

ULTRASTRUCTURE OF MIDGUT CELLS OF NORMAL AND IRRADIATED POTATO TUBER WORM *PHTHORIMAEA OPERCULELLA* ZELLER (LEPIDOPTERA, GELECIIDAE)

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INTRODUCTION

The potato tuber worm, *Phthorimaea operculella* Zeller (Lepidoptera, Geleciidae), represents one of the most important insect pests, especially in the developing countries, as well as many other parts of the world. It is one of the destructive pests of potato plants and tubers *Solanum tuberosum* L., both in the field and in storage. It may also attack other solanaceous truck crops in Egypt, such as eggplant (*Solanum melanogena* L.).

The efficient control and removal of insects from food commodities had long been the goal of producers and processors. Irradiation could offer a suitable solution, and its use is cheaper, safer and more reliable than chemical methods, and is a good method for the protection and conservation of stored products (Haiba, 1990).

Previous studies reported that, the larval mid gut of some Lepidoptera especially the epithelial cells were sensitive to the gamma irradiation causing a damage including destruction of regenerative nidi, lack of brush border and vacuolation of epithelial cells (Szezepanik and Ignatowicz, 1995).

The aim of the present work is to study the effects of sterilizing dose of gamma radiation on the anterior and posterior regions of midgut cells in the female potato tuber moth, also to estimate the damage that could occur.

MATERIAL AND METHODS

The strain of the potato tuber moth, *Ph. operculella* was reared and maintained in the laboratory of Entomology at the National Center for Radiation Research and Technology (NCRRT), as described by Haiba (1990).

Five-day old female pupae were exposed to sterilizing dose (previously tested and was found to cause sterilization to the species used) of gamma irradiation, 150 Gy using ^{60}CO source with dose rate 3.7 rad/ sec.

Electron microscopic study

Four-days old (non irradiated and irradiated) females were dissected in a prefixative solution (3% gluteraldehyde in 0.076 M cacodylate buffer pH 7.2 containing 29 mM sucrose). The anterior and posterior regions of midguts were taken and left in the prefixative for 2 hrs at 4°C, thoroughly rinsed overnight at 4°C in cacodylate buffer pH 7.2, then postfixed in 1% osmium tetroxide in cacodylate buffer. The specimens were washed in distilled water and dehydrated in a graded series of acetone, left in filtration medium (acetone, epon resin 1:1) for several hrs in slow revolving drum. Specimens were embedded in epon resin followed by polymerization for 2 days at 60 °C. Ultrathin sections were made and stained with saturated uranyl acetate and counterstained with lead citrate. Sections were examined using a Philips TEM 301 at 80 and 100 KV.

RESULTS AND DISCUSSION

Ultrastructure of normal (non-irradiated) midgut cells

Functionally, the midgut cells were attributed to be responsible for the synthesis and/ or secretion of digestive enzymes, absorption and transport of products of digestion (Kamel *et al.*, 1994; Mahmoud, 1997).

The fine structure of midgut cells of normal 4-days old (non-irradiated) *Ph. operculella* is generally similar to other insect midgut cells (Ryerse *et al.*, 1992, Kamel *et al.*, 1994). In the female, the midgut cells need 3-4 days after emergence to reach full differentiation (Smith *et al.*, 1969, Kamel *et al.*, 1994), so the present study dealt with four days old female, to ensure full differentiation of midgut cells would be complete. Nonetheless, a variety of differences related to the metabolic function of the midgut in both anterior and posterior regions have been observed.

The present result showed that, the apically folded plasma membrane, microvilli of the *Ph. operculella* midgut cells are arranged in a regular array in the anterior region and it is irregular and somewhat broken in the posterior region of the midgut (Figs. 1, 7). The surface of microvilli is coated with a fine layer of intermediate electron density, the glycocalyx, it is similar to that observed in other lepidopterans; *Ephestia kuchniella* and *Pectinophora gossypiella* (Smith *et al.*, 1969, kamel *et al.*, 1994), and it was found rich in glycoprotein (Santos *et al.*, 1984). The role of the glycocalyx was suggested to work as a filter to keep large particle away from approaching the plasma membrane (Novikoff and Holtzman, 1970). In addition to increasing the surface area, the microvilli play a role in the movement of substrates from within the midgut to the epithelial cells and eventually to the haemolymph (Hecker *et al.*, 1971).

