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Ommatidial structure in relation to turnover of photoreceptor membrane in the locust

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Summary. In the compound eye of the locust, *Locusta,* the cross-sectional area of the rhabdoms increases at "dusk" by 4.7-fold due to the rapid assembly of new microvillar membrane, and decreases at "dawn" by a corresponding amount as a result of pinocytotic shedding from the microvilli. The rhabdoms at night have more and longer photoreceptor microvilli than rhabdoms during the day. The orientations of the six rhabdomeres that comprise the distal rhabdom also change. The density of intramembrane particles on the P-face of the microvillar membrane, putatively representing mostly rhodopsin molecules, or aggregates thereof, does not change.

An alteration in the size of the ommatidial field-stop, produced by the primary pigment cells, is concomitant with the change in rhabdom size. At night the increase in size of the field-stop must widen the angular acceptance of a rhabdom, increasing the capture of photons from an extended field. Conversely, during the day, when photons are more abundant, its decrease must narrow the acceptance angle, increasing angular resolution. Because of the presence of this field-stop, the optics of the ommatidium would not be greatly affected if the rhabdom were to remain always at its night size. It is argued, therefore, that the variable-size rhabdom must have resulted from some demand other than that of light/dark adaptation.

Changes in size and organisation of the rhabdoms in response to various light regimes indicate that: (1) Rapid shedding of photoreceptor membrane is induced by the onset of light, but shedding also occurs slowly in darkness during the day. (2) Microvillar assembly is initiated by the onset of darkness, but also occurs at the normal time of dusk without a change in ambient lighting, provided there has been some light during the day. Therefore, both shedding

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and assembly of microvillar membrane are affected by the state of illumination, but also appear to be under some endogenous control.

Key words: Photoreceptors – Microvilli – Membrane turnover – Freeze fracturing – Biological clock – Grasshopper

Following the original electron-microscopical studies of the effects of light and darkness on rhabdom size in the larval mosquito eye by White (1967), it has been established that the rhabdoms of many invertebrates decrease in width (and in some cases length also) at the onset of light (normally dawn), and increase at the onset of darkness (normally dusk). "Variable-size rhabdoms" of this kind have been found in: (1) Invertebrates exclusive of arthropods: holothurian ocelli (Yamamoto and Yoshida 1978), seastar ocelli (Eakin and Brandenburger 1979), cephalopod molluscs (Young 1962); (2) Crustaceans: shrimps (Debaisieux 1944; Itaya 1976; Hertel 1980), crabs (Nässel and Waterman 1979; Stowe 1980a); (3) Chelicerates: spiders (Blest 1978; Blest and Day 1978; Blest et al. 1980), harvestmen (Schliwa 1979), *Limulus* lateral eye (Behrens 1974; Miller and Cawthon 1974); (4) Insects: larval mosquito ocelli (White and Lord 1975), adult mosquito compound eye (Sato et al. 1957; Brammer and Clarin 1976; Brammer et al. 1978), mycetophilid larval (glowworm) ocelli and adult compound eye (Meyer-Rochow and Waldvogel 1979), adult tipulid (Williams 1980a), mantid and locust (Horridge et al. 1981). The change in rhabdom size is achieved by the daily turnover of large amounts of photoreceptor membrane and a separation of the peaks of the shedding and assembly phases of the turnover (Blest 1980); the lateral eye of *Limulus* possibly provides an exception (Chamberlain and Barlow 1979; but see also discussion by Stowe 1981).

Notwithstanding reports that locusts have variable-size rhabdoms (Horridge and Blest 1980; Horridge et al. 1981), the daily changes in rhabdom structure have not been described in detail. The present paper examines the rhabdom structure and associated optics of the ommatidium in *Locusta,* in relation to time of day and state of illumination. The photoreceptor cells of locusts are amenable to intracellular recording for long periods of time, and are therefore highly suitable for investigating the functional effects of photoreceptor membrane turnover (Horridge et al. 1981). The following description provides essential background for such studies. In addition, it gives insight into the control of photoreceptor membrane turnover.

Materials and methods

Adult *Locusta migratoria* L. and *Valanga irregularis* (Walk.) were taken from a laboratory culture that was fed bran and wheat and maintained on a 12 h light: 12 h dark cycle (lights on, i.e. "dawn", at 06.00 h; "dusk" at 18.00 h) at 25–35° C, for at least two weeks prior to experimentation. Light was provided by a 60 W incandescent bulb in each cage (36 × 36 × 46 cm), and two 40 W daylight-fluorescent lights shared by three cages (illuminance in the centre of the cage was 1,500 lux). At night, lights were off and no light entered through any windows.

Compound eyes of *Locusta* were fixed at particular times on the normal light regime and on five experimental regimes (as shown in Fig. 8). During at least 12 h immediately prior to fixation, animals were kept at 25 ± 1° C and 48 ± 1% relative humidity, and under the following illumination: for light

adaptation, under daylight fluorescent lights of similar intensity to that used during the day previously; for dark adaptation, the only lighting was a Kodak 1A safelight (15 W bulb). The eyes were dissected under the appropriate illumination (fluorescent lights or the safelight). Dissections were made so as to leave the central region of the eye (Fig. 1) intact and undistorted. The head was cut in half, one half was discarded, then a fresh razor blade was used to slice through the surface of the remaining eye. The slice was initiated through the cornea at about the midline, and directed ventrally, in such a way that no cut was made through the basement membrane, and all the ommatidia dorsal to the cut were undistorted. The half-head was then quickly immersed in primary fixative: either 2.5% glutaraldehyde + 2% paraformaldehyde buffered in 0.08 M sodium dihydrogen orthophosphate-NaOH plus 0.06 M D-glucose, or 2.5% glutaraldehyde buffered in 0.08 M sodium cacodylate plus 0.11 M sucrose. No calcium was added to the buffer solutions (Williams 1980b). After 3–12 h primary fixation at 4° C, samples were transferred to buffer without fixative, and the dissection was completed so that only a small block of the ommatidia, lying immediately dorsal to the initial slice, remained (Fig. 1). Following this method of fixation, no whorls of membrane, described by Horridge and Barnard (1965) as "onion bodies", were evident in the retinular cells.

For conventional microscopy, tissues were osmicated in the same buffer, dehydrated through an ethanol series and propylene oxide, and embedded in Araldite. Thin sections were collected on formvar-coated slot grids and stained with uranyl acetate followed by Reynolds' lead citrate. They were examined in a Zeiss 109 electron microscope. Measurements of the cross-sectional areas of the rhabdoms were made with a Kontron MOP-AMO 3 image analyser, from micrographs of rhabdoms in true transverse section (e.g., Fig. 2), magnified 3,300 times.

Adult *Valanga* were used in the freeze-fracture experiments since no *Locusta* were available at the time. To prepare replicas, glycerol was slowly added to the wash solution, containing pieces of retina that were dissected and fixed as above, to give a concentration of 25–30%. Blocks of retinae were frozen in Freon-22, stored in liquid nitrogen, and fractured at $-115°$ to $-105°$ C and 10^{-6} to 10^{-7} torr in a Balzers BAF 300 freeze-etch unit. Specimens were not etched before platinum/carbon coating, which was controlled with a quartz thin-film monitor. Replicas were cleaned in chromic acid (5% sodium dichromate in 50% sulphuric acid), rinsed in distilled water, collected on formvar-coated slot grids, and examined in an Hitachi H500 microscope. The particle density on the P-face of the microvillar membrane was measured from prints magnified at least 100,000 times, using the Kontron MOP-AMO 3 image analyser. Particles in only the central 50-nm of the microvillar profile were considered. To compare particle densities between day and night-state eyes about 200 particles were counted from each of 30 rhabdoms sampled from four day-adapted animals and 30 rhabdoms sampled from four night-adapted animals.

To test the significance of differences in rhabdom size, intramembrane particle density, and microvillar diameter, the two-sample *t*-test (two tail) was used. Usage of this test was justified on the grounds that the two data samples were unrelated, had similar variances, and were from populations that did not deviate too strongly from a normal distribution (e.g., were not obviously skewed).

