ABSORPTION OF LIGHT BY VISUAL PIGMENTS: A REVIEW OF THEORETICAL ANALYSES

B. D. GUPTA† and I. C. GOYAL
Department of Physics, Indian Institute of Technology, New Delhi 110 016 (India)
(Received March 7, 1984; in revised form October 2, 1984)

Summary

In this paper we review studies of the absorption of light by visual pigments *in vitro*, *in situ* and *in vivo*. In these experiments two types of light source, *i.e.* flash and steady state, were used to bleach the visual pigments. Plane-polarized light was also used in some experiments. The theoretical analyses of the results of these experiments have been used in the determination of various important features of the visual pigment and its photoproducts (*e.g.* photosensitivity, quantum efficiency, rate constant, principal absorption axes of the molecule and coefficient of viscosity of the environment).

1. Introduction

Studies of visual pigments in various environments have increased exponentially during the past two decades. Their ability to absorb light, which is their most important property, has been used as the principal tool for their identification and characterization. The physical and chemical features (photosensitivity, quantum efficiency, rate constants, kinetic activation parameters, principal absorption axes of the molecule, orientation of the chromophores, diffusion coefficients, presence and absence of intermediates in the reaction etc.) of the visual pigments and their intermediates in various environments have been obtained by analysing the results of investigations of their absorption of light. Although these results have been reported in a large number of review articles [1 - 17], the methods used to analyse the experimental data and hence determine these properties have not been the subject of a detailed review. Therefore the methods used to analyse experimental light absorption data for the visual pigments *in vitro*, *in situ* and *in vivo* are reviewed in this paper. The mathematical techniques already used and those which may be useful for further progress in investigations of visual pigments and visual photoreceptors are described.

†Associated with the Biochemistry Cell.

0047-2670/85/$3.30

The vertebrate retina, which is one of the most important parts of the eye, contains two types of photoreceptor, *i.e.* rods and cones. A photoreceptor can be divided into a further two parts, an inner segment and an outer segment. The outer segment contains a large number of disc-shaped membranes with planes perpendicular to the axis of the photoreceptor which are enclosed by the plasma membrane (Fig. 1). The visual pigments, which play an important role in vision, are located in the disc membranes. These molecules undergo both rotational and translational diffusion in the plane of the disc membrane [18 - 22].

The visual pigment of the rod photoreceptors is called rhodopsin. It consists of a specific protein (opsin) and a chromophore (11-*cis*-retinal), and accounts for approximately 85% of the total membrane protein [23 - 28]. The chromophoric group (the primary light-absorbing structure) of rhodopsin lies almost parallel to the plane of the disc membrane [3, 29 - 34]. The visual pigment of the cone photoreceptors is called iodopsin. In this review we shall confine ourselves to a description of the absorption of light

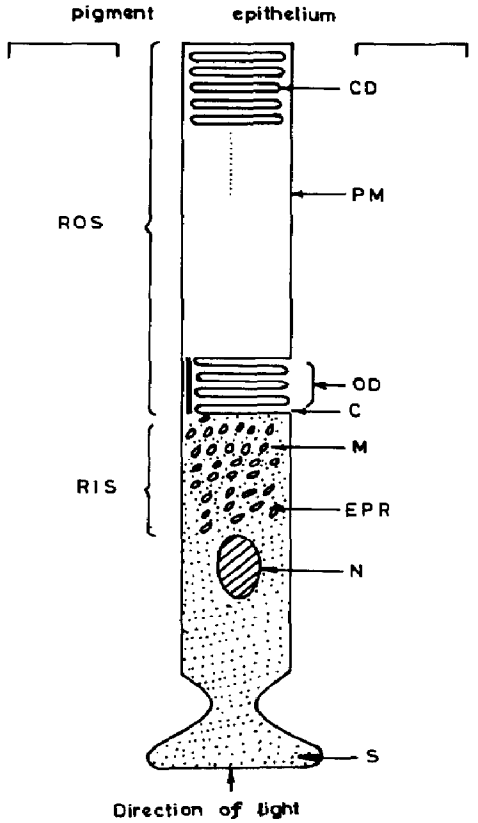


Fig. 1. Diagram illustrating the structure of a rod photoreceptor: ROS, rod outer segment; RIS, rod inner segment; PM, plasma membrane; CD, closed discs; OD, open discs; C, connecting cilium; M, mitochondria; EPR, endoplasmatic reticulum; N, nucleus; S, synaptic terminal.

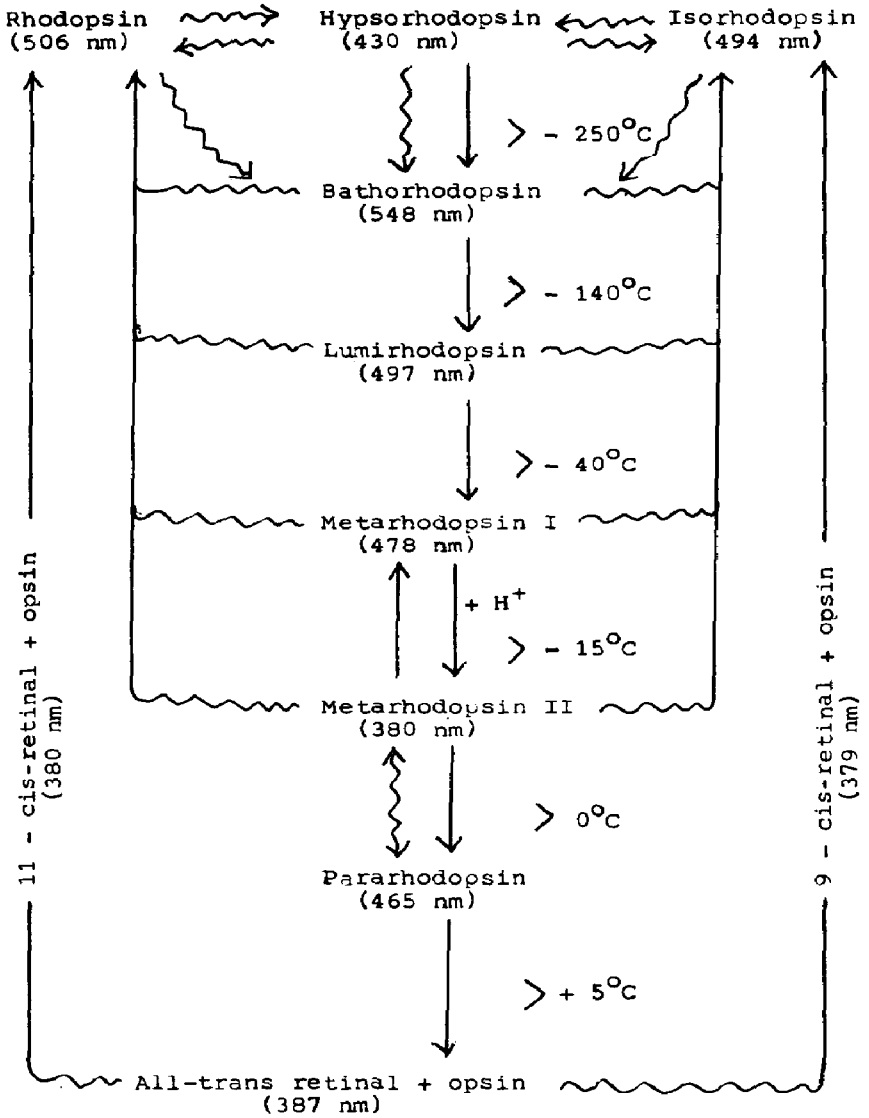


Fig. 2. Stages in the bleaching of cattle rhodopsin: ⇝, changes upon absorption of light; →, thermal changes. The numbers in parentheses denote the maximum absorption wavelength λ_{max}. The λ_{max} for rhodopsin, hypsorhodopsin, isorhodopsin and bathorhodopsin are at −268 °C, those for lumirhodopsin and metarhodopsin I are at −65 °C and those for other intermediates are at about 0 °C. The reactions can only proceed at temperatures above these values.

by the rhodopsin molecules only. The main reason for this is that, at present, much more information is available for rods than for cones.

The visual mechanism in a photoreceptor begins with the absorption of light by the visual pigments. The absorption of a photon by a rhodopsin molecule isomerizes the chromophore attached to the protein [35, 36] and leads to a series of thermal changes (Fig. 2) [37]. In vertebrates the chromophore separates from the protein and subsequently recombines with it during

a regeneration process. The intermediates involved in the reaction can also absorb light. The intermediates up to metarhodopsin I are highly unstable at physiological temperatures. These intermediates have been studied either at low temperatures or by the flash photolysis technique. The remaining intermediates (*i.e.* the long-lived intermediates) have been studied at room temperature after bleaching the visual pigment system with a light flash.

2. Steady light source

2.1. Visual pigments in solution

A substantial amount of research on the absorption of light by solutions of the visual pigments has been reported [36 - 69]. Visual pigments in solution exhibit diffusional motion in the optical cell and their diffusion coefficient depends upon various conditions such as the temperature of the solution, the viscosity of the solvent etc. Theoretical analysis of the absorption of light by the visual pigment solutions has been performed for the following two limiting cases: (i) the diffusion coefficient is very large (*i.e.* fluid sample); (ii) the diffusion coefficient is zero (*i.e.* solid sample).

The effect of temperature on the decomposition of the visual pigment will not be considered for these two cases. Since visual pigments can decompose in the absence of light if the temperature of the solution is increased we shall consider the effect of temperature on the absorption of light by the visual pigment solution and denote it case (iii).

2.1.1. Large diffusion coefficient

Dartnall *et al.* [38, 39] have extensively studied the absorption of light by visual pigment solutions and have obtained many new results from theoretical analysis of the experimental data. Their work can be subdivided further into the following cases.

2.1.1.1. No impurity is present in the solution and the intermediates do not absorb light. This was the simplest case described by Dartnall *et al.* [38]. Consider an optical cell containing visual pigment and having plane parallel faces at right angles to the direction of propagation of monochromatic light. The sample, of thickness l, can be regarded as a pile of very thin identical plates of thickness dx (Fig. 3). If the intensity (expressed as the number of photons per unit area per unit time) of light incident on one of these plates is I then a portion dI will be absorbed by the visual pigments and the remainder will be transmitted to the next plate. The fraction dI/I of light absorbed depends on the thickness dx of the plate, the pigment concentration C (in number of molecules per unit volume) and the pigment extinction coefficient $\alpha(\lambda)$. Thus we can write

$$-\frac{dI}{I} = \alpha(\lambda)\, C\, dx \qquad (1)$$

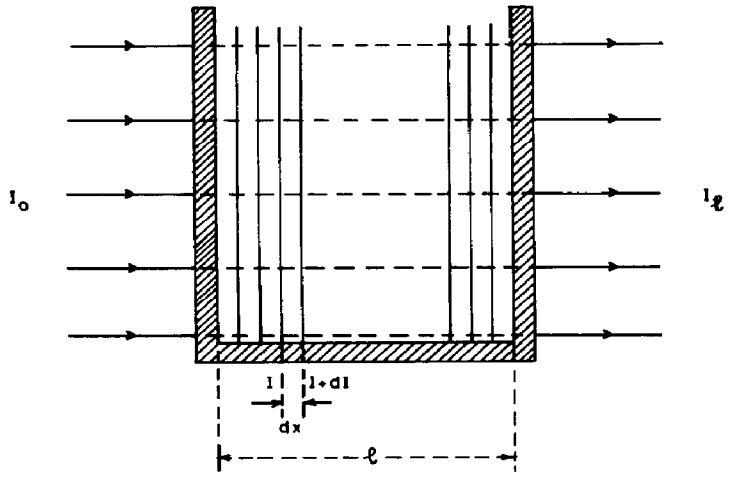


Fig. 3. Cross section of an optical cell containing a solution of visual pigments. The sample, of thickness l, can be regarded as a pile of very thin plates of thickness dx.

Integration of eqn. (1) gives

$$I_\ell = I_0 \exp\{-\alpha(\lambda) C l\} \tag{2}$$

where I_0 represents the intensity of the incident beam on the front surface of the cell and I_ℓ is the intensity of the transmitted light. Thus the number of photons absorbed per unit area per unit time by the visual pigments is given by

$$J(\lambda, t) = I_0[1 - \exp\{-\alpha(\lambda) C(t) l\}] \tag{3}$$

Now the rate of the reaction (*i.e.* the number of pigment molecules bleached per unit volume of the solution per unit time) can be written as

$$\frac{dC}{dt} = -\frac{\gamma J(\lambda, t)}{l} \tag{4}$$

where γ represents the quantum efficiency of the photochemical reaction which is defined as the ratio of the number of molecules bleached to the number of photons absorbed by the visual pigments. Using eqns. (2) - (4) we obtain

$$\ln\left(\frac{I_\ell}{I_0 - I_\ell}\right) = \gamma\alpha(\lambda) I_0 t + \text{constant} \tag{5}$$

The transmitted intensity I_ℓ is a function of time because the concentration C changes with time. It can be seen from eqn. (5) that if the intensity of the incident beam and the time variation of the intensity of the transmitted light are known experimentally the value of the product $\gamma\alpha(\lambda)$ (*i.e.* the photosensitivity of the visual pigments) can be determined from the plot of $\ln\{I_\ell/(I_0 - I_\ell)\}$ against the exposure $I_0 t$. The plot is a straight line and its

slope is equal to $\gamma\alpha(\lambda)$. This method was used to determine the photosensitivity of the visual pigments.

