

Characteristics of *Drosophila* Rhodopsin in Wild-Type and *norpA* Vision Transduction Mutants

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ABSTRACT The properties of the major visual pigment of *Drosophila melanogaster* were evaluated. The visual pigment was isolated from other protein components using acrylamide gel electrophoresis and spectral identification. Sodium dodecyl sulfate (SDS) acrylamide gels of the isolated visual pigment gave a single protein subunit with a mol wt of 37,000 daltons. The rhodopsin₄₈₀ molar extinction coefficient was 35,000 liter/mol-cm (\pm 2,700 SE). The metarhodopsin₅₈₀ molar extinction coefficient was \sim 56,000 liter/mol-cm. Microspectrophotometry was used to compare the rhodopsin concentrations in wild-type flies and *norpA* vision transduction mutants. At 2 days of age (12 h dark-12 h light cycle, 19°C) all of the *norpA* flies exhibited a similar rhodopsin concentration (75% of the wild-type strain). By 21 days of age some of the *norpA* alleles showed substantially reduced rhodopsin concentrations (16–43% of normal), whereas others showed no major age-dependent decreases (68–77%). Temperature and light-dark cycle affected the reduction. Alleles with no receptor potential exhibited the largest decreases in rhodopsin concentration. The data indicate that the *norpA* phototransduction mutant has a defect in the system responsible for maintaining the rhodopsin₄₈₀ concentration. This defect in the rhodopsin maintenance system does not appear to be the cause of the reduced electroretinogram (ERG) amplitude observed in some of these mutants, but instead is a consequence of the decrease in ERG amplitude, or the flaw(s) responsible for the decrease in ERG amplitude.

INTRODUCTION

The *norpA* group of mutants of *Drosophila melanogaster* results from a single gene lesion on the X chromosome (chromosomal map location 6.5 ± 0.7 ; Pak, 1975) and is characterized by a defect in the visual transduction process. More extreme alleles of this mutation have no detectable receptor potential (Alawi et al., 1972). Other alleles have measurable receptor potentials, including some with receptor potentials of normal amplitude, but in all of these alleles the time-course of the potential is prolonged (Alawi et al., 1972). The quantum bumps of the *norpA* alleles exhibit a normal time-course and amplitude but an abnormal latency dispersion (Pak et al., 1976). The defect responsible for the altered receptor potential of the *norpA* mutant is not yet known.

The major visual pigment of *Drosophila* (the visual pigment of retinula cells 1–6) is a rhodopsin₄₈₀ which is photoconvertible to a stable metarhodopsin₅₈₀

(Ostroy et al., 1974; Pak and Lidington, 1974; Harris et al., 1976). The *Drosophila* rhodopsin exhibits the same photochemical properties as other muscoid insects (Hamdorf et al., 1973; Hamdorf and Rosner, 1973; Stavenga et al., 1973). Compared to the four or five spectrally distinguished states of the vertebrate rhodopsin which would be observed under these conditions, most of the insect visual pigments appear to exhibit a very much simplified (and often reversible) response to light. It is, therefore, of some interest to investigate the molecular characteristics of the insect visual pigment to evaluate their possible role in the spectral intermediate sequence. As reported here, the molecular weight and extinction coefficient of the *Drosophila* rhodopsin are similar to those of the vertebrate rhodopsin.

A major value of the use of genetic mutants is that one can select the mutant to affect a process of interest (such as phototransduction) even when one does not know the detailed steps involved in that process. In the visual system the detailed steps associated with the processes of phototransduction, adaptation, maintenance, and metabolism are largely unresolved. In addition, the possible interrelationships between these processes are also largely unknown. The data presented in this paper show that *norpA* mutants (which were selected to have defects in the phototransduction process) also exhibit defects in the system responsible for renewing the rhodopsin₄₈₀ concentration after illumination. The defect in the renewal system seems related to the defect in the phototransduction process, because those alleles with the smallest receptor potentials exhibit the least ability to renew their rhodopsin. Also, the defect in the renewal system seems related to some slow processes in the photoreceptor cell because the effects become more pronounced as the fly becomes older. Because the decrease in the rhodopsin₄₈₀ concentration develops slowly with age, whereas the receptor potentials of the fly do not exhibit any age dependence (and therefore can be zero at a time when the fly has its full complement of rhodopsin concentration), the present data affirm the indirect data which suggested that the changes in rhodopsin concentration were not responsible for the altered receptor potentials of these mutants (see Discussion).

Although *norpA* is a single gene mutation, we have already observed concentration changes in two eye protein subunits. On sodium dodecyl sulfate (SDS) acrylamide gels, a component of 51,000 daltons was increased in concentration and a component of 46,000 daltons was decreased in concentration, with the degree of change dependent on the particular allele (Ostroy and Pak, 1973, 1974). The molecular weight of the *Drosophila* rhodopsin had not been determined previously. The molecular weight of the one insect visual pigment which had been determined earlier had a value which was close enough to the altered components of the *Drosophila* eye so that one could not a priori conclude that the altered components were not the *Drosophila* rhodopsin (35,000 daltons, *Ascalaphus macaronius*; Paulsen and Schwemer, 1973). Therefore it was important to determine the molecular weight of the *Drosophila* rhodopsin. The results presented here show that the protein concentration changes observed in the SDS gels of *norpA* were not rhodopsin₄₈₀. The opsin component has an apparent mol wt of 37,000 daltons and under the conditions of those experiments did not exhibit major changes in concentration.

MATERIALS AND METHODS

For the acrylamide gel electrophoresis of native visual pigment, 1,000 eyes (from flies which had been dark adapted for 24 h) were dissected under dim red light ($\lambda > 620$ nm). To avoid contaminants from other portions of the head, only those parts of the eye that could be removed completely free of other parts of the head were dissected. The sample was crushed and washed twice with 1 ml of cold 0.07 M Tris buffer solution, pH 8.3, and the precipitate was recovered after each wash by centrifugation for 30 min at 27,000 *g* (Sorvall RC2B centrifuge, DuPont Instruments-Sorvall, DuPont Co., Wilmington, Del.). The visual pigment was extracted by crushing and resuspending the precipitate in 0.2 ml of a 1.6% digitonin, 0.07 M Tris solution, and stirring for 20 min. The sample was then centrifuged at 27,000 *g* for 30 min. After adding 1–3 mg of sucrose, the supernate was added to a 3.5 or 7% acrylamide disc gel (the gel buffer was 0.09 M Tris, pH 8.3; the electrode buffer was 0.07 M Tris, pH 8.3). The sample was electrophoresed at 5°C for 1 h 40 min at 2 mA per gel. The gel diameter was 0.5 cm. After the electrophoresis, the sample was placed on a temperature-controlled holder in a Cary 118 recording spectrophotometer (Varian Associates, Instrument Div., Palo Alto, Calif.). The gel was kept at 11°C. The illumination of the sample was carried out using a 1/4 × 12 inches light pipe (American Optical LG 1/4, American Optical Corp., Southbridge, Mass.), fiber optics illuminator (American Optical Illuminator type K150), and a broad band 420- to 470-nm interference filter (Balzer K-2, Balzers High Vacuum Corp., Santa Ana, Calif.).

