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THE VISUAL PIGMENTS OF RODS AND CONES IN THE RHESUS MONKEY, *MACACA MULATTA*

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SUMMARY

1. New microspectrophotometric measurements have been made of the photopigments of individual rods and cones from the retina of the rhesus monkey (*Macaca mulatta*). The measuring beam was passed transversely through isolated outer segments.

2. The transverse absorbance for rods ranged from 0·02 to 0·04 and that for cones from 0·01 to 0·03.

3. The mean absorbance spectrum for rods ($n = 25$) had a peak at $502 \pm 2{\cdot}7$ nm. A digitonin extract from the same group of eyes gave a $\lambda_{max.}$ of 499 ± 1 nm.

4. Of a sample of 82 cones, 40 were 'red' (P565 nm) and 42 were 'green' (P536 nm). The mean absorbance spectrum for the green cones is very similar to the Dartnall nomogram, but that for the red cones is narrower.

5. No bleachable, blue-sensitive outer segments were recorded, although structures were found that absorbed at short wave-lengths and were neither photosensitive nor dichroic.

6. If the long wave-length and middle wave-length cone pigments of the rhesus monkey are assumed to be identical to those of man and if additional assumptions are made about the lengths of human outer segments and about prereceptoral absorption, it is possible to derive psychophysical sensitivities that closely resemble the π_5 and π_4 mechanisms of W. S. Stiles.

INTRODUCTION

It is now more than a decade since the first publication of microspectrophotometric measurements of the photopigments of individual primate rods and cones, but few further measurements have followed the justly celebrated though preliminary reports of Marks, Dobelle & MacNichol (1964) and Brown & Wald (1964). Initially, since the end-on absorbance of receptors was thought to be very low, microspectrophotometry was carried out with a circular measuring beam passing axially through the outer segment of the receptor. Marks *et al.* (1964) used a beam 2 μm in diameter and reported results from 10 primate cones: a red and a blue

human cone and one blue, four green and three red cones from rhesus monkeys. The 'green' and 'red' cones were maximally sensitive at 540 and 577 nm respectively; the $\lambda_{max.}$ of the human 'blue' cone was 460 nm and that of the rhesus monkey 'blue' cone was 440 nm (we adopt the improper, but convenient, fiction of referring to red, green and blue cones when we mean cones that in fact are maximally sensitive to yellow, green and violet light respectively). The human blue cone absorbed only 0·4% at maximum, an absorbance of only 0·0017. The peak absorbances of the other cones ranged from 0·013 to 0·027. At about the same time, Brown & Wald (1964) and Wald & Brown (1965) reported axial measurements from two blue, four green and three red human cones as well as from two rods. They obtained difference spectra with $\lambda_{max.}$ 440–450, 525–530 and 555–565 nm. The maximum changes in absorbance ranged from 0·009 to 0·025. For rods the $\lambda_{max.}$ was 505 nm and the maximum change in absorbance was 0·05.

These early axial recordings suffered from the difficulty of ensuring that the measuring beam passed through the length of the outer segment without passing through neighbouring receptor cells. Given a measuring beam with a diameter of 2 μm and cone outer segments with a length of 35 μm and a diameter of only 1–2 μm, this problem is unavoidable. Thus axial records could give apparent indications of a mixture of pigments in a single cell, such as a mixture of green- and red-absorbing pigments in single foveal cones. The very low maximum absorbances do indeed suggest that considerable leakage of light did occur. Liebman (1972) reviews the difficulties of interpreting the early microspectrophotometric data and concludes that 'at best, they suggest the existence of three cone pigments in separate cones; the data alone cannot be regarded as accurate to better than 20–30 nm, and published densities cannot be regarded as indications in the least of what exists in the living eye'.

Some of these difficulties of microspectrophotometric measurements can be avoided by recording with a light beam passing transversely through the outer segment. Using a rectangular beam adapted to the shape and size of the outer segment, leakage of light can be largely avoided and it is possible to be certain that only a single outer segment is in the beam. The disadvantage of this method, however, is that the possible path-length is reduced from 30–40 μm to only 1–2 μm, and the absorbance per μm is reduced to two thirds because of the dichroism of the visual pigment within the receptor membranes (Schmidt, 1938). However, the loss of absorbance due to dichroism can be off-set by measuring with light polarized perpendicular to the long axis of the outer segment.

The first published spectrum obtained from such transverse measurements of a primate cone was that of a green cone from a rhesus monkey (Dobelle *et al.* 1969). They did not determine a precise $\lambda_{max.}$ but recorded a specific absorbance of 0·008 μm^{-1}, suggesting an end-on absorbance of 0·28 for a 35 μm-long outer segment. Liebman (1972) also reported obtaining transverse records from man and from rhesus monkey. He gave a transverse absorbance of 0·015 and listed $\lambda_{max.}$ of 440, 535 and 575 nm, but gave no spectra.

Up to the present, then, as few as twenty primate cones have been measured. Peak sensitivities for the blue, green and red cones have ranged from 440 to 460, from 525 to 540 and from 555 to 577 nm respectively; and end-on absorbances

have ranged from 0·002 to 0·28. Since existing measurements can therefore be regarded only as preliminary; since electronic amplifiers have improved; and since the advantages of transverse measurements are clear, we have made fresh measurements of a large sample of receptor cells from the rhesus monkey. The microspectrophotometer we used was designed by P. A. Liebman and it would seem that the results do not justify his cautious pessimism: by combining multiple records from this instrument it appears possible to locate the $\lambda_{max.}$ of the primate visual pigments to within ± 3 nm, to calculate their specific absorbances to within $\pm 0{\cdot}003$ μm^{-1} and to specify the band widths of absorbance spectra at 50% absorbance to within ± 200 cm^{-1}.

Behavioural experiments suggest that the colour vision of macaques is trichromatic and very similar to that of man. Comparisons of macaque and human observers on spectral sensitivity, on the anomaloscope and on wave-length discrimination are given by De Valois, Morgan, Polson, Mead & Hull (1974). We note in particular that the species used here, *Macaca mulatta* (rhesus monkey) is shown not to be tritanopic by measurements of increment thresholds (Sperling, Sidley, Dockens & Jolliffe, 1968).

There is evidence that some visual pigments in solution have a $\lambda_{max.}$ different from that found when measurements are made *in situ* (Wald & Brown, 1958; Bowmaker, 1973; Bowmaker, Loew & Liebman, 1975). A further purpose of the present study, therefore, was to compare measurements of rhodopsin made by microspectrophotometry with measurements of a digitonin extract derived from the same group of retinae.

METHODS

The eyes were taken from male rhesus monkeys (*Macaca mulatta*), ranging in weight from 5 to 12 kg and in age from 6 to 11 years. These animals had served in neuropsychological experiments on learning and cross-modal matching in the Cambridge Psychological Laboratory, after having undergone localized extirpations of non-visual areas of cerebral cortex. Control experiments had shown that their post-operative visual acuity and visual discrimination of objects were normal, although their colour vision had not specifically been tested (for details of lesions and controls, see Petrides, 1977). The animals were first given a sub-lethal dose of sodium pentobarbitone. To allow dark adaptation to occur, they were then placed in a dimly-lit room with their eyes covered. After 1 hr they were given a lethal dose of sodium pentobarbitone and the eyes were immediately removed, either in dim white light or in the red light of a photographic safe-light. Either immediately or after 30 min at room temperature, the eyes were placed on ice and were taken from Cambridge to Sussex University.

