Light Transmittance of Ocular Media in Living Rabbit Eyes

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Purpose. The purpose of this study was to measure the transmittance of electromagnetic radiation, particularly visible light, through the ocular media of living and whole rabbit eyes. Previous determinations have been carried out on excised cadaver eyes.

Methods. A specially designed fiberoptic probe (outer diameter, 0.9 mm) was placed in the vitreous in front of the retina using a microsurgical technique. In eight living albino rabbits (under general anesthesia), ocular transmittance was determined in the wavelength range 350 to 1100 nm using a reversed beam path (from vitreous to cornea).

Results. A maximum optical transmittance of 94% to 96% (standard deviation, 2%–3%) was found between 630 and 730 nm (reflection losses in the cornea-air interface excluded). In the blue portion of the spectrum, transmittance decreased rapidly for shorter wavelengths, and was 50% at 400 nm and less than 1% at 380 nm. In the infrared part of the spectrum, transmittance was close to 90% up to 900 nm but declined at longer wavelengths, coinciding with the absorption in pure water. Calibration recordings showed a 1% to 2% accuracy of the method.

Conclusions. This experimental technique using an intraocular fiberoptic probe yields a high accuracy and indicates that light transmittance is very high in vivo and superior to that reported from cadaver eyes. Invest Ophthalmol Vis Sci. 1993;34:349–354.

Studies on ocular transmittance characteristics with respect to electromagnetic radiation1–8 have formed the basis for interpretation of photoeffects on the eye.9 Some reports are compatible with each other, whereas others do not agree. All measurements up until now have, however, been carried out on dead (enucleated) eyes, and it has not been fully investigated how early postmortem changes affect the optical characteristics of the eye. Boynton et al3 and Demott et al5 found considerable time dependence in postmortem changes, whereas Boettner and Wolter6 reported a minor, almost negligible time dependence. One possible explanation of these incongruities may be the varying drying effects in the specimen due to different handling techniques.

To overcome these discrepancies, we worked out an in vivo method for measurement of transmittance of ocular media. A special fiberoptic probe was developed for insertion into the vitreous cavity of a living eye. With this intraocular fiberoptic technique, it is possible to perform several types of experiments, including measurements of radiation transmittance, and studies of fluorescence phenomena and optical scattering in different parts of the ocular media. In the current report, we describe the results of intraocular light transmittance in the cornea, aqueous, lens, and vitreous using this fiberoptic probe technique.

MATERIALS AND METHODS

Instrumentation

A fiberoptic probe, designed and developed for these experiments, was inserted into the vitreous cavity close to the retina and adjusted to the optical axis of the eye.

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FIGURE 1. Location of fiberoptic probe (outer diameter, 0.9 mm) inside the vitreous cavity, close to the retina, centered to the optical axis of eye. Test light enters the eye through the optical fiber and is reflected from the tip of the probe in the vitreous to exit through the pupil and cornea.

The fiberoptic cable was connected to a test light source, a halogen lamp (Intralux 250 HL Volpi AG, Zurich, Switzerland) or He-Ne laser (model 106-1, 10 mW; Spectra Physics, Carlsbad, CA). The intraocular fiberoptic probe makes it possible, and preferable, to use a reversed beam path through the eye because this greatly facilitates the optical alignment in living eyes. The beam path from the tip of the fiberoptic probe was directed anteriorly through the pupil to exit the eye through the cornea (Fig. 1).

Basically, the probe consists of a standard, multimode optical fiber (HCG-365, numerical aperture = 0.2; Ensign Bickford, Avon, CT). The optical parts (core and cladding) of this fiber are made of pure quartz, resulting in very high transmittance within the wavelength range of 300 to 2000 nm.

The fiber was first fixed into an approximately 50-mm long injection steel needle with an outer diameter of 0.9 mm. The needle was bent to an appropriate radius to fit the rabbit eye retinal curvature. A critical part of the probe manufacturing was the preparation of the optical needle end. To achieve a well confined beam output, it was necessary to cut and polish the fiber tip to an angle of 45° with respect to the optical axis of the fiber. To serve as a mirror, this surface had to be coated with aluminum to avoid leakage of radiation in unwanted directions (Fig. 2). The outcoupling surface of the fiber tip also was made flat to achieve a well confined beam lobe with low divergence. The half-angle of the output lobe was 14° in air and less than 10° (numerical aperture = 0.18) in water (vitreous), which is small enough to avoid any obstruction of the beam path by the dilated eye pupil aperture during the measurements.

