J Comp Physiol A (1984) 154:535–547

Journal of Comparative Physiology A Sensory, Neural, and Behavioral Physiology

Renewal of visual pigment in photoreceptors of the blowfly

Joachim Schwemer
Institut für Tierphysiologie, Ruhr-Universität, Postfach 102148, D-4630 Bochum 1, Federal Republic of Germany

Accepted January 10, 1984

Summary. Spectrophotometric measurements of photoreceptors 1–6 in the blowfly demonstrate that rhodopsin undergoes a continuous renewal. This involves, in the dark, the slow degradation of rhodopsin whereas metarhodopsin is degraded at a much faster rate. The effect of light is to reduce the rate at which metarhodopsin is degraded, i.e. the rate is inversely related to the intensity of the light. Rhodopsin synthesis is dependent on the presence of 11-*cis* retinal which is formed via a photoreaction from all-*trans* retinal resulting from the breakdown of rhodopsin and/or metarhodopsin: the biosynthesis of rhodopsin is therefore a light dependent process. Light of the blue/violet spectral range was found to mediate the isomerization of all-*trans* retinal into the 11-*cis* form. It is proposed that this stereospecificity is the result of all-*trans* retinal being bound to a protein. On the basis of the results a visual pigment cycle is proposed.

Introduction

The pioneering studies of Hubbard and St. George (1958), Brown and Brown (1958), and Kropf et al. (1959) demonstrated that cephalopod metarhodopsin is thermostable, relative to vertebrate metarhodopsins, and that rhodopsin can be regenerated from metarhodopsin by light in a reaction known as photoregeneration. Subsequent investigations of visual pigment systems in different invertebrates have been mainly concerned with the photochemistry of the pigments. In general, these systems are all characterized by the presence of a thermostable metarhodopsin which can be converted to rhodopsin by photoregeneration (e.g. Schwemer 1969; Hamdorf et al. 1971a; Schwemer et al. 1971; Brown and White 1972; Goldsmith and Bruno 1973; Stavenga et al. 1973; for review see Hamdorf 1979; Stavenga and Schwemer, in press). The physiological significance of this fast photoregeneration was first recognized from the results of electrophysiological studies on insect photoreceptors (Hamdorf et al. 1971b, 1973) which, together with similar results from other invertebrate photoreceptors, led to the conclusion that photoregeneration is the main mechanism by which the rhodopsin content, and thus the receptor sensitivity, is maintained at a high level (for review see Hamdorf and Schwemer 1975; Hamdorf 1979; Hillman et al. 1983; Stavenga and Schwemer, in press).

That the visual pigment of invertebrates might also be regenerated by a process of membrane turnover was indicated by the results of early morphological studies of photoreceptor cells (White 1964, 1967, 1968) which showed that the volume of the rhabdom is reduced in the light and increased again in darkness. However, these results and those of other morphological investigations (e.g. Eguchi 1965; Eguchi and Waterman 1966, 1967; Blest 1978, 1980) provided only indirect evidence for the renewal of visual pigment. Similarly, radioautographic experiments demonstrated that labelled amino acids are incorporated into proteins which, in part, could be found within the rhabdoms (Burnel et al. 1970; Perrelet 1972; Tuurala and Lehtinen 1974), but in none of these cases was the labelled protein shown to be opsin.

Microspectrophotometric measurements of visual pigment in a moth showed that the recovery of rhodopsin required several days after the animals were exposed to daylight, suggesting that a renewal of photoreceptor membrane is involved (Goldman et al. 1975). Evidence that opsin undergoes a light dependent turnover in the mosquito

was presented by Stein et al. (1979). More recently, the biosynthesis of rhodopsin and, its counterpart in the visual cycle, the breakdown of visual pigment have been clearly demonstrated in adult blowflies (Schwemer 1983). The presence of 11-*cis* retinal, the chromophore of rhodopsin, was found to be a prerequisite for the biosynthesis of rhodopsin. Furthermore, it was shown that all-*trans* retinal cannot be isomerized in the dark to the 11-*cis* form, nor could metarhodopsin be regenerated in the dark to form rhodopsin.

The latter results formed the basis for the present study on the degradation of rhodopsin and metarhodopsin and the biosynthesis of rhodopsin, processes which are tightly coupled to the isomerization of all-*trans* retinal. The relatively simple methods used, of determining the amount of visual pigment in situ after experimentally manipulating blowflies by subjecting them to carefully designed light regimes, allows one to effectively 'dissect' the visual cycle to reveal the mechanisms operating within it.

Materials and methods

White-eyed blowflies, *Calliphora erythrocephala* Meig. 'chalky', were used in this study. In order to obtain flies with a consistently high level of visual pigment, the larvae were raised on bovine liver which is known to contain large amounts of vitamin A-derivatives.

Adaptation of the flies. Flies were adapted to different lights in a wooden cabinet which was divided into four compartments, each panelled with aluminum foil. A large window in the top of each compartment was covered by a filter (see below) and the whole cabinet illuminated with artificial daylight from fluorescent tubes mounted 1.5 m above it (Philips TL, U 25; their relative spectral flux is shown in Fig. 1 a).

The flies were kept in lucite boxes containing supplies of sugar and water and the boxes placed in each of the compartments of the cabinet. Different lighting conditions, including

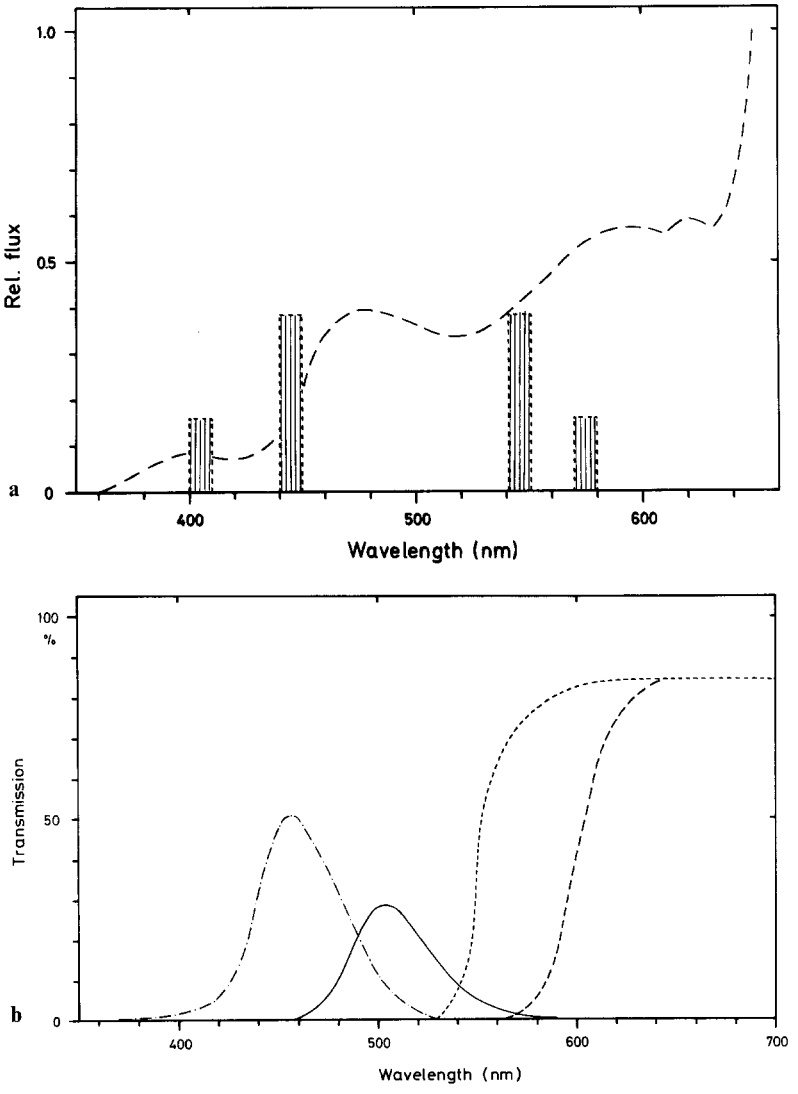

Fig. 1. a Relative spectral flux of the fluorescent tubes (dashed line) used to illuminate the flies. Bars represent narrow emission peaks in the spectrum whose energy content has been recalculated for a 10 nm range in order to bring them within the scale of the graph.
b Spectral tansmission of coloured, acrylic glass filters used for setting up specific lighting conditions. –·–·– blue; —— green; ---- yellow; ——— red

