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Turnover of membrane and opsin in visual receptors of normal and mutant *Drosophila*

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Summary

Electron microscopy was used to investigate membrane turnover in the photoreceptors of *Drosphila*. Coated pits and vesicles, multivesicular bodies, primary lysosomes, multilamellate bodies, residual bodies and Golgi complexes are present throughout a light/dark cycle. Serial sections reveal that the membrane bounding of multivesicular bodies is only seen at an optimal plane of section. The temperature-sensitive *shi*bire (*shi*^{ts}) mutant has a defect in conversion of coated pits into vesicles which may also affect visual receptors. We used monoclonal antibodies to Rh1 in R1–6 receptors in the compound eye (also to Rh2 in *ocellar* receptors in the simple eyes) ro relate turnover processes at the visual pigment compared with membrane levels. Compound eye rhabdomeres but not rhabdomere caps stained selectively. Immunogold labelling was equivocal in multivesicular bodies. Further, early in the process of carotenoid replacement therapy, labelling is high in the rough endoplasmic reticulum, demonstrating *de novo* opsin synthesis.

Introduction

Turnover of photoreceptor membrane has been central to the study of function and pathology of arthropod visual cells since the pioneering studies of White (1964, 1968). Only in the past few years has this topic been addressed in *Drosophila* (Stark *et al.*, 1988) and in mutants with faulty maintenance (Stark & Sapp, 1987, 1989; Matsumoto *et al.*, 1988; Stark *et al.*, 1989a; Matsumoto-Suzuki *et al.*, 1989; Suzuki *et al.*, 1990). *Drosophila* is particularly important because of the potential for a neurogenetic approach and the possibility of a facilitated molecular analysis. Membrane turnover in *Drosophila* shares many features with the better studied arthropods (Stark *et al.*, 1988). Coated pits originate from rhabdomeric microvilli and plasmalemma and form into coated vesicles. Coated vesicles merge into multivesicular bodies (MVBs) which are attacked by primary lysosomes.

Here, we extend the analysis of Stark and coworkers (1988) to include micrographs from throughout a 12-h light/12-h dark (L/D) cycle. Also we now show several of the steps in MVB reduction from multilamellate bodies (MLBs) to residual bodies (RBs). In this regard, our use of white-eyed *Drosophila* is advantageous because of the lack of intraretinular eye colour pigment granules which could otherwise be confused with RBs (Stark & Sapp, 1988). We present analyses of a mutant, *shi*^{ts}(*shibire*) which has a temperature sensitive defect in the formation of coated vesicles from pits (Kosaka & Ikeda, 1983); this mutant should prove useful in genetic dissection of photoreceptor autophagy. We use serial sections and the high voltage electron microscope (HVEM) (Stark & Carlson, 1986) to visualize membrane-bounded MVBs and the unusual coated pits of *shi*. Finally, we used immunocytochemistry with monoclonal antibodies to examine the synthesis, degradation and organelle specificity of opsin. Some of this material has been reported in preliminary form (Sapp, 1990; Sapp *et al.*, 1990a, b).

Animals

Drosophila melanogaster were raised on our standard medium, adequate for full visual development (Stark, 1977). The yellow cornmeal used was probably a sufficient source of carotenoids; however, we supplemented our fly food with β-carotene at a level of 0.125 mg ml^{-1} which was just adequate to produce flies with maximal sensitivity when reared from egg to adult on a medium which was otherwise carotenoid deficient (Stark *et al.*, 1977). Carotenoid deprivation was achieved by rearing flies from egg to adult on

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Sang's medium according to previously published techniques (Stark, 1977; Stark *et al.*, 1977). Carotenoid replacement 'therapy' was performed according to Stark and co-workers (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 most studies. Flies were reared on a 12 h light/12 h dark cycle of laboratory fluorescent (Phillips 30 W Cool White) lighting at an intensity of 75–80 lux (Lutron LX 101 digital lux meter). The temperature sensitive mutant *Shibire* (shi^{ts}) or the white-eyed version with yellow body marker ($y\ w\ shi^{ts-1}$) was used in a number of studies. These stocks were maintained in a healthy condition in our laboratory by rearing at 18° C, well within the permissive range. For heat treatments at restrictive temperatures, the duration and temperature at the restrictive temperature is given in the text and figure legends. In addition, a specimen of *w shi* was exposed to enough blue light to maximally convert rhodopsin to metarhodopsin (450 nm at 15.7 log quanta cm^{-2} for 10 s) and maintained in the dark for 1.33 h at 29° C before dissection at room temperature. In one set of experiments, the *trp* (transient receptor potential mutant) (e.g. Chen & Stark, 1983; Stark & Sapp, 1989) was examined.

Electron microscopy

Electron microscopy (EM) fixation was as follows: prefix (1% formaldehyde, 1% gluteraldehyde, 1% acrolein, in pH 7.2, 0.1 M Sorensen's phosphate buffered 2% sucrose), rinse in buffered 3% sucrose, postfix (buffered 1% osmium with 2% sucrose); heads were dehydrated in ethanol and embedded in Spurr resin. Sections were stained with uranyl acetate and lead citrate (Stark *et al.*, 1989b). The bismuth poststaining technique, done according to the methods of Ainsworth and Karnovsky (1972), was used in several experiments to enhance visibility of coated membranes and pits, although we noticed little improvement. We photographed our sections on a Siemens 101 transmission electron microscope. For serial section work, sections of about 150–190 nm thick were mounted on Formvar-coated slot grids and viewed at a million volts on the HVEM at the NIH Integrated Microscopy Resource in Madison WI. These techniques are detailed elsewhere (Stark & Carlson, 1986). Serial reconstruction was done by focusing an RCA Hi Pot SA video camera (with a Fujicon HF35A-2 lens) onto the negative (on a Colour Control light box) and feeding the image into an AIC (Analytical Imaging Concepts Model IM) image analysis system operated on a Northgate 286 computer. Grey levels were selected to highlight subcellular structures and the outlines were exported to the 3-D serial reconstruction software.

Immunocytochemistry

White-eyed *Drosophila* heads were dissected and fixed for immunocytochemistry. The fixative was gentler than our usual EM fixation: here we used 2.85% paraformaldehyde and 0.15% glutaraldehyde in Sorensen's phosphate buffer (pH 7.2) with 2% sucrose added, for 2.75 h at room temperature. The tissue was rinsed in Sorensen's buffer for 1 h and subsequently dehydrated through a graded series of ethanol diluted in water (30–90%). Finally, the tissue was transferred to the acrylic resin L. R. White. The resin was heat polymerized at 55° C for 24 h. Thin sections were cut with a diamond knife and picked up on Formvar-coated grids. These sections were used for immunocytochemistry.

Figs. 1–10. Some aspects of turnover in *w* (white) *Drosophila* fixed at various times on a 12-h light/12-h dark cycle.

Fig. 1. Two R1–6 cells 2 h after light onset. In this field, quite a few aspects of cycling can be seen, including coated pits (arrows; from plasmalemma, and from rhabdomere, R, a fully formed MVB surrounded with primary lysosomes, a nascent (loosely packed) MVB (hollow arrowhead), and a Golgi complex (G). A pigment granule (PG) with its obvious membrane bounding, invaginates into one retinula cell from a narrow area of secondary pigment cell between the two photoreceptors. Several mitochondria are also seen. ×36 800.

Fig. 2. Section through R2 from a specimen fixed 6 h after lights on. Two MVBs are seen next to each other, with the upper one being somewhat denser. The much denser bodies near the rhabdomere are probably RBs as, in white (*w*) mutants, no intraretinular pigment granules of similar size, density and location would be present to confound identification at this location. Several membrane-bounded secondary cell pigment granules are seen, with one (PG) being noted for its long invaginating stalk. Mitochondria also abound. ×39 600.

Fig. 3. Section showing an MVB next to the nucleus (asterisk) in an R1–6 cell of a fly fixed 7 h after light onset. ×56 000.

Fig. 4. R5 from a fly fixed 7 h after light onset shows a multilaminate body (MLB) in addition to the obvious MVB with primary lysosomes. Note the coated pit originating from the plasmalemma (arrow). ×25 600.

Fig. 5. Another field with an MLB in R3 from a fly fixed 8 h after light onset. An MVB with a normal appearance is seen, with another MVB which is becoming dense. Several mitochondria are also seen. ×40 000.

