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Possible Energy Transfer Mechanism in the Partially Bleached Eye Excised from the Albino Rat

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Abstract

Five different series of kinetic studies on the fast photovoltage (FPV's) from the rhodopsin, intermediates B and C of the excised eye of the albino rat were performed at room temperature. The FPV was decreasing according to a first-order decay scheme when a series of consecutive blue flashes were given to the dark-adapted eye. The apparent decreased fraction of the signal per blue flash was found to be a function of the time interval between two flashes. This anomalous result was explained by a mechanism in which the energy transfer occurs from the electronically excited intermediate B to the unexcited rhodopsin. The anomalous results of other experiments were also explained by this mechanism. Theoretical consideration on the possibility and analysis of the energy transfer were developed.

Introduction

The rhodopsin and three intermediates A, B, and C (hereafter they will be abbreviated as Rh, A, B, and C) in the bleaching process of the eye have been shown to have their individual characteristics (shapes, magnitudes, spectral sensitivities, half-lives, *etc.*) of the fast photovaltages (FPV's) induced by a flash of light.** The magnitudes of those signals, if isolated from each other, were found to be proportional to the quantities of the respective species exposed to the light (Cone, 1965, 1967; Pak and Ebrey, 1966). Several kinetic studies have clarified the bleaching mechanisms and the nature of the intermediates (Ebrey, 1968; Hosoya, 1969). This is because these FPV's are directly related to certain molecular changes in the retina. In this sense each of these electrical signals can be called as a spectrum. On the other hand, some crucial experiments and considerations have been tried to get positive

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** Originally called as the early receptor potential (ERP) (Brown and Murakami, 1964). The nomenclature of FPV is by Hagins and McGaughy (1967). For additional references see the preceding paper (Hosoya, 1969).

or negative evidence for the transfer of energy between different light-absorbing units, *i. e.*, pigment molecules (Hagins and Jennings, 1959; Kropf, 1967; Pak and Helmrich, 1968), in the retinal rod. This idea was suggested and encouraged by the extensive studies on the anomaly of emission spectra of organic molecules in solutions and crystals, in which simple spectral linearity or additivity fails due to the energy transfer between the adjacent molecules.* The present paper reports some kinetic study of FPV from the excised eye of the albino rat with a particular reference to the phenomena in which a simple additivity rule is broken down. Although conclusive evidence for the energy transfer is still lacking, this idea is one of the most possible candidates for the explanation of the anomalous experimental results obtained. Theoretical background for the energy transfer in the retinal rod is discussed and analysis for interpreting the experimental results is also developed.

Experimental

Eyes were excised from the dark-adapted albino rat (Sprague-Dawley, male, about 150 g), and mounted in between two wick electrodes, one on the cornea and the other on the retina. Measurements were performed 10 min after the excision lest the wave forms of the signals should be distorted by the *a* and *b* waves of the ERG. In order to suppress the artefact the electrodes were prevented from being exposed to the flash light. The details of the equipments are described elsewhere (Ebrey, 1968; Hosoya, 1969). The room temperature was kept at about $23° \pm 1°C$.

For the sake of clarity and simplicity, let us adopt simple notations for several different types of operations as in the previous paper.

Bleach: The operation *Bleach* means that a strong continuous 100 W tungsten light was focussed into an exised eye in order to bleach all of the Rh. The wavelengths of the light above 900 and below 480 nm were cut off with Corning CS 1-69 and CS 3-71 filters in order to prevent the temperature from rising and to prevent B from absorbing the light to decompose. All of the Rh was bleached at least by a continuous illumination for 15 sec. By use of various combinations of Wratten neutral density filters the ratio of the bleached Rh to the total number of Rh exposed to the light can be changed. This operation is denoted as *Partial bleach.*

Blue: The operation *Blue* means that a 0.5 msec blue test flash was directed into the eye with a Honeywell Strobonar 600 electronic flash, a Corning CS 7-51 narrow band-path filter (365 ± 30 nm) and the diffuser.

* For the references see the reviews by Förster (1960) and by Streyer (1960).

An electrical response was recorded across the eye.

Green: The operation *Green* is the same as *Blue* except a green filter (Kodak 58B, 530±30 nm) instead of a blue filter.

Yellow: This operation gives almost the same effect as the operation *Green*. A Corning CS 3-71 yellow filter was used (>480 nm).

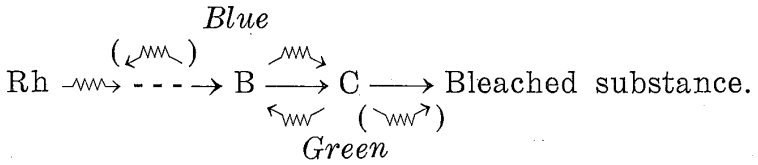
Δt: The operation *Δt* means to wait for a certain length of time *t* between two operations.

Bracket: A pair of solid brackets means to repeat the processes or operations in them several times.

Underline: An underline means that several series of experiments were done by changing the underlined operation.

