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An Ultrastructural Study of the Hepatopancreas of Indian White Prawn *Penaeus indicus* H. Milne Edwards

M. HEMAMBIKA and R. PAUL RAJ*
Central Marine Fisheries Research Institute, Kochi
*Madras Research Centre of CMFRI, 68/1 Greams Road, Chennai - 600 006,India.

Abstract

Electron microscopic studies of the hepatopancreatic tubule revealed four distinct cell types. The Embryonic (E-cells) were undifferentiated with a large nucleus and cytoplasm surrounding it. Golgi complex and free ribosomes were abundant. Fibrillar (F-cells) contained extensive rough endoplasmic reticulum with ribosome - studded cisternae. The golgi cisternae were always dilated with numerous zymogen granules. Secretory (B-cells) showed many vesicles and a large vacuole. Microvillar border was prominent. Absorptive (R-cells) were characterized by the presence of lipid material. The cytoplasm showed a number of mitochondria, rough endoplasmic reticulum and golgi complex through out the cell. These cellular structural make-up reflected the complex functions of the digestive gland in food absorption, secretion of digestive enzymes, storage and depot of lipids, glycogen and minerals.

Introduction

The crustacean hepatopancreas has attracted the interest of scientists for at least a century and a half, yet as Loizzi (1966) commented, "the total information accumulated on this organ is meagre, when compared to other areas of biology". That the hepatopancreas is a vital and major organ in the Crustacea is beyond doubt, involved in diverse metabolic activities, it is primarily responsible for the synthesis and secretion of digestive enzymes and subsequent uptake of nutrient materials, but is also implicated in excretion, the moulting cycle, the storage of inorganic reserves, and in the lipid and carbohydrate metabolism (Gibson and Barker, 1979). Recently cytology of the hepatopancreas of decapod crustaceans has been studied extensively by light and electron microscopy.

Early histological studies defined two cell types but, since the classification of Hirsch and Jacobs (1928), the E-, R-, F- and B-cells have been described in many decapods. Recent studies on the Reptantia or walking forms of decapods (Hopkin and Nott, 1979, 1980; Erri-Babu *et al.,* 1982), have revealed further cytological characteristics and confirmed the earlier observations that the tubule epithelium contains four cell types. The Natantia or swimming forms have been largely neglected, except for the work of Pillai (1960) and Dall (1967), wherein they have identified four and three types of cells in *Caridina laevis* and *Metapenaeus benaettae* respectively. Papathanassiou and King (1984) and Al-Mohanna *et al.,* (1985) have distinguished four types by electron microscopy.

The Indian white prawn, *Penaeus indicus* is an ecologically important and widely distributed species which has potential for economic exploitation. It is of interest, therefore to, examine the ultrastructural make up of the complex organ - hepatopancreas.

Materials and Methods

Specimens of *Penaeus indicus* of size 100 - 120 mm collected from the culture ponds of Central Marine Fisheries Research Station at Narakkal were maintained in the laboratory for 2 to 3 days. Animals in their intermoult stages were selected and dissected live in ice. The gland as a whole was fixed in ice-cold 5% glutaraldehyde solution in phosphate buffer of pH 7.2. A few glands dissected were fixed in 5% glutaraldehyde solution in buffered sodium cacodylate (pH 7.2) for 1 hour at 0-4°C. The glands cut into small pieces were then washed in several changes of buffer solution followed by post fixation in 0.5% Osmium tetroxide solution for 1 hour at 0-4°C. After dehydration in graded levels of cold acetone 25%, 75%, 95%, 100%), the material was embedded in Spurr resin. Sections were cut with a ultramicrotome (LKB Bromma 8800 ultrotome) and mounted on copper grids. The ultrathin (600°A) sections were then double stained in 30% uranyl acetate (30 minutes) followed by lead citrate (10 minutes) and viewed in a Carl Zeiss Transmission Electron Microscope 109 R - at an accelerating voltage of 80 Kv). Electron micrographs were taken using AGFA Ortho 25 film.

Results and Discussions

General morphology of the hepatopancreas

The reddish brown hepatopancreas, is a large compact organ which occupies the greater part of the cephalothoracic cavity, posterior to the cardiac portion of the stomach. It has two separate lobes which are composed of compact arrays of blindended tubules; each lobe connects ventrally with the gut at the junction of the pyloric stomach and the anterior end of the midgut.

Each tubule under light microscopic studies consists of four cells types which can be classified as E-, F-, B-, and R-cells according to the scheme of Jacobs (1928). The E- cells,

'Embryonic cells' are undifferentiated and localized at the blind end of the tubule. F and R- cells occupy mid distal region. B cells occupy the mid region and are mostly interspersed with R- cells. The proximal portion of the tubule is lined predominantly by R- cells with few F- cells in between (Hemambika, 1989).

There are four cell types in hepatopancreas of *P. indicus* as observed under electron microscope (Plates - 1 and 2).

