On the effect of darkness on the fine structure of the phalangid eye

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In common with that of other arthropods, the phalangid eye, a uni-lenticular ocellus, shows a microvillar arrangement of photoreceptor membranes in the rhabdoms (Fig. 1.). The latter are cylindrical structures at the centre of each retinula, which comprises one central and three peripheral cells. The central cell has three peripheral processes alongside the rhabdom and a central process projecting up the base of the rhabdom. The retinulae pack in a near-hexagonal array to form the retina, in which they are directed towards the dioptric apparatus consisting of a cuticular lens surmounting a glassy body of transparent, columnar lentigen cells. Ultrastructural features of the phalangid eye in the light adapted state have been described by Curtis (1969; 1970)

The general organisation of the retinulae of *Oligolophus agrestis* (Meade) is similar to that seen in other phalangids, such as *Mitopus morio* (Fabricius) (Fig. 1). Retinula cell features include a proximal nucleus, electron dense pigment granules and large clear vesicles or vacuoles (0.2–0.8μm dia.), mitochondria (1–2μm long, 150nm dia.) as well as dense bodies, multilamellate bodies and occasional multivesicular bodies. Golgi complexes occur only in the proximal cytoplasm and have lamellae 1–2μm long with associated vesicles of diameter 30–50nm. The rhabdoms are roughly cylindrical and about 30μm long. The microvilli constituting the rhabdoms are near-cylindrical foldings of the retinula cell plasma membranes and have diameters of the order of 80nm. Their lengths are mostly in the region of 1.1–1.5μm. The microvilli are hexagonally packed roughly perpendicular to the rhabdom axis (Figs. 2A, B). Some regions of lamellae with intra-lamellar separations of 25–50nm occur at the edges of the rhabdoms and these membranes are continuous with those of the microvilli. The peri-rhabdomeric vesicles (50–80nm dia.) which surround the rhabdom are usually few in number but are more plentiful in distal regions, particularly near the areas of rhabdom fusion. They are also abundant in the central process. Small tubules and lamellae are interspersed between the peri-rhabdomeric vesicles and all these structures form a complex of membranes surrounding the rhabdom.

Features of the photoreceptor cells of *O. agrestis* which had been subjected to 24 hours darkness are reported here. The experimental animals were kept in a moist atmosphere in complete darkness for 24 hours and then the eyes were excised and quickly placed in fixative at room temperature. This was performed in dim red light from a Kodak Wratten No. 1 safelight filter. Light adapted eyes were also excised and fixed in identical solutions. Both light and dark adapted eyes were fixed for 1 hour in 1% osmium tetroxide; the fixed tissue blocks then acetone dehydrated, embedded in Araldite and sectioned on a LKB Ultrotome. The tissues were stained with uranyl acetate and lead citrate.

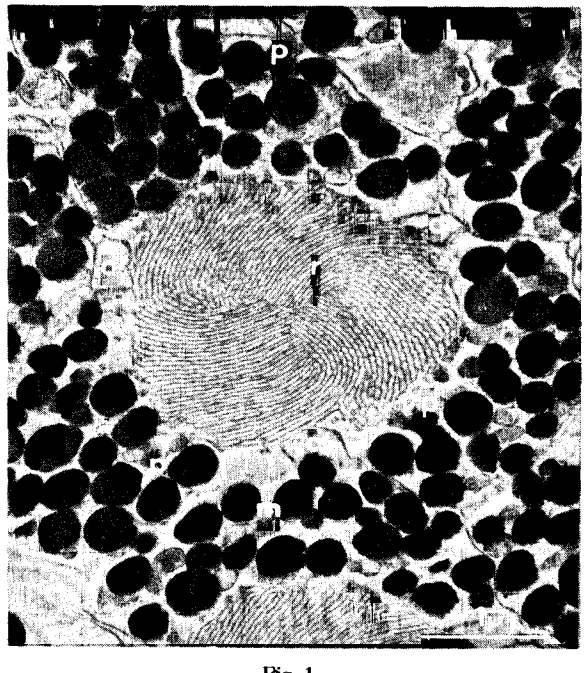

Fig. 1

Mitopus morio. T.S. retinula showing tightly packed microvilli in rhabdom (r). c: central cell process; p: peripheral cell; m: mitochondrion.

