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Histochemical localization of energy reserves in the mussel *Musculista senhausia*

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Abstract

Histochemical changes of energy rich organic compounds like protein, glycogen and lipids in the gonads of *Musculista senhausia* (Bivalvia:Mytilidae) from Cochin backwaters, west coast of India were studied for the first time. These fractions exhibited marked differences in male and female gonads during various developmental stages. Protein and lipid were found to have an important role as storage materials for utilization during gametogenesis. It is evident that gametogenesis and embryogenesis are two distinct energy demanding processes.

Keywords- Protein, lipid and glycogen reserves, Gametogenesis, mussel

Introduction

A great deal of work has been done on different aspects of reproduction in Mytilid species from Indian as well as other waters, of which Wilson and Hodkin (1967), George and Nair (1973), Pipe (1987), Morton (1988), King *et al.* (1989) and Barkati and Ahmed (1990) are worth mentioning. But very few contributing reports have been made on the histochemical nature of the reproductive stages. The gametogenic cycle involves the formation, growth and ripening of the spermatocytes and oocytes , including the process of vitellogenesis in the female and their release. Intimately linked with this cycle, the metabolites in the form of protein, glycogen and lipid are stored and subsequently utilized in the production of gametes when the metabolic demand is high.

Here, an attempt has been made to furnish some details of the histochemical changes in the gonad of *Musculista senhausia* occurring in the Cochin backwaters of Kerala during different reproductive stages. The aim of the present study is to locate the vital compounds such as protein, glycogen and lipid in the gonad and their reported changes during gametogenesis.

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Material and methods

Samples of *M. senhausia* were collected from Cochin backwaters. Individuals of different stages of maturity viz. developing, mature and spawning were used for

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the histochemical localization of protein, glycogen and lipid. The sex and stages of gonad development were determined by macroscopical and microscopical observations. The tissues were fixed every month to follow the histochemical changes. For glycogen, Periodic acid Schiff technique (Humason, 1972); for protein, Mercury Bromophenol blue (Humason, 1972) and for lipid Sudan black B (Pearse,1968) were adopted. Cryostat (5030 microtome) was used for sectioning.

Results and discussion

M. senhausia showed different prominent gonadal stages throughout the year, as germ cells at various developmental stages could be seen in the follicles. It has been elucidated that energy reserves like protein, glycogen and lipid displayed marked quantitative changes during development (Sreedhar and Radhakrishnan, 1995). Developing stages of oocytes presented a positive change with Mercury bromophenol blue (MBB). The cytoplasm of the oocyte was comparatively heavily stained than the other components. The nucleolus was found to be more stained. A clear perinuclear ring rather stained could be seen (Fig.1). This may be an indication of the transfer of nuclear material into the cytoplasm. The staining was deeper in mature ova. In the spawning stage also tissues showed a positive but mild reaction (Fig.2). An increase in the affinity for the stain may be due to the accumulation of the basic proteins at these sites. In male the spermatogonia, spermatocytes, spermatids and spermatozoa exhibited higher stainability. In developing male, the mantle epithelium was found to be more stained in comparison with others, but the spent gonads stained light (Fig.3). The gonadal tissue showed slight increase in protein content in mature condition (Fig.4). Eventhough there was a rise in protein content in gonadal tissues, such an increase was not seen in mantle epithelium. This phenomenon explains the fact that with the build up of proteins in the developing oocytes and spermatocytes, there may not be a simultaneous rise in the quantity of proteins in mantle epithelium. In the spent gonad, the staining property was highly diminished during spawning stage because of the depletion of protein correlated with the release of gametes.

Periodic acid Schiff (PAS) reaction was employed to localize glycogen in the gonad. Colour intensity was more pronounced in the oocytes than in the spermatocytes. In the developing stage, oogonia, oocytes, spermatognia and spermatocytes presented a mild reaction with PAS. In mature oocytes, the cytoplasm showed a strong affinity for PAS. Glycogen appeared to be concentrated near the oolemma (Figs.5 & 6). The male gonad showed slightly less stainability with PAS. Spermatozoa were found to be little more intensively stained (Fig.7). It may be because of the fact that either the acrosome reaction or the development of the acrosome requires an endogenous supply of energy. The mantle epithelium in both sexes stained intensively. According to Bayne (1975,1976) the glycogen stored in