Results

Ommatidial structure and optics

Each compound eye of an adult *Locusta* has about 8,500 ommatidia packed in an hexagonal array (Shaw 1978). A region of higher acuity, where the interommatidial angle is smaller, accepts light from in front of the animal (Horridge 1978). The present paper examines ommatidia from the centre of the eye (Fig. 1), where the interommatidial angle is fairly constant. The ommatidial organisation has been described by Wilson et al. (1978). Beneath the lenslet, four cone cells taper proximally (Fig. 3) and penetrate about 10 μm into the centre of the rhabdom. The rhabdom is about 300 μm long. Two primary pigment cells surround the cone cells, and secondary pigment cells ensheath the entire ommatidium. Light is brought to focus near the most distal extremity of the rhabdom (McIntyre 1982), and here the

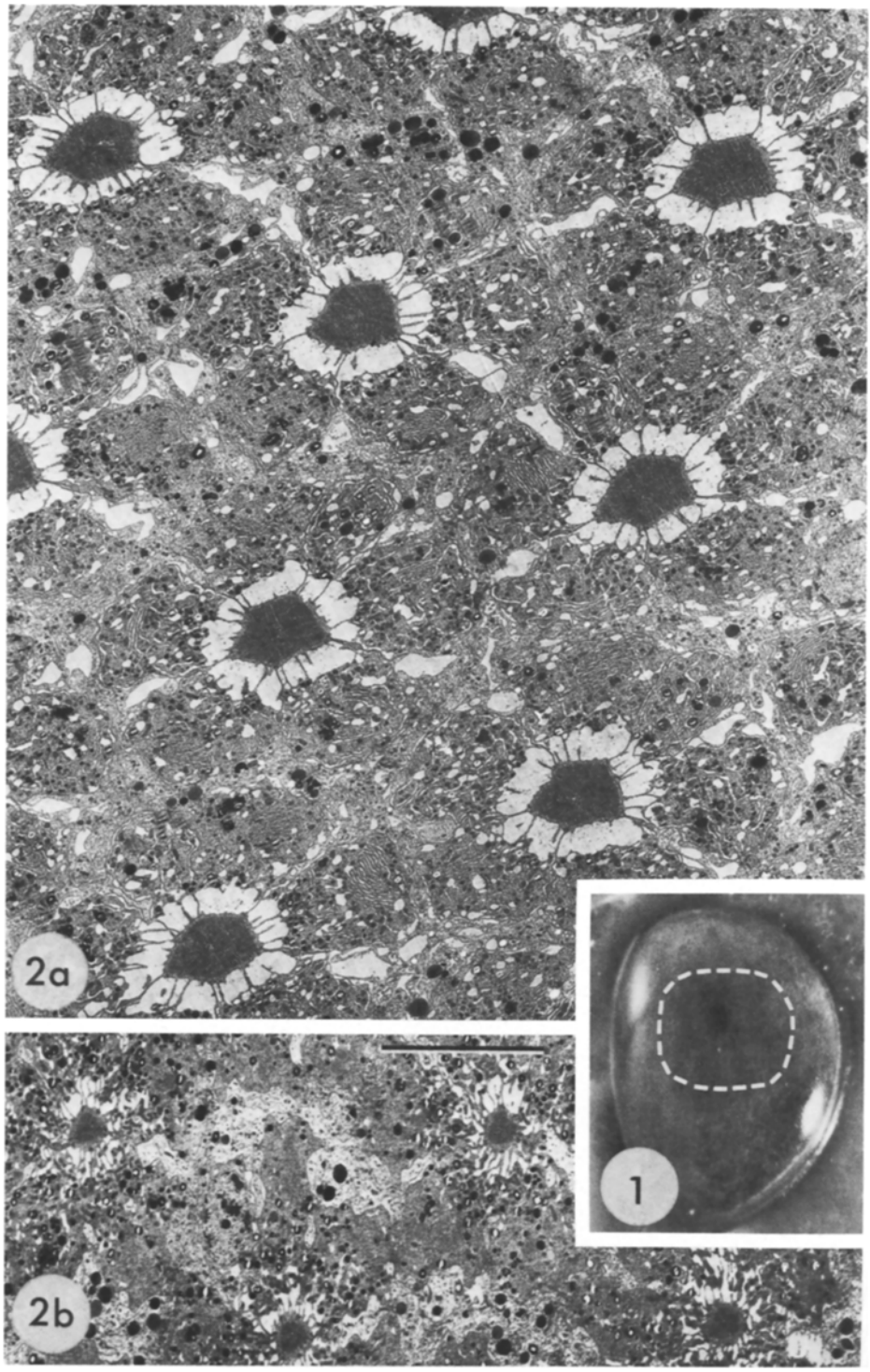
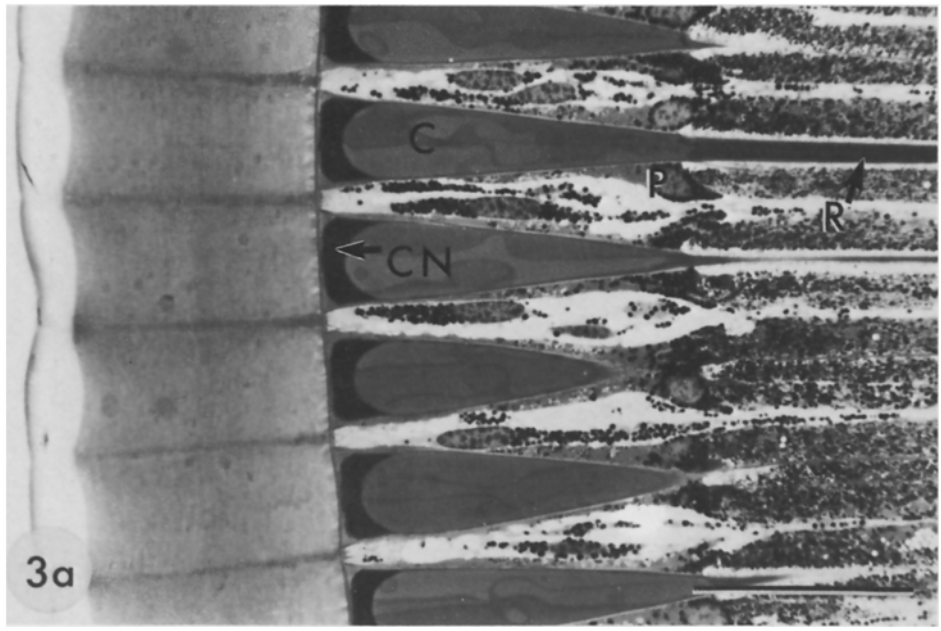


Fig. 1. Photograph of left compound eye of *Locusta migratoria*. The present paper considers ommatidia from the region enclosed by the white dashes. ×15

Fig. 2. Transverse sections through the retina in the central region of a compound eye of *Locusta*. Typical of the sections used for measurements of rhabdom cross-sectional areas: **a** normal night-state; **b** normal day-state. **a, b** Scale bar 10 μm; ×2,300



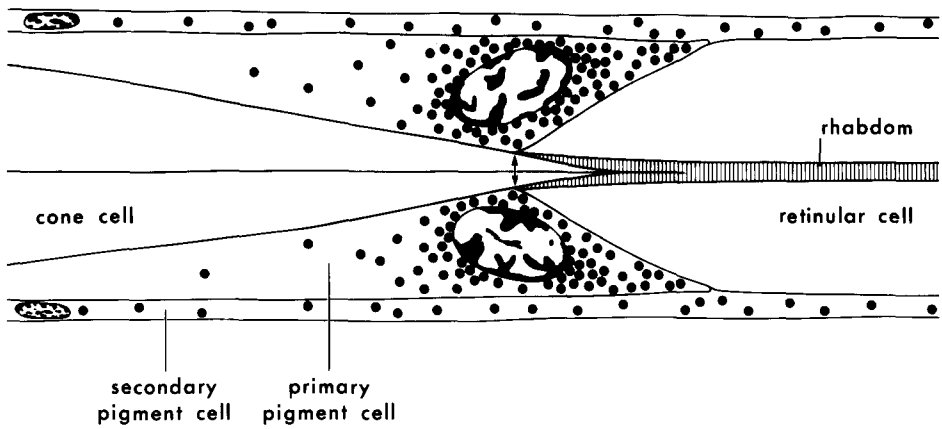


Fig. 3. a Light micrograph of longitudinal section through lenslets, the cone (*C*), and distal end of the rhabdom (*R*). The nuclei of the cone cells (*CN*) cap each cone. *P* primary pigment cell. Scale bar 50 μm; ×600. **b** Diagram of base of cone and distal end of rhabdom. The primary pigment cells effect a field-stop, which is indicated by the *double-headed arrow*

primary pigment cells provide the ommatidium with a field-stop (Fig. 3b). This field-stop is about 1.5 times greater in diameter than the rhabdom at the level of the distal nuclear layer (about 50–100 μm proximal to the field-stop), irrespective of the size of the rhabdom. The position of the field-stop is the same at night as during the day; it is 80–85 μm from the flat distal end of the cone cells (Fig. 3a). The secondary pigment cells do not change markedly between light and dark adaptation.

