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***Research article***

# ULTRA STRUCTURE OF THE MID-GUT OF THE THIRD INSTAR LARVAE OF *SARCOPHAGA AEGYPTIACA* (DIPTERA: SARCOPHAGIDAE)

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**ABSTRACT:** The mid-gut of third instar larvae of *Sarcophag aegyptiaca* was divided into anterior, middle and posterior mid-gut regions. A well-developed peritrophic membrane appeared in the apical part of the anterior and middle portions of mid-gut while it is absent from the posterior portion. The microvilli differs in 3 portions, appeared as apical membrane in the anterior portion, sparse in middle portion to long slender compact microvilli in posterior portion. Numerous organelles were observed throughout the cytoplasm of the 3 portions; lipid spheres, rough endoplasmic reticulum, seceretory vesicles and mitochondria. A large apically nucleus appeared in the 3 portion of mid-gut. A basal labyrinth was observed in both anterior and posterior regions of mid-gut while it was absent in middle portion of mid-gut. Thin basement membrane was observed in the 3 portions of mid-gut of third instar larvae of *Sarcophag aegyptiaca.*

**Key words:** *Sarcophaga aegyptiaca*, Maggots, Ultra structure, Mid-gut.

## INTRODUCTION

The alimentary canal of insects is a tube, normally straight or coiled, which extends from the mouth to the anus, with three main sections having different embryonic origins: the fore-gut or stomodaeum; the mid-gut, made up of the ventriculus and gastric caeca; and the hind-gut or proctodaeum (Uvarov, 1966; Borror and De Long, 1969; Belkin, 1976; Maranhão, 1976). The mid-gut, which has different cell types, is the main organ of an insect's digestive tract, which also has an absorptive function (Cavalcante and Cruz-Landim, 1999). The gastric caeca contains bacteria and other digestive tube microorganisms that produce enzymes and vitamins. In addition, it is through this organ that water is absorbed and nutrients are digested (Gallo *et al.*, 2002). In certain insects, digestion commences in the foregut by virtue of salivary gland secretions or enzymes regurgitation from the midgut. Rare instances of extra-intestinal digestion have also been reported in some insects (Chapman, 2013). Flies in the family Sarcophagidae are commonly known as flesh flies. They differ from most flies in that they are ovoviviparous, opportunistically depositing hatched maggots instead of eggs on carrion, dung, decaying material, or open wounds of mammals, hence their common name. Some flesh fly larvae are internal parasites of other insects such as Orthoptera (Richards and Davies, 1977). The *Sarcophaga* species belong to the family Sarcophagidae, and are found worldwide in various environments (Roback, 1956). The adult flesh fly is approximately 13 mm long and is characterized by a black and grey striped body. The *Sarcophaga* species feed on dead and decaying matter; however, not all species of the flesh fly consume decomposed flesh. In addition, their life cycle is temperature dependent (Woodmorappe, 1998), and their larvae goes through three instars before developing into pupae.

The aim of the present study is to clarify the structure of midgut cells in *Sarcophaga aegyptiaca* and correlate it with the physiological state of the insect to study the physiology of digestion of this insect.

## MATERIALS AND METHODS

### 1- Rearing of insect

A colony of *Sarcophaga aegyptiaca* originating from field-collected specimens was established in the laboratory at 28±1°C and 65±5% R.H. The adults were fed 10% sucrose solution. A piece (~100 g) of fresh beef was offered as a larviposition medium and also as a diet for larvae. Prior to pupation, the larval rearing medium was covered with a thin layer of sawdust for pupariation**.**

### 2- Dissection of organs

Mid-guts of third instars larvae of *S. aegyptiaca* were dissected using fine entomological needles under a stereoscopic microscope at 4X magnification in phosphate-buffered saline [PBS; 10 mM $Na_2SO_4$, 145 mM NaCl (pH 7.2)] and transferred to a micro centrifuge tube with a small volume of PBS. The mid-guts were divided in to anterior, middle and posterior portions.

