Chapter 4

Photosensitivity

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With 8 Figures

Contents

I. Introduction

In photochemical reactions, light can be regarded as one of the reactants, the other being the absorbing molecules. An important property of these reactions is the *quantum efficiency*, which is defined as the ratio of the number of molecules changed to the number of photons absorbed.

In one sense the quantum efficiency is always unity, for a photon excites the molecule that absorbs it. But the overall quantum efficiency, as measured by some permanent change, depends on the consequences of the excitation.

In some cases all the excited molecules may return to the ground state by radiationless transfers of their energies, the net result being no permanent change but only a rise in temperature of the system. For such molecules (photostable pigments) the overall efficiency is zero. Alternatively a part of the acquired energy may be re-radiated (at a longer wavelength than that of the exciting radiation) as fluorescence or phosphorescence. Again there is no permanent change to the system,

E. W. Abrahamson et al., *Photochemistry of Vision*

and the quantum efficiency is still zero, though one could measure the quantum efficiency of fluorescence (or phosphorescence) as the ratio of the number of photons emitted (of wavelength λ_2) to the number of those absorbed from the incident light (λ_1).

Sometimes the absorbing molecule, though not itself permanently affected, may pass its energy to another molecular species which, as a result, is changed. This is photosensitization, and the appropriate measure of quantum efficiency would be the ratio of the number of molecules changed to the number of photons absorbed by the unaffected species.

In yet other cases excitation may be followed by a permanent change to the molecule that absorbs the photon. This might occur either directly (e.g. because the molecule dissociates) or indirectly because during its brief life in the excited state the molecule meets another (of the same or different species) with which it can react. Such are the photosensitive molecules for which the quantum efficiency may range from values as low as 0.01 (where deactivating processes are dominant) to several thousands, where energy or molecular chain reactions are initiated.

In some photochemical experiments it is possible to arrange the concentration of the reactant to be so high that – throughout the experiment – all incident light is absorbed. In this event the total photon dose received by the system is simply the product of the average incident intensity (in photons per second) and the duration of the exposure. The number of molecules changed is ascertained by chemical analysis, and the quantum efficiency can then be calculated.

Such a course is not generally open for the study of the visual pigments. These are normally obtained as a few millilitres of extract, about 10^{-5}M in pigment, and with an optical density at the absorbance maximum that rarely exceeds unity in the thin optical cells (e.g. 0.5 cm) commonly used. Thus even at the beginning of an experiment only 90% of the incident light would be absorbed and, as the extract bleached, this percentage would become progressively less, approaching zero for a fully-bleached solution. Moreover, even if it were practicable to prepare an extract of very high density (and this can be done in some cases) and to irradiate it for a limited period so that the part-bleached extract still had a sufficiently high density at the end to absorb practically all the incident light, there would remain the problem of assessing how much of the pigment had been bleached. There are no chemical methods of assaying the visual pigments and the spectrophotometric method (except at very long wavelengths, where extinctions are low and not reliably known) is not applicable – one could hardly distinguish between, for example, an original pigment density of 5 (99.999% absorption) and a final density of 3 (99.9% absorption) even though 40% of the pigment had bleached.

These difficulties are avoided by the *method of photometric curves* (Dartnall, Goodeve and Lythgoe, 1936) which, though especially devised for the visual pigments, can be applied to any photochemical system that undergoes measurable changes in transmissivity when irradiated.

In the method, which is described in some detail later on, the transmissivity of the photosensitive solution is recorded at convenient intervals during its exposure to a steady light. The data, when plotted as a certain function of the transmissivity against time, yield a straight line of slope equal to $\alpha\gamma I_i$, where α is the extinction (absorbance) coefficient of the pigment for the wavelength of the

bleaching light, γ is the quantum efficiency, and I_i is the intensity of the incident light. Since the slope and the intensity are measured the value of $\alpha\gamma$ can be obtained.

The product $\alpha\gamma$ consists of two efficiency terms. One, α, the extinction or absorbance, is a measure of the efficiency with which the bleaching light is absorbed; the other, γ, the efficiency with which the absorbed light causes the change that is measured. The method cannot separate these factors but it does allow their product to be measured with precision – in spite of the accumulation of absorbing products, and moreover, in preparations of unknown concentration. It is thus of particular value for the visual pigments and has the additional general advantage of requiring only a knowledge of the intensity of the *incident* light – as in those instances when arrangements can be made for all the incident light to be absorbed.

The method focused attention on the product $\alpha\gamma$ and in 1938 GOODEVE and WOOD proposed that a special name be given to it – "photosensitivity". It has a similar role in the reaction kinetics of photochemical processes to that of the "velocity constant" of thermal reactions. Since the quantum efficiency, γ, is a ratio it is dimensionless. Hence photosensitivity has the same dimensions as absorbance, i.e. $[L]^2$.

II. The Coefficient of Extinction (Absorbance)

A. Significance and Physical Dimensions

The loss of intensity that a beam of light suffers as it passes through an absorbent material can be interpreted in two ways. In the wave theory the loss is due to a progressive reduction in amplitude as the energy of the wave motion is transferred to the absorbent material. On this basis every ray in the beam is attenuated, but is never wholly extinguished no matter how thick the absorbent. Alternatively, in the quantum theory the light beam (as regards its interactions with matter) behaves as a stream of energy "particles" called photons. Each photon, in its passage, either "collides" with an absorption centre – and suffers extinction, or else it misses every such centre – and emerges unscathed.

Both theories lead to the same expected relation between incident and transmitted intensities, and it is only in special cases that philosophical distinctions arise. To take an extreme example, the wave theory suggests that every ray of sunlight that strikes the Earth is represented (though reduced to an infinitesimal fraction) in a seam of the deepest coal mine; the quantum theory, on the other hand, suggests that in this situation there are long periods of utter darkness, punctuated every 10^x years on average by the arrival of a lucky photon or two that shot through without losing anything. On either theory, however, the energy transmitted – steadily on the wave theory, or intermittently on the quantum theory – is the same when integrated over a sufficiently long time.

The relation between the incident and transmitted light intensities, and the properties of the absorbing medium can be easily derived. In the present context it is relevant to consider the absorptive properties of a solution (e.g. an extract of visual pigment) but the following treatment is equally valid for solids and gases.

Consider, then, a sample of an extract in a transparent optical cell that is bounded by plane parallel faces, and coaxial with a beam of parallel monochromatic light (Fig. 1). We require to find the relation between I_i, the light that enters the first surface AA, and I_t, the light that reaches the second surface BB. The extract, of thickness l, can be regarded as a pile of very thin identical plates dl. Then, if the intensity of light incident on one of these plates is I, a portion dI will be absorbed, the remainder being transmitted to the next plate. The fraction of

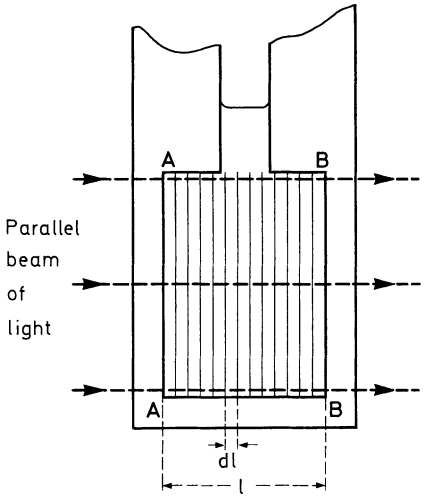


Fig. 1. Cross section of optical cell and contents as arranged for the measurement of optical densities. The solution, of thickness l, can be regarded as a pile of an infinite number of discs of thickness dl (DARTNALL, 1957)

light absorbed, dI/I, is independent of the intensity I and depends only on the plate thickness dl, the pigment concentration c, and a coefficient α_λ peculiar to the absorptive power of the pigment and the wavelength of the light. Thus we may write

$$-dI/I = \alpha_\lambda \cdot c \cdot dl$$

(the minus sign arising because absorption is a decrement in I). By integrating this between the limits $I = I_i$ and $I = I_t$ we obtain

$$\log_e (I_i/I_t) = \alpha_\lambda \cdot c \cdot l. \tag{1}$$

The quantity $\log_e (I_i/I_t)$ is the optical density[1], and α_λ is the *extinction (absorbance)*