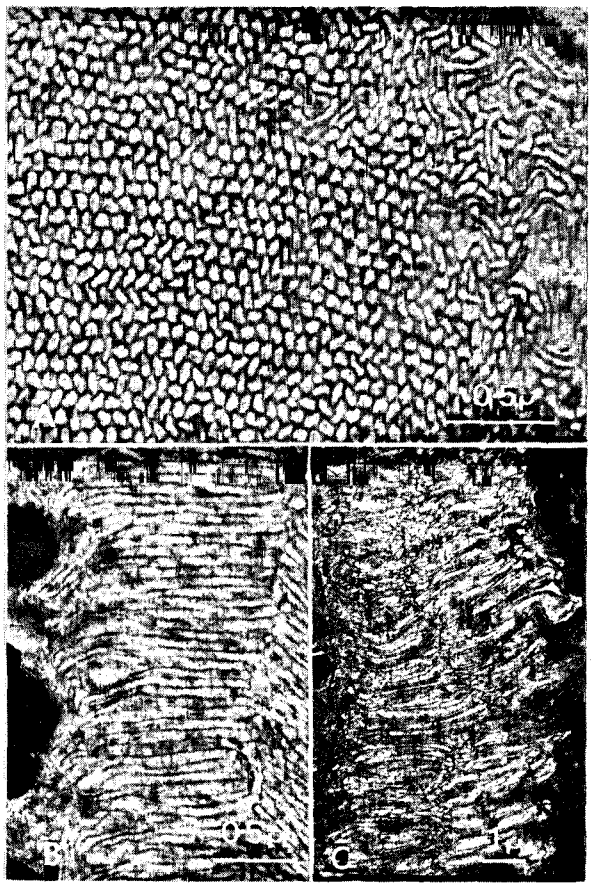

Fig. 2

Oligolophus agrestis. A: Light adapted microvilli in T.S. showing hexagonal array; some lamellar membranes near edge of rhabdom. B: Light adapted microvilli in L.S. showing orientation perpendicular to rhabdom axis. C: Dark adapted rhabdom, L.S. at low magnification, showing extensive lamellae etc. which replace the microvilli.

Observations

The most striking feature observed in the dark adapted eyes was the change in the organisation of the rhabdoms (Fig. 3). After 24 hours of darkness, the microvilli of many of the rhabdoms have broken down and the membrane system of the rhabdom incorporates large fields of lamellae (Figs. 2C, 4A). These appear as spirals or concentric circles in the transverse plane and roughly parallel profiles in the longitudinal plane. Some rhabdoms are almost completely disarrayed, others appear intact, while some show regions of lamellar organisation and regions of microvilli. In the latter rhabdoms, the concentric or spiral lamellar formations can be seen to correspond with the cross-sectional profiles of the microvilli, while the parallel lamellae correspond with longitudinally sectioned microvilli (Fig. 4A). The microvillar diameters are mainly of the order of 65nm, compared to 80nm in light adapted eyes.

A possible interpretation of this phenomenon is that the microvillar arrangement of the photoreceptor membrane is disturbed to produce lamellae which are still oriented in approximately the same way as the microvilli. The lamellae are curved, forming concentric or spiralled groups, and each is about 10–20nm wide. Their membranes are similar to those of the microvilli: two electron dense layers of about 2.5nm separated by a less dense zone of 2.5nm giving a total thickness of 7.5nm. In some areas of the

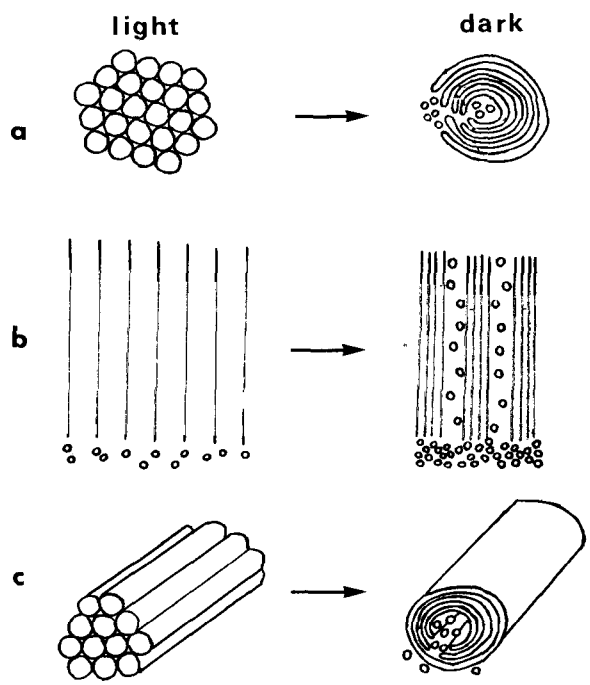

Fig. 3

Diagram of transition of photoreceptor membranes from light to dark adapted state. (a) T.S. hexagonally packed microvilli change to concentric membranes plus vesicles. (b) L.S. parallel profiles of microvillar membranes change to lamellar stacks and vesicles. (c) Stereogram of the change.

rhabdoms continuity was seen between the membranes of the microvilli and those of adjacent lamellae.

