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Seasonal Ultrastructural Modulations of the Molting gland -Y organ in the fresh water crab "Barytelphusa Guerini"

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Abstract
The Y-organ in crustaceans plays a crucial role in regulating molting through the synthesis and secretion of ecdysteroid hormones. The present study investigates seasonal ultrastructural variations in the Y-organ of the freshwater crab Barytelphusa guerini to understand its functional adaptations under changing environmental conditions. Specimens were collected across different seasons, and Y-organs were processed for transmission electron microscopy to examine cellular and subcellular alterations. Ultrastructural observations revealed significant seasonal modulation in organelle organization, including variations in mitochondrial density, smooth endoplasmic reticulum proliferation, nuclear morphology, and secretory vesicle abundance. Enhanced development of mitochondria and smooth endoplasmic reticulum during pre-reproductive and reproductive period indicates elevated steroidogenic activity associated with active molting phases. Conversely, reduced organelle complexity and increased cytoplasmic inclusions during post reproductive period suggest lowered metabolic and endocrine activity. These findings demonstrate that the Y-organ exhibits pronounced environmental fluctuations and molting physiology. The study provides insights into endocrine regulation and adaptive strategies of freshwater crabs, contributing to a better understanding of crustacean reproductive and growth cycles in fluctuating freshwater ecosystems.

Keywords: Barytelphusa guerini, Y organ, Ultrastructure, Steroidal nature. Moulting.

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Introduction
Crustaceans represent one of the most diverse and ecologically significant groups within the phylum Arthropoda, inhabiting a broad spectrum of aquatic environments. With approximately 30,000 described species, including crabs, prawns, lobsters, shrimps, and numerous planktonic forms, crustaceans constitute a substantial component of global aquatic biodiversity. Their extensive distribution, ecological adaptability, and physiological complexity make them important model organisms for studies in ecology, physiology, and environmental biology.

In aquatic ecosystems, crustaceans perform essential ecological functions and contribute significantly to ecosystem stability and productivity. They occupy a wide range of ecological niches and exhibit highly regulated physiological processes mediated through neuroendocrine signaling pathways that enable adaptive responses to environmental variability and internal physiological demands (Le Blanc, 2007). Larger decapod crustaceans such as shrimps, lobsters, and crabs are of considerable economic importance as globally traded seafood commodities, whereas planktonic crustaceans, including copepods and cladocerans, serve as critical trophic intermediaries linking primary producers to higher consumers within aquatic food webs.

The remarkable diversity in crustacean morphology, physiology, reproductive strategies, and life-history adaptations has enabled successful colonization of nearly all aquatic habitats (Gruner et al., 1993). These organisms occur in marine and freshwater ecosystems, including lakes, rivers, groundwater systems, and subterranean cave environments. Within these habitats, crustaceans occupy both benthic and pelagic ecological zones, reflecting their high degree of ecological plasticity.

Beyond their ecological roles, crustaceans are widely employed as biomonitors and bioindicators in aquatic environmental assessments. Their sensitivity to environmental perturbations allows effective interpretation of ecological data and facilitates the development of ecotoxicological endpoints for monitoring pollution and ecosystem health.

The endocrine system of crustaceans comprises epithelial endocrine glands and neuroendocrine structures derived from nervous tissue, which collectively regulate a wide array of physiological and developmental processes. Hormonal control governs moulting, hydromineral balance, sexual differentiation, reproduction, chromatophore activity, and cardiac function. Crustacean physiology is particularly complex due to the interaction between overlapping biological cycles, notably the recurrent moulting cycle and the reproductive cycle, both of which significantly influence adult physiological states (Skinner, 1985; Keller, 1992; Chang, 1993,1997; Webster, 1986).

Understanding crustacean endocrinology is therefore essential not only for elucidating fundamental biological mechanisms but also for advancing aquaculture practices. Insights into hormonal regulation can enhance growth management, reproductive control, and culture efficiency of commercially important species, thereby contributing to sustainable seafood production (Reddy and Ramamurthi, 1999).

