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A Biochemical Analysis of Oocytes andFollicular Cells of Beetle CybisterTripuctatus

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Abstract*- Process of vitellogenesis is evident with cytological changes in the oocyte, Trophocytes and follicular epithelium in the adult female. The yolk deposition is evident in the terminal oocytes of 4 day old beetles And it is continued till the 8- day old beetle. the yolk material is composed of proteins, lipids and carbohydrates as revealed by histochemical studies. the yolk material is transported from haemolymph to the vitellogenic oocyte through intrafollicular spaces. As soon as the vitellogenesis is over, the follicular epithelium secretes the Vitelline membrane and the chorion. The trophocytes become completely Degenerated.*

I- INTRODUCTION

Yolk proteins are accumulated and stored in mature yolk bodies ofEgg until the initiation of embryonic development, when they are utilized. ThusThe mature yolk body can be viewed as a delayed lysosomalCompartment particular to oocytes. <oreover, evidence suggests that yolkBodies posses a unique set of enzymes and they have a specialized lysosomalFunction (Railhel, 1984; Wall and Maleka, 1985; Cho et al., 1991, Van Antwerpen,1993). After the completion of vitellogensis, the vitelline membrane and chorionAre developed, respectively. In Orthoptera and Lepidoptera, the vitellineMembrane is produced by oocyte itself (Raikhel and Lea, 1991). But in manyInsects, the follicle cells secrete material which forms, the vitelline membrane(Hoffmann,1995). In both the cases, vitelline membrane production isProceeded by an extensive production and is preceded by an extensiveHypertrophy of rough endoplasmic reticulum. It is followed by formation of GolgiComplexes and accumulation of secretory granules in the apical zone of the Follicle cells. At the end of vitellogenesis the previtelline membrane secretorySubstances begin to be deposited between the follicle cells and the oocyte. TheSecretory material then coalesce and gradually form the vitilline membrane whichIs composed of two components, the vitelline membrane bodies secreted by theOocyte and the fine granular material secreted by the follicle cells. In the ButterflyCalpodesethlius, a distinct vitelline membrane is detectable at the endOf the yolk phase. It has an electron dense layer away from the oocyte and

An electron lucent layer apposing the oocyte. Both the oocyte and the follicleCells contain coated vesicles, which appear to be in the process of exocytosis.
The electron dense material seems to be exocytosed from the follicle cells whereCoated vesicles at the oocyte surface exocytose the electron lucent granularMaterial. Once fully formed, the vitelline membrane is completely electron dense(Grinffith and Li-fook, 1986; Yamamoto and Takahashi, 1993.

The chorin is secreted by the follicle cells and is composed ofTwo layers, the inner endochorion and outer exochorinon. The follicle cells areMesodermal in origin but the chorion is cuticle-like in nature and contains layersOf proteins and lipoproteins, which are tanned by polyphenolic substancesReleased by the follicle cells. After the secretion of chorion, the follicle cells die.The chorion protects the oocyte form mechanical and environmental stressesAnd at the same time permits gas exchange and sperm penetration

(Regier andKafatos, 1985; Pascual et al., 1990, Belles et al., 1993; Anderew and Tembhare,
1995). In some species, a wax layer is formed below the endochorion. This waxLayer is secreted as oil droplets by the follicle cells which make the chorionWaterproof. The inner chorion layer, the crystalline chorion, is flexible but Restricts the volume of the oocyte. It also helps in gas exchange through plastronRespiration. This layer is followed by trabeculate layer which is characterized byPresence of cavities and pores. The cavities may interconnect and formExtensive channels which serve as air spaces and help in gas exchange. The Outermost layer of the endochorion is characterized by the presence of lamellaeBased on the hexicoidal arrangement of stacks of fibrils. In many Lepidoptera,
The lamellar layer serves as the outer portion of the egg shell. The exochorionMay house channels to termit entry of sperm and lines of weakness to faciliatateHatching. The process of choriogensis probably depends on brain neurohormone,Juvenile hormone and 20- hydroxyecdsone (Raabe, 1989; Pascual, 1990,Belles, 1993).

II-MATERIAL AND METHOD:

BIOCHEMICAL TECHNIQES

In order to estimate total ovarian lipid, protein and carbohydrateConcentration in the ovary and colleterial gland of the adult female beetles, theyWere sacrificed every day from emergence to the first ovipositio and ovariesAnd other structures were dissected out and processed for biochemicalEstimation.

PREPERATION OF EXTRACT

The ovaries and colleterial glands were removed gently after cutting thetergites of terminal region of the abdomen and immediately transferred to icecold insect Ringer's solution. The fat bodies, trachea, nerves and othertissues were carefully removed and each ovary and colletrial gland were weighedto ± 1 m.g. Both the organs were thoroughly washed in ice cold Ringer's solutionand then homogenized in ice cold citrate phosphate buffer (pH 6.8) and madeupto 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 10_0C untilrequired.

The following biochemical techniques were used for the estimationOf total protein, lipid and carbohydrate concentration in the overies 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).

