AGE AND HUMAN LENTICULAR FLUORESCENCE

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ABSTRACT

This critical survey of studies on the fluorescence of human eye lenses covers basic problems of metrology and their relation to comparative measurements on extracts, on *in vivo*, and on *in vitro* material. Attention is drawn to the effects of lenticular refraction and absorbance, and to potential photic involvement on the part of ambient illumination. The extent to which fluorescence may interfere with vision is also reviewed. © *1996 Society of Photo-Optical Instrumentation Engineers.*

Keywords light scatter and fluorescence; lenticular fluorescence; lenticular absorbance; ocular aging; age; haze.

1 VALUE OF LENTICULAR FLUORESCENCE STUDIES

Lenticular fluorescence has been known for the last 150 years or so, and, though important qualitative observations were reported earlier in the twentieth century,[1] quantitative analysis began only after the Second World War,[2] probably following the development of photoelectric devices.

Vogt drew attention to the changes in the coloration of lenticular fluorescence which correlate with age, but did not distinguish between forward and backward fluorescence.[3] Qualitative studies evidently could not distinguish between effects due to multiple fluorphors on the one hand and complications due to pigment absorption on the other. Klang[2] was able to demonstrate the presence of at least two different fluorophors and thereby established the analytic value of fluorescence studies.

Interest in lenticular fluorescence further developed with the realization that it increases with age, and also following the discovery that it is distributed nonuniformly in the lenticular matrix.[4]

Information *in vivo* was not obtained till the last decade or two. This is probably due to the increase in the sensitivity of radiometric methods, but Klang had demonstrated the feasibility of *in vivo* measurements when photoelectric techniques were being developed, and visual photometry might have been available had the interest been there.

Consequently *in vivo* lenticular fluorescence has only recently become a tool suitable for comparative measurements within and between populations, and its value, especially in connection with diabetic lens problems, has only lately been recognized without being fully exploited even now.

1.1 METROLOGY

Since the measurement of fluorescence entails the use of an exciting beam with a short-wavelength radiation and the detection of emitted radiation invariably with a longer wavelength, the separation of the two is important. Except when forward fluorescence is studied, the separation can be achieved by the physical segregation of the two beams, as is true in *in vivo* when a careful distinction between the two directions has to be ensured. It is supplemented optically. Because of the relatively low intensity of the emitted light, spectral filters rather than the preferable monochromatic radiations have to be employed to provide an adequate signal-to-noise ratio. As they have finite bandwidths, overlap between them needs firm control. Only a few workers in the field (see Ref. 5) have measured the extended absorbances of the filters they have used in order to assure themselves of the absence of spectral ''feet'' which treacherously transmit in distant spectral regions, potentially vitiating their results.

In vivo measurements suffer from a further uncertainty that is frequently overlooked. Although the exciting beam is usually defined with respect to its point of entry and the pupillary center, and, like the emitted beam, regarding the angle it includes with the visual axis, the point at which emission takes place is a matter of unconfirmed surmise. There are several reasons for this. The depth of the anterior chamber is not recorded; it decreases systematically with age. Consequently the point of entry of the exciting beam into the lens becomes indeterminate, with the result that a detector of the radiation emitted over a wide volume can offer no certainty about the locus of provenance. It has to be said that this

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result of the gradient of the lenticular refractive index[6] is not peculiar to fluorescence measurements; it introduces a great deal of uncertainty also into conclusions drawn from topographic studies of the lens, for example, by means of Scheimpflug photography.

Detailed spectral analyses of the emitted light are rare (see Refs. 7 and 8). There are two attendant problems, one of which is partly related to the difficulty addressed above. Lenticular coloration affects the spectral distribution of the emitted light in two ways. First, the exciting light is absorbed and causes fluorescence, depending on the absorbance of the relevant fluorophor; and second the emitted light is absorbed en route to the lens surface from which it emerges again, depending on the absorbance of any pigment it traverses. Hence the collected emitted light in all probability consists of a mix of radiations from different fluorophors, located in different parts of the lenticular interior. This raises important points.

No detailed action spectrum appears to have been published for human lens fluorescence, even though this can be attempted in principle. To be specific, what is required, for different age groups, is the spectral distribution of exciting radiations, based on an equal-quantum spectrum, and eliciting a constant emission spectrum. Here constancy refers both to amplitude and spectral distribution. While this would not of itself overcome the difficulty of the location of one or more fluorophors, it would assist in the definition of their spectral efficacy, and help to relate them to known chromophores. It is likely that this approach would signally refine the existing data, for example, on the variation of fluorescence as a function both of age and of the duration of diabetes.

1.2 AGE

In industrialized countries with obligatory registrations of birth, there is no metrological problem attached to age that has not been mentioned in the previous section. The interpretation of measurements could be refined by the application of correction factors, if not, indeed, ad hoc measurements, of such variables as the depth of the anterior chamber, lens thickness, and lenticular coloration, which are all age dependent. At present, studies indicate a great deal of variability (see Figure 1), and it remains to be seen whether refinements in measuring techniques justify the application of allowances for the above variables.

1.3 DISEASE

This applies also to *in vivo* studies of lens color in pathological conditions, such as diabetic and other cataracts. However, the simple ''one-spectral-band'' measurement used in all population studies so far needs replacing by a method involving at least two spectral measuring bands. The reason is that the absorbance of the pigment accumulating in the lens as a result, for example, of diabetes, is distinguishable from that of other pigments accumulating merely due to aging processes; the use of more than one spectral band would render this difference quantifiable and also provide a specific measure for the abnormal fluorescence.

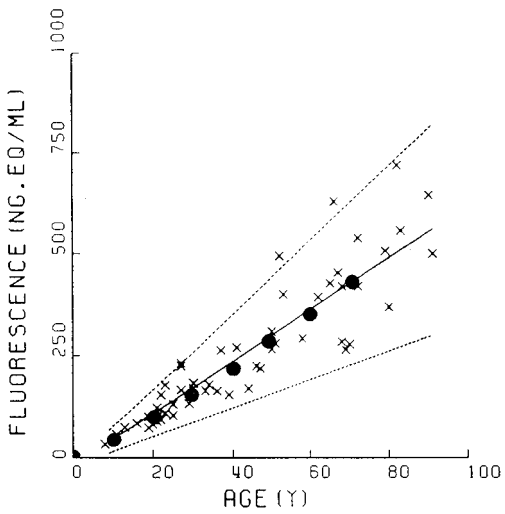

Fig. 1 Peak fluorescence (cf. Fig. 8) as a function of age of healthy subjects. Solid line, regression; dotted lines, 95% confidence limits; filled circles, values predicted on the basis of absorbance and a variable conversion efficiency. (After Refs. 3 and 29.)

