ROTATIONAL DIFFUSION OF RHODOPSIN-DIGITONIN MICELLES STUDIED BY TRANSIENT PHOTODICHROISM

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ABSTRACT The transient photodichroism induced by a 0.80 sec plane-polarized light flash in a rhodopsin-digitonin mixture at about −70°C was compared with a theoretical description of the effect. It was concluded that the transient dichroism is entirely due to rotational diffusion of the pigment molecules. When the rhodopsin-digitonin micelles are assumed to be rotationally symmetric it was found from the observed relaxation time that the axial ratio is probably less than 2. The initial photodichroism after each flash as a function of the number of flashes was shown to obey an equation derived for the photochemical equilibrium reaction between rhodopsin, lumirhodopsin, and isorhodopsin. The absolute quantum efficiency of the transition of rhodopsin to lumirhodopsin was found to be equal, within experimental error, to the quantum efficiency of bleaching rhodopsin at room temperature.

INTRODUCTION

During a recent study of the photodichroism of rhodopsin-digitonin extracts diluted with glycerol at low temperatures, it was observed that irradiation of these mixtures at about −70°C with plane-polarized light induces a transient dichroism. From the lack of any stable photodichroism at all stages of the bleaching process and at all wavelengths in the interval between 450 and 520 nm, it was concluded that the transient effect is not caused by a "dark" reaction such as the decay of prelumirhodopsin to lumirhodopsin, a stable bleaching product of rhodopsin at this temperature. Furthermore it is apparent from flash photolysis experiments with visual pigment solutions (Wulff et al., 1958; Grellmann et al., 1962; Pratt et al., 1964) that this reaction, even at the low temperatures considered here, is too fast by at least one order of magnitude to be observed in this experiment. A preliminary investigation indicated that the transient photodichroism must be attributed to rotational diffusion of the pigment molecules in the highly viscous aqueous glycerol

mixture. In the present communication the results of a more detailed study of this effect will be reported.

METHOD

Rhodopsin solutions were obtained in the usual way by extraction of rod outer segments from cattle retinas, separated according to Saito's flotation method in 40% (w/v) sucrose solution (Saito, 1938), with 2% digitonin in 0.1 M phosphate buffer (pH 6.9). The extinction ratios $\epsilon_{400}/\epsilon_{500}$ were in the range from 0.25 to 0.35. No attempt was made to purify the crude extracts from foreign proteins. The rhodopsin solutions, diluted with glycerol in a ratio of 1:3, were irradiated in a Lucite cell of 10 mm light path mounted in the photodichroism measuring equipment described previously (Strackee, 1970). The temperature cell of this equipment was cooled with dry ice–isopropyl alcohol and remained at constant temperature within 0.2°C. The recording of the photodichroism signal started within 0.5 sec after the 0.80 sec plane-polarized light flashes. Rhodopsin concentrations were determined spectrophotometrically at room temperature using $\epsilon = 40.6 \times 10^6$ cm$^2 \cdot$mole^{-1} as the molecular extinction (Wald and Brown, 1952).

The viscosity of the rhodopsin-glycerol mixtures at low temperature was measured in the temperature cell according to the method of Stokes by observing the rate of fall of a ball through the medium. In order to minimize the influence of the somewhat uncertain value of the density of the fluid at the low temperature and to increase the rate of fall, the material of the balls was chosen as gold. Care was taken to ensure that the whole system at the start of the experiment was in temperature equilibrium. The viscosity was calculated from Stokes's formula, corrected for the finite dimensions of the tube (Ladenburg and Faxen, 1932), and is estimated as

$$\gamma = 172 \times 10^3 \text{ poise}$$

at −72.0°C.

The intensity of the bleaching light was measured as described previously.[1]

RESULTS

The transient photodichroism induced by plane-polarized light in an aqueous rhodopsin-glycerol mixture at about −70°C is shown typically in Fig. 1. In most experiments the wavelength of the 0.80 sec bleaching flashes was 549 nm, other wavelengths giving qualitatively comparable results. The principal features of the transient photodichroism can be summarized as follows.

At all wavelengths of the measuring light in the interval between 450 and 520 nm the initial photodichroism $D_{\Delta B}$ after each light flash is negative,[2] indicating that

[1] Strackee, L. *Photochem. Photobiol.* In press.

