TISSUE & CELL 1979 11 (3) 457 465
Published by Longman Group Ltd. Printed in Great Britain

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THE FINE STRUCTURE OF THE LIGHT ORGAN OF THE NEW ZEALAND GLOW-WORM *ARACHNOCAMPA LUMINOSA* (DIPTERA: MYCETOPHILIDAE)

ABSTRACT. The swollen distal tips of the Malpighian tubules of the glow-worm *Arachnocampa luminosa* constitute the light organ. The ventral and lateral surfaces are covered by a tracheal 'reflector' and the nervous supply to the light organ comes from the ganglion in the penultimate segment. Fine nerve terminals, axons, and glial cells can be seen in close proximity to the basal surface of the cells of the light organ. The epithelial cells of the light organ are large, the cytoplasm dense, homogeneous and acidophilic. The cytoplasm gives a strong positive reaction for protein. The cytoplasm contains a high density of free ribosomes, patches of dense material, smooth endoplasmic reticulum, glycogen and scattered microtubules. Mitochondria are numerous; they are large, randomly distributed and packed with fine cristae. These cells lack the features characteristic of Malpighian tubule epithelial cells; infolding of the apical and basal cell surfaces is reduced and the cytoplasm contains few organelles. These cells do not contain secretory or photocyte granules and the grainy cell matrix is thought to be the luciferin substrate. Oxygen is supplied via the tracheal layer (which may have secondary reflecting properties) and light production controlled by neurosecretory excitation either directly via synapses, or by hormones. There are no other reports of Malpighian tubules of insects producing light and the fine structure of these cells is distinct. Thus, the swollen distal tips of the Malpighian tubules of the glow-worm undoubtedly constitute a unique luminescent organ.

Introduction

THE New Zealand glow-worm *Arachnocampa luminosa* is the larva of a midge-like insect of the Mycetophilidae or fungus fly family (Order: Diptera) and is well known from the early descriptions of its habits and life history (Meyrick, 1886; Hudson, 1886, 1887). Edwards (1924) described the spectacular display by the glow-worm population in the Waitomo Caves as 'the radiance of such a massed body of glow-worms as cannot be found anywhere else in the world, utterly incalculable as to numbers and merging their individual lights in a nirvana of pure sheen'. Their taxonomy and behaviour have been treated in more recent works (Richards, 1960; Harrison, 1966; Stringer, 1967). As a result a reasonably good account of the habits and behaviour of the glow-worm now exists. A number of biological studies of this insect have been reported (Edwards, 1924; Harvey, 1952; Norris, 1970) and accounts of the anatomy and histology of the larva were presented by Gatenby (1959, 1960) and Ganguly (1960) respectively.

Wheeler and Williams (1915) were the first to identify the terminal ends of each of the four Malpighian tubules as being the source of the characteristic luminescent glow. They suggested that this in itself was of interest both morphologically and physiologically. There are no reports of the Malpighian tubules of insects other than *Arachnocampa* producing light.

The histology and ultrastructure of the

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Received 20 March 1979.

light organ of the glow-worm is described and compared with the well-documented system in the firefly and in another member of the Mycetophilidae, *Platyura fultoni*.

Methods

Larval specimens of *Arachnocampa luminosa* were collected from natural populations in limestone outcrops 135 km north of Auckland, New Zealand. For observations of gross anatomy larvae were dissected live in physiological saline. For routine histological examination, animals were fixed whole in Bouin's solution either immediately or a few hours after collection, and tissue was stored in 70% ethanol. Material was dehydrated in Cellosolve (ethylene glycol monoethyl ether) and embedded in ester wax (melting point 45–50 C) according to the method of Steedman (1960). Sections cut on a Cambridge Rocker microtome at 5–8 μm were stained with Mallory and Heidenhain and mounted in DPX on glass slides.

Additional material, fixed in 6% glutaraldehyde in phosphate buffer and embedded in Epon 812 for electron microscopy as described below, was examined for light microscopy after staining with Loeffer's methylene blue. Further sections of Epon embedded material were prepared for histochemical tests according to the method of Snodgress *et al.* (1932) and stained for protein using bromophenol blue (Humason, 1972).

Specimens of larval *A. luminosa* to be used for ultrastructural studies were dissected live in Insect Ringer and the Malpighian tubules removed, separated into their four distinct regions and fixed immediately in 6% glutaraldehyde in Millonig's phosphate buffer. After fixation for 1 hr, material was rinsed several times in buffer and post-fixed for 1 hr in 1% osmium tetroxide. Tissue was dehydrated in an acetone series and embedded in Epon 812. Sections were cut on a Reichert OM U2 microtome, picked up on uncoated copper grids, double stained with uranyl acetate and lead citrate and examined and photographed on a Philips EM 301 electron microscope.