1.4 COMPOSITION

The chemical analysis of fluorescent compounds derived from the human lens is carried out on solutions (see later discussion) and does not present any significant radiometric problem. Van den Berg[9] has measured the quantal conversion efficiency *in vivo*, and there is little doubt that, once they are obtained, *in vitro* values are likely to be appreciably greater. The reason is that the configurations of molecules *in situ* tend to differ from those in solution: in the latter instance they tend to unravel, with more photosensitive sites becoming exposed.[8,10] The result is that a fixed exciting flux is liable to convert to a larger quantal output than in *in situ*.

1.5 RELATIVE ADVANTAGES OF DIFFERENT TYPES OF MEASUREMENT ON LENSES

The disadvantages of studying intact lenses, whether *in situ* or not, have been spelled out with special reference to the uncertainty of the loci of photoconversion and also regarding spectral analysis of the emitted radiation. In young lenses it makes little difference whether axial measurements are done forward or backward,[3] but it can be shown that this no longer holds for older lenses. For example, whereas age leads to a redward shift of the emergent fluorescent light in the forward case, it is blueward in the backward variety.

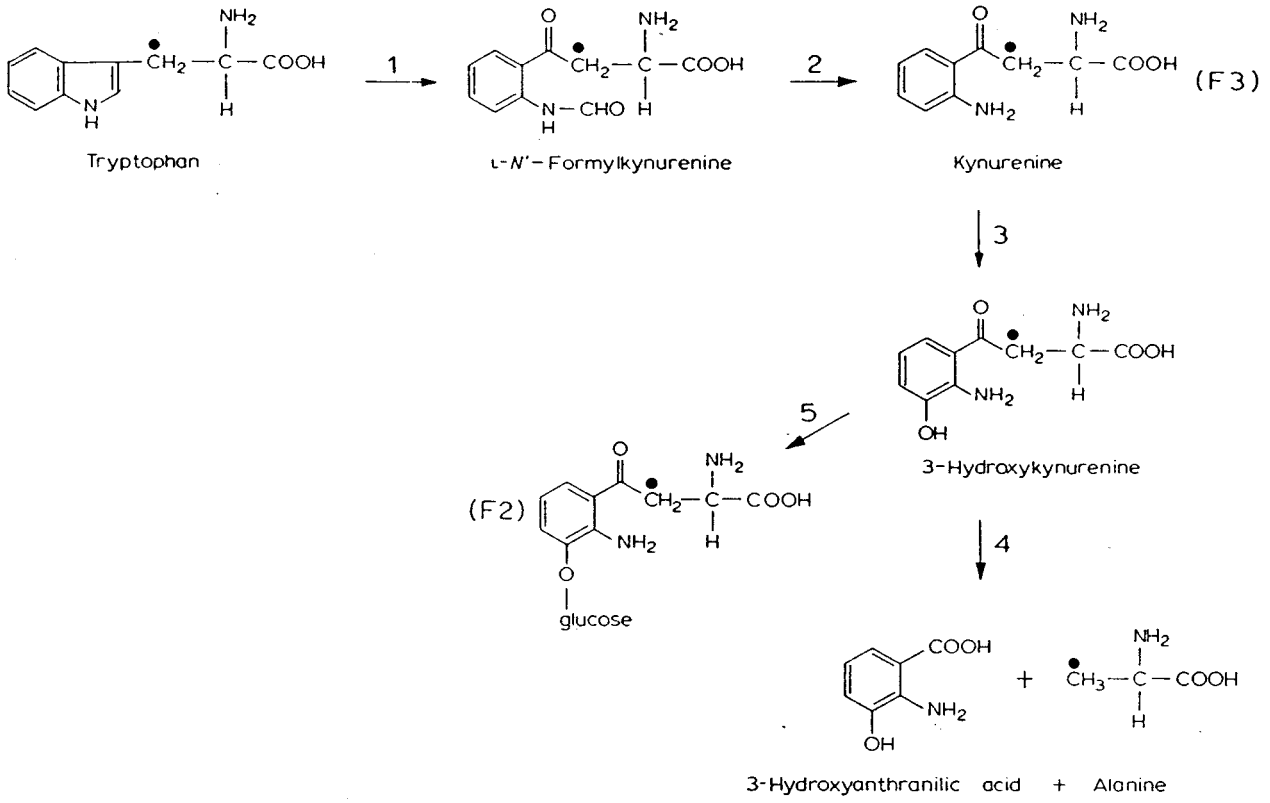

Fig. 2 Metabolism of [^{14}C] tryptophan (labeled carbon atom marked with dot): F2 is the *O*-β-D-glucoside of 3-hydroxy-L-kynurenine. (Reproduced courtesy of R. Van Heyningen and Ciba Foundation.)

Access to the nucleus is achieved at present only by section, although Pierscionek's[11] keyhole approach could prove very valuable, for example, in demonstrating whether the highly transient fluorescent complex arising from flash illumination of the lens surface[12] is detectable also in the nucleus.

2 MEASUREMENTS ON FLUOROPHORES

It has been known for a long time that fluorescence can be excited in the lens in more than one spectral region, and that more than one fluorophor is likely to be involved. Generally one distinguishes between tryptophan and nontryptophan fluorescence. Tryptophan, an aromatic amino acid, absorbs maximally at ~270 nm and fluoresces between 332 and 342 nm when bound to protein, but at 350 to 360 nm when free.[13] Since the cornea absorbs 50% of the incident radiation at 320 nm, the lenticular conversion of natural energy even by bound tryptophan is not going to be negligible. However, the lens also contains fluorescent material in specific proteins, excited at 360 nm and emitting in the range of 420 to 440 nm. A compound dialyzed mainly from younger human lenses and fluorescing with blue light[14] probably belongs to this group (but see later discussion). Other compounds absorbing in the violet part of the spectrum and fluorescing at ~500 nm are likely to have a more powerful adverse visual effect.