[2] The sign of the photodichroism D follows from the definition

$$D = \frac{T_\parallel - T_\perp}{T_\parallel + T_\perp}$$

where $T_\parallel$ and $T_\perp$ are the transmitted light intensities when the electrical vectors of the measuring light and bleaching light are parallel and perpendicular respectively.

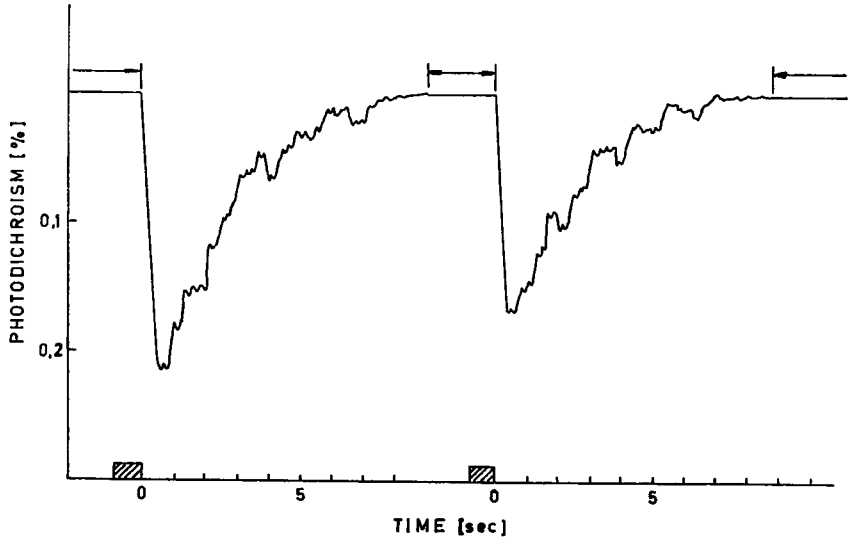

FIGURE 1 Transient photodichroism induced in a rhodopsin-glycerol mixture at −70.0°C by plane-polarized light flashes (λ = 549 nm) of 0.80 sec duration (part of the recording). Measuring light 500 nm. The integration time constant of the measuring equipment was 0.3 sec. During the intervals marked with arrows a protective shutter in front of the photomultiplier was closed.

the photoproduct first formed absorbs more strongly in this spectral region than rhodopsin itself and can be identified therefore as lumirhodopsin (Hubbard et al., 1959). At progressive bleaching $D_{\Delta B}$ diminishes at first approximately exponentially as a function of the number of light flashes, and reaches after exhaustive flashing a small but finite value of the opposite sign (Fig. 2).

By graphical analysis of the transient curves it was found that the photodichroism decays within experimental error as a single exponential function. The decay constant is strongly dependent on the temperature and is estimated as (0.33 ±0.02) sec^{-1} at −72.6°C, as (0.35 ±0.02) sec^{-1} at −72.0°C, and as (0.53 ±0.02) sec^{-1} at −70.0°C (the indicated errors are the standard deviations of the mean of five plots). The measuring equipment used did not allow extension of the temperature range.

The general aspects of the observed phenomena can be readily understood if it is assumed that the rhodopsin and the ensuing pigment molecules are subject to rotational diffusion and further that the subsequent absorption of light by lumirhodopsin produces either rhodopsin or isorhodopsin according to the photochemical equilibrium reaction

$$\text{rhodopsin} \leftrightarrows \text{lumirhodopsin} \leftrightarrows \text{isorhodopsin}$$

