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ULTRASTRUCTURE OF MID-GUT OF THE THIRD LARVAL INSTAR OF GASTEROPHILUS INTESTINALIS (DIPTERA: GASTEROPHILIDAE)

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SUMMARY

Mid-gut of both early and late third larval instar of *Gasterophilus intestinalis* was investigated by electronmicroscop. Electronmicrographs of early and late larval instars illustrate difference in the structures of apical border; the microvilli, basal lamina, mitochondria and rough endoplasmic reticulum. Vacuoles of trilamellate layer bearing secretory granules are a characteristic feature of late instar. Also a lot of multivesicular bodies resulting from the degenerating of neighboring organelles.

INTRODUCTION

The survival of insects depends mainly on the physiology of the digestion and absorption of nutrients. The midgut of insects comprises the functional part of the digestive tract that deals primarily with digestion of foodstuffs and absorption of nutrients. In case of endoparasitic insects, the survival of the parasite depends on the structural function of the midgut cells. The architectural structure of insect midgut cells in relation to their function has been studied by many authors (Hecker *et al.* 1971, Brown 1980 and Billingsley 1990). The aim of the present work was to investigate the structural changes in the epithelial cell of the midgut of both early and late third larval instars of *G. intestinalis* and their correlation with the physiological state of the insect.

MATERIALS AND METHODS

Third larval instars of *Gasterophilus* were collected from the stomach of freshly slaughtered donkies and horses in the Zoo, Giza, Egypt. They were identified according to Zumpt (1965).

Ultrastructural preparation of the mid-gut carried

out on 20 specimens taken from both early and late larval instars of *G. intestinalis*. The freshly collected larvae were washed in several changes of phosphate-buffered saline (pH 7.2) to remove debris.

Transmission electron microscope preparation

Larvae were dissected in 3% glutaradehyde in 0.076 M cacodylate buffer pH 7.2, containing 29mM sucrose for two hours at 4°C then was thoroughly rinsed over night at 4°C in 0.2 M cacodylate buffer containing 29mM sucrose. The specimens were post fixed in 1% osmium tetraoxide for 30 minutes, washed in distilled water for 2 minutes, followed by dehydration in a graded series of acetone. Embedding in Epon takes place, and then section were stained with saturated uranyl acetate for 15 minutes and counterstained in lead citrate for 20 minutes (Harely and. Fergusen 1990). Sections were examined in a C M 100 microscope. (Histology Department Faculty of Medicine, Ein-Shams University)

Scanning electron microscope preparations

The mid-gut were fixed in 2.5% glutarldehyde (pH 7.2) for 24 hours at 4°C, then posfixed in 1% osmium teteraoxide for 1 hour at room temperature (Harley and Fergusen, 1990). The specimens were then dehydrated with acetone; the mi-gut was splitted, critical points dried, and finally sputter coated with gold. The examination and photographing were done through ALx 30 Scanning Electron Microscope . (Histology Department, Faculty of Medicine, Ein-Shams University) .

RESULTS

In the early third larval instar, the cells of the anterior mid-gut show a very well developed brush border consisting of numerous slender and long microvilli extended epically into the mid-gut lumen. In some cells the microvilli were more uniform than in other cells, covered with a distinct glycocalyx (Fig. 3). The microvilli in posterior mid-gut cells are less dense, less uniform and also less compact (Fig 11).

The basal plasma membrane is deeply folded and has occasional openings into the haemocele (Fig 9). Fibers of circular and longitudinal muscles forming a very well-developed muscle layer and also a very well-developed thick basal lamina which are clear in both regions of the mid-gut. (Fig 2, 6, 8). The basal lamina consists of an amorphous substrate into which are embedded fibrils or beadlike lamellae. These beadlike substructures followed a course parallel to the basal plasma membrane (Fig 8). The basal lamina appears folded probably as a result of muscular contraction.

The anterior region of the early third larval instar showed the presence of numerous dark-staining granules, which are few below the microvilli, api-

cal part of the cell and increase in density towards basal part (Fig 2). Both regions of the mid-gut are characterized by the presence of numerous polymorphic, elongated also fusing mitochondria, which appear mainly in posterior region of the gut, which may account for formation of multivesicular bodies. Also mitochondria cristae seem to be very compact, (Fig. 4, 5, 6, 7, 8, 9, 10, 11, 12, 13).

Stacks of rough endoplasmic reticulum (rer) and a lot of free ribosomes appear throughout the cell, well dispersed in the cytoplasm (Fig. 1, 3, 4, 5). Large and small vesicles are present nearly in all parts of the mid-gut; these vesicles are dilated rer filled with an amorphous substance. (Fig 4). These dilated rer appear fusing together especially in the posterior part of the mid-gut forming multivesicular bodies. Multivesicular bodies appear especially in basal part of posterior mid-gut cells as a result of fusing neighboring cell organelles. (Fig.7, 8, 9, 10).

Few lysosomes and whorls of myelin bodies appear in the early instar (Fig.5, 11).

In the late third larval instar of *G. intestinalis*, the anterior mid-gut region shows very well developed apical border formed by well-developed microvilli, strongly compact and thick (Fig 15).

Cells characterized by apical extrusions into the mid-gut lumen were found in the posterior region of the late third larval instar of *G. intestinalis* (Fig. 18). The microvilli have lost their uniformity.

The epithelial cells of the late third larval instar of *G. intestinalis* of both the anterior and posterior mid-gut regions showed that all the cell organelles were in a deteriorated state.

The mitochondria seemed to be swollen and the cristae appeared to have lost their uniformity, which is clear in the anterior part of the mid-gut of this late instar (Fig 14, 16). Myelin bodies, lysosomes and old multivesicular bodies also appear in the anterior region of mid-gut of this instar. (Fig 15, 16) Vacuoles of trilamellate membranes, free ribosomes and glycogen droplets are prominent in the posterior mid-gut region of the late third larval instar of *G. intestinalis* (Fig 17, 18, 19).

The basal cell membrane and the basal lamina appeared poorly developed and not as dense as in the mid-gut cells of the early instar. Tracheal Cells seemed degenerated. (Fig 20).

The transmission electron micrographs showed clearly the tracheal cells embedded between the basal lamina and muscle layer (Fig. 6, 9). Also

scanning electron micrograph, showed clearly the well-developed tracheal system, which is closely connected with the mid-gut region in *G. intestinalis* larvae. The main truncks give branches, which fuse between the muscle layer and basal lamina (Figs. 21, 22, 23, 24, 25, 26, 27).

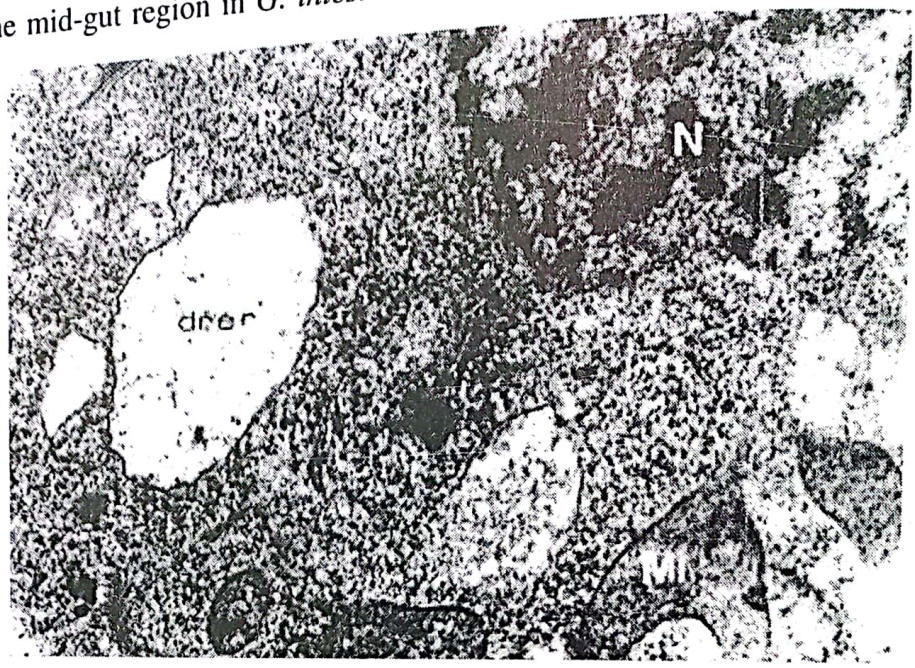

Fig. 1: Electron micrograph from the anterior mid-gut region of the early third larval instar of *G.intestinalis* showing Nucleus (N) ,stacks of rough endoplasmic reticulum (rer) dilated rough endoplasmic reticulum (drer), free ribosomes (R) and polymorphic mitochondria (Mi) X 14000.

