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TRANSMISSION ELECTRON MICROSCOPIC STUDY ON THE CYTOMORPHOLOGY AND ADAPTIVE ALTERATION OF DIGESTIVE GLAND CELLS IN AN INTERTIDAL MARINE BRACHIOPOD *LINGULA ANATINA* (LAMARCK, 1801) WITH TIDAL FLUCTUATIONS

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ABSTRACT

The intertidal fauna are subjected to alternative episodes of ebb and tide and has to accommodate with the changes in the environmental conditions of the habitat. In consequence, their feeding cycles and intracellular digestion are also orchestrated accordingly. In case of the intertidal brachiopod, (*Lingula anatina*), feeding is initiated by the entry of the food particle containing tidal sea water into the holes in the intertidal mud that serves as the niche for *Lingula anatina*. However, the process of digestion continues till the food particles in the tidal water in the mud tunnel are finished since the water is retained in the holes after the tide withdraws and ebb sets in. Fresh food resources are brought in by the advancement of the next tide. in brachiopods, the midgut gland or the digestive diverticula has attracted attention from various authors like, Barfurth (1883), Graham (1939), Bogen and Farley (1974), Steele-Petrovic (1976), Chaki *et al*. (1982), James *et a*l. (1992), Chaki and Misra (2004), Aldana *et al.* (2008) and others. There exists difference of opinion among the cell types that are involved in the midgut gland or diverticula as well as to the possible changes that may be phasic with changes in the feeding activity.

Microscopic as well as transmission electron microscopic studies of the midgut gland of *Lingula anatina* reveals the presence of three basic types of cells, namely two types of digestive cells(types A and B) and secretory (type C cell). Comparative analysis between the ultrastructural details of the digestive as well as he secretory cells of the midgut glands in *Lingula anatina* in the pre feeding as well as the post feeding conditions demonstrate a sequential as well as a continuous process of renewal of the cells and is utilization in the digestive process. In other words, it provides evidence of a phasic alteration in relation to the diurnal fluctuation of the ebb and tide and it appears that the process of digestion in the intertidal brachiopods is actually a continuous process.

Keywords: *Lingula Anatina* , Midgut Gland, Cell Types, Ultrastructure, Phasic Change,

I. INTRODUCTION

The brachiopod digestive system is devoid of the wide spectrum of remarkable diversity and elaboration unlike the molluscs with the predatory cephalopods having exclusively extracellular digestion that differs distinctly from the gastropods or bivalves that have particulate feeding mechanism (,Hyman, 1959, Williams,1997). The digestive diverticula or the midgut glands are the main site of intracellular digestion in brachiopods and are composed of a cluster of acini connected to the stomach by a series of ciliated branching ducts (Chuang, 1959; Storch and Welcsh, 1975; D'Hondt and Boucaud-Camou, 1982). Although three cell types have been reported in the digestive diverticula of *Lingula anatina* (Storch and Welcsh, 1975), and two cell types exist in articulated species (Stelle-Petrovic, 1976), the above cell types could not be differentiated in studies with acinar cells of the digestive diverticula of *Lingula anatina*, and it is not clear whether those cells are the different phasic states of a same type (James, 1997).

Present available evidence indicates the presence of a number of cell types in brachiopod digestive system categorized for comparative purposes (Samanta et al, 2014). The luminal lining of stomach in *Lingula anatina* contains a type of secretory cell that has no parallel in any other part of the digestive system. Hence, it seems clear from the reported observations that there is a need for systematic ultrastructural and histochemical investigations of the brachiopod digestive system (James, 1992, 1997). Light microscopic observations reveals that there are basically three types of cells, namely Type A cells that are long, cylindrical, located at the peripheral region of the acinus with clear or electron lucent cytoplasm, type B cells which are larger than type A, are vase like in shape with dense cytoplasm and located towards the type A cells and the secretory cells(type C) with characteristic darker cytoplasm and are elongated than types A and B digestive cell. (Chuang.1959a,1960).