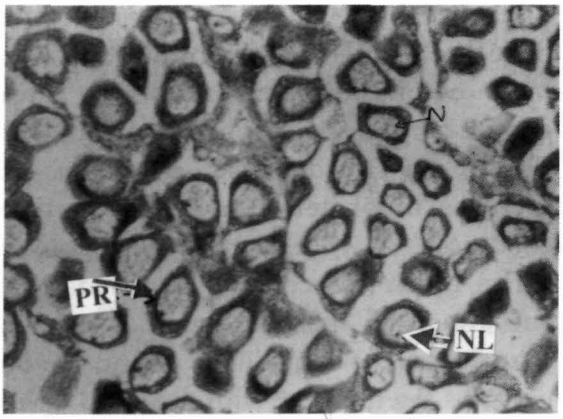

Fig.1. *Mature oocytes of* M. senhausia *showing comparatively heavily stained oocyte cytoplasm with a clear perinuclear ring (PR) x400. (NL – nucleolus)*

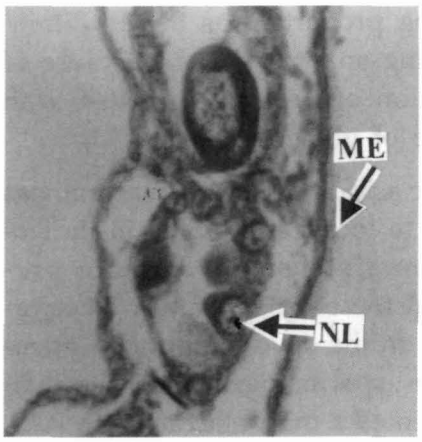

Fig.2. *Spawning gonad* (M. senhausia) *showing more stained mantle epithelium (ME) x400.*

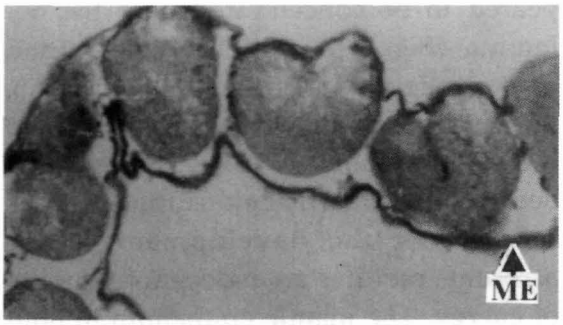

Fig.3. *Developing male gonad* (M. senhausia) *showing strongly stained mantle epithelium (ME) x400.*

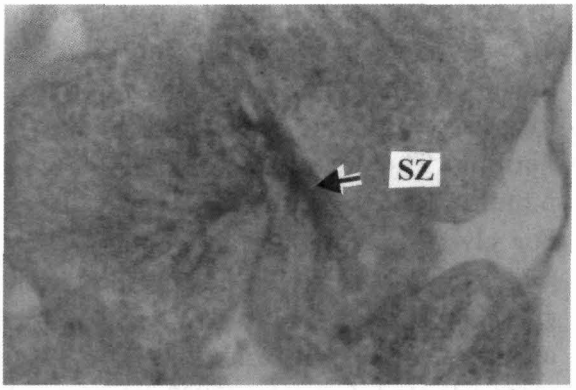

Fig.4. *Mature male gonad* (M. senhausia). *High protein concentration in the region of spermatozoa (SZ) x1000*

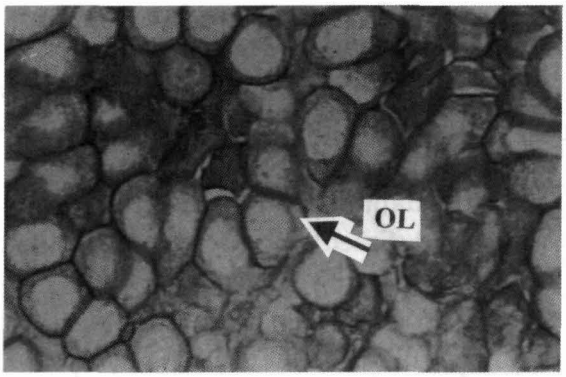

Fig.5. *Mature female gonad* (M. senhausia) *with strongly stained oocyte cytoplasm. High concentration of glycogen is seen near oolemma (OL) x400.*