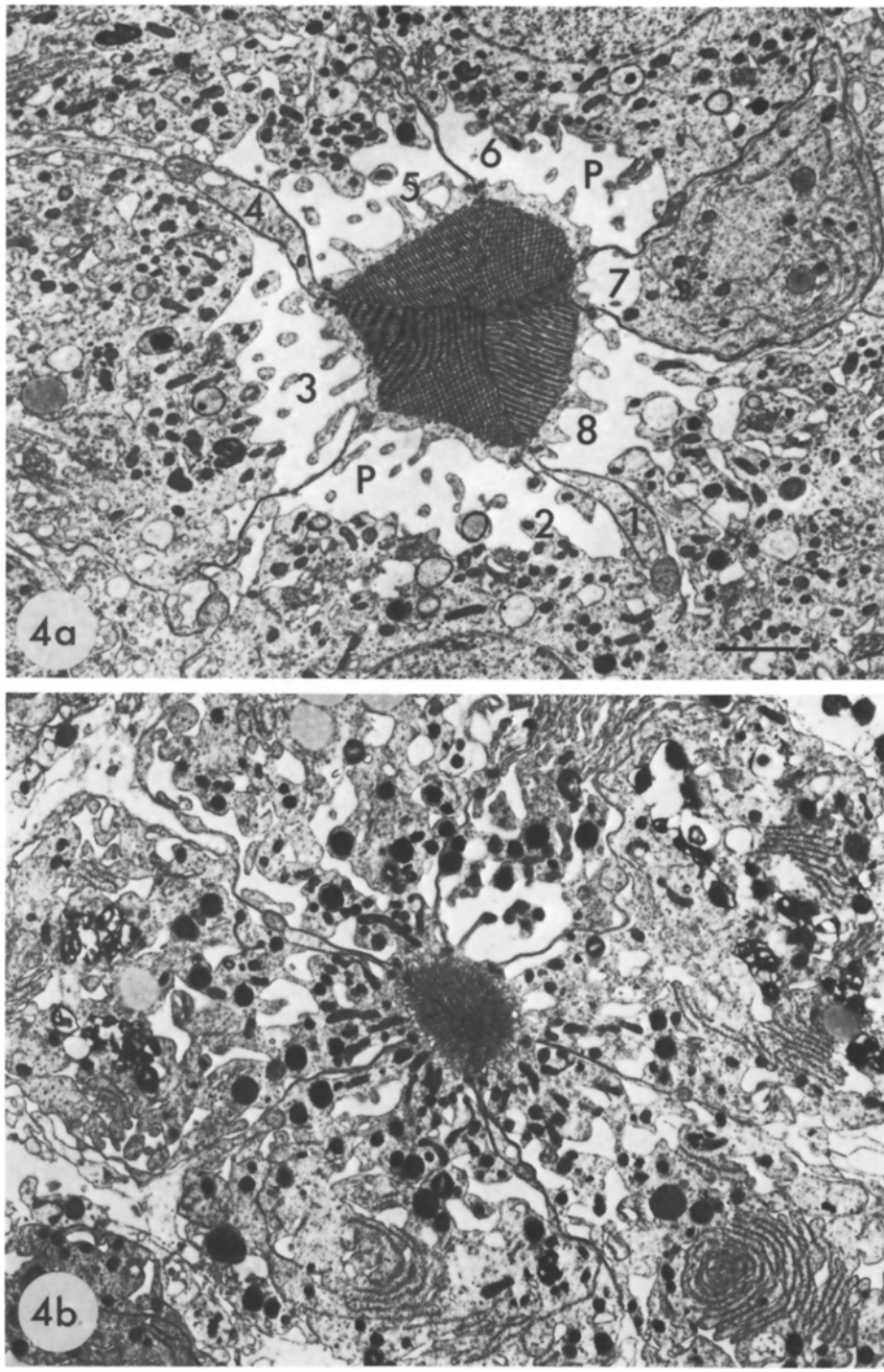


Fig. 4. Transverse sections through the distal part of an ommatidium in **a** normal night-state, **b** normal day-state. Retinular cells in **a** are numbered. *P* perirhabdomal palisade. a, b Scale bar 2 μm; ×6,500

Table 1. Rhabdom structure during night and day

	Rhabdom cross-sectional area[a] (μm^2)	Microvillar diameter[b] (nm)	Intramembrane particle density[b] (μm^{-2})
Day	3.6 ± 0.58	68.0 ± 1.55	6401 ± 373
Night	17.0 ± 1.37	66.8 ± 1.19	6089 ± 424
Probability of no difference[c]	< 0.001	0.5 (n.s.d)	0.3 (n.s.d)

[a] From Fig. 8a
[b] Means of 30 measurements of different rhabdoms sampled from 4 animals
[c] *t*-test (two tail); n.s.d., no significant difference
Error is ± 2 standard errors of the mean

In addition to the field-stop, the "palisade" of vacuoles of endoplasmic reticulum, which surrounds the rhabdom when dark-adapted (Horridge and Barnard 1965) (Figs. 2a, 4a), should also affect the amount of light captured by the rhabdom. Some doubt concerning the effectiveness of the palisade has been expressed by Shaw (1978), who found it to persist in light-adapted ommatidia. In the present study, the distal regions of retinae fixed within a few hours after the onset of light were found to have their perirhabdomal palisades replaced by dense cytoplasm containing pigment granules and mitochondria. This condition was not so obvious in eyes that had been light-adapted for some time (e.g., fixed in the afternoon on a normal light cycle). An interplay between a clear palisade and dense contents of the cytoplasm should affect the light absorption of the rhabdom by changing its light-guiding properties (Horridge and Barnard 1965), and is probably important as a short-term means of adaptation when illumination changes (cf. the longitudinal pupil mechanism found in many other insects: review by Stavenga 1979).

Rhabdom structure on a daily cycle

Between night and day the cross-sectional area of the distal rhabdom changes 4.7-fold (Fig. 4, Table 1). The distal part of the rhabdom contains a central cone thread and tapers. Therefore, for reasons of accuracy, measurements of the cross-sectional area of rhabdoms were made proximal to the central cone thread and distal to, or including the distal nuclear layer; i.e. along a length of rhabdom of about 30 μm with an almost uniform diameter.

Cycle *a* in Fig. 8 shows measurements of rhabdom cross-sectional area in *Locusta* at various stages throughout a normal daily cycle. During a period of 1 h after the onset of light at "dawn", the cross-sectional area is reduced by 79% from its "night-state" size to its "day-state" size (Table 1; cf. Fig. 2a with 2b, and 4a with 4b), after which there is no significant change until the following dusk. The post-"dawn" reduction is achieved by massive pinocytosis of membrane from the bases of the microvilli (Fig. 5). The basal parts of the microvilli initially become disorganised. Coated pits, which pinch off as coated vesicles, form in the disordered