(Hubbard et al., 1965). The highly unstable precursor of lumirhodopsin, pre-

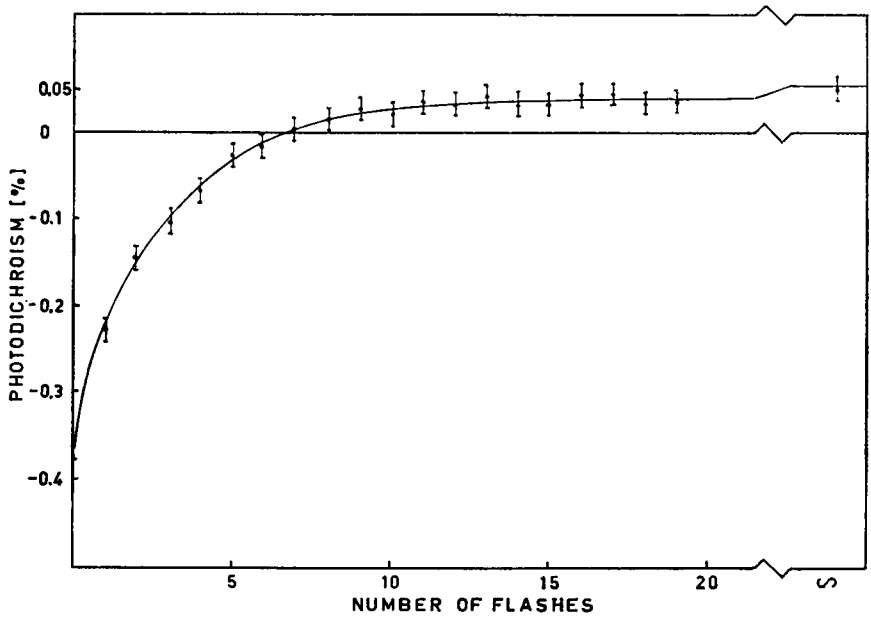

FIGURE 2 Photodichroism induced in an aqueous rhodopsin-glycerol mixture at −73.3°C by plane-polarized light flashes (λ = 549 nm), extrapolated to the end of the flashes and corrected for the decrease of dichroism during the irradiation as a function of the number of flashes. After reaching the final value of the opposite sign, light flashes irrespective of their duration produced always the same transient photodichroism.

lumirhodopsin, has been left out of this scheme since this pigment can not be observed under the conditions of the experiment.

Let us first consider the rotational diffusion. It is well-known that rhodopsin, solubilized by digitonin, is contained in a micelle that is composed of about 200 digitonin molecules per rhodopsin molecule (Hubbard, 1954). The molecular weight of this complex has been estimated from the sedimentation constant in the ultracentrifuge by Hecht and Pickels (1938) as about 270,000, and more recently by Hubbard (1954) as 260,000–290,000, and by Wolken (1956) as 290,000. An assumed molecular weight of 280,000 seems a reasonable compromise among the reported values. Furthermore we shall assume that the micelle can be considered as an ellipsoid of revolution the shape of which is not affected when the pigment molecules are interconverted by light.

Generally the molecules can rotate both about their symmetry (*a*) axis and about an equatorial (*b*) axis, thus yielding two rotational diffusion constants θ_a and θ_b. Since the decrease of the photodichroism after each flash is characterized by a single exponential function we may conclude that the principal axis of the absorption ellipsoids of the pigments, assumed to be also rotationally symmetric, coincides with the *a*-axis. In that case we do not need to consider rotation about this axis.

The rotational diffusion constant θ_b is given by the Perrin equations (see e.g. Cohn and Edsall, 1943).

$$\theta_b = \frac{KT}{4\gamma V}\frac{q^2}{1-q^4}\left\{\frac{2-q^2}{\sqrt{1-q^2}}\ln\left(\frac{1+\sqrt{1-q^2}}{q}\right)-1\right\} \quad \text{for} \quad q<1, \quad (1)$$

and

$$\theta_b = \frac{KT}{4\gamma V}\frac{q^2}{q^4-1}\left\{\frac{q^2-2}{\sqrt{q^2-1}}\arctan\left(\sqrt{q^2-1}\right)+1\right\} \quad \text{for} \quad q>1, \quad (2)$$

where is K = constant of Boltzmann, T = absolute temperature, $V = (4/3)\pi ab^2$, volume of the ellipsoid, γ = viscosity of the solvent, and $q = b/a$, axial ratio.

The relation between the observed relaxation time τ, defined as the time that the photodichroism decays to a fraction $1/e = 0.368$ of its initial value, and the rotational diffusion constant can be derived directly from the work of Bênoit (1951) on the electrooptical Kerr effect. When the permanent electric dipole moment vector and the electrical and optical polarizabilities coincide with the rotational symmetry axis of the molecules, it can be shown that the relaxation time of the birefringence signal after removal of the electric field is given by

$$\tau = \frac{1}{6\theta}. \quad (3)$$

Since the orientation distribution induced by the electrical field can be described by a relation that has the same mathematical form as the equation that relates the orientation distribution of the photochemically bleached molecules to the flash exposure, we may use equation 3 without modification.