darkness, could be set up in each compartment according to the choice of filter used to cover them. Blue, green, yellow, and red filters were used (acrylic glas, Röhm GmbH, W. Germany; their transmission spectra are shown in Fig. 1b). The absolute intensities, determined by a thermopile within the compartment, were 8.2×10^{-5} W cm^{-2} for 'white', 2.7×10^{-5} W cm^{-2} for red, 4.5×10^{-5} W cm^{-2} for yellow, 7.8×10^{-6} W cm^{-2} for green and 1×10^{-5} W cm^{-2} for blue light. Intensities were reduced by grey filters (acrylic glas, Röhm GmbH, W. Germany). The room temperature was kept constant at 21 °C.

Determination of rhodopsin and metarhodopsin content. The rhodopsin content of photoreceptors 1–6 was determined spectrophotometrically in eye cup preparations consisting of the cornea and underlying retina. A detailed description of the isolation of the eye and the mounting of the eye cup has already been published (Schwemer 1983).

The total rhodopsin content of the photoreceptors was determined as follows. The eye cups of flies which had been subjected to darkness or various light conditions were transferred to the spectrophotometer (Hitachi, model 356) in dim red light. Within the spectrophotometer, the eye cup was further adapted to monochromatic red light (635 nm; interference filter, Schott & Gen., W. Germany; Xenon arc light source, XBO 150 W). This adaptation converted any metarhodopsin remaining in the photoreceptor membranes into rhodopsin. The eye cup was then adapted to monochromatic blue light (472 nm; interference filter, Schott & Gen., W. Germany) and the absorbance increase measured simultaneously at 580 nm. This adaptation was continued until no further change in absorbance was recorded, i.e., a photoequilibrium between rhodopsin ($\lambda_{max} =$ 490 nm) and metarhodopsin ($\lambda_{max} = 570$ nm) was established which, under the conditions used, was about 30:70% (Hamdorf et al. 1973). Thus the absorbance increase measured at 580 nm was always due to 70% of the total rhodopsin content (i.e. the sum of the original rhodopsin and metarhodopsin content) being converted into metarhodopsin. The total rhodopsin content in the eyes of flies subjected to various experimental conditions was related to that measured at the start of the experiment in control flies (taken as 1.0) to give the 'relative rhodopsin content'.

This procedure does not, however, allow the fraction of rhodopsin (f_R) and the fraction of metarhodopsin (f_M) present in the eye at the time the animals were sacrificed to be determined. In order to determine the metarhodopsin content (results presented in Fig. 4), the preparation of the eyes was carried out under infrared light with the aid of an infrared converter. After transferring the preparation to the spectrophotometer, the measuring beam (set at 580 nm) was switched on and, after marking the absorbance with the recorder, the red adapting light (635 nm) switched on. The total decrease in absorbance was recorded, related to the absorbance decrease in control flies (70% metarhodopsin) and plotted as relative metarhodopsin content.

Injection of 11-cis retinal. The eyes of some animals were injected with 11-*cis* retinal to assess the physiological state of the photoreceptor cells. The injection procedure is described by Schwemer (1983).

Results

Degradation of rhodopsin in the dark

The total rhodopsin content of flies after pupation was found to increase each day by about 5% of the maximum level which was usually reached on the fourth day. Thereafter the total rhodopsin content remained fairly constant throughout the five week life of the flies when they were kept under a 12/12 h cycle of 'white' room light and darkness.

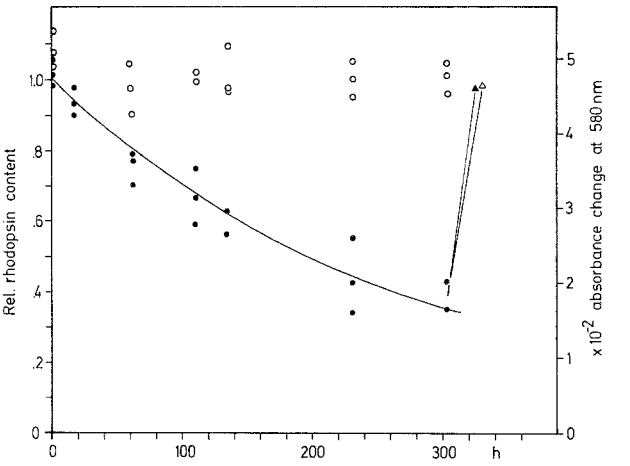

Fig. 2. Relative rhodopsin content in photoreceptors 1–6 of flies kept under a 12/12 h cycle of light and darkness (open circles) and continuous darkness (filled circles). Before the total rhodopsin content was measured, the metarhodopsin present was converted into rhodopsin with red light. – Each symbol represents a single fly in this and the following figures, unless otherwise stated. Solid line represents the least-square approximation to a first-order degradation of rhodopsin with a rate constant $k = (3.7 \pm 0.3) \times 10^{-3} h^{-1}$. The same procedure of approximation has been used in the other figures. An increase of rhodopsin content was found after injection of 11-*cis* retinal into flies (filled triangle; mean of 2 animals) and after transferring the flies to 'white' light (open triangle; mean of 3 animals). Right-hand ordinate: Absorbance changes measured at 580 nm caused by irradiation with blue light (472 nm)

A culture of flies was divided into one control group which was kept on the 12/12 h cycle of light and darkness and a second group of flies which were first adapted to red light for two hours, in order to convert metarhodopsin to rhodopsin, and then kept in the dark. Measurements of the total rhodopsin content after different times showed that it steadily decreased with time in flies kept in the dark (Fig. 2), whereas control animals maintained a consistently high rhodopsin content. The time-course of the rhodopsin decrease fits that of a first-order reaction with a rate constant $k = 3.7 \times 10^{-3} h^{-1}$. To ensure that this decrease in rhodopsin content was not due to the degeneration of the photoreceptor cells in darkness, flies were taken from the dark and injected with 11-*cis* retinal under dim red light and then returned to darkness. As can be seen from Fig. 2, the amount of rhodopsin in the eyes of these animals returned to its original value (that of the controls) within about 20 h of the injection.

This result indicates that the decrease of rhodopsin was not due to a degeneration of the photoreceptor cells since they were capable of synthesizing the same amount of rhodopsin as control eyes, if provided with exogenous 11-*cis* retinal which they evidently lacked.

Despite the fact that adult flies cannot isomerize all-*trans* retinal in the dark (i.e. enzymatically) yet require 11-*cis* retinal for the synthesis of rhodopsin (Schwemer 1983), they can nevertheless maintain a high rhodopsin content when kept in the light (see Fig. 2, controls). Therefore, light seems in some way to be involved in the isomerization process. This was evident from the results of returning flies into 'white' light after they had been in the dark for 300 h: as with injection of 11-*cis* retinal, the total rhodopsin content of the photoreceptors increased rapidly showing that rhodopsin synthesis occurred in the light. Thus, the decrease in rhodopsin content, which probably resulted from the degradation of rhodopsin, was made good for by a light-induced synthesis.

