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Human visual pigments: microspectrophotometric results from the eyes of seven persons

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The material for this work was obtained from seven eyes removed because of malignant growths. Foveal and parafoveal samples of the retinas were taken and transverse measurements were made of the absorbance spectra of the outer segments of the rods and cones, using a Liebman microspectrophotometer.

Four kinds of spectra were obtained with absorbance peaks at the following wavelengths: rods, 496.3 ± 2.3 nm ($n = 39$); red cones, 558.4 ± 5.2 nm ($n = 58$); green cones, 530.8 ± 3.5 nm ($n = 45$); blue cones, 419.0 ± 3.6 nm ($n = 5$).

The distribution of the peaks was unimodal for the rods. For the red and green cones, however, there was evidence for bimodal distributions, with sub-population maxima at 563.2 ± 3.1 nm ($n = 27$) and 554.2 ± 2.3 nm ($n = 31$) for the reds and at 533.7 ± 2.1 nm ($n = 23$) and 527.8 ± 1.8 nm ($n = 22$) for the greens.

A substantial difference in mean spectral location of the red cones was observed between patient 1 (561 nm) and patient 4 (553 nm). Both patients were classified as normal trichromats by all clinical tests of colour vision but there was a clear difference in their relative sensitivities to long-wave fields. In both direction and magnitude, this difference proved to be that required by the microspectrophotometric results.

INTRODUCTION

In a previous paper (Bowmaker & Dartnall 1980), microspectrophotometric results were reported from a human eye removed because of the presence of a melanoma. Foveal and parafoveal samples were taken from the retina of this eye, and transverse measurements made of the absorbance spectra of the outer segments of the rods and cones.

Because of the ready collaboration of the surgeon (and of his patients) we embarked on a programme of the examination of similar eyes. Only eyes in which a substantial portion of the retina (including fovea) was uninvaded by the tumour were suitable for our purpose. We assumed that the visual pigment was not altered in the tissue used, despite the malignancy elsewhere in the eye. Between February and August 1979, a further six suitable eyes were obtained. After that date eyes

became less frequently available for a number of reasons and, because the apparatus was also changed then by coupling it to a computer (a change that entailed a slightly different method of processing the data), we have decided to publish the results obtained on these first seven eyes.

In this sample of eyes we did not encounter a dichromatic or clearly anomalous retina, but in two subjects the red-sensitive cones seemed sufficiently different to prompt us to compare the psychophysical sensitivities of the remaining eyes of these subjects. We found a difference between them, in line with the microspectrophotometric measurements.

Methods

Microspectrophotometry

As reported in the previous paper (Bowmaker & Dartnall 1980) the first eye was examined in Sussex, and the first microspectrophotometric measurements on it were made about 3 h after completion of the London operation. The other six eyes were examined in London, the microspectrophotometer having been moved to Queen Mary College. Because of this the interval between the end of the operation and the beginning of the microspectrophotometric examination was shortened to between 45 and 90 min, except in one case where 24 h elapsed before examination. This was because two eyes were obtained together from two consecutive operations. Time does not appear to be crucial, provided the retina is in good condition, for measurements were continued throughout the day and, in favourable circumstances, even for a second day. The eyes were kept on ice.

All patients wore a bandage over the diseased eye and this bandage (applied the evening before) was not removed until the operation began. The first eye was removed under red light, while eyes 4 and 7 were exenterations and were taken complete with eyelids sutured together. The other four eyes were removed under the full glare of the operating lights.

In the laboratory the eyes were dissected in the dark room under deep red light. The fovea was located by a momentary exposure to dim blue light. Pieces of retina, approximately 1 mm^2 in size, from the foveal region were removed from time to time for examination, the eye-cup in the meantime being kept in darkness in ice-cold Ringer solution. Each piece of retina was prepared on a slide as previously described for the cynomolgus monkey (Bowmaker *et al.* 1980). All visual examinations of these preparations and the lining up of individual cells for measurement were done in infrared. An image converter was used to make them visible.

The Liebman microspectrophotometer, of dual beam design, was the same as used before (for description see Knowles & Dartnall 1977).

At the start of each session the two equal beams of the microspectrophotometer were set at 2×1 μm, but they were adjusted equally as needed to suit the dimensions of the outer segments of the photoreceptors. Measurements were generally made with the e-vector of the beam perpendicular to the long axis of the outer segment to maximize its absorbance.

With one beam wholly in the chosen outer segment and the other in a clear space in the preparation a record was run from 700 to 350 nm, and then back to 700 nm.

This double scan, taking 20 s, was a check that no significant bleaching or other change, such as movement of the cell, had occurred during the measurements. The preparation was then moved a little, so that both beams now fell in clear spaces, and a 'baseline' record was run from 700 to 350 nm and back.

Each receptor record was analysed individually in the following way. At various wavelengths visual estimates were made of the values of points that lay midway within the 'noise band' of the record. This was done for both cell and baseline traces. The baseline values were subtracted from the corresponding cell values, and the differences used to construct the absorbance spectrum. This was normalized, and its λ_{max} estimated in the way described by Knowles & Dartnall (1977, pp. 79–80). An appropriate nomogram was used for comparison. For the rod and the green cone spectra the rhodopsin data were used; for blue cones, the frog green-rod data were used and for the red cones the chicken red cone data were used (Knowles & Dartnall 1977, p. 76).

