University of St Andrews



Full metadata for this thesis is available in St Andrews Research Repository at:

http://research-repository.st-andrews.ac.uk/

This thesis is protected by original copyright

THE ORGANIZATION OF SPERMATOZOA IN Acilius sulcatus AND CERTAIN OTHER DYTISCID WATER BEETLES

by

J.B. Mackie

A thesis submitted for the degree of M.Sc. in the University of St. Andrews

1973



Th 7051

THE ORGANIZATION OF SPERMATOZOA IN Acilius sulcatus AND CERTAIN OTHER DYTISCID WATER BEETLES.

J. B. Mackie, Zoology Department, University of St. Andrews.

Abstract

This study deals with the changes in fine structural organization which occur during sperm development in four Dytiscid water beetles: Acilius sulcatus, Dytiscus marginalis, Colymbetes fuscus, and Agabus bipustulatus.

Sperm pairing in <u>Acilius</u> is a separate process from sperm development.

Paired sperm in <u>Acilius</u> are not produced from the same secondary spermatocyte (sister cells). Sperm-pairs in <u>Acilius</u> have only been observed in vasa deferentia.

Pairing does not occur in the testes. A layer of coarse granules between paired sperm heads may act as a 'cement'. This 'cement' contains periodic acid reactive carbohydrates. Paired sperm heads are not mirror images of each other or "right" and "left" handed. The two heads in a pair have identical shapes which are such that the two heads fit closely against and around each other. <u>Dvtiscus</u> sperm pair in a similar manner to <u>Acilius</u> sperm. <u>Colymbetes</u> sperm form groups with their heads arranged around a mass of 'cement' which contains periodic acid reactive carbohydrates. In the groups of sperm which are formed in <u>Agabus</u> vasa deferentia there is no specific arrangement of the sperm heads in relation to each other. The 'cementing' materials found in <u>Acilius</u>, <u>Dvtiscus</u> and <u>Colymbetes</u> are absent in <u>Agabus</u>.

All the dytiscid sperm heads which have been studied are highly asymmetrically shaped.

Treatment with detergents, proteolytic enzymes and special histochemical staining techniques have demonstrated that the accessory fibres and central tubules in <u>Acilius</u> sperm tails are similar to each other and that they differ

from doublet fibres. The paracrystalline material in mitochondrial derivatives contains periodic acid reactive carbohydrates and protein.

An XO system of sex chromosomes has been demonstrated for Acilius.

The fine structure of synaptinemal complexes and polycomplexes in Acilius is described in detail.



- (A) Acilius sulcatus male
- (B) Acilius sulcatus female
- (C) Dytiscus marginalis male
- (D) Dytiscus marginalis female

CONTENTS

	Page
PREFACE	
THE RESERVE BY AS A STATE OF THE PARTY OF TH	
INTRODUCTION	1
(2) Materials and Methods	5
The Animals	5
Male reproductive system	6
Light Microscopy	7
(a) Heidenhain's iron haematoxylin staining of	
paraffin wax sections	7
(b) Feulgen staining	8
(i) Wax sections (ii) Squashed cells	
(c) Light microscopical examination of living sperm	9
(d) Photomicroscopy	9
Electron Microscopy	10
(a) Sectioned material	10
(b) 'Negatively' stained material	11
(c) Sodium lauryl sulphate treatment of sperm from	
the vasa deferentia of Acilius	11
(d) Sarkosyl treatment of sperm in the vasa deferentia of Acilius	12
(e) Silver methenamine staining of carbohydrates in thin sections	12
	12
(f) Trypsin treatment of sperm from the vasa deferentia of <u>Acilius</u>	13
(g) Pronase digestion of sectioned sperm	13

	Page
(3) Spermatogenesis in Acilius	14
Mitosis	14
Primary spermatocytes	14
Synaptinemal complexes	15
Polycomplexes	16
Meiotic metaphase I	16
Meiotic anaphase I	16
Meiotic telophase I	17
Meiotic metaphase II	18
Initial development of the flagellum	18
Meiotic anaphase II	18
Meiotic divisions	19
Fine Structure of Spermatids	
(a) Stage 1 Spermatids	19
(b) Stage 2 Spermatids	20
(c) Stage 3 Spermatids	20
(d) Stage 4 Spermatids	21
(e) Stage 5 Spermatids	21
(f) Stage 6 Spermatids	22
(g) Stage 7 Spermatids	22
(h) Stage 8 Spermatids	23
Late spermatids and mature sperm in the testis	23

	Pag
DISCUSSION	. 25
Sex chromosomes	2 5
Spermatogenesis and sperm pairing	. 25
Centricles	. 26
Basal body and centriole adjunct	. 27
Synaptinemal complex	. 28
Spindle and mitochondria-associated microtubules	29
Mitochondrial derivatives	. 31
Chromatin condensation	. 32
Sperm flagellum	
(4) Mature Acilius sperm from the vasa deferentia	• 35
Sperm movement and tail shape	. 40
DISCUSSION	. 42
Mature sperm heads and sperm pairing	. 42
Acilius sperm tails	. 44
Flagellum	. 44
Mitochondrial derivatives	. 46
Sperm movement	. 48

	rage
(5) Experimental treatment of <u>Acilius</u> sperm from vasa deferentia	49
Sodium lauryl sulphate treatment	49
Sarkosyl treatment	49
Silver nitrate methenamine treatment	5 1
Trypsin treatment	5 1
Pronase	52
DISCUSSION	53
(6) Structure of the mature sperm in <u>Dytiscus</u> , <u>Colymbetes</u> and <u>Agabus</u>	55
Dytiscus sperm	55
Colymbetes sperm	56
Agabus sperm	58
Discussion	59
Summary	62
References	64
Key to figures	80
Key to abbreviations used in labelling	81
Text figures	

The spermatozoa of most animals exhibit a high degree of structural complexity. This complexity is more elaborate than that of most of the other cell types in an organism. Yet, spermatozoa develop from spermatogonial cells which are by comparison structurally much less intricate. Furthermore, in certain dytiscid water beetles the heads of the mature sperm contained in the vasa deferentia are shaped so that they fit together in pairs in a close and intimate fashion. The following account deals with an investigation of the developmental processes involved in the production of spermatozoa in one such beetle. Acilius sulcatus, as revealed by light and electron microscopy. The fine structure of mature Acilius spermatozoa is described in detail. This description has been augmented by an analysis of the composition of several components of these spermatozoa. Treatment with detergents and proteolytic enzymes, as well as special histochemical staining techniques have been used in this analysis. Mature spermatozoa, and certain aspects of their development, in three other dytiscid beetles have also been examined to compare their organization with those of Acilius. While these comparisons have revealed similarities in sperm composition they have also shown striking differences in the ways in which the spermatozoa are structurally associated with each other inside the vasa deferentia in these closely related beetles.

I wish to express my sincere gratitude to Professor H.G. Callan for allowing me the time to undertake this project and use of the facilities in the Department of Zoology. I am indebted to Professor H.C. Macgregor, Department of Zoology, Leicester University for his initial encouragement and supervision, to Dr. J.B. Tucker for his supervision in the preparation of the text, and to Dr. M.H. Walker for her useful comments and suggestions. I thank Mr. J. Calder, Mr. D. Cunningham and Mr. I. Johnston for taking some extra responsibilities for technical duties in the department while I was engaged in this investigation. I am also grateful to all the other members of the department who have given useful criticism and encouragement.

INTRODUCTION

Certain dytiscid water beetle spermatozoa are distinct from those of most other animals because groups of spermatozoa are often joined together at their heads. Pairs of spermatozoa are the most common grouping encountered. Paired spermatozoa are not only found in the vasa deferentia of males but also in the receptaculum seminis of female beetles after copulation. The first detailed account of such associations was provided by Emil Ballowitz (1895) in his extensive report on 'The Double Spermatozoa of Dytiscids'. Since then similar sperm-pairs have been reported in one other insect order (Thysamura) and in certain myriapods, gastropods and marsupials.

During fertilisation, most sperm swim, and penetrate eggs as separate individuals. Ballowitz's study raises issues such as: how do pairs and other groupings of sperm perform these activities, and how are such groupings accomplished during spermatogenesis? Ballowitz advanced certain speculations and conclusions concerning these and other questions posed by his findings. He found pairs of sperm in Acilius and Dytiscus but only single sperm in Agabus. In Colymbetes he found groupings of 3 to 8 sperm with their heads attached around a 'cementing substance'. He regarded sperm grouped in threes as precursors of larger aggregations. Ballowitz asserted that the cementing substance was the only material responsible for bringing about union of sperm heads. Sperm heads are sometimes very intimately

associated. For example, in the sperm-pairs of Acilius, the curved side of each head passes around the thickened edge of the other head. The possibility that sperm-pairs represent sister cells was discounted by Ballowitz because immediately following the division of each secondary spermatocyte, sister spermatids are separated and can become associated with other spermatids in their proximity. The origin of the two partners in each sperm-pair intrigued Ballowitz. He questioned whether there is 'an original duality of the sperm, a qualitative difference, or a type of polar opposites!.. In 'The Cell in Development and Heredity * E.B. Wilson (1924) pointed out that 'During the period of their association the two sperms are of different but corresponding shape, being mirror figures of each other, or "right" and "left" handed'. Selenka (1887) makes a similar claim for sperm-pairs in the marsupial Didelphys. Can 'mirror image sperm' develop from germinal cells which are genetically identical? I have given special attention to this and evidence will be presented showing that for dytiscid beetles such differentiation of sperm is not in fact the case.

Dytiscid spermatozoa have not been examined since Ballowitz studied them in 1895. However, the sperm-pairs of certain marsupials have been studied recently by Phillips (1970). Paired spermatozoa have also been described in a gastropod mollusc (Retzius, 1906), in a thysanuran (Bawa, 1964), and in certain myriapods (Reger and Cooper, 1968; Horstmann and Breucker, 1969). These sperm-pairs were

found in spermatic ducts, seminal vesicles, epididymi, vasa deferentia, and seminal receptacles. The sperm-pairs are usually closely associated in the head region, but in the case of the thysanuran Thermobia domestica Pack the tails are intertwined near their tips.

Ballowitz necessarily had to rely on light microscopy during his studies. He examined fresh sperm isolated in physiological saline. He also examined air dried sperm, sperm fixed with osmium tetroxide fumes (sometimes subsequently stained with gentian violet). and sperm in paraffin wax sections of vasa deferentia stained with alum carmine and haematoxylin. In the present study, four of the species (each from a different genus) examined by Ballowitz have been re-examined using modern microscopical techniques to investigate some of the questions posed by Ballowitz in greater detail. Ultrathin sections of sperm and negatively stained sperm have been examined using transmission electron microscopy. Living sperm have been examined using Nomarski Interference Contrast and Phase Contrast microscopy. Treatment of sperm with proteolytic enzymes and detergents, and certain histochemical techniques, have also been used to analyse and compare the composition of some of the sperm components. Structural changes occurring during spermatogenesis and the structure of mature sperm have been investigated in detail in an attempt to clear up some of the points raised by Ballowitz. In particular the following issues have been dealt with. Are sperm partnered in pairs structurally distinct from each other? Are the two sperm in a pair descended from

the same secondary spermatocyte (i.e. are they sister cells?)? In what ways are the heads of sperm-pairs, triplets and larger groupings associated? How do these associations develop and what is the nature and role of the cement between sperm heads? Do sperm-pairs show any unusual locomotory features? Are the sperm of Agabus always unassociated or do they ever occur as pairs and even larger groupings as they do in other dytiscid beetles? Do sperm heads differ radically in structure and composition for different dytiscid genera and if so are such differences correlated with the ways in which heads become associated?

In addition to dealing with the questions outlined above, many previously undescribed details of the fine structure and composition of the spermatocytes, spermatids, and mature sperm of dytiscid beetles are reported in this dissertation. In several places, I have tried to interpret the organization, and changes in the organization, of mature sperm and developing sperm, in terms of the developmental processes occurring during spermatogenesis and the functional role of mature sperm. The significance of certain differences and similarities between the sperm of the four beetles examined have also been considered. Finally I should point out that my investigations have been concentrated on the sperm of Acilius which usually occur as sperm-pairs. Only certain aspects of sperm organization, selected as being the most rewarding for comparative purposes, have been examined for Agabus (single sperm), Dytiscus (pairs) and Colymbetes (triplets and larger groupings).

(2) MATERIALS AND METHODS

The Animals

The water beetles used in this study were all from the family Dytiscidae. The classification of these beetles gives some indication of the relationships between the beetles used.

Order Coleoptera

Group Hydradephaga

Family Dytiscidae

Tribe Colymbetini

Genera Agabus

Colymbetes

Tribe Dytiscini

Genera Dytiscus

Acilius

The four species used were <u>Agabus bipustulatus</u> Linnaeus, <u>Colymbetes fuscus</u> Linnaeus, <u>Dytiscus marginalis</u> Linnaeus and <u>Acilius</u> sulcatus Linnaeus.

The beetles were collected from a pond in Tentsmuir Forest, Fife, Scotland. This pond is stagnant and the water has a pH of about 7.0. The beetles were caught with a hand net. The highest yields were obtained during the warmer months (April - September). They were kept in transparent polystyrene tanks at 18°C in tap water with a little pond weed (Elodea canadiensis). Dytiscus, and sometimes the other beetles, were kept one to a tank to avoid cannibalism. They were fed with Tubifex rivulorum.

Male and female beetles were easily distinguished. Males of Acilius and Dytiscus have smooth elytra; females have sulcated elytra. All males have modified tarsi; prothoracic male tarsi are broader than those of females and have rows of suckers on the three most distal segments. Meso-thoracic male tarsi are similarly modified in Agabus and Colymbetes.

Male reproductive system

The testes and vasa deferentia and their contents are the only reproductive tissues which have been examined. The testes and vasa deferentia are easily distinguished in dissected beetles. The gross form and dimensions of the main reproductive tissues in Acilius are shown in Fig. 1. The testes (TS), vasa deferentia (VD) and accessory glands (AG) are all paired. Each testis is simple, tubular, and closely coiled. The anterior extremity of each testis is positioned below one of the large post-coxae. The region of each testis which is filled with spermatids and sperm is a creamy white colour. The remainder of each testis is a very pale white. The unction of each testis with a vas deferens is usually marked by a yellow band of material. Each vas deferens is coiled and is creamy white. There is a dilation of each vas deferens before it enters the accessory gland mear the point where the two accessory glands meet to form the single median ejaculatory duct (EJ). Each accessory gland has a rounded free end and is coiled clockwise three or four times. The free end of each accessory gland is very pale white, this is then followed by

small yellow coloured band, and the remainder of the gland is white in colour. The ejaculatory duct (6 mm.) and the aedeagus (3 mm.) are short and somewhat flattened dorsoventrally.

Light Microscopy

(a) Heidenhain's iron haematoxylin staining of paraffin wax sections.

Testes and vasa deferentia were dissected from Acilius and fixed immediately in San Felice's fixative for 12 hr. The fixative was prepared by mixing equal volumes of two solutions immediately before use (solution A: 100 ml 2% chromic acid. 60 ml acetic acid. Solution B: 100 ml 40% formaldehyde. 60 ml water). After washing for 12 hr with water they were placed in 70% ethanol. Then they were immersed for 30 seconds in a 1% solution of light green stain in 70% ethanol. After this treatment they were stained sufficiently darkly so that they could be seen and located in the wax during embedding. After further dehydration in ethanol they were embedded in wax (M.P. 55°C). Sections 10µ thick were cut and mounted on glass slides. The sections were hydrated to water and then mordanted in 3% iron alum for 1 hr, stained in Heidenhain's iron haematoxylin for 1 hr, and differentiated in 1.5% iron alum (1-2 min) until only cell nuclei were darkly stained. Sections were then dehydrated with ethanol, cleared in xylene, and mounted in neutral balsam.

(b) Feulgen staining

(i) Wax sections

Testes and vasa deferentia were fixed as described above and stored in 95% ethanol. After being taken down to water, they were hydrolyzed in 1N HCl at 60°C for 20 min, and then stained in Feulgen's reagent for 2 hr. The Feulgen reagent was prepared using the method described by Swift (1955). After three changes of SO₂ water (10 ml N HCl; 10 ml 5% potassium metabisulphite; 180 ml H₂0), the tissues were dehydrated with ethanol. Wax sections (10 μm) were prepared as described above.

(ii) Squashed cells

Testes and vasa deferentia were fixed for 3 hr in a freshly prepared mixture of 3 parts 100% ethanol and one part glacial acetic acid. After 5 (10 min) washes with 70% ethanol they were taken down to water, hydrolyzed in 1N HCl at 60°C for 10 min and stained in Feulgen's reagent for 2 hr. After washing in SO₂ water as described above they were placed into 45% Glacial Acetic acid for about 30 min. They were then squashed between slides and coverslips and frozen with solid CO₂. The coverslips were removed, and the squashed preparations were dehydrated starting with 95% ethanol, cleared in xylene, and mounted in neutral balsam for examination.

(c) Light microscopical examination of living sperm

The vasa deferentia from freshly killed beetles were placed into liquid paraffin on a slide and teased apart. A coverslip was then placed on top of each such preparation and the movements of sperm inside the vasa deferentia were examined.

The testes and vasa deferentia from freshly killed beetles were removed and placed into insect saline (aqueous 0.75% NaCl). Sperm were taken from either the testes or vasa deferentia and diluted in a drop of clean saline on a slide. A coverslip was then placed on top and sealed at the edges with rubber solution.

(d) Photomicroscopy

Sections, squashed cells, and living sperm were all examined with a Carl Zeiss Photomicroscope. Planapo objectives were used for bright-field examinations. Micrographs were taken using Ilford Pan F 35 mm film which was developed in Agfa Rhodinal (1 ml in 100 ml of water) for 20 min, and fixed in Metafix for 10 min. This microscope was also used for examining living sperm using phase-contrast Neofluar objectives and Nomarski Interference-Contrast attachments. Micrographs of living sperm were taken using a Zeiss electronic flash device and Ilford Pan F film.

Electron Microscopy

(a) Sectioned material

The testes and vasa deferentia were dissected from a freshly killed beetle and fixed initially in a 10% solution of glutaraldehyde buffered with a phosphate buffer (0.066 M. pH 7.2) for 5 min. During this time the material was cut into short lengths of a few millimetres. Three washes (1 min) with phosphate buffer (0.066 M, pH 7.2) followed. The material was then post-fixed in a solution of 1% osmium tetroxide in veronal acetate buffer (0.14 M, pH 7.2) (Palade, 1957) for 10 min. After washing with distilled water the material was dehydrated with an acetone series (70%, 90%, 100%) and embedded in Vestopal W. Araldite was also used for embedding. This was preceded by dehydration with an alcohol series (70%, 95%, 100%). Silver and grey Vestopal and Araldite sections were cut with glass knives on a Huxley Ultramicrotome (Cambridge Instrument Co.) and mounted on Athene 483 grids (Smethurst Highlight, Ltd.) without supporting films. The Vestopal sections were stained with 2% aqueous (W/V) uranyl acetate for 5 min followed by lead citrate (Reynolds, 1963) for 2 min. Araldite sections were stained for 1 hr in 2% uranyl acetate and 5 min in lead citrate. The sections were examined with a Siemens Elmiskop I operating at 80 Kv with a 50 µm objective aperture. Plates (Ilford EM4) were taken at magnifications ranging from 5,000 - 40,000 and developed at 20°C for 21 min in Ilford PQ Universal developer.

(b) 'Negatively' stained material

Whole sperm from the vas deferens of all the species used in this study were examined after having been 'negatively' stained with phosphotungstic acid. A vas deferens was removed from a freshly killed beetle and placed into saline (aqueous 0.75% NaCl). The vas deferens was pulled apart releasing a thick suspension of sperm. Sperm were picked up using New 200 grids (Smethurst Highlight Ltd.) coated with a film of Formvar and an evaporated carbon film. Grids were touched onto the surface of the suspension, washed in distilled water for 1 min and then promptly drained of water by touching the edge of the grid against a piece of filter paper. This was immediately followed by a drop of filtered phosphotungstic acid (pH 6.5) which was discharged onto the grid from a hypodermic syringe needle (20 gauge). The needle was attached to a millipore filter holder containing a filter of 0.45 µm pore-size and the phosphotungstic acid was pushed through the filter with a hypodermic syringe. After 1 min the stain was drained off with filter paper and the grid then allowed to dry before examination in the electron microscope.

(c) Sodium lauryl sulphate treatment of sperm from the vasa deferentia of Acilius

A freshly excised vas deferens was placed in insect saline (aqueous 0.75% NaCl) and teased open with fine forceps. Sperm from this suspension, with as little accompanying saline as possible, was

pipetted into a watch glass containing a few drops of a 0.025% solution of sodium lauryl sulphate. After 2 min the sperm were picked up on grids (coated as described above) at 1 min intervals for a period of 13 min. The grids were then washed for 1 min with distilled water and 'negatively' stained for electron microscopy as described above.

(d) Sarkosyl treatment of sperm in the vasa deferentia of Acilius

Sarkosyl (NL35, Geigy Industrial Chemicals) was diluted with insect saline (aqueous 0.75% NaCl) to give final concentrations of 0.05%, 0.025%, 0.5%, 1.0%, 1.5% and 2% Sarkosyl. To every 500 ml of the diluted Sarkosyl 0.84 gm of E.D.T.A. (Versene) was added.

Parts of a freshly excised vas deferens were placed in the Sarkosyl solutions for 1 hr. Each Sarkosyl solution was then pipetted off and the material was fixed initially in 10% Glutaraldehyde and subsequently with osmium tetroxide (the same fixatives as those described earlier). Dehydration with acetone was followed by embedding in Vestopal W.

(e) Silver methenamine staining of carbohydrates in thin sections

Vasa deferentia from <u>Acilius</u>, <u>Dytiscus</u> and <u>Colymbetes</u> were fixed with glutaraldehyde and osmium tetroxide and embedded in Araldite (see section (a)). Thin sections were picked-up with titanium grids (Ernest F. Fulham Inc.) and stained for 45 min with

silver methenamine (Rambourg, 1967). Titanium grids were used because silver methenamine does not react with titanium to produce electron-dense reaction products which contaminate sections if they are supported on conventional copper grids.

(f) Trypsin treatment of sperm from the vasa deferentia of Acilius

A freshly excised vas deferens was placed in saline (aqueous 0.75% NaCl). A drop of sperm suspension was then placed in a few drops of 0.5% aqueous trypsin. Formvar and carbon coated grids were touched onto the surface of the suspension of sperm in trypsin solution at intervals ranging from 5-90 min after the start of the trypsin treatment. The grids were then washed with distilled water for 1 min and 'negatively' stained with phosphotungstic acid at pH 6.5 for 1 min.

(g) Pronase digestion of sectioned sperm

Thin sections of Araldite embedded vasa deferentia (fixed as described previously) were picked up on titanium grids. Grids were then immersed in 0.5% aqueous pronase (pH 7.4; adjusted with 0.01 M NaOH) (Monneron and Bernhard, 1966). After 6 hr digestion at 37°C the grids were washed with distilled water for 5-10 min and this was followed by immersion in cold 5% aqueous trichloroacetic acid (T.C.A.) for 5 min and washing in distilled water for 2 min. Subsequently the sections were stained with uranyl acetate and lead citrate (see section (a)).

(3) Spermatogenesis in Acilius

Testes in beetles collected during mid-summer usually contain mature sperm and cells at all stages involved in the development and maturation of sperm from apical testis cells (Figs. 3, 4, 5, 6, 7, 9, 10). Each vas deferens is filled with mature sperm (MS) (Fig. 7). The main features occurring during this maturation are summarised in Fig. 2.

Mitosis

Each apical cell divides mitotically to produce two spermatogonia (Fig. 3). The mitotic apparatus occupies most of the cell during metaphase (MM) as it does at anaphase (MA) when the two groups of separating sister chromosomes are clearly visible (Figs. 9. 10).

Primary spermatocytes

Primary spermatocytes vary considerably in size. Their diameters range from about 5 μm (Fig. 11) to about 9 μm (Fig. 13). In a small spermatocyte the nucleus (N) fills most of the cell (Fig. 11). The chromatin (CN) is condensed and structures called <u>synaptinemal complexes</u> (SC) are present (Fig. 11). Within some nuclei there is a mass of material (DN) which stains more densely than the remainder of the chromatin (Fig. 11). In the granular cytoplasm there are a few elongate mitochondria (M) and a Golgi body (G). Rough endoplasmic reticulum is also present (Fig. 11). In larger spermatocytes the chromatin (CN) is even more highly condensed in preparation for the

first meiotic division (Figs. 12, 13) indicating that the larger spermatocytes are at a later stage than the smaller ones. The contents of the cytoplasm are similar to those of smaller spermatocytes; they are larger because the volume of the cytoplasm has increased. Some of the membranes associated with the Golgi bodies (G) are more densely stained than is usual for Golgi membranes (Figs. 13, 14). Paired centricles, oriented at right angles to each other, also occur in cells at this stage. These centricles have a typical arrangement of 9 sets of triplet microtubules (Fig. 15).

Synaptinemal Complexes

Synaptinemal complexes (SC) have been found in cells at meiotic prophase (Figs. 16, 18, 19, 20, 21). Dense lateral elements (L) oriented parallel to the longitudinal axis of each complex occur, although their shapes are partly masked by the close proximity of dense chromosomal material (CL) (Figs. 18, 19, 20). The thicknesses of the lateral elements in longitudinal sections of complexes varies between 16.6 and 20 nm. The central element (CE) of each complex has a ladder-like appearance and it runs more or less parallel to the lateral elements (L) (Figs. 19, 20). Periodic transverse striations (M.J. Moses, 1968), thickness about 5 nm, pass obliquely across each central element (CE) with a periodicity of about 17 nm (Figs. 19, 20). Transverse filaments (TF) extend from each central element (CE), they are arranged in register with, and their longitudinal

axes are parallel to, the transverse striations (PT) (Figs. 19, 20).

Sections grazing the edge of a complex (SC) longitudinally cut the filaments (TF) in cross-section and reveal that they are indeed circular filaments rather than flat strips of material (Fig. 20).

In many cases one end of each synaptinemal complex contacts the nuclear envelope (NE) (Figs. 18, 20). At these contact points the membranes of the nuclear envelope are much closer together and stain more densely than elsewhere (Figs. 18, 20).

Polycomplexes

Polycomplexes (P) of two or three synaptinemal complexes fused together side by side have also been found in primary spermatocytes undertaking the 1st meiotic division (Fig. 17). In some cases chromosomal material (CL) lies on both sides of a polycomplex (Fig. 17), in others on one side only. Such chromosomal material (CL) is more highly condensed than that which surrounds synaptinemal complexes and indicates that polycomplexes occur at a later stage of division than synaptinemal complexes and are presumably formed by association of synaptinemal complexes.

Meiotic metaphase I

In cells at meiotic metaphase I (MM¹) the chromosomes (CH) are arranged on the equator of the spindle (Figs. 21-30, 32, 33) except for a single X chromosome (X) which has already been segregated

from the main bundle of chromosomes (Figs. 30, 31). Bundles of spindle tubules (ST) extend from the kinetochores (K) of the chromosomes (CH) towards the poles of the cell (Fig. 35) where centrioles (C) are situated 0.5 µm to 1 µm from the cell membrane (CM) (Fig. 33). Mitochondria (M) are elongate and are positioned at the periphery of the mitotic apparatus (Figs. 33, 24). The nuclear envelope has broken down completely at this stage (Figs. 33, 34, 35).

Meiotic anaphase I

During anaphase the groups of chromosomes become more distantly separated on the spindle (Figs. 23, 24, 31).

Meiotic telophase I

During early telophase (MT¹) the two groups of clumped chromosomes (CH) are concentrated at opposite ends of the dividing cell (Figs. 23, 24, 36). Very elongated mitochondria (M) run between them (Fig. 36). Spindle tubules (ST) lie closely alongside the mitochondria (M) (Fig. 38) and at the equator of the cell the cell membrane (CM) furrows in towards the mitochondria (Fig. 36). By late telophase the cell membrane (CM) has separated two daughter cells (Figs. 23, 27). The contents of the cytoplasm are similar to those described for early telophase. Centricles (C) are positioned close to the chromosomes (CH) (Fig. 37).

Meiotic metaphase II

In some meiotic metaphase II cells an X chromosome is situated at each spindle pole (Fig. 31). Therefore an XO system of sex chromosomes exists in <u>Acilius</u> males because only one X chromosome (which is not a bivalent) and no Y chromosomes have been detected in meiotic metaphase I cells (Figs. 30, 31).

Initial development of the flagellum

Stages in the initial development of the flagellum are present in meiotic metaphase II cells (Figs. 39-43). One centricle (C) of a pair is associated with a cup-shaped vesicle (CV); flagellar microtubules extend from each centricle and project into a vesicle (Figs. 39-43). Centricles have vesicles (CV) attached to them even while they are still associated with the spindle poles (SP) (Figs. 39, 42, 43). Sections show that in at least some cases the vesicle membrane (CV) is continuous with the cell membrane (CM) (Figs. 42, 43). In sections vesicles (CV) often appear clear and empty (Fig. 41) but sometimes they have granular contents (Figs. 39, 42). A granular mass (GM) lies at the tips of some vesicles (Figs. 42, 43).

Meiotic anaphase II

During early anaphase (EA²) the chromosomes move towards the poles of the spindles (Figs. 25, 29). By late anaphase (LA²) the chromosomes (CH) are positioned at the spindle poles (Figs. 26, 29, 44), the mitochondria (M) are elongate, and Golgi bodies (G) are present (Fig. 44).

Meiotic divisions

Sections show that the number of cells undertaking their 1st meiotic division always greatly exceeds those undertaking the 2nd meiotic division. This indicates that meiosis I is of greater duration than meiosis II. The arrangement of 1st and 2nd meiotic division figures in sections of testes are apparently not related spatially in any way to each other or to the general structure of testes (Figs. 3, 22-29).

Fine Structure of Spermatids

Eight distinct stages in the development of mature sperm from spermatids have been distinguished. These will be referred to as stage 1-8 spermatids respectively.

(a) Stage 1 Spermatids

Groups of early spermatids (SD) are present amongst meiotic cells (Figs. 3, 29). Early spermatids (SD) do not have any specific arrangement within a group (Fig. 4, 45), and the spermatids shapes are irregular (Figs. 47, 48, 49). Sectioned nuclei exhibit circular or oval profiles with densely stained chromatin (CN) lying near the nuclear envelope (Figs. 4, 45, 48, 49). In each spermatid the mitochondria are grouped together to form a nebenkern (NK) (Figs. 47, 48, 49) close to the nucleus. Numerous vesicles (V) and Golgi bodies (G) are present in the cytoplasm (Fig. 49). Early stages in the outgrowth of flagella (F),

already with a 9 + 2 arrangement of tubules are present at this stage (Fig. 48).

(b) Stage 2 Spermatids

At this stage of maturation spermatid nuclei are situated at the periphery of each spermatid cyst (Figs. 4, 45). The nuclei are still spherical with densely stained chromatin adjacent to nuclear envelopes (Fig. 46).

(c) Stage 3 Spermatids

Spermatid nuclei are arranged in a 'horseshoe configuration'

(H) at the periphery of the cysts (Figs. 4, 45). In these nuclei further condensation of the chromatin (CN) has taken place (Figs. 50, 51). A proacrosomal granule (PA) is situated close to each nucleus (Fig. 50). Adjacent to each nuclear envelope (NE) where densely stained material is clumped against the envelope there is a centricle which serves as a basal body (B) for a flagellum (Fig. 51). Two mitochondrial derivatives (MD) are formed by the separation of each nebenkern and they are aligned on either side of each flagellum (F) (Figs. 50, 51). A 9 + 2 arrangement of microtubules is present in each flagellum (F) but a curved structure (CD) extends from subfibre B (SB) or each doublet (D) (Fig. 50). Tubules (MT) (diameter about MITOCHONORIAL 20 nm) are present in the vicinity of the flagella (F) and Aderivatives (MD) (Fig. 50). Developing sperm tails (T) are directed to the

opposite side of the cyst and point away from the 'horseshoe' formation of nuclei (Fig. 45). Golgi bodies (G) are present in the cytoplasm of spermatids at this stage (Figs. 50, 51).

(d) Stage 4 Spermatids

Groups of spermatids (SD) are positioned with their nuclei towards the epithelial border (E) of each testis (Fig. 58).

(e) Stage 5 Spermatids

Densely stained chromatin (CN) fills most of each nucleus (Figs. 5, 53, 54, 59, 60). Where regions of nuclear envelopes have densely stained deposits of material on their inner surfaces, microtubules lie closely against the outer surface of the envelope (Figs. 53, 54). An acrosome (A) is closely associated with each nucleus and is situated at the presumptive anterior tip of the spermatid (Fig. 53). The volume of cytoplasm which surrounds the nuclei is much less than it is in stage 3 spermatids (Figs. 52-54). A fibrous centriole adjunct (CA) surrounds each basal body (B) (Fig. 52). Each flagellum lies between the two mitochondrial derivatives (MD) with its longitudinal axis parallel to that of the derivatives; a flattened membrane-bounded vesicle (DL) separates each flagellum from the two adjacent derivatives (Fig. 56). The curved structures (CD) (Fig. 50) have become C-shaped (CS) (Fig. 56). Numerous vesicles (V) are present in the cytoplasm of some developing sperm tails (Fig. 56).

(f) Stage 6 Spermatids

while the densely staining regions of the nuclear envelope (NE) extended along the internal surface, as seen in transverse sections, there was a corresponding increase in the extent of external surface associated with microtubules (MT) (Fig. 55). During this stage the C-shaped structures separate from the doublets (D) and form accessory fibres (AF) (Fig. 57). Sections show that the mitochondrial derivatives (MD) are much smaller, than they are in stage 5 spermatids and in each Aderivative there is a densely stained region of material (Fig. 57). Microtubules (MT) associated with the derivatives (MD) are still present (Fig. 57).

