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Flash Photolysis of Rhodopsin in Rabbit*

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Abstract. Flash photolysis of rhodopsin in rabbit's retina has been analysed theoretically, and the results are found to be in good agreement with the experimental results of Hagins (1957). We have also obtained the variation of relative concentrations of rhodopsin, lumirhodopsin, isorhodopsin and metarhodopsin I during the period of the flash corresponding to two different intensities of the flash. It has been found that the quantum efficiencies of conversion of lumirhodopsin into rhodopsin and isorhodopsin will lie in the range 0.24–0.45 and 0.20–0.44 respectively; quantum efficiencies of conversion of metarhodopsin I into rhodopsin and isorhodopsin are found to have values greater than 0.52 and 0.45 respectively and the quantum efficiency of conversion of isorhodopsin into lumirhodopsin has been found to be approximately 0.865. Also the maximum value of the rate constant of the reaction metarhodopsin I → metarhodopsin II at 37° C has been determined in decerebrated eye and it has been found that it is of the same order as found by Pugh (1975) in the case of human eye.

Key words: Flash photolysis – Rhodopsin – Quantum efficiency – Rate constant.

Introduction

When a rhodopsin molecule absorbs a photon, photoisomerization of its retinal takes place and it is converted to prelumirhodopsin (Yoshizawa and Wald, 1963; Busch et al., 1972). Prelumirhodopsin further decomposes thermally over a series of intermediates – lumirhodopsin, metarhodopsin I, metarhodopsin II and para rhodopsin – to all-trans-retinal and opsin (Yoshizawa, 1972). The half lives of these intermediates increase from prelumirhodopsin to final photo product. Apart from decaying thermally, these intermediates can undergo photoreversal to form rhodopsin or isorhodopsin. Thus there is a competition between two reactions which are taking place in the presence of light; one is the thermal reaction and the other is the

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photoreversal of the photoproducts. Significant photoreversal can only occur if these photoproducts can absorb sufficient amount of light before they decay. This can only happen if intense flashes of short duration are used to bleach the rhodopsin; a method often called flash photolysis.

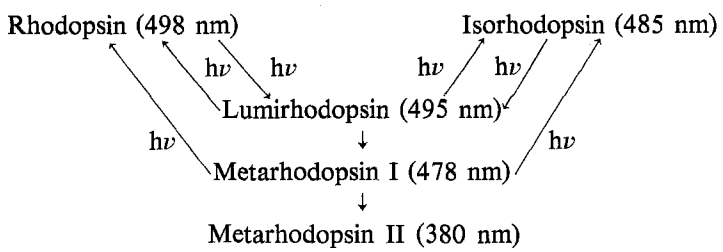
Hagins (1957) observed that if a flash of 20 μs duration falls on rabbit's retina, it can not bleach more than half the rhodopsin molecules; this is independent of the intensity of the flash. Baumann and Ernst (1970) studied the flash photolysis of rhodopsin in case of frog using flashes of 2 ns duration and Pugh (1975) studied flash photolysis of rhodopsin in man using flashes of 600 μs duration.

If the duration of the flash is very small (≈ few nano seconds) then the concentrations of later products formed during the flash will be very small and therefore photoreversal will take place mainly from prelumirhodopsin. As the duration of the flash is increased, earlier products decay and the concentrations of later products increase. In the flash of 600 μs duration, since the half life of prelumirhodopsin and lumirhodopsin is much less than the duration of the flash, they soon decay to later products. However, the half life of metarhodopsin I is large compared to 600 μs therefore only small amount of metarhodopsin II will be formed during the flash. Thus most of the photoreversal takes place from metarhodopsin I. This is the reason why Pugh (1975) in his theoretical analysis of experimental results did not consider prelumirhodopsin and lumirhodopsin in the decay scheme. In Hagins' (1957) flash photolysis experiment, the flash of 20 μs duration is large compared to the half life of prelumirhodopsin but is small compared to the half life of lumirhodopsin, therefore a significant photoreversal will take place from lumirhodopsin.