Premises

The following facts are known and important for all the discussions in this paper (Hagins, 1956, 1957; Matthews *et al.*, 1963-1964; Cone, 1965, 1967; Ebrey, 1968; Hosoya, 1969). At room temperature a Rh molecule, when it absorbs light, changes into B through prelumi-rhodopsin, lumi-rhodopsin, and A, at the latest, 1 msec after absorbing the light; the green 500 nm light being the most effective. The absorption peak of B is at 380 nm. It thermally decomposes into C with a half-life of about 2 min at room temperature. This C has an absorption peak at about 465 nm and its estimated half-life is an hour or so at room temperature. A blue light predominantly photo-decomposes B into C, while very small portion of B is photo-regenerated back to Rh. A green or yellow light on C predominantly photo-regenerates B, while very few portion of C is photo-decomposed into the bleached substance, *i. e.*, all-trans-retinal and opsin. The scheme is illustrated in the following diagram:*



At room temperature an operation *Blue* on Rh and B, respectively, gives a biphasic FPV (cornea-negative R2 and positive R1 components) and a cornea-positive B-signal, whereas C responds very weakly to *Blue* (by a factor of about 0.1). Since the R2 component overwhelms the R1 at room temperature, hereafter a special attention is focussed on R2. With the apparatus used in this experiment, both the maximum amplitudes of the R2 from an unbleached eye and of the B-signal from

* Straight arrows and wavy arrows, represent thermal and photo-chemical reactions. Wavy lines in the brackets mean that the reaction is of minor importance.

a bleached eye are about 50 μV.* A single operation of *Blue* bleaches about 10 per cent of Rh.** The operation of *Green* or *Yellow* on Rh and C, respectively give FPV (the same shape as in the case of *Blue*) and a cornea-negative C-signal, whereas almost no response comes out from B.*** With the apparatus used in this experiment, the maximum amplitudes of the R2 and the C-signal responding to the green flash are, respectively, 500 and 50 μV.** The above-mentioned knowledges are illustrated in Fig. 1.

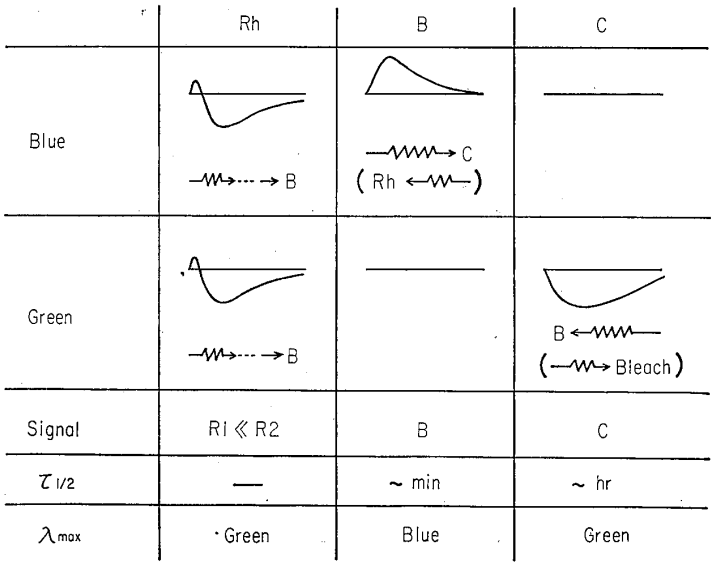


Fig. 1. Illustrations of several characteristics of Rh, B, and C of the albino rat eye and their photoelectric responses and reactions. Signal shapes are schematic. See also the footnote 3. Explanations for the items in the first column are: Blue (Green), photoelectric responses to and reactions by a blue (green) flash; Signal, the names of those signals and components; $\tau_{1/2}$, half-lives of the intermediates B and C in the dark at room temperature; λ_{max}, the most sensitive wavelength regions of the species to give the signals.

Results and Discussion

The experiments are classified into "blue-flash" and "green (or yellow-) flash" experiments. Hopefully, they are complementary to each other, since B is sensitive to a blue flash and C to a green flash.

* At room temperature almost 80 per cent of the Rh is changed into and still remaining as B even 45 sec after a continuous exposure (15 sec) to a strong yellow light whose wavelengths are longer than 480 nm. No photo-chemical reaction occurs in or from B.

** Judging from the figure the efficiency of the present apparatus (the ratio of the magnitude of the signal to the energy of the flash light) seems to be worse than the apparatuses used by the other authors (Cone, 1065, 1967; Ebrey, 1968).

*** At room temperature more than 90 per cent of Rh is changed into and remaining as C, 10 min after the exposure to a strong continuous light. See also the footnote *.

However, more reliance and emphasis is put on the former class of experiments from the following two reasons. i) The signal responding to a green flash seems to be changing in its shape (see, for example, Fig. 7), whereas a blue flash gives a stable signal for a fairly long period of time, and ii) The fact that a green flash on C regenerates B makes the overall mechanisms terribly complicated.

Blue flash experiments

Experiment i) [*Blue* $\underline{\Delta t}$].* If a series of consecutive blue flashes were given to a dark-adapted eye with a constant time interval t, a normal biphasic FPV was decreasing monotonously. The signal shape (R1 and R2) was unchanged during the experiment, although a slight mixing of the B-signal to the normal FPV is expected to change the signal shape especially in its nodal part (see, for example, Fig. 3). A plot of the log of the maximum intensity of each signal versus time (or the number of flashing) gave a straight line, indicating this is a first order decay. From the slope of this line an apparent decreased fraction α of the R2 per blue flash was determined. The time interval t was changed from 20 sec to 4 min and the observed α's are shown in Fig. 2. As far as the experiment could be done there seem to exist upper and lower limits for the value of α.** Between these two limits

