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Ultrastructure of the Larval Endocrine Glands of *Spodoptera litura* (Fabricius) *

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The ultrastructural features of the endocrine glands, viz., the median neurosecretory cells the corpora allata and the prothoracic glands, of the last instar *Spodoptera litura* (Fabricius) are described. The median neurosecretory cells show cyclical activity in synthesising and releasing the neurosecretion. The intermoult period is the most active stage characterised by the presence of large amount of secretory droplets (1100–2600 Å diam.), rough endoplasmic recticulum and golgi apparatus. The secretory product is released from the median neurosecretory cells, a day before prepupal stage. The corpora allata are active immediately after the last larval moult and degenerate at the end of last instar. The prothoracic gland cells of *S. litura* have ultrastructural similarities with those of the silkmoth, *Antheraea pernyi*. The gland sheath had pore canals involved in the movement of substances between haemolymph and the gland.

Key Words: *Spodoptera litura*, **Neurosecretion, Ultrastructure, Corpus allatum, Prothoracic gland**

Introduction

The importance of endocrine glands in the regulation of post-embryonic development of insects is well known, and this has been exhaustively reviewed by Doane (1973), Slama (1974), Gilbert and King (1974) and Novak (1975). While the histological techniques of staining enable us to understand the gross morphology and certain aspects of cell activity, the finer details of the cytology leading to correlation of structural changes with physiological events are better understood through ultrastructure studies (Rowell 1976). Ultrastructural features

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of endocrine glands viz., median neurosecretory cells of brain (MNC) of several insects species, corpus allatum (CA) of *Celerio lineata* (Schultze 1960), *Hyalophora cecropia* (L.) (Waku & Gilbert 1964), *Leucophaea maderae* F. (Scharrer 1964a), *Drosophila melanogaster* Meign (King et al. 1966) and *Schistocerca gregaria* Forskal (Odhiambo 1966a, b) and prothoracic gland (PG) of *L. maderae* (Scharrer 1964b), *Antheraea pernyi* Guerin (Beaulaton 1968) and *H. cecropia* (Herman 1967) have been described in detail. However, none of the Indian economic lepidopterans, has been subjected to such detailed studies. This paper describes in detail, the ultrastructural features of the endocrine glands of the tobacco caterpillar, *Spodoptera litura* (Fabricius) during the last larval instar to bring out the changes preceding the larval-pupal transformation.

Materials and Methods

The test insect was reared on castor leaves at 27±2°C and 75% relative humidity according to Pawar and Ramakrishnan (1971). The larval stage lasted for 13 days and had 6 instars of which the last instar was of 4 days duration. Samples of larvae for fixation of endocrine glands were obtained from the stock culture at 24 hr interval from the 8th day till pupation. Brain, CA and PG were dissected out in cold phosphate buffer (0.1 M, *p*H 7.2) in the shortest time possible and fixed in fresh, 6.25% glutaraldehyde (prepared in 0.1 M phosphate buffer, *p*H 7.2 having 0.2 M sucrose) for 4 hr at 5–10°C. These tissues were washed in buffer and post-fixed in 1% osmium tetroxide (prepared in 0.1 M phosphate buffer *p*H 7.2 having 0.2 M sucrose) for 8 hr at 5–10°C. This schedule was finally adopted as rapid fixation (Hayat 1972) led to inadequate preservation of brain tissue owing to the fact that the thick nerve sheath investing the brain of this insect acted as a barrier for penetration of the fixatives. The tissues were dehydrated in graded series of acetone and resin mixture, impregnated and embedded in Epon (812) — Araldite (6005) (Ladd Research Industries, USA) mixture according to Hayat (1972). Care was taken to orient *Pars intercerebralis* of the brain horizontally below the tip of the block before curing.

Ultrathin sections (300 Å) of the tissues under study were cut on ultramicrotome (LKB model 4801) using freshly prepared glass knives and mounted on farmvar coated copper grids. Sections were double stained with uranyl acetate and lead citrate according to Hayat (1972). The grids were examined in transmission electron microscope (Philips 300) operated at 40 or 60 KV accelerating voltage. Direct electron micrographs of selected areas of the tissue preparations were taken and enlarged as per requirements.

