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SPERMATOGENESIS AND SPERMATOPHORE FORMATION IN THE INDIAN WHITE PRAWN *PENAEUS INDICUS* H. MILNE EDWARDS

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ABSTRACT

The origin and formation of the spermatozoa and spermatophore in the penaeid prawn *Penaeus indicus* has been described. Spermatogenesis takes place in the lumen of the testicular acini which have a peripheral germinal zone. Spermatogenesis involves the progressive reduction of the cytoplasm to produce a spherical sperm having an electron dense cap region containing the acrosomal complex from which extends a single spike. Histochemical studies revealed that the spermatozoa had a remarkable abundance of polysaccharides and basic proteins such as arginine and lysine, but was poor in lipids. Spermatophore formation took place inside the vas-deferens with the aid of secretory epithelial cells lining the ducts. The mid vas-deferens was responsible for the deposition of the sperm matrix, spermatophore layer I and the wing, while the distal was deferens secreted the outer bounding layer (layer II). The terminal ampoule served to mould the spermatophore into its characteristic oval shape and for its forceful ejaculation during copulation. The complete spermatophore was electroejaculated and its structural morphology and chemical composition of the acelluar bounding layers was studied.

INTRODUCTION

IN CRUSTACEANS the male reproductive biology and physiology is not as well understood as that of females (Aiken and Waddy, 1980). Studies on the male reproductive organs and process of spermatogenesis in penaeid prawns are very few and therefore information on the male reproductive physiology is limited (King, 1948 ; Subrahmanyam, 1965 ; Lu *et al.*, 1973). In most crustaceans including penaeids, sperm transfer during copulation is accomplished by making use of a specialised sperm packet called spermatophore. The spermatophore of penaeids have been studied by only very few investigators (Shaikhmahmud and Tembe, 1958 Malek and Bawab, 1974 a, b) in spite of its diversity in structure and morphology among different species. The formation of the spermatophore in the different ducts of temperate penaeids has been recently studied by Ro *et al.* (1990) and Chow *et al.* (1991).

This paper reports the observations on the process of spermatogenesis and spermatophore formation in the Indian white prawn *Penaeus indicus*. A histochemical study of the testis and the electroejaculated spermatophore is also made to understand the organic nature of the sperm cells and the acellular bounding layers of spermatophores.

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Materials and Methods

Male *P. indicus* (100-170 mm TL) used in the study were collected from the sea off Cochin using short duration otter trawls. They were then taken to the laboratory in transportation bags and kept in plastic pools with continuous aeration until use.

Prawns of different sizes were dissected in crustacean saline (3.4% NaCl) and the exposed testes and seminal ducts were examined under a dissection microscope. For general histological studies, parts of the testicular lobes, proximal vas-deferens, mid vas-deferens, distal vas-deferens and terminal ampoule were fixed in Bouin's fluid for 24-48 h. The tissues were then processed, embedded in paraffin and serial sections cut at 8 μm and stained with Harris haematoxylin and eosin and Mallory's triple stain. For studying the structural details of the sperm and spermatophore, spermatophore extrusion was induced by electrically stimulating the base of the fifth walking legs (where the gonopores open) with a 12 V current delivered through the electrodes of an electrocautery apparatus. Upon stimulation, a single spermatophore was extruded from the terminal ampoules and these were subjected to gross morphological observations and then fixed in Bouin's fluid for detailed histological studies.

Histochemical tests for detecting proteins, nucleic acids, carbohydrates and lipids in the testis and spermatophore were carried out as per methods described by Pearse (1968). Further the electroejaculated spermatophore was fixed in 4% glutaraldehyde and processed for examination in a JEOL JEM 100 CX II electron microscope at 80 KV.

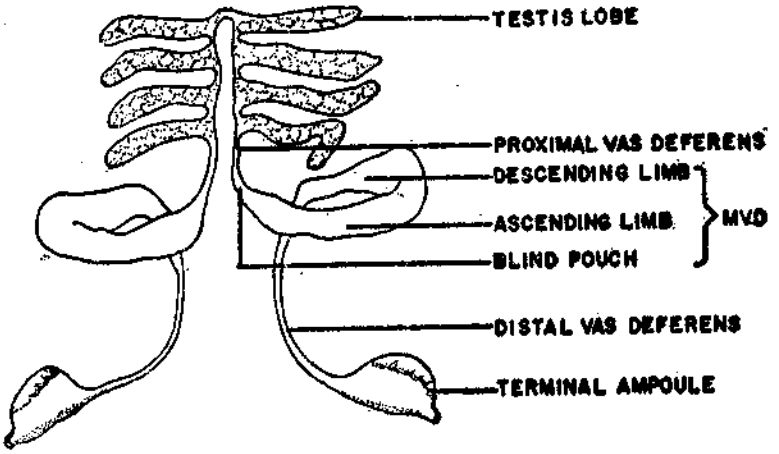

Fig. 1. Diagrammatic representation of male reproductive system in *P. indicus*.

