Transmission of the ocular media

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The spectral transmittance of ultraviolet, visible, and near infrared light through the ocular media of humans has been measured. Using freshly enucleated eyes, the transmittances of each component part (cornea, aqueous humor, lens, vitreous humor) were determined for the wavelength range from 0.22 to 2.8 μ. To date, 9 eyes have been measured, from persons ranging in age from 4 weeks to 75 years. Two types of measurements were made, the first to measure the total light transmitted (direct and scattered) at each wavelength, and the second to measure the per cent transmittance of that light passing directly through the various media without absorption or scattering. The results show that: (a) The transmission of ultraviolet radiation decreases with the age of the eye. (b) The transmission of infrared radiation appears to be independent of the age. (c) The maximum total transmittance of the whole eye, which is about 84 per cent, is obtained in the region from 650 to 850 μ.

Knowledge of the transmission characteristics of the eye to radiation in the electromagnetic spectrum is of importance in two general ways. First, it is information which one must have to assess the possible effect of intense radiation sources on the various parts of the eye. Second, such data are required if one desires to learn something about the physiologic mechanisms excited by any portion of the spectrum, because, in such experiments, the spectrophotometric characteristics of the radiation can be measured only at the cornea, while excitation occurs after the light passes to the retina. One of the first investigators to concern himself with the transmission was Brucke, who investigated the reason for the invisibility of ultraviolet rays. Later others investigated the visible and infrared portions of the spectrum. Duke-Elder1 summarized the work in this field up to 1952 very thoroughly in both Volumes 1 and 4 of his textbook.

Most of the measurements have been made on animal eyes, especially those of the rabbit.7

Ludvig and McCarthy2 in 1938 reported measurements in the visible region on 4 human eyes of average age 62 years, all with sarcoma of the choroid. Recently, Geeraets and co-workers3 measured 7 eyes, only 2 of which could be termed normal. Both of these papers were concerned with the measurement of the whole eye, rather than its components.

Because of the dearth of information on human eyes, a research program has been instituted, the aim of which is to determine the transmittance characteristics of the components of as many eyes as possible. In this way, it is hoped that a good statistical sampling of various ages can be obtained. The present report is a description of data on 9 human eyes.

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The measurements were made in the ultraviolet, visible, and near infrared portions of the spectrum, covering the wavelength region from 220 to 2,800 nm. The components measured were the cornea, aqueous humor, lens, and vitreous humor. When electromagnetic radiation passes through a medium, several things can happen to the radiation:

1. It can pass directly through the medium (called direct transmittance in this report).
2. It can be reflected by the medium.
3. It can be scattered by the medium. In this case, all the radiation passing to the medium also emerges, but in random directions.
4. It can be absorbed by the medium, in which case the energy is used to ionize atoms, to heat the atoms, to cause them to fluoresce, etc.

All four of these phenomena take place when radiation passes through the eye. Some of the energy passes directly through to form an image in the retina. Some is scattered by the media, resulting in a general illumination within the eye. Some is absorbed and reradiated at longer wavelengths. A small amount is reflected by surfaces separating media of different refractive indices. Most of the reflection takes place at the anterior surface of the cornea, which is the boundary with the greatest change in index of refraction.

In this report, two sets of transmittance measurements of the individual components are described. One is a measure of direct transmittance and the other is a measure of both the direct and a portion of the scattered. These two sets of data can be better defined by referring to Fig. 1. The aperture which limits the direct transmittance measurements is so arranged that it accepts only the rays of the direct beam and those within about 1 degree of the direct. The total transmittance reading is a measure of all the radiation emerging over a cone of about 170 degrees centered about the optical axis. This measurement includes that radiation which is forward scattered.

It does not include the portion of the radiation that is back scattered (that portion which is reflected back into the same hemisphere as the original incoming beam).

The eyes used were normal human eyes from patients varying in age from 4 weeks to 75 years. The pathologic conditions of the eyes are described in the section, Evaluation of Data. The mounting and measuring techniques were first worked out with the use of the eyes of rhesus monkeys. Monkey eyes were also used for preliminary observations of the effect of time after enucleation on the transmittance measurements.

**Procedure**

The freshly enucleated eye was moved directly from the operating room to the operating room annex, where it was immediately divided into its components.

