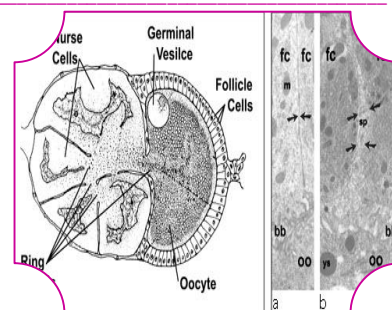




HISTOLOGICAL, HISTOCHEMICAL AND BIOCHEMICAL STUDIES ON THE OVARY IN THE BEETLE, *CYBISTER TRIPUNCTATUS* OL. (COLEOPTERA: DYTISCIDAE)

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ABSTRACT

A pair of large ovaries are situated on either side of the alimentary canal occupying most of the abdominal cavity. Each ovary is composed of large number of ovarioles. The ovarioles are differentiated Antero posteriorly into four regions: terminal filament, germarium, vitellarium and pedicel. The vitellarium consists of a series of developing oocytes in linear fashion, each accompanied with a group of 15 nurse cells and thus representing polytropic – Meroistic type. The terminal oocyte undergo development through a series of consecutive 5 vitellogenic stages such as pre, early, mid, late and maturational stages. The deposition of yolk starts at the early vitellogenic stage during which transport of fine granules from follicular cells to the oocyte is well evident. During mid and late vitellogenic stages the oocyte grows in size and filled with the yolk bodies. The nurse cells initially transport RNA to the vitellogenic oocyte and undergo degeneration prior to late vitellogenic stage. The histochemical and biochemical studies show protein lipid and carbohydrate composition of yolk material.

KEYWORDS: vitellogenesis, RNA, Yolk

INTRODUCTION

Female reproductive system and mechanism of oocyte development and vitellogenesis is of prime importance. The present work has, therefore, been undertaken to study female reproductive system in *Cybister tripunctatus* with special reference to the following aspects-

1. Morphology of female reproductive system;
2. Cytological changes occurring in developing oocytes, nurse cells and follicular epithelial cells;
3. Histochemical demonstration of synthesis, accumulation and transport of DNA, RNA, protein, carbohydrate and lipid during oogenesis;
4. histochemical and biochemical analysis of the yolk material and secre-

Tory material of the colleterial gland and Thorough study of the process of vitellogenesis. Become surrounded by follicular epithelium and connected by the trophic cord. The young oocytes occur in previtellogenic stage during which the trophocytes transport material to the oocytes needed for their development. The nutritive Cord collapse during vitellogenesis. The terminal oocytes undergo the process of Vitellogenesis and finally the follicular epithelium secretes a vitelline membrane and the chorion. The number of ovarioles vary between species from one in some Scarabaeinae to about thousands in *Meloprosarabaeus* (Engelman, 1979; Raabe, 1986).

The base of each ovariole forms a pedicel which unites and opens into a lateral oviduct. The lateral oviducts join to form a single median oviduct which is generally muscular and ectodermal in origin.

Cytological changes are observed in the cell lining of the oviducts. In *Oncopeltus fasciatus* the cells of the anterior region of oviduct show secretory Activity, whereas, the cells of the posterior part help in the deposition of cuticle (Chen et al., 1962). The distal end of the common oviduct forms the gonopore Which opens into the genital chamber. The genital chamber consists of the Vagina which is present between the gonopore the external vulva. The vagina is Covered with thick muscle layer and lined by a cuticular intima. The genital Chamber is modified forming the bursa copulatrix which stores The spermatophores. In *Lepidoptera*, the bursa is located on the 8th abdominal Segment and is connected with the vagina by the seminal duct. In some butterflies e.g. *Danus*, tooth-like cuticular denticles are present in the bursa copulatrix which help to cut-open the spermatophores. A pair of ectodermal glands are found associated with the genital chamber i.e. the spermathecae. The paired accessory glands or colleterial glands open into the vagina (Callahan and Chapin, 1960; Matsuda, 1976). The morphology and number of spermathecae vary from species to species. In most insects, it is a single spherical organ and may have its distal end specialized for secretion to from the spermathecal accessory gland. The Secretion of spermathecal gland is mucoprotein or muco-polysaccharide and it is An energy source to the spermatozoa stored in the spermatheca (Weaver and Edwards, 1990). Differentiation of spermatheca occurs in the late pupal stage And it is under the control of the hormone, 20-hydroxyecdysone (Raabe, 1986).

In the *Orthoptera*, *Chorthippus curtipennis* and *Gomphocerus rufa*, the Differentiation is under the control of juvenile hormone (Kaulenas, 1992). In *Rhodnius prolixus* secretion of the median neurosecretory cells control the Differentiation of spermatheca (Davey, 1985). The newly laid eggs in many insects are covered with a protective Coating. In *Lepidoptera*, the eggs are individually coated with a proteinaceous

1. MATERIAL AND METHOD

The present work is carried out on the aquatic beetle, *Cybister tripunctatus* OL.

