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Ultrastructural Changes In the Midgut During Metamorphosis in *Apis Cerana Indica.*

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The midgut epithelium of Indian honey bee *Apis cerana indica* is consist of digestive cells and small regenerative cells. The regenerative cells are placed in the nests scattered among the digestive cells. During metamorphosis the midgut of *Apis cerana indica* is remodeled. The larval midgut epithelium and muscular sheath digested partially at the end of larval period and thrown out in the form of debris in the lumen. The new epithelium is formed by the proliferation of the regenerative cells and during pupation reorganization of midgut layer occurs.

The ultrastuctural studies shows that the regenerative cells are in contact with degenerative cells by the cytoplasmic extension which have many septed and gap junctions in the fifth instar larvae. In developing pupae reorganization of the midgut epithelium is continued whereas in the pharate adult the midgut wall shows, characteristic of adult midgut epithelium with pycnotic nuclei in some cells.

Key words: *Apis cerana indica, midgut, histology, ultrastructure, metamorphosis*

Introduction

Apis cerana indica is a small honey bee commonly known as Indian hive bee. In India it is used for apiculture or bee keeping practices since ancient period. The larval and adult forms of *Apis cerana indica* differs drastically both in morphology and physiology. This difference is found to be adaptive for adult life and change in environment. The simultaneous changes during the process of metamorphosis includes degradation of larval tissue and differentiation of new organs that are suitable for adulthood preferably due to expression of different sets of genes which are under the control of environmental condition and hormones like ecdysteroids and juvenile hormone (Martins,1969; Rachinsky *et al* .,1990; Tata,1994).

The midgut epithelium of bees is formed by the digestive cells, which serves to secrete enzymes besides nutrition and absorption. The small regenerative cells that are placed in nests scattered among the digestive cells. During metamorphosis the larval midgut epithelium degenerates and a new adult midgut epithelium is built by differentiation of regenerative cells (Martins *et al.,* 1969). In the process of pupation, reabsorption of larval organs and differentiation of adult organs take place (Cruz-Landim and Cavalcante, 2003). The midgut epithelium of last larval instar shows programmed cell death during metamorphosis and leads into reorganization of adult midgut (Gregorc and Bowen, 1997).

In *Apis cerana indica,* alimentary canal passes through remodeling by means of metamorphosis, but the cytomorphological and ultrastuctural changes during remodeling of midgut is not known. Therefore the present work has been undertaken to know sub cellular and ultrastructural changes in the midgut epithelial cells of larvae and pupae in *Apis cerana indica.*

Materials and Methods

Histological technique

The larvae and pupae of worker honeybees required were collected from the colony of *Apis cerana indica* that has been established at the garden of the Department of Zoology, RTM Nagpur University Campus, Nagpur.

The alimentary canal was dissected from developing larvae and pupae in Ringr's solution and then midgut region was separated from alimentary canal and fixed in freshly prepared Bouins fixative for histological studies.

