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Pathways of Visual Pigment Regeneration in Fly Photoreceptor Cells*

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Abstract. From three possible pathways of rhodopsin regeneration which were investigated, only one, biosynthesis of rhodopsin, is shown to occur in fly photoreceptors 1–6. The prerequisite of this biosynthesis is the availability of 11-*cis* retinal which obviously starts opsin synthesis. This pathway seems to be part of a renewal process since metarhodopsin is found to be degraded in fly photoreceptors.

A dark-regeneration of rhodopsin from metarhodopsin as well as a biosynthesis of metarhodopsin and/or rhodopsin from all-trans retinal is not observed. These results indicate that all-trans retinal is not isomerized enzymatically to the 11-*cis* form, neither when it is bound to opsin (metarhodopsin) nor in its free form.

This leaves two pathways to regenerate rhodopsin in fly photoreceptors, *1*) the well-known photoregeneration, and *2*) the biosynthesis of rhodopsin.

Key words: Fly photoreceptors – Rhodopsin regeneration – Biosynthesis of rhodopsin – Visual pigment degradation

Introduction

Since the absorption of a light quantum by a rhodopsin molecule leads finally to photoproducts which no longer elicit a receptor response, and, since protein molecules, in general, have a limited lifetime, visual pigment molecules must be regenerated in order to maintain photoreceptor function. The term "regeneration" is used in this paper in its broadest sense which includes the restoration of the visual pigment molecule itself as well as the renewal of rhodopsin by biosynthesis. A more specific description of different pathways of regeneration based on the mechanisms involved will be given below.

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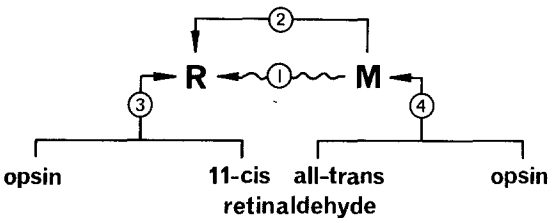

Fig. 1. Possible pathways of visual pigment regeneration in invertebrate photoreceptor cells

In vertebrates, rhodopsin "bleaches" as a result of light absorption, i.e., the molecule hydrolyzes into all-*trans* retinal and opsin. The bleached rhodopsin molecules are restored by a mechanism called "dark-regeneration" which involves an enzymatic isomerization of the all-trans chromophore into the 11-*cis* configuration. The 11-*cis* isomer then reacts spontaneously with opsin to form rhodopsin (for review see Baumann 1972; Bridges 1976; Knowles and Dartnall 1977). A second process, known as "renewal of photoreceptor membrane", includes the biosynthesis of rhodopsin which is incorporated into newly synthesized disk membrane (for review see Young 1975).

In invertebrates, the situation is less clear. Rhodopsin is not bleached by light but is converted to a stable photoproduct, metarhodopsin, which can be reconverted to rhodopsin by light. This peculiarity has first been demonstrated in cephalopods (Hubbard and St. George 1958; Brown and Brown 1958) and, subsequently, has been shown to occur in all invertebrate visual systems that have been studied. From electrophysiological experiments it became clear that this mechanism, "photoregeneration", plays an essential role in maintaining a high receptor sensitivity (for review see Hamdorf and Schwemer 1975; Hamdorf 1979). In addition to the well-characterized photoregeneration, a conversion of metarhodopsin to rhodopsin in the dark has been reported for a cephalopod (Schwemer 1969), some insects (Stavenga et al. 1973; Stavenga 1975; Goldman et al. 1975; Bernard 1979) and crustacea (Goldsmith and Bruno 1973; Bruno et al. 1977). In order to explain the regeneration of rhodopsin from metarhodopsin in the dark, an isomerase has been postulated that converts all-*trans* retinal into the 11-*cis* form. However, a dark-regeneration of rhodopsin was not found in ocelli of larval mosquito (Brown and White 1972) or in the compound eyes of *Calliphora* (Hamdorf et al. 1973) and *Drosophila* (Pak and Lidington 1974).

Mechanisms other than photo- and dark-regeneration that may be involved in maintaining photoreceptor function such as a biosynthesis of rhodopsin have not yet been clearly demonstrated.