Results

The male reproductive system of *P. indicus* was found to consist of internal organs *viz.* paired testes, vas-deferens and terminal ampoules and external organs *viz.* a petasma and a pair of appendix masculina. The testis is an unpigmented translucent organ composed of four lateral lobes located in the cardiac region dorsal to the hepatopancreas. The testicular lobes are connected to each other at their inner ends and lead to the vasa-deferentia (Fig. 1). Each vas-deferens consists of three portions ; a short and narrow proximal vas

deferens (PVD), a thick and large mid vas-deferens (MVD) and a long and narrow distal vas-deferens (DVD). The MVD consists of a blind pouch, descending limb and an ascending limb (Fig. 1).

The testes were observed as extremely delicate transparent structures in young prawns, while in larger males the testes appeared bigger in size and somewhat opaque. Internally, testis was composed of minute convoluted seminiferous tubules or acini in which developing sperm cells were seen.

Spermatogenesis

Spermatogenesis takes place in the lumen of the testicular acini. In cross sections, a strand-like germinal zone with spermatogonia and nurse cells was apparent adjacent to the acinar wall (Pl. I A, B). Each spermatogonium passes through a period of quick growth to become a primary spermatocyte. A meiotic division results in two secondary spermato cytes. These divide again to produce four spermatids which develop without further division into spermatozoa.

Since spermatogenesis involves the progressive reduction of cytoplasm, the spermatogonial cells were observed as larger than spermatocytes which in turn were larger than spermatids. The spermatogonial cells had a round vesicular nucleus (5-7 μm) with diffused chromatin matter which stained weakly with haematoxylin (Pl. I A). Nurse cells were found dispersed in between spermatogonial cells (Pl. I B). These small elongate cells were seen to have prominent nuclei and measured 8-12 μm in length and 3-5 μm in width. As they were closely associated with spermatogonial cells, it is assumed that they have a nutritive and supportive role. The nuclei of spermatocytes measured 4-5 μm and that of spermatids was 3-4 μm. The spermatozoa developed from these cells through cellular differentiation without further reduction in size. The mature sperm cells were almost spherical in outline with diffuse chromatin matter. A 'Y' shaped acrosome vesicle and a short spike were apparent at the apical region of the main body.

Ultrastructure of sperm

The sperm was composed of a spherical main body that was partially encompassed by an electron dense cap region containing the acrosomal complex, from which extends a single spike (Pl. I C). The main body had a diffuse nuclear region containing chromatin and this was partially surrounded by a cytoplasmic band having small vesicles. The acrosomal complex consisted of an electron dense plate and a less electron dense latticed matrix. The electron dense spike measured 5-7 μm in length. The cap and spike were bound by a double plasma membrane.

Histochemistry of testis

The histochemical tests applied to the testis revealed the complex chemical nature of the sperm cells in *P. indicus*. The cytoplasm of the spermatogonial cells were negative to PAS test, but the nucleus showed moderate positive reaction due to the presence of 1, 2 glycol groups (Table 1). The presence of glycogen in the nucleus was revealed by its positive reaction to Best's Carmine. Both PAS and Carmine positivity increased substantially in spermatocytes and spermatids, with the maxima observed in spermatids and spermatozoa. With the AB-PAS test all the spermatogenic stages except spermatozoa gave a magenta reaction indicating the presence of neutral mucopolysaccharides. In the sperm cells only the acrosome gave the magenta reaction, the nucleus gave a blue reaction denoting the presence of acid mucopolysaccharides (AMPs). Alcianophilia at low molar concentrations with the critical electrolyte method confirmed the presence of AMPs.

The cytoplasm of spermatogonial cells were intensely positive to the test for —SH proteins

(Table 2). The cytoplasm was also weakly pyroninophilic indicating the presence of RNA, which however, was not detected in the nucleus. The nuclei were intensely positive to tests for basic and acidic proteins. Due to the diffuse nature of the chromatin, Feulgen reaction was very weak in these cells. Spermatocytes and spermatids recorded decreased positive reaction to all tests for protein end groups except the alkaline fast green test for basic proteins. acidic, neutral and phospholipids. In general the spermatozoa of *P. indicus* had an abundance of polysaccharides and basic proteins like arginine and lysine.

Spermatophore formation

Histological studies on the vas-deferens revealed the detailed mechanism of spermatophore formation. This process was found to

TABLE 1. *Histochemical responses for carbohydrates in the testis of* P. indicus

Histochemical tests	Epithelial tissue	Spermatogonial cells		Spermatocytes	Spermatids	Spermatozoa	Nurse cells
		Cytoplasm	Nucleus	Nucleus	Nucleus		
Schiff Alone ..	—	—	—	—	—	+	±
Periodic Acid Schiff (PAS) ..	+++	—	+	++	+++	+++	++
Deamination ..	++	—	+	++	+++	+++	+
Acetylation ..	—	—	—	—	—	±	—
Deacetylation ..	+++	—	+	++	+++	+++	++
Delipidation	+++	—	+	++	+++	+++	++
Diastase digestion	++	—	±	++	++	++	+
Best's Carmine Test	—	—	++	+++	+++	+++	+
Diastase digestion ..	—	—	+	+	+	+	±
Alcian blue C-E-C Method							
0.1 M	±	±	+	+	±	±	±
0.2 M ..	—	—	—	+	++	+	+
0.6 M ..	—	±	—	±	±	—	—
0.8 M ..	—	—	—	—	—	—	—
1.0 M ..	—	—	—	—	—	—	—
Alcian Blue—PAS (AB-PAS)	++(M)	—	+(M)	++(M)	+(M)	+++(B) ++(M) Acrosome	++(M)

M—Magenta. B—Blue.

