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Visible and near infrared light absorption in pigment epithelium and choroid*

Absorption de l'énergie lumineuse visible et du proche infrarouge par l'épithélium pigmenté et la choroïde

Absorción de la luz infrarroja cercana y visible en el epitelio pigmentario y coroides

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The pigment epithelium, in addition to its important physiological functions, has a dominant role in photocoagulation by virtue of its concentration of light-absorbing melanin and its critical position between neural retina and choroid; thermal lesions in the fundus thus arise primarily in the pigment epithelium and can lead to retinopexy by scarring. For optimization of the photocoagulation parameters, such as wavelength and exposure time and increased reproducibility, it is therefore necessary to know more about the absorption and its variation over the fundus.

The first quantitative measurements of the light absorption of these structures of the eye were made by Ham and Geeraets and their co-workers. They measured the transmission of flat preparations of the pigment epithelium and choroid together by means of a spectrophotometer. We have extended their measurements with a more sophisticated experimental technique to collect more detailed information about the absorption characteristics of the fundus, in particular: (1) the spectral absorption characteristics in the visible and near infrared range of the pigment epithelium and choroid separately in the rabbit, rhesus monkey and human (notably the existing data in the IR contradictory); (2) variations in the absorption in one distinct area of the fundus at a given wavelength using a spot size of about 50 μm; (3) the dependence of absorption in the location within the fundus in comparison to the ophthalmoscopic appearance of the fundus; (4) the distribution of individual absorptions among humans.

MATERIAL AND METHODS

Flat preparations of pigment epithelium and choroid of different regions of the fundus of rabbits, rhesus monkeys and humans were prepared for measurement of light absorption. We assumed that regional differences in pigmentation in the fundus would roughly have rotational symmetry about the posterior pole of the eye. A strip of material (Fig. 1) reaching from the papilla to the ora serrata was therefore taken as representative. We could divide these strips into successive areas, following the accepted clinical definitions of the fundus areas by measuring the distances from the fovea. We removed the neural retina from globes fixed in glutaraldehyde and peeled the pigment epithelium off by microdissection in the

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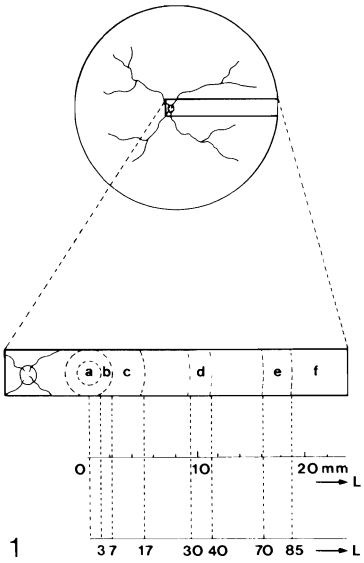


Fig. 1 Division of the fundus in successive areas for preparation of pigment epithelium and choroid. a = macula, b = paramacula, c = posterior pole, d = mid-periphery, e = equator, f = oral periphery.

specified regions. Thereafter it was possible to remove the choroid in small pieces. All specimens were mounted on histological slides and covered as usual (Fig. 2).

A microspectrophotometer (Fig. 3) was used to determine the transmission of these flat preparations. Monochromatic substage illumination was provided for a transmission microscope and the light transmitted by the specimen was collected by a × 100 objective with a N.A. of 1.32 and measured with a photomultiplier; the spatial resolution was 50 μm.

Preliminary experiments on the scattering of the pigment epithelium were performed to determine what portion of transmitted light was detected. Figure 4 shows a differential scattering distribution of light of two different wavelengths transmitted through the pigment epithelium. We found that over 95% of all transmitted light within the wavelength range of interest was gathered by the objective used.

Fig. 2 Microphotograph of a flat preparation of pigment epithelium.

