

Renewal of Opsin in the Photoreceptor Cells of the Mosquito

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ABSTRACT Mosquito rhodopsin is a digitonin-soluble membrane protein of molecular weight 39,000 daltons, as determined by sodium dodecyl sulfate gel electrophoresis. The rhodopsin undergoes a spectral transition from R515-520 to M480 after orange illumination. The visual pigment apoprotein, opsin, is the major membrane protein in the eye. Protein synthesis in the photoreceptor cells occurs in the perinuclear cytoplasm and the newly made protein is transported to the rhabdom. Light adaptation increases the rate of turnover of this rhabdomal protein. The turnover of electrophoretically isolated opsin is also stimulated by light adaptation. The changes observed in protein metabolism, biochemically, are consistent with previous morphological observations of photoreceptor membrane turnover. The results agree with the hypothesis that the newly synthesized rhabdomal protein is opsin.

INTRODUCTION

Studies of protein metabolism in invertebrate photoreceptor cells have been reported for few Arthropod classes (Insecta, Merostomata, Crustacea). These light and electron microscope autoradiographical investigations demonstrated that most of the newly synthesized protein ultimately became associated with the extensive areas of microvillar membrane (rhabdom) which characterize invertebrate photoreceptor cells (Burnel et al., 1970; Perrelet, 1972; Krauhs et al., 1976; Hafner and Bok, 1977). Studies that monitored the effect of light and dark adaptation on the metabolism of this rhabdomal protein reported that the rate of protein turnover was influenced, if not controlled, by the conditions of ambient illumination (Burnel et al., 1970; Krauhs et al., 1976; Hafner and Bok, 1977). Similar results have been obtained in morphological studies of rhabdomal membrane degradation in the mosquito, *Aedes aegypti* (White and Lord, 1975; Brammer and Clarin, 1976; Brammer et al., 1978). Because the rhabdomal membrane of invertebrates is known to contain the visual pigment molecules, it has been assumed that the newly synthesized protein must be opsin, the apoprotein of the visual pigment. However, no direct evidence has been presented to support this hypothesis. In contrast, studies of the vertebrate rod photoreceptor cells have shown that opsin

accounts for as much as 80–85% of the newly synthesized protein in the photoreceptor membrane (Hall et al., 1969). In the vertebrate rod, renewal of the photoreceptor membrane has been shown to be a continuous process (Young, 1967; Young and Droz, 1968). The rate of this renewal process is influenced by the conditions of illumination (Besharse et al., 1977) in a manner analogous to that described for invertebrate photoreceptor membrane.

The purpose of this study was to elucidate the molecular basis for the turnover of rhabdomal membrane in mosquito photoreceptor cells and to examine the influence of light and dark adaptation on this process. To accomplish this, the pattern and kinetics of protein synthesis were studied autoradiographically by following the incorporation of [³H]leucine into photoreceptor cell protein under different conditions of light and dark adaptation. The visual pigment was extracted and the turnover of the apoprotein, opsin, was studied under similar conditions of light and dark adaptation using time-sequence extraction of [³H]leucine-labelled opsin. The results are discussed with respect to both the morphology and physiology of the insect photoreceptor cell.

MATERIALS AND METHODS

Mosquitoes, *Aedes aegypti* (Rockefeller strain, wild-type or white-eye) were raised from egg sheets¹ which were vacuum hatched in distilled water and the larvae were maintained in an environmental chamber (air temperature 29°C and water temperature 26°C). Hartz Mountain Corp. Dog Yummies (Harrison, N.J.) were crumbled into the water to serve as a nonsterile food source for the larvae. At all stages of development, prior to experiments, the mosquitoes were maintained on a 12-h light–12-h dark photoperiod.

Autoradiography

The pattern and extent of protein synthesis was studied autoradiographically under several different light-dark adaptation schedules. Since previous morphological studies showed that changes in ambient illumination result in striking changes in photoreceptor ultrastructure, the mosquitoes were either light-adapted or dark-adapted for 36–48 h before isotope injection. After injection with ~0.1 µl of [³H]leucine (31.9 Ci/mmol, New England Nuclear, Boston, Mass.), the mosquitoes were maintained in the preinjection light or dark condition for 15 min equilibration. After equilibration, the animals were placed in the appropriate light or dark environment. Five animals were obtained for each of the incorporation times listed in Table I A. At the end of the incorporation time, the heads were removed in Veronal buffer (54 mM sodium acetate and 62 mM sodium barbiturate) halved longitudinally and fixed for one hour in 1% osmium (buffered with 27 mM sodium acetate and 31 mM sodium barbiturate). Dehydration was accomplished through a graded series of acetone concentrations. The eyes were embedded in an Epon-Araldite mixture (Shell Chemical Co., New York) (Mollenhauer, 1964) and hardened for 24-h periods sequentially in 40°, 60°, and 90°C ovens. 1-µm sections were taken so that four longitudinally cut rhabdoms were obtained in four sections from one eye in each of five animals of each treatment group. Thus, 16 rhabdoms per animal and 80 rhabdoms per treatment group were

¹ Egg sheets were provided by Dr. George B. Craig, Jr., Department of Biology, University of Notre Dame.

examined.² The plastic sections were mounted on clean glass slides and coated with Kodak NTB-2 nuclear track emulsion (Eastman Kodak Co., Rochester, N.Y.), melted at 40°C and diluted 1:1 with distilled water. After a 21-d exposure (4°C), the emulsion coated sections were developed at 17°C with Kodak Dektol Developer (2 min), followed by a stop bath (1.4% acetic acid), and Kodak Acid Fixer (17 min). The sections were stained through the emulsion with 1% toluidine blue – 1% sodium borate. All grain counts were made by the same individual (without knowledge of the light-dark treatment of the specimen sections). Grains were counted in 49- μm^2 regions of the rhabdom and the perinuclear cytoplasm by positioning each section under a calibrated ocular micrometer. The results were expressed as the grain density in the rhabdom divided by the sum of the grain density in the rhabdom and perinuclear cytoplasm. The data were fitted by computer to an exponential model using nonlinear

TABLE I
EXPERIMENTAL TREATMENTS

A. Autoradiography protocol Preinjection adaptation	Injection	Equilibrium	Incorporation
			<i>h</i>
36–48 h dark	Dark	15 min dark	0, $\frac{1}{4}$, $\frac{1}{2}$, 1, 2, 6 dark (dark-dark)
36–48 h dark	Dark	15 min dark	$\frac{1}{4}$, $\frac{1}{2}$, 1, 2, 6 light (dark-light)
36–48 h light	Light	15 min light	0, $\frac{1}{4}$, $\frac{1}{2}$, 1, 2, 6 light (light-light)
36–48 h light	Light	15 min light	$\frac{1}{4}$, $\frac{1}{2}$, 1, 2, 6 dark (light-dark)

B. Opsin extraction protocol Preinjection adaptation	Injection	Incorporation
36–48 h dark	Dark	1, 2 dark (dark-dark)
36–48 h dark	Dark	1, 2 light (light-dark)
36–48 h light	Light	1, 2 light (light-light)
36–48 h light	Light	1, 2 dark (light-dark)

regression analysis (SPSS Nonlinear, Madison Academic Computing Center, University of Wisconsin).

