Photoregeneration and Sensitivity Control of Photoreceptors of Invertebrates

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It is a well known fact that the metarhodopsins of cephalopods are thermostable and photoreconvertible into rhodopsin (Fig. 1). In the octopus species Eledone moschata the wavelength maxima of the rhodopsin (R) and acid metarhodopsin (M) are separated by about 50 nm. Due to the large distance between the wavelength maxima, this R-M-system is well suited for photokinetic studies (1-3). Monochromatic illumination causes a photoequilibrium between R (11-cis R, isoR) and its acid M. The equilibrium is determined solely by the ratio betweeen the absorption coefficients of the two pigments. Illumination at the isosbestic point yields 50% R and 50% M. Minimal R-concentration (38%) is obtained at 440 nm. At longer wavelength the M is quantitatively reconverted to R.

Thermostability and photoreconversion have been demonstrated for several photoreceptors of the rhabdomeric type. The present paper concerns the photoreceptors of three insects. The frontal eye of the neuropter Ascalaphus macaronius is sensitive to ultraviolet light only (Fig. 2) (4). Absorption of an UV quantum by the extracted pigment causes the formation of a photoproduct absorbing at long wavelengths (5-8). This pigment has a high molar absorbance. Long wavelength illumination

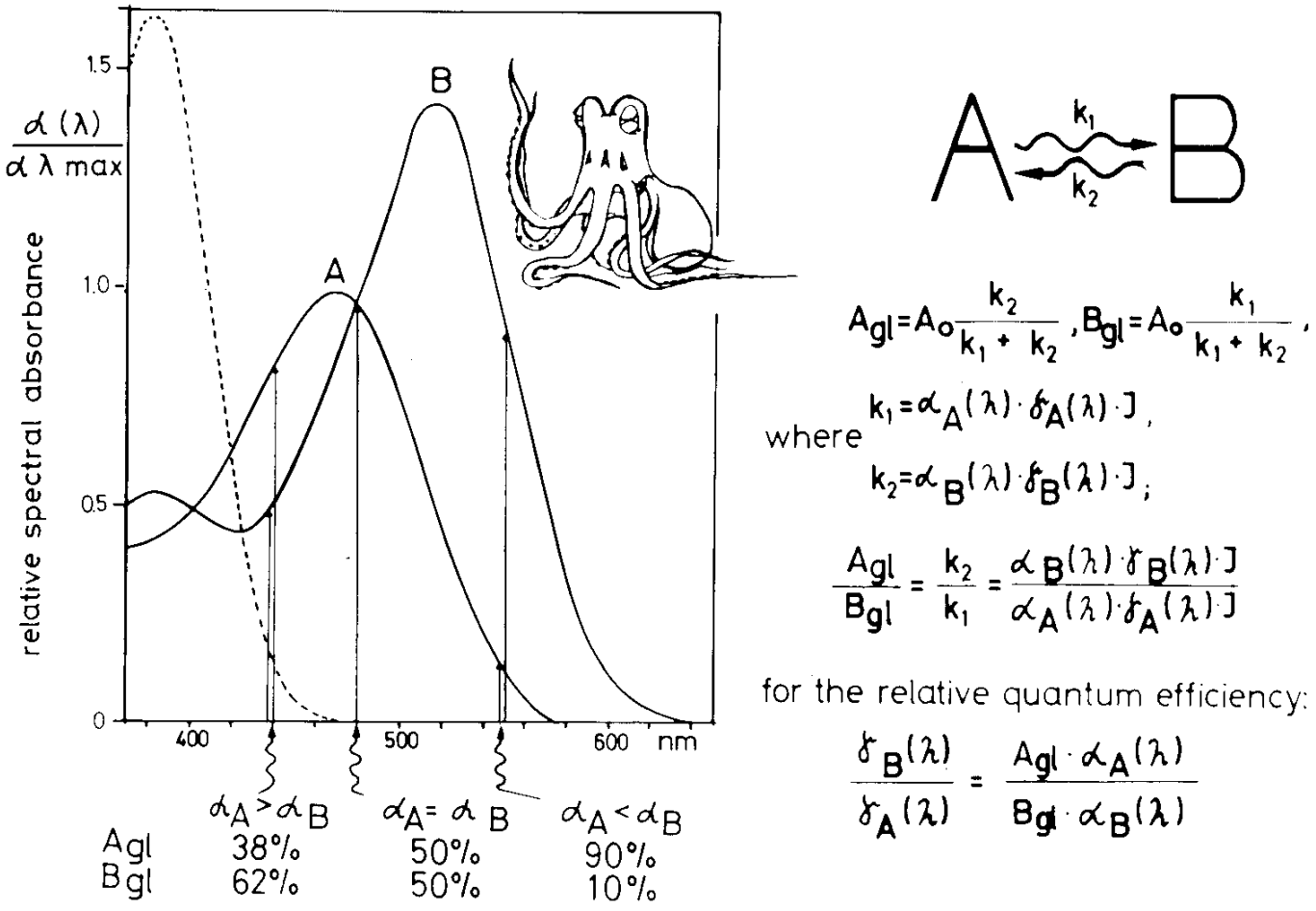
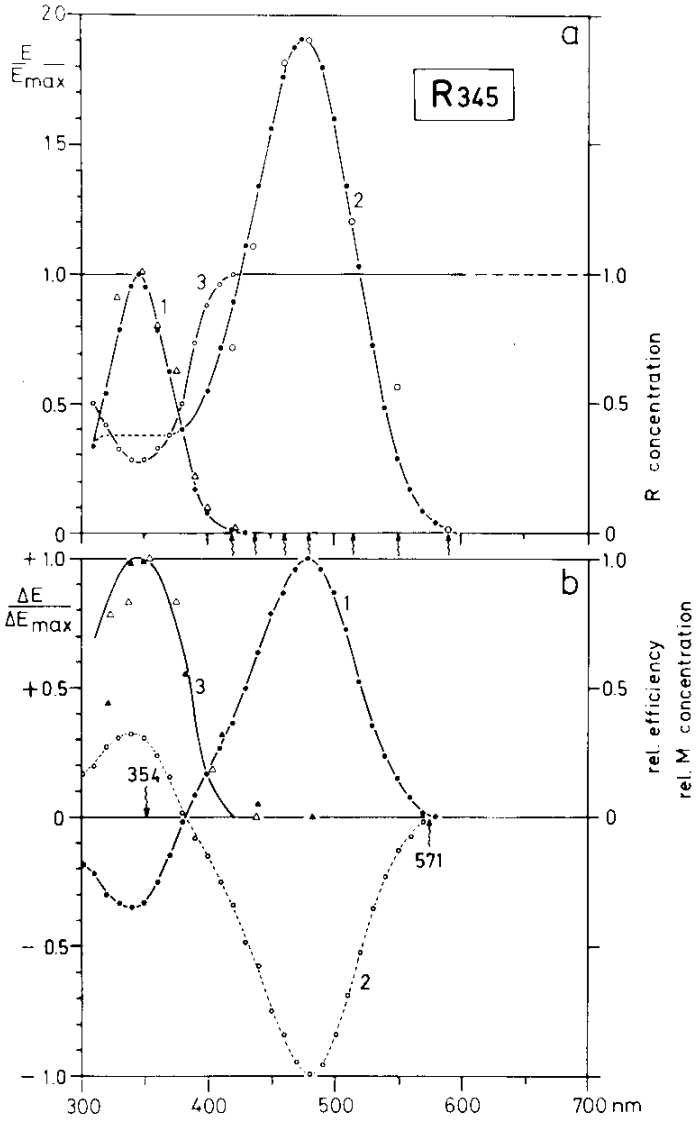


