A Biochemical Analysis of Oocytes and Follicular Cells of Beetle Cybister Tripuctatus

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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 of Egg until the initiation of embryonic development, when they are utilized. Thus the mature yolk body can be viewed as a delayed lysosomal compartment particular to oocytes. Moreover, evidence suggests that yolk bodies posses a unique set of enzymes and they have a specialized lysosomal function (Raikhel, 1984; Wall and Maleka, 1985; Cho et al., 1991, Van Antwerpen, 1993). After the completion of vitellogenesis, the vitelline membrane and chorion are developed, respectively. In Orthoptera and Lepidoptera, the vitelline membrane is produced by the oocyte itself (Raikhel and Lea, 1991). But in many insects, the follicle cells secrete material which forms the vitelline membrane (Hoffmann, 1995). In both cases, vitelline membrane production is preceded by an extensive hypertrophy of rough endoplasmic reticulum. It is followed by formation of Golgi Complexes and accumulation of secretory granules in the apical zone of the Follicle cells. At the end of vitellogenesis the previtelline membrane secretory substances begin to be deposited between the follicle cells and the oocyte. The secretory material then coalesces and gradually forms the vitelline membrane which is composed of two components, the vitelline membrane bodies secreted by the oocyte and the fine granular material secreted by the follicle cells. In the Butterfly Calpodes ethlius, a distinct vitelline membrane is detectable at the end of 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 follicle cells contain coated vesicles, which appear to be in the process of exocytosis. The electron dense material seems to be exocytosed from the follicle cells where coated vesicles at the oocyte surface exocytose the electron lucent granular material. Once fully formed, the vitelline membrane is completely electron dense (Griffith and Li-fook, 1986; Yamamoto and Takahashi, 1993).

The chorion is secreted by the follicle cells and is composed of two layers, the inner endochorion and the outer exochorion. The follicle cells are mesodermal in origin but the chorion is cuticle-like in nature and contains layers of proteins and lipoproteins, which are tanned by polyphenolic substances released by the follicle cells. After the secretion of chorion, the follicle cells die. The chorion protects the oocyte form mechanical and environmental stresses and at the same time permits gas exchange and sperm penetration.
The following biochemical techniques were used for the estimation of total protein, lipid and carbohydrate concentration in the ovaries and colleteral 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

II-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 colleteral gland were separated, the weight was taken individually and the organs were processed for biochemical techniques described above.

STATISTICAL METHODS

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

Students ‘t’ test was made use of for testing the significance of differences between the means of reading of experimental and control groups using 5 percent level of significance.

III- OBSERVATIONS

Total protein, lipid and carbohydrate concentration in the ovary during oocyte development and vitellogenesis have been estimated and summarized in the Table – 1

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

<table>
<thead>
<tr>
<th>Days</th>
<th>Vitellogenic Stage</th>
<th>Protein (mg/100mg)</th>
<th>Lipid (mg/100mg)</th>
<th>Carbohydrate (mg/100mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Newly emerged</td>
<td>0.81 ± 0.0.4</td>
<td>3.62 ± 0.198</td>
<td>0.23 ± 0.02</td>
</tr>
<tr>
<td>2</td>
<td>PV</td>
<td>13.16 ± 0.33</td>
<td>4.16 ± 0.512</td>
<td>0.58 ± 0.05</td>
</tr>
<tr>
<td>4</td>
<td>EV</td>
<td>25.56 ± 0.42</td>
<td>4.22 ± 0.30</td>
<td>0.94 ± 0.06</td>
</tr>
<tr>
<td>6</td>
<td>MV</td>
<td>30.53 ± 0.65</td>
<td>5.34 ± 0.60</td>
<td>1.81 ± 0.04</td>
</tr>
<tr>
<td>8</td>
<td>LV</td>
<td>38.41 ± 0.24</td>
<td>7.20 ± 0.40</td>
<td>2.09 ± 0.03</td>
</tr>
<tr>
<td>10</td>
<td>MS</td>
<td>41.00 ± 0.17</td>
<td>7.35 ± 0.136</td>
<td>2.53 ± 0.07</td>
</tr>
</tbody>
</table>
Total ovarian protein concentration (TOPC) measures about 9.81 ± 0.08 mg/100 mg in the newly emerged adult female beetle. Gradual rise in TOPC occurs from early vitellogenic from 25.56 ± 0.42 mg/100 mg to the 41.00 ± 0.17 mg/100 mg during maturation stage.

Total ovarian lipid concentration (TOLC) measures about 3.2 ± 0.1 mg/100 mg in the newly emerged adult female beetle. Significant 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/100 mg during maturation stage.

Total ovarian carbohydrate concentration (TOCC) measures about 0.23 ± 0.02 mg/100 mg in the newly emerged adult female beetle, which rises gradually at the late vitellogenic stage measuring about 2.97 ± 2.03 mg/100 mg and to the maximum level about 2.53 ± 0.07 mg/100 mg 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 involved in RNA synthesis in a number of species (Anderson, 1964; Ray and Ramamurty, 1978). In Cybustertripunctatus, follicular cells contain rich quantity of RNA suggesting their role in protein synthesis, in order to secrete egg membranes, vitelline and chorion. It is now apparent in several species that once the oocyte is fully-developed, the follicular epithelium secretes 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; Koepp et al., 1981; Irie and Yamashita, 1983; Regier and Kafatos, 1985). In Cybustertripunctatus, the egg membrane, i.e., the vitelline 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 membranes 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 significance of the interfollicular spaces is reported by earlier workers (Wigglesworth, 1943; Bonhag, 1958; Shigematsu, 1958; Telfer, 1960, 1981).

REFERENCES