Visual Pigment Extraction

Live adult female mosquitoes (white-eye) were dark-adapted for a minimum of 24 h and then placed in a freezer at -20°C until needed (within 1 wk). Routinely, 1,400 dark-adapted mosquito heads (2,800 eyes) were crushed and washed twice with 1 ml of cold 0.07 M Tris [tris (hydroxymethyl) aminoethane] solution (pH 8.3). The visual pigment was extracted with 0.3 ml of 2% digitonin buffered with 0.07 M Tris. Spectra

² In 7 of the 22 groups, less than 80 rhabdoms were counted: light-dark 2 h (*n* = 48), 6 h (*n* = 32); dark-light $\frac{1}{4}$ h (*n* = 64), 6 h (*n* = 64); dark-dark $\frac{1}{4}$ h (*n* = 64), $\frac{1}{2}$ h (*n* = 64), 1 h (*n* = 64).

were recorded with a Cary 118 recording spectrophotometer (Varian Associates, Palo Alto, Calif.).

Acrylamide Gel Electrophoresis—Digitonin Extracts

In order to separate the mosquito visual pigment from contaminating proteins, extracts of mosquito heads were subjected to polyacrylamide disc gel electrophoresis. Heads from 500 female mosquitoes (dark-adapted and frozen as above) were crushed, then extracted with 0.3 ml of 2% digitonin-Tris solution. Sucrose was added to obtain a 4% (wt/vol) solution and the sample was layered onto a 3.5% acrylamide gel. The gel consisted of 0.49 M acrylamide; 0.006 M methylenebisacrylamide; 0.09 M Tris; 0.005 M ammonium persulfate, and 0.14% *N*, *N*, *N'*, *N'*-tetramethylethylenediamine. The electrode buffer was 0.07 M Tris; 0.002 M Na₂EDTA (ethylenediamine tetra-acetic acid), and 0.007 M H₃BO₃. The sample was electrophoresed at 2°C in the dark for 75 min at 2 mA per gel. Spectra of the visual pigment were taken in the gel immediately after electrophoresis while the gel was maintained at 10–11°C. Rhodopsin was bleached using an American Optical Corp. (Southbridge, Mass.) fiber optics illuminator (type 150K) with four heat filters (Edmund Scientific Co., Barrington, N.J., No. 4333) and a Corning CS3-68 yellow filter (>527 nm) (Corning Glass Works, Science Products Div., Corning, N.Y.). After the rhodopsin was located spectrophotometrically on the acrylamide gel, the region containing the visual pigment was cut out, and the protein was eluted with 0.1 ml of a solution containing 5% sodium dodecyl sulfate (SDS) and 0.01 M phosphate buffer (pH 7.0), in preparation for molecular weight determination by SDS gel electrophoresis.

SDS Gel Electrophoresis

The sample extract described above was electrophoresed on a 10% acrylamide disc gel according to the method of Weber and Osborn, 1969. The gels were stained with 0.25% Coomassie Blue in 9.2% acetic acid and 45% methanol for 24 h and destained electrophoretically for 7–8 h. The mobility of mosquito opsin was measured relative to the mobility of glyceraldehyde-3-phosphate dehydrogenase (mol wt 36,000 daltons) or aldolase (mol wt 40,000 daltons) (Sigma Chemical Co., St. Louis, Mo.).

SDS gel electrophoresis was also performed on membrane fractions of whole mosquito heads, excised eyes, and a cornea-cuticle preparation. Whole heads and excised eyes were washed three times with 0.01 M phosphate buffer (pH 7.0) to eliminate soluble proteins, prior to extraction with 1% SDS 0.01 M phosphate buffer. The cornea-cuticle extract was prepared by passing a buffer-washed (10 times) homogenate of mosquito eyes through a coarse metal mesh (0.5-mm grid). The material retained by the mesh was extracted with 1% SDS-phosphate buffer. The gels were run at 8 mA per tube for 9 h and stained according to the procedure of Fairbanks et al., 1971.

[³H]Leucine Labelling—Time-Sequence Opsin Extraction

The kinetics of opsin turnover were studied by examining the extent of [³H]leucine incorporation into electrophoretically separated opsin after 1- and 2-h incorporation periods under different light and dark adaptation conditions. Four groups of 200 female mosquitoes were dark-adapted for 36–48 h and four groups were similarly light-adapted. At the end of the adaptation period, 25 mosquitoes from each group were injected with [³H]leucine (40 Ci/mmol, New England Nuclear). Approximately 0.1 μl (0.1 μCi) was pressure-injected into each animal through a drawn-out glass capillary. After injection, the 25 injected mosquitoes and their 175 uninjected coun-