Fig. 1. Photoequilibrium in rhodopsin system of Eledone. The ratio between rhodopsin (A) and acid metarhodopsin (B) in photoequilibrium is given by equation A_{gl}/B_{gl} (right half)



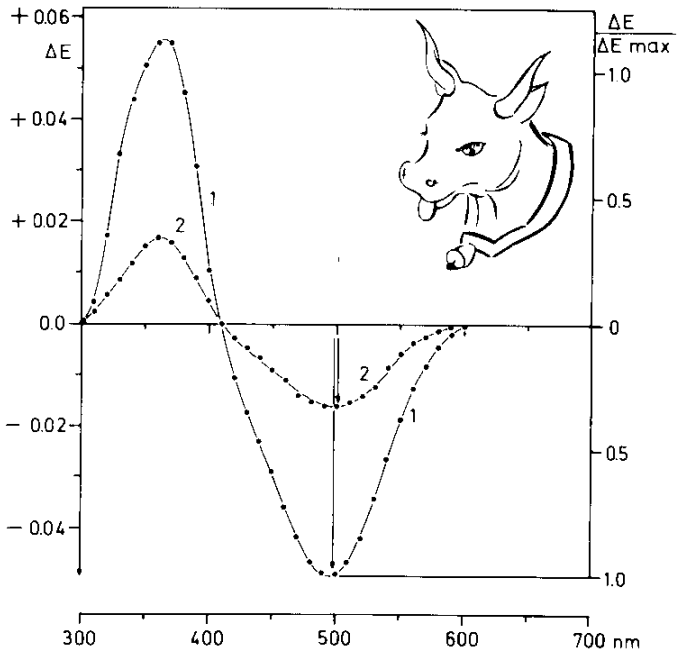


Fig. 3. Cattle rhodopsin synthesized from chromophore of UV pigment in Ascalaphus. Difference spectra show bleaching of synthesized cattle rhodopsin in presence of NH_2OH. Cattle rhodopsin formed from denatured R 345 (1). - Cattle rhodopsin formed from UV (354 nm) irradiated R 345 (2). In the second experiment only one third of the previously formed cattle rhodopsin is formed

Fig. 2. Visual pigment system of Ascalaphus.
a) Spectral absorbance of R 345 (1) and its acid M 475 (2), and concentration of R 345 in photoequilibrium in relation to wavelength of illumination (3). - Open triangles: Relative spectral sensitivity of the frontal eye. Open circles: Spectral efficiency for photoreconversion based on electrophysiological data like that in Fig. 6.
b) Change in absorbance in R 345 extract after UV (1) and after subsequent blue irradiation (2). Filled triangles: Experimental data from extract. Open triangles: Experimental data from the retina

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completely reconverts this thermostable pigment into the UV visual pigment. In contrast, UV light leads to a photochemical equilibrium between the UV pigment and its photoproduct. Like in Eledone, the equilibrium is directly proportional to the ratio between the absorption coefficients for the two pigments. The content of the UV pigment and its photoproduct after monochromatic illumination can be calculated from this ratio (Fig. 2a). There is good agreement between the calculated contents, and the contents found in measurements on pigment extract and in microspectrophotometric measurements of the pigments in situ (Fig. 2b).

The UV visual pigment is an 11-cis retinal proteid (9). The chromophore can be separated from the pigment molecule and combined with cattle opsin. Bleaching of this "synthesized" cattle rhodopsin in the presence of NH_2OH leads to the difference spectrum of high amplitude shown in Fig. 3. When the same experiment is carried out on a UV irradiated pigment, only one third of the amount of cattle rhodopsin is formed. This value corresponds to the content of 11-cis retinal which is to be expected at the photochemical equilibrium between R 345 and its thermostable M 475.

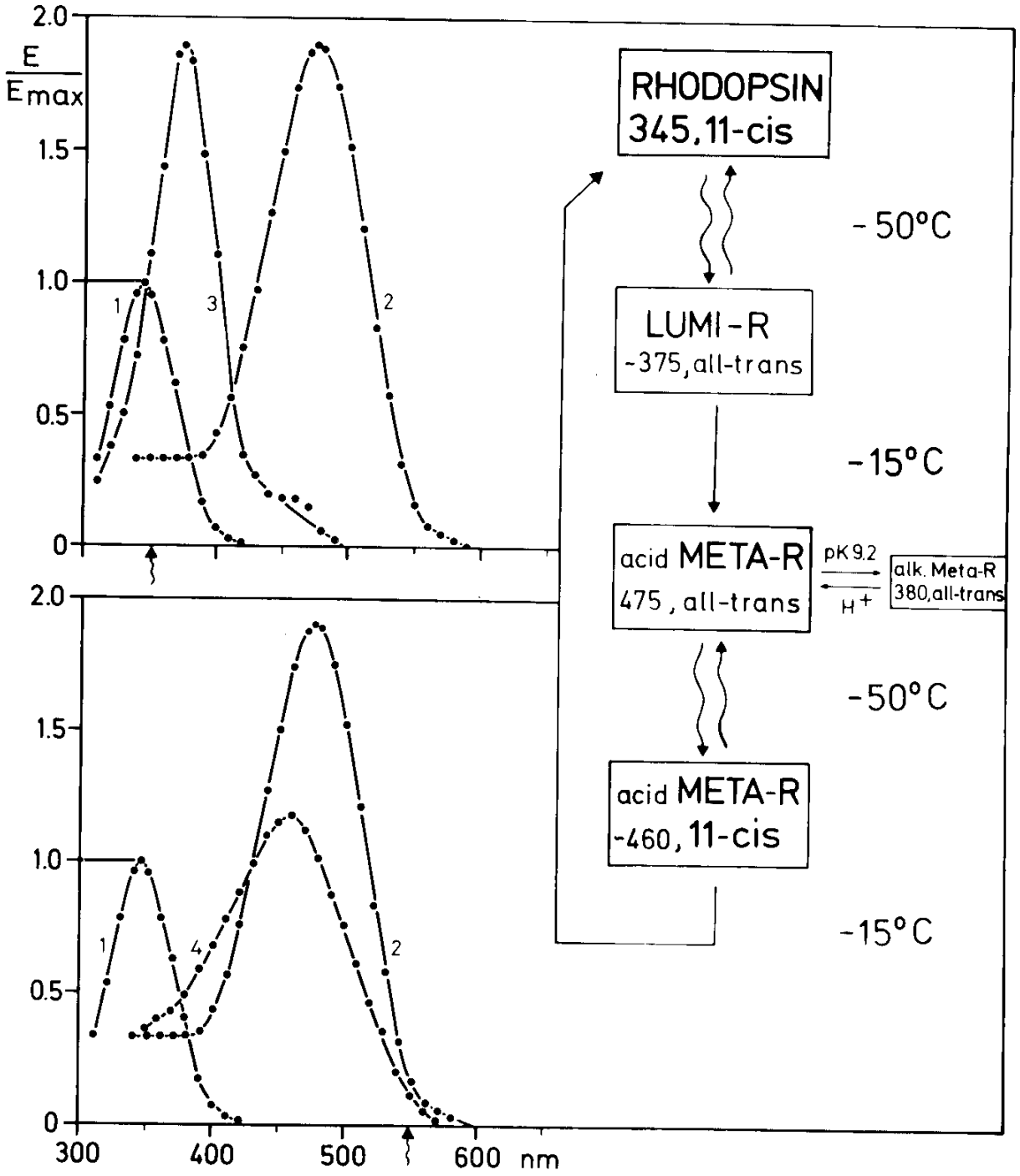

Fig. 4. Light and dark reactions of the R 345-system of Ascalaphus.
Left: Spectral absorbance of rhodopsin 345 (1), acid metarhodopsin 475 (2), lumirhodopsin (3), and 11-cis metarhodopsin (4). Spectra of intermediates (3 and 4) are calculated from difference spectra.
Right: Scheme of photoreactions (wavy lines) and dark reactions (straight lines). The alkaline metarhodopsin does not exist under physiological pH conditions, its spectral absorbance is not shown

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In UV light at -50°C, R 345 converts into an all-trans pigment of high absorbance in the UV region (Fig. 4). This intermediate, probably lumirhodopsin, when warmed converts into acid M 475. Long wavelength illumination of acid M 475 at -50°C leads to another intermediate. The protein configuration of this compound should be similar to that of the all-trans M 475. However, the chromophore is 11-cis retinal, because upon warming in darkness the intermediate converts into R 345. The intermediate is therefore designated as acid 11-cis metarhodopsin. The same intermediate occurs in Eledone during photoreconversion. (In this species also the alkaline all-trans M converts into a corresponding alkaline 11-cis M).

