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VITELLOGENESIS IN *LEPISMA SACCHARINA* L., A CYTOCHEMICAL STUDY

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The ovary in *L. saccharina* is of panoistic type. Each ovariole has been divided into six zones - Z_1 to Z_6. Z1 includes the terminal filament; Z2 comprises the germarium; Z3 comprises primary oocytes lying in pairs and each measuring about 45 μm x 600 μm; in Z4 the oocyte measures from 60 μm x 60 μm to 75 μm x 375 μm and the oocytes lie in a single row; in Z_5 the oocytes measure about 90 μm x 480 μm and the follicular epithelium covering the oocytes secretes the chorion; Z_6 is the pedicel. Cytochemical studies of the oocytes in the various zones and origin of yolk have been attempted.

INTRODUCTION

Except for some morphological and histochemical accounts on panoistic ovary (Bonhag, 1959; Anderson, 1964; Nath, 1968; Nirmala & Rajasekarasetty, 1972; Postlethwait & Giorgi, 1985) not much is known about the origin, functions and composition of lipids, proteins, nucleic acids and carbohydrates arising during oogenesis. The present investigations were undertaken to know about the functioning of various metabolites in the panoistic ovary of *Lepisma saccharina* L.

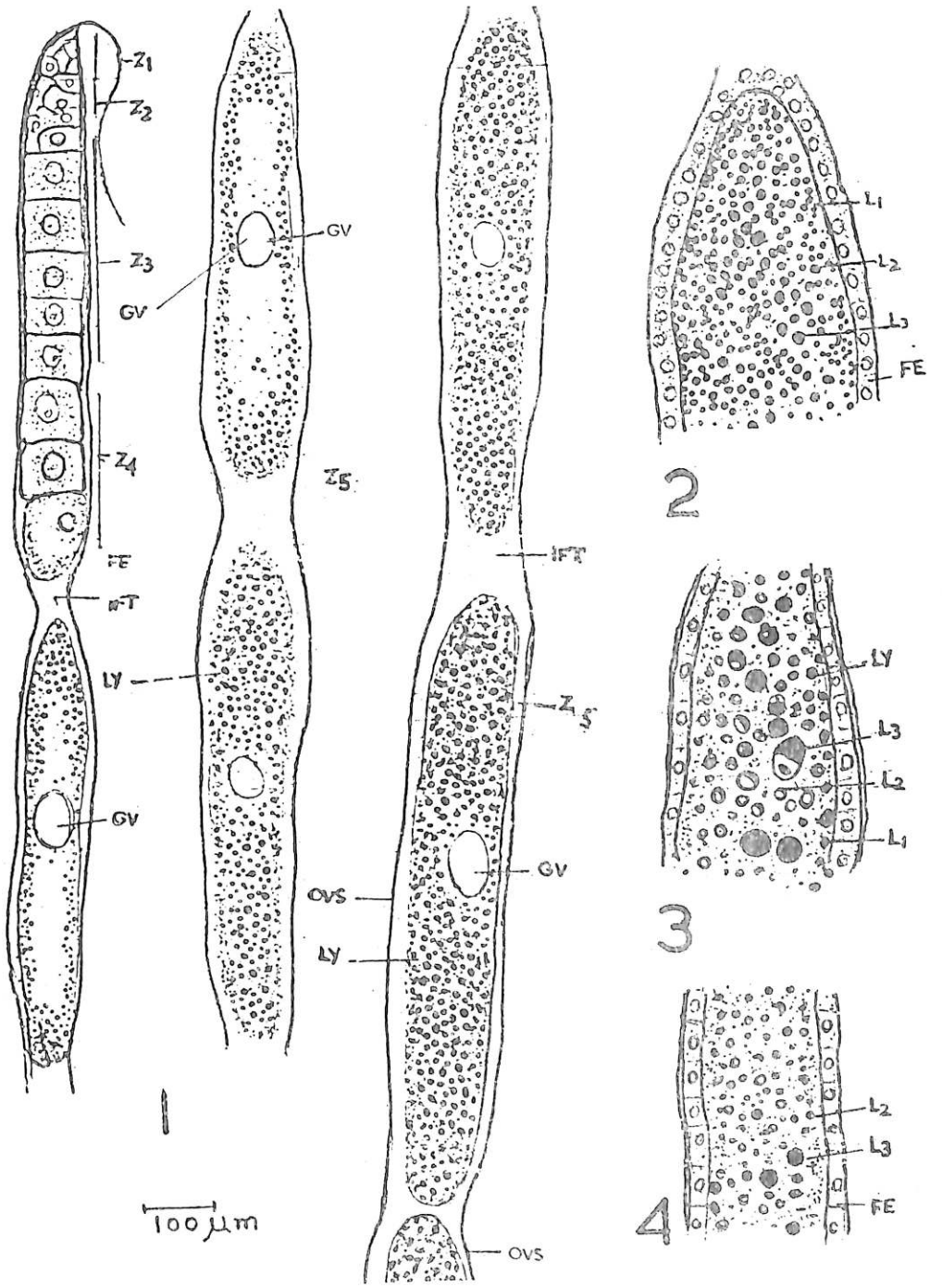
MATERIAL AND METHODS

L. saccharina were collected at Chandigarh. The ovaries were dissected out in physiological saline and fixed in Zenker, Carnoy, Bouin, weak Bouin and formaldehyde calcium. The various histochemical tests employed are the same as described in the previous report (Sareen & Kaur, 1987).

OBSERVATIONS

Structure of the ovary: A pair of ovaries in *L. saccharina* is lodged in the abdominal cavity from first to sixth segment. Each ovary consists of five panoistic ovarioles. They are attached at the thoracic peritoneal membrane by terminal filament. They are supplied all over by tracheal tubes. The adult ovariole is an elongated tapering unit, measuring about 3.22 mm in length and is covered by tunica propria and an external ovariole sheath. Following Bonhag (1959), the ovariole has been divided into six zones. Zone I includes the terminal filament measuring about 0.52 mm in length; it is cylindrical, syncytial with nuclei arranged almost in two columns. The membranous tunica propria applied to the terminal filament is continuous with rest of the ovariole. The terminal filament is separated from rest of the germarium by a tranverse septum (Fig. 1).

