Dark Regeneration of Rhodopsin in Crayfish Photoreceptors

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ABSTRACT The eyes of crayfish were exposed to lights of known spectral composition, and the course of regeneration was followed in the dark by measuring the content of rhodopsin and metarhodopsin in single rhabdoms isolated at various times after the adaptation, using an assay that is based on the fluorescence of metarhodopsin. Complete recovery requires several days in the dark after intense adaptation to orange light, but requires <2 d after blue light exposure. Following an orange light exposure with blue produces recovery kinetics characteristic of the blue light exposure alone. This quickening of recovery occurs whether the receptors are exposed to blue light either immediately or many hours after the original exposure to orange. Conversely, following blue light adaptation with orange leads to slow recovery, which is characteristic of orange alone. Recovery from long-wavelength adaptation is slower principally because many rhabdoms seem to delay the onset of regeneration. We suggest that the regeneration system is itself photosensitive, and after orange light adaptation the supply of active chromophore (presumably 11-cis retinal) limits the rate of recovery. Once started, recovery proceeds slowly and continuously, and the total pigment concentration (rhodopsin plus metarhodopsin) in the rhabdomeric membrane remains approximately constant. Within hours after intense adapting exposures, the rhabdoms become altered in appearance, the surfaces become coated with accessory pigment, and the bands of microvilli are less distinct. These changes persist until recovery of rhodopsin proceeds, which suggests that visual pigment regeneration results from addition of newly synthesized rhodopsin associated with membrane turnover.

INTRODUCTION

The visual pigments of invertebrates characteristically shuttle between two photoconvertible, stable forms. The isomeric form that predominates in dark-adapted receptors and initiates phototransduction is usually called rhodopsin, whereas the alternative stable form is metarhodopsin. Although rhodopsin can be photoregenerated from metarhodopsin (Hamdorf et al., 1973; Hamdorf and Schwemer, 1975), in some species dark regeneration of rhodopsin also takes...
place. In general, the means by which this dark regeneration occurs are not understood, but the process is known to proceed slowly at room temperature, with published half-times ranging from 5 min to >10 h (Stavenga et al., 1973; Stavenga, 1975; Bruno et al., 1977; Bernard, 1983a, b; Schwemer, 1983).

Morphological observations of arthropod photoreceptors have revealed a large amount of membrane synthetic activity (Blest, 1978; Nässel and Waterman, 1979; Stowe, 1980; reviewed in Waterman, 1982). It is tempting to assign the entire process of regeneration to renewal of membrane containing newly synthesized visual pigment, especially because the rates of the two processes seem to fall into the same range (Kong and Goldsmith, 1977; Stein et al., 1979; Schwemer, 1983). However, regeneration could also be a result of reisomerization of the chromophore in situ, of replacement of just the retinaldehyde chromophore (Bernard, 1983a), or of replacement of metarhodopsin in the membrane with rhodopsin from the cytoplasm without renewal of other constituents of the membrane.

Crayfish are known to regenerate rhodopsin in the dark (Cronin and Goldsmith, 1982a). They also show evidence of membrane cycling in their photoreceptors (Eguchi and Waterman, 1966, 1976, 1979; Hafner et al., 1980, 1982), and newly synthesized protein is continuously added to the rhabdom (Hafner and Bok, 1977). Crayfish rhodopsin has a $\lambda_{\text{max}}$ at 530 nm and metarhodopsin at 510 nm. The metarhodopsin (but not the rhodopsin) fluoresces, and by an appropriate measurement of the fluorescence of single rhabdoms, it is possible both to estimate the total concentration of pigment in the organelle and to determine the proportions present as rhodopsin and metarhodopsin (Cronin and Goldsmith, 1981, 1982a, b). In the present experiments, compound eyes of living crayfish were exposed to various lights of known spectral content, at exposures sufficient to produce predictable mixtures of rhodopsin and metarhodopsin in all receptors. The animals were then allowed to recover in the dark, and the visual pigment composition of their rhabdoms was measured at intervals, allowing us to trace the course of recovery. The results show that the time course of the regeneration process is not only longer than for any previously described case, but it is also markedly dependent on the conditions of the initial photoconverting exposure.