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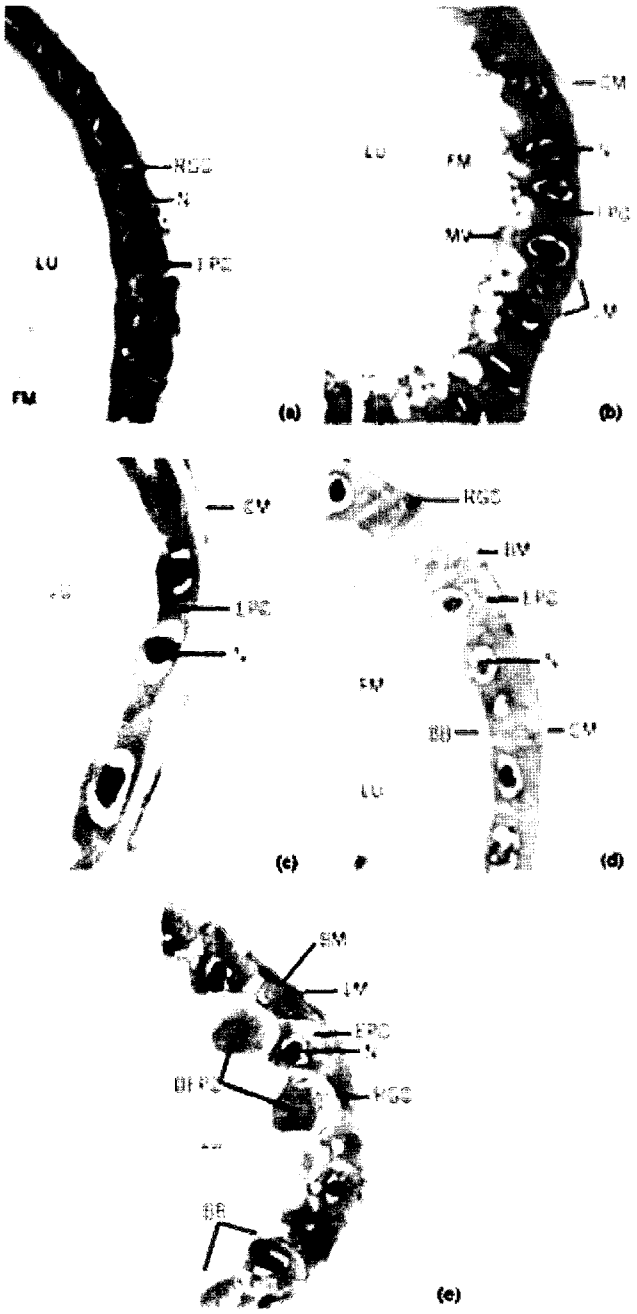

Fig. 1. Light microscopy of the larval midgut in *Apis cerana indica*. (a) Histological structure of MG of I[st] instar showing epithelial cells (EPC), regenerative cells (RGC) at the base and circular muscle (CM) HE × 400. (b) cross section of midgut of II[nd] instar showing epithelial cells (EPC) with membranous vesicles (MV) food material (FM) in the lumen (Lu) HE × 300. (c) cross section of midgut of III[rd] instar larva showing epithelial cells (EPC) and circular muscle layer (CM) HE × 400. (d) cross section of midgut of IV[th] instar showing epithelial cells (EPC) with brush border (BB), group of regenerative cell (RGC), basement membrane (BM), food material (FM) in lumen (LU) and cicular muscles (CM) HE × 400. (e) cross section of midgut of V[th] instar showing newly formed epithelial cells (NEPC) degenerative epithelial cells (DEPC) towards lumen, group of regenerative cells (RGC) HE × 300.

Thereafter the tissue was dehydrated, cleared in xylene and embedded in paraffin wax at 58 - 60°C. Blocks were sectioned at 3 - 5 μm thickness and stained with Eherlich Haematoxylene-Eosin (HE) staining technique (Humason 1962).

Transmission Electron Microscopy (TEM)

The alimentary canal were dissected out from the fifth instar larvae and pupae, fixed in the Karnovsky's fixative for 18 hrs and post fixed in 1% osmium tetraoxide.Then dehydrated in acetone and embedded in embedding medium No-1(Mixture of ARALDITE, CY212,DDSA, DMP-30,MNA).Semithin section were stained in methylene blue and ultrathin section were double stained with uranyl acetate and lead citrate. The ultra thin sections (60 - 150 nm) were viewed on the Morgagni-200 E Electron microscope at All India Institute of Medical Sciences (AIIMS) New Delhi, India.

Results

Histological observations

The midgut wall of developing larvae from first to last instar shows well developed midgut with apparently uniform population of epithelial cells. On the external surface of midgut is distinct striated border present, covered with a thin transparent peritoneal membrane. The layer of circular muscles present in between epithelial cells and longitudinal muscle layer (Fig. 1(a)-(c)). The regenerative cells with large nuclei, scanty cytoplasm and thin cell wall were seen in close vicinity to circular muscle layer in fourth and fifth larval stages (Fig. 1(d)-(e)).