### 3- Transmission electron microscope preparations:

Approximately 20 specimens of 3 day old larvae were removed from the rearing box and individually dissected in phosphate buffer pH of 7.4 under a binocular dissecting microscope (Olympus®, Japan). The mid-gut portions were separated from alimentary canal. The dissected mid-guts were transferred from the phosphate buffer and prefixed with 2.5% glutaraldehyde in phosphate buffer solution at a pH of 7.4 at 4 °C for 24 h to accomplish primary fixation. Then rinsed twice with phosphate buffer solution at 10 minutes intervals. Rinsed specimens were treated with 1% osmium tetroxide at room temperature for 30 minutes for post-fixation. Post-fixation was followed by rinsing twice with phosphate buffer solution and dehydrating with alcohol. To replace the water in the specimens with alcohol, they were subjected to ascending series of alcohol. After that, organ specimens were placed in acetone for 10 minutes before transferring into ratios of resin to acetone of 1:3 for 24 h, 1:1 for 24 h, and 3:1 for 24 h, sequentially. This was followed by treatment with pure resin twice for 3 h. Each sample was then embedded in Epon resin by placing them into a plastic block and by incubating at 70°C for 24 h. Semi-thin section (0.5 µ) of each sample was made with a glass knife on an ultra microtome (Boeckeler®, USA). This was followed by staining with 1% methylene blue mixed with 1% Azure II (1:1) to view under a light microscope (Olympus®, Japan). The ultrathin sections (90 nm) were stained with uranyl acetate and lead citrate then examined with the ZEISS EM 10 electron microscope (Germany).

## RESULTS

The mid-gut was divided into anterior, middle and posterior mid-gut regions. In the anterior part of the mid-gut of third instars larvae of *S. aegyptiaca*, a well-developed peritrophic membrane appeared in the apical part (Plate 1, Fig 1a, b, c, d). Microvilli appeared compact in parts of anterior midgut (Plate 1 Fig. 1a) while in other parts appeared as an apical membrane that is compressed by numerous rough endoplasmic reticulums (rer) (Plate 1 Fig. 1c, d). A large well-developed nucleus was observed apically (Plate 1 Fig. 1b). A lateral cell membrane was also observed separating two cells in anterior portion of mid-gut of third instar larvae of *S. aegyptiaca* (Plate 1 Fig. 1e). The basal part of anterior mid-gut of third instar larvae of *S. aegyptiaca* possessed thin basement membrane with well-developed musculature and tracheae insertions (Plate 1 Fig. 1f). A basal labyrinth was observed in the basal part of anterior mid-gut of third instars larvae of *S. aegyptiaca* (Plate 1 Fig. f). Numerous small lipid spheres, mitochondria and secretory vesicles were observed in apical part of the anterior portion of mid-gut of third instars larvae of *S. aegyptiaca* (Plate 1 Fig. a, b).

The middle portion of mid-gut of third instars larvae of *S. aegyptiaca* showed apically two layers of peritrophic membrane one thick and the other thin (Plate 2 Fig. a, c). Microvilli were observed but are sparse and less developed (Plate 2 Fig. b, c). A large well-developed nucleus was observed apically (Plate 2 Fig. a). The basal part possessed thin basement membrane with well-developed muscles and tracheae (Plate 2 Fig. d). Numerous organelles were observed throughout the cytoplasm; mitochondria, secretory vesicles, dense granules and rer (Plate 2 Figs. a, b, c, d). No basal labyrinth was observed in the middle portion of mid-gut of third instars larvae of *S. aegyptiaca*.

The posterior portion of mid-gut showed apically a very well-developed striated border represented by long slender compact microvilli (Plate 3 Fig. a, b, c). In other part this long slender compact microvilli became wide and appeared just as an apical membrane (Plate 3 Fig. b, c, d). Beneath this apical membrane, a lot of secretory material appeared that is ready to be discharged in the lumen (Plate 3 Fig. b, c, 3).

A lot of secretory vesicles with different sizes, rer, dense bodies, polymorphic mitochondria and numerous lipid spheres with different sizes appeared throughout the cytoplasm (Plate 3 Fig. a, b, c, d). Some of these lipid spheres can be seen in the lumen of mid-gut cells (Plate 3 Fig. d). A very large well-developed nucleus appeared near apical part of posterior portion of mid-gut of third instar larvae of *S. aegyptiaca* (Plate 3 Fig. b, d). The basal part of posterior portion of mid-gut of third instars larvae of *S. aegyptiaca* possessed thin basement membrane with well-developed musculature and tracheae insertions (Plate 3 Fig. f, g, h). A well-developed basal labyrinth was observed in the basal part of posterior mid-gut (Plate 3 Fig. g).

