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A New Mechanism for Transitory, Local Endocytosis in Photoreceptors of a Spider, *Dinopis*

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Summary. Intermediate segment outgrowths (ISOs) are transitory specialisations of the plasma membrane of intermediate segments of the posterior median photoreceptors of *Dinopis*. Local regions form outgrowths into the glial partitions separating the receptors and remain connected to their parent intermediate segments by narrow necks. ISOs, only a few μm in diameter, are sites of intense endocytosis. Coated pits in their plasma membranes give rise to saccular internalisations. Unusual, slender, endocytotic tubules either pinch off coated vesicles, or become detached to yield a tubular detritus. Products of endocytosis are assembled to yield multivesicular and dense bodies, which are usually surrounded by smooth saccules derived from the endoplasmic reticulum of the intermediate segment. ISOs also contain arrays of tubules, thought to be stacks of haemocyanin molecules. There are usually at least 10 times the number of empty ISOs as full and active outgrowths. The number of active ISOs increases rapidly at dawn to peak at about 3 h after sunrise and then rapidly declines. The present sample suggests that the number of empty ISOs increases steadily throughout the day. Thus, ISOs turn over and are probably rapidly formed and short-lived. The contents of ISOs are observed to be evicted into the intermediate segments, where they presumably join the population of secondary lysosomes and are digested. The cyclical activity of ISOs is greatest after shedding of rhabdomeral membrane at dawn (Blest 1978). ISOs are thought to be concerned with the return to the receptors of a minor fraction of rhabdomeral material lost to extracellular space during shedding of the microvillar membrane.

Key words: Transitory membrane specialisations – Endocytosis – Photoreceptor membrane turnover – Diurnal cycle – Spider, *Dinopis*.

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Photoreceptor membrane in posterior median (pm) eyes of the spider *Dinopis* is destroyed in bulk at dawn (Blest 1978). Most of the membrane is shed within the receptors by endocytosis at the bases of the rhabdomeral microvilli in the manner originally described for ocelli of mosquito larvae by White (1967, 1968).

Nevertheless, some arthropod photoreceptors shed the greater part of their rhabdomeral membrane to the extracellular space during turnover (Blest and Maples 1979; Williams and Blest 1980), a mechanism also found in photoreceptors of a holothurian (Yamamoto and Yoshida 1978) and a seastar (Eakin and Brandenburger 1979).

Intermediate segments of *Dinopis* retinal receptors are swollen axons 22 μm in diameter and 150–250 μm in length. They contain the organelles that sustain the catabolism of internalised membrane after dawn, and the synthesis of new microvilli at dusk (Blest et al. 1978b). An intermediate segment is ensheathed by the processes of much-divided pigmented glial cells. Between these glial laminae lie follicles, 1–6 μm in diameter, that are outgrowths of the intermediate segments and connected to them each by a narrow neck (Blest 1980). In the present paper, we show that the follicles are specialised transitory compartments of the receptor axons presumed to be concerned with the endocytosis of materials from extracellular space. Their activity and frequency of incidence are correlated to some degree with the normal daily light cycle. They will be termed *I*ntermediate *S*egment *O*utgrowths (ISOs).

Materials and Methods

Methods of obtaining and handling *Dinopis* for electron microscopical examination of the retinae have been previously described (Blest 1978). Most of the material analysed in the present investigation was derived from Araldite-embedded blocks of conventionally fixed material prepared in 1977, and from a later study of acid phosphatase cytochemistry (Blest et al. 1979). The latter observations were repeated with especial reference to the ISOs, using technical improvements to the method found to be advantageous in another context (Blest et al. 1980). Observations were also made following a modified zinc iodide-osmium tetroxide (ZIO) impregnation (Blest et al. 1978b). All preparations were made from the posterior median retinae of adult female spiders, and all animals were maintained under a normal, environmental light regime. Silver-grey sections taken from Araldite-embedded blocks were stained with uranyl acetate and Reynold's lead citrate and examined in a JEOL JEM 100C electron microscope. For quantitative estimates of the frequencies of ISOs and their organelles at different times of the day, longitudinal sections of receptors were taken from the region around the retinal poles. Sections were made at 5–10 μm intervals, and ISOs and their contents scored as the numbers of ISOs in 50 complete longitudinal glial profiles, avoiding sections with *en face* profiles of glial partitions. Samples included most of the 24 h cycle.

Results

Anatomy of Intermediate Segment Outgrowths (ISOs)

An ISO consists of a saccular outgrowth of an intermediate segment that remains connected to its parent axon by a narrow neck (Figs. 1–3). Usually, an ISO is single, or two or three may lie close together. In some sections, but only infrequently and most often at a level immediately below the dense pigment layer, multilocular ISOs may be observed (Fig. 15); they are more loosely organized than single outgrowths,

