Reports

Red fluorescence in older and brunescent human lenses. NAI-TENG YU, JOHN F. R. KUCK, JR.* AND CARL C. ASKREN.

Brunescent lenses and normal human lenses more than 70 years old exhibit red fluorescence due to a fluorophor with emission maximum at 672 nm under excitation by the 647.1 nm line of a krypton ion laser. The properties and mode of occurrence of this fluorophor suggest that its formation is highly pertinent to senile nuclear pathology.

The appearance of fluorescent materials (fluorophors) in the aging human lens and especially in brunesceence is of widespread current interest because of the possibility that they may be involved in cataract formation, particularly the type called senile nuclear cataract which is characterized by greatly increased color and fluorescence.1-7

We report here the discovery of a red fluorophor in the nucleus of the older human lens, whose accumulation appears to parallel the development of brunesceence. Its appearance may be regarded as a sign of incipient brunesceence for four reasons: (1) it is not normally present before the seventh decade, (2) its concentration increases with age in a highly cooperative manner (i.e., sharp transition), (3) its accumulation is remarkably higher (~10^3 times) in brunescent lenses than in normal lenses of comparable age, and (4) its distribution has a maximum near the center of the nucleus. In these properties it differs from the blue fluorophor of the lens which increases gradually with age and is only slightly elevated above normal in brunescent lenses. Thus the red fluorophor does possess the important properties expected of a substance involved in nuclear pathology.

The red fluorophor has an emission maximum at 672 nm (full width at half maximum: ~2000 cm⁻¹) with excitation at 647.1 nm; the lens cortex is highly transparent to both wavelengths, so that the fluorescence of the nucleus may be measured without interference from cortical absorption. This situation is in sharp contrast to that involving the measurement of blue fluorescence (maximum at ~440 nm) with an ultraviolet (UV) excitation at ~345 nm. There the fluorescence of an intact lens is primarily that of the cortex and the outer nucleus; thus the change in blue fluorescence intensity with age does not faithfully reflect the age-related accumulation of fluorophor in the nucleus.

A laser Raman spectrometer⁸ was employed to investigate the emission properties of intact lenses and normal human lenses (24, 64, 75 and 84 years old) and a brunescent lens (68 years old) with excitation at 647.1 nm. Experimental conditions for the first three curves were the same, i.e., laser power (p), 300 mW; spectral split width (Δω), 10 cm⁻¹; sensitivity (S), 3 x 10^3 counts/sec full scale. The fourth curve was recorded with p, 250 mW; σ, 8 cm⁻¹; and S, 1 x 10⁶ cps. The bottom curve was obtained with p, 300 mW, σ, 6 cm⁻¹ and S, 1 x 10⁶ cps. The Raman peaks (<1 x 10⁵ cps) in the bottom curve are completely buried in the overwhelming fluorescence background (~1 x 10⁶ cps at 672 nm). This curve was recorded with a much reduced scale. Three horizontal lines adjacent to curves 1 to 3 represent the baselines in the absence of light. The zero baselines for the bottom two curves are much below the scale and thus not indicated in the figure.

Fig. 1. Raman and fluorescence spectra of normal human lenses (24, 64, 75 and 84 years old) and a brunescent lens (68 years old) with excitation at 647.1 nm. Experimental conditions for the first three curves were the same, i.e., laser power (p), 300 mW; spectral split width (Δω), 10 cm⁻¹; sensitivity (S), 3 x 10^3 counts/sec full scale. The fourth curve was recorded with p, 250 mW; σ, 8 cm⁻¹; and S, 1 x 10⁶ cps. The bottom curve was obtained with p, 300 mW, σ, 6 cm⁻¹ and S, 1 x 10⁶ cps. The Raman peaks (<1 x 10⁵ cps) in the bottom curve are completely buried in the overwhelming fluorescence background (~1 x 10⁶ cps at 672 nm). This curve was recorded with a much reduced scale. Three horizontal lines adjacent to curves 1 to 3 represent the baselines in the absence of light. The zero baselines for the bottom two curves are much below the scale and thus not indicated in the figure.
human lenses with ages between 1 and 95. Since both Raman emission and fluorescence may be recorded simultaneously, it becomes possible to determine the relative fluorescence intensity, with Raman signals used as the internal standard. The 647.1 nm line of a krypton ion laser was the exciting wavelength. Intact lenses were preserved in a balanced salt solution in a glass cuvette and mounted on an X-Y translation stage equipped with two micrometers. The laser beam path was along the visual axis of the lens, with its focus at the center of the lens. The radiation from only a small volume (~10⁻³ ml) near the focus was admitted to the spectrometer for analysis (90° geometry). Raman lines, being independent of exciting wavelength and very sharp, can readily be distinguished from fluorescence emission which is very broad and wavelength-dependent.