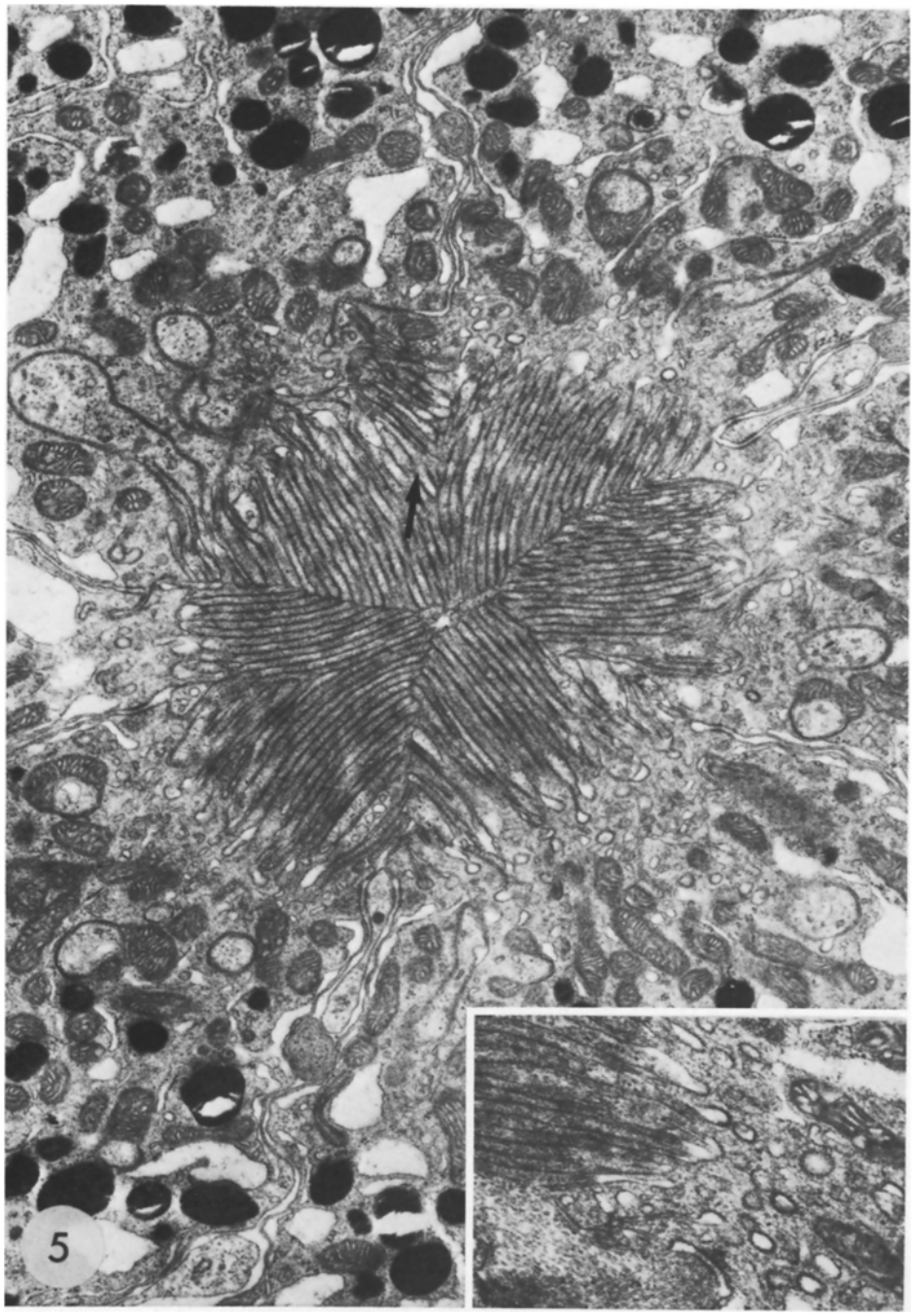


Fig. 5. Rhabdom on normal cycle fixed 15 min after "lights-on". At their bases the microvilli are disordered and vesiculating. The shorter microvilli at one margin of each rhabdomere (e.g., *arrow*) are shed in toto as rhabdomeral orientation changes. × 17,800. *Inset*: Higher magnification of vesiculation at the bases of microvilli about 30 min after lights-on. × 32,400

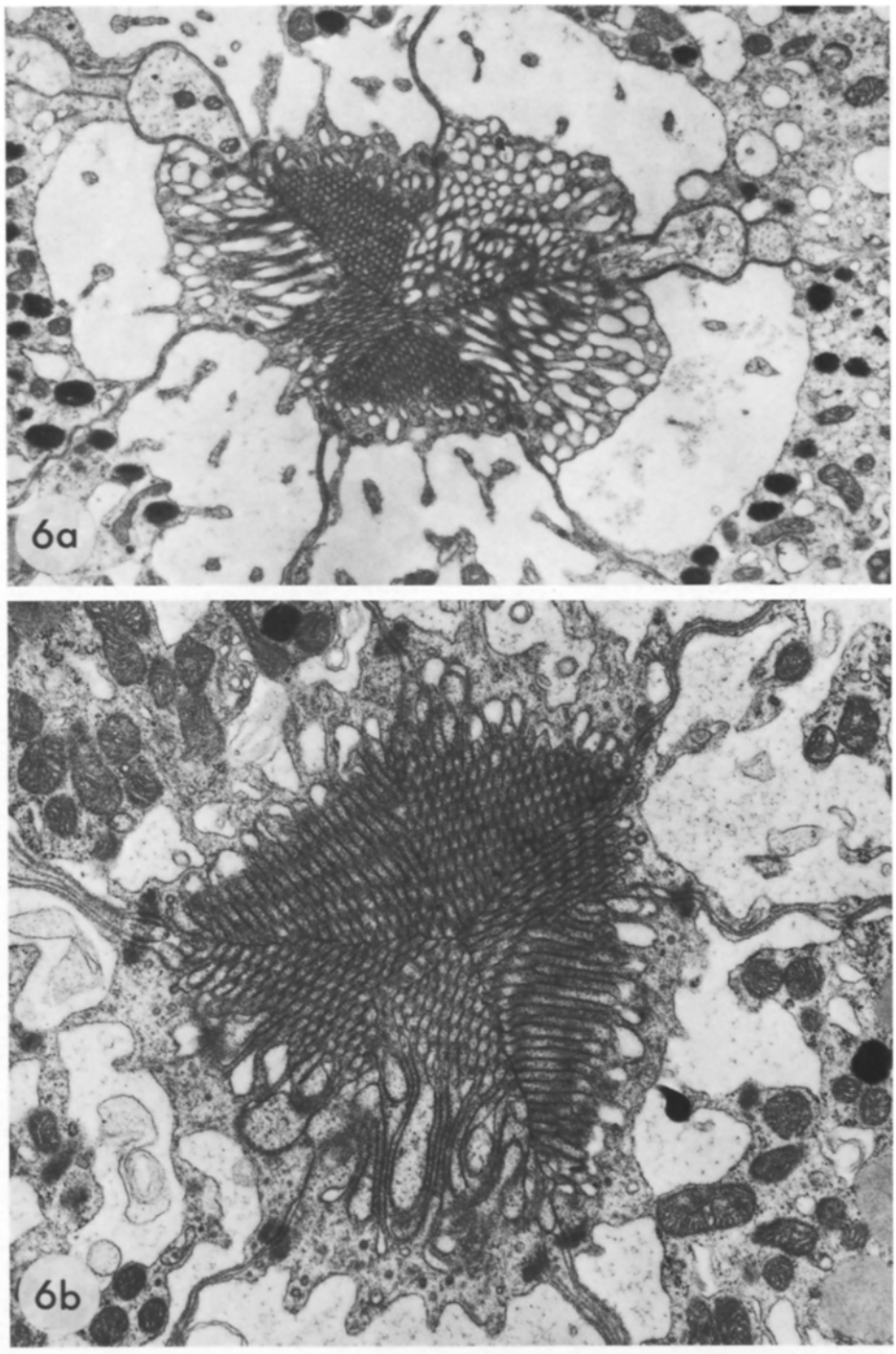


Fig. 6. Disarrayed rhabdomeres during the assembly of new microvillar membrane to form the night rhabdom immediately after "dusk". **a** Three rhabdomeres are disordered during an early stage of reassembly. ×11,000. **b** Except for two rhabdomeres (*bottom, left*), assembly is nearly completed. ×17,000

photoreceptor membrane and some quite large segments of membrane are sloughed from the rhabdom. After about 30 min, the rhabdom has resumed an ordered state and membrane continues to be shed by the pinocytosis of coated pits, as in most other arthropods (e.g., Eguchi and Waterman 1967; White 1968; Blest 1978; Martin and Hafner 1981). If exposed to a natural sunrise of gradually increasing light intensity, the microvilli remain strictly aligned throughout the entire dawn period and pinocytotic diminution of the rhabdom proceeds more slowly. At sunrise, about 1 h after the first noticeable light of dawn in the east (midsummer, 35° latitude), the cross-sectional area of the rhabdoms is midway between night-state and day-state size (20 rhabdoms from each of two animals averaged 9.6 ± 0.49 (2 S.E.) μm^2). By 1 h after sunrise, rhabdoms have reached their normal day-state (Figs. 2b, 4b).

Immediately after the onset of darkness, the night rhabdom is constructed by the assembly of new photoreceptor membrane. During construction the rhabdomeres are characteristically disarrayed (Fig. 6). By 1–3 h after "dusk", ordered night rhabdoms (Figs. 2a, 4a) are evident, and they remain in this state until dawn. In cycle *a* (Fig. 8), measurements of rhabdom size after dusk are shown only for ordered rhabdoms in the night-state.

Between night and day, a change in orientation of microvilli in the distal rhabdom is associated with the change in rhabdom size. In the day-state, the distal rhabdom typically comprises six rhabdomeres with pairs of adjacent rhabdomeres having nearly the same microvillar orientation. The result is a triradiate pattern (Fig. 4b) (Wilson et al. 1978). Proximally, this pattern is lost as rhabdomeres twist (Williams 1981, for discussion). In the night rhabdom, however, the triradiate pattern is not present, for rhabdomeres have independent orientations even in the distal rhabdom (Fig. 4a). At dawn, the shorter microvilli at one lateral margin of each night rhabdomere are shed entirely to give rise to the triradiate configuration (Fig. 5).

Microvillar structure

Only the length and number of microvilli have been found to vary between day and night in *Locusta*. Firstly, unlike in *Limulus* (Behrens and Krebs 1976), the mosquito (White 1967), and a crab (Nässel and Waterman 1979), the diameter of the microvilli was constant (Table 1). Secondly, the intramembrane particles found on the P-face of the microvilli did not change in density (Table 1; Fig. 7), and their size appeared to remain at about 8 nm in diameter.

Effects of illumination and time of day on rhabdom structure

Fig. 8b–f illustrates the changes in rhabdom size in response to abnormal lighting regimes.