In the present investigation, the structural organization of the Y-organ was analyzed using transmission electron microscopy to elucidate its ultrastructural characteristics in relation to cellular secretory activity and the reproductive cycle. Furthermore, experimental manipulations involving eyestalk ablation and eyestalk extract injection were carried out to assess their regulatory effects on the morphology and functional activity of the Y-organ.

Materials and Methods

2.1 Selection of Experimental Animal

The freshwater field crab *Barytelphusa guerini* was selected as the experimental model in the present investigation due to its ecological relevance, economic importance, and year-round availability (Gangotri et al., 1978). This species is an annual breeder exhibiting a well-defined reproductive cycle that can be categorized into three distinct phases based on breeding activity: the pre-reproductive period (January–April), reproductive period (May–August), and post-reproductive period (September–December).

Barytelphusa guerini constitutes an important edible crustacean species widely consumed by local populations, serving as a significant source of dietary protein in rural communities and a preferred food delicacy in urban regions. Ecologically, the species forms an integral component of paddy field ecosystems, contributing to nutrient cycling and trophic interactions

2.2 Procurement and Maintenance of Animals

Adult male and female crabs with a carapace width of 50–55 mm and body weight ranging between 35–45 g was collected from rice field habitats in the Attapur and Rajendranagar regions. The animals were transported to the laboratory and acclimatized under controlled conditions for one week prior to experimentation. Crabs were maintained in plastic troughs containing adequate freshwater, with water replaced on alternate days to maintain optimal environmental conditions. During acclimatization, animals were fed with grass. However, feeding was discontinued 24 h prior to experimentation to eliminate physiological variability arising from differential feeding status. Only healthy individuals with intact appendages were selected for experimental use. Each experimental group consisted of samples obtained from six animals.

2.3 Transmission Electron Microscopy

For transmission electron microscopy (TEM) analysis, the Y-organ was carefully dissected and immediately fixed in **3% glutaraldehyde** prepared in **0.05 M phosphate buffer (pH 7.2)** for 24 h at 4°C. This primary fixation was followed by post-fixation in **2% aqueous osmium tetroxide** to enhance membrane preservation and ultrastructural contrast.

Subsequently, tissues were dehydrated through a graded ascending series of ethanol concentrations and embedded in **Araldite 6005 resin** following the procedures described by Glauert et al. (1958) and Mollenhauer et al. (1959). The embedded tissues were sectioned into ultrathin sections of approximately **50–70 nm thickness** using an ultramicrotome.

The sections were post-stained with **uranyl acetate** and **lead citrate** to improve electron density and contrast. Prepared sections were examined at various magnifications using a **Hitachi H-7500 transmission electron microscope** at Ruska Laboratories, Sri Venkateswara Veterinary University (SVVU), Rajendranagar, Hyderabad. TEM observations were conducted on control animals representing all three phases of the annual reproductive cycle.

Ultrastructural Studies of The Y-Organ: A Review Of Previous Investigation

The Y-organ is an epithelial endocrine gland responsible for regulating moulting, reproductive activity, calcium metabolism, and several associated physiological processes in crustaceans (Knowles and Carlisle, 1956). It functions as the primary moulting gland by secreting α-ecdysone, which is subsequently converted into β-ecdysone, the biologically active moulting

Histologically, Y-organ cells exhibit ultrastructural characteristics comparable to vertebrate steroid-secreting endocrine cells (Christiansen and Fawcett, 1965). Seasonal histological variations in the Y-organ have been documented in both male (Hussain and

Vasantha, 1985) and female (Bharathi, 1987) *Barytelphusa guerini*, suggesting endocrine modulation associated with reproductive periodicity.

