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The Effect of Age upon Colour Matching and Colour Discrimination

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LBSTRACT.

A short account is given of previous work, both on age changes in colour vision response and on some related physical and chemical age changes in the visual system. The aims of the present investigation are then discussed.

Statistical information on the effect of age upon a number of colorimetric functions is recorded and discussed in Chapters III to VI inclusive. The functions are: wavelength discrimination; colour matching of a 'white light' test field and of a spectral test field; the relative luminosities of the matching stimuli of the W.D.W chromaticity co-ordinate system; and the relative luminous efficiency of selected wavelengths in the spectrum. Some four hundred observers with normal colour vision took part in these investigations.

The variation of the functions with age has been investigated statistically and the conclusion reached that age changes which occur are due to the variation in transmission of the optical media of the eye. Known and postulated variations in the media have been applied, by calculation, to the present data. As a result of these calculations, it appears that the lens is the most probable cause of the age changes in transmission of light through the eye to the retina.

Light scatter is suggested as the physical cause of the transmission changes with age, since the wavelength dependence of the changes closely follows Rayleigh's scattering law.

Some measurements of light scattering in the human eye, as a

function of wavelength, have been made. These measurements tend to confirm that an increase in Rayleigh scattering, located in the lens, occurs with increasing age.

Results obtained by Verriest and by Birch have been examined in the light of the present work, and are found to accord satisfactorily with the conclusions drawn in the thesis.

Data on the spectral absorption curve of the macular pigment is given in Chapter IX and this is shown to differ somewhat from previous data.

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Chapter I

<u>Previous Work on Changes in Colour Vision Response with Age</u> and Plan of the Present Investigation.

A number of previous investigations of the change in colour vision response with age have been made, but they have usually had fairly limited objectives. The techniques used have been of two olasses; either a survey has been made to investigate the visual response as a whole, or the study has been directed at the age changes in some particular element of the visual system. The present work is primarily of the former class, although the nature of the measurements has allowed some analytical investigation into the causes of age changes.

Colour Vision and Age Surveys.

A number of surveys have been carried out, usually with some type of colour screening chart and with the aims of detecting changes in colour discrimination with age. Chapanis (1950) found a slightly significant relationship between age and colour vision, but this was due solely to the poor performance of individuals under 15 years of age. 547 observers took part in the survey, but they were not pre-screened for colour defectiveness. As a result, histograms recording errors in reading the plates were bi-modal in form. The data were obtained for six sets of colour testing charts. Chapanis also investigated visual acuity as a function of age and found a curvilinear relationship between them. Gilbert (1957) used 355 subjects in an investigation of the changes in colour discrimination with age. 48 coloured discs provided a disorimination test, but as they were not always viewed in the standard illumination (daylight) the data were somewhat unreliable. Differences between different age groups in the number of mistakes made in arranging the discs were significant at .01 level, the older groups making a greater number of errors. The results were also analyzed with respect to sex difference, and women were found to show better disorimination than men, significant at .05 level. As the observers were not pre-selected, this latter result is presumably due to the greater number of congenitally defective men which would be tested. The author reported that at all ages, blue and green were less well discriminated than yellow and red, which suggests that the discs used to measure discrimination were not equally spaced in terms of chromaticity steps.

Lakowski (1958) carried out a survey using a Pickford anomaloscope, and suggested increasing macular pigment thickness as the cause of changes with age which he observed. In a further paper (1961) he reports anomaloscope findings for some 900 observers, with an age range 5 years to 95 years, as well as a group of aphakic observers. Three pairs of stimuli provided the test fields for matching, these being violet and blue-green, yellow and blue and red and green. The anomaloscope settings showed a variation with age, both in terms of the mean values and the standard deviation. This was more marked in the first two of the pairs enumerated above. Lakowski interpreted his data in terms of a change in wavelength discrimination and showed that it could be simulated in young observers, by placing filters before the eye. These filters were yellow and of approximately similar spectral distribution of optical density to that involved in lens ageing, as measured by Said & Weale (1959). The filters required were of greater overall density than given by Said & Weale, however, and this was interpreted as an indication of an increase of macular pigment concentration with age.

The conclusions drawn from these results are open to some objections. The use of relatively broad-band filters with a continuous source means that wavelength discrimination is not being investigated, if discrimination is the correct description of measurements on this anomaloscope. The fact that filters change the anomaloscope settings indicate that changes in luminance are being measured. Further, Said and Weale's data are applicable only to a fully dilated eye pupil and must be corrected for exit pupil size in practice (Weale 1961b). As in this case, the natural eye: pupil was used at relatively low luminance level, the differences in lens transmission will be exaggerated due to variation in eye pupil size with age. The aphakic observers showed the lens to be at least partly responsible for the age changes obtained.

Perhaps the best controlled experimental survey was carried out by Verriest, Valdergine and Valderdonck (1962), and by Verriest (1963 who measured the change of hue discrimination with age. In this experiment, the Farnsworth-Munsell 100 hue test, Farnsworth (1943), was used, illuminated always by illuminant C. Some of the results have been illustrated in Fig. 1 and the reduction of blue-green

FIG.1



Error Scores of 100-hue test for different age groups. Results of Verriest.



NORMALIZED DISTRIBUTIONS OF ERROR SCORES OBTAINED WITH THE MUNSELL-FARNSWORTH 100-HUE TEST (DATA OF BIRCH). discrimination with age is noticeable. The 480 observers were not closely screened for anomalous colour vision before the experiment, and although obviously defective observers were omitted from the results, this does mean, as Verriest points out, that some mildly defective observers may have been used in the work . Verriest ouncluded that there was a loss of discrimination of the hues with age, significant at .01 level. This loss of discrimination he could partly simulate by the use of filters with absorption similar to that of the lens. However, he points out that other causes, such as senile macular degeneration, reduction of retinal illumination in the old eye to the mesopic range, and permanent selective retinal adaptation could be of importance.

These results, which will be discussed again later in the thesis, were substantially confirmed by the screening tests carried out at inperial College by Mrs. Birch, with the 100 hue test, as reported the Medical Research Council. The results are illustrated in Figure 2. It is important to note that the blue-green region is always more difficult to discriminate, even for young observers, and hence this suggests that the hue spacing of the 100 hue test is not even. This may be due to variable mounts of macular pigmentation amongst observers, which would effectively alter the energy distribution of light reflected from the broad-band pigments used in this test. The increase in confusion of the hues, as age increases, could then be due to increase in macular density, but any other absorption of blue-green wavelengths could have a similar effect.

Kelly (1958) used the metamerism of a pair of Granville Greys

(one grey being of simple linear reflection characteristics throughout the spectrum, the other having a series of peaks and troughs). Depending upon the source of illumination, the simple grey canappear either redder or greener than the complex one. Kelly showed that the balance point (expressed in terms of the inverse source colour temperature in μ rd), where the tiles appeared to be matched in chromaticity, was different for a 2° and 10° angular subtense.

It was, calculated from Stiles (1955) 2° colour mixture data and also for Stiles (1955) pilot 10° colour mixture data. 39 observers found the red-green balance point for the two grey tiles, with both 2° and 10° angular subtense for the pair of tiles. The colour temperature at which the balance occurred was found, and this was taken as a measure of the pigmentation of the observer. The results for the 39 observers are shown for the two field sizes, Fig. 3. As can be seen, a trend in the red-green balance point with age occurs. which is statistically significant at .01 level, for both field conditions. However, the 2° and 10° data yield two curves which are approximately parallel and differences between these two curves were found to have no significant correlation with age. From these results, Kelly deduced that macular pigmentation did not change with age. This conclusion is, perhaps, not of great significance, as the yellow macular pigment can vary greatly in density amongst observers of the same age. (Wright 1928-29).

Stiles and Burch (1959) have also provided data on the possible light losses in the lens and the macular pigmentation. This was deduced from consideration of colour matching data which they had

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obtained for a group of 49 observers. Variations in the colour mixture functions were correlated with lens absorption and with macular pigment absorption. Significant variations due to lens absorption were demonstrated, by considering differences in colour matching functions between two wavelengths where macular absorption is equal. No such evidence could be obtained in the case of macular pigment variation. As regards ageing, at any given wavelength, pre-receptoral light losses should affect all three colour mixture functions equally and this was found to be so, except for the blue stimulus, rod intrusion being suggested as the reason for this irregularity. As logs of the colour matching functions were plotted, a single pigment should produce a set of curves separated from each otherby a constant difference (Lambert and Beer's laws). From Fig. 4, the authors showed that this was not so, and suggested that both lens and macular absorption changes may have occurred with age. Said & Weale (1959) have since shown that the lens absorption is not of a single pigment form, which may explain Stiles and Burch's findings. Weale(1961b) has shown that the results of Stiles and Burch are in good agreement with the lens ageing data, except in the macular absorption region of the spectrum.

Warburton (1954) took a survey of 247 observers who were not pre-screened to eliminate colour defectives, but the results did not include one or two observers who deviated wildly from the average range. The test consisted of matching one of a set of dichroic patterns with a standard, slightly dichroic sample. The samples lay approximately along a straight line in a uniform chromaticity space, and illuminant 'B' was used for matching. Warburton showed that



change from illuminant S_A to S_B was equivalent to a change in coular pigmentation, and also that it lead to a different member of the dichroic series being matched to the standard. He thus interpreted different matches as being equivalent to different degrees of ocular pigmentation. An obvious shift in the average match occurred with age, and this was interpreted as equivalent to a change in pre-receptoral pigmentation.

Chemical & Physical Measurement of Age Changes.

A number of investigations have been made into the chemical and physical changes in the eye which are associated with ageing. Of these, a few have been reviewed, being relevant to the question of colour vision changes with age.

Transmission of the Human Crystalline Lens.

Said and Weale (1959) measured the change in optical density, with age, of the human crystalline lens, as a function of wavelength. The method consisted of photographic measurement of the intensities of the 'Purkinje Images' produced by reflection of light from the front and back surfaces of the lens in vivo. A dilated weye pupil was used to increase the amount of light reflected and a Xenon arc, with coloured filters, provided the high light intensities required for accurate measurement of the image intensity. The results show that, after the age of 20 years, transmission of the lens decreases steadily with age, this decrease being more marked at shorter wavelenths Fig. 5. Weale (private communication) has indicated to the author that, especially amongst older observers, irregularities



of the back surface of the lens make it impossible to obtain a clear reflection image, hence the observers were carefully chosen so as to avoid this. As a result, Said & Weale's data may not be fully representative. The results for the young lenses, howeyer, seem to agree fairly well with the data of Ludvigh & McCarthy (1938) for the transmission of the ocular media as a whole. This was measured by placing a small reflecting glass plate in contact with the vitreous body. The sclerotic, choroid and retina were removed from the foveal region used, and the reflection from the glass plate measured in a monochromator.

The data were obtained for observers of different ages but unfortunately were not published, except in a reduced form for young observers. The differences between the two sets of data could presumably be due to light losses in the cornea and the vitreous and aqueous humours, which would be included in thedata for the whole ocular media, Fig. 6. Le Grand (1948) states that the lens absorption is variable between people of the same age, although no evidence for this is given. Wald (1949) also mentions variations in lens density within a small age range, and also published an optical density curve for a 68 year old lens. This was compared to the lens absorption for a young observer, and with the density of an 8 year old rhesus monkey. The data for the young (20 year old) observer was deduced from measurements on aphakic and normal observer rod sensitivity, 8° from the foven, Fig. 7. Weale (1961b) has criticized the absorption curve for the 68 year old lens on the grounds that it may have become cloudy after extirpation.



- Ludvigh and McCarthys data for total refractive media
- Said and Weale's data for the lens
 (young eye).





In discussing the chemical and metabolic changes which occur in the human eye, Fischer (1948) states that the lens is the main source of change with age. The water content of the lens decreases with age, as first shown by Priestley-Smith, and soluble proteins decrease, whereas insoluble proteins are accumulated, a fact also pointed out by Weale (1960). Mazow (1960) states that in a body such as the lens, with a low rate of metabolism, changes with age would occur at a regular rate, and this is borne out by Said & Weale's results.

Van Heyningen (1962) states that, with increasing age, the rate of growth of the lens decreases, although this occurs virtually throughout the life history. This growth, which is in the form of new fibres, causes increasing compression in the central nucleus, as the older cells are displaced towards the centre. Woolf (1954) also mentions this, and points out that the lens fibres shrink with increasing age, and their refractive index increases. Their surfaces also become irregular. Woolf also states that the envelope of the lens becomes thicker with age, and acquires striations.

Weale (1961a) indicates that some increase with age in light scatter may occur, both in the cornea and the vitreous humour, the latter becoming 'yellowish' with age.

The effect of age upon the retina and its neural connections is virtually unknown, at least as regards colour response. Fischer (1948) states that the retina does not change with age, from the general viewpoint of its metabolism, although Mazow (1960) reports that the retina becomes thinner and optically denser with age. Weale (1961b) has calculated the change in absolute visual threshold due to increases in lens absorption with age and found

that the calculated changes fitted quite well to experimental results, including those of Luria (1960) and Robertson and Yudkin (1944-45). This is illustrated in Fig. 8. An experiment corroborating this conclusion is quoted by Chapanis (1950). Ferrara found that old aphakic observers had a lower absolute threshold for blue light than young normal observers. These results suggest that the rod response, at least, is unaffected by age.

A final measurement of some importance is that of Wright (1946) who, at the ages of 22 years and 38 years, measured the chromaticity of the white source, S_B , in the W.D.W. co-ordinate system. The results showed that a marked shift in chromaticity occurred, such as to indicate increase in pre-receptoral pigmentation. Due to the direction of shift in the chromaticity chart, Wright suggested that an increase in macular pigment density was the cause of the change in chromaticity. The result is shown in Fig. 9.

General Conclusions from Previous Work.

From the above resume of previous work it is clear that changes do occur in the response of the eye to colour as age increases, but the possible causes of this are not resolved. That the lens yellows with age has been ably demonstrated by Said & Weale, and as the results were obtained in vivo this seems fairly reliable data. The vitreous humour also becomes yellower with age, although Weale (1961a) is of the opinion that this has little photometric significance. The modular pigmentation may increase in concentration, or thickness, as age increases, and as previously mentioned, Wright first proposed this as a cause of age changes. Most other workers have suggested



that the macular pigment may be a contributory factor in ageing, although this has never been satisfactorily demonstrated. The chief difficulty is that the macular pigment varies so greatly in density amongst observers of the same age. This was found by Wright (1928-29) from analysis of white point chromaticities, and by chemical means by Wald (1945). Hence, a large number of observers are required if a statistical survey is to separate different components of pre-receptoral absorption increases. Finally, the colour response of the receptors, as a function of age, is virtually unknown.

Plan of the Present Investigation.

The present work consists of a survey of colour vision characteristics, measured colorimetrically. A large number of observers took part in this survey, these being pre-screened to eliminate observers with abnormal colour response. The observers were grouped by age and the results investigated for statistically significant dependence upon age. As far as possible, the experimental procedure was such as to permit an analytic investigation of the causes of any changes which did occur with age.

The tests performed included colour matching, measuring the relative luminosities of the matching stimuli, wavelength discrimination, and relative spectral sensitivity. These are more fully described in the following chapters. In analyzing the results, it was necessary to consider the effect of variations in prereceptoral light absorption on colour matching or on the relative spectral sensitivity.

Treatment of Pre-receptoral Absorption as a Filter.

If we consider a standard response system e.g. the 1931 C.I.E 2° observer or the Stiles-Speranskaya 10° observer, (C.I.E. 1959), the effect of an increase in optical density of the lens or of the macular pigment can be predicted. Firstly, one has to obtain a transformation from the C.I.E. system into the W.D.W. system in which all work in this thesis has been plotted.

Transformation of C.I.E. to W.D.W. co-ordinate system.

The W.D.W. system is based on matching stimuli of $460 \text{ m}\mu$, 530m μ and 650m μ , the intensities of the two former required for a match of wavelength 494m μ being arbitrarily made equal as are the intensities of the latter two in a match of wavelength 582.5m μ .

In general, we have (for C.I.E. matching stimuli X, Y and Z)

C = x(X) + y(Y) + z(Z)(1) for any spectral wavelength, and from three such equations as this for the W.D.W. matching stimuli we can obtain another set of equations of the type

 $(X) = x_r(B) + x_g(G) + x_b(B) \qquad \dots \dots (2)$ and similarly for (Y) and (Z)

R etc. are the matching stimuli of the W.D.W. system. If we now express wavelengths 494m µ and 582.5m µ in the C.I.E system in the form of (1), equation (2) can be applied to yield:-

$$C_{494} = x_{494}(x_{\mathbf{R}}(\mathbf{R}) + x_{\mathbf{g}}(\mathbf{G}) + x_{\mathbf{b}}(\mathbf{B})) + y_{494}$$
$$(y_{\mathbf{r}}(\mathbf{R}) + y_{\mathbf{g}}(\mathbf{G}) + y_{\mathbf{b}}(\mathbf{B})) + z_{494}(z_{\mathbf{r}}(\mathbf{R}') + z_{\mathbf{g}}(\mathbf{G}) \div z_{\mathbf{b}}(\mathbf{H}))$$

This can now be expressed as

 $C_{494} = r_{494}(R) + g_{494}(G) + b_{494}(B)$ and to transform to primaries of the Wright system we apply a factor $\frac{g_{494}}{b_{494}}$ to $b_{4,4}(R)$. The process is repeated for 582.5m u correcting b_{494} this time the red coefficient to be equal to the green, and by substituting back into (2) we obtain (X) etc. in terms of a W.D.W. primary system

$$(X) = (g) x_r (R) + x_g (G) + (g) x_b (B) \dots (3)$$

and similarly for (Y) and (Z). Expressing this fully in the W.D.W. system it becomes:-

$$(X) = a x_r \frac{g_{582.5}}{r_{582.5}} (R) + x_g(G) + x_b \frac{g_{494}}{b_{494}} (B) \dots (4)$$

'a' is required to normalize the equation, as(X) is a 'T-unit' in the C.I.E. system. This normalization is not necessary for pure transformation computations.

Any colour, as expressed by (1), can now be transformed by equations (3), and then normalized to give (C) in the W.D.W. system.

The transformation from C.I.E. to W.D.W. co-ordinates is given by:-

$$(X) = 'a' (.8183(R) - .504(G) + .0187(B))$$

$$(Y) = 'a' (-.1503(R) + 1.3354(G) - .0495(B))$$

$$(Z) = 'a' (-.1371(R) + .0398(G) + .9370(B))$$

where 'a' is the normalizing constant.

Representation of Increased Light Absorption with Age.

Considering the increased absorption of, for example, the

lens with age, consider the optical density to be D at age 30 years and D' at some greater age, a years.

Then $D_a^{\prime} = D + D_a$

and D_{α} , a function of λ can be considered as the effect of age upon the system. For spectral wavelengths, this will cause only an intensity change, but for a continuous energy distribution, such as the white source S_{B} , it will result in a change in the energy distribution and hence in the chromaticity of the source. From D_{α} , the transmission t_{α} of the lens ageing can be calculated directly.

Calculation of Chromaticity Change due to Pre-receptoral Absorption.

If the white source has energy distribution E_{λ} , its chromaticity is computed in the C.I.E. system as:-

 $x = \Sigma E_{\lambda} \overline{x}_{\lambda} , \quad y = \Sigma E_{\lambda} \overline{y}_{\lambda} , \quad z = \Sigma E_{\lambda} \overline{z}_{\lambda}$ where $\Lambda = \sum_{x \neq z} (\Sigma E_{\lambda} \overline{x}_{\lambda})$

and x $_{\lambda}$ etc. are the distribution coefficients of the C.I.E. standard observer.

To find the effect of the change in transmission t_a , the normal computation methods are used:-

 $X = \sum_{\lambda \in \lambda} t_a(\lambda) \times t_{\lambda}$ etc.

are determined and normalized to give the chromaticity co-ordinates.

The co-ordinates obtained can then be transformed by equations 2 into the W.D.W. co-ordinate system. Therefore, the effect of pre-receptoral absorption upon the chromaticity co-ordinates of

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a continuous source can be calculated.

Calculation of Changes in V₂ due to Variation in Pre-receptoral Absorption.

Relative spectral luminous efficiency (V_{λ}) is directly related to changes in transmission of the pre-receptoral ocular media. If the change in transmission is by a factor $t_a(\lambda)$, at wavelength λ , then:-

 $\overline{\mathbf{v}}_{\lambda} = \mathbf{v}_{\lambda} \mathbf{t}_{a}(\lambda)$

where \overline{v}_{λ} is the value for an observer of a years and v_{λ} the value for a young observer.

This treatment of pre-receptoral absorption assumes that the retinal function does not change with age and if changes do occur in receptoral response the treatment is invalidated. The present investigation has also been aimed at measuring receptoral changes,

Hence, the present work was intended to evaluate both qualitatively and quantitatively, the degree to which known or postulated pre-receptoral absorption can predict the change of the visual system with age. An attempt has also been made to detect any change in receptoral response with increasing age.

CHAPTER II.

Apparatus, General Experimental Methods and Observers.

The apparatus used for the experimental investigation was the Wright tri-chromatic colorimeter (Fig. 10) which has been fully described by the designer, Wright (1927-28). For the sake of further references to Fig. 10, it is worth noting certain points. The two spectra are formed at I and IJ spectrum I providing the test and desaturation stimuli, and spectrum II providing the three matching stimuli. Throughout the work on ageing, the three matching simuli used have been those of the W.D.W. system i.e. $650m \mu$, $530m \mu$ and $460m \mu$ called the 'red', 'green' and 'blue' stimuli and provided by small reflecting prisms placed in the spectrum.

The standard white source is at A, this being used for flicker photometry and in conjunction with a filter to provide a comparison field of known colour temperature for colour matching. The adaptation source, which can also be used to provide a fixation point, is at B. A white sector, for providing the flicker source, is rotated at C, being illuminated by the lamp at A. The sector is a 90° soutor and coated with white, matt paint. For colour matching, using the standard source, a magnesium exide reflector is placed at C, which reflects light from the standard source into the test half of the field.

The colour temperature of the standard lamp was 2854°K, this being calibrated on a photometer bench with a Lummer-Brodhun contrast head. A substitution method was used, in which the comparison source was a sub-standard N.P.L source. This was then converted Fig. 10

OPTICAL SYSTEM OF WRIGHT



to the C.I.E. standard source S_B by use of a Ronis B2:1 filter. This is a glass filter, made of threepieces of coloured glass, the wavelength transmission of which is illustrated in Fig. 11. Also illustrated is the distribution of energy in the experimental S_B obtained with this filter, compared with the C.I.E. source S_3 .

In order to obtain a 10° field for colour matching, the widefield attachment designed by Clarke (1963) was used. This again has been fully described by its designer and will not be further described here. The only difference in the present condition was that the field was viewed continuously, hence the paddle provided to interrupt the field, was not required.

Calibration of the Instrument.

In an experiment of the present type, in which quantitative comparison is to be made between data collected at different times over a total interval of three years, the calibration of the instrument is of great importance.

The colorimeter calibrations include that of the wavelength scales of the two spectra, the intensity scales of the photometric wedg the luminance levels of the visual fields and the energy output of the lamp, measured at the exit pupil of the instrument.

Wavelength Calibration of the Colorimeter.

The wavelength scales were calibrated by substituting a line source at S and using an auxiliary lens to view the position in the exit pupil of the lines reflected from the spectrum. This technique was used for all the wavelength scales, including the matching stimuli. Mercury and Cadmium sources were used to provide the



spectral lines, and the Cornu-Hartmann equation was used to interpolate between the lines. This calibration was checked rather infrequently (about four times during the total work), although a visual comparison of the hue of any point in the yellow region for the two spectra provided a fairly sensitive, and easily performed check at more frequent intervals. In actual fact, little variation was found in this calibration.

Calibration of the Photometer Wedge Scales.

The transmissions of the three photometric wedges, placed before the matching stimuli, were calibrated visually in terms of the relative logarithmic intensity of the light reaching the eye. This was done in the usual way Wright (1946), by setting the test reflector at the same wavelength as the matching stimulus being calibrated, and matching the primary stimulus against different intensities of the test stimulus. The test stimulus was varied in intensity by means of a series of sectors of known angle, and the reading on thephotometer wedge scale was noted. In terms of logs of the intensities, a good linear plot was obtained for each photometer wedge. These were checked about six times during the course of the work and were fairly constant, except on accasions when the gelatine contracted , leaving an air gop. In such cases, a new wedge was substituted.