Degradation of metarhodopsin in the dark

A similar experimental design was used to investigate whether or not the presence of metarhodopsin influences the rate at which the measured total rhodopsin content decreases in darkness. Thus one group of flies was adapted to red light and a second group adapted to blue light for two hours which converted 70% of the total rhodopsin content into metarhodopsin. Thereafter, both groups were kept in the dark. At different times flies were removed and the total rhodopsin content was measured after first converting metarhodopsin into rhodopsin by red light (635 nm). In control animals adapted to red light the total rhodopsin content decreased steadily as shown in Fig. 2 with a rate constant $k = 5 \times 10^{-3} h^{-1}$ (Fig. 3). In contrast, the total rhodopsin content of flies adapted to blue light decreased rapidly until approximately 35% remained, after which the rate of decrease was markedly slower (Fig. 3). The total rhodopsin content was reduced to half approximately 2.5 times faster that in red adapted flies which contained no measurable amount of rhodopsin. A simple exponential decay ($k = 14 \times 10^{-3} h^{-1}$) calculated on the basis of the first four values (time 0 to 72 h) does not fit the remaining data (time 94 to 192 h). This result indicates that at least two processes with different rate constants are involved, conceivably the breakdown of metarhodopsin and rhodopsin respectively. However, since it was the total rhodopsin content that was measured (i.e., any metarhodopsin present was first converted into rhodopsin), no conclusion can be drawn from this result specifically concerning the rate constant of metarhodopsin breakdown.

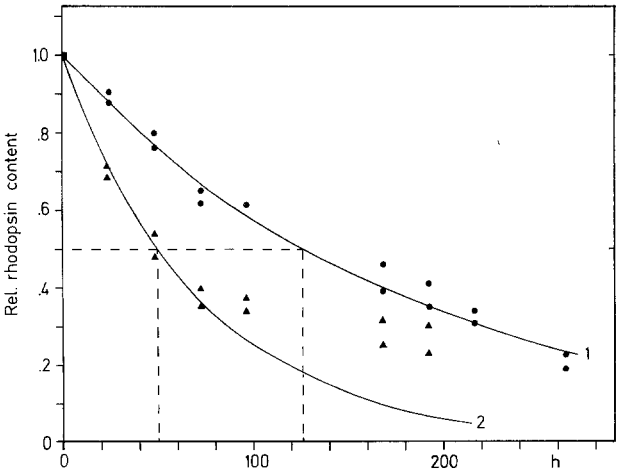

Fig. 3. Relative rhodopsin content in photoreceptors of flies kept in darkness after adaptation to red light (circles) or blue light (triangles). In the former case, the rhodopsin content decreased exponentially with a rate constant of $k_1 = (5.3 \pm 0.3) \times 10^{-3} h^{-1}$. Data obtained from blue adapted flies represent combined effects of rhodopsin *and* metarhodopsin decay (any metarhodopsin present before the measurement was first converted into rhodopsin with red light) and cannot be approximated to by a single exponential decay. Solid line represents an exponential decay with a rate constant $k_2 = (13.7 \pm 0.8) \times 10^{-3} h^{-1}$ which fits only the first four values plotted (time 0 to 72 h). Dashed lines indicate halftimes of decay of rhodopsin (curve 1) and decay of rhodopsin and metarhodopsin together (curve 2)

To investigate the degradation of metarhodopsin, the decrease in metarhodopsin content was measured in a group of flies adapted to blue light for two hours and then kept in the dark. This time, however, the eye cups were prepared in infrared light in order not to change the content of metarhodopsin which was then determined by irradiating with red light (635 nm). The results are presented in Fig. 4 and clearly demonstrate that the decrease in metarhodopsin content occurred at a high rate with the kinetic of a first-order reaction ($k = 306 \times 10^{-3} h^{-1}$) which is approximately 60 times faster than the observed rate of rhodopsin decrease (as shown in Figs. 2 and 3). This preferential breakdown of metarhodopsin as revealed by direct measurement of the metarhodopsin content is also evident from the results of the previous experiment where the total rhodopsin content was measured in blue adapted flies, after converting the remaining metarhodopsin into rhodopsin (Fig. 3). In that case, the total rhodopsin content fell rapidly to a level of about 35%, which can be attributed to the fast degradation of metarho-

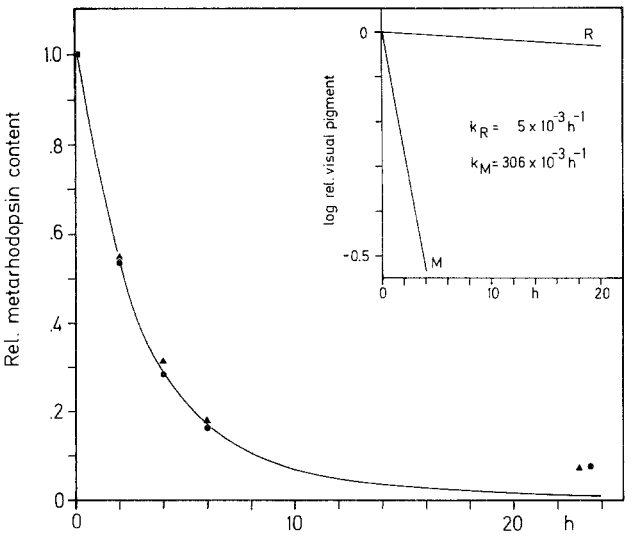

Fig. 4. Degradation of metarhodopsin in the dark. Before being transferred into darkness, flies were adapted to blue light which converts 70% of the total rhodopsin into metarhodopsin (photoequilibrium). Metarhodopsin decreased continuously (circles; mean of 3 animals). Solid line is a least-square fit of this decay with a rate constant $k = (306.0 \pm 4.3) \times 10^{-3} h^{-1}$. The decay of metarhodopsin in flies which were adapted to green light (528 nm; producing about 33% metarhodopsin), shown by the triangles (mean of 3 animals) fits well the exponential calculated for 70% metarhodopsin. Inset shows a comparison of the degradation (log rel. visual pigment) of rhodopsin (*R*) and metarhodopsin (*M*) in darkness

dopsin, and thereafter decreased slowly, reflecting the degradation of rhodopsin.

It has already been shown that some rhodopsin is synthesized during the breakdown (Schwemer 1983). This observation was confirmed here by the results of measuring the total rhodopsin content in the eyes which had been used for the direct measurement of metarhodopsin content. The latter measurements showed that virtually all metarhodopsin had been degraded after about 20 h. Since this accounted for 70% of the total rhodopsin content, the remaining rhodopsin content is expected to amount to approximately 30% (neglecting the breakdown of rhodopsin during this time). In fact, the total rhodopsin content was found to fall to only about 50% during this time, the roughly 20% in excess of the expected value being due to the synthesis of rhodopsin during that time. It appears therefore that the photoreceptor cell contains a store of 11-*cis* retinal which amounts to about 20% of the total rhodopsin content (see also Fig. 5 in Schwemer 1983). The possibility that rhodopsin is regenerated from metarhodopsin in the dark can be excluded (Schwemer 1983).

In order to investigate whether or not the degradation of metarhodopsin is influenced by its fraction present, flies were adapted to green light (528 nm) which produces a photoequilibrium between rhodopsin and metarhodopsin of 67:33%. The result was that the decay of 33% metarhodopsin followed the first-order exponential decay (Fig. 4) calculated from the results of the previous experiment when the initial fraction of metarhodopsin was 70%. It is therefore concluded that the rate of metarhodopsin degradation in the dark depends only on the fraction of metarhodopsin present in the photoreceptor membranes.