The germarium comprises the second zone measuring about 30 μm in length. It contains prefollicular tissue, oogonia and young primary oocytes. The prefollicular nuclei are found primarily at the periphery of the germarium; most of them are interspersed amongst the spaces between the newly differentiated oocytes. The oogonia occupy the anterior part of the adult germarium and the cell boundaries of the densely packed cells which assume polygonal shapes. Posterior to the oogonia are the young



Figs. 1 - 4. 1. L.S. of an ovariole showing the distribution of lipids, FCa-PC/SBB; 2. showing the distribution of L1, L2 and L3 bodies, FCa-PC/SBB; 3. showing the lipid bodies of different shapes and sizes, FCa-PC/SBB; 4. showing the distribution of L1, L2 and L3 bodies, FCa-PC/SBB;.

(C = Cytoplasm, CL = Calyx, CY = Compound yolk, FE = Follicular epithelium, FN = Follicle cell nucleus, GV = Germinal vesicle, IFT = Interfollicular tissue, L1, L2 and L3 = Lipid bodies, LY = Lipid yolk, N = Nucleus, NE = Nucleolar extrusions, OVS = Ovariole sheath, Z1-Z6 = Zones of ovariole)

oocytes which are larger than the oogonia from which they have been differentiated (Figs. 1 & 5).

The third zone contains primary oocytes measuring about 45 μm x 600 μm. Some of them lie in pairs across the ovariole. The prefollicular nuclei concentrate around the oocytes forming a rudiment of the eventual follicular epithelium (Figs. 5 & 6). The oocytes of this zone increase in size. The oocyte-nucleus in Zone III is characterized by a gradual increase in size and a state of dispersion of chromatin. The chromatin bodies are concentrated towards the periphery of the nucleus.

In the fourth zone the oocytes are arranged in a single row and each oocyte has its own follicular epithelium. The oocyte is squarish in the beginning of zone IV, then barrel-like and elongating in accordance with the long axis of the ovariole. Ultimately it is elliptical and remains so in zone V. The first oocyte in zone IV measures about 65 μm x 70 μm while the last one measures about 375 μm x 75 μm. The nuclei of the oogonia measuring about 15 μm increase to about 52 μm in the mature oocytes. The nucleoli show fragmentation in the vitellogenic oocytes. In the distal oocytes of zone IV granular yolk precursors are seen in the cortical ooplasm (Fig. 7). At this stage the nucleus is seen almost in the centre of the oocyte. In the cortical region of the oocyte adjoining the follicular epithelium accumulate small granules of yolk. It appears that the granular yolk is continuously being synthesised at the cortical ooplasm (Fig. 7).