Observations

(a) Median neurosecretory cells of the brain (MNC)

The MNC are irregularly oval and surrounded by a thick layer of glial processes. The nucleus is almost round, rich in chromatin and has a highly convoluted double layered nuclear membrane (plate 1, figures A,B).

Changes in the activity of MNC: A day prior to the last larval moult (8-day old larvae), the perikaryon of MNC had smooth as well as rough (granular) endoplasmic reticulum (SER and RER) seen in close proximity with

Golgi apparatus (GA). The GA is compact and ellipsoidal having multilayered cristae and small vesicles. The neurosecretory droplets (NSD) near GA are granular whereas the ones in the axons and clusters of granules in the cytoplasm are electron dense. The matrix of mitochondria is light and cristae are not very prominent. The electron dense granules are 1240–2100 Å in diameter whereas the less dense granules that are devoid of the membranes are only 1000–1500 Å in diameter (plate 1, figure C). Axon terminals of these secretory cells are found to contain several electron dense NSD. On the 9th day, i.e., a few hours after the last larval moult, the MNC were devoid of NSD as well as clearly resolved endoplasmic reticulum. However, numerous free ribosomes, small filaments of the endoplasmic reticulum are discernible. Mitochondria with distinct cristae and GA are predominant. The lamellar system of Golgi cisternae are dispersed over a large area (plate 1, figure D).

With the advancement of larval age by one more day (10-day old larvae) there is a phenomenal change in the secretory activity of the MNC (plate 1, figures E,F). At this stage the cytoplasm is completely occupied by NSD most of which are electron dense, almost round, membrane bound and are 1870–2370 Å in diameter. A few droplets, whose boundaries are not well resolved are also present and measured 1100–1450 Å. The NSD are found interspersed with long filaments of RER. The filaments of RER are parallel to each other having ribosomes deposited on the surface representing the typical ergastoplasm. The GA are widely dispersed in the cytoplasm with their vesicles almost anastamosing with the tips of RER.

The same structural features are observed in the MNC of 11-day old larvae also. In addition, mitochondria are in abundance. In many secretory cells there was profuse production of NSD that were found orienting towards the axon terminals and as such several axon terminals of these cells were filled with NSD (plate 2, figure A).

With the advancement to prepupal stage, the cell activity drastically changed in that, the MNC are almost devoid of neurosecretion and most of the cell cytoplasm is occupied by free ribosomes. Some cells in addition had vacuoles, myelin figures and lysosome-like bodies at the periphery (plate 2, figures B, C).

(b) Corpus cardiacum (CC) and corpus allatum (CA)

The CC in *S. litura* larva is the sac where the axons of the secretory cells of brain terminate and acts as storage release organ. No intrinsic secretory cells are observed in CC. Structurally the CA can be differentiated into 2 regions: (a) the basal vacuolar region where some of the secretory axons having NSD terminate, and (b) the terminal region having the intrinsic secretory cells having a close net work of cellular structure. These cells lack definite cell boundaries and can be regarded as syncytial having scattered nuclei (plate 3, figure A). The ultrastructure of these cells in 9-day old larvae shows the presence of small but highly convoluted nuclei having evenly dispersed chromatin and cytoplasm having a wide network of highly folded plasma membranes and SER. The SER can rarely be recognised from the cytoplasmic plasma membranes. Quite interestingly the mitochondria are seen accumulated in the

peripheral cells of the gland below the acellular gland sheath. The mitochondria are highly pleomorphic and lack clearly resolved cristae. This zone is further dominated by the presence of several ribosomes and a few electron dense vesicles (plate 3, figure B). On the contrary, the ultrastructure of these cells at the prepupal stage has quite distinct features that are different from the above. The cytoplasm is lacking the fine network of membranes, SER and mitochondria but in their place electron dense material, several empty vesicles and vacuoles are seen (plate 3, figure C).