Columnar epithelial cells were more or less cuboidal with internal brush border. The nucleus is large, oval and centrally placed. Change in the nuclei localization from center to the luminar surface can be seen during fifth instar stage. The large number of vacuoles appeared in the cytoplasm towards the luminar surface indicating the process of histolysis (Fig. 2(a)). Some semidigested degenerating epithelial cells also observed in front of epithelial layer in the lumen of last instar larva while, it appear as a layer of semi degenerative epithelial cells (LDEPC) in the lumen of early pupae (Fig. 2(b)). Group of regenerative cells, were present at the base whereas newly formed epithelial cells forms almost uniform layer of digestive cells. In pink eye midpupa the newly formed epithelial cells were became elongated with oval nuclei. Membranous vesicles were extended in the lumen arising from free end of epithelial cells (Fig. 2(c)). In late pupae *i.e.* brown eye with brown body midgut epithelium resembling adult structure but some cells shows very pycnotic nuclei (Fig.

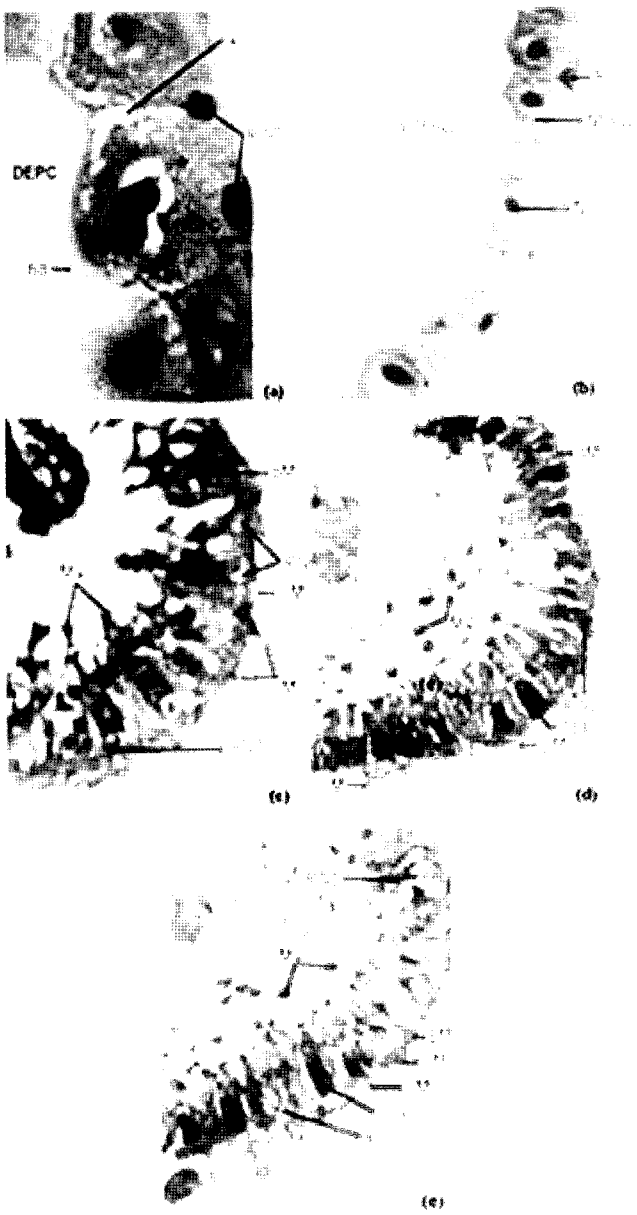

Fig. 2. Light microscopy of the larval-pupal midgut in *Apis cerana indica.* (a) Magnified view of midgut of Vth instar showing newly formed epithelial cells (NEPC) vacuolated cytoplasm (VC), with brush border (BB) and semi-digested degenerative epithelial cells (DEPC) HE×300. (b) cross section of midgut of early pupa showing newly constructed epithelial cells (NEPC), group of regenerative cells (RGC) at the base. The layer of semi digested degenerative epithelial cells (LDPC) appeared in the lumen HE×400. (c) cross section of midgut of mid pupa (pink eye) showing nests of regenerative cells (RGC) at the base and membranous vesicle (MV) from degenerative cells in the lumen HE×533. (d) cross section of midgut of late pupa (brown eye) showing columnar epithelial cells (CEPC), regenerative cells (RGC) and membranous vesicle (MV) toward the lumen HE×400. (e) cross section of midgut of late pupa (brown eye with brown body) showing columnar epithelial cell (CEPC), regenerative cells (RGC) circular muscle layer (CM) and membranous vesicle (MV) in the lumen HE×400.

