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Carotenoid Replacement Therapy in *Drosophila*: Recovery of Membrane, Opsin and Visual Pigment

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Rhabdomeres are substantially smaller and visual pigment is nearly eliminated when *Drosophila* are carotenoid-deprived from egg to adult. Rhabdomeres enlarge and visual pigment increases with carotenoid replacement in adults using carrot juice. We used a monoclonal antibody to the opsin in R1-6 receptors in the compound eye to further quantify opsin recovery in such carotenoid replacement therapy. Density of immunogold, specific to R1-6 (vs. R7), increases between days 1 and 3 of replacement as visual pigment and rhabdomeres recover. In summary, visual pigment, opsin and the opsin-containing organelle recover during carotenoid replacement therapy in carotenoid-deprived *Drosophila*.

Key words: carotenoids; opsin; rhodopsin; *Drosophila*; vitamin A; immunocytochemistry; electron microscopy.

1. Introduction

Carotenoid deprivation has been a very important way to manipulate the visual system of flies since the classic studies of Goldsmith and Fernandez (1966). In the mid-1970's, using *Drosophila*, Stark and coworkers rediscovered the usefulness of deprivation for decreasing visual pigment (Harris et al., 1977; Stark, 1977; Stark, Ivanyshyn and Greenberg, 1977; Stark, Ivanyshyn and Hu, 1976). Carotenoids apparently control expression of fly opsin synthesis in deprived *Drosophila*; opsin is absent in SDS gels (deCouet and Tanimura, 1987); deployment into the microvillar rhabdomere, the visual pigment organelle, is also disrupted in deprived flies (Boschek and Hamdorf, 1976; Harris et al., 1977), and a similar finding has been made in the moth (White and Bennett, 1989). Rhabdomeric P-face particles seen in freeze fracture electron microscopy (most of which are likely opsin) are reduced to one-fourth and spectrophotometrically measured visual pigment as well as electroretinographic (ERG) sensitivity are reduced considerably more, likely 100-fold, without disrupting microvilli (Harris et al., 1977). Corresponding work with the blowfly *Calliphora* led to an investigation of the usefulness of recovery when vitamin A was provided to deprived flies (Schwemer, 1983; Schwemer, 1984). This laboratory's interest in turnover of photoreceptive membrane and visual pigment led us to initiate investigations of carotenoid replacement therapy in *Drosophila* (Stark et al., 1988). The purpose of this paper is to thoroughly document the effects of carotenoid replacement therapy on the recovery of visual membrane and visual pigment using microspectrophotometry, electron microscopy, morphometry and immunocytochemistry. An abstract of these findings has been published (Sapp et al., 1990).

2. Materials and Methods

Animals

Drosophila melanogaster were raised on our standard medium, adequate for full visual development (Stark, Walker and Eidel, 1985). The use of yellow cornmeal probably, by itself, serves as an adequate source of carotenoids; however, we supplemented our fly food with β-carotene at 0·125 mg ml^{-1}, the minimum dose found to maximize sensitivity for flies reared from egg to adult on an otherwise carotenoid deficient medium (Stark et al., 1977). Carotenoid deprivation was achieved by rearing flies from egg to adult on Sang's medium according to previously published techniques (Stark, 1977; Stark et al., 1977). Carotenoid replacement 'therapy' was performed according to Stark et al. (1988) by placing deprived adults into a vial containing a Kimwipe soaked with carotenoid rich carrot juice.

White-eyed flies (w = white) were used for microspectrophotometry since the lack of eye color allows for visual pigment investigation unconfounded by the spectral effects of screening pigments. Red-eyed (+ = wild-type) flies were used for all electron microscopic studies. Flies were reared on a 12 hr light/12 hr dark (L/D) cycle of laboratory fluorescent (Phillips 30 W Cool White) lighting at an intensity of 75–80 lux (Lutron LX 101 digital lux meter).

Deep Pseudopupil Microspectrophotometry

Techniques for averaging visual pigment measurements from R1-6 rhabdomeres from large numbers of live *Drosophila* have been used extensively and

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modified (Stark and Johnson, 1980; Stark et al., 1988). The measurement depends on the non-bleaching photointerconversion of a 480 nm absorbing rhodopsin with its 570 nm-absorbing metarhodopsin. (Incidentally, this situation justifies our consistent use of the term 'visual pigment' rather than 'rhodopsin' to name the spectrophotometrically measureable entity since the visual pigment is a rhodopsin-metarhodopsin system.)

