The effect of light history on the photolysability of human visual pigments in situ

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The foveae of two subjects were studied by the method of rapid fundus reflectometry (RFR). Difference spectra were obtained after the eye had dark-adapted for at least 12 min and then been exposed to a bleaching light of variable intensity and spectral composition. It is found that if the period of dark-adaptation is preceded by an exposure to intense light, designed to eliminate the previous light history of the retina, the density spectrum is lower than in the absence of such a clearing exposure, provided the latter is such as to furnish on average more than one quantum per visual pigment molecule within approximately 500 msec. It is shown that, within limits, cone pigments studied with RFR show properties similar to those obtained with visual purple in vitro.

INTRODUCTION

All attempts to explain visual functions in terms of photochemical reactions implicitly or explicitly make one assumption: namely, that the only effect that photic exposure has on a visual pigment is to reduce its concentration. On at least three recent occasions, however, it has been suggested that, when the isomerizing exposure is high, the photosensitivity of visual pigment solutions may decrease (Hubbard & Kropf 1958; Bridges 1961; Williams 1964). The photosensitivity $\phi$ is defined as the product of the extinction coefficient $\varepsilon$ and the quantum efficiency $\gamma$, the latter being the proportion of molecules of pigment isomerized per quantum of wavelength $\lambda$ absorbed. The present paper shows that such an apparent decrease in photo-sensitivity (or photo-reversal) can occur in the living human eye (see p. 100 for an elaboration of this view). It is only fair to add that the experiments now reported were not designed to discover whether or not the effect could be detected in vivo, and only led to this conclusion and the execution of some ad hoc experiments as a result of an accidental observation reported below.

The original problem was as follows. A large number of experimental data have been accumulated by means of the method of rapid fundus reflectometry (Brindley & Rushton 1955; cf. bibliographies in Rushton 1963, 1964; Weale 1965): when the density change $\Delta D(2)$ measured at the human fovea with light of wavelength $\lambda = 560$ nm is plotted against the intensity of the bleaching light (figure 1) the two quantities show an expected correlation (Weale 1965). In point of fact, when a similar plot is made for an extra-foveal part of the retina, poor in cones, the data agree well with the relation

$$\text{fraction of pigment bleached} = 1 - \exp \left(-\frac{I_B}{I_{Be}}\right).$$

[ 96 ]
where $I_B$ is the bleaching intensity and $I_{Be}$ that intensity that reduces the pigment concentration to $1/e$ of its maximum. This relation is plotted in figure 1 and, while it describes the data adequately at lower isomerizing intensities (allowance being made for the fact that the experimental conditions were not the same throughout), there is a bleaching deficit above a level of 6.2 log photopic trolands (log phot. td), the exposure time being 30 s in every case. This deficit interested us and we accordingly wished to explore this intensity range in detail, and to find out whether products of isomerization might not perhaps interfere with the measurements, e.g. by accelerating the mechanism of regeneration.

![Temperature Graph](image)

**Figure 1.** The density change $\Delta D(2)$ following double-transit of the measuring light of wavelength $\lambda = 560$ nm through the retina as a function of the intensity of the bleaching radiation. The wavelength of maximum transmission of the filters in the bleaching beam increases from left to right: Ilford 601 is a violet filter, and 206 a deep-red one. The curve represents an exponential bleaching function (after Weale 1965).

**Method**

**Apparatus**

The apparatus has been described previously (Weale 1959; Ripps & Weale 1964) and was altered in one respect only. The bleaching light was passed through one arm of a trichromatic colour-matching apparatus, to wit, the test-arm. The other arm, which provided facilities for trichromatic matching, is of no consequence in the present context and can be ignored. In effect, then, the bleaching beam was obtained from the xenon arc as before but it passed through some additional
optics. Also, the arc formed an image that was intercepted by means of an aperture, in turn imaged in the plane of the subject's pupil: this image had a diameter of approximately 2 mm but the arc image occupied a small part thereof.

Procedure

Subjects A and B were used as before. Dark-adaptation was never less than 12 min and only the fovea was studied. At the end of the period of dark-adaptation when the pigments could be expected to be fully regenerated (Weale 1959), a reflection spectrum of the fundus was recorded. Thereupon the eye was exposed for 30 s either to a green or to a blue light (figure 2) of intensity $I_B$ and a new reflexion spectrum recorded immediately on cessation of the bleaching exposure—in effect within 2 s.