The functional role of the Y-organ in regulating moulting has been well established in crustaceans. Several investigations have documented histomorphological and ultrastructural changes occurring in the Y-organ during the moult cycle in various decapod species, including *Palaemon paucidens* and *Pandalus kessleri* (Aoto et al., 1974), the isopod, *Palaemon serratus* (Le Roux, 1977), and *Cancer anthonyi* (McConaugha, 1980).

Detailed ultrastructural organization of the Y-organ has also been described in numerous crustacean taxa such as *Carcinus maenas* (Knowles, 1965; Chassard-Bouchand and Hubert, 1975), *Pachygrapsus crassipes* (King, 1967), *Pachygrapsus marmoratus* (Bressac, 1973), *Astacus astacus* (Burghause, 1975), *Carcinus mediterraneus* (Zerbib et al., 1986), *Libinia emarginata* (Hinsch and Hajj, 1975; Charmantier and Trilles, 1979). These studies collectively demonstrate species-specific ultrastructural adaptations associated with endocrine regulation of moulting processes.

Result and Discussion

Generalised structure of the cells the electron photo micrographs of Y - organ reveals four types of cells based on the shape of the cell and shape of the nucleus. The shape of the cell corresponds to the shape of the nucleus.

4.1 Pre -Reproductive period

A –TYPE OF CELL : The electron photo micrographs of A-Type of cell shows a rounded cell with a rounded nucleus in both males and females.The cytoplasm of the cell is rich with polymorphic vacuoles of large and small sizes.The mitochondria are tubular rounded and club shaped with membranous profiles.Smooth endoplasmic reticulum,lipid droplets ,vesicles and secretory granules are observed.Nucleus is heterochromatic in nature with blocks of chromatin in perinucleolar and internucleolar pattern.Nucleolus is small and rounded.

B-TYPE OF CELL: These cells are characterized by irregular shaped nucleus with a round nucleolus in both males and females. The cytoplasm shows polymorphic vacuoles of varying sizes that is, small medium and large. The mitochondria are membranous rounded, tubular and club shaped. Few vesicles are observed. Rough endoplasmic reticulum is scarce, smooth endoplasmic reticulum is richly developed in the form of agranular profiles. Secretory granules and lipid droplets are observed in large numbers and distributed throughout the gland. Nucleus is heterochromatic in nature with blocks of chromatin arranged in internucleolar and perinucleolar pattern. Nucleolus is small and rounded.

C-TYPE OF CELL: These cells are characterized by an elongated shaped nucleus. The cytoplasm shows polymorphic vacuoles of varying sizes, that is small medium and large. The mitochondria are membranous tubular, rounded and club shaped, rough endoplasmic reticulum is scarce, smooth endoplasmic reticulum is richly developed in the form of agranular profiles. Lipid droplets and secretory granules are prominently observed. Nucleus is hetrochromatic in nature with blocks of chromatin arranged in perinucleolar and internucleolar pattern.

D- TYPE OF CELL: These cells are characterized by crescent shaped nucleus both in males and females. The cytoplasm shows membranous mitochondria with rounded, tubular and club shaped structure, polymorphic vacuoles of varying sizes are present, smooth endoplasmic reticulum in the form of fine granules are distributed throughout the cytoplasm. Lipid droplets and secretory granules are prominently observed. Nucleus is crescent shaped and hetrochromatic in nature with blocks of chromatin in perinucleolar and internucleolar pattern Nucleolus is small and rounded. The above features were observed during the pre- reproductive period.

4.2 Legend for Figure

Magnification- 5500 X: Electron photo micrograph of Male and female Y-organ in pre-reproductive period showing, A-Cell. B-Cell, C-Cell and D-Cell.