In the anterior and posterior regions of the midgut of *Ph. operculella*, very minute shapes and sizes are associated with microvilli (Figs. 1, 7). These are similar to that observed and described by Baerwald and Delcarpio (1983) as microtubules. Large number of vesicles and blebs appear to slough from the microvilli in the posterior midgut region and fewer appear in the anterior region of the adult *Ph. operculella*. They were suggested as "Fuzz bearing" vesicles arise as nipped of microvillar tips (Santos *et al.*, 1984). Other forms of secretion along the microvilli observed in the posterior midgut region of *Ph. operculella*, were suggested by Ryerse *et al.*(1992), as profiles through the tips of the intact microvilli out of the plane of the midgut section.

Raes *et al.* (1994), reported that the ultrastructural studies of the midgut epithelium of adult honeybee revealed that the microvilli of the regenerative crypt cells develop within a spherical extracellular space where glycosaminoglycans are secreted. These microvilli are supported by microfilaments that extend into the cortical cytoplasm of the cell (Cruz-Landim *et al.* 1997).

Ph. Operculella adult female, the lateral cell membranes of the adjacent cells are separated by more or less constant intercellular spaces, which in apical and middle regions is connected by septate junction, while the gap junctions could be detected in the apical and basal regions of the midgut epithelium (Figs 2, 3 & 8). These findings are in agreement with Hecker (1977), and Rubin and Hecker (1982).

The present work revealed that, the basal region of the columnar cells is deeply infolded forming the basal labyrinth filled with mitochondria, and bound externally by muscle and tracheal cells (Figs 5, 6, 11& 12).

Mitochondria are observed scattering through the cytoplasm in larger number in the anterior midgut than that of the posterior one (Figs. 4, 6& 8), indicating that the anterior part is actively involved in the absorption and transportation of nutrients across the epithelial cells (Kamel, *et al.*, 1994; El-Sherif and Koura, 1995). The extensive infoldings of the basal membrane in the midgut were also reported in various other insects (De Priester, 1971; Houk, 1977; Dimitriadis and Kastritsis, 1984; Dimitriadis and Papamanoli, 1992).

In agreement with the report issued by Dimitriadis and Kastritsis (1984), Santos *et al.* (1984), and Kamel *et al.* (1994), the nuclei of the midgut cells appear oval, quite large. The nucleus possesses two nuclear membranes, traversed by nuclear pores. Ribosomes are found associated with the nuclear membranes. The nucleoplasm is floccular in appearance, and the nuclear chromatin is clumped into patches of varying densities (see Figs. 4, 9).

The rod-like cisternae of the rough endoplasmic reticulum is found mainly in the vicinity of the nucleus of the anterior and posterior midgut cells; its outer membranes are covered with abundant ribosomes. Narrow smooth endoplasmic reticulum was also detected (Figs. 3& 8). A rather floccular appearance in the cytoplasm, are the free ribosomes, which are found in small groups, polysomes, or as a single particles, monosome (Figs. 3, 8). The whorls of the RER were also observed, and are considered as a means of increasing the synthesis of protein digesting enzymes (Hecker, *et al.*, 1971; Kamel *et al.*, 1994; Serrao and Cruz-Landim, 1996).

In accordance with Smith *et al.* (1969); Young (1979); Dimitriadis (1985); kamel, *et al.*(1994) and Mahmoud (1997), large number of primary, secondary lysosomes, dense bodies and coated vesicles were seen accumulated in the cells of the anterior and posterior midgut regions of *Ph. operculella* (Figs. 4, 9). The dense bodies observed in the midgut cells of *Ph. operculella*, was previously illustrated in the midgut cells of *S. eridania*, they were suggested to play a prominent role in the involution at the prepupal stage, presumably the *in vivo* mobilization of the enzymes released from lysosomes is under hormonal control (Young, 1979).

The lysosome concept encompasses a variety of mechanisms and structures unified by the environment of acid hydrolysis secreted within a cell, in the intracellular digestion either of segregated portions of the cell or droplets of the extracellular milieu ingested by endocytosis (Smith *et al.*, 1969).