[1] The quantities I_i and I_t as defined above cannot be directly measured because of the reflexions that occur at the two cell/air interfaces and the two cell/liquid interfaces. This practical difficulty can be solved in the case of solutions by having, as a control or reference point, a second identical cell filled with solvent alone. The two cells are moved successively into the light beam and readings taken of the light intensities leaving the rear surface of the reference cell (I_r) and of the sample cell (I_s) respectively. It can be shown (taking first order reflexions into account but omitting the negligible effect of second and higher order reflexions) that the density of the solute (pigment) is given by

$$D_p = \log_e I_r/I_s$$

coefficient — a characteristic of the pigment and the wavelength of the light. When the concentration c is in number, n, of molecules per cubic centimetre, and the path length, l, is in centimetres, Eq. (1) becomes

$$\log_e (I_i/I_t) = \alpha_\lambda \cdot n/A$$

where A is the area exposed to light. Since the left hand side of this equation (optical density) is a numeric, the right hand side must also be dimensionless. From this it follows that α_λ has the dimensions of an area, and is expressed in square centimetres.

In the measurement of an extinction coefficient we are performing an operation that is analogous to firing bullets from random positions at right angles to a vertical plane (AA) that encloses a number of identical and impenetrable targets. We take note of the number of rounds fired (the incident intensity I_i) and of those that miss the targets and consequently hit the butts behind (the transmitted intensity I_t). From these numbers we can deduce the area of the targets (the absorption centres in molecules). Thus when all the targets lie in one plane, the ratio of $(I_i - I_t)$ to I_i, i.e. $\Delta I/I$, gives the fraction of the total shooting area that is occupied by the targets. When, on the other hand, the targets are distributed randomly in three dimensions (as are solute molecules in solution) the appropriate measure of target area is $\int dI/I$ which, as we have seen, is $\log_e (I_i/I_t)$, an expression that takes care of the screening effects of targets on each other.

In the concentration units mentioned above α_λ is the *molecular* (or, more precisely, the *chromophoric*) extinction coefficient, and is related to the effective physical area of a single absorption site (the chromophore) multiplied by the probability that a photon arriving within this area shall be absorbed. The change with wavelength of α_λ — the absorbance spectrum — can thus be construed as the variation of the probability term within its limits of zero and unity.

According to BRAUDE (1945) the maximum area of a chromophore is likely to lie between the orders 10^{-16} and 10^{-15} cm^2, which led him to suggest that there is a theoretical limit of this order for the extinction coefficient of a chromophore, assuming the probability term is unity at the absorbance maximum. Rhodopsin has an α_{max} of 1.56×10^{-16} cm^2 per chromophore. In 1936 DARTNALL, GOODEVE and LYTHGOE reported that a partial search of the literature on substances having continuous simple spectra in the visible or near ultra-violet had failed to reveal any with α_{max} in excess of 1.2×10^{-16} cm^2. One substance overlooked by them in this search is astacene, a carotenoid which, untypically, has a simple absorption band, and one of a shape and spectral position almost identical with that of rhodopsin. The α_{max} of astacene is 3.3×10^{-16} cm^2, which is in keeping with the fact that its conjugated chain is about twice the length of the prosthetic group of rhodopsin.

Thus the maximum values of α_{max} are consistent with BRAUDE'S hypothesis. Moreover the values cited relate to measurements in solution, in which the molecules are randomly oriented in space. It is shown below that when a molecule is oriented so that its dipole lies in the plane of the electric vector of the light, its extinction coefficient is three times as great as the average value for an unoriented molecule. Thus the appropriate value in BRAUDE'S sense for the α_{max} of rhodopsin is 4.68×10^{-16} cm^2, and for astacene is 9.9×10^{-16} cm^2. These are in good agreement with his hypothesis, and suggest that for these substances the probability term approaches unity at α_{max}.

The units in which the extinction coefficient has so far been expressed are not the usual ones. Usually the *molar absorbance*, ε_λ, is quoted, a quantity that is

defined by the equation

$$\log_{10}(I_i/I_t) = \varepsilon_\lambda \cdot c \cdot l$$

where the concentration, c, is in moles (gramme-molecular weight) per litre, the path length, l, is in centimetres (as before), and the optical density is in decadic (not napierian) logarithms. In these units the value of ε_{max} for rhodopsin in solution is 4.06×10^4 litre per cm mole (WALD and BROWN, 1953). Since these units are not homogeneous, however, the figure does not give the area occupied by a mole of chromophores. This area can be obtained by adjusting the figure to the base of napierian logarithms (multiply by 2.303) and to concentration in moles/cm^3 instead of moles/litre (multiply by $c.1000$). The result is 9.35×10^7 cm^2 per mole and this is the effective area occupied by a mole of randomly oriented rhodopsin chromophores for, on dividing it by Avogadro's Number (6.02×10^{23}) – the number in a mole – we obtain 1.56×10^{-16} cm^2, as before, for the chromophoric extinction coefficient.

B. Directional Properties (Dichroism)

The absorptive properties of the visual pigment molecule are highly directional. Only that component of the electric vector of light that is parallel to the long axis of the chromophoric group is absorbed in the principal band. If the electric vector of a particular ray of light makes an angle θ with this axis, then the effective amplitude of the vector is $\cos\theta$ and its intensity is the square of this. Thus if α' is the extinction coefficient of a molecule that is parallel to the electric vector, then the effective extinction coefficient for one that makes an angle θ with the vector is $\alpha' \cos^2\theta$.

In an extract of visual pigment the molecules are random; in the retina they are partly organised. Thus in the photoreceptors the molecules lie within a series of planes (the lamellae) that are at right angles to the axis of the photoreceptor[2]. Within each plane, however, the molecules are randomly disposed. Consider, first, a beam of plane polarized light falling axially on the photoreceptor. The electric vectors in this beam are all parallel and at right angles to the photoreceptor axis. They consequently lie in the planes and make all angles with the molecules. If we consider a quadrant it is clear (since all angles are equally likely) that for every molecule that makes an angle θ with an electric vector there exists, on average, another molecule that makes the angle $(90° - \theta)$. Thus the effective extinction coefficient of *two* such molecules is given by

$$\begin{aligned} &\alpha' (\cos^2\theta + \cos^2(90° - \theta)) \\ &= \alpha' (\cos^2\theta + \sin^2\theta) \\ &= \alpha' \end{aligned}$$

Since all the molecules can be paired in this way it follows that the effective extinction coefficient for an assemblage of molecules randomly disposed in a series of planes at right angles to the direction of a light beam is $\alpha'/2$. Since ordinary light

[2] The dichroism so caused is not complete, however, the average orientation of the electric dipole responsible for the absorption having axial and transverse components in the ratio 1:9. (For discussion see Chapter 7, Section IB).

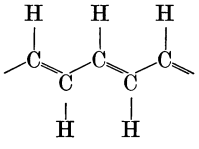
can be regarded as a mixture of polarized light of all angles of polarization (to each of which the above argument can be applied) it follows that the effective extinction coefficient is $\alpha'/2$ for unpolarized light also.

It can be similarly shown that in a random assemblage of molecules (e.g. as in an extract of visual pigment or a suspension of photoreceptor outer limbs) the effective extinction coefficient, whether for polarized or for unpolarized light, is $\alpha'/3$. Thus in the partially organized arrangement of molecules in photoreceptors the extinction is 50% greater than in a random assemblage (DENTON, 1959) (see also Chapter 7).

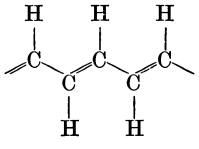
C. Variation with Wavelength

This section is intended only as a guide to the interpretation of the absorbance spectra of some conjugated polyenes. For a more rigorous treatment of some of the problems involved the reader is referred to Chapters 1 and 3.