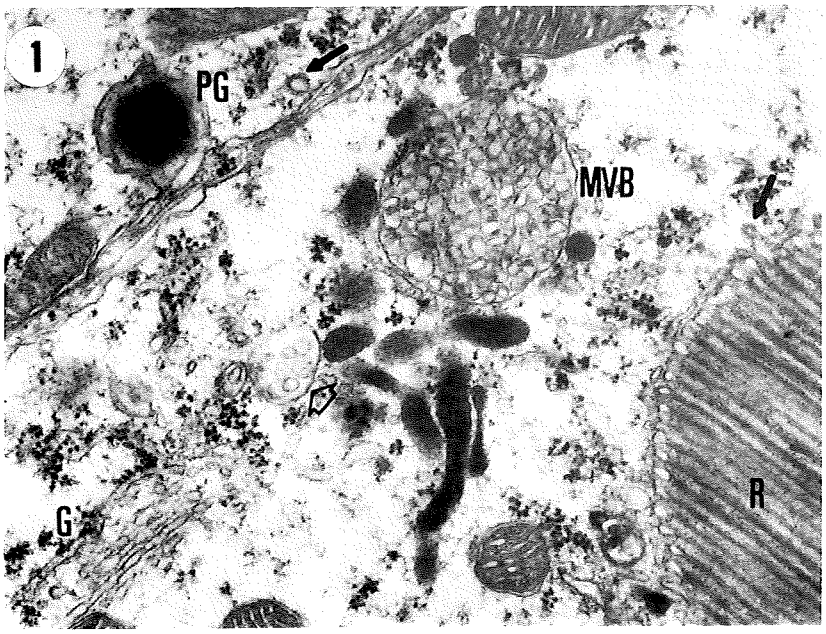
Fig. 6. Section from R2 of a specimen fixed 11 h after light onset. An MVB is clearly seen between the nucleus (asterisk) and the rhabdomere. Two sets of Golgi apparatus (G) are seen. Note the coated pit originating from the rhabdomere (arrow). ×19 299.

Fig. 7. Field from a fly fixed at 14.5 h (2.5 h after light offset). Two MVBs (top) and two MLBs (middle) are seen in an R1–6 cell. A rhabdomere and a mitochondrion are seen. ×52 000.

Fig. 8. An RB is centred in this field from R6 at 18.5 h. An MVB, mitochondria and a pigment granule are seen. ×52 000.

Fig. 9. In a fly at 23 h, two R1–6 cells show Golgi apparatus while R7, at this deep plane of section just an axon, shows an MVB. ×34 500.

Fig. 10. Coated pits from plasmalemma (arrows) and an MVB are seen at 23 h. ×16 000.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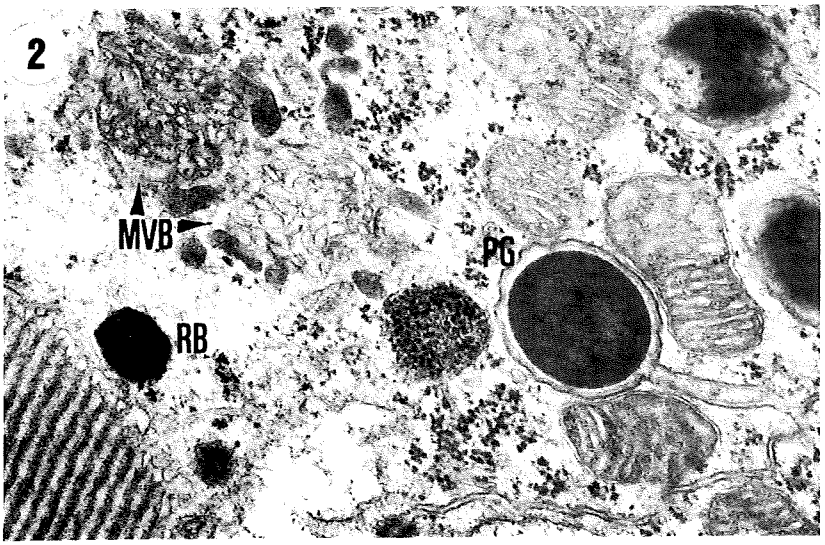
PG
MVB
R
G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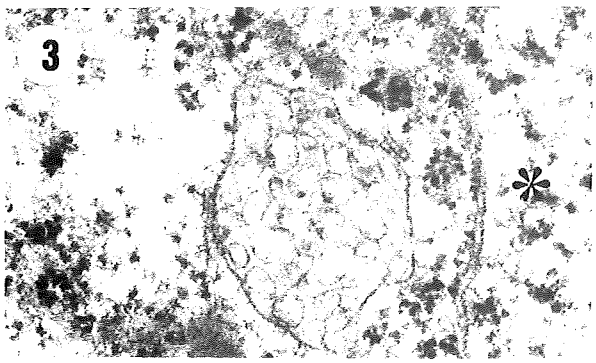
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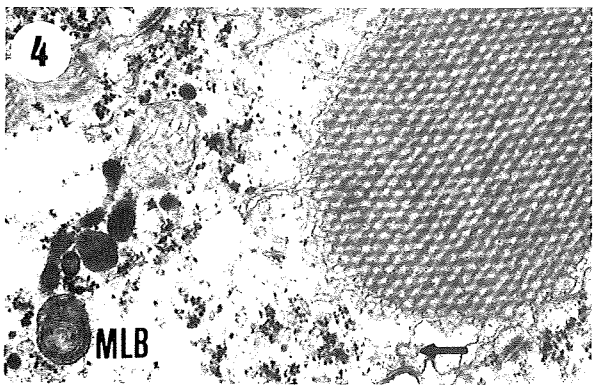
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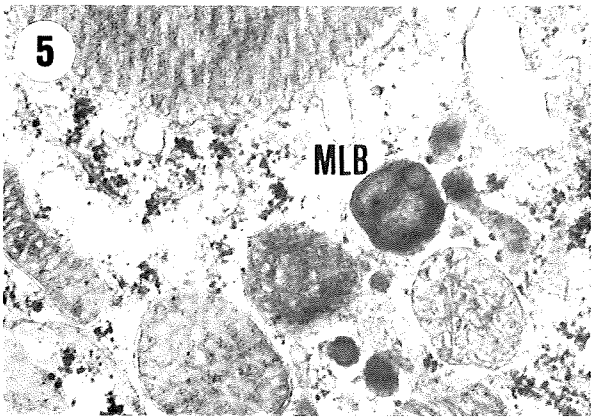
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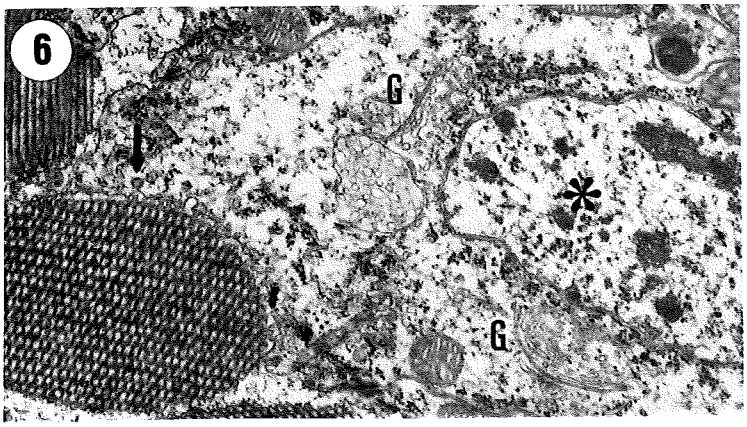
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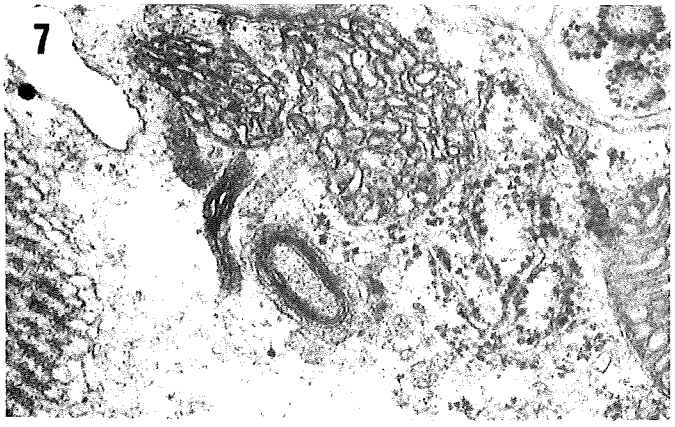
G
G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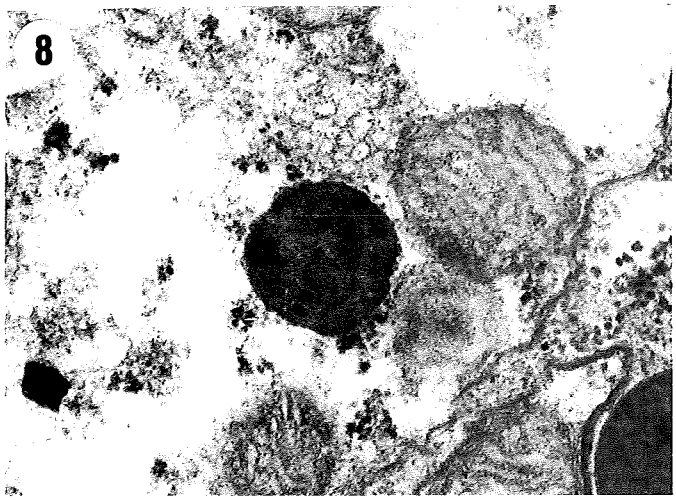
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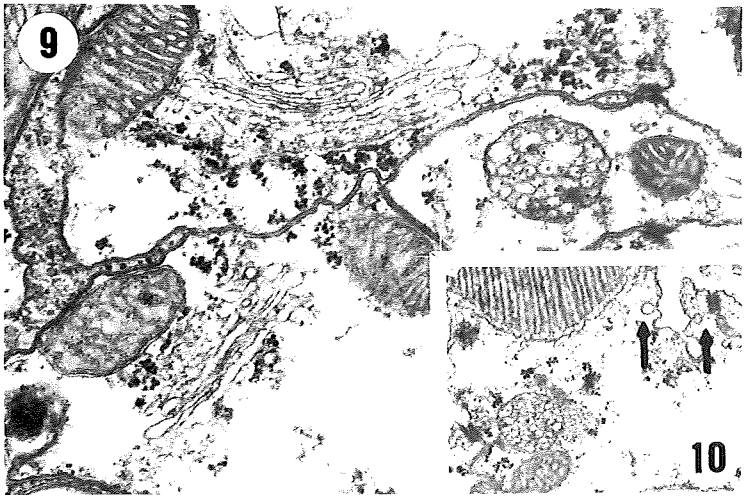
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Immunogold staining was carried out by incubating grids on the monoclonal IgG antibody against Rh1 (deCouet & Tanimura, 1987) diluted 1:100 in PBS (phosphate buffered saline) containing 0.05% Tween 20 to quench background labelling for 30 min. This was followed by several rinses of PBST (phosphate buffered saline – thimerisol)-Tween 20. Ten nm gold conjugated goat anti-mouse IgG (Sigma Chemicals) was diluted 1:5 in PBS-Tween 20 and incubated for 30 min followed by several rinses of PBST-Tween with a final rinse in distilled water. Sections were then post-stained in uranyl acetate for 4 min and lead citrate for 1 min.