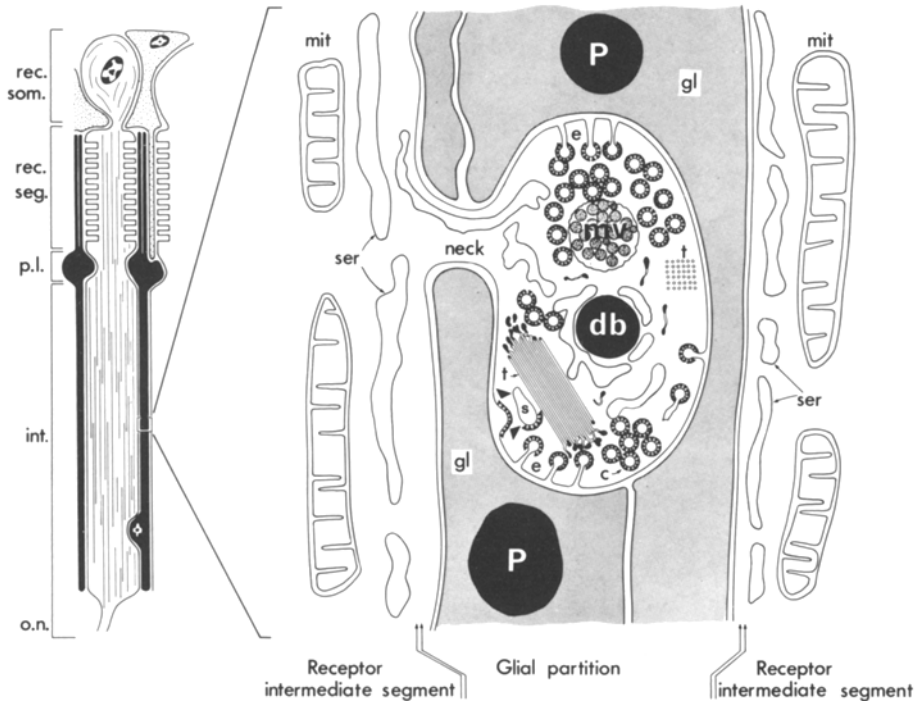


Fig. 1. Schematic representation of the anatomy of an intermediate segment outgrowth in a posterior median retina of *Dinopis*. *Left:* A single receptor. The soma (*rec. som.*) lies distally, nearest to the lens (not shown); behind it a receptive segment (*rec. seg.*) some 55 μm long contains rhabdomeral microvilli and is backed by a pigment layer (*p. l.*). An intermediate segment (*int.*) 250 μm in length narrows proximally to form an optic nerve fibre (*o.n.*). Glial partitions separating the receptor intermediate segments are shown in black. *Right:* Organisation of an intermediate segment outgrowth. The outgrowth lies in a partition composed of glial processes (*gl*) and is connected to its intermediate segment by a narrow neck. It contains tubules (*t*) possibly representing stacks of haemocyanin molecules, coated vesicles (*c*) derived from endocytotic tubules (*e*), a dense body (*db*) surrounded by saccules of smooth endoplasmic reticulum derived from those in the intermediate segment (*ser*) and a multivesicular body (*mv*). A single endocytotic pit is shown at the bottom left of the ISO (*arrowheads*) with an adjacent partially coated saccule(*s*); *mit* mitochondria; *P* glial pigment granule

and the compartments can be seen to communicate with each other. All ISOs lie within the much-divided glial sheets that ensheath the intermediate segments. A single ISO was observed to invade an adjacent intermediate segment, so that the two axonal membranes were apposed, but this relationship resulted from an abnormal absence of the glial partition that normally separates them. The maximum diameter of a single ISO is approximately 6 μm. Of the contents, only the dense and multivesicular bodies and possibly the tubular structures to be described below most likely stain intensely with toluidine blue, and all these organelles are of the same order of size as the pigment granules in the glial processes. This explains our inability to identify ISOs in semithin sections, and the fact that they can only be counted under the electron microscope. The narrowness of the necks of ISOs means that few profiles show the continuity between ISO and intermediate segment.

ISOs are found to be filled with a number of characteristic organelles, often at high density, or to be empty. Compartments appearing to be empty ISOs are greater in abundance than filled ISOs, but their continuity with intermediate segments has not been observed.

The organelles are summarised in Fig. 1:

(i) *Small multivesicular bodies* (Figs. 2, 3) are dense and quite distinct in appearance from the large bodies that result from rhabdomere breakdown at dawn (Blest 1978; Blest et al. 1978a). They appear to transform to:

(ii) *Dense bodies.* After conventional staining for electron microscopical examination, sections of these structures are highly electron dense (Figs. 2–6).

(iii) *Saccules of smooth endoplasmic reticulum* (Figs. 3, 5) that often fully or partially envelop profiles of the secondary lysosomes, can be seen within the neck of an ISO, suggesting that they are derived from the ER system in the main body of the intermediate segment (Fig. 2).

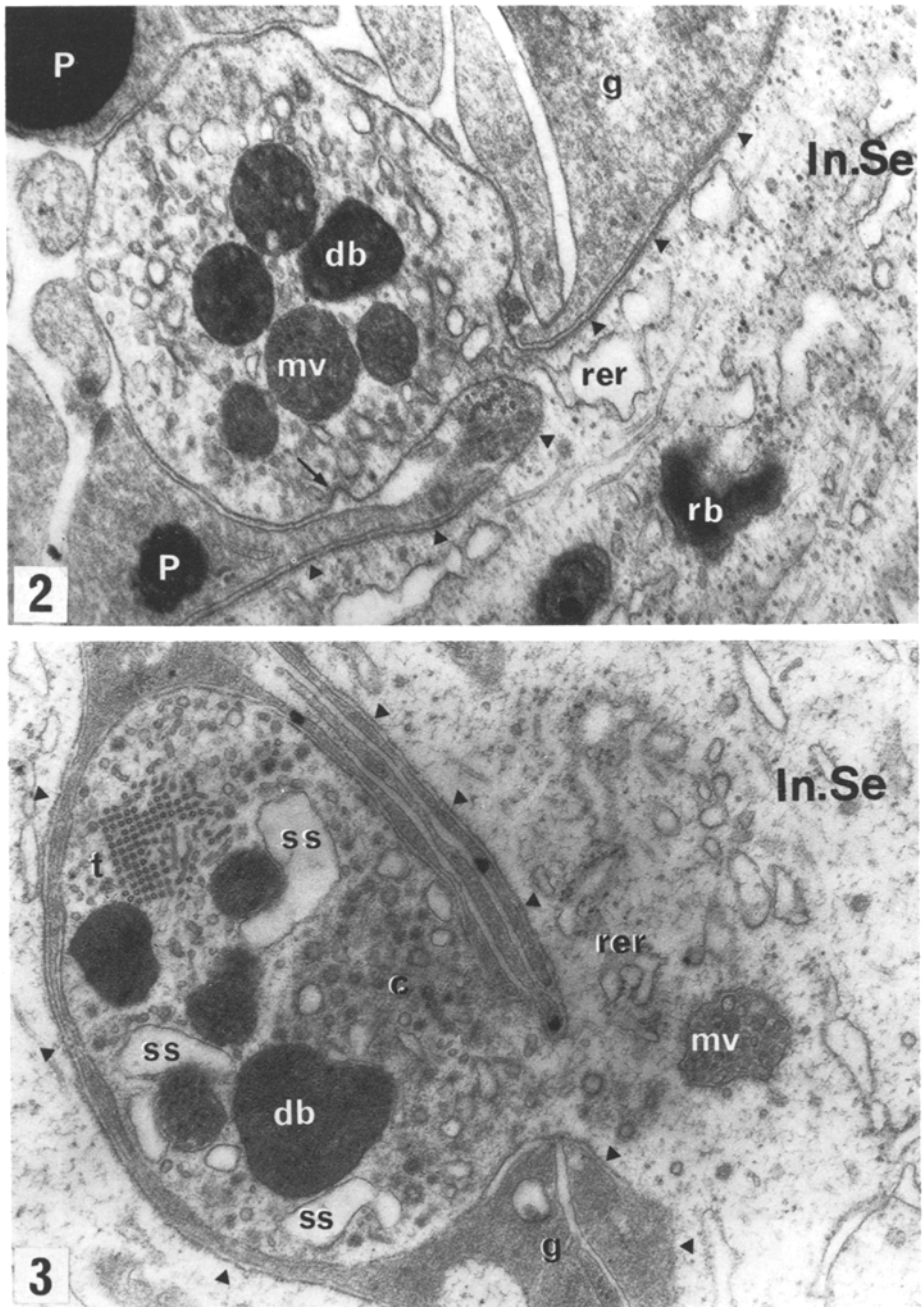
(iv) *Coated vesicles* (Figs. 3, 4) can occupy a large part of a profile of an ISO. They are of various sizes and some profiles can be best described as:

(v) *Coated saccules* (Figs. 4, 5). Large coated saccules and profiles that suggest vesicles of comparable dimensions can also pack the ISO.