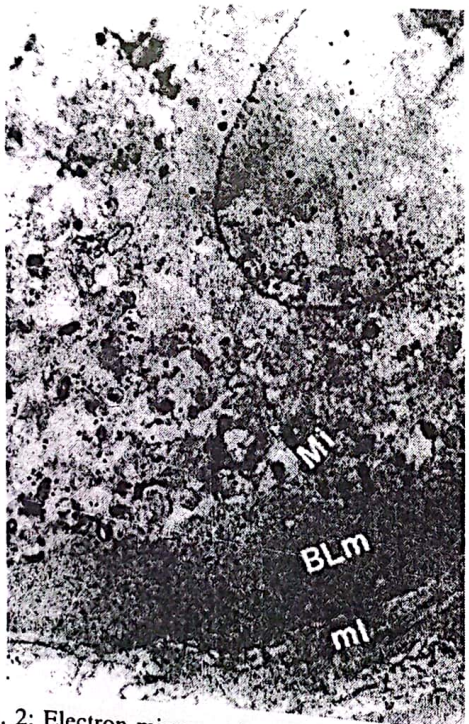

Fig. 2: Electron micrograph from the anterior mid-gut region of the early third larval instar of *G.intestinalis* showing small polymorphic mitochondria (Mi), dense secretory granules (Circles), thick basal lamina (BLm), and muscle layer (ml) X 3810.

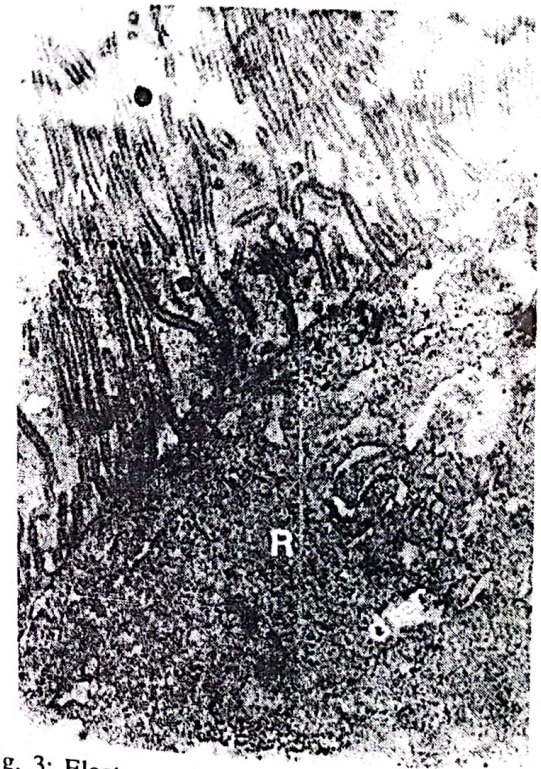

Fig. 3: Electron micrograph from the anterior mid-gut region of the early third larval instar of *G.intestinalis* showing microvilli (MV) coated with glycocalyx (arrow) , cytoplasm densely granulated with ribosomes (R), and dilated rough endoplasmic reticulum (drer) X 9800.

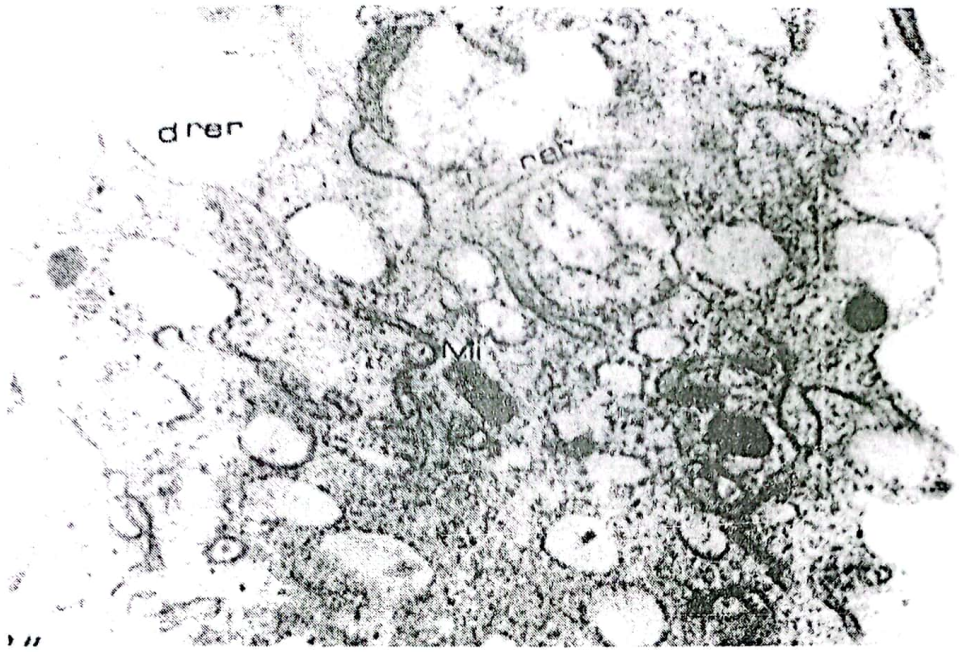

Fig. 4: Electron micrograph from the anterior mid-gut region of the early third larval instar of *G. intestinalis* showing mitochondria (Mi) with dense compact cristae, rough endoplasmic reticulum (rer), dilated rough endoplasmic reticulum (drer) X 14000

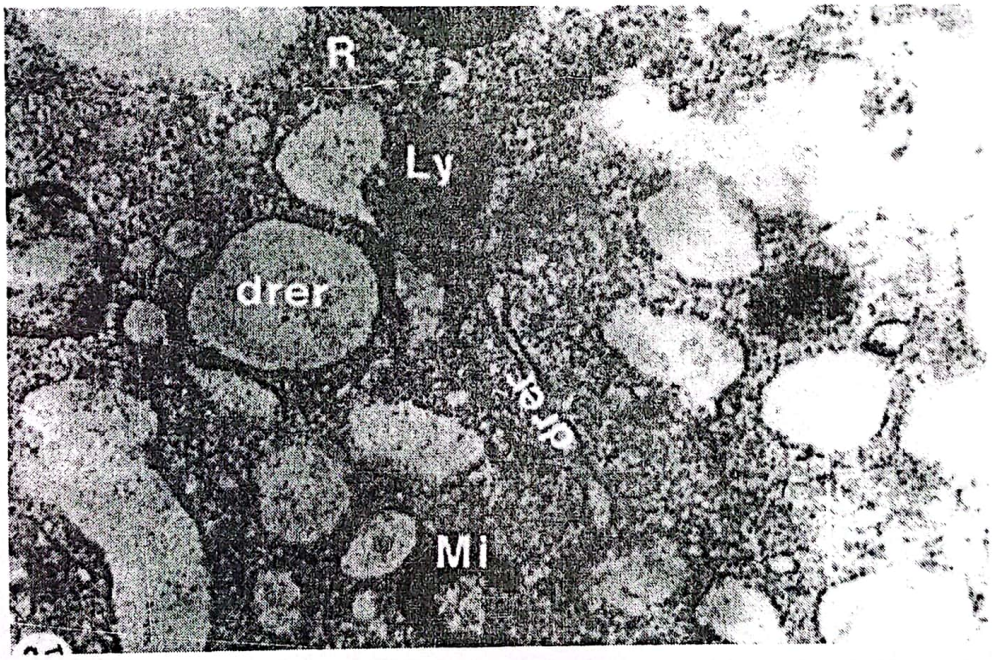

Fig. 5: Electron micrograph from the anterior mid-gut region of the early third larval instar of *G. intestinalis* showing dilated rough endoplasmic reticulum (drer) , elongated mitochondria (Mi) with dense compact cristae and lysosomes (Ly) , X 14000