Observations at the electron microscopic level is indicative that they can be categorized into two types of cells, namely the digestive cells undergoing phasic alterations throughout the prefeeding and the post feeding stages along with the secretory cells. Thus, the cell types of the midgut digestive glands of Lingula anatina exemplifies an evolutionary adaptive mechanism where the cellular physiology as well as its genetic program controlling the cell cycle is under control of the environmental conditions .

II. MATERIAL AND METHODS

In order to obtain a feeding as well as post feeding condition of the specimens, the collection time had to be co ordinate with the timings of ebb and tide.. The digestive glands were collected from *Lingula anatina* and fixed using 4% glutaraldehyde and 2% Paraformaldehyde as prefixatives along with 0.1M cacodylate buffer (ph 7.2). Following overnight washing in the same buffer and dehydration in graded acetone along with post-osmicated in buffered 1-2% osmium tetraoxide.(Carr and Toner,1982). This was followed by dehydration and post fixation with final embedding in araldite after having gradually moved through graded propylene oxide and araldite mixture. The prepared tissues were sectioned in LKB ultratome. (LKB BROMMA 2088)., the sections were stained with 0.5% uranyl acetate and observed by transmission electron microscopic (MORGAGNI 268D operated at 80 kV and TECNAI G2 operated at 120 kV).Semithin sections were stained with Toluidine blue for light microscopic observations. (Dykstra J. Michael, 1993).

III. OBSERVATIONS

Transmission electron microscopic observation in feeding stage during tide

Under Transmission electron microscopic observation, the digestive diverticula of *Lingula anatina* under TEM shows closely packed elongated cells arranged in a digitate pattern with distinct cell junctions. In relation to the light microscopic sections, type A, type B and type C cells are also observable under TEM observation .Both the types A and B cells can be can be regarded as the phases of digestive cell, while the cell type C can be regarded as the secretory cell but all types can function in digestion on basis of the cytoplasmic consistency. (Figures1, 2).The exact differentiation of the cell types is confusing.

Digestive cell (type A)

The cell type A has a reduced dimension than the other two and is more numerous in number. The cells are about 6.95µm x 1.3 µm in dimension and smaller in size than the type B and type C cells. The cell distribution is formed by interdigitating pattern of closely packed cells. The plasma membrane of type A cells show the different types of cell junctions, namely the tight junctions and gap junctions and desmosomes. Long and pleated septate junctions and tight junctions with the cell types B follow the zona adherens. The three cell types and the open at the lateral surfaces of the apical regions of the lumen of the gland together.(Figures 3).

The luminal surface of the Cell type A is produced into microvilli towards the luminal surface. These cells are characteristically impregnated with secretory rounded or elongated structures and mitochondria towards the luminal region. Cell nucleus and nucleolus is visible. The cytoplasm of the cell is provided with numerous vesicular structures. In comparison to that o cell type C, the nature of the cytoplasm is electron lucent.(Figure 4) .

Digestive cell (Cell type B)

The cell type B is with elongated and broader in shape than the type A and measures about 10.86 µm x 2.6 µm TEM observation reveals the presence of distinct ciliary rootlets that are almost alternating in position in B cells associated with dense aggregations of digestive vesicles. The cells with the apical ciliary rootlet alternate with a slender cell type, tapering at both ends, whose apical region do not open at the luminal end and is fused with the plasma membrane of the two adjoining cells opening to the lumen on both sides. The plasma membrane of the cell type B is also provided with desmosomes and tight and gap junctions. The apical end of the plasma membrane of the cell type B in its final stages of development extends into the luminal region as microvilli. These cytoplasm of the cells characteristically contain less granulation and lower concentration of heteromorphic mitochondrial structures accumulated towards the luminal end of the cells.(Figures 5). The cytoplasm of the type B cells is provided with numerous vesicles of different shapes, vacuoles, vesicles. The cell is with extensive Golgi complex and associated vesicular structures like GERL system (Figures 6,7).The cell cytoplasm is provided with different vesicles that alter in content as it passes through different digestive phases. In the feeding condition, the digestive cell type shows the presence of distinct types of vesicles that can be classified as four types(Figure8).

