ULTRA STRUCTURAL STUDY OF CROP AND MIDGUT IN *APIS CERANA INDICA* F.(HYMENOPTERA: APIDAE)

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Abstract: The honey bee (*Apis cerana indica* F.) is common bee in India. The morphology of honey bee extensively studied, but less attention has been given ultramicroscopic studies on the alimentary canal i.e. crop and midgut. The aim of the current study was to describe the ultrastructure study of crop and midgut of the honey bee *Apis cerana indica*. The crop consist of nucleus of epithelial cell is prominent. Epicuticle is well defined. Between epicuticle and outfolded pleats there is a space which is continuous upto the crop. Nuclei are spherical and present in the cytosole. Interstitial out folded pleats traced to stored nectar in the lumen. Midgut consist digestive columnar epithelial cells are seen with oval nucleus, surrounded by endoplasmic reticulum and compactly arranged Golgi body into the cytoplasm. Membranous vesicles are loosely arranged. Position of mitochondria is on apical side of the cell. Columnar cell showed extensively large vacuole with apical cytoplasm. The nucleus was prominent with dense chromatin material. Rough endoplasmic reticulum were seen along the side of nuclear surface. Digestive vacuoles were seen in cytoplasm along with the nucleus. Some regions were also seen with free ribosome, short fragments of rough endoplasmic reticulum. The regenerative cells were cuboidal with large number of vacuoles. Lumen were found with packed densely dead material. The regenerative cells live close to the basal membrane of epithelium and have the function of regeneration. Regenerative cells produce mitotically and differentiation takes place. Usually they are solitary.

I Introduction

The crop of the honey bee is a specially adapted bag in the alimentary canal that serves to carry the collected load to the hive. In this honey stomach and in the hive the floral nectar is modified with various metabolic processes enzymatic action, activity of microorganisms into the invaluable honey (Oliver, 2007; De-Grandi-Hoffman *et al.,* 2013; Morais *et al.,* 2013). Crop in insect shows stretchable epithelium and muscles for contraction to throughout the content. Crop was divided into two regions namely proventricular bulb and stomodeal valve posteriorly invaginated in midgut.

Internally midgut is lined with thin layer of peritrophic membrane which separates food from midgut epithelium. Peritrophic membrane plays a key role in mechanical action of food, physical barrier for micro-organism etc. (Terra 1988).

This study was undertaken to reveal for the first time the ultra-structural study of crop and midgut in *A. c. indica* which is one of the useful honey bees in Japan, China, India and other East Asian countries. It is widely distributed in India.

II Materials and methods

The honey bee *A. cerana indica* were used for TEM study. The alimentary canal dissected out washed in PB and fixed in 2.5% glutaraldehyde containing 2% paraformaldehyde in 0.1M PB (pH 7.4,). Tissues were washed in PB, then post fixed in 1% osmium tetraoxide for 2 hrs. at 4ºC. After post-fixation, tissue washed in PB, dehydrated through ascending grades of acetone, cleared in toluene and embedded in epoxyresin.

Sections of semi thin 1µm have been stained with toluidine blue and microscopically examined by light microscope. Ultrathin sections of uranyl acetate and lead citrate have been stained and examined under Philips-10 transmission electron microscope at the All India institute of Medical Sciences (AIIMS), New Delhi.

III Result

3.1 CROP.

In transmission electron microscopy of honey bee crop of *A. cerana indica* an interstitial fluid is present. Nucleus of epithelial cell is prominent. Epicuticle is well defined. Spaces are seen between the epicuticles of an outfolded pleat is continuous with the crop. Spherical nuclei were seen in cytosole when it was highly compressed. Interstitial spaces between outfolded pleats traced to stored nectar in the lumen. The crop of honey bee shows both type of muscles invested in cords of muscles that are numerous enough, in both longitudinal and latitudinal ways, to fully enclose and confine the underlying, cuticle-lined epithelium. Inner wall of epithelium undergo extensive pleating. Epithelial layer and procuticle undergo extreme compression to maintain pleats for balancing the pressure exerted by the volume of crop contents. Pleats unfold as needed. Pleats are appressed, with presence of interstitial fluid. (Fig. 1 a, b).

