CHAPTER FIVE

HISTOLOGICAL AND HISTOCHEMICAL OBSERVATIONS AND DISCUSSION ON MUCOSUBSTANCES IN GENITAL DUCTS (SPERM-OVIDUCT, OVIDUCT, VAGINA AND VAS DEFERENS AND SPERMATHECA OF *ONCIDIUM VERRACULATUM*

GENITAL DUCTS AND SPERMATHECA

A short review of histochemical and biochemical work on the sperm oviduct, oviduct, vagina, vas deferens and spermatheca

The process of reproduction in the land pulmonates has been studied for a very long time. The seasonally influenced changes in the reproductive system have been studied from the anatomical and histological points of view by many investigators (Bett, 1960; Hunter, 1968) who have attributed one or the other functional role to the secretions of the various parts in the different reproductive activities. From the critical review of the literature, it appears that though the histochemical studies of the male reproductive parts and the glands of these snails have been fully critically studied from the functional point of view (Hollande, 1967, 1969; Nanaware and Varute, 1973a, 1973b), very few attempts have been made to analyse these above mentioned chemicals of the male and female genital ducts and their glands (Bayne, 1967; Fantin and Vigo, 1968; Plesch et al., 1971 and Nanaware and Varute, 1974). A brief survey of such histochemical studies in the male and female genital ducts and their associated glands is given below.

Mucosubstances

Mucus secretions from the female genital duct are known

to play an important role during the egg shell or egg coat formation (Bayne, 1967). It also serves as a passage for movement of sperm strands or spermatophores (Beeman, 1970; Lind, 1973) and serves as a reservoir for eggs before they are laid. The results of some histochemical investigations on this organ show that acid mucopolysaccharides are secreted in the female portion of the sperm oviduct of the land snail, Ariophanta ligulata (Rangrao, 1963), a sulfated acid mucopolysaccharide was present in the common duct region of the slug Ariolimax columbianis (Meenakshi and Scheer, 1968) and both acid mucopolysaccharides and neutral mucopolysaccharide secreting eleven different types of cells were reported in the female part of the snail, Lymnaca stagnalis (Plesch et al., 1971). Els, W.J. (1974) histochemically demonstrated mixture of sulfomucins and non-sulfated acid mucopolysaccharides within the individual cells of sperm oviduct of the slug Deroceras laevis. According to Nanaware and Varute (1975) gland cells in the female duct of a land pulmonate Semperula maculata elaborated neutral mucins, glycogen, weakly and strongly sulfated sulfomucins and sialomucins, each elaborated by a different type of cells. Except for these reports, there is no further study of histochemical mucin characterisation and localization in different cell types. Since in very recent years acidic mucosubstances have been reported in the secretion of these organs our efforts in the present investigation aim

at a detailed histochemical characterisation of these mucosubstances.

A small sac, associated with the female genital tract variously called as spermatheca, seminal receptacle, receptaculum seminis, gametolytic gland or bursa copulatrix, receives the sperms at copulation and opens by its slender duct into the vagina of the female duct before it reaches the female gonopore (Hyman, 1967; Runham and Hunter, 1970). From the studies on spermatheca it seems that it plays very significant role in reproductive physiology of the hermaphroditic pulmonates and other gastropods which generally practice reciprocal copulation with exchange of sperms. The spermatheca plays interesting role in many ways - 1) The outer material of spermatophores and sperm strands which it receives at copulation is digested releasing the sperms free. 2) Though some sperms pass upto the common duct, some are returned and retained in it. 3) The sperm mass from the spermatheca stimulates ovulation in the mating partner, since the mated animals ovulate much earlier than the unmated ones; if the spermathecal duct is cut before mating, no stimulus for ovulation occurs, cutting spermathecal duct after copulation had no effect. 4) The remaining sperms within the spermatheca are broken down by secretions from its wall and reabsorbed. The most striking fundamental problem in these cross fertilizing