(g) Stage 7 Spermatids

As the chromatin condenses the nuclei elongate and become spindle-shaped (Figs. 6, 61, 62). In sections of nuclei the less dense areas are larger than they are in stage 6 spermatids. There are only small regions of nuclear envelope (NE) which are not densely stained (Fig. 62). Within a cup-shaped indentation of each nucleus, adjacent to a modified region of nuclear envelope a centricle (C)

MITOCHONDRIAL and Aderivatives (MD) are situated (Fig. 62). The volume of cytoplasm surrounding the nuclei and tails is much less than it is in stage 6 spermatids (Fig. 62). Granular material (GA) and microtubules (MT) are situated close to each flagellum (F) (Fig. 62). A double membrane (DE) surrounds each tail (Fig. 62). Sections show a distinct difference

in size between the two mitochondrial derivatives (MD) in each tail (Fig. 62).

(h) Stage 8 spermatids

With further condensation of the chromatin (CN) the nuclei become more flattened (Fig. 63). Dense material fills accessory fibres (AF) (Fig. 63). The granular material (GA) situated beside each flagellum has become a V-shaped rib with the point directed away from the flagellum (Fig. 63).

Late spermatids and mature sperm in the testes

In the final stages of maturation further condensation of the chromatin (CN) takes place (Figs. 64, 66, 68-71) until each nucleus is filled with densely stained chromatin (Fig. 67). The nuclei flatten (Figs. 64, 66, 67) and lengthen (Figs. 68-71) until they are shaped like mature sperm heads (MS) (Figs. 7, 67, 68-71). Each nucleus at this stage is slender and spindle shaped (Figs. 70, 71). In transverse sections through nuclei at certain levels there are slit-like (SL) regions which open out into the tail area (T) (Fig. 67). Microtubules (MT) are still associated with the nuclear envelope (Fig. 64). As maturation progresses the tubules (MT) disappear on the dorsal side of each nucleus and the cell membrane (CM) comes into contact with the nuclear envelope (Fig. 66). In each tail the V-shaped

rib (GA) is longer than it is in stage 8 spermatids (Fig. 65). Two densely stained bars (DB) of material are present between each MITOCHONDRIAL flagellum and the adjacent pair of Aderivatives (MD) (Fig. 65). The MITOCHONDRIAL region of densely stained material in each Aderivative is crossstriated (CR) (Fig. 65) and more mature derivatives exhibit a honeycomb-like pattern (HC) (Fig. 66). Tubules (MT) are present in each tail until maturation is almost complete (Fig. 67). Sections show that testicular sperm (MS) are still arranged in groups (Figs. 68, 69) and their tails exhibit a wave form (Fig. 68).

Testicular sperm are not tightly paired together (Fig. 67).

DISCUSSION

Sex chromosomes

Sex determination in certain Coleopterans (Smith, 1960), and in many other insects is achieved by an "XO" system where males are XO and females XX, and in which the male produces an equal number of gametes with and without an X chromosome. There are two ways in which an XO system operates during spermatogenesis. In one type the X chromosome divides into separate chromatids at 1st meiotic anaphase, with segregation following at the 2nd meiotic anaphase. In the second type the X chromosome moves to one pole at 1st meiotic anaphase and divides into separate chromatids at the 2nd meiotic anaphase. It is the second type that appears to apply in Acilius.

Spermatogenesis and sperm pairing

The study of sperm development in <u>Acilius</u> shows that secondary spermatocytes divide and form two completely separate cells and that the resulting sister spermatids are not at this stage associated in any particular way or in pairs. Furthermore, mature sperm in testes are closely packed together in groups but they are not associated in pairs. Therefore there is no apparent mechanism present during the development of sperm which might produce associations between sperm in testes. Therefore it would appear that sperm pairing is a separate process from sperm development, that there is no reason to

suppose that long lasting association of sister spermatids and sperm is the basis for pairing, and that sperm pairing takes place more or less randomly between adjacent sperm in the vas deferens. It seems unlikely that any of the situations which Ballowitz suggested regarding 'an original duality, a qualitative difference or a type of polar opposites' can exist in <u>Acilius</u>. Ballowitz (1895) also observed that the division of secondary spermatocytes produces separate sister spermatids and single unpaired sperm in the testes.

Centrioles

Typical centrioles, with 9 tubule triplets in their walls, are present in spermatocytes and spermatids of Acilius. They are similar to those already described in other insects (Anderson and André, 1968; Hoage and Kessel, 1968; Phillips, 1970a). In Acilius as in many other organisms, the triplet tubules on one side of each centricle are cut transversely and on the other side they are cut obliquely, suggesting that the triplets may have a long pitched helical course (Anderson and André; 1968; André and Bernhard, 1964; Fawcett, 1966; Hoage and Kessel, 1968; Phillips, 1970a). Prior to division, cells at meiotic metaphase II have paired centrioles. One centriole of each pair becomes associated with a cup-shaped vesicle and the initial development of the flagellum takes place within the cup of the vesicle which does not have any attachment to the cell membrane at first.

At a later stage these vesicles do become attached to the cell membrane.

In this respect the initial development of a flagellum in Acilius is similar to that described for a water mould (Renaud and Swift, 1964); certain mammals (Martinez and Deams, 1968; Sorokin, 1962, 1968) and some insects for example (Friedlander and Wahrman, 1966; Phillips, 1970a). Fritz-Niggli and Suda (1972) however have presented evidence that in Drosophila the cup-shaped vesicles are attached to the cell membrane throughout the meiotic divisions. I have not found any evidence to suggest that this situation exists in Acilius.

Basal body and centricle adjunct

Centrioles form the basal bodies of growing flagella and they are associated with modified regions of nuclear envelope. This modification of the nuclear envelope has also been noted by Phillips (1970a) and Warner (1971). A mass of material which surrounds each basal body and lies adjacent to the nuclear envelope was first termed the centriole adjunct by Gatenby and Tahmisian (1959). Since then some workers have used this term but others have referred to it as a juxtamuclear body (Sotelo and Trujillo-Cenoz, 1958), a basophilic centriole (Kaye, 1962), a flagellar accessory structure (Kaye and Kaye, 1966), a perinuclear granular aggregate (Kessel, 1967), a granular material (Kessel, 1966), a dense fibrous material (Phillips, 1966a), a dense material (Anderson, 1967) and a ring centriole (De Robertis, Nowinski and Saez, 1965).

In the blowfly <u>Sarcophaga bullata</u> Warner (1971) demonstrated that the centricle adjunct is granular in early spermatids and later the adjunct is transformed into filaments. As spermatid development proceeds the filaments become granular and eventually disappear. I have only observed granular centricle adjuncts in Acilius.

Synaptinemal complex

Synaptinemal complexes were first observed in the electron microscope by Moses (1956) and Fawcett (1956). The highly organized structure which they observed lying longitudinally between homologous chromosomes were ribbon-like with a tripartite backbone. The function and composition of synaptinemal complexes are not clearly understood yet but the complexes are intimately associated with pairing and crossing over of chromosomes. A fully developed synaptinemal complex has two lateral elements and a central element. The central element can be a relatively simple dense line or it can have a lattice-like organization with transverse periodic structures and triple longitudinal divisions (Wettstein and Sotelo, 1970). Fine transverse filaments pass from the lateral elements to the central element. Synaptinemal complexes in Acilius contain three parallel longitudinal components similar to those described by Sotelo and Wettstein (1966) and Gassner (1969). Fine transverse filaments pass between the lateral elements and the central element. The central elements have two walls and fine periodic striations pass between them. In Acilius,

as in other insects, synaptinemal complexes contact densely stained regions of nuclear envelope (Maillet and Folliot, 1965; Moens, 1969a; Moses, 1968; Sotelo and Wettstein, 1966).

In his review of synaptinemal complexes Moses (1968) defined polycomplexes as being "regular, three dimensional stacks of fused complexes, any unit of which resembles the synaptinemal complex in dimensions and fine structure; usually found in gametocyte prophase of certain insects". Multiple or polycomplexes have been observed in several insects, for example the cricket (Jaworskia and Lima-de-Faria, 1969; Schin, 1965; Guenin, 1965; Wettstein and Sotelo, 1967), the frog-hopper (Folliot and Maillet, 1966), the grasshopper (Moens, 1969b). The composition of polycomplexes in Acilius are similar to those which have been observed in other insects in that the units of a complex resemble a synaptinemal complex in dimensions and fine structure.

Spindle and mitochondria-associated microtubules

In most organisms cell division is associated with the formation of a spindle-shaped structure of microtubules called the mitotic apparatus. Chromosomes become firmly attached to some of the spindle microtubules at localized regions on the chromosomes (Harris, 1965). The attachment site or kinetochore lies in an indentation of each chromosome called the primary constriction or centromere (Jokelainen,

1967). Very little is known about the mechanism which brings about the attachment of spindle microtubules at this site (Bajer and Mole-Bajer. 1970). During the first meiotic division there is one kinetochore for each homologue (Nicklas. 1967). Direct chromosomal movement seems to coincide with the attachment of the chromosomes to the spindle. Separation of the chromosomes to their respective poles then takes place. This is followed by a progressive cytoplasmic constriction across the plane of the metaphase plate which divides the cell (Dupraw, 1970). During division the mitochondria appear to be pinched in half by the cytoplasmic constriction or cleavage furrow (Meves, 1907: Duesberg, 1911: Bowen 1920: Johnson, 1931). It has been shown that microtubules are located in close proximity to the elongated mitochondria and run parallel to their axes (Behnke and Forer, 1966; La Fountain, 1972). Numerous observations of living spermatocytes have shown the alignment of mitochondria around the spindle (Novikoff. 1951). According to La Fountain the orientation of the mitochondria ensures equal distribution of energy-producing machinery to all four spermatids. The explanation for the ordered arrangement of mitochondria around the spindle and their distribution is that the mitochondriaassociated microtubules perform both a supportive and motile function. La Fountain drew these conclusions after careful consideration of the evidence so far available concerning the function of microtubules in producing and maintaining cell form and in bringing about movements

within cells. The observation of mitochondria-associated microtubules and the distribution of mitochondria in spermatocytes of <u>Acilius</u> suggests that the microtubules may play a similar role to those described in <u>Nephrotoma</u> spermatocytes by La Fountain.

Mitochondrial derivatives

The transformation of numerous small mitochondria into relatively large mitochondrial derivatives during spermatogenesis has been described by several investigators. The most complete studies are those of Bowen (1920, 1922a, b, 1924), Pollister (1930), and Johnson (1931). In most animals the mitochondria aggregate soon after meiosis and form a large spherical mass which was termed the nebenkern by Retzius (1904). Since the advent of the electron microscope detailed studies have been made of mitochondrial development, progressive fusion of mitochondria into two interwoven nebenkernen and their rearrangement and segregation. The most complete studies have been made for the butterfly Pieris (André, 1959, 1962), for the stinkbug Murgantia (Pratt 1967, 1968, 1970). The process of nebenkern development and dissolution in Acilius is apparently identical to that described for other insects.

Chromatin condensation

In <u>Acilius</u> the granular chromatin condenses in uneven clumps.

Chromatin fibres have been reported in grasshopper <u>Chorthippus</u>

(Das and Ris, 1958) and in cricket <u>Acheta</u> (Kaye and Kaye, 1966).

Chromatin fibres have not been observed in <u>Acilius</u>. In the bush katydid <u>Scudderia</u>, Phillips (1970a) observed that in the anterior regions of spermatid nuclei, chromatin was present in the form of thin lamellae but in the posterior regions occurred as several clumps.

At the onset of chromatin condensation and elongation of spermatid nuclei of Acilius, microtubules become closely associated with regions of outer surface of the nuclear envelope. At the same time a densely stained material accumulates against the inside of the envelope. As chromatin condensation and nuclear elongation progress the densely stained material and microtubules continue to accumulate against the nuclear envelope. However, during maturation spermatid nuclei are never completely surrounded by densely stained material and microtubules. Microtubules begin to disappear in the later stages of maturation and there are no tubules present around nuclei of mature sperm.

Microtubules have been observed encircling spermatid nuclei in many other insects (Kessel, 1966, 1967, 1970; Phillips, 1966a; Shoup, 1967; Werner, 1966). Several layers of microtubules (the manchette)

surround the caudal pole of each spermatid nucleus in mammals (Burgos and Fawcett, 1955: Fawcett and Phillips, 1967); birds (McKintosh and Porter. 1967): reptiles (Boisson and Mattei. 1965): annelids (Anderson. Weisman and Ellis, 1967; Anderson and Ellis, 1968; Bradke, 1963), platyhelminthes (Silviera and Porter, 1964), and polychaetes (Potswald, 1967). In insects the single layer of microtubules is believed to perform the same functions as the multi-layered manchettes of other animals. In the dragonfly Aeschna extra-nuclear microtubules are associated with regions of nuclear envelope which have a densely stained material deposited on the inside of the envelope throughout chromatin condensation and nuclear elongation. Although there is strong evidence to suggest that microtubules play an important role in elongation. morphological observations alone are not sufficient (Kessel, 1970). Kessel suggests that, in addition, the deposits on the nuclear envelope might also play an active role in the elongation process. A similar situation exists in Acilius. The depositing of densely stained material against the nuclear envelope might be the initial step in the process of chromatin condensation. I have not made any observations which would support or reject the idea that microtubules might play an active role in the elongation of spermatid nuclei. The idea that the shape of sperm heads may be largely determined from within the nuclei by a specifically controlled aggregation of DNA and protein during chromatin condensation was suggested by Fawcett, Anderson and Phillips (1971). This specific aggregation may

produce the diversity of external form of sperm heads found in different animals. These workers concluded that microtubules may be essential for the redistribution of cytoplasm, but that they are probably not directly involved in the shaping of the nucleus. This theory was substantiated by observations made on spermiogenesis in the opposum Caluromys philander (Phillips, 1970b) where the sperm head flattens in a plane perpendicular to the axis of the tail and the manchette and the manchette tubules are not packed around the envelope as they are in the spermatids of other animals where flattening and compression occur in planes parallel to manchette tubules. In most animals, the first deformation of a spermatid nucleus towards its final shape is always associated with the onset of chromatin condensation (Fawcett, Anderson and Phillips 1971). This is also the case in Acilius.

Sperm flagellum

A 9 + 2 pattern of tubules characterizes flagella of many plant and animal species (Manton, 1952; Fawcett and Porter, 1954; Afzelius, 1959; Gibbons and Grimstone, 1960). Most insect sperm flagella however possess nine outer singlet tubules in addition to the 9 + 2 pattern of tubules. The nine outer tubules have sometimes been referred to as accessory fibres (Kaye, 1964; Cameron, 1965). Cameron reported that an arm extends from the B tubule of each doublet, lengthens, becomes C-shaped, and finally detaches from the doublet. Then the gap in the C-shaped structure closes to form a complete tubule. Accessory fibres

develop in an identical fashion in <u>Acilius</u> spermatids. Cameron was the first to recognize that arms were precursors of accessory fibres although they had already been observed by others (Kaye, 1964; Kessel, 1967; Phillips, 1966b). The formation of accessory fibres by growth against B tubules has been reported for many animal types (Fawcett, 1970; Kessel, 1970; Kiefer, 1970; Phillips, 1970a; and Warner, 1971).

(4) Mature Acilius Sperm from the Vasa Deferentia

Mature Acilius sperm are all similar and occur mostly in pairs with their heads (HD) closely associated (Figs. 72, 73, 74, 76, 78). Groups of paired sperm with their heads (HD) more or less aligned parallel to each other are present (Fig. 77). Groups of sperm are not bound together in cysts as they are in testes (Figs. 8, 78). Single unpaired sperm have been found in saline preparations (Fig. 75). Acilius sperm are approximately 340 µm long (Figs. 72, 73). The approximate lengths of the constituent parts are as follows: acrosome 0.6 μm, head 9.2 μm, initial segment 55 μm, posterior portion 265 μm, end piece 12 µm (Fig. 79). Due to the highly asymmetrical shape of Acilius sperm heads I have termed the surfaces which are always apposed to each other in pairs as being ventral (Fig. 81). The acrosome (A) is situated at the anterior of each head (Fig. 81). I have termed the side where the tail extends from as the short side and the opposite side as the long side (Fig. 81). The acrosome (A) is granular, is bounded by the cell membrane (CM), and separated from

the head by the nuclear envelope (NE) (Figs. 87-90). Each acrosome (A) lies against the apex of a head (Figs. 79, 80, 82, 83). Sperm heads (HD) are composed mostly of Feulgen positive nuclear material (Fig. 77) which is granular and stains densely (Figs. 87-96). Each head is enclosed by the cell membrane (CM) and nuclear envelope (NE) and coating the outside of the cell membrane is a layer of densely stained material (DA) (Figs. 87-96). In some instances this material appears in the form of short parallel bars (BR) (Fig. 90). Between most paired heads there is a layer of coarse granular material (DM) (Figs. 91-96, 111, 112, 113). The apex of one sperm head of a pair is at a slightly different level from the other one (Figs. 76, 83). Acilius sperm heads are highly asymmetrical in shape (Figs. 79, 80, 82, 83). There is a groove (GR) present in the short side of each head (Figs. 79, 80, 82, 90, 91, 92). An invagination occurs (SI) in the posterior region of the ventral surface of each head (Figs. 79, 80, 82, 83, 96). In some paired sperm the long side of each head is curved and curls around the short side of the other head (Figs. 111, 112). In transverse sections through the lower regions of each head the invagination (SI) is usually positioned at the most anterior part of the curved edge (Fig. 111). There is a hood (HO) formed within the posterior region of each head (Figs. 80, 82, 83). Figs. 94, 95, 96 show transverse sections through hoods at different levels from the anterior to posterior of the hoods. In sections paired sperm heads are separated by distances ranging from about 11-100 nm. A sperm tail

extends out from a position near the posterior of the groove in each head (Figs. 79. 80. 82. 83. 92-96). Surrounding the tail there is a layer of densely stained material (DA) which is similar to the material adjacent to the heads (Figs. 92-96). In transverse sections, at certain levels through the initial segment of each tail, the centriole adjunct (CA). flagellum (F). mitochondrial derivatives (MD). microtubules (MT) and 'microtubular border' are present (Figs. 92-97). Flagellar tubules (FT) emerge from the centricle adjunct (Fig. 94). The derivatives contain a paracrystalline material (PC) and at the periphery of each one there is a densely stained area of material (Fig. 93). In each MITOCHONDRIAL tail one of the derivatives is always larger than the other derivative (Figs. 94, 95, 96). In the anterior region of the initial segment the accessory fibres (AF) do not contain a central element (Figs. 95. 96). Lobes (LO) of granular material extend out from the mitochondrial derivatives (MD) on opposite sides from each flagellum (F) (Figs. 95. 96). In this region each tail is no longer attached directly to the head (Figs. 95, 96). In transverse sections through tails in this region characteristic 9 + 9 + 2 patterns of flagellar tubules are present (Figs. 95, 96). Accessory fibres (AF) are 28 nm in diameter and the central tubules (CT) are 25 nm in diameter and they have a centre to centre spacing of about 28 nm. Accessory fibres (AF) and central tubules (CT) possess a central element (Fig. 96). Accessory fibre walls are formed of subunits of about 7 nm diameter with a 4.5 nm centre to centre spacing (Fig. 108). The plane of symmetry is

determined by the position of the mitochondrial derivatives. When transverse sections of flagella are viewed (looking from flagellum MITOCHONDRIAL base to tip) and the derivatives are towards the observer, a line through the centre of the central tubules in all sperm tails passes through approximately 'eleven o'clock and five o'clock' (Figs. 96, 97). Transverse sections through tails at a more posterior level than in Figs. 95 and 96 exhibit a 'microtubular border' which extends out from the vicinity of each flagellum (Fig. 97). Between each flagellum and the MITOCHONDRIAL adjacent derivatives there is a 'membranous sheath' (MH) (Fig. 97). MITOCHONDRIAL derivatives are larger in this region and in some transverse sections a honey-comb (HC) pattern can be seen (Fig. 97). Negatively stained MITCHONDRA derivatives exhibit rod-like subunits with a spacing of about 10 nm arranged in a zig-zag array. The zig-zag (Z) array has a repeat of about 26.8 nm and a wave (W) array has a major repeat of 57.5 nm. There is also a 6.2 nm transverse periodicity (TP) visible which corresponds to the image seen in thin sections (Figs. 85, 109, 110). Cristae (CB) in mitochondrial derivatives are present in negatively stained material spaced at intervals of about 41 nm (Fig. 84). Areas in which cristae are present in negatively stained material correspond to the lobes of material present in transverse sections. The 'microtubular border' disappears at approximately 55 µm from the head and this signifies the end of the initial segment and the start of the posterior portion.

In the posterior portion of each tail the further away from the initial segment the mitochondrial derivatives (MD) are the smaller MITOCHONDRIAL they become (Figs. 98-106). The derivatives terminate approximately 320 µm posteriorly from the head. The spacing of mitochondrial cristae in the initial segment is about 41 nm but it is only about 20 nm near the end piece (Fig. 86). The alignment and size of the subunits in derivatives are the same throughout their length (Figs. 98-106). MITOCHONDRIAL While the derivatives become smaller along the length of the posterior portion, flagellar axonemes remain the same size (Figs. 97-106). There is a reduction in the size of the 'membranous sheath' (MH) as it passes from the anterior to posterior of each posterior portion (Figs. 98-106). At different positions along the length of each posterior portion the central element in each accessory fibre terminates. starting with accessory fibre 8 (Fig. 99). In sections of flagella which are viewed from the base to the tip (i.e with the arms of subfibre A of each doublet directed clockwise) a straight line through the central tubules passes through one of the doublets. The accessory fibre associated with this doublet is termed accessory fibre 1 (Fig. 99).

The end piece (EP) of each tail is straight and pointed (Figs. 80, 86) and contains only flagellar tubules and accessory fibres.

The accessory fibres and central tubules terminate before the doublets (Fig. 107). Prior to termination of the doublets, C-shaped fibres are present (Fig. 107)

The lumen of each vas deferens is surrounded by a layer of cuboidal epithelial cells (EC) which are linked by tortuous intercellular membranes. Short, irregular microvillii (MV) form the apical membrane of the border. A granular material (GL) of medium electron density occupies the space between sperm in the vasa deferentia (Fig. 113).

In sections a few examples of tails are present with an extra flagellum (F), or an extra mitochondrial derivative (MD), or extras of both, or with only one derivative (Figs. 114-117). In one other example several disrupted tails are present within the same cell membrane (CM) (Fig. 118).

Sperm Movement and Tail Shape

Flagella in Acilius sperm tails have an oscillating appearance during movement. The initial segments are rigid and the flagellar movement is initiated in the region of the heads (Figs. 72-76, 78).

Flagellar helicos flow in a rapid sequence from anterior to posterior and they appear as short waves when viewed from above or from the side (Figs. 74, 75, 76). In the posterior regions of the initial segment the waves are slightly longer and the flow is also reduced. The posterior portion of each tail moves as a whole and it has a regular coarse helical shape (Figs. 72, 73). Regular waves pass along the length of each posterior portion in the same way as the flagellar movement of the posterior of the initial segment. The waves have a

lower frequency and longer wave length as they near the end piece.

The end piece is straight and rigid. Posterior portions of some tails are coiled like a spring in a clockwise direction and flagellar helices flow along them. In some cases the coarse helices are absent and in others only the flagellar movement in the initial segment exists. Where the posterior portion is coiled like a spring the only visible movement is in the form of a cart-wheel with the head remaining more or less in the same position. In double sperm the movement of individual tails can be fully independent of each other.

Acilius sperm placed in liquid paraffin move forward with the flattened sides of each head remaining in a horizontal position. Helices are present in the initial segment and posterior portion. In each posterior portion the coarse waves are restricted in their ability to coil, having only a few waves which oscillate irregularly. Many whorls of sperm are formed by the movement of the sperm. There are no spring-like coils present. Individual sperm can move in the same way as double sperm.

DISCUSSION

Mature sperm heads and sperm pairing

Acilius sperm in vasa deferentia are associated in pairs. This association takes place in the region of the heads which are highly asymmetrical. In sperm pairs the flat surfaces are always apposed against each other and in many cases the long side of each head curls around the short side of the other head. The acrosome of one sperm head is always out of alignment with the other acrosome. Acilius sperm are surrounded by a granular material of medium electron density and between the sperm heads there is a layer of coarse granules of high electron density. As these coarse granules are only present in vasa deferentia it is possible that they might play some role in the production of paired sperm. Two types of sperm associations have been described in two sub-orders of Orthoptera by Cantacuzene (1968). In some Ensifera, mucopolysaccharidic secretions are ejected into the lumen of the genital ducts from the glandular cells of its walls and form a "muff" around each bundle of sperm. In certain Caelifera the spermatid heads become coated with a mucoproteic secretion in the nurse cells and when sperm mature this secretion forms the 'cap' of a sperm bundle which groups about 200 sperm together. In Acilius the vasa deferentia walls are composed of a thick layer of epithelial cells and the apical membrane is formed of microvillii. In comparison the testes walls are very thin and there are no microvillii present in the apical

border. This suggests that the epithelial cells forming the vasa deferentia walls may be capable of secreting mucopolysaccharides or mucoproteins which could facilitate sperm pairing. Therefore a similar situation may exist in Acilius to that described for some Ensifera in so far as the accumulation of a densely stained layer of granules between paired sperm heads may act as a 'cement'. It might also be that Acilius sperm pair as a result of their movement and the highly asymmetrical shape of their heads, so that when pairing occurs they are held together by the 'cement'. In Acilius pairs the heads do not come into contact with each other whereas in Caluromys (Phillips, 1970c) the plasma membranes near the acrosome form a close junction. There are similarities between the paired sperm of Acilius and certain other animals in that they usually pair after leaving the testis and in most cases their heads are closely associated. In each animal the sperm heads have a highly asymmetrical shape and are always packed against each other in a specific manner.

As a result of serially sectioning paired sperm heads and subsequently constructing models of the heads it can be demonstrated
without doubt that paired sperm heads are not mirror images of each
other or "right" and "left" handed as Wilson (1924) suggested. Each
head in a pair has an identical shape to its partner. In heads from
testes the long sides are either flat or slightly curved. However in
vasa deferentia the long sides of paired heads are either slightly
curved or they are curved to the extent that curling around each short side

takes place. Therefore it would appear that the curling occurs after pairing. Although the curling occurs in vasa deferentia I do not know how soon it takes place after entry of the sperm into vasa deferentia.

Acilius sperm tails

The classic zonation of sperm tails based on the presence of a mitochondrial sheath cannot be applied in <u>Acilius</u> due to the presence of a 'microtubular border'. I have therefore termed the anterior section of each tail the initial segment. The 'microtubular border' runs along the length of the initial segment and with its termination the posterior portion begins. The posterior portion of each tail contains two mitochondrial derivatives and a flagellum. With the termination of the derivatives the posterior portion ends and it is followed by an end piece.

Flagellum

According to Phillips (1970s) there are no centrioles present in mature sperm because typical centriole triplets have not been observed. This conclusion was made after studying the mature sperm of many species of insects and the descriptions of other investigators. However Perotti (1970) demonstrated in <u>Drosophila</u> by serial sectioning that centriole triplets are still present in mature sperm. Warner (1971) also demonstrated centriole triplets and stated that the entire

transition from a central pair of tubules and triplets through to a flagellar axoneme with a complete complement of tubules takes place in less than 200 nm along its base. I have assumed that due to the lack of triplets being observed in the proximal portion of the flagellar axoneme that centrioles are not present in mature sperm of Acilius. A typical insect sperm flagellum is present in each Acilius sperm. The outer 9 singlet accessory fibres are similar in morphology to the central pair of singlet tubules except that their diameters are slightly greater. Accessory fibres and central bubules in Sepsis (Phillips. 1966c) and Tenebrio (Cameron. 1965) contain a central tubule in place of a rod. A similar situation is present in Acilius. The comparisons which have been made between accessory fibres and central tubules demonstrate that there is a wide interspecies variation although their topological relationship to one another is rather constant. Behnke and Forer (1967) presented evidence suggesting that there is a chemical as well as morphological similarity between accessory and central tubules. This will be discussed at a later stage. The accessory fibres in Acilius terminate in the end piece before the doublets and central pair in a similar fashion to those of Sepsis (Phillips, 1966 b. c). In Acilius as in most other insects the doublets are composed of subfibres A and B. Subfibre A has two small arms directed towards the adjacent doublet (Gibbons and Grimstone, 1960). Flagellar doublets are unchanging in character from species to species (Phillips. 1970a). In transverse sections through the end piece of

flagella in some insects the doublets are dissociated from each other into nine complete tubules and nine fibres with C-shaped profiles (Phillips. 1966c). Phillips also states that it is likely that the doublets in insect sperm are composed of one complete tubule and another one that is incomplete and shares a portion of the wall that is common to both. The dissociation of the tubules also takes place in Acilius. Flagellar tubule walls appear to be composed of regular subunits (Andre. 1961: Kaye. 1964). Accessory fibres and central tubules have 13 evenly spaced subunits in their walls which have an approximate diameter of 7 nm (Phillips. 1966c: Ringo. 1967) and that cytoplasmic microtubules are composed of 12 or 13 subunits (Ledbetter and Porter, 1964; Gall, 1965, 1966; Behnke and Zelander, 1967; Fuge, 1968). Flagellar tubule subunits in Acilius are similar to those which have already been observed. According to Phillips (1970d) the accessory elements in insects and mammals are both formed as outgrowths of the 9 doublets and may have a similar role in motility. The role of accessory elements in sperm motility is not clearly understood as yet. Accessory elements are apparently not essential for motility as in some insect sperm flagella they are absent.

Mitochondrial derivatives

In <u>Acilius</u> as in many other species of insects, sperm possess two mitochondrial derivatives of unequal size and one extends further anteriorly than the other. This is also the case in some other insect

sperm. for example Thompson and Blum (1967). In many species of insects there is a paracrystalline component present in each derivative. component has been described as a honeycomb-like structure when viewed in transverse section (Phillips. 1966a), a periodically striated structure in longitudinal section (André 1962) and a herring-bone pattern structure (Meyer, 1964, 1966; Hess and Meyer, 1968). Phillips (1970a) and Bairati and Perotti (1970) have described the paracrystalline component as consisting of hexagonally packed roundish subunits, whereas Warner (1971) describes the subunits as rods. In longitudinal sections the herring-bone pattern has a periodicity of about 45 nm (Meyer 1964. 1966: Hess and Meyer. 1968: Phillips. 1970a) and a transverse period of about 6.2 nm (Warner, 1971). In negatively stained derivatives the subunits are aligned in such a way that they exhibit a wave-like array with a major period of 49.6 nm and a zig-zag pattern with a major MITOCHONDRIAL period of 24.8 nm. The derivatives also exhibit a 10.0 nm spacing of the rods (Warner, 1971). The major period of the zig-zag pattern and wave form, the transverse period and spacing of the subunits and their MITOCHONDRIAL appearance in derivatives of Acilius sperm are similar to those of other insects. Mitochondrial cristae can only be seen at the edges of derivatives in many insects (Phillips, 1970d), and this is also the case in Acilius. Bairati and Perotti (1970) have suggested that the intricate organization of the paracrystalline material must be related to complex biochemical functions. Mitochondrial derivatives of mature sperm assume a species-specific shape.

Sperm movement

In sperm pairs of <u>Caluromys</u>, the tails beat in synchrony (Phillips, 1970c). This synchronisation has also been noticed by Phillips in sperm from other mammalian species where chance contact has been established in the head region. Sperm pairs of <u>Acilius</u> also beat in synchrony. However they can also beat independently of each other. Single sperm can move in the same way as paired sperm.

Pairing of sperm is not essential for their passage through vasa deferentia as this is probably effected by muscular contractions. Pairing however may be necessary during the fertilization process. The fact that in paired sperm the heads are always slightly out of alignment with each other may result in the most anterior heads being protected in some way during their passage through vasa deferentia. The initial segment of <u>Acilius</u> sperm tails is usually rigid. This rigidity is probably due to the presence of a 'microtubular border' along the length of each initial segment.

As far as I am aware there are no other sperm tails which exhibit a 'microtubular border' except for <u>Dytiscus</u> which I have also studied. The rigidity of the initial segment might reduce the chances of separation of paired heads by restricting flagellar movement and coiling in this region next to the heads.

(5) Experimental treatment of Acilius sperm from vasa deferentia

Sodium lauryl sulphate treatment

The treatment of sperm with sodium lauryl sulphate shows that the degree of dissociation of flagellar components is directly proportional to the concentration of sodium lauryl sulphate used. These observations were made on flagella which had been treated with a range of concentrations of sodium lauryl sulphate. Treatment with a 0.025% sodium lauryl sulphate demonstrates that subfibre B (SB) of each doublet is more sensitive to sodium lauryl sulphate than subfibre A (SA) (Fig. 119). Doublets (D) are also more sensitive than accessory fibres (AF) and central tubules (Figs. 119, 120). The walls of accessory fibres and doublets are made up of subunits (SU) 4.6 ± 0.5 nm in diameter (Figs. 119, 120).