Degradation and synthesis of visual pigment in the light

The total rhodopsin content of flies kept under a 12 h cycle of light and darkness remains at a high level whereas that of flies kept in the dark decreases (see Fig. 2), indicating that light plays an important role in the regulation of the rhodopsin content. This was investigated further by subjecting flies to different intensities of continuous 'white' light. The photoequilibrium between rhodopsin and metarhodopsin is *not* dependent upon the intensity of the light and therefore remained constant throughout the experiment (approximately 75:25% under the conditions used).

The total rhodopsin content was measured after flies had been kept for different lengths of time in white light: before the measurement, the metarhodopsin present was converted into rhodopsin with red light. The rhodopsin content was found to remain fairly constant over a period of 192 h in flies kept at relative intensities of 1.0, 0.3, 0.2 and 0.06 (Fig. 5). However, when the relative intensity was reduced to 0.04 the total rhodopsin content decreased slowly to reach approximately 70% after 192 h. When the intensity was further reduced to 0.01 the total rhodopsin content decreased steadily to reach about 8% after 192 h (Fig. 5). During the same period of time, the rhodopsin content of dark controls decreased to approximately 45%. At such low intensities (0.04 and 0.01) there is evidently insufficient light to maintain a high rhodopsin content, most probably because rhodopsin synthesis requires 11-*cis* retinal to be isomerized from the all-*trans* form which results from the breakdown of rhodopsin and metarhodopsin (there being no evidence for the existence of any larger store of 11-*cis* retinal from which rhodopsin is synthesized that would offset its decay to levels as low as 10%). The results therefore suggest that the isomerization of all-*trans* retinal is a light-dependent process (see also Fig. 2). The results also show that the intensity can be var-

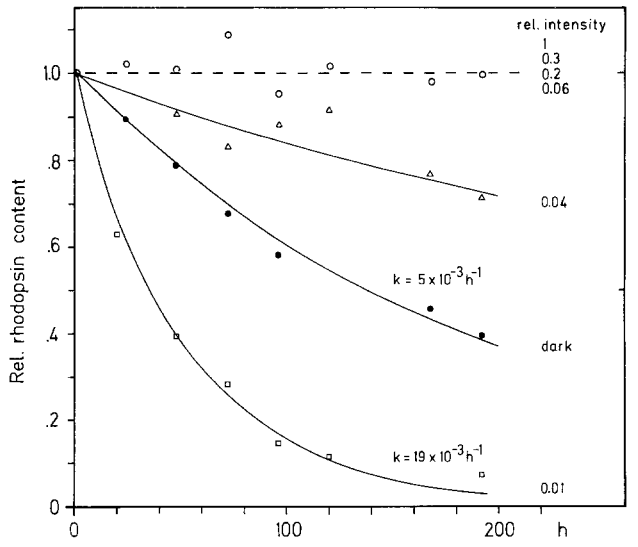

Fig. 5. Rhodopsin content in photoreceptors of flies kept in different intensities of continuous 'white' light (open symbols) and in continuous darkness (filled circles). No decrease in rhodopsin could be found in flies kept at relative intensities of 1.00 to 0.06 and thus the individual measurements have not been plotted separately. A slow decrease in rhodopsin content was found at a relative intensity of 0.04 (open triangles; mean of 2 or 3 animals), but a rapid and continuous decrease occurred at a relative intensity of 0.01 (open squares). Solid lines are least-square fits of an exponential degradation with $k = (5.0 \pm 0.3) \times 10^{-3} h^{-1}$ for darkness, and $k = (18.7 \pm 0.8) \times 10^{-3} h^{-1}$ for a relative intensity of 0.01. – For these experiments relative intensity of 1.00 was 1×10^{-5} W cm^{-1}

ied over a wide range without affecting the rhodopsin content but at relative intensities ≤ 0.01 the photoisomerization cannot provide sufficient 11-*cis* retinal for the biosynthesis of rhodopsin to compensate for the breakdown of rhodopsin and metarhodopsin, primarily metarhodopsin.

The time-course of the observed decrease in the total rhodopsin content at relative intensity 0.01 approximates that of a first-order reaction with a rate constant $k = 19 \times 10^{-3} h^{-1}$, which is about four times greater than that found for rhodopsin in the control animals which were kept in the dark (Fig. 5; $k = 5 \times 10^{-3} h^{-1}$). This high rate is apparently due to the constant fraction of metarhodopsin (25%) present in the photoreceptor membrane throughout the experiment which is degraded faster than rhodopsin (see Figs. 3 and 4).

The next series of experiments was designed to examine the influence of metarhodopsin on the rate of degradation *in the light* and, at the same time, to determine the colour of light which causes the isomerization of retinal. For this, flies were kept in continuous yellow, green or blue light by placing the appropriate acrylic glass filters (see Fig. 1 b for their transmission properties) over the windows in the cabinet.

The intensity of light incident on the flies was adjusted so that the electrophysiologically recorded response of the retina was the same for each colour of light. Thus, the isolated head of a fly was mounted on a holder and recording electrodes positioned and connected to an oscilloscope. The mounted head was then placed inside the blue compartment of the cabinet and allowed to become adapted to that light, thereby setting up a specific photoequilibrium between rhodopsin and metarhodopsin (30:70% for this blue filter). After a 5 min period of dark adaptation, the electroretinogram was recorded in response to blue test flashes produced by interposing a camera shutter between the light source and the cabinet, different intensities being obtained by placing grey filters over the window of the compartment. The results were plotted as an amplitude/log intensity function. The whole process was then repeated using green and yellow filters. Finally, the appropriate grey filters were placed over each compartment so that light incident on the flies elicited a response of the same amplitude in each case, which was within the linear range of the amplitude/log intensity function. Final equilibration was in fact performed using a single fly by exchanging the filters. The use of such physiologically equilibrated light meant that changes in the total rhodopsin content could not be attributed to differences in the physiological state of the photoreceptor cells due to their differing sensitivity to light of different colours.

Groups of flies were kept for different lengths of time under these equilibrated lighting conditions after which the total rhodopsin content was measured having first converted the metarhodopsin present into rhodopsin. The results (Fig. 6) demonstrate that the total rhodopsin content of flies kept in yellow and green light respectively decreased at quite different rates which are proportional to the fractions of metarhodopsin present. In yellow light, the total rhodopsin content decreased steadily to approximately 38% after 120 h, whereas in green light it was reduced to approximately 2% after the same period. A comparison between the rate constants ($k_{yellow} = 7 \times 10^{-3} h^{-1}$ and $k_{green} = 30 \times 10^{-3} h^{-1}$) and the fraction of metarhodopsin established by the different colours of light (13% in yellow and 48% in green light) seems to suggest that a direct relationship exists between the rate constant and the fraction of metarhodopsin present. However, this is a consequence of the way the data have been plotted. The rate constants are about equal, if the data are plotted with respect

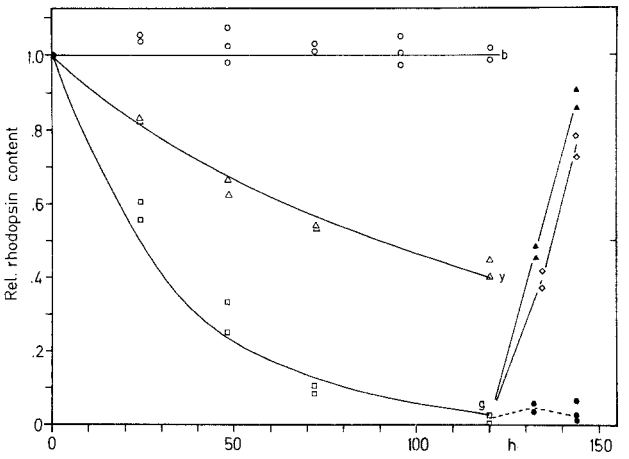

Fig. 6. Rhodopsin content in photoreceptors of flies kept in continuous yellow (open triangles), green (open squares) and blue (open circles) light. Rate constants: yellow light, $k=(7.3\pm 0.5)\times 10^{-3}h^{-1}$ and green light, $k=(29.9\pm 2.7)\times 10^{-3}h^{-1}$. No change in rhodopsin content was found when flies kept for 120 h in green light were transferred into darkness (filled circles) whereas it increased after injection of 11-*cis* retinal (filled triangles) or when the flies were returned to 'white' light (open diamonds). In contrast to the effects of yellow or green light, no change in rhodopsin content was found in flies kept in continuous blue light (open circles)

to the actual metarhodopsin content against time instead of the total rhodopsin content.