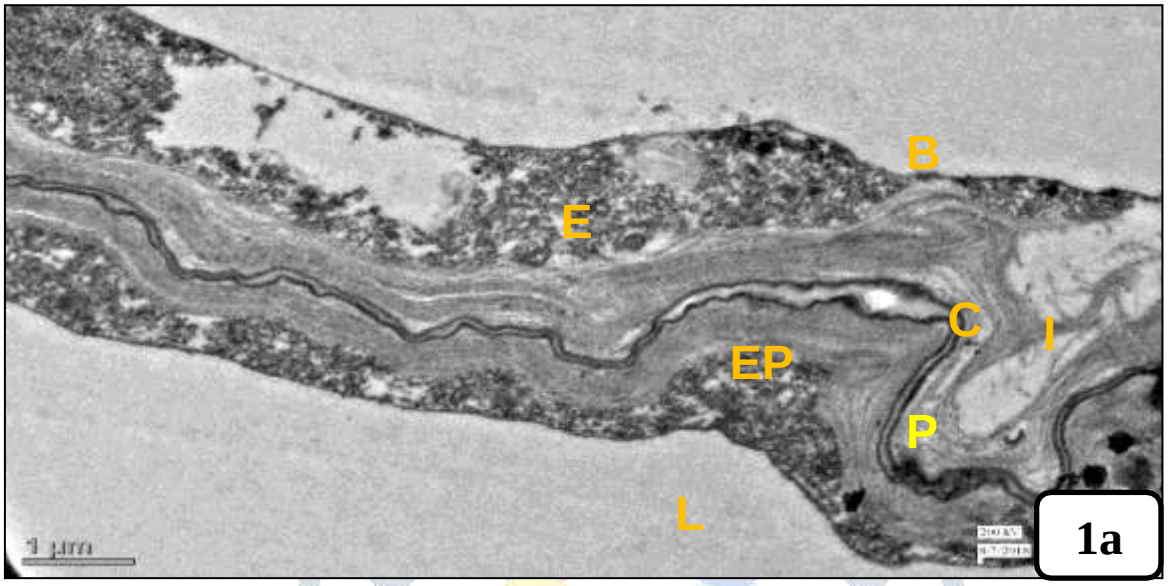


Fig.: 1 Transmission electron microscopic (TEM) photograph of the crop of *A. cerana indica*