Some fluorescent compounds, identified in human lens extracts and showing significant variations with age, have low molecular weights. They include L-kynurenine, the *O*-β-D-glucoside of 3-hydroxy-L-kynurenine, and one related to the latter, but with the α-amino group protected or absent.[15] Their compositions are shown in Figure 2. Kynurenine is derived from tryptophan and the glucoside from both tryptophan and glucose, and physiological concentrations of the compounds can act as photosensitizers (see Ref. 16). Another substance with an absorption spectrum similar to the glucoside appears to be so photosensitive as to be extractable best in the dark:[17] no attempt seems to have been made by a noninvasive method to detect its presence in the living human eye, for example, after a night's sleep. However, Lerman et al.[18] were able to induce blue fluorescence in a 3-day-old lens following UV irradiation even though no fluorescence could be detected in the lens of a newborn baby. If confirmed, this observation could mean that photosensitization is effected by the lens being exposed to some part of the (solar) spectrum.

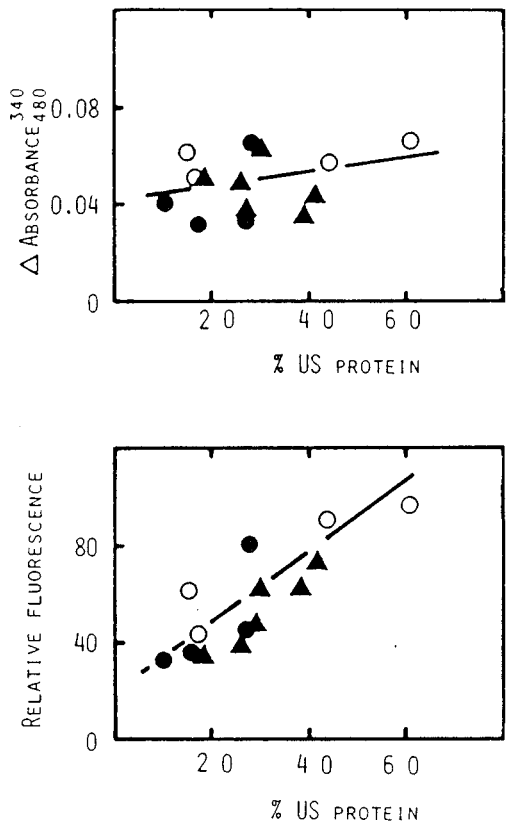

Fig. 3 Ordinate: (top) absorbance; (bottom) relative fluorescence; abscissa: percentage of urea-soluble protein. (After Ref. 20.)

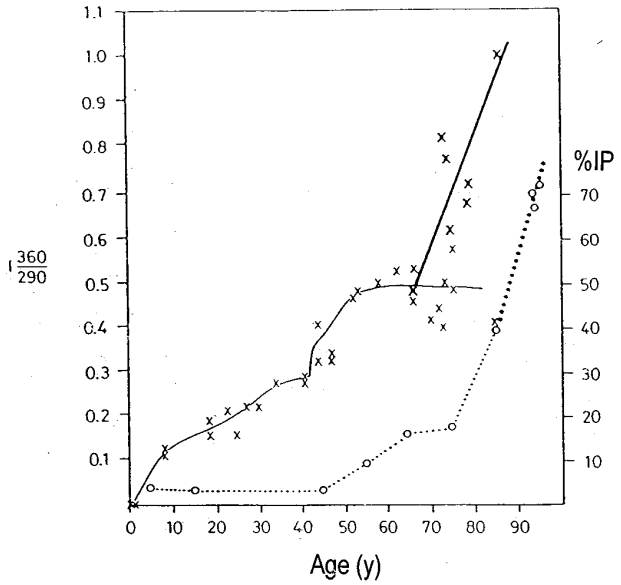

Fig. 4 Left ordinate: ratio of fluorescence intensity emitted at 420 to 440 nm (excited by 360 nm) to fluorescence intensity emitted at 332 nm (excited by 290 nm); solid line, normal eyes; heavy line, nuclear cataracts. Right ordinate: percentage of insoluble protein (IP); dotted line, normal eyes; heavy dots, nuclear cataracts. Abscissa: age in years. The lines were drawn by hand. [Reproduced courtesy of S. Lerman and R. Borkman (Ref. 21) and *Ophthalmic Res.*]

The spectral fluorescent band is not characteristic of any one group of compounds. For example, we noted above that a soluble compound, found mainly in young lenses, fluoresces with blue light. But this also applies to some insoluble proteins, the concentration and degree of fluorescence of which systematically increase with age. This is true of both normal and cataractous lenses.[19,20]

The latter authors illuminated ''cryostat-thin'' sections of lenses with a band of 300 to 400 nm, and distinguished the cortex from the nucleus in material that had not been improved as a result of being kept for about 1 month at −20 °C. Extracts were separated into water-soluble (ws), urea-soluble (us), and urea-insoluble (ui) moieties. Although a cortex would occasionally fluoresce blue, fluorescence of aged normal and cataractous lenses was confined to the colored nucleus. The relative fluorescence of whole normal lenses, and the cortices of cataractous ones, increased linearly with the fraction of us protein (Figure 3). It is not obvious why the cortices of lenses with nuclear cataracts should behave in such a manner, but slicing the material may involve contamination, and no control of this has been reported.

This comment is underlined by the observation that, in intact lenses, the increase in fluorescence observed during the first four decades (Figure 4) occurs in the absence of any increase in insoluble (is) protein, the concentration of which starts rising only during the fifth decade. Similarly, nuclear cataracts contained in intact lenses (Figure 4) show a rise in fluorescence some 20 years prior to a rise in the concentration of is protein.[21]

There appears to be a real problem here since work on extracts from normal lenses,[22] extended to brunescent cataracts,[19] links an increase in fluorescence with a decrease in water-soluble protein. When Satoh, Bando, and Nakajima[22] excited the (ui) moiety with 340 nm and measured at 420 nm, fluorescence showed a steep rise after the age of 20 years, with that of the soluble material rising at only about 14% of that rate. There was also a systematic shift in the wavelength of maximum emission: γ-crystallin showed the shortest, with β-crystallin, α-cystallin and (us) showing progressively longer maximal wavelengths.

