STUDIES ON THE THELYCUM AND SPERMATOPHORE OF THE PRAWN *PENAEUS INDICUS* H. MILNE EDWARDS.

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C E R T I F I C A T E

This is to certify that this dissertation is a bonafide record of work carried out by Kum. Laxmi Latha.P. under my supervision and that no part thereof has been presented before for any other degree.

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C O N T E N T S

P R E F A C E

The world wide interest in the culture of penaeid prawns has stimulated keen interest in the reproductive biology of these prawns with a view to breeding them in captivity for producing prawn seed on a large scale in hatcheries. The reproductive strategies adopted by the penaeid prawns are vastly different from those adopted by the fish. Mating and spawning acts are simultaneous in fish, the male fish shedding the milt while the female fish releases the mature ova into the water where the motile sperms fertilize the ova. In penaeid prawns, on the other hand, mating and spawning are two different processes separated by a time interval. The sperms are non-motile and packed in sperm bags called spermatophores which are transferred by the male to the female thelycum during the mating act. The sperms are stored inside the thelycum till the female spawns, simultaneously releasing the ova from the ovary and the sperms from the thelycum into the seawater where fertilization takes place. Surprisingly very little is known about the detailed structure of the thelycum and spermatophore and consequently the reproductive mechanisms in penaeid prawns are poorly understood.

The aim of the present work is to study the detailed morphology, histology and histochemistry of the spermatophore and thelycum of Penaeus indicus, a very important cultivable

species of penaeid prawn. Such a study is essential for understanding the functions of these two structures in reproduction and is basic to solving the various problems encountered in induced breeding of these prawns in hatcheries.

The spermatophores were extracted by electrostimulation from the living male and examined. Paraffin sections of the spermatophore were used for studying the histology. Histochemical techniques were used to visualize the chemical nature of the component parts of the spermatophore. The structure of the thelycum was studied after clearing it in saturated solution of potassium hydroxide. Paraffin sections of the thelycum were used for histological and histochemical studies.

These investigations have brought to light the extremely complex structure of the thelycum of Penaeus indicus. For the first time, the existence of specialised epithelium in the thelycum for secreting acid mucopolysaccharides has been demonstrated. The acid mucopolysaccharides seems to have an anti-microbial role, protecting the sperms from bacterial and fungal infection during their prolonged storage inside the thelycum. The spermatophore has a chitinous sperm bag inside which the sperms are embedded in a matrix of acid mucopolysaccharides. The sperms seem to have a store of glycogen inside them to provide energy for their metabolic needs. To the sperm

bag is attached a chitinous "wing" which is quickly worn off after the spermatophore is transferred to the thelycum of the female.

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I N T R O D U C T I O N

Study of the reproductive strategies adopted by the animals belonging to the Class Crustacea has been attracting the attention of the scientists in recent years. Crustacean reproduction has certain unique features such as the non-motile nature of the spermatozoa, the prevalence of the practice of packaging these male gametes in spermatophores before they are transferred to the female and the capacity of the female to store the spermatozoa in a viable condition for long periods. An understanding of the complex reproductive mechanisms in the edible decapod crustaceans like prawns, lobsters and crabs is a prerequisite for breeding them on a large scale.

The first description of the spermatophore of a decapod was given by Kolliker (1841) for Pagurus bernhardus. Since then, the spermatophores of a number of other decapods have been described. Dudenhausen and Talbot (1983) have reviewed the extensive literature on the spermatophores of decapod crustaceans. They have listed 47 anomurans, macrurans and brachyurans in which spermatophores have been investigated. They have classified the decapod spermatophores into three categories viz, the vesicular, pedunculate and tubular types. The simplest is the vesicular type common in brachyuran crabs where the spermatophore is small, spherical or ellipsoid with a thin wall covering the sperm mass. The second type is the pedunculate spermatophore of anomurans in

which the sperm mass is packed into one or more spermatophores elevated on stalks which are attached to a common gelatinous base. The third type is the tubular spermatophore equipped with several distinct investing layers surrounding the sperm mass arranged in a cord-like manner. This type is common among the macrurans such as astacids, homarids, nephropsids, panulirids and carideans.

The origin, nature and development of the spermatophoric mass of the spiny lobster Panulirus penicillatus, the rock lobster Parribacus antarticus and the nephropsid lobster Enoplometopus occidentalis have been studied by Mathews (1951, 1954 a and 1954 b). The spermatophoric mass of Palinurus gilchristi and other South African spiny lobsters have been described by Berry (1969) and Berry and Heydorn (1970). The tubular spermatophore of the American lobster Homarus americanus has been studied in great detail by Maria and Talbot (1982) and Kooda-Cisco and Talbot (1982, 1983). Histochemical observations on the tubular spermatophore of the spiny lobster Panulirus homarus revealed the presence of neutral mucopolysaccharides in the spermatophoric wall whereas the sperm mass and gelatinous matrix are rich in acidic mucopolysaccharides (Radha and Subramoniam, 1985).

The pedunculate spermatophores of anomurans have been described by various authors. In Diogenes pugilator only a single spermatophore is attached with its gelatinous base to the sternum of the female (Bloch, 1935). In Pagurus bernhardus 4-5 spermatophores are placed on each gelatinous strip and the attachment is only through the peduncle; the ampoules of the spermatophore are

not bound to one another (Jackson, 1913; Bloch, 1935). The development of the pedunculate spermatophore of the hermit crab Dardanus asper has been recorded by Mathews (1953). In the sand crabs Emerita talpoida, E.analoga and Hippa pacifica, ribbon like spermatophores have been observed by Wharton (1942), Mac Ginitie and Mac Ginitie (1949) and Mathews (1956 a) respectively. Subramoniam (1977) found that the ribbon like spermatophoric mass in Emerita asiatica is actually composed of a row of numerous tightly packed dimorphic, pedunculate spermatophores attached by the peduncle end to membranous strands and that the whole ribbon is embedded in a gelatinous matrix. The formation of the spermatophoric mass and the histochemical nature of the component parts were also investigated by Subramoniam (1984) who found that various types of mucopolysaccharides are present in the ribbon. The sand crab Albunea symnista differs from the other anomurans in possessing a non-pedunculate, tubular spermatophore, more akin to those of the lobsters; the sperms are packed inside a highly convoluted tube embedded in a gelatinous matrix (Subramoniam 1984). Mucopolysaccharides are the main components in the spermatophoric mass of A.symnista also (Subramoniam 1984).

Among the brachyurans, the vesicular spermatophores of the crab Carcinus maenus have been studied by Spalding (1942) and Hinsch and Walker (1974). The histochemical characteristics of the vesicular spermatophores of Scylla serrata have been investigated by Uma and Subramoniam (1979).

The Natantia appear to possess a wide variety of spermatophore types. The spermatophores of the caridean prawns belonging to the genus Macrobrachium resemble the tubular spermatophores of lobsters and are reported to consist of sperm mass and adhesive and protective gelatinous material (Chow et al 1982; Sandifer et al 1981). Among penaeid prawns the minute, elliptical spermatophores of Parapenaeopsis stylifera with the rod-like spermatozoa packed inside have been figured by Shaikmahamud and Tembe (1955) and Tirmizi (1968). They appear to resemble the vesicular type of spermatophores of the Brachyura. In contrast, the spermatophores of the penaeid prawns belonging to the sub-genus Litopenaeus are large and have elaborate processes and expansions. These highly complex structures are described in great detail by Perez Farfante (1975) for the five American species Penaeus (Litopenaeus) setiferus, P.(L) schmitti, P.(L) vannamei, P.(L) stylirostris and P.(L) occidentalis. She has also illustrated the equally complex spermatophores of some, solenocerid prawns such as Pleoticus robustus, P.muelleri and Mesopenaeus tropicalis (Perez Farfante, 1977). Compared to the elaborate spermatophores of these American species of penaeoids those of the Japanese prawn Penaeus (Marsupenaeus) japonicus are relatively simple with wing-like expansions (Hudinaga, 1942; Tirmizi, 1958). The formation of the closely similar spermatophore of Penaeus (Melicertus) kerathurus has been described by Malek and Bawab (1970, 1971, 1974 a and b).

The spermatophore of Penaeus (Fenneropenaeus) merguiensis has been photographed by Tuma (1967).

The spermatophore or sperm cord of the stomatopod crustacean Squilla holoschista has been studied by Deecaraman and Subramonium (1980) who reported that the spermatozoa are bound together with acid mucopolysaccharides to form the sperm cord; there are no investing membranes. The spermatophores are found in the lesser crustaceans also and the literature on the subject has been reviewed by Mann (1984).

At the time of mating the spermatophores are usually transferred to the thelycum-the external genetalia of female decapod crustaceans where the spermatophores are stored until the period of spawning, when both the spermatozoa and the ova are released simultaneously by the female. The anomuran crabs have no specialized regions that can be designated as the thelycum; the spermatophore ribbons of the sand crabs are deposited in the pleopodal region of the female and are protected by the tucked-in telson. In the hermit crabs Diogenes pugilator and Pagurus bernhardus the pedunculate spermatophores are attached to the sternum of the female (Bloch 1935). In Clibanarius olivaceous Kamalaveni (1947) has reported certain grooves in the sternum and coxa of the thoracic appendages which which appear to channel the spermatozoa into the oviduct.

In the Brachyura there is no thelycum and the spermatophores are injected into the oviduct of the female by the male at the time of mating and the spermatophores are stored in an enlarged part of the oviduct called the spermatheca (Ezhilarasi and Subramoniam 1980). Similar internal spermatheca or vaginal pouch is said to be present in the stomatopod Squilla holoschista (Deecaraman and Subramoniam 1980).

It is in the Macrura that the thelycum reaches its highest complexity. In its simplest form,the thelycum merely consists of a series of prominences, depressions, grooves or plates on the sternites of the sixth to the eighth thoracic segments to which the spermatophores are superficially attached by cementing substances. This "open type" of thelycum is found in spiny lobsters (Berry 1969) in caridean shrimp such as Macrobrachium rosenbergi,(Sandifer et al 1981) and in Aristidae (De Man, 1911; Kubo, 1949) Solenoceridae (Kubo 1949; Perez Farfante and Bullis, 1973 and Perez Farfante 1977), and the American species of the sub-genus Litopenaeus (Perez Farfante 1969, 1971) among the penaeoid prawns. However, in the majority of prawns belonging to the families Penaeidae and Sicyonidae the thelycum is of the "closed type" with seminal receptacles to store the spermatophores (Alcock, 1906; Kubo, 1949; Hall, 1962; Racek and Dall, 1965; Dall, 1962; Starobogatov, 1972). As all these workers were taxonomists, the descriptions of the thelycum were concerned only with the external appearance of the thelycum

which is species-specific and hence of great value in the identification of the species. However the earliest of the workers to illustrate the seminal receptacles of penaeid prawns was Kishinouye (1900) for the Japanese species. Subsequently, Burkenroad (1934 and 1939) discussed the structure of the thelycum of some penaeid prawns in greater detail. Hudinaga (1942) and Tirmizi (1958) showed the relationship of the implanted spermatophore to the thelycum in P.japonicus. In the American species of the genus Penaeus, Perez Farfante (1969 & 1970) figured for the first time the posterior horns of the median protuberance which are normally hidden under the lateral plates of the thelycum. She also published clear illustrations of the paired seminal receptacles of the American species of Trachypenaeus (Perez Farfante, 1971). Tirmizi (1968, 1969) figured the paired seminal receptacles of Parapenaeopsis stylifera and P.hardwickii while George and Muthu (1968) indicated the presence of paired seminal receptacles in Metapenaeopsis stidulans and M.barbata. A "closed type" thelycum with seminal receptacle appears to be present in the homarid lobsters also (Aiken et al 1984).

Even the Euphausiacea have complex thelyca in which the stalk of the pedunculate spermatophores are implanted during mating (Einarsson 1942, 1945; Sebastian 1966; Costanzo and Guglielmo 1976).

From the foregoing review of the literature, it is clear that we have practically no information on the detailed structure of the male spermatophore and the female thelycum of the Indian species of penaeid prawns which are currently being used for aquaculture purposes. For successful culture of any animal that is grown in captivity, it is essential to breed them under artificial conditions and this is possible only when the reproductive mechanism of the animal is understood properly. It is with this objective in view that the present investigation was taken up. Penaeus indicus, the Indian white prawn is a prime candidate species for aquaculture in coastal waters. The present study deals with the morphology, histology and histochemistry of the spermatophore and thelycum of P.indicus to understand their role in the reproduction of this prawn. The study has revealed for the first time, the highly complex nature of the thelycum of P.indicus, which appears to be more than a mere passive receptacle for keeping the spermatophore received during mating. The results are presented and discussed in this dissertation.

MATERIALS AND METHODS

Live specimens of Penaeus indicus collected from the Marine Prawn Hatchery Laboratory, Narakkal and from the sea were used for the study.

Spermatophore :

To study the structure of the spermatophore, the spermatophore situated within the terminal ampoule at the base of the fifth walking leg, was electroejaculated by applying an electric stimulus (4-6 V) to the base of the fifth walking leg. The spermatophore freshly obtained in this manner was observed under the stereoscopic microscope to study the structure. The extruded spermatophored was also allowed to remain in sea water for a few minutes and again observed to discover other structural details and response of the spermatophore to sea water.

For histological study, the spermatophore within the terminal ampoule and the electrically extracted spermatophore were fixed in Bouin's fixative for 24 hrs, dehydrated in graded series of alcohol from 70 % to 100 %, cleared in xylene and embedded in paraffin wax in a hot air oven at 60°C. The blocks prepared were sectioned at 6 µ and spread on micro-glass slides. Different stains such as Mallory's triple, Harris' Haematoxylin-Eosin and Polychrome (Leishman's Eosin-Methylene Blue) were tried, to stain the sections after dewaxing with xylene. The stained

slides were dehydrated in graded alcohol series cleared in xylene and mounted in DPX. Sections stained with Polychrome gave the best histological details and differentiation.

Thelycum :

The thelycum located between the fifth to eighth sternal plates of females was cut and removed carefully. In order to study the structural details, the thelycum was cleared in a saturated solution of potassium hydroxide so as to dissolve the adhering tissue. The cleared, translucent thelycum was then studied under a stereoscopic binocular microscope. The thelycum from females of different size groups in the inter moult stage and impregnated females were studied in this manner.

The thelycum from live females were cut and removed carefully and fixed in different fixatives. Thelycum removed from females of different sizes in the intermoult stage and from the impregnated females were used for this type of study. After initial fixation in Bouin's fixative for 24 hrs, decalcifying agents such as De Castro's fluid, Perenyi's fluid, and Jenkin's fluid were tried for different durations of decalcification such as 6 hrs, 18 hrs and 24 hrs. Direct decalcification in fixatives such as Perenyi's fixative and Zenker's fixative was also tried. Zenker's fixative proved to be the most suitable agent and was subsequently used for further study.

The thelycum fixed in Zenker's fluid for 18 hrs, was washed in running tap water overnight to remove the mercury pigment. Dehydration was done in different grades of alcohol from 70 % to 100 %. The material was cleared in xylene-alcohol mixture for 1 hr and in pure xylene for 1 hr and then embedded in paraffin wax in a hot air oven at 60°C. The blocks were sectioned at 6-8 μ and spread on glass slides.

Staining, after dewaxing in xylene and gradual hydration, was tried using different stains such as Mallory's triple, Harris' Haematoxylin-Eosin combination, Polychrome (Leishman's Eosin-Methylene Blue) Toluidine Blue, and Paraldehyde Fuchsin. Of these Polychrome and Haematoxylin-Eosin gave best histological details.

The stained sections were photographed in a Olympus (Model Vanox PM 10) microscope with automatic photomicrographic attachment, using Kodacolor 100 ASA and Ilford 100 ASA black & white, 35 mm films.

Histochemistry :

For histochemical studies, the spermatophore and thelycum were fixed in 10 % neutral, buffered formaldehyde and processed as done for histological study. The histochemical staining procedures were mainly from Pearse (1968) and Subramonium (1982).

Histochemical identification and characterization of proteins were made by

a) Mercurie Bromophenol Blue test for Proteins
b) Aqueous Bromophenol Blue test for Basic proteins
c) Ninhydrin-Schiff test for amino groups
d) Toluidine Blue test for acidic groups
e) Ferric-ferricyanide method for -SH groups
f) Thioglycollic ferric-ferricyanide method for -SS groups
g) Millon's test for tyrosine
h) DMAB-nitrite method for tryptophan

Histochemical staining procedures adopted for carbohydrates were

a) Periodic acid-Schiff technique for carbohydrates
b) Bests' carmine test for glycogen
c) Toluidine Blue at different pH for acid mucopolysaccharides
d) Critical electrolyte concentration (CEC) method for acid mucopolysaccharide
e) Alcian Blue-PAS test for neutral and acid mucopolysaccharide

Histochemical characterisation of lipids was made by

a) Sudan Black B test for lipids
b) Nile Blue method for neutral and acidic lipids

c) Nile Blue sulphate method for phospholipids

d) Oil red 'O' method for neutral lipids

e) UV-Schiff reaction for unsaturated lipids

f) Sudan Black 'B' method for masked lipids

In addition, tests for chitin and nucleic acids were also performed by using the Chitosan test and Pyronin G test respectively.

Suitable blocking procedures (controls) were also used for each test to prove the presence of specific reactive groups. The blocking procedures included deamination, methylation, demethylation, mercaptide, iodination, formaldehyde thioglycollate reduction, acetylation, deacetylation, chloroform-methanol extraction, and Taka-diastase treatment.

The histochemical observations for protein, carbohydrate and lipid obtained for spermatophore and thelycum were tabulated indicating the intensity of reaction.

R E S U L T S

MORPHOLOGY OF THE SPERMATOPHORE :

When a voltage of 4-6 V is applied at the base of the fifth pair of legs, the male extrudes a pair of spermatophores, one from each terminal ampoule. The two freshly extruded spermatophores are elliptical in shape and stick together as they come out of the genital opening. If they are left in sea water for sometime the "wing" of the spermatophore spreads out like a crumpled handkerchief. The wing is attached along the outer margin of the membranous bag which contains the sperm mass inside (Fig. 1). The region of attachment of the wing to the sperm bag is tough and fibrous. On the anteromedial corner of each sperm bag, externally, is found a sticky mass of granules which bind the two spermatophores into one unit. Inside the sperm bag the sperms are embedded in a spongy matrix. The sperms are transparent, roundish and possess a "spike" as long as the sperm body (Fig. 13). The wing sticks out of the thelycum of impregnated female and gets worn off quickly.