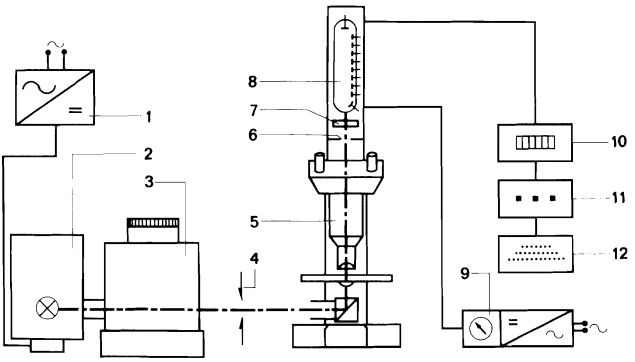


Fig. 3 Diagram of the set-up for microspectrophotometry. 1, 2, 3: light source and monochromator; 4, 5, 6, 7: transmission microscope with aperture; 8, 9: photomultiplier; 10, 11, 12: voltmeter and data processing.

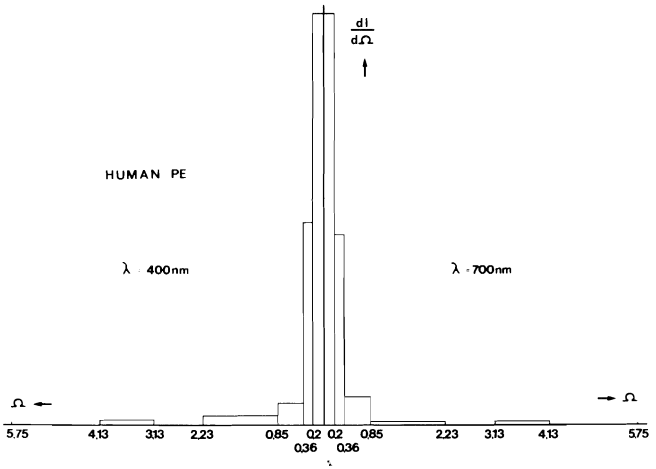


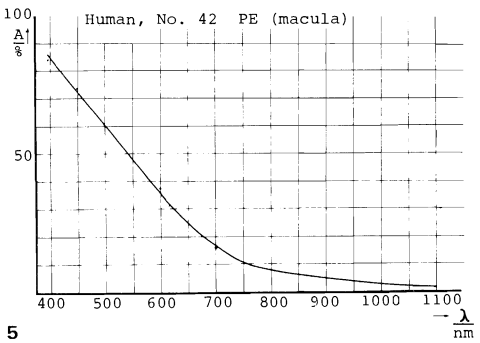
Fig. 4 Differential scattering distribution of light at 400 and 700 nm, transmitted through the pigment epithelium.

About 250 specimens of rabbit, rhesus monkey and human eyes were measured and 45,000 data points evaluated by computer.

RESULTS

In the following, only the absorption of human pigment epithelium as a function of wavelength in the range 400 – 1100 nm, depending on both location in the fundus and on individual variations, will be reported.

Figure 5 shows the typical wavelength dependence of the absorption of all 16 human macular preparations. The absorption decreases monotonically with increasing wavelength and for the most part matches that of the absorbing melanin. At wavelengths beyond 800 nm the absorption of most preparations is less than 5% and measurement accuracy limits the determination of the absorption to a relative uncertainty of 100% or more. Thus, in order to extrapolate the average curve for humans to the region where inaccuracy is intolerably large,



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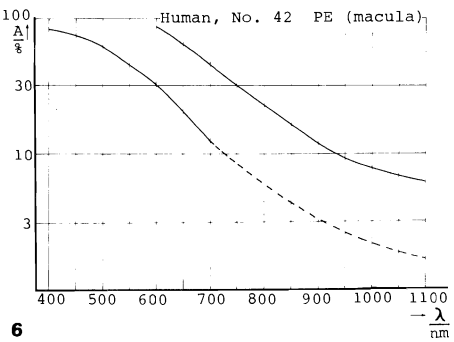

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Fig. 5 Absorption for human pigment epithelium as a function of wavelength.