In order to exclude the possibility that the observed decrease in total rhodopsin content was due to the continuous light damaging the photoreceptor cells, flies in which the total rhodopsin content was reduced by 98% were transferred into (1) darkness, following injection of 11-*cis* retinal into the eye under red light, and (2) 'white' lighting on a 12 h cycle of light and darkness. In addition, some animals were transferred into the dark after exposure to green light in order to determine whether or not all-*trans* retinal had been isomerized by green light to 11-*cis* retinal which led to rhodopsin synthesis during the ensuing period in the dark.

The results of these control experiments (Fig. 6) show that the rhodopsin content of flies which were kept in the dark after continuous green light remained at a low level, indicating that no 11-*cis* retinal was present. However, the total rhodopsin content of animals injected with 11-*cis* retinal increased rapidly in the dark. From these data it is concluded that the rapid decrease in total rhodopsin content caused by green light is not due to the light damaging the receptor cells but to the reduced biosynthesis of rhodopsin. This in turn results from a lack of 11-*cis* retinal that can be made up for by supplying exogenous 11-*cis* retinal which leads to the restoration of the original rhodopsin content. This can also be achieved by simply transferring the flies from green to 'white' light (Fig. 6), with the result that all-*trans* retinal is isomerized by this light to 11-*cis* retinal, which then leads to the synthesis of rhodopsin. This result also indicates that all-*trans* retinal is not lost from the retina as a result of the breakdown of rhodopsin and metarhodopsin but is stored and recycled. Furthermore, the decrease in rhodopsin content with yellow and green light implies that such light cannot isomerize retinal. The observation that almost no increase in rhodopsin content was found when animals adapted to green light were transferred into darkness excludes the possibility that the degradation of rhodopsin and metarhodopsin occasioned by green light occurs at a much faster rate than isomerization and biosynthesis. The same is true with respect to yellow light.

Since the rate of the decrease of the total rhodopsin content depends on the fraction of metarhodopsin present, blue light which produces the highest fraction of metarhodopsin (70%) would be expected to result in the fastest decrease of the total rhodopsin content. However, as can be seen from Fig. 6, the total rhodopsin content remains at the original value throughout the experiment. This apparent contradiction can be explained if, and only if, blue light is also the most effective in isomerizing all-*trans* retinal into 11-*cis* retinal, which thus ensures the biosynthesis of rhodopsin.

According to this, it is only with blue light that the isomerization of all-*trans* retinal into the 11-*cis* form does not represent a rate limiting step in the renewal of rhodopsin, i.e. the rate of rhodopsin biosynthesis must be higher than the rate of rhodopsin and metarhodopsin breakdown under this lighting condition.

The time-course of rhodopsin synthesis was studied in flies that had been kept in green light to reduce the total rhodopsin content. These animals were transferred into 'white' light which converts only about 25% of the rhodopsin synthesized into metarhodopsin with the result that the rate of degradation during the biosynthesis of rhodopsin was slower than if the flies had been kept in blue light. The results (Fig. 7) demonstrate that the total rhodopsin content increased rapidly in the light with a rate constant $k=77\times 10^{-3}h^{-1}$ which is very close to the value obtained for the biosynthesis of rhodopsin in the dark following injection of 11-*cis* retinal into the eyes of vitamin A-deprived flies ($k=70\times 10^{-3}h^{-1}$; Schwemer 1983).

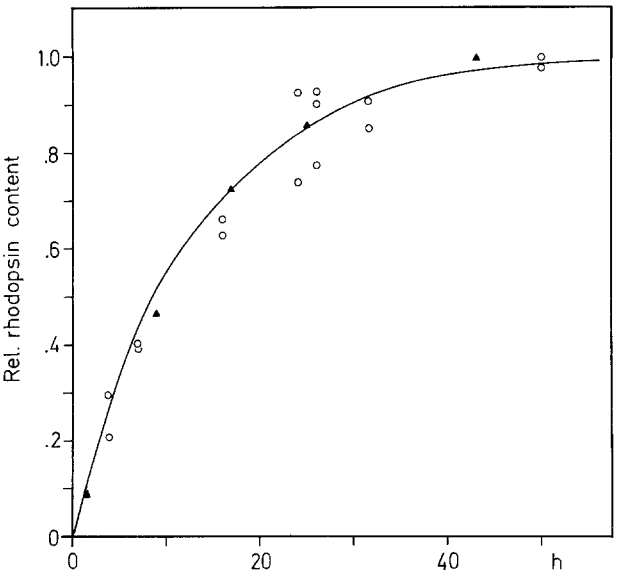

Fig. 7. Increase of rhodopsin content (open circles) in photoreceptors of flies in 'white' light (intensity 0.5×10^{-5} W cm^{-2}) after it had been reduced by keeping the animals in green light. Any metarhodopsin present was converted into rhodopsin before rhodopsin content was measured. The rhodopsin increased with a rate constant of $k = (77 \pm 10) \times 10^{-3} h^{-1}$. For comparison, synthesis of rhodopsin after injection of 11-*cis* retinal into eyes of vitamin A-deficient flies is also shown (filled triangles; mean values taken from Schwemer 1983)

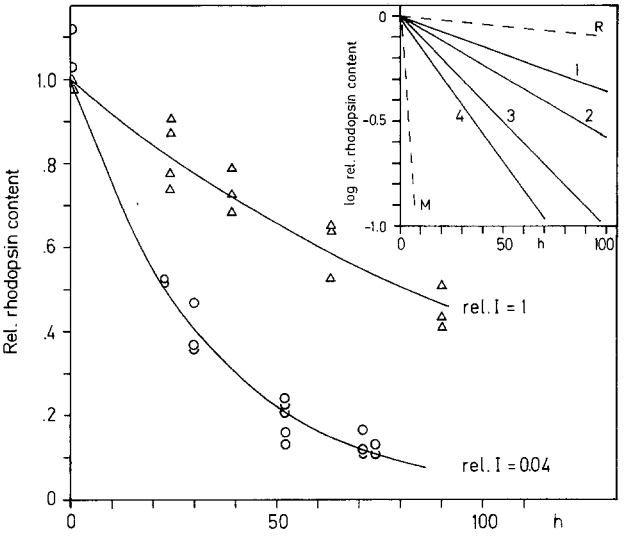

Fig. 8. Rhodopsin decrease in photoreceptors of flies kept in different intensities of green light. The exponential decay is shown for relative intensities of 1.00 and 0.04. – Inset shows the semi-log plot (rel. rhodopsin content) for each of intensities used (solid lines). For comparison, dashed lines indicate decrease of rhodopsin (*R*) and metarhodopsin (*M*) in darkness. Rate constants are: 1: $I = 1.00$ $k_1 = (8.4 \pm 0.7) \times 10^{-3} h^{-1}$; 2: $I = 0.30$ $k_2 = (13.5 \pm 0.9) \times 10^{-3} h^{-1}$; 3: $I = 0.10$ $k_3 = (22.7 \pm 1.4) \times 10^{-3} h^{-1}$; 4: $I = 0.04$ $k_4 = (29.6 \pm 1.5) \times 10^{-3} h^{-1}$; *R*: darkness $k_R = (5.0 \pm 0.3) \times 10^{-3} h^{-1}$; *M*: darkness $k_M = (306.0 \pm 4.3) \times 10^{-3} h^{-1}$. Relative intensity of 1.00 corresponds to 7.8×10^{-6} W cm^{-2}

