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AGE AND THE TRANSMITTANCE OF THE HUMAN CRYSTALLINE LENS

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SUMMARY

1. Conflicting data both on the transmission of the human crystalline lens in the ultra-violet part of the spectrum and on its variation with age necessitate a re-examination of the subject.

2. Twenty-four excised lenses in the age range of 0–85 years were studied with a Perkin–Elmer spectrophotometer between 327 and 700 nm.

3. Some lenses were homogenized and the homogenates were similarly examined.

4. A systematic increase in absorbance with age was observed both in the visible and the ultra-violet parts of the spectrum.

5. An exponential function describes the data, facilitating comparison with, and prediction of, other values.

6. The absorbance of homogenized material was found to be predictable from that of intact lenses, and does not support the notion that mechanical trauma may account for high values in earlier studies.

INTRODUCTION

The crystalline lens controls radiation flux within the eye in two ways. First, it acts as an image-forming device in conjunction with the cornea; by virtue of its accommodation it assists in the production of detailed retinal images. Secondly, it acts as a filter. Its transmission band in the visible part of the spectrum is narrower than that of any of the other ocular media, and becomes more confined with advancing age.

However, the absorbance of the lens in the range of approximately 320–380 nm (UVA) is of topical interest. If the hypothesis that it protects the retina from noxious radiations is true, then manufacturers of lens implants ought to know which filtering properties of the normal lens have to be mimicked, so that when a cataract operation necessitates its removal appropriate compensation may be provided.

The visible part of the spectrum has been studied repeatedly (cf. Weale, 1982), but information on the UVA is sparser. Boettner & Wolter (1962), well aware of the difficulties of measuring absorbances much in excess of 3, published UVA data on one 4·5-year-old lens which have been taken by others to be representative of all ages. The curve is characterized by a small window of transmission at 320 nm, which is separated from the main transmission maximum in the visible by an absorption band peaking approximately at 360 nm. Cooper & Robson (1969) have published data based on measurements of thin frozen lens slices, total values being calculated from the lens thickness and the assumption that Beer's and Lambert's laws are valid (but cf. Zigman, 1978). Their absorbances are very high in the UVA, yet fail to show the usual variation with age in the visible. Lerman (1984, 1986) and Lerman & Borkman (1976) have published data which differ from one another to a remarkable degree: this has, however, failed to attract the authors' attention. They have shown age to cause a systematic decrease in transmittance in the visible part of the spectrum, but there is constancy in the UVA above the age of 25 years.

The inconsistencies amongst the several studies have prompted a re-examination of the subject.

METHODS

Material

The lenses were obtained in general from eyes used for corneal transplants, and the help given by the surgeons of Moorfields Eye Hospital in providing them is gratefully acknowledged. Earlier studies had shown that if the material was refrigerated, no significant change in either the image-forming properties of the lens (Weale, 1983) or in its transmissivity (Weale, 1985*a*) could be detected. Moreover, storage of the lenses in castor oil had shown that these and other optical properties failed to lead to any detectable variation with the time post mortem (within a few days) at which measurements were made (Weale, 1979, 1985*a*). The present need for all material to be tested for the presence of AIDS viruses has made it impossible to use lenses immediately after removal from the orbit: the mean time elapsing post mortem before measurements averaged $31{\cdot}5 \pm 8{\cdot}5$ h(S.D.).

The lens was removed from the eye as previously described, carefully cleaned, and placed in castor oil (Weale, 1983). An hour was allowed to elapse between this manipulative procedure and spectral measurement so as to minimize the effects of cold cataract, and to allow the disappearance of effects due to occasional minor reversible trauma resulting even from the most careful handling. Only lenses that appeared to be perfectly transparent were used.

Apparatus

A Perkin–Elmer 552 UV–VIS spectrophotometer was used in the range of 327–700 nm. Two suitable cuvettes were marked so that one was always used for the sample, the other for the blank. As the path length was 1 cm, but lenticular thickness is always below this (Weale, 1982), L-shaped 'Perspex' inserts were made which fulfilled two functions. Being 5 mm thick, they adequately reduced the path length for most lenses, so that these were in general slightly applanated. Some of the lenses were thinner than 5 mm: particular care was taken to ensure they remained upright, and a correction was applied for the absorbance of the space occupied by castor oil. The blank did not contain any castor oil, which did not significantly affect any of the experimental results. The lower part of the insert served as a rest for the lenses, so that their height in the cuvette could be adjusted if necessary.