hermaphrodite animals, is, how the ova are selectively fertilised by the foreign sperms through the great mass of the animal's own sperm which already packs the whole hermaphrodite duct, is not still clearly understood and is not completely solved. In order to understand this fundamental physiological problem in such animals, some investigators have studied this gland from various points of view. de Jorge et al., (1965) and de Jorge and Haeser (1968) studied biochemically certain organic constituents in spermatheca of snail, Strophocheilus oblongus musculus (Becquaert). Bayne (1970) observed sperm breaking processes within this gland in the Ariolimax reticulatus under phase contrast and electron microscopes and discussed the complexity of the flagellum and relative simplicity of the acrosome in the light of the ecology of the spermatozoan and opened many problems for further investigations concerning the functional physiology of the sperm organelles. There are a few histochemical observations (Smith, 1965; Kugler, 1965; Dembski, 1968). Dembski (1968) was able to show glucose and polysaccharides released by disintegrating oligopyrene and some of the eupyrene sperms in the brood pouch and seminal receptacle. According to him these substances might supply nutrition for eupyrene sperms when they move towards oviduct, albumen gland and seminal receptacle where they are stored and later on fertilize the eggs. Nemeth and Kovacs (1972) studied some

ultrastructural features of epithelium of seminal receptacle of edible snail Helix pomatia during different seasons of the year and they found that the golgi apparatus and heterophagic vacuoles contained acid hydrolases and the lumen contained some disintegrating spermatozoa and an acid phosphatase-positive substance which was also observed inside the heterophagic vacuoles. They suggested probable functional role of lysosomal enzymes in decomposition of sperms and the digestion of the resulting debris by the heterophagic vacuoles of the epithelial cells. Rogers et al. (1980) performed ultrastructural analysis of the morphology and functions of the spermatheca of the pulmonate snail Sonorella santaritana. According to them spermatheca wall can be divided into three distinct zones and the layer adjacent to the lumen shows 2 cell types i.e., with and without microvilli, the next layer has also 2 cell types i.e., muscle cell and pigment cells and outermost layer contains large glycogen containing cells. They also suggest that spermathecal functions are both digestive and absorptive. From the critical survey of the allied literature on this gland, it is seen that except for the above mentioned studies, neither histochemical nor biochemical investigations have been carried out, nor is the work available to know the chemistry of carbohydrates mucins and other metabolites in the different parts of this gland.

OBSERVATIONS

I. Morphological

After giving off a finger-like whitish vasicle called the vesicula seminalis the common or hermaphrodite duct becomes somewhat thicker in form and passes imperceptibly into a greatly coiled glandular tube, the sperm-oviduct. The sperm-oviduct divides distally into two unequal tubes. The thinner one from these tubes is the vas deferens and the thicker one is the oviduct. The duct from the albumen gland opens into the proximal portion of the sperm-oviduct. The oviduct runs posteriorly below the pulmonary chamber, it receives a duct from spermatheca. Later part of the oviduct is termed as vagina; it enters the floor of the body cavity and opens to the exterior on the ventral side, just near the anus. The vas deferens runs to a short distance along with the oviduct, but instead of opening to the exterior at the posterior end, it penetrates the floor of the body cavity, turns cephalad, proceeds anteriorly through the floor tissue, and emerges again into the body cavity near the oral end on the right side. It then forms a long coil in the body cavity and ultimately enters into the penis.

II. Histological

Sperm-oviduct

The general histological picture of the female duct showed that the duct was semi-circular in surface, which was bounded by a very thin and delicate epithelium followed by epithelial glandular tissue with elongated polygonal cells - mucocytes - packed in many irregular secretory material. In the epithelium goblet cells are scattered on the surface. These glandular cells had distinct round nuclei, staining blue in HE technique. The lumen of the duct was lined by a single layer of ciliated columnar epithelium, which exhibited pink stain in HE technique. This layer produced numerous longitudinal folds projecting inwards increasing the glandular surface.

