

“Histomorphological Changes In The Structure Of Y-OrganA Moulting Gland During The Annual Reproductive Cycle In The Fresh Water Crab *Barytelphusa Guerini*”

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Abstract:

Crustaceans are important constituents of aquatic ecosystems that provide a variety of ecological and economic services. Many crustaceans live in marine environments ,others in various freshwater systems and may be used as biomonitors and bioindicators in various aquatic systems. The Y-organs of crustaceans are classical non-neural endocrine glands . They are paired and lobulated structures and are located ventrolaterally to the eyesockets. The Y-organs of crustaceans secrete ecdysteroids (moulting hormone) which play a crucial role in the moulting and growth of crustaceans .The present study reveals the structural and histomorphological pattern of the Y-organ during the annual reproductive cycle.

Keywords : *Barytelphusa guerini*, Y-organ, Histomorphology, Light Microscopy .

INTRODUCTION

Among arthropods crustaceans are a major group inhabiting a variety of habitats. About 30,000 species of crustaceans like Crabs,Prawns, Lobsters, Shrimps etc constitute major portion of aquatic biota. They are important from both biological and ecological point of view and hence offer great scope for investigation. The crustaceans occupy diverse ecological niches and regulate neuroendocrine signaling cascades and physiology in response to environmental and internal cues (Le Blanc , 2007).

The larger crustaceans (Shrimps, Lobsters and Crabs) are used as food throughout the world and therefore important to human economy . Some planktonic crustaceans such as Copepods, water fleas etc are major links in food chains .The crustaceans are used as biomonitors and bioindicators in various aquatic systems . Using these organisms ,interpretation of data from bioindicator studies and the development of ecotoxicological end points can be made .The Y-organs of crustaceans are classical non-neural endocrine glands . They are paired and lobulated structures and are located ventrolaterally to the eyesockets. The histological characteristics of these glands are similar to the prothoracic glands. The Y-organs of crustaceans secrete ecdysteroids (moulting hormone) which play a crucial role in the moulting and growth of crustaceans and also shows structural changes during the course of the moult cycle. These glands are the source of alpha-ecdysone , which is converted to beta ecdysone ,the moulting hormone (Gabe, 1953; Chang O'Connor , 1977;

Keller and Schmid , 1979 ; Reddy and Ramamurthy,1999) .Growth and moulting alternates in the life of crustaceans (Yudin *et al .* , 1980) . Hormones regulate crustacean moulting and regeneration (Skinner ,1985 ; Chang *et al .* , 1993 ; Lachaise *et al .* , 1993 ; Reddy and Ramamurthy , 1999) .

Gabe (1953, 1956) observed a pair of glands in the cephalothorax of malacostracans and named them Y- organs and compared them with the moulting gland of insects based on its histomorphology. The Y-organ is an epithelial endocrine gland which controls moulting, reproduction, calcium distribution and other physiological activities (Knowles and Carlisle , 1956). The Y- organs represent the moulting gland in crustaceans which secretes alpha – ecdysone as a primary secretory product that is converted to beta-ecdysone, the moulting hormone (Gabe, 1953; Chang and O'Connor ,1977) .The location of the Y-organ was reported in the cray fish *Cambarus affinis* by (Chaudonneret ,1956), *Carcinus maenas* (Echalier,1959) , *Procamarus sp* (Durand ,1960) , Shrimp *Pandalus danae* (Hoffman,1966,1967) , Isopod *Palaemon serratus* (Le Roux,1968,1974), *Cambarellus shuffeldti* (Connell ,1970) , *Varuna litterata* (Madhyastha and Ranganekar ,1972) ,*Sphaeroma serratum* (Charmantier and Trilles,1979), Indian white Shrimp *Fenneropenaeus indicus* (Lachaise *et al .* ,1993).