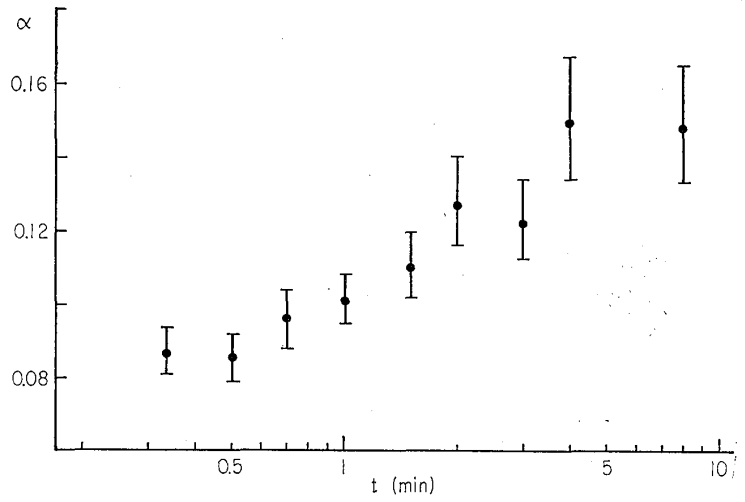


Fig. 2. The apparent fraction α of the R2 component of the FPV from the dark-adapted eye of the albino rat observed with a series of blue flashes with a time interval of t. *Experiment i.*

* Arden *et al.* (1966) reported a change of the FPV value by a series of successive white flashes. However, their test flash is so strong that about 70 per cent of Rh is bleached by a single flash. When the FPV is weakened to about one-tenth of this original value, the C-signal appears, which in turn is decomposing more slowly. This is actually observed by them as in their Fig. 2.

** The existence of the lower limit excludes the possibility of incomplete charge-up of the strobonar for shorter time interval experiments.

α is increasing with the increase of t. This is indeed a strange result, if the value α exactly represents the fraction of the Rh bleached by a single blue flash. Namely, i) if a blue flash gives only a normal FPV and no other reaction is involved, the value of α is independent of t. ii) If the surviving B gives the B-signal responding to a blue flash, α will be larger for smaller t experiments, because of the opposite polarity of the signals B and R2. On the other hand, the photo-regeneration of Rh from B may account for the slower decay for the smaller t experiments, if this regeneration is large enough. However, this possibility was rejected by the following preliminary experiment. That is, with the present equipments, less than a few per cent of Rh can be regenerated by a blue flash from the system in which B is predominant. Note the difference in the several condition from what were used by Cone (1967) to show the photo-regeneration of Rh from B. Thus all these three mechanisms cannot explain the results obtained.

At room temperature B has a half-life of about 2 min, which just corresponds to the value of t at which the plot in Fig. 2 shows the sharpest change. This gives one an attractive idea that before B decays into C the energy is transferred from a photo-excited B to one of the unexcited Rh's surrounding the excited B. In the Theoretical section argument on the possibility of this energy transfer mechanism will be given. In this mechanism B is a so-called sensitizer and Rh an acceptor. In other words, in a partially bleached eye the sensitivity of Rh to a blue light is enhanced by the surrounding B, which absorbs more efficiency the blue light than Rh and give that energy to one of the neighboring unexcited Rh's. The efficiency of the energy transfer is dependent on the concentrations of B and unexcited Rh. If the ratio of Rh and B can be changed at will, several experiments can be designed to give conclusive evidence for the energy transfer, as have been successful in many spectroscopic studies. The next experiment was tried along this line.

Experiment ii) *Partial bleach Blue.* We can change the ratio of Rh and B in the retina by a continuous illumination (15 sec) of the yellow light with different intensity by use of various combinations of neutral density filters. Shortly after this operation (45 sec) an electrical signal responding to a blue flash was measured. Depending on the degree of the bleaching the resultant signal shape is gradually changing from the normal R1-R2 signal to the B-signal as in Fig. 3. The signals in the intermediate stage were turned out to be the mixing of these components. If we fix the value of the abscissa on the oscilloscope, say 2 msec after the trigger, the magnitude of the signal S is increasing with the optical density D of the neutral density filter inserted in front of the bleaching light. However, since one eye can

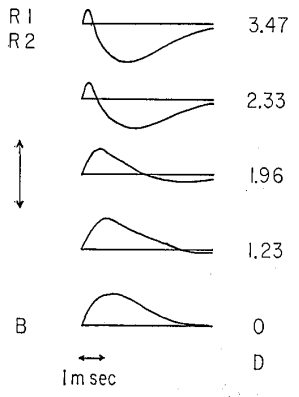


Fig. 3. The signal shapes from partially bleached eye of the albino rat responding to a blue flash. The scales of the signal intensities, which are about 50 μV, are not given, since they are obtained from different eyes. The D values in the figure are the optical densities of the neutral density filters inserted between the eye and the continuous yellow light for bleaching. *Experiment ii.*