Besides this general structure, there are some histological regional differences in the sperm-oviduct which include the thickness of the sections, the presence or absence of histologically and histochemically different reactive cells and their secretion. On the basis of the histological differences, the sperm-oviduct could be divided into four regions, columnar epithelial cells, goblet cells, mucocytes and the luminal contents.

Oviduct

The general histological picture of the oviduct showed that the duct was semi-circular in surface, which was bounded by muscle coat. The lumen of the duct was lined by a single layer of ciliated columnar epithelium. This layer produced numerous longitudinal folds projecting inwards. Some glandular cells were observed just below the inner lining of epithelium.

Vagina

The general histological picture of the vagina showed that the duct was circular in surface which was bounded by muscle coat. The lumen of the duct was lined by tall columnar epithelium with long cilia. The epithelial lining produces numerous longitudinal folds projecting inwards. The nuclei of the epithelial cells are basally situated. Some glandular cells are observed just below the inner lining.

Vas deferens

Vas deferens is very thin and circular in its surface. The inner lining was found to be formed of columnar epithelial cells which are short and having long cilia. The outer muscle coat includes the longitudinal as well as circular muscles.

Spermatheca

The spermatheca is thin walled, hollow, oval or rounded

vesicle. It has fairly long, big tubular stalk called spermathecal duct, which opens in the vagina and receives the sperms at the time of copulation. The spermatheca has a big rounded lumen which usually contained yellow or orange coloured material, which is generally responsible for the pigmentation of the whole gland. It also contains disintegrating sperms, some granules and albuminous secretion mixed together. Histologically the spermatheca showed the outer layer of thin epithelium, middle layer of connective tissue with a thin band of muscular tissue and an inner-most layer of non-ciliated columnar epithelial cells. Our observations are quite similar to the observations made by Rogers et al. (1980) on spermathecal structure of Sonorella santaritana.

III. Histochemical

The histochemical data on some important staining reactions employed in the present investigation of the different cell types in the sperm-oviduct, oviduct, vagina, vas deferens and spermatheca are recorded in Tables No. 4 & 5 according to the visually estimated intensity and shade with four plus (++++) representing the strongest activity. The histochemical results requiring further description and consideration are presented hereafter along with the interpretations of the histochemical staining reactions.

1) Elaboration of Mucosubstances by Sperm-Oviduct

The histochemical results are photomicrographically illustrated in the Plate No. 3, Figs. 1 to 6.

i) Elaboration of Mucosubstances by Epithelial Cells

The columnar epithelial cells of the sperm-oviduct exhibited moderate PAS reactivity which was diminished due to diastase or saliva digestion and was abolished by prior phenylhydrazine treatment indicating glycogen and neutral mucins. These cells show alcianophilia with AB pH 1.0 but do not with AB pH 2.5. Sequential staining procedures with AB (pH 1.0, 2.5)-PAS suggest presence of weak sulfomucins. The graded conc. of Mg^{++} technique also supported the presence of sulfomucins. Thus, the columnar epithelium contains glycogen, neutral mucins and weak sulfated acidic mucins.

ii) Elaboration of Mucosubstances by Goblet Cells

These are scattered among the epithelial cells. These cells exhibited moderately intense PAS reactivity which was slightly diminished by prior diastase digestion and completely abolished by prior phenylhydrazine treatment, indicating the presence of neutral mucins. These cells show intense alcianophilia to both AB (pH 1.0) and AB (pH 2.5). The degree of

intensity of these two levels being practically identical. Sequential staining procedures with AB (pH 1.0, 2.5)-PAS gives intense blue colouration indicating the presence of only acidic mucosubstance. In these cells mild methylation was without any effect on the alcianophilia at pH 2.5 but active methylation effected a complete and irreversible loss of the alcianophilia. Acid hydrolysis failed to abolish the alcianophilia at pH 2.5. These staining reactions indicated presence of sulfomucin in these cells and absence of carboxyl containing mucins. This was further confirmed by the studies on the extinction of the alcianophilia by addition of graded concentration of Mg^{++} to the staining solution. The alcianophilic reaction remained unaffected by the addition of 0.1 M and 0.2 M Mg^{++} but any further addition of Mg^{++} led to an extinction of the alcianophilia. Absence of sialic and hyaluronic acids in the mucosubstances elaborated by these cells, was evidenced by the absence of any effects in sialidase and hyaluronidase treated sections on the alcianophilia both at pH 1.0 and 2.5.