Sarkosyl treatment

Sperm in vasa deferentia treated with Sarkosyl (0.05% aqueous) are similar to sperm in a control specimen. In sections of 0.25% Sarkosyl-treated vasa deferentia the cell membrane of each sperm is either badly disrupted or has disintegrated (Fig. 121). The components of each tail remain intact except that some accessory fibres (AF) are displaced from their normal position adjacent to the doublets (D) (Fig. 121). In 0.5% Sarkosyl-treated vasa deferentia the arms which extend from subfibre A of each doublet are no longer present and the

microtubules adjacent to the mitochondrial derivatives are less distinct than they are in 0.25% Sarkosyl material. In material treated with 1% Sarkosyl the central pair of tubules (CT) in each flagellum are displaced and in some cases they are more distantly spaced from each other than they are in control specimens (Figs. 122. 123. 124). In each end piece, subfibres A (SA) and B (SB) have separated in some doublets (Fig. 125). Transverse sections through sperm heads treated with 1% Sarkosyl shows that the chromatin (CN) has some fibrous material associated with it (Fig. 126). Sperm in vasa deferentia treated with 1.5% Sarkosyl demonstrate a further deterioration in the structure of their flagella (Figs. 127, 128). For example, in some doublets subfibre B (SB) is completely separate from subfibre A (SA) (Fig. 128). In some cases the central pair of tubules (CT) are wider apart than is normally the case (Fig. 127). The radial links (RL) between the doublets and central pair of tubules are not as distinct as they are in material treated with weaker concentrations of Sarkosyl. The 'microtubular border' (MB) is still present in each sperm tail. In sperm treated with 2% Sarkosyl there is a greater degree of spreading of the components of each flagellum than in sperm treated with weaker concentrations. Subfibres A (SA) and B (SB) of each doublet are separate from each other (Fig. 129) and the radial links are no longer present (Fig. 129). Fig. 130 shows the normal content and arrangement of components in untreated sperm tails. In sperm from vasa deferentia treated with varying concentrations of Sarkosyl, it always seems to be

the case that accessory fibres and central tubules are more resistant to the effects of Sarkosyl than are doublet tubules.

Silver nitrate methenamine treatment

Sections of Acilius vasa deferentia treated with silver nitrate methenamine for periodic acid reactive carbohydrates exhibit specificity of staining. In each flagellum accessory fibres (AF) and central tubules (CT) are densely stained whereas the doublets (D) are not as densely stained (Figs. 132, 133). The layer of material (DM) between paired sperm heads and the acrosome, acrosomal and nuclear membranes (NM), nuclear envelope, mitochondrial derivatives (MD) and 'microtubular border' (MB) are also densely stained (Figs. 131, 132, 133). The material surrounding the sperm is not densely stained (Fig. 131). In the epithelial cells of vasa deferentia walls the granular material has both a high and a low electron density (Fig. 133). The nuclei and spaces surrounding flagella and mitochondrial derivatives are unstained (Figs. 131, 132, 133). In muscle, which surrounds the epithelial cells of vasa deferentia, staining of myo-fibrils (MF), mitochondria (M) and glycogen (GC) occurs (Fig. 134).

Trypsin treatment

The internal components of each flagella are separated from each other following short periods of treatment with Trypsin (0.5% aqueous).

As the duration of treatment is increased doublet tubules (D) of each flagellum collapse and their subunits (SU) fray out prior to the collapse of the accessory fibres (AF) and central tubules (CT) (Figs. 135-138). The subunits are about 7.0 nm in diameter (Figs. 135-138). In most preparations of sperm treated with Trypsin for 30 mins there are many bundles of tubules which resemble frayed out subunits (Figs. 139-140).

Pronase

In sections of vasa deferentia treated with pronase the chromatin, accessory fibres (AF), central tubules (CT) and the paracrystalline material (PC) in mitochondrial derivatives (MD), are digested (Figs. 141, 142). The doublets (D) in each flagellum are only slightly digested (Figs. 141, 142). The layer of densely stained granular material between each pair of sperm heads is not digested. In transverse sections through control specimens the profiles of flagella are more distinct than they are in sections which have been treated in the normal way (see section (a) electron microscopy). Fig. 143 indicates that T.C.A. solubilizes some of the material between the flagellar fibres.

DISCUSSION

Accessory fibres and central tubules are not only similar to each other in morphological detail but they react in a similar manner to treatment with detergents. proteolytic enzymes and special histochemical staining techniques. However doublets differ from accessory fibres and central tubules in their morphology and in their response to the several treatments which have been used. Treatment with sodium lauryl sulphate, sarkosyl, and trypsin, induces breakdown of doublets before that of either accessory fibres or central tubules. The response of doublet fibres to digestion and silver nitrate methenamine staining is much less than it is in accessory fibres and central tubules. treated doublets subfibre B collapses before subfibre A. Behnke and Forer (1967) and Bigliardi, Baccetti, Burrini and Pallini (1970) have also demonstrated that accessory fibres and central tubules are similar to each other but differ from doublets in morphology and chemical composition. Behnke and Forer (1967) also observed that in doublet tubules, subfibre B collapses before subfibre A. Silver nitrate methenamine staining and enzyme digestion shows that accessory fibres and central tubules in Acilius are composed of both periodic acid reactive carbohydrates and proteins. Behnke and Forer (1967) demonstrated the presence of proteins in accessory fibres, central, tubules, and doublet tubules of Nephrotoma. Bigliardi, Baccetti, Burrini and Pallini (1970) observed that in Bacillus accessory fibres

and central tubules are composed of succinic - dehydrogenase and cytochrome oxidase but that the doublets are composed of glucose-6-

phosphatase. Accessory fibres and central tubules in Ceratitis are similar to those in <u>Bacillus</u>. They also possess small amounts of polysaccharide and a diffuse content of proteins. The doublets in <u>Ceratitis</u> are entirely proteinaceous.

In Acilius the paracrystalline material in mitochondrial derivatives is formed of periodic acid reactive carbohydrates and protein.
MITOCHONDRIAL
ABerivatives have also been shown to exhibit glucose-6-phosphatase
activity (Anderson, 1968). They also contain glycogen (Anderson,
1968; Anderson and Personne, 1970). UTPase and cytochrome oxidase
(Bigliardi, Baccetti, Burrini and Pallini, 1970) and respiratory
proteins (André, 1962; Mayer, 1964).

Cantacuzène (1968) suggested that secretions of mucopolysaccharides and mucoproteins are responsible for the associations of sperm in certain ensiferans and caeliferans. As the densely stained layer of granules between each pair of sperm heads is composed of periodic acid reactive carbohydrates a similar type of situation may exist in Acilius to those described by Cantacuzène. Ballowitz (1895) suggested that a 'cementing substance' was responsible for bringing about union of sperm heads. It may be that the densely stained layer of carbohydrate granules described here between paired sperm heads acts as a 'cement'.

(6) Structure of the mature sperm in Dytiscus. Colymbetes and Agabus

Dytiscus sperm

Dytiscus sperm differ from Acilius sperm in several ways. The acrosomes in Dytiscus are surrounded by a granular material (GL) of medium electron density (Fig. 154). Dytiscus sperm heads are highly asymmetrical and they possess a BARB (HK) which extends out from the flat surface of each head in the posterior region (Figs. 145-150). The main part of each BARS (HK) is formed of nuclear material and a wedge-shaped structure (WS) of material which is situated between the membranes of the nuclear envelope (Figs. 148, 155, 156, 157). In all the sectioned material examined of paired heads the Beeks are never seen in contact with each other. Situated between paired heads in Dytiscus there are two layers of coarse granular material (DM) which is electron dense (Fig. 155). The initial segment (IS) is straighter and more rigid in Dytiscus than it is in Acilius (Fig. 144). The 'microtubular border' (MB) in each initial segment is wider and thicker than it is in Acilius (Fig. 159). These 'microtubular' structures have also been examined in longitudinal sections (Fig. 158) and in negatively stained whole mount preparations (Fig. 153). The central elements (CE) in the accessory fibres (AF) are present throughout the length of the posterior portion of each sperm (Fig. 160).

Treatment of sectioned <u>Dytiscus</u> sperm with silver nitrate methenamine gave similar results to those obtained for <u>Acilius</u>. The

layers of coarse granular material (DM) between paired sperm heads stains intensely (Fig. 161).

Treatment of <u>Dytiscus</u> sperm with pronase gave similar results to those obtained for <u>Acilius</u>. The coarse granular material (DM) between paired sperm heads is not digested (Fig. 162) whereas accessory fibres (AF) and central tubules (CT) are digested (Fig. 163). The wedge-shaped structure (WS) in each **BARB** (HK) is partially solvenized with periodic acid in control specimens (Fig. 164).

Colymbetes sperm

Colvmbetes sperm differ from those of Acilius in that the sperm are usually found in groups with their heads (HD) arranged around a densely stained mass of granular material (DM) (Figs. 165, 168, 172, 173, 178). Pairs and single sperm are present in saline preparations (Figs. 166, 167). Colvmbetes sperm heads are highly asymmetrical and different in shape from Acilius heads (Fig. 169). In the acrosomal region the granular mass (DM) has a medium electron density but posterior to this region it has a high electron density (Figs. 172, 173, 176, 177). The complex system of membranes in the acrosomal region can be seen in Figs. 170 and 172. The heads in each group of sperm are always aligned in such a way that the acrosomes (A) are associated with the granular mass (DM) and so that the hoods (HO) are always directed away from the mass (Figs. 172, 173, 177). A granular mass (GM) of medium electron density extends out from each hood

(Figs. 171, 174). There is no initial segment in <u>Colymbetes</u> sperm, only a posterior portion (PP) and an end piece (EP) (Fig. 165). The central elements (CE) in accessory fibres (AF) are present in the end piece of each tail (Fig. 175). There is a coating of granular material (GL) on the inside of the cell membrane (CM) in each end piece (Fig. 175).

The helices and flagellar waves are more pronounced in <u>Columbetes</u> than they are in <u>Acilius</u> (Figs. 165, 168). There is no straight rigid region in the anterior of each tail in <u>Columbetes</u> (Figs. 165-168).

The tails in a group of sperm beat at the same rate.

Sections of <u>Columbetes</u> vasa deferentia treated with silver nitrate methenamine gave similar results to those obtained in <u>Acilius</u> (Figs. 178, 179, 180). The material (DM) of high electron density between sperm heads and the granular material (DM) of medium electron density surrounding the acrosomal region are intensely stained (Figs. 178, 179).

Treatment with pronase of <u>Colymbetes</u> material resulted in the digestion of the high electron dense material (DM) between heads but the granular material (DM) of medium electron density is not digested (Figs. 181, 182). Digestion of other sperm components was similar to those in <u>Acilius</u>; the central regions of accessory and central pair fibres were digested before those of doublet fibres (Figs. 181, 182, 183). Periodic acid partially somputates some of the sperm material in control specimens (Fig. 184).

Agabus sperm

Agabus sperm differ from those of Acilius in that pairing is only detectable in saline preparations (Figs. 186, 187). Sperm heads are not closely apposed along their lengths as they are in Acilius but are only attached near their tips (Fig. 187). There is no specific arrangement of the sperm within the groups which occur in Agabus (Figs. 189, 190, 191). In Agabus there is a GARB (HK) which extends out from the posterior region of each head and it is similar to the BARBS in Dytiscus (Figs. 185. 187. 188. 189). Each BARB (HK) is surrounded by a granular mass (GM) of material which extends out from the hood (HO) (Fig. 189, 191). There is a diffuse layer of material (DF), which is unevely distributed and is adjacent to the heads in Agabus (Figs. 189. 190. 191). Each tail consists of only a posterior portion and an end piece. The central elements (CE) in the accessory fibres (AF) do not disappear at certain levels in Agabus as they do in Acilius (Figs. 192, 193). In each end piece (EP) there is a coating of granular material (GL) between the flagellum and cell membrane (CM) similar to that seen in Colymbetes (Fig. 193).

In the anterior region of each tail in <u>Agabus</u> there is no straight rigid region and the helices and flagellar waves are more pronounced than they are in <u>Acilius</u> (Fig. 187). Due to the absence of any specific arrangement of the sperm heads within a group or a 'cementing' material in <u>Agabus</u>, treatments with silver nitrate methenamine and pronase were not carried out.

DISCUSSION

Certain similarities and differences between the Dytiscid sperm studied have been observed. The sperm heads are all highly asymmetrical with an acrosome at the anterior tip and a hood in the posterior region of each head. The main structural differences between the heads are the presence of a BARB in each head in Dytiscus and Agabus and a mass of material which extends out from the hood in Agabus and Colymbetes. The fact that in Dytiscus the 68RBS in paired sperm do not come into contact with each other in vasa deferentia suggests that they do not play a signficant role during the formation of sperm-pairs or in maintaining pairs, once they have been established. This suggestion is supported by the finding that in Agabus sperm there are also BARBS present in their heads although pairing does not occur. Dytiscus and Acilius both have initial segments in the anterior region of their tails due to the presence of a 'microtubular border'. The initial segment in Dytiscus is straighter and more rigid than it is in Acilius. This may be due to the larger size of the 'microtubular border' in Dytiscus. It is also interesting to note that sperm of the closely related genera Acilius and Dytiscus both form definite sperm-pairs, whereas Colymbetes and Agabus sperm do not. The presence of a 'microtubular border' in Acilius and Dytiscus sperm may help in the production of paired sperm due to the restriction of the flagellar movement and coiling in the initial segment. It appears that the

presence of a 'microtubular border' is essential for the rigidity of the initial segment in Acilius and Dytiscus. There is no rigid region in the anterior region of sperm tails in Colymbetes and Agabus which both lack a 'border'. The groups of sperm are different in Colymbetes and Agabus. In Colymbetes the sperm heads are associated with a mass of material in a specific manner to form clearly defined groups. There is apparently no specific arrangement of heads in the groups of Agabus sperm or an association with either a mass of material or a layer of granules similar to those in Acilius and Dytiscus. The granular layer of material between paired Dvtiscus sperm heads contains periodic acid reactive carbohydrates and is not digested with pronase in a similar way to the granular layer of Acilius. The material of medium and high electron density with which the sperm heads in Colymbetes are associated also contains periodic acid reactive carbohydrates. The high electron dense material also contains protein as it is digested with pronase. However the medium electron dense material is not digested. Therefore the composition of the granular layer of material in Acilius and Dytiscus is similar to the material of medium electron density in Colymbetes but is different from the material of high electron density in Colymbetes. Therefore it may be that the presence or absence of a 'cementing' material and its composition is in some way responsible for the different types of associations produced between the Dytiscid sperm which have been studied. In two subclasses

of orthopterans Cantacuzene (1968) observed associations between sperm in genital ducts and testicular follicles. The structure and chemical nature of the secretions associated with the sperm heads are different. The 'cementing' substances in dytiscids also show differences in their chemical composition where pairing and grouping only occurs in vasa deferentia. In their study of several families belonging to the homopteran suborder Auchenorhyncha Folliot and Maillet (1970) observed that in some families sperm bundles are formed with their heads embedded in a protein material and that in other families sperm bundles are not formed. My observations indicate that pairing and grouping in dytiscids is largely influenced by the highly asymmetrical structure of the sperm heads combined with the movement of the sperm and on the composition of the 'cementing' material.

Summary

Sperm pairing in Acilius is a separate process from sperm development. Paired sperm in Acilius are not produced from the same secondary spermatocyte (sister cells). Sperm-pairs in Acilius have only been observed in vasa deferentia. Pairing does not occur in the testes. A layer of coarse granules between paired sperm heads may act as a 'cement'. This 'cement' contains periodic acid reactive carbohydrates. Paired sperm heads are not mirror images of each other or "right" and "left" handed. The two heads in a pair have identical shapes which are such that the two heads fit closely against and around each other. Dytiscus sperm pair in a similar manner to Acilius sperm. Colymbetes sperm form groups with their heads arranged around a mass of 'cement' which contains periodic acid reactive carbohydrates. In the groups of sperm which are formed in Axabus vasa deferentia there is no specific arrangement of the sperm heads in relation to each other. The 'cementing' materials found in Acilius, Dytiscus and Colymbetes are absent in Agabus.

All the dytiscid sperm heads which have been studied are highly asymmetrically shaped.

Treatment with detergents, proteolytic enzymes and special histochemical staining techniques have demonstrated that the accessory

fibres and central tubules in <u>Acilius</u> sperm tails are similar to each other and that they differ from doublet fibres. The paracrystalline material in mitochondrial derivatives contains periodic acid reactive carbohydrates and protein.

An X0 system of sex chromosomes has been demonstrated for Acilius.

The fine structure of synaptinemal complexes and polycomplexes in Acilius is described in detail.

REFERENCES

- Afzelius, B., 1959. Electron microscopy of the sperm tail. Results obtained with a new fixative.

 J. biophys. biochem. Cytol., 5, 269-279.
- Anderson, W.A., 1967. Cytodifferentiation of spermatozoa in

 <u>Drosophila melanogaster:</u> The effect of elevated temperature on spermiogenesis.

 Molec. Gen. Genet., 99, 257-273.
- Anderson, W.A., 1968. Intramitochondrial localization of Glycogen, Glucose-6-phosphatase, and Adenosine triphosphatase activity in spermatozoa of Paracentrotus lividus.

 J. Ultrastruct. Res., 24, 398-411.
- Anderson, W.A. and André, J., 1968. The extraction of some cell components with pronase and pepsin from thin sections of tissue embedded in an Epon-Araldite mixture.

 J. Microscopie, Z., 343.
- Anderson, W.A. and Ellis, R.A., 1968. Acrosome morphogenesis in <u>Lumbricus terrestris</u>.

 Z. Zellforsch mikrosk. Anat., 85, 398-407.
- Anderson, W.A. and Personne, P., 1970. Recent cytochemical studies on spermatozoa of some invertebrate and vertebrate species.

 In <u>Comparative Spermatology</u> (Baccetti, B., editor)

 Academic Press, New York-London, 431-449.

- Anderson, W.A., Weisman, A. and Ellis, R.A., 1967. Cytodifferentiation during spermiogenesis in Lumbricus terrestris. J. Cell Biol., 32, 11-26.
- André. J., 1959. Etude au microscope électronique de l'evolution du chondriome pendant la spermatogenese Papillon du Chou Pieris brassicae. Ann. Sci. nat. Zool. Biol. Animale, 1, 283.
- André. J., 1961. Sur quelques détails nouvellement connus de l'ultrastructure des organites vibratiles. J. Ultrastruct. Res., 5, 86-108.
- Andre, J., 1962. Contribution a la connaissance du chondriome. Etude de ses modifications ultrastructurales pendant la spermatogenese. J. Ultrastruct. Res., Suppl., 3, 1-185.
- Andre, J. and Bernhard, W., 1964. The centriole and the centriolar region.
 - XI International Congress of Cell Biology, Providence R.I. 9.
- Bairati, A. and Perotti, M.E., 1970. Some comparative considerations of the ultrastructure of Drosophila melanogaster spermatozoon. In Comparative Spermatology (Baccetti, B., editor) Academic Press, New York-London. 333-345.
- Bajer, A. and Mole-Bajer, J., 1971. Architecture and function of the mitotic spindle. In Advances in Cell and Molecular Biology (Dupraw, E.J. editor) Academic Press, New York-London. Vol. 1, 213-266.

- Ballowitz, E., 1895. Die Dopplespermatozoa der Dytisciden. Z. wiss. Zool. 60, 458-499.
- Bawa, S.R., 1964. Electron microscope study of spermiogenesis in a firebrat insect, <u>Thermobia domestica</u> pack.

 J. Cell Biol., <u>23</u>, 431-446.
- Behnke, O. and Forer, A., 1966. Some aspects of microtubules in spermatocyte meiosis in a crane fly Nephrotoma suturalis (Loew): intranuclear and intrachromosomal microtubules.

 C.r. Trav. Lab. Carlsberg. 35. 437-455.
- Behnke, O. and Forer, A., 1967. Evidence for four classes of microtubules in individual cells.

 J. Cell Sci., 2, 169-192.
- Behnke, O. and Zelander, T., 1967. Filamentous substructure of microtubules of the marginal bundle of mammalian blood platelets. J. Ultrastruct. Res., 19, 147-165.
- Bigliardi, E., Baccetti, B., Burrini, A.G. and Pallini, V., 1970.

 The spermatozoon of Arthropoda XII. The distribution of some enzymes in the insect sperm tail. In Comparative Spermatology (Baccetti, B., editor) Academic Press, New York-London. 451-463.
- Boisson, C. and Mattei, X., 1965. Sur la spermiogenèse de <u>Python sebae</u> (Gmelin) étudiée au microscope électronique.

 C.r. Séanc. Soc. Biol. Filiales, <u>159</u>, 1192-1194.
- Bowen, R.H., 1920. Studies on insect spermatogenesis. I. The history of the cytoplasmic components of the sperm in Hemiptera. Biol. Bull. mar. biol. Lab., Woods Hole, 39, 316-362.

- Bowen, R.H., 1922a. Studies on insect spermatogenesis. II. The components of the spermatid and their role in the formation of the sperm in Hemiptera.

 J. Morph., 37, 79-193.
- Bowen, R.H., 1922b. Studies on insect spermatogenesis. III. On the structure of the nebenkern in the insect spermatid and the origin of nebenkern patterns.

 Biol. Bull. mar. biol. Lab. Woods Hole, 42, 53-85.
- Bowen, R.H., 1924. Studies on insect spermatogenesis. VI. Notes on the formation of the sperm in Coleoptera and Aptera, with a general discussion of flagellate sperms.

 J. Morph., 39, 351-414.
- Bradke, D.L., 1963. The origin and development of the manchette during spermatogenesis.

 Anat. Rec., 145, 210.
- Burgos, M.H. and Fawcett, D.W., 1955. Studies on the fine structure of the mammalian testis: I. Differentiation of the spermatids in the cat (Felis domestica).

 J. biophys. biochem. Cytol., 1, 287-300.
- Cameron, M.L., 1965. Some details of ultrastructure in the development of flagellar fibres of the <u>Tenebrio</u> sperm.

 Can. J. Zool., <u>43</u>, 1005-1010.
- Cantacuzene, A.M., 1968. Recherches morphologiques et physiologiques sur les glandes annexes males des Orthopteres. III.

 Modes d'association des spermatozoïde d'Orthoptères.

 Z. Zellforsch. mikrosk. Anat., 90, 113-126.

- Das, C.M.S. and Ris. H., 1958. Submicroscopic organization of the nucleus during spermiogenesis in the grasshopper.

 J. biophys. biochem. Cytol., 4, 129-132.
- De Robertis, E.D.P., Nowinski, W.W. and Saez, F.A., 1965.
 In Cell Biology, W.B. Saunders Company, Philadelphia.
- Duesberg, J., 1911. Nouvelles recherches sur l'appareil mitochondrial des cellules séminales.

 Arch. Zellforsch., 6, 40-139.
- Dupraw, E.J. 1970. DNA and Chromosomes, Holt, Rinehard and Winston, Inc. New York.
- Fawcett, D.W., 1956. The fine structure of chromosomes in the meiotic prophase of vertebrate spermatocytes.

 J. biophys. biochem. Cytol., 2, 403-406.
- Fawcett, D.W., 1966. The Cell: Its Organelles and Inclusions.
 W.B. Saunders Company, Philadelphia.
- Fawcett, D.W., 1970. A comparative view of sperm ultrastructure. Biol. Reprod., Suppl. 2, 90-127.
- Fawcett, D.W., Anderson, W.A. and Phillips, D.M., 1971. Morphogenetic factors influencing the shape of the sperm head. 1.

 Developmental Biology, 26, 220-251.
- Fawcett, D.W. and Phillips, D.M., 1967. Further observations on mammalian spermiogenesis.

 J. Cell Biol., 35, 152A, 316.

- Fawcett, D.W. and Porter, K.R., 1954. A study of the fine structure of ciliated epithelia.

 J. Morph., 94, 221.
- Folliot, R. and Maillet, P.L., 1966. Sur l'indépendance morphologique des complexes synaptonematiques a l'égard des chromosomes du cours de la méiose de (Philaenus spumaris) L. male (Hômoptère, Cercopidae).

 C.r. hebd. Séanc. Acad. Sc. Paris, 262, 394-396.
- Folliot, R. and Maillet, P.L., 1970. Ultrastructure de la spermiogenese et du spermatozoïde de divers insectes Homoptères.

 In <u>Comparative Spermatology</u> (Baccetti, B., editor)

 Academic Press, New York-London. 431-449.
- Friedlander, M. and Wahrman, J., 1966. Giant centrioles in Neuropteran meiosis.

 J. Cell Sci., 1, 129-144.
- Fritz-Niggli, H. and Suda, T., 1972. Bildung und bedentung der zentriolen: Eine studie und neuinterpretation der meiose von <u>Drosophila</u>.

 Cytobiologie, 5, 12-41.
- Fuge, H., 1968. Zum feinbau des periplasten und der geissel von <u>Trypanosoma brucei</u> und <u>Trypanosoma gambiense</u>.

 Z. Zellforsch. mikrosk. Anat., <u>89</u>, 201-211.
- Gall, J.G., 1965. Fine structure of microtubules. J. Cell Biol., 27, 32A, 59.
- Gall, J.G., 1966. Microtubule fine structure. J. Cell Biol., 31, 639-643.

- Gassner, G., 1969. Synaptinemal complexes in the achiasmatic spermatogenesis of <u>Bolbe nigra</u> Giglio-Tos (Mantoidea). Chromosoma, 26, 22-34.
- Gatenby, J.B. and Tahmisian, T.N., 1959. Centricle adjunct, centricles, mitochondria, and ergastoplasm in Orthopteran spermatogenesis. An electron microscope study.

 Cellule, 60, 105-134.
- Gibbons, I.R. and Grimstone, A.V., 1960. On flagellar structure in certain flagellates.

 J. biophys. biochem. Cytol. 7, 697-716.
- Guenin, H.A., 1965. Observations sur la structure submicroscopique du complexe axial dans les chromosomes meiotiques chez Gryllus campestris L. et G. bimaculatus De Geer (Orthopt. Gryll.).

 J. Microscopie, 4, 749-758.
- Harris, P., 1965. Some observations concerning metakinesis in sea urchin eggs.

 J. Cell Biol., 25, (Suppl.) 73-78.
- Hess, O. and Meyer, G., 1968. Genetic activities of the Y chromosome in <u>Drosophila</u> during spermatogenesis.

 Advan. Genet., 14, 171-223.
- Hoage, T.R. and Kessel, R.G., 1968. An electron microscope study of the process of differentiation during spermatogenesis in the drone honey bee (Aphis mellifera L.) with special reference to centriole replication and elimination.

 J. Ultrastruct. Res., 24, 6-32.

- Horstmann, E., 1970. The acrosome complex of the Diplopode

 Spirostreptus sp.

 In Comparative Spermatology (Baccetti, B., editor)

 Academic Press. New York-London. 255-262.
- Horstmann, E. and Breucker, H., 1969. Spermatozoen und spermiohistogenese von <u>Graphidostreptus</u> spec. (Myriapoda, Diplopoda).
 - I. Die reifen spermatozoen.
 - Z. Zellforsch. mikrosk. Anat., 96, 505-520.
- Jaworskia, H. and Lima-de-Faria, A., 1969. Multiple synaptinemal complexes at the region of gene amplication in <u>Acheta</u>. Chromosoma, <u>28</u>, 309-327.
- Johnson, H.H., 1931. Centricles and other cytoplasmic components of male germ cells of the Gryllidae.

 Z. Wiss. Zool., 140, 115-166.
- Jokelainen, P., 1967. The ultrastructure and spatial organisation of the metaphase kinetochore in mitotic rat cells.

 J. Ultrastruct. Res., 19, 19-44.
- Kaye, J.S., 1962. Acrosome formation in the house cricket. J. Cell Biol., 12, 411-433.
- Kaye, J.S., 1964. Fine structure of flagella in spermatids of the house cricket.
 J. Cell Biol., 22, 710-714.
- Kaye, J.S. and Kaye, R.M., 1966. The fine structure and chemical composition of nuclei during spermiogenesis in the house cricket. I. Initial stages of differentiation and the loss of nonhistone protein. J. Cell Biol., 31, 159-179.

- Kessel, R.G., 1966. The association between microtubules and nuclei during spermiogenesis in the dragonfly. J. Ultrastruct. Res., 16, 293-304.
- Kessel, R.G., 1967. An electron microscope study of spermiogenesis in the grasshopper with particular reference to the development of microtubular systems during differentiation. J. Ultrastruct. Res., 18, 677-694.
- Kessel, R.G., 1970. Spermiogenesis in the dragonfly with special reference to a consideration of the mechanisms involved in the development of cellular asymmetry.

 In <u>Comparative Spermatology</u> (Baccetti, B., editor)

 Academic Press, New York-London, 531-552.
- Kiefer, B.I., 1970. Development, organization, and degeneration of the <u>Drosophila</u> sperm flagellum. J. Cell Sci., <u>6</u>, 177-194.
- La Fountain, J.R., 1972. An association between microtubules and aligned mitochondria in Nephrotoma spermatocytes.

 Expl. Cell Res., 71, 325-328.
- Ledbetter, M.C. and Porter, K.R., 1964. Morphology of microtubules of plant cells.

 Science (Washington), 144, 872-874.
- Maillet, P.L. and Folliot, R., 1965. Sur les ultrastructures chromosomiques de la méiose chez (Philaenus spumaris" L. male (Homoptera, Cercopidae).

 C.r. hebd. Séanc. Acad. Sci. (Paris), 260, 3486-3489.

- Manton, I., 1952. The fine structure of plant cilia. Symp. Soc. Exp. Biol., 6, 306-319.
- Martinez, P.M. and Deams, M. TH., 1968. Les phases précoces de la formation des cils et le problème de l'origine du corpuscle basal.
 - Z. Zellforsch. mikrosk. Anat., 87, 46-68.
- McKintosh, J.R. and Porter, K.R., 1967. Microtubules in the spermatids of the domestic fowl.

 J. Cell Biol., 35, 153-173.
- Meves, F., 1907. Die spermatocytenteilungen bei der honigbiene

 <u>Apis mellifica L.</u>), nebst bemerkungen uber chromatinreduktion.

 Arch, mikrosk. Anat. Entw. Mech., 70, 414-491.
- Meyer, G.F., 1964. Die parakristallinen körper in den spermienschwänzen von <u>Drosophila</u>.

 Z. Zellforsch. mikrosk. Anat., 62, 762-784.
- Meyer, G.F., 1966. Crystalline mitochondrial derivatives in sperm.

 In <u>Proceedings of the 6th International Congress of</u>

 Electron Microscopy, Kyoto. 2, 629-630.
- Meyer, G.F., 1968. Spermiogenese in normalen und y-defizient mannchen von <u>Drosophila melanogaster</u> and <u>D. hydei</u>.

 Z. Zellforsch. mikrosk. Anat., <u>84</u>, 141-175.

- Moens, P.B., 1969a. The fine structure of meiotic chromosome polarization and pairing in <u>Locusta migratoria</u> spermatocytes. Chromosoma. 28. 1-25.
- Moens, P.B., 1969b. Multiple core complexes in grasshopper spermatocytes and spermatids.

 J. Cell Biol., 40, 542-551.
- Monneron A. and Bernhard, W., 1966. Action de certaines enzymes sur des tissus inclus en Epon.

 J. Microscopie, 5, 697-714.
- Moses, M.J., 1956. Chromosomal structures in crayfish spermatocytes. J. biophys. biochem. Cytol., 2, 215-218.
- Moses, M.J., 1968. Synaptinemal complex.

 Annual Review of Genetics, 2, 363-412.
- Nicklas, R.B., 1967. Chromosome micromanipulation. II. Induced reorientation and the experimental control of segregation in meiosis.

 Chromosoma, 21, 17-50.
- Novikoff, A.B., 1961. Mitochondria (Chondriosomes).

 In <u>The Cell</u> (Brachet, J. and Mirsky, A.E. editors)

 Academic Press Inc., New York. 2, 299-421.
- Perotti, M.E., 1970. The neck region in mature sperm of <u>Drosophila</u>

 <u>melanogaster</u>.

 In <u>Comparative spermatology</u> (Baccetti, B., editor)

 Academic Press, New York-London. 523-529.

- Phillips, D.M., 1966a. Observations on spermiogenesis in the fungus gnat Sciara coprophila.
 - J. Cell Biol., 30, 477-497.
- Phillips, D.M., 1966b. Fine structure of Sciara coprophila sperm.

 J. Cell Biol., 30, 499-517.
- Phillips, D.M., 1966c. Substructure of flagellar tubules. J. Cell Biol., 31, 635-638.
- Phillips, D.M., 1970a. Insect sperm: Their structure and morphogenesis.

 J. Cell Biol., 44, 243-277.
- Phillips, D.M., 1970b. Development of spermatozoa in the wooly opossum with special reference to the shaping of the sperm head.
 - J. Ultrastruct. Res., 33, 369-330.
- Phillips, D.M., 1970c. Ultrastructure of spermatozoa of the wooly opossum <u>Caluromys philander</u>.

 J. Ultrastruct. Res., 33, 381-397.
- Phillips, D.M., 1970d. Insect flagellar tubule patterns: Theme and variations.
 - In <u>Comparative Spermatology</u> (Baccetti, B. editor) Academic Press, New York-London. 263-273.
- Pollister, A.W., 1930. Cytoplasmic phenomena in the spermatogenesis of Gerris.
 - J. Morph., 49, 455-507.

- Potswald, H.E., 1967. An electron microscope study of spermiogenesis in <u>Spirobis (Laeospira) morchi</u> Levinsen (Polychaeta).

 Z. Zellforsch. mikrosk. Anat., <u>83</u>, 231-248.
- Pratt, S.A., 1967. An electron microscope study of nebenkern formation and differentiation in spermatids of <u>Murgantia histrionica</u> (Hemiptera, Pentatomidae).

 J. Cell Biol., 35, 106A, 219.
- Pratt, S.A. 1968. An electron microscope study of nebenkern formation and differentiation in spermatids of <u>Murgantia histrionica</u> (Hemiptera, Pentatomidae).