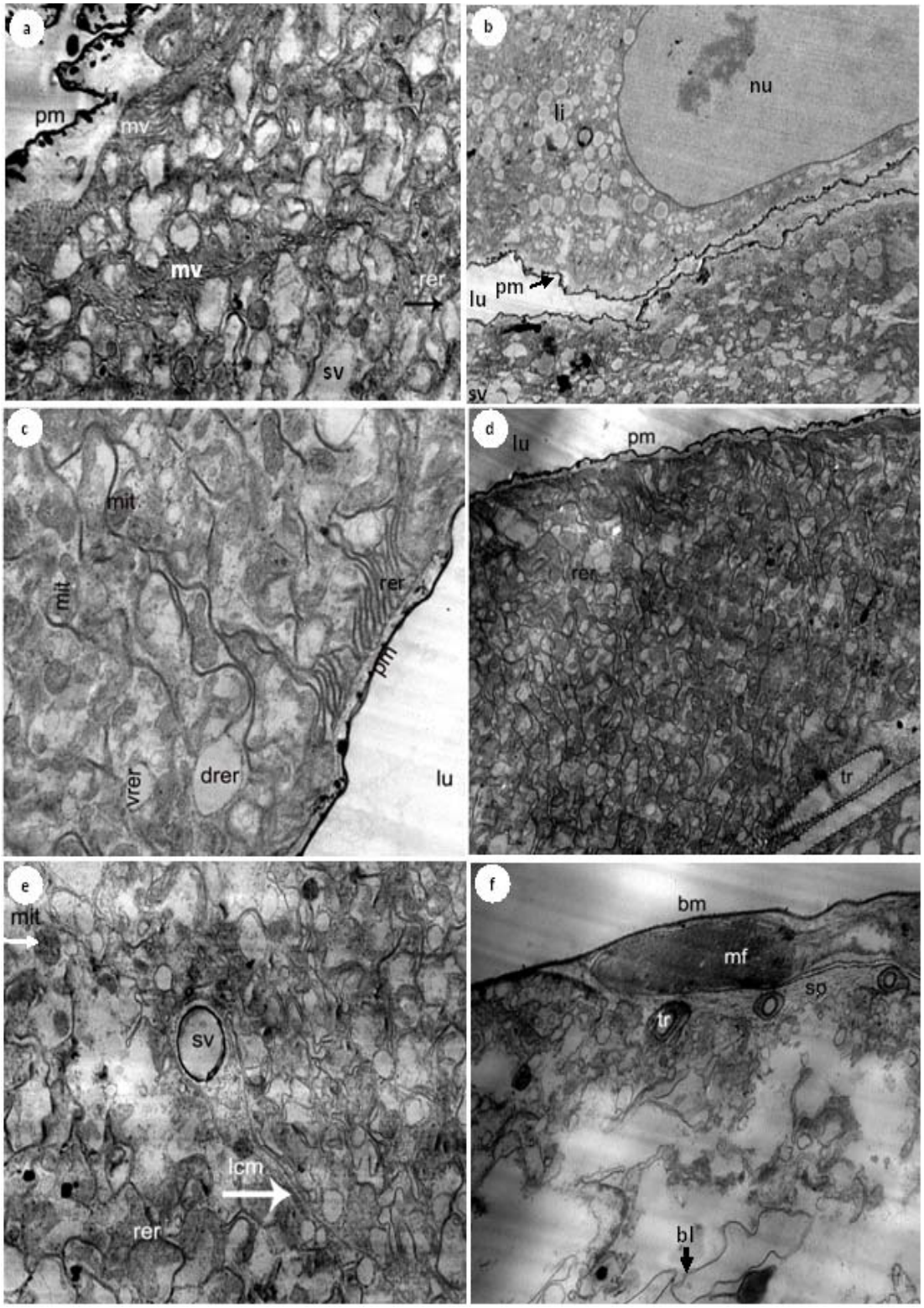


**Plate (1): Electron micrograph of anterior midgut of third instar larvae of *Chrysomya megacephala*: Showing apically microvilli (mv), peritrophic membrane (pm), secretory vesicles (sv) Fig. (a); lipid spheres (li), large nucleus (nu), lumen (lu) Fig. (b); apical membrane (arrow), rough endoplasmic reticulum (rer), mitochondria (mi), vesicles of rough endoplasmic reticulum (vrer) Fig.