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CONE PIGMENTS IN HUMAN DEUTAN COLOUR VISION DEFECTS

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SUMMARY

1. The Nagel anomaloscope, neutral points and dichromatic matches to a spectral green light identified a population of seventy red-green dichromats.

2. The anomaloscope settings allow the calculation of the relative action spectrum of the match at the wave-length of the red (645 nm) and green (535 nm) primaries. The distribution of this ratio is bimodal; there are two clusters with a gap of about 0·75 log units between. Among the thirty-eight deuteranopes there are wide differences in anomaloscope matches; similar differences appear among the thirty-two protanopes.

3. Retinal densitometry of the foveas of fifteen of the deuteranopes is compared and contrasted with measurements on trichromats. In the former, only one photolabile pigment is found in the red-green region of the spectrum; normals always have two. The view of Rushton (1965*a*) that deuteranopes have erythrolabe but no measurable chlorolabe is confirmed for each member of this group.

4. Simple deuteranomalous show two red-green cone pigments. The difference spectra of extreme deuteranomalous are very similar to those found in deuteranopia.

5. Individual difference in kinetics (photosensitivity, time constant of regeneration) and in the density and λ_{max} of the difference spectrum of erythrolabe in deuteranopia are appreciable; the reasons for these differences are not clear.

INTRODUCTION

The development of modern views of foveal trichromacy has relied heavily on the suggestion advocated in a crude form by Thomas Young

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(1807) and elaborated first by Maxwell (1860) and then by Koenig & Dieterici (1886), that both varieties of red-green dichromacy are 'reduction' forms of normal colour vision. The hypothesis restated in terms of current concepts is essentially that protanopes have the normal short and medium wave-length sensitive cone visual pigments (i.e. cyanolabe and chlorolabe, respectively) and lack erythrolabe (the normal long wave sensitive cone visual pigment), while deuteranopes (lacking chlorolabe) have normal erythrolabe and cyanolabe.

An alternative, usually attributed to Leber (1873) and Fick (1879), though it was first proposed by Aitken (1873), is that all three normal cone visual pigments are found in the dichromatic fovea and that the dichromacy is a consequence of a neural (or a chemical) 'fusion'. One form of the 'fusion' hypothesis, for example, is the proposal (Rushton, 1958*a*, *b*) that deuteranopes have in every cone normally sensitive to the long and medium wave-lengths a fixed mixture of chlorolabe and erythrolabe.

Although the arguments – and much of the evidence – in support of 'reduction' for deuteranopia and protanopia are quite symmetrical, the credence with which it is maintained as a satisfactory explanation of protanopia is a good deal stronger than is the case for deuteranopia (Pitt, 1944; Willmer, 1949; Rushton, 1958*a*, *b*; Le Grand, 1956; Wyszecki & Stiles, 1967). The matter would appear settled by the direct densitometer demonstration by Rushton (1965*a*) that in a deuteranope the difference spectrum after a 'red' bleach was identical to that after a 'blue-green' as in normals it certainly was not. However clear this result, universal agreement has not followed with it. Serious students (Hurvich, 1972; Le Grand, 1968, 1969, 1970, 1972) retain fusion as an alternative explanation of deuteranopia even in the face of Mitchell & Rushton's (1971) experimental equation of the action spectrum of a 50% bleach of the visual pigment in the fovea of a deuteranope with that of his subjective luminosity.

Doubts raised with regard to this work are of two kinds: the first questions its generality, the second its sensitivity.

(i) *Generality.* The rather large individual differences in the colour vision of different deuteranopes (Hecht & Shlaer, 1936; Farnsworth, 1961; Wald, 1966; Judd, 1966; Nimeroff, 1970) suggest the possibility that there are two different kinds, a 'reduction' form and a 'fusion' form (Willmer, 1949). While it is possible to show, as Alpern, Mindell & Torii (1968) have, that a given deuteranope fulfilling all of Willmer's criteria of 'fusion deuteranopia' has only one foveal cone visual pigment in the red-green spectral range, this does not in itself exclude the possibility that other deuteranopes have two. In a personal communication to one of

us (M.A.), Le Grand has recently again proposed this duplex explanation in order to maintain the viability of the 'fusion' hypothesis in the face of Rushton's (1965*a*) densitometer evidence to the contrary.

(ii) *Sensitivity.* Hurvich remains sceptical of densitometric evidence about 'reduction' as applied to deuteranopia on quite different grounds. He cites Rushton's (1965*d*) reference to (unpublished) experiments which failed to distinguish deuteranomalous trichromats from deuteranopes with the retinal densitometer. It is argued that since deuteranomalous must have three cone visual pigments the densitometer proof that deuteranopes have lost one is '. . . seriously flawed' (Hurvich, 1972).

Weale (1968) points out that the problem '. . . seems to demand a repetition of this excellent and important [i.e. densitometer] work, with a larger number of observers, a test field more nearly commensurate with the extent of the rod free area of the retina as pictured at the present time, and consideration of preretinal filters . . .'.