A probable source of systematic error in the determination of the absorbance spectra by these methods stems from (slight) errors in the position of the baseline records relative to those of the cells. If they are too close the differences will give an absorbance spectrum that is erroneously narrow and, conversely, if the baseline is too far away, the estimated spectrum will be too broad. Such errors in positioning can arise if the chosen cell is slightly more, or slightly less, cloudy than the 'clear' space against which it is measured. They can also arise, when the baseline is being drawn, if the two beams are not in places of exactly equal clarity. Consideration of these points has led us to modify slightly the procedure for the estimation of λ_{max} from that used in the previous paper (Bowmaker & Dartnall 1980).

In the previous paper the mean λ_{max} of each record was obtained by comparison with the appropriate nomogram over a range extending from 80% absorbance on the short-wave side of the maximum to 30% on the long-wave side (Knowles & Dartnall 1977, pp. 79–80). However, to minimize the effect of error in baseline placement on the estimated λ_{max} it is clearly necessary to use a portion of the spectrum that is symmetrical about the maximum, say from 70% on the short-wave side to 70% on the long-wave, and this has been done for all the results reported in this paper. Recalculation of the results on the previous eye to the new basis gave mean values of λ_{max} ranging from 1.0 to 1.9 nm lower (see table 1) than reported previously (Bowmaker & Dartnall 1980).

Psychophysical tests

Two of the patients, 1 and 4 (see table 1), were tested psychophysically in Cambridge. Each completed a number of clinical tests of colour vision comprising:

(i) pseudoisochromatic plates, including the Ishihara (ninth edn), the Okuma (Amoriex Co., Tokyo), the Farnsworth tritan plate, and an unpublished tritan plate kindly provided by J. Birch-Cox;

(ii) the City University test (first edn), a booklet version of the Farnsworth Panel D15 test;

(iii) the Farnsworth–Munsell 100 hue test; and

(iv) the Nagel anomaloscope.

For details of several of these tests see Pokorny *et al.* 1979. Tests (i)–(iii) were

administered under C.I.E. illuminant C. The primaries of the anomaloscope, as measured immediately before the testing, were 541, 666 and 589 nm. Since the microspectrophotometric measurements were dominated by foveolar samples, the anomaloscope settings were made both with a large field (3.1° of visual angle) and with a small field (1.2°). Ten settings were made at each field size. Two known normal trichromats (Observers J.D.M. and P.G.P. of Mollon & Polden 1977) were tested at the same time as each patient and their mean settings were used for the derivation of anomalous quotients.

A reduced version of Stiles' field-sensitivity method (Stiles 1978) was used to obtain a rapid psychophysical estimate of the spectral location of the long-wavelength pigment in the two patients. Detection thresholds were first measured for 666 nm targets presented in Maxwellian view to the dark-adapted fovea. The targets were disc-shaped, subtended 0.85° of visual angle and lasted 10 ms. The measurements were under computer control: the patient indicated by push-buttons whether or not he had seen a given flash, and the flash intensity was adjusted according to a random double staircase procedure (Cornsweet 1962). When the absolute threshold had been established, the 666 nm target was set to be 1 lg unit above the absolute threshold and a steady adapting field, subtending 6.25° of visual angle, was introduced. In successive runs the wavelength of the adapting field was either 650 nm or 555 nm and in each run the intensity of the field was adjusted, according to a single staircase procedure, to estimate the intensity at which 50 % of targets were detected. The inter-flash interval was 5 s and there were 68 flashes in each run, the estimate of threshold being based on the last 32 responses. The step-size was 0.1 lg unit. Four runs were devoted to each field wavelength.

For general details of the apparatus used in the measurement of the field sensitivities, see Mollon & Polden (1977). A flash duration of 10 ms was adopted because of recent evidence that brief targets favour a spectrally non-opponent mode of detection and are thus more likely to yield the spectral sensitivity of the receptors themselves (King-Smith & Carden 1976; Wandell & Pugh 1980).

Results

Microspectrophotometric measurements

General

From the retinas of the seven eyes 173 absorbance spectra of different outer segments were obtained. It was easy to distinguish the outer segments of rods from those of cones, and the 44 examples of the former all had spectra with λ_{max} (wavelength of peak absorption) close to 496 nm. The outer segments of the cones were assigned to three classes simply according to their λ_{max}.

Thus 69 of them peaked between 550 and 570 nm in the greenish yellow, and were designated reds; 49 were greens (peaked at 520–540 nm in the green) and 11 were blues (415–425 nm in the violet). Since the cones could not be morphologically distinguished from each other, these 129 records are probably a random sample (from the vicinity of the fovea) and hence may reflect roughly the proportions of the three cone types present there.

Twenty-six (15%) of the records were rejected from precise computations of absorbance spectra. This was either because their signal:noise ratios were very low (maximum pigment density < 0.01) or because the traces or baselines were irregular (because of drifting of cells into or out of the beams). The acceptable 147 records that remained comprised 39 rods, 58 red cones, 45 green cones and five blue cones.