The influence of light intensity on the degradation

It has been shown that the total rhodopsin content decreases rapidly when flies are kept under low intensities of 'white' light (Fig. 5). To investigate the possible influence of light intensity on the rate of rhodopsin and metarhodopsin breakdown, groups of flies were kept under different intensities of green light such that their relative intensities were 1.00, 0.3, 0.1 and 0.04. Green light was used for two reasons. Firstly, there is no measurable synthesis of rhodopsin (Fig. 6), and therefore no major isomerization of all-*trans* retinal into the 11-*cis* isomer, at least at the intensities used in this study. Secondly, green light establishes a photoequilibrium between rhodopsin and metarhodopsin of approximately 50:50% in each case. Thus, at the beginning of the experiment, the absolute concentration of rhodopsin and metarhodopsin was the same in each group of the flies and during the experiment the photoequilibrium between rhodopsin and metarhodopsin remained constant. Flies were taken from the compartments at different times and their total rhodopsin content determined after converting the metarhodopsin present (50%) into rhodopsin. The results (Fig. 8) clearly demonstrate that the rate of the decrease of the total rhodopsin content (primarily due to the breakdown of metarhodopsin) depends on the intensity. It increases with decreasing intensities of illumination: the calculated rate constant was $k = 8 \times 10^{-3} h^{-1}$ for relative intensity 1.00 and $k = 30 \times 10^{-3} h^{-1}$ for the lowest relative intensity of 0.04. The rate constants found for relative intensities 0.3 and 0.1 were $k = 13 \times 10^{-3} h^{-1}$ and $23 \times 10^{-3} h^{-1}$, respectively.

To summarize, *in the dark* rhodopsin is degraded slowly ($k = 5 \times 10^{-3} h^{-1}$) whereas metarhodopsin is degraded rapidly ($k = 306 \times 10^{-3} h^{-1}$). *In the light* the rate of metarhodopsin degradation also depends on the intensity of light, the lower the intensity, the faster the rate of breakdown: the upper and lower limits for the rate of metarhodopsin breakdown in the light represent the rate of metarhodopsin and rhodopsin degradation in the dark, respectively (see inset to Fig. 8).

Discussion

The results presented here relate to spectrophotometric measurements of the chromophore content

(i.e. rhodopsin and metarhodopsin) and not to the content of opsin. However, it is justified to speak of the results as showing changes in the content of the whole molecule of rhodopsin and/or metarhodopsin (i.e. chromophore *and* protein moiety) for the following reasons. Flies with no rhodopsin also have no opsin in the photoreceptor membrane, as demonstrated by electrophoresis (Paulsen and Schwemer 1979), i.e., there is no opsin without chromophore being present. Furthermore, flies with little rhodopsin have few particles (opsin) in the receptor membrane (Boschek and Hamdorf 1976; Harris et al. 1977). Moreover, recent freeze fracture studies show that a decrease in the total rhodopsin content is accompanied by a decrease in the number of membrane particles (Schwemer and Henning 1984).

The results presented in this study demonstrate that the rhodopsin of photoreceptors 1–6 in the blowfly ommatidium undergoes a continuous renewal involving its breakdown and resynthesis. It should be stressed that in normal flies one cannot detect any change in the total rhodopsin content spectrophotometrically during the course of a day (or over weeks), giving the impression that no renewal occurs. It has, however, been made manifest through the 'dissection' of the visual cycle by the experimental manipulation of flies and the relative simple procedure of measuring the rhodopsin and/or metarhodopsin content spectrophotometrically.

A key factor in the regulation of rhodopsin renewal is the role of 11-*cis* retinal (Schwemer 1983): it is a prerequisite for rhodopsin biosynthesis, which does not occur in its absence. The formation of 11-*cis* retinal from all-*trans* retinal represents a major rate limiting factor in the synthesis of rhodopsin.

The results show that rhodopsin decays in the dark at a slow rate with a half-time of approximately 5 days. This implies that there is no compensating synthesis of rhodopsin which, in turn, implies that there is a lack of 11-*cis* retinal, even though the decay of rhodopsin might be expected to yield 11-*cis* retinal. Rather, this lack can be explained if the chromophore of rhodopsin is *thermally* isomerized to all-*trans* retinal before or during the breakdown process because the total rhodopsin content continues to decrease exponentially even after 300 h in the dark by which time one would expect any 11-*cis* retinal present to have led to an increase in rhodopsin content. This observation also reflects the inability of the fly's eye to isomerize all-*trans* retinal into the 11-*cis* form in the dark and thus supports earlier data (Schwemer 1983).

The results further show that metarhodopsin is degraded in the dark at a fast rate with a half-time of approximately 20 h which is about 60 times faster than the decay of rhodopsin, i.e. metarhodopsin is selectively degraded. A comparison of the rates at which metarhodopsin is degraded in the dark after different photoequilibria between rhodopsin and metarhodopsin were established in the eye (with blue and green light producing 70 and 33% metarhodopsin respectively: Fig. 4) demonstrates that in both cases the degradation of metarhodopsin followed a first-order exponential decay with the same rate constant. A similar decay of metarhodopsin has been reported to occur in butterflies (Bernard 1983), although at a much higher rate than that reported here for the fly photoreceptors.

An exponential decrease of the total rhodopsin content also occurs in the light which is primarily due to the selective degradation of metarhodopsin. The rate of degradation is inversely proportional to the intensity, so that a high level of illumination results in a slow rate of degradation and vice versa. This inverse proportionality presumably relates to the fact that the higher the intensity of light the shorter the length of time a metarhodopsin molecule exists before it absorbs a photon and is converted into rhodopsin. On the other hand, it has been estimated from the results, that, at the fastest rate of degradation, a metarhodopsin molecule exists for a much longer average time before it is degraded. Taking this into consideration, the effects of light on the rate of metarhodopsin breakdown can be explained according to the following hypothesis, which is schematized as

$$R \overset{\sim\sim}{\leftarrow} M \xrightarrow{t} M_u$$

Thus, metarhodopsin exists in two spectrally identical states, the 'normal' stable state (M) and an 'unstable' state (M_u) which is degraded. Metarhodopsin is converted into the unstable state by a thermal reaction (t) and the absorption of light by metarhodopsin in either of these states leads to the photoregeneration of rhodopsin (wavy lines to R). It follows that the fraction of M_u present is determined by the balance between the rate at which it is formed and the rate at which it is converted into rhodopsin. Thus, after a certain time in the dark (during which no photoregeneration occurs), all metarhodopsin exists in the unstable state and is therefore degraded at the maximum rate. In the light, photoregeneration of rhodopsin reduces the fraction of unstable metarhodopsin present to an extent proportional to the intensity of light so that the higher the intensity, the less

unstable metarhodopsin is present and its rate of degradation is proportionally slower. This hypothetical scheme is fully consistent with the results presented here. The unstable state of metarhodopsin postulated above may correspond to metarhodopsin which is fully phosphorylated (Paulsen and Bentrop, submitted).