In the fifth zone the oocytes measuring about 480 μm x 90 μm are laden with yolk (Figs. 1 &13). In this zone the follicular epithelium secretes the chorion. The oocyte at the end of ovariole is provided at the distal end facing the oviduct, with a mass of follicular epithlium forming the plug. This marks the beginning of the ovariole pedicel opening into the oviduct. The terminal oocyte increases many fold than the penultimate oocyte which grows further only when the terminal oocyte is ovulated.

Lipid yolk : The lipid bodies appear in the form of small granules at the peripheral ooplasm of the zone III oocytes measuring about 50 μm x 60 μm. Their size as well as number show increase with the advancement of vitellogenesis. In the terminal filament, prefollicular cells interspersed between germ cells and follicular cells of zones II and III oocytes, the lipid bodies are in the form of granules (Fig. 1). These granules have been named L_1 bodies; they are stained intense black with SBB and blue with NBS. It reveals that the L_1 bodies contain phospholipids. In the oocytes of zone IV the lipid granules increase in size and number (Figs. 1-4). They start migrating towards the anterior and posterior poles of the oocyte and around the nucleus. These lipid bodies are sandwiched amongst the yolk spheres (Fig. 13). These are the L_2 bodies which can be differentiated from the L_1 bodies, being stained violet in NBS, indicating the presence of triglycerides alongwith phospholipids.

As the oocyte grows further in the zone V the lipid bodies are deposited more at the cortical ooplasm during late stages while the cortical ooplasm gets occupied by yolk spherules (Figs. 1, 8- 11). The big lipid bodies are the L_3 bodies. By further growth the L_3 bodies increase in size and measure 0.11 to 0.19 mm. They are crescents and rings in SBB preparations and are differentiated from the L_2 bodies in being stained pink with NBS, thereby showing their triglyceride nature. In Sudan III and IV preparations these are not coloured uniformly as in SBB; some of them have their cortices dark coloured while medullae are lightly coloured; others show one, two or several vacuoles; still others show one, two or several vacuoles; still others show medullae darkly and cortices lightly coloured. Some L_3 bodies also show irregular contours. They are negative to acid haematein test. These tests reveal that the L_3 bodies contain triglycerides.