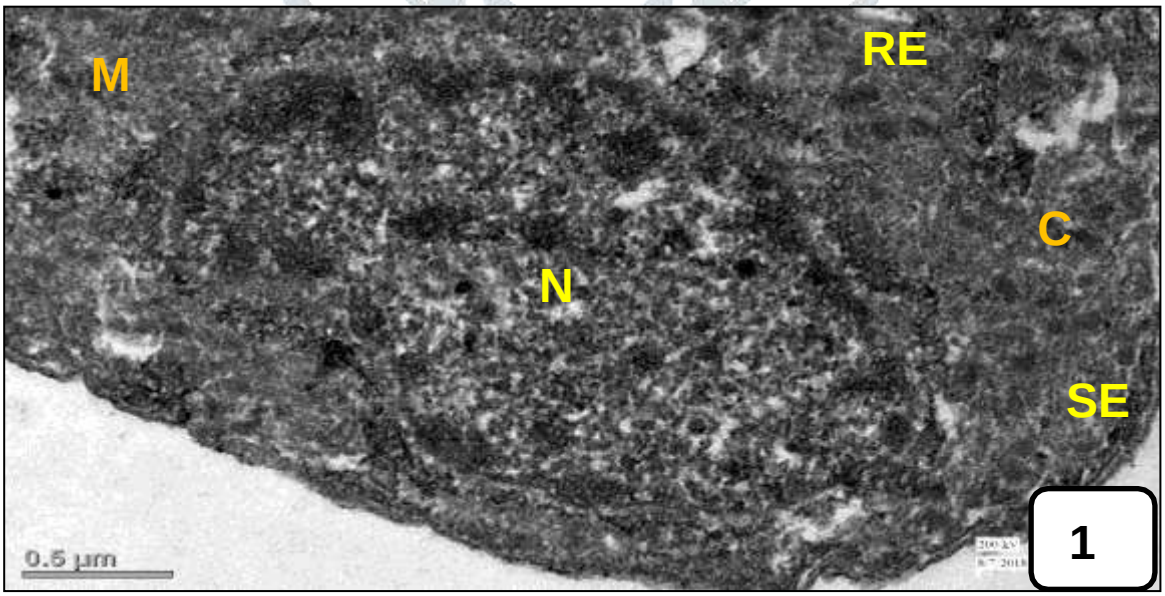


Fig.1a: Showing lumen (LU), epithelial cell (EC), epicuticle (EPC), pleat (PL), cuticle (CU), interstitial fluid (IF), basement membrane (BM), TEM: scale bar 1 µm.

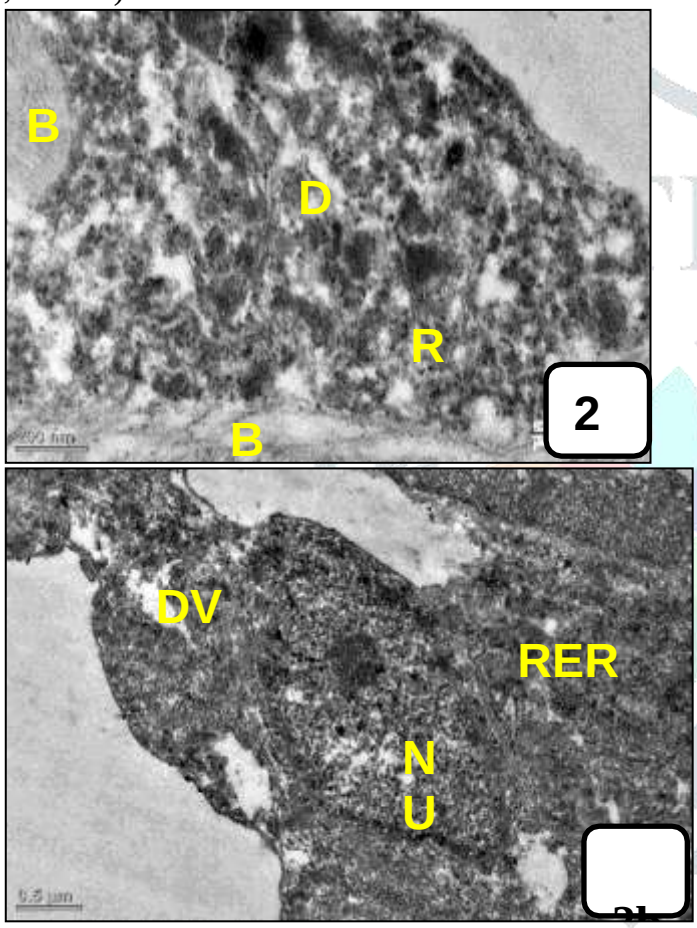
Fig.1b: Showing Epithelial cell of crop. It consist of rough endoplasmic reticulum (RER), cytoplasm (CP), nucleus (NU), mitochondria (MC). TEM: scale bar 0.5 µm

3.2 MIDGUT:

In the microscope, we found that the wall of midgut was surrounded by double layer of muscles oriented in various directions. In this study digestive columnar epithelial cells are seen with oval nucleus, surrounded by endoplasmic reticulum and compactly arranged Golgi body into the cytoplasm. Membranous vesicles are loosely arranged. Position of mitochondria is on apical side of the cell. Columnar cell showed extensively large vacuole with apical cytoplasm. The nucleus was prominent with dense chromatin material. Rough endoplsmic reticulums were seen along the side of nuclear surface. Digestive vacuoles were seen in cytoplasm along with the nucleus. Some regions were also seen with free ribosome, short fragments of rough endoplasmic reticulum.

The regenerative cells were cuboidal with large number of vacuoles. Lumen were found with packed densely dead material. Midgut also shows, elliptical nucleus and well organized cytoplasmic contents.

The regenerative cells live close to the basal membrane of epithelium and have the function of regeneration. Usually they are solitary. Regenerative cells produce mitotically and differentiation takes place. (Fig.2 a, b and c).



