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THE REFLEXION OF LIGHT FROM THE MACULAR AND PERIPHERAL FUNDUS OCULI IN MAN

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It has long been disputed whether the yellow pigment of the human macula is present in life or is a post-mortem artifact: most physiologists have taken the former view, but Gullstrand (1906, 1907) and Hartridge (1947) have argued in favour of the latter. Subjective tests on the differences between the spectral sensitivity of the macula as compared with the periphery, as made by Abney (1895), Wald (1945) and others, are all open to the objection that the receptors present in the two fields may differ in their spectral sensitivities or in their contribution to luminosity.

A difference in colour between the macular and peripheral parts of the fundus oculi is certainly difficult to see by ordinary direct ophthalmoscopy but it is not impossible, and it can be seen more easily if a red-absorbing filter is used. The experiments here described were designed to measure this difference and obtain spectral reflexion curves for macular and peripheral regions of the fundus, using an optical system similar to that employed in retinoscopy to measure the refraction of the eye, but substituting a single oblique glass plate for the perforated retinoscopy mirror in order to avoid the gap in the retinal image caused by the hole, and incidentally (though this has no advantage over the reverse arrangement and was dictated only by mechanical considerations in the apparatus available) interchanging the positions of the light source and the observer.

METHODS

The apparatus, which was set up in a dark room, is shown in Fig. 1. The light source was a 6 V., 18 amp. ribbon filament lamp (*A*) run from the a.c. mains with a transformer, the voltage being controlled by a rheostat in the primary circuit. An image of the filament was formed by the lens (*B*) on the input slit (*C*) of a Hilger single monochromator. An image of the exit slit (*F*) of the monochromator 10 mm. in width and about 30 mm. in height was formed by the lens combination (*G*) in the plane of the pupil of the subject's eye (*M*), the light passing through the glass plate (*K*) at an angle of 49° to the normal. The subject's head was held in position by a rigidly clamped dental impression. The pupil of the eye used was dilated with homatropine and cocaine.

The field of light seen by the subject was a circle of 1° 11′ diameter determined by the stop (*D*) symmetrically truncated by two vertical straight lines 51′ apart determined by the edges (E_1) and

(E_2) of the monochromator prism (E). The image of the stop (D) formed by the lenses (N) and (G) was in the plane (H), 65 cm. from (M) and those of the edges (E_1) and (E_2) were very close to this plane. The spectacle lens (L) corrected to refraction of (M) for this distance, i.e. allowed an image of the plane (H) to be formed on (M)'s retina. Since 65 cm. was also the distance of the image of (M) in (K) from (O), an image of the illuminated area of (M)'s retina was formed at (O).

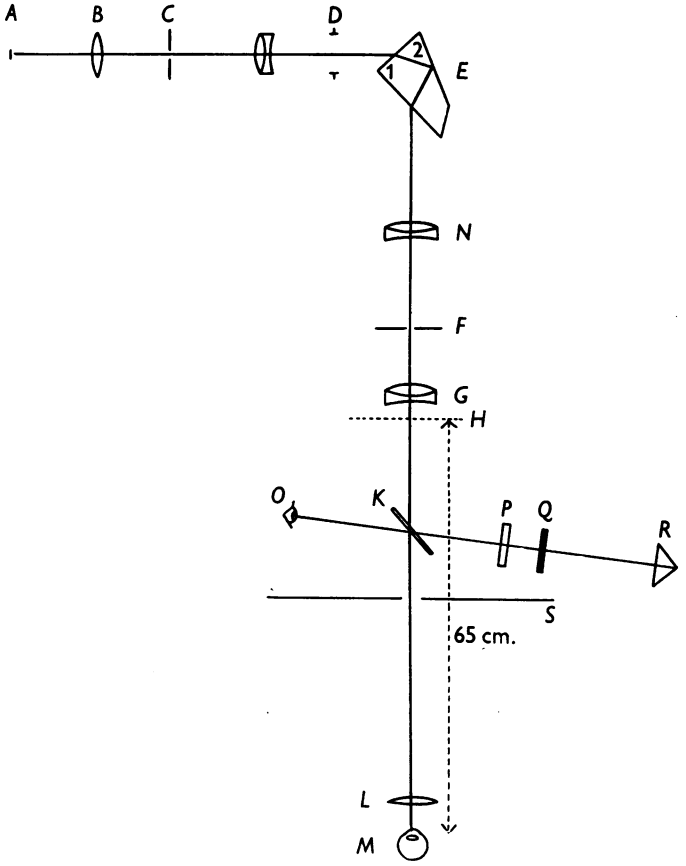

Fig. 1. Diagram of the apparatus. A, light source, ribbon filament lamp; B, lens; C, entrance slit of monochromator; D, stop; E, prism; F, exit slit of monochromator; G, lens; H, plane of image of stop D; K, microscope slide; L, lens for correcting refraction of eye; M, eye of subject; N, lens; O, eye of observer; P, neutral wedge; Q, neutral filter; R, right-angled prism; S, screen.