Thus, these cells are endowed with a capacity to elaborate strong sulfomucins along with the neutral mucins.

iii) Elaboration of Mucosubstances by Mucocytes

There are a large number of cells secreting mucin called

as mucocytes. These cells exhibited intense PAS reactivity. It was reduced by prior diastase or saliva digestion and prior phenylhydrazine treatment indicating the presence of glycogen. These cells show intense alcianophilia to AB (pH 1.0) than AB (pH 2.5), sequential staining procedures with AB (pH 1.0, 2.5)-PAS showing both pink and blue reactivities separately and distinctly. With aldehyde fuchsin it gives dark purple reactivity and with sequential staining AF-AB it shows purple and blue colouration separately. Mild methylation was without any effect on the alcianophilia at pH 2.5, but active methylation effected complete and irreversible loss of alcianophilia at pH 2.5. Acid hydrolysis abolishes the alcianophilia at pH 2.5 indicating the presence of sulfomucins and carboxymucins. This was further confirmed by the studies on the extinction of the alcianophilia by the addition of graded concentration of the Mg^{++} to the staining solution. The alcianophilic reaction remains unaffected upto the addition of 0.5 M Mg^{++} suggesting the presence of strong sulfated mucosubstances. Presence of sialic acid in the mucosubstances elaborated by these cells, was evidenced by observing effect of sialidase treatment. In case of sialidase treated sections the alcianophilia decreases both at pH 1.0 and pH 2.5.

From the above discussion it is clear that these cells contain glycogen, strong sulfated as well as carboxyl mucins with sialic acid.

iv) Elaboration of Mucosubstances by Luminal Contents

The luminal contents of the sperm-oviduct exhibit moderately intense PAS reactivity. It was slightly reduced by prior phenylhydrazine treatment and was completely diminished by diastase or saliva digestion. Luminal contents show no alcianophilia to both AB (pH 1.0) and AB (pH 2.5). Sequential staining procedures with AB (pH 1.0, 2.5)-PAS gave only pink colouration and there was no trace of alcianophilia. All other histochemical technique reactivities are found to be negative. Thus, luminal contents contain glycogen and neutral mucins.

2) Elaboration of Mucosubstances by Oviduct

The histochemical results are photomicrographically illustrated in the Plate No. 4, Figs. 7 and 8.

i) Elaboration of Mucosubstances by Inner Epithelium

The columnar epithelial cells exhibited moderate PAS reactivity which was reduced by diastase or saliva digestion and completely abolished by prior phenylhydrazine treatment indicating the presence of glycogen. These cells show very poor alcianophilia with AB (pH 1.0) and weak alcianophilia with AB (pH 2.5). Sequential staining procedures with AB (pH 1.0, 2.5)-PAS these cells stain only pink. Mild

methylation was without any effect on the alcianophilia at pH 2.5 but active methylation effected a complete and irreversible loss of the alcianophilia. Acid hydrolysis failed to abolish alcianophilia at pH 2.5. These staining reactions indicated the presence of weak sulfomucins. This was further confirmed by the studies on the extinction of the alcianophilia by addition of graded concentration of Mg^{++} to the staining solution. The alcianophilic reaction remained unaffected by the addition of 0.1 M and 0.2 M Mg^{++} but any further addition led to an extinction of the alcianophilia.

Thus epithelial cells contain glycogen and weak sulfomucins.