In the derivation of eqn. (5) it has been assumed that the only absorbing substance in the optical cell is the visual pigment. However, this is only true for the ideal case. In practice there will be some impurity in the cell which also absorbs light and eqn. (5) cannot be used to determine the photosensitivity.

2.1.1.2. Impurities are present but the intermediates do not absorb light. Let us further assume that the impurities present in the solution are photostable. Let $C_1, C_2, C_3, \ldots$ and $\alpha_1, \alpha_2, \alpha_3, \ldots$ be the concentrations and extinction coefficients respectively of the impurities. In the presence of impurities eqns. (2) and (3) can be written as

$$I_\ell = I_0 \exp[-\{\alpha(\lambda)C(t) + K(\lambda)\}l] \tag{6}$$

and

$$J(\lambda, t) = I_0(1 - \exp[-\{\alpha(\lambda)C(t) + K(\lambda)\}l]) \frac{\alpha(\lambda)C(t)l}{\alpha(\lambda)C(t)l + K(\lambda)l} \tag{7}$$

where

$$K(\lambda) = \alpha_1(\lambda)C_1 + \alpha_2(\lambda)C_2 + \ldots$$

and the rate of the reaction in the presence of impurities is given by eqn. (4). Solving eqns. (4), (6) and (7) gives

$$\frac{dI_\ell}{dt} = \gamma\alpha(\lambda)I_0 I_\ell \frac{\ln(I_f/I_\ell)}{\ln(I_0/I_\ell)} \frac{I_0 - I_\ell}{I_0} \tag{8}$$

where $I_f = I_0 \exp\{-K(\lambda)l\}$ is the intensity of the transmitted light at $t = \infty$ when all the molecules are bleached (*i.e.* $C(t) = 0$). It has been found experimentally [38] that

$$\ln\left(\frac{I_\ell}{I_f - I_\ell}\right) = mt + \text{constant} \tag{9}$$

where

$$m \neq \gamma\alpha(\lambda)I_0$$

Equation (9) can also be written as

$$\frac{dI_\ell}{dt} = \frac{mI_\ell(I_f - I_\ell)}{I_f} \tag{10}$$

Comparison of eqns. (8) and (10) gives

$$m = \gamma\alpha(\lambda)I_0\phi$$

where

$$\phi = \frac{I_f}{I_f - I_\varrho} \frac{I_0 - I_\varrho}{I_0} \frac{\ln(I_f/I_\varrho)}{\ln(I_0/I_\varrho)} \tag{11}$$

Thus when impurities are present eqn. (5) becomes

$$\ln\left(\frac{I_\varrho}{I_f - I_\varrho}\right) = \gamma\alpha(\lambda)I_0\phi t + \text{constant} \tag{12}$$

It should be noted that ϕ is a function of time because it contains I_ϱ. However, it has been found experimentally [38] that the value of ϕ in any one experiment varies very slowly as I_ϱ changes from its initial value to its final value I_f. It has been reported [38] that in the majority of experiments the total change in ϕ is less than 1% and therefore its mean value can be used without introducing a significant error. Thus, if the values of I_0, I_ϱ and I_f are known, the value of $\gamma\alpha(\lambda)$ can be determined from the plot of $\ln\{I_\varrho/(I_f - I_\varrho)\}$ against $I_0\phi t$. Crescitelli and Karvaly [66] have recently used this method to determine the photosensitivity of the visual pigment of the gecko.

2.1.1.3. Impurities are present and the intermediates absorb light but are photostable. Until now it has been assumed that the photoproducts do not absorb light appreciably. The analyses given in Sections 2.1.1.1 and 2.1.1.2 are still valid even though the photoproducts absorb light if they decompose to a colourless (non-absorbing) substance at a much faster rate than that of the photodecomposition of the visual pigment. However, this is not always true in practice. In the case where photoproducts absorb light and are stable the optical density of the solution at any time t can be written as

$$\ln\left(\frac{I_0}{I_\varrho}\right) = \alpha(\lambda)C(t)l + z'\alpha'\{C_0 - C(t)\}l + K(\lambda)l \tag{13}$$

where

$$z'\alpha' = a'\alpha_a(\lambda) + b'\alpha_b(\lambda) + \ldots$$

$\alpha_a, \alpha_b, \ldots$ are the extinction coefficients of the photoproducts of the bleaching process, $a', b', \ldots$ are their stoichiometric relations with the visual pigment (*e.g.* a' is the ratio of the number of molecules of type A formed to the number of molecules of the visual pigment bleached), $K(\lambda)l$ is the optical density of the stable impurities present and C_0 is the concentration of the visual pigment when none of the molecules are bleached (*i.e.* at $t = 0$).

The time dependence $C(t)$ of the concentration of the visual pigment can be obtained from the kinetic equation

$$\frac{dC}{dt} = -\frac{\gamma}{l} \frac{\alpha(\lambda)C(t)l(I_0 - I_\varrho)}{\alpha(\lambda)C(t)l + z'\alpha'\{C_0 - C(t)\}l + K(\lambda)l} \tag{14}$$

Solving eqns. (13) and (14) gives

$$\ln\left(\frac{I_\varrho}{I_f - I_\varrho}\right) = \gamma\alpha(\lambda) I_0 t\phi + \text{constant} \tag{15}$$

where ϕ is given by eqn. (11). In this case also ϕ has been found to be almost constant [39]. Thus if the values of I_0, I_f and I_ϱ are known, the photosensitivity of the visual pigment can be found from eqn. (15).

2.1.2. Zero diffusion coefficient

In Section 2.1.1 it was assumed that the molecules undergo diffusional motion in the optical cell. Therefore the concentration of the unbleached pigment is the same everywhere throughout the solution at all times during the bleaching process. If the visual pigments do not undergo diffusional motion in the cell, *i.e.* if the visual pigment system is a solid layer, the kinetic equations change owing to the axial dependence of the concentration of the unbleached pigment in the cell. Various cases are considered below.

2.1.2.1. No impurities are present and the intermediates do not absorb light. In this case the decrease in the intensity of the light beam in the layer and the disappearance of the visual pigments can be described by the following set of differential equations:

$$\frac{dI(x, t)}{dx} = -\alpha(\lambda) C(x, t) I(x, t) \tag{16}$$

$$\frac{dC(x, t)}{dt} = -\gamma\alpha(\lambda) I(x, t) C(x, t) \tag{17}$$

where $I(x, t)$ and $C(x, t)$ represent the light intensity and the concentration respectively of the visual pigments at depth x and exposure time t. Equations (16) and (17) give

$$\frac{d^2 \ln I(x, t)}{dx\, dt} = -\gamma\alpha(\lambda) \frac{dI(x, t)}{dx} \tag{18}$$

When the condition $I(x, t) = I_0$ at $x = 0$ is applied, eqn. (18) simplifies to

$$\frac{d \ln I(x, t)}{dt} = \gamma\alpha(\lambda)\{I_0 - I(x, t)\} \tag{19}$$

Integration of eqn. (19) gives

$$I(x, t) = \frac{I_0}{1 + [\exp\{\alpha(\lambda) C_0 x\} - 1] \exp\{-\gamma\alpha(\lambda) I_0 t\}} \tag{20}$$

where C_0 is the initial concentration of the visual pigment. Equation (20) can also be written in the form

$$\ln\left\{\frac{I(l, t)}{I_0 - I(l, t)}\right\} = \gamma\alpha(\lambda) I_0 t + \text{constant} \tag{21}$$

which is identical with eqn. (5) obtained for the case of a fluid sample where diffusional motion is present. It should be noted that I_ϱ in eqn. (5) is the same as $I(l, t)$ in eqn. (21). An equation similar to eqn. (21) was obtained by Rabinovitch [70] for an unstirred sample with a non-absorbing product. Thus, in the ideal case eqn. (5) can be used to determine the photosensitivity of visual pigments regardless of whether they undergo diffusion.

2.1.2.2. Impurities are present and the intermediate absorbs light. In this case eqn. (17) remains the same and eqn. (16) is replaced by

$$\frac{dI(x, t)}{dx} = -[\alpha(\lambda)C(x, t) + \alpha_p(\lambda)\{C_0 - C(x, t)\} + K(\lambda)]I(x, t) \tag{22}$$

where $\alpha_p(\lambda)$ is the extinction coefficient of the photoproduct. It can be seen that eqns. (17) and (22) cannot be solved in a simple way. Onderdelinden and Strackee [71] solved them numerically and plotted the quantity $\{I(l, \infty) - I(l, t)\}/I(l, t)$ as a function of $\gamma\alpha(\lambda)I_0t$ for various values of

$$\rho = \frac{\alpha_p C_0 + K(\lambda)}{\alpha(\lambda)C_0}$$

The results obtained for an initial transmission $I(l, 0)/I_0$ of 0.20 are plotted in Fig. 4 which also includes the results obtained for a fluid model (*i.e.* eqn. (15)). It can be seen that the theoretical bleaching curves for a rigid layer model deviate from linearity. Therefore, in the absence of diffusion the photosensitivity of the visual pigments cannot be obtained from the slope of the theoretical bleaching curves. A correction to the equation derived by Dartnall *et al.* [38] is needed to obtain the photosensitivity in the case of a rigid layer.

Rabinovitch [70] has discussed the analytical solution of eqn. (22) under very simplifying assumptions. He assumed that there was no impurity present, *i.e.* $K(\lambda) = 0$, and gave the following solution for eqn. (22):

$$I(x, t) = \frac{C(x, t)}{C_0} \frac{I_i \exp\{\gamma\alpha(\lambda)I_0t\}}{\alpha(\lambda)\{C_0 - C(x, t)\}} \left[\{\alpha(\lambda) - \alpha_p(\lambda)\}\{C_0 - C(x, t)\} + \right.$$

$$\left. + \alpha_p(\lambda)C_0 \ln\left\{\frac{C_0}{C(x, t)}\right\}\right] \tag{23}$$

where $I_i = I(l, t = 0)$. Assuming further that

$$\frac{I_f - I_i}{I_f - I_\varrho} \gg \frac{\alpha}{\alpha_p}$$

he obtained

$$\frac{I_i}{I_f - I_i}\left(\frac{I_f}{I_\varrho} - 1\right) - \frac{\beta}{1 + \beta} = \frac{1}{1 + \beta} \exp\{-\gamma\alpha(\lambda)I_0t\} \tag{24}$$

where

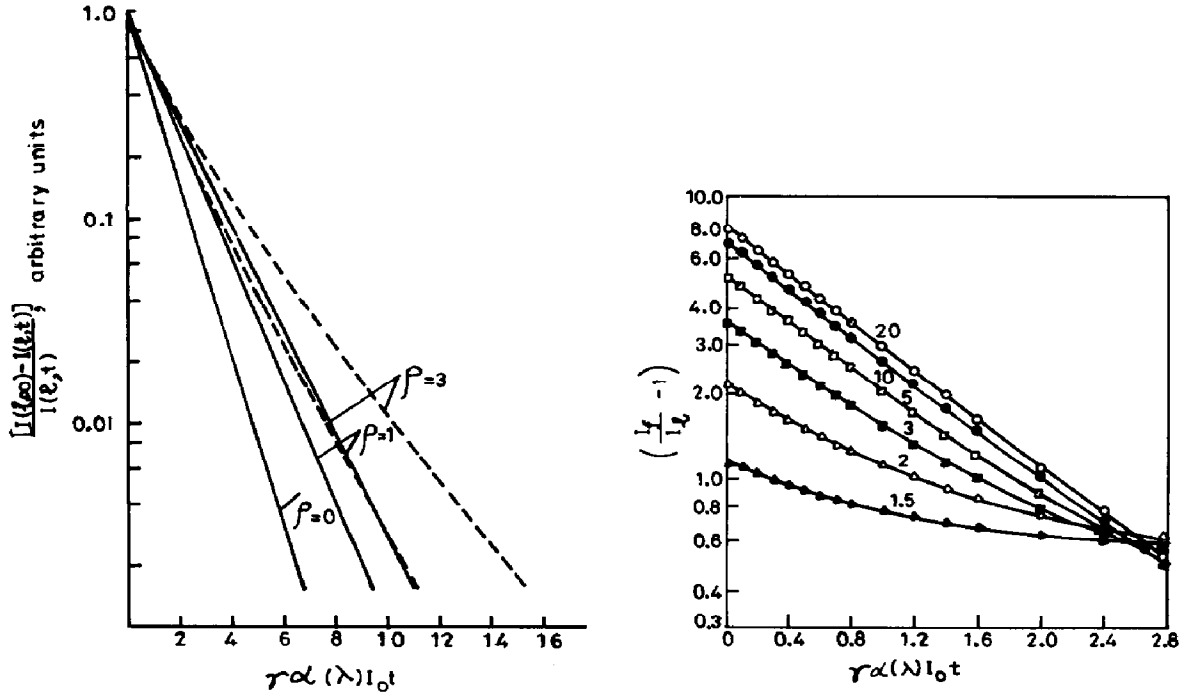


Fig. 4. The calculated values of $\{I(l, \infty) - I(l, t)\}/I(l, t)$ as a function of $\gamma\alpha(\lambda)I_0 t$ for various values of ρ and an initial transmission of 0.20: ——, fluid model; — — —, rigid-layer model. The values are normalized to unity at $t = 0$.

