SOME ASPECTS OF THE REPRODUCTIVE BIOLOGY OF *FUSITRITON OREGONENSIS* (REDFIELD) (GASTROPODA, PROSOBRANCHIA)

by

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ABSTRACT

Little is known of the reproductive biology of the prosobranch *Fusitriton oregonensis* (Redfield). Therefore this thesis describes the morphology and histology of the male and female reproductive tracts, the spermatogenesis of all types of sperm and their structure.

Both the male and female reproductive systems are in an advanced mesogastropod state, approaching the neogastropod condition. Primitive features of the male system are an open prostate gland and an open sperm groove extending along the phallus. The least advanced feature of the female system is the seminal receptacle which is merely a dilation of the distal portion of the oviduct.

Three types of sperm develop in the testes of *F. oregonensis* continually throughout the year. Maximum production however, occurs during February, March and April. Two sperm lines develop, (1) the eupyrene or fertilizing sperm and (2) the apyrene anucleate sperm, of which there are two types, the carrier and the lancet. Development of eupyrene sperm is typical but complex; one spermatogonium produces four haploid sperm after two meiotic divisions. In contrast, development of apyrene is atypical and direct; one spermatogonium produces one sperm. The nucleus of the spermatogonium fragments and degenerates within the cell. Cellular differentiation is not complex.

The most numerous and active of all three types are the eupyrene sperm. They are long and filamentous, similar to other gastropod sperm. The acrosome is

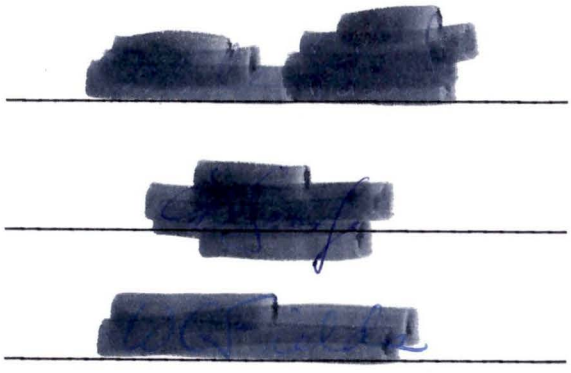
conical and hollow. A rod extends through the subacrosomal space which also contains a subacrosomal granule. Posterior to the acrosome is a modified centriole from which extends an axoneme of the standard 9+2 pattern. The nucleus is cylindrical, encircling the axoneme distal to the acrosome. Distal to the nucleus is a mitochondrial spiral also encircling the axoneme and further distal is the glycogen region. Grouped glycogen rosettes having a characteristic pattern extend to the end of the tail piece where the axoneme emerges free for a short distance.

The slow-moving carrier apyrene sperm is short and fusiform. Through its centre runs a core of axonemes of standard configuration, each of which extends from an anterior modified centriole. Encircling the core are tightly packed large droplets containing acid mucopolysaccharide complexed with protein. Dispersed amongst these are glycogen rosettes and a few mitochondria. Fifty or more eupyrene sperm attach to one carrier sperm forming a spermatozeugma. Carrier sperm are probably a nutrient source for eupyrene sperm. There may be other less obvious functions for the spermatozeugmata.

Lancet apyrene sperm are long and so named because of their shape. They are less active than the carrier sperm and eupyrene sperm do not attach to them. Several standard axonemes arise from apically placed modified centrioles and extend beneath the plasmalemma distally to the end of the sperm; single microtubules lie in a similar position between the axonemes. Disposed anteriorly in the cytoplasm are mitochondria and numerous small droplets containing acid mucopolysaccharides complexed

with protein. Posterior are several large mucus-containing droplets. Scattered throughout the cytoplasm are glycogen rosettes. The roles of the lancet sperm are less obvious than those of the carrier.

After copulation, the three types of sperm are stored for a short time in the female copulatory bursa. Most eupyrene sperm move to the seminal receptacle for some time prior to fertilization. Apyrene sperm remain in the viscous fluid in the copulatory bursa. Lancet sperm appear to begin degeneration and entrap any other sperm remaining in the bursa, producing plasmodial-like formations. The significance of this is uncertain.



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INTRODUCTION

The hairy Oregon whelk, *Fusitriton oregonensis* (Redfield)[1] is a common, subtidal prosobranch gastropod of the northeastern Pacific (Kozloff, 1973). Preliminary observations on its seminal fluid had revealed large, unusual cells to which were attached several filiform sperm.[2] This thesis demonstrates that there are two morphologically and functionally distinct atypical sperm in the semen which are apyrene and non-fertilizing. Additionally it documents the morphology and development of these sperm and of the long slender fertilizing sperm, and describes other related aspects of the reproductive biology of *F. oregonensis*.

Although sperm dimorphism or polymorphism is a common phenomenon in prosobranchs (Fretter and Graham, 1962; Hyman, 1967; Grassé, 1968) no published information on the sperm of *F. oregonensis* exists, and literature on its reproductive biology is scanty. Philpott (1921-25) described oogenesis and oviposition of this species and Eaton (1971)

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[1] *F. oregonensis* fits into the following taxonomic position, according to Keen and Coan (1963):

Class	Gastropoda
Subclass	Prosobranchia
Order	Monotocardia
Suborder	Taenioglossa
Superfamily	Tonnacea
Family	Cymatiidae

[2] Atypical sperm were observed in the seminal fluid of *F. oregonensis* by students in an undergraduate invertebrate laboratory session I was demonstrating.

documented its mating, oviposition and egg brooding. Modern studies of prosobranch sperm are few, particularly any on the structure and function of atypical sperm. Furthermore, existing work needs clarification.

LITERATURE REVIEW

This literature review covers the most significant papers dealing with structure, function and development of atypical sperm in prosobranch molluscs. Less pertinent papers are also listed, but not summarized. Key papers dealing with typical sperm ultrastructure and development in general are given in Appendix I. Selection of citation in this category was necessary because of the volume of existing literature.

I. Atypical Sperm of Prosobranchs

Cases of spermic dimorphism and polymorphism are abundant in the literature. The following authors give comprehensive lists of the literature to the dates of their publications: Reinke, 1914; Ankel, 1930; Neahaus, 1959; Dupuoy, 1964.

In 1836, von Siebold discovered spermic dimorphism in *Paludina vivipara* (= *Viviparus viviparus*) a freshwater prosobranch. Duval (1879a) continued the study and showed definitely that two types of sperm were present in the seminal fluid, a filiform type (eupyrene) and a worm-like type (oligopyrene), that they had two separate lines of development, and that the oligopyrene sperm was neither a parasite nor a spermatophore. The work on sperm of *P. vivipara* was resumed by Meves (1903) who studied spermatogenesis of both types of sperm and showed the worm-like ones to be oligopyrene; *i.e.*, containing a smaller

amount of chromatin than the regular haploid amount of eupyrene (fertilizing) sperm.[3] In this case, eupyrene sperm do not attach to oligopyrene sperm although the latter contain granules. Meves rejected the idea that there was nutrient absorption by the eupyrene sperm. He concluded that oligopyrene sperm have no role in the fertilization of eggs, but suggested that atypical sperm may stimulate eggs to develop prior to fertilization.

Spermic dimorphism was later found in *Strombus bitubereulatus*, by Reinke (1912) who gave a brief account of development of apyrene (chromatin lacking) atypical sperm, which contain large "yolk" droplets. He also showed that eupyrene sperm attach to nurse-cells in the testes of *Littorina anguilifera* and *L. nebulosa*. In a succeeding paper (1914) he gave a more detailed account of spermatogenesis of the two types of sperm of *S. bitubereulatus*. Reinke suggested that the apyrene sperm might: 1) act as nurse cells to eupyrene sperm within the female after copulation; or by secretions stimulate 2) either eupyrene sperm or eggs during fertilization; or 3) direct eupyrene sperm to eggs. He stated emphatically that apyrene sperm do not participate in fertilization.

Schitz (1920a) showed that *Cerithium vulgatum, Turritella triphiata* and *Bittium reticulatum* all produce eupyrene and apyrene sperm. The

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[3]Dupuoy (1960) was concerned with reclassifying the types of sperm according to cytoplasmic instead of chromatin content and proposed abandoning the terms eupyrene, oligopyrene and hyperpyrene (having more than the regular haploid chromatin content), to basing it on cytoplasmic content. Secondly, (1964) he suggested that a nomenclature based on the shape of sperm might be useful, and proposed that "filiform" and "worm-like" replace the terms above. Neither of his proposals has been accepted and the original terminology has been retained.

apyrene sperm of these prosobranchs are similar in structure, *i.e.*, they contain "yolk" droplets encircling an axial core and posteriorly extending "tail filaments" which actively beat to move the sperm forward.

Schitz (1920b) also described the structure and spermatogenesis of apyrene sperm of a second group of prosobranchs: *Murex trunculus, Aporrhais pespelicani, Fusus* sp., and *Nassa reticulata*. These sperm are all long and slender, wider anteriorly; they have "yolk" droplets in the cytoplasm and intracellular "fibrils" lying at their surfaces.

The case of sperm polymorphism in *Fasciolaria tulipa* is unusual (Hyman, 1923). Three oligopyrene sperm are produced, which are like each other and eupyrene sperm except for small size differences and differing chromatin content. Only one type of oligopyrene sperm is present in the semen at one time; no suggestion is made as to their significance.

Ankel's (1924; 1926; 1930; 1933) contributions to the study of spermic dimorphism are the most careful and significant up to the application of electron microscopy. In 1924 he noted two atypical sperm in *Bithynia tentaculata*. This prosobranch is peculiar because it produces a hyperpyrene (atypical sperm having more than the typical haploid amount of chromatin) sperm which is immediately resorbed by the testis, while a second type, oligopyrene, is formed and liberated into the semen. He described spermatogenesis of both atypical types of sperm.

Ankel (1926) also described the spectacular apyrene sperm of *Janthina* and *Scala*. These sperm are truly outstanding because of their

large size, each being close to 1 mm in length. They are composed of anterior oval, flat locomotory pieces, and posterior narrow tail pieces filled with "yolk" droplets. Hundreds of eupyrene sperm attach to this tail piece forming a complex, the "spermatozeugma". Subsequently Ankel (1930) documented spermatogenesis of these apyrene sperm.

Because of Tuzet's (1930) divergent views on spermatogenesis of atypical sperm, Ankel's final paper (1933) on this subject is an elaborate discussion and defence of his former work. He stressed the point that all atypical sperm studied have a similar development which often parallels that of eggs. His thinking was coloured by contemporary developmental ideas concerning a balance of "maleness" and "femaleness" that now have been generally disregarded. Nevertheless, a wealth of details makes the paper valuable. Other papers on atypical sperm which also contribute to the same erroneous developmental theories, by Kuschakewitz (1913), Goldschmidt (1916) and Portmann (1930) are not reviewed here.

Aspects of the reproductive biology of *Goniobasis laqueata* (Woodard, 1935) are unusual. There is spermic dimorphism, but a spermatophore is formed which includes no atypical sperm, *i.e.*, they never reach the female. Their development begins after the eupyrene sperm are mature. Woodard's view is that apyrene sperm are functionless in fertilization or in eupyrene nutrition.

Neahaus (1959) redescribed the atypical sperm of *Bithynia tentaculata*, noting that they were absorbed by epithelial cells of the seminal receptacle. Consequently he suggested such sperm were an important source of nutrient.

Bulnheim (1962) described the development of typical and atypical sperm of *Opalia crenimarginata*. The atypical sperm are similar to those of *Janthina* and *Scala* in shape and size, and also form the bases for spermatozeugmata. They lack RNA and DNA but have glycoproteins in the mid piece. Bulnheim (1962) also found that apyrene sperm degenerate in the seminal receptacle of the female, perhaps contributing nutrition and a stimulus to eupyrene sperm. *Epitonium tinctum* also has atypical sperm with similar characteristics (Bulnheim, 1968).

Surprisingly little electron microscopy has been applied to the study of development or structure of atypical sperm types. Hanson, Randall and Bayley (1952) studies the ultrastructure of *Viviparus viviparus*. They established that the oligopyrene sperm contains one anteriorly disposed chromosome, that the core of the sperm is filled with axonemes similar to those seen in eupyrene sperm tails and that the bodies encircling the axial core in the midregion contain polysaccharides.

Yasuzumi and Tanaka (1958) studied ultrastructure and development of atypical sperm of *Cipangopaludina malleata*. They followed degeneration of the nucleus in development and found that the apyrene sperm also contains an axial core of filaments surrounded by droplets of various sizes. Yasuzumi *et al.*, (1967) later followed the break-down of nuclear material in atypical sperm of *C. malleata*.

The most significant electron microscope study of atypical sperm made was by Gall (1961) on *Viviparus contectus*. He carefully followed development of the atypical sperm with particular emphasis on the development of centrioles. This is a classic study on these organelles.

He describes their structure and that of their associated axial filaments. Also, he carried out histochemical tests, finding that the peripheral droplets of the cytoplasm were PAS positive and that there was no DNA or RNA in the cytoplasm. Dembski (1968) showed that the cytoplasm of the mid piece of oligopyrene sperm of *V. contectus* also contained polysaccharides (was PAS positive) and glucose.

Bulnheim (1962) described the ultrastructure of the axonemes and tail piece of the apyrene sperm of *Opalia crenimarginata*.

Atypical or aberrant sperm are not restricted to the prosobranchs, but are fairly common throughout the animal kingdom (Roosen-Runge, 1973). This wider literature is not reviewed here.

II. Eupyrene Sperm

Franzen (1956) reviewed sperm structure and biology of fertilization amongst invertebrates, stressing phylogenetic relationships. He investigated spermiogenesis in molluscs (1955), cephalopod molluscs (1967) and recently has considered the phylogenetic significance of sperm morphology throughout the animal kingdom (1969). Thompson and Bebbington (1970) have redescribed the structure of aplyssid opisthobranch sperm at the light microscope level.

The papers already cited for atypical spermatogenesis in gastropods also considered eupyrene development and need not be cited again.

The literature of typical sperm ultrastructure is voluminous. Therefore, this review is limited to descriptions of invertebrate sperm types which have significance to the present study. Some key vertebrate

papers are included. This literature is cited phylogenetically in Appendix I.

III. General Reviews

Baccetti (1970) edited a valuable collection of ultrastructural studies on various aspects of vertebrate and invertebrate sperm, while Idelman (1967) and Fawcett (1970) have contributed important comparative papers on the ultrastructure of sperm. Phillips (1970) published a general paper on the structure of insect sperm and more recently a comparative review of spermiogenesis (1974). Both of the latter have excellent bibliographies on the topic of the ultrastructure of sperm.

MATERIALS AND METHODS

I. Collecting and Holding; Reproductive Cycles

I collected monthly samples of 8 to 10 specimens of *F. oregonensis* (September, 1972 to September, 1973) from the outside surface of the Ogden Point Breakwater at the entrance to Victoria Harbour, by SCUBA diving at depths of 5 to 12 meters. As soon as possible I checked gametes of each male and female snail to determine the stage of maturity of each. In the procedure used, shells were cracked and removed, and sizes of the exposed gonads noted. The organs were pierced and contents drawn from them with disposable glass pipettes. Male and female gametes were handled in the same way. A drop of gonadal fluid was mixed with 10 drops of cold, filtered seawater in a small glass vial. This was covered and kept at 6°C until slide preparations were made. Such samples were taken from 3 or 4 regions of the gonad, posterior to anterior. A drop of the diluted fluid was placed on one end of a previously refrigerated slide, smeared with a coverslip, covered and observed wet.

In February, 1974, I collected 35 individuals at the same location and placed them in cold isosmotic magnesium chloride-seawater ($MgCl_2$-SW) solution (7.2% $MgCl_2$: seawater; 1 : 1) for 20 to 30 minutes, to retard the contractile response to handling and to changes in light intensity, and to reduce the number of withdrawals into the shell during the "righting" process. I placed each triton "on its back", after which it withdrew into its shell before slowly beginning the righting process.

During righting, a snail extends its foot first, then the tentacles, palps and finally the head. Once the head protrudes the animal can be sexed. If male, the base of the phallus is obvious behind the right tentacle.

I tagged male tritons with low and females with high numbers, so that each sex was recognizable. After other tagging methods failed, I found the use of "spaghetti" tags (numbered plastic cylinders) successful. Two small holes 1.5 mm in diameter were drilled in the outer lip of the aperture of each shell. A fine string was threaded through the tag, through one of the holes, along the inside of the shell's lip, through the second hold and knotted. The tag sat externally, paralleling the outer lip, and did not interfere with the snail's normal activities.

All snails were held in a tank of recirculating seawater at Sealand of the Pacific. During weekly visits from February to May, I made observations of any sexual behaviour.

II. Gross Morphology

I made several dissections to reveal the gross morphology of the reproductive tracts of both male and female *F. oregonensis*. Animals were relaxed in $MgCl_2$-SW for 20 to 60 minutes, unless their sperm were needed for microscopic study. The snails' shells were broken with a hammer and removed, leaving the animals intact for observation. The tracts were exposed by making a median, longitudinal incision in the dorsal mantle and folding back each flap.

III. Light Microscopy

A. "Live" method

I made wet preparations of sperm as previously described, and studied them with phase contrast and Zeiss Nomarski interference optics. Care was taken to keep the preparation cool for as long as possible. Phase contrast gave details of cell outlines while Nomarski gave more information on cell contents.

B. Paraffin method

I fixed tissues from each portion of both reproductive tracts in Bouin's, which proved superior to either 10% seawater-formalin or susa fixation. After 24 hour fixation, tissues were dehydrated in ethanol and xylene, and embedded in Paraplast. Generally 6μ sections were cut, except for some 2μ sections of testis and seminal vesicle.

For routine observations, I stained sections with Delafield's haematoxylin-eosin and mounted them with DPX mounting medium (British Drug House).

C. Histochemistry

I carried out histochemical tests on mature sperm taken from seminal vesicles of males. For all tests, seminal fluid was treated in a similar manner. "Dry" sperm was diluted one to 10 with cold, filtered seawater as previously described. A drop of diluted seminal fluid was placed on an acid-alcohol cleaned coverslip and a smear preparation was made. Coverslips were cleaned to enhance self adhesion of sperm to the glass.

The coverslip bearing the drop of sperm was placed on a horizontal surface and another coverslip was held at a 45° angle so that its edge touched the drop and spread it across the underlying slip. At this point, the angled coverslip was quickly drawn across the underlying one. This usually gave a uniform smear with the correct cell density and little or no cell breakage. Before the smears dried, the slips were floated, sperm downward in the desired fixative in a petri dish.

For all methods but the dinitrofluorobenzoylation test for protein, the fixative used was non-buffered 10% formalin. (No obvious disruption of sperm membranes was observed.) The fixative for the protein test was 5% acetic acid in 100% ethanol.

Following fixation but prior to the actual test series, coverslips were washed with distilled water to remove any salt crystals.

The tests used are listed in Table 1. Initially tests were employed to obtain information on the nature of the various inclusions in the sperm types. These tests included general ones for protein, carbohydrate, lipid and DNA. Because the more important inclusions proved to be of carbohydrate nature, a number of carbohydrate tests were additionally performed (Table 1).

TABLE 1

HISTOCHEMICAL TESTS

TEST	REFERENCE	DIAGNOSIS
Periodic acid - Schiff (PAS) reaction	Pearse, 1968	1-2 glycol groups of carbohydrates
Acetylated PAS	Pearse, 1968	blockade of PAS reactive sites to confirm carbohydrate nature
Standard toluidine blue	Chayen, *et al.*, 1969	metachromasy
Toluidine blue extinction (at pH 4.2, 5.0, 5.6)	Chayen, *et al.*, 1969	basiphilia (isoelectric point)
Alcian blue (AB)	Chayen, *et al.*,	selective for acid mucopolysaccharides
Best's carmine	Pearse, 1968	specific for glycogen
Sudan IV	Humason, 1972	lipids in general
Dinitrofluorobenzene (DNFB)	Pearse, 1968	specific for tyrosine, also SH groups; general for protein
Feulgen reaction	Pearse, 1968	specific for DNA

D. Photomicrography

I took photographs with a 35 mm Leica photomicrographic camera and used Plus-X film for photos of live sperm and Panatomic-X for photos of paraffin and histochemical preparations. A substage electronic flash attachment operated at 30 or 60 watt seconds was a necessity when photographing live sperm.

IV. Electron Microscopy

The greatest difficulty I encountered during this investigation was finding a good method of fixation for sperm ultrastructure. Both the seminal fluid of male *F. oregonensis* and fluids in the reproductive storage organs of females were extremely viscous, probably due to their mucus component. The fixatives could not penetrate these fluids quickly. Fixation proved to be an extremely difficult and time consuming task, one which I have not completed perfectly.

Of all fixation methods tried, the following two gave the best results:

1. Fix in 5.0% paraformaldehyde, 1.0% acrolein, 0.75% glutaraldehyde, 3.0% NaCl and 3.5% sucrose in 0.1 M phosphate buffer (pH 7.0-7.2) for 4 to 16 hours. Wash in 8.0% sucrose in 0.1 M phosphate buffer (pH 7.0-7.2). Postfix in 2.0% osmium tetroxide in 0.4 M phosphate buffer for 1.5 to 2.0 hours. This is a fixative used successfully by Fahrenbach (1973) on sperm of *Limulus*, the horseshoe crab.

2. Fix in 5.0% glutaraldehyde and 0.4 M Milloning's phosphate buffer (pH 7.4) mixed 1:1. Postfix in 2.0% osmium tetroxide, 2.5% sodium bicarbonate solution (pH 7.4) for 1.5 hours. This method was taken from Cloney and Florey (1968).

A third method, a variant of the second was to postfix in 1.0% osmium tetroxide in 0.1 M phosphate buffer (pH 7.4) for 1.5 hours.

Each of these solutions was osmotically balanced to 960 milliosmoles using NaCl. All fixatives were initially cold (at 6°C), but fixation was carried out at room temperature. Any microtubular structures should have been protected from degradation which occurs below 4°C.

Following dehydration in a graded series of ethanol to propylene oxide, tissues were embedded in Epon 812.

Thin sections, silver and gold, were cut on a Reichert ultramicrotome using glass knives. I made no attempt to cut grey sections because a slightly thicker section has proven to be better for study of sperm and their organelles (Fahrenbach, personal communication). Once cut, sections were mounted on uncoated, 300 mesh, copper grids.

Sections were stained (Fahrenbach, personal communication) with uranyl acetate and lead citrate as follows:

1. Stain 30 to 45 seconds in 0.2% lead citrate.
2. Wash using dip method in double distilled water.
3. Stain 2 to 3 minutes in 2.0% uranyl acetate (pH 4.5), kept in dark.
4. Wash (as above).
5. Stain 30 to 45 seconds in 0.2% lead citrate.
6. Wash.

For best results, I filtered both stains using a 0.45μ millepore filter to remove any precipitate or dirt. Typically, the shorter

staining time of the range given above, gave better results on mature eupyrene sperm due to the affinity of the nucleus to the stain. The longer times gave better results on developing eupyrene sperm, in which the nucleus is in a different chemical state, and on apyrene sperm types which are anucleate.

Stained preparations were studied with a Philips 300 electron microscope at 60 KV. Lower magnification photos had better resolution. Thick sections (.5 - 1.0μ) were stained with Richardson's stain and viewed for orientation of blocks and some sperm study.

OBSERVATIONS

The results of my investigations into the reproductive biology of *F. oregonensis* fall into six categories: (1) the morphology and histology of male and female reproductive tracts; (2) the light microscopy and histochemistry of the sperm; (3) the ultrastructure of the mature sperm; (4) a brief synopsis of spermatogenesis of sperm based on light and electron microscopic data; (5) general observations of reproductive behaviour and cycles; (6) sperm degeneration in the female reproductive tract.

I. Reproductive Tracts: Morphology and Histology

The reproductive systems of *F. oregonensis* follow a plan exhibited by many prosobranchs (Fretter, 1946; Fretter and Graham, 1962; Grassé, 1968). Female tracts show more complexity and variability than male tracts (Fretter and Graham, 1962).

A. Male reproductive system

The testis is bright orange, grainy in texture, and overlies the spiralling digestive gland (fig. 1). It is composed of many branched, blind-ending tubules and encased in a muscular coat of variously oriented fibres. This is externally covered by a columnar epithelium. The spermatic tubules ramify through a connective tissue stroma.

In section, a tubule (fig. 3) is encircled by a single layer of basal cells which may be a syncytium. Lumenally this is covered by a basement membrane on which rest the germinal cells. Sperm in early stages of spermatogenesis are peripherally placed, while those

in later stages are found lumenally. Eupyrene sperm tails extend into the centre of the tubule (fig. 4). Mature apyrene sperm may extend from the basal lamina to the lumen or be found free there.

Mature sperm collect in a region of the testis termed the " seminal vesicle" (fig. 1 and 2). This is histologically similar to the testis (fig. 4), but grossly it is different. The seminal vesicle is cream coloured, due to the mature sperm stored here, and smooth in texture. It probably has a spermatogenic capacity early in the production cycle of the testis; however, it functionally differentiates as a holding structure or seminal vesicle as more mature sperm are produced by the testis.

The seminal vesicle always contains some mature sperm, but becomes swollen as more accumulate during the breeding season. Eupyrene sperm are attached to carrier apyrene sperm here.

A closed sperm duct runs from the seminal vesicle beside the columellar muscle to the prostate (fig. 1 and 2). It is lined with ciliated columnar cells interspersed with secretory cells (fig. 5). Their product appears to be mucus.

Anterior to the columellar muscle, where the mantle extends free from the snail's head, is a laterally flattened pouch (fig. 1 and 2) termed the prostate (Laxton, 1969). It is attached basally to the triton's neck. Its roof rests against the mantle flap and extends a short distance over the pouch, making the prostate open anteriorly. The closed sperm duct enters the right prostate wall (fig. 2), emptying into the pouch. The prostate is lined with an epithelium

made up of two cell types (fig. 6), tall narrow ciliated cells with large centrally located nuclei, interspersed with non-ciliated secretory cells with basally placed nuclei; the latter do not appear to rest on the underlying basal lamina.

More anteriorly the floor of the prostate extends beyond the roof forming an open groove, the sperm groove, which is a continuing sperm duct (fig. 2). Two ridges run laterally along the groove to the base of the phallus (fig. 2) where they fade, the groove continuing along the outer edge of the phallus to the bifid tip. The sperm groove is lined with high columnar ciliated cells (fig. 7). A few secretory cells are intermingled with these. Internally blood sinuses run through the muscle layers of the phallus.

B. Female reproductive system

The organs of the female reproductive tract will be considered in succession from the gonopore to ovaries. Sperm is received through the female genital aperture (fig. 8) which is controlled by a sphincter muscle. Apparently the bilobed tip of the male phallus fits around this opening so that the sperm groove lines up with it. The aperture opens into a short "vagina" which in turn leads into a blind bulbous region (fig. 8 and 9). This complex is the copulatory bursa, that region of the female reproductive system which receives sperm and prostatic fluid (Fretter and Graham, 1962). It is extremely muscular, the walls being composed of an outer thick layer of circular muscle and an inner layer of longitudinal muscle. The copulatory bursa is lined with tall narrow, ciliated cells (fig. 15) containing long oval

nuclei. Amongst these cells are a few goblet-shaped secretory cells, which have rounded nuclei located closer to the basal lamina.

The walls of the copulatory bursa are convoluted; in its lumen is a jelly-like substance. Apyrene sperm, which never leave this structure, plus some remaining eupyrene sperm are caught in this substance and form clumps (fig. 14). The copulatory bursa receives all sperm types and, as indicated, holds eupyrene sperm for a short time. Its elongated vaginal region opens medially to the ventral region of the vestibule, the anterior portion of the capsule gland (fig. 9). This opening is not always evident and must be muscularly controlled.

Only eupyrene sperm leave the copulatory bursa to enter the vestibule. They then follow the ventral channel of the capsule gland posteriorly. The capsule gland is long (fig. 8 and 9) and appears to be a folded sheet resulting in two lateral walls which are fused dorsally and ventrally enclosing a central hollow chamber. Histologically the gland has an acinar structure. Several secretory cells comprise an extended lobe, which in turn is surrounded by connective tissue. Small ducts run through each lobe, receiving secretions of the surrounding cells and carrying them to the inner epithelium of the chamber (fig. 13). The lobes of the gland stain in either of two ways with haematoxylin-eosin (purple or red), suggesting the production of two types of secretions.

The ventral sperm channel is ciliated and runs the length of the gland complex. Ciliary tracts lining the inner walls of prosobranch

capsule glands are responsible for capsule formation (Fretter and Graham, 1962; Buckland-Nicks, 1974). Such tracts exist in the capsule gland of *F. oregonensis*, but their distribution was not determined.

The albumen gland and capsule gland are joined by a thin sheet of connective tissue. Lateral sperm channels leave the ventral channel at this point and run dorsally encircling the gland (fig. 8 and 9). Histologically the albumen gland is similar to the capsule gland (fig. 12), but its secretions are different. It supplies a protein (albumen) coat to each fertilized egg; the capsule gland secretes a complex mixture of polysaccharide, lipid, and protein (Stickle and Mrozek, 1973).

The oviduct enters the gland complex where the lateral channels extend from the main ventral channel (fig. 8 and 9); these channels are continuations of the oviduct (Fretter and Graham, 1962). The oviduct is lined with a cuboidal epithelium at this point which develops into a tall columnar epithelium closer to the ovary. The apical portions of the cells are filled with granules in some cases and clear vacuoles in others. They are ciliated and rest on a basal lamina. The duct is encircled with muscle. In the lower dilated portion of the oviduct thousands of eupyrene sperm can be seen (fig. 11) with their heads on the epithelial cells, and their tails directed lumenally. This region is called the seminal receptacle (Fretter and Graham, 1962).

The ovary is olive green and occupies the same relative position as the testis, covering the snail's spiralled viscera. It is "ripe"

from April to August. Several tubules comprise the ovary. They empty into collecting branches which in turn empty into the main oviduct.

The ovarian tubules are surrounded by a thin layer of basal cells. Oogonia and maturing oocytes (fig. 10) lie on a basal lamina which rests on the basal layer of cells. The more mature oocytes contain numerous yolk droplets. The nuclei are round, conspicuous and often contain nucleoli.

I suggest that eggs leave the ovary, are fertilized in the seminal receptacle and pass into the gland system. They must be carried via the lateral sperm channels and ciliary currents through the albumen gland where they are coated, and then to the capsule gland. Thousands of albumen-coated eggs are found encapsulated here. The capsule must pass anteriorly to the vestibule, through the vaginal area of the copulatory bursa and be deposited through the female aperture. This aperture is highly dilated in females just after oviposition, suggesting that the musculature controlling the opening is capable of great extension to allow for the extrusion of a large capsule.

The capsules are box-like, wider at the top than bottom and flattened, resembling kernels of corn. They are laid in counter clockwise whorls by the female who starts at the centre of the whorl and winds her way out (Philpott, 1921-25; Howard, 1962; Eaton, 1971).

II. Characterization and Histochemistry of the Types of Sperm

Mature sperm were always collected from seminal vesicles of males. A study of smears showed that there are 3 types of sperm in the seminal fluid of *F. oregonensis*.

A. Eupyrene sperm

The eupyrene or fertilizing sperm (fig. 16 and 17) are most numerous and are about 200μ in length and about 1μ in diameter. They undulate at high frequencies, which I could not measure with the available equipment.[4] These sperm do not move in a "cork-screw pattern". Eupyrene sperm quickly detach from carrier apyrene sperm during observation and their anterior tips stick to the glass slide. This portion of the sperm is termed the acrosome and appears as a small knob in figure 23. Unfortunately I never observed an "acrosome reaction". Posterior to the acrosome is the nucleus, approximately 26μ in length, the mitochondrial region, 36μ long, and finally the tail piece which extends for 130μ and tapers to a thin end piece (fig. 23).

B. Carrier sperm

The carrier is much shorter than the eupyrene sperm and about 36μ long and 7μ in diameter (fig. 18 and 24). A core of fibrils runs through the centre of the carrier, on either side of which are large droplets (fig. 18). The core often appears to be disposed "ventrally" while "dorsally" it is covered by droplets.

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[4]A contemporary area of study related to sperm ultrastructure is the motility of sperm. Relating structure with energetics and movement patterns is beyond the scope of this study. Papers by the following authors will give a good introduction to the topic: Rikmenspoel, 1965; Fray, Hoffer and Fawcett, 1972; Gilula and Satir, 1972; Lindemann and Rikmenspoel, 1972; Bergstrom, Henley and Costello, 1973; Brokaw, 1973; Brokaw and Josslin, 1973; Costello, 1973a and b; Rikmenspoel and Rudd, 1973.

I have termed this sperm the carrier because about 50 eupyrene sperm attach to it (fig. 20, 21, and 22), producing a spermatozeugma. Eupyrene sperm in figure 20 are most normally arranged around the carrier; the heads of eupyrene sperm are clearer in figure 21 where, however, they are abnormally arranged around the carrier sperm. Eupyrene sperm in figure 22 are attached anteriorly on the carrier sperm which is often the case. Movement of the unattached fusiform carrier sperm is slow; it flexes from side to side in large C-shaped curves. When eupyrene sperm are attached to it, its movement is minimal, any propulsion being supplied by the attached undulating eupyrene sperm. The movement of the several eupyrene sperm tails is synchronized in such a way that they never entangle. This characteristic is known as phase inhibition. The adhesion of eupyrene sperm to the carrier is tenuous and temporary; in a wet preparation eupyrene sperm quickly detach and swim off.

C. Lancet sperm

The second apyrene sperm I have termed the lancet, because of its shape. Anteriorly it is small and rounded and broadens to 5μ (fig. 19 and 25). It then tapers to a thin, flat, elongated(115μ) cell. Its lateral edges are permanently undulated. Longitudinally running fibrils are evident at its surfaces (fig. 19) and inclusions of various sizes and shapes are found centrally. The lancet is less motile than the carrier. The anterior third flicks up and down in a dorso-ventral plane because it usually lies with a flat surface toward the glass surface. No other sperm adhere to the lancet.

D. Histochemistry

To obtain information about the chemical composition of each type of sperm, histochemical tests were applied as shown in table 2. Certain regions of the sperm reacted characteristically with specific tests and the results were tabulated on this basis. I hoped these analyses might indicate the usefulness of inclusions in apyrene sperm, possibly as significant nutritional stores.

The following is a brief explanation of some of the significant results shown in table 2. The tail piece of eupyrene sperm shows a PAS positive reaction, thus this region contains carbohydrate, probably polysaccharide (Pearse, 1968). The cytoplasm surrounding the fibrillar core of carrier apyrene sperm is also rich in polysaccharides, confined to the large droplets found here. Likewise positive results for the anterior and mid droplet zone of the lancet sperm indicate that the droplets found here also contain polysaccharides.

Metachromasy is produced when a polymerized dye stuff is attracted to a substrate with regularly arranged anionic charges (Pearse, 1968). Mucopolysaccharides are molecules bearing regularly and densely arranged sulphate groups which are electronegatively charged. Toluidine blue during pH changes will polymerize firstly to the beta state (purple) and further to the gamma state (red). The regularly arranged polar groups of the polymerized dyestuff are attracted to the anionic surfaces of the substrate (mucopolysaccharide) producing a purple to red colour. The standard toluidine blue test (pH 4.4) produced no metachromasy (ortho or blue staining is considered a negative result).

The extinction test was then followed to see if metachromasy could be attained with pH changes. Droplets of the anterior portion of lancet sperm showed some metachromasy while so did droplets of the mid portion of this sperm; *i.e.*, these regions contain mucopolysaccharides.

Alcian blue is known as a specific stain for mucins, while in practice it usually stains acid mucopolysaccharides, but not mucoproteins (Pearse, 1968). The droplets of the carrier and of lancet sperm are in part acid mucopolysaccharide, particularly those of the mid region of the lancet (table 2).

Results of Best's test for glycogen show that the tail piece of eupyrene sperm is rich in this compound (fig. 23). Glycogen also is found in abundance in the cytoplasm surrounding droplets of carrier sperm and in a smaller amount in the cytoplasm of lancet sperm (fig. 24 and 25). Negative results for the test for lipid (Sudan IV) show that it is not present in any of the sperm.

Positive results for the DNFB test verify the presence of protein in a variety of places, particularly in the tail piece of eupyrene sperm. It is also found in association with the droplets of both apyrene sperm.

The positive Feulgen reaction verified the position of the nucleus in eupyrene sperm and showed there was no DNA present in either type of apyrene sperm.

III. Ultrastructure of Mature Sperm Types

Due to the size of the sperm of *F. oregonensis*, particularly the eupyrene and lancet type, it was impossible to produce micrographs of

TABLE 2. HISTOCHEMICAL RESULTS

Sperm type	Region	PAS rx	Acetylated PAS	Toluidine blue (metachromasy)	Toluidine blue Extinction pH 4.2	Toluidine blue Extinction pH 5.0	Toluidine blue Extinction pH 5.6	Alcian blue	Best's carmine	Sudan IV	DNFB	Feulgen rx
Eupyrene	Nucleus	-	-	-	ortho	ortho	ortho	-	-	-	-	++++
	Mitochondria	-	-	-	-	-	-	-	+	-	++	-
	Tail piece	++++	-	-	-	-	-	-	++++	-	++++	-
	End piece	-	-	-	-	-	-	-	+	-	+	-
Carrier	Peripheral droplet zone	++++	-	-	-	-	ortho	some droplets +++	cytoplasm around droplets +++	-	++++	-
	Fibrillar core	-	-	-	-	faint ortho	ortho	-	-	-	-	-
Lancet	Anterior droplet zone	++++	-	-	-	-	beta	some droplets +++	small droplets +	-	small droplets ++++, large -	-
	Mid droplet zone	+++	-	-	-	-	gamma	++++	some large droplets -	-	small droplets ++++	-
	Posterior zone	-	-	-	-	-	ortho	+	++	-	small droplets +++	-
	Peripheral fibrillar zone	-	-	-	-	-	-	-	-	-	-	-

Legend: ++++, very strong reaction
++, weak reaction
-, no reaction

longitudinal sections of the entire sperm. I have included composite drawings reconstructed from several electron micrographs to illustrate the salient details.

A. Eupyrene sperm

The term acrosome, coined by Lenhossek (1898) means "tipbody" or "apical body"; this term now, however, refers to a characteristic sperm structure derived from the Golgi apparatus (Franklin, 1970). In eupyrene sperm of *F. oregonensis* the acrosome is conical, the apex of the cone is anterior and the base posterior (fig. 26A, 27, 28 and 29). The acrosome surrounds the subacrosomal space which is filled with a relatively electron transparent substance; within this space lies the subacrosomal granule (Kaye, 1962) (fig. 27). Extending through the acrosomal granule is the subacrosomal rod (Rousset-Galangau, 1972; Longo and Dornfield, 1967; Walker and McGregor, 1968) (fig. 27). The acrosome proper is enclosed in a membrane. The outer sperm membrane or plasmalemma encases this (Fig. 26A, 28 and 29). It is often swollen in appearance, a characteristic of the plasmalemma surrounding the acrosome (Fawcett, 1970). Anteriorly the plasmalemma is markedly swollen forming a bulb (fig. 27), a feature common to other prosobranch sperm (Walker and McGregor, 1968; Garreau de Loubresse, 1971; Giusti and Mazzini, 1973; Buckland-Nicks, 1973).

A broken membrane, referred to as the ragged membrane (Walker and McGregor, 1968; Buckland-Nicks, 1973) runs laterally from the tip of the acrosome to its base (Fig. 26A and 29). It continues posteriorly beyond the acrosome as a solid membrane (fig. 29). I am uncertain of

its fate beyond this point. It may stop or fuse with the anterior portion of the nuclear membrane as in the case of *Nucella* sperm (Walker and McGregor, 1968).

Posterior to the acrosome is a mass which forms an inverted cup (fig. 30 and 70) the centriolar cap, shown in cross section in fig. 31. In the centre of its base is a knob which pushes anteriorly into the subacrosomal space (fig. 28) forming an indenting fossa. It is overlain by the nuclear membrane which now is termed the interstitial membrane (Kaye, 1962) because it separates the acrosome from the nucleus-axoneme complex (fig. 28). The walls of the cap extend down over the distal centriole (fig. 26A, 30 and 70). A similar structure is found in other prosobranchs. It has been termed a modified proximal centriole (Rousset-Galangau, 1972) and a basal body (Buckland-Nicks, 1973), but was not described by Walker and McGregor and Giusti and Mazzini (1973).

Figure 26 includes a reconstruction of the region of the distal centriole. This region of the eupyrene sperm was poorly fixed with every technique tried, therefore the drawing and following description are largely interpretation. The base of the centriole lies inside the previously described cap. The distal centriole is certainly modified. There are no central tubules[5] extending into its base. The base is a dense inner

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[5]The original terminology established for the parts of the sperm flagella by Afzelius (1959) and for flagellar structure of protozoa by Gibbons and Grimstone (1960) will be slightly modified here. The central fibres will be referred to as central tubules; the outer doublet fibres will be referred to as outer doublet tubules and the fibres of the centriole will be referred to as tubules. The convention of labelling the armed tubule of the outer doublets "a" and the adjacent one "b" will be retained, while a third one adjacent to "b" in the centriole will still be named "c".

ring or annulus at the periphery of which are placed evenly 9 tubules (see fig 26-a2). Posterior to this region the cap terminates and the 9 single tubules appear to become doublets which project at acute angles from the inner ring (fig. 26-a3 and 32). The doublets are dense and I think represent tubules "b" and "c". The inner ring may represent nine fused "a" tubules. Two central tubules appear at this point (fig. 26-a2, a3). Move posteriorly and the "c" tubule fades and "a" tubules form (fig. 26-a4) producing the standard axonemal pattern described early in the history of ultrastructure (Afzelius, 1959; Gibbons and Grimstone, 1960) for cilia and flagella (Gibbons, 1961) and sperm tails (Idelman, 1967; Fawcett, 1970). The pattern is two central tubules surrounded by nine peripheral pairs of "a" and "b" tubules.

The central tubules are joined by a bridge (Costello, 1973a) and encircled by a central sheath (Gibbons, 1963) (fig. 26, 35, 37 and 38). From the central sheath extend nine radial links (Gibbons, 1963) or spokes (Costello, 1973a) to the "a" tubule of the peripheral doublets (fig. 36 and 37). A swelling known as the secondary fibre (Gibbons, 1963) can at times be seen midway along the spokes (fig. 26-a4, 26-b and 37). The existence of arms extending from the "b" tubule of doublet 6, as in the standard axonemal plan, is debatable in sperm of *F. oregonensis*. Presumably the central pair of microtubules fade prior to axonemal termination, but micrographs of this were not obtained.

The mature nucleus is a dense cylindrical sleeve. It encircles the axoneme, and is about 27μ in length and 120nm in width posteriorly. It is encased in a closely adhering nuclear membrane which in turn is

covered by the plasmalemma (fig. 26B and 33). The base of the acrosomal core rests on the anterior portion of the nucleus. The nucleus is thickened at this point (fig. 28) forming a postacrosomal dense lamina (Fawcett, 1970), the nuclear lamina.

Posterior to the nucleus is the mitochondrial cylinder, about 36μ in length. There is dense material (fig. 33) between these structures which may hold them together. The cylinder is encased in a membrane, which is also covered by the plasmalemma (fig. 33). The cylinder is composed of one or two mitochondria spiralled around the axoneme (fig. 36). The cristae are almost perpendicular to the axoneme (fig. 26-a3 and 33) and may be rectangular or rounded.

Posterior to the mitochondrion, the axoneme emerges surrounded by glycogen deposits (fig. 26C, c and 37) (Walker and McGregor, 1968; Anderson and Personne, 1970). The glycogen rosettes are arranged in nine wedge-shaped groups; the narrow part of each wedge lies opposite each peripheral doublet of the axoneme (fig. 26-b and 37). Bars run from each outer doublet through the centre of the glycogen wedge (fig. 26-c and 36) to the outer sperm membrane. These are aligned with the spokes of the intra-axonemal space, but are not continuous longitudinally and resemble rungs of a ladder (fig. 26C and arrows, 34). Where connected to the outer doublet the bar forms a "Y", one branch to each doublet (fig. 26-c and 36). At the outer membrane the bar divides into two or three branches to connect to the plasmalemma. Here the outer membrane invaginates, making it appear undulated.

The connecting bars shorten posteriorly and the glycogen disappears. The end piece is the standard nine plus two axoneme (fig. 26-d and 38).

B. Carrier sperm

The carrier sperm is a relatively simple cell at the ultrastructural level. The fibrillar core seen with the light microscope is resolved as a group of axonemes, ranging in number from 30 to over 100 (fig. 41, 42 and 44).

Apically the carrier is rounded and contains a dense granular mass, in which the centrioles are embedded (fig. 39, 41 and 43). As in the eupyrene sperm, the centrioles are modified. Basally they look like rootlets and are solid (fig. 41). The diameter increases posteriorly and a space appears in its centre. At this point, the peripheral mass has an outer shape similar to the triplet formation of a "standard" centriole (fig. 43). However, tubules do not appear hollow or empty in cross section. The centriole broadens further and takes on the formation described for the eupyrene sperm; *i.e.*, 9 pairs of tubules resting on a dense inner ring. Finally 9 peripheral doublets appear and more posteriorly a central pair of tubules emerge (fig. 41). The flagella extend from the modified centrioles which are 40mμ in length. The axonemes run the length of the sperm and have the standard 9 plus 2 pattern. The axonemes together form a median band; its width is vertically oriented and extends from the dorsal to the ventral surface of the carrier sperm. Interspersed among the axonemes are single microtubules (fig. 45) which also run longitudinally in the carrier. A few mitochondria and many glycogen particles in the beta form (Fawcett, 1966) are also found in the cytoplasm (fig. 39 and 43).

On either side of the axonemal cores are numerous large, dense, membrane-bound polysaccharide droplets (fig. 39, 42 and 44). They are absent from the tips of the carrier. The peripheral droplets are in close apposition with the plasmalemma (fig. 42 and 44). There are no other prominent cell organelles, a nucleus is definitely absent.

There is an amorphous substance between the plasmalemmas of the carrier sperm and attached eupyrene sperm. I find no other attachment structures in the membranes or between them (fig. 42). This may explain why the attachment is so tenuous.

C. Lancet sperm

As in carrier sperm, the apical tip of the lancet contains a granular mass into which extend the centrioles (fig. 40 and 46). They are fewer, but otherwise similar to those of the carrier. Axonemes extending from the centrioles have the 9 plus 2 pattern and always number 15 to 17 (fig. 49 and 50).

The location of the axonemes in the lancet is different from the carrier. Posterior to the centrioles, the axonemes diverge toward the periphery and lie just under the plasmalemma (fig. 48, 49, 50, 51 and 52), forming a "cage" of flagella. This accounts for striations seen in the lancet with the light microscope. Between each axoneme, also just under the plasmalemma and running longitudinally are numerous single microtubules (fig. 49). These also add to the cage formation.

Most mitochondria are found just posterior to the centrioles in the lancet (fig. 49). Droplets also begin to appear in the cytoplasm

at this point. In the anterior section where it broadens to form shoulders, the lancet is dorso-ventrally flattened, and small dense polysaccharide droplets are most numerous (fig. 47 and 50). The posterior portion of the sperm is round and narrow in cross section (fig. 51 and 52). Here a second type of larger droplet is more numerous (fig. 48, 51 and 52). Their contents are flocculent in appearance, suggesting that they are mucus. Glycogen deposits, in the beta form, are also found interspersed amongst the droplets in the cytoplasm (fig. 48). Towards the posterior tip of the lancet the axonemes terminate, leaving a peripheral band of single microtubules extending to the end of the sperm (fig. 52, inset). Usually the droplets do not extend this far. The lancet also has no nucleus (fig. 40).

IV. Spermatogenesis

Two sperm lines develop in the testis, the eupyrene and the apyrene.

A. Eupyrene

Figure 53 shows schematically eupyrene spermatogenesis. There appears to be a single type of spermatogonium and several of these cells may be found at the periphery of a testis tubule. These cells are small, about 14μ in diameter, with dense nuclei (fig. 54, 55 and 56). The nuclei characteristically contain a flocculent "sap" through which are distributed many dense chromosomes (fig. 64). The scanty cytoplasm is homogenous and filled with ribosomes. Mitochondria and Golgi are not always evident in the spermatogonia.

After a growth period, the spermatogonium develops into the primary spermatocyte, about 22μ in diameter in *F. oregonensis* (fig.

54, b and 55). Nuclei of spermatocytes are 16μ in diameter, rounded and larger than those of the spermatogonia. Often their chromosomes are found in the spireme formation (fig. 55) or equatorially aligned. Synaptonemal complexes or chromosome cores are evident in some nuclei (fig. 65) of primary spermatocytes presumably indicating meiotic pairing (King and Akai, 1971b).

The cytoplasm of primary spermatocytes is less opaque than that of spermatogonia. A Golgi apparatus is located in a juxtanuclear position (fig. 65); a pair of centrioles is often found in association with it. Smooth endoplasmic reticulum (SER), glycogen and a few mitochondria are dispersed throughout the cytoplasm.

Pairs of cells which I suspect are secondary spermatocytes (fig. 54, c and 58), are transient and never found in abundance in paraffin sections. If not found as pairs early after division of the primary spermatocyte secondary spermatocytes can easily be confused with growing spermatogonia. However, secondary spermatocytes often are placed more lumenally than spermatogonia. Secondary spermatocytes are about 18μ in diameter, the nuclei 11μ. The cytoplasm to nucleus ratio in the secondary spermatocyte is higher than in the spermatogonium. Insufficient ultrastructural evidence was obtained to give fine structural details of the secondary spermatocyte. Presumably it divides meiotically yielding two haploid spermatids.

The spermatids usually are in groups located near the lumen of a testis tubule. Their very round nuclei, about 6μ in diameter, are a characteristic feature (fig. 54-d, 57 and 58). The nucleus of an

early spermatid is ringed with a dense band and centrally is lightly staining; this is observable with both the light and electron microscope (fig. 59 and 66). The cytoplasm of the spermatid is vacuolated and large in volume. The total cell diameter is about 13μ.

Spermiogenesis or the maturation of the spermatid is a complex process. A description of the development of each sperm organelle follows in outline.

1. Acrosome. The acrosome develops from a vesicle (acrosomal vesicle) which rests in the open end of the large horseshoe-shaped Golgi (fig. 67). The acrosomal vesicle enlarges producing the acrosomal cone. By the time the centriole has migrated through the nucleus, the acrosome is rectangular with an apical knob (fig. 71). Running longitudinally around the acrosomal membrane is the ragged membrane, a cylinder of granules which looks like a string of beads in section. Within this cylinder also running longitudinally are microfilaments (fig. 72 and 73). A basal plate develops between the acrosome and nucleus (fig. 71). The plate appears to develop in association with the Golgi external to the acrosomal vesicle. As development proceeds the plate elongates anteriorly pushing up into the acrosome, possibly producing the acrosomal rod in the subacrosomal space. Simultaneously the acrosome takes on its shape.

2. Nucleus. In the newly formed spermatid, the nucleus is round with a peripheral dense ring and an inner less opaque "sap" area as previously described. The dense zone is finely particulate and the

inner area flocculent. The centriole, which appears to be single (fig. 66) lies next to the nucleus. In this region the nuclear membrane is thickened into a band, the nuclear lamina, also noted in spermatids of *Littorina* (Buckland-Nicks, 1974). During centriolar migration through the nucleus, the nucleus fills with the dense chromatin material (fig. 68 and 69).

As the spermatid elongates (fig. 60), the nuclear chromatin forms longitudinal grainy strings or strands (fig. 72 and 73). These begin to thicken and spiral (fig. 74) producing "beaded" strands which fuse radially to produce lamellae or cylindrical sheets (fig. 75). These finally fuse producing a non-granular, homogeneous cylinder, the mature nucleus (fig. 35).

3. Centriole and axoneme. It appears that there is a single centriole in the early spermatid. There is evidence that a centriolar satellite complex develops around the centriole prior to migration (fig. 68). It persists seemingly until the migration is complete (fig. 71) and then disappears. It is composed of 9 arms extending from the peripheral triplets (or doublets as in this case) ending in "Y" shaped connectives on the plasmalemma.

The centriole begins migration at the location of the nuclear lamina pushing it up through the centre of the nucleus. A hollow nuclear canal is formed (fig. 69) and the lamina comes to lie below the acrosome (fig. 28). The centriole definitely bears its cap, the apical modification described previously, during migration (fig. 70 and 71). The axoneme is continually elongating, possibly as proposed

by Gall (1961) and Fulton (1971), by the addition of protein distal to the centriole. This in itself suggests an interesting problem as the complex is moving anteriorly and elongating posteriorly.

4. Mitochondrion. The early spermatid contains 6 to 8 large mitochondria which aggregate juxtanuclearly in one area of cytoplasm (fig. 66). Whether these are Nebenkerne, produced by the fusion of several smaller mitochondria (Phillips, 1970), has not yet been revealed in spermatids of *F. oregonensis*.

The large mitochondria eventually form a ring around the developing axoneme, posterior to the nucleus (fig. 76). Before the mitochondria elongate, the membranes between them dissolve and the mass becomes enclosed in a single membrane (fig. 71). On this basis I refer to the mature mitochondrial spiral as a mitochondrion, however, two mitochondrial masses may be present. I suggest that the mitochondrion forms a closed crescent around the axoneme. One end then begins to spiral around the axoneme posteriorly as the mass elongates.

5. Tail piece. As the axoneme extends posteriorly beyond the mitochondrion, the connecting bars of the mature sperm develop, 9 ladders projecting from the outer droplets of the axoneme. The glycogen rosettes later accumulate around these bars forming 9 wedge-shaped longitudinal bands (fig. 77). Still unknown to me are the mechanics of glycogen formation and final organization in the tail piece. Myelin bodies in the region of the tail piece (fig. 77) are evidence for extra cytoplasm being destroyed.

B. Apyrene

Figures 61, 62 and 63 show schematically spermatogenesis of the carrier and lancet sperm. No meiotic divisions occur during apyrene sperm development. As stated, apyrene sperm develop from a spermatogonium which is not different in appearance from those giving rise to eupyrene sperm. Soon after growth the two types are separable at the ultrastructural level. The cytoplasm of the apyrene spermatogonium becomes "frothy"; *i.e.*, the lumina of the smooth endoplasmic reticulum, SER, fill with an opaque material (fig. 78). Otherwise the cell is similar to the eupyrene spermatogonium (fig. 63-a): the dense grainy cytoplasm contains mitochondria. As growth of the apyrene spermatogonium continues, the nucleus takes on a crescent shape and the chromatin begins to "ball up" (fig. 63-b and c). This is noticeable with both light and electron microscopes (fig. 55 and 79). I refer to this stage as the apyrene spermatocyte. The cytoplasm of the growing apyrene spermatocyte has distinct fine structural characteristics. Cristae of the SER are swollen; one or more dictyosomes are prominent. Often dense bodies can be seen in association with the Golgi (fig. 79). The cytoplasm is dense due to many granules which may be glycogen; mitochondria are present but often difficult to detect in the dense cytoplasm.

As the spermatocyte differentiates into the mature apyrene sperm it undergoes tremendous growth. Simultaneously, three processes are taking place and on the basis of these the apyrene development diverges into the carrier and lancet lines. These processes are best observed

in cells of preparations made for the light microscope. The nucleus never undergoes a division; instead it fragments and the nuclear material becomes vacuolated (fig. 57 and 63-b, c, and d). This nuclear degeneration is the first process in apyrene development. The second is the formation of cilia which extend, like an artist's brush, from the spermatocyte (fig. 63-d). As the cilia are developing they are also extending posteriorly into the cell (fig. 63-e). The third process is the formation of cytoplasmic droplets. This occurs on either side of the advancing ciliary core and gives the cytoplasm a "bubbly" appearance (fig. 63-c, d, 80 and 81). It should be noted that these three processes are dynamic and occurring simultaneously; therefore, it is difficult and artificial to assign stages to the spermatocyte during differentiation.

The vacuolated chromatin is grainy and usually dense (fig. 80 and 81), when viewed with the electron microscope. The chromatin is not expelled but seems to be degraded within the cell. When the cilia begin to form, the carrier and lancet spermatocyte become distinguishable, the carrier having 30 or more centrioles and cilia, the lancet having 15 to 17 (fig. 83, 84 and 85). There is evidence (fig. 81) that the centrioles have the nine peripheral triplet tubules, but the unusual rootlet bases are formed at the spermatocyte stage. A consideration of their formation follows in the discussion.

At the base of the advancing ciliary core, filamentous strands can be observed with the light microscope (fig. 57 and 63-d). They may be microfilaments, but were not observed by electron microscopy.

In the developing spermatocyte, Golgi and SER are very prominent (fig. 79 and 80). As stated the cristae of the SER fill during droplet

formation producing membrane-bound deposits. The droplets were never observed forming in association with the Golgi. In the carrier the peripheral large polysaccharide droplets appear around the ciliary core (fig. 56 and 82) and eventually fill the cytoplasm. Mitochondria can be seen dividing in these cells (fig. 83).

In the lancet (fig. 59 and 60), two types of droplets form. The small peripheral droplets are surrounded with SER and there is some indication that they may fragment from a large droplet. The mucous droplets are surrounded with rough endoplasmic reticulum (RER) initially (fig. 84 and 85). It then becomes a smooth membrane at maturation when the droplets appear lightly staining and flocculent (fig. 85).

The lancet differs from the carrier in that the axonemes or cilia separate from the core arrangement, move through the cytoplasm to lie at the cell periphery, forming a "cage" around the cytoplasm. How this process takes place is unclear and the development of the associated microtubules was not followed.

The observations on development of the apyrene sperm are sketchy. More work is needed on the origins of the two lines (apyrene) and also on the final stages of lancet maturation.

V. General Observations on Reproductive Biology

A. Maturation cycles

Seminal vesicles of male *F. oregonensis* always held some mature sperm, but were extremely swollen during May, June and the first half of July, almost empty in the later half of July and August, and in various states of "filling" throughout the remaining months. The

testis contained developmental stages of sperm throughout the year, but noticeably began to fill up in March and remained in that state till early or mid July.

Females showed a pronounced ovarian cycle. Samples taken from "plump" ovaries during May, June, July and early August contained large, yolky oocytes. More females contained ripe ovaries from June to early August. Ovaries checked September through December were not productive and contained few immature oocytes. Their contents build up from December to May. This agrees with Philpott (1921-25) who found ripe females June, July and August.

B. Courtship and mating

These observations were made on the tagged *F. oregonensis* held at "Sealand". The tagging method worked well. The tags remained clear of debris and only 2 (out of 52) were lost. Six (11%) of the population in captivity died between February and August.

F. oregonensis reportedly begins courtship by pairing as early as 5 months prior to oviposition; *i.e.*, as early as March; however, most pairing occurs in May (Eaton, 1971). I have noted pairs at Ogden Point in February, but it is questionable if this is courtship as *F. oregonensis* can often be found in clumps or pairs, not only males on females, but males on males or females on females. Clustering and clinging was observed to occur amongst the tritons in the holding tank as well. The captive whelks began true pairing in March; 3 to 4 pairs were observed at each weekly visit. These were not permanent pairs. In April there were 5 to 6 pairs at each weekly visit while in

May there were 6 to 9 pairs. Not all snails in captivity took part in pairing. However, some (males and females) paired 2 or 3 times. The longest time I observed one pair to remain together was 3 weeks. Eaton (1971) found that mates stayed together for longer times (1 to 2 months) and this applied to many pairs. The conditions of the holding facility I used may have caused this discrepancy in behaviour.

Male *F. oregonensis* mount the female shell from the rear and copulation takes place as described by Eaton (1971). The male finds the correct area or opening in the female's mantle with his tentacles and slips his long phallus under the female's mantle to align with her gonadal aperture. The open sperm groove in the male's phallus is pressed to the female's head forming a "closed" channel. The hairy periostracum of the whelks, particularly of females deteriorated during mating, but began to reappear in August.

C. Sexuality

F. oregonensis are hermaphroditic displaying consecutive sexuality. Of the 52 animals tagged 4 had changed sex from male to female while one had reversed from female to male. At the time of pairing it was noted that males were invariably smaller than their female counterparts. This was also observed by Eaton (1971) and implies that they are protandic.

D. Oviposition

In late June, July and August sperm was readily collected from the copulatory bursa. Oviposition has been described for *F. oregonensis* in

detail by Philpott (1921-25), Howard (1962) and Eaton (1971). Locally it begins in late June at Ogden Point and continues until mid August. Veligers hatch out in September. Egg masses, a spiral mass of "corn kernels", are laid on the vertical stone faces locally at a depth of 3 to 8 metres. The female broods her eggs until they hatch. Eaton (1971) has made some detailed observations on this behaviour.

VI. Sperm Degeneration in Females

As shown, mature eupyrene sperm are stored in the seminal receptacle while mature apyrene sperm remain in the copulatory bursa. I have indicated that any sperm remaining in the copulatory bursa become entangled in its fluids and begin to degenerate. Ultrastructural evidence shows that the lancets are active in initiating plasmodial formations, which entangle other degenerating sperm (eupyrene and carrier) (fig. 86 and 87). The lancets seem to split and change their shape, sending out arms which entangle other sperm. The significance of this is uncertain and the fate of the plasmodia is unknown.

DISCUSSION

I. Morphology and General Reproductive Biology

There is a trend in the Mollusca towards an increasing complexity of the reproductive systems. They change from simple systems, lacking elaborations of efferent ducts (exhibited by amphineurans, bivalves and some prosobranchs) to complex, with diverse elaborations of efferent ducts (exhibited by prosobranchs, opisthobranchs, pulmonates and cephalopods). Concomitantly there is a change from external to internal fertilization. There is also a trend for the once associated coeloms (pericardial, renal and gonadal) to lose their association with one another. This is true for *F. oregonensis*.

The male reproductive system in *F. oregonensis* is comparable to those of other mesogastropods including *Bithynia* (Lilly, 1953), *Buccinum* (Grassé, 1968) and *Littorina* (Linke, 1934; Fretter and Graham, 1962; Buckland-Nicks, 1974). Morphologically the reproductive tract is advanced for a mesogastropod, except for the open prostate gland and grooved sperm duct extending from the prostate to phallus tip, which are both primitive features (Fretter and Graham, 1962). In *Littorina sitkana, L. scutulata* (Buckland-Nicks, 1974) and *Bithynia tentaculata* (Lilly, 1953), the prostate is closed and consists of tubules lined with secretory cells. Some epithelial cells lining the prostate of *F. oregonensis* are secretory. An open sperm duct is also found in *L. sitkana, L.scutulata* (Buckland-Nicks, 1974), *L. littorea, L. obtusata, L. nudis* (Linke, 1934) and *L. irrorata* (Bingham, 1972).

B. tentaculata exhibits the most advanced state of the mesogastropods, with a closed and internal sperm duct in the penis (Lilly, 1953). The phallus of *F. oregonensis* is long, adapted to reach the copulatory bursa of the female, flat, to fit under the female's mantle, and with the sperm groove so placed that it is sealed by the female's head during copulation. It can be extended by hemolymph engorgement (due to internal blood sinuses) and is also muscularly controlled. It lacks any associated organs such as the mammiliform glands found in *L. sitkana* (Buckland-Nicks, 1974) or a secondary gland as found in *B. tentaculata* (Lilly, 1953), but secretory cells contributing to the seminal fluid are distributed in the lining of the groove.

Advanced characteristics found in the male tract of *F. oregonensis* are: the small storage organ for mature sperm (the seminal vesicle); the closed sperm duct which enters the prostate gland; the presence of a prostate gland; secretory and ciliated cells lining the entire tract; and the well adapted phallus. "Primitive" characteristics are the open prostate and sperm groove. Nevertheless the reproductive tract of male *F. oregonensis* is advanced, approaching the neogastropod state. Laxton (1969) also holds this view for other cymatiids.

The female reproductive tract also exhibits some advanced characteristics being similar in some respects to that of the neogastropod *Nucella lapillus*. The position of the seminal receptacle, however, differs. In *N. lapillus* it is a small pouch posterior to the albumen gland. In *F. oregonensis* and in another

mesogastropod *Pomatius elegans* (Fretter and Graham, 1962), the upper part of the oviduct serves as the seminal receptacle. I suggest that this is an advantage because eupyrene sperm there are in position to fertilize mature eggs before they become enclosed by albumen or capsule gland secretions. *F. oregonensis* is peculiar among the cymatiids with respect to the seminal receptacle. In other cymatiids, the seminal receptacle is an outpocketing of the albumen gland (Laxton, 1969).

Fertilized eggs pass to the albumen gland where they are coated with fluid albumen. Presumably *F. oregonensis* is similar to other cymatiids in that two secretions are produced in the albumen gland (Laxton, 1969). By contrast three types are produced in neogastropods (Fretter and Graham, 1962).

On the basis of stained preparations I suggest that the capsule gland of *F. oregonensis* produces two secretions, as do those of New Zealand cymatiids (Laxton, 1969) and *N. lapillus* (Fretter and Graham, 1962). Egg capsules of *F. oregonensis* are composed of protein (24%), lipid (11%) and polysaccharide (2%) (Stickle and Mrozek, 1973). The female genital aperture is capable of great dilation to allow for oviposition of large capsules.

The copulatory bursa is another organ for storage of sperm; however, it is morphologically distinct from the seminal receptacle. Their relationship in the molluscs is interesting, and has been reviewed by Thompson and Bebbington (1969), Beeman (1970) and Schmekel (1971) in opisthobranchs, Duncan (1958) in pulmonates, and Fretter (1941), Fretter

and Graham (1962) and Ghiselin and Wilson (1966) in stenoglossans. The copulatory bursa of *F. oregonensis* receives and stores all three types of sperm for a short time, whereas the seminal receptacle receives and stores only one, the eupyrene.

Functionally the copulatory bursa both stores and sorts sperm. Only eupyrene sperm leave it to ascend the female tract; apyrene sperm never reach the eggs. Cells lining the bursa are secretory, their products perhaps inhibiting motion of apyrene sperm and effectively trapping them. Only active eupyrene sperm can enter the capsule gland. The viscous bursal secretion may act also as a minor food source for eupyrene sperm.

Apyrene sperm degenerate in the bursa. Electron micrographs show autolysis taking place in apyrene sperm; the bursal secretion may also contain a membrane lysin to assist in this degeneration. It has been suggested that epithelial cells of the copulatory bursa of *L. scutulata* and *L. sitkana* ingest apyrene sperm and break-down products (Buckland-Nicks, 1974); no evidence of this was found in *F. oregonensis*.

Thus the female reproductive tract is a mixture of advanced and less advanced characteristics. The seminal receptacle for the storage of eupyrene sperm, although not a newly elaborated organ as in neogastropods (Fretter and Graham, 1962) is a modification of an existing structure. The copulatory bursa is functionally and structurally advanced. It sorts sperm, and is divided into a receiving region and a holding area. The gland complex is elaborate as in neogastropods, although the albumen gland apparently produces one less secretion. This may be a

more primitive mesogastropod condition. The capsule gland appears to be equally as complex as that of neogastropods, both histologically and functionally.

Associated with internal fertilization, which is a direct result of elaborate reproductive systems, is a tendency toward a more complex sexual behaviour. Undoubtedly this is epitomized by the pulmonates and cephalopods (Galtsoff, 1961). The cymatiids are prosobranchs which do exhibit mating behaviour, but it is less elaborate than that of the previous two classes. *F. oregonensis* undergoes courtship and pairing three to four months prior to copulation, with pairing lasting up to a month. I found that the incidence of pairing and therefore probably mating increased during May. This coincides with the gonadal maturation cycles. Although females are often fertilized before their oocytes are mature, sperm can be stored in the seminal receptacle until the oocytes are ready to be fertilized. *F. oregonensis* also shows pair fidelity; *i.e.*, the propensity of a male to return to his original mate after separation (Eaton, 1971).

After egg laying, females brood the capsules for eight to nine weeks or until veligers hatch (Eaton, 1971). Brooding has also been observed in New Zealand cymatiids (Laxton, 1969). It does not aid in the development of the eggs (Laxton, 1969), but is a protective gesture, keeping would-be predators away from eggs (Eaton, 1971). This adds to the complexity of the sexual behaviour. *F. oregonensis* is certainly hermaphroditic exhibiting consecutive sexuality, but it may in fact be protandic.

II. Eupyrene Spermatogenesis and Structure of Mature Sperm

Development of eupyrene sperm in *F. oregonensis* is essentially similar to that process in other invertebrates and particularly other prosobranchs. Four motile sperm are produced from a single spermatogonial cell. The following is a consideration of the genesis and structure of important eupyrene sperm parts.

A. Acrosome

The method of acrosomal formation observed in sperm of *F. oregonensis* is found in developing sperm of many gastropods (Rousset-Galangau, 1972; Giusti and Mazzini, 1973). In all, the acrosome forms in the Golgi. In the pulmonate *Nerita senegalensis*, acrosomal development is claimed to take place without Golgi influence (Garreau de Loubresse, 1971). This is a remarkable exception if the observations are correct.

The acrosome of *F. oregonensis* sperm develops from one granule, rather than by the coalescence of several as in the bivalves *Mytilus* (Longo and Anderson, 1967), *Spisula* (Longo and Anderson, 1969) and *Crassostrea* (Daniels *et al.*,1971). The basal plate lying under the developing acrosome forms simultaneously with the cone and may contribute to the subacrosomal structures. Filaments have been implicated in cell elongation and motility (Wessels *et al.*, 1971). Their presence at the periphery of the developing acrosome could account for its elongation. The granules lying alongside the length of the acrosomal membrane probably become the ragged membrane of the mature sperm.

Despite the diversity of acrosomal structures described, Franklin (1970) points out that there is a plan of organization basic to all.

Acrosomes are invariably bipartite, consisting of a membrane-bound cap portion and an underlying structured or unstructured granular mass (Idelman, 1967; Franklin, 1970). Austin (1968) suggests the cap portion be termed the acrosome and the underlying material, subacrosomal substance. I have adopted this terminology.

Gastropod acrosomes generally are similar to those of bivalves (Longo and Anderson, 1967, 1969; Daniels *et al.*, 1971), while the structure in cephalopods is distinct (Longo and Anderson, 1970; Maxwell, 1974; Fields and Thompson, 1976). Eupyrene sperm of *F. oregonensis* have a typical gastropod acrosome comparable with those of several species of the class (Yasuzumi and Tanaka, 1958; Walker and McGregor, 1968; Giusti, 1969; Garreau de Loubresse, 1971; Giusti and Mazzini, 1973; Buckland-Nicks, 1973).

The acrosomal region in sperm of *F. oregonensis* is most similar to that found in cirripedes (Munn and Barnes, 1970) and polychaetes (Potswald, 1967; Fallon and Austin, 1967), apart from other molluscs.

B. Nucleus

The type of nuclear development found in eupyrene sperm of *F. oregonensis* is not uncommon and has been documented for other gastropods (Walker and McGregor, 1968; Ohsako, 1971; Rousset-Galangau, 1972; Buckland-Nicks, 1973; Giusti and Mazzini, 1973; Henley, 1973 and Takahashi, Nishimura and Yamagashi, 1973) although descriptions vary in some details. Rebuhn (1957) may have been the first to describe the process of nuclear condensation. Walker and McGregor (1968) have given the least confusing report of nuclear condensation in a gastropod,

Nucella, which compares favourably with Rebuhn's description. During nuclear elongation the chromatin condenses, forming interlocking strands arranged longitudinally with respect to the flagellar axis. The strands take on a helical configuration and fuse to form lamellae radially arranged around the axoneme. Finally the lamellae condense producing a dense nuclear sleeve.

The process of nuclear shaping has yet to be clarified. Manchettes composed of microtubules have been observed in sperm of *Acheta domesticus* (Kaye, 1962), *Aeschna grandis*, a dragonfly (Kessel, 1966), *Lumbricus terrestris* (Anderson *et al.*,1967), *Melanoplus d. differentialis* (Kessel, *et al.*, 1967) and *Eledone cirrhosa* (Maxwell, 1974) around the spermatid nucleus, prior to elongation. Therefore, it has been suggested that the tubules play a role in the lengthening and shaping of the nucleus. Bloch and Hew (1960) found that in sperm of *Helix aspersa* fine electron-dense filaments formed in the nucleus just prior to the final nuclear shaping. They also found that there was a simultaneous change from a lysine-rich to an arginine-rich dominant histone in the nucleus and concluded that a change in proteins produced the structural change. Fawcett *et al.* (1971) proposed a theory, based on the previous work (Bloch and Hew, 1960) that the shape of the nucleus may be determined from within by a genetically controlled pattern of nucleoprotein aggregation. Bergstrom and Arnold (1974) have shown that condensation of chromatin appears directly opposite the microtubules of the manchette in *Loligo* and suggest this is also true for *Drosophila*, *Lumbricus*, and *Aeschna*. They propose that genetic control of nucleoprotein aggregation and microtubule influence

shape the nucleus. A single theory explaining nuclear shaping has yet to be proven as many sperm nuclei such as those of *F. oregonensis* develop without the presence of microtubules.

C. Centriole and Axoneme

The centriolar arrangement found in primitive sperm (as defined by Franzen, 1969) is considered the standard plan of organization; *i.e.*, two centrioles, the distal giving rise to the axoneme and the proximal lying perpendicular to it, located at the tail end of the head piece. However, there are many species in which differences from this standard centriolar arrangement occur.

Some sperm have two centrioles, but their arrangement is not as described above, *e.g.*, in sperm of the urchin *Lytechinus variegatus* (Marshall and Luykx, 1973), the horseshoe crab *Limulus polyphemus* (Fahrenbach, 1973) and scorpions of the Family Vejovidae (Jespersen and Hartwick, 1973). Furthermore, secondary structures may be found in association with the centrioles, which themselves often have an unusual arrangement; *e.g.*, in the sperm of insects *Melanoplus* and *Neombius* (Gatenby and Tahmisian, 1960) and in sperm of the cockroach *Eublaberes* (Lindsey and Biesle, 1970). Phillips (1970) reviewed insect sperm structure, suggesting there is a pair of centrioles in sperm of most species. This suggestion may be incorrect, since Friedlander and Wahrman (1966, 1971) have shown that many insect sperm have a single centriole.

It is difficult to describe the centriolar arrangement for the gastropods. Descriptions in past publications are not always clear;

however, one common point shown by them all is that the centriolar arrangement is aberrant. A single centriole (Gall, 1961) exists in eupyrene sperm of *Viviparus* but it has the classical centriolar substructure (Fulton, 1971). A single centriole is described in *Helix* sperm (Anderson and Personne, 1967). It has an auxiliary modification, a dense conical basal body wedged into the nucleus; the centriole lacks the standard structure but retains its inherent ability to initiate movement in the axoneme. *Nucella* (Walker and McGregor, 1968) has a single centriole of standard structure which supposedly never replicates. Ohsako (1971) described a modified complex in *Radix* sperm. The flagellum extends from a distal centriole which has a cap lying against the nucleus. The cap is thought to be a derivative of the proximal centriole. Such an arrangement has also been reported for sperm of *Nerita* (Garreau de Loubresse, 1971), *Milax* and *Agriolimax* (Rousset-Galangau, 1972), and *Littorina* (Buckland-Nicks, 1973). Buckland-Nicks (1974) suggests that a procentriole which lies perpendicular to the distal centriole in the spermatid migrates to a position anterior to the distal centriole prior to centriolar migration, producing a cap. Sperm of *Truncatella* at maturity have a single but unconventional centriole like a ciliary rootlet.

Examples of one centriole producing the axoneme and the second being modified as an accessory structure are not limited to the gastropods but are found in cirripedes (Munn and Barnes, 1970), teleosts (Grier, 1973) and Mammals (Fawcett and Ito, 1965).

This evidence points out that modified structure and arrangements of centrioles are far from unusual, particularly in gastropods. The single modified centriole in eupyrene sperm of *F. oregonensis* is similar to those of *Radix, Nerita, Agriolimax, Milax* and *Littorina*. The homology and origin of its cap-like structure is yet uncertain. It could be a modified proximal centriole as suggested for the previously mentioned examples, but two centrioles were never observed in developing spermatids. This point needs clarification by further study.

I previously suggested that the inner indistinct ring of the existing distal centriole may represent nine fused "a" tubules. There are connections between the outer doublet tubules in sections of cilia of *Tetrahymena* (Allen, 1968); possibly the ring is a fusion of such connections plus "a" tubules.

At the late spermatid stage prior to centriolar migration, there is a satellite structure surrounding the distal centriole. It compares with the centriolar satellite complex listed for various sperm by Summers (1972) and for holothurian sperm by Atwood and Chia (1974). The satellite complex is non-existent in the mature sperm of *F. oregonensis* and presumably disappears during elongation. A transient satellite complex has been noted in *Strongylocentrotus* (Longo and Anderson, 1969). It may correspond to the "ring centriole" of light microscopists.

Szollosi (1964) proposed that the satellite complex provided structural support by anchoring the distal centriole to the plasmalemma. Summers (1972) suggests that it may function to coordinate sperm

flagellar movement. Nelson (1967) proposes that it may function in the transport of ATP from the mitochondrion to the flagellum or may itself contain a contractile protein. The function of the satellite complex in sperm of *F. oregonensis* is unknown as it disappears before sperm maturity. It might conceivably function in anchorage during the initial stages of flagellar growth.

D. Mitochondrion and Tail Piece

Walker and McGregor (1968) and Buckland-Nicks (1973) report that several mitochondria fuse to form a Nebenkern prior to spermatid elongation in prosobranchs. This may occur in maturing sperm of *F. oregonensis*, however, I did not observe the entire process. The existing large mitochondria formed a ring at the base of the nucleus. Outer membranes appeared to fuse producing one or possibly two large mitochondrial pieces in ring formation. One free end wound spirally around the extending flagellum. The cristae tended to align themselves perpendicularly to the axis of the axoneme.

Spiral or helical mitochondria are common in sperm and are found in insects (Pratt, 1968; Phillips, 1970; Jespersen and Hartwick, 1973) and mammals (Fawcett and Ito, 1965; Fawcett, 1970; Phillips, 1974) as well as in many prosobranchs (Yasuzumi and Tanaka, 1958; Gall, 1961; Walker and McGregor, 1968; Garreau de Loubresse, 1971; Giusti, 1971; Buckland-Nicks, 1973; Giusti and Mazzini, 1973), opisthobranchs (Holman, 1972), pulmonates (Anderson and Personne, 1967; Ohsako, 1971) and cephalopods (Longo and Anderson, 1970). The significance of the spiral conformation is unknown. Buckland-Nicks (1973) suggests that

it increases the surface area of the mitochondria to absorb more oxygen from the surrounding medium. Fawcett and Ito (1965) propose that the close and lengthy opposition of the spiral mitochondria to the contractile longitudinal fibres of the tail ensures a short diffusion path for ATP required for motility. It is known that in cilia and sperm tails the energy produced by mitochondria and derived from the dephosphorylation of ATP induces axonemal motility. The dynein arms (Gibbons, 1963) of the outer axonemal doublets are sites of ATPase activity (Gibbons and Rowe, 1965; Gibbons, 1966). Anderson and Personne (1969) also proved the existence of sites of ATPase activity on the outer doublets of the axoneme of *Helix* sperm; such activity has also been shown in the central sheath of lung-fluke sperm (Burton, 1973).

The origin of glycogen deposits (verified using Best's carmine technique) in the tail piece of sperm of *F. oregonensis* is uncertain. Glycogen granules can be seen in the cytoplasm of all developmental stages of these sperm. Intra or extra-axonemally placed glycogen deposits are common in a variety of sperm (Anderson and Personne, 1970; Giusti, 1969; Giusti, 1971; Ohsako, 1971; Silveira, 1973; Giusti and Mazzini, 1973; Buckland-Nicks, 1973; Maxwell, 1974). Glycogen is arranged in a similar pattern to that of *F. oregonensis* in the prosobranchs *Bythinella, Pseudomnicola, Cerithium* (Giusti, 1971) and *Truncatella* (Giusti and Mazzini, 1973); *i.e.*, wedge-shaped aggregates surround connecting bars running from the outer axonemal doublets to the plasmalemma.

Glycogen stores in all sperm are an important endogenous energy source (Anderson and Personne, 1970). Satir (1968) suggests the enzymes regulating synthesis and degradation of a reserve metabolite would be available in the flagellar matrix. Active phosphorylase has been located in the glycogen compartment of *Helix* sperm (Personne, 1966; Personne and Anderson, 1969) and glucose-6-phosphatase activity has been detected in glycogen-containing mitochondria (Anderson, 1968). This suggests that pathways for glycogen utilization are available in sperm. Similarly Bogitsch (1973) suggests the glycogen can be used in the sperm of the gastropod *Biomphalaria* because the necessary enzyme systems are present.

The glycogen reserve in sperm of *F. oregonensis* could serve as a metabolite in the production of ATP. This would be significant during storage in the seminal receptacle. There is some indication that sperm with large glycogen reserves can "switch over" to anaerobic production of ATP by glycolysis (Anderson and Personne, 1970). This may be true for eupyrene sperm of *F. oregonensis* stored in the seminal receptacle.

I can ascribe no function to the connecting bars running through the glycogen aggregates. A strongly positive result for the DNFB reaction suggests that protein is present in large quantities here.

III. Apyrene Spermatogenesis and Structure of Mature Sperm

After reviewing earlier studies by light microscopists of prosobranch apyrene sperm, I find that carrier apyrene sperm of

F. oregonensis closely resemble those of *Strombus bituberculatus* and have structural similarities with apyrene sperm of the whelks *Cerithium vulgatum, Turritella triphiata* and *Bittium reticulatum*. The lancet apyrene sperm of *F. oregonensis* are like the apyrene sperm of *Murex trunculus, Aporrhais pespelicani, Fusus* sp. and *Nassa reticulata*. All of these prosobranchs except *F. oregonensis*, however, have only one type of apyrene sperm.

Apyrene sperm development is direct; *i.e.*, one spermatogonium produces one mature apyrene sperm, as also found in *Strombus bituberculatus* (Reinke, 1914) and *Goniobasis laqueata* (Woodard, 1935). The spermatogonia producing the two lines (apyrene and eupyrene) must be cytochemically different although similar in appearance. A difference between the types of sperm is not perceptible until the abortive nuclear division begins; the apyrene line is thereafter easily recognized. Soon after, droplets and cilia begin to form. The differences in structure and organization of the droplets and cilia distinguishes the two types of sperm of the apyrene line from one another. This raises a question as to how the three types of sperm are produced. Are there originally three spermatogonial cell lines or a single line which is modified to produce three types of sperm?

A. Nucleus

I saw no evidence to indicate that products of nuclear degeneration are extruded from the developing sperm. Yasuzumi *et al.*, (1967) found diphosphatase activity in the Golgi of developing atypical sperm of *Cipangopaludina*. If this is true for apyrene sperm of *F. oregonensis*,

the Golgi apparatus may be the site of hydrolysis of nucleic acids and protein which could be converted at the same site to polysaccharides or other metabolites.

B. Droplets

During the nuclear fragmentation stage, a high cytoplasmic RNA content has been found in apyrene sperm of *Opalia crenimarginata* (Bulnheim, 1962) and *Epitonium tinctum* (Bulnheim, 1968). At maturity the RNA disappears. The presence of RNA was not detected in immature apyrene sperm of *F. oregonensis* so speculation as to function of RNA in droplet formation cannot be made.

There are several Golgi areas in both types of developing apyrene sperm and the cytoplasm is filled with both SER and RER. In both types of sperm all droplets apparently develop in the cisternae of the ER rather than in association with the Golgi. The large polysaccharide droplets of the carrier sperm are enclosed in SER and so are the small dense polysaccharide droplets of the lancets. The larger mucous droplets in the lancet are enclosed in RER.

The droplets of the carrier are polysaccharide complexed with protein as shown by PAS (+) and DNFB (+) reactions. A fairly strong AB (+) reaction suggests that the polysaccharide is an acid mucopolysaccharide. The cytoplasm around the droplets and axonemes contains glycogen.

The small dense droplets of the lancet sperm are PAS (+), DNFB (+), exhibit metachromasy with toluidine blue and are AB (+). The evidence strongly suggests that these droplets contain acid mucopolysaccharides complexed with protein. The large droplets

appear to be mucus from their physical appearance in electron micrographs. It may be a neutral mucin since these droplets have a faint reaction with AB and little reaction with DNFB. There also is glycogen in the cytoplasm of these cells.

The mucopolysaccharide droplets of the carrier sperm are full in sperm taken from the testis and seminal vesicle of male *F. oregonensis*. On the other hand, droplets in carriers taken from the copulatory bursa of females are usually completely empty or show degradation. Polysaccharide complexes of a similar type are found in the yolk bodies of oocytes (Bulnheim, 1962, 1968). I suggest that these droplets in carrier sperm are a nutrient source for the attached eupyrene sperm, and that the latter draw on it from the time of maturity in the testis until they leave the female's copulatory bursa. Apyrene sperm of other gastropods *Viviparus viviparus* (Hanson *et al.*, 1952), *Cipangopaludina malleata* (Yasuzumi and Tanaka, 1958), *Opalia crenimarginata* (Bulnheim, 1962), *Epitonium tinctum* (Bulnheim, 1968) and *Viviparus contectus* (Dembski, 1968) also contain droplets of a similar chemical nature which also presumably can supply a nutrient source for eupyrene sperm.

The small dense droplets of the lancet sperm also appear to be a foodstuff. These are liberated in the copulatory bursa of the female when the lancets begin to break down; the eupyrene sperm could take advantage of this alternate food source. Simultaneously, the mucous droplets also are dispersed. Their contents contribute to the fluids of the copulatory bursa. The significance of the mucus is uncertain, but it may stimulate eupyrene sperm to leave the bursa. I think it

more probable that this is another endogenous energy source for the lancets or upon liberation, a minor food source for eupyrene sperm.

C. Centrioles, axonemes and microtubules

The axonemal core and cage of the two atypical sperm are most interesting. Other atypical sperm reportedly have axonemal cores, *e.g.*, those of the gastropods *Viviparus viviparus* (Hanson, Randall and Bayley, 1952), *Cipangopaludina malleata* (Yasuzumi and Tanaka, 1958), *Viviparus malleatus* (Gall, 1961) and the tail pieces of atypical sperm of *Opalia crenimarginata* (Bulnheim, 1962) and *Epitonium tinctum* (Bulnheim, 1968). Multiple centrioles develop which produce the extending cilia of the developing sperm. It was difficult to follow the development and migration of the many centrioles due to their small size and modified structure. Other workers have had similar difficulty. Friedlander and Wahrman (1970) attribute difficulties in discerning centriolar structure of atypical sperm of the moth *Bombyx* to the presence of additional material which masks the basic pattern of nine microtubular triplets. This appears to be true for apyrene sperm of *F. oregonensis*. Friedlander and Wahrman (1971) state that the structure of centrioles is labile and changes with the cell cycle, and these factors make it difficult to determine the structure of centriole. They also suggest that most published micrographs of centrioles are carefully chosen to fit the prototype structure. Another explanation for the structure of such modified centrioles has been proposed by Ash and Stephens (1975). When studying developing bivalve gill tissue they found that maturing centrioles did not go through the intermediary stages found in other tissues and that the mature centrioles were

modified. They suggested that centrioles of the gill tissue are primitive and never have the standard centriolar structure.

The mode of production of the many centrioles is obscure. Gall (1961) described the development of centrioles in apyrene sperm of *Viviparus malleatus* which have a multiflagellar core. Each spermatocyte has two "normal" centrioles around which forms a ring of procentrioles. These mature to yield centrioles having a standard structure, which become bases for the flagella.

Sororkin (1968) found that in rat lung, centrioles were produced in the manner described by Gall, but without the presence of "parent" centrioles and without a range of precursors. Dirksen and Crocker (1966) also described a similar acentriolar development for basal bodies in mammalian respiratory epithelium. Fibrous granules, precursors of centrioles, may develop into centrioles in monkey oviduct (Anderson and Brenner, 1971) in the absence of a parent centriole, while "induced" production also takes place. The latter authors propose that there may be a fundamental organizer present which initiates development of fibrous granules (centriolar precursors) which may be found associated with existing centrioles or in the cell cytoplasm. "Although this type of centriole multiplication from fibro-granular material,...the so-called *de novo* formation, superficially appears to be different from replication of centrioles in preparation to mitosis, all pathways leading to the production of centrioles presumably start from the same molecular precursors" (Friedlander and Wahrman, 1971).

Fibrous material or fibrogranules are common in the cytoplasm of early apyrene spermatocytes of *F. oregonensis*. Whether one or two parent centrioles are present in these cells is as yet uncertain. The type of centriolar production first outlined; *i.e.*, induction of procentrioles by the parent centriole, is a conceivable method of development for the lancet apyrene, as fifteen to seventeen centrioles only are produced. However, thirty to one hundred centrioles are produced in the carrier. Fifteen to fifty procentrioles would therefore have to develop around each parent centriole if there were two, or a succession takes place whereby procentrioles continually form around new centrioles. I suggest that in the carrier apyrene sperm the second method described; *i.e.*, "acentriolar" production takes place. Centrioles may be formed from fibrogranule precursors in the cytoplasm in this case. This distinction may not be significant if there is a common fundamental mode of development.

The apyrene centrioles migrate through the cell cytoplasm as do eupyrene centrioles; the modified centriole structure may be related to this. However, there is a difference in arrangement of axonemes in the two apyrene sperm. The carrier contains an axonemal core, while the lancet has an axonemal cage. This may account for the difference in their motility patterns, the carrier "wiggles" while the lancet is less active and flexes from side to side.

Also related to this difference in motility may be the ring of single microtubules which run longitudinally between the axonemes in the lancet. Microtubules have been implicated in morphogenesis, the

shaping of cells and organelles (Byers and Porter, 1964; Tilney and Gibbons, 1969; Wessels *et al.*, 1971; Hasskare *et al.*, 1973) and in intracellular support (Silveira and Porter, 1964; Anderson *et al.*, 1967), providing a contractile though rigid cytoskeleton (Anderson *et al.*, 1967).

During early development in the lancet, sixteen to seventeen axonemes are centrally placed in the cell, while no microtubules are conspicuous. The tubules apparently form when the axonemes move to the cell periphery and may, in fact, take part in this process. They may also act as a cytoskeleton. The "flicking" motion of the lancet may be a result of the structural combination of axonemes and microtubules, the latter providing a semi-rigid skeleton for the cell, while the axonemes undulate. This motion may be significant in the testis, and may in part be responsible for liberating developing sperm cells from the blind tubules into the main lumen.

IV. Spermatozeugmata and the Function of Apyrene Sperm

Eupyrene sperm of *F. oregonensis* attach to carrier apyrene sperm in the testis and remain attached until the seminal fluid reaches the copulatory bursa in a female. The acrosome of eupyrenes is not responsible for the attachment. It has been suggested (Buckland-Nicks, 1973) that the "attachment power" or stickiness is inherent in the bulbous tip of eupyrene sperm. This may be true for *F. oregonensis*, but there is some selectivity of attachment. Eupyrenes never attach to lancets. This suggests that the surface membranes of carrier sperm may be modified in certain areas, providing attachment sites.

Buckland-Nicks (1974) describes septa running through an amorphous zone between nurse cells and attaching eupyrene sperm in *Littorina*. A dense area exists between attaching eupyrene heads and carrier sperm of *F. oregonensis* but there are no septa.

As outlined in the literature review, various functions have been ascribed to atypical sperm of prosobranchs. These include: providing a stimulus to oocytes to complete development; providing a nutrient source for eupyrene sperm; producing a secretion to direct eupyrene sperm to maturing oocytes or to stimulate eupyrene sperm to fertilize eggs; and providing, after breaking down, a nutrient source for epithelial cells of semen-holding structures.

Carrier apyrene sperm of *F. oregonensis* contain polysaccharide-rich droplets. These are empty when the carrier sperm reach the copulatory bursa of females. Eupyrene sperm attach to carriers in the testis, may be stored there for some time (up to six months), and remain attached when the semen passes through the lengthy male reproductive tract to the copulatory bursa of the female. Eupyrene sperm are metabolically active throughout this time. I suggest that the carrier is acting as a nurse cell for eupyrene sperm, the close association of eupyrene sperm heads to the nutrient-containing droplets of the carrier reinforce this hypothesis.

Although fertilization is internal in *F. oregonensis*, the sperm duct is an open groove, which may allow some sperm loss. *F. oregonensis* does not produce spermatophores but, rather spermatozeugmata. Sperm loss may be minimized by transferring these motile sperm "packets" instead of single sperm.

Lancet apyrene sperm may, as suggested earlier, help to release eupyrene spermatids to the lumens of testis tubules. Lancets also contain polysaccharide-rich droplets plus a mucus-containing droplet. Both substances are released into the copulatory bursa when the lancets begin to break down. The polysaccharide droplets could provide nutrients for eupyrene sperm which have not left the copulatory bursa, or to the epithelial cells of this structure. The mucus also contributes to the viscous liquid contents of the bursa, possibly providing a stimulus to eupyrene sperm to leave this temporary holding structure. During degeneration of any apyrene or eupyrene sperm remaining in the bursa, the lancets initiate a plasmodial formation or gathering up of cellular debris. It is uncertain whether this clumped debris is ejected or absorbed by the lining of the copulatory bursa.

The functional significance of apyrene sperm remains obscure. The ideology supported by Goldschmidt (1916) that apyrene sperm are somehow an expression of "femaleness" lacks factual proof, but has been supported until very recently (Bulnheim, 1962; 1968). Apyrene sperm of *F. oregonensis* are structurally complex and may have several functions, primarily acting as accessory nutrient sources for eupyrene sperm. Their functional adaptations do not fit readily into the Goldschmidt theory.

Roosen-Runge (1973) suggests that deviant cell development occurs during spermatogenesis commonly in the animal kingdom. The deviant cells have some chromosomal defects, displayed at either the first or second meiotic divisions, which produce aberrant sperm. The resultant cells

usually degenerate. This loss serves to select and remove gametes which are not suitable for propagation (Roosen-Runge, 1973). Furthermore he suggests the apyrene sperm of gastropods have evolved from such aberrant sperm. Apyrene sperm are morphologically and functionally adapted to enhance fertilization though they themselves are incapable of the fertilizing act.

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APPENDIX I

Sperm Ultrastructure

I.	Coelenterata		
	A. Hydrozoa	*Phialidium gregarium*	Szollosi, 1964
		Pennaria tiarella	Summers, 1970
		Hydra cauliculata (spermatogenesis)	Moore and Dixon, 1972
		Eudendrium ramosum	Summers, 1972
	B. Scyphozoa	*Nausithoë*	Afzelius and Franzen, 1971
	C. Anthozoa	*Bunodosoma cavernata* (spermatogenesis)	Dewell and Clark, 1972
II.	Ctenophora		
	A. Nuda	*Beroë ovata* (spermatogenesis)	Franc, 1973
III.	Platyhelminthes		
	A. Turbellaria	*Geoplana pasipha*,	Silveira, 1973
		G. leucophryna,	
		Geobia subterranea,	
		Dugesia tigrina	
	B. Trematoda	*Microcotyle erythrini*,	Tuzet, Kjari, 1971
		M. mormyri	

B. Trematoda, cont'd.	*Diclidophora* sp.,	Burton, 1973
	Choricotyle pagelli,	
	Erpocotyle catenulata,	
	Plectanocotyle gurnardi,	
	Trochopus pini	
C. Nematoda	*Deontostoma californicum*	Wright, Hope, Jones, 1973
IV. Annelida		
A. Polychaeta	*Spirorbis niorchi* (spermiogenesis)	Potswald, 1967
	Sabella penicillum (spermiogenesis)	Graebner, Kryvi, 1973
B. Oligochaeta	*Lumbricus terrestris* (spermiogenesis)	Anderson, Weissman, Ellis, 1967
	Lumbricus terrestris (spermiogenesis)	Henley, 1973
V. Mollusca	*Cipangopaludina malleata* (spermiogenesis)	Yasuzumi and Tanaka, 1958
A. Gastropoda	*Viviparus viviparus* (spermiogenesis)	Gall, 1961
B. Prosobranchia	*Nucella lapillus* (spermiogenesis)	Walker and McGregor, 1968
	Sadleriana caprai	Giusti, 1969
	Nerita senegalensis (spermiogenesis)	Garreau de Loubresse, 1971

B.	Prosobranchia Cont'd.	*Bythinella opaca*,	Giusti, 1971
		Pseudamnicola sp.,	
		Hydrobia acuta,	
		Belgrandia caprai,	
		Cerithium vulgatum	
		Littorina scutulata	Buckland-Nicks, 1973
		Truncatella subcylindrica	Giusti, Mazzini, 1973
C.	Opisthobranchia	*Acanthodoris pilosa*	Holman, 1972
D.	Pulmonata	*Helix aspersa* (spermiogenesis)	Anderson, Personne, 1967
		Agriolimax reticulatus	Bayne, 1970
		Radix japonica	Ohsako, 1971
		Milax gagates,	Rousset-Galangau, 1972
		Agriolimax agrestis (spermiogenesis)	
		Limax elavus (spermiogenesis)	Takahashi, Nishimura and Yamagashi, 1973
E.	Bivalvia	*Crassostrea viriginica*	Galtsoff and Philpott, 1960
		Mytilus edulis (spermiogenesis)	Longo and Dornfield, 1967
		Spisula solidissima (spermiogenesis)	Longo and Anderson, 1969a
		Crassostrea viriginica	Daniels *et al.*, 1971
F.	Cephalopoda	*Octopus bimaculatus*	Longo and Anderson, 1970
		Loligo pealei (spermiogenesis)	Bergstrom and Arnold, 1974
		Eledone cirrhosa (spermiogenesis)	Maxwell, 1974

VI. Arthropoda

A. Meratostomata	*Limulus polyphemus* (spermiogenesis)	Fahrenbach, 1973
B. Arachnida	*Tityus bahiensis*	Cruz, Landim and Ferreira, 1973
	Hadrurus arizonensis, *Vejovis puritanus*, *Anuroctonus phaeodactylus*, *Uroctonus mordax* (spermiogenesis)	Jespersen and Hartwick, 1973
C. Pycnogonida	*Nymphon leptocheles*	van Deurs, 1973
D. Crustacea	*Balanus balanus*, *B. perforatus*	Munn and Barnes, 1970
	Astacus leptodactilus	Studitsky and Elyakova, 1970
E. Insecta	*Neombius* sp.,	Gatenby and Tahmisian, 1960
	Melanoplus (spermiogenesis)	
	Melanoplus d. differentialis (spermiogenesis)	Kessel, 1967
	Peregrinus maidis	Herold and Munz, 1967
	Apis mellifera (spermiogenesis)	Hoage and Kessel, 1968
	Eublaberus posticus	Lindsey and Biesele, 1970
	Drosophila melanogaster (spermiogenesis)	Tokuyasu, 1974

E. Insecta, Cont'd.	*Telmatoscopus albipunctatus*,	Baccetti, Dallai and Burrini, 1973
	Psychoda cinerea,	
	Psychoda sp.,	
	Psychoda alternata	
	Acerentulus tragardhi,	Baccetti, Dallai and Fratello, 1973
	Acerentomon majus,	
	Eosentomon transiforium	
	Odagmia pontina,	Baccetti *et al.*, 1974
	Odagmia ornata,	
	Willelmia mediterranea	
VII. Echinodermata		
A. Holothuroidea	*Leptosynapta clarki*	Atwood, 1974
B. Echinoidea	*Arbacia punctulata*,	Longo and Anderson, 1969b
	Strongylocentrotus purpuratus (spermiogenesis)	
	Lytechinus variegatus	Marshall and Luykx, 1973
VIII. Pogonophora	*Siboglinum ekmani*	Franzen, 1973
IX. Chordata		
A. Ascideacea	*Ascidia nigra*	Schabtach and Ursprung, 1965
B. Teleostomi	*Hyperopisus bebe*,	Mattei, Mattei, Reiger and Chevalier, 1972
	Mormyrus rume,	

B.	Teleostomi, Cont'd.	*Gnathonemus niger*,	
		G. senegalensis,	
		Petrocephalus bovei	
		Poecilia latipinna (spermiogenesis)	Grier, 1973
C.	Mammalia	*Myotis lucifugus*,	Fawcett and Ito, 1965
		Eptesicus fuscus	

Figure 1. Gross morphology of the male reproductive system.

cm - columellar muscle; dg - digestive gland; ki - kidney;
ph - phallus; pr - prostate; sd - closed sperm duct;
sg - sperm groove; sv - seminal vesicle; te - testis.

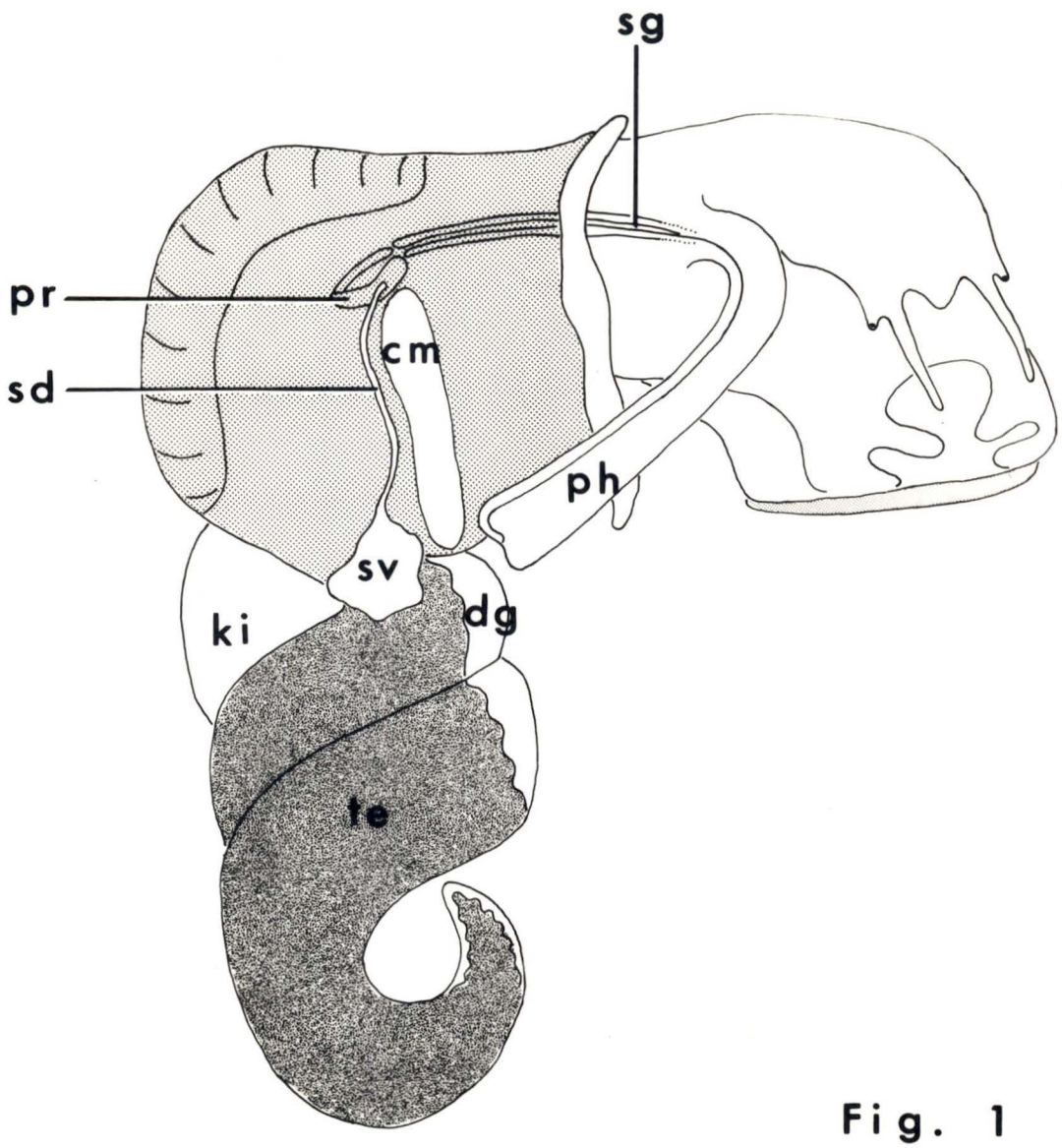


Fig. 1

Figure 2. Parts of the male reproductive system.

a - section through the posterior portion of the prostate at the entrance of the sperm duct; b - section through the anterior portion of the prostate; c - section through the open sperm groove on the snail's head; d - section through the sperm groove on the phallus. pr - prostate; sd - closed sperm duct; sg - sperm groove; sv - seminal vesicle; te - testis.

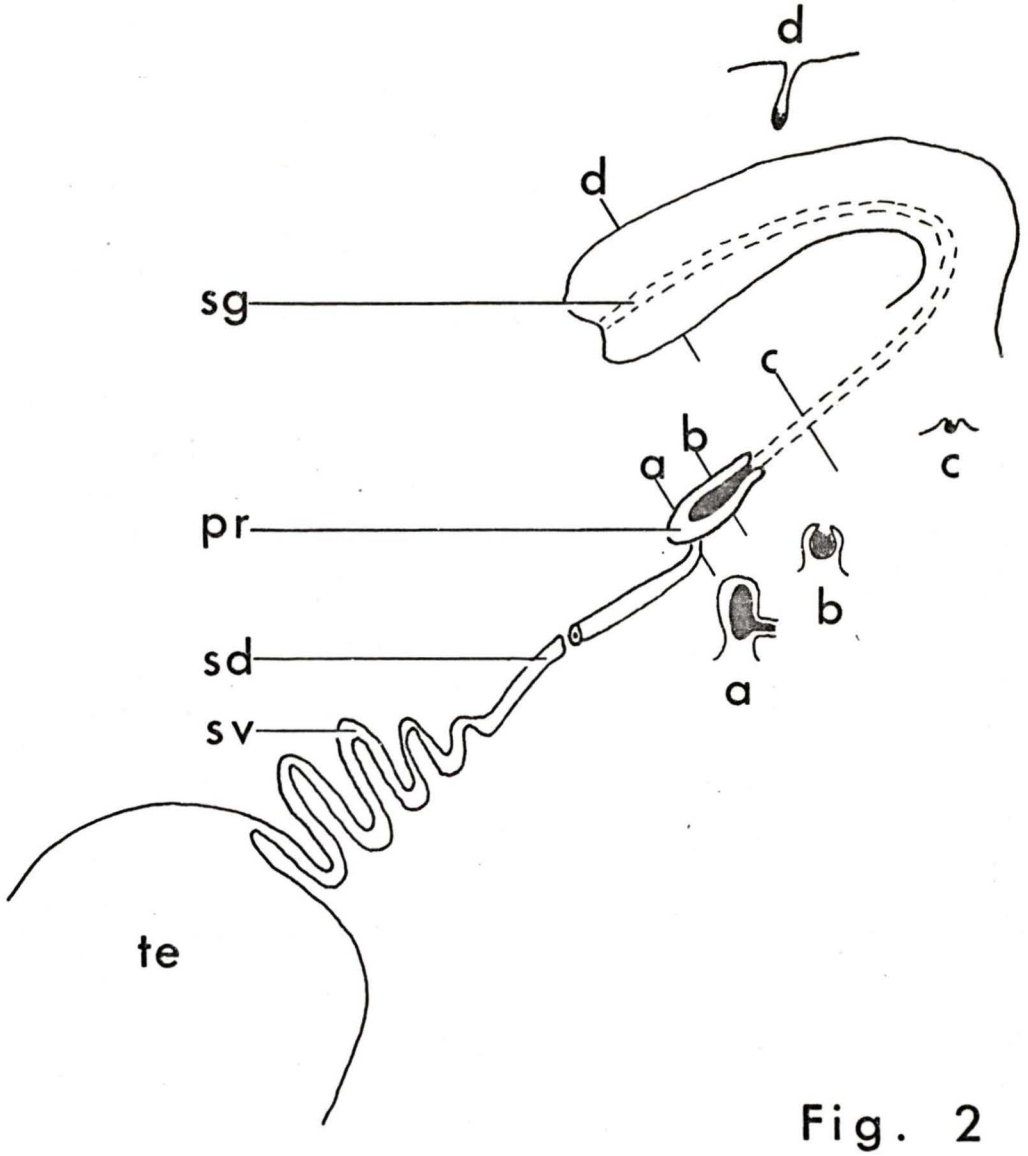


Fig. 2

Figures 3 to 7 are photomicrographs of paraffin sections, stained with haematoxylin-eosin.

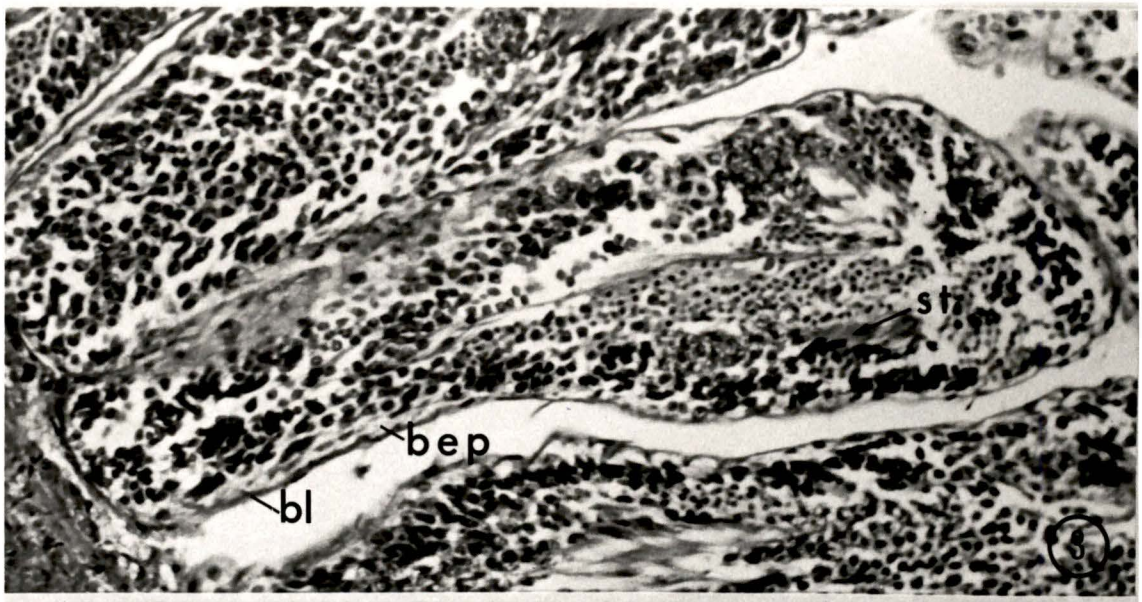
Figure 3. Grazing section through a testis tubule. Spermatids and their tails are in the center of the tubule. bep - basal epithelial cells; bl - basal lamina; st - spermatids. (X 640)

Figure 4. Section through part of a seminal vesicle tubule. as - apyrene sperm; es - eupyrene sperm. (X 1100)

Figure 5. Enlargement of the cells lining the closed sperm duct. Note the cells are ciliated and some are secretory (se). (X 6200)

Figure 6. Section through the anterior part of the prostate. bl - basal lamina; ep - ciliated epithelial cells; arrowheads mark secretory packets. (X 370)

Figure 7. Section through the sperm groove of the phallus. gr - groove; se - secretory packets. (X 460)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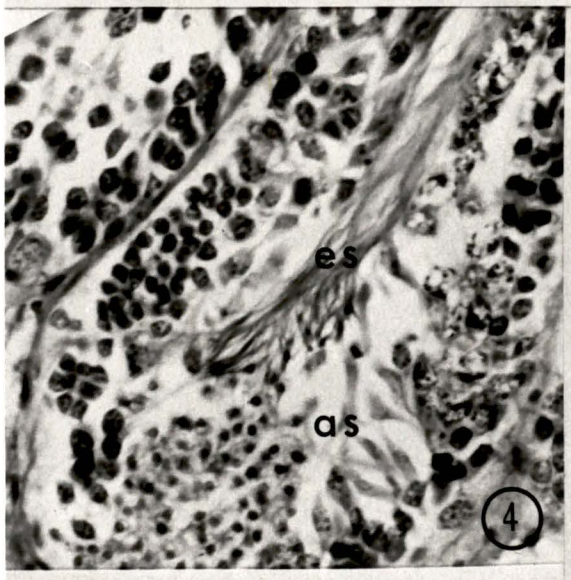
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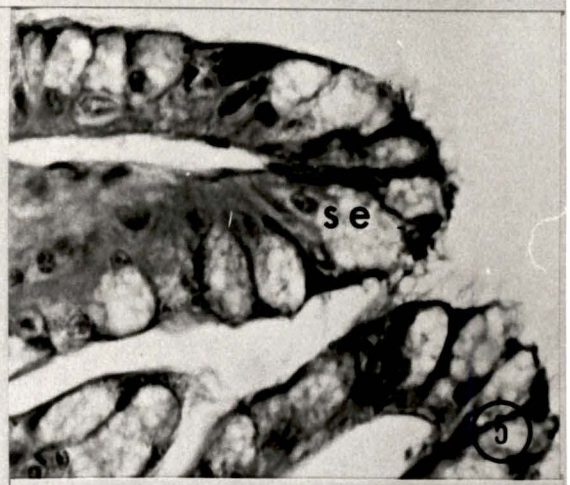
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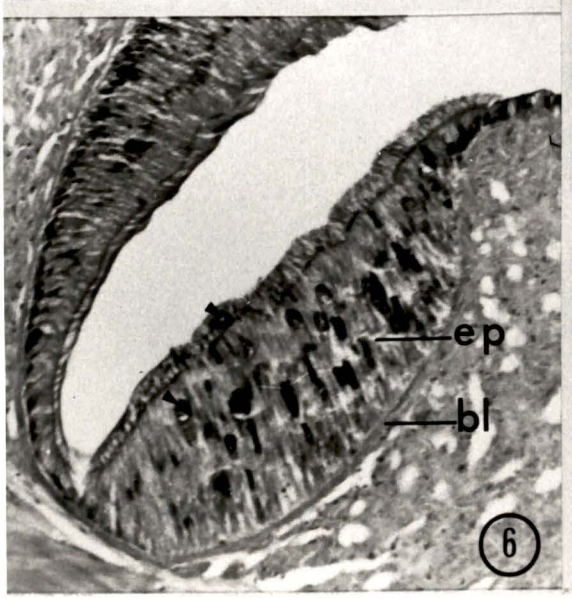
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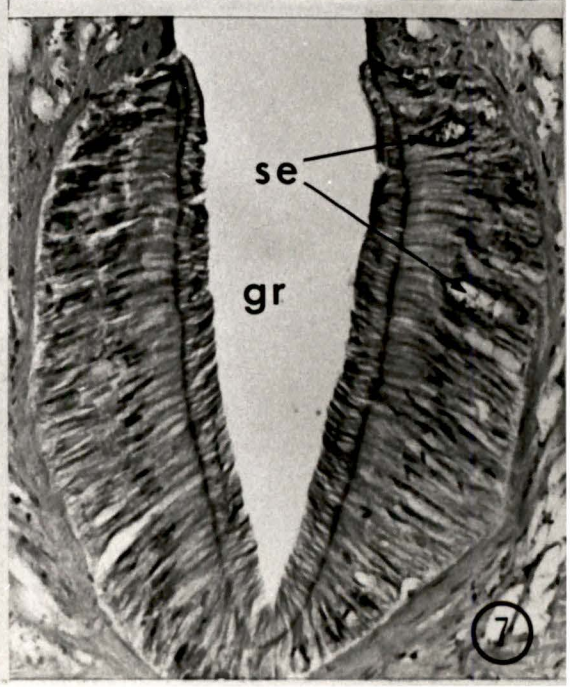
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Figure 8. Gross morphology of the female reproductive system.

ag - albumen gland; cb - copulatory bursa; cg - capsule gland; go - genital aperture; ki - kidney; od - oviduct; ov - ovary; sc - lateral sperm channels; sr - seminal receptacle; vb - vestibule; vc - ventral sperm channel.

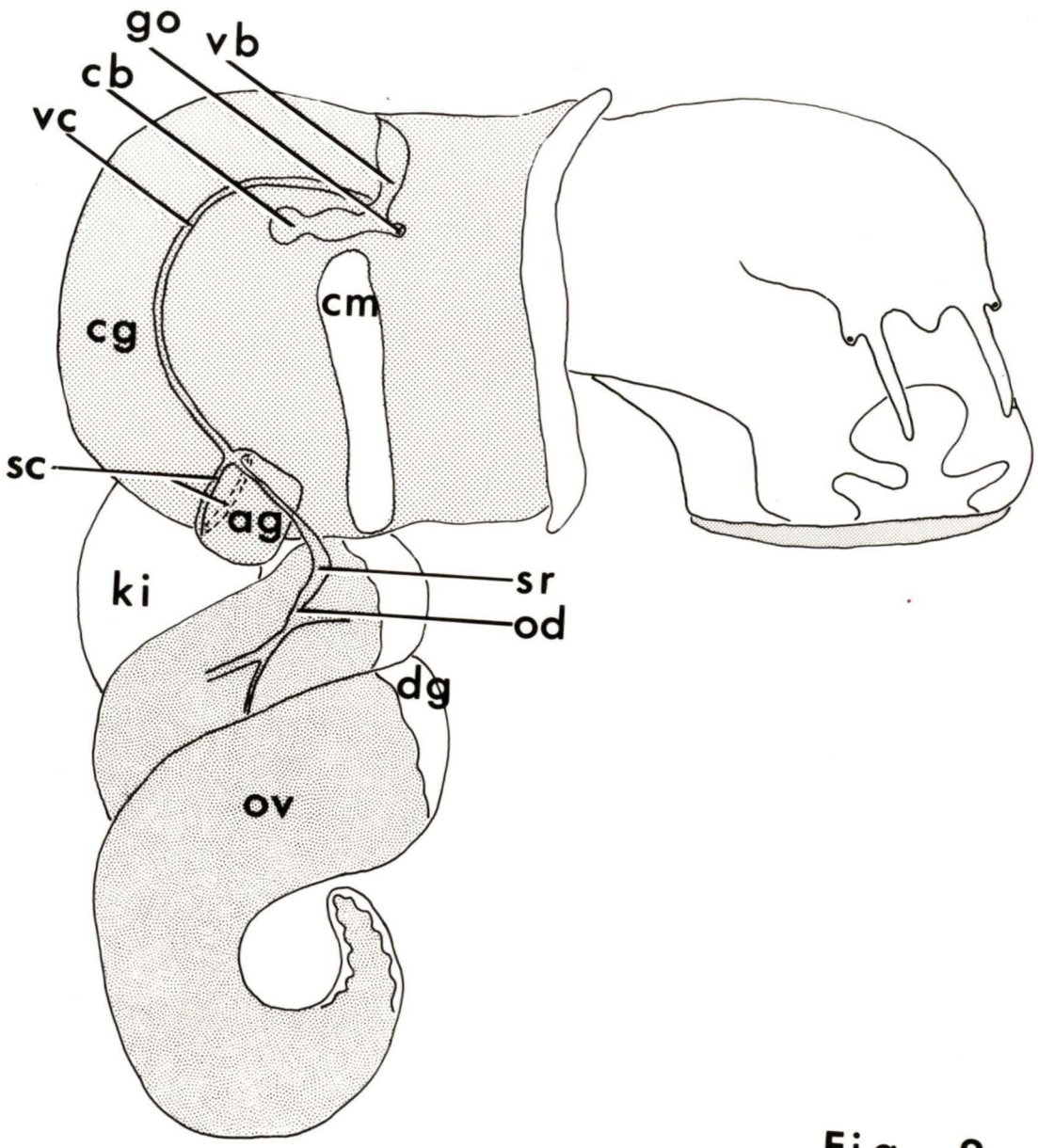


Fig. 8

Figure 9. Parts of the female reproductive system.

a - section through the albumen gland; b - section through the capsule gland and copulatory bursa; c - section through the anterior of the capsule gland. cb - copulatory bursa; go - genital aperture; od - oviduct; ov - ovary; sr - seminal receptacle; vc - ventral sperm channel.

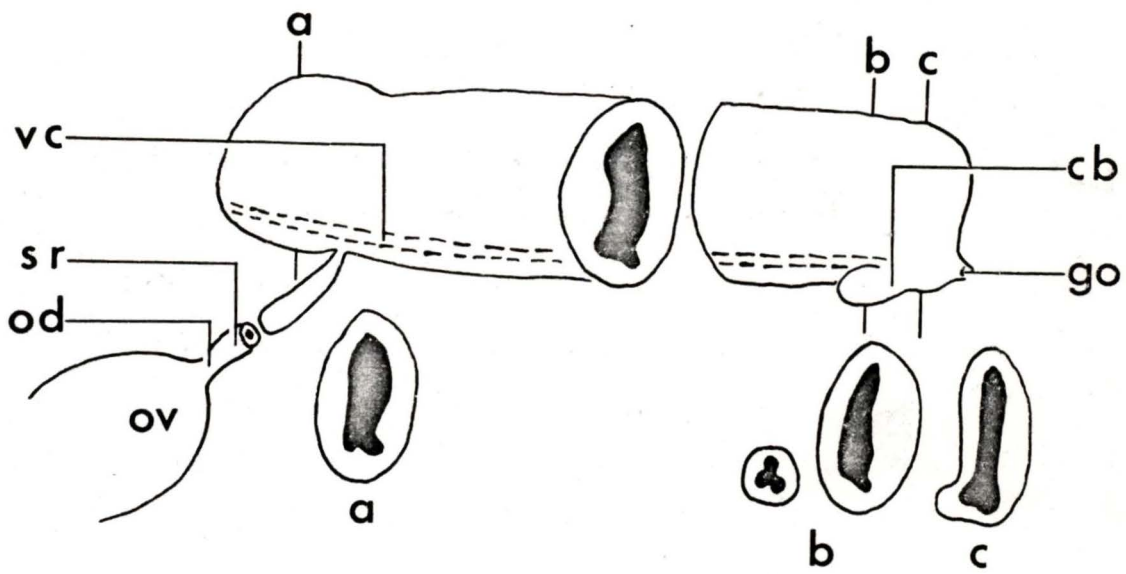


Fig. 9

Figures 10 to 15 are photomicrographs of paraffin sections, stained with haematoxylin-eosin.

Figure 10. Section through part of the ovary.
oc - growing oocyte, larger ones are filled with yolk droplets; og - oogonium. (X 400)

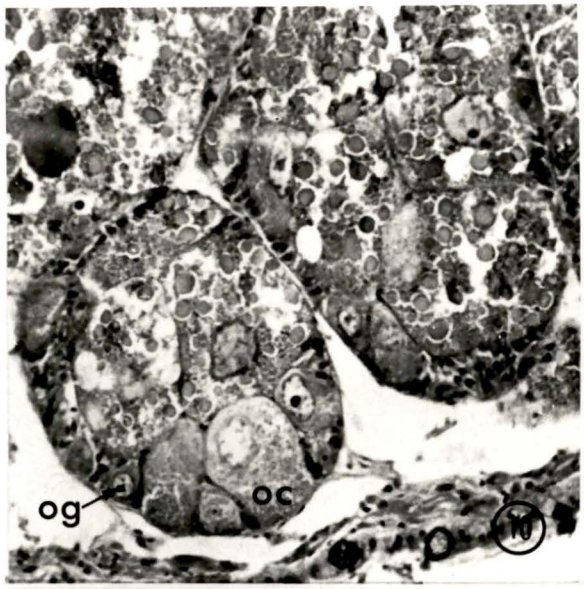
Figure 11. The seminal receptacle filled with eupyrene sperm. Note all the eupyrene sperm are aligned with their heads toward the epithelium.
ep - epithelium; es - eupyrene sperm. (X 1500)

Figure 12. Section through the ventral portion of the albumen gland.
lu - lumen of gland; vc - ventral channel. (X 150)

Figure 13. Portion of the capsule gland. Note the secretory cells (arrowheads) lining the lumen (lu) of the gland complex. (X 500)

Figure 14. Cross section through the copulatory bursa. Its walls are highly folded and it is filled with a viscous fluid. Clumps of degenerating sperm can be seen within it (arrowheads). (X 90)

Figure 15. An enlargement of the epithelium (ep) lining the copulatory bursa. Note the secretory cells (se).
bl - basal lamina. (X 100)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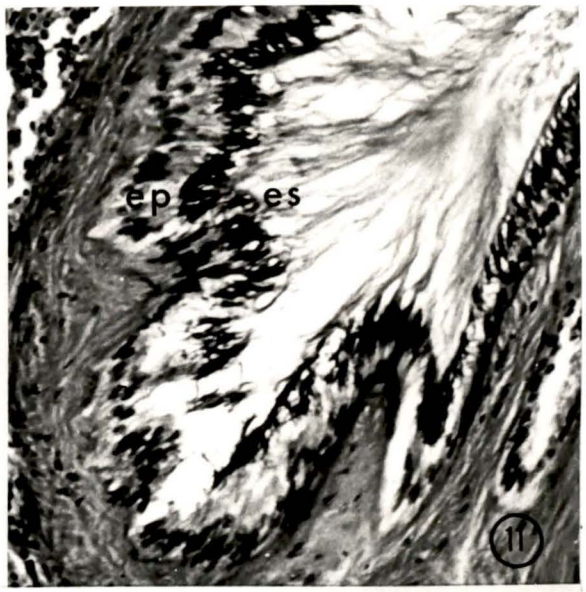
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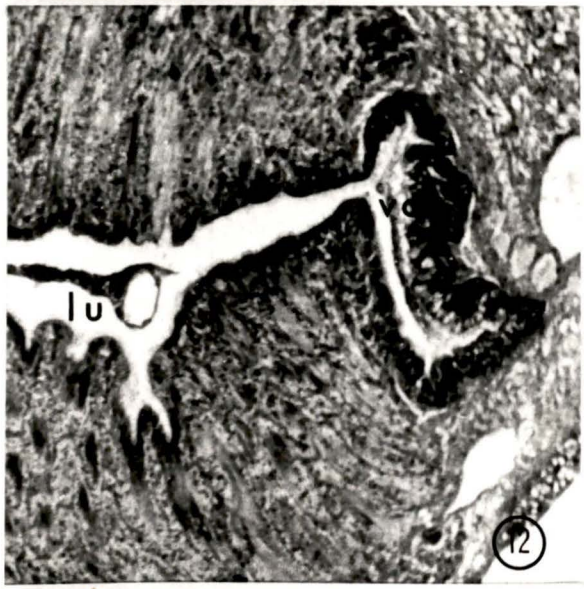
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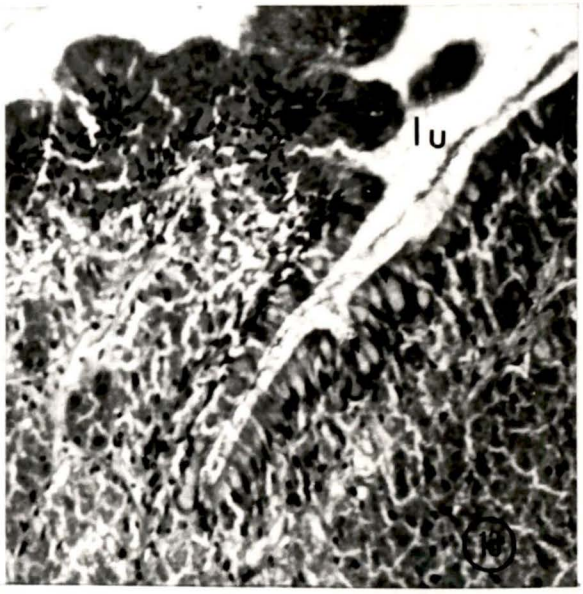
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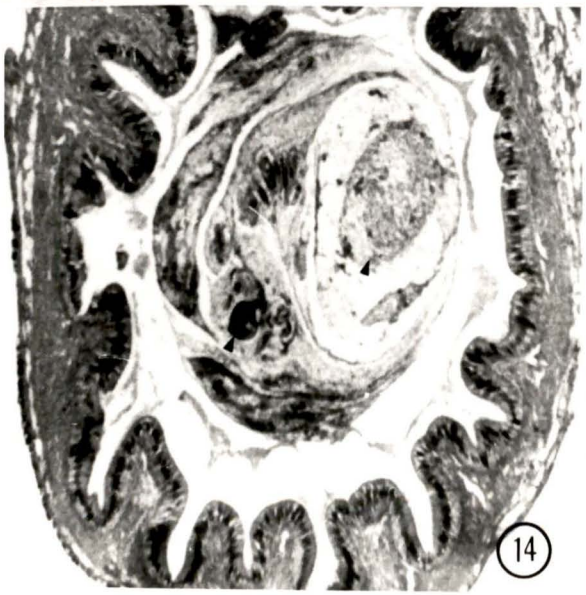
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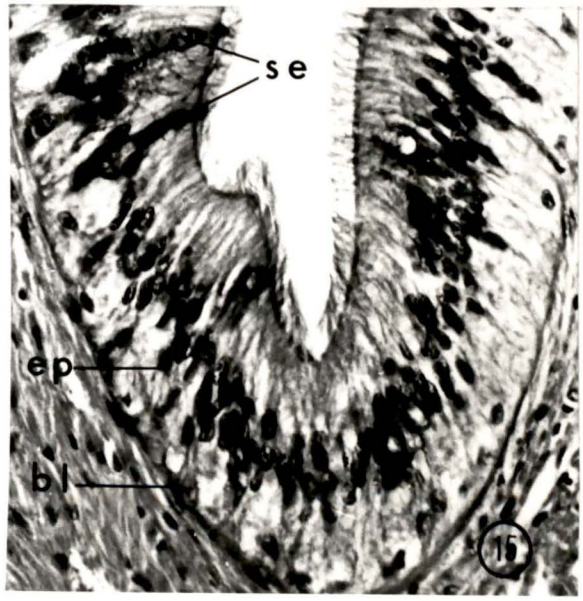
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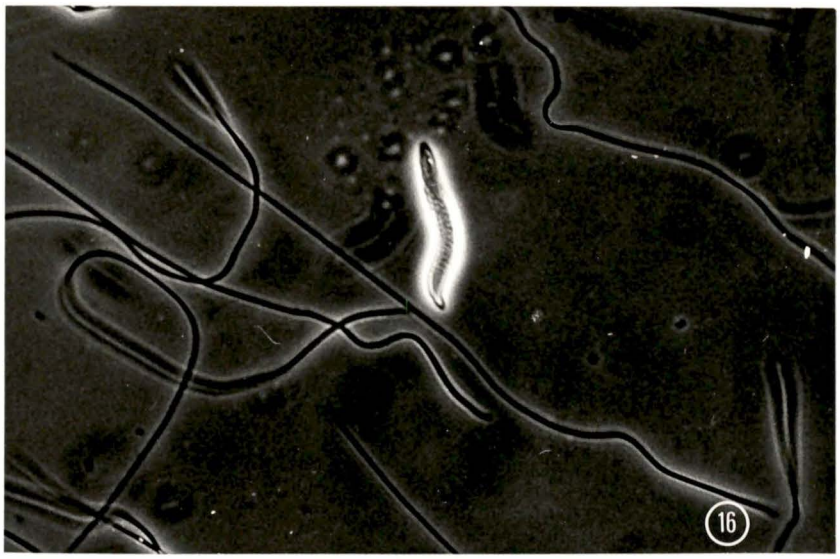
Figures 16 to 19 are photomicrographs of living sperm from smear preparations.

Figure 16. A carrier apyrene sperm (in centre) surrounded by long thin eupyrene sperm. (Phase contrast, X 550)

Figure 17. A eupyrene sperm is central in the photograph; above it is a carrier, in which droplets can be seen regularly arranged. Below it is a lancet sperm, with droplets irregularly dispersed throughout. (Nomarski, X 550)

Figure 18. A carrier sperm. The axial core is just visible, with large droplets on either side. (Nomarski, X 700)

Figure 19. A lancet sperm. Its longitudinal axonemes can be seen as dark bands; it contains some droplets. (Phase contrast, X 1000)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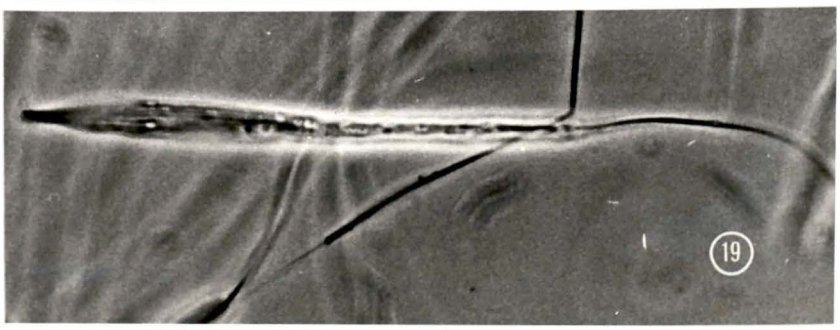
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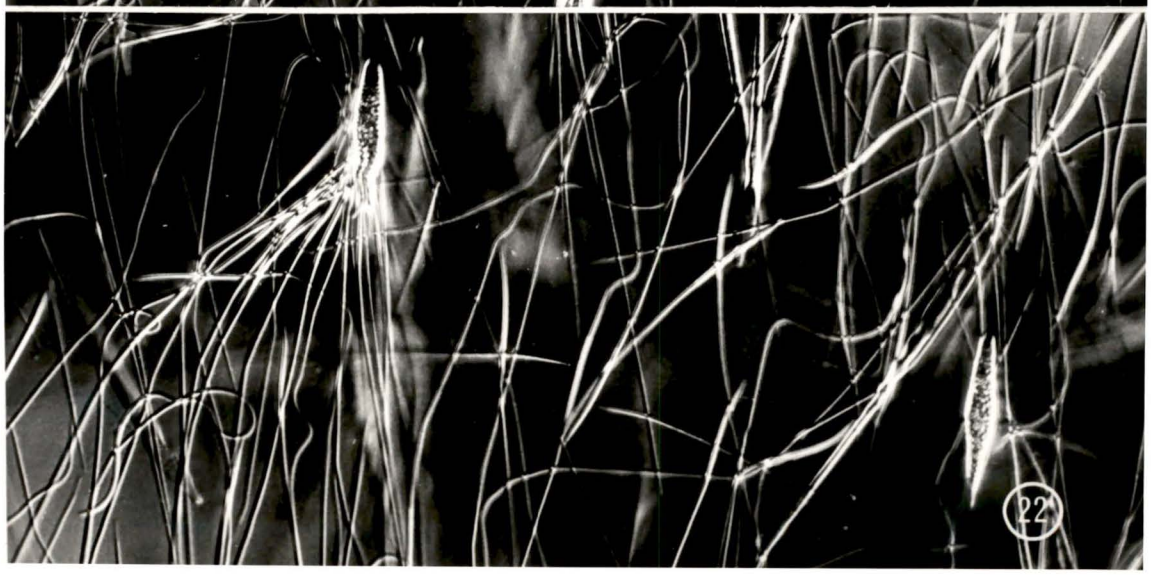
Figures 20, 21 and 22. Spermatozeugmata. Many long slender eupyrene sperm are attached to a carrier sperm. ("Live" preparations; Nomarski, X 700)


20


21


22

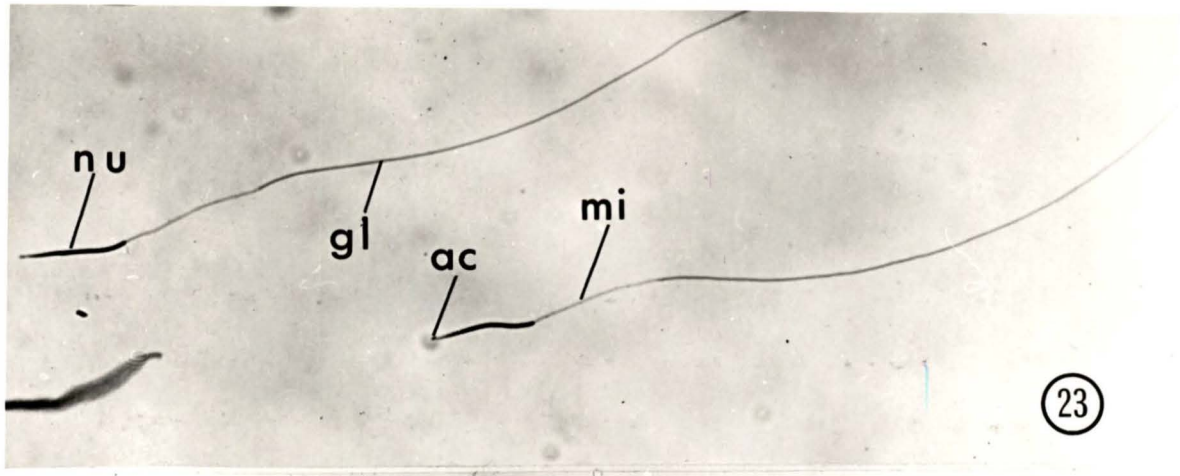
Figures 23 to 25 are photomicrographs of sperm from formalin-fixed smears. Sperm parts were differentiated with Best's carmine and sperm were counterstained with Mayer's haemalum.

Figure 23. Eupyrene sperm. The tail piece is positive for glycogen.

Figure 24. Carrier apyrene sperm. Its cytoplasm is strongly positive for glycogen.

Figure 25. Lancet apyrene sperm. The cytoplasm is less positive for glycogen than cytoplasm of the carrier.

ac - acrosome; ca - carrier sperm; gl - glycogen region of eupyrene sperm tail piece; la - lancet sperm; mi - mitochondrion; nu - nucleus. (X 550)



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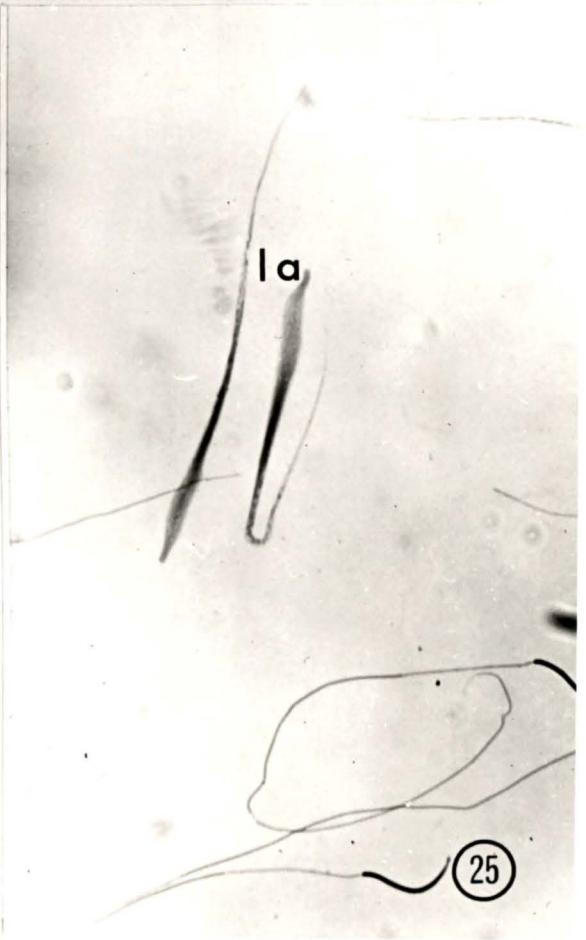
24



25

Figure 26. A diagrammatic representation of the fine structure of the eupyrene sperm.

A - enlargement of a longitudinal section through the acrosome and centriolar region.

a1 - cross section through the nucleus and cap only.

a2 - cross section through the nucleus and the base of the centriole.

a3 - cross section through the nucleus and centriole which is beginning to take on the subtriplet structure; the central tubules are also visible.

a4 - cross section through the nucleus and axoneme.

Stippling in the centriole and tubules indicates that the structure is indistinct.

B - enlargement of a longitudinal section of the junction between the nucleus and mitochondrion.

b - cross section through the mitochondrion.

C - enlargement of a longitudinal section through the junction between the mitochondrion and glycogen region.

c - cross section through the glycogen region of the tail piece.

d - cross section through the end piece

ac - acrosome; ag - subacrosomal granule; ar - subacrosomal rod; ca - cap; ce - centriole; ep - end piece; gr - glycogen region of tail; im - interstitial membrane; mi - mitochondrion; nu - nucleus; rm - ragged membrane.

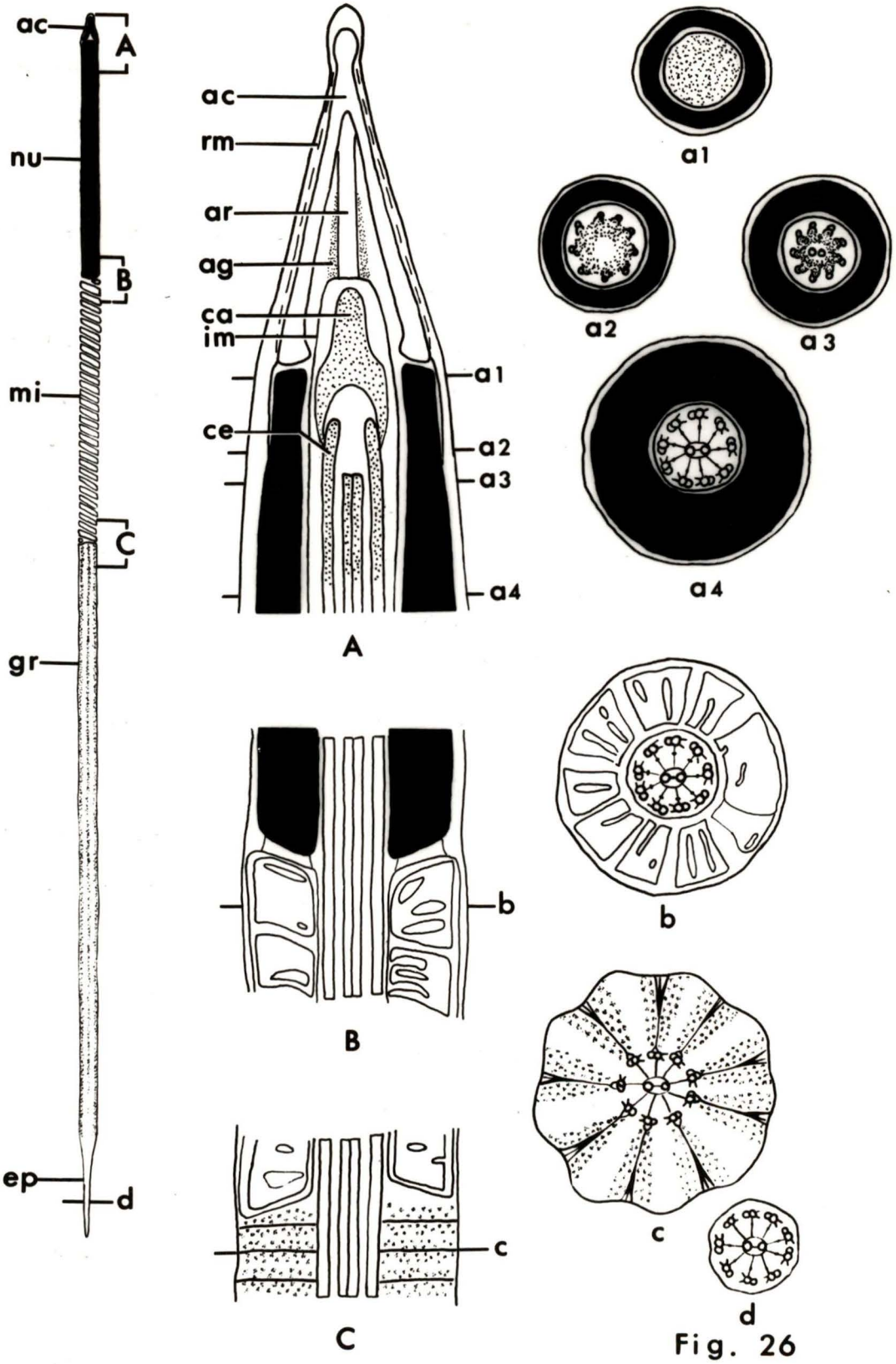

ac
A
nu
B
mi
C
gr
ep
d
ac
rm
ar
ag
ca
im
ce
a1
a2
a3
a4
A
a1
a2
a3
a4
b
B
c
C
d

Fig. 26

Figure 27. Longitudinal section through the acrosome of a eupyrene sperm; particularly note the subacrosomal space. sar - subacrosomal rod; sag - subacrosomal granule. (X 51200)

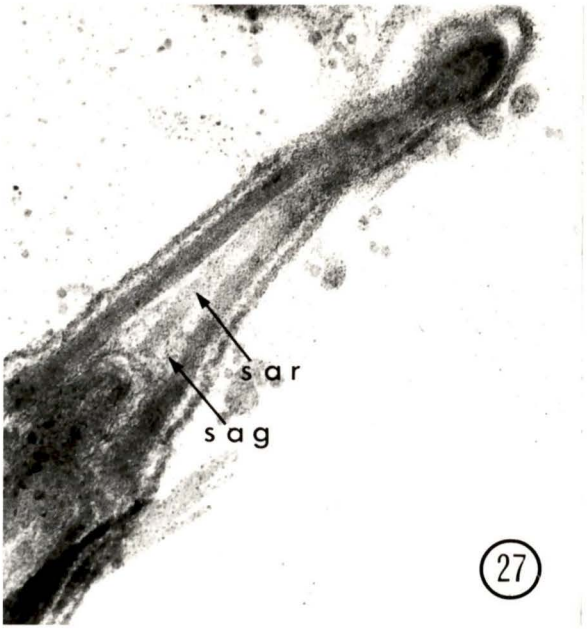
Figure 28. Longitudinal section through the junction of the acrosome and nucleus. Note the knob on the centriolar cap protruding into the subacrosomal space. ac - acrosome; im - interstitial membrane; nl - nuclear lamina. (X 53100)

Figure 29. Longitudinal section through the acrosome and apex of nucleus.
ac - acrosome; rm - basal portion of the ragged membrane. (X 51200)

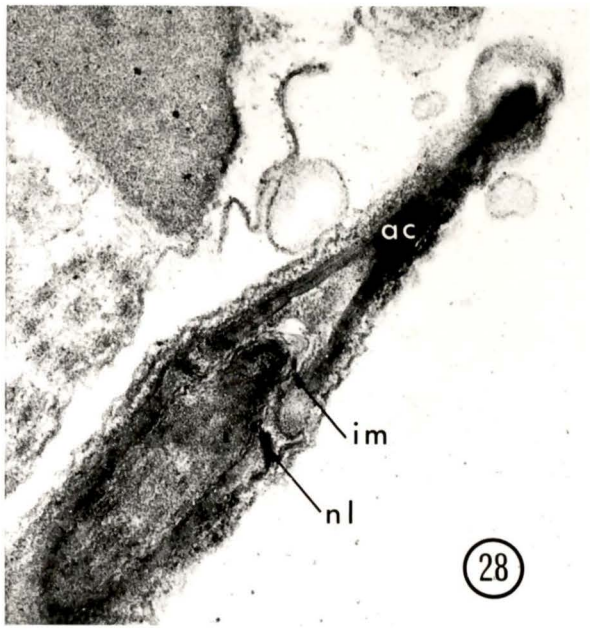
Figure 30. Grazing section through the centriole (ce).
nu - nucleus. (X 89900)

Figure 31. Cross section through the apex of the nucleus, and centriolar cap (ca). (X 211000)

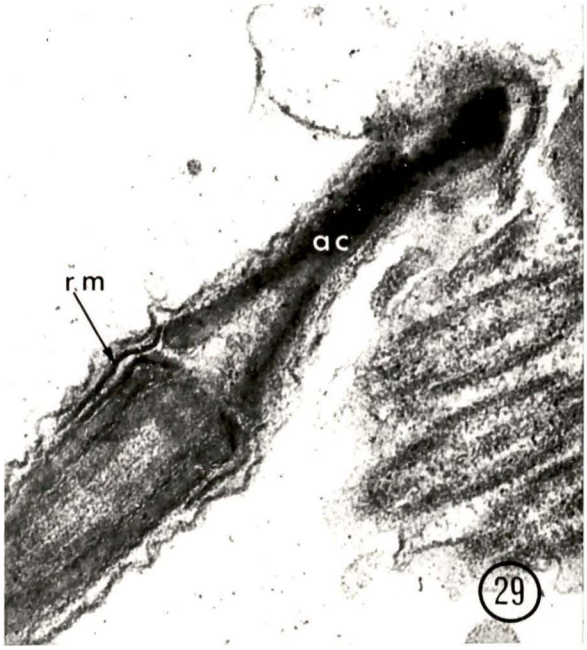
Figure 32. Cross section through the posterior portion of the centriole (ce). This is in a developing spermatid. (X 211000)



27



28



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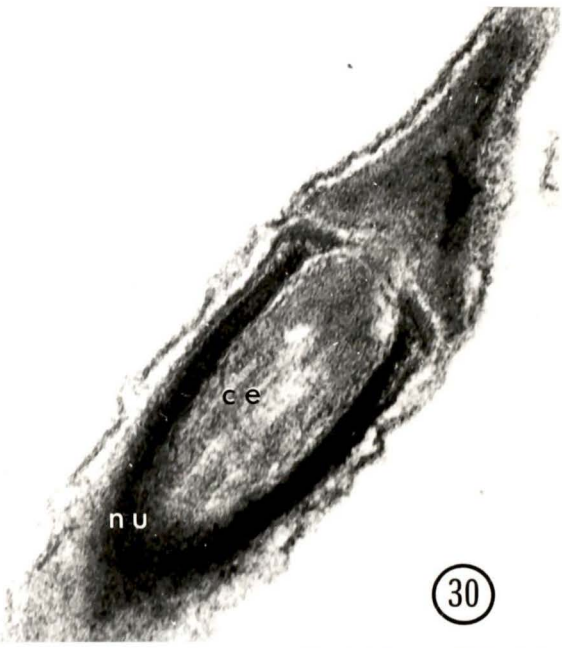
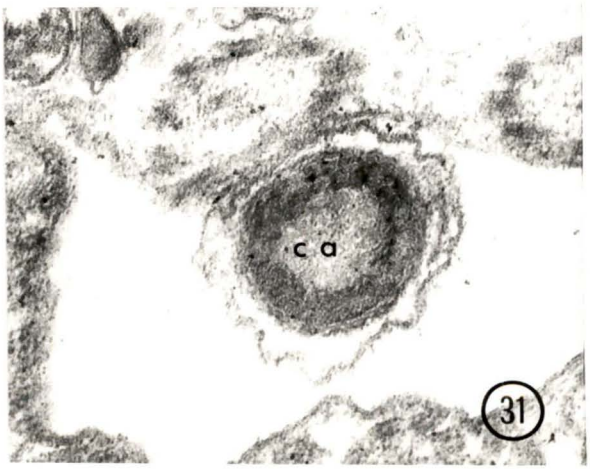
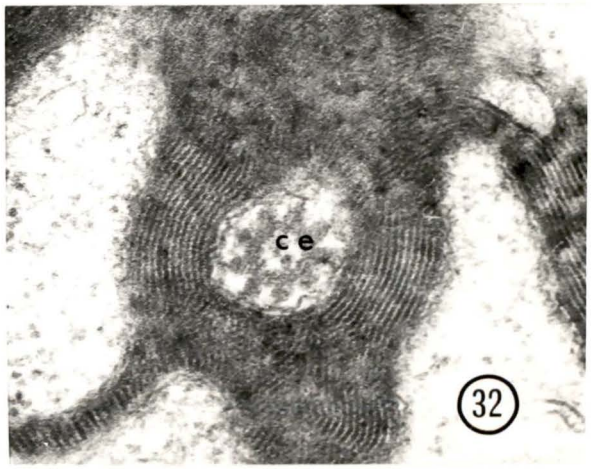


30



31



32

Figure 33. Longitudinal section through the junction of nucleus and mitochondrion; arrowheads point to the plasmalemma and the single arrow points to the mitochondrial membrane. (X 24400)

Figure 34. Longitudinal section through the junction of the mitochondrion and glycogen region of the tail piece. Arrows indicate sections through connecting bars. (X 24400)

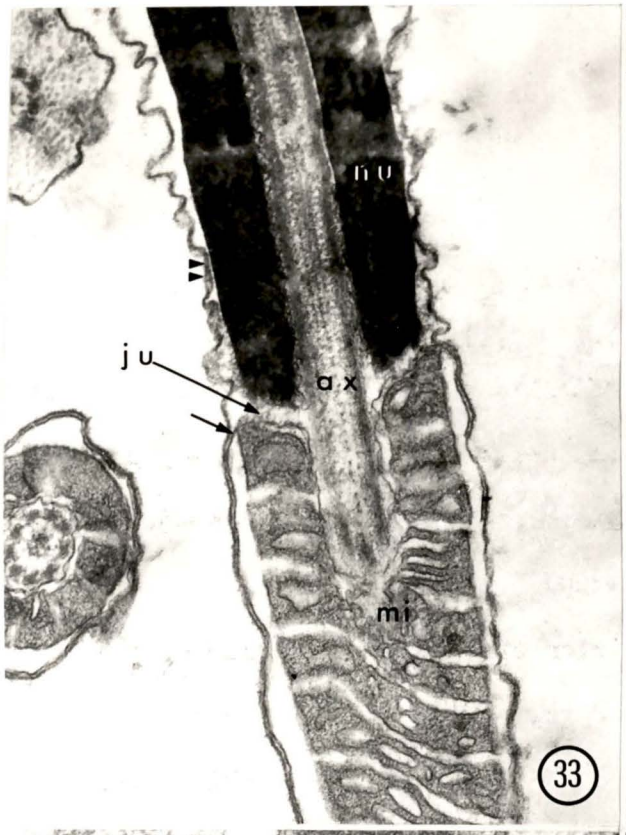
Figure 35. Cross section through the nucleus. (X 44300)

Figure 36. Cross sections through the mitochondrion and glycogen region. Arrow indicates a connecting bar which is branching to attach to theplasmalemma. (X 12000)

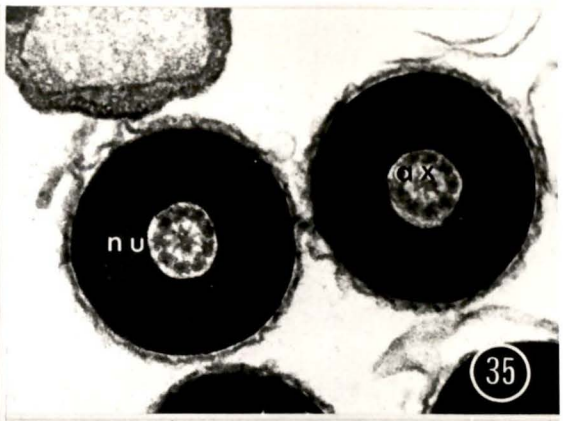
Figure 37. Cross section of the glycogen region. (X 37600)

Figure 38. Cross section through the end piece. (X 97400)

ax - axoneme; gl - glycogen; ju - junction between nucleus and mitochondrion; mi - mitochondrion; nu - nucleus.



33



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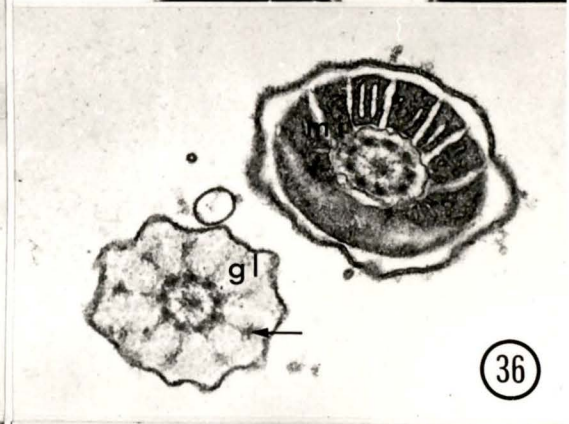
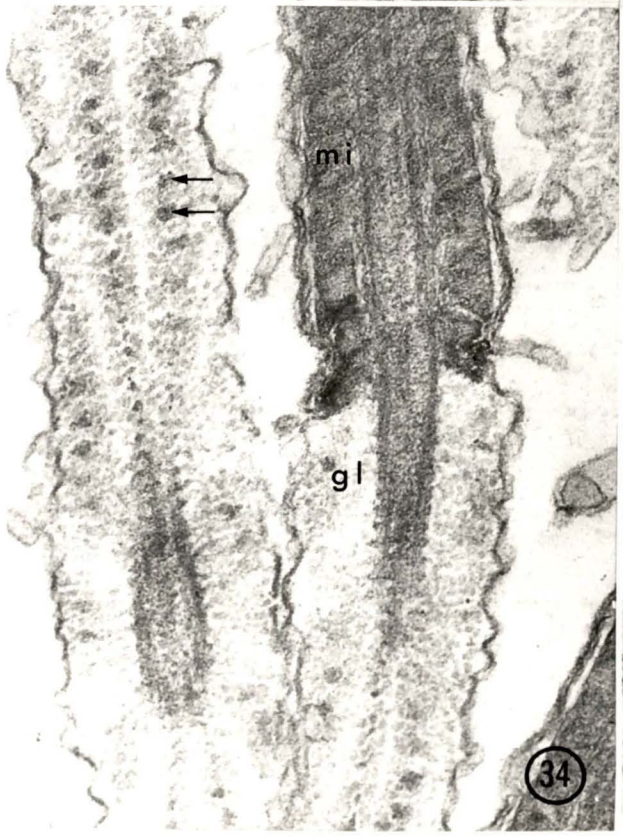
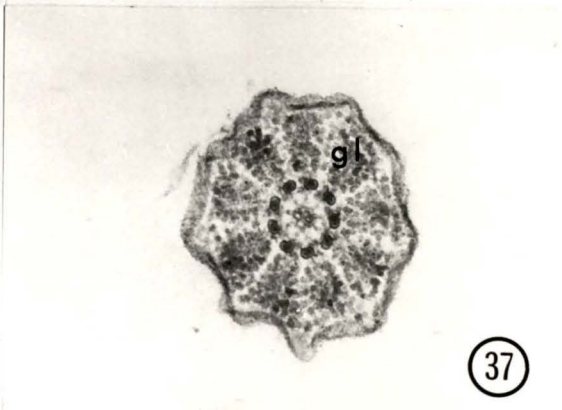


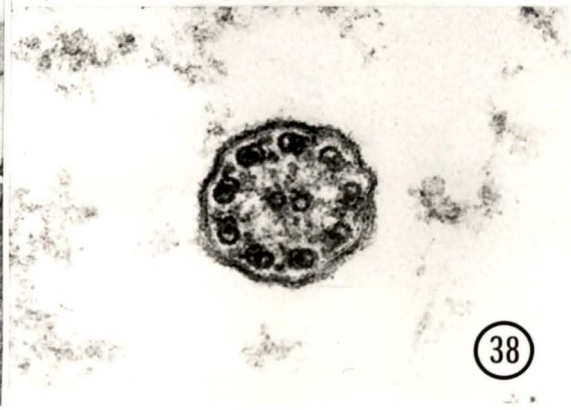
36



34



37



38

Figure 39. Representation of the fine structure of the carrier apyrene sperm.

ap - apical portion of sperm containing dense mass; ax - axoneme; ce - centriole; gl - glycogen rosettes; lpd - large polysaccharide droplets; mi - mitochondrion; mt - microtubule.

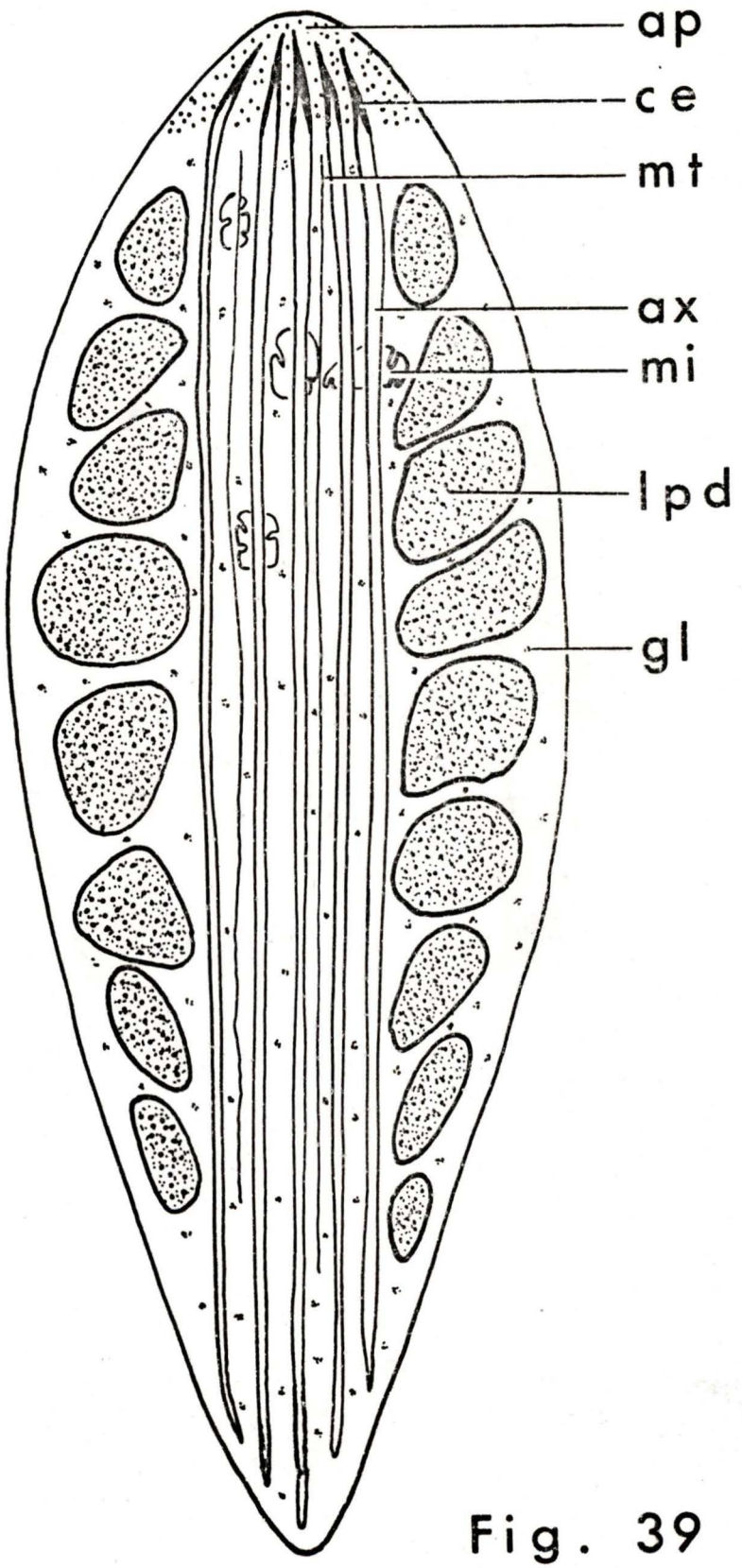


Fig. 39

Figure 40. Representation of the fine structure of the lancet apyrene sperm.

ap - apical portion of sperm containing dense mass; ax - axoneme; ce - centriole; gl - glycogen rosettes; md - mucous droplets; mi - mitochondrion; mt - microtubules; spd - small polysaccharide droplets.

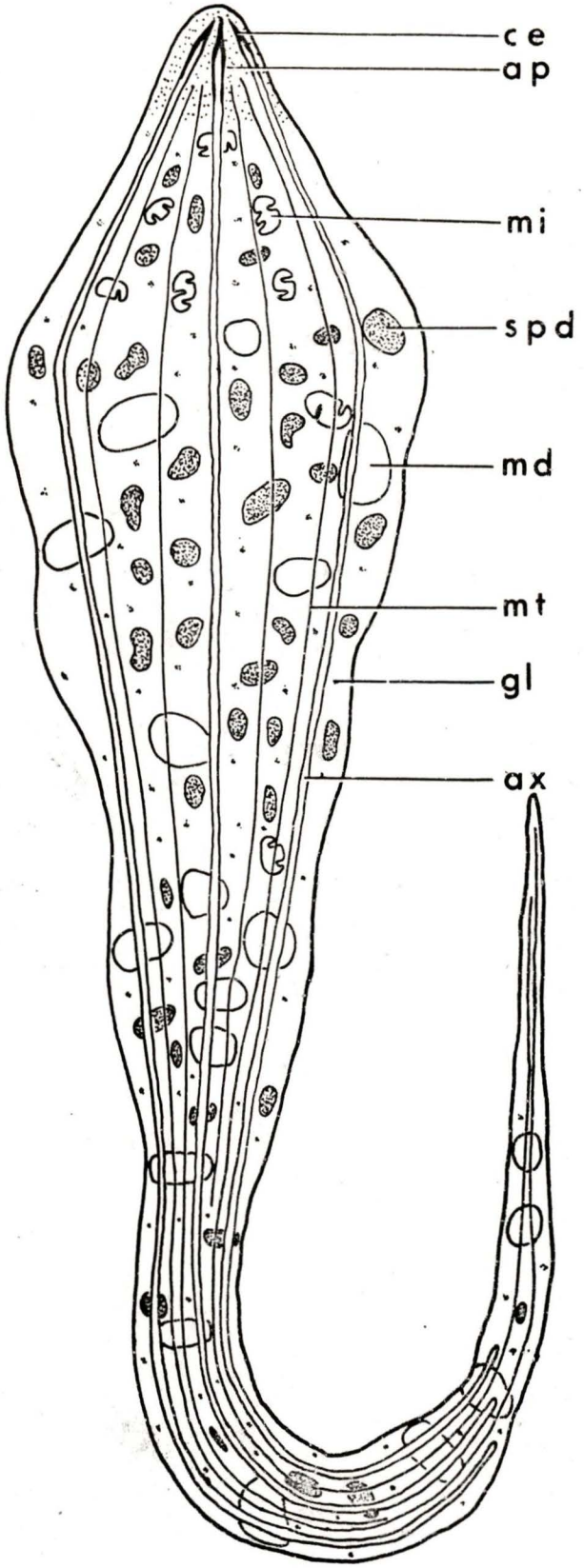


Fig. 40

Figure 41. Grazing section through the apical portion of a carrier sperm, demonstrating the "rootlets" (inset) of the centrioles.

ax - axonemes; lpd - large polysaccharide droplets; rb - rootlets of modified centrioles. (X 6500; inset X 36900)

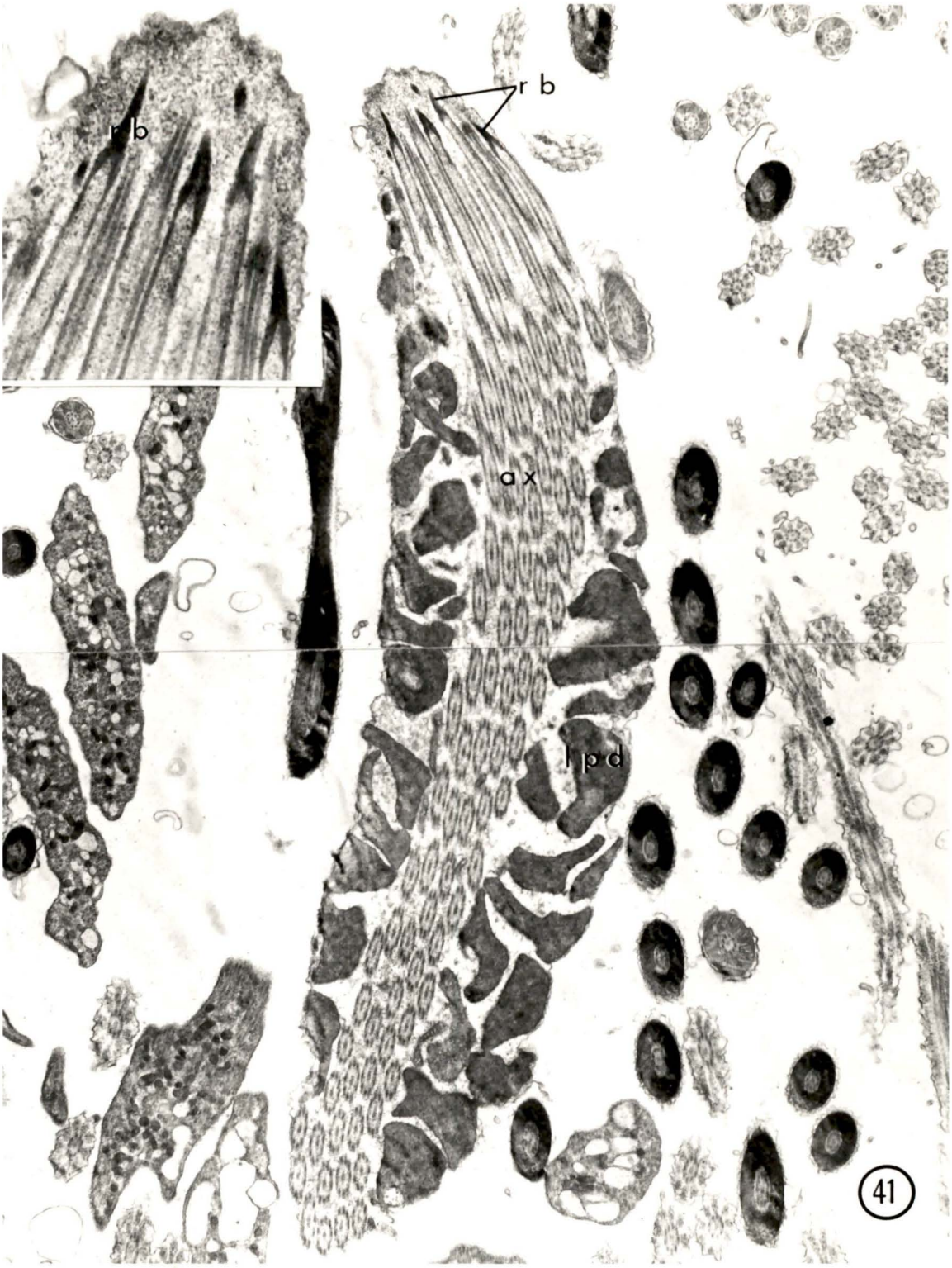

r b
r b
a x
l p d
41

Figure 42. Grazing section through a carrier sperm with a eupyrene sperm (es) attached to it. Inset shows an enlargement of a similar attachment; note the dense material between the two sperm membranes (arrowhead).

ax - axonemes; lpd - large polysaccharide droplets.
(X 11800; inset X 47200)

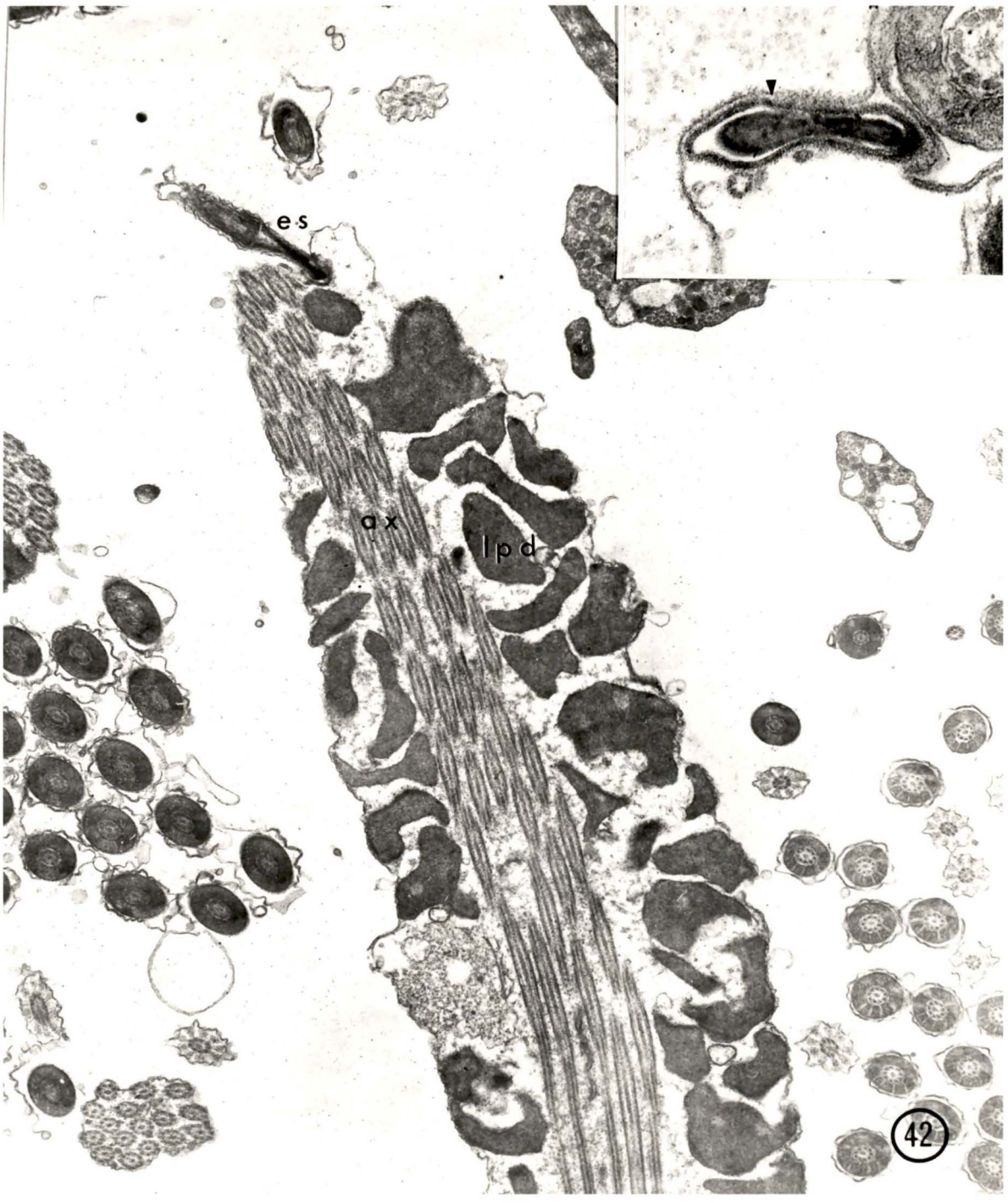

es
ax
lpd
42

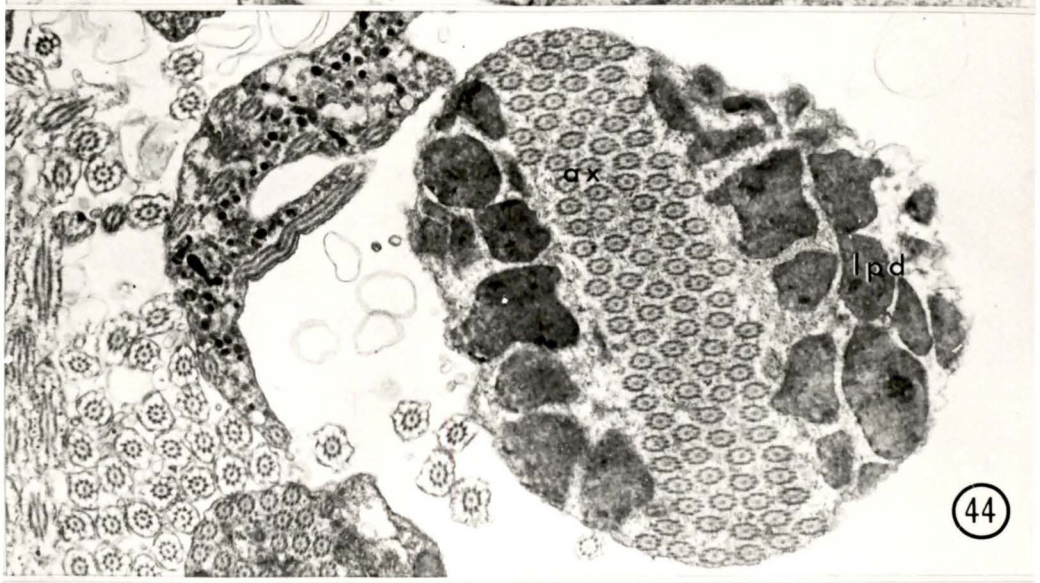
Figure 43. Section through the centriolar region of the carrier. (X 10900)

Figure 44. Section through the mid region of the carrier. (X 10400)

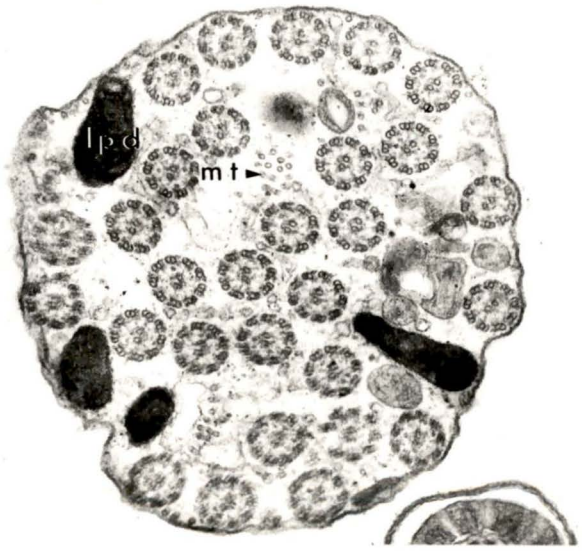
Figure 45. Section through the posterior portion of the carrier. (X 10900)

ax - axonemes; br - rootlet base of the modified centriole; ce - centriole; gl - glycogen; lpd - large polysaccharide droplets; od - portion posterior to the centriole before the central tubules emerge; mt - microtubules.

43



44



45

Figure 46. Grazing section through the apical portion of a lancet sperm. (X 10300) Inset shows a cross section through the centriolar region. (X 24300)

Figure 47. Longitudinal section through the mid portion of the lancet. (X 10300)

Figure 48. Longitudinal section through the posterior portion of the lancet. (X 36500)

ax - axonemes; ce - centriole; rb - rootlet or basal portion of modified centriole; gl - glycogen; md - mucous droplets; spd - small polysaccharide droplets.

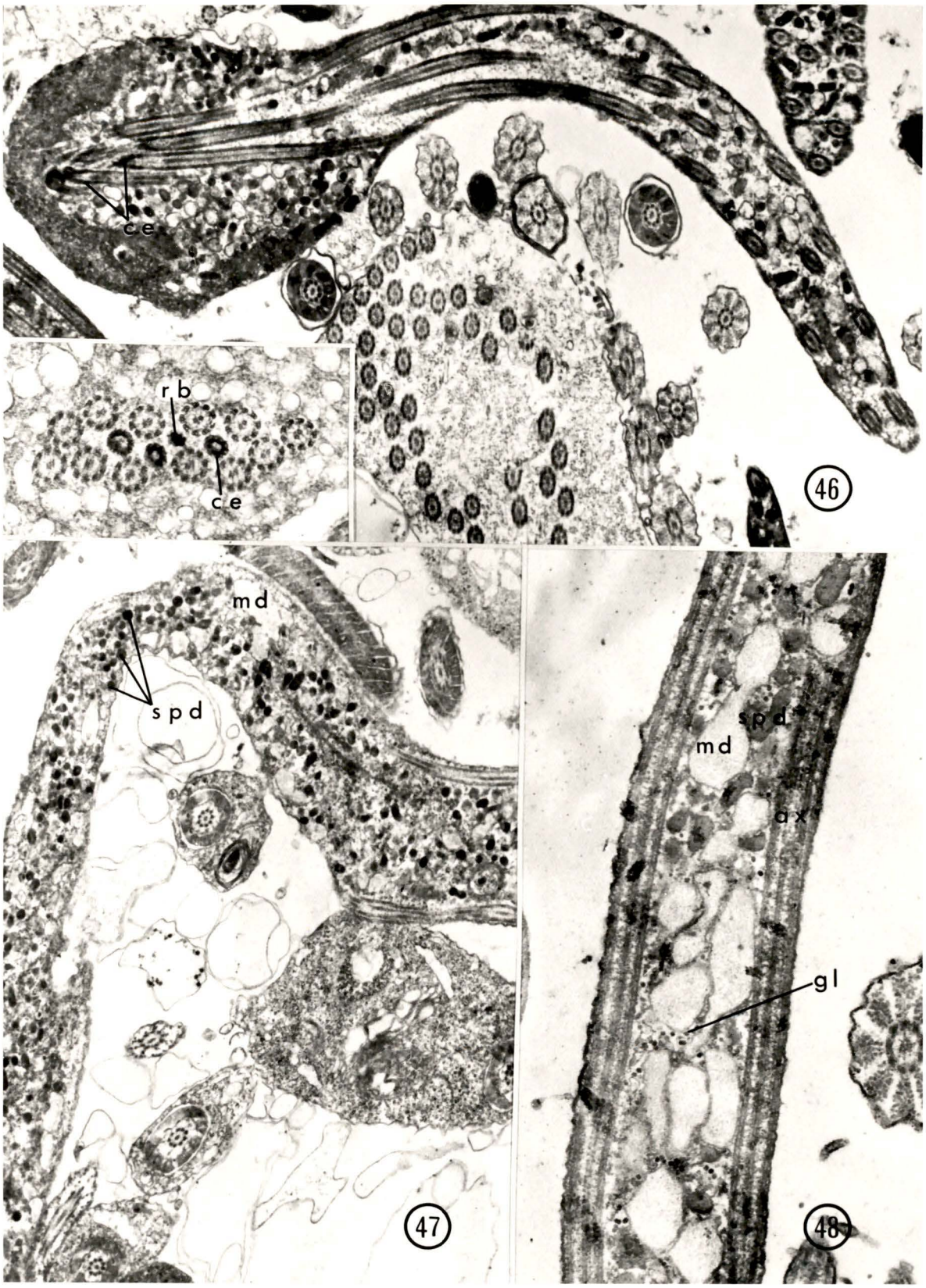

ce
rb
ce
46
md
spd
47
spd
md
ax
gl
48

Figure 49. Cross section through the lancet posterior to the centriolar region. (X 30800)

Figure 50. Cross section through the mid portion of the lancet. (X 16700)

Figures 51 and 52. Cross sections through the lancet moving posteriorly. The axonemes are fading out. (X 23200) Inset shows the end piece of the lancet where only microtubules (arrowhead) ring the periphery of the sperm. (X 30600)

ax - axonemes; md - mucous droplets; mi - mitochondria; mt - microtubules; spd - small polysaccharide droplets.

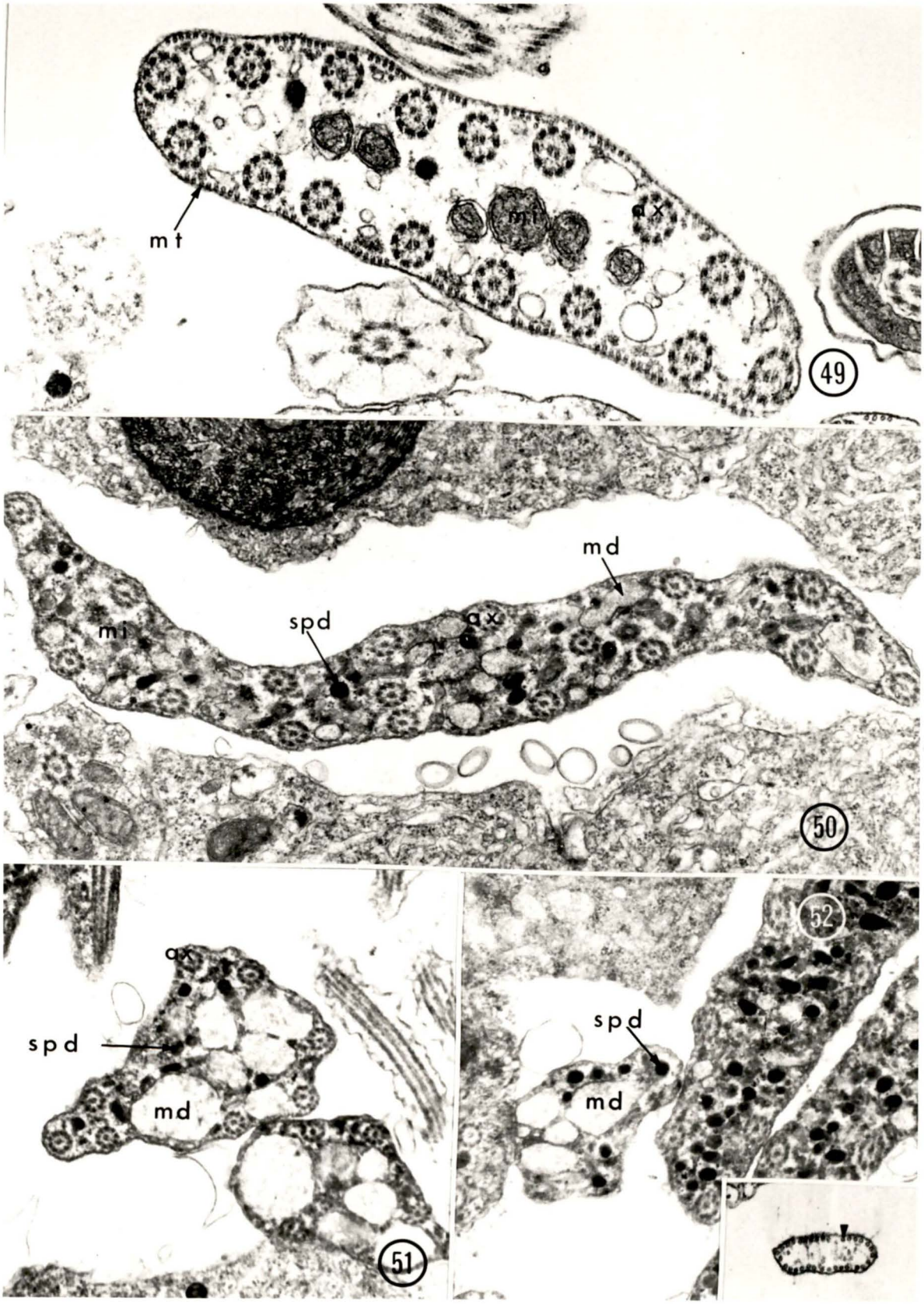

mt
mi
ax
49
md
spd
mi
ax
50
ax
spd
md
51
spd
md
52

Figure 53. Schematic representation of eupyrene spermatogenesis; note that there are two meiotic divisions.

psc - primary spermatocyte; sg - spermatogonium; sp - mature sperm; ssc - secondary spermatocyte; st - spermatid.

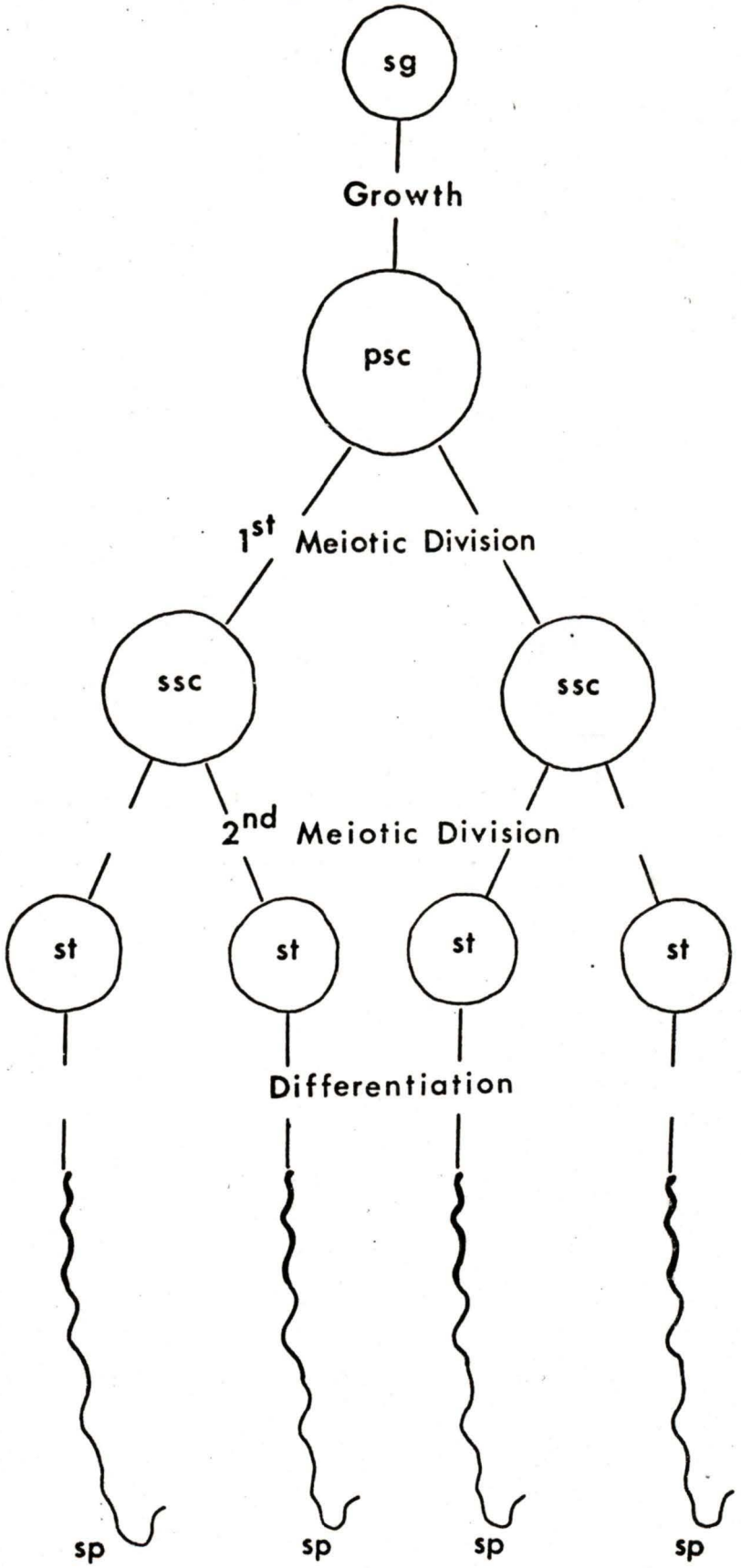
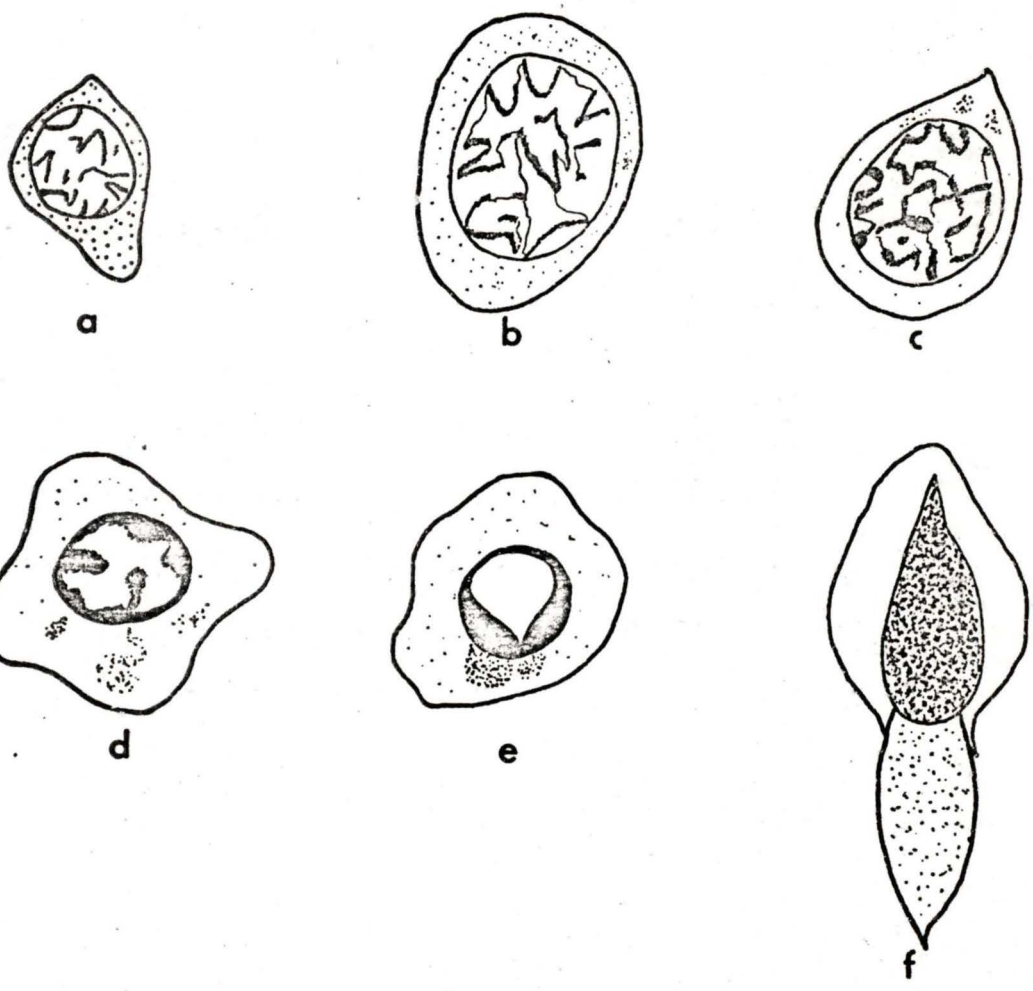

sg
Growth
psc
1st Meiotic Division
ssc
ssc
2nd Meiotic Division
st
st
st
st
Differentiation
sp
sp
sp
sp

Fig. 53

Figure 54. Diagrams of stages of eupyrene spermatogenesis as seen at the light microscope level.

a - spermatogonium; b - primary spermatocyte; c - secondary spermatocyte; d - early spermatid; e - later spermatid; f - spermatid elongating, the nucleus is the heavily stippled anterior portion and the mitochondrion is posterior to it.


a
b
c
d
e
f

Figures 55 to 60 are photomicrographs of paraffin sections, stained with haematoxylin-eosin.

Figures 55, 56, 57, 58, 59 and 60. Stages in both eupyrene and apyrene spermatogenesis. (X 6000)

asc - apyrene spermatocyte; ca - carrier sperm;
la - lancet sperm; sc 1 - primary spermatocyte;
sc 2 - secondary spermatocyte; sg - spermatogonium;
st - spermatid; vn - vacuolated nuclear fragment.

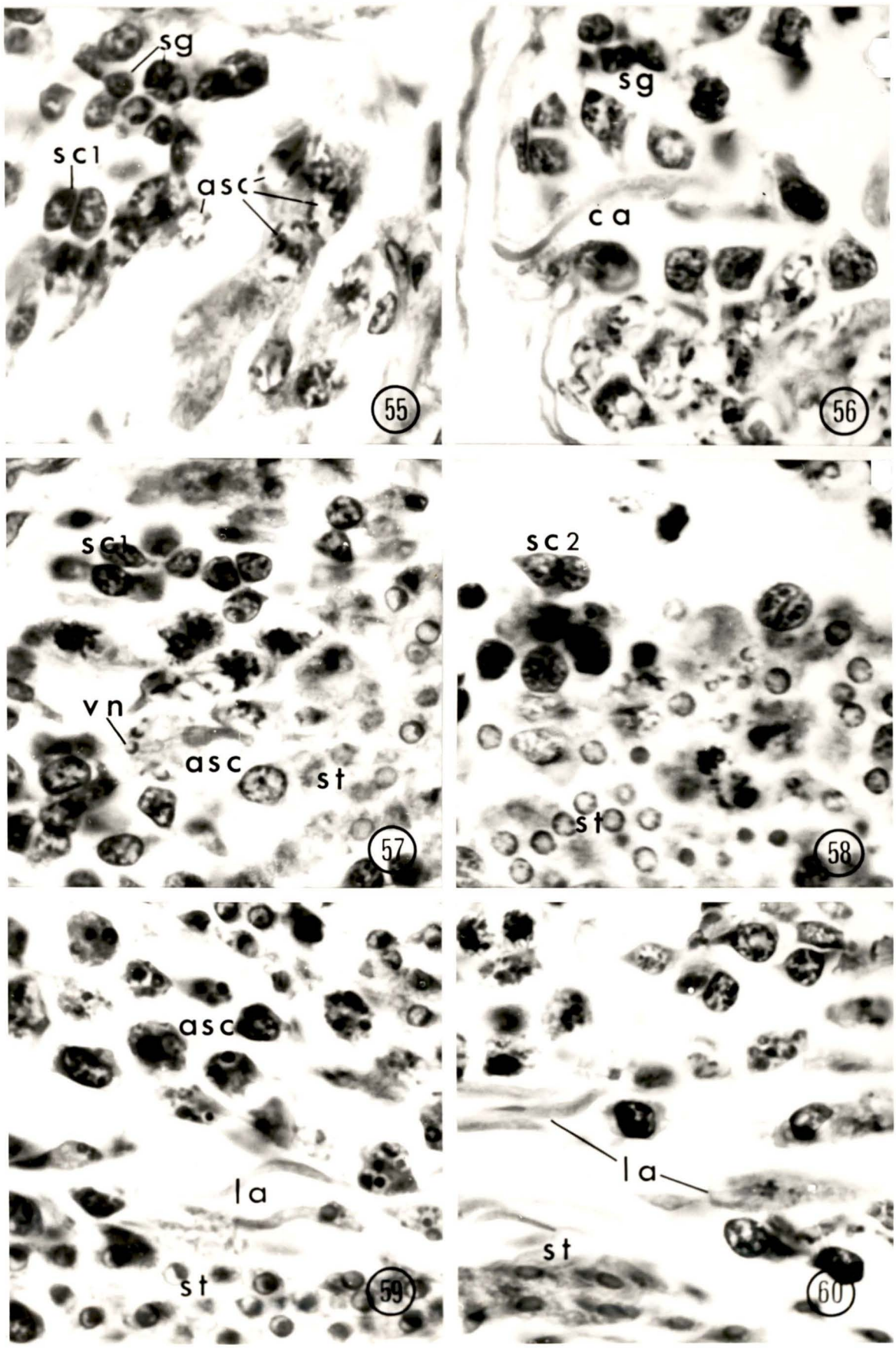
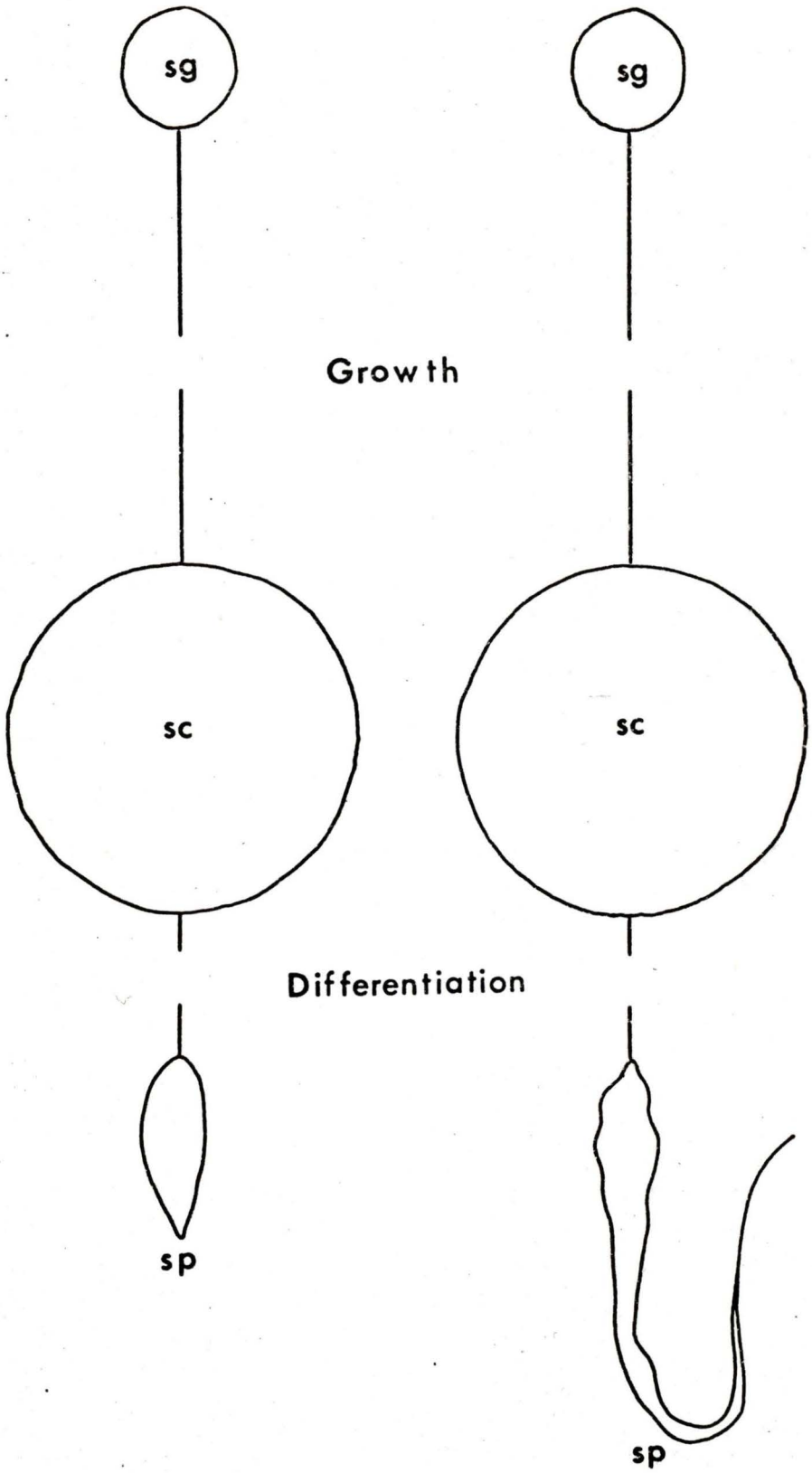

sg
sc1
asc
55
sg
ca
56
sc1
vn
asc
st
57
sc2
st
58
asc
la
st
59
la
st
60

Figure 61. Schematic representation of carrier spermatogenesis.

Figure 62. Schematic representation of lancet spermatogenesis. In both apyrene types of sperm development is direct, there are no divisions.

sc - spermatocyte; sg - spermatogonium; sp - mature sperm.



Figs. 61 and 62

Figure 63. Stages of apyrene spermatogenesis.

a - spermatogonium; b, c - early spermatocytes, note that chromosomes clump rather than line up for division; d - spermatocyte after ciliary extension: ax - axonemes, dr - droplet developing in the cytoplasm, vn - vacuolated nuclear fragments; e - later stage of "d".

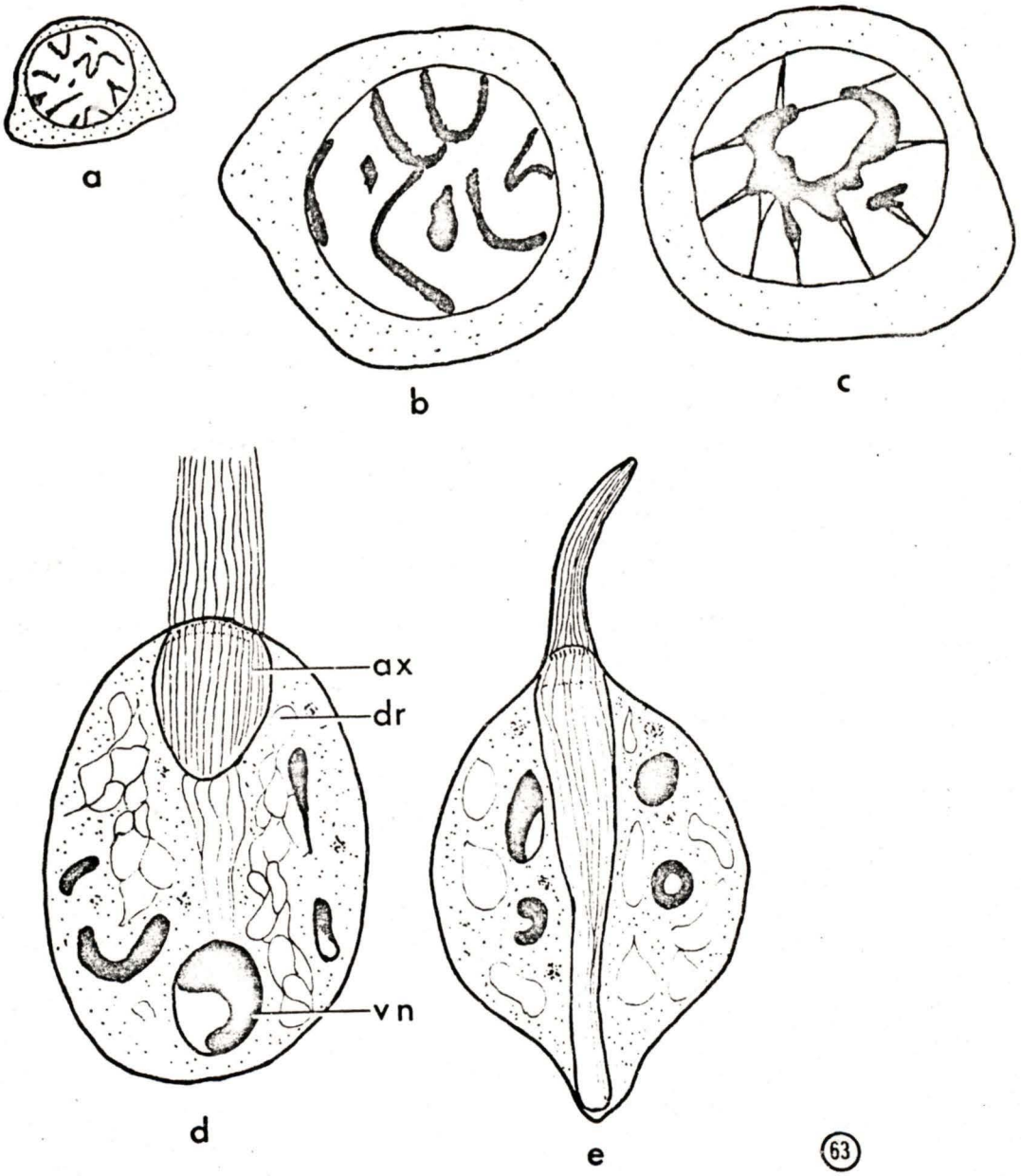
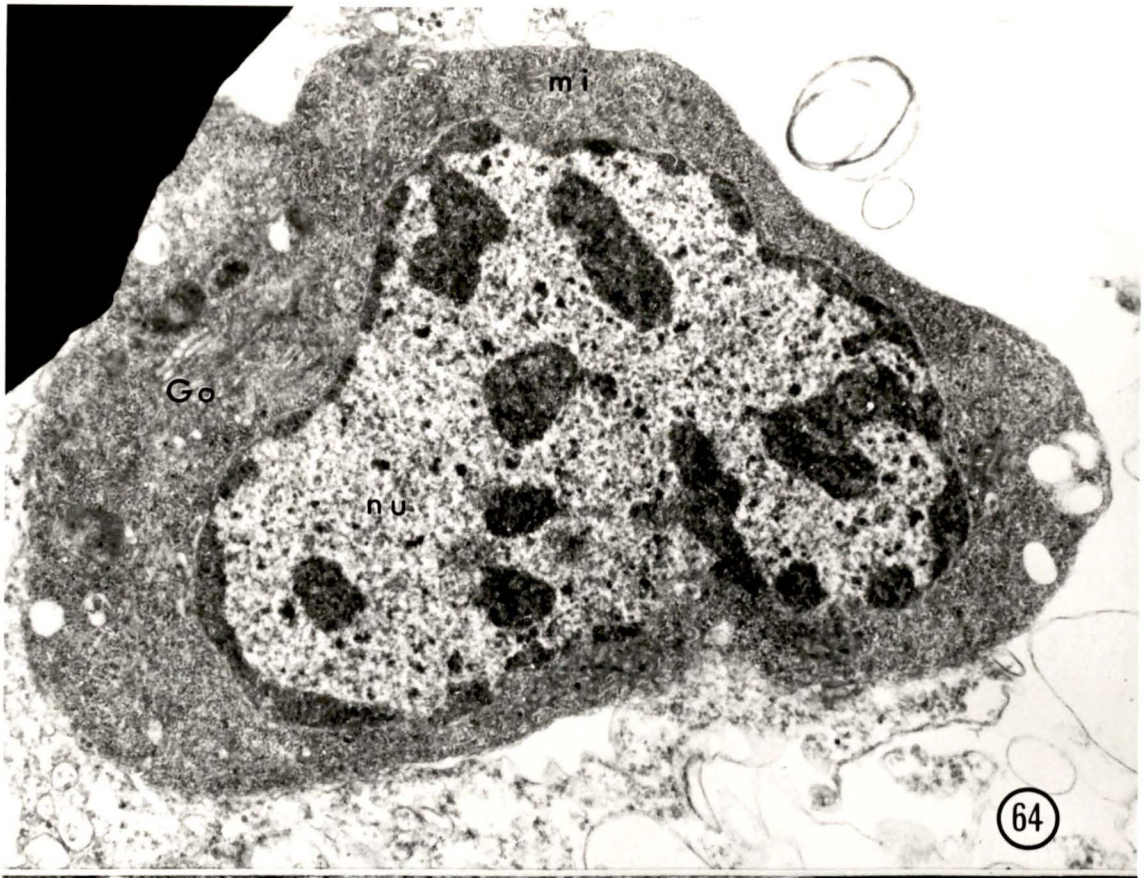

a
b
c
ax
dr
vn
d
e

Figure 64. Section through a spermatogonium. Note the dense, homogeneous cytoplasm. (X 18500)

Figure 65. Section through a primary spermatocyte. Its cytoplasm is not as dense as in the figure above. (X 13000)

cc - chromosome core; cp - centriole pair; Go - Golgi; mi - mitochondrion; nu - nucleus.


mi
Go
nu
64

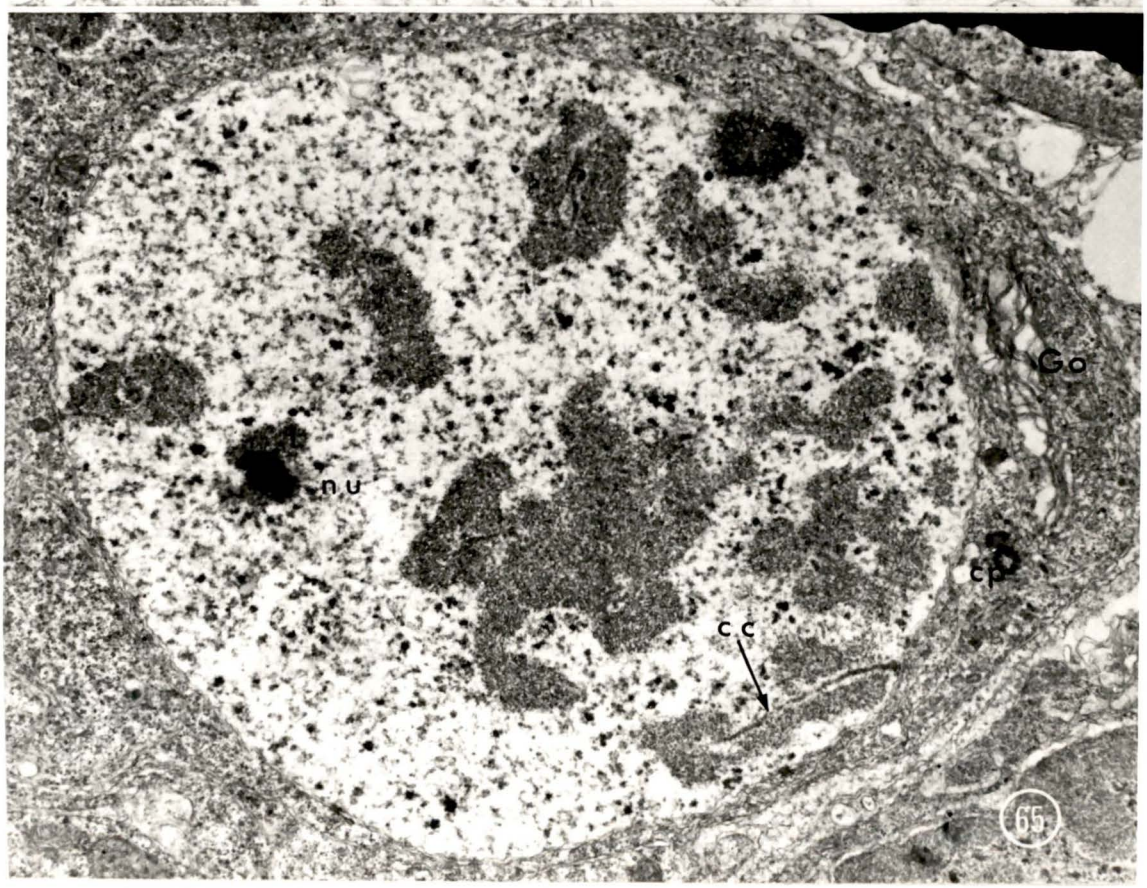
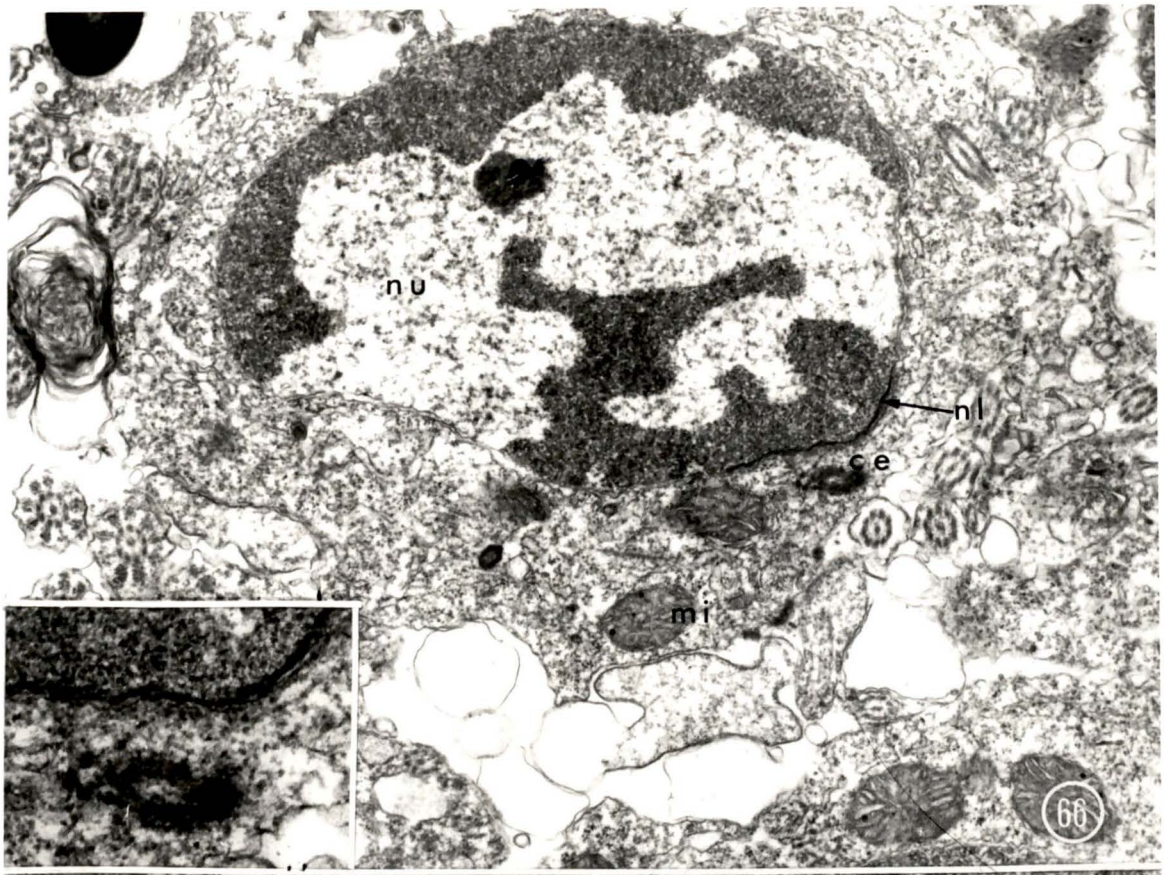
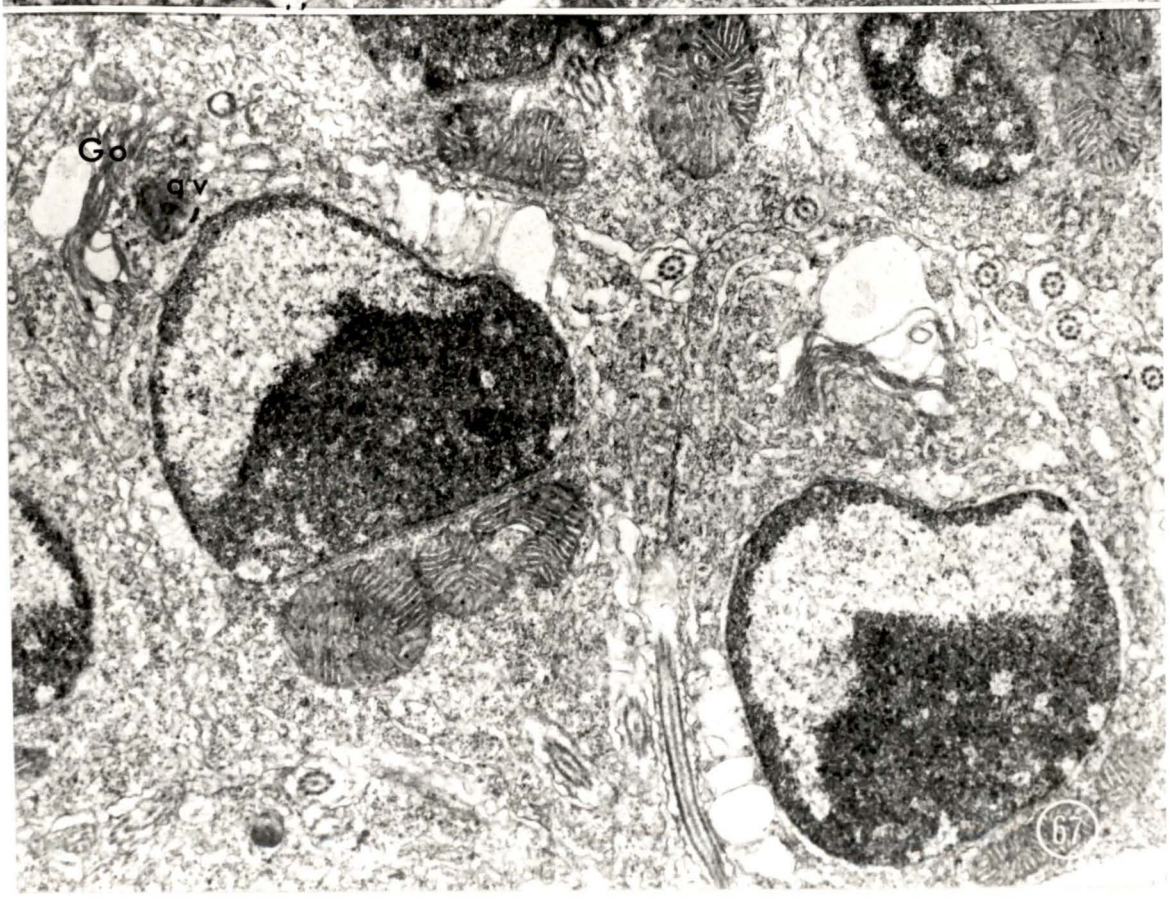

Go
nu
cp
cc
65

Figure 66. Section through an early spermatid. (X 27100)
Inset shows an enlargement of the centriole.
(X 76000)

Figure 67. Eupyrene spermatid showing acrosomal development.
(X 27900)

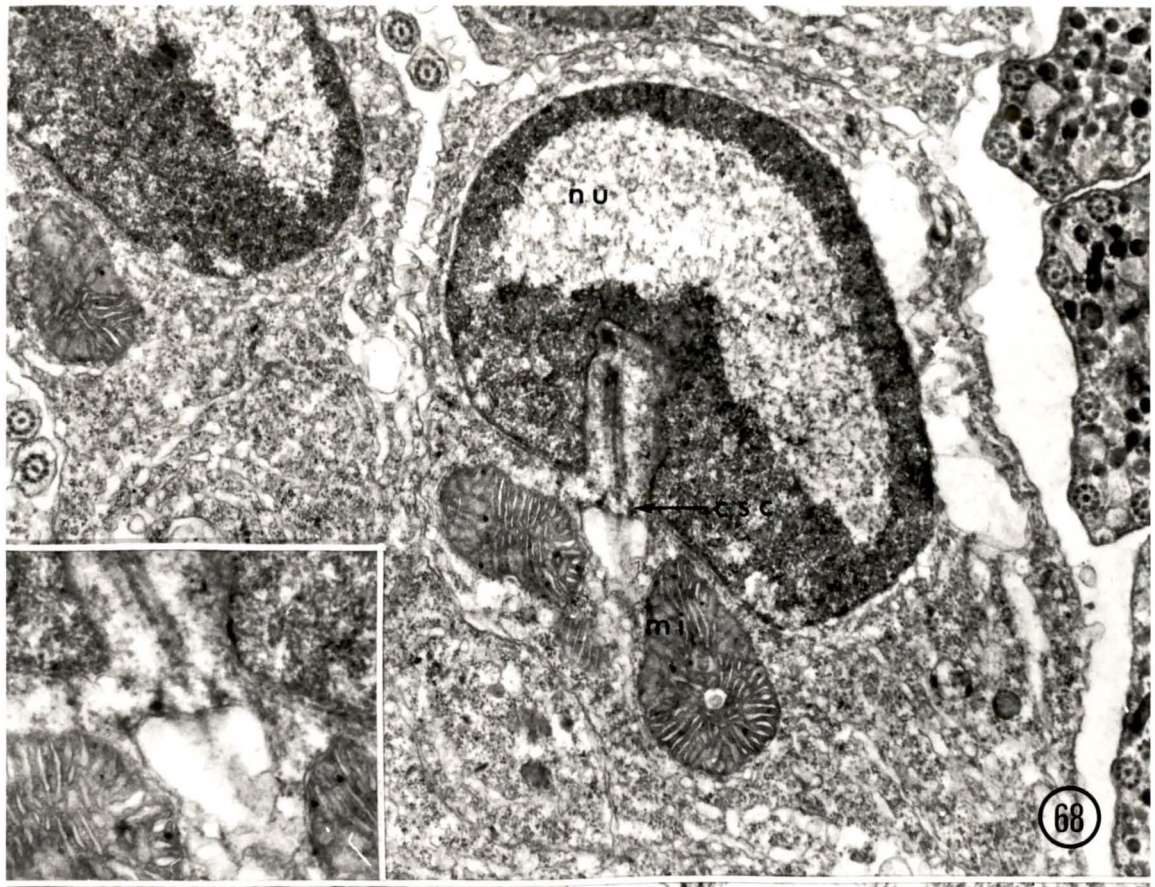
av - acrosomal vesicle; ce - centriole; Go - Golgi;
mi - mitochondrion; nl - nuclear lamina; nu - nucleus.


nu
nl
ce
mi
66


Go
qv
67

Figures 68 and 69. Developing spermatids showing centriolar migration through the nucleus. Inset (figure 68) an enlargement of the centriolar satellite complex. (68 - X 15300, inset - X 31600; 69 - X 17900)

csc - centriolar satellite complex; mi - mitochondrion; nc - channel made through the nucleus by the migrating centriole; nu - nucleus.


nu
csc
mi
68

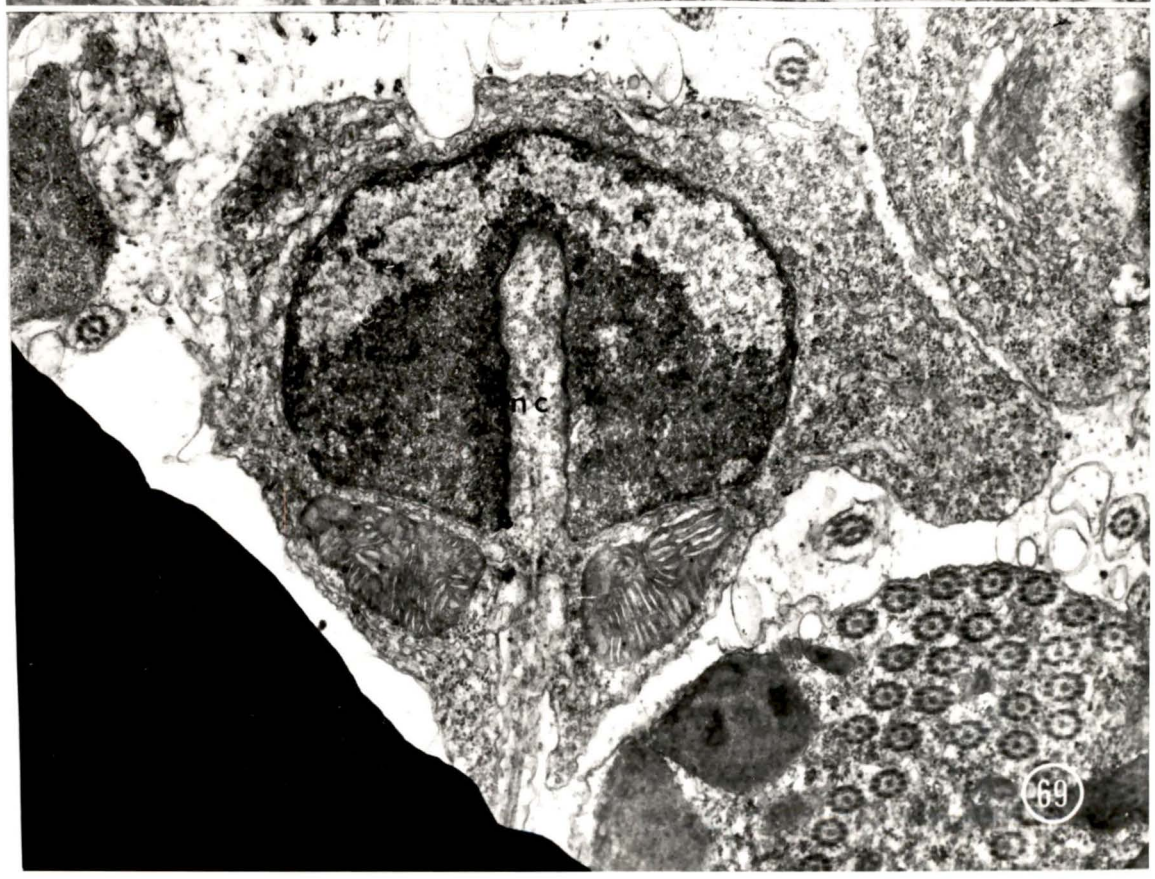

mc
69

Figure 70. Longitudinal section through a spermatid showing the centriole and accessory structure. (X 25800)
Inset, enlargement of the centriole and cap.
(X 68900)

ax - axoneme; ca - cap; ce - centriole.

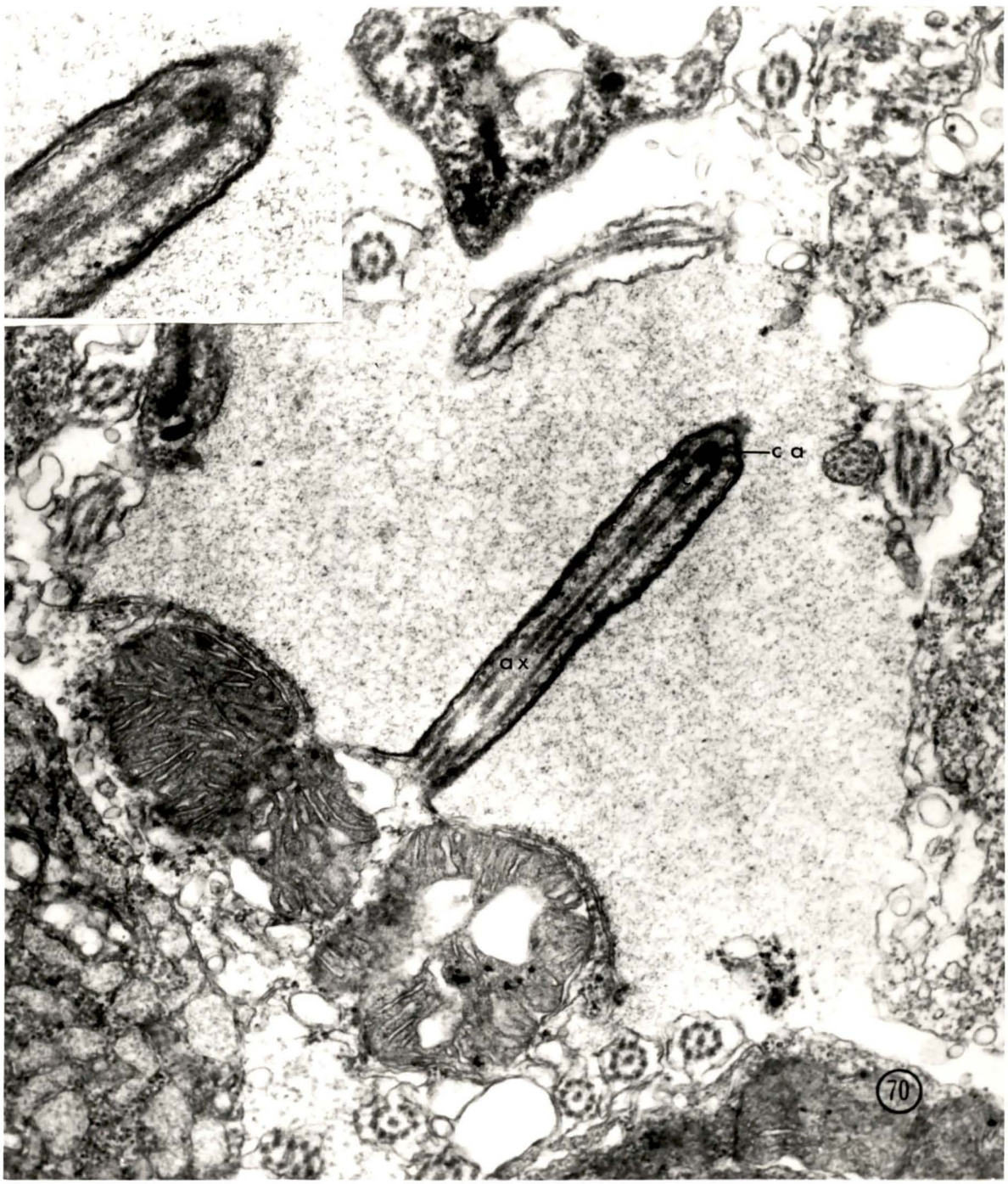

c a
a x
70

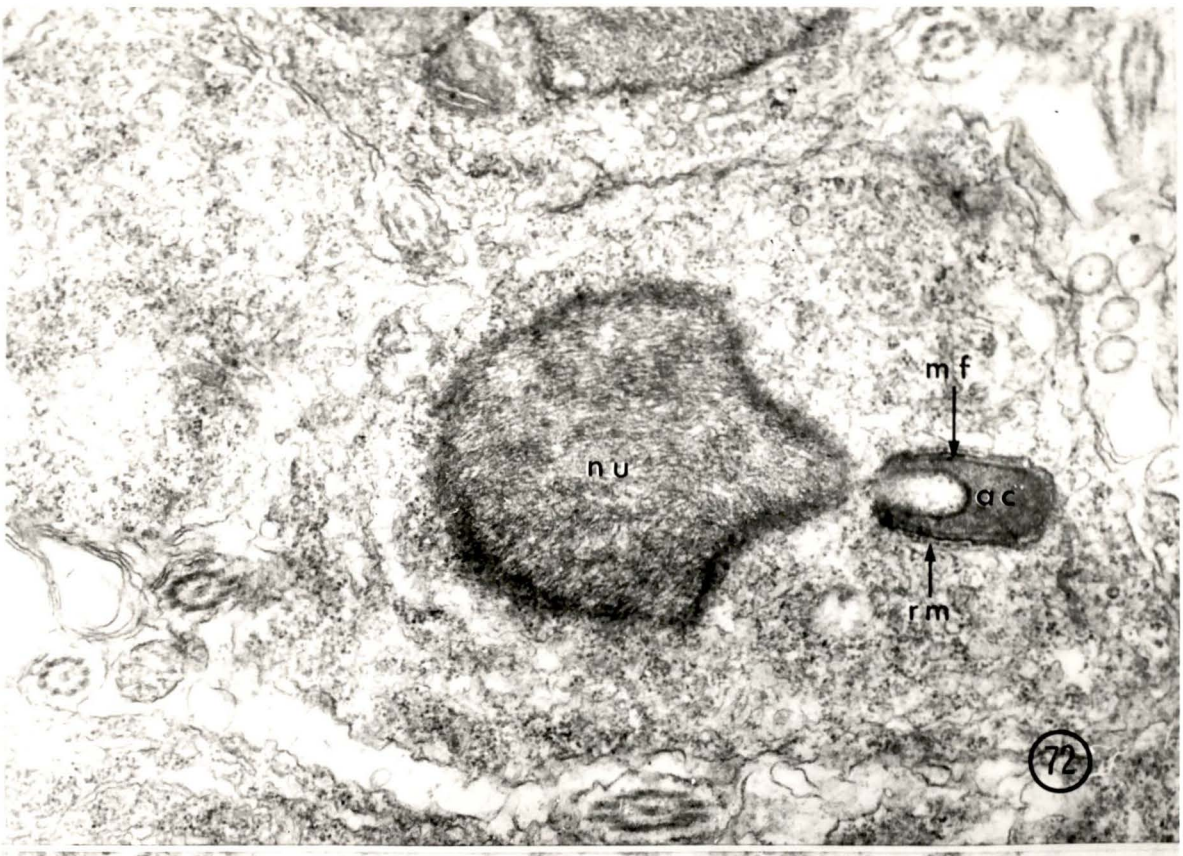
Figure 71. A longitudinal section through a spermatid showing all important parts. (X 20700)

ac - acrosome; bp - basal plate; ca - cap; ce - centriole; mi - mitochondrion; nu - nucleus.


ac
ca
bp
ce
nu
mi

Figures 72 and 73. Further stages of nuclear and acrosomal development in spermatids. (72 - X 25600; 73 - X 53000)

ac - acrosome; ce - centriole; mf - microfilaments; nu - nucleus; rm - ragged membrane.


mf
nu
ac
rm
72

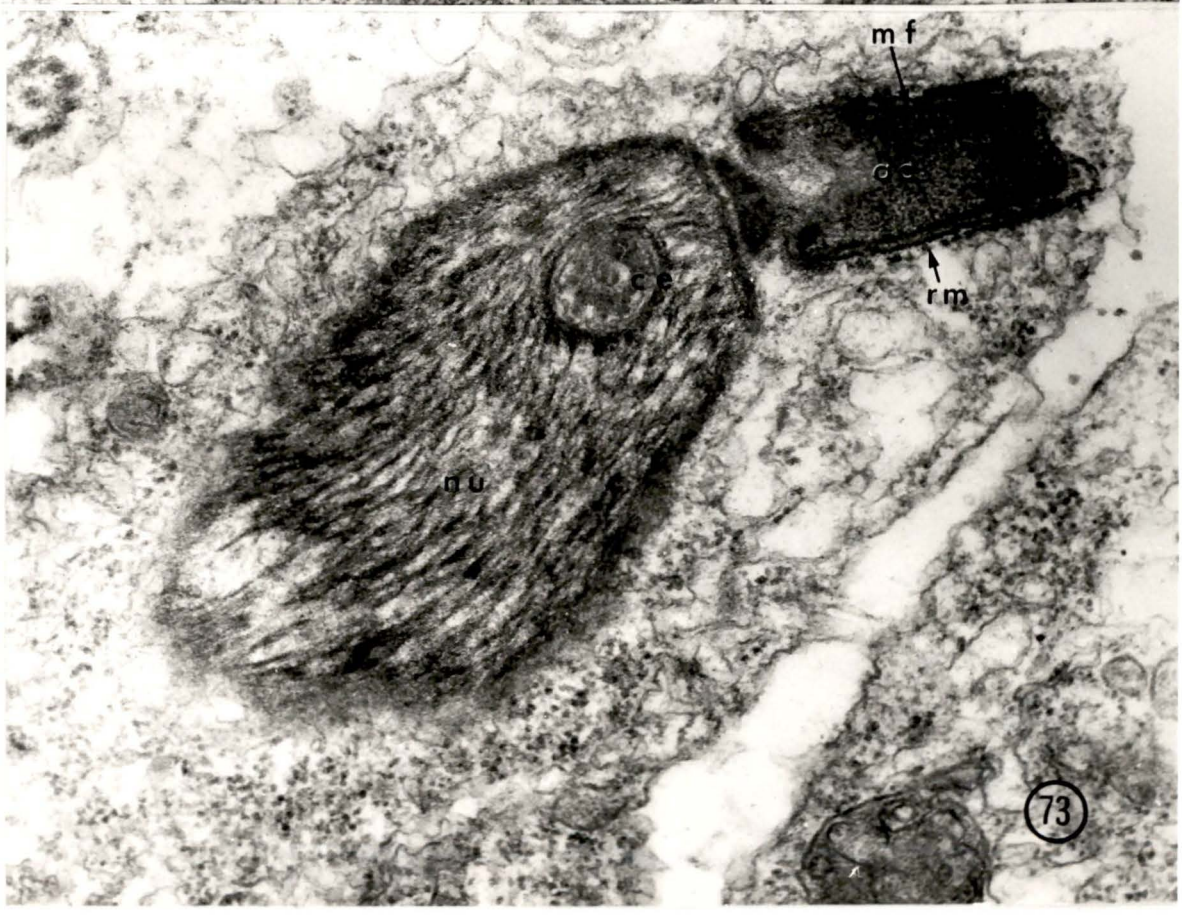
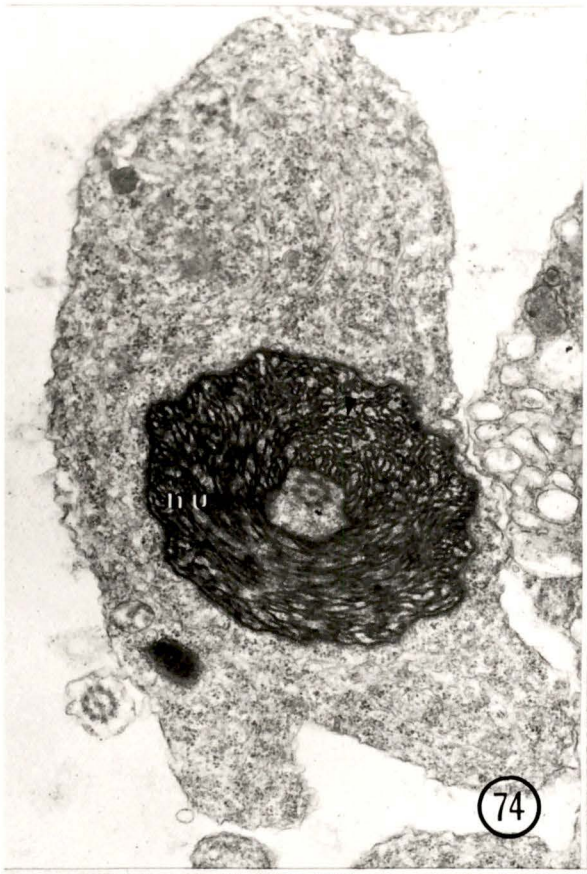

mf
ac
rm
ce
nu
73

Figure 74. Cross section through a developing nucleus (nu) in the beaded strand stage (arrowhead). (X 22800)

Figure 75. Cross section of developing nucleus (nu) in the lamellar fusion stage (arrowheads point to the lamellae). (X 20400)

Figure 76. Cross section through a developing mitochondrial spiral, separate mitochondria (mi) are fusing. (X 30900)

Figure 77. Cross section through the developing tail piece. The connecting bars (arrowhead) are present and glycogen (gl) is accumulating around them. Excess cytoplasm is being sloughed off as evidenced by the myelin bodies (my). (X 54100)



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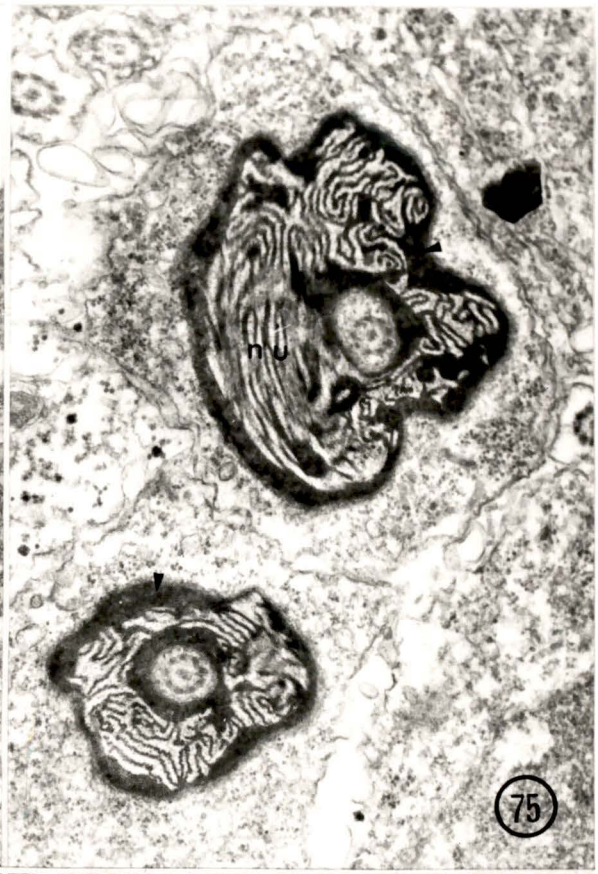

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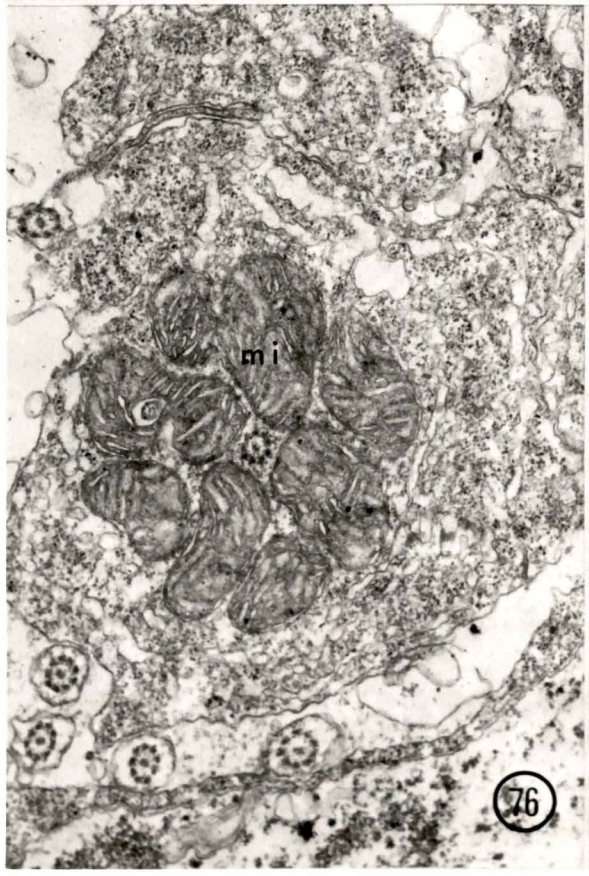

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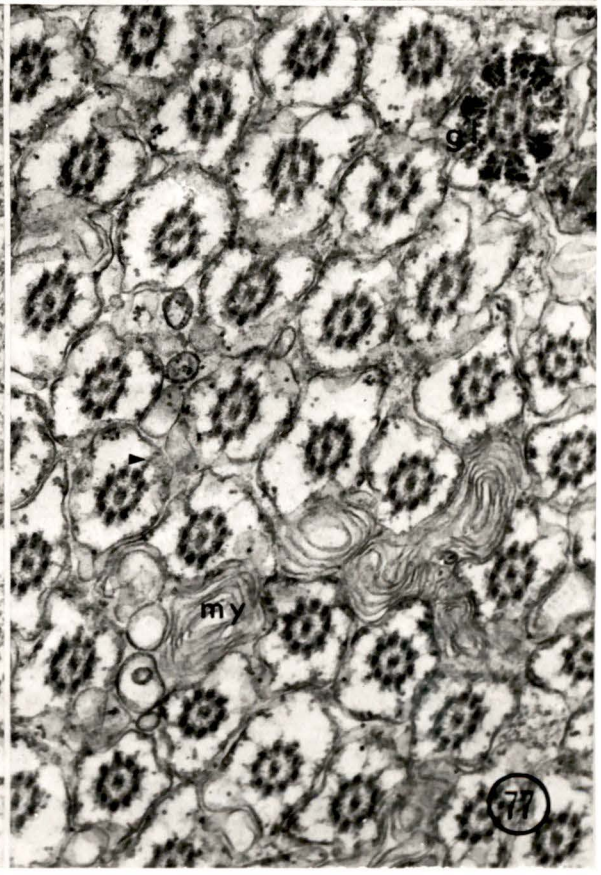
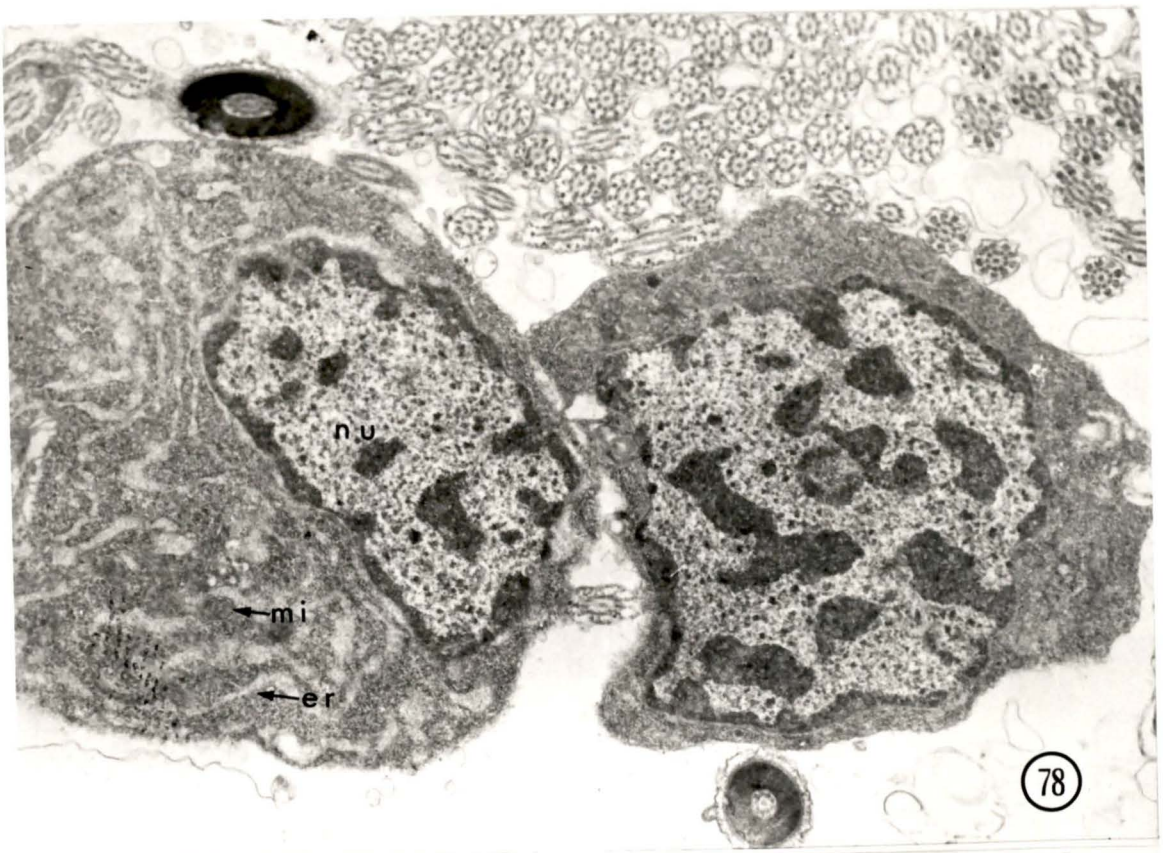


77

Figure 78. Spermatogomium already showing apyrene characteristics, frothy cytoplasm. (X 12500)

Figure 79. Early apyrene spermatocyte stage, the nucleus has become cresenteric. (X 17700)

db - dense bodies; er - smooth endoplasmic reticulum; Go - Golgi; mi - mitochondrion; nu - nucleus.



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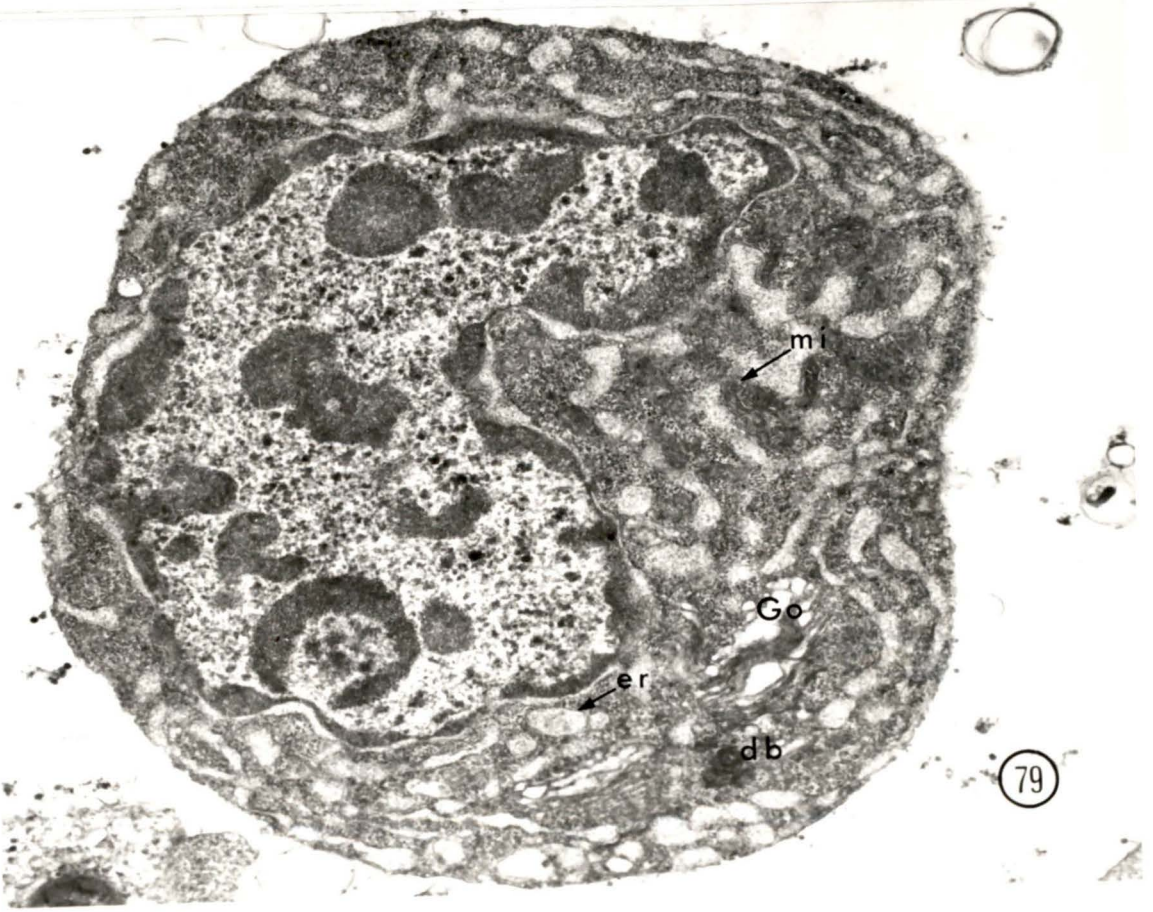
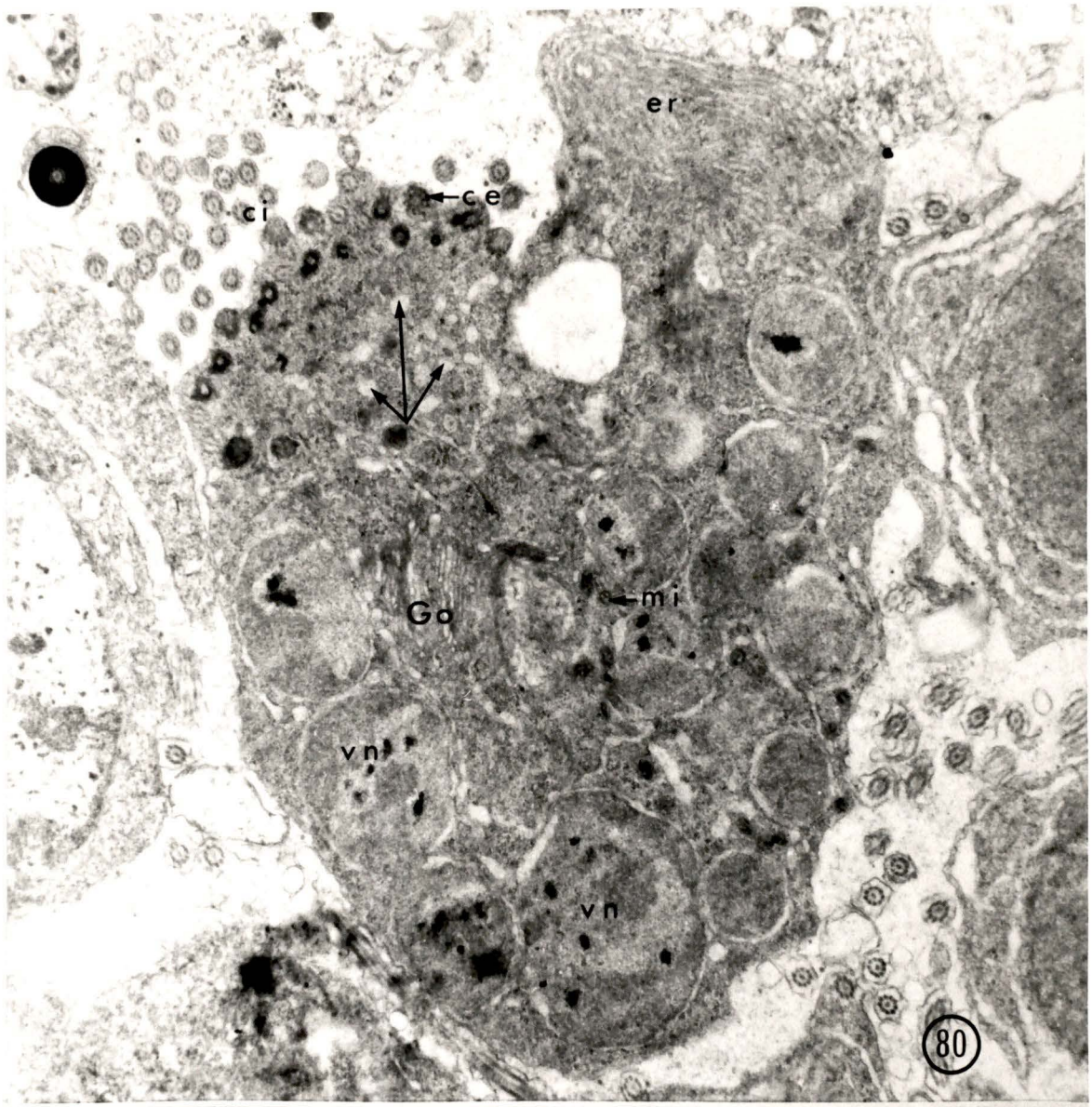


79

Figure 80. A later apyrene spermatocyte. (X 12600)

Figure 81. Enlargement of the centriolar region of the extending and developing cilia. (X 30400)

ce - centrioles; ci - cilia; er - smooth endoplasmic reticulum; Go - Golgi; mi - mitochondrion; rb - "rootlet" base of a centriole; vn - vacuolated nuclear fragment; arrows indicate "bubbles", small, developing droplets.


er
ci
ce
Go
mi
vn
vn
80

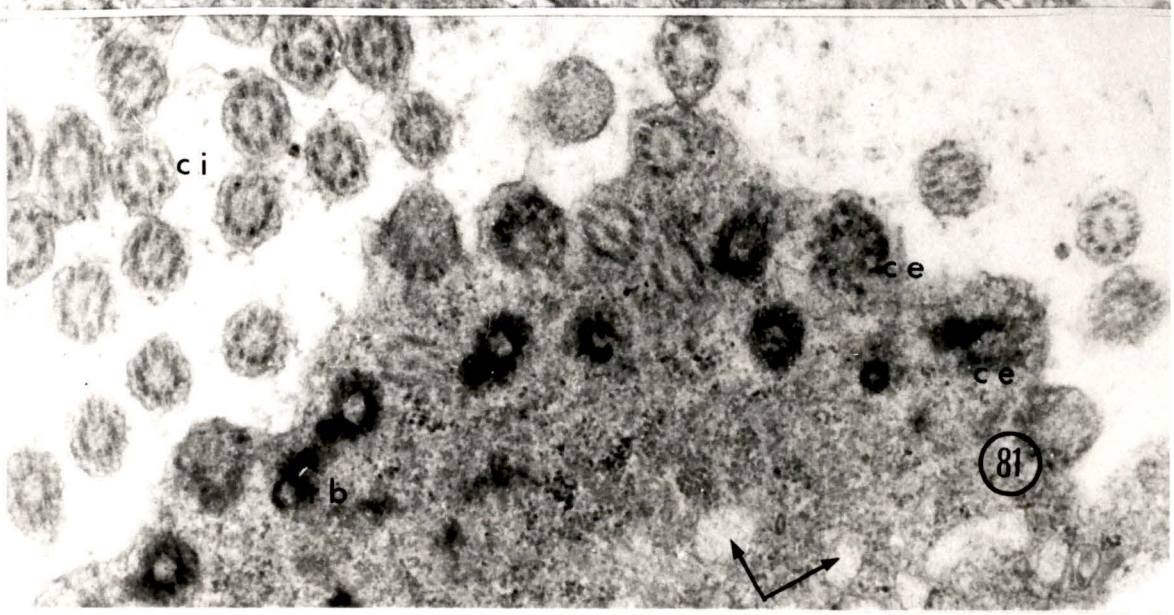
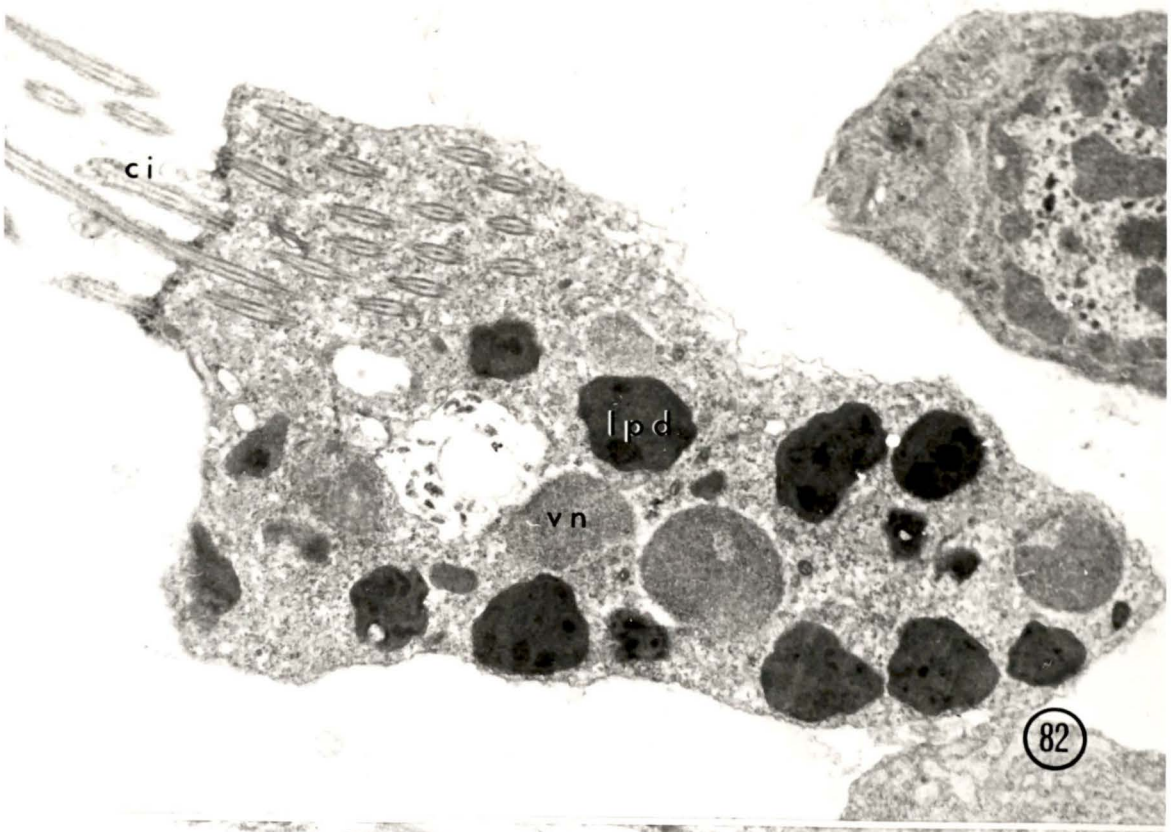

ci
ce
ce
b
81

Figure 82. Longitudinal grazing section through a developing carrier sperm. (X 25700)

Figure 83. Cross section through a developing carrier sperm; arrowhead points to a dividing mitochondrion. (X 12200)

ax - axonemes; ci - cilia; mi - mitochondria; lpd - large polysaccharide droplets; vn - vacuolated nuclear fragments.



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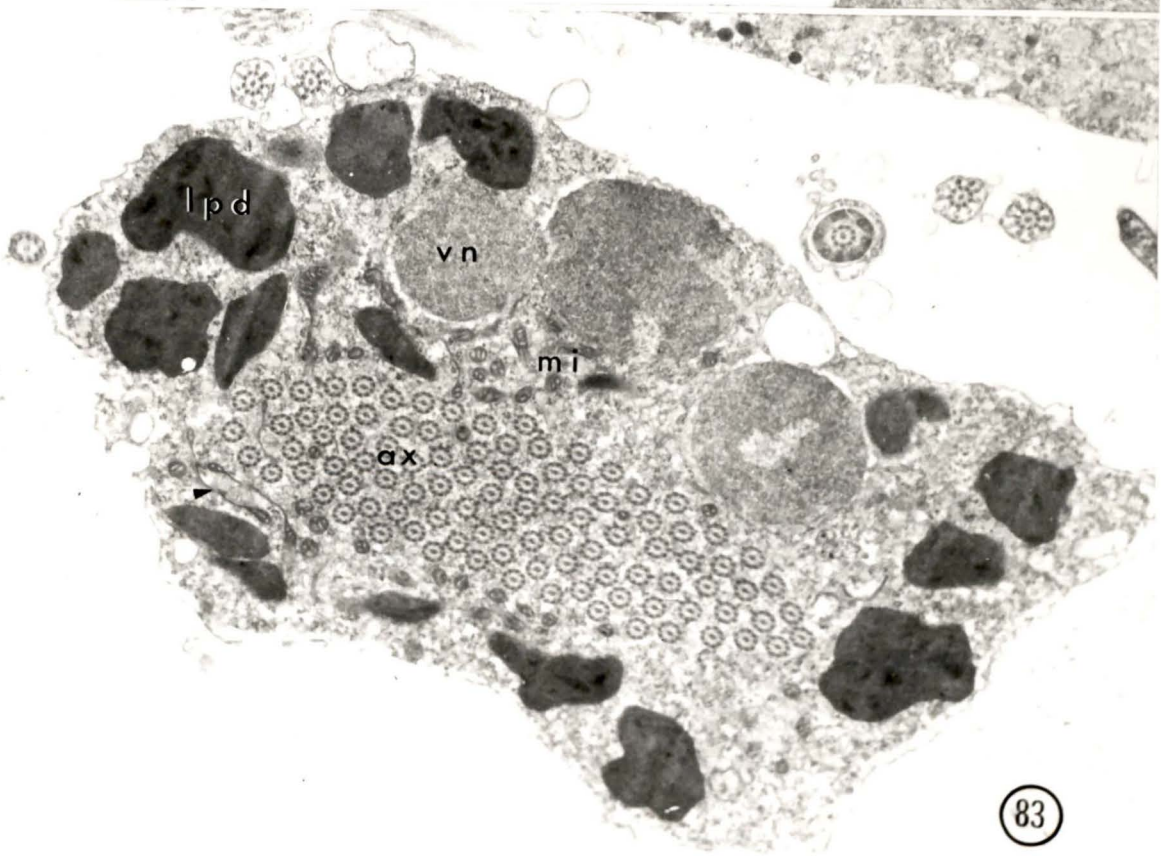
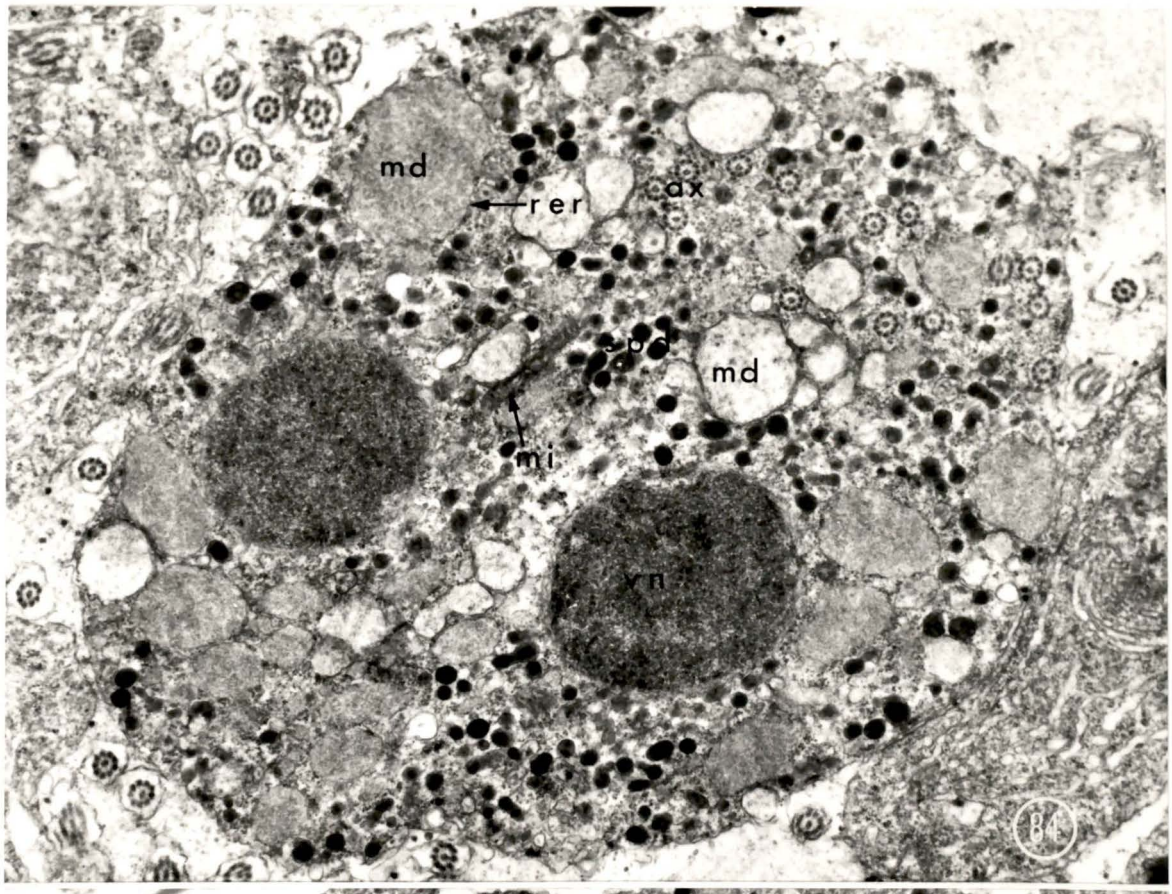


83

Figures 84 and 85. Maturing stages of lancet sperm. Axonemes have not yet migrated to the cell peripheries. (84 - X 14000; 85 - X 11100)

ax - axonemes; md - mucous droplets; mi - mitochondria; rer - rough endoplasmic reticulum; spd - small polysaccharide droplets; vn - vacuolated nuclear fragments.


md
rer
ax
spd
md
mi
vn
84

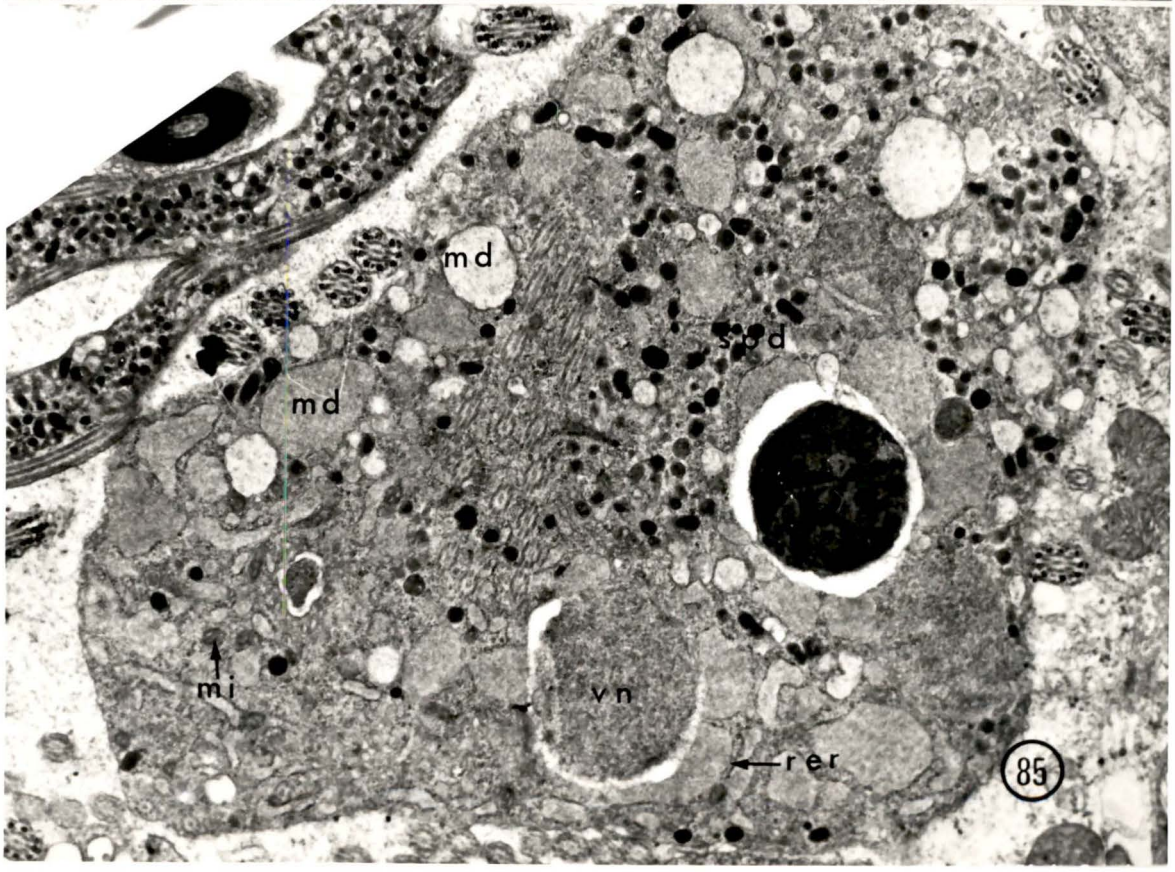
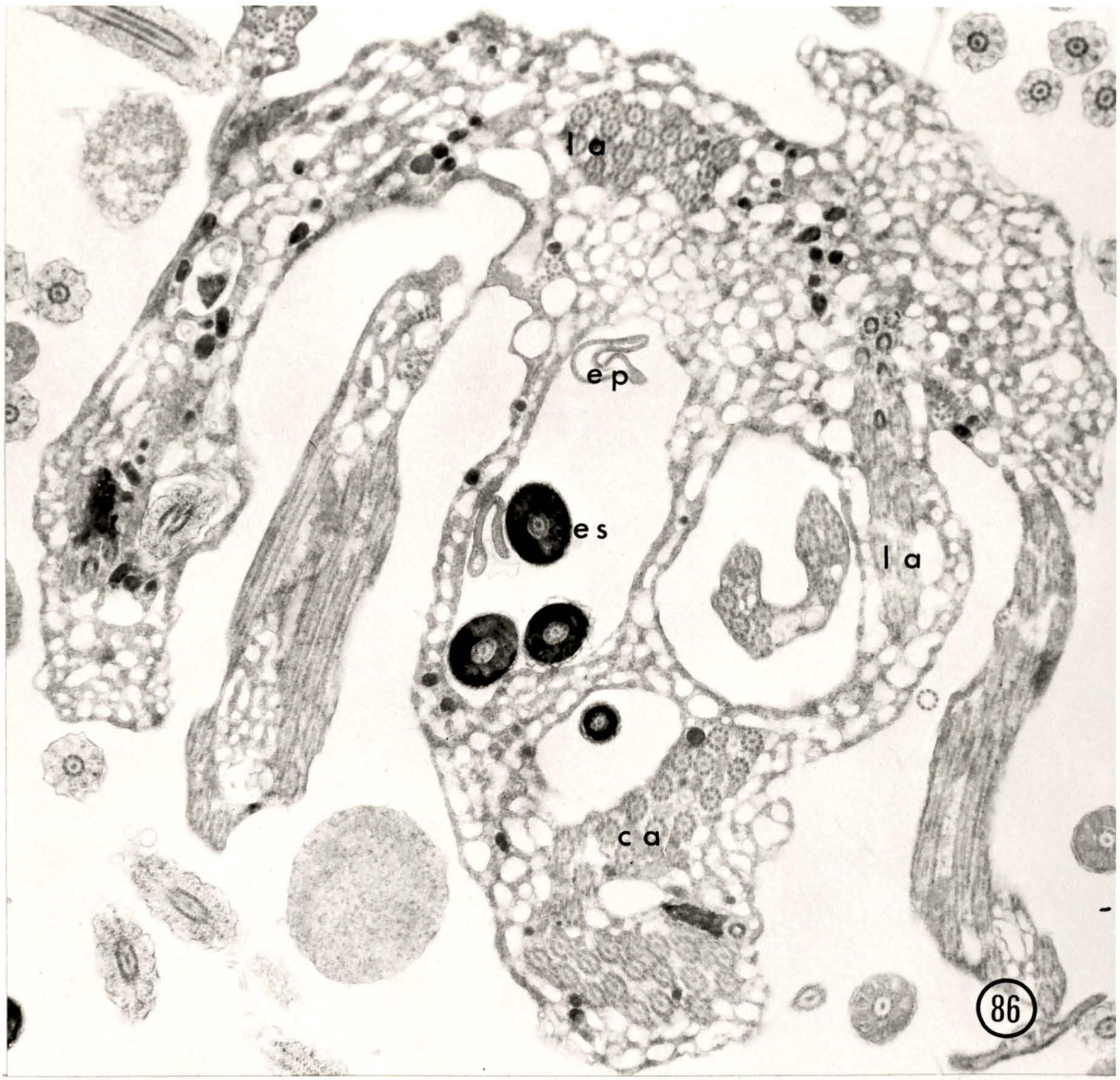
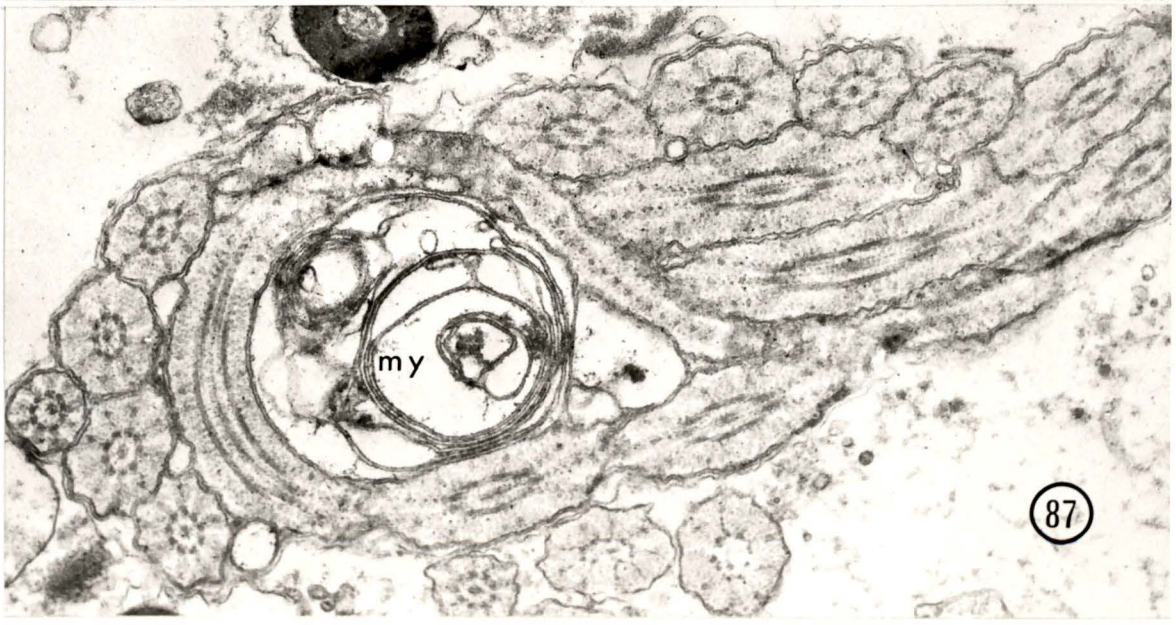

md
spd
md
mi
vn
rer
85

Figure 86. A plasmodial formation initiated by a lancet, taken from the copulatory bursa. It has engulfed eupyrene sperm (es), other lancets (la) and carrier (ca) sperm. (X 11700)

ep - end piece of a lancet sperm.

Figure 87. Degenerating eupyrene sperm in the copulatory bursa, note the myelin body (my) and clumping. (X 33900)


la
ep
es
la
ca
86


my
87

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Mackie, G.O., D.H. Paul, C.M. Singla, M.A. Sleigh and D.E. Williams, 1974. Branchial innervation and ciliary control in the ascidian *Corella*. Proc. R. Soc. Lond. B 187:1-35.

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SOME ASPECTS OF THE REPRODUCTIVE BIOLOGY OF

FUSITRITON OREGONENSIS (REDFIELD) (GASTROPODA, PROSOBRANCHIA)

Author [redacted]

DAPHNE EIRI WILLIAMS

May 3, 1976