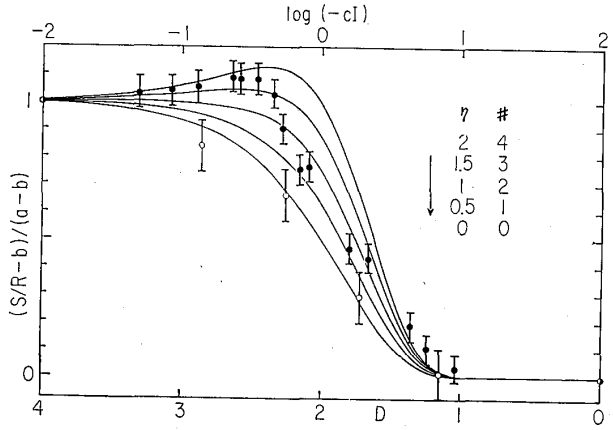


Fig. 4. The results showing the energy transfer between B and Rh. Five curves (# 0–4) are the plots of Equation (6) with different values of η, showing the predicted relation between the signal intensity and the bleaching light intensity. Read the top abscissa. The filled circles are the observed values of $(S/R-b)/(a-b)$ from the partially bleached eye of the albino rat responding to a blue flash; $a=0.7$, $b=-0.7$. S is obtained with a blue flash 45 sec after the partial bleaching, while R is the reference R2 value from the same eye obtained with the same blue flash 10 min before the bleaching. The eye was bleached by a continuous 15 sec illumination of a yellow light with a different combination of neutral density filters, with the optical density of D. Read the bottom abscissa. The coincidence of the top and bottom scales at the value of one is accidental. The open circles were obtained similarly to the filled circles except that the second test blue flash was given 10 min after the partial bleaching, $a=0.7$, $b=-0.2$. Both the points at the far right and left represent filled and open circles. All the values R and S are the readings on the oscilloscope at 2 msec after the trigger. *Experiments ii* and *iii.*

be used only for one measurement, S should be normalized. The filled circles in Fig. 4 are the plots of the values of S/R versus D, where R is the magnitude of the reference R2 signal (taken at 2 msec after the trigger), which was measured with the same blue flash 10 min before the partial bleaching. The length of the time 10 min was chosen to suppress the contaminating effect of B responding to the test blue flash. Actually in Fig. 4 the ordinate is regraduated from 0 to 1 in order to compare with the theoretical values (see Theoretical). It should be noted that several points are above the left asymptote of the curve 0 and that the observed curve abruptly begins to fall off at $D=2.5$ and reaches the bottom within one and a half unit change of D.

As will be shown in the Theoretical part the plot should give a sigmoid curve like $x=\exp(-cI)$ as the curve 0 in Fig. 4 (Equation (4)), if there is no energy transfer between the pigment molecules. The

region of the critical change has about two and a half log units of the abscissa. Since the constant c is unknown, a parallel transformation of the curve along the abscissa was tried so that the best coincidence with the observed points is attained. However, the observed points seem to deviate appreciably from the curve 0 but rather to follow the curve 2 or 3. The group of curves 1-4 in Fig. 4 were calculated from Equation (6) on the basis of the energy transfer, where the efficiency of the energy transfer is increasing in this order (see Theoretical). A reference experiments are needed to know to what extent the curve 0 is followed by the observed points which are obtained under such a condition that the energy transfer is not expected. The following two *Experiments iii* and *iv* were designed along this line.

Experiment iii) Partial bleach *Δt Blue*. This is different from *Experiment ii* only in that the operation *Blue* is delayed by 10 min after the partial bleaching. During this period of time more than 90 per cent of B is supposed to decay into C or bleached substance, neither of which responds to the blue light used. In this experiment the expected signal is almost the R2 component, whose magnitude is proportional to the amount of the remaining Rh, and therefore the energy transfer is not expected. The open circles in Fig. 4 are the plots of the S/R versus D followed by a re-normalization. The values S and R have the same meanings as in *Experiment ii*, but they are averaged values over several measurements. Since the signal shape is sensitive to the temperature change and it was difficult to maintain a constant temperature for a long period of time, the observed S/R values are more erroneous than those in *Experiment ii*. However, they seem to fit the curve 0 rather well. This in turn supports the statement that something anomalous is happening in *Experiment ii*.

Green (or Yellow) flash experiments

In the next two experiments, a yellow or green flash was used instead of a blue flash as in *Experiments i-iii*. Originally *Experiments iv* and *v* were performed in order to get supporting evidence for the mechanism that the energy absorbed by B is transferred to Rh. However, it was found that *Experiment iv* really supports this mechanism, while *Experiment v* does not. In the last part of this section the interrelationships among all of the five experiments in this paper will be discussed.

Experiment iv) Partial bleach *Yellow*. This experiment differs from *Experiment ii* only in that a yellow test flash was given to a partially bleached eye instead of a blue flash, a pure FPV signal being recorded. Since the yellow light is not absorbed by B, the magnitude of the R2 signal, S, responding to this yellow flash should be proportional to the

amount of the unbleached Rh remaining after the partial bleaching. The filled circles in Fig. 5 are the plots of the observed quantity $(S/R-b)/(a-b)$, where R is the value of the R2 signal observed with a blue flash 10 min before the partial bleaching. Both the values S and R are the readings on the oscilloscope at 2 msec after the trigger. These points were found to follow very closely the curve of Equation (4). This result, together with that of *Experiment iii*, shows that the simple exponential relation (4) is observed for the case where energy transfer is not expected and that the deviation of the points of *Experiment ii* from the curve 0, or Equation (4), is much larger than the experimental error and therefore is meaningful.