This was presumably due to the high arginine and lysine content in them. Maximum DNA content was observed in the spermatid and spermatozoa stages.

All stages including spermatogonia, spermatocyte and spermatids were poor in lipids (Table 3). The spermatozoa were moderately sucarophilic and weakly positive to tests for

take place in the vas-deferens with the help of secretions from the glandular epithelial cells. The histological structure of the proximal, mid and distal vas-deferens and the terminal ampoule is described.

Proximal vas-deferens : The PVD was observed to be a short slender tube leaving

the posterior margin of the testis (Fig. 1). The duct has an outer connective tissue sheath and measured 1.0-1.5 mm in diameter. Thick (350 μm) circular muscle fibres were seen lining the inner wall of the duct. No glandular cells were observed in this portion of the deferent duct. Sperm cells were rarely seen and most of the time the lumen was empty. It appeared glandular epithelium was observed. The MVD at this point measured 4-5 mm in diameter.

The ascending limb of the MVD was divided internally into two unequal ducts by a connective tissue septum (Pl. I D). The larger of the ducts, the sperm duct, measured 2-3 mm in diameter and the

TABLE 2. *Histochemical responses of proteins and nucleic acids in the testis of* P. indicus

Histochemical tests	Epithelial tissue	Spermatogonial cells		Spermato-cytes	Sperma-tids	Sperma-tozoa	Nurse cells
		Cytoplasm	Nucleus	Nucleus	Nucleus		
Mercuric Bromophenol Blue	+++	+	+++	++	++	+	++
Aq. Bromophenol Blue	+	—	+++	+++	++	++	++
Deamination	—	—	++	++	+	—	+
Aq. Toluidine Blue	—	—	+++	+++	++	+	++
Methylation	—	-	-	-	-	-	-
Ninhydrin Schiff Test	—	—	++	+	+	++	-
Deamination	-	-	-	-	-	-	-
Ferric Ferricyanide Test	+	+++	+	++(PN)	++(PN)	+	-
Mercaptide	—	+	—	+	+	+	-
Performic Acid Alcian Blue	±	±	—	—	—	—	±
Alcian Blue Alone	—	—	—	—	—	—	—
Millon's Test	—	—	++	+	+	—	—
Iodination	—	—	—	—	—	—	—
DMAB-Nitrite Test	—	±	+	±	±	-	-
Formaldehyde	-	-	-	-	-	-	-
Methyl Green Pyronin	+(R)	+(R)	++(G)	++(G)	+++(G)	+++(G)	+(G)
10% Perchloric	+(R)	-	+(G)	+(G)	++(G)	++(G)	+(G)
Fuelgen reaction	—	—	+	++	+++	+	+++
Alkaline Fast green	—	—	+	++	+++	+	++

PN—Perinuclear, R—Red, G—Green

from the histological structure that the PVD's function was to transport the mature sperms from the testis to the MVD through peristaltic movements.

Mid vas-deferens : The PVD dilates to form the blind pouch of the MVD (Fig. 1) which in turn continues anteriorly as the ascending limb. The blind pouch functions as a storage site for the sperms. No specialised sperm mass passes through this for the deposition of spermatophore layers (Pl. I E). The sperm duct was lined with glandular epithelial cells (13 ± 3 μm diameter) which secrete the spermatophore layers (Pl. I F). The cytoplasm of these cells were granular and vacuolated and possessed a vesicular nucleus with an eccentric nucleoli. The glandular cells had a basophilic secretion which was deposited around the sperm mass as