For ocelli, red-eyed *Drosophila* heads were particularly useful for ascertaining that the sections passed through these tiny eyes because the ocelli contain brown ommochrome pigments (Stark *et al.*, 1989b). For immunogold labelling, the grids were incubated on the anti-Rh2 monoclonal IgG antibody (hybridoma supernatant, used undiluted) (Shieh *et al.*, 1989) for 45 min. Following a rinse in PBS, the secondary antibody (goat anti-mouse), conjugated with 10 nm gold (Sigma Chemicals) and diluted 1:5 with PBS, was added for 25 min.

Results

While most anthropods studied have distinct rhabdom degradation and construction phases, Williams (1982) claimed that turnover is continuous in the fly. Figs 1–10 document evidence substantiating this claim in *w* (white) *Drosophila*. Fig. 1 is a cross-section of 2 R cells fixed 2 h after light onset. Several organelles associated with the cycling process, including MVBs, primary lysosomes, Golgi apparatus and coated pits are shown. Fig. 2 is a section through R2 of a fly fixed 6 h after light onset. Two MVBs are found adjacent to one another in this field. While both have many surrounding primary lysosomes, one is notably more dense and is presumably already in the process of lysosomal degradation. A much more electron-dense body is present close to the rhabdomere. This is probably an RB because the mutant *w* does not have intraretinular pigment granules, which are similar in size, shape, electron density and location (Stark & Sapp, 1988). Fig. 3 is a higher magnification of a nascent MVB next to the nucleus of an R1–6 cell of a specimen fixed 7 h after light onset. This MVB appears to have larger vesicles and is less densely packed than others. Fig. 4 is also from as fly fixed 7 h after light onset. This micrograph depicts an R5 cell with an MLB next to a typical MVB and primary lysosomes. Fig. 5 also shows an MLB but this time the fly was fixed 8 h after light onset. Also present in this R3 cell are two MVBs with different degrees of densification. A fly fixed 11 h after light onset is shown (Fig. 6). Several characteristic organelles are present in this R2 cell including two Golgi complexes and an MVB. Also note a coated pit originating from the microvilli of the rhabdomere. Two MVBs and two MLBs are present in Fig. 7 which is taken from a fly fixed at 14.5 h (2.5 h after light offset). In Fig. 8, an RB is seen in an animal fixed at 18.5 h. At the top is an MVB adjacent to the RB. Figs 9 and 10 are both taken from animals fixed at 23 h. Fig. 9 is a micrograph with two R cells each having Golgi apparatus, while R7 shows an MVB. Fig. 10 shows coated pits invaginating from the plasmalemma; also note the MVB with lysosomes.

Because *shi*(*shibire*) mutant *Drosophila* have a defect in the formation of coated vesicles (Kosaka & Ikeda, 1983), a thorough portrayal of autophagy in the eye is useful. Figs 11 and 12 are taken from control *shi* flies that were maintained at a permissive temperature and fixed in the light at room temperature within 5 min of removal from an incubator kept at 18° C. Fig. 11 shows

Figs 11–18. Some aspects of the turnover in *shi* (*shibire*) mutant *Drosophila*.

Fig. 11. Control (permissive temperature) *shi* fly (fixed in the light at room temperature within 5 min of removal from an incubator kept at 18° C). Note the apparent coated vesicle (arrow) in retinula cell. Across the belt desmosome, the numerous dense bodies near the rhabdomere are intraretinular pigment granules in this red eyed fly. ×44 800.

Fig. 12. Control *shi* fly as in Fig. 11. Here, two coated pits seem to be on anastomosing stalks (arrow). An MVB is also labelled. ×60 800.

Fig. 13. Coated pit on a stalk (arrow) with several long stalks to the right (presumably with coated pits outside of the plane of section). Specimen is *w shi* exposed to enough blue light to maximally convert rhodopsin to metarhodopsin (450 nm at 15.7 log quanta cm^{-2} for 10 s) and maintained in the dark for 1.33 h at 29° C before dissection at room temperature. ×68 400.

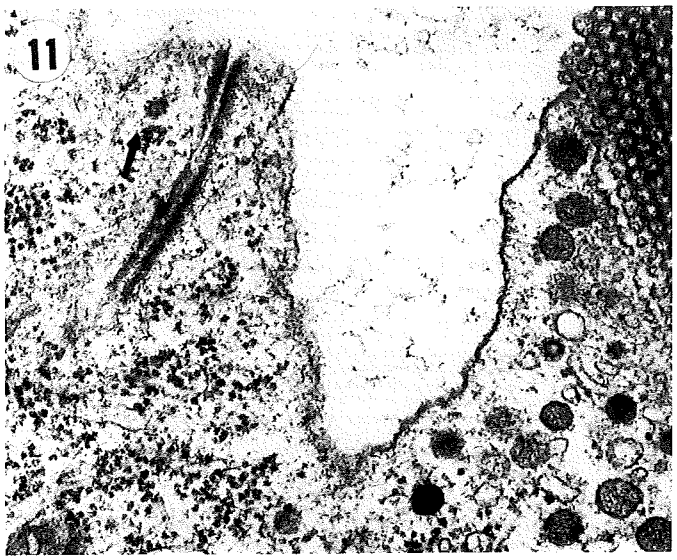
Fig. 14. Stalked coated pits (arrows) in *shi* maintained for 2 h at 28.4° C before fixation. Staining is with bismuth. The dense body on the left is a granule of a secondary pigment cell while the smaller one is an intraretinular granule in this red eyed fly. ×86 000.