To determine the molecular weight of the visual pigment, the acrylamide gel was left unstained, and a center portion of the visual pigment band (spectrally determined) was cut from the gel (~ 2 mm of the 6 mm visual pigment band). The gel section was dissolved in a 5% SDS solution in 0.01 M phosphate buffer, pH 7.0. 5 μ l of β -mercaptoethanol was then added and the sample was incubated at 80°C for 20 min. The supernate was then added to the top of a 1% SDS gel (Weber and Osborn, 1969) and the sample was electrophoresed at 1.5 mA/gel for 15 min and at 8 mA/gel for 6 h. The sample was then stained with Coomassie Blue, destained, and the absorbance of the gel was measured as described previously (Ostroy and Pak, 1973, 1974).

To determine the extinction coefficient of the *Drosophila* rhodopsin, the method of Wald and Brown (1953) was used. 1,000 heads were dissected (under dim red light) from the white-eyed wild-type strain (Oregon-R, brown, scarlet; see end of Materials and Methods). The flies had been dark-adapted for 24 h and kept frozen before use. The heads were then crushed and suspended in a saline solution (140 mM NaCl, 4 mM KCl, 2 mM CaCl₂, 2 mM MgCl₂, 1.7 mM NaH₂PO₄, 4.3 mM Na₂HPO₄, pH 7.1). After stirring for 10 min, the mixture was centrifuged at 27,000 *g* for 30 min. The precipitate was then resuspended in the saline solution and the procedure was repeated. The visual pigment was extracted by adding 1 ml of a 1% Triton X-100/saline solution to the precipitate and stirring for 15 min. The solution was then centrifuged at 27,000 *g* for 30 min and the supernate was filtered through a syringe filter. Just before the experiment, a neutralized solution of hydroxylamine was added so that the final hydroxylamine concentration was 0.2 M. Triton X-100 was used instead of digitonin because the metarhodopsin₃₈₀ was thermally unstable in this solvent, and the hydroxylamine reacted with the retinal. We could not find comparable conditions in digitonin extracts. The extinction coefficient for the retinal-oxime in Triton X-100 was determined by comparing the absorbances of the retinal oximes formed in digitonin and Triton X-100 using the same retinal concentrations. The extinction coefficient for the retinal-oxime in Triton X-100 was 54,700 liter/mol-cm ($\pm 4,000$ SE; $\lambda_{\text{max}} = 367$ nm; $n = 3$). This is not very different from the value in digitonin: 51,600 liter/mol-cm (Wald and Brown, 1953).

The microspectrophotometric measurements were made on a modified Cary model 14 (Strehler et al., 1963; Ostroy et al., 1974). For these measurements, single flies were

placed on ice, their heads were removed, and the sample beam of the spectrophotometer was focused on one of the eyes (using 580-nm light). An initial spectrum was then taken. The eye was then illuminated for 5 min with an 80-W fiber optic illuminator (American Optical, type II-80) containing two heat filters and a 490-nm interference filter (Ditric Optics, Inc., Malboro, Mass.). Another spectrum was then taken. After completion of the spectrum, a 5-min illumination with orange light was carried out using a 580-nm interference filter (Ditric Optics, Inc.). A third spectrum was then taken. This was followed by a second blue illumination, fourth spectrum, another orange illumination, and a fifth final spectrum. Difference spectra were then taken between each of the succeeding spectra, resulting in four difference spectra illustrating the rhodopsin₄₈₀ to metarhodopsin₅₈₀ transition and its reversal. The intensity of the 490-nm illumination was 1.7×10^{-4} W/cm² and the intensity of the 580-nm illumination was 4.2×10^{-4} W/cm². The measuring beam was 5.0×10^{-7} W/cm² at 500 nm. At the scanning speed used for the microspectrophotometry, 20 full spectra of a dark-adapted preparation (always long wavelengths to short wavelengths) did not exhibit any measurable bleaching (limit of resolution, 0.25% bleaching per scan). To determine the relative rhodopsin₄₈₀ concentration, the peak absorbance at 580 nm was determined for each of the difference spectra and the average absorbance was computed from those spectra which exhibited the characteristic difference spectra (normally three to four spectra). The values presented in the results are a further average of a minimum of three independent experiments of the type described. The absorption peak of the metarhodopsin₅₈₀ was used as a measure of the relative rhodopsin₄₈₀ concentration for two major reasons. In the *Drosophila* eye, the absorbance changes at 580 nm are approximately three times larger than the changes at 480 nm (Ostroy et al., 1974). Also, the rhodopsin of central cell no. 7 has a peak at 370 nm and it is transformed photochemically to a metarhodopsin at 480 nm (Harris et al., 1976). In whole eye spectra this transition is probably observed to only a minor degree compared to the rhodopsin₄₈₀ ⇌ metarhodopsin₅₈₀ transition of the peripheral cells 1-6 (Harris et al., 1976; Ostroy et al., 1974). However, it could cause some difficulties in the 480-nm region of the spectrum. In those experiments where differences at both 580 and 480 nm were measured, the changes have been quantitatively proportional. We have found this type of spectrophotometry to be quantitatively reproducible (as illustrated by the small standard errors reported in Table I) and not subject to the geometry and pupil effects which can alter the absorbance in deep-pseudopupil measurements (Stavenga et al., 1973; Stavenga, 1976; Lo, 1977). However, our technique exhibits absorbance changes which are about a factor of two smaller than those observed using the deep-pseudopupil (Lo, 1977). This is presumably because some of our incident light goes through nonvisual pigment areas of the eye.

Both the wild-type strain (Oregon-R) and *norpA* mutant strains used in this work were genetically placed on a white-eyed background to avoid difficulties due to screening pigments (by a combination of brown and scarlet mutations on the second and third chromosomes, respectively; Pak et al., 1969). These genes do not appear to alter the electroretinograms (ERGs) of the flies except for an increase in their sensitivity to light (Pak et al., 1969). Before eclosion, the pupae were kept on a 12 h light-12 h dark cycle. For adult flies on the light-dark cycle, the timing was continued throughout adulthood except that the flies were dark adapted for 24 h just before use. Flies that were to be maintained in the dark as adults were placed into the dark as pupae 24 h before the collection of newly emerged flies. The adult flies were treated as described in the legends. As pupae, Oregon-R flies and the *norpA* alleles were maintained at 19°C, except for *norpA*^{P13}, *norpA*^{P41}, and *norpA*^{P24}, which were incubated at 24°C, 24 h before the collection of newly emerged flies. These particular flies were placed at the higher temperature for the limited period of time to induce eclosion.