The light beam exiting the pupil was directed, using a multimode quartz fiber, through a monochromator (Oriel [Stratford, CT] 77200, with gratings 1200 lines/mm #77998 and 600 lines/mm #77999) to a photodiode (Photodyne XL-88 [Westlake Village, CA] with detector #250) and an RG XYt recorder (PM 8277; Philips, Eindhoven, Holland). The experimental set-up is shown in Figure 3.

Surgical Technique

to place the fiberoptic probe (outer diameter, 0.9 mm) into the vitreous cavity, a microsurgical technique using an operating microscope was adopted. The pupil of the eye was maximally dilated with eye drops (atropine, phenylephrine). General anesthesia was induced with intramuscular injections of ketamine (35 mg/kg body weight) and xylazine (5 mg/kg body

FIGURE 2. Appearance of the tip of the fiberoptic probe. Optical fiber inside a 20 G steel needle ends in a mirrored surface that reflects light in the direction of the optical axis of the eye. Divergence angle (α) is 10° in water.
weight) and, if necessary, additional anesthetics were given every 30 to 45 min.

The conjunctiva was cut free (limbal incision) from the upper lateral quadrant of the eye. The eye wall was perforated with a stiletto (sclerotomy 1.4 mm) in the region of the equator of the eye and the stiletto was directed toward the posterior pole of the eye. The fiberoptic probe (20 G) fits tightly to the sclerotomy. The probe was inserted into the vitreous and guided to the posterior pole of the eye, close to the retina, under microscopic control (using a corneal contact lens). The front surface of the probe was positioned less than 2 mm from the retina and centered to the optical axis. The position of the mirror of the probe was checked by connecting the fiberoptic cable to a He-Ne laser that illuminates the mirror in a “retrograde” direction. The probe, attached to a flexible arm fixture, was fixed in such a position that the light beam entering the eye pupil was focused to the optical tip of the probe. This position was maintained throughout the experiment.

Care was taken to avoid drying of the corneal epithelium, and balanced salt solution was dropped regularly on the cornea. The transparency of the ocular media remained excellent. No hypotony occurred because the sclerotomy remained water-tight. Experiments were run over a 2-hr time course without complications. Intraocular hemorrhages or retinal detachment did not occur during the experiments. When the fiberoptic probe was removed from the eye, the sclerotomy and conjunctiva were sutured.

**Animals**

Measurements were performed on 12 eyes from 12 New Zealand white (albino) rabbits. The rabbits’ ages ranged from 4 mo to 2.5 yr (median, 9 months). The rabbits were handled in accordance with the ARVO Resolution on the Use of Animals in Research. We were able successfully to examine eight eyes. In four cases, the recordings were not satisfactory, partly due to insufficient immobilization of the animal, with variations in breathing movements.

**Measurement Procedure**

The measurements were divided into two procedures. First, the relative spectral transmission of the eye was recorded over the whole spectral range (350–1100 nm). After that, the absolute spectral transmittance at a specific wavelength (633 nm) was measured.

In the first procedure, we used the white light source (halogen lamp) and a scanning monochromator for measurement of the optical power transmission as a function of the wavelength with \( P_{\text{eye}}(\lambda) \) and without \( P_{\text{sys}}(\lambda) \) the eye present in the optical system. The optical power as a function of wavelength was recorded graphically with the XY recorder. The slit opening of the monochromator was set to 0.8 mm, corresponding to an optical bandwidth of 4 nm. A sequence always started with a calibration, recording the spectral characteristics of the complete system without the eye \( P_{\text{sys}}(\lambda) \). Immediately after this calibration, we inserted the fiberoptic probe into the already prepared rabbit eye and made a new recording \( P_{\text{eye}}(\lambda) \). The relative spectral transmission \( T_{\text{rel}}(\lambda) \) of the rabbit eye then was calculated as the ratio of the transmission values with and without the eye:

\[
T_{\text{rel}}(\lambda) = C_{\text{rel}} \times \frac{P_{\text{eye}}(\lambda)}{P_{\text{sys}}(\lambda)} \quad (1)
\]

where \( C_{\text{rel}} \) is a wavelength-independent scale factor.
The constant \(C_{\text{rel}}\) differs from eye to eye due to minor deviations in the beam path alignment and detector amplification adjustments.