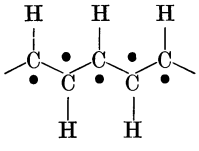
In the structural formulae of chemistry each single-valency bond represents two electrons, more or less equally shared between the two atoms forming the bond. Similarly a double bond represents four shared electrons. When the molecule is conjugated, i.e. has a chain of alternate single and double bonds, a special situation can arise. For example, the formula for the conjugated fragment



can equally well be written



provided the terminal groups of the chain permit this rearrangement of valencies. In such cases most of the molecules can be regarded as existing in an intermediate condition that we can represent by the formula



in which the black dots are unpaired electrons.

The "π" electrons that remain (five in the present example), after allocation of a single bond linkage between adjacent atoms, are not limited to the spaces between atoms. Instead they occupy a series of molecular orbitals that embrace the whole length of the conjugated chain. By PAULI's exclusion principle each orbit can only accommodate one electron (of given spin). Consequently the more electrons there are (i.e. the longer the chain of conjugation) the more ground-state orbits have to be filled, and less energy (i.e. a photon of lower frequency) is needed to raise the "nearest" electron to an excited-state orbit.

As the length of the conjugated chain increases, therefore, the spectral location of the absorption band advances to longer wavelengths (where the quantal energy is less). Thus in the diphenylpolyenes, $C_6H_5-(CH=CH)_n-C_6H_5$, the simplest members ($n = 1$ and 2) absorb in the ultraviolet, and are colourless. When $n = 3$ the polyene is pale yellow; when $n = 5$ it is orange; when $n = 11$ it is violet, and when $n = 15$ it is green – the absorption bands being respectively centred in the violet, blue, green, and red regions of the spectrum.

The most familiar conjugated substances are the carotenoids, which exist in many varieties and are widely dispersed in nature. They contain forty carbon atoms per molecule, and can be regarded as formed from eight isoprene ($CH_2=CH-C(CH_3)=CH_2$) units.

As exemplified by β-carotene (Fig. 2) the carotenoids absorb light in three spectral regions. The main band lies in the visible (centred usually between 400 and 500 nm), a subsidiary band is found in the near ultraviolet (centred at 340 nm) – the region of *cis* peaks, and another in the far ultraviolet (centred at 275 nm). The following interpretation of these spectra is based on the ideas of Lewis and Calvin (1939), Pauling (1939) and Zechmeister (1944).

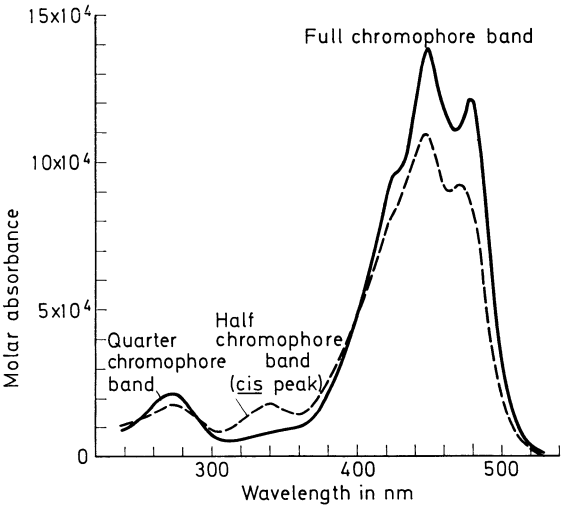


Fig. 2. Absorbance spectra of two molecular conformations of β-carotene. The continuous curve gives the spectral absorbance of *trans*-β-carotene, the dashed curve that of *cis*-β-carotene

The oscillating electric vector of light induces π-electrons to surge backwards and forwards along the conjugated chain. When the light is of a frequency to correspond with the rhythm of these movements it is strongly absorbed. This gives rise to the fundamental band in the visible. The intensity (absorbance) of the band is proportional to the square of the regularly alternating dipole moment of the molecule, and hence to the square of the length of the conjugated chain.

The *cis*-peak (at 340 nm) is attributed to induced oscillations of π-electrons from the two ends towards the middle of the conjugated chain, and back. The dipoles of these "half" oscillations are opposed. Consequently in "straight" (all-

trans) isomers absorption is negligible in this spectral region. In *cis*-isomers, however, the molecule is bent, and consequently has a resultant dipole due to these half-oscillations at right angles to the main axis of the chain. This dipole gives rise to a moderately intense absorption in the *cis*-, or "half-chromophore" band (Fig. 2).

In *cis*-isomers the intensity of absorption in the fundamental band is less than that for the all-*trans* isomer. A *cis* bond has its greatest effect in this respect when it is located in the middle of the chain, for it then reduces the chromophore length by the factor cos 30°, and hence decreases the intensity of the "full-chromophore" band to $\cos^2 30° = 0.75$ times that for the *trans* isomer (Fig. 2).

The band at 275 nm in the ultraviolet is attributed to induced concentrations of π-electrons alternately in the first and third and in the second and fourth quarters of the chain. These quarter oscillations confer an overall dipole moment on the molecule that is maximal for the all-*trans* isomer and less for any *cis* form (Fig. 2).

The maxima of these three bands in carotenoids (450 nm, 340 nm and 275 nm for β-carotene) are separated by roughly equal frequency intervals (about 7,100 wave numbers).

The spectra of the vitamins A, i.e. the retinols and 3-dehydroretinols, and of their corresponding aldehydes the retinals and 3-dehydroretinals can be similarly interpreted. Since these substances have conjugated chains only half as long as those of the carotenoids, however, their main absorption bands are in the near ultraviolet, and (when present) their *cis* peaks and "quarter oscillation" bands are displaced to the further ultraviolet (see Chapter 2).

The visual pigment molecule consists of a protein (in the "opsin" series) in combination with a single prosthetic group (HUBBARD, 1954) that is based either on vitamin A_1 or on vitamin A_2. Apparently the prosthetic group is always in the 11-*cis* conformation (WALD, 1958). Because of the close association between opsin and prosthetic group the main absorption band of the visible pigment is displaced to longer waves by comparison with that for the detached prosthetic group (retinal or 3-dehydroretinal). No entirely satisfactory explanation of this bathochromic shift has been advanced (for a discussion see Chapter 3, Section IIIC, p. 91). Thus in the A_1 series the spectral location of maximum absorbance ("λ_{max}") ranges from 433 nm for the green-rod pigment of the frog (DARTNALL, 1967) to 575 nm for the cone iodopsin of the frog (LIEBMAN and ENTINE, 1968). Fewer pigments are known in the A_2 series but the λ_{max} ranges from 438 nm for the green-rod pigment of the frog tadpole to 620 nm for the cone cyanopsin of the same species (LIEBMAN and ENTINE, 1968).

Within these ranges several pigments have been characterized. There is evidence from surveys of nearly 200 animal species that the λ_{max} of visual pigments are not randomly distributed but tend to occur at certain points in the spectrum, one set of positions for the A_1 pigments (DARTNALL and LYTHGOE, 1965a, b) and another for the A_2 pigments (BRIDGES, 1964, 1965).

The absorbance spectrum of a typical visual pigment, bovine rhodopsin, is shown in Fig. 3. In addition to the principal band (at 498 nm in this case) there is a secondary ("*cis*") band at about 340 nm and considerable absorption, largely due to the opsin moiety, in the ultraviolet. On bleaching there is little change in this region. HUBBARD (1969) reports that the absorbance of rhodopsin at 280 nm de-

creases by only about 3% when it is bleached by light and, from this and other evidence considers that the prosthetic group chromophore contributes but little to the absorbance of rhodopsin in this far-ultraviolet region.