The Y-organ varies in gross morphology and location in different species of crustaceans. It is conical in the Branchyuran *Cancer pagurus*, tenticular in Natantia *Palaemon paucidens*, foliaceous in Isopoda *Sphaeroma serratu* (Spindler and O' Connor , 1980). The histomorphological pattern of the Y-organ has been studied in a number of decapod crustaceans, in *Protunus trituberculatus* (Miyawaki and Taketomi,1971), *Pachygraspus marmoratus* (Bressac,1973) , *Palaemon paucidens* (Aoto *et al.*,1974) In *Libinia emarginata* (Hinsch and Hajj,1975), *Carcinus maenas* (Chassard Bouchand and Hubert,1975,1976), *Palaemon serratus* (Le Roux,1977 and Chang O'Connor,1977), *Cancer anthonyi* (Vernet *et al.*,1978 and Mc Conaughy ,1980), Indian white Shrimp *Fenneropenaeus indicus* (Vijayan *et al.*,2003). The Y-organ has the cell pattern which is similar to the vertebrate cells associated with steroid secretions (Christiansen and Fawcett ,1965). Seasonal histological variations were reported in the Y-organ of male *Barytelphusa guerini*, (Hussain and Vasantha,1985) and in female *Barytelphusa guerini*, (Bharathi,1987). Earlier some aspects on the morphology and histomorphology of the Y-organ of male *Barytelphusa guerini* was done by Hussain (1987) and on female by Bharathi (1987). In the present investigation structure of the Y-organ was observed under dissection microscope and light microscope in relation to the reproductive cycle .Also the effect of eye stalk ablation and eye stalk extract injection was also studied.

MATERIAL AND METHODS

SELECTION OF TEST ANIMAL

In the present investigation , the fresh water field crab , *Barytelphusa guerini* was selected as the test animal because of its economic importance and easy availability throughout the year (Gangotri *et al .* , 1978). It is an annual breeder and the annual reproductive cycle has been differentiated into 3 different stages/ periods depending on their breeding behavior .

1. Prereproductive period (January to April)
2. Reproductive period (May to August)
3. Post reproductive period (September to December)

These field crabs are important edible species of local population . They form major source of proteinaceous food for rural population and delicacy for urban people. They are also an important component of paddy field ecosystem.

PROCUREMENT AND MAINTENANCE OF THE ANIMAL

Mature adult male and female crabs with a carapace width of 50mm to 55mm, weighing

35g to 45g were collected from the rice fields of Attapur and Rajendranagar area. They were maintained in the laboratory and acclimatized to the laboratory conditions for one week by maintaining them in plastic troughs with sufficient water. Water was changed on alternate days and they were fed with grass. They were not fed one day prior to the experimentation to avoid differences, if any due to differential feeding.

DISSECTION MICROSCOPY

Isolated samples of Y-organ were kept in physiological saline (Grau and Crooke 1992, Toullec *et al.*, 1996) observed under a dissecting microscope, and photographed with a Nikon digital camera.

LIGHT MICROSCOPY

For the present investigation the Y-organ of the experimental animals were fixed in 2.5%-3% gluteraldehyde in 0.1M phosphate buffer (pH-7.2) for 24 hours at 4°C and post fixed in 2% aqueous osmium tetroxide in the same buffer for 2 hours. Dehydrated in series of graded alcohols, infiltrated and embedded in Araldite 6005 resin (Glauert *et al.*, 1958 and Mollenhauer *et al.*, 1959). Semi thin sections (1200mm) were made with a glass knife on ultra microtome (Leica ultra cut UCT-GA-D/E-1/00) mounted on glass slide and stained with 5% Toluidineblue + AzurII in borex buffer (Bozzola and Russel, 1998). Required magnifications as per the standard procedure at Ruska Labs College of Veterinary Sciences, SVVU, Rajendranagar, Hyderabad, India.

RESULTS

DISSECTION MICROSCOPY

The Y-organ of *Barytelphusa guerini* is a paired glandular lobulated structure situated ventrolateral to the eye sockets between the mandibular external adductor muscles and at the anterolateral edge of the branchial chamber. It is ovoidal, translucent, creamish in colour with a notch at the inner posterior end measuring about 0.44 – 0.46 mm in diameter and 0.80 -0.85 mm in length in the sexually mature male and female crabs. The length and the diameter of the gland of male and female is same during the three periods of annual reproductive cycle (fig-1,2).