The deep pseudopupil was viewed with 579 nm, near metarhodopsin's absorbance maximum. This image was localized into a closely fitting aperture in front of a photomultiplier. Maximal rhodopsin was created by applying a 620 nm stimulus for 10 sec at 16·2 log quanta cm^{-2} s^{-1}. Then transmission of 579 nm light through the deep pseudopupil was measured. To maximize the metarhodopsin, 10 sec of 450 nm light at 15·8 log quanta cm^{-2} s^{-1} was applied, after which the tranmission was again measured. The log of the ratio of these two transmissions (after 620 nm vs. after 450 nm) is the absorbance difference.

Electron Microscopy

We fixed fly heads for EM morphometry using aldehyde prefix, osmium postfix, and lead and uranyl staining, described adequately elsewhere (Stark and Carlson, 1984; Stark and Sapp, 1988; 1989; Stark et al., 1988; 1989a; 1989b). Although in earlier studies xylene was used to expand sections while they were afloat in the wake of the diamond knife, this step was omitted lest it introduce unmeasurable variability. For morphometry, sections were photographed at 8000× on a Siemens transmission electron microscope. Because the rhabdomeres are known to taper, we always photographed at one plane in the distal-proximal extent, namely the R1-6 nuclear layer where most ommatidia showed at least 2 nuclei. For further control, we only looked in sagittally sectioned heads at rhabdomeres near the equator which were well cross-sectioned. These controls, while important, restricted the numbers of rhabdomeres for morphometry.

Immunocytochemistry

White-eyed *Drosophila* heads were dissected and fixed for immunocytochemistry. The fixative was better for preserving tissue antigenicity than our usual EM fixation in that it lacked acrolein and had lower glutaraldehyde: 2·85% paraformaldehyde and 0·15% glutaraldehyde in Sorensen's phosphate buffer (pH = 7·2) with 2% sucrose added for 2·75 hr at room temperature. The tissue was rinsed in Sorensen's buffer for 1 hr and subsequently dehydrated through a graded series of ethanol: water (30–90%). Finally, the tissue was transferred to the acrylic resin L. R. White (London Resin Company). The resin was heat polymerized at 55°C for 24 hr. Thin sections were cut on a diamond knife and picked up on formvar coated grids.

Immunogold staining was carried out by incubating grids on the monoclonal antibody against Rh1, the rhodopsin of R1-6 receptors, (deCouet and Tanimura, 1987) diluted 1:100 in PBS (phosphate buffered saline) containing 0·05% Tween 20 to quench background labeling for 30 min. This was followed by several rinses of PBST (phosphate buffered saline-thimerisol) -Tween 20. A secondary antibody conjugated with 10 nm colloidal gold particles permitted opsin identification and quantification of the increase in rhabdomeric opsin. Ten nanometers gold conjugated goat-anti-mouse IgG (Sigma Chem. Corp.) was diluted 1:5 in PBS-Tween 20 and incubated for 30 min followed by several rinses of PBST-Tween and a final rinse in distilled water. Sections were then post-stained in uranyl acetate for 4 min and lead citrate for 1 min.

Morphometry

Morphometry was done by focussing an RCA Hi Pot SA video camera (with a Fujicon HF35A-2 lens) onto EM negatives (on a Color Control light box) and feeding the image into an AIC (Analytical Imaging Concepts) model IM morphometric and densitometric system operated on a Northgate 286 computer. The perimeters of rhabdomeres were manually drawn for exact area determination, measured on the single object provision.

It was necessary to manually count immunogold particles because clumps of label (a common situation when monoclonal antibodies are used) could not be resolved adequately by our morphometry system. The particles were counted in areas isolated by using an ocular grid mounted in a Nikon Diaphot inverted microscope (35×). The particle density was determined from the count in all areas summing to the cross-sectional area of one ommatidium's rhabdomeres divided by the total area of those rhabdomeres.