The *cis* band has λ_{max} ranging from about 340 to 380 nm in different pigments, being separated by about 1000 wave numbers from the main band λ_{max}. The fact that the photosensitivity spectrum of frog rhodopsin (Fig. 3) also possesses a *cis* peak is evidence that it belongs to the same chromophore as the main band

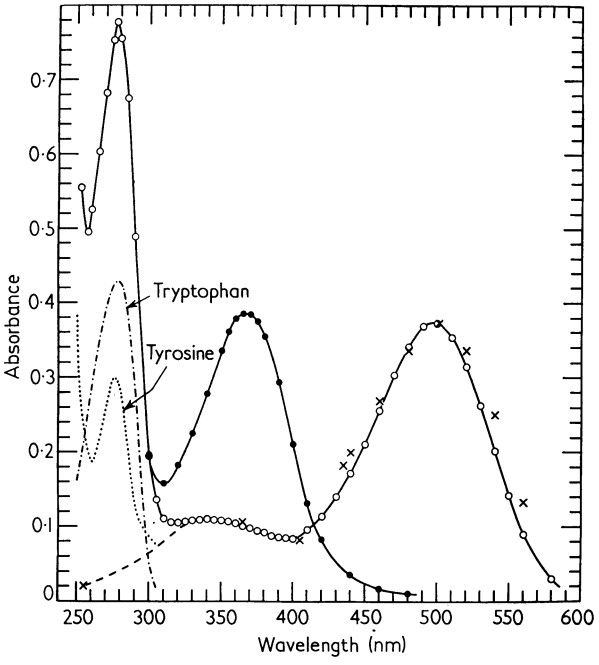


Fig. 3. Absorbance and photosensitivity spectra of typical visual pigments. The curve through the plain circles gives the absorbance spectrum of an extract of bovine rhodopsin (pigment 498_1) at pH 9.2; curve through filled circles, the same after bleaching. Note that the main band at 498 nm and the *cis* band at 340 nm are replaced by a photoproduct band at 370 nm but that the "protein band" at 278 nm is little affected by the bleaching. The protein band is due largely to tyrosine and tryptophan. The crosses are photosensitivity data from SCHNEIDER, GOODEVE and LYTHGOE (1939) and GOODEVE, LYTHGOE and SCHNEIDER (1942) for frog rhodopsin (pigment 502_1) scaled to agree with the bovine rhodopsin absorbance curve at 500 nm. (Modified from COLLINS, LOVE and MORTON, 1952)

(GOODEVE, LYTHGOE and SCHNEIDER, 1942). HELLER (1968a, b), however, has recently published a spectrum of purified bovine rhodopsin that lacks the *cis* peak and has an A_{280}/A_{500} ratio of 1.55–1.68 instead of the usual 2.2. HELLER suggested that the *cis* peak is an artifact due to photoproduct contamination. This view is difficult to accept, however, not only because of the photosensitivity data but also because frog rhodopsin shows in this region a very marked circular dichroic absorption (see Chapter 6) that disappears when the pigment is bleached (CRESCITELLI, MOMMAERTS and SHAW, 1966). Possibly the stringent purification

methods used by HELLER resulted in some "surgery" to the visual pigment molecule without causing it to lose its photosensitivity and the other properties that we associate with visual pigments. In any case HELLER'S important observations and methods should be extended.

III. The Bleaching Kinetics of Visual Pigments

When exposed to a continuous beam of light a visual pigment preparation "bleaches", i.e. its transmissivity of most wavelengths increases until a final value for the fully-bleached condition is reached. Some typical transmission/time curves for room-temperature bleachings of extracts are shown in Fig. 4 for various visual pigments and bleaching wavelengths.[3]

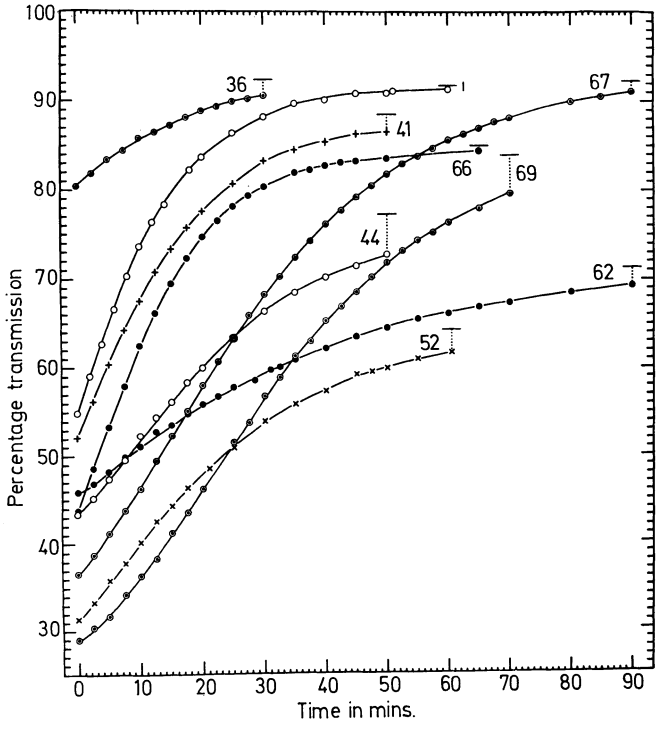


Fig. 4. Transmission/time curves for the bleaching of visual pigment extracts from various species. Plain circles are for frog extracts (502_1), diagonal crosses for a gurnard extract (492_1), centred circles for conger eel extracts (486_1), upright crosses for a carp extract (523_2) and filled circles for tench extracts (530_2). The short horizontal lines, connected by dots to the last readings, indicate the final transmissions, I_f, after prolonged exposure. Temperature 25° C, pH 8—9, no hydroxylamine present. Various bleaching wavelengths were used, as follows: Experiment 44; 460 nm: Experiments 52, 62 and 69; 480 nm: Experiments 36, 67; 510 nm: Experiment 1 and 66; 540 nm: Experiment 41; 560 nm. (DARTNALL, 1958)

[3] When bleaching is carried out by light of less than the isosbestic wavelength the preparation becomes less transmitting as it "bleaches", and $I_f - I_t$ on the left hand side of Eq. (2) must be replaced by $I_t - I_f$ (GOODEVE, LYTHGOE, and SCHNEIDER, 1942).

A. Method of Photometric Curves

It has been shown (DARTNALL, GOODEVE and LYTHGOE, 1936, 1938; DARTNALL, 1958, 1968) that the equation

$$\log_e \frac{I_t}{I_f - I_t} = \phi \cdot \alpha_\lambda \cdot \gamma \cdot I_i \cdot t + \text{constant} \qquad (2)$$

describes the bleaching kinetics of all visual pigments tested, under all conditions – provided certain requirements are met.

In Eq. (2) the symbols have the following meanings.

I_i, the intensity (in number of quanta per sec. per cm^2) of a constant, uniform beam of parallel monochromatic light incident on the front surface of an extract contained in an optical cell having plane parallel faces at right angles to the light.

I_t, the intensity of light (in any convenient units) transmitted through the rear surface of the bleaching extract at the time t.

I_f, the intensity of light (in the same convenient units) finally transmitted through the fully-bleached extract.

t, the time (in seconds) from the initial exposure to light.

γ, the quantum efficiency of the photochemical change, a ratio defined as

$$\frac{\text{number of chromophores destroyed}}{\text{quanta absorbed by the chromophores}}$$

α_λ, the extinction coefficient (cm^2) of a single chromophore for the bleaching light of wavelength λ, as defined by the equation

$$\log_e \frac{I_i}{I_t} = \alpha_\lambda \cdot c \cdot l$$

where c is the chromophore concentration (number per cm^3) and l is the length (cm) of light path through the extract.

The validity of equation (2) is not affected by the presence of other light-absorbing species (e.g. impurities that may have accompanied the visual pigment into the extract) provided they are stable and do not act as catalysts or photosensitizers. This follows from the derivation of the equation (DARTNALL, GOODEVE and LYTHGOE, 1936, 1938) and has also been shown by practical tests (DARTNALL, unpublished observations) in which the presence or absence of added inert pigment has made no difference to the value obtained for the photosensitivity, $\alpha_\lambda\gamma$, of the visual pigment. The light absorbed by impurities is generally small, for modern methods of extraction yield preparations of high spectrophotometric purity. But when the bleachings are carried out with shortwave light, the absorption in the protein band (Fig. 3) of the visual pigment itself can be appreciable. However, to the extent that no energy transfer to the prosthetic group chromophore occurs, this absorption by the opsin moiety has the same effect as though a stable impurity were present, and is similarly compensated by the factor ϕ (see below).