♦ M – Mitochondria ♦ SG – Secretory granules ♦ LD – Lipid droplets ♦ V – Vacuoles ♦ SER – Smooth endoplasmic reticulum ♦ N – Nucleus ♦ NL- Nucleolus ♦ NM-Nuclear membrane ♦ NP-Nuclear pore ♦ CH – Chromatin ♦ CJ – Cell junction ♦ VS - Vesicle

FIG-1 MALE

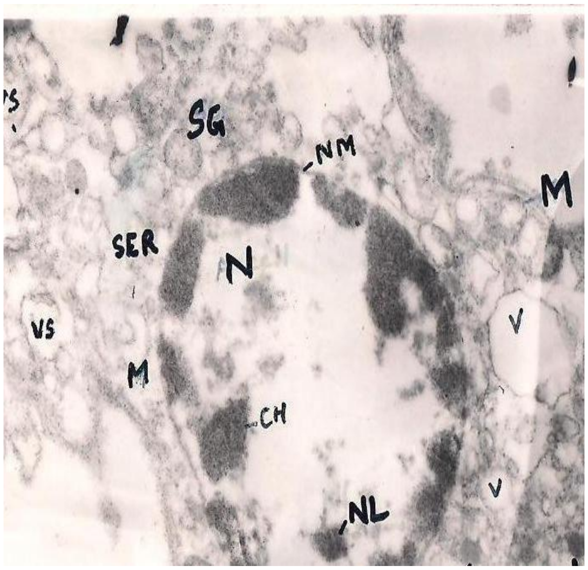
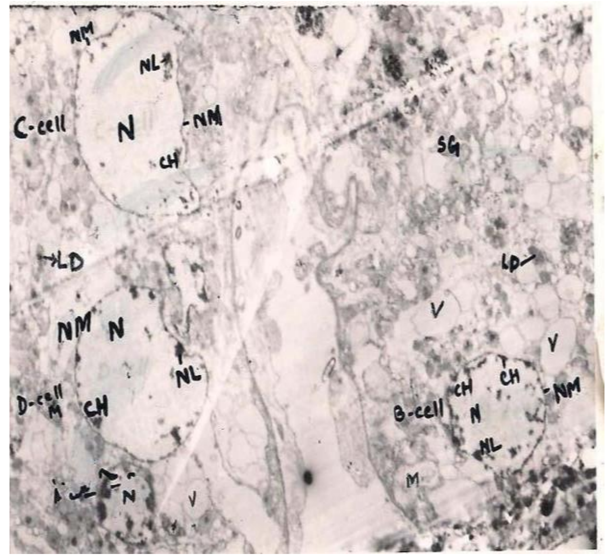


FIG-2 FEMALE



4.3 Reproductive Period

A-TYPE OF CELL; During the reproductive period the cells are richly loaded with secretory granules. Mitochondria are rounded, tubular and club shaped distributed throughout the cytoplasm. Smooth endoplasmic reticulum is present in the form of agranular profiles. Vacuoles are few in number. Prominent vesicles are distributed throughout the cytoplasm. Nucleus is heterochromatic with round nucleolus. Chromatin is arranged in the form of perinucleolar and internucleolar pattern.

B-TYPE OF CELL: These cells with irregular nucleus are more in number during the reproductive period. The cytoplasm is rich in secretory granules; few vacuoles are also seen. Mitochondria are rounded, tubular and club shaped, smooth endoplasmic reticulum is present in the form of agranular profiles. Lipid droplets are few in number in the form of granules, prominent vesicles were distributed throughout the cytoplasm. Nucleus is irregular in shape and heterochromatic in nature with blocks of chromatin in nucleoplasm, nucleolus is small and rounded.

C-TYPE OF CELL: The cells show an elongated nucleus. Cytoplasm is rich in secretory material. Vacuoles are few in number, lipid droplets are few in number. Mitochondria are rounded, tubular and club shaped. Smooth endoplasmic reticulum is scarce, prominent vesicles are distributed throughout the cytoplasm. Nucleus is heterochromatic with round nucleolus.