terparts were placed in the appropriate conditions of illumination so that eight groups were obtained as outlined in Table I B. At the end of the incorporation period, the injected and uninjected mosquitoes were frozen at -20°C. As soon as possible after freezing, the heads of the 200 mosquitoes from each group were removed and homogenized in 0.01 M phosphate buffer (pH 7.0). The homogenate was centrifuged for 30 min at 15,000 rpm (Damon/IEC, Needham Heights, Mass., No. 870). After two additional buffer washes, the pellet was reground with 1% SDS-phosphate buffer and extracted for 2 h at 25°C. Low speed centrifugation (2,200 rpm) pelleted debris and an aliquot was electrophoresed on a 10% disc gel (as described above). The absorbance profiles of the Coomassie-stained gels were obtained by scanning at 600 nm with a Gilford model 2400 spectrophotometer and model 2410 linear transport accessory (Gilford Instrument Laboratories Inc., Oberlin, Ohio). The gels were frozen with powdered dry ice and sliced with a Mickle gel slicer (Brinkmann Instruments, Inc., Westbury, N.Y.). The 1-mm slices were placed into glass scintillation vials, air-dried (overnight at 25°C) and then solubilized in 0.1 ml of 30% H₂O₂ in a 60°C oven for 2-4 h (Tischler and Epstein, 1965). After cooling, 15 ml of toluene-based cocktail (250 ml Triton X-100 [Rohm & Haas Co., Philadelphia], 750 ml toluene; 50 mg/liter 1,4-bis-2-[5-phenyloxazolyl]-benzene; 4 g/liter 2,5-diphenyloxazole) were added to each vial (Stein and Borun, 1972). The gel slices were counted for radioactivity in a Packard Tricarb liquid scintillation spectrometer (58% efficiency) (Packard Instrument Co., Downers Grove, Ill.). The amount of radioactivity in opsin was determined by planimetrically measuring the area under the peak at 39,000 daltons from the radioactivity profile. The amount of protein in the opsin band was determined by planimetrically measuring the area under the 39,000 dalton peak on the absorbance profile determined for the Coomassie-stained gel. The specific activity of opsin was the ratio of the two measured areas. The range of protein concentrations employed was in the linear-binding range of Coomassie Blue in the SDS gel for the 39,000 dalton component.

RESULTS

Protein synthesis, as indicated autoradiographically by the incorporation of [³H]leucine, was observed in the eyes of mosquitoes under all conditions of light and dark adaptation studied. Fig. 1 shows a typical time series of autoradiographs from the eyes of animals dark-adapted before and after isotope injection. The most striking incorporation within the eye was invariably in the photoreceptor cells, although uptake was also observed in the corneal lens, cone, and pigment cells. In the photoreceptor cells, very little label was ever observed in association with nuclei, pigment granules, or lipid droplets. Fig. 1 shows that, with increasing incorporation time, the most striking uptake was always associated with the rhabdom. This phenomenon was observed in dark adaptation as well as in all other adaptation conditions studied. The proportion of label associated with the rhabdom was altered by light and dark adaptation. In light adaptation, the proportion of label associated with the rhabdom is much more pronounced at the earlier incorporation times than it is in dark adaptation (see Fig. 2). Although there is a slow increase in isotope uptake in nonrhabdomal areas of the cell with time, there is a much greater accumulation into the rhabdom. Fig. 3 shows that particularly heavy labelling can also be observed in the perinuclear cytoplasm. The perinuclear cytoplasm is an area typically found to be rich in rough

endoplasmic reticulum (Brammer, 1970). Heavy labelling of this region was seen in all adaptation conditions but not in all sections.

The quantitative data presented in Fig. 4 support the qualitative observations that the synthesis of rhabdomal protein represents the majority of the protein synthesized in the photoreceptor cell and that the turnover of this protein is dependent upon the conditions of adaptation. Fig. 4 shows that at

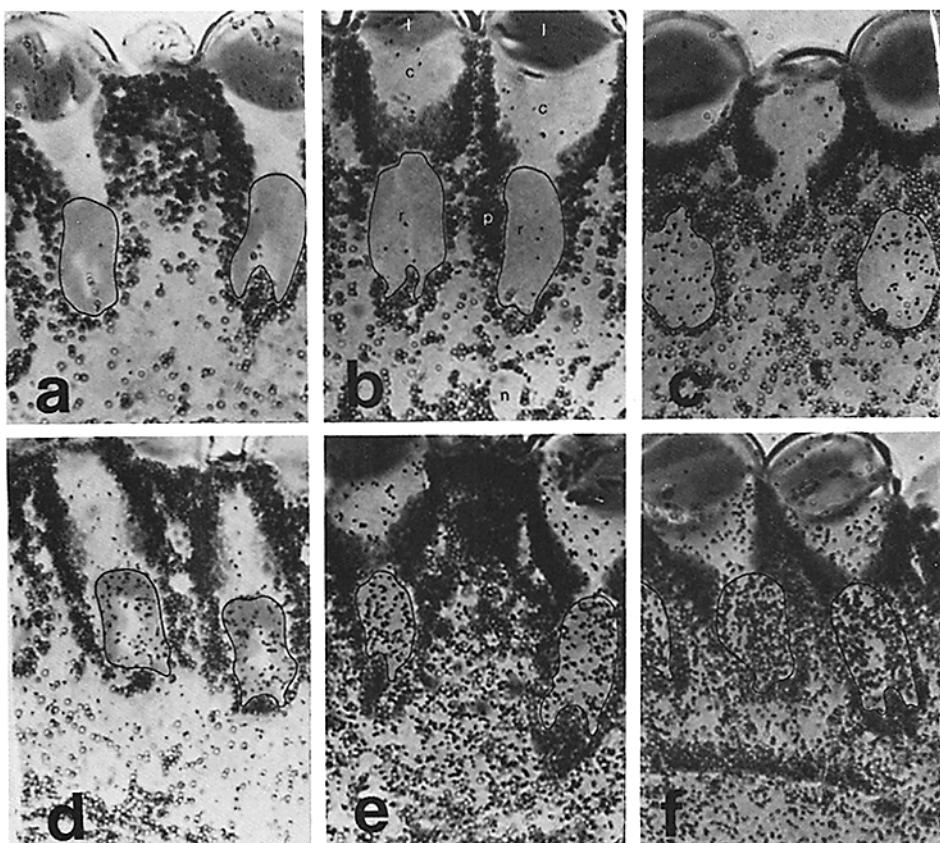


FIGURE 1. Dark-dark autoradiographic series. Note increasing proportion of label associated with the rhabdom with increasing incorporation time. Rhabdom area is outlined in black. (a) 0 min postequilibration. (b) 15 min postequilibration (r, rhabdom; c, cone cell; l, lens; p, pigment granules; n, nucleus). (c) 30 min postequilibration. (d) 1 h postequilibration. (e) 2 h postequilibration. (f) 6 h postequilibration.

the longer incorporation times, regardless of adaptation state, 70–80% of the grains are associated with the rhabdom. It is also obvious that the proportion of grains associated with the rhabdom increases with time until a plateau is reached. These data, along with the observation that cytoplasmic labelling increased more slowly than rhabdomal labelling, imply that the protein synthesized in the perinuclear cytoplasm accumulates in the rhabdom and