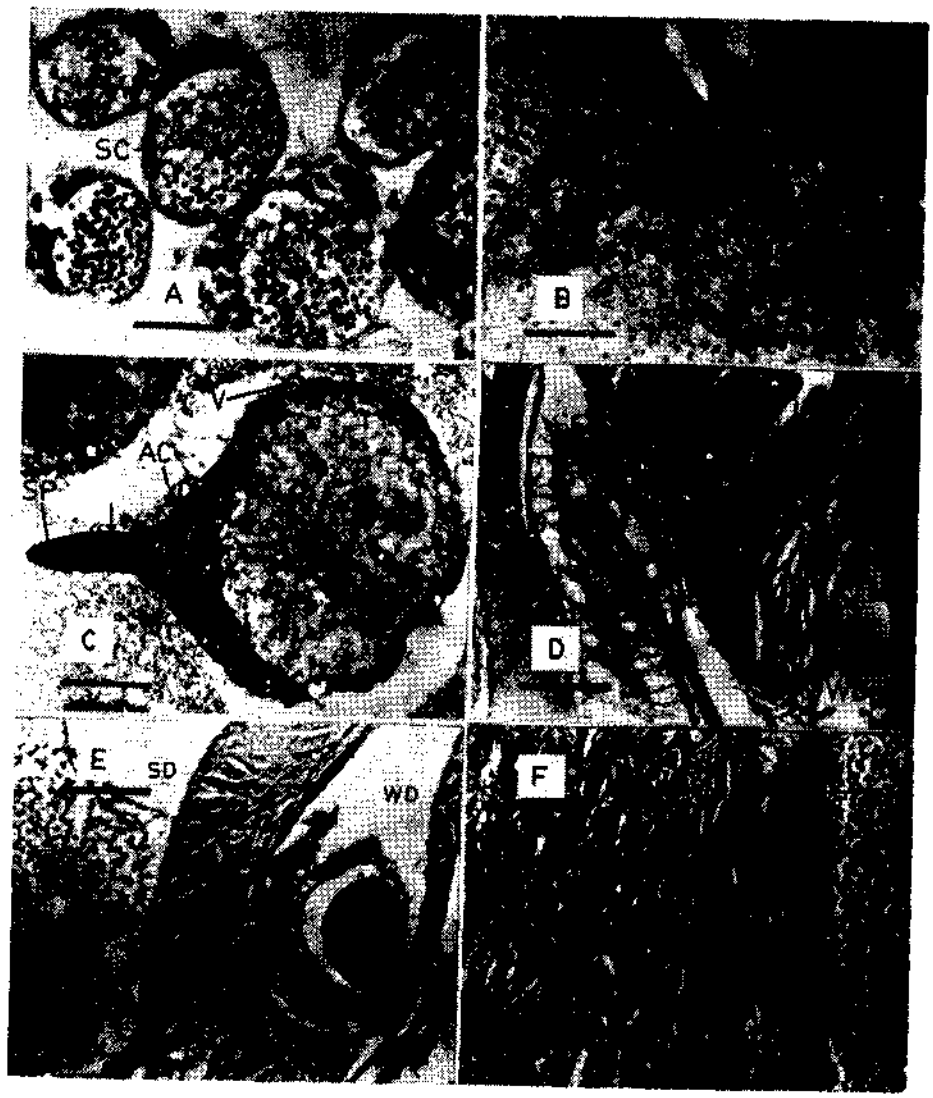

PLATE I. A. Cross section of the testicular acini with peripheral germinal zone (GZ) containing spermatogonia and lumen containing spermatocytes (SC). H & E, Bar = 50 μm, B. Cross section of the acini showing spermatogonia (SG), nurse cells (NC), spermatocytes (SC) and spermatids (SD). H & E, Bar = 25 μm; C. Electron micrograph of the unistellate sperm in the sperm matrix of the spermatophore. SP-spike, AC-acrosome, V-vesicle. Bar = 1 μm; D. Cross section of the ascending limb showing sperm duct (SD) and wing duct (WD) separated by the connective tissue septum (SE). H & E, Bar = 100 μm; E. The sperm duct (SD) and wing duct (WD) with secretory epithelial cell (SC) lining. S-sperms, W-wing and small (T) typholosole in the wing duct. H & E, Bar = 100 μm and F. the basophilic secretion of the secretory cells (SC) forms the spermatophore layer I (SML). S-sperms. H & E, Bar = 25 μm.