Four possible pathways of visual pigment regeneration are summarized in Fig. 1: *1* photoregeneration; *2* dark-regeneration, i.e., conversion of meta-rhodopsin to rhodopsin in the dark; *3* biosynthesis of rhodopsin from 11-*cis* retinal and opsin; and *4* biosynthesis of metarhodopsin from all-*trans* retinal and opsin which then could be converted to rhodopsin by absorbing light (Whittle 1976); if however rhodopsin is formed from all-*trans* retinal in the dark, this would indicate that the fly has an isomerase. The purpose of this study is to investigate the last three pathways; the first, photoregeneration, has been

already well characterized (Hamdorf 1979). In addition, it will be shown that visual pigment undergoes degradation in photoreceptors of a fly.

Flies seemed to be very well suited for this study because they can easily be raised on a Vitamin A-deficient diet (Goldsmith et al. 1964) which leads to rather low amounts of rhodopsin in the photoreceptor membrane (Schwemer, see Razmjoo and Hamdorf 1976, for *Calliphora;* Harris et al. 1977, for *Drosophila*). These Vitamin A-deficient animals were used to examine pathways 3 and 4, whereas pathway 2 as well as the degradation were studied in flies with "normal" rhodopsin content.

Materials and Methods

The animal used in this study was the blowfly, *Calliphora erythrocephala* chalky, a white-eyed mutant which lacks all screening pigments (Langer 1962).

Larvae were raised on either bovine liver or bovine heart meat which are known to contain a high and a very low amount of retinol derivatives, respectively. It has been demonstrated spectrophotometrically that the rhabdomeres of photoreceptors 1–6 of adult flies fed bovine liver contain a "normal" amount of rhodopsin, while those fed bovine heart meat contain a low amount (1–5% of that of normal flies). According to the rhodopsin content, the flies will be termed R^+- flies and R^-- flies.

Measurement of Rhodopsin Content

In order to estimate the rhodopsin content of photoreceptors 1–6, the eye was sectioned from the head with a razor blade, and the remaining parts of the brain and ganglia were removed with fine forceps. Thus, the resulting preparation consisted of the cornea and, attached to it, the retina. The isolated eye was then mounted in insect Ringer's (Ephrussi and Beadle 1936) in a holder which was covered on both sides with cover slips. The preparations were usually performed in red light. In all experiments where changes in the state of the visual pigment caused by light had to be avoided, dissection and mounting of the eyes were performed in infrared light with the help of an infrared converter. The sample was then transferred to a spectrophotometer (Hitachi, Model 356), whose measuring beam passed through the central part of the eye; the diameter of the area measured was 0.8 mm. The sample was irradiated with red light (635 nm; actinic light source: xenon arc, 150 W) in order to convert all metarhodopsin (λ_{max} 570 nm) present in the photoreceptor membranes to rhodopsin (λ_{max} 490 nm). This "dark-adapted" eye was then irradiated with blue light (472 nm) while, at the same time, the absorbance change at 580 nm was recorded. When no further increase in absorbance could be observed, it was assumed that the photoequilibrium between rhodopsin and metarhodopsin (being about 30 : 70% for this particular wavelength) was reached. The total absorbance increase at 580 nm, which was due to the conversion of 70% rhodopsin into metarhodopsin, was then used as a measure of the rhodopsin content of the photoreceptors. Possible contributions of the central photoreceptors 7 and 8 were neglected

because neither their rhodopsins nor their metarhodopsins have a considerable absorbance at 580 nm.

All filters used in this study were interference filters (Schott & Gen., Mainz, FRG).

Injection Experiments

Injection of retinal into one eye of each fly was performed with a glass capillary (tip diameter approximately 70 μm) which was connected to a microliter syringe (10 μl) via a polyethylene tube. After the system was filled with water, retinal was taken up via the glass capillary. Reproducible injection volumes (0.2 μl) were obtained by using a repeating dispenser. Under the dissecting microscope, the capillary was directed towards the dorsal corner of the eye of an immobilized fly with a micromanipulator. The cornea was penetrated, and the sample was injected. All injections were performed in dim red light in order to prevent isomerization of retinal. After the injection, the animals were returned to cages and kept in the dark until they were used for experiments.