In this paper, we have made a theoretical analysis of the experimental results of Hagins' flash photolysis experiment. The theoretical results obtained by the adjustment of the quantum efficiencies ($\gamma_{L,R}$, $\gamma_{L,I}$, $\gamma_{I,L}$, $\gamma_{M_1,R}$ and $\gamma_{M_1,I}$) are in good agreement with Hagins' experimental results on variation of relative concentration of unbleached photopigment with the intensity of the flash. However, the quantum efficiencies (except $\gamma_{L,R}$) which we have found out for rabbit's retina are much different from those in case of cattle (Hubbard and Kropf, 1958; Strackee, 1971). Also we have found out that the maximum photosensitivity of isorhodopsin is greater than that of rhodopsin in the case of rabbit and we have determined the value of the rate constant $K_{M_1 \to M_2}$ at 37° C in the case of decerebrated eye.

Theoretical Analysis

We have considered the following reaction model for 20 μs flash bleach:



In this model we have not taken prelumirhodopsin because of its short half life as compared to the duration of the flash. Due to small rate constant of metarhodopsin II, the products (after metarhodopsin II) do not form in an appreciable amount during the flash. Therefore we have also not taken them in the reaction model.

Assuming the conservation of total number of molecules, i.e.

$r(t) + l(t) + i(t) + m_1(t) + m_2(t) = 1,$

the kinetic equations can be written as

$$R_0 \frac{dr}{dt} = -\gamma_{R,\,L} J_R(t) + \gamma_{L,\,R} J_L(t) + \gamma_{M_1,\,R} J_{M_1}(t) \tag{1}$$

$$R_0 \frac{dl}{dt} = \gamma_{R,\,L} J_R(t) - (\gamma_{L,\,R} + \gamma_{L,\,I}) J_L(t) + \gamma_{I,\,L} J_I(t) - K_{L \to M_1} l(t) \tag{2}$$

$$R_0 \frac{di}{dt} = \gamma_{L,\,I} J_L(t) - \gamma_{I,\,L} J_I(t) + \gamma_{M_1,\,I} J_{M_1}(t) \tag{3}$$

$$R_0 \frac{dm_1}{dt} = -(\gamma_{M_1,\,R} + \gamma_{M_1,\,I}) J_{M_1}(t) + K_{L \to M_1} l(t) - K_{M_1 \to M_2} m_1(t) \tag{4}$$

$$R_0 \frac{dm_2}{dt} = K_{M_1 \to M_2} m_1(t) \tag{5}$$

where R_0 is the concentration (in chromophore per cm^3) of rhodopsin molecules when all the molecules are unbleached; $r = R/R_0$, $l = L/R_0$, $i = I/R_0$, $m_1 = M_1/R_0$ and $m_2 = M_2/R_0$ represent the instantaneous relative concentrations of rhodopsin, lumirhodopsin, isorhodopsin, metarhodopsin I and metarhodopsin II respectively during the reaction; R, L, I, M_1 and M_2 represent the concentrations (in chromophore per cm^3) of respective molecules at any instant t; $\gamma_{P,\,Q}$ represents quantum efficiency of conversion of P type of molecule into Q type of molecule, $K_{A \to B}$ represents the rate constant (in s^{-1}) of the reaction A to B and $J_X(t)$ represents the absorption rate of species X at time t (in photons absorbed per cm^3 per s) and is given by

$$J_X(t) = \int_{\lambda_1}^{\lambda_2} J_X(\lambda, t)\, d\lambda \tag{6}$$

where λ_1 to λ_2 is the range of wavelength of bleaching light and $J_X(\lambda, t)\, d\lambda$ represents the number of photons with wavelength lying between λ and $\lambda + d\lambda$ absorbed by X-type of molecules per cm^3 per s.

It can be easily shown that (Hagins, 1957)

$$J_X(\lambda, t) = \frac{\alpha_X(\lambda)\, X(t)\, I_n(\lambda, t)}{H} (1 - e^{-H})\,(1 + be^{-H}) \tag{7}$$

where

$$H = \{\alpha_R(\lambda)\, r(t) + \alpha_L(\lambda)\, l(t) + \alpha_I(\lambda)\, i(t) + \alpha_{M_1}(\lambda)\, m_1(t)\} R_0 d \tag{8}$$

$\alpha_X(\lambda)$ represents the extinction coefficient of the species X at wavelength λ (in cm^2 per chromophore); $I_n(\lambda, t)\, d\lambda$ represents spectral distribution of the intensity of the incident light falling on the retina with wavelength lying between λ and $\lambda + d\lambda$ (in

number of photons per cm^2 per s); b represents the reflectivity of the sclera and d represents the length of the eye receptor containing the photopigment (in cm).