**MATERIALS AND METHODS**

**Animals**

Adult *Procambarus clarkii* (Carolina Biological Supply Co., Burlington, NC) were used for all experiments. Animals were maintained at room temperature in a 12 h light/12 h dark cycle (light phase 0600–1800 h EST, except for the experiments of Fig. 5, during which the light phase was 0900–2100 h), and were fed dried dog food pellets twice weekly. All crayfish were given at least 4 d to adjust to laboratory conditions, and most experiments began within 1 wk of arrival. In any single experiment, all animals were of similar size and were usually from the same original shipment.

**Adapting Exposures**

In order to irradiate all receptors as uniformly as possible during the photoconverting exposure, the eyes of living animals were placed at the focus of a parabolic reflector
obtained from the front of a standard flashlight. Correct placement of the eye was facilitated by removing the antennae, antennal scales, and antennules. Timed exposures of usually 30 s were normally begun 3 h after the onset of the dark phase; between 20 and 60 min was required to expose the eyes of all animals. The source of light was a 100-W tungsten-halogen lamp (Oriel Corp. of America, Stamford, CT) filtered by a KG 3 heat-absorbing filter (Schott Optical Glass Inc., Duryea, PA) and then by either a colored glass filter (Corning Glass Works, Medfield, MA) or a narrow-band interference filter (~10 nm bandwidth at half-maximum transmission). For “blue” light exposures, a Corning 5-56 filter was used (peak transmission at 420 nm, 200 nm bandwidth at half-peak), and for “orange” exposures, a Corning 3-66 filter (long-pass filter, 50% transmission at 570 nm). Typically, photon flux at the location of the reflector was of the order of $10^{16}$ quanta cm$^{-2}$ s$^{-1}$, measured with a thermopile (Eppley Laboratory, Inc., Newport, RI). Flux at the surface of the eye, estimated from the ratios of the squares of the diameters of the reflector (39 mm) and the crayfish eye (3 mm), and assuming 50% loss of light, was of the order of $10^{18}$ quanta cm$^{-2}$ s$^{-1}$. This will be referred to as the “standard” intensity. Such a high intensity was required to effect a photosteady state within 30 s in all of the eye’s receptors. Occasionally, other adapting intensities were used; these are specified in the Results section. During the adapting procedure, the laboratory was illuminated with dim red light.

After the photoconverting exposure, animals were maintained in darkness at room temperature and allowed to recover for various lengths of time. Single isolated rhabdoms were assayed for the fractions of their visual pigment present as rhodopsin and metarhodopsin.

**Determination of the Rhodopsin and Metarhodopsin Content of Single Rhabdoms**

Preparation of isolated rhabdoms for measurement of fluorescence time courses was as previously described (Cronin and Goldsmith, 1981). At predetermined sampling times, usually at daily intervals but more frequently in some experiments, one eye was removed from each of two different animals and prepared for analysis. Suspensions containing isolated rhabdoms were fixed in 0.75% formaldehyde (from paraformaldehyde) in pH 7.5 crayfish saline (van Harreveld, 1936) and maintained at 0°C. 20 isolated rhabdoms from each eye were individually exposed to a 510-nm excitation beam on the stage of a compound microscope (Carl Zeiss, Inc., Thornwood, NY) equipped for epifluorescence, and the time courses of changes in fluorescence during these exposures were measured with a photomultiplier tube, photon-counting electronics, and a computer (for examples, see Cronin and Goldsmith, 1981, 1982a). The intensity of the excitation beam was adjusted to give a time constant for the photoconversion of ~0.75 s, and emission measurements were averaged over each 0.1 s. Each time course was fitted with a single-exponential function, and the values of the initial fluorescence and average final steady state fluorescence were calculated from this function. In the cases in which the steady state metarhodopsin concentration was very near the initial concentration, a linear regression was performed on the first 10 measured points to find the initial proportion of metarhodopsin (see also Cronin and Goldsmith, 1982a).