Light continued after day (Fig. 8b). Rhabdoms of animals kept in the light and fixed 1, 2 and 4 h after the normal time of "dusk", possessed the distal triradiate configuration, and were significantly smaller than normal night-state rhabdoms ($p < 0.001$), but those fixed 1 and 2 h after 18.00 h were significantly larger (mean

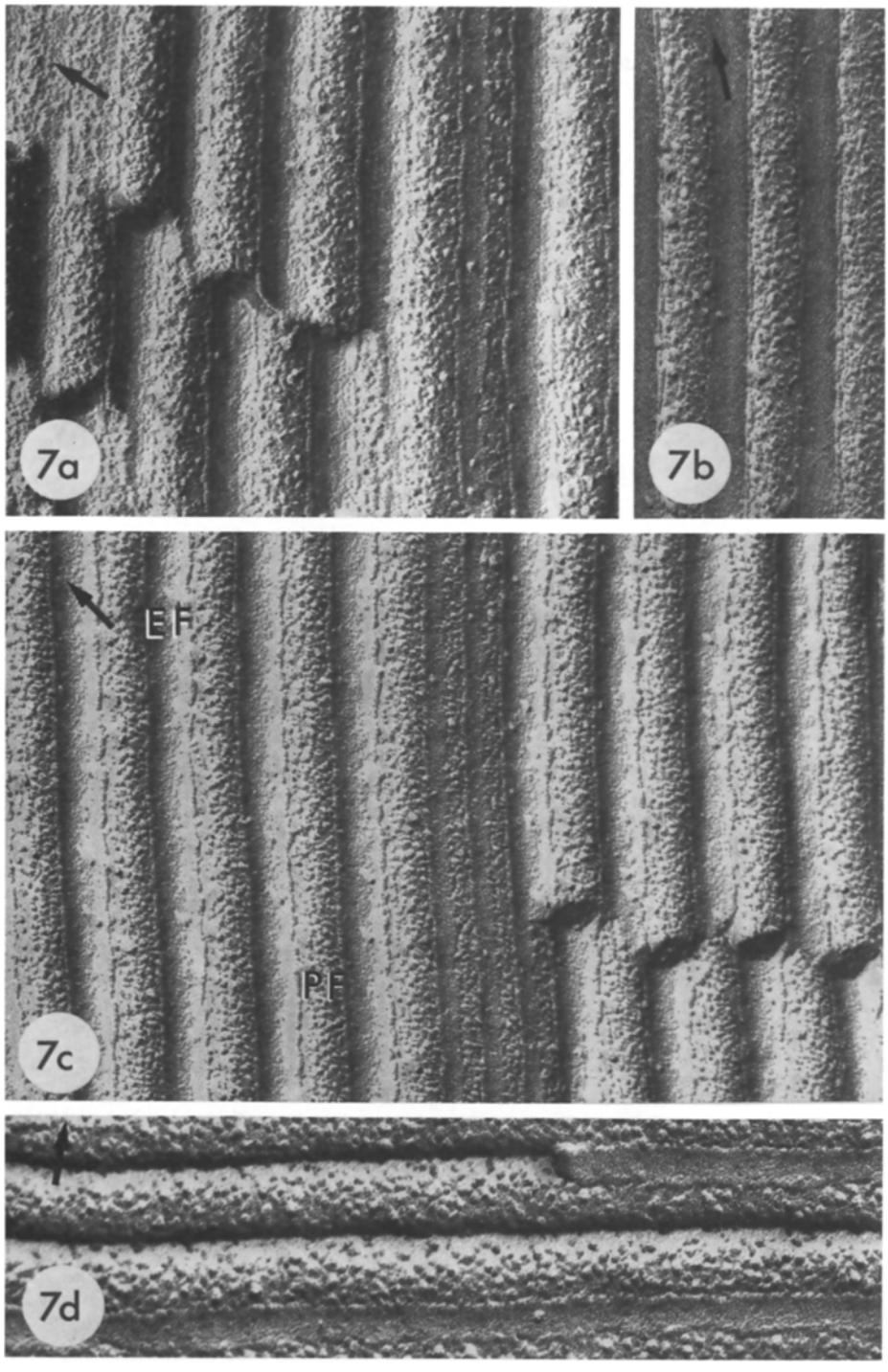


Fig. 7. Electron micrographs of freeze-fracture replicas of photoreceptor microvilli from *Valanga*. The intramembrane particles on the cytoplasmic face (*PF*) probably represent mostly rhodopsin molecules, or aggregates thereof. *EF* face of outer membrane layer. *Arrows* indicate direction of platinum coating. **a, b** Fixed at midday on normal light cycle; **c, d** at midnight. a–c ×100,000; d ×150,000

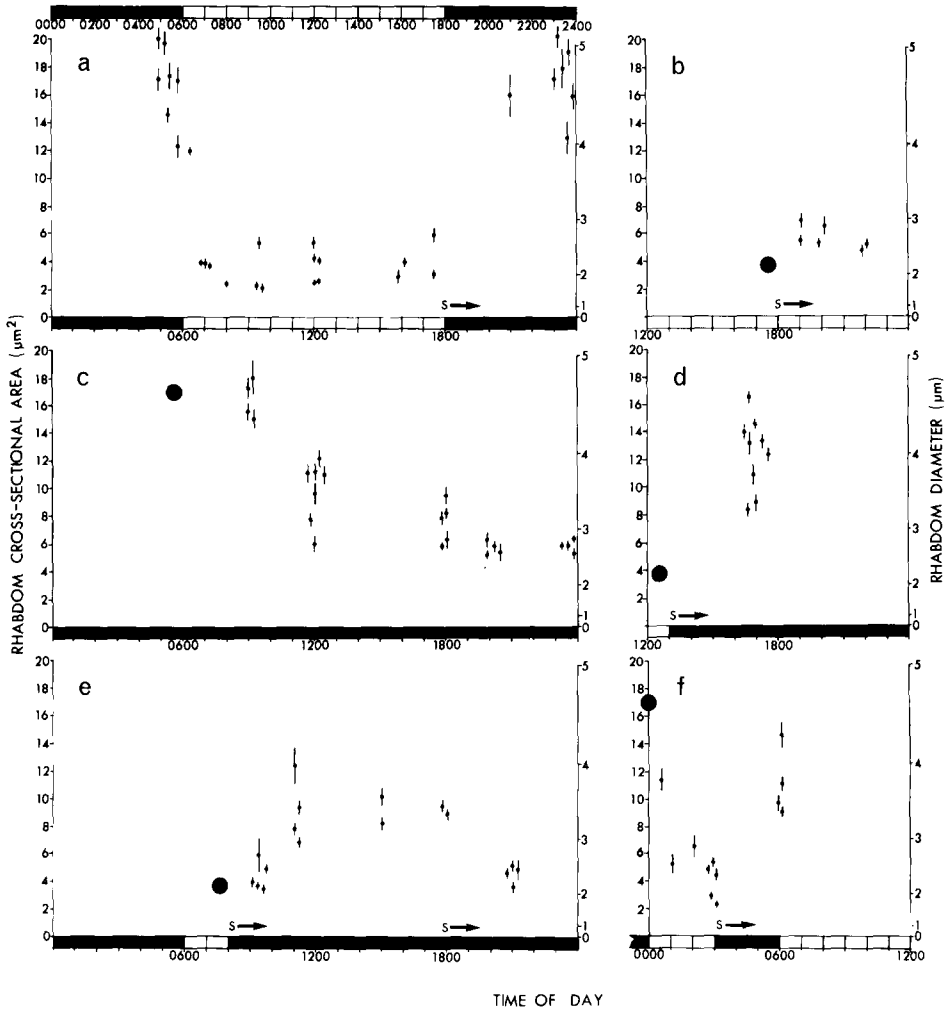


Fig. 8. Cross-sectional area (and diameter for circular shape) of rhabdoms in the central region of a compound eye in *Locusta*, in relation to time of day and lighting conditions. Each point represents the mean size of 15–30 rhabdoms from one animal. Vertical bars extend 2 standard errors of the mean. Large dots represent mean size on the normal cycle (**a**) before the start of the abnormal lighting. Microvillar assembly (Fig. 6) was observed at times indicated by S→. For at least 2 weeks prior to the day of fixation, animals were exposed to a 12 h light: 12 h dark cycle (uppermost). Lighting conditions on the experimental day (beginning midnight) were as follows: **a** normal cycle continued; **b** light continued into night-time; **c** darkness during the day; **d** "dusk" 5 h early; **e** "dusk" 10 h early; **f** 3 h light after midnight

size: 6.0 ± 0.79 (2 S.E.) μm^2) than the normal day-state ($p < 0.002$). Most rhabdoms in two eyes fixed at 20.00 h had one or two rhabdomeres in a disarrayed state (Fig. 9), indicative of assembly of new microvillar membrane. However, large numbers of coated pinocytotic pits and vesicles were present at the bases of the microvilli, and in and around the assembling receptor membrane, implying that much of the newly synthesised microvillar membrane was rapidly being shed, thus