Fig. 15. Stalked coated pits (arrows) in blue adapted *w shi* (as in Fig. 13). Note loosely packed MVB. ×54 400.

Fig. 16. Unusual membranes at the bases of the microvilli in *shi* maintained for 3 h at 30° C. ×58 000.

Fig. 17. Portion of an optic cartridge in the lamina ganglionaris of blue adapted *w shi* (as in Fig. 13). At the top are R terminals noted for their intermediate electron density and round or stalked capitate projections (Stark & Carlson, 1986). The bottom right shows the more lucent postsynaptic L cells. Here we observe unusual strings of what appear to be coated pits (arrows). Note the rare T synaptic ribbon in one L cell. ×32 400.

Fig. 18. Another field from a blue adapted *w shi* cartridge with coated pits (arrow). ×52 800.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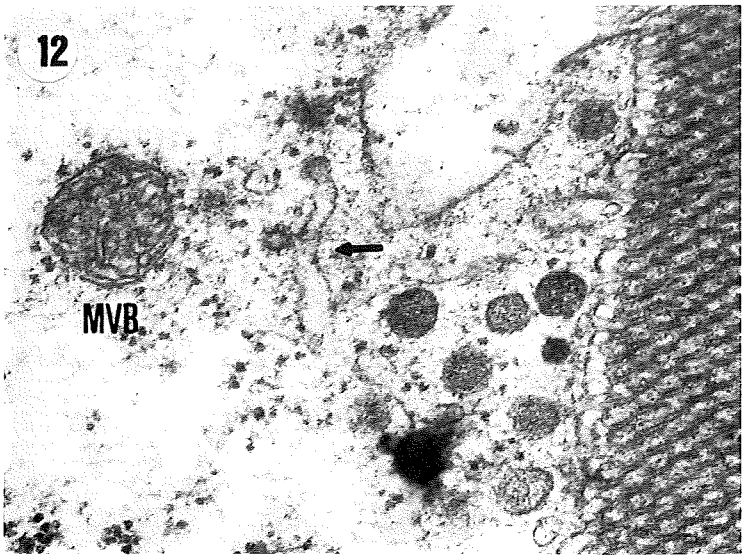
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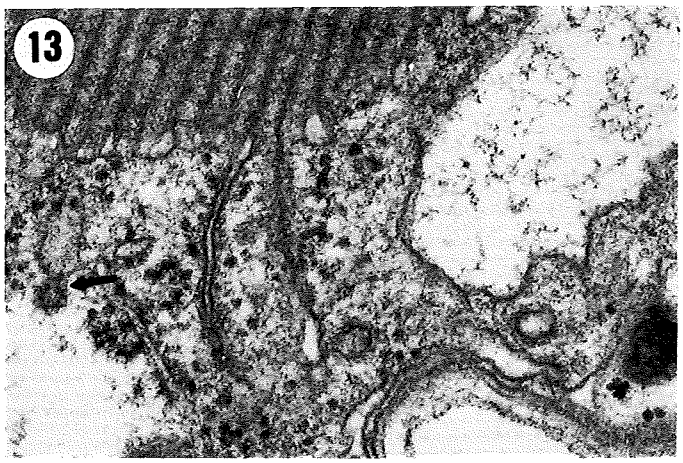
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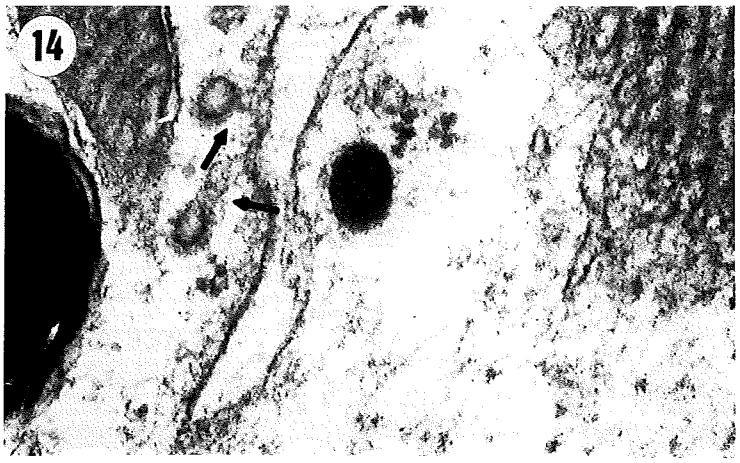
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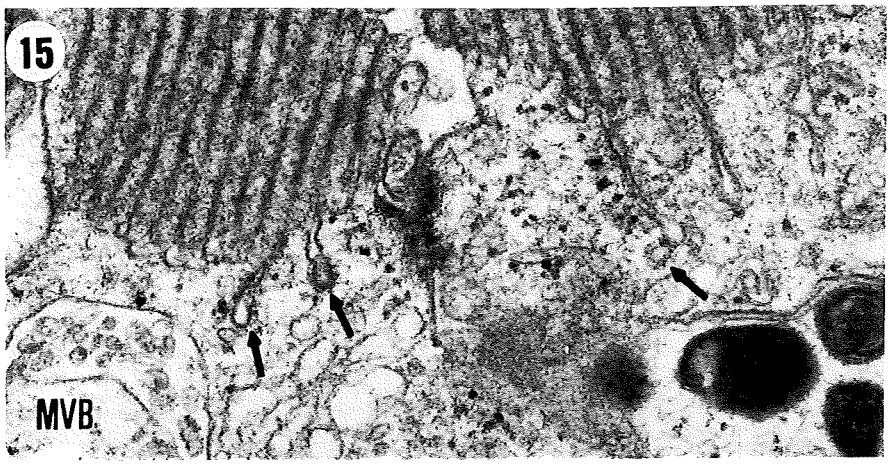
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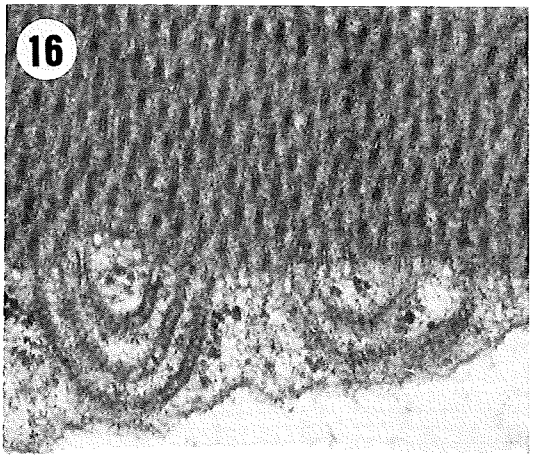
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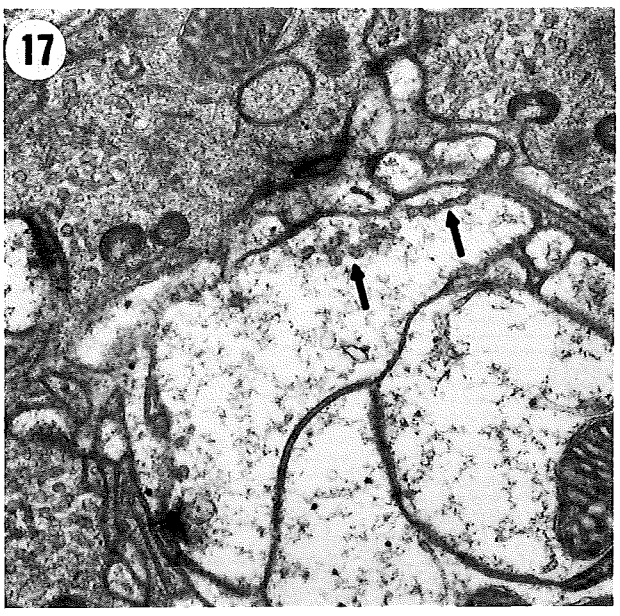
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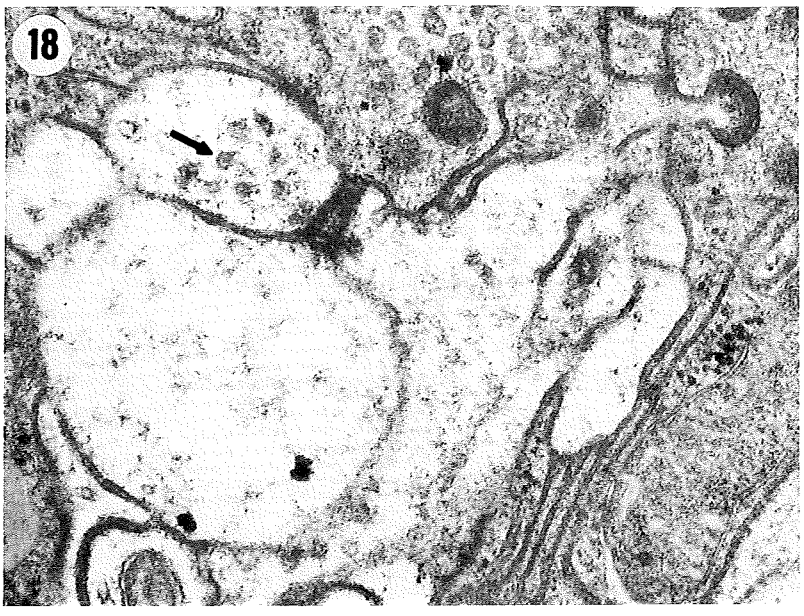
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a coated vesicle from the plasmalemma and, across the belt desmosome, intraretinular pigment granules are seen in this red eyed fly. In Fig. 12, coated pits seem to be on extended stalks. A coated pit on a stalk is depicted in Fig. 13 with a *w shi* fly exposed to enough blue light to maximally convert rhodopsin to metarhodopsin (Stark & Johnson, 1980) and then maintained in the dark for 1.33 h at 29° C before dissection at room temperature. This strategy was based on the finding that fly metarhodopsin is more labile than rhodopsin (Schwemer, 1983, 1984) and the anticipation that coated pits might carry visual pigment from the rhabdomere. However, we have evidence from *Drosophila* that the former does not occur, at least over a short time frame (Stark & Johnson, 1980; Zinkl *et al.*, 1990). Coated pits on stalks are shown in Fig. 14 which is taken from an animal maintained for 2 h at 28.4° C. Fig. 15 shows stalks with coated pits in blue adapted *w shi* under the same conditions as Fig. 13. Also there is a nascent MVB. Unusual membranes are present at the base of a rhabdomere in Fig. 16; this fly was maintained for 3 h at 30° C. Fig. 17 is from the lamina ganglionaris of a blue adapted *w shi* maintained at 1.33 h at 29° C. Here, in the optic cartridge, are R1–6 terminals of intermediate electron density and rounded capitate projections. At the bottom of the figure are more electron-lucent postsynaptic L cells. Note here what appears to be an elaboration of coated pits, rarely observed at this location. Also a T synaptic ribbon is shown in one L cell, rarely seen in *Drosophila* (except see Meinertzhagan & O'Neil, 1988). Fig. 18 also reveals endocytotic pits from the lamina of a blue adapted *w shi* fly also under the same conditions as Fig. 17.