The extent of the two (vertical) beams was far in excess of the diameter of the lens. Accordingly they were reduced on both sample and blank cuvette by means of steel washers (external diameter = 14 mm, internal diameter = 7 mm) so that the lens was transilluminated effectively along a diameter. In order to ensure that no leakage of light occurred over the top of the washers an appropriate cardboard mask was placed across both apertures.

The apparatus was carefully inspected for stray light. None was detected, presumably because the instrumental stray-light filters were fulfilling their purpose.

Procedure

This followed the manufacturers' instructions as regards determining the baseline ('back-correction') of the instrument, attention to the possibility of backlash in the control of the wavelength settings, etc. The automatic recorder was not used, because it may lead to some systematic errors being undetected. If, for example, there had been a change in absorbance resulting from the exposure to some of the ultra-violet measuring wavelengths, even repeat tracings of the absorbance spectrum would have failed to detect this. Accordingly, wavelengths of choice were set manually, the absorbance determined in each case, with snap measurements being made in other parts of the spectrum to test whether any change had occurred. When a spectral run was completed at the short-wavelength end, a number of stops for control measurements were made on the return to the long-wavelength start of the run. After the instrument had settled down, the temperature in the space containing the lens was about 30 °C.

Homogenization

Cooper & Robson (1969) had, as already noted, reported very high calculated absorbances: could they have been artifacts due to their lenses having been sliced? It is well known that severe trauma can produce irreversible opacification: while this might have escaped notice in thin slices, multiplication of the effect in the subsequent computation might have caused the high values for the absorbance. Accordingly, a number of lenses were measured twice: first as described above, and secondly after homogenization. For this purpose, the lens was transferred into a low-volume cuvette of 1 cm path length. Perspex plungers were inserted into both sample and blank cuvette. In the former it served to homogenize the lens, and to ensure that it filled the available volume. Care was taken to avoid producing air bubbles. No castor oil was used in this part of the study.

RESULTS

Figure 1 shows data for 13- and a 63-year-old lens. Both sets reveal the well-known absorption band at about 360 nm. The pronounced 'window' at 330 nm, present in the younger set, is nearly obliterated at the higher age. However, this feature was variable: a pair of 25-year-old lenses exhibited an even more rudimentary inflexion, while curves from older ones might dip towards 330 nm.

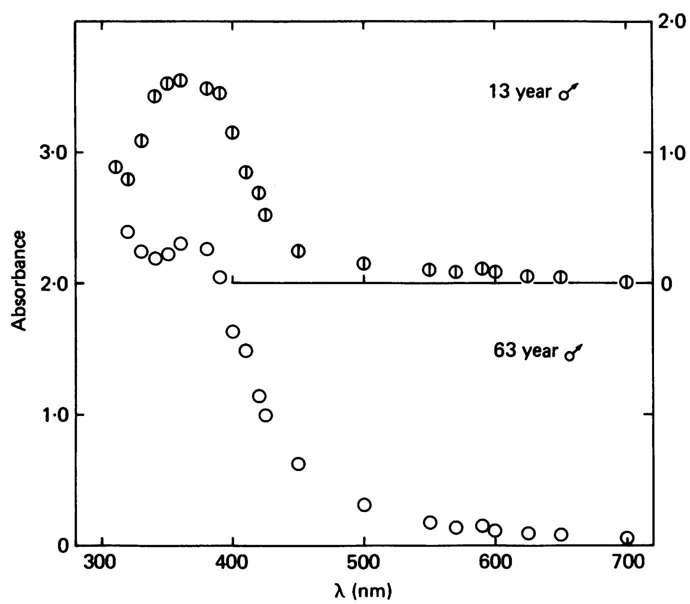

Fig. 1. Absorbance as a function of wavelength. The right axis refers to the data for the 13-year-old lens.

The absorbance at 590 nm tended to be above that at the lower wavelength of 570 nm, which is why this otherwise uninteresting spectral region was examined in some detail. Though small, this dip is significant with $P < 0{\cdot}01$ when compared with the trend of the data at 550 and 600 nm. It is unlikely to affect vision; whether the