It may be noted that we have not taken metarhodopsin II into consideration in Equation (8); this is due to the fact that the extinction coefficient of metarhodopsin II is zero in the region of wavelength λ_1 to λ_2.

From the results of Hagins' experiment on reflection density at 20° C for $\lambda =$ 516 nm, the value of H is found to be 0.1 at $t = 0$ and it decreases at later times. Further, the initial value of H for $\lambda > 516$ nm would always be less than 0.1 because the extinction coefficient decreases with wavelength for $\lambda > 516$ nm. Since the flash contains wavelength greater than 520 nm, the value of H is always less than 0.1. Therefore we can expand exponential terms in Equation (7) and neglect terms of higher orders; thus

$$J_X(\lambda, t) = \alpha_X(\lambda)\ x(t)\ R_0 I_n(\lambda, t)\ \{(1 + b) - \tfrac{1}{2}(1 + 3b)\ \mathrm{H}\}\,. \tag{9}$$

It is clear from Equation (9) that Equations (1)–(4) are non-linear coupled differential equations. These equations can not be solved analytically. We have solved them numerically by using Runge-Kutta method (Scarborough, 1966) with the following initial conditions: at $t = 0$; $r = 1$; $l = i = m_1 = m_2 = 0$.

Calculations

The photosensitivity of rhodopsin in situ in the rabbit's retina has been reported to be 1.03×10^{-16} cm^2 per chromophore at λ_{max} (Hagins, 1954). Since the quantum efficiency of rhodopsin is close to 0.67 (Dartnall, 1968), the extinction coefficient of rhodopsin in Rabbit's eye at λ_{max} will be 1.537×10^{-16} cm^2 per chromophore. Using this value of α_R (λ_{max}), the extinction coefficient of rhodopsin at $\lambda = 516$ nm can be found from its absorption spectrum (Hagins, 1957) to be 1.45×10^{-16} cm^2 per chromophore and using Equation (8) the value of $R_0\, d$ is found to be 6.89×10^{14} chromophore per cm^2.

In the present analysis, we have assumed that the absorption spectrum of rhodopsin, lumirhodopsin, isorhodopsin and metarhodopsin I are identical in shape; the only difference is in the values of λ_{max} and extinction coefficient at λ_{max}. We have used the following values for extinction coefficients at λ_{max} of various photoproducts:

$$\left.\begin{aligned} \alpha_L(\lambda_{max})/\alpha_R(\lambda_{max}) &= 1.18 \\ \alpha_I(\lambda_{max})/\alpha_R(\lambda_{max}) &= 1.13 \\ \alpha_{M1}(\lambda_{max})/\alpha_R(\lambda_{max}) &= 1.06 \end{aligned}\right\} \begin{array}{l}\text{(Yoshizawa, 1972)} \\ \text{(Hubbard and Kropf, 1958).}\end{array}$$

The absorption spectrum of rhodopsin (Hagins, 1957) can be fitted well by the following simple relation for $\lambda \geqslant Z$:

$$\alpha_X(\lambda) = \alpha_X(\lambda = Z) \exp\left\{-\left(\frac{\lambda - Z}{48}\right)^2\right\}, \tag{10}$$

where $Z = 505$ nm for rhodopsin. The values of Z for lumirhodopsin, isorhodopsin and metarhodopsin I are 502 nm, 492 nm and 485 nm respectively. Similarly, the

variation of intensity of the flash with time can be fitted by the following relations:

$$I_n(\lambda, t) = I_0(\lambda)\left\{1 - \exp\left(-\frac{t}{1.7}\right)\right\} \quad 0 \leqslant t \leqslant 8\ \mu s$$
$$= I_0(\lambda)\exp\left\{-\left(\frac{t-8.0}{10.4}\right)^2\right\} \quad t > 8\ \mu s\,. \tag{11}$$

The spectral distribution of the intensity of the bleaching, i.e. $I_0(\lambda)$ can be approximated by the following relation (Hagins, 1957):

$$I_0(\lambda) = I_0 \quad \lambda \geqslant 520\ nm$$
$$= 0 \quad \lambda < 520\ nm\,. \tag{12}$$

Since $\alpha_X(\lambda)$ decreases very rapidly as λ increases [Eq. (10)]; we may, without committing any appreciable error, replace λ_2 by ∞ in Equation (6); this simplifies our calculation. The value of λ_1 is 520 nm.