This is the first of three papers on deutan colour vision defects. The subsequent papers strongly depend on the proposition that the deuteranopes under study lack a foveal cone pigment in the red-green part of the spectrum. Hence, this one addresses the issues raised by those unconvinced by the densitometer demonstration of Rushton (1965*a*) that deuteranopes have only a single foveal red-green cone pigment. It evaluates the generality of Rushton's result by measuring difference spectra after red and green partial bleaches on fifteen deuteranopes, and by comparing and contrasting the results with those obtained by identical procedures, on the foveas of an equal number of normal trichromats. It studies sensitivity of the densitometer in resolving two visual pigments in the red-green part of the spectrum by similar measurements on nine deuteranomalous trichromats. Before either of these could be done, however, the colour defective subjects had to be screened, the diagnosis established unequivocally, and a representative sample of widely different deuteranopes selected for further study. The methods of doing this and the results obtained by using these procedures are described in the first part of this paper. The second part is devoted to the densitometer analysis.

PART I. SELECTION OF DICHROMATS

METHODS

Advertisements for colour defectives in the student newspaper yielded a supply of subjects who were further evaluated by bichromatic colour matching of spectral lights. Most tests do not draw a sharp distinction between dichromacy and extreme anomalous trichromacy. The present purpose required a very conservative

dichromatic diagnosis; evidence of trichromacy in any one of the following tests was sufficient to exclude the subject from further consideration as a dichromat.

(i) *Nagel (1907) anomaloscope (model 1).* Normal subjects vary the proportion (α) of red (645 nm) and green (535 nm) primaries to match a monochromatic yellow (589 nm) light of intensity β. That an exact match can invariably be made with these two knobs means that normal trichromats are dichromatic in this part of the spectrum. Not surprisingly red-green dichromats are monochromatic there; they match the yellow to either the red or the green primary, or to any mixture of the two, merely by adjusting β, the intensity of the yellow. But the converse is not so. A monochromatic match with the three wave-lengths studied here does not insure red-green dichromacy, despite numerous authorities (Committee on Colorimetry of the Optical Society of America, 1953; Linksz, 1964; Kalmus, 1965; Cameron, 1967; Cruz-Coke, 1970; Jaeger, 1972; Krill, 1972; Birch, 1974; among others). Birch (1974) in fact regards the ability of the anomaloscope to differentiate dichromats from anomalous trichromats as its major *raison d'être*. That the anomaloscope cannot properly do this is seen in Table 1. In this Table the correct diagnosis of the defects of eighty-three subjects from the present survey diagnosed as dichromats by the anomaloscope are summarized.

TABLE 1. Diagnosis of red-green defectives classified as dichromats by the anomaloscope

	Protan	Deutan	Total
Monochromats	0	1	1
Dichromats	32	38	70
Trichromats	7	5	12
Total	39	44	83

Evidently more than 15% of the sample were erroneously classified. For trichromats, the difficulty appears to be that though two cone visual pigments in the same retina may, at the three wave-lengths of the anomaloscope primaries, have identical absorbances (when suitably scaled) elsewhere in the spectrum that will not be so. To exclude such subjects two dichromatic matches were made in the region of the spectrum for which hue discrimination of red-green colour abnormal subjects is especially good.

(ii) *A dichromatic match with a long (535 nm), and a short (460 nm) wave primary (the choice is arbitrary) and a spectral light somewhere between (about 500 nm).* Many trichromats behave as dichromats on this test but most of these (Nelson, 1938; McKeon & Wright, 1940) are diagonsed correctly by the anomaloscope. On the other hand, all but two of the twelve trichromats classified as dichromats by the anomaloscope could not make this dichromatic match. Those two were correctly diagnosed with the third test, selected to exclude the possibility that the choice of the spectral wave-length of the match was fortuitous.

(iii) *Neutral point.* Subjects adjusted the wave-length settings of a Hilger–Watts constant deviation prism monochromator to find one wave-length which could be precisely matched to C.I.E. Illuminant C by adjusting its intensity alone. Of the twelve trichromats diagnosed as dichromats by the anomaloscope, ten were properly classified by this procedure. Two improperly classified by both, were unable to make the dichromatic matches described in (ii).

Trichromats diagnosed as dichromats by one of these tests merit further study. The constraints imposed by their color matches suggest interesting properties of the three cone visual pigments upon which trichromacy must depend; but no analysis of these properties has yet been made.

It is not generally appreciated that the diagnosis of dichromacy by any one of these tests is not sufficient to guarantee that diagnosis by every other. However, carefully measuring colour matching functions at 5 nm wave-length intervals in the spectrum on more than twenty dichromats demonstrates that a person diagnosed as a dichromat by all three of these tests will behave as dichromats under every other foveal testing condition.

RESULTS

Seventy red-green dichromats were selected by this procedure. The next step was to establish the diagnosis and obtain a quantitative estimate of the spectral sensitivity of each in the red-green part of the spectrum. Their anomaloscope matches do this. In anomaloscope screening, each subject was required to adjust (β) the intensity of the yellow primary to match different proportions (α) in the mixture of the red and green primaries extending over the entire range.

In Fig. 1 the settings of two dichromats are plotted as open and filled circles respectively; each set of results was obtained in a single experimental session of about 10 min duration. The Figure shows the plot of β as a function of α. Alpern (1974) has shown that results plotted in this way fall on a straight line and that the ratio of the slope to the intercept of this line can be used to calculate the ratio of spectral sensitivities (at the cornea) at the wave-length of the red and green primaries. The straight lines drawn through the two sets of results in this Figure were calculated by minimizing the squares of the deviations; the high coefficient of correlation (+0·965 and −0·985 in the illustrated cases) almost invariably found is good evidence that such lines describe these matches with precision.