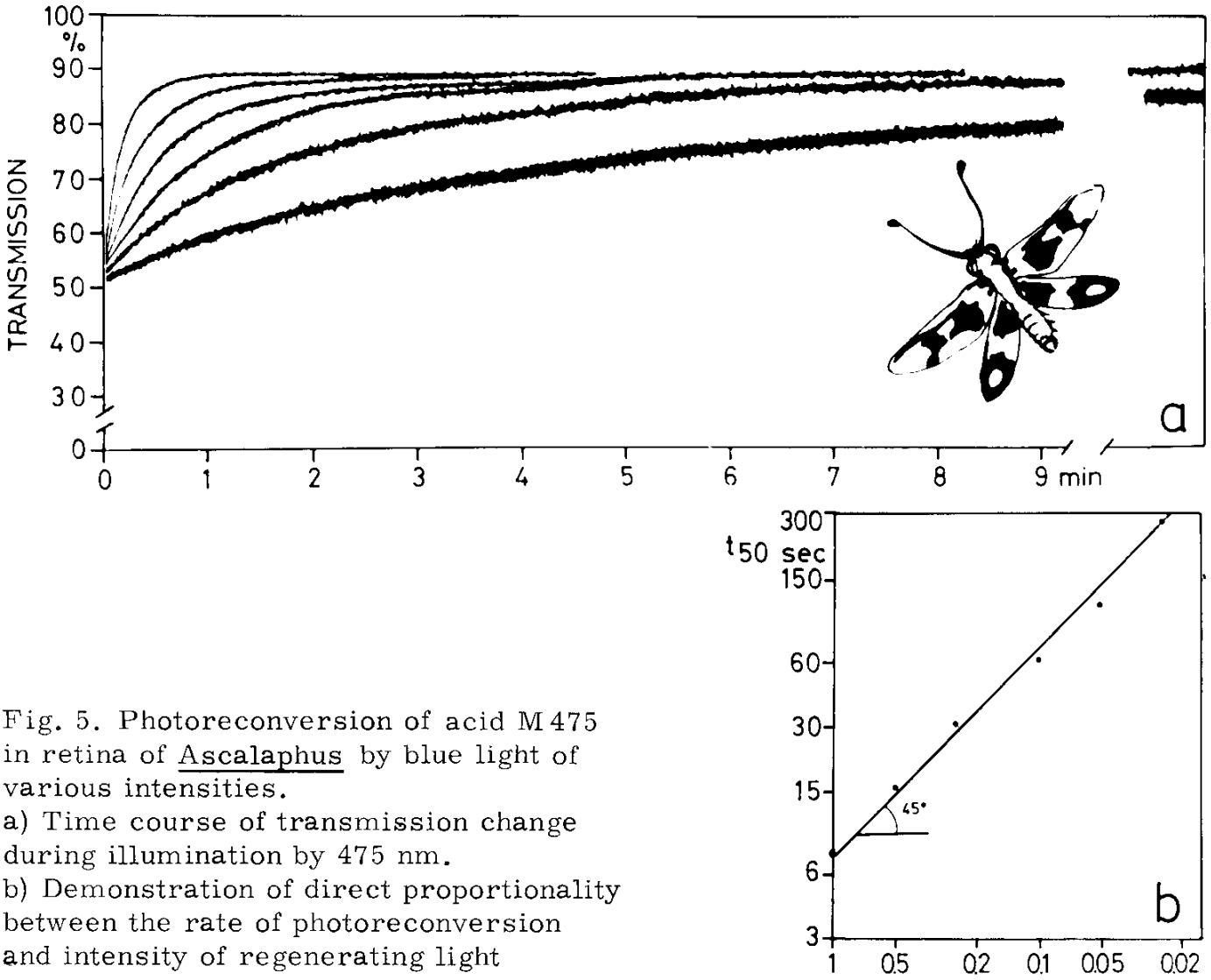

Fig. 5. Photoreconversion of acid M475 in retina of Ascalaphus by blue light of various intensities.
a) Time course of transmission change during illumination by 475 nm.
b) Demonstration of direct proportionality between the rate of photoreconversion and intensity of regenerating light

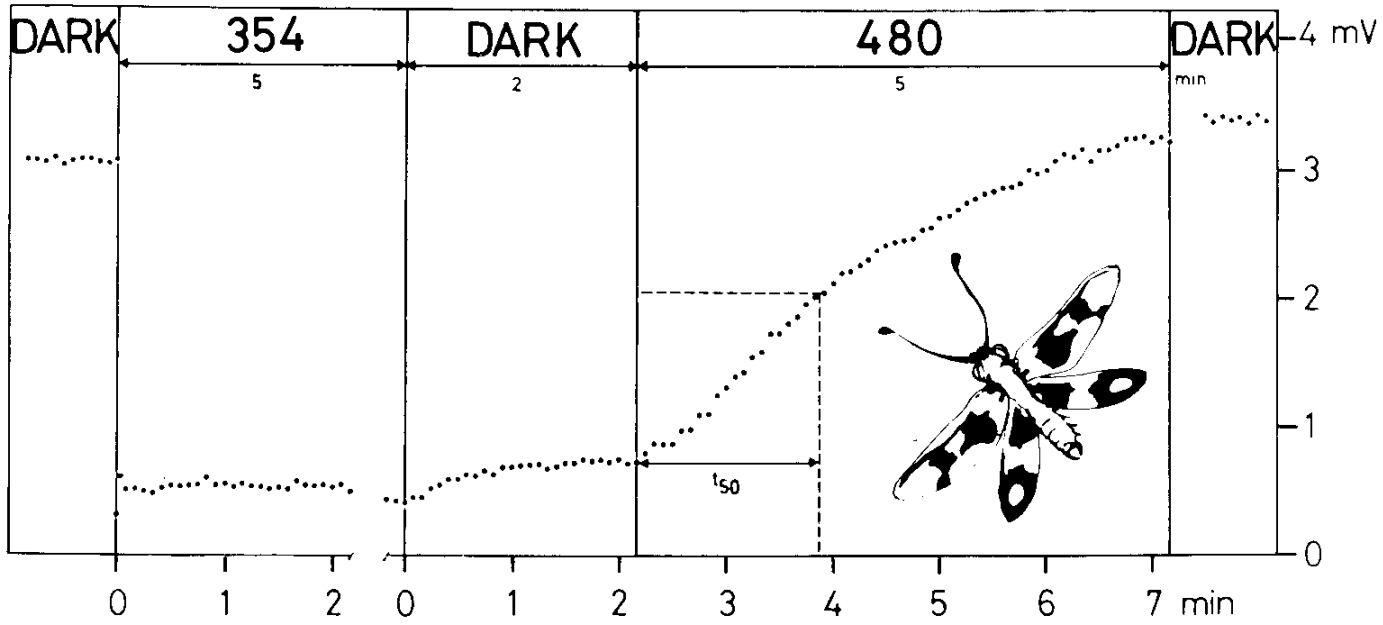

Fig. 6. Regeneration of retina sensitivity by blue light (Ascalaphus): Amplitude of extracellularly recorded receptor potential (dots) to low intensity UV test-flash in darkness, during bright UV adaptation (354 nm), and during illumination with blue light (480 nm). The rate of sensitivity increase (t_{50}) depends on wavelength, using light of equal quanta