A certain amount of amorphous material is present in some rhabdomal regions. This material is fairly electron dense and tends to fill the spaces between the stacks of lamellae, though this may be clear in other areas of the rhabdoms. Large numbers of vesicles, 30–80nm dia., are associated with the lamellae. They are bound by a unit membrane similar in thickness to the membranes of the lamellae. The material constituting these lamellar and vesicular membranes may be derived from the membrane constituents of the disrupted microvilli. There are many more peri-rhabdomeric vesicles in the dark adapted retinula cells than in those of light adapted eyes. These are of similar size and appearance to the vesicles in the rhabdoms and their increase in numbers may possibly be correlated with the production of the vesicles along with the lamellae.

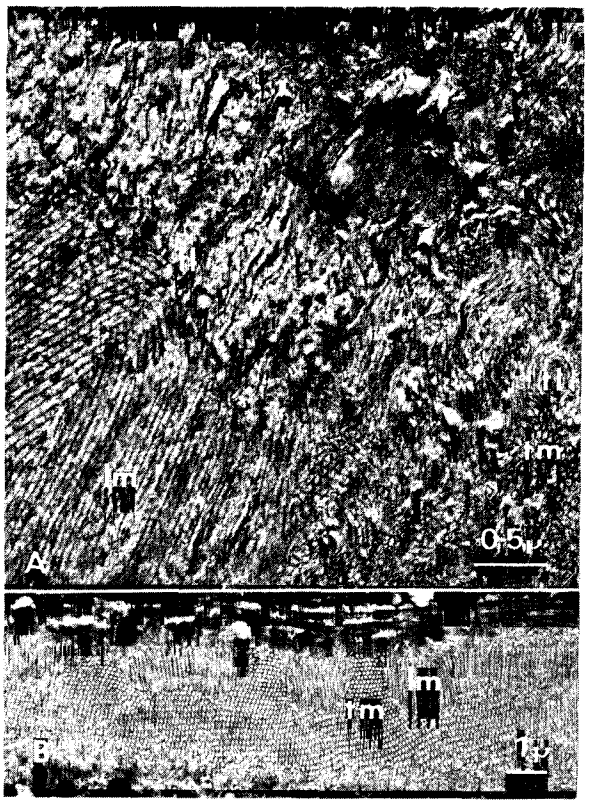

Fig. 4

A: *Oligolophus agrestis.* Field from dark adapted rhabdom which includes some intact microvilli. Parallel profiles of lamellae in L.S. (11) correspond with microvilli in L.S. (1m), while the spiral or concentric profiles of lamellae in T.S. (t1) correspond with microvilli in T.S. (tm). Dense amorphous material and vesicles (v) also present.

B: *Opilio parietinus.* Light adapted rhabdom in L.S. showing alternating, perpendicular stacks of parallel microvilli sectioned transversely (tm) and longitudinally (1m).

Discussion

Similar disruptive effects of darkness have been reported in other arthropod eyes. Eguchi (1965) and Eguchi & Waterman (1966) observed comparable changes in the eyes of several crustaceans including *Procambarus* and *Artemia.* In these the rhabdomal microvilli were disrupted after continuous darkness for 3 months. These changes were similar to the changes in the phalangid eye after only 24h. Horridge & Barnard (1965) observed collection of cisternae of the endoplasmic reticulum near the rhabdom of dark-adapted locust eyes, but no disruption of structure. Dilation of the microvilli in the rhabdoms of mosquito larvae reared in continual darkness has been reported by White (1967).

The fixative employed can effect the appearance of the rhabdoms. Kabuta, Tominaga & Kuwabara (1968) noted disruption of the rhabdom in several arthropods when fixed with osmic acid, but not with glutaraldehyde/osmic acid fixation. Röhlich & Török (1962) and Röhlich & Tar (1968) observed disruptive effects in the visual cells of flatworms only when osmium tetroxide alone was used for fixation and not when glutaraldehyde/osmic acid was used. They consider that these different responses to fixative are meaningful and indicate changes in the photoreceptor membrane. In the retinal clubs of the flatworms, vesicular material replaced the microvilli which were almost completely lost after 3 weeks darkness but recovered when returned to light conditions after 4 weeks in the dark.