Secretory cell (Cell type C)

The type C cells are the secretory cells and correlates with the large triangular dense cytoplasmic cells as observed in light microscopy. (Figure 9). The cells are about 17.33 µm x 6 µm in dimension with a nuclear size of about 2.17µ X1.59µ -appears narrow, constricted at middle with vacuolations that has pushed the

cytoplasm towards the periphery and the vacuoles occupy a major portion of the cytoplasm. Plasma membrane demonstrates the presence of adherens junctions and luminal microvilli.. Magnified views of secretory cell reveal the presence of very dense electron opaque substance in the cytoplasm. The cell junction is distinctly visible that separates it from another digestive cell with numerous vesicular inclusions. There are vacuoles that appear to be partially filled with dense secretory material. The secretory cell numerous secretory vesicles with secretory material that are released from the luminal surface by apocrine mechanism. The nucleus with the nucleolus is easily distinguishable the cytoplasm is granular and much darker than other cells and the rough endoplasmic reticulum is extensively developed with bloated regions indicating highly increased protein synthesis. (Figure 10).

Regarding the cell types, it may be possible that cell type B is derived from cell type A or both are the same cell types in different stages of maturation, and the cells might be exhibiting different morphofunctional alterations according to digestive activity. Luminal regions show high pinocytic activity in all the cells. The zona adherens and the lateral plasma membrane are also characteristic.

Transmission electron microscopic observation in Post- Feeding stage during ebb

Digestive cell: type A

The digestive cell: type A) shows similarity in membrane structures to that of the feeding condition. The presence of tight and gap junctions, desmosomes are present. The nucleus is epibasally located, enlarged with a differential shape that deviated from the typical oblong or round nucleus in the feeding condition. The mitochondria are less prominent than that of the feeding stage and are not apically concentrated towards the luminal region. The Cytoplasm of this cell type contains numerous inclusions, mostly phagic vesicles and vacuolar structures with floccules inclusions.. The type A cell with a central vacuole filled with digested material (residual bodies) is present (Figure 11)

Digestive cell: Type B

The digestive cell type B has similar membrane structural characters to that of the prefeeding condition and maintains the location with respect to the cell types A and secretory cell . The cell size is at the post feeding condition is larger than that of the feeding type and contains numerous vesicles with homogenous or heterogeneous materials. The presence of heterolysosome and multivesicular bodies are evident. The changes in the digestive cells are obvious at this stage processes.. The cell types here are both nucleated as well as anucleated indicating a possible phasic change in the functional as well as morphology of the digestive cells with time. The digestive cells are with a loss of structural consistency and the cellular ultrastructure seems to be damaged and undergoing atrophy. (Figures 12, 13,14,15)

Secretory cell (cell type C)

In contrast to the feeding condition, observation of the secretory cell shows the presence of an active nucleus with rough endoplasmic reticulum. The cell cytoplasm is filled with numerous vacuoles and vesicles containing secreted material and electron dense granules .The nucleus is different in location and shape.

The secretory cell appear to have an basal nucleus at this stage while the electron lucent cells (Digestive cells A and B) are either with a bloated cytoplasm containing residual material and devoid of nucleus, or with a basally placed nucleus with large number of membrane bound structures, digestive vesicles and secretory structures.