The *biosynthesis of rhodopsin* has been shown to occur in adult flies raised on a vitamin A-deficient diet during their larval development with the result that they have a total rhodopsin content of only 2–5% of that of normal flies (Schwemer 1983). The present study provides evidence of rhodopsin biosynthesis in flies with a normal rhodopsin content which occurs as part of a continuous cycle of degradation and synthesis involved in the renewal of rhodopsin. These results corroborate the conclusions of earlier work (Schwemer 1983) showing that 11-*cis* retinal is a prerequisite and a trigger for rhodopsin synthesis, whereas all-*trans* retinal does not elicit the biosynthesis of rhodopsin or incorporation of free opsin (if synthesized) into the photoreceptor membrane (Schwemer 1983; Paulsen and Schwemer 1983). It is by virtue of this, and the fact that the isomerization of all-*trans* retinal into the 11-*cis* form requires light, that rhodopsin synthesis is a light-dependent process limited by the rate of photoisomerization. This dependency also represents a link in the chain which can be broken under specific experimental conditions, thereby enabling one to effectively dissect the rhodopsin cycle. In 'normal' daylight, photoisomerization of all-*trans* retinal is not a rate limiting step in the rhodopsin cycle because the blue/violet component is sufficiently intense to provide enough 11-*cis* retinal for rhodopsin synthesis to replace the metarhodopsin which is degraded. Green and yellow light, however, do not lead to photoisomerization and therefore, under these conditions, a degradation of rhodopsin and/or metarhodopsin is observed (Fig. 6). The rate of rhodopsin synthesis promoted by 'white' light is almost exactly the same as that obtained after injecting 11-*cis* retinal into eyes of vitamin A-deficient flies (Fig. 7), showing that the photoisomerization is a highly stereospecific mechanism.

Concerning possible mechanisms of photoisomerization, ultraviolet light can isomerize free (i.e. unbound) retinal giving a mixture of at least four isomers, including 11-*cis* and 9-*cis* retinal. Even though the dioptric apparatus of the eye transmits ultraviolet light, this possibility is unlikely to be the basis of the observed light-induced isomerization since the intensity of the near ultraviolet (380 nm) reaching the flies was extremely low in these experiments (see Fig. 1a, b). Furthermore, visual pigments based on 9-*cis* retinal (isorhodopsin) have not been detected in the eyes of insects, although injection of 9-*cis* retinal into vitamin A-deficient flies does lead to the formation of isorhodopsin (Schwemer, in preparation). The possibility that free retinal was isomerized by blue/violet light (450 nm), under which rhodopsin synthesis and thus photoisomerization was most efficient (Fig. 6), is unlikely because of the low absorbance probability of all-*trans* retinal in this spectral range. It is more likely that retinal is bound in some way since firstly, free retinal is very reactive and secondly, this would account for the fact that retinal is not lost from the retina as a result of rhodopsin and/or metarhodopsin breakdown (Figs. 6, 7). Moreover, the spectral range most effective for photoisomerization does not coincide with the absorption spectrum of free retinal, but is shifted to that spectral range in which protonated Schiff's bases of retinal absorb. It is therefore hypothesized that all-*trans* retinal is bound to a component, possibly a protein, which could also account for the observed stereospecificity.

A retinal-binding protein from the honeybee retina, which was first described by Goldsmith (1958), was recently re-investigated and shown to consist of a protein (molecular weight 27,000 dalton) to which all-*trans* retinal is bound, probably via a Schiff's base. This pigment (λ_{max} 440 nm) is converted by light into a photoproduct with λ_{max} 370 nm (Pepe et al. 1982). High performance liquid chromatography revealed that light converted the bound all-*trans* retinal almost exclusively into the 11-*cis* isomer (Schwemer et al. 1984). These observations represent a precedent supporting the hypothesis that all-*trans* retinal is bound to a protein in the photoreceptors of the fly. This view is further supported by results relating to a comparable retinal-binding protein which can be isolated from the retina of cephalopods. In these animals, all-*trans* retinal is bound to a protein moiety forming the pigment retinochrome which can be converted by light into metaretinochrome with the 11-*cis* retinal as chromophore (Hara and Hara 1965, 1972). The 11-*cis* retinal provided by retinochrome can then be used for reconstituting rhodopsin (Seki et al. 1980).

According to the view presented above, the visual cycle in fly photoreceptors can be summarized by the following scheme (Fig. 9). In this visual pigment cycle, rhodopsin (R) is converted by light to metarhodopsin (M and M_u) which can be reconverted into rhodopsin following the absorption of light (photoregeneration). Thus a photoequili-

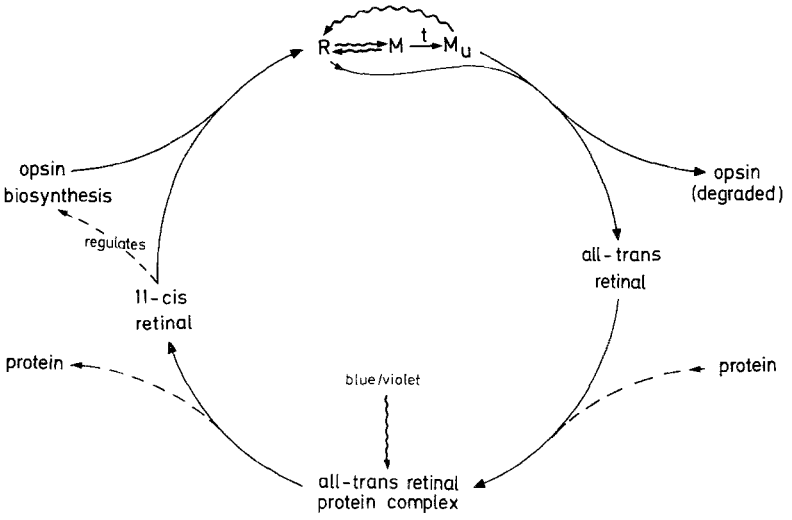

Fig. 9. Visual pigment cycle in photoreceptors of the blowfly. Reactions requiring light are shown by wavy arrows; dashed lines represent hypothetical reactions. For explanation see text

brium is set up under continuous illumination in which the ratio of rhodopsin to metarhodopsin depends on the spectral composition of the light source and the absorbance coefficients of rhodopsin and metarhodopsin and whose kinetic depends on the intensity of the light (for review see Hamdorf and Schwemer 1975; Hamdorf 1979; Hillman et al. 1983; Stavenga and Schwemer, in press). Besides this, rhodopsin is removed from the photoreceptor membrane, but this contributes little to the bulk degradation effected by the selective breakdown of the unstable state of metarhodopsin M_u at a rate which is inversely proportional to the intensity of illumination (see above). The degradation of M_u results in opsin, which is further broken down, and all-*trans* retinal which is bound by a retinal-binding protein and recycled by absorbing light (blue/violet), thereby converting all-*trans* into 11-*cis* retinal which leads to the biosynthesis of rhodopsin. Newly synthesized rhodopsin is incorporated into the photoreceptor membrane. The retinal-binding protein could be either degraded or recycled by binding another molecule of all-*trans* retinal, as seems to be the case with the retinal-binding protein from the honeybee retina (Schwemer et al. 1984).

The visual pigment cycle proposed here is further supported by recent experiments in which blowflies with a 'normal' rhodopsin content were injected with labelled amino acids and kept in blue, 'white' or red light. The largest fraction of labelled opsin was found in the flies kept in blue light whereas none could be detected in the flies kept in red light; the total opsin content of the photoreceptor membranes did not change (Paulsen and Schwemer 1983). These results are fully consistent with the expected results based on the visual pigment cycle proposed here. They also testify to the direct relationship between the content of chromophore and opsin as argued for in the first paragraph of the Discussion section.