The autophagic vacuoles, lysosomes, and multivesicular bodies were observed and similarly described in many other insect midgut cells as in: nurse bees (Serror and Cruz-Landim, 1996), *C. tarsalis* (Houk, 1977), *A. aegypti* (Hecker, *et al.*, 1971), *C. erythrocephala* (De Priester, 1971), *E. ello* (Santos *et al.*, 1984); *D. auraria* (Dimitriadis, 1985), and *E. megacephalus* (Mahmoud, 1997). Coated vesicles were found to originate from pinocytotic activity at the base of the midgut microvilli, fuses with primary lysosomal structures, producing the profile of the multivesicular bodies. Acid and alkaline hydrolase activities have been detected in the multivesicular bodies in support of this hypothesis (Locke and Sykes, 1975).

The present investigation also has shown the existence of a closed type of endocrine cells, which are localized in the basal region of the midgut of adult female *Ph. operculella* (Fig. 5). Similar observations were reported in the midgut of both *E. ello* and *E. megacephalus* (Santos *et al.*, 1984 & Mahmoud, 1997). The endocrine cells play a role in the elucidation of hormone function and phylogeny (Brown *et al.*, 1985).

In the posterior region of the midgut of female adult *Ph. operculella*, few regenerative cells were observed at the base of the epithelium (also termed basal cells), with irregular outline, large space surrounding them, and smaller mitochondria (Fig. 10).

In the present study, the ultrastructural examination showed a few number of spheroidal lipids are distributed throughout cytoplasm of the *Ph. operculella* midgut cells (Figs. 5, 9). Similar result was observed in *Anopheles* as the storage products (Hecker, 1977). While, in many other insect midgut cells numerous small lipid droplets or areas of lipid storage as in *Lutzomyia longipalpis*, *Drosophila auraria* larva and *Eristalis megacephalus* (Rubin and Hecker, 1982; Dimitriadis and Kastritsis, 1984; Mahmoud, 1997, respectively) indicating that the cells, in which they are found, are absorbing them from the gut lumen.

Turunen and Chippendale (1989) showed that, the anterior midgut of *Diatraea grandiosella* is the main site of absorption and transport of lipids while middle and posterior midgut are simple storage sites. Billingsley and Downe (1989) proposed that the excess of lipid storage in the crop of *R. prolixus* may be required for egg production and could be important in long term survival of starved insects.

In the posterior midgut region *Ph. operculella*, regenerative cells (nidi) were observed with rounded or ovoid nuclei containing characteristic inclusions similar to that previously described in many insect species (Smith *et al.*, 1969; De

Priester, 1971; Houk, 1977). El-Sherif and Koura (1995) reported the presence of well-developed Golgi complex accompanied by some secretory granules in the regenerative cells indicating that, these cells are involved in the process of secretion of digestive enzymes essential for the food of termites.

The muscle surrounding the midgut in both anterior and posterior regions of *Ph. operculella* are well-developed circular and the longitudinal muscles contain lateral nuclei, mitochondria, lamellar bodies and tracheoles and T-system in longitudinal muscle (Figs. 6& 11). This structure of the midgut muscles supports the theory of Smith (1968), which revealed that smaller visceral fibers may be activated by the relatively slow passage of ions from the haemolymph through the plasma membrane of the muscle nucleus, reducing the need for elaborating sarcotubular system, while larger fibers probably do rely on a sarcotubular system. So, the function of the longitudinal muscle may be necessary for the excitation of the muscle cell.

The results of the present study for midgut muscles support the observations of Dimitriadis and papamanoli (1992) in the crop epithelium of *D. auraria*. Recently, it was found that the muscular activity of the hoverfly midgut in both larvae and adults is confined to a churning action and a slight peristaltic movement that could be generated by any two- muscle layers (Mahmoud, 1997). It seems most likely that the circular and longitudinal muscle layers are the main determinant of the gut movements.

Ultrastructure of irradiated midgut cells

Various ultrastructural changes caused by the sterilizing gamma dose (150 Gy), in adult female *Ph. operculella* sacrificed four days post irradiation. The microvilli of the anterior and posterior regions appeared irregular and broken, and large amount of cell debris were found including cell organelles (Figs. 13, 20). The outer membrane appears to slough from the microvilli into the lumen in various broken blebs (Figs. 13, 19).