RESULTS

Properties of the Drosophila Rhodopsin₄₈₀

To isolate rhodopsin₄₈₀ from other eye proteins, digitonin extracts of the water-insoluble proteins from excised eyes were electrophoresed on acrylamide gels. As shown in Fig. 1, only two major bands were evident when the general protein stain, amido black, was used. When the unstained gels were scanned at 480 nm, a single peak was observed. That peak was sensitive to illumination (Fig. 1) and exhibited the spectral changes characteristic of the rhodopsin₄₈₀ ⇌ metarhodopsin₅₈₀ transition (Fig. 2). The approximate molecular weight of the rhodopsin₄₈₀ was determined by cutting the rhodopsin₄₈₀ band from the acrylamide gel and re-electrophoresing it on an SDS acrylamide gel. An example

TABLE I
EFFECT OF AGE ON THE *DROSOPHILA* RHODOSPIN

Species	ERG amplitude* (from Ostroy and Pak, 1973)	Absorbance value (±SE)					
		2 days	4 days	6 days	8 days	14 days	21 days
Wild-type	20 mV (±1.4)	0.037 (±0.002)	0.042 (±0.002)	0.042 (±0.0008)	0.040 (±0.005)	0.044 (±0.005)	0.044 (±0.005)
<i>norpA^{P16}</i>	17.7 mV (±1.0)	0.029 (±0.004)	0.040 (±0.002)	0.041 (±0.005)	0.038 (±0.002)	0.038 (±0.002)	0.034 (±0.003)
<i>norpA^{H5}</i>	13.7 mV (±1.1)	0.030 (±0.003)	0.045 (±0.0009)	0.041 (±0.004)	0.025 (±0.002)	0.036 (±0.0008)	0.031 (±0.002)
<i>norpA^{P12}</i>	2 mV (±0.2)	0.027 (±0.003)	0.026 (±0.0009)	0.031 (±0.008)	0.026 (±0.005)	0.026 (±0.0006)	0.030 (±0.004)
<i>norpA^{H44}</i>	0	0.026 (±0.001)	0.026 (±0.003)	0.036 (±0.005)	0.033 (±0.002)	0.027 (±0.002)	0.019 (±0.002)
<i>norpA^{P13}</i>	1.4 mV (±0.3)	0.030 (±0.005)	0.033 (±0.0004)	0.030 (±0.002)	0.019 (±0.0004)	0.017 (±0.003)	0.015 (±0.005)
<i>norpA^{P41}</i>	0	0.027 (±0.002)	0.031 (±0.004)	0.036 (±0.001)	0.025 (±0.004)	0.017 (±0.005)	0.009 (±0.004)
<i>norpA^{P24}</i>	0	0.026 (±0.002)	0.025 (±0.002)	0.023 (±0.004)	0.021 (±0.002)	0.018 (±0.004)	0.007 (±0.003)

Flies maintained at 19°C, 12 h light-12 h dark cycle and then dark-adapted for 24 h before the measurement. Microspectrophotometric measurements of whole eyes. Absorbance change of the metarhodopsin₅₈₀ is presented as an indication of the rhodopsin₄₈₀ concentration (See Materials and Methods).

* The amplitude of the sustained negative wave of the ERG was measured in response to a 500-nm flash with an intensity of 4.25×10^{12} $\frac{\text{photons}}{\text{cm}^2 \cdot \text{s}}$ and duration of 0.5 s.

of the results is shown in Fig. 3. Single bands were obtained with an approximate mol wt of 37,000 daltons and a mobility of $0.422 (\pm 0.008)$.

The molar extinction coefficient of the rhodopsin was determined using the techniques of Wald and Brown (1953). The technique involves measuring the absorbance of the retinal-oxime formed after bleaching a detergent extract of rhodopsin in the presence of hydroxylamine. A representative experiment is shown in Fig. 4. The value of the rhodopsin₄₈₀ extinction coefficient was 35,000 liter/mol-cm ($\pm 2,700$ SE; $\lambda_{\text{max}} = 480$ nm; $n = 3$). This value should be considered indistinguishable from the value of 33,000 liter/mol-cm reported by Harris et al. (1976). They estimated the extinction coefficient of the *Drosophila*

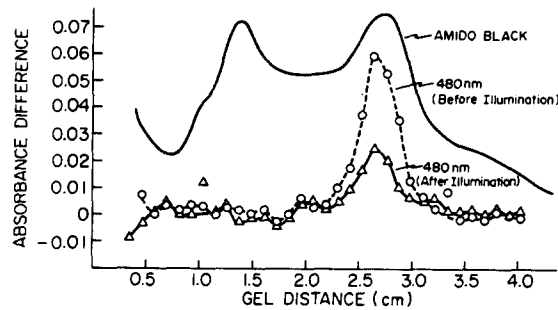


FIGURE 1. Proteins observed in acrylamide gels with a digitonin extract of *Drosophila* eyes and isolation of the rhodopsin₄₈₀ band. A representative experiment from 32 runs. Lower curves are the 480-nm absorbances of the unstained gel before and after illumination. The upper curve is the absorbance of the same gel after staining with amido black. The gel distance is the distance of the amido black-stained gel. To correct for the gel expansion on staining, the abscissas of the lower curves were multiplied by 1.15. The absorbance scale is the scale for the unstained gel. The amido black-stained gel shows relative absorbances only.

rhodopsin in a solution homogenate by measuring the amount of rhodopsin converted to metarhodopsin by a specified light intensity. The agreement between our values and theirs is quite remarkable, considering the differences in technique. The determination of the rhodopsin extinction coefficient presented here assumes that the β -band of the rhodopsin is negligible compared to the absorbance at its spectral maximum. This assumption is consistent with the spectral data which have been obtained in the *Drosophila* up to the present (Fig. 2 *b*; Ostroy et al., 1974; Harris et al., 1977), but some β -band absorbance may still be present. Absorbance by a β -band in the 360-nm region of the spectrum which decreases upon illumination would cause our calculated value for the rhodopsin extinction coefficient to be smaller than the actual value.