TABLE 1. DETAILS OF THE RESULTS

(Mean absorbance is the mean transverse absorbance at λ_{max}.)

eye number	1	2	3	4	5	6	7	all
sex	male	male	male	male	female	female	female	
age/years	46	43	58	70	34	74	63	
				rod outer segments				
number	11	5	1	13	1	8	0	39
mean λ_{max}/nm	496.5	497.2	496.0	496.2	494.0	495.9	—	496.3±2.3
mean absorbance	0.035	0.027	0.025	0.044	0.033	0.045	—	0.039±0.011
				blue cone outer segments				
number	3	1	0	1	0	0	0	5
mean λ_{max}/nm	419.3	419.0	—	418.0	—	—	—	419.0±3.6
mean absorbance	0.037	0.024	—	0.023	—	—	—	0.032±0.011
				green cone outer segments				
number	11	2	5	13	2	9	3	45
mean λ_{max}/nm	532.7	532.5	527.6	530.7	534.5	529.0	530.7	530.8±3.5
mean absorbance	0.033	0.026	0.040	0.036	0.032	0.040	0.030	0.035±0.008
				red cone outer segments				
number	20	5	3	8	2	9	11	58
mean λ_{max}/nm	560.9	561.4	552.0	553.1	556.0	556.3	560.0	558.4±5.2
mean absorbance	0.027	0.021	0.029	0.033	0.029	0.034	0.023	0.028±0.007

The mean results are itemized for each subject in table 1. This table should be read in conjunction with figure 1 in which all the 147 results are shown individually in histogram form.

It is clear from figure 1 that in all seven eyes the λ_{max} of every photoreceptor can be placed in one of four spectrally separated groups clustered around means at 419.0 nm (blue cones), 496.3 nm (rods), 530.8 nm (green cones) or 558.4 nm (red cones). These means are shown as interrupted vertical lines in figure 1.

It appears that there are no significant differences between the eyes from male (1, 2, 3 and 4) and female (5, 6 and 7) subjects. The absence of the relatively rare blue cones from the eyes of the three females is only apparent, not real; in one female case (eye 6) two examples of the blue cone were found, but the records were not good enough for the computation of precise spectra.

Also of interest are the values of the transverse absorbance (at λ_{max}) of the different receptors. The mean values for the seven eyes are given in table 1. Eye 1 was enucleated under deep red light while eyes 4 and 7, though removed under the unfiltered operating lights, were exenterations, that is were removed complete with eyelids sutured together. Thus eyes 1, 4 and 7 were protected from the glare of the operating lights while eyes 2, 3, 5 and 6 were not. It may have been expected

therefore that the transverse absorbances of the various receptors would be greater for the protected eyes. But such was not the case. The mean values for rods, green cones and red cones respectively were 0.040, 0.034 and 0.027 for the 'protected' eyes, and much the same, namely 0.037, 0.038 and 0.029 for the unprotected ones. Thus, as we have previously noted for the cynomolgous monkey (Bowmaker *et al.* 1980), the traditional practice of enucleating eyes in dim red light may not be necessary for microspectrophotometry of primate pigments.

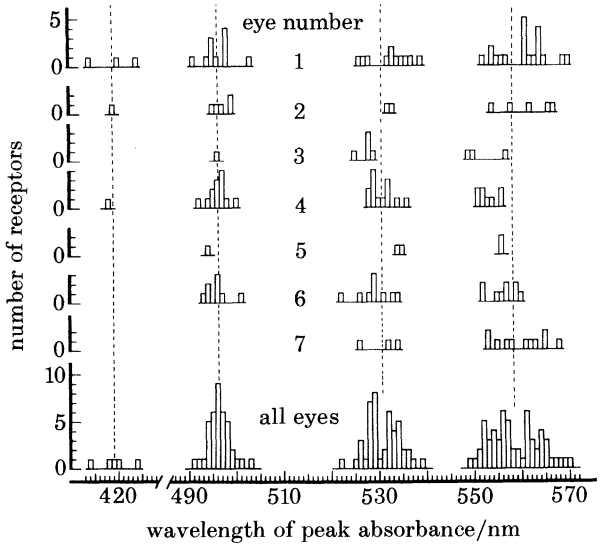

FIGURE 1. Spectral distributions of the human photoreceptors. The vertical dashed lines give the mean values of λ_{max} for the four kinds of receptor. See also table 1.

The mean absorbance spectra

The mean relative absorbance values at 10 nm intervals are listed in table 2 for each of the four types of photoreceptor outer segments. Every record, after conversion to a maximum of 100, was given equal weight, irrespective of its actual peak absorbance or, indeed, of its quality. In the case of the rod values (for example) each datum is, in general, the mean of 39 determinations from the 39 records, and correspondingly in the other cases. The qualification 'in general' is made since values towards the tail ends of the spectra were not obtained from every record. The peak positions of curves drawn through these mean data, namely 496.7, 419.0, 531.1 and 558.7 nm for rods, and blue, green and red cones respectively are nearly the same as the values obtained by averaging the λ_{max} estimates of individual curves, namely 496.3, 419.0, 530.8 and 558.4 nm (figure 1 and table 1).