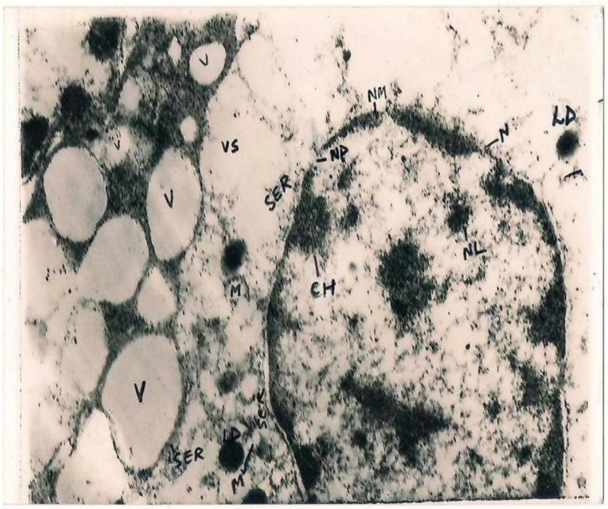
D – TYPE OF CELL: The D-Type of cells with a crescent shaped nucleus are less in number during this period in both males and females. The cytoplasm shows secretory granules. Lipid droplets and vacuoles are few in number, smooth endoplasmic reticulum is scarce. Mitochondria are rounded, tubular and club shaped, prominent vesicles were distributed throughout the cytoplasm. Nucleus is heterochromatic with round nucleolus

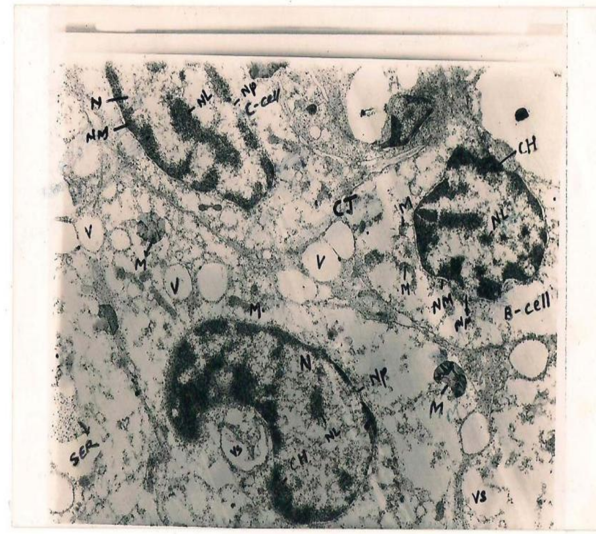
4.4 Legend for Figure

Electron photo micrograph of male Y-organ in reproductive period showing, A-Cell, B-Cell, C-Cell and D-Cell
Magnification- 5500 X

- M – Mitochondria, SG – Secretory granules, V – Vacuoles, N – Nucleus, NL- Nucleolus
NM-Nuclear membrane, CH – Chromatin, FG – Fat globules.

FIG-3 MALE FIG-4 FEMALE***4.5 POST*** **Reproductive Period**





A-TYPE OF CELL: The A- Type of cells are rounded in shape with a round nucleus. The cytoplasm of the cell possesses polymorphic vacuoles of large and small sizes. Mitochondria are tubular, rounded and club shaped with membranous cristae. Fat globules are prominently seen, smooth endoplasmic reticulum in the form of agranular profiles are observed. Secretory granules are few in number. Nucleus is heterochromatic with chromatin arranged in perinucleolar pattern. Nucleolus is small and rounded.

B-TYPE OF CELL***:*** The B-Type of cells with irregular shape are less in number in both males and females during this period. The cytoplasm of the cell shows many polymorphic vacuoles of large and small sizes. A tubular, rounded and club shaped mitochondria with membranous cristae are seen. Prominent fat globules are seen, agranular profiles of smooth endoplasmic reticulum were observed Secretory granules are few in number. Nucleus is heterochromatic with chromatin spread in dense nucleoplasm, with a round. Nucleolus

C-TYPE OF CELL: The C-Type of cells with an elongated nucleus are more in number in both males and females during this period. The number of vacuoles increases and prominent fat globules are observed. Secretory granules are few in number. Mitochondria are tubular, rounded and club shaped with membranous cristae, agranular profiles of smooth endoplasmic reticulum were observed. Nucleus is heterochromatic with chromatin arranged in perinucleolar pattern. Nucleolus is small and rounded.