In some experiments dark-adaptation was preceded by a clearing bleach with white (xenon arc) light of intensity $I_C$ lasting 30 s.

![Figure 2. The spectral transmissivity of the blue (Ilford 621) and green (Ilford 604) filters used in the bleaching experiments.](image)

Light intensity

The bleaching lights were calibrated by heterochromatic luminance matching. Light from a lamp (colour temperature 2545 °K), calibrated at the National Physical Laboratory, Teddington, Middlesex, was scattered by a magnesium oxide surface and viewed through an artificial pupil coincident with the final image of the arc mentioned above. The luminance of the surface could be computed from a knowledge of the candle-power of the lamp and the geometry of the situation, and was also measured by means of an SEI photometer. The two estimates agreed to within 5% of each other. The intensities were expressed in log photopic trolands:

<table>
<thead>
<tr>
<th>Filter</th>
<th>$I_B$ (log photopic trolands)</th>
<th>$I_C$ (log photopic trolands)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ilford 604</td>
<td>4.99</td>
<td>7.59</td>
</tr>
<tr>
<td>Ilford 621</td>
<td>5.48</td>
<td>6.87</td>
</tr>
<tr>
<td>Ilford 604</td>
<td>5.93</td>
<td>5.52</td>
</tr>
</tbody>
</table>

The last entry in each column represents the maximum obtainable with the existing arrangement.
RESULTS

Difference spectra were calculated for every experiment and the average computed from 6 to 12 determinations or more if they happened to have been obtained in parallel experiments. A smooth curve was drawn through the data. Allowance was made for the small oedema effect where necessary (cf. Ripps & Weale 1964) and the density changes read off for wavelengths 450, 520, 560 and 580 nm.

Figure 3 shows the results obtained at \( \lambda = 560 \) nm with a green bleaching light: the data measured in the present series are shown with black symbols, those previously obtained with white ones (cf. Ripps & Weale 1963; Weale 1965). Similar data were obtained with the blue bleaching light.

![Figure 3](http://rspb.royalsocietypublishing.org/Downloaded from http://rspb.royalsocietypublishing.org/)

**Figure 3.** Comparison between bleaching data obtained with and without a clearing bleach. The density change \( \Delta D(2) \), measured for double transit through the retina, relates to the wavelength of 560 nm. The clearing and bleaching exposures lasted 30 s. White clearing exposure in phot. td:

- Subject A: \( \square \)
- Subject B: \( \bigcirc \)

Dark adaptation 12 min.

DISCUSSION

The most striking feature of figure 3 is the difference between the intensities required to bleach the fovea with green light in the past and more recently respectively. At low intensities the light required previously was 4 to 5 times as high to produce a given density difference and at high intensities this factor rose to 10. While one would not be surprised at a photometric error of 2, one of between 5 and 10 is beyond what can be tolerated. It was also clear that no
error had crept into recent photometric measurements, repeated on several occasions. It then occurred to us that there had been a change in procedure, which, though unlikely to account for the difference in photolysability of the fovea, merited some attention. In all the earlier work (Weale 1959, 1962a; Ripps & Weale 1963, 1964) the period of dark-adaptation t, during which the pigment was allowed to reach its maximum concentration, was preceded by a clearing bleach with white light of known high intensity \( I_C \). The object of this torture was to wipe out all the previous light history of the retina (Thomson 1949). We thought, however, that, as regards the photo-chemistry of the eye, this was perhaps an unnecessary precaution, for if the concentration reached a maximum when \( 6 \text{ min} < t < 12 \text{ min} \) (Weale 1959), any effect of the clearing bleach would, at best, affect neural factors of the visual system. On account of the shift in the curve shown in figure 3 we returned to the older procedure involving \( I_C = 0 \), even though it was not obvious at first how the clearing intensity was to affect the photochemical reactions being studied at least 12 min after its cessation.