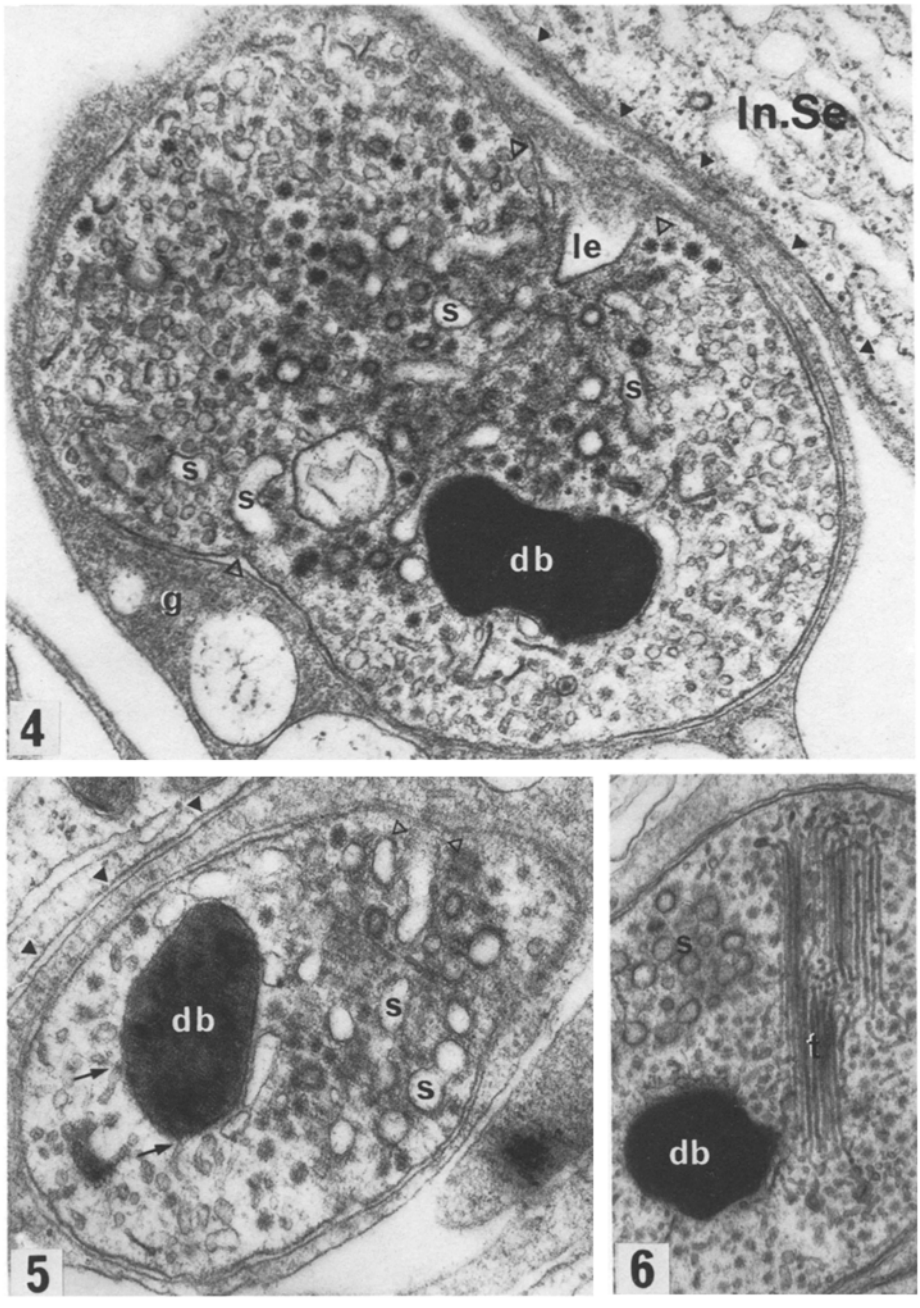
(vi) *Tubules.* Arrays or tangles of tubules of about the same diameter as the microtubules found in the intermediate segments and axons of the optic nerve are a common component of ISO profiles (Figs. 3, 7), and in some instances are seen to fill them completely (Fig. 11). That they are not composed of tubulin is shown by their consistently strong reaction to the ZIO procedure (Fig. 12) to which microtubules in the axons do not respond at all. In conventionally fixed material (either in cacodylate or PIPES buffer) they form arrays of parallel tubules that resemble the tight assemblies of tubules described by Schönenberger et al. (1980) in the compound eye of a crustacean and identified as haemocyanin by immunofluorescence: tubular diameters (within the range 175–200 Å) are consonant with those of *Limulus* haemocyanin (190 Å; Fahrenbach 1970). The arrays give the appearance of "fraying" at the ends of the tubules. Fragmented tubular material free in the ISOs (Fig. 6) and fusing with dense bodies (Fig. 5) is assumed to be derived from them. Although tubular fragments are often greater in width than the tubules in the arrays, they also respond strongly to the ZIO procedure, which often reveals close relationships between disintegrating arrays and adjacent multivesicular and dense bodies (Fig. 12). The ends of the tubules are not in association with polyribosomes, as seen by Fahrenbach (1970) in cyanoblasts of *Limulus,* so they are not being synthesised in situ. Whether all the tubular debris is derived from disintegrating arrays. or whether some of it represents separating haemocyanin in the process of aggregation is not clear.

After brief fixation (30–40 min in 2.5% glutaraldehyde in PIPES buffer at pH 7.2) and incubation during cytochemical procedures (acid hydrolase media with TRIS-maleate buffer at pH 5.0 and sucrose), the regular arrays of tubules often disassociate to some extent (Fig. 11) and become tangled. In control media at pH 5.0 they tend to bind lead, a reaction microtubules do not show.