III-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. Thefemale reproductive organs were dissected out. The ovaries and a colleterial glandwere separated, the weight was taken individualy and the organs were processedfor biochemical techniques describedabove.

STATISTICAL METHODS

All statistics presented in this study are mean ± standard errors.

Students 't' test was made use of for testing the significance of differencesbetween the means of reading of experimental and control groups using 5percent level of significance.

III- OBSERVATIONS

Total protein, lipid and carbohydrate concentration in the ovaryDuring oocyte development and vitellogenesis have been estimated andSummarized in the Table – 1

Table 1 : Total ovarian protein, lipid and carbohydrate during oocyte Development and vitellogenesis in Cybistertripunctatus

Days	Vitellogenic Stage	Protein (mg/100mg)	Lipid (mg/100mg)	Carbobydrate (mg/100mg)
0	Newly emerged	0.81 ± 0.0.4	3.62 ± 0.198	0.23 ± 0.02
2	PV	13.16± 0.33	4.16 ± 0.512	0.58 ± 0.05
4	EV	25.56 ± 0.42	4.22 ± 0.30	0.94 ± 0.06
6	MV	30.53 ± 0.65	5.34 ± 0.60	1.81 ± 0.04
8	LV	38.41 ± 0.24	7.20 ± 0.40	2.09 ± 0.03
10	MS	41.00 ± 0.17	7.35 ± 0.136	2.53 ± 0.07

Abb. :PV - previtellogenic stage
MS - maturation stage
EV - earlyvitellogenic state
TC - trophocyte
MV - midvitellogenic stage
NE - Newly emerged
LV - late vitellogenic stage
± - standard error.

Total ovarian protein concentration

Total ovarian protein concentration (TOPC) measures about 9.81 ± 0.08 mg/100 mg in the mewly emerged dult female beetle. Gradual rise in TOPC occurs from early vitellogenic from 25.56 ± 0.42 mg/100mg to the 41.00 ± 0.17mg/100mg during maturation stage.

Total ovarian lipid concentration

Total ovarian lipid concentration (TOLC) measures about 3.2± 0.1 mg /100mg in the newly emerged adult female beetle. Singnificant rise in TOLC concentration is found about 5.34 ± 0.6 mg/100 mg at mid vitellogenic Stage which is elevated to 7.35 ± 0.13 mg/100mg during maturatrion stage

Total ovarian carbohydrate concentration

Total ovarian carbohydrate concentration (TOCC) measures about 0.23 ± 0.02 mg/100 in the newly emerged adult female beetle, which risesGradually at the late vitellogenic stage measuring about 2.97 ± 2.03 mg/100mg And to the maximum level about 2.53 ± 0.07 mg/ 100mg at the maturation stage.

The colleterial gland

The colleterial gland is the female accessory sex gland composed of A single layer of columnar epithelial cells which show cyclical changes during Oocyte development and vitellogenesis.

IV-DISCUSSION

Besides the trophocytes, the follicular cells are also reported to be InvolvedinRNA synthesis in a number of species (Anderson, 1964; Ray and Ramamurty, 1978). In Cybistertripunctatus, follicular cells contain rich Quantity of RNA suggesting their role in protein synthesis, in order to secrete Egg membranes, vitelline and chrion. It is now apparent in several species that once the oocyte is Fully-developed, the follicular epithelium secrets the egg chorion (Machida, 1941; Hsu, 1953; Nath et al., 1958; Telfer , 1960; King and Koch, 1963; King, 1964; Matsuzaki,1968; Beams and Kessel, 1969; Anderson And Telfer,1970; Engelmann, 1970; Miya and Kurihara, 1970; Chruickshank,1971,1972; Ona et al., 1975; Bast and Telfer, 1976; Akutsu and Yoshitake, 1977; Koeppe et al., 1981; Irie and Yamashita, 1983; Regier and Kafatos,1985) In Cybistertripunctatus, the egg membrane.i.e. thevatelline membrane and the Chorion are formed at the late vitellogenic maturation stages mostly from the Proteins and lipids supplied by the investing epithelium as evident from the Proteins and lipids supplied by the investing epithelium as evident from the Histochemical studies. Similar pattern of egg membernae formation has been Noticed by Bonhag (1955b) in Oncopeltus, Miya and Kurihar (1966) in Bombyx, Tembhare and Thakare (1975) in Orthetrum and Sidhra et al., (1984) in Mylabris. The present histological and histochemical observations reveal that The terminal oocyte in the adult female is without yolk material till the 4th day of Post-emergence representing the pre-vitellogenic stage. Deposition of yolk is Initiated with the inception of carbohydrates, proteins and lipids consecutively In the perioplasm on the 4th day of post-emergence representing the early Vitellogenic stage. These yolk components increase rapidly during the Successive stages of vitellogenesis to occupy the whole oocyte. The investing Follicular epithelial cells and interfollicular spaces are initially filled with Protein bodies suggesting the transport of protein yolk into the oocyte from Haemolymph through the interfollicular space. Similar functional singnificance Of the interfollicular spaces is reported by earlier worlers (Wigglesworth,1943; Bonhag,1958; Shigematsu,1958; Telfer, 1960, 1981)

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