The results of the ultrastructure clearly emphasized and confirmed the cellular damage occurs in the midgut cells of 4 days-old female *Ph. operculella*, following gamma irradiation at 150 Gy. The apical cell membrane showed varying degrees of irregularity, appeared as breaks in the microvilli border into the lumen in the form of blebs, large quantities of cell debris were expelled in the lumen. These pathological changes due to irradiation have been recognized in most insects e.g.:

denudation of microvilli followed by release of whole cells or cell organelles into the lumen (Ducoff, 1972; Read, 1980).

The nuclei in the anterior and posterior midgut regions showed irregular shapes, some of the nuclear edges were more rippled, and the outer nuclear membrane was distinctly granular (Figs. 14, 23). As a result of shrinkage of the cells, the nuclei were observed close to each other and appeared almost aggregated. In the anterior and posterior regions of the midgut, the chromatin appeared distributed in large and small clumps (Figs. 14& 23). Similar results were reported including swollen or shedding of the nuclei into the lumen and coagulation of the chromatin (Jafri and Ismail, 1977).

In deteriorating cells, irregularities in the mitochondrial structure could be observed in the anterior and posterior midgut regions, including membrane swelling, fusion of mitochondria, disarray of mitochondrial cristae with more or less disintegration (Figs. 15, 21). Similar results of the mitochondrial damage were observed in midgut epithelium of *D. melanogaster* (Gartner, 1973); *Aedes aegypti* (Read, 1980) and *G. palpalis* (Stiles *et al.*, 1989). It is assumed that, this damage of mitochondria is accompanied with a considerable reduction in the supply ATP to the basal membrane, which would affect the active transport in this region and could therefore affect the nutrient absorption.

Distinct variation in the lysosomal structure was found in the irradiated female *Ph. operculella*. The large secondary lysosomes (autophagic vacuoles or residual bodies) were distinctly accumulated in the middle region of the cell, appeared continuous with the RER cisternae and packed with the cellular debris (Figs. 15, 22, 23).

The autophagic vacuoles in the midgut epithelium of irradiated *Ph. operculella* appeared larger in size, and the cytoplasm of the anterior and posterior midgut cells showed extensive vacuolation, which could be due to the formation of secondary lysosomes. It is believed that there is an increase in the free lysosomal enzyme content of the cell following irradiation due to the membrane damage; this leakage of lysosomal enzymes may cause degradation of most cellular macromolecules and result in cell death (Abdel-Meguid, 1982). Some of the large autophagic vacuoles were observed to contain cell debris, which may be also expelled in the lumen.

In agreement with Stiles *et al.* (1989) concerning *G. palpalis* midgut cells, many epithelial midgut cells of irradiated *Ph. operculella*, were found to contain large number of residual bodies and showed increase in electron dense materials.

In comparison to the gut of the normal adult moth, the basal cell membrane appeared more folded almost reaching approximately up to the middle region of the cells (Figs. 17, 21). While, the basal labyrinth becomes narrower and form irregular invaginations extending quite far into the cells (Figs. 15, 22). On the other hand, no evident damage to the basal lamina was observed in either the anterior or posterior region of the midgut (Figs. 17, 23).