From the straight line in this figure the ratio of the spectral sensitivities at the anomaloscope primaries (645 nm and 535 nm) of the dichromat whose matches are given by the open circles was calculated to be 0·16; for the filled circles it was 0·0138. The histogram in Fig. 2 summarizes the results of measurements of the ratio of sensitivities obtained in this way from all seventy dichromats. Note that the logarithms of the ratios have been plotted. The distribution is clearly bimodal; evidently the action spectra underlying dichromatic anomaloscope matches fall into one of two distinct groups, those from one group peaking at a good deal longer wave-length than those from the other. The two sets of results in Fig. 1 represent individual matches by one member of each of these groups; the open circles, from one of the former, operationally defined as deuteranopes, the filled circles, from one of the latter, called protanopes.

In Fig. 1 the two straight lines intersect within a rectangle outlining the range of the acceptable match of one normal trichromat. If this relationship held not only for the anomaloscope yellow (i.e. 589 nm) but for every

other wave-length as well, it would mean that this protanope and this deuteranope accepted the trichromat's matches. That is the expected behaviour according to the reduction hypothesis of dichromacy (i.e. the proposal that a single foveal cone pigment underlies the matches of each dichromat in this part of the spectrum, the deuteranope having only the long wave sensitive, and the protanope only the middle wave sensitive, cone visual pigment of this particular normal trichromat).

The two clusters in the bimodal distribution in Fig. 2 are separated by a

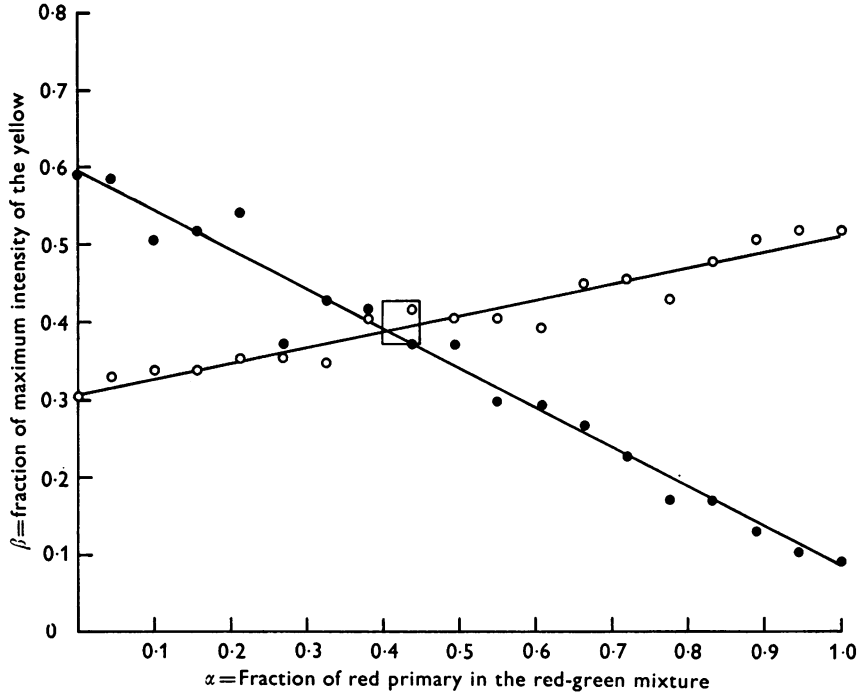

Fig. 1. Anomaloscope plot of the settings of two red-green dichromats matching (β) the intensity of monochromatic yellow (plotted linearly on the ordinate) as a function of various proportions (α) of the mixture of the red and green primaries plotted linearly on the abscissa (1 being all red, 0 being all green). Filled circles show the settings of one protanope; the least-squares line drawn through them has the equation $\beta = 0{\cdot}594 - 0{\cdot}509\alpha$; the Pierson product moment correlation is $r = -0{\cdot}985$ and the ratio of sensitivity of the red to the green primary calculated from this result is 0·0138 (the calculation of this ratio from the constants of the straight line has been described elsewhere (Alpern, 1974). The open circles show the settings of one deuteranope; the least-squares line drawn through them has the equation $\beta = 0{\cdot}306 + 0{\cdot}204\alpha$; the correlation coefficient of these measurements is $r = +0{\cdot}965$ and the calculated ratio of the sensitivities of the anomaloscope primaries is 0·16. The rectangle near the intersection of the two lines shows the range of acceptable anomaloscope matches of one normal trichromat.

gap of 0·75 $\log_{10}$ units. There is no evidence in this population for dichromats intermediate between protanopes and deuteranopes, a possibility suggested by de Vries (1948). He found a dichromat who rejected normal trichromatic matches but accepted all matches of one deuteranomalous and all but one of a single protanomalous. De Vries proposed that this dichromat was one whose matches in the red-green spectral range depended on a single cone visual pigment which was neither the normal long wave, nor the normal middle wave, pigment. Rather this dichromat's matches