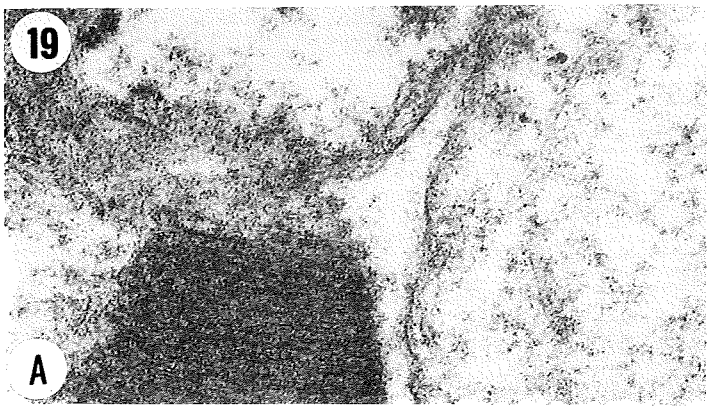
Serial section analyses by HVEM thick sections with 3-D reconstruction yields certain answers unavailable with single plane vistas. Autophagic bodies in *shi* (Fig. 19) and *trp* (Fig. 20) were seen. Fig. 19 is a high magnification of serial-sectioned *shi* maintained 3.5 h at 30° C. Each micrograph traverses through an area of coated pits from the plasmalemma. Fig. 19E is a computerized 3-D reconstruction of the area. By presenting a serial traverse through what might, at one plane of section, appear to be coated vesicles, we show that they are, in fact, confluent with the plasmalemma and are therefore actually coated pits. Fig. 20 is from a 3-week-old *trp* fly and shows serial sections through an area with a coated vesicle and a primary lysosome merging into an MVB. Our presentation of material from *trp*, which has normal MVBs (Stark & Sapp, 1989), is intended to demonstrate typical features and is arbitrary, based on our having obtained a good series from this mutant. Note that the limiting membrane of the MVB is not visible except in the two most optimal section planes. MVBs appear to exist with or without limiting membranes. This series also adds to the literature in which coated vesicles are seen in close proximity, and presumably merging with, MVBs in flies (Williams, 1982; Schwemer & Henning, 1984; Stark *et al.*, 1988). Again Fig. 20E is a 3-D reconstruction of the above serial sections.

By determining which cellular constituents are reactive for opsin immunocytochemically, we can better relate turnover at membrane compared with visual pigment levels. Figs 21–25 show our observations on white flies. Fig. 21 shows a proximal cross-sectioned ommatidium which displays gold particles on the outer (R1–6) rhabdomeres but not the central (R8) rhabdomere. This selectivity is the result of the antibody being specific to the type of rhodopsin (Rh1) unique to the outer rhabdomeres and not found in the central photoreceptors. Note that the gold particles are each discrete 10 nm circles but exhibit the typical clumpy distribution of monoclonals. Fig. 22 is a very distal longitudinal section and shows gold labelling of two R1–6 rhabdomeres but not of the rhabdomeric caps. Fig. 23 shows immunogold labelling localized in a rhabdomere as well as a small amount in an MVB. This would suggest that some opsin immunoreactivity remains in the membranes that are sloughed from the microvilli of the rhabdomere or the plasmalemma and that these membranes may contain some opsin. Fig. 24 is from a fly that has been supplemented with carotenoid for 24 h after being raised from egg to adult without a source of carotenoids. There is an usually high level of staining in the rough endoplasmic reticulum indicating that new opsin is being synthesized. Fig. 25 shows a glimpse of an ocellar rhabdomere stained with an Rh2 antibody which was less selective in its staining characteristics. Work on ocelli was not pursued further, but descriptions of ocellar structure can be found elsewhere (Stark *et al.*, 1989b).

Figs 19 and 20. Serial section analyses of autophagic bodies in *Drosophila*.

Fig. 19. Serial section through an area of coated pits (CP's), joined together and confluent with the intraommatidial cavity (IOM) and near the rhabdomere (R) in *shi* (treated 3.5 h at 30.5° C). (A–D) HVEM serial sections judged by their purple colour to be about 150–190 nm thick. (E) Reconstruction of the major structures shown. ×62 500.