For a given bleaching intensity, the amount of foveal pigment was found to be less than in the absence of the clearing exposure. An analogous experiment on an extra-foveal part of the retina had led to a similar result (Weale 1962b), but its significance had not been appreciated at the time. Just as in those experiments, we alternated experiments with and without the clearing exposure and found that the effect of the latter bleach was to reduce the density change obtained with a fixed bleaching exposure. In addition, we re-examined some of our earlier experimental results in order to discover the relation between the density change \( \Delta D(2) \) recorded at 450 and 560 nm with green or white bleaching light of intensity \( 5 \cdot 48 \log \text{phot. td} \), the quantity of the clearing exposure \( \log_{10} I_C t \), where \( t \) is the exposure time, forming a variable (figure 4). The dates of the experiments are entered at the top and clearly the correlation of \( \Delta D(2) \) with \( \log_{10} I_C t \) is higher than with the date of the experiment. The exposure time \( t \) was 30 s except in the right-hand point for the para-fovea when it was almost 7 min (cf. Weale 1962b). This latter measurement refers to the wavelength of the corresponding maximum density difference, namely, 510 nm. Figure 4 is consistent with the view that if the exposure of the clearing intensity exceeds \( 7 \cdot 7 \log (\text{phot. td \times sec}) \) \( (= 6 \cdot 2 \log \text{phot. td} + 1 \cdot 48 \log \text{sec}) \), the photolysability of the retinal pigments is reduced. Now this is the exposure that corresponds approximately to the intensity giving a full bleach in 30 s (figure 3) and therefore presumably to one quantum per molecule in 30 s. Moreover, it is at this intensity that the aforementioned deficit begins when the eye has been exposed to a strong clearing light (figure 3). We may suggest tentatively, therefore, that when the pigment has been subject to quantal saturation two effects occur. First, the photolysability of the pigment is reduced by a factor of 4 or 5 (figure 3). It might be argued that it is the photosensitivity \( \phi \) that is so diminished, but there is no direct support for this view. We prefer to use the term photolysability \( (\psi) \) because we take it to imply not sensitivity to radiation as does the term photo-sensitivity, but rather the ability of the visual pigment molecule to complete the reaction initiated by the radiation absorbed. It is obvious that the
pigments are fully regenerated because it is possible to produce approximately equal maximum density changes with and without the clearing exposure. Consequently, we may think of what happens in terms of quenching. The reduction in photolysability persists for some 20 to 25 min. It is during this period that some four times more quanta are required to bleach the pigment than if there has been no clearing exposure, and it follows that the discrepancy between figure 1 and Rushton’s corrected data (Weale 1965) is fully accounted for.

![Figure 4](http://rspb.royalsocietypublishing.org/)

**Figure 4.** The density change at 560 nm following a bleach of intensity $I_B = 5.48 \log$ td and 30 s duration plotted as a function of the clearing intensity $I_C$. For curve see text.

Secondly, once a high clearing exposure has reduced the photolysability of the retinal pigments, bleaching exposures in excess of $6.2 \log$ phot. td are associated with a bleaching deficit. As this intensity is equivalent to at least one quantum per molecule per 30 s, the excess exposure must involve double hits or, more generally, every molecule receives on average more than one quantum. Williams (1964) has shown that if quantal saturation is exceeded within a millisecond or so, photo-reversal may take place and the effective photosensitivity be reduced from unity to one half. Our exposures of 30 s were, of course, some four orders of magnitude higher than this; consequently our phenomena may have nothing to do with photo-reversal. Alternatively, the solid state structure of the receptor may have a much higher integration time on account of the greatly reduced mobility of the visual pigment molecules.

Assuming for a start that the intensity of $6.2 \log$ phot. td corresponds to one of 1 quantum mol$^{-1}$ 30 s$^{-1}$, we can calculate the average time interval $t$ between two quanta arriving at one molecule in the course of exposures to higher intensities. By means of Poisson integrals it is then easy to calculate the percentage of molecules receiving a single and a double hit within 0 to, say, 8 s. Triple and higher multiple hits are likely to occur with sufficient rarity for them to be neglected. It is then found that the curve shown in figure 4 approximately follows the
density changes recorded with green light of intensity $5.48 \log \text{phot.td}$ plotted as a function of the clearing intensity $I_c$ on the following assumptions. (i) The saturation intensity under normal conditions is more nearly $5.8 \log \text{phot.td}$, a value consistent with the data in figure 3. (ii) If a molecule receives a double hit within $0.42 \text{s}$ or, say, $500 \text{ms}$, its photolysability is lowered by a factor of 4 or 5, i.e. the energy required to achieve a given small change in concentration must be multiplied by a factor of 4 to 5. (iii) This reduction in $\psi$ persists for some 20 min.