(c); tracheae (tr), Fig. (d); lateral cell membrane (lcm) Fig. (e); thin basement membrane (bm), muscle fiber (mf) and basal labyrinth (bl) Fig. (f).**

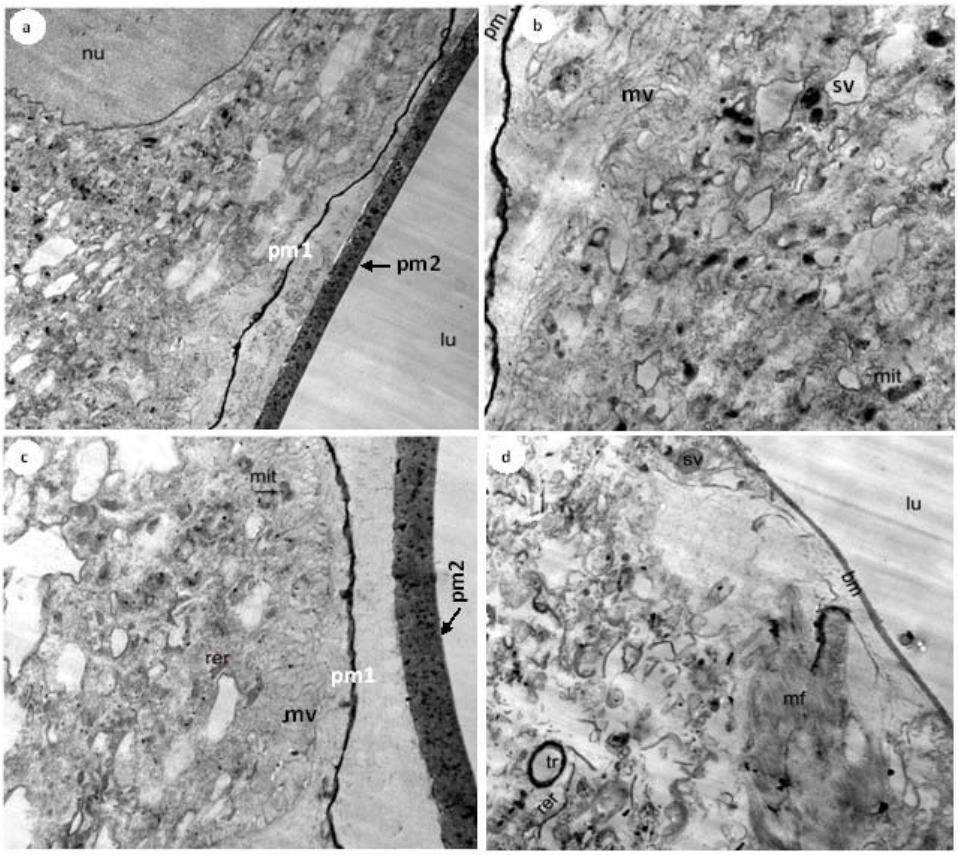


**Plate (2): Showing apically two layers of peritrophic membrane (pm1, pm2) Fig. (a); nucleus (nu), sparse microvilli (mv), secretory vesicles (sv), mitochondria (mi), Fig.(b,c); thin basement membrane (bm), muscle fiber (mf), tracheae (tr), rer Fig.(d).**

