

CHAPTER FIVE

HISTOLOGICAL AND HISTOCHEMICAL OBSERVATIONS
AND DISCUSSION ON MUCOSUBSTANCES IN SPERM
DUCT OF CYPRAEA ARABICA ARABICA

A very simple condition of the male system is seen in small prosobranchs, in which the sperm duct runs from the testis to the male gonopore located near the anus, without showing any differentiations along its course. Usually, however, the sperm duct, shortly after leaving the testis, enlarges and is thrown into numerous convolutions that act as seminal vesicles for sperm storage. Often, some part of the sperm duct is altered into a prostate gland. After the prostatic region the sperm duct narrows again and runs in the floor of the mantle cavity to the male gonopore.

A short review of histochemical work done on
the sperm duct

The sperm duct of gastropods has been a target organ of several investigations for long time. The morphological details and functional role of its different cells has been studied by Guzman, M. Amir and M. Delpin in 1972 and differentiated the zone of sperm duct which corresponds to the seminal vesicle.

West (1978) studied reproductive biology of Colus stimpsoni and showed that the epithelium of the seminal vesicle is composed of ciliated and microvillar cells. The epithelium of the vas deferens contains only ciliated cells. Thomas and Ronald (1975)

studied the reproductive system of Bursatella leachiplei with special reference to histology and showed that the anterior portion of hermaphrodite duct functions as prostate gland. Els (1974) studied histochemical demonstration of mucosubstances in the glandular layer of the spermooviduct of the slug Deroceras laevis and an attempt was made to differentiate them. Mixtures of sulfomucins and non-sulfated acid mucopolysaccharides within the individual cells are reported.

OBSERVATIONS

I. Histological Observations

The follicles of the testis are connected with the small branches of sperm duct. The sperm duct is a narrow, whitish brown thin walled tube which carried the sperms. Its first half portion is much convoluted and forms seminal vesicle, but after the portion of seminal vesicle it becomes more or less straight. The histological picture visualised by the thin sectioning and histologically stained preparations, showed that the sperm duct is round and hollow. The wall of the sperm duct consisted of two layers of cells. An outermost layer is composed of very much flattened epithelium and an inner layer is composed of conspicuously ciliated short columnar epithelium. The cilia are very long and project in the cavity of the duct. There are round

and very prominent nuclei situated at the centre of the cells. As the animals were collected in the month of August, the lumen contained sperm mass in this season. Some nutritive cells were present at the periphery of the lumen.

II. Histochemical Observations

The histochemical data on some important staining reactions employed in the present investigation of the different cell types in the sperm duct are recorded in Table No. 3 according to the visually estimated intensity and shade with four plus (++++) representing the strongest activity. The localization of different mucosubstances in the different cell types in the sperm duct are photomicrographically illustrated in the Plate No. 3, Figs. 1 to 9. The histochemical results requiring further description and consideration are presented hereafter along with the interpretation of histochemical staining reactions.

Elaboration of Mucosubstances by Outer

Epithelial Layer

These cells form the outermost covering of the sperm-duct and exhibited intense PAS reactivity which was slightly diminished by prior diastase digestion and 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. These cells also show moderate reaction towards the AF-AB (pH 2.5). 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 alcianophilia 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.

Elaboration of Mucosubstances by ciliated epithelium

The ciliated epithelium cells showed moderate PAS reactivity which was slightly diminished by diastase or saliva digestion and which was abolished by prior phenylhydrazine treatment. These cells exhibit alcianophilia both at pH 1.0 and pH 2.5 in the combined sequential staining techniques with AB (pH 1.0, 2.5)-PAS the cells reacted only with PAS giving a pink staining, there being no trace of blue colouration. Mild and active methylation, acid hydrolysis and suppression of alcianophilia by $MgCl_2$ showed negative staining towards their techniques.

The above histochemical reactions indicate that these cells contain mainly neutral mucins and the glycogen.

Elaboration of Mucosubstances by Sperms

In the present investigation the animals were collected in the month of August, the lumen of the sperm duct contained the mature sperms. The sperm showed intense reactivity towards these histochemical techniques. The head and tail of sperm showed different reactivities.

Elaboration of Mucosubstances by Sperm Head

The mature sperm found in bundle showed positive reaction towards the histochemical techniques employed in this investigation.

The sperm head shows intense PAS reactivity which was slightly reduced after prior treatment of phenylhydrazine and also diastase or saliva digestion. AB (pH 1.0) and AB (pH 2.5) alcianophilia was observed which was abolished by mild methylation whereas saponification could restore little of the original. The histochemical reactions, thus, indicate the presence of carboxy mucins. Acid hydrolysis effected a complete loss of alcianophilia; sialidase digestion could practically abolish the alcianophilia from these sites. The histochemical reactions, thus, indicated presence of sialic acid in carboxymucins. Such sialomucins seem to be bound with protein, since proteolysis is necessary for the visualisation of their basophilia.

