

Biophysics

FLASH PHOTOLYSIS OF RHODOPSIN : EFFECT OF TEMPORAL DISTRIBUTION OF THE FLASH INTENSITY ON BLEACHING KINETICS*

B. D. GUPTA** and I. C. GOYAL

Physics Department, Indian Institute of Technology, New Delhi-110 029

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In the present paper, the effect of temporal distribution of the flash intensity on bleaching kinetics of rhodopsin has been studied. The solutions of the kinetic equations describing a flash bleach experiment have been determined under the assumption that the temporal distribution of the flash [$f(t)$] is given by

$$f(t) = f_0 = \text{a constant,} \quad 0 \leq t \leq \tau \\ = 0, \quad t > \tau$$

where τ represents the duration of the flash. The results obtained using these solutions have been compared with those obtained by solving kinetic equations numerically using Runge-Kutta method for an arbitrary temporal distribution of the flash. It has been shown that for an arbitrary temporal distribution of the flash, the solutions obtained using above assumption cannot be used at all temperatures for an arbitrary intensity of the flash. Some cases have been reported in which the analysis can be made simpler. The transient concentrations of the reactants have also been determined after substituting

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

in the solutions of the kinetic equations. The results so obtained are in good agreement with those obtained by solving the equations numerically.

INTRODUCTION

FLASH photolysis technique is widely used to study the formation of the short lived photo-products of visual pigments and their light absorbing and thermal properties (e.g., quantum efficiencies, absorption ratios between the principal absorption axes of the molecules, rate constants etc.) at physiological temperatures (Hagins, 1956; Wulff *et al.*, 1958; Bridges, 1961a, b; Goldstein & Williams, 1966; Goldstein, 1967; Ebrey, 1968; Rushton & Henry, 1968; Williams & Breil, 1968; Baumann, 1970; Williams, 1970, 1974; Busch *et al.*, 1972; Cone, 1972; Eder & Williams, 1973; Pugh, 1975; Bensasson *et al.*, 1975, 1977; Gupta *et al.*, 1978). The importance of this technique is that it can also be used in the case of living eyes. In the theoretical analysis of the flash photolysis experiments, the temporal distribution of the flash intensity which is an important parameter is, generally, not considered (Ebrey, 1968; Mainster *et al.*, 1971). In the present paper, its effect on the bleaching kinetics of

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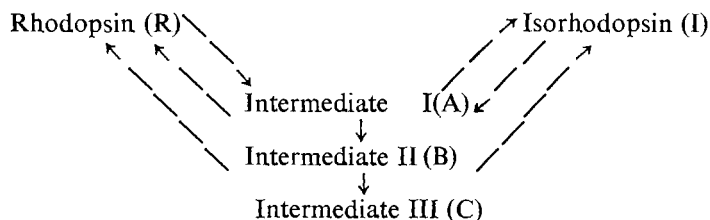
the rhodopsin molecule has been studied. The solutions of the kinetic equations describing a flash bleach experiment have been determined under the assumption that the intensity of the flash remains constant throughout the time of the flash ($0 \leq t \leq \tau$) and the temporal distribution of the flash* ($f(t)$) is replaced by

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

where τ represents the duration of the flash. The results obtained using these solutions have been compared with those obtained by solving kinetic equations numerically using the Runge-Kutta method for an arbitrary TDF. It has been shown that for an arbitrary bleaching source the solutions obtained using eqn. (1) cannot be used at all temperatures for an arbitrary intensity of the flash. Some special cases have been described in which these solutions can be used.

MATHEMATICAL ANALYSIS

(i) *Photochemical Reaction Model* — To study the effect of TDF on bleaching kinetics of rhodopsin, the following general reaction model for a flash bleach experiment has been considered here



Intermediates I, II and III in above reaction model depend on flash duration and temperature of the sample.

(ii) *Rate Equations and their Solutions* — Let N_1 , N_2 , N_3 , N_4 and N_5 represent the instantaneous concentrations of R, A, I, B and C molecules respectively. For an arbitrary bleaching source, the kinetic equations which describe the photochemical reaction model can be written as

$$\frac{dN_1}{dt} = -\gamma_{R,A} J_R(t) + \gamma_{A,R} J_A(t) + \gamma_{B,R} J_B(t) \quad \dots(2)$$

$$\frac{dN_2}{dt} = \gamma_{R,A} J_R(t) - (\gamma_{A,R} + \gamma_{A,I}) J_A(t) + \gamma_{I,A} J_I(t) - K_{A \rightarrow B} N_2(t) \quad \dots(3)$$

$$\frac{dN_3}{dt} = \gamma_{A,I} J_A(t) - \gamma_{I,A} J_I(t) + \gamma_{B,I} J_B(t) \quad \dots(4)$$