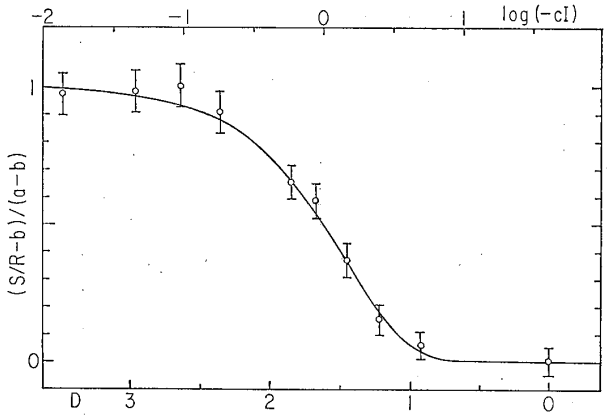


Fig. 5. A reference experiment to the result of Fig. 4. The values of S (R2 component) were obtained with a yellow flash on a partially bleached eye of the albino rat 45 sec after the bleaching. R is the reference R2 value taken from the same eye with a blue flash 10 min before the bleaching. The bleaching was done just in the same way as the experiment in Fig. 4. All the values R and S are the readings on the oscilloscope at 2 msec after the trigger and a and b are, respectively, 13.0 and 0. Read the bottom abscissa. The curve is drawn according to Equation (4) where no energy transfer mechanism is involved. Read the top abscissa. *Experiment iv*.

Results similar to *Experiment iv* were obtained from the experiment in which a green test flash was used instead of the yellow flash.

Experiment v) [*Green* $\underline{\Delta t}$]. By flashing consecutively green test flashes with a constant time interval t, the size of the FPV signal is decreasing exponentially. From the slope of the plots, log of the maximum amplitude of the R2 component versus time, a fraction α is obtained by which the FPV signal is decreasing. A series of experiments were performed with different values of t from 30 sec to 4 min. Similarly to *Experiment i*, the value α is changing with t as in Fig. 6.

This experiment was originally designed to provide a reference to *Experiment i*, since a series of green flashes instead of blue flashes was thought not to give B a chance to absorb light but induces only Rh excitation as long as the resultant C is much fewer than Rh. However, the shape of the FPV signal was found to be changing appreciably by a successive flashing of the green light. The longer the time interval t, the larger the change is. The result of the experiment with $t=8$ min is shown in Fig. 7 but excluded from the plot in Fig. 6. Thus the effect of the C-signal is shown not to be negligible.

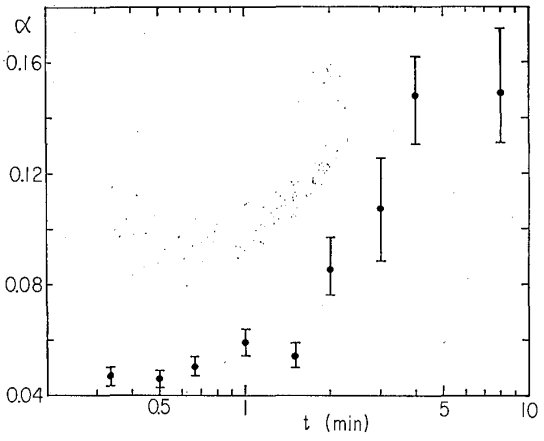


Fig. 6. The apparent fraction α of the R2 component of the FPV from the dark adapted albino rat eye observed with a series of green flashes with a time interval of t. *Experimeut v.*

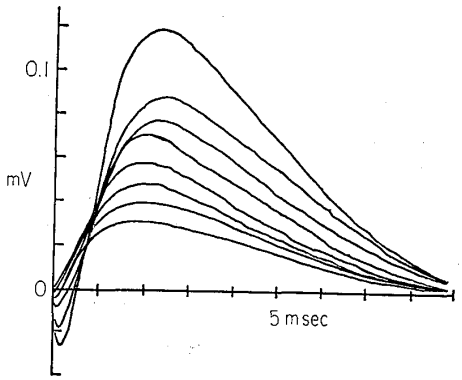


Fig. 7. The change of the FPV signal of the dark adapted albino rat eye responding to a series of successive green flashes. The time interval is 8 min. Note that a biphasic signal is decreasing its intensity and losing the R1 component. *Experiment v.*

Further the C-signal itself was also found to be changing the shape slowly. A kinetic analysis would be terribly complicated from several reasons, *e. g.*, photo-regeneration of B from C (Cone, 1867 ; Hosoya, 1969), a possible energy transfer from C to Rh, *etc.* Therefore further discussion or conjecture on this experiment is pending.

Relations among the experiments and other possible mechanisms

Let us summarize the interrelationships among these five experiments. *Experiments iii* and *iv* support the statement that the energy transfer from B to Rh can explain the anomalous result of *Experiment ii.* On the other hand, if we discard the result of *Experiment v* because of the tangling of so many complicated factors, the results of *Experiment i* are in favor of the energy transfer mechanism from B to Rh.

However, there is another possibility that both the results of *Experiments i* and *v* might be explained by the following mechanism. Suppose that the efficiency of the electrical signals, the ratio of the magnitude of the signal to the number of the pigments excited, is a function of ordering of the pigments. Recall the following two facts in the Premises. i) A blue flash induces almost the same magnitudes of the R2 component and the B-signal from the eyes in which Rh and B are, respectively, predominant in spite of the fact that B is far more sensitive to a blue light than Rh. ii) Although Rh and C have similar sensitivities to a green light, the maximum amplitude of the R2 component is by far larger than the C-signal responding to the same green flash. It is highly possible that a dark-adapted eye has a structure of ordered Rh, whereas in the eye where B or C is predominant the

pigments are disordered to a considerable extent. Then the larger the time interval t between two flashes, the more the ordered structure of the pigments is lost and therefore the decreased fraction α of the signal per flash increases with t. It might also be inferred that an unknown mechanism of adaptation is responsible to the anomalous results of *Experiments* i and v.