LEGEND FOR FIGURE

Fig 1 - Structure of the whole gland Y-organ in male under dissection microscope .
Magnification-10 X

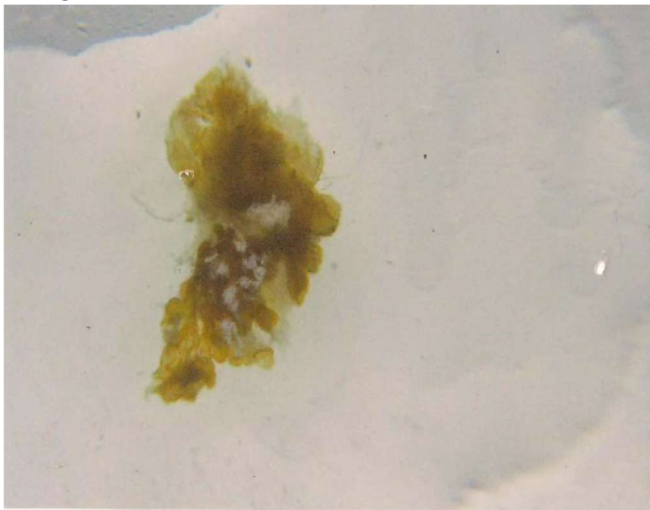
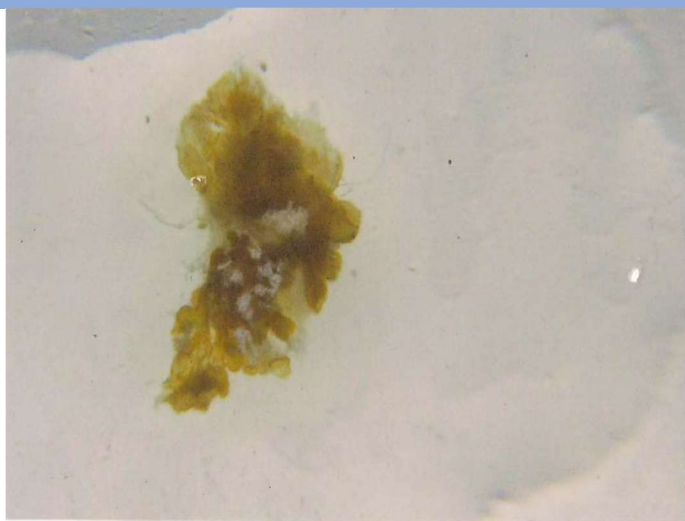


Fig 2 - Structure of the whole gland Y-organ in female under dissection microscope .
Magnification-10 X



LIGHT MICROSCOPY

Generalised structure of the gland

The light photo micrographs revealed that the cells of the Y-organ are closely packed, arranged in lobes separated by stromal layer with sparse cytoplasm. Four types of cells are distinguished based on the shape and the size of the nucleus .

A TYPE OF CELL - These cells are characterized by small round nucleus.

B TYPE OF CELL - These cells are characterized by large irregular shaped nucleus.

C TYPE OF CELL - These cells are characterized by large and elongated nucleus.

D TYPE OF CELL - These cells are characterized by crescent shaped nucleus.

PRE-REPRODUCTIVE PERIOD - (fig - 3,4)

During this period the cells are closely packed in a dense connective tissue with intercellular spaces in both males and females . The A-Cells and B-Cells were found to be more in number and uniformly distributed throughout the gland, whereas the C-Cells and D-Cells are less in number ,mainly seen at the anterior proximities of the cell.

REPRODUCTIVE PERIOD- (fig -5,6) During the reproductive period the cells are closely packed in a dense connective with less intercellular spaces in both males and females .The A Cells and B-Cells are more in number uniformly distributed throughout the gland, whereas the C Cells and D-Cells are least in number seen at the proximities of the cell.

POST REPRODUCTIVE PERIOD - (fig - 7,8)

During the post reproductive period the cells are loosely packed, connective tissue is scarce , with large intercellular spaces in both males and females .The number of A-Cells are same as observed in the other periods ,B-Cells are least in number, whereas the C-Cells and D-Cells are more in number during this period.

LEGEND FOR FIGURE

Fig 3,4 - Light photo micrograph of the male and female Y-organ during the pre reproductive period showing four types of cells