*Hereafter we will name it TDF.

$$\frac{dN_4}{dt} = -(\gamma_{B \rightarrow R} + \gamma_{B \rightarrow I}) J_B(t) + K_{A \rightarrow B} N_2(t) - K_{B \rightarrow C} N_4(t) \quad \dots(5)$$

$$\frac{dN_5}{dt} = K_{B \rightarrow C} N_4(t), \quad \dots(6)$$

where $J_x(t)$, the absorption rate of the species X at time t , is given by

$$J_X(t) = \int_{\lambda_1}^{\lambda_2} J_X(\lambda, t) d\lambda. \quad \dots(7)$$

The symbols occurring in eqns. (2) to (7) have their usual meaning (Gupta *et al.*, 1978).

Since the absorbance of the system is generally small (Strackee, 1970, 1972; Gupta *et al.*, 1978),

$$J_X(\lambda, t) = \alpha_X(\lambda) N_X(t) I_n(\lambda, t) \quad \dots(8)$$

The above equation will be applicable if the experiment is performed on the solution of rhodopsin or on the retina. In living eyes, it will be applicable only if the reflectivity of the sclera is zero or negligible. The initial conditions for solving eqns. (2) to (6) are

$$N_1 = R_0, N_2 = N_3 = N_4 = N_5 = 0, \text{ at } t = 0, \quad \dots(9)$$

where R_0 represents the concentration of rhodopsin when all the molecules are unbleached.

If the spectral distribution of the flash is independent of time t , then

$$I_n(\lambda, t) = f(t) I(\lambda). \quad \dots(10)$$

The analytical solutions of eqns. (2)–(6) cannot be obtained for an arbitrary function $f(t)$. Therefore, for specific cases of $f(t)$ [eqns. (17) and (18)], these equations have been solved numerically using Runge-Kutta method (Scarborough, 1966). If $f(t)$ is assumed to have a constant value f_0 [eqn. (1)], then eqns. (2)–(6) can be solved analytically. The solutions obtained are the following :

$$\begin{aligned} N_1(t) = R_0 e^{-at} + \sum_{i=1}^4 S_i \left[\frac{1}{m_i + a} \left(b + \frac{gK_{A \rightarrow B}}{m_i + z} \right) \left(e^{m_i t} - e^{-at} \right) \right. \\ \left. + \frac{gK_{A \rightarrow B}}{(m_i + z)(z - a)} \left(e^{-zt} - e^{-at} \right) \right] \quad \dots(11) \end{aligned}$$

$$N_2(t) = \sum_{i=1}^4 S_i e^{m_i t} \quad \dots(12)$$

$$N_3(t) = \sum_{i=1}^4 S_i \left[\frac{1}{m_i + d} \left(c + \frac{h K_{A \rightarrow B}}{m_i + z} \right) \left(e^{m_i t} - e^{-dt} \right) + \frac{h K_{A \rightarrow B}}{(m_i + z)(z - d)} \left(e^{-zt} - e^{-dt} \right) \right] \quad \dots(13)$$

$$N_4(t) = K_{A \rightarrow B} \sum_{i=1}^4 \frac{S_i}{m_i + z} \left(e^{m_i t} - e^{-zt} \right) \quad \dots(14)$$

and

$$N_5(t) = K_{A \rightarrow B} K_{B \rightarrow C} \sum_{i=1}^4 \frac{S_i}{m_i + z} \left[(e^{m_i t} - 1)/m_i + (e^{-zt} - 1)/z \right] \quad \dots(15)$$

where

$$S_i = \frac{a R_0 [x_1 + (a + x_2)(x_2 + m_j + m_k + m_l) + m_j m_k + m_j m_l + m_k m_l]}{(m_i - m_j)(m_i - m_k)(m_i - m_l)}$$