The green photometer wedge was used for the relative luminosity measurements at a number of spectral wavelengths, and hence the problem of the non-neutrality of this wedge arose. In effect, the non-neutrality meant that, to a first approximation, the wedge . (calibration of **log** (intensity) against scale reading would have a different slope at different wavelengths. Hence, a linear factor could be used to give an approximate correction to the calibration at 530m µ, but in actual fact, as only a limited number of wavelengths were used, the wedge was calibrated separately at each of these wavelengths. This was done to limit errors due to the use of a number of wedges during the total experiment, for which the variations from non-linearity could have introduced systematic errors.

Calibration of Retinal Illumination Level.

The illumination level at which the measurements on the colorimeter were obtained was measured in terms of retinal illumination units, trolands. This was achieved by means of the sub-standard source, which was calibrated for candle power as well as colour temperature. If the output of the lamp is I and the light is reflected from the white sector at S into the eyepiece, the reflected light can be flickered for equal brightness against the light received from the photometer wedges. The illumination of the sector is

$$E = I \frac{\cos \beta}{r^2}$$

where r is the distance of the source from the sector, β the angle of incidence of the light on the sector. In this case, $\beta = 45^{\circ}$ and r = .86 metres.

The luminance of the sector, B, is thus:-

$$\frac{B = p I \cos\beta}{r^2 \pi}$$

where p is the reflection factor of the sector.

The retinal illumination then becomes

$$R = \frac{a p I \cos \beta}{\sqrt{r^2}}$$

where a is the area of the exit pupil.

The calibration was performed with a sub-standard lamp of known candle power, and was not checked frequently, as only the approximate value of retinal illumination was required.

Wavelength Calibration of the Large-Field Attachment.

The large field attachment was calibrated as was suggested by Clarke (1963). Again, the source S was replaced by a line source (this time a sodium source) and the reflectors were adjusted on the calibrated test and 'green' wedge scales so as to reflect the D lines. The lines were then observed lying across the pin-hole of the wide-field attachment with a magnifying glass. It was found useful to illuminate, dimly, the pin-hole from the viewing end of the colorimeter, (D of figure 10), as it became more easily visible. The pin-hole was then adjusted until the relevant D-line was centred in the instrument. This was a somewhat difficult procedure to be performed accurately, due to the natural width of the spectral lines and to the difficulty of judging when the line was centrally placed in the pin-hole. It was considered that accuracy of setting was, at the best; $\pm 1\%$ m u.

Calibration of the Relative Energy Output of the Lamp.

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The lamp was calibrated by placing a collimating tube and a photocell at the viewing end of the instrument. The relative sensitivity of the photocell to different wavelengths was known from the National Physical Laboratory calibration, and hence the relative energy could be found directly from the photo-cell output. A G.E.C. photo-cell and measuring unit were used to obtain the

readings. The energy from the 'green' reflector was measured at 10m µ steps through the spectrum. It was found that if the colorimeter beams were allowed to strike the cell directly, the results were very variable, especially at the red end of the spectrum, where the cell has low sensitivity. This was presumably due to variations in response over the photo-sensitive surface, and a negative lens was placed just beyond the instrumental exit pupil in order to diverge the beam to cover a larger surface of photo-cell. The lens was chosen in power so that menther the blue nor red beams quite covered the area of the cell's sensitive surface, to avoid errors due to different spreading of the two beams. No correction was made for light loss due to this extra lens in the measuring system, since this would be virtually independent of wavelength.

The calibration was performed only for the 'green' matching stimulus reflector of the colorimeter, as this was used at all wavelengths for measuring the V_A curve, for which the energy calibration is required.

Of all calibrations, the lamp output was most variable in time. This was due partly to 'pitting' of the tungsten ribbon, due to evaporation, which leads to higher resistance and, as the applied voltage was kept constant, variable output. Further, with use, the tungsten was deposited on the side of the lamp case, which left a black residue on the wall of the glass case. This lead to a drop in overall light intensity and, from transmission measurements on a Beckman spectrophotometer, it was found that the deposit was not neutral, but absorbed more heavily in the red region of the spectrum. To overcome this, a device seen during an Open Day at the National Physical Laboratory was utilized. This is illustrated in Fig. 12. A small can was attached with analdite on to the lamp case, near the top, and cool air blown over the top of the lamp. Presumably due to convection currents within the lamp, this causes most of the tungsten to evaporate on to the cap of the lamp case, out of the optical path of the colorimeter. The lamp was calibrated every four to five weeks of active use. The relative light energy of wavelength λ , incident at the cornea, is thus:-

$$E_{\lambda b} = E_{\lambda a} \left\{ \frac{KI_{\lambda b}}{KI_{\lambda a}} \right\}$$

where $E_{i_{A}}$ is the energy measured for a fixed position 'a' of the green wedge, and E_{Ab} is the energy at some other wedge position 'b'. The term in brackets gives the ratio of the intensities transmitted to the exit pupil by the wedge at positions 'a', at which the calibration was made, and position 'b'. This ratio is found from the wedge calibrations.

General Experimental Arrangements.

Observers were allowed about five minutes dark adaptation, during which they became accustomed to the instrument controls. The heads of the observers were supported by a chin rest, and although this was less satisfactory than the dental clamp method, the large number of observers made the latter an unrealistic proposition. The rest was adjusted so that the eye suffered no transverse chromatic aberration when viewing a mixture of the three
FIG. 12



MECHANISM FOR MAINTAINING A CONSTANT LAMP OUTPUT. matching stimuli, thus ensuring that light entered through the centre of the exit pupil of the eye. This is of importance in view of the Stiles-Crewford effect (Stiles & Crawford 1933). Observers wore correcting spectacles of the required power to overcome any deficiency of refraction in the eye. Normally, this was provided by the observer, but if the dividing line between the fields could not be clearly distinguished by an observer, using his own spectacles, a set of auxiliary lenses were available, and could be attached beyond the exit pupil. The field was 1° 20' in size, divided horizontally into two halves, and viewed through a 2 m.m. exit pupil in Maxwellian view.

The observations were performed in the order colour matching, luminosity measurements and wavelength discrimination. Normally, two sessions were required for each observer, giving a total observation time of $1\frac{1}{4}$ hours to 2 hours per observer, depending on how adept the observer was at performing the tests.

Observers.

About 400 observers took part in the colorimetric measurements, as far a possible equally divided into 5 year age groups. The total range was 16 years to 72 years, although in the group above the age of sixty, few observers with age greater than 65 years were obtained, and the mean age of this group was only 64 years, although all observers over the age of 61 years were included in its values.

The distribution of observers was not equal in the groups, due to the difficulty in obtaining 'volunteers' in the older age groups, and the relatively small number of these people attached to Imperial College.

Observers were screened for any congenital colour defects, using colour screening plates for this purpose. These plates included the Ishihara and the American Optical (Hardy-Ritter-Rand) plates.

The criterion used for rejection of an observer was that given by Belcher, Greenshields and Wright (1958) for the Ishihara plates.

CHAPTER III

Wavelength Discrimination and Age.

Introduction.

In order to investigate change with age in the light receptoral system, measurements of wavelength discrimination were taken. When a single wavelength or narrow range band of wavelengths is incident upon the eye, an interposed filter of any spectral transmission characteristic will alter the intensity, but not the frequency, of the light quanta (excluding fluorescent filters). As the initial light reaction in the retina is a photo-chemical reaction, energised by light quanta of energy hv, a monochromatic light beam is changed only in intensity by any absorbing medium in front of the receptors, such as the crystalline lens or the macular pigment.

Hence, discrimination between two wavelengths is independent of pre-receptoral absorption, assuming that equality of brightness between the beams can be maintained, and that the loss of intensity is not sufficient to affect the discrimination. Consequently, changes in wavelength discrimination are symptomatic of changes in the receptoral system of the eye. Defective colour vision is an extreme example of variation in wavelength discrimination due to abnormal processes in the retina or higher centres.

The wavelength band received by the eye in the colorimeter varies from about 12m µ in the red end to about 3m µ in the blue end of the spectrum. Wright (1946) found that the eye integrates this band, so that discrimination is effectively as between two monochromatic lines, as would be expected from the near linearity of the spectrum locus over short wavelength steps.

Technique for Measuring Wavelength Discrimination.

In testing the large number of observers, the technique utilized for measurement of wavelength discrimination was the just noticeable difference (J.N.D.) method. The observer controls the wavelength of one of the fields, and the comparison field is set at a fixed wavelength. A means of controlling the brightness of one of the fields is provided, so that equality of brightness in the two fields can be maintained. The observer is requested to change the wavelength of the controllable field until a difference in colour between the two fields can be perceived. The difference in wavelength between the two fields is then taken as a measure of the J.N.D. wavelength discrimination step, at the wavelength of the fixed field. In practice, the mean of the steps taken on either wavelength side of the fixed field is taken as the discrimination step.

This technique is not completely satisfactory, because of the lack of objectivity in defining a 'just noticeable difference'. This leads to some uncertainty in interpretation of the results, because the criterion will depend upon the mental approach of the observer. For example, the author found that in his own case, the J.N.D. became much smaller with practice. Further, the results of observers who are unaccustomed to critical decisions are somewhat more susceptible to variation. The observers were inhomogeneous in distribution through the age spectrum, regarding scientific or technical training, there being a much higher ratio of people so trained in the younger age groups. However, the technique has the advantage of being relatively quick, an important factor where the time available for each observer is strictly limited. Further, it has been the method most frequently adopted by other investigators. <u>Experimental</u>.

The 'green' wedge of the colorimeter was utilized to provide the comporison beam from spectrum II and the test reflector in spectrum I was used to obtain the difference step. The field size was 1° 20' square, divided horizontally into two halves, and five minutes dark adaptation preceded the measurements. The observer was initially briefed to distinguish between brightness changes and hue changes. it being requested that each time a hus 'ifference was obtained, the observer should rematch the beams for brightness, using the control of the 'green' photometer wedge. When, on rematching for brightness, a hue difference still persisted, this was taken as the J.N.D. The observer was asked to obtain the J.N.D. four or five times, on both wavelength sides of the comparison beam. Measurements were taken on both sides of the comparison wavelength in order to eliminate the effect of a small error in setting the green wedge. The J.N.D. was taken as equal to the mean of the two steps.

Three wavelengths were used for the comparison beam, at 590m µ, at 530 m µ and 490 m µ, these being two minima and a maximum of the wavelength discrimination function for a normal observer, Wright & Pitt (1934). These wavelengths were chosen because it is required to obtain information on colour perfection through the whole range

of sensitivity. Although the relation between discrimination and the trichromatic response systems is not known, the two must clearly be linked and this assumption has formed the basis of theoretical work on colour discrimination by Helmholtz and by Schrödinger (both quoted by Stiles (1946)), and by Stiles himself (1946). If it is accepted that the fundamental response curves of the trichromatic system represent approximately the three independent response systems, it would appear that the minimum at 590m μ will be governed by the 'red' and 'green' response systems, and that at 490m μ by the 'green' and 'blue' systems. Hence, discrimination at these two wavelengths is related to the three response processes, the wavelength at 530m μ being used for comparison of sizes of minima and maxima in the discrimination curves.

The brightness level was kept approximately constant at 100 trolands, neutral filters of appropriate density being inserted in spectrum I to achieve this.

Results.

The results obtained for the J.N.D. have been summarized in Table I and in Fig. 13. As in all other results in this work, the light has been defined in terms of its wavelength at the cornea. This has been done to facilitate comparison with the results of other authors, although frequency is probably a better variable to use, being a constant as the light passes through the eye.

Fig. 13 gives the mean J.N.D. steps for each of the age groups for the three wavelengths, and the J.N.D. distributions are grouped in Tables 2 - 4. In all, 349 observers took part in the investigatio

Table	Variati	on of	Wavele	ngth D	iscrim	inatio	n with	Age		
Age Group	16 -20	21 -25	26 -30	31 - 35	36 -40	41 -45	46 - 50	51 -55	56 -60	61 →
No. of subjects	40	43	40	40	34	36	34	30	32	30
$\frac{\text{Mean Discriminati}}{\text{Step } \Delta \lambda}$	on									
Wa v elength 590mμ Δλ 590	3,25	2.90	2.90	3.00	3.05	3.20	3.10	4.00	3.40	3.20
530mμ Δλ 530	7.10	5.90	6.75	6.80	7.75	6.75	7.00	8.50	7.60	7.30
490mμ Δλ ₄₉₀	4.20	3.30	3.50	3.80	4.10	3.85	4.10	4.75	4.40	4.40

Table 2 Variation	on of I	Distril	oution	of J.N	1.D.s v	vith Ag	ge for	$\lambda = 49$)Omµ		
Age Group	16 -20	21 -25	26 - 30	31 -35	36 -40	41 -45	46 -50	51 - 55	56 - 60	61 →	Total
$\frac{\text{Discrimination}}{\text{Step in m}\mu \ (\Delta \lambda)}$				No. c	of sub;	jects					
1. 25mµ	0	0	0	0	0	0	0	0	0	0	0
	3	6	3	2	1	2	3	0	2	0	22
2.50mµ	3	14	13	7	6	10	4	1	3	3	64
	8	9	8	10	7	8	8	4	4	6	72
3.75mu	12	4	6	12	6	5	3	4	4	2	58
- ··· ·	3	6	2	3	3	4	7	6	4	2	40 -
5.00mµ	3	1	5	2	5	1	1	4	7	3	32
	3	1	1	2	2	1	3	4	3	2	22
6.25mu	1	1	1	0	2	3	1	2	1	2	14
	2	1	0	0	0	1	3	1	2	0	10
7•50mji	1	0	0	2	2	0	0	3	1	0	·9
Total	39	43	39	40	34	35	33	29	31	20	343

Table 3	Varia	tior	<u>ı of</u>	dist	ribu	utior	ı of	J.N.	D.s	with	
	Age	λ=	590n	ութ							
Age Grou	2 16	21	26	31	36	41	46	51	56	61	Total
	-20	-25	-30	-35	<u>4</u> 0	- 45	- 50	- 55	- 60		
<u>Discrimin</u> <u>Step in</u>	natior mµ (¢	<u>1</u> (λ)	No	. of	sub	ject	S				
]. Отµ	2	6	5	3	4	5	4	ı	4	3	37
	· 13	8	14	15	10	8	7	3	5	8	91
3 . 0-тµ	7	14	10	12	8	8	11	6	7	3	86
	8	7	3	6	6	6	6	6	6	1	5 5
5 . 0mµ	3	6	5	2	5	5	5	7	2	1	41
	6	2	2	l	1	2	0	3	2	2	21
7.0mµ	0	0	0	0	0	0	l	4	4	0	9
	l	0	l	l	0	2	0	0	1	2	8
Total	40	43	40	40	34	3 6	34	30	31	20	3 48
			reje	cted	1						

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.

Table 4	Vari	atio	<u>n of</u>	dis	trib	<u>utio</u>	n of	J.N	.D.s	wit	h
	Age	for	<u>λ= 5</u>	30mµ	·						
Age Group	16	21	26	31	36	41	46	51	56	61	Total
	-21	25	-30	-35	- 40	- 45	-50	-55	-60	-65	
Discrimina	ation	1	No.	of	suhi	ects					
Step in :	mμ (🕰 λ	.)			Dubj	0000	•				
1.625mµ	0	0	l	0	0	0	0	0	0	0	1
	l	l	1	l	0	0	1	0	0	0	5
3.25mµ	l	2	3	2	0	1	0	0	2	0	11
	2	3	7	5	3	8	6	0	2	2	38
4.875mµ	4	10	6	7	6	4	4	3	3	2	49
	5	11	6	5	8	9	7	4	3	3	61
6.5mµ	12	6	3	6	4	4	3	6	5	3	52
	4	5	8	6	4	2	1	4	8	4	46
8 .125 nµ	8	2	1	4	4	3	4	3	2	2	33
	l	1	l	l	2	2	2	3	2.	l	16
9.75mµ	2	0	l	2	2	1	4	4	2	2	20
	0	l	1	l	l	l	2	3	3	l	14
Total	40	42	39	40	34	35	34	30	32	20	346
			~~		+	7					
			т Т	elec	tea	י ד					
Table 5	-		(elat	jive	J . N .	D.S	-	<i>с</i> -	· • •		· ¬
Age Gro	up .	1.6 2				06 4) L
	-2	20 -2	25 - 3	λ- 	ック 4	-0 -4	-5 -5	-5	05 - 0	0	
		Rati	Los -	$\frac{1}{\lambda_2}$							
^λ 590	• 4	491	• 4	60	• 4	67	• 4	78	•46	54	
^λ 530		• <u>-</u>	552	4	-59	• 4	97	• 4	-97	• 42	28
^λ 590	{	322	• 7	75 [.] 5	. 8	34.9	•7	'19	•80)4	
^λ 490		*	963	.	325	•)19	• દ	85	. 80	04
<u>λ</u> 490		, 522	.	486	.• '	196.	•	556	.5	13	
^λ 530)	• ¹⁵	560		433	•	48 0	·•	51,1	$\bullet I_t$	86



Fig. 14 shows the distribution of steps for the total population of observers.

Discussion.

The plot of average wavelength discrimination step πs a function of age shows a slight trend with age. A minimum of discrimination occurs at all three wavelengths for the 21 - 25 age group, with other peaks and minima occurring at various ages. The correlation coefficients between the J.N.D steps and age, for each age group, were calculated from the Tables 2 - 4 of grouped data.

In treating the results, a few observers were found to give results which lay a long way outside a range of 2.5 to 3 - for the age group. Hence, the results for these observers were rejected, and the number of rejected results for each group has been noted. In actual fact, the distribution of the J.N.D's was not Gaussian, either for the single groups or for the total population (Fig. 14). This is almost certainly a result of the technique used to obtain the results. The existence of a finite discrimination step places a fixed lower limit on the step which must be taken to obtain a difference in colour between the two fields. Although this step may vary somewhat from observer to observer, this does give a lower limit to the J.N.D. which could be attained if the observer were sufficiently critical in approach. However, many observers require a more 'positive' difference before fixing the step and this means that the upper limit is not finite. The resulting bias demonstrated in Fig. 14 results from this. As the distributions are single peaked and not too markedly skew, Gaussian statistics have been

continuous curve:- total data for all age groups broken curve:- data for 21-25 yr group



used to estimate error ranges, etc. No correction was made for grouping of results when the correlation coefficients were calculated. The mean J.N.D's were calculated from ungrouped data.

The correlation coefficients between the wavelength discrimination step and age were calculated from the formula:correlation coefficient $r = \sum_{i=1}^{n} (xy)$ and y being values $(i\sum(x^2) \sum(y^2))^{\frac{1}{2}}$ relative to their means x and y being the variables, in this case age and J.N.D. steps. The correlation coefficients were calculated from the grouped data and the values found were:-

 $r_{590} = + .10 \pm .05$; $r_{530} = + .18 \pm .05$, $r_{490} = + .21 \pm .05$ As can be seen, the coefficients are small and positive in each case. The standard error of the correlation coefficients were calculated from the formula:-

$$\mathbf{s} = \left\{ \frac{1 - \mathbf{r}^2}{n^2} \right\}$$

where 'n' is the number of observers. These coefficients are significant at .01 level for wavelengths 530m μ and 490m μ , but not for wavelength 590m μ . This suggests that there is a decrease with age in overall wavelength discrimination (increase in $\Lambda \lambda$), which is selectively more marked in the green and blue-green regions of the spectrum.

Relative J.N.D. steps.

In view of the uncertainty in the J.N.D technique for measurement of wavelength discrimination, the data were re-investigated, using a reduced form of the J.N.D. In this treatment, the ratios of pairs of the steps for each observer were calculated, and plotted as a function of age (Fig. 15). They are also tabulated in Tables 5 - 8. This reduced form was investigated because it helps to allow for variation in the criterion used by observers, when finding the J.N.D. For example, a cautious observer might be expected to take a larger step at each wavelength and the ratios between these steps would be a better measure of discrimination. Further, it would certainly be a better indicator of a change of discrimination in one part of the spectrum, relative to that of another part.

Fig. 15 shows that the ration between the three steps are random in their maxima and minima, when these steps are plotted as a function of age, and this result is confirmed by the very small correlation coefficients with age which were all less than 1.04.

Hence, summing the results obtained from the experiments on 349 observers, it appears that the absolute wavelength discrimination step, as measured by the J.N.D. technique, is slightly significantly correlated with age in the green and blue-green regions of the spectrum, an increase in step size occurring as age increases. However, reservations regarding the technique make a positive assertion of this correlation impossible. The relative steps at each wavelength, on the other hand, show no correlation with age, indicating that relative wavelength discrimination steps are not dependent upon age.

<u>Table 6 D</u>	istri	buti	lon (of re	educe	ed J	N.D	. ste	eps v	vith_	Age
	for	λ ₅₉₀) rel	lativ	re to	λ	530				
Age Group	16	21	26	31	36	41	46	51	56	61	Total
	-20	-25	-30	-35	-40	-45	- 50	-55	-60)	
Δλ <u>590</u> Δλ ₅₃₀			No.	of s	subj€	ects					
.22	4	0	4	2	3	l	l	l	0	2.	18
	7	3	3	7	6	2	6	2	4	3	43
36	5	5	3	5	3	5	7	3	3	5	44
	3	5	9	8	6	5	4	4	7	3	54
•50	5	4	8	4	6	7	4	8	7	0	53
	4	9	4	7	4	5	3	3	5	2	46
•64	3	5	4	2	l	3	3	1	2	2	26
	3	6	l	2	1	3	2	2	2	2	24
•78	3	2	2	0	l	2	2	3	0	0	15
	2	0	0	2	2	l	1	2	1	0	11
•92	0	4	1	0	1	1	1	1	0	1	10
Total	39	43	39	39	34	35	34	30	31	20	344

Table 7 (I	Dist	ribu	tion	of :	reduc	ced a	J.N.I) <u> </u>	teps	with	Age
	for	λ ₄₉ () rel	ati.	ve to	D 753	50				
Age Group	16	21	26	31	3 6	41	46	51	56	61	Total
	- 20	-25	-30	-35	- 40	-45	- 50	-55	- 60		
<u>∧</u> λ∴90			~-								
۵ ^λ 530			No.	of	sub	jects	5				
.32	2	0	2	4	4	2	l	1	2	3	21
	8	5	10	11	6	8	6	8	6	5	73
44	6	7	7	5	6	12	8	4	5	l	61
	8	15	10	8	3	2	3	7	8	4	68
. 56	8	4	7	5	6	8	8	2	4	4	56
	5	3	2	4	4	l	l	2	3	2.	27
. 68	2.	4	0	3	3	l	5	l	3	l	23
	l	1	l	0	2	0	l	l	l	0	8
_ 80	0	2	0	0	0	0	l	2	0	0	5
	0	2	0	0	0	0	0	l	0	O	3
	40	43	39	40	34	34	34	29	32	20	345

.

Table 8 D	istri	buti	.on c	of ro	duce	:d J.	<u>N.</u> D.	ste	eps w	ith	Age
	for	λ ₅₉₀	, rel	ati.	re to	ολ ₄ α	90°				
							-				
Age Group	16	21	2 6	31	36	41	46	51	56	61	Total
	-20	-25	-30	-35	- 40	-45	-50	-55	-60		
Δ^{λ} 590											
Δ ^λ 490											
• 45	8	1	7	9	4	3	2	2	3	3	42
	б	2	2	8	11	5	13	2	6	4	59
•75	7	8	13	9	б	10	5	8	7	7	80
	5	3	9	6	4	8	4	6	7	2	54
1.05	9	12	4	2	5	5	4	4	6	2	53
	2	8	3	3	2	1	2	3	1	1	26
1.35	1	5	l	0	1	l	1	2	1	l	14
	l	0	0	3	0	1	1	1	0	0	7
1.65	1	1	1	0	0	l	2	l	0	0	7
	0	1	0	0	l	l	0	0	0	0	3
Total	40	41	40	40	34	36	34	29	31	20	345





Forced Choice Technique for Wavelength Discrimination.

The investigation of the changes with age in the J.N.D. wavelength discrimination step lead to the conclusion that discrimination becomes somewhat impaired with age, although the relative discrimination steps at different points in the spectrum do not change with age. However, as was previously mentioned, the size of the absolute J.N.D. is subject to much variation, and so a limited investigation of wavelength discrimination was made, using a sounder experimental technique.

Obserfers.

Six observers were used, three aged 18-26 years, one aged 44 years and two over sixty. These were chosen from the main group of observers and their results from J.N.D. measurements have been given, for comparison with the means of their respective age groups. The size of the observer group in these experiments was limited, due to the time required to obtain results.

