CHAPTER ONE

INTRODUCTION

REVIEW OF THE LITERATURE

REASONS FOR UNDERTAKING THE PRESENT INVESTIGATION

AND

PLAN OF THE PROPOSED WORK

INTRODUCTION

The wealth of biochemical and histochemical data of four classes of molluscs which has been reviewed by Hyman (1967), provides both a background and a stimulus for further histochemical research on any facet of the phylum. In the gastropod molluscs, wherein great diversity in taxonomy and ecology is observed and wherein organ systems show greatest complexities, the great interest of researchers, both in the biochemistry and histochemistry has been aroused since last century. In the course of a critical review of biochemical and histochemical data obtained in gastropods, many physiological and biochemical gaps in our information and the resulting lack of certainty come to light. Such studies have provided reliable information about the different chemical constituents, both organic and inorganic, and their natural cytochemical architecture within the cells and consequently in the organs to explain probable functional roles of these constituents in various functions performed by these organs. The results of such studies provide substantial background for wider researches in comparative morphology, anatomy and physiology. A critical review of the work on the reproductive organs in molluscs and their physiology, shows that only in the recent years the biochemical aspects of these organs and their involvement in the reproductive physiology are being studied in some greater details. Though the structural aspects of the

reproductive organs have been investigated satisfactorily, the metabolic aspects are comparatively less understood. The present work was, therefore, undertaken to get a clear picture of the metabolic aspects of mucosubstances in the reproductive physiology of a marine slug Oncidium verraculatum. A brief review of the work on mucosubstances in the reproductive tract of different gastropods, it is hoped, will provide basic information for discussion and comparison of the results obtained in the present investigation. It is with this view, therefore, it is proposed to review briefly the recent addition to our knowledge of mucosubstances in the reproductive tract of the gastropods.

(A) A Brief Review of the Mucosubstances in the Reproductive Tract of Gastropods

1. Glycogen

Since physiologically important fluctuations in glycogen content are localized in certain organs, tissues, or even in different ultrastructural regions of the cells, a combination of histochemical and microbiochemical analysis yields the most fruitful information. This may be especially true in genital organs, eggs and developing embryos. Mancuso (1956) studied the distribution of glycogen in the egg development of

Phallusia mamillata. Ramamoorthi (1959) reported that the glycogen content in the oocyte during the development of Pila virens decreased, while its accumulation increased in the shell gland area per unit weight of embryo. Emerson (1965) studied glycogen contents of the seven species of west coast intertidal prosobranch snails. In these gastropods, glycogen was the main polysaccharide in both the sexes which showed seasonal variations and the glycogen content was more in herbivores than the carnivores, indicating close relationship with the diet. Kugler (1965) determined glycogen of the reproductive system of the slug Philomycus carolinianus (Bose). Goudsmit (1964) studied metabolism of glycogen by the pulmonate snails, Bulimnaea megasoma and Helix pomatia in both embryos and adults. Meenakshi and Scheer (1968), along with other carbohydrate contents, studied glycogen in the other slug, Ariolimax columbianis, with special reference to its distribution in the reproductive system. They found glycogen in very small quantities in the albumen gland. Taylor and Anderson (1969) studied changes during differentiation of oocytes in a gastropod, Ilyanassa obsoleta by light and electron microscopy. They observed that among the differentiating oocytes and follicle cells were leydig cells which were characterized by the presence of a large vacuole containing glycogen. Anderson and Personne (1970) carried out the microbiochemical analysis of glycogen stores within mitochondrial derivative of sperm

of pulmonate gastropod by electron microscopic study. Couteaux-Bargeton (1947) followed the glycogen content of gonads, the digestive complex, and the labial palps in Crassostrea angulata through a calendar year. The gonads had the greatest loss during the breeding season, but made a rapid recovery. The relative size of these structures made it likely, that the recovery of glycogen in the gonads during the winter months was at the expense of the glycogen in the other organs. Some observations on seasonal changes in total polysaccharides in Helix pomatia are presented by Chaigne (1934), but Von Brand (1931) worked out these changes in Helix in greater details. The changes occurring in hibernation and starvation reported before 1927 have been adequately reviewed by Simroth and Hoffman (1927). Later papers have recorded greater differences in glycogen content of different organs. But unless reproductive changes are occurring, glycogen appears to be more easily metabolized than fat. In Chitons, Katherina tunicata and Mopalia hindsii, the glycogen content in both species increased in the gonads in the breeding season (Giese and George, 1962). They further remarked that the type of nutrients stored and the changes in the amount during breeding could form, to some extent, a relation to the habits of the animals. Similarly, Nimitz (1964) and Nimitz and Giese (1964) studied histochemical changes in Chiton in