The cytoplasm contains lysosomes in different stages of activity, numerous secretory vesicles, poor endoplasmic reticulum, and residual bodies indicating intracellular digestion and reduced protein synthesis**.** (Figure16)
The cell cytoplasm is also packed with glycogen granules obtained from the digestion of the feeding condition. However, an elaborate and intricate arrangement of GERL as well as numerous vesicles, secretory bodies, and lysosomes are found.. In addition, there are stream of membrane bound vesicles or possibly digestive vesicles with digested contents in the elongated portion of the cell cytoplasm bounded by cell junction The Golgi bodies appear to be less organized in the post feeding condition and the dense accumulation of spherules in the cytoplasm of the cell is very conspicuous. There are large and dense aggregations of secretory deposits in the cell cytoplasm of the digestive diverticula in the post feeding condition, even with loss of the nucleus in certain cases (Figures 17, 18).

IV. DISCUSSION

The digestive cell in any organism is a highly specialized cell that is very active and has to undertake a variety of functions that are essential for the survival of the species. As the cells are located in the digestive system, they are primarily focused on the process of digestion and absorption, and it is thus evident that high endocytic, pinocytic activity and lysosomal system. In brachiopods, the digestive gland functions as the seat of synthesis and release of the digestive secretions those are used for lubrication and solubilisation of the food particles. It appears that the cellular density changes can be attributed to the process of intracellular digestion and secretory activities of the digestive and secretory cells. In *Lingula anatina*, there appears to be three different types of cells with possible functional divergence .It may be also that they are different stages of the phasic activity that might be present in case of the digestive cells in this organism. This becomes evident from the light microscopic observation of the cell types where the three types of cells are clearly distinguishable on basis of the cytoplasmic lucency. Of the three types, one type (classified as type -A- , has a clear cytoplasm, the second type B has a slight darker cytoplasm than the type and the third type is characteristically more homogenous and distinctly darker than the other two types and classified as -C--.From the light microscopic observation, it becomes evident that the different cell types *Lingula anatina* do offer substantial basis to consider the activity of diverse cell types in the physiology and functional aspect of the midgut gland and it also indicated towards the possibility of phasic alteration of the cell type or types that occurs in different stages of the intracellular digestion. It is difficult to predict the exact changes or alterations in congruence with the light microscopic observations. However, the observations do indicate subtle changes or alterations that might be considered important in understanding the functional anatomy of the cells of the midgut gland or digestive diverticula and are a common occurrence in these types of cells. In the pre and the post feeding stages, there is perceptible alterations in the nuclear and cell sizes which clearly indicate a phasic nature of the cells.
Thus in case of both the digestive as well as the secretory cells, a cyclical activity marks the cellular lifecycles where there is a generation, elongation and increase in functional activity. As it proceeds towards the lumen, liberation of the contents into the lumen to be followed by final atrophy..an aspect reflective of the phasic activity of the midgut digestive in marine gastropods as has been reported by different workers like Bogen et al,(1974),Sreenevasan 1995) and others. It is interesting to note that the secretory cell remains in a constant

phase of activity showing well both in feeding as well as the post feeding conditions with minor changes unlike the digestive cell that undergoes apoptosis. This phasic change is a continuous process that occurs throughout the life span of the organism and such there is no resting phase in the digestive midgut gland cells in the brachiopods that has adapted itself with the cyclical and activity of a hydrological phenomena. The results are articulate and supportive of the fluctuations and of the climate along with the diverse physio-geographiocal parameters(Chakraborty ,2013) that have aided to the evolution of a synchronous cellular function.