In Fig. 1 we present the spectra of four normal lenses, ages 24, 64, 75, and 84, and one dark, but clear, brunescent lens (68 years old). Spectral features of normal lenses between 1 and 24 years old are virtually identical and thus not shown. The first three spectra display well-resolved, sharp Raman lines from lens crystallins. On the basis of previous studies on animal lenses and a 6-month-old human lens, the intense line at 1004 cm⁻¹ is assigned to phenylalanine residues, the so-called amide I and III lines at 1672 and 1240 cm⁻¹ are indicative of antiparallel β-pleated sheet structure of the globular lens crystallins; tryptophan and tyrosine residues give rise to peaks at 760 and 644 cm⁻¹, respectively. No major conformation changes were noted in proteins of normal lenses between 1 and 75 years old. It is of interest to note that the baseline, as indicated by dashed curves, shows an increasing slope with age. This is interpreted as indicating the appearance of a trace fluorescence which may be readily identified as the peak at 672 nm in the spectra of the 84-year-old normal lens and the brunescent lens. This red fluorescence in the 68-year-old brunescent lens (bottom curve) was estimated to have been elevated above normal by approximately 3 orders of magnitude. Six other lenses with varying degrees of brunescence also presented the strong red fluorescence at 672 nm.

Although the optical density of the lenses was not measured, a correlation was noted between the depth of color and the intensity of red fluorescence. This parallelism was particularly striking in the comparison of an old pale lens and a lens which was younger but brunescent; here the difference in red fluorescence intensity was at least 2 orders of magnitude, showing that aging of itself is not sufficient to enhance the accumulation of the red fluorophor.

A plot of intensity ratio between fluorescence and Raman signal for Phe at 1004 cm⁻¹ in the normal lens as a function of age is shown in Fig. 2. For quantitative intensity measurements, laser power and slit width were fixed at 250 mW and 8 cm⁻¹, respectively. The fluorescence intensity at 672 nm was corrected for the background (~1000 counts/
sharp transition occurs at ~81 years of age. In contrast, the blue fluorophor usually studied exhibits a gradual increase with age. The intensity of blue fluorescence in brunescent nuclear cataract was reported to be elevated above normal only within a factor of 2. The 1000-fold enhancement observed here for the red fluorescence of brunescent lenses emphasizes the importance of studies focusing on the nucleus and those of its constituents which are markedly increased in old age and especially in brunescence.

Not all the normal lenses above 80 years of age exhibit strong red fluorescence at 672 nm. In fact, a notable exception was a clear, lightly pigmented 95-year-old lens whose spectrum resembled that of the 75-year-old lens (Fig. 1) containing only an extremely low level of red fluorophor.

The various fluorophors in the human lens are of more than academic interest because their presence in abnormal amounts may be indicative of metabolic aberrations involved in lens pathology. Our investigations of older and brunescent human lenses have revealed the presence of three fluorophors heretofore unreported: orange 568/591, near-red 568/633, and the red 647/672 discussed above. It is interesting to note that excitation at a single wavelength 568.2 nm produces fluorescence simultaneously at both 591 (orange) and 633 nm (near-red). The intensity of the near-red fluorophor increases relative to that of the orange fluorophor as one goes from the anterior cortex into the nucleus.

The red fluorophor, in particular, appears to be characteristically elevated in brunescence. This situation is of potential clinical application because fluorescence, being a form of radiation, can be measured in vivo as the emission resulting from an excitation beam of wavelength harmless to the eye. The beam may be directed into the eye at such an angle that light passing through the lens impinges on the retina far from the macula and moreover is out of focus. The monitoring of the first appearance and increase in concentration with age of the red fluorophor would give an unparalleled opportunity to study this type of pathological condition in the same patient’s lenses over a period of time.

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Photically induced experimental exophthalmos: role of Harderian and pituitary glands. DOUGLAS D. JOHNSON, P. KEVIN RUDEEN, AND W. KEITH O'STEEN.

Exposure of adult male and female albino rats to high-intensity illumination for 17.5 hr results in a marked, transient exophthalmos, which persists for approximately 48 hr after the light exposure. Upon examination of the