D-TYPE OF CELL: The D-Type of cell with crescent shaped nucleus are more in number in both males and females. The cytoplasmic details are same as that of B-cell. The number of vacuoles increases and prominent fat globules are observed. Secretory granules are few in number. Mitochondria are tubular, rounded and club shaped with membranous cristae. Nucleus is heterochromatic with chromatin arranged in perinucleolar pattern. Nucleolus is small and rounded.

4.6 Legend for Figure

Electron photo micrograph of male and female Y-organ in Post reproductive period showing, A-Cell, B-Cell, C-Cell and D -Cell.
Magnification- 5500 X

- M – Mitochondria, SG – Secretory granules, V – Vacuoles, N – Nucleus, NL- Nucleolus
NM-Nuclear membrane, CH – Chromatin, FG – Fat globules.

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FIG -5 MALE

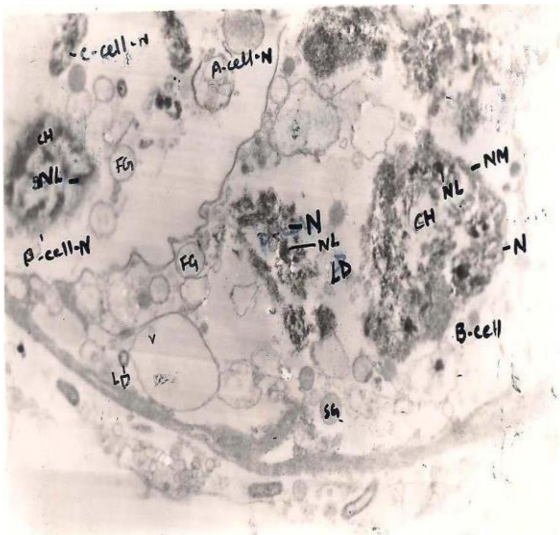
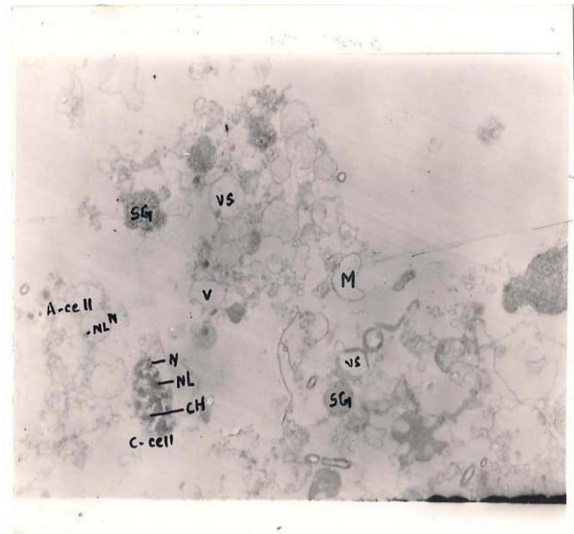


FIG -6 FEMALE



Discussion and Conclusion

The Y-organ, functionally recognized as the moulting or ecdysial gland, represents a paired endocrine structure of ectodermal origin (Lachaise and Lerox, 1993). Earlier investigations by Hussain (1987) in males and Bharthi (1987) in females of *Barytelphusa guerini* localized the gland ventrolateral to the eye sockets, positioned between the mandibular external adductor muscles at the anterolateral margin of the branchial chamber.