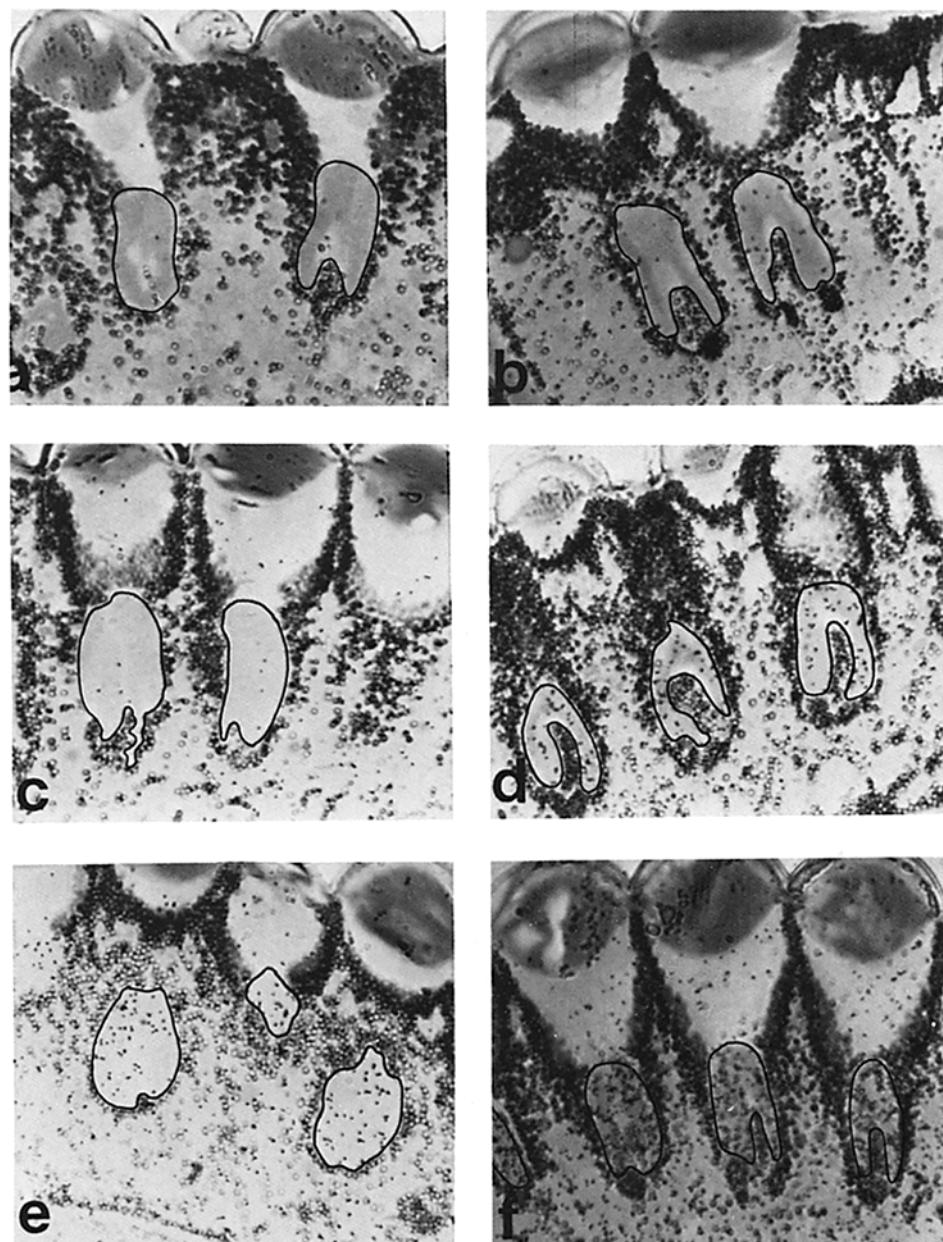


FIGURE 2. Leucine incorporation at early incorporation times in light and dark adaptation. Sections a, c, and e show incorporation at 0, 15, and 30 min in dark-dark-treated eyes. Sections b, d, and f show that there is greater incorporation in the rhabdom in light-light-treated eyes at the same time periods.

represents the bulk of the protein synthesized. Since rhabdomal protein turnover is faster than cytoplasmic turnover, the percentage associated with the rhabdom is an underestimate of the proportion of total synthesis associated with this structure. The smooth curves in Fig. 4 are the computer-predicted fit to the data points. The *inset* to Fig. 4 shows comparisons of the rates of incorporation for various treatments analysed as kinetic plots. The 95% confidence interval (CI) for the slopes are shown. The half-lives (\pm SEM) of leucine incorporation are: dark-dark = 39.0 ± 3.6 min; light-light = 15.1

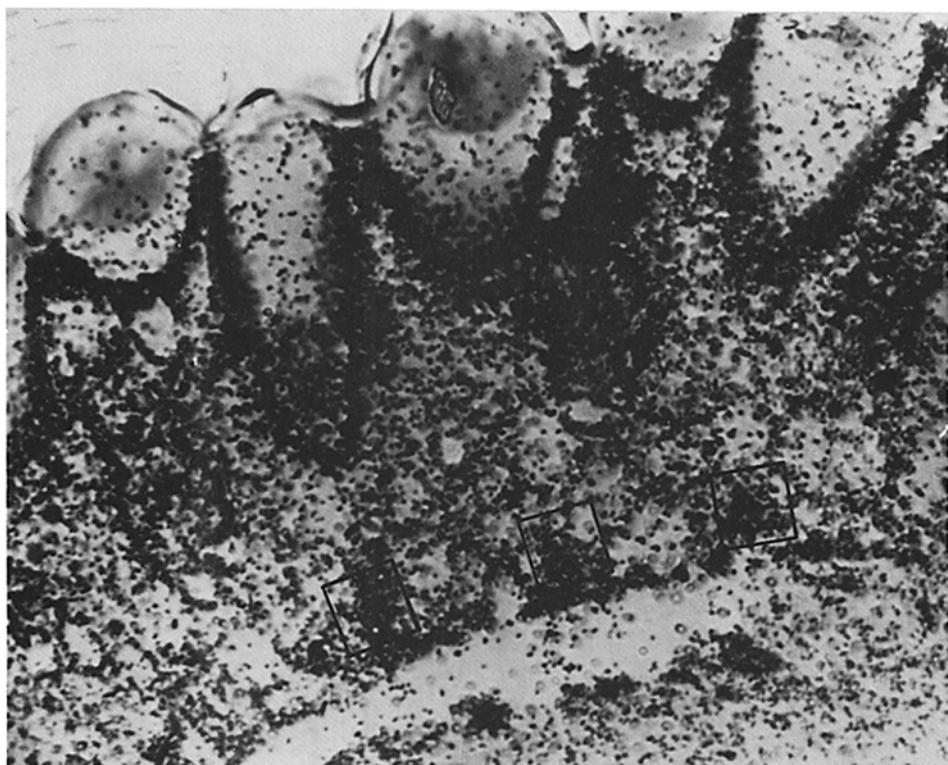


FIGURE 3. Autoradiograph of a 6-h light-light-treated eye. Note areas of heavy incorporation in the perinuclear cytoplasm (enclosed by black squares).