 J. Morph., 126, 31-36.
- Pratt, S.A., 1970. Formation and differentiation of the nebenkern in spermatids of an Hemipteran insect <u>Murgantia histrionica</u>. In <u>Comparative Spermatology</u> (Baccetti, B., editor) Academic Press, New York-London. 301-310.
- Rambourg, A., 1967. An improved silver methenamine technique for the detection of periodic acid-reactive complex carbohydrates with the electron microscope.
 - J. Histochem. Cytochem., 15, 409-412.
- Reger, J.F. and Cooper, D.P., 1968. Studies on the fine structure of spermatids and spermatozoa from the Millipede Polydesmus sp.
 - J. Ultrastruct. Res., 23, 60-70.
- Renaud, F.L. and Swift, H., 1964. The development of basal bodies and flagella in Allomyces arbusculus.

 J. Cell Biol., 23, 339-361.

- Retzius, G., 1904. Zur kenntnis der spermien der Evertebraten. I. Biol. Unters., 11, 1-32.
- Retzius, G., 1906. Die spermien der Gastropoden. Biol. Unters., 13, 1-36.
- Reynolds, E.S., 1963. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy.

 J. Cell Biol., 17, 208-212.
- Ringo, D.L., 1967. The arrangement of subunits in flagellar fibres.

 J. Ultrastruct. Res., 17, 266-277.
- Schin, K.S., 1965. Core strukturen in den meiotischen und postmeiotischen kernen der spermatogenese von <u>Gryllus domesticus</u>.
 Chromosoma, <u>16</u>, 436-452.
- Selenka, E., 1887. Studien uber entwicklungsgeschicte der theire.

 Viertes heft. das opposum (<u>Didelphys virginiana</u>).

 C.W. Kreidels Verlag, Wiesbaden.
- Shoup, J.R., 1967. Spermiogenesis in wild type and in a male sterility mutant of <u>Drosophila melanogaster</u>.

 J. Cell Biol., 32, 663-675.
- Silviera, M. and Porter, K.R., 1964. The spermatozoids of flatworms and their microtubular systems.

 Protoplasma, 59, 240-265.
- Smith, S.G., 1960. Chromosome numbers of Coleoptera. II. Can. J. Genet. Cytol., 2, 66-88.

- Sorokin, S.P., 1962. Centrioles and the formation of rudimentary cilia by fibroblasts and smooth muscle cells.

 J. Cell Biol., 15, 363-377.
- Sorokin, S.P., 1968. Reconstructions of centriole formation and ciliogenesis in mammalian lungs.

 J. Cell Sci., 3, 207-230.
- Sotelo, J.R. and Trujillo-Cenoz, O., 1958. Electron microscope study of the kinetic apparatus in animal sperm cells.

 Z. Zellforsch. mikrosk. Anat., 48, 565-601.
- Sotelo, J.R. and Wettstein, R., 1966. Fine structure of meiotic chromosomes comparative study of nine species of insects. Chromosoma, 20, 234-250.
- Swift, H., 1955. In <u>The Nucleic Acids</u>. Edited by Chargaff, E. and Davidson, J.N. Academic Press, New York. 2, 51.
- Thompson, T.E. and Blum, M.S., 1967. Structure and behaviour of spermatozoa of the fire ant <u>Solenopsis saevissima</u> (Hymenoptera: Formicidae).

 Ann. Entomol. Soc. Amer., <u>60</u>, 632-642.
- Warner, F.D., 1971. Spermatid differentiation in the blowfly

 Sarcophaga bullata with particular reference to flagellar morphogenesis.

 J. Ultrastruct. Res., 35, 210-232.
- Werner, G., 1966. Untersuchengen uber die spermicgenese bei einem laufkäfer <u>Carabus catenulatus</u> scop. und der skorpion wasserwanze <u>Nepa rubra</u> L.

 Z. Zellforsch. mikrosk. Anat., 73, 576-599.

- Wettstein, R. and Sotelo, J.R., 1967. Electron microscope serial reconstruction of the spermatocyte I nuclei at pachytene. J. Microscopie, <u>6</u>, 557-576.
- Wettstein, R. and Sotelo, J.R., 1970. The modecular architecture of synaptinemal complexes.

 In Advances in Cell and molecular biology. Du Praw, E.J. editor. Academic Press, New York-London. Vol. 1, 109-152.
- Wilson, E.B., 1924. The Cell in Development and Heredity. 305.

Figures 1-71 are micrographs of material from <u>Acilius</u> testes except for figures 15-21, 44, 50 and 51 which are micrographs from <u>Agabus</u> testes. Figures 72-143 are micrographs of sperm from <u>Acilius</u> vasa deferentia. Figures 144-164 are of <u>Dytiscus</u> sperm, figures 165-184 are of <u>Colymbetes</u> and Figures 185-193 are of <u>Agabus</u> sperm.

Key to abbreviations used in labelling

A	acrosome	DE	double membrane
AF	accessory fibre	DF	diffuse layer of material
AG	accessory gland	DL	a flattened membrane-bounded vesicle
В	basal body	DM	coarse granular material in Acilius and Dytiscus
BR	parallel bars	DM	densely stained mass of granular material in Colymbetes
C	centriole	DN	darker staining nuclear material
CA	centricle adjunct		
СВ	cristae	DS	dorsal side
CD	curved structure	E	epithelial border
		$_{\rm EA}$ 2	2nd meiotic anaphase (early)
CE	central element	EC	epithelial cells
CF	coarse fibre	EJ	ejaculatory duct
CH	chromosome	97	
CL	chromosomal material	EP	end piece
CM	cell membrane	F	flagellum
CN	chromatin	FT	flagellar tubules
		G	Golgi body
CR	cross striations	GA	granular area
CS	C-shaped structures	GC	glycogen
CT	central tubules	370	
CA	cup-like vesicle	GL	granular material
D	doublet	GМ	granular mass
		GR	a groove
DA	coating of densely stained material	H	horseshoe configuration
DB	dense bars	HC	Honeycomb-like structure

***	1.5.3	NE	nuclear envleope
HD	head	NK	nebenkern
HK	BARB		
HO	hood	MM	nuclear membranes
IS	initial segment	P	polycomplex
K	kinetochore	PA	proacrosomal granule
L	lateral elements	PC	paracrystalline material
LA ²	2nd meiotic anaphase (late)	PP	posterior portion
LK	linkages	PT	periodic transverse striations
FO	a lobe of granular material	R	rough endoplasmic reticulum
LS	spindle-shaped nucleus	RL	radial links
M	mitochondria	s	sperm
MA	mitotic anaphase	SA	subfibre A
MA ¹	1st meiotic anaphase	SB	subfibre B
MB	'microtubular border'	SC	synaptinemal complex
MD	mitochondrial derivative	SD +	(early) spermatids
MF	myo-fibrils	sī	an invagination
MH	membranous sheath	SL	slit-like opening
MM	mitotic metaphase	SP	spindle poles
MM1	1st meiotic metaphase	ST	spindle tubules
m ²	2nd meiotic metaphase	SU	subunits
MS	mature sperm	T	tails
MT	microtubules	TF	transverse filaments
MT1	1st meiotic telophase	TP	transverse period
MV	microvilli	TS	testis
N	nucleus	٧	vesicles

- VD vas deferens
- W wave array
- WS wedge-shaped structure
- X X chromosome
- Z zig-zag array
- +SD¹ stage 1 spermatid
- +SD² stage 2 spermatid
- +SD4 stage 4 spermatid
- +SD⁵ stage 5 spermatid

Fig. 1. Diagram to show the gross form of the main reproductive tissues in <u>Acilius</u>. The testes (T), vasa deferentia (VD) and accessory glands (AG) are all paired. There is a single median ejaculatory duct (EJ).

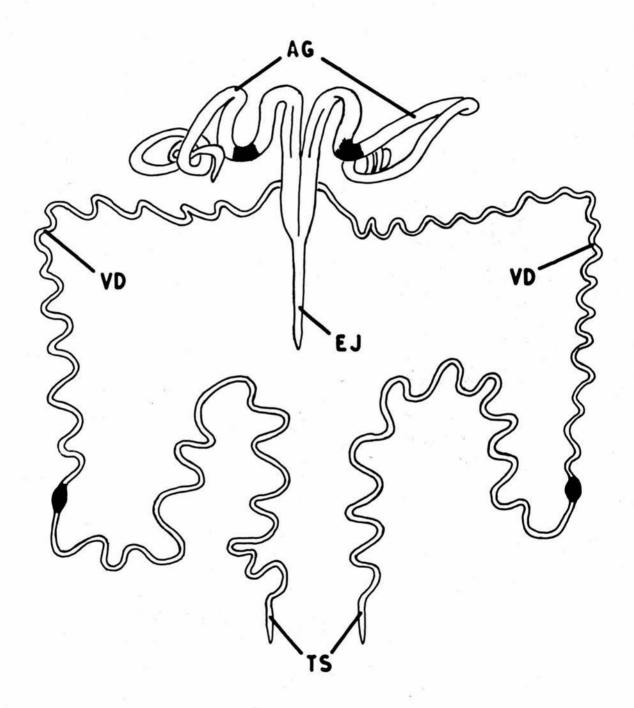
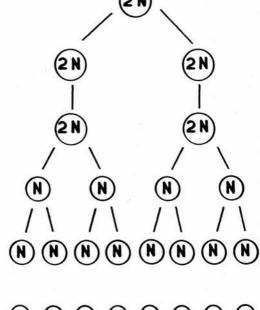


Fig. 2. The main features which occur during spermatogenesis are illustrated in this diagram.

MITOSIS

MEIOSIS IST MEIOTIC DIVISION

2mMEIOTIC DIVISION



777777

INTERPHASE

MITOTIC METAPHASE MITOTIC ANAPHASE

SPERMATOGONIA

PRIMARY SPERMAT-OCYTES METAPHASE I ANAPHASE I AND TELOPHASE I

SECONDARY SPERMATOCYTES
METAPHASE JANAPHASE
I AND TELOPHASE I

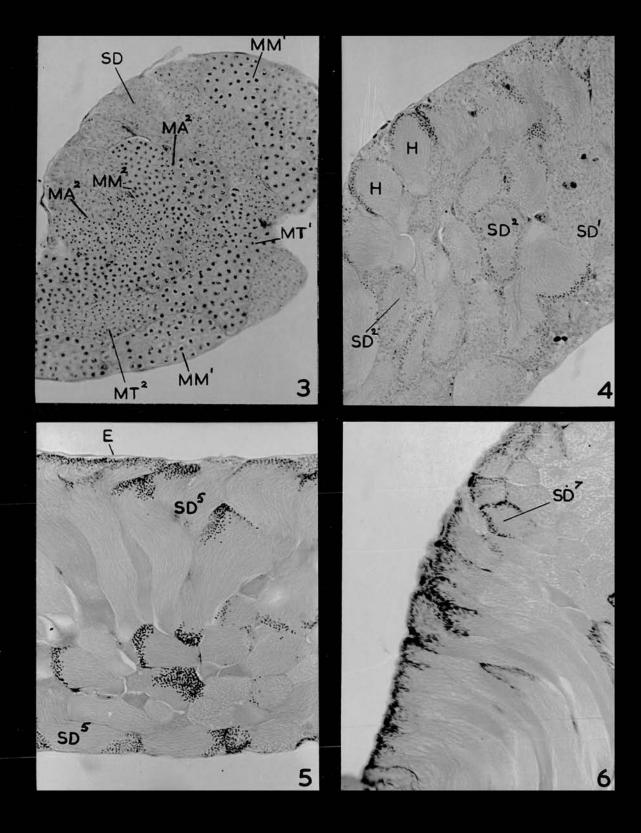
SPERMATIDS

SPERMATOZOA

Figs. 3-6 show longitudinal sections through testes of Acilius stained with Heidenhain's haematoxylin. Bright field microscopy.

- Fig. 3 A section showing cells at meiotic metaphase I (MM¹), meiotic telophase I (MT¹), meiotic metaphase II (MM²), meiotic anaphase II (MA²) and early spermatids (SD¹). x 216.
- Fig. 4. In this section stage 1 spermatids (SD¹) and stage 2 spermatids (SD²) are present as well as cysts of spermatids with their nuclei arranged in a 'horseshoe configuration' (H). x 216.
- Fig. 5. In stage 5 spermatids (SD⁵) the nuclei are filled with densely stained chromatin and in some cases they are situated near the epithelial border (E) of testes.

 x 216.
- Fig. 6. The nuclei in stage 7 spermatids (SD⁷) are spindle-shaped. x 216.



Figs. 7-9 show longitudinal sections through Acilius testes and a vas deferens stained with Heidenhain's haematoxylin. Bright field microscopy.

- Fig. 7. A section through a testis to show mature sperm (MS) arranged in groups. x 216.
- Fig. 8. Mature sperm (MS) in vasa deferentia are not bound together in cysts as they are in testes. The lumen of vasa deferentia are surrounded by a layer of cuboidal epithelial cells (EC). x 600.
- Fig. 9. In mitotic metaphase (MM) cells the mitotic apparatus occupies most of the cell. Separating sister chromosomes are clearly visible in cells at meiotic anaphase (MA). x 600.
- Fig. 10. This is a similar section to the one in Fig. 9 but it has been treated with Feulgen reagent. Chromosomes in mitotic metaphase (MM) and anaphase (MA) cells are Feulgen positive. x 600.

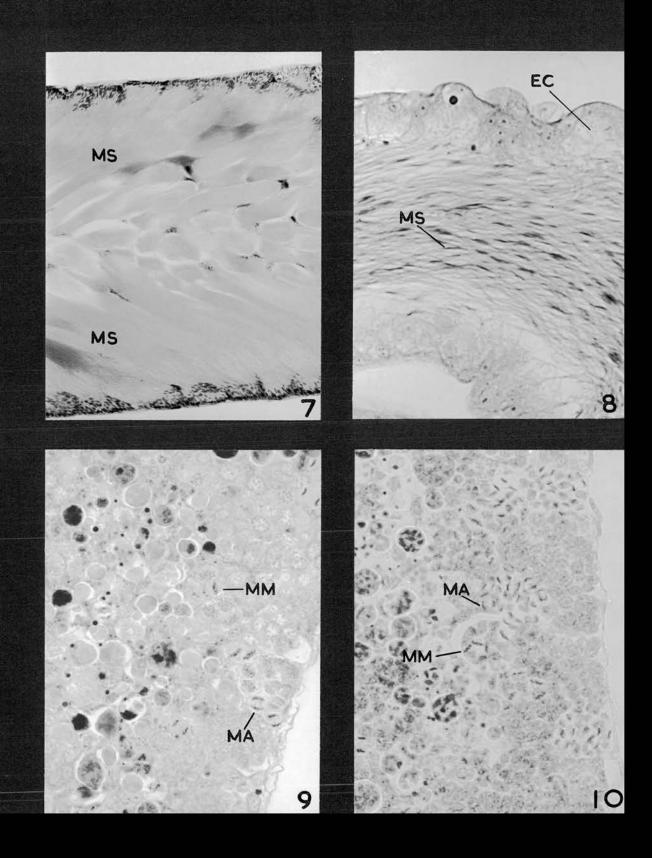


Fig. 11. A transverse section through a group of primary spermatocytes at early pachytene. The nucleus (N) fills most of each cell. Chromatin (CN), synaptinemal complexes (SC) and a darker staining nuclear material (DN) are present within some nuclei. In the cytoplasm surrounding the nuclei in these cells rough endoplasmic reticulum (R), mitochondria (M) and Golgi bodies (G) are present.

x 12,500.

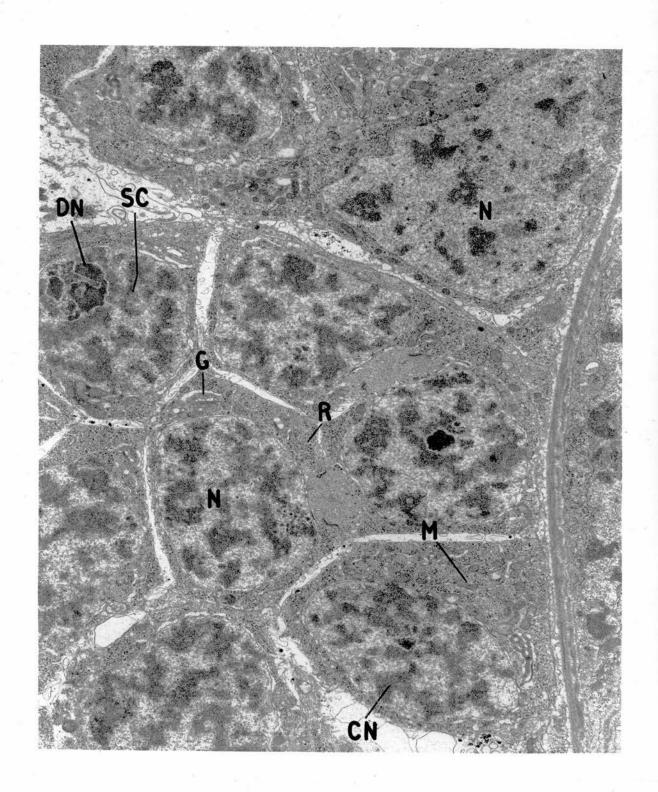
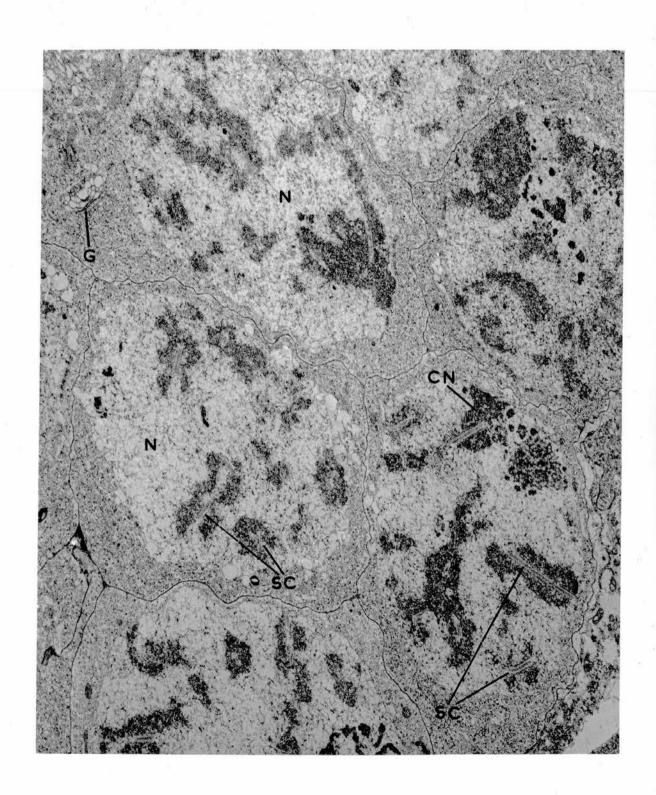
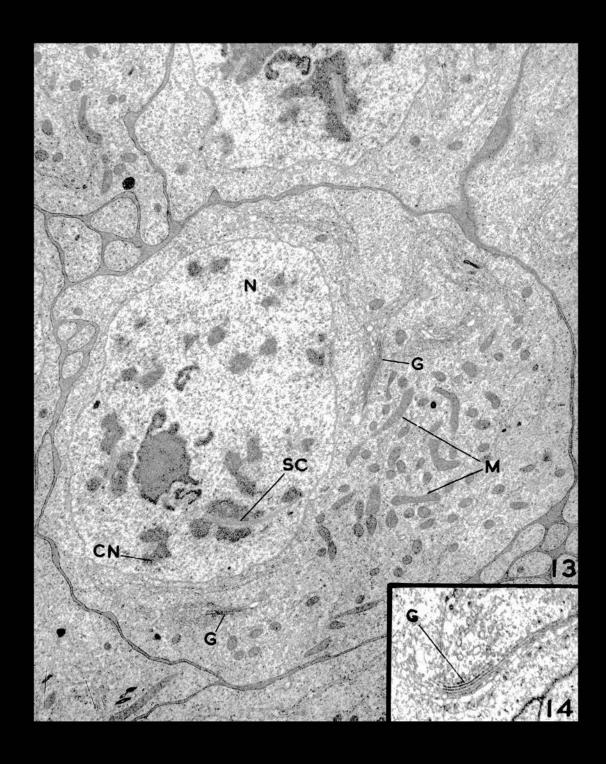


Fig. 12. In spermatocytes at a later stage than those in Fig. 11

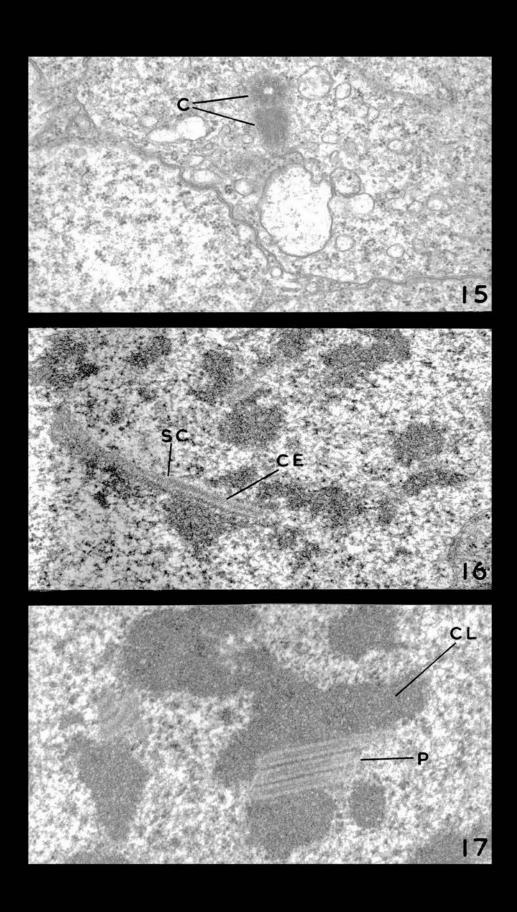
the chromatin (CN) is more condensed and the synaptinemal complexes (SC) are clearly visible. Golgi bodies (G) are present in the cytoplasm. x 12,500.



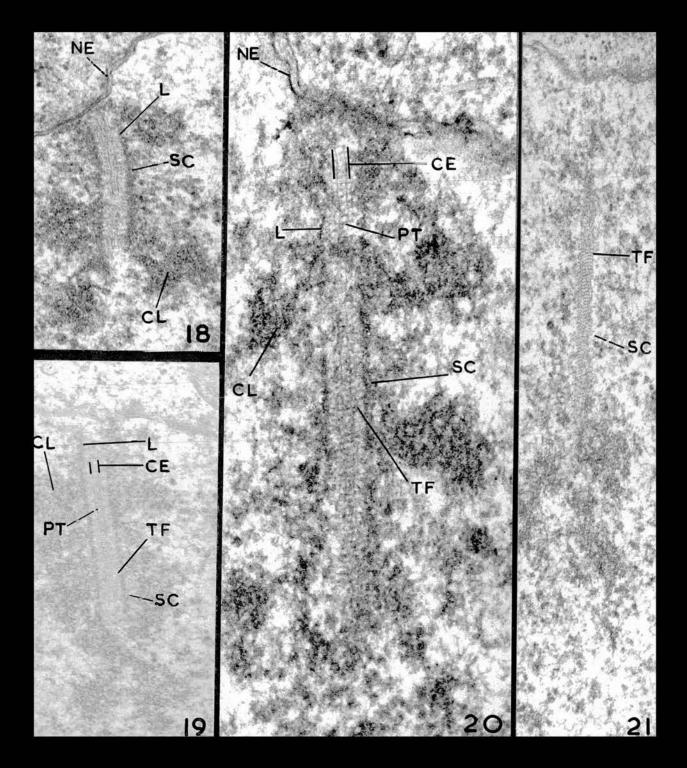
- Fig. 13. Transverse section through a spermatocyte showing the chromatin (CN) and synaptinemal complexes (SC) within the nucleus (N). Golgi bodies with densely stained membranes and elongate mitochondria (M) are present in the cytoplasm. x 12,500.
- Fig. 14. A Golgi body (G) at a higher magnification to show the densely stained membranes that are associated with some Golgi bodies. x 20,000.



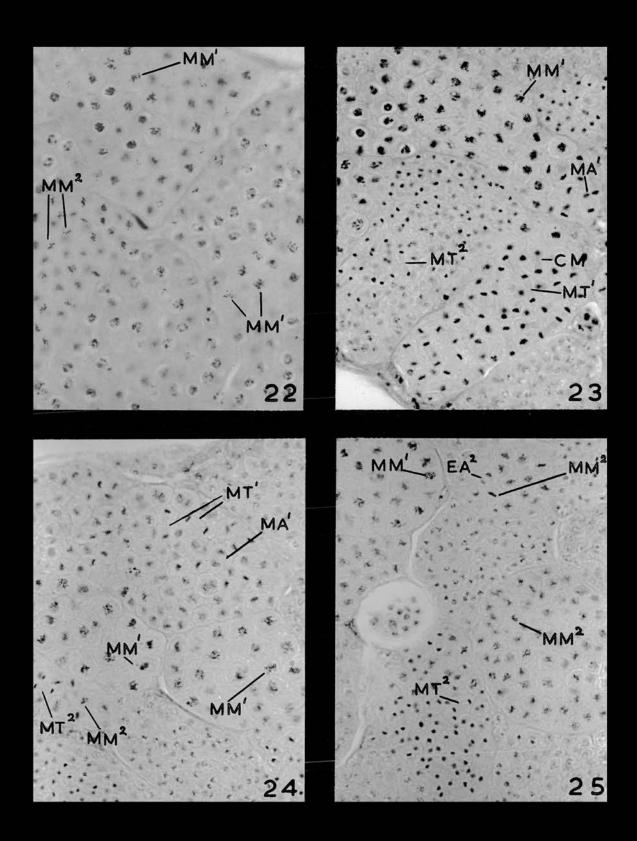
- Fig. 15. Paired centrioles (C) which occur in spermatocytes exhibit a typical arrangement of 9 sets of triplet microtubules when cut transversely. x 40,000.
- Fig. 16. Synaptinemal complexes (SC) are found in nuclei of meiotic prophase cells. This synaptinenal complex has been cut longitudinally showing part of the central element (CE). x 40,000.
- Fig. 17. A longitudinally sectioned polycomplex (P) of three synaptinemal complexes fused together with chromosomal material (CL) lying on both sides of the polycomplex. x 40,000.



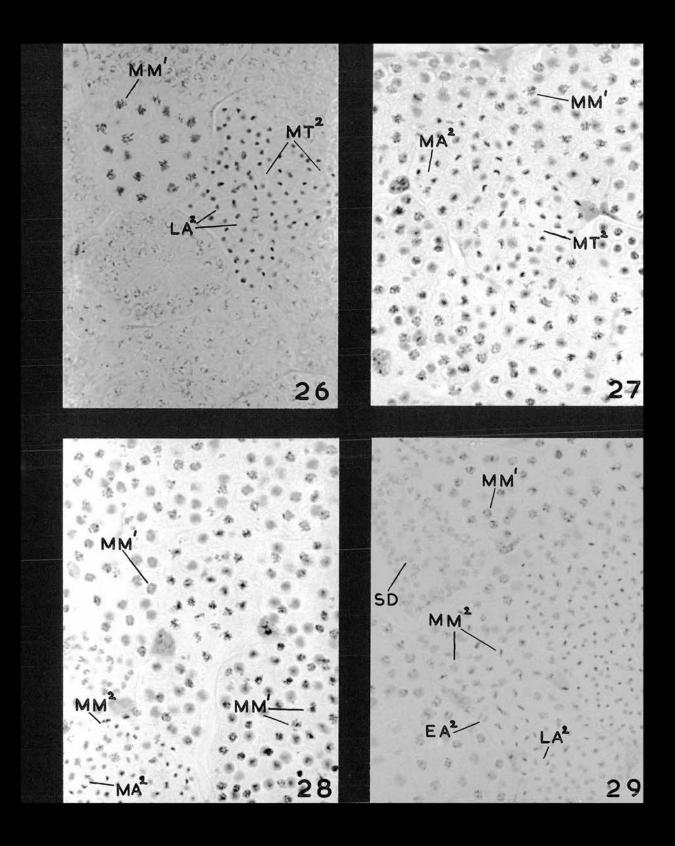
- Figs. 18-21 are all longitudinal sections through synaptinemal complexes (SC) from cells at meiotic prophase.
- Fig. 18. A synaptinemal complex (SC) in contact with the nuclear envelope (NE) in a region where the membranes are closer together and stain more densely than elsewhere. The lateral elements (L) are marked by the chromosomal material (CL). x 70,000.
- Fig. 19. Synaptinemal complex (SC) showing the dense lateral elements (L) closely associated with chromosomal material (CL) and between the lateral elements there is a central element (CE). Periodic transverse striations (PT) pass obliquely across the central element and in register with and parallel to the longitudinal axes of the striations transverse filaments (TF) extend from the central element to the lateral elements. x 60,000.
- Fig. 20. A similar view of a synaptinemal complex (SC) to the one in Figs. 18 and 19 showing the lateral elements (L), central element (CE), periodic transverse striations (PT) and transverse filaments (TF). The synaptinemal complex is in contact with the nuclear envelope (NE). Chromosomal material (CL) is situated in the close proximity of the lateral elements. x 120,000.
- Fig. 21. A section grazing the edge of a complex (SC) shows that the transverse filaments (TF) are circular. x 60,000.



- Figs. 22-25 are longitudinal sections through testes stained with Heidenhain's haematoxylin. Bright field microscopy.
- Fig. 22. Meiotic metaphase I (MM¹) and metaphase II (MM²) cells have their chromosomes arranged on the equator of the spindle. x 600.
- Fig. 23. Cells at meiotic metaphase I (MM¹), anaphase I (MA¹), telophase I (MT¹) and telophase II (MT²). In late meiotic telophase I (MT¹) the cell membrane (CM) separates the two daughter cells. x 600.
- Fig. 24. A similar view to Fig. 23 showing cells at meiotic metaphase I (MM¹), anaphase I (MA¹), telophase I (MT¹), metaphase II (MM²) and telophase II (MT²) x 600.
- Fig. 25. This section shows meiotic metaphase I (MM^1) , metaphase II (MM^2) , early anaphase II (EA^2) and telophase II (MT^2) cells. x 600.



- Fig. 26. Longitudinal section through a testis showing cells at meiotic metaphase I (MM¹), late anaphase II (LA²) and telophase II (MT²) stained with Heidenhain's haematoxylin. Bright field microscopy. x 600.
- Figs. 27-29 are longitudinal sections through testes treated with Feulgen reagent. The chromatin is Feulgen positive and it is densely stained. Bright field microscopy.
- Fig. 27. Illustrated in this section are meiotic metaphase I (MM¹), anaphase II (MA²) and telophase II (MT²) cells. X 600.
- Fig. 28. A similar view to Fig. 27 showing cells at meiotic metaphase I (MM^2) , metaphase II (MM^2) and anaphase II (MA^2) . x 600.
- Fig. 29. This section illustrates cells at meiotic metaphase I (MM¹), metaphase II (MM²), early anaphase II (EA²), late anaphase II (LA²) and early spermatids (SD). x 600.



- Figs. 30-32 are squash preparations of Feulgen treated testes.
- Fig. 30. This squash shows meiotic metaphase I (MM¹) cells with their chromosomes arranged on the equator of the spindle except for the single X chromosome (X) which is segregated from the main bundle of chromosomes. x 1,400.
- Fig. 31. A similar view showing meiotic metaphase I (MM¹) and anaphase I (MA¹) chromosomes arranged on the spindle.

 The X chromosomes (X) are separate from the main bundle of chromosomes. x 1,400.
- Fig. 32. In meiotic metaphase II (MM²) cells there are two X chromosomes (X) with one at each spindle pole.
 x 1,400.

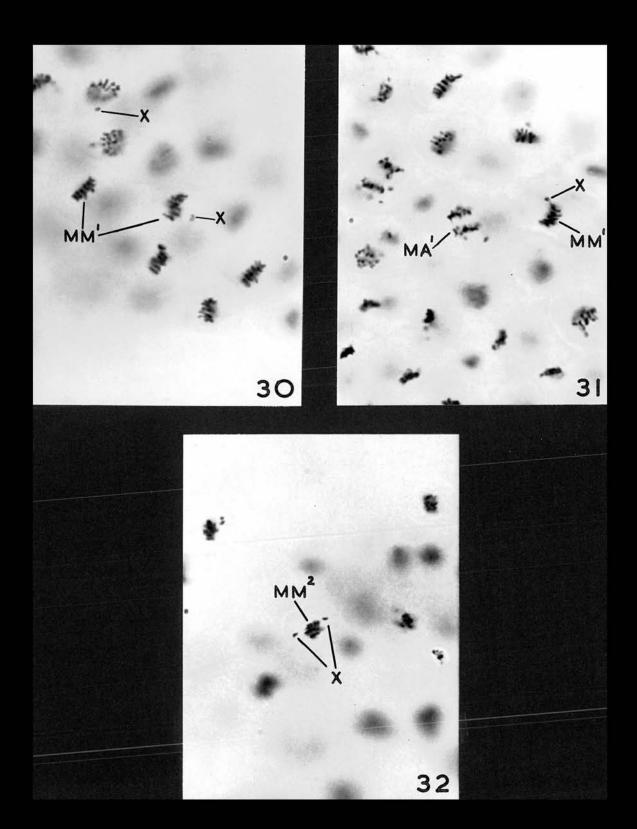


Fig. 33. A longitudinal section through a meiotic metaphase I cell showing the chromosomes (CH) arranged at the equator of the spindle and close to the cell membrane (CM) and spindle pole (SP) a centriole (C) is situated. At the periphery of the mitotic apparatus there are several elongated mitochondria (M). At this stage the nuclear envelope is completely broken down. x 15,000.