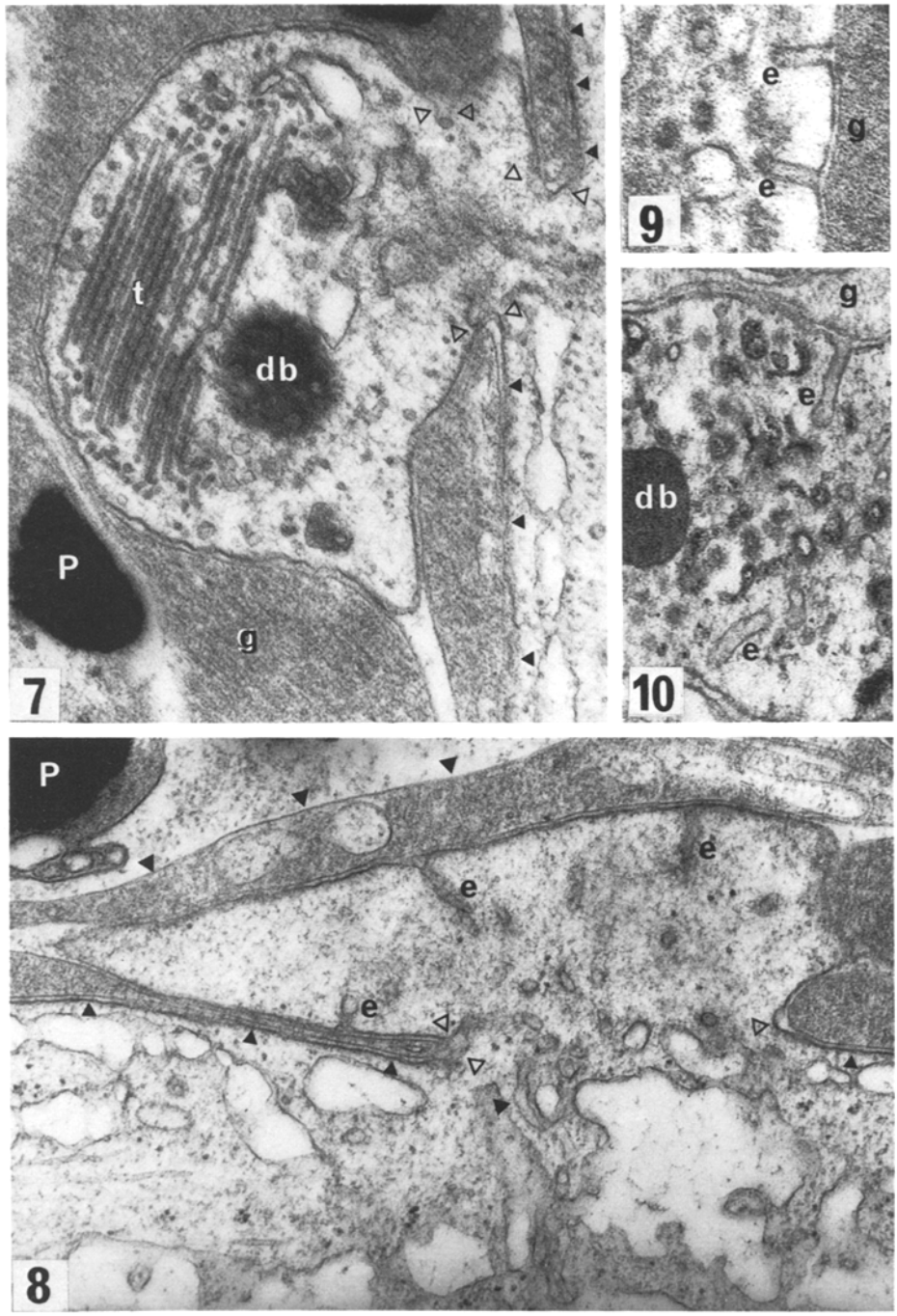
The contents of a given profile of an ISO can be diverse and the components are not always seen together in a single section. It is, therefore, not known with certainty whether all ISOs contain all components. To determine that this is the case



Figs. 2 and 3. ISOs sectioned to show necks and continuity with intermediate segments (*InSe*); *db* dense body; *mv* multivesicular body; *rer* rough endoplasmic reticulum; *t* array of tubules; *ss* smooth saccules; *c* coated vesicles; *g* glial process; *P* pigment granule; *rb* residual body. *Solid arrowheads* indicate plasma membranes of intermediate segments. *Arrow* (**Fig. 2**) indicates coated pit. Smooth saccules within the ISO shown in Fig. 2 seem to arise from saccules of rer passing through the neck of the outgrowth. × 42,000



Figs. 4–6. Typical profiles of ISOs to show density of contents. Coated saccules appear to be derived from large endocytotic pits and saccules (*le*) in continuity with the ISO plasma membrane (*open arrowheads*). There is also a large population of small coated vesicles, and (**Fig. 6**) a tubular array (*t*). *In. Se.* intermediate segment; *db* dense body; *s* coated saccules. *Solid arrowheads* indicate plasma membrane of the intermediate segments. *Arrows* (**Fig. 5**) indicate continuities between tubules and a dense body, interpreted as fusion. ×30,000



Figs. 7 and 8. ISOs, to show close association and possible continuities (*open arrowheads*) between ISO plasma membranes at the neck and endoplasmic reticulum of the intermediate segments. *Solid arrowheads* indicate the plasma membrane of the intermediate segment. Array of tubules in longitudinal section shown in Fig. 1. Fig. 7: ×50,000; Fig. 8: ×35,000

Fig. 9. Two endocytotic tubules. Acid phosphatase medium without substrate. Modified from Blest (1980). ×61,000

Fig. 10. Endocytotic tubules and ISO saccules after incubation with acid phosphatase medium (β-glycerophosphate). Note absence of reaction in tubules and dense body, lead deposits associated with saccules; *db* dense body; *e* endocytotic tubules; *g* glia; *P* pigment granule. ×65,000