Expressing the apparent molecular volume V as

$$V = \frac{M}{dL}h(w), \quad (4)$$

where M is the molecular weight, L Avogadro's number, d the density of the micelle, and $h(w)$ a factor that accounts for the hydration of the micelle, we obtain at once for the relaxation time as a function of the axial ratio q and the hydration w

$$\tau(q, w) = \frac{\gamma M}{KTLd}f(q)h(w),$$

with

$$f(q) = \frac{2}{3}\frac{1-q^4}{\frac{q^2(2-q^2)}{\sqrt{1-q^2}}\ln\left(\frac{1+\sqrt{1-q^2}}{q}\right)-q^2} \quad \text{if} \quad q<1,$$

$$f(q) = \frac{2}{3} \frac{q^4 - 1}{\frac{q^2(q^2 - 2)}{\sqrt{q^2 - 1}} \arctan(\sqrt{q^2 - 1}) + q^2} \quad \text{if} \quad q > 1,$$

and

$$f(q) = 1 \quad \text{if} \quad q = 1. \tag{5}$$

We note that $f(q)\, h(w)$ is the rotational diffusion equivalent of the frictional ratio used in translational diffusion studies. Substitution of the numerical values[3] in equation 5, taking $d = 1.30$ g/cm^3 (Hubbard, 1954), and comparing the calculated values of τ with the experimental results gives the result

$$f(q)\, h(w) = 1.27,$$

with an estimated error of $\pm 5\%$.

A detailed interpretation of this result is difficult since the degree of hydration of the rhodopsin-digitonin micelle is not known; however, assuming the reasonable value of 0.30 (grams of water bound by one gram of the micelles) for the hydration, $h(w)$ becomes about 1.1 and $f(q)$ consequently also 1.1. This means that the axial ratio q is about 0.7 when the ellipsoid of revolution is elongated or about 2 when the ellipsoid is flattened. Although these figures indicate that the micelle is probably not spherical, the deviation from sphericality is small, even when a much lower degree of hydration is assumed.

A second experimental datum is the initial photodichroism $D_{\Delta E}(n)$ after each flash of duration Δt, as a function of the over-all exposure of the sample, that is the number of flashes n, as shown in Fig. 2. When the following conditions are satisfied—the flash duration is small compared to the relaxation time, the fractional bleaching caused by each flash is small, and the number of flashes per unit time is low—it can be shown (see Appendix) that $D_{\Delta E}(n)$ for the photochemical equilibrium reaction between rhodopsin, lumirhodopsin, and isorhodopsin is given approximately by equation 6:

$$D_{\Delta E}(n) = \sum_k^3 M_k \exp(S_k n \Delta E), \tag{6}$$

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[3] The viscosities at temperatures other than -72.0°C were calculated with the empirical relation (see e.g. Glasstone, 1956)

$$\gamma(T_1) = \gamma(T_2) \exp\left(B \frac{T_2 - T_1}{T_1 T_2}\right),$$

where the constant B was estimated from the variation of the photodichroism decay constant with the temperature.

where M_k and S_k are functions of the fractional photosensitivities l_j and the axial ratio κ of the absorption ellipsoids of the pigments involved. These functions are defined by the equations A 4, A 5, and A 11 of the Appendix.

Although the first condition under which equation 6 strictly applies is not fulfilled in the present experiments, Δt is of the same order of magnitude as τ, we still may use equation 6 because at the light intensities employed ($I = 0.75 \times 10^{16}$ photons·cm^{-2}sec^{-1} at $\lambda = 549$ nm) the chance that a pigment molecule will absorb more than 1 photon during one flash is less than about 3%. In that case the observed value of $D_{\Delta E}(n)$, extrapolated to the end of the flash, can be corrected simply for the photodichroism decay during the irradiation.

Inserting now the values of the absorption coefficient as reported by Hubbard et al. (1959), the relative quantum efficiencies and the axial ratio κ of the absorption ellipsoid taken from a previous publication (Strackee, 1970), summarized in Table I, gives

$$D_{\Delta E}(n) = 10^{-17}\{-0.141 \exp(-1.66\,\eta_r n\Delta E) - 0.014 \exp(-0.18\,\eta_r n\Delta E) + 0.016\}N_0, \quad (7)$$

where η_r is the absolute quantum efficiency of the interconversion of rhodopsin to lumirhodopsin in the -70°C temperature range (strictly speaking the absolute quantum efficiency of the transition of rhodopsin to prelumirhodopsin at this temperature) and N_0 the initial rhodopsin density.