Technique.

As previously stated, a forced choice technique was used to measure the wavelength discrimination step. Two fields are provided, one of fixed wavelength and the other adjusted by the experimenter. The observer is requested to name the colour difference between the two fields e.g. for a yellow comparison field, he would be asked to say which of the two fields were greener or redder in colour. The observer is not allowed to state that the two fields are equal, even if they appear so, but is forced to make a choice of the difference between the fields. The experimenter presents the controllable field a large number of times, at a series of wavelengths, ranging from equality between the two fields to about one J.N.D. step on either side of the fixed wavelength , the order of presentation being randomized. The observers choice of, say, the redder field in the example above was recorded and plotted.

Experimental.

The 'green' wedge was again used to provide the comparison field and the test reflector the variable field. In order to make the test as decisive as possible, the change in brightness which occurs as the reflector is moved through the spectrum was compensated for by placing a neutral density wedge in the path of spectrum I. The change of density of this wedge along its length was such as to just compensate changes in brightness through the spectrum in the particular region investigated, as calibrated for a typical observer. Commercial wedges were used, although individual observer differences necessitated that every observer re-set the brightness match for each observation. However, the re-setting required was much reduced by use of the wedges in the spectrum.

Five wavelengths were investigated. 590m µ, 530m µ, 490m µ, 455m µ and 445m µ. The number of pairs of wavelengths presented at each wavelength was governed partly by the size of the discrimination step and partly by the dispersion of the spectrum e.g. at long wavelengths, dispersion severely limits the number of pairs which can be used. Each wavelength pair was presented sixteen times, the test field being obliterated whilst being changed in wavelength. No limit was set on observation time, although observers were encouraged to make rapid decisions. Presentation of the pairs was randomized, using a numbered card system, these cards being shuffled betweeneach set of readings.

Results.

The results are summarized in Figs. 16 - 20. Each curve represents the frequency with which the observer called the test half of the field by the longer of the two hues to be named e.g. red in the case of yellow (590m µ) discrimination. The J.N.D. steps and the steps obtained by the present method have been tabulated in Table 9. The forced choice discrimination step has been taken, arbitrarily, as half the wavelength range between a single error in naming the folour differences on either side of the test wavelength.

Discussion.

The wavelength discrimination steps obtained by this forced choice method show marked differences from the J.N.D's previously obtained. The steps are much smaller than the J.N.D's and, further, are much closer to the steps given by Wright and Pitt (1934) for wavelength discrimination with a $1^{\circ} \ge 2^{\circ}$ test field. Although the small number of observers make it difficult to detect a trend in discrimination step sizes with age, no such trend seems to occur. The observers used for this test were not atypical of the age groups which they represented in the size of the J.N.D's recorded during the main experiment, as can be seen from Table 9.

These results are indicative of the difficulties inherent

Table 9 Wavelength discrimination as measured b	oy
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	J.N.	D.s an	d 'For	ced Ch	oice'	techni	que		
Subject	Age								
		J.N.D	<u>.</u> (mµ)		Δλ by'Forced Choice'				
		^λ 590	^λ 530	^λ 490	^λ 590	^λ 530	^λ 490		
K.H.R.	24	2.5	4.8	2.1	.90	1.30	1.18		
J.M.A.	20	3.0	6.0	2.6	.65	1.91	1.15		
J.A.S.	26	3.6	9.0	3.3	.85	1.66	.85		
W.H.K.	42	2.9	7.0	2.8	.80	2.20	1.21		
C.R.R.	72	2.9	7.5	5.1	.78	2.05	1.35		
V.I.R.	60	2.8	9.0	5.2	.85	1.70	1.20		
	Δ	<u>λ by '</u>	Forced	Choic	e' tec	hnique	(mµ.).		

		^λ 455	^λ 445
K.H.R.	24	2.6	1 .8
J.M.A.	20	3.3	2-~9
J.A.S.	26	2.2	1.7
W.H.K	42	2.9	2.0
V.I.R.	60	3.7	2.0





let the second

f-number of occasions on which the field appears of longer wevelength



of longer wavelength λ .



number of occasions on which the field appears of 1 longer wavelength



in a technique, such as the J.N.D. technique, when applied to untrained observers. The result is dependent both upon the visual response of the observer and upon the decision of what constitutes a 'Just Noticeable Difference'. The forced choice technique gives results which indicate that the J.N.D. steps may be a result of two processes of which the decision process is subject to change with age or training, for as previously mentioned, the groups were not homogeneous as regards their scientific training. The small number of observers used in the forced choice experiments makes generalization difficult, because it could be that the old observers used were exceptional in the reduction of the size of the discrimination step between the two methods. However, considering the small size of the correlation coefficient of the J.N.D's with age, coupled with the results of the reduced J.N.D's and of the forced choice method, the conclusion is drawn that any change in wavelength discrimination with age is negligible, if any does in fact occur.

The Shape of the Forced Choice Discrimination Curves.

The curves obtained (Figs. 16 - 20) by smoothing the results of forced choice discrimination measurements are of interest, as in general they may well throw some light on the discrimination mechanisms. The present results are too few in number, and based on too small a number of observations, to merit close attention, but some discussion of their origin has been made.

Fluctuations in the Response System.

In general, one would expect the signals in the optic nerve,

generated by a light beam to be subject to fluctuations. Courses of this include, possibly, the quantum fluctuations of the photon beam (Bouman and Walraven (1961), Bouman et al (1963)), the existence of thermal decomposition in the photo-pigments (Barlow (1957)) and spontaneous discharge in the neural bodies (Grant 1955). Of these, the first is a Poisson distribution, which would tend to become Gaussian for large numbers of signals and the other two would give rise to some form of random signal. Hence, whether one considers the optic nerve fibres to be carrying discrete signals or a current, it will be subject to fluctuations. The method of coding hue information is not known, except that it must be corried in at least three separate channels. However these three signals are compared e.g. by taking their ratios, the result will still be subject to an uncertainty. Changing the hue will change the signals and hence the possibility of discrimination will be equivalent to discriminating between two Gaussian distributions with different/values, assuming the fluctuations in the signals to be random in nature. The Gauss curves represent the spectrum of the possible values of the signal, which values will be separated in time. Considering Fig. 21, if both Gauss curves are normalized, the hatched area A is equal to

$$A = 2 \left(1 - \frac{2}{\sqrt{2\pi} h} \int_{-\infty}^{\infty} e^{-\frac{x^2}{2h^2}} d \right)$$

where 'h' is a constant. This assumes that the two Gauss curves have the same standard deviation.

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Hence:-

 $A = 2 \operatorname{erf}(\mathbf{X})$

when $\mathbf{A} = 0$, probability or correct discrimination is .5, and when A = 2 it is 1. Assuming that the intermediate 'values of the probability of discrimination are proportional to A, $p = \frac{1}{2} (1 + erf(x))$ is the normalized probability. If curve P is considered as representing the longer wavelength and curve Q the shorter, the hatched area represents the probability of detecting one field as being of longer wavelength than the other. The assumption that the Gauss curves have equal standard deviation is probably justified in considering changes in signal over very short wavelength steps. Hue and wavelength discrimination have been similarly considered as equivalent over this short range of wavelength.

Results.

The forced choice discrimination curves have been fitted by function p for two observers, chosen randomly from the six. The results are shown in Figs. 22 and 23. The results for 590m µ have not been used in this comparison, as so few readings were taken at this wavelength. The curves were normalized for equality at a probability of discriminating of .84 (equal to the probability at a value equal to the standard deviation of the fluctuations).

The agreement between the experimental and theoretical curves is fairly good, except at high probabilities of correct discrimination, where the experimental values are greater than the theoretical values. This may well be due to the naive form F14.21



of (n) is a normal distribution.





of signal-noise ratio used, although, also, the extreme tails of the Gauss curves represent probabilities which would be equivalent to much less than 1 in 16 chance of not discriminating the two fields correctly. Further, the assumption of complete randomness of fluctuations can only be an approximation to the time distribution which could cause the tails of the Gauss curves to be non-exist nt.

The curves show a tendency to be asymmetric at 455m µ, probably due to the relatively large discrimination step, which could result in a difference in the changes in nervous signals with change in wavelength on the two sides of the test wavelength. This theory does not include the possibility of random guesses by the observer, although these will be at least partially allowed for in smoothing the probability curves.

CHAPTER 1V

The Relative Luminosities of the Matching Stimuli Introduction.

In the preceding chapter, it was shown that the functioning of the receptoral system, in terms of wavelength discrimination, changes little if at all, with age. This, however, does not necessarily provide a complete description of the receptoral response to colour. Wright (1946) has pointed out that the wavelength discrimination curve is not directly related to the colour mixture curves. In fact, from Helmholtz's treatment of the wavelength discrimination curve, it was impossible to find a set of fundamental colour mixture curves which would fit both theory and experiment. Stiles (1946) was more successful in predicting, theoretically, both the wavelength discrimination and chromaticity discrimination.

It does seem, however, that the relative luminosity of the matching stimuli provide information on the functioning of the fundamental response curves in a more direct fashion than is given by the wavelength discrimination. The variation with age of the relative luminosity of the matching stimuli of the W.D.W. chromaticity co-ordinate system was therefore measured. The magnitude of these luminosities will depend on the relative contributions of the three component colour mixture curves to wavelengths 494m µ and 582.5m µ when the colour mixture curves are plotted on an equal energy basis. Thus, if these colour mixture curves are changed, either in their wavelength distribution being distorted or by one curve being changed in overall size relative
to the other two, the relative luminosities of the matching stimuli would be altered.

In the W.D.W. system the scales of the red (650 m, u) and the green (530 m, u) stimuli are arbitrarily adjusted to give equal values in a match of yellow (582.5 m, u). Similarly, the green and blue (460 m, u) values are adjusted to be equal when matching against a blue-green stimulus of wavelength 494 m μ . The luminosity measurements on the three stimuli are then made in terms of their scales, adjusted, as explained, for matching 494 m μ and 582.5 m μ wavelengths.

Dependence of Relative Luminosities of the Matching Stimuli upon Variation in Pre-receptoral Absorption.

Suppose an observer, having previously measured the relative luminosities of the matching stimuli, has a filter, with transmission $t_B \approx 460 \text{ m}$ and $t_G \approx 530 \text{ m}$, placed before his eyes. Then the intensity of blue light reaching the retina will change by a factor $\frac{t_B}{t_G}$ relative to the green light. The gaale of the blue stimulus will therefore require to be multiplied by a factor $\frac{t_G}{t_B}$ to keep the amounts of blue and green stimuli equal in the blue-green (494m m) match. Similarly, the flux of the blue stimulus will be changed by a factor $\frac{t_B}{t_G}$ relative to the green. However, applying the colour matching 'correction' factor to the blue intensity scale, the net change in the blue luminosity recorded will be:-

$$\frac{\mathbf{t}_{\mathrm{B}}}{\mathbf{t}_{\mathrm{G}}} \quad \frac{\mathbf{t}_{\mathrm{G}}}{\mathbf{b}_{\mathrm{B}}} = \mathbf{1}$$

that is, the relative luminosities of the matching stimuli remain

independent of pre-receptoral absorption.

Experimental.

The colour matches of 494m µ and 582.5m µ were made, using the matching stimuli 650m µ, 530m µ and 460m µ. The 494m µ test field was desaturated with sufficient red desaturation stimulus to make the match possible, whereas the yellow match did not, in general, require any desaturation, as observers were insufficiently practised to be sensitive to the small amounts of blue desaturation normally required at this wavelength.

Each match consisted of four settings of the controls of the photometer wedges. The matching stinuli were then flickered against a white field, provided by a 90° white sector. In this measurement, spectrum I was entirely obliterated by a screen. The flicker was performed in two parts, the red and green stinuli being flickered against white in one case, and blue and green in the other. This was done because of the large difference in energy available in the blue and red ends of the spectra, in the colorimeter. Although there is some risk of increasing errors by performing two measurements instead of one, it was decided that this was off-set by the greater ease with which the red and green pair could be set for elimination of the flicker. The blue stinulus was flickered at a level of about 15 trolands, whereas the red was flickered at a level of about 150 trolands.

The criterion for elimination of flicker was not always easy to obtain in the case of the blue stimulus, and a few people felt unable to perform this part of the experiment. However, as this almost always occurred when the lamp was old and of reduced light output, and happened in general in all age groups, it was felt that no selectivity was introduced in not having the results of these observers available. Measurements were made on only about 300 of the observers in this part of the work.

Results.

The results are summarized in Fig. 24 and Tables 10-11. The results show fair agreement with the data given by Wright (1928-29) for a 2° field, the red stimulus having virtually equal values of relative luminosity in the two cases, although the blue stimulus in the present work has a higher value. This may be due to the change in field size, although in view of the tendency of the fovea to become tritenopic for small fields (Willmer & Wright, 1945) this would be surprizing. Alternative reasons could be that Wright's seven observers were not fully representative of the total population, or that the present work was not performed with a luminous surround to the field.

The data show virtually no variation with age, as can be seen from Fig. 24. The correlation coefficients of the relative luminosities with age are also statistically negligible. These were calculated from the grouped data of the Tables 10-11.

Colour Matching Data for the Spectral Test Wavelength 494m_u Introduction.

In obtaining the relative luminosities of the matching stimuli, it was necessary to obtain colour matches of the spectral wavelengths 494m µ and 582.5m µ. As explained in the previous

Table 10	Relative	e Lumir	losity	of the	Blue	Matchi	ng Sti	mulus	(460mp	<u>.)</u>	
Age Group	16 -20	21 -25	26 -30	31 -35	36 -40	41 -45	46 -50	51 - 55	56 -60	61 →	Total
V _b				<u>No. c</u>	of subj	jects					
3	1	0	1	0	1	2	2	0	1	2	10
4	4	8	3	3	6	4	1	1	1	3	34
5	12	9	7	11	3	9	11	10	6	3	81
6	11	9	11	10	11	8	8	5	7	7	87
7	2	4	6	7	5	5	4	2	2	2	39
8	3	3	5	5	1	3	3	1	2	1	27
9	6	4	2	4	2	1	0	0	1	1	21
10	0	0	1	0	2	0	1	1	0	1	6
11	0	0	0	0	0	1	0	0	0	0	1
Total	39	37	36	40	31	33	30	20	20	20	306
Mean V _b	6.6	6.6	6.9	6.6	6.6	6.5	6.4	6.4	6.4	6.3	
Table 11	Relativ	ve Lumi	nosity	r of th	.e Red	Matchi	ng Sti	mulus	(650mµ	.)	
Age Group	16 -20	21 - 25	26 - 30	31 - 35	36 -40	41 -45	46 -50	51 - 55	56 -60	61 7	Total
Vr				No. c	f subj	jects					
30	1	0	3	1	0	0	0	0	0	0	5
40	4	9	5	3	6	5	5	4	5	2	48
50	11	9	12	15	10	9	9	6	6	5	92
60	15	8	8	13	8	11	11	6	6	10	96
70	8	8	8	5	6	8	3	4	5	3	58
80	3	5	1	1	0	1	3	1	0	1	16
90	0	1	0	2	1	0	1	0	1	1	7
Total	42	40	37	40	31	34	32	21	23	22	322
Mean V r	62.5	63.0	58.4	62.5	60.0	63.0	62.7	61.5	60.9	64.9	

E16.24

RELATIVE LUMINANCES OF THE MATCHING STIMULI



section, it was found that all observers required desaturation of the 494m µ with 650m µ wavelength stimulus in order to obtain a colour match, whereas most of the observers were insensitive to blue desaturation of the test field 582.5m µ. It was therefore possible to calculate the chromaticity co-ordinate of the 494m µ match in the W.D.W. co-ordinate system and to use the co-ordinate values as a further check on the relationship between the receptoral response of the eye and age. Because the W.D.W. chromaticity co-ordinate system is based upon spectral colour matches, the spectrum locus is independent of variation in pre-receptoral light transmission. Any variation of the co-ordinate values for the spectral wavelengths can hence be ascribed to variation in the receptoral response.

The experimental details have been given in the previous section.

Results.

The results are given in Tables 32 and 13. Table 12 gives the mean values of the red chromaticity co-ordinate of 494m µ for each age group and Table 13 the distribution of the co-ordinate values within the age groups. As the blue and green co-ordinates are adjusted to be equal at this wavelength, the chromaticity co-ordinates are fully described by the red co-ordinate alone. Its mean values are plotted as a function of age in Fig. 25.

Discussion.

As can be seen from Fig. 25 the values of the red chromaticity co-ordinate of wavelength 494m µ show little consistent trend with

Tablo 12 Re	ed ch	roma	tici	ty c	0-01	dine	ate c	f 49	14mµ.		
Age Group	16	21	26	31	36	41	46	51	56	61	
	-20	-25	-30	-35	-40	-45	-50	-55	-60		
		-		-							
Mean	121		.119		.140) -			.121		
values		120		.109	_	.13		.126	-	.124	
	-					-					
Tablel3 Dis	strib	utio	n of	red	<u>co-</u>	ordi	inate	val	ues		
Age Group	16	21	26	31	36	41	46	51	56	61	Total
-	-20	-25	-30	-35	-40	-45	-50	-55	- 60		
T	No	. of	ohs	erve	rg		-				
<u> </u>		• • • •	່ ບັນນີ້ 1	6	- 0	٦	2	г	0	г	ר 3
02	1. -7	-	- -	0	0	т Т	<u> </u>	ـلـ ح	0	-L- -1	
	3	1	2	2	0	2	2	1.	Ľ	1	10
06	3	4	5	5	1	3	3	1	2	4	31
	3	5	4	6	4	3	7	2	2	1	37
-1.00	12	11	8	9	4	7	10	5	9	4	79
	9	12	8	7	8	6	3	5	5	6	67
-1.40	8	3	11	4	10	9	4	3	5	2	59
	6	4	1	4	2.	3	4	4	2	3	33
-1.80	0	1	2	2	4	4	1	2	1	2	19
	l	1	0	3	1	2	0	0	2	1	11
Total	46	42	42	48	34	40	36	22	30	25	3 65



age. This is confirmed by calculation of the correlation coefficient from Table 13, this coefficient having a value of + .03, which is not statistically significant.

Conclusions

Both sets of data reported in this chapter appear to be independent of age. As both are independent of variations in ocular light absorption, the results can be interpreted as indicating that there is no mean change with age in the receptoral response to colour.

CHAPTER V

Colour Matching Data with White Light Source S_B Introduction.

The results of the previous chapters served to allow an investigation of the change in receptor response as a function of age. In order to find out what changes occur in the pre-receptoral absorption of the eye, colour matching data for white light source $S_{\rm p}$ were collected for 373 observers. The likely causes of agedependent absorption are the lens (Said & Weale's 1959 data) and the macular pigmentation for foveal fields (Wright's 1946 data). Weale (1961a) indicates that no other factor is likely to be of much importance in measurements performed with an artificial exit pupil.

As explained by Wright (1946), the W.D.W system of co-ordinates permits measurement of spectral chromaticity co-ordinates which are independent of pre-receptoral absorption, whereas the co-ordinates of a continuous light source will depend upon the degree of prereceptoral absorption. The co-ordinates of a white point in the chromaticity chart will give an indication of the degree of pre-retinal absorption in the observer and if a large number of observers are taken in a series of age steps, the shift of the mean value for the white point chromaticity will indicate a change in pre-receptoral absorption with age.

The deductions made in such a way are complicated by two factors. Firstly, the white points in a given age group will vary between individual observers due to variations in pre-receptoral

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absorption. Wright (1928-29) showed that modular pignentation varies greatly amongst observers of the same age, whereas Said & Weale (1959) state that lens absorption is approximately constant in observers of the same age (excluding subjects with cataracts). Hence, in order to obtain a representative mean value of the white point chromaticity, a large number of observers must be used.

The second factor to be borne in mind in analysis of the mean white-point chromaticities of each age group is that they will also be affected by variations in receptoral response. Hence, individual variations will again have to be cancelled out by using a large number of observers to obtain a representative mean for each age group, and these means will depend on any changes in receptoral response withage. It has been argued from the results in the previous chapters that the receptoral response remains constant with age. If this is correct, it follows that if the number of observers is sufficiently large, the mean chromaticity co-ordinates of the white points for each age group will depend only upon the prereceptoral absorption variations with age.

The results so obtained can be compared with the calculated chromaticity changes caused by increase in lens absorption or macular pigment.

Experimental.

The white point, S_B, provided the test field. This was chosen, as it was the source used for previous work on age changes in colour matching by Wright (1946). The white source was calibrated as described in Chapter II, and a smoked magnesium

oxide surface provided the reflector at position C of Fig. 10. The matching beam was provided by the three matching stimuli of the W.D.W. system. The spectral matches of 494m µ and 582.5m µ were made by each observer, as described in the chapter on the relative luminosities of the matching stimuli. Each match was made four or five times by every observer, the experimenter upsetting the matches between each setting. 373 observers took part in these measurements.

<u>Results</u>.

The mean chromaticity co-ordinates of the white points at different ages are given in Fig. 26 and Table 14. The distribution of the results within eachage group is summarized in Tables 15-17, which give the distribution of each of the chromaticity co-ordinates of the white point as a function of age. The mean values for each age group in Table 14, were computed from the results of individual observers, and not the data of Tables 15-17. Given with Tables 15-1/ are the correlation coefficients between age and each of the white point co-ordinates. The standard error of the coefficients is also given.

Discussion of Results.

The mean white points for each age group, plotted in the chromaticity diagram, show a definite trend with age. If each of the white point co-ordinates, grouped in 10 year age spans, is plotted, a series of curves is obtained in which the peak is seen to shift regularly with age, apart from the two youngest groups, which are closely similar (Fig. 27). The curves were fitted by cye to hist-ograms, and 10 year age groups were used in order to Table14 Mean White Point (SB) Chromaticities

<u>Age Group</u> 16 21 26 31 36 41 46 51 56 61 <u>Mean</u> -20 -25 -30 -35 -40 -45 -50 -55 -60

Coefficients

r	.247	.248	•279	.284	. 298	.277
		.256	.254 .	281 .	.298 .32	5
g	.405	.3 98	.424	•426	.440	.423
		.415	.407 .	429	•4 33 • 45	5
Ъ	•348	• 354	. 297	.290	. 262	300
						* 200

.329 .349 .290 .269 .220

Table 15 Distributions of Green Co-ordinate of White Point

Age Group	16	21	26	31	36	41	46	51	56	61	Totals
	-20	-25	-30	·~35	-40	-45	-50	55	6 0		
g			No.	of s	subje	ects					
.300	2	1	3	0	0	0	0	0	0	0	6
	3	2	0	0	0	0	0	0	0	0	5
.340	3	2	3	2	3	2	0	0	1	0	16
	1	2	6	9	2	l	1	2	2	0	26
.380	8	6	9	5	5	4	11	2	2	3	55
	6	12	9	14	4	11	11	8	3	4	82
•42.0	9	10	5	4	7	9	4	5	13	4.	70
	6	3	3	5	8	7	4	8	5	6	55
. 460	2	3	1	l	5	3	6	3	4	3	31
	0	l	1	0	2	2	l	2	2	3	14
. 500	0	1	0	0	0	2	2	0	. l	7	13
Totals	40	43	40	40	36	41	40	30	33	30	373

Table 16	Distribution	With /	lge of	Blue	Co-ord	inate d	of Whit	e Poin	t Chro	matici	ties
Age Group	2 16 -20	21 -25	26 -30	31 -35	36 -40	41 -45	46 -50	51 -55	56 -60	61 - ``	Totals
b				No.	of Su	bjects					
0.080	0	0	0	0	0	0	0	0	0	2	2
0.120	0	0	0	0	0	0	1	1	1	3	6
.160	0	0	0	0	3	4	4	4	6	6	27
.200	2	3	4	3	5	4	6	9	3	8	47
.240	4	4	2	4	4	8	4	5	10	3	48
.280	10	10	6	8	9	9	12	5	8	4	81
•320	10	11	6	10	6	11	11	3	1	3	72
•360	5	6	10	10	2	3	2	2	3	1	44
.400	1	4	9	4	5	2	0	1	1	0	27
•440	6	3	0	1	2	0	0	0	0	0	12
.480	2	1	3	0	0	0	0	0	0	0	6
•520	0	1	0	0	0	0	0	0	0	0	1
Total	40	43	40	40	36	41	40	30	33	30	3 73

Table17 Distributions of the Red co-ordinate of white point

Age gro	<u>up</u> 16	21	26	31	36	41	46	51	56	61	Total
	-20	-25	-30	-35	-40	-45	- 50	-55	- 60		
r		Nc	. of	sut	ject	s					
.16	l	l	l	0	0	0	0	0	0	0	3
	3	l	l	2_	0	0	0	0	0	0	7
.20	. 6	7	7	2	l	2	0	0	7	0	28
	6	8	12	12	5.	2	3	3	2	0	53
. 24	9	12	7	9	5	7	6	2	4	3	64
	5	5	3	7	7	14	.8	4	3	3	59
. 28	8	5	5	4	9	6	13	7	5	2	64
	2	3	3	3	2	7	6	2	6	Ą.	38
•32	0	l	0	I	4	2	2	8	7	8	33
	0	0	1	0	3	l	1	2	l	4	13
.36	0	0	0	0	0	0	l	2	2	5	10
	0	0	0	0	0	0	0	0	0	1	l
Total	40	43	40	40	3 6	41	40	30	33	30	373

Correlation coefficients of blue co-ordinate = $-.49 \stackrel{+}{-} .04$ of red co-ordinate = $+.49 \stackrel{+}{-} .04$ of green co-ordinate = $+.38 \stackrel{\pm}{-} .04$ FIG.26







give reasonable frequency in the number of observations for each ordinate on the curves.

