Fluorescence intensity profile of human lens sections. Roger Jacobs* and David L. Krohn.

The fluorescence intensity profiles of thin cross-sections of human lenses were determined as a function of age by scanning microspectrofluorimetry with blue light excitation close to that available in conventional slit-lamp examination (filter transmission maximum 410 nm). The fluorescence intensity of the entire nuclear region increased with age. The profile of the adult lens was manifested as a plateau, with a depressed inner region. The anterior slope (representing cortex) was nearly always more gradual than the more precipitous posterior slope. The peak fluorescence intensity was usually located at the posterior juxtacortical region. An additional shoulder occasionally appeared in the anterior juxtacortical nucleus. The fluorescence maximum for all ages occurred at 530 nm.

The fluorescence intensity of the blue band in human lens nucleus has been shown to increase as a function of age. The literature, however, is sparse concerning nuclear fluorescence excited by bands of longer wavelengths, such as the broad 430 nm band passing the Wratten 47A or 47B blue filter of standard slit lamps. The human lens nucleus has been shown to increase as a function of age. The fluorescence intensity profile of a human lens was shown to increase as a function of age. The fluorescence intensity profile of a human lens was shown to increase as a function of age.

The purpose of this investigation was to measure the regional fluorescence intensity profile of human lens excited by a relatively narrow band spectrally close to the blue band available in standard slit lamps and to assess some of the characteristics of this emission. The regional backscatter of white light in the human lens has been studied. Fluorescence localization within the lens, however, has not been previously reported.

Materials and methods. Eyes obtained from the Eye Bank for Sight Restoration, New York, N. Y., were kept refrigerated in a saline moist chamber and used within 24 hr of death of the donor. Lenses, which were all free of gross cataractous changes were carefully removed from eyes and placed in a special clear Lucite holder (miter) for sectioning. The holder consisted of three parts, a center spacer and two outer parts, held together with press-fit stainless steel pins. The complete miter (Scientific Machine Specialties, Hawthorne, N. Y.) contained a lens-shaped cavity. Different miters were used as required by the size and shape of the lens. The miter was designed to yield a cleanly cut section through the entire lens in the antero-posterior central axis of a uniform 1.5 mm thickness. This lens section was transferred with minimal manipulation to a glass microscope slide under continuously hydrated conditions (0.9M NaCl). A cover slip was mounted over the preparation, and excess saline was removed. Sections were made only along the anterior-posterior central axis and not along the equatorial axis, excluding possible influence of cell nuclear activity.

The lens section in its slide spacer cover slip assembly was placed on the stage of a Zeiss Standard 18 epifluorescence microscope. The excitation source was filtered with a Corning 5-58 blue bandpass filter. The microscope contained a type 510 dichroic mirror and a removable Corning 3-68 yellow barrier filter (removed during measurement) for visual positioning of the lens section. A Zeiss type 45, 1.5X objective, with its extension tube removed, was used for all observations.

The microscope was equipped with a Gamma Scientific (San Diego, Calif.) scanning photometric eyepiece containing a 450 µm diameter fiberoptic pickup probe (at the image plane) which replaced one ocular in the binocular head. Light entering the probe was conveyed via a 46 cm fiberoptic cable to a type NM3H (Gamma Scientific) monochromator (half-power bandwidth 5.8 nm) operated by a SC-1 control unit with a digital readout. Light passing through the monochromator impinged upon an RCA 4840 photomultiplier in a housing attached to the monochromator. The photomultiplier housing assembly was cooled with solid carbon dioxide. The signal from the photomultiplier was amplified by a Gamma Model 2900 autophotometer, which also supplied the operating potential (set to 1 kV) for the photomultiplier. The output of the autophotometer was used to drive the y-axis of an Esterline-Angus Model xy-530 recorder. For convenience, the time constant of this signal was increased by placement of 1 µfd capacitor across the y-input of the recorder. For representation of the position of the probe on the x-axis, information was derived from a potentiometer that was mechanically linked to the probe and supplied with electrical potential from a mercury cell. The x-axis (wavelength) of the recorder was driven by the monochromator control unit.

Spectral scans were made with the photometric probe positioned at three points on the image of
Fig. 1. Typical plots of the fluorescence intensity of human lens sections measured as a function of the position of a 450 μm diameter observation optical probe on the image of the section. The ordinate is the fluorescence intensity at 530 nm in relative units. Ages in years of lenses are solid line, 73; dotted line, 50; dashed line, 29; alternate dotted and dashed line, 7.