In the present study, having measured the extinction coefficient of the rhodopsin₄₈₀, and measuring the isosbestic point of the rhodopsin₄₈₀-metarhodopsin₅₈₀ conversion (509 nm (\pm 2 nm) for digitonin extracts, $n = 9$), should allow us to estimate the extinction coefficient for the metarhodopsin₅₈₀. From the Dartnall nomogram (Dartnall, 1953) at the isosbestic point, the extinction of the rhodopsin is $\sim 75\%$ of the extinction at the 480-nm peak. From the Ebrey and Honig nomogram (Ebrey and Honig, 1977) the isosbestic is at $\sim 47\%$ of the 580-nm peak extinction (Fig 2 *b*). Therefore, because the extinction coefficient for each species is equal at the isosbestic point, one can estimate the metarhodopsin₅₈₀ extinction coefficient as follows:

$$\begin{aligned} A_{\text{rhodopsin}}^{509 \text{ nm}} &= A_{\text{metarhodopsin}}^{509 \text{ nm}} \\ 0.75 \epsilon_{\text{rhodopsin}}^{480} (l) (c) &= 0.47 \epsilon_{\text{metarhodopsin}}^{580} (l) (c) \\ (0.75) (35,000) &= (0.47) \epsilon_{\text{metarhodopsin}}^{580} \\ \epsilon_{\text{metarhodopsin}}^{580} &\cong 56,000 \text{ liter/mol-cm.} \end{aligned}$$

This value is somewhat higher than the 43,000 liter/mol-cm determined by Harris et al. (1976). However, considering the approximations involved in both

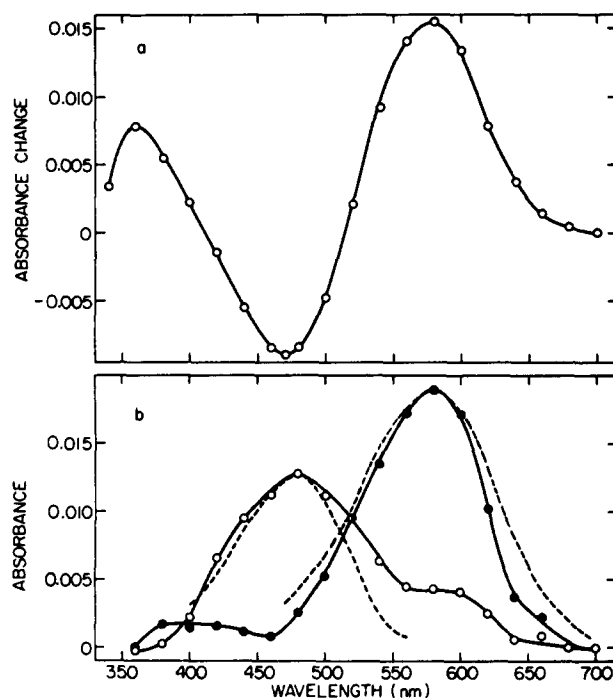


FIGURE 2. Spectral changes of the rhodopsin₄₈₀ and metarhodopsin₅₈₀ in acrylamide gels. This gel was not stained with amido black as was the gel shown in Fig. 1, but the rhodopsin section was later cut from the gel and rerun on an SDS gel (Fig. 3). A representative experiment from 13 runs. Temp = 11°C. The upper curve (a) shows the difference spectrum obtained after illumination with blue light (420–470 nm). It was obtained by subtracting the spectrum taken before illumination from one taken immediately after illumination. An increase in absorbance in the 360- to 380-nm region seems characteristic of the reaction and appears to represent the release of some free retinal. The isosbestic point for the rhodopsin₄₈₀ to metarhodopsin₅₈₀ conversion seems shifted to longer wavelengths in acrylamide gels and in microspectrophotometric experiments (Fig. 6; Ostroy et al., 1974) when compared to digitonin extracts (see Results; Ostroy et al., 1974). This shift to longer wavelengths may result from the presence or formation of components other than rhodopsin₄₈₀ or metarhodopsin₅₈₀, or may be a result of the inhomogeneity of the sample. The lower curves (b) show the rhodopsin₄₈₀ and metarhodopsin₅₈₀ spectra derived in the following way: The rhodopsin₄₈₀ curve was obtained by subtracting the absorbance of the gel in a protein-free portion from the spectrum taken before illumination. The metarhodopsin₅₈₀ curve was obtained by subtracting the absorbance of the gel in a protein-free portion from the first spectrum taken immediately after illumination. The relative heights of the curves are the actual data obtained and do not necessarily reflect the relative extinction coefficient of the rhodopsin₄₈₀ and metarhodopsin₅₈₀. The dashed curves are the respective nomograms for visual pigments absorbing maximally at 480 nm (Dartnall, 1953) and 580 nm (Ebrey and Honig, 1977). The long wavelength region of the rhodopsin spectrum exhibits increased absorbance compared to the nomogram. It seems apparent that some metarhodopsin₅₈₀ was present in the unbleached preparation, though it does not affect the results or conclusions.

calculations, this difference cannot be regarded too seriously. In the present calculation within the standard error of the isosbestic point, the metarhodopsin₅₈₀ extinction coefficients would be 49,000 liter/mol-cm (511 nm) to 59,000 liter/mol-cm (507 nm).

Rhodopsin Changes in *norpA* Mutants

The rhodopsin₄₈₀ concentration effects caused by the *norpA* mutation were studied in seven *norpA* alleles and a wild-type strain. The data are presented in Table I for flies kept at 19°C on a 12 h light-12 h dark cycle. The data for a few

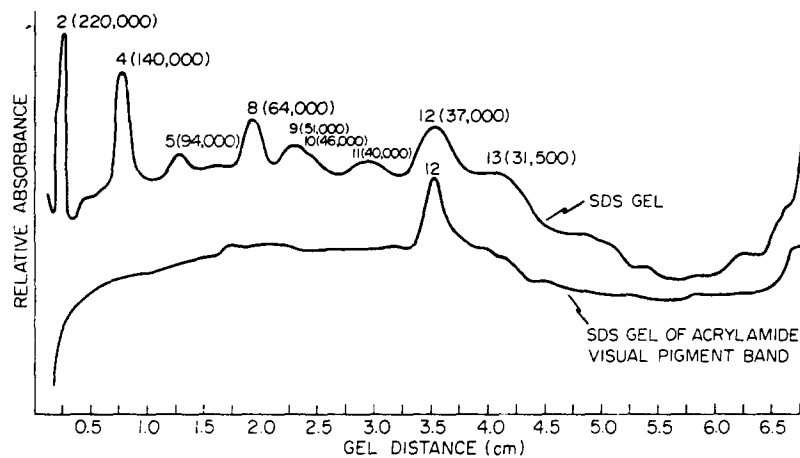


FIGURE 3. Re-electrophoresis of the rhodopsin band on SDS gels. Lower curve is the SDS gel resulting from re-electrophoresis of the rhodopsin band cut from the gel shown in Fig. 2. A representative experiment from a total of 13. Upper curve shows the SDS gel of a digitonin extract from whole eyes. Each band is designated by a band number and the approximate molecular weight (inside the parentheses) using the identifications from Ostroy and Pak (1974).