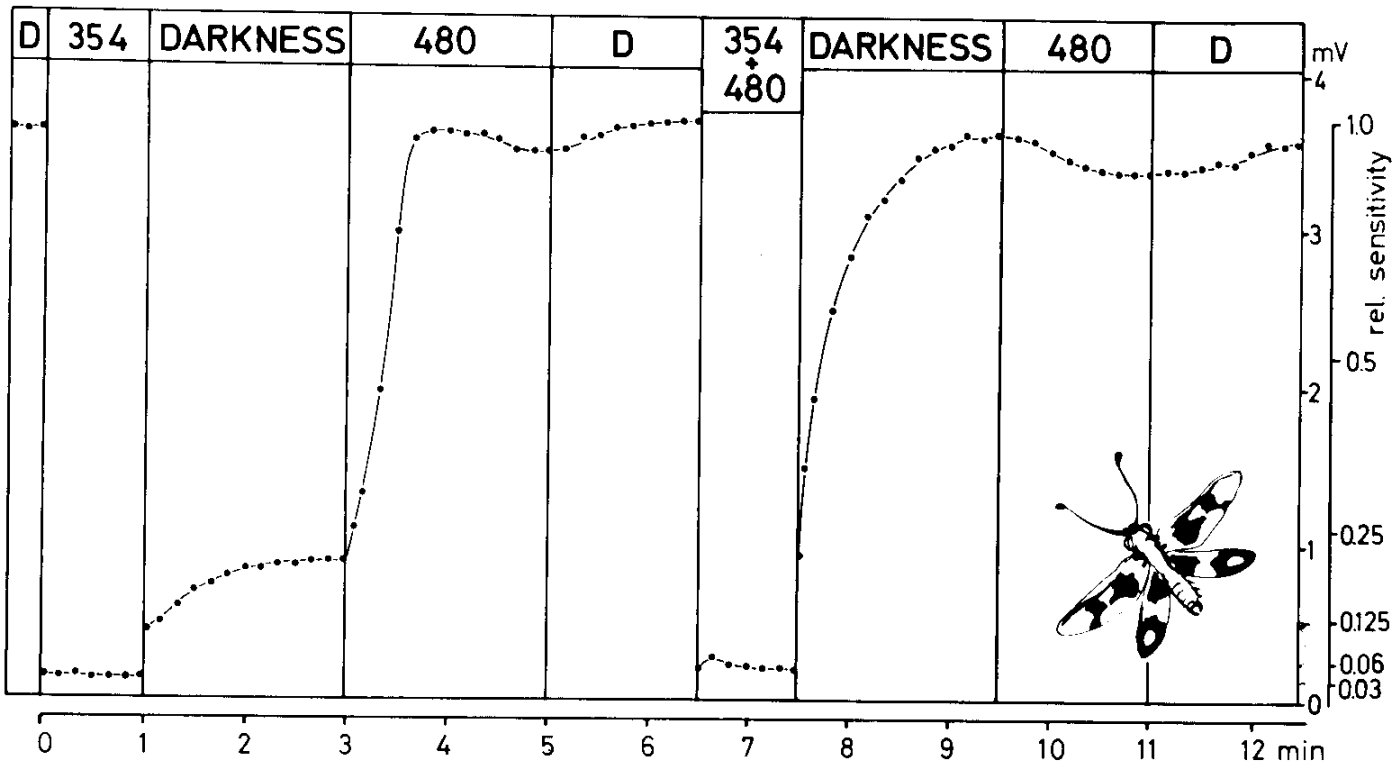

Fig. 7. Regeneration of retina sensitivity by blue light (Ascalaphus): Amplitude of extracellularly recorded receptor potential (dots) to low intensity UV test-flash in darkness, during bright UV adaptation (354 nm), during illumination by blue light (480 nm), and by a combination of UV light (354 nm) and blue light (480 nm). - Right ordinate: Relative sensitivity at each response amplitude

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The rate of photoregeneration from acid M to R 345 depends on the intensity of illumination, as shown by microphotometric measurements on the retina (Fig. 5). The rate of transmission change at 475 nm was determined after saturating UV illumination. The rate increases with the intensity of the regenerating light. Fig. 5b shows a direct proportionality between the intensity of the regenerating light and the rate of photoreconversion.

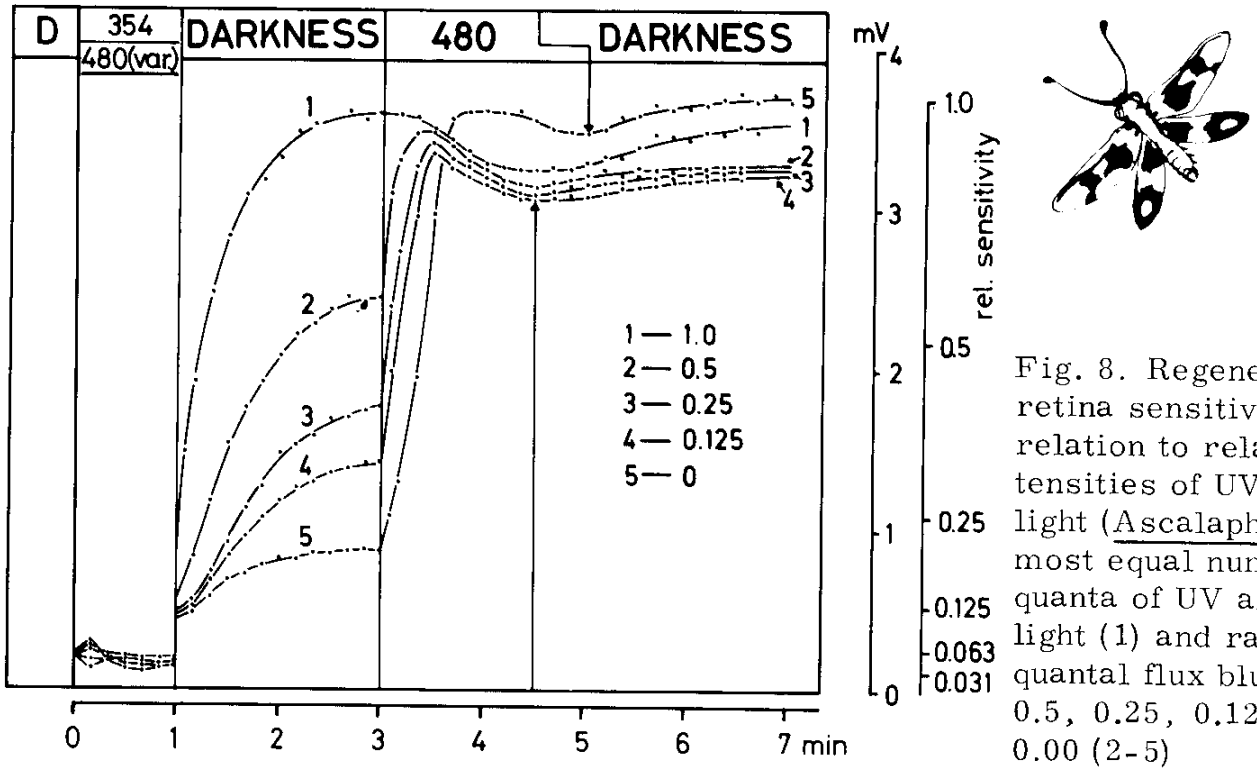

Fig. 8. Regeneration of retina sensitivity in relation to relative intensities of UV and blue light (Ascalaphus): Almost equal number of quanta of UV and blue light (1) and ratios of quantal flux blue/UV: 0.5, 0.25, 0.125, and 0.00 (2-5)