The mean data of table 2 are plotted in figure 2 that thus shows the mean absorbance spectra for the four receptor types. It has become normal practice over the years to plot the absorbance spectra of the visual pigments against a scale of frequencies rather than one of wavelength. This practice stems partly from quantum theory considerations and partly from the publication of a visual

TABLE 2. THE MEAN ABSORBANCE DATA (max = 100) FOR THE FOUR CLASSES OF PHOTORECEPTOR

wavelength nm	rods $n = 39$	blue cones $n = 5$	green cones $n = 45$	red cones $n = 58$
370	—	59.3	—	—
380	—	65.2	—	—
390	—	74.4	—	—
400	38.4	87.8	39.7	43.4
410	40.1	97.2	38.7	41.9
420	42.1	99.8	38.2	40.2
430	45.7	95.7	37.9	38.3
440	51.5	85.4	39.6	37.7
450	60.5	69.5	42.9	36.3
460	71.8	50.5	48.1	38.0
470	83.0	35.5	55.5	41.0
480	92.4	24.8	63.7	46.1
490	98.2	15.9	73.1	52.1
500	99.5	10.6	82.3	59.4
510	95.0	5.3	90.4	67.0
520	85.5	3.0	96.9	75.7
530	72.5	—	99.9	84.4
540	58.1	—	98.6	92.2
550	43.4	—	92.4	97.9
560	31.5	—	82.6	99.9
570	22.7	—	70.2	97.7
580	15.8	—	56.0	91.4
590	11.0	—	42.7	82.2
600	7.3	—	31.7	70.9
610	4.7	—	23.2	58.9
620	3.7	—	15.5	44.9
630	—	—	11.2	31.8
640	—	—	7.5	21.4
650	—	—	—	12.7

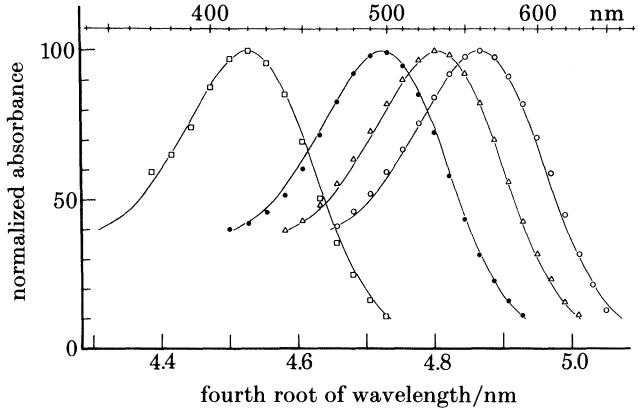

FIGURE 2. The mean absorbance spectra of the four human photoreceptors. Squares, the blue cones ($\lambda_{max} = 419.0 \pm 3.6$ nm, mean of 5); filled circles, the rods ($\lambda_{max} = 496.3 \pm 2.3$ nm, mean of 39); triangles, the green cones ($\lambda_{max} = 530.8 \pm 3.5$ nm, mean of 45); plain circles, the red cones ($\lambda_{max} = 558.4 \pm 5.2$ nm, mean of 58). The curves are all exactly the same shape and were constructed from the mean human data of table 4. Note inset scale of wavelengths.

pigment nomogram (Dartnall 1953) that was based on the supposition that on the frequency basis all visual pigments had the same shape of absorbance spectrum. However, in 1968 Liebman & Entine showed that the spectra of the pigments in frog and tadpole photoreceptors became progressively narrower on the frequency scale as the λ_{max} advanced to longer wavelengths. We found this also to be the case for the receptors of the cynomolgous monkey when we showed that the breadth of the absorbance spectrum varied linearly with the spectral location of the peak (Bowmaker *et al.* 1980).

Recently Barlow (1982) used our cynomolgous results and made the observation that the shapes of the spectra of all four classes of receptor (λ_{max} at 416, 500, 533 and 567 nm) can be made very similar by plotting them in another way – namely to an abscissal scale of the fourth root of wavelength. This also works well with the present human data, as is shown in figure 2. In the figure the continuous curves drawn through the four sets of mean data all have exactly the same shape, a single curve having been appropriately displaced sideways to give best fits. The meaning of this empirical observation is not clear at present but it is a better generalization than the nomogram based on a frequency plot.

Variation of λ_{max} within classes

It is clear from figure 1 that the values of λ_{max} within each class of receptor are spread over substantial ranges. Thus the λ_{max} values for blue cones extend from 414 to 424 nm, for rods from 491 to 503 nm, for green cones from 522 to 539 nm, and for red cones from 549 to 570 nm. In this section we consider whether the populations in the different classes follow normal distributions or whether there is any evidence for the existence of sub-populations.

Blue cones. The five records give a mean λ_{max} value of 419.0 nm with standard deviation of ± 3.6 nm. With so few observations further analysis is hardly possible.

Rods. The 39 records give a mean λ_{max} value of 496.3 nm with a standard deviation of ± 2.3 nm. As figure 1 shows the mean value is also the commonest value. In figure 3 the distribution histogram is shown on a larger scale together with a dashed-line curve giving the expected distribution values, 39 (number of observations) $\times Q$, where Q is calculated from the expression

$$Q = \frac{1}{\sigma\sqrt{(2\pi)}} \exp\left(-\frac{1}{2}\left(\frac{x-\mu}{\sigma}\right)^2\right),$$

where σ is the standard deviation (± 2.3 nm), x the variable λ_{max} (491 to 503 nm), and μ the mean value (496.3 nm).