The eyes were maintained on ice in the dark. Measurements typically began within 6 hr of death but retinae were used up to 48 hr after death, with no apparent effect on the experimental results. Each eye was prepared under dim red light (Kodak safe-light No. 2). An equatorial section was made of the globe and the anterior half was removed. Care being taken not to displace the retina, the vitreous was then removed and the eye-cup was placed in ice-cold mammalian Ringer solution, pH 7·1. The fovea was located and a small piece of retina (approximately 1 mm^2), which either contained the fovea or came from an area adjacent to the fovea, was then removed. The tissue was placed on a cover slip, teased apart and mounted in the Ringer solution (to which 5% Dextran had been added) and then squashed under a second, smaller cover slip. The preparation was sealed with paraffin wax and mounted on the microscope stage of the microspectrophotometer.

The microspectrophotometer is of dual-beam design and is similar in most respects to that previously described by Liebman & Entine (1964) and by Liebman (1972). At the start of each session, the measurement and reference beams were usually adjusted to be 2×1 μm, but both

were continuously variable and could be further adjusted equally as desired. All measurements were made transversely and to increase the proportion of light absorbed by the dichroic outer segment of the receptor cell, all measurements were made with the e-vector of the measuring beam perpendicular to the long axis of the outer segment. Previous experiments have shown that there is little, if any, difference between 'parallel' and 'perpendicular' measurements of either $\lambda_{max.}$ or band width (Bowmaker *et al.* 1975; Harosi, 1975). Wave-length calibration of the MSP was carried out using a mercury-cadmium lamp as previously described (Bowmaker *et al.* 1975): wave-lengths given below are accurate to 1 nm.

All lining up of cells was carried out under infra-red illumination. The absorbance spectrum was obtained by scanning from 700 to 400 nm and back to 700 nm, so as to check that no significant bleaching occurred during the recording. The total time for the double scan was about 20 sec. Bleaching of receptors was carried out by passing white light along the measuring beam.

For the analysis of absorbance curves, records were selected that had a relatively high signal-to-noise ratio, that is records showing a transverse absorbance of at least 0·015. Transverse absorbances were normally in the range 0·02–0·03, although some large parafoveal cones had transverse absorbances of up to 0·04. The selected records were analysed individually by visually estimating, at 10 nm intervals, points lying midway within the noise band for both the absorbance and baseline traces. The base line value was subtracted from the absorbance curve value and the difference used to compute a normalized point-curve. The $\lambda_{max.}$ was estimated by the method of Dartnall, Lander & Munz (1961) as amplified by Bridges (1967) and involved 10 separate estimates based on the A_1 nomogram of Dartnall (Wyszecki & Stiles, 1967, p. 584). The precision of this analytical method and the reproducibility of absorbance measurements for the MSP have been shown previously (Bowmaker, Loew & Liebman, 1975). The mean and S.D. of the $\lambda_{max.}$ and a mean absorbance spectrum for each class of receptor was then determined.

RESULTS

Rods. Absorbance spectra were recorded from a total of twenty-five rod outer segments from four animals. One such record is shown in Fig. 1. The continuous line 3 is a base line drawn by eye through the noise and line 1 is a nomogram curve constructed upon it and having a $\lambda_{max.}$ of 502 nm. Line 2 was obtained after a 15 sec bleach with white light and is presumed to be composed of metarhodopsin III ($\lambda_{max.}$ 470–475 nm) and 'retinal' ($\lambda_{max.}$ 385–390 nm) (Baumann & Bender, 1973), both orientated perpendicularly to the long axis of the outer segment.

The outer segments ranged in diameter from 1 to 2·5 μm and the transverse absorbances ranged from 0·020 to 0·040, giving a specific absorbance of $0{\cdot}019 \pm 0{\cdot}004$ μm^{-1} ($n = 25$). The mean $\lambda_{max.}$ of the outer segments was $501{\cdot}9 \pm 2{\cdot}7$ nm and the mean absorbance curve, determined at 10 nm intervals, is shown in Fig. 2 and compared to a nomogram curve with a $\lambda_{max.}$ of 502 nm. The values of $\lambda_{max.}$ determined for each of the four animals showed no significant variation and are listed in Table 1.

Cones. Absorbance spectra were recorded from eighty-two cone outer segments from five animals. Of the 82, 42 had absorbance maxima at wave-lengths between 530 and 545 nm and 40 between 560 and 570 nm.

Fig. 3 shows one of the best records, which was obtained from the outer segment of a large, parafoveal, green cone with a diameter of about 2·5 μm. The continuous line 3 is a base line drawn by eye through the noise and line 1 is a nomogram curve for $\lambda_{max.}$ at 534 nm, constructed on the base line. The majority of outer segments of green cones had diameters in the range 0·7–2 μm; transverse absorbances ranged from 0·010 to 0·030, giving a mean specific absorbance of $0{\cdot}015 \pm 0{\cdot}004$ μm^{-1}. The

mean $\lambda_{max.}$, determined from the nineteen best records, was $536 \pm 3{\cdot}5$ nm. The mean absorbances at 10 nm intervals are shown in Fig. 4 and compared with a nomogram curve placed with its $\lambda_{max.}$ at 536 nm.

A record from a red cone with a transverse absorbance of 0·03 is shown in Fig. 5. The continuous lines 1 and 3 are comparable with those in Fig. 3, but trace 2 was

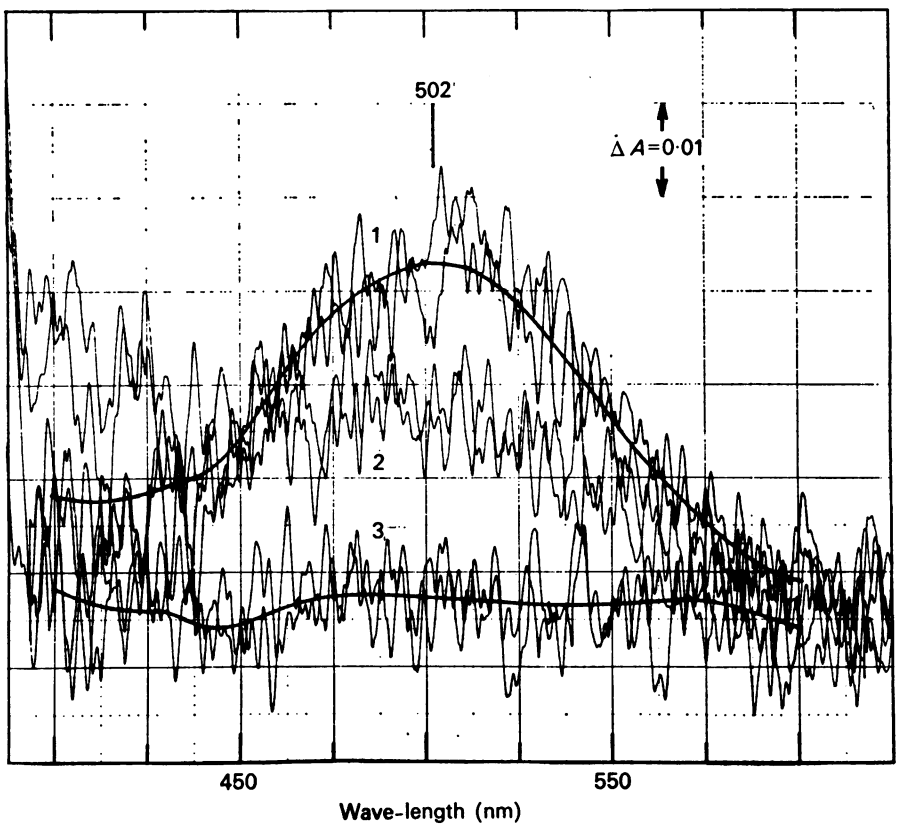

Fig. 1. Absorbance spectrum of a rhesus rod outer segment measured with the microspectrophotometer. Trace 1, before bleaching; trace 2, after a 15 sec bleach with white light; trace 3, instrumental base line. The continuous line drawn through trace 1 represents the absorbance of a rhodopsin of $\lambda_{max.} = 502$ nm calculated from the Dartnall monogram and constructed upon the smoothed base line drawn through trace 3.