Evidence from the effects of darkness on vertebrate photoreceptor membranes suggests alterations in the membrane structure and composition. Adams (1967) showed changes (quantitative rather than qualitative) in the lipids of frog retinal rod membranes; the lipids were more easily extracted

from dark-adapted eyes. Clark & Branton (1968) used freeze-etching techniques to demonstrate some structural changes in the membranes of outer segments of the guinea pig when dark adapted. They also observed altered structure under hypotonic conditions, suggesting that the membrane is effected by osmotic conditions.

This effect of osmotic changes may have some bearing on the changes reported here. Eguchi (1965) has also shown destruction of photoreceptor organisation by excess Ca^{++} and excess K^{+}. It is possible that the microvillar derangement in the phalangid eye in the dark could be an osmotic effect. Fixatives of different tonicity have been shown to influence the appearance of the rhabdomal membranes, producing whorled lamellae, vesicles and amorphous material (Curtis, 1968). In a period of darkness the tonicity of the medium surrounding the rhabdoms could possibly change to an extent deleterious to the microvilli. Alternatively, and perhaps more likely, the organisation of the microvillar membranes at a macromolecular level could change so that they lose their stability under the prevailing osmotic conditions.

A possible functional role might be served by the vesicles produced in the dark adapted rhabdom of *Oligolophus agrestis*. They are similar in size and appearance to peri-rhabdomeric vesicles and if they have similar histochemical properties, i.e. contain acetylcholinesterase (Curtis, 1969), they could serve as an amplifying system in the dark adapted eye. Stimulation of a photoreceptor membrane could have a response in the greater number of vesicles which are present and thus for a given small quantity of light a larger physiological response could be obtained. This could be of use to the predominantly nocturnal phalangids. However, the membranes of the microvilli do not exhibit acetylcholinesterase activity and so, if the vesicles of the dark adapted rhabdom are derived directly from the microvilli, it is unlikely that they will have the enzyme. More investigation of the time-course of membrane changes would be needed to study this possible mechanism. The eyes of some insects (as well as the human eye) have been shown to be more sensitive in the dark-adapted state than when light-adapted, but this increase in sensitivity is regarded as a neural effect (Cosens, 1966).

The short length of time required for rhabdom disruption is in contrast with the long periods of darkness required for the effects reported by the authors mentioned above. The microvilli represent a more ordered configuration of photoreceptor membrane than the dark adapted array, and even an orderly arrangement of microvilli into alternating, perpendicular layers of parallel microvilli has been observed in the eye of the phalangid, *Opilio parietinus* (De Geer) (Fig. 4B). Perhaps the layered arrangement of microvilli occurs under conditions of high light intensity and there could be changes in the rhabdomal membrane configuration from one extreme to the other. It is tempting to postulate a diurnal cycle of change in the rhabdomal membranes of the phalangid eye, in view of the apparent rapidity of disruption in the dark and it would be interesting to find out whether the fine structure of the photoreceptor cells conformed to a dynamic rhythm of changing structure.

Summary

The effects of 24h. complete darkness on the photoreceptor fine structure in *Oligolophus agrestis* include disruption of the rhabdomal microvilli which constitute the photoreceptor membranes.

The light adapted rhabdom contains hexagonally packed microvilli (80nm dia.) which are replaced in the dark by spiral or concentrically arranged cylindrical lamellae (10–20nm wide), vesicles and amorphous material. Some microvilli (mainly 65nm dia.) remain in the dark adapted rhabdoms. Peri-rhabdomeric vesicles (50–80nm dia.) lie around the rhabdoms and are greatly increased in number in the dark.

These changes in rhabdomal organisation on dark adaptation are not dissimilar to those which have been observed in other invertebrates, but they occur very much more rapidly in the phalangid eye. They could be the result of osmotic changes in the eyes, or of lability of the photoreceptor membranes.

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A fossil crab spider from Pliocene sediments in Western Alaska

A recent paper (Leech & Matthews, 1971) describes the fossil cymbium and palpal organ of a Thomisid spider, which is named *Xysticus archaeopalpus*. It was found in Alaska in peat beds which were overlaid by basalt during the Pliocene period and dating, by the potassium-argon method, to 5.7 million years ago. Yet this palp requires care to distinguish it from *X.britcheri* Gertsch inhabiting that region today!

Accompanying plant and insect fossils show that the environment in which the spider lived was a "shrubby opening within a forest dominated by *Picea* and *Betula*, but containing also *Pinus*, *Tsuga* and *Corylus*", quite different from the tundra occurring there today.

The authors raise the questions: was *X.archaeopalpus* the progenitor of *X.britcheri?* Were they both existing in the Pliocene? Has *X.archaeopalpus* become extinct or does it still exist thereabouts, yet to be rediscovered?

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