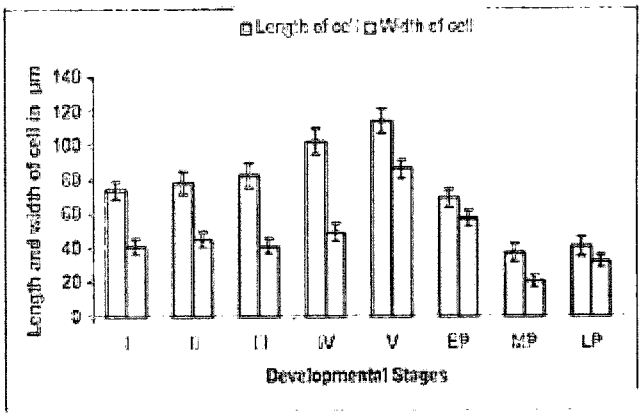

Fig. 3. Cell size during post-embryonic development.

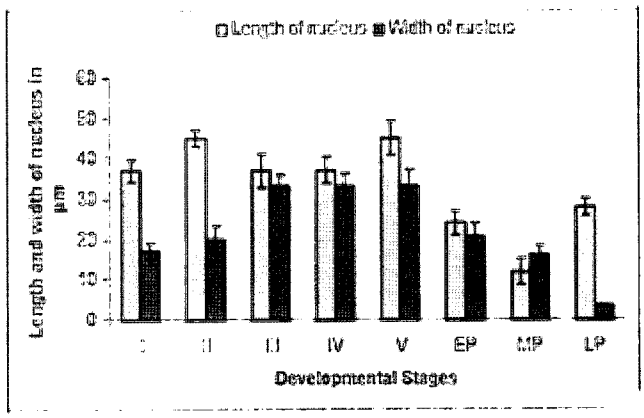

Fig. 4. Nucleus size of midgut cells during post-embryonic development.
Abbri : I, II, III, IV, V-Larval stages / EP-Early pupa / MP-Mid pupa / LP-Late pupa

2(d)-(e)). During the post embryonic development of *Apis cerana indica* the midgut cell and nucleus size increase significantly up to fifth instar larvae and decreased in mid-pupae and again increase in latepupae (Fig. 3 and Fig. 4).

Ultrastructural observations

TEM study of midgut in fifth instar shows digestive columnar epithelial cells with, oval, elliptical, nucleus surrounded by, endoplasmic reticulum and densely packed Golgi complex in the cytoplasm. The mitochondria are seen towards the apical region while the membranous vesicles are seen loosely arranged, in the lumen of gut (Fig. 5(a)). The degenerating cells shows the apical cytoplasm of the digestive columnar cells were extensively vacuolated. The microvilli had almost disappeared and the curved, elongated mitochondria were scattered in the cytoplasm (Fig. 5(b)). The nucleus was intact with dense chromatin material and portion of rough endoplasmic reticulum were observed along the side of nuclear surface. Digestive vacuoles were seen in cytoplasm as well as in

