KARL GEORG WINGSTRAND

COMPARATIVE SPERMATOLOGY OF A PENTASTOMID, *RAILLIETIELLA HEMIDACTYLI*, AND A BRANCHIURAN CRUSTACEAN, *ARGULUS FOLIACEUS*, WITH A DISCUSSION OF PENTASTOMID RELATIONSHIPS

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Synopsis

The structure and development of the spermatozoa of the pentastomid *Raillietiella hemidactyli* Hett, a lung parasite of lizards, are described on the basis of light and electron microscopical techniques. The mature spermatozoon is 100–130 μ long, and the anterior 35 μ forms a "pseudoacrosome", which differs fundamentally from true acrosomes in its development. The following part, called "body" has a uniform structure throughout its length and contains a filiform nucleus, three filiform mitochondria, and an axonema, all arranged in a strictly symmetrical pattern. Several specialized structures, particularly the pseudoacrosome, some complicated sheaths around the axonema, and details of the latter were believed to be completely unique in the animal kingdom, until an identical pattern was found in the spermatozoa of the branchiuran crustacean *Argulus foliaceus* (L.).

A comparison between the spermatozoa of *Raillietiella* and *Argulus* is presented and shows that identity is extended to surprising details, both in the structure of the mature spermatozoon and in developing spermatids. Comparisons show that most of the specialized features are unknown in other animals. The most prominent difference between *Raillietiella* and *Argulus* is the transient appearance of a true acrosome and an acrosome filament in the latter. These structures are reduced before maturation, but their existence together with the pseudoacrosome shows definitely that the latter is a unique structure without relation to normal acrosomes.

The spermatozoa of *Argulus* and *Raillietiella* share so many specialized features which are unknown in other animals that independent development and evolutional hazards are, in practice, excluded as explanations. It is therefore concluded that the Pentastomida and the Branchiura are more closely related to each other than to other animals, and it is suggested that the Pentastomida be placed as a parasitic and strongly modified sub-group of the Branchiura in the zoological system. Their situation will then be analogous to that of the Rhizocephalia within the crustacean group Cirripedia. The Rhizocephalia, like the Pentastomida, are strongly modified parasites which are difficult to place in the system on the basis of adult morphology, but their spermatozoa are of the same very characteristic type as that of the free-living Cirripedia.

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CONTENTS

I. INTRODUCTION

During morphological and embryological investigations of Pentastomida, performed in collaboration with Dr. A. Nørrevang (see Nørrevang 1972), it was noticed that pentastomid spermatozoa have a rather exceptional structure. Since this might give a clue to the systematic affinities of this enigmatic group, I undertook a structural and developmental analysis of the spermatozoa in the pentastomid *Raillietiella hemidactyli* Hett, 1934. For a long time these appeared to have a completely unique structure, and comparisons with other animals were not very successful. The theoretical evaluation of the results therefore made little progress until, in the summer of 1971, material of *Argulus foliaceus* (L.) was obtained. The spermatozoa of *Argulus* turned out to be identical with those of *Raillietiella* in general structure and also in many details. Moreover, the spermatogenesis in the two forms proved identical in many important and partly surprising respects.

Since many of the features common to the spermatozoa of *Raillietiella* and *Argulus* are completely unknown in other animals, a close phylogenetic relationship between the two forms is indicated. I realize that many biologists will find it hard to accept a conclusion like this and that substantial documentation is necessary. Therefore, as a basis for the final discussion, the structure and development of the spermatozoa in the two forms is described in great detail in the present paper.

Documentation rests on the electron micrographs reproduced in the Plates 1–23. In order to facilitate comparison, corresponding sections and stages of *Raillietiella* and *Argulus* are shown side by side, when this has been possible. This means that pictures from the two species are completely mixed and could be confused by the reader. Therefore an "R" for *Raillietiella* or an "A" for *Argulus* is added after the number of each figure.

II. MATERIAL AND METHODS

All material of *Raillietiella* was obtained from the lungs of an agamid lizard, *Calotes versicolor* Daudin, imported from Thailand (Bangkok). On an average, one out of five or six lizards contained the parasites, which agree with Hett's (1934) description of *R. hemidactyli* in most respects: Posterior hooks blunt and much larger than anterior ones, head strongly tapering from posterior hooks to mouth segment (Plate

1:1), females 10–16 mm long (Hett: 13–17 mm). But the present females have only 25–27 rather indistinct abdominal rings, not 28–30 as stated by Hett; The mature males are only 3.5–4.0 mm long (hundreds have been seen), not 12–13 mm as stated by Hett. Further, the present animals have double frontal papillae (sensu Heymons 1935) and dorso-lateral papillae (Plate 1:2). Hett does not describe papillae in this species. Identification of the material with Hett's *R. hemidactyli* is supported by the fact that Hett reported the species from the same host, *Calotes versicolor*, from Burma, and that the blunt posterior hooks are regarded as distinctive characters. At any rate, the species belongs to the *R. geckonis* group of Heymons (1935) (See Self 1969).

For fixation, the testicle, vesicula seminalis or receptaculum seminis was removed from the living animal and immersed in cold fixative.

All material of *Argulus* belongs to *A. foliaceus* (L.) (Det. U. Røen), and was obtained from the lake around Frederiksborg castle, Hillerød, Denmark. The living animals were killed by immersion in cold fixative, and the abdominal lobes with testicles or receptacula were immediately cut off. The said organs, sometimes also the vesicula seminalis, were rapidly exposed to the fixative by partial removal of the cuticle.

For electron microscopy, fixation was performed in cold Palade's (1952) osmic, or in cold, phosphate-buffered 2% glutaraldehyde, followed by washing and post-osmification. Epon sections from a Reichert Om U2 microtome were contrasted with uranyl acetate and lead citrate and were examined in a Zeiss Em 9S-2 microscope. In general, Palade's osmic gave the most reliable pictures of the general morphology. Glutaraldehyde worked well for mature sperm and preserved microtubules best, but caused exaggerated vacuolization and shrinkage of spermatocytes and spermatids in many specimens. These artifacts could not be eliminated by varying the phosphate concentration.

Most light microscopy was performed on thick (1–2 μ) epon sections of the material used for electron microscopy. The sections were stained with toluidine blue – borax. The males of *Raillietiella* were also studied in paraffin sections (fixation:Bouin; staining: Ehrlich's hematoxylin-eosin or Feulgen-light green).

Important observations on whole spermatozoa were made with phase contrast on living sperm, suspended from the receptaculum seminis in mammalian Ringer solution. Osmium-fixed spermatozoa were also spread in distilled water on coated grids and dried to allow EM-observations of intact spermatozoa.

Comparisons were made with material of the tardigrade *Macrobiotus hufelandi* Schultze (testicles, receptaculum), and the thysanurans *Lepisma saccarina* L. (testicles, vesicula seminalis), and *Petrobius brevistylis* Carp. (testicles, vesicula seminalis). This material was examined essentially as the *Raillietiella* and *Argulus* specimens. The results obtained with *Petrobius* are original and will be published in the future.

Im am obliged to several persons who have helped to supply me with material: U. Røen and F. Møller Hansen *(Argulus)*, the Drøbak Station in Norway, particularly Finn Walvig, and also Å. Jespersen and J. Lützen *(Petrobius)*, T. Hallas and B. Theisen *(Macrobiotus)*. The scanning pictures were taken with a Cambridge Stereoscan

microscope in the Institute for Historical Geology and Paleontology (prof. T. Birkelund and Dr. J. Hansen).

The work was supported by a grant from the Danish Research Council.

III. *RAILLIETIELLA HEMIDACTYLI* HETT

V. Haffner (1924) described the development of the sperm cysts in the pentastomid *Nettorhynchus* (*Armillifer*) *moniliformis* (Diesing 1935) and mentions that the mature spermatozoa are filiform as seen with the light microscope (see also v. Haffner 1922). The development of spermatocysts as well as the filiform appearance of the spermatozoa was confirmed by Doucet (1964, 1965) for *Nettorhynchus (Armillifer) armillatus* (Wyman 1847), *Raillietiella boulongeri* (Vaney & Sambon 1910) and *Sebekia wedli* Giglioli 1922. Doucet also published two good EM pictures of cross sections of spermatozoa of *Raillietiella boulongeri*. These pictures show the interesting structure but were insufficient for a detailed interpretation.

The structure of the testicle

The testicle of *Raillietiella* is an unpaired, thin-walled sac, lying in the posterior half of the abdomen, connected with the dorsal body wall by an unpaired, median ligament (See Heymons 1935, Doucet 1965). Anteriorly it is connected with the large, ovoid vesicula seminalis through a funnel-shaped passage.

The wall of the testicle is a single-layered epithelium, standing on a thick (0.3–0.5 μ) basement membrane, which has the indistinct structure and medium contrast of a mucopolysaccarid coat (Plates 1:3, 3:6,8). In many places this basement membrane projects with tongue-like processes into the intercellular spaces between the epithelial cells.

The testicular epithelium consists of two types of cells: 1) the predominant phagocytic vegetative cells, and 2) the germinal cells (primary spermatogonia). The two types probably correspond to the "nutritive" and "germinative" cells described by v. Haffner (1924) in the testicular wall of *Nettorhynchus*.

The vegetative cells contain few ribosomes, few ergastoplasmic vesicles and a moderate number of mitochondria (Plate 3:6,8). Many of the cells show pronounced indications of phagocytosis and may be more or less filled with large, phagocytotic vesicles. The material taken up by the phagocytes is clearly the cellular debris suspended in the testicular lumen. The newly formed vacuoles often contain recognizable pieces of spermatozoa and sometimes clumps of debris, surrounded by non-nucleated cytoplasm (Plate 3:8). Such clumps are common in the testicular fluid and can sometimes be seen half-engulfed on the surface of vegetative cells. The non-nucleated cytoplasm of these clumps probably derives from the follicular wall of disintegrated sper-

matocysts (see below). V. Haffner (1924) suggested that the vegetative cells in *Nettorhynchus* secrete nutrients into the testicular lumen, to be used by the developing spermatocysts. Such a function is not distinctly reflected in the cytology of the vegetative cells of *Raillietiella*, although some cells contain some empty-looking vacuoles which may be concerned with secretion.

The germinative cells or primary spermatogonia are solitary cells, characterized by a rounded shape, a large nucleus, abundant free ribosomes and many mitochondria, but complete absence of phagocytic vacuoles (Plate 3:6). When typically developed, these germinal cells are always overgrown and separated from the testicular lumen by plasmatic processes from the neighbouring vegetative cells.

The development of the spermatocysts

The solitary germinal cells in the epithelium are the origin of the spermatocysts. Each cell divides into 2, 4, 8, 16, etc., daughter cells, and electron microscopy as well as light microscopy show that all divisions within a cyst are simultaneous. The cell clump thus formed bulges out into the testicular lumen, still covered by a cellular sheath (cyst wall) derived from the vegetative cells (Plate 3:6). About the time maturation begins, the cyst is pinched off from the wall and floats free in the lumen, still surrounded by the thin plasmatic cyst wall (Plates 1:3, 2:4, 3:6–8). Meiotic divisions have only been seen in free-swimming spermatocysts.

The cyst wall is obviously formed by several surrounding vegetative cells, for several cell limits are seen around the periphery of a single cyst. However, I have never seen a nucleus in this sheath, so it must be concluded that only cytoplasmatic parts are detached from the neighbour cells when a cyst is pinched off. The cyst wall is in most places less than 1 μ thick and may be as thin as 500 Å locally (Plate 3:7). The thicker parts contain some mitochondria, a small number of vacuoles, and rather numerous microtubules, oriented parallel to the outer surface (Fig. 21:106).

The entire spermatogenesis takes place within these cysts. According to v. Haffner (1924), working with *Nettorhynchus*, maturation begins when the 64-cell stage has been reached in the cysts. With two subsequent meiotic divisions this makes 256 spermatozoa in each cyst. Doucet (1965), working with *Raillietiella boulongeri*, states that maturation begins after the 32-cell stage has been reached. This would give 128 spermatozoa in each cyst.

Both possibilities are realized in *R. hemidactyli*. Counting of spermatids is fairly precise in EM-pictures showing cross sections of advanced spermatocysts, because the filiform spermatids are oriented parallel to each other (Plate 3:7). A series of countings gave the following numbers of spermatids for individual cysts:

256, 254, 255, 249, 248, 249, and

128, 128, 128, and 128 + 7 pseudoacrosomes.

Theoretically, only the exact figures 128 and 256 could result from simultaneous divisions of this kind. In the 256 series it is probable, therefore, that some spermatids

have either been dislocated or failed to develop. The 7 extra pseudoacrosomes in the last cyst of the 128-series were situated along the wall of the cyst and had obviously been bent and doubled back.

In cysts with early spermatids there is a narrow cleft-like lumen between the cells in the center of the slightly elongate body. When flagellae appear, these always grow into this central lumen and bend towards the same end of the cyst. This end soon becomes pointed, so the cyst becomes drop-like. Later, when the long pseudoacrosomes grow out from the opposite end of the spermatids, they are directed towards the other end of the cyst, which then becomes spindle-shaped (see Plate 1:3). Inside these more advanced cysts the spermatids are parallel, but the bundle of spermatids is slightly coiled. The spermatids are interconnected by plasmatic bridges until very late stages, i.e., the final separation following meiotic divisions is much retarded.

The cysts finally open and the mature spermatozoa are liberated. Exactly how the spermatozoa were liberated and transferred to the vesicula seminalis was not observed. At any rate, the transfer must be selective, for the vesicula contains no cellular debris or abnormal spermatozoa, only a tangle of non-orientated, normal spermatozoa. The wall of the vesicula is strongly phagocytotic, with many vacuoles containing recognizable remnants of spermatozoa.

The contents of the testicle are rather varied. In addition to spermatocysts and normal spermatozoa there are always some abnormal spermatozoa, cellular debris, and non-nucleated clumps of protoplasm. The non-nucleated plasma balls usually contain large vacuoles with inclusions, and sometimes degenerated spermatozoa. These balls are probably formed by the non-nucleated walls of the cysts when these disintegrate.

For the said reasons, squash preparations of testicles are not very useful in studies of spermatogenesis. The normal spermatids keep together in the spermatocysts and are difficult to spread, so the squash is usually dominated by abnormal spermatozoa and spermatids in addition to undefinable debris.

In some males, the testicle contains mainly normal spermatocysts in different stages of development, and the amount of suspended matter is moderate (Plate 1:3). In other males a great proportion of the spermatocysts show strong vacuolization and atrophy of the spermatids, and the fluid is crowded with abnormal spermatids, plasma balls with large inclusions, and undefinable debris (Plate 2:1). The latter picture is perhaps abnormal, indicating that the hosts were not kept well. On the other hand, such a picture could be a normal physiological condition if the spermatogenesis is under control and is checked when the vesicula seminalis is filled up.

The mature spermatozoa

External features, regional differentiation

The mature spermatozoa from the vesicula seminalis of the male or from the two receptacula of the female are filiform, 100–130 μ long (Fig. 1, Plate 4). The thickness is greatest about the middle, ca. 0.8 μ, tapering slightly towards both ends. When internal structure is considered, only two regions can be distinguished (Fig. 1): 1) a "pseudoacrosome", occupying the anterior ca. 35 μ of the spermatozoon, and 2) a "body" of uniform structure extending from the base of the pseudoacrosome to the posterior end.

The term "*pseudoacrosome*" is introduced here to designate the anterior part of the *Raillietiella* spermatozoon, which could be mistaken for a true acrosome because of its situation and external morphology, but which differs from true acrosomes in internal structure and development. Conditions in *Argulus*, where a similar pseudoacrosome and a true acrosome exist side by side during development, show that this nomenclature is justified.

The pseudoacrosome is thinner than the body, about 0.35 μ at the base and 0.15 μ at the extreme point. It is stiff and usually arched like the periphery of a half-circle in living material and often preserves this shape in fixed specimens (Fig. 1, Plate 4:10). The transition from the pseudoacrosome to the somewhat thicker body is abrupt and can be seen in living sperm with phase contrast. EM-pictures of intact spermatozoa show this sudden change of thickness distinctly, so measurements of pseudoacrosome and body can be obtained with great accuracy (Plate 4:10).

Internal structure of body

As seen in cross sections, the body is bilaterally symmetrical (Fig. 1:B, Plate 5:16). It contains: 1) a filiform nucleus, cylindrical or slightly flattened in cross section, 2) three mitochondrial rods, arranged symmetrically in relation to the median plane, 3) a typical axonemal complex of the 9+2 type, 4) a system of high-contrast sheaths around axonema and lateral mitochondria, called dorsal and ventral ribbons,

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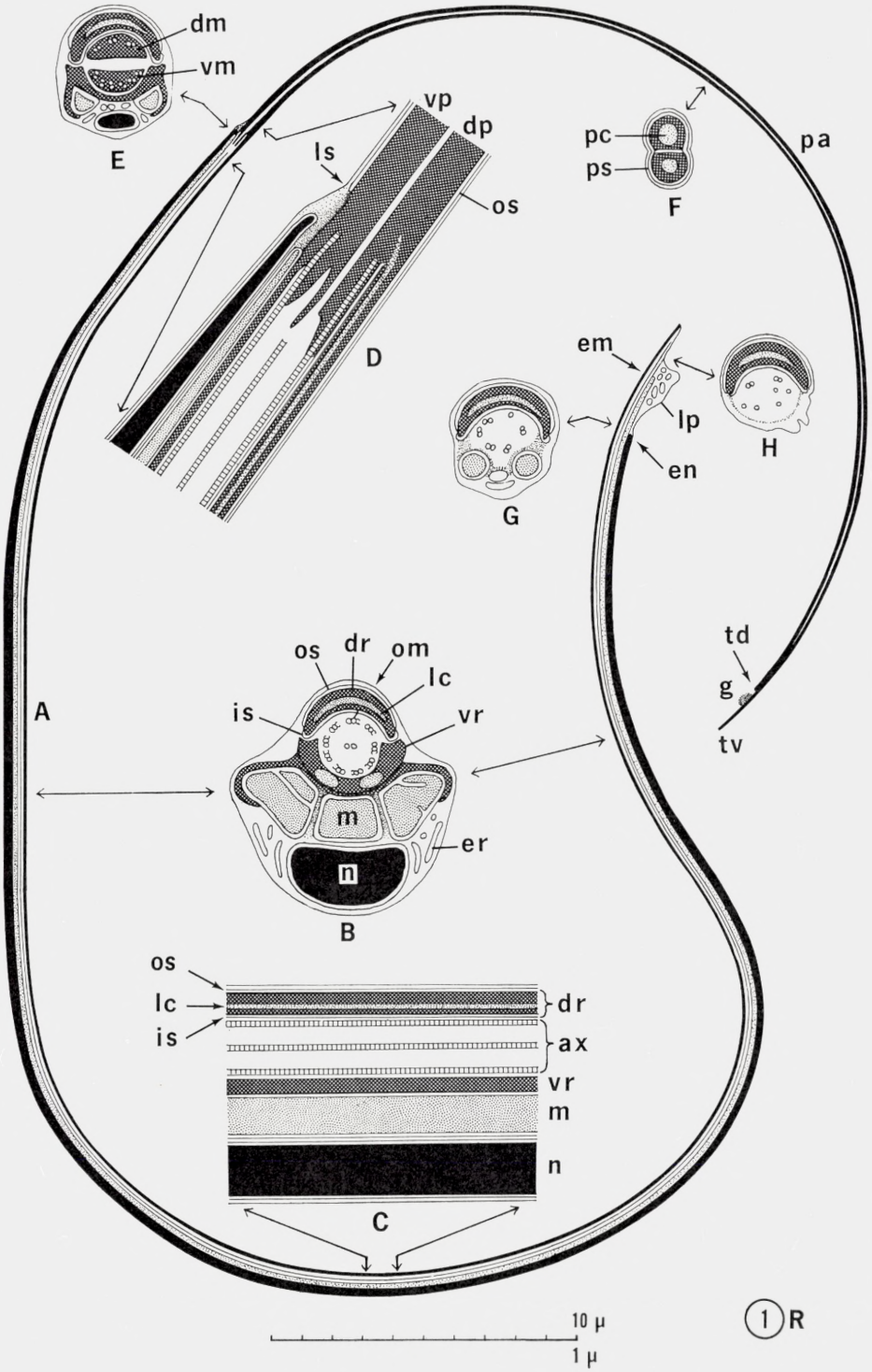
Fig. 1. *Raillietiella*. Diagram of the mature spermatozoon, based on numerous total preparations and sections like those in Plates 4–7. The scale is 10 μ when used for the intact spermatozoon (A) and 1 μ when used for the details (B–H).

A. Entire spermatozoon. — B. Cross section of body. — C. Median section of body. — D. Median section of transitional region between body and pseudoacrosome. — E. Cross section through anterior end of nucleus. — F. Cross section of pseudoacrosome. — G and H. Cross sections of posterior end.

ax = axonema, dm = dorsal mass of pseudoacrosomal granular matter, investing filaments nos. 9, 1 and 2, dp = dorsal rod of pseudoacrosome, dr = dorsal ribbon, em = posterior end of mitochondria, en = posterior end of nucleus, er = smooth endoplasmic reticulum, g = granulosome, is = inner membranous sac of dorsal ribbon, lc = light core of dorsal ribbon, lp = lump of vesicular plasm, ls = limit of membranous sac on the ventral side of the pseudoacrosome, m = mitochondria, n = nucleus, om = oblique membrane between axonemal doublet 1 and dorsal ribbon, os = outer membranous sac of dorsal ribbon, pa = pseudoacrosome, pc = light core of dorsal rod of pseudoacrosome, ps = pseudoacrosomal membranous sac, td = top of dorsal rod of pseudoacrosome, tv = top of ventral rod of pseudoacrosome, vm = ventral mass of pseudoacrosomal granular matter, investing filaments no. 3–8, vp = ventral rod of pseudoacrosome, vr = ventral ribbon.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and 5) minimal amounts of cytoplasm with remnants of smooth endoplasmic reticulum and a superficial cell membrane.

For descriptive purposes the axonemal aspect of the spermatozoon is called dorsal, the nuclear aspect ventral (see Folliot and Maillet 1970 p. 297).

The nucleus (Fig. 1:B–E, Plates 5:16, 6:18) has a highly condensed chromatin core, and appears compact black or indistinctly granular in EM-pictures. It is surrounded by the two usual nuclear membranes.

The mitochondria are three filiform, continuous rods, extending throughout the entire body. The median rod lies between axonema and nucleus, the other two form a pair of symmetrical wings in the cross section, closely attached to the median one (Fig. 1:B, Plate 4:16). The mitochondria are bounded by two unit membranes like normal mitochondria, but the cristae are poorly developed. The median mitochondrion has no cristae at all in the mature spermatozoon, whereas one or two folds of the inner membrane are seen in cross sections of the lateral mitochondria (Plate 6:18, Plate 17:83).

The axonema has the 9+2 pattern (Plates 5:16, 17:83). The central pair is always symmetrically placed, one on each side of the median plane, and the peripheral doublet 1 is dorso-median. An astonishing feature, observed by Doucet (1965), is the constant presence of a dark line, connecting the dorsal peripheral doublet (no. 1) with the high-contrast sheaths surrounding the axonema. Since this line is constant in good cross sections, it must represent a continous membrane, extending from the filament no. 1 to the sheath. The membrane is always obliquely oriented, being the only asymmetrical structure in the spermatozoon, if the arms on the axonemal doublets are excepted (Plates 5:16, 17:83). From its attachment to the doublet, the membrane is inclined to that side where the arms of doublet 1 are situated.

The high-contrast sheaths around the axonema have a complex origin, which will be described later. The main components are two half-cylindrical sheaths, which together form a complete, tube-like investment of the axonema (Fig. 1:B). These two half-tubules will be called *dorsal and ventral ribbons* in the subsequent descriptions. The dorsal ribbon covers the dorsal half of the periphery of the axonema. Its free margins are sharp edges, which fit into furrows in the thickened free margins of the ventral ribbon (Fig. 1:B, Plates 5:16, 17:83). The core of the ribbons is a dark-staining granular substance, in which some light areas are found in constant location: One sickle-shaped median area in the dorsal ribbon, and a symmetrical pair of light spots in the ventral ribbon.

The outer surfaces of the dorsal ribbon are formed by double unit membranes, both on the convex and the concave sides (Fig. 1:B). They are the remnants of two flat membrane-bound sacs, which, in spermatids, surround the growing ribbon from the dorsal and ventral sides (Plates 16:80, 17:83). The two membranes are only visible in very thin and fortunate sections of the mature spermatozoon, because the lumen has disappeared and the two membranes are closely attached to each other. The ventral ribbon is not associated with membranes of this kind.

The ventral ribbon is continuous with thin lamellae of dark matter, which cover the dorsal and lateral aspects of the lateral mitochondria (Fig. 1, Plate 5:16). Although these sheaths sometimes can be resolved into two tightly attached dark lines, they are probably not built of unit membranes, for distinct membranous sacs are not observed here during spermatogenesis.

The ventral ribbon is also continuous with the more or less distinct dark matter which fills the narrow space between the lateral and median mitochondria on each side (Plate 17:83, compare Fig. 1:B).

The cytoplasm is not abundant and is covered by a seemingly normal cell membrane, forming the outer surface of the spermatozoon. In cross sections, a few flattened sacs can be seen on each side in the narrow space between lateral mitochondrion and nucleus. These sacs are remnants of the smooth endoplasmic reticulum, which is well developed in spermatids (Plates 5:16, 17:83).

Towards the *posterior end* the body gets thinner, depending on decrease in thickness of nucleus and mitochondria. However, the typical cross section with nucleus, three mitochondria, axonema, ribbons, etc., extends to a point about 4–5 μ from the extreme end. At this point the nucleus ends, and the ventral ribbon disappears at about the same level (Fig. 1:A, G, and H, Plates 4:14, 7:29). The mitochondria extend a little further but become very narrow. The filaments of the axonema begin to become irregular and fewer in number from the level where the nucleus ends, but a few axonemal filaments together with the dorsal ribbon form the very tip of the spermatozoon. A free flagellum with normal structure can therefore not be recognized. Irregular clumps of protoplasm, often with some large vacuoles, are regularly seen bulging out from this terminal part of the spermatozoon (Plates 4:14, 7:29).

The anterior end of the body is attached to the long pseudoacrosome. In the transitional region the axonema continues into the base of the pseudoacrosome as a strongly modified centriole (Fig. 1:D,E). The nucleus, at this level very thin and consisting mainly of the narrow tube of nuclear membranes, ends at the level of the pseudoacrosomal base, and the mitochondria reach approximately the same point. The dorsal ribbon is directly continuous with the "dorsal rod" of the pseudoacrosome (see below). The ventral ribbon seems to be continuous with the "ventral rod" of the pseudoacrosome, but development shows that this connection is secondary and is established by the plentiful dark-staining matter, which tends to obscure the region of transition.

The original triplets of the centriole change to doublets at an early stage of spermatogenesis, as will be described in a separate chapter. In cross sections of mature spermatozoa these doublets are seen as pairs of light spots in the plentiful dark matter in which they are embedded (Fig. 1:E, Plate 7:21,22). Although this matter obscures some details, a very constant pattern is repeated in all sections through the transitional region. The three dorsal doublets (nos. 9, 1 and 2) converge and are embedded in a common dorsal mass of the dark pseudoacrosomal matter, continuous with the dorsal rod of the pseudoacrosome. The six ventral doublets (nos. 3–8) are in a similar way cemented together by pseudoacrosomal granular substance continuous with the ventral

rod of the pseudoacrosome (see also Plates 6:20, 22:114–116). Details will be described in connection with spermatogenesis.

Internal structure of pseudoacrosome

The long, arched pseudoacrosome is covered by an ordinary cell membrane, immediately followed by two more unit membranes, which are continuous all around the cross section (Fig. 1:F, Plate 7:23–24). The latter are the remnants of a smooth endoplasmic reticular sac, which surrounds the growing pseudoacrosome in the spermatids. The space inside the membranes is occupied by the two pseudoacrosomal rods, a dorsal and a ventral one, which look almost identical in cross sections of mature spermatozoa. Both consist of dark-staining, granular matter which surrounds a light-staining central area (Fig. 7:24).