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

$$x_1 = a^2 + ab + cd$$

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

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

$$a = \gamma_{R,A} z_R f_0 \quad ; \quad b = \gamma_{A,R} z_A f_0$$

$$c = \gamma_{A,I} z_A f_0 \quad ; \quad d = \gamma_{I,A} z_I f_0$$

$$g = \gamma_{B,R} z_B f_0 \quad ; \quad h = \gamma_{B,I} z_B f_0$$

$$z_X = \int_{\lambda_1}^{\lambda_2} \alpha_X(\lambda) I(\lambda) d\lambda$$

$$m_1, m_2, m_3 \text{ and } m_4 \text{ are the roots of the following equation} \\ m^4 + \alpha m^3 + \beta m^2 + \gamma m + \delta = 0, \quad \dots(16)$$

where

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

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

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

$$\delta = ad K_{A \rightarrow B} K_{B \rightarrow C}$$

The roots of eqn. (16) are the following (Turnbull, 1952) :

$$m_1 = u^+ + v^+ ; \quad m_2 = u^+ - v^+ \\ m_3 = u^- + v^- ; \quad m_4 = u^- - v^-$$

where

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

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

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

$$N = \left(\frac{\alpha\beta}{12} - \frac{\gamma}{2} + \alpha x\right) / 2M,$$

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

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

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

and

$$\omega_2 = (9\alpha\beta\gamma - 27\alpha^2\delta + 72\beta\delta - 2\beta^3 - 27\gamma^2)/432.$$

(iii) *Selection of parameters* — In the present analysis, the calculations have been performed for 2m sec and 20 μ sec flash durations. In the case of 2 m sec flash duration and at physiological temperatures intermediates I and II in the reaction model will represent metarhodopsin I (M_1) and metarhodopsin II (M_2) respectively (Pugh, 1975). If the flash used is such that it does not contain ultraviolet light then the photoreversal from metarhodopsin II will not take place. The photoreversal will take place only from metarhodopsin I.

In the case of 20 μ sec flash duration and at physiological temperatures, intermediates I, II and III in the reaction model will represent lumirhodopsin (L), metarhodopsin I and metarhodopsin II respectively (Gupta *et al.*, 1978). The calculations for $\tau < 20 \mu$ sec, in which the main photoequilibrium intermediate will be prelumirhodopsin, have not been done. This is because for $\tau < 20 \mu$ sec ($\sim 10^{-8}$ sec)* the effect of TDF on the bleaching kinetics will be similar to that in the case of 20 μ sec flash due to the similar reactions. Only the reactants will be different.

The spectral distribution of the flash (i.e., $I(\lambda)$) and the values of λ_1 and λ_2 have been chosen to be those used by Williams and Breil (1968) in their flash photolysis experiments. If we assume that the absorption spectra ($\alpha_X(\lambda)$) of rhodopsin, lumirhodopsin, isorhodopsin and metarhodopsin I are identical to the absorption spectra obtained corresponding to their λ_{\max} from the nomogram (Dartnall, 1953; Ebrey & Honig, 1977) then, using the values of $\alpha_X(\lambda_{\max})$, we obtain

$$\begin{array}{ll} Z_R = 59.139 \eta & ; \\ Z_I = 57.732 \eta & \text{and} \end{array} \quad \begin{array}{l} Z_L = 68.397 \eta; \\ Z_{M_1} = 57.752 \eta, \end{array}$$

*In this case, metarhodopsin I will not form in an appreciable amount and because of this the photoreversal from it can be neglected.

where η is a parameter independent of wavelength and decides the intensity of the flash.

The TDF of 2m sec* and 20 μ sec flash durations have been chosen to be the following :

$$\begin{aligned} f(t) &= 1 - \exp(-t/5.5 \times 10^{-5}) & 0 < t < 0.25 \times 10^{-3} \text{ sec} \\ &= \exp[-(t - 0.25 \times 10^{-3})/0.534 \times 10^{-3}] & t \geq 0.25 \times 10^{-3} \text{ sec} \\ &\text{(for 2 m sec flash)} & \dots \quad (17) \end{aligned}$$

$$\begin{aligned} f(t) &= 1 - \exp(-t/5.5 \times 10^{-7}) & 0 < t < 0.25 \times 10^{-5} \text{ sec} \\ &= \exp[-(t - 0.25 \times 10^{-5})/0.534 \times 10^{-5}] & t \geq 0.25 \times 10^{-5} \text{ sec} \\ &\text{(for 20 } \mu \text{ sec flash)} & \dots \quad (18) \end{aligned}$$

The quantum efficiency of the forward reaction ($\gamma_{R,L}$ or γ_{R,M_1}) has been chosen to be 0.67 (Kropf, 1967; Dartnall, 1968). The other quantum efficiencies estimated relative to this and used in the present analysis are :

$$\gamma_{L,R} = 0.415; \gamma_{L,I} = 0.067; \gamma_{I,L} = 0.167 \quad (\text{Strackee, 1971})$$

$$\gamma = 0.335; \gamma_{M,I} = 0.067; \gamma_{I,M_1} = 0.201 \quad (\text{Hubbard \& Kropf, 1958})$$

The two remaining parameters $K_{L \rightarrow M_1}$ and $K_{M_1 \rightarrow M_2}$ have different values at different temperatures and therefore the results have been obtained for their different values.