Results

Dark-Regeneration

R^+-flies were adapted to blue light (470 nm) for 1 h in order to convert 70% of the rhodopsin into metarhodopsin. Afterwards, the animals were kept in the dark. At certain time intervals, flies were taken from this batch, and their eyes were isolated and mounted in infrared light. Immediately after transferring the sample to the spectrophotometer, the measuring beam was switched on. Shortly afterwards, the eye was irradiated with red light (635 nm) in order to convert all metarhodopsin present in the photoreceptor membrane to rhodopsin. At the same time, the absorbance decrease was recorded as a measure of the fraction of metarhodopsin. When no further change in absorbance could be observed, the retina was irradiated with blue light (472 nm) in order to again establish the photoequilibrium which was present before the animals were dark adapted. The difference between the absorbance changes caused by red light (metarhodopsin $\xrightarrow{635\text{ nm}}$ rhodopsin) and blue light (rhodopsin $\xrightarrow{472\text{ nm}}$ metarhodopsin) then indicated the fraction of metarhodopsin which was converted in the dark.

Figure 2, 1 shows the result of a control animal which has been measured at the end of adaptation to blue light. The absorbance decrease found during irradiation with red light corresponds exactly to the subsequent absorbance increase caused by blue light. Since the absolute absorbance changes are identical, it is concluded that the photoequilibrium between rhodopsin and metarhodopsin (30 : 70%) has indeed been obtained by adapting the live flies to blue light. The following pairs of absorbance changes (Fig. 2; pairs 2 and 3) were recorded after 30 and 60 min of dark adaptation, respectively. In both cases,

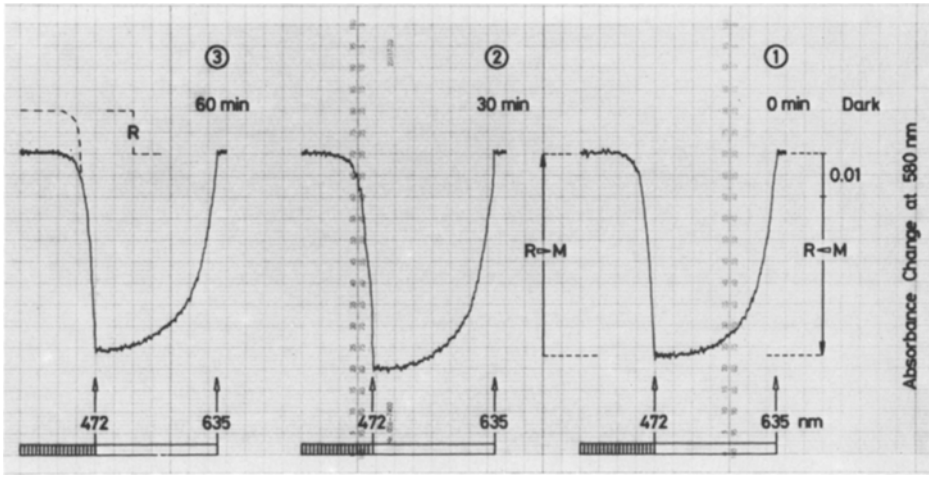

Fig. 2. Metarhodopsin content in photoreceptors of R^+-flies after dark adaptation. The absorbance changes (read from right to left) caused by actinic irradiation with light of the wavelengths 635 and 472 nm, respectively, are measured at 580 nm. Onsets of actinic irradiation are marked by arrows. 1: content of metarhodopsin immediately after 1 h of adaptation to blue light; 2 and 3: content of metarhodopsin following a dark period of 30 and 60 min, respectively

irradiation of the retina with blue light caused exactly the same absorbance changes as the preceding irradiation with red light.

The results demonstrate that the fraction of metarhodopsin remained unchanged within 1 h of dark adaptation, i.e., these results clearly do not support the hypothesis of a dark-regeneration within 1 h.

If some metarhodopsin had been converted to rhodopsin in the dark, the absorbance decrease due to red light would have been smaller than the absorbance increase caused by the subsequent irradiation with blue light. Such a result is indicated by the dashed line in example 3 of Fig. 2; the difference between the absorbance changes (R) would have indicated the fraction of metarhodopsin that had been reconverted to rhodopsin.

Biosynthesis of Rhodopsin Following Injection of 11-cis Retinal

The question of whether adult flies are able to synthesize rhodopsin was of basic interest.