Table I. Fluorescence intensity* at 530 nm for three nuclear positions arranged in order of age

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>No. of lenses</th>
<th>Anterior peak</th>
<th>Center</th>
<th>Posterior peak</th>
</tr>
</thead>
<tbody>
<tr>
<td>75</td>
<td>1</td>
<td>2.000</td>
<td>1.800</td>
<td>2.000</td>
</tr>
<tr>
<td>74</td>
<td>2</td>
<td>2.350 ± 0.050†</td>
<td>2.000 ± 0.200</td>
<td>2.450 ± 0.075</td>
</tr>
<tr>
<td>73</td>
<td>2</td>
<td>1.800 ± 0.200</td>
<td>1.600 ± 0.100</td>
<td>1.925 ± 0.150</td>
</tr>
<tr>
<td>69</td>
<td>1</td>
<td>1.375</td>
<td>1.100</td>
<td>1.400</td>
</tr>
<tr>
<td>67</td>
<td>2</td>
<td>1.125 ± 0.125</td>
<td>1.125 ± 0.075</td>
<td>1.325 ± 0.225</td>
</tr>
<tr>
<td>57</td>
<td>2</td>
<td>0.875 ± 0.025</td>
<td>0.850 ± 0.050</td>
<td>0.975 ± 0.025</td>
</tr>
<tr>
<td>50</td>
<td>1</td>
<td>1.100</td>
<td>1.100</td>
<td>1.250</td>
</tr>
<tr>
<td>41</td>
<td>1</td>
<td>0.675</td>
<td>0.600</td>
<td>0.650</td>
</tr>
<tr>
<td>35</td>
<td>1</td>
<td>0.525</td>
<td>0.480</td>
<td>0.925</td>
</tr>
<tr>
<td>29</td>
<td>3</td>
<td>0.498 ± 0.032</td>
<td>0.400 ± 0.047</td>
<td>0.617 ± 0.108</td>
</tr>
<tr>
<td>7</td>
<td>1</td>
<td>0.060</td>
<td>0.125</td>
<td>0.080</td>
</tr>
</tbody>
</table>

*Expressed in relative units.
†Mean deviation.

the lens section: at the center and the anterior and posterior nuclear regions. Location of the latter two relative to cortex were selected by both fluorescence intensity characteristics and inspection. Spectral scanning from these three locations was designed to supply fluorescence emission data for each lens to determine the monochromator setting appropriate to the subsequent sweep. Spectral scanning however, indicated that the fluorescence maximum occurred at 530 nm in all parts of the nuclear region of all lenses, independent of probe position or donor age, on blue excitation peaking at 410 nm (Corning 5-58 filter). For all sweeps across the lens section, therefore, the monochromator was set at 530 nm. Sweeps were made through the central lens cross-section at a slow and uniform rate (approximating 0.2 mm/sec).

Results. In Fig. 1, typical sweeps of lenses from donors 7, 29, 50, and 73 years of age are shown. The x-axis is the position of the probe in the ocular field, and the y-axis is the fluorescence intensity at 530 nm. The fluorescence intensity profile of the adult lens was manifested as a plateau with a depressed central region. The anterior slope was
nearly always more gradual than the more precipitous posterior slope.

Because the probe position in the ocular field in the section was always in the same proportion to the pen position on the chart paper, the distance of a particular feature of the lens fluorescence from another point of interest could be determined. However, the linear extent of a feature had to be considered in relative terms, since there was variation with donor age, lens thickness, and perhaps with the condition of the section at the time of observation. In a sweep of a 57-year-old lens in which, by inspection, the division between cortex and nucleus was particularly sharp, the "edge peaks" clearly represented fluorescence from within the nucleus, the initial and terminal transients developing as the probe moved across cortical lens material.

The peak fluorescence intensity was usually located at the posterior juxta-cortical nuclear region. In some lens sections, an additional shoulder appeared in the anterior juxta-cortical nuclear region.

Table I contains relative fluorescence intensity values measured at 530 nm, corresponding to measurements of anterior peak, central, and posterior peak fluorescence intensities. These values are presented as averages ± mean deviation where more than one lens was available. All maxima are included in Table I. For the population studied, the fluorescence intensity of the entire nuclear region increased as a function of age, independent of location.