TABLE 1. Visual pigments obtained from individual animals

	Rods			Green cone			Red cone			Total number of cones	
Animal	$\lambda_{max.}$	S.D.	n	$\lambda_{max.}$	S.D.	n	$\lambda_{max.}$	S.D.	n	Green	Red
18/11	501·5	3·1	8	533·1	1·7	4	566·1	2·5	7	6	8
22/11/1	501·5	2·3	5	537·3	5·2	3	562·3	—	1	5	3
22/11/2	501·6	2·5	7	539·4	3·4	3	561·8	0·6	2	5	3
29/11	503·5	2·8	5	536·0	2·5	7	562·9	1·6	6	18	17
2/12	—	—	—	533·0	1·1	2	566·8	1·6	2	8	9
Mean	501·9	2·7	25	535·8	3·5	19	564·5	2·6	18	42	40

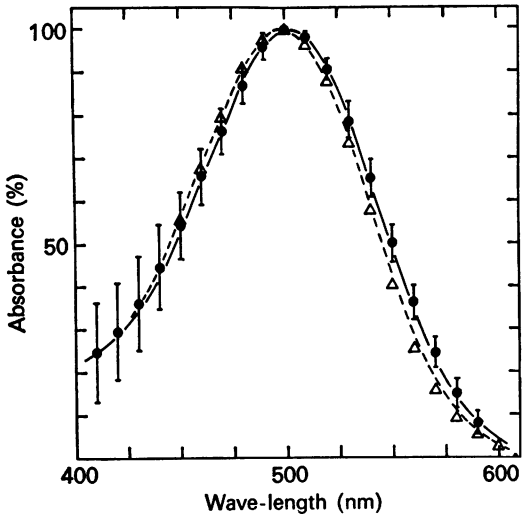

Fig. 2. Absorbance spectra of rhesus rhodopsin. Filled circles, mean absorbance ± S.D. of twenty-five rod outer segments recorded by MSP; open triangles, mean absorbance of three digitonin extracts of rhesus retinae. Continuous line is the Dartnall nomogram with $\lambda_{max.}$ = 502 nm and dashed line is the nomogram with $\lambda_{max.}$ = 499 nm.

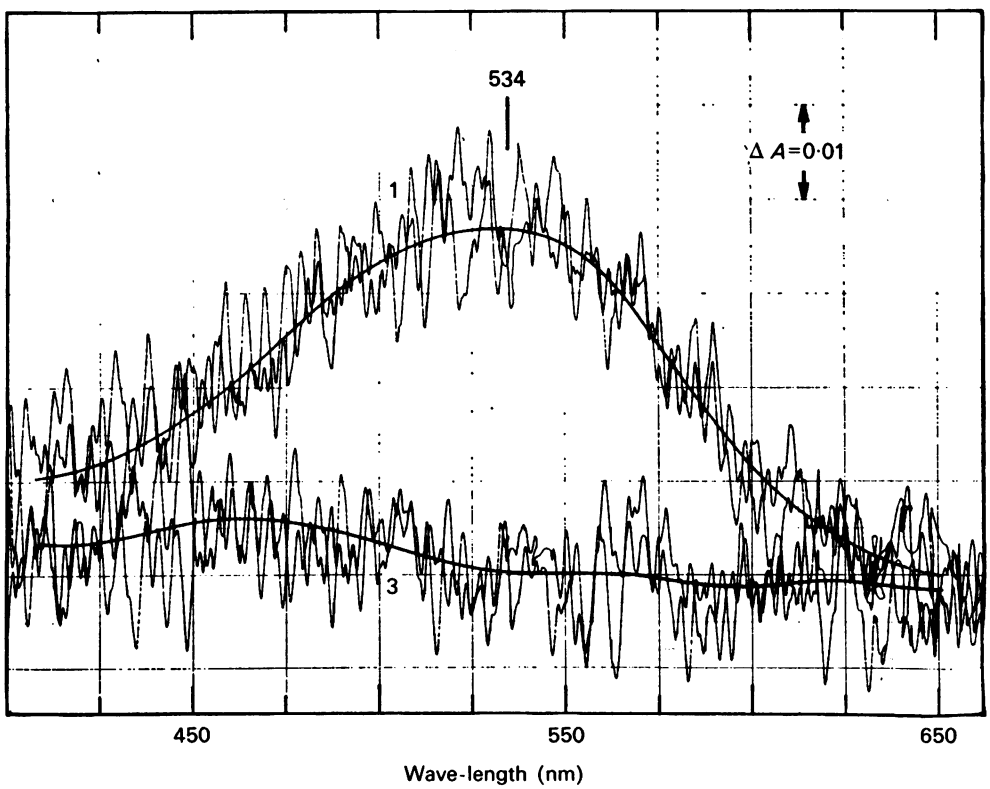

Fig. 3. Absorbance spectrum of a rhesus green-cone outer segment measured with the MSP. Trace 1, before bleaching; trace 3, instrumental base line. The continuous curve drawn through trace 1 represents the absorbance of a visual pigment of $\lambda_{max.}$ 534 nm calculated from the Dartnall nomogram and constructed upon the smoothed base line drawn through trace 3.

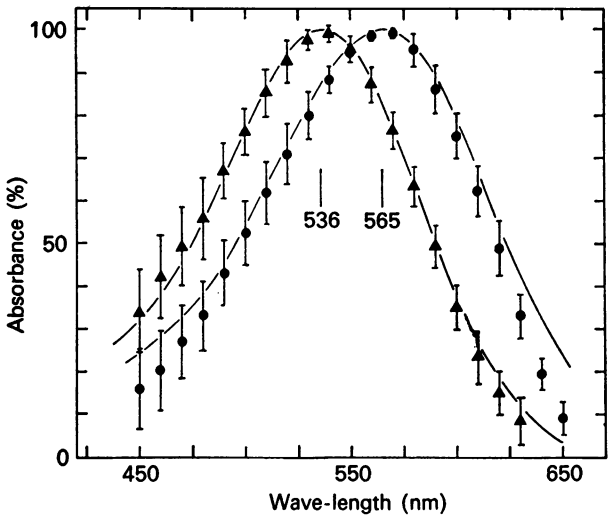

Fig. 4. Absorbance spectra of rhesus cone visual pigments. Circles, mean absorbance ± S.D. of eighteen red-cone outer segments measured by MSP; triangles, mean absorbance ± S.D. of nineteen green-cone outer segments measured by MSP. The continuous lines are Dartnall nomograms with $\lambda_{max.}$ = 565 and 536 nm as indicated.