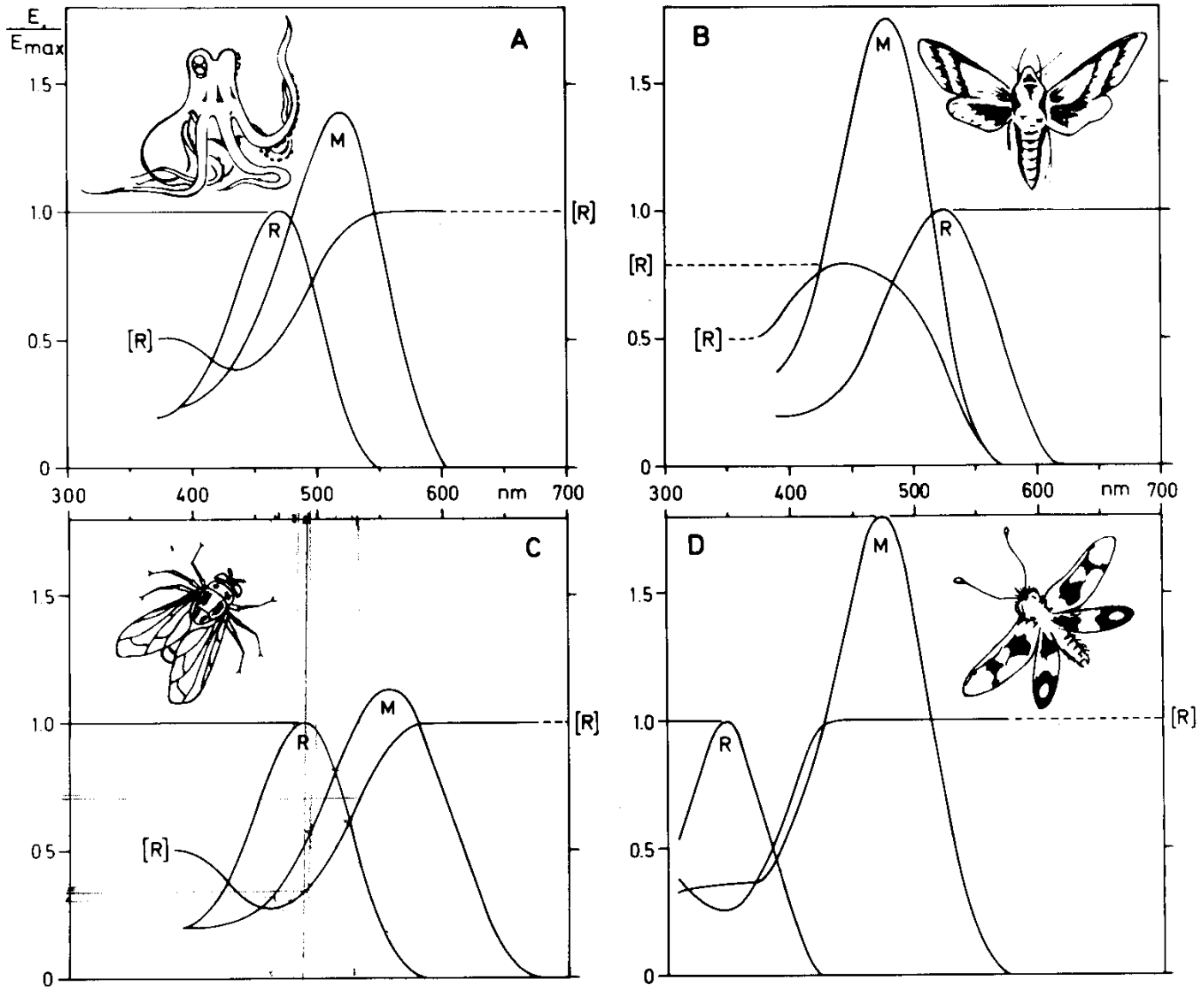

Fig. 9. Photoconvertible visual pigment systems of four invertebrates: A) Eledone moschata, B) Deilephila elpenor, C) Calliphora erythrocephala, D) Ascalaphus macaronius - R: Rhodopsin; M: Acid metarhodopsin. R : Content of rhodopsin in receptor in relation to wavelength of monochromatic adapting light, calculated for relative quantum efficiency of one

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The proportionality between light intensity and rate of regeneration can also be demonstrated electrophysiologically (8). Exposure of the eye in Ascalaphus to UV light causes a considerable decrease in the response amplitude (Fig. 6). The amplitude increases only slightly after cessation of the adapting light. During exposure to blue light, the amplitude increases to the original value. The rate of this sensitivity increase is directly proportional to the intensity of the blue light. - The rate of the sensitivity increase varies with the wavelength of the regenerating light (8). Using irradiation of equal quanta in the wavelength range 400 nm to 589 nm, the regeneration rate was found to be highest at 475 nm (Fig. 2a, open circles). The spectral efficiency for the electrophysiologically recorded sensitivity increase is directly proportional to the absorbance spectrum of the acid M 475.

Information about the sensitivity regulation for Ascalaphus in its natural environment was obtained by simultaneously exposing the eye to about an equal number of quanta of UV and blue light (Fig. 7). The maximal sensitivity was recorded immediately after cessation of the adapting light. The sensitivity increase is determined by the intensity of the blue light relative to that of the UV light (Fig. 8) (10).