TABLE I

ABSORPTION COEFFICIENTS OF RHODOPSIN, LUMIRHODOPSIN, AND ISORHODOPSIN AND THE QUANTUM EFFICIENCIES OF THE TRANSITIONS RELATIVE TO THE RHODOPSIN-LUMIRHODOPSIN CONVERSION AT -70°C USED FOR THE COMPUTATION OF THE CURVE DRAWN IN FIG. 2

Pigment	Absorption coefficient	
	500 nm	549 nm
	$cm^2 \times 10^{17}$	$cm^2 \times 10^{17}$
Rhodopsin (rh)	18	6.1
Lumirhodopsin (lumirh)	21	6.1
Isorhodopsin (isorh)	15	3.0

Transition	Relative quantum efficiency
Rh → lumirh	(1.00)
Lumirh → rh	0.62
Isorh → lumirh	0.25
Lumirh → isorh	0.10

Estimating $\Delta E = I\Delta t$ as 0.61×10^{16} photons·cm^{-2} per flash and inserting $N_0 = 6.8 \times 10^{17}$ cm^{-3}, the calculated curve may be fitted to the experimental values by adjusting η_r. As shown in Fig. 2 a fair agreement is obtained when η_r has the value 0.62.

Since the bleaching of rhodopsin at room temperature is governed, according to the model proposed by Yoshizawa and Wald (1963), by the conversion of rhodopsin to prelumirhodopsin by light, the stepwise decay of the latter pigment through a series of intermediates proceeding in the dark, we may compare η_r to the over-all quantum efficiency η of bleaching as reported recently by Dartnall (1968). His estimate of this quantity ($\eta \approx \frac{2}{3}$), which was shown to be independent of the origin of the visual pigments studied, is within experimental error equal to the value that is derived from transient photodichroism measurements at −70°C. We may conclude therefore that the quantum efficiency of bleaching rhodopsin is independent of the temperature in this range.

DISCUSSION

The agreement between the theoretical transient photodichroism in the −70°C range and the experimental results may be considered as a direct proof of the correctness of the assumptions underlying the calculations both with regard to the rotational diffusion hypothesis and with regard to the photochemical equilibrium reaction (equation 1). Since the initial value of the transient photodichroism after each flash depends critically on the axial ratio κ of the absorption ellipsoids of the pigments involved, the accordance supports furthermore the assumed value of $\kappa = 5$ (elongated ellipsoid of revolution). The shape of the rhodopsin-digitonin micelles, that follows from the rotational diffusion constant as determined by transient photodichroism measurements, is, regarding the accuracy of the estimate of the "frictional ratio" $f(q)h(w)$, compatible with the results from translational diffusion measurements (Hubbard, 1954). Unfortunately it can not be decided from either of these methods whether the geometrical shape of the micelles is an elongated or a flattened ellipsoid. We may remark that a careful comparison between rotational and translational diffusion data might settle this problem in principle, provided that the degree of hydration is known.

When the directions of incidence of the bleaching and the measuring light are not parallel it can be shown easily that linear polarization of the bleaching light is not a necessary condition for the induction of photodichroism. This is in particular so when both directions are mutually perpendicular, an arrangement that is used preferentially in flash-bleaching experiments. Transient photodichroism, or more generally time-dependent absorption changes due to rotational motions, may therefore affect the results obtained from these experiments and should be considered. In this respect it would be of interest to reexamine the thermal decay of prelumirhodopsin to lumirhodopsin as studied by Grellmann et al. (1962) from

flash photolytic experiments with special reference to the slower phases of this process.