In the second procedure, we used the He-Ne laser (633 nm) as a light source. This facilitated the visual alignment and measurement procedures. The reason for using a laser instead of a conventional light source was that it is much easier to launch high optical power confined in a narrow wavelength band into an optical fiber with a laser. The absolute calibration reference level of the optical transmission was measured for the complete optical system with the fiberoptic probe immersed in 1 mm pure water \(P_{\text{sys}}(\lambda = 633)\). Immediately after the calibration in water, the probe was inserted into the eye and the optical power was measured again \(P_{\text{eye}}(\lambda = 633)\). The absolute transmittance \(T_{\text{abs}}(\lambda = 633)\) is simply the quotient \(\frac{P_{\text{eye}}(\lambda = 633)}{P_{\text{sys}}(\lambda = 633)}\). Note that the absolute transmittance depends only on absorption and scattering of light inside the eye because we cancel out the effects of reflections in the interfaces between probe tip and vitreous humor as well as the interface between the cornea and air using the calibration procedure with the probe immersed in pure water. The influence of absorption and scattering in water with a path length of 1 mm at 633 nm can be ignored safely. The refractive index of the cornea and vitreous humor is very close the refractive index of water \((n = 1.33)\). Thus, the transmittances presented in this report do not include the effects of reflection of light in the cornea-air interface, which was calculated to be 2% according to the well known formula for optical power reflection \((R)\) at perpendicular incidence:

\[
R = \left(\frac{n_1 - n_0}{n_1 + n_0}\right)^2
\]

where \(n_1 = 1.33\) for water and cornea and \(n_0 = 1\) for air.

The absolute transmittance for any wavelength in the measured range for a specific eye can now be calculated using the scale factor \(C_{\text{abs}}\) according to the following expressions:

\[
T_{\text{abs}}(\lambda) = C_{\text{abs}} \times T_{\text{rel}}(\lambda)
\]

\[
T_{\text{rel}}(\lambda) = C_{\text{rel}} \times \left(\frac{P_{\text{eye}}(\lambda)}{P_{\text{sys}}(\lambda)}\right)
\]

\[
C_{\text{abs}} = \frac{P_{\text{eye}}(\lambda = 633)}{P_{\text{sys}}(\lambda = 633)} \times \left(\frac{P_{\text{sys}}(\lambda = 633)}{P_{\text{eye}}(\lambda = 633)}\right) \times \frac{1}{C_{\text{rel}}}
\]

**Accuracy of the Method**

Repeated calibration recordings indicated an accuracy of 1% to 2% for the whole system. In some recordings with the optical probe inside a living rabbit eye, however, the accuracy typically could be 3% to 6% because of disturbances caused by the rabbit’s breathing movements. The depth of the anesthesia was found to be a critical factor.

**RESULTS**

**Visible Light**

The averaged maximum optical transmittance of eight living rabbit eyes was measured to be as high as 96% at 700 nm (standard deviation [SD], 1.3%). An average transmittance of 94% to 96% was found between 630 and 730 nm. The transmittance exceeds 90% in the wavelength range from 525 to 860 nm (SD 2%-5%). The averaged readings over 25 nm or shorter intervals plotted on a graph are shown in Figure 4.

**Ultraviolet Radiation**

In the ultraviolet part of the spectrum, the transmittance decreased rapidly from 50% at 400 nm down to less than 1% below 378 nm. In the blue part of spectrum, the measurements showed a larger deviation of the readings and, below 450 nm, the SD increased to approximately 15% (Fig. 4). The high SD could be caused by several factors, such as variations in absorption and pronounced scattering of light.

**Near-Infrared Radiation**

In the infrared part of spectrum, the transmittance of the eye was close to 90% up to about 900 nm. At longer wavelengths, there was a decline of transmittance to approximately 60% in the range of 960 to 1000 nm. This drop seemed to coincide with the absorption curve in pure water. For wavelengths up to about 1050 nm, the transmittance of the whole eye (from cornea to retina) was close to that for water of the same interaction length (Fig. 5). The SD values were markedly increased for wavelengths above 1050 nm, however, indicating some inaccuracy in the measurements in this part of spectrum (Fig. 4).