HISTOLOGY OF THE SPERMATOPHORE :

The membranous wall of the sperm bag stains pinkish red with Polychrome stain and appears to be single layered (Figs. 2 & 12). The wing stains pinkish red like the spermatophore wall (Fig. 2). The point of attachment of the wing to the sperm bag is clearly seen in Fig. 10 . At the anteromedial corner of the spermato-

phore the wall invaginates to form a shallow cup (Fig. 11) which contains the sticky mass of granules that stain a dark purple with Polychrome (Figs. 3&4). The spongy matrix adjacent to the inner wall of the spermatophore (Fig. 2) stains purple. The material of the spongy matrix forms a lamellar network (Figs. 4 & 5). Spermatozoa are present in a mass at the centre of the spermatophore and stain purple like the spongy matrix. The sperm mass is invaded by the spongy matrix in a number of places, so much so the sperm mass appears like islands in the spongy matrix (Fig. 5).

The wing, the wall of the sperm bag, the sticky mass of granules, the spongy matrix and the sperm mass could also be clearly recognised in sections of the terminal ampoule containing the spermatophore (Figs. 6, 7, 8 & 9). The staining properties of these various components is also the same as in the sections of the extruded spermatophore; only the spongy matrix stains a lighter shade of purple and the lamellar network structure is not apparent (Figs. 7, 8 & 9). The highly developed secretory epithelium of the terminal ampoule which seems to secrete the spermatophore wall, the wings and the sticky mass of granules is also clearly seen in these sections.

HISTOCHEMISTRY OF THE SPERMATOPHORE :

The results of the histochemical tests performed on the spermatophore sections are summarised in Table I (a, b & c).

Spermatophore wall and wings :

They are positive to chitosan test indicating the presence of chitin in these structures. The intense positive reaction with aqueous Bromophenol blue shows that they are rich in basic proteins which also seem to contain tyrosyl groups (Millon positive). While the wall may contain some acidic groups, the wings were negative to the Toluidine blue test. The -SH, -SS and tryptophanyl groups are conspicuous by their absence in the protein of these structures.

The purplish red colour with PAS which is not extinguished by deacetylation indicates the presence of muco substances. The blue colour with Best's carmine which disappears after diastase treatment may indicate carbohydrates other than glycogen. Blue colour with AB-PAS shows the presence of acid mucopolysaccharides (AMP). Blue colour with AB in low CEC strongly suggests that the AMP is carboxylated. Sulphated groups appear to be absent, as shown by the negative response to Bracco-Curti's test. Lipids appear to be totally absent.

Sticky mass of granules :

Appear to contain basic proteins with tyrosyl groups. The strong positivity with TB may indicate acidic mucopolysaccharides rather than acidic groups in the protein. Moderate positively with PAS even after deacetylation and blue colour with Best's cormine may be indicative of mucopolysaccharides. Blue colour with AB-PAS suggest that they are acid mucopolysaccharides. Intense positivity to Bracco-Curti's test shows that the AMP is sulphated. But it is not confirmed by the TB test in low pH and high CEC. It is negative to all tests for lipids. Positivity to chitosan test is rather perplexing.

Spongy matrix :

It is only moderately positive to protein tests, totally negative to lipid tests and appears to be entirely made up of carboxylated acid mucopolysaccharides. Chitosan positivity is perplexing.

Sperm mass :

It is also only moderately positive to proteins and totally negative to lipids. The very intense positivity to PAS and the other tests for carbohydrates appear to indicate the presence of glycogen along with the occurrence of carboxylated AMP. Intense chitosan positivity is most perplexing.

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Table - I (a)

P.indicus - Histochemical characterization of spermatophore :

Proteins

S. No.	Histochemical Test	Sperm mass	sac wing		spongy matrix	sticky mass of granules	Indicates
1	2	3	4	5	6	7	8
1. a)	Mercurie Bromophenol blue (MBB)	+ DB	++ DB	+ DB	+ DB	+ DB	Proteins
2. a)	Aqueous Bromophenol Blue (ABB)	+ B	+++ B	+++ B	+ B	++ B	Basic proteins
b)	ABB after deamination	-	-	-	-	-	
3. a)	Ninhydrin-Schiff test (NS)	±	±	±	±	±	Amino groups
b)	NS after deamination	-	-	-	-	-	
4. a)	Toluidine Blue test (TB)	+++ B	+ B	-	++ B	+++ B	Acidic groups
b)	TB after methylation	-	-	-	-	-	
5. a)	Ferric-ferricyanide test (FF)	-	-	-	-	-	-SH groups
b)	FF test after mercaptide	-	-	-	-	-	
6. a)	Thioglycollate FF test (TFF)	-	-	-	-	-	-SS group
b)	TFF test after Thioglycollate reduction	-	-	-	-	-	

contd...

Table-I (a) contd.

1	2	3	4	5	6	7	8
7. a)	Millon's test (MT)	♦ R	♦ R	♦ R	♦ R	♦ R	Tyrosine
b)	MT after Iodination	-	-	-	-	-	
8. a)	DMAB-nitrite method	-	-	-	-	-	Tryptophan
b)	DMAB-nitrite method after formeldehyde	-	-	-	-	-	

DB - Dark Blue

B - Blue

R - Red

♦ - Moderately positive

♦♦ - Positive

♦♦♦ - Intensely positive

Table - I (b)

P.indicus - Histochemical characterization of spermatophore

Carbohydrates

S. No.	Histochemical test	sperm mass	sac	wing	spongy matrix	sticky mass of granules	Indicates
1	2	3	4	5	6	7	8
1.	a) Periodic-Acid-Schiff technique (PAS)	+++ M	+ M	+ M	++ M	+ M	Glycogen
	b) PAS after deacetylation	+ M	+ M	+ M	+ M	+ M	
	c) PAS after deamination	-	-	-	-	-	
2.	a) Best's Carmine test (BC)	++ B	++ B	++ B	++ B	++ B	Glycogen
	b) BC after Taka diastase	-	-	-	-	-	
3.	Toluidine Blue at						
	pH 1	+ B	+ B	+ B	+ B	+ B	$-SO_4$ groups of AMP
	pH 2	+	+	+	+	+	$-SO_4$ groups of AMP
	pH 3	+	+	+	+	+	$-PO_4$ & SO_4 group of AM
	pH 4	+++	+	+	++	++	-COOH group of AMP

contd...

Table-I (b) contd.

1	2	3	4	5	6	7	8
4.	Critical electrolyte concentration (CEC) method						
	0.1 M	+++ B	++ B	++ B	+ B	-	-COOH groups of AMP
	0.2 M	+	+	+	+	-	-COOH groups
	0.6 M	-	-	-	-	-	-COOH groups
	0.8 M	-	-	-	-	-	$-SO_4$ groups
	1.0 M	-	-	-	-	-	$-SO_4$ groups of AMP
5.	Bracco-curti's test	-	-	-	-	+++	$-SO_4$ groups of AMP
6.	Chitosan Test	+++ P	+ P	+ P	++ P	++ P	Chitin
7.	Alcian Blue-PAS test	+++ B	++ B	++ B	++ B	++ B	Acid & Neutral AMP

M - Magenta

B - Blue

P - Purple

\+ - Moderately positive

++ - Positive

+++ - Intensely positive

\- - Negative

Table - I (c)

P.indicus - Histochemical characterization of spermatophore :

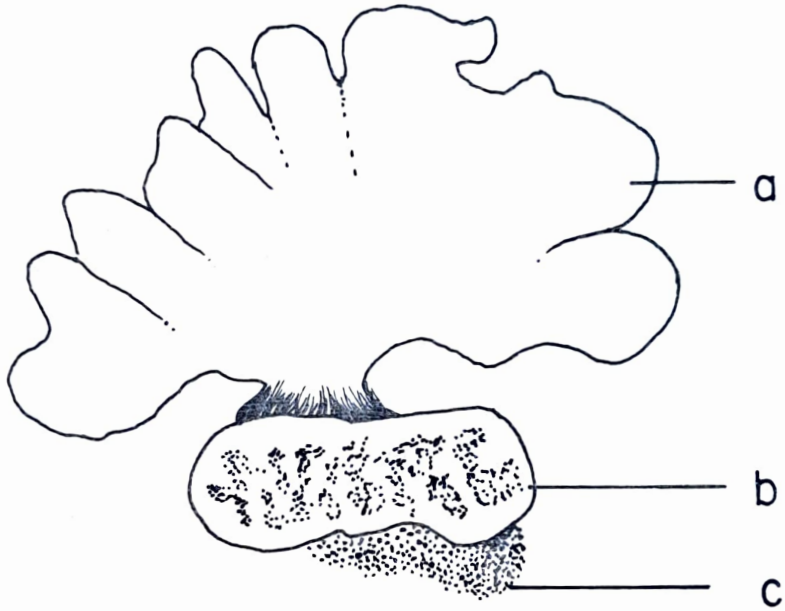
Lipids

S. No.	Histochemical test	sperm mass	sac wing	spongy matrix	sticky mass of granules	Indicates
1.	a) Sudan Black B test	-	- -	-	-	Lipids
	b) SBB after chloroform methanol extraction	-	- -	-	-	
2.	a) Nile Blue method	-	- -	-	-	Neutral & Acidic lipids
	b) Nile Blue after chloroform methanol extraction	-	- -	-	-	
3.	a) Nile Blue sulphate method (NB)	-	- -	-	-	Phospholipids
	b) NB after chloroform methanol extraction		- -	-	-	Neutral lipids
4.	a) Oil Red 'O' method	-	- -	-	-	
	b) Oil Red 'O' after pyridine extraction	-	- -	-	-	
5.	a) UV-Schiff reaction	-	- -	-	-	Unsaturated lipids
	b) UV-Schiff after pyridine extraction	-	- -	-	-	
6.	a) Sudan Black B (SBB)	-	- -	-	-	Masked lipids
	b) SBB after pyridine extraction	-	- -	-	-	
7.	Pyronin G test	+++ R	+++ R	+++ R	+++ R	+++ R	RNA & DNA

R - Red

\- - Negative

Spermatophore



2 mm

a - wing

b - sperm bag

c - granules

Fig: 1.

Fig. 2.

Section through extruded spermatophore stained in Polychrome, showing wall (Wa) of sperm bag, a portion of the wing (Wg), spongy matrix (SPM) and sperm mass (SM) (x 100).

Fig. 3.

Section through extruded spermatophore stained in Polychrome showing sticky mass of granules (SMG) (x 100).

Fig. 2

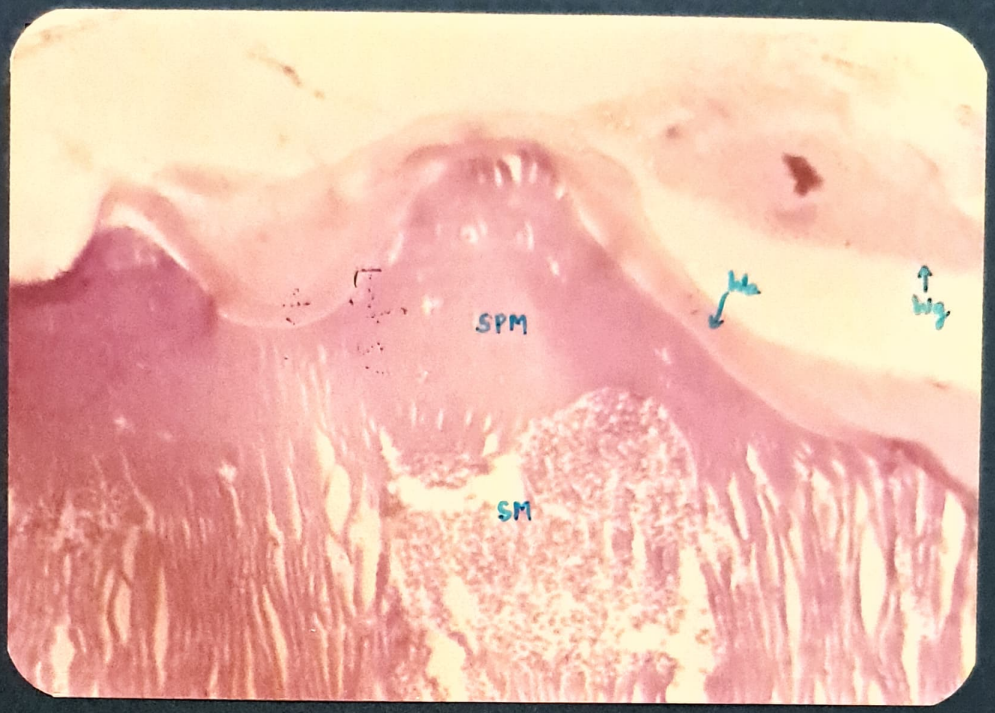


Fig. 3

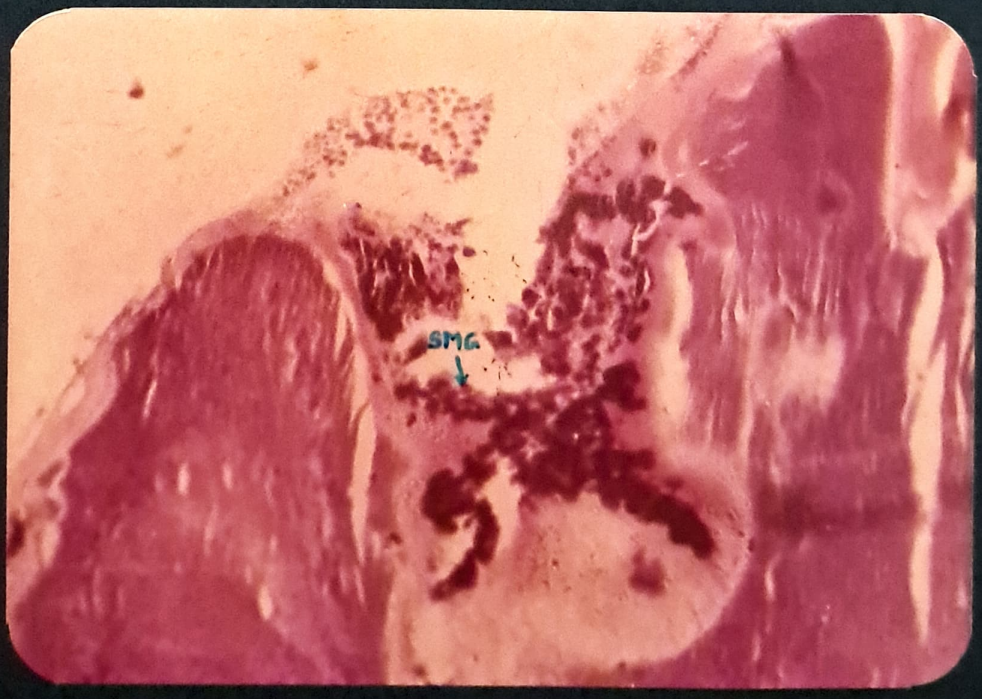


Fig. 4.

Enlarged view of section through sticky mass of granules; Polychrome stain (x 200).

Fig. 5.

Section through centre of sperm bag showing sperm mass (SM) embedded in spongy matrix; Polychrome stain (x 100).

Fig. 4

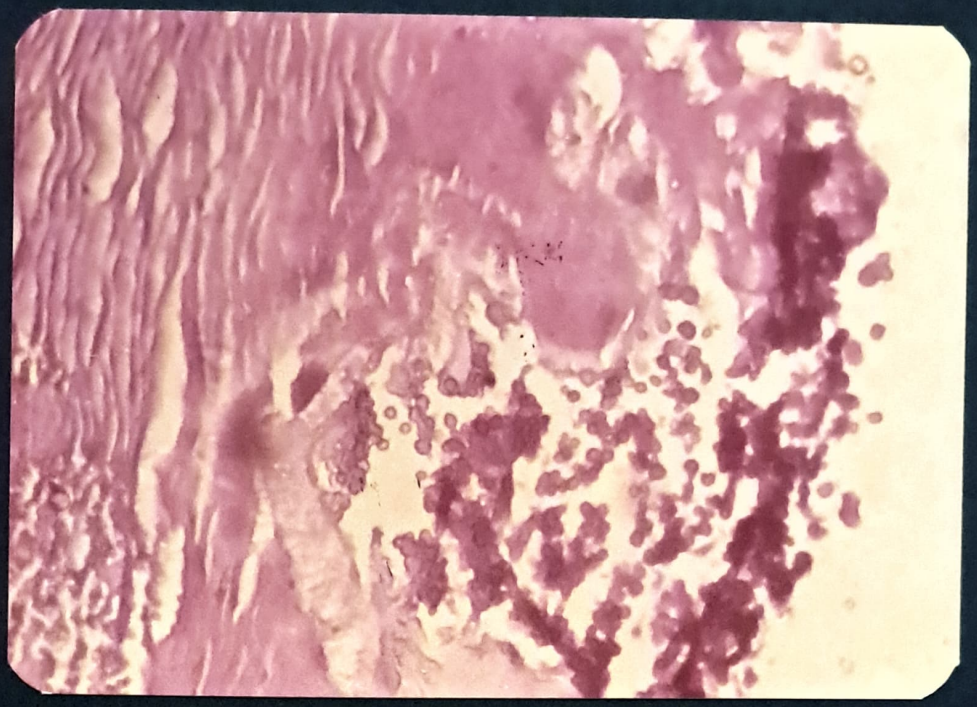


Fig. 5

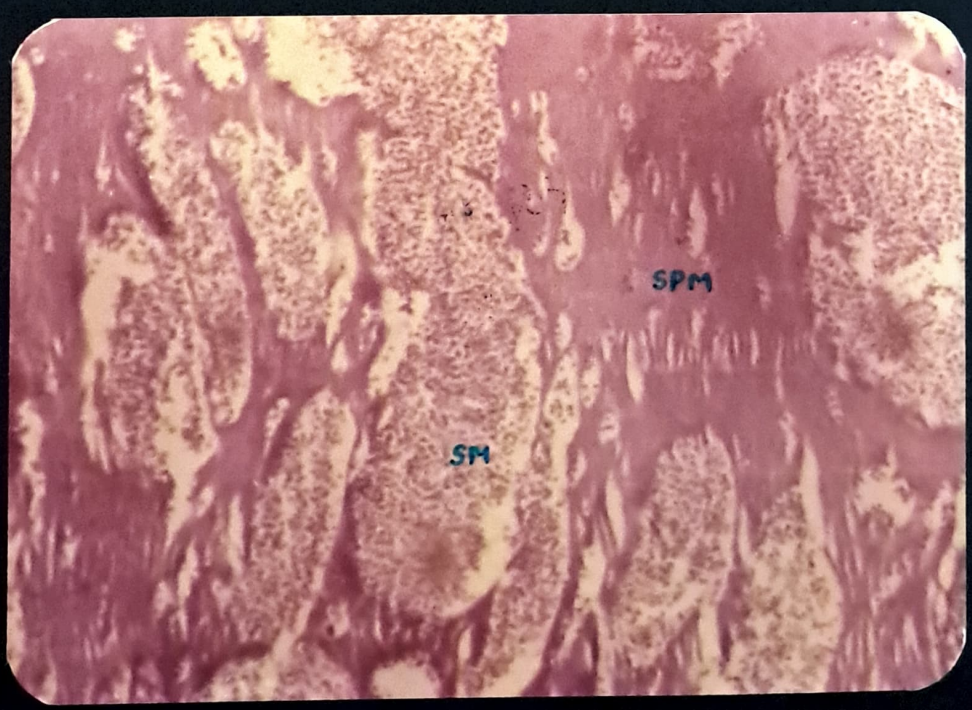


Fig. 6.