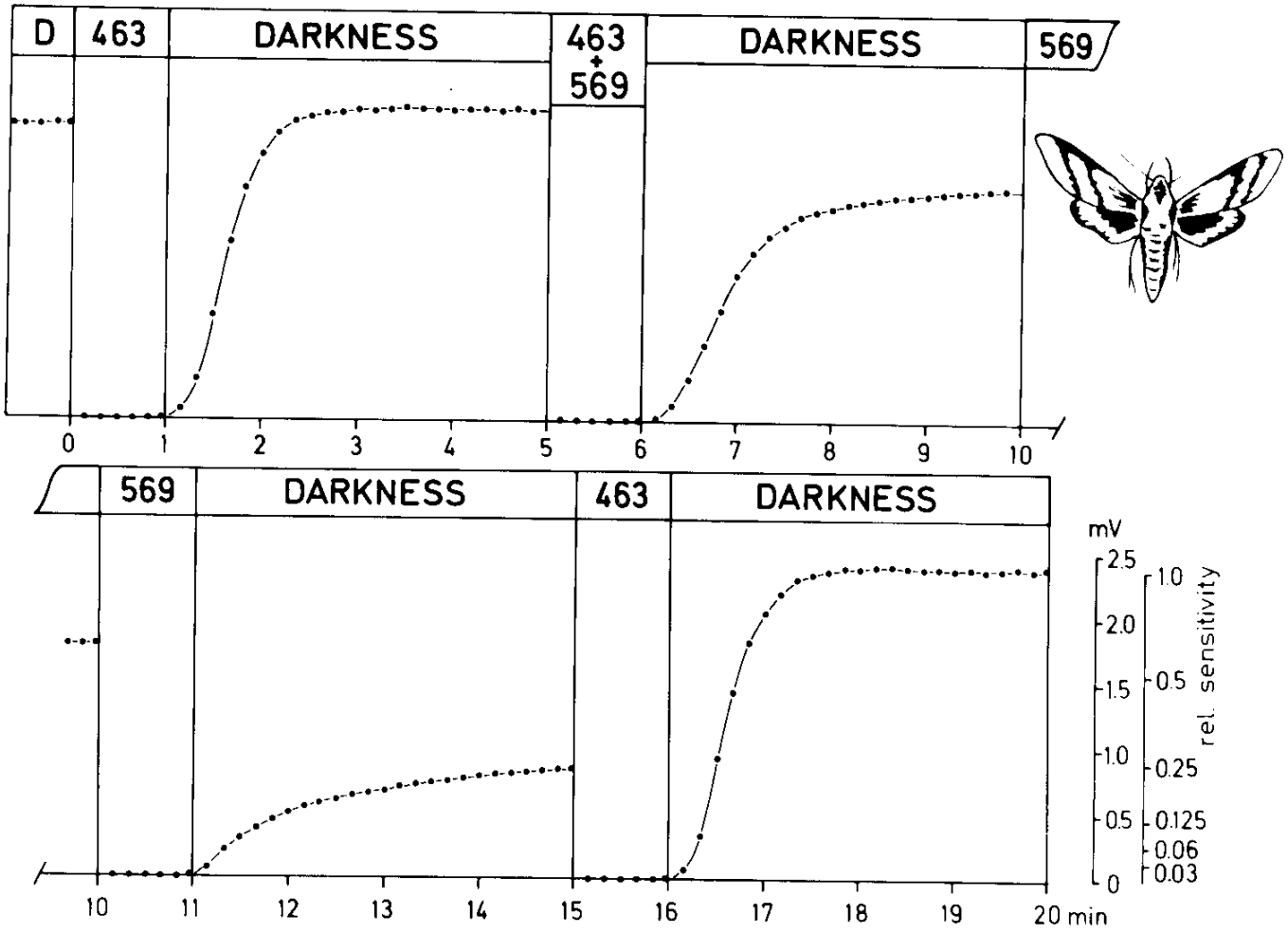

Fig. 10. Effect of blue (463 nm) and yellow (569 nm) adapting light, and combination of blue and yellow light on retina sensitivity of Deilephila. After cessation of blue adapting light the electrical response of the retina to a short stimulus (40 msec, 608 nm) increases within 2 min to a maximal value. In contrast, after yellow adapting light the sensitivity increases only slightly. After the combination of blue and yellow the response increases nearly to the maximal value

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The spectral content of the light from the sky has a maximum in the blue region, and another in the UV (8). The high content of blue light, and the high absorption of the acid M 475 in this region, causes almost all M to be reconverted into R 345. Calculations show that the R content is about 90%. The sensitivity to UV light is therefore almost nearly maximal.

Rhodopsins absorbing maximally in the green region of the spectrum occur in the moth Deilephila elpenor (11,12) and the fly Calliphora erythrocephala (13). In Deilephila the M absorbs at wavelengths shorter than the absorption maximum of R (11,12) while in Calliphora the maxima are reversed (Fig. 9C). Both metarhodopsins are reconverted by light. The variation in R concentration with wavelength is shown in Fig. 9. It is to be expected that in Deilephila blue light reconverts the M into R, and that long wavelengths completely convert the R into its M. In the fly, on the other hand, long wavelengths should regenerate, and blue light should cause a maximal conversion into M. Experimental results confirm this hypothesis: In Deilephila after cessation of a blue adapting light (Fig. 10), the sensitivity increases to the original value in less than two minutes. In contrast, the sensitivity increases much less after adapting to long wavelengths. After subsequent exposure to blue light the sensitivity increases again to its maximal value. After a combination of blue and long wavelength adapting light, the sensitivity is almost equal to that after blue light alone, in spite of the higher photon flux during the light adaptation (14).

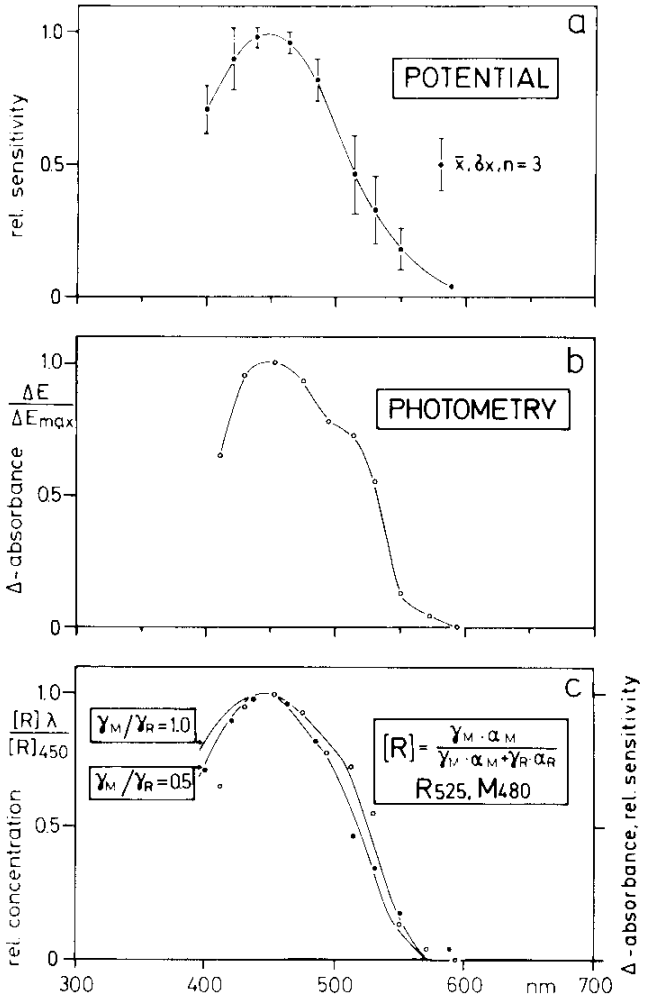

Fig. 11. Photoequilibrium and sensitivity in Deilephila.
a) Relative sensitivity of retina 2 min after monochromatic light adaptation (equal quantal flux). Abscissa: Wavelength of irradiation.
b) Change in absorbance during monochromatic light adaptation obtained from measurements on isolated retina. Values are proportional to R concentration.
c) Comparison between theoretical function of R content in photoequilibrium (calculated for relative quantum efficiencies, $\gamma M/\gamma R$, 1.0 and 0.5), and empirical data from a) and b)