Several EM-pictures of total spermatozoa show that the ventral rod, as defined by the proximal attachment to the ventral filaments of the centriole, follows the convex side of the curvature of the pseudoacrosome. At the point, the dorsal rod ends 0.4–0.9 μ before the ventral one, so the very tip is supported by the ventral rod alone (Plate 4:12). On the dorsal side of this single rod is a granule of moderate contrast, about 0.2 μ in diameter. It has been seen and photographed in five different spermatozoa which had been spread on grids after osmic fixation and had their anterior ends intact (Fig. 1:A, Plate 4:12). Development shows that this granule is a derivative of the so-called granulosome in spermatids (see spermatogenesis).

Movements

Spermatozoa spread in Ringer solution from the receptaculum seminis survive for 15 minutes or more. They swim rapidly by undulating movements of the body, whereas the pseudoacrosome is kept stiff and is bent to one side, usually in the half-circular fashion seen in Fig. 1:A. This asymmetry results in constant deviation to one side, when the undulations push the spermatozoon forwards. The path described is therefore more or less circular. The spermatozoa were studied with phase contrast. As they were mounted under a cover glass and only had possibilities for movements in two directions, it is unknown how they behave in free liquid.

Spermatogenesis

Spermatogenesis in *Raillietiella* has been analyzed in considerable detail in order to facilitate interpretation and characterization of the many strange structures. The analysis was greatly facilitated by the simultaneous development of all spermatids in each cyst: numerous sections at different levels could be obtained of spermatids of a defined stage. Nevertheless, between 50 and 150 EM-pictures were often necessary to give a reasonably complete picture of a particular stage.

In the following, the general course of spermatogenesis will be described stage by stage with emphasis on features characteristic of *Raillietiella*. At the end of the chapter,

a few pages will be devoted to the development of the complicated transitional region between pseudoacrosome and body.

From spermatogonia to early spermatids

This part of spermatogenesis is of an ordinary type. The regular, polygonal cells (Plates 3:6,8, 8:32) reach maximum size with a diameter of ca. 10 μ as spermatocytes 1, just before maturation starts. Spermatogonia and early spermatids are smaller, 6–7 μ. The nuclear diameter is large in spermatogonia and spermatocytes 1 (4.5–5.7 μ) but is reduced to 3–3.9 μ in early spermatids. The cytological picture during this development is characteristic of growth and synthesis: numerous nuclear pores, numerous free ribosomes or polysomes in the plasm, and a large (1.3–1.9 μ) nucleolus.

In early spermatids the nuclear pores and free ribosomes are still abundant, but the nucleolus is smaller, about 0.6–0.7 μ.

Two structures are of particular interest for the subsequent development: mitochondria and centriolar structures.

The mitochondria are rod-shaped or tubular in spermatogonia and spermatocytes 1, with a diameter of 0.2–0.3 μ in spermatogonia and 0.3–0.4 μ in spermatocytes (Plate 9:46). The length is difficult to ascertain in sections, but pieces as long as 6 μ have been measured. At the end of meiosis the mitochondria change drastically. They become spherical with a diameter of ca. 1 μ and are concentrated to a group of eight or more in the neighbourhood of the nuclear membrane of early spermatids (Plate 9:48, Fig. 2). Some mitochondria probably degenerate during this process, but definite proof is difficult to obtain. In spermatocytes and during meiosis dark-staining rods are seen between the scattered, tubular mitochondria. Some of the dark rods have double outer membranes and crista-like folds of the inner membrane, indicating a mitochondrial origin. The majority of these dark bodies have simple membranes and a granular or multivesicular content. In spermatids such bodies are present but lack all mitochondrial characteristics and are seen as a group of dark, membrane-bound bodies, distinctly separated from the group of mitochondria (Figs. 2–5).

The centrioles. Two centrioles, oriented perpendicular to each other, were seen in several spermatocytes 1 (Plate 8:38). This stage could be definitely identified with the help of mitochondrial and cellular size and the presence of well-developed synaptonemal complexes in the nucleus. Obviously no duplication of the centriole takes place after the 2nd meiotic division, for only one centriole was found in numerous early spermatids 1, which were studied in several spermatocysts. The length of the centriole is 0.8–1.0 μ in early spermatids and in 2nd meiotic cells (Plate 8:39,40), whereas shorter centrioles measuring only about 0.6 μ were encountered in spermatocytes 1 (Plate 8:38).

The bilateral structure, characteristic of the centriolar complex of spermatids, develops already before the 2nd meiotic division. Cross sections of 2nd meiotic centrioles show two dark outer rods, following one side of the centriole (Plate 8:32). This side is the prospective dorsal side of the centriole, and the rods are the first anlage of the

dorsal ribbon. In early spermatids the two rods are still present and are covered by a dark membrane, formed by a flat membranous sac continuous with the smooth endoplasmic reticulum (Plate 8:33). This sac is the prospective outer sac of the dorsal ribbon. The dark rods and the outer sac are definitely not present in spermatocytes 1, so they must be formed during meiosis.

The characteristic structures at the prospective proximal end of the centriole also appear during or just before meiosis. These structures are: 1) a body of granular substance, here called "*granulosome*", attached to the proximal end of the centriole, and 2) the "*apical membrane*", which is a dark, membrane-like condensation of the granular material across the end of the centriole (Figs. 2–4 and 11, Plate 8:41). The granulosome may be traced but is not so well defined during meiosis and in spermatocytes 1 (Plate 8:38,39). The apical membrane is distinctly present in 2nd meiosis centrioles (Plates 8:39), but probably begins to form already in late spermatocytes 1 (Plate 8:38). In these early stages the apical membrane is ring-like, covering only the free edges of the centriolar tube (see also Plate 8:40).

"Granulosome" and "apical membrane" are tentative names, chosen to avoid premature identification with previously described structures such as "centriole adjunct", "acroblast", "acrosome granule", "post-nuclear body", etc.

The granulosome and the apical membrane, from which the pseudoacrosome later develops, as well as the anlage of the dorsal ribbon, are thus present as pericentriolar structures already in meiotic cells. They develop in close contact with the centriole without direct contact or interference of the nucleus. These structures make the centriolar complex bilaterally symmetrical already in late meiosis, so the prospective dorsal and ventral sides, as well as proximal and distal end, can be recognized already at this early stage.

Spermatid 1; with several mitochondria

Sections of early spermatids, often with remnants of meiotic spindles, always show the above-mentioned group of spherical *mitochondria* near the nucleus (Fig. 2, Plate 9:48). Usually a single section shows five to eight mitochondria in the group, so there must obviously be more than eight in many cases.

The centriole in these cells is always in contact with the cell membrane by one end. Usually the membrane is indented to form a pit at the point of contact. A short flagellar rudiment may be formed from the bottom of the pit, but it is purely plasmatic, without axonemal filaments (Fig. 2, Plate 11:56).

As mentioned above, the centriole has developed a distinct granulosome, apical membrane, two dark dorsal fibers and a dorsal, (outer) membranous sac (Plate 8:33, 11:56). The proximal end with the granulosome lies free in the plasm although it sometimes is near the nucleus, as in Plate 11:56. The centriole has thus not established the contact with nucleus and mitochondria so typical of later stages.

The nucleus has many pores and a rather large nucleolus. The cytoplasm contains the above-mentioned group of dark, membranebound bodies (Fig. 2), and is

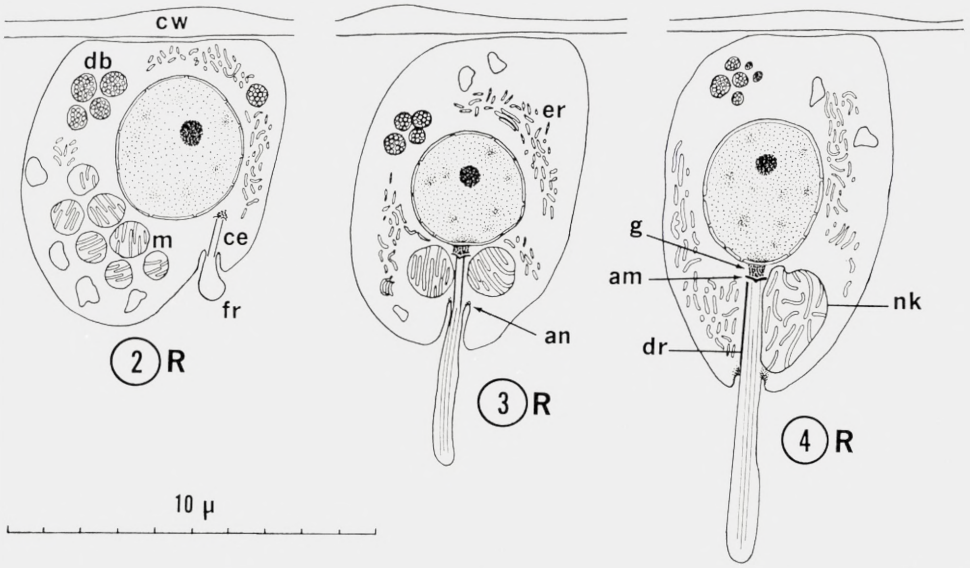

Fig. 2. *Raillietiella*, spermatid, stage 1. with numerous mitochondria.
Fig. 3. *Raillietiella*, spermatid, stage 2. with three mitochondria.
Fig. 4. *Raillietiella*, spermatid, stage 3. with "Nebenkern". All diagrams based on light microscopical pictures for gross dimensions and on numerous EM pictures like those in Plates 8–11 for details.
am = apical membrane, an = annulus-like thickening around the flagellum in the bottom of the flagellar pit, ce = centriole, cw = wall of spermatocyst, db = dark bodies, perhaps in part degenerating mitochondria, dr = dorsal ribbon, er = smooth endoplasmic reticulum, fr = flagellar rudiment, g = granulosome, m = mitochondria, nk = "Nebenkern".

rich in smooth endoplasmic reticulum, which forms irregular tubules and vesicles. Rough endoplasmic reticulum with ribosomes is very rare and, when seen, of little extension. Stacks of Golgi vesicles have been seen a few times but are rare and very small. They have no relation to the structures described here. Free ribosomes, usually in small clusters, are abundant (Plate 11:56).

Spermatid 2; with three spherical mitochondria

Mitochondria. The three spherical mitochondria of this stage are large, 1.3–1.7 μ, and have numerous, distinct cristae (Plates 9:50, 10:54, 11:59). They are arranged in a triangular fashion in contact with the nucleus and touching each other two and two. The contact between the mitochondria is characterized by slight flattening of the membranes and accumulation of medium-contrast matter in the interspace (Plate 9:50).

The final reduction of the spherical mitochondria from more than eight in the preceeding stage to three in the present stage probably takes place by fusion. This is indicated by pictures which are believed to show stages of this process: Two distinctly different, almost spherical mitochondria in contact with each other, with common

outer membrane but separate inner membranes (Plate 9:47). The other possibility, that some mitochondria degenerate, is practically ruled out by the absence of degenerative symptoms within the group of spherical mitochondria.

The axonema grows out from the centriole into the pre-formed plasmatic flagellum during this stage (Fig. 3, Plate 11:57,59). No transverse disc or similar structure has been seen between the centriole and axonema at any stage of development. This makes the shift from centriole to axonema rather indistinct, so a definition is required. In the following, all parts lying proximal to the point where the central filaments begin are called centriole.

As seen in the figures (Plate 11:57,59), the axonema is restricted to the free flagellum at this stage: The central filaments begin at the level of the bottom of the flagellar pit. Here, around the base of the flagellum, there is a thickening of the cell membrane which probably maintains the shape of the pit. This thickening is called the annulus, although it is associated with very little dark matter in the inside plasm (Plates 11:59, 8:41).

The centriole complex has changed in some important respects. The membranous sac, covering its dorsal aspect, is still present and its inner wall is thickened to form a dark membrane, which arches over the centriole in a cross section (Plate 8:34). But under this membrane there are three dark rods, not two as in previous stages. The change from two to three dark rods during this developmental period is well documented but details are not well understood (compare Plate 8:32–34).

The three dark fibers under the membranous sac correspond to the three dorsal filaments of the axoneme: nos. 9, 1 and 2. Dark outer fibers have also appeared outside the other filaments (Plate 8:34). Of these, nos. 3 and 8 could not be followed further, but nos. 4–7 are often distinct in later stages and are included in the ventral ribbon (Plate 16:76, 80).

During meiosis and in spermatid 1 the filaments of the centriole appear as more or less distinct, obliquely oriented triplets. This picture has changed in spermatids 2, where well-separated doublets are found in cross sections (Plate 8:33,34).

The relation between nucleus, centriole and mitochondria is established during this stage. Early spermatids 2 show nucleus, mitochondria and centriole lying independent of each other (Plate 10:54). In other, more advanced spermatids 2, the granulosome is distinctly attached to the nuclear membrane and the centriole lies in the furrow between two of the mitochondria (Plates 8:34,41, 11:57,59). At the contact point with the granulosome there is an accumulation of dark matter on the inner side of the inner nuclear membrane (Fig. 3, Plate 8:41).

The centriole-axonema always passes from the flagellum to the nucleus in the cleft between two of the mitochondria. In later stages the prospective dorsal ribbon with the endoplasmic membranous sac is always on that side of the axonema which is opposite to the mitochondrial triplet, as seen in Plates 8:34 and 10:52. In the present stage this relation is variable, and the anlage of the dorsal ribbon is sometimes on the side facing the mitochondria. This must mean that the orientation of the centriole

and the dorsal ribbon takes place after the contact with the mitochondria is established.

Other cytoplasmic organelles are not very different from those of spermatids 1.

Spermatid 3; with mitochondrial "Nebenkern"

Mitochondria. The single mitochondrial "Nebenkern" seen with the light microscope in spermatids of this stage consists of the three mitochondria of the previous stage, which have been packed together to form a single body (Fig. 4, Plate 10:52). The Nebenkern is about 2.5 μ in diameter and is bilaterally symmetrical in relation to a median plane through the centriole. The limits of the original mitochondria are only faintly indicated as furrows on the outer surface but are distinctly marked by the intact mitochondrial membranes inside the body. Thus, the three giant mitochondria maintain their individuality and never fuse to one body in the way described for many arthropods and molluscs (Favard and André 1970). The space between the mitochondria is filled with a medium-contrast matter, making the attachment desmosome-like (Plate 10:52).

The Nebenkern with its single median and two symmetrical lateral mitochondria has a shallow furrow for the centriole and axonema. In this furrow all three mitochondria face the centriole-axonema with rather sharp, straight edges (Plates 10:52). These edges of all three mitochondria extend from the granulosome to near the annulus, where the axonema enters the free flagellum (Fig. 4).

Centriole-axonema. In earlier stages the centriole entends to the annulus, and the axonema, when it grows out, is restricted to the free flagellum (Fig. 3). In spermatids 3, more and more of the axonema is included in the cell body proper, so the annulus moves away from the end of the centriole, and the posterior part of the cell behind the nucleus is elongated (Fig. 4). This posterior migration of the annulus continues to the end of spermatogenesis. When the axonema inside the cell body grows, the anlages of the dorsal ribbon and the mitochondria grow correspondingly, so their tips keep contact with the annulus but never enter the free flagellum (Figs. 4–10, Plate 11:58).

Spermatid 4; anterior dislocation of centriole

The flagellum grows out from that wall of the cell which faces the inner, cleft-like lumen of the spermatocyst. This end of the cell is the prospective posterior one, whereas the end in contact with the cyst wall is the prospective anterior one. The point of contact of the granulosome-centriole is originally on the posterior surface of the nucleus (Fig. 3). After formation of the "Nebenkern" the point of attachment shifts over to the side and is finally found near the anterior end of the now more elongate nucleus (Fig. 5 and 6, Plate 12:60,61). After this has occurred, the centriole-axonema, like the dorsal ribbon and mitochondria, extends along one side of the nucleus to reach the anteriorly situated point of attachment.

This dislocation of the centriole could be explained by assuming that the nucleus rotates, as suggested for *Lepisma*, in which a similar dislocation takes place (Werner

1964). Another possibility is that the granulosome-centriole slides along the nuclear surface. Both possibilities are in fact open in *Raillietiella*. Conditions in *Argulus*, where a true acrosome is present as a fixed point on the nuclear surface, are definitely in favour of the sliding alternative.

Mitochondria. During dislocation, the centriole-axonema migrates up along the prospective dorsal side of the nucelus, with the dorsal ribbon on the ab-nuclear side (Fig. 4, 5 and 6). The main bulk of the mitochondrial Nebenkern remains behind the nucleus, but all three mitochondria are extended as narrow tongues between the axoneme and the nucleus, their tips remaining in contact with the centriole (Plate 15:72). The mitochondrial tongues are symmetrically situated with one median and a pair of lateral ones like the mitochondria of the Nebenkern (compare Plate 13:65).

The mitochondria are also prolonged as narrow tongues posteriorly, along the ventral aspect of the axonema. These posterior mitochondrial tongues, like the anterior ones, are arranged symmetrically in relation to the median plane (Plates 15:73, 16:76). The posterior tongues grow in proportion to the lengthening of the cell, so the tips remain in the neighbourhood of the flagellar pit.

Dorsal ribbon. During this stage a definite dorsal ribbon begins to form above the centriole and anterior axonema. The three large dark fibers above the flagellum fuse and form a dark plate, covered by the dark sheath formed by the outer membraneous sac of endoplasmic reticulum (Plates 10:52, 16:76,80. Compare Figs. 11:B,C and 12). A second flat membranous sac appears between the dark core of the dorsal ribbon and the axonema, with lateral connections to the endoplasmic reticulum. One of the walls of this inner sac forms another dark sheath, covering the core of the dorsal ribbon from the ventral side. The growing dorsal ribbon therefore consists of a granular core, formed by the dark outer fibers 9, 1, and 2, delimited by two dark sheaths, formed by the outer and inner membranous sacs (Fig. 12:F).

Differentiation of the dorsal ribbon appears to proceed from the centriole backwards, so very immature stages with three dark fibers can be seen near the flagellar pit. The other outer fibers are also prolonged in a posterior direction to the neighbourhood of the flagellar pit, where particularly the fibers 4–7 can be very distinct (Plate 16:76).

Centriolar apparatus. During dislocation of the centriole the apical membrane appears to be detached, while the granulosome remains attached to its anterior surface (Figs. 5,6,11 B, Plate 12:60,61). The granulosome loses its contact with the nucleus and is seen as a granular pellet on the extreme top of the growing pseudoacrosome of later stages (Figs. 6–10). New granular matter appears behind the apical membrane, in the space delimited by this membrane, the nucleus, the centriole and the mitochondria (pm in Figs. 5–7). This matter plays an important role in the development of the pseudoacrosome, and will therefore be called "pseudoacrosomal granular matter" or PGM. It condenses to form a dark pellet on the posterior side of the apical membrane (pm in Fig. 11), whereas a more diffuse "ventral portion" fills out the space near nucleus and mitochondria (vm in Plate 12:61).

The centriole is covered by a developing dorsal ribbon like the axonema, with inner and outer membranous sacs and granular core. The inner sac, like the granular

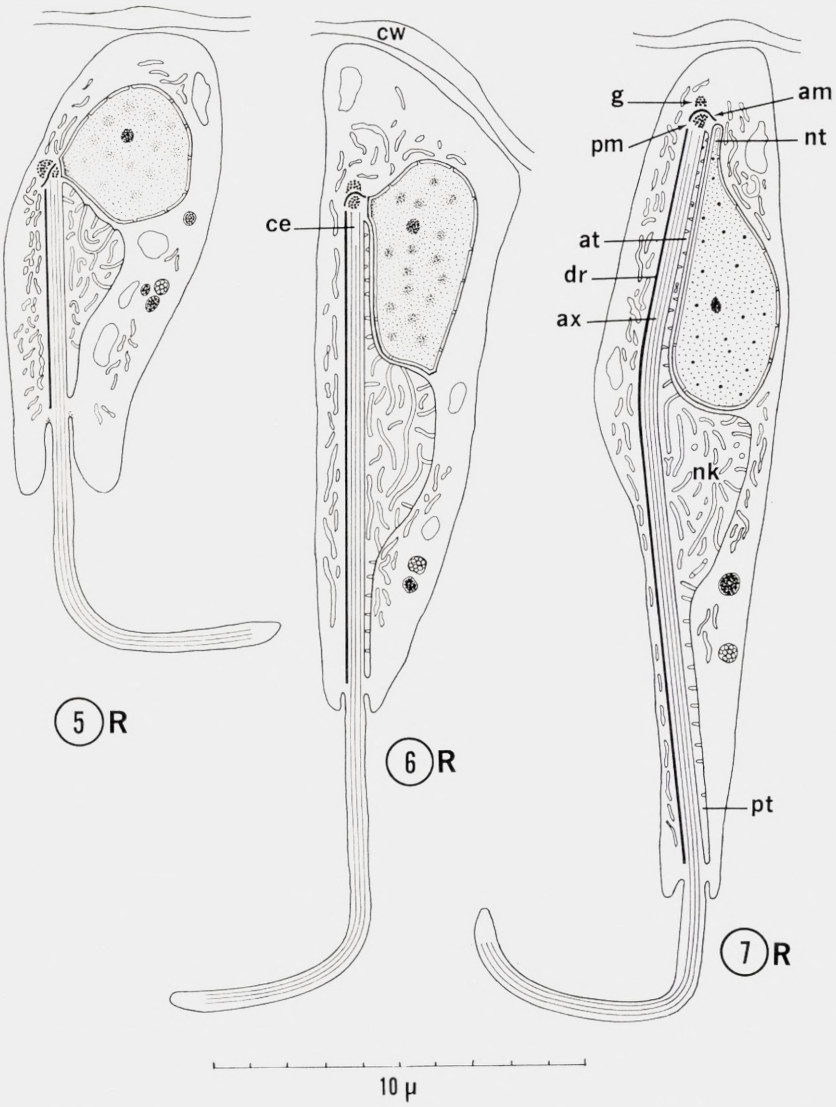

Fig. 5. *Raillietiella*, spermatid stage 4, beginning migration of centriolar complex.
Fig. 6. *Raillietiella*, spermatid, late stage 4.
Fig. 7. *Raillietiella*, spermatid, early stage 5, development of the nuclear tongue (nt). All diagrams are based on light microscopical sections for gross dimensions and on numerous EM pictures like those on Plates 12, 13, 15 and 16 for details.
am = apical membrane, at = anterior tongues of mitochondria, ax = axonema, ce = centriole, cw = cyst wall, dr = dorsal ribbon, g = granulosome, nk = "Nebenkern", nt = anterior nuclear tongue, pm = pseudoacrosomal granular matter, pt = posterior tongues of mitochondria.

core, ends above the anterior part of the centriole, but the outer sac spreads to cover the anterior surface of the apical membrane (Fig. 11:B). This membranous sac excepted, there is little continuity between the structures of the dorsal ribbon and the apical membrane (Plate 12:61, Fig. 11).

Spermatid 5; formation of nuclear tongue and of pseudoacrosome

After the centriole is dislocated forwards, it is connected with the nucleus by the ventral portion of the PGM (Fig. 6, 11:C). The contact area is situated on a slight elevation of the nuclear membrane. The further development includes excessive growth of this elevation, which develops into an anteriorly directed nuclear tongue, with the contact point at the top (Figs. 7, 8, 11:C–D). The dorsal ribbon, the axonema, and the three mitochondrial tongues follow this elongation, so a narrow rostrum containing all these structures is formed (Figs. 7–8, Plate 13:64,65). The main bulk of the mitochondrial Nebenkern still remains behind the thicker posterior part of the nucleus.

The pseudoacrosome begins to grow out from the anterior rostrum, all the time carrying the granulosome on its top (Figs. 8–9, Plates 13:65, 23:117). The granulosome sits on a transverse membrane, which is identical with all or part of the previously described apical membrane (Figs. 8–10, Plates 12:61, 13:64,65, 23:117). The growing pseudoacrosome is surrounded by a flat membranous sac in communication with the endoplasmic reticulum (Plates 20:99–101, 21:106–107). This sac develops as a forward extension of the outer sac of the dorsal ribbon, which spreads over the apical membrane and down the sides of the growing pseudoacrosome, which finally is completely surrounded (ps in Fig. 12). Below the anterior end of the centriole, this sac fails to fuse ventrally, so there is an opening in the membranes in front of the nucleus (Fig. 12:A, D).

Granular matter (PGM) condenses on the inner side of the pseudoacrosomal sac to form two, eventually half-cylindrical, dark sheaths. These are called *dorsal and ventral sheaths* in the following (Fig. 12:B, C, Plate 20:99–101). The space inside the sheaths is filled with PGM, which is remodelled several times during the subsequent development.

The ventral sheath is directly continuous with the apical membrane. In early stages of pseudoacrosome development, the apical membrane appears to be bent over on the ventral side, and the half-cylindrical ventral sheath seems to grow posteriorly from its ventral margin (Fig. 12:A, Plate 13:64). At the base of the pseudoacrosome the ventral sheath is open in the midline and continues as two wing-like plates to the sides of the centriole (Fig. 12:D, Plate 22:114–116).

The dorsal sheath is originally a narrow, band-like accumulation of granular substance, continuous with the outer dark sheath of the dorsal ribbon (Fig. 12, Plate

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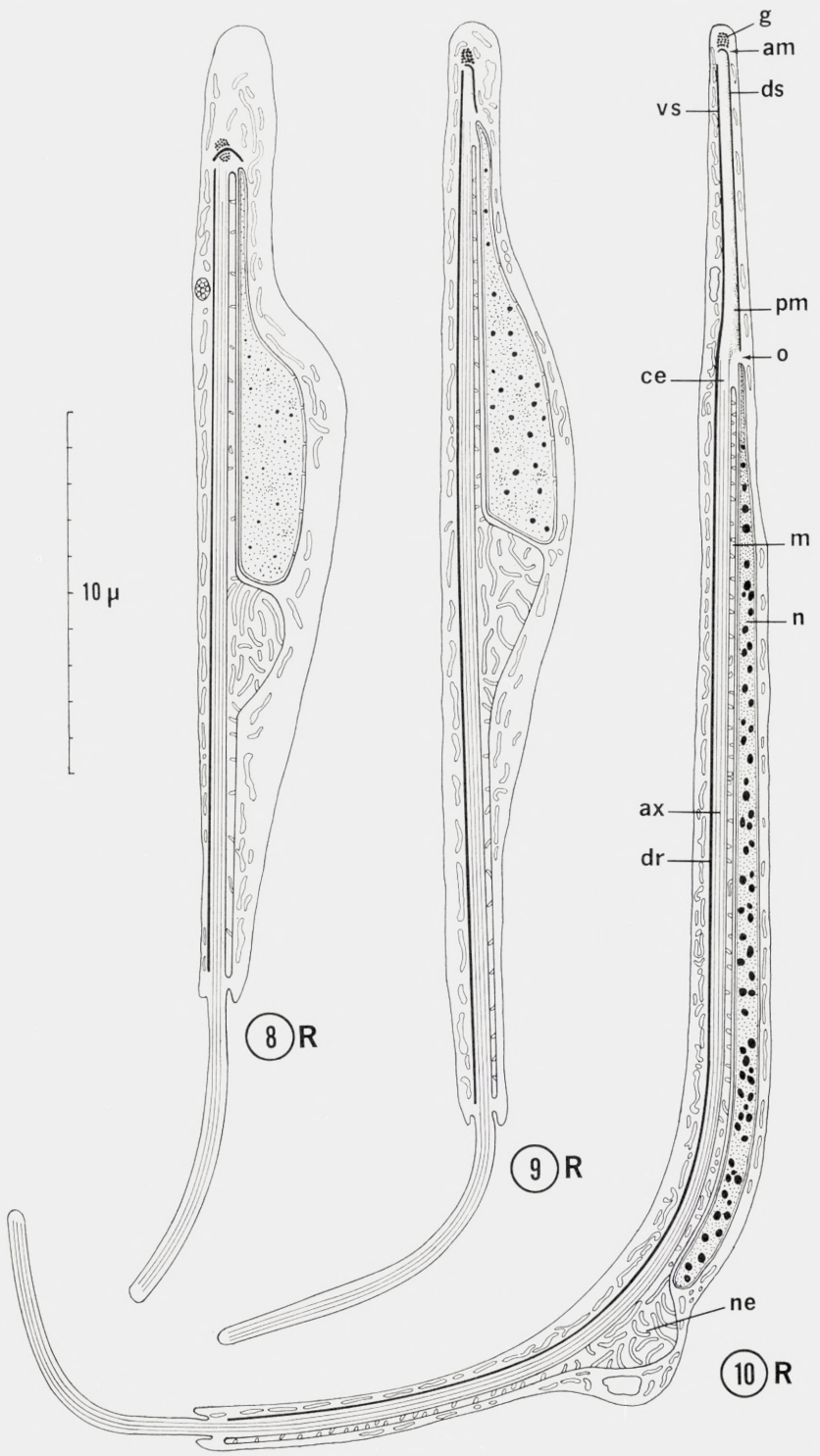
Fig. 8. *Raillietiella*, spermatid stage 5, additional lengthening of nucleus.
Fig. 9. *Raillietiella*, spermatid, late stage 5, beginning formation of pseudoacrosome.
Fig. 10. *Raillietiella*, spermatid stage 6. Final elongation of nucleus, pseudoacrosome and mitochondria. All diagrams are based on light microscopical sections for gross dimensions and on numerous EM pictures like those on Plates 13, 17, and 20–23 for details.
am = apical membrane, ax = axonema, ce = centriole, dr = dorsal ribbon, ds = ventral sheath of pseudoacrosome, g = granulosome, m = mitochondria, n = nucleus, nk = "Nebenkern", o = opening between anterior end of nucleus and posterior margin of ventral acrosomal sheath, pm = pseudoacrosomal granular matter, vs = dorsal sheath of pseudoacrosome.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20:99–101). Later the dorsal sheath, like the ventral one, grows out to become half-cylindrical (Plate 21:106–107). For a long time it remains somewhat smaller than the ventral one, and thins out before reaching the apical membrane.