of the mutants are illustrated in Figs. 5 and 6. At 2 days of age all of the *norpA* mutants had a similar concentration of rhodopsin amounting to an average absorbance of 0.028 (± 0.0007 SE). This represents 75% of the amount of rhodopsin found in the wild type. At 4–6 days of age, increases in rhodopsin concentration were observed in the wild-type strain and some of the mutants. At 4–6 days of age, the rhodopsin concentration of *norpA*^{P16} and *norpA*^{H5} appear indistinguishable from those of the wild-type strain, whereas the other *norpA* mutants still exhibit a somewhat lower concentration (55–86% of the concentration in the wild-type strain). By 6 days of age, *norpA*^{P24} (which will eventually exhibit the lowest rhodopsin concentration at 21 days of age) has begun to show decreases in rhodopsin concentration. Decreases in rhodopsin concentration are observed in all of the mutants by 8 days of age. By 21 days of age some of the *norpA* alleles show dramatic age-dependent decreases in rhodopsin concentration, while others showed no major age-dependent decreases. The most severely affected mutants are *norpA*^{P24}, *norpA*^{P41}, *norpA*^{P13}, and *norpA*^{H44}, which at 21 days

of age, respectively exhibit 16, 20, 34, and 43% of the rhodopsin concentration in the wild type. Less severely affected are *norpA^{P12}*, *norpA^{H5}*, and *norpA^{P16}*, which, at 21 days of age, exhibit 68, 70, and 77% of the concentration observed in the wild-type strain. Fig. 6 presents some representative spectra from the wild-type, *norpA^{P12}* and *norpA^{H44}* flies. Only slight shape changes in the difference spectra are observed, suggesting that no major new or altered visual pigments are formed during this decrease.

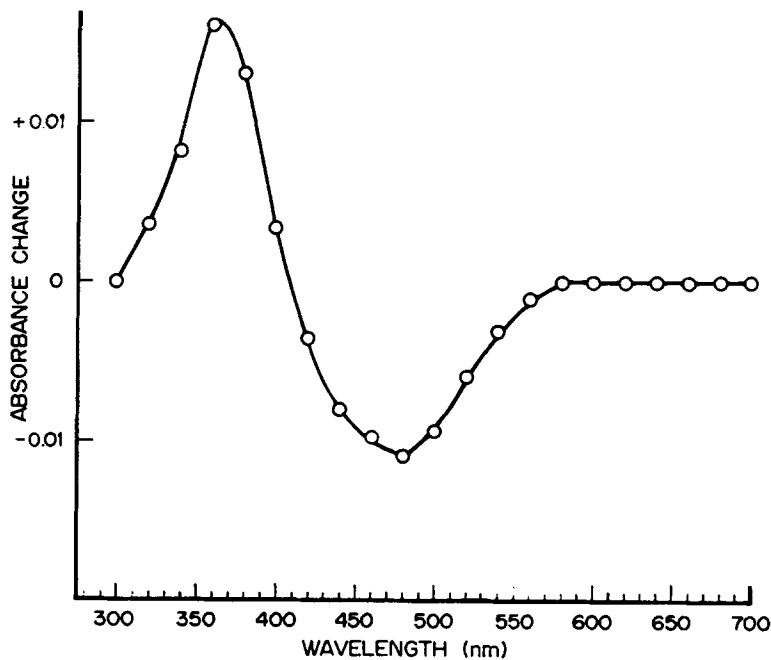


FIGURE 4. Determination of the extinction coefficient of rhodopsin. Curve shown is the difference spectra obtained on bleaching the *Drosophila* rhodopsin in a Triton X-100 extract in the presence of 0.2 M hydroxylamine ($n = 3$). The increase in absorbance at 360 nm is the retinal-oxime. For the experiment shown, the calculated rhodopsin extinction coefficient would be:

$$\frac{0.011 A_{\text{rhodopsin}}}{0.017 A_{\text{retinal-oxime}}} \times 54,700 \epsilon_{\text{oxime}} = 35,394 \text{ liter/mol-cm.}$$

The changes in rhodopsin concentration may be compared with the ERG amplitudes normally associated with these flies (Ostroy and Pak, 1973). As presented in Table I, those *norpA* mutants which showed the largest age-dependent decreases in rhodopsin concentration also exhibited either no ERG (*norpA^{P24}*, *norpA^{P41}*, and *norpA^{H44}*), or an ERG of very small amplitude (*norpA^{P13}*); those mutants which showed no major age-dependent decrease in rhodopsin concentration exhibited a slightly larger ERG amplitude (*norpA^{P12}*) or an ERG amplitude close to normal (*norpA^{H5}* and *norpA^{P16}*).

Increasing the environmental temperature of the flies to 24°C caused some

dramatic changes in the rhodopsin concentrations. Fig. 7 compares the effect of temperature on wild-type flies and the mutants *norpA^{P12}* and *norpA^{H44}*. The higher environmental temperature caused some increases in rhodopsin₄₈₀ concentration at 2 days of age. However, by 21 days of age, decreases in rhodopsin concentration were observed in all of the flies tested. At 21 days of age, the wild-type flies exhibited only 40% of their 2-day-old concentration. The *norpA^{P12}* flies exhibited 30% of their 2-day-old concentration and no rhodopsin could be detected in the *norpA^{H44}* flies at 14 or 21 days of age (limit of detection, 0.001 absorbance units).

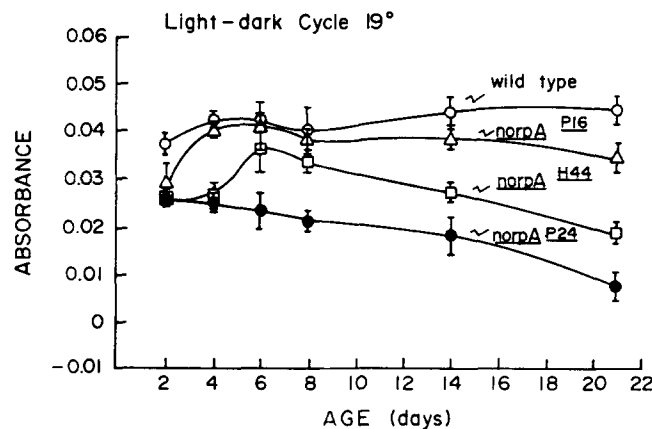


FIGURE 5. The effect of age on the rhodopsin concentration in *Drosophila* eyes. Selected microspectrophotometric data from Table I. Each point represents the average from at least three flies where the value for each fly results from an average of 3 to 4 spectra (see Materials and Methods). Error bars are standard error.