relation to the reproductive cycle. Their results showed that the germinal epithelial cells of gonads stored glycogen-like material, which diminished in quantity during gametogenesis. Nagbhushanam and Kulkarni (1971) studied carbohydrate, protein and fat changes in the reproductive tract of the land slug, Laevicaulis alte. Maxwell (1980) studied the distribution of glycogen deposits in 2 euthyneuran sperm tails by stereological analysis in Arion hortensis and Lymnaea peregra; according to him the distal part of the sperm tail contains significantly more glycogen than the region nearer the sperm tail. Goudsmit (1975) studied albumen gland in the Helix pomatia and suggested that glycogen accumulates in preparation of winter hibernation. Raghupathiramireddy (1967) and Visser (1965) studied different types of tissues in active and aestivating snail Pila. But their observations were contradictory. Raghupathiramireddy observed progressive decrease in glycogen during aestivation in snails, while Visser observed that the mono, oligo and polysaccharide contents of the tissues during aestivation were increased. In support of his observation, Visser commented that such increase might be due to a conversion of lipid material in carbohydrates. The fat seems to be the major storage material in active snails, while in the aestivating individuals this role is taken over by carbohydrates.

2. Galactogen

Galactogen is a high molecular weight homopolymer of galactose. It is largely confined to the albumen gland of adult pulmonate snails and to the albumen gland pallial oviduct of some prosobranchs. The albumen gland secretes the perivitelline fluid immediately bathing the zygote. This fluid, composed of galactogen and proteins but not of lipid, is metabolized by the developing embryo (Horstman, 1956a) or utilized after hatching (Horstman, 1956b). The large amount of galactogen in the eggs and its utilization by the embryos or freshly hatched snails has led many to believe that this polysaccharide must have a considerable influence upon embryonic development. Aside from Helix and Limnaea, galactogen has been looked for and found in the albumen glands of the following pulmonates: Oxychilus cellarius (Rigby, 1963), Achatina fulica (Ghose, 1963), Aplexa nitens and Otala lactea (McMahon et al., 1957), Bulimnaea megasoma (Goudsmit, 1964), Semperula maculata (Nanaware and Varute, 1974). Galactogen has been identified in the prosobranchs Pila virens and Viviparous (Meenakshi, 1954). The chemistry of galactogen has been worked out by different investigators such as Horstmann (1964), Correa et al. (1967), and Fleitz and Horstmann (1967). The histochemical reactivities of galactogen have been studied by Grainger and Shillitoe (1952) and in

recent years reliable histochemical demonstration of galactogen was described by Varute and Nanaware (1972a-d; 1973). Recently, Chatterjee et al. (1979) studied immunochemically the proteogalactan of albumen gland in Achatina fulica and showed that it consists of 90% sugar, galactans in the albumen gland and eggs of snails do not occur as pure polysaccharide but as proteo-galactan. Galactogen content increases in the albumen gland of the adult snails prior to egg laying (May, 1934b, Goudsmit, 1975) and it is subsequently deposited with the eggs. After egg laying the galactogen content remains low. After the animal enters hibernation, the glycogen is alowly used up and galactogen is utilized as an emergency food source only after all the glycogen had been used up. Meenakshi and Scheer (1969) showed seasonal variations of galactogen content of the albumen gland of the slug, A. columbianus. They found that the galactogen content is highest during the breeding season and lowest during the period following egg laying. They also studied whether the galactogen synthesis in the albumen gland is controlled by the optic tentacles. The present evidence suggests that the growth of the albumen gland is under endocrine control from a centre in the optic tentacles and that endocrine effects on synthesis of galactogen are a consequence of the effect of growth. Emerson (1965) studied galactogen content of the

prosobranch snails which showed wide seasonal variations.

3. Mucopolysaccharides

Mucopolysaccharides are important components of animal mucins, connective tissue and skin. They provide mechanical or protective support and lubrication. In the oviparous molluscs, they are of further significance in the composition of egg jellies and capsules. Mucins production by molluscs is a well known trait. Land snails crawl along a slime path. Fresh water pulmonates travel from substrate to surface along mucoid tight ropes. Mucus figures dramatically in the courtship behaviour of slugs (Hyman, 1967). From the histochemical and biochemical literature, it seems that very few workers have concentrated their attention towards the detection of mucins in the reproductive organs. Rangrao (1963) histochemically studied the reproductive system of the land snail, Ariophanta ligulata with respect to the formation of egg capsules. He found acid mucopolysaccharides in the female portion of the sperm-oviduct and when these results were compared with the reports of different layers of the egg capsules, it was seen that the capsular fluid was secreted by albumen gland. Hence he also suggested a role of mucopolysaccharide as a chelating agent in the physiological process of calcification of egg shell which has been suggested by Simkiss and Tyler (1958, 1959) in birds and reptiles. Kugler (1965) determined