± 1.3 min; light-dark = 39.7 ± 4.1 min; dark-light = 17.8 ± 1.5 min. Statistical comparisons of the differences between the half-lives for leucine incorporation for dark-dark vs. light-light, dark-dark vs. dark-light, and light-dark vs. light-light all showed that the dark incorporation rate was significantly slower than the light incorporation rate (Scheffe procedure, $P < 0.05$) (Scheffe, 1970). The rate of incorporation was ~ 2.6 -fold faster in constant light than in constant dark.

The spectral changes observed upon illumination of a digitonin extract of mosquito rhodopsin are illustrated in Fig. 5. Spectra 1 and 2 are difference

spectra showing mosquito rhodopsin and metarhodopsin, respectively. The rhodopsin has its λ_{max} at 515–520 nm and is the difference between the native spectrum and the final spectrum after illumination and warming of the sample. The metarhodopsin difference spectrum (spectrum 2) has λ_{max} , between 470 and 480 nm. This spectrum is the difference between the spectrum after illumination with >527 nm light and the final, warmed spectrum. Fig. 6 shows difference spectra of mosquito visual pigment after gel electrophoresis of a digitonin extract of 500 heads (1,000 eyes). Spectra 1 and 2 show the λ_{max}

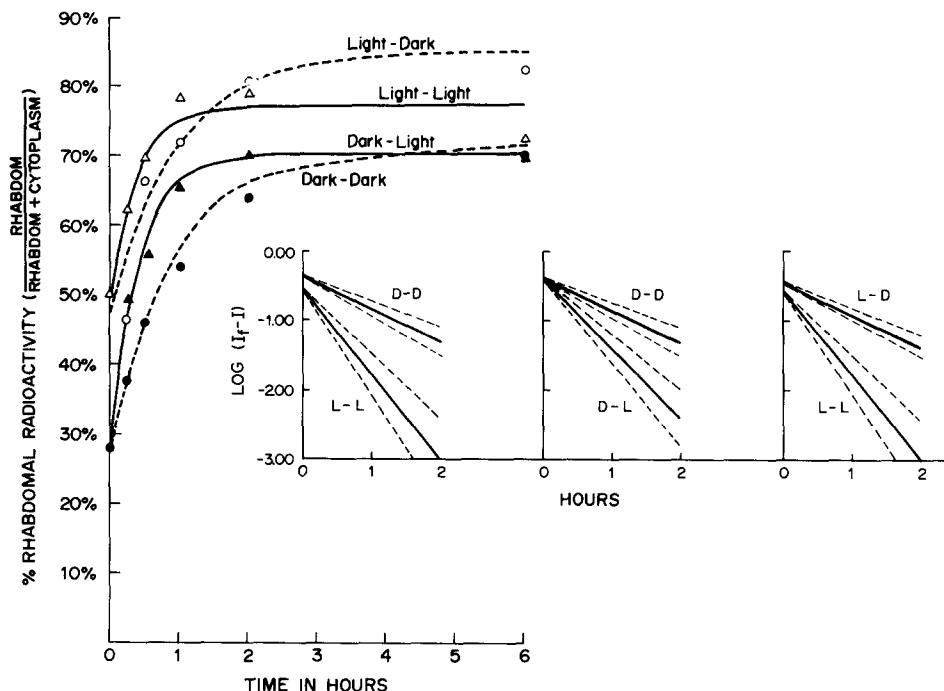


FIGURE 4. Leucine incorporation into the rhabdom. Light-dark (○); dark-dark (●); light-light (Δ); dark-light (▲). The symbols represent the data points obtained as described in the text. The smooth lines are the computer-derived fit to those data. Inset illustrates the computer-predicted plots of the rates (\pm CI) for treatments of interest. I_f , final grain density; I , grain density at a given time.

of the rhodopsin and metarhodopsin, respectively. The spectra are similar to those seen in the previous figure. The rhodopsin λ_{max} is at 520–530 nm and the metarhodopsin λ_{max} is at 480 nm. It should be noted that some difficulties were encountered in preparing successful extracts for spectrophotometric characterization (with or without prior electrophoresis). Procedures to remove soluble proteins—water, buffer, and acetone washes, or the use of isolated eyes rather than heads—significantly reduced the yield of spectrally intact visual pigment. In addition, the metarhodopsin obtained after photoisomerization proved to be unstable.

Fig. 7 shows the results of an experiment in which the photosensitive band (from the experiment in Fig. 6) was cut out of the acrylamide gel, the protein was eluted and reelectrophoresed on an SDS gel. The approximate molecular weight of the major protein was 39,000 daltons. In a total of five band transfer experiments, this molecular weight component always appeared as the major protein. However, in three of the five experiments, a protein band of molecular weight 30,000 daltons appeared in concentrations approaching that of the 39,000 dalton band.