Fig. 5. Time dependence of a reaction with an absorbing product and its variation with the relative values of the extinction coefficient (the numbers on the curves are the values of α/α_p). The initial optical density is taken to be 1.0.

$I_f = I(l, t = \infty)$

$I_\ell = I(l, t)$

and

$$\beta = \frac{\alpha_p(\lambda) I_i}{\{\alpha(\lambda) - \alpha_p(\lambda)\} I_f}$$

Thus if $\ln(I_f/I_\ell - 1)$ is plotted against time, the constant β, and thus α/α_p, can be evaluated. The time dependence of a reaction corresponding to eqn. (24) is given in Fig. 5 [70]. It can be seen that as the value of α/α_p decreases the plot of $\ln(I_f/I_\ell - 1)$ as a function of time deviates more from linearity.

2.1.3. Effect of temperature on the photochemical bleaching of visual pigment solutions

In the absence of light visual pigments may decompose if the temperature of the visual pigment solution is increased. Therefore at high temperatures a correction term for the thermal decomposition should be included in the kinetic equation of bleaching. Williams and Milby [72] reported data for the rates of thermal decomposition of visual pigments from a variety of

species. The data obtained were consistent with a first-order rate process. Thus, in the presence of thermal decomposition, the kinetic equation can be written as

$$\frac{dC}{dt} = -\frac{\gamma}{l}\frac{\alpha(\lambda)C(t)l(I_0 - I_\ell)}{\alpha(\lambda)C(t)l + z'\alpha'\{C_0 - C(t)\}l + K(\lambda)l} - k_1C(t) \tag{25}$$

where we have assumed that impurities are present and intermediates absorb light but are photostable, k_1 is the rate constant and the remaining symbols have already been defined. The optical density of the solution at any time t is given by eqn. (13). Equations (13) and (25) give

$$\ln\left(\frac{I_\ell}{I_f - I_\ell}\right) = \phi\gamma\alpha(\lambda)I_0t + k_1\int_0^t \frac{I_f}{I_f - I_\ell}\ln\left(\frac{I_f}{I_\ell}\right)dt + \text{constant} \tag{26}$$

where ϕ is given by eqn. (11). This equation was obtained by Dartnall *et al.* [39]. At low concentrations of visual pigments, eqn. (26) can be written as

$$\ln\left(\frac{I_\ell}{I_f - I_\ell}\right) = \{\phi\gamma\alpha(\lambda)I_0 + k_1\}t + \text{constant} \tag{27}$$

Thus a plot of $\ln\{I_\ell/(I_f - I_\ell)\}$ as a function of time will give a straight line with a slope

$$m = \phi\gamma\alpha(\lambda)I_0 + k_1 \tag{28}$$

from which the photosensitivity can be obtained by substituting the values of ϕ, I_0 and k_1. However, if the concentration of visual pigments in the solution is high and the thermal decomposition is appreciable a straight line will not be obtained.

2.2. Visual pigments in isolated photoreceptors

The following three types of experiments have been performed on visual photoreceptors [3, 18, 19, 29 - 34, 73 - 103]: (a) light is incident along the axis of the photoreceptor (longitudinal incidence); (b) light is incident perpendicular to the axis of the photoreceptor (transverse incidence); (c) light is incident on the rod outer segment suspension. Since little theoretical work has been done on rod outer segment suspension, we shall discuss only the first two cases in detail.

2.2.1. Longitudinal incidence

In the preceding sections the intensity distribution of the bleaching light in the plane perpendicular to the direction of propagation was considered to be uniform. However, this is true only if the visual pigments are in solution. If the visual pigments are in the photoreceptors this assumption is not valid. This is because visual photoreceptors behave like optical waveguides [104 - 106] and allow light to propagate through them only in the form of transversely varying intensity patterns (modes). As in the solid sample, the instantaneous concentration of the unbleached pigments along

the direction of the incident light (*i.e.* the photoreceptor axis) will also vary in the case of visual photoreceptors. This is because the visual pigments are located in the disc membranes whose planes are perpendicular to the axis of the photoreceptor and therefore they cannot move along this axis. Therefore in the case of visual photoreceptors the light intensity, and hence the concentration of unbleached pigment, will exhibit both axial and radial dependence. Thus the amount of the light absorbed by the photoreceptor will be affected by the waveguide property of the photoreceptor. Since visual pigments undergo translational diffusion in the plane of the disc membrane [20 - 22], the contribution of the waveguide property will depend on the translational diffusion of the molecules. Gupta and Sharma [107] studied the effect of the waveguide property on the absorption of light in photoreceptors. The following reaction model was used for the analysis:

visual pigment $\rightsquigarrow$ photoproduct (29)

The kinetic equation which describes the above reaction model is

$$\frac{\partial C(r, x, t)}{\partial t} = D_T\left\{\frac{\partial^2 C(r, x, t)}{\partial r^2} + \frac{1}{r}\frac{\partial C(r, x, t)}{\partial r}\right\} - \gamma\alpha(\lambda) I(r, x, t) C(r, x, t) \quad (30)$$

where

$$I(r, x, t) = I(r, 0, 0) \exp\left\{-\int_0^x \alpha(\lambda) C(r, x, t)\, dx\right\} \quad (31)$$

$C(r, x, t)$ represents the instantaneous concentration of visual pigment at an axial distance x from the inner segment–outer segment junction and a radial distance r from the axis of the outer segment, $I(r, x, t)$ represents the instantaneous intensity of the incident beam at an axial distance x and a radial distance r, $I(r, 0, 0)$ represents the intensity distribution at the inner segment–outer segment junction and is assumed to be independent of time and D_T represents the translational diffusion coefficient of the visual pigment. It can be seen that eqn. (30) cannot be solved analytically. Therefore Gupta and Sharma [107] studied the maximum effect of the waveguide property on the absorption of light in photoreceptors and analysed the following two cases: (i) there is no transverse diffusion; (ii) the transverse diffusion is infinitely rapid (this is equivalent to the assumption that the photoreceptor does not behave like an optical waveguide, *i.e.* there is no transverse dependence of the intensity).

The difference between the number of photons absorbed in the outer segment in time t in these two cases is given by

$$\Delta F = \frac{\pi}{\gamma\alpha(\lambda)}\left\{2\int_0^{r_0} \ln(1 + \exp(-\beta')[\exp\{\gamma\alpha(\lambda) I(r, 0, 0) t\} - 1]) r\, dr - \right.$$
$$\left. - r_0^2 \ln(1 + \exp(-\beta')[\exp\{\gamma\alpha(\lambda) I_{av} t\} - 1])\right\} \quad (32)$$

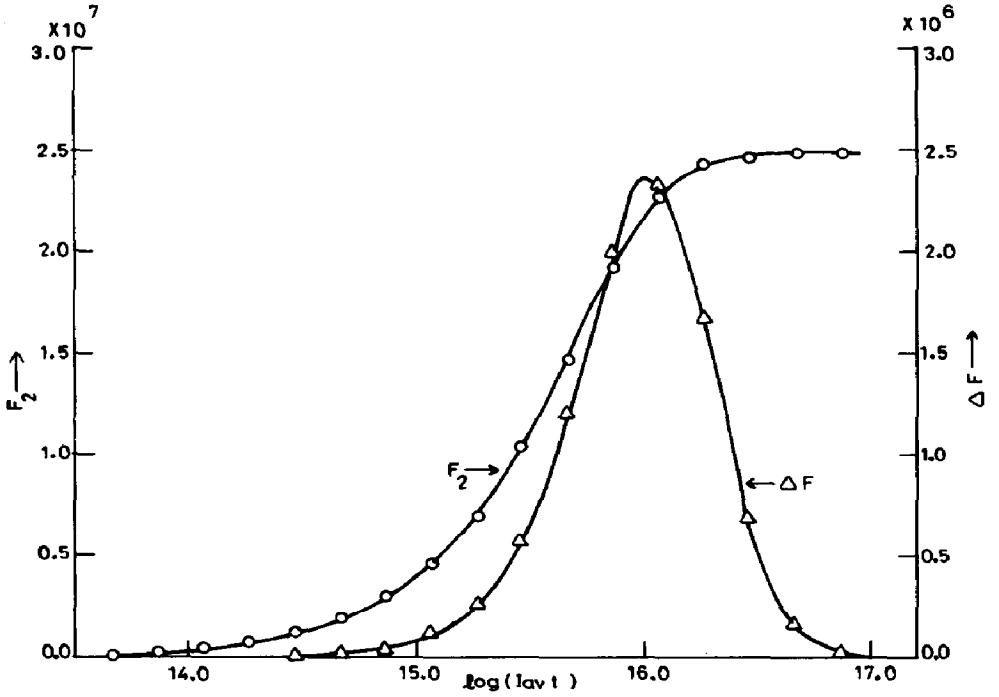


Fig. 6. Variation of the number F_2 of photons absorbed when there is an infinitely rapid molecular diffusion or when the transverse dependence of the intensity is absent and the difference ΔF between the number of photons absorbed with the exposure $I_{av}t$: ○, F_2; △, ΔF.

where

$$I(r, 0, 0) = AJ_0^2\left(U\frac{r}{r_0}\right)$$

$$I_{av} = \frac{2}{r_0^2}\int_0^{r_0} I(r, 0, 0)r\,dr = A\{J_0^2(U) + J_1^2(U)\}$$

$$\beta' = \alpha(\lambda)C_0 l$$

r_0 and l represent the radius and length respectively of the outer segment of the photoreceptor, U represents the normalized propagation constant of the fundamental mode, J_0 and J_1 are Bessel functions of orders zero and unity respectively and A is a constant.

After selecting the appropriate values of the unknown parameters, Gupta and Sharma [107] plotted ΔF and F_2 (*i.e.* the number of photons absorbed in the outer segment in time t in Section 2.1.2) as a function of the exposure $I_{av}t$ for a human rod photoreceptor (Fig. 6). It was found that for $I_{av}t \leqslant 10^{15}$ the transverse dependence of the intensity, *i.e.* the waveguide property of the photoreceptor, does not play a major role in the absorption of light in the photoreceptor. As the value of $I_{av}t$ increases, the effect of the

transverse dependence of the intensity increases. The maximum effect was about 11% for $I_{av}t = 10^{16}$. From this it was concluded that, if the photoreceptor does not behave like an optical waveguide, the assumption will be valid if the value of the exposure is small. As its value increases up to some critical value the validity of the assumption decreases. When $I_{av}t$ exceeds the critical value the validity of the assumption increases. Since the translational diffusion of the visual pigments in the disc membrane is finite, the maximum effect will be less than 11% in the actual case.

2.2.2. Transverse incidence

In the case of transverse incidence, the kinetic equations or expressions for the absorption rates become very complicated owing to the cylindrical geometry of the photoreceptor. Gupta [108] studied the absorption of light in photoreceptors when it is incident perpendicular to the photoreceptor axis for various species under various conditions and suggested simple expressions for the absorption rates for some of the species. The reaction model described in Section 2.1 was used for the analysis. Since the visual pigments diffuse in the disc membranes the following two cases were considered in the analysis: (i) transverse diffusion is present; (ii) transverse diffusion is absent.

(i) This case occurs when the photoreceptor is at room temperature. The kinetic equation for light incident perpendicular to the photoreceptor axis is

$$V\frac{d\bar{A}}{dt} = -\gamma J_{PD}(\lambda, t) \tag{33}$$

where (Fig. 7)†

$$J_{PD}(\lambda, t) = I_0 \Delta l \int_{-r_0}^{r_0} [1 - \exp\{-\alpha_\perp(\lambda)\bar{A}(t)u(x)\}]\, dx$$
$$u(x) = 2(r_0^2 - x^2)^{1/2} \tag{34}$$

$\bar{A}(t)$ represents the instantaneous concentration of visual pigments in the disc membrane, Δl and r_0 represent the thickness and radius of the disc respectively and $\alpha_\perp(\lambda)$ is the extinction coefficient of the visual pigments located in the disc membrane for light of wavelength λ incident perpendicular to the photoreceptor axis. Integration of eqn. (34) gives

$$J_{PD}(\lambda, t) = -\pi I_0 \Delta l r_0 [I_1\{-\mu(\lambda, t)\} + L_1\{-\mu(\lambda, t)\}] \tag{35}$$

where

$$\mu(\lambda, t) = 2\alpha_\perp(\lambda)\bar{A}(t)r_0$$

†In Fig. 7 (x, y, z) is a coordinate system associated with a disc membrane and its z axis is perpendicular to the plane of the disc. The light is assumed to be incident along the y axis.