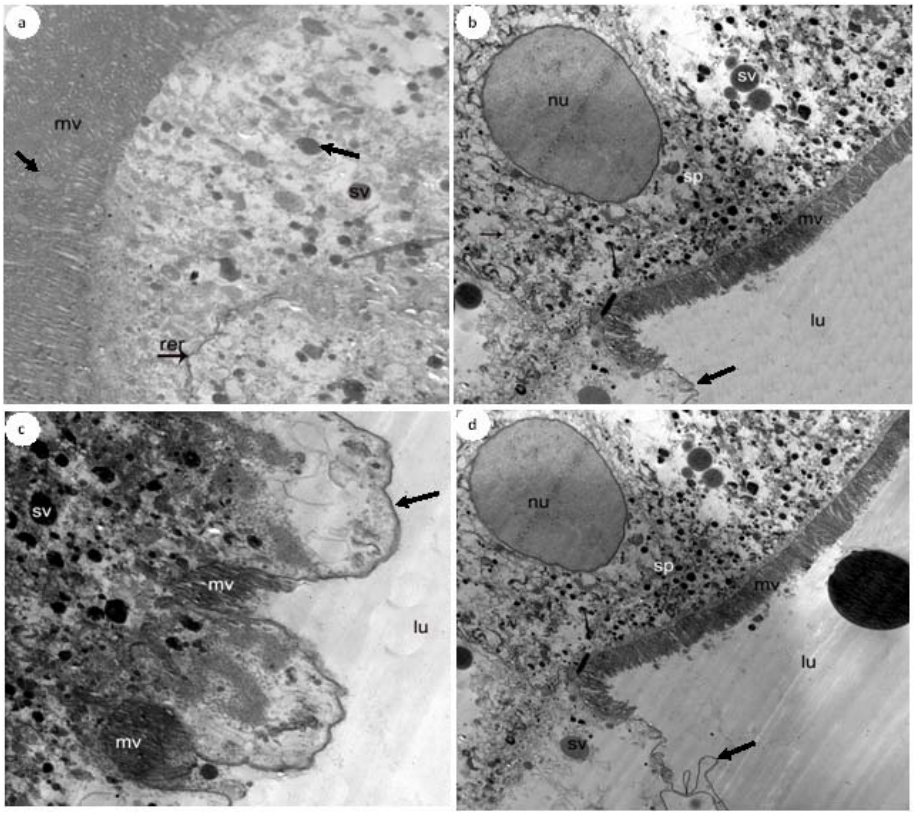


Plate-3

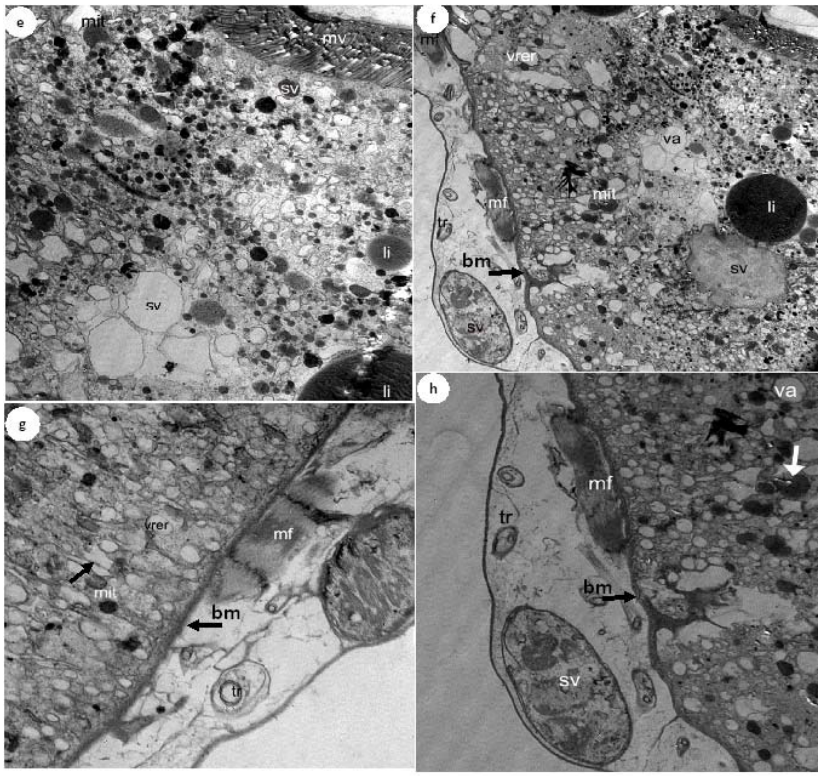


**Plate (3): Electron micrograph of middle midgut of third instar larvae of *Chrysomya megacephala*: Showing apically microvilli (mv), vesicles (arrow), rer Fig. (a); nucleus (nu), long, slender microvilli (mv), vesicles (sv), apical membrane (arrow), secreotry product (sp) Fig.(b,c); lipid sphere in the lumen Fig. (d); microvilli (mv), secretory vesicles (sv), lipid spheres (li), mitochondria Fig. (e); thin basement membrane (bm), muscle fibers (mf), tracheae (tr) Fig. (f,h); basaal labyrinth (arrows) Fig. (g).**

## DISCUSSION

The insect mid-gut is the chief site both for the digestion of food and absorption of nutrition, and the epithelium is responsible both for the production of many digestive enzymes and/or the uptake and transfer of nutrients to the haemolymph (Wigglesworth, 1965).

Aspects of the fine structure of mid-gut cells have been described in several electron microscopic studies, including the mid-gut epithelium of *Calliphora* by Waterhouse and Wright (1960), and the ultrastructure and physiology of the mid-gut epithelium of *Ephestia* by Smith *et al*. (1969). Ferreira *et al*. (1981) examined the mid-gut intercellular structure of the larvae of *Rhynchyosciara* fly and Dimitriadis (1991) examined the mid-gut cells of the adult *Drosophila auraria*. Also Taha *et al.* (2010) studied the mid-gut cells of the larvae of *Chrysomya megacephala*.

The architecture of the mid-gut of early third instar larvae of *S. aegyptiaca* is the same as those reported for *Drosophila* (Dimitriadis, 1991), and similar to those of other dipteran insects: *Musca domestica* (Sohal *et al*., 1977), *Calliphora erythrocephala* (Priester, 1971), *Aedes aegypti* (Rudin and Hecker, 1979), *Anopheles gambiae*, *A. stephensi*, *Culex pipien fatigans* (Hecker, 1977), *Lutzomya longipalpis* (Rudin and Hecker, 1982) and *Chrysomya megacephala* (Taha *et al.*, 2010).