The light-dark cycle of the fly also had an effect on the rhodopsin concentration. The data are presented in Fig. 8 and compare flies which had been maintained continuously in the dark with ones on a 12 h light-12 h dark cycle. For the wild-type fly there are no significant differences in rhodopsin concentration for flies kept in the dark when compared to ones on the 12 h light-12 h dark cycle. Up to 8 days of age, the same is true of *norpA^{P12}* and *norpA^{H44}*. However, in these mutants, at ages above 8 days, the flies kept in the dark exhibited a significantly higher concentration of rhodopsin than ones kept on the 12 h light-12 h dark cycle.

DISCUSSION

Properties of the Drosophila Rhodopsin

The properties of the *Drosophila* rhodopsin which have been presented seem consistent with the properties of other visual pigments. The molecular weight of 37,000 daltons as determined by SDS gels is similar to the molecular weight of the other insect visual pigment which has been determined—*Ascalaphus macaro-*

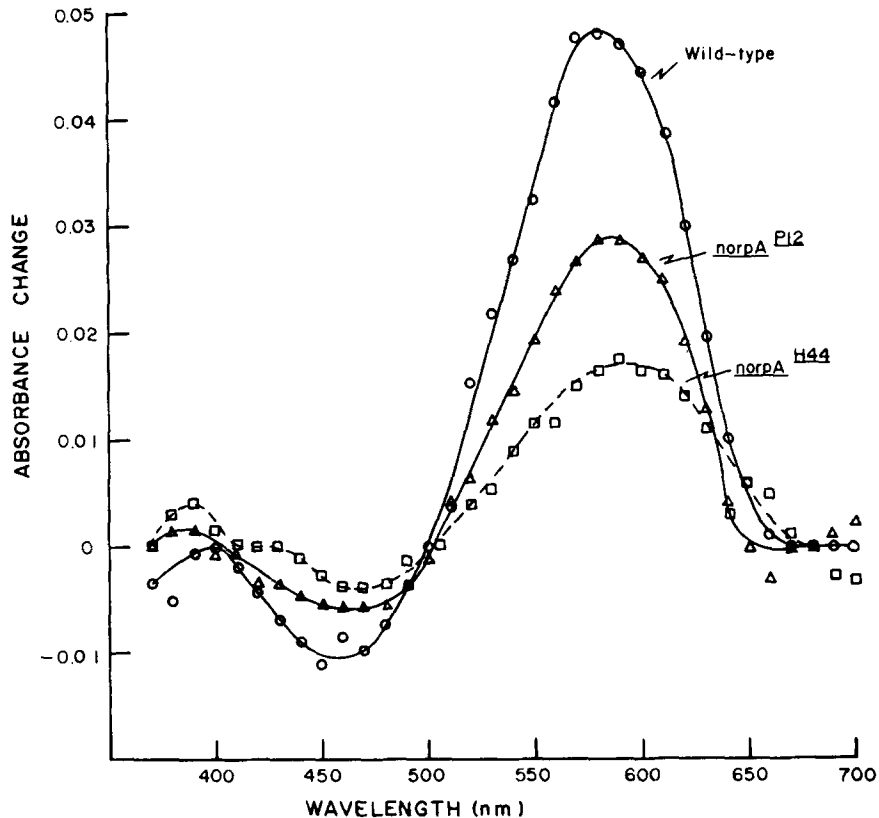


FIGURE 6. Representative microspectrophotometric measurements of whole eyes of *Drosophila*. The curves show the difference spectra obtained immediately after illumination. All flies were maintained on 12 h light-12 h dark cycle at 19°C. Spectra were taken at 24°C. Wild type and *norpA*^{H44} were 21 days of age. *NorpA*^{P12} was 28 days of age. In these measurements the absorbance change observed at 580 nm compared to the absorbance change observed at 480 nm is somewhat larger than one could expect from the ratio of extinction coefficients and the broadness of the spectra. Because a 580-nm pigment exhibits ~30% of its peak absorbance at 480 nm whereas a 480 nm pigment exhibits only 3% of its peak absorbance at 580 nm, the factor of 1.6 nm in extinction coefficients becomes a factor of 3 in measured absorbance changes. The observed ratios in this figure are approximately four to five. The reasons for this are not clear but may be related to the phenomena presented in the Fig. 2 a legend.

nius, 35,000 daltons (Paulsen and Schwemer, 1973). It is also consistent with another insect visual pigment that we have recently investigated, *Aedes aegypti*, 39,000 daltons.¹ The molecular weight is also similar to the molecular weight of vertebrate rhodopsin (35,000–40,000; Abrahamson and Fager, 1973). When one considers the apparent differences in photochemical behavior of these visual pigments, the similarity of the molecular weights is surprising. It is also most

¹ Stein, P. L., S. E. Ostroy, and J. D. Brammer. Manuscript in preparation.

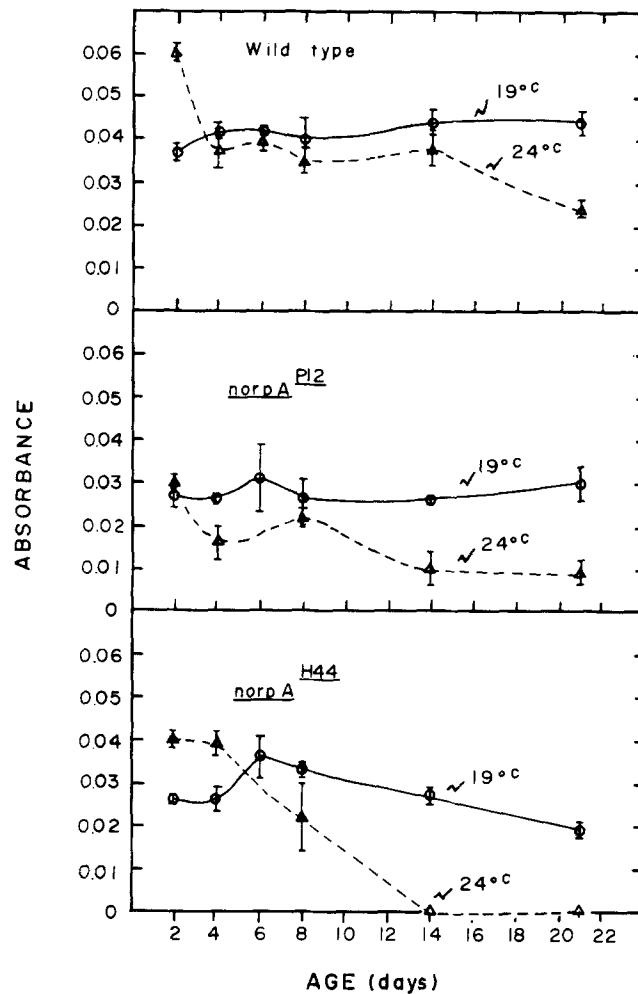


FIGURE 7. The effect of environmental temperature on the rhodopsin₄₈₀ concentration in *Drosophila*. Microspectrophotometric measurements from whole eyes of *Drosophila* comparing flies kept on a 12 h light-12 h dark cycle. Error bars are standard error.

interesting that the extinction coefficient for the *Drosophila* rhodopsin is so similar to that of the vertebrate (40,600; Wald and Brown, 1953), particularly in light of the other differences in photochemical behavior. The higher extinction coefficient for the *Drosophila* metarhodopsin₅₈₀, which is similarly observed in the vertebrate metarhodopsin I₄₇₈ (Matthews et al., 1963) is another parallel between the properties of the *Drosophila* and vertebrate rhodopsins.