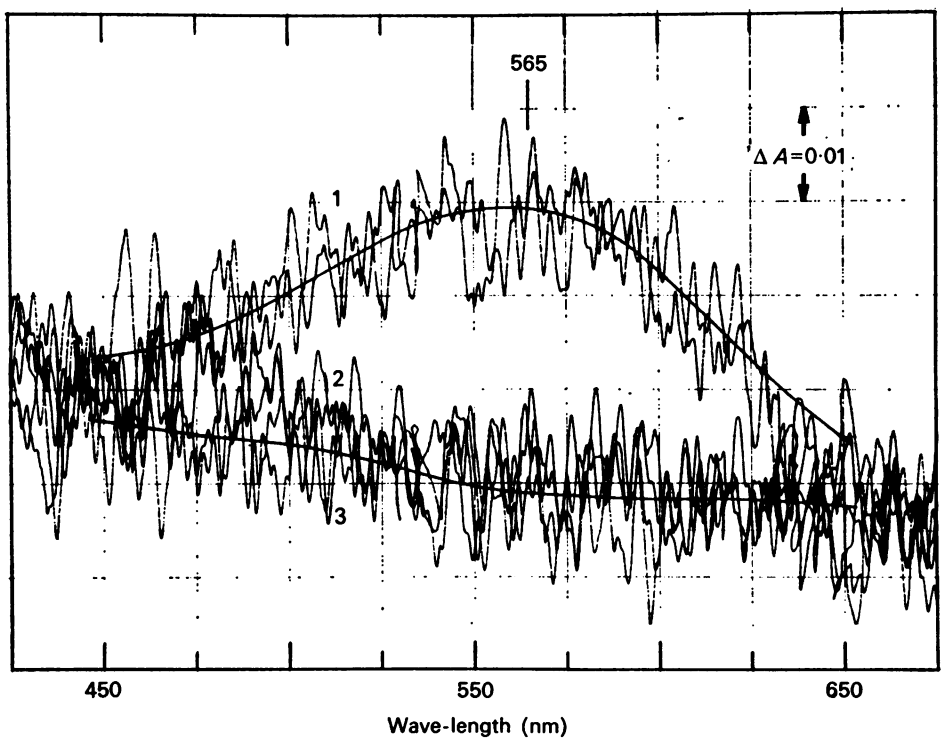

Fig. 5. Absorbance spectrum of a rhesus red-cone outer segment measured with the MSP. Trace 1, before bleaching; trace 2, after 15 sec bleach with white light; trace 3, instrumental base line. The continuous curve drawn through trace 1 represents the absorbance of a visual pigment of $\lambda_{max.}$ = 565 nm calculated from the Dartnall nomogram and constructed upon the smoothed base line drawn through trace 3.

obtained after a 15 sec bleach with white light. In contrast to the post-bleach spectrum of the rod (Fig. 1, line 2) there is no indication of photoproducts orientated perpendicularly to the long axis of the outer segment. The red cones were similar in diameter to the green and had a specific absorbance of $0{\cdot}013 \pm 0{\cdot}002\ \mu m^{-1}$. The mean $\lambda_{max.}$, determined from the best eighteen records, was $564{\cdot}5 \pm 2{\cdot}5$ nm. The mean absorbances at 10 nm intervals are shown in Fig. 4 and compared with a nomogram curve placed with its $\lambda_{max.}$ at 565 nm.

The sensitivities shown graphically in Figs. 2 and 4 are tabulated in Table 4. (For the convenience of the psychophysicists we also tabulate the sensitivities predicted at the human cornea if the visual pigments are the same in man and macaque; but we emphasize that these latter sensitivities depend on a number of debatable assumptions, which are discussed below.)

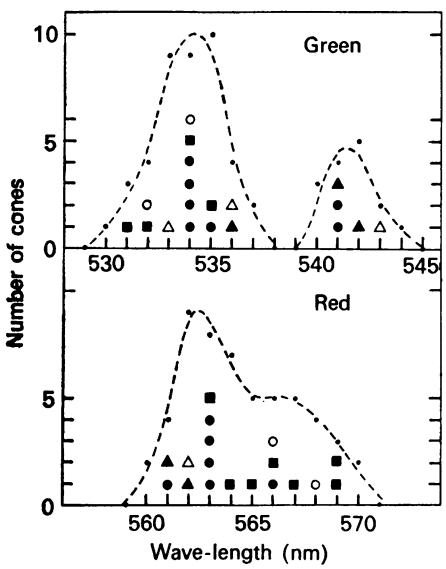

Fig. 6. Distribution of the $\lambda_{max.}$ of nineteen green and eighteen red cones of rhesus. The $\lambda_{max.}$ of individual cones have been corrected to the nearest nm. The symbols represent cones from the five different animals: squares, 18/11; open triangles, 22/11/1; filled triangles, 22/11/2; filled circles, 29/11; open circles, 2/12 (see Table 1). The dashed lines are 'running average' curves obtained by averaging the frequencies of cones over three consecutive wave-lengths.

Table 1 lists the mean values of $\lambda_{max.}$ for the red and green cones of individual animals and shows the number of records used in each case: it is apparent that there was no significant variation between the animals.

However, an interesting complication arises from the MSP data on the green cones in that the nineteen records that were analysed to obtain the mean $\lambda_{max.}$ and absorbance spectrum appear to fall into two distinct groups. In Fig. 6 the values of $\lambda_{max.}$ for the individual records, corrected to the nearest nm, are plotted on a wave-length scale and it can be seen that the majority of cones cluster at about 534 nm while the remainder cluster around 541–542 nm with none falling between 537 and 540 nm. Such a division in $\lambda_{max.}$ is not seen within the eighteen red cones

(Fig. 6). The differences in $\lambda_{max.}$ of the green cones is not a result of variability between individuals since, as shown in Fig. 6, representatives of both groups of cones were found within individual animals. Clearly, with the type of records obtained from cone outer segments with a diameter of only 1–2 μm, a sample of nineteen is insufficient to show whether such a division definitely does occur. Alpern & Moeller (1977) have suggested that there are individual variations in the peak sensitivities of 'erythrolabe' and 'chlorolabe' of normal human observers and that in anomalous trichromats both long-wavelength pigments are drawn from the same parent class. In the light of this proposal, further measurements of the primate pigments are desirable.

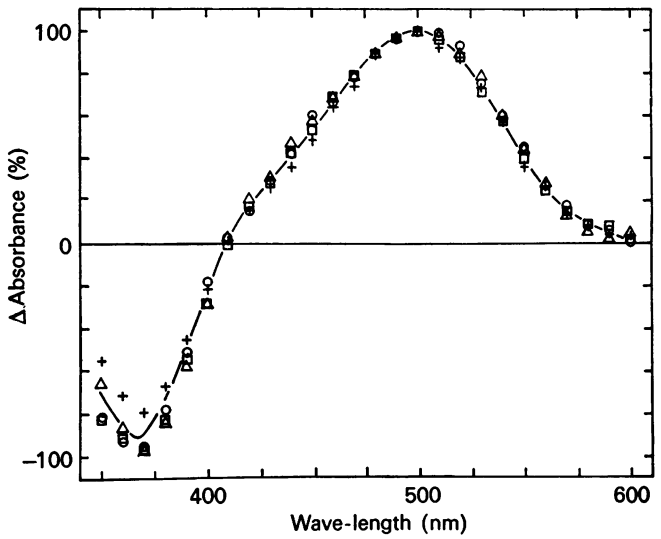

Fig. 7. Difference spectra, normalized to 100 %, for four successive partial bleaches, in the presence of hydroxylamine, of a digitonin extract of rhesus retinae. Crosses and circles, first and second bleaches respectively, both of 0·5 min; squares, third bleach of 2 min; triangles, fourth bleach of 7 min. The full line is the difference spectrum of the total bleach and has a maximum density loss at 499 nm.