Using high-pressure gel permeation chromatography, Bessems et al.[23] were able to show that nontryptophan (nt) fluorescence increases with age by virtue of the age-related increase in the concentration of all the native crystallins with the exception of γ_2. They separated the cortex on the assumption that the inner 30% of the lens mass is the nucleus (Niesel, Kräuchi and Bachmann[24] put the nuclear mass more nearly at 12%). The presence of pigments was monitored in the supernatants following homogenization of the lenses by absorbance measurements at 280 nm (which would evidently miss

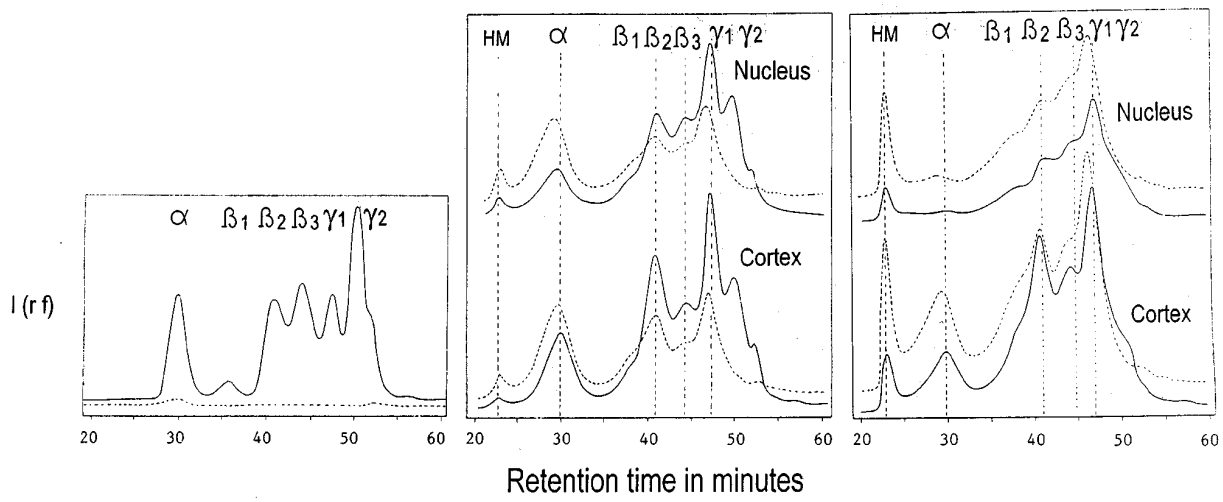

Fig. 5 Left: High-pressure gel permeation chromatography (HPGPC) of the water-soluble fraction from the lens of a newborn baby. Continuous line, absorbance measured at 280 nm; broken line, relative fluorescence intensity excited with 360 nm, and measured with 420 nm. Center: HPGPC of the nuclear and cortical water-soluble fractions of a 29-year-old lens. Right: HPGPC of the nuclear and cortical water-soluble fractions for a clear 70-year-old lens. (After Ref. 23.)

pigments not showing any absorbance at this wavelength; cf. Ref. 25), and comparing this with the degree of fluorescence corresponding to calibrated molecular weights. Fluorescence was excited by 360 nm and measured at 420 nm. Thus Fig. 5 (left to right) show traces for the two functions, with high-molecular-weight constitutents being on the left and low ones on the right for lenses of different ages. Note the swamping of γ_2 in Figure 5 (right), and the marked accumulation of high-molecular-weight components after infancy.

Figure 6 summarizes the results for different crystallins on the left and shows that the age-related increase in nuclear fluorescence progresses at a faster rate than in the cortex. On the right, the dominant involvement of the nucleus is shown for nuclear cataracts: the onset of brunescence appears to be a watershed for an enhancement of fluorescence (see Ref. 21). The fluorescence of such lenses is partly attributable to the formation of 3,3′-bityrosine and anthranillic acid.[25]

The use of both intrinsic (i) and extrinsic (e) probes enabled Liang[8] to study the microenvironment of fluorophors under observation; (i) included probes for both tryptophan (t) and nontryptophan (nt) fluorescence, while (e) included 4-(*N*-iodoacetoxy)*N*-methylamino-7-nitrobenz-2-oxa-1,3-diazole (IANBD) and 6-(*O*-toluidinyl)naphthalene-2-sulfonate (TNS). Measurements were obtained for emission maxima, relative quantum yields ϕ (which are proportional to the conversion efficiency s, see below), and polarization P. The latter is a function of the ratio of parallel to perpendicular polarization of the emitted light, the exciting light being vertically polarized. The (t) probe revealed constant values of ϕ and P as a function of age, but decreases of the former, and increases in the latter

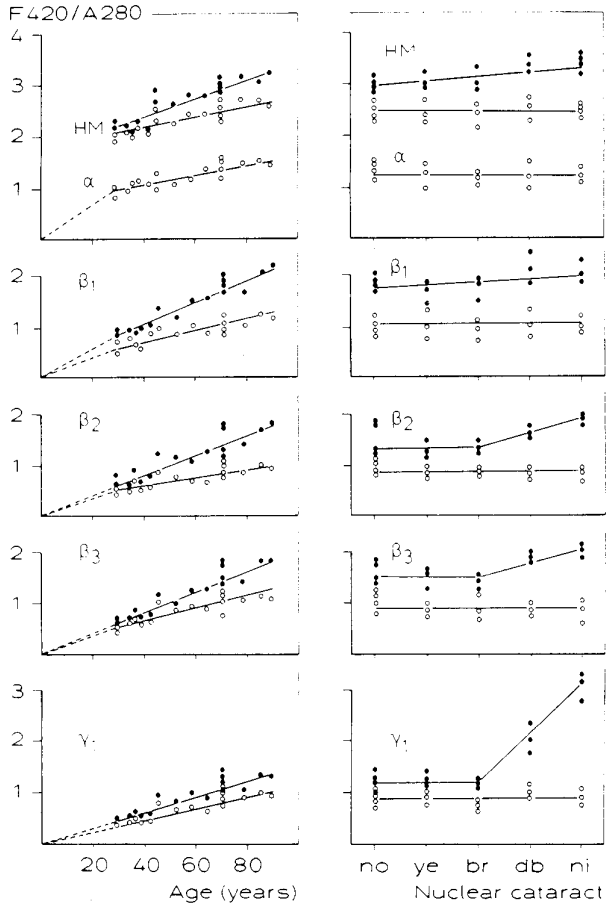

Fig. 6 Variation in the ratio of the relative fluorescence intensity measured at 420 nm to the absorbance at 280 nm for water-soluble protein in normal lenses (left) and nuclear cataracts (right). Empty circles, cortex; filled circles, nucleus. no, normal; ye, yellow; br, brown; db, dark brown; and ni, nigra. [Reproduced courtesy G. J. H. Bessems et al. (Ref. 23) and *Invest. Ophthal. Vis. Sci.*]