1.1 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*

1.1 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 filiform 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 sickle-shaped 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.

1.2 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.

1.3 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.

1.4 REARING

The larvae and adult beetles were kept in well aerated aquarium in The laboratory. The muddy water and small stones having crevices wer 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'Solution under stereoscopic binocular microscope. The organs were fixed inBouin's fluid for 18-24 hrs for histology and in 6 to 12 hours in Carnoy's fixativeFor 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.

2.2.3) BIOCHEMICAL TECHNIQUES

In order to estimate total ovarian lipid, protein and carbohydrate Concentration in the ovary and colleterial gland of the adult female beetles, they Were sacrificed every day from emergence to the first ovipositio and ovaries And other structures were dissected out and processed for biochemical Estimation.

PREPERATION OF EXTRACT

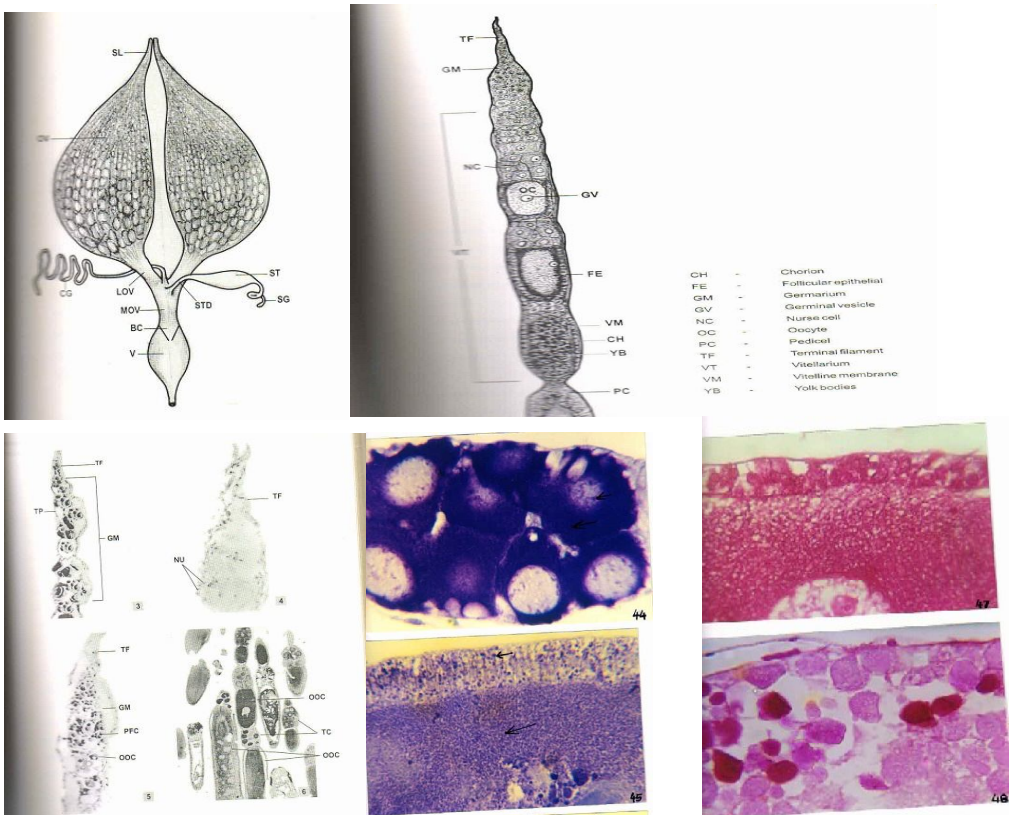
The ovaries and colleterial glands were removed gently after cutting the tergites of terminal region of the abdomen and immediately transferred to ice cold insect Ringer’s solution. The fat bodies, trachea, nerves and other tissues were carefully removed and each ovary and colleterial gland were weighed to ± 1 m.g. Both the organs were thoroughly washed in ice cold Ringer’s solution and then homogenized in ice cold citrate phosphate buffer (pH 6.8) and made upto 1 ml. The homogenate was centrifuged for 15 min. about 2000 revs/ min. The clear supernatant was stored under a drop of toluene at about 10oC until required.

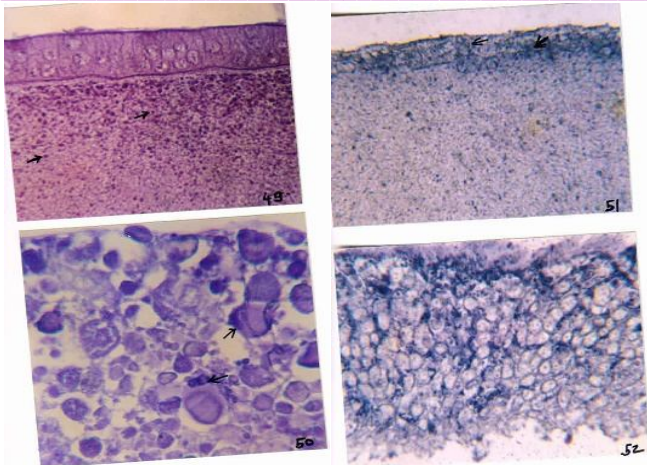
The following biochemical techniques were used for the estimation Of total protein, lipid and carbohydrate concentration in the ovaries and colleterial Gland-

- 1] Total protein estimation by Layne’s technique (Layne 1957).
- 2] Total lipid estimation by the Folch et al., method (Folch et al., 1957) and
- 3] Total carbohydrate estimation by Hawk’s method (Hawk et al: 1954).

