flux a pupil of larger area, $A_e = \pi d_e^2/4$, is required.

The ratio of the light flux for large to small pupils is given by the ratio of the large to small effective pupil areas. This ratio at four wavelengths is compared with that in red light (620 nm) and Table 1 shows by what factor the scotopic visibility curve should be multiplied to correct for viewing through a 2 mm pupil. As the table shows, the correction is small except at short wavelengths. These corrections lead to a spectral shift of the peak of the curve towards longer wavelengths, but it is small, probably no more than 1 nm.

TABLE 1

λ (nm)	factor
400	0.865
450	0.945
500	0.985
550	0.995
620	1.000

Factor to modify scotopic visibility curve for 2 mm pupil viewing.

Acknowledgements I should like to thank Mr. R. F. FISHER for help with this study, and Mrs. B. PETTET for technical assistance.

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Abstract-The light transmission of freshly excised normal human lenses has been measured at five visible wavelengths. The lens profile has also been recorded and used to calculate light loss per unit pathlength of lens. This loss, although made up of absorption and scatter the contributions of which cannot be quantified, may be regarded as due to a hypothetical absorbing pigment. The loss is directly proportional to pigment concentration and remains constant throughout the central 7-8 mm of the lens and between the ages of 20 and 60. Because of this constancy, senile lenticular yellowing is attributed to increasing lens thickness. The visual importance of this light loss in the lens is briefly considered.