mucopolysaccharides of the reproductive system of the slug Philomycus carolinianus. Smith (1965) investigated mucin contents of the reproductive tract of another garden slug, Arion ater, from the anatomical and histochemical points of view. Meenakshi and Scheer (1968) studied carbohydrates of the slug, Ariolimax columbianis with special reference to their distribution in the reproductive system. They found a sulfated acid mucopolysaccharide located in the common duct region. Fantin and Vigo (1968) histochemically investigated glands of the reproductive tract of Lymnaea stagnalis and found strongly acid mucopolysaccharide (non-sulfated) in the large cells of the muciparous glands, whereas sulfated mucopolysaccharide containing small cells were scattered among these cells. De Jong Brink et al. (1979) were able to locate thirteen different secretory cell types in the female part, majority of them produce different types of acid mucopolysaccharides and the male part appeared to contain twelve secretory cell types. Nanaware and Varute (1973a,b) characterized cytochemical nature of these mucosubstances in the cells of the multifidal gland and in the cells of the penial glands of a land stylommatophoran snail, Semperula maculata. Their results showed that the multifidal gland (dart gland) elaborated mucosubstances consisting of neutral mucins, sulfomucins and sialomucins and these mucosubstances were located in five different cell types, which showed seasonal

variations in their mucin contents. Similarly, penial glands were shown to be composed of sulfomucin secreting and sialomucin secreting unicellular glands at the outer surface of penis and multicellular glands in the penial sheath of the penis, which also showed seasonal changes in their mucin contents. In their study they have commented on the importance of these mucins in the reproductive physiology of the snails.

Molluscan zygotes are commonly enclosed in nutritive and protective layers and perhaps by a gelatinous or hard capsule. The composition of these layers and their origin in various glands of the reproductive tract have been the subject matter of several investigations. Rangrao (1963) studied histochemically polysaccharides in the egg capsules of Ariophanta ligulata and found that acid mucopolysaccharides were scattered in the jelly of the egg capsule which was secreted by the sperm-oviduct. Hunt (1966) observed carbohydrate nature of the egg capsule of the whelk, Buccinum undatum. Bayne (1966) repeated investigation for a search of different components of the egg layers in a grey field slug, Ariolimax reticulatus, by thin layer chromatography, gel filtration and electrophoresis. He found that the most complex nutritive layer surrounding the embryos contained glycoproteins and galactogen as carbohydrates along with proteins, water, aminoacids, calcium and other inorganic ions but no lipids. Bayne (1968) in his

comparative study on carbohydrates in the egg capsules of some species of the prosobranchs, opisthobranchs and pulmonates, showed that all the animals under investigation contained carbohydrates in every layer of all egg masses. Neutral polysaccharides were abundant especially in nutritive fluids providing nurishment to the embryos and the acid mucopolysaccharides were common in the supporting layers with the exception of the prosobranch capsule.

Except glycogen and galactogen, no other carbohydrate has been studied seriously in the reproductive organs of the gastropod snails or slugs. Though mucopolysaccharides play very important role in the reproductive physiology of the gastropod snails or slugs, very few investigators have devoted their attention to these important chemical constituents in these organs. The following few investigators have worked out distribution of mucopolysaccharides in the different organs of the reproductive tract of gastropod molluscs. Els (1974, 1978) studied the mucosubstances secreted by the genital tract of the slug Deroceras laeve and suggested that certain glands secrete acid mucosubstances rich in sialic acid. He also studied sperm-oviduct and suggested the presence of highly acidic sultomucins, sialic acid and non-sulfated mucins. Fantin and Vigo (1968) studied the mucosubstances in the glands which are associated with the reproductive tract of Lymnaea

stagnalis in different seasons of the year. Nanaware and Varute (1973a, 1973b and 1974) studied the distribution and seasonal mucosubstance changes in the breeding-aestivation reproductive cycle of a land pulmonate, Semperula maculata. Their studies have added a great deal of knowledge to the physiology of reproduction of the land gastropod snails in which mucosubstances play important role.

(B) Reasons that stimulated the Undertaking of the Present Investigation

A critical evaluation of the available literature on the animal mucosubstances in general and reproductive tract mucosubstances in particular, reveals that there are several avenues open for young scientists working in the field of histochemistry and biochemistry of mucosubstances in the invertebrate reproductive tract. The literature on animal mucosubstances significantly shows that:

1) Though the various tissues and organ systems such as mantle folds, alimentary tract etc. have been elaborately investigated with a view to finding out their localization, histochemical and biochemical characterization isolation and purification of mucosubstances and wherever possible some physiological roles have been attributed to the mucosubstances, comparatively our status of knowledge on mucosubstances in the

invertebrate reproductive tract is poor.