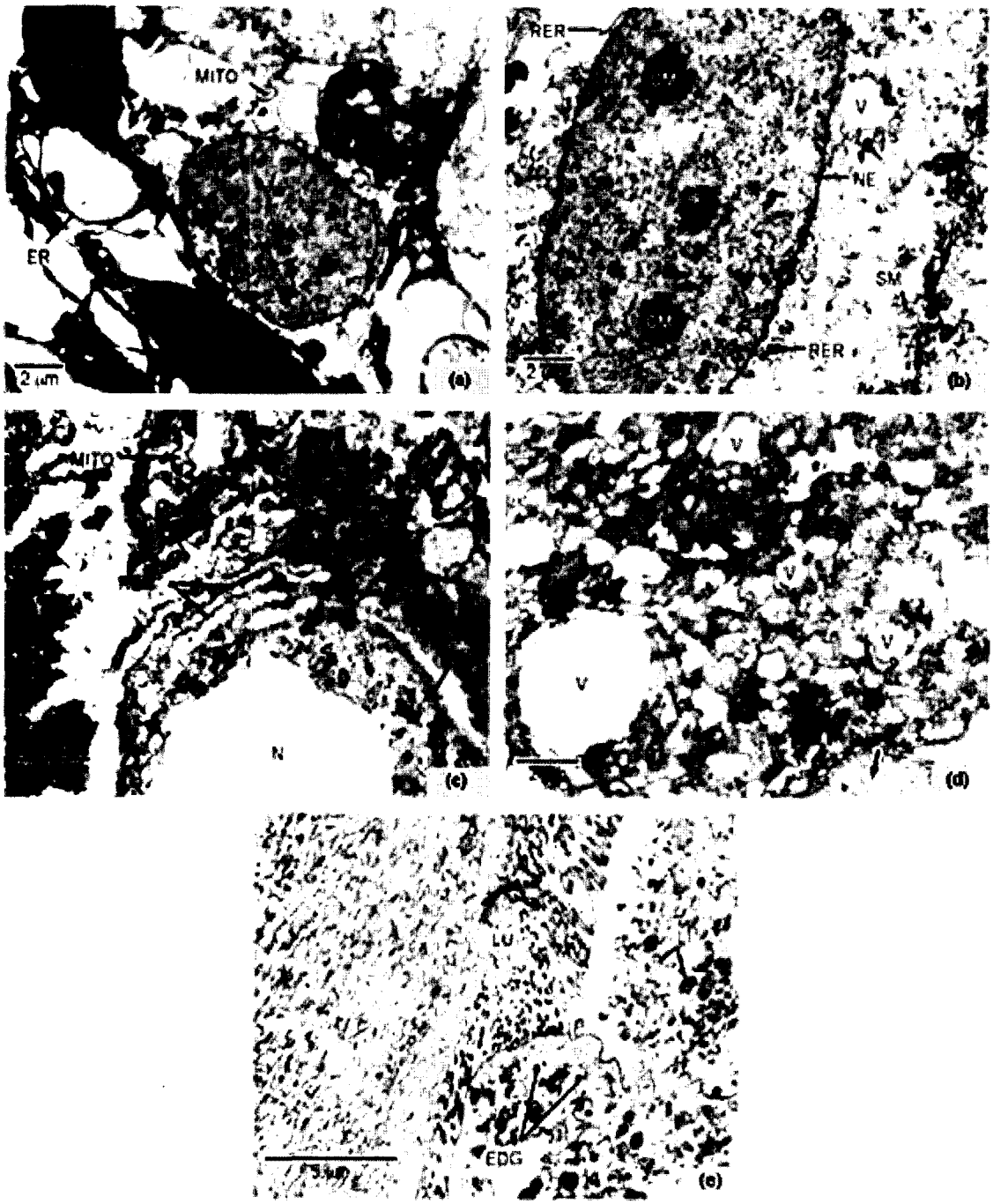

Fig. 5. Transmission electron microscopy of the midgut of *Apis cerana indica*. (a) Midgut columnar epithelial cell of V^{th} instar showing nucleus, endoplasmic reticulum, Golgi complex, mitochondria and membranous vesicles. (b) disorganized digestive cell of V^{th} instar showing loss of microvilli vacuolated cytoplasm and nucleus with dense chromatin material (CM). (c) semidigested degenerative cell showing nucleus with RER. (d) disorganized digestive cell showing loss of microvilli (arrow) and extensively vacuolated cytoplasm. (e) disorganized digestive cell in early pupa showing electron dense granules (EDG) and cytoplasmic contents.

Abbri : CM-chromatin material, EDG-electron dense granule, ER-endoplasmic reticulum, GC-Golgi Complex, MITO-Mitochondria, MV-membranous vesicles, N-nucleus, NE-nucleus membrane, SM-swollen mitochondria, V-vacuoles.

the nucleus. The chromatin material in the nucleus migrated towards the peripheral margin of the nucleus at the onset of pupation (Fig. 5(c)-(d)).