Retinal extract. A digitonin extract was obtained from the retinal remains from twelve eyes, including those used in the MSP study, that were collected over a period of two weeks and frozen. The extract was divided into three samples, which had maximum absorbances of 0·094 and 0·072 and 0·189. The first sample was bleached for 10 min in the presence of hydroxylamine, using light of wave-lengths longer than 490 nm (Corning filter 3·70): this procedure yielded a difference spectrum with $\lambda_{max.}$ 498·8 ± 1·2 nm. The other two samples, after the addition of hydroxylamine, were partially bleached using a combination of two red filters (Corning 2·63 and 3·66) that effectively cut off light of wave-lengths less than 590 nm. This combination of filters is used routinely (A. Knowles, personal communication) to separate rhodopsin and iso-rhodopsin in flash-bleached extracts of cattle retinae. The exposure sequence using these filters was 0·5, 0·5, 2, 7 and 20 min, and was followed by a 10 min bleach with yellow light (Corning filter 3·70). The difference spectra obtained from the third sample are shown in Fig. 7, normalized to 100 %.

The values of $\Delta D_{max.}$ for the five red bleaches were 0·049, 0·035, 0·065, 0·046, and 0·004 respectively; the yellow exposure produced no further change in absorbance. The agreement between the spectra shows that only a single pigment, with $\lambda_{max.}$ 499 nm, is present in detectable quantities, a result that might be expected for this predominantly rod retina. The partial bleaching of the second sample followed a very similar pattern. The mean difference spectrum from the full bleach of the three extract samples at wave-lengths above 450 nm is shown in Fig. 2 (open triangles) and is compared with a 499 nm nomogram curve and with the MSP data for the outer segments of rods.

TABLE 2. $\lambda_{max.}$ of human and rhesus rhodopsin

	Extract $\lambda_{max.}$		*In situ* $\lambda_{max.}$		
Source	Man	Rhesus	Man	Rhesus	Method
Crescitelli & Dartnall, 1953	497*	—	—	—	—
Wald & Brown, 1958	493	—	500	—	Suspension
			502·5		Retinal area
Bridges, 1959	—	497	—	—	—
Brown & Wald, 1963	—	—	500	503	Retinal area
Brown & Wald, 1964	—	—	505†	—	MSP
Liebman, 1972	—	—	498‡	498‡	MSP
Baumann & Bender, 1973	—	—	500	—	Retinal area
Bridges, 1973	494	—	—	—	—
Present	—	499 ± 1	—	502 ± 2·5	MSP
					$n = 25$

* Possibly too long because of photoproduct interference since no hydroxylamine present.
† Axial measurements from only two rods.
‡ Number of cells measured not recorded.

DISCUSSION

Rods. Table 2 summarizes measurements of human and rhesus rhodopsin and includes data derived from measurements of extracts and suspensions of outer segments, from spectrophotometry of small regions of retina and from axial and transverse microspectrophotometry of individual rods. The first MSP results were those of Brown & Wald (1964), who made axial measurements of two single human rods and obtained a $\lambda_{max.}$ of 505 nm and a maximum absorbance difference of 0·05. Using a transverse measuring beam of about $0·7 \times 4\ \mu m$, Dobelle, Marks & MacNichol (1969) obtained for rhesus rods an absorbance spectrum with a specific absorbance of $0·008\ \mu m^{-1}$, but did not publish a precise $\lambda_{max.}$ Liebman (1972) also reports measurements for rods from man and rhesus: in both cases the $\lambda_{max.}$ was 498 nm and the transverse absorbance was 0·015.

The data for the rhesus reported in the present paper are the most comprehensive so far and give a $\lambda_{max.}$ for rods of $502 \pm 2·5$ nm ($n = 25$), with a specific absorbance of $0·019 \pm 0·004\ \mu m^{-1}$. A $\lambda_{max.}$ of 499 ± 1 nm is obtained for the difference spectrum of a digitonin extract in the presence of hydroxylamine. Thus the $\lambda_{max.}$ in the intact outer segment is at a slightly longer wave-length than that of the pigment in solution. The difference between the two is about 3 nm and is similar to that found for frog rhodopsin (Bowmaker, 1973; Bowmaker *et al.* 1975). A hypsochromic

shift of this kind is not universal, since measurements of chicken rhodopsin give a $\lambda_{max.}$ of 506 nm both in solution (Knowles, 1976) and in the rod outer segment (Bowmaker & Knowles, 1977). The shift found for primate rhodopsin is difficult to assess, owing to the low signal-to-noise ratios in MSP studies and to the possible interference by photoproducts, cone pigments and isorhodopsin in measurements of extracts; but *prima facie* the MSP measurements are the most appropriate for psychophysical comparisons.

There is an indication in Table 2 that the rhodopsin of the rhesus monkey has a maximum absorbance at a slightly longer wave-length than that of man: the extract data for man and rhesus give peak absorbances at 494 and 498 nm respectively whereas measurements *in situ* give 500 and 502 nm respectively. However, the same problems that make it difficult to interpret the hypsochromic shift make it difficult to assess this second discrepancy.

TABLE 3. $\lambda_{max.}$ of human and rhesus cone visual pigments

		Blue			Green			Red		
Source		$\lambda_{max.}$	End on absorbance	n	$\lambda_{max.}$	End on absorbance	n	$\lambda_{max.}$	End on absorbance	n
Brown & Wald, 1963	M	—	—	—	535	0·015	Foveal area	565	0·03	Foveal area
	R	—	—	—	527	0·015	Foveal area	565	0·03	Foveal area
Marks *et al.* 1964	M	460	0·002	1	—	—	—	570	0·02	1
	R	440	0·02	1	540	0·02	4	577	0·02	3
Brown & Wald, 1964	M	450	0·025	1	525	0·025	2	555	0·013	1
Wald & Brown, 1965	M	440	0·009	1	529	0·015	2	565	0·019	2
Murray, 1968	R	—	—	—	526	Foveal sonicate		573	Foveal sonicate	
Dobelle *et al.* 1969	R	—	—	—	not given	0·28*	?	—	—	—
Liebman, 1972	M/R	440	0·015	?	535	0·015	?	575	0·015	?
Present	R	—	—	0	536 ± 3·5	0·525**	42	565 ± 2·5	0·525**	40

* Specific absorbance of 0·008 μm^{-1}; ** specific absorbance of 0·015 μm^{-1}; both assuming a 35 μm long outer segment.

R = Rhesus monkey; M = man.