Rhodopsin Changes in the norpA Mutants

The *norpA* mutation appears to have had an effect on the system responsible for maintaining the rhodopsin concentration of the *Drosophila* eye. When the *norpA*

flies (*P12* and *H44*) were kept continuously in the dark, they were able to maintain their concentration of rhodopsin (between 71 and 93% of the wild-type concentration, Fig. 8). However, when additional demands were made on the rhodopsin maintenance system by placing the flies on a light-dark cycle, some of the *norpA* alleles (such as *H44*) were not able to maintain their rhodopsin concentration with increasing age (Table I and Figs. 5 and 6). The effect was more pronounced at an environmental temperature of 24°C (Fig. 7). The defect in the maintenance of rhodopsin appears to involve the integrity of the photoreceptor membrane, or the retinal concentration of the photoreceptor, or the structure of the opsin or some other factor involved in the retinal-opsin

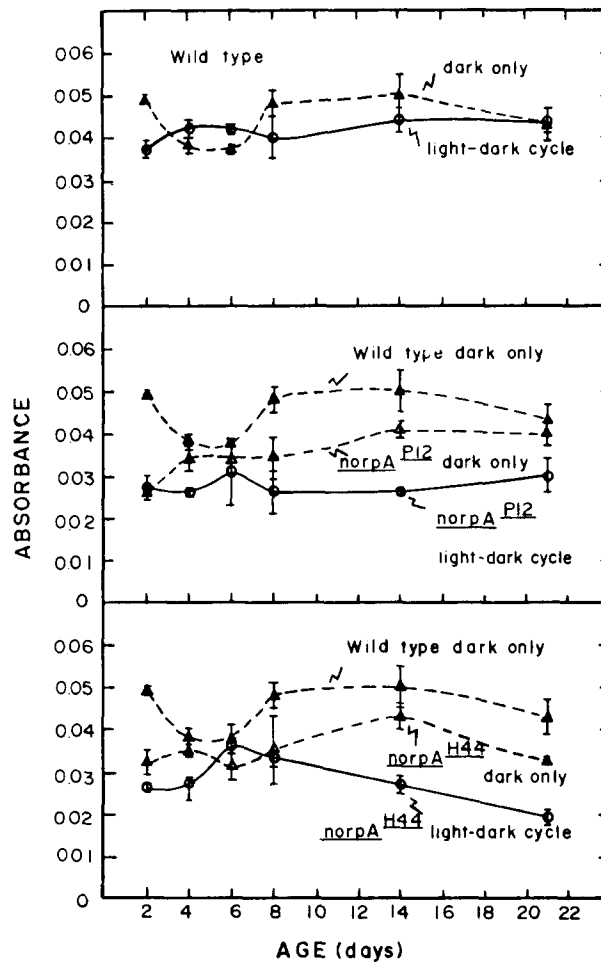


FIGURE 8. The effect of light-dark cycle on the rhodopsin₄₈₀ concentration in *Drosophila*. Microspectrophotometric measurements from whole eyes of *Drosophila* comparing flies kept in a 12 h light-12 h dark cycle (Table I) with ones kept continuously in the dark ($n \geq 3$). Flies maintained at an environmental temperature of 19°C. Error bars are standard error.

reaction. We have not observed major decreases in opsin concentration under conditions where the spectral differences are quite evident (Ostroy and Pak, 1974).

The inability of certain *norpA* alleles to maintain their rhodopsin concentration seems to bear some causal relationship to the decreased ERG amplitude observed in these mutants (Table I). However, a variety of data suggests that the defect in the rhodopsin maintenance system was not the cause of the decrease in ERG amplitude, but instead was a consequence of the decrease in ERG amplitude, or the defect responsible for that decrease. At 2 days of age all of the *norpA* alleles exhibited a similar concentration of rhodopsin even though their respective ERG amplitudes were quite different. In fact, the ERG amplitudes did not exhibit any age or light-dark cycle dependencies. In the temperature-sensitive allele *norpA^{H52}*, under temperature conditions where no receptor potential could be obtained, the fly exhibited a concentration of rhodopsin which was very similar to the concentration in the wild-type fly (Pak et al., 1976). Finally, it is not clear that even the most severe age-dependent reductions in visual pigment observed in some of the *norpA* alleles (57–86%) could account for elimination of the ERG. In vertebrates, maximum receptor potential amplitudes can be obtained upon bleaching <0.1% of the native rhodopsin (Fain and Dowling, 1973), and bleaching 80% of the available pigment raises the threshold by only 2 log units (Grabowski and Pak, 1975). In the insect (although in these types of experiments it is difficult to be certain of the number of quanta impinging on the photoreceptor) there is no indication of major shifts in the sensitivity compared to the vertebrate (Reichardt, 1965; Pak and Lidington, 1974; Razmjoo and Hamdorf, 1976). In *Drosophila*, exciting only a small fraction of the available pigment appears sufficient to produce receptor potentials of saturated amplitude. Flies deprived of vitamin A in their diet exhibit sensitivity shifts of approximately 2.3 log units but display maximum receptor potentials of normal amplitude (Stark and Zitzmann, 1976). Recently Harris et al. (1977) have studied another group of flies deprived of vitamin A. In that case, the ERG sensitivity was reduced by only 80-fold even though the amount of rhodopsin, in the deprived fly, was <1.3% of the control. Certain *Drosophila* mutants which contain only 10–15% of the photopigment observed in wild-type also exhibit maximum receptor potentials of normal amplitude (W. L. Pak, personal communication). This light sensitivity in the insect appears to depend linearly on the concentration of pigment (Razmjoo and Hamdorf, 1976).