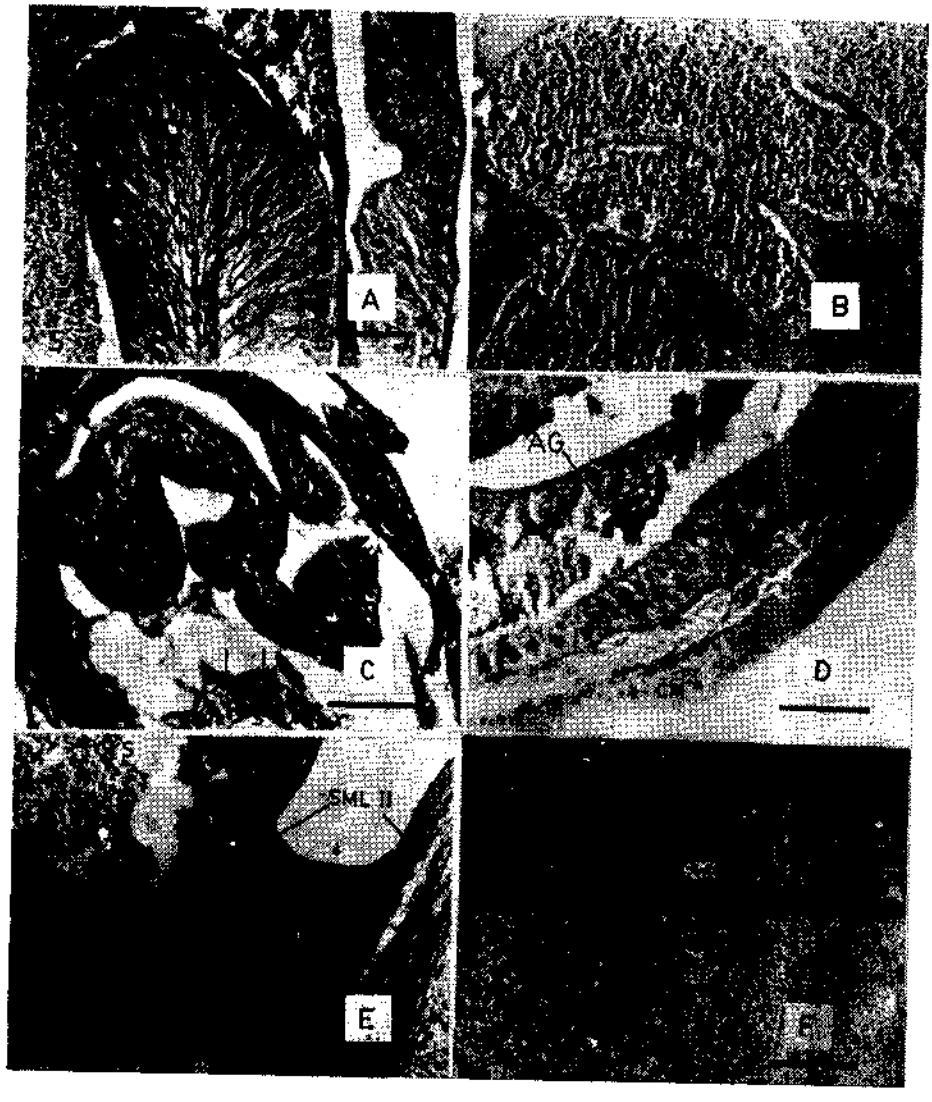

PLATE II. A. Cross section of the large typholosole (T) in the sperm duct. Arrows indicate blood vessels. SC-secretory cells. H & E, Bar = 50 μm. B. Cross section of descending limb of MVD where sperm mass is arranged in pouches. SM — sperm mass. H & E, Bar = 50 μm; C. Cross section of DVD with typholosoles (T) secreting spermatophore layer II (arrow head) S — sperms. Mallory's triple stain. Bar = 100 μm. D. Cross Section of the periphery of terminal ampoule showing outer circular muscles (CM), inner longitudinal muslce (LM) and secretory epithelial cells (SE) secreting adhesive globules (AG). Mallory's triple stain. Bar = 100 μm; E. LS of stalk (ST) of spematophore which attaches body with swing (W). S-sperms, SML II-spermatophore layer II. Mallory's triple stain. Bar = 100 μm and F. Fine structure of spermatophore layers I and II (SML I and II.) Note difference in densities. Bar = 5 μm.

spermatophore layer I (Pl. I F). In order to provide more surface area for the glandular secretory activity, a large typhlosole with blood vessels was observed in the sperm duct (Pl. II A). In the descending limb, after the formation of layer I, the sperm mass was arranged in pouches (Pl. II B).

The smaller wing duct (0.5 mm diameter) in the ascending limb of the MVD had glandular secretory epithelial cells similar in structure to that found in the MVD. The secretion of the outer spermatophore layer II takes place in this duct with the help of highly convoluted typhlosoles. With Mallory's triple stain, layer II was deeply acid fuchsin positive in contrast to the aniline blue positivity of layer I.

Terminal ampoule : The DVD dilates to form the TA or the ejaculatory duct which is

TABLE 3. *Histochemical responses for lipids in the testis of* P. indicus

Histochemical tests	Epithelial tissue	Spermatogonial cells		Spermato-cytes	Sperma-tids	Sperma-tozoa	Nurse cells
		Cytoplasm	Nucleus	Nucleus	Nucleus		
Sudan Black B Test	∓	—	+	+	++	++	+
Delipidation	—	—	—	—	—	—	—
Nile Blue method	∓	—	+(B)	++(B)	++(B)	+++(B)	+(B)
Delipidation	—	—	—	—	—	++	—
Baker's Acid Hematein	∓	—	±	±	±	+	+
Pyridine extraction	—	—	—	—	±	—	—
Oil Red O method	∓	—	+	+	+	+	±
Delipidation	—	—	—	—	—	—	—
UV Schiff Reaction	—	—	+	+	+	+	±
Non UV	—	—	+	±	+	±	±

B –Blue

epithelial cells identical to that found in the sperm duct along with smaller typhlosoles (Pl. I E). These are involved in the secretion of the wing of the spermatophore. The septum which divides the wing and sperm duct was open at the end of the descending limb to facilitate the attachment of the wing to the body of the spermatophore. The descending limb of the MVD gets constricted and becomes narrow to continue as DVD.

Distal vas-deferens : This long slender tube proceeding to the terminal ampoule measured 0.5-1.0 mm in diameter. In transverse sections circular muscle fibres were evident beneath the outer connective tissue sheath (Pl. II C). The inner wall of the DVD was lined with embedded in the coxal muscles of the 5th periopod. The final and complete moulding of the spermatophore takes place in this ampoule. A very thick outer layer of circular muscles and an inner layer of longitudinal muscles formed the walls of the TA, the inside of which was lined with secretory epithelial cells (Pl. II D). While the muscular walls of the TA help in the forceful ejaculation of the spermatophore, the secretion of the epithelial cells forms the adhesive globules required to cement and join parts of the spermatophore from the TA on either side.