RESULTS

(1) *2m Sec Flash Duration*—In this case, A and B will represent metarhodopsin I and metarhodopsin II respectively and $K_{B \rightarrow C} = 0 = \gamma_{B,R} = \gamma_{B,I}$. The variations of the concentrations of the reactants at the end of the flash ($t = \tau = 2$ msec) have been plotted as a function of the number of quanta per sample area per flash** (Q) for $K_{M_1 \rightarrow M_2} = 100 \text{ sec}^{-1}$ and 900 sec^{-1} in Figs. 1(a) and 1(b) respectively. The continuous curves correspond to eqn. (1) while the dotted curves correspond to eqn. (17). It can be seen from these figures that for large values of Q , the results obtained are independent of TDF while the middle portion of the curves depend on it. For small values of Q , the concentrations of rhodopsin and isorhodopsin obtained after the flash are independent of TDF while the concentrations of metarhodopsin I and metarhodopsin II depend on it. Also it can be seen from these figures that if the value of the rate constant $K_{M_1 \rightarrow M_2}$ (or temperature) increases, the dependence of the results on TDF increases.

*This expression approximately matches with the TDF of the flash used by Williams and Breil (1968).

**The number of quanta per sample area per flash is given by

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

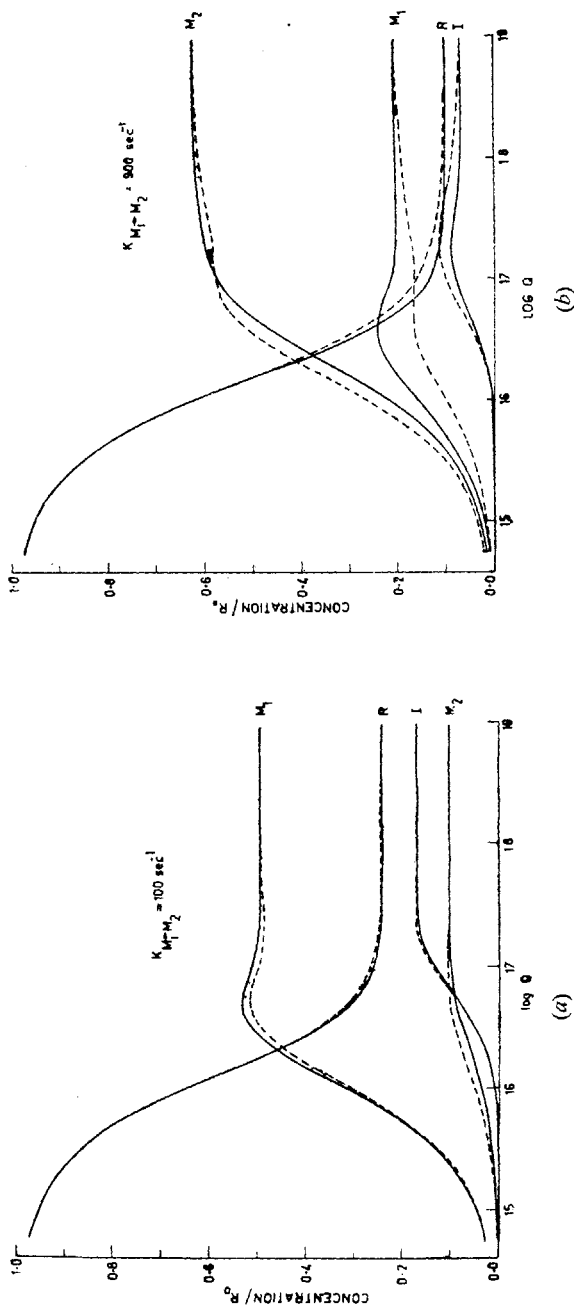


FIG. 1. Variation of the concentrations of rhodopsin (R), metarhodopsin I (M_1), isorhodopsin (I) and metarhodopsin II (M_2) remaining after exposure to 2 m sec flash with the number of quanta per sample area per flash (Q) for (a) $K_{M_1 \rightarrow M_2} = 100 \text{ sec}^{-1}$ and (b) $K_{M_1 \rightarrow M_2} = 900 \text{ sec}^{-1}$. The continuous curves correspond to eqn. (1) while the dotted curves correspond to eqn. (17).