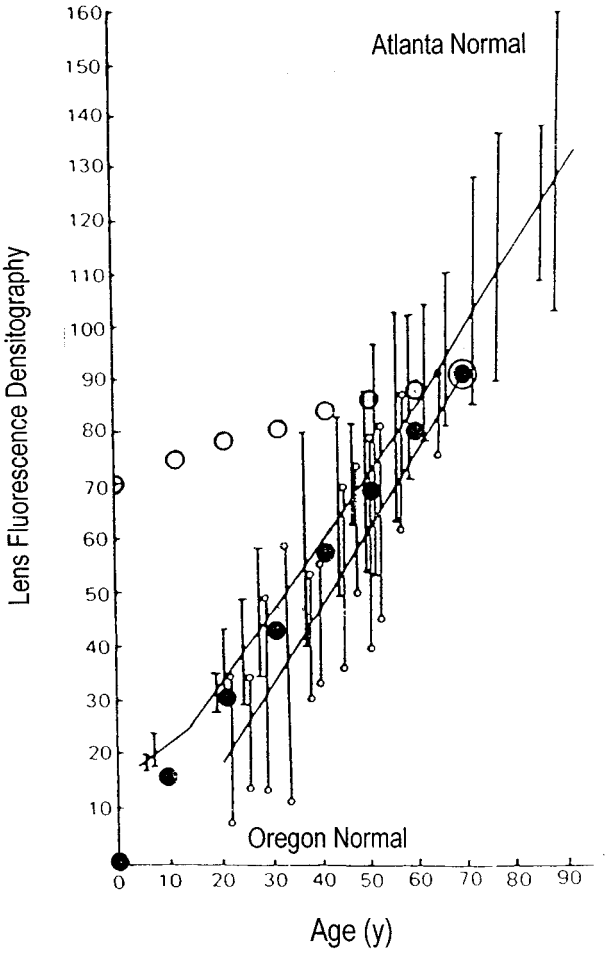

Fig. 7 Ordinate: lens fluorescence index, abscissa: age in years. The error bars ending in circles pertain to measurements in Oregon, the others to those in Atlanta. Filled circles, values predicted on the basis of absorbance and a variable conversion efficiency; empty circles, values predicted on the basis of absorbance and a constant conversion efficiency. (After Refs. 3 and 33.)

in glycated and high-molecular-weight α-crystallin. In contrast, these compounds responded to (nt) with a rise in ϕ and a reduction in P. IANBD revealed a marked age-related rise in ϕ.

Liang interprets this in terms of an unfolding of the parent molecule[10] so that increasing numbers of fluorophor sites become exposed. By assuming that the conversion efficiency $s=1-\exp(-\beta A)$, where A is the age in years and β is the main term governing the increase in the age-related absorbance,[26] good agreement can be obtained between theory and experiment as shown in Figures 1 and 7.[3] The sparseness of Liang's age groups does not permit one to press the comparison, but, such as it is, the correlation between ϕ and s is good (0.996). As predicted,[27,28] s and ϕ increase with age.

This puts a complexion on the effects of lenticular senescence which has its attractions. The age-related increase in fluorescence which Bleeker et al.[29] attribute to a continued exposure to ambient short-wavelength radiation (see also Ref. 13) is not damaging itself, and, in the absence of any pathology, is not a sign of damage. But, as a mark of the molecule being unfolded, it is a harbinger of an increased vulnerability. And, that, after all, is one of the corollaries of senescence.

3 *IN VIVO* STUDIES

A number of informative studies on age-related variation in fluorescence have been published. For example, Scheimpflug biomicroscopy was used for living eyes between 17 and 85 years, although Hockwin and Lerman[30] do not show a (linear) increase beyond 66 years. The traces produced by this method are less informative than those yielded by the methods listed below, but demonstrate nonetheless the presence of at least two fluorophores which were studied in more detail *in vitro* (see Sec. 4). Also, the peculiar transmissivity data (which changed systematically in the course of three publications spanning a decade) obtained from this laboratory, particularly for the ultraviolet part of the spectrum, raise some doubt about the radiometric reliability of this apparatus.

The spatially analytical quantification of fluorescence in the living human lens was introduced by Zeimer and Noth,[31] and developed by subsequent workers. Commercially available fluorophotometers were used. The method is sketched in Figure 8. A short-waveband exciting beam enters the eye at a known distance from the cornea, and the resulting emitted beam is passed through a barrier filter that is nominally opaque to the exciting waveband. The intensity of the emergent beam is recorded. The distance between source and cornea is varied systematically so that a trace relating intensity to putative distance of penetration into the lens can be recorded. This is referred to as the lens profile (Figure 8). It is characterized by a marked proximal and a weak distal peak, the difference between which is barely noticeable when the ordinate is logarithmic.[32] Measurements on thin lens slices[4] suggest that this may be due to an artifact, possibly absorption: with one exception, the posterior peak was at all ages higher than the frontal one.