Theoretical

First in this section a possibility of the energy transfer in the rod outer segment is discussed from theoretical view point, secondly it is predicted how the energy transfer breaks the simple relation between the fraction of the bleached Rh versus the bleaching light intensity, and finally it is shown that this relation also holds in the case where Rh's are clustered into small group (multimer) and therefore the inspection of the observed relation of these two quantities cannot differentiate the number of Rh's, if ever, in each cluster.

Calculation of the critical distance of the energy transfer

Let us first examine the possibility of the energy transfer in the rod with the theory of resonance transfer by Förster (1948, 1960). The critical distance R_0 is determined by the following equation,

$$R_0 = \sqrt[6]{\frac{9\times10^6(\ln 10)^2\kappa^2 c\tau_S J}{16\pi^4 n^2 N^2 \nu_0^2}}$$

at which an electronically excited sensitizer S has a 50-50 chance of giving its energy to an acceptor A. J is an overlap integral between the emission spectrum $\varepsilon_A(\nu)$ of A and the absorption spectrum $f_S(\nu)$ of S in the wave number scale and is approximated as in the far-right side of the following expression, where ν_0 is the wave number of the 0-0 band of the spectrum of S.

$$J=\int_0^\infty f_S(\nu)\varepsilon_A(\nu)d\nu=\int_0^\infty \varepsilon_S(2\nu_0-\nu)\varepsilon_A(\nu)d\nu$$

The quantity κ is an angular factor depending on the relative orientation of the transition dipoles of S and A and the average over a random orientation yields $\kappa^2=2/3$. The fluorescence life-time of S in the medium concerned (with a refractive index of n) is τ_S, which is the product of the quantum yield η_S of the emission and the natural radiative life-time τ_S^0. If the emission is *via* a singlet excited state of S (*i.e.*, fluorescence), τ_S^0 is in the order of nanoseconds. The universal constants c and N are, respectively, the velocity of light in cm sec^{-1} and the Avogadro's number. If n is assumed to be 1.3, R_0 is turned out to be

$$R_0 = \sqrt[6]{\frac{10^{15}\tau_S J}{\nu_0^2}} \quad \text{(in Å)}.$$

Hagins and Jennings (1859) evaluated the R_0 value for a pair of excited and unexcited Rh's to be 19Å which is much smaller than the mean distance between two neighboring Rh's (55Å in the rabbit rod) even if η_S was assumed to be unity. Their conclusion is that energy transfer among Rh's is unlikely to occur. This small probability of the energy transfer is mainly due to the very small value of the integral J between the absorption and fluorescence spectrum of Rh.* On the other hand the fact that the intermediate B absorbs light maximumly at about 380 nm gives a reasonable conjecture that the fluorescence spectrum of B and the absorption spectrum of Rh almost coincide as shown in Fig. 8. A rough estimate of this overlap integral J (for a pair of B*-Rh) is larger than the case of Rh*-Rh by a factor of 10^3, giving R_0 almost as large as the mean distance between two neighboring pigment molecules. If τ_S^0 is assumed to be one nanosecond and ν_0 is taken as $5\times10^4\,\mathrm{cm}^{-1}$, the values of R_0's are calculated as 43, 29, and 20Å, respectively, for the values of $\eta_S = 1.0$, 0.1, and 0.01.** In the rat rod the average distance between two Rh's is estimated to be about 50Å as are the cases for the rabbit (Hagins and Jennings, 1959), cattle and frog (Wolken, 1963). Several intermediates in the bleaching process might be less rigidly bound in the bed of the opsin and might have a chance to get closer to other pigment molecules. Therefore it is very probable that energy absorbed by B

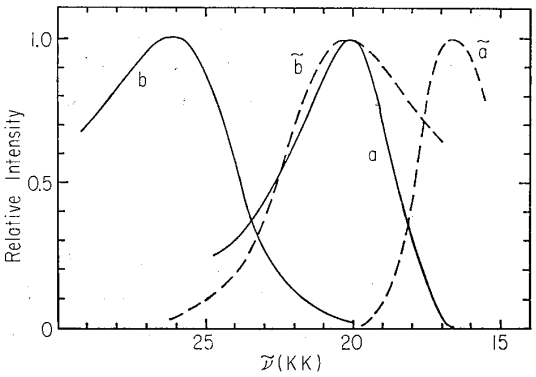


Fig. 8. Relations between the absorption and fluorescence spectra of Rh and B. Intensities are normalized to unity. a: Absorption spectrum of Rh of the ox (Collins, F.D., Love, R.M. and Morton, R.A. (1952). *Biochem. J.* 51, 292.). ã: Fluorescence spectrum of Rh of the cattle (Guzzo and Pool, 1968). b: Absorption spectrum of B of the cattle (Matthews *et al.*, 1963-1964). b̃: Mirror image of b, the Stokes shift being assumed as 6000 cm^{-1}. No wavelength correction is made. Both the maximum molar extinction coefficients of the Rh and B absorptions are estimated to be 42000 by Matthews *et al.* (1963-1964).