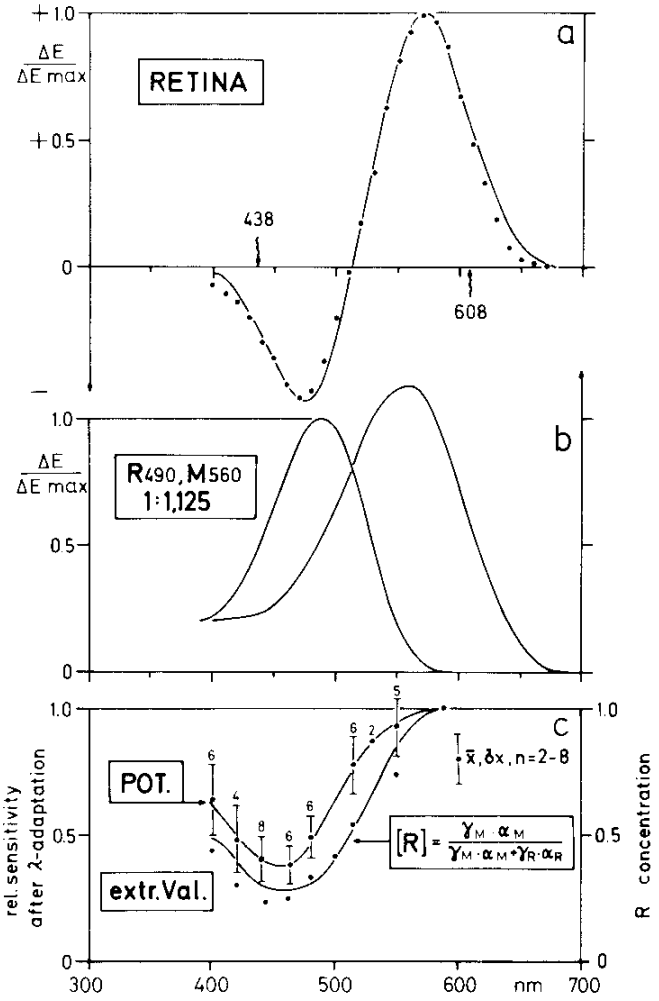

Fig. 12. Photoequilibrium and sensitivity in Calliphora.
a) Difference in absorbance caused by alternating illumination (608 nm and 438 nm) of retina.
b) Calculated spectra of R 490 and M 560 (from a).
c) Comparison between calculated R content in photoequilibrium (relative quantum efficiency 1.0), and sensitivity of retina 2 min after monochromatic light adaptation. Dots with bars: mean values of the electrophysiological data. Dots without bars: extreme values of a single electrophysiological experiment showing good fit with calculations of R content

The sensitivity increase after light adaptation varies with the wavelength of the adapting light (Fig. 11 a). The increase is maximal at 450 nm. The spectral efficiency for the sensitivity increase is very similar to the photometrically measured reconversion of M into R. Maximal reconversion into R 525 is caused by light of 450 nm wavelength (Fig. 11 b). The R concentration at various wavelengths can be calculated from the absorbance spectra (11). Fig. 11 c shows the R concentration at a relative quantum efficiency (M/R) of 1.0 and 0.5. There is a good agreement with the data from the photometrical and electrophysiological measurements. Therefore, it can be calculated that during 2 min dark-adaptation the sensitivity depends only on the probability for quantum absorption by the visual pigment.

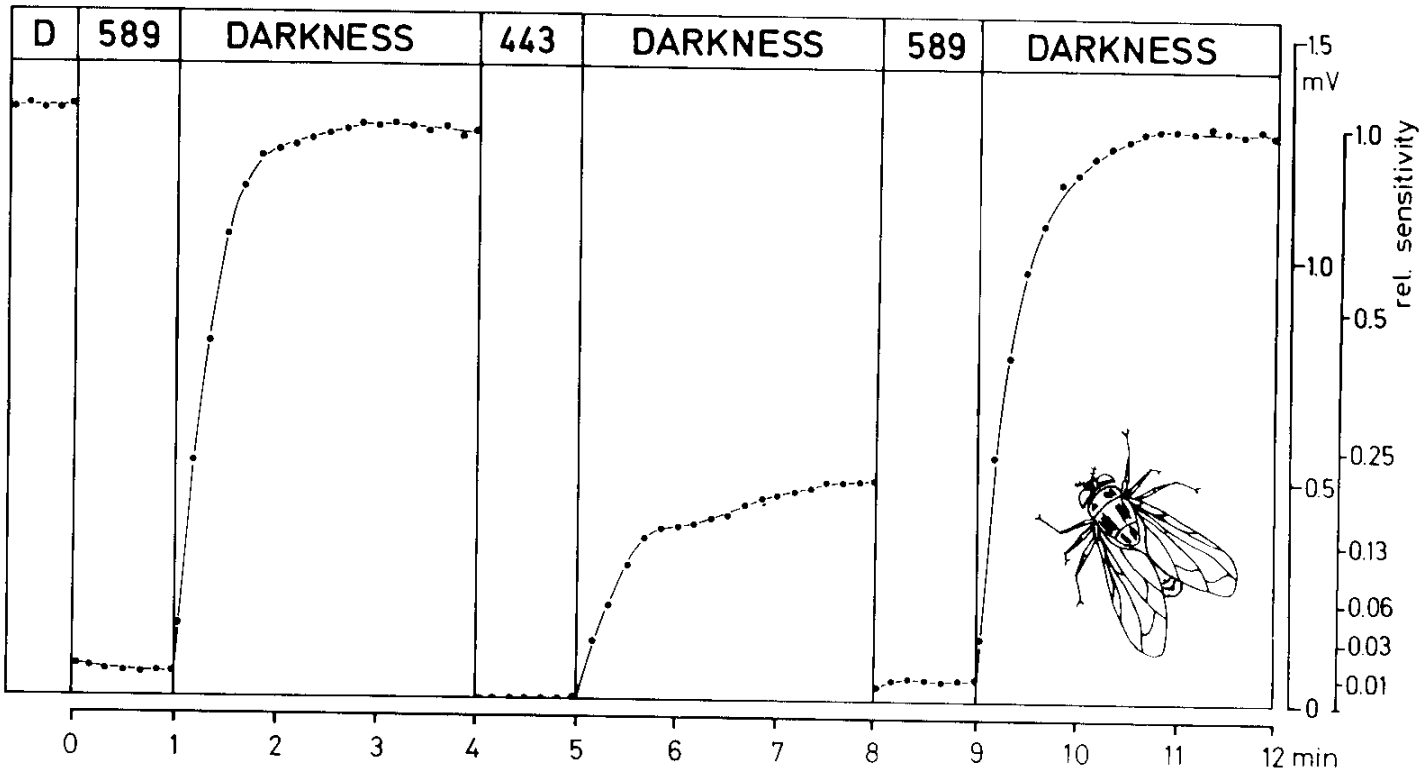

Fig. 13. Effect of blue (443 nm) and yellow (589 nm) adapting light on retina sensitivity of Calliphora. After cessation of yellow, the electrical response to a test flash increases to a maximal value within 2 min. After blue adaptation the sensitivity is strongly reduced. Subsequent yellow illumination causes maximal sensitivity

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Exposure of the retina in Calliphora to light of short wavelength, followed by exposure to light of long wavelength, gives the difference spectrum shown in Fig. 12 a. Calliphora is maximally sensitive to light of wavelength 490 nm. The visual pigment therefore absorbs maximally at this wavelength. The wavelength maximum of the acid M, and its absorbance relative to that of R, can be calculated from the difference spectrum and the spectral sensitivity (Fig. 12 b). Assuming that the acid M is completely reisomerized by light, the variation in R concentration with wavelength can be calculated (Fig. 12 c). The regulation of the sensitivity by light was demonstrated similarly for Calliphora as for Ascalaphus and Deilephila. The eye was exposed to light of wavelength 443 nm (Fig. 13). After illumination, the sensitivity increased only slightly. Following subsequent exposure to long wavelength illumination the sensitivity rapidly increased by about 2 log units. The spectral efficiency of sensitivity increase is similar to the calculated spectral variation in the photoequilibrium between R 490 and M 560 (Fig. 12c) (15).