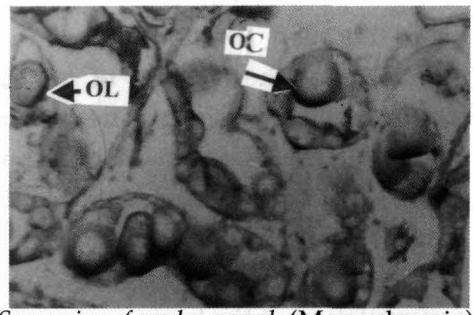

Fig.6. *Spawning female gonad* (M. senhausia) *showing the presence of glycogen in oocyte cytoplasm (OC), oolemma (OL) and mantle epithelium (ME) x400.*

the female mantle is conserved to some extent for use in the synthesis of yolk. During later stages the mantle exhibited less stainability, and the follicle wall also stained less. In *Mytilus edulis* Bayne *et al*, (1982) and Lowe *et al.* (1982) observed the transfer of the yolk precursor substances from the connective tissue to the developing oocytes in the follicles. In comparison with proteins, glycogen appeared to be stored in small quantities. In the ovary, glycogen accumulated directly in the cytoplasm of the oocytes, whereas in the testes it occurred mostly in the mantle epithelium and to a lesser extent in the tissues. The spent gonad was found to be moderately stained with PAS. Connective tissues and mantle epitheilium were seen to contain relatively more glycogen (Fig.8).

The oocytes of maturing and mature gonad was found to be positive to sudan black B (SBB) indicating the presence of lipid in the oocyte. The cytoplasm of these inclusions was observed to be filled with sudanophilic substances. In stalked oocyte, especially in the region of stalk, comparatively high staining intensity was noticed (Fig.9). This may lead to the conclusion that other than the lipid stored in the connective tissue, it is also contributed from other tissues. Compared to glycogen the lipid content was found to be higher in gonad tissue . The high level can be correlated to the storage of lipid in the ripening gametes as reserves to be used subsequently in early embryonic development. This agreed with the results of biochemical analysis of *M.senhausia* (Sreedhar and Radhakrishnan,1995). Lubet *et al.* (1976) observed the appearance of more sudanophilic granules in mature oocyte and spermatocyte in *Mytilus edulis.* In male, the staining property of the gonadal tissue showed differential reactions at different stages of maturity. In developing and mature conditions more sudanopholic substances could be observed in spermatogonia and spermatocytes (Fig.10). In both sexes, the mantle epithelium and follicular wall showed stainability in spawning stage (Figs.11&12), whereas in mature gonad, the follicular epithelium took lesser stain. Histochemical investigations revealed an accumulation of lipid with the advancement of maturity in both sexes.

The distribution of glycogen, protein and lipid showed variation in different tissues during the various developmental stages of the gonad. It showed a close relationship between reproductive cycle and the accumulation-consumption cycle of these energy reserves (Gabbot,1975). Thus it is evident that these reserves are utilized for gametogenesis. This clearly agreed with the observations made on the quantitative study of these energy reserves (Sreedhar and Radhakrishnan, 1995). Protein, glycogen and lipid are seen distributed in different tissues of the gonad. Among these three energy reserves, lipid and protein were found to be a little higher in concentration. Mantle epithelium, which is glycolipoprotein in nature, also showed differences in stainability. The observations made by Ajithakumar (1984) on *Perna indica* and *P.viridis* agreed with these facts. These results revealed that

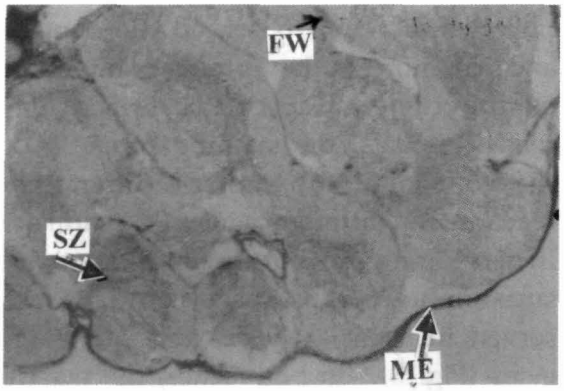

Fig.7. *Mature male gonad* (M. senhausia) *with strongly stained spermatozoa (SZ) and mantle epithelium (ME) x400*