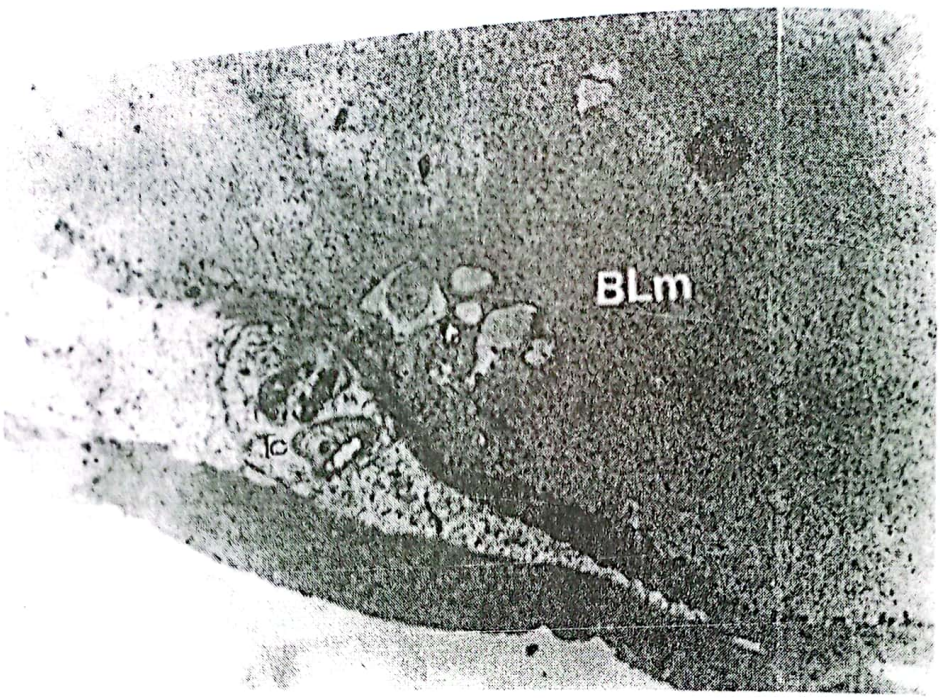

Fig. 6: Electron micrograph from the anterior mid-gut region of the early third larval instar of *G. intestinalis* showing thick basal lamina (BLm) , and tracheal cell (Tc) embedded in the musculo connective envelope of the mid-gut . X 3810.

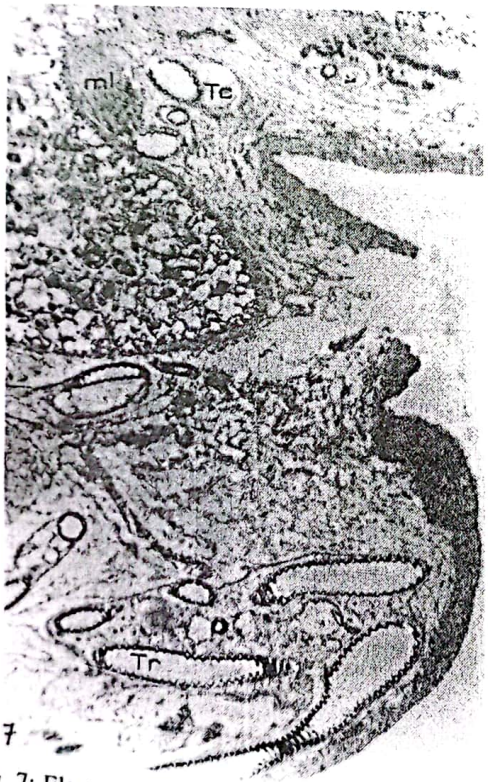

Fig. 7: Electron micrograph from the posterior mid-gut region of the early third larval instar of *G. intestinalis* showing dense polymorphic mitochondria (Mi) between folds of basal cell membrane , Muscle layer (ml), Tracheal cell (Tc) , and tracheae (Tr) . X 2750

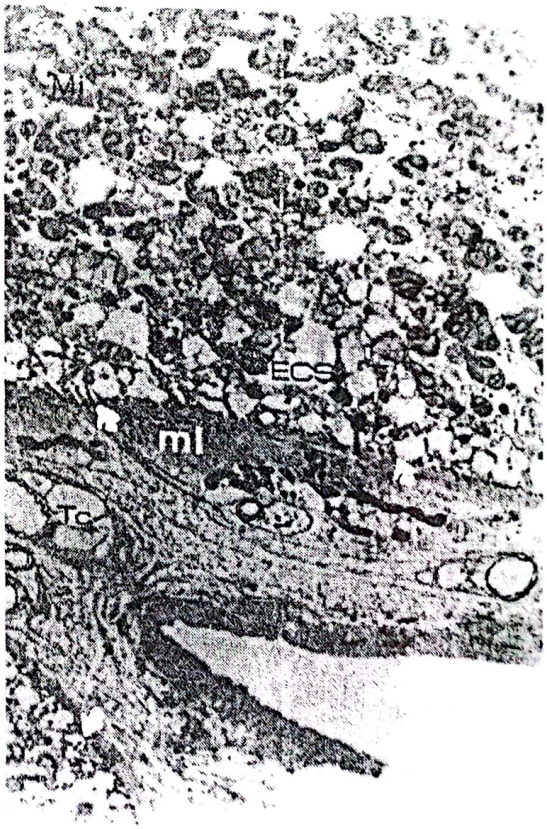

Fig. 8: Electron micrograph from the posterior mid-gut region of the early third larval instar of *G. intestinalis* showing dense polymorphic mitochondria (Mi) , Extracellular space (ECS) , bead ñ like structure at the base of basal cell membrane (arrows), Muscle layer, (ml), and Tracheal cell (Tc) embedded in the musculo -connect envelope of the mid-gut . X 3180

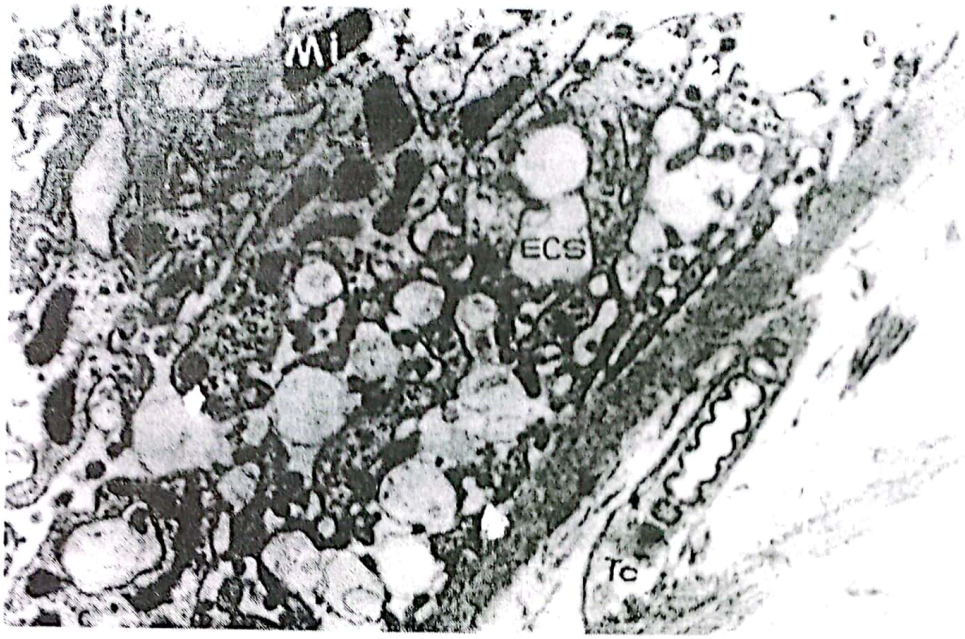

Fig. 9: Electron micrograph from the posterior mid-gut region of the early third larval instar of *G. intestinalis* showing Tracheal cell (Tc) embedded in the musculo connective envelope of the mid-gut , basal lamina (BLm), narrow openings to the haemocele (red arrows) , Extracellular space (ECS) , mitochondria (Mi) , and dense secretorygranules (green arrows) in newly formed multivesicular bodies .X 9800.

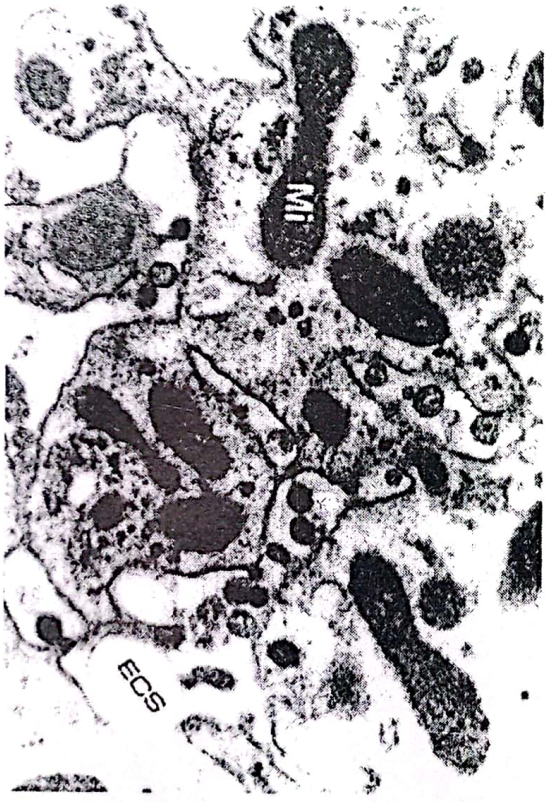

Fig. 10: Electron micrograph from the posterior mid-gut region of the early third larval instar of *G. intestinalis* showing polymorphic mitochondria (Mi), dense secretory granules (arrows) in groups of small and large newly formed multivesicular bodies , and Extracellular space (ECS) . X 18000.