The other parameters which will be used in the analysis are the reflectivity of the sclera, the rate constants and quantum efficiencies. The value of the reflectivity of the sclera of the excised eye is nearly 0.41 (Hagins, 1957). Treating the thermal reaction as simple first order process, the rate constant $K_{L \to M_1}$ in solution at −20° C has been reported to be 4.5×10^{-4} s^{-1} and the enthalpy of activation (ΔH) to be 60 kcal/mole (Matthews and Wald; as quoted by Hubbard et al., 1965). The reported values of $K_{M_1 \to M_2}$ for rabbit in situ are 30 s^{-1} at 12° C and 600 s^{-1} at 26° C; the value of ΔH is 37.6 kcal/mole (Abrahamson and Wiesenfeld, 1972). Using Arrhenius equation (Hagins, 1957) and the values of various parameters as given above, we find, $K_{L \to M_1} = 0.20 \times 10^2$ s^{-1} and $K_{M_1 \to M_2} = 5.6$ s^{-1} at 5° C, and $K_{L \to M_1} = 5.23 \times 10^3$ s^{-1} and $K_{M_1 \to M_2} = 1.83 \times 10^2$ s^{-1} at 20° C.

Results of Theoretical Analysis

1. Quantum Efficiencies:

To the knowledge of authors, there are no data available about the concentration of isorhodopsin formed after a flash of 20 μs duration (having saturated intensity)[1] on the rabbit eye in situ. Therefore we have considered different values of the ratio (ϱ) of isorhodopsin to rhodopsin concentrations just after the flash of saturated intensity.

Case I. When no Isorhodopsin Forms (i.e. $\gamma_{L,I} = \gamma_{M_1,I} = \gamma_{I,L} = 0$). In this case, there are two unknown parameters ($\gamma_{L,R}$ and $\gamma_{M_1,R}$). In case of cattle, albino rat and man, the quantum efficiency, $\gamma_{M_1,R}$, has been found to be in the range 0.33–0.67 (Hubbard and Kropf, 1958; Ebrey, 1968; Pugh, 1975). Assuming $\gamma_{M_1,R}$ to lie between 0.33 and 0.67 and solving Equations (1), (2) and (4) at 5° C, we find that a

[1] In flash photolysis experiment the relative concentration of any reactant just after the flash depends upon the intensity of the flash. However, this relative concentration attains a constant value at some intensity of the flash beyond which if the intensity is increased the relative concentration does not change. This intensity is called saturated intensity

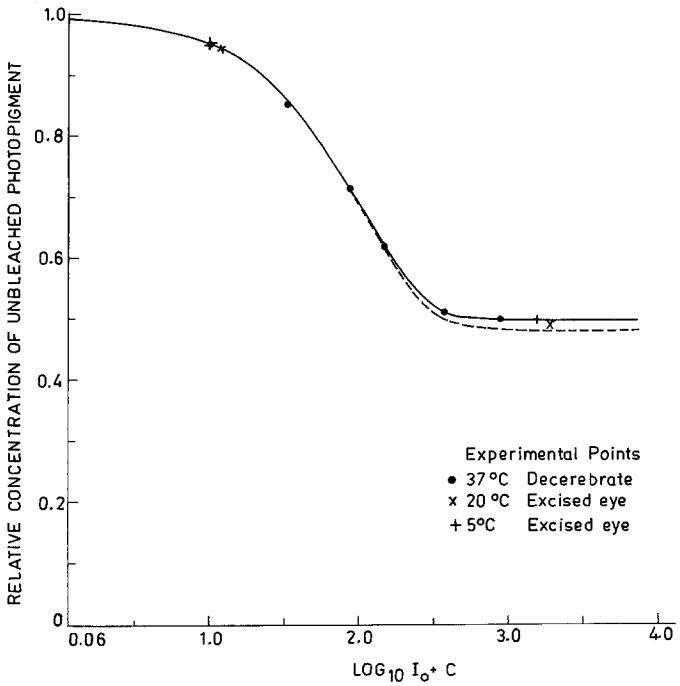


Fig. 1. Variation of relative concentration of unbleached photopigment remaining after exposure to 20 μs flash with the peak intensity (I_0) of the flash. C is a constant and has value 16.94 for excised eye. Curves are theoretical. Dotted curve corresponds to $K_{L \to M1} = 1.48 \times 10^6$ s^{-1} and $K_{M1 \to M2} = 6.29 \times 10^3$ s^{-1}