Cones. Table 3 summarizes measurements of human and rhesus cone pigments derived from partial bleaching of foveal preparations and from axial and transverse MSP of single cones. In the present study of the rhesus monkey we have recorded from eighty-two cones, forty-two green and forty red, but no blue cones. The values of $\lambda_{max.}$ for these receptors were 536 ± 3·5 and 565 ± 2·5 nm respectively. The mean absorbance spectrum for the eighteen best red cones (Fig. 4) is significantly narrower than the nomogram, having a band width at 50% absorbance of about 3900 cm^{-1} compared with the nomogram's 4150 cm^{-1}. The narrowness of absorbance spectra with maxima at longer wave-lengths appears to be a general characteristic, having

been observed in frog (Liebman & Entine, 1968), turtle (Liebman & Granda, 1971), and in birds (Bowmaker & Knowles, 1977; Bowmaker, 1977*b*). The mean absorbance spectrum of the green cone is, however, very similar in shape to the nomogram. The variation with wave-length of the band width of visual pigments has been reviewed recently by Ebrey & Honig (1977) and if the approximately linear relationship that they propose is correct, then the green cone probably should have a band width closer to 4000 cm^{-1} than to 4150 cm^{-1}. However, owing to the relatively poor signal-to-noise ratio of MSP data from the small outer segments of primate cones, it is possible to estimate a bandwidth only to about ± 200 cm^{-1}.

TABLE 4. Absorbance and calculated relative absorptance of rhesus receptors

Wave-length (nm)	Rod		Green cone		Red cone	
	Absorbance (%)	Log relative absorptance*	Absorbance (%)	Log relative absorptance†	Absorbance (%)	Log relative absorptance†
650	—	—	(2·2)	(0·573)	9·0	1·167
40	—	—	(4·5)	(0·878)	19·3	1·472
30	—	—	8·6	1·148	33·1	1·672
20	—	—	15·2	1·379	49·0	1·804
10	(2·4)	(0·591)	23·5	1·547	62·4	1·878
600	(4·5)	(0·859)	35·1	1·693	75·2	1·930
90	7·9	1·095	49·4	1·807	86·1	1·965
80	14·8	1·352	63·5	1·883	95·0	1·988
70	24·4	1·547	76·6	1·935	98·7	1·997
60	36·1	1·691	87·3	1·968	98·2	1·996
550	49·8	1·800	95·5	1·990	94·3	1·987
40	65·0	1·884	98·5	1·997	88·2	1·971
30	78·8	1·939	97·4	1·984	80·0	1·936
20	90·3	1·975	92·6	1·932	71·1	1·865
10	97·7	1·994	85·4	1·833	61·9	1·746
500	99·7	1·999	76·4	1·659	52·5	1·551
90	95·6	1·974	67·3	1·475	43·0	1·337
80	86·7	1·934	56·1	1·401	33·0	1·226
70	76·1	1·879	49·5	1·312	26·8	1·101
60	65·6	1·811	42·3	1·186	20·2	0·920
450	54·2	1·728	34·2	1·124	15·8	0·834
40	44·6	1·619	(27·3)	(1·058)	(13·5)	(0·787)
30	36·2	1·447	(23·5)	(0·937)	(12·0)	(0·674)
20	29·5	1·193	(21·2)	(0·781)	(11·3)	(0·535)
10	24·7	0·856	(19·8)	(0·627)	(11·0)	(0·394)
400	(21·5)	(0·423)	(19·5)	(0·316)	—	—

* based on an end-on absorbance of 0·475 (25 μm long outer segment with specific absorbance of 0·019 μm^{-1}) and corrected for lens absorbance as detailed in text.

† based on an end-on absorbance of 0·525 (35 μm long outer segment with specific absorbance of 0·015 μm^{-1}) and corrected for lens and macular absorbance as detailed in text.

Values in brackets are extrapolations of the experimentally determined curves, those of rod and green cone based on the nomogram.

Absence of blue-sensitive receptors. Although considerable effort was made in searching for blue cones in areas of the retina both adjacent to and at some distance from the fovea, no structures were observed that contained a blue-absorbing photosensitive pigment. In addition, all structures that could be identified as outer

segments were found to contain either the green or yellow photosensitive pigment and no outer segments were found from which, because of their small size or because of an absence of pigment, records could not be obtained. However, a number of structures were found that contained a blue-absorbing pigment that was neither photosensitive nor dichroic. Fig. 8 shows records from two such structures that resembled outer segments and that had transverse absorbances of 0·018 and 0·026. The continuous lines in Fig. 8 represent nomogram curves with $\lambda_{max.} = 430$ nm, constructed upon baselines drawn by eye through the experimental base lines. Although the shape of neither spectrum conforms to the nomogram shape to the same extent as do the absorbance spectra of the red and green cones, these structures might easily have been misinterpreted as the outer segments of blue cones if the lack of photosensitivity and of dichroism had not been established. In both the cases shown in Fig. 8, the structure was exposed to light for 60 sec without effect.

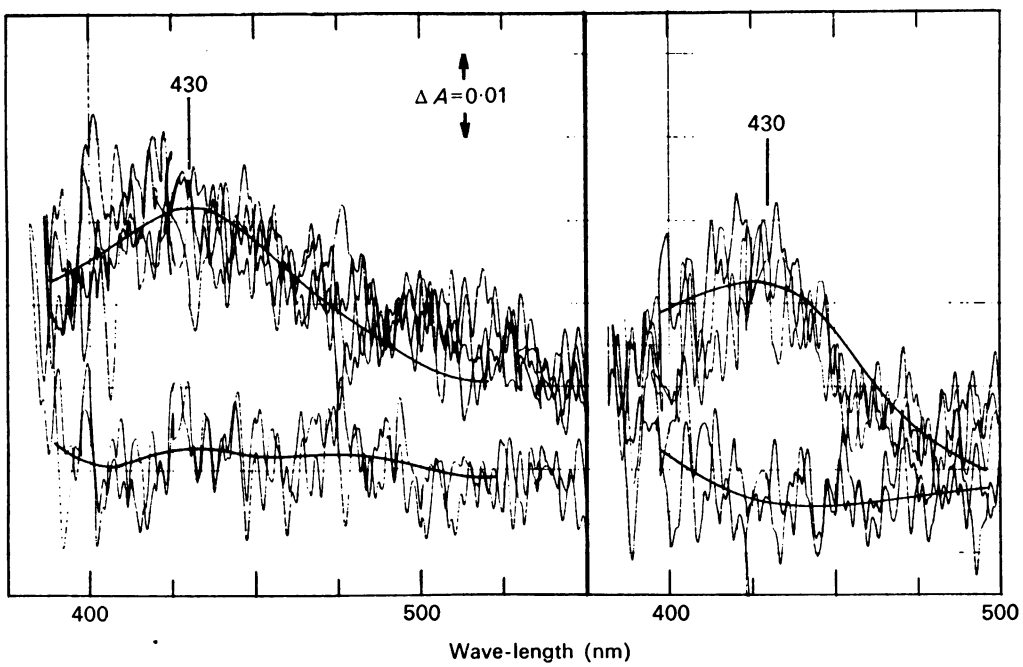

Fig. 8. Absorbance spectra of two photostable, blue-absorbing structures measured by MSP. The continuous curves through the upper traces represent the absorbance of a visual pigment of $\lambda_{max.} = 430$ nm calculated from the Dartnall nomogram and constructed on the smoothed base lines drawn through the lower traces.

Our failure to record blue-sensitive outer segments in a sample of eighty-two may have a number of explanations. First, there may indeed be no blue cones. The psychophysically measured blue mechanism may, for example, represent the subtraction of the π_4 signal from the rod signal, as proposed by Willmer (1961). However, this hypothesis has always faced difficulties, notably in the failure of photopic matches at scotopic levels and in the Stiles-Crawford effect shown by the psychophysical blue mechanism (Stiles, 1939).