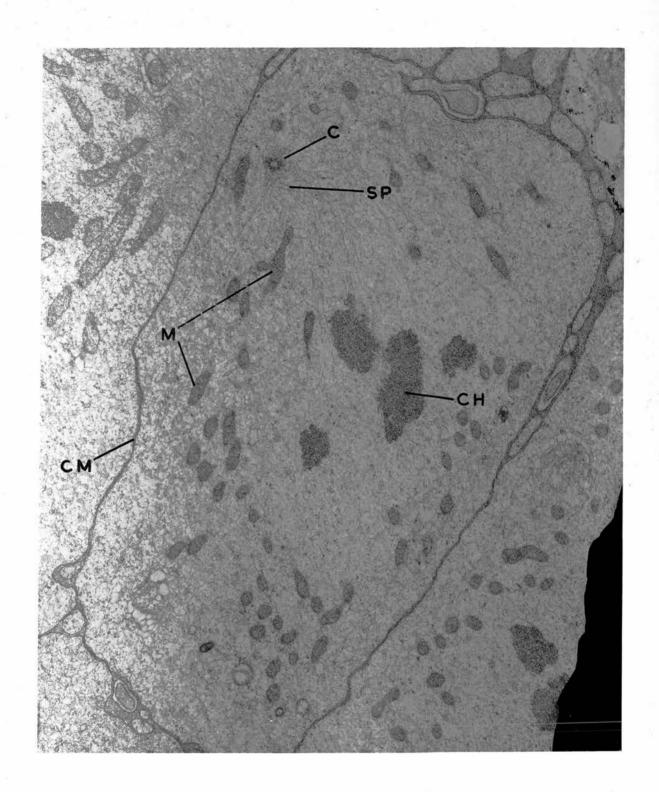


Fig. 34. A longitudinal section through a meiotic metaphase I cell showing the chromosomes (CH) arranged at the equator of the spindle. The nuclear envelope is completely broken down. x 12,500.

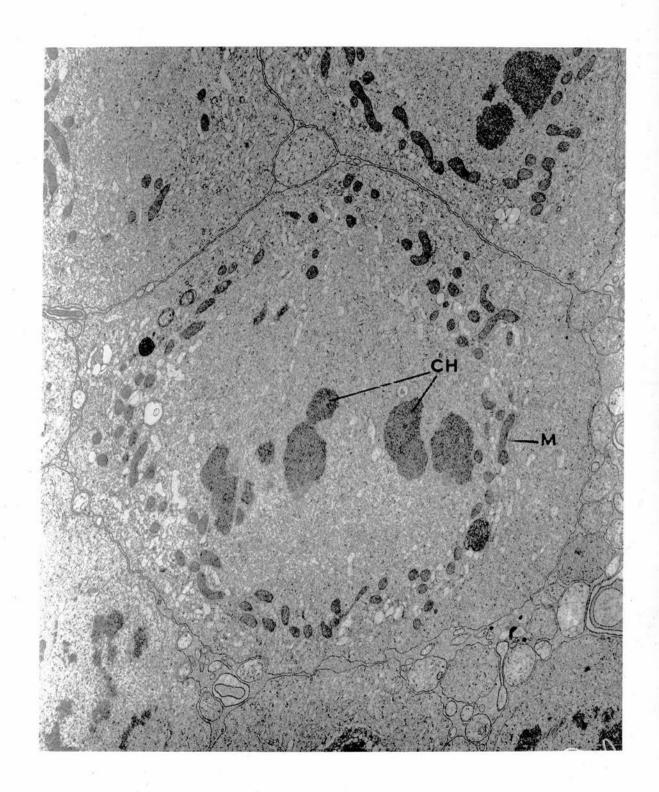
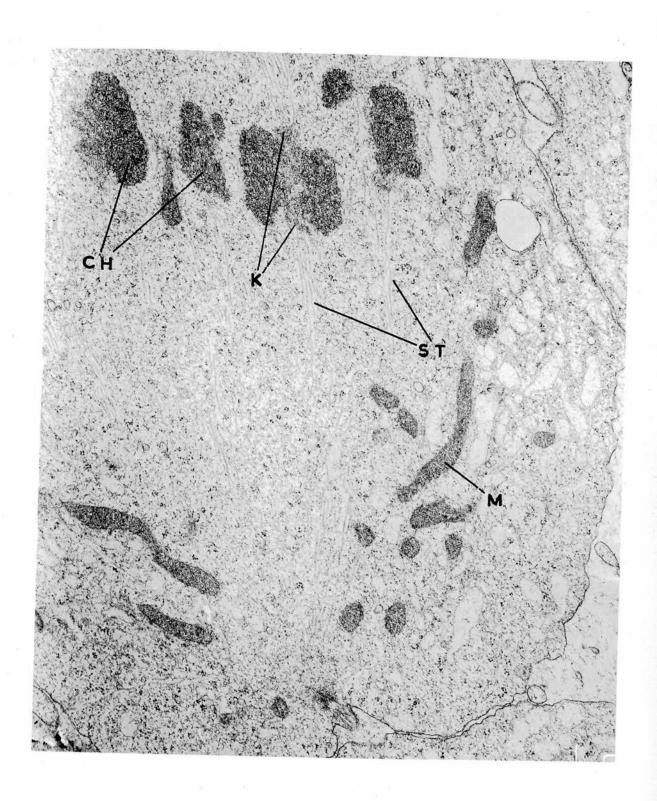


Fig. 35. A longitudinal section to show the fine structure of the spindle tubules (ST) and their attachment points to the chromosomes (CH) at the kinetochores (K). Elongated mitochondria (M) are situated at the periphery of the mitotic apparatus. x 25,000.



- Fig. 36. In meiotic telophase I cells chromosomes (CH) are clumped at opposite ends of the cell and elongate mitochondria (M) pass between them. The cell membrane (CM) furrows in towards the mitochondria at the equator of the cell.

 Golgi bodies (G) are present in the cytoplasm. x 4,000.
- Fig. 37. A longitudinal section through a cell at late telophase
 I showing the separation of the cell into two daughter
 cells by the cell membrane (CM). Chromosomes (CH),
 mitochondria(M), Golgi bodies (G) and a centriole (C)
 are also present in this micrograph. x 6,000.

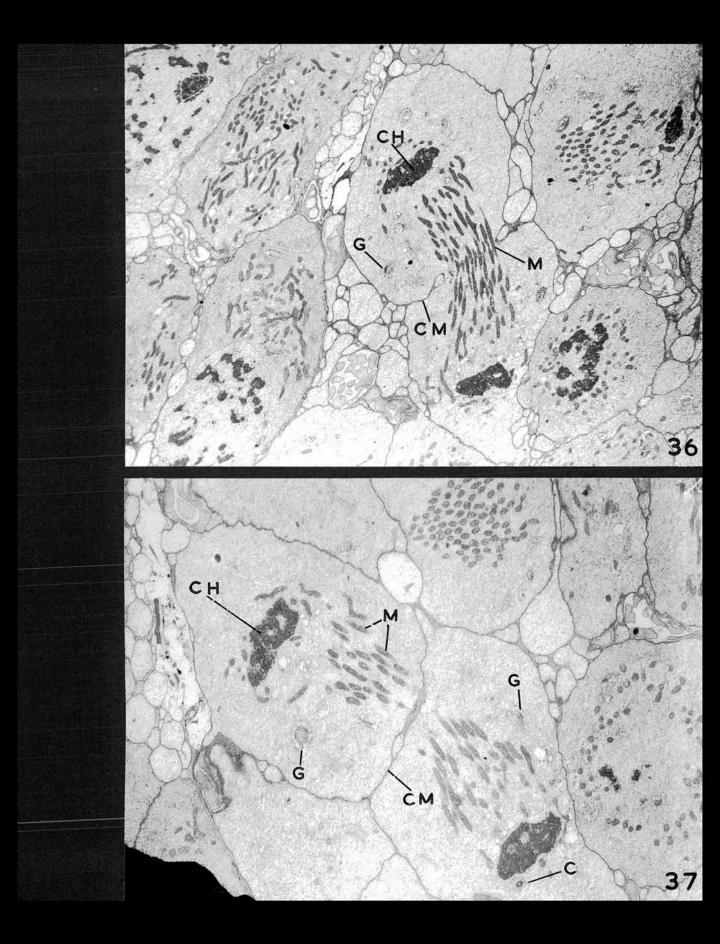
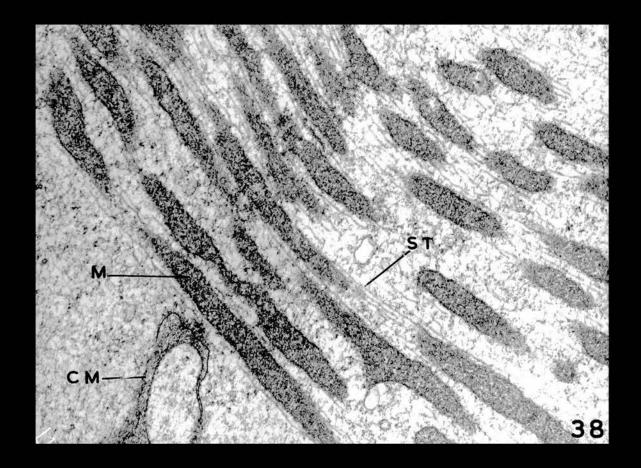


Fig. 38. Part of the section illustrated in Fig. 36 at a higher magnification to show the spindle tubules and their close associations with the elongated mitochondria (M) in meiotic telophase I cells. Part of the cell membrane (CM) which furrows in towards the mitochondria is also present. x 37,500.



- Figs. 39-43 represent stages in the initial development of the flagellum present in meiotic metaphase II cells.
- Fig. 39. A longitudinal section through a centriole (C) and a cup-shaped vesicle (CV). Flagellar microtubules extend from the centriole and project into the vesicle. This centriole is situated near the spindle pole (SP). x 40,000.
- Fig. 40. An oblique section through a cup-shaped vesicle (CV) and the centriole microtubules (C). x 20,000.
- Fig. 41. A pair of centrioles (C) which have been cut longitudinally showing the orientation of the centrioles to each other and to the cup-shaped vesicle (CV). The vesicle in this case appears to be clear and empty. x 20,000.
- Fig. 42. The cup-shaped vesicle (CV) in this section is continuous with the cell membrane (CM). There is a granular mass (GM) at the tip of the vesicle. The flagellar microtubules from one centriole (C) project into the vesicle (CV) which the other centriole (C) is orientated at right angles. Both centrioles are situated near the spindle pole (SP). x 40,000.
- Fig. 43. This section is similar to Fig. 42 showing the centrioles (C), and a cup-shaped vesicle (CV) and granular mass (GM) which are continuous with the cell membrane (CM). The centrioles are situated in the region of the spindle pole (SP). x 20,000.

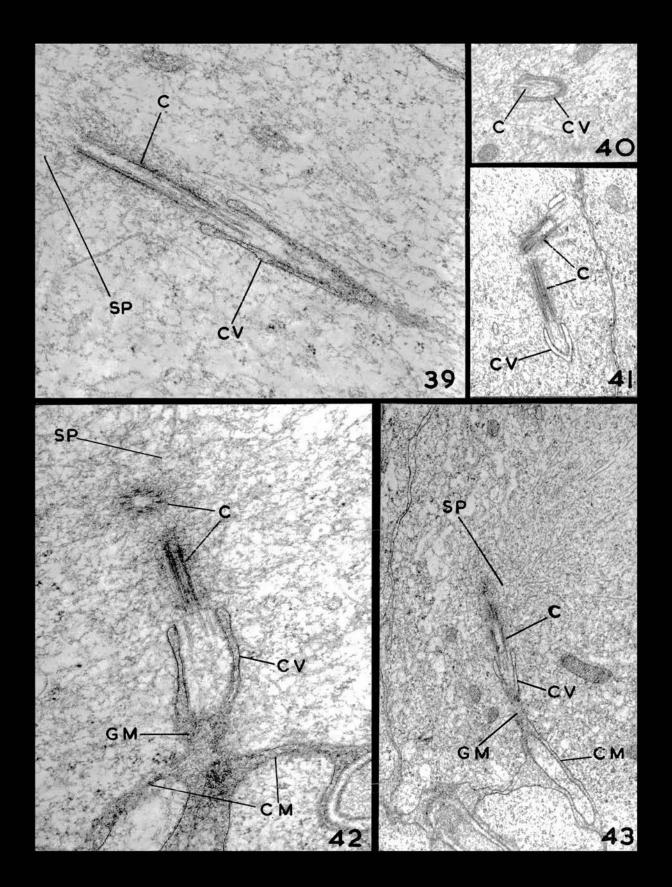
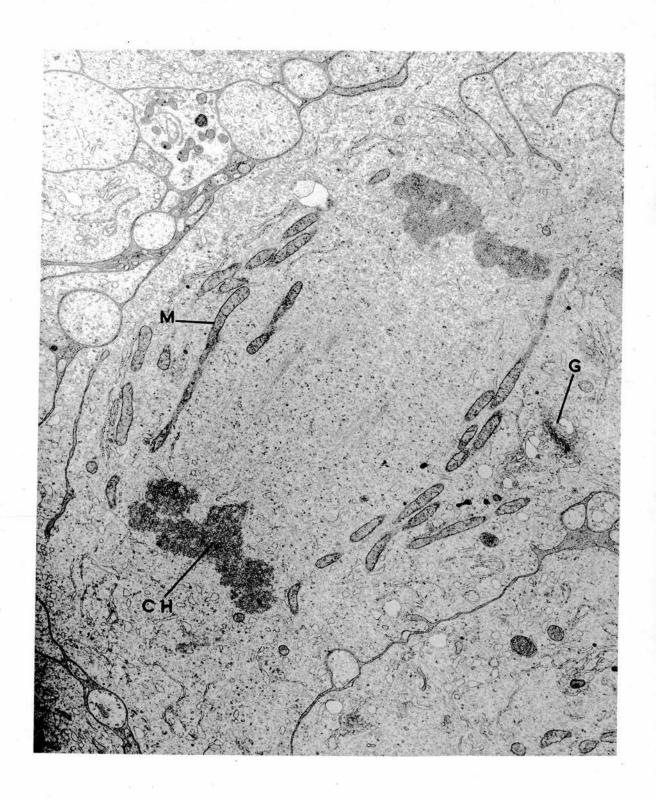
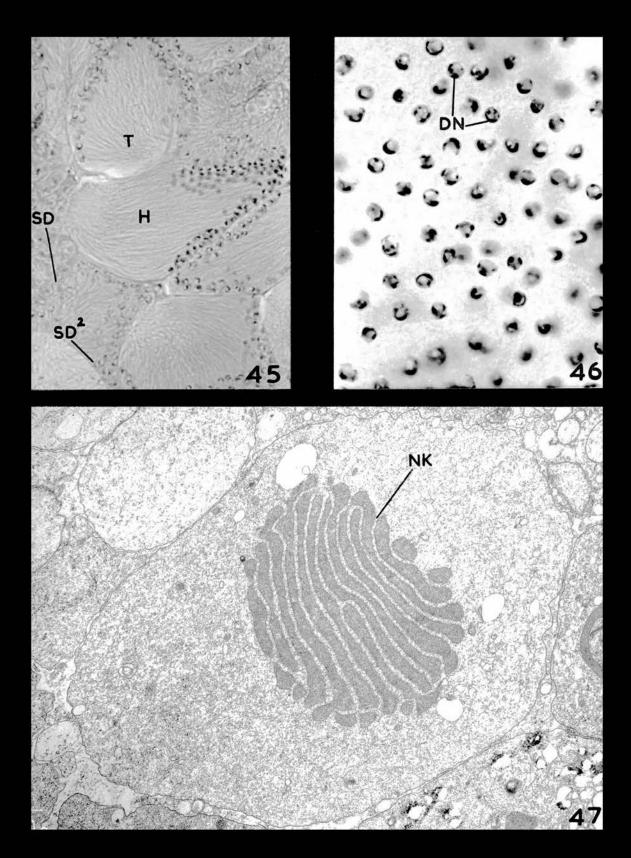


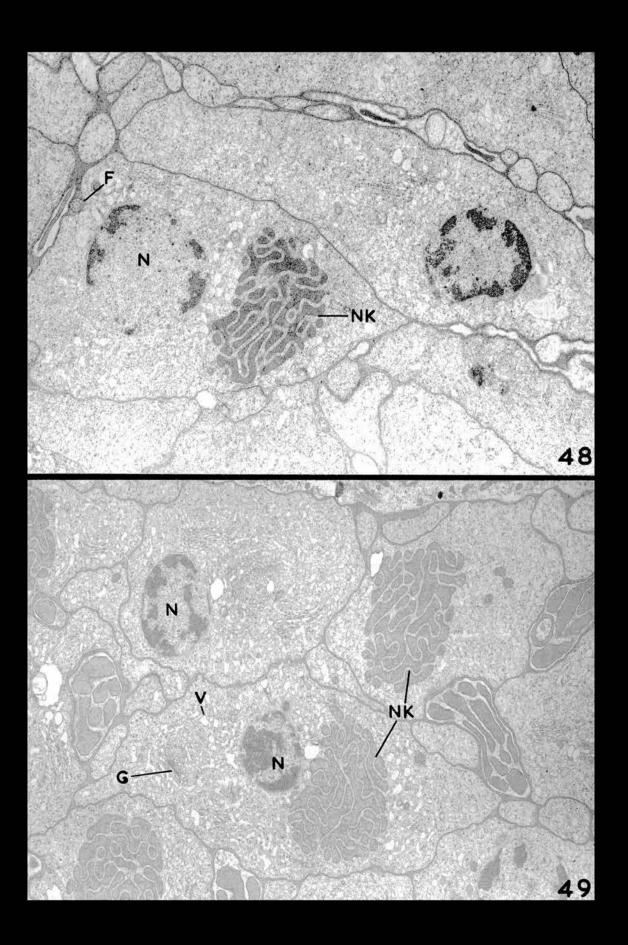
Fig. 44. The chromosomes (CH) are positioned at the spindle poles in late meiotic anaphase II cells. The mitochondria (M) are elongate and they are situated at the periphery of the spindle. Golgi complexes (G) can be seen in the cytoplasm. x 15,000.



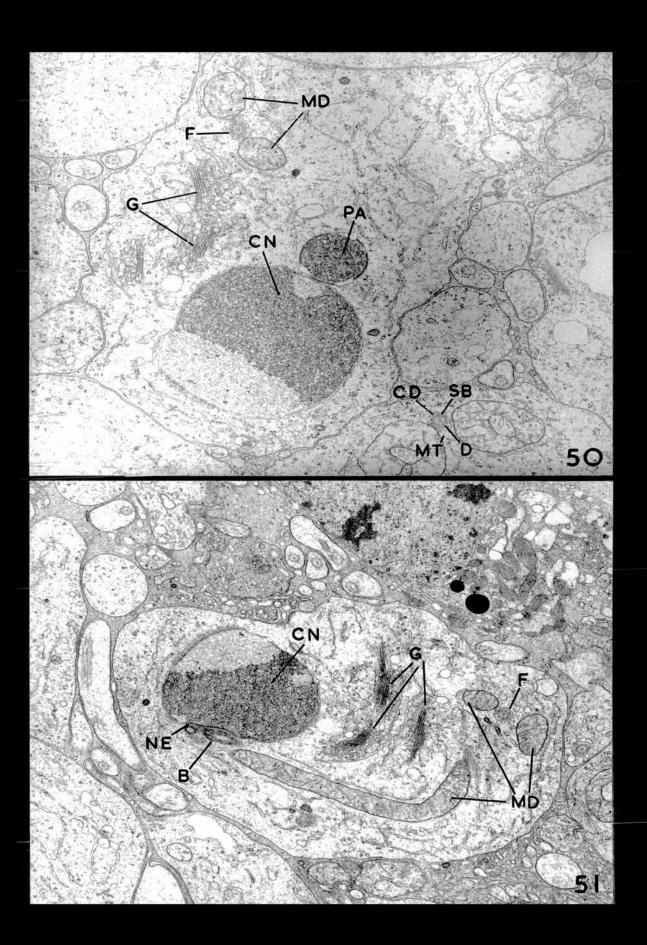
- Fig. 45. Longitudinal section through part of a testis to show that early spermatids (SD) are not specifically arranged within a cyst and that stage 2 spermatids (SD²) are situated at the periphery of a cyst. Stage 3 spermatid nuclei are arranged in a 'horseshoe configuration' (H) at the periphery of the cyst and the tails (T) are directed away from the 'horseshoe' formation. Stained with Heidenhain's haematoxylin. Bright field microscopy. x 600.
- Fig. 46. Stage 2 spermatid nuclei are spherical with densely stained chromatin (DN) adjacent to the nuclear envelopes. Feulgen squash preparation and bright field microscopy. x 1,400.
- Fig. 47. Transverse section through the nebenkern (NK) of an early spermatid which has been formed by the aggregation of the mitochondria. x 16,000.



- Fig. 48. A similar view to Fig. 47 showing the nucleus (N) and the nebenkern (NK). An early stage in the development of a flagellum (F) with a 9 + 2 arrangement of tubules is also present. x 10,000.
- Fig. 49. Section through several early spermatids showing their irregular shape. Numerous vesicles (V) and a Golgi complex (G) are present in the cytoplasm. In each nucleus (N) the densely stained chromatin mainly lies near the nuclear envelope. The nebenkern (NK) is closely situated to the nucleus. x 10,000.



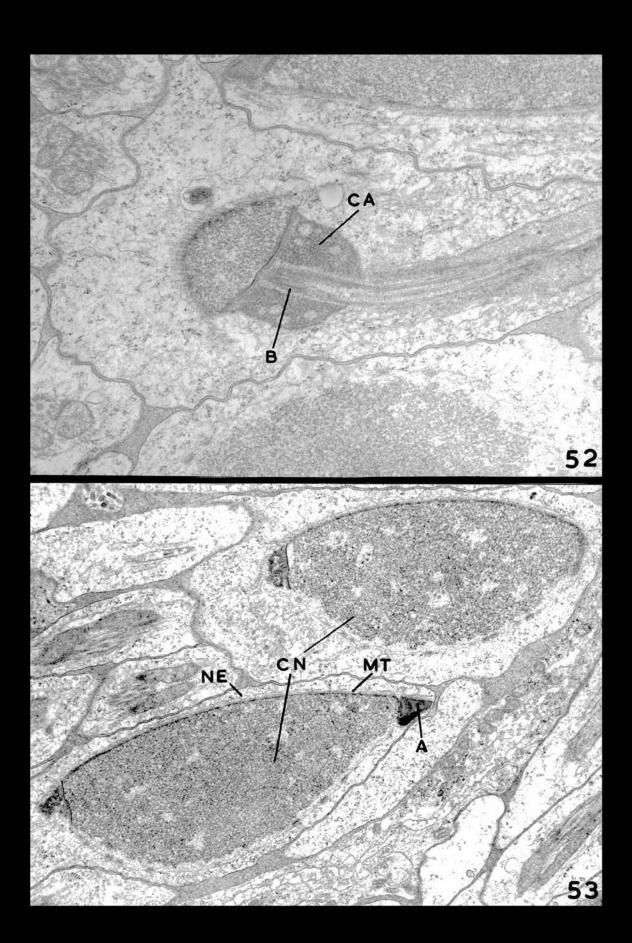
- Fig. 50. The chromatin (CN) in stage 3 spermatids is more condensed than in earlier stages and a proacrosomal (PA) granule is situated close to the nucleus. Several Golgi bodies (G), two mitochondrial derivatives (MD) and a flagellum (F) are present in the cytoplasm. The flagellum contains a 9 + 2 arrangement of microtubules and a curved structure (CD) extends from subfibre B (SB) of each doublet (D). Microtubules are present in the vicinity of the flagellum and mitochondrial derivatives. x 20,000.
- Fig. 51. A section through a similar stage to Fig. 50 but passing through a region of the nuclear envelope (NE) which has a densely stained material clumped against it. Adjacent to this region there is a basal body (B). Chromatin (CN), Golgi bodies (G), mitochondrial derivatives (MD) and a flagellum (F) are also present. x 16,000.



- Fig. 52. An oblique section through the base of a nucleus in a stage 5 spermatid passing through the fibrous centriole adjunct (CA) which surrounds the basal body (B) of the flagellum. x 30,000.
- Fig. 53. A longitudinal section through two stage 5 spermatids.

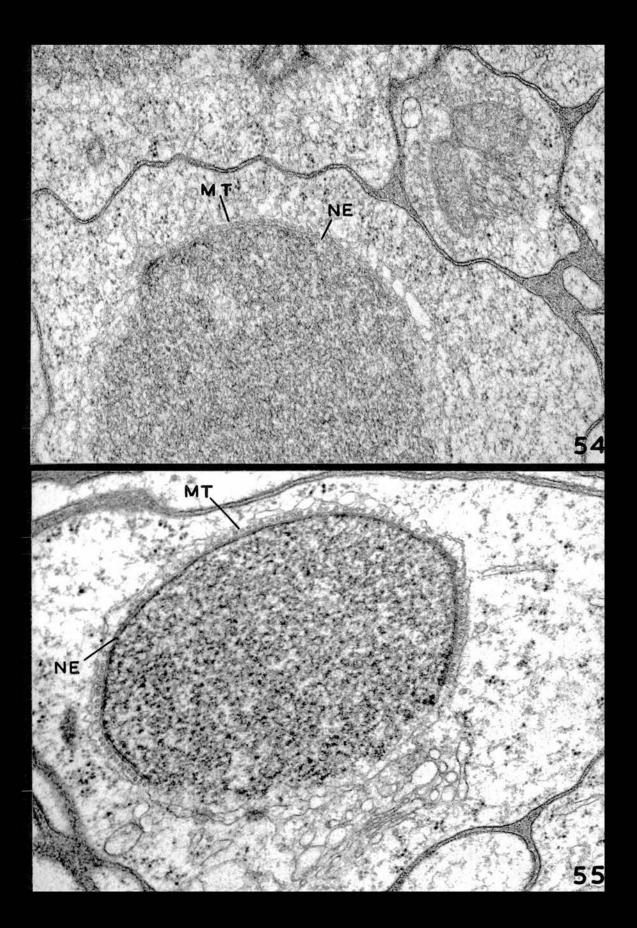
 Densely stained chromatin (CN) fills most of each
 nucleus. Microtubules (MT) lie closely against the
 outside of the nuclear envelope (NE) in regions where
 there are densely stained deposits on the inner surface
 of the envelope. At the anterior tip of the nucleus an
 acrosome (A) is closely associated with the nucleus.

 x 20,000.

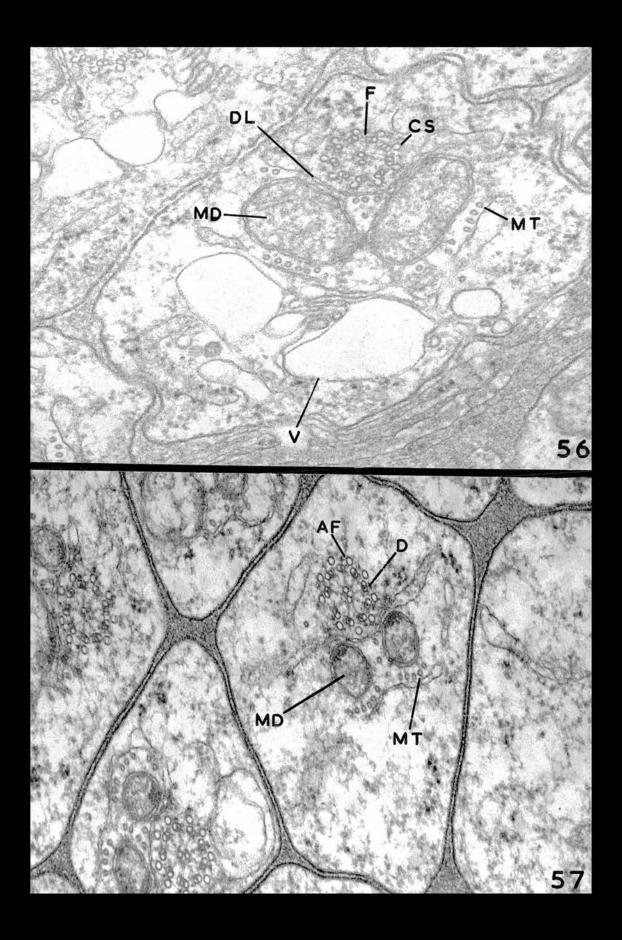


- Fig. 54. Transverse section through part of a nucleus to show the microtubular (MT) associations with the nuclear envelope (NE) in stage 5 spermatids. x 50,000.
- Fig. 55. A similar transverse section through a nucleus of a stage 6 spermatid shows that the densely stained regions of the nuclear envelope have extended along the internal surface of the nuclear envelope (NE). The microtubules (MT) increase to the same extent on the external surface of the nuclear envelope. x 60,000.

: - E



- Fig. 56. A sperm tail of a stage 5 spermatid transversely cut to show the spatial arrangement of the flagellum (F) and the mitochondrial derivatives (MD). The curved structures found in earlier stages of spermatids are now C-shaped (CS). A flattened membrane-bounded vesicle (DL) separates the flagellum (F) from the mitochondrial derivatives (MD). Several microtubules (MT) can be seen in the vicinity of the flagellum and mitochondrial derivatives. There are many vesicles (V) in the cytoplasm. x 80,000.
- Fig. 57. In stage 6 spermatid tails the C-shaped structures have separated from the doublets (D) and formed accessory fibres (AF). The mitochondrial derivatives (MD) are smaller than they are in stage 5 spermatids. In each derivative there is a densely stained region. Microtubules (MT) are present in the vicinity of the Aderivatives and flagellum. x 80.000.



- Fig. 58. Longitudinal section through part of a testis showing a group of stage 4 spermatids (SD⁴) with their nuclei adjacent to the epithelial border (E). Spermatids at stage 2 (SD²) and 3 (H) are also present in this section. x 600.
- Fig. 59. A Feulgen squash preparation of stage 5 (SD⁵) nuclei shows that the densely stained chromatin (CN) fills most of each nucleus. Bright field microscopy. x 1,400.
- Fig. 60. Stage 5 spermatids (SD⁵) aligned along the epithelial border of a testis with their nuclei filled with densely stained chromatin (CN). Stained with Heidenhain's haematoxylin. Bright field microscopy. x 600.
- Fig. 61. As the chromatin condenses in stage 7 spermatids the nuclei become spindle-shaped (IS). x 600.

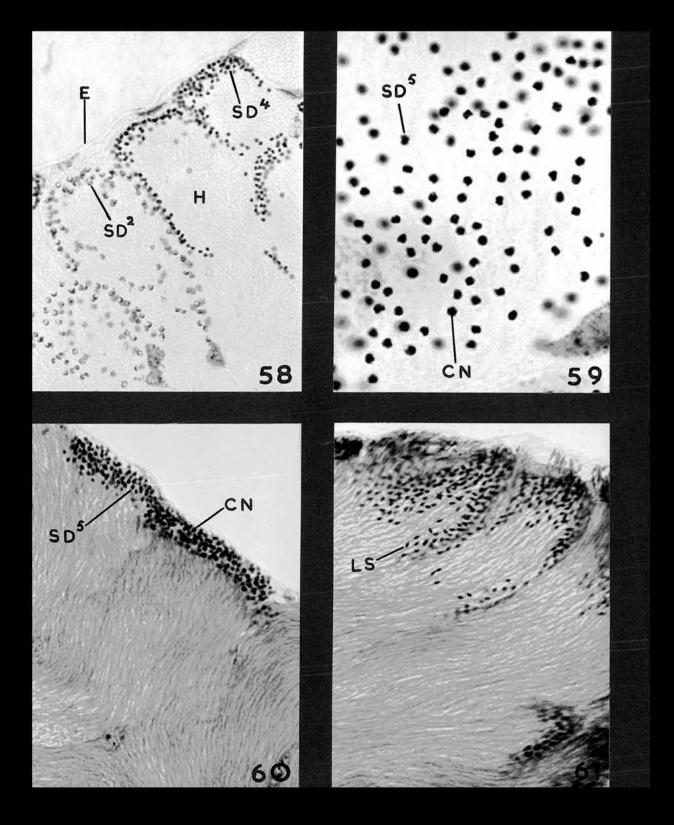


Fig. 62. Transverse section through several stage 7 spermatids.

In the nuclei there are many less dense areas and there are only small regions of nuclear envelope (NE) which are not densely stained. Within a cup-shaped indentation of each nucleus a centriole (C) and mitochondrial derivatives (MD) are situated. Microtubules (NT) are associated with regions of nuclear envelope (NE) which are densely stained. A double membrane (DE) surrounds the flagellum (F), mitochondrial derivatives (MD) and granular area (GA). There are several microtubules (NT) in the vicinity of these components. x 50,000.



- Fig. 63. The nuclei are more flattened in stage 8 spermatids than they are in stage 7 spermatids and the chromatin (CN) is more condensed. The granular area (GA) is now a V-shaped rib. The accessory fibres (AF) are filled with a densely stained material. x 16,000.
- Fig. 64. In late spermatid nuclei the chromatin (CN) is more condensed than in earlier spermatids. Microtubules (MT) are still associated with the nuclei. x 32,000.