good fit of our theoretical results with Hagins' data occurs when $\gamma_{L,R} \approx 0.629$ (Fig. 1). Thus at 5° C a change in the value of $\gamma_{M_1,R}$ from 0.33–0.67 does not affect the value of $\gamma_{L,R}$. This is due to the fact that in case of excised eye at 5° C, the rate constant is so small that during 20 μs flash the concentration of metarhodopsin I formed is very small and thus a negligible amount of photoreversal takes place from metarhodopsin I to rhodopsin.

If the temperature of the excised eye is 20° C, then the rate constant $K_{L \to M_1}$ will not be small; therefore, an appreciable amount of metarhodopsin I will form during the flash. Thus taking $\gamma_{L,R} = 0.629$ and rate constants at 20° C we find that for $\gamma_{M_1,R} \approx 1.391$, one obtains a good fit with Hagins' experimental data (Fig. 1). It may be mentioned here that this value of $\gamma_{M_1,R}$ is beyond the range 0.33–0.67 mentioned before, however it has been verified that if $\gamma_{M_1,R}$ is taken to be 1.391 at 5° C, it does not affect the value of $\gamma_{L,R}$.

Case II. When Isorhodopsin Forms (i.e. $\gamma_{L,I} > 0$, $\gamma_{M_1,I} > 0$ and $\gamma_{I,L} > 0$). In the present case when isorhodopsin is also formed the measuring beam of 516 nm [as used in Hagins' (1957) experiment] will be absorbed by rhodopsin as well as isorhodopsin. Therefore, the ordinate in Figure 1 does not represent the relative concentration of rhodopsin as has been assumed in the experiment. A little consideration will show that it should represent $r + i\,[\alpha_I(\lambda = 516\text{ nm})/\alpha_R(\lambda = 516\text{ nm})]$ in which the second term gives the effective concentration of isorhodopsin. As has been done in

the previous case, we have found out the values of $\gamma_{L,R}$, $\gamma_{L,I}$ and $\gamma_{I,L}$ from the experimental data at 5° C and $\gamma_{M_1,R}$ and $\gamma_{M_1,I}$ from the experimental data at 20° C for different values of ϱ (Fig. 1).

The values of the quantum efficiencies so obtained for different values of ϱ show that as ϱ increases $\gamma_{L,R}$ and $\gamma_{M_1,R}$ decrease; $\gamma_{L,I}$ and $\gamma_{M_1,I}$ increase while $\gamma_{I,L}$ remains constant. Since the quantum efficiency can not be greater than unity, only a restricted range of the values of ϱ (0.5–2.1) is allowed and this gives the ranges for various quantum efficiencies. Thus $\gamma_{L,R}$ should lie in the range 0.24–0.45; $\gamma_{L,I}$ should lie in the range 0.20–0.44; $\gamma_{M_1,R}$ and $\gamma_{M_1,I}$ should have values greater than 0.52 and 0.45 respectively and $\gamma_{I,L}$ has the value 0.865. In the case of cattle, the reported values of $\gamma_{L,R}$, $\gamma_{L,I}$ and $\gamma_{I,L}$ are 0.38, 0.07 and 0.17 respectively (Strackee, 1971) and that of $\gamma_{M1,R}$ and $\gamma_{M1,I}$ are 0.33 and 0.07 respectively (Hubbard and Kropf, 1958). Thus the quantum efficiencies in the case of rabbit are quite different (except $\gamma_{L,R}$) from those in the case of cattle.