Although at the present time we do not know enough about the *norpA* defect or the rhodopsin maintenance system to understand the mechanism of their interaction, one possible explanation for the observed age-dependent decrease in rhodopsin in *norpA* might be the following: In the insect there is a light-induced degradation and resynthesis of opsin-containing photoreceptor membrane (White and Lord, 1975; Stein et al.¹). If the membrane turnover is important for the synthesis of rhodopsin, and if the turnover is dependent on the presence of a receptor potential (Pepe and Baumann, 1972), then elimination of the receptor potential in certain *norpA* alleles would lead to problems in maintaining the rhodopsin concentration and probably in maintaining the integrity of the membrane.

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REFERENCES

- ABRAHAMSON, E. W., and R. S. FAGER. 1973. The chemistry of vertebrate and invertebrate visual photoreceptors. *Curr. Top. Bioenerg.* **5**:125-200.
- ALAWI, A. A., V. JENNINGS, J. GROSSFIELD, and W. L. PAK. 1972. Phototransduction mutants of *Drosophila melanogaster*. *Adv. Exp. Med. Biol.* **24**:1-21.
- DARTNALL, H. J. A. 1953. The interpretation of spectral sensitivity curves. *Br. Med. Bull.* **9**:24-30.
- EBREY, T. G., and B. HONIG. 1977. New wavelength dependent visual pigment nomograms.
- FAIN, G. L., and J. E. DOWLING. 1973. Intracellular recordings from single rods and cones in the mud puppy retina. *Science (Wash. D. C.)*. **180**:1178-1181.
- GRABOWSKI, S. R., and W. L. PAK. 1975. Intracellular recordings of rod responses during dark adaptation. *J. Physiol. (Lond.)*. **247**:363-391.
- HAMDORF, K., R. PAULSEN, and J. SCHWEMER. 1973. Photoregeneration and sensitivity control of photoreceptors of invertebrates. In *Biochemistry and Physiology of Visual Pigments*. H. Langer, editor. Springer-Verlag New York, Inc., New York. 155-165.
- HAMDORF, K., and G. ROSNER. 1973. Adaptation and photoregeneration in the eye of the blowfly. *J. Comp. Physiol.* **86**:281-292.
- HARRIS, W. A., D. F. READY, E. D. LIPSON, A. J. HUPSPETH, and W. S. STARK. 1977. Vitamin A deprivation and *Drosophila* photopigments. *Nature (Lond.)*. **266**:648-650.
- HARRIS, W. A., W. S. STARK, and J. A. WALKER. 1976. Genetic dissection of the photoreceptor system in the compound eye of *Drosophila melanogaster*. *J. Physiol. (Lond.)*. **256**:415-439.
- LO, M-V. C. 1977. Darkening of deep-pseudopupil in *Drosophila*: An optical indication of inactivation of the peripheral photoreceptors. Ph.D. Dissertation. Purdue University, West Lafayette, Ind.
- MATTHEWS, R. G., R. HUBBARD, P. K. BROWN, and G. WALD. 1963. Tautomeric forms of metarhodopsin. *J. Gen. Physiol.* **47**:215-240.
- OSTROY, S. E., and W. L. PAK. 1973. Protein differences associated with a phototransduction mutant of *Drosophila*. *Nat. New Biol.* **243**:120-121.
- OSTROY, S. E., and W. L. PAK. 1974. Protein and electroretinogram changes in the alleles of the *norpA^{P12}* *Drosophila* phototransduction mutant. *Biochim. Biophys. Acta.* **368**:259-268.
- OSTROY, S. E., M. WILSON, and W. L. PAK. 1974. *Drosophila* rhodopsin: Photochemistry, extraction and differences in the *norpA^{P12}* phototransduction mutant. *Biochem. Biophys. Res. Commun.* **59**:960-966.
- PAK, W. L. 1975. Mutants affecting the vision of *Drosophila melanogaster*. In *Handbook of Genetics*. R. C. King, editor. Plenum Publishing Corp., New York. **3**:703-733.
- PAK, W. L., J. GROSSFIELD, and N. V. WHITE. 1969. Nonphototactic mutants in a study

- of vision of *Drosophila*. *Nature (Lond.)*. **222**:351-354.
- PAK, W. L., and K. J. LIDINGTON. 1974. Fast electrical potential from a long-lived, long-wavelength photoproduct of fly visual pigment. *J. Gen. Physiol.* **63**:740-756.
- PAK, W. L., S. E. OSTROY, M. C. DELAND, and C. E. WU. 1976. Photoreceptor mutant of *Drosophila*: Is protein involved in intermediate steps of phototransduction? *Science (Wash. D. C.)*. **194**:956-959.
- PAULSEN, R., and J. SCHWEMER. 1973. Proteins of invertebrate photoreceptor membranes. Characterization of visual pigment preparations by gel electrophoresis. *Eur. J. Biochem.* **40**:577-583.
- PEPE, I. M., and F. BAUMANN. 1972. Incorporation of H³-labelled leucine into the protein fraction in the retina of the honeybee drone. *J. Neurochem.* **19**:507-512.
- RAZMJOO, S., and K. HAMDORF. 1976. Visual sensitivity and the variation of total photopigment content in the blowfly photoreceptor membrane. *J. Comp. Physiol.* **105**:279-286.
- REICHARDT, W. E. 1965. Quantum sensitivity of light receptors in the compound eye of the fly *Musca*. *Cold Spring Harbor Symp. Quant. Biol.* **30**:505-515.
- STARK, W. S., and W. G. ZITZMANN. 1976. Isolation of adaptation mechanisms and photopigment spectra by vitamin A deprivation in *Drosophila*. *J. Comp. Physiol.* **105**:15-27.
- STAVENGA, D. G. 1976. Fly visual pigments. Difference in visual pigments of blowfly and dronefly peripheral retinula cells. *J. Comp. Physiol.* **111**:137-152.
- STAVENGA, D. G., A. ZANTEMA, and J. W. KUIPER. 1973. Rhodopsin processes and function of the pupil mechanism in flies. In *Biochemistry and Physiology of Visual Pigments*. H. Langer, editor. Springer-Verlag New York, Inc., New York. 175-180.
- STREHLER, B. L., I. R. KONIGSBERG, and J. E. T. KELLEY. 1963. Ploidy of myotube nuclei developing *in vitro* as determined with a recording double beam microspectrophotometer. *Exp. Cell Res.* **32**:232-241.
- WALD, G., and P. K. BROWN. 1953. The molar extinction of rhodopsin. *J. Gen. Physiol.* **37**:189-200.
- WEBER, K., and M. OSBORN. 1969. The reliability of molecular weight determinations by dodecylsulfate-polyacrylamide gel electrophoresis. *J. Biol. Chem.* **244**:4406-4412.
- WHITE, R. H., and E. LORD. 1975. Diminution and enlargement of the mosquito rhabdom in light and darkness. *J. Gen. Physiol.* **65**:583-598.