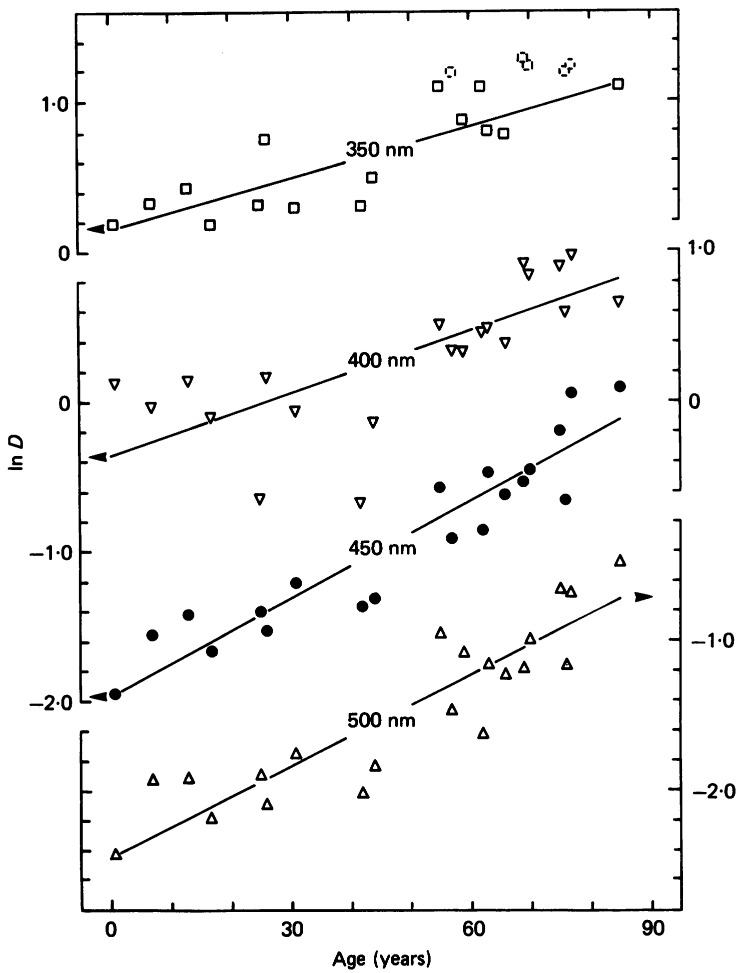

Fig. 2. The natural logarithm of the absorbance (D) as a function of age. Parameter: wavelength. The arrows point at the appropriate scales. The lines are regressions. The dashed readings (350 nm) were noisy and not used in the calculation of the regression.

underlying chromophore is linked to the type of long-wavelength fluorescence reported by Yu, Kuck & Askren (1979) remains to be determined.

It is instructive to plot absorbance (D) *vs.* age. Linear-regression analysis gave the least mean square when the natural logarithm of the absorbance was used, seven functions being tested. Typical results are shown in Fig. 2. Similar linear regressions were calculated for a number of other wavelengths, the form of each being.

$$\ln D(A) = \ln D(0) + \beta A, \qquad (1A)$$

TABLE 1. Statistical analysis of data obtained at seven wavelengths for which the natural logarithm of the absorbance was regressed on age (cf. eqn (1)), and the correlation R between the data and the regression was calculated, the F value giving the ratio of the total to the residual variance

λ (nm)	330	350	400	450	500	550	600
N	18	15	21	20	21	21	21
R^2 (%)	28	68	51	88	81	64	36
F	6·213	28·03	19·518	128·152	81·568	33·141	11·020
P	0·024	0·00015	0·0003	0·00000	0·00000	0·00002	0·0036

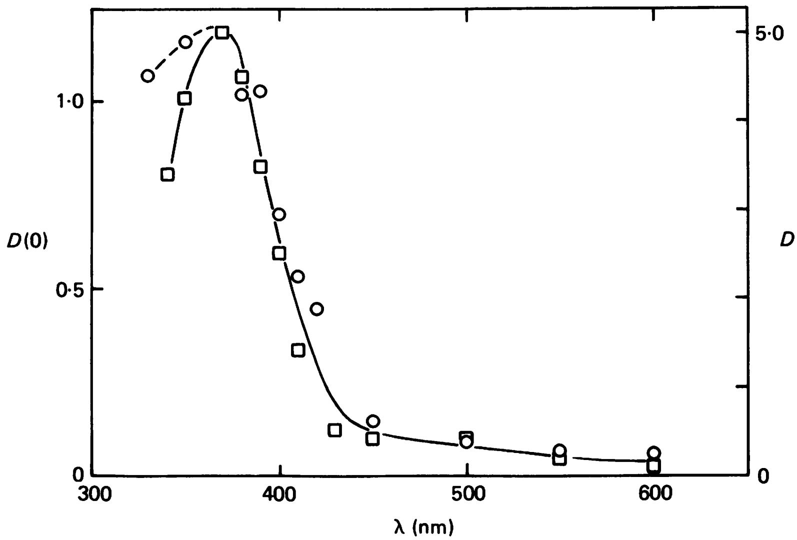

Fig. 3. Comparison of absorbance determined from the regressions (○) (cf. Fig. 2 and eqn (1) and results (□: right-hand scale) due to Cooper & Robson (1969). The curves are drawn free hand. Neonate.