Magnification- 70 X

Fig 3 - MALE

- ☐ A - Cell
- ☐ B - Cell
- ☐ C- Cell
- ☐ D- Cell

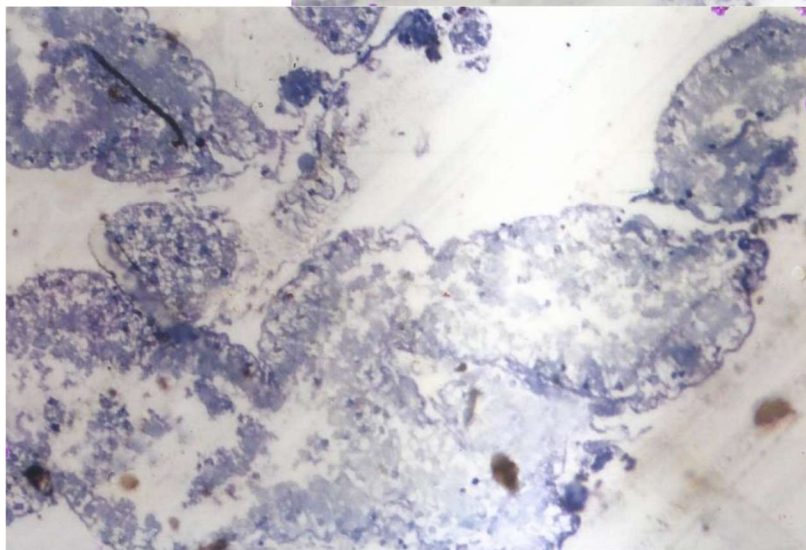
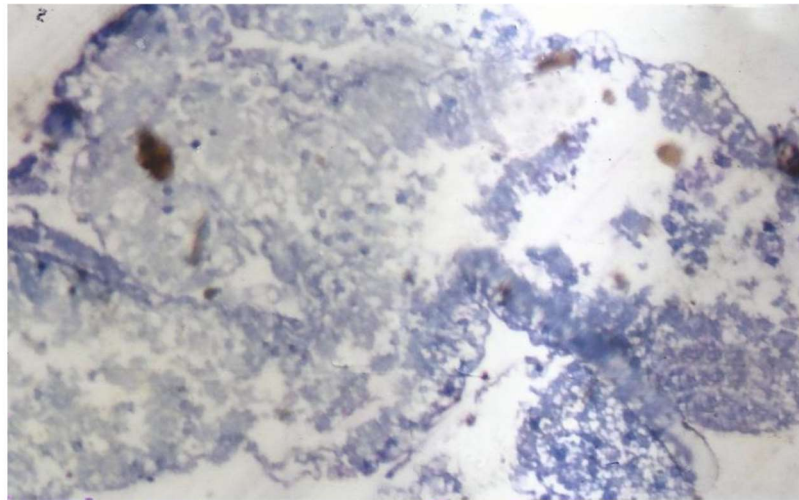


Fig 4 - FEMALE

LEGEND FOR FIGURE

Fig 5,6 - Light photo micrograph of the male Y-organ during the reproductive period showing four types of cells
Magnification- 70 X

- ☐ ☐ A - Cell
- ☐ ☐ B - Cell
- ☐ ☐ C- Cell
- ☐ ☐ D- Cell

Fig 5 - MALE

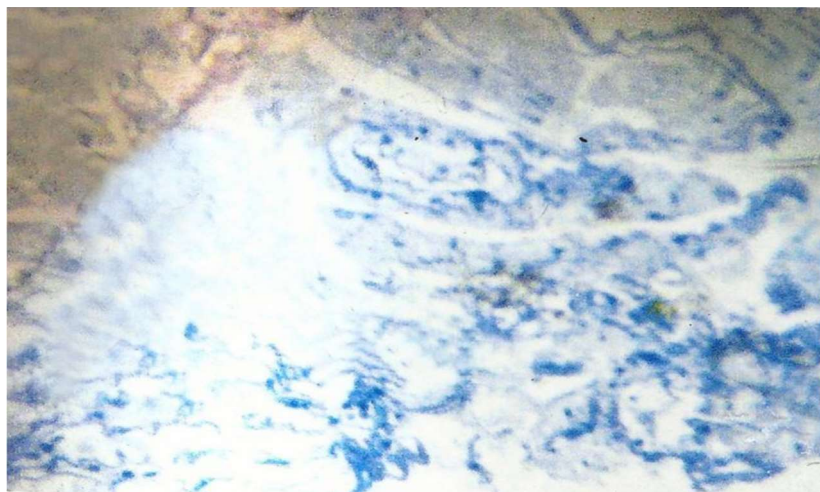
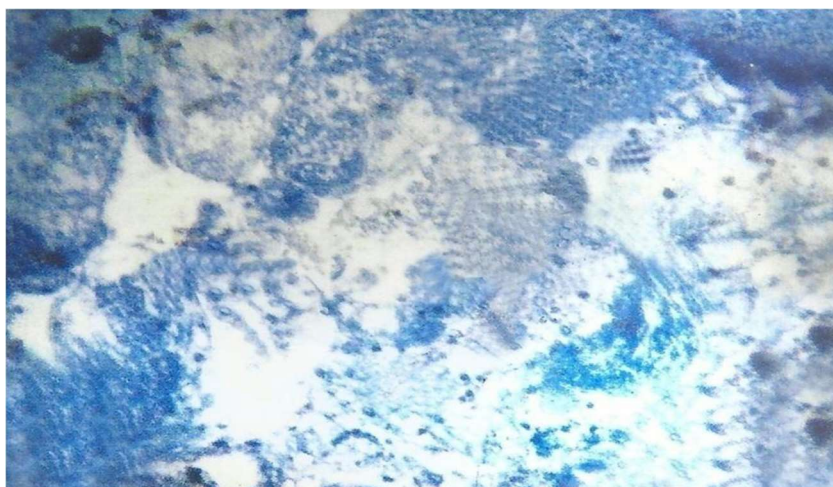