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Plates

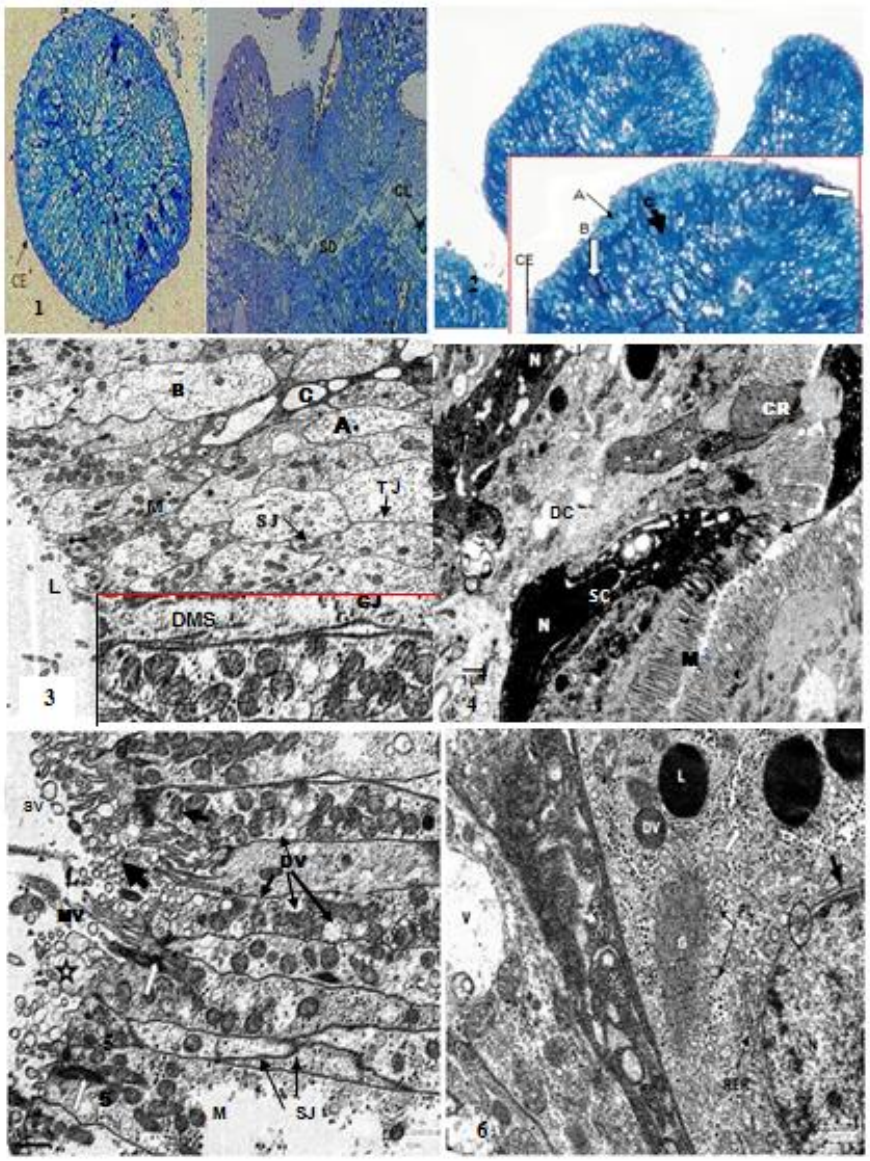

Figure 1 *: Thin section of digestive gland,(LM section) showing single acinus , CE coelomic epithelium, SD secondary duct, CL=central lumen(X40).* ***Figure2****: Cell types of digestive gland (X 40.* ***Figure 3****:TEM structure of midgut gland showing disposition and types of digestive cells A,B(digestive) and CS(secretory), L- lumen of gland ,DS desmosomes, GJ gap junctions, T J tight junctions, M- mitochondria aggregated towards luminal side .* ***Figure 4****. TEM section showing microvilli (M) black arrows, ciliary rootlet (CR), Digestive cell(DC) and secretory cell(SC).****Figure 5****:TEM section of secretory B cells alternating with ciliated cells(white arrow). Extensive accumulation of mitochondria towards lumen (M). Degranulation of digestive cells by exocytosis releasing digestive products as secretory vesicles (SV), direction of exocytosis (black arrow).* ***Figure 6****.Tem section of cytoplasm of B cells showing accumulation of numerous digestive vesicles, nucleus with nuclear membrane GEL system, with primary lysosomes .G-Golgi, Pl -primary lysosomes, N- nucleus, M-mitochondria*