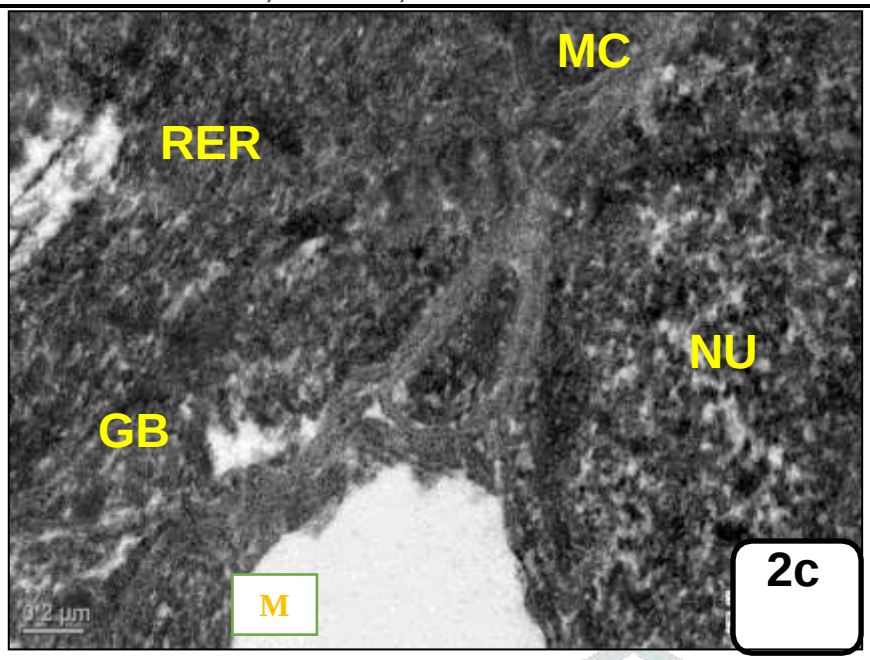


Fig.: 2 Transmission electron microscopic photograph (TEM) of the midgut region of *A. cerana indica*

Fig. 2a: Shows basal membrane (BM), regenerative cell (RC), Digestive cell (DC), brush border cell (BB); scale bar- 200 nm.

Fig.2b: Shows magnified digestive cell with nucleus (NU), rough endoplasmic reticulum (RER), digestive vacuole (DV): scale bar- 0.5 µm.

Fig.2c: Shows nucleus (NU), mitochondria (MC), rough endoplasmic reticulum (RER), Golgi body (GB), muscle (M). scale bar- 0.2 µm.

The digestive cells are larger than the endocrine cells with prominent nucleus. The regenerative cells were found near to the endocrine cells, but not inside and shows no relationship exist between them.
The plasma membrane is continuous without extensive basal folds. Large autophagic vacuole were seen alog with the rough endoplasmic reticulum with well-developed Golgi apparatus. Numerous mitochondria were seen. Midgut epithelium were composed of regenerative cells, endocrine cells, and pleomorphic columnar cells. Regions of the midgut were encountered in which the cytogeny of the columnar cells, the content of discharged vesicles, and the structure of the peritrophic membrane varied.

IV Conclusion

The crop is present inbetween the esophagous and proventriculus and the main function is to store the food (Snodgrass 1956). It also has been concerned with predigestion (Baker *et al.*, 1984) and absorption (Hoffman and Downer 1976; Joshi and Agarwal 1976, 1977).
Schmid-Hempel *et al*., (1985) have determined that honey bees continue the nectar collection though their crops are not full, due to flight-cost budgeting. If this is the case, then reserve capacity for emergency long distance foraging is well served anatomically. The size of the crop and its capacity for enlargement also might be a necessity during swarming or absconding activity, when bees are carrying all their food reserves in their bodies to establish a new colony. This extra food is needed to activate the wax glands that produce beeswax, the material bees use for their nest.
The peritrophic membrane is a thin layer on the inside of the midgut that separates the food from the midgut epithelium in many insect orders. Various functions, such as protecting the epithelium against the mechanical action of food, serving as a physical barrier against infection by microorganisms, and preventing excretion of digestive enzymes, have been suggested for this structure (Terra 1988). The regenerative cells divide synchronically, and due to the short chromosome condensation period during mitosis, cell proliferation may have been underestimated in the midgut sections analysed Rost (2006). Another possible hypothesis would be that undifferentiated cells from the hemolymph could migrate across the basal membrane to participate in the epithelium (Baldwin & Hahim 1991). Various researches have focused on the reorganization of the bee midgut during metamorphosis (Neves *et al.*, 2002, 2003, Cruz-Landim & Cavalcante 2003, Martins *et al.*, 2005). Morphological studies of insect midgut epithelial cells are rare, especially those concerning the distinct metamorphosis phases of the midgut. Various studies involve ultrastructural analysis in insect orders such as