(c) Prothoracic gland (PG)

The ultrastructure of PG cells of the late last instar (11-day old larvae) of *S. litura*, that represents the most active stage of the gland, is studied. The bead-like cells of the gland are enclosed in a peripheral membrane, the tunica propria (TP), that had characteristic finger-like projections contributing to increase surface area of the gland. The TP has numerous fine pore canals that run across its thickness into the gland. Electron dense material is observed on the surface of the gland as well as in the pore canals of TP. The cytoplasm is having a wide network of lacunae, especially at the peripheral zone, that are surrounded by parenchymal processes. Some of the lacunae contain electron dense material. Bundles of microtubules, originating at the base of TP run perpendicular to its surface. Further, the cytoplasm surrounding the nucleus represents the association of tubular mitochondria with vacuolar spaces and fragments of SER. Neither secretory axons nor trachaeae are observed in the PG cells (plate 4, figures A to D).

Discussion

The ultrastructure of the MNC of *S. Litura* at moult and intermoult periods of last instar clearly shows cyclical activity which is evident not only by the presence of neurosecretion but also by the activity of cellular organelles, especially of endoplasmic reticulum, GA and ribosomes. At the end of 5th instar and immediately after the last larval moult, a few NSD are present in cell cytoplasm along with SER and sparsely distributed ribosomes. This represents a relatively inactive phase of MNC after the release of neurosecretion which promoted the last larval moult. The regain of secretory activity is seen in MNC of 9-day old larvae that contained the dispersed GA as against the compact one in 8-day old larvae. It is established that the dispersed GA is more active than the compact GA (Fawcett 1966). No sooner the larval age advanced and the feeding was initiated after the last larval moult, the cell activity changed leading to the synthesis of neurosecretion. A clearly defined RER was formed due to the orientation of ribosomes on the surface of endoplasm. A very high amount of NSD was seen in the cytoplasm during intermoult period (10 and 11-day old larvae) indicating that the MNC were active in synthesising the neurosecretion. Further, the accumulation of NSD at axonal ends and complete loss of NSD in the MNC of 12-day old larvae indicate the release of neurosecretion before prepupal stage that eventually triggered the events of morphogenesis. This coincided with cessation of feeding prior to pupation.

That insect endocrines show phases of secretory activity in each larval instar and at metamorphic moult is well established in many insects, especially in