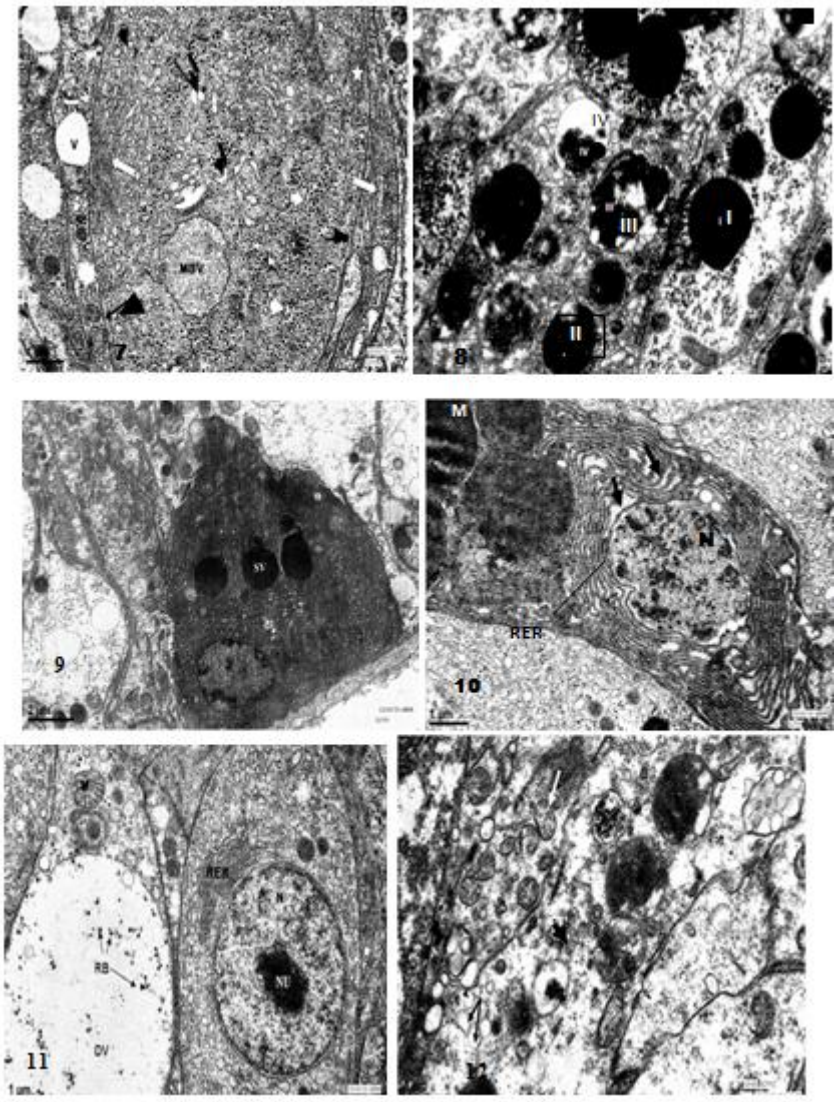

***Figure** 7 : TEM section of digestive cell type B showing extensive Golgi network and hyperactivity in cell,(white arrow) numerous vesicles (black arrowheads), MVB , V=vesicle, Multivesicular body. **Figure8**: TEM section of digestive cell type B in feeding condition showing presence o 4 types of digestive vesicles characterized as I, II, III and IV respectively. **Figure 9**:. TEM section of secretory cell (type C)in feeding condition with basal nucleus, triangular shape and absence of secretory deposits showing **Figure 10:** TEM section of secretory cell in feeding condition showing extensive RER looping and hyperactivity in feeding condition. RER_ rough endoplasmic reticulum, M= mitochondria, N= nucleus. **Figure 11**:.TEM section of digestive cell (type A) in post feeding condition showing a digestive cell and a secretory cell in two phasic states. The digestive cell has released its contents while the secretory cell continues to function,-DV-digestive vesicle RB-residual bodies, V-vesicle, NU nucleus, RER- rough endoplasmic reticulum.**Figure12**: TEM section of the digestive cell (type B) undergoing degenerative changes in the post feeding condition. ,different type of digestive vesicles- (arrowhead)*