The granular matter (PGM) inside the sheaths is condensed to form an irregular tube-like structure in early pseudoacrosomes (Plate 20:99, 100). Later the tube collapses and the granular matter typically forms a median lamella, attached to the dorsal sheath and partly stuck in the half-cylindrical ventral sheath (Plate 20:101). In still more advanced pseudoacrosomes of stages 6 and 7 the PGM is divided into two portions, which fill out the dorsal and ventral sheaths, respectively, and are separated by a horizontal cleft (Plate 21:106–107). These sheaths with their contents are then transformed to the dorsal and ventral rods of the mature spermatozoon.

The nucleus still has a small nucleolus when it begins to emit the anterior tongue, and several nuclear pores are present. These structures have not been seen after the pseudoacrosome has begun to grow out. Instead several small, compact accumulations of chromatin are seen as black dots in the nucleoplasm as a first sign of nuclear condensation (Plate 13:64, 65, Figs. 8, 9).

The cytoplasm of stage 5 spermatids contains numerous small smooth-surfaced vesicles and sacs of endoplasmic reticulum, while rough-surfaced vesicles and Golgi-stacks are very rare and small, as in earlier stages. Ribosomes and polysomes are still numerous. A system of longitudinal microtubules has developed as an almost continuous layer along the growing pseudoacrosome and, as more scattered tubules, along the elongating anterior end of the nucleus (Plate 20:99–101).

Spermatid 6; elongation of nucleus and mitochondria, formation of ventral ribbon

The nucleus. During previous stages the main bulk of the nucleus is a thick ovoid body, from which the narrow anterior tongue grows out (Figs. 7, 8). In the present stage the main body of the nucleus elongates strongly and extends backwards below the axonema (Fig. 10). The Nebenkern remains in the position behind the end of the nucleus and decreases in size, while the mitochondrial rods along the axonema increase in length.

The chromatin of the nucleus is in a state of progressive condensation. The small clumps of chromatin seen in stage 5 grow to large round bodies, which in later stages collapse to form the compact nuclear rod of the mature spermatozoon (Fig. 10, Plate 17:82–83).

The ventral ribbon forms between the axonema and the mitochondria. Differentiation appears to proceed from the centriolar region backwards, for differentiation is generally less advanced near the posterior end. Orginally the outer dark fibers 4–7 are situated in the region where the ribbon develops, whereas fibers 3 and 8 seem to disappear (Plate 16:76). In more advanced stages these dark fibers are less distinct and a dark lamella of unknown origin appears on the ventral aspect of the axonema and extends over the dorsal surface of the lateral mitochondria (Plate 16:80, Fig. 12:F). This lamella together with the outer mitochondrial membranes delimits a space in

which the four dark fibers and an increasing amount of dark-staining matter is present (Fig. 12:F). Finally all these dark-staining components fuse to form the ventral ribbon which, therefore, is supposed to contain the outer dark fibers 4–7 of the axonema. The dark lamella which was mentioned as one of the components is probably not a unit membrane, although it has approximately the same thickness. It is not sharply delimited as a typical unit membrane, tri-lamination has never been seen, and no connection with endoplasmic reticulum has been observed. The light cores which become visible in the final stages of spermatogenesis could well be the dark outer fibers of doublets 5 and 6, just like the light core of the dorsal ribbon appears to be formed by the fused outer fibers 9, 1 and 2.

The dorsal ribbon extends laterally down the sides of the axonema but final contact with the ventral ribbon is not obtained until very late stages. More dark matter is deposited in contact with the inner and outer sacs, forming the compact dark cortex of the mature ribbon. The granular core, originally formed by outer dark fibers 9, 1 and 2, appears to persist and to change stainability, forming the light core of the mature ribbon (Fig. 12:F, Plates 16:80, 5:16, 22:116).

In the *pseudoacrosome* the granular matter fills the half-tubular sheaths and is divided by a horizontal cleft, so the dorsal and ventral rods are distinctly separated (Plate 21:106–107).

Numerous *microtubules* are seen along all parts of the growing spermatid of this and the following stage.

Spermatid 7; final transformation to mature spermatozoon

The development from stage 6 to mature spermatozoon includes additional elongation of all parts (compare Figs. 10 and 1). At all levels the spermatid is surrounded by numerous microtubules, which must be eliminated before final maturation, for they are absent in the free mature spermatozoa (Plates 21:107, 17:83).

Nucleus, ribbons, and mitochondria extend to near the posterior end of the axonema, and the free flagellum is eliminated. Details of this process have not been studied.

The *pseudoacrosome* is reorganized at the top, so the ventral rod overshoots the dorsal one with a few microns, and the granulosome slides over to the dorsal side of the single terminal rod. That this takes place before liberation of the spermatozoa could be seen in fortunate sections of a nearly mature spermatocyst, in which some granulosomes remained terminal on the top of the rods, whereas others had slid over on the side of one of the rods (Plate 23:118).

Detailed analysis of the pseudoacrosome and of the transitional region between pseudoacrosome and body

The precise derivation of the pseudoacrosome and its relation to other structures is of fundamental interest for comparisons. Its development will therefore be considered separately here.

In spermatids 4–5, just before pseudoacrosome development starts, there is an anterior rostrum containing the nuclear tongue, the three mitochondrial tongues, the centriole, and the dorsal ribbon (Fig. 11:C, D). The anterior ends of these structures are at approximately the same level; only the mitochondria are a little shorter. The centriole, the mitochondria, and the nucleus are in contact with the pseudoacrosomal granular matter (PGM), which has condensed to form a dark granule at the end of the centriole, whereas the more scattered ventral portion of PGM is in contact with nucleus and mitochondria (Cf. Plate 12:61). Anteriorly the PGM is covered by the apical membrane, over which the flat membranous sac of the dorsal ribbon extends. The apical membrane carries the granulosome on its anterior surface.

When the pseudoacrosome grows out, it is quite obvious that its dorsal sheath is formed directly as an anterior prolongation of the outer sheath of the dorsal ribbon. The outer endoplasmic reticular sac of the latter grows forwards, and dark matter, continuous with that of the ribbon, accumulates on its inner surface to form the dorsal sheath (Fig. 12:A, C–E).

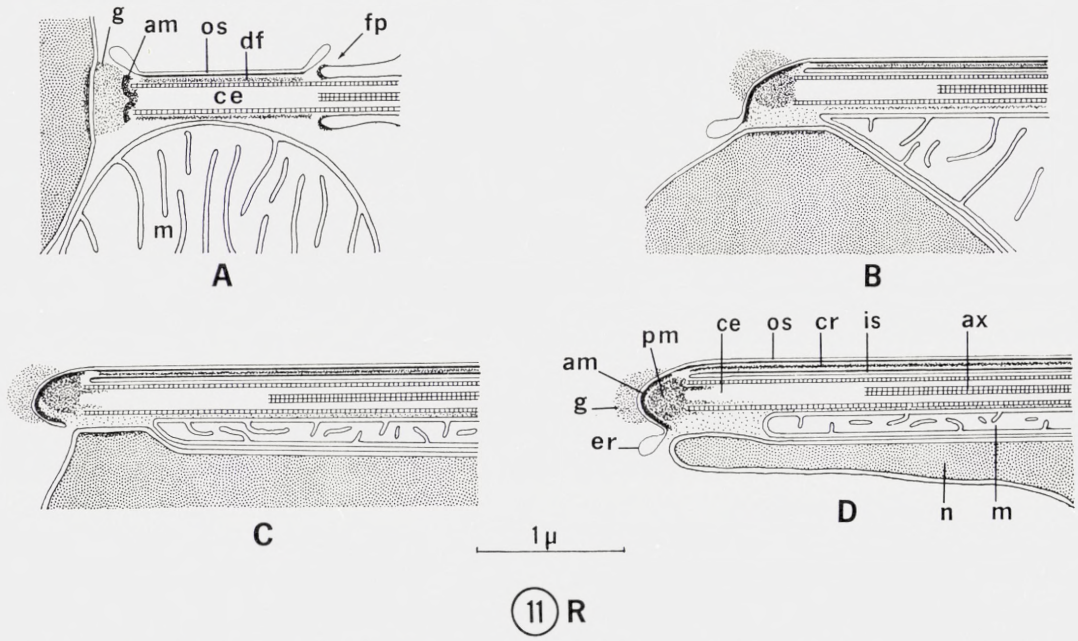

Fig. 11. *Raillietiella*, diagrams showing the development of the centriolar region from spermatid stage 2 (Fig. A), through early stage 4 (Fig. B) and late stage 4 (Fig. C) to stage 5 (Fig. D)
Originally, the apical membrane is not associated with membranous sacs (Fig. A), but later the outer sac of the dorsal ribbon appears to spread over its surface (Figs. B–D). In Fig. A only the outer sac of the dorsal ribbon is present. In Figs. B–D the inner sac has appeared between the axonema and the dark matter formed by the dark outer fibers.
am = apical membrane, ax = axonema, ce = centriole, cr = granular core of dorsal ribbon, df = dark outer filaments of centriole, er = endoplasmic reticulum, fp = flagellar pit, g = granulosome, is = inner membranous sac of dorsal ribbon, m = mitochondria, n = nucleus, os = outer membranous sac of dorsal ribbon, pm = pseudoacrosomal granular matter.

At an early stage the apical membrane grows out to form a pair of posteriorly directed wing-like flaps on the sides of the centriole. These are also formed on the inner side of membranous sacs, which are connected both with the inner sac of the dorsal ribbon and, anteriorly, with the common sac of the pseudoacrosome (Fig. 12:D, Plate 20:104). When the top of the pseudoacrosome grows forwards, the ventral sheath is formed as a forward continuation of the wing-like flaps and remains in continuity with the apical membrane (Fig. 12:A, B, C).

The PGM appears behind the apical membrane when this is detached from the end of the centriole (Fig. 11:A, B). At this stage a formation of PGM directly from the nucleus could seem possible, but later development makes this interpretation doubtful. During development the acrosome grows out to a 35 μ long rod, and the general rule is that the morphological maturation of the PGM is most advanced at the base, whereas it is less advanced just behind the apical membrane. Thus, immediately behind the membrane, there is always an unorganized mass of PGM (Plate 23:117), a little further

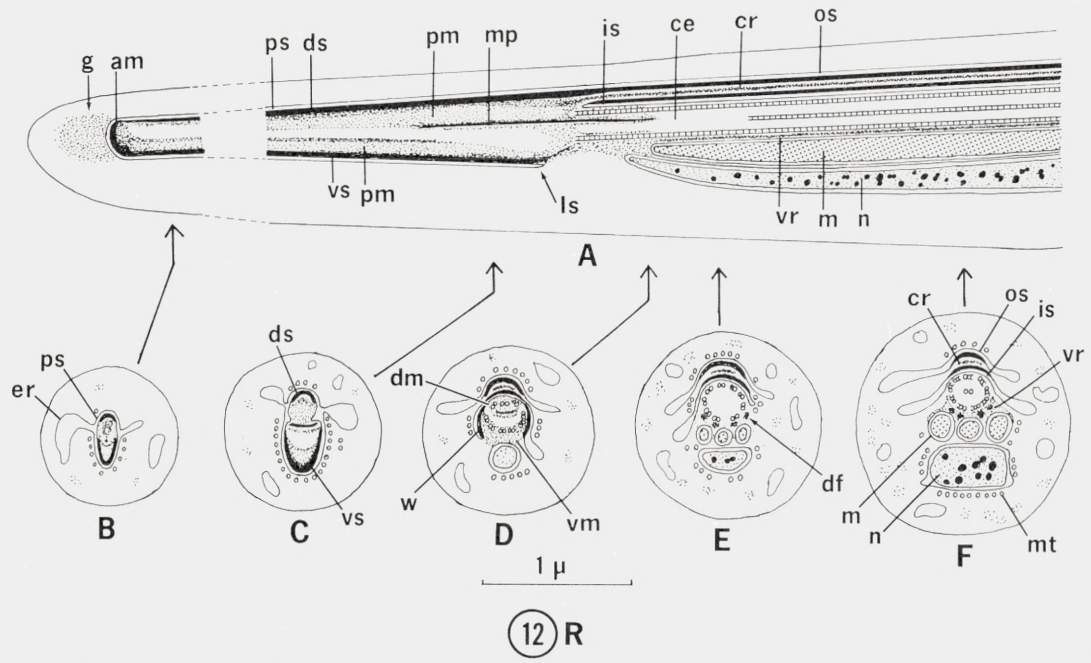

Fig. 12. *Raillietiella*, spermatid, stage 6. Diagrams of median section (A) and of cross sections from different regions (B–F). Compare Plates 16 and 20–23. The pseudoacrosome is formed exclusively by the pericentriolar structures shown in Fig. 11.

am = apical membrane, ce = centriole, cr = granular core of dorsal ribbon, df = dark outer fibers of axonema, dm = dorsal extension of the pseudoacrosomal granular matter, ds = dorsal sheath of pseudoacrosome, er = smooth endoplasmic reticulum, g = granulosome, is = inner membranous sac of dorsal ribbon, ls = posterior limit of the membranous sac on the ventral side of the pseudoacrosome, m = mitochondria, mp = membrane-like condensation of PGM, mt = longitudinal microtubules, n = nucleus, os = outer membranous sac of dorsal ribbon, pm = pseudoacrosomal granular matter (PGM), ps = membranous sac of pseudoacrosome, vm = ventral extension of the PGM, vr = ventral ribbon, vs = ventral sheath of pseudoacrosome, w = posterior wing-like extensions of the ventral sheath on the sides of the centriole.

back it may be condensed to a median lamella attached to the dorsal sheath, and at the base the PGM may have formed almost compact rods by filling the dorsal and ventral sheaths. This indicates that addition of new substances takes place from the anterior end, and that the apical membrane or granulosome, perhaps also dorsal and ventral sheaths, constitute the synthetic apparatus.

This means that all structures taking part in the formation of the pseudoacrosome have a peri-centriolar origin: The dorsal ribbon, the granulosome, and the apical membrane are present before the centriole is attached to the nucleus, and the PGM is most probably formed by these structures. The very poorly developed Golgi apparatus has not been observed to have any connection to the developing structures, in contrast to conditions in *Argulus*, in which the Golgi apparatus is better developed.

Since the pseudoacrosome has a purely centriolar origin, it is completely different from typical acrosomes, which form without connection with the centriole, as derivatives of the Golgi apparatus, and in intimate contact with the nucleus, at least in later stages.

The transitional region. When the pseudoacrosome forms, the centriole is directly included in its base. This inclusion of the centriole can be distinctly followed in the sections. Already in stages 5 and 6 some PGM invades the anterior end of the centriole and is condensed to form a dark plate under the dorsal filaments 9, 1 and 2 (Fig. 12:A, C, D). These filaments converge a little and are embedded in additional dark PGM, so a complex dorsal portion of PGM is formed below the inner sac of the dorsal ribbon (Plate 22:115–117). This dorsal portion with the three dorsal centriolar filaments fuses anteriorly with the core of the dorsal rod of the pseudoacrosome.

The six ventral filaments of the centriole are in a similar way surrounded by the more scattered ventral portion of PGM, which tends to condense around the filaments (Plate 22:115–117). This ventral portion fills out the space between the top of the nucleus and the mitochondria and the posterior margin of the ventral sheath, and is covered from the sides by the wing-like posterior extensions of the latter (Fig. 12:A, D). Actually therefore, the six ventral filaments are connected with the ventral rod of the pseudoacrosome.

The gap between the posterior margin of the ventral sheath and the top of the nucleus is preserved also in mature spermatozoa, although the ventral portion of PGM becomes denser (Fig. 1).

IV. *ARGULUS FOLIACEUS* (L.), COMPARED WITH *RAILLIETIELLA*

It was Leydig (1850) who discovered the testicles of *Argulus* and described their position in the vestigial, bilobed abdomen. He was also able to see and describe the spermatozoa, although these are only 0.2–0.5 μ thick. He writes that they are filiform, about "0.05‴ lang". In modern units this corresponds to about 110 μ, which is surpris-

ingly accurate, for in the present study values between 110 and 120 μ have been obtained both with phase contast and electron microscopy in the same species.

The structure of the testicle was correctly described by Grobben (1908). In his admirable studies of spermatozoa, Retzius (1909) confirmed Leydig's and Grobben's statement that those of *Argulus* are filiform, and could not distinguish any regional differentiation. He demonstrated clearly that the body consists of at least three thin longitudinal fibers. Later light microscopists (Martin 1932, Debaisieux 1953) could, for obvious reasons, get no further.

Recently Brown (1966, 1970) included material of *Argulus* sp. from the garfish *Lepisosteus platyrhinus* Dekay in a combined light- and electron microscopical study of crustacean spermatozoa. He described the body of the spermatozoon and noticed many of the unique features: the three mitochondrial rods, the dorsal ribbon (called U-shaped body), and the symmetrical position of the central filaments of the axonema on each side of the median plane. Several sections of the pseudoacrosome are seen in the pictures in the thesis from 1966. They are correctly marked "sperm ends" but are not further commented on.

Brown's results, which directed my attention to *Argulus*, will be referred to in the following descriptions. These will also include comparisons with *Raillietiella* on all relevant points.

The structure of the testicle and the general course of spermatogenesis

As described by Leydig (1850), Grobben (1908) and Martin (1932), the testicles of *Argulus* are a pair of ovoid bodies, situated in the hemocoelic space of the two lobes of the vestigial abdomen (Plate 2:5). A vas efferens goes from each testicle forwards to the seminal vesicle, which is situated in the posterior part of the cephalothorax.

In a cross section, each testicle is approximately circular, with an external basement membrane and a thick epithelium. This surrounds an internal cavity, which is continuous anteriorly with the lumen of the vas efferens. The epithelium is thinner, 2–3 cell layers only, in the dorsal midline (Plate 2:5). According to Grobben this is the germinal zone, from which spermatogonia are given off to the surrounding walls. Spermatogenesis takes place while the cells move down the walls of the testicles to the ventral midline, where the most advanced spermatids are found. The spermatocytes 1 form very thick layers on the upper part of medial and lateral walls; the zone of spermatocytes 2 is very narrow, and the spermatids occupy a multicellular layer on the lower part of the wall on each side.

About halfway down the walls, some cells with light-staining plasm appear along the basement membrane. These are the prospective Sertoli cells. As soon as a pseudoacrosome is formed in the spermatids it becomes stuck in the plasm of these Sertoli cells. Further down the sides, where the spermatids become filamentous, they are seen in tufts, each associated with one Sertoli cell.

In the electron microscope the long pseudoacrosomal ends of the spermatids are

seen sticking in deep narrow pockets in the distal end of the Sertoli cells (Plate 3:9). These pockets are lined by the plasma membrane of the Sertoli cells, which is separated from that of the spermatids by a narrow cleft, measuring 200–400 Å (Plate 20:102). No membrane fusion between spermatids and Sertoli cells has been observed.

Near the ventral midline the Sertoli cells elongate strongly, standing on the base ment membrane with a narrow stalk, and with their thick distal end with the nucleus just below the center of the testicle (Plate 2:5). These long Sertoli cells bear mature or nearly mature spermatids, which are liberated into the lumen. Actually there are two groups of such high Sertoli cells in a cross section, one derived from the medial side of the testicle and the other from the lateral side. They are separated by degenerating cells which have liberated their spermatozoa.

All this is fundamentally different from the organization of the testicle in *Raillietiella*. The most striking differences are:

1. In *Raillietiella* there are typical, free-swimming spermatocysts containing 128 or 256 synchronously developing spermatids and surrounded by a plasmatic cyst wall. In *Argulus* no cysts or cyst walls are formed, only poorly defined groups of synchronously developing cells.

2. In *Argulus* the advanced spermatids develop while their pseudoacrosomes stick deep into the plasm of typical Sertoli cells. No Sertoli cells are present in *Raillietiella*. The pseudoacrosomes are free. They may be in contact with the wall of the spermatocyst but this wall is smooth, without pockets for the spermatid ends (Plate 23: 118).

3. The migration along the wall during development, from a relatively small dorsal germinal area to a maturation zone near the ventral mid-line, is peculiar to *Argulus*. In *Raillietiella* no such migration takes place, and proliferation of new cysts is seen from all walls, with the possible exception of a mid-ventral area.

In view of these fundamental differences it may seem astonishing that spermatogenesis and sperm structure is closely related in the two forms. There is, however, one point in which they agree: The sperm is transferred from the vesicula seminalis of the male to the receptaculum seminis of the female in the form of seminal fluid, not as spermatophores, which are common in arthropods. However, Fryer (1958) has found spermatophores in *Dolops ranarum* (Stuhlmann) and *D. geayi* (Bouvier), which are typical branchiurans, placed in the same family as *Argulus*.

The mature spermatozoa

The structure of the mature spermatozoa in *Argulus* is so similar to that of *Raillietiella*, that the same terminology can be used throughout.

External features, regional differentiation

The mature spermatozoa from the female receptaculum or from the vesicula seminalis of the male are filiform, 110–125 μ long (Fig. 13A, Plate 4:11). Four intact spermatozoa, fixed in osmic acid and lying flat on grids were measured with great accuracy, and the figures 110, 119, 121 and 124 μ were obtained. Fresh spermatozoa from the receptaculum were photograped in phase contrast, just after the movements had stopped. A slight coiling caused a small degree of uncertainty but values between 110 and 120 μ were obtained all the time. These values agree with those obtained by Leyding in 1850.

The diameter (height) of the spermatozoa varies from ca. 0.55 μ about the middle of the body to 0.2 μ in the anterior part of the pseudoacrosome.

The pseudoacrosome was measured in 13 intact, osmium-fixed specimens, photographed with the electron microscope, and all values fell within the range 27 μ to 33 μ. The transition from the anterior end of the body, where the height is 4–4.5 μ, to the 3 μ thick base of the pseudoacrosome is not so distinct as in *Raillietiella* but sudden enough to allow reasonable accuracy when EM-pictures of pseudoacrosomes are measured (Plate 4:11). The pseudoacrosome is stiff in living specimens but is not so regularly arched as in *Raillietiella*. Usually it is almost straight or slightly curved, but half-circular bending has sometimes been seen, both in fresh and in fixed specimens.

The spermatozoa of *Argulus* and *Raillietiella* are thus almost identical with regard to regional differentiation and dimensions:

	Argulus	*Raillietiella*
Total length	110–124 μ	100–130 μ
Length of pseudoacrosome	27– 33 μ	ca. 35 μ
Maximal diameter	0.55 μ	0.8 μ

The spermatozoa of *Argulus* are a little thinner (Plate 5:16,17), and there may be a small difference in the average shape of the pseudoacrosome.

In a different (unidentified) *Argulus* species from *Lepisosteus*, Brown (1966, 1970) found spermatozoa which are said to be about 350 μ long, or three times as long as those of *Argulus foliaceus*. Brown (1966) also describes a spiral structure in the spermatozoa of his *Argulus* species, and one of his phase contrast pictures shows this coiling distinctly. There are about 5–6 μ between the coils. In *Argulus foliaceus* no such dense coiling is present. EM-pictures of whole spermatozoa fixed in different ways show no coiling at all (Plate 4:11). Phase contrast microscopy of fresh sperm of *A. foliaceus*, studied as soon as movements become slow, show the body bent into three to four coils, but this may as well be an artifact caused by contraction.

Internal structure of body

Cross sections of the body of *Argulus* spermatozoa differ very little from corresponding sections of *Raillietiella* spermatozoa (Figs. 1:B and 13:B, Plate 5:16,17). *Argulus* spermatozoa are more compressed from the sides, but the general pattern is the same.

The nucleus is more flattened dorso-ventrally in *Argulus* than in *Raillietiella*. More significant is perhaps, that there are at least one, sometimes more, nuclear membranes inside the two usual ones present in *Raillietiella*. In *Argulus* there is also a dark, asymmetrical thickening on one side, associated with the nuclear membranes above the nucleus proper ("af" in Fig. 13:B, Plate 5:17). This structure is discussed in the chapter on spermatogenesis as a probable remnant of the acrosome filament.

The mitochondria are three continuous rods, situated symmetrically between the nucleus and the axonema, as in *Raillietiella*. Typical cristae are present in all three mitochondria, but they are small. This was the case also in Brown's (1966) species of *Argulus*.

The axonema is of the 9 + 2 pattern and agrees on two very specific points with that of *Raillietiella:* 1) The central filaments are symmetrically situated on each side of the median plane, the peripheral doublet 1 being dorso-median, and 2) the doublet 1 is connected with the dorsal ribbon by an obliquely orinted membrane, which is inclined to the side where the arms of doublet 1 are, just as in *Raillietiella* (Plate 5:16,17).

In addition, the doublets 3 and 7 in *Argulus* are attached to the inner wall of the inner membranous sac of the dorsal ribbon (Fig. 13:B, Plate 5:17). In *Raillietiella* there is no such attachment (Plate 5:16).

Brown noticed the exceptional symmetrical position of the central filaments also in his species of *Argulus* (Brown 1966, 1970).

The dorsal and ventral ribbons are nearly identical with those of *Raillietiella*, and the sharp edges of the dorsal ribbon fit into furrows in the free edge of the ventral ribbon as in this species (Plate 5:16,17). Also the dark but thin lamellae, covering the dorsal and lateral aspects of the lateral mitochondria, are present in *Argulus*. Differences have been found in a few details: 1) The ventral ribbon has tightly set transverse ridges

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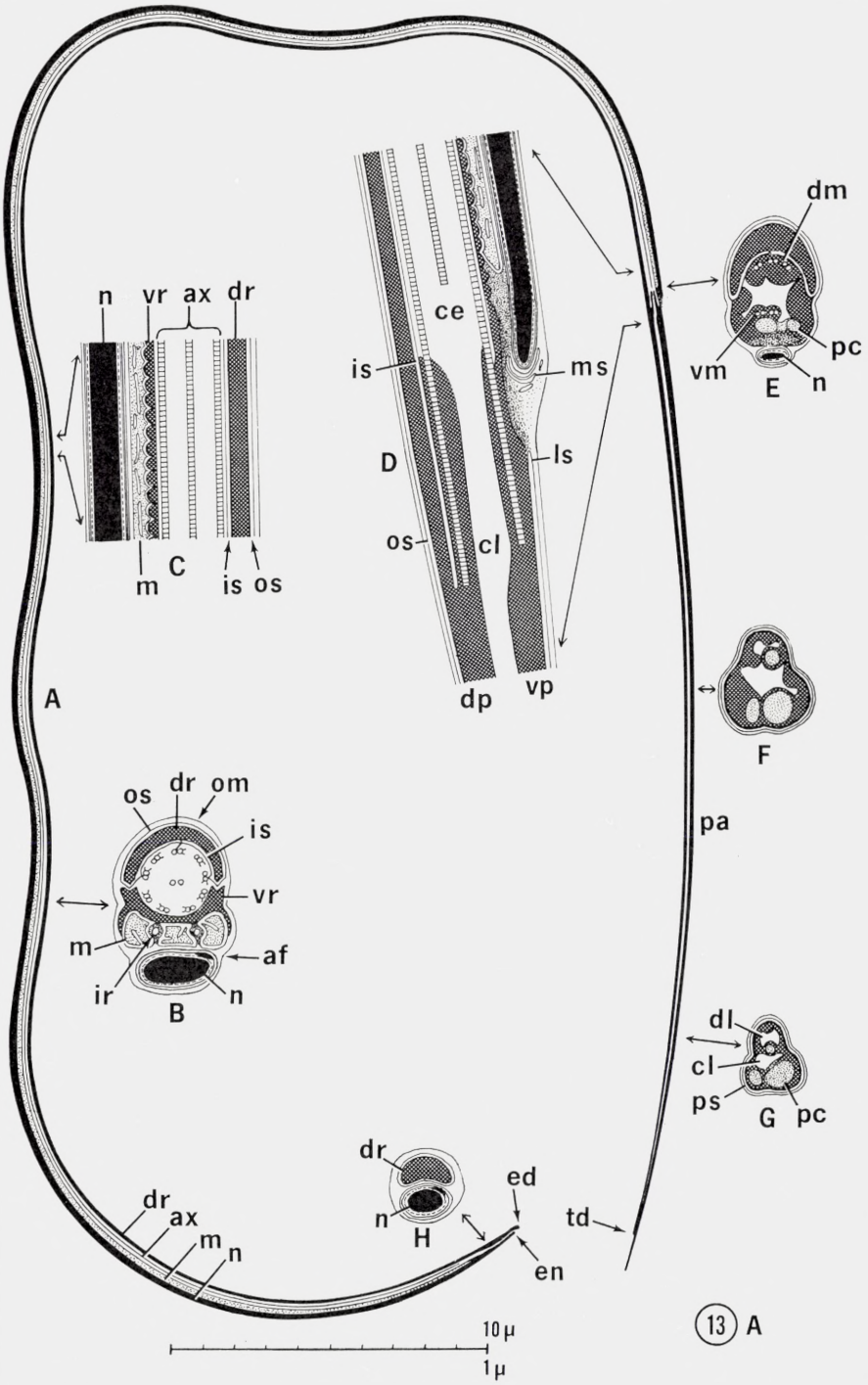
Fig. 13. *Argulus*. Diagram of the mature spermatozoon, based on numerous total preparations and sections like those in Plates 4–7. The scale is 10 μ when used for the intact spermatozoon (A) and 1 μ when used for the details (B–H).