The transient concentrations have been calculated in two ways :

(a) Firstly, the results have been obtained assuming f_0 independent of time. These results have been compared with those obtained by solving kinetic equations numerically using Runge-Kutta method for the TDF given by eqn. (17). The calculations have been done for $K_{M_1 \rightarrow M_2} = 250 \text{ sec}^{-1}$ and $Q = 10^{17}$ and 10^{18} and the results have been shown in Figs. 2(a) and 2(b) respectively. It can be seen from these figures that for large values of Q the variation is independent of TDF. As the value of Q decreases the dependence on TDF increases. The TDF dependence of transient concentrations is more noticeable at higher temperatures (see Fig. 3).

(b) Secondly, the calculations have been done assuming f_0 is given by*

$$f_0 = f_0(t) = \frac{1}{t} \int_0^t f(t') dt' \quad \dots (19)$$

Fig. 4 shows the transient concentration of rhodopsin calculated from eqn. (11) with f_0 given by eqn. (19) for $K_{M_1 \rightarrow M_2} = 10^3 \text{ sec}^{-1}$ and $Q = 10^{16}$, 10^{17} and 10^{18} . These results have been compared with those obtained (i) from eqn. (11) with f_0 independent of time, and (ii) by solving eqn. (2) numerically using actual TDF $f(t)$. It can be seen from this figure that these results agree better with the exact calculation but they still differ with the exact calculation for some values of Q . At higher intensity of the flash, however, the variation of concentrations of the reactants comes out to be the same by all the three methods of calculation. Calculations for other pigments show similar behaviour.

(2) *20 μ Sec Flash Duration* — In this case, A, B and C will represent lumirhodopsin, metarhodopsin I and metarhodopsin II respectively. Similar to the previous case, the concentrations of the reactants at the end of the flash have been plotted as a function of Q for $K_{L \rightarrow M_1} = 10^3 \text{ sec}^{-1}$ and $K_{M_1 \rightarrow M_2} = 50 \text{ sec}^{-1}$ in Fig. 5(a) & 5b and for $K_{L \rightarrow M_1} = 10^6 \text{ sec}^{-1}$ and $K_{M_1 \rightarrow M_2} = 5 \times 10^3 \text{ sec}^{-1}$ in Fig. 5(b)†. Fig. 5(a) shows that for small values of $K_{L \rightarrow M_1}$ and $K_{M_1 \rightarrow M_2}$, the results obtained are independent of TDF for any intensity of the flash.

Fig. 5(b) shows that for large values of $K_{L \rightarrow M_1}$ and $K_{M_1 \rightarrow M_2}$ the concentrations of the lumirhodopsin and metarhodopsin I obtained at the end of the flash depend very much on TDF for the values of Q lying between 10^{16} to 10^{19} for which the calculations have been done. It can also be seen from these figures that the concentration of isorhodopsin does not depend on TDF for any values of Q , $K_{L \rightarrow M_1}$ and $K_{M_1 \rightarrow M_2}$. However, for low intensity, the concentration of rhodopsin does not depend on TDF.

The time variations of the concentrations of the reactants for $K_{L \rightarrow M_1} = 10^6 \text{ sec}^{-1}$ and $K_{M_1 \rightarrow M_2} = 5 \times 10^3 \text{ sec}^{-1}$ have been shown in Fig. 6(a) for $Q = 10^{17}$ and

*In this way, the number of quanta incident on the sample at any time t are the same as in the actual flash of duration t .

†In some of the figures, the concentrations of some of the reactants have not been shown. This is because their concentrations are very small in comparison to that of other reactants.