**DISCUSSION**

The experiments show that in the living rabbit eye, the transmittance of radiation is very high, exceeding 90% from about 525 to 860 nm, with a peak of 96% at 700 nm. In previous reports on light transmittance in cadaver eyes, Ludvigh and McCarthy\(^1\) found a maximum transmittance of 75% at 700 nm and Boettner and Wolter\(^6\) a maximum of 85% at 770 nm. When the measurements were carried out 15 to 45 min after enucleation of the eye, Wiesinger et al\(^4\) recorded a maximum light transmission of 95% at 680 nm. In our experiments, the living eye maintained a healthy corneal epithelium and an intact circulation. It is conceivable, but
Light Transmittance in Living Eyes

FIGURE 4. Radiation transmittance, average of eight rabbit eyes. Transmittance (circles) is 94% to 96% between 630 and 730 nm. Within these wavelengths, the SD (crosses) is 2% to 3% (plotted at base of graph).

not shown in this study, that the optical transparency of the living eye is superior to that postmortem.

The current method enables a thorough positioning of the detector probe, the tip of which is adjusted in the vitreous under microscopic control. In addition, a He-Ne laser beam was used as an adjunct measure to monitor the accurate positioning of the probe. The output lobe of the fiberoptic probe subtends a half-angle of 10° in water, and delivers the light beam through the eye pupil and cornea. This experimental design yields an accurate alignment of the test light to the recording system with minimal losses of radiation. The main reflection loss (calculated to be 2%) emanates from the interface between the cornea and air. It

FIGURE 5. Radiation absorption in 12 mm of pure water (circles) and transmittance of rabbit eye (crosses). The two curves coincide up to about 1050 nm.
is justifiable to use a reversed beam path given the assumption that the absorption and scattering processes in the eye are isotropic. Measurements of ocular transmission using radiations that traversed the eye in a posteroanterior direction have been performed in the past.²

In the extremes of the spectrum, the readings were associated with a high SD. This indicates that several factors may influence the results here. In the infrared part of spectrum, ocular transmittance coincided with absorption in water up to about 1050 nm. For longer wavelengths, ocular transmittance declined and the curve deviated from that of water absorption. The poor correspondence above 1050 nm can be explained by the rapid decrease in sensitivity of the optical detector for wavelengths in this part of spectrum.

It is well known that age-related changes in the crystalline lens reduce transmittance, particularly of blue light.¹⁰¹¹ The rabbit lens is comparatively thick (6–7 mm in optical axis) in relation to the size of the eye and certainly has a great impact on ocular light transmittance. Thus, individual or age-related variations in the crystalline lens cannot be ruled out. With this intraocular fiberoptic technique, it is possible not only to measure transmittance, but also to study fluorescence phenomena and optical scattering in different parts of the ocular media. A most interesting part of the spectrum is the wavelength region below 450 nm where the scattering effects become very significant. The transmittance decreases with increasing absorption and scattering of light. The influence of scattering effects on the total transmittance has not been fully investigated. Boettner and Wolter measured direct and total transmittance in an attempt to separate the scattering effects from pure absorption. The direct transmittance was much lower than the total transmittance, especially at short wavelengths, which indicates a pronounced influence of scattering.

To distinguish between scattering and pure absorption effects, however, it is necessary to perform measurements on light scattering per se, which require an accurate definition of the direct beam, a very small optical aperture at the detector fiber optic tip, and a well defined size of the retinal image. This kind of measurement does not seem to have been done properly in living or cadaver eyes. With the current fiberoptic probe technique, such measurements can be made when the system has been slightly modified (eg, the probe must be equipped with a very small optical aperture at its tip).

It is conceivable that the human eye has light transmittance that is as good as that of the rabbit. To prove this hypothesis, experiments have to be extended to primate eyes and, when the opportunity arises, to human eyes.

Key Words

intravitreal probe, light transmittance, living eyes, quartz optical fiber, wavelength, whole eyes.

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