Some glandular cells observed just below the epithelium show the same histochemical reactivities like that of the epithelial cells and were found to contain glycogen and weak sulfated mucosubstances.

ii) Elaboration of Mucosubstances by Muscles

Muscular layer exhibited moderately intense PAS reactivity, which was reduced by diastase or saliva digestion and completely abolished by prior phenylhydrazine treatment. This layer shows poor alcianophilia with both AB (pH 1.0) and AB (pH 2.5), sequential staining procedures with AB (pH 1.0, 2.5)-PAS muscles stained pink only. Mild methylation was without any effect on alcianophilia but active methylation effected a

complete and irreversible loss of alcianophilia at pH 2.5. Acid hydrolysis failed to abolish alcianophilia at pH 2.5. Further studies on the extinction of the alcianophilia by addition of graded concentration of Mg^{++} to the staining solution. The alcianophilic reaction remained unaffected by the addition of 0.1 M Mg but it decreases with increasing concentration and was completely abolished at .4 M Mg^{++} concentration. All these histochemical reactivities confirm the presence of weak sulfomucins.

Thus, the muscle layer of oviduct contained glycogen, neutral mucins and weak sulfomucins.

iii) Elaboration of Mucosubstances by Luminal Contents

Luminal contents exhibit intense PAS reactivity which was much reduced by prior phenylhydrazine treatment and remained unaffected by the diastase or saliva digestion. It showed poor alcianophilia with AB (pH 1.0) but intense alcianophilia with AB (pH 2.5). With sequential staining techniques with AB (pH 1.0)-PAS and AB (pH 2.5)-PAS it showed differential staining, with the former it showed pink staining and with the latter it showed blue staining indicating the presence of acidic mucosubstances containing sialic acid which was further confirmed by sialidase digestion technique.

3) Elaboration of Mucosubstances by Vagina

The histochemical results are photomicrographically illustrated in the Plate No. 3, Figs. 7 and 8.

i) Elaboration of Mucosubstances by Epithelial Layer

The epithelium exhibited moderate reactivity with PAS which was not diminished by diastase and saliva digestion and completely abolished by the prior phenylhydrazine treatment. These cells were without any alcianophilia with both AB (pH 1.0) and AB (pH 2.5). Sequential staining procedures with AB (pH 1.0, 2.5)-PAS they stained moderately pink only. All other histochemical techniques show negative reactivity. Thus, this epithelial layer contains only glycogen and neutral mucosubstances.

ii) Elaboration of Mucosubstances by Glandular Cells

These cells exhibit moderately intense reactivity with PAS which was reduced by diastase or saliva digestion and was completely abolished by prior phenylhydrazine treatment. These cells did not show alcianophilia with AB (pH 1.0) but with AB (pH 2.5) show weak alcianophilia. Sequential staining procedures with AB (pH 1.0)-PAS and AB (pH 2.5)-PAS these cells stained pink and pink-blue respectively. Mild methylation abolishes the alcianophilia at pH 2.5, but it was again

visible after saponification to the fullest extent. Acid hydrolysis completely abolishes the alcianophilia. Thus it indicates the presence of sialic acid in carboxy mucosubstances. The presence of sialic acid was further confirmed by the sialidase digestion.

Thus, these glandular cells contain glycogen and carboxy mucins containing sialic acid.

iii) Elaboration of Mucosubstances by Muscles

Histochemical reactivities with different methods employed here are more or less similar to that of epithelial layer. The muscles exhibited moderate PAS reactivity which was reduced by prior diastase or saliva digestion and was completely abolished by prior phenylhydrazine treatment. The muscles were without any alcianophilia with both AB (pH 1.0) and AB (pH 2.5). Sequential staining procedures with AB (pH 1.0, 2.5)-PAS show only pink colouration and there was no trace of blue colouration. All the other histochemical techniques gave negative reactivities.

Thus the muscle of vagina contains glycogen and neutral mucins.