Discussion. Age-dependent fluorescence excitation in the lens nucleus has been reported by most investigators to be characteristic of the region from about 325 nm to 360 nm. Little information is available concerning nuclear fluorescence excited by excitation bands at longer wavelengths. Kurzel et al. 6 however, have shown that emission monitored at 500 nm shows excitation peaks for both normal and nuclear cataractous lenses, higher for the latter, extending from 410 nm through 430 nm. If the emission is monitored at 550 nm, a red shift in the cataractous lens to 430 nm by Bando et al. 7 showed greatest absorption and therefore greatest protein concentration centrally. In addition, quantitative measurements of regional backscatter as a function of age made by Sigelman et al. 8 showed no suggestion of edge peaks in the nucleus. It would therefore seem likely that the depressed central plateau is an absorption phenomenon related to centrally accumulating pigments, which may either fluoresce poorly on excitation peaking at 430 nm, or are sufficiently concentrated to produce a quenching effect despite the minimal thickness of the segments scanned. Such an explanation would, however, be more consistent with a curve reaching a nadir in the central nucleus than with the plateau effect that was found. It is clear that further studies are required to explain the depressed plateau and also the relative steepness of the posterior terminal slope as compared to the anterior.

Although the exact nature of the fluorogens excited by long-wavelength blue light is in some doubt, it is of interest that both nuclear fluorescence and backscatter may increase strikingly in intensity relative to youthful baselines without loss of central visual acuity as conventionally measured.

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From the Department of Ophthalmology, New York University Medical Center, New York, N. Y. Supported in part by grants CA 18705 from the National Cancer Institute and EY 02428 and EY 02872 from the National Eye Institute, National Institutes of Health, U. S. Public
In the cornea, label was found in the epithelium and in the connective tissue cells and blood vessel endothelial cells of the stroma. Prior administration of a 350-fold excess of nonlabeled hormone completely suppressed this localization, indicating that these cells are specific glucocorticoid target cells. There was no localization found in the corneal endothelium. Accumulation of silver grains between corneal stroma and Descemet's membrane suggests that this membrane acts as a partial barrier to diffusion of the steroid.

In a prior study, dexamethasone was localized radioautographically to the nuclei of target cells of the rabbit eye after intravenous administration of the labeled steroid. Specifically bound steroid was found in the nuclei of stromal and endothelial cells of the outflow pathway region and conjunctiva, with no apparent localization in the cornea. The absence of binding in the cornea could have been related to either the lack of specific target cells in this tissue or to insufficient exposure of the cornea to the labeled steroid during the time course of the experiment. The presence of a significant blood-aqueous barrier to steroid hormones in the normal eye could have limited exposure of the cornea to the labeled hormone. Since glucocorticoids are widely used in the treatment of corneal-conjunctival disorders, it is important to determine whether the cornea contains glucocorticoid target cells. In order to bypass the blood-aqueous barrier, labeled dexamethasone was administered topically, the most common therapeutic route of administration of the steroid. A method of dry-mount radioautography suitable for diffusible substances was used to localize the labeled hormone.

**Materials and methods.** Female albino New Zealand rabbits, weighing 2.2 to 2.5 kg, were used. $^3$H-dexamethasone (6.25 μCi; sp. act. 26.4 Ci/mmol; New England Nuclear) in 0.025 ml of saline was topically administered to one eye. Control animals received the same dose of labeled dexamethasone 10 min after treatment of the eye with 32 μg of nonlabeled dexamethasone (Decadron; Merck, Sharp & Dohme) in 0.05 ml of saline. Approximately 1 hr later the animals were anesthetized with sodium pentobarbital (intravenously), and the eye was enucleated. Tissue samples from cornea and conjunctiva were dissected, frozen in Freon over liquid nitrogen, and stored in liquid nitrogen. Cryostat sections, 6 μm thick, were freeze-dried in a cryopump (ThermoVac Industries, Inc., Copiague, N.Y.) at −35°C with a prevacuum produced by an oil-diffusion pump backed by a mechanical pump. Freeze-dried sections were pressed to glass slides that had previously been coated with nuclear track emulsion (NTB-3; Kodak) which had been allowed to dry. After 4 to 6 months of exposure, radioautographs


Topically administered $^3$H-dexamethasone was localized radioautographically to the nuclei of several cell types in the cornea and conjunctiva. In the cornea, label was found in the epithelium and keratocytes. In the conjunctiva, label was found in the epithelium and in the connective tissue cells and blood vessel endothelial cells of the stroma. Prior administration of a 350-fold excess of nonlabeled hormone completely suppressed this localization, indicating that these cells are specific glucocorticoid target cells. There was no localization found in the corneal endothelium. Accumulation of silver grains between corneal stroma and Descemet's membrane suggests that this membrane acts as a partial barrier to diffusion of the steroid.

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