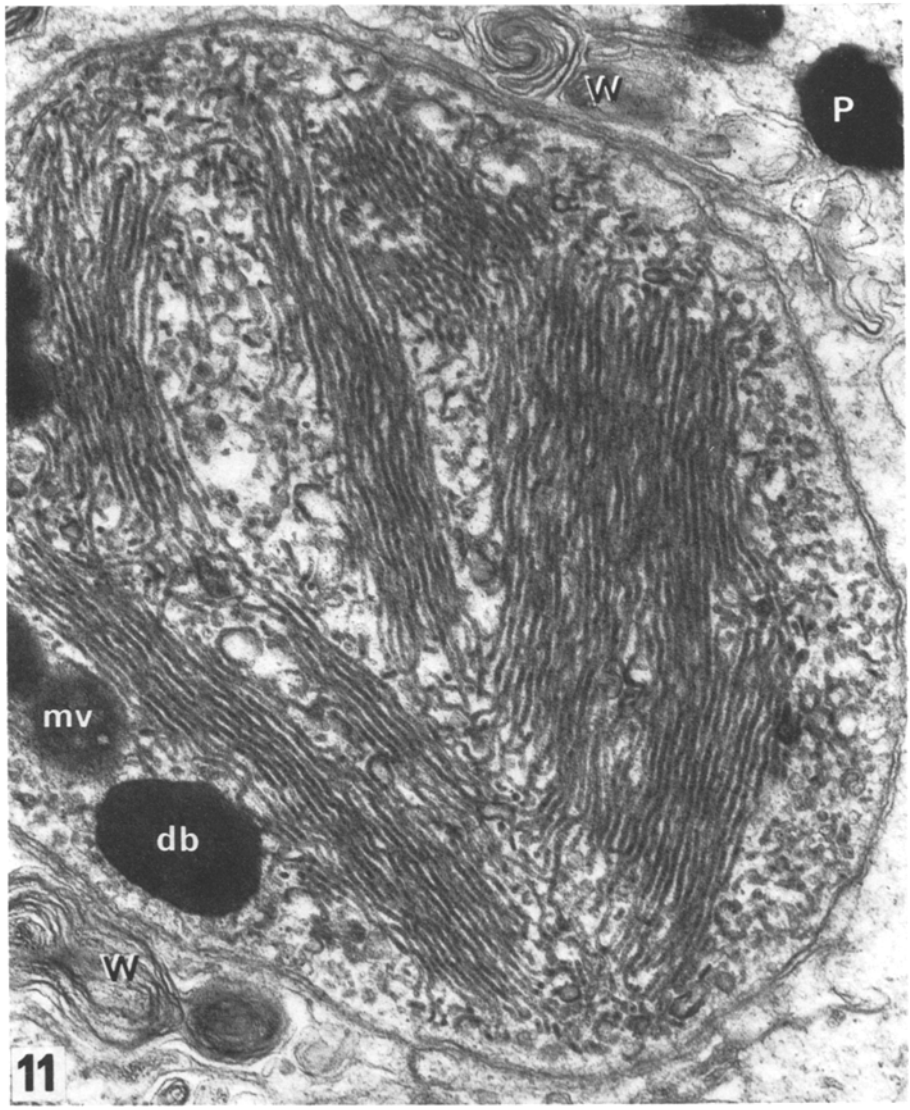
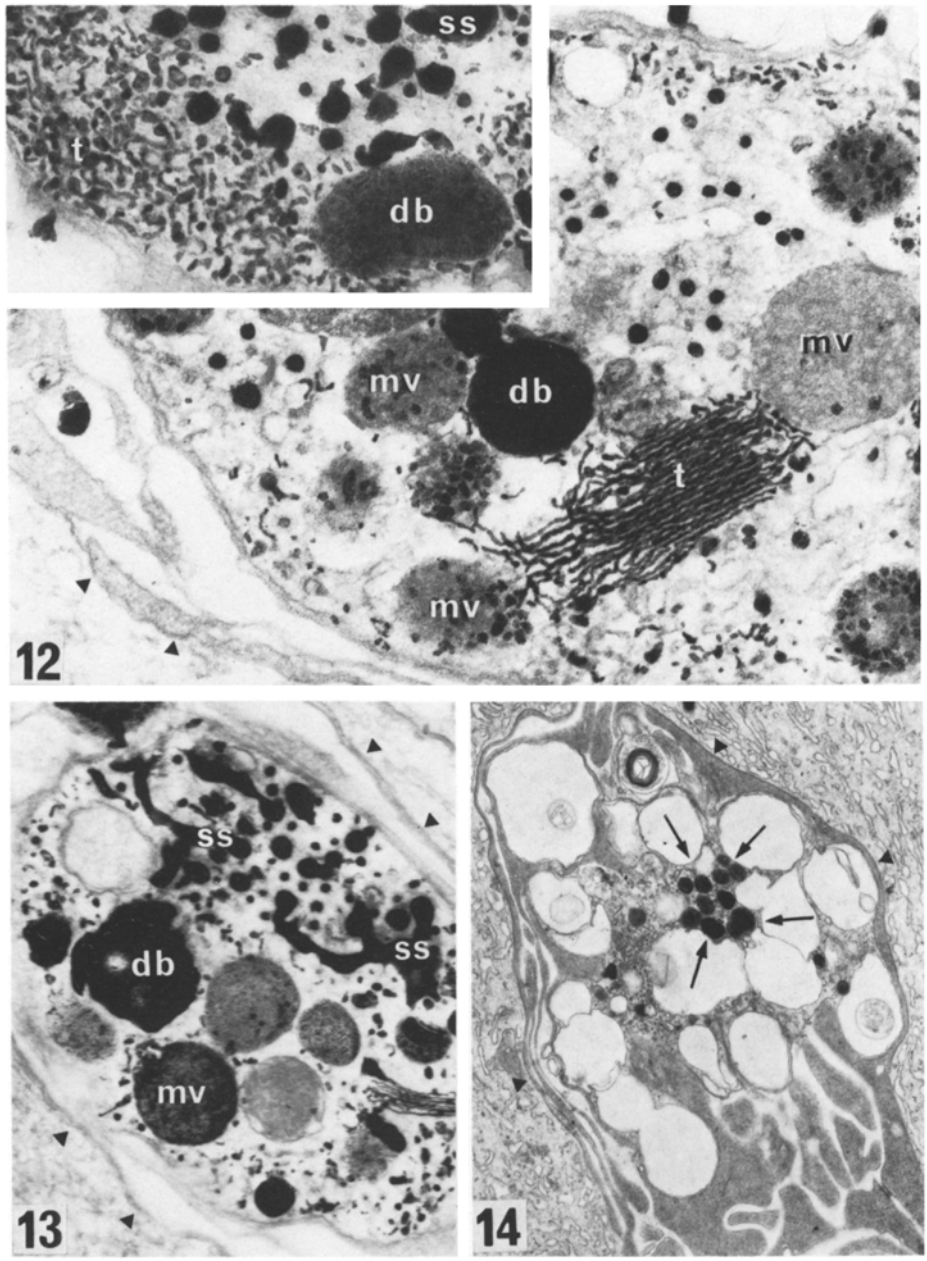


Fig. 11. ISO filled with arrays of tubules, after incubation in acid phosphatase medium without substrate; *db* dense body; *mv* multivesicular body; *P* pigment granule. Membrane whorls (*W*) in glial cells are an artifact of incubation. ×42,000

would necessitate the serial sectioning of ISOs, but their low density and the impossibility of identifying them in semithin sections preclude this approach.