Secondly, blue receptors may exist, but our procedure may select against them.

For example, their outer segments might adhere to the pigment epithelium or their dark adaptation might be impaired by the anaesthetic administered to the monkeys before death. The blue mechanism is known to be disproportionately vulnerable to disease or toxins (Verriest, 1974; Adams, Brown, Haegerstrom-Portnoy, Flom & Jones, 1976), though these may be neural failures rather than pigment loss. However, as stated above, no 'empty' outer segments were found. It is also unlikely that our monkeys were all tritanopic, since they were of different ages and came from different sources.

A third possibility is that the 'artifacts' described above (Fig. 8) are indeed the blue receptors. For example, a 'fixed-filter' hypothesis can be postulated that would suppose a filter in front of a sub-set of, say, the green cones and a subsequent neural subtraction of the signals from filtered and unfiltered receptors. Such a mechanism appears to function in birds to give sensitivity maximal between 420 and 440 nm (Yazulla & Granda, 1973) and it has been suggested (Bowmaker & Knowles, 1977; Bowmaker, 1977*b*) that cones containing a long wave-length visual pigment ($\lambda_{max.}$ 565–570) are divided into two populations, one containing oil droplets that cut off short wave-length light and a second containing clear oil droplets. A second hypothesis in this class would suppose, first, that the blue-sensitive visual pigment has a relatively stable photoproduct with an absorbance spectrum similar to that of the visual pigment and, secondly, that neither pigment is dichroic. A photoproduct with a $\lambda_{max.}$ at about 445 nm (that can be equated with metarhodopsin III) has been identified in the blue receptor, the so-called green rod, of the frog that contains a P437, though in this receptor both pigments are dichroic (Bowmaker, 1977*a*). A third hypothesis could be one in which visual excitation is achieved without bleaching, perhaps by photosensitization. Hypotheses of this type, however, imply that the blue-sensitive mechanism in primates is fundamentally different from that of other vertebrates, for blue receptors containing typical photosensitive pigments based on retinae have been found in fish, amphibians, reptiles and birds.

The fourth, and most likely, possibility is that photosensitive blue cones exist but are rare. The quantum efficiency of the psychophysical blue mechanism is almost 100 times poorer than that of the long wave-length mechanisms (Barlow, 1957) and this would be compatible with a frequency of 0·5% of all cones, although the over-all quantum efficiency will be in part reduced by the greater pre-retinal absorbance at short wave-lengths. If the frequency of blue cones is as little as 1%, there is a probability of 0·44 that none would be recorded in a sample of eighty-two, but if the frequency were as high as 10%, this probability falls to the (very significant) level of 0·00018. The absence of blue cones in our sample of eighty-two suggests that the blue cone population comprises less than 3·5% of the cone population at the 0·05 probability level.

In the light of our failure to record any blue cones, it is interesting that four of the twenty cones originally reported by Marks *et al.* (1964), Brown & Wald (1964) and Wald & Brown (1965) were identified as blue cones. Of the two blue cones reported by Marks *et al.* that from man had a maximum absorbance of only 0·0017 and neither cone was specifically reported to have been bleachable. Similarly, Liebman (1972) reports only 'some evidence for a pigment $\lambda_{max.}$ = 440 nm', a

description that might be applied to our Fig. 8. On the other hand, the two blue cones reported by Wald & Brown were identified from difference spectra produced by bleaching. However, a problem that can occur with both axial and transverse MSP is that the receptor under investigation can move fractionally so that the measuring beam no longer passes completely through the outer segment. If such a movement were to occur during exposure to light, an apparent loss of a photosensitive pigment would be recorded, though in fact no bleaching had occurred.

Scotopic sensitivity. The relative spectral sensitivity of rods can be determined from the absorbance spectrum and specific absorbance of the rhodopsin. On the assumption that the rod outer segments are about 25 μm long and have a specific absorbance of 0·019 μm^{-1}, the end-on absorbance of the outer segment will be about 0·475, so that the absorptance spectrum (% of light absorbed) will be considerably broader than the nomogram. A value of 0·475 is higher than recent psychophysical estimates (Zwas & Alpern, 1976).

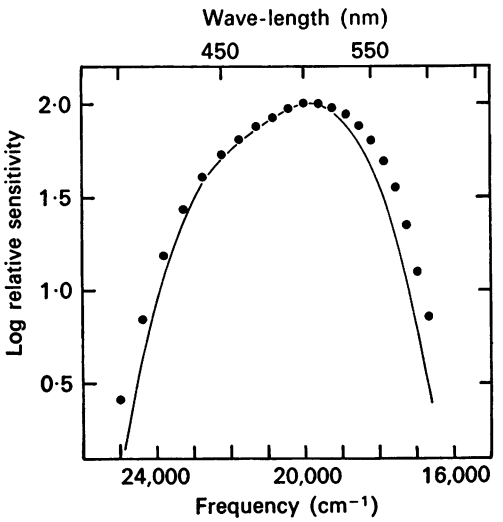

Fig. 9. Comparison of the relative spectral sensitivity of rhesus rods (circles) with the CIE scotopic function for man plotted on an equal quantum basis (continuous line). The rod sensitivity is based on an end-on absorbance of 0·475 and corrected for lens transmission as detailed in the text.

To compare the present data with psychophysically measured scotopic sensitivities, the pigment curve has to be corrected for selective transmission by pre-retinal material such as the lens and vitreous. It would appear that the scotopic sensitivities of macaque and man are very similar (De Valois *et al.* 1974) and that the values of $\lambda_{max.}$ for their rhodopsins are also very similar (Table 2), so that it is of interest to correct our macaque rod data with the data for human lens transmission and to compare the result with the CIE scotopic luminosity function.

Measurements of the transmission of the ocular media in man are very variable, owing in part to the age-dependent decrease in transmission of short-wave light by the lens and, when measurements are made of material removed after death, to a post-mortem increase in scattering. It would seem that the best method to

determine lens transmission is to compare the spectral sensitivities of normal and aphakic observers (Wald, 1945). These data show that the transmission of the lens remains uniform for wave-lengths down to about 500 nm, below which it decreases to only about 5–10% at 400 nm (for a review of transmission by human ocular media see Norren & Vos, 1974). We have, therefore, used the data from Wyszecki & Stiles, 1967, p. 216) for wave-lengths below 500 nm and have assumed that no selective transmission occurs at longer wave-lengths.

A relative scotopic spectral sensitivity determined according to the above considerations is shown in Fig. 9 (filled circles) and compared with the CIE scotopic sensitivity function expressed on a quantum basis. The log relative absorptance values for the rods are listed in Table 4. Although the maxima agree, the CIE function is considerably narrower than the sensitivity determined from the present data and the discrepancy is difficult to account for. In the past, the narrowness of the CIE curve has been explained by assuming a very low end-on absorbance for the rods of less than 0·1. However, the present data for the specific absorbance of the rhodopsin make this assumption untenable. Even a difference between the $\lambda_{max.}$ of rhesus and human rhodopsin of 4 nm (Table 2) will not significantly alter the shape of the predicted sensitivity. We note that a recent determination of the scotopic sensitivity of macaques (n.b., not rhesus) (De Valois *et al.* 1974) gave a function similar to the CIE scotopic function but showing higher sensitivity (about 0·3 log units) at wave-lengths above the maximum. This function is closer to the sensitivity predicted by the present data, but we do not know how much weight to place on it since De Valois *et al.* give a scotopic sensitivity for man that differs from the CIE function in the same way.