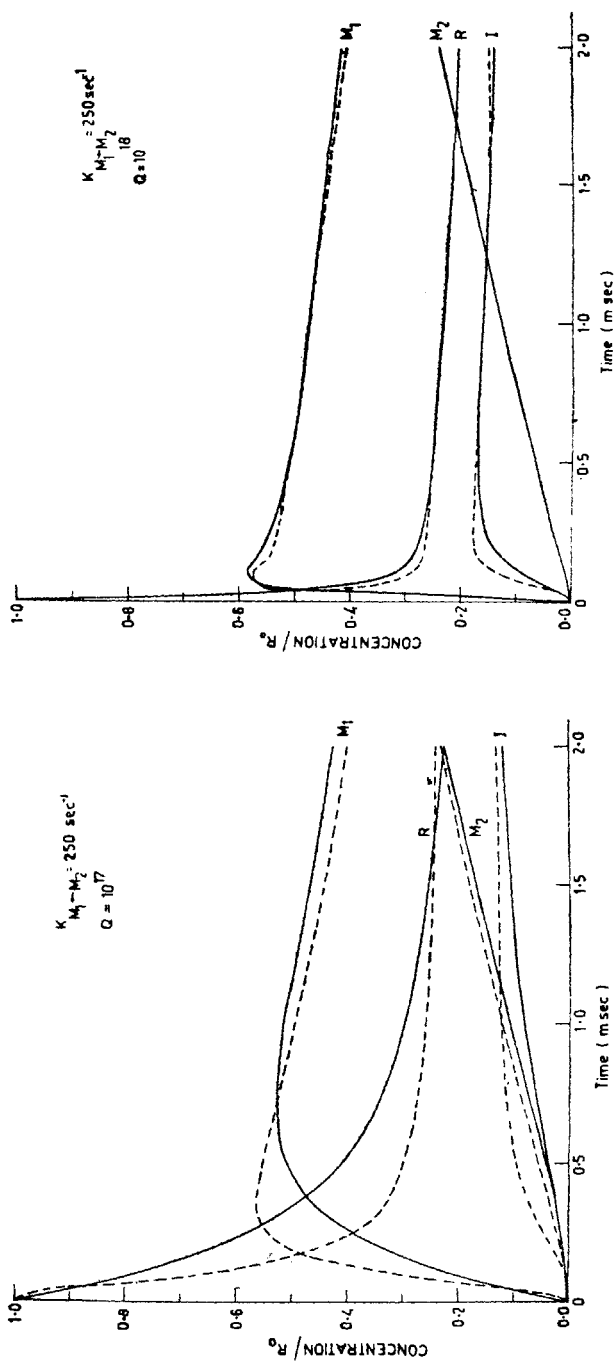


FIG. 2. Time variation of the concentrations of rhodopsin (R), metarhodopsin I (M_1), isorhodopsin (I) and metarhodopsin II (M_2) during the period of the flash for (a) $K_{M_1 \rightarrow M_2} = 250 \text{ sec}^{-1}$ and $Q = 10^{17}$, (b) $K_{M_1 \rightarrow M_2} = 250 \text{ sec}^{-1}$ and $Q = 10^{18}$. The continuous curves correspond to eqn. (1) while the dotted ones to eqn. (17).

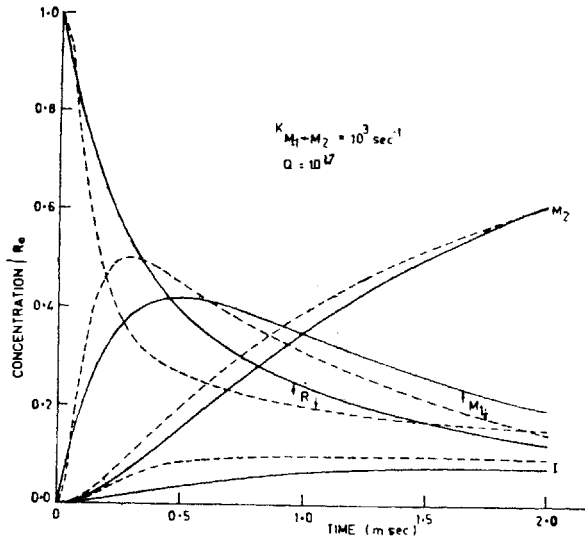


FIG. 3. Time variation of the concentrations of rhodopsin (R), metarhodopsin I (M_1), isorhodopsin (I) and metarhodopsin II (M_2) during the period of the flash for $K_{M_1 \rightarrow M_2} = 10^3 \text{ sec}^{-1}$ and $Q = 10^{17}$. The continuous curves correspond to eqn. (1) while the dotted curves correspond to eqn. (17).