Results

Morphology

As in other Diptera, there are four Malpighian tubules in *Arachnocampa luminosa*. Each tubule is divided into four morphologically distinct regions and each region is comprised of a different cell type. The structure and function of cell Types I, II, and III from Parts 1, 2, and 3 respectively is the subject of separate publications (in preparation). This paper deals only with Part 4, the light organ, and its Type IV cells.

In the anterior part of the terminal abdominal segment the Malpighian tubules increase in diameter and these swollen distal tips of the tubule (0·9 mm in length) form the light organ. Both the ventral and lateral surfaces of the light organ are covered by a layer formed from hypertrophied tissue of the tracheal epithelium (Fig. 1). The two main longitudinal tracheal branches enter this layer on the ventral surface, each dividing immediately into three smaller longitudinal branches. From these, fine terminal branches form a network of tracheoles encasing these swollen ends of the Malpighian tubules.

The nervous supply to the eighth abdominal segment containing the light organ comes from the ganglion in the penultimate segment. The nerve from this ganglion branches; one branch supplies the musculature, the other continues down to the region of the light organ where fine branches supply the scolophore organs of the anal papillae and the Malpighian tubules.

Histology

The epithelial cells lining the tubule of the light organ are large and cuboidal. The cytoplasm is dense, homogeneous and acidophilic and small spherical vacuoles can be seen near the inner margins of the cells. The cytoplasm gave a strong positive reaction for protein (bromophenol blue). Infolding of the basal cell membrane remains distinct although not extensive, and the luminal brush border of microvilli is greatly reduced. Nuclei are circular and small and the cytoplasm contains densely packed mitochondria (Figs. 1, 2).

Ultrastructure

The large cells of the light organ are approximately 250 μm long by 120 μm wide. Cells are between 25 and 30 μm deep and three or four cells normally line the tubule lumen. The lumen is large (60 μm diameter) and round.

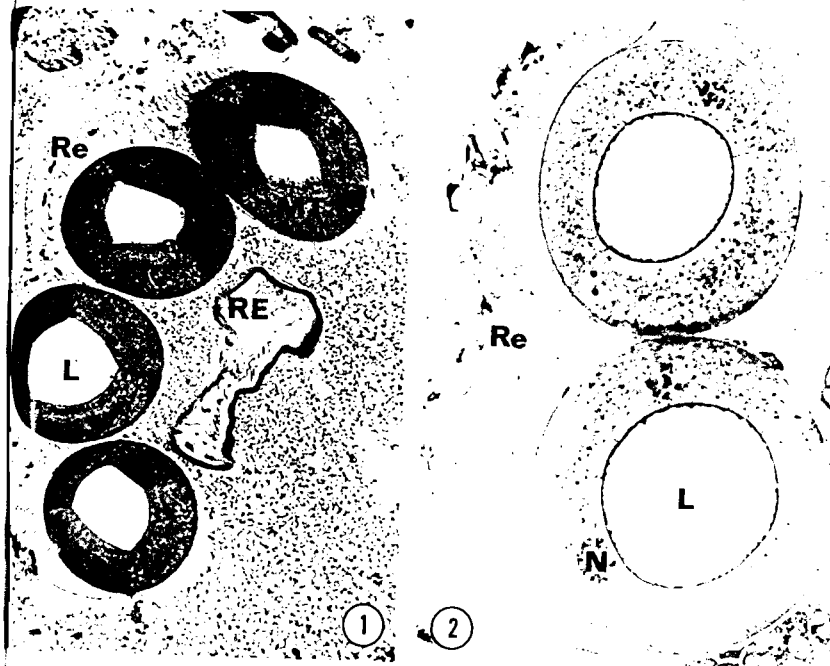


Fig. 1. Transverse section of Part 4, the light organ, of the glow-worm Malpighian tubule. A tracheal reflector (Re) covers the ventral surface of the tubules (MT). The tubule lumen (L) is large. The rectal epithelium (RE) is also shown. ×210. Bouin's fixed, wax embedded and Mallory–Heidenhain's stain.