The correlation coefficients calculated from the grouped data are all significant at .01 level of significance. This suggests a definite change in pre-receptoral absorption of light with increasing age.

Distribution of Co-ordinates within Age Groups.

Two of the three linearly dependent co-ordinates were investigated, namely the blue and red cc-ordinates. As explained in the introductory remarks to the colour matching data, the spread of co-ordinate values in each age group will be caused by variations between individual observors in the receptoral response and in macular pignent density. Individual errors of observation are also of importance, especially with untrained observers, but no attempt was made to allow for these. A few results from each age group were analysed to determine the individual observer errors and these were found to be, in general, much less than the size of the co-ordinate ranges used in the Tables 15 - 17 (i.e. .020 in the red and green co-ordinate, and .040 in the blue).

The data, illustrated in Fig. 27, appear fairly close in shape to Gauss curves. This fonclusion was confirmed, using the X^2 test, in which the 'closeness of fit' of the data to a Gaussian distribution was investigated at .05 significance level. The values of X^2 are given in Table 18, from which it appears that the data are not significantly different from a Gauss distribution. The single exception to this is the blue co-ordinate for the

Table 18	Values	s of X ²	calculated	for the	he red	and	green
_	<u>co-o</u> 2	rdinates	of S_{B}				
Age	x_r^2	x ² .05,cr	x ² _b	^{x2} .05,	cr.		
16-25	4.91	9•49	10.54 .68	9.49 3.68			
26-35	8.35	9.49	5.3	9.49			
36-45	5.30	7.83	58	9.49			
46-55	4.28	7.83	5.93	7.83			
56 -	7.53	9.49	4.04	7.83			

 $x^2_{.05,cr.}$ is the critical value for a 5% level of significance, for the appropriate no. of degrees of statistical freedom.

16 - 26 year age group. In this one case, regrouping of results into pairs led to the second value of X^2 , which again indicates a Gaussian distribution. The curve-fitting is illustrated for the red co-ordinate of the 16 - 26 year age group, Fig. 28.

Comparison between Co-ordinate Distributions in different Age Groups.

The spread in the chromaticity co-ordinates of any particular age group depends upon variations in receptoral response and macular pignentation density between individual observers. As receptoral response appears to remain constant with age changes, any difference in the spread of chromaticity co-ordinates between age groups could be indicative of a difference in macular pignentation.

The standard deviations used for calculating the Gauss curves were of the same order for each age group, but some veriation did exist. To investigate this more closely, the X^2 test for consistency of sampling was used (Grow et al). A correction was applied to the data of Tables 16 and 17, such that the mean co-ordinate values for each age group were equal to that of the 16 - 20 age group. The factor added or subtracted to each mean value was then applied to give Tables 19 - 20. The value of X^2 for each co-ordinate is given with the appropriate table, and at .05 level the results appear to be consistent for sampling. Hence, apart from a mean chromaticity co-ordinate shift, there is no difference with age changes in the co-ordinate distributions. It can therefore be assumed that the only change in macular pigmentation with age which could occur is

FIG. 28



N frequency of 'r's data for 16-25yr age group

Table 19 Red o	:o-ord	inate	of S	p cor	rected	d for a	re changes
Age Group	16	26	36	46	56	Total	
	-25	-35	-45	-55	••••••••		
r	No	. of	subjed	ets			
.160	2	1	2]	0	4]	9	
.180	4 6	314	517	5 5	4} ⁸	21	
	13	9	10	9	8	49	
.220	14	24	18	1 6	8	80	$X^2 = 13.0$
	21	16	17	15	17	86	x^{2} 05 24-36 4
. 260	10	10	12	16	13	61	•0,24-,0•4
	13	9	7	5_	5	3 9	
•300	5	6]	4	3	3	21	
	ıj ⁶	2	216	1,4	ב∳ ⁴	7	
Total	83	80	7'7	70	63	373	
Table 20 Blue	e co-c	rdina	te of	S _D c	orrec	ted for	age changes
<u>Table 20 Blue</u> Age group	<u>e co-c</u> 16	ordina 26	te of 30	<u> </u>	orrec 56	ted for Total	age changes
Table 20 Blue Age group	<u> co-c</u> 16 -25	ordina 26 - 35	<u>te of</u> 3ύ -45	<u>కాం</u> 46 -55	orrec 56	ted for Tot al	age changes
Table 20 Blue Age group	<u>e co-c</u> 16 -25	ordina 26 -35	<u>te of</u> 30 -45	<u>Sp</u> c 46 -55	<u>orrec</u> 56 -	ted for Total	age changes
<u>Table 20 Blue</u> <u>Age group</u> <u>b</u>	e co-c 16 -25 Nc	ordina 26 -35 . of	te of 30 -45 subje	<u>Sp</u> 46 -55 cts	<u>orrec</u> 56	ted for Total	age changes
<u>Table 20 Blue</u> <u>Age group</u> <u>b</u> .200	e co-c 16 -25 No 5	ordina 26 -35 0. of 7 6	<u>te of</u> 30 -45 subje 4	<u>Sp</u> 46 -55 cts 5	orrec 56 - 3	ted for Total 24	age changes
<u>Table 20 Blue</u> <u>Age group</u> <u>b</u> .200	e co-c 16 -25 No 5 8	ordina 26 -35 0. of 7 6	<u>te of</u> 30 -45 subje 4 10	<u>Spe</u> 46 -55 cts 5 8	<u>orrec</u> 56 - 3 8	<u>ted for</u> Total 24 40	age changes
<u>Table 20 Blue</u> <u>Age group</u> <u>b</u> .200 .280	e co-c 16 -25 No 5 8 20	26 -35 . of 7 6 14	<u>te of</u> 30 -45 subje 4 10 13	<u>Sp</u> 46 -55 cts 5 8 13	orrec 56 - 3 8 10	<u>ted for</u> Total 24 40 70	age changes $X^{2} = 25.5$
<u>Table 20 Blue</u> <u>Age group</u> <u>b</u> .200 .280	e co-c 16 -25 No 5 8 20 21	26 -35 . of 7 6 14 16	<u>te of</u> 30 -45 subje 4 10 13 14	$\frac{S_{T}}{46}$ -55 cts 5 13 12 12	orrec 56 - 3 8 10 19	ted for Total 24 40 70 82 78	<u>age changes</u> $x^{2} = 25.5$ $x^{2}_{.05,24} = 36.4$
<u>Table 20 Blue</u> <u>Age group</u> <u>b</u> .200 .280 .360	e co-c 16 -25 No 5 8 20 21 11	ordina 26 -35 0. of 7 6 14 16 20	<u>te of</u> 30 -45 subje 4 10 13 14 19	Sp. 2 46 -55 cts 5 8 13 12 18	orrec 56 3 8 10 19 10	ted for Total 24 40 70 82 78 42	<u>age changes</u> $X^{2} = 25.5$ $X^{2}_{.05,24} = 36.4$
<u>Table 20 Blue</u> <u>Age group</u> <u>b</u> .200 .280 .360	20 21 11 5 8 20 21 11	26 -35 0. of 7 6 14 16 20 13	te of 30 -45 subje 4 10 13 14 19 7	$\frac{S_{p}}{46}$ -55 cts 5 13 12 18 11	orrec 56 - 3 8 10 19 10 6 5	ted for Total 24 40 70 82 78 42	age changes $X^{2} = 25.5$ $X^{2}_{.05,24} = 36.4$
<u>Table 20 Blue</u> <u>Age group</u> <u>b</u> .200 .280 .360 .440	20 21 11 5 8 20 21 11 5	ordina 26 -35 0. of 7 6 14 16 20 13 1 1	<u>te of</u> 30 -45 subje 4 10 13 14 19 7 7	$ \frac{S_{p}}{46} $ -55 cts 5 13 12 18 11 3	orrec 56 - 10 19 10 6 5	ted for Total 24 40 70 82 78 42	age changes $x^{2} = 25.5$ $x^{2}_{.05,24} = 36.4$
<u>Table 20 Blue</u> <u>Age group</u> <u>b</u> .200 .280 .360 .440	20 21 11 5 20 21 21 21 21 21 21 21 21 21 21 21 21 21	ordina 26 -35 0. of 7 6 14 16 20 13 13 13 5 34	<u>te of</u> 30 -45 subje 4 10 13 14 19 7 7 310	$ \frac{S_{T}}{46} - 55 cts 5 13 12 18 11 3 0 3 $	orrec 56 3 10 19 10 6 5 17	ted for Total 24 40 70 82 78 42 37	<u>age changes</u> $x^{2} = 25.5$ $x^{2}_{.05,24} = 36.4$
<u>Table 20 Blue</u> <u>Age group</u> .200 .280 .360 .440 .520	20 21 11 5 3 20 21 11 5 3 3 11	ordina 26 -35 0. of 7 6 14 16 20 13 13 13 3 4 0	<u>te of</u> 30 -45 subje 4 10 13 14 19 7 7 7 310 0	Sp e 46 -55 ets 5 8 13 12 18 11 3 0 3 0	orrec 56 3 8 10 19 10 6 5 1 7 1	ted for Total 24 40 70 82 78 42 37	<u>age changes</u> $X^{2} = 25.5$ $X^{2}_{.05,24} = 36.4$

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a steady accumulation of density in all observers.

Shift of the Mean White Points with Age.

The general direction of the change in chromaticity with increasing age is towards the yellow part of the spectrum locus. This suggests that the change in pre-receptoral transmission responsible for the change in chromaticity is such as to cause loss of blue light from the retinal image. That the correlation co-efficients with age of the chromaticity co-ordinates **ene** significant has previously been mentioned.

The best straight line through the means was found by the nethod of least squares and is given in the r - g co-ordinate plane in Fig. 29. A linear relationship between the means can be seen to be a good approximation to the chromaticity changes as age increases, and the dominant wavelength of this line is 579.5m μ in the C.I.E 2° spectrum locus.

Comparison between the Colour Matching Data and Transmission Changes of the Ocular Media.

In Fig. 30, the changes in white point chromaticity deduced from the lens absorption data of Said & Weale (1959) are shown. The method of calculation was indicated in Chapter I, and the actual values for changes in lens transmission with age were obtained, graphically, from the data of Said and Weale. Calculation was performed between the wavelength limits of 400m μ and 700m μ in lOm μ steps. A correction factor, for the instrumental pupil size, was applied to the lens data, as these were obtained with a dilated eye pupil. The correction was taken from the curve given by Weale (1961b). Figure 31 shows the experimental white points of the colour matching compared with increase in macular pigment density. The pigment absorption curve was obtained from the results of Wald (1945) and a lower wavelength limit of 400m μ was used, with 5m μ steps being taken for calculation.

Although the present data agree fairly closely with the falculation based on the lens ageing data, the statistical significance of the differences between the two lines was investigated The difference in slove of the two lines would not provide a useful means of doing this, as that for the present results is based only on the means of each age group, and carries no information on the considerable scatter within the groups. Hence, the t - test (Crow et al) for testing the significance of differences between the expected and the obtained values of the differences in the means of two samples was utilized. The test requires that samples should be of the same standard deviation and with normal distribution. These limitations have been seen to be approximately obeyed by the white point chronaticity co-ordinates for the 10 year groups, and this has been assumed to be true for five year groups also. Five year groups were used to investigate changes in order to utilize the full co-ordinate change, as obtained from the present data.

$$t = \frac{x_1 - x_2 - d}{s_0 \left(\frac{1}{n_1} + \frac{1}{n_2}\right)^{1/2}}$$
was computed

where x_1 and x_2 are the means of the two samples, 'd' the expected difference in the means and n_1 and n_2 the number of observations in







each sample. $\underset{O}{s}$ is the standard deviation of the combined samples and

$$\mathbf{s}_{0}^{2} = \frac{n_{1}}{\sum (\mathbf{x}_{1}j - \mathbf{x}_{1})^{2}} + \frac{n_{2}}{\sum (\mathbf{x}_{2}j - \mathbf{x}_{2})^{2}} (n_{1} + n_{2} - 2)$$

Values of t were computed for the 41 - 45 age group and the 61 - 65 age group combined in each case with the 16 - 20 age group. The values of 'd' were taken from Said & Weale's data, as being the predicted change in each of the co-ordinates. t values computed are:-

red: t₄₅=.99, t₆₅=1.68. blue: t₄₅=1.1, t₆₅=1.92: t_{.05} =1.99 the critical t-value is for a 5% level of significance. From the results it appears that we cannot reject the hypothesis that the present data are consistent with the lens ageing data. In comparing these results, it must be remembered that no error has been ascribed to the results calculated from Said and Weale's data, and as they are based on a small number of observers, they may not be fully representative. However, as can be seen from the Fig. 30 and the statistical tests, the agreement between the two sets of data is quite satisfactory, especially as the data for lens ageing is calculated on the basis of a hypothetical standard observer. Hence, for the average results of a large number of observers, the lens ageing data of Said and Weale, with a correction factor for the pupil size, satisfactorily predicts the change in colour matching with age. The experimental data do not change with age at a constant rate, and some age groups, especially the younger ones, have mean values which are in reverse of the general age trend.

The data of Shid and Weale showed a steady change of lens transmission with age, after the age of 20 years. In view of the relatively small change between adjacent age groups, compared with the large chromaticity spread within the age groups, it is, however, unlikely that these reversals have any significance.

Comparison with the change in chromaticity due to increase in macular pignent concentration is shown in Fig. 31. Again, the direction of change of chromaticity in the chart is close to that yielded by the present data with increasing age. The results are therefore not really capable of distinguishing between the two types of absorption. The fact that lens data for ageing, obtained by an objective physical measurement, predict fairly closely the present data, suggests that this is more probably the cause of the change in white point chromaticity with increasing age, as observed in the present work.

Large Field Data.

In an effort to remove, as far as possible, the effect of macular pigmentation upon the chromaticities of the white points, it was decided to perform a subsidiary colour matching experiment, using a 10° field of view, these matches to be performed by a group of young and a group of old observers. The 10° matches will be relatively free of the effect of the high concentrations of macular pigment, especially if the central area of the field is ignored in making the colour match . Hence, changes in chromaticity between the two groups will be due to lens absorption, assuming that sufficient observers are used to give a reliable mean and that, they represent an average receptoral response.

Experimental.

The colorimeter was used in conjunction with the 10° attachment of Clarke (1963). This was calibrated as described in Chapter II. The white points S_A and S_B were both used as test fields for the young age group, S_B alone being used for the old group. The three matching stimuli of the Wright system were used and the matching was performed with continuous viewing conditions. Observers were requested to ignore any central non-uniformity in the matches, especially noticeable in matching the 494m μ wavelength, which was desaturated with 650m μ radiation. The matches were repeated four times each, the experimeter changing the wedge settings between each match.

The matching level was about 150 trolands for S_A and the spectral wavelengths and 50 trolands for S_B . The white point S_B was also measured again for field size $1^{\circ} 20'$, the total matching session occupying about an hour.

Results.

The chromaticity co-ordinates for S_B with the 10° field conditions are given in Fig. 32. The 1° 20' co-ordinates are given in Fig. 33. 22 observers provided the results, twelve in the young group and ten in theold group. The mean age of the young group was 24 years and that of the old group was 62 years.

Discussion of Large Field Data.

The results of Figs. 32 - 33 show the white (S_B) points for



FIG. 33 FOR 58. (2°) CHROMATICI CO ORDS. Ĵ 5 -4 .3 .2 •2 •3 •4 15 4 -

results for young observers
x results for old observers.
C.I.E. 2° observer.

1° 20' fields and 10° fields. The small field results show the expected wide scatter of chromaticities and the two sets of data, for the young and old groups, overlap in a considerable range. The

S_B calculated for lens ageing is shown in the diagram and that predicted by the C.I.E. standard observer data is also given. This point is fairly centrally placed within the observed results for small fields. The small field white points are distributed over a wide range, especially for the young observers, as these were chosen so as to provide both lowly and highly pigmented observers of the young age group.

Comparison with C. I.E. Proposed 10° Data.

The 10° field results, by comparison, form two quite distinct groups of results, for the young and old observers. The scatter between individual results is still fairly considerable, almost sufficient to cause overlap between the groups. The calculated S_B from the C.I.E 10° data is given (30 years cl4) and the expected change of this point with lens ageing is also given. In this case, the C.I.E. proposed 10° data do not predict the results obtained experimentally, for although relatively few observers were utilized in this part of the experiment, the group of results shows a very real divergence from the predicted result, both for young and old observers.

There could be a number of reasons for this disagreement. As was mentioned in Chapter II, the wavelength adjustment of the colorimeter with the widefield attachment presents some difficulty, and a tolerance of about $\pm 1\%$ µ was accepted at 590m µ. Further, the wavelength band received at the eye was about 10m μ for the wide-field viewing (at 590m μ), which is a considerable range, and the assumption of single wavelengths incident upon the eye, made in the transformation of data for the 10° observer, is not valid, and may be a source of error. A further point is that the level of retinal illumination was very low and application of the C.I.E data could lead to incorrect results under such conditions.

These hypotheses were tested by applying an error of 3m µ to the setting of the pin-hole at the wavelength of the sodium D-lines, and testing the effect upon the chromaticity predicted by the 10 $^{
m o}$ observer. 3m µ was perhaps rather greater than the expected error et the D-lines, but it provided fairly 'round number' corrections to be applied to the other wavelengths used in transforming C.I.E to W.D.W co-ordinates. These corrections were, at 650m µ, 4.5m µ, at 530m µ,2.5m µ at 494m µ,1.5m µ and at 460m µ, 1m µ. The results of these corrections are shown in Fig. 34, which gives the result for the average and oldest observers. As can be seen, a considerable shift in the S_B chromaticity occurs as a result of this correction, and is in such a direction as to give better agreement with the present data. Although the magnitude of the correction made is somewhat larger than would have been expected necessary by the author, a smaller correction would also have resulted in better agreement between predicted and observed chromaticities. It was therefore decided that the disagreement initially found between observed and predicted results was due to an error in calibration. Confirmation of this was given by the fact that source S_A , which was matched by a number of the young observers, similarly corrected



by using the new transformation (Fig. 25), gave better agreement with the experimental data. The scatter between individual results is largely independent of nacular variations, observers being asked to ignore the 'Maxwell Spot' effect which occurred in the centre of the field. Further, according to Said and Weale, lens absorption is fairly constant at any particular age and hence the scatter for the white points should represent only variations in receptor response. If the trichromatic system is viewed in terms of three 'fundamental' matching wavelengths, the calculations of lithe shift in chromaticity with errors of the wavelength setting in the colorimeter show that the range of fundamental stimuli required to produce such observer variations is not large. The somewhat larger range of chromaticities for the old observers may be indicative of some variation in lens absorption between observers.

As can be seen from Fig. 34, the agreement between the present data for the two age groups and the chromaticities calculated from the lens ageing are in fair agreement, assuming that a correction has to be made for mis-calibration of the 10° attachment. This tends to confirm that the lens ageing can predict, adequately, the changes in colour matching functions with age.



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<u>Transformation Equ tions for W.D.W. and C.I.E. proposed</u> <u>10⁰ Data</u>

1). jormal Transformation

$$(X) = .7634(R) - .5872(G) + .0281(B)$$

$$(Y) = -.2021(R) + 1.4637(G) - .0701(B)$$

$$(Z) = -.1173(R) - .0057(G) + 1.7992(B)$$

3). Transformation in the case of a -3mm error at

 $\frac{590 \text{m}\mu \text{ in the wavelength calibration}}{(X) = .895 (R) - .600 (G) + .019 (B)}$ (Y) = -.214 (R) + 1.476 (G) - .072 (B)(Z) = -.141 (R) + .002 (G) + 1.575 (B)

CHAPTER V1

Relative Luminosity Function and Age

Introduction.

The relative sensitivity of the eye to different wavelengths $(V_{\lambda} \text{ curve})$ depends both upon the receptoral response and the light losses which occur between the cornea and the receptors. For any wavelength λ ;-

 $\overline{v}_{\lambda} = v_{\lambda} t$ (1)

where χ is the relative luminance when a filter of transmission t χ is placed before an eye having real relative luminosity curve V_{λ} . Hence, if the lens and nacular pigment are considered as filters, the variation of V_{λ} values with age will give information on the change in pre-receptoral light loss with age at those wavelengths for which the values of V_{λ} are found. Such measurements abe necessary, for although the data of chapters III, 1V and V suggest the age changes are probably due to pre-receptoral absorption, only the broadest indication of the type of absorption can be deduced. Thus, the change in chromaticity of S_B with increasing light absorption in the ocular media is practically the same for macular absorption or for lens absorption in spite of the obvious differences in wavelength dependence of these two absorptions.

The V_{λ} values can be analyzed in a similar way to the colour matching data of chapter V and the average receptoral response system for each age group is again assumed to be independent of age. The individual values of V_{λ} in each age group may, however,

show variations due to observer differences both in degree of ocular light absorption and receptoral response. The work of Coblentz and Emerson (1918) indicates just how great are the variations in χ between individual observers. Their results were obtained under different conditions from those of the present investigation, the 2° field having a large surround field of about equal luminance, but the dataare valuable for indication of the variability to be expected in determining V_{λ}

Experimental.

The wavelengths investigated were $600m \mu$, $560m \mu$, $530m \mu$, $515m \mu$, $460m \mu$ and $420m \mu$. The first four wevelengths were investigated using flicker photonetry and the two latter using heterochromatic brightness matching. The results were normalized at $560m \mu$ hence providing 5 measurements relative to the normalizing value. The flicker measurements were performed using the 'green' photometer wedge of the colorimeter spectrum II (Fig. 10) to provide the test beam, an illuminated sector being rotated at C to interrupt the beam. The field was not provided with a luminous surround, as is usually recommended. The observer was requested to adjust the sector speed and the control of the photometer wedge until a single wedge position gave a minimum sensation of flicker. Use of a minimum rather than total extinction of the flicker was found to give more consistent results.

The heterochromatic brightness matching was carried out using the test spectrum to provide the comparison field and the 'green' reflector provided the adjustable field. Matching was performed with 515m µ as a comparison field for 460m µ and 460m µ as a comparison field for 420m µ, errors in individual results being as much as 4 times greater, for a given observer, than in the method of flicker photometry. A few observers were unable to perform the experiments, being unable to form any criterion of equal brightness of different hues. Without doubt, the criterion to be used could not satisfactorily be indicated to observers, and the difficulty of separating the effects of colour and brightness is well known under the title 'the Helmholtz-Kohlrausch' effect. In the case of brightness matching, the field was viewed in conditions of dark adaptation, with about 5 minutes pre-dark adaptation.

The levels of illumination used were about 100 trolands for flicker photometry, about 60 trolands for the $515m \mu - 460m \mu$ match and about 5 trolands for the 420m μ match. The very low level of retinal illumination in the last case was due to the low output of short wavelength from the tungsten lanp used as the light source.

<u>Results.</u>

The results of the measurements of V_{λ} as a function of age are given in tables 21 - 26 and the correlation coefficients of the V_{λ} values with age have been given with the tables. These latter were calculated from the grouped data of the tables. The mean values of V_{λ} for each age group, with their standard deviations, are given in table 21 and the distribution of the values amongst different groups are indicated, for some wavelengths, in Fig. 36. The mean values are plotted in Figs. 37 - 40.