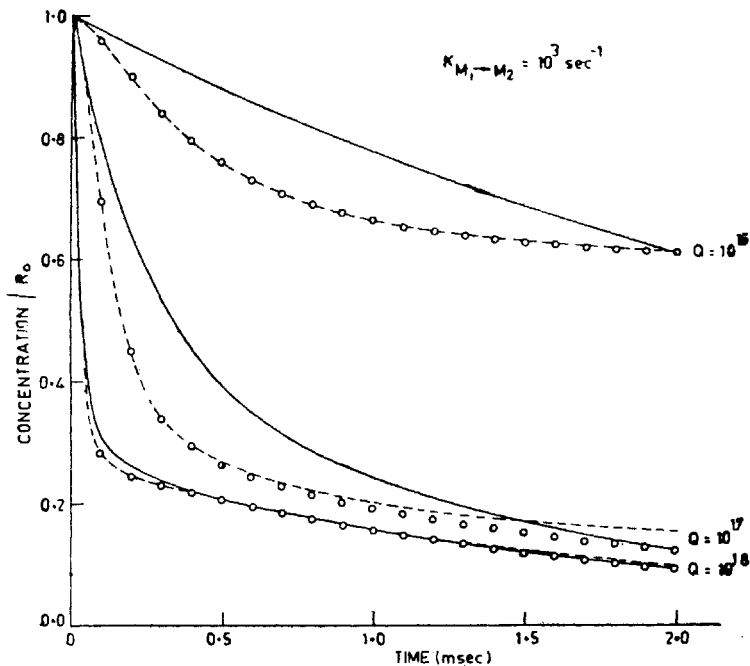
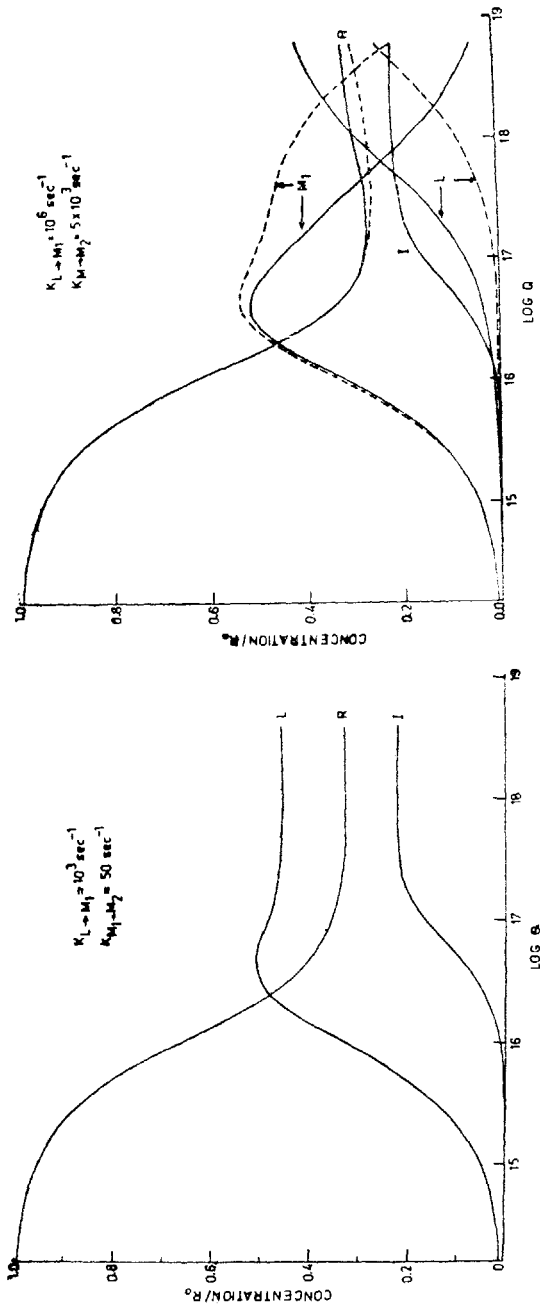


FIG. 4. Time variation of the concentration of rhodopsin during the period of the flash determined in the following three ways for $K_{M_1 \rightarrow M_2} = 10^3 \text{ sec}^{-1}$ and $Q = 10^{16}, 10^{17}$ and 10^{18} : (i) from eqn. (11) assuming f_0 to be a constant during the flash (continuous curves), (ii) from eqn. (11) assuming f_0 to be given by eqn. (19) (circles) and (iii) by solving eqn. (2) numerically using actual TDF (dotted curves).



(a) (b)
 Fig. 5. Variation of the concentrations of rhodopsin (R), lumirhodopsin (L), isorhodopsin (I) and metarhodopsin I (M_1) remaining after exposure to 20μ sec flash with the number of quanta per sample area per flash (O) for (a) $K_{L \rightarrow M_1} = 10^3 \text{ sec}^{-1}$ and $K_{M_1 \rightarrow M_2} = 50 \text{ sec}^{-1}$ and (b) $K_{L \rightarrow M_1} = 10^6 \text{ sec}^{-1}$ and $K_{M_1 \rightarrow M_2} = 5 \times 10^3 \text{ sec}^{-1}$. The continuous curves correspond to eqn. (1) while the dotted curves correspond to eqn. (18).