In order to test the possibility of a spontaneous formation of rhodopsin in R^--flies, excised retinae from R^--flies were incubated with 11-*cis* retinal in the dark for up to two hours at room temperature. These experiments, however, did not yield measurable amounts of rhodopsin.

Therefore, 11-*cis* retinal was injected into one of the eyes of live R^--flies which were then kept in the dark. Figure 3 shows that injection of this compound leads to the formation of a photosensitive pigment. In Fig. 3a, the difference spectra from the non-injected eye (squares) and the injected eye (circles) of a

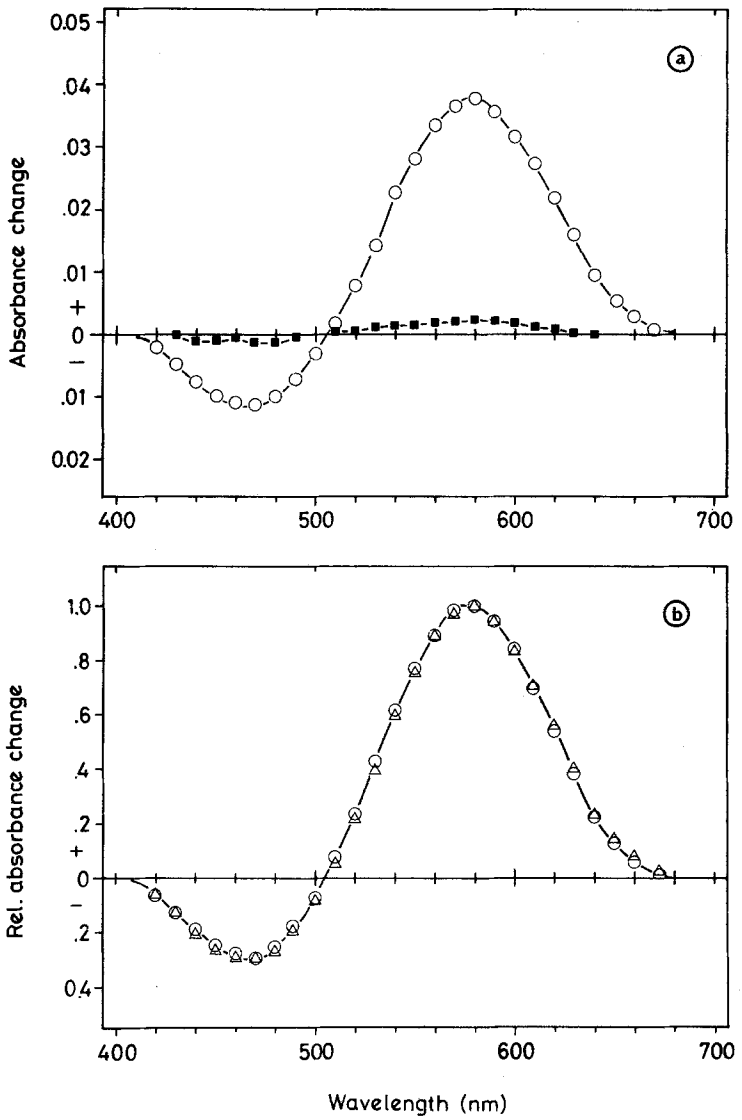

Fig. 3 a and b. Rhodopsin formation in photoreceptors of R^--flies after injection of 11-*cis* retinal. **a** Absorbance changes (difference spectrum) in isolated eyes of a R^--fly. One eye (circles) was injected with 11-*cis* retinal about 30 h prior to the measurement, while the other eye (squares) was not. Both difference spectra were recorded subsequently to an actinic irradiation with light of 472 nm. **b** Comparison of difference spectra recorded from an eye of a R^+-fly (circles) and an eye from a R^--fly which was injected with 11-*cis* retinal (triangles)

R^--fly are shown. The spectra were obtained approximately 30 h after the injection. Whereas irradiation with blue light leads only to small absorbance changes in the non-injected eye, fairly large changes peaking at about 470 nm (decrease) and 580 nm (increase) can be seen in the injected eye. The shape of this difference spectrum is identical with that obtained from an isolated eye of a

R^+-fly indicating that the photosensitive pigment formed after injection is rhodopsin.

