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Histochemistry Of the Male Reproductive Tissue and Their Secretions in The Marine Hermit Crab *Diogenes Costatus* (Hilgendorf- 1893)

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Abstract

Histochemical analysis of the testis and vas deferens of the hermit crab *Diogenes costatus* reveals that the testis, PVD, MVD and DVD secrete unique secretions. These secretions are utilized in the synthesize of ampulla, stalk and pedestal of spermatophore and the seminal plasma. Histochemical characterization elucidates the possible origin of these substances from the epithelial cells of the vas deferens and the testis of *D.costatus*. Substance A, the secretion from the testis is a glycoprotein with basic group of proteins. The substance B & C secreted by the anterior and posterior part of the proximal vas deferens respectively. Substance D of MVD and substance E of DVD are of aromatic proteins and mucopolysaccharides in nature. The ampulla, stalk and pedestal are glycoproteins with aromatic group. Similarities in the histochemical composition of the spermatophore and the secretions of the vas deferens show the possible origin of components of spermatophore from the secretions of vas deferens

Keywords
Hermit crab Testis, Vas deferens, Spermatophore, Histochemistry.

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INTRODUCTION

Vas deferens of Crustaceans in general is lined internally by a secretory epithelium and externally by connective tissue. In some instances, in between the epithelium and the connective tissue is a layer of muscle cells. Male gametes are released from the testis into the anterior most part of the vas deferens which in some cases is divided into small tubules called 'collecting ducts' proximal to the testis. In the hermit crab *Pagurus novae - zealandiae* the collecting duct extends the whole length of the testis. The wall of the collecting duct is made of cuboidal secretory cells with a large centrally placed nucleus. Musculature is absent and the movement of the semen along the lumen of the collecting duct is apparently by built-up pressure. The spermatozoa within the duct are embedded in epithelial secretion (Greenwood, 1972). The epithelium of the collecting

ducts of the hermit crab *Dardanus asper* (Matthews, 1953) and the penaeid prawn *Penaeus kerathurus* (Malek and Bawab, 1974 a) are non-secretory.

The vas deferens in crustaceans is divided into several portions. Each plays a role in the assemblage of masses of sperm into spermatophores and the production of seminal fluid. Dahlgren and Kepner (1908) described a seminal fluid sheath around the sperm mass of a cray fish and a lobster (not named), which stimulated a renewed interest in spermatophore which culminated in extensive studies by Mouchet (1930, 1931) on the male reproductive systems and its products in many Crustacea. The structure and function of the various regions of the vas deferens show some apparent species differences. Mouchet (1931) assigned nine regions of activity to the vas deferens of *Diogenes pugilator.* Since 1930, male reproductive systems have been studied ia several groups of Crustacea. In *Callinectes* (Cronin, 1947), *Carcinus* (Spaulding, 1942), *Portunus* (Ryan, 1967) and *Libinia* (Hinsch and Walker, 1974). Matthew's work includes studies on *Dardanus* (1953, 1956 b), *Hippa* (1956 a), *Coenobita* and *Birgus* (1956 b) and *Aniculus* (1957). Matthews (1953) observed the gradual morphological changes in the vas deferens *of Dardanus asper* and are paralleled by physiological changes. Greenwood (1972) observed only seven functional regions in the hermit crab Pagurus *novae - zealandiae* out of nine as mentioned by Mouchet (1931) in D.*pugilator.* Mouchet (1931) found two opposed coils in the proximal vas deferens of *D. pugilator* and she stated that in these coils' fragmentation of the continuous sheath into discrete capsule formation of the spermatophore occurs. According to Greenwood (1972) there is only one coiled region iu the vas deferens of P. *novae - zealandiae,* the fragmentation of the continuous sheath into discrete capsule formation is a result of muscular activity rather than the change in curvature of the vas deferens. Fasten (1917) suggested that synthesis and secretion of the wall begins in the proximal region of the vas deferens. The anterior vas deferens is apparently the site of formation of the spermatophore. Spermatophore and production of seminal fluids occurs in the more posterior regions.

MATERIALS AND METHODS

For histochemical detection of proteins, carbohydrates and lipids, the testis, three different regions of the vas deferens namely PVD, MVD and DVD of the hermit crab *D. Costatus* were fixed in 5% cold neutral buffered formalin. After fixation, they were washed in water, dehydrated in alcohol and embedded in paraffin wax (melting point 58 - 60°C) and sections of 8|µm thickness were taken. The tissue sections after deparaffinization and hydration were used to test the chemical nature of the testis vas deferens and spermatophores

Tests for Protein

Histochemical procedure of Pearse (1968) was adopted for determining the basic proteins. Acidic protein in the testis and vas deferens were found out by adopting the method of Mazia *et. al.* (1953). Tyrosyl group in the tissue sections was detected by Million's test (Pearse, 1968). DMAB - nitrite method (Pearse, 1968) was followed to observe the trytophanyl group.