It can be concluded that in all three species the sensitivity after a short dark period depends only on the concentration of R in the receptor membrane. The absolute sensitivity is nearly directly proportional to the R concentration and is determined only by the probability for light absorption by rhodopsin. The concentration of the acid M thus does not influence the sensitivity. In all three receptors the light from the sky keeps the R concentration above 50%. Thereby an absolute sensitivity of 50% or more is attained after a very short period in darkness. It is questionable if there is chemical regeneration from M to R. Such a chemical regeneration seems unnecessary in diurnal insects because a chemical regeneration to 100% R only doubles the absolute sensitivity.

A chemical regeneration may be of importance to animals which, like Deilephila, are active at low light intensities. This reconversion can be very slow. Fig. 14 il-

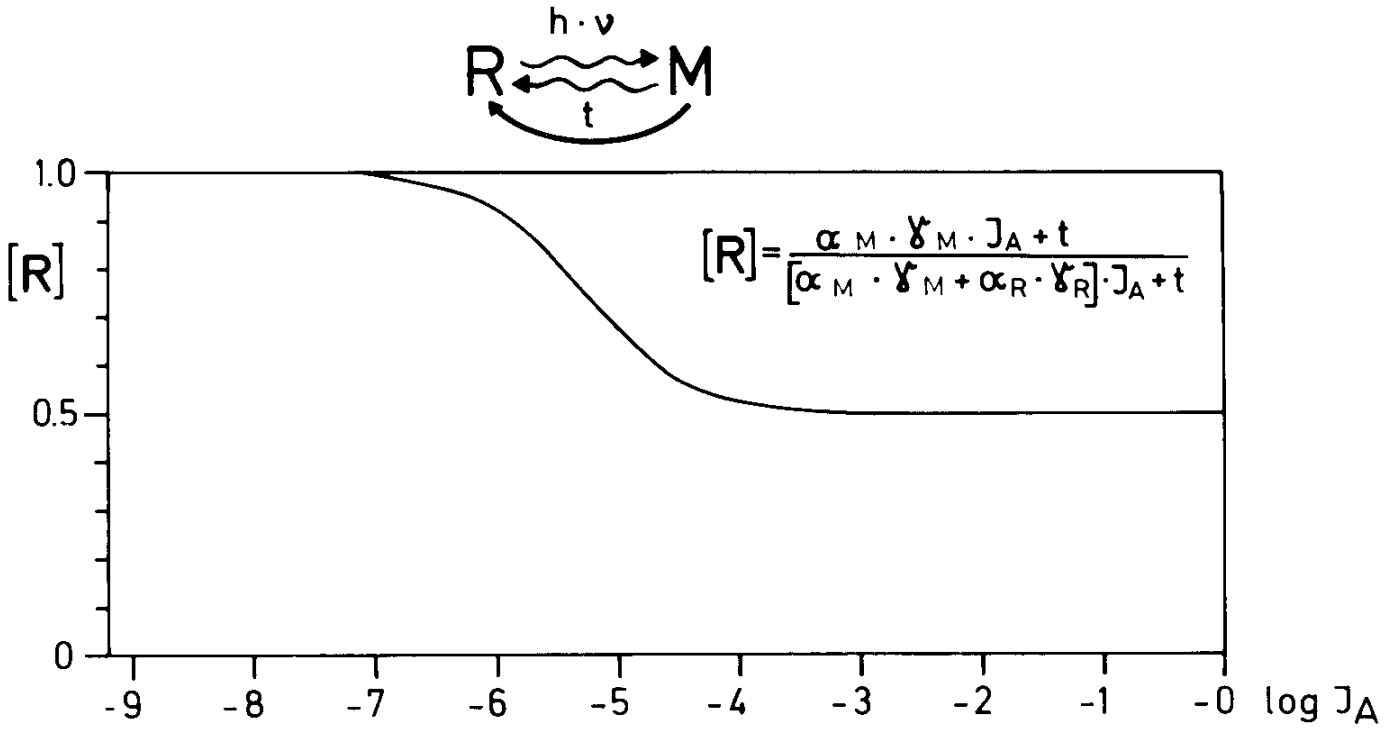

Fig. 14. R concentration in a photoreceptor with low rate of chemical regeneratio in relation to intensity of adapting light. - The photoequilibrium becomes 0.5 R b illumination with monochromatic light at the isosbestic point

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lustrates the variation in R concentration with the quantal flux of the adapting light It is assumed that the probability for chemical regeneration of a M molecule is independent of the light intensity. The R concentration in the receptor is nearly 100 at light intensities that are so low that the quantal absorption by the pigment is les than the probability for chemical reconversion of the photoproducts. A photochemical equilibrium between R and M occurs at the intensity range at which the photochemical reconversion becomes almost equal to the chemical regeneration. The equilibrium concentrations remain constant when the adapting light is further increased.

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Discussion

T. P. Williams: First, I should like to say that your spectrum of the 11-cis metarhodopsin is excellent. Much better than the one I obtained. Next, I would like to ask if you measured the rate at which it is converted to rhodopsin? Is this experiment possible with your system?

K. Hamdorf: Between 11-cis metarhodopsin and all-trans metarhodopsin there exists a photoequilibrium depending on the wavelength of irradiation. Our present measurements indicated that all the 11-cis metarhodopsin formed changes into rhodopsin. Kinetic data for this thermal transition have not yet been determined. Technically these measurements can be carried out with our equipment.

T. Ebrey: You stated that irradiating metarhodopsin at -50°C can, at least partially, produce a species, "11-cis" metarhodopsin, which can, upon warming, yield rhodopsin. Isn't it also possible that other isomers of retinal may be present on the illuminated metarhodopsin? Some of these could be stable (9-cis?) but others probably would not be stable and thus difficult to identify with your techniques.

K. Hamdorf: It may be possible that several retinal isomers are produced by illumination. Yet, the experimental results on the UV-rhodopsin extracted from Ascalaphus show that in this case only the 11-cis configuration is formed in provable quantities, not only at -50°C, but also in the physiological temperature range: You can see this best in the experiment described in Fig. 3: If other isomers were produced in considerable amounts at physiological temperature, a shift of λ_{max} of the synthesized cattle rhodopsin were to be expected. Furthermore, we might have been able to detect these other isomers by our TLC-experiments (in any case the 9-cis isomer).

G. Wald: In an eye that depends upon photoregeneration, would it not be an advantage for the visual pigment and its photoproduct to overlap maximally - as they nearly do in the American squid Loligo pealii - so that there would be no "bleaching" without regeneration?

K. Hamdorf: It is impossible to give a generalizing answer to this question. In the case of total overlapping of R and M, the photoequilibrium between the two pigments is constant, independent from the spectral distribution of the light source. The concentration of R and M in this equilibrium is determined only by the ratio of the molar absorption coefficients at λ_{max}. It depends on the behaviour and environment of Loligo, whether this system is an advantage; this should be examined.
In the case of different λ_{max} of R and M, the photoequilibrium is determined by the spectral emission as well as by the molar absorption coefficients. As shown for Ascalaphus, the strong separation of the two pigments provides a very high R concentration under natural light conditions. This system would allow a change in R content according to the particular conditions of illumination. Furthermore, in the Deilephila-system, which will be described by Dr. Höglund (12), the separation of R and M surely is an advantage for the constancy of color discrimination in trichromatic vision: The blue light of the sky absorbed equally by all the three metarhodopsins. Therefore, it is to be concluded that the ratio of absolute sensitivities is constant for the three receptor types.

W. de Grip: Have you ever performed similar measurements on night insects?

K. Hamdorf: No, so far experiments have been carried out only on the moth Deilephila elpenor, which is active in twilight.