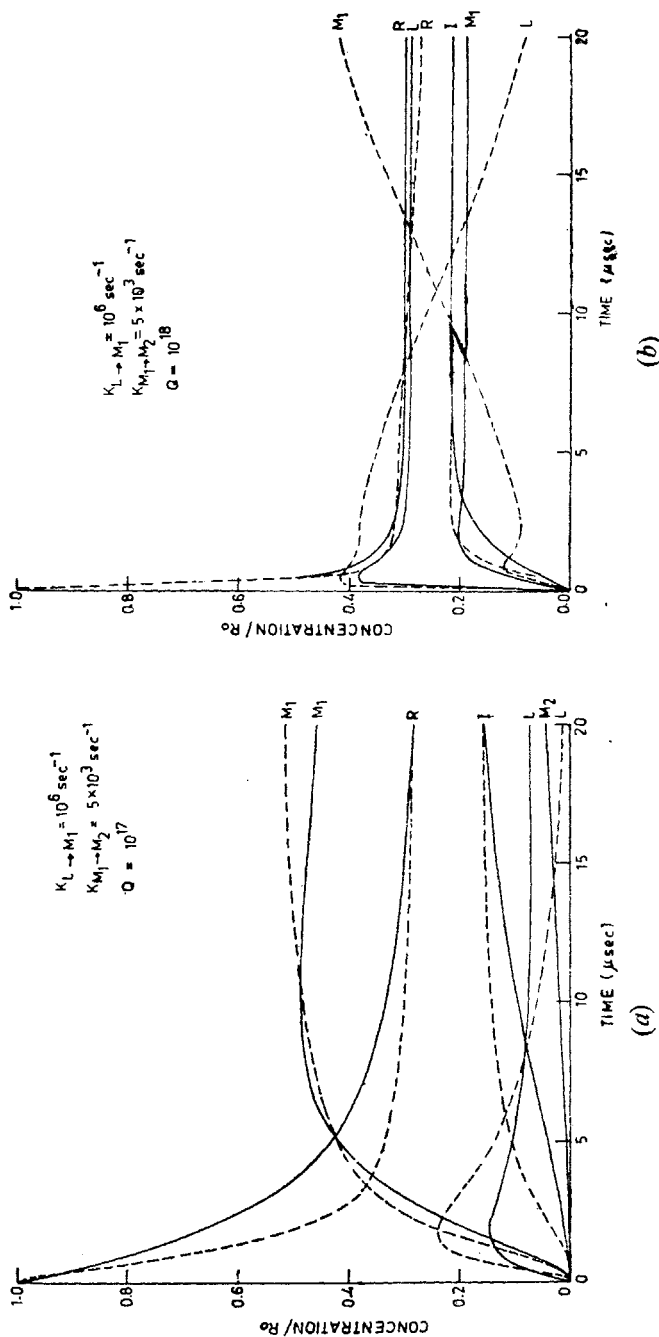


Fig. 6. Time variation of the concentrations of rhodopsin (R), lumirhodopsin (L), isorhodopsin (I), metarhodopsin I (M_1) and metarhodopsin II (M_2) during the period of the flash for $K_L \rightarrow M_1 = 10^6 \text{ sec}^{-1}$, $K_{M_1 \rightarrow M_2} = 5 \times 10^3 \text{ sec}^{-1}$ and (a) $Q = 10^{17}$, (b) $Q = 10^{18}$. The continuous curves correspond to eqn. (1) while the dotted curves correspond to eqn. (18).

in Fig. 6(b) for $Q = 10^{18}$ as in the case of 2 m sec flash. Here also, one can draw similar conclusions as in the case of 2 m sec flash. At higher intensities, unlike the case of 2 m sec flash, the effect of TDF is appreciable in the concentrations of lumirhodopsin and metarhodopsin I although negligible in rhodopsin and isorhodopsin (Fig. 6(b)).

Therefore, to make the analysis of flash photolysis experiments simpler, the following points are necessary :

(1) At low temperatures, the concentrations of the reactants at the end of the flash do not depend on TDF for any value of the peak intensity of the flash (see Figs. 1(a) and 5(a)). As the temperature increases the dependence of the results on TDF increases. At a particular temperature, the dependence on TDF is more for intermediate intensities of the flash.

(2) For low and high intensities of the flash, the concentrations of rhodopsin and isorhodopsin at the end of the flash do not depend on TDF while the concentrations of other reactants depend on TDF. However, if the flash duration is such that the photoreversal takes place mainly from one intermediate then at high intensities of the flash, its concentration at the end of the flash also does not depend on TDF.

(3) Transient concentrations of the reactants, in general, depend on TDF. The transient concentrations of the reactants obtained from the solutions of the kinetic equations after substituting,

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

for TDF are in good agreement with the actual concentrations.

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