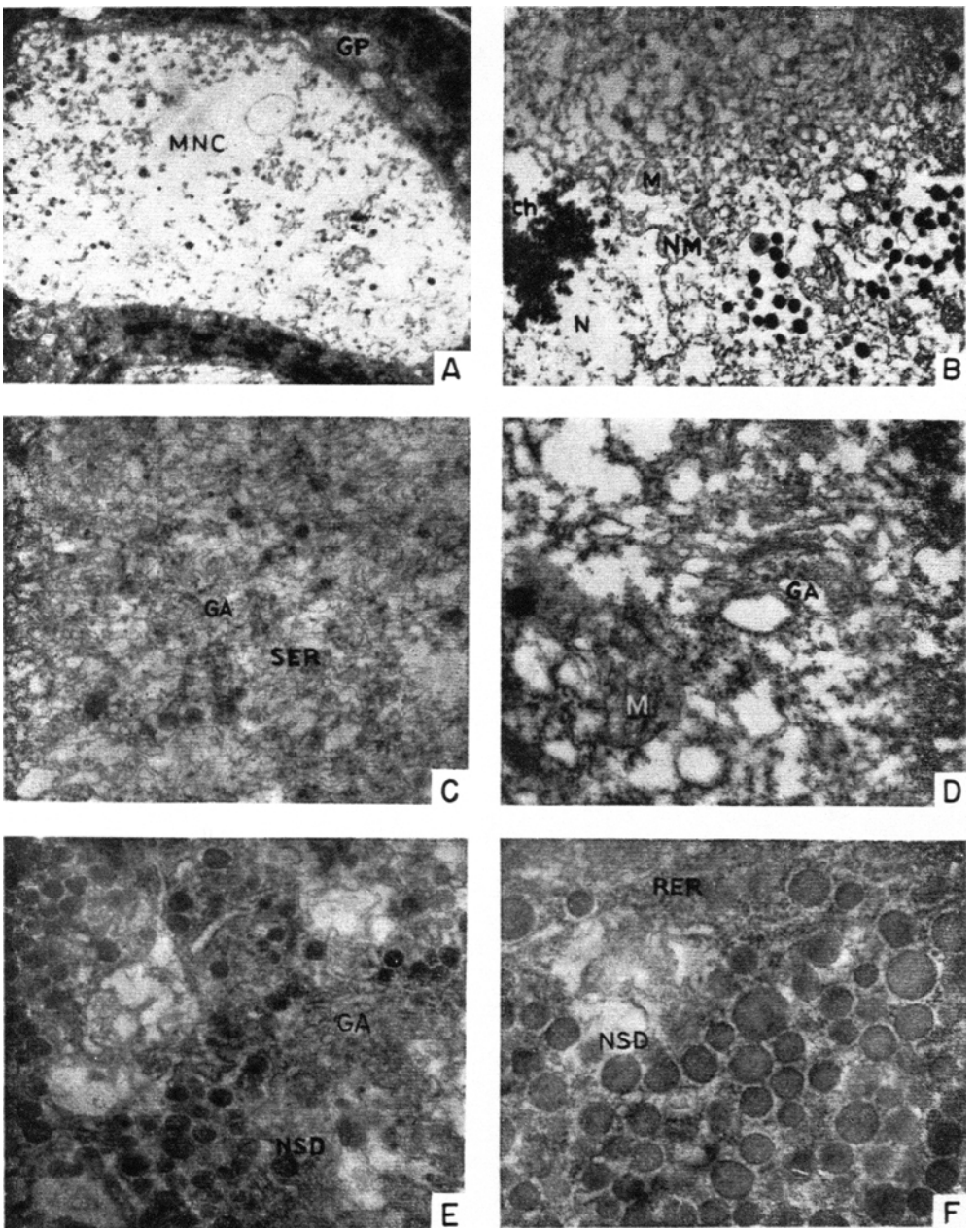
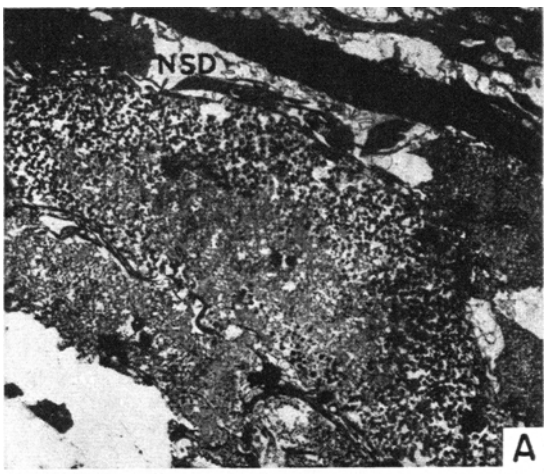
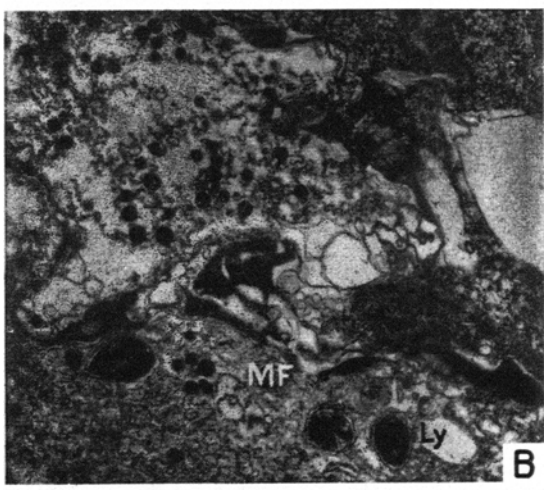
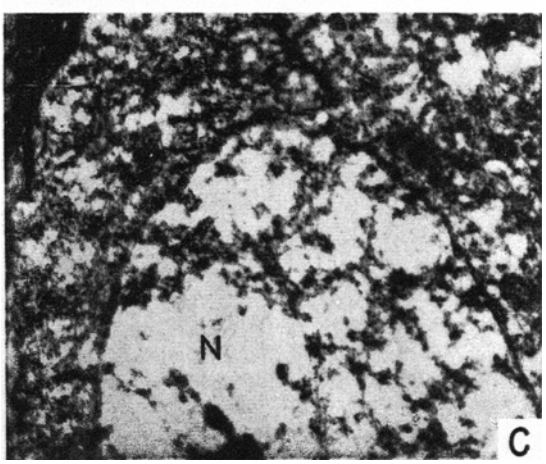