4) Elaboration of Mucosubstances by Vas-deferens

The histochemical results are photomicrographically

illustrated in the Plate No. 3, Figs. 7 and 8.

i) Elaboration of Mucosubstances by Epithelial Cells

The columnar epithelial cells of the inner lining of the vas deferens exhibited moderate PAS reactivity which was diminished by prior diastase or saliva digestion and was completely abolished by prior phenylhydrazine treatment. These cells were without any alcianophilia with both the AB (pH 1.0) and AB (pH 2.5) sequential staining procedures with AB (pH 1.0, 2.5)-PAS and show only pink colouration. All the other histochemical reaction gave negative reactivities.

Thus, from the above reactivities it is clear that ciliated columnar epithelial cells of the inner lining of vas deferens contains only glycogen, neutral mucins.

ii) Elaboration of Mucosubstances by Muscles

Histochemical reactivities with different methods employed here are more or less similar to that of epithelial layer. The muscles exhibited moderate PAS reactivity which was reduced by prior diastase or saliva digestion and was completely abolished by prior phenylhydrazine treatment. The muscles were without any alcianophilia with both AB (pH 1.0) and AB (pH 2.5). Sequential staining procedures with AB (pH 1.0, 2.5)-PAS show only pink colouration and there was

no trace of blue colouration. All the other histochemical techniques gave negative reactivities.

Thus, from the above discussion it is clear that muscle layer of vas deferens contains only glycogen and neutral mucins.

5) Elaboration of Mucosubstances by the Spermatheca

The histochemical results are photomicrographically illustrated in Plate No. 4, Figs. 1 to 6.

i) Elaboration of Mucosubstances by Columnar Epithelium

Only the inner border of the columnar epithelial cells exhibited PAS reactivity, which was slightly resistant to prolonged diastase digestion but it is practically abolished by prior phenylhydrazine treatment. These initial histochemical reactions indicated a possible presence of the acidic mucosubstances in these sites. With the combined sequential staining procedures, AB (pH 1.0, 2.5)-PAS, this border gave faint blue staining, indicating the occurrence of acidic mucins in it. This border exhibited alcianophilia with AB both at pH 1.0 and 2.5, the degree of intensity being practically identical. Mild methylation was without any effect on the alcianophilic staining at pH 2.5,but active methylation effected a complete and irreversible loss of the alcianophilia.

Acid hydrolysis failed to abolish the alcianophilia at pH 2.5. These staining reactions, thus, indicated presence of weak sulfomucins in this border. This was further confirmed by the studies on the extinction of alcianophilia, by addition of graded concentration of Mg^{++} to the staining solution. The alcianophilic reaction remained unaffected by the addition of 0.1 M and 0.2 M Mg^{++} but any further addition of Mg^{++} led to an extinction of the alcianophilia. Absence of sialic and hyaluronic acids in the mucosubstances at this border, was evidenced by the absence of any effects of the sialidase and hyaluronidase digestion on the alcianophilia both at pH 1.0 and 2.5. Thus the above reactivities indicated that the weak sulfomucins are present in these cells.

ii) Elaboration of Mucosubstances by Connective Tissue and Muscles

The histochemical reactions of the connective tissue were very much identical with those of the inner border of columnar epithelial cells. The muscles exhibited alcianophilic reactivities upto the 0.2 M Mg^{++} concentration which indicated presence of weak sulfated mucosubstances in it. The mucosubstances localised in the muscles showed partial loss of PAS reactivity by diastase or saliva digestion and almost completely abolished by prior phenylhydrazine treatment which

indicated the presence of glycogen in it. Thus, the muscles of spermatheca contained weak sulfomucins and glycogen in it.

iii) Elaboration of Mucosubstances by External Layer or Adventitia

The external layer of large cells exhibited intense PAS reactivity which was completely abolished by prior phenylhydrazine and diastase digestion. There was no alcianophilia with both AB pH 1.0 and 2.5 and in the sequential staining with AB (pH 1.0 and 2.5)-PAS giving only pink colouration indicating the presence of only glycogen in them.