Cell type I :

E- Cell (Embryonic Cell):

These are undifferentiated cell (Plate 1b) and have high nuclear to cytoplasmic ratio. They contain few mitochondria having randomly oriented cristae. Proximal to the nucleus there are Glogi Complexes and moderate amounts of rough endoplasmic reticulum (Plate 1b). The cytoplasm possess abundant free ribosomes and single membrane recticulum. Microvilli are present on the border adjacent to the lumen (Plate 1a,b).

The E- cell at the blind ends of the tubule in decapods are generally believed to be involved in mitotic activity (Gibson and Barker, 1979) for the production of other cell types which comprise the tubule epithelium. The E- Cell of *Penaeus indicus* as observed in the present study is similar to the E - Cell observed in other crustaceans (Van Weel, 1955; Davis and Burnett, 1964; Bunt, 1968; Stanier *et al.*, 1968; Gibson and Barker, 1979; Papathanassiou and King, 1984; Al-Mohanna *et al.*, 1985).

Cell Type - II :

F-Cells (Fibrillar cell)

The cell (Plate 1a) contain extensive rough endoplasmic reticulum with long, narrow ribosome studded cisternae. The golgi cisternae in the cells are always dilated and the entire complex is ringed by small dense vesicles (Plate 1b and d). The cytoplasm contains abundant free ribosomes but fewer mitochondria. The structure of these cells resemble that of the F-cells in the Crayfish hepatopancreas (Loizzi, 1971). Extremely electron dense cytoplasmic inclusions are occasionally observable in the fibrillar cells. Each inclusion is enclosed within a membranous vacuole and consists of granular material which is concentrated centrally to form a rather solid core surrounded by irregular clumps of the dense material. These are the zymogen granules. Based on these structural observations in cells it may

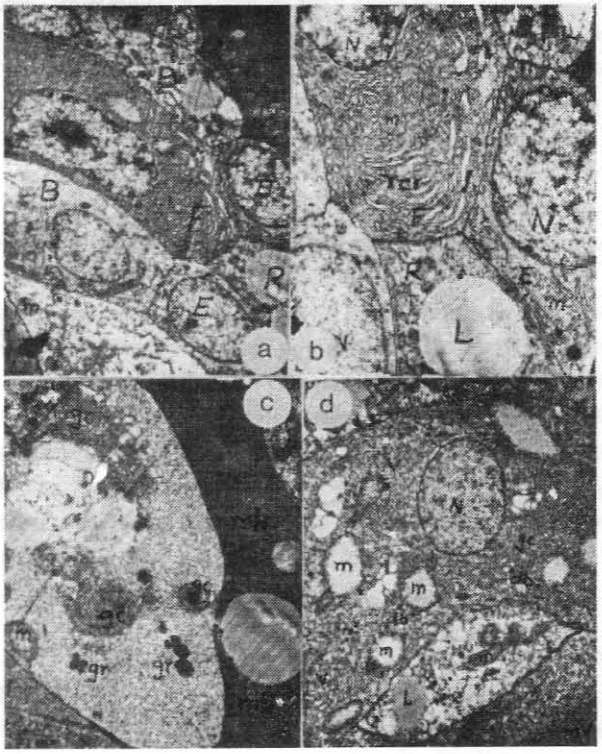

Plate 1

a) Electron micrograph of hepatopancreas of Penaeus indicus showing the different cell types (E, F, B & R cells) x 3,000

b) F, R, E cells with rough endoplasmic reliculum, Golgi cisternae. N-nucleus, L-lipid, g-globule, m-mitochondria x 7000.

c) E. Embryonic cell with Golgi complexes (gc), rough endoplasmic recticulum in the cytoplasm and vesicles of smooth endoplasmic reticulum.

d) F-Fibrillar cell with vesicles (V), golgi cisternae (gc), few metochondria (m) and a centrally located nucleus (n), Note the abundant rough endoplasmic reticulum (rer) db-dense bodies. X 3000.