2) Moreover, whatever histochemical work has been done on reproductive tract, shows that vertebrates are the focal point for such studies. As compared to the mucosubstances in the vertebrate reproductive tract very little is known about these components in the reproductive tract of invertebrates.

3) Whatever work has been reported on the mucosubstances in the invertebrate molluscan reproductive tract, some investigators have selected one organ of a given animal others have selected altogether a different organ of a different animal. Hence, as the literature on the mucosubstances in the reproductive tract stands today, there is very scanty literature on detailed investigation on mucosubstances in all the organs of the reproductive tract of one and the same animal.

4) Most of the animals selected for the histochemical work were from the land and fresh water habitat but very few marine animals were selected for such studies.

5) Among the molluscan group bivalves are mostly studied but gastropods are generally neglected.

The above critical evaluation of the existing literature on invertebrate molluscan reproductive tract mucosubstances

makes it very clear that a detailed investigation of mucosubstances in invertebrate molluscan reproductive tract is essential to augment their nature and role played in the physiology of the reproductive tract and also to augment the understanding of animal mucosubstances in general. It is with this view, therefore, that an extensive research project has been undertaken in this laboratory to study the mucosubstances of the various organs of the reproductive tract of different pulmonate molluscs. When the entire research project will be completed, most of the lacunae in our present day understanding of molluscan reproductive tract mucosubstances will be satisfactorily removed. The work included in the present thesis forms a part of this extensive research scheme and concerns with the mucosubstances, their histochemical characterization and distribution in various organs of the reproductive tract of the Oncidium verraculatum. A battery of recently developed and recommended histochemical techniques has been employed to achieve a technical and methodological perfection.

(C) Plan of the Present Investigation

Keeping in view the aforementioned points and meagre amount of work done on the reproductive tract mucosubstances in gastropod molluscs, it was decided to work out the histology

of the various organs and distribution and characterization of mucosubstances in the reproductive tract of the Oncidium verracutatum.

(a) Choice of the Animal

While selecting the animal care was taken to select such gastropod mollusc wherein no work has been carried out on mucosubstances in any organ of the reproductive tract. Secondly the animals should be available in required number throughout the course of investigation. Therefore, Oncidium verraculatum was found most suitable for the present investigation.

(b) Choice of Techniques

Since the present investigation aims at a detailed study of the nature of mucosubstances present in different cellular sites in the reproductive tract from ovotestis to the penis and penial gland complex and to characterise them histochemically recent and well established histochemical techniques were employed. More than twenty different techniques involving PAS reactivity, modifications in PAS reactivity following diastase digestion and phenylhydrazing pretreatment, alcianophilia at different pH levels, aldehyde fuchsin staining, sequential staining techniques such as AB pH 1.0-PAS, AB pH 2.5-PAS, AF-AB pH 2.5, metachromasia at various pH levels,

critical electrolytic concentration, methylation, saponification, acid hydrolysis, enzyme digestions such as hyaluronidase digestion, neuraminidase digestion and pepsin digestion have been employed. Such histochemical techniques have an advantage over biochemical techniques in the fact that, though the latter techniques gave reliable data on the quantitative presence, they are not of much use in illustrating the tissue and cellular localization. From this point the histochemical techniques are better. In the present investigation, the staining timings were kept constant throughout the work, and difference, if any, in the intensity of staining was taken as reflection of differences in the concentrations of the different types of mucosubstances.

(C) Critical Evaluation of the Observations

It was decided to analyse critically the results obtained in the present investigation on the reproductive tract of *Oncidium verraculatum* in relation to:

(1) Histology of the various organs such as ovotestis, hermaphrodite duct, seminal vesicle, albumen gland, prostate gland, genital ducts (sperm-oviduct, oviduct, vasdeferens, vagina), spermatheca, penis and penial glands with their end sacs.

(2) Histochemical characterization of the mucosubstances in different histological parts in different organs of the reproductive tract.

(3) The distribution of mucosubstances in different histological parts of the reproductive tract.

(4) To compare results obtained in the present investigation and the existing literature on other gastropod molluscs especially palmonates so as to find out similarities or differences, if any.

(5) To find out cellular specializations, if any, in a given organ of the reproductive tract.

(6) To project idea about the functional significance of mucosubstances in the various organs of the reproductive tract based on circumstantial evidences.