The role played by each region of the vas deferens in the formation of the spermatophore is summarised in Table 4.

Structure and chemical composition of spermatophore

The freshly extruded spermatophore was roughly oval in shape and measured approximately 3.5-4.0 mm in length and 2.0-2.5 mm in breadth. Externally the body of the spermatophore had a dirty cream colour and was thrown into folds resembling a bag full of grapes (Fig. 2). The translucent parachute-like wing was found to be 9 mm long and 9-10 mm wide and was connected to the body by means of a short stalk.

bounding layer was 21 μm thick and deeply acid fuchsin positive (Pl. II E). On the lateral edge of the spermatophore a sheathed trough was present in which aniline blue positive adhesive globules (8 μm diameter) were seen (Fig. 2). The sheath was acid fuchsin positive and identical to layer II. A stout stalk attached the wing to the body (Pl. II E). The wing had an amorphouss tructure and was weakly stained.

Ultrastructural studies showed that the moderately electron lucent layer I was composed

TABLE 4. *Functions of the vas deferens during spermatophore formation in* Penaeus indicus

Region of vas-deferens	Role played in spermatophore formation
Proximal vas deferens	— Transport of sperms from testis to MVD by peristaltic movements
Mid Vas Deferens	
Blind pouch	— Accumulation and storage of sperms
Ascending and Descending limbs	—
Sperm duct	— Secretion of the spermatophore layer I (Sph. I) and sperm matrix, packing of sperm mass into lobules.
Wing duct	— Secretion of the amorphous wing of the spermatophore.
Distal Vas Deferens	— Secretion of the seramatophore layer II (Sph. II)
Terminal Ampoule	— Secretion of the adhesive globules final moulding of the spematophore and ejaculation.

Longitudinal sections of the spermatophore stained with Mallory's triple stain revealed the complex structure inside. The sperm mass was embedded in an aniline blue positive matrix in distinct oval lobules which measured 302±72 μm in diameter. Spermatophore layer I was contiguous with this matrix and formed a protective coat around the sperm mass. The spermatophore layer II which is the outer

of a homogenous flocculent material (Pl. II F), while layer II was structurally electron dense composed of small filaments which were arranged compactly.

Histochemically, both the spermatophore layers were PAS positive, the outer layer being intensely so (Table 5). The positive reaction was mainly due to the presence of 1, 2 glycol

groups. Layer II was also strongly carmine positive whereas the inner layer I was carmine negative. The outer wall of the spermatophore was mainly composed of neutral mucopolysaccharides as evident by the magenta reaction with AB-PAS combination. Both sulphated and carboxylated mucosubstances were demonstrated particularly in the matrix by alcianophilla positive to Millon's test indicating the presence of minute quantity of tyrosine. An accumulation of amino groups in the adhesive globules was demonstrated by the Ninhydrin Schiff test. Spermatophore layers were deeply acid hematein positive indicating the strong presence of phospholipids (Table 7). Phospholipids were also indicated in the adhesive globules.

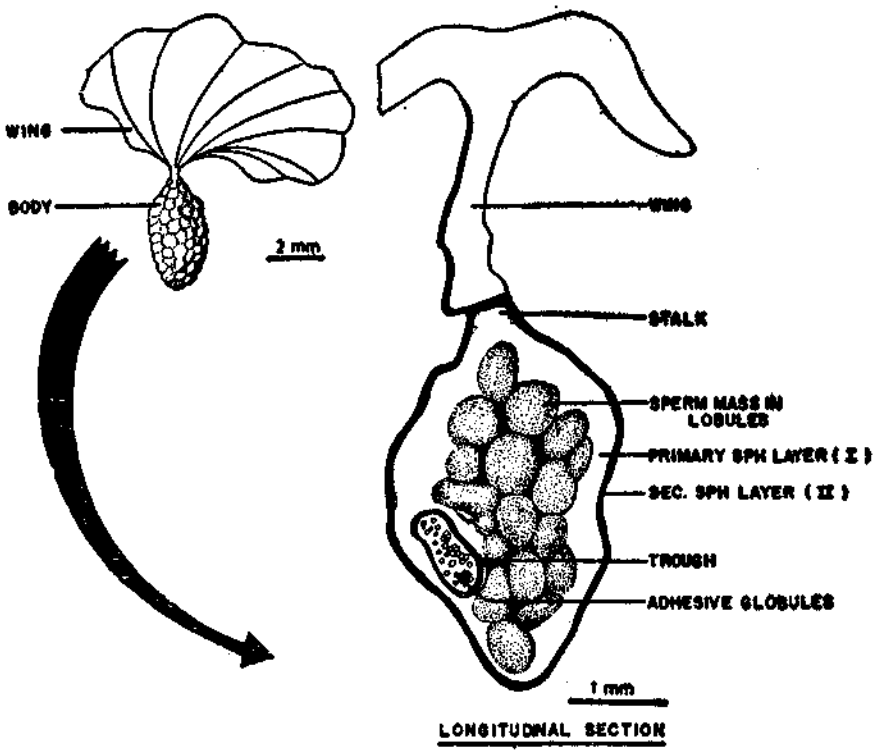

FIG. 2. Structure of the extruded spermatophore in *P. indicus*.

at low molar concentrations. The wing did not respond to any of the carbohydrate tests applied. The adhesive globules were intensely PAS positive even after acetylation, diastase digestion and delipidation, suggesting the conjugation of glycols with proteins and lipids.