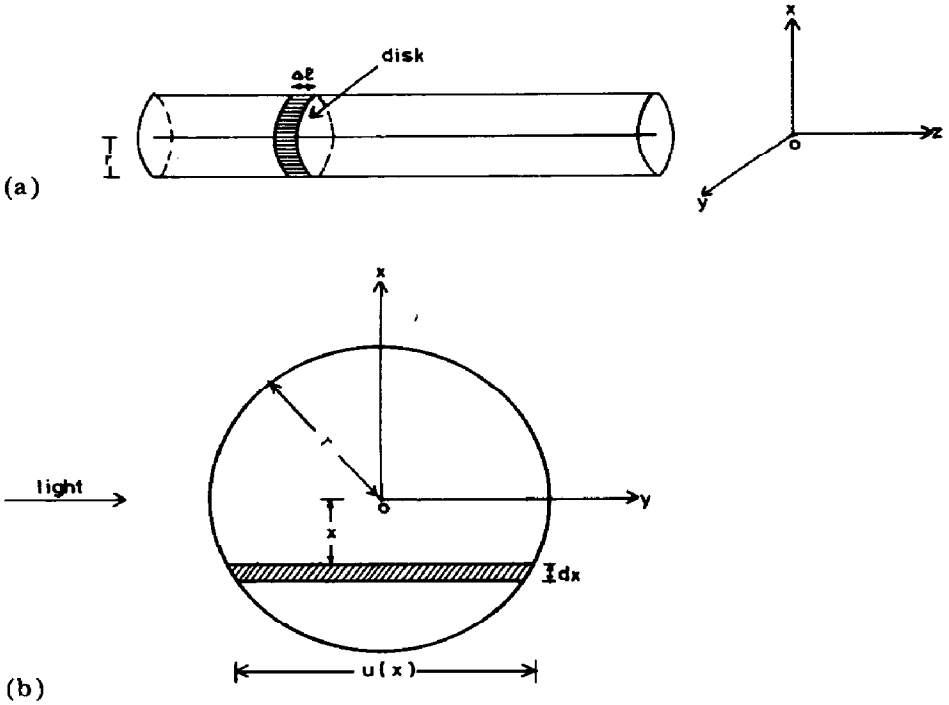


Fig. 7. Coordinate system (x, y, z) adopted for a disc membrane of radius r and thickness Δl: (a) rod outer segment; (b) disc membrane. The z axis is perpendicular to the plane of the disc and the light is incident along the y axis.

$I_1\{\ \}$ is a modified Bessel function of the first kind of order unity and $L_1\{\ \}$ is a modified Struve function.

(ii) This case occurs when the photoreceptor is at a low temperature or if it is treated with glutaraldehyde [18]. In this case the concentration of the visual pigment will vary depending on its position in the disc membrane. Therefore the expression for the absorption rate is

$$J_{AD}(\lambda, t) = 2I_0\Delta l\int_0^{r_0} \frac{[\exp\{\alpha_\perp(\lambda)A_0u(x)\} - 1]\exp\{-\gamma\alpha_\perp(\lambda)I_0t\}\,dx}{1 + [\exp\{\alpha_\perp(\lambda)A_0u(x)\} - 1]\exp\{-\gamma\alpha_\perp(\lambda)I_0t\}} \tag{36}$$

It can be seen that eqns. (35) and (36) are complicated, and therefore the absorption rates in these two cases must be calculated numerically.

If the photoreceptor is assumed to have a square rather than a circular cross section (say, case (iii)), the following simple expression is obtained for the absorption rate in both the presence and absence of diffusion:

$$J_S(\lambda, t) = \frac{I_0a\Delta l[\exp\{\alpha_\perp(\lambda)A_0a\} - 1]\exp\{-\gamma\alpha_\perp(\lambda)I_0t\}}{1 + [\exp\{\alpha_\perp(\lambda)A_0a\} - 1]\exp\{-\gamma\alpha_\perp(\lambda)I_0t\}} \tag{37}$$

where

$$a = \pi^{1/2}r_0$$

Equation (37) is the simplest of the three expressions for the absorption rate.

Gupta [108] substituted appropriate values for the unknown parameters and plotted the absorption rates as a function of time for $I_0 = 10^{16}$ photons cm^{-2} s^{-1} in the three different cases for human, cattle and frog photoreceptors (Figs. 8, 9 and 10 respectively). From these figures it was concluded that the absorption rates for the human and cattle photoreceptors are the same at all times in the three different cases, whereas those for the frog photoreceptors vary. The difference between cases (ii) and (iii) is smaller than the difference between cases (i) and (ii). The following conclusions were drawn from this study [108].

(1) In investigations of the mechanism of human and cattle visual photoreceptors when light is incident perpendicular to the photoreceptor axis the photoreceptor can be assumed to have a square cross section of area πr_0^2 regardless of its temperature or whether it has been treated with glutaraldehyde. However, the cross section of frog photoreceptors can be assumed to be square only if the temperature is very low or if the photo-

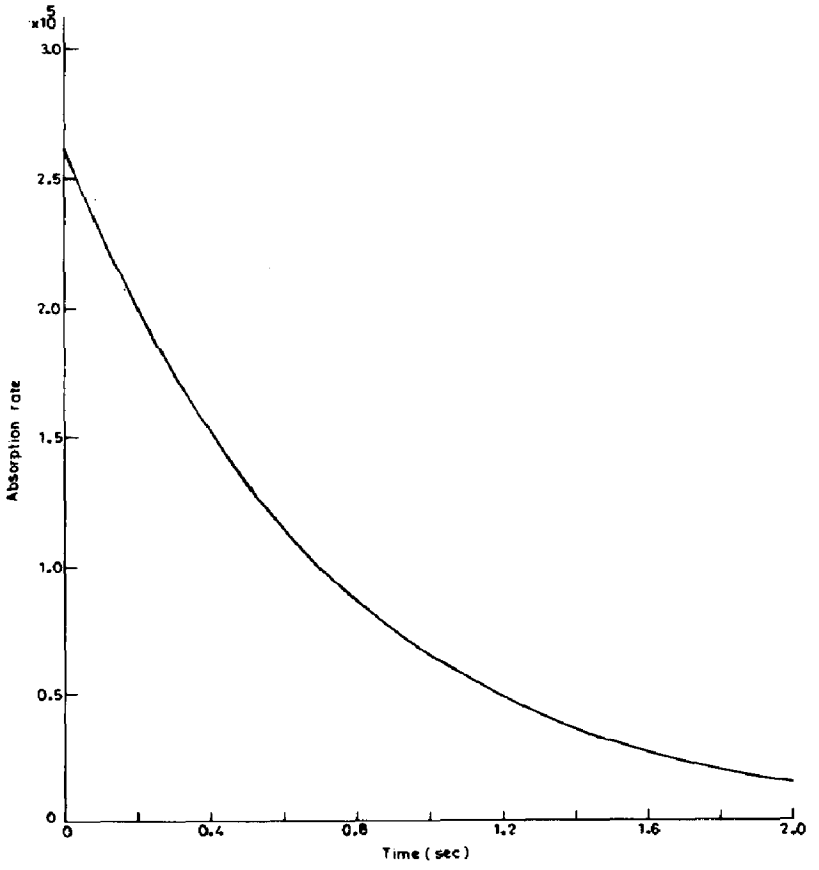


Fig. 8. Variation of the absorption rate for human photoreceptors ($I_0 = 10^{16}$ photons $cm^2 s^{-1}$).

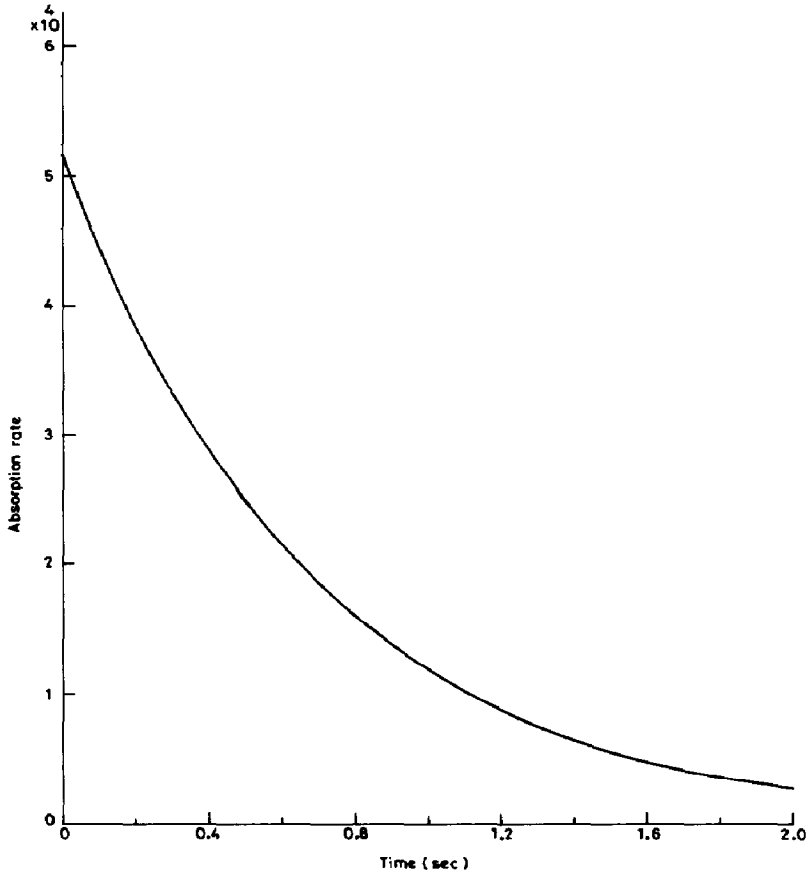


Fig. 9. Variation of the absorption rate for cattle photoreceptors ($I_0 = 10^{16}$ photons $cm^2 s^{-1}$).

receptor has been treated with glutaraldehyde. The expression for the absorption rate becomes very simple for photoreceptors with square cross sections.

(2) The cross sections of frog photoreceptors cannot be assumed to be square at room temperature (*i.e.* if the molecules have diffusional motion in the disc membrane). Therefore the analysis of absorption in frog photoreceptors in the presence of diffusion will become very complicated.

2.2.3. Rod outer segment suspension

A large number of investigations of the absorption of light by the rod outer segment suspensions have been reported in the literature. It has generally been assumed that the absorption of light in rod outer segment suspensions and visual pigment solutions is the same. However, this may not be true for all species. This is because the density of visual pigments in the rod outer segment suspension in an optical cell is inhomogeneous whereas their distribution is homogeneous in aqueous detergent solutions. The difference between the two will depend on the absorbance of a single rod outer

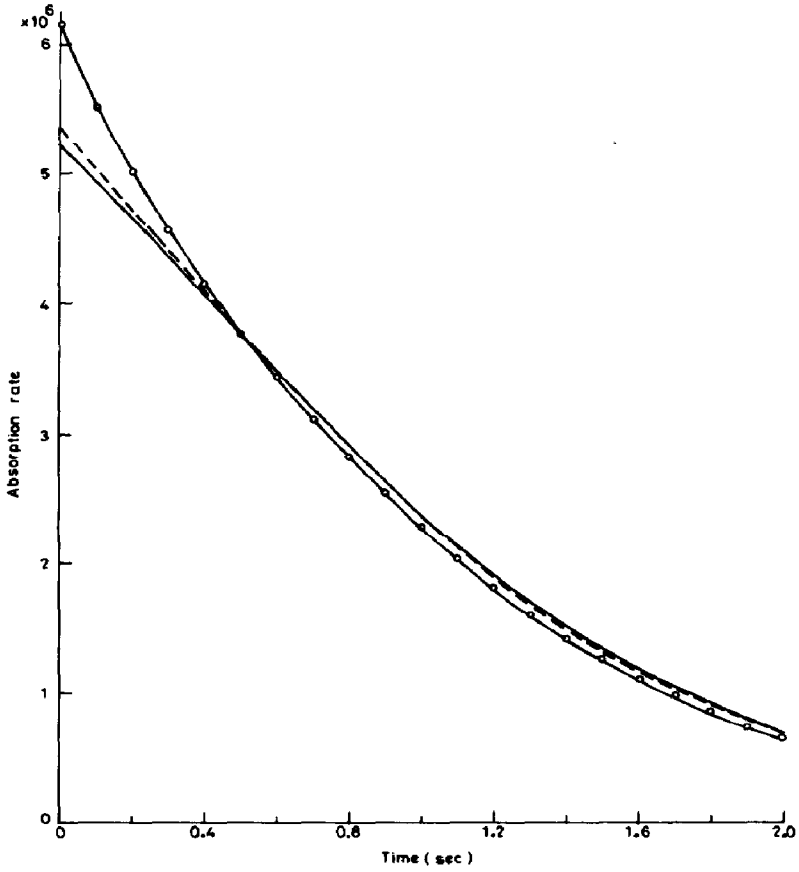


Fig. 10. Variation of the absorption rate for frog photoreceptors ($I_0 = 10^{16}$ photons cm^2 s^{-1}): ○, $J_{PD}(t)$; – – –, $J_{AD}(t)$; ——, $J_S(t)$.