Fig 6 - FEMALE

LEGEND FOR FIGURE

Fig 7,8 - Light photo micrograph of the male Y-organ during the Post reproductive period showing four types of cells

Magnification- 70 X

- A - Cell
- B - Cell
- C- Cell
- D- Cell

Fig 7 - Male

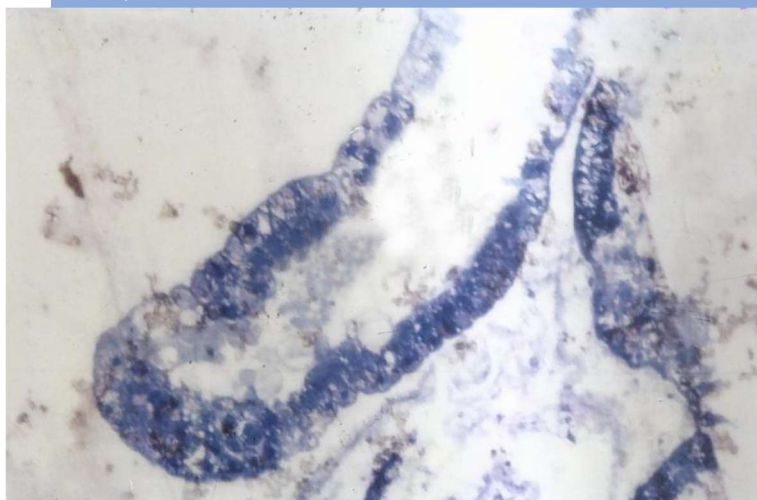
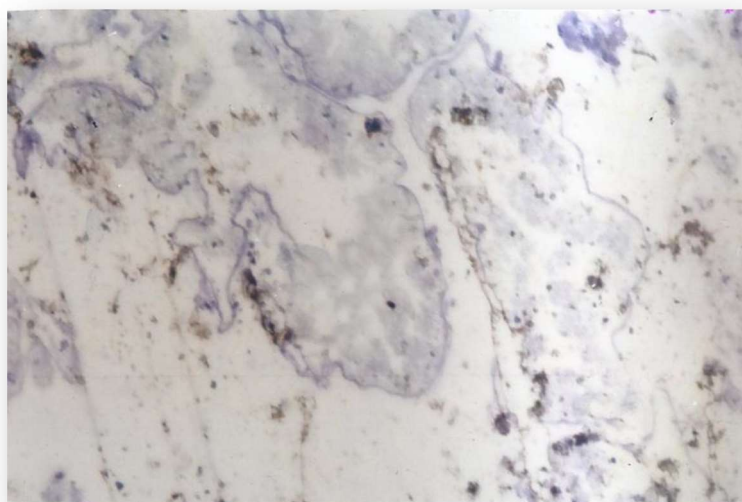


