



**HISTOLOGICAL AND HISTOCHEMICAL STUDIES ON THE COLLETERIAL GLAND IN THE BEETLE,
CYBISTER TRIPUNCTATUS OL. (COLEOPTERA: DYTISCIDAE)**

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ABSTRACT:

A long-coiled thread like colleterial gland opens into the common oviduct. It consists of a wall of single layered epithelium enclosing a large lumen at the centre. The nuclei are stained with Feulgen and Toluidine reaction suggests synthesis of DNA and RNA respectively while the secretory material stored in the cells and lumen stained with Hg- Bromophenol blue suggests that protein nature of the secretory material. The secretory material does not react with PAS and Sudan B suggesting the absence of carbohydrate and lipids from the secretory material respectively.

KEYWORDS: Lipid, Bromophenol blue, PAS.

INTRODUCTION:

The colleterial gland is about $65.60 \pm \text{standard error} \mu\text{m}$ in diameter while the lumen is about $49.20 \pm \mu\text{m}$ in diameter. The wall of the gland composed of a layer of columnar epithelial cells with large spherical nuclei at the center and cytoplasmic inclusion in the cell bodies. The cell and nuclear diameter of the epithelial cells measure about 16.40 and $12.30 \pm \mu\text{m}$ respectively. Externally they are covered with a thin peritoneal membrane. The epithelium is internally lined with a thin layer of cuticular intima. The epithelial cells undergo cyclical changes during secretory phase, the nuclei become large the perikarya are initially containing secretory granule around the nuclei and later on disbursed towards the periphery. The secretory The present work is carried out on the aquatic beetle, *Cybister tripunctatus* OL. CLASSIFICATION (Richards and Davis 1977) Systematic position of the aquatic beetle, *Cybister tripunctatus* OL is given below.

Class – Insecta

Subclass – Pterygota

Division – Endopterygota

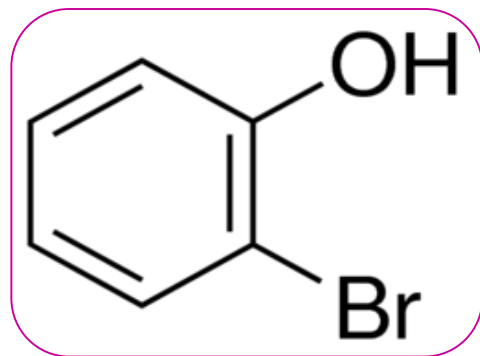
Order – Coleoptera

Suborder – Adephaga

Family – Dytiscidae

Genus – *Cybister*

Species – *tripunctatus* (OL)



CHARACTERS

1.2.1] In aquatic beetles *Cybister tripunctatus* OL, sexes are separate and sexual dimorphism is well marked as the forelegs of male beetles show presence of adhesive pads, while such structures are absent in the females.

1.2.2] It possesses filliform antennae.

1.2.3] Hind legs are notatorial, functioning as swimming organs, Flattened and fringed with large hairs.

1.2.4] Larvae are with long sickleshaped mandibles.

1.2.5] Last two abdominal segments along with abdominal lobes are Fringed with hairs.

1.2.6] Elytra store air beneath them. It is the source of oxygen which is Supplied to tracheal system by last two pairs of abdominal Spiracles, during diving in deep water.

• SELECTION

Aquatic beetle, *Cybistertripunctatus*. OL, is selected for the present Work because of the following reasons-

1.3.1] It is easily available, and commonly found in local ponds in all Seasons.

1.3.2] It can easily be collected by fishing nets or hand nets in ample Quantity.

1.3.3] It can be acclimatized under the laboratory conditions for a long Duration due to their sturdy nature and their quick adaptability to New environment.

1.3.4] It can be maintained by feeding small fishes and crustaceans.

1.3.5] It is of convenient size to handle and easy for experimentation.

• SOURCE

The aquatic carnivorous beetles were collected from the ponds Located Pavani, Disti.Bhandara (MS). The beetles were reared in laboratory Throughout the year to carry out the present studies.