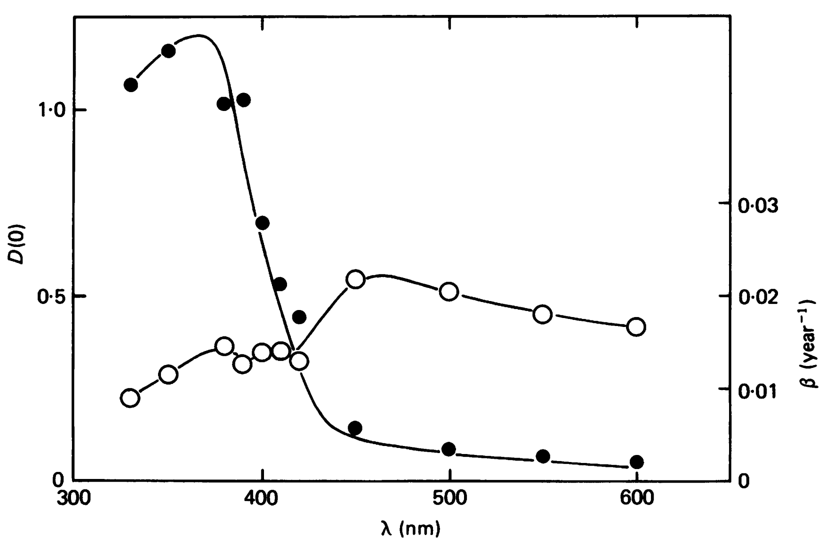

Fig. 4. The constants $D(0)$ (●) and β (○) as a function of wavelength as determined from eqn (1). The $D(0)$ curve is that shown with the dashed branch in Fig. 3.

equivalent to

$$D(A) = D(0)e^{\beta A}, \tag{1B}$$

where A is the age in years and β a constant with the dimension of year^{-1}. It will be seen below that $D(0)$ varies greatly with wavelength, being a sort of basic absorption spectrum, whereas β does so to a smaller extent.

The applicability of eqn (1) is examined for seven sample wavelengths in Table 1. Inspection of plots of the residual errors against predicted values failed to reveal any systematic trend: this and the data in the Table suggest the eqn (1) suffices for the description of the present data.

No significance should be read into the function being exponential: the value of β is partly due to the growth of the lens (cf. Weale, 1982).

A plot of $D(0)$ *vs.* wavelength should represent the neonatal absorbance spectrum. This had been determined by Cooper & Robson (1969), and Fig. 3 shows that calculation and observation agree if the two are scaled. The problem of the different scales is considered below.

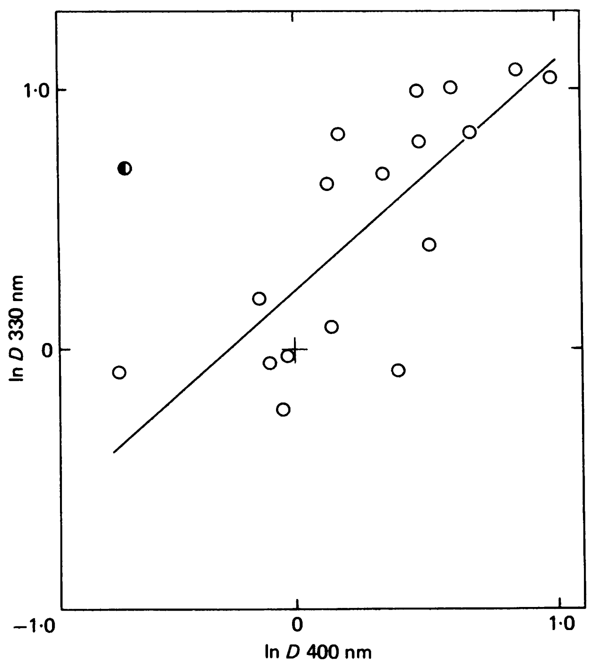

Fig. 5. Correlation between results obtained for 330 and 400 nm. The line is the regression (excluding the outlier, ◐). The cross indicates the origin.