Sulphydryl and disulphide groups were the only amino acids detected in abundance in both the spermatophore layers and matrix (Table 6). The outer layer alone was weakly

DISCUSSION

Unlike female penaeids, males provide no grossly visible clues as to the physiological state of the gonad. Therefore, very few workers have described any well defined maturity stages in males. Using the small variation in opacity and size of the testes with size of the animal, Subrahmanyam (1965) had described 5 maturity stages in *P. indicus*. In the present study however, no such distinction

was made as histological investigations showed that adult males had all the spermatogenic stages in each testicular acini. The process of spermatogenesis observed in *P. indicus* is similar to that reported in other crustaceans by Pochon-Masson (1983). A general feature of the process was the reduction in cytoplasmic volume. Only few cytological studies have been made on the testes of penaeids (King, 1948 ; Subrahmanyam, 1965 ; Lu *et al.*, 1973) and their results bear close resemblance to that observed in the present study.

TABLE 5. *Histochemical responses for Carbohydrates in the extruded spermatophore (SPH) of* P. indicus

Histochemical tests	SPH Layer I	SPH Layer II	SPH Matrix	SPH Wing	Adhesive globules
Schiff alone	–	–	–	–	–
Periodic Acid Schiff (PAS)	+	+++	++	–	+++
Deamination	+	++	+	–	++
Acetylation	–	–	–	–	++
Deacetylation	+	+++	++	–	+++
Delipidation	+	+++	++	–	++
Diastase digestion	±	++	+	–	+
Best's Carmine Test	–	++	+	–	+++
Diastase digestion	–	–	–	–	++
Alcian Blue C-E-C Method					
0.1 M	+++	++	+	±	–
0.2 M	++	+	+	–	–
0.6 M	–	–	–	–	–
0.8 M	–	–	–	–	–
1.0 M	–	–	+	–	–
Alcian Blue—PAS (AB-PAS)	+(M)	++(M)	+(B)	±(B)	+(M)

M—Magenta B—Blue

Studies on the fine structure of the sperm in *P. indicus* showed that it possessed a spike which is unlike a flagellum and therefore was non-motile. In other penaeids like *Sicyonia ingentis* (Kleve *et al.*, 1980) and *P. setiferus* (Lu *et al.*, 1973) similar non-motile sperms were reported. Surprisingly no organelles were observed in *P. indicus* sperm. This may be due to the fact that among Malacostraca itself, the decapod spermatozoa show considerable modification and loss of organelles (Adiyodi, 1985). The degeneration of organelles such as mitochondria, Golgi complex and centrioles has been reported in the spermatozoa of *Procambarus clarkii* (Moses, 1961). The penaeid sperm differs even from the pleocyematan sperm in the absence of radiating nuclear arms. The present investigations indicate that the *P. indicus* sperm belongs to the altered vesicular type, which is different from other flagellate and non-flagellate gametes in crustaceans (Pochon-Masson, 1983).

The cytochemical study on spermatogenesis revealed an abundance of polysaccharides and basic proteins in the sperms. The spermatogonial cells and spermatocytes have an intense metabolism as revealed by the presence of

RNA granules and protein in their cytoplasm. The amount of RNA and proteins decreased in spermatids and spermatozoa, a phenomenon apparently common in Crustacea (Descamps, 1969 ; Pochon-Masson, 1983). Usually gamete DNA is linked to basic proteins (Pochon-Masson, 1983) and this therefore explains the significant presence of nuclear histones in *P. indicus* sperm. In *P. indicus*, spermatozoa are stored in the thelycum of the female during the short period extending between copulation and ovulation. Transmoult retention of sperms in females is common among crustaceans (Pochon-Masson, 1983). Histochemical evidences indicate that glycogen and glycoproteins are the principal reserve nutrient material for the sperms in *P. indicus*. In cirrepede sperms, ascorbic acid and carbohydrates are reported to be used as reserve material (Barnes and Finlayson, 1962).

The vas deferens in *P. indicus* serves not only to transport sperms from the testis to the outside, but also to package them into spermatophores. The protective envelope of the spermatophore was secreted by the epithelial cells lining the wall of the vas-deferens. The vas-deferens is reported to function in a similar manner in other decapods like *Macrobrachium rosenbergii* (Chow *et al.*, 1982) and *Panulirus homarus* (Radha and Subramoniam, 1985) and in other penaeids like *P. kerathurus* (Malek and Bawab, 1974 a, b), *P. setiferus* (Ro *et al.*, 1990 ; Chow *et al.*, 1991) and *P. vannamei* (Chow *et al.*, 1991). Typhlosoles observed in the lumen of MVD and DVD served to increase

TABLE 6. *Histochemical responses for proteins and nucleic acids in the extruded spermatophore (SPH of* P. indicus