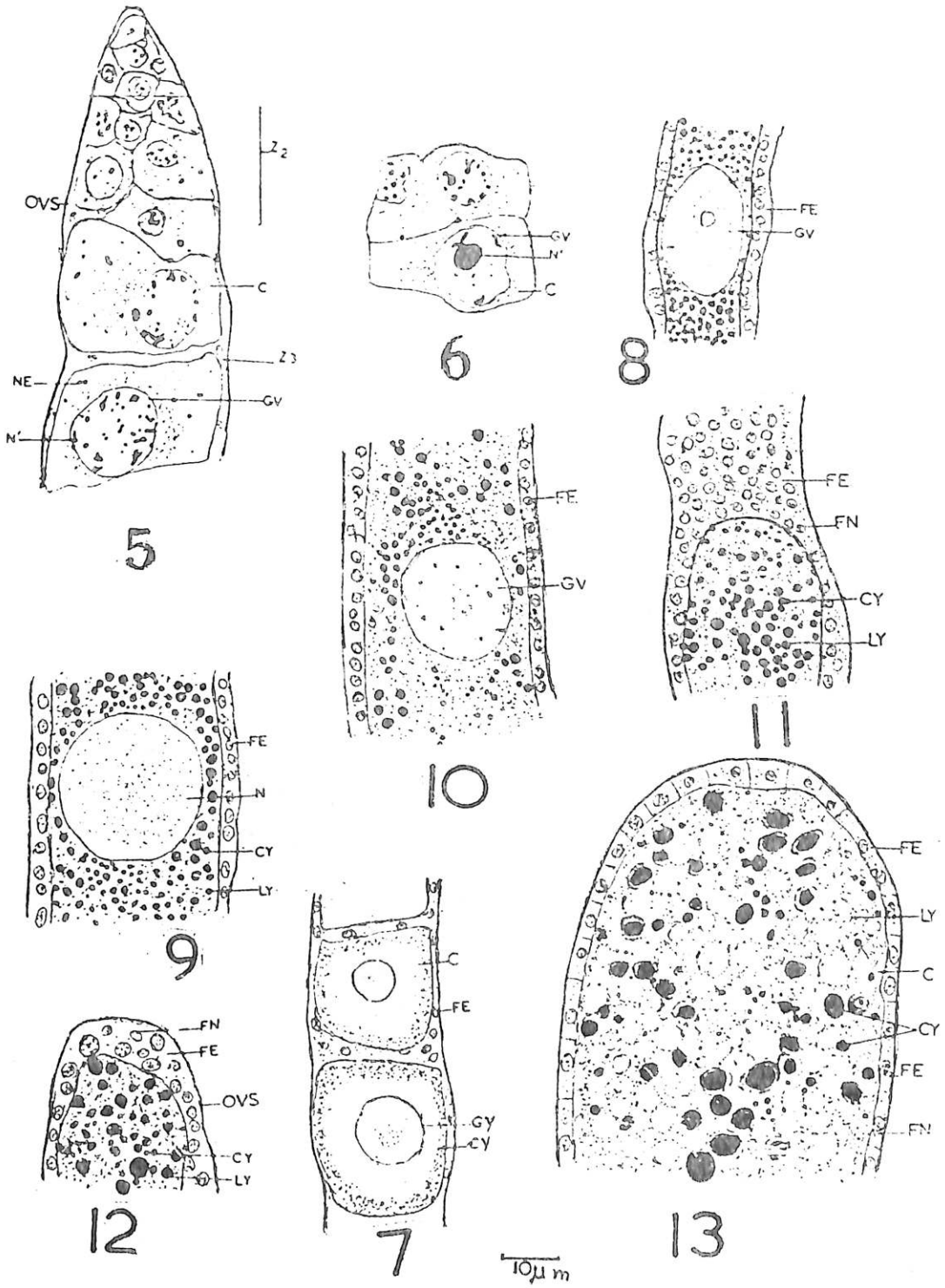


Fig. 5-13. 5. L.S. of young oocytes in zones I and II, Zenker/IH; 6. section through oocytes of zone III, Bouin/Hg-BPB; 7. section through oocytes of zones IV and V showing yolk granules appearing first in the peripheral ooplasm, Carnoy/Hines and Moriber; 8-12. sections through oocytes of zones IV and V showing almost uniform distribution of yolk globules, Zenker/Himes and Moriber; 13. a section through a portion of a mature oocyte of zone V showing the yolk globules sandwiched with the lipid yolk bodies, Bouin/PAS.

Protein yolk globules : The first appearance of yolk is in the form of granules, in the follicular epithelium and cortical ooplasm of zone IV oocytes (Fig. 7). Some of the granules are also seen in the central ooplasm where they grow into yolk globules. In zone IV where the oocytes are nearly twice as long as broad, the yolk globules come to occupy almost the whole of ooplasm of the oocyte measuring about 375 μm x 75 μm (Fig. 13). The lipid bodies at this stage are sandwiched amongst the yolk globules. In zone V the oocyte measuring about 435μm x 90μm shows concentration of lipid bodies in the central ooplasm while the cortical ooplasm is occupied by growing yolk globules (Figs. 8-11). In the penultimate oocyte of zone V there are more lipid bodies than the yolk globules. In mature oocyte measuring about 480 μm x 90 μm the yolk globules do not stain homogeneously but give froathy appearance (Fig. 13). They are PAS positive in paraffin sections of Bouin and Carnoy fixed material (negative after acetylation and positive after KOH reversal). They give gamma metachromasia with toluidine blue and are positive with alcian blue. They stain intense pink with Himes and Moriber's test. They are positive to Hg-BPB and performic acid-Schiff tests. These reactions suggest that the yolk globules are carbohydrate-protein complex; the carbohydrate component gives positive tests for acid mucopolysaccharides.

Cytoplasm and nucleus : The cytoplasm of zones II and III oocytes and associated follicular epithelial cells stains positive in Hg-BPB for proteins, intensely pyroninophil and negative after pretreatment of RNAase and yellow in Himes and Moriber's technique for proteins. The pyroninophilia due to RNA declines in concentration in the oocytes of zones IV and V. The nucleoli are also intensely pyroninophil and show budding. The chromatin bodies of the follicular epithelial cells and the oocytes are stained pink in Feulgen's reaction (negative after pretreatment with perchloric acid).

DISCUSSION

Each ovariole in *L. saccharina* matures a single oocyte at a time. The terminal oocyte increases many times as compared to the penultimate oocyte. In the panoistic ovary of *Blatella germanica*, the terminal oocyte increases in volume 180 fold over a period of 10 days, while the penultimate oocyte increases only three times (Postlethwait & Giorgi, 1985). In the panoistic ovary of stick insect removal of the terminal follicle results in the resumption of the vitellogenic growth in the penultimate follicle, but only if the tissue connecting the two adjacent follicles remains intact (Mesnier, 1980).