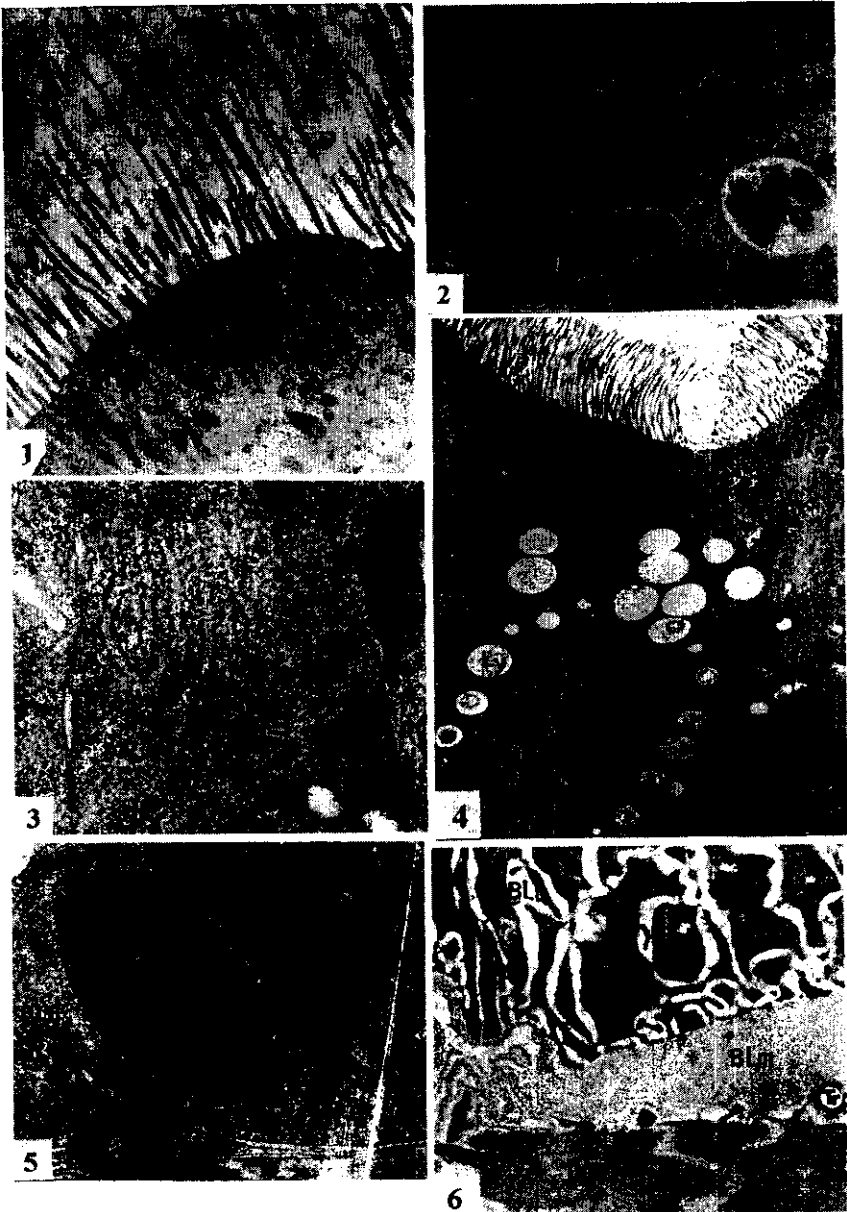
The regenerative nidi of all insect species studied were found to be very susceptible to irradiation and damage induced to them results in the loss of midgut epithelium as in lepidopteran *P. interpunctella* (Szczepanik and Ignatowick, 1995), *S. gregaria* (Quereshi *et al.*, 1975), and *T. confusum* ((Szczepanik and Ignatowick, 1994).

Variable degree of damage of gut musculature was observed after irradiation, irregularities were observed in both circular and longitudinal muscle layers with the presence of large number of polymorphic, swollen mitochondria, tracheoles, lysosomal and few lipid spheres (Figs. 18, 24).