In early pupae, the midgut cell had an expanded apical cytoplasm with bubbles deprived of organelles and filled with fine granular material. The lumen of midgut contain cellular debris and some fine electron dense granules (Fig. 5(e)). In pink eye pupae the cells were flatter with numer-

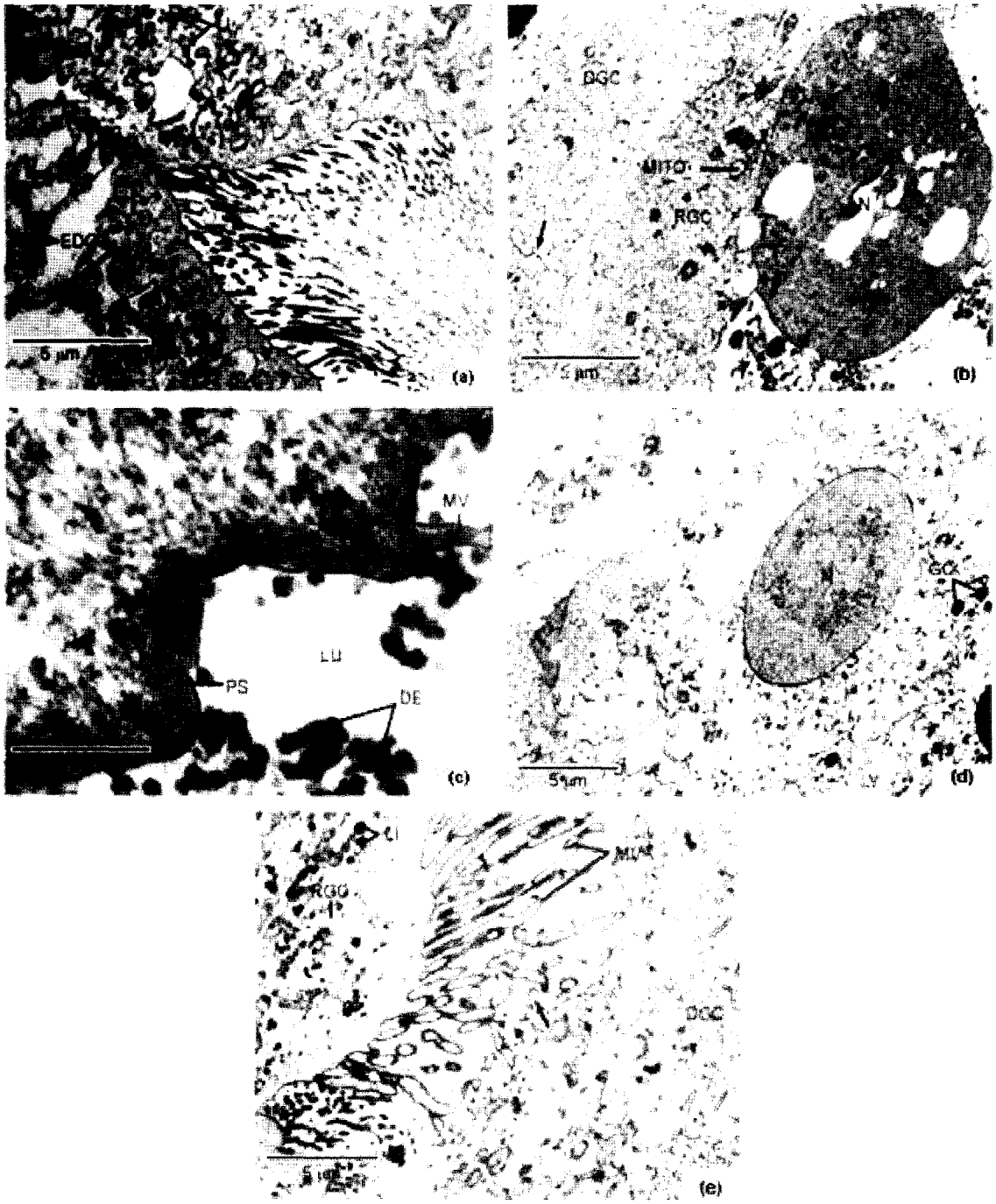

Fig. 6. Transmission electron microscopy of the midgut of *Apis cerana indica.* (a) Apical region of degenerative cell in early pupa showing lipid deposits and polysome towards the lumen. (b) a cell being shaded into lumen and below the regenerative cell arrow point to simuous cell contact of DGC and RGC. (c) magnification of apex of the digestive cell in a brown eye pupa with microvilli, debris found in the lumen. (d) completely formed pharate adult midgut epithelial cells. (e) apical section of digestive cell in pharate abult showing lipid deposition and mitochondria, microvilli with swollen tip in the lumen (arrow).
Abbri : DE-debris, DGC-degenerative epithelial cell, EDG-electron dense granule, LI-lipid deposition, LU-lumen, LY-lysosome, MITO-Mitochondria, MV-membranous, PS-polysome, RGC-regenerative cell, V-vacuoles.