The observer at (O) saw two fields of light. One was the image of the pupil of the subject's eye (M) in (K). This appeared uniformly illuminated except for the corneal reflex, which was not found troublesome except when the field was very dim. The other field, rectangular in shape and of about the same size, was formed by light which had first been reflected by (K), then returned along its own path by the right-angled prism (R) and transmitted by (K) on its return, passing twice through the neutral wedge (P) and the neutral filter (Q). The position of (R) was so adjusted that the two fields touched but did not substantially overlap. The observer matched the two in brightness by adjusting the wedge (P), the filter (Q) being kept always the same.

Now if the fundus were, like the prism R, a perfect specularly reflecting surface returning all incident light back along its own path without loss of polarization, the paths providing the two fields would be equivalent except for loss by reflexion and absorption, and would match in intensity when the fractions lost by reflexion and absorption were the same in each. The true state of affairs departs from this ideal in three ways.

(i) Most of the light incident on the fundus is absorbed and only a small fraction reflected.

(ii) Of the light reflected not all is returned along its own path.

(iii) Any polarization of the incident light is, as far as we have been able to detect, completely lost on reflexion. In these circumstances the two fields will match when

$$\frac{\text{Fraction transmitted by the absorbing components in the path } K\text{–}R\text{–}K}{\text{Fraction transmitted by the absorbing components in the path } K\text{–}M\text{–}K}$$

= (Fraction of light incident on the fundus which is reflected)
× (Fraction of the total reflected light which is returned through the pupil)
× (Correction for loss of the partial polarization due to the plate K on reflexion by M).

Let us abbreviate this equation to '$X_1 = X_2\ X_3\ X_4$', the symbols representing the factors in the order in which they have been written. Our task, in this notation, was to determine X_2. To do it, we made three assumptions:

(i) That losses by reflexion and absorption at the surfaces and in the media of the eye M were equal to those due to the front surface and glass of the prism R, so that $X_1 = (T_p^2\ T_q^2)/T_l^2$, where T_p, T_q and T_l are the fractions transmitted at the wave-length used by the wedge P, the filter Q and the lens L respectively. This assumption is probably nearly correct under the conditions of the experiment; it would not be so for shorter wave-lengths or for older subjects.

(ii) That the fundus is a perfect diffusing surface, i.e. that the amount of light reflected by a small element of area δA into a small element of solid angle $\delta\omega$ at $\theta°$ from the normal is proportional to $\delta A\ \delta\omega \cos\theta$. From this it can easily be shown that to a close approximation $X_3 = r^2/d^2$, where r is the radius of the pupil as measured by orthogonal optical projection through the cornea and d is the distance from the reflecting surface of the fundus to the anterior surface of the cornea. A value of 23 mm. was assumed for d.

The perfectly diffusing property of the fundus was roughly tested by narrowing the slit F until only a small part of the centre or one margin of the pupil was illuminated. No departure from uniformity in the illumination of the pupil by reflected light could be seen from O whether M's foveal centre or periphery was used.

(iii) That the partial polarization of light transmitted through K was completely destroyed by reflexion from the fundus of M, but that of light reflected by K was unchanged by reflexion from the prism R. We confirmed experimentally that this is very nearly true. It can be deduced that the relative intensity as seen from O of the field which suffers complete loss of polarization on reflexion is greater than it would be if it suffered no loss by a factor

$$\left[\frac{2}{\alpha_r + 1} + \frac{2(\alpha_r - 1)}{(\alpha_r + 1)\ ([1/\alpha_t] + 1)}\right],$$

where α_r is the ratio of reflexion fractions and α_t that of transmission fractions of K for vertically and horizontally polarized light. α_r and α_t were determined experimentally and the correction factor found to be 0·85, i.e. not sufficiently different from 1 for any possible errors in its determination to be of great importance.

It may be noted that any inaccuracies introduced by our three assumptions, though they affect the values of the reflexion fractions themselves, do not affect the ratios of macular to peripheral reflexion fractions from which properties of macular pigment are inferred.

Introducing now the values given by the three assumptions, the equation from which reflexion fractions were calculated is:

$$X_2 = \frac{T_p^2\ T_q^2}{T_l^2}\frac{d^2}{r^2}\left[\frac{2}{\alpha_r + 1} + \frac{2(\alpha_r - 1)}{(\alpha_r + 1)([1/\alpha_t] + 1)}\right] = \frac{T_p^2\ T_q^2}{T_l^2} \times \frac{23^2}{r^2} \times 0{\cdot}85,$$

where r is in mm.