At 510 nm, the photosteady state proportion of metarhodopsin in the mixture is 0.486 (Cronin and Goldsmith, 1982b). Thus, the initial metarhodopsin fraction is given by 

\[(0.486) \times (\text{initial fluorescence} - \text{background fluorescence}) / (\text{steady state fluorescence} - \text{background fluorescence}).\]

By definition, the fractional compositions of rhodopsin and metarhodopsin sum to 1.

Background was determined for a number of rhabdoms by measuring the residual signal after bleaching all rhodopsin and metarhodopsin at pH 9 with wavelengths >520 nm (Goldsmith, 1978). The relationship between the steady state fluorescence produced
by 510-nm light and the background associated with the same exciting beam was empirically fit by linear regression, and separate regression equations were developed for two generalized classes of rhabdom: clean, well-organized organelles and heavily pigmented, disorganized ones (see Results). With these equations, it was subsequently possible to calculate the metarhodopsin content in other rhabdoms to within 2% without having to perform a time-consuming (>20 min) total photobleach on each organelle.

In Procambarus clarkii, unlike Orconectes rusticus, fully dark-adapted rhabdoms usually retain a small amount of fluorescence, which, like the fluorescence of metarhodopsin, can be photobleached at higher intensities. We do not know the origin of this fluorescence; it could emanate from a residual amount (~5%) of metarhodopsin in the dark-adapted receptor or from some other source. Because of this residual fluorescence, we assumed complete regeneration of rhodopsin had occurred in eyes when the estimated fraction of metarhodopsin was consistently <0.1.

RESULTS

Morphological Appearance of Rhabdoms Following Photoisomerizing Exposures

At the time of the initial saturating exposure, the dark-adapted rhabdoms had a very regular appearance, with well-marked microvillar bands, and were devoid of external pigmentation (Fig. 1A). Within hours, however, many rhabdoms appeared highly disorganized and had become covered with granules of secondary screening pigment (Fig. 1B). The numbers of rhabdoms that continued to exhibit this disorganized condition many hours after the adapting exposure depended strongly on the wavelength of the adapting light, as will be described in detail below. During recovery, some rhabdoms retained these new characteristics, while others gradually resumed the appearance of fully dark-adapted receptors, often exhibiting intermediate aspects between the two extreme types. In the graphs that follow, we have classified the rhabdoms into two categories, depending on how close they appeared to the illustrative examples in Fig. 1. Because of the existence of intermediate types, it is useful to remember that such a dichotomous classification is, of necessity, somewhat arbitrary. On the other hand, the classification of rhabdoms by morphological type was made prior to, and is therefore independent of, measurements of metarhodopsin content.

Recovery from Photoisomerizing Adaptations to Blue Light

Receptors given a saturating exposure to broad-band blue light had ~56% of their pigment driven to metarhodopsin, and the metarhodopsin required >1 d to disappear (Fig. 2). Few or no darkly pigmented, disorganized rhabdoms were seen on the day after the exposure, and all rhabdoms seemed to recover at about the same rate.

During recovery, the total pigment (rhodopsin plus metarhodopsin) per unit area of membrane remained constant; this can be seen by the absence of any regular pattern of change from day to day in the strength of the fluorescence signal in the 510-nm photosteady state (Fig. 3). Consequently, reappearance of rhodopsin occurred in step with the disappearance of metarhodopsin.

Fig. 4 shows recovery from a photoisomerizing exposure to a narrow band of wavelengths centered at 514 nm and half the standard intensity. The initial
photosteady state and the time course of recovery are similar to those produced by blue light (Fig. 2). Because the intervals between samples were shorter, it can also be seen more clearly that recovery is continuous rather than episodic. Many disrupted, pigmented rhabdoms were observed early in recovery (Fig. 4, X's); they were not observed in the experiment of Fig. 2, perhaps because in that experiment fewer observations were made during the early phase of recovery. Thus, their appearance in the experiment of Fig. 4 may not be due to the use of blue-green rather than blue adapting light.

**Figure 1.** Appearances of isolated rhabdoms as seen in Nomarski optics. The scale bar in A indicates 20 μm. (A) Rhabdom of fully dark-adapted crayfish. (B) Rhabdom of intensely light-adapted crayfish.