Plate 1 A–F Ultrastructure of the median neurosecretory cells of last instar *S. litura* larva. *A*, Low magnification view of MNC from 8-day-old larva (×7386.3); *B*, Part of MNC from 8-day-old larva showing ultrastructural details (×11271.5); *C*, Perikaryon of MNC from 8-day-old larva showing formation of SER, GA and NSD (×18037.5); *D*, Perikaryon of MNC from 9-day-old larva showing the dispersed golgi apparatus (×25065.6); *E*, *F*, Area in the MNC of 10-day-old larva showing the formation of membrane bound NSD interspersed with GA and RER (× 1878.7 and 18037.5 respectively)







lepidopterans like *Cerura venula* (Gilbert & King 1973). Further, Kono (1975) observed similar cycle of MNC activity in *Pieris brassicae* L. larvae based on ultrastructure study. The present study indicates that MNC of *S. litura* are active in synthesising neurosecretion at the last intermoult period and release it a day prior to prepupal stage. Light microscopic study, adopting *in situ* aldehyde fuchsin staining, also indicated similar phases of activity of MNC of *S. litura* that coincides with moult cycle (Subrahmanyam 1979). The appearance of parallel arrays of RER and the orientation of GA near RER indicate their role in the synthesis of the membrane bound proteinaceous neurosecretion, as earlier reviewed by Slama (1974). In fact, in the active phase of synthesis, the MNC of cultured brains as well as the ones directly taken from *Manduca sexta* Joh. also had parallel arrays of RER that terminate near GA (Borg & Marks 1973). Further, the involvement of GA in packing the secretory product as membrane bound granules is clearly observed in the present study.

Based on the size of NSD (1100–2600Å in diam.) it can be stated that the MNC of *S. liura* represent the classical A-type secretory neurones according to the classification of Scharrer as detailed by Doane (1973). While the less electron dense granules of 1100-1500 Å in diam. represent the elementary granules,

Plate 2 A–C Ultrastructure of MNC of last instar *S. litura* larva; *A*, Low magnification view of MNC from 11-day old larva whose cytoplasm is completely filled with NSD (× 3555.5); *B*, MNC from 12-day old larva showing lysosome-like bodies and myelin figures at the periphery of the cell (×11400); *C*, MNC from prepupal stage devoid of NSD (× 14358.5)