2.3 EXPERIMENTAL DESIGN

The adult female beetles of known age viz; newly emerged (0-day),2,4,6,8 and 10-day old beetles, were taken out from each aquarium. The female reproductive organs were dissected out. The ovaries and a colleterial gland were separated, the weight was taken individually and the organs were processed for various histological, histochemical and biochemical techniques described above.





IV. OBSERVATIONS
THE FEMALE REPRODUCTIVE SYSTEM
1. MORPHOLOGY

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

1. a pair of ovaries ;
2. a pair of lateral oviduct;
3. a common oviduct;
4. a vagina;
5. a spermatheca with a spermathecal gland and
6. 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	-	Suspensary 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 fully 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 Gland opens into the common oviduct.

2. HISTOLOGY

2.1 The ovarioles

The ovarioles are long, tubular structures (Fig.3). They are Anteroposteriorly differentiated into following four regions, (Fig.2) :

1. a terminal filament;
2. a germarium;
3. a vitellarium and
4. a pedicel

2.1.1 The Terminal filament

(Fig.3-4)

Apical region of each ovariole contains stem line, the terminal filament. The terminal filament is anterior-most thread-like, slender, fine structure of the ovariole. The wall of the terminal filament is composed of a mass of small spherical cells with oval nuclei. The cells are almost devoid of

Ch	-	Chorion
FE	-	Follicular epithelial
GM	-	Germarium
GV	-	Germinal vesicle'
NC	-	Nurse Cell
OC	-	Oocyte'
PC	-	Pedicel
TF	-	Terminal filament
VT	-	Vitellarium
VM	-	Vitelline membrane
YB	-	Yolk bodies

Fig. 2: Ovariole in *Cybistertripunctatus* (Diagrammatic)

cytoplasm. Internally a large lumen is present. Externally, the terminal filament is covered with a fine layer of tunica propria. A transverse septum lies in between the terminal filament and the germarium. The terminal filament of all ovarioles of an ovary unite together to form a thick cord, the suspensory ligament.

2.1.2] The germarium

(Fig.5)

It is a small region clearly marked from the terminal filament by a transverse septum. It lies between the terminal filament and the vitellarium. It contains some cystoblasts, cystocysts and profollicular cells. The anterior Part of the germarium is exclusively filled with cystoblasts. The cystoblasts are Spherical in shape and contain dense cytoplasm and large spherical nuclei at the Centre. The cystoblasts produce cystocysts after undergoing mitosis. The cystocysts are closely packed in the posterior region of germarium. The Posterior most region of germarium contains a germinal cyst forming an egg Chamber.

The posterior region of the germarium in the matured beetles Contains large number of trophocysts or nutritive cells, arranged in the several Tiers along with a small basal oocyte, migrating to the vitellarium.

EXPLANATION OF FIGURES

Fig. 3 HLS passing through early oocyte showing apical region of the ovariole differentiated into terminal filament and germarium IHE X 60

Fig. 4 HLS passing through terminal filament IHE X 128

Fig. 5 HLS passing through germarium IHE X 128

Fig.6 HLS passing through vitellarium of ovariole IHE X 16

Abbreviations:

TF	-	Terminal filament
GM	-	Germarium
TP	-	Oocyte
OOC	-	Trophocyte
TC	-	Nuclea
NU	-	Pre-follicular cells

DISCUSSION

The number of ovarioles in each ovary vary greatly in Coleoptera. There is only one, ovariole per ovary in Scarabaeus and coprini (Heymons 1929, 1930), two per ovary in Pasalidae (Reyes Casillo and Richer, 1973), and Curculionidae (Stein, 1847, Munro 1990-12, Bisell, 1937, Lenkova, 1949, Cram, 1958, Burke 1959, Vernier 1970, Garthe 1970, Stone et al 1971, 56 in Ctenicera (Zacharuk, 1958 a) and numerous in more than 329 species of beetles belonging To 45 families (Robgertson, 1961). In *Cybistertripunctatus* also 23-25 large number Of ovarioles are observed in each ovary.

According to Stein (1847) the ovaries in Coleoptera are of three major Types -1. The ovary with pedicel of individual ovarioles, 2. The ovary with a Common central pedicel and 3. The ovary with lateral pedicel, and in *Cybister Tripunctatus* the ovaries represent the first type.

Histology and development of the ovaries in *Dytiscus* is described By Korschelt (1886) and Demandt (1912) among Dytiscidae, while the present Study is perhaps first on *Cybistertripunctatus*.

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