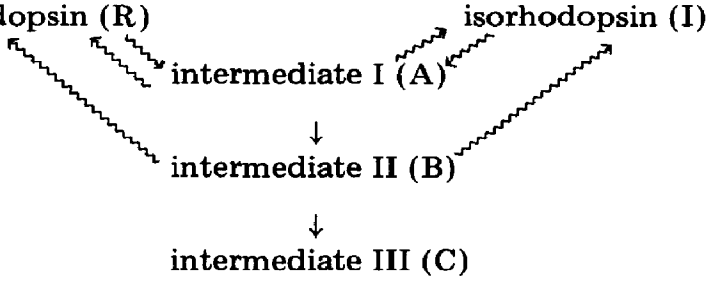
segment suspension which will be different for different species. Since no theory has been proposed so far we shall not discuss this in the present review.

3. Flash photolysis of visual pigments

3.1. Visual pigments in solution or in the retina

In the preceding discussion the bleaching source was assumed to be a steady monochromatic light. A large number of studies in which the bleaching source was a high intensity flash have been reported [19, 40, 42 - 46, 48 - 51, 53 - 57, 60 - 62, 73 - 75, 109 - 119]. The advantage of using a flash is that the formation and properties of short-lived intermediates can be studied at physiological temperatures. In flash photolysis experiments a flash of high intensity and short duration is delivered to the sample and the resultant change in light absorption by the sample is studied using another monochromatic beam of very low intensity. In most flash photolysis investigations of visual pigments the concentration of unbleached visual pigments at the

end of the flash is obtained as a function of the flash intensity [49, 54, 113, 120]. This concentration can be determined from the measured change in the absorbance of the sample at the end of the flash. The amount of visual pigment present at the end of the flash depends on the light-absorbing properties (*e.g.* the quantum efficiency and extinction coefficient) and the thermal properties of the visual pigment and its intermediates which form and decay during the flash. This is because these intermediates can regenerate visual pigment when they absorb light. The amount of photoregeneration will depend on the rate constants of the intermediates and hence on the temperature of the sample. Thus there is competition between the two reactions in flash photolysis experiments. If the temperature increases photoregeneration decreases. In this section we present a theoretical analysis of a general flash bleach experiment on rhodopsin in solution or in isolated retinas. We use the following general model of the reaction for the analysis [121]:



The wavy arrows represent the reaction due to the absorption of light and the straight arrows represent the thermal reactions. In a typical experiment intermediates I, II and III depend on the duration of the flash and the temperature of the sample. For example, if the duration of the flash is of the order of 1 μs, intermediates I, II and III in the above reaction model at physiological temperatures are lumirhodopsin, metarhodopsin I and metarhodopsin II respectively [122]. However, if the duration of the flash is 1 ms, intermediates I and II will be metarhodopsin I and metarhodopsin II respectively and the presence of intermediate III can be neglected [113].

If it is assumed that the total number of molecules is conserved, *i.e.* $N_1 + N_2 + N_3 + N_4 + N_5 = R_0$, the kinetic equations describing the above reaction model are

$$\frac{dN_1}{dt} = -\gamma_{R,A} J_R(t) + \gamma_{A,R} J_A(t) + \gamma_{B,R} J_B(t) \tag{38}$$

$$\frac{dN_2}{dt} = \gamma_{R,A} J_R(t) - (\gamma_{A,R} + \gamma_{A,I}) J_A(t) + \gamma_{I,A} J_I(t) - K_{A \to B} N_2(t) \tag{39}$$

$$\frac{dN_3}{dt} = \gamma_{A,I} J_A(t) - \gamma_{I,A} J_I(t) + \gamma_{B,I} J_B(t) \tag{40}$$

$$\frac{dN_4}{dt} = -(\gamma_{B,R} + \gamma_{B,I})J_B(t) + K_{A\rightarrow B}N_2(t) - K_{B\rightarrow C}N_4(t) \quad (41)$$

$$\frac{dN_5}{dt} = K_{B\rightarrow C}N_4(t) \quad (42)$$

where R_0 is the concentration in chromophores per cubic centimetre of rhodopsin molecules when all the molecules are unbleached, N_1, N_2, N_3, N_4 and N_5 represent the instantaneous concentrations of rhodopsin, intermediate I, isorhodopsin, intermediate II and intermediate III respectively, $\gamma_{P,Q}$ represents the quantum efficiency of the conversion of a molecule of type P into a molecule of type Q and $K_{X\rightarrow Y}$ represents the rate constant in reciprocal seconds of the reaction $X \rightarrow Y$. $J_X(t)$ represents the absorption rate of species X at time t in photons absorbed per cubic centimetre per second and is given by

$$J_X(t) = \int_{\lambda_1}^{\lambda_2} J_X(\lambda, t)\, d\lambda \quad (43)$$

where $\lambda_1 - \lambda_2$ is the wavelength range of the bleaching light and $J_X(\lambda, t)\, d\lambda$ represents the number of photons with wavelengths lying between λ and $\lambda + d\lambda$ absorbed by molecules of type X per cubic centimetre per second.

If the absorbance of the system is low, we can write

$$J_X(\lambda, t) = \alpha_X(\lambda)N_X(t)I_{inc}(\lambda, t) \quad (44)$$

where $\alpha_X(\lambda)$ represents the extinction coefficient in photons per square centimetre per second of species X at wavelength λ and $I_{inc}(\lambda, t)\, d\lambda$ represents the spectral and temporal distribution of the flash intensity with wavelength lying between λ and $\lambda + d\lambda$. The initial conditions for solving eqns. (38) - (42) are $N_1 = R_0$ and $N_2 = N_3 = N_4 = N_5 = 0$ at $t = 0$. If the spectral distribution of the flash is independent of time, *i.e.* if it does not change during the experiment, we can write

$$I_{inc}(\lambda, t) = f(t)I(\lambda) \quad (45)$$

where $I(\lambda)$ and $f(t)$ represent the spectral and temporal distributions respectively of the flash intensity.

The analytical solutions of eqns. (38) - (42) cannot be obtained for an arbitrary function $f(t)$. If $f(t)$ has a constant value f_0 then eqns. (38) - (42) can be solved analytically. The solutions obtained after substituting

$$\begin{aligned} f(t) &= f_0 = \text{constant} \qquad 0 \leqslant t \leqslant \tau \\ &= 0 \qquad\qquad\qquad\quad t > \tau \end{aligned} \quad (46)$$

where τ is the duration of the flash, are as follows:

$$N_1(t) = R_0 \exp(-at) + \sum_{i=1}^{4} S_i \left[\frac{1}{m_i + a} \left(b + \frac{gK_{A \to B}}{m_i + z} \right) \{\exp(m_i t) - \exp(-at)\} + \right.$$

$$\left. + \frac{gK_{A \to B}}{(m_i + z)(z - a)} \{\exp(-zt) - \exp(-at)\} \right] \tag{47}$$

$$N_2(t) = \sum_{i=1}^{4} S_i \exp(m_i t) \tag{48}$$

$$N_3(t) = \sum_{i=1}^{4} S_i \left[\frac{1}{m_i + d} \left(c + \frac{hK_{A \to B}}{m_i + z} \right) \{\exp(m_i t) - \exp(-dt)\} + \right.$$

$$\left. + \frac{hK_{A \to B}}{(m_i + z)(z - d)} \{\exp(-zt) - \exp(-dt)\} \right] \tag{49}$$

$$N_4(t) = K_{A \to B} \sum_{i=1}^{4} \frac{S_i}{m_i + z} \{\exp(m_i t) - \exp(-zt)\} \tag{50}$$

$$N_5(t) = K_{A \to B} K_{B \to C} \sum_{i=1}^{4} \frac{S_i}{m_i + z} \left\{ \frac{\exp(m_i t) - 1}{m_i} + \frac{\exp(-zt) - 1}{z} \right\} \tag{51}$$

where

$$S_i = \frac{aR_0\{x_1 + (a + x_2)(x_2 + m_j + m_k + m_l) + m_j m_k + m_j m_l + m_k m_l\}}{(m_i - m_j)(m_i - m_k)(m_i - m_l)}$$

i, j, k and l are all different and have values between 1 and 4.

$x_1 = a^2 + ab + cd$

$x_2 = b + c + K_{A \to B}$

$z = g + h + K_{B \to C}$

$a = \gamma_{R,A} Z_R f_0$ $\qquad$ $b = \gamma_{A,R} Z_A f_0$

$c = \gamma_{A,I} Z_A f_0$ $\qquad$ $d = \gamma_{I,A} Z_I f_0$

$g = \gamma_{B,R} Z_B f_0$ $\qquad$ $h = \gamma_{B,I} Z_B f_0$

$$Z_X = \int_{\lambda_1}^{\lambda_2} \alpha_X(\lambda) I(\lambda)\, d\lambda \tag{52}$$

m_1, m_2, m_3 and m_4 are the roots of the equation

$$m^4 + \alpha m^3 + \beta m^2 + \gamma m + \delta = 0 \tag{53}$$

where

$$\alpha = a + d + x_2 + z$$

$$\beta = a(c + d + K_{A\to B}) + d(b + K_{A\to B}) + z(a + d + x_2)$$

$$\gamma = K_{A\to B}\{K_{B\to C}(a + d) + a(d + h) + gd\} + z(ad + ac + bd)$$

$$\delta = adK_{A\to B}K_{B\to C}$$

The roots of eqn. (53) are

$$m_1 = u^+ + v^+ \qquad m_2 = u^+ - v^+$$

$$m_3 = u^- + v^- \qquad m_4 = u^- - v^-$$

where

$$u^{\pm} = -\left(\frac{\alpha}{4} \pm M\right)$$

$$v^{\pm} = \left\{\left(\frac{\alpha}{4} \pm M\right)^2 - \left(\frac{\beta}{6} + 2x \pm N\right)\right\}^{1/2}$$

$$M = \left(\frac{\alpha^2}{16} - \frac{\beta}{6} + x\right)^{1/2}$$

$$N = \frac{\alpha\beta/12 - \gamma/2 + \alpha x}{2M}$$

$$x = \left(\frac{\omega_1}{3}\right)^{1/2} \cos\left(\frac{\theta}{3}\right)$$

$$\theta = \cos^{-1}\left\{-\frac{3\omega_2}{\omega_1(\omega_1/3)^{1/2}}\right\}$$

$$\omega_1 = \delta - \frac{\alpha\gamma}{4} + \frac{\beta^2}{12}$$

$$\omega_2 = \frac{9\alpha\beta\gamma - 27\alpha^2\delta + 72\beta\delta - 2\beta^3 - 27\gamma^2}{432}$$

As we have mentioned earlier, solutions (47) - (51) of eqns. (38) - (42) were obtained by assuming $f(t) = f_0$ = constant. To determine the validity of this assumption, Gupta and Goyal [121] performed the calculations for flash durations of 2 ms and 20 μs and compared the results obtained from eqns. (47) - (51) with those obtained by solving the kinetic equations numerically for the actual temporal distribution of the flash (TDF). The spectral distribution $I(\lambda)$ of the flash and the values of λ_1 and λ_2 were chosen to be those used by Williams and Breil [49] in their flash photolysis experiments.