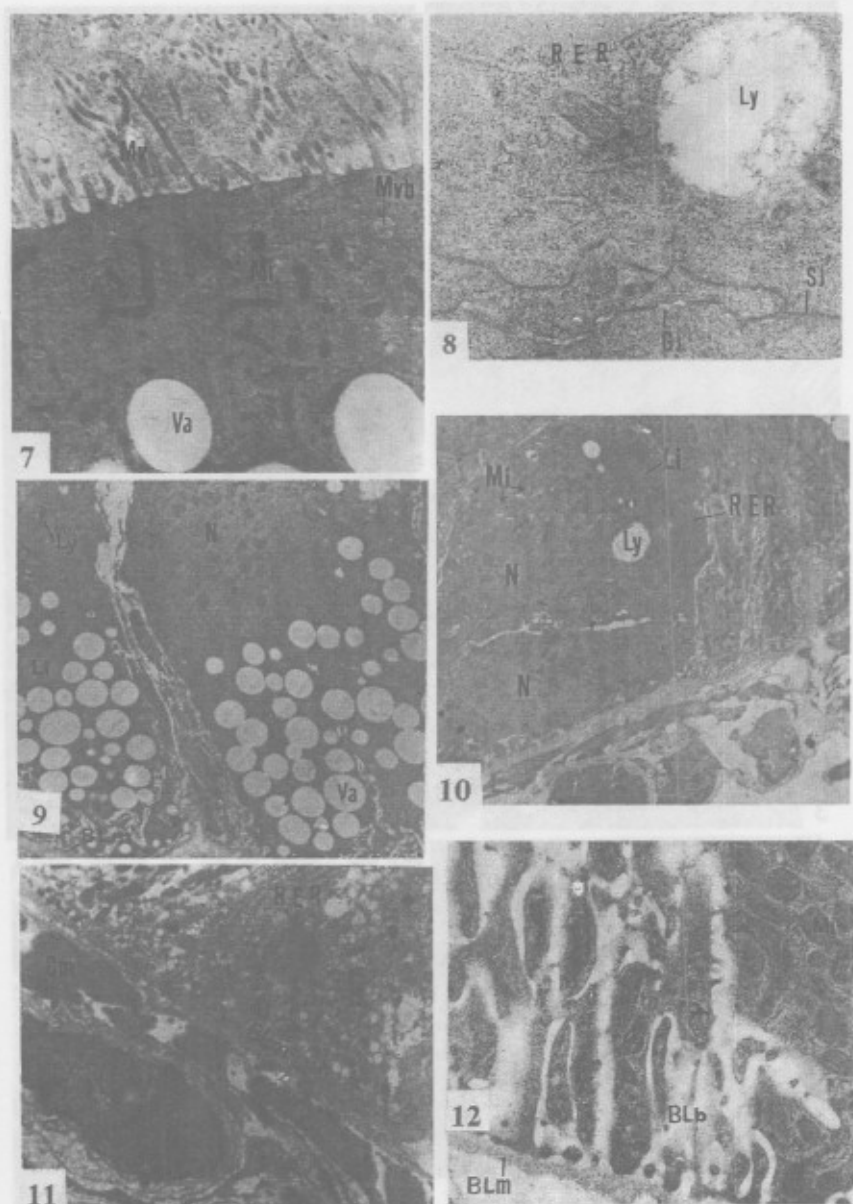
The irregularities of the muscularies in the midgut cells of irradiated *Ph. operculella* were observed in both circular and longitudinal muscle layers. This is in agreement with other authors; Prasad *et al.* (1980) on *D. dorsalis* and Szczepanik and Ignatowicz (1994) on *T. confusum*. Who confirmed that the midgut muscle layer seems to be very resistant to irradiation.

It was suggested that after initiation of the primary lesions by irradiation, a number of injury repair reactions are possible and the extent of the damage can cope with the injury, the extent of which is dependent on the level of radiation (Read, 1980).