A well-developed peritrophic membrane was observed running over microvilli in both anterior and middle sections of mid-gut of early third instar larvae of *S. aegyptiaca* while it was absent from posterior section. The mid-gut epithelium of insects lacks a permanent uniform intima (cuticle) (Snodgrass, 1935). In many insects, a peritrophic membrane exists between the food bolus and the mid-gut epithelium. This structure secreted by the mid-gut cells is believed to protect the mid-gut cells from mechanical damage caused by food particles (Smith, 1968). In the present study two layers of peritrophic membrane were observed in the middle section of mid-gut. Several layers are always resolved in other insects, in the mid-gut of *Drosophila* and fruit fly peritrophic membrane contains two layers (Hung *et al*., 2000), and in blowfly there is a three-layer membrane (Waterhouse and Wright, 1960). The peritrophic envelope functions as a barrier, separating the midgut cells from the ingested food. In this way it can protect the gut wall from damage by ingested material, including abrasive food particles, pathogens and certain toxins.

In addition, the peritrophic envelope separates the gut lumen into two compartments: the endo-peritrophic space (between the peritrophic envelope and the midgut cells) and ecto-peritrophic space (in the gut lumen), so permitting compartmentalization of enzyme activities and the generation of a countercurrent flow of fluids that increases the efficiency of absorption (Chapman, 2013). The third instar larvae of *S. aegyptiaca* possessed two ecto-peritrophic spaces due to presence of two layers of peritrophic membranes.