A. Total spermatozoon. — B. Cross section of body. — C. Median section of body. — D. Median section of transitional region between body and pseudoacrosome. — E. Cross section through anterior end of nucleus. — F. Cross section of the pseudoacrosome, basal part. — G. Cross section of the pseudoacrosome, terminal part. — H. Cross section through posterior end of body.

af = remnants of acrosome filament in the nuclear membranes, ax = axonema, ce = centriole, dl = dorsal lumen of pseudoacrosome, dm = dorsal extension of pseudoacrosomal granular matter, containing axonemal filaments nos. 9, 1, and 2, dp = dorsal rod of pseudoacrosome, dr = dorsal ribbon, en = posterior end of nucleus, is = inner membranous sac of dorsal ribbon, ir = intermitochondrial light rods, ls = limit of pseudoacrosomal membranous sac on the ventral side, m = mitochondria, n = nucleus, o = oblique membrane between axonemal doublet 1 and dorsal sheath, os = outer membranous sac of dorsal ribbon, pa = pseudoacrosome, ps = pseudoacrosomal membranous sac, td = top of dorsal rod of pseudoacrosome, pc = light cores in pseudoacrosome, vm = ventral extension of pseudoacrosomal granular matter, including axonemal filaments 4 and 5, vp = ventral rod of pseudoacrosome, vr = ventrale ribbon.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on the outer side in *Argulus* but not in *Raillietiella* (Plates 6:18,19, 18:89,90). 2) The light cores in the dorsal and ventral ribbons, present in *Raillietiella*, could only be demonstrated in the centriolar region of *Argulus* (Plates 5:16,17, 7:25,26). 3) In *Argulus*, but not in *Raillietiella*, a pair of very distinct light rods with 350 Å diameter are situated in the interspaces between median and lateral mitochondria (Figs. 1:B, 13:B, Plate 5: 16,17). Spermatogenesis indicates that these rods are the homologues of the light cores of the ventral ribbon in *Raillietiella*.

The cytoplasm of the spermatozoon is more reduced in *Argulus* than in *Raillietiella*, and remnants of the smooth endoplasmic reticulum, as seen in the latter species, have not been seen in *Argulus*. Brown (1966, 1970) describes what he calls a lateral organelle in his species of *Argulus:* a membrane-bound sac with granular content, situated asymmetrically on one side in contact with the lateral mitochondrion. In a few sections this organelle was present on both sides, so Brown suggests that there are two, one on each side, which overlap in a short region. Such lateral organelles are found in non-mature spermatids of *A. foliaceus*, but they are completely reduced in the mature spermatozoa (Plates 17:86,87, 18:88, 5:17). They will be described in the chapter on spermatogenesis.

The posterior end has some significant features in common in the two forms: There is no free flagellum, and the dorsal ribbon continues into the extreme posterior tip. But constant differences are present in the posterior extension of other components: In *Argulus* the nucleus extends as far as the dorsal ribbon, whereas mitochondria, ventral ribbon and axonema terminate some microns from the end (Fig. 13:A, H, Plates 4:15, 7:30,31). In *Raillietiella* the nucleus is the first structure to end, followed by mitochondria and ventral ribbon, whereas filaments of the axonema can be followed into the very tip (Fig. 1:A,G,H, Plates 4:14, 7:29). In *Argulus* the terminal part of the dorsal ribbon often has a remarkable, swollen appearance.

The anterior end of the body carries the long pseudoacrosome, and the transitional area has essentially the same pattern in both forms. In *Argulus* as in *Raillietiella* the dorsal ribbon is directly continuous with the dorsal rod of the pseudoacrosome, and its outer membranous sac continues forwards as the pseudoacrosomal sac (Fig. 13:D). The inner sac of the ribbon ends at the level of the anterior end of the centriole (Plate 22:111). The behaviour of the three dorsal centriolar filaments, nos. 9, 1, and 2, is particularly significant. In *Argulus* as in *Raillietiella* they converge and are embedded in the dorsal portion of dark PGM, which is continuous with the dorsal rod of the pseudoacrosome (Plates 22:111, 6:20, 7:21, 22, 25, 26).

The ventral filaments are in both species embedded in the ventral portion of PGM, which connects with the ventral rod of the pseudoacrosome. But in *Argulus* this is true only for the two medioventral ones, for the other four (nos. 3,4,7, and 8) disappear in the centriolar region. In *Raillietiella* all six ventral filaments (nos. 3–8) are connected with the ventral rod in this way (Figs. 1 and 13, Plates 7:21,22,25,26, and 22:112–126).

In *Argulus* as in *Raillietiella* there is a deficiency in the ventral pseudoacrosomal wall in front of the nucleus, and the ventral sheath forks posteriorly into a pair of wing-

like plates, which cover the centriole from the sides. This is best seen before final maturation of the spermatids (Plate 22:112–116).

Internal structure of pseudoacrosome

The pseudoacrosome as such, a unique structure not known from other animals, is an important identical feature in *Argulus* and *Raillietiella*. However, its internal structure presents considerable differences in details (Figs. 1:F, 13:F,G).

The cross section of the pseudoacrosome in *Argulus* deviates distinctly from a bilateral symmetry. This asymmetry is constant and is particularly pronounced in the much broader ventral part of the section. Development shows clearly that this ventral part is homologous to the ventral rod in *Raillietiella*, whereas the more narrow dorsal part corresponds to the dorsal rod (Plate 21).

The pseudoacrosome in *Argulus* is surrounded by a flat sac of the endoplasmic reticulum, which is best seen before maturation (Plates 20:102,103, 21:108–110). As in *Raillietiella*, this sac collapses later and forms two closely attached unit membranes all around the pseudoacrosome, but they are often difficult to resolve in the mature sperm.

The dorsal rod has a central lumen, surrounded by dark PGM. This dark substance also forms a transverse bridge, which delimits the dorsal lumen from the central lumen, situated mainly in the ventral rod (Fig. 13:F,G, Plate 7:27,28). In the bridge there is a longitudinal light rod which, near the base of the pseudoacrosome, has an asymmetrical position.

The dark PGM of the ventral rod bulges out into the central lumen as two unequal ridges, which are roughly half-cylindrical and partly fuse when they meet. Each ridge contains a light rod, one thicker than the other. The behaviour of the three light rods at the base of the pseudoacrosome is complicated and has not been followed in detail. It was stated, however, that the dorsal lumen communicates with the central lumen not far from the base, and that the central lumen is continuous with the lumen of the centriole as shown in Fig. 13:D.

The inner structure of the two pseudoacrosomal rods in *Argulus* is thus completely different from the pattern in *Raillietiella*, where the rods are symmetrical, simple, with a light core in the center (Fig. 1:F, Plate 7:23,24).

Intact ends of pseudoacrosomes of *Argulus* spermatozoa have been seen many times in EM-pictures (Plate 4:13). The terminal part to a point ca. 2 μ from the end is extremely narrow, less than 0.1 μ, and consists of one rod as in *Raillietiella*. No indication of a granulosome like the one in *Raillietiella* could be found although it is present during development (see Plates 4:12, 23:117–120). The negative statement is perhaps not definite, for technical hazards are difficult to exclude completely.

Movements

Like Brown (1966, 1970), I observed violent movements in sperm suspended from the vesicula seminalis in mammalian Ringer. The movements were so rapid that an analysis was impossible, but came to a sudden stop after a few minutes. Then only abnormal bendings remained.

Spermatogenesis

Spermatogenesis in *Argulus* follows the same general lines as in *Raillietiella*. It is therefore possible to subdivide spermatogenesis into the same stages as those used for *Raillietiella*, with minor modifications. The analysis of spermatogenesis in *Argulus* has only been carried as far as was thought necessary for a comparison with *Raillietiella*, and has been focused on the anterior ends of the spermatids, which contain the most interesting structures.

From spermatogonia to early spermatids

This part of the spermatogenesis is of an ordinary type in *Argulus* as well as in *Raillietiella*. The cells are regular, polygonal, with numerous free ribosomes and polysomes in the plasm. The smooth endoplasmic reticulum is poorly developed in spermatogonia but develops strongly in the spermatids as numerous small sacs and vesicles. Stacks of Golgi vesicles are often seen in the spermatocytes and are in general more numerous and better developed than in *Raillietiella*. This is true also for early spermatids.

The mitochondria are rod-shaped or tubular with a diameter of about 0.5 μ in the spermatocytes 1 and during 1st meiotic division. Two anaphase cells with numerous remnants of synaptonemal complexes still had small scattered mitochondria. Late spermatocytes 2, classified on the basis of nuclear size and presence of two centrioles, already had more spherical mitochondria, and cells classified as early spermatids 1 had all mitochondria concentrated into one group.

The centrioles. Four centrioles lying in a group were seen in several spermatocytes 1, and these, like single centrioles seen in cells of this stage, did not show remarkable features. During meiosis two dark outer fibers appear along one side of the centriole, just as in *Raillietiella*. In *Argulus* this must occur already during 1st meiotic division for the above-mentioned cells in 1st anaphase had already two distinct dark rods. Late spermatocytes 2, classified on the basis of nuclear size, rounded and large mitochondria and the presence of two centrioles, always have two distinct outer fibers, covered by a darkened flat sac of endoplasmic reticulum (Plate 8:35,36). Thus in *Argulus* as in *Raillietiella*, the anlages of the dorsal ribbon develop already during meiosis, giving the centriole a bilateral symmetry.

Spermatid 1; with several mitochondria

Very few cells of this stage were seen. The mitochondria form one group near the nucleus and have contracted to irregularly rounded bodies with many cristae and a diameter of more than 1 μ (Plate 9:49). The centriole has no contact with the nucleus but in one case was seen attached to the cell membrane and having formed an axonema in a short flagellum.

Spermatid 2; with three spherical mitochondria

The three large, spherical *mitochondria* of this stage are up to 2 μ in diameter and have numerous cristae. The mitochondria are in contact with each other in a triangular

fashion (Plate 9:51). The arrangement is strikingly similar to that seen in the corresponding *Raillietiella* spermatids (Plate 9:50).

The centriole at this stage has a distinct granulosome attached to the proximal end of the tube, as in *Raillietiella* (Plate 8:44). A dark, membrane-like condensation seen across the top of the centriole is undoubtedly homologous to the apical membrane in *Raillietiella* (Plate 8:41,45). However, in *Argulus* the dark matter penetrates into the terminal part of the lumen of the centriole, so the "apical membrane" looks more complicated.

A flat sac of endoplasmic reticulum covers the centriole on the prospective dorsal side and is associated with much dark matter. The outer dark fibers under this sac are now three in number. Thus, in *Argulus* as in *Raillietiella* there is a change from two to three dark outer fibers under the membranous sac in the period from meiosis to spermatid 2 (Plate 8:32–37,42,43).

Relation between nucleus, mitochondria and centriole. In spermatids 2 of *Argulus* I have never seen the granulosome in contact with the nucleus, although many cells have been photographed. This contact appears to be established relatively later in *Argulus* than in *Raillietiella*, after the mitochondria have formed a typical Nebenkern. A contact between the mitochondrial triplet and the centriole has been seen in several spermatids 2, the centriole lying in the furrow between two of the mitochondria (Plate 8:37, 45).

In other spermatids 2 such contact has not yet been established; the mitochondria, the nucleus, and the centriole are independent as in corresponding stages of *Raillietiella* (Plate 10:54,55).

The flagellum is well developed and contains a fairly long axonema in all spermatids of this stage, but the distal end of the centriole is still in contact with the flagellar pit, so there is no axonema within the cell body proper.

Spermatid 3; with mitochondrial "Nebenkern"

In spermatids with a Nebenkern, the granulosome has always obtained contact with the nuclear membrane, so a relationship between centriole, mitochondria and nucleus like that in *Raillietiella* is established (Fig. 14, Plate 12:62). In *Argulus* this contact of the granulosome with the nucleus appears to be established late and has never been seen in spermatids 2 with spherical mitochondria. Further, the contact appears to be asymmetrical from the beginning in *Argulus*, as seen in Plate 12:62 and Fig. 14. Attachment to the posterior pole, as seen in *Raillietiella* (Figs. 3,4, Plate 11:59), was never encountered in *Argulus* although about 20 spermatids 2 and 3 were seen in more or less perfect longitudinal sections.

Mitochondria. The Nebenkern of *Argulus* is practically identical with that of *Raillietiella:* The three large mitochondria do not really fuse, their membranes remaining intact when the spheres are packed together to form a single body (Plate 10:53). All three mitochondria have numerous distinct cristae and are in contact with the ovoid, slightly irregular nucleus (Fig. 14, Plate 12:62,63). Along the prospective dorsal side

of the Nebenkern all three mitochondria are in contact with the centriole with straight, rather sharp edges as in *Raillietiella* (Plates 10:52,53, 8:42,43).

The centriole. The original triplets of the centriole probably change to doublets during this stage, for triplets have not been seen in subsequent stages, but documentation is admittedly too poor to allow detailed analysis. The three outer dark fibers 9, 1 and 2 are large and distinct and are covered by the outer sac of the prospective ribbon (Plate 8:42,43). This sac forms a dark outer sheath covering the fibers and bends down to the sides of the centriole. In the anterior direction the sac has covered the apical membrane (Plates 12:63, 20:105). The apical membrane has grown posteriorly as a pair of dark plates, attached to the sides of the centriole (Plate 8:42,43). The granulosome is attached to the apical membrane but is not so well defined as in *Raillietiella* (Plate 12:63, 20:105).

In *Argulus*, the apical membrane is not only detached but is rather far anterior to the end of the centriole in this stage, and plentiful pseudoacrosomal granular matter has appeared (Fig. 14). This matter consists of two portions as in *Raillietiella*, but proportions are a little different. In *Argulus*, the very dark condensation of PGM is shaped like a longitudinal rod between the end of the centriole and the apical membrane, whereas in *Raillietiella* it is an almost isodiametrical pellet (Plates 12:63,61, 20:105). The ventral portion of PGM is less dense and delimited by nucleus, mitochondria, and apical membrane as in *Raillietiella*, but it is located in a distinct pit between nucleus and Nebenkern (vm in Plates 12:63 and 20:105).

If the mentioned differences in proportions are excepted, there is an almost complete identity of details in the centriolar complex of *Raillietiella* and *Argulus* at this stage (Plates 12:60–63, 20:105).

The axonema is no longer restricted to the flagellum proper but has a short inner segment between the flagellar pit and the end of the centriole (Plate 12:62).

The true acrosome begins its development in spermatids of this stage. Stacks of Golgi vesicles are rather common in the anterior end of the spermatids, and some of them form a bowl-shaped body at the anterior end of the nucleus, with the concave side facing the nuclear membrane. The acrosomal rudiment is first seen in the center of this half-spherical Golgi apparatus, in contact with the nuclear membrane (Fig. 14, Plate 14:67–69). When first seen, it consists of a small vesicle, covering a dark granule which is attached to the outer nuclear membrane (Plate 14:67). Later development shows that the vesicle should be regarded as an acrosomal vesicle, and the granule as a post-acrosomal one. The granule soon grows to a short plug, the top of which is covered by the cap-like invaginated acrosomal vesicle (Plate 14:68,69).

Spermatid 4; anterior migration of centriole, formation of acrosome filament

The migration of the centriolar complex up along the dorsal aspect of the nucleus to a position near its anterior end takes place in the same way as in *Raillietiella* (Figs. 14–15 and 5–7). The three mitochondria of the Nebenkern emit anterior tongues which keep contact with the centriole with their anterior ends (Plate 15:74). The three mito-

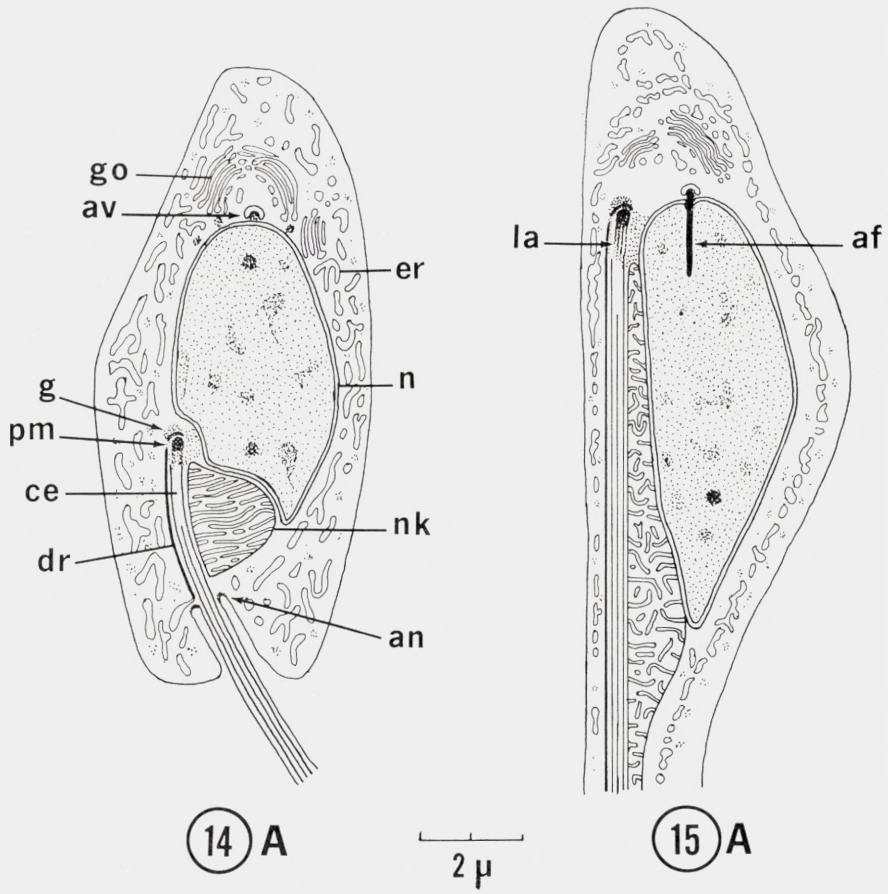

Fig. 14. *Argulus*. Diagram of spermatid, early stage 4.
Fig. 15. *Argulus*. Diagram of spermatid, late stage 4. Compare Plates 8, 10, and 12–16.
af = acrosome filament, an = annulus-like thickening of cell membrane in the bottom of the flagellar pit, av = acrosome vesicle, ce = centriole, dr = dorsal ribbon, er = smooth endoplasmic reticulum, g = granulosome, go = Golgi apparatus, la = horizontal lamellae, formed by the PGM, n = nucleus, nk = mitochondrial "Nebenkern", p = pseudoacrosomal granular matter (PGM).

chondrial tongues are symmetrically situated between the axonema and the nucleus (Plate 16:78,79). Similar tongues are emitted by the Nebenkern in a posterior direction along the ventral aspect of the axonema (Plate 16:77).

While this dislocation takes place, the shape of the nucleus changes in a characteristic way and is different from that of *Raillietiella*. The anterior end of the nucleus is broad and rounded with the true acrosome planted in a central position (Figs. 14–15, Plates 14:67–70, 15:74). The posterior end of the nucleus is tapering and projects below the mitochondrial Nebenkern, where finally a posterior nuclear tongue grows out (Plate 15:75). The fact that the true acrosome remains at the anterior end of the

nucleus during dislocation of the centriole makes it unlikely that the nucleus rotates during this process, as suggested for *Lepisma* by Werner (1964). It appears more probable that the centriole slides up along the side of the nucleus.

The *centriole* and the pericentriolar structures change considerably during this stage. The apical membrane is still more removed from the end of the centriole, and the dark rod of PGM seen in previous stages elongates and differentiates into several horizontal lamellae in its posterior part (Fig. 16, Plate 19:93–98). There is still no ventral pseudoacrosomal sheath, but a dorsal one developed as an anterior continuation of the dorsal ribbon is present, at least as an anlage. The apical membrane extends posteriorly as two wing-like lateral plates, which are very long and reach the anterior part of the centriole, which is laterally compressed (Plate 19:94). The posterior part of the centriole remains circular in cross sections (Plate 19:93).

In the region of the centriole and axonema an inner sac has appeared between the granular core of the dorsal ribbon and the axonema (Plate 16:77,78, 19:93,94). This sac ends at the anterior end of the centriole, but the sac communicates in a rather complicated way with the pseudoacrosomal sac in this region (Plate 19:95,96).

In most details these centriolar structures are as in *Raillietiella*, but there are a few differences: In *Argulus* the apical membrane is farther away from the centriole, and the horizontal lamellae formed by the PGM are specific to this species (Compare Figs. 11 and 16).

The true acrosome. The acrosomal vesicle remains almost unchanged from the previous stage, but the postacrosomal granule grows out to form an acrosome filament, which penetrates deep into the nucleus, surrounded by a deep, tube-like pocket formed

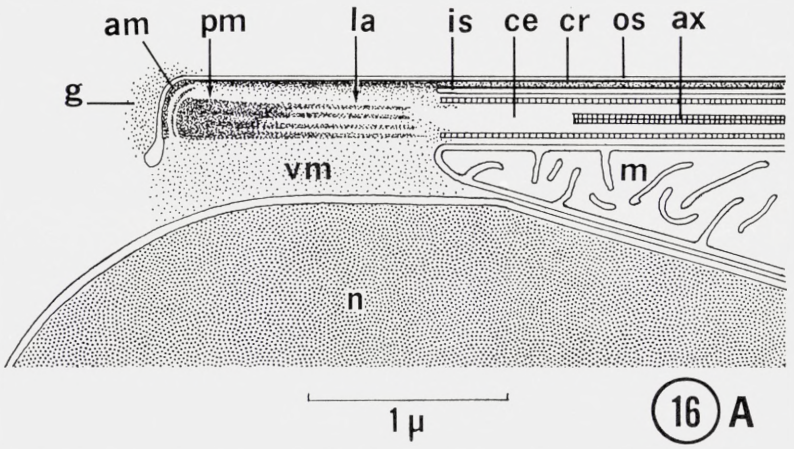

Fig. 16. *Argulus.* Diagram showing development of pseudoacrosome in spermatid, early stage 5. Compare Plates 12, 19, 20 and 23. Plate 19 shows cross sections of this particular stage.
am = apical membrane, ax = axonema, ce = centriole, cr = granular core of dorsal ribbon, g = granulosome, is = inner membranous sac of dorsal ribbon, la = horisontal lamellae formed by the PGM, m = mitochondria, n = nucleus, os = outer membranous sac of the dorsal ribbon, pm = pseudoacrosomal granular matter (PGM), vm = ventral extension of loose granular matter between nucleus and pseudoacrosome.

by the nuclear membranes. In spermatids of this stage the acrosome filament does not reach more than halfway through the length of the nucleus (Fig. 15, Plate 14:70).

Spermatid 5; formation of pseudoacrosome

In *Argulus* the pseudoacrosome begins to grow out long before the narrow anterior nuclear tongue is formed; in *Raillietiella* the nuclear tongue develops first (Figs. 17,8,9).

The components forming the pseudoacrosome are the same as in *Raillietiella:* The dorsal ribbon and its outer membranous sac, the granulosome, the apical membrane, and the pseudoacrosomal granular matter (PGM). The apical membrane with the granulosome is always on the top of the growing pseudoacrosome, but the granulosome appears to be less compact than in *Raillietiella* (Plate 23:119,120). The dorsal sheath of the pseudoacrosome is a direct anterior prolongation of the outer sheath of the dorsal ribbon and is originally small, half-circular in cross sections (Plate 20:102,103). The ventral sheath is formed in direct continuity with the apical membrane and is U-shaped, rather narrow in cross sections (Plate 20:102,103). The posterior end of the ventral sheath is forked into the two previously mentioned wing-like plates, which extend to the sides of the centriole. This forked posterior part of the ventral sheath appears to be longer than in *Raillietiella*, so a considerable part of the base of the pseudoacrosome is open ventrally (Figs. 17,18). The growing pseudoacrosome is completely surrounded by the membranous sac ("ps" in Plate 20:102,103).

At the base the pseudoacrosomal membranous sac is directly continuous with the outer sac of the dorsal ribbon, but it is also connected with the inner sac by two flattened ducts which cover the wing-like plates behind the ventral sheath proper (Plate 19: 94–98). The median, transverse part of the inner sac ends at the level of the anterior end of the centriole, and only its lateral diverticula continue forwards as the mentioned membranes on the wing-like plates.

The PGM behaves rather differently in *Argulus* and *Raillietiella*. In *Argulus* spermatids of stage 5 there is an undifferentiated dark mass just behind the apical membrane, but further back the PGM has condensed to form three horizontal lamellae, which give the cross section a ladder-like appearance (Plates 23:120, 20:102,103). These lamellae are located in the ventral sheath and between its posterior paired extensions. The most dorsal of these lamellae is fused in a T-shaped fashion to a median lamella which bridges the space between the two sheaths and is surrounded on the dorsal side by the dorsal sheath. This median lamella looks very much like the one seen in corresponding stages of *Raillietiella* (Plate 20:101,102,103).

The Golgi apparatus is still rather well developed at this stage and appears to have a functional connection with the membranous sacs of the dorsal ribbon and pseudoacrosome. As seen in Plate 18:92, the Golgi apparatus is separated by a zone of small vesicles from a sac of endoplasmic reticulum which is directly continuous with the outer sac of the dorsal ribbon. Such connections have also been seen at the level of the pseudoacrosome. In *Raillietiella* no such connections have been seen, and the Golgi apparatus is on the whole very little developed.