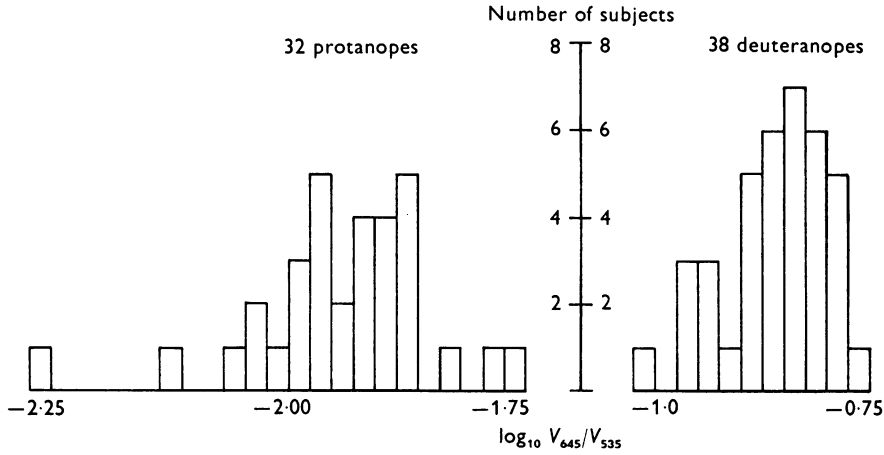

Fig. 2. Histogram of the logarithm of the ratio of the spectral sensitivities at the anomaloscope primaries (645 nm and 535 nm) of all seventy red-green dichromats of this study. To condense the entire distribution to a single Figure, the hiatus in the abscissa between the two clusters has been drawn to a scale reduced by 0·22 that of the rest of the Figure.

depended upon the anomalous (i.e. the middle wave sensitive cone) pigment of the deuteranomalous which, according to the theory of Schouten (1937) was also the long wave sensitive cone pigment of the protanomalous. The failure, however, to discover observers of this kind in a sample of only seventy dichromats is insufficient to exclude their existence with any statistical confidence.

Within each cluster of Fig. 2 there is a fair amount of individual difference. The spread of the protan cluster is wider than that of the deutan cluster. This does not necessarily imply that the variability of the action spectrum among different protanopes is greater than it is among different deuteranopes, since the green reference primary of the anomaloscope (535 nm) is very near to the λ_{max} of most protanopes luminosity curve (Alpern & Torii, 1968*a*). On the other hand, for deuteranopes,

(Alpern & Torii, 1968*b*), 535 nm is on the short wave side of the λ_{max} of the foveal spectral sensitivity function and 645 nm is on its long wave side.

TABLE 2. Precision of deuteranope anomaloscope matches V(645)/V(535)

Deuteranope	N	$\bar{X}$	± 1 S.E. of mean
1	4	0·173	± 0·010
3	5	0·163	± 0·002
6	23	0·160	± 0·006
8	10	0·157	± 0·005
9	6	0·157	± 0·005
11	30	0·154	± 0·003
12	28	0·151	± 0·003
18	4	0·143	± 0·002
19	26	0·142	± 0·002
23	5	0·135	± 0·009
27	8	0·132	± 0·002
30	5	0·126	± 0·003
32	12	0·117	± 0·002
35	14	0·111	± 0·003
36	8	0·111	± 0·005
38	17	0·100	± 0·001

The typically high correlation coefficients shown in Fig. 1 are good evidence for the accuracy with which the anomaloscope can be used to estimate the ratio of sensitivities at the wave-lengths of its primaries, but how repeatable are such measurements?

Table 2 shows the average ratio of red to green sensitivities ± 1 S.E. of mean for those (sixteen) deuteranopes from whom at least four anomaloscope repetitions were obtained. The first column identifies the deuteranope in terms of his position in the distribution (one being farthest from, and thirty-eight closest to, the protanopic distribution); the second column lists the number of experimental repetitions; the third column, the average ratio; and the final column, the standard error of the means. Evidently, the variability illustrated among the deuteranopes in Fig. 2 cannot be attributed to the variability of individual settings alone but must be interpreted as genuine differences among different deuteranopes. For example, deuteranope 32 is clearly different from his slightly younger brother (deuteranope 38).

These results confirm that there is a fair amount of variability among different deuteranopes. But they shed no light on whether the deuteranopic matches depend upon the absorption of light in a single cone pigment or in two, or indeed on the possibility that in *some* deuteranopes it depends upon the absorption of light in one cone pigment while in *others* it depends upon absorption in two.

This is the matter disposed of in the next part of this paper.

PART II. DENSITOMETER ANALYSIS OF DEUTAN RED-GREEN CONE PIGMENTS

APPARATUS AND METHODS

The Florida retinal densitometer of Hood & Rushton (1971) was modified in a way already described (Alpern, Maaseidvaag & Ohba, 1971). Sixteen deuteranopes were studied. In all but one, difference spectra following one full and two partial bleaches were measured. The partial bleaches were obtained with a red (650 nm) light which normally bleached about half the measurable erythrolabe in the eyes of deuteranopes (and no measurable chlorolabe in the eyes of protanopes). The second partial bleaching was by a 515 nm light which bleached for some deuteranopes exactly the same amount of erythrolabe at equilibrium as the 650 nm light (for others, it was slightly more, or slightly less, effective).