Recovery from Photoisomerizing Exposures to Orange Light

Receptors in eyes given a 30-s exposure to bright orange light initially had ~75% of their visual pigment converted to metarhodopsin (Fig. 5). Such a large proportion of metarhodopsin is formed in the photosteady state because crayfish rhodopsin absorbs more strongly at long wavelengths than does the metarhodopsin (Cronin and Goldsmith, 1982b). In contrast to recovery from photoisomerizing adaptations to blue or blue-green light, dark recovery from orange light requires several days (Fig. 5A). Moreover, some rhabdoms seemed to recover
relatively rapidly, while others showed little or no loss of metarhodopsin for several days. Consequently, after several days the rhabdoms fell roughly into two classes, depending on whether they had started to recover, and with succeeding days the number of rhabdoms that failed to show recovery decreased.

On the day after orange exposure, most rhabdoms had become pigmented and disorganized (filled circles in Fig. 5). On succeeding days, fewer rhabdoms had this appearance, and the rhabdoms that maintained the disorganized, pigmented structure typically had the most prolonged recovery. Conversely, rhabdoms that recovered rhodopsin sooner also resumed the appearance of dark-

![Figure 2](image-url)

**Figure 2.** The course of recovery of rhodopsin in eyes of crayfish initially exposed to blue light and then maintained in the dark. The initial exposure took place at 2000 h on the first day (indicated by the arrow B), and two eyes (from different animals) were immediately sampled. Pairs of eyes were sampled at 0600 h on each succeeding day. For clarity, the data from each of the two eyes are shown slightly displaced from each other on the time axis, and as indicated by the bracket under the arrow B, the first samples were taken after the blue adapting exposure. Open circles: well-organized rhabdoms with little or no attached pigmentation (Fig. 1A). Filled circle: disorganized, pigmented rhabdom (Fig. 1B). Each point is an average of rhabdoms from the same eye; the area of the symbol is proportional to the number of rhabdoms included in the mean. (This number is also given next to each data point.) Error bars indicate ± 1 SD (when n > 2). Where a filled and an open circle occur at the same location on the time axis, they represent rhabdoms from the same eye. Marks on the abscissa are placed at 12-h intervals; the larger marks occur at each midnight.

adapted organelles earlier (open circles in Fig. 5). The appearance of two classes of rhabdoms at intermediate stages of recovery is more evident in Fig. 5B than in Fig. 5A (see also Figs. 7–9).

The evidence for constancy of total pigment concentration during recovery from orange adaptation is more difficult to interpret unambiguously than it is for experiments with blue adapting exposures. Fig. 6A shows the intensity of fluorescence per unit area of membrane at the 510-nm photosteady state for the same rhabdoms whose metarhodopsin content is shown in Fig. 5A. Clearly, the
FIGURE 3. Fluorescence per unit area (arbitrary units) from individual rhabdoms during dark recovery after a photoisomerizing exposure to blue light. Fluorescence was measured after driving the pigment to the 510-nm photosteady state and is thus (in the absence of absorption by granules of screening pigment) proportional to total concentration of visual pigment. Same rhabdoms as in Fig. 2; symbols and abscissa as described for Fig. 2.