• REARING

The larvae and adult beetles were kept in well aerated aquarium in The laboratory. The muddy water and small stones having crevices were Kept in aquarium to maintain natural condition. The small fishes were kept as a Food of the beetles. The stones were kept to provide place for egg laying. Some Times, they lay the eggs on the inner side of the wall of aquarium also. The Aquarium was covered to prevent escaping of beetles from the aquarium. The Fresh water was added for sufficient supply of oxygen.

The larvae and beetles were acclimatized and reared in laboratory Under normal condition of photoperiod 12L : 12 D and 24°C temperature. The Mating occurred mostly during daytime. The mated female laid eggs in a capsule Like case which hatched within 3-4 days depending upon environmental Conditions. The food was supplied once every day. The first instar larvae Underwent two moults. The well developed third instar larvae were trasfered Into another aquarium.

The larvae lastly constructed the pupal chambers in a soil. Newly Emerged adults of bothe sexes were separated and kept into individual glass jars. The date and time of emergence of the adult beetles were recorded.

2. METHODS

2.1 DISSECTON, FIXATION AND SECTIONING

The female reproductive organs dissected in insect Ringer's Solution under stereoscopic binocular microscope. The organs were fixed in Bouin's fluid for 18-24 hrs for histology and in 6 to 12 hours in Carnoy's fixative For DNA, RNA. Protein and carbohydrate histochemistry. The fixed tissues were, Dehydrated and embedded in paraffin wax at 60-62. The sections were cut at 4 And 10 um thickness on the microtome for histological and histochemical Staining techniques respectively.

For histochemistry of lipids, the ovaries, colleterial gland and Spermathecal gland were dissected gently, fixed in Baker's calcium formal Fixative and sections of 10-15um thickness were cut on the cryocut (Leica, U.S.A.).

2.2 TECHNIQUES

2.2.1 HISTOLOGICAL TECHNIQUES

Following histological techniques (Humason, 1962) were used-
1] Ehrlich's Haematoxylin-eosin (HE)

- 2] Heidenhain’s Iron-Haematoxylin-orange G (FeH)
- 3] Mallory’s triple stain.

2.2.2 HISTOCHEMICAL TECHNIQUES

The histochemical techniques are given in the table-1.

Table 1:- Histochemical staining techniques.

• **OBSERVATIONS**

IV. OBSERVATIONS

THE FEMALE REPRODUCTIVE SYSTEM

MORPHOLOGY

The female reproductive system is well developed in the adult beetle *Cybistertripunctatus* (OL). It consists of (Fig.1) :

- a pair of ovaries ;
- a pair of lateral oviduct ;
- a common oviduct ;
- a vagina ;
- a spermatheca with a sp-ermathecal gland and a colleterial gland.

The female reproductive organs are located in the abdominal cavity occupying the region comprising 1st to 8th abdominal segments. The reproductive organs are intermingled with the fat body and trachea. In the immature beetle, the ovaries are small laying ventral to the Alimentary canal in the posterior 4th and 5th segments of the abdomen. In the Matured beetles, the ovaries develop extensively and they occupy.

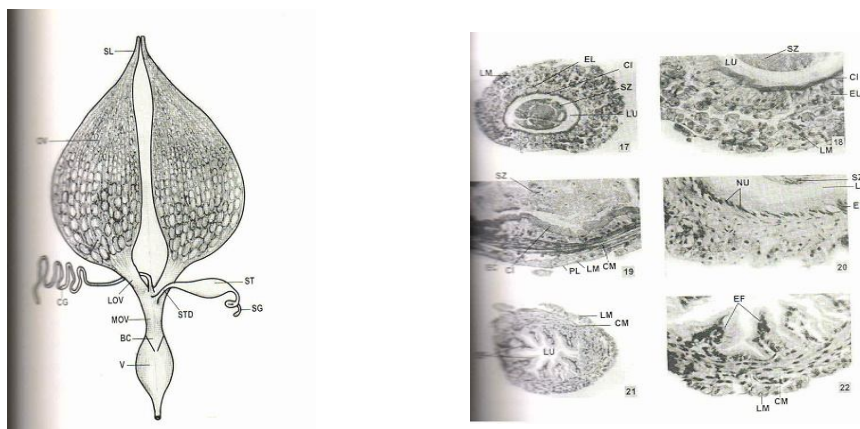


Fig 1 : Female Reproductive system in *Cybistertripunctatus* (Diagrammatic)