Fig. 2. The cells of the light organ are large and normally only two or three cells line the tubule lumen (L). Infolding of the basal and apical plasma membranes is not extensive. Nuclei (N) are circular and small. The reflector (Re) covers the ventral surface of the tubules. ×500. Glutaraldehyde fixed, Epon embedded and methylene blue stain.

An extremely narrow basal lamina covers the outer surface of the tubule. The tracheal layer is closely apposed to the basal lamina on the ventral and lateral surfaces. Tracheal end cells are randomly distributed through the compact regular array of tracheoles that constitute the 'reflector' (Fig. 3). Although no intracellular tracheoles exist, the tracheoles are in very close apposition to the basal surface of the Malpighian tubule cells (Fig. 4). Fine nerves containing both opaque (diameter approximately 50 nm) and clear (diameter approximately 100 nm) vesicles presumed to be neurosecretory and neurotransmitter respectively (Figs. 5, 6), axons with neural tubules and mitochondria (Fig. 7), and glial cells (Fig. 8) are found flush with, or in proximity to, the basal cell surface. The haemocoelic space between the tubules is filled with an amorphous matrix (Fig. 8).

Type IV cells show a marked reduction in

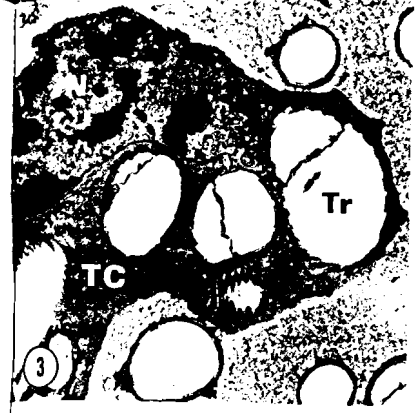
the extent of infolding of the basal membrane although the canalicular spaces still remain distinct. These extracellular channels are 45 nm wide, 3·0 μm long and extend across one-tenth of the cell depth. The cellular infoldings contain a grainy amorphous cytoplasm, few associated mitochondria and scattered pieces of smooth endoplasmic reticulum (Fig. 9).

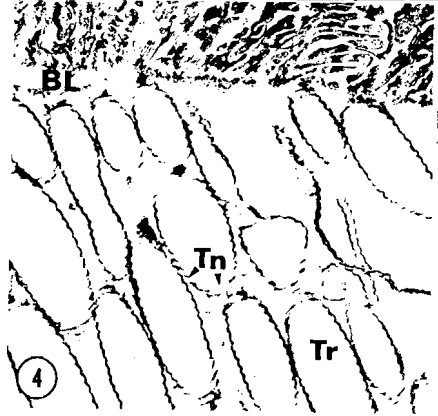
The cell cytoplasm is quite distinct, with few organelles. It is uniform in appearance, contains a high density of free ribosomes and patches of randomly distributed electron-dense, grainy material. Smooth endoplasmic reticulum, glycogen particles and scattered microtubules also occur in the cytoplasm (Figs. 10, 11). Spherical vacuoles are often seen near the inner margins of the cell. The absence of Golgi and rough endoplasmic reticulum is notable but the most striking feature of this cell type is the mitochondria. They are large, randomly distributed and packed with fine cristae (Fig. 10).

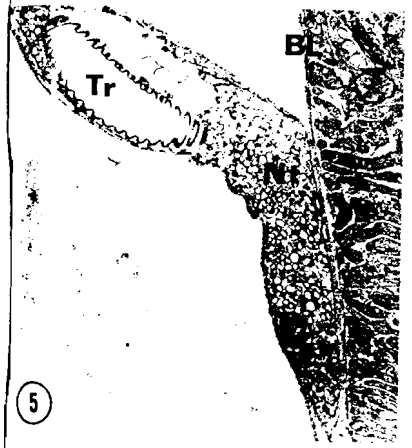
Microvilli are greatly reduced and form a short border on the luminal cell surface and constitute only one-twelth of the cell depth. They do not contain mitochondria (Figs. 11, 12).