FIGURE 4. The continuous course of recovery in the dark after exposure to blue-green light (514-nm narrow-band interference filter). The initial exposure took place at 2100 h; samples were taken immediately, at 2400 h, and at 4-h intervals thereafter. In this experiment, 15 rhabdoms from each eye were assayed and the data are plotted individually. Open circles: well-organized rhabdoms. X's: pigmented rhabdoms. The vertical lines are drawn at each midnight.
signal is significantly lower for the pigmented, disrupted organelles, particularly on days 2–4, than it is for the rhabdoms with more organized morphology. This might be interpreted to mean that there was a 50% loss of pigment from the rhabdoms during this phase of the recovery process. Note, however, that the fraction of pigment present as metarhodopsin remained high and nearly constant on days 2–4 (Fig. 5A); therefore, if the decrease in the total amplitude of fluorescence signal shown in Fig. 6A for days 2–4 is to be interpreted as a loss of pigment, we must conclude that metarhodopsin was not lost from the rhabdoms selectively. In other words, both rhodopsin and metarhodopsin must have been lost in direct proportion to the concentrations that were present at the end of the adapting exposure.
We doubt, however, that the decreased signal on days 2–4 is due to loss of visual pigment. First, the effect is not always seen, even after orange adapting exposures. Fig. 6B shows an example where there is only a suggestion of a dip in the strength of the fluorescence signal during recovery. (The changes in pigment composition for these same rhabdons are shown in Fig. 9A.) Second, when the effect is large, as in Fig. 6A, it clearly correlates in time with the existence of rhabdons whose surfaces are irregularly coated with various amounts of secondary screening pigment. These pigment granules will inevitably absorb some of the 510-nm excitation light as well as some of the emitted fluorescence. Because of the irregular nature of this pigmentation (and the somewhat arbitrary nature of the dichotomous classification of rhabdons as either pigmented or not), it is difficult to quantify this effect. Note, however,
that a 50% loss of signal, which could be interpreted as a 50% loss of visual pigment, could be caused by an average absorbance of screening pigment of <0.3. For these reasons, we conclude that there is no persuasive evidence for a net loss of visual pigment from the rhabdoms at any time during recovery.

It seemed possible that the protracted recovery seen after saturating orange light adaptation was at least partly caused by confining the animals to the dark; in other words, a normal light/dark cycle might have been necessary to initiate rapid recovery. But when animals continued to experience the normal 12 h light/12 h dark cycle of room light after the orange adapting exposure (Fig. 5B), recovery followed a time course similar to that of the animals in constant darkness (Fig. 5A). Note that ambient room light during the solar day did appear to speed the recovery of some of the rhabdoms, a conclusion that is supported by a statistical analysis of the data in Fig. 5. In a two-way analysis of variance, the effects of the L/D and constant dark treatments are significantly different at the 0.01 level. On the other hand, the ambient room light had only a modest effect on the recovery rate compared with the effect of a saturating blue adaptation, either given initially (Fig. 2) or after orange light (Fig. 7, to be discussed next).

Effects of Sequential Exposures

The greatly extended recovery following orange exposure, but not blue, could originate in several ways. One possibility is that the high-intensity exposure used to saturate the visual pigment system damages the recovery mechanism directly, causing a delay while repairs are effected. If this were the case, it would not be possible to hasten recovery once the orange light exposure occurred.

To test the hypothesis that photodamage is the cause of prolonged recovery, we presented sequential exposures of orange and blue light to crayfish eyes and monitored the restoration of rhodopsin. The orange exposure was for 30 s at the standard intensity, but to overcome the effects of screening pigment migration, the subsequent blue exposure (which followed within 1 min) was given at five times the standard flux. Results were unequivocal (Fig. 7). After orange saturation, the blue stimulus brought the photosteady state mixture to ~55% metarhodopsin, although a few receptors were left far from the second steady state (Fig. 7B). Subsequent recovery was complete by the second day in the dark. Only a few pigmented, disorganized rhabdoms were seen on the day after exposure, and the recovery followed a single time course, unlike the control animals exposed to orange light alone (Fig. 7A). When the reciprocal stimulus sequence was applied, with long-wavelength light following short-wavelength light, recovery exhibited a time course and features more characteristic of the long-wavelength exposure alone (Fig. 8). These results make it clear that it is not photodamage that prolongs recovery, but some wavelength-specific effect on the photoreceptors.