Section through terminal ampoule showing secretory epithelium (EP) of ampoule wall, portion of the wing (Wg), and wall (Wa) of sperm bag; Polychrome stain (x 100).

Fig. 7.

Section through terminal ampoule showing wing (Wg) and sperm bag (SB) separated by a partition with secretory epithelium (EP) on both sides and connective tissue (CT) in the middle. MW = Outer muscular wall; OEp = Outer epithelium; Polychrome stain (x 100).

Fig. 6

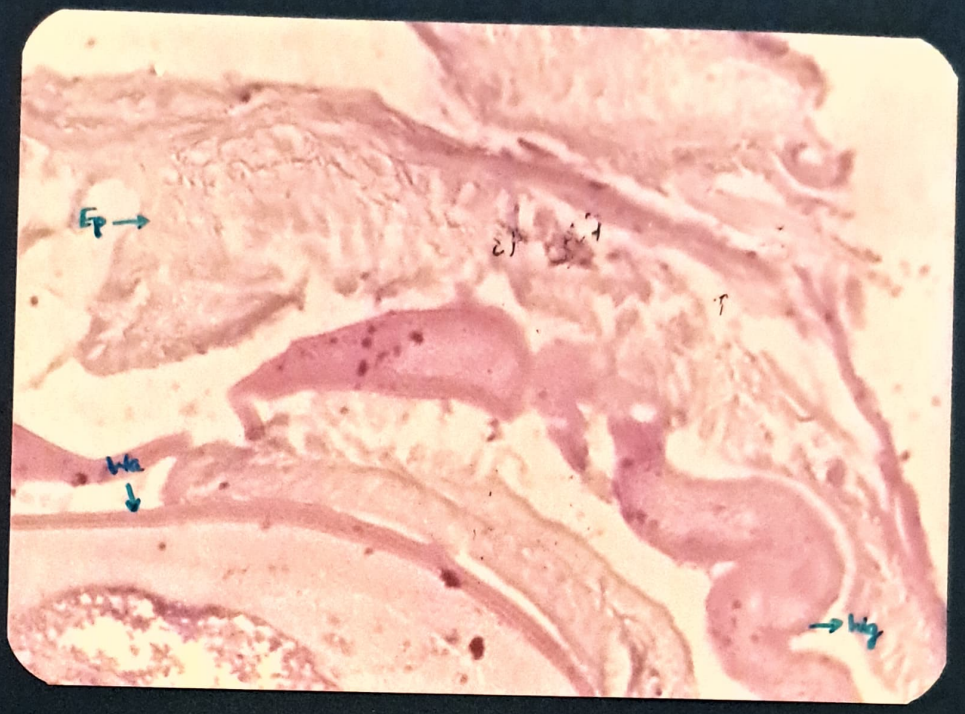


Fig. 7

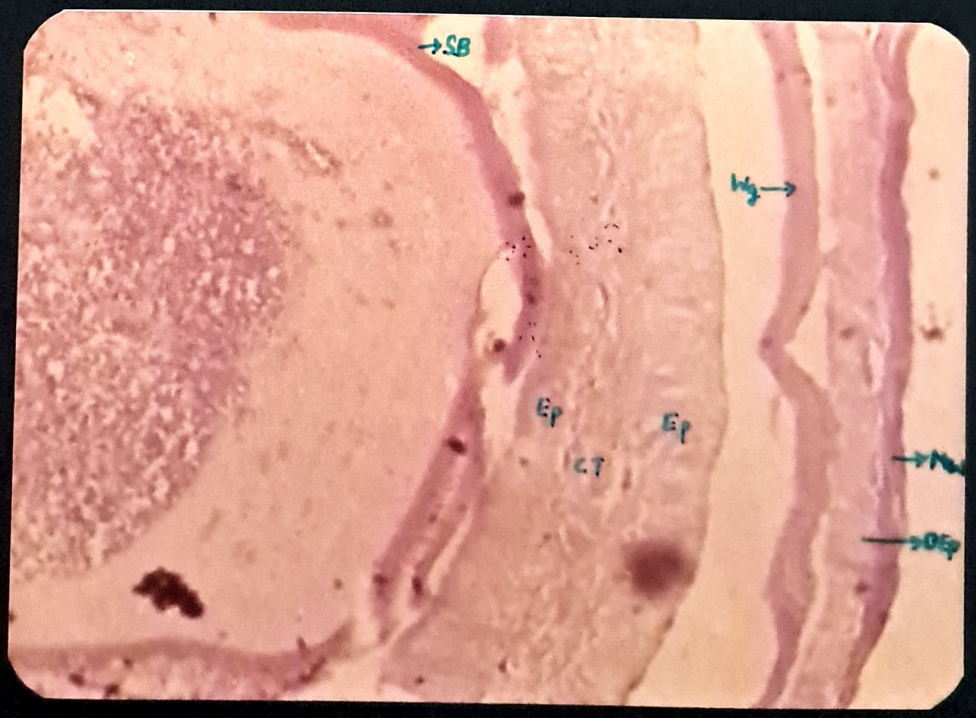


Fig. 8.

Section through terminal ampoule showing outer muscular wall (MW) secretory epithelium (Ep), sticky mass of granules (SMG), wall (Wa) of sperm bag, spongy matrix (SPM) and sperm mass (SM); Polychrome stain (x 100).

Fig. 9.

Section through terminal ampoule showing sticky mass of granules (SMG), wall (Wa) of sperm bag, spongy matrix (SPM) and sperm mass (SM); Polychrome stain (x 100).

Fig. 8

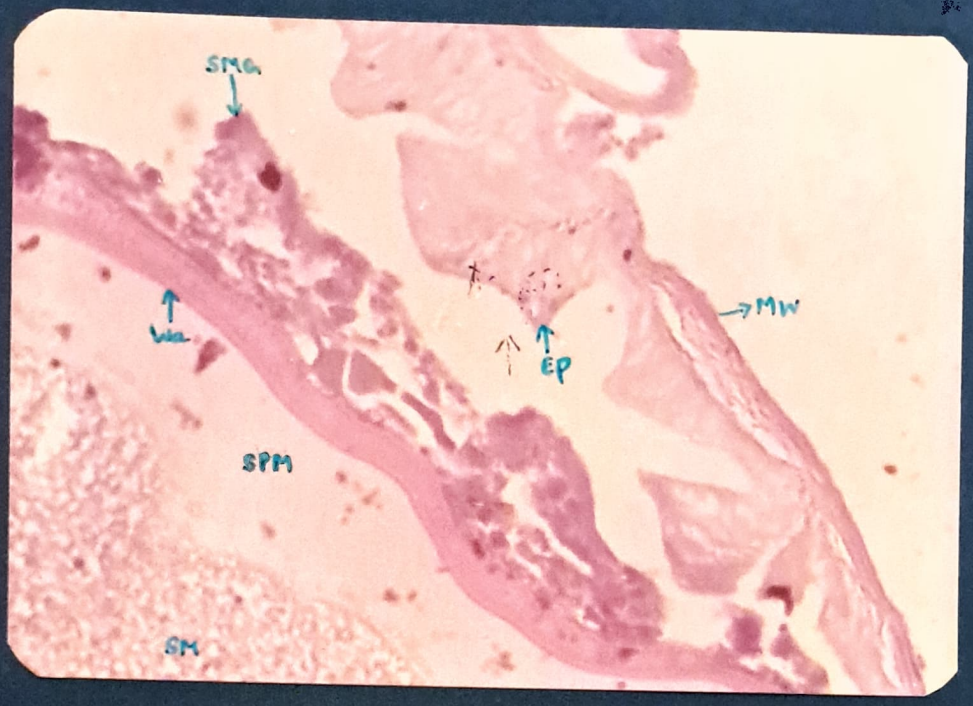


Fig. 9

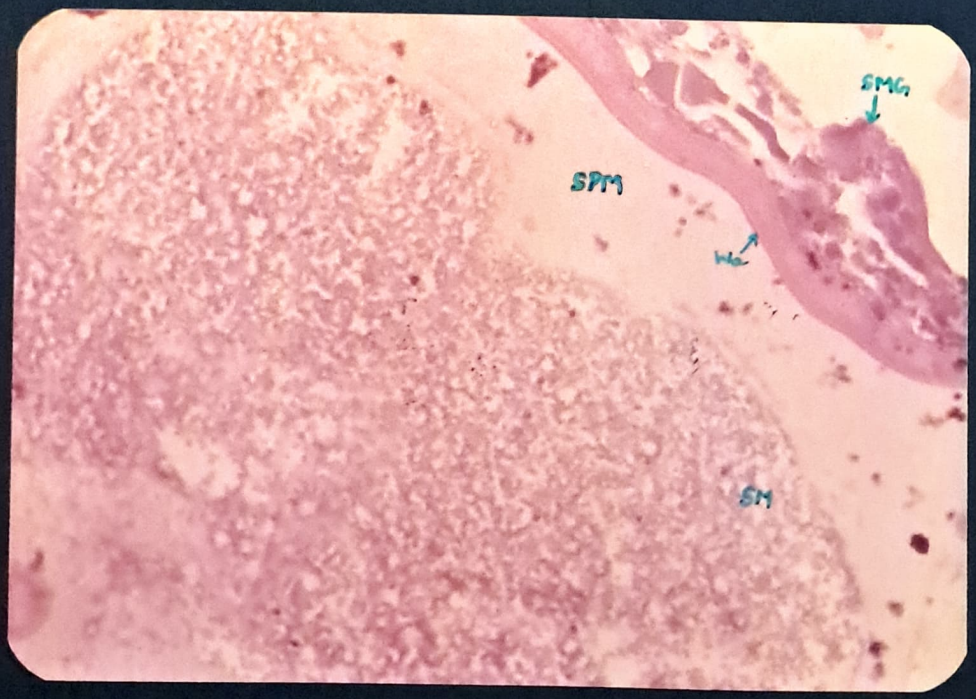


Fig. 10.

Section through extruded spermatophore, showing attachment of wing (Wg) to wall (Wa) of sperm bag (x 100).

Fig. 11.

Section through extruded spermatophore showing shallow cup containing sticky mass of granules (SMG) (x 100).

Fig. 10

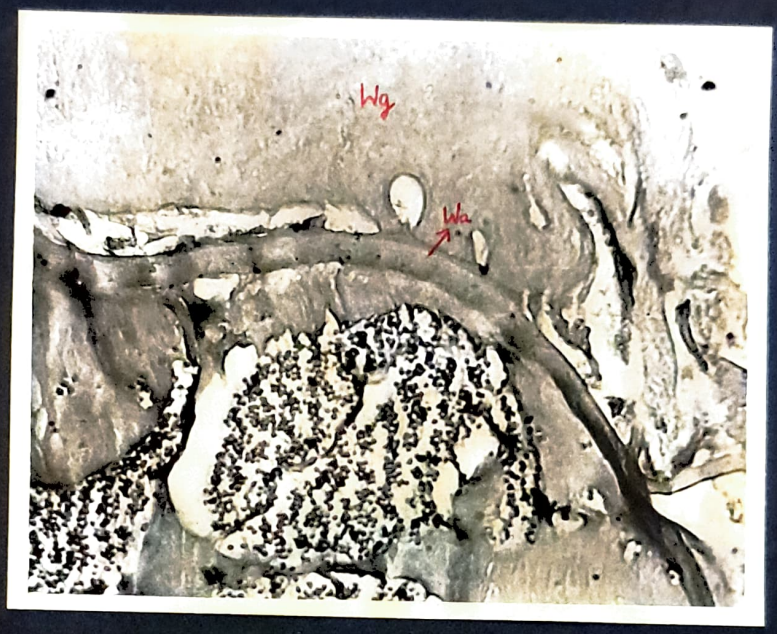


Fig. 11

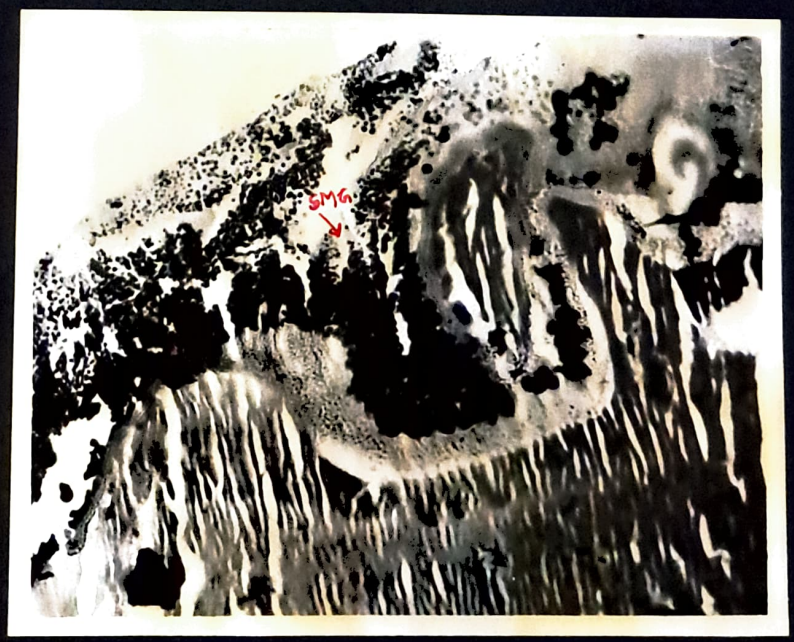


Fig. 12.

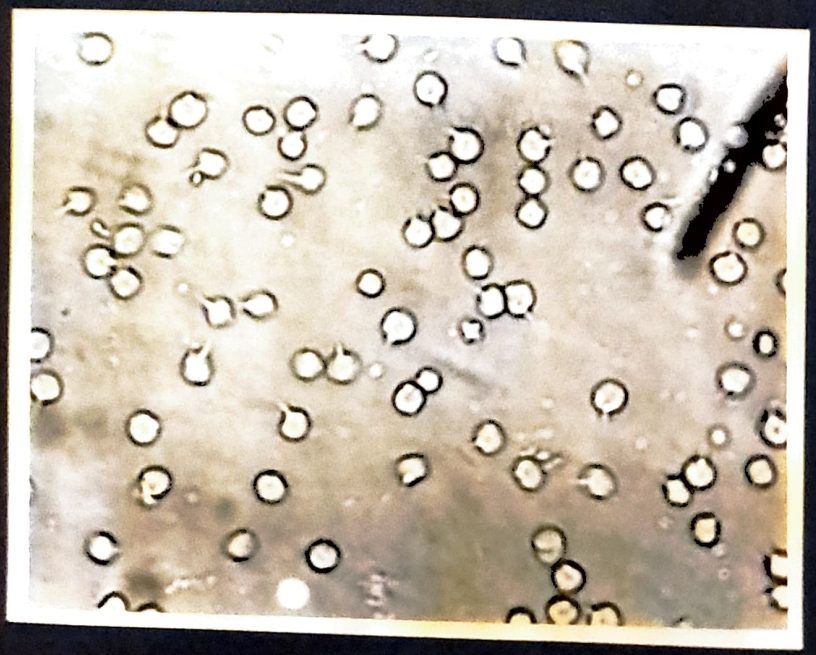
Section through extruded spermatophore showing wall (Wa) of sperm bag, portion of wing (Wg), spongy matrix (SPM) and sperm mass (SM) (x 100).

Fig. 13.

Spermatozoa freshly removed from extruded spermatophore (x 400).

Fig. 12

Fig. 13



MORPHOLOGY OF THE THELYCUM :

When the thelycum, cleared in saturated potassium hydroxide solution is viewed from the ventral side (Figs 16 & 17), one can see the small slightly conical anterior process between the 4th pair of walking legs on sternite XIII and the two lateral plates forming an almost circular area (slightly narrower anteriorly) between the 5th pair of walking legs on sternite XIV. The two lateral plates meet each other along their median margin which is reflected ventrally to form the slightly tumid lips. Anteriorly the two lips embrace the median keel arising in the middle of the anterior process. A closer examination of the lips clearly shows the presence of numerous delicate villi on their vertical medial surface. The dorsal edge of the medial wall of each lip ends in a row of stiff rounded teeth. The gap between the two rows of stiff teeth is the entrance to the single chambered seminal receptacle. The cuticle and epithelium of the lips after rounding the row of stiff teeth get reflected laterally below the lateral plates to form the floor of the seminal receptacle. After reaching the lateral edge of the thelycal region this cuticle again gets reflected dorsally and medially to form the dorsal wall of the seminal receptacle (Fig. 14). When this dorsal wall is cut away the two rows of rounded teeth are clearly visible (Fig. 18).

If the lateral plates are carefully cut away along the outer margin, a "trident" formed of two lateral horns and a median broadly conical process is seen to project posteriorly from the posterior margin of sternite XIII into the thelycum (Fig. 15). The trident is actually a posterior extension of the anterior process of the thelycum. The keel on the anterior process is continued posteriorly on the median conical process of the trident. In adult females the ventral surface of the median conical process is covered with blunt villi, broader and shorter than the villi on the medial wall of the lips. The ventral surface of the lateral horns of the trident are covered with a few minute setae.

In young females the anterior process and the trident originate as upheavals on sternite XIII which gradually grow in size to assume the definitive shape in the adult female. The lateral plates arise as posterolateral folds on sternite XIV, which grow medially and anteriorly as the female grows and finally meet each other to form the lips and also cover the anterior shoulder of the trident and a portion of the anterior process, leaving a minute opening at the level of the sternite on each side at the base of the conical anterior process. The spermatozoa appear to come out of the seminal receptacle through these openings.