It is possible to show that the bleaching deficit shown in figure 3 can be accounted for with only one additional hypothesis. We return for this purpose to the Poisson integrals and calculate the probability that a molecule shall absorb 0, 1, 2, 3, ... quanta in $500 \text{ms}$ if the average number in this interval of time is $\bar{q}$. It is therefore possible to determine the fraction of molecules ($P_1$) receiving 1 quantum and that receiving 2 quanta ($P_2$), larger numbers being negligible. Then, if the hypothesis underlying the curve in figure 4 is valid, the number of quanta $N$ needed to bleach any given fraction of the pigment is proportional to

$$N = P_1 + 7P_2$$

(2)

If pre-exposure necessitated the use of such high intensities for the pigments to be bleached as to involve a sizeable number of double-hits within $400$ to $500 \text{ms}$, then an estimate of the fractional increase in the number of quanta required is

$$1 + (7P_2/P_1)$$

(3)

Consequently, if this increase is allowed for, the data should more nearly obey expression (1) and figure 5 shows that this is, in fact, the case.

Figure 6 throws some light on the question as to whether the clearing bleach does more than just to reduce the photolysability. All four difference spectra were obtained by means of a bleaching intensity of $5.5 \log \text{phot.td}$ acting for $30 \text{s}$ and set to zero at $670 \text{nm}$. However, the black symbols represent the averages for experiments done when the clearing intensity $I_c$ was $10^{6.1} \text{phot.td} \times \text{sec}$ or zero, whereas the white symbols stand for the average data obtained for clearing intensities between $10^{8.5}$ and $10^{9.1} \text{phot.td} \times \text{sec}$ (cf. figure 4). The continuous curves were drawn free-hand through the latter data and the dashed curves were scaled from the continuous ones by a factor of 1.83 for subject A (top) and one of 2 for subject B (bottom). The scaled curves provide a good fit for the data obtained following the low clearing intensity except at short wavelengths where the experimental results are higher than the computed ones. We may conclude that, at any rate as regards the long wavelength part of the difference spectrum, these results do not indicate a spectral variation in the reduction of photolysability.

**Conclusion**

It would appear that photo-reversal can be involved to account for the present data only if, in the living eye, the primary or secondary product of isomerization can exist for as long as $500 \text{ms}$. This is much longer than the corresponding time of about $1 \text{ms}$ mentioned by Williams (1964). However, even he found, albeit at
Figure 5. The data of figure 1 after a correction has been applied for quantal saturation.

Figure 6. Difference spectra obtained from experiments with (white) and without (black) clearing exposures. For details see text.
a temperature of 2-5 °C, that two flashes absorbed within 500 ms bleach only some 0-52 of the available light-sensitive pigment. As the maximum amount that would be bleached in his experiments was 0-59 and a single flash bleached 0-36, the drop in \( \Delta D \) was 50% (his figure 5) as compared with some 50% or less in ours (figure 4). We would not wish to press the comparison beyond saying that we are dealing with cone-pigments in vivo at body temperature, whereas Williams dealt with a rod-pigment in solution at 2-5 °C. In any case, Williams's extrapolation to low intervals of time can easily lead to values of \( \phi \) less than 50%.

It would be premature to speculate on the nature of the mechanism involved in the reduction of the photolysability of the human cone-pigments by a factor of 4 to 5 beyond saying that it can be accounted for formally in view of the photo-sensitivities of cone and rod pigments being similar and the rate of regeneration of the former 2 to 3 times greater than that of the latter. The idea of molecular quenching furnishes no more than a description of the observation that the photolysability of visual pigments remains reduced by 75 or 80% following a high clearing exposure and that a subsequent double-hit can reduce it even further. The usefulness of this mechanism for the protection of the visual pathway is self-evident.

The change in \( \psi \) illustrated in figure 3 bears on the comparison of our work with that of two other investigations. Attention was drawn (Weale 1965) to the discrepancy between the data shown in figure 1 and the data of Rushton (1963). This is now eliminated. We can say that, to a first approximation, the photo-sensitivities of the cone pigments and visual purple are substantially similar. Secondly, the light quantities required to produce the Brücke–Bézold phenomenon by subjective and objective means (Weale 1964) showed a discrepancy of a factor of more than an order of magnitude in comparison with the data of Cornsweet et al. (1958). This is now also reduced to dimensions attributable to technical differences.

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References