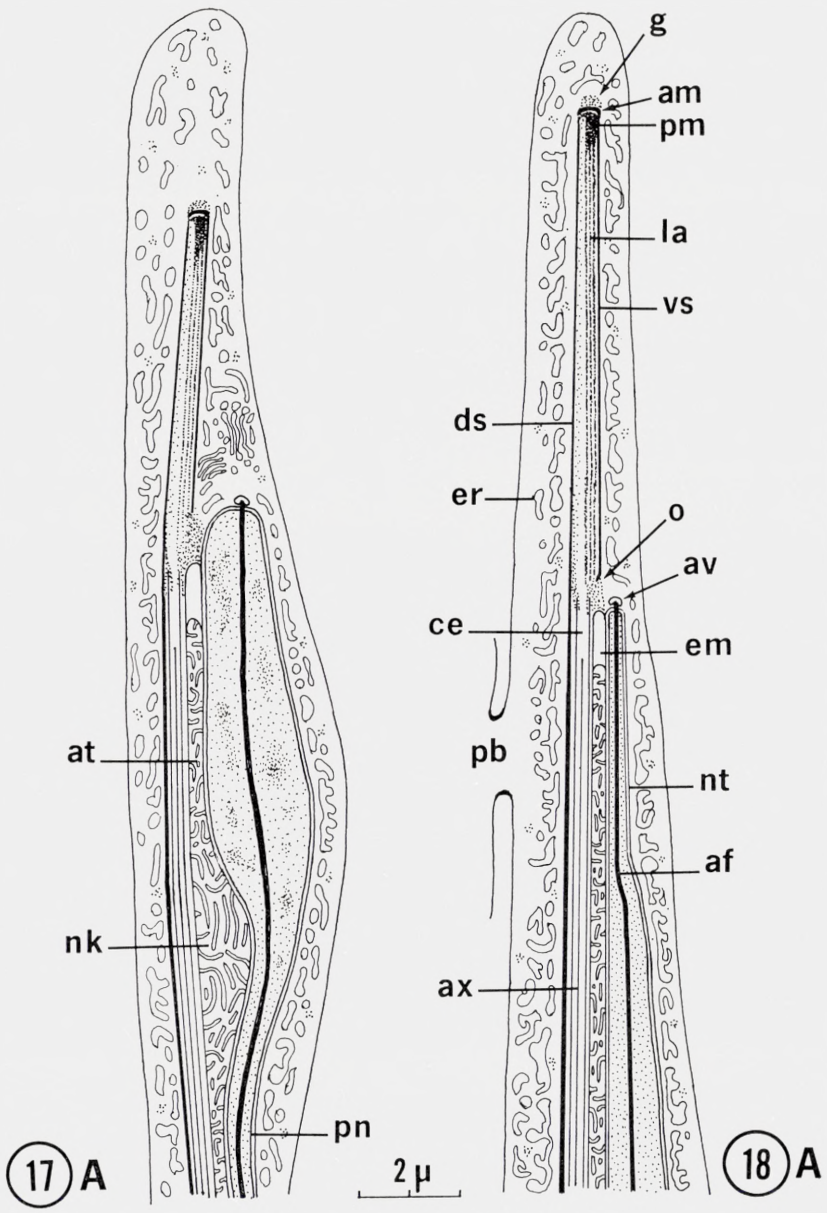

Fig. 17. *Argulus*. Spermatid, stage 5.

Fig. 18. *Argulus*. Spermatid, stage 6. Compare Plate 13–17, 19, 20, 22 and 23.

af = acrosome filament, am = apical membrane, at = anterior tongues of mitochondria, av = acrosome vesicle, ax = axonema, ce = centriole, ds = dorsal sheath of pseudoacrosome, em = anterior point of median mitochondrion, from which the inner membrane has retracted, er = smooth endoplasmic reticulum, g = granulosome, la = horizontal lamellae formed by the PGM, nk = mitochondrial "Nebenkern", nt = anterior nuclear tongue, o = opening between ventral ribbon and anterior end of nucleus, pb = plasmatic bridge to neighbour cell, indicating very slow separation of cytoplasm after 2nd meiotic division, pm = pseudoacrosomal granular matter (PGM), pn = posterior nuclear tongue, vs = ventral sheath of pseudoacrosome.

The nucleus. The blunt, originally rounded anterior end of the nucleus grows out to a narrow tongue with the true acrosome on the top (Fig. 18, Plate 14:71). The top remains all the time at the level of the centriole, and the space between nucleus, mitochondrial ends, centriole and ventral sheath of pseudoacrosome is filled with the ventral portion of PGM (Fig. 18).

In *Argulus* but not in *Raillietiella*, the nucleus also develops a posterior tongue along the ventral surface of the mitochondria. This posterior tongue does not, however, reach the flagellar pit but is rather short. The bulk of the Nebenkern is thus not situated behind the nucleus as in *Raillietiella* but lies in a depression of the dorsal surface of the nucleus (Fig. 17, Plate 15:75).

The true acrosome is very close to the base of the pseudoacrosome in this stage, because of the attenuation of the anterior end of the nucleus and its close contact with the centriole (Plate 14:71). The acrosome vesicle is still quite typical, sitting like a cap on and partly surrounding the anterior end of the acrosome filament (Plate 14:71). The filament itself has grown through the entire nucleus to its posterior end, all the way surrounded by a tube of nuclear membranes (Plate 16:79).

The presence of this unmistakable, true acrosome and acrosome filament, separate from the pseudoacrosome, shows of course that the latter has nothing to do with an acrosome in the normal sense.

Longitudinal microtubules are particularly common around the growing pseudoacrosome but are also present along other regions of the spermatid (Plate 20:102,103).

Spermatid 6; elongation of nucleus and mitochondria, formation of ventral ribbon

During this phase of the development the nucleus and the mitochondria are stretched to form long, filamentous structures, and the bulge on the nucleus as well the Nebenkern bulge on the mitochondria disappear. The entire spermatid is greatly elongated. There is still a free flagellum, but it is short compared to the rest of the spermatid.

The nucleus and the acrosome filament change drastically during this development. The nucleus is markedly flattened and is asymmetrically disposed. In a cross section one margin of the flattened body is seen in contact with the median mitochondrion, whereas the other margin is displaced to the lateral part of the cell (Plate 16:81, 17:84). As far as stated, the nucleus is always dislocated to that side of the median plane where the arms on axonemal filament 1 are situated.

Inside this nucleus the greatly swollen membranous tube of the acrosome filament is always asymmetrically situated, near the lateral nuclear margin (Plates 16:81, 17:84,85). The filament itself appears to disappear. It is still visible in Plate 16:81, poorly visible in Plate 17:84 and absent in Plate 17:85.

Chromatin condensation begins and is continued during stage 7. It differs completely from chromatin condensation in *Raillietiella* (Plates 16:81, 17:82–87). In *Argulus* the first signs of condensation are some small threads or lamellae of chromatin, attached like a dark tuft to the medial side of the tube of the acrosome filament (Fig.

16:81). This tuft grows out to form an irregular body of granular or fibrous material (Plate 17:84). It never fills out the fairly voluminous nuclear space. In more advanced spermatids of this stage and stage 7, the chromatin body becomes flattened and the originally rough surface is covered on the dorsal and ventral sides by membrane-like condensations of dark matter, whereas the lateral and medial margins remain "open" for a longer period (Plate 17:85). In more advanced spermatids 7 the dark, compact chromatin body is covered completely by this smooth surface (Plates 17:86,87, 18:88).

When the first signs of chromatin condensation are visible, a row of darker bodies is seen along the inner nuclear membrane all around the nucleus. In more advanced stages a membrane is present here between the original nuclear membranes and the smooth surface of the chromatin body (Plates 16:81, 17:84–87, 18:88). It is probable therefore, that the secondary membrane is formed by the row of dark bodies seen in earlier stages. In some sections, probably from other levels of the spermatozoon, there are several membranes, and probable stages of formation have been seen as concentric rows of dots inside the nuclear membranes in earlier stages (Plates 18:88).

The membranous tube of the acrosome filament remains outside the chromatin body and is probably enclosed between primary and secondary nuclear membranes. The tube does not collapse definitely until stage 7, and details in the degeneration of the acrosome filament tubes have been difficult to follow. It appears probable that the dark body seen in cross sections of mature spermatozoa are remnants of the tube (Plate 5:17). This body, like the acrosome filament tube of later spermatids, is situated on the same side of the median plane as that where the arms on axonemal filament 1 are situated.

The ventral ribbon is formed as in *Raillietiella*, as an accumulation of dark matter between axonemal doublets 4–7 and mitochondria. As in *Raillietiella* the surface of the early anlage is marked by a membrane-like condensation of matter, but true unit membranes are probably not present (Plates 16:80,81, 17:84,85). It is probable that the dark outer fibers 4–7 are included in this ventral ribbon, but they have been rather indistinct in all sections seen. In Plate 17:84,85 there is a pair of dark dots in the ventral ribbon, probably corresponding to outer dark fibers 5 and 6, but nos. 4 and 7 are indicated only in some sections of the centriole (Plate 8:37,42). Further development including additional accumulation of dark matter, formation of sheaths on the lateral mitochondria, and formation of the furrow in the free margins is as in *Raillietiella*. *Argulus* differs only in the development of the light intermitochondrial rods (Plates 17:84–87, 5:17). If the interpretation of the dark spots in the ventral ribbon in Plate 17:84,85 as outer filaments 5 and 6 is correct, it is probable that these filaments form the intermitochondrial rods. In Plate 17:85 the dark spots have moved down into the interspace between the mitochondria, in 17:86 the intermitochondrial rods are formed but are only seen as bulges on the ventral ribbon, and in 17:87 they are in their final location. The rods are dark in relation to their surroundings in early stages. In later stages they loose contrast and are seen as light spots.

If the intermitochondrial rods are formed by outer dense fibers 5 and 6, the ab-

sence of light cores in the ventral ribbon can be understood, for in *Raillietiella* these dense fibers probably form the light cores.

The pseudoacrosome increases in length and develops a distinct asymmetry in spermatids of this stage. The transitional region is still approximately symmetrical, but in front of the centriole the ventral sheath tips over to one side (Plate 21:108–110). The two sheaths are rather far from each other in the earlier stages, connected only by the common flat sac of endoplasmic reticulum. The PGM inside the sheaths is remodelled and the ladder-like pattern of horizontal lamellae is broken down. Instead the dark rods characteristic of the mature stage appear in a strongly asymmetrical way, the asymmetry being most pronounced in the ventral rod (Plate 21:110). Details of this process, which is completed during stage 7, have not been followed.

This asymmetry, and also the type of remodelling of the granular matter, is very different from anything seen in *Raillietiella*. However, Plate 21:106–110 shows that the differences appear at a late stage and that the homologies of the dorsal and ventral rods in the two forms can be established with full certainty in earlier stages.

Spermatid 7; final transformation to mature spermatozoon, transcient appearance of lateral organelles and a row of flat vesicles

As in *Raillietiella* the final development of the spermatozoon includes additional elongation of all structures, elimination of the free flagellum, and reduction of cytoplasm. In *Argulus foliaceus* maturation also includes symmetrization of the nucleus, final reduction of the true acrosome, and appearance of two organelles which soon disappear again: the lateral organelles and the row of flat vesicles.

The elongation of the sperm is accompanied by the appearance of numerous longitudinal microtubules as in *Raillietiella*. These microtubules disappear completely during final maturation, for they are not present in the mature spermatozoa (Plates 18:88, 21:110, 22:112).

The elimination of the free flagellum has not been studied.

The true acrosome. Reduction of the acrosome filament begins already in stage 6 spermatids and is far advanced before the end of this stage. The acrosome vesicle at the anterior pole of the nucleus persists longer. In earlier stage 7 spermatids there is still a doublewalled, i.e., invaginated, vesicle below the tips of the mitochondria (Plate 22:112–113). Longitudinal sections of the anterior nuclear end in late spermatids 7 and mature spermatozoa show only some irregular membranes and nothing like a recognizable acrosome vesicle. This degeration is confirmed by EM pictures of total mature spermatozoa, in which nothing like an acrosomal top or even bulge is seen in the transitional region (Plates 22:111, 4:11).

It must therefore be concluded that the true acrosome and acrosome filament develops progessively from about stage 3 to stage 5 and is established as a rather large and complicated structure, which is reduced to insignificant remnants before final maturation. No trace of a true acrosome or acrosome filament was ever seen in *Raillietiella*.

The lateral organelles, described by Brown (1966, 1970) in another *Argulus* species are present in stage 7 spermatids of *Argulus foliaceus.* In cross sections they are seen as membrane-bound sacs with medium-contrast, coarsely granular content, in contract with the lateral mitochondrion. Usually the cross sections contain only one lateral organelle, and this is invariably on the side opposite to the one where the dislocated nucleus is situated (Plates 17:86, 87, 18:88). In a few sections lateral organelles are present on both sides, but then the one on the nuclear side is smaller, almost vestigial (Plate 17:86). In longitudinal sections it is seen that the sacs of the lateral organelle are comparatively short, arranged in a row.

The morphological significance of this organelle is uncertain. Its anlage can be seen in some cross sections of late stage 6 and early stage 7 spermatids as empty sacs, communicating with the sacs of endoplasmic reticulum. This communication is seen also later, when the sacs are filled with granular matter, but is so narrow that it is doubtful wether the lumina are in open connection or not.

A lateral organelle of this kind was described by Brown (1966, 1970) in *Argulus* sp., in which they are said to be present also in the mature spermatozoa. In *A. foliaceus* there is certainly nothing left of these organelles in mature spermatozoa (Plate 5:17). Brown believed that lateral organelles are developed on both sides, although at different levels, and that they overlap only in a short region. This does not seem probable in *A. foliaceus,* for in this species the organelle is restricted to, or by far best developed, on the same well-defined side of the spermatozoon. This is the side opposite the one to which the nucleus is dislocated, i.e., the side opposite the arms of axonemal filament 1.

A typical lateral organelle is not present in *Raillietiella,* but membrane-bound vesicles, empty or filled with granular matter, are often seen in cross sections of late spermatids. However, the location and appearance is not constant enough to allow safe comparison with the organelles of *Argulus.*

The row of flat vesicles is a short-lived structure, present only in the stage 7 spermatids. It is a straight rod, consisting of flat circular sacs packed close together like coins in a roll. It is only present during the asymmetrical stage, and is adjacent to the medial margin of the nucleus and to the ventral surface of the lateral mitochondrion on that side where no nucleus is present (Plate 18:88–90). The structure is best seen in longitudinal sections: Numerous, flat, empty vesicles, closely packed but without communication of their lumina. In cross sections the lumen often appears to contain some dense material, but this obviously depends on the almost unavoidable inclusion of one or more of the flat vesicle walls in the section. The organelle disappears completely before final maturation. No structure of this kind was seen in *Raillietiella.*

Ventral ribbon. The annular thickenings of the ventral ribbon are present as fairly independent rods in early stage 7 spermatids (Plate 18:90). Later the rods fuse and are seen as ridges on the ventral ribbon in longitudinal sections (Plate 18:89). The origin of the rods has not been followed.

V. GENERAL COMPARISON WITH OTHER SPERMTOZOA

The descriptive chapters have revealed that the spermatozoa of *Raillietiella* and *Argulus* are closely related with regard to structure and development. In the following, points of identity and difference will be summarized, and a comparison will be made with spermatozoa of other animals, particularly arthropods. The main problem to be solved is whether the mutual similarities between *Raillietiella* and *Argulus* are shared with other animals or not, and if systematic and phylogenetic conclusions are indicated by this material.

General structure of the testicle

With regards to testicular structure and general course of spermatogenesis, *Raillietiella* and *Argulus* are different throughout.

In *Raillietiella* the spermatogenesis takes place inside spermatocysts: groups of simultaneously dividing and developing germ cells, surrounded by a plasmatic cyst wall.

Similar spermatocysts are well-known from the majority of insects (Phillips 1970a), including the thysanurans (Werner 1964). In insects, as in *Raillietiella*, the divisions are synchronous and result in a fixed number of spermatids, being a power of 2. In *Raillietiella* the number is either 128 (2^7) or 256 (2^8). These numbers are not uncommon in insects (Phillips 1970a).

However, a more careful consideration leaves some doubt about the significance of these superficial similarities. That groups of male germ cells divide and develop simultaneously is a wide-spread and common phenomenon in animals and is therefore no significant point of similarity between *Raillietiella* and insects. Actually such groups are present also in *Argulus*, although they are not so distinct because of the absence of cyst walls.

More important is the identity or non-identity of the cyst wall. In *Raillietiella* this is formed by several vegetative wall cells, which extend over the growing group of spermatogonia. The wall of the cyst is therefore a mosaic of non-nucleated plasmatic plates which are pinched off from the wall cells when the cyst becomes free. In insects the cyst wall consists of nucleated cells, regarded as abortive germ cells, which *in toto* attach to the group of spermatogenia (Baccetti and Bairati 1964, Bairati and Baccetti 1964, Phillips 1970b). The structure of the cyst wall is therefore different in insects and *Raillietiella* and the homology is somewhat uncertian.

In chelicerates and crustaceans groups or bundles of simultaneously developing spermatids is a common feature, and in some cases a kind of cyst wall is said to be present, for instance in scorpions (Gilson 1885, Sokolow 1913), pseudoscorpions (Boissin and Manier 1966–67), isopod crustaceans (Fain-Maurel 1970), and, perhaps, araneids and opilionids (Gilson 1885, Bösenberg 1905). Details are poorly known for

many groups. Conditions in isopods, in which plasmatic extensions from large, polyvalent wall cells of the testicle surround the islands of simultaneously developing spermatids, are perhaps more comparable to conditions in *Raillietiella* than are the spermatocyst patterns in insects (Fain-Maurel 1970).

Structure and development of the spermatocysts in *Raillietiella* are therefore hardly decisive with regard to the affinities of the group Pentastomida.

In *Argulus* the clumps of simultaneously developing germinal cells move ventrally along the testicular wall and are not surrounded by cyst walls. They are finally attached in bundles to typical Sertoli cells, the pseudoacrosomes being buried in deep pouches developed from the cell wall of the Sertoli cells. This looks very much like the relation between Sertoli cells and spermatids in the vertebrates, but, of course, in the vertebrates the true acrosomes stick in the depressions of the Sertoli cells.

Nutritive cells similar to Sertoli cells are known from many invertebrates, and are particularly typical in gastropods (Yasuzumi et al. 1960). In arthropods it appears difficult to find unquestioned homologues to the Sertoli cells of *Argulus*. In insects, the anterior ends of the spermatids usually stick in depressions in the large polyvalent cells of the cyst wall (Phillips 1970 a). This could suggest a spermatid-Sertoli cell relationship but occurs inside a spermatocyst. In *Raillietiella* the spermatids remain free inside the cysts; their anterior ends are not surrounded by the protoplasm of the cyst wall. In cirriped crustaceans the bundles of spermatids are attached to the testicle wall, but specialized Sertoli cells do not seem to have been described. In some malacostracans and acarids there are nutritive cells (nurse cells, sustentacular cells) of rather special appearance (Fain-Maurel 1970, Moses 1961, Langreth 1969, Reger 1961).

Altogether the structure of the testicle is specialized in very different ways in *Argulus* and *Raillietiella*, and is little useful in discussions of the phylogeny of these groups within the Arthropoda.

The pseudoacrosome

The pseudoacrosome, occupying the anterior fourth or third of the spermatozoon, is the most outstanding and unique feature in the spermatozoa of *Argulus* and *Raillietiella*. The development of the pseudoacrosome is practically identical in the two forms. The structures involved can be readily homologized and are all intimately related to the centriole: The dorsal ribbon, the granulosome, the apical membrane and the pseudoacrosomal granular matter (PGM).

The development from pericentriolar structures in both forms makes a homology with a true acrosome improbable, and this non-identity is settled in a decisive way in *Argulus*, in which a typical true acrosome and a pseudoacrosome exist side by side during development.

Differences in the structure of the pseudoacrosomes between *Argulus* and *Raillietiella* concern the final differentiation of the PGM into rods, particularly its asymmetrical way of condensation in *Argulus foliaceus*. Most differences appear very late during

spermatogenesis, and do not appear to be fundamentally significant. Actually the variation within the genus *Argulus* appears to be great enough to cover even such differences as those between *Argulus foliaceus* and *Raillietiella*, for the *Argulus* species examined by Brown (1966) appears to have a pseudoacrosome of the *Raillietiella* type. Brown only refers to this structure as "sperm end" in the legends to his Fig. 31, but most probably the sections shown are of symmetrical pseudoacrosomes similar to those of *Raillietiella*.

A pseudoacrosome of this kind is not known from other animals, and it is even difficult to find homologues to the single components involved in its development. These components will now be considered separately.

The granulosome develops in contact with the centriole in a region where granular bodies have been described in many animal species: Centriole adjunct, post-nuclear body, annexe centriolaire, granular material, juxta-nuclear body, etc. (see Gatenby and Tamishian 1959, Breland et al. 1966, Phillips 1970 a, Cantacuzène 1970). The granulosome is hardly homologous with these structures, for it develops in contact with the proximal end of the centriole, not around the centriole as the typical centriole adjunct. Moreover, the typical centriole adjunct is formed after the centriole has obtained contact with the nucleus, whereas the granulosome is present at the end of the centriole long before this has happened, and actually mediates the contact between the centriole and the nuclear membrane in later stages.

The situation and early presence of the granulosome would, perhaps, invite a comparison with the proximal centriole, which is located between flagellar centriole and nucleus in many animals. In some animals, e.g., the spider *Pisaurina sp.*, the proximal centriole appears to lose its characteristic structure early so that it looks like a rather unorganized clump in the figures (Reger 1970 b). But no proximal centriole has ever been seen in spermatids of *Argulus* and *Rallietiella*, and is certainly not present in the latter species, of which numerous early spermatids have been seen. The early appearance of the granulosome and apical membrane at one end of the centriole before last meiotic division makes such an interpretation completely untenable.

Actually the granulosome could better be compared with the matter forming around the centrioles of certain urodeles before these attain contact with the nucleus (Werner 1970), but criteria for such a homology are weak and the systematic position of the animals makes the comparison purely theoretical.

The best interpretation of the granulosome and the apical membrane is that they are homologues to the "couronne osmophile" attached to the proximal end of the flagellar centriole in the cirriped *Trypetesa nassarioides* Turquier (Turquier and Pochon-Masson 1969, p. 461). This ring-formed crown of dark matter looks very much like the early anlage of the apical membrane in *Raillietiella*, but nothing similar to the granulosome proper appears to be present in the cirriped.

The apical membrane is present in *Argulus* and *Raillietiella*, and develops during meiosis as a condensation in the granulosome material across the free edges of the centriole. In *Raillietiella* it can be recognized already in late spermatocytes 1. The only

probable homologue is the above-mentioned dark ring at the end of the centriole in the cirriped *Trypetesa*. Although this homology seems reasonable, the differences are considerable. In *Trypetesa* the "couronne osmophile" remains as a dark ring at the end of the centriole in mature spermatozoa, whereas the apical membrane in *Argulus* and *Raillietiella* develops dramatically as one of the constituents of the pseudoacrosome.

Other membranous structures have been described at the proximal end of the centriole in species of arthropods but are more or less clearly derived from the anterior, vestigial end of the nucleus, for instance in the thysanuran *Lepisma saccharina* (Werner 1964), the beetle *Cicindela campestris* (Werner 1965), the mystacocarid *Derocheilocaris typicus* (Brown and Metz 1967, Brown 1970), and cirripeds (Turquier and Pochon-Masson 1969, Pochon-Masson et al. 1970). It is then supposed that the "collar" described by Munn and Barnes (1970) in *Balanus balanus*, *B. perforatus*, and *B. balanoides* is identical with the flat nuclear diverticulum described by Turquier and Pochon-Masson (1969) and Pochon-Masson et al. (1970).

The pseudoacrosomal granular matter (PGM) is clearly homologous in *Raillietiella* and *Argulus*, being formed in identical areas and subdivided in the same way into a dense dorsal part and a more scattered ventral portion. It is obviously part of the centriolar annexes, for in abnormal spermatids with two centrioles, each is associated with its own portion of PGM (Plate 18:91). I have not been able to find any homologues in other animals.

The dorsal ribbon and its membranous sacs take part in the formation of the pseudoacrosome, but are discussed below.

The general organization of the body

Development of the nucleus and the mitochondria into long, filamentous structures lying parallel with the axonema is in no way a unique feature in *Argulus* and *Raillietiella*, for it is a fairly common feature in arthropods (see Baccetti 1968, 1970a, Phillips 1970a). Even dislocation of the centriole to a level near the anterior end of the nucleus, and the filamentous development of the latter, is known in some arthropods, particularly in *Lepisma saccharina* (Werner 1964), *Thermobia domestica* (Bawa 1960, 1964a,b), *Cicindela campestris* (Werner 1965), *Panorpa annexa* and *P. germanica* (Baccetti et al. 1969), *Derocheilocaris typicus* (Brown 1966, Brown and Metz 1967, Brown 1970), and cirripeds (Brown 1966, 1970, Turquier and Pochon-Masson 1969, Pochon-Masson 1970, Munn and Barnes 1970). Such an anterior dislocation of the centriole is also known from non-articulates, belonging to widely different phyla: some flatworms (Silveria and Porter 1964, Hendelberg 1970), the chaetognath *Spadella cephaloptera* (v. Deurs 1972), the fish *Polypterus senegalus* (Mattei 1969, 1970), and some amphibians (Austin and Baker 1964). The filamentous shape of nucleus and mitochondria and the anterior dislocation of the centriole are thus far from specific to *Argulus* and *Raillietiella* and are little useful in discussions on phylogenetic relationships.

The nearly perfect bilateral symmetry of the spermatozoan body is a characteristic feature of *Argulus* and *Raillietiella*, although the spermatids of the former pass through an asymmetrical stage before final maturation. Similar symmetry is common in insect spermatozoa, as far as mitochondria and axonema are concerned (Phillips 1970a). In forms with an anteriorly dislocated centriole such as *Lepisma*, the cross section of the body can be similar to that of *Raillietiella* and *Argulus*, with nucleus, mitochondria and axonema symmetrically disposed (Werner 1964). The flagellate crustacean spermatozoa found in the mystacocarids and cirripeds are strongly asymmetrical (Brown and Metz 1967, Brown 1966, 1970, Turquier and Pochon-Masson 1969, Pochon-Masson et al. 1970, Munn and Barnes 1970). The large spermatozoa of ostracods are bilaterally symmetrical but are aflagellate and can hardly be compared to those of *Argulus* and *Raillietiella* because of their highly specialized and unique structure (Reger 1970a). A distinctly symmetrical pattern including axonema, filiform mitochondria and a filiform nucleus is not present in chelicerates, myriapods or diplopods (André 1959, 1965, Rosati et al. 1970, Reger 1961, 1962, 1963, 1969, 1970b, Grassé et al. 1965, Reger and Cooper 1968, Horstmann 1968, 1970). It may thus be concluded that *Argulus* differs strikingly from other crustaceans with regard to the bilateral symmetry of the spermatozoa. However, the presence of similar patterns in some insects makes it difficult to use the strictly identical symmetry of *Raillietiella* and *Argulus* for phylogenetic conclusions.

Complete reduction of the free flagellum is typical of *Raillietiella* and *Argulus* and is possibly a fairly specific feature. In other articulate spermatozoa of filiform type there is a short free flagellum also in the mature spermatozoon, e.g., in *Lepisma* (Werner 1964), *Derocheilocaris* (Brown and Metz 1967) and Cirripeds (Pochon-Masson et al 1970). However, the morphology of this end of the spermatozoon is often incompletely known in the arthropods, so the uniqueness of *Argulus* and *Raillietiella* cannot be regarded as established with regard to this feature.

The axonema

Both in *Argulus* and *Raillietiella* the axonema is of the 9+2 type and presents some very specific features, common to both species: 1) the symmetrical situation of the central filaments on each side of the median plane, 2) the dorso-median situation of peripheral doublet no. 1, 3) the presence of a minute membrane, connecting doublet 1 with the dorsal ribbon, and 4) the oblique situation of this membrane which, in cross sections, is inclined to the side where the arms of doublet 1 are situated.

The constant symmetrical situation of the central filaments on each side of the median plane appears to be a feature restricted to *Argulus* and *Raillietiella*. The plane of the central filaments thus forms an angle of 90° with the median plane in these forms. In other arthropods with symmetrical spermatozoa, i.e., a number of insects, the plane through the central filaments is more or less vertical, forming a small angle with the median plane (Phillips 1970b). Even in the symmetrical spermatozoa of

Lepisma (Werner 1964, own observations) and *Petrobius* (own observations) the central filaments are oriented as in other insects, i.e., they are nearly but not exactly median. Numerous EM pictures in the literature, particularly in *Comparative Spermatology* (Baccetti 1970b) confirm these statements.

The exceptional orientation of the central filaments may indicate that the bending movements of the spermatozoids of *Argulus* and *Raillietiella* are dorso-ventral, not more or less lateral as is supposed for other arthropods (see Fawcett and Porter 1954, Gibbons and Grimstone 1960, Phillips 1970a).

The orientation of the central filaments has, by definition, the consequence that doublet 1 must be median, but there are two possibilities: dorso-median or ventro-median. Point 2) above says that the first alternative is realized both in *Argulus* and *Raillietiella*.

The small membrane connecting outer doublet 1 with the dorsal ribbon is also a feature restricted to *Argulus* and *Raillietiella*. In cross sections the membrane is inclined to the same side in both species, i.e., the side where the arms of doublet 1 are situated.