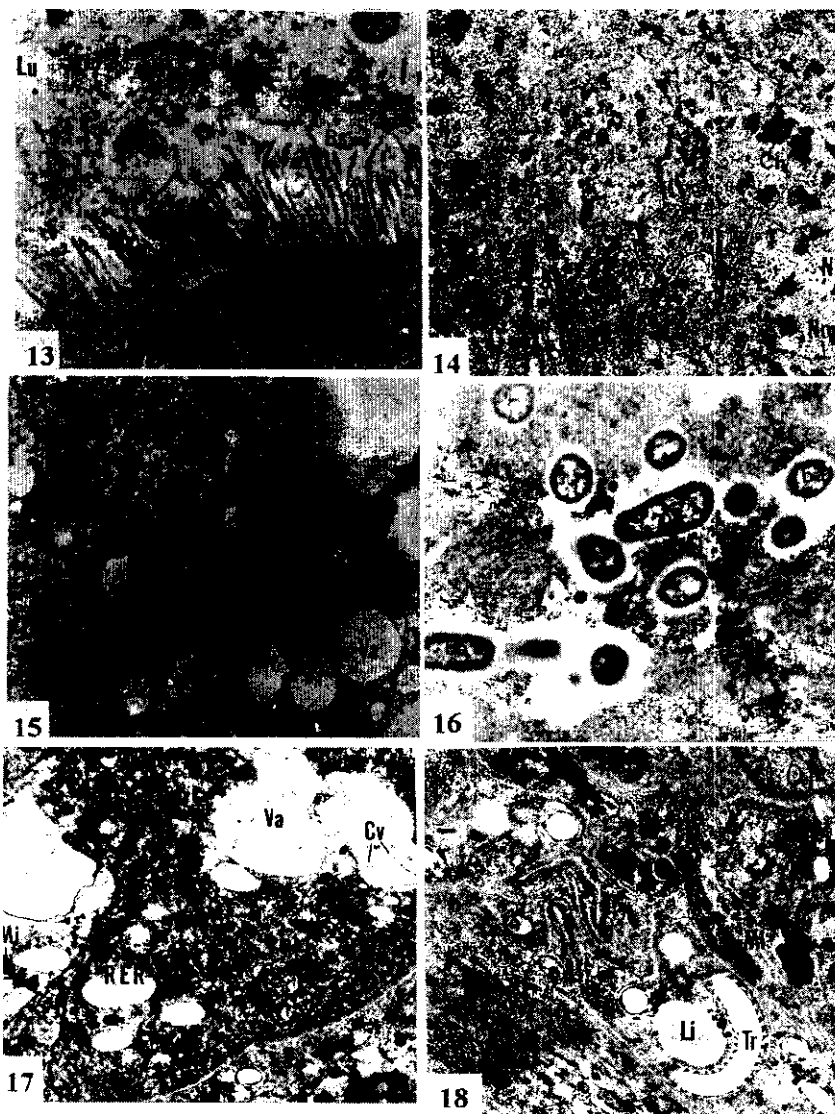
In the anterior midgut, the basal region of the cell is swollen, extremely vacuolated and contained coated vesicles and dense granules (Fig. 17). Likewise, this region revealed varying forms of bacteria in different stages of development (Fig. 16). Similar results were observed in midguts of other irradiated insect, infected with bacteria or different microorganisms as in *G. palpalis* (Stiles *et al.*, 1989) and *D. cucurbitae* (Sakurai *et al.*, 1996).



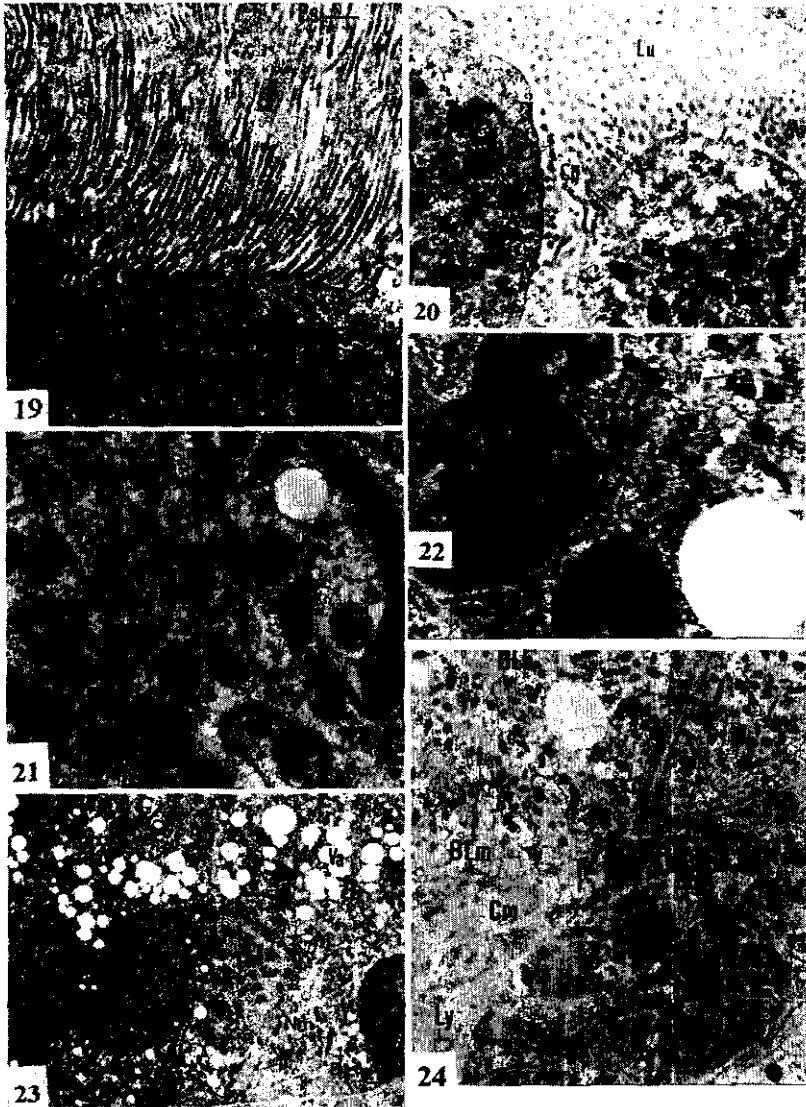
Figs. 1-6: Electron micrographs of epithelial cells from anterior midgut regions of non-irradiated 4-day old female, *Ph. Operculella*