The regeneration of rhodopsin in the fly's eye occurs via two pathways, photoregeneration and the renewal of rhodopsin. Both these processes depend on light. A dark regeneration of rhodopsin, as suggested by Bernard (1983), does not occur in flies (Schwemer 1983): rhodopsin can be *synthesized* in the dark but only when 11-*cis* retinal is made available by the light-dependent process of isomerization. The existence of photoregeneration effectively allows the rhodopsin content to be maintained at a high level and at less cost (energetically speaking) than would otherwise be possible if it were supported only by the much slower process of renewal.

The question remains unanswered as to whether the incorporation of newly synthesized rhodopsin into existing rhabdomeres is based on a molecular exchange mechanism or occurs in association with membrane renewal which is perhaps more likely (for review see Blest 1980; Autrum 1981), although no significant differences in the area of the rhabdomeres in light and dark adapted photoreceptors have been found (Williams 1982). Recent evidence has demonstrated the regeneration of rhodopsin in eyes of crayfish following the exposure to orange light, which converts rhodopsin to metarhodopsin (Cronin and Goldsmith, submitted). In contrast to the results presented for the fly, complete recovery occurs in the dark, but it requires several days. However, irradiation with blue light shortens the time-course of recovery to less than two days. The authors find that the regeneration is itself photosensitive and presume that after irra-

diation with orange light the recovery of rhodopsin is limited by the supply of 11-*cis* retinal. Since blue light accelerates the recovery it implies that the system in crayfish is similar to that in flies where 11-*cis* retinal is formed by the action of blue/violet light.

Acknowledgements. I thank Ms. C. Nagel and Ms. E. Wenz for their valuable technical assistance, Paul K. Brown for his generous gift of 11-*cis* retinal and Dr. P. Schlecht for his help with the computer analysis of the data. I am also indebted to Profs. Dr. K. Hamdorf, T.H. Goldsmith, D.G. Stavenga and A.C. Whittle for critical discussions. This work was supported by the Deutsche Forschungsgemeinschaft through SFB 114 and through a Heisenberg Stipendium.

References

Autrum H (1981) Light and dark adaptation in invertebrates. In: Autrum H (ed) Comparative physiology and evolution of vision in invertebrates. (Handbook of sensory physiology, vol VII/6C) Springer, Berlin Heidelberg New York, pp 1–91

Bernard DG (1983) Dark-processes following photoconversion of butterfly rhodopsins. Biophys Struct Mech 9:277–286

Blest AD (1978) The rapid synthesis and destruction of photoreceptor membrane by a dinopid spider: a daily cycle. Proc R Soc London Ser B 200:463–483

Blest AD (1980) Photoreceptor membrane turnover in arthropods: comparative studies of breakdown processes and their implications. In: Williams TP, Baker BN (eds) The effect of constant light on visual processes. Plenum, New York London, pp 217–245

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

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

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

Burnel M, Mahler HR, Moore WJ (1970) Protein synthesis in visual cells of *Limulus*. J Neurochem 17:1493–1499

Eguchi E (1965) Rhabdom structure and receptor potentials in single crayfish retinular cells. J Cell Comp Physiol 66:411–430

Eguchi E, Waterman TH (1966) Fine structure patterns in crustacean rhabdoms. In: Bernhard CG (ed) Functional organization of the compound eye. Wenner Green Internatl Symp Series 7. Pergamon Press, Oxford, pp 105–124

Eguchi E, Waterman TH (1967) Changes in retinal fine structure induced in the crab *Libinia* by light and dark adaptation. Z Zellforsch 79:209–229

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

Goldsmith TH (1958) The visual system of the honeybee. Proc Natl Acad Sci USA 44:123–126

Goldsmith TH, Bruno M (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. (Handbook of sensory physiol, vol VII/6A) Springer, Berlin Heidelberg New York, pp 145–224

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

Hamdorf K, Schwemer J, Gogala M (1971a) Insect visual pigment sensitive to ultraviolet light. Nature 231:458–459

Hamdorf K, Gogala M, Schwemer J (1971b) Beschleunigung der Dunkeladaptation eines UV-Rezeptors durch sichtbare Strahlung. Z Vergl Physiol 75:189–199

Hamdorf K, Paulsen R, Schwemer J, Täuber U (1972) Photoreconversion of invertebrate visual pigments. In: Wehner R (ed) Information processing in the visual system of arthropods. Springer, Berlin Heidelberg New York, pp 97–108

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

Hara T, Hara R (1965) New photosensitive pigment found in the retina of the squid *Ommastrephes*. Nature 206:1331–1334

Hara T, Hara R (1972) Cephalopod retinochrome. In: Dartnall HJA (ed) Photochemistry of vision. (Handbook of sensory physiology, vol VII/1) Springer, Berlin Heidelberg New York, pp 720–746

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

Hillman P, Hochstein S, Minke B (1983) Transduction in invertebrate photoreceptors: role of pigment bistability. Physiol Rev 63:668–772

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

Kropf A, Brown PK, Hubbard (1959) Lumi- and meta-rhodopsin of squid and octopus. Nature 183:446–448

Paulsen R, Schwemer J (1979) Vitamin A-deficiency reduces the concentration of visual pigment protein within blowfly photoreceptor membranes. Biochim Biophys Acta 557:385–390

Paulsen R, Schwemer J (1983) Biogenesis of blowfly photoreceptor membranes is regulated by 11-*cis* retinal. Eur J Biochem 137:609–614

Pepe IM, Schwemer J, Paulsen R (1982) Characteristics of retinal-binding proteins from the honeybee retina. Vision Res 22:775–781

Perrelet A (1972) Protein synthesis in the visual cells of the honeybee drone as studied with electron microscope radioautography. J Cell Biol 55:595–605

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

Schwemer J (1983) Pathways of visual pigment regeneration in fly photoreceptor cells. Biophys Struct Mech 9:287–298

Schwemer J, Gogala M, Hamdorf K (1971) Der UV-Sehfarbstoff der Insekten: Photochemie in vitro und in vivo. Z Vergl Physiol 75:174–188

Schwemer J, Henning U (in press) Morphological correlates of visual pigment turnover in fly photoreceptors. Cell Tissue Res

Schwemer J, Pepe IM, Paulsen R, Cugnoli C (1984) Light-activated *trans-cis* isomerization of retinal by a protein from honeybee retina. J Comp Physiol A 154:549–554

Seki T, Hara R, Hara T (1980) Reconstitution of squid rhodopsin in rhabdomal membranes. Photochem Photobiol 32:469–479

Stavenga DG, Schwemer J (in press) Visual pigments of invertebrates. In: Ali MA (ed) Photoreception and vision in invertebrates. Plenum Press, New York

Stavenga DG, Zantema A, Kuiper JW (1973) Rhodopsin processes and the function of the pupil mechanism in flies. In: Langer H (ed) Biochemistry and physiology of visual pigments. Springer, Berlin Heidelberg New York, pp 175–180

Stein PJ, Brammer JD, Ostroy SE (1979) Renewal of opsin in the photoreceptor cells of the mosquito. J Gen Physiol 74:565–582

Tuurala O, Lehtinen A (1974) Inkorporierung des tritiummarkierten Leucins in die Sehzellen von *Oniscus asellus* L. (Isopoda, Oniscoidea). Ann Zool Fennici 11:135–140

White RH (1964) The effect of light upon the ultrastructure of the mosquito eye. Am Zool 4:433

White RH (1967) The effect of light deprivation upon the ultrastructure of larval mosquito ey. II. The rhabdom. J Exp Zool 166:405–425

White RH (1968) The effect of light and light deprivation upon the ultrastructure of the larval mosquito eye. III. Multivesicular bodies and protein uptake. J Exp Zool 169:261–278

Williams DS (1982) Rhabdom size and photoreceptor membrane turnover in a muscoid fly. Cell Tissue Res 226:629–639