Histochemical tests	SPH Layer I	SPH Layer II	SPH Matrix	SPH Wing	Adhesive globules
Mercuric Bromophenol Blue	+	+	+	+(PP)	+
Aq. Bromophenol Blue	—	—	—	+	—
Deamination	—	—	—	—	—
Aq. Toluidine Blue	—	—	—	—	—
Methylation	—	—	—	—	—
Ninhydrin Schiff Test	+	—	—	—	+++
Deamination	—	—	—	—	—
Ferric Ferricyanide Test	+++	++	++	+	++(PP
Mercaptide	+	+	+	—	+
Performic Acid Alcian Blue	+++	++	++	—	—
Alcian Blue alone	—	—	—	—	—
Millon's Test	±	±	—	—	—
Iodination	—	—	—	—	—
DMAB– Nitrite Test	—	—	—	—	—
Formaldehyde	—	—	—	—	—
Methyl Green Pyronin	± (R)	+++(R)	++(R)	—	+++(RR)
10% Perchloric	—	++(R)	+(R)	—	+++(R)
Fuelgen Reaction	—	—	—	—	—

PP—Peripheral R—Red

the surface area of the epithelial cells and the presence of rich blood supply indicated the high metabolic rate of these cells. Similar typhlosoles were observed in the vasa-deferentia of the lobster *P. homarus* (Berry and Heydorn, 1970 ; Radha and Subramoniam, 1985) and the mole crab *Emerita asiatica* (Subramoniam, 1984). Clearly demarked functions were attributed to each segment of the vas-deferens for spermatophore formation in *P. indicus*.

Two acellular layers were seen to encompass the spermatophore in *P. indicus* as opposed to three in the lobster *Homarus americanus* (Kooda-Cisco and Talbot, 1982). The inner layer of flocculent material and an outer layer

TABLE 7. *Histochemical responses for lipids in the extruded spermatophore (SPH) of* P. indicus

Histochemical tests	SPH Layer I	SPH Layer II	SPH Matrix	SPH Wing	Adhesive globules
Sudan Black B Test	−	−	+	−	±
Delipidation	−	−	−	−	−
Nile Blue Method	±	−	+	−	+++(B)
Delipidation	−	−	+	−	+++
Baker's Acid Hematein	+++	+++	+	−	+++(PP)
Pyridine extraction	+	+	±	−	+
Oil Red O Method	±	−	±	−	−
Delipidation	−	−	−	−	−
UV Schiff reaction	±	±	±	−	+
non UV	±	±	±	−	+

B—Blue, PP—Peripheral

Most workers have investigated spermatophores of crustaceans using material teased out from the distal vas-deferens (Radha and Subramoniam, 1985 ; Ro *et al.*, 1990 ; Chow *et al.*, 1991). The technique of electroejaculation of spermatophores is a recent one and has greatly helped in understanding the structure of the fully formed spermatophore (Kooda-Cisco and Talbot, 1982). The fully formed extruded spermatophore of penaeids has been subjected to study for the first time. The presence of a parachute-like wing and an adhesive mechanism to join spermatophores ejaculated from both left and right TAs is apparently unique to penaeids and has not been observed in other crustaceans like lobster (Kooda-Cisco and Talbot, 1982 ; Radha and Subramoniam, 1985). The wing seemingly helps in anchoring the spermatophore inside the thelycum of the female after which it dissolves in the surrounding sea water.

of dense fibrils observed presently were strikingly similar to that observed in *H. americanus*, although an intermediate layer was lacking. As in *H. americanus*, adhesive properties could be ascribed to the outer bounding layer, since this forms a bond between the cuticular wall of the thelycum and spermatophore.

Mucopolysaccharides were found to be the principal components of the spermatophore layers of *P. indicus*. Based on a biochemical investigation, Sasikala and Subramoniam (1987) reported the predominance of AMP like chondroitin sulfate and hyaluronic acid in the spermatophore of *P. indicus*. The present study reveals that the outer bounding layer is com-

posed of a mucoprotein, with cystine and cysteine being the main amino acid components. Both layer I and II also possessed sulphated and carboxylated AMP. Such a mucopolysaccharide heterogeneity has been reported in Crustacea by Subramoniam (1984) in the anomuran crabs *Albunea symnista* and *E. asiatica*. The gel forming and antimicrobial properties of AMPs are well known (Itow and Sekiguchi, 1984) and hence these layers may have an adhesive and protective role. The glycogen content observed in the layers might have a nutritive function during the short storage in the female thelycum. In *E. asiatica* and *A. symnista*, Subramoniam (1984) reported the sperm mass substance or sperm matrix to be an AMP and the gelatinous cord to be a neutral mucopolysaccharide.

The presence of tyrosine-rich proteins was taken as indirect evidence for phenolic tanning in the spermatophore of *P. trisulcatus* by Malek and Bawab (1971). Subramoniam (1984) also detected tyrosyl groups and the enzyme phenolase in anomurans to support this view. However, in *P. indicus*, such evidence was lacking as the amount of tyrosine detected in the layers was small. Perhaps tropical penaeids do not need to have a hardened spermatophore, since the duration between moults is comparatively short. With each moult, the impregnated thelycum is lost and fresh copulation is necessary for a renewed supply of sperms.

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