Subjects. Two subjects were selected out of four examined, the others being rejected on account of a tigroid pattern of fundus background. G.S.B. and C.H. had both almost uniformly coloured fundus background except for macular pigment. G.S.B. was aged 25 and C.H. 20 years. G.S.B. was about 1D. hypermetropic, requiring at (*L*), when homatropinized, +3D. at wave-length 680 mμ. and +2D. at wave-length 460 mμ.; C.H., practically emmetropic, requiring +2D. at 680 mμ, and +1D. at 460 mμ.

RESULTS

The light-adapted eye

Observations were made on the light-adapted eye throughout the spectrum from wave-length 680–460 mμ., using alternately the foveal centre and an area 9° 30′ above and nasal to it. With the shorter wave-lengths the entry slit (*C*) of the monochromator was widened to compensate for the greater dispersion of the prism (*E*). Wave-lengths shorter than 460 mμ. could not be used owing to the low intensity of violet light emitted by the lamp and the low reflecting power of the macula in this part of the spectrum. The results are plotted in Fig. 2 in the form $-\log_{10}$ (fraction reflected), i.e.

$$-\log_{10}\left[\frac{(T_p^2\, T_q^2)}{T_l^2} \times \frac{23^2}{r^2} \times 0{\cdot}85\right].$$

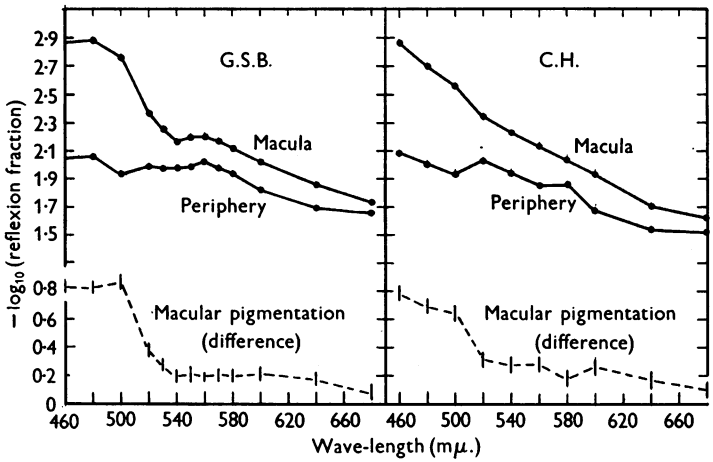

Fig. 2. Continuous lines: the absorption of light by the macula and periphery respectively in two subjects, G.S.B. and C.H. Broken lines: the differences between the fractions reflected by the macula and the periphery, i.e. the absorption caused by macular pigmentation. The total length of each vertical straight line is twice the standard error of the difference of means.

For subject G.S.B. each point is derived from the mean of from four to ten observations made in three separate experiments. For subject C.H. each is derived from the mean of three observations made in a single experiment. The difference between macular and peripheral log reflexion fractions for each wave-length is plotted below and the standard error of each difference of means indicated. The random errors of observation are probably larger than errors of

any other kind affecting the *differences*, but for the log reflexion fractions themselves, the assumptions made concerning absorption within the eye and the perfectly diffusing property of the fundus may have introduced errors larger than those of observation.

The dark-adapted eye

An attempt was made by two methods to detect the bleaching of visual purple in the dark-adapted peripheral retina of G.S.B. First, the log reflexion fraction at 510 mμ. was determined for one area of the peripheral fundus 9° 30′ from the foveal centre in the light-adapted eye, and again after dark-adaptation for 1 hr. No significant difference was found. It was thought likely that, during the time taken for the measurement of the reflexion fraction in the dark-adapted eye, the brilliant illumination of the area being measured (i.e. about 3×10^8 times scotopic threshold) might have destroyed all its dark-adaptation.

A second method was therefore used. Five fixation points were marked on the screen (S), so that when the subject fixed on them in turn, five areas of peripheral fundus arranged round the foveal centre at distances of 7° to 10° could be investigated. The subject's left eye was dark-adapted for at least 1 hr. (in one experiment for 10 hr.) and matches made rapidly for each of the five regions in turn, at less than 10, at 30 and at 60 sec. after the subject had begun to fix on the appropriate mark. By this method the mean reflexion fractions at 500 and at 540 mμ., 30 sec. after beginning light-adaptation, were found to exceed those at < 10 sec. by a small but significant amount, and those at 60 sec. to exceed those at 30 sec. by a very small and probably insignificant amount. The mean values of (log reflexion fraction at < 10 sec.) $-$ (log reflexion fraction at 60 sec.) were:

at 500 mμ. $0{\cdot}032 \pm 0{\cdot}007$ (S.E. of mean of 15 observations);
540 mμ. $0{\cdot}027 \pm 0{\cdot}009$ (S.E. of mean of 10 observations);
580 mμ. $0{\cdot}022 \pm 0{\cdot}012$ (S.E. of mean of 5 observations).