If, in the initial experiment, it was found that the usual equation of red and green bleaching lights was appreciably in error for a given deuteranope a psychophysical experiment was used to make it more exact. The two bleaching lights were used in turn as a background and the energy of each required to raise the threshold of a 1° circular test in the densitometer measuring beam by a factor of 10 over its dark value was determined. For the test the tungsten light source was attenuated by a red Wratten gelatin (no. 70 dominant wave-length λ = 678 nm) and flashed for 50 msec. A constant stimulus method was used to measure threshold; a typical single determination required five judgements for each of at least five different test intensities to establish the psychometric curve. The subsequent measurements of bleaching with the intensities of the two lights equated in this way confirmed the equation within the precision of the measurement.

The transmissivity T_D of a calibrated wedge in the red measuring beam was used to balance the fundus reflexion at each of eight wave-lengths (500, 520, 545, 565, 585, 605, 614·5 and 638 nm) after full dark adaptation. The average of five settings at each wave-length was usually obtained. The process was repeated obtaining T_R after bleaching to equilibrium with a deep red (650 nm) light. In the usual case its intensity was 14·05 log quanta sec^{-1} cm^{-2} (of retina, at the cornea). The subject was dark adapted once more and then the process was repeated bleaching to equilibrium with a green (515 nm) light (usually of intensity 13·6 log quanta cm^{-2} sec^{-1}) to measure the values of T_G at the eight wave-lengths. The process was next repeated with a long full bleach (about 6·2 log photopic td of white light) to find T_o. Finally recovery was followed (at one wave-length) as a function of time in the dark after the full bleaching light was extinguished.

With this information, one calculates first $(T_R - T_D)/T_o$ the difference spectrum caused by the red bleach, then $T_G - T_R)/T_o$, the amount of further bleaching (if any) by the green light whose intensity was matched to the red by the action spectrum of the deuteranope's fovea in the red-green part of the spectrum, and finally the difference spectrum after a full bleach, $(T_o - T_D)/T_o$.

The circular measuring field was 2° in diameter. A field stop further reduced (concentrically) the foveal area of the measuring field sampled by the photomultiplier tube by an amount somewhere between $\frac{1}{2}$ and $\frac{2}{3}$ of the illuminated area of retina. (The concentric bleaching field was 10°). Subjects were especially cautioned to maintain good fixation during the 7 sec required for a wedge adjustment and settings made while they were unable to do so were discarded. None of the measurements reveal evidence of contamination by rhodopsin.

Because of the individual differences, a limited study of the variability of erythrolabe kinetics was undertaken in deuteranopes. This was done in the manner of

Rushton (1958, 1965*b*), bleaching first with a 10 sec flash followed immediately by a dimmer light, estimated to bleach about the same amount at equilibrium as the flash. If the estimation was accurate, the measurements, as a function of time after the onset of the dimmer light, hovered within the experimental error about the constant level; if inaccurate, they slowly increased or decreased depending upon whether the estimation was too small, or too large. In any event, a study of the measurements as a function of time following the onset of the dimmer light gave (i) the amount bleached at equilibrium by a light of known wave-length (562 nm) and quantum content and (ii) the amount bleached by the 10 sec flash. Since the results are well described by first-order kinetics, it is possible from these data to determine (Q_e) the photosensitivity of the pigment and the constant I_o in the steady-state equation $p = I_o/(I+I_o)$ (the details are described elsewhere (Rushton, 1958, 1963, 1965*b*; Rushton & Henry, 1968; Alpern *et al.* 1971)).

Regeneration in the dark after long full bleaches followed the equation $p = 1-\exp(-t/t_0)$, in keeping with first order kinetics; the regeneration can thus be described by a single constant t_0. Since p is the fraction of pigment present, the results, when plotted as $(1-p)$ on a logarithmic ordinate scale as a function of time in the dark (plotted linearly) fall on a straight line passing through $(1-p) = 1$ at $t = 0$. The slope of this line varied with t_o, which could be read directly from the point on the line at which $(1-p) = e^{-1}$.

RESULTS

A. Difference spectra of normal trichromats and dichromats. The difference spectra results are clear and unequivocal. In none of the fifteen deuteranopes, and in every one (of about the same number) of normal trichromats studied so far, are there two foveal photolabile pigments measurable in the red-green part of the spectrum. This is most readily seen in Fig. 3 which compares and contrasts difference spectra obtained on deuteranopes and normals under identical conditions.

This Figure contains twenty-one graphs showing the characteristic results from fourteen deuteranopes (left hand and middle columns) and seven normal trichromats (right hand column). Each graph displays the average results of a single subject. The deuteranope's position in the distribution of Fig. 2 is indicated by a number on his (or her) graph (one being the subject furthest from, thirty-eight being the deuteranope closest to, the protanope distribution). Every plot in this figure contains three difference spectra. The circles indicate the value $(T_R-T_D)/T_o$ the results of bleaching with the deep red light, the squares show $(T_G-T_R)/T_o$ the effect of further bleaching by the green (equated as closely as possible to the red for the deuteranopes action spectrum) and the triangles the difference spectrum after a full bleach $(T_o-T_D)/T_o$.