3. Results

Carotenoid Deprivation

Figures 1 and 2 show micrographs revealing that carotenoid deprivation drastically reduces rhabdomere size, a finding not anticipated from the seeming normality of all aspects except P-face particle density in freeze fracture (Boschek and Hamdorf, 1976; Harris et al., 1977; Schinz et al., 1982). All flies used in Figs 1 and 2 are aged 10 days post-eclosion. TEMs compare carotenoid replete animals (Fig. 1) to deprived animals (Fig. 2). Shown in Fig. 1 is a cross-sectioned ommatidium taken from the distal retina at the level of the nucleus (also close to the equator). All sections were obtained strictly from this area to keep the variability as low as possible. The replete fly has

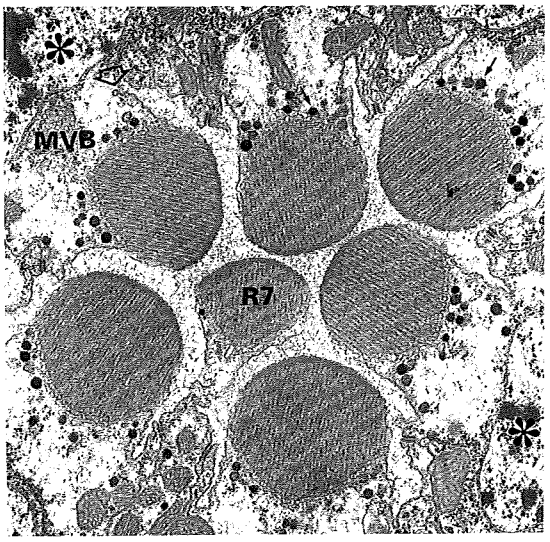

FIG. 1. Cross-sectioned ommatidium from normal wild-type (+) fly reared on a medium high in carotenoid. R7's rhabdomere (R7) inserts from the 7 o'clock position with R1-6 numbered counterclockwise from there. Note that the intraretinular pigment granules (arrows) are in the fully light adapted position, i.e. juxtaposed to the rhabdomeres, as expected since fixation was in the light. All sections were obtained from the distal retina at the level of the nuclei (*), and next to the equator to insure consistency. A coated pit (hollow arrowhead) and a multivesicular body (MVB) are shown. ×12400.

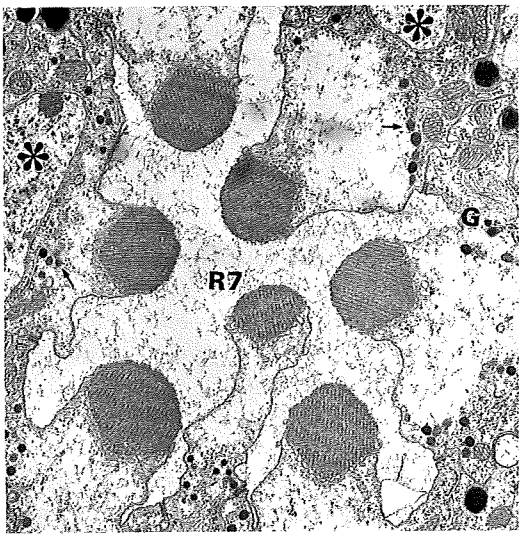

FIG. 2. Cross-sectioned ommatidium (again note nuclei, *, indicative of distal plane of section) from a fly reared on a medium lacking carotenoid. Note here that the size of the rhabdomeres is significantly smaller than in the Fig. 1 control fly. Structural integrity of the microvilli seems to have been preserved. All other conditions, including lighting, are the same as for the control fly (Fig. 1) except for diet. Note that the intraretinular pigment granules (arrows) are dispersed to a dark adapted position away from the rhadomeres which may reflect the lower visual sensitivity in the deprived fly. ×12400

rhabdomeres that are notably larger in area than the deprived fly (Fig. 2).

Figure 3 shows morphometric data quantifying the observations typified by the TEMs of Figs 1 and 2: rhabdomeres are largest for normal diet (0·125 mg ml^{-1} β-carotene + yellow cornmeal), smaller for Sang's medium (the deprivational food) with β-carotene added (0·125 mg ml^{-1}, despite the finding that this dose maximized sensitivity when flies were reared on an otherwise deprivational diet, Stark et al., 1977), and for Sang's medium lacking all carotenoids. Data for R1-6 and R7 were treated separately because R7 is smaller than R1-6.