The lateral plates of the thelycum are covered with a smooth cuticle having a few, very minute, denticle-like setae (Fig. 19). The cuticle covering the keel, the villi on the lips and on the ventral surface of the median conical process of the trident is very thin and in surface view appears to be raised into minute rounded papillae; some indications of cuticular pores among the papillae are also seen (Fig. 20). Numerous, distinct cuticular pores are seen along the entire length of the lips on the ventral margin (Figs. 21 & 22).

HISTOLOGY OF THE THELYCUM :

Serial transverse sections of the thelycum were examined to study the histology of the highly complex thelycum.

The epithelium of the lip villi (Fig. 31), the keel (Fig. 32), the ventral surface of the median conical process of the trident (Fig. 33), the lateral horns (Fig. 34) and of stiff rounded teeth (Fig. 35) is clearly specialised and appears to be highly secretory in function, as evidenced by the hypertrophied and syncitial nature of the epithelium and the large size of the nuclei. The normal (non-secretory) epithelium of the thelycum seen on the outer wall of the lips (Fig. 31), the outer wall of the lateral plates (Fig. 36) the dorsal wall of the conical median process of the trident (Fig. 37) and the roof of the seminal receptacle (Fig. 36) has small cuboidal cells with normal nuclei.

The epithelium of the villi and the keel practically occupies the entire space inside them, leaving only a narrow lumen between the two epithelial layers (Figs. 31 &). The nuclei are large and rounded, occupying a major portion of the cell volume (Fig. 23). The hypertrophied epithelia of the lateral horn and the stiff rounded teeth, resemble each other closely and have closely packed, elongated nuclei (Fig. 24).

The inner core of the base of the trident has loose connective tissue containing granules staining blue with Polychrome (Figs. 25 & 38). A different type of granules which appear to be enclosed inside a follicle are found at the base of the stiff rounded teeth (Figs. 26 & 27).

The spaces between the loose connective tissue of the lips appear to be occupied by a mucilagenous substance that stains purple with Polychrome (Figs. 28 & 31).

In impregnated females, prominent, deep staining oval patches are seen inside the lip (Fig. 39) and at the base of the keel (Fig. 32). Whether they are sub-epithelial tegumental glands or accumulations of secretory material produced by the epithelial cells, is not clear. They stain deep purple with Polychrome.

The cuticle covering the villi and keel is very thin and crenulated and stains blue with Polychrome (Figs. 31 & 32). In marked contrast to this, the cuticle of the lateral horns and the stiff rounded teeth are thick, a thin inner layer adjacent to the epithelium staining deep blue and the thicker outer layer staining light purple with Polychrome (Figs. 34 & 35). The cuticle of the ventral and dorsal walls of the seminal receptacle (Fig. 36) and the dorsal wall of the median conical process of the trident (Fig. 37) are thicker and distinctly lamellar, staining blue with Polychrome.

Broken bits of the spermatophores are found inside the seminal receptacle of the impregnated females (Figs. 40, 41, 42 & 43). The spermatophore wall, spongy matrix and the sperm mass are clearly visible in these sections. Whether the spermatophores are broken at the time of section cutting or whether they were already broken inside the thelycum before it was fixed, is not clear. While the cuticle lining the thelycum stains blue, the chitinous wall of the spermatophore stains red with Polychrome.

In juvenile females the villi on the lips are few and short (Figs. 29 & 30) while in the adult, impregnated ones the villi are numerous and long.

HISTOCHEMISTRY OF THE THELYCUM :

The results of the histochemical tests performed on the transverse sections of the thelycum are summarised in Table II (a, b & c).

The cuticle and epidermis of the lip region, trident and lateral plates are positive to basic proteins containing amino, acidic and tyrosyl groups. The staining for the basic proteins is most intense in the lip region, lesser in the trident and least in the lateral plates.

The tests for carbohydrates revealed that these regions are positive to acid mucopolysaccharides and that the AMPs are carboxylated. The intense blue colour of the epidermis with Best's Carmine is indicative of active nuclei. PAS positivity even after deacetylation was more intense in the lip region than in the other regions, indicating the concentration of AMPs in this region. The cuticle and epidermis of all the regions was positive to chitosan test.

The lipid tests were negative in all the regions.

The dark staining patches in the lips and at the base of the keel in impregnated females appear to be almost pure carboxylated acid mucopolysaccharides, reacting very intensely to PAS, Best's Carmine, TB in high pH and low CEC, and very weakly to protein tests and not at all to lipid tests.

Table - II (a)

P.indicus - Histochemical characterization of Thelycum :

Proteins

S. No.	Histochemical test	Cuticle			Epidermis			Dark staining areas in the lip
		Lips	Keel	Lateral horns	Lips	Keel	Lateral horns	
1	2	3	4	5	6	7	8	9
1.	Mercurie Bromophenol Blue test	+++ DB	++ B	++ B	+++ DB	++ B	+ B	±
2.	a) Aqueous Bromophenol Blue Test (ABB)	+++ B	++ B	++ B	+++ B	++ B	+ B	±
	b) ABB after deamination	-	-	-	-	-	-	-
3.	a) Ninhydrin-Schiff Test (NS)	++ M	++ M	++ M	++ M	++ M	++ M	±
	b) NS after deamination	-	-	-	-	-	-	-
4.	a) Toluidine Blue test (TB)	+ B	+ B	+ B	+ B	+ B	+ B	+++ DB
	b) TB after methylation	-	-	-	-	-	-	-
5.	a) Ferric-Ferricyanid test (FF)	-	-	-	-	-	-	-
	b) FF after mercaptide	-	-	-	-	-	-	-
6.	a) Thioglycollate Ferric-ferricyanide test (TFF)	-	-	-	-	-	-	-
	b) TFF after thioglycollate reduction	-	-	-	-	-	-	-

contd..

Table-II (a). contd..

1	2	3	4	5	6	7	8	9
7.	a) Millon's test (MT)	+ R	+ R	+ R	+ R	+ R	+ R	+ R
	b) MT after iodination	-	-	-	-	-	-	-
8.	a) DMAB - nitrite method	-	-	-	-	-	-	-
	b) DMAB - nitrite after formaldehyde	-	-	-	-	-	-	-

DB	-	Deep Blue
B	-	Blue
M	-	Magenta
R	-	Red
+	-	Moderately positive
++	-	Positive
+++	-	Intensely positive
-	-	Negative

Table - II (b)

P.indicus - Histochemical characterization of thelycum :

Carbohydrates

S. No.	Histochemical test	Cuticle			Epidermis			Dark staining areas in the lip
		Lips	Keel	Lateral horns	Lips	Keel	Lateral horns	
1	2	3	4	5	6	7	8	9
1. a)	Periodic Acid-Schiff technique (PAS)	++ DM	++ DM	++ DM	++ DM	++ DM	++ DM	++ PR
b)	PAS after acetylation	-	-	-	-	-	-	-
c)	PAS after deacetylation	++ M	+ M	++ M	+ M	+ M	+ M	+ M
2. a)	Best's carmine test (BC)	++ B	++ B	++ B	+++ B	+++ B	+++ B	+++ DB
b)	BC after Taka diastase	-	-	-	-	-	-	-
3.	Toluidine Blue Test at							
	pH - 1	-	-	-	-	-	-	-
	pH - 3	+	+	+	+	+	+	+
	pH - 4	++	++	++	++	++	++	++
	pH - 7	+++ B	+++ B	+++ B	+++ B	+++ B	+++ B	++ P
4.	Critical Electrolyte concentration (CEC)							
	0.1 M	+++ B	+++ B	+++ B	+++ B	+++ B	+++ B	+++ B
	0.2 M	++	++	++	++	++	++	+
	0.6 M	+	+	+	+	+	+	+
	0.8 M	-	-	-	-	-	-	+
	1.0 M	-	-	-	-	-	-	-

contd...

Table-II (b) contd.

1	2	3	4	5	6	7	8	9
5.	Bracco-cruti's method	±	±	±	±	±	±	±
6.	Alcian Blue-PAS test (AB-PAS)	++ B	++ B	++ B	++ P	++ P	++ P	++ P
7.	Chitosan test	++ P	++ P	++ P	++ P	++ P	++ P	+ P
8.	Pyronin G Test	+++ R	+++ R	+++ R	+++ R	+++ R	+++ R	+++ R

DM - Deep Magenta

PR - Purplish Red

M - Magenta

DB - Dark Blue

P - Purple

B - Blue

R - Red

\+ - Moderately positive

++ - Positive

+++ - Intensely positive

\- - Negative

± - Doubtful

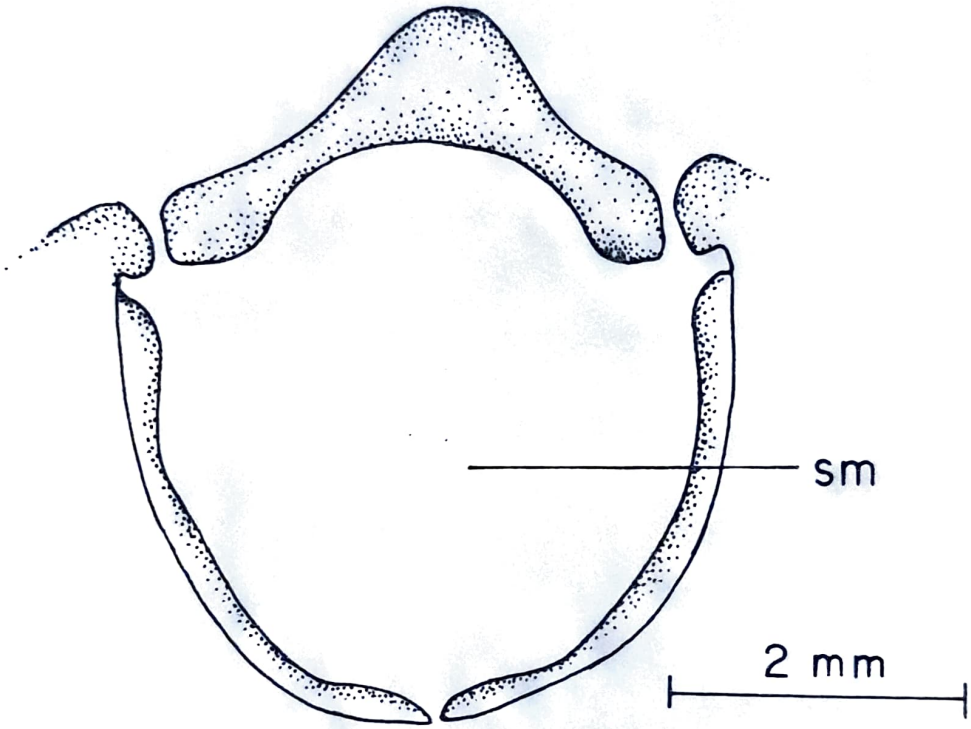
Table - II (c)

P.indicus - Histochemical characterisation of thelycum :

Lipids

S. No.	Histochemical test	Cuticle			Epidermis			Dark staining areas in the lip
		Lips	Keel	Lateral horns	Lips	Keel	Lateral horns	
1. a)	Sudan Black B test (SBB)	-	-	-	-	-	-	-
b)	SBB after chloroform methanol extraction	-	-	-	-	-	-	-
2. a)	Nile Blue method (NB)	-	-	-	-	-	-	-
b)	NB method after chrolo-form-methanol extraction	-	-	-	-	-	-	-
3. a)	Nile Blue Sulphate method (NBS)	-	-	-	-	-	-	-
b)	NBS after chloroform methanol extraction	-	-	-	-	-	-	-
4. a)	Oil Red 'O' method	-	-	-	-	-	-	-
b)	Oil Red 'O' after chloroform methanol extraction	-	-	-	-	-	-	-
5. a)	UV-Schiff reaction	-	-	-	-	-	-	-
b)	UV-Schiff after pyridine extraction	-	-	-	-	-	-	-
6. a)	Sudan Black 'B' (SBB) method	-	-	-	-	-	-	-
b)	SBB after pyridine extraction	-	-	-	-	-	-	-

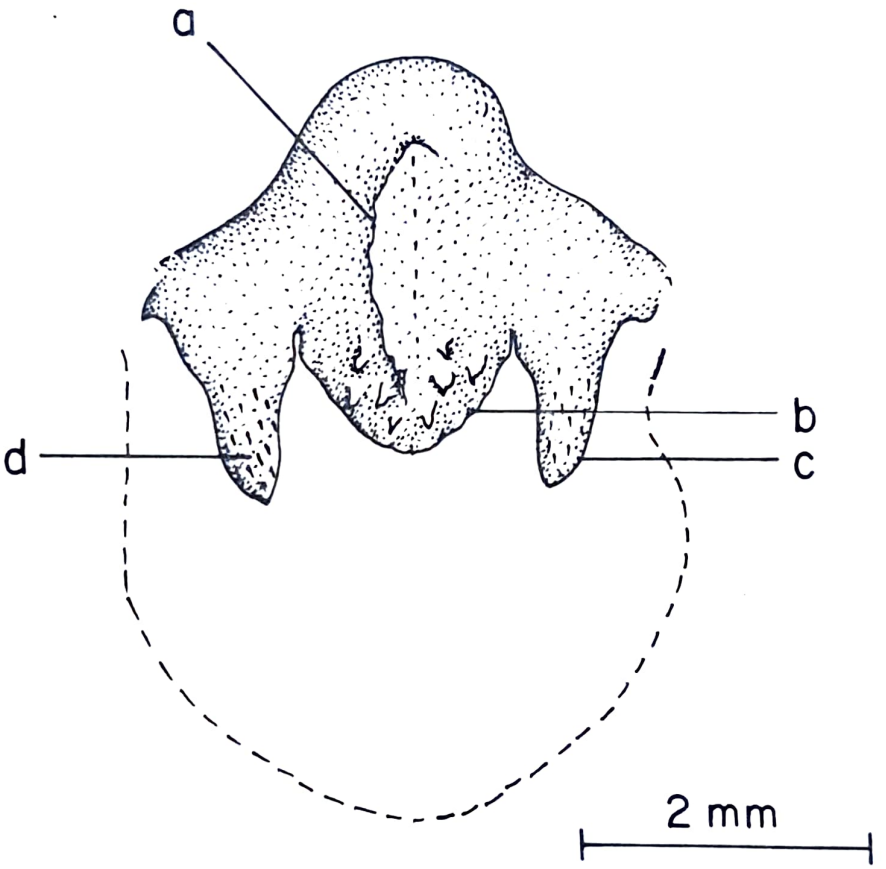
Dorsal view of thelycum



sm- Seminal receptacle

Fig: 14.

Thelycum – Trident



a - Keel

b - Conical median process

c - Lateral horn

d - Short setae

Fig: 15.

Fig. 16.

Ventral view of thelycum showing anterior process (AP), lateral plates (LP) and lips (L). Note villi on the lips (x 10.8).

Fig. 17.

Ventral view of moulted thelycum. Lips slightly pulled apart to expose keel and villi on median conical process. Lateral horns (LH) clearly visible through lateral plates (LP). AP = Anterior process (x 10.8).

Fig. 16

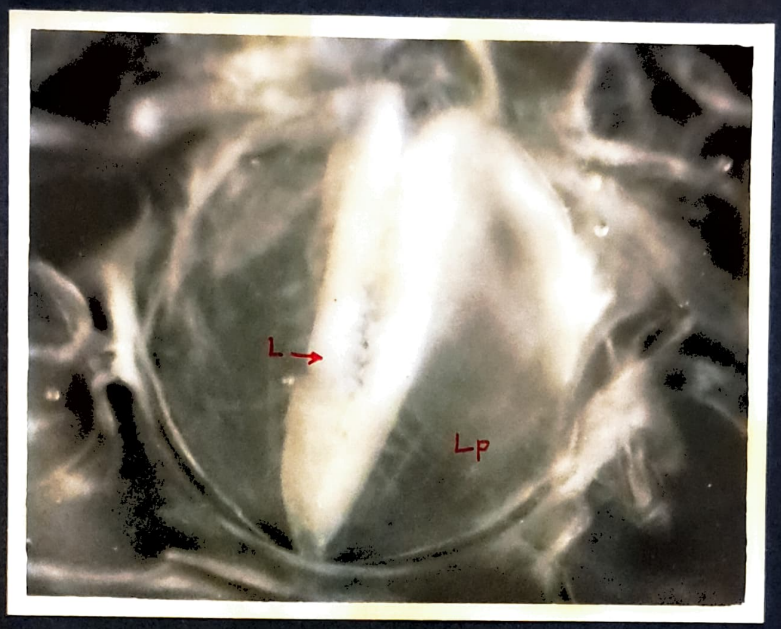


Fig. 17

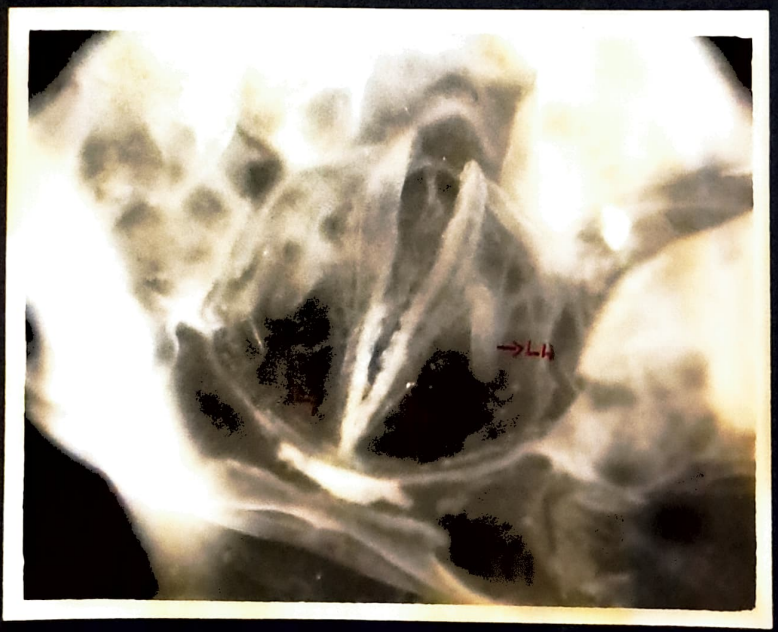


Fig. 18.

Dorsal view of thelycum with dorsal wall of seminal receptacle removed to expose two rows of stiff rounded teeth (RT) and median conical process (MCP) of trident (x 10.8).

Fig. 19.

Surface view of cuticle covering lateral plate, showing minute setae (x 200).