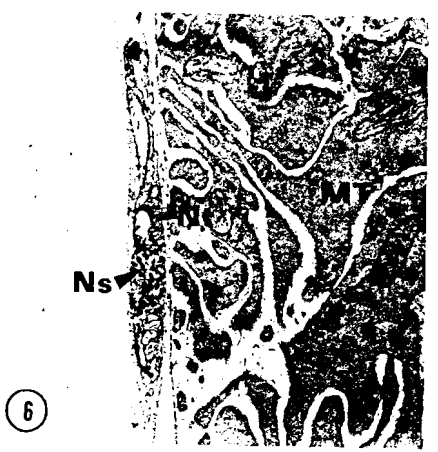
Discussion

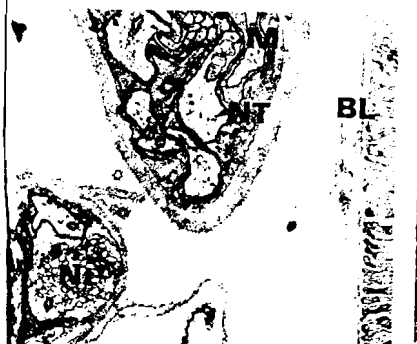
The phenomenon of bioluminescence has received considerable attention and there are various accounts of the origin and history of bioluminescence (Seliger, 1975; Harvey, 1952), and of the biochemical processes involved in light production in a wide range

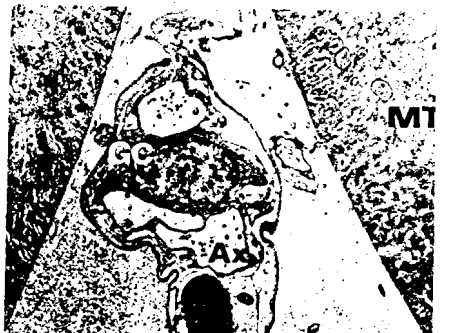












Figs. 3–8. The tracheal and nervous supply to the light organ.

Fig. 3. Tracheal end cells (TC) are randomly distributed throughout the tracheal reflector. A large nucleus (N) and numerous tracheoles (Tr) can be seen. × 11,200.

Fig. 4. The compact array of tracheoles constituting the ventral reflector is apposed to the basal surface (BL) of the Type IV cells of the light organ. Spiral taenidial thickenings (Tn) of the tracheoles (Tr) can be seen in tangential section. × 11,050.

Fig. 5. A nerve with neurotransmitter vesicles (Nt) and a small tracheole (Tr) can be seen flush with the basal surface (BL) of the tubule × 11,200.

Fig. 6. Fine nerve terminals containing both neurotransmitter (Nt) and dense core neurosecretory vesicles (Ns) can be found apposed to the basal surface of the Type IV cells (MT) of the light organ. × 20,850.

Fig. 7. In some nerves at the basal cell surface (BL), axons (Ax) containing mitochondria (M) and neural tubules (NT) can be resolved. Neurotransmitter vesicles (Nt) are prominent. × 13,100.

Fig. 8. Between the basal surfaces of two tubules (MT) a nerve containing a glial cell (GC) and axons (Ax) can be seen. × 2700.

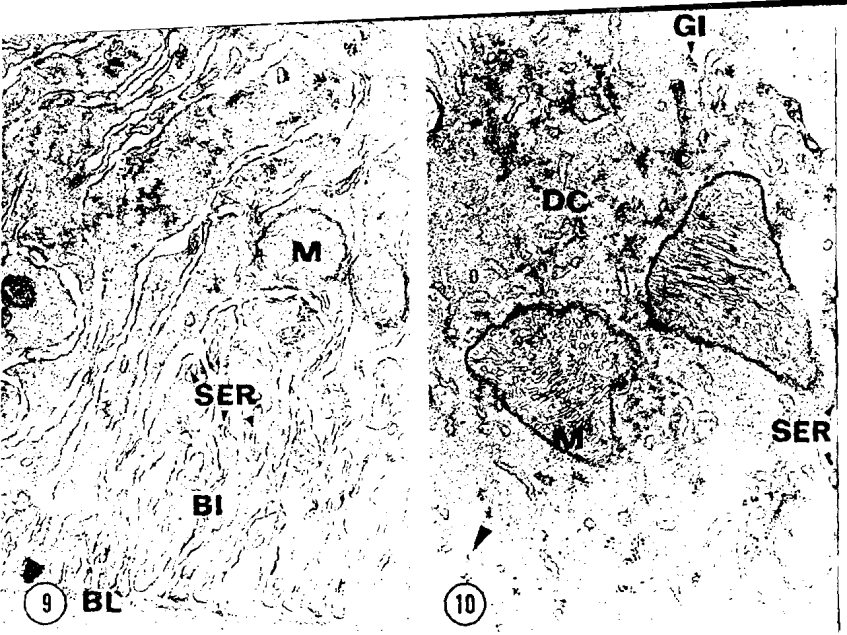
Figs. 9–12. Type IV cells of the light organ.