Tests for Carbohydrates

Periodic acid - Schiff s technique (Hotchkiss, 1948) was adopted for detecting carbohydrates containing 1, 2 – glycols. The presence of glycogen in the sections was determined by adopting Best's carmine method (Pearse, 1968). Toluidine blue test (Pearse, 1968) was followed for finding the presence of sulphated and carboxylated acid mucopolysaccharides in the tissue sections. The presence of chitin in the tissue sections was determined by the Chitosan method as modified by Peters (1972) and by Rajeswari and Ravindranath (1975).

Tests for Lipids

Sudan black B method of Casselman (1954) was followed to ascertain the presence of lipid in the tissue sections. Neutral lipids in the tissue sections were determined by staining with oil red O stain (Pearse, 1968).

RESULTS

Testis and Collecting Tubule

Histochemical analysis of the different regions of the vas deferens and the testes revealed that the secretion (A) of the testis of the hermit crab *D. costatus* is used to agglutinate the sperm to form sperm mass in the collecting tubule. This secretion gives positive result with aqueous bromophenol blue indicates the presence of basic groups. Positive reaction with PAS and Best's Carmine reveals the presence of carbohydrate substances (Tables 1,2). Absence of lipids have been shown by negative results with Sudan black B and oil red 'O' (Table -3).

Proximal Vas deferens

In the proximal vas deferens of D. *costatus, the* lumen includes not only the secretory products of their own but also the substances released from the testis. The anterior most part of the PVD secretes a fluid substance which is referred to as substance B.

Basic groups, tryphtophanyl and tyrosyl groups of proteins are present both in the epithelial cells and substance B. Negative reaction of substance B with PAS and Best's Carmine shows the absence of glycogen, but glycogen is present in the epithelial cells and muscular layer. Substance B is devoid of lipids (Table-3).

The secretory product of the posterior part of PVD is referred to as substance C. The histochemical properties of substance C reveal the presence of basic and aromatic group of proteins (Table-1). Positive reaction of PAS and Best's carmine indicate the presence of glycogen. Presence of acid mucopolysaccharides is indicated by the positive reaction with toludine blue. Histochemical tests for lipids indicate that the substance is devoid of lipids (tables 2,3).

Middle vas deferens

The middle vas deferens of *D.costatus* species secretes substance D. The histochemical properties of epithelium, substance D and spermatophore reveal the presence of basic and aromatic group of proteins. PAS and Best's Carmine test shows positive result for the presence of glycogen. Staining reaction of toludine blue shows the sulphated acid mucopolysaccharides. Lipid histochemical tests indicates that the substance D is devoid of lipds (Tables 1,2,3).

Distal vas deferens

The distal vas deferens of *D. costatus* secretes the substance E. Histochemical properties of epithelial cells and substance E reveal the presence of tryptophanyl group. Positive reaction with toluidine blue at higher pH indicates the presence of carboxylated acid mucopolysaccharides (Tables 1, 2). Negative results of spermatphores with chitosan test indicate the absence of chitin. Sudan black B and Oil red O tests for lipids show that the substance E is devoid of lipid.

Spermatophore

Spermatophore wall of *D. costatus* shows positive results with DMAB and Millon's test indicate the presence of tryptophanyl and tyrosyl groups of proteins. Positive reactions of sperm mass substance, spermtophore wall, stalk and pedestal with toludine blue show the presence of mucopolysaccharides (table). Sperm mass substance, spermatophore wall, connecting cord and gelatinous matrix show positive reaction with toludine blue indicate the presence of mucopolysaccharides. Negative result of chitosan test with spermatophore wall indicates the absence of chitin (Tables 1, 2, 3).

DISCUSSION

In decapod crustaceans, spermatophores play a major role in sperm transfer and storage. Spermatophores not only serve to protect the spermatozoa during transmission to females but also serve the function of providing energy rich substrates for prolonged storage in the females (Subramoniam, 1991). The walls of the spermatophores and the substrate matrix are secreted by the epithilium of the vas deferens and the non-germinal cells of the testis. Histochemical tests of the testis, vas deferens and spermatophore reveal their chemical composition. It is found that in *D.costaus,* the main componenet of spermatophore is the mucopolysaccharide complexed with proteins. Similarity in the histochemical results of spermatophoric components with the secretory materials of the testis, PVD, MVD, DVD reveals the possible transformation of these materials into the spermatophoric components. Presence of tyrosyl group in the spermatophore wall of *D. costatus* suggest the possible occurrence of phenolic tanning in the spermatophore of *D. costatus* (Table1). Phenolic tanning was reported by Malek and Bawab (1971) in *Penaeus kerathurus* and by Subramoniam (1984) in A*lbunea symnista.*