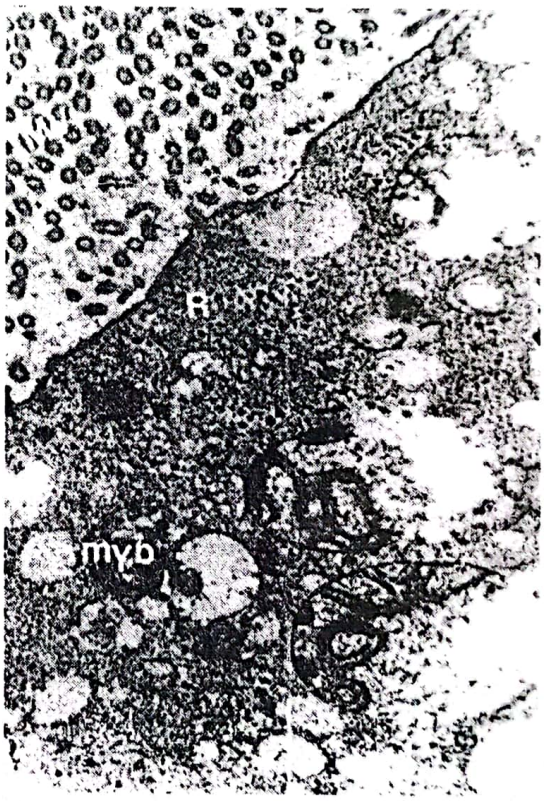

Fig. 11: Electron micrograph from the posterior mid-gut region of the early third larval instar of *G. intestinalis* showing Microvilli (MV) , dense free ribosomes (R) , and Newly formed myelin bodies (myb) propably from degenerating mitochondria . X 14000.

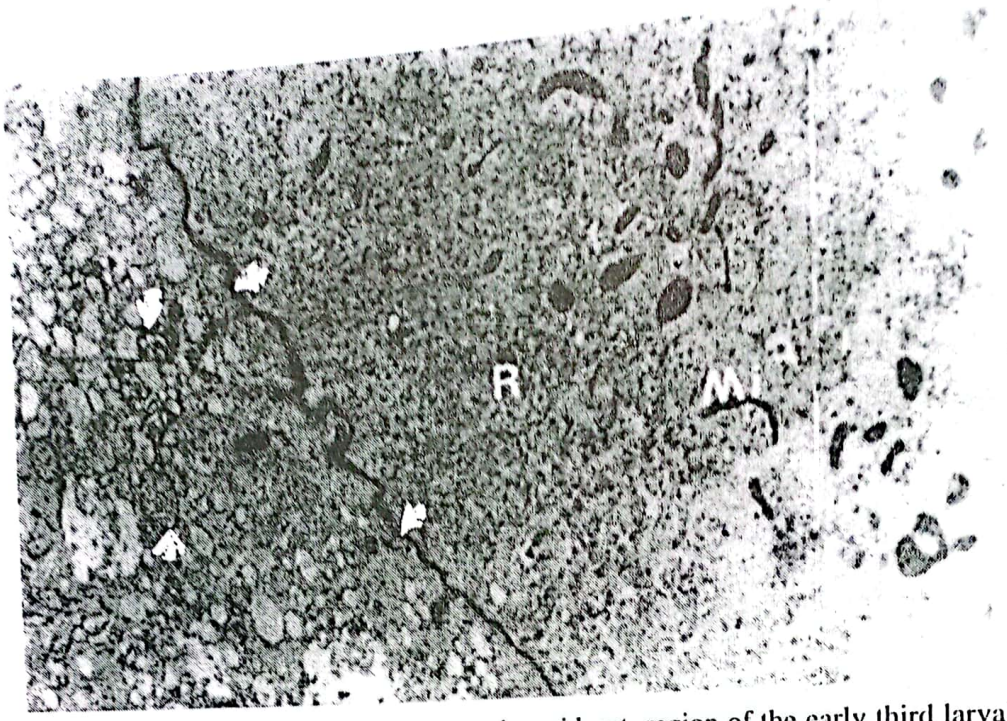

Fig. 12: Electron micrograph from the posterior mid-gut region of the early third larval instar of *G. intestinalis* showing lateral cell membrane (red arrows), polymorphic mitochondria (Mi) , free ribosomes (R) , and appearance of degenerating mitochondria in process of forming multivesicular bodies (green, arrows) . X 3810.

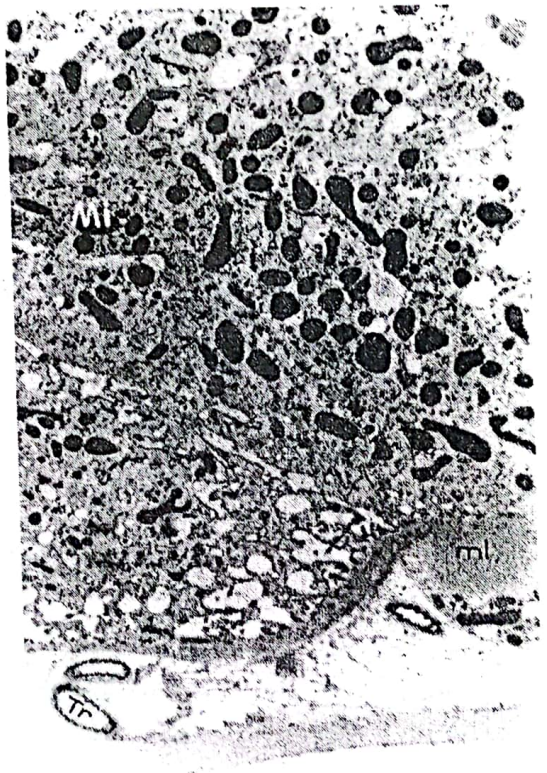

Fig. 13: Electron micrograph from the posterior mid-gut region of the early third larval instar of *G. intestinalis* showing dense the mitochondria (Mi), Muscle layer (ml) , and Tracheae (Tr) embedded in musculo connective envelope of the midgut . X3810.

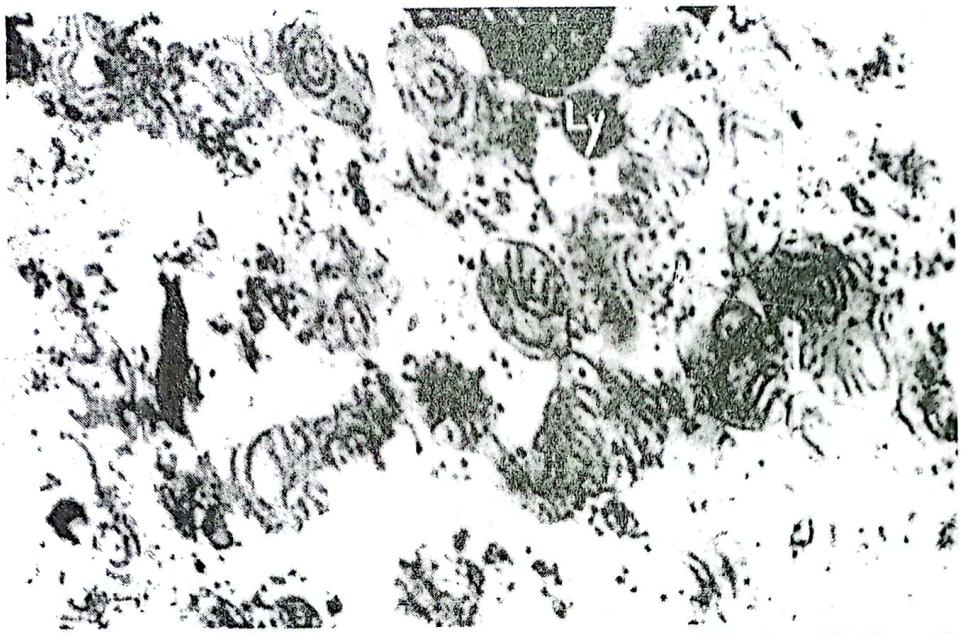

Fig. 14: Electron micrograph from the anterior mid-gut region of the late third larval instar of *G. intestinalis* showing degenerating mitochondria (Mi) , and densely stained lysosomes (Ly) . X 15500.