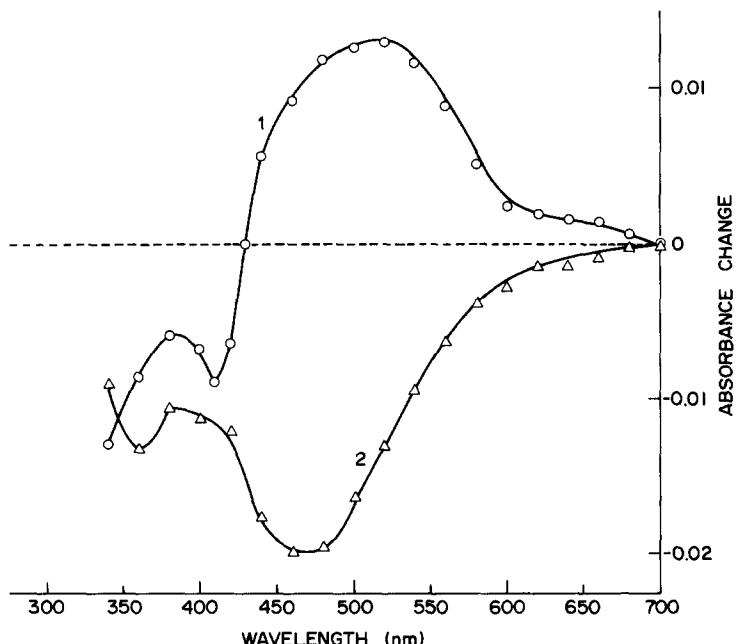


FIGURE 5. Spectral characteristics of a digitonin extract of mosquito rhodopsin. Spectrum 1. Spectrum 1 is the difference between the initial spectrum and a final spectrum taken at 25°C, 42 min after the illumination and 18 min after raising the sample temperature to 25°C. It shows the spectrum of mosquito rhodopsin with a $\lambda_{\text{max}} \approx 520$ nm. Spectrum 2 is the difference between a spectrum started 1 min after the end of the illumination and the final spectrum taken at 25°C, 42 min after the illumination and 18 min after raising the temperature to 25°C. It shows mosquito metarhodopsin with $\lambda_{\text{max}} \approx 480$ nm.

SDS gels of extracts of buffer-washed mosquito heads contain the 30,000 and 39,000 dalton proteins described above as well as many other proteins, (see Fig. 8 A). Electrophoretic analysis of SDS extracts of carefully excised, buffer-washed eyes reveal that the concentration of the 39,000 dalton band increased, while that of the 30,000 band decreased (see Fig. 8 B). The 30,000 dalton component was also present in SDS extracts of an extensively washed cornea-cuticle preparation (see Fig. 8 C). The 39,000 dalton component is missing from this fraction. If the absorbance profile of the 30,000 dalton component and the other proteins present in the corne-acuticle fraction were

subtracted from the absorbance profile of the eye extract, the 39,000 dalton component would be, by far, the major protein component.³

The specific activity of the electrophoretically isolated opsin bands is shown in Table II. A typical radioactivity-absorbance profile is illustrated in Fig. 9. Those groups of mosquitoes which incorporated isotope in the light (light-

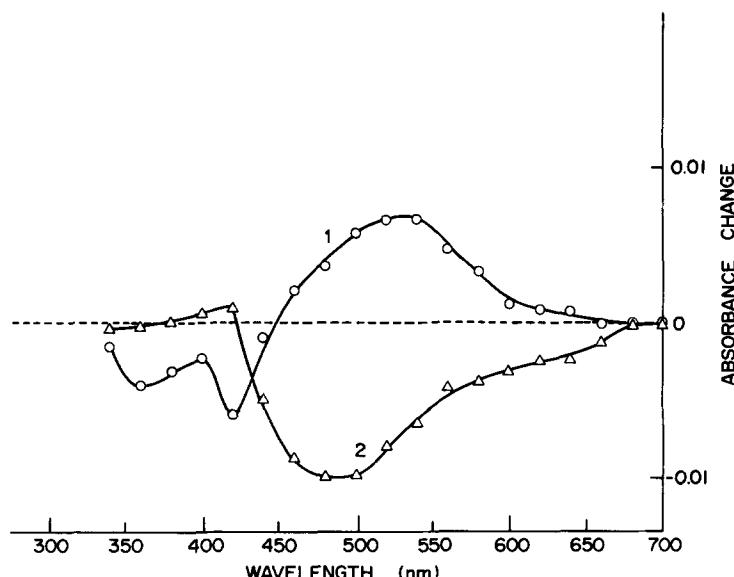


FIGURE 6. Spectral characteristics of a digitonin extract of mosquito rhodopsin on an acrylamide gel. Spectrum 1 is the difference spectrum between the initial spectrum and a final spectrum taken at 25°C, 20 min after the illumination and 10 min after raising the sample to 25°C. It shows the mosquito rhodopsin with λ_{max} at 520–530 nm. Spectrum 2 is the difference between the spectrum started 1 min after the end of the illumination and the final spectrum taken at 25°C, 110 min after the illumination and 47 min after raising the sample temperature to 25°C. It shows the mosquito metarhodopsin with λ_{max} at ~ 480 nm.

light and light-dark) showed an increase of 16.7% and a decrease of 2.7%, respectively. The dark incorporating groups (dark-dark and light-dark) showed increases of 86.1% and 75.5%, respectively. A comparison of the percent change in specific activity between the light and dark incorporating

³ We believe that the 39,000 dalton band represents a single protein subunit. There was no evidence in any of our SDS gels of band splitting or broadening at 39,000 daltons. In addition, the fact that the 39,000 dalton component disappeared when only cornea-cuticle material was electrophoresed (yet all other proteins remained) would require that there be two or more proteins of identical molecular weight, intrinsic to the membrane present in the photoreceptor cells but not in the cornea-cuticle. This would mean that the proteins of the mosquito photoreceptor membrane would be unique in that opsin was not the major protein present as it is in all photoreceptors studied to date.

groups revealed that there was a significantly greater (Student's *t*, $P < 0.05$) increase in specific activity of the dark incorporating groups. The data indicate that the dark incorporating groups continue to accumulate isotope through 2 h of incorporation while incorporation into the light groups is reduced. Thus, it appears that maximal labelling is achieved after 2 h in the dark incorporating groups whereas it is achieved before 2 h in the light incorporating groups. Under conditions of continuous labelling, as were assumed to be in effect, the time to maximal labelling is an indication of the turnover rate (Goldberg and Dice, 1974). Therefore, light adaptation increases the turnover rate of opsin.

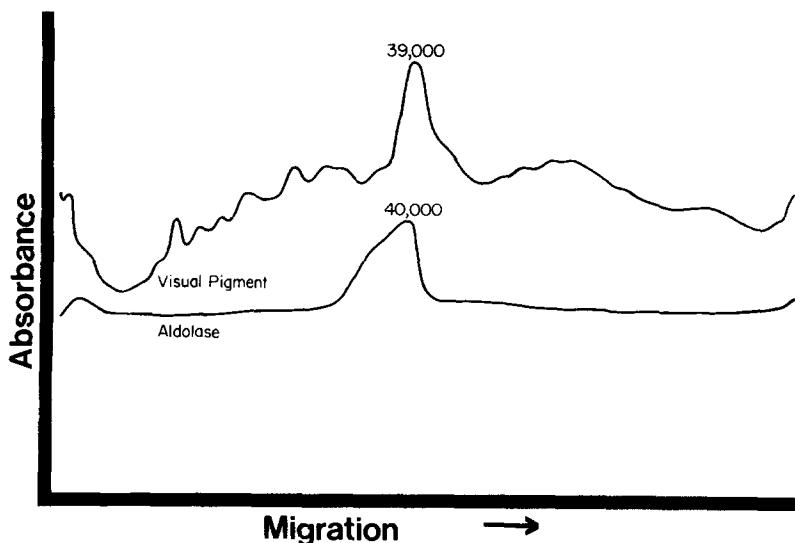


FIGURE 7. SDS acrylamide gel of the extract of the visual pigment band. Note a single major band with approximate molecular weight 39,000 daltons. Aldolase standard (40,000 daltons) run simultaneously on a separate gel.