With the exception of the smooth endoplasmic reticulum, the contents of ISOs are unlikely to be derived from adjacent axonal cytoplasm. Aggregates of coated vesicles ("*Nebenkerne*") derived from endoplasmic reticulum are found in the main axonal compartment, but their morphology and fates differ from those of the vesicles seen in the ISOs (Blest et al. 1978b). "*Nebenkerne*" (a) do not appear to



Figs. 12 and 13. ISOs after reaction with ZIO; *db* dense body; *mv* multivesicular body; *t* tubular array; *ss* smooth saccules. Note reactions in vesicles. *Solid arrowheads* indicate plasma membranes of the intermediate segments. **Fig. 12:** ×35,000; *insert* (*top left*) shows tubular debris possibly derived from tubular arrays. **Fig. 13:** ×20,000

Fig. 14. Glial partition to show profiles of empty, multilocular ISOs. Some compressed ISOs are still active, as indicated by the cluster of 10 dense bodies at the centre (*arrows*). ×7,300

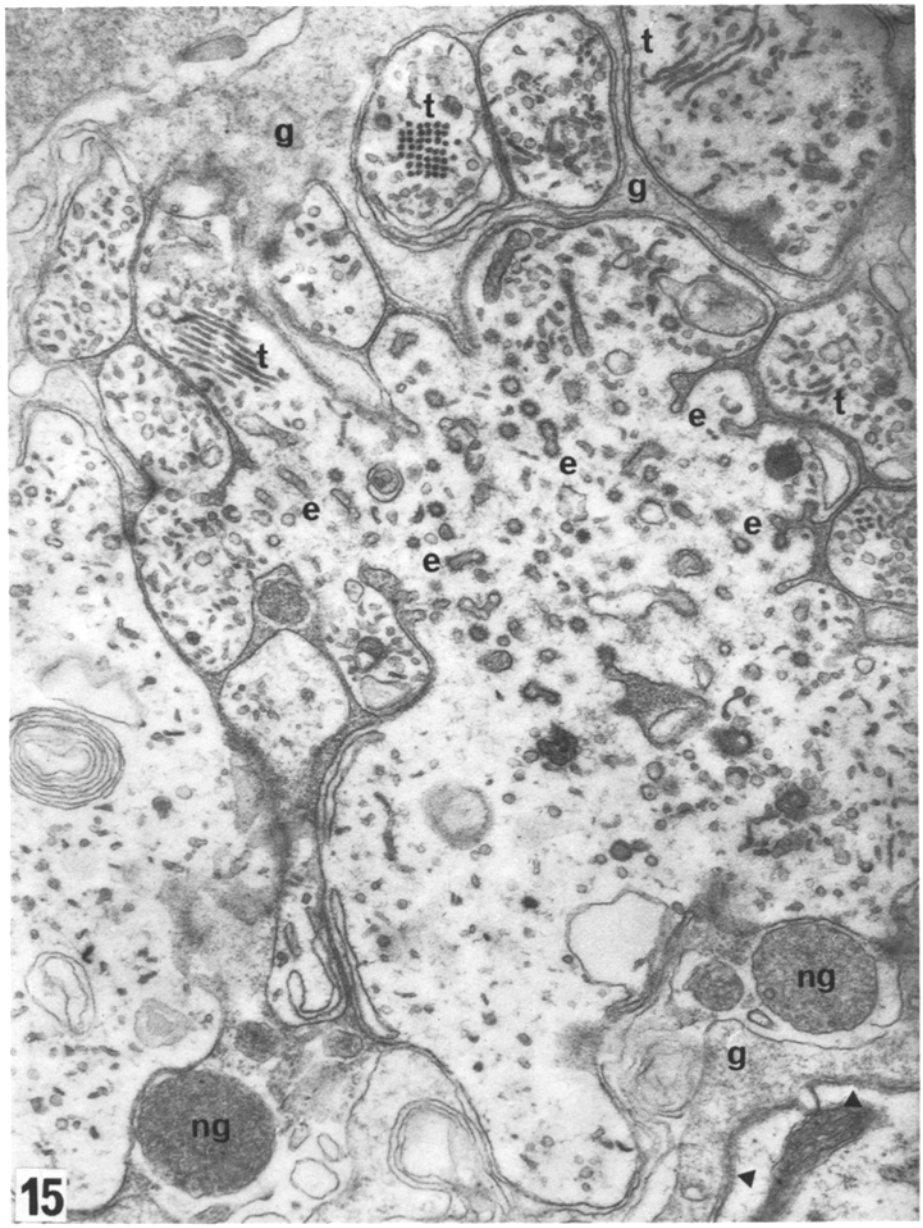


Fig. 15. Part of a large, multilocular ISO immediately below the pigment layer. Note active, small endocytotic systems (*e*) composed of tubules with coated tips, tubular arrays (*t*) and coated vesicles and tubular fragments in the cytoplasm; *g* glial partition derived from pigmented glia; *ng* strand of non-pigmented glia extending proximally from receptive layer. × 36,000

transform to multivesicular bodies; (b) fuse with residual bodies of much larger size and different appearance from those of the ISOs (Blest et al. 1978a); (c) are not seen migrating to the periphery of the intermediate segments. Further, the multivesicular bodies of the ISOs are morphologically distinct from those derived by pinocytosis from rhabdomeral microvilli (Blest 1978; Blest et al. 1978a). The plasma membrane of the intermediate segments does not show endocytotic activity except within the ISOs, where it is intense.

Two types of endocytotic structures are seen: (i) Narrow endocytotic tubules dilate at their tips and give rise to small coated vesicles by pinocytosis (Figs. 8–10, 15). Coats at the tips of the tubules are barely discernible in Figs. 8–10, but can be seen in the complexes of tubules and vesicles labelled "*e*" in Fig. 15. (ii) Endocytotic pits, sometimes extended in a way that suggests a larger version of the narrow tubules, give rise to the large coated saccules and saccular vesicles (Figs. 4, 5). The regularity with which structures of both kinds are seen implies that these internalising systems are in a constant state of activity during the life of each ISO. Lumina of endocytotic pits are electron-lucent (Fig. 4), and perhaps they merely transfer extracellular fluid to the ISOs. Coated vesicles, on the other hand, and the tubules from which they arise, often seem to have cores and lumina that are markedly electron-dense, suggesting that they sequester extracellular material for transfer to the photoreceptors.

Cyclical Behaviour of Intermediate Segment Outgrowths (ISOs)

The concentration and types of organelles within ISOs show that they are very active structures. At dusk, *Dinopis* synthesises rhabdomeral membrane and assembles it into microvilli rapidly over a period of 1–2 h; at dawn, nocturnal membrane is rapidly internalised and, later, lysed (Blest 1978). Blest and Day (1978) suggested that a proportion of rhabdomeral membrane is lost to the extracellular space during turnover by the spider *Dolomedes* and returned to the photoreceptor intermediate segments by transitory endocytotic clefts.