Gaussian expressions were used for the absorption spectra $\alpha_X(\lambda)$ of rhodopsin and its intermediates. The advantage of using the gaussian expressions is that the integral in eqn. (52) can be evaluated [123]. The quantum efficiencies of the reactions used in the calculation were taken from refs. 36, 124 and 125, and the results were obtained for various values of the rate constants and of the number

$$Q = \int_{\lambda_1}^{\lambda_2} I(\lambda)\, d\lambda \int_0^{\tau} f(t)\, dt$$

of quanta per sample area per flash. It was found that at low temperatures the concentrations of the reactions at the end of the flash do not depend on the temporal distribution of the flash for any value of the peak intensity of the flash (Figs. 11 and 12) if we substitute

$$f_0 = \frac{1}{\tau} \int_0^{\tau} f(t)\, dt \qquad (54)$$

As the temperature increases the dependence of the results on the temporal distribution of the flash increases (Figs. 13 and 14). At a given temperature

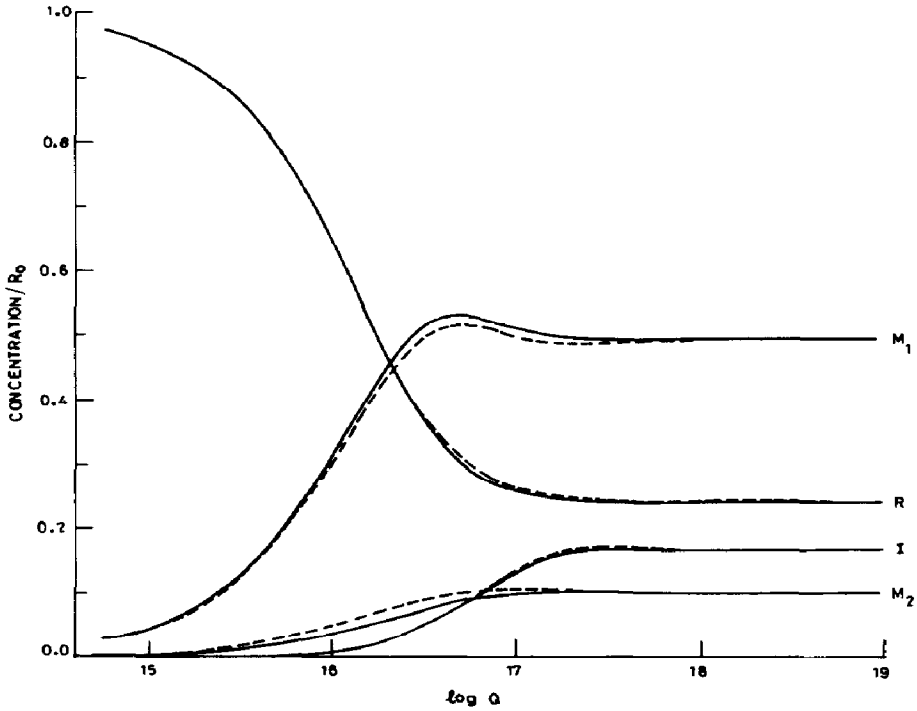


Fig. 11. Variation of the concentrations of rhodopsin (R), metarhodopsin I (M_1), isorhodopsin (I) and metarhodopsin II (M_2) remaining after exposure to a 2 ms flash with the number of quanta per sample area per flash for $K_{M_1 \rightarrow M_2} = 100\ s^{-1}$: ———, eqn. (54); – – –, actual TDF.

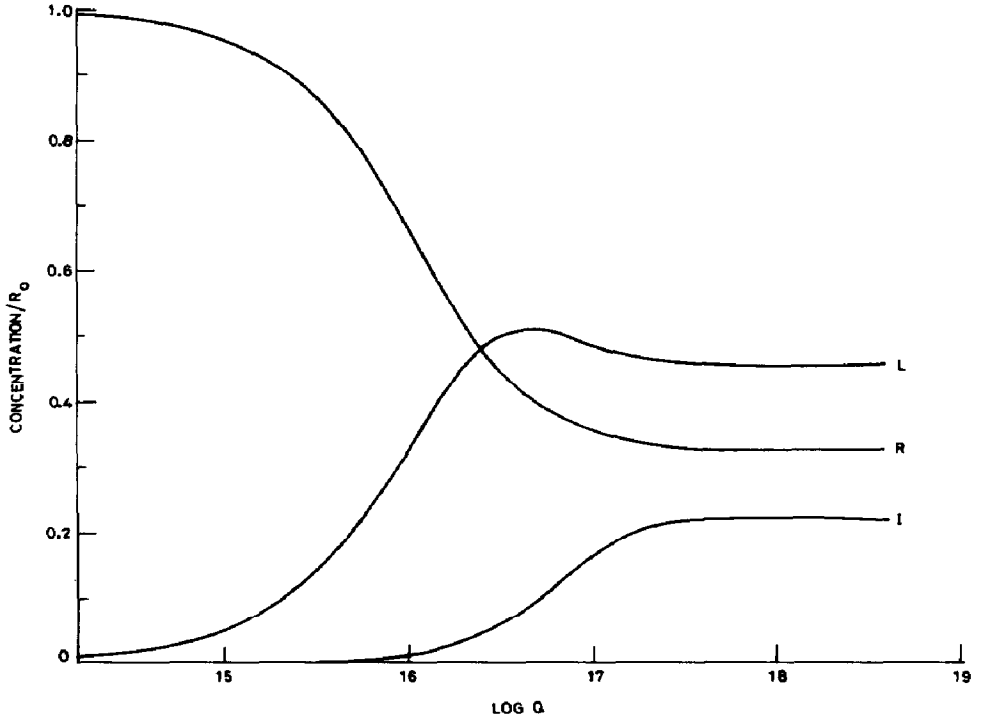


Fig. 12. Variation of the concentrations of rhodopsin (R), lumirhodopsin (L) and isorhodopsin (I) remaining after exposure to a 20 μs flash with the number of quanta per sample area per flash for $K_{L\rightarrow M_1} = 10^3\ s^{-1}$ and $K_{M_1\rightarrow M_2} = 50\ s^{-1}$: ——, eqn. (54).

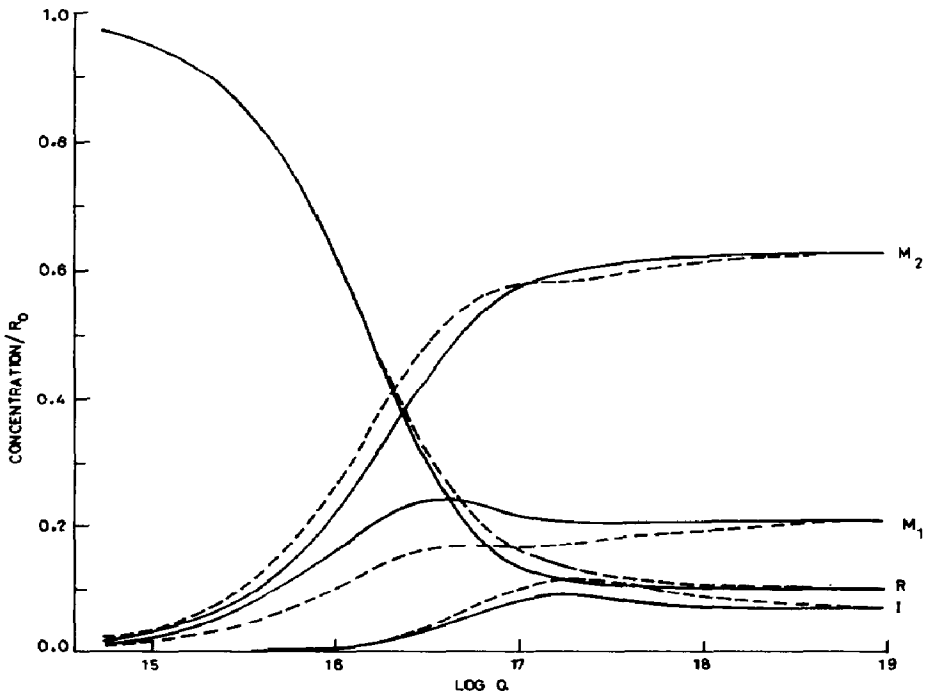


Fig. 13. As Fig. 11 except that $K_{M_1\rightarrow M_2} = 900\ s^{-1}$.

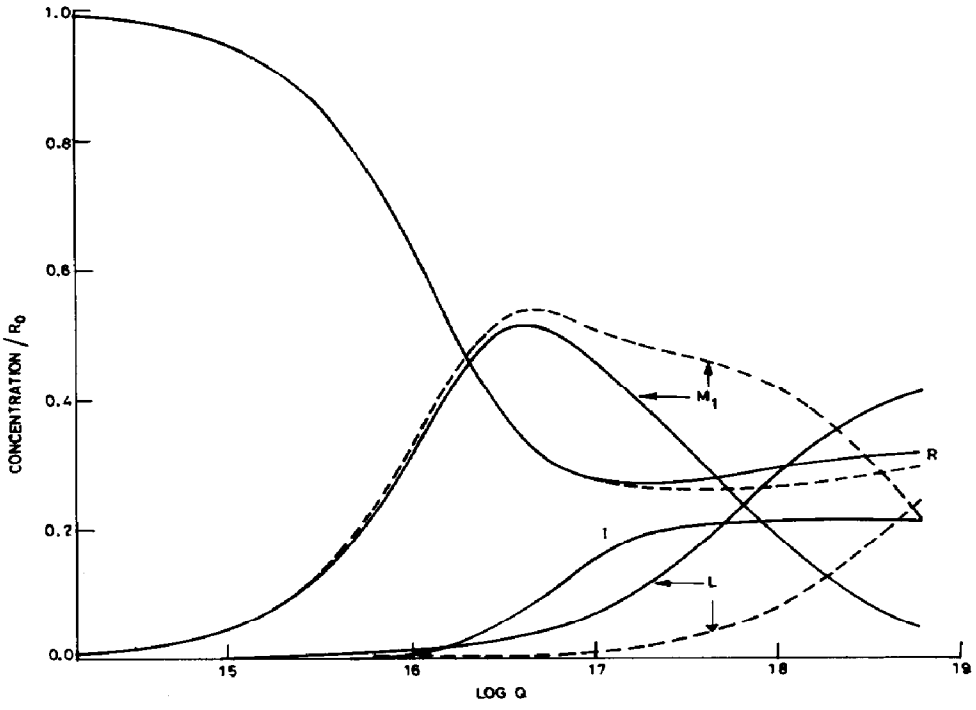


Fig. 14. As Fig. 12 except that $K_{L \rightarrow M_1} = 10^6\ s^{-1}$ and $K_{M_1 \rightarrow M_2} = 5 \times 10^3\ s^{-1}$.

the dependence on the TDF was found to be greatest for intermediate flash intensities.

The transient concentrations of the reactants during the flash were found to depend on the TDF. However, when

$$f_0 = \frac{1}{t}\int_0^t f(t')\,dt' \tag{55}$$

is substituted in the solutions of the kinetic equations (eqns. (47) - (51)), the transient concentrations obtained were in good agreement with the exact concentrations of the reactants (Fig. 15). This analysis can be usefully applied to the experimental observations of the flash photolysis experiments. The results of this analysis were used by Gupta and Goyal [126] to determine the kinetic activation parameters of the various reactions.

3.2. Visual pigments in the intact eye

The absorption of light by the visual pigments in the intact eye has been studied by means of the retinal densitometer which is also called the fundus reflectometer [113, 127 - 136]. As in the ophthalmoscope, light is shone into the eye, some is absorbed in the lens and vitreous humour, some is absorbed in the retina and a small fraction is reflected by the back layer. This reflected light is weakened by further absorption in the retina, the vitreous humour and the lens, and its intensity can be measured after it has

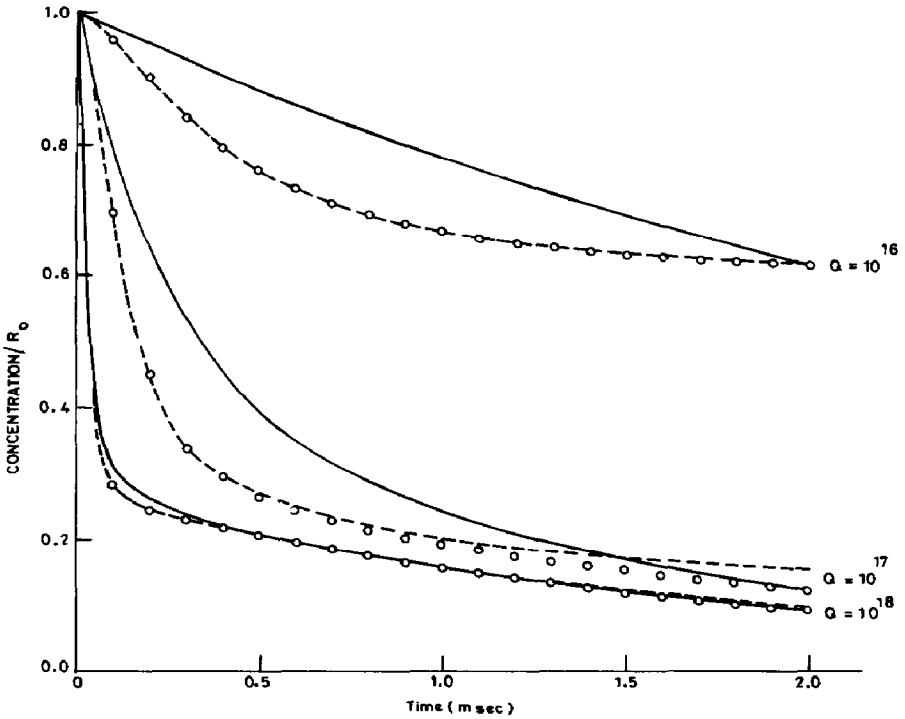


Fig. 15. Variation of the concentration of rhodopsin with time during the flash determined in the three ways for $K_{M_1 \to M_2} = 10^3 s^{-1}$ and Q values of 10^{16}, 10^{17} and 10^{18}: ——, from eqn. (47) assuming f_0 to be constant during the flash; ○, from eqn. (47) assuming f_0 to be given by eqn. (55); – – –, by solving eqn. (38) numerically using the actual TDF.

emerged from the eye. Since the components of the eye apart from the retina are photostable, this emergent intensity will depend on the state of adaptation of the eye. In the case of an eye which is adapted to the dark the emerging light will be weaker than if the retina has been exposed to a bright light which bleaches it. Consequently, if measurements are made with light of any wavelength before and after bleaching, the difference between the two will be due entirely to changes in absorption by the visual pigments and this can be used to determine the density of the pigments present in the eye. In a similar manner flash photolysis experiments have been performed on living eyes. The visual pigments were bleached by flashes of short duration and various intensities and their concentration was determined from the intensity of the reflected measuring light. In such cases the analysis of the flash photolysis experiment will be similar to that described in Section 3.1 except that the expression for the absorption rate of species X will be given by

$$J_X(\lambda, t) = \frac{\alpha_X(\lambda) X(t) I_{inc}(\lambda, t) \eta(\lambda)}{H(\lambda, t)} [1 - \exp\{-H(\lambda, t)\}] \times$$

$$\times [1 + b(\lambda) \exp\{-H(\lambda, t)\}] \qquad (56)$$

where

$$H(\lambda, t) = \sum_X \alpha_X(\lambda) X(t) l$$

$I_{inc}(\lambda, t)$ represents the intensity of light incident on the cornea, $\eta(\lambda)$ is the fraction of light of wavelength λ that reaches the photoreceptive layer, $b(\lambda)$ represents the reflectivity of the sclera for light of wavelength λ, $X(t)$ represents the instantaneous concentration of species X and l is the length of the outer segment. In the above equation $\eta(\lambda)$ and $b(\lambda)$ were determined experimentally. Pugh [113] used the data of Boettner and Wolter [137] for $\eta(\lambda)$ in the case of the human eye where $b(\lambda) \approx 0$. Hagins [120] determined $b(\lambda)$ experimentally for excised albino eyes. Gupta *et al.* [122] used eqn. (56) to determine the quantum efficiencies and rate constants of various reactions in rabbit eyes by analysing the experimental data reported by Hagins [120] and using the analysis given in Section 3.1.