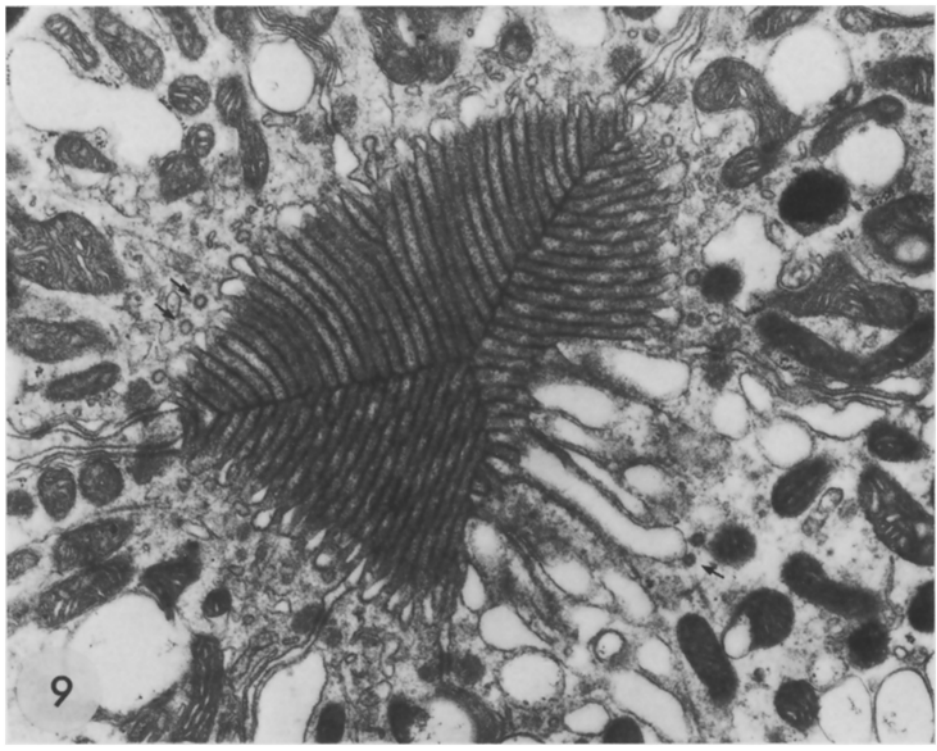


Fig. 9. Rhabdom of *Locusta* fixed after being kept in light 2 h after normal time of dusk. One rhabdomere is undergoing reassembly. Pinocytotic vesicles (e.g., *arrows*) are budding off from much of the rhabdom. × 25,000

maintaining a small rhabdom. A similar process has been described in a crab under this light regime (Stowe 1981).

Darkness continued after night (Fig. 8c). The sudden burst of photoreceptor membrane shedding at the time of "dawn" does not occur in the absence of light (Fig. 10), but eventually, in the dark, rhabdom size does decrease. By 12.00 h rhabdoms (mean size: 9.8 ± 1.67 (2 S.E.) μm^2) were found to be significantly smaller than at night ($p < 0.001$), but the distal triradiate configuration was not attained until 20.00 h. Occasional coated vesicles and secondary lysosomes, similar to those found in photoreceptor cells fixed after exposure to light at dawn, were observed in photoreceptors fixed on this regime. Hence, the rhabdom diminution that occurs during the day while locusts are held in darkness appears to be achieved by the same mechanism as normal dawn shedding, albeit on a very much smaller scale.

Darkness before dusk (Fig. 8d, e). Assembly of new photoreceptor microvilli occurs whenever a locust is placed in darkness during daytime for a few hours. The ultrastructure of rhabdoms 1–2 h after the onset of darkness on regime *d* or *e* was similar to that in normal cycle retinae immediately after "dusk", with many

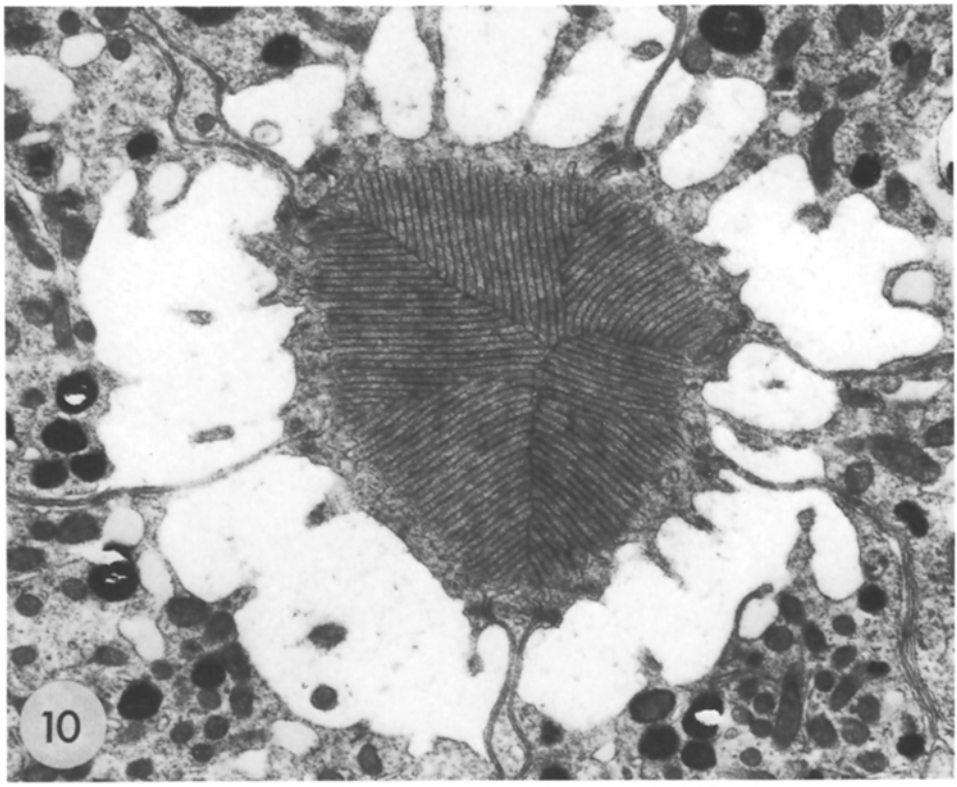


Fig. 10. Rhabdom of *Locusta* fixed after being held in darkness 6 h after normal time of dawn. ×15,600

rhabdomeres in the transitional disarrayed state. Nevertheless, a full night-state rhabdom did not result from microvillar assembly at these times. Rhabdoms fixed between 11.00 h and 18.00 h on regime *e* (put in darkness at 08.00 h) had lost their distal triradiate configuration but were significantly smaller (mean size: 9.25± 1.18 (2 S.E.) μm^2) than in the normal night-state ($p < 0.001$). The size of rhabdoms 3.5–4.5 h after being put in darkness at 13.00 h (mean 12.6± 1.78 (2 S.E.) μm^2) was also significantly smaller ($p < 0.002$).

Darkness before and beyond time of dusk (Fig. 8c, e). In two eyes of the four sampled at the normal time of dusk on regime *e*, rhabdoms (not measured) were found to be reassembling their microvilli. Yet rhabdoms measured after 18.00 h on this regime were smaller (mean size: 4.7± 0.74 (2 S.E.) μm^2) than those measured before 18.00 h (mean size: 9.25± 1.18 (2 S.E.) μm^2) ($p < 0.002$). No rhabdoms from the four eyes fixed on regime *c* at the normal time of dusk were found to be undergoing microvillar assembly, and rhabdoms fixed later still were of a size that could be expected from a continuation of the gradual diminution that had begun almost 12 h earlier (Fig. 8c). Assembly of new microvilli therefore appears to occur at the usual time of dusk on regime *e* but not *c*, and on regime *e* it results in rhabdoms that are *reduced* in size.