Two other hypotheses occurred to us that could explain the differences between recoveries after long- or short-wavelength exposure. The orange light effect could simply be related to the greater proportion of metarhodopsin in the photosteady state after orange exposure (75–80% metarhodopsin after orange vs. 55% after blue). In other words, the amount of visual pigment to be
Figure 7. Recovery in the dark following exposures to orange light alone or orange light followed by blue light. Symbols and axes as for Fig. 2, except in C, day 2, where for clarity the data from the two eyes sampled before the blue exposure have been combined, as have the data for the two eyes sampled after the blue exposure. All initial exposures were made at 2100 h; samples were taken immediately and at 0900 h on each succeeding day. (A) Recovery following exposure to orange light (arrow labeled O) alone. (B) Recovery following exposure to orange light, immediately followed by blue light (arrows O and B). (C) Recovery following exposure to orange light (arrow O), with a blue light exposure provided at 1200 h on the second day of the experiment (arrow B).
regenerated might overwhelm the receptors' regenerating system. Alternatively, the wavelength of the adapting exposure might affect not only the visual pigment photosystem, but also a photosensitive regenerating system, such that short-wavelength exposure leaves more replacement capacity in the photoreceptor than does long-wavelength exposure. After an exposure to long-wavelength light, for example, regeneration of rhodopsin might be limited by the rate at which

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**Figure 8.** Recovery in the dark following exposure to (A) blue-green light alone (514-nm narrow-band interference filter), (B) orange-red light alone (611-nm narrow-band interference filter), or (C) blue-green followed immediately by orange-red. Symbols and axes as in Figs. 2 and 7. The original exposures were presented at 2100 h; samples were taken immediately and on each succeeding day at 0900 h.
new chromophore is made available. In this case, the actual store of chromophore would be photosensitive and therefore subject to manipulation by chromatic exposure independently of the visual pigments.

If a pool of photoconvertible chromophore resides in the eye, then under the second hypothesis an exposure to blue light delivered during recovery from orange adaptation should convert some chromophore back to the active form, speeding subsequent recovery. By presenting the second (blue) irradiation at a time when recovery was expected to have brought the population of rhabdoms

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\begin{align*}
&\text{FIGURE 9. Recovery in the dark following exposure to orange light alone (A) or orange light followed by blue light on the second day (B). Symbols as for Fig. 2, except (as in Fig. 7 C) in B, day 2, where data for pairs of eyes have been combined. The original adapting exposure was at 2100 h; samples were taken immediately and at 0900 h on each successive day. In B the blue light exposure was made at 1800 h on the second day (arrow B). Two eyes were sampled immediately before this exposure was presented and their data are plotted slightly to the left of the time indicated by the arrow; two more were sampled immediately after the exposure and are plotted to the right.}
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to the same average proportion of metarhodopsin as is present in the blue light photosteady state, i.e., ~55%, one should be able to discriminate between the two hypotheses.

The results of presenting a 4X standard blue exposure (see Methods) at 1200 h on the second day, 15 h after the original (orange) adapting exposure, are shown in Fig. 7C. The variation between batches of animals made it impossible to predict accurately the time at which the exposure to blue light should be given. In this case, the blue light treatment caught the rhabdoms while the average fraction of metarhodopsin was still close to 67% and lowered it to ~60%. Recovery was promoted (compare Figs. 7A and 7C) and was complete 2 d later. It seems unlikely that this enhancement was due only to the small reduction in metarhodopsin content. The second stimulus occurred 3 h after the 0900 h census on day 2, so there would already have been some further dark decrease in metarhodopsin from the data points at 0900 h. Moreover, 1 d after the blue exposure, there were very few pigmented, disorganized rhabdoms in the blue-treated group (Fig. 7C), whereas they still dominated the control group, which had received only orange light (Fig. 7A). Finally, the control group lagged at least 1 d behind the experimental group, even though its metarhodopsin content was ~60% on the third day of the experiment (Fig. 7A).

In order to deliver blue irradiation at a time when dark recovery had lowered the fractional composition of metarhodopsin to a value closer to 56%, this experiment was repeated. As the results show (Fig. 9B), this time the average metarhodopsin content of the controls had fallen to ~64%, and the blue stimulus caused a change in metarhodopsin content to 53%. Subsequent recovery was once again more rapid compared with the controls (Fig. 9A).

**DISCUSSION**

**Turnover Processes in Arthropod Photoreceptors**

Several kinds of observations relate to possible mechanisms of rhodopsin regeneration in invertebrate (particularly arthropod) eyes. Direct photoregeneration of rhodopsin from metarhodopsin is clearly important in some systems (Hamdorf et al., 1973; Hamdorf and Schwemer, 1975). Photoregeneration is not a factor in the present study, however, since in all experiments, except that shown in Fig. 5B, recovery took place entirely in the dark.