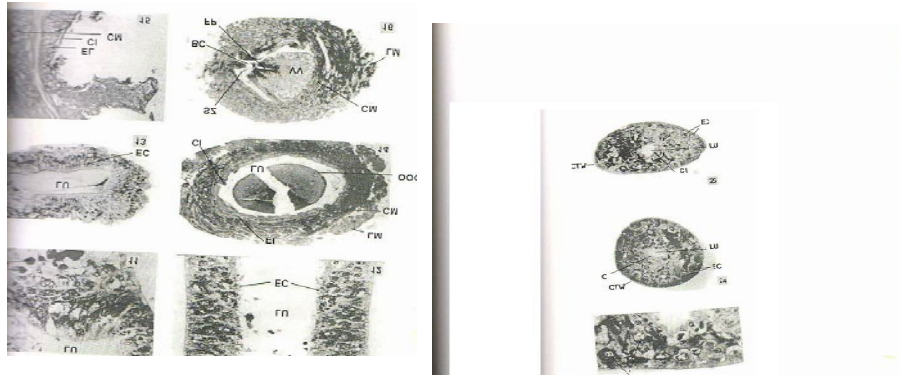
- | | | | | | |
|-----|---|-------------------|-----|---|---------------------|
| BC | - | Bursa copulatrix | SG | - | Spermathecal gland |
| CG | - | Colleterial gland | SL | - | Suspensory ligament |
| LOV | - | Lateral oviduct | ST | - | Spermatheca |
| MOV | - | median oviduct | STD | - | Spermathecal duct |
| OV | - | Ovary | V | - | Vagina |

most of the region of the abdominal cavity from 1st to 6th abdominal segments. Each ovary is a large and oval in shape consisting about 20 to 25 Ovarioles. Each ovariole is about 1.5 cm in length. An immature ovary measures About 8 to 12 mg and the fully matured one about 46 to 54 mg in weight.

The ovaries are externally covered with a thin peritoneal sheath. The ovaries are attached anteriorly to the inner surface of the wall of the 1st Abdominal segment by a suspensory ligament. They open posteriorly into the Lateral oviducts. The lateral oviducts are short and tubular structures. They run Latero-medially from

the 6th to 7th abdominal segments. Both the lateral oviducts unite together forming common oviduct. The common oviduct is a large Tubular and convoluted structure. The posterior part of the common oviduct is Modified into the bulbous vagina.

Large spindle shaped spermatheca is well-developed and located in The lateral region of the 4th to 6th abdominal segments. It opens into the common Oviduct at junction of the lateral oviducts by a fine elongated Spermathecal duct. Terminally it bears a distinct tubular spermathecal gland. The spermatheca is full formed in the matured female and differentiated into two Region; the receptaculumseminis and laguna. A long thread-like, enormously coiled collateral gland is present in The right lateral region of the abdominal cavity, below the ovary. The collateral



• **EXPLANATION OF FIGURES**

- Fig.23 T.S. passing through colleterial gland IHE X 85
- Fig.24 T.S. Passing through colleterial gland IHE X 192
- Fig.25 T.S. Passing through colleterial gland IHE X 480

Abbreviations :

- CI - Cuticular intima
- CTW - Connective tissue wall
- EC - Epithelial cells
- LU - Lumen
- NU - Nucleas

material is well stained by counterstains. It is stored into the lumen during post copulation period. The presence of intracellular as well as inter cellular spaces in the epithelial cells can be seen distinctly.