More surprising, perhaps, is the fact that neither the setting up of a concentration gradient in the unstirred extract as bleaching proceeds, nor the formation of light-absorbing photoproduct seriously disturbs the validity of Eq. (2). For a consideration of these points the reader is referred to the literature (DARTNALL, 1936, 1957; DARTNALL, GOODEVE and LYTHGOE, 1936, 1938; GOODEVE, LYTHGOE and SCHNEIDER, 1942; SCHNEIDER, GOODEVE and LYTHGOE, 1939).

The presence of inert impurities, and the accumulation, as bleaching proceeds, of stable photoproduct result, of course, in a slower rate of visual-pigment bleaching than would otherwise be the case, for these other pigments compete for the light. But the reduction in rate from either or both these causes is precisely allowed for by the slope-compensating factor, ϕ, in Eq. (2).

The function ϕ is given by

$$\phi = \frac{I_f}{I_f - I_t} \cdot \frac{I_i - I_t}{I_i} \cdot \frac{\log I_f/I_t}{\log I_i/I_t}$$

and hence, since it contains I_t, is a variable. However the value of ϕ in any one experiment alters only very slowly as I_t changes from its initial value of I_i to its final value of I_f. In the majority of actual experiments the total change in ϕ is less than 1%, and mean values can be used without introducing significant error[4].

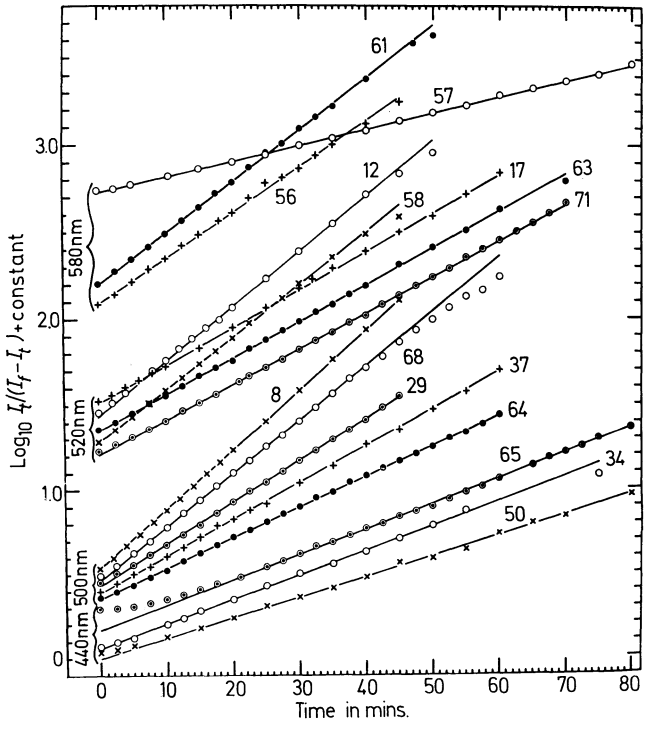


Fig. 5. Examples of the linear plots obtained in the bleaching experiments by the method of photometric curves. The visual pigment extracts were from various species (frog, gurnard, conger eel, carp and tench as symbolized in Fig. 4), and the bleachings were carried out at 25° C and pH 8—9 by monochromatic lights of various wavelengths, as indicated (DARTNALL, 1958)

[4] In practice a correction must be made to I_i to allow for the light reflected back into the visual pigment extract from the rear surfaces of the optical cell (usually about 5%). This reflected light increases as the extract becomes more transmitting, thus acting in the opposite sense to the decrease in ϕ that occurs in these conditions. The two effects are also opposed when the extract becomes less transmitting as it bleaches, i.e. when bleached with light shorter than the isosbestic wavelength.

Since ϕ is effectively constant in any one experiment it follows from Eq. (2) that the values of the function $\log I_t/(I_f - I_t)$ should lie on a straight line when plotted against the time. This found to be so, provided certain conditions are met, and Fig. 5 illustrates the acceptably linear plots that can be obtained.

From Eq. (2) the slopes, S, of such lines are given by

$$S = \phi \cdot \alpha_\lambda \gamma \cdot I_i$$

and hence, since ϕ and the intensity I_i are known, it is possible to calculate the value of $\alpha_\lambda \gamma$, the photosensitivity of the visual pigment at λ, the bleaching wavelength.

B. Applicability of the Method

Eq. (2) was derived for the simple photochemical reaction,

$$A \xrightarrow{h\nu} B$$

in which a photosensitive substance A is converted to a photoproduct B that is both thermally and photochemically stable. The equation would also apply if the immediate photoproduct B were very unstable and decomposed by an extremely rapid thermal process to a stable product C, for in this event the amount of C present at any time would be a strict measure of the total amount of B that had been formed, and of the total amount of A that had been photolysed. The number of unstable intermediate stages before a stable end product is reached is clearly immaterial, and Eq. (2) applies to the general case

$$A \xrightarrow{h\nu} B \longrightarrow C \longrightarrow D \longrightarrow \ldots X$$

unstable intermediates — Stable end product

provided that the amounts of the unstable intermediates are vanishingly small at all times during the bleaching. This requires that all the intermediates shall be very unstable, and also that the rate of production of B, the first of them, shall be low. This, in turn, means that the intensity of the bleaching light must be relatively low. In summary we may say that Eq. (2) will apply provided the rate-limiting factor of the total reaction is the initiating photolytic process.

The bleaching of a visual pigment is just such a complex process — initiated by light and followed by a sequence of purely thermal reactions. The complexities of this sequence are the subject of some controversy (for discussion see Chapter 3, Section IVB) but for our purpose the scheme of Ostroy, Erhardt and Abrahamson (1966) for rhodopsin, as set out in Fig. 6, will suffice.

Many satisfactory experiments by the method of photometric curves have been carried out at room temperatures and above under mildly alkaline conditions (and with bleaching light intensities of $10^{13}-10^{14}$ quanta $\sec^{-1}\mathrm{cm}^{-2}$)[5]. In these circumstances the "final" product is the alkaline form of N-retinylidene opsin, which is only moderately stable and slowly hydrolyses to retinal (Fig. 6). Fortunately, however, the spectra of these two substances are not very dissimilar, and the "end point" is sufficiently sharp for Eq. (2) to apply, particularly at the longer

[5] In these orders of intensity, photoreversal (see Chapter 5, Sections V and VI) is calculated to be negligible.

wavelengths where the absorbances of N-retinylidene opsin and retinal are low. It is better, however, to carry out the bleachings in the presence of 0.02–0.06 M hydroxylamine (DARTNALL, 1968) for then the final product is the stable retinal oxime, irrespective of pH, and there is the additional advantage that hydroxylamine, by capturing prosthetic-group retinal as oxime, shortens the lifetimes of the intermediates (BRIDGES, 1962).

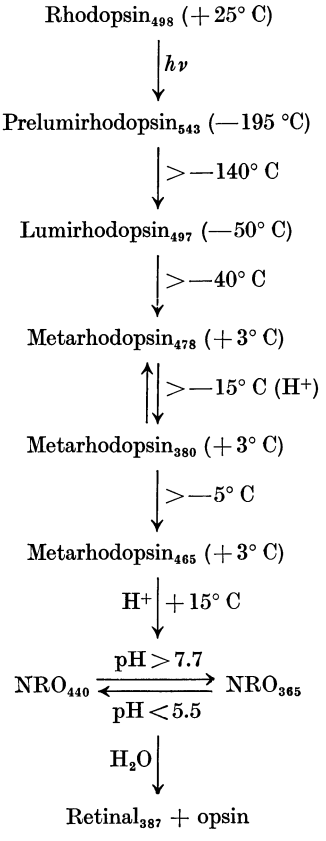


Fig. 6. Thermal reactions following photolysis of bovine rhodopsin according to OSTROY, ERHARDT and ABRAHAMSON (1966). (Compare with Fig. 14 in Chapter 3). The subscript figures give λ_{max} of the various intermediates at the temperatures given in brackets. The metarhodopsins 478, 380 and 465 are also called metarhodopsin I, II and III respectively, and the last is also known as pararhodopsin. NRO denotes N-retinylidene opsin. Prelumirhodopsin is also known as bathorhodopsin to distinguish it from hypsorhodopsin (see YOSHIZAWA, Chapter 5, Fig. 1)

Although linear plots of log $I_t/(I_f - I_t)$ against time are generally obtained in the absence of hydroxylamine it had been noticed in a few cases (particularly with short-wave bleachings of the 486_1 pigment of the conger eel) that there was an appreciable divergence from linearity in the early part of the experiment, as though "the bleaching took some time to get under way" (DARTNALL, 1958). A clear example of this is given by experiment 65 in Fig. 5.