The sperm head contains neutral mucins, glycogen and sialic acid in carboxymucins.

Elaboration of Mucosubstances by Sperm Tail

The sperm tail exhibits moderate PAS reactivity which was completely lost after prior saliva or diastase and phenylhydrazine

treatment. Towards other histochemical techniques, these mucosubstances showed negative reactivities indicating the presence of only glycogen in these sites. Thus, the sperm tails contained only glycogen in them.

DISCUSSION

The investigation of the mucosubstances elaborated by the sperm duct of the marine cypraea, was undertaken with a view to augmenting 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 cypraea in particular. Until recently, the secretion of the genital duct was variously described as mucins (Lusis, 1961), acid mucopolysaccharides (Rangrao, 1963). 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 genital duct and 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 etc.

Another interesting fact of the elaboration of these

mucosubstances indicates that, for the secretory function the sperm duct seems to have evolved a specialised division of labour at cellular level. The mucosubstance contents and their various concentrations in the sperm duct bring out interesting functional features of the sperm duct.

Epithelial Layer

The present investigation of marine cypraea Arabica arabica, the epithelial cells seem to contain neutral mucosubstances and glycogen in them. But in addition the epithelium contains weakly sulfated acidic mucins. From the existing literature it appears that there are some differences in mucin contents in these cells of the sperm duct in fresh water, marine and land molluscs. But differences cannot be interpreted on the basis of habitat with the scanty information or studies available.

Ciliated Epithelium

The ciliated epithelium shows capacity of elaboration of both the types of mucosubstances i.e., neutral mucosubstances, weakly sulfated acidic mucosubstances and glycogen. From the present investigation the mucosubstances elaborated by the ciliated epithelium shows similarity with the mucosubstances elaborated by outer epithelium layer. The functional role of

ciliated epithelium is also similar with the outer epithelium, in addition to that the ciliated epithelium functions as transfer of sperms to the gonopore.

Nurse Cells

In the present investigation of marine cypreae, the nurse cells seem to contain neutral mucosubstances and glycogen in them. While other mucins are completely absent in nurse cells, indicating the nurse cells provide nutrient to the mature sperms.

Sperms

Very few attempts have been made to understand the nature of mucins in the different parts of the sperm from the non-stylommatophoran and stylommatophoran gastropoda. Bradbury and Meek (1963) observed glycogen in mitochondria of sperm. PAS-positive granules were observed in atypical sperms and in sperm tails (Bulnheim, 1968). Nanaware and Varute (1975) have found sialic acid in sperm head and glycogen in tails of sperms. In the present investigation, we also found sialic acid glycogen in sperm head and tails respectively.

The presence of sialic acid in the sperm heads helps in

the smooth entry of the sperm strands in the gonopore of copulating partner at the time of copulation. It should be noted that the sialic acid containing mucosubstances are localised at the tip of the head of the sperms, thus indicating their probable presence in the acrosome of the sperm. Such a presence of sialic acid in the acrosome indicates its probable importance in acrosome reaction in the process of fertilization. The glycogen from the tails might serve as a reserve food for the sperms.

The tail of sperm contains only neutral mucins and glycogen, while other mucosubstances are completely absent in tail of sperm, indicating that the mucin from the tail region serves food for the sperm.

Captions to Figures

Plate No. 3

T.S. of sperm duct stained with eosin haematoxyline showing histology, sperm (sp), outer epithelium (EP), ciliated epithelium (CE) x 400.

T.S. of sperm duct stained with PAS showing intense reactivity. Sperm (sp), Nutritive cells (NU) x 400.

T.S. of sperm duct stained with di-PAS showing effect on PAS reactivity of the sperm (sp), epithelium (EP), nutritive cells (NU) x 400.

T.S. of sperm duct stained with PAS showing intense staining to the sperm (sp), outer epithelium (EP) x 400.

T.S. of sperm duct stained with PAS, sperm (sp) nutritive cells (NU), epithelium (EP) x 900.

T.S. of sperm duct stained with AB pH 1.0-PAS showing PAS reactivity to the sperm mass (SP) and alcianophilia to epithelium (EP) and ciliated epithelium (CE) x 900.

T.S. of sperm duct stained with PAS. Sperm (sp), nutritive cells (NU) x 900.

T.S. of sperm duct stained with AB pH 2.5-PAS showing PAS reaction to the sperms (sp) and alcianophilia to epithelium (EP) x 900.

T.S. of sperm duct stained with PAS showing sperms (sp) x 900.