The spermatophores of the hermit crab *D.costaus,* shows negative result with Chitosan test indicate the absence of chitin. Gelatinous matrix of the hermit crab gives metachromasia with toludine blue at high pH indicates the presence of carboxylated AMP (Table 2). Subramoniam (1984) reported the presence of carboxylated AMP in the protective gelatinous matrix of *A. symnista*

Heterogeneity in the various spermatophoric components of the hermit crab may be correlated to their protective as well as structural functions (Jeanloz, 1970; Montgomery,1970).

Table 1. Histochemical tests of proteins in the testis and different regions of vas deferens of *D. costatus.*

Tests	Testis		PVD				MVD			DVD			To Indicate
	SM	SA	EL	SM	SB	SC	EL	SP	SD	EL	SE	SP	
Aqueous Bromophenol blue	+++	++	+	+++	++	++	+	++	++	+	+	++	Basic group of proteins
Deamination+ABB	-	-	-	-	-	-	-	-	-	-	-	-	
Mercuric Bromophenol blue	+++	++	+	+++	++	`++``	+	++	++	+	+	+	Basic and acidic group of proteins.
Methylation+MBB	-	-	-	-	-	-	-	-	-	-	-	-	
Millon's test	+	+	++	+	++	+++	+	++	++	+	-	++	Hydroxyl groups
Bromination+ Millon's test	-	-	-	-	-	-	-	-	-	-	-	-	Tyrosine
DMAB test	++	++	+	++	+++	++	+	++	++	+	+++	++	Tryptophan
40% Formaldehyde +DMAB	-	-	-	-	-	-	-	-	-	-	-	-	

Table 2. Histochemical tests of Carbohydrates in the testis and different regions of vas deferens of *D.costatus.*

Tests	Testis		PVD				MVD			DVD			To Indicate
	SM	SA	EL	SM	SB	SC	EL	SP	SD	EL	SE	SP	
Best's Carmine	++	+	+	++	+	+++	++	+	++	++	+	+	Glycogen and Mucopolysaccharides.
Diastase + Best's Carmine	-	±	+	+	±	+	+	±	+	+	+		
Schiff alone	-	-	-	-	-	-	-	-	-	-	++	-	Free aldehydes
Periodic acid Schiff (PAS)	+++	+	+++	+++	-	++	+++	++	++	+++	++	++	Glycogen, Viccinyl glycols, Mucopolysaccharides
Distase + PAS	±	±	+	+	-	++	+	±	+	+	+	±	Glycogen
Toludine Blue pH1	++ V	+ B	++ B	++ V	-	-	++ B	++ V	++ V	++ B	++ V	++ V	Carboxylic Phosphate and sulphate groups of acid Mucopolysaccharides.
pH2	++ V	+ B	++ B	++ V	-	-	++ B	++ V	++ V	++ B	++ V	++ V	
pH3	++ B	+ B	+ B	++ B	+ B	+ B	+ B	++ V	++ V	++ B	++ V	++ V	
pH4	++ B	+ B	+ B	++ B	++ B	++ B	+ B	++ V	++ V	+ V	++ V	++ V	
pH7	+ B	+ B	+ B	+ B	+ B	++ B	+ B	++ V	++ V	+ V	++ V	++ V	
Chitosan	-	-	-	-	-	-	-	-	-	-	-	-	Chitin

Table 3. Histochemical tests of Lipids in the testis and different regions of vas deferens of *D.costatus.*

Tests	Testis		PVD				MVD			DVD			To Indicate
	SM	SA	EL	SM	SB	SC	EL	SP	SD	EL	SE	SP	
Sudan Black B	+ Bb	-	+ Bb	+ Bb	-	-	+ Bb	+ Bb	-	+ Bb	-	+ Bb	General Lipids
Acetone + SBB	-	-	-	-	-	-	-	-	-	-	-	-	
Oil Red 'O'	+ R	-	-	+ R	-	-	-	+ R	-	-	-	+ R	Neutral Lipids.
Chroroform+ Methanol extraction + Oil Red'O'	-	-	-	-	-	-	-	-	-	-	-	-	

Legends in the histochemistry tables:

SM	**Sperm mass**
SA	**Substance A**
EL	**Epithlial layer**
SB	**Substance B**
SC	**Substance C**
SP	**Spermatophore**
SD	**Substance D**
SE	**Substance E**
PVD	**Proximal Vas deferens**
MVD	**Middle Vas deferens**
DVD	**Distal Vas deferens**

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