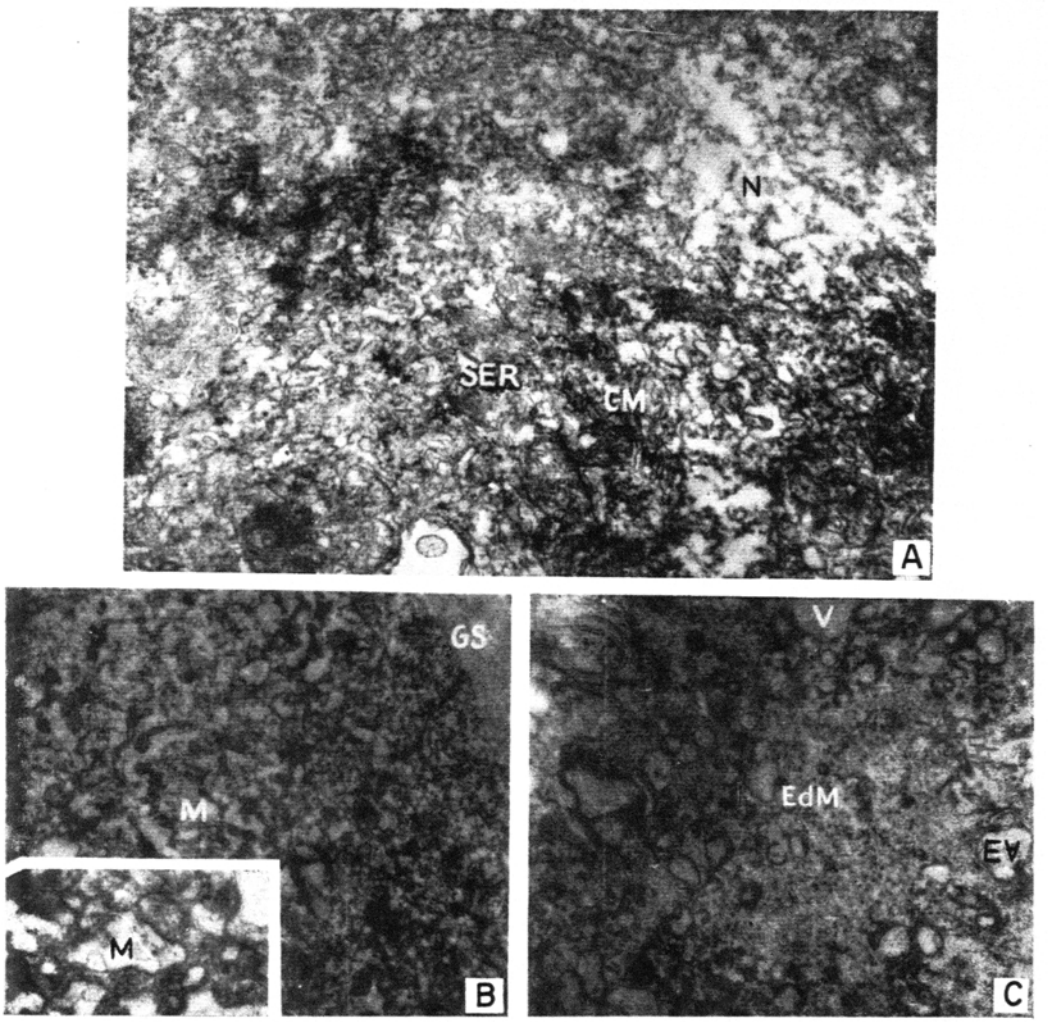


Plate 3 A–C Ultrastructure of corpus allatum of last instar *S. litura*. *A*, Intrinsic secretory cell from CA of 9-day old larva (× 31308); *B*, Section through periphery of active CA, showing accumulation of mitochondria (× 12347.2). Inset pleomorphic mitochondrion (×35809); *C*, Area from CA of late last instar showing degeneration of ultrastructure (× 20832)