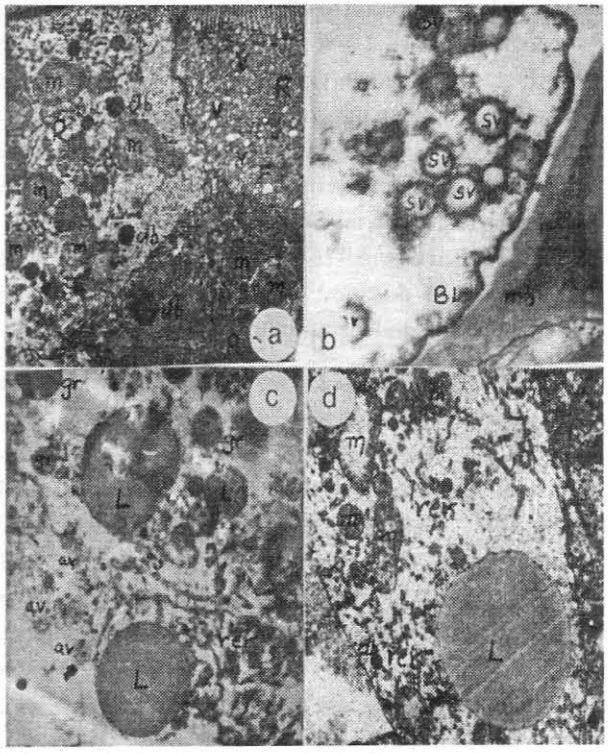

Plate 2

a) Electron micrograph showing the special membrane of the B and R cells - Mv. microvilli, db - dense bodies, lu - lumen between the adjacent cells x 7000.

b) A portion of secretary cell (B) showing the vesicles filled with proteinaceous substances at the periphery X 12000.

c) Absorptive R cell with lipid droplets and dense inclusions and autophagic vacuoles (av). X 7000.

d) R-cell with lipid droplet (l) and mitochondrion (m) and cisternae. X 7000.

be noted that they are probably designed for manufacture of digestive enzymes. Similar supporting observations have been made by Bunt (1968) and Stanier *et al.*, (1968) in other crustaceans. It may be recalled that the sequence of cell transformation as put forth by Jacobs (1928) for Astacus as E-cell > F-cell>B-cell is very evident in this species, also by the increased amount of rough endoplasmic reticulum and again the secretory cells, secreting their products into the lumen.

Loizzi (1971) suggested that F-cells in cray fish are involved in synthesis of digestive enzymes because of their rich concentrations of rough endoplasmic reticulum. Here again their cisternae are filled with a flocculent material (Plate 1b). They may be actively involved in digestive enzyme synthesis which the cell sequester them in a supranuclear vacuole which enlarges by pinocytic intake of luminal nutrients and fluids. Such kinds of cells are also reported in vertebrate pancreas (Fawcett, 1966). Within the F-cells of *P. indicus* also active endoplasmic reticulum and Golgi complex are seen supporting their role in enzyme secretion. Inside the Golgi vesicles are seen certain moderately electron dense, somewhat flocculent material which may be interpreted as secretory products of proteinaceous nature, again indicative of active protein synthesis.

Cell Type - III :

B - Cells (Secretory Cells)

These cells contain large number of vesicles, which initially occupy a considerable part of the cell and eventually occlude it (Plate 2a and b) or sometime contain a single large vacuole enclosed by a thin shell of cytoplasm. Separating the vocuole from the lumen is an apical complex consisting of a microvillar border, dense cytoplasm. Small mitochondria and a meagre surface enteric coat. These are the secretory cells and resemble the B-cells of Davis and Burnett (1964) and Stanier *et al.*, (1968).

These cells are sometimes characterised by a single large vacuole, and may be considered to be essentially an F-cell in which the small secretory vacuoles have coalesced (Plate 2b) to form a single large vacuole and a compressed basal nucleus. The contents of the vacuole have obviously been affected by solvents used during dehydration and embedding. Loizzi (1971) has attributed that in crayfish these cells are secretory in nature and B-cell secretion involves pinching off the apical complex followed by extrusion of the enzyme-rich vacuolar contents. Some what similar components are also observed in the B-cell of P. indicus indicating their functional significance in secretion.

Cell Type-IV:

R-Cell (Absorptive Cell)

These cells are characterized by the presence of lipid material (Plate 2c and d). The cytoplasm contains a number of mitochondria, some of which are concentrated below the apical plasma membrane (Plate 1a). The nucleus is centrally or basally located and nucleolus is present. The apical membrane of the cells have microvilli, the surface of which are covered by short filaments. Rough endoplasmic reticulum and golgi complex occur through out the cell. Smooth endoplasmic reticulum is restricted to the basal region of the cell. Apically, the cell resembles a vertebrate intestinal absorptive epithelium with a dense brush border and an organelle - free region lying below the microvilli. Autophagic vacuoles containing material of different configurations with low electron density are present in the cytoplasm (Plate 2d). These cells are similar to those described as R-cells in the Crayfish (Loizzi, 1971).

The function of lipid storage may be attributed to this cell, because of the presence of striated apical border, believed to be involved in food absorption. Again light microscopic observation of histochemical tests reported dense concentration of lipids in membrane bound vacuoles. Hence, their role in storage and metabolism of glycogen and lipids. Gopalakrishnan (1957) also noted similar cells in *P. indicus* under light microscopic observations. However, he could only distinguish secretory, storage and basal cells. In the present investigation, ultrastructural details could be well delineated on account of higher resolution and this lead to the identification of four type of cell in the tubular epithelium of hepatopancreas. This finding support the work of Papathanassiou and King (1984), Al-Mohanna *et al.*, (1985) on Palaemon serratus and *Paenaeus semisulcatus* respectively.

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