Table 21	Mean Relative V, values for each Age Group	
Lge Group	16 21 26 31 36 41 46 51 56 61	
	-20 -25 -30 -35 -40 -45 -50 -55 -60	
<u> Հ (пµ)</u>	.700 .665 .696 .720 .707	
600	.676 .693 .705 .739 .697	
530	.864 .901 .890 .858 .822	
<i></i>	. 884 . 893 . 860 . 860 . 858	
515	. 560 . 581 . 550 . 547 . 509	
	.610 .578 .54 <u>6</u> .523 .516	
460	.086 .104 .082 .069 .061	
100	.095 .092 .074 .057 .057	
420	.034 .039 .028 .023 .019	
720	.042 .035 .027 .018 .017	
560mµ	Normalizing value .995	
	9 Standard deviation of mean V. of each age group	(s)
		· · · ·
Age Grou	7 16 21 26 31 36 41 46 51 56 61	
Age Grou	-20 -25 -30 -35 -40 -45 -50 -55 -60	
Age Group	16 21 26 31 36 41 46 51 56 61 -20 -25 -30 -35 -40 -45 -50 -55 -60	
Age Group λ(mμ) 600	$\begin{array}{cccccccccccccccccccccccccccccccccccc$	
Age Group λ(mμ) 600 530	16 21 26 31 36 41 46 51 56 61 -20 -25 -30 -35 -40 -45 -50 -55 -60 8.0 7.5 7.2 7.8 6.8 7.4 7.9 9.1 8.2 6.6 9.4 8.0 6.7 6.2 7.5 8.0 8.3 8.1 8.9 8.0	
Age Group λ(mμ) 6.00 530 515	$\begin{array}{cccccccccccccccccccccccccccccccccccc$	
Age Group λ(mμ) 6.00 530 515	$\begin{array}{cccccccccccccccccccccccccccccccccccc$	
Age Grouy λ(mμ) 6.00 530 515 460	$\begin{array}{cccccccccccccccccccccccccccccccccccc$	
Age Group λ(mμ) 6.00 530 515 460	$\begin{array}{c} & & & & & & & & & & & & & & & & & & &$	
Age Group λ(mμ) 6.00 530 515 460 420	$\begin{array}{c} & & & & & & & & & & & & & & & & & & &$	
Age Group λ(mμ) 6.00 530 515 460 420	$\begin{array}{c} & & & & & & & & & & & & & & & & & & &$	
Age Group λ(mμ) 6.00 530 515 460 420	16 21 26 31 36 41 46 51 56 61 -20 -25 -30 -35 -40 -45 -50 -55 -60 8.0 7.5 7.2 7.8 6.8 7.4 7.9 9.1 8.2 6.6 9.4 8.0 6.7 6.2 7.5 8.0 8.3 8.1 8.9 8.0 13.2 11.4 14.2 12.2 10.6 10.4 11.6 13.6 13.9 10.5 37.2 32.7 34.1 39.1 36.1 35.1 43.4 41.9 60.0 28.1 52.9 38.5 35.7 47.8 36.8 46.3 51.4 40.7 50.0 35.3	
Age Group λ(mμ) 6.00 530 515 460 420	16 21 26 31 36 41 46 51 56 61 -20 -25 -30 -35 -40 -45 -50 -55 -60 8.0 7.5 7.2 7.8 6.8 7.4 7.9 9.1 8.2 6.6 9.4 8.0 6.7 6.2 7.5 8.0 8.3 8.1 8.9 8.0 13.2 11.4 14.2 12.2 10.6 10.4 11.6 13.6 13.9 10.5 37.2 32.7 34.1 39.1 36.1 35.1 43.4 41.9 60.0 28.1 52.9 38.5 35.7 47.8 36.8 46.3 51.4 40.7 50.0 35.3 Somparison with the standard C.I.E. V, values	
Age Group λ(mμ) 6.00 530 515 460 420 	$\begin{array}{c} & & & & & \\ 16 & 21 & 26 & 31 & 36 & 41 & 46 & 51 & 56 & 61 \\ -20 & -25 & -30 & -35 & -40 & -45 & -50 & -55 & -60 \\ \hline & & & & & & & & & & & & & & & & & &$	
Age Group λ(mμ) 6.00 530 515 460 420 Δ C.I.E.	$\begin{array}{c} & & & & & & & & & & & & & & & & & & &$	
Age Group λ(mμ) 6.00 530 515 460 420 <u>λ</u> <u>C.I.E.</u> Present	$\begin{array}{c} & & & & & & & & & & & & & & & & & & &$	
Age Group λ(mμ) 6.00 530 515 460 420 <u>λ</u> <u>C.I.E.</u> Present data	$\begin{array}{c} & & & & & & & & & & & & & & & & & & &$	

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Table 22	Mea	in ve	alues	<u>s of</u>	<u>v,</u>	for)	<u> </u>	500mi	Ļ.		
Age Group	16	21	2.6	31	3 6	41	46	51	56	61	Total
	-20	-25	-30	· - 35	- 40	- 45	 50	- 55	-60		
V ₆₀₀			No.	of	obset	cvers	3				
.600	3	8	10	8	2	4	5	3	4	4	51
" 640	11	13	15	5	11	7	4	4	3	4	77
" 680	10	10	12	15	8	11	10	7	10	8	101
.720	11	J.O	2	9	5	12	7	,5	5	5	71
~ 760	3	1	l	2	2	2	5	3	4	3	26
.80 0	2	0	l	1	2	2	3	6	2	0	19
Total	40	42	41	4 J	30	3 8	3 4	28	28	24	345
Table 23	Mea	an ve	alves	s of	V_{λ}	for)	= 5	5 3 0mµ	L		
Age Group	16	21	26	31	36	41	46	51	56	61	Total
	-20	-25	-30	-35	40	-45	- 50	~ 55	60		
V ₅₃₀			No.	of (obsei	rvers	3				
. 660	2	0	0	0	0	l	1	0	2	l	7
	1	1	1	0	0	0	0	l	4	0	8
.740	2	2	0	1	1	2	5	3	3	2	21
	5	7	3	4	5	7	5	5	7	1	49
.820											
.020	8	6	4	3	6	9	3	8	5	8	60
	8 8	6 9	4 12	3 12	6 8	9 9	3 11	8 6	5 4	8 7	60 86

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Total

40 42 41 40 30 38

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Table 24	M∋a	n va	lues	of	$V_{\lambda} f$	or λ	<u>= 5</u>	<u>15:::µ</u>			
Age Group	16	21	26	31	36	41	46	51	56	61	Total
	-20	-25	-30 .	-35	-40	- 45	-50	-55	-60		
V.515			No.	of	ob s e	rver	s				
. 360	1	Ũ	0	0	0	0	0	0	2	1	4
	l	1	l	0	1	2	l	5,	l	2	15
•440	2	0	3	3	4	5	6	4	3	3	33
	11	9	4	5	7	8	6	6	12	3	71
•520	5	15	7	10	2	8	5	5	6	11	74
	9	4	11	6	5	7	8	2	l	4	57
•600	6	10	6	9	4	2	6	4	3	0	50
	l	2	3	4	3	3].	l	0	0	18
. 680	4	0	б	0	3	3	l	l	0	0	18
	0	l	0	3	l	0	0	0.	0	0	5
Total	40	42	41	40	30	38	34	28	2 8	24	345
Table 25	Me	an v	alue	s of	V	for	λ =	420m	щ.		
<u>Table 25</u> Age Group	<u>Me</u> 16	<u>an v</u> 21	alue 26	<u>s of</u> 31	V 36	<u>for</u> 41	<u>λ</u> = . 46	<u>420m</u> 51	<u>щ</u> . 56	61	Total
Table 25 Age Group	<u>Me</u> 16 -20	<u>an v</u> 21 -25	<u>alue</u> 26 -30	<u>s of</u> 31 -35	V ₂ 36 -40	<u>for</u> 41 -45	$\frac{\lambda}{46} = \frac{1}{50}$	<u>420m</u> 51 -55	<u>н</u> . 56 -50	61	Total
<u>Table 25</u> <u>Age Group</u> V ₄₂₀	<u>Me</u> 16 -20	<u>an v</u> 21 -25	<u>alue</u> 26 -30 No	<u>s of</u> 31 -35 . of	V 36 -40 obs	for 41 -45 erve	$\lambda = 46$ $5 -50$ ers	<u>420m</u> 51 -55	<u>uu</u> 56 -50	61	Total
<u>Table 25</u> <u>Age Group</u> V ₄₂₀ .00	<u>Me</u> 16 -20 0	<u>an v</u> 21 –25	<u>alue</u> 26 -30 No 0	<u>s of</u> 31 -35 . of 0	V ₂ 36 -40 obs 0	for 41 -45 erve 2	$\lambda = 46$ -50 ers 6	<u>420m</u> 51 -55 7	<u>щ</u> 56 –60	61 4	Total 25
<u>Table 25</u> <u>Age Group</u> V ₄₂₀ .00	<u>Me</u> 16 -20 0 3	<u>an v</u> 21 -25 1 6	<u>alue</u> 26 -30 No 0 1	<u>s of</u> 31 -35 . of 0 13	V 36 -40 obs 0 9	for 41 -45 erve 2 11	$\lambda = 46$ -50 ers 6 10	<u>420m</u> 51 -55 7 ,9	9 <u>44</u> -50 5 14	61 4 15	Total 25 91
<u>Table 25</u> <u>Age Group</u> V ₄₂₀ .00 .02	<u>Me</u> 16 -20 0 3 9	an v 21 -25 1 6 11	<u>alue</u> 26 -30 No 0 1 8	<u>s of</u> 31 -35 . of 0 13 4	V ₂ 36 -40 obs 0 9 9	for 41 -45 erve 2 11 13	$\lambda = 46$ -50 ers 2 6 10 5 10	<u>420m</u> 51 -55 7 9	9 <u>44</u> -50 5 14 5	61 4 15 3	Total 25 91 80
<u>Table 25</u> <u>Age Group</u> V ₄₂₀ .00 .02	<u>Me</u> 16 -20 0 3 9	an v 21 -25 1 6 11 9	alue: 26 -30 No 0 1 8 17	<u>s of</u> 31 -35 . of 0 13 4 13	V ₂ -40 obs 0 9 9	for 41 -45 erve 2 11 13 5	$\lambda = 46$ 5 -50 2 6 10 5 10	<u>420m</u> 51 -55 7 9 8	<u>44</u> -56 -50 5 14 5 1	61 4 15 3 0	Total 25 91 80 67
<u>Table 25</u> <u>Age Group</u> V ₄₂₀ .00 .02 .04	<u>Me</u> 16 -20 0 3 9 8 11	an v 21 -25 1 6 11 9 2	alue 26 -30 No 0 1 8 17 3	<u>s of</u> 31 -35 . of 0 13 .4 13 4	V ₂ -40 obs 0 9 7 1	for 41 -45 erve 2 11 13 5	$\lambda = 46$ 5 -50 2 6 10 5 10 5 5 0	<u>420m</u> 51 -55 7 9 8 2	<u>4</u> -50 -50 5 14 5 14 5 1	61 4 15 3 0 0	Total 25 91 80 67 25
<u>Table 25</u> <u>Age Group</u> V ₄₂₀ .00 .02 .04	<u>Me</u> 16 -20 0 3 9 8 11 3	an v 21 -25 1 6 11 9 2 4	alue 26 -30 No 0 1 8 17 3 3	<u>s of</u> 31 -35 0f 13 4 13 4	V -40 obs 0 9 9 7 1 2	for 41 -45 erve 11 13 5 2	$\lambda =$ 46 5 -50 ers 6 10 5 10 5 0 6 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	<u>420m</u> 51 -55 7 9 8 2 1	<u>111</u> -50 -50 14 5 14 5 1 0	61 4 15 3 0 0 0	Total 25 91 80 67 25 18
<u>Table 25</u> <u>Age Group</u> V ₄₂₀ .00 .02 .04 .04 .05	Me 16 -20 0 3 9 8 11 3 2	an v 21 -25 1 6 11 9 2 4 5	alue: 26 -30 No 0 1 8 17 3 3 6	<u>s of</u> 31 -35 0f 13 4 13 4 13	V ₂ -40 obs 0 9 7 1 2 1	for 41 -45 erve 11 13 5 2 2	$\lambda =$ 46 5 -50 ers 6 10 5 10 5 0 6 10 6 1 0 0 0	<u>420m</u> 51 -55 7 8 2 1 1 0	₩ -50 -50 14 5 14 5 1 0 - 1 0	61 4 15 3 0 0 0	Total 25 91 80 67 25 18 16
<u>Table 25</u> <u>Age Group</u> V ₄₂₀ .00 .02 .04 .04 .06	Me 16 -20 0 3 9 8 11 3 2 0	an v 21 -25 1 6 11 9 2 4 5 3	alue: 26 -30 No 0 1 8 17 3 3 6 2	<u>s of</u> 31 -35 0f 13 4 13 4 1 1	V ₂ -40 obs 0 9 7 1 2 1	for 41 -45 erve 11 13 5 2 2 0 0	$\lambda = 46$ 5 -50 2 6 10 5 10 5 0 2 1 0 0 1	<u>420m</u> 51 -55 7 9 8 2 1 1 0 0	94 -50 -50 -5 14 5 14 5 1 0 0 0 0	61 4 15 3 0 0 0 1 0	Total 25 91 80 67 25 18 16 8
<u>Table 25</u> <u>Age Group</u> V ₄₂₀ .00 .02 .04 .06 .08	<u>Me</u> 16 -20 0 3 9 8 11 3 2 0 1	an v 21 -25 1 6 11 9 2 4 5 3 1	alue 26 -30 No 0 1 8 17 3 6 2 0	<u>s of</u> 31 -35 0f 13 4 13 4 1 1 2	V ₂ -40 obs 0 9 7 1 2 1 1	for 41 -45 erve 11 13 5 2 2 0 0 0 0 0	$\lambda = 46$ 5 -50 ers 10 5 10 5 0 10 5 0 10 0 0 1 0 0 10 0 0 10 0 0 10 0 0 10 0 0 10 0 0 10 0 0 10 0 0 10 0 0 0 0 0 0 0 0 0	<u>420m</u> 51 -55 7 9 8 2 1 1 0 0 0	<u>4</u> -50 -50 -50 -5 -5 -5 -5 -5 -5 -5 -5 -5 -5 -5 -5 -5	61 4 15 3 0 0 0 1 0 0	Total 25 91 80 67 25 18 16 8 4
<u>Table 25</u> <u>Age Group</u> V ₄₂₀ .00 .02 .04 .04 .06 .08	Me 16 -20 0 3 9 8 11 3 2 0 1 1	an v 21 -25 1 6 11 9 2 4 5 3 1 0	alue 26 -30 No 0 1 8 17 3 6 2 0 0	<u>s of</u> 31 -35 of 0 13 4 13 4 1 1 2 1	V -40 obs 0 9 7 1 2 1 1 0 0	for 41 -45 erve 11 13 5 2 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	$\lambda =$ 46 5 -50 ers 6 10 5 10 5 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	<u>420m</u> 51 -55 7 8 2 1 1 0 0 0 0 0	± 56 -50 5 14 5 14 5 1 0 0 0 0 0 0 0 0 0	61 4 15 3 0 0 0 1 0 0 0	Total 25 91 80 67 25 18 16 8 4 2
<u>Table 25</u> <u>Age Group</u> V ₄₂₀ .00 .02 .04 .04 .06 .08 .10	Me 16 -20 0 3 9 8 11 3 2 0 1 2	an v 21 -25 1 6 11 9 2 4 5 3 1 0 0	alue 26 -30 No 0 1 8 17 3 6 2 0 0 0	<u>s of</u> 31 -35 0f 0 13 4 13 4 1 1 2 1 0	V -40 obs 0 9 7 1 2 1 1 0 0 0	for 41 -45 erve 11 13 5 2 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	$\begin{array}{c} \lambda = \\ 46 \\ -50 \\ rs \\ 6 \\ 10 \\ 5 \\ 10 \\ 5 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ $	<u>420m</u> 51 -55 7 9 8 2 1 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	± 56 -50 5 14 5 14 5 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	61 4 15 3 0 0 0 1 0 0 0 0 0	Total 25 91 80 67 25 18 16 8 4 2 2

r

Table 26 Distribution with Age of V_{λ} 's for $\lambda = 460m\mu$											
Age Group	16 -20	21 -25	26 -30	31 -35	36 40	41 -45	46 -50	51 -55	56 -60	61 - 	Totals
v				No. o	f subj	ects					
.010	о	1	0	0	1	2	1	4	2	1	12
.030	7	3	2	7	4	7	10	9	10	10	69
.050	5	12	5	12	9	10	9	7	8	10	87
.070	13	10	9	3	4	6	4	4	3	0	56
.090	6	10	13	10	7	6	8	3	1	1	65
.110	3	2	3	2	2	4	1	1	1	1	20
.130	3	1	3	0	2	1	0	0	0	0	10
.150	2	0	1	1	0	0	0	0	1	0	5
.170	1	0	2	3	0	0	0	0	0	0	6
.190	0	2	2	1	0	0	0	0	0	0	5
.210	0	1	0	1	1	0	0	0	0	0	3
Total	40	42	40	40	30	36	33	28	26	23	338
		Corre	lation	coeff	icient	s of V	$\lambda^{s \text{ wit}}$	h age			
				λ (mµ)		r					
				600 530 515 460 420	+. 	156 22 25 39 49					
Table 27		<u>'</u> B	est Es	stimate	' valu	es of	<u>ν</u> _λ				
Age Group		21 -25	26 -30	31 - 35	36 40	41 -45	46 -50	51 - 55	56 60	61 →	• <u>•</u> •
$\lambda(m\mu)$											
600		.674	.680	•687	•693	.700	•708	.716	•723	•731	
530		.901	.890	.878	.869	.860	.852	.844	.838	•833	
515		•586	•575	•562	•552	•542	•533	•525	.518	•503	
460		.116	.105	.088	.081	.074	.069	.065	.060	•057	
420		.042	.038	.034	.030	.026	.023	.020	.018	.017	













Discussion of Results.

The mean values of V_{λ} show variation with age in each case. As all the results are relative to a fixed value of V_{λ} at 560m µ, they effectively measure the change with age in pre-receptoral transmission relative to any change at 560m µ. The mean V_{λ} 's are positively correlated with age at 600m µ and at wavelengths shorter than 600m µ are negatively correlated. All correlation coefficients are significant at .01 level. The correlation coefficients increase progressively as the wavelength decreases, suggesting that if the change in V_{λ} with age is indeed due to loss of pre-retinal absorption, this absorption increases for shorter wavelengths.

Distribution of Vy 's within each Age Group.

The standard deviations of the results have been given with Table 21 and the distributions are indicated in Fig. \mathcal{B} . The distributions for the other wavelengths were not shown, as the groups are not sufficiently different to make graphical representation worth-while. The distributions are similar in shape for each age group at any wavelength, becoming markedly more skow in shape for the short wavelength results. The values of the standard deviations, expressed as a percentage of the mean, seen to be quite independent of age, or at least show no discernable trend with increasing age. In calculating the standard deviations, a few deviations were not included at 420m μ and 460m μ , as they were greater than three times the average deviation, and hence completely dominated the values of s². The readings were not rejected in taking the means, however. The values of **s** are of some interest, as they reflect the

variation in response at the different wavelengths investigated. The large values of s at 420m μ and 460m μ are probably due, in part, to the method of investigation, although the work of Coblentz and Emerson (1918) indicates large variations in the values of

for their 125 normal trichromats at the short wavelength end Ч of the scale, a range of about .02 - .10 for 460m µ being recorded. Their measurements, of course, were not grouped by age and thus would include the total variation over all the groups of the present work. The values of s for 600n µ, 530n µ and 515n µ are almost certainly representative of the variations in response, as they were obtained by flicker photometry. The values of s at 525m µ are significantly higher than those at 530 and 600m µ for all age groups. This could be due to greater variations in receptor response between individuals at 515n µ, but if the author's work on nacular pigmentation (1963) is correct, the increase in s may well be due to the variation of macular pignent density between observers. The results of Coblentz and Emerson showed more spread in the yellow - red than the blue - green part of the spectrum, which is somewhat at variance with the present data. This may be due to the fact that in the case of these workers, the V_{λ} curves were plotted with a peak value of 100, whereas the present results have been normalized at 560m µ.

Comparison with Other Determinations of V_{λ}

The mean values for V_{λ} are also worth comparing with the C.I.E data (C.I.E proceedings 1924). The values at 420m μ and 460m μ are very different from those of the C.I.E data, as can be

seen from table 21 (the mean values given are for the 26-30 age group). This was not unexpected in view of some recent investigations, and of these, a few values are plotted. Those of Stiles (1955), deduced from colour mixture data, are nearest in value to those of the present data. Figs. (41-42) illustrate this agreement both in terms of the relative \mathtt{V}_λ curve for wavelengths shorter than 460m µ and for the same region normalized to unity at 460m µ. Results of Ishak and Teele quoted by Stiles (1955) are also shown. The other wavelengths are in approximate agreement with the C.I.E data, although the values for $\lambda = 600n \mu$ are higher than those given by the C.I.E curve at all ages. Considerable variations in the V_{λ} curve have been found by different investigators, as the review by Le Grand (1948) clearly shows. Some of the disagreement between the present data and the C.I.E curve could be due to the experimental conditions of small field (1° 20') and dark surround.

Estimation of 'Best Values' of Va as a Function of Age.

The mean values of V $_{\lambda}$, plotted as a function of age, show fairly steady trends, although the results for individual age groups show irregular peaks in value. Consequently, smooth curves have been drawn by eye through the mean V $_{\lambda}$'s and an estimated value of V $_{\lambda}$ for each age group has been obtained (table 27). In drawing such a curve, one assumes that the age changes occur at a steady rate, and the results for the two shortest wavelengths, where change with age is most marked, appear to justify this assumption. No estimate has been made for the youngest age group, as for this group the trend in change of V with age was unaccountably reversed

V_{λ} values and Colour Matching.

From the change in V_{λ} with age, values for the change of transmission through the ocular media can be calculated (equation 1). The results of this calculation are shown in Fig. 43, where values at wavelengths greater than 600m μ have beentaken from Said and Weale's data, corrected for pupil size. This is justified, because the present results are likely to be principally a result of changes in lens transmission (Chapter V). From the transmission curve of Fig. 43 the change in chromaticity co-ordinates of the white point S_B can be calculated. This calculation shows that the change in chromaticity co-ordinates of S_B with age, estimated from the V_{λ} data, is in excellent agreement with the experimental colour matching data (Fig. 44). This indicates that the V_{λ} and colour matching data are self-consistent, assuming that age changes in both are due to pre-receptoral light absorption.

Comparison of Age Changes in \mathtt{V}_λ with Transmission changes of the

Ocular Media.

The values of the correlation coefficients of V_{λ} with age suggest that if the age changes in V_{λ} are caused by a loss in light transmission in the ocular media, this loss must be more marked at shorter wavelengths. The values for lens ageing obtained by Said and Weale were applied to the present data. The values of change in lens transmission for a 2n.m. exit pupil were applied



FIG.43







to equation 1, using the values of V_{λ} for the 26-30 age group as normalizing values. In order to comply with the fact that the V_{λ} data were normalized at 560m μ for all age groups, the V_{χ} values calculated from lens ageing data were similarly normalized. Theresults are summarized in Figs. 45-49 and as can be seen, the general agreement between the calculated V_{λ} 's and the 'best estimate' set is quite good. There are divergences, however, for at 600m u, 530m μ and 515m μ lens ageing gives larger changes in V than are found experimentally, whereas at 460m µ and 420m µ the reverse is true. Statistical investigation of the differences was somewhat difficult, due to the skew nature of the distribution of V_{λ} values in individual age groups (Fig. 36). However, assuming the distributions to be 'not too skew' (Crow et al), the t^2 test, as described in Chapter V, was applied to the 21-25 and the 61-70 age groups. The object was to test whether the differences in ${\tt V}_{\!\!\lambda}$ values between these groups, as measured esperimentally and as calculated from lens ageing, were statistically significant. It was found that there was a statistical difference at all wavelengths at .01 level of significance.

This may be due to the fact that the 'best estimate' values of V_{λ} are not accurate, but considering the degree of consistency between the colour matching data and the V_{λ} data, there seems to be a small, though real, deviation from the lens ageing data. The general trend of wavelength dependence and the order of magnitude of the age effect is, however, much the same for the lens and for the colorimetric data.