Similar membranes, connecting peripheral doublets with surrounding sheaths or fibers, have been described in many different animals, for instance mammals (Fawcett and Phillips 1970), and fish (Mattei 1969). They are probably present in some arthropods (see figures in Phillips 1970b). However, the restriction of this membranous connection to doublet 1, and its very characteristic and constant oblique course, are features only found in *Argulus* and *Raillietiella*. The attachment of the membranous envelopes of the dorsal ribbon to doublets 3 and 8, as seen in *Argulus* only, would appear to be a less unique feature. A (probably analogous) attachment of these doublets to outer fibers and sheaths is seen in some mammals (Fawcett and Phillips 1970, Pedersen 1970).

Altogether, the details in axonemal structure common to *Argulus* and *Raillietiella* are so fundamental and so different from anything known in other animals, that they have to be considered earnestly in phylogenetic discussions.

The dorsal and ventral ribbons

The identical structure and development of the ribbons in *Argulus* and *Raillietiella* was described in pages 32 and 36–44. Identity is extended to numerous details like 1) the sharp lateral edges of the dorsal ribbon, fitting into furrows in the broad margins of the ventral ribbon, 2) the presence of membranous sacs around the dorsal ribbon, not around the ventral one, 3) extension of the ventral ribbon over the dorsal and lateral surfaces of the lateral mitochondria, 4) the early development of the anlage of the dorsal ribbon before 2nd meiotic division in both forms, as a flat membranous sac with underlying dark rods on one side of the centriole, 5) the shift from two to three dark rods in the early anlage of both forms, the latter three rods corresponding to outer dark filaments 9, 1 and 2.

The most astonishing of all these common features is perhaps that the centriole becomes bilaterally symmetrical by the appearance of a rudimental dorsal ribbon long before meiosis is completed.

The only difference of importance between the two forms is the development of a pair of distinct "intermitochondrial rods" in *Argulus*, probably homologous with outer dark fibers nos. 5 and 6. In *Raillietiella* these dark fibers together with nos. 4 and 7 are included in the ventral ribbon and probably form the light cores.

Development of dark outer fibers or outer filaments, peripheral to the doublets, is not rare in arthropods (Baccetti 1970a, Phillips 1970a,b, Rosati et al. 1970), but fusion of such fibers to form something like the dorsal and ventral ribbons of *Argulus* and *Raillietiella* does not appear to have been described.

Other dark-staining rods, situated outside the dark outer fibers along the axonema, have been described in some insects, e.g., culicids (Breland et al. 1966), certain homopterans (Folliot and Maillet 1970), *Lepisma* (Werner 1964) and *Petrobius* (own observations). They are said to be derived from a "centriole adjunct" or "postnukleärer Körper" and may be paired, symmetrically situated as in the homopterans and *Petrobius* or unpaired as in *Lepisma*. Neither the structure nor the development of these bodies supports a homology with the ribbons of *Raillietiella* and *Argulus*, in which no typical centriole adjunct is formed.

The only well-established affinity in the case of dorsal and ventral ribbons was derived from comparisons with the spermatogenesis of cirripeds as described by Bocquet-Védrine and Pochon-Masson (1969) and Pochon-Masson et al. (1970). In cirriped spermatids they found a bilamellar structure, attached to one side of the axonema, often situated between the axonema and the single mitochondrion. They believe that the two lamellae are derivatives of the endoplasmic reticulum. Although the situation on the mitochondrial side of the flagellum is somewhat problematic, the structure looks very much like the endoplasmic sacs of the dorsal ribbon in *Argulus* and *Raillietiella*. In the cirripeds this bilamellar structure or "lamelle paraflagellaire" is only present during development and was not seen in mature spermatozoa.

Another possible homologue to the sacs of the dorsal ribbon is the "Lamelle" described by Werner (1965) in spermatids of *Cicindela*. It is a half-cylindrical piece of membrane, situated in the plasm near the centriole, and disappears before maturation. Although a homology is possible, I hesitate to suggest it, because the structure in *Cicindela* has a variable orientation and location.

Thus, with the exception of a possible presence in early spermatids of cirripeds of a homologue of reticular sacs of the dorsal ribbon, this ribbon, like the ventral one, appears to be highly characteristic of *Raillietiella* and *Argulus*, with no real counterparts in other articulates. This is true also of the numerous details of these complicated and composite structures.

The mitochondria

Three mitochondrial rods, with vestigial cristae, situated symmetrically between the ventral ribbon and the nucleus, are typical of the mature spermatozoon of *Argulus* and *Raillietiella*. Development is identical: The tubular mitochondria of the spermatocytes are reduced in number and become spherical. They fuse in early spermatids to form three large spheres in contact with each other. These large, mitochondrial balls are packed together into a single Nebenkern in which the three composite mitochondria remain separated by intact mitochondrial walls. Three mitochondrial tongues are emitted from this Nebenkern along the ventral aspect of the flagellum, first in the anterior direction, a little later also in a posterior direction. When these mitochondrial tongues have attained their definite length the Nebenkern is completely reduced.

The difference between the two forms is inconsiderable: Absence of cristae in the median mitochondrion of *Raillietiella*, and retraction of the inner mitochondrial membrane from the anterior end of the same mitochondrion in *Argulus*.

Long, filamentous mitochondria are common in insects (Baccetti 1968, Phillips 1970a, Favard and André 1970), but the normal number is two or one. Only collembolans have more: in addition to two long ones there may be six to seven small ones in the region of the neck (Dallai 1967). In *Anurida maritima* (Guerin) three long filamentous mitochondria extend along the axonema, the median one being shorter than the other two (Dallai 1970). Cirripeds have only one long mitochondrion (Pochon-Masson et al. 1970). Mystacocarids are not well known but the *Argulus* pattern is certainly not present (Brown and Metz 1967). Other crustaceans have such specialized spermatozoa that a comparison with *Argulus* has little purpose (Brown 1970, Baccetti 1970). Among chelicerates, *Limulus* (= *Xiphosura*) *polyphemus* has simple mitochondria of the polychaete type (André 1965), and the spermatozoa of other chelicerates and myriapods vary with regard to mitochondria, but nothing like an *Argulus* pattern has been described (Reger 1961, 1962, 1963, 1969, 1970b, Horstmann 1968, 1970, Breucker and Horstmann 1968, Rosati et al. 1970, Baccetti 1970a).

With regard to transformations during development, the mitochondria of *Argulus* and *Raillietiella* follow a pattern known mainly from insects and gastropods (André 1962, Favard and André 1970). In insects the large Nebenkern is formed by several mitochondria, which are transformed into a single continuous network of mitochondrial tubes by membrane fusion (de Robertis and Raffo 1957, Favard and André 1970, Pratt 1970). This body may later be subdivided into the two mitochondrial derivatives usually present in mature spermatozoa. Details are known only for few species, but if this description holds for insects in general, it may be concluded that *Argulus* and *Raillietiella* are different with regard to the formation of the Nebenkern. In these forms the initial mitochondrial fusion results in formation of three spherical bodies, which remain separated by double membranes in the Nebenkern.

Thus, with regard to the mitochondria, *Raillietiella* and *Argulus* follow a pattern

common in arthropods, particularly in insects. The only unique feature is the number of mitochondrial rods, which is three, whereas other filiform arthropod spermatozoa have two or one. Apart from the Branchiura and Pentastomida, three mitochondrial rods are known only from the collembolan *Anurida maritima*. In other respects the collembolan spermatozoa are so different from those of *Argulus* and *Raillietiella* that comparisons are very difficult.

The nucleus

The long, filamentous nucleus is almost identical in mature spermatozoa of *Argulus* and *Raillietiella*. The development of the nucleus is, however, different in details. In *Argulus* both the anterior and the posterior ends of the ovoid nucleus are drawn out to form nuclear tongues, whereas only the anterior end is drawn out in *Raillietiella*. Eventually the thick part of the nucleus is attenuated in both species, and the final result is the same. Condensation of the chromatin is different: In *Raillietiella* many small granules grow to large clumps which coalesce, in *Argulus* there is a rod-like condensation along the acrosome filament. Further, the extra membranes formed inside the original nuclear membranes appear to be peculiar to *Argulus*.

A long, filamentous nucleus is a common feature in arthropods (Baccetti 1968, Phillips 1970a), but it is rarely extended to the extreme posterior end as in *Argulus* and *Raillietiella* (see also Thompson and Blum 1967). The condensation of chromatin takes place in so many and different ways in different species, that general statements with phylogenetic significance are excluded.

Thus, while the general features of the nucleus are very much the same in *Argulus* and *Raillietiella* and fit well in the phylum Arthropoda, there are no unique features with great significance in phylogenetic discussions as far as the nucleus is concerned.

The true acrosome

With regard to the true acrosome, *Argulus* and *Raillietiella* spermatozoa are nearly identical in the mature state, in which the structure is absent *(Raillietiella)* or present only as an insignificant vestige *(Argulus)*. In *Raillietiella* no trace of a true acrosome has been seen during development, whereas spermatids of *Argulus* develop a distinct acrosome vesicle and acrosome filament ("perforatorium"), which eventually disappear.

The acrosome apparatus of *Argulus* spermatids is of a generalized type with a bowl-shaped acrosome vesicle covering the top of an acrosome filament, which extends throughout the long nucleus, lying in a tube-like invagination of the nuclear membranes. Among arthropods, a very similar apparatus is found in *Limulus* (André 1965, Philpott and Shaw 1959), which is supposed to have the most primitive spermatozoa of all arthropods (Baccetti 1970a). In other arthropods there are modifications of the acrosome vesicle or of the acrosome filament or of both. In crustaceans a fairly

typical acrosome vesicle covering a rod-like acrosome filament is found in the cirripeds, but the filament does not penetrate into the nucleus (Pochon-Masson et al, 1970. Munn and Barnes 1970). The acrosome of the mystacocarid *Derocheilocaris* is described as an electron-dense through-like structure, and no acrosome filament is mentioned (Brown and Metz 1967). Other crustaceans are very strongly modified with regard to acrosome development (Yasuzumi 1960, Yasuzumi et al. 1961, Hollande and Fain 1964, Reger 1964a,b, 1966, 1970a, Brown 1966, 1970, Fain-Maurel 1966, 1970, Pochon-Masson 1969).

Chelicerates are not very well known with regard to acrosome development, but the araneids certainly have a fairly compact acrosome vesicle and an acrosome filament (Reger 1970b, Rosati et al. 1970). The chilopod *Geophilus linearis* Koch has a long compact acrosome (Horstmann 1968), whereas pauropods lack an acrosome and the symphylans and diplopods have a strongly modified one (Rosati et al. 1970, Reger and Cooper 1968, Horstmann 1970). No acrosome filament appears to be present in these millipedes and centipedes.

Insect acrosomes are very compact throughout development and there is no acrosome filament penetrating into the nucleus (Phillips 1970a). Interesting exceptions from this rule are found in the collembolan *Anurida maritima* (Dallai 1970) and the thysanur *Petrobius maritimus* (author's unpublished material). In *Anurida* the acrosome filament appears to be permanent. In *Petrobius*, a compact acrosome vesicle and a very large acrosome filament develops during spermatogenesis. The filament penetrates the very long nucleus and ends near the nuclear membrane in the region of the centriole, but disappears completely before final maturation of the spermatozoon. The acrosome vesicle develops into an extremely long and narrow acrosome of the insect type, and persists in the mature spermatozoon.

When the variation of the acrosomal apparatus within the arthropods is taken into consideration, it appears probable that the ancestral arthropods have had an open acrosome vesicle and a long acrosome filament like those seen in spermatozoa of *Limulus* and, in more modified forms, in araneids and in spermatids of *Petrobius* and *Argulus*. This is also the type of acrosomal apparatus found in annelids with fertilization in the water like *Hydroides hexagonus* (Colwin and Colwin 1961a,b) and *Nereis japonica* (Takashima and Takashima 1963).

The transcient appearance of such an apparatus during spermatogenesis in *Argulus* is particularly interesting, since the structures are reduced before final maturation and therefore cannot be used for their original function: to penetrate the egg surface. This reminds strongly of ontogenetical recapitulation, here illustrated on the cytological level. Following this line up, one would assume that the genes regulating the development of the acrosomal apparatus have been partly suppressed in *Argulus* and completely suppressed in *Raillietiella*, in which no trace of a true acrosome has been found. The latter should, therefore, be regarded as more advanced in this respect. The appearance and later reduction of the acrosome filament in *Petrobius* can probably be viewed in a similar way.

The lateral organelles

The lateral organelles are present only in late spermatids of *Argulus* and disappear in *A. foliaceus* before final maturation. In the other *Argulus* species examined by Brown (1966 and 1970) these organelles are said to be present in mature sperm. Membrane-bound vesicles with granular content have been seen in spermatids of *Raillietiella*, but do not have the constant localization typical of lateral organelles. Homologization of these more variable vesicles with the row of elongate sacs in *Argulus* therefore remains uncertain. A possible homologue of the lateral organelles is the "vesicule" in cirriped spermatids, described by Bocquet-Védrine and Pochon-Masson (1969), Turquier and Pochon-Masson (1969) and Pochon-Masson et al. (1970). Like the lateral organelles this "vesicule" is supposed to be developed from the smooth endoplasmic reticulum, but the criteria for a homologization of the two structures are admittedly not very strong.

VI. PHYLOGENETIC AND SYSTEMATIC IMPLICATIONS

Main conclusions

The detailed comparisons in chapter IV show that the spermatozoa of *Raillietiella* and *Argulus* are nearly identical with regard to structure and development. The analysis in chapter V shows that the *Raillietiella-Argulus* type of spermatozoon is highly specialized and represents a type of its own, not encountered in other animals. A long series of fundamental features common to the spermatozoa of *Raillietiella* and *Argulus* has no counterpart in other spermatozoa. This justifies the conclusion that the Pentastomida and the Branchiura are closely related.

The conclusion is of course based on the great over-all similarity between the spermatozoa of the two animals, but is specifically supported by the following features which have only been found in *Raillietiella* and *Argulus:*

1) The presence of a ca. 30 μ long pseudoacrosome, developed as two half-tubular sheaths with granular contents and originating in well-defined peri-centriolar structures which can be homologized in the two species: granulosome, apical membrane, dorsal ribbon, and PGM.

2) The very similar structure of the transitional region between pseudoacrosome and body in the two forms: The dorsal rod of the pseudoacrosome is continuous with the dorsal ribbon of the body, and is connected with the centriolar filaments nos. 9, 1, and 2; The ventral rod of the pseudoacrosome is continuous with some of the ventral filaments of the centriole and its outer sheath is forked posteriorly into a pair of wing-like flaps which cover the sides of the centriole.

3) The plane of the central filaments of the axonema is perpendicular to the median plane of the body. The peripheral doublet no. 1 is medio-dorsal and differs from

the other eight doublets in being connected with the dorsal ribbon by a minute, obliquely oriented membrane. This membrane is inclined to the side where the arms of doublet no. 1 are situated.

4) The presence and structure of the dorsal and ventral ribbons, which together form a tubular investment of the axonema: The dorsal ribbon is half-cylindrical with sharp edges which fit into furrows in the thicker free edges of the ventral ribbon. The latter covers the dorsal aspect of the mitochondria and extends with thin lamellae over the lateral aspects of the lateral mitochondria. The dorsal ribbon, but not the ventral one, is surrounded by narrow membranous sacs, developed from an inner and an outer sac in the spermatids. The dark outer fibers 9, 1, and 2 are included in the dorsal ribbon.

5) The rudiment of the dorsal ribbon is present before the 2nd meiotic division in both species and can be recognized as a pair of dark rods along one side of the centriole, covered by a small sac of smooth endoplasmic reticulum (this sac may be traced also in the Cirripedia). Later, in the early spermatids, there are not two but three dark rods under the endoplasmic reticular sac, and these three rods are the outer dark fibers 9, 1, and 2 of the centriole-axonema. The shift from two to three rods is well documented in both species.

6) The presence of *three* filamentous mitochondria, symmetrically situated between nucleus and axonema. This feature is perhaps not completely unique, for three mitochondrial rods are present in the collembolan *Anurida maritima*.

The most important *difference* between the spermatozoa of *Argulus* and *Raillietiella* is the transient appearance of an acrosome vesicle and an acrosome filament in spermatids of the former. Other differences are hardly greater than what could be expected between different species of a single genus. The most striking ones are found in the pseudoacrosome, which is strongly asymmetrical in *Argulus foliaceus* and strictly symmetrical in *Raillietiella*. However, the *Argulus sp.* studied by Brown (1966) appears to have a symmetrical pseudoacrosome of the same appearance as that of *Raillietiella*, i.e., if my interpretation, that the "sperm ends" in Brown's figures are pseudoacrosomes, is correct.

In view of the numerous points of identity between the spermatozoa of *Raillietiella* and *Argulus*, and the absence of most of these features in other animals, it must be concluded that convergence and evolutional hazards are not good explanations. The identical features must therefore depend on a close phylogenetic relationship in the sense that the Pentastomida and the Branchiura are more closely related to each other than to other animal groups.

It is therefore suggested that the Pentastomida be placed as a sub-group of the Branchiura in the zoological system. The subclass Branchiura can be maintained and can be divided into two orders: The Argulida and the Pentastomida.

It is admitted that adult and larval morphology appear to give little support to such a rearrangement of the system, but negative evidence is of little weight in a case like this, when one of the groups, the Pentastomida, are specialized endoparasites, and

the other, the Argulida, show distinct specializations for an ectoparasitic life. Parallel cases are known, in which the adult morphology of parasites is inconclusive with regard to systematic affinities: e.g., Rhizocephalia among the Cirripedia and the genus *Enteroxenos* among the Gastropoda. In these cases the larval forms finally revealed the true relationships. In the case of *Raillietiella* and *Argulus*, the embryology and internal anatomy of the larval forms is poorly known, and a comparison between the two forms does not seem to have been attempted. This is left for future research.

The present conclusion in the light of crustacean spermatology

Considerable support to the conclusions above can be derived from present knowledge of crustacean spermatozoa. As pointed out particularly by Brown (1970), each major crustacean group is characterized by its own type of spermatozoa, and a classification of the Crustacea based on the spermatozoa alone would fit very well with the system for decapods suggested by Borradaile (1907). Within the Malacostraca, all spermatozoa are non-flagellate, and the group Peracarida stands out beautifully because Isopoda, Amphipoda, Cumacea and Mysidacea have spermatozoa which can be referred to a common type, although there are differences in details between the groups. This indicates strongly that spermatozoan morphology is conservative enough to be a useful diagnostic criterion for the larger systematic units within the crustaceans.

This is also beautifully illustrated by the Cirripedia, which are of interest because they are well investigated, and because they are often believed to be somewhat related to the Branchiura. Both free-living forms such as *Balanus, Chthamalus, Lepas* and *Scalpellum* and strongly modified parasites such as the acrothoracid *Trypetesa* and the rhizocephalian *Sacculina* have been examined after the electron microscope became available (Brown 1966, 1970, Turquier and Pochon-Masson 1969, Pochon-Masson et al. 1970, Munn and Barnes 1970). It was stated that the spermatozoa of all investigated species were modifications of a characteristic "cirriped type" of spermatozoon, in which the typical relations between acrosome, nucleus, axonema and mitochondrion remained constant (Pochon-Masson et al. 1970). This type is not like any spermatozoa encountered in other crustaceans. It appears that a classification of crustaceans based exclusively on the spermatozoa would include a group Cirripedia, very distinct from other crustaceans, and also containing the strongly modified parasites *Trypetesa* and *Sacculina*, which have been difficult to place on the basis of adult morphology. In fact, the situation of *Sacculina* within the Cirripedia reminds very much of that of Pentastomida within the Branchiura, if the system suggested above is accepted.

The expected similarities between Branchiura and Cirripedia on the spermatological level turned out to be very slight and partly doubtful: 1) Presence in spermatids of cirripeds of a "lamelle paraflagellaire" along the axonema, obviously homologous with the membranous sacs of the dorsal ribbon in the *Argulus-Raillietiella* spermatid (see p. 53), 2) Presence in *Trypetesa* of dark material at the end of the centriole, perhaps homologous with a granulosome or apical membrane (p. 49), and 3) Presence in

cirripeds of a "vesicule", perhaps homologous with the lateral organelles in *Argulus* (p. 57).

Although these similarities between cirripeds and *Argulus-Raillietiella* may seem insignificant, they are the only positive result of the search for homologies of the many unique structures in the spermatozoa of *Raillietiella* and *Argulus*. It is admitted, however, that these similarities to cirripeds are not great enough to be conclusive in systematic discussions.

The present conclusions and general articulate spermatology

All recent investigators of Pentastomida agree that the morphology and larval development definitely show that they are articulates (Heymons 1935, Beklemischev 1958, 1969, Kaestner 1954/55, 1965, Doucet 1965, Legendre 1967, v. Haffner 1971). For this reason, mainly articulate spermatozoa have been considered in the comparative parts of the present work. Actually the Annelida are rarely mentioned, partly because the ultrastructural investigations are few. However, the numerous light microscopical investigations, particularly those of Franzén (1956, 1958, 1962, 1970), show that the majority of polychaete spermatozoa are of the primitive type characteristic of animals with external fertilization in the water. Presence of the advanced pattern characteristic of the *Raillietiella-Argulus* spermatozoa is therefore excluded. In a few polychaete families, and in the Hirudinea, Oligochaeta and Archiannelida there are more specialized spermatozoa, which in a few cases can be truly filiform. In practically all cases light microscopy has revealed sufficient detail to show that they are fundamentally different from the *Raillietiella-Argulus* type (see Franzén 1956, 1970). Electron microscopy of the spermatozoa of *Hydroides hexagonus* (Colwin and Colwin 1961 a, b), *Nereis japonica* (Takashima and Takashima 1963), *Spirorbis moerchi* (Postwald 1967), *Lumbricus spp.* (Gatenby and Dalton 1959, Bradke 1963, Anderson et al. 1967), *Enchytraeus albidus* (Reger 1967), and *Hirudo medicinalis* (Pastisson 1966) give no reason to change this statement. The studies of annelid spermatology therefore give us no reason to believe that *Argulus* and *Raillietiella* should have inherited their remarkable spermatozoa directly from annelid-like ancestors.

The situation appears to be much the same for arthropods in general. Their sperm structure, which is exceptionally well studied, indicates that specializalion of the spermatozoa, perhaps also development of internal fertilization, has taken place independently within several evolutional lines leading up to the major groups of recent arthropods. A striking argument is furnished by *Limulus* (= *Xiphosura*) *polyphemus*, which has external fertilization and a primitive type of spermatozoon (Philpott and Shaw 1959, André 1965). This spermatozoon is almost identical with that found in many polychaetes, molluscs, echinoderms and other animals with external fertilization (Franzén 1956, 1970). This makes it most improbable that *Limulus* or its ancestors ever had internal fertilization and specialized spermatozoa, for then the "primitive" type of

sperm had to evolve independently. A more probable interpretation is that *Limulus* inherited the mode of fertilization and the primitive spermatozoa directly from the pro-articulates. This means that the pro-arthropod (if the group Arthropoda is monophyletic) or at least the pro-chelicerate must have had external fertilization and simple spermatozoa, and that internal fertilization and specialization of the spermatozoa must have evolved independently within several arthropod lines.

Recent chelicerate spermatozoa are also very different from one group to another, and the features common to several groups are seemingly restricted to those present in the primitive spermatozoa of *Limulus* (i.e., symplesiomorphous). This would be expected if specialization for internal fertilization has been largely independent within each evolutional line and has started with a *Limulus* type of sperm. Facts concerning the structure of chelicerate spermatozoa have been supplied for *Limulus* (Philpott and Shaw 1959, André 1965), scorpions (Tuzet 1938, André 1959), araneids (Reger 1970b, Rosati et al. 1970), opilionids (Sotelo et al. 1958, Reger 1969), pseudoscorpions (Boissin and Manier 1966a,b, 1967), and acarids (Reger 1961, 1962, 1963, Breucker and Horstmann 1968).

The very great differences between the spermatozoa of the crustacean groups also support the view that specialization for internal fertilizalion has been largely independent within each evolutional line (for literature see Brown 1966, 1970, Baccetti 1968, 1970a). If all crustaceans had started with a specialized spermatozoon in a common ancestor, some of the specializations would be expected to occur in several recent groups, and a kind of "crustacean type" of spermatozoon could be constructed. This is certainly not possible. The round, non-flagellate anostracan spermatozoa, the enormous motile but non-flagellate and complicated ostracod spermatozoa, the ovoid non-flagellate copepod spermatozoa, the flagellate branchiuran spermatozoa with their pseudoacrosome, the flagellate cirriped spermatozoa with true acrosome and anteriorly displaced centriole, and the several types of non-flagellate malacostracan spermatozoa have little in common except features which are present in a primitive cell. Brown (1966, 1970) suggests that the spermatozoids of the cephalocarid *Hutchinsoniella macracantha* have some features in common with malacostracan spermatozoa, and that those of the mystacocarid *Derocheilocaris typicus* show affinity to cirriped and branchiuran spermatozoa, but other generalizalions are difficult to find.

Insects appear much more uniform with regard to the spermatozoa, and a standard type can be imagined in spite of considerable variations (Baccetti 1968, 1970a, Phillips 1970a).

Very strong specializations are found in the different groups of millipedes and centipedes (Grassé et al. 1965, Horstmann 1968, 1970, Deschamps 1969, Chevallier 1970, Reger and Cooper 1968, Rosati et al. 1970).

Of particular interest are the spermatozoa of Onychophora and Tardigrada, since these animals have played a role as possible relatives of the Pentastomida. Light microscopical investigations of *Peripatus* spermatozoa (Montgomery 1900, Gatenby 1925)

indicate that these are almost filiform with "head", "middle-piece" and "tail" after each other in normal sequence. The spermatozoa of the tardigrade *Macrobiotus hufelandi* are comparatively little specialized, with a pointed acrosome, a spiral nucleus, a sheath of metamorphosed mitochondria around the anterior part of the axonema, and a long free flagellum (Baccetti, in print, and author's own material). No pentastomid characters could be discovered.

Although the articulate system and particularly the arthropods are well covered by ultrastructural investigations of spermatozoa, no structures comparable to the specialized features in the spermatozoa of *Raillietiella* and *Argulus* have been found.

It is admitted that the present chapter is theoretical and may be superficial in many respects, but it is supposed to show that placing the Pentastomida outside the Branchiura, e.g., within Chelicerata, or together with Myriapoda, or together with Tardigrada and Onychophora, or deriving them directly from Annelida, is strongly contradicted by the spermatological picture. In these cases, the common ancestor of *Argulus* and *Raillietiella* would be either a pro-articulate, a pro-arthropod, or a pro-mandibulate, hypothetical forms which must be supposed to have had primitive spermatozoa and, most probably, external fertilization. At any rate these hypothetical ancestors cannot have had the specialized *Argulus-Raillietiella* type of spermatozoon, for no trace of such specialized features is found in other descendants of these ancestral forms. Evolution of the highly specialized spermatozoa would therefore have taken place independently within the pentastomid line and the branchiuran line and resulted in the same structure with a long series of identical specialized details. This cannot be accepted as probable.

Comments on previous opinions about pentastomid relationships

Since a relation between pentastomids and branchiuran crustaceans has never been suggested before, the conclusions in this paper must be in conflict with the majority of the numerous ideas published on the pentastomid problem (see reviews in Heymons 1926/27, 1935, Osche 1963, Doucet 1965, Self 1969, v. Haffner 1971).

Among the old ideas, those dealing with affinities to nematodes, trematodes and cestodes did not survive when Leuckart's (1860) admirable monograph appeared. Leuckart himself regarded the pentastomids as related to acarids, particularly eriophyids ("Phytoptus") which have two pairs of anteriorly placed limbs and an annulate body, superficially resembling pentastomid hooks and segmentation.

An affinity of the pentastomids to crustaceans was mainly suggested by v. Beneden (1849), who compared the primary larva with a crustacean nauplius. As probable relatives of the pentastomids he also mentioned the pantopods (pycnogonids), by v. Beneden regarded as crustaceans but now usually placed near the Chelicerata. Although this comes near the conclusions of the present paper it is admitted that v. Beneden's arguments are untenable (see criticism in Leuckart 1860, Osche 1963, v. Haffner 1971).