iv) Elaboration of Mucosubstances by Luminal Contents

The mucosubstances localised in the luminal contents exhibited strong PAS reactivity, which was completely abolished by prior phenylhydrazine treatment and was slightly diminished after diastase or saliva digestion. In the combined sequential staining with AB (pH 1.0, 2.5)-PAS, these mucosubstances reacted only with PAS giving a pink staining to the watery luminal contents. The above histochemical reactions indicate presence of neutral mucosubstances and glycogen in the watery luminal contents of the spermatheca. In this uniform pink staining of PAS some small dots of blue staining were found to be scattered which were easily observable

in the combined sequential staining with AB (pH 1.0)-PAS and AB (pH 2.5)-PAS. The alcianophilia was found to be resistant upto .5 M Mg^{++} concentration, suggesting the strong sulfomucins. With AF-AB (2.5) they stained purple. Thus, the contents containing a water fluid elaborating neutral mucosubstances and glycogen and also some particular matter containing sulfomucins and sialomucins were observed.

DISCUSSION

1) Sperm-oviduct, Oviduct and Vagina

The investigation of the mucosubstances elaborated by the columnar epithelial cells in the sperm-oviduct of the marine slug, was undertaken with a view to augment the understanding of the histochemical nature of mucosubstances in general and their localization in this part and their probable role in physiology of reproduction of the slugs in particular. Until recently, the secretion of the sperm-oviduct was variously described as mucins (Lusis, 1961), acid mucopolysaccharides (Rangrao, 1963), strongly acidic polysaccharides containing sulfuric acid esters (Smith, 1965). It was not known in which cells these mucosubstances are elaborated. The present detailed histochemical investigation confirms the presence of sulfomucins as the major constituent of the mucosubstances elaborated by the sperm-oviduct and in

addition shows that the sulfomucins though they form a major constituent are not the only mucosubstances elaborated by this duct. In addition to sulfomucins, the duct also elaborates glycogen, neutral mucins, carboxyl group containing sialic acid mucin, but at a comparative level, they occur in much lesser concentration. Hence, presently it has to be consided that the mucosubstances secretion by various parts of the sperm-oviduct are as follows: columnar epithelial cells elaborate glycogen, neutral mucins and weakly sulfated acidic mucins, goblet cells contain neutral mucins and strongly sulfated acidic mucins, mucocytes secreted glycogen, sialomucins and sulfated mucins and luminal contents elaborated glycogen and neutral mucins.

Another interesting fact of the elaboration of these mucosubstances indicates that for the secretory function the sperm-oviduct seems to have evolved a specialised division of labour at cellular level. Certain cells being specialised to elaborate only sulfomucins, others neutral mucins, some secreting only sialomucins and still others only glycogen. The sperm-oviduct of marine slug, thus, affords an excellent example of biochemical division of labour at cell level for its secretory function.

The mucosubstance contents and their various concentrations in the oviduct bring out interesting functional

features of the oviduct. The neutral mucosubstances and glycogen are in high concentration in the lumen, indicating that during maturation processes they supply continuous source of nutrition to the sex cells (Dembski, 1968). Acidic mucins in the duct probably might be utilised for the formation of egg-coats (Smith, 1965; Bayne, 1967) or probably acts as a stimulus for the activation of these glandular cells in the duct.

These acidic mucins also function as lubricant in the tract (Nanaware, 1974), for outward passage of eggs during oviposition (Kugler, 1965). Such a role of lubrication has been attributed to the molluscan mucosubstances by Goudsmit (1972). It also serves as fluid medium in which sperms from a copulating partner are drawn into the vagina (Kugler, 1965). The exact significance of sialomucins which were observed in this duct is not understood.

2) Vas deferens

As expected, very interestingly only two mucin types — glycogen and neutral mucosubstances — were found in the vas deferens of the marine slug, *Oncidium verraculatum*. The columnar epithelial cells have capacity to synthesise and elaborate the two types of mucins, naturally these mucins are secreted into the lumen of this tube. This tube is filled

with sperm-mass. The histochemical reactivities were similar to those observed in the ovotestis for sperms.