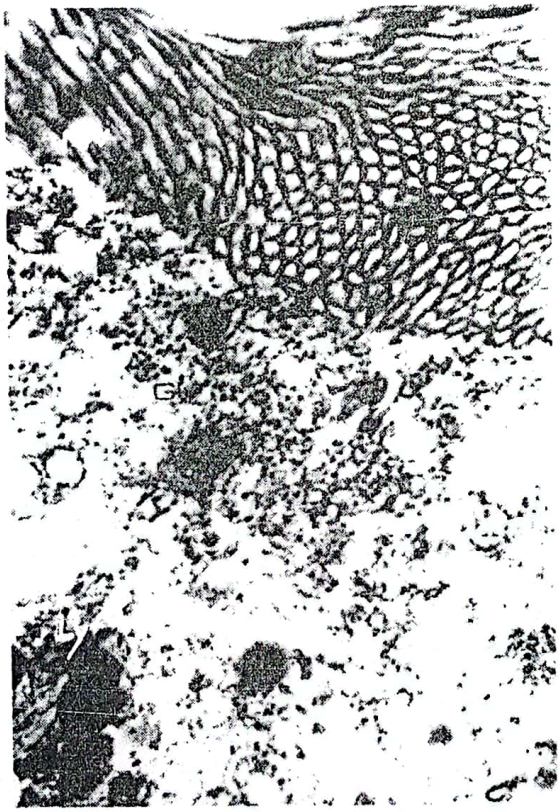

Fig. 15:Electron micrograph from the anterior mid-gut region of the late third larval instar of *G. intestinalis* showing compact microvilli (MV) , densely stained old lysosomal bodies (Ly) , and glycogen droplets (Gly). X 15500.

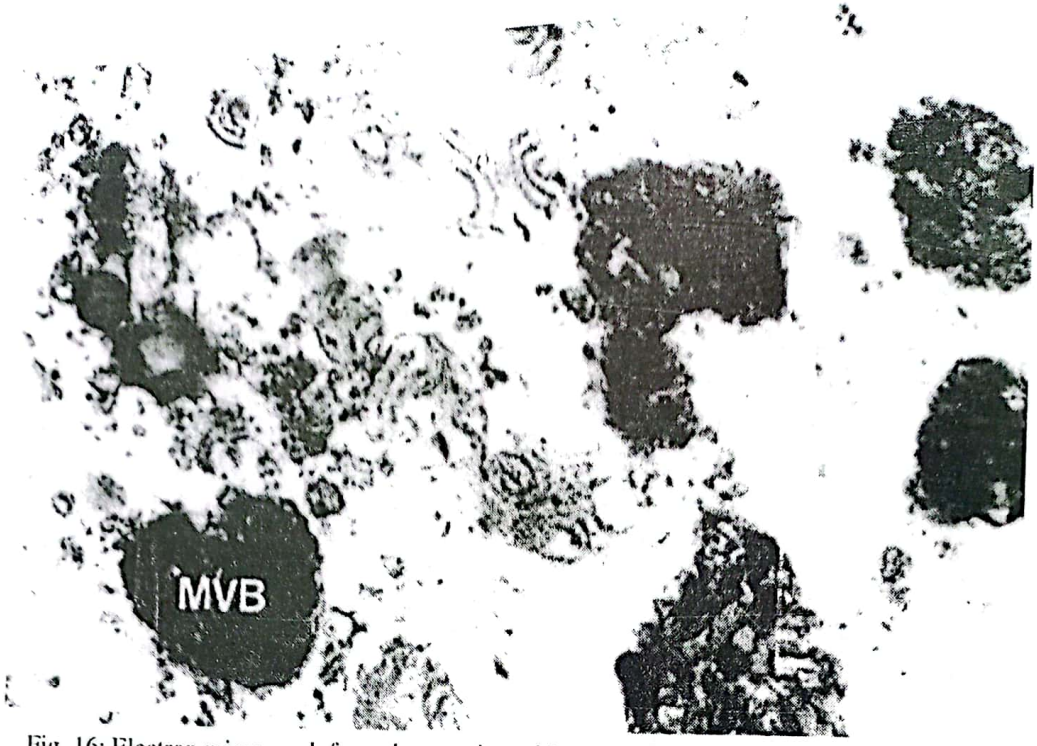

Fig. 16: Electron micrograph from the anterior mid-gut region of the late third larval instar of *G. intestinalis* showing degenerating mitochondria (Mi), and multivesicular bodies (MVB) X 15500.

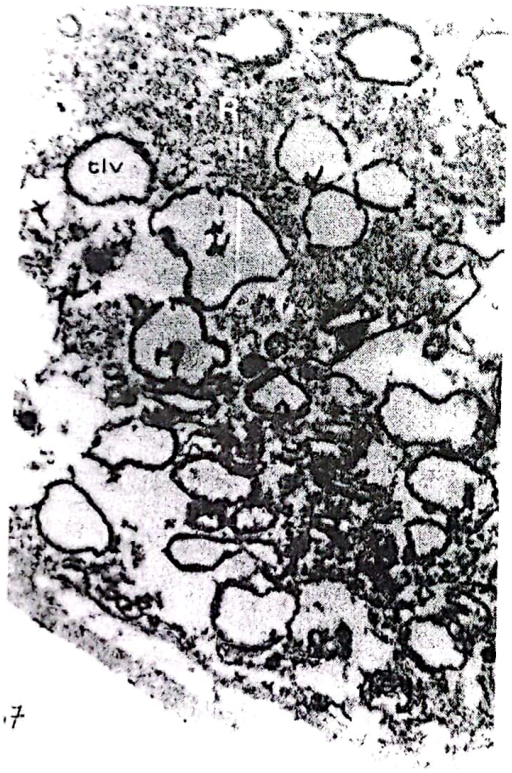

Fig. 17: Electron micrograph from the posterior mid-gut region of the late third larval instar of *G. intestinalis* showing trilamellate vacuoles (tlv) , and ribosomes (R) X 8900.

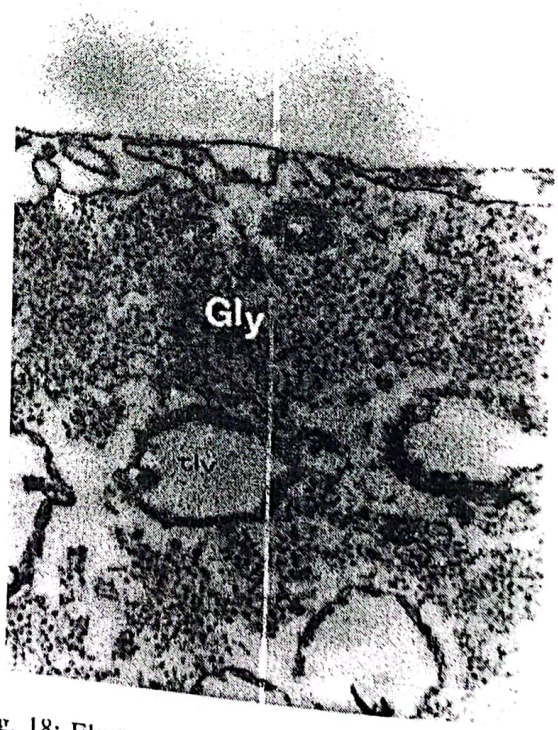

Fig. 18: Electron micrograph from the posterior mid-gut region of the late third larval instar of *G. intestinalis* showing apical extrusions in the gut lumen (arrows) , Glycogen droplets (Gly) ,and trilamellate vacuoles (tlv) X 15500.