In a later investigation (DARTNALL, 1968) it was found that the addition of hydroxylamine removed this anomalous behaviour, and perfect straight lines for the whole period of the experiment were obtained with all the visual pigments examined. Another effect of the presence of hydroxylamine was to increase the slopes of the straight lines, i.e. to increase the values of the photosensitivities. The magnitude of this increase was found to vary with different visual pigments (though it was always the same for any one pigment). Thus in the presence of hydroxylamine the photosensitivity of the 520_1 pigment of the freshwater fish gwyniad is raised by 4%, that of the 502_1 pigment of the common frog by 16% and that of the conger 486_1 pigment by no less than 31%.

More significant is the fact that the maximum photosensitivities of all the retinal-based pigments examined were found to be the same, within experimental error, when measured in hydroxylamine whereas without it they showed some variation (Fig. 8).

At the time it was thought probable that the effect of hydroxylamine was due to inhibition of visual pigment regeneration, but a subsequent investigation (DARTNALL, unpublished observations) showed that it was due simply to its property of drastically shortening the lifetimes of the intermediates (and thus validating the assumptions made in deriving Eq. (2)). This result was obtained in the following way. A series of bleaching experiments was carried out at 25° C on frog rhodopsin extracts without hydroxylamine but, instead of exposing the extracts continuously to the light, they were exposed for periods of two minutes only, followed by ten minutes in darkness. They were then re-exposed for another two minutes followed again by ten minutes in the dark. The experiments were continued in this way until bleaching was complete. It was found that there was appreciable loss of optical density in the dark periods. From such experiments carried out with bleaching lights of various wavelengths between 440 and 560 nm it was possible to construct the difference spectrum of the substance that was fading in the dark periods. It had λ_{max} at about 480 nm and was, presumably, principally due to the fading of metarhodopsin III (Fig. 6). It had not been anticipated that this intermediate would interfere with the applicability of Eq. (2) at a temperature as high as 25° C.

When the functions $\log I_t(I_f - I_t)$ were plotted against time for these discontinous-exposure experiments (ignoring the ten minute dark periods) the *same* slopes were obtained as in continuous-exposure experiments carried out in the presence of hydroxylamine. This indicates that the effect of hydroxylamine is simply to hasten the decomposition of interfering intermediates, and explains the varying of this effect from pigment to pigment as due to variations in the thermal stability of the metarhodopsins. It also suggests that thermal regeneration of visual pigment from intermediates (BRIDGES, 1960) is negligible under these experimental conditions.

By bleaching extracts in discontinuous bursts, viz. by short exposures to light followed by lengthy periods in darkness to allow unstable intermediates to be cleared from the extract, the applicability of the method of photometric curves can be extended to quite low temperatures. With the technique[6] Mr E. D.

[6] This technique was first used by BAUMANN (1965) in his photochemical study of the rate of bleaching of the perfused frog-retina.

FOLLAND and I (unpublished observations in 1968) have obtained satisfactorily linear plots at temperatures down to $-15°$ C using extract/glycerol mixtures to prevent freezing. In some of these experiments only two 2-minute light exposures were made each day, the intervening darkness periods having to be very long (alternately 6 hours and an "overnight" 18 hours) to allow the very slow thermal changes following exposure to be completed before the next exposure was made. Such experiments took well over a week to complete, yet yielded results as precise as the fast ones at room temperature.

IV. Photosensitivities of the Visual Pigments

A. Extracts

1. In the Visible

In the first use of the method of photometric curves (DARTNALL, GOODEVE and LYTHGOE, 1936, 1938) measurements were made on frog rhodopsin at a wavelength close (506 nm) to the absorbance maximum, and the value 9.1×10^{-17} cm^2 was obtained for the photosensitivity, $\alpha_{506}\gamma$, in absolute units. This corresponds to an $\varepsilon_{max}\gamma$ of 2.39×10^4 litre per cm mole, where ε_{max} is the molar absorbance at the maximum (502 nm). This result was confirmed by SCHNEIDER, GOODEVE and

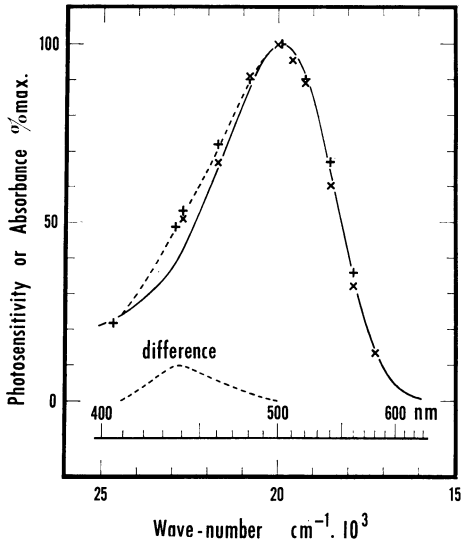


Fig. 7. Comparison between the absorbance spectrum of pure frog rhodopsin (full-line curve) and the photosensitivity spectrum as determined (in the absence of hydroxylamine) on retinal extracts. The upright crosses are SCHNEIDER, GOODEVE and LYTHGOE's (1939) data (436—560 nm) and the value at 405 nm is from GOODEVE, LYTHGOE and SCHNEIDER (1942). The diagonal crosses are derived from DARTNALL's (1958) data. Note the discrepancy (dashed portion of curve) between absorbance and photosensitivity, maximal at 430—440 nm, and separately plotted as a difference. This is probably due to the presence of green-rod pigment, $\lambda_{max} = 433$ nm, in the hydroxylamine-free extracts used for the photosensitivity measurements (DARTNALL, 1968)

LYTHGOE (1939) who in addition measured the photosensitivity to lights between 436 and 560 nm, and found that it varied with wavelength in practically the same way as did the absorbance. This indicated that γ, the quantum efficiency, is independent of wavelength. A comparison of their results with a modern absorbance spectrum for pure frog rhodopsin is made in Fig. 7. This confirms the correspondence between the wavelength variations of photosensitivity and of absorbance on the long-wave side of the maximum but there is a clear discrepancy on the short-wave side, maximal at 430–440 nm. This is due to the presence in the rhodopsin extracts of the 433_1 pigment, the pigment of the green rods. Frog extracts contain about 91% rhodopsin and 9% green-rod pigment (DARTNALL, 1967) and the unsuspected presence of the latter would lead to spuriously high values for the photosensitivity of rhodopsin at wavelengths below 500 nm. There seem no grounds, therefore, for doubting that the wavelength variation of the photosensitivity of pure rhodopsin exactly matches that of its absorbance, and hence that γ, the quantum efficiency is invariant, at least for wavelengths >436 nm.

In 1958 DARTNALL reported the results of photosensitivity measurements at various wavelengths on some other visual pigments using frog rhodopsin for comparison. The pigments used were the 486_1 pigment of the conger eel, the 493_1 pigment of the gurnard and, in the A_2 series, the 523_2 pigment of the common carp and the 530_2 pigment of the tench. These four pigments were all found to have photosensitivities comparable to that of frog rhodopsin, the values at their respective maxima ranging from 66 to 104% of that for the frog pigment.