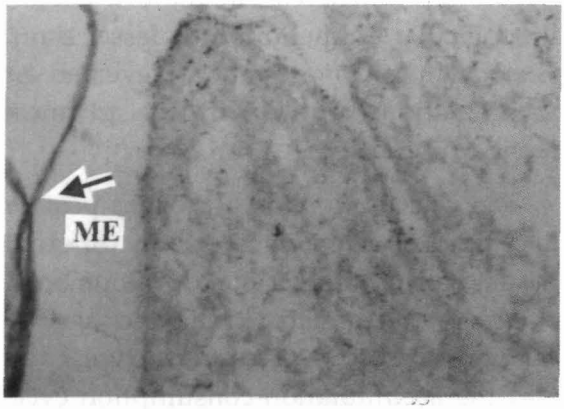

Fig.8. *Spawning male gonad* (M. senhausia) *showing relatively more glycogen in mantle epithelium (ME) x1000.*

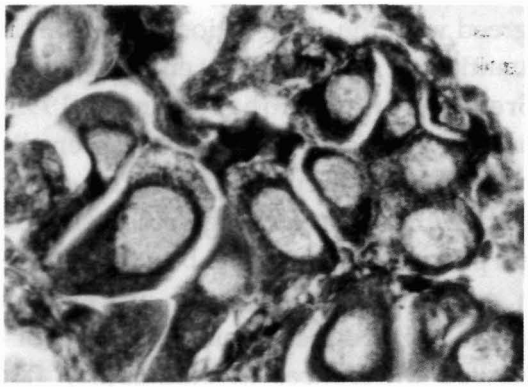

Fig. 9. *Mature female gonad* (M. senhausia) *showing comparatively high staining intensity in the region of stalk x1000*

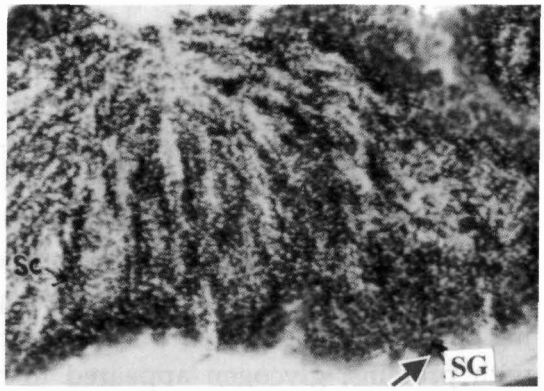

Fig.10.*Mature male gonad* (M. senhausia) *with high lipid content in spermatogonia (SG) and spermatocytes (SC) x1000*

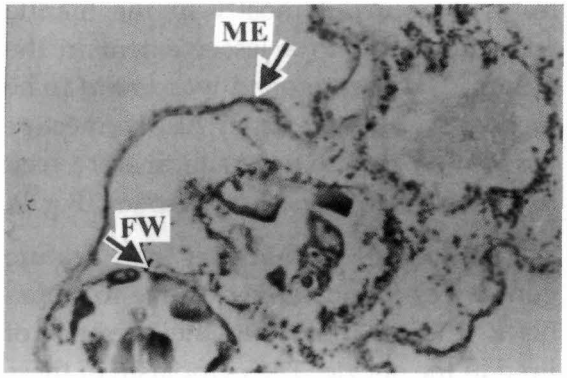

Fig.11. *Spawning female gonad* (M. senhausia) *showing strongly stained mantle epithelium (ME) and follicular wall (FW) x400*

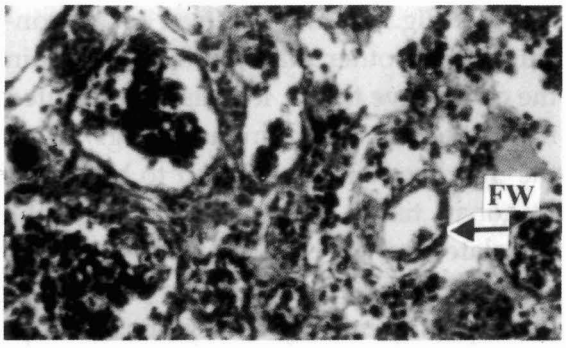

Fig.12. *Spawning male gonad* (M. senhausia) *showing strongly stained mantle epithelium (ME) and follicular wall (FW) x1000*

gamatogenesis and embryogenesis are two distinct energy demanding processes.

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