The investigation of mucosubstances from the points of their localisation and chemical nature of the Vas deferens in the marine slug has first time been thoroughly investigated.

The mucosubstances in the Vas deferens have only one function i.e., to supply nourishment to the sperms passing through it.

3) Spermatheca

With a view to augmenting the understanding of the histochemical nature of mucosubstances in general and their distribution in the spermatheca and their probable role in physiology of reproduction of the marine gastropod slugs in particular, the present investigation was undertaken. Till recent times, some polysaccharides, glucose, glycogen and Alcian blue reactive material have been shown to be present in various regions and in the luminal contents of the spermatheca. But no attempt had yet been made to localise the mucosubstances in various parts of the spermatheca of the marine slugs. The present detailed histochemical investigation on spermatheca points out the presence of sulfomucins in columnar epithelial cells and in connective

tissue, glycogen and sulfomucins in the muscles, neutral mucosubstances, glycogen and a few scattered particulate sulfomucin granules in the luminal contents.

The role of apocrine secretion of the spermatheca in Philomycus carolinianus was stated by Kugler (1965), in which he attributed the roles of sperm loosening from the sperm thread received from a mating partner and of preparation of passage for the sperms upto the fertilization chamber of the genital tract. Meisenheimer (1907) believed that the spermathecal secretion in Helix pomatia loosened sperms from the spermatophores received in reciprocal copulation. The tailless sperms are then passively conveyed to the spermatheca where they are stored until the time of oviposition. Ikeda (1929) found that if the slugs were kept isolated, the sperms produced by a single animal migrated individually to the spermatheca where they lost their flagella and eventually fertilised the eggs in the fertilization chamber. Some other functions have been attributed to the spermatheca in which the most important role of the columnar epithelial cells is to break down the remaining sperms by their secretion and resorbed the material formed during the process. Very recently Kruglov (1980) has shown similar function of autosperm resorption of spermatheca in twentyseven fresh water pulmonate molluscs. He also pointed out the role of spermatheca in the

transition of two phases of sexual maturity, i.e., male sexual maturity and hermaphroditic sexual maturity. The spermatheca and copulation play an important role in the transition of first phase to another. The spermatheca reabsorps allo and autosperm. The products of resorption switch on the endocrine system controlling sexual reproduction. Dembski (1968) has attributed nutritive role to the polysaccharide and glucose secretion of spermatheca.

The synthesis and elaboration of the sulfomucins in the columnar epithelium, sulfomucins and glycogen in connective tissue and muscles showed intense reactivities when sperm mass was filled in the spermathecal lumen. The sperms are temporarily stored and they were released for the fertilization of the eggs into the genital tract. The stored sperms require nutrition for their survival which might be supplied by the glycogen synthesized in the muscles. Similar role is assigned to the spermatheca in land snail, Semperula maculata (Nanaware, 1974). The presence of glycogen in lumen was evident. Since sperm tails were dropped or separated in the spermatheca and they were degraded and degenerating processes take place. Since the sperm tails contain only glycogen in them (Nanaware and Varute, 1974; Maxwell, 1980) which was useful and helpful for stored sperms as nutrition. The presence of sulfomucins in different parts and in the luminal

content, perhaps might help in pushing and moving the tailless sperms. The sulfomucin containing inner border of the spermatheca may be of same functional significance. It is fully established that some sperms are broken down in the lumen of the spermatheca and the cell debris, thus created, is endocytosed by these epithelial cells. Such debris has been reported in these cells at ultrastructural level. In the mechanism of such phagocytosis, the cell border generally referred to as glycocalyx, plays an important role. Such glycocalyx functions as the binding site of the material to be phagocytosed. In several other cells performing such function of phagocytosis, such glycocalyx has been shown to be composed of sulfomucins. Thus, the columnar epithelial sulfomucins are important in the intracellular degradation of the sperm debris.