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SPECTRAL PROPERTIES OF INDIVIDUAL CELLS OF THE RETINA OF THE HUMAN EYE

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OUR visual sensations allow us to distinguish the shapes of objects in our environment, the brightness of radiation, and its color. Experiments on the mixing of radiations of varying spectral composition, the phenomena of colored after-images, the phenomena of color contrast, and anomalies in color perception (color-blindness, etc.) have led to the hypothesis that the eye contains at least three types of light-sensitive elements. The simultaneous excitation of these elements as a whole must produce the sensation of "color," permitting us to see the world, not in a "black-and-white" representation, but in all of its richest variety of colors, shades, half-tones, and transitions.

The retina of the eye contains two types of light-sensitive cells, which are called rods and cones. The outer segments of the rods contain a photochemically sensitive substance, visual purple or rhodopsin. This pigment is decomposed by light, is bleached, and acts on the nerve fibers transmitting the excitation to the cerebral centers. In the dark, the reverse process of regeneration of visual purple occurs. The spectral sensitivity of the rods has been well studied; it has the form of a bell-shaped curve with a maximum at ~ 507 mμ. The high sensitivity of the rods to light permits the eye to perceive light of very low intensity, but this "twilight" vision is achromatic; the rods permit us only to perceive brightness differences. As the light intensity is increased, all of the visual purple is bleached, and the rods become "blind."

Under these conditions, the other light-sensitive elements of the retina, or cones, begin to play a role. They make possible "daytime" vision. Here the capability of distinguishing colors arises.

Attempts at anatomical or physiological discovery of differences in the properties of individual cones have been undertaken for a long time. Thus, for example, Granit and his associates (1939) used microelectrodes, and measured the "action currents" in individual nerve fibers in the frog's retina when illuminated by monochromatic light. On shifting the electrodes, they found that the spectral sensitivity differed in different parts of the retina: some regions reacted better to red light, others to green, and still others gave a maximum response to the blue-violet. On the contrary, the fine histological observations of other authors led to the idea that the three different light-sensitive pigments occur in each cone.

In their most recent study, P. Brown and G. Wald[1] were able to measure the absorption spectra directly in individual cells of the retina, and to demonstrate the actual existence of three types of cones having differing spectral properties.

They performed the measurements on a retina taken from a human eye and kept in the dark and cold. A piece of the retina containing the foveal region* was fastened in a microcuvette filled with 55% glycerol solution in NaCl solution (0.9%). The cuvette containing the preparation was placed on the stage of a microscope having a 100-power apochromatic objective and a 20-power ocular in such a way that the visual cells were aligned vertically (parallel to the light path). The image of the retinal preparation was projected in weak red light (680—690 mμ) on a screen on the front surface of the housing of a photocell. A small round aperture was cut in the screen so that the light falling on the photoelement had passed through only one cell, a rod or a cone. The preparation being studied could be examined visually at the same time through the ocular. The crosshairs in the ocular had previously been set so that their intersection corresponded to the central hole in the screen. In order to put the retinal preparation in a required position, it sufficed simply to bring the image of an individual cell to the intersection of the crosshairs. The microscope was attached to a Cary 14 spectrophotometer. The comparison beam passed through a compensating arrangement of lenses and stops that permitted them to make the intensities of the two beams approximately equal. The light source was a powerful quartz-iodine lamp (DWL, 600 W), and a high enough voltage was applied to the photocell (R-136) to permit the use of very narrow slits during the recording of the spectra. The spectra were automatically recorded at a rate of 25 Å per second.

The recording was first made from 650 to 380 mμ and then immediately in the opposite direction (this permitted them to eliminate errors involving the gradual bleaching of pigment). The mean of the two curves gave the correct spectrum.

After the absorption spectrum of any dark-adapted retinal cell had been recorded, the powerful lamp was flashed, the retina was bleached, and the spectrum was recorded again. By subtracting the "bleached"

*The region of clearest vision involves the so-called yellow spot (macula lutea). A depression in it, or central pit, is called the fovea centralis. There are almost no rods at this site; it is filled almost solidly with cones.

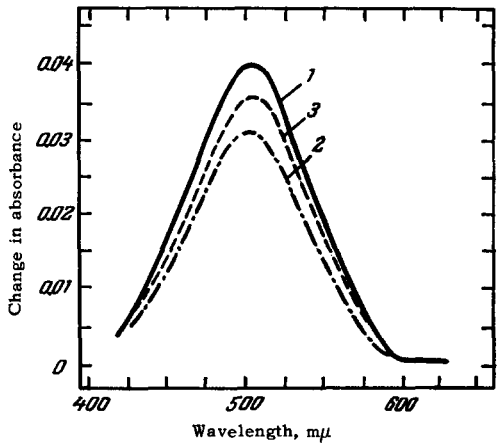

FIG. 1. Difference spectra of the visual pigment in a single rod in the parafoveal region of a human retina. Spectrum 1 was measured from 650 to 380 mμ, and spectrum 2 in the reverse direction. Spectrum 3 is the mean curve having $\lambda_{max} \sim 505$ mμ.

spectra from the "dark" spectra, they obtained the "difference," or differential spectra. The light of the flash lamp was directed onto a small region of the retina in such a way that the experiment could be repeated by shifting the preparation to other receptors of the same specimen.