DISCUSSION

If it may be assumed: (1) that most of the light reflected from the fundus is not merely reflected from the retina, but has passed through the retina, been reflected by the pigment epithelium and choroid and then returned through the retina; and (2) that the reflecting properties of the pigment epithelium and choroid are the same in macula and periphery, then the difference in log reflexion fractions between macula and periphery represents twice the difference in optical density between macular and peripheral retina. The first of these assumptions is well supported by ophthalmoscopic appearances. The second can be justified only by the absence of any anatomical evidence suggesting the

contrary, and the good agreement in both magnitude and spectral distribution between our estimate of the difference between macular and peripheral retinal light absorption and other estimates obtained by methods whose potential errors are of quite different kinds. Such estimates would include those of Abney (1895), Krawkow (1925) and Wald (1945), who compared macular and peripheral photopic luminosity curves, of Thomson (1951) who compared the foveal luminosity curves of different individuals, of Stiles (1950, personal communication) who compared the sensitivity of the 'blue mechanism' in the fovea and in the periphery, and of Sachs (1891) who measured the absorption spectrum of maculae excised within 3 hr. of death.

It is unlikely that the differences which we have found are entirely due to one pigment. The xanthophyll extracted by Wald from human maculae, which seems likely to be responsible for most of the large difference in the blue, cannot account for the smaller difference throughout the rest of the spectrum. It is possible that this difference is due to a difference in the pigment epithelium or choroid, contrary to our assumption. On the other hand, it may well be due to another pigment present in the macular retina in addition to Wald's xanthophyll, and Wald's own estimate of macular pigment by comparison of macular and peripheral luminosity curves gives some support to this view. The presence of blood in retinal capillaries in the periphery and possibly of visual purple even in the light-adapted eye provide further reasons why no estimate of the differences in absorption spectrum between macular and peripheral retina, however accurate, can be expected to agree exactly with the spectrum of a pure substance extractable from the macula.

From the reflexion fraction for the peripheral fundus we can estimate the magnitude of one of the sources of stray light in the eye, namely the light reflected from other parts of the fundus. For uniform illumination of a perfectly diffusing fundus this would be a fraction:

$$\left[\frac{2\pi - \text{solid angle subtended by pupil, iris and ora serrata}}{2\pi} \times \text{reflexion fraction}\right]$$

of the retinal illumination, and for non-uniform illumination very roughly this same fraction of the *mean* retinal illumination. For either of the two eyes examined this would be roughly 1/60 of the mean retinal illumination for blue or green light, 1/50 for yellow, and 1/20 to 1/30 for red. Since also its visual effectiveness must be reduced by its obliquity, owing to the Stiles-Crawford effect, it seems that this kind of stray light can rarely be significant in the normal eye.

The values obtained for the difference in density of visual purple between the dark-adapted and light-adapted periphery are surprisingly small, representing only density 0·016 or 3·8% absorption of 500 mμ. for a single journey through the retina. The total density of visual purple in the dark-adapted eye

must be greater than this, for Hecht, Shlaer & Pirenne (1942) showed that their subjects could see on 60% of trials flashes containing 54–148 quanta at 510 mμ. at the cornea, and that the frequency-of-seeing functions were inconsistent with less than 6 quanta being required for seeing a flash. Thus, allowing for about 50% loss by reflexion, absorption and scattering in the eye, at least 8–22% of light at 510 mμ. incident on the dark-adapted peripheral retina must be absorbed by visual purple. If then the present results could be accepted as giving the whole difference in density of visual purple between the dark-adapted and light-adapted retina, they would imply that dark-adaptation was not mainly due to an increase in the amount of visual purple in the retina. However, our estimate is almost certainly too low, because some of the visual purple must be bleached before the first of our three readings can be taken, and possibly some remains still to be bleached after the last; though subsequent readings did not differ significantly from those taken at 60 sec. It is not impossible that improvements in technique might give values for the difference between absorption by the dark and the light-adapted retina which would be consistent with the bleaching of all the visual purple during light-adaptation.

SUMMARY

1. Estimates of macular pigmentation in the living eye have been made by comparing the reflexion of light from the macula with that from a peripheral area. The results are in general agreement with previous estimates obtained by other means.

2. Attempts by the same technique to assess the difference in amount of visual purple present in the dark-adapted and in the light-adapted eye gave a surprisingly small difference.

3. It is concluded that the amount of light which under normal conditions is reflected within the eye and which could stimulate other parts of the retina is a very small fraction of the incident light.

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