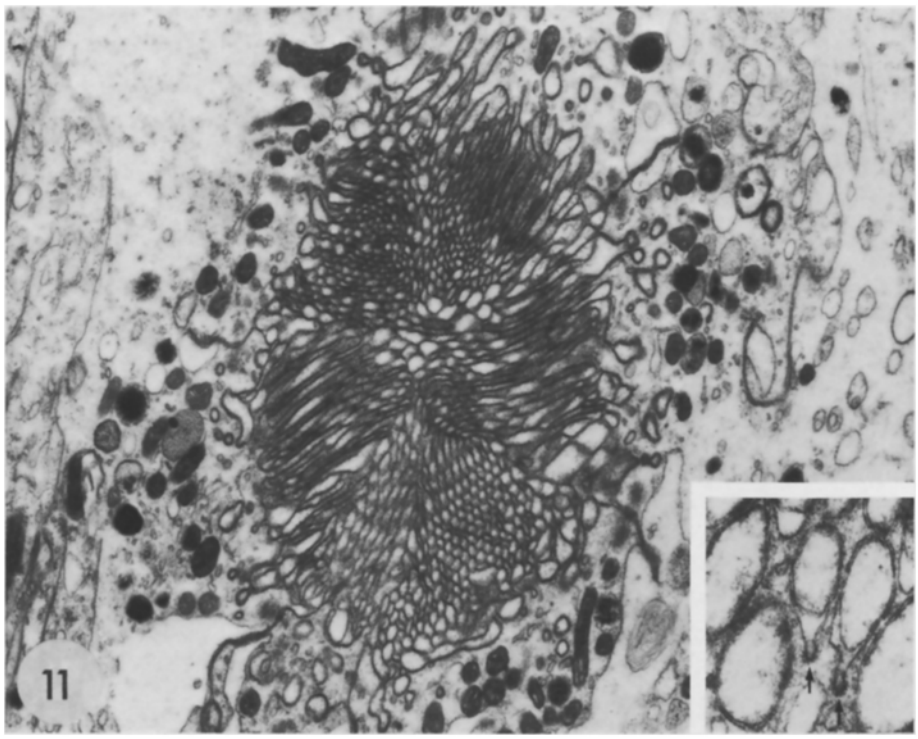


Fig. 11. Rhabdom of *Locusta* fixed after exposure to 0.5 h light at midnight. ×12,600. *Inset:* Higher magnification of coated pits (*arrows*) forming in loops of disordered membrane in a rhabdom after exposure to 2 h light at night. ×37,000

Light at night (Fig. 8f). When the same daylight-fluorescent lighting used for normal daytime (see Materials and methods) was switched on at midnight, eyes fixed between 00.30 h and 02.00 h had many rhabdoms with microvilli that had lost their normal orderly configuration (cf. Horridge et al. 1981) (Fig. 11). Particularly at their bases, membranes of adjacent microvilli collapsed together to form dilated "loops" of membrane. The presence of coated pits in these membranous loops (Fig. 11) suggest that membrane is being internalised by pinocytosis. About 3 h after the onset of light, the rhabdoms had assumed a configuration and size (mean: 3.9 ± 1.24 (2 S.E.) μm^2) that was indistinguishable from the normal day-state (size: $p = 0.7$).

After being returned to the dark for 3 h after 3 h of light, rhabdom size (mean: 11.3 ± 2.57 (2 S.E.) μm^2) had increased over that immediately prior to darkness, and the distal triradiate configuration was lost, showing that new membrane had been assembled. Rhabdom size was, however, significantly smaller than that of normal night-state ($p < 0.002$). Some rhabdoms had disarrayed rhabdomeres, indicating that they were still in the process of microvillar assembly.

Comparisons with Valanga

Rhabdoms from the central region of the compound eyes of 12 adults of another locust, *Valanga irregularis*, were examined according to regimes (as in Fig. 8): *a* (2

day-state, 2 night-state), *c* (2 at 09.00 h, 2 at 12.00 h), *d* (2 at 17.30 h), and *f* (2 at 03.00 h). In all cases rhabdom structure was similar to the corresponding *Locusta* rhabdoms, even approximately to the extent of absolute size of the rhabdom.

Discussion

Changes in ommatidial structure

Rhabdom structure. Even after 24 h of dark adaptation, Horridge and Barnard (1965) found the rhabdoms of *Locusta migratoria* that were reared in constant light to be the same size as those of light-adapted locusts, although they stated that there was a greater "variability of cross-sectional area at the 0.5 per cent level of significance". Horridge et al. (1981) found, however, that the rhabdoms of the locust, *Valanga,* are larger at night than during the day. The present results show that rhabdom size changes on a daily cycle in *Locusta* also (Fig. 8a). In addition, it has been shown that (1) rhabdomeral orientation changes; (2) the size of the ommatidial field-stop changes in coordination with rhabdom size; but (3) the diameter and intramembrane particle density of the microvilli are constant.

Effects on angular sensitivity. Unlike in the compound eyes of some other arthropods (e.g., the bug, *Lethocerus,* Walcott 1971; tipulid fly, *Ptilogyna,* Williams 1980a; *Limulus* ventral eye, Behrens 1974 and Miller and Cawthon 1974), the distal end of the rhabdom in *Locusta* was not found to move a significant distance distally into an out-of-focus position at night. Therefore, given that the function of the dioptric system can be reasonably explained in terms of geometrical optics (McIntyre 1982), the number of photons impinging on the rhabdom from an extended field at a given time is directly dependent on the area of the field-stop. Since the areas of the pigment field-stop and the rhabdom appear to change in tandem, changes in the area of the field-stop are indicated by the more accurately determined changes in cross-sectional area of the rhabdom. The 4.7-fold increase in cross-sectional area of the rhabdoms at night therefore indicates a 4.7-fold increase in the number of photons entering the rhabdom from an extended source. Because rhodopsin concentration is inferred to remain constant between day and night (see below), the proportion of captured photons absorbed is unchanged for a given rhodopsin/metarhodopsin equilibrium. Therefore, the photon absorption by a rhabdom from an extended source should be 4.7 times greater in a dark-adapted eye at night than in an eye dark-adapted during the day for 15–20 min: i.e., before the assembly of new membrane, but after the formation of the palisade (Horridge and Barnard 1965).

Because this increase in photon absorption is achieved by increasing the angular acceptance of the rhabdoms, it is at the expense of the spatial acuity of the eye. "Trading off" a fine visual angle to increase sensitivity, whether at the photoreceptor level or at a higher level (e.g., Barlow et al. 1957), is a common strategy of dark adaptation. Its advantage has been discussed by many authors (see Snyder 1979, for review).

Implications for photoreceptor membrane turnover. That some arthropods with constant-size rhabdoms appear to turn over photoreceptor membrane (PRM) (Blest and Maples 1979; Williams 1982), and indeed that most of all surface membranes turn over (Poste and Nicholson 1976), implies that the variable-size rhabdom has evolved from a basic cellular mechanism. To achieve a significant variation in size, however, a rhabdom must daily turn over a large proportion of its membrane, in addition to having the shedding and assembly phases of its turnover at least partially separated. Turnover of so much PRM would seem to be costly in terms of energy requirements for PRM synthesis and degradation. Blest (1978) considered that although *Dinopis* should benefit from an increase in visual sensitivity due to more PRM at night than during the day, this increase is small and the primary reason for turning over PRM so quickly was probably not in order to effect changes in sensitivity. The present results from *Locusta* support this viewpoint.

In *Locusta,* sensitivity is controlled by the field-stop of pigment and not rhabdom size. Consequently, it should make little optical difference if the larger night rhabdom was kept during the day: the smaller angular acceptance of the rhabdom would still be obtained by the narrowing of the field-stop. This argument also holds for many other insects (e.g., *Lethocerus,* Walcott 1971; *Ptilogyna,* Williams 1980a), many crustaceans (e.g., Stowe 1980a) and *Limulus* (Behrens 1974; Miller and Cawthon 1974), all of which are capable of stopping down light entering their rhabdoms from an extended field by several log units with screening pigment, and yet still have variable-size rhabdoms. The variable-size rhabdom, with its large daily amount of turnover, therefore, must have resulted from some demand other than that of light/dark adaptation. Perhaps, it is more economical for variable-size rhabdoms to turn over large amounts of PRM each day than to maintain PRM that is not essential to the performing eye. The rapid turnover of variable-size rhabdoms contrasts with the comparatively slow turnover and constant size (cf. Basinger et al. 1976) of the rod outer segments of vertebrates: the life-span of rod outer segment discs is 10 days in rats and mice and considerably longer in amphibians and goldfish (Young 1967; for review, see Kinney and Fisher 1978). The daily rate of bulk turnover of PRM in constant-size rhabdoms is not known (cf. Williams 1982).

Effects on polarisation sensitivity. The change in orientation of the dichroic microvilli in the distal rhabdom between day and night states should change the vector of polarization sensitivity of a photoreceptor cell by up to 15°. This change has particular relevance to a current theory on electrical coupling between locust photoreceptors. When recording intracellularly from a receptor, Lillywhite (1978) found very small discrete depolarisations in membrane potential, which he called "small bumps" and attributed to single photon absorptions by a receptor neighbouring the impaled cell. Lillywhite hypothesized that these photon absorptions were recorded in the impaled cell because neighbouring photoreceptors are naturally electrically coupled. In support he reported that polarisation sensitivity curves for small bumps matched what would be expected for responses from neighbouring cells, based on the triradiate rhabdom configuration. However, his locusts were dark-adapted for several hours in the afternoon, and therefore probably had night-state rhabdoms, lacking the distal triradiate configuration.