The V_{λ} data were also compared with absorption due to possible density increases in nacular pigment with age. The work of Wald (1945) was used for this purpose and this indicates that increase in macular pigment density should only be detected in the 460m m and 420m m V_{λ} values of the colorimetric data. The change in the C.I.E. V_{λ} values due to a 100% increase in the pigment given by Wald is shown in Fig. 50.

Consider the values of V at $460n \mu$ and $420n \mu$. If the transmission of a filter placed before the eye is t_{420} at $420n \mu$ and t_{460} at $460n \mu$

$\frac{v_{420}}{v_{460}} = \frac{t_{420}}{t_{460}} \left(\frac{v_{420}}{v_{460}} \right)$

gives the relative change in the V_{λ} curve due to this filter at the two wavelengths. V_{420} etc. refer to the values of V_{λ} with the filter in place. From a combination of Beer's and Lambert's laws:-

$$t_{420} = 10^{-k_{420} c x}$$

where c is the concentration of absorbing pignent in the filter, x the pigment thickness and k_{420} is the nolecular extinction coefficient.

Hence:-

$$t_{420}/t_{460} = 10^{-cx(k_{420}-k_{460})}$$
(2)

If $k_{420} < k_{460}$, the ratio of (2) will be greater than unity for all positive values of c, and so the ratio will increase with increase in c.

From the absorption curve of crystalline xanthophyll, from that of extracted macular pignetit (Wald 1945) and from the work of the



author (Ruddoch 1963), it is seen that $k_{420} < k_{460}$. Referring to equation 2, it can be seen immediately that the ratio $\frac{V_{420}}{V_{460}}$ should increase with age, if this is accompanied by increasing macular pigment concentration, whereas in fact, a steady decrease occurs (Fig. 51). In this figure, ratios calculated for the present data were colculated from actual results (not 'best estimate') and the effect of macular pigment change was calculated assuming the change in V_{460} to be due to change in macular pigment density. The change in the ratio V_{420}/V_{460} due to lens ageing is also shown, and it can be seen that this agrees fairly well with the present data. Further, the divergence from lens ageing is in an opposite sense to a divergence caused by macular pigment increase accompanying the increase in lens absorption.

Cause of Differences in Present Data and Lens Ageing Data.

Both the colour matching data and the V_{λ} data diverged somewhat from the predictions based on age changes in the crystalline lens. However, only the differences in the V_{λ} values were statistically significant. As previously mentioned, this could be due purely to the fact that the 'best estimate' values of V_{λ} did not really represent, accurately, the change with age. However, at 460m µ and 420m µ, the actual V_{λ} values lie very closely to the 'best estimate' values and hence reasons for the disagreement should be sought. The fact the Ludwigh & McCarthy's data show greater absorption in the blue end of the spectrum, relative to the red, compared with the lens data of Said & Weale (Fig.6) suggests other sources of pre-receptoral light loss. The cornea and the vibreous humour seen the nost probable media for such a light loss, and the latter is reported as becoming yellowish with age, Weale (1961a). It could be, therefore, that the numerical divergence between the present data and lens ageing data is partially caused by light loss in the ocular media other than the lens, most probably in the vitreous humour. This effect would have to be small compared with the lens ageing, however, as this latter is of sufficient magnitude to give changes comparable in magnitude to the changes measured in the colorimetric data. Further, this does not explain the fact that the lens data predicts larger changes with age in V_{λ} at 600m μ , 530m μ and 515m μ than are actually found.

Physical Basis of the Pre-receptoral Light losses.

In discussing the physical basis of light losses which occur with age, it must be borne in mind that the lens is the cause of the majority of this light loss, Hence, the discussion of Said and Weale (1959) will be reviewed in the light of the present data.

From the combination of Beer's and Lambert's laws :-

$$I = I_0 10^{-x} e^{-c}$$

where I is the transmitted flux and I_0 the flux incident through an absorbing modium of concentration c, thickness x and nolecular extinction coefficient $\boldsymbol{\epsilon}$. Hence:-

$$-\log \underline{I} = D = x \boldsymbol{\ell} \boldsymbol{c}$$

where D is the optical density of the medium.

Therefore

 $\log D = \log x + \log c + \log \epsilon$

which yields an arithmetic increase in logD at all wavelengths for a change in concentration.

In the crystalline lens, the thickness changes with age, and so:-

 $\log\left(\frac{D}{2}\right)$ plotted for different ages should give a series of parallel lines, if age changes were due to increase in concentration of a single pignent. Said & Weale showed that the lens optical density, plotted as above, did not give a series of parallel curves at different ages. A number of pignents, each contributing to the age changes in transmission, could explain the curves, but Said and Weale came to the conclusion that Rayleigh-type scattering must play at least some part in the transmission changes.

The present data has been examined for evidence of age changes occurring due to light scatter.

Rayleigh's law states that, for a small centre scattering light waves :-

$$I_{S} = \frac{k}{\lambda^4} I_{C}$$

where I_s is the intensity of the scattered light and I_o that of the original beam, λ the wavelength of the light and k a constant. For absorption in a medium, giving Rayleigh-type scattering in medium of thickness dx:-

$$dI = -KIdx$$

is the light lost in the medium from a beam of intensity I.

Hence, for thickness me-

$$\left[h_{\text{og I}} \right]_{I_{\text{o}}}^{\text{I}} \qquad \left\{ -K_{\text{X}} \right\}_{\text{o}}^{\text{X}}$$

and $K = -\frac{k}{\lambda^{4}}$ for Rayleigh scattering $\log \frac{I}{I_{0}} = \log_{e} t = -\frac{kx}{\lambda^{4}}$ or $\log_{10} t = -\frac{xK'}{\lambda^{4}}$,.....(3)

hence, as x changes with age, $\frac{\log_{10} t}{x}$ should give a straight line, against $1/\lambda^4$.

Equation (3) was applied to the present data, to find how closely changes in ocular transmission with age were described by it. The values of Said and Weale for lens ageing were taken as the change in transmission at 600h μ . The ocular transmission values obtained from the V_{λ} data were then plotted as a function of age, relative to the 600m μ values (Fig. 52). For two wavelengths λ_{1} and λ_{2} , the changes in transmission in a scattering medium, from equation (3), becomes:-

$$\frac{\log_{10} t_1}{\log_{10} t_2} = \frac{\lambda 2^4}{\lambda 1^4}$$

which is independent of x.

Using the above equation, calculating relative to the values of log (transmission) at 600m μ , the transmission of the optical media at other wavelengths was calculated. The results of the calculation are plotted in Fig. 52, together with the values of log (transmission) deduced from the V_{λ} data. As can be seen, the two sets of data are very similar, both in their wavelength dependence and in the magnitude of the changes in transmission.

It seems, therefore, that the age changes in the ocular media are, at least in part, due to scattering, which must arise mainly in the lens. Scattering could also be of some importance in the vitreous humour, where carbohydrates(polysaccharides) are known to



crosses - log t, calculated from Rayleigh's law

be deposited with age.

It must be noted that in calculating the transmission values shown in Fig. 52, the wavelength λ at the cornea was substituted in equation (3). In fact, a term $\left(\frac{n}{\lambda}\right)_{4}^{4}$ should be used instead of $\left\{\frac{1}{\lambda}\right\}_{4}^{1}$, as scattering would occur mostly in the lens, of refractive index about 1.4. No data on the variation with wavelength of the refractive index of the lens could be found, but assuming it to follow the normal type of variation with wavelength, the correction would be too small to invalidate the present conclusions (about a 4% increase in $\frac{1}{\lambda}_{4}$ in the violet end of the spectrum, relative to the red end). This correction would, however, increase the quantity of scattered light by a factor of about 4.

Scattering in the Ageing Lens.

The conclusion that the scattering may be the main cause of increasing light losses in the ageing eye implies increase of scatter in the ageing lens. Loss of water, increase in the number of fibres and hence in weight, change in refractive index and irregular surfaces of the fibres and depositing of insoluble proteins were all mentioned in chapter I as occurring in the ageing eye. These could all contribute to increased light scattering, as they all tend to give rise to irregularities of refractive index. Salmony and Weale (1960), in a brief note, state that they could not find differences in pignent concentrations, taken from crushed young and old human lenses to account for the changes measured in the lens transmission. No numerical details were given, however. The lens pignent has been variously identified as a melanin pignent

by Fischer (1948) and a urochrone, McEwen (1959).

Whether scattering in the lens should be Rayleigh-type is another question which needs consideration. Rayleigh originally derived his law for scattering by small, independent light scattering sources. Scattering in solids and liquids can be of the Rayleigh type, although the theory requires modification. Theiner & Lell (1953) published results of scatter neasurements in optical glasses. They found that glass with imperfections gave much larger scattering than ordinary glass, and maxima in the wavelength dependence of the scatter were sometimes found. Mueller (1938), in theoretical grounds, suggested mechanical strains in glass as a cause of the large scattering sometimes found. Finally, Stacey (1956) points out that Rayleigh-type scattering can occur for particles with one dimension of the order of wavelength of light (the others being very small), although this leads to asymmetrical scattering, instead of Rayleigh's classical case of symmetrical scattering.

Applying the above considerations to changes in lens structure with age, it seems not unlikely that increased light scatter could result from these changes. The decreased water content and build up in the number of fibres could well lead to irregularities of refractive index. Further, the depositing of insoluble protein particles would also give a larger number of scattering centres in the lens. It is of interest that light scattering is used as a means of measuring molecular weights of proteins and viruses (Stacey 1956).

Chapter V11

Results for 3 Individuals.

Results were obtained for three observers, for whom earlier data exists.

1. <u>W.D.W</u>

Results were recorded for colour matching of the white point, S_B, for which previous data of W.D.W were available, at the ages of 22 years and 38 years (fig. 9). The experimental conditions were modified from those described in chapter V, a field size 2° square being used in conjunction with a lm.m. exit pupil. This change of field condition made the experimental conditions comparable with those used in the previous measurements by W.D.W.

Results.

The chromaticity of S_B was measured as:-

.275 (R) + .420 (G) + .305 (B)

The result is illustrated in Fig. 53a. As can be seen, the data for W.D.W do not conform to the general trend found in Chapter V, because the chromaticity of S_{g} is virtually unchanged between the ages of 38 years and 57 years. The difference between the observer W.D.W and the average data is further illustrated in Fig. 53b.

This disagreement between the results of an individual and the statistical data of the large group is difficult to explain. That receptoral response has not changed in the case of W.D.W. was checked by calculation of the chronaticity co-ordinates of wavelength 494n µ, which were found as:-



(494) = -.116(R) + .558(G) + .558(B)

which is identical to the data given for W.D.W at the age of 38 yrs.

This result may be indicative of irregular changes with increasing age of transmission in the ocular media of a given individual, rather than the steady **de**crease which appeared to occur for the average of the large samples of Chapters V and VL.

2. <u>F.L.W</u>

Results were again taken for colour notching of S_B , and again the modified field conditions described in the previous section were utilized. The relative luminosities of the matching stimuli for observer F.L.W were also neasured, as described in Chapter 1V.

Results.

The chromaticity co-ordinates of SB were found as

.310(R) + .435(G) + .255(B).

compared with

.272(R) + .376(G) + .352(B)

for a measurement 30 years previous.

The result is illustrated in Fig. 53a and shows that the chromaticity of S_B has progressed towards the spectrum locus with increase in age. The magnitude of the change is approximately as would be expected from the data of Chapter V, although the direction of the shift is somewhat different. It is of interest to note that the change in chromaticity of white point S_B is closer to the shift measured in the present work than to that calculated from the lens ageing data (fig. 30). This appears to confirm that



CHROMATICITY COORDINATES OF W.D.W. COMPARED WITH THE COORDINATE DISTRIBUTION FOR THE RESPECTIVE AGE GROUP.

lines give the values for U.D.W. 'n' - fractional distribution of co-ords for the age groups.

the data on the wavelength dependence of transmission changes with age given in chapter Vl, are fairly reliable.

The relative luminosity of the matching stimuli were found as :-

 $V_{(R)} = 79.6; V_G = 100; V_B = 5.3$

compared with

 $V_{(R)} = 71.5; V_{G} = 100; V_{B} = 4.9$

There is some divergence in the two results for the red stimulus. Although the difference is small compared with the normal range of values of V_R (chapter 1V), it is rather larger than would be expected from experimental error. None the less, the data do seem to confirm that the receptoral response remains unchanged with age. The results for F.L.W were taken from Martin et al (1933) and from private communication.

3. <u>A.G.G</u>.

The chronaticity co-ordinates of S_B were found for A.G.G., an aphakic observer. In this case, the field conditions were as for the general survey, viz. 1°20' square field and a 2n.m exit pupil.

Results.

The chromaticity of S_B was found as

.127(R) + .216(G) + .657(B)

and this is plotted in Fig. 54. In a paper by the observer (Gaydon (1938) it is stated that, before renoval of the lens, his colour vision was normal. This statement was based on measurements by Pitt and hence it is presumed that the chromaticity of $S_{\rm B}$ lay within the normal range, before removal of the lens. Fig. 54 shows


that the present S_B lies well outside the range for normal observers of the age group to which A.G.G. belongs (46-50 year group). In fact, the result indicates that a yellow filter has effectively been removed from before the receptors, as would be expected. The result of removing the lens is sufficient to give A.G.G. less pre-receptoral yellow pignent than the extreme of the youngest age groups. This result is indicative of the great effect which the lens has upon the chronaticity co-ordinates of S_B . More quantitative analysis was not attempted, because of the great enhancement in aphakic observers to the blue sensitivity by ultra-violet light. Gaydon reports this (1938), as does Wald (1949), and this effect would invalidate comparison between colour matches of aphakic and normal trichromatic observers.

Conclusions.

The results reported in this chapter provide a check as to whether or not the data of the survey are applicable to individual observers. As the previous data have been fairly well explained by the data on lens ageing of eleven observers, as measured by Said and Weale, it was expected that individual results would show agreement with the data of the survey.

The conclusion that the receptor response remains unchanged with age is substantially confirmed by the colour match of 494m u for W.D.W and the values of the relative luminosities of the matching stimuli of F.L.W. The transmission changes with age in the ocular media are not so well borne out by the present results. However, as was pointed out, the results do tend to confirm the

fact that the lens data of Said and Weale yield an incorrect prediction of the change in chronaticity of S_B with age. It appears that the shift with age in chromaticity is towards a shorter wavelength in the spectrum locus than is predicted by Said and Weale's data. The data of the aphakic observer show the extent to which the lens absorption affects colour matching data.

CHAPTER V111

Variation with Age in Performance on the Farnsworth-Munsell 100 hue Test.

As previously mentioned (Chapter I) Verriest (1963) and Verriest et al (1962) found that the results of the 100-hue test for hue discrimination were significantly correlated with age. These observations were confirmed in the screening tests carried out by Mrs. Birch, preliminary to the present colorimetric investigation.

The test, designed by Farnsworth (1943) as a diagnostic test for defective colour vision, uses 85 of the Munsell colour chips of equal value (lightness) and chroma (saturation), which have to be arranged in order of successive hues. The chromaticity of the colour samples, which depends upon the type of illumination used, will vary with any coloured filters interposed between the observer and the test. It was shown by Verriest that performance on the 100-hue test was affected by yellow filters placed before the eye, and the change in lens transmission with age must therefore affect the results of the test.

The work reparted in this section was an effort to predict the general form of the change of colour response with age, as measured with the 100-hue test.

Experimental.

The spectral reflection curves of the Munsell 100-hue discs were neasured, using a Beckman D.K. recording spectrophotometer. The discs were sufficiently large to reflect the whole of the spectrophotometer beam, and were mounted behind a sheet of black cardboard, into which a hole was cut for the disc. Magnesium oxide was used as a comparison surface and 100% and zero lines were checked before each measurement. A few results are shown in Fig. 55.

The energy distribution of source C was taken as the spectral composition of the illuminant, this being the approximate source used for both Verriest's and Birch's investigations. The effect of ageing on the visual system was taken as equal to the lens ageing measured by Said and Weale, this being virtually equal to the age changes deduced from the present colorimetric investigation. The computation was performed for a 3m.m. exit pupil, that is, for a condition of light adaptation. Under such conditions, the variation of pupil size with age produces virtually no variation in the correction to the lens ageing data for pupil size (Weale 1961b). This assumption of virtual light adaptation was true for the colour matching cabinet used by Mrs Birch.

The tristinulus values of the Munsell chips were then computed as

and similarly for Y and Z, these being the tristimulus values. $\overline{\mathbf{x}}_{\lambda}$ is the distribution coefficient of the 2° C.I.E. observer data; P_{λ} the relative energy of source C; β_{λ} the spectral luminance factor of the sample and t_{λ} a factor representing the change in lens transmission with age. λ represents wavelength in each case. Computation was performed in the range 400 - 700m μ , this being the range in which lens ageing data is availabe, and



computation was performed at 10m µ intervals. The tristinulus values of equation (1) were normalized to give chromaticity co-ordinates and these were transformed to the Breckenridge and Schaub (1939) uniform chromaticity system. The equations used for this were:-

$$x'' = \frac{.823 (x + y - 1)}{1.0x - 7.053y - 1.64} \qquad x' = .075 - x''$$
a)
$$y'' = \frac{3.697x - 5.077y - 1.369}{1.0x - 7.053y - 1.64} \qquad y' = y'' - .50$$

where x and y are the C.I.E chromaticity co-ordinates, and equations b) adjust the U.C.S. such that the equal energy point is central.

The computation was performed using an Elliot 803 computor belonging to the Imperial College optics department. Three sets of values were computed, with values of t_{λ} corresponding to lens ageing at 45 years and 65 years and for the normal C.I.E. observer, that is with t_{λ} equal to unity, to represent the young observer. <u>Results</u>.

The results are illustrated in Figs. 56 - 57. Figure 56 gives the C.I.E co-ordinates for the three age groups, a continuous curve having been drawn between the samples. The locus of co-ordinates for the standard C.I.E. observer shows close identity with the results given by Granville, Nickerson and Foss (1943) in their description of the 100-hue test. Figure 57 shows the position of the coloured discs in the uniform chromaticity scale and every fifth sample co-ordinate has been numbered.

Discussion.

A first inspection of the results immediately shows either



FIG. 51



that the 100-hue test does not consist of equally spaced hues or that the Breckenridge and Schaub chart is not a truly uniform chart. Close grouping occurs in the red (81 - 10) and the blue-green (38 - 54) regions. Further, this close grouping becomes more marked as age increases, in agreement with the loss of discrimination in these regions as age increases, reported by Verriest et al (1962) and by Birch.

The following quantitative investigation of the results has been attempted on the basis of certain assumptions, in particular that the Breckenridge and Schaub chart is effectively uniform. This is probably justified for the fairly restricted chromaticity range in the central area of the chart, where the Farnsworth-Munsell discs are located. On this assumption, the probability of discrimination occurring between two points is then . proportional to the distance between then in the chart. The method of scoring the proofs on the Farnsworth-Munsell 100-hue test does not give a direct measurement of the discrimination sensitivity of the observer, but it has been assumed that the ratio of error scores for different regions of the Munsell locus is directly proportional to the ratio of the chromaticity separation of the hue samples in these regions.

Fig. 58 shows the inverse of the distance between every five discs, as measured in the U.C.S. chart, i.e. the distance between 85 and 5, 5 and 10 etc., for the standard C.I.E. observer. They therefore give an estimate of how the colour confusions in the 100-hue test are distributed for young observers. The envelope



of Verriest's results for the 26 - 30 age group has also been plotted with an arbitrary zero. Fig. 59 shows the results of Birch for the 26-30 group, analyzed to give the error scores in every set of five discs. In Fig. 53,the curve has been normalized with present calculation at the peak value, and the agreement between calculation and experiment is fairly good, at least as regards the position of maxima in the error score. The one region of disagreement is in the red region, Nos. 1 - 10, where actual discrimination is better than would be expected from calculation. This may be due to lack of perfect uniformity in the Breckenridge and Schaub chart.

To estimate the effect of age, inverse ratios between distances in the U.C.S. chart for the young and the two older age groups were taken, again for distances between every fifth disc. These ratios are given in Fig. 58 - 59 and as can be seen they predict that a loss in discrimination should occur, with increasing age, in the red and blue-green hue regions. This is as found experimentally by Verriest and by Birch, and the results of the latter investigator have been analyzed to provide a comparison. The ratios of the error scores for the young and old age groups are plotted in Fig. 59. These data show good agreement with the calculation, although they require to be normalized to the calculated values to give accurate agreement. This requirement indicates the main source of disagreement between the calculated and experimental age changes, namely that the old observers should show better discriminating power in certain regions of the 100-huelocus (e.g. Nos. 70 - 75), whereas, in actual fact, there is an overall

FIG. 59 ESTIMATE OF MUNSELL SLORES

5 - value of Munsell' scores for the two age groups, relative to the 26-30gr age group (data obtained from survey of Birch).

> Broken curve gives the values of this relative score, calculated from lens ageing data.





loss in discrimination with age.

There are two possible explanations of this disagreement. One is that there is an overall loss of hue discrimination with age, as could be inferred from the present work on the J.N.D. wavelength discrimination steps. However, the non-homogeneity of the observers referred to in that case was also true in the case of the 100-hue tests carried out by Birch. The results of the 46-50 yr old age group, which was composed almost equally of observers with scientific and technical training and those without are shown in Fig. 60, plotting the two groups separately. The difference in the number of errors made by the two groups is very marked and as nearly all the younger age group possessed scientific or technical training, this could well cause the apparent general loss of discrimination with age. Such an explanation is in keeping with the discrimination data obtained by the forced choice technique in the present work. The overall loss in light transmission due to the lens is unlikely, at least in the case of the results of Birch, to lower the illumination to mesopic levels, in which conditions the age changes in the 100-hue test are simulated by young observers (Verriest et al 1963).

Conclusions.

It has been shown that the selective nature of the loss of hue discrimination with age, as measured on the 100-hue test, is predicted qualitatively and quantitatively by consideration of the change in lens transmission with age. An explanation for the general loss in discrimination with age throughout the 100-hue test, has also been suggested.

Chapter 1X

Light Transmission Properties of the Macular Pignent and the

Human Crystalline Lens.

1). <u>Macular Pigmentation</u>.

Introduction.

In assessing the changes with age in transmission of the optical media of the eye, the possibility of increase with age in nacular pigment density was examined. Although there was no indication that such an increase did occur, the present colorinetric survey did provide an opportunity of investigating the wavelength dependence of macular pigment absorption.

Wright (1928-29) published obsorvations on the chromaticity co-ordinates of the white source S_B , for 31 normal trichromats. The large variations observed in these white points was attributed, at least in part, to variations in the density of nacular pignent in different observers. It was pointed out, howerver, that receptoral variations would also cause differences between observers in the white point chromaticities. The macular pignent, a yellow pignent found in the central forea of dissected human retinae, was first observed by Soormering. Gullstrand (1906) claimed that the pignent, which he identified as a carotenoid, was a post-morten effect. Wald (1945; 1949) identified the pignent as a carotenoid and also published an absorption curve, which was very similar to that of crystalline zanthophyll, with which he tentatively identified the macular pignent. Wald also found that the pignent occurred in variable anounts in different observers, from no pignent in some observers to quite high densities in other observers. This fact fitted Wright's experimental observations very satisfactorily, and also renders the 'post-morten effect' explanation difficult, because of the fact that some dissected eyes had no detectable pigment. Rushton (private communication) reports that the pigment is visible under a slip lamp illumination.

Walls and Mathews (1952) suggested that the cause of the difference in colour-vision response of the foveal and parafoveal regions of the eye was a difference in distribution of receptors in the different retinal regions. This distribution was supposed to be variable in different observers. Brindley (1960), however, pointed out that such an explanation would necessitate a variation in hue of monochromatic light at different retinal locations.

Although the pignent distribution in the retina is not known exactly, the post-mortem yellow spot is restricted to the fovea. Wright (1946) measured the chronaticity co-ordinates of the white point S_B at retinal locations away from the central fovea and found that the white point moved away from the yellow part of the spectrum locus as it was located further from the fovea (up to 6° away). This he interpreted as being due to the removal of the screening macular pignent, as the field was shifted from the fovea.

Ains of the Present Investigation.

The present work has been carried out with two ains. Firstly, in order to find the extent to which the change in colour vision response with change in retinal location is due to macular pignent changes and to what extent it is due to variation in receptor response. The second ain was to deduce an absorption curve for the macular pignent from colour matching data.

Colour Matching for White Point S_B in the Parafovea and Fovea. Observers.