There are numerous electron microscope studies of turnover of arthropod photoreceptor membrane; in some species this turnover is rapid and massive (Blest, 1978; Chamberlain and Barlow, 1979; Nässel and Waterman, 1979; Stowe, 1980, 1981; Williams, 1982a), while in others the process appears slow and continuous (White and Lord, 1975; Eguchi and Waterman, 1976, 1979; Hafner et al., 1982). Efforts to relate these morphological changes to patterns of protein synthesis have begun (Hafner and Bok, 1977; Stein et al., 1979).

Regeneration of rhodopsin in the dark has been measured spectrophotometrically. Half-times for recovery at room temperature are ~25 min for lobster (Bruno et al., 1977) and blowfly (Stavenga et al., 1973), and range from 5 min to >4 h in butterflies (Stavenga, 1975; Bernard, 1983a, b). We find that in crayfish, recovery of rhodopsin is slow and continuous, requiring hours or days
for completion. Moreover, under some conditions the onset of recovery of some of the receptors may be delayed for several days. These results are in concordance with numerous anatomical investigations of crayfish retinas, which consistently find evidence of processes that are slow or have relatively little diel variation. Such processes include membrane cycling (Eguchi and Waterman, 1976, 1979; Hafner et al., 1980), synthesis of protein (Hafner and Bok, 1977), and movements of retinal hemocytes (Waterman and Piekos, 1981). Thus, the results of numerous previous studies, as well as the present work, support the interpretation that turnover of photoreceptor proteins and membranes in the crayfish eye is a gradual and not a catastrophic process.

Rhabdom Morphology and Regeneration

On the day after exposure to long- but not to short-wavelength light, many isolated rhabdons were heavily pigmented and had disorganized structures (Fig. 1B). These altered rhabdons often persisted for several days. Since rhabdons exposed to short wavelengths can also become pigmented for several hours after the exposure (Figs. 4 and 8A), we assume that the pigmentation itself is simply a consequence of light adaptation and results from movement of pigment within the retinular cells (see also Tsutsumi et al., 1981). Electron microscopy of light-adapted tissue will be necessary to understand its basis.

The disrupted appearance of the microvillar layers is not simply a mechanical result of metarhodopsin formation, as the disruption takes several hours to appear. In white-eyed mutant crayfish, room light intensities induce rhabdom degeneration (Kong and Goldsmith, 1977; Hafner et al., 1982), and similar effects are observed in deep-water crustacea upon illumination at even lower intensities (Loew, 1976; Meyer-Rochow, 1981; Nilsson, 1982). Our results, which employ unusually high light exposures and normally pigmented animals, are consistent with earlier conclusions that the accessory screening pigments are important in controlling the balance between synthetic and degradative processes in the photoreceptors.

By the second day after long-wavelength saturation, some receptors appeared fully recovered, while others still contained the initial metarhodopsin content (Figs. 5 and 7–9). The approximately parallel relationship between recovery of rhodopsin and of normal rhabdom morphology implicates more extensive biochemical processes than simply chromophore isomerization or replacement. Specifically, it suggests that recovery awaits the insertion of new rhodopsin molecules, which implies synthesis of opsins and perhaps other components of the membrane as well. This interpretation is supported by the evidence from white-eyed crayfish (Kong and Goldsmith, 1977) and mosquito (Stein et al., 1979) that synthesis of new membrane involves incorporation of new rhodopsin molecules.

Mechanisms of Regeneration

Although the details by which crayfish rhodopsin is renewed remain uncertain, there appears to be a stoichiometric replacement of metarhodopsin with rhodopsin, with the metarhodopsin remaining within the rhabdomeric membranes until the replacement occurs. Any loss of pigment that may occur is not a selective
removal of metarhodopsin (Figs. 5–6). Bernard (1983a, b) found a rather different situation in butterfly eyes, where the metarhodopsin disappeared with a rapid exponential decay, leaving <10% within the receptor 30 min after the isomerizing flash. Rhodopsin returned much more slowly. Similarly, Schwemer (1983) has found that in the eyes of flies, after photoisomerizing exposures that convert 70% of the visual pigment to metarhodopsin, there is a preferential loss of metarhodopsin from the rhabdomeres. The three species are similar in that the metarhodopsin is not biochemically (as opposed to photochemically) recycled in situ to rhodopsin. They differ in the length of time the metarhodopsin is retained in the photoreceptor membrane.