4. Bleaching by plane-polarized light

The primary light-absorbing structure of visual pigment, *i.e.* 11-*cis*-retinal, is an anisotropic absorber and therefore the absorption of light by visual pigment will depend on its orientation with respect to the electric vector associated with the incident light. Therefore plane-polarized light has been widely used as a bleaching source for the determination of various structural and directional properties of visual pigment and photoreceptors [18, 19, 31, 52, 76, 125, 138]. Some studies have suggested that it is a planar molecule [139, 140] and is probably bent and twisted [141]. The results for the structural and directional analysis of visual pigments have been obtained from theoretical analysis of the experimental data for the absorption of plane-polarized light by visual pigments in various environments.

The light-absorbing properties of a molecule can, in general, be represented by three extinction coefficients $\alpha_{x'}$, $\alpha_{y'}$ and $\alpha_{z'}$ corresponding to the three mutually perpendicular axes x', y' and z' of the coordinate system fixed within the molecule. However, the effective extinction coefficient will depend on the orientation of the molecule (represented by the x', y' and z' axes) with respect to the coordinate system (x, y, z) in which the direction of polarization of the bleaching light is fixed. The orientation can be expressed in terms of the three eulerian angles θ, ϕ and ψ defined in Fig. 16 [139]. If the electric vector of the plane-polarized light propagating along the x axis is assumed to be parallel to the z axis, the effective extinction coefficient of a molecule with orientation specified by the angles θ, ϕ and ψ is given by

$$\alpha(\theta, \psi, \lambda) = \alpha_{z'}(\lambda)(K_1 \sin^2\theta \sin^2\psi + K_2 \sin^2\theta \cos^2\psi + \cos^2\theta) \quad (57)$$

where

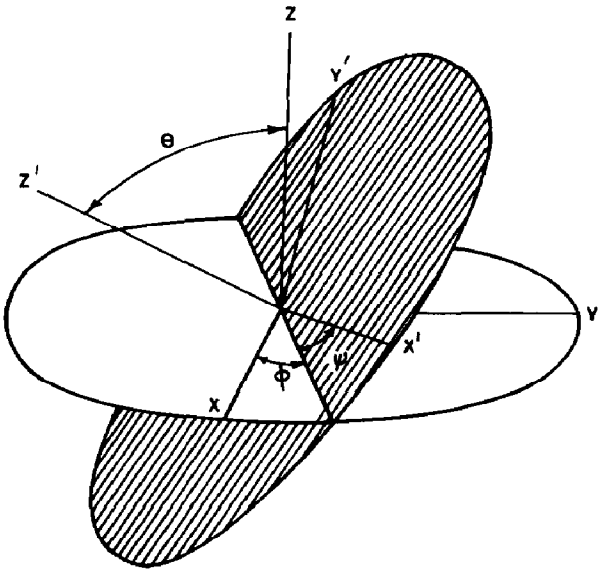


Fig. 16. Coordinate systems defining the eulerian angles.

$$K_1 = \frac{\alpha_{x'}}{\alpha_{z'}} \qquad K_2 = \frac{\alpha_{y'}}{\alpha_{z'}}$$

$\alpha(\theta, \psi, \lambda)$ is independent of ϕ because of the rotational symmetry about the z axis.

In the case of the retina with the light falling along the photoreceptor axis (*i.e.* the x axis) eqn. (57) becomes

$$\alpha(\theta, \lambda) = \alpha_{z'}(\lambda)(K \sin^2\theta + \cos^2\theta) \tag{58}$$

where

$$K = \frac{\alpha_{y'}}{\alpha_{z'}}$$

If the retina is mounted in hydroxylamine the absorption of light results in reaction (29). The kinetic equation which describes this reaction for bleaching with plane-polarized light is

$$\frac{\partial C(\theta, t)}{\partial t} = D_R \frac{\partial^2 C(\theta, t)}{\partial \theta^2} - \gamma\alpha(\theta, \lambda) I_0 C(\theta, t) \tag{59}$$

where $C(\theta, t)$ represents the instantaneous concentration of unbleached visual pigment and is a function of the orientation, *i.e.* θ, and D_R is the rotational diffusion coefficient of the molecule. In eqn. (59) the effect of the waveguide property is neglected and it is assumed that the absorbance is small. In general, eqn. (59) cannot be solved analytically. However, analytical solutions can be obtained in the following two cases.

(i) If the photoreceptor is treated with glutaraldehyde or if the temperature is very low D_R will be zero.

(ii) If the retina is at room temperature and has not been treated with glutaraldehyde then eqn. (59) can be solved if it is bleached by plane-polarized light with a very short flash duration. The duration should be so small that the molecules do not change orientation appreciably during the bleaching time. In such cases the bleaching source will be used to induce the concentration gradient and hence it can be assumed that I_0 is zero and eqn. (59) can be solved.

Case (i). The solution of eqn. (59) is given by

$$C(\theta, t) = \frac{C_0}{2\pi} \exp\{-\gamma\alpha(\theta, \lambda)I_0 t\} \tag{60}$$

The absorbance of a system of this type measured using plane-polarized light of wavelength λ_0 with its electric vector along the z axis is

$$A_z(\lambda_0, t) = l\int_0^{2\pi} \alpha_{z'}(\lambda_0)(\cos^2\theta + K \sin^2\theta)C(\theta, t)\, d\theta \tag{61}$$

If the electric vector of the measuring light is along the y axis it will be

$$A_y(\lambda_0, t) = l\int_0^{2\pi} \alpha_{z'}(\lambda_0)(\sin^2\theta + K \cos^2\theta)C(\theta, t)\, d\theta \tag{62}$$

The difference between $A_z(\lambda_0, t)$ and $A_y(\lambda_0, t)$ gives the photodichroism of the system. In this case it is

$$D(\lambda_0, t) = C_0\alpha_{z'}(\lambda_0)l(1 - K) \exp\{-(1 + K)f(\lambda, t)\}I_1\{(1 - K)f(\lambda, t)\} \tag{63}$$

where

$$f(\lambda, t) = \frac{\gamma\alpha_{z'}(\lambda)I_0 t}{2}$$

This type of analysis was performed by Strackee [80] for dichroism in the retina at −196 °C. The only difference was in the reaction model used for the analysis. Since Strackee [80] performed experiments at −196 °C, he used the following reaction instead of reaction (29):

rhodopsin ⇄ prelumirhodopsin ⇄ isorhodopsin

From a comparison of the theoretical analysis with the experimental data he reported the value of the absorption ratio K between the absorption axes of rhodopsin and its photoproducts in the plane of the retina. For the visual pigment solution at low temperature the expressions for photodichroism and absorbance of the system bleached by plane-polarized light are very complicated owing to the complexity of the expression for the effective extinction coefficient (see eqn. (57)). The integrals in the final expressions for photodichroism and the absorbance of the visual pigment solution at low temperature were evaluated numerically by Strackee [52] and Gupta *et al.* [139] respectively by using a simplified model of rhodopsin and its photoproducts.

The theoretical results were compared with the experimental data. From the comparison Strackee [52] and Gupta *et al.* [139] reported the values of the absorption ratios of rhodopsin, prelumirhodopsin and isorhodopsin. Later, Gupta [140] performed a more general analysis of the system and reported individual values of the absorption ratios for rhodopsin and prelumirhodopsin.

Case (ii). The solution of eqn. (59) is given by

$$C(\theta, t) = u\{1 + K + (1 - K) \exp(-4D_R t) \cos 2\theta\} \tag{64}$$

where u is a constant. Cone [19] studied the dichroic ratio in the retina after bleaching it with plane-polarized light. The dichroic ratio is given by

$$D(t) = \frac{\int_0^{\pi/2} C(\theta, t)(\cos^2\theta + K \sin^2\theta)\, d\theta}{\int_0^{\pi/2} C(\theta, t)(\sin^2\theta + K \cos^2\theta)\, d\theta} \tag{65}$$

Substituting eqn. (64) in eqn. (65) gives

$$D(t) = \frac{2(1 + K)^2 + f(1 - K)^2 \exp(-4D_R t)}{2(1 + K)^2 - f(1 - K)^2 \exp(-4D_R t)} \tag{66}$$

where $f \leqslant 1$ is an empirical factor introduced to account for polarization loss caused by the optics and by light scattering in the experiment. Equation (66) was derived by Cone [19] for $K = 0$. The value of f was obtained from the experimentally determined dichroic ratio at time $t = 0$, and the value of the rotational diffusion coefficient D_R was obtained from the agreement of the time dependence of the experimentally recorded dichroic ratio with the time dependence given by eqn. (66). Cone [19] determined the coefficient of viscosity of the fluid of the disc membrane from this value of D_R.

5. Conclusion

From the studies reported above, it can be concluded that mathematical analyses are very useful for the determination of the photosensitivity [38, 39, 66, 70, 71], the quantum efficiency, the rate constant [113, 122], the kinetic activation parameters [126], the principal absorption axes of the molecule [52, 80, 125, 139, 140], the orientation of the chromophore in the disc membrane [139, 140], the rotational diffusion coefficient of the visual pigment in the disc membrane [19] etc. In addition the formation and decay of an intermediate in the bleaching reaction of the visual pigment can be studied by comparing the theoretical results with experimental data for the absorption of light by visual pigment systems [142]. These analyses may also be useful for studying various properties of other photosensitive molecules.