The lipid bodies in *Lepisma* are of three types, *viz.* L_1, L_2 and L_3. A conversion of L_1 into L_2 and L_3 has been suggested in various insects (Nath, 1968). The cytochemical picture of the distribution of lipids in the ovarian follicles indicates that there is an increase in phospholipid content in young oocytes as it grows. Once the peak is reached, a decline in phospholipid distribution takes place. A similar sequence in phospholipid synthesis has been reported in panoistic ovary of other insect species. The progressive diminution of phospholipid content in *Lepisma* oocyte can be explained by assuming that phospholipids are utilized or converted by the oocyte during final stages of yolk formation. The RNA content of the oocyte shows a progressive increase in the growth period until the maximum level is reached and this is maintained upto the entire growth phase even though the basophilia declines as a result of dilution of cytoplasmic RNA in the enlarging oocytes. (Bier, 1954; Colombo, 1953; Gresson & Threadgold, 1962). DeLoof & Lagasse (1970) believe that the low concentration of RNA in the yolk synthesising oocytes suggests that protein is no longer being synthesised by the oocyte. The appearance of PAS positive yolk precursors initially at the periphery of the cell suggests the possibility of some extraovarian contribution to the developing oocyte through the follicle cells. During the vitellogenesis in the stick-insect (Mazzini and Giorgi, 1984) it has been observed that differentiation of the follicular epithelium is a process temporally well coordinated with oocyte development and it is suggested that cell-to-cell communication through gap junctions is an essential feature of the

developing follicle for such a coordination to occur. Postvitellogenic development entails deposition of the endochorionic material in the extracellular space facing the oocyte surface. The completion of this layer brings the endocytic activity on the oocyte oolemma to a complete stop.

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REFERENCES

ANDERSON, E. 1964. Oocyte differentiation and vitellogenesis in the roach, *Periplaneta americana*. *J. Cell Biol. 20* : 131-135.

BIER, K. 1954. Uber phasen gestergerter protein and kohlehydratein-la-gerung and die fettverteilung in Hymenopteran ovar. *Dtsch. Zool. Ges. 18* : 422-429.

BONHAG, P.F. 1959. Histological and histochemical studies on the ovary of the American cockroach, *Periplaneta americana L. Univ. Calif. Publs. Ent. 16* : 81-124.

COLOMBO, G. 1953. L' oogenesi negil ortotteri. I. Richerche istologische e Entometriche in *Anacridium aegyptium* L. dalla Sehinsa all' imagine. *Acta Zool. 34* : 191-232.

DeLOOF, A. & LAGASSE, A. 1970. The ultrastructure of the follicle cells of the ovary of Colorado beetle in relation to yolk formation. *J. Insect Physiol. 16* : 211-220.

GRESSON, R.A.R. & THREADGOLD, L.T. 1962. Extrusion of nuclear material in Blatta orientalis. *Quart J. micr. Sci. 103* : 141- 145.

MAZZINI, M. & GIORGI, F. 1984. Vitellogenesis in the stick-insect *Bacillus rossius* (Rossi) (Phasmatodea, Bacillidae). I. Ultrastructural observations on ovarian follicle cells. Monitore Zool. Ital. (N.S.) *18* : 239-257.

MESNIER, M. 1980. Study of oocyte growth within the ovariole in a stick-insect, *Clitumnus extrodentatus*. *J. Insect Physiol 26* : 59- 65.

NATH, V. 1968. *Animal Gametes (Female)*. Asia Publishing House, Bombay.

NIRMALA, N.A. & RAJASEKARASETTY, M.R. 1972. Origin, distribution and chemical nature of polysaccharides in the oocytes of the grass-hopper, *Acrida turrita* (Acrididae : Orthoptera). *J. Mysore Univ.* (Sect. B) *Sci. Med. Eng. 25* : 103-125.

POSTLETHWAIT, J.H. & GIORGI, F. 1985. Vitellogenesis in insects. In : *Developmental Biology,* Vol. I. Oogenesis (L.W. Browder Ed.). Plenum Press, New York.

SAREEN, M.L. & KAUR, K. 1987. Morphological and histochemical studies on the spermatogenesis in the bug, *Halys parvus* (Pentatomidae : Hemiptera). *Res. Bull. Panjab Univ. 38* : 91-97.

SESHACHAR, B.R. & BAGGA, S. 1963. A cytochemical study of oogenesis in the dragonfly , *Pantala flavescens* (Fabr.). *Growth 27* : 225-246.