A more precise comparison between the photosensitivities of different visual pigments was made possible by carrying out the experiments in the presence of hydroxylamine. In this investigation (DARTNALL, 1968), already mentioned above (p. 137), the use of hydroxylamine revealed a dichotomy of photosensitivity corresponding to the A_1/A_2 dichotomy of the prosthetic group. Thus all seven A_1 (retinaldehyde-based) pigments examined had maximum photosensitivities within the range $2.63-2.85 \times 10^4$ litre per cm mole; the five A_2 (3-dehydroretinal-based) pigments values between 1.89 and 1.95×10^4 litre per cm mole, i.e. about 70% of the A_1 values (see Fig. 8 and Table 1). These results are linked to the value of 2.78×10^4 litre per cm mole for frog rhodopsin, obtained on increasing by 16% the previous figure of 2.39×10^4 litre per cm mole (DARTNALL, GOODEVE and LYTHGOE, 1938; SCHNEIDER, GOODEVE and LYTHGOE, 1939) (see p. 137). KROPF (1967) measured the photosensitivity of frog rhodopsin *(Rana pipiens)* to light of 436 nm by a different method in which potassium ferrioxalate was used as an actinometer (HATCHARD and PARKER, 1956). This was done in the presence of 0.1 M hydroxylamine so the result is directly comparable with those just quoted. He obtained the value 1.14×10^4 litre per cm mole for the photosensitivity at 436 nm. To relate this with the value for the photosensitivity at λ_{max} we need the value of the ratio $\varepsilon_{502}/\varepsilon_{436}$. Unfortunately this is not known very precisely but KROPF (1967) takes ε_{436} for rhodopsin as 1.76×10^4 litre per cm mole. Since ε_{502} is 4.06×10^4 litre per cm mole (WALD and BROWN, 1953) the ratio $\varepsilon_{502}/\varepsilon_{436}$ is 2.31 on this reckoning. The writer, on the other hand, (DARTNALL, 1968) has estimated this ratio to be 2.58. If we use the mean, 2.44, and multiply KROPF's photosensitivity value by it we obtain 2.78×10^4 litre per cm mole as the photosensitivity at λ_{max}. This is the same figure as was obtained in the photometric

curves experiments when hydroxylamine was present (DARTNALL, 1968) and reinforces SCHNEIDER, GOODEVE and LYTHGOE's conclusion that the quantum efficiency is independent of wavelength in the visible spectrum.

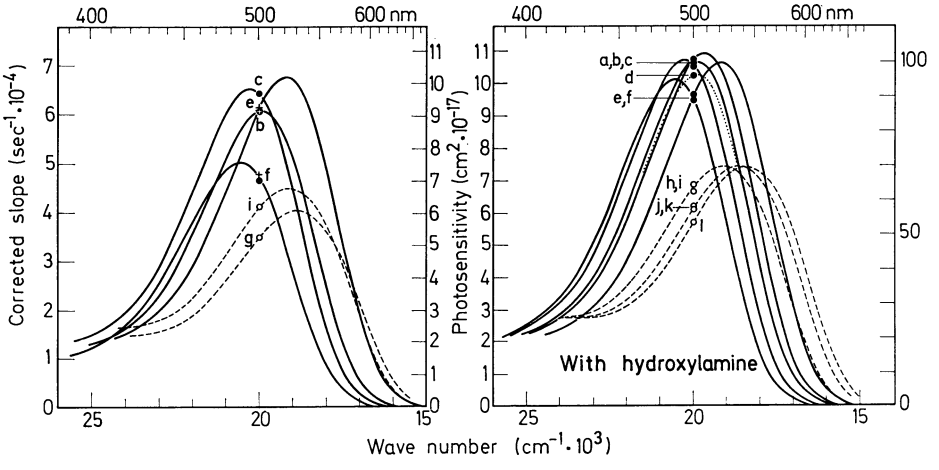


Fig. 8. The dichotomy of photosensitivity corresponding to the dichotomy of prosthetic group in the visual pigments. Measurements at 25° C in the absence and presence of hydroxylamine, as indicated. Left ordinate scale, slopes of the straight lines obtained by photometric curve analyses: centre ordinate scale, photosensitivities ($\alpha\gamma$) in absolute units: right ordinate scale, photosensitivities as percentages of that for *Rana temporaria* at λ_{max}. Filled circles give the photosensitivity values at 500 nm (20,000 wave-numbers) of A_1-based pigments; plain circles those of A_2-based pigments. The curves, full-line for A_1 pigments (dotted-line in one case to avoid confusion) and dashed-line for A_2 pigments are absorbance spectra scaled to pass through these values. The data on the left (filled and plain circles) were obtained in the 1958 work and agree with those of the 1968 work (crosses) that were also obtained without hydroxylamine. The data on the right were obtained in the presence of hydroxylamine (1968 work), and fall into two classes having maximum photosensitivities of either 10.1 — 10.9 $\times$ 10^{-17} cm^2 per chromophore (A_1 pigments) or 7.4 $\times$ 10^{-17} cm^2 per chromophore (A_2 pigments). Key to letters as follows: a *Salvelinus alpinus* (508_1); b *Rana temporaria* (502_1); c *Trigla cuculus* (493_1); d *Rana cancrivora* and *Galago crassicaudatus agisymbanus* (502_2 and 501_1 respectively); e *Coregonus clupeoides pennantii* (520_1); f *Conger conger* (486_1); g *Tinca tinca* (530_2); h *Carassius carassius* (523_2); i *Cyprinus carpio* (523_2); j *Coregonus clupeoides clupeoides* (536_2); k *Rutilus rutilus* (536_2); l *Osmerus eperlanus* (543_2) (DARTNALL, 1968)

2. In the Ultraviolet

GOODEVE, LYTHGOE and SCHNEIDER (1942) extended the method of photometric curves to the violet and ultraviolet by making measurements of the photosensitivity of rhodopsin at 405, 365 and 254 nm. Their values at 405 and 365 nm were in line with what we know to be the variation of absorbance with wavelength in this region (Fig. 3) and indicate that absorption in the β-band of rhodopsin is just as effective as in the α-band in promoting bleaching, i.e. that the quantum yield is invariant down to 365 nm at least. Their value at 254 nm however was very low (Fig. 3) though absorption in this region — the γ-band of rhodopsin — is very high indeed. This suggested that energy absorbed by the "protein moiety" of the molecule was not available to the chromophore in the prosthetic group.

Recently, however, KROPF (1967) has measured the photosensitivity of frog rhodopsin in the ultraviolet (at 254 and 280 nm) and has obtained evidence that there is a substantial intramolecular transfer of energy absorbed in the protein part of the molecule to the prosthetic part carrying the retinal-based chromophore. KROPF established that bleaching by irradiation with ultraviolet light was "normal", i.e. that the prosthetic group product of bleaching, as with visible light, was in the all-trans form (HUBBARD and WALD, 1952) and that the free opsin was capable of regenerating rhodopsin, a criterion for native opsin (HUBBARD, 1958).

KROPF'S values for the photosensitivities to 254 and 280 nm radiation are $1.10 \pm 0.40 \times 10^4$ and $2.30 \pm 0.50 \times 10^4$ litre per cm mole respectively, (compare with 2.78×10^4 litre per cm mole for the photosensitivity at λ_{max}).

In this work KROPF tacitly assumed that the rhodopsin chromophore has no absorption in the 254–280 nm region, i.e. that all the ultraviolet is absorbed by the opsin moiety. HUBBARD (1969) considers that the contribution from the chromophore must be small for the "absorption of rhodopsin at 280 nm decreases[7] by only about 3% when it is bleached by light to all-*trans* retinal and opsin, and the molar absorbance of all-*trans* retinal is only about 4% that of rhodopsin at 280 nm".

B. In Situ

In 1954 HAGINS reported measurements of the photosensitivity of mammalian rhodopsin *in situ*. By following the time course of the rise in retinal reflectivity of the excised eye (of a dark-adapted albino rabbit) as it bleached in light of known intensity and wavelength (516 nm) he was able to determine the photosensitivity as a rate constant. He calculated his result to the λ_{max} for rabbit rhodopsin (498 nm) and reported $\varepsilon_{max}\gamma$ as 2.7×10^4 litre/cm mole (corresponding to $\alpha_{max}\gamma = 10.3 \times 10^{-17}$ cm^2/mole). Digitonin extracts of rabbit rhodopsin, solidified as agar gels and treated in the same way gave a distinctly lower result, 1.7×10^4 litre/cm mole (corresponding to $\alpha_{max}\gamma = 6.5 \times 10^{-17}$ cm^2/mole). The difference between these results is nicely accounted for by the 50% enhancement of absorbance that one would expect from orientation of the *in situ* pigment. It is less easy, however, to relate these results to the values found in extracts by conventional methods (Table 1), mainly because of the uncertainties in the light losses by pre-retinal absorption and reflection.