In the present study, the Y-organ was observed as a paired, ventrally situated gland lateral to the ocular peduncles. Morphologically, the gland is highly lobulated and ovoid, exhibiting a distinct posterior inner notch and a creamish appearance. Morphometric analysis revealed dimensions of approximately 0.44–0.46 mm in diameter and 0.83–0.87 mm in length in males, whereas females measured 0.45–0.47 mm in diameter and 0.82–0.85 mm in length, indicating negligible sexual dimorphism in gland size.

Histological examination revealed four morphologically distinct cellular populations differentiated primarily on nuclear morphology:

- **A-cells:** small cells possessing a compact, spherical nucleus.
- **B-cells:** Irregularly contoured cells with pleomorphic nuclei.
- **C-cells:** large elongated cells characterized by elongated nuclei.
- **D-cells:** large cells containing crescent-shaped nuclei.

5.1 Ultrastructural Characteristics

During the **pre-reproductive phase**, the cytoplasm exhibited extensive polymorphic vacuolation with vacuoles of variable dimensions distributed throughout the cytoplasmic matrix. Mitochondria displayed marked polymorphism, occurring in tubular, rounded, and club-shaped forms with well-defined membranous profiles. Abundant smooth endoplasmic reticulum (SER), lipid droplets, and prominent secretory vesicles were observed. The nucleus appeared heterochromatic with chromatin aggregation in perinucleolar and internucleolar arrangements, accompanied by a small, spherical nucleolus.

In the **reproductive phase**, cells demonstrated enhanced secretory activity characterized by dense accumulation of secretory granules. Mitochondria remained polymorphic and uniformly dispersed throughout the cytoplasm. The SER occurred predominantly as agranular tubular profiles, whereas cytoplasmic vacuoles were comparatively reduced. Numerous vesicular structures were evident, suggesting elevated endocrine activity. Nuclear architecture remained heterochromatic with a distinct rounded nucleolus and organized chromatin distribution.

During the **post-reproductive phase**, cytoplasmic polymorphic vacuoles reappeared prominently. Mitochondria retained tubular and rounded conformations with clearly defined cristae. Lipid inclusions and fat globules were conspicuous, while SER profiles persisted in agranular form. Secretory granules were markedly reduced, indicating diminished secretory activity. Nuclear chromatin was predominantly arranged in a perinucleolar pattern with a small rounded nucleolus.

5.2 Functional and Cytophysiological Interpretation

Steroidogenic cells are typically characterized by extensive smooth endoplasmic reticulum, polymorphic mitochondria, lipid inclusions, secretory vesicles, and heterochromatic nuclei. The ultrastructural attributes documented in the present investigation closely conform to these diagnostic features, strongly supporting the steroidogenic nature of Y-organ cells.

The abundance of SER suggests active lipid metabolism and steroid biosynthesis pathways, facilitating intracellular transport and enzymatic conversion processes. Mitochondrial structural polymorphism observed herein corroborates the established relationship between mitochondrial morphology and steroidogenic activity (Gemmel et al., 1977; Nussdorfer et al., 1978), consistent with observations reported in *Carcinus maenas* (Knowles, 1965).

Lipid droplets and fat globules likely function as metabolic reserves required for steroid hormone synthesis, similar to findings in *Libinia emarginata* (Hinsch and Hajj, 1975). The presence of abundant secretory vesicles and heterochromatic nuclei comparable to those reported in *Carcinus maenas* (Knowles, 1965) and *Carcinus mediterraneus* (Zerbib et al., 1975) further substantiates endocrine specialization of the gland.

5.3 Physiological Implications

The present findings confirm the steroidogenic nature of the Y-organ in *Barytelphusa guerini*. Among the identified cellular components, B-cells are presumed to constitute the principal ecdysone-secreting elements responsible for regulation of moulting processes. The increased prevalence of B-cells during the reproductive phase suggests a functional association between ecdysteroid synthesis and reproductive physiology, indicating a possible trophic influence of moulting hormones on reproductive activity.

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