Photopic sensitivities. Spectral sensitivities can be derived for the red and green cones in a similar manner to that for rods. Outer segments of foveal cones are about 35 μm long (Polyak, 1941) and have a specific absorbance of about 0·015 μm^{-1}, so having an end-on absorbance of about 0·525. However, in addition to correcting their absorptance spectra for lens transmission, a correction also has to be made for absorption by macular pigment. As with lens transmission, measurements of macular pigment are very variable so that for simplicity we have used the data of Wyszecki & Stiles (1967, p. 219) where the maximum absorbance of the macular is taken as 0·5. As in the case of rods, we have made no lens correction at wave-lengths longer than 500 nm, and in this context we note that three of the four observers who served in the determination of the π mechanisms of W. S. Stiles (Stiles, 1977) were aged 20–30 yr. Observers of this age show little variation in transmission at wave-lengths above 500 nm (Wyszecki & Stiles, 1967, Figs. 2, 9).

The relative spectral sensitivity determined for the red cone, $\lambda_{max.}$ 565 nm, based on the above assumptions, is shown in Fig. 10 (filled circles) and compared with the Stiles π_5 function. The fit is extremely good over the entire spectral range measured for the cone pigment (450–630 nm).

A similar comparison, but of the green cone and the Stiles π_4 function, is shown in Fig. 11. Although the maxima agree, the π_4 function is slightly narrower than the calculated sensitivity of the green cone. At wave-lengths below 450 nm the discrepancy (about 0·20 log units) may be due, at least to some extent, to an unsatisfactory correction for macular absorbance. The log relative absorptance

values for the red and green cones used in the comparison with the π functions are listed in Table 4. Suggestions have been made (Smith & Pokorny, 1975) that the end-on absorbance of the green cone is less than that of the red cone by about 0·1 and if correct this would narrow slightly the sensitivity function for the green cones, bringing it close to the π_4 function. However, since the specific absorbance of the green cone is similar to that of the red, a lower end-on absorbance would suggest a shorter outer segment (25–30 μm): no measurements of the lengths of outer segments were made in the present study but the two classes of cone were impossible to differentiate by eye under the miscroscope.

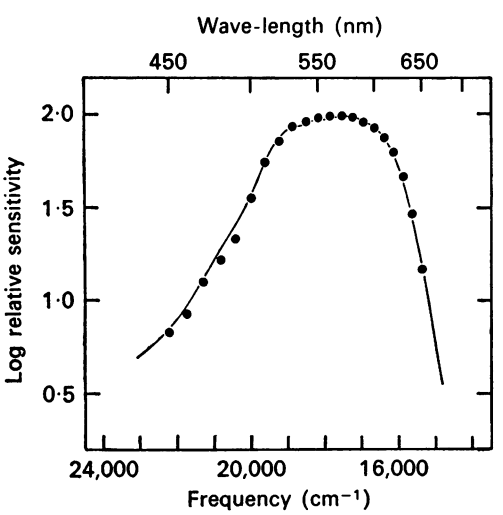

Fig. 10

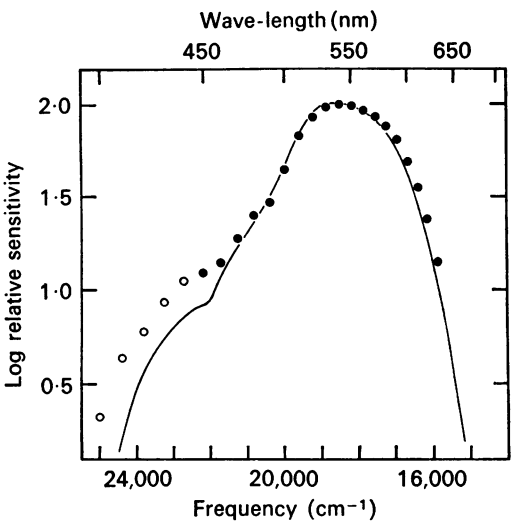

Fig. 11

Fig. 10. Comparison of the relative spectral sensitivity of the red cone of the rhesus (circles) with the π_5 mechanism of Stiles (continuous line). The sensitivity of the red cone is based on an end-on absorbance of 0·525 and corrected for lens transmission and macular absorbance as detailed in the text.

Fig. 11. Comparison of the relative spectral sensitivity of the green cone of the rhesus (circles) with the π_4 mechanism of Stiles (continuous line). The sensitivity of the green cone is based on an end-on absorbance of 0·525 and corrected for lens transmission and macular absorbance as detailed in the text. The open circles are an extrapolation of the experimental data based on the Dartnall nomogram.

The striking comparisons of Figs. 10 and 11 depend upon assumptions about pre-receptoral absorptions and the reader may properly question the particular values we have adopted for these factors. A more secure comparison is provided by Fig. 12, where we have plotted the ratio of the calculated sensitivities of the green and red cones and compared this with the ratio of the sensitivities of π_4 and π_5. This comparison is independent of assumptions about absorption by the ocular media and the macular pigment, provided only that we admit the minimal assumption that pre-receptoral absorption is similar for the two classes of cone. We are still required to assume a value for the end-on density of the cones. Given the disparate sources of the data, Fig. 12 shows a remarkable agreement between the two functions: for wave-lengths above 470 nm the discrepancy never exceeds 0·1 $\log_{10}$ unit. At

short wave-lengths there remains a small but systematic divergence and to account for it we can no longer turn to the macular pigment for a catch-all explanation. It may reflect the influence of photoproducts in the microspectrophotometric measurements.

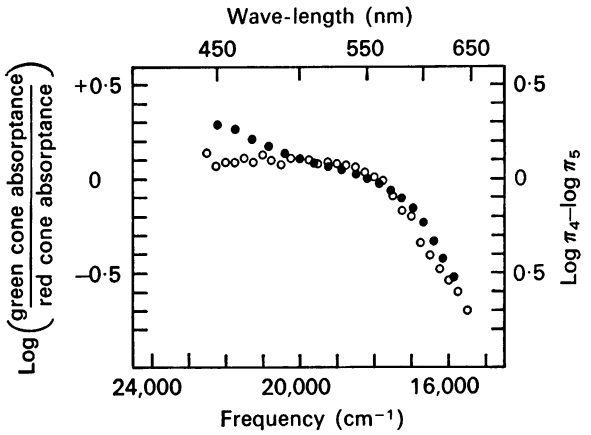

Fig. 12. Comparison of the ratio of the calculated sensitivities of the red and green cones with the ratio of the sensitivities of π_5 and π_4. Filled circles, ratio of the sensitivities of the green and red cones. Open circles, ratio of π_4 and π_5 (data from Wyszecki & Stiles, p. 579, 1967).

On the assumption that colour vision, at least in terms of the red and green mechanisms, is similar in macaque and man (Norren, 1972; De Valois *et al.* 1974), it may be concluded that the π_5 and π_4 functions represent retinal mechanisms based on single foveal cones containing visual pigments with $\lambda_{max.}$ either 565 or 536 nm. Although we have not shown specifically that the visual pigment content of each cone is homogeneous, no visual receptor cells, other than those of some fish and amphibians that contain mixtures of retinal- and 3-dehydroretinal-based pigments, have ever been found to contain more than one visual pigment.

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