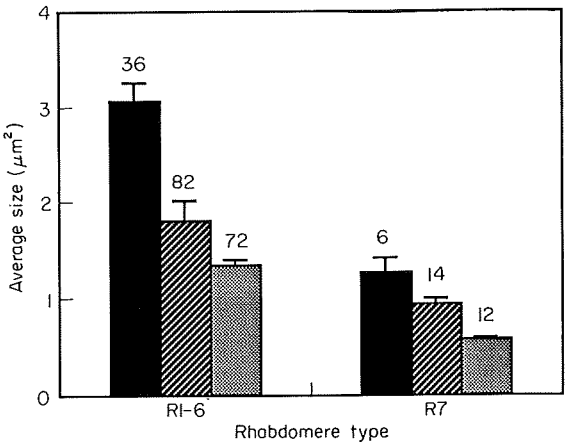

FIG. 3. Rhabdomere area of R1–6 and R7 determined by computerized morphometric analysis is shown for 3 different dietary conditions; ■, flies on a normal diet of carotenoid (0·125 mg ml^{-1} + cornmeal) ($n = 6$ ommatidia from 2 animals); ▨, flies reared on a high β-carotene dose in otherwise deprivational medium (0·125 mg ml^{-1}) ($n = 14$ ommatidia for 2 animals); ▩, flies on a medium lacking carotenoid ($n = 12$ ommatidia for 2 animals). On the left half is a graph obtained from rhabdomeres of the R1-6 subset only. The graph on the right shows relative sizes of rhabdomeres from the R7 subset of photoreceptors. They were treated separately because R7 is smaller than R1-6. Notice here the larger area of rhabdomeres from flies reared on a high carotenoid diet. Standard deviation, calculated from the rhabdomere areas averaged, is shown as bars over each plot. The numbers above each bar are n values and indicate the number of rhabdomeres measured.

Carotinoid Replacement Therapy

Earlier, we had shown that supplementing *Drosophila*, which had been carotenoid deprived from egg to adult for one generation, with carrot juice, a source rich in carotenoids, caused a recovery of visual pigment to normal levels (Stark et al., 1987; Stark et al., 1988). In this study, we (1) replicated this earlier microspectrophotometric result with a higher n (= 25) using males and females; (2) showed that rhabdomeres recover in size during replacement therapy; and (3) plotted the recovery of opsin using EM immunocytochemistry. We had hypothesized that females might recover faster than males since their abdomens became noticeably more orange earlier on the replacement time scale presumably since they were eating more for the sake of vitellogenesis.

The graphs in Fig. 4 obtained by microspectrophotometry show recovery of visual pigment as a

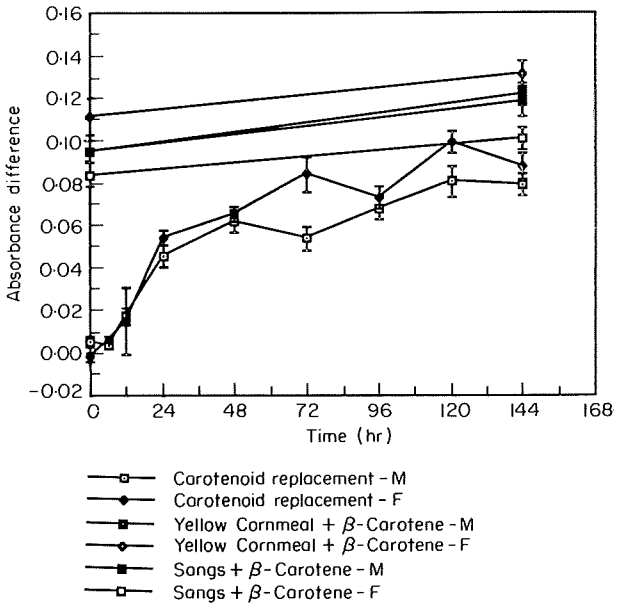

FIG. 4. Shown here is a graph determined by microspectrophotometry showing recovery of visual pigment as a function of time. Both males and females were carotenoid deprived from egg to adult for one generation and then supplemented with a diet of carrot juice as a function of time. One curve is from male flies and shows a rapid increase in levels of visual pigment in the first 24 hr and then a steady increase to a maximum at approximately 5 days. The other curve shows measurements taken from female flies yielding results very similar to the above graph. Control data points from both males and females are from deprived, supplemented (Sang's + β-carotene) and normal food (β-carotene-+yellow cornmeal). $n = 25$ for each data point with S.E.S shown.