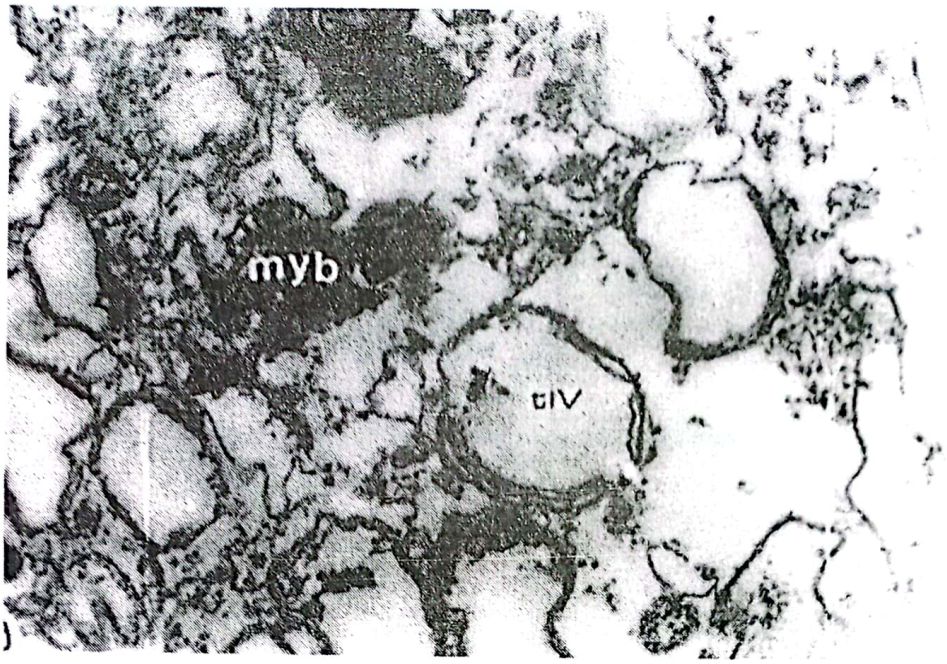

Fig. 19: Electron micrograph from the posterior mid-gut region of the late third larval instar of *G. intestinalis* showing old myelin bodies (myb), and trilamellate vacuoles (tlv) X 15500.

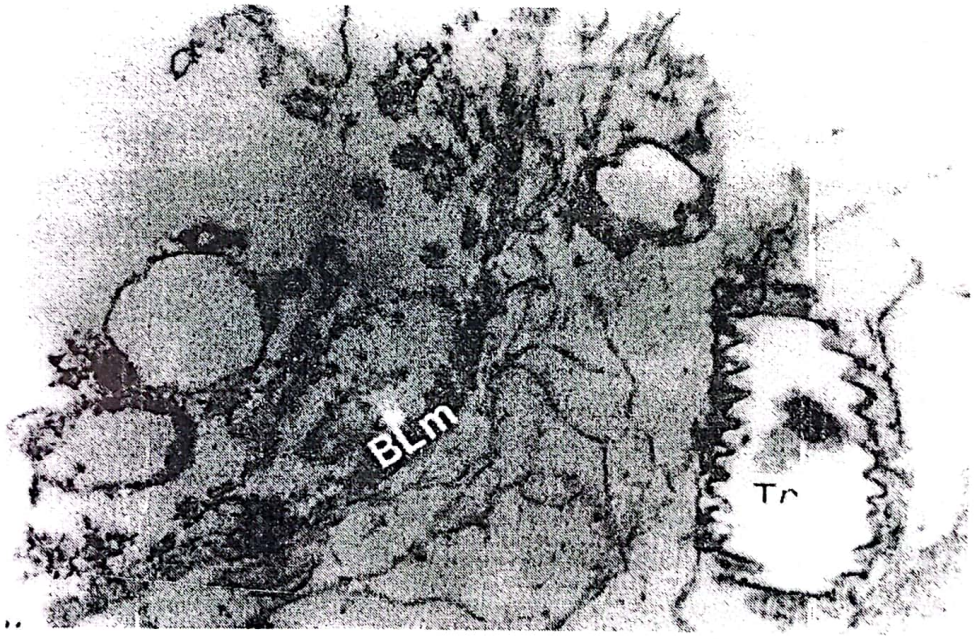

Fig. 20: Electron micrograph from the posterior mid-gut region of the late third larval instar of *G. intestinalis* showing weak basal lamina (BLm) and poorly developed , Tracheae (Tr) X 31.000.

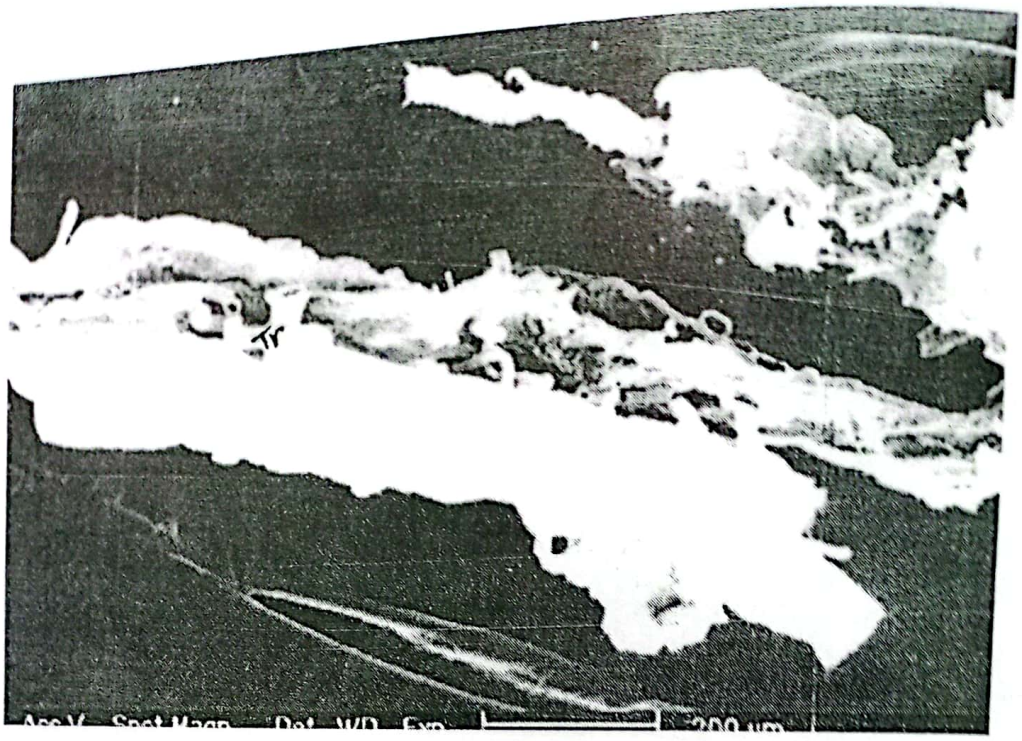

Fig. 21:S E M from the mid-gut of the third larval instar of *G. intestinalis* showing trachea closely attached to outside of mid-gut . X 100.

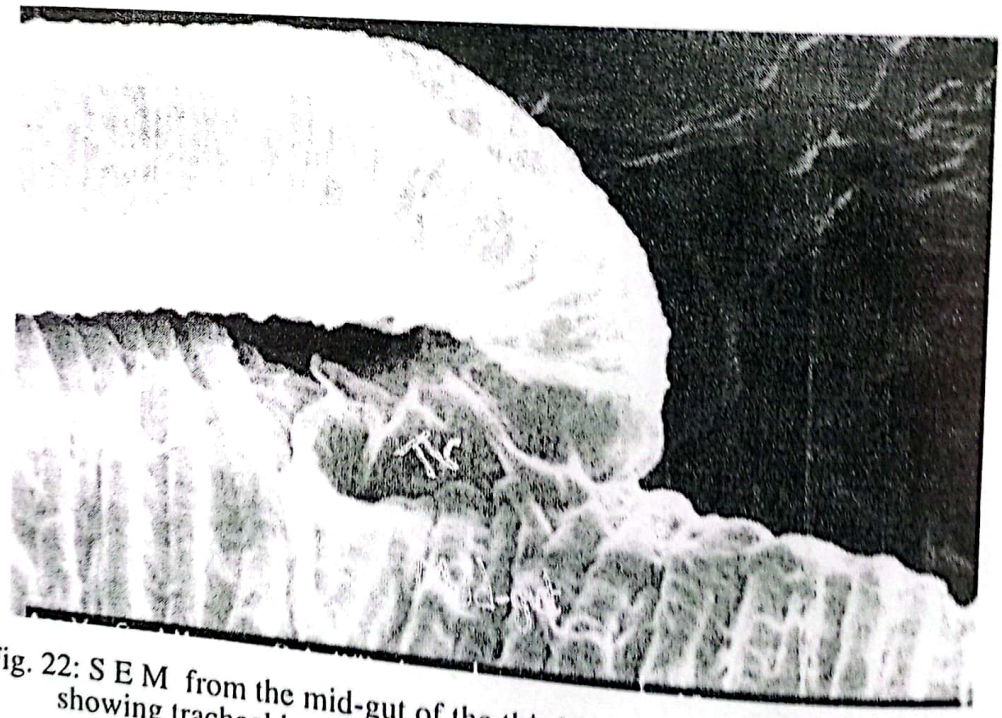

Fig. 22: S E M from the mid-gut of the third larval instar of *G. intestinalis* showing tracheal branch fusing into mid-gut X 2000.