The continuous curve has been drawn by eye to approximate best the trends shown by each set of triangles. For a given deuteranope the same curve appropriately scaled down has been drawn through the filled circles. The amount of scaling indicates the fraction of total erythrolabe

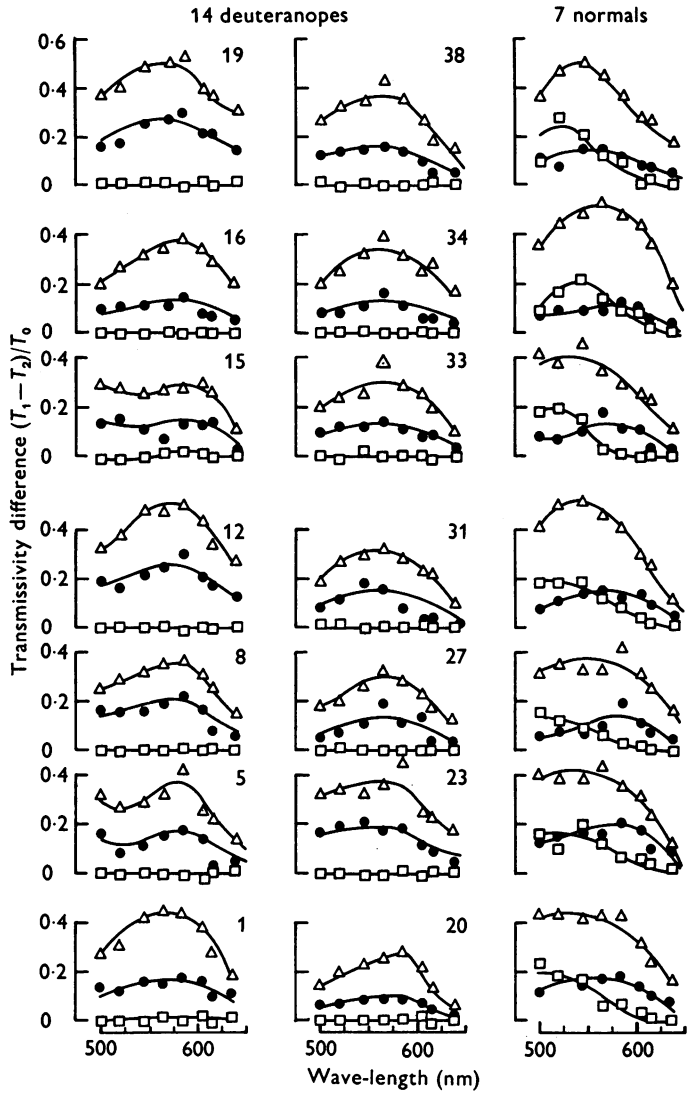

Fig. 3. Transmissivity difference spectra on fourteen deuteranopes (left hand and middle columns) and seven normal trichromats (right hand column). Each graph shows three transmissivity differences plotted as a function of wave-length. The circles show $(T_R-T_D)/T_o$, the squares $(T_G-T_R)/T_o$ and the triangles $(T_o-T_D)/T_o$. The number on each deuteranope's graph designates his position in the distribution of Fig. 2. The curve through the triangles is an eye fit continuous curve drawn to illustrate the trends; that through a given deuteranopes' filled circles is the curve through the same subject's triangles appropriately scaled down.

bleached at equilibrium by the red light. Not only does the scaled curve reasonably describe the bleaching with red, but the green equated to it produces no further bleaching. There is no evidence of a second photolabile pigment in this part of the spectrum although the same experiment reveals substantial concentration of another one (chlorolabe) in the fovea of every normal trichromat.

There is a certain amount of individual differences in the λ_{max} of deuteranopic difference spectra which only approximately correlates with the shape of the action spectra. This may be seen both in Fig. 3 and in Table 3 which summarizes the difference spectra results as well as the measurements of the kinetics on all deuteranopes studied in this laboratory.

The first column in this Table gives the anomaloscope rank, the second the ratio of sensitivities at the wave-lengths of the anomaloscope primaries. The next column gives the λ_{max} of the difference spectrum. There is a general tendency for deuteranopes with increased sensitivity in the red to have a difference spectrum which peaks at longer wave-lengths in agreement with expectation, but the correlation is by no means perfect. The deviations from expectation are most likely due to individual differences in the fraction of the densitometer measuring light which has not passed twice through the pigment (Rushton, 1965*c*).

TABLE 3. Foveal retinal densitometry on sixteen deuteranopes

Anomaloscope		Difference spectrum			Regeneration		Bleaching	
Rank	$\frac{r_{645}}{r_{535}}$	λ_{max}	n	$(T_o - T_D)/T_o$ at λ_{max}	n	t_o	log Q_e	log I_o
1	0·173	565	2	0·458	7	117 ± 12·2	14·92 ± 0·2	12·74 ± 0·13
5	0·162	585	2	0·419	4	121	14·7	12·6
6	0·160	605	1	0·719	3	132	14·41	12·21
8	0·157	585	14	0·398 ± 0·022	14	111 ± 8·1	14·66	12·57
12	0·151	585	5	0·498 ± 0·053	7	146 ± 8·4	—	—
15	0·147	605	2	0·296	2	88	—	—
16	0·146	585	5	0·388 ± 0·039	5	84 ± 10·9	—	—
19	0·142	585	6	0·520 ± 0·026	10	123 ± 5·9	14·69 ± 0·03	12·70 ± 0·06
20	0·141	585	2	0·276	—	—	—	—
23	0·135	585	3	0·454	5	126 ± 4·5	14·78	12·71
27	0·132	565	1	0·333	7	113 ± 6·8	—	—
31	0·120	565	4	0·324	5	103 ± 5·8	—	—
32	0·117	—	—	0·454	4	116	15·05	13·11
33	0·117	565	2	0·386	2	109	—	—
34	0·114	565	5	0·397 ± 0·019	9	108 ± 5·8	15·76	13·59 ± 0·04
38	0·100	565	3	0·418	9	127 ± 4·8	14·80 ± 0·06	12·76 ± 0·08