Fig. 18

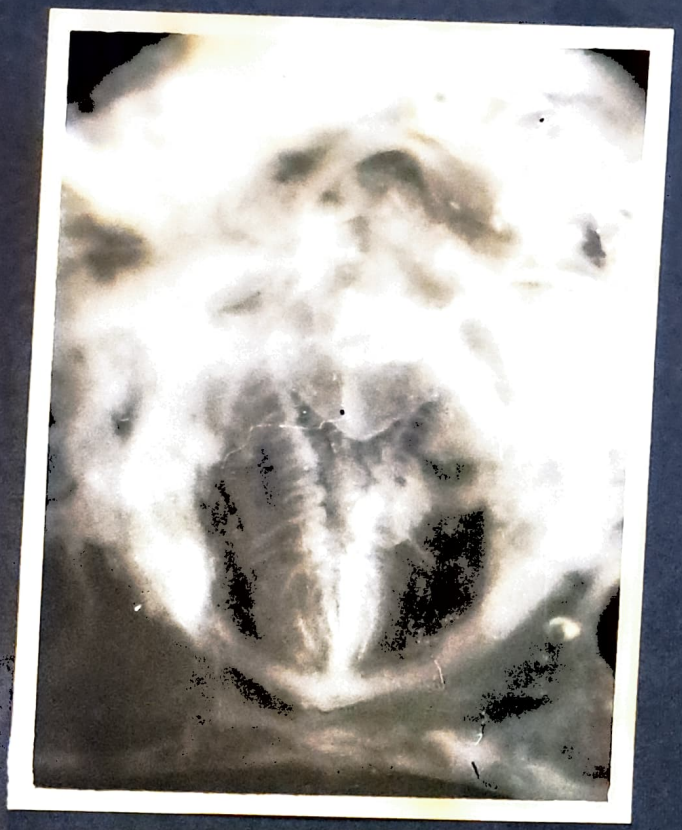


Fig. 19

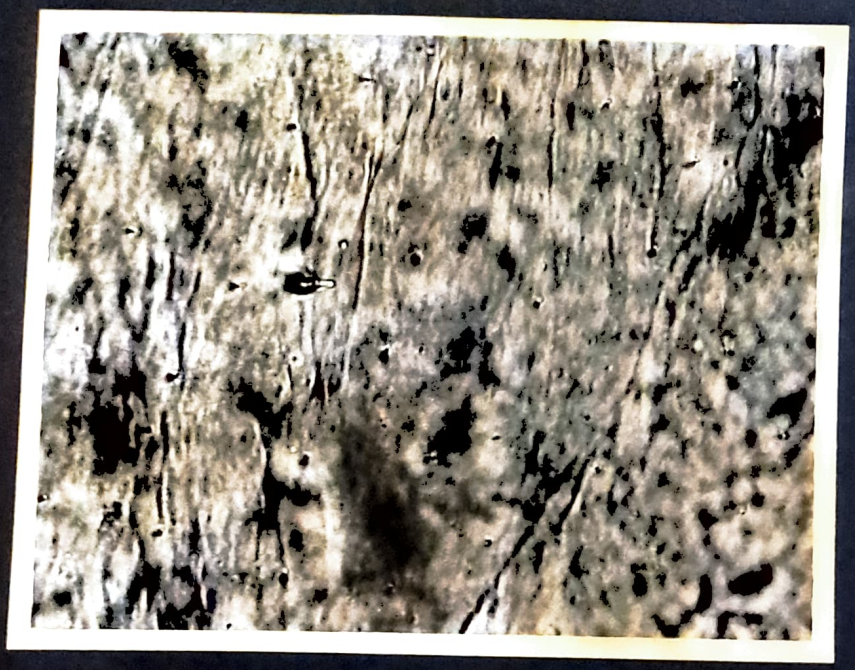


Fig. 20.

Surface view of cuticle over lip villi. Note small rounded papillae and indication of pores in centre of photograph (x 400).

Fig. 21.

Cuticular pores on cuticle covering ventral margin of lips; Epithelium removed (x 400).

Fig. 20

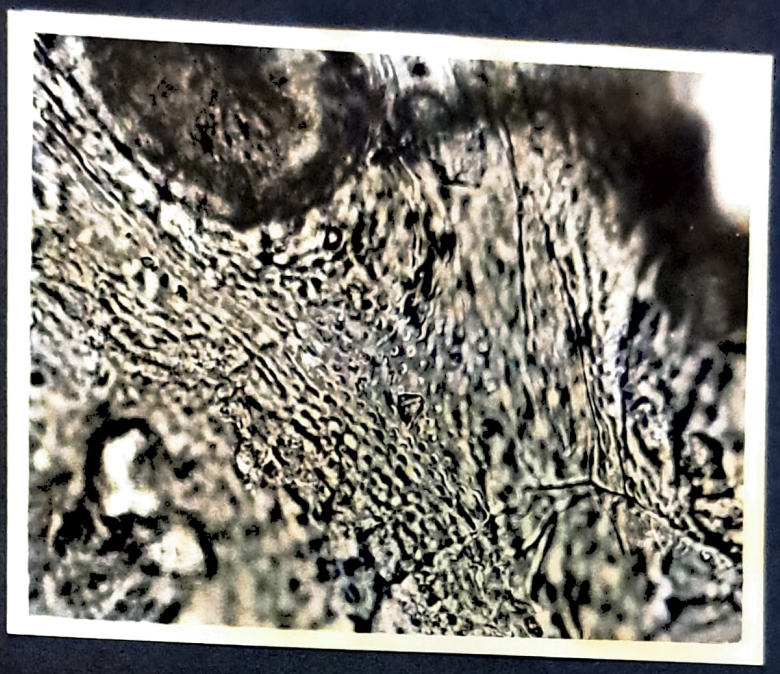


Fig. 21

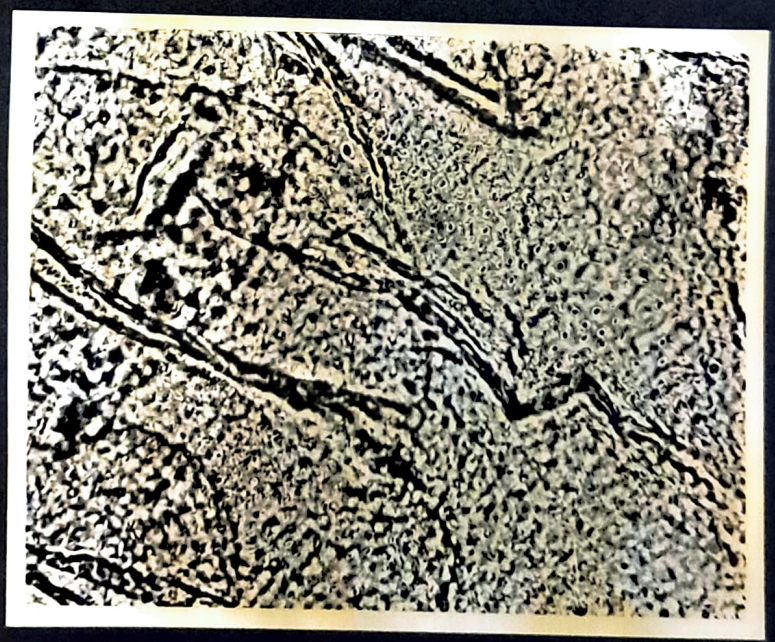


Fig. 22.

Cuticular pores on cuticle covering ventral margin of lips. Epithelium intact (x 400).

Fig. 23.

Section of villi on the lips. Note crenulated cuticle and large rounded nuclei of secretory epithelium (x 400).

Fig. 22

Fig. 23

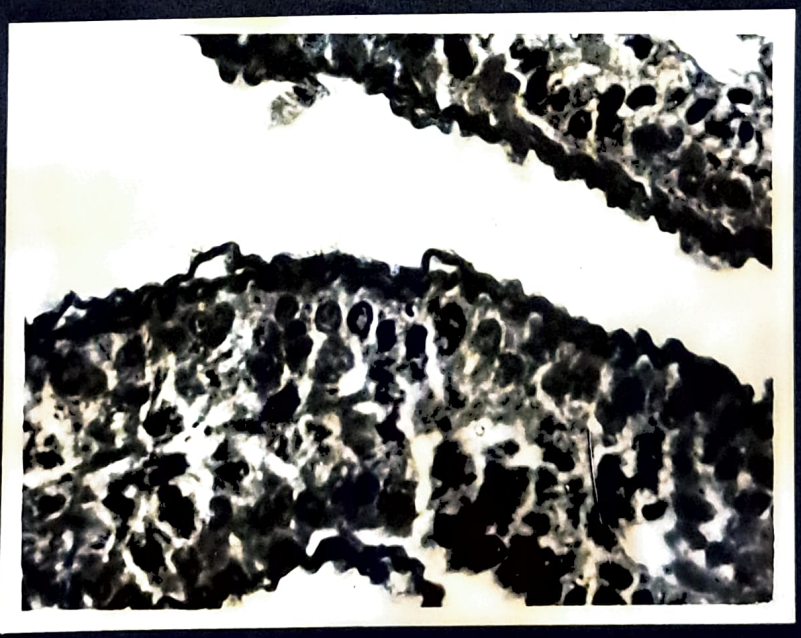


Fig. 24.

Section of stiff rounded tooth. Note elongated rod-like nuclei of secretory epithelium (x 400).

Fig. 25.

Section through base of trident showing rounded granules (G) in connective tissue (x 100).

Fig. 24

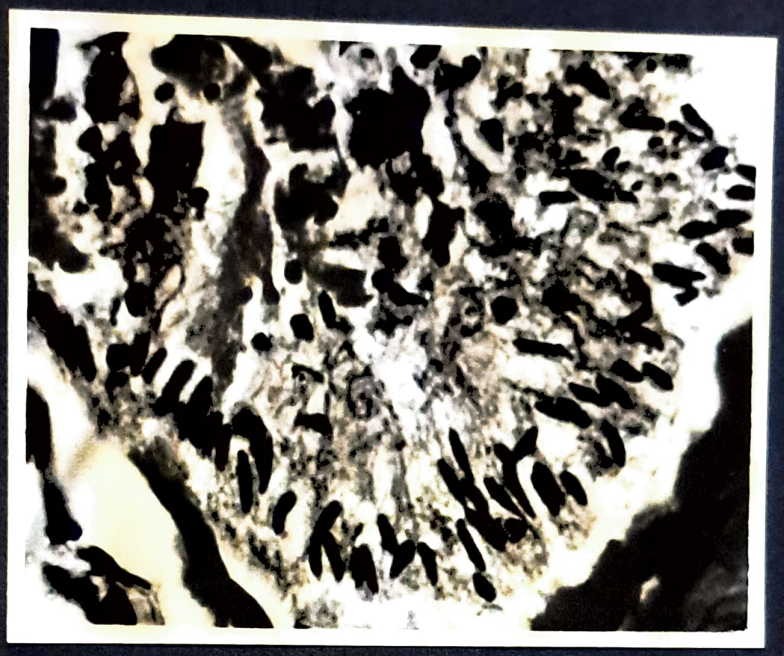


Fig. 25

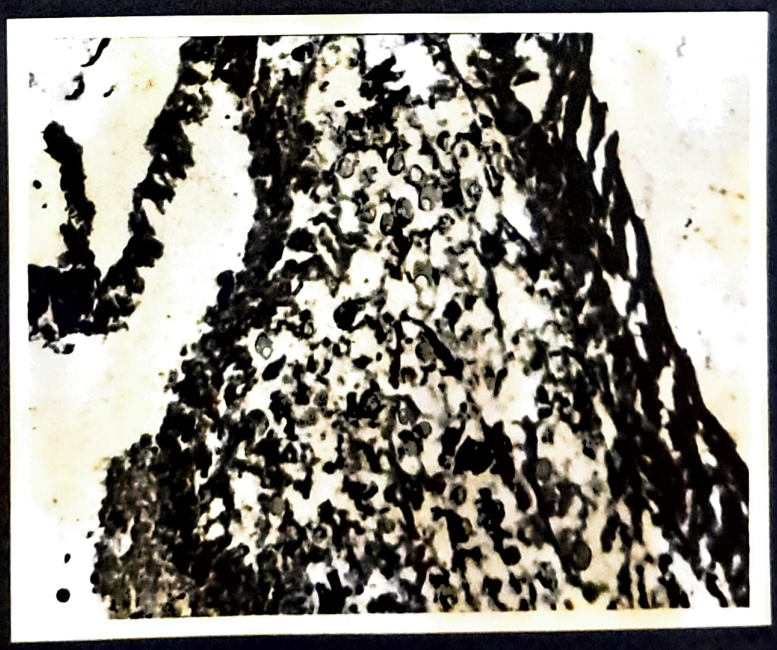


Fig. 26.

Section through stiff rounded tooth showing secretory epithelium (EP) of tooth, connective tissue (CT) and round granules (G) at the base (x 100).

Fig. 27.

Enlargement of round granule lodged inside a follicle (F) (x 400).

Fig. 26

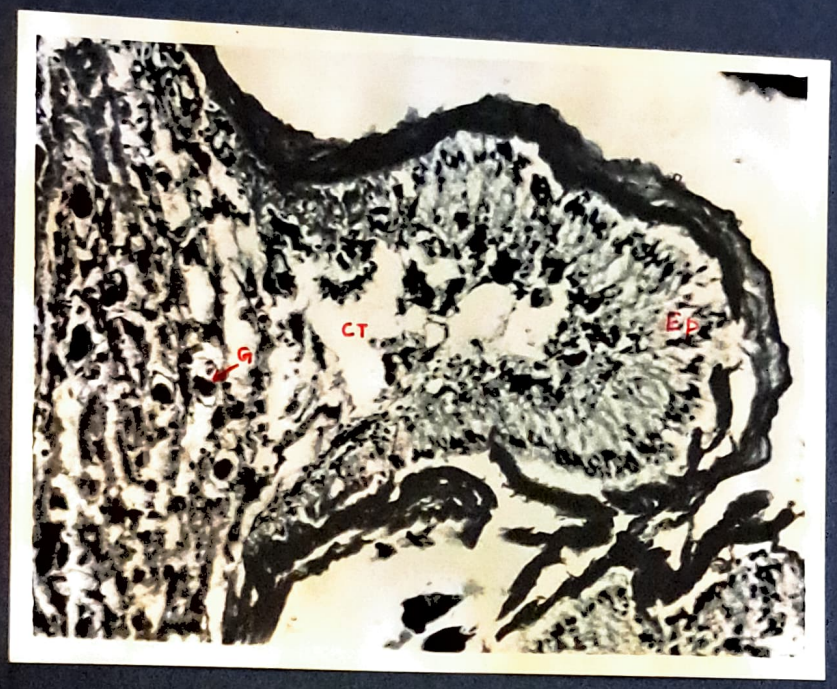


Fig. 27

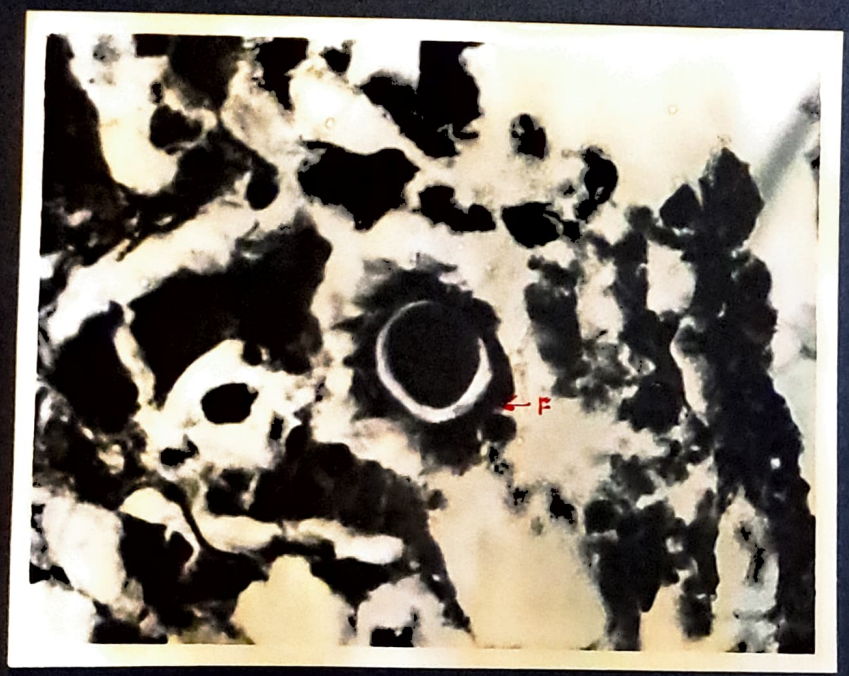


Fig. 28.

Section through lip region showing villi (V), stiff rounded tooth (RT) and portion of lateral plate (LP). Note mucus-like substance (M) in loose connective tissue (x 100).

Fig. 29.

Section through lip (L) and keel (K) on the median conical process (MCP) of a juvenile female. Note short and few villi (V) (x 100).

Fig. 30.

Enlarged view of section through keel (K) and short villi (V) of a juvenile female (x 200).

Fig. 28

Fig. 29

Fig. 30

Fig. 31.

Section through lips showing two long villi, showing secretory epithelium (SEP) of villi and normal epithelium (EP) of outer wall of lip. CT = Connective tissue, M = Mucilagenous substance, C = Crenulated cuticle; Polychrome stain (x 100).

Fig. 32.

Section through keel showing secretory epithelium (SEP), thin crenulated cuticle and dark staining patch (DSP) at base of keel; Polychrome stain (x 100).