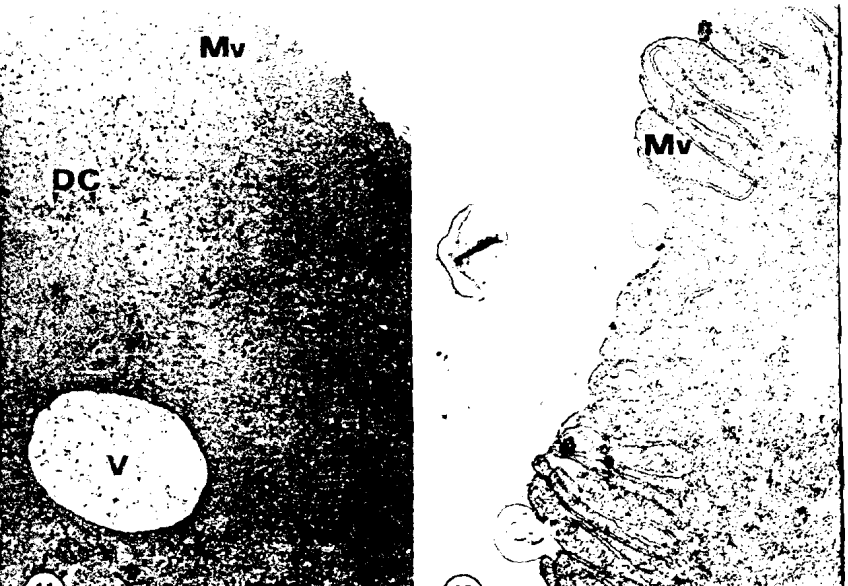
Fig. 9. The basal surface of the Type IV cells is covered by a thin undifferentiated basal lamina (BL). Infolding of the basal membrane (BI) is not extensive and the cytoplasmic areas contain a grainy matrix, few mitochondria (M) and smooth endoplasmic reticulum (SER). × 33,600.

Fig. 10. The cytoplasm of the Type IV cells of the light organ is grainy with denser patches (DC), scattered glycogen (GI), microtubules (arrow) and smooth endoplasmic reticulum (SER). Mitochondria (M) are large and have numerous fine cristae. × 33,600.

Fig. 11. The cell matrix is very grainy with denser patches (DC) and scattered glycogen particles (GI). Spherical vacuoles (V) can often be seen at the inner margin of the cells. Microvilli (Mv) form an extremely short luminal border. × 7400.

Fig. 12. Microvilli (Mv) are short, irregular in shape and do not contain mitochondria. × 18,700.





of different animals (reviewed by Seliger and McElroy, 1965, 1966). Among the Insecta, the only orders that are known to have bioluminescent members are the Coleoptera, Diptera, Collembola and Hemiptera (Harvey, 1952; McElroy *et al.*, 1974). The firefly, *Photuris pennsylvanica*, has attracted a great deal of attention and the biochemical processes involved in light production in this insect have been largely determined (reviewed by McElroy *et al.*, 1974). The firefly lantern is thought to originate from mesodermal fat body cells which differentiate to produce a bilayered organ consisting of a ventral light-producing epithelium, backed by a dorsal layer of cells (Harvey, 1952). A detailed account of the ultrastructural organization and innervation of the light organ of the adult firefly was presented by Smith (1963). The ultrastructure of the light organ of the larval firefly has been described by Peterson (1970). As in the adult, the dorsal reflecting layer contains urate granules, mitochondria and glycogen while the ventral photogenic layer contains the photocyte cells with their photocyte granules. The tracheolar organs, thought to control the flash of the adult light, are absent from the larva. Within the Mycetophilidae, the larva of *Platyura fultoni* is luminescent. Here, light is produced in close proximity to large 'black bodies'; these contain secretory granules, the extrusion of which is correlated with luminous activity (Bassot, 1978).

Bioluminescence in the glow-worm has been of interest since the first description of the larva by Meyrick (1886). Early reports on the bioluminescent processes in the New Zealand glow-worm were the result of investigations by Shimomura *et al.* (1966). The most recent work is that of Lee (1976) who investigated the biochemical processes involved in light production in the Australian glow-worm *Arachnocampa richardsae*, a close relative of *A. luminosa* (Harrison, 1961). Lee's results are consistent with those of McElroy *et al.* (1974) for the firefly, where the reaction is known to proceed by the action of ATP on the substrate luciferin in the presence of magnesium and luciferase. This is followed by oxidation to give light (Lee, 1976).