Figure 4 shows plots of both $D(0)$ and β; they can be used to predict absorbances as functions of age and wavelength within acceptable limits of accuracy (cf. p. 584). They are empirical and the values of β have to be reduced by approximately 0·004 year^{-1} (to allow for lens growth) if a comparison were to be made e.g. with lens extracts.

While failing to note any variation in absorbance with age for wavelengths longer than 400 nm, Cooper & Robson (1969) reported both an increase in the absorbance in the UVA, and a systematic shift of the absorption maximum originally located at approximately 360 nm (cf. Fig. 3). This could be due e.g. to an increase in Raleigh scatter (Said & Weale, 1959; but cf. Zigman, Groff, Yulo & Griess, 1976). In addition to missing any effect due to age in the UVA, Lerman and his school overlooked the presence of the absorption band at or near 360 nm, let alone any spectral shift it might manifest.

These matters are tested (Fig. 5) in the correlation between results obtained here for 330 and 400 nm, values of absorbance > 3 being omitted (cf. Fig. 2). In view of the outlier (which represents the mean of two fellow lenses exhibiting the aforementioned rudimentary inflexion in lieu of a well-marked transmission 'window' at 330 nm), regressions were calculated both with and without it. Both intercepts are non-zero ($P < 0{\cdot}05$), supporting the notion of a spectral shift toward shorter wavelengths as age advances.

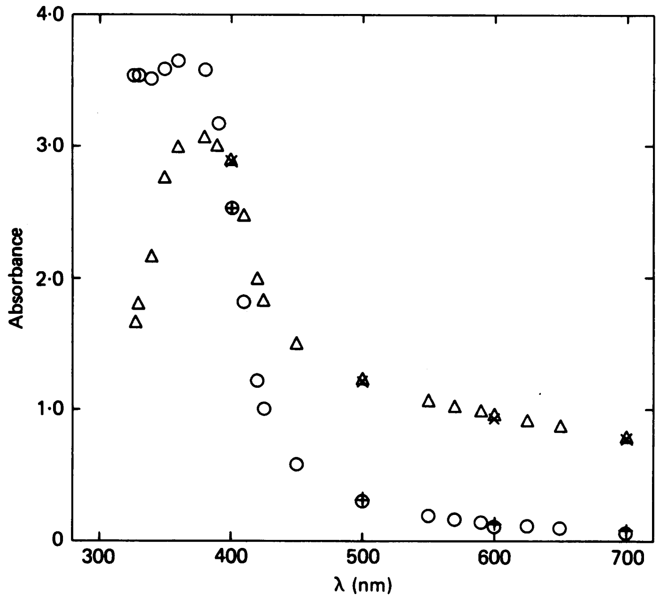

Fig. 6. Absorbance spectra of a lens before (○) and after (△) homogenization. + and × represent repeat measurement. 69-year-old man.

The present data consequently fall between Cooper and Robson's results on the one hand and those of Lerman and his collaborators on the other. The latter show no UVA variation with age above 25 years but do so at longer wavelengths; the present results reveal a significant rise in absorbance throughout both the UVA and the visible parts of the spectrum; and Cooper & Robson (1969) note a rise in absorbance in the UVA only, and also observe a spectral shift of the absorbance peak.

Figure 6 gives results for a 69-year-old lens, measured both before and after homogenization. The baseline of the second run is raised, which suggests that the process had produced turbidity; this was observed in all such experiments. But though the peak absorbance value is not far removed from the original, the absorbance relative to the new baseline is approximately halved. Whether or not this halving is to be accepted at face value is discussed below.

Some authors gauge lenticular senescence from the annual change in absorbance. This depends on both the wavelength and the age range covered. There are significant variations e.g. as between the values given by Said & Weale (1959) on the one hand and those of Zeimer & Noth (1984) and the present data on the other. However, it can be shown that eqn (1) explains them.

DISCUSSION

The results offer a picture of lenticular senescence because eqn (1) describes them with reasonable accuracy. They agree with earlier ones obtained with filtered light (Weale, 1985*b*) in the visible part of the spectrum and with other studies. They can be tested by comparing predictions based on the constants shown in Fig. 4 with appropriate data obtained in various ways and published during the last few decades. These estimates depend essentially on threshold measurements or on evoked potentials. For example, Wright (1951) determined the spectral sensitivities of normal and aphakic observers: the difference between their logarithms gives a measure of the absorbance of the lens. Zrenner & Lund (1984) and Werner & Hardenberg (1983) compared phakic eyes with fellow eyes carrying a lens implant. Werner (1982) compared thresholds for visually evoked potentials with the absorption spectrum of visual purple; but his data need correcting for senile miosis and for corneal pigmentation (the latter with data obtained by Lerman, 1984).