Fig 8 - FEMALE



Discussion

The Y-organ has been thoroughly investigated in *Carcinus maenas* (Gabe,1953 and Echailer , 1959) and interpreted as the ecdysial gland of crustacea . Gabe (1953) considered that in all malcostracans Y-organ is maxillary in position .Echalier (1959) reported that the Y-organ in *Carcinus maenas* is located at the junction of branchial and pre branchial mandibular adductor muscles and just above the junction of branchiostegite with lateral body cuticle . Aoto *et al* (1974) found the Y-organ in *Palaemon paucidens* and *Pandalus kessleri* located at the junction of prebranchial and branchial chambers just beneath the inner wall of branchiostegite . Hinsch and Hajj (1975) located the ecdysial gland of spider crab, *Libinia emarginata* posterior to the mandibular region . Le Roux (1977) described the Y-organ of *Palaemon serratus* as a strip of epidermis situated a little below the branchiostegite ,spreading in to the maxillary segment . Mc.Conaugha (1980) located the Y-organ in *Cancer anthonyi* just anterior to the junction of branchial and pre branchial chambers and ventral and posterior to the base of antennules. The Y-organ was termed as the moulting gland or ecdysial gland consisting of paired organs,ectodermal in origin , (Lachaise and Lerox ,1993) . Hussain (1987) in male *Barytelphusa guerini* and Bharthi (1987) in female identified the Y-organ as a pair of glands situated ventrolateral to the eyesockets between the mandibular external adductor muscles and at the anterolateral edge

of the branchial chamber. In the present investigation the Y-organ of *Barytelphusa guerini* is identified as a paired organ ventral in position and lateral to the eye sockets. It is highly lobulated, ovoid structure with a notch at the inner posterior end. It is creamish in colour measuring about 0.44-0.46 mm in diameter and 0.83-0.87 mm in length in males, whereas in females it measures about 0.45-0.47 mm in diameter and 0.82-0.85 mm in length. Earlier literature on the histological structure of the Y-organ of the crustaceans suggests the presence of one type of cell (Knowles and Carlisle , 1956); *Carcinus mediterraneus* (Zerbib *et al.*, 1975) ; *Hemigraspus nudus* and *Carcinus maenas* (Buchhloz and Adelung , 1980). But in *Varuna litterata* , there are two type of cells i.e small and large. The small cells are more abundant, with a distinctly staining roughly spherical nucleus. It is characterized by a single , centrally placed or peripherally situated nucleolus. The chromatin material is in the form of small particles, seen on the inner surface of nuclear membrane. The large cells are scarce. The nucleus possess a central nucleolus and chromatin material is uniformly scattered in the nucleoplasm. The occurrence of two types of cells suggests the possibility of secreting two types of hormones (Madhyastha and Rangnekar 1972) . Aoto *et al.*, (1974) reported that in *Palaemon paucidens* the Y- organ is composed of many lobulated cell masses. Each lobule consists of 10-20 cells with poor cytoplasm and the cell boundaries are hardly detectable. The nucleus is spherical or ovoidal with aggregated chromatin. The Y-organ of *Sphaeroma serratum* (Charmantier and Trilles, 1979) shows a large nucleus in its cells. The Y-organ of male *Cancer antennarius* (Hinsch *et al.*, 1980) is lobulated, epitheloid structure embedded in brain fatty tissue cells in periphery, extending processes to the connective tissue capsule . Cytoplasm is scarce relative to nuclear volume , while in *Cancer anthonyi* (Mc Conaughy , 1980) scarce cytoplasm with indistinct boundaries is reported . In female *Barytelphusa guerini* (Bharathi, 1987) , the Y-organ is composed of single type of closely packed subspherical cells, scattered in dense connective tissue stroma . The nucleus is large, prominent, roughly spherical filled with distinctly stainable material . Cytoplasm is scarce and cell boundaries are poorly visible. In the present study the Y-organ of male & female *Barytelphusa guerini* showed the presence of 4-types of cells based on the size and shape of the nucleus.

A-Type of cell is smaller in size with a small round nucleus.

B-Type of cell is irregular in shape with irregular nucleus.

C-Type of cell is large and elongated with elongated shaped nucleus.

D-Type of cell is large with crescent shaped nucleus.

In the present investigation the histomorphological structure of Y-organ of *Barytelphusa guerini* shows, the cytoplasm of the cell is rich with polymorphic vacuoles of large and small sizes distributed throughout the cytoplasm. During pre-reproductive period A-cells are fewer in number whereas the B-cells are numerous . In reproductive period the A-cells were absent, and B-cells increased to a great extent. In post reproductive period A-cells were seen occasionally , whereas the number of B-cells reduced. The variations in the cells during the annual reproductive cycle showed that these cells might have been involved in the secretory activity. Thus the present study of the light microscopic details confirms four types of cells in the Y-organ i.e, A-Cells, B-Cells, C-Cells and D-Cells. It is presumed that the B-Cells in particular may be the source of ecdysone and play an important role in moulting. The ecdysteroids also play a role in reproduction, therefore the fact that B-Cells are more in reproductive period is may be due to ecdysteroids, and which may have some tropic effect , which influences reproductive activity .

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