In view of the present findings, the question of interreceptor coupling in the locust retina should be re-examined.

Constancy of rhodopsin concentration

It is now considered that the intramembrane particles on the P-face of photoreceptor microvilli probably represent mostly rhodopsin molecules, or at least aggregates of the photopigment molecule (see discussions by Fernandez and Nickel 1976; Nickel and Menzel 1976). Accordingly, because the intramembrane particle size and density in the freeze-fracture replicas of day- and night-state locust rhabdoms are the same, it can be concluded that the rhodopsin concentration is probably unchanged between these two states. In the bull ant, Nickel and Menzel (1976) also found no change in intramembrane particle density between dark- and light-adapted rhabdoms.

Horridge and Blest (1980) noted that if rhodopsin was inserted into preformed membrane during synthesis of PRM, rhodopsin concentration could be manipulated according to day or night, and PRM would thus be enriched with rhodopsin during dark adaptation in order to increase photon absorption efficiency. Their prediction is not supported by the present results, or those of Nickel and Menzel (1976). The possibility of this mechanism of PRM synthesis and its exploitation in this manner for light/dark adaptation by some animals should not be ruled out, however. The rhabdoms of many arthropods, such as *Locusta,* are long (300 μm in adult *Locusta*), so that their absorption efficiency of light during the day is already extremely high at the maximally absorbed wavelength (cf. Kirschfeld 1969), and any increase in rhodopsin density could only significantly increase the absorption of photons whose wavelength is distant from peak wavelength (450–500 nm in *Locusta:* Lillywhite 1978). Unless this effect is important, it would be unlikely that, in arthropods with long rhabdoms, rhodopsin would be increased at night over that during the day, even if the means to do so were available.

Photoreceptor membrane turnover and its control

On a normal light cycle, microvillar formation in *Locusta* is discontinuous, occurring over only a few hours after dusk. Since no reduction in size was detected in the day- or night-state rhabdoms once they were established, by basal rate of shedding must be low. Accordingly, microvillar assembly and shedding are practically completely separated, and a change in rhabdom size usually indicates the occurrence of one of these two phases of PRM turnover. This situation differs from that in the larval mosquito eye, where rhabdom size is the result of a certain balance between shedding, which ceases only in darkness, and continual assembly (White and Lord 1975; White et al. 1980).

Accompanied by observations on cytological changes that indicate when microvillar assembly was in process (Fig. 6), changes in the size of the locust rhabdom (Fig. 8) indicate the following: (1) Light induces shedding, and can elicit complete shedding even at night. Shedding also occurs slowly in darkness after the time of dawn. The rapid shedding that Horridge et al. (1981) believed to occur in locusts held in darkness throughout and after dawn has not been confirmed by the present study. (2) Microvillar assembly is triggered by the onset of darkness. It also

Table 2. Morning shedding in darkness

Animal	Time sampled (hours after "dawn")	% Normal shedding[a]	Reference
Dinopis (spider)	2	"none"	Blest (1978)
Limulus	not stated	"none"	Chamberlain and Barlow (1979)
Grapsus (crab)	4	47%	Fig. 6 in Nässel and Waterman (1979)
Procambarus (crayfish)	1	30%	Fig. 8 in Hafner et al. (1980)
Locusta (insect)	3	0%	Fig. 8c in present report
	6	44%	
	12	61%	
Rana (frog)	6 and 14	"none"	Basinger et al. (1976)
Xenopus (tadpole)	4	20%	Figs. 18, 22 in Besharse et al. (1977a)
	8	42%	
Albino rat	1–9	100%	LaVail (1976)

[a] Shedding determined in invertebrates by decrease in rhabdom diameter, and in vertebrates by increase in number of phagosomes from rod outer segments per unit length of the retinal pigment epithelium

occurs at the normal time of dusk without a change in ambient lighting, provided there has been some light during the day.

Shedding

On a normal light cycle, shedding of variable-size rhabdoms (Blest 1978) and rod outer segments (LaVail 1976; Basinger et al. 1976) peaks at dawn. Now it is recognized that light is essential for full dawn shedding of rhabdoms in representatives from all major groups of arthropods, and of rod outer segments in amphibians but not rats (Table 2). Endogenous effects on shedding are apparent, however. The shedding that occurs in darkness during daytime (Table 2) probably results from an endogenous stimulus. Even in frogs, where no spontaneous shedding is evident during one day without light, after constant darkness of several days some outer segments eventually shed (Basinger et al. 1976). In *Limulus*, efferent nervous input of the retina has been identified as a source of endogenous stimulus. Although light is essential for dawn shedding, the efferent input must also be intact (Chamberlain and Barlow 1979). In rats, rod outer segment shedding follows a circadian rhythm (LaVail 1980). Horridge et al. (1981) emphasised the endogenous component of shedding in the locust, *Valanga;* however, their conclusion seems to be based on a misinterpretation. Their figure (Fig. 1 D), which they state shows a rhabdom spontaneously shedding after being kept in darkness for 4 h past the normal time of dawn, depicts entire microvilli that are dilated and somewhat disarrayed, and thus is not typical of a locust rhabdom undergoing normal membrane shedding; in fact, it appears more like a rhabdom assembling new microvilli (cf. Fig. 6a). The four *Valanga* eyes and the *Locusta* eyes (Fig. 8c) that were sampled in the present study after they had been held in darkness throughout and after dawn, had their microvilli in the same ordered configuration as in the normal night-state (Fig. 10). In partial agreement with Horridge et al., it

was found that some shedding does occur in locusts during the morning in darkness (Fig. 8c; Table 2); but there was no indication that this shedding was achieved by any other method than a smaller and more protracted version of the normal shedding mechanism, involving pinocytosis from the bases of the microvilli. A second observation, reported by Horridge et al. to show endogenous control of shedding, was that rhabdoms exposed to light at night remained larger than normal day-state rhabdoms. In the present study, no significant difference was found between the size of rhabdoms after 3 h under the same light intensity during the day or night in *Locusta* (Fig. 8f) ($p = 0.7$) or *Valanga*. Nevertheless, changes in rhabdom structure induced by light at night are quite distinct from those at "dawn" (cf. Figs. 5, 11). Similarly, in *Dinopis,* during the breakdown of PRM after exposure to light at night, normal internalisation by pinocytosis does not take place (Blest 1980).

Therefore, shedding of PRM in locusts, as in many animals, is induced by light, but there is also clearly some endogenous control.

Microvillar assembly

Assembly of new PRM in mosquitoes (White and Lord 1975; Brammer et al. 1978) and amphibians (Besharse et al. 1977b) proceeds more gradually than in a locust. In locusts this process is similar to that in the crab, *Leptograpsus,* where it is obviously discontinuous, and involves the dissolution of the existing rhabdom, followed by assembly of entirely new microvilli (Stowe 1980b). A major distinction between the crab and locust appears to be that new membrane forms directly from the endoplasmic reticulum in crab (Stowe 1980b), whereas in the locust, the Golgi apparatus is implicated as the immediate precursor of microvillar membrane (own unpublished observations).

Microvillar assembly occurred at 18.00 h without any change in lighting and in near constant temperature and humidity, when there had been some light during the day: it occurred on regime *b* (constant light throughout dusk) and *e* (2 h light from dawn followed by darkness), but not on *c* (darkness all day). That assembly is initiated at the normal time of dusk under these conditions indicates that it is under some endogenous control, although the strongest evidence for this view would involve showing a circadian nature (cf. Brady 1974).

As in crabs (Nässel and Waterman 1979; Stowe 1981) and *Dinopis* (Blest 1978), the assembly of new microvilli was found to be triggered by the premature onset of darkness, but a full-sized night rhabdom was not achieved if the locust was exposed to darkness much earlier than the normal time of dusk. Stowe (1981) noted in *Leptograpsus* that the smaller rhabdom resulting from an "early dusk" did not seem to be due to an inability to carry out any particular synthetic step, and was likely to be the result of a shortage of material(s). At 21.00 h on regime *e* (when the eye had been in darkness since morning), rhabdom size (from a preliminary sample) was reduced from that before 18.00 h ($p < 0.002$) apparently by the assembly of new microvilli. This reduction could result from a reassembly program involving the disposal of the existing rhabdom and assembly of a new one (cf. Stowe 1980b), combined with a low supply of materials for the assembly of new microvilli at the time of dusk: microvillar assembly had already occurred once since the previous dusk.

In conclusion, initiation of microvillar assembly, like that of shedding, is influenced by ambient lighting and an element of endogenous control. In addition, the extent of assembly appears to be dependent on a prior build-up of materials.

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