Zeimer and Noth[31] stated that the ratio of the two amplitudes is equal to the transmissivity of the lens for the mean of the exciting and emitted wavebands. However, a spatial analysis of the fluorescence lens profile shows that, unless the exciting and emitted beams are collinear, the ratio is rather a measure of the transmissivity for the exciting radiation.[3]

The height of the first maximum is taken as a measure of fluorescence, and both Bleeker et al.[29] and Occhipinti, Mosier and Burnstein[32] calibrated amplitude in terms of equivalents of fluorescein concentration. Its variation with age is shown in Figure 1. The black circles indicate theoretical values predicted on the basis of absorbance measure-

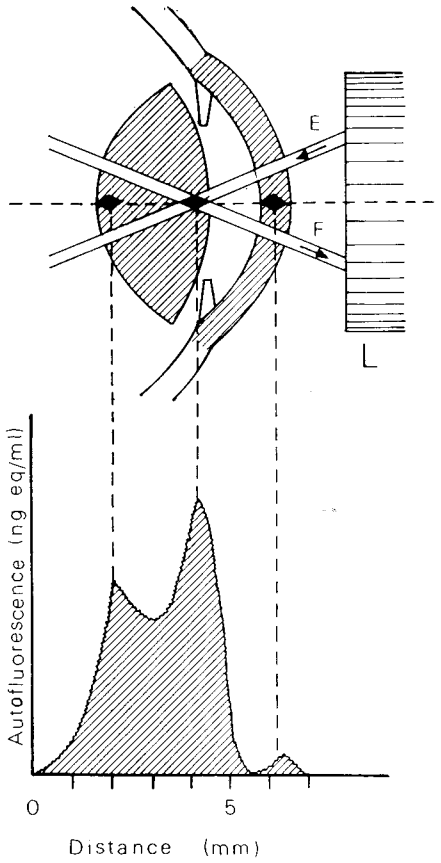

Fig. 8 *In vivo* fluorimetry. Top: E, exciting beam; F, measuring beam; and L, imaging system of fluorophotometer. Bottom: autofluorescence in nanogram equivalent of fluorescein per milliliter as a function of notional distance along the optic axis of the eye. [Courtesy J. C. Bleeker et al. (Ref. 29) and *Invest. Ophthal. Vis. Sci.*]

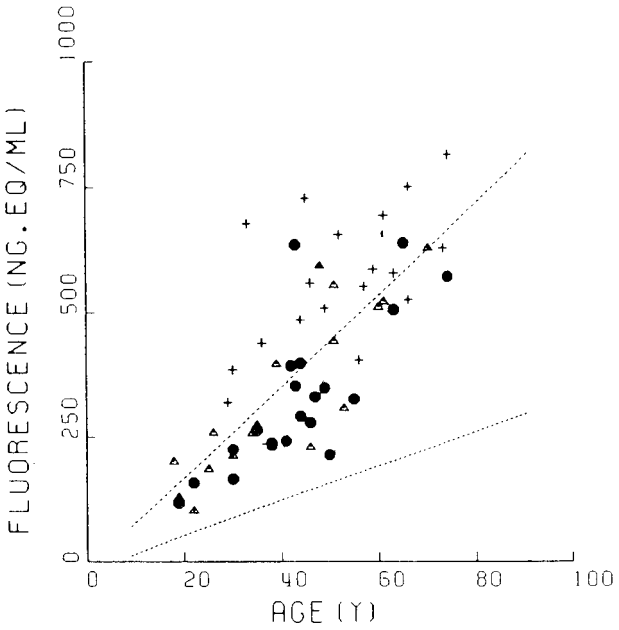

Fig. 9 Peak fluorescence (cf. Fig. 8) as a function of age of diabetic subjects. Dotted lines, 95% confidence limits for normal subjects (cf. Fig. 1); filled circles, duration of condition <6 *y*; triangles, 6 to 15 *y*;+>15 *y*. (After Ref. 29.)

ments on excised lenses.[3,26] Analogous results obtained for diabetic patients differ significantly (Figure 9): there is no simple model that would predict their data in terms of normal lens absorbance as in Fig. 1, and the reason is almost certainly that the diabetic pigment differs from the purely ''aging'' ones.

Lerman[33] extended the procedure by defining the lens fluorescence index (LFI) in terms of the area of the lens profile rather than just in those of some of its features. He measured the LFI in two populations, namely, inhabitants of Atlanta and Oregon, respectively. The difference between the two (Figure 7) is attributed to the greater sunshine prevailing in Atlanta. The study is less valuable than it might be because it contains no information on whether diabetics were excluded.

Van Best et al.[34] and Occhipinti, Mosier and Burnstein[32] followed Zeimer and Noth in the belief that the ratio of the amplitudes of the second to the first peak of the lens profile provides a measure of the transmissivity of the lens. While such values can evidently be computed, they tend to yield transmissivities that are substantially higher than those determined by direct methods. Several reasons may be responsible for this. For example, the data may be contaminated by stray light,[29] and the filters used may not cut off with the necessary efficiency. However, the relative ease with which data may be obtained makes the method very suitable for comparative measurements for aging lenses, and also for normal and pathological conditions.

4 OBSERVATIONS *IN VITRO*

The first detailed attempt at segregating the effects of absorbance on fluorescence measurements was undertaken by Jacobs and Krohn,[4] who studied excised lenses under three conditions: (1) they illuminated the lens along the axis, and measured the fluorescence of the light emerging along an equatorial radius; (2) the lens was illuminated at 45 deg to the axis, and the light emerging from the same point at 90 deg to this was measured, i.e., mainly cortical fluorescence was sampled; and (3) the lens was hemisected through the optic axis, and the arrangement of (2) applied to the central part of the nucleus.