1. Oocyte Development and Vitellogenesis

The terminal oocyte undergo development periodically. Repeated cycles of oocyte development and subsequent cycles of oviposition occur in the adult female *Cybistertripunctatus*. Development of the terminal Oocyte shows consecutive stages of vitellogenesis.

During development the terminal follicles show remarkable changes in the oocyte shape, size, cytological structure, deposition of yolk material and formation of egg-membranes along with the cytomorphological change in the Trophocytes and follicular epithelium. The entire process of vitellogenesis can be

- **Divided into following five stages :**
 - 1) Pre-vitellogenic;
 - 2) Early-vitellogenic;
 - 3) Mid-vitellogenic;
 - 4) Late-vitellogenic and
 - 5) Maturation stage.

1.1 Histomorphological changes during vitellogenesis

1.1.1 Pre-vitellogenic stage

(Fig. 26-31)

In the newly emerged females. The ovaries are small thread like structures measuring about 20.00 ± 2.00 mg in weight. The follicle is filled with cytocysts and the oocytes are undifferentiated. In two day-old females, differentiation of nurse cells and oocyte become distinct. Most of the region of follicle is occupied by the nurse cells, and the oocyte is very small, lying ventrally. The oocyte bears centrally placed large germinal vesicle. The cytoplasm of oocyte is granular. The nurse cells are large and their nuclei are lobulated. The nurse cells measure about 96.10 ± 0.48 μm in diameter. They discharge secretory material into the oocyte through the radial canals. The follicular epithelium of the previtellogenic oocyte is composed of squamous epithelial cells. They possess large spherical nuclei at the centre measuring about 8.10 ± 0.5 μm in diameter. The pre-vitellogenic oocyte grows up to $201 \pm \mu\text{m}$ in length.

The transport of secretory material from the nurse cells to the previtellogenic oocytes is well evident. The pre-vitellogenic oocytes are filled

DISCUSSION

A colleterial gland is well developed in Dermastidae and Elateridae in Coleoptera (Beckers, 1956, a) and it is also well developed in *Cybister tripunctatus*.

In *Cybister tripunctatus*, the structural organization of the ovarioles suggest the polytrophic type of ovary, a characteristic feature of Adephagan Coleoptera in contrast to the telotrophic ovaries in Polyphaga (Davies, 1977).

Although in some Polyphaga, nutritive cords are indistinct (Datta Gupta and Kumar 1963, Berneet and Helms, 1972; Gundevia and Ramamurthy, 1972 b; Rajendram and Ramalingam, 1979 a; Monga and Sareen, 1980; Sareen and Sharma, 1981; Inamdar and Joshi, 1984), the trophocytes with well defined nutritive cords are of common occurrence in majority of polyphagous beetles (Krause, 1946; Schlottman and Bonhag, 1956; Zacharuk, 1958; Cerezke, 1964; Gupta and Riley, 1967; Gerber et al., 1971; Buning, 1972, 1978; Grodowitz and Brewer, 1987; Ma and Ramaswamy, 1987).

The terminal oocyte passes through various stages of oogenesis in Coleoptera: 3 in *Cylas formicarius* (Maruthi et al., 1987), 9 in *Tenebrio molitor* (Ullmann, 1973), 5 in *Epilachna vigintioctomaculata* (Kurihara, 1975), *Mylabris pustulata* (Sidhara et al., 1984), and *Dermestes maculatus* (Khalifa, 1986). In *Cybister tripunctatus* also the developing oocyte shows five stage of vitellogenesis.

Follicular epithelium undergoes cytological modifications during the development, facilitating the entry of exogenous yolk components and plays an important role in the formation of the egg membranes (Bonhag, 1958; De Wilds, 1964; Telfer, 1965; Engelmann, 1970; Mahowald, 1972; Brennan et al., 1982; Margaritis, 1985; Regier and Kafatos, 1985; Kurihara and Matsuzaki, 1989).

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