When the number of observations is relatively small (not more than 50) the normality of distribution can be conveniently assessed by Shapiro & Wilk's (1965) analysis of variance test. Application of the test to our rod data showed no significant evidence of non-normality ($W = 0.969$, p (probability of normality) ≈ 0.5).

Green cones. The 45 records give a mean λ_{max} value of 530.8 nm and standard deviation ± 3.5 nm. But the mean value is not the commonest one. Indeed, as shown in figure 1, there is actually a trough in the population histogram in this region. In figure 3, which shows the distribution histogram on a larger scale, the

calculated distribution values (dashed curve) do not seem to be a satisfactory description of the actual results. Does this mean that the distribution is not normal and that (for example) there are sub-populations of 'long' and 'short' greens?

If we divide the 45 greens into two groups according to whether λ_{max} lies above or below 530.5 nm we obtain 23 'longs' with mean $\lambda_{max} = 533.7 \pm 2.1$ and 22 'shorts' with mean $\lambda_{max} = 527.8 \pm 1.8$. The expected distribution for such a population is given by the continuous double-humped curve for greens in figure 3, and it might be thought that it describes the actual distribution better than the single-humped dashed curve.

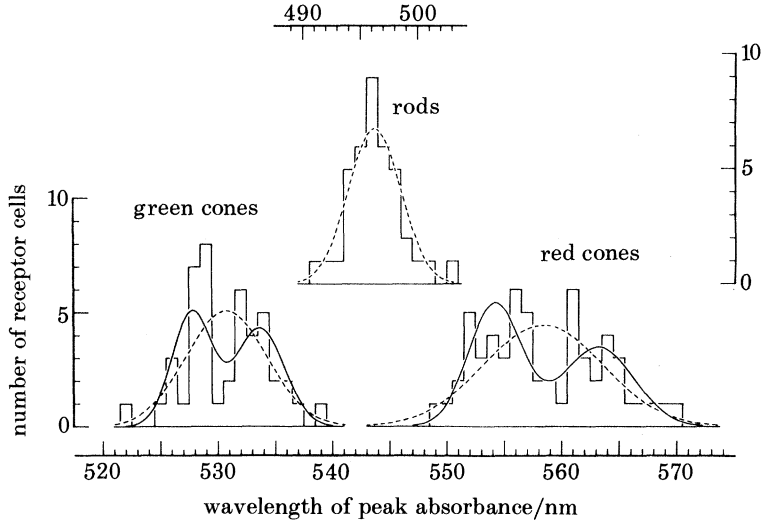

FIGURE 3. Spectral distributions of the human rods, green cones and red cones interpreted in terms of their statistics. The dashed-line curves give the normal distributions expected from the relevant values of the means and standard deviations. The double-hump continuous curves drawn through the green and red cone data give the expectations if the respective populations are regarded as containing 'long' and 'short' sub-populations. For further explanation see text.

But on the application of the Shapiro & Wilk test to the data, no evidence of non-normality was obtained ($W = 0.976$, $p = 0.5$). This was an unexpected result and we therefore tried the specific test for bimodality devised by Giacomelli *et al.* (1971). In this test a statistic, termed the 'dip intensity' is calculated from the data. Large values of the statistic indicate bi- (or multi-) modality. The test is stated to be most effective where there are only two modes, and to be unduly conservative with three or more. When applied to the 45 'greens' the test yielded a dip intensity of 2.40. This (high) value indicates a probability of less than 10% that the sample could have come from a unimodal distribution.

Red cones. The 58 records give a mean λ_{max} of 558.4 nm and a standard deviation of ± 5.2 nm. Figure 1 shows that the mean is not the commonest value, the dip in this region being even more pronounced than in the case of the green cones. In figure 3, which shows the distribution histogram on a larger scale, the expected

normal distribution of 58 values of $\lambda_{max} = 558.4 \pm 5.2$ nm is given by the dashed-line curve. It does not seem to be a satisfactory description of the actual distribution.

To apply the Shapiro & Wilk test it is necessary to reduce the sample size to 50, for those authors tabulated coefficients for their W test only up to that number of observations. For the purposes of this test, therefore, we discarded the eight records of poorest quality. This left 50 with a mean of 558.8 nm and standard deviation of ± 5.1 nm, close to the values for the full sample, and yielding a W-value of 0.940. This confirms our expectation that the distribution of the 'red' population is very significantly non-normal (for $W = 0.938$, $p = 0.02$).

If we divide the 58 reds into two groups according to whether the λ_{max} lies above or below 558.4 nm, we obtain 27 'longs' with mean $\lambda_{max} = 563.2 \pm 3.1$ nm and 31 'shorts' with mean $\lambda_{max} = 554.2 \pm 2.3$ nm. The expected distribution for such a population is given by the continuous double-hump curve for reds in figure 3. It seems to be a fair approximation to the actual distribution, and we anticipated that the bimodality test would yield a highly significant result. This was not the case, however; the calculated dip intensity was only 1.85, indicating a roughly 50 % chance that the sample could have come from a unimodal distribution. This result was so unexpected that, as a check, the test was applied to a set of 58 reds tailored to fit the bimodal curve in figure 3 exactly (rounding-off to the nearest whole numbers of cells). Even these data gave a dip intensity of only 2.03; this indicates that the chance that this perfectly bimodal sample could still have come from a unimodal distribution was as high as between 50 and 20 %.