In order to assess the possibility that the ISOs of *Dinopis* serve a similar function, counts were made of full (active) and empty follicles throughout the daily cycle. The twenty blocks which were sectioned and scored (see Methods), each sampling one eye of a different spider, consisted of those remaining from the study made in 1977. Multilocular or closely adjacent ISOs were scored as single occurrences.

Fig. 16 shows that the number of active ISOs appears, within the limits of the sample, to follow the diurnal cycle. After dusk and in the early morning there are few ISOs, but in the 3 h after sunrise at 05.30 h their numbers mount, to decline to their former frequency by about 6 h after dawn.

The much greater population of empty ISOs follows a less well-defined course (Fig. 16). The data available suggest that the number of empty ISOs rises after dawn, but does not decline with the subsequent fall in the number of active ISOs, although it is implied that the number of empty ISOs must drop during the hours of darkness. The relationship between the numbers of active and empty ISOs is plotted separately in Fig. 16 as a double logarithmic scattergram, in order to display it more clearly.

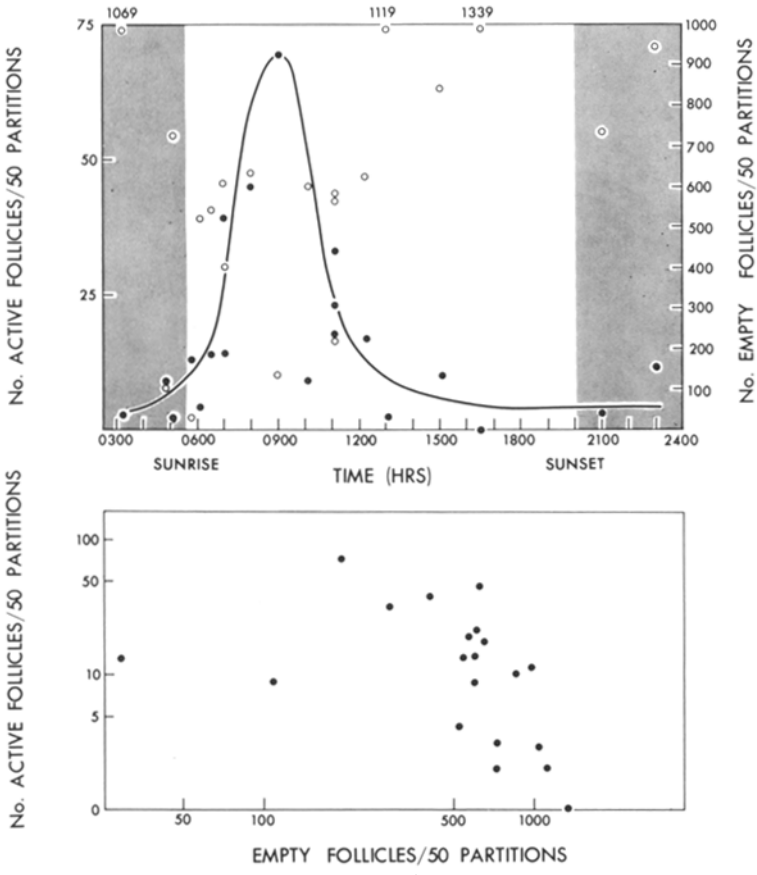


Fig. 16. *Top:* Relations between population levels of active ISOs (*solid points*) and empty ISOs (*open points*) and the daily light cycle, for 20 retinal poles, each from a different spider. Line for active ISOs drawn by eye. *Bottom:* Scattergram relating numbers of active and inactive ISOs, from the same data. Logarithmic scales are employed for both axes

Evidence for Lysosomal Systems in Intermediate Segment Outgrowths (ISOs)

Photoreceptors of *Dinopis* are not readily amenable to standard techniques for the localisation of acid hydrolases (Blest et al. 1979); while interpretable results can be obtained from the main compartments of the receptors, it has proven much more difficult to obtain results from ISOs. Of the component organelles, the arrays of tubules markedly bind lead from control media, but do not give any stronger reactions when incubated in media containing substrates (β-glycerophosphate and *p*-nitrophenylphosphate). Although endocytotic tubules and pits fail to respond, in favourable preparations, some reaction product is seen in the saccular structures and large vesicles that surround dense bodies (Fig. 10).

The response of ISOs to the ZIO procedure is striking; the arrays of tubules react intensely, and so do the smooth ER saccules surrounding the dense bodies and many of the coated vesicles and saccules (Figs. 12, 13). A proportion of the vesicles

within multivesicular bodies also respond, although the matrices do not, and dense bodies, which in normally prepared ISOs show no vesicular substructure, stain intensely. These strong reactions, typical of the contents of ISOs, are not shared by structures in the adjacent intermediate segment cytoplasm, save for the scattered reactions in the structures of GERL type described by Blest et al. (1978a). The latter paper reviews possible interpretations of ZIO reactions, and notes that there is evidence that they may correlate with sites where hydrolytic enzymes are in close association with lipid. Blest et al. (1979) showed that acid phosphatases can be localised at the appropriate sites, but that ZIO also stains clusters of vesicles derived by pinocytosis from the rhabdomeral microvilli. In the present context, it seems that vesicles and saccules derived by endocytosis from the ISO plasma membrane and the arrays of tubules also respond, and are unlikely to represent sites for primary lysosomal enzymes. Dense bodies and smooth ER saccules are plausible sites for hydrolases, but the erratic responsiveness of these compartments to techniques for identifying sites of acid phosphatase activity does not allow definite identification of a primary lysosomal system within the ISOs.

Discussion

The understanding of ISOs has been impeded by misinterpretation of their nature and of changes in the apparent pigmentation of the glial sheaths during the daily cycle. Blest (1978) showed that the pigmentation of the intermediate segments underwent cyclical changes, and attributed them to movement of the pigment granules in the glial investiture. ISOs were not described, because the narrow necks connecting them to their intermediate segments were not seen, and they were assumed to be a compartment of the glia. Furthermore, dense bodies are of the same size and electron density as glial pigment granules, adding to the confusion. Later (Blest 1980) it was suggested that the turnover pattern of ISOs might generate the changes in pigmentation, and the quantitative aspects of the present study test this possibility. The present data show that while ISOs undergo a marked daily cycle, the latter does not correlate with the changes in pigmentation; generally, intermediate segments are darkly and uniformly pigmented throughout the day, while after dusk a distal zone of variable extent becomes paler. ISOs, on the other hand, achieve their highest population density some 3 h after dawn and only a small number remain after midday.

Secondly, the peak of ISO activity is not concurrent with the peak of microvillar pinocytosis. Rhabdomere breakdown begins at sunrise (ca. 05.45 h) and is complete by 09.00 h (Blest 1978). The build-up of ISOs appears from the present small sample to peak at around 09.00 h, *after* the nocturnal rhabdom has been destroyed; it is not, therefore, merely an unusual extension of the rapid pinocytosis taking place in the receptive segments at dawn.