In a further study, the time-course of rhodopsin formation was followed. At various times in the dark, injected R^--flies were removed from the cage, and their eyes were dissected in dim red light. As before, the amount of visual pigment formed was measured by simple determining the absorbance increase at 580 nm during irradiation with blue light. The total absorbance change caused by this irradiation is equivalent to 70% of the rhodopsin. Fig. 4 (dots) demonstrates that the rhodopsin content of photoreceptors 1–6 increases with time and reaches a maximal amount approximately 40 h after injection. The time-course of rhodopsin formation was approximated by a first order kinetic with a rate constant $k = 70 \times 10^{-3}\,h^{-1}$. No increase in rhodopsin was found in the non-injected eye of each fly indicating that, at least during the time of the experiment, retinal was not transported from one eye to the other.

Biosynthesis of Metarhodopsin and/or Rhodopsin Following Injection of All-Trans Retinal

The injection experiments were performed in the same way as just described, except that all-*trans* retinal was used for injection and the preparation was performed in infrared light in order to prevent conversion of any metarhodopsin that could have been created from injected all-trans retinal. In order to detect metarhodopsin, the isolated eyes were first irradiated with red light which shifts metarhodopsin to rhodopsin. Subsequently, the samples were irradiated with blue light in order to measure the rhodopsin content.

The results obtained in this series of experiments (Fig. 4, open circles) demonstrate that neither red nor blue light induced major absorbance changes at 580 nm. This clearly indicates that neither metarhodopsin nor rhodopsin were formed after the injection of all-*trans* retinal. Even 70 h after injection, the rhodopsin content did not exceed that which was present before injection (Fig. 4, triangle at zero time). Therefore, the data suggest, that, in the fly retina, all-*trans* retinal is not bound to opsin to yield metarhodopsin and, furthermore, there is no enzyme present that can isomerize all-*trans* retinal in the dark. Such an enzyme could have conceivably led to rhodopsin formation.

Visual Pigment Degradation

The rhodopsin formation found in R^--flies could also occur in R^+-flies as part of a renewal of visual pigment. In other words, if there is a formation of rhodopsin in flies with "normal" rhodopsin content, there should also be a degradation of visual pigment. If flies degrade their pigment, it should be evident during dark adaptation, because all-*trans* retinal is not isomerized under this condition and thus, rhodopsin formation is blocked.

Therefore, R^+-flies were exposed to blue light for 1 h and then kept in the dark. Again, all manipulations were performed in infrared light. First, the

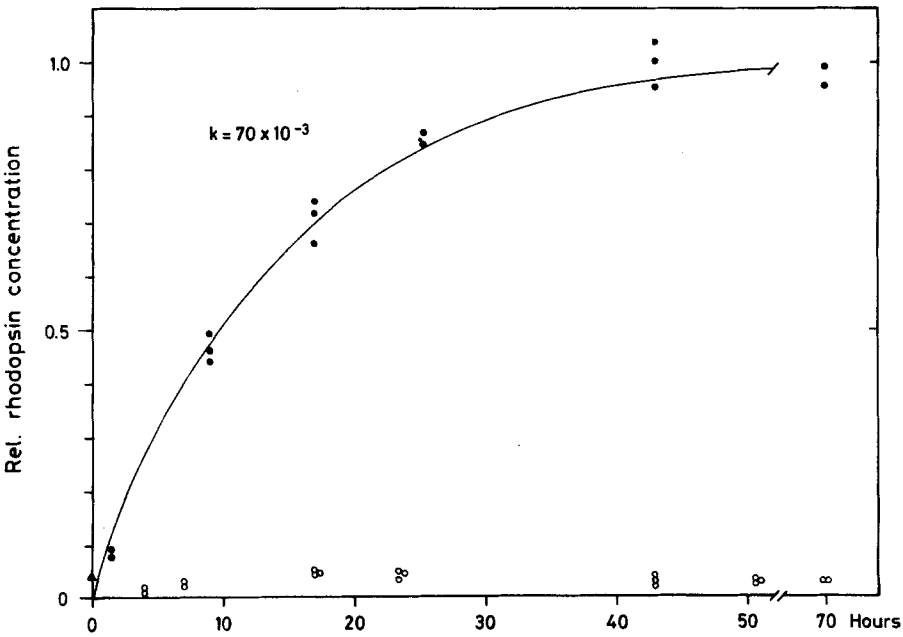

Fig. 4. Rhodopsin content of photoreceptors of R^--flies at different times after injection of 11-*cis* retinal (dots) and all-*trans* retinal (circles). The rhodopsin increase was approximated by a first order reaction with a rate constant $k = 70 \times 10^{-3}\ h^{-1}$ (solid line). Each symbol represents a measurement from a single animal