The equivocal performances of the Shapiro & Wilk test for normality and of the Giacomelli *et al.* (1971) test for bimodality, when applied to the green and red cone samples, are further considered in the Discussion.

Psychophysical measurements

When, in the course of this investigation, the examination of eye 4 had been completed it was thought that the results obtained (and displayed in figure 1) showed a sufficient difference in the spectral locations of the red cones from those of eye 1 to warrant measurements of red sensitivity in the remaining eyes of the two patients. The tests described in the Methods section were therefore carried out.

Both patients gave normal responses to all the pseudoisochromatic plates, and neither made any errors in the City University test. In the Farnsworth–Munsell test patient 1 had a total error score of 89 on the first attempt and 40 on the second while patient 4 had scores of 115 and 51. All these scores are within the ranges for normal observers of the appropriate age groups (Verriest 1963). As figure 4 shows, neither patient showed the clustered pattern of errors characteristic of colour defective subjects.

For patient 1 the anomalous quotient on the Nagel instrument was 1.09 for the 3.1° field and 1.04 for the 1.2° field. For patient 4 the values were 0.98 and 1.08 respectively. All these values are within the range for normal trichromats, given as approximately 0.74–1.33 by Pokorny *et al.* (1979). The standard deviations of successive settings of the $G{:}R$ ratio were low, namely 1.42 and 0.95 scale units for patient 1 and 1.23 and 2.21 for patient 4 (the instrument has a scale of 0–89). The

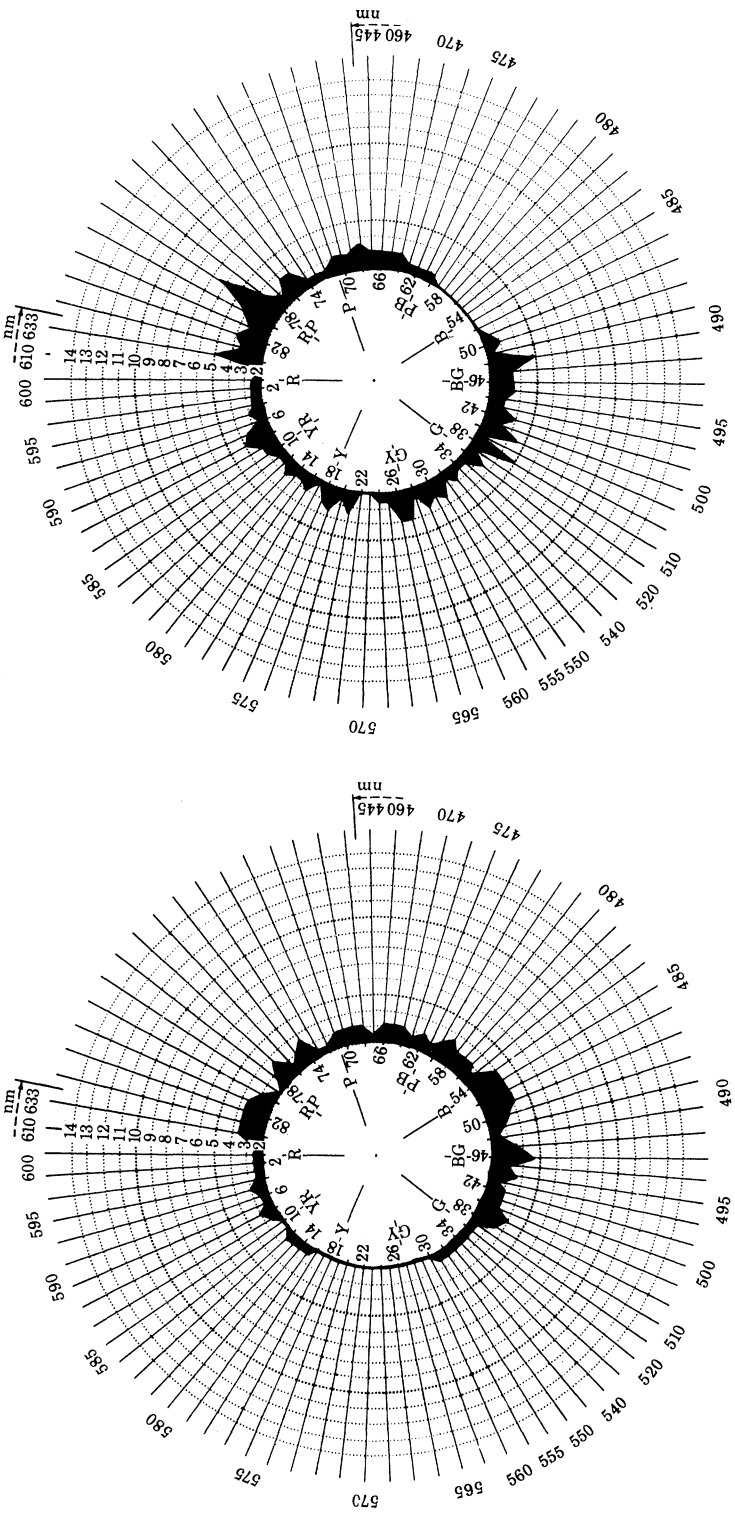

FIGURE 4. The performances of patients numbers 1 and 4 on the Farnsworth–Munsell 100 hue test. Both are normal trichromats. For the spectral distributions of their photoreceptors see figure 1.

performance of patient 4 had one odd feature. In adjusting the intensity of the monochromatic yellow field to achieve a complete match between the two fields of the anomaloscope he showed a high variability. Thus the standard deviation of his settings was 8.42 scale units, compared with 1.17 and 1.51 for J.D.M. and P.G.P., the two known normal trichromats of Mollon & Polden (1977).