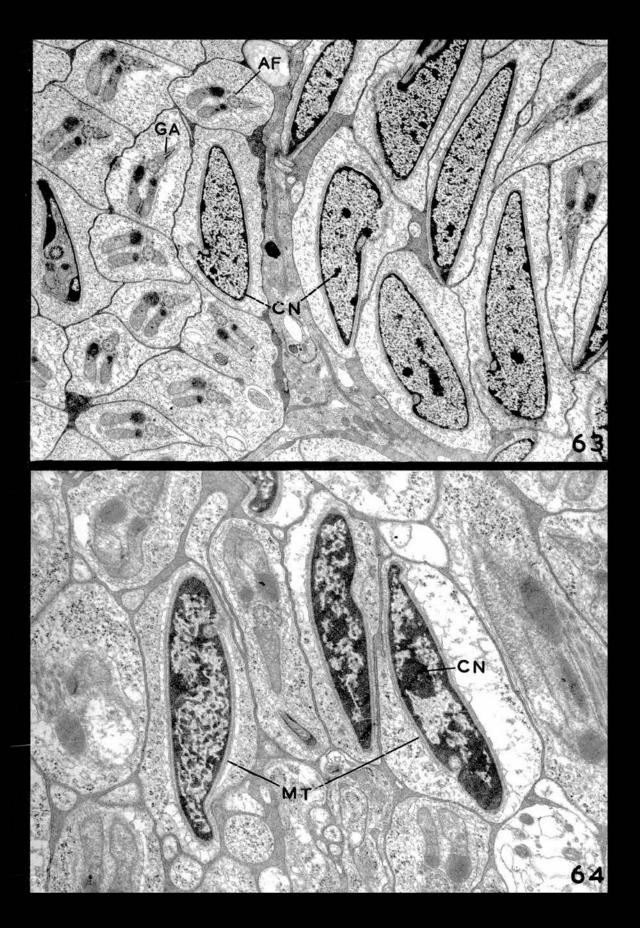
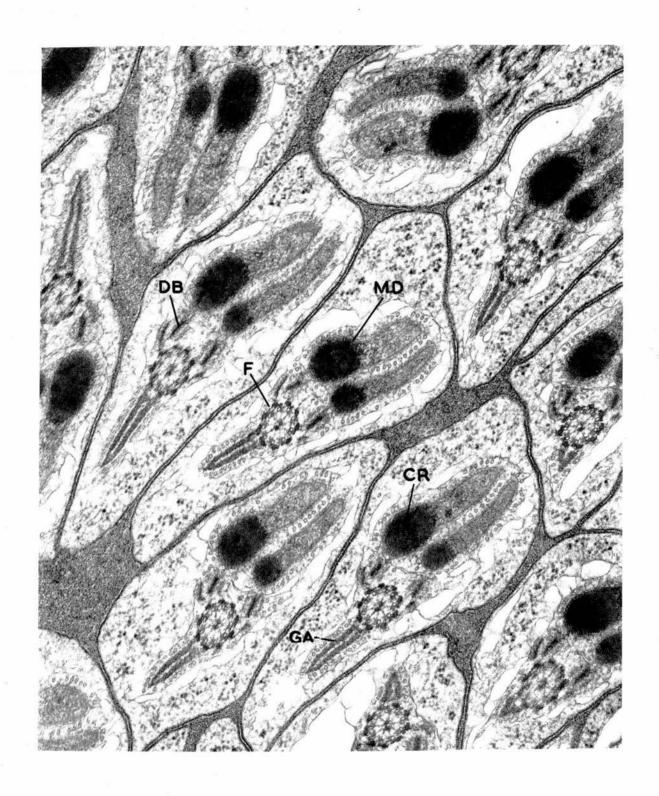


Fig. 65. A transverse section through late spermatid tails shows
a V-shaped rib (GA), two densely stained bars (DB),
a flagellum (F) and mitochondrial derivatives (MD)
and that the region of densely stained material in the MITOCHONDRIAL
derivatives is cross-striated (CR). x 50,000.



- Fig. 66. The nucleus of a spermatid at a later stage than in

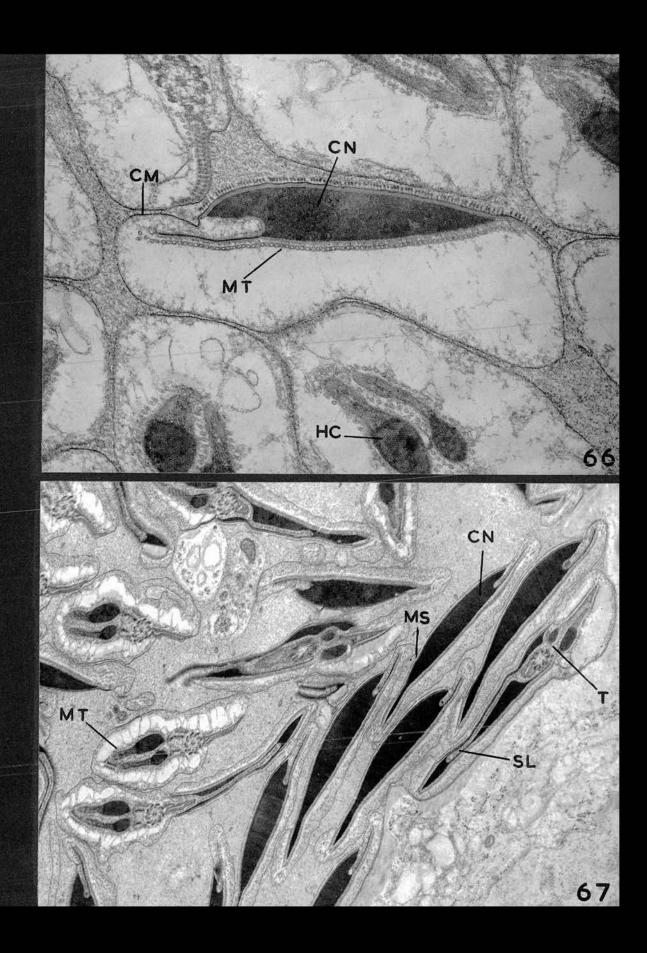
 Fig. 65 is shaped like a mature sperm head. The

 chromatin (CN) is fully condensed and evenly distri
 buted. The cell membrane (CM) is closely associated

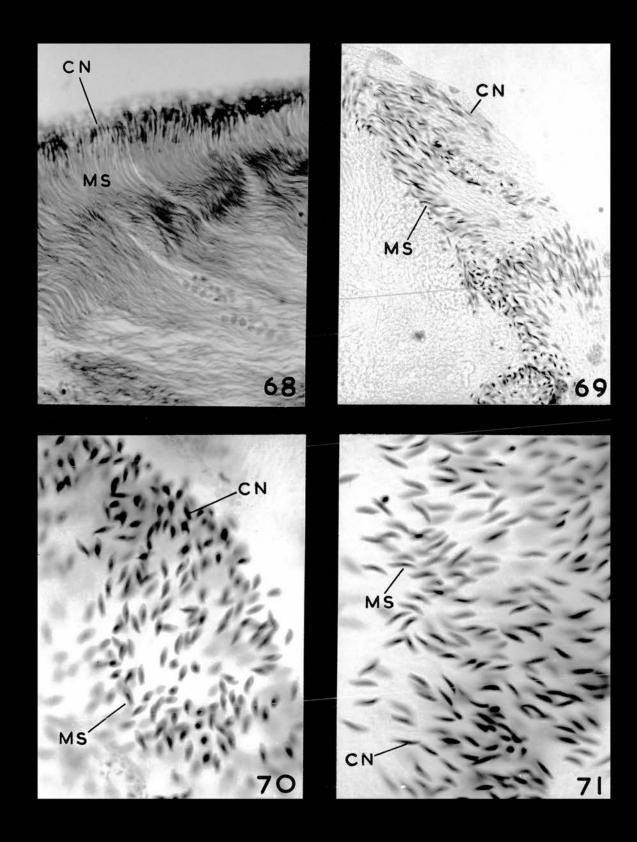
 with part of the nucleus while microtubules (MT) are

 associated with other parts of the nucleus. Mitochon
 drial derivatives exhibit a honey-comb-like pattern

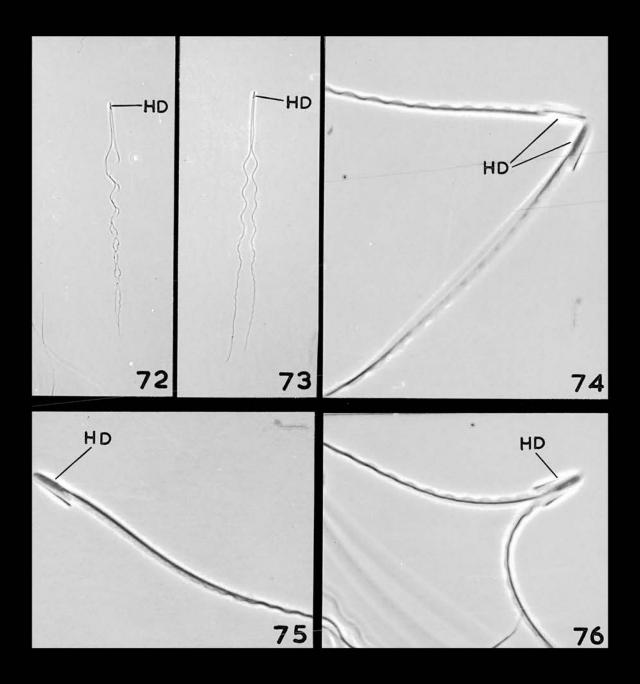
 (HC). x 60,000.
- Fig. 67. Transverse section through several mature sperm (MS) from part of a testis. The nuclei are filled with densely stained chromatin (CN). At certain levels in the nuclei slit-like (SL) regions open out into the tail area (T). Microtubules (MT) can still be seen in the tails. Testicular sperm are not paired. x 20,000.



- Fig. 68. A longitudinal section through part of a testis showing the densely stained chromatin (CN) in the nuclei of mature sperm (MS). Testicular sperm are still arranged in groups and their tails are in a wave form. Stained with Heidenhain's haematoxylin. Bright field microscopy. x 600.
- Fig. 69. A section of part of a testis treated with Feulgen reagent to show that the densely stained chromatin (CN) in mature sperm (MS) nuclei is Feulgen positive. x 600.
- Fig. 70. In a Feulgen treated squash preparation the mature sperm (MS) nuclei are spindle-shaped and the chromatin is Feulgen positive. x 1,400.
- Fig. 71. A similar view to Fig. 70 showing that the mature sperm (MS) nuclei are composed of Feulgen positive chromatin. x 1,400.



- Figs. 72-76 are photographs of live sperm from <u>Acilius</u> vasa deferentia using phase contrast microscopy.
- Fig. 72. A pair of sperm with their heads (HD) closely associaated. The initial segment in the anterior region of each tail is rigid and the posterior portion has a coarse helical shape. x 600.
- Fig. 73. A similar photomicrograph showing a pair of sperm with their heads (HD) closely associated. The initial segments are rigid and the posterior portions have a helical shape. The end piece is pointed. x 600.
- Fig. 74. A pair of loosely associated sperm heads viewed from above and the side. The flagellar helices appear as short waves. x 1,400.
- Fig. 75 Single sperm showing the shape of the head (HD) and the flagellar helices in the initial segment of the tail. x 1,400.
- Fig. 76. The apex of one sperm head (HD) is at a slightly different level from the other one. The flagellar helices are present as short waves in the initial segment of the tail. x 1,400.



- Fig. 77. Squash preparation treated with Feulgen reagent to show that the heads in mature sperm from vasa deferentia are composed mostly of Feulgen positive nuclear material. x 1,400.
- Fig. 78. Sperm from vasa deferentia place in liquid paraffin form whorls of sperm. The sperm heads (HD) are closely associated and the sperm move forwards with the flattened sides of the heads in a horizontal position. x 600.

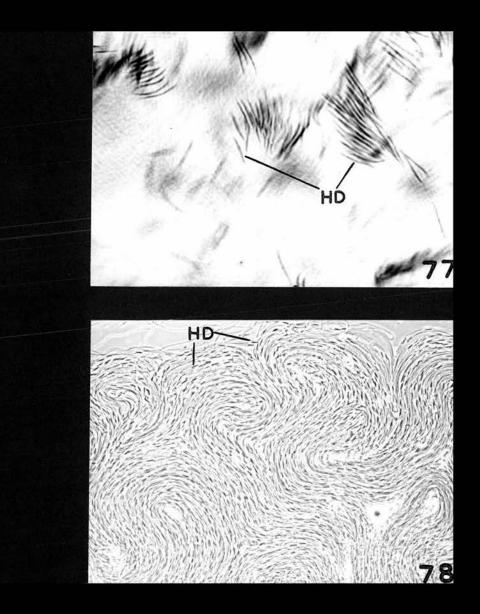


Fig. 79. A schematic diagram of a mature Acilius sperm illustrating the main components of the sperm. Diagrams of transverse sections at certain levels are also illustrated and their levels are indicated. An acrosome (A) is attached to the anterior tip of the head and there is a groove (GR) in the short side. An invagination (SI) occurs in the ventral surface of the head. The initial segment of the tail contains a 'microtubular border' (MB), flagellum (F) and two mitochondrial derivatives (MD) of mnequal size. The 'microtubular border' disappears at approximately 55 µm and the posterior portion begins. There is an apparent reduction in the size of the mitochondrial derivatives (MD) along the length of the posterior portion and they terminate approximately 320 µm from the head. The end piece contains only the flagellum (F).

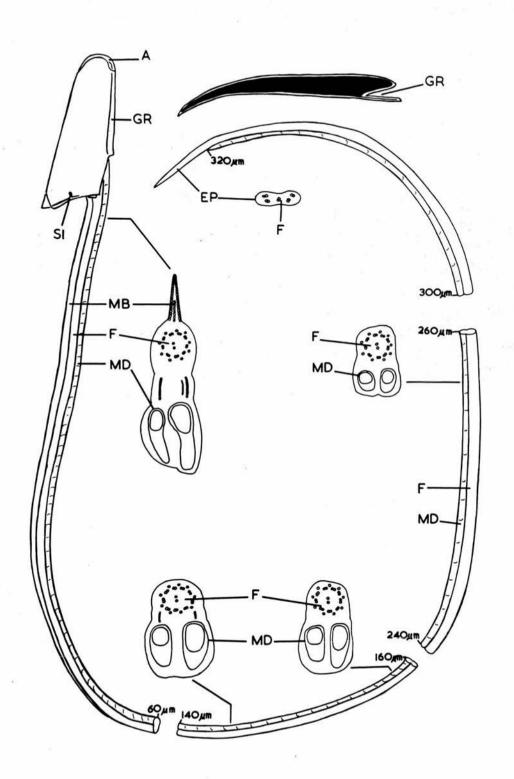


Fig. 80. Schematic diagram of an <u>Acilius</u> sperm head to illustrate the shape of the head and its special features and the position of the tail in relation to the head. Diagrams 1-8 represent transverse sections through a head and their levels are indicated in the diagram of the head. The features of the head which are illustrated are the acrosome (A), nucleus (N), groove (GR), centriole adjunct, (CA), mitochondrial derivatives (MD), hood (HO), flagellum (F), an invagination (SI) and microtubules (MT).

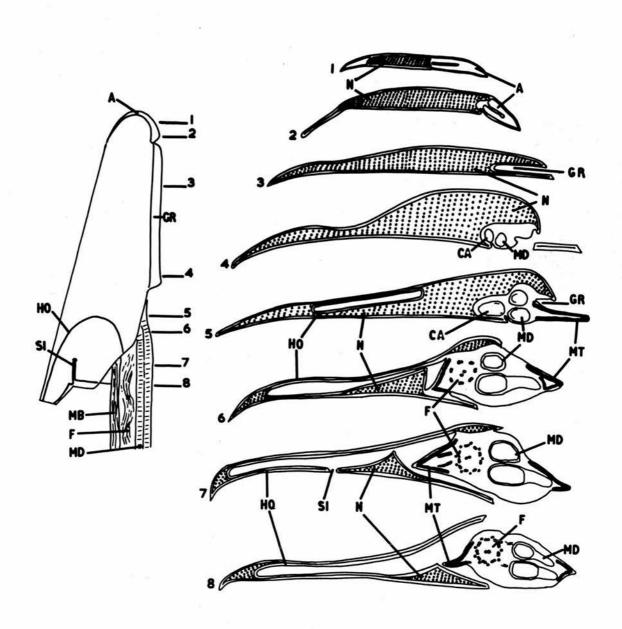
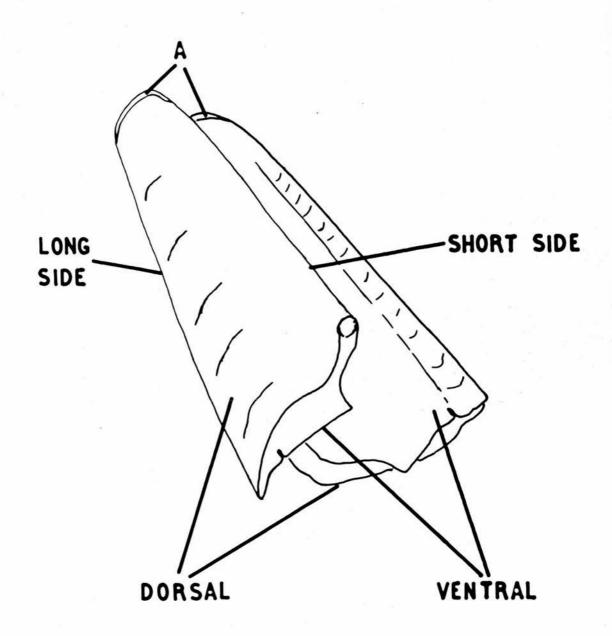
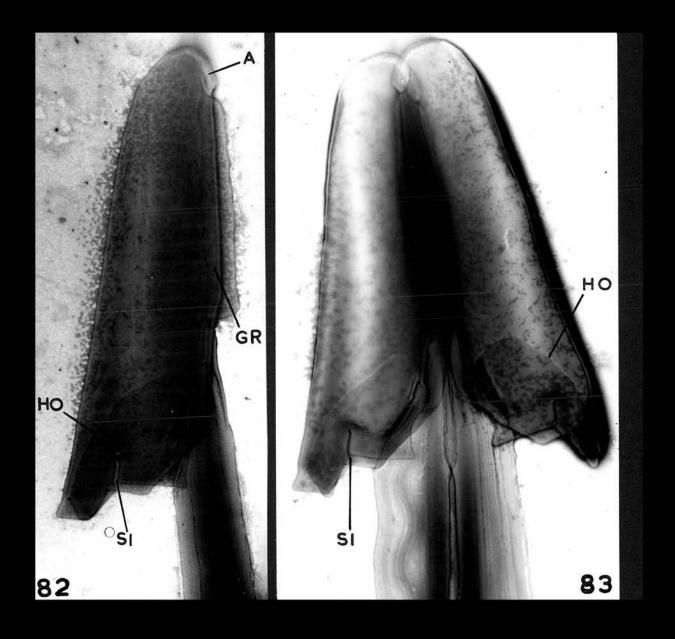


Fig. 81. A schematic diagram to illustrate the terms which I have used to describe the highly asymmetrical shape of the head. The surfaces of the head which are always apposed to each other are ventral and the acrosome is situated at the anterior of each head. The tail extends out from the short side.

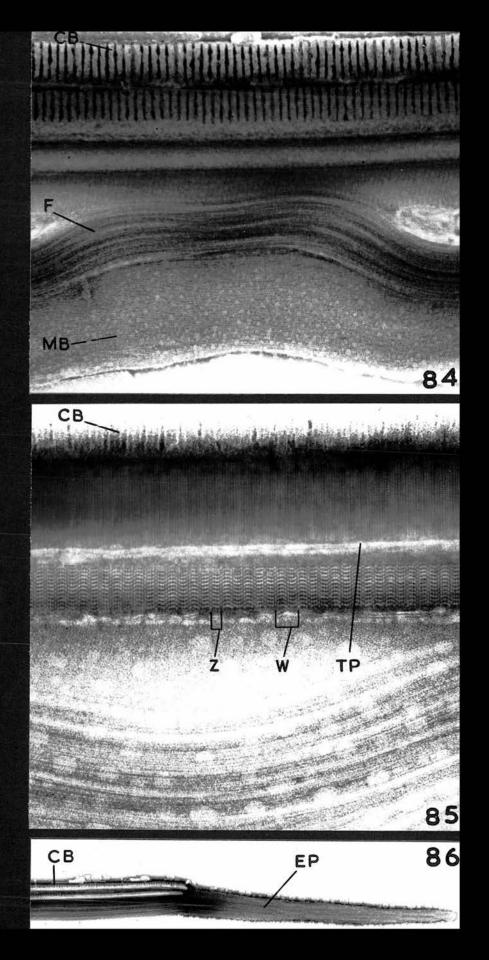


- Fig. 82. An electron micrograph of a negatively stained sperm head showing the outline of the head with the groove (GR) on the short side and an invagination (SI) in the posterior region of the head. The acrosome (A) is outlined at the anterior of the head. The cavity formed by the hood (HO) can be seen in the posterior region of the head. x 15,000.
- Fig. 83. A pair of negatively stained sperm heads showing that the apex of one sperm head is at a different level from the other one. The hoods (H) and invaginations (SI) are visible in the posterior region of the heads. x 12,500.



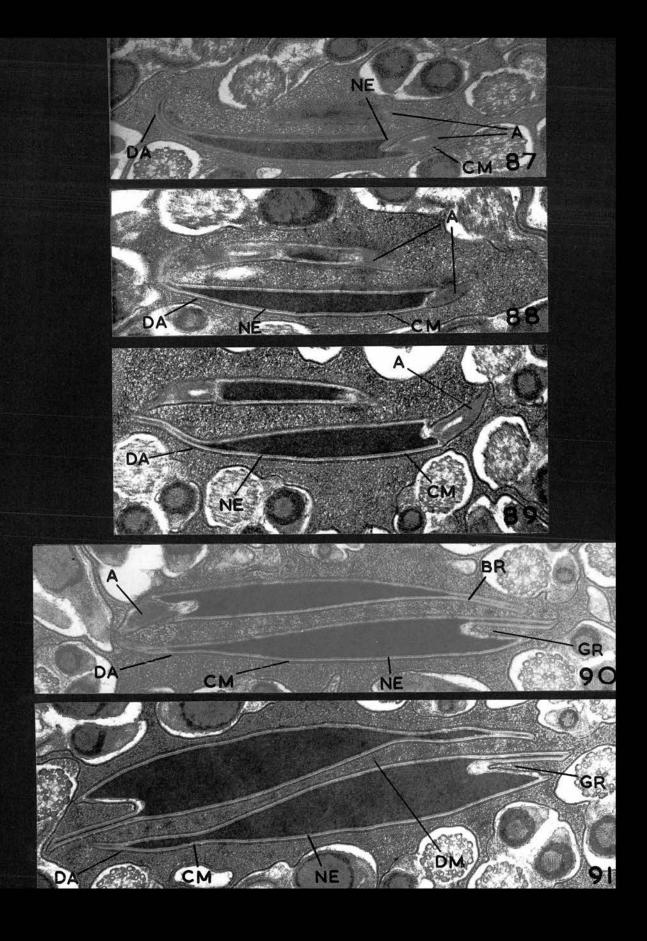
- Fig. 84. Part of the initial segment of a negatively stained tail showing the 'microtubular border' (MB), flagellum (F) and cristae (CB) in the mitochondrial derivatives.

 x 50,000.
- Fig. 85. Some mitochondrial derivatives exhibit zig-zag (Z) arrays, wave (W) arrays and a transverse periodicity (TP). The cristae (CB) are spaced at regular intervals. x 100,000.
- Fig. 86. A region of posterior portion and an end piece of a sperm tail. The beginning of the end piece is clearly marked by the termination of the mitochondrial derivatives in the posterior portion. Cristae (CB) are present along MITOCHONDRIAL the length of the derivatives. x 12,500.



- Figs. 87-91 are electron micrographs of transverse sections through paired sperm heads.
- Fig. 87. The acrosome (A) is granular and is bound by the cell membrane (CM) and separated from the head by the nuclear envelope (NE). There is a densely stained material (DA) coating the outside of the cell membrane. x 60,000.
- Fig. 88. A similar section to Fig. 87 but cut at a more posterior level. x 60.000.
- Fig. 89. A section through a more posterior level than Fig. 88. x 60,000.
- Fig. 90. The groove (GR) in the short side of the head appears at a level below the posterior region of the acrosome. The densely stained material (DA) which coats the cell membrane (CM) appears as short parallel bars (BR) in some cases. x 60,000.
- Fig. 91. The sperm heads are surrounded by the cell membrane (CM) and nuclear envelope (NE) and a densely stained material (DA) coats the outside of the cell membrane. Between the heads there is a layer of coarse granular material.

 x 60,000.

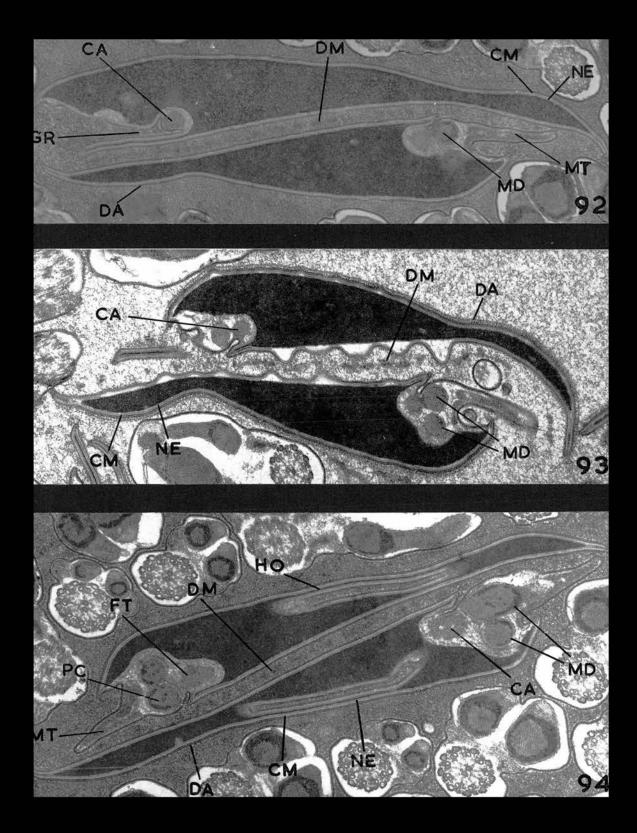


Figs. 92-94 are electron micrographs of transverse sections through paired sperm heads. The heads are enclosed by the cell membrane (CM) and nuclear envelope (NE) and a densely stained material (DA) coats the outside of the cell membrane. There is a layer of coarse granular material between the heads. The position of the tail in relation to the head can be seen.

- Fig. 92. At the anterior tip of each tail there is a centriole adjunct (CA) and one mitochondrial derivative (MD).

 NITOCHONDRIAL
 Adjacent to the derivative, microtubules (MT) are present. x 60,000.
- Fig. 93. There are two mitochondrial derivatives (MD) in sections which are cut at a more posterior level than in Fig. 92.

 The centricle adjunct is still visible. The coarse granular material (DM) between the heads is clearly shown in this section. x 60,000.
- Fig. 94. Sections through the anterior region of the hood (HO) appear as slits in the head. Flagellar tubules (FT) are present in the granular material of the centricle adjuncts (CA) and there is a paracrystalline (PC) material in the mitochondrial derivatives (MD). x 60,000.



Figs. 95 and 96 are transverse sections through the posterior regions of paired sperm heads showing the cell membrane (CM), nuclear envelope (NE), densely stained material (DA) coating the outside of the cell membrane and the hood (HO). The tails contain a 'microtubular border' (MB), flagellum (F), mitochondrial derivatives (MD) and microtubules (MT) adjacent to the derivatives.

- Fig. 95. The hood is shown opening out into the tail region with the tail attached to the head and when it has separated from the head. A lobe (LO) of granular material extends out from each mitochondrial derivative and one derivative is larger than the other in each tail. Accessory fibres (AF) are present in the anterior region of the flagellum. x 60,000.
- Fig. 96. A similar view to Fig. 95 showing the invagination (SI) in the ventral surface of one head. Accessory fibres (AF), doublets (D) and central tubules (CT) are clearly visible in the flagellum (F). Linkages (LK) between the doublets and central tubules are present as well as coarse fibres (CF) between the accessory fibres.

 x 60,000.

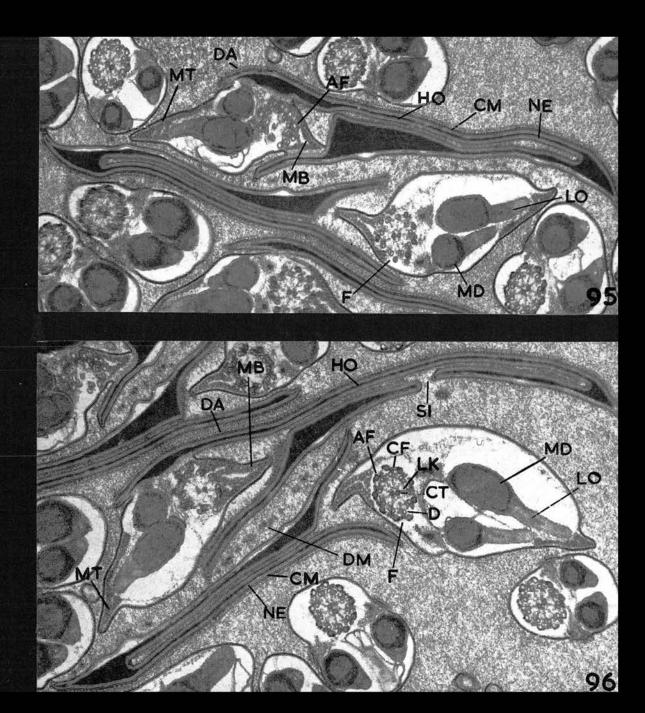
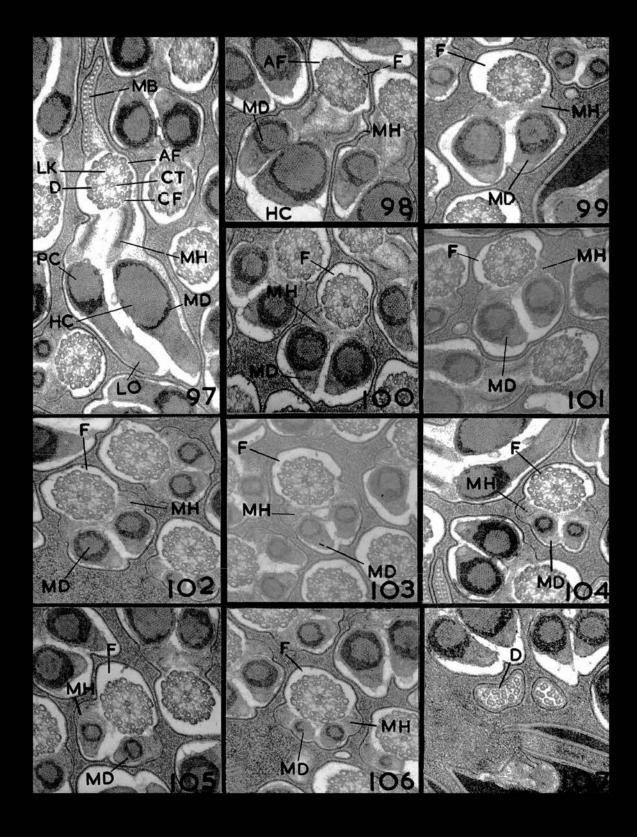


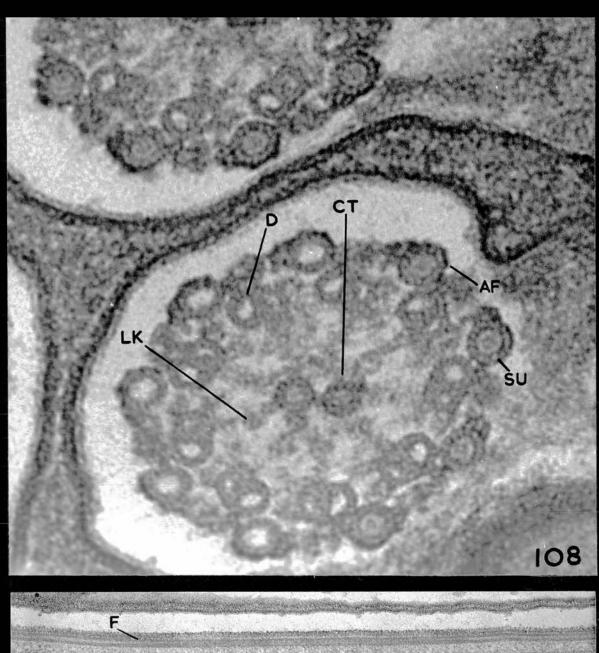
Fig. 97. Transverse section through an initial segment showing the 'microtubular border' (MB), flagellum, membranous sheath (MH) and the mitochondrial derivatives (MD). Accessory fibres (AF), doublets (D), central tubules (CT), coarse fibres (CF) and linkages (LK) are present in the flagellum. The membranous sheath is positioned between the flagellum and the derivatives. The paracrystalline material (PC) in the mitochondrial derivatives has a honey-comb (HC) pattern. The lobes (LO) of material extending from the MITOCHONDRIAL derivatives corresponds to the region where cristae are present. x 60,000.

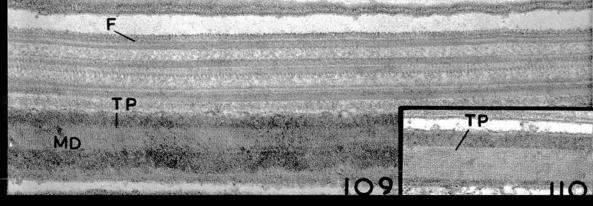
Figs. 98-106 are transverse sections through the posterior portion of the tail from the anterior to the posterior. The closer the mitochondrial derivatives (MD) are to the end piece the smaller they become. The flagellum (F) remains the same size. There is also a reduction in the size of the membranous sheath (MH) along the length of the posterior portion.