The aqueous humor was removed by means of a 26 gauge hypodermic needle and syringe. The
Fig. 2. Cells used to contain the ocular media.

needle was inserted into the anterior chamber through the corneoscleral limbus and the aqueous withdrawn. It was then transferred immediately to a modified short pathlength Beckman No. 92134 liquid cell. This cell was modified as shown in Fig. 2, A by replacing the conventional 0.5 mm. shim by one which is 1.5 mm. thick, but has its cross-section reduced to a minimum so that the amount of solution that is required is only 0.12 ml. Except for the smaller infant eye, the volume of aqueous removed was always sufficient to fill the cell. Next, the cornea was removed by cutting around the corneoscleral limbus with Aebli's corneal scissors. The cornea was washed briefly in saline solution and then placed between two cell windows of 1 mm. thick fused quartz. This sandwich was then placed in a cell holder as shown in Fig. 2, B, with care to use only sufficient pressure on the windows to flatten the cornea without compressing it.

To obtain the lens the zonule was first broken by using the Kirby lens loop with a small amount of pressure on the sclera. Then the same instrument was used to lift the lens and remove it.

The lens was mounted in a cell containing a supporting shim about 0.2 mm. less in thickness than the axial thickness of the lens (Fig. 2, C). This permitted the lens to be flattened without breaking the lens capsule. The reason for flattening the lens surface was to remove as much of the optical lens effect as possible. However, a complete removal by this technique was not possible because of the varying index of refraction of the inner portions of the lens.

The vitreous was removed from the scleral shell by using a hypodermic syringe with a large opening. This was inserted through the area of the pars plana of the ciliary body. This was found to be the best way to avoid contaminating the vitreous with bits of pigment from the posterior iris surface. The spacers used in the vitreous cell (Fig. 2, D) had a diameter of 13 mm. and a thickness of 12 mm. so that the vitreous from a single eye was sufficient to fill a cell and still permit a measurement at a thickness approaching that of the same medium in the eye.

The transmittances of the individual components were measured with two spectrophotometers. A Beckman DK-2 spectrophotometer was used to measure the direct transmittance. This instrument is a double-beam spectrophotometer where the detecting phototube compares the intensity of the sample beam with that of the reference beam, with the ratio of the two intensities recorded as per cent transmittance. Here, the cells containing the individual components were placed in the sample beam, and a quartz plate was placed in the reference beam. This latter plate cancelled out the effects of reflection and absorption by the quartz windows in the sample cells. Two diaphragms of equal diameter (6 mm.) were placed in the two radiation beams (sample and reference) so that the incident beam of radiation was smaller than the smallest component to be measured.

The total transmittance measurements were made with the use of a Beckman DK-1 spectrophotometer. This differs from the DK-2 instrument only in that the radiation from the mono-
chromometer terminates in an integrating sphere, where it illuminates the sphere wall. The sphere is illuminated alternately by the reference beam and the sample beam. The detecting photocell measures the wall illumination of the sphere, rather than the direct beams. By placing the eye specimen holder immediately before the sample beam part of the sphere, all of the radiation transmitted by the sample, both direct and forward scattered, enters the sphere and contributes to the wall illumination, and therefore to the phototube signal.

The preliminary work on this project, to test both the cells and the measuring techniques, was done with the eyes of rhesus monkeys immediately after death. These eyes, although only 20 mm. in diameter as compared with 25 mm. for the human eye, appear anatomically similar to the human eye. One of the first experiments performed with these eyes was to determine the effect on transmission of the length of time between enucleation and the measurements. Some measurements were started as soon as 15 minutes after the eye was removed. Repeat measurements extended the test to as much as 4 hours. The results showed only minor changes in transmission. They are discussed in the next section with a similar test on human eyes.

Results

Nine human eyes, at ages 4 weeks, 2, 4½, 23, 42, 51, 53, 63, and 75 years respectively, have been measured. Figs. 4 and 6 show the transmittances of the aqueous and the vitreous humors. These data have been averaged from the measurements on several eyes. The curves of the cornea and the lens (Figs. 3 and 5) are of specific eyes in order to show the effect of age on the transmittances.

Cornea. This component transmits radiation from 300 μm in the ultraviolet to 2,500 μm in the infrared (Fig. 3). The total transmittance increases rapidly from 300 μm and reaches about 80 per cent at 380 μm, and from 500 μm to 1,300 μm is greater than 90 per cent. Beyond 1,300 μm, two absorption bands of water appear (1,430 and 1,950 μm) but the transmission between the bands remains high.