Diptera (Nopanitaya & Misch 1974, Wood & Lehane 1991, Andrade-Coelho *et al.,* 2001), Hemiptera (Billingsley 1988, Ranjini & Mohamed 2000) and Lepidoptera (Cioffi 1979, Lello *et al.,* 1984, Santos *et al.,* 1984).

Midgut endocrine cells of insects are especially showing the presence of clear cytoplasm, secretory granules mainly in the basal area, cytoplasmic processes reaching the intestinal lumen, and absence of basal plasma membrane infoldings since they are scattered in the base of the digestive cells (Priester, 1971; Brown *et al*., 1985; Serrao & Cruz-Landim, 1996). However, few differences in relation to the number, type, and distribution of these cells are observed among the studied species, possibly indicating important differences in the digestive physiology of these insects (Andries & Tramu, 1985). In Hymenoptera, cells with vesicles observed in *Apis mellifera* (Raes & Verbeke, 1994) have larger and less electrondense vesicles than the secretory granules found in *M. quadrifasciata anthidioides.* Among the Hymenoptera, earlier ultrastructural reports on midgut endocrine cells were limited to a description of a few small pyramidal cells, without basal plasma membrane infolding and with electrondense granules, in the midgut of *Apis mellifera* (Jimenez & Gilliam, 1990). Later, still in *Apis mellifera*, two open-endocrine cell types were described in the posterior midgut region. These cells were called granular cells and contained electron dense granules of 100-200 nm diameter and vesicular cells having large electron lucent vesicles of variable diameter (Raes & Verbeke, 1994).

Ultrastructural studies of the secretory granules in the endocrine cells can be related to the peptide they produce. However, this classification is hindered by morphological variations of these granules as a result of several factors (Grube & Forssmann, 1979).

Endocrine cells are granulated cells, were characterized as found in low number, with clearer cytoplasm as that of the digestive cells, electrondense granules, and without basal labyrinth, and are the same as the "probable endocrine cells" described by Platzer Shutltz & Welsch (1970).

The *F. schrottkyi* midgut is lined by a single layer of columnar digestive cells with well-developed striated border and two muscle layers showing the same pattern as other Hymenoptera, Serrao, J.E.; Cruz-Landim, C. (1996). The presence of RER, Golgi complex, and vesicles in the cells of the anterior midgut region in all species analysed here are features of cells that synthesize proteins. However, only in *E. townsendi* are these proteins released into the midgut lumen inside the vesicles, as occurs in other midgut regions of bees (Serrao and Cruz-Landim 1996b).

The anterior midgut region of *A. mellifera,* besides its role in water absorption, seems to play a role in ion accumulation because it commonly has spherocrystals in the cytoplasm. Spherocrystals are typical structures of Malpighian tubule cells and their presence in the apical portion of the digestive cells suggests that these cells have an excretory function, as well as in the regulation of ion concentration in the cytoplasm (Gouranton 1968, Martoja and Ballan-Dufranc¸ais 1984, Cruz-Landim 1985). In comparison to these findings we observed in crop nucleus of epithelial cell is prominent, epicuticle is well defined. Interstitial between outfolded pleats are there to stored nectar in the lumen. The crop of honey bee is invested in cords of muscles that are numerous enough, in both longitudinal and latitudinal directions, to fully enclose and confine the underlying, cuticle-lined epithelium. Inner wall of epithelium undergoes extensive pleating. In our study we examined the ultra-structure of midgut, epithelial cells with oval nucleus, surrounded by endoplasmic reticulum and compactly arranged Golgi body into the cytoplasm. Vesicles are loosely arranged. Mitochondria is on apical side of the cell. Columnar cells with large vacuole. Nucleus was prominent with dense chromatin material.

V References

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