Ingeniously, the authors did not select for the experiments the central depression (the fovea) of the yellow spot, where the cones lie very closely packed, but instead, the parafoveal regions of the retina. In this region, the cones are more widely spaced, and the measurements can give in particular the absorption spectrum of isolated cells.

Figures 1 and 2 show the curves obtained from

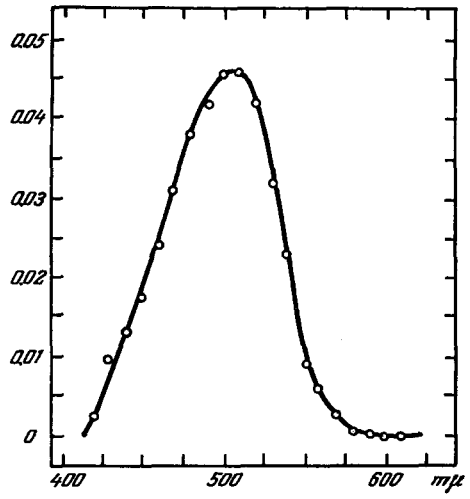

FIG. 2. The difference spectrum of the visual pigment in a single rod in a human retina in the parafoveal region. The spectral curve was first recorded in the dark, and then after bleaching (with a flash of yellow light). The difference values are shown in Fig. 2. The curve has a maximum near 505 mμ and closely matches the difference spectrum of human rhodopsin, which the same authors had previously measured in suspensions of outer segments of rods and in relatively large retinal regions.

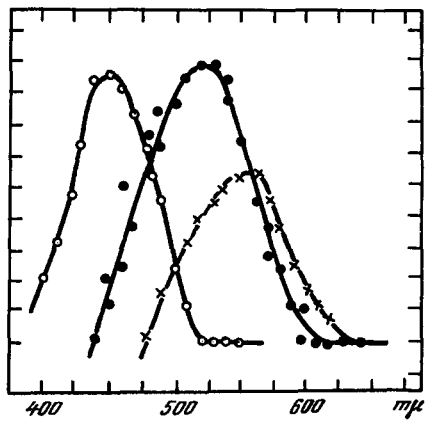

FIG. 3. Difference spectra of the visual pigments of single cones in the parafoveal region of a human retina. The absorption spectra were measured in the dark from 650 to 380 mμ and then again after bleaching. The differences between these spectra are plotted as curves. One of the cones, apparently a blue-receptor, gives a spectrum having $\lambda_{max} \sim 450$ mμ; two others, apparently green-receptors, gave $\lambda_{max} \sim 525$ mμ; and one, probably a red-receptor, had $\lambda_{max} \sim 555$ mμ.

individual rods. The averaged absorption spectra agree well with the differential spectrum of the rhodopsin of the human eye, as had been previously measured in suspensions of rods.[2] One notes only a small shift of the peak toward the red (~ 5 mμ) as compared with the rhodopsin spectrum. The authors ascribe this shift to the formation of the yellow pigment retinene as the rhodopsin is bleached.

Figure 3 shows the difference curves of the light-sensitive pigments of four cones. These spectra clearly show that the retina contains cones having varying spectroscopic properties. Curve 1 corresponds to a cone with a "blue-sensitive" receptor, 2 and 3 are green-sensitive, and 4 corresponds to the red. The peaks of these curves occur at 450, 525, and 555 mμ. The obtained data are close to the earlier results of Brown and Wald, in which they measured the spectra of relatively large regions of the retina. We observe an appreciable displacement only when comparing the spectra of the blue-sensitive receptors.

The earlier studies of Brown and Wald[3,4] showed that the rhodopsin of the human eye and the green- and red-sensitive pigments consist of the same chromophore, 11-cis-retinene, combined with different protein structures. Retinene easily combines with opsin and other proteins, forming light-sensitive products.

If the bleaching of the blue-sensitive pigment of the cones leads to the formation of retinene, then the difference spectrum should be somewhat narrowed and shifted toward the red. Thus the authors explain certain discrepancies in the course of the curves of the blue-sensitive pigment. The effect of the retinene should be less pronounced for the green- and red-sensitive pigments.

[1] P. K. Brown and G. Wald, Science **144**, 45 (1964).
[2] G. Wald and P. K. Brown, Science **127**, 222 (1958).
[3] P. K. Brown and G. Wald, Nature **200**, 37 (1963).
[4] Wald, Brown, and Gibbons, J. Opt. Soc. Am. **53**, 20 (1963).

Translated by M. V. King