The total transmittance curve is representative of 6 eyes, with the spread in the values at 700 μm of less than 3 per cent with no age trend.
Two direct transmittance curves are shown, with the lower near the average of 8 eyes and the upper curve (1) the best transmittance observed. The maximum transmittance of the direct measurements is at 1,100 m/μ. The cornea is the only component having its maximum this far in the infrared.

Aqueous humor. This component begins transmitting at 220 m/μ in the ultraviolet and continues to 2,400 m/μ in the infrared (Fig. 4). In the ultraviolet, it has a strong absorption band at 265 m/μ which some investigators have attributed to nucleoprotein. Through the visible region, the aqueous has a high transmittance, slightly less than that of an equal thickness of water. Its transmittance in the infrared is decreased by water absorption bands at 980, 1,200, 1,430, and 1,950 m/μ. The transmittance at 2,200 m/μ is only 0.1 per cent and the complete absorption beyond 2,400 m/μ is due to water. No differences in transmittance due to age were noted for this medium. Only the direct transmittance is reported since no difference could be found between the direct and total transmittance measurements. Visual observation of the aqueous after removal from the anterior chamber also shows no evidence of light scattering. However, a small amount of scattering (less than 2 per cent) could be present and still escape detection by either method.

Lens. Transmittance through the lens extends from the ultraviolet to an upper limit at 1,900 m/μ in the infrared (Fig. 5). The ultraviolet and short wavelength visible light transmittance varies considerably with the age of the eye. The lens of the young child begins transmitting at 300 m/μ; however, an absorption band centered at 360 m/μ reduces the transmittance to a very low value below 390 m/μ. Because of this absorption band, the lens of a child has a transmitting band centered at 320 m/μ of about 5 per cent under 5 years and less than 0.1 per cent by the age of 22 years. This same transmittance band was found in the lenses obtained from monkey eyes. The total transmittance of the young eye begins increasing rapidly about 390 m/μ, and reaches 90 per cent at 430 m/μ. The rate of increase is considerably slower for the older lens, e.g., a 63-year-old lens begins transmitting at 400 m/μ but does not reach 90 per cent total transmittance until 540 m/μ. In addition, the light scattering by the older lens is much higher. The direct transmittance of the young lens at 700 m/μ is about 88 per cent, while the 75 year lens measured only 41 per cent. The lens continues to have a high transmittance to 1,400 m/μ in the infrared and demonstrates the usual water bands at 980, 1,200, and 1,430 m/μ.

The age dependence in the visible region is in fairly good agreement with the findings of Said and Weale on the direct transmittance of the lens in the living eye. For example, their curve for the 21 year lens is very close to our data (curve 1) in the region from 450 to 600 m/μ. Likewise, their data for the older lens lie between our curves for the 53 year and 75 year eyes. However, it has been our experience that the older eye shows a larger variation in the amount of transmitted radiation in the visible. As an example of this variation, the lens of a 71 year eye measured recently had a direct transmittance better than that of the 53 year eye (curve 2 of Fig. 5), and the suggestion by Said and
Weale that the age can be determined by the transmittance measurements is doubtful on the basis of our experience to date.

Vitreous humor. The vitreous transmits from 300 μm in the ultraviolet to 1,400 μm in the infrared (Fig. 6). Its ultraviolet total transmittance increases rapidly to 80 per cent at 350 μm. The total transmittance in the visible region is greater than 90 per cent, but begins dropping rapidly in the infrared. The water bands at 980 and 1,200 μm are very strong, and no transmittance is noted beyond 1,400 μm. No differences in transmittance due to age were noted.

Transmittance of the entire eye. The data shown in Figs. 3 to 6 were used to compute the successive transmittances as radiation passes through the whole eye. The resulting curves are shown in Figs. 7 and 8. In making this computation, the loss due to reflection of normally incident radiation at the interface between air and the cornea was included. Reflection losses between the other surfaces (e.g., aqueous-lens) were neglected as they total less than 0.3 per cent.

These data are representative of the child or young adult eye except in the ultraviolet (less than 380 μm) where the transmission is that of a child's eye. For example, the 4 per cent incident on the vitreous (Fig. 7) at 320 μm would be completely absorbed by the lens in the adult eye.

The maximum transmittance through an entire eye is calculated as 83.5 per cent. Recently, a technique was developed in which a window was cut into the posterior of a whole eye to permit the measure of the transmittance through all the ocular media. With the use of this technique, the total transmittances of 2 eyes have been measured to date, with values of 82 per cent and 79 per cent at 700 μm.