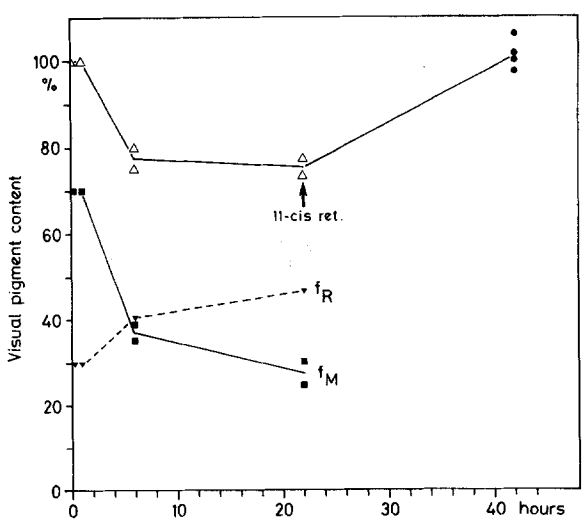

Fig. 5. Visual pigment content in photoreceptors of R^+-flies after various times in the dark. Prior to dark adaptation, the animals were exposed to blue light for 1 h. The total visual pigment content (open triangles, $f_M + f_R$) is shown as well as the fractions of metarhodopsin (f_M, squares) and rhodopsin (f_R, dashed line). – After 22 h in the dark, 11-*cis* retinal was injected into the eyes of some animals; dots indicate the total visual pigment of these eyes which was maximal by 20 h after injection. Each symbol represents a measurement of a single animal

fraction of metarhodopsin was determined (irradiation with 635 nm), and then the total amount of visual pigment was measured (irradiation with 472 nm). Fig. 5 shows that, at the beginning of the dark period, the total amount of visual pigment (100%; open triangles) consisted of 70% metarhodopsin (f_M; squares) and 30% rhodopsin (f_R; filled triangles). Whereas no changes could be seen after

1 h, a decrease of f_M by about 32% was found after 6 h of dark adaptation. f_M continued to decrease, reaching about 28% after 22 h of dark adaptation. During the same time, the total visual pigment content was only reduced to 78 and 75%. If only metarhodopsin was degraded during that time, the decrease of the total visual pigment content should have been the same as the decrease found for f_M. The reason for this difference is given by f_R which did not remain constant (30%) but increased within 22 h by about 17%. Nevertheless, the data clearly demonstrate a significant loss of metarhodopsin.

After 22 h in the dark, some animals were injected with 11-*cis* retinal. Twenty hours after injection, the visual pigment content was again measured. As can be seen from Fig. 5 (dots), the original amount of visual pigment was restored during that time. From this result, it is concluded that the loss of visual pigment is not due to a deterioration of the photoreceptor cells caused by the prolonged dark period, but to a lack of 11-*cis* retinal during these experimental conditions.

Discussion

The results presented in this paper indicate that, in addition to photoregeneration, only one of the three pathways studied, biosynthesis of rhodopsin, can occur in photoreceptors of adult flies. The prerequisite of this rhodopsin formation was shown to be the availability of 11-*cis* retinal. Freeze fracture studies of photoreceptor membranes of R^+- and R^--flies have shown that Vitamin A-deficiency causes not only a reduction of visual pigment, but also a drastic decrease of particle density on P-faces of microvillar membranes (Boschek and Hamdorf 1976; Harris et al. 1977). Direct evidence, that photoreceptor membranes of R^--flies contain indeed only a low amount of the visual pigment protein opsin came from SDS polyacrylamide gel electrophoresis (Paulsen and Schwemer 1979) indicating that opsin was not synthesized in the absence of retinal. Therefore, it is concluded that the formation of rhodopsin after injection of 11-*cis* retinal is due to a de novo synthesis, and furthermore, that opsin synthesis strongly depends on the presence of 11-*cis* retinal. The maximal amount of rhodopsin corresponding to that of R^+-flies, is reached at approximately 40 h after injection.