Fig. 20. Serial sections through a MVB in *trp* (aged 3 weeks). Nearby structures include a coated vesicle (CV) merging with the MVB, primary lysosomes (L) and mitochondria (M). A–E and HVEM details as in Fig. 19. ×50 000.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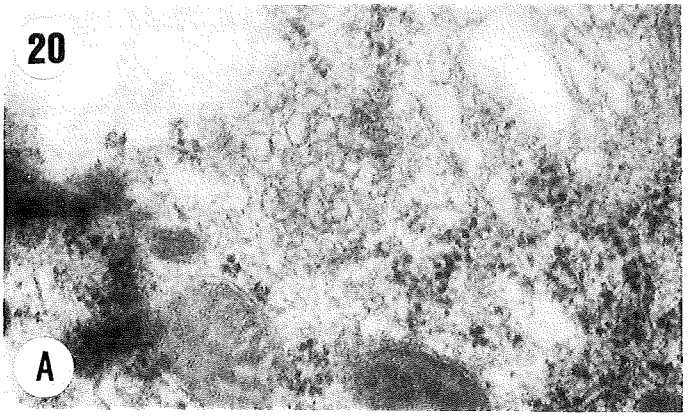
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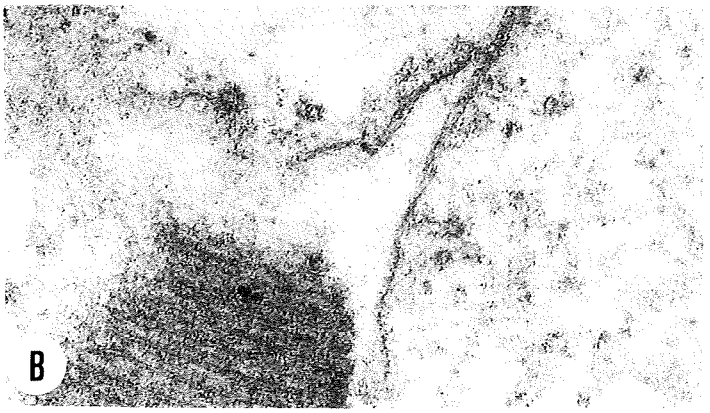
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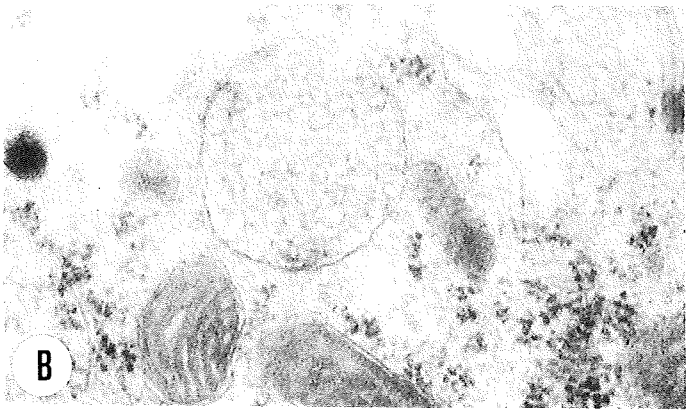
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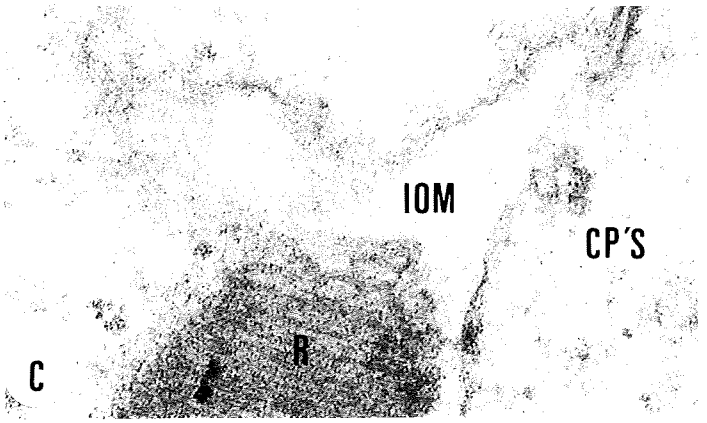
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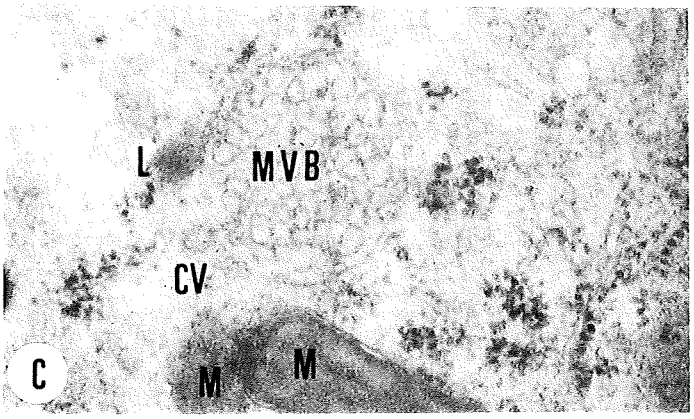
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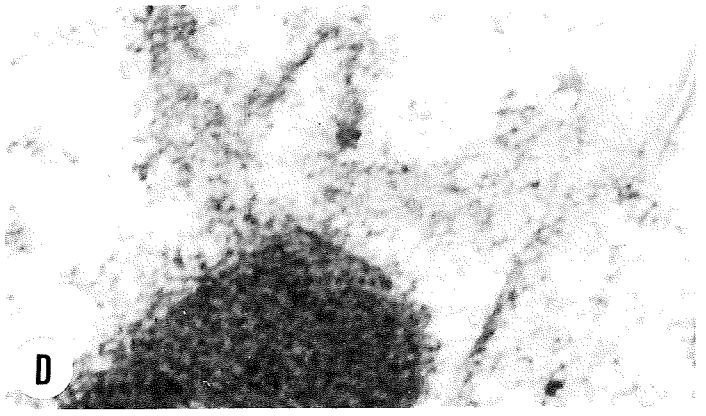
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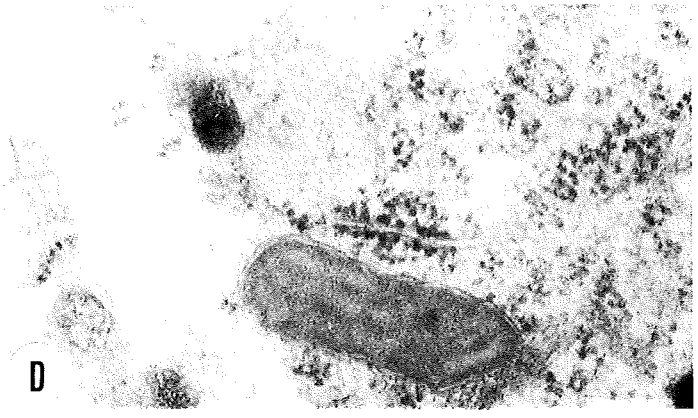
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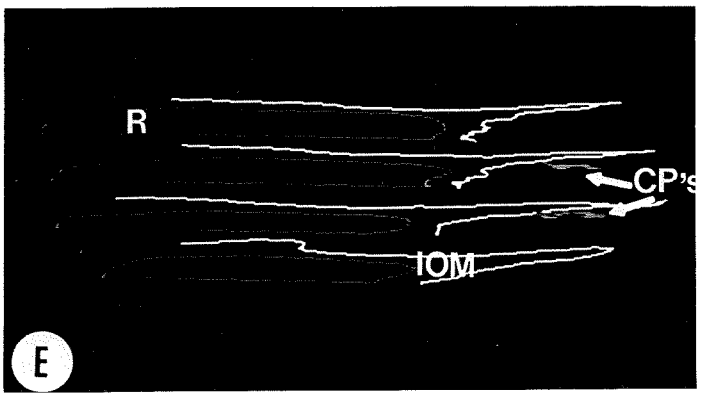
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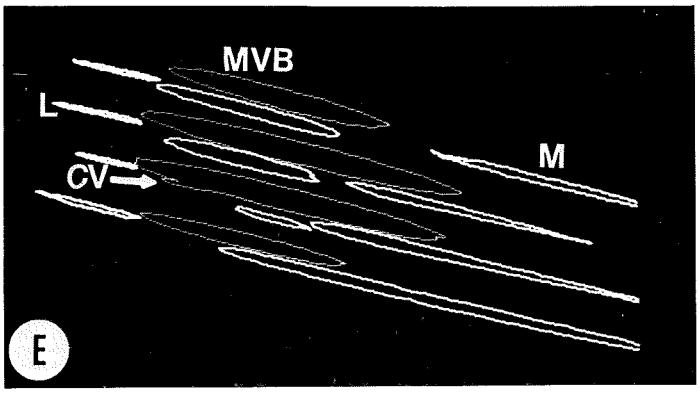
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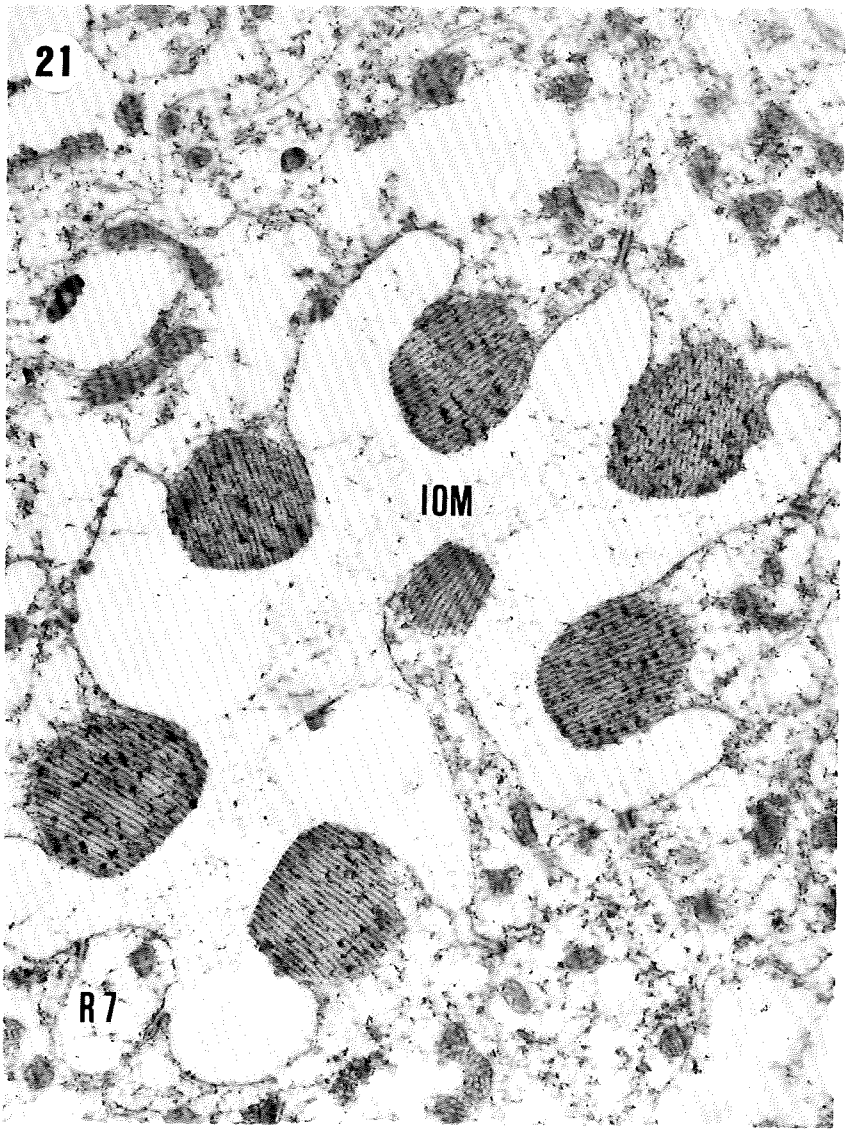
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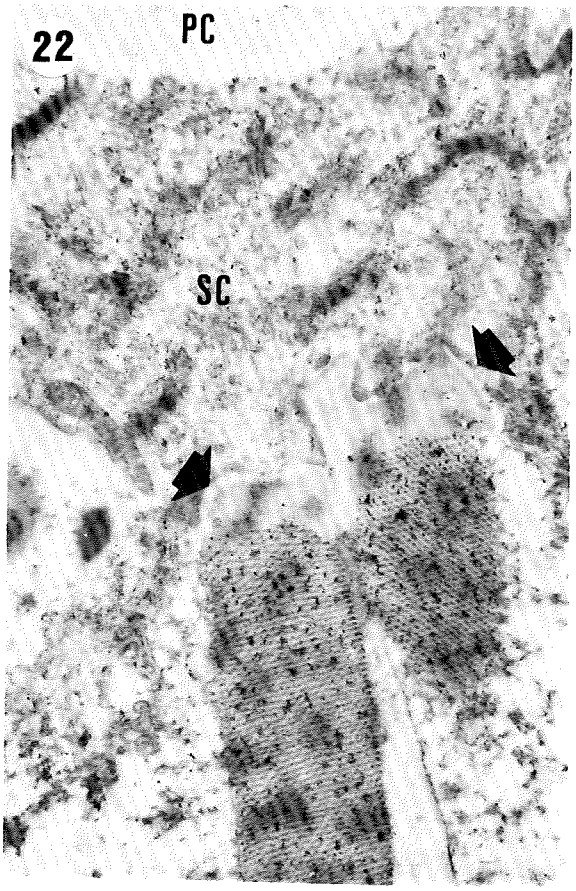
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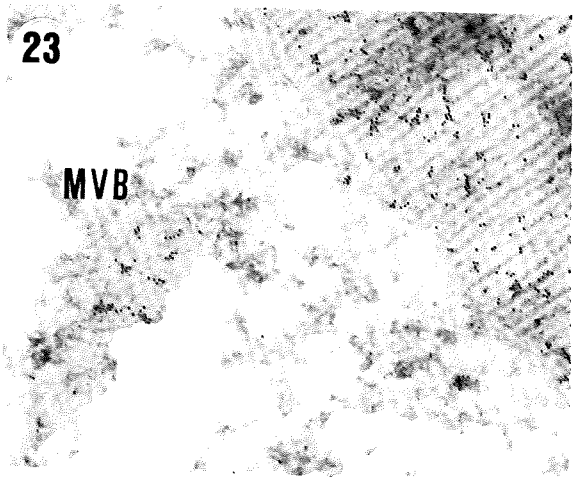
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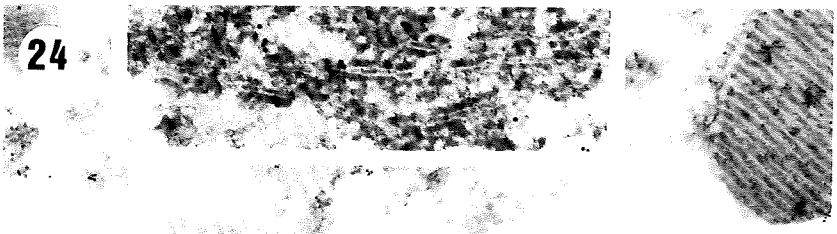
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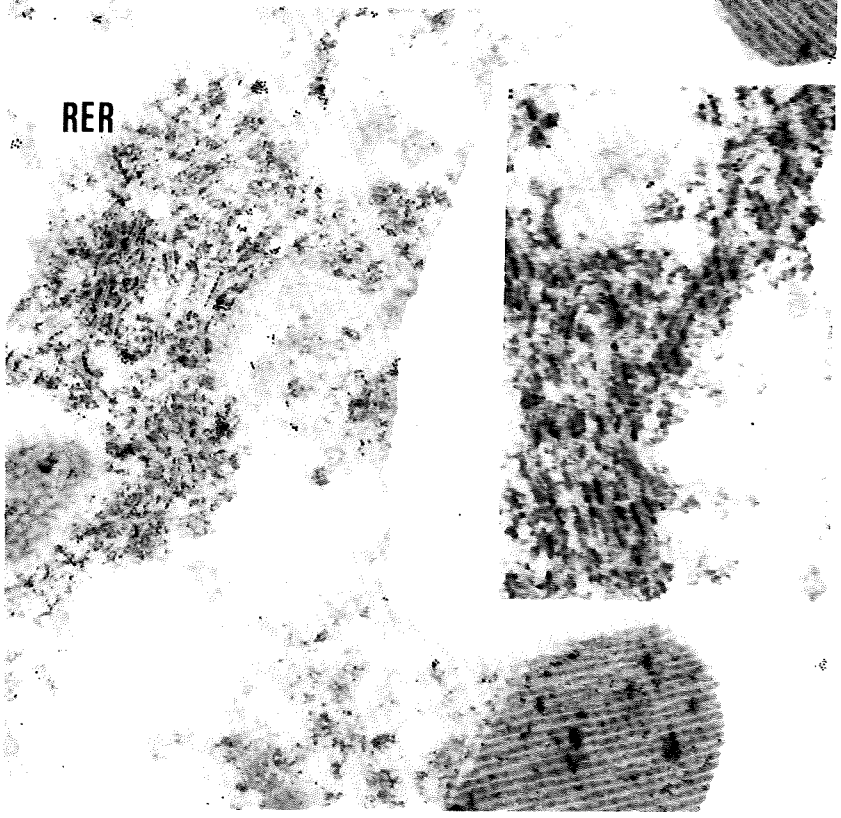
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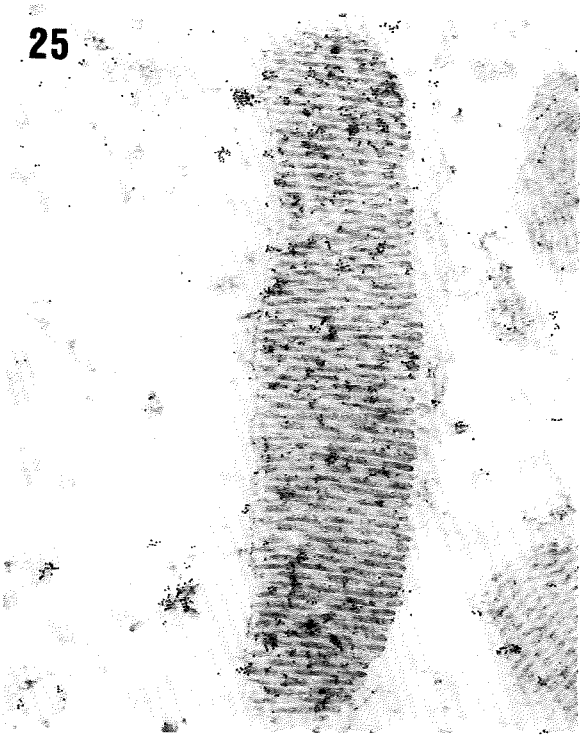
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Discussion