The more specified ideas from the last few decades may be grouped as follows:

1. The Pentastomida are related to the Tardigrada and the Onychophora. The idea originates in a paper on *Myzostoma* by v. Graff (1877), in which myzostomids, tardigrades and pentastomids are compared. Cuenot (1952), Vandel (1949), and Weber (1949) created a particular group including the Pentastomida, the Tardigrada, and the Onychophora and called it Malacopoda, Pararthropoda or Onchopoda. The most important argument for keeping the groups together was the structure of limbs and claws (hooks).
2. Most recent authors hesitate with regard to the affinities to tardigrades or directly reject them. It is stated that the pentastomids have a mixture of arthropod and annelid features with some preponderance of the former. Since no distinct and specific relation to any articulate group is found, the pentastomids are derived directly from annelid-like ancestors or pro-arcitulates, independently or in some relation to tardigrades (Kaestner 1954/55, 1965, Beklemischew 1958, 1969, v. Haffner 1971).
3. On the basis of new information derived from embryos of the pentastomid *Reighardia sternae*, Osche (1963) concluded that the pentastomids are tracheate arthropods, probably related to myriapods.

It is obvious that all three alternatives are in serious conflict with the conclusions in the preceding pages, for all three will imply that the complicated and distinctly synapomorphous pattern of characters in the spermatozoa of *Argulus* and *Raillietiella* must have arisen independently within the two lines.

The first and second alternatives are not so fundamentally different, for all authors are somewhat hesitant with regard to the significance of those characters which indicate tardigrade relationship (limbs, hooks, nervous system). As stated and documented by v. Haffner (1971) and Osche (1963), this affinity to tardigrades cannot really be regarded as established. Kaestner, Beklemischew and v. Haffner, being unable to accept the affinities to myriapods suggested by Osche, were therefore left with the statement that available knowledge of pentastomids does not reveal distinct and specific affinity to any arthropod or annelid group. The logical consequence is that the pentastomids are regarded as an isolated group and are placed between annelids and arthropods because of their supposed mixture of general characters from both phyla.

When now a specific and obviously synapomorphous set of characters in the spermatozoa indicates a close relation between the pentastomids and the branchiuran crustaceans, there is consequently no alternative theory, and the new findings must be regarded as decisive. Some interesting consequences of the new position of the pentastomids in the system must be discussed, however.

The immediate consequence of the suggested position of the Pentastomida as a subdivision of the Branchiura is that they must have undergone a strong regressive development during their adaption to a parasitic mode of life. Kaestner (1965) admits that such a posibility is open but not proved. V. Haffner (1971) is much more reluctant

with regard to possible reductions in the body of pentastomids. He is of the opinion that the pentastomids never reached the level of fully developed arthropods, mainly because of the presence of a number of characters regarded as specific to annelids. The most important of these are 1) the seemingly unsegmented supraoesophageal ganglion, 2) the non-development of a composite suboesophageal ganglion, 3) the presence of an outer layer of ring muscles in the body wall, and 4) the presence of segmental, paired sensory organs along the body sides (''lateral line organs'').

It is obvious that presence of specific and indisputable annelid characters in pentastomids would make it difficult to derive the group from branchiurans, which must be supposed to have lost these characters. The significance of the four points above will therefore be considered critically.

1) *The supraoesophageal ganglion.* Pentastomids appear to have a simple pair of preoesophageal ganglia like annelids, whereas mandibulate arthropods have a proto- and deutocerebrum. Osche (1963), however, on the basis of his observations in *Reighardia* embryos, supposes that the protocerebrum fails to develop in pentastomids, and that the paired preoral ganglion is a deutocerebrum, because it is associated with a pair of appendage-like processes, interpreted as first antennae. Osche refers to the reduction of the protocerebrum which can be seen in blind arthropods, particularly in the blind chilopod *Scolioplanes hirtipes,* which appears to lack the protocerebrum completely (Beklemischew 1969: fig. 54C). It is possible that a similar reduction of the protocerebrum has taken place in the blind, parasitic pentastomids, for *Argulus* appears to have well-developed proto- and deutocerebrum (Martin 1932). A possible rudiment of the protocerebrum is an ectodermal thickening at the anterior pole of the embryo called ''Archicerebrum'' in Osche's (1963) figures 12 and 13, but Osche admits that the interpretation of this structure is uncertain.

Von Haffner's interpretation of the single preoral pair of ganglia in pentastomids as an undivided annelid brain has the advantage of being simple and direct. On the other hand, Osche's more circumstantial interpretation is certainly also possible. The former gives a slightly modified annelid type of brain, the latter gives a strongly modified arthropod type of brain. Since both possibilities are open, the structure of the brain cannot be regarded as an absolute annelid character.

2) *The suboesophageal ganglion* of mandibulate arthropods usually consists of the fused ganglia of the of the mandibular, first maxillular and second maxillular segments, whereas the anterior ganglia of the ventral cord are free in primitive pentastomids and annelids. Failing fusion of the ganglia is not an absolute annelid character, however, for it has been described in several crustaceans, particularly in phyllopods (Horridge 1965). Moreover, the corresponding limbs in pentastomids are certainly strongly modified and reduced, if the animals have evolved from branchiuran crustaceans. This alone could explain why the ganglia fail to fuse into a composite suboesophageal ganglion, which is regarded as a coordinating center for the mouth parts.

3) *The ring muscles under the epidermis,* present in pentastomids, certainly look like the ring muscles of the annelid body wall (v. Haffner 1971). Their function appears

to be the same in both groups: to exert a pressure on the body fluid so the body can be extended. In other respects, e.g., with regard to cross striation and subdivision of the longitudinal muscles into separate bundles, the musculature of the pentastomids is arthropod-like. The question therefore arises whether the circular muscles could have developed secondarily in connexion with annelid-like movements, when the parasites were adapted to their particular habitat. Parasitic crustaceans often have strongly modified muscles. The most astonishing example is described by Claus (1887) in the copepod *Lerneascus nematoxys*, in which the longitudinal muscles form a layer inside the cuticle as in nematodes. Obvious cases of secondary development of subhypodermal muscles with a circular direction are also known in arthropods. Larval house flies, which move very much like pentastomids, have three pairs of flat muscles in each segment, lying under the hypodermis and probably functioning as the ring muscles of pentastomids (Hewitt 1910). In the Chilopoda epimorpha there is an almost continuous coat of circular muscles under the cuticle in the pleural region (Beklemischew 1969).

It may therefore be concluded that the ring muscles in the body wall of pentastomids are similar to those of annelids, but it cannot be excluded that they are a result of secondary adaptation to a parasitic mode of life.

4) *The "lateral line organs"* of pentastomids have the same structure as arthropod sense organs. The annelid-like feature is restricted to their situation along the sides of the abdomen, metamerically repeated on each segment, often in a longitudinal pigmented "lateral line". This arrangement has also been observed in several annelids (v. Haffner 1971). But it is possible that incomplete knowledge of this detail of arthropod anatomy is the reason why the arrangement appears to be an annelid feature. In *Lepisma saccharina*, groups of sensory organs are metamerically repeated on the abdominal segments, forming one ventro-median and a pair of lateral rows on the sternites and a pair of lateral rows on the tergites (K. Birket-Smith, personal communication). Actually it appears quite reasonable that sense organs are metamerically arranged if they are situated on a metameric part of the body. The annelid-like feature is therefore reduced to the presence of sense organs on the sides of the abdomen and their relation to a lateral pigmented zone. It appears to me that independent development of such a sensory system in an endoparasitic group is far from excluded.

In the absence of other arguments, the four characters dealt with above could, with some degree of probability, be interpreted as annelid-like features inherited from some pro-articulates (plesiomorphous, sensu Hennig 1966). On the other hand, it cannot be excluded that these characters could be present in an euarthropod, adapted to an entoparasitic form of life. Placing the pentastomids within the Branchiura as a strongly modified, parasitic sub-group will therefore not meet with serious difficulties. It is in good harmony with the majority of anatomical features in pentastomids, which are distinctly arthropod-like (v. Haffner 1971, p. 92–93), and with the spermatological features which must be regarded as strictly synapomorphous (sensu Hennig 1966): numerous, highly specialized features, identical in detail in the two forms and unknown in other animals.

Placing the pentastomids outside the Branchiura has the absurd consequence that the spermatozoa must have undergone independent and extreme specialization in each line separately and that they by accident happened to be identical in all the new features evolved, even in minute details. This is certainly excluded on the basis of statistical probability.

Osche (1963), after a step-wise argumentation, partly on the basis of his own findings in embryos, comes to the conclusion that the pentastomids are mandibulate arthropods. This result agrees very well with the present findings. From the view of the conclusions in the present paper, the critical point in Osche's argumentation comes when he has to choose between the crustaceans and the antennates (insects and myriapods) as probable relatives of the pentastomids. Osche prefers the latter alternative, although more or less admitting that the anatomical criteria are not immediately convincing: absence of a digestive gland in pentastomids, poor development of the 2nd antenna in pentastomids, development of a distinct head in pentastomids and antennates, rarely in crustaceans. Osche's main argument is that pentastomids infest the respiratory tract of amniotes and should, therefore, be drived from terrestrial arthropods, not from crustaceans. Although indicative, this argument does not exclude the other possibility, viz., that they have evolved from crustaceans. If, for example, the ancestors were *Argulus*-like anthropods, infesting gills and perhaps the lung of crossopterygians, they could easily be imagined to evolve into specialized parasites of the lungs, while their hosts developed into terrestrial animals. The absence of pentastomids (as adults) in the small remnants of the amphibian group surviving today is certainly no strong argument against such a possibility.

I have tried not to over-emphasize the importance of the spermatological data in this discussion, for I realize that such data are not more conclusive than other morphological features. On the contrary, I have tried to deal with the spermatological features in the same way as with other morphological arguments used in the phylogenetic discussions. From this point of view, it is obvious that an almost identical set of highly specialized structures in *Argulus* and *Raillietiella*, like that found in the spermatozoa, must be regarded as a decisive argument for a close relationship, when the same features are unknown in other animals. Since no other arguments are in serious conflict with such a conclusion, I do not hesitate to take the consequence and suggest that the pentastomids should be placed within the Branchiura.

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References

Anderson, W. A., A. Weissman and R. A. Ellis, 1967: Cytodifferentiation during spermiogenesis in Lumbricus terrestris. *J. Cell Biol. 32:* 11–26.

André, J., 1959: Étude au microscope électronique de l'évolution du chondriome pendant la spermatogénèse du scorpion Euscorpius flavicaudis. *J. Ultrastruct. Res. 2:* 288–308.

— 1962: Contribution à la connaissance du chondriome. Étude de ses modifications ultrastructurales pendant la spermatogénèse. *J. Ultrastruct. Res. Suppl. 3.* Academic Press, N. Y. – London.

— 1965: A propos d'une lecon sur la limule. *Ann. Fac. Sci., Clermont-Ferrand 26:* 27–38.

Austin, C. R., and C. L. Baker, 1964: Spermatozoon of Pseudobranchus striatus axanthus. *J. Reprod. Fert. 7:* 123–125.

Baccetti, B., 1968: Spermatologia comparata degli arthropodi. *Atti VII. Congresso naz. Ital. di Entomologia, Verona, 1967:* 1–32.

— 1970a: The spermatozoon of Arthropoda. IX. The sperm cell as an index of arthropod phylogenesis. In *Comparative spermatology* (ed. B. Baccetti): 169–181. Academic Press, N. Y. – London.

— 1970b (editor): *Comparative spermatology.* Proc. int. Symp. Rome and Siena, 1–5 July 1969. Academic Press, N. Y. – London.

Baccetti, B., and A. Bairati, 1964: Indagini sull'ultrastruttura delle cellule germinale maschili in Dacus oleae Gmel. *Redia 49:* 1–29.

Baccetti, B., R. Dallai, and F. Rosati, 1969: The spermatozoon of Arthropoda. III. The lowest holometabolic insects. *J. Microscopie 8:* 233–248.

Bairati, A., and B. Baccetti, 1964: Observations on the ultrastructure of the male germ cells of Drosophila melanogaster and Dacus oleae. In *Electron microscopy – Proc. 3rd Europ. reg. Conf., Prague* (ed. M. Titlbach), *vol. B:* 441–442.

Bawa, S. R., 1960: Atypical spermiogenesis in Thermobia domestica, a thysanuran insect. *Nature, Lond. 188:* 1132.

— 1964a: Electron microscopy of double spermatozoa of an insect, Thermobia domestica Pack. In *Electron microscopy – Proc. 3rd Europ. reg. Conf., Prague* (ed. M. Titlbach), *vol. B:* 445–446.

— 1964b: Electron microscope study of spermiogenesis in a fire-brat insect, Thermobia domestica Pack. I. Mature spermatozoon. *J. Cell Biol. 23:* 431–446.

Beklemischew, W. N., 1958: *Grundlagen der vergleichenden Anatomie der Wirbellosen. Bd. 1.* VEB Deutscher Verlag der Wiss., Berlin.

— (1964) 1969: *Principles of comparative anatomy of invertebrates. I.* Oliver and Boyd, Edinburgh.

Beneden, P. J. van, 1849: Recherches sur l'organisation et de développement des Linguatules (Pentastoma Rud.), accompagnées de la description d'une espèce nouvelle provenant d'un mandrill. *Mém. Acad. r. Sci. Lett. Belg., Ser. III, Zool., 23:* 1–38.

Bocquet-Védrine, J., and J. Pochon-Masson, 1969: Cytodifférenciation d'une vesicule de sécrétion au cours de la speriogenèse chez Balanus perforatus Brug. (Crustacé Cirripède). *Archs. Zool. exp. gén. 110:* 595–616.

Bösenberg, H., 1905: Beiträge zur Kenntnis der Spermatogenese bei den Arachnoiden. *Zool. Jb., Abt. f. Anat. 21:* 515–570.

Boissin, L. and J.-F. Manier, 1966a: Spermatogenèse d'Hysterochelifer meridianus (L. Koch) (Arachnide, Pseudoscorpion, Cheliferidae). 1. Étude caryologique. *Bull. Soc. zool. Fr. 91:* 469–476.

— 1966b: Spermatogenèse d'Hysterochelifer meridianus (L. Koch). II. Étude de l'evolution du chondriome. *Bull. Soc. zool. Fr. 91:* 697–706.

— 1967: Spermatogenèse d'Hysterochelifer meridianus (L. Koch) (Arachnide, Pseudoscorpion, Cheliferidae). *Bull. Soc. zool. Fr. 92:* 705–712.

Borradaile, L. A., 1907: On the classification of the decapod crustaceans. *Ann. Mag. nat. Hist. 19:* 457–486.

Bradke, D. L., 1963: Special features of spermatogenesis in Lumbricus terrestris. *Anat. Rec. 145:* 360.

Breland, O. P., G. Gassner, R. W. Reiss, and J. J. Biesele, 1966: Certain aspects of the centriole adjunct, spermiogenesis and the mature sperm of insects. *Can. J. Genet. Cytol. 8:* 759–773.

Breucker, H., and E. Horstmann, 1968: Die Spermatozoen der Zecke Ornithodorus moubata (Murr.). *Z. Zellforsch. mikrosk. Anat. 88:* 1–22.

Brown, G. G., 1966: *Ultrastructural studies on Grustacean spermatozoa and fertilization.* Doctoral thesis, Univ. of Miami. University microfilms, Inc., Ann Arbor, No. 66 – 12,996.

— 1970: Some comparative aspects of selected crustacean spermatozoa and crustacean phylogeny. In *Comparative Spermatology* (ed. B. Baccetti): 183–203. Academic Press, N. Y. – London.

Brown, G. G., and C. B. Metz, 1967: Ultrastructural studies on the two primitive crustaceans Hutchinsoniella macracantha and Derocheilocaris typicus. *Z. Zellforsch. mikr. Anat. 80:* 78–92.

Cantacuzène, A.-M., 1970: L'annexe centriolaire du spermatozoide des insectes. In *Comparative Spermatology* (ed. B. Baccetti): 553–563. Academic Press, N. Y. – London.

Chevallier, P., 1970: Le noyau du spermatozoide et son évolution au cours de la spermatogenèse. In *Comparative Spermatology* (ed. B. Baccetti): 499–514. Academic Press, N.Y.– London.

Claus C., 1887: Über Lernaeascus nematoxys, Cls, und die Familie der Philichthyiden. *Arb. zool. Inst. Wien 9:* 55–59 and 71–75.

Colwin, A. L., and L. H. Colwin, 1961a: Fine structure of the spermatozoon of Hydroides hexagonus (Annelida), with special reference to the acrosomal region. *J. biophys. biochem. Cytol. 10:* 211–230.

— 1961b: Changes in the spermatozoon during fertilization in Hydroides hexagonus (Annelida). *J. biophys. biochem. Cytol. 10:* 231–274.

Cuenot, L., 1952: Phylogenèse de règne animal. In *Traité de Zoologi* (ed. P.-P. Grassé), *Tome 1:* 1–33. Masson and Cie, Paris.

Dallai, R., 1967: Lo spermatozoo degli arthropodi. I. Anurida maritima (Guérin) et Orchesella villosa (Geoffroy) (Insecta, Collembola). *Atti Accad. Fisiocr. Siena, Ser. 13, Vol. 16:* 468–476.

— 1970: The spermatozoon of Arthropoda. XI. Further observations on Collembola. In *Comparative Spermatology* (ed. B. Baccetti) 275–279. Academic Press, N. Y. – London.

Debaisieux, P., 1953: Histologie et histogenèse chez Argulus foliaceus L. (Crustacé, Branchiure). *Cellule 55:* 245–290.

Deschamps, M., 1969: Étude cytologique de la spermatogenese chez Lithobius forficatus L. (Myriapode chilopode). *Archs. Zool. exp. gén. 110:* 349–361.

Deurs, B. van, 1972: On the ultrastructure of the mature spermatozoon of a chaetognath, Spadella cephaloptera. *Acta zool. Stockh. 53:* 93–104.

Doucet, J., 1964: Données nouvelles sur l'anatomie et histologie des Pentastomes. *Proc. 1st internat. Congr. Parasitology, Roma, Vol. 1:* 621. Pergamon Press, Oxford – London.

— 1965: *Contributions a l'étude anatomique, histologique et histochimique des pentastomes (Pentastomida).* Doctoral thesis, Univ. Paris. Sér. A, No. 4554. No. d'ordre: 5401. Orstom, Paris.

Fain-Maurel, M. A., 1966: Contribution a l'histologie et la caryologie de quelques isopodes. Spermatogenèse et infrastructure du spermatozoide des oniscoidés et cymothoidés. *Annls. Sci. nat., Zool. ser. 12, tome 8:* 1–188.

— 1970: Le spermatozoide des isopodes. In *Comparative Spermatology* (ed. B. Baccetti): 221–236. Academic Press, N. Y. – London.

Favard, P., and J. André, 1970: The mitochondria of spermatozoa. In *Comparative Spermatology* (ed. B. Baccetti): 415–429. Academic Press, N. Y. – London.

Fawcett, D. W., and K. R. Porter, 1954: A study of the fine structure of ciliated epithelia. *J. Morph. 94:* 221–282.

Fawcett, D. W. and D. M. Phillips, 1970: Recent observations on the ultrastructure and development of the mammalian spermatozoa. In *Comparative Spermatology* (ed. B. Baccetti): 13–28. Academic Press, N. Y. – London.

Folliot, R., and P.-L. Maillet, 1970: Ultrastructure de la spermatogénèse et du spermatozoide de divers insectes homoptères. In *Comparative Spermatology* (ed. B. Baccetti): 289–300. Academic Press, N. Y. – London.

Franzén, Å., 1956: On spermiogenesis, morphology of the spermatozoon, and biology of fertilization among invertebrates. *Zool. Bidr. Upps. 31:* 355–482.

— 1958: On sperm morphology and acrosome filament formation in some Annelida, Echiuroida and Tunicata. *Zool. Bidr. Upps. 33:* 1–28.

— 1962: Notes on the morphology and histology of Xironogiton instabila (Moore 1893) (Fam. Branchiobdellidae) with special reference to the muscle cells. *Zool. Bidr. Upps. 35:* 369–383.

— 1970: Phylogenetic aspects of the morphology of spermatozoa and spermiogenesis. In *Comparative Spermatology* (ed. B. Baccetti): 29–46. Academic Press, N. Y. – London.

Fryer, G., 1958: Occurrence of spermatophores in the genus Dolops (Crustacea: Branchiura). *Nature, Lond. 181:* 1011–1012.

Gatenby, J. B., 1925: A reinvestigation of the spermatogenesis of Peripatus. *Q. Jl microsc. Sci. 66:* 1–48.

Gatenby, J. B., and J. Dalton, 1959: Spermiogenesis in Lumbricus hercules. An electron microscope study. *J. biophys. biochem. Cytol. 6:* 45–52.

Gatenby, J. B., and T. N. Tamisian, 1959: Centriole adjunct, centrioles, mitochondria and ergastoplasma in orthopteran spermatogenesis. An electron microscope study. *Cellule 60:* 103–134.

Gibbons, I. R., and A. V. Grimstone, 1960: On flagellar structure in certain flagellates. *J. biophys. biochem. Cytol. 7:* 697–715.

Gilson, G., 1885–1888: Étude comparée de la spermatogenèse chez les arthropodes. *Cellule 1* (1885): 7–188; *2* (1886): 81–237; *4* (1888): 5–93.

Graff, L. von, 1877: *Das Genus Myzostoma F. S. Leuckart.* Engelmann, Leipzig.

Grassé, P.-P., O. Tuzet, and N. Carasso, 1965: Ultrastructure d'un spermatozoide aberrant d'un Iule, Spirostreptes castaneus Attems (Myriapode, diplopode), avec des remarques sur les chromosomes. *C. r. hebd. Séanc. Acad. Sci. Paris 243:* 337–343.

Grobben, K., 1908: Beiträge zur Kenntnis des Baues und der systematischen Stellung der Arguliden. *Sber. Akad. Wiss. Wien., Math.-naturw. Klasse 117 (1):* 191–233.

Haffner, K. von, 1922: Beiträge zur Kenntnis der Linguatuliden II. Zur Eireifung von Porocephalus armillatus (Wyman). *Zool. Anz. 54:* 170–177.

— 1924: Beiträge zur Kenntnis der Linguatuliden III. Über den Hoden und die Spermatocystenbildung von Porocephalus moniliformis. *Zool. Anz. 58:* 285–290.

HAFFNER, K. VON, 1971: Das Pentastomidenproblem (Tagmosis, Metamerie, Organisation, Evolution, systematische Stellung). *Mitt. hamb. zool. Mus. Inst. 67:* 53–107.

HENDELBERG, J., 1970: On the number and ultrastructure of the flagella of flatworm spermatozoa. In *Comparative Spermatology* (ed. B. Baccetti): 367–374. Academic Press, N. Y. – London.

HENNIG, W., 1966: *Phylogenetic systematics.* Univ. of Illinois Press, Urbana – Chicago – London.

HETT, M. L., 1934: On a collection of linguatulids (Pentastomida) from Burma, with description of a new subgenus. *Proc. R. Soc. London 1934:* 425–431.

HEWITT, C. G., 1910: *The house fly, Musca domestica, Linnaeus.* Sherratt and Hughes, Manchester – London.

HEYMONS, R., 1926/27: Pentastomida. In *Handbuch der Zoologie* (ed. Kükenthal – Krumbach), *Bd. 3, 1:* 69–131. Walter de Gruyter, Berlin – Leipzig.

— 1935: Pentastomida. In *Bronn's Klassen und Ordnungen des Tierreichs, 5. Bd Arthropoda, IV. Abt. Arachnoidea, 1. Buch Pentastomida.* Akademische Verlagsgesellschaft, Leipzig.

HOLLANDE, A., and M. FAIN, 1964: Structure et ultrastructure du spermatozoide des Cymothoidés. *C. r. hebd. Séanc. Acad. Sci. Paris 258:* 5063–5066.

HORRIDGE, G. A., 1965: Arthropoda: General Anatomy. In *Structure and Function of the Nervous System of Invertebrates* (ed. Th. H. Bullock and G. A. Horridge), *vol. 2:* 801–964. W. H. Freeman, San Francisco – London.

HORSTMANN, E., 1968: Die Spermatozoen von Geophilus linearis Koch (Chilopoda). *Z. Zellforsch. mikr. Anat. 89:* 410–429.

— 1970: The acrosome complex of the diplopode Spirostreptes sp. In *Comparative Spermatology* (ed. B. Baccetti): 255–262. Academic Press, N. Y. London.

KAESTNER, A., 1954/1965: *Lehrbuch der speziellen Zoologie, Bd. 1: 1.* 1st ed. 1954, 2nd. ed. 1965. Gustav Fischer, Jena.

LANGRETH, S. G., 1969: Spermiogenesis in Cancer crabs. *J. Cell Biol. 43:* 575–603.

LEGENDRE, R., 1967: Morphologie, histologie er développement des pentastomides. *Fortschr. Zool. 18:* 337–349.

LEUCKART, R., 1860: *Bau und Entwicklungsgeschichte der Pentastomen nach Untersuchungen besonders von Pent. taenioides und P. denticulatum.* C. F. Winter, Leipzig – Heidelberg.

LEYDIG, F., 1850: Über Argulus foliaceus. Ein Beitrag zur Anatomie, Histologie und Entwicklungsgeschichte dieses Tieres. *Z. wiss. Zool. 2:* 323–349.

MARTIN, F. M., 1932: On the morphology and classification of Argulus (Crustacea). *Proc. zool. Soc. Lond. 1932:* 771–806.

MATTEI, X., 1969: *Contribution â l'etude de la spermiogenèse et des spermatozoides de poissons par les methodes de la microscopie électronique.* Doctoral thesis, Univ. Montpellier, Faculté Sci. C. N. R. S., no. A. O. 3263.

— Spermiogénèse comparée des poissons. In *Comparative Spermatology* (ed. B. Baccetti): 57–69. Academic Press, N. Y. – London.

MONTGOMERY, T. H., 1900: The spermatogenesis of Peripatus (Peripatopsis) balfouri up to the formation of the spermatid. *Zool. Jb., Abt. fur Anatomie 40:* 277–368.

MOSES, M. J., 1961: Spermiogenesis in the crayfish (Procambarus clarkii) II. Description of stages. *J. biophys. biochem. Cytol. 10:* 301–333.

MUNN, E. A. and H. BARNES, 1970: The fine structure of the spermatozoa of some cirripeds. *J. exp. marine Biol. Ecol. 4:* 261–286.

NØRREVANG, A., 1972: Oogenesis in Pentastomids. *Acta zool., Stockh. 53.* In press.

NØRREVANG, A., and K. G. WINGSTRAND, 1970: On the occurrence and structure of choanocyte-like cells in some echinoderms. *Acta zool. Stockholm 51:* 249–270.

OSCHE, G., 1959: »Arthropodencharaktere« bei einem Pentastomiden-embryo (Reighardia sternae). *Zool. Anz. 163:* 169–178.

Osche, G., 1963: Die systematische Stellung und Phylogenie der Pentastomida. Embryologische und vergleichend-anatomische Studien. *Z. Morph. Okol. Tiere 52:* 487–596.

Palade, G. E., 1952: A study of fixation for electron microscopy. *J. exp. Med. 95:* 285–298.

Pastisson, C., 1966: Anatomie ultrastructurale du spermatozoide du sangsue, Hirudo medicinalis L. *Annls Univ. (Reims) et Arers 4:* 67–75.

Pedersen, H., 1970: Ultrastructure of the ejaculated human sperm. In *Comparative spermatology* (ed. B. Baccetti): 133–142. Academic Press, N. Y. – London.

Phillips, D. M., 1970a: Insect sperm: their structure and morphogenesis. *J. Cell Biol. 44:* 243–277.

— 1970b: Insect flagellar tubule patterns – theme and variations. In *Comparative spermatology* (ed. B. Baccetti): 263–273. Academic Press, N. Y. – London.

Philpott, D. E., and W. N. Shaw, 1959: An electron microscopic study of the sperm of Limulus polyphemus. *Biol. Bull. mar. biol. Lab., Woods Hole, 117:* 423–424.

Pochon-Masson, J., 1969: Infrastructure de spermatozoide de Palaemon elegans (De Man) (Crustacé décapode). *Archs Zool. exp. gén. 110:* 363–372.

Pochon-Masson, J., J. Bocquet-Védrine, and Y. Turquier, 1970: Contribution à l'étude du spermatozoide des crustacés cirripèdes. In *Comparative Spermatology* (ed. B. Baccetti): 205–219. Academic Press, N. Y. – London.

Potswald, H. E., 1967: An electron microscope study of spermiogenesis in Spirorbis (Laeospira) mörchi Levinsen (Polychaeta). *Z. Zellforsch. mikrosk. Anat. 83:* 231–298.