It is almost impossible to trace a biosynthesis of rhodopsin in R^+-flies using only spectrophotometry. Since such a biosynthesis in R^+-flies can be considered to be part of a renewal of rhodopsin, it was more feasible to look for a visual pigment degradation. Figure 5 not only demonstrates that visual pigment is lost in the dark, but that mainly metarhodopsin is broken down. Whereas the content of metarhodopsin decreases from 70% to about 28%, the total visual pigment content ($f_R + f_M$) is reduced by only 25%. If mainly metarhodopsin was degraded and f_R remained constant at 30% (the fraction resulting from the initial photoequilibrium) the decrease in the total visual pigment content should have paralleled the decrease of f_M. The difference between these two functions, f_R, shows that the rhodopsin content is increased by about 17% during the 22-h' dark period. Since all-*trans* retinal is not isomerized under these experimental

conditions, the increase of rhodopsin was probably due to biosynthesis of rhodopsin caused by a limited amount of 11-*cis* retinal present in the photoreceptor cells. Thus, this experiment does demonstrate both a break down of metarhodopsin and a simultaneous biosynthesis of rhodopsin. A more detailed study on the turnover of visual pigment in fly photoreceptors will be published elsewhere (Schwemer, in prep.).

In contrast to 11-*cis* retinal, the all-*trans* isomer did not lead to a synthesis of metarhodopsin (pathway 4) as was proposed by Whittle (1976). Since rhodopsin was also not formed, it is concluded that an enzymatic isomerization does not occur. This result coincides with the finding that metarhodopsin was not reconverted to rhodopsin in the dark (pathway 2). It has been concluded from photochemical studies (Hamdorf et al. 1973), that such a conversion initially requires the isomerization of all-*trans* retinal which then triggers conformational changes of opsin. This first step, the isomerization of all-*trans* retinal when it is still bound to the protein moiety did not occur in the dark. This finding also supports the hypothesis that the increase of f_R in photoreceptors of R^+-flies (Fig. 5) in the dark is caused by a de novo synthesis of rhodopsin.

This particular result seems to contradict studies where a dark-regeneration has been postulated. However, the data obtained with flies (Stavenga et al. 1973) can probably be explained on the basis of the results presented here. This is especially likely since the life-times for the absorbance increase in the blue spectral range (= increase of rhodopsin) and the decrease in the yellow spectral range (= decrease of metarhodopsin) found in that study were not identical. In addition, earlier findings in butterflies which were interpreted as a dark-regeneration (Stavenga 1975; Bernard 1977) are most likely due to a degradation of metarhodopsin and a concomitant synthesis of rhodopsin. Here again, the kinetics of the decay of metarhodopsin and the recovery of rhodopsin are different, but both processes seem to be faster than those found in fly photoreceptors (Bernard 1983). For a moth, it was reported that rhodopsin regeneration required several days after the animals were exposed to daylight for a few minutes (Goldman et al. 1975). Because of the slow rate of this process, the authors concluded that a renewal of photoreceptor membrane is involved in this moth. Whether the quite different rates which were observed are due to experimental differences or, a more likely possibility, depend on the species, remains to be demonstrated by future studies.

A recovery of rhodopsin from metarhodopsin was also found in the lobster (Goldsmith and Bruno 1973; Bruno et al. 1977); possible mechanisms discussed were a dark-regeneration and an exchange of chromophore. However, the authors conceded that the recovery of rhodopsin may be due to a membrane turnover of photoreceptor membrane which, again, would involve a biosynthesis of rhodopsin.

In conclusion, similarly as in vertebrates, flies, and probably most insects, are provided with only two pathways of visual pigment regeneration: *1)* the fast mechanism of photoregeneration, which is responsible for a high rhodopsin content on a short term basis, and *2)* a biosynthesis of rhodopsin, which is part of a renewal process ensuring photoreceptor function over a long period of time.