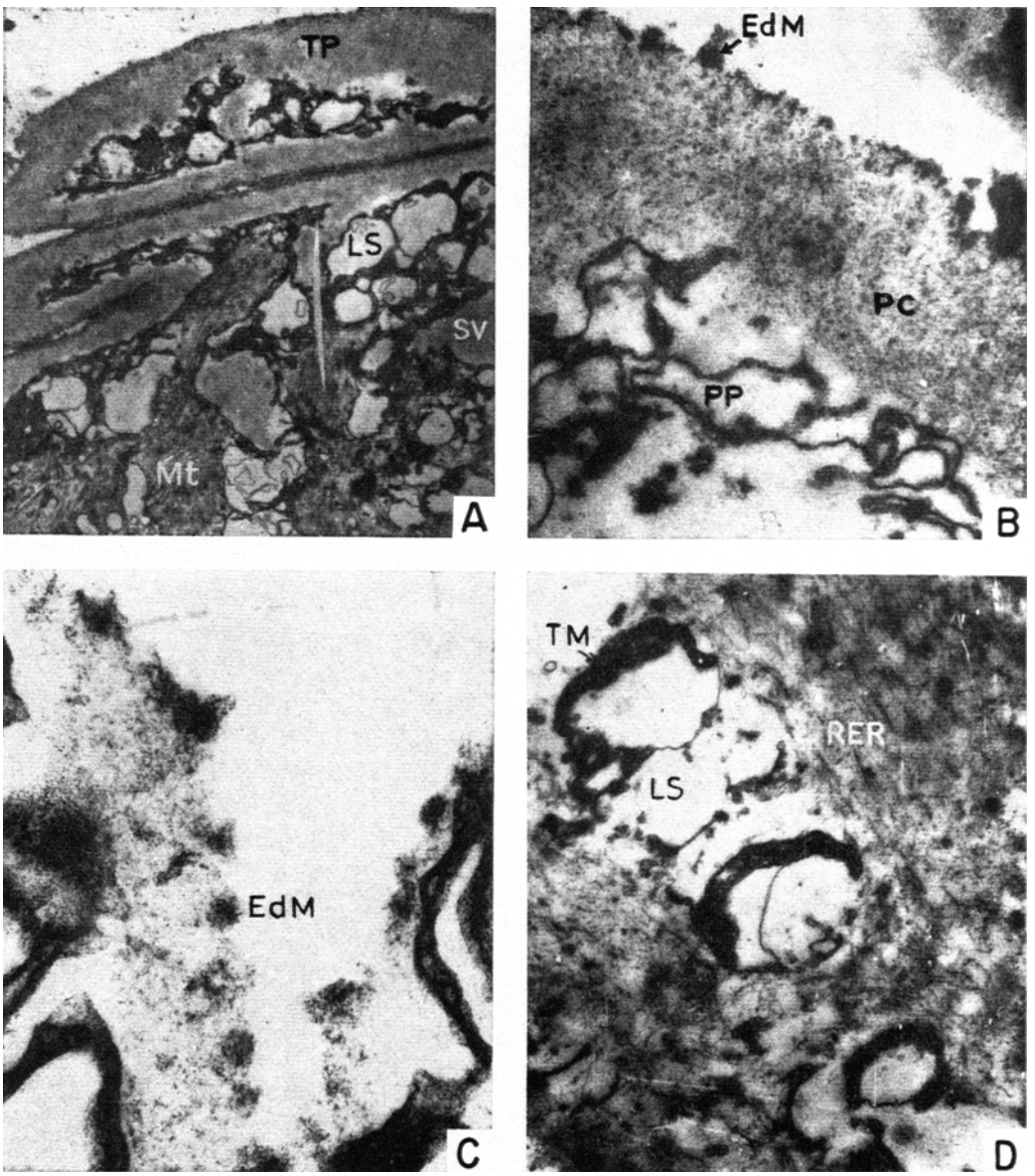


Plate 4 A–D Ultrastructure of active prothoracic gland (PG) cells of late last instar *S. litura* larva. *A*, Section through periphery of PG cell showing finger-like projections of tunica propria, microtubules and lacuna system (× 6897); *B*, Magnified view of tunica propria showing pore canals (× 36583); *C*, The diffusion of electron dense material into the gland (× 82419); *D*, Perinuclear zone of PG cell showing the association of mitochondria, RER and lacuna system (× 22706.6)

the dense granules of 2100–2600 Å in diam. represent the final form of the NSD.

The secretory cells of CA of 9-day old *S. litura* larvae are characterised by the presence of SER and membranes in the cytoplasm together with the orientation of mitochondria at the periphery of the gland. The SER is involved in juvenile hormone (JH) synthesis in *L. maderae* (Scharrer 1961), *H. cecropia* (Waku & Gilbert 1974) and *S. gregaria* (Odhiambo 1966a, b). The highly infolded plasma membranes of the glandular cells of CA further indicate the activity of the gland as observed in *C. lineata* by Schultze (1960). The accumulation of mitochondria in peripheral cells of active CA was observed in *H. cecropia* (Waku & Gilbert 1964) and *S. gregaria* (Odhiambo 1966a). The presence of mitochondria at the periphery of the gland probably indicates their role in release of JH into the haemolymph. The activity of CA as seen in the present study is further strengthened by the detection of JH peak in the haemolymph of 10-day old *S. litura* larvae (Subrahmanyam & Ramakrishnan 1980). The CA of late last instar of *S. litura* may be regarded inactive as SER is absent and may be in the process of structural degeneration favouring pupal moult.

The ultrastructure of PG cells of *S. lituru* during the late last instar bears similarities in several respects to the active PG cells of silk moths viz., *A. pernyi*, *Samia cynthia* and *H. cecropia* as reviewed by Herman (1967). This is especially so with reference to the tunica propria having finger-like projections leading to increased surface area of the gland. In addition, the present study clearly shows the fine pore canals in the vertical plane of TP and the presence of electron dense material on its surface as well as in the interior indicating the transportation of substance (probably cholesterol which is a precursor of ecdysone) of haemolymph origin into the gland. The lacunae developed immediately below the TP and the association of mitochondria with SER and vacuoles are similar to those observed in the active PG of *A. pernyi* by Herman (1967) and Beaulaton (1968) who suggested that the secretory product of steroidal nature, that was not preserved adequately by the fixative, might be present in the lacunae system that migrate towards the periphery of the gland.

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