DISCUSSION

Mosquito rhodopsin is a digitonin-soluble membrane protein which can be photoisomerized from rhodopsin (λ_{max} 515–520 nm) to metarhodopsin (λ_{max} 470–480 nm). The absorbance maxima obtained for the digitonin-extracted protein, whether in solution or acrylamide gel, match those obtained in the intact larval mosquito ocellus (R515–520, M480), as determined microspectrophotometrically (Brown and White, 1972). Similarly, the spectral sensitivity of the larval mosquito ocellus showed a maximum at 515–520 nm (Seldin et al., 1972). A study of the spectral sensitivity in the adult mosquito compound eye showed a maximum at 500 nm (Brammer and Clarin, 1976). However, in this study the 515–520 peak may well have been missed because of the filters employed: i.e., 466 nm, 500 nm, 533 nm.

The minimum molecular weight of mosquito opsin is very close to the

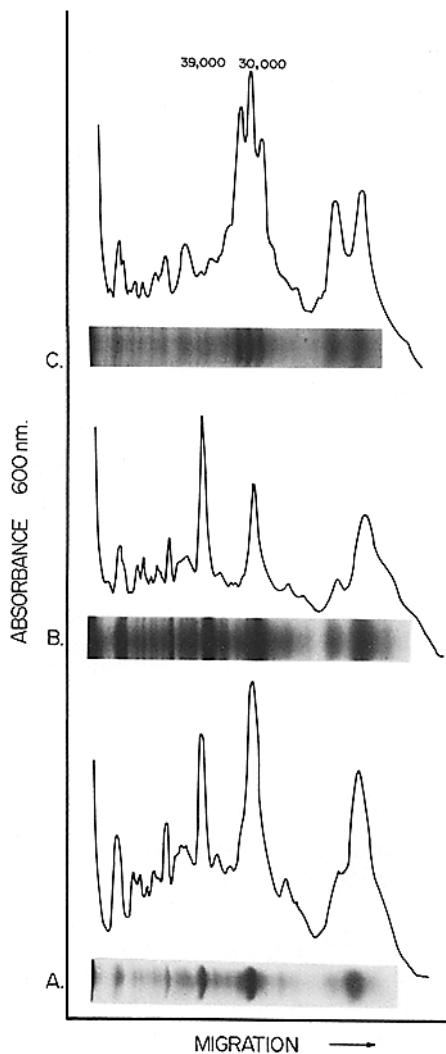


FIGURE 8. (A) SDS acrylamide gel of an extract of a buffer-washed pellet of mosquito heads. Note the appearance of at least 15 bands including a 39,000 ($R_f = 0.33$) and more prominent 30,000 dalton band. (B) SDS acrylamide gel of an extract of buffer-washed, excised mosquito eyes. Note the increase in relative concentration of the 39,000 dalton band and decrease in concentration of the 30,000 band. (C) SDS acrylamide gel of a buffer-washed preparation of cornea-cuticle from excised eyes. Note the increase in concentration of the material in the 30,000 dalton region and the absence of the band at 39,000 daltons.

molecular weights reported for the two other insect opsins which have been characterized: *Drosophila melanogaster*—37,000 daltons (Ostroy, 1978) and *Ascalaphus macaronius*—35,000 (Paulsen and Schwemer, 1973). In comparison, the molecular weight of most vertebrate opsins have been reported to fall between

35,000 and 40,000 daltons (Frank and Rodbard, 1975). Electrophoretic analysis of proteins in SDS extracts of mosquito heads, eyes, and the cornea-cuticle fraction showed that opsin is the predominant membrane protein in the photoreceptor. Opin has also been shown to be the major membrane protein in the photoreceptors of *Loligo pealii* (Hagins, 1973), *Ascalaphus macaronius* (Paulsen and Schwemer, 1973), and *Rana catesbeiana* (Bownds et al., 1971).

The pattern of incorporation of [³H]leucine into the photoreceptor cells of the mosquito indicates that protein is synthesized in the perinuclear cytoplasm and subsequently transported to the rhabdom.⁴ The appearance of label in a region rich in rough endoplasmic reticulum followed by transport to the photoreceptor membrane is similar to the pattern of labelling observed in other invertebrates (Burnell et al., 1970 Perrelet, 1972; Hafner and Bok, 1977; Krauhs et al., 1977) as well as in vertebrates (Young, 1967; Young and Droz, 1968). It seems clear to us from examination of more than 1,500 ommatidia that most of the newly synthesized protein in the photoreceptor cells ultimately becomes associated with the rhabdom. As in most other invertebrates, there is no order apparent in the entrance of label into the rhabdomal membrane.

TABLE II
[³H]LEUCINE INCORPORATION INTO OPSIN

Treatment	Specific activity	Specific activity	Δ%
	(2-h incorporation)	(2-h incorporation)	
	cpm/unit protein absorbance	cpm/unit protein absorbance	
Dark-dark	0.94	1.75	+86.1
Dark-light	1.82	1.77	-2.7
Light-light	1.12	1.31	+16.7
Light-dark	0.90	1.58	+75.5

Random appearance of label into photoreceptor membrane has also been reported for vertebrate cone photoreceptors (Bok and Young, 1972). In contrast, vertebrate rod photoreceptors show a highly ordered entrance and migration of labelled protein along the long axis of the outer segment (Young, 1967). It cannot be determined whether the random entrance of label into the mosquito rhabdom implies the random insertion of newly made protein molecules or rapid diffusional redistribution of protein molecules inserted in a more ordered fashion.