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References

Baumann Ch (1972) The regeneration and renewal of visual pigment in vertebrates. In: Dartnall HJA (ed) Photochemistry of vision. Springer, Berlin Heidelberg New York (Handbook of sensory physiology, vol VII/1, pp 395–416)

Bernard DG (1979) Red-absorbing visual pigment of butterflies. Science 203: 1125–1127

Bernard GD (1983) Bleaching of photoreceptors in eyes of intact butterflies. Science (in press)

Boschek BC, Hamdorf K (1976) Rhodopsin particles in the photoreceptor membrane of an insect. Z Naturforsch 31c: 763

Bridges CDB (1976) Vitamin A and the role of the pigment epithelium during bleaching and regeneration of rhodopsin in the frog eye. Exp Eye Res 22: 435–455

Brown PK, Brown PS (1958) Visual pigments of the octopus and cuttlefish. Nature (London) 182: 1288–1290

Brown PK, White RH (1972) Rhodopsin of the larval mosquito. J Gen Physiol 59: 401–414

Bruno MS, Barnes SN, Goldsmith TH (1977) The visual pigment and visual cycle of the lobster, Homarus. J Comp Physiol 120: 123–142

Ephrussi B, Beadle GW (1936) A technique of transplantation for Drosophila. Am Nat 70: 218–225

Goldman LJ, Barnes SN, Goldsmith TH (1975) Microspectrophotometry of rhodopsin and metarhodopsin in the moth Galleria. J Gen Physiol 66: 383–404

Goldsmith TH, Barker RJ, Cohen CF (1964) Sensitivity of visual receptors of carotenoid-depleted flies: a Vitamin A deficiency in an invertebrate. Science 146: 65–67

Goldsmith TH, Bruno MS (1973) Behavior of rhodopsin and metarhodopsin in isolated rhabdoms of crabs and lobster. In: Langer H (ed) Biochemistry and physiology of visual pigments. Springer, Berlin Heidelberg New York, pp 147–153

Hamdorf K (1979) The physiology of invertebrate visual pigments. In: Autrum H (ed) Invertebrate photoreceptors. Springer, Berlin Heidelberg New York (Handbook of sensory physiology, vol VII/6A, pp 145–224)

Hamdorf K, Paulsen R, Schwemer J (1973) Photoregeneration and sensitivity control of photoreceptors of invertebrates. In: Langer H (ed) Biochemistry and physiology of visual pigments. Springer, Berlin Heidelberg New York, pp 155–166

Hamdorf K, Schwemer J (1975) Photoregeneration and the adaptation process in insect photoreceptors. In: Snyder AW, Menzel R (eds) Photoreceptor optics. Springer, Berlin Heidelberg New York, pp 263–289

Harris WA, Ready DF, Lipson ED, Hudspeth AJ, Stark WS (1977) Vitamin A deprivation and *Drosophila* photopigments. Nature (London) 266: 648–650

Hubbard R, St George RCC (1958) The rhodopsin system of the squid. J Gen Physiol 41: 501–528

Knowles A, Dartnall HJA (1977) The Photobiology of vision. In: Davson H (ed) The eye, vol 2B. Academic Press, New York London

Langer H (1962) A new eye colour mutation in Calliphora erythrocephala Meig. Nature (London) 194: 111–112

Pak WL, Lidington KJ (1974) Fast electrical potential from a long-lived, long wavelength photoproduct of fly visual pigment. J Gen Physiol 63: 740–756

Razmjoo S, Hamdorf K (1976) Visual sensitivity and the variation of total photopigment content in the blowfly photoreceptor membrane. J Comp Physiol 105: 279–286

Schwemer J (1969) Der Sehfarbstoff von Eledone moschata und seine Umsetzungen in der lebenden Netzhaut. Z Vergl Physiol 62: 121–152

Stavenga DG (1975) Dark regeneration of invertebrate visual pigments. In: Snyder AW, Menzel R (eds) Photoreceptor optics. Springer, Berlin Heidelberg New York, pp 290–295
Stavenga DG, Zantema A, Kuiper JW (1973) Rhodopsin processes and the function of the pupil mechaqnism in flies. In: Langer H (ed) Biochemistry and physiology of visual pigments. Springer, Berlin Heidelberg New York, pp 175–180
Whittle A (1976) Reticular spezialisations in photoreceptors: a review. Zool Scr 5: 191–206
Young RW (1975) Visual cells and the concept of renewal. Invest Ophthalmol Vis Sci 15: 700–725

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