Fig. 31

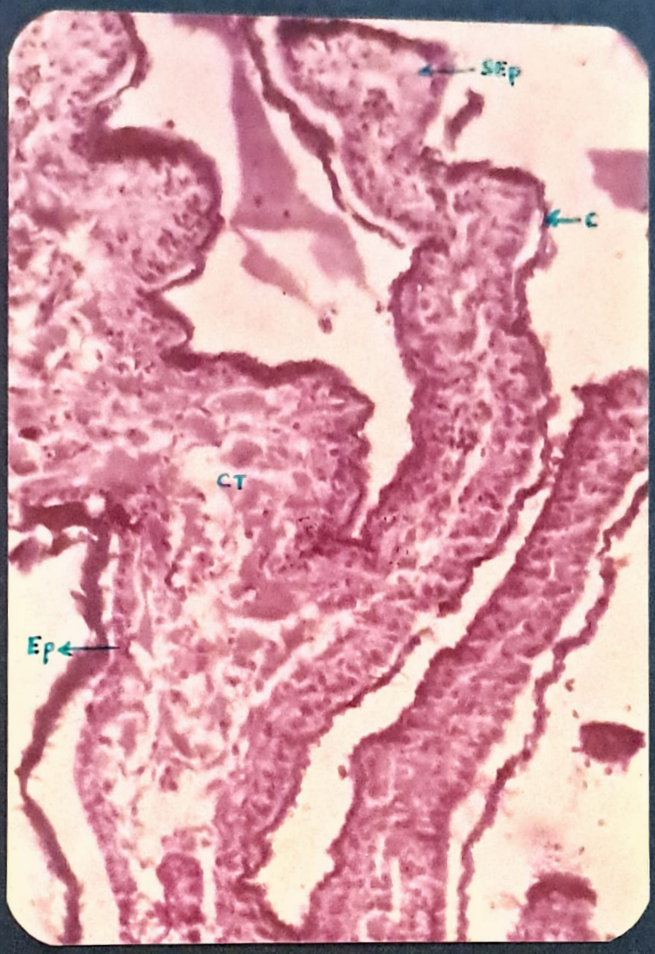


Fig. 32

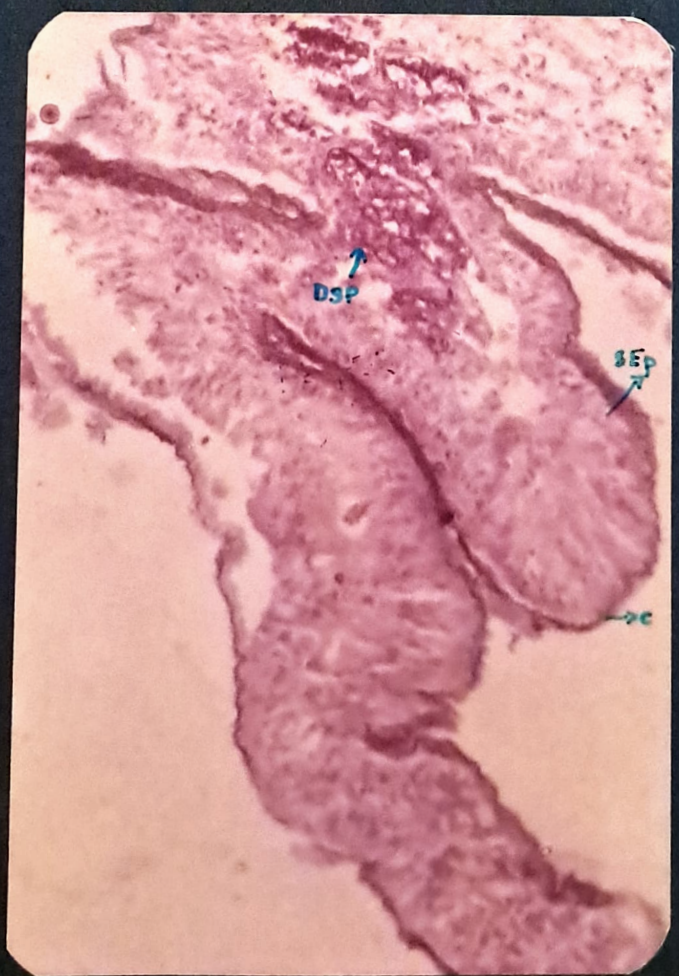


Fig. 33.

Section through base of trident and lip villi. SEP = Secretory epithelium on ventral wall of median conical process of trident. V = Villi; Haematoxylin-Eosin (x 100).

Fig. 34.

Section through lateral horn showing secretory epithelium (SEP), thick cuticle (C) and core of connective tissue (CT); Polychrome stain (x 100).

Fig. 33

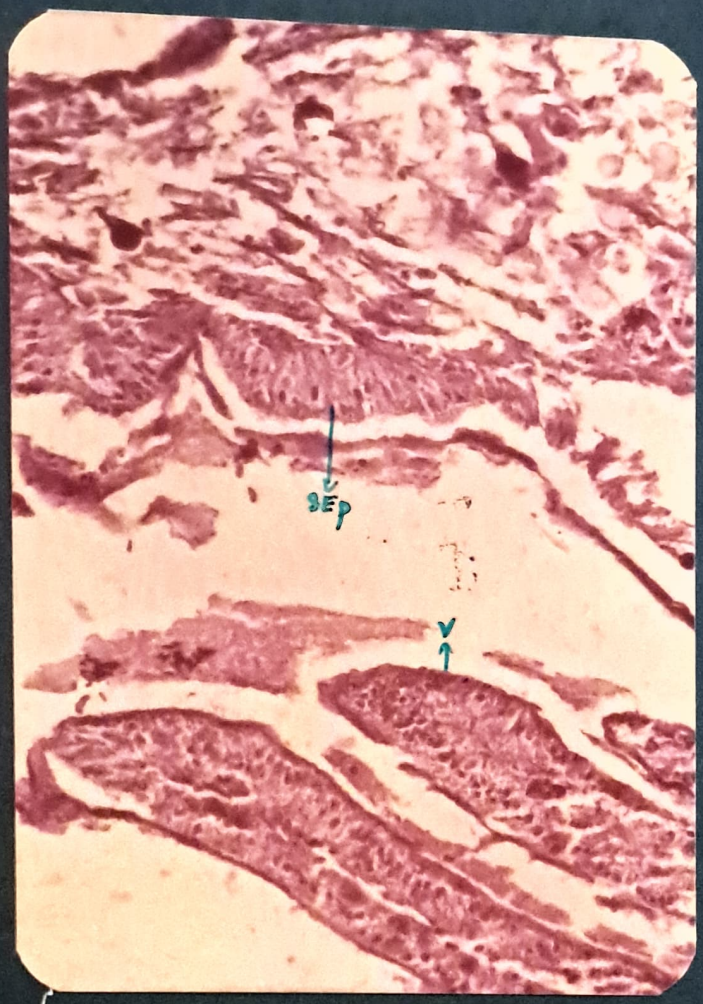


Fig. 34

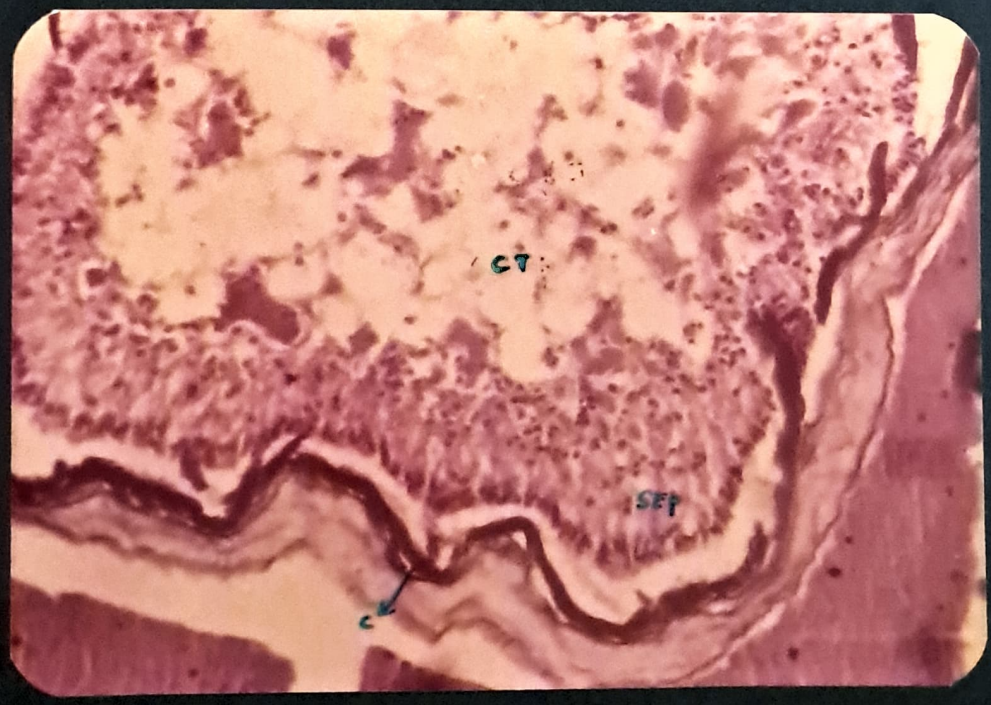


Fig. 35.

Section through stiff rounded tooth showing secretory epithelium (SEP), thick cuticle (C) and connective tissue at the base (CT); Polychrome stain (x 100).

Fig. 36.

Section through posterior region of thelycum showing dorsal wall (DW) and ventral wall (VW) of seminal receptacle (SR), loose connective tissue in lateral plate (CT) and outer wall of lateral plate (OW); Polychrome stain (x 100).

Fig. 35

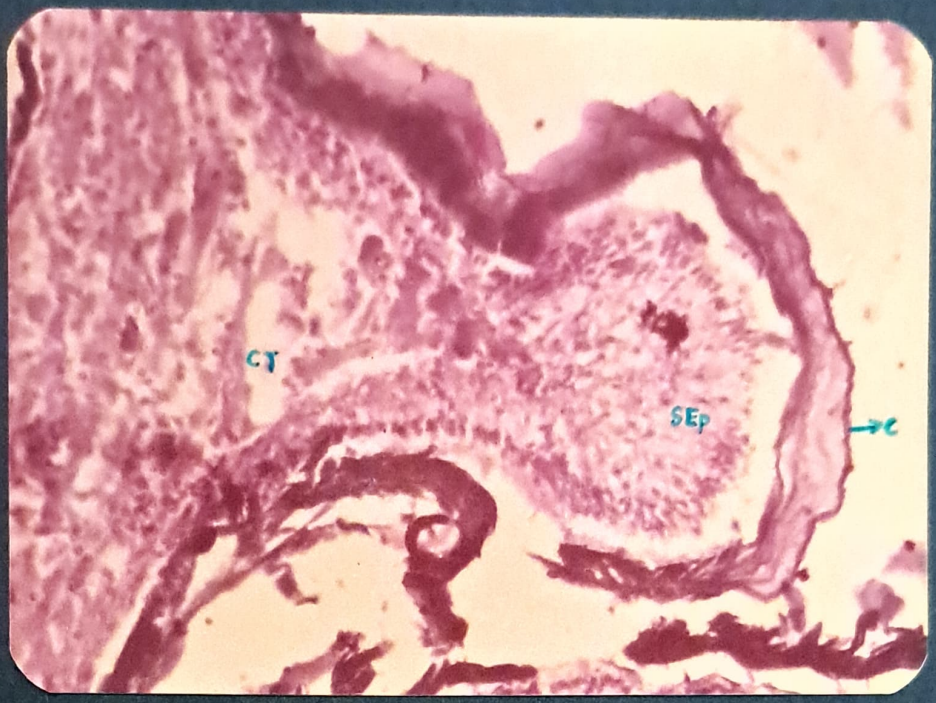


Fig. 36

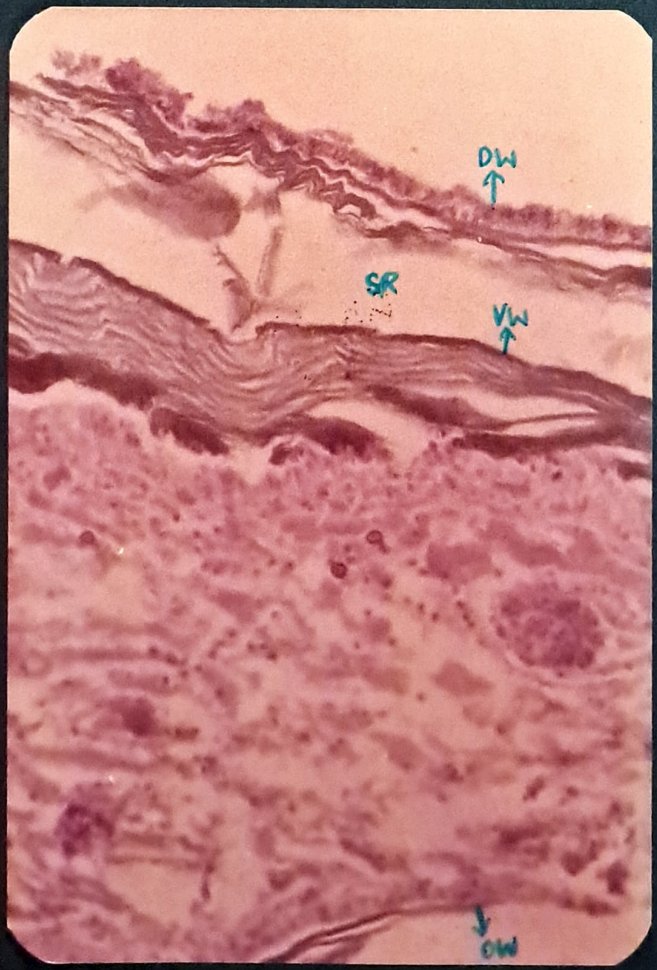


Fig. 37.

Section through base of keel showing laminated cuticle (LC) covering dorsal wall of conical median process and dark staining patches (DSP); Polychrome stain (x 100).

Fig. 38.

Section through base of trident, showing blue staining granules in loose connective tissue; Polychrome stain (x 100).

Fig. 37

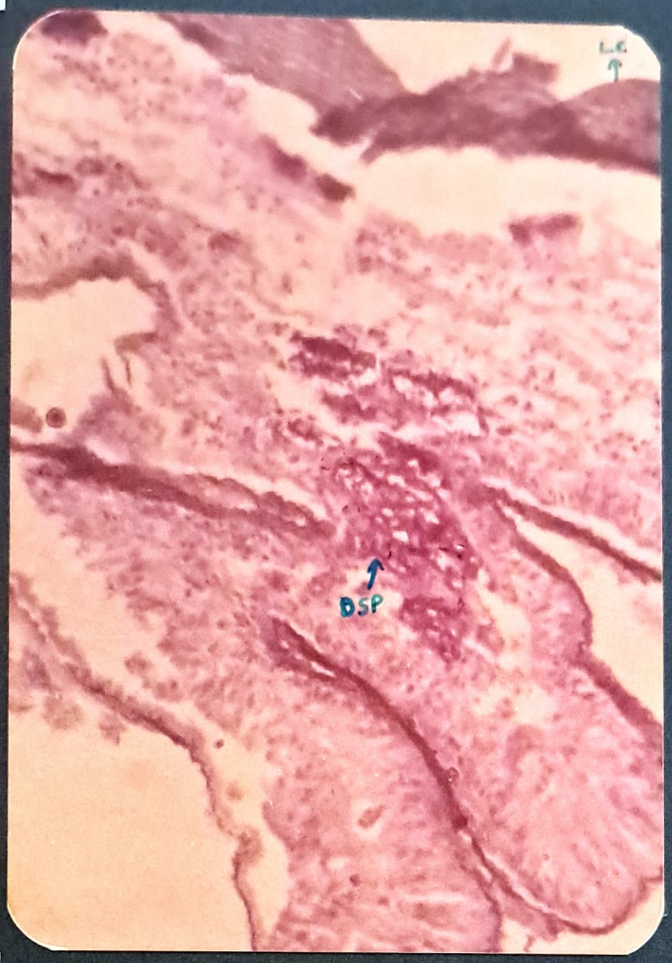


Fig. 38

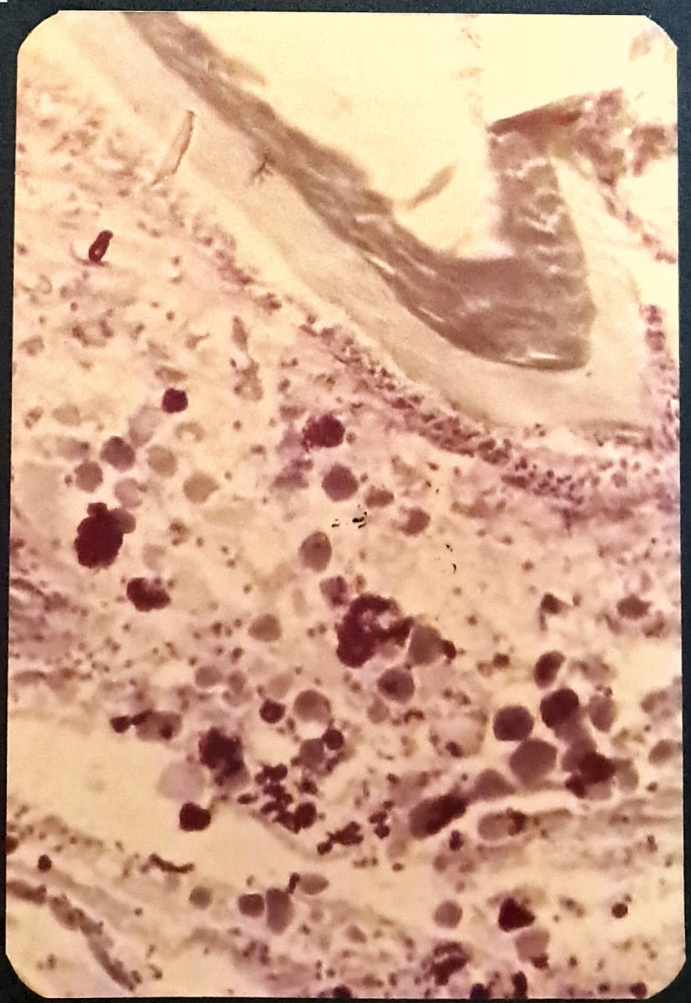


Fig. 39.

Section through base of lip and portion of lateral plate. Showing deep staining patches (DSP) inside lip, loose connective tissue (CT) and outer wall (OW) of lateral plate; Polychrome stain (x 100).

Fig. 40.

Section through impregnated thelycum showing spermatophore wall (Wa), spongy matrix (SPM), and sperm mass (SM) of stored spermatophore; Polychrome stain (x 100).