Soon afterwards the photosensitivity of rhodopsin in the living human eye was measured by RUSHTON (1956) who calculated from his results a value for the photosensitivity at 507 nm that was 1.7 times that reported by DARTNALL, GOODEVE and LYTHGOE (1938) for extracted frog rhodopsin at 506 nm. This was after allowances for the effect of orientation, and for the pre-retinal light losses (LUDVIGH and MCCARTHY, 1938; WEALE, 1954). This can be regarded as tolerable agreement considering that the bleaching was done by white light (the calculations consequently involving the use of the scotopic luminosity function and the appropriate relative spectral energy distribution for the colour temperature (2750° K) of the white light), and the assumption of the value of 44% for light losses in his subject's

[7] KROPF'S (1967) spectra show a slight *increase* in absorbance at 280 nm on bleaching. The A_{280}/A_{500} ratio for his rhodopsin extracts was about 4.3, considerably more than the 2.2 for a pure specimen (WALD, 1949).

eye. Nevertheless, RUSHTON drew attention to the possibility that the discrepancy might arise from an approximately 2:1 concentration of light intensity incident on the pigment in the outer segments by the funnelling action of the inner segments.

Most of the uncertainties inherent in the above measurements were avoided by BAUMANN (1965) in his study of the rate of bleaching of the perfused isolated retina of the edible frog, *Rana esculenta*. He used light of wavelength 504 nm and intensity 2.57×10^{14} quanta per second per cm^2 of retina and measured the transmissivity of the retina at various times. In order to avoid the complication that would otherwise be caused by the relatively slower thermal decomposition *in situ* of the intermediate products he invented the discontinuous exposure technique that was described in Section III B, employing 15–30 sec exposures followed by 20–30 min in darkness. He analysed his results by the method of photometric curves, using the stable values for the transmissivities at the end of the dark periods, and found a photosensitivity at 504 nm for rhodopsin of 14.6 ± 1.8 (S. D.) $\times 10^{-17}$ cm^2 per chromophore (equivalent to an $\varepsilon_{max}\gamma$ of $3.81 \pm 0.47 \times 10^4$ litre per cm mole).

The value for unoriented frog pigment in the extracted condition is 10.4×10^{-17} cm^2 per chromophore. The absorbance spectra of extracted and *in situ* rhodopsin are identical (DARTNALL, 1961) and hence if their only difference is in the partial organisation of the latter (Section II B) one would expected an $\alpha_{max}\gamma$ for *in situ* pigment of 15.6×10^{-17} and this, in fact, is well within one standard deviation of BAUMANN'S value.

V. Quantum Efficiency of Bleaching

The parameter photosensitivity is the product of the extinction coefficient (α or ε) and the quantum efficiency γ, and the measurements of it described in the previous pages can only give indirect information about its component factors. Thus in the original and early papers it was concluded (from the constancy of photosensitivity over ranges of temperature, concentration and pH, and from certain photochemical considerations) that the quantum efficiency for the bleaching of frog rhodopsin "is equal to or not much less than unity" (DARTNALL, GOODEVE and LYTHGOE, 1936, 1938) and again "not less than 0.6" (SCHNEIDER, GOODEVE and LYTHGOE, 1939). No more precise statements from these results seem possible.

We are delivered from this uncertainty, however, by the measurements of extinction coefficients that have been independently made, both for A_1- and A_2-based pigments. Thus cattle rhodopsin ($\lambda_{max} = 498$ nm) has an absorbance of 4.06×10^4 litre per cm mole (WALD and BROWN, 1953) assuming one retinal equivalent per molecule (HUBBARD, 1954), and chicken iodopsin ($\lambda_{max} = 562$ nm) practically the same value (WALD, BROWN and SMITH, 1955). The only published information on A_2-based pigments is the statement in BROWN, GIBBONS and WALD (1963) that "the molar extinction of porphyropsin is about 30,000[8] (WALD, BROWN

[8] This measurement was made on a digitonin extract (to which hydroxylamine had been added) of the yellow perch, *Perca flavescens*, and the molarity of the pigment was computed, as in the case of rhodopsin, by reference to the spectrum of the oxime formed on bleaching (private communication from Prof. WALD).

and BROWN, unpublished observations)". In none of these species has the pigment photosensitivity been measured but it is clear from the many published spectra that the α-band intensities of the visual pigments stand in nearly constant relation to those of their retinal (or dehydroretinal) oximes after bleaching. Thus the ε_{max} values of 4.06 and 3.00 $\times$ 10^4 litre per cm mole are applicable (at least approximately) to all A_1- and A_2-based pigments respectively. The quantum efficiencies can be obtained, therefore, by dividing the values of $\varepsilon_{max}\gamma$ by the appropriate figure. The results of doing this are set out in Table 1, which shows that the quantum efficiency is practically the same (2/3) for all visual pigments, A_1- and A_2-based alike.

Table 1. *The photosensitivities of the visual pigments and the quantum efficiencies of their bleaching at 25° C in the presence of hydroxylamine* (DARTNALL, 1968)

Species	Pigment	Photosensitivity			Quantum efficiency γ
		% frog pigment	$\alpha_{max}\gamma$ [a]	$\varepsilon_{max}\gamma$ [b]	
Conger conger	486_1	94·7	10·1	2·63	0·65
Trigla cuculus	493_1	100·6	10·7	2·80	0·69
Galago crassicaudatus agisymbanus	501_1	96·4	10·2	2·68	0·66
Rana temporaria	502_1	100·0	10·6	2·78	0·68
R. cancrivora	502_1	96·4	10·2	2·68	0·66
Salvelinus alpinus	508_1	102·6	10·9	2·85	0·70
Coregonus clupeoides pennantii	520_1	100·0	10·6	2·78	0·68
	Mean for A_1-based pigments		10·5	2·74	0·67
Cyprinus carpio	523_2	67·9	7·2	1·89	0·63
Carassius carassius	523_2	69·8	7·4	1·94	0·65
Rutilus rutilus	536_2	69·4	7·4	1·93	0.64
Coregonus clupeoides clupeoides	536_2	70·3	7·5	1·95	0·65
Osmerus eperlanus	543_2	69·7	7·4	1·94	0·65
	Mean for A_2-based pigments		7·4	1·93	0·64

[a] in $cm^2 \times 10^{-17}$ per chromophore (napierian base).
[b] in litre $cm^{-1} \times 10^4$ per mole of chromophore (decadic base).

Recently a much lower value for ε_{500}, namely 2.31 $\pm$ 0.80 $\times$ 10^4 litre per cm mole has been reported by HELLER (1968a) for bovine rhodopsin. This value, were it confirmed for all A_1-based pigments, would suggest a γ of near unity but, as mentioned above (Section IIC, p. 131) HELLER's absorbance spectrum lacked a *cis* peak, and there is doubt as to the relevance of his preparation to the present purpose. In any case, three groups of workers have since confirmed WALD and BROWN's (1953) original measurement. Thus SHICHI, LEWIS, IRREVERRE and STONE (1969) have reported 4.2 $\times$ 10^4 litre per cm mole for the maximum absorbance of bovine rhodopsin, BRIDGES (1970) the value of 4.19 $\times$ 10^4 for frog rhodopsin and DAEMEN, BORGGREVEN and BONTING (1970) the value 4.30 $\pm$ 0.70 $\times$ 10^4 for bovine rhodopsin.

Thus there seems little doubt about the molar absorbances of the visual pigments and consequently a strong suggestion from the photosensitivity values (Table 1) that the quantum efficiency of bleaching both in extracts and *in situ* is distinctly below unity. GUZZO and POOL (1968) have reported that the quantum efficiency of fluorescence of rhodopsin is only 0.005, so the one in three absorbed quanta that do not lead to bleaching cannot be accounted for in this way.

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