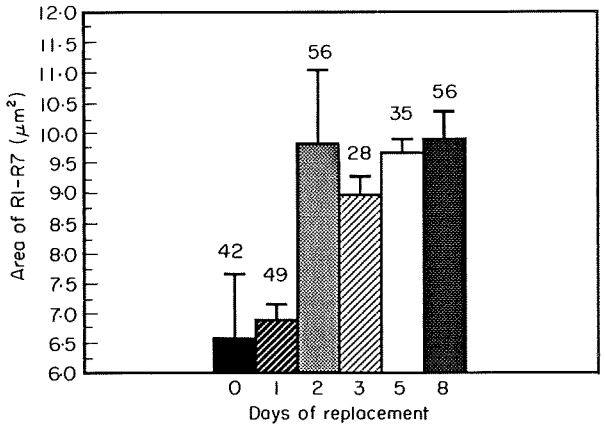

FIG. 5. Image analysis of rhabdomere area (μm^2) as a function of days of replacement therapy on carrot juice. To expedite this analysis, area was taken as an average from R1-7 rhabdomeres pooled together. Each bar represents two animals except for 3 day replacement which is only one animal. Numbers above each bar are n values and indicate number of rhabdomeres measured. Error bars represent standard deviation.

function of days on the replacement medium. Flies had been carotenoid deprived from egg to adult for one generation and then placed on carrot juice. Curves from male and female flies show similar rapid increases

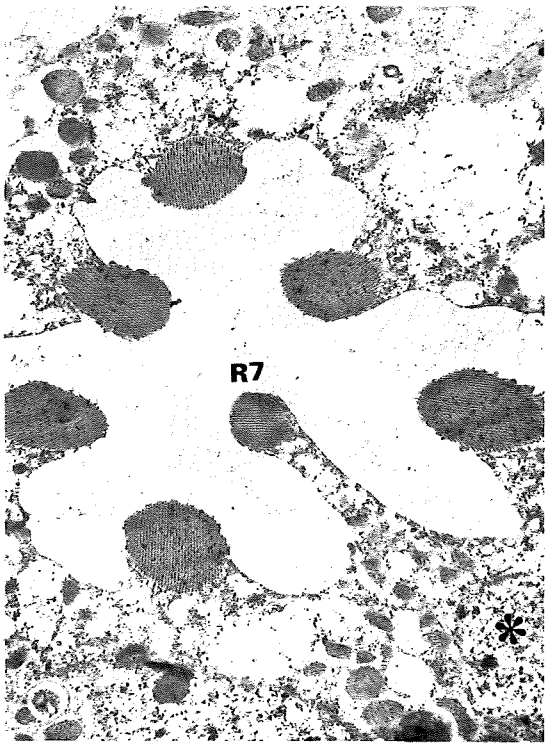

FIG. 6. TEM of a cross-sectioned ommatidium from a fly on replacement therapy for 1 day stained for immunocytochemistry. The antibody is against the Rh1 type of opsin unique to the R1-6 subset of photoreceptors. This micrograph shows one ommatidium with a few gold particles present only on the R1-6 rhabdomeres. Note that R7 is almost devoid of gold particles and provides an excellent control since it has a different type of opsin. Labeling in considerably lower than controls (Sapp, 1990) which are carotenoid replete (cf. Fig. 12). ×12400.

in visual pigment in the first 24 hr followed by steady increases to maxima at approximately 120 hr or 5 days. Control lines are plotted for 4 conditions: males and females on normal diet (yellow cornmeal + β-carotene) and Sangs + β-carotene. Lines connect 2 data points (newly emerged and 6 day old) for each of the 4 control experiments.

Figure 5 is a graph obtained by image analysis of rhabdomere area in square microns (μm^2) as a function of days of replacement time on carrot juice. Total area was taken as an average from R1-7 rhabdomeres pooled together to expedite data handling. Note an increase to asymptote reached around day 2 of carotenoid replacement.

Figures 6 and 7 show the *Drosophila* retina stained selectively for opsin for 1 day replacement and deprived respectively. Figure 6, a TEM of a cross-sectioned ommatidium (replacement, 1 day), shows labeling for the Rh1 type of opsin unique to the R1–6 subset of photoreceptors. Gold particles are present only on the R1-6 rhabdomeres. Note that R7 is almost devoid of gold particles and provides an excellent control since R7 has different types of opsin, namely Rh3 and Rh4 (Montell et al., 1987; Zuker et al., 1987). Labeling is considerably lower than in controls which are carotenoid replete (Sapp, 1990); hence