Fig. 39

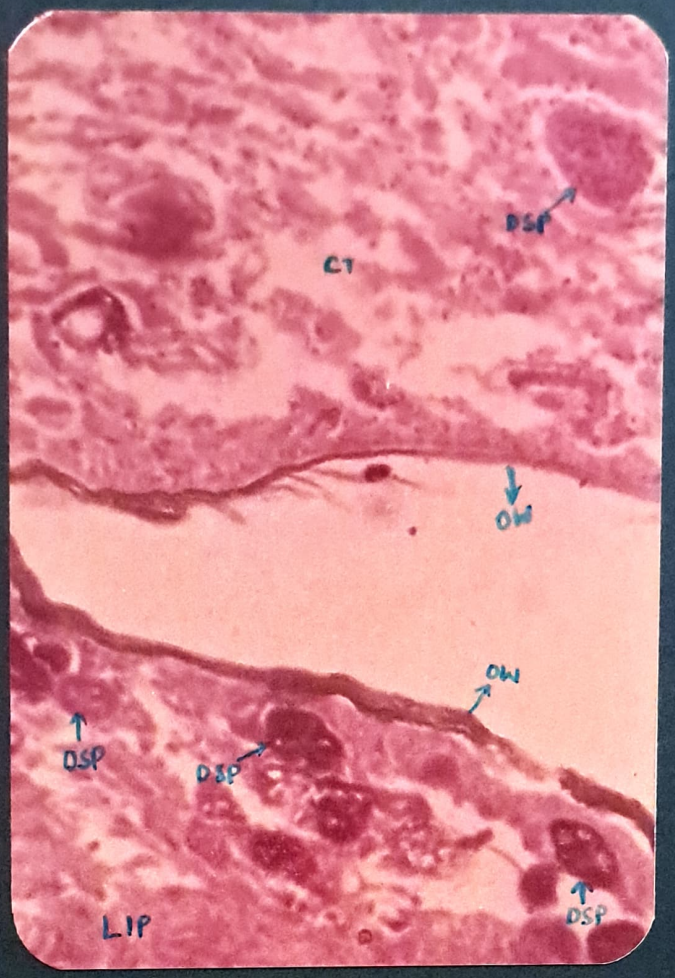


Fig. 40

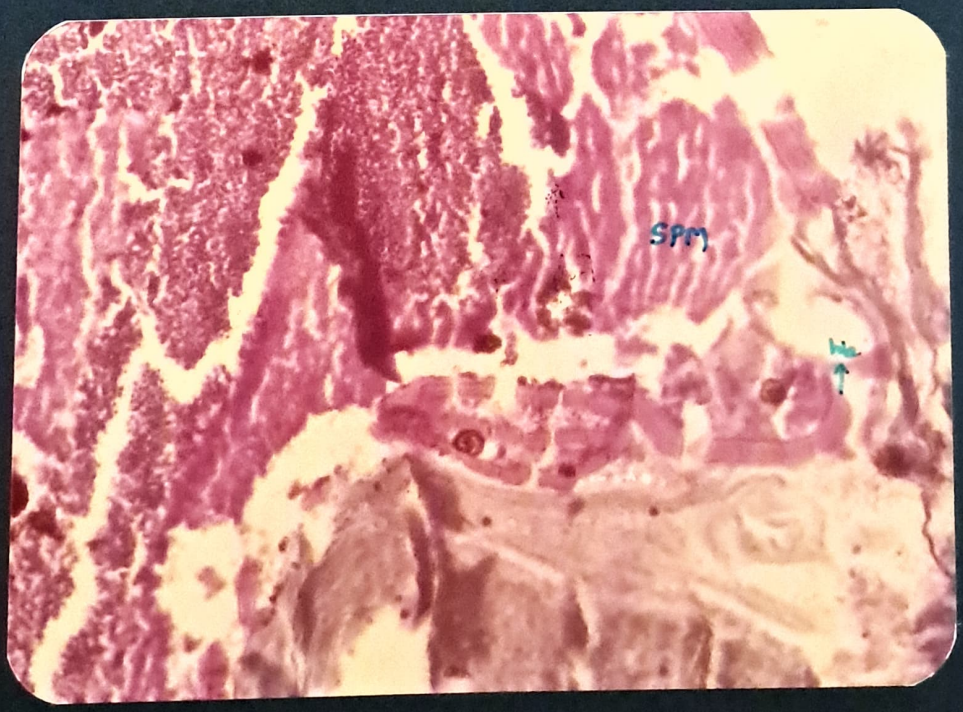


Fig. 41.

Section through impregnated female showing spermatophore wall (Wa) and sperm mass (SM) of stored spermatophore; Polychrome stain (x 100).

Fig. 42.

Section through impregnated thelycum showing spermatophore wall (Wa) and sperm mass (SM) of stored spermatophore; Polychrome stain (x 100).

Fig. 41

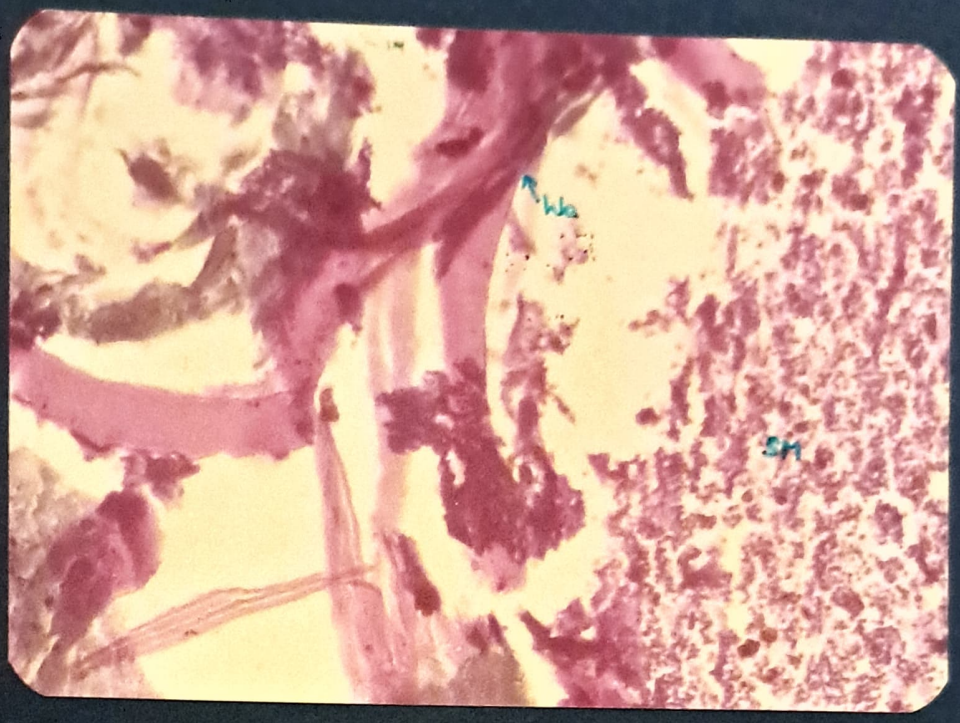


Fig. 42

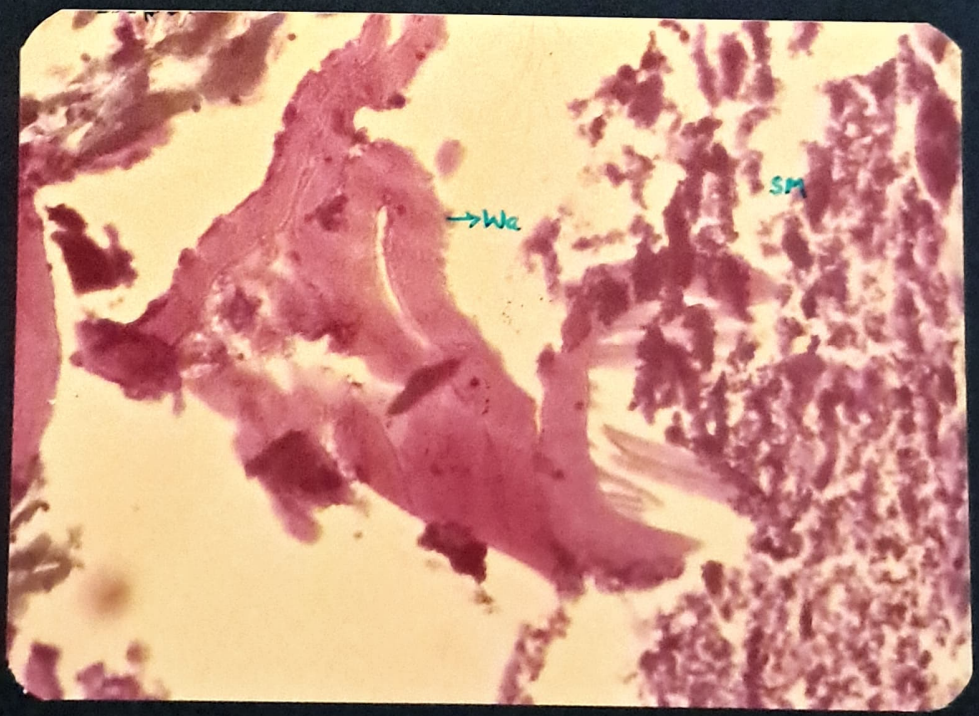
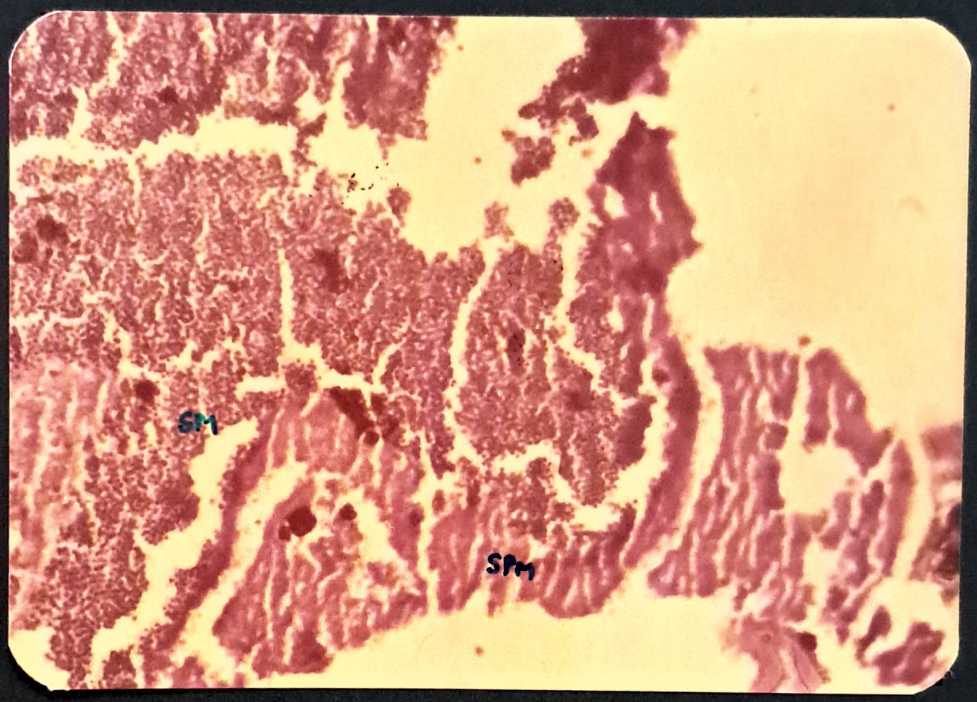


Fig. 43.

Section through impregnated thelycum showing spongy matrix (SPM) and sperm mass (SM) of stored spermatophore; Polychrome stain (x 100).

Fig. 43



D I S C U S S I O N

This is the first time that the spermatophore and thelycum of *Penaeus indicus* has been studied from the point of view of morphology, histology and histochemistry. The spermatophore structure of *P.indicus* does not fit into any of the three types or categories into which the decapod spermatophores have been classified by Dudenhausen and Talbot (1983). It is neither vesicular, pedunculate nor tubular. Although the sperms are packed inside a sperm bag as in brachyuran crabs, the bag is large and chitinous and has a chitinous wing in *P.indicus* while in the brachyuran *Scylla serrate*, sperm sacs are minute, with non-chitinous wall and float in the seminal plasma (Uma & Subramoniam 1979). This difference in structure is clearly associated with the difference in the reproductive strategies adopted by *P.indicus* and *S.serrata*. The spermatophores are introduced into the oviduct of the female during mating and stored safely inside the body in an enlarged portion of the oviduct in *S.serrata* while in *P.indicus* the spermatophores are lodged inside the seminal receptacle of the thelycum which is practically external to the body and hence need greater protection from the influence of the environment. The chitinous wall of the sperm bag in *P.indicus* may be the answer to this problem. The sand crabs *Emerita asiatica*, *Albunea symnista* and the panulirid lobster *Panulirus homarus* which deposit the spermatophores epizoically on

the body of the female protect the sperms from the harmful influences of the environment by enclosing them in a non-chitinous, membranous tube of neutral mucopolysaccharide and embedding the convoluted spermatophore tube in a thick gelatinous matrix of acid mucopolysaccharides which may harden into a hard substance in lobsters and Albunea symnista or remain gelatinous in Emerita asiatica (Subramonium, 1985). In P.indicus the spermatophore is not embedded in a gelatinous matrix of AMP for protection, but the wall of the sperm bag itself appears to be made up of AMP and stabilised by chitin. The presence of tyrosine containing protein in the wall of the sperm bag suggests the posibility of phenolic tanning to further strengthen the wall. But since the occurrence of phenolase in the wall was not tested for during the present study, this cannot be confirmed. However, Malek and Bawab (1971) have reported phenolic tanning in the spermatophore of Penaeus kerathurus. Chitin has been reported in the spermatophore of Penaeus setiferus also (King, 1948). These findings suggest that the spermatophore wall of Penaeus is similar to the cuticle of crustaceans. But the wall of the sperm bag in P.indicus stains red while the cuticle of the thelycum of this prawn stains blue with Polychrome. A further difference between the two structures is that the wall of the sperm bag is homogenous without any lamellations while the cuticle is lamellar (except in the lip region).

The sperm bag of P.indicus has a sticky mass of granules attached externally to the anteromedial region. The substance appears to be a sulphated AMP which may be useful for cementing the two spermatophores into a single mass as they come out of the male genital opening at the base of the 5th, walking leg so that they can be easily handled by the male at the time of transferring the spermatophores into the thelycum of the female.

Inside the sperm bag the sperm mass is embedded in a spongy matrix which presents a lamellar network pattern in paraffin sections. The material of the spongy matrix is positive to histochemical tests for carboxylated AMP. The sperm mass itself is intensely positive to carboxylated AMP. The abundant presence of mucopolysaccharides complexed with proteins has been clearly demonstrated in the spermatophoric ribbon of the sand crabs and the spermatophoric mass of rock lobsters by Subramoniam (1984) and Radha and Subramoniam (1985) respectively. The latter authors, using polyacrylamide and agarose gel electrophoresis have isolated a AMP fraction which has been positively identified as chondroitin sulphate. They suggest that the chondroitin sulphate may have a dominant role in the hardening of the lobster spermatophore after it is attached to the sternum of the female, as well as in maintaining a micro-environment within the spermatophoric tube for prolonged sperm survival.

The function of the carboxylated AMP in the spongy matrix and sperm mass inside the sperm bag of P.indicus is not known. Carboxylated AMP has been reported in the gelatinous matrix of the spermatophoric ribbon of Emerita asiatica which does not undergo hardening on exposure to sea water. A protective anti-microbial defense function has been suggested for the AMPs (Pigman and Horton 1970). It is likely that the spongy matrix physically protects the sperm mass from the environments and that the carboxylated AMP may prevent microbial damage to the spermatozoa.

The intense PAS positivity of the sperm may be indicative of the presence of glycogen in the spermatozoa. Glycogen has been demonstrated in the spermatozoa of Scylla serrata by Uma and Subramoniam (1979) and in the sperm mass of the sand crabs by Subramoniam (1984) who suggest that the crustacean spermatozoa are provided with a glycogen store for endogenous energy metabolism, and that they do not depend upon the spermatophoric components for their nutrition.

A perplexing feature of the spermatophore of P.indicus is the intense positivity of the sperm mass to the chitosan test; the spongy matrix and sticky mass of granules are also positive to the chitosan test. Surprisingly the wall of the sperm bag and the wings are only moderately positive to chitosan. Whether the sperm mass and the spongy matrix really contain chitin will have to be further carefully investigated.

The present study has revealed the great complexity of the thelycum of P.indicus. Although Perez Farfante (1969) has illustrated the posterior horns of the median protuberance of some American species of Penaeus, the "trident" (which appears to be homologous to the "posterior horns") has been described here for the first time in P.indicus and may be present in all the Indo-pacific species of Penaeus as well. The villi on the lips and on the ventral surface of the median conical process of the trident, the row of rounded teeth at the dorsal margin of the lips guarding the opening to the seminal receptacle, the minute setae on the ventral surface of the lateral horns and the cuticular pores along the ventral margin of the lips have also been brought to light by this study. The secretory nature of the epithelium lining the villi, the keel, the ventral surface of the median conical process of the trident, the lateral horns and the rounded teeth, is evident from the hypertrophied and syncitial nature of the epithelium and the enlarged nuclei. The long villi on the lips, the keel and the short villi on the median conical process are developed, obviously for increasing the surface area of this secretory epithelium. These epithelial surfaces are associated with a very thin, crenulated cuticle, overlying them. It will be highly interesting to study this cuticle surface using SEM techniques. Even under the high power optical microscope, indications of pores are visible. Whether they are real cuticular pores or optical illusions can be verified only by scanning electron microscopy. If the pores really exist they may serve as

outlets for the epithelial secretions. However, distinct cuticular pores were noticed along the entire length of the lips on the ventral margin. They undoubtedly are the channels through which the secretions from the villi and the rounded teeth reach the external surface of the lips.

The histochemical studies suggest that the epithelial secretions are rich in carboxylated acid mucopolysaccharides. In impregnated females, transverse sections of the lips and the base of the keel reveal numerous dark staining patches which appear to be accumulations of acid mucopolysaccharides produced by the secretory epithelium or tegumental glands which secrete these AMPs. However, no cellular structure, or nuclei could be discerned in these areas. These dark staining patches are characteristic of impregnated thelycum and have not been found in sections of the thelycum of unmated females. This suggests that the production of acid mucopolysaccharides by the secretory epithelium of the thelycum is accelerated by the presence of the spermatophore inside the thelycum. The copious production of the secretions containing AMPs may result in accumulation of these substances inside the lips and at the base of the keel, leading to the formation of the dark staining patches.

It is probable that the AMPs secreted by the thelycal epithelium come out through the pores in the cuticle and cover the lips, the trident and perhaps also spread to the interior of the thelycum and in some way protect the stored spermatozoa in the

seminal receptacle. A thick mucus coating on the lips and the ventral surface of the trident could effectively block the entrance to the seminal vesicle preventing loss of the spermatozoa from the thelycum. Further, since the AMPs are known to have antimicrobial properties (Pigman and Horton, 1970), these secretions may also prevent bacterial and fungal infection of the spermatozoa during prolonged storage inside the thelycum.

The nature of the blue-staining granules in the connective tissue at the core of the lateral horns and at the base of the stiff rounded teeth, is not clear. The function of the minute setae on the ventral surface of the lateral horns is also not clear. The setae may be sensory, and may respond to the presence of spermatophores inside the impregnated thelycum, triggering the accelerated secretion of AMPs and possibly hormones etc., connected with the reproductive processes. Electron microscopic studies may throw more light on the structure and function of these setae on the lateral horns.

Dehisence of the pedunculate spermatophores of Emerita asiatica deposited on the pleopod region of the female has been observed to occur only when the egg mass comes in contact with them and Subramonium (1977) has suggested that an oviducal secretion is responsible for the digestion of the cementing material closing the lip of the spermatophore. In Albunea symnista and the spiny lobsters where the convoluted tubular spermatophore is embedded in a thick gelatinous matrix which hardens into a thick

putty-like mass, the female is said to use its powerful chelae to remove the protective matrix and then gouge the spermatophoric tube open, to release the spermatozoa at the time of fertilization (Fielder, 1964; Berry, 1969; Subramoniam, 1984). In the case of P.indicus, since the spermatophores are inside the thelycum, the oviducal secretions cannot act on them, nor could the appendages of the female reach them to tear open the sperm bag. The possibility of one of the thelycal secretions acting on the wall of the sperm bag and dissolving it is suggested by the present study. But this needs further investigation.

Even if some thelycal enzymes dissolve the spermatophore wall and liberate the spermatozoa inside the thelycum, how is the female able to synchronise their release from the thelycum with the spawning of the ova. These questions cannot be answered at present.

How do the spermatozoa escape from the thelycum ? The seminal receptacle is open on the ventral side through the lips. However, the numerous villi, coated with a thick layer of the viscous mucus secretions from the thelycal epithelium can effectively block this passage. The only other way through which the spermatozoa can come out are the two small openings on either side of the anterior process of the thelycum left by the anterior extension of the lateral plates that cover the shoulder of the lateral horns of the trident. If the male gametes come out of these openings they will

be close to the oviducal openings on the coxa of the 3rd walking legs through which the ova are released. Hence they have a good chance to get attached to the ova and fertilize them. However, the mechanism of release of the spermatozoa, first from the sperm bag and then from the thelycum needs further study.

SUMMARY

1. The morphology, histology and histochemistry of the spermatophore and thelycum of Penaeus indicus has been studied in great detail to understand the structure and role of these organs in reproduction.

2. The spermatophore of Penaeus indicus consists of a chitinous sperm bag and wings, with a sticky mass of granules rich in sulphated AMP at the anteriomedial corner of the sperm bag. The sticky substance serves to cement the two spermatophores as they issue out of the male genital openings, into one unit. Inside the sperm bag the sperm mass is embedded in a spongy matrix rich in carboxylated AMP. The spermatozoa appear to have a glycogen store for endogenous energy metabolism.

3. The thelycum of P.indicus is very complex. The lateral plates hide the posterior projections of the folds of the sternite XIII which form a "trident" consisting of a conical median process having a median ventral keel, and two lateral horns covered with minute setae on the ventral side. The lateral plates meet in the centre to form the raised lips, the medial surfaces of which are covered with numerous villi. The ventral surface of the conical median

process of the trident also bears short villi in adult females. The dorsal margin of the lips bear a row of stiff rounded teeth which guard the entrance to the seminal receptacle which is single chambered.

4. The villi on the lips and median conical process, and the keel have well developed secretory epithelium and are covered with very thin cuticle, which, in surface view appears to be raised into minute rounded papillae.

5. The stiff blunt teeth and the lateral horns also have a secretory epithelium, but have thick cuticle.

6. On the ventral margin of the lips numerous minute cuticular pores are seen. The secretions produced by the thelycal epithelium appear to come out through these pores.

7. In transverse sections of impregnated thelucum, dark staining patches are observed inside the lips and at the base of the keel. They appear to be accumulations of the epithelial secretions.

8. The epithelial secretions of the thelycum are rich in carboxylated AMP which seem to protect the stored spermatozoa from bacterial and fungal infections. These secretions could also effectively block the entrance to the seminal receptacle and prevent the loss of spermatozoa from the thelycum.

REFERENCES

Aiken, D.E., S.L.Waddy, K.Moreland and S.M.Polar. 1984. Electrically induced ejaculation and artificial insemination of the American lobster Homarus americanus. Jour. Crust. Biol., 4 (4): 519-527.

Alcocu, A.W. 1906. The prawns of the Penaeus group. Catalogue of the Indian Decapod Crustacea in the collection of the Indian museum; Part III - Macrura, Indian museum, Calcutta. p 55.

Bauer, R.T. 1976. Mating behaviour and spermatophore transfer in the shrimp Heptacarpus pictus (Stumpson) (Decapoda, Caridea: Hippolytidae). Jour. Nat. Hist., London 10: 415-440.

Berry, P.F. 1969. Occurrence of an external spermatophoric mass in the spiny lobster Palinurus gilchristi Crustaceana, 17: 223-224.

Berry, P.F. and A.E.F.Heydorn. 1970. A comparison of spermatophoric masses and mechanism of fertilization in South African spiny lobsters (Palinuridae). Invest. Rep. Oceanogr. Res. Inst. S.Africa, 25: 1-18.

Bloch, F. 1935. Contribution al'etude des gametes et da la fecondation chez les crustaces decapodes. Trav. Stn. Zool Wimereux 12: 185-270.

Burkenroad, M.D. 1934. The penaeidae of Louisiana with a discussion of their world relationships. Bull. Am. Mus. Nat. Hist., 68: 61-143.

Burkenroad, M.D. 1939. Further observations on Penaeidae of the northern Gulf of Mexico. Bull. Bingham Oceangr. Collect., 6 (6): 1-62.

Calman, W.T. 1909. A Treatise on zoology, Part VII Appendiculate (Third fascicle: crustacea), R.Lankester, ed. Idam and Charles Black, London. pp 346.

Chow, S., Yoshimitsu Ogasawara and Yasuhiko Taki. 1982. Male reproductive system and fertilization of the Palaemonid shrimp Macrobrachium rosenbergi. Bull. Jap. Soc. Sci. Fish., 48 (2): 177-183.

Costanzo, G. and L.Guglielmo. 1976. Diagnostic value of the thelycum in Euphausids Mediterranean species. Crustaceana, 31: pp 45-52 and pp 178-180.

Criel, G. 1980. Morphology of the female genital apparatus of Artemia: a review In The Brine shrimp Artemia: Vol. I - Morphology, Genetics and Radiobiology and Texicology; pp 75-86.

Dall, W. 1957. A revision of the Australian species of Penaeinae - (Crustacea: Decapoda: Penaeidae). Inst. J. Mar. Freshw. Res. 8: 136-231.

Dall, W. 1965. Studies on the Physiology of a shrimp, Metapenaeus sp. (Crustacea: Decapoda: Penaeidae); III. Composition and structure of the integument. Aust. Jour. Mar. Freshw. Res., 16: 13-23.

Dall, W. and A.A.Racek. 1965. Littoral Penaeinae (Crustacea, Decapoda) from Northern Australia, New Guinea and adjacent waters. N.V. Noord-Hollandsche Uitgeversmatschappij; Amsterdam.

Deecaraman, M. and T.Subramoniam. 1980. Male reproductive tract and accessory glands of a stomatopod Squilla holoschita. Int. Jour. Inv. Repr. 2: 175-188.

Drury, R.A.B., E.A.Wallington and Sir Roy Cameron. Carleton's histological technique. Oxford University Press, London (4th ed.).

Dudenhausen, E. and P.Talbot. 1979. Ultrastructural examination of sperm and spermatophores from the crayfish, Pacifastacus lenuisculus. Second International Symposium on Invertebrate Reproduction, Proceedings.

Dudenhausen, E. and P.Talbot. 1983. An Ultrastructural comparison of soft and hardened spermatophores from the crayfish Pacifastacus leniusculus Dana. Can. J. Zool., 61: 182-194.

Einarsson, H. 1942. Notes on Euphausiacea I-III - On the systematic value of the spermatozoa, on sexual dimorphism in Neutoscelis and on the male in Bentheuphausia. Vidansk Medd Fra Dansk naturhistorisk Forening i Kobenharen, 104: 263-286.

Einarsson, H. 1945. Euphausiacea 1. Northern Atlantic species Rept; 27: 1-185.

Ezhilarasi, S. and T.Subramoniam. 1980. Development of spermatheca and its importance in the maturation of the ovary in Scylla serrata Forskal. Prog. Invert. Reprod. Aquacult. Madras University: 50-55.

Fielder, D.R. 1964. I - The spiny lobster, Jasus lalandei (H.Milne Edwards) in South Australia: Aust. J. Mar. Freshw. Res., 15: 77-92.

Fielder, D.R. 1964. II - The spiny lobster, Jasus lalandei (H.Milne Edwards) in South Australia. Aust. J. Mar. Freshw. Res., 15: 33-44.

Fyhn, U.E.H. and J.D.Costlow. 1977. Histology and histochemistry of the ovary and oogenesis in Balanus amphitrite. L and B. eburneus Gould (Cirripedia, Crustacea). Biol. Bull., 152: 351-359.

George, M.J. and M.S.Muthu. 1968. On the occurrence of Metapenaeus barbata (De Haan) (Decapoda: Penaeidae) in Indian waters with Taxonomic notes on the Genus. Jour. Mar. Biol. Ass., India 10 (2): 286-291.

Haley, R. Samuel. 1984. Spermatogenesis and spermatophore production in the Hawaiian Red lobster Enoplometopus occidentalis (Randall) (Crustacea, Nephropidae). J. Morph., 180 (3): 181-193.

Hinsch, G.W. and M.H. Walker. 1974. The Vas deferens of the spider crab, Libinia emarginata. J. Morph. 143: 1-20.

Hudinaga, M. 1942. Reproduction, development and rearing of Penaeus japonicus Bate. Jap. Jour. Zool. Vol.10: 305-393.

Jackson, H.G. 1913. Eupagurus. L.M.B.C. Mem. typ. Br. Mar. Pl. Anim., 21: 495-573.

Kamaladevi, S. 1947. Biology of Clibanarius olivaceous. M.Sc. thesis, Madras University.

King, J.E. 1948. A study of the reproductive organs of the common marine shrimp, Penaeus setiferus (Linnaeus). Biol. Bull. (Woods Hole), 94: 244-262.

Kishinouye, K. 1900. Japanese species of the genus Penaeus. J. Fish. Bur., Tokyo 8: 1-29.

Kooda-Cisco, M.J. and P.Talbot. 1982. A structural analysis of the freshly extruded spermatophore from the lobster, Homarus americanus. Jour. Morph., 172 (2): 193-207.

Kooda-Cisco, M.J. and P.Talbot. 1983. A technique for electrically stimulating extrusion of spermatophores from the lobster, Homarus americanus. Aquaculture 30: 221-228.

Kölliker, A. 1841. Beiträge zue Kenntnis der Geschlechts - Verhältnissi and der Samenflüssigkeit Wirbeloser Thiere, nebst einem versuch über das wesen und die Bedeutung der Sogenanntan Samenthiere. W.Logier, Berlin, S. 88.

Kubo, I. 1949. Studies on Penaeids of Japanese and its adjacent waters. J. Tokyo Coll. Fish., 6 (1): 47.

Linder, H.J. 1959. Studies on the freshwater fairy shrimp Chirocephalus bundyi (Forbes). I. Structure and histochemistry of ovary and accessory reproductive tissues. Jour. Morph., 104: 1-60.

Linder, H.J. 1960. Studies on the freshwater fairy shrimp Chirocephalus bundyi (Forbes). II. Histochemistry of egg-shell formation. Jour. Morph., 107: 259-284.

Mac Ginitie, G.E. 1938. Movements and mating habits of the sand crab, Emerita analoga. Am. Midl. Nat., 19: 471-481.

Malek, S.R.A. and F.M.Bawab. 1971. Tanning in the spermatophore of a crustacean Penaeus trisulcatus. Experimentia 27: 1098.

Malek, S.R.A. and F.M.Bawab. 1974. The formation of the spermatophore of Penaeus kerathurus (Forskal 1775) (Decapoda-Penaeidae); 1) The initial formation of sperm mass. Crustaceana 26: 272-275.

Malek, S.R.A. and F.M.Bawab. 1974. The formation of the spermatophore in Penaeus kerathurus (Forskal 1775) (Decapoda: Penaeidae). 2) The deposition of the main layers of the body and of the wing. Crustaceana 27 (1): 73-83.

Man, J.G., De. 1911. The Decapoda of the Siboga Expedition - Part I. Family Penaeidae. Siboga - Exped. Monogra. 39 a: 131.

Mann, Thaddeus. 1984. Spermatophores. Zoophysiology Vol.15 Springer-Verlag, Berlin. 217.

Mathews, D.C. 1957. The origin, development and nature of the spermatophoric mass of the spiny lobster, Panulirus penicillatus (Oliver). Pac. Sci., 5: 359-371.

Mathews, D.C. 1953. The development of the pedunculate spermatophore of the hermit crab, Dardanus asper (De Haan). Pac. Sci., 7: 255-266.

Mathews, D.C. 1956. The origin of the spermatophoric mass of sand crab, Hippa pacifica. Q.J. Microsc. Sci., 97: 257-268.

Mittal, P.K. 1976. Morphological and cytochemical studies on the yolk nucleus during vitellogenesis in a Macruran, Panulirus homarus (Macrura, Crustacea). Jour. Mar. Biol. Ass., 18 (1): 165-167.

Motoh, Hiroshi. 1980. Development of external genitalia of the giant prawn, Penaeus monodon. Bull. Jap. Soc. Sci. Fish 46 (2): 149-156.

Pearse, A.G.E. 1968. Histochemistry, Theoretical and Applied, Vol. I J & A Churchill, London.

Perez Farfante, I. 1969. Western Atlantic Shrimps of the genus Penaeus. Fish. Bull., 67 (3): 461-591.

Perez Farfante, I. 1970. Diagnostic characters of juveniles of the shrimps Penaeus aztecus aztecus, P.duorarum duorarum and P.brasiliensis (Crustacea) (Decapoda, Penaeidae) U.S. Fish. Wildℓ. Serv. Spec. Sci. Rep. Fish., No.599.

Perez Farfante, I. 1971. A key to the American Pacific shrimps of the Genus Trachypenaeus (Decapoda, Penaeidae) with the description of new species. Fish. Bull. 69 (3): 635-646.

Perez Farfante, I. and H.R.Bullis Jr. 1973. Western Atlantic shrimps of the genus Solenocera with description of a new species (Crustacea, Decapoda, Penaeidae). Smithson contrib. Zool., 153: 33.

Perez Farfante, I. 1975. Spermatophores and thelyca of the American white shrimps, genus Penaeus Subgenus Litopenaeus. Fish.Bull., 73: 463-486.

Perez Farfante, I. 1977. American Solenocerid shrimps of the genera Hymenopenaeus, Haliporoides, Pleoticus, Hadropenaeus genus and Mesopenaeus new genus. Fish. Bull., 75 (2): 261-346.

Pigman, W. and D.Horton. 1970. The carbohydrates, Vol. 2 B, New York Academic Press. pp 592-604.

Sandifer, P.A. and T.W.Lynn. 1981. Artificial insemination of caridean shrimp. In. W.H.Clark Jr. and T.S.Adams, eds. Developments in Endocrinology Vol. II; 271-288; Elsevier/North Holland, New York.

Sebastian, M.J. 1966. Euphausiacea from Indian seas; Systematics and general considerations. In "Symposium on Crustacea". Vol. I: 233-354. Mar. Biol. Ass., India.

Shaikhmahmud, F.S. and V.B.Tembe. 1958. Study of Bombay prawns - the reproductive organs of Parapenaeopsis stylifera (M.Edwards). J. Uni., Bombay 27(3): 100-110.

Starobogatov, Y.I. 1972. Penaeidae (Crustacea, Decapoda) of the Tonking Gulf. In. The fauna of the Tonking Gulf and conditions of life in it. Acad. Sci. U.R.R.S., Zool. Inst; Explorations of the fauna of the seas. 10 (18): 359-415. (In Russian).

Spalding, J.F. 1942. The nature and formation of the spermatophore and sperm plug in Carcinus maenas. Q. J. Microsc. Sci. 83: 399-422.

Subramoniam, T. 1977 a. Aspects of sexual biology of anomuran crab, Emerita asiatica. Mar. Biol., 43: 369-377.

Subramoniam, T. 1977 b. Continuous breeding in the tropical anomuran crab Emerita asiatica Milne Edwards from Madras coast. In. Advances in Invertebrate Reproduction - K.G. Adiyodi & R.G. Adiyodi, Eds. Peralam - Kenoth, Kerala, India.

Subramoniam, T. 1984. Spermatophore formation in two intertidal crabs, Albunea Symnista and Emerita asiatica (Decapoda: Anomura) Biol. Bull., 166: 78-95.

Tirmizi, M. Nasima. 1958. A study of some developmental stages of the thelycum and its relation to the spermatophores in the prawn Penaeus japonicus Bate. Proc. Zool. Soc. London 231-444.

Tirmizi, M.Nasima. 1968. Parapenaeopsis hardwickii (Miers) from the Arabean sea (Decapoda Penaeidae): Crustaceana, 15: 137-140.

Tirmizi, M. Nasima. 1968. On the structure and some developmental stages of the genitalia in the prawn, Parapenaeopsis stylifera (H.Milne Edwards) (Decapoda, Panaeidae). Crustaceana, 15: 193-203.

Travis, D.F. 1955. The moulting cycle of the spiny lobster Panulirus argus Latreille II - Pre-ecdysial histological and histochemical changes in the hepatopancreas and integumental tissues. Biol. Bull. Wood's Hole, 108: 88-112.

Travis, D.F. 1957. The moulting cycle of the spiny lobster Panulirus argus Latreille IV - Post ecdysial histological and histochemical changes in the hepatopancreas and integumental tissues. Biol. Bull. Wood's Hole 113: 451-479.

Tuma, D.J. 1967. A description of the development of primary and secondary sexual characters in the Banana prawn, Penaeus merguiensis De Man (Crustacea, Decapoda, Penaeidae). Inst. Jour. Mar. Freshw. Res., 18: 73-88.

Uma, K. and T.Subramoniam. 1979. Histochemical characteristics of spermatophore layers of Scylla serrata (Forskal) Decapoda, Portunidae) Int. J. Invertbr. Repr., 1: 31-41.

Wolfe, A.F. 1971. A histological and histochemical study of male reproductive system of Artemia. J. morph., 135: 51-70.

Young. E., Janice. 1974. Variations in the timing of spermatogenesis in Uca pugnax (Smith) and possible effectors (Decapoda, Brachyuran Ocypodidae). Crustaceana: 27 (1): 68-72.