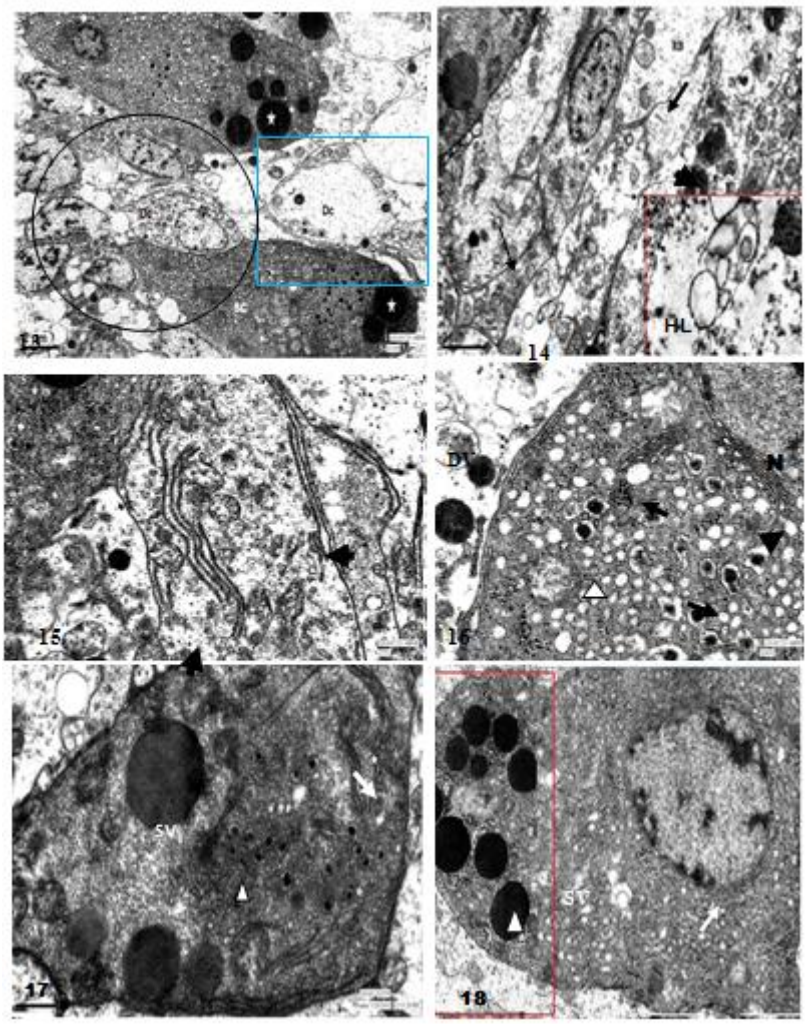

Figure 13 *: TEM section of digestive cells in post feeding condition , both nucleated , with digestive vesicles (black circle,) as well as anucleated condition (blue square) of the cells show differential phasic stages.* ***Figure 14****.TEM-showing formation of extensive digestive vesicles, lysosomal structures and heterolysosome in digestive cell type B in post feeding condition.* ***Figure 15****.TEM of digestive cell type B showing loss of structural features as disorganized Golgi and cytoplasmic contents (black arrows).* ***Figure 16****. TEM of secretory cell type C in post feeding condition showing active nucleus, rough endoplasmic reticulum (black arrowhead), different types of secretory and other vesicles (black arrow), DV digestive vesicle, N- nucleus.* ***Figure 17, 18*** *.TEM section of secretory cell type C in post feeding condition showing dense secretory deposited in cytoplasm with t without nucleus .(white arrowhead)*

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