2. *Rate Constants*

We have also calculated the rate constant $K_{M_1 \to M_2}$ for the decerebrated eye at 37° C using Hagins' (1957) experimental data and assuming that the quantum efficiencies are same as those in the excised eye. Further, if we assume that the rate constants in decerebrated eye are also the same as those in the excised eye, then at 37° C we will have

$$\begin{aligned} K_{L \to M_1} &= 1.48 \times 10^6 \text{ s}^{-1} \\ K_{M_1 \to M_2} &= 6.29 \times 10^3 \text{ s}^{-1} \end{aligned} \tag{13}$$

However, with these values of rate constants, it is found that the theoretical curve deviates much from the experimental data (see dotted curve in Fig. 1). Therefore, we adjust the two rate constants in such a way that experimental data are best explained. Since there are two adjustable parameters, $K_{L \to M_1}$ and $K_{M_1 \to M_2}$, therefore we have chosen $K_{L \to M_1}$ arbitrarily and obtained the corresponding value of $K_{M_1 \to M_2}$ for best fitting (Fig. 1). The values so obtained are given in Table 1. It can be seen that $K_{M_1 \to M_2}$ is much different from that for an excised eye [see Eq. (13)]. However, it is of the same order as found by Pugh (1975) in the case of human eye. In these calculations for the decerebrated eye, we have taken $\alpha_R(\lambda_{max})$ to be the same as that in the excised eye. However, any change in its value would only

Table 1. Values of $K_{M_1 \to M_2}$ for different values of $K_{L \to M_1}$ at 37° C for good agreement

S. No.	$K_{L \to M_1}$ (s^{-1})	$K_{M_1 \to M_2}$ (s^{-1})
1	1.48×10^6	3×10^2
2	10^6	3.5×10^2
3	5×10^5	4×10^2
4	10^5	6×10^2

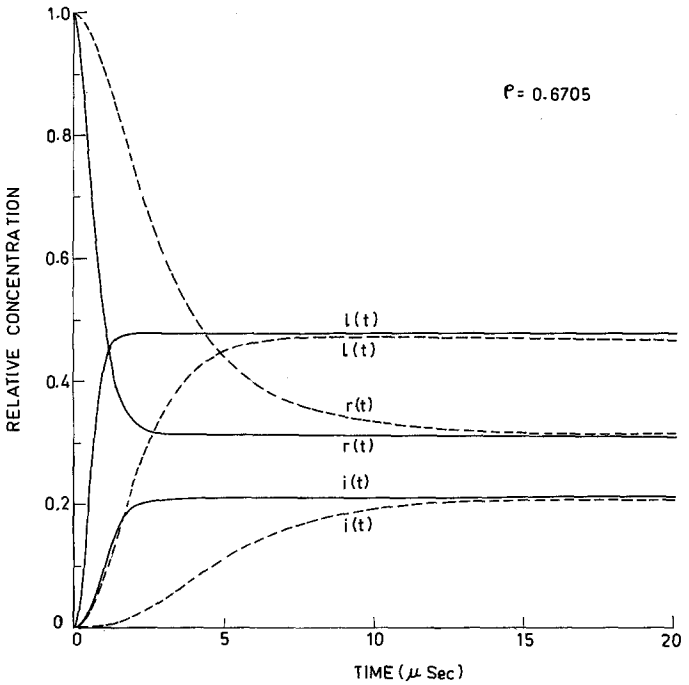


Fig. 2. a Time variation of relative concentrations of rhodopsin, lumirhodopsin and isorhodopsin during the period of the flash for $I_0 = 10^{20}$ (dotted curve) and 10^{21} (continuous curve) photons cm^{-2} s^{-1} for excised eye at 20° C

shift the theoretical curve laterally and would not affect the asymptotic level of bleaching.

We have thus found the ranges for the values of various quantum efficiencies and the value of $K_{M_1 \to M_2}$ at 37° C in decerebrated eye. However, the correct values of the quantum efficiencies can not be ascertained unless we know the value of ϱ. It is therefore suggested that in experiments ϱ should be determined by taking measuring light of two or more different wavelengths between the range 500–600 nm and finding the reflection density at some time after the flash, so that lumirhodopsin and metarhodopsin I would have decayed to metarhodopsin II. This is because in the region 500–600 nm the absorption of measuring light will be mainly due to rhodopsin and isorhodopsin and not due to metarhodopsin II.