No obvious differences in retinula cell ultrastructure or in rhabdomere size (Chen *et al.*, 1990) were observed in animals at various times throughout the L/D cycle. This supports the claim of Williams (1982) that turnover in the fly might be a continuous process even though visual pigment turnover is not continuous (Stark *et al.*, 1988; Zinkl *et al.*, 1990). By using the mutant *w* (white) which lacks intraretinular pigment granules but is otherwise normal, we were able to identify MLBs and RBs with confidence. We tentatively claim, on the basis of our entire collection of micrographs, that there may be a slightly greater number of loosely packed MVBs in the first 2–4 h in the photophase as expected because visual pigment decreases at that time (Stark *et al.*, 1988; Zinkl *et al.*, 1990). The loosely packed MVBs are not surrounded by lysosomes suggesting that the term 'nascent' is, most likely, appropriate. In preliminary analyses, we counted MVBs from two to 13 ommatidia from one to two animals for nine time points distributed through the photophase and found no significant differences in MVBs per ommatidium (an average of 1.5 with large variances, range 0.5–2.6, with the ommatidial area being about 80 μm^2). Although quantitative analysis of other turnover structures relies heavily on subjective judgments, Golgi apparatus was more noticeable after about 6 h into the photophase. Golgi apparatus is involved in rhabdomere degradation through its involvement in forming primary lysosomes but also presumably in renewal, as Golgi may well be involved in opsin synthesis and deployment (Schwemer, 1985).