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APPENDIX

When three pigments I, II, and III are interconvertible by the action of light according to

$$(\mathrm{I}) \underset{\lambda_1}{\overset{\lambda_4}{\leftrightarrows}} (\mathrm{II}) \underset{\lambda_2}{\overset{\lambda_3}{\leftrightarrows}} (\mathrm{III}), \tag{A 1}$$

it can be shown[1] that the photodichroism $D(E)$, that results from irradiation of a rigidly frozen solution of these pigments with plane-polarized light as a function of the light exposure E, is given by

$$D(E) = K(s_1, s_2)G(x_1, \kappa) + K(s_2, s_1)G(x_2, \kappa);$$
$$x_1 = -3\, s_1 E; \quad x_2 = -3\, s_2 E. \tag{A 2}$$

The constant $K(s_1, s_2)$ can be written as

$$K(s_1, s_2) = \sum_i^3 N_{i0}\beta_i(s_1, s_2),$$

where N_{i0} ($i = 1, 2, 3$) are the initial concentrations of the pigments at $t = 0$ and

$$\beta_1(s_1, s_2) = \frac{1}{s_1(s_1 - s_2)}[{}_m\bar{\alpha}_1\{s_1^2 + s_1(l_2 + l_3 + l_4) + l_3 l_4\} + {}_m\bar{\alpha}_2(s_1 + l_3)l_1 + {}_m\bar{\alpha}_3 l_1 l_2];$$

$$\beta_2(s_1, s_2) = \frac{1}{s_1(s_1 - s_2)}\{{}_m\bar{\alpha}_1(s_1 + l_3)l_4 + {}_m\bar{\alpha}_2(s_1 + l_1)(s_1 + l_3) + {}_m\bar{\alpha}_3(s_1 + l_1)l_2\};$$

$$\beta_3(s_1, s_2) = \frac{1}{s_1(s_1 - s_2)}[{}_m\bar{\alpha}_1 l_3 l_4 + {}_m\bar{\alpha}_2(s_1 + l_1)l_3 + {}_m\bar{\alpha}_3\{s_1^2 + s_1(l_1 + l_2 + l_4) + l_1 l_2\}]. \tag{A 4}$$

l_j ($j = 1, 2, 3, 4$) are the fractional photosensitivities defined as $l_1 = {}_b\bar{\alpha}_1\eta_1$, $l_2 = {}_b\bar{\alpha}_2\eta_2$, $l_3 = {}_b\bar{\alpha}_3\eta_3$, and $l_4 = {}_b\bar{\alpha}_2\eta_4$. ${}_b\bar{\alpha}_i$ and ${}_m\bar{\alpha}_i$ are the average absorption coefficients of the pigments at the wavelength of the bleaching and measuring light respectively, and η_j the quantum efficiencies of relevant transitions in the equilibrium reaction A 1. The corresponding expression for $K(s_2, s_1)$ is found by interchanging the roots s_1 and s_2 of the equation

$$p^2 + p(l_1 + l_2 + l_3 + l_4) + l_1 l_2 + l_1 l_3 + l_3 l_4 = 0. \tag{A 5}$$

The function $G(x, \kappa)$ appearing in equation A 2 is defined by

$$G(x, \kappa) = \frac{3}{2}\frac{1-\kappa}{2+\kappa}\int_0^{\pi}\frac{3\cos^2\theta - 1}{2} \cdot \exp\left\{-(\sin^2\theta + \kappa\cos^2\theta)\frac{x}{2+\kappa}\right\}\sin\theta\, d\theta, \quad (A\,6)$$

where κ is the axial ratio of the absorption ellipsoids of the pigments, assumed to be rotationally symmetric. It is to be noted that the photodichroism equation A 2 applies only to the case in which the axial ratios of the pigments involved are all equal and furthermore that the rotational symmetry axes of the absorption ellipsoids of each set of three interconvertible pigment molecules coincide in space. Let the values of $G(x_1, k)$ and $G(x_2, k)$ for an exposure ΔE, delivered in a flash that is short compared to the rotational relaxation time τ, be G_1 and G_2. When the over-all exposure rate is much slower compared to τ the initial value $D_{\Delta E}(n)$ of the photodichroism after each flash as a function of the number of flashes n becomes

$$D_{\Delta E}(n) = G_1\sum_i^3 N_{i0}(n)\beta_i(s_1, s_2) + G_2\sum_i^3 N_{i0}(n)\beta_i(s_2, s_1). \quad (A\,7)$$