References

1 R. A. Morton and G. A. J. Pitt, *Fortschr. Chem. Org. Naturst., 44* (1957) 244.
2 W. A. H. Rushton, *Prog. Biophys. Biophys. Chem., 9* (1959) 239.
3 G. Wald, P. K. Brown and I. R. Gibbons, *J. Opt. Soc. Am., 53* (1963) 20.
4 C. D. B. Bridges, *Compr. Biochem., 27* (1967) 31.
5 E. W. Abrahamson and S. E. Ostroy, *Prog. Biophys. Mol. Biol., 17* (1967) 179.
6 G. Wald, *Nature (London), 219* (1968) 800.
7 R. A. Morton and G. A. J. Pitt, *Adv. Enzymol. Rel. Areas Mol. Biol., 32* (1969) 97.
8 S. L. Bonting, *Curr. Top. Bioenerg., 3* (1969) 351.
9 E. W. Abrahamson and R. S. Fager, *Curr. Top. Bioenerg., 5* (1973) 125.
10 B. Honig and T. G. Ebrey, *Annu. Rev. Biophys. Bioeng., 3* (1974) 151.
11 E. W. Abrahamson, R. S. Fager and W. T. Mason, *Exp. Eye Res., 18* (1974) 51.
12 M. Montal and J. I. Korenbrot, in A. Martinosi (ed.), *The Enzymes of Biological Membranes*, Vol. 4, Plenum, New York, 1976, p. 365.
13 S. E. Ostroy, *Biochim. Biophys. Acta, 463* (1977) 91.
14 R. R. Birge, *Annu. Rev. Biophys. Bioeng., 10* (1981) 315.
15 R. Uhl and E. W. Abrahamson, *Chem. Rev., 81* (1981) 291.
16 A. Maeda and T. Yoshizawa, *Photochem. Photobiol., 35* (1982) 891.
17 T. Yoshizawa and Y. Shichida, in L. Packer (ed.), *Methods in Enzymology*, Vol. 81, Part H, Academic Press, New York, 1982, p. 333.
18 P. K. Brown, *Nature (London) New Biol., 236* (1972) 35.
19 R. A. Cone, *Nature (London) New Biol., 236* (1972) 39.
20 M. M. Poo and R. A. Cone, *Exp. Eye Res., 17* (1973) 503.
21 M. M. Poo and R. A. Cone, *Nature (London), 247* (1974) 438.
22 P. A. Liebman and G. Entine, *Science, 185* (1974) 457.
23 M. O. Hall, D. Bok and A. D. E. Bacharach, *J. Mol. Biol., 45* (1969) 397.
24 D. Bownds, A. Gordon-Walker, A. C. Gaide-Huguenin and W. Robinson, *J. Gen. Physiol., 58* (1971) 225.
25 H. Heitzmann, *Nature (London) New Biol., 235* (1972) 114.
26 W. E. Robinson, A. Gordon-Walker and D. Bownds, *Nature (London) New Biol., 235* (1972) 112.
27 F. J. M. Daemen, W. J. de Grip and P. A. A. Jansen, *Biochim. Biophys. Acta, 271* (1972) 419.
28 D. S. Papermaster and W. J. Dreyer, *Biochemistry, 13* (1974) 2438.
29 W. J. Schmidt, *Kolloid-Z., 85* (1938) 137.
30 E. J. Denton, *Proc. R. Soc. London, Ser. B, 150* (1959) 78.
31 W. A. Hagins and W. H. Jennings, *Discuss. Faraday Soc., 27* (1959) 180.
32 P. A. Leibman, *Biophys. J., 2* (1962) 161.
33 R. A. Weale, *Vision Res., 11* (1971) 1373.
34 F. I. Harosi and E. F. MacNichol, Jr., *J. Opt. Soc. Am., 64* (1974) 903.
35 R. Hubbard and G. Wald, *J. Gen. Physiol., 36* (1952) 269.
36 R. Hubbard and A. Kropf, *Prof. Natl. Acad. Sci. U.S.A., 44* (1958) 130.
37 T. Yoshizawa, in H. J. A. Dartnall (ed.), *Handbook of Sensory Physiology*, Vol. 7/1, Springer, Berlin, 1972, p. 147.
38 H. J. A. Dartnall, C. F. Goodeve and R. J. Lythgoe, *Proc. R. Soc. London, Ser. A, 156* (1936) 158.
39 H. J. A. Dartnall, C. F. Goodeve and R. J. Lythgoe, *Proc. R. Soc. London, Ser. A, 164* (1938) 216.
40 V. J. Wulff, R. G. Adams, H. Linschitz and E. W. Abrahamson, *Ann. N. Y. Acad. Sci., 74* (1958) 281.
41 R. Hubbard, P. K. Brown and A. Kropf, *Nature (London), 183* (1959) 442.
42 E. W. Abrahamson, J. Marquisee, P. Gavuzzi and J. Roubie, *Z. Elektrochem., 64* (1960) 177.
43 C. D. B. Bridges, *Biochem. J., 79* (1961) 128.

44 C. D. B. Bridges, *Biochem. J., 79* (1961) 135.
45 C. D. B. Bridges, *Vision Res., 2* (1962) 201.
46 K. H. Grellmann, R. Livingston and D. Pratt, *Nature (London), 193* (1962) 1258.
47 T. Yoshizawa and G. Wald, *Nature (London), 197* (1963) 1279.
48 T. P. Williams, *Vision Res., 8* (1968) 1457.
49 T. P. Williams and S. J. Breil, *Vision Res., 8* (1968) 777.
50 T. P. Williams, *Vision Res., 10* (1970) 525.
51 B. N. Baker and T. P. Williams, *Vision Res., 11* (1971) 449.
52 L. Strackee, *Photochem. Photobiol., 15* (1972) 253.
53 D. J. Eder and T. P. Williams, *Am. J. Optom. Arch. Am. Acad. Optom., 50* (1973) 765.
54 T. P. Williams, *Vision Res., 14* (1974) 603.
55 R. Bensasson, E. J. Land and T. G. Truscott, *Nature (London), 258* (1975) 768.
56 J. G. Stewart, B. N. Baker, E. O. Plante and T. P. Williams, *Arch. Biochem. Biophys., 172* (1976) 246.
57 B. H. Green, T. G. Monger, R. R. Alfano, B. Aton and R. H. Callender, *Nature (London), 269* (1977) 179.
58 J. B. Hurley, T. J. Ebrey, B. Honig and M. Ottolenghi, *Nature (London), 270* (1977) 540.
59 S. Kawamura, F. Tokunaga and T. Yoshizawa, *Vision Res., 17* (1977) 991.
60 V. Sundstrom, P. M. Rentzepis, K. Peters and M. L. Applebury, *Nature (London), 267* (1977) 645.
61 T. Kobayashi, *FEBS Lett., 106* (1979) 313.
62 T. Kobayashi, *Photochem. Photobiol., 32* (1980) 207.
63 N. Sasaki, F. Tokunaga and T. Yoshizawa, *FEBS Lett., 114* (1980) 1.
64 T. Suzuki and R. H. Callender, *Biophys. J., 34* (1981) 261.
65 E. J. R. Sudhölter, W. J. deGrip and J. B. F. N. Engberts, *J. Am. Chem. Soc., 104* (1982) 1069.
66 F. Crescitelli and B. Karvaly, *Proc. R. Soc. London, Ser. B, 220* (1983) 69.
67 T. Yoshizawa, S. Horiuchi, F. Tokunaga and N. Sasaki, *FEBS Lett., 163* (1983) 165.
68 C. Blazynski and S. E. Ostroy, *Vision Res., 24* (1984) 459.
69 W. H. Waddell, J. Lecomte, J. L. West and U. E. Younes, *Photochem. Photobiol., 39* (1984) 213.
70 B. Rabinovitch, *Photochem. Photobiol., 17* (1973) 479.
71 D. Onderdelinden and L. Strackee, *Vision Res., 13* (1973) 1297.
72 T. P. Williams and S. E. Milby, *Vision Res., 8* (1968) 359.
73 W. A. Hagins, *Nature (London), 177* (1956) 989.
74 T. G. Ebrey, *Vision Res., 8* (1968) 965.
75 C. Baumann, *Vision Res., 10* (1970) 789.
76 E. J. Denton, *J. Physiol. (London), 124* (1954) 17.
77 R. N. Frank, *Vision Res., 9* (1969) 1415.
78 H. Ripps and R. A. Weale, *J. Physiol. (London), 200* (1969) 151.
79 C. Baumann and W. Ernst, *J. Physiol. (London), 210* (1970) 156P.
80 L. Strackee, *Vision Res., 10* (1970) 925.
81 R. A. Weale, *Vision Res., 11* (1971) 1373.
82 C. Baumann, *J. Physiol. (London), 222* (1972) 643.
83 C. Baumann and S. Bender, *J. Physiol. (London), 235* (1973) 761.
84 C. Baumann and R. Reinheimer, in H. Langer (ed.), *Biochemistry and Physiology of Visual Pigments*, Springer, Berlin, 1973, p. 89.
85 G. Gyllenberg, T. Reuter and H. Sippel, *Vision Res., 14* (1974) 1349.
86 K. O. Donner and S. Hemila, *Vision Res., 15* (1975) 985.
87 C. Baumann, *J. Physiol. (London), 259* (1976) 357.
88 T. Reuter, *Vision Res., 16* (1976) 909.
89 F. Tokunaga, S. Kawamura and T. Yoshizawa, *Vision Res., 16* (1976) 633.
90 J. K. Bowmaker, *Vision Res., 17* (1977) 17.

91 K. P. Brin and H. Ripps, *J. Gen. Physiol., 69* (1977) 97.
92 S. Kawamura, F. Tokunaga and T. Yoshizawa, *Vision Res., 17* (1977) 991.
93 C. Baumann, *J. Physiol. (London), 279* (1978) 71.
94 S. Horiuchi, F. Tokunaga and T. Yoshizawa, *Biochim. Biophys. Acta, 503* (1978) 402.
95 W. S. Jagger, *Vision Res., 19* (1979) 159.
96 W. S. Jagger, *Vision Res., 19* (1979) 381.
97 D. A. Baylor, T. D. Lamb and K. W. Yau, *J. Physiol. (London), 288* (1979) 589.
98 M. Chabre and J. Breton, *Vision Res., 19* (1979) 1005.
99 K. Azuma and M. Azuma, *Photochem. Photobiol., 32* (1980) 529.
100 C. Baumann and W. Zeppenfeld, *J. Physiol. (London), 317* (1981) 347.
101 N. Sasaki and T. Yoshizawa, *Photobiochem. Photobiophys., 2* (1981) 365.
102 M. Michel-Villaz, R. Roche and M. Chabre, *Biophys. J., 37* (1982) 603.
103 N. Sasaki, F. Tokunaga and T. Yoshizawa, *Biochim. Biophys. Acta, 722* (1983) 80.
104 J. M. Enoch, *J. Opt. Soc. Am., 50* (1960) 1025.
105 J. M. Enoch, *J. Opt. Soc. Am., 51* (1961) 1122.
106 J. M. Enoch, *J. Opt. Soc. Am., 53* (1963) 71.
107 B. D. Gupta and A. Sharma, *Biophys. Struct. Mech., 6* (1980) 227.
108 B. D. Gupta, *Biophys. Struct. Mech., 8* (1981) 35.
109 E. B. Goldstein and T. P. Williams, *J. Opt. Soc. Am., 56* (1966) 543.
110 E. B. Goldstein, *Vision Res., 7* (1967) 837.
111 W. A. H. Rushton and G. H. Henry, *Vision Res., 8* (1968) 617.
112 G. E. Busch, M. L. Applebury, A. A. Lamola and P. M. Rentzepis, *Proc. Natl. Acad. Sci. U.S.A., 69* (1972) 2802.
113 E. N. Pugh, *J. Physiol. (London), 248* (1975) 393.
114 R. Bensasson, E. J. Land and T. G. Truscott, *Photochem. Photobiol., 26* (1977) 610.
115 J. W. Lewis, J. S. Winterle, M. A. Powers, D. S. Kliger and E. A. Dratz, *Photochem. Photobiol., 34* (1981) 375.
116 J. Buchert, V. Stefancic, A. G. Doukas, R. R. Alfano, R. H. Callender, J. Pande, H. Akita, V. Balogh-Nair and K. Nakanishi, *Biophys. J., 43* (1983) 279.
117 M. Catt, W. Ernst and C. M. Kemp, *Vision Res., 23* (1983) 971.
118 W. J. DeGrip, J. Olive and P. H. M. Bovee-Geurts, *Biochim. Biophys. Acta, 734* (1983) 168.
119 C. Pfister, H. Kuhn and M. Chabre, *Eur. J. Biochem., 136* (1983) 489.
120 W. A. Hagins, Rhodopsin in a mammalian retina, *Ph.D. Thesis*, Cambridge University, 1957.
121 B. D. Gupta and I. C. Goyal, *Proc. Indian Natl. Sci. Acad., Part A, 46* (1980) 29.
122 B. D. Gupta, I. C. Goyal and A. K. Ghatak, *Biophys. Struct. Mech., 4* (1978) 129.
123 B. D. Gupta, *J. Photochem., 24* (1984) 407.
124 H. J. A. Dartnall, *Vision Res., 8* (1968) 339.
125 L. Strackee, *Biophys. J., 11* (1971) 728.
126 B. D. Gupta and I. C. Goyal, *J. Photochem., 11* (1979) 419.
127 W. A. H. Rushton, F. W. Campbell, W. A. Hagins and G. S. Brindley, *Opt. Acta, 1* (1955) 183.
128 W. A. H. Rushton, *J. Physiol. (London), 134* (1956) 11.
129 R. A. Weale, *Cold Spring Harbor Symp. Quant. Biol., 30* (1965) 335.
130 H. Ripps and R. A. Weale, *J. Physiol. (London), 196* (1968) 67P.
131 M. Alpern, *J. Physiol. (London), 217* (1971) 447.
132 M. Alpern and E. N. Pugh, *J. Physiol. (London), 237* (1974) 341.
133 A. B. Bonds and D. I. A. MacLeod, *J. Physiol. (London), 242* (1974) 237.
134 E. N. Pugh, *J. Physiol. (London), 248* (1975) 413.
135 I. Perlman, *J. Physiol. (London), 278* (1978) 141.
136 H. Ripps, L. Mahaffey III, I. M. Siegel, W. Ernst and C. M. Kemp, *J. Gen. Physiol., 77* (1981) 295.
137 E. A. Boettner and J. R. Wolter, *J. Invest. Ophthalmol., 1* (1962) 776.

138 T. Yoshizawa and S. Horiuchi, in H. Langer (ed.), *Biochemistry and Physiology of Visual Pigments*, Springer, Berlin, 1973, p. 69.
139 B. D. Gupta, A. Sharma and I. C. Goyal, *Biophys. Struct. Mech.*, *5* (1979) 321.
140 B. D. Gupta, *Biophys. Struct. Mech.*, *7* (1980) 97.
141 W. Sperling and C. N. Rafferty, *Nature (London)*, *224* (1969) 591.
142 B. D. Gupta and I. C. Goyal, *Photochem. Photobiol.*, *29* (1979) 619.