- Fig. 98. The accessory fibres (AF) in the anterior region of the posterior portion all contain central elements. x 60,000.
- Fig. 99. The central element in accessory fibre 8 has terminated. x 60,000.
- Fig. 100. This view shows that the central elements in accessory fibres 7, 8, and 9 are missing. x 60,000.
- Fig. 101. A similar view to Fig. 100 showing that the central element in accessory fibre 6 is also missing. x 60,000.
- Fig. 102. The central elements in 5 of the accessory fibres have terminated in this section. These fibres are number 1, 6, 7, 8 and 9. x 60,000.
- Fig. 103. A similar view to Fig. 102 with 6 accessory fibres without central elements. These fibres are in positions 1, 5, 6, 7, 8 and 9. x 60,000.
- Fig. 104. Accessory fibres 3 and 4 are the only accessory fibres which have a central element present. x 60,000.
- Fig. 105. The last accessory fibre to lose its central element is number 4. x 60,000.
- Fig. 106. A view showing all the accessory fibres without central elements. x 60,000.
- Fig. 107. Transverse section through two end pieces showing 9 doublets (D) in one end piece and the separation of the doublets in the other end piece. x 60,000.



- Fig. 108. A high power micrograph showing the fine structure of the accessory fibres (AF), doublets (D), central tubules (CT) and the linkages (LK) between the doublets and central tubules. The profiles of the subunits (SU) are clearly visible. x 400,000.
- Fig. 109. A longitudinal section through a tail showing the transverse periodicity (TP) in the mitochondrial derivative (MD) and the flagellum (F). x 75,000.
- Fig. 110. Part of a longitudinally cut mitochondrial derivative showing the transverse periodicity (TP) in the paracrystalline material. x 75,000.





- Fig. 111. Transverse section through the posterior region of a pair of heads showing the invagination (SI) positioned at the anterior of the curved edge. x 60,000.
- Fig. 112. The layer of coarse granular material (DM) found between most paired heads is clearly shown in this transverse section. x 60,000.

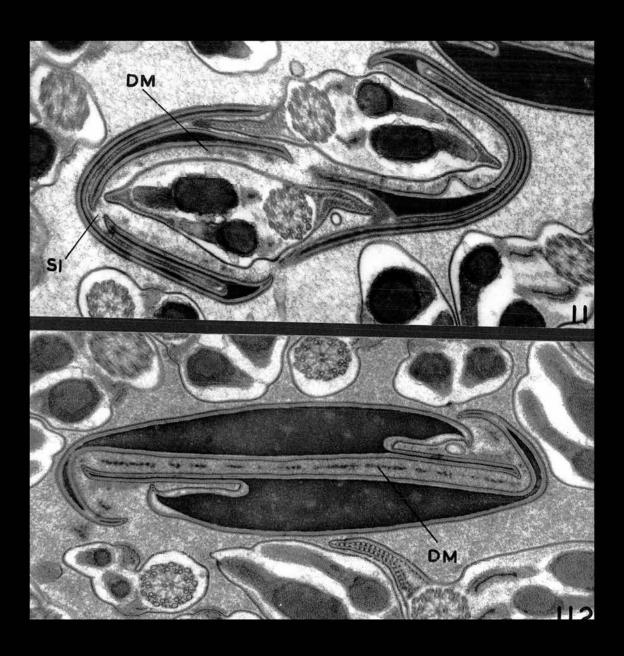
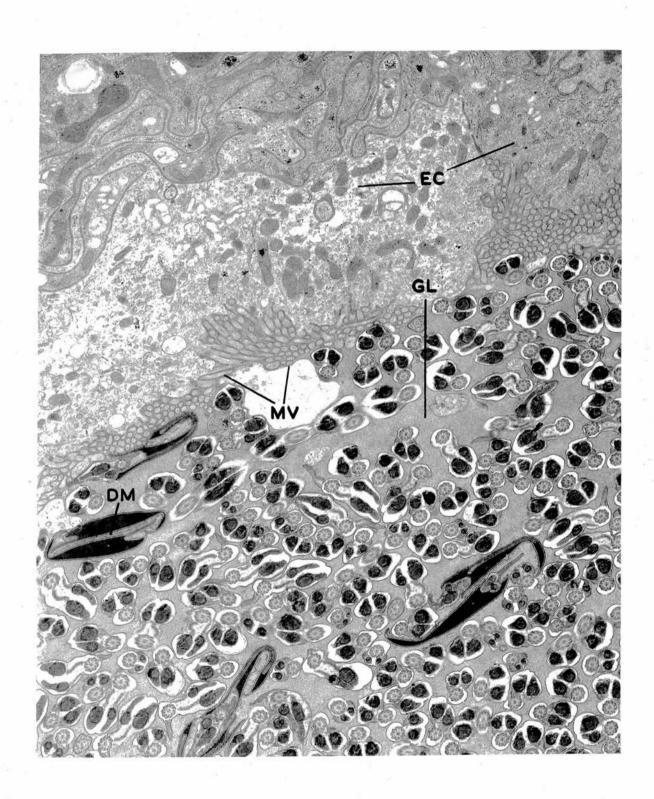
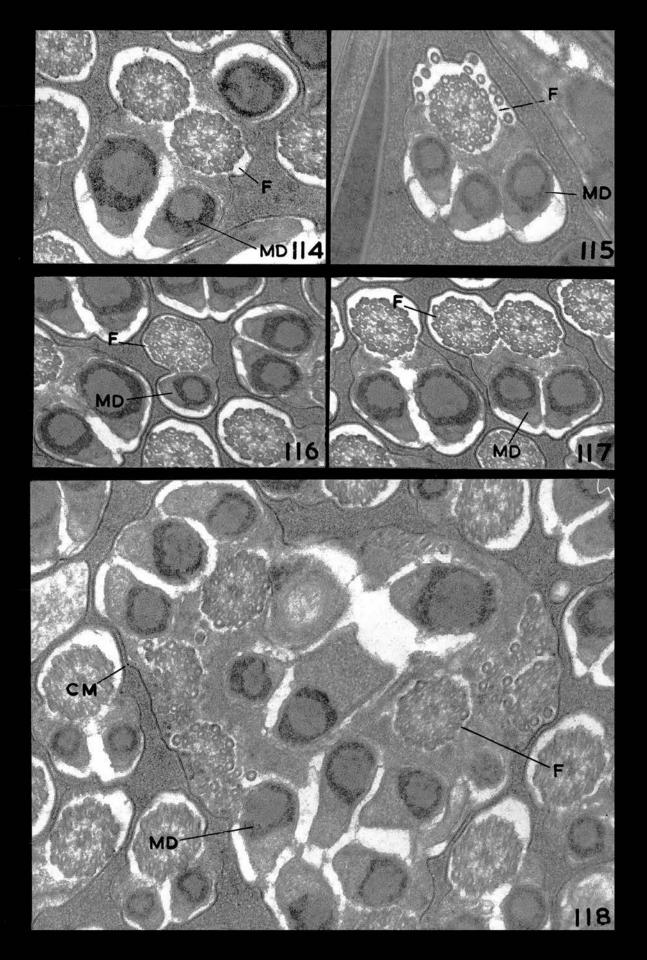


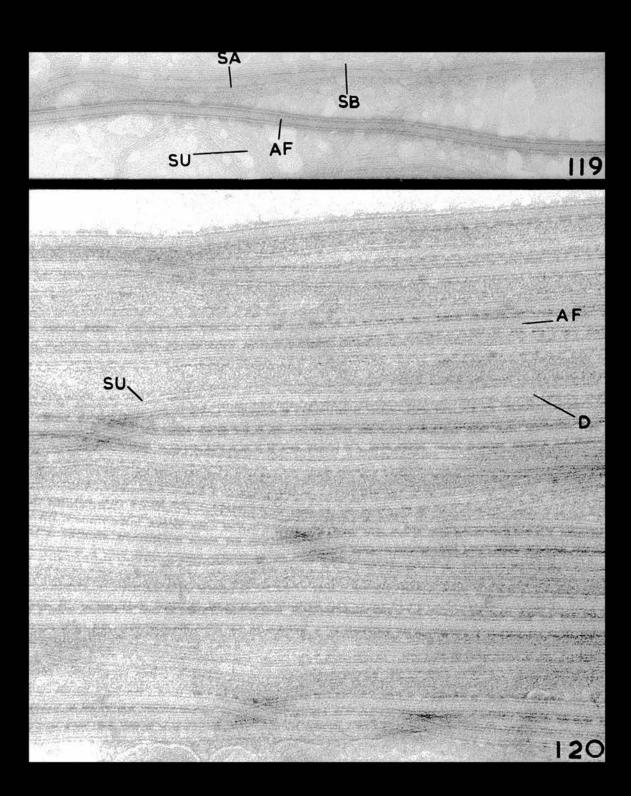
Fig. 113. Transverse section through part of a vas deferens showing the epithelial cells (EC) and the short, irregular microvillii (MV) which surround the lumen. A granular material (GL) of medium electron density occupies the space between sperm in the lumen. The coarse granular material (DM) between paired heads is also present in this section. x 15,000.



- Fig. 114. A transverse section through a tail containing 2 flagella (F) and three mitochondrial derivatives (MD). x 80,000.
- Fig. 115. Transverse section showing a tail with three mitochondrial derivatives (MD) and a flagellum (F). x 80,000.
 - Fig. 116. A sperm tail with a flagellum (F) and one mitochondrial derivative (ND). x 80,000.
 - Fig. 117. A transverse section through a tail containing an extra flagellum (F). x 80,000.
 - Fig. 118. Several disrupted tails are contained within the same cell membrane (CM) in this transverse section. x 80,000.



- Fig. 119. Part of a flagellar axoneme which has been treated with sodium lauryl sulphate and then negatively stained showing an accessory fibre (AF) and subfibres A (SA) and B (SB) of a doublet. The subunits (SU) can be distinguished in the accessory fibres and doublets. x 60,000.
- Fig. 120. A view of part of a flagellum treated with sodium lauryl sulphate and then negatively stained to show that the doublets (D) are more sensitive than the accessory fibres (AF). The subunits (SU) in the accessory fibres and doublets can be clearly distinguished. 140,000.



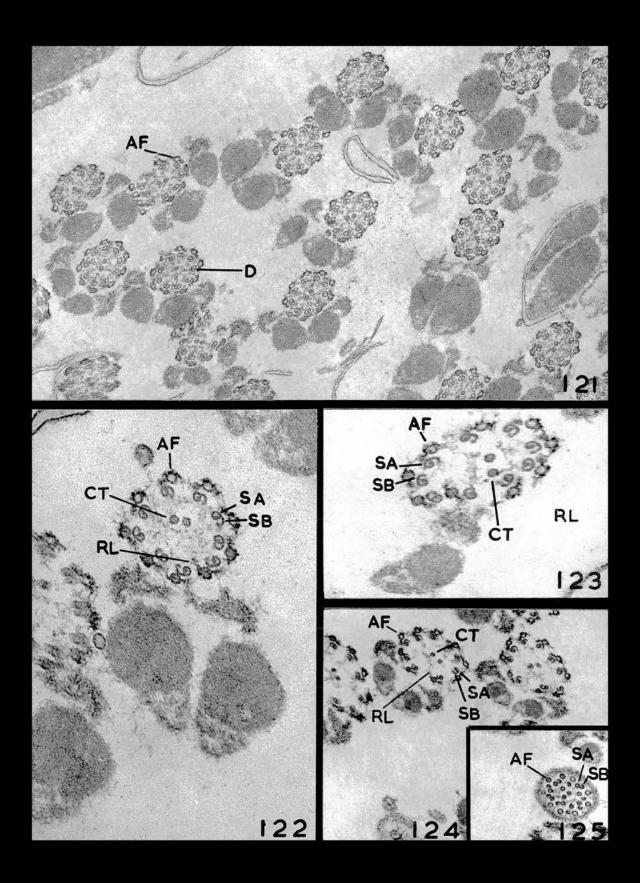
- Fig. 121. In 0.25% Sarkosyl treated sperm the cell membrane surrounding the tails disintegrates and some of the
 accessory fibres (AF) are displaced from their normal
 position adjacent to the doublets (D). x 60,000.
- Fig. 122. Transverse section through a tail which has been treated with 1% Sarkosyl showing some doublets with subfibre B (SB) partially separated from subfibre A (SA). The central tubules (CT) are displaced from their central position and the radial links (RL) are indistinct.

 x 120,000.
- Fig. 123. A similar view to Fig. 122 with the central tubules even more displaced than they are in Fig. 122.

 x 120,000.
- Fig. 124. A similar view to Figs. 122 and 123 showing the partial separation of the doublets and the displacement of the central tubules (CT). x 60,000.
- Fig. 125. A transverse section through an end piece treated with

 1% Sarkosyl showing the separation of subfibres A (SA)

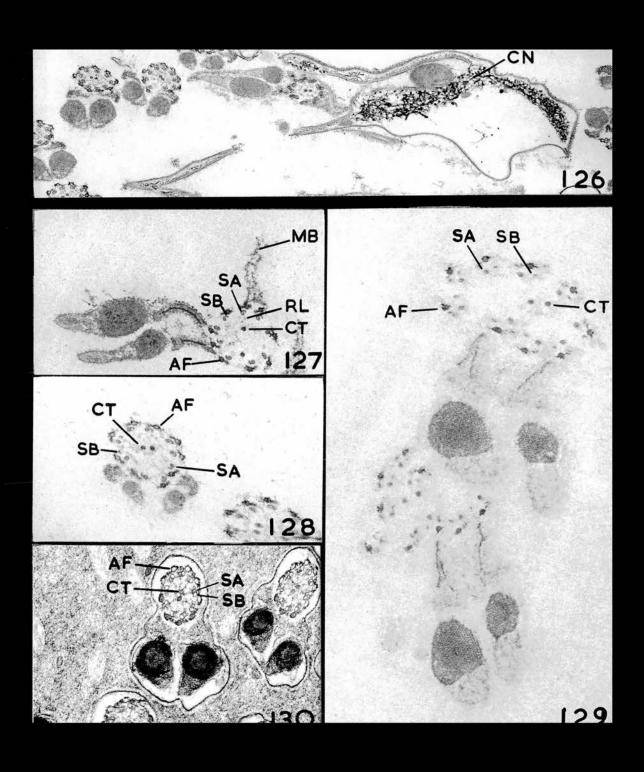
 and B (SB) in the doublets at a more anterior level than
 they would be in an untreated end piece. x 60,000.



- Fig. 126 The chromatin (CN) in heads treated with 1% Sarkosyl has some fibrous material associated with it. x 40,000.
- Fig. 127. Transverse section through the initial segment of a tail treated with 1.5% Sarkosyl showing a further deterioration in the structure of subfibres A (SA) and B (SB), accessory fibres (AF), central tubules (CT) and the 'microtubular border' (MB), x 60,000.
- Fig. 128. In the posterior portion subfibre B (SB) has separated completely from subfibre A (SA) in some doublets. The central tubules (CT) are not central and the accessory fibres (AF) are not as distinct as they are in sperm treated with weaker concentrations of Sarkosyl.

 x 60,000.
- Fig. 129. In flagellar axonemes which have been treated with 2% Sarkosyl subfibres A (SA) and B (SB) in all the doublets are completely separate from each other. The radial links are absent and the central tubules (CT) are no longer central. x 60,000.
- Fig. 130. Control specimen showing the normal content and arrangement of the accessory fibres (AF), central tubules (CT) and subfibres A (SA) and B (SB) in the doublets.

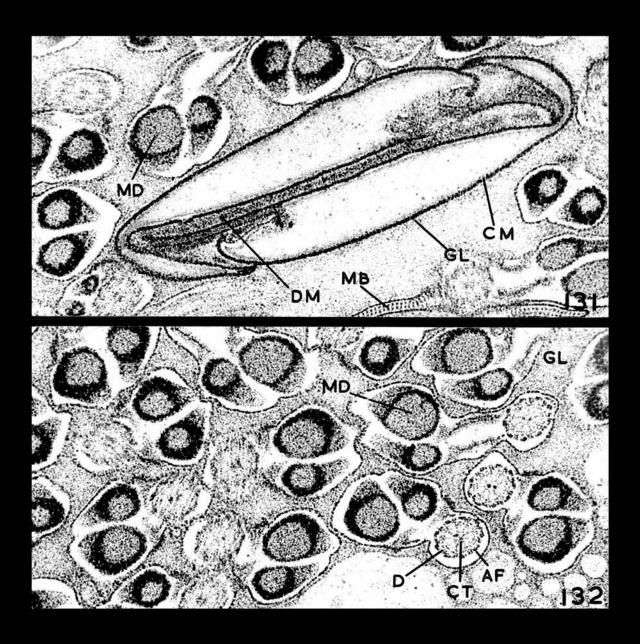
 x 60,000.



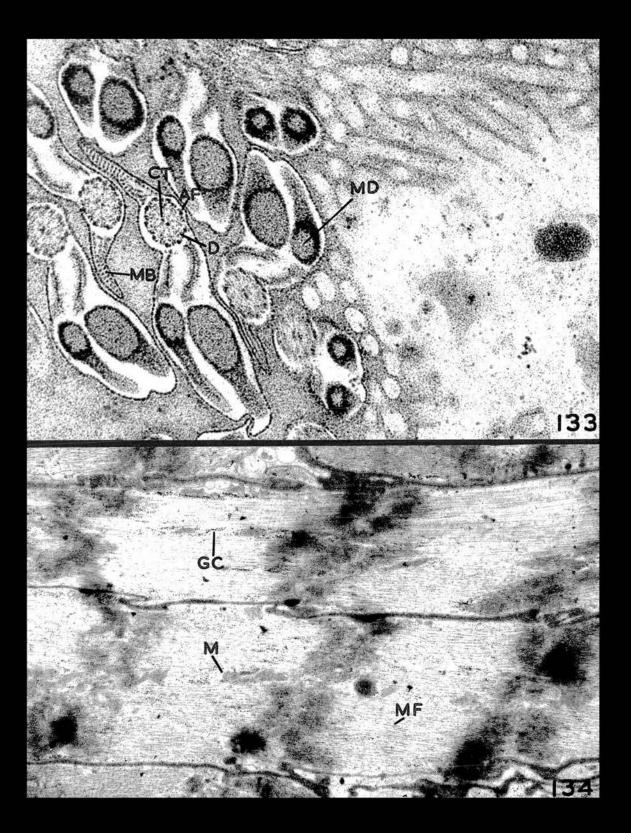
- Fig. 131. A silver nitrate methenamine treated section which shows that the layer of material (DM) between paired sperm heads is specifically stained as well as the cell membranes (CM), mitochondrial derivatives (MD),

 'microtubular border' (MB) and the granular material between sperm. The nuclei are unstained. x 50,000.
- Fig. 132. Accessory fibres (AF) and central tubules (CT) stain more intensely than the doublets (D) in flagella which have been stained with silver nitrate methenamine.

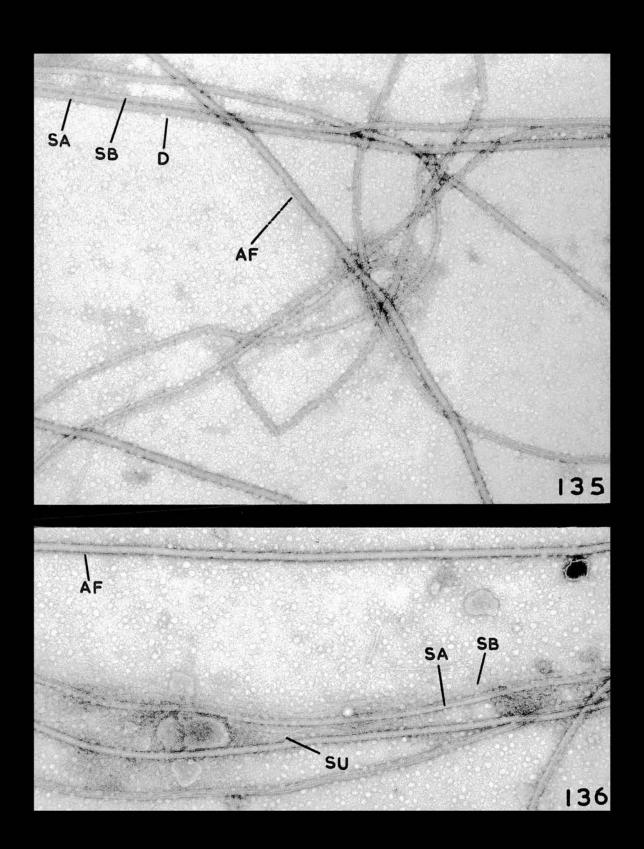
 x 50,000.



- Fig. 133. Granular materials of high and low electron density are present in epithelial cells (EC). The specific staining of accessory fibres (AF), central tubules (CT), doublets (D), mitochondrial derivatives (MD), 'microtubular border' (MB) and the granular material (GL) between sperm is also shown. x 50,000.
- Fig. 134. Treatment of smooth muscle with silver nitrate methenamine stains the myo-fibrils (MF) mitochondria (M) and glycogen (GC). x 12,500.

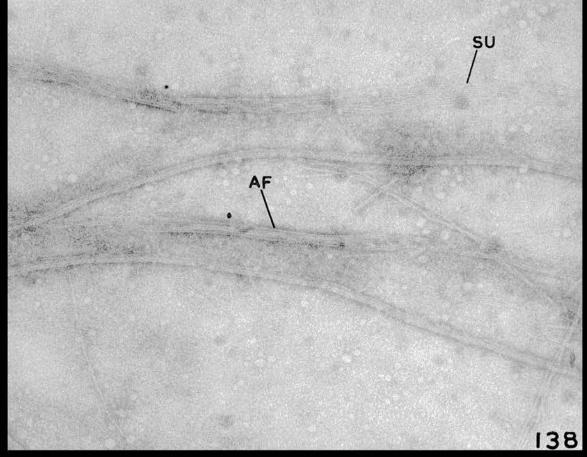


- Fig. 135. Doublets (D) are more sensitive to Trypsin (0.5% aqueous) than the accessory fibres (AF). In some doublets subfibre B (SB) separates completely from subfibre A (SA). Subunits (SU) frayed out from the doublet fibres are also present. 10 mins Trypsin treatment. x 62,500.
- Fig. 136. A similar view to Fig. 135 showing the subunits (SU) in subfibres A (SA) and B (SB). 10 mins Trypsin treatment. x 62,500.

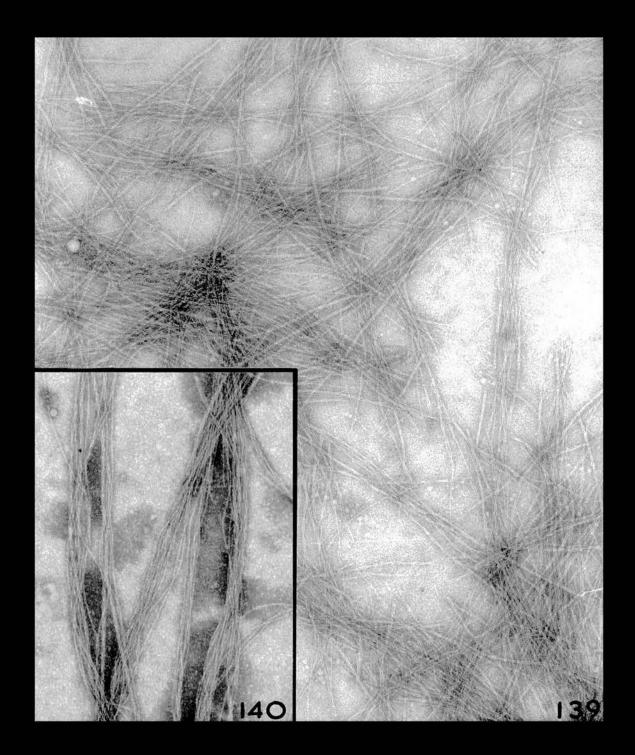


- Fig. 137. A Trypsin treated accessory fibre (AF) showing the frayed out subunits (SU). 10 mins Trypsin treatment. x 100,000.
- Fig. 138. Parts of accessory fibres (AF) remain intact after 30 mins treatment with Trypsin while the remainder fray out into subunits (SU). x 100,000.

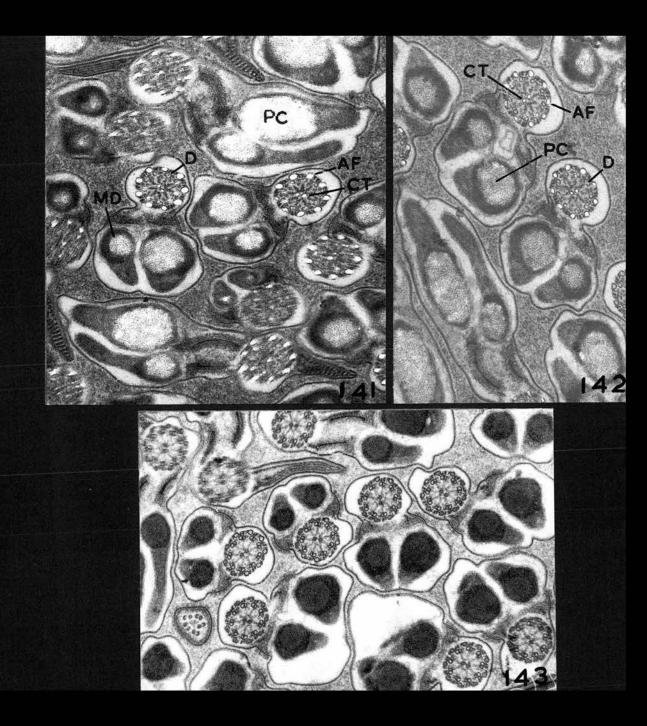




- Fig. 139. Bundles of tubules resembling subunits are found in most preparations of sperm treated with Trypsin for 30 mins. x 62,500.
- Fig. 140. Bundles of tubules similar to those in Fig. 139. x 75,000.



- Fig. 141. Accessory fibres (AF), central tubules (CT) and the paracrystalline (PC) material in mitochondrial derivatives (MD) are digested with pronase. The doublets (D) are only slightly digested. x 50,000.
- Fig. 142. A similar view showing the digestion of the accessory fibres (AF), central tubules (CT), doublets (D) and the paracrystalline(PC) material in the mitochondrial derivatives (MD). x 55,000.
- Fig. 143. Control specimen to show that T.C.A. SOLUBKIZESaway some of the material between the flagellar fibres. x 50,000.



- Figs. 144-146 are photomicrographs of living sperm from vasa deferentia of <u>Dytiscus</u>. Saline preparations and phase contrast microscopy.
- Fig. 144. A single sperm showing the head (HD) and the initial segment (IS) which is straight and rigid. The flagellar helices in the posterior portion are also present.

 x 600.
- Fig. 145. A side view of a pair of heads which are only closely associated in the anterior region of the heads showing a Bake (HK) in the posterior region of each head.

 x 1,400.
- Fig. 146. Paired sperm viewed from above showing the BARBS (HK) in the posterior region of the heads and the flagellar waves in the rigid initial segment. x 1,400.
- Fig. 147. A Feulgen squash preparation to show that the heads are composed of Feulgen positive material. The Beaks (HK) can also be distinguished. x 1,400.

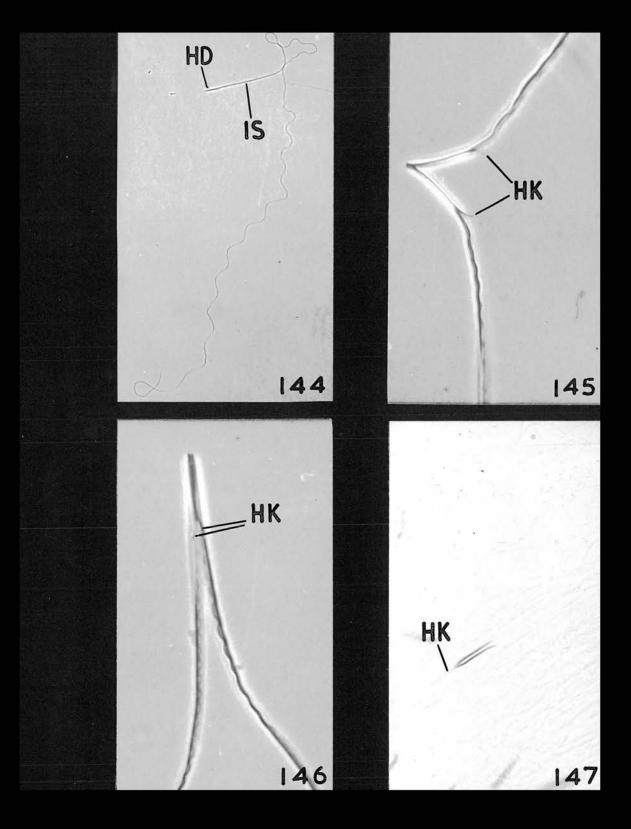
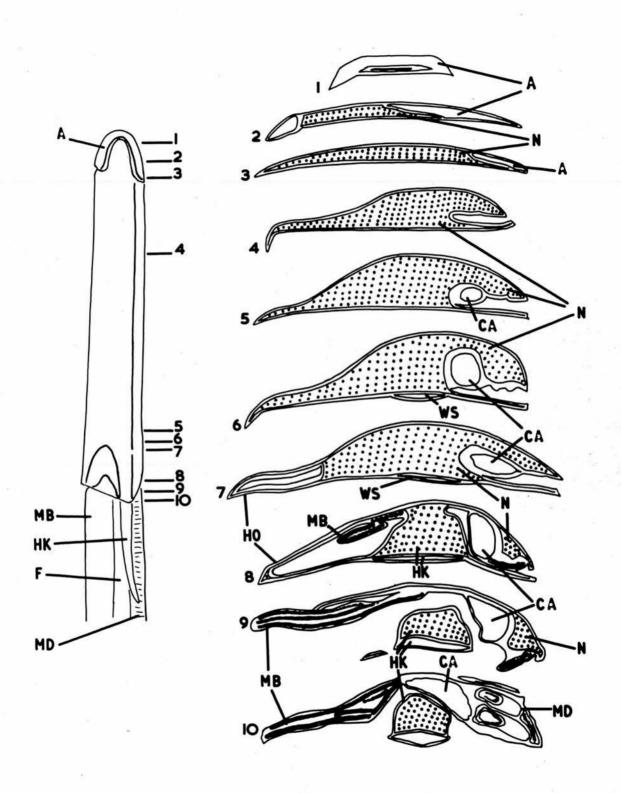


Fig. 148. Schematic diagram to show the outline and main features of a <u>Dytiscus</u> sperm head and diagrams of transverse sections through a head at certain levels to give some impression of the three dimensional structure. The levels at which the sections were cut have been indicated in the diagram. The main features which have been illustrated are the acrosome (A), nucleus (N), wedgeshaped structure (WS), hood (HO), 'microtubular border' (MB), centriole adjunct (CA) and mitochondrial derivatives (MD).



- Fig. 149. A negatively stained head showing the outline of the acrosome (A) in the anterior region and the BARB (HK) in the posterior region. x 10,000.
- Fig. 150. A negatively stained preparation showing that the heads are on different levels from each other and that the curved **Basks** (HK) are not in contact with each other. x 10,000.
- Fig. 151. The posterior portion of a negatively stained tail showing the flagellum (F) and mitochondrial derivatives (MD). x 12,500.
- Fig. 152. The mitochondrial derivatives (MD) terminate in the posterior portion and the end piece (EP) only contains a flagellum (F). x 12,500.

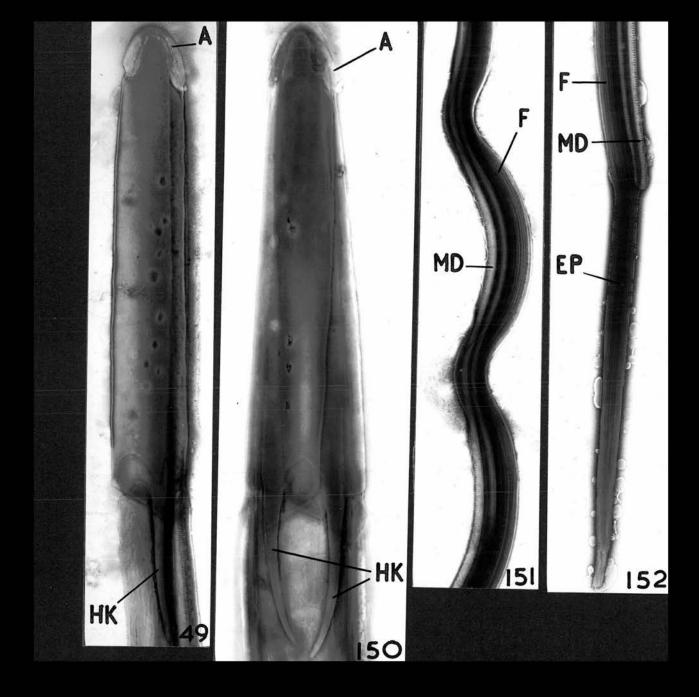
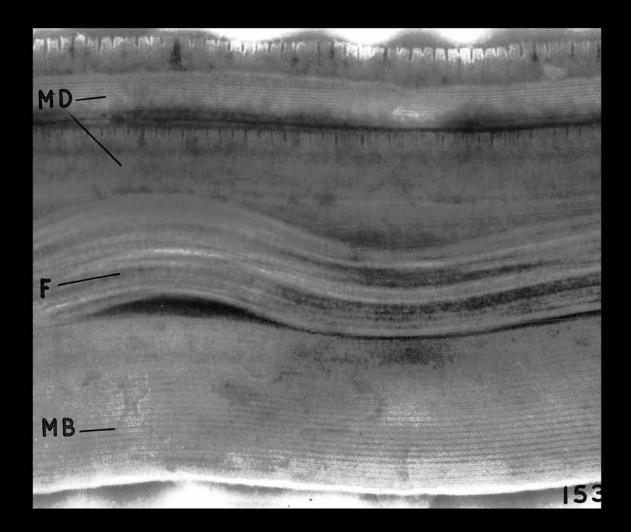
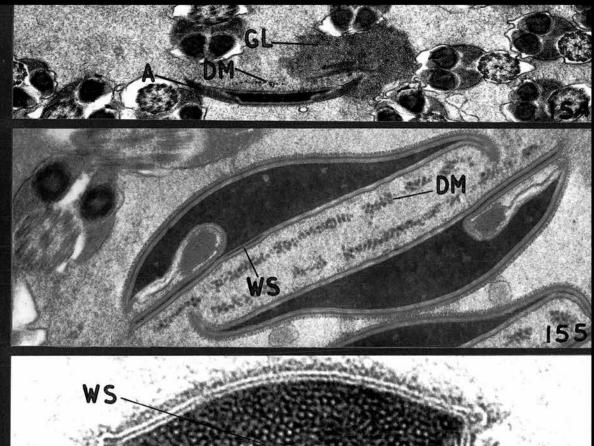


Fig. 153. Part of the initial segment of a negatively stained tail showing the 'microtubular border' (MB), flagellum (F) and mitochondrial derivatives (MD). x 70,000.