It has to be said that the physical controls were unconventional. For example, the slit widths of the spectrophotofluorimeter were kept constant, which can have ensured neither quantal nor bandwidth constancy. For each lens, the wavelength of the exciting beam was varied ''in order to determine the exciting wavelength at which the maximum fluorescence occurred.''[4] It was shown in Sec. 1.1 that

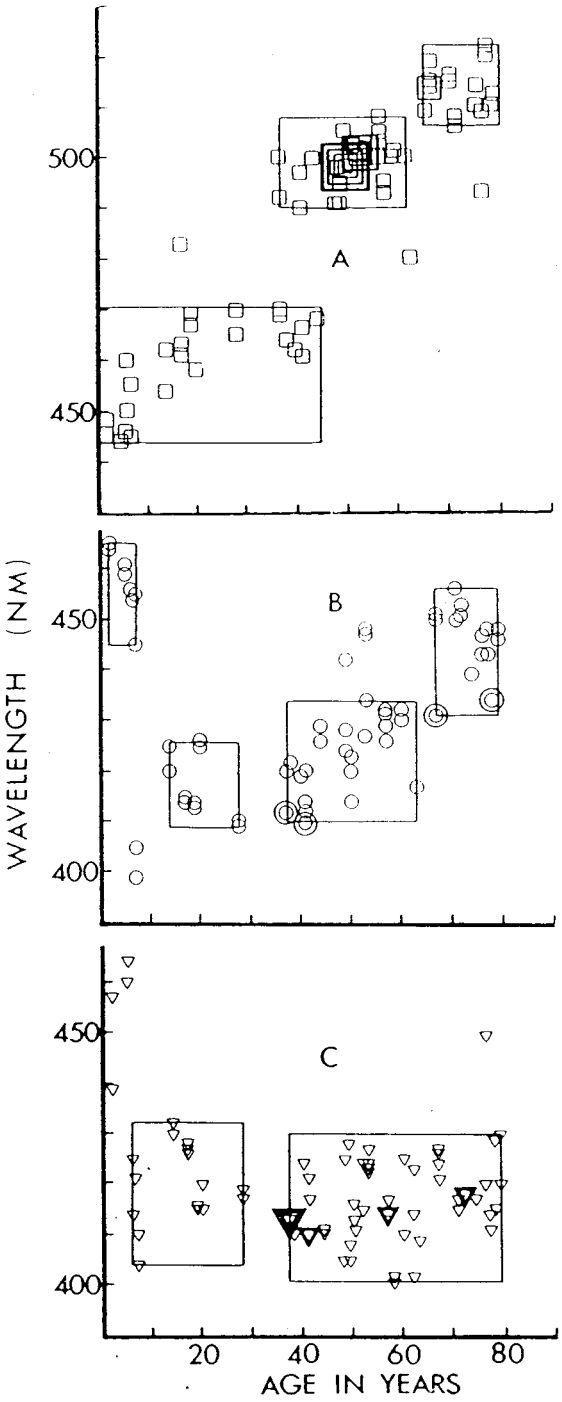

Fig. 10 (A) Wavelength of fluorescence maxima in whole human lenses as a function of age. The frames delineate clusters. Coincident values are indicated by concentric symbols. (B) The same data for the nucleus and (C) for the cortex. [Reproduced courtesy R. Jacobs and D. L. Krohn (Ref. 4) and *J. Geront.*]

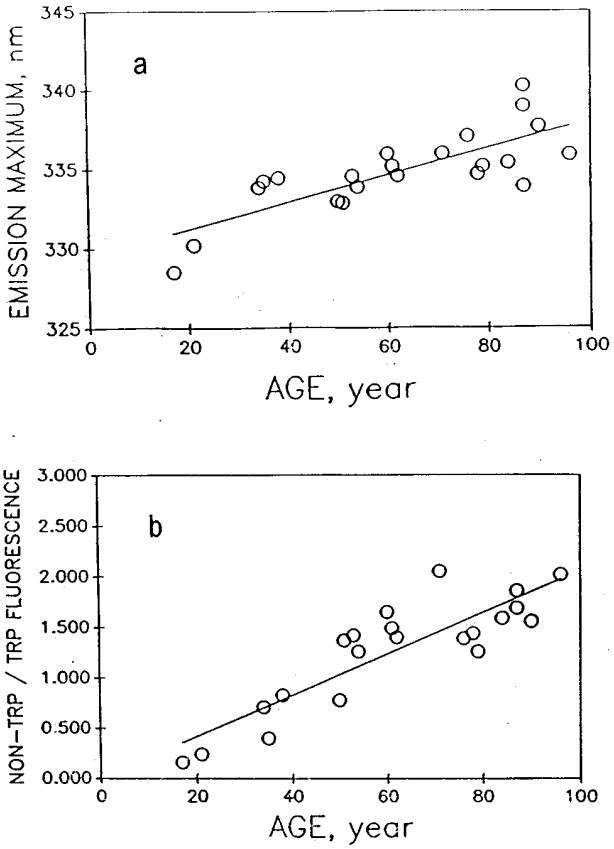

Fig. 11 Ratio of non-trp to trp fluorescence as a function of age; p=0.0001. (After Ref. 35.)

this is unlikely to lead to unequivocal results. Nevertheless, Figure 10, which shows plots of the wavelengths of maximal fluorescence as a function of age for the above three conditions, emphasizes the need to distinguish cortical from nuclear portions at least regarding the type of fluorophor. It suggests that the major part of the age-related change revealed in whole lenses is to be attributed to those occurring in the nucleus, and that the situation is relatively constant in the cortex. Jacobs and Krohn also give results for fluorescence intensity, but their manipulation of the data does not permit their results to be compared with others obtained later.

Though also adhering to apparently constant slit widths, Liang[35] allowed for variations in spectral emittance and detector response, and also distinguished between fluorescence due to tryptophan (trp) and other substances (ntrp) respectively (see Sec. 4). The lenses were bisected through the equator and the flat (nuclear) surfaces studied. Figure 11(a) shows that the peak emission wavelength of (trp) fluorescence increases linearly with age, and its intensity predominates over (ntrp) only in young lenses (Fig. 11b).

It is difficult to assess the results obtained by Kurzel, Wolbarsht and Yamanash.[7] Overlooking the fact that Cooper and Robson[14] reported absorbance data on human lenses which ''are extrapolations of measurements made on thin layers of lens material'', and that the UV absorption maximum dropped in a sigmoid fashion, Kurzel and coworkers confined their attention to intact lenses in