⁴ It is important to note that changes of [³H]leucine incorporation in either the autoradiographic or opsin-labelling experiments could result from changes in the leucine precursor pool. Preliminary experiments indicate that, in the mosquito, changes in the trichloroacetic acid-soluble radioactivity pool do not appear to account for changes in opsin or rhabdomal protein labelling. Despite the uncertainty concerning the influence of pool size, morphological changes characteristic of protein synthesis, and transport are known to occur shortly after the exposure of dark-adapted larval mosquito ocelli to light (White and Sundeep, 1967). We believe the formation of the organelles of protein synthesis and transport without an increase in protein synthesis to be highly unlikely, and we are confident that the changes in [³H]leucine incorporation we observe are indicative of changes in protein metabolism.

The quantitative data from the autoradiographic experiment shows that light adaptation prior to [^3H]leucine incorporation results in more extensive uptake into the rhabdom than does dark adaptation, and that the half-time of leucine incorporation is smaller by a factor of 2.6 in light-adapted eyes. These results are in accordance with the hypothesis that light increases the turnover of photoreceptor membrane in mosquito photoreceptor cells. Morphological analysis of membrane turnover indicates that the turnover of

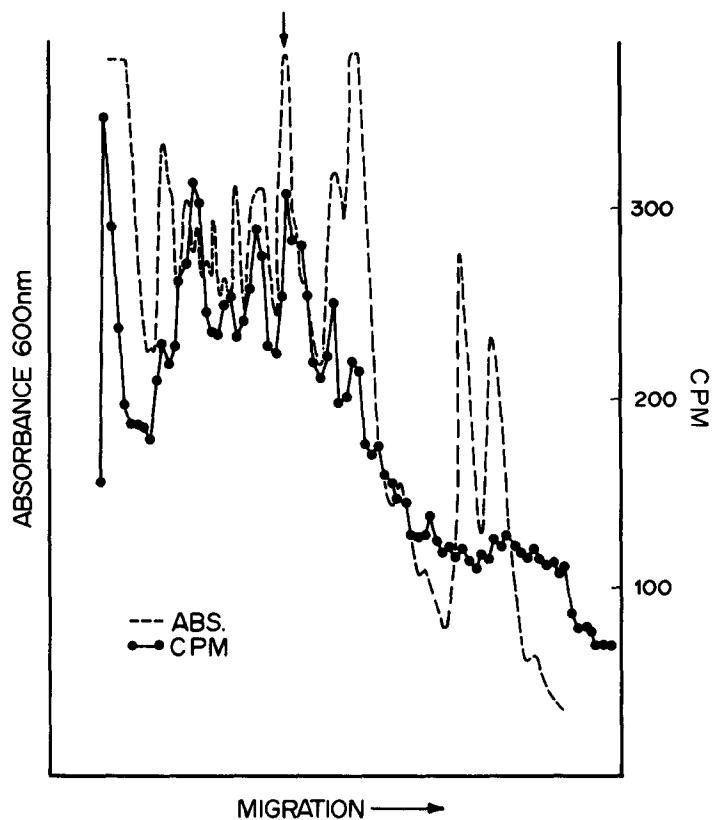


FIGURE 9. Labelled-opsin extraction—absorbance and radioactivity profiles. SDS extract of labelled mosquito heads (1-h light-light gel). The arrow marks the opsin band at molecular weight 39,000 daltons.

membrane occurs 2.9 times faster in light-adapted than dark-adapted eyes (Brammer et al., 1978). The results of the opsin-labelling experiment showed that the turnover rate of opsin also increased in light adaptation. Thus, both the turnover rate of opsin and the turnover of newly made protein are increased by light adaptation. However, a direct comparison between these two experiments may not be fully justified. In the opsin-labelling experiment, opsin is solubilized from all parts of the cell by the detergent, whereas the autoradiographic analysis is based only on the labelled protein that has been

transported to the rhabdom. The fact that light adaptation does increase the turnover rates of both rhabdomal protein and opsin is consistent with the hypothesis that the protein observed autoradiographically is opsin, but our inability to recover purified rhabdomal membranes (despite numerous attempts) has prevented us from obtaining the definitive data necessary to clearly establish that the labelled rhabdomal protein is opsin.

The finding that light enhanced the turnover of both rhabdomal protein and opsin agrees well with results obtained in morphological studies of membrane turnover in mosquitoes (White and Lord, 1975; Brammer and Clarin, 1976; Brammer et al., 1978). These studies showed that light adaptation results in a rapid initial decrease in the amount of photoreceptor membrane. After 1–2 h in the light, the amount of membrane became stabilized at a new lower level. It seems most probable to us that at the onset of illumination, both the rate of rhabdom membrane degradation and synthesis are increased, but that there is a time lag (for transport) before newly made protein can be incorporated into membrane. Thus, membrane degradation initially outstrips synthesis and the amount of membrane decreases. However, after 1–2 h of light adaptation, the amount of membrane is stabilized by incorporation of the recently transported protein into membrane. Substantial accumulation of labelled protein in the rhabdom is observable in autoradiographs after 1–2 h. Dark adaptation is accompanied by an initial rapid increase in the amount of rhabdom membrane (White and Lord, 1975) followed by a slower, more gradual increase at longer times of dark adaptation (Brammer et al., 1978). We believe that dark adaptation results in a virtual elimination of membrane degradation and a slowing in the rate of synthesis. The rapid increase in the amount of membrane presumably results from the incorporation of newly made protein (from the previous light period) into rhabdom membrane. The more gradual increase in membrane is accounted for by the low level of protein synthesis which persists in the dark. Thus, alterations in protein synthesis and degradation caused by light and dark adaptation could well account for the changes in photoreceptor membrane which have been described morphologically.

The relationship between opsin synthesis and rhodopsin recovery is a question which has yet to be resolved. It has been suggested that *de novo* synthesis of membrane protein is essential to the recovery of rhodopsin following photoisomerization (Goldman et al., 1975). Brown and White (1972) reported that they were unable to measure any regeneration of rhodopsin after 1–2 h of dark adaptation following photoisomerization of larval mosquito rhodopsin. Slow recovery of rhodopsin has also been reported for *Drosophila melanogaster* (Pak and Lidington, 1974) and *Galleria mellonella* (Goldman et al., 1975). However, it appears that in the adult mosquito, substantial amounts of membrane protein (presumably including opsin) have been synthesized at a time when rhodopsin recovery is not yet observable. This would imply that the rate-limiting step for the recovery of rhodopsin is not synthesis of new membrane but rather the addition of chromophore to newly made opsin and/or the chemical regeneration of rhodopsin from metarhodopsin. The relation-

ship between opsin turnover and rhodopsin recovery and the mechanisms by which light and dark adaptation control the biochemical events within the photoreceptor cells remain important areas for further investigation.

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