λ_{max} = wave-length of maximum measurement.
Q_e = photosensitivity at 562 nm in $h\nu.cm^{-2}$.
I_o = intensity (in $h\nu$ (562 nm) sec^{-1} cm^{-2}) which bleaches 50 % at equilibrium.
t_o = time constant of regeneration in sec means ± 1 S.E. of means.

B. Differences in erythrolabe kinetics among deuteranopes. Individual differences in the measured regeneration time constant (t_o), photosensitivity (Q_e) and I_o (the number of quanta (562 nm) sec^{-1} cm^{-2} of retina incident at the cornea required to bleach half the pigment at

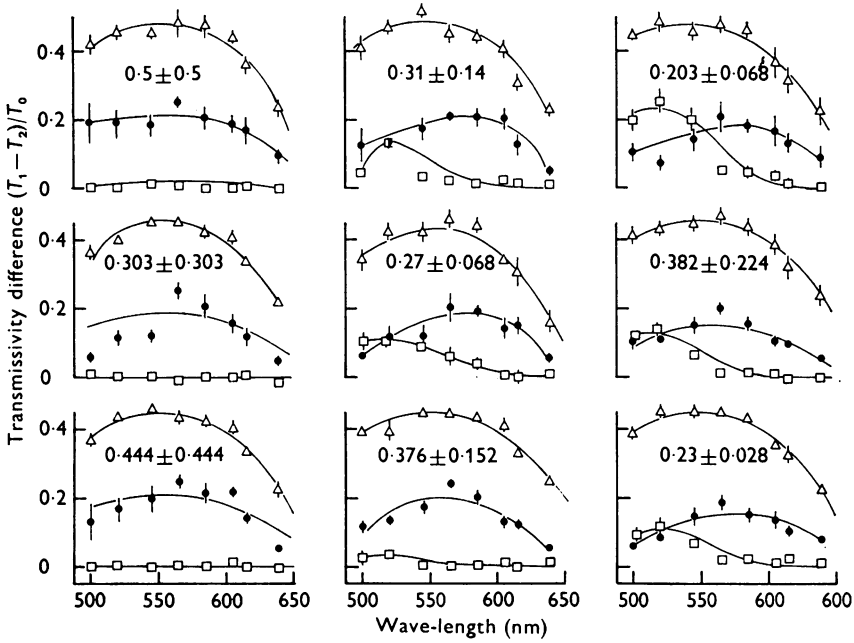

Fig. 4. Transmissivity difference spectra on nine deuteranomalous. The plotted values are the means ± 1 S.E. of mean of the results of at least five (in all but two cases, six) experimental repetitions. The symbols, and the graphs are identical to those shown in Fig. 3. Each graph represents a different deuteranomalous. The number indicates the midpoint and range of acceptable proportions of the red primary in the red-green mixture matched to a yellow in the Nagel anomaloscope. Normal trichromats match within the region 0·5 ± 0·1 with a normal acceptable range for any given observer equal to or less than 0·05. The left hand column are all extreme deuteranomalous; the others are simple deuteranomalous. The curve through the triangles is an eye fit continuous curve drawn to illustrate best the trends. That through the filled circles for the three extreme anomalous is the same curve appropriately scaled down.

equilibrium) are all greater than the precision of the measurement. They indicate clear kinetic differences in the erythrolabes of different deuteranopes although there is no obvious relation between these variables and the ratio of red to green sensitivity as measured by the anomaloscope.

Individual differences among the deuteranopes in the value of $(T_o - T_D)/T_o$ at the λ_{max} are also evident in Fig. 3 and Table 3. Such differences

are important in evaluating why deuteranopes' foveal action spectra vary in the red-green part of the spectrum but discussion of this problem will be deferred until the following paper (Alpern & Pugh, 1977).

C. Difference spectra in deuteranomalous trichromacy. The results of similar measurements on nine deuteranomalous are summarized by the difference spectra in Fig. 4 in which the graphs as well as the symbols on them have the same meaning as in Fig. 3. Each graph illustrates the means ± 1 S.E. of mean of six (or in two cases, five) measurements on a different subject. Also shown on the graph is the subject's setting of the red-green mixture (midpoint ± the range of acceptable matches) in the Nagel anomaloscope match to a fixed monochromatic yellow (normal trichromats always match in the range 0·4–0·6 with a precision of 0·05 or less, in this instrument).