* Since no fluorescence data of Rh was available at that time, they estimated the J value from the spectra of vitamin A. Although they reported J to be $1.4\times10^{10}\,\mathrm{cm}^3\,\mathrm{mmole}^{-2}$, this is obviously an overestimation by a factor of ten.

** To the author's awareness the only information on η of the pigments in the rod is by Guzzo and Pool (1968). They report the η of Rh to be 0.05. The value for B might be larger than this.

is transferred to Rh in the rod outer segment.

The above discussion is based on the assumption that the energy difference between the maxima of the absorption and fluorescence spectra (Stokes shift) of B is about 6000 cm^{-1}. According to this result even if the Stokes shift is 4000 or 8000 cm^{-1} (namely, the energy difference between the maxima of the fluorescence spectrum of B and the absorption spectrum of Rh is ± 2000 cm^{-1}), the integral J is reduced only by a factor of 0.7 giving a negligible effect ($\times 0.94$) on R_0.

Similar thing might happen between a pair of molecules, an excited C and an unexcited Rh (C*-Rh), from the following argument. The former species is reported to have higher excitation energy (465 nm) than the absorption spectrum of Rh but the difference is still 2000 cm^{-1} making the energy transfer possible. There is also a possibility of the energy transfer between a pair of B*-C. However, further discussion will be deferred until more quantitative knowledges on C are accumulated. As has been mentioned earlier in this paper, the phenomena in which C is involved to a considerable extent are rather complicated owing to the several factors, *e. g.*, photo-regeneration to B, possibility of a mixture of several species (recall that the shape of the C-signal is changing in some stage of FPV kinetics), *etc.* In conclusion the following point should be emphasized. Although there is no *a priori* reason for or against the energy transfer in the rod, the transfer from some bleaching intermediate, especially B, to Rh is much more probable than the transfer among Rh's, and, if so, it might be involved in the light-adaptation mechanism.

Prediction of the physical quantity in the energy transfer mechanism

The next problem is to predict how the physical quantity is affected by the energy transfer. The relation between the fraction x of unbleached Rh's and the light intensity I is known as

$$x = \exp(-cI) \tag{1}$$

where c is a positive constant pertinent to the apparatus and the wavelength of the light used (Hagins, 1957 ; Cone, 1963). It can easily be shown that the curve (1), if it is plotted in the $x - \log I$ scale, is identical to the curve $x = \exp(-I)$ after a parallel transformation along the abscissa. Therefore we do not need the value of c. Suppose that shortly after the partial bleaching of Rh with a yellow light, a blue flash is shone and the electrical response is measured (*Experiment ii*). Since both of the biphasic FPV and cornea-positive B-signal respond to the blue light, the total electrical signal shape is their mixture as in Fig. 3. Assume that for the system in which more than two different kinds of pigments p's are coexisting, each p contributes to an

electrical signal independently from others by an amount S_p proportional to the number of the excited molecules, whose life-time is long enough to induce the change of the surrounding ionic species and hence the electrical response. Especially for *Experiment ii* we have the R2 and B component as

$$\begin{aligned} S_{R2}/R &= ax \\ S_B/R &= b(1-x) \end{aligned} \tag{2}$$

where a and b are constants.* The magnitudes of the signals S should be divided by a reference signal value R (the R2 component of the FPV), which has been obtained before the bleaching, since the data taken from different eyes are to be compared with each other. Furthermore it is understood that all the signal values are the readings on the oscilloscope at a certain time, *e.g.*, 2 msec, after the trigger.

In the case where energy is not transferred, the total signal intensity S^0/R is expressed as

$$\begin{aligned} S^0/R &= (S_{R2}+S_B)/R \\ &= b+(a-b)\exp(-cI) \end{aligned} \tag{3}$$

or

$$(S^0/R-b)/(a-b)=\exp(-cI)\,. \tag{4}$$

The curve 0 in Fig. 4 was drawn according to Equation (4). The values of a and b can be obtained from the two experiments in which no bleaching light ($x=1$) and the strongest bleaching light ($x=0$) are given, respectively. Then the plot of $(S/R-b)/(a-b)$ versus a quantity linearly related to $\log I$ (*e.g.*, the optical density D of the neutral density filter used for the partial bleaching experiment; $D=\log I_0-\log I$, I_0 being the intensity of the bleaching light without any neutral density filter) should fit the curve 0 in Fig. 4, by a parallel transformation along the abscissa.

If a simple mass action law is assumed to hold in the energy transfer mechanism, the probability of the energy transfer from B to the unbleached Rh is proportional to the product of the fraction of the two components, $\eta x(1-x)$. The fractions of Rh and B are then changed ihto $x+\eta x(1-x)$ and $(1-x)-\eta x(1-x)$, respectively, where η is a positive constant. The magnitude of the electrical signal S^{et}/R as the result of the energy transfer is then

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* During the time between the *Bleach* and *Blue* (actually 45 sec in this experiment) a $1-2^{-t/\tau}$ portion of B decays into C and the bleached substance, both of which are insensitive to the blue light used. τ is the half-life of B. Therefore the quantity S_B/R in Equation (2) should be $2^{-t/\tau}\cdot b(1-x)$. However, this effect is omitted from the present discussion for the sake of simplicity.

$$S^{et}/R = a\{x+\eta x(1-x)\}+b\{(1-x)-\eta x(1-x)\}$$
$$= S^0/R+\eta x(a-b)(1-x)\,. \tag{5}$$

or

$$(S^{et}/R-b)/(a-b) = \exp(-cI)+\eta x(1-x)$$
$$= \exp(-cI)\{1+\eta(1-\exp(-cI))\}\,. \tag{6}$$

In Fig. 4 calculated values of $(S^{et}/R-b)/(a-b)$ are plotted with several positive values for η.