The apical part of anterior midgut possessed microvilli in the form of apical membrane due to pressing of rough endoplasmic reticulum. This structure was previously described in the epithelium of salivary glands of *Chrysomya megacephala* larvae (Taha, 2015). In the middle section of the mid-gut microvilli appear sparse while in the posterior section, microvilli appeared compact, long and slender in part and other part appeared as apical membrane due to pressing lipid spheres that project into the lumen, as previously described by Abdel-Meguid *et al.* (2013). These microvilli provide an enormous surface area for absorbing material from the lumen (Romoser, 1996). Many insects show interesting variations in the basic theme of microvillar fine structure. In certain Diptera the striated border sometimes shows unusual structure, being formed of lamellae instead of microvilli (Waterhouse and Wright, 1960) .Waterhouse and Wright (1960) stated that in the middle mid-gut of *Lucilia*, the lumen borders of both the lipophilic and cuprophilic cells differ strikingly from the usual picture of a striated border.

A very large well-developed nucleus was observed apically in the three sections of mid-gut of early third instar of *S. aegyptiaca*. The large nucleus is a characteristic of active cells, in which large quantities of nucleic acids move in and out of the nucleus to generate synthesis and secretion of proteins (Evangelista and Leite, 2007)

The cytoplasm of anterior section of mid-gut of early third instar of *S. aegyptiaca* is full of rough endoplasmic reticulum with its different shapes stacks and vesicles. Bertram and Bird (1961) suggested that the rough endoplasmic reticulum might be responsible for all the secretory and absorptive functions ascribed to the mid-gut. Most authors interpret the phenomenon of vesicles of rough endoplasmic reticulum as a transition of the cell synthetic apparatus into a more active state (Filimonova, 1989).

These rough endoplasmic reticulums are directed apically in the cell, preparing to release their products through microvillus. This may explain the appearance of microvilli as an apical membrane due to pressure of secretory products. Numerous mitochondria and secretory vesicles were seen throughout the cytoplasm of anterior section of mid-gut of early third instar of *S. aegyptiaca* and between rough endoplasmic reticulum. This may be due to that high energy is needed to carry out protein synthesis (Boonsriwong *et al.,* 2012). Some vesicles are distributed in the microvilli of apical part of the cells of the posterior mid-gut in the studied species, they may be released into the gut lumen through the apical plasma membrane by exocytosis. Microvillar vesiculation was found in *Calliphora* (Priester, 1971) and in *Chrysomya megacephala* (Taha *et al.,* 2010). Different routes of enzyme secretion have been reported. Membrane-bound vesicles may move to the periphery of the cell, fuse with the cell membrane and release their contents into the gut lumen by exocytosis. The gut principal cells have also been interpreted to display apocrine secretion, *i.e.,* the pinching-off of cytoplasmic extensions of the cell, carrying their contents into the gut lumen and ultimately releasing them, but this putative process is contentious because the principal evidence is microscopical and could be an artifact of fixation methods. Intracellular vesicles containing enzymes may also be transported to the microvilli, from which they bud off before releasing their contents. Different enzymes may leave a single cell by different routes, and different methods of secretion may occur in one insect (Chapman, 2013). Therefore the enzyme secretion in third instar larvae of *S. aegyptiaca* may take place by exocytosis and intracellular vesicles.

The cytoplasm of posterior section of mid-gut of early third instar of *S. aegyptiaca* is full of lipid droplets, secretory vesicles and a lot of dense granules. These lipid droplets press microvilli and projects into the lumen. Numerous lipid inclusions suggest that posterior section of mid-gut of early third instar of *Sarcophaga aegyptiaca* plays a major role in lipid absorption and energy storage (Abdel-Meguid *et al.,* 2013). The three sections of mid-gut of third instar of *S. aegyptiaca* possessed thin basement membrane. Well- developed tracheae and muscle insertions were observed in basal part of the three sections of mid-gut. Probably more tracheae were observed in cytoplasm of anterior region of mid-gut of third instar of *S. aegyptiaca.* This may reflect that a high level of oxygen was supplied from the respiratory system (Boonsriwong *et al.,* 2012).A basal labyrinth was observed in both anterior and posterior sections of mid-gut of third instar of *S. aegyptiaca*. This aspect is common to the Diptera insects which could be attributed to the transport of components (Lehane and Billingsley, 1996).

## CONCLUSION

It is clear from the previous results and discussion that the anterior midgut of the early third instar of *Sarcophaga aegyptiaca* plays a major role in production of enzymes and digestion of food, while posterior part is concerned more with the lipid absorption and energy storage.

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