Figs. 7-12: Electron micrographs of epithelial cells from posterior midgut regions of non-irradiated 4-day old female, *Ph. operculella*.



Figs. 13-18: Electron micrographs of epithelial cells from anterior midgut regions of irradiated 4-day old female, *Ph. operculella* exposed to sterilizing dose (150 Gy).



Figs. 19-24: Electron micrographs of epithelial cells from posterior midgut regions of irradiated 4-day old female, *Ph. operculella* exposed to sterilizing dose (150 Gy).

Where, Av: autophagic vacuole, BLb: basal labyrinth, BLm: basal lamina, Bs: blebs, Cd: cell debris, Ch: chromatin, Cm: cuticular muscle, Cv: coated vesicles, Gj: gap junction, Li: lipid, Lm: longitudinal muscle, Lu: lumen, Ly: lysosomes, Mi: mitochondria, Mv: microvilli, Mvb: multivesicular body, N: nucleus, Nm: nuclear membrane, Nu: nucleolus, R: ribosome, Rb: residual bodies, RER: rough endoplasmic reticulum, Si: septate junction, T: T- system, Tr: tracheoles, V: vesicle, Va: vacuole.

Srivastava and Kumar (1990) noted that the differentiation of epithelial cells of midgut and hindgut of *Parasarcophaga ruficornis* were inhibited by X-rays and UV rays followed by arresting of chitinous intima secretion and digestion of yellow body. Severe changes were also observed in the *T. confusum* adults irradiated with lower doses, including vacuolisation of the epithelial cells, or fading of epithelial boundaries. While the higher tested caused the complete disintegration of epithelial cells, and loss of basement membrane and muscle layer, this damage was increased with the intents of the gamma doses (Szczepanik and Ignatowick, 1994).

Vinson *et al.* (1969) also observed that, changes in the midgut of *Heliothis virescens* may be contributed to the early death of the insect. Deformation of the functional midgut epithelium and destruction of the peritrophic membrane, which started to appear a few hours after irradiation, indicated changes in the digestive and absorptive processes. Observations made at 72 hrs after irradiation suggested inhibition of cell division in the midgut epithelium and enlargement of the regenerative cells. Twelve and eighteenth days after irradiation, an increase in cell size was evident, and some disintegration of the nucleus took place. Moreover, Prasad *et al.* (1980) detected that, compete histolysis of midgut epithelium could result from high doses of radiation, bringing into prominence the muscular layer in *Plodia. interpunctella* and *Dacus dorsalis*.

SUMMARY

Ultrastructure examination of midgut cells of (normal and irradiated) 4-day old female *Phthorimaea operculella* was carried out. The electron micrographs of midgut cells in the normal female moth clearly showed the cell organelles, which are generally similar to other insect midgut cells, but nonetheless a variety of differences related to the metabolic function of the midgut in both anterior and posterior regions have been observed.

The results of the ultrastructure clearly emphasized and confirmed that, the cellular damage occur in the midgut cells of 4-day old female *Ph. operculella*, following gamma irradiation with sterilizing dose (150 Gy). The damage of the cells appeared as ruptures in the microvillar border into the lumen as blebs and expulsion of large quantities of cell debris in the gut lumen. High vacuolation, more or less indistinct cell boundaries, damage of the nuclei, mitochondrial deterioration and large number of bacteria were observed also as a form of cell damage. Small

secondary lysosomal bodies, irregular shaped mitochondria were observed as a result of swelling of membranes. Irregularities of muscularies were also observed.

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