The variation of relative concentrations of rhodopsin, lumirhodopsin and isorhodopsin during the reaction for an excised eye at 20° C have been shown in Figure 2a and that of metarhodopsin I in Figure 2b for $I_0 = 10^{20}$ and 10^{21} photons per cm^2/s. It may be seen from Figure 2a that the concentrations of rhodopsin, lumirhodopsin and isorhodopsin saturate earlier if the intensity of the flash is increased. It may further be noted from Figure 2b that the concentration of metarhodopsin I does not saturate during the duration of the flash and the variation of the concentration of metarhodopsin I depends on the value of the intensity of the flash. For $I_0 = 10^{21}$ photons/cm^2 s the relative concentration of metarhodopsin I increases as the duration of the flash having variation of the intensity with time given by Equation (11)

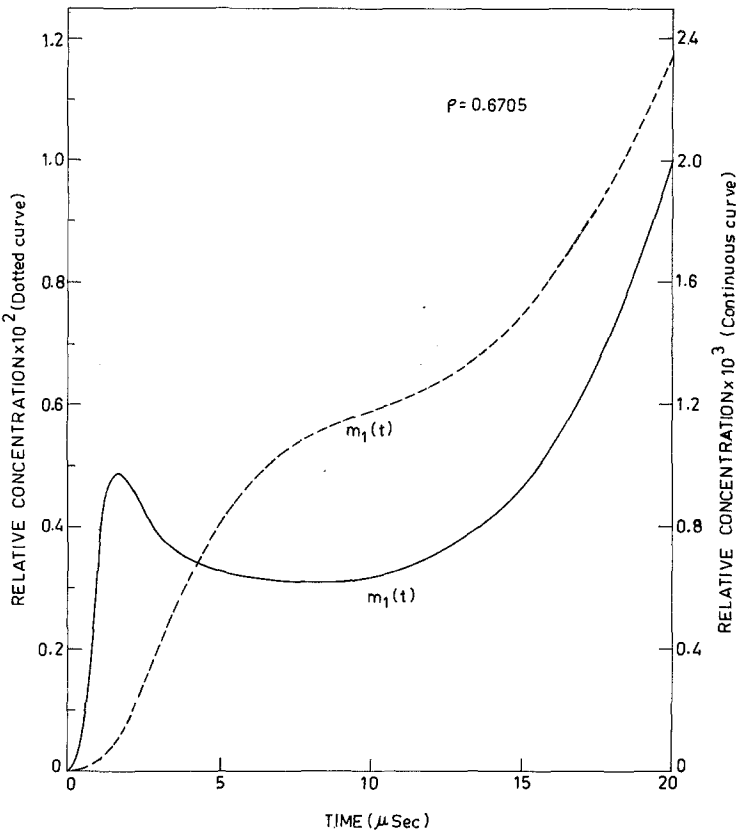


Fig. 2. b Time variation of relative concentration of metarhodopsin I during the period of the flash for $I_0 = 10^{20}$ (dotted curve) and 10^{21} (continuous curve) photons cm^{-2} s^{-1} for excised eye at 20° C

increases. This is due to the conversion of lumirhodopsin into metarhodopsin I by a thermal process. But metarhodopsin I will also absorb some intensity and thus the photoreversal will take place from metarhodopsin I to rhodopsin and isorhodopsin. The photoreversal increases as the intensity is increased. Initially the rate of photoreversal from metarhodopsin I is less than the rate of conversion of lumirhodopsin into metarhodopsin I. But after some duration of the flash, the concentration of lumirhodopsin becomes constant and therefore the rate of conversion of lumirhodopsin into metarhodopsin I becomes constant. But the intensity is still increasing upto its maximum value; therefore, the photoreversal increases and thus the relative concentration of metarhodopsin I decreases upto the time corresponding to maximum intensity. Now if time increases then the intensity decreases and therefore the rate of photoreversal decreases and the relative concentration of metarhodopsin I increases. However, the variation is different for $I_0 = 10^{20}$ photons/cm^2 s. This is because of the fact that the concentration of lumirhodopsin does not saturate before the time corresponding to the maximum intensity of the flash and therefore the rate of conversion of lumirhodopsin into metarhodopsin I remains greater than the rate of photoreversal from metarhodopsin I.

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