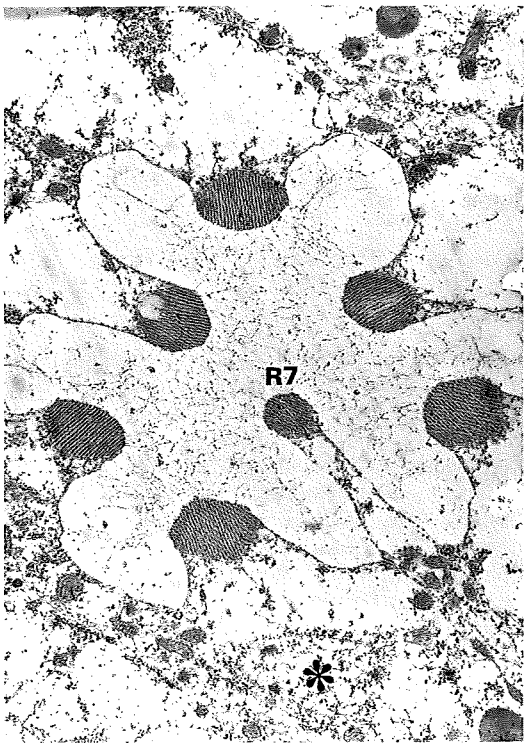

FIG. 7. This is a TEM of a cross-sectioned ommatidium from a fly that is carotenoid deprived and also stained for immunocytochemistry. There are very few gold particles even on the R1-6 rhabdomeres. ×12400.

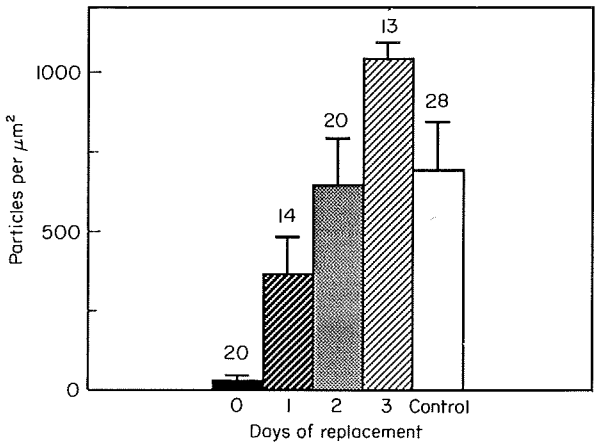

FIG. 8 Immunocytochemistry and morphometry were used to quantify the amount of opsin per square micron in R1-6. Measurements were made on flies that were originally deprived of carotenoid and later supplemented with carotenoid for 1–3 days. To compensate for any nonspecific staining in R1-6, the number of particles in R7 was substracted from the values obtained for R1-6 because R7 contains a different type of opsin than R1-6; therefore any particles in R7 would present background staining. Data are from 3 separate experiments: 0–1 day, 1-2-3 day and control (replete). Normalization was necessary to adjust the 0 day reading to the 1-2-3 day data and this is described in the results. We had no data to normalize the control to the other two experiments. Each bar represents one fly, except three flies were used in control experiments (replete = yellow cornmeal + β-carotene). The number above each bar indicates the number of rhabdomeres sampled. Standard deviation bars are also shown for each experiment.

controls contain greater levels of opsin. There are very few gold particles even on the R1-6 or R7 rhabdomeres in Fig. 7 (deprived). In summary, opsin is increased as the carotenoid is replaced. In other work on immunocytochemistry (Sapp, 1990; Sapp et al., 1990), we showed that early in replacement, labeling is particularly high in rough endoplasmic reticulum.

Figure 8 is a graph representing the amount of opsin immunogold per square micron of rhabdomere. The immunocytochemical staining was done for 1, 2 and 3 day replacement flies at one time and, unfortunately, the deprived and replete preparations were not stained in the same experimental session. Thus, when we stained 0 day, we also stained 1 day again so the 1 day count could be used to normalize the 0 day to the 1-2-3 day counts. This was achieved by comparing the two 1 day replacements and adjusting the 0 day count by the ratio accordingly. In this way, the 0, 1, 2 and 3 days could be compared fairly. The control fly (replete) was also stained in a different experimental session than the 1-2-3 day labeling, but in this case, no overlap for normalization purposes was available. Furthermore, the plane of sectioning was different (more proximal) which could have created slight errors for several reasons including the smaller area of the rhabdomeres. Despite these compromises, the graph does reveal an increase in opsin as labeled with immunocytochemistry, and this increase is *probably* largely completed to control level by 2 or 3 days.

4. Discussion

In this current work the investigators have discovered a difference in the size of photoreceptors in control vs. carotenoid-deprived *Drosophila*. Carotenoid-deprived flies have rhabdomeres which are considerably diminished, a difference overlooked in previous studies (Boschek and Hamdorf, 1976; Harris et al., 1977; Schinz et al., 1982) although rhabdomeres looked slightly different optically (optical neutralization technique) and in light microscopy (Stark et al., 1977) though, at that earlier time, the authors failed to emphasize the difference.