The acquisition of specialized functional roles by the Malpighian tubule cells of insects is not uncommon and the part they play in the storage of lime and production of silk has been noted (Wigglesworth, 1965). However, only in *Arachnocampa* are the Malpighian tubules involved in light production.

The swollen distal tips of the Malpighian tubules, which form the light organ, are comprised of an epithelial cell type markedly different from those of the other three regions of the tubule (in preparation). They lack the features characteristic of transporting epithelial cells; slight infolding of the apical and basal cell membrane occurs but the microvilli are very short and contain no mitochondria, and the basal infoldings are greatly reduced. In contrast to the firefly, these cells contain no photocyte granules. Secretory granules, as seen in the 'black bodies' of *Platyura* (Bassot, 1978), are also absent. Instead they contain large amounts of a very grainy cytoplasm with denser patches, smooth endoplasmic reticulum, glycogen and mitochondria with dense cristae. The dense grainy matrix of the Type IV cell cytoplasm stains positively for protein and at high magnification numerous free ribosomes can be resolved (their presence is normally masked by the density of the cytoplasm). These results suggest that in the glow-worm the grainy matrix may contain luciferin and perhaps be produced by the abundant free ribosomes. Luciferin is irreversibly expended during the production of light (McElroy *et al.*, 1974) and must be replenished. The mitochondria of the Type IV cells contain a large number of very fine cristae, in detail most resembling the mitochondria of mammalian heart cells described by Munn (1974). Mitochondria are the sites of ATP production, a reactant in the luminescent system, so their large size and developed cristae are indicative of an extremely active cell. The even distribution of these mitochondria throughout the cell supports the proposal that the grainy matrix in the cell cytoplasm may be the luciferin substrate. Glycogen in the cells (also seen in the firefly photocyte cells (Smith, 1963)) may also be metabolized for use in light production.

Although the precise location of the reactants of the light-producing system in the firefly have not been demonstrated, it has been suggested that the photocyte granules provide the luciferin substrate (Smith, 1963). In *P. pennsylvanica* the matrix of the dorsal region of the photocyte cells is similar to that

of the Type IV cells of the glow-worm light organ. The mitochondria of the Type IV cells are similar in appearance to the mitochondria around the intracellular tracheoles in the photocyte cells of the firefly. These features suggest that the Type IV cells of *A. luminosa* and the photocyte cells of the firefly have a similar function.

Oxygen is required for light production and the oxygen reaction rate limiting (Lee, 1976). Although there are no intracellular tracheoles in the glow-worm, a continuous direct supply of oxygen is assured to the system through the extensive ventral tracheal layer. As well as supplying oxygen, this tracheal cup may be a layer to reflect light, as does the tracheal tapetum of the insect eye. Horridge *et al.* (1972) found that light entering the rhabdom columns of the compound eye of the skipper butterfly is reflected back out of the rhabdom by the parallel regular cross bars (taenidial thickenings) of the trachea of the basal tapetum. He suggested that such an arrangement of trachea, if extremely regular, could reflect light of some wavelengths but not others. Although the characteristic blue-green light of the glow-worm is thought to be produced by luciferin-luciferase reaction (Lee, 1976), certain wavelengths could be intensified by selective reflection from the tracheal cup.

Fine nerves at the base of the cells contain both neurosecretory and transmitter vesicles. From the early work of Gatenby and Ganguly (1957) it is known that the glow-worm turns the light off through nervous stimulation. Light production may be controlled by secretion of substances from the nerve axons which are apposed to the basal lamina surrounding the tubule cells. The tracheal and nervous supply to the light organ of the glow-worm, however, does not appear as complex as that described for the firefly (Smith, 1963).

Malpighian tubule secretion is known to be controlled by hormones produced by neurosecretory cells. The site of hormone production differs between species. From the site of production hormones travel down axons to sites of storage and release, the neurohaemal areas. The release of hormone occurs in response to the arrival of a nerve impulse at the neurosecretory axon end. Hormone is normally released into the haemolymph. Thus, hormonal control of light production by the terminal ends of the glow-worm Malpighian tubules cannot be disregarded.

Thus, Type IV cells of the glow-worm Malpighian tubules have become highly modified for the secondary function of light production. They lack many of the features characteristic of transporting epithelial cells but exhibit cellular modifications consistent with their acquired role in light production.

Acknowledgements

The author wishes to thank Associate Professors S. Bullivant and P. R. Bergquist for interest throughout the course of this work and Dr T. Hughes for helpful criticism of the manuscript.

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