TABLE 2. Ratio observed and predicted values for the lenticular absorbance in three UVA regions

Source	Observed	Predicted	Mean age (years)	350 nm	~ 360 nm	~ 400 nm
Boettner & Wolter (1962)	2·4	1·27	4·5	—	1·89	—
Wald (1949)	2·72	1·53	20	—	1·78	—
Werner (1982)	1·27	1·63	0	—	—	0·78
	2·37	2·30	60	—	—	1·03
Werner & Hardenberg (1983)	1·67	1·63	30	1·02	—	—
Wright (1951)	3·15	1·66	27	—	1·89	—
	1·1	1·06	—	—	—	1·04
Zrenner & Lund (1984)	1·3	1·63	59	—	—	0·82

Table 2 shows ratios of observed/predicted values (O/P) for three wavelength ranges in the UV part of the spectrum. Their mean ($1{\cdot}28 \pm 0{\cdot}5$ S.D.) does not differ significantly from unity, but one cannot rule out that some underestimate may be produced. On the other hand, the values due to Wald (1949) and Wright (1951) were based in part on an unexplained deficit of the aphakic spectral sensitivity at $\lambda = 550$ nm in relation to that of the normal eye; this greatly increases the O/P value.

The general agreement shown in Table 2 and the successful prediction of the shape of the neonatal absorbance spectrum (Fig. 3) buttress the validity of the new experimental data. The course of events may be as follows. At birth, there is present a pigment characterized by $D(0)$, probably 3-hydroxy-L-kynurenine (van Heyningen, 1973). Figure 4 and eqn (1) suggest that it continues accumulating after birth. However, another chromophore, absorbing mainly at about 460 nm, is added later, and causes the occasional shoulder in the sixties-and-over groups. Care was taken to determine whether this and the minute band at ≈ 590 nm may not perhaps be due to instrumental artifacts: gelatine filters with absorbance spectra similar to lenticular ones were examined in a strictly analogous manner, but their absorbance spectra were smooth in the critical regions.

Lerman & Borkman (1978) have postulated the addition of a later chromophore to explain changes in lenticular fluorescence with age. The relevant excitation spectrum is unknown, but it is likely to dominate the violet part of the spectrum.

Table 2 supports the validity of the absorbance scales noted in these experiments; by implications it questions the low ones due to Lerman & Borkman (1976), and the high ones due to Cooper & Robson (1969). May trauma explain the latter? Let us assume that Beer's and Lambert's laws apply, some evidence to the contrary notwithstanding (Zigman, 1978); also that homogenization is unlikely to change the extinction coefficient, while it may affect the concentration (and orientation) of the chromophores and the effective path length p of the test beam. If the suffix t stands for traumatized and n for normal, we obtain

$$D_t D_n = [V_n/p_t]/[V_t/p_n], \tag{2}$$

where V_t represents the volume of the whole lens, and V_n that of the nucleus: it is here that pigmentation is paramount. According to Niesel, Kräuchli & Bachmann (1976) the principal diameters of the nucleus are approximately one-half those of the lens: hence its volume is about one-eighth. The ratio p_t/p_n of the effective path lengths is about 4. Thus the predicted value of the above ratio of the densities is 0·5, in approximate agreement with Fig. 6. The type of trauma produced here therefore does not seem to lead to the type of increase suggested by a comparison between the present data and those due to Cooper & Robson (1969) (cf. Fig. 4), and it may be that a freezing procedure is needed for this to be achieved.

The present study has shown that the lenticular absorbance in the UVA varies with age much as it does in the visible parts of the spectrum, even though the rate of change shows a statistically significant spectral variation. This implies that, in senescence, the potential protection of the retina from phototoxic radiations is likely to be increased; by the same token, however, the absorption of radiation by the lens which may harm it is also increased. This may appear to be partly mitigated by an age-linked increase in lenticular fluorescence, which rises between the ages of 40 and 80 by about twofold. However, the fraction of the incident light absorbed by the lens at these ages at $\lambda = 350$ nm is 98·5 and 99·9% respectively, so that risk and mitigating factor seem to be linked only tenuously if at all.

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