ous well defined microvilli at the luminar surface. The apical zone of cell cytoplasm contains some free ribosomes, short fragments of rough endoplasmic reticulum and deposition of lipid (Fig. 6(a)). In the brown eye pupae the shedding of some rounded degenerative digestive cells were observed. The regenerative cells were cuboidal, vacuolated with apical expansion (Fig. 6(b)). Polysomes were observed towards the apical margin of cell while in the lumen densly packed dead material (debris) were found (Fig. 6(c)). In the pharate adult midgut epithelial cell

show, elliptical nucleus and well organized cytoplasmic contents similar to the adult epithelial cells (Fig. 6(d)-(e)).

Discussion

The alimentary canal of honey bee larvae is relatively simple in structure. The histomorphological studies on the alimentary canal during the post embryonic development in Indian honey bee, *Apis cerana indica* shows the similar structure to that of *Apis mellifera* (Snodgrass, 1956; Crailshem and Past, 1990; Cruz-Landim and Cavalcante; 2003) and *Apis florae* (Timande, 2005).

During the post embryonic development, the midgut is long tubular structure consisting, outer layer of longitudinal muscles, middle layer of circular muscle and inner layer of epithelium. The epithelium latter on differentiated into two types of cells, the minute regenerative cells, forming the basal crypsts and tall columnar cells similar to that of in other honey bees. (Snodgrass, 1956; Wigglesworth, 1965; Chapmann, 1985 a, b; Cruz-Landinm and Cavalcante, 2003; Martin *et al.*, 2006).

In *Apis cerana indica* the successive development of midgut epithelial cells were observed and replacement of larval midgut epithelium found in the early fifth instar while in late fifth instar, midgut epithelium was partially degenerated and replaced by new epithelium as found in *Apis mellifera* (Gregorc and Boven, 1997; Cruz -Landim and Cavalcante , 2003).

In *Apis cerana indica* the midgut cells of last instar larvae shows the sign of degeneration such as nuclear chromatin condensation and appearance of digestive vacuoles in the cytoplasm at the apical region of cell. However in *Apis mellifera*, metamorphosis itself begins in the prepupal stage (Cruz-Landim and Cavalcante, 2003) and involves the shedding of larval epithelium into the lumen and the formation of a new epithelium, mainly by mitosis of the regenerative cells. The greatest damage was seen in the cells apices; which appeared vacuolated and forming bulbous projections towards lumen. It is possible that apices of these cells are casted off and the remaining basal part preserved to participate in the new regenerated epithelium together with the new cells produced by mitosis of regenerative cells (Cruz-Landim and Cavalcante, 2003), similar shedding of the epithelial cells and coming of regenerative cells forming a new epithelium was observed during larval-pupal metamorphosis in *Apis cerana indica.*

The present study shows chromatin condensation in nuclei and the digested degenerative cells (damage cell) shedded into the lumen. The apoptic body has been observed in the larval midgut of *Apis mellifera* (Jimenz and Gilliam, 1990; Gregorc and Bowen, 1997; Cruz-Landim C, 1985; Tata , 1994) similar debris were observed in the lumen of *Apis cerana indica* may represents entire cells or part of them.

In *Apis mellifera* during the post embryonic development anterior and posterior part of midgut where the apical cells extrusions are more significant in the primary activation for destroying the replacing epithelial cells because of cell secretion. (Jimenz and Gilliam, 1990). In the *Apis cerana indica* morphological sign of high intensive secretion is of secretory granules in the midgut epithelial cells may be coincide with programmed cell death in larval and pupal metamorphosis.

The regenerative cells live close to the basal membrane of epithelium and have the function of regeneration; usually they are solitary and some time two or three together in a group. Regenerative cell produce mitotically and differentiation takes place in daughter cells (De Priester, 1971; Willie, 1983). The similar regenerative cells division, differentiation and propagation were observed in *Apis cerana indica,* during present study.

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