The results in the left hand column of graphs on Fig. 4 establish that difference spectra measured on the foveas of extreme deuteranomalous trichromats (i.e. those who can match the green anomaloscope primary to the yellow) resemble similar measurements on the deuteranope fovea. This confirms Rushton's description of the results of his unpublished experiments on extreme anomalous (Rushton, 1965*d*). But it is misleading to interpret this as a serious limitation on the ability of densitometry to detect two foveal pigments in the red-green part of the spectrum. That is evidenced by the fact that these extreme anomalous are quite different in this respect than most deuteranomalous, whose difference spectra fall somewhere between those of deuteranopes, and those of normals. The remaining graphs in Fig. 4 verify that all six of the simple deuteranomalous (i.e. anomalous who never see the green and yellow anomaloscope primaries as metameric) show measurable (if sometimes small) quantities of two foveal red-green photolabile pigments. (There is, in fact, a subtle suggestion of two pigments in the extreme anomalous with the narrowest matching range; his difference spectrum after a red bleach is much less adequately described by the scaled down version of the curve fit to his full bleach spectrum than is the case in deuteranopia.)

DISCUSSION

It is difficult to interpret the results in Fig. 4 as demonstrating a 'serious flaw' (Hurvich, 1972) in the densitometric proof that deuteranopes have only one foveal red-green pigment. What is quite clear from the present experiments is that all normal, and most deuteranomalous, trichromats have two measurable foveal red-green photolabile pigments and all deuteranopes studied so far have but one. That the densitometer spectral resolution with eight interference filters is too coarse to detect

what must be very subtle differences between some extreme deuteranomalous and deuteranopes, is perhaps not surpising once it is recognized that not even the anomaloscope, by far the most sensitive single test, can always do that (see for example the results in Table 1). Expecting a degree of discrimination with this somewhat crude objective measurement equal to that achieved with a combination of three very precise subjective tests asks too much of the present state of the art of foveal reflexion densitometry.

To maintain Hurvich's scepticism in the face of the results in Fig. 4 one is pressed to the position that if deuteranopes do in fact have two foveal red-green pigments, one of them is by no means 'normal'. It can neither be detected in reflexion densitometry (as all normal and most deuteranomalous red-green cone pigments clearly can be), nor does it make any contribution to colour matching (in the way that the densitometrically undetectable extreme anomalous cone pigment clearly does). But if it does neither of these things is there any serious reason for continuing to believe in its existence? The importance of these results on extreme anomalous is not the 'serious flaw' in the proof of the loss of a foveal pigment in deuteranopia, but in the hint it gives us about the foveal pigments in extreme deuteranomalous trichromats. The clear suggestion is that the absorption characteristics of two of their visual pigments are so nearly the same in the red-green part of the spectrum that the densitometer is unable to separate them as it can in the foveas of most other deuteranomalous, and all normal, trichromats. This is a point to which we will return in the final paper in this series (Alpern & Moeller, 1977).

The results in Fig. 2 confirm previous reports of large individual differences in color vision among different deuteranopes (Hecht & Shlaer, 1936; Willmer, 1949; Farnsworth, 1961; Wald, 1966; Judd, 1966; Nimeroff, 1970). In the following paper, Alpern & Pugh (1977), the analysis of this variation (including consideration of preretinal filters, referred to in the quotation from Weale, 1968) begins with the assumption that all these deuteranopes are missing a cone visual pigment sensitive to medium wave-length light (chlorolabe) always found in the normal fovea. That analysis would collapse if some (or any one) of the deuteranopes had normal concentrations of two foveal photolabile pigments in the red-green part of the spectrum such as predicted by one form of the 'fusion' explanation of deuteranopia. The results in Fig. 3 show that for none of the deuteranopes in this study is that the case. Though they cannot exclude the possibility that some dichromat, somewhere, may have all three normal foveal cone visual pigments in normal concentrations, the fact remains that every densitometer attempt (Rushton, 1965*a*; Mitchell &

Rushton, 1971; and the present study) as well as those with the Stiles (1939) foveal two colour increment thresholds (Rushton, 1958; Boynton & Wagner, 1961; Speelman & Krauskopf, 1963; Wald, 1966; Alpern & Torii, 1968*b*; Alpern *et al.* 1968; Piantanida & Sperling, 1973, among others) have so far failed to uncover evidence for them.

Although the reasons for the variability shown in Fig. 2 are discussed in the following paper one possibility relates to the results in Fig. 3 and merits brief consideration. It is the suggestion that the variability is due to different mixtures of a modest amount of chlorolabe with one fixed erythrolabe in different deuteranope long wave sensitive foveal cones. This proposal is analogous to one suggested (and rejected) by Bowmaker, Loew & Liebman (1975) to account for variability in the λ_{max} of rhodopsin in the rods of different frog retinas (i.e. they propose a mixture of porphyropsin and rhodopsin). If a very small and variable amount of chlorolabe mixed with erythrolabe in the outer segments of foveal cones of different deuteranopes is the reason why the action spectra of the long wave cones of different deuteranopes differ, one expects a progressive build up of a green sensitive difference spectrum $(T_G - T_R)/T_o$ as one moves successively from the bottom left plot to the top plot in the middle column of Fig. 3. No such phenomenon is to be found in these results. If such an effect occurs it is much too small to be detected with the densitometer.

Since differences of exactly this kind are, in fact, observed among different anomalous trichromats (Fig. 4), the technique is quite capable of making more subtle distinctions in the variability in concentration of a second ('green') cone pigment than are to be found among the results in Fig. 3. The fact that it does not do so makes this hypothesis quite unlikely, if still not impossible.

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