Pratt, S., 1970: Formation and differentiation of the Nebenkern in spermatids of an hemipteran insect, Murgantia histrionica. In *Comparative Spermatology* (ed. B. Baccetti): 301–310. Academic Press, N. Y. – London.

Reger, J. F., 1961: The fine structure of spermatids from the tick, Amblyomma dissimili. *J. Ultrastruct. Res. 5:* 584–599.

— 1962: A fine-structure study on spermiogenesis in the tick Amblyomma dissimili, with special reference to the development of motile processes. *J. Ultrastr. Res. 7:* 550–565.

— 1963: Spermiogenesis in the tick, Amblyomma dissimili, as revealed by electron microscopy. *J. Ultrastr. Res. 11:* 181–192.

— 1964a: A study on the fine structure of developing spermatozoa from the isopod, Asellus militaris (Hay). *J. Microscopie 3:* 559–572.

— 1964b: The fine structure of spermatozoa from the isopod Asellus militaris (Hay). *J. Ultrastr. Res. 11:* 181–192.

— 1966: A comparative study on the fine structure of developing spermatozoa in the isopod, Oniscus asellus, and the amphipod, Orchestia sp. *Z. Zellforsch. mikr. Anat. 75:* 579–590.

— 1967: A study on the fine structure of developing spermatozoa from the oligochaete, Enchytraeus albidus. *Z. Zellforsch. mikr. Anat. 82:* 257–269.

— 1969: A fine structure study on spermiogenesis in the Arachnida, Leiobunum sp. (Phalangida: Harvestmen). *J. Ultrastr. Res. 28:* 422–434.

— 1970a: Some aspects of the fine structure of filiform spermatozoa (Ostracod, Cypridopsis sp.) lacking tubule sub-structure. In *Comparative Spermatology* (ed. B. Baccetti): 237–245. Academic Press, N. Y. – London.

— 1970b: Spermiogenesis in the spider, Pisaurina sp.: A fine structure study. *J. Morph. 130:* 421–434.

Reger, J. F., and D. P. Cooper, 1968: Studies on the fine structure of spermatids and spermatozoa from the milliped Polydesmus sp. *J. Ultrastr. Res. 23:* 60–70.

Retzius, G., 1909: Die Spermien der Crustaceen. In *Biologische Untersuchungen von G. Retzius, N. F., 14:* 1–54. Gustav Fischer, Jena.

Robertis, E. de, and H. F. Raffo, 1957: Submicroscopic organization of the mitochondrial body and other cytoplasmic structures in the insect testis. *Expl. Cell Res. 12:* 66–79.

Rosati, F., B. Baccetti, and R. Dallai, 1970: The spermatozoon of Arthropoda. X. Araneids and the lowest myriapods. In *Comparative Spermatology* (ed. B. Baccetti): 247–254. Academic Press, N. Y. – London.

Self, J. T., 1969: Biological relationships of the Pentastomida; A biblography on the Pentastomida. *Experiment. Parasitol. 24:* 63–119.

Silveria, M., and K. R. Porter, 1964: The spermatozoids of flatworms and their microtubular system. *Protoplasma 59:* 240–265.

Sokolow, S., 1913: Untersuchungen über die Spermatogenese bei den Arachniden. I. Über die Spermatogenese der Skorpione. *Arch. Zellforsch. 9:* 399–432.

Sotelo, J. R., and O. Trujillo-Cenóz, 1958: Electron microscope study of the kinetic apparatus in animal sperm cells. *Z. Zellforsch. mikr. Anat. 48:* 565–601.

Takashima, R., and Y. Takashima, 1963: Electron microscope studies on the fine structure of Nereis spermatozoa before and after the acrosomal reaction. *Tokushima J. exp. Med. 10:* 117–123.

Thompson, T. E., and M. S. Blum, 1967: Structure and behaviour of spermatozoa of the fire ant Solenopsis saevissima (Hymenoptera: Formicidae). *Ann. ent. soc. Am., 60:* 632–642.

Turquier, Y., and J. Pochon-Masson, 1969: L'infrastructure du spermatozoide de Trypetesa (=Alcippe) nassaroides Turquier (Cirripede acrothoracique). *Archs Zool. exp. gén. 110:* 453–470.

Tuzet, O., 1938: Sur la spermatogénèse de Buthus occitanus (Amor.). *Archs Zool. exp. gén. 80:* 335–351.

Vandel, A., 1949: Généralités – Composition de l'embranchement. In *Traité de Zoologie (ed. P.-P. Grassé), VI:* 79–158. Masson and Cie, Paris.

Weber, H., 1949: *Grundriss der Insektenkunde.* Gustav Fischer, Jena, 1949 and 1954.

Werner, G., 1964: Untersuchungen über die Spermatogenese beim Silberfischchen, Lepisma saccharina L. *Z. Zellforsch. mikr. Anat. 63:* 880–912.

— 1965: Untersuchungen über die Spermatogenese beim Sandläufer, Cicindela campestris L. *Z. Zellforsch. mikr. Anat. 66:* 255–275.

— 1970: On the development and structure of the neck in urodele sperm. In *Comparative Spermatology* (ed. B. Baccetti): 85–91. Academic Press, N. Y. – London.

Yasuzumi, G., 1960: Spermatogenesis in animals as revealed by electron microscopy. VII. Spermatid differentiation in the crab, Eriocheir japonicus. *J. biophys. biochem. Cytol. 7:* 73–78.

Yasuzumi, G., G. I. Kaye, G. D. Pappas, H. Yamamoto, and I. Tsubo, 1961: Nuclear and cytoplasmic differentiation in the developing sperm of the crayfish, Cambaroides japonicus. *Z. Zellforsch. mikr. Anat. 53:* 141–158.

Yasuzumi, G., H. Tanaka, and O. Tezuka, 1960: Spermatogenesis in animals as revealed by electron microscopy. VIII. Relation between the nutritive cells and the developing spermatids in a pond snail, Cipangopaludina malleata Reeve. *J. biophys. biochem. Cytol. 7:* 499–504.

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PLATES

PLATE 1

Fig. 1. *Raillietiella ?hemidactyli* Hett. Anterior end of ♀ seen from the ventral side. Arrows indicate mouth opening and anterior hooks. Note blunt posterior hooks and tapering anterior end. Scanning microscope picture of specimen from *Calotes versicolor*, fixed in formalin and post-fixed in 1 % Osmic over night. Dried with benzene as described in Norrevang and Wingstrand 1970.

Fig. 2. Same specimen as Fig. 1, seen from the anterior end to show dorso-lateral papillae (dl), frontal papillae (arrow) and mouth opening (arrow). The frontal papillae are in fact double.

Fig. 3. *Raillietiella*. Light microscopice cross section of testicle. Small, still attached spermatocysts bulge out from the wall at yc, the lumen is filled with free spermatocysts in different stages of development. mc indicates cyst with nearly mature sperm. No degenerative changes.

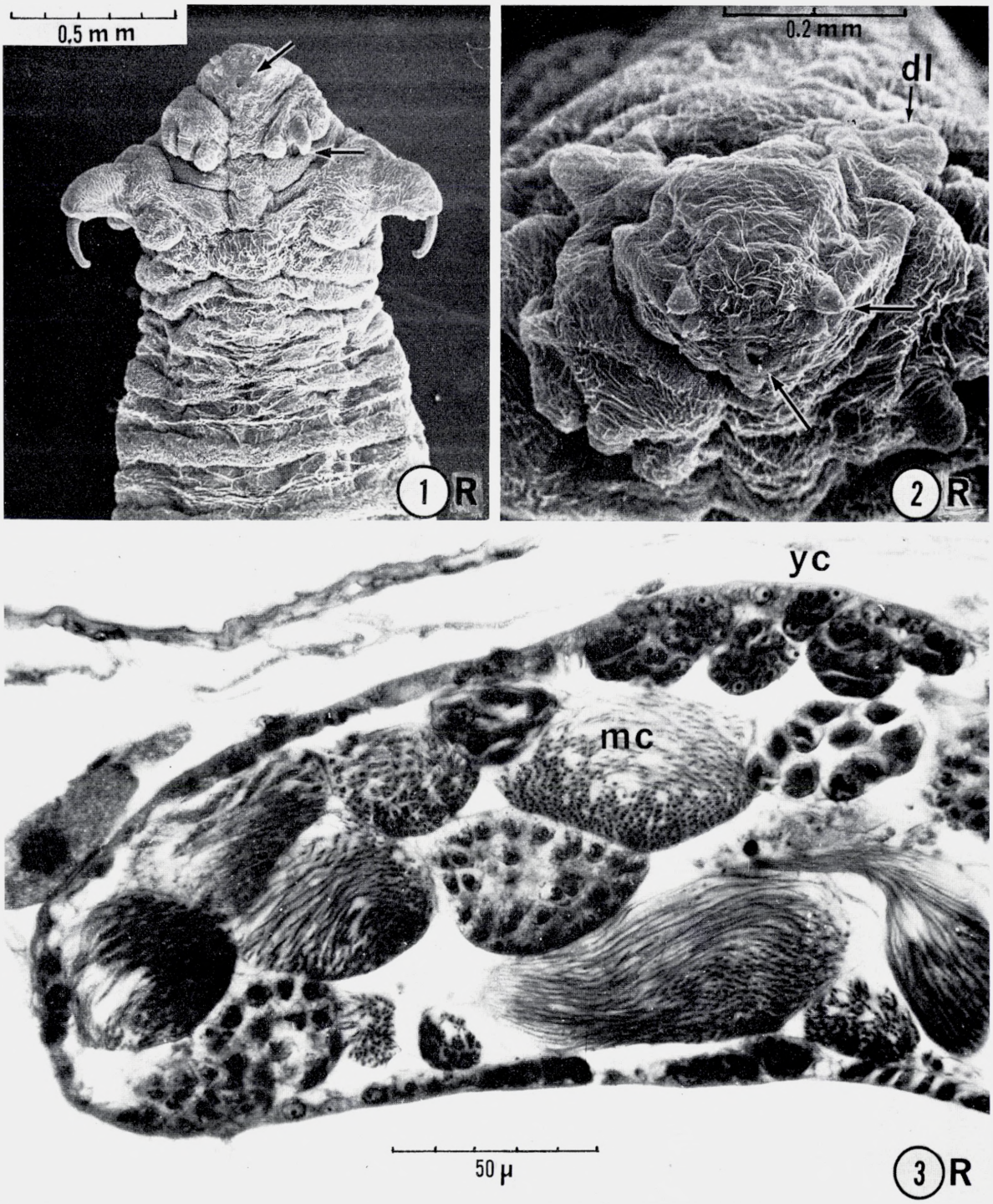

0,5 mm
1 R
0.2 mm
dl
2 R
yc
mc
50 µ
3 R

PLATE 2

Fig. 4. *Raillietiella*. Thick epon section (2 μ, stained with toluidin blue) of testicle with degenerate features. The lumen is filled with abnormal spermatozoa and debris, some cysts (dc) show abnormal development. A few normal spermatocysts: young, still attached ones at yc, two cysts with stage 4 spermatids at s4, and one with stage 1 spermatids at sl.

Fig. 5. *Argulus*. Transverse section of one testicle (2 μ epon section, stained with toluidin blue). Median plane left. The germinal epithelium is the thin plate above sp, down along median and lateral walls are spermatocytes (spc) and spermatids (spt). Ventrally the large Sertoli cells (sc) are attached, carrying large tufts of advanced spermatids. sp = mature sperm in lumen.


50 µ
yc
dc
s4
sI
dc
4 R

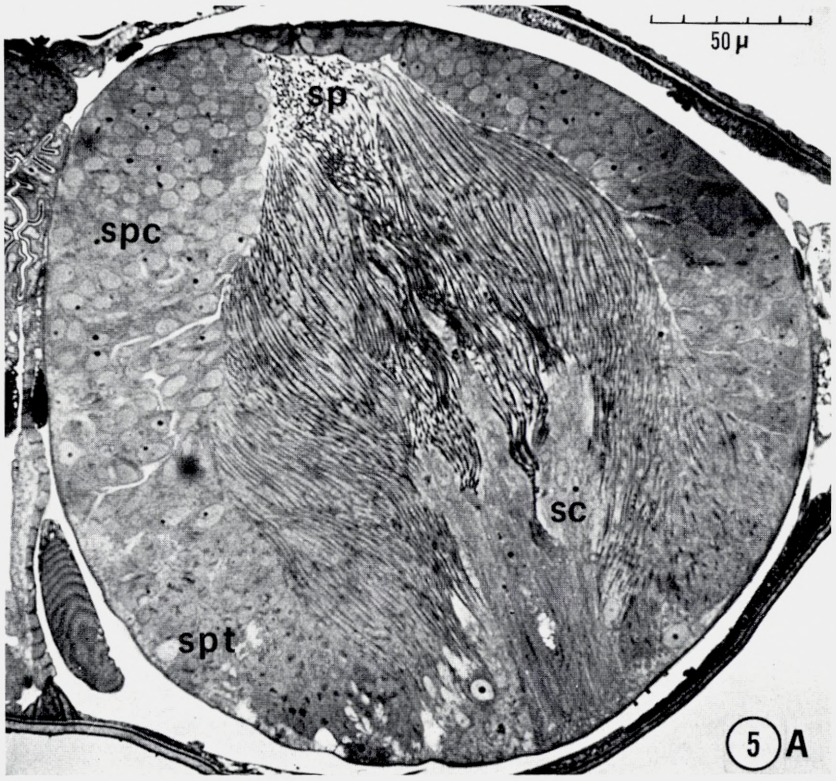

50 µ
sp
spc
sc
spt
5 A

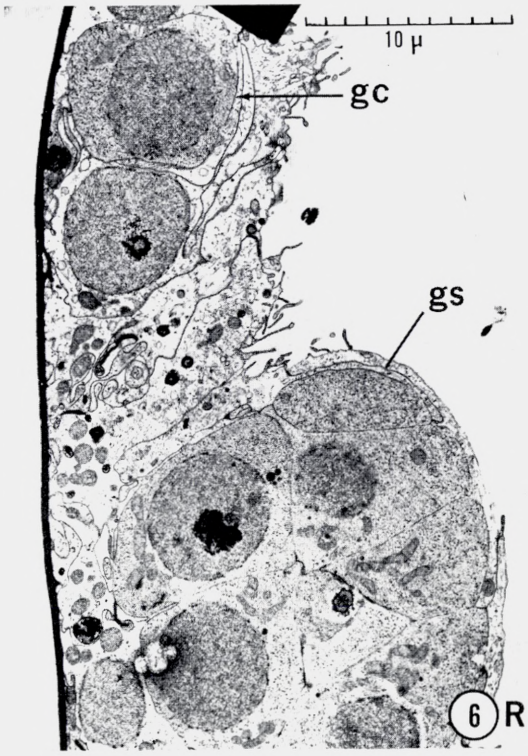
PLATE 3

Fig. 6. *Raillietiella*. Part of testicular wall. The basement membrane is black. gc = germinal cell, overgrown by the neighbouring vegetative cells. gs = growing spermatocyst, containing spermatogonia with dark plasm and covered by plasmatic extensions from the vegetative cells.

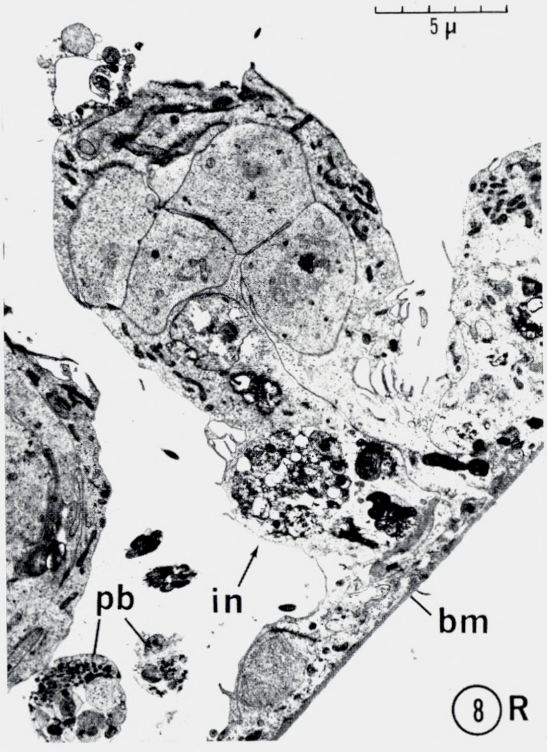
Fig. 7. *Raillietiella*. Cross section of free spermatocyst, containing 128 spermatids of stage 6. Note the plasmatic cyst wall.

Fig. 8. *Raillietiella*. Tangential section of spermatocyst ready to be pinched off from the testicular wall. Four distinct spermatocytes are seen, surrounded by plasm which is continuous with that of vegetative cells at the base. in = large inclusion in vegetative cell. pb = plasmatic balls with cellular debris in the lumen. bm = basement membrane.

Fig. 9. *Argulus*. Sertoli cells. Three large nuclei are seen. The lower part of the picture shows stalk-like basal parts of the cells, the upper part of the picture shows distal ends with many pseudoacrosomes sticking in the plasm (dark spots). Compare Plate 20: 102.


10 μ
gc
gs
6 R


10 μ
7 R


5 μ
pb
in
bm
8 R

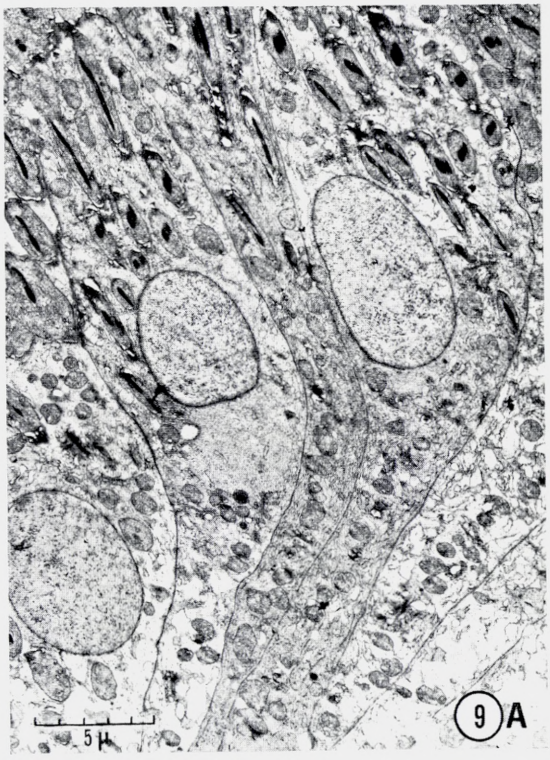

5 μ
9 A

PLATE 4

EM pictures of osmium-fixed, intact spermatozoa, spread on coated grids and dried.

Fig. 10. *Raillietiella*. Entire spermatozoon. Transition from body to pseudoacrosome (arrow) is sudden and distinct.

Fig. 11. *Argulus*. Transition from body to pseudoacrosome (arrow) is marked by a rather slow decrease in diameter.

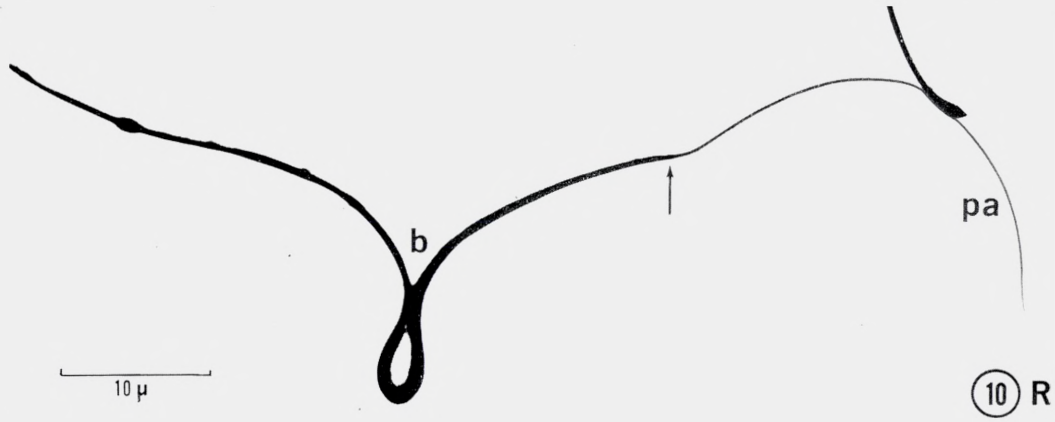
Fig. 12. *Raillietiella*. Top of pseudoacrosome with granulosome (g). The dorsal rod ends at the arrow.

Fig. 13. *Argulus*. Top of pseudoacrosome. There is no granulosome. The small granule seen in this picture is not present in other specimens. Dorsal rod ends at the arrow.

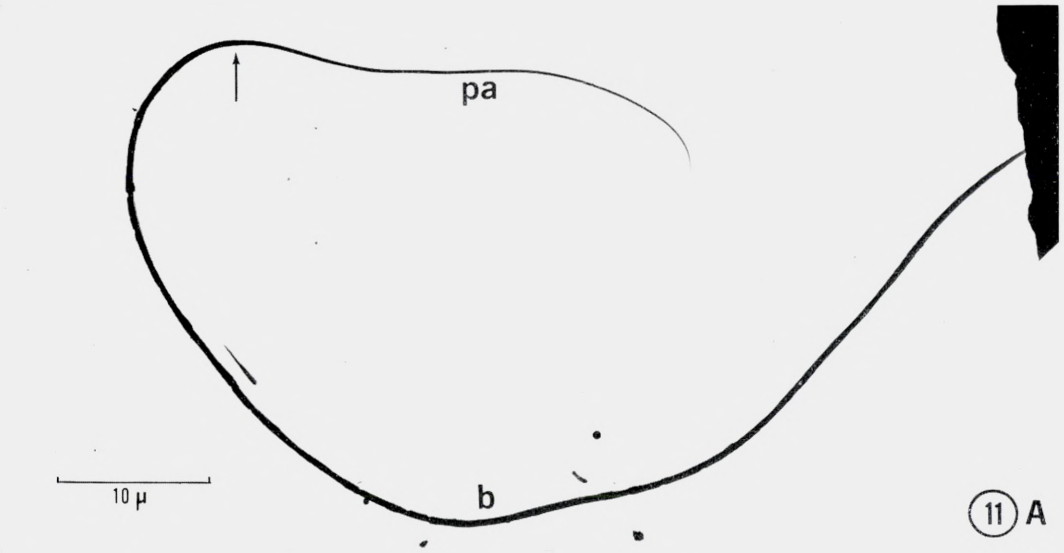
Fig. 14. *Raillietiella*. Posterior end of spermatozoon. Compare Plate 7.

Fig. 15. *Argulus*. Posterior end of spermatozoon. Compare Plate 7.

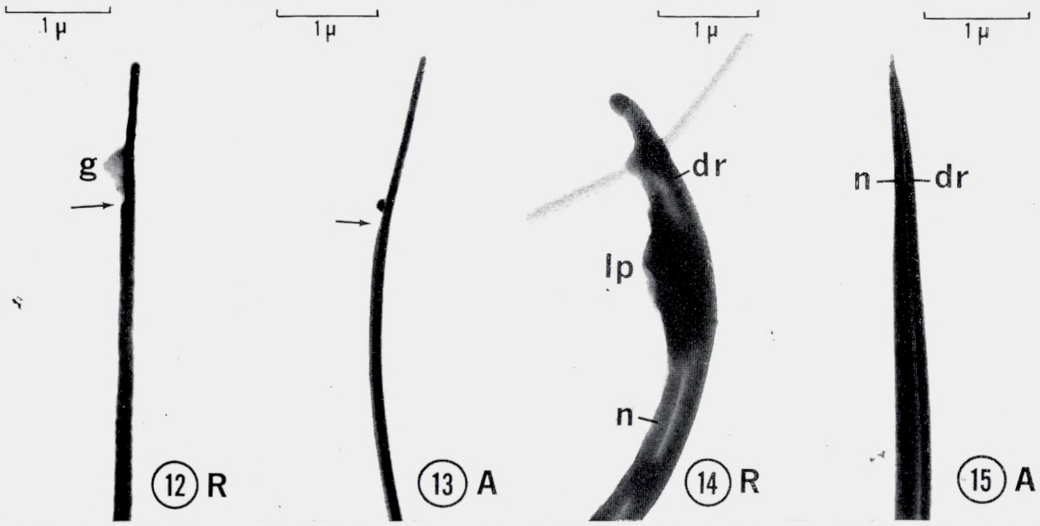
b = body of spermatozoon, dr = dorsal ribbon, g = granulosome, lp = lump of vesicular plasm near posterior end in *Raillietiella*, n = nucleus, pa = pseudoacrosome.



10 R



11 A



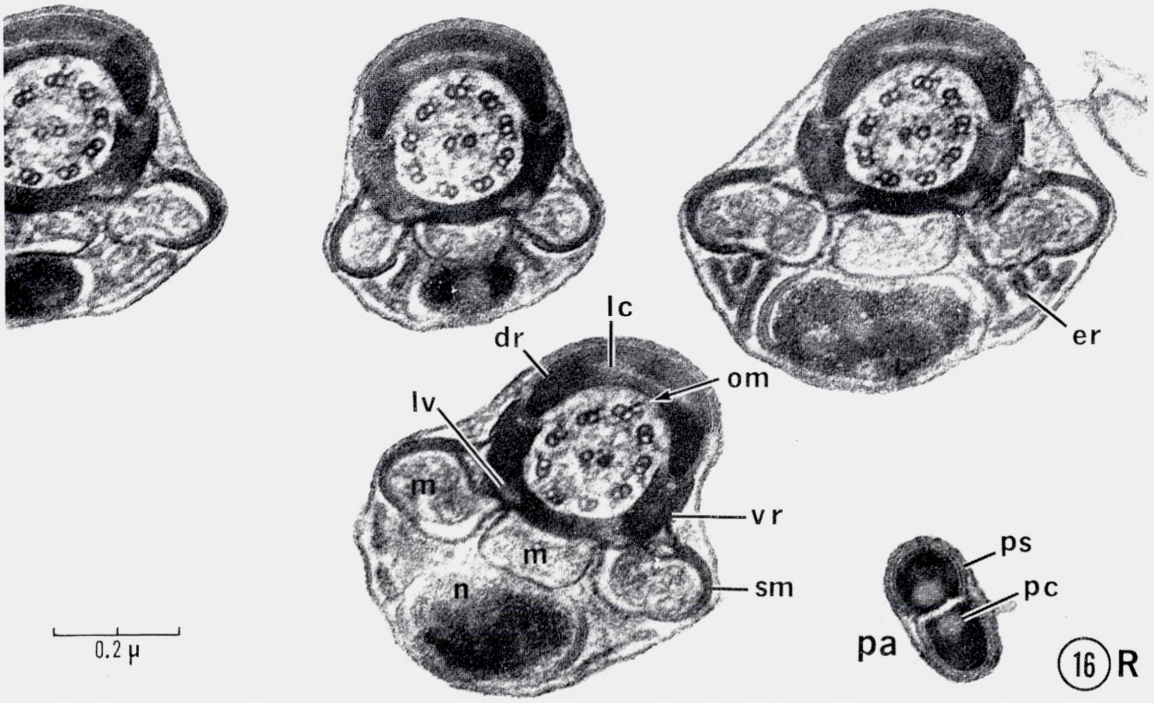
12 R 13 A 14 R 15 A

PLATE 5

Fig. 16. *Raillietiella*. Cross sections of the body and of a pseudoacrosome (pa).

Fig. 17. *Argulus*. Cross sections of several spermatozoan bodies and of one posterior end (pe).

af = remnant of acrosome filament in the nuclear membranes of *Argulus*, always asymmetrical, ax = axonemal space, cut just behind the posterior ends of the filaments in *Argulus*, dr = dorsal ribbon, er = smooth endoplasmic reticulum, ir = intermitochondrial rods in *Argulus*, is = inner membranous sac of dorsal ribbon, attached to doublets 3 and 8 in *Argulus*, lc = light core of dorsal ribbon, lv = light rods in ventral ribbon of *Raillietiella*, m = mitochondria, n = nucleus, om = oblique membrane between axonemal doublet 1 and dorsal ribbon, pa = pseudoacrosome, pe = posterior end of body, pc = light core of dorsal pseudoacrosomal rod in *Raillietiella*, ps = membranous sac of pseudoacrosome, sm = dark sheath covering lateral mitochondria, vr = ventral ribbon.


lc
dr
om
lv
m
vr