A Dynamic Model of Intermediate Segment Outgrowth (ISO) Turnover

How the functional role of the ISO can be interpreted depends on how effectively it internalises extracellular materials; this, in turn, will depend upon the rate of turnover of the ISOs themselves. At present, interpretation must be tentative, and

depends largely upon how the ratio between empty and full ISOs is regarded. Fig. 16 shows that there are usually, at all points in the daily cycle, at least 10 times the number of empty ISOs as there are full outgrowths. It is not certain that the number of empty ISOs undergoes a daily cycle, and this can only be tested by examining a larger sample than is currently available; the evidence suggests that empty ISOs accumulate during the day, and are removed before dawn. If they do accumulate in this manner, the disparity in numbers between full and empty outgrowths implies that an individual ISO forms rapidly, has a short life-span, and involutes just as quickly. Thus, the number of empty ISOs present late in the day may be the most meaningful indication of the number of outgrowths active in the course of the preceding period, and suggests populations in excess of 1000 outgrowths/50 profiles of glial partitions. In terms of this model, the ISO becomes a major mechanism of endocytosis by the photoreceptor, for an intermediate segment 250 μm long and 22 μm in diameter might be expected to deploy over 100 outgrowths. The ease with which the endocytotic mechanism can be observed in sections and the rich accumulation of endocytotic products favours the assumption that there is rapid growth and turnover of ISOs.

The ISO internalises very large areas of membrane, and the plasma membrane at the site of a forming ISO must balloon outwards as it grows. What is the source of this plasma membrane? Some possibilities can be excluded. The Golgi apparatus is poorly developed in *Dinopis* (Blest et al. 1978b); it is not seen to be active in the vicinity of ISOs, nor are coated vesicles derived from it seen outside the necks of ISOs at any stage. Rough and smooth endoplasmic reticulum is abundantly present in the intermediate segments near ISOs, and repeatedly, smooth saccules are seen passing through the necks of ISOs in a way that suggests that they give rise to the smooth saccules surrounding the dense bodies (Figs. 2, 3). The ISO plasma membrane often appears poorly defined at the neck of the outgrowth and seems to be in continuity with smooth ER membranes (Figs. 7, 8). In a crab, ER membrane has been shown to give rise to microvillar plasma membrane after a sequence of transformations, without passing through the Golgi compartment (Stowe 1980). Less detailed observations suggest that this is also true for photoreceptors of spiders (unpublished data). It would not be surprising, therefore, if addition of plasma membrane elsewhere in the photoreceptor proceeded in an analogous manner.

Since all coated vesicles and saccules appear to originate from within the ISO, it seems that the neck of the ISO is the most plausible site of membrane addition. Such a possibility requires that membrane is redistributed over the surface of the outgrowth by membrane flow, a mechanism that is also implicit in a recent model for the ultrarapid assembly of rhabdomeral microvilli (Stowe 1980).

The two mechanisms of endocytosis found within ISOs do not occur on the plasma membrane of the main compartment of the intermediate segment, so that mass endocytosis is confined to the outgrowths, the plasma membrane of which must be specialised for this purpose. Pfeiffer et al. (1980) and Walter et al. (1980) demonstrated that the distribution of coated pits on the surfaces of mouse macrophages in culture is not random. They are clustered, and after colchicine treatment, which induces lobular outgrowths of the cells, pits become concentrated on the surfaces of the outgrowths and at their necks. These authors propose a model implying that pits or their molecular precursors in the membrane move either by

diffusion or by membrane flow and are trapped through events generated by local configurations of the membrane. In the present case, it seems more likely that the plasma membrane synthesised and differentiated to yield an outgrowth is of different composition to that of the main compartment, and is specially designed to promote endocytosis driven by the formation of coated structures (cf. Heuser and Evans 1980, for a discussion).

What happens to the contents of ISOs when the outgrowths become empty? Occasionally, small multivesicular and dense bodies, and on one occasion an array of tubules, were seen in an intermediate segment, outside the dilated necks of outgrowths presumed to be voiding their contents. In the absence of a nearby ISO in the same plane of section, however, the non-tubular organelles would be difficult to distinguish reliably from small secondary lysosomes derived from the rhabdomeres. It is likely that some ISO contents are digested within the outgrowth, and others discharged to join the secondary lysosomal system of the intermediate segment (Blest et al. 1978a). Despite the very large number of observations of empty ISOs, at this stage they were never seen to be in continuity with the intermediate segments; presumably, they are pinched off after emptying, and perhaps digested at a later stage by the glia, although no evidence has been seen of this.

Function of Intermediate Segment Outgrowths (ISOs)

The function of ISOs is obscure. Blest and Day (1978) saw evidence of a minor extracellular route of turnover in a nocturnal spider *Dolomedes*. During membrane shedding, extracellular spaces filled with electron-dense material, and this appeared to be returned to the photoreceptors *via* large, transitory endocytotic clefts in the intermediate segments. In *Dinopis*, there is no obvious accumulation of breakdown products extracellularly, although some of the profiles of endocytotic tubules suggest that their lumina are filled with material. The anterior lateral eyes of some salticid spiders shed rhabdomeral membrane to the extracellular space, from which it is retrieved by glial processes. In the supposedly primitive *Lyssomanes,* however, compartments of the receptors resembling ISOs engage in rapid pinocytosis and fill with coated vesicles (Blest, in preparation).

Why spiders should have evolved so great a diversity of turnover mechanisms in their photoreceptors is not understood, nor is the significance of the arrays of tubules known. It is likely that they consist of stacks of haemocyanin molecules, by analogy with haemocyanin crystals in the cyanoblasts of *Limulus* (Fahrenbach 1970) and crustacea (Schönenberger et al. 1980), and they are not, therefore, likely to be concerned with the sequestration of components of the rhabdomeral membranes that are scarce and need to be conserved (Blest 1980). They are not synthesised within the ISOs, for the latter do not contain polyribosomes, and such large molecules cannot diffuse through the plasma membrane (Fernandez-Moran et al. 1966; Fahrenbach 1970). Haemocyanin could enter the ISO from the intermediate segment and aggregate under the influence of the local environment. It does not seem likely that it is derived from the extracellular fluid, because it could only reach the cytosol of the ISO if endocytosis and the assembly of multivesicular bodies were "leaky" processes. The inferred presence of haemocyanin within the

receptors is of interest, because it may relate to the respiratory transport problems of very large cells in which the pattern of membrane turnover must impose severe metabolic demands during the daily cycle.

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