We also observed a difference in the microspectrophotometrically determined amount of visual pigment and opsin amounts as determined by immunocytochemistry. The levels of visual pigment and opsin are very low in carotenoid-deprived flies. However, we find that rhabdomeres enlarge, visual pigment level increases and opsin increases virtually in unison when adults are sustained on a diet consisting only of carrot juice. Immunocytochemistry is useful to quantify the opsin level and microspectrophotometry is an accepted method to give an alternative source of quantification in the form of spectrophotometrically assayed visual pigment level. Both methods gave similar results and provided a useful means of obtaining an extra degree of certainty concerning our visual pigment measurements.

The situation in vertebrates is strikingly different. In rat rod outer segments, the finding can be summarized as follows: vitamin A deprivation for 10, 18 and 26 weeks after weaning decreases rhodopsin quantity but not opsin density (Katz et al., 1990) and thus, these two entities can be distinguished operationally. The rhodopsin was assayed spectrophotometrically before vs. after bleaching. Opsin density per square micron was quantified by P-face particle density in cross-fractured outer segment discs and by EM immunocytochemistry.

This present study indicates that membrane area, visual pigment and opsin recover quickly after the commencement of carotenoid replacement therapy (considering that the flies are consuming the carrot juice and thus that digestion and circulation intervene). Visual pigment and opsin increases start from virtually zero values while membrane area increases start from about half normal size. Finer grain comparisons of time course and amount of increase are beyond the scope of these data. Although part of the increasing area of rhabdomeric membrane may be a direct consequence of the space occupied by newly synthesized and deployed opsin, we estimate that only 0·07 μm^2 of the 3 μm^2 area of each R1–6 rhabdomere (cf. Fig. 3) is occupied by opsin. Our logic and assumptions are as follows. Opsin density is roughly 3000 μm^{-2}. This, however, may be lower bound since it assumes one opsin per freeze fracture bump and that none of the freeze fracture bumps remaining in carotenoid-deprived flies (1030 μm^{-2}) is opsin (Harris et al., 1977). We use the area occupied by opsin of 7·5 nm^2 per opsin (Liebman, Parker and Dratz, 1987) which is probably a fairly safe estimate.

Immunocytochemistry was also used to ascertain the location of other 'extra-rhabdomeric' or intracellular opsin (Sapp, 1990; Sapp et al., 1990). Opsin may be sequestered in structures of turnover-breakdown (autophagy of plasmalemma and rhabdomere, Stark et al., 1988) though Rh1 staining (and hence opsin content or opsin antigenicity) is low in multivesicular bodies. After one day of replacement, as opsin is just beginning to recover in rhabdomeres, immunogold labeling is high in rough endoplasmic reticulum (Sapp, 1990; Sapp et al., 1990). While opsin regulation in *Drosophila* could conceivably be at the levels of transcription, translation or post-translational modifications, witnessing this early biosynthetic step and acknowledging the lack of opsin on gels in deprived *Drosophila* (deCouet and Tanimura, 1987) favors the possibility that the carotenoid regulates opsin gene expression.

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References

Boschek, C. B. and Hamdorf, K. (1976). Rhodopsin particles in the photoreceptor membrane of an insect. *Z. Naturforsch.* **31C**, 763.

deCouet, H. G. and Tanimura, T. (1987). Monoclonal antibodies provide evidence that rhodopsin in the outer rhabdomeres of *Drosophila melanogaster* is not glycosylated. *Eur. J. Cell Biol.* **44**, 50–56.

Goldsmith, T. H. and Fernandez, H. R. (1966). Some photochemical and physiological aspects of visual excitation in compounds eyes. In *The Functional Organization of the Compound Eye* (Ed. Bernhard, C. G.). Pp. 125–144. Pergamon Press: Oxford; New York.

Harris, W. A., Ready, D. F., Lipson, E. D., Hudspeth, A. J. and Stark, W. S. (1977). Vitamin A deprivation and *Drosophila* photopigments. *Nature (Lond.)* **266**, 648–650.

Katz, M. L., Stark, W. S., White, R. H., Gao, C.-L. and Kutryb, M. (1990). Influence of dietary vitamin A on opsin density in the photoreceptor outer segment disc membranes. *Invest. Ophthalmol. Vis. Sci. Suppl.* **31**, 77.