We have considered two explanations for the observation that recovery after orange light adaptation is much slower than that after blue adaptation. As orange light produces a larger amount of metarhodopsin in the photosteady state, it is conceivable that there is a direct causal relationship such that the larger the concentration of metarhodopsin, the slower the recovery. Two observations lead us to believe that this is an unlikely explanation. Although all rhabdoms contain similar amounts of metarhodopsin after orange light, individual rhabdoms recover at widely different times (Figs. 5 and 7–9). This implies that there are factors in addition to the concentration of metarhodopsin that determine the time of recovery. Second, photoconverting blue exposures that were delivered during the first day of recovery and which reduced the average content of metarhodopsin from 64–67% to 53–60% (Figs. 7 and 9) immediately produced recovery profiles characteristic of blue rather than orange light adaptations. If there is a direct dependence of recovery rate on metarhodopsin content, the relationship must be sharply nonlinear, with very different effects of metarhodopsin concentration below 60% and above 64%.

The alternative is that there exist two separate, photoconvertible systems in crayfish photoreceptors. The first, and most accessible, is the visual pigment system, which is shifted to photosteady states containing increasing amounts of metarhodopsin as the adapting wavelength increases beyond 450 nm (Cronin and Goldsmith, 1982a, b). The hypothetical second system is a regenerating system, in which the photosensitive unit is likely to be protein-bound replacement chromophore, which is suggestive of retinochrome of cephalopod mollusks (Hara and Hara, 1972). Such a light-sensitive regenerating system is also present in the eyes of flies (J. Schwemer, personal communication) and bees (Pepe and Cugnoli, 1980; Schwemer et al., 1984).

The postulated regenerating system of crayfish operates more effectively after exposure to short-wavelength (blue) light than to long-wavelength (orange) light, which in turn suggests that its photosteady state decreases in its proportion of “useful” chromophore (presumably 11-cis retinal) with increasing wavelength. If such is the case, the saturation spectra of both the visual pigment and the regenerating systems should be very similar. We argued above that dark recovery of rhodopsin involves the synthesis of new opsin. The differential effects of blue and orange adapting lights in turn suggest that the rate of assembly of new rhodopsin molecules can additionally be limited by the availability of 11-cis retinal.
If the chromophore reserve is photosensitive, there is a ready explanation for the range in recovery rates following orange adaptation exhibited by different rhabdoms from the same eye. Should various amounts of reserve chromophore be left in individual receptors after the orange exposure, because the reserve is relatively insensitive to the exposure and thus driven to saturation much more slowly and heterogeneously than the visual pigment system, individual recovery rates would vary despite an originally homogeneous distribution of reserve. In the locust, membrane turnover varies among ommatidia depending on their initial exposure to light (Williams, 1982b).

The times required for recovery of crayfish rhodopsin are longer than have been measured in most other systems. For example, in their work with another decapod crustacean, the lobster, Bruno et al. (1977) found half-times of recovery of ~25 min at room temperature. But species doubtless differ in their reserves of chromophore. For example, lobster eyes are known to contain such extensive stores of 11-cis retinol (Wald and Burg, 1957) that chromophore is unlikely to become limiting under conditions equivalent to those employed in the present work. In the crayfish, however, no matter how much recovery is slowed by long-wavelength adaptation, it eventually proceeds to completion. This contrasts with the blowfly, where there is an absolute light requirement for the restoration of 11-cis chromophore (Schwemer, 1983). Thus, in the crayfish, either dark processes for enzymatically restoring 11-cis chromophore exist, or reserves of chromophore are ultimately mobilized for synthesis of rhodopsin.

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