It is evident that the amount of scattered radiation through the young whole eye, represented by the difference between curve 4 in Fig. 7 and in Fig. 8, decreases with wavelength, from about 55 per cent at 450 μm in the visible to 30 per cent in the infrared. Generally the scattering in the older eye starts at a higher figure (70 per cent or more) in the visible, but proceeds at a more rapid rate of decrease into the infrared.

Evaluation of data

Several factors may affect the validity of the results, including the condition of the eyes, the length of time between enucleation and the measurements, and the accuracy of the instrumental methods and measuring techniques. As stated previously, only eyes having normal refracting media were used in this study. Four eyes
had melanoma of the choroid, with the refracting media appearing normal in all respects. The other 5 eyes had no pathologic abnormalities. All measurements were made in the time interval between 15 and 210 minutes after enucleation.

The effect of time after enucleation was carefully studied on 3 monkey eyes and 2 human specimens. Contrary to the results of Boynton and Demott and their associates, only a little time effect was found. However, they attribute the change to a drying out of the specimen. In the work described here, the components were placed in sealed cells so that evaporation was negligible.

The only measurable change with time was noted in the transmittance of the aqueous humor in the vicinity of 230 m\(\mu\) in the ultraviolet. There is an isolated transmission band at this wavelength (Fig. 4, A) separated from the longer transmitting wavelengths by an absorption band at 265 m\(\mu\). The amount of absorption at this latter wavelength decreases with time, resulting in an increase in the height and a slight shifting to longer wavelengths of the transmission band at 230 m\(\mu\). For example, the aqueous of one monkey eye measured 1.9 per cent at 232 m\(\mu\) 30 minutes after enucleation. A subsequent measurement at 3 hours showed a transmittance of 4.2 per cent at the peak, which shifted to 236 m\(\mu\). Likewise, one human eye measured 1.1 per cent at 235 m\(\mu\) 40 minutes after enucleation and 1.9 per cent at 240 m\(\mu\) at 4 hours.

Through the remainder of the spectrum any change with time was less than the reproducibility of the measuring technique. It should be noted that any transmission change in the first few minutes after enucleation would not have been detected in these experiments as the first readings were made at 15 minutes or more after enucleation.

Tests were carried out on the technique of measuring the cornea, because of Maurice's observation that stressing the cornea could increase the amount of light scattering, thereby decreasing the direct transmittance. Measuring the cornea in two ways, first by mounting it without any distortion, and, second, by using our technique of gently flattening it, no change in direct transmittance was noted. Because of the fragile nature of the thin quartz windows, no attempt was made to apply excessive pressure to substantiate the observations of Maurice.

The accuracy of the spectrophotometer readings depends on the instruments, the amount the radiation beams must be masked to match the size of the specimens, the cleanliness of the cell windows, and the ability to match the window in the reference beam with those in the cells. The instrumental deviations were determined by making repeat runs on glass samples using diaphragms of the sizes necessary to measure the eye components. From these measurements it was determined that the mean deviation for the direct transmittance of the cornea, aqueous, and vitreous was 1.2 per cent before thickness corrections. That for the lens was 1.5 per cent. The mean deviation of the total transmittance data was 1.5 per cent except for the lens. The mean deviation of the lens was 2 per cent before thickness correction.

Some of the measurements were made at thicknesses less than found in the eye to have sufficient medium to fill the cell. This was true principally of the aqueous and vitreous humors, which were measured at 1.5 mm. and 12 mm., respectively. The measured transmittances were converted to the transmittance for the average thickness of the component in the eye of a normal adult by using Bouguer's absorption law. The thicknesses used are as follows: lens, 3.2 mm.; aqueous, 3.0 mm.; vitreous, 15.0 mm.

The number of specimens measured was not sufficient to obtain as good a statistical accuracy as one would like, especially to evaluate more accurately the effect of age on the direct light transmission of the eye. However, in measuring the spectral transmittance of the ocular media of 9 human
eyes, much useful data were obtained, including the effect of age on the total transmittance in the ultraviolet, visible, and infrared. In addition, these data confirm the conclusions of recent workers\textsuperscript{3-5} measuring the transmittances of the eyes of animals and occasional single observations on human eyes, that the transmittance of human eyes does not vary appreciably from that of several other mammals such as rabbits, cats, monkeys, and cattle.

REFERENCES