Liebman, P. A., Parker, K. R. and Dratz, E. A. (1987). The molecular mechanism of visual excitation and its relation to the structure and composition of the rod outer segment. *Ann. Rev. Physiol.* **49**, 765–91.

Montell, C., Jones, K., Zuker, C. and Rubin, G. (1987). A second opsin gene expressed in the ultraviolet-sensitive R7 photoreceptor cells of *Drosophila melanogaster*. *J. Neurosci.* **71**, 1558–1566.

Sapp, R. J. (1990). Maintenance of photoreceptor membrane and visual pigment in *Drosophila* compound eyes. MA Thesis, The University of Missouri, Columbia.

Sapp, R. J., Stark, W. S., Christianson, J. S., Maier, L. and Studer, K. (1990). Immunocytochemistry and morphometry of carotenoid replacement in *Drosophila*. *Invest. Ophthalmol. Vis. Sci. Suppl.* **31**, 77.

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

Schwemer, J. (1984). Renewal of visual pigment in photoreceptors of the blowfly. *J. Comp. Physiol.* **154**, 535–547.

Stark, W. S. (1977). Diet, vitamin A and vision in *Drosophila*. *Drosoph. Inform. Serv.* **52**, 47.

Stark, W. S. and Carlson, S. D. (1984). Blue and ultraviolet light induced damage to the *Drosophila* retina: ultrastructure. *Curr. Eye Res.* **3**, 1441–1454.

Stark, W. S., Hartman, C. R., Sapp, R. J., Carlson, S. D., Claude, P. and Bhattacharyya, A. (1987). Vitamin A replacement therapy in *Drosophila*. *Dros. Inf. Serv.* **66**, 136–137.

Stark, W. S., Ivanyshyn, A. M. and Greenberg, R. M. (1977). Sensitivity and photopigments of R1-6, a two-peaked photoreceptor, in *Drosophila*, *Calliphora* and *Musca*. *J. Comp. Physiol.* **121**, 289–305.

Stark, W. S., Ivanyshyn, A. M. and Hu, K. G. (1976). Spectral sensitivities and photopigments in adaptation of fly visual receptors. *Naturwissen.* **63**, 513–518.

Stark, W. S. and Johnson, M. A. (1980). Microspectrophotometry of *Drosophila* visual pigments: Determinations of conversion efficiency in R1-6 receptors. *J. Comp. Physiol.* **140**, 275–286.

Stark, W. S. and Sapp, R. (1988). Eye color pigment granules in wild-type and mutant *Drosophila melanogaster*. *Can. J. Zool.* **66**, 1301–1308.

Stark, W. S. and Sapp, R. J. (1989). Retinal degeneration and photoreceptor maintenance in *Drosophila*: *rdgB* and its interaction with other mutants. In *Inherited and Environmentally Induced Retinal Degenerations* (Ed. LaVail, M. M., Anderson, R. E. and Hollyfield, J. G.). Pp. 467–489. Liss: New York.

Stark, W. S., Sapp, R. J. and Carlson, S. D. (1989a). Ultrastructure of the ocellar visual system in normal and mutant *Drosophila melanogaster*. *J. Neurogenet.* **5**, 127–153.

Stark, W. S., Sapp, R. J. and Haymer, D. S. (1989b). Eye color pigment granules in *Drosophila mauritiana*: Mosaics produced by excision of a transposable element. *Pig. Cell Res.* **2**, 86–92.

Stark, W. S., Sapp, R. J. and Schilly, D. (1988). Rhabdomere turnover and rhodopsin cycle: maintenance of retinula cells in *Drosophila melanogaster*. *J. Neurocytol* **17**, 499–509.

Stark, W. S., Walker, K. D. and Eidel, J. M. (1985). Ultraviolet and blue light induced damage to the *Drosophila* retina: microspectrophotometry and electrophysiology. *Curr. Eye Res.* **4**, 1059–1075.

White, R. H. and Bennett, R. R. (1989). Ultrastructure of carotenoid deprivation in photoreceptors of *Manduca sexta*: myeloid bodies and intracellular microvilli. *Cell Tiss. Res.* **257**, 519–528.

Zuker, C. S., Montell, C., Jones, K., Laverty, T. and Rubin, G. M. (1987). A rhodopsin gene expressed in photoreceptor cell R7 of the *Drosophila* eye: Homologies with other signal-transducing molecules. *J. Neurosci.* **7**, 1550–1557.