$N_{i0}(n)$, the pigment concentrations after the over-all exposure $n\Delta E$, are easily found as the solutions of the set of the simultaneous differential equations

$$\begin{aligned}\frac{dN_1}{dt} &= -Il_1N_1 + Il_4N_2,\\ \frac{dN_2}{dt} &= -I(l_2 + l_4)N_2 + Il_1N_1 + Il_3N_3,\\ \frac{dN_3}{dt} &= -Il_3N_3 + Il_2N_2,\end{aligned} \quad (A\,8)$$

describing the photochemical reaction (equation A 1). Since the exposure rate was assumed to be slow the orientation-dependent reaction constants λ_j may be replaced by Il_j (I is the intensity of the bleaching light). The solutions of equations A 8 can be written conveniently as ($s_3 = 0$)

$$N_{i0}(n) = \sum_k^3 L_{ik}\exp(S_knI\Delta t), \quad (A\,9)$$

where L_{ik} are specified functions (see below) of l_j, s_1, and s_2, and Δt the duration of the flashes. Evidently $\Delta E = I\Delta t$. Substitution of equation A 9 into equation A 7 and introducing the fictitious quantity $G_3 = 0$ yields immediately

$$D_{\Delta E}(n) = \sum_m^3\sum_i^3\sum_k^3 L_{ik}\beta_{im}G_m\exp(s_k n\Delta E) = \sum_k^3 M_k\exp(s_k n\Delta E) \quad (A\,10)$$

$$M_k = \sum_m^3\sum_i^3 L_{ik}\beta_{im}G_m$$

with $\beta_{i1} = \beta_i(s_1, s_2)$, $B_{i2} = \beta_i(s_2, s_1)$, and $\beta_{i3} = 0$.

Starting the experiment with a pure solution of the pigment I with a concentration N_0 we find

$$L_{11} = \frac{s_1(s_1 + l_2 + l_3 + l_4) + l_3 l_4}{s_1(s_1 - s_2)} N_0, \qquad L_{13} = \frac{l_3 l_4}{s_1 s_2} N_0,$$

$$L_{21} = \frac{l_1(s_1 + l_3)}{s_1(s_1 - s_2)} N_0, \qquad L_{23} = \frac{l_1 l_3}{s_1 s_2} N_0,$$

$$L_{31} = \frac{l_1 l_2}{s_1(s_1 - s_2)} N_0, \qquad L_{33} = \frac{l_1 l_2}{s_1 s_2} N_0, \qquad \text{(A 11)}$$

(L_{i2} follows from L_{i1} by interchanging s_1 and s_2.)

REFERENCES

BÉNOIT, P. 1951. *Ann. Phys. (Paris).* **6**:561.

COHN, E. J., and J. T. EDSALL. 1943. Proteins, Amino Acids and Peptides. Reinhold Publishing Corp., New York.

DARTNALL, H. J. A. 1968. *Vision Res.* **8**:339.

GLASSTONE, S. 1956. Textbook of Physical Chemistry. Macmillan and Co. Ltd., London.

GRELLMANN, K. H., R. LIVINGSTON, and D. PRATT. 1962. *Nature (London).* **193**:1258.

HECHT, S., and E. G. PICKELS. 1938. *Proc. Nat. Acad. Sci. U. S. A.* **85**:172.

HUBBARD, R. 1954. *J. Gen. Physiol.* **37**:381.

HUBBARD, R., D. BOWNDS, and T. YOSHIZAWA. 1965. *Cold Spring Harbor Symp. Quant. Biol.* **30**:301.

HUBBARD, R., P. K. BROWN, and A. KROPF. 1959. *Nature (London).* **183**:442.

LADENBURG, R., and H. FAXEN. 1932. *In* Handbuch der Experimentalphysik. L. Flüge, editor. Akademische Verlagsgesellschaft mbH, Frankfurt. **4**(2):339.

PRATT, D. C., R. LIVINGSTON, and K. H. GRELLMANN. 1964. *Photochem. Photobiol.* **3**:121.

SAITO, Z. 1938. *Tohoku J. Exp. Med.* **32**:432.

STRACKEE, L. 1970. *Vision Res.* **10**:925.

WALD, G., and P. K. BROWN. 1952. *J. Gen. Physiol.* **35**:797.

WOLKEN, J. J. 1956. *J. Cell. Comp. Physiol.* **48**:349.

WULFF, V. J., R. G. ADAMS, H. LINSHITZ, and E. W. ABRAHAMSON. 1958. *Ann. N. Y. Acad. Sci.* **74**:281.

YOSHIZAWA, T., and G. WALD. 1963. *Nature (London).* **197**:1279.