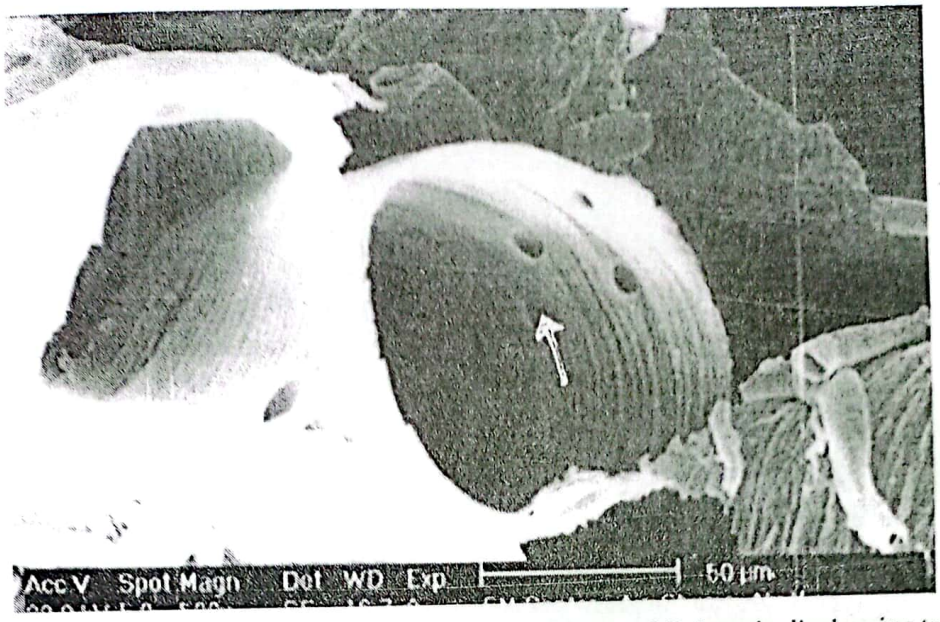

Fig. 23: S E M from the mid-gut of the third larval instar of *G. intestinalis* showing tracheal trunks branching into mid-gut X 500.

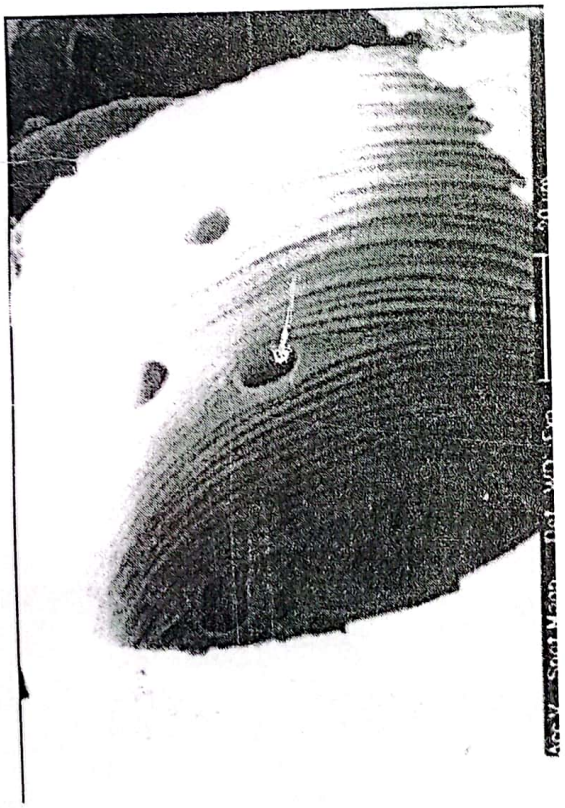

Fig. 24: S E M from the mid-gut of the third larval instar of *G. intestinalis* showing openings indicating the origin of the branching site from the tracheal trunks . X 1000 .

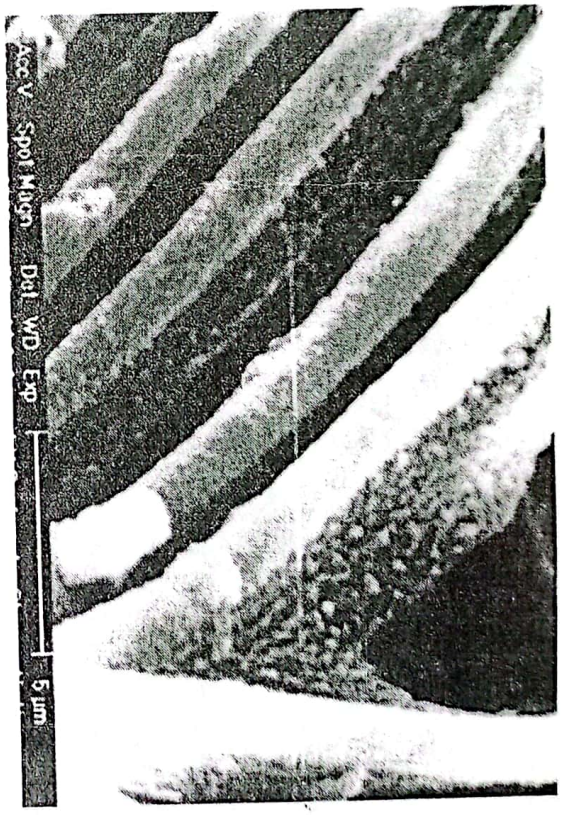

Fig. 25: S E M from the mid-gut of the third larval instar of *G. intestinalis* showing microvilli X 6500.

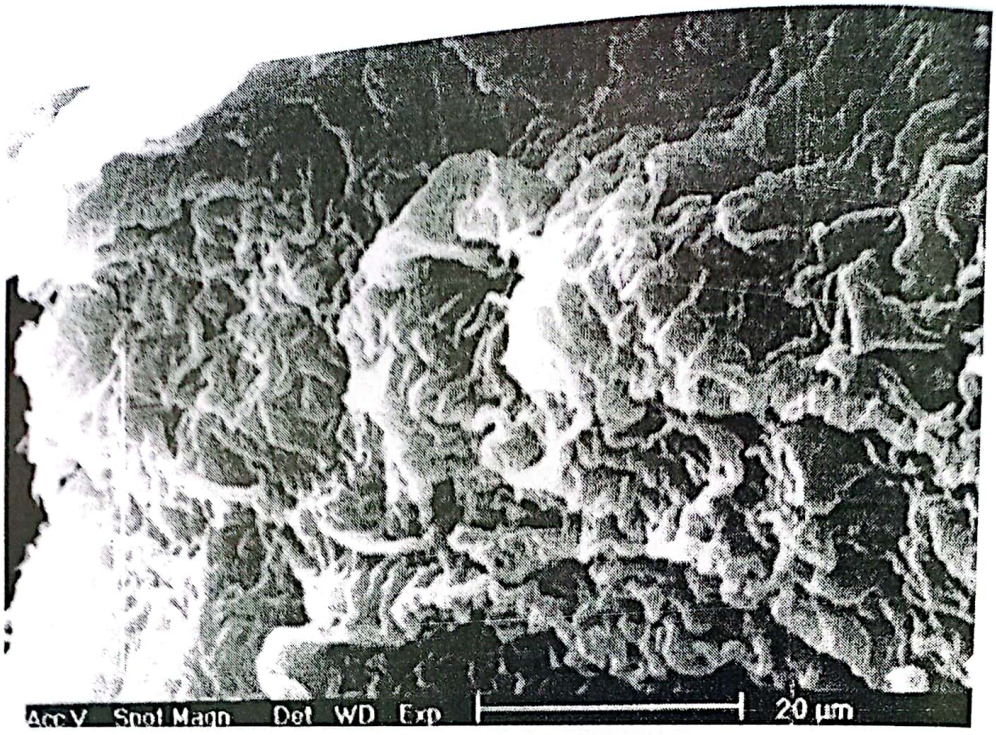

Fig. 26: S E M from the mid-gut of the third larval instar of *G. intestinalis* showing internal folds of mid-gut. X 1500.

Fig. 27: S E M from the mid-gut of the third larval instar of *G. intestinalis* showing internal folds and tubes in mid-gut . X 1000.

DISCUSSION

The mid-gut may be grossly differentiated into anterior mid-gut and posterior mid-gut, correlated with this the cells may appear to be different, or they may appear the same, or different cell types may be interspersed either randomly or in some regular manner.

In general, the anterior and posterior regions of the mid-gut epithelium of the early third larval instar of *G. intestinalis* consists of a single layer of cells which is characterized by: microvillar border, randomly distributed mitochondria, well developed infoldings of the basal plasma membrane, nucleus situated centrally and well developed rough endoplasmic reticulum ramifying extensively in the cytoplasm.

The insect mid-gut is a dynamic organ where the active transport of ion, water and nutrients transverse to the haemolymph. Active transport of ions across the epithelial cells is crucial in controlling many of these fluxes (Andreson and Harvey 1966).