Four observers were chosen from the 150 young observers taking part in the colour vision and age survey. From Fig. 61 it can be seen that observers matching the white point S_B near point K will be observers with small concentrations of macular pigment, whereas those close to L will have large concentrations. Observer K.H.R possessed heavy pigmentation and R.A.H. about average amounts, whereas J.A.S and M.G. both possessed little if any pigment. As previously mentioned, variations in receptoral response will also cause changes in the white point chromaticity co-ordinates. To check the similarity in response systems of the four observers, the following may be noted.

1) All four observers possessed normal wavelength discrimination (some results for K.H.R and J.A.S are given in Chapter 111).

ii) The spectrum loci of the four observers were all near the normal. A comparison for K.H.R and J.A.S is given in Fig. 62.

iii) The relative luminances of the matching stimuli of the W.D.W system were

<u>Red stinulus</u> (650n هر)		<u>Blue Stimulus</u> (460m u)
M₊G	62.4	5.5
R.A.H	50.0	5.0
J.A.S	79.6	5.1
K.H.R	66.0	7.0



FIG. UChromaticity co-ordinates of the spectrum for ten observers as measured by Wright. Shown also are the chromaticities obtained for the white point S_B by the present author from observers who took part in the initial survey.





Results relative to a green stimulus (530m µ) luminosity of 100.

Although these are variable from observer to observer, they are well within the range of normal observers given in Chapter IV, and do not show any consistent trend with the estimated macular . pigment concentrations.

As the three sets of observer data discussed above are all independent of pre-receptoral absorption, it seens unlikely that differences in the receptor system of the observers will greatly affect comparison of the data for the four observers.

Experimental.

The colour matching was performed, as before, with the Wright colorimeter. The Romis filter, described in Chapter 11, was used with a sub-standard S_A source to give the standard source S_B . A red fixation spot was provided by the adapting source of Fig. 10, which was reflected into the eye by means of a reflector in the eye-piece. As each observer used the right eye, the field was always situated on the temporal side of the forea.

For extra-foweal matching, a flash and recovery cycle was used to overcome Troxler's effect and progessive adaptation in the matches. Following the work of Clarke (1960) a fixed number (15) of flashes was used to obtain a match, the cycle being of 1 sec. exposure and 3 sec. darkness. No separation of the fields was required for the small eccentricities used in this work. Some 15 min. dark adaptation preceded each series of measurements, matching sessions lasting for about 1 hour.

Results.

Figures 63 - 66 show the results of colour matching using a $1^{\circ}20^{\circ}$ field at various eccentricities and also a 10° centrally fixed field. The points are plotted in the W.D.W. system of units, using the wavelengths 494n µ, and 582.5n µ as the fixed points of the system, with 650n µ, 530n µ and 460n µ as the instrumental matching stimuli (R), (G) and (B). Each point was measured on three or four separate occasions for each observer. Rather than plot a single value with it s standard deviation, it was preferred to show the means of each individual matching session and take the areas enclosed by these points as respresentative of the error associated with the measurement.

Discussion.

The author considers the present measurements as strong evidence of a pigment existing in the forea of the living eye, the optical density of which can vary considerably from observer to observer. A difference in receptor response would be an extremely unlikely explanation of the foreal differences in the chromaticities of S_A and S_B as matched by K.H.R and J.A.S or M.G.as has been explained in the introduction.

The macular pignent absorption data given by Wald (1949) has been used to calculate the dominant wavelength of the macular pignent for sources S_A and S_B . The calculation was performed by considering the pignent as a filter situated before the response system. The distribution coefficients were obtained from experimental results for K.H.R and have been used in the calculation. For R.A.H.,



FIG. **b** The chromaticities of the white points S_A and S_B as matched at different retinal locations by K.H.R. The eccentricities of the centre of the field is given in degrees with each set of matches. Also shown are the white point changes due to changes in optical density of xanthophyll.

- — chromaticities of S_A for 1°20′ fields. — chromaticities of S_B for 1°20′ fields.
- -chromaticities of S_A for 10° fields.
- —chromaticities of S_B for 10° fields. M
- -chromaticities of white points due to changes in xanthophyll optical density.







FIG. 45 The chromaticities of the white points S_A and S_B as matched at different retinal locations by R.A.H. Symbols as for Fig. 43.



FIG. **61**. The chromaticities of white points S_A and S_B as matched at different retinal locations by J.A.S. Symbols as for Fig. **6.3**.

howover, the C.I.E standard observer data were assumed (Commission Internationale de l'Eclairage, 1931 proceedings).

The results for the non-pigmented observers J.A.S and M.G indicate that little change in response occurs within the para-foveal area investigated. Therefore, the changes in chromaticity observed for K.H.R and R.A.H should be due to changes in concentration or thickness of the macular pigmentation. In that case, the chromaticity changes should correlate closely with the dominant wavelength curves of xanthophyll. In the case of R.A.H agreement is quite good for both sources S_h and SB, whereas for K,H.R agreement is quite good for source S_R but not for S_A . The results of the latter observer are widely separated in the chromaticity chart and hence any disagreement between the calculated and experimentally measured results is more easily detected. A further notable point is that the directions of calculated shift in chronaticity due to variations in xanthophyll density are closely similar for K.H.R and the standard observer. This demonstrates that the trichromatic response systems for the two cases are very similar.

The 10° white points for the "pigmentless" observers agree closely with those for the small field matches, whereas for the other observers the 10° point is located in the chromaticity chart close to the small field white points measured at 2.5° and 5° from the forea. These results suggest that the differences in chromaticity between the two field sizes, yiewed foreally, can be ascribed, in the main, to the filtering effect of the macular pigmentation upon the small field. For R.A.H and K.H.R the large field showed a lack of

uniformity in the blue-green matches, having a marked central "Maxwell's Spot". This was ignored by the observers, when matching, and apparently did not exist for the two other observers. Hence, for a given observer, the comparison of the chromaticities of white matches for small and large fields is a good indicator of the density of macular pigmentation within the eye, assuming "Maxwell's Spot" is ignored in the 10° case.

The fact that such large variations in nacular pigment density occur from observer to observer makes any functional explanation of the pigment, such as thatattempted by Dartnall and Thomson (1949), difficult to accept, especially as some observers appear to have virtually no pigment. The hypothesis of Judd (1952), namely that the pigment protects the fovea from over-stimulation by blue light such as causes long-lasting after-images, may be valid. It is hoped that further experiments will be carried out to investigate his suggestion.

The Spectral Absorption Curve of the Macular Pigment.

The measurements of the previous section showed that, in the cases of J.A.S. and M.G., the proportions of (B) to (G) required to match 494m μ were constant with change in retinal location of the colorimeter field. For K.H.R. however, a large change in this ratio was noted, and a similar but small change in the case of R.A.H. This variation in the matches of the second pair of observers was attributed to the change in optical density of the macular pigment with change in retinal location. Further, it was noticed that a variation also occurred in the proportions of (R) and (G) in the

582.5m μ match, although the effect was less marked than for the blue-green match. If this latter observation were also due to the pigment absorption, it is at variance with the chemical identification of Wald, since the absorption by xanthophyll of 530m μ or 650m μ is negligible (Fig. 67). If similar measurements are carried out with other sets of matching stimuli and test colours, it is possible to deduce the absorption curve of the macular pigment.

Thus, consider two points of the retina with the same cone response, of which one point is covered with macular pigment and the other clear of pigment. Let F_1 and F_2 be the luminous flux of the wavelengths λ_1 and λ_2 required to match a wavelength λ at the pigment free point and F_1 ' and F_2 ' their flux for a match at the other point. Further, let the transmission of the pigment at λ_1 and λ_2 be T_1 and T_2 . Then:

$$F_{1}/F_{2} = F_{1}'T_{1}/F_{2}'T_{2}$$

which expressed log-rithmically becomes:-

$$\log F_1 - \log F_2 = \log F_1' - d_1 - \log F_2' + d_2$$
(1)

where d_1 and d_2 are the pignent optical densities at λ_1 and λ_2 , logs being expressed to the base 10.

But from experiment we have:-

$$\log F_1 - \log F_2 = \delta_2 \text{ and}$$
$$\log F_1' - \log F_2' = \delta_1$$

Where $\log F_1$, etc., are obtained directly from the instrument scales, which are calibrated logarithmically, therefore

$$(\log F_1 - \log F_2) - (\log F_1' - \log F_2') = -(\delta_1 - \delta_2)$$

and from (1)
$$d_1 - d_2 = \delta_1 - \delta_2$$
(2)



If an observer possessed no macular pigment.d1 and d2 would be zero and hence, from (2), $\delta_1 - \delta_2$ should also be zero. This calculation has assumed equal cone response at different retinal locations, and the validity of this assumption has been checked by finding the values of ($\delta_1 - \delta_2$) for an observer with negligible macular pigmentation.

Expression (2) gives the difference in optical density of the pignent at two wavelengths, assuming that one of the retinal locations at which the measurements were taken is free from pigment. If, as is almost certainly true for the present work, meither point is clear of pigment, the optical densities obtained will be proportional to the difference in concentration of the pigment at the two points. Such a means of measuring the optical density of the macular pigment depends solely on changes in physical stimuli and is independent of any subjective assessment of colour quality.

This principle has been applied using matching stimuli over the range 580m μ to 440m μ . It was not possible to extend the range of measurements beyond 440m μ as there was insufficient light at wavelengths shorter than this for them to act as matching stimuli. Instead, the changes in a heterochromatic brightness match at different retinal locations were used to obtain the optical density of the pigment in the short-wave region. In this case, δ is the logarithmic change in light flux required to maintain the brightness match between λ_1 and λ_2 as the field is located at different positions on the retina. This method could have been used to obtain the rest of the density curve, but heterochromatic brightness matching is a less reliable technique than colour matching.

Experimental.

Two observers, K.H.R and J.A.S., were used for the measurements. The results of J.A.S. were compared at one or two points with the results given by M.G. in the first part of this work.

Wavelengths between 590m μ and 520m μ , taken in steps of 10m μ , were in turn used as one of the matching stimuli, 650m μ providing the other matching stimulus. The comparison stimulus was in each case provided by an intermediate wavelength, this latter being chosen such that it was as sensitive as possible to changes in both the matching stimuli. This region of the spectrum locus is sufficiently dichromatic to require no desaturation for matching. Wavelengths between 450m μ and 490m μ , again taken in steps of 10m μ , were used as one matching stimulus with 530m μ providing the other, and similarly wavelengths between 520m μ and 490m μ were mixed with 460m μ . In the two latter sets of matches the intermediate matching stimulus was provided with a minimal amount of red desaturation.

The trichromatic nature of the match was ignored for purposes of calculation, a red matching stimulus being used merely to obtain a satisfactory match.

The field was again presented in a flash and recovery cycle, this time only eight flashes being utilized, so as to minimize any progressive adaptation in the matches. The retinal positions investigated were at the foven and 2° eccentrically from the foven, the field again being situated on the temporal side of the fovea in the latter case.

<u>Results</u>.

The results are summarized in Fig. 68. Each point represents the mean of five readings taken in one session. Each wavelength was matched on at least three separate occasions. The point at 490n μ shows rather greater spread in value than other wavelengths, due to the fact that for reasons of colour mixture and geometry of the instrument, it was difficult to devise a sensitive match using this wavelength. The curve shown is a composite curve, the points between 440m μ and 490m μ having been obtained relative to the value at 530m μ and those between 520m μ and 490m μ relative to the value at 460m μ . Further, the points between 400m μ and 440m μ were determined relative to the values at 450m μ .

Discussion.

The interpretation of the curve in terms of macular pignent absorption seens well founded, as the observers M.G. and J.A.S. show practically no variation in the function plotted. Further, the point at 520m μ yields values for the optical density of the pignent, relative to the absorption at both 460m μ and 650m μ which are selfconsistent, an extremely unlikely occurrence if receptor variations were the cause of changes in matching.

The results show an absorption curve for the macular pigmentation which is of an appearance suggesting a carotenoid absorption, and the pigment has been identified as a carotenoid both by Wald and Gullstrand. Wald produced an absorption curve of extracted macular which was coupled with visual observations. These visual results were obtained by taking the difference in cone threshold at the



FIG\$8. Estimate of the optical density of the macular pigment
→Values of δ₂-δ₁ for observer K.H.R.
→Values of δ₂-δ₁ for observer J.A.S.
→Wald's visual estimate of macular pigment optical density. Wavelength, λ, in mμ.

forea and 8° from the forea, and to some extent supported his identification of the pigment as xanthophyll. H is method does presume, of course, that the cone response is constant as far $c.s8^{\circ}$ from the forea. Wald's visual results have been plotted against the present curve and it may be noted that a point which he obtained at 545.5m μ agrees well with the present curve, as do his other visual results which lie within the wavelength range of the present investigation (Fig. 68).

However, the differences between the absorption curve obtained for Wald's extracted chemical and that of the present investigation suggests that further work is required to identify the pignent. Evidence does exist for naturally occurring carotenoids which have absorption extending to 560m µ, as can be seen by examining the absorption curves given by Karrer and Jucke. (1948) for substances such as rhodoxanthin.

Other identifications of the macular pignont have been carried out by Sachs, by Kugelburg and by Hanströn (1940), the last working on macaca nonkeys. All these investigators obtained absorption above the upper wavelength absorption limit of xanthophyll, although the spectrophotometric curves of Sachs and of Hanströn did not yield absorption peaks typical of carotenoids. The maximum of the curve for K.H.R at 490m µ is somewhat doubtful, although it was found that a match of 470m µ, made by mixing 450m µ and 490m µ, was acceptable to all observers, hence indicating that the absorption of the pigment must have a similar value at these three wavelengths.

This derived absorption curve was used to calculate the change in chromaticity of S_A and S_B with variation of pignent concentration,

Figs. 69 and 70. This was done for observer K.H.R., and as the observer's distribution coefficients were again used for the calculation, the results are independent of any assumed data, except for the wavelength distribution of the energy of source S_A . The agreement between the calculated data and observed changes in the chromaticities at different retinal locations is good, supporting the conclusion that these changes are due to variation in the macular pigment density at different retinal locations. As the point of zero pigment density is not known, percentages have been expressed relative to a 100% absorption between 0° and 2° off the forea.

The following may be noted:

(a) the agreement between calculation and experimental results is much better than the comparison based on Wald's pignent absorption data

(b) the change of pigment density (between the foven and the different retinal locations investigated) required to give the chromaticities of both S_A and S_B at different retinal locations is consistent for the two cases. Only at 5° is there some discrepancy and this is not great in view of the greater error associated with the measurements at this point.

(c) there is a disparity in the calculated and measured chromaticities for the foweal location. The trichromatic distribution coefficients involve the measured values of the relative luminosity curve, the chromaticity co-ordinates and the relative luminances of the matching stimuli on which the chromaticities are based. From these measurements, the accumulated error in the



FIG19. \bigcirc —Values of the chromaticity of the white point S_4 for different concentrations of the macular pigment. The results are calculated for K.H.R. using the optical density curve of Fig18. The percentage change in the pigment (relative to its value at the central fovea) is given with each point.

Also shown are the matches at various foveal eccentricities obtained by K.H.R., and the calculated change in chromaticity due to changes in concentration of xanthophyll - -...





calculated chromaticities of sources S_A and S_B was estimated at about \pm .015 in the red co-ordinate and \pm 0.020 in the green co-ordinate for S_B and \pm 0.020 in either co-ordinate for S_A . This range of error is greater than the difference in the experimental and calculated points.

(d) a distribution curve for the mocular pignent concentration or thickness variation across the fovea can be deduced (Fig. 71). This curve shows the variation across the retine, the optical density of the pignent being directly proportional to its concentration (Beer's low). The shape of the eurve between the measured points is of little significance, serving only to connect these points.

In calculating the chronaticities of extra-foveal matches, additivity has been assumed throughout this work. Time did not permit an investigation of the validity of this assumption. Previous work for small fields by Clarke (1960) showed that additivity does not hold at 10° from the fovea. However, the results for the nonpigmented observers seen to indicate that additivity holds for the white points under the present conditions, at least approximately. If the non-linear retinal processes are indeed due to rods interacting with the cones, it would be expected that the effects of non-additivity would become more noticeable, the further from the fovea the field is situated. Considering the distribution of rods across the retina, as given by Osterberg (1935), the breakdown even at 5° would be much smaller than at Ω° .




2) Scattering of Light in the Human Lens.

Introduction.

Measurements on the effect of age upon colour vision indicated that the main cause of age changes was an increase in absorption of the pre-receptoral media. This absorption was such as to follow fairly closely Rayleigh's law, regarding its wavelength dependence, and the possibility of such scattering arising in the lens was discussed. It was decided to attempt to detect such scattering directly by experiment and to make some semi-quantitative measurements.

Previous work on scattering in the eye has been concerned, in the main, with the effect of scatter as a cause of glare. Such work includes that of Stiles (1929) on the veiling effect of a glare source. Wright (1946) mentions scatter of light in the ocular media as a contributory cause of glare effects. A number of papers have been written on scatter in the eye by Boynton and coworkers. Boynton (1953) reports the effect of scattered light on the b-wave of the human E.R.G. Comparison between the E.R.G. produced by snall fields and large fields gave an indication of the effect of the scattered light (from the small field condition) and the equivalent direct light intensity required to simulate the effect of scatter (from the large field condition). Boynton found some indication of greater efficiency of blue light in producing the b-wave, which he attributed to Rayleigh-type scatter. De Mott and Boynton (1953a) found that for an excised steer eye, there was not a marked wavelength dependence in the intensity of the scattered

light, for although the blue end of the spectrum was scattered markedly more than the green, so was the red end. De Mott and Boynton (1958b) report that in the excised steer eye, the cornea and edges and core of the lens give rise to most of the scatter, the cornea giving rise to about 70% and the lens 30% of the scatter. Vos (1962) published a photograph for the human eye, in which the cornea and outer regions of the lens appeared to be giving rise to the light scatter. By consideration of the Stiles-Crawford effect. upon glare sources for varying glare angles, he deduced that scatter of light is virtually the same for all glare angles. He attributed 30% of light scatter to the cornea. Boynton and Clarke (1964) attributed 25% of entoptic light scatter to the cornea and the remainder to vitreous and lenticular scatter. The source of disagreement between the last two results and the data of Delott and Boynton is not certain. Although a likely cause is the existence of rapid post-norten changes in the excised steer eye. Le Grand (1957) reports that the effect of glare sources upon contrast sensitivity is not dependent upon wavelength. Gindy (1963) examined the possibility that scatter of light within the eye was contributing to colour contrast phenomena. On this assumption, he found that blue light would have to bescattered nuch more efficiently than other wavelengths to explain his experimental data.

Hence, from past work, there is only slight evidence for the existence of Rayleigh-type scattering within the eye. Only Boynton (1953) and Gindy (1963) have found some evidence for its existence.

Apparatus.

A diagramatic representation of the apparatus is shown in Fig. 72. The source used was a 1,000 watt tungsten filament lamp at S, focused on to a pin-hole by lens L_1 . In order to prevent formation of an image of the tungsten coils, the lamp was turned so that these coils were virtually continuous in the image at the pin-hole, and the beam was slightly defocussed at the pin-hole to further prevent any such image formation. The light from the pinhole was collimated by L_2 , an achronat, and re-focussed by L_3 , another achromatic doublet. The lens L_4 , of low converging power, was used to provide a fine adjustment of the position of the focus. Filters could be placed at point G in the system.

The exit bean was divided by a semi-silvered mirror placed at R. Beam A entered the observers eye, obliquely, at an angle of about 75° to the line of sight. The observer's head was fixed by a dental clamp, although in order to position the beam correctly in the eye, it was necessary to provide further support for the forehead. A fixation spot, D, was provided for the observer's eye, in order to fix the angle at which the light beam entered the eye. Beam B was reflected by a mirror at T, through a neutral density wedge V and on to a white reflecting surface at W. All light energing from the instrument, other than the converging light beam, was excluded.

Method of Measurement.

The observer was requested to view alternately the reflecting



surface W and the fixation spot and to adjust the brightness of the surface, with the wedge V, to be equal to that of the scattered light visible in the visual field around the fixation spot. A number of rapid glances were used to obtain the brightness match. The measurement was performed for a series of coloured filters placed at G.

Sources of Error.

The relatively simple technique outlined above is subject to several difficulties. The greatest is the existence of rapid adaptation in the eye, which causes the apparent brightness of the scattered light to change rapidly. No attempt was made to overcome this, except by using only short times of viewing the scatter. The adaptation was apparently due to the incident light bean causing the scatter, although the observer was virtually unaware of the incident beam itself. Use of a binocular matfhing technique could have helped to maintain fixed levels of adaptation. The variation of adaptation level rendered the experiment no more than semiquantitative in nature. The adaptation change was confirmed by observation of the eye pupil of the observer.

The quality of light scattered on to the central foves and that received from the reflector was not the same. Broad-band filters were used to provide the different 'wavelengths' (fig. 73), in order to give sufficient light to yield visible scatter. In scattering, a colour difference between the scattered light and the reflector was apparent, making it difficult to obtain a satisfactory brightness match.



Finally, localization of any measured effect in the lens was difficult, because the light has to pass through the total ocular media. The oblique angle of incidence ensured that the effect of corneal scatter was minimized, but the vitreous humour would give rise to scatter (Weale 1961a) as would the retina, although the latter is not likely to be of importance. The eye was initially dark-adapted, so that eye pupil size was fairly large.

Observers.

Five observers took part in the work. Two of these were of age 24 years (K.H.R., W.R.W), two 41 years (W.H.K; C.C.) and one was 60 years of age (V.I.R).

Results.

The results are shown in Fig. 74, each point representing the mean of four settings of the wedge. The intensity of the scattered light has been plotted as a function of λ . The intensity of the scatter was measured as the flux required at W to give equality of brightness between the scattered light and W. It was therefore taken in terms of the calibrated intensity scale of the neutral density wedge.

The intensity of the scatterd light should be proportional to $1/\lambda^4$. Due to the errors involved in the technique, the wavelength of the transmission peak of each filter was considered a sufficiently good approximation to the wavelength of the scattered light. The age dependent loss of transmission through theoptical media also affects the light reflected into the eye from the surface W and the results have to be corrected to allow for this. This correction



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to the results of the older observers was made by colculating the effect of lens ageing data of Said and Weale upon the intensity of the light beam from W.

Discussion.

The intensity of scattered light at the fovea appears to be nore marked for blue than red light, even in the case of young observers. The data were not considered to be of sufficient accuracy to merit calculation based on Rayleigh's law, but the intensity does appear to be inversely dependent upon wavelength.

The scattering also appears to increase with æge, the results for the old observers being significantly different from those of the young observers, despite large scatter of individual results. The intensity of the scattered light, expressed logarithmically, has been normalized for all observers for the red beam in Fig. 74. This helps in comparison of the wavelength dependence of the scatter for different **age groups**, which is the point of interest in the investigation.

Conclusions.

The results of this investigation appear to indicate the existence of wavelength dependent light scatter, which is of Rayleigh form in as much that it is more marked at short wavelengths than at long. Further, this scatter appears to increase with age at short wavelengths, relative to the scatter at long wavelengths. This is as would be expected, if the number of scattering centres were increasing with age. These data, if accurate, provide good agreement with the conclusions drawn from the survey of colour vision and age.

Chapter X

General Conclusions and Comments.

Measurements on 400 observers have provided statistical data concerning the variation of colour vision response with increasing age. In discussing the general importance and appliability of the data, at least one limitation of the observer sampling has to be borne in mind. The observers all had healthy vision, even those over sixty years of æge. Fischer (1948) has given data which shows that the incidence of pathological conditions rises significantly with increasing æge. Hence, it should be noted that the data pertain to observers who are selected both for normal colour response and for healthy condition of the eyes.

The results of Chapters III and 1V suggest that the receptoral system does not change in colour response with age, at least in terms of relative response to different wavelengths. That wavelength discrimination is independent of age is not an entirely definite conclusion, as it rests to some extent on results obtained for a very limited sample (six observers only).

The effect of age upon colour matching and the relative spectral sensitivity curve of the eye was also measured (chapters V and V1). Assuming the response system of the eye to remain constant with increasing age, the effect of lens ageing upon both the colour matching and the V_{λ} curve was calculated. It was found that the lens ageing was such as to give fairly good qualitative and quantitative agreement with the experimental change in the two functions. Such differences as did exist were thought to be due possibly to age changes in other parts of the ocular media of the

eye, particularly the vitreous humour. The data of Wald (1949), suggests that Said and Weale's data may not be strictly accurate in its wavelength variation. However, the absolute density of Wald's data (fig.7) is much higher than indicated by the present results, which renders his data somewhat unreliable. No effect of age upon the macular pigmentation, however, has been detected, and indeed, the data are such as to suggest that no change in macular pigment density with age does occur (fig.51). Thus, it appears that the lens ageing, occurring without any accompanying change in "We receptoral response, can successfully predict the effect of age upon colour vision.