Effect of sub-units

Suppose that all the N Rh's in the rod are clustered into m n-mers, $N=nm$ $(n>1)$. By the exposure to a bleaching light a fraction x is left unbleached. Further suppose that all the bleached Rh's remain as B, the total numbers of Rh and B being, Nx and $N(1-x)$, respectively. In the individual sub-units the number of the unbleached Rh, i, out of n pigments varies from 0 to n with the probability

$$P_{n,x}(i) = {}_nC_i x^i(1-x)^{n-i}\,.$$

Assume that the number of B's (or Rh's) which donate (or accept) the energy to Rh's (or B's) within the same sub-unit is proportional to the product of the numbers of both the components

$$n^{et}(i) = n\eta\frac{i}{n}\frac{n-i}{n} = \eta i(n-i)/n\,,$$

where η is the same constant as in Eq. (5). The total number N_n^{et} of the energy transfer is

$$N_n^{et} = \sum_{i=0}^{n} mP_{n,x}(i)n^{et}(i)$$
$$= \frac{m\eta}{n}\sum_{i=0}^{n}\frac{n!}{i!\,(n-i)!}i(n-i)x^i(1-x)^{n-i}$$
$$= m\eta(n-1)x(1-x)\sum_{i=1}^{n-1}\frac{(n-2)!}{(i-1)!\,(n-i-1)!}x^{i-1}(1-x)^{n-i-1}$$
$$= m\eta(n-1)x(1-x)\sum_{j=0}^{k}\frac{k!}{j!\,(k-j)!}x^j(1-x)^{k-j}$$
$$= N\eta\frac{n-1}{n}x(1-x) \qquad (n>1)\,, \tag{7}$$

while for the case in which there is no sub-unit the total number of the energy transfer N^{et} is

$$N^{et} = n\eta x(1-x) \tag{8}$$

By analogy to the derivation of Equation (5) the signal S_n^{et}/R expected from the n-mer sub-unit system is

$$S_n^{st}/R = S^0/R + \eta x(a-b)(1-x)(1-1/n) \qquad (n>1)\,. \tag{9}$$

However, there is no way of differentiation between groups of curves (5) and (9), since η in (5) and $\eta(1-1/n)$ in (9) are the constants to be determined from the experiments.

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References

Arden, G.B., Ikeda, H. and Siegel, I.M. (1966). Effects of light adaptation on the early receptor potential. *Vision Res.* **6**, 357–371.

Brown, K.T. and Murakami, M. (1964). A new receptor potential of the monkey retina with no detectable latency. *Nature, Lond.* **201**, 626–628.

Cone, R.A. (1963). Quantum relations of the rat electroretinogram. *J. gen. Physiol.* **46**, 1267–1286.

Cone, R.A. (1965). The early receptor potential of the vertebrate eye. *Cold Spring Harb. Symp. quant. Biol.* **30**, 483–491.

Cone, R.A. (1967). Early receptor potential: Photoreversible charge displacement in rhodopsin. *Science, N.Y.* **155**, 1128–1131.

Ebrey, T.G. (1968). The thermal decay of the intermediates of rhodopsin in situ. *Vision Res.* **8**.

Förster, Th. (1948). Zwischenmolekulare Energiewanderung und Fluoreszenze. *Ann. Physik.* **2**, 55–75.

Förster, Th. (1960). Transfer mechanisms of electronic excitation energy. *Radiation Res. Supplement.* **2**, 326–339.

Guzzo, A.V. and Pool, G.L. (1968). *Science, N.Y.*

Hagins, W.A. (1956). Flash photolysis of rhodopsin in the retina. *Nature, Lond.* **177**, 989–990.

Hagins, W.A. (1957). Rhodopsin in a mammalian retina. Thesis, University of Cambridge, England.

Hagins, W.A. and Jennings, W.H. (1959). Radiationless migration of electronic excitation in retinal rods. *Discussion Faraday Soc.* **27**, 180–190.

Hagins, W.A. and McGaughy, R.E. (1967). Membrane origin of the fast photovoltage of squid retina. *Science, N.Y.* **159**, 213–215.

Hosoya, H. (1969). Direct measure of the rate of decay of intermediate B in the bleaching of rhodopsin in the excised eye of the albino rat. *This Report,* **20**, No. 2, 45–52.

Kropf, A. (1967). Intramolecular energy transfer in rhodopsin. *Vision Res.* **7**, 811–818.

Matthews, R., Hubbard, R., Brown, P.K. and Wald, G. (1963). Tautomeric forms of metarhodopsin. *J. gen. Physiol.* **47**, 215–240.

Pak, W.L. and Ebrey, T.G. (1966). Early receptor potentials of rods and cones in rodents. *J. gen. Physiol.* **49**, 1199–1208.

Pak, W.L. and Helmrich, H.G. (1968). Absence of photodichroism in the retinal receptors. *Vision Res.* **8**, 585–589.

Streyer, L. (1960). Energy transfer in proteins and polypetides. *Radiation Res. Supplement.* **2**, 432–451.

Wolken, J.J. (1963). Structure and molecular organization of retinal photoreceptors. *J. opt. Soc. Amer.* **53**, 1–19.