The apical cell membrane of the anterior and posterior regions of the mid-gut of the early third larval instar of *G. intestinalis* is represented by a great number of cylindrical processes or microvilli that project into the gut lumen. The microvillar border is long, slender, well developed and covered externally with the glycocalyx. Similar microvillar structures have been observed in other insects as in *Ephestia Kuhniella* and in *Pectinophora gossypiella* (Smith et al., 1969 and Kamel et al., 1994). The external surface of microvilli is covered with a fuzzy material, which has been referred to as cell coat that is rich in glycoproteins (Santos et al., 1984). The chief role suggested for the glycocalyx is a filter, keeping large particles in the lumen from approaching the plasma membrane (Novikoff and Holtzman, 1970).

Hecker et al. (1971) and Kamel et al. (1994) described that the presence of microvilli, means an enormous increase of the surface area for absorption in the mid-gut epithelia of *Aedes aegypti and P. gossypiella respectively.*

Amino acids are absorbed with potassium ions by a transport mechanism located at the microvillar membranes of Lepidoptera mid-gut cells (Giordana et al., 1982).

The mid-gut cells of the early third larval instar of *G. intestinalis* also revealed that the basal region showed deep infoldings of the basal labyrinth, which are characterized by openings to the haemocele and numerous mitochondria. These mitochondria are numerous, with various sizes and shapes in the posterior mid-gut than that in the anterior mid-gut. This indicates clearly that the posterior region of the mid-gut of this insect is active-

ly involved in the absorption and transportation of nutrients across the epithelial cells to the haemolymph than the anterior region of mid-gut. The extensive infoldings of the basal membrane in the mid-gut which appear in the present study were also reported in various other insects: *Lucilia* larvae, *Calliphora erythrocephala*, non-blood fed *Culex tarsalis* and *Drosophila auraria* by Waterhouse and Wright (1960); De Priester (1971); Houk (1977); Dimitriadis and kastritsis (1984); Dimitriadis and Papamanoli (1992) respectively.

The present study also revealed that, these infoldings of the basal labyrinth in the posterior mid-gut cells of the early third larval instar of *G. intestinalis* have closely associated with mitochondria and extra cellular space than the anterior region. This also may indicate that the posterior mid-gut region of the early third larval instars of G. intestinalis is involved in the absorption and transportation of solutes and nutrients.

Brown (1980) stated that infoldings of the basal labyrinth in the anterior mid-gut cells of *Glossina morsitans* have closely associated mitochondria, which may assist transcellular transport of water and ions by forming an extracellular space within the cell. This space is in contact with the apical and basal regions and is open to the haemolymph via a restricted number of openings .The spaces may be important in setting up diffusion gradients extracellularly, providing for the movement of water and solutes. The fact that the basal plasma membrane infoldings constitute an extracellular compartment, with restricted access to the haemolymph enables the cells from the posterior mid-gut region to concentrate solutes in that compartment, to create an osmotic pressure gradient between that compartment and lumen, which might assist the absorption of water (Berridge, 1970). In the active larvae of *Pectinophora*, the developed basal labyrinth associated with many mitochondria play an important role in the absorption and transport of food substances (Kamel et al., 1994). Recently, El-Sherif and Koura (1995) suggested that the vacuolation of the basal cytoplasm and the presence of well-developed infoldings of the basal plasma membrane closely associated with mitochondria might indicate that the mid-gut is involved in the absorption of nutrients in Anacanthotermes ochraceus.

The basement membrane is very thick and finely granular under the mid- gut epithelium, of the early instar larvae of *G. intestinalis*. This is similar to that in the adult of Rhodinus prolixus (Pacheco, 1970), of the larvae of *Ephestia kuhniella* (Smith *et al.*, 1969) and of *Cantharis fusca* (Holter, 1970).

The results revealed that the cytoplasm of mid-gut cells of the early third larval instar of *G. intestinalis* is packed with free ribosomes, especially that of anterior region. Richards (1975) indicated that

ribosomes are generally thought to be responsible for protein synthesis. And it has been suggested that ribosomes synthesizing digestive enzymes must be attached to endoplasmic reticulum to keep the enzymes segregated to prevent digestion of the cells producing the enzymes. This can be correlated with the fact that the mid-gut cells have much rough endoplasmic reticulum.

Both regions of the mid-gut of the early third larval instar of *G. intestinalis* possess many small and large vesicles of rough membrane. These vesicles appear to be dilated rough endoplasmic reticulum, which indicates high activity of the cell as stated by Richards, (1975).

The marked dilation of rough endoplasmic reticulum was also observed in B cells and acinar cells of STZ - diabetic rats after 6 weeks in accordance with Efart *et al.*, (1990) in diabetic mice , Taniyama et al., (1993) in spontaneous diabetic young cattle and El- Agouza et al., (2000) in diabetic rats

The mid-gut of the early third larval instar of G. intestinalis possessed many dense secretory granules especially in the posterior mid-gut region. The cells of the midgut epithelium with dense granules were described as midgut endocrine cells and contain enzymes (El- Sherif and Koura 1995, Nishutsutsuji and Endo1981 and Stoffolano et al. 1989).

The muscle layer surrounding the mid-gut in both anterior and posterior regions of the early third larval instar of *G. intestinalis* are well developed and the peripheral sarcoplasm contains few mitochondria and numerous trachaea.

It seems that both regions of the mid-gut in the early third larval instar of *G. intestinalis* are involved in digestion and absorption.

The anterior mid-gut in Culicidae is a typical absorptive epithelium, responsible for the absorption of sugars passed into the anterior mid-gut from the gut diverticulum. Also the presence of glycosidases is suggested to support a saccharides digestive role for this region of mid-gut (Billingsley, 1989). Bertram and Bird (1961) stated that the posterior mid-gut in Culicidae is storage, synthetic, secretory and absorptive in functions.

Billingsley (1990) stated that the anterior mid-gut in Glossinidae is responsible for temporary storage of blood meal, regulation of water and ions and for the active transport of water and ions while the posterior mid-gut cells are absorptive and temporary storage epithelium.

The apical plasma membrane of the posterior mid-gut of the late third larval instar of *G. intestinalis* is characterized by thick and very compact degenerating microvilli, while the posterior mid-gut is characterized by apical extrusions into the

mid-gut lumen.

Apical extrusions are suggested as final stages of a secretory process, as a result of degenerative changes induced by starvation or artifacts of fixation (Khan and Ford 1962 and Brunnings and De-Priester 1971, Wigglesworth. 1972).

The appearance of apical extrusions in the posterior mid-gut of the late third larval instar of *G. intestinalis* may be due to ageing process or pupal development.

The anterior mid-gut of the late third larval instar of *G. intestinalis* is characterized by mitochondria that increase in size, become more osmophilic, their cristae disarranged and cristae ñ free areas appear. Anton-Erxleben (1983) stated that these changes might be due to increasing age. The anterior mid-gut also is characterized by numerous old multivesicular bodies which resulted from fusing neighboring degenerating organelles.

The posterior mid-gut of the late third larval instar of *G. intestinalis* is characterized by the presence of trilamellate vacuoles containing amorphous substance. The musculature and tracheation seems to be less developed than that of the early instar in both regions.

Glycogen droplets characterize both regions of this late instar. Wigglesworth. (1972), stated that glycogen in the larva of *Gasterophilus* at one stage of growth may reach 31 % of dry weight, and that it converts store of glycogen into fat to utilize the oxygen set free for energy production. The oxygen is conveyed from the host to the tissues, and the CO_2 resulting from the oxidation in the tissues is eliminated.

The great majority of insects breathe by means of tracheal tubes, which usually open at the surface of the body through a number of spiracles and convey air directly to the tissues. Wigglesworth (1972) stated that the tracheae are invaginations of the cuticle, which branch everywhere among the tissues. In most insects spiracle gives rise to a tracheal tree, and the tracheae anastomose freely to form longitudinal and transverse trunks. There are many modifications of the cuticle to meet special needs .In case of the tracheae; the cuticle lining is thrown into folds, which commonly run a spiral course round the tube. Often the folds become filled with cuticular substance to give rise to a spiral thread or taeniduim.. Also Chapman (1998) stated that in *Gasterophilus* larvae running from the posterior spiracles are four pairs of tracheal trunks which taper and give off short branches at intervals along their lengths. Each branch breaks up into numerous tracheols, which are functionally, if not structurally, within a tracheal cell. This above description of tracheae is true in case of *Gasterophilus* larvae as shown by scanning electron micrographs, which clearly

showed the close relation of the trachea with the mid-gut.

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