Turnover involves coated pits forming into coated vesicles and merging into membrane bounded MVBs, presumably filling and packing from loosely packed putative nascent MVBs. The MVBs are then attacked by primary lysosomes. Our serial sectioned tissue and 3-D reconstructions (many series in addition to Fig. 20) verify that primary lysosomes and coated pits are intimately related with MVBs. The lysosomes spill their hydrolytic enzymes into the MVB and begin to digest its contents. An intermediate step, the MLB, is now revealed as well as the material that cannot be digested, the RB.

We examined the *shi* mutant, which has a long but unclear history in genetic dissection of behaviour. Discovered as a temperature sensitive paralytic (Kelly & Suzuki, 1974), one might think that it has a synaptic exocytosis defect. However, recent data show that *shi* has a defect in the formation of coated vesicles from pits in the garland cell, a cell which exhibits considerable endocytosis (Kosaka & Ikeda, 1983). Possibly the apparent synaptic failure follows from a membrane recycling deficit. In our hands, even in control flies kept at 18° C until 5 min before fixation, pits seem to extend on stalks and do not form coated vesicles. This is especially true for experimental animals that were maintained for 1–3 h at restrictive temperatures (30° C). HVEM serial sectioning and reconstruction was performed to ascertain whether bodies which might look like coated vesicles at one plane of section were really coated pits, still confluent with extracellular space by stalks.

It is important to note that many, but not all, visual mutants studied to date have MVBs, and thus have at least one aspect of membrane turnover being ostensibly normal. Here we show MVBs in *shi* and *trp*, the latter result replicating findings of Stark and Sapp (1989). The *norpA* (no receptor potential) mutant has MVBs (Stark *et al.*, 1989a; Stark & Sapp, 1989; Zinkl *et al.*, 1990), despite the lack of a receptor potential. The *rdgB* (retinal degeneration) mutant also has MVBs before the receptor somata show cell death (Stark & Sapp, 1989). The outer rhabdomeres (R1–6) in the mutant *ora* (outer rhabdomeres absent) do not have MVBs even though flies eclose with small rhabdomeres which are gradually lost because of this mutation in the R1–6 opsin gene (Stark & Sapp, 1987).

Using the protocol of deCouet and Tanimura (1987) and Rh1 antibody, we confirmed specific labelling in R1–6 and not R7 , with different opsins, Rh3 and Rh4 (Zuker *et al.*, 1987) (not shown), or R8. Thus, the fly ommatidium offers an excellent internal control. We had hoped this technique might offer better insight into the specific pathway of visual pigment degra-

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Figs 21–25. Immunocytochemistry with monoclonal antibodies to *Drosophila* rhodopsins.

Fig. 21. Proximally cross-sectioned ommatidium in *w* control diet. Rh1 antibody labels the rhabdomeres of R1–6 surrounding the intraommatidial matrix (IOM) but not that of R8 (central). At this plane of section, R7, identified on figure, is just an axon, and R1–6 are counterclockwise from R7 with R8 between R1 and R2. ×20 800.

Fig. 22. Distal longitudinal section in *w* control diet reveals label in R1–6 rhabdomeres but not in rhabdomere caps (arrowheads). Semper cells (SC) and pseudocone (PC) are distal to rhabdomeres. ×18 400.

Fig. 23. R1–6 cell in *w* control diet showing rhabdomere (upper right) and an MVB. The 10 nm gold particles are present on the rhabdomere with a few in the MVB. ×44 800.

Fig. 24. R1–6 cells in *w* after 24 h of carotenoid replacement therapy show high labelling in the rough endoplasmic reticulum (RER). ×38 400. Upper insert, RER from another retinula cell. ×75 000. Right insert, RER from yet another retinula cell. ×60 000.

Fig. 25. Ocellar rhabdomere in wild type labelled with Rh2 antibody. ×31 200.

dation. Assuming that opsin is internalized stoichiometrically with membrane, early MVBs should contain substantial opsin as witnessed by freeze fracture in the crayfish (Eguchi & Waterman, 1976). Low immunogold labelling of MVBs (Fig.23) could reflect a lack of antigenicity rather than of opsin. By contrast, in the vertebrate phagolysosomal system, Feeney-Burns and co-workers (1988) showed that opsin immunoreactivity remains in early phagosomes from rods in retinal pigment epithelial cells. Also, EM immunocytochemistry lacks sufficient resolution to show opsin being endocytosed via coated pits. Thus, we were disappointed with the vistas provided by immunocytochemistry relevant to opsin autophagy.

Carotenoid replacement synchronizes a massive biosynthesis and deployment of opsin. This optimized visualization of immunocytochemical labelling in the rough endoplasmic reticulum. In the crab, Stowe (1980) observed membranes associated with rhabdom build-up at dusk. Although we had proposed that opsin was routed through such membrane vesicles (Stark *et al.*, 1988) via Golgi apparatus, we saw no labelled vesicles or Golgi. Such a route would be consistent with the vertebrate mechanism: Papermaster and co-workers (1985) provided immunocytochemical and autoradiographic evidence in frog that newly synthesized opsin is transported from the Golgi toward the rod outer segment in vesicles.

In the rat, vitamin A deprivation (10, 18 and 26 weeks after weaning) decreases spectrophotometrically measured rhodopsin, protein with chromophore; yet opsin density remains high in rod outer segment disc membranes as determined by P-face particle and anti-opsin EM immunogold counts (Katz *et al.*, 1991). The *Drosophila* situation is strikingly different. Deprivation of carotenoid from egg to adult decreases ERG sensitivity 100-fold (Stark *et al.*, 1976) by lowering rhodopsin (spectrophotometrically measured) along with opsin (deduced from freeze fracture electron micrographs, Harris *et al.*, 1977). Sapp and co-workers (1990a,b) showed in *Drosophila* that opsin and rhodopsin recover in unison during carotenoid replacement (EM immunocytochemistry and microspectrophotometry). We explain the difference between rat and fly with the proposal that carotenoids regulate opsin synthesis (at the level of transcription, translation or post-translational modifications) in the fly but not the rat. This is logical from the respective photochemistries. The fly's photointerconvertible, non-bleaching rhodopsin 480-metarhodopsin 570 system need only be synthesized once except for some daily turnover (Stark *et al.*, 1988). In rat rods, light bleaches rhodopsin into opsin and chromophore. Chromophore is recycled through different cells, the supportive retinal pigment epithelium. As the reisomerized chromophore is reapplied to the outer segments for rhodopsin recovery, it would not be adaptive to hold the earlier opsin synthesis hostage to the availability of chromophore in the rod inner segment.

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