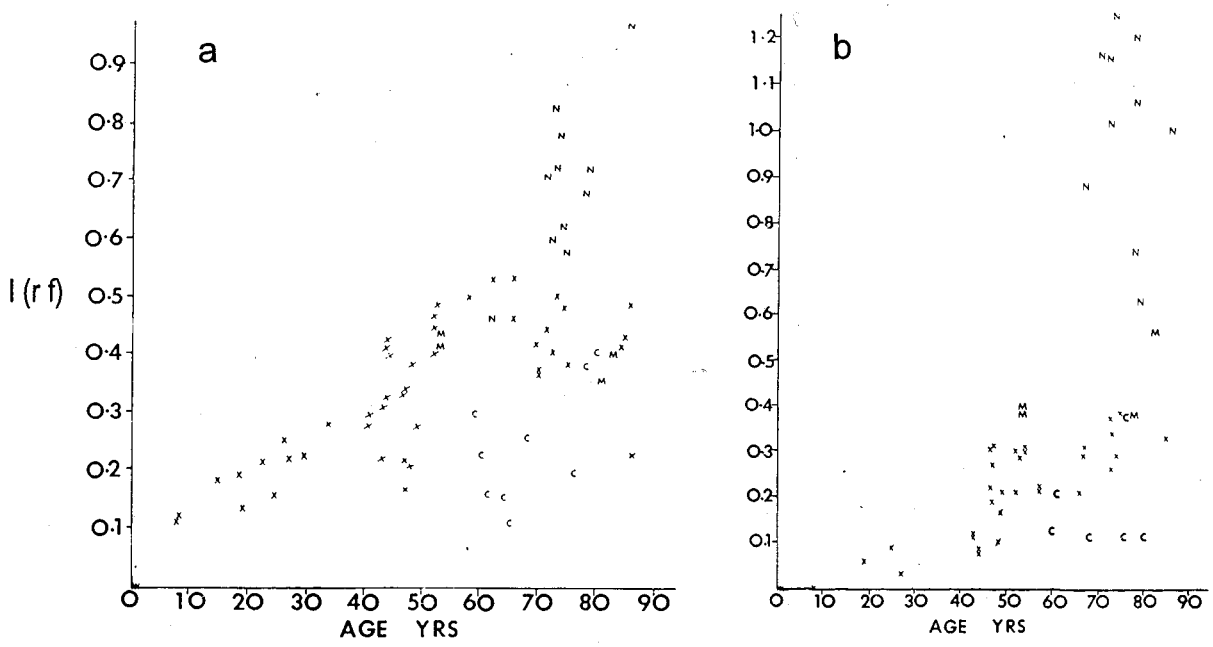

Fig. 12 Relative fluorescence I(rf) *in vivo* as a function of age: (a) ratio of fluorescence intensity emitted at 440 nm (excited by 360 nm) to fluorescence intensity emitted at 332 nm (excited by 295 nm); and (b) ratio of fluorescence intensity emitted at 500 to 520 nm (excited by 435 nm) to fluorescence intensity emitted at 332 nm (excited by 295 nm). In both cases the denominator relates to tryptophan fluorescence (trp). x, normal; N, nuclear; C, cortical; M, mixed cataracts. (After Ref. 21.)

the age range of 39 to 81 years, and are unique in having ''found no variation in the relative contributions of the spectral features with respect to age.'' It would, of course, have been interesting to learn what, if any, absolute changes occurred. No information is given on scaling constants relating to different emission wavelengths. Within the spectral range of 300 to 470 nm, the authors claim to have detected the presence of 4 to 5 principal chromophores [fluorophors?]. However, fluorophors can be found at longer wavelengths, for example, at 480 nm and 500 to 510 nm, depending on the exciting wavebands[36] (see Ref. 13).

The effect of light scatter is liable to interfere with fluorescence measurements, a problem that is aggravated when fluorescence is measured in a spectral range which also shows much scatter. Lerman and Borkman[13] attempted to surmount this difficulty, accentuated in the presence of cortical cataracts, by expressing their results as ratios of the fluorescence intensity measured in the visible part of the spectrum and that due to tryptophan fluorescence measured at ~330 nm. The rationale of the method is not explained; it is not immediately obvious how the experimental *addition* of two radiation intensities, due, for example, to a signal and noise, can be allowed for by a correction based on the *ratio* of intensities. It is also relevant to point out that there exists some confusion about Lerman and Borkman's work. They have corrected a reprint of Ref. 13 in my possession, which makes it clear that the graph appearing above the legend to Figure 2 is, in fact, Fig. 4; and vice versa (see Ref. 21). Further inked-in corrections relate to stimulus parameters.

That said, Lerman and Borkman found two fluorophors. One with an emission band in the blue-green (500 to 520 nm) accumulates faster after birth (Figure 12a) than does the other with an emission band at 440 nm (Fig. 12b). Since this spectral range can excite the fluorescence recorded in Figure 12a,[36] there arises the question of whether secondary fluorescence may not perhaps have occurred and so contaminated the data: this has not been faced. Note that nuclear, brunescent cataracts present greatly accentuated fluorescence, but that cortical ones tend to be subnormal. This cannot come as a surprise if the absorption of light by a chromophore is a prerequisite for the occurrence of fluorescence: cortical cataracts represent discontinuities that have so far have not been associated with any pigmentation.

5 LENTICULAR FLUORESCENCE AS NOISE

It is remarkable how little attention the role of lenticular fluorescence in everyday vision has received. Dodt and Walther[37] showed that electroretinographic responses of the cat are significantly affected by it, and that it is liable to vitiate the results of measurements. How does this happen? The stimulus to retinal responses has to be measured externally to the eye; hence an additional stimulus in the form of fluorescent, visually effective light

sneaking into the system within the eye after the measurement has been made will facilitate a retinal response at a lower measured level than could be the case in its absence.

The question is one of magnitude and significance. It was shown in 1981, if not earlier, that the celebrated data on the age-related variation in human dark adaptation[38] contain a paradox. When corrected for lenticular absorbance, they suggest that the oldest observers have a lower cone threshold than, say, middle-aged ones. Once fluorescence had been measured in an axially forward direction, and its visual equivalent had been computed from the data,[36,39] Domey and McFarland's data for the highest age groups were found to be in error by an order of magnitude. This is of the same order as Van den Berg's[9] more detailed data computed for a shorter wavelength range. Given the best available corrections for lenticular absorbance and fluorescence, the human visual threshold turns out to be remarkably constant over the major part of our lives.[40]

From an evolutionary point of view, it is noteworthy that the coloration of the lens assists in the reduction of chromatic aberration of the eye.[41] However, the validity of this depends on a trade off between an illumination that optimizes visual acuity and minimizes untoward effects of lenticular fluorescence. A low level of fluorescence during the first half of our lives is helpful in that respect.

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