The wavelength dependence of the differences with age in the transmission of the ocular media closely follows Rayleigh's law (fig.52). It is possible that two or more pigments, each increasing in density with age, could cause a similar light loss. However, Rayleigh-type light scattering has been demonstrated to occur in the eye. As the intensity of the scattered light was found to increase with age, this was presumed to be the same effect as that giving rise to variations with age in colour matching etc. That the age dependent light loss in the ocular media should be due to scatter rather than pigment absorption has the advantage that it could account for the loss of visual acuity with age.

Loss of light from the retinal image could affect wavelength discrimination (Thomson and Trezona 1951), but this is unlikely to be detected unless very low levels of retinal illumination were used (Bedford and Wyszecki, 1958). In view of the level of light intensity used in neasurement of wavelength discrimination in the

present work, this effect is not of importance.

The effect of age upon the visual colour response can, therefore, be calculated solely in terms of pre-receptoral absorption. Either the lens ageing data or the modified curve obtained from the present data (fig. 43) can be used to represent the transmission changes of the optical media of the eye. An example of this type of calculation has been successfully carried out, and is given in chapter VIII. The importance of ageing will, however, depend to some extent upon the experimental conditions. For example, if only a small number of observers are represented in a given set of data, the age variations are liable to masked for small (2°) fields, by variations in macular pigment density (e.g. see Fig. 33) For large (10°) fields, however, the differences between different age groups become relatively more important (e.g. data of Stiles and Burch (1959), data of Fig.32). The fact that colour vision response does vary with age should, however, be generally recognised.

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Statistical Significance tables were taken from the second and third general references.

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EVIDENCE FOR MACULAR PIGMENTATION FROM COLOUR MATCHING DATA

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Abstract—Measurements of the chromaticities of two white stimuli have been made for four observers. From changes in chromaticity which occur as the field is moved from the fovea, evidence for the existence of a macular pigment in the living eye has been obtained.

From a further series of colour matches, in which the matching stimuli were varied and the comparison field was provided by a spectral band, the variation of optical density of the pigment with respect to wavelength was deduced. Calculations of the chromaticity of the white stimuli based on this optical density curve were found to be in good agreement with the experimentally observed results. The variation in concentration or thickness of the pigment across the fovea has been indicated.

Résumé—On mesure sur quatre observateurs les chromaticités de deux stimuli blancs. Le pigment maculaire est mis en évidence dans l'œil vivant par les changements de chromaticité qui se produisent quand le champ sort de la fovea.

Avec de nouvelle séries d'égalisations colorées avec des stimuli variés et un champ de comparaison obtenu par une bande spectrale, on déduit la variation de la densité optique du pigment en fonction de la longeur d'onde. On trouve un bon accord entre les calculs de chromaticité des stimuli blancs fondés sur cette courbe de densité optique et les résultats expérimentaux observés. On obtient des indications sur la variation en concentration ou en épaisseur du pigment à travers la fovea.

Zusammenfassung—An drei Beobachtern wurde die Farbigkeit zweier weisser Reize gemessen. Aus den Anderungen der Farbigkeit, die bei der Bewegung des Reizfeldes von der Netzhautgrube weg auftreten, wurde auf ein Maculapigment im lebenden Auge geschlossen.

Aus einer weiteren Reihe von Farbtesten, bei denen die Reize variiert und das Vergleichsfeld von einer Spektrallinie beleuchtet wurde, wurde die Variation der optischen Dichte des Pigments mit der Wellenlänge abgeleitet. Berechnungen der Farbigkeit weisser Reize, die auf dieser optischen Spektralkurve beruhten, zeigten eine gute Übereinstimmung mit den experimentell beobachteten Ergebnissen. Auf die Variation der Konzentration oder Dichte des Pigments über die Fovea wird hingewiesen.

INTRODUCTION

THE W.D.W. method of plotting chromaticities (WRIGHT, 1928–9) makes it possible to separate the effects of pre-retinal absorption variation from those of receptor variation. Spectral wavelengths, if matched trichromatically, yield chromaticities dependent only on receptor response, whereas a match of a continuous energy distribution will vary with differences in both the receptors and the pre-receptoral absorption. WRIGHT (1928–9) determined the white point S_B for a large number of observers and found a large variation in their chromaticities, which he attributed to the absorption of blue light by a yellow macular pigment of variable density. Fig. 1 shows similar results obtained in a survey by the present author. In a further experiment, WRIGHT (1946) measured his own white point for a 2° field at various points on the parafoveal region and found that a shift in chromaticity occurred which was consistent with a decrease in yellow pigment as the field was located further from the fovea. As mentioned by Wright, these results also depend on any variation in the receptors which may occur at the different retinal locations. GILBERT (1950) also studied the parafoveal region and obtained results similar to those of Wright, but further suggested that there was little variation in cone response within 5° of the fovea. ISHAK (1951) used this method of analysis in treating the results of his Egyptian observers and found variations between observers consistent with a variation in density of a yellow macular pigment. Further, his results were in agreement with WALD's (1949) identification of the pigment as xanthophyll.



FIG. 1. Chromaticity co-ordinates of the spectrum for ten observers as measured by Wright. Shown also are the chromaticities obtained for the white point S_B by the present author from observers who took part in the initial survey.

Some disagreement has occurred in the past as to whether or not the yellow pigment found in the retina of the eye is a post mortem effect. GULLSTRAND (1906 and 1907) believed that it was and also identified the pigment as a carotenoid. However, differences between observers in white point chromaticities and relative luminosity functions have generally been accepted as evidence of the existence of the pigment in the living eye. A geometrical factor causing reduction in blue light reaching the central fovea is a possible explanation of these differences, assuming that such a factor could vary between observers. However, the post mortem existence of a yellow pigment in the macular, coupled with Wald's data, is strong evidence for the pigment theory. WALLS and MATHEWS (1952), however, have suggested that a receptor density variation across the retina, which is different for different observers, is the cause of variation in colour response attributed to macular pigment differences.

PROGRAMME OF THE PRESENT INVESTIGATION

The aims of the present investigation were twofold. Firstly, an attempt was made to find the extent to which changes in chromaticity of white points at different retinal locations, such as were measured by Wright, are caused by differences in the receptors and to what extent by differences in macular pigment concentration at the retinal locations studied. For this purpose, matches were performed foveally and at eccentricities of 1°, 2.5° and 5° from the fovea, the field size being $1^{\circ}20' \times 1^{\circ}20'$ angle of subtense in each case. Chromaticities were also obtained for a foveally fixated 10° field, for comparison with the small field data.

The further aim of the investigation was to deduce an absorption curve for the macular pigment from colour-matching data. In this case, the matching stimuli were varied and spectral wavebands used to provide the comparison field. From changes in these matches as the field was transferred to different points on the retina, a quantitative measurement of the pigment absorption at different wavelengths was made.

OBSERVERS

Four observers were used, all of whom were normal trichromats. These were chosen from some 150 young observers who had taken part in a colour vision survey entailing colour matching, luminosity and wavelength discrimination measurements. From Fig. 1 it is seen that subjects with low pigmentation will match S_B to give chromaticities in the region of point K, whereas those with heavy pigmentation will give chromaticities near point L. This simple analysis is subject to complication due to receptor variation, but if observers are chosen with similar spectrum loci, this will eliminate to a great extent the effect of receptor variation. All four observers do yield a normal spectrum locus, but K.H.R. possessed heavy pigmentation, R.A.H. possessed average pigment density and M.G. and J.A.S. were observers with little, if any, macular pigment. The 150 young observers fully confirmed the large range of chromaticities for white point matches found by Wright, and substantially confirmed the mean direction of shift towards the spectrum locus.

APPARATUS

The apparatus used for colour matching was the Wright trichromatic colorimeter (WRIGHT, 1927-8) in which the field is a square 1°20' in size and divided horizontally into two halves. A large matching field, 10° in size, was also provided, using the wide field attachment of Clarke. Calibration was performed in the usual way, especial care being taken to ensure that the wide field attachment was accurately calibrated, as any error in setting would invalidate the comparison of the two sets of data for the large and small fields. The white sources used were the standard sources S_A and S_B . Source S_A was obtained directly by calibrating a tungsten lamp for colour temperature on a photometer bench, using an N.P.L. sub-standard white source for comparison. Source S_B was obtained by using the S_A source in conjunction with a Ronis B 2 : 1 filter, and for purposes of calculation, the transmission of the filter was combined with the energy distribution of S_A to give the experimental S_B . The transmission of the filter was measured on the Wright photoelectric spectrophotometer.

EXPERIMENTAL

Colour matching was performed in the usual way for foveal matches, a dental clamp being used to fix the observer's eye centrally with respect to the field. For extra-foveal matching, a flash and recovery cycle was utilized to overcome Troxler's effect and progressive



FIG. 2. The chromaticities of the white points S_A and S_B as matched at different retinal locations by K.H.R. The eccentricities of the centre of the field is given in degrees with each set of matches. Also shown are the white point changes due to changes in optical density of xanthophyll.

- \bigcirc —chromaticities of S_A for 1°20′ fields.
- —chromaticities of S_B for 1°20′ fields. □ —chromaticities of S_A for 10° fields.
- \blacksquare —chromaticities of S_B for 10° fields.
- - chromaticities of white points due to changes in xanthophyll optical density.



FIG. 3. The chromaticities of white points S_A and S_B as matched at different retinal locations by M.G. Symbols as for Fig. 2.

adaptation in the matches. Following the work of CLARKE (1960) a fixed number (15) of flashes was used to obtain a match, a cycle of 1 sec exposure and 3 sec darkness being used. Approximate matches were obtained before the field was cycled, and the match was slightly disturbed between each match. For the small angles of eccentricity used in the matches, no separation of the fields was required, although at 5° the accuracy of the matches would have been increased somewhat by such a separation.

A red fixation spot was provided for fixing the eccentricity of the field and as the right eye was used in each case, the field was always situated on the temporal side of the fovea. Some 15 min dark-adaptation preceded each series of measurements, matching sessions normally continuing for about 1 hr.

The illumination level was about 600 trolands for the small field of view and about 60 trolands for the large field.



FIG. 4. The chromaticities of the white points S_A and S_B as matched at different retinal locations by R.A.H. Symbols as for Fig. 2.

RESULTS

Figures 2 to 5 show the results of colour matching using a 1°20' field at various eccentricities and also a 10° centrally fixed field. The points are plotted in the W.D.W. system of units, using the wavelengths 494 m μ , and 582.5 m μ as the fixed points of the system, with 650 m μ , 530 m μ and 460 m μ as the instrumental matching stimuli (R) (G) and (B). Each point was measured on three or four separate occasions for each observer. Rather than plot a single value with its standard deviation, it was preferred to show the means of each individual matching session and take the areas enclosed by these points as representative of the error associated with the measurement.

DISCUSSION

The author considers the present measurements as almost certain evidence of a pigment existing in the fovea of the living eye, the optical density of which can vary considerably from observer to observer. A difference in receptor response would be an extremely

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unlikely explanation of the foveal differences in the chromaticities of S_A and S_B as matched by K.H.R. and J.A.S. or M.G., as all three observers show normal wavelength discrimination and similar spectral chromaticity co-ordinates (Fig. 6). These last two properties are independent of pre-retinal absorption, but do depend upon variations in the receptor system.



FIG. 5. The chromaticities of white points S_A and S_B as matched at different retinal locations by J.A.S. Symbols as for Fig. 2.

The macular pigment absorption data given by WALD (1949) has been used to calculate the dominant wavelength of the macular pigment for sources S_A and S_B . The calculation was performed by considering the pigment as a filter situated before the response system. The distribution coefficients were obtained from experimental results for K.H.R. and have been used in the calculation. For R.A.H., however, the C.I.E. standard observer data were assumed (Commission Internationale de l'Éclairage, 1931 proceedings).



FIG. 6. The spectral chromaticity co-ordinates for observer K.H.R. are shown by the continuous line. The broken line shows the co-ordinates for J.A.S. The co-ordinates are in terms of the matching primaries R, G and B plotted against wavelength, λ , in m μ .

The results for the non-pigmented observers J.A.S. and M.G. indicate that little change in response occurs within the para-foveal area investigated. Therefore, the changes in chromaticity observed for K.H.R. and R.A.H. should be due to changes in concentration or thickness of the macular pigmentation. In that case, the chromaticity changes should correlate closely with the dominant wavelength curves of xanthophyll. In the case of R.A.H. agreement is quite good for both sources S_A and S_B , whereas for K.H.R., agreement is quite good for source S_B but not for S_A . The results of the latter observer are widely separated in the chromaticity chart and hence any disagreement between the calculated and experimentally measured results is more easily detected. A further notable point is that the directions of calculated shift in chromaticity due to variations in xanthophyll density are closely similar for K.H.R. and the standard observer. This demonstrates that the trichromatic response systems for the two cases are very similar.

The 10° white points for the "pigmentless" observers agree closely with those for the small field matches, whereas for the other observers the 10° point is located in the chromaticity chart close to the small field white points measured at 2.5° and 5° from the fovea. These results suggest that the differences in chromaticity between the two field sizes viewed foveally can be ascribed, in the main, to the filtering effect of the macular pigmentation upon the small field. For R.A.H. and K.H.R. the large field showed a lack of uniformity in the blue-green matches, having a marked central "Maxwell's Spot". This was ignored by the observers, when matching, and apparently did not exist for the two other observers. Hence for a given observer, the comparison of the chromaticities of white matches for small and large fields is a good indicator of the density of macular pigmentation within the eye, assuming "Maxwell's Spot" is ignored in the 10° case.

The fact that such large variations in macular pigment density occur from observer to observer makes any functional explanation of the pigment, such as that attempted by DARTNALL and THOMSON (1949), difficult to accept, especially as some observers appear to have virtually no pigment. The hypothesis of JUDD (1952), namely that the pigment protects the fovea from over-stimulation by blue light such as causes long-lasting afterimages, may be valid. It is hoped that further experiments will be carried out to investigate his suggestion.



FIG. 7. The optical density curve of xanthophyll. Wavelength, λ , in m μ .

THE SPECTRAL ABSORPTION CURVE OF THE MACULAR PIGMENT

The measurements of the previous section showed that in the cases of J.A.S. and M.G. the proportions of (B) to (G) required to match 494 m μ were constant with change in retinal location of the colorimeter field. For K.H.R., however, a large change in this ratio was noted, and a similar but small change in the case of R.A.H. This variation in the matches of the

second pair of observers was attributed to the change in optical density of the macular pigment with change in retinal location. Further, it was noticed that a variation also occurred in the proportions of (R) and (G) in the 582.5 m μ match, although the effect was less marked than for the blue-green match. If this latter observation were also due to the pigment absorption, it is at variance with the chemical identification of Wald, since the absorption by xanthophyll of 530 m μ or 650 m μ is negligible (Fig. 7). If similar measurements are carried out with other sets of matching stimuli and test colours, it is possible to deduce the absorption curve of the macular pigment.

Thus, consider two points of the retina with the same cone response, of which one point is covered with macular pigment and the other clear of pigment. Let F_1 and F_2 be the luminous flux of the wavelengths λ_1 and λ_2 required to match a wavelength λ at the pigment free point and F_1' and F_2' their flux for a match at the other point. Further, let the transmission of the pigment at λ_1 and λ_2 be T_1 and T_2 . Then:

$$F_1/F_2 = F_1'T_1/F_2'T_2$$

which expressed logarithmically becomes:

$$\log F_1 - \log F_2 = \log F_1' - d_1 - \log F_2' + d_2 \tag{1}$$

where d_1 and d_2 are the pigment optical densities at λ_1 and λ_2 , logs being expressed to the base 10.

But from experiment we have:

$$\log F_1 - \log F_2 = \delta_2 \text{ and}$$
$$\log F_1' - \log F_2' = \delta_1$$

where log F_1 , etc., are obtained directly from the instrument scales which are calibrated logarithmically, therefore

$$(\log F_1 - \log F_2) - (\log F_1' - \log F_2') = -(\delta_1 - \delta_2)$$

$$d_1 - d_2 = \delta_1 - \delta_2$$
(2)

and from (1)

If an observer possessed no macular pigment d_1 and d_2 would be zero and hence, from (2), $\delta_1 - \delta_2$ should also be zero. This model has assumed equal cone response at different retinal locations, and the validity of this assumption has been checked by finding the values of $(\delta_1 - \delta_2)$ for an observer with negligible macular pigmentation.

Expression (2) gives the difference in optical density of the pigment at two wavelengths, assuming that one of the retinal locations at which the measurements were taken is free from pigment. If, as is almost certainly true for the present work, neither point is clear of pigment, the optical densities obtained will be proportional to the difference in concentration of the pigment at the two points. Such a means of measuring the optical density of the macular pigment depends solely on changes in physical stimuli and is independent of any subjective assessment of colour quantity.

This principle has been applied using matching stimuli over the range 580 m μ to 440 m μ . It was not possible to extend the range of measurements beyond 440 m μ as there was insufficient light at wavelengths shorter than this for them to act as matching stimuli. Instead the changes in a heterochromatic brightness match at different retinal locations were used to obtain the optical density of the pigment in the short-wave region. In this case, δ is the logarithmic change in light flux required to maintain the brightness match between λ_1 and λ_2 as the field is located at different positions on the retina. This method could have been used to obtain the rest of the density curve, but heterochromatic brightness matching is a less reliable technique than colour matching.

EXPERIMENTAL

Two observers, K.H.R. and J.A.S., were used for the measurements. The results of J.A.S. were compared at one or two points with the results given by M.G. in the first part of this work.

Wavelengths between 590 m μ and 520 m μ , taken in steps of 10 m μ , were in turn used as one of the matching stimuli, 650 m μ providing the other matching stimulus. The comparison stimulus was in each case provided by an intermediate wavelength, this latter being chosen such that it was as sensitive as possible to changes in both the matching stimuli. This region



of the spectrum locus is sufficiently dichromatic to require no desaturation for matching. Wavelengths between 450 m μ and 490 m μ , again taken in steps of 10 m μ , were used as one matching stimulus with 530 m μ providing the other, and similarly wavelengths between 520 m μ and 490 m μ were mixed with 460 m μ . In the two latter sets of matches the intermediate matching stimulus was provided with a minimal amount of red desaturation.

The trichromatic nature of the match was ignored for purposes of calculation, a red matching stimulus being used merely to obtain a satisfactory match.

The field was again presented in a flash and recovery cycle, this time only eight flashes being utilized, so as to minimize any progressive adaptation in the matches. The retinal positions investigated were at the fovea and 2° eccentrically from the fovea, the field again being situated on the temporal side of the fovea in the latter case.

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RESULTS

The results are summarized in Fig. 8. Each point represents the mean of five readings taken in one session. Each wavelength was matched on at least three separate occasions. The point at 490 m μ shows rather greater spread in value than other wavelengths, due to the fact that for reasons of colour mixture and geometry of the instrument, it was difficult to devise a sensitive match using this wavelength. The curve shown is a composite curve, the points between 440 m μ and 490 m μ having been obtained relative to the value at 530 m μ and those between 520 m μ and 490 m μ relative to the value at 460 m μ . Further, the points between 400 m μ and 440 m μ were determined relative to the values at 450 m μ .



FIG. 9. \bigcirc —Values of the chromaticity of the white point S_A for different concentrations of the macular pigment. The results are calculated for K.H.R. using the optical density curve of Fig. 8. The percentage change in the pigment (relative to its value at the central fovca) is given with each point.

Also shown are the matches at various fovcal eccentricities obtained by K.H.R., and the calculated change in chromaticity due to changes in concentration of xanthophyll - -...

DISCUSSION

The interpretation of the curve in terms of macular pigment absorption seems well founded, as the observers M.G. and J.A.S. show practically no variation in the function plotted. Further, the point at 520 m μ yields values for the optical density of the pigment, relative to the absorption at both 460 m μ and 650 m μ which are self-consistent, an extremely unlikely occurrence if receptor variations were the cause of changes in matching.

The results show an absorption curve for the macular pigmentation which is of an appearance suggesting a carotenoid absorption, and the pigment has been identified as a carotenoid both by Wald and Gullstrand. Wald produced an absorption curve of extracted macular which was coupled with visual observations. These visual results were obtained by taking the difference in cone threshold at the fovea and 8° from the fovea, and to some extent supported his identification of the pigment as xanthophyll. This method does presume, of course, that the cone response is constant as far as 8° from the fovea. Wald's visual results have been plotted against the present curve and it may be noted that a point which he

obtained at 545.5 m μ agrees well with the present curve, as do his other visual results which lie within the wavelength range of the present investigation (Fig. 8).

However, the differences between the absorption curve obtained for Wald's extracted chemical and that of the present investigation suggests that further work is required to identify the pigment. Evidence does exist for naturally occurring carotenoids which have absorption extending to 560 m μ , as can be seen by examining the absorption curves given by KARRER and JUCKER (1948) for substances such as rhodoxanthin.



FIG. 10. \bigcirc —Values of the chromaticity of the white point S_B for different concentrations of the macular pigment. For further explanation see Fig. 9.

Other identifications of the macular pigment have been carried out by SACHS, by KUGELBURG and by HANSTRÖM (1940), the last working on macaca monkeys. All these investigators obtained absorption above the upper wavelength absorption limit of xanthophyll, although the spectrophotometric curves of Sachs and of Hanström did not yield absorption peaks typical of carotenoids. The maximum of the curve for K.H.R. at 490 m μ is somewhat doubtful, although it was found that a match of 470 m μ , made by mixing 450 m μ and 490 m μ , was acceptable to all observers, hence indicating that the absorption of the pigment must have a similar value at these three wavelengths.

This derived absorption curve was used to calculate the change in chromaticity of S_A and S_B with variation of pigment concentration, Figs. 9 and 10. This was done for observer K.H.R., and as the observer's distribution coefficients were again used for the calculation, the results are independent of any assumed data, except for the wavelength distribution of the energy of source S_A . The agreement between the calculated data and observed changes in the chromaticities at different retinal locations is good, supporting the conclusion that these changes are due to variation in the macular pigment density at different retinal locations.

The following may be noted:

(a) the agreement between calculation and experimental results is much better than the comparison based on Wald's pigment absorption data

(b) the change of pigment density (between the fovea and the different retinal locations investigated) required to give the chromaticities of both S_A and S_B at different retinal

locations is consistent for the two cases. Only at 5° is there some discrepancy and this is not great in view of the greater error associated with the measurements at this point.

(c) there is a disparity in the calculated and measured chromaticities for the foveal location. The trichromatic distribution coefficients involve the measured values of the relative luminosity curve, the chromaticity co-ordinates and the relative luminances of the matching stimuli on which the chromaticities are based. From these measurements, the accumulated error in the calculated chromaticities of sources S_A and S_B was estimated at about ± 0.015 in the red co-ordinate and ± 0.020 in the green co-ordinate for S_B and ± 0.020 in either co-ordinate for S_A . This range of error is greater than the difference in the experimental and calculated points.

(d) a distribution curve for the macular pigment concentration or thickness variation across the fovea can be deduced (Fig. 11). This curve shows the variation across the retina, the optical density of the pigment being directly proportional to its concentration (Beer's law). The shape of the curve between the measured points is of little significance, serving only to connect these points.



FIG. 11. ○—Percentage decrease of macular pigment (relative to the value at the central fovea) required to give the chromaticity of S_A at different retinal locations.
●—Percentage decrease of the macular pigment required to give the chromaticity of S_B at different retinal locations. The results are derived from Figs. 9 and 10.

In calculating the chromaticities of extra-foveal matches, additivity has been assumed throughout this work. Time did not permit an investigation of the validity of this assumption. Previous work for small fields by CLARKE (1960) showed that additivity does not hold at 10° from the fovea. However, the results for the non-pigmented observers seem to indicate that additivity holds for the white points under the present conditions, at least approximately. If the non-linear retinal processes are indeed due to rods interacting with the cones, it would be expected that the effects of non-additivity would become more noticeable, the further from the fovea the field is situated. Considering the distribution of rods across the retina, as given by ØSTERBERG (1935), the breakdown even at 5° would be much smaller than at 10° .

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