Fig. 6 Absorption for human and rhesus monkey (upper line) pigment epithelium and its extrapolation to the near infrared.

we selected 6 preparations (from eyes of rhesus monkeys) with high absorption and correspondingly smaller inaccuracy. The optical density of the function of λ for the wavelength range 700 – 1100 nm was measured and averaged for this group of samples. Figure 6 shows it in a logarithmic scale. The general function of D [λ] was then used to extrapolate absorption curves of lower absorption to the wavelength range 700 – 1100 nm, with a fit at the wavelength in the overlap range of 600, 650 and 700 nm.

The variation of the absorption as a function of the location within the fundus was another point of interest. Figure 7 shows such a distribution for one individual at 500 nm. The height of the column indicates the average absorption at that site for each region from which a sample was taken. The systematic variation of absorption with location far exceeds the random variation (indicated by the standard deviation lines in each column) caused mainly by non-homogenous pigmentation. The general course of this spatial dependence in one individual is typical for all 16 cases investigated.

Figure 8 shows the relative frequency distribution among 16 caucasian individuals at the posterior pole for 500 nm. It is somewhat interesting that differences in absorption up to a factor of 4 were found.

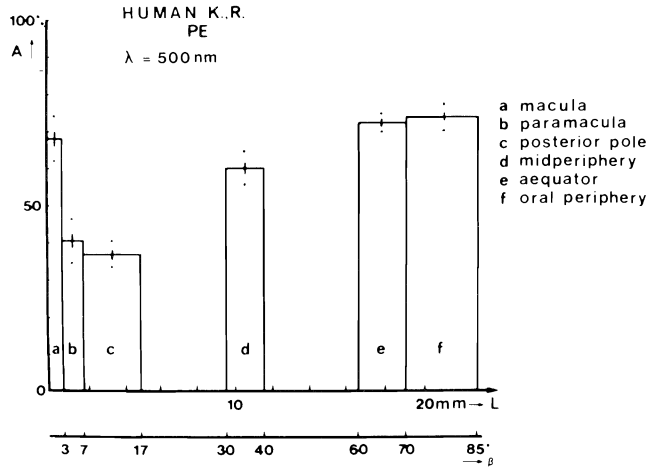

Fig. 7 Distribution of absorption as a function of location within the fundus.

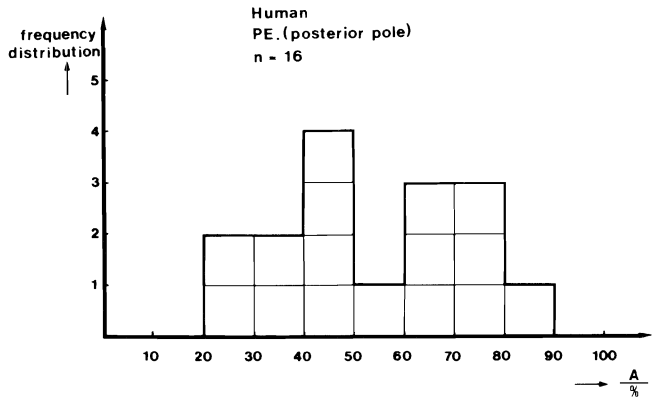

Fig. 8 Frequency distribution of absorption among 16 individuals.

The absorption measurements of the flat preparations of the choroid gave a similar wavelength dependence and a less pronounced distribution at the location with a much higher variation within one location.

CONSEQUENCES FOR PHOTOCOAGULATION

1. The spectral absorption characteristic decreases monotonically with increasing wavelength. This indicates that light beyond 700 nm (50% of xenon arc lamp emission) does not contribute to photocoagulation.

2. The random variation of absorption in a given area is exceeded by far by the systematic spatial variation. This means that a prediction of local light absorption would be possible with an individual measurement of absorption in one area.

3. The individual difference in local light absorption of the pigment epithelium for 16 caucasian eyes varied by a factor of 4. This helps to explain the large differences in the dosage needed to produce clinical argon laser lesions of similar degree.

4. Because the absorbing structures in the choroid are about 30 times thicker than in the pigment epithelium, the optical density of the pigmentation and therefore the absorbed light energy per unit volume is 30 times less than in the pigment epithelium.

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