TABLE 3. COMPARISON BETWEEN THE lg PSYCHOPHYSICAL RATIO (DIFFERENCE BETWEEN lg FIELD SENSITIVITIES AT 555 nm AND 650 nm) AS DETERMINED BY PSYCHOPHYSICAL MEASUREMENT AND BY CALCULATION FROM THE MICROSPECTROPHOTOMETRIC MEASUREMENTS OF RED CONE ABSORBANCE AT THE TWO WAVELENGTHS AND ASSUMING AN AXIAL ABSORBANCE AT λ_{max} OF 0.525

	lg psychophysical ratio	
patient	measured	calculated from microspectrophotometric data
1	0.56	0.58
4	0.75	0.71

Although patients 1 and 4 were classified as normal trichromats by all clinical tests of colour vision, there was a clear difference in their relative sensitivity to long-wave fields under conditions designed (see Methods) to isolate the long-wave pigment. Table 3 shows for each patient the lg psychophysical ratio (difference between lg field sensitivities at 555 and 650 nm) compared with the values expected from the microspectrophotometric results. In calculating the expected values from the microspectrophotometrically measured absorbances at 555 and 650 nm, it was assumed that the red cones in the central fovea had a length of 35 μm and a specific absorbance of 0.015 μm^{-1}, giving them an axial absorbance at λ_{max} of 0.525 (see Bowmaker & Dartnall 1980). There was assumed to be no difference in the pre-receptoral absorption at the two wavelengths.

DISCUSSION

The $\lambda^{\frac{1}{4}}$ rule

We have already remarked on the near-constancy in shape of the absorbance spectra of all four kinds of human receptor when plotted with an abscissal scale of the fourth root of wavelength. In table 4 values of the function $(\lambda_A^{\frac{1}{4}} - \lambda_{max}^{\frac{1}{4}})$ are listed at various absorbance (A) levels for the different receptors of man and rhesus monkey, and also for the extracted pigments cattle rhodopsin (Collins *et al.* 1952) and chicken iodopsin (Wald *et al.* 1955). These values can be used, where appropriate, for constructing absorbance spectra for given values of λ_{max}.

The constant-shape curves in figure 2 were constructed from the mean values of the function for the human receptors. A scrutiny of figure 2 reveals that the agreement of the curves with the individual spectra (symbols), though close, is not exact. In fact the spectra, on the $\lambda^{\frac{1}{4}}$ basis, tend to become broader as the position

TABLE 4. VALUES OF THE FUNCTION $(\lambda_A^{\frac{1}{4}} - \lambda_{max}^{\frac{1}{4}})$, WHERE λ_A IS THE WAVELENGTH AT WHICH THE ABSORBANCE IS A% OF MAXIMUM, FOR VARIOUS PHOTORECEPTORS OF MAN AND RHESUS MONKEY, AND FOR THE TWO EXTRACTED PIGMENTS, CATTLE RHODOPSIN AND CHICKEN IODOPSIN

	photoreceptors									
	man					rhesus monkey			extracts	
absorbance % max	blue cones 419 nm	rods 496 nm	green cones 531 nm	red cones 558 nm	mean	rods 503 nm	green cones 535 nm	red cones 566 nm	cattle rhodopsin 497 nm	chicken iodopsin 563 nm
40	—	−0.221	−0.216	−0.213	−0.217	−0.167	−0.192	−0.194	−0.159	−0.174
50	—	−0.146	−0.162	−0.164	−0.157	−0.137	−0.157	−0.163	−0.131	−0.147
60	−0.134	−0.116	−0.131	−0.131	−0.128	−0.113	−0.128	−0.136	−0.107	−0.122
70	−0.095	−0.094	−0.103	−0.101	−0.098	−0.092	−0.104	−0.111	−0.087	−0.099
80	−0.068	−0.072	−0.078	−0.075	−0.073	−0.070	−0.078	−0.085	−0.067	−0.078
90	−0.046	−0.047	−0.050	−0.048	−0.048	−0.047	−0.050	−0.057	−0.044	−0.054
95	−0.031	−0.030	−0.032	−0.031	−0.031	−0.031	−0.033	−0.037	−0.030	−0.038
95	0.032	0.031	0.035	0.036	0.033	0.032	0.036	0.035	0.029	0.038
90	0.045	0.045	0.048	0.050	0.047	0.046	0.050	0.048	0.042	0.053
80	0.066	0.064	0.069	0.071	0.067	0.065	0.070	0.069	0.062	0.073
70	0.081	0.082	0.086	0.089	0.085	0.080	0.087	0.086	0.077	0.089
60	0.094	0.097	0.101	0.104	0.099	0.095	0.102	0.101	0.091	0.103
50	0.108	0.111	0.116	0.120	0.114	0.110	0.118	0.116	0.104	0.116
40	0.124	0.128	0.133	0.135	0.130	0.125	0.134	0.130	0.119	0.130
30	0.144	0.148	0.153	0.152	0.149	0.142	0.151	0.145	0.133	0.144
20	0.171	0.175	0.178	0.171	0.174	0.163	0.172	0.162	0.152	0.164
10	0.205	0.212	0.215	—	0.211	0.191	0.201	0.181	0.179	0.174

The values for the human photoreceptors were derived from the data listed in table 2, those for the rhesus receptors from the mean data of Bowmaker *et al.* (1978) augmented by subsequent measurements to a total of 80 rods ($\lambda_{max} = 502.8 \pm 3.0$ nm), 127 green cones ($\lambda_{max} = 534.8 \pm 4.8$ nm) and 92 red cones ($\lambda_{max} = 566.2 \pm 4.8$ nm). The values for the extracted pigments were calculated from the spectra of cattle rhodopsin (Collins *et al.* 1952) and chicken iodopsin (Wald *et al.* 1955).

of λ_{max} advances towards the long-wave end of the spectrum. This is true also for the three receptors of the rhesus monkey, and the same tendency is shown by the extracted pigments, the values of the function (table 4) being greater for iodopsin than for rhodopsin.

At equal absorbance levels the agreement between the values of the function for the human and monkey receptors is close, particularly if comparison is made between receptors of similar λ_{max}. The agreement with the values for the extracted pigments is not so good but the differences, which are most marked at the spectral extremes, could be ascribed to a scattering component in the photoreceptor spectra that increases towards the short-wave end of the spectrum. Although there would be least scattering at long wavelengths, its effect could be considerable there for the true absorbance of the photopigment is also low in this region.

In our view the data in table 4 do not necessarily provide evidence that the spectra of extracted pigments differ in shape from those of pigments in the photoreceptors. We consider that these latter may be more or less 'contaminated' by the effects of scattering.

Variability

The results presented here suggest that there is a true variability in the spectral positions of the normal human green and red pigments. Alpern & Moeller (1977), on the basis of psychophysical results for dichromats and anomalous trichromats, have already suggested that the spectral position of the red pigment may vary, from one normal observer to another, over a range of several nanometres (and that deuteranomalous trichromacy occurs when the green and red pigments are both drawn from the same group – the 'erythrolabe cluster'). The present data suggest the similar, but distinct, possibility that there are discrete sub-populations within each major class of cone, and that normal observers may draw more or less receptors from each of them.

The histograms of figure 3 recall qualitatively the 'clustering' hypothesis of Dartnall & Lythgoe (1965). These authors, after surveying the vitamin A_1-based pigments that had been extracted from retinas, suggested that chemical factors constrain visual pigments to have their absorption peaks only at certain spectral locations, these being separated by approximately constant intervals of 6.5–8 nm (see also Knowles & Dartnall 1977). In view of this it is disappointing that the statistical tests on the human data have yielded equivocal, not to say conflicting, results. On the one hand the Shapiro & Wilk (1965) test has indicated that the distribution of the greens is not significantly different from normal (even chance), and that the distribution of the reds is very significantly different (50 to 1 against normality). On the other hand, the bimodality test of Giacomelli *et al.* (1971) gives the converse results, the green distribution being bimodal (10 to 1 against unimodality) and the red one not significantly different from unimodality.

These unexpected results are not necessarily because of any shortcomings of the tests. They arise from the fact that in certain circumstances two or more normally distributed populations can add to give a distribution that is still unimodal, and even normal. It can be shown, for example, that two approximately equal normal distributions, with maxima close together in relation to their

standard deviations, will add to give a population of intermediate maximum and a distribution closely resembling the normal. In such cases, Shapiro & Wilk's test is bound to indicate normality in spite of the underlying sub-populations. In similar circumstances the bimodality test of Giacomelli *et al.* (1971) will also fail, for there would be no 'dip', on which the test depends. Moreover even with a constructed example, based on the 'red' data but with a perfect bimodal distribution and a well-defined dip between the peaks, the test – as we have seen – fails to indicate a significant difference from unimodality.

We incline to the view that the distributions of the green and red receptors are bimodal, but that the peaks of the respective sub-populations are too close together (6–9 nm) in relation to their standard deviations (2–3 nm) for a definitive conclusion to be reached. To settle the matter it would seem to be necessary either to increase greatly the number of observations or, by an improvement in experimental technique, to reduce the standard deviation. As regards the latter, while it is certain that known sources of microspectrophotometric error, such as scattering and the presence of photo-products (Liebman 1972; MacNichol *et al.* 1973) must contribute to our recorded variance, part of it may well be because of a residual variability of the putative sub-populations.

These matters apart, the main reason for our belief that the observed variability is not wholly experimental in origin rests on the following fact. Struck by the difference in spectral distributions of the red pigments of patients 1 and 4 we were led to obtain independent psychophysical measurements for these patients. In direction and magnitude the psychophysical difference in long-wave sensitivity proved to be that required by the microspectrophotometric results.

Nevertheless, it is curious that the spectral separation of the red and green pigments can be reduced to 22 nm (patient 4, see table 1) without obvious impairment of hue discrimination (figure 4).

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