- Fig. 154. A transverse section through the anterior region of a pair of heads. There is a granular material (GL) surrounding one of the acrosomes (A) and a layer of coarse granular material (DM) adjacent to the head. x 40,000.
- Fig. 155. In this transverse section the anterior region of the wedge-shaped structure (WS) of a **Beak** has been cut through. Situated between the paired heads are two layers of coarse granular material (DM). x 60,000.
- Fig. 156. A transverse section through a BARS (HK) to show that the wedge-shaped structure (WS) is situated between the membranes of the nuclear envelope (NE) and the association between the cell membrane (CM) and the nuclear envelope (NE). x 374,400.



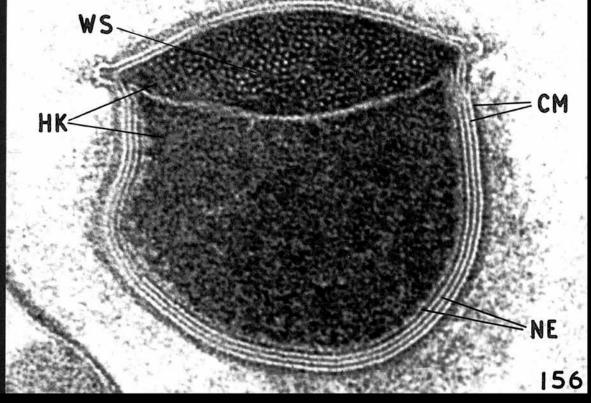
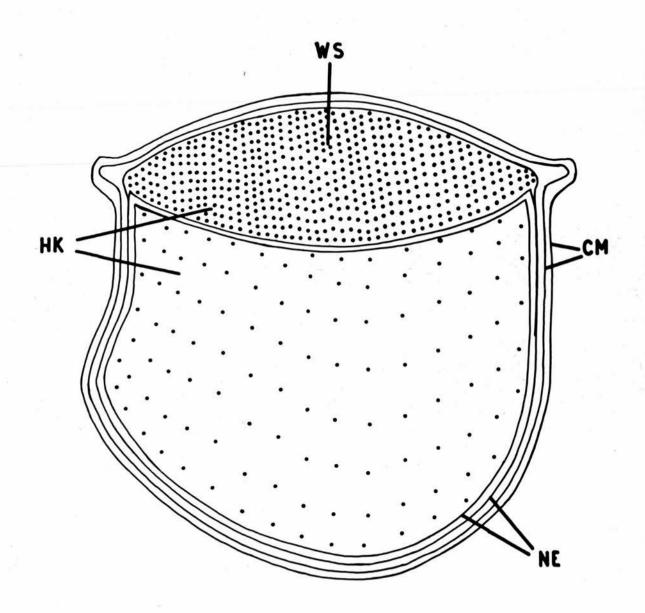


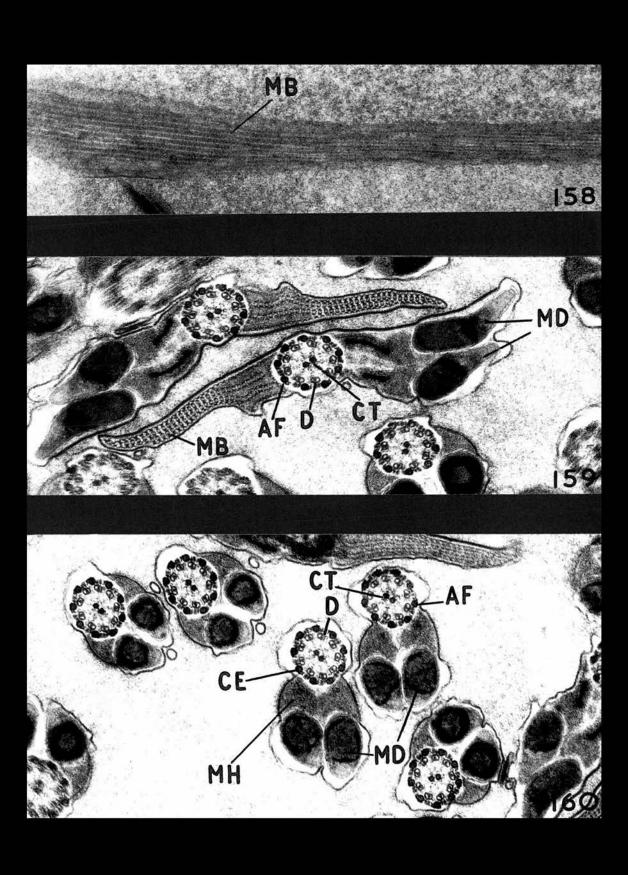
Fig. 157. A schematic diagram to show the organization of the cell membrane (CM) and nuclear envelope (NE) surrounding the nuclear material of the BARB (HK) and wedgeshaped structure (WS).



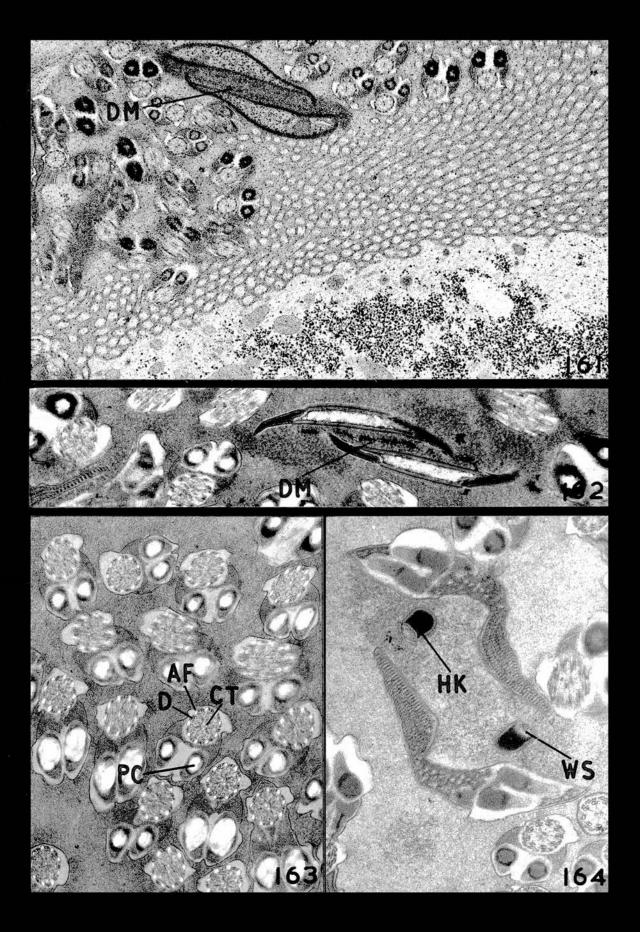
- Fig. 158. Longitudinal section through part of a 'microtubular border' (MB). x 70,000.
- Fig. 159. Transverse section through the initial segment of two tails showing the 'microtubular border' (MB) and the accessory fibres (AF), doublets (D) and central tubules (CT) in the flagella, and the mitochondrial derivatives (MD). x 70,000.
- Fig. 160. Transversely cut sperm tails through the posterior portion showing that the 'microtubular border' is not present.

 The central elements (CE) are present in accessory fibres (AF) throughout the length of the posterior portion. The doublets (D), central tubules (CT), membranous sheath (MH) and mitochondrial derivatives (MD) are also shown.

 x 70,000.



- Fig. 161. The coarse granular material (DM) between paired sperm heads stains intensely when treated with silver nitrate methenamine. x 20.000.
- Fig. 162. Pronase digestion of sectioned sperm heads reveals that the coarse granular material (DM) between paired heads is not digested. The nuclear material forming the head is digested. x 40,000.
- Fig. 163. Digestion of sperm tails with pronase shows that accessory fibres (AF) and central tubules (CT) are digested whereas the doublets (D) are only slightly digested. The paracrystalline (PC) material in the mitochondrial derivatives is also digested. x 40,000.
- Fig. 164. A control specimen showing that periodic acid partially somewize the wedge-shaped structure (WS) in each GRAR (HK). This indicates that the wedge-shaped structure (WS) and nuclear material are composed of different materials. x 60,000.



- Figs. 165-168 are photomicrographs of living sperm from Colymbetes vasa deferentia. Saline preparations and phase contrast microscopy.
- Fig. 165. A group of <u>Colymbetes</u> sperm associated in the head (HD) region. Flagellar helices are present in the posterior portions (PP) and the end pieces (EP) can be clearly distinguished. x 600.
- Fig. 166. A pair of <u>Colymbetes</u> sperm which are associated by their heads (HD). x 1,400.
- Fig. 167. A single sperm head (HD). x 1,400.
- Fig. 168. A group of sperm showing the heads (HD) which are closely associated and flagellar helices in the posterior portion of the tails. x 1.400.

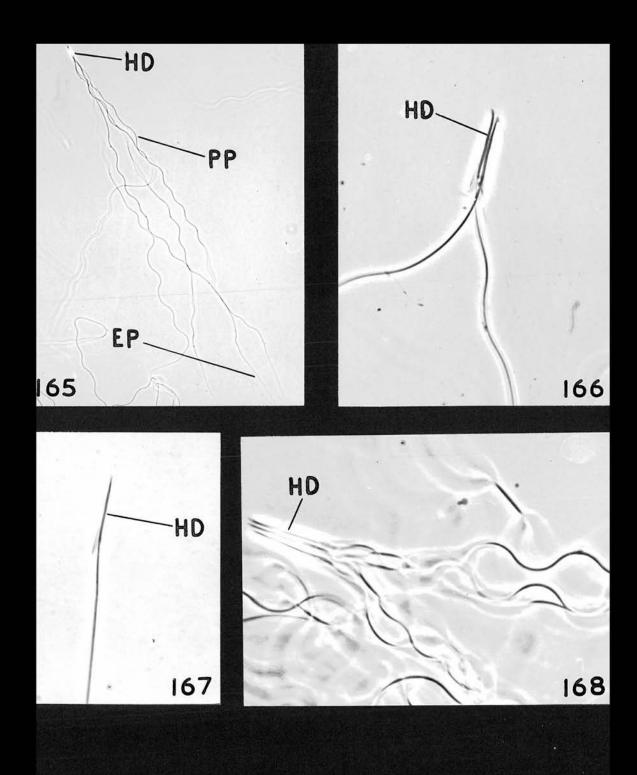


Fig. 169. Schematic diagram showing the outline of a <u>Colymbetes</u> sperm head and sections through certain levels of a head. The section levels 1-7 are indicated opposite the diagram of the sperm head. The structures which have been illustrated are the acrosome (A), nucleus (N), hood (HO), mitochondrial derivatives (MD) and flagellum (F).

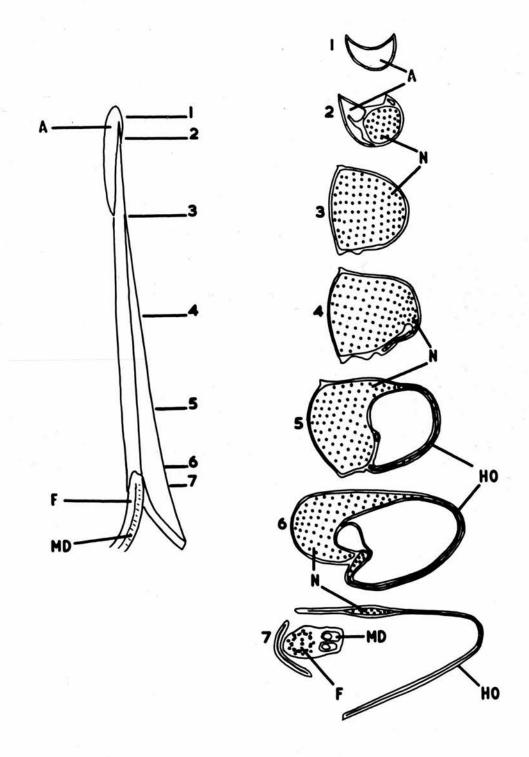
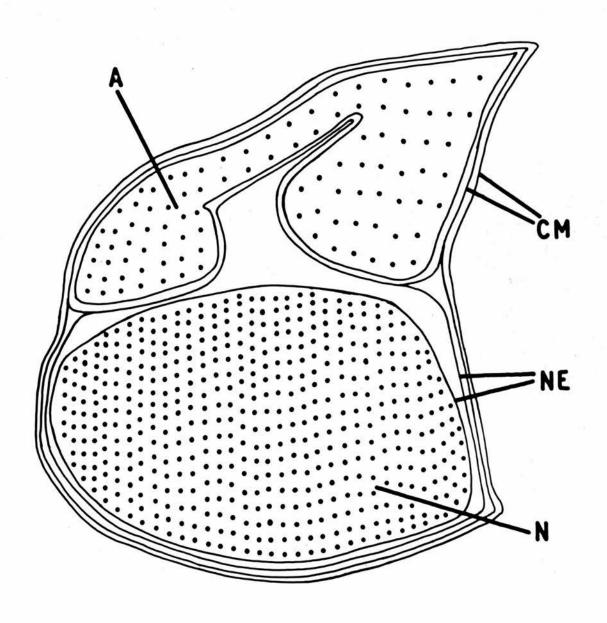


Fig. 170. A schematic diagram of a transverse section through the anterior region of a <u>Colymbetes</u> head to show the complex system of cell membranes (CM) and nuclear envelope (NE) which surround the acrosome (A) and nucleus (N).



- Fig. 171. A negatively stained preparation of a <u>Colymbetes</u> sperm head showing the outline of the acrosome (A), head and granular mass (GM) which extends from the hood.

 x 10,000.
- Fig. 172. Transverse section through the acrosomal region of a group of sperm showing the acrosome (A) and nucleus (N) in each head (HD). The heads are arranged around a granular mass (DM) of medium electron density. x 60,000.

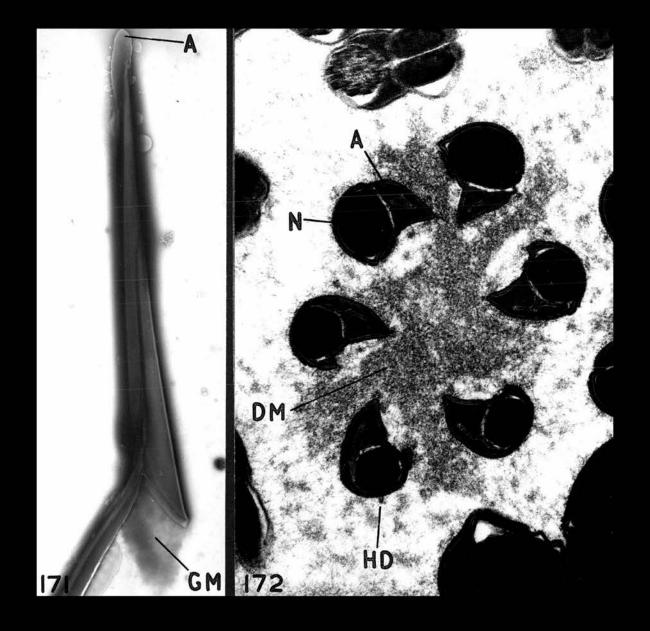
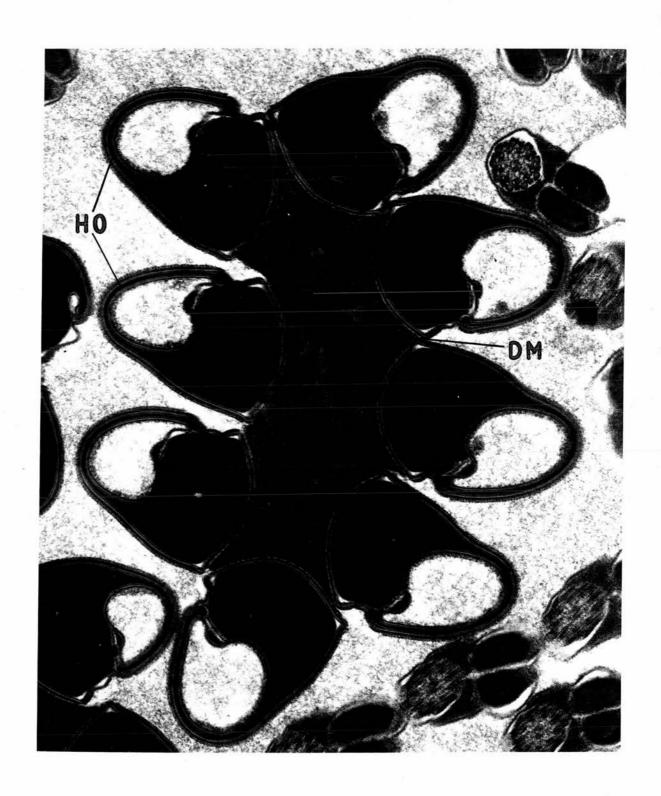


Fig. 173. Transverse section through the posterior region of a group of sperm heads showing the hoods (HO) and a granular mass (DM) which has a high electron density. x 60,000.



- Fig. 174. Transverse section through the posterior region of several hoods showing the granular masses (GM) which extend out from the hoods. x 30,000.
- Fig. 175. Transverse section of an end piece (EP) with accessory fibres (AF), doublets (D) and central tubules (CT) still present in the flagellar axonome. The central elements (CE) in accessory fibres can also be distinguished and there is a coating of granular material (GL) on the inside of the cell membrane (CM). 120,000.
- Fig. 176. Longitudinal section to show the change in electron density of the granular mass (DM) around which the sperm are arranged. x 15,000.

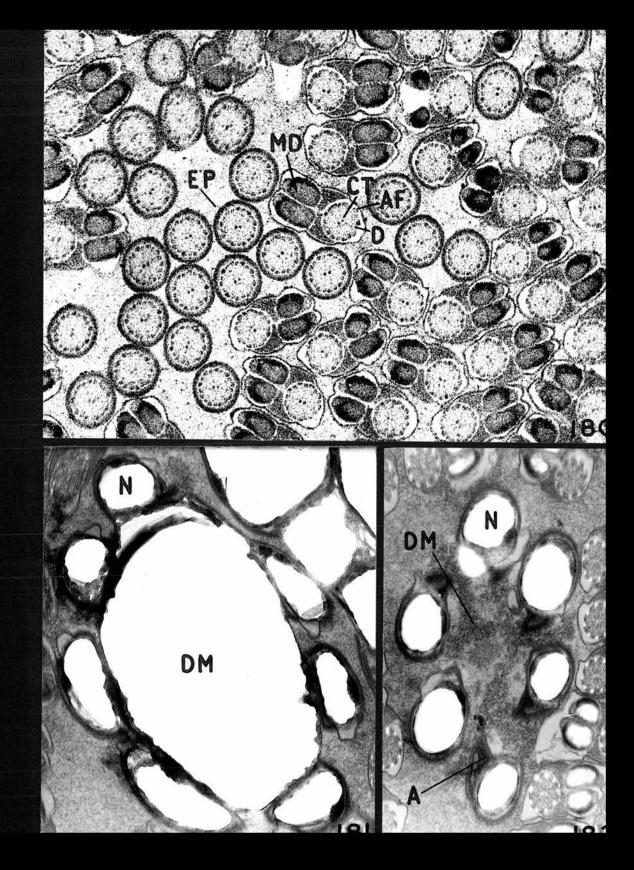


- Fig. 177. A view of several groups of sperm cut transversely showing the arrangement of sperm heads around the granular masses (DM). x 12,000.
- Fig. 178. Transverse section through a group of heads which have been treated with silver nitrate methenamine showing that the granular mass (DM) stains intensely.

 x 20,000.
- Fig. 179. A similar section to Fig. 178 showing the granular mass (DM) intensely stained. x 20,000.



- Fig. 180. Transverse section through the posterior portion and end piece (EP) of several tails which have been treated with silver nitrate methenamine. Accessory fibres (AF) and central tubules (CT) stain more intensely than doublets (D). The mitochondrial derivatives are also intensely stained. x 40.000.
- Fig. 181. A transverse section which has been treated with pronase to show that the granular mass (DM) of high electron density and the nuclei (N) are digested. x 40,000.
- Fig. 182. Transverse section through the acrosomal region of a group of heads showing that the granular mass (DM) of medium electron density and the acrosomes (A) are not digested. The nuclei (N) are digested. x 40,000.



- Fig. 183. A promase treated transverse section through several tails to show that accessory fibres (AF) and central tubules (CT) are digested before doublets (D). The paracrystalline (PC) material in the mitochondrial derivatives is also digested. x40,000.
- Fig. 184. PERIOOK partially sourcessome of the material in control sections. The structure of the accessory fibres (AF), doublets (D), central tubules (CT) and mitochondrial derivatives (MD) is more clearly seen than in untreated sperm. x 40,000.

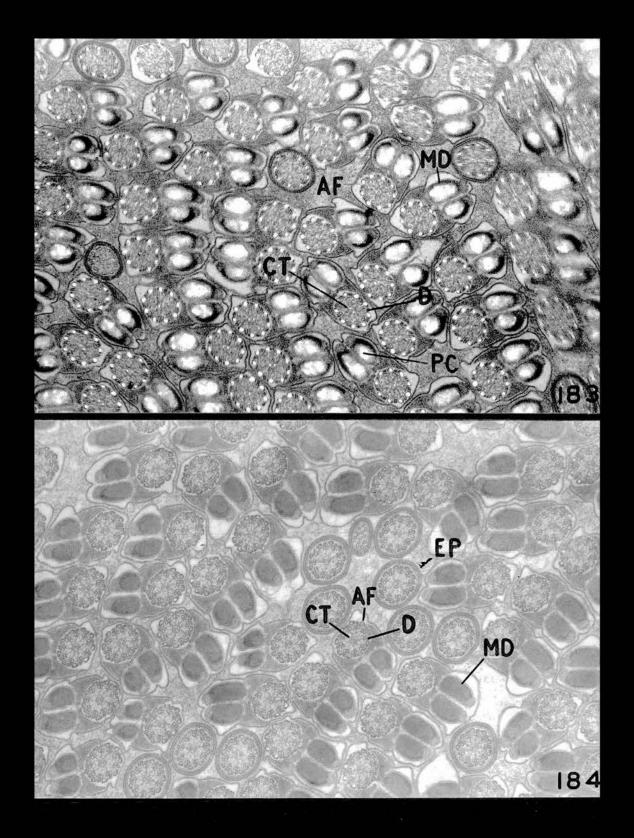
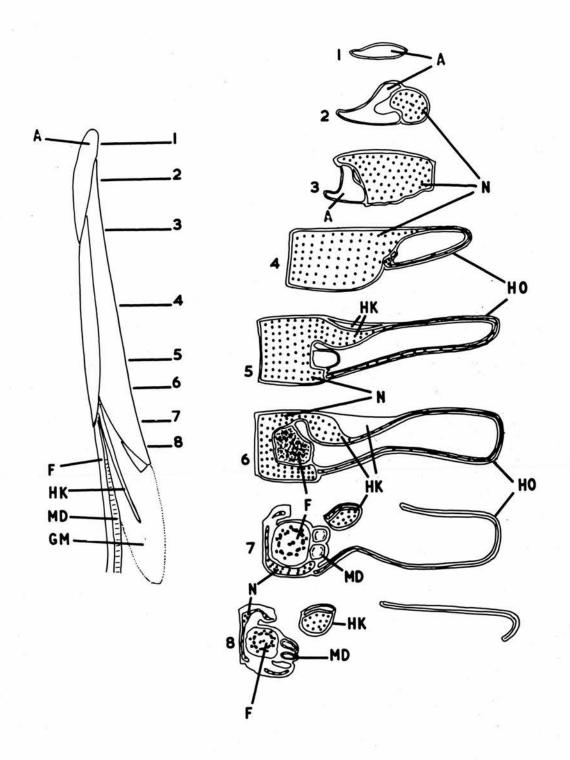


Fig. 185. Schematic diagram to show the outline and main features of an Agabus sperm head. The levels of the transverse sections are indicated opposite the diagram of the head. The features of the head which have been illustrated are the acrosome (A), nucleus (N), hood (HO), flagellum (F), mitochondrial derivatives (MD) and the granular mass (GM) which extends out from the hood.



- Fig. 186. A pair of <u>Agabus</u> sperm from a saline preparation of sperm from vasa deferentia. The heads (HD) are not closely associated. Phase contrast microscopy.

 x 600.
- Fig. 187. A pair of heads (HD) attached at their tips with a

 RAR (HK) visible in one of the heads. Flagellar helices

 are present in the anterior region of the posterior

 portion in the tails. Saline preparation and phase

 contrast microscopy. x 1,400.
- Fig. 188. A negatively stained Agabus head showing the outline of the acrosome (A), head (HD) and BARS (HK). There is also a granular mass (GM) of material surrounding the BARS. x 10,000.

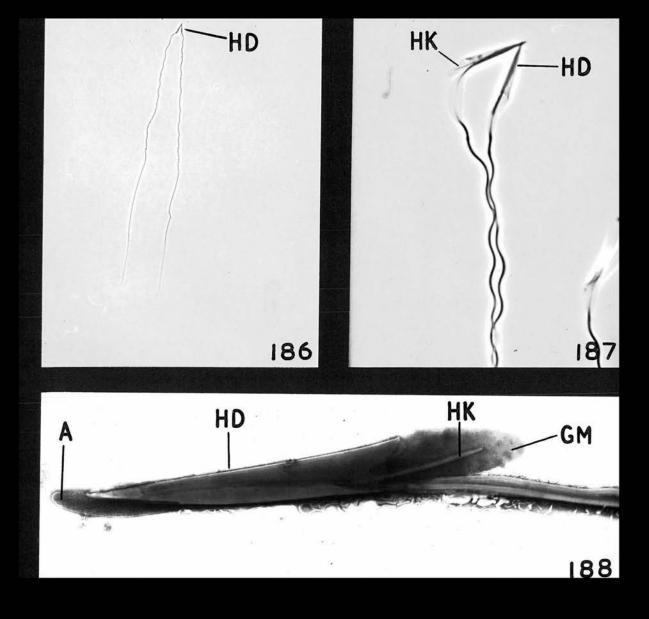


Fig. 189. Transverse section through several Agabus sperm heads to show that there is no specific arrangement of the sperm into pairs or groups associated with a granular material. Present in these sections are acrosomes (A), BARBS (HK), hoods (HO) and granular masses (GM) of material which surround each BARB. x 10,000.

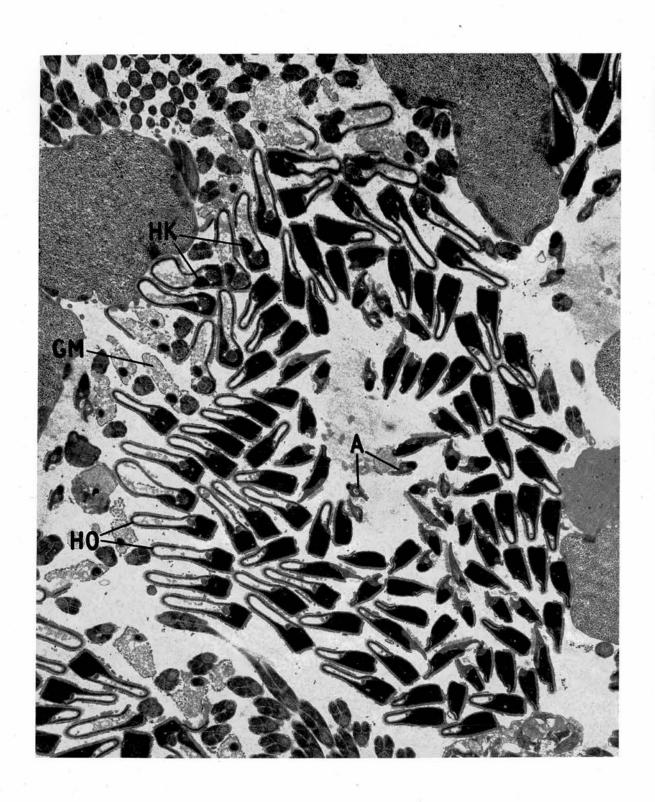


Fig. 190. A high power electronmicrograph of several heads showing the acrosomes (A) and nuclei (N) in detail. In some regions there is a diffuse layer (DF) of material adjacent to the heads. x 50,000.

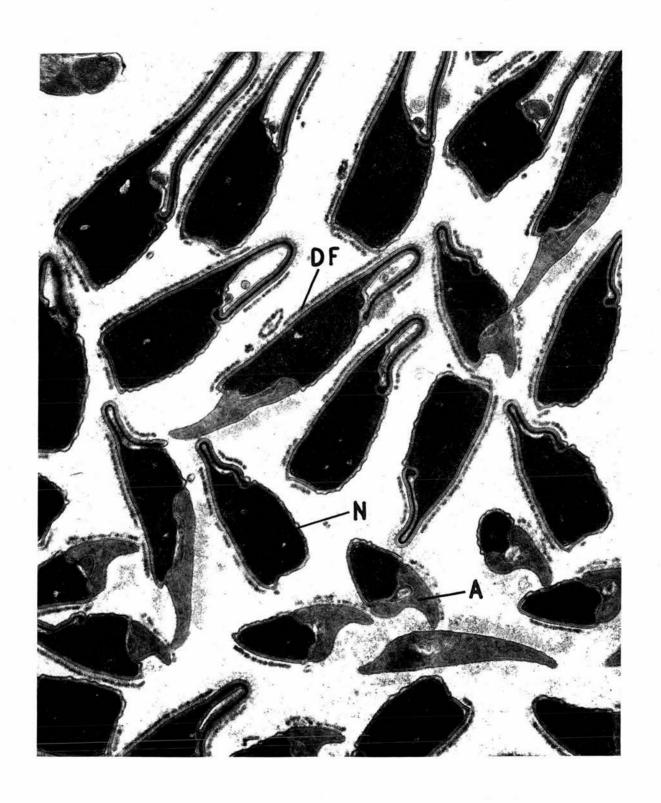
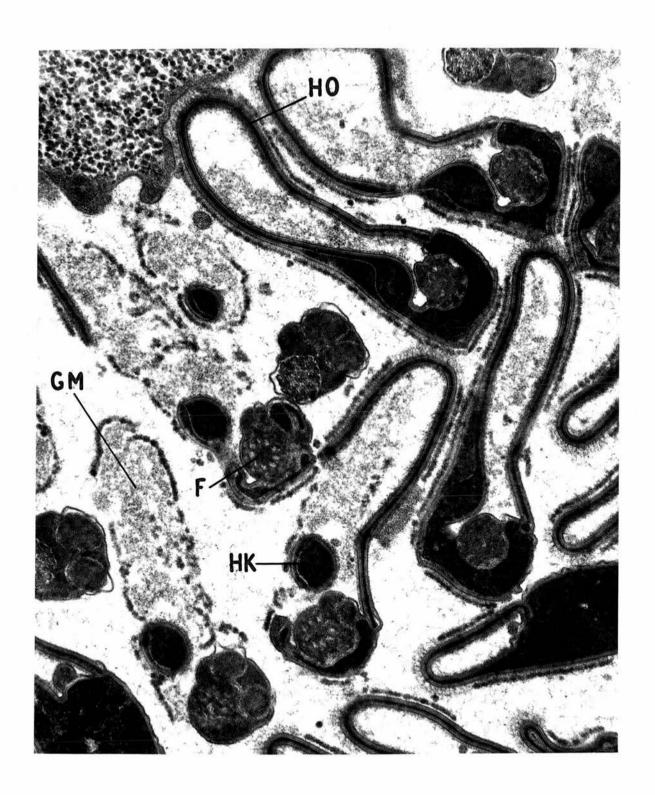


Fig. 191. Transverse section through the posterior region of several heads showing the hood (HO), BARB (HK), flagellum (F) and the granular mass (GM) of material surrounding each hook. x 60,000.



- Fig. 192. Sperm tails cut transversely through the posterior portion.

 Central elements (CE) are present in the accessory fibres

 (AF). Doublets (D), central tubules (CT), mitochondrial

 derivatives (MD) and membranous sheaths (MH) are also

 present in this view. x 120,000.
- Fig. 193. Transverse section through several end pieces (EP) with the central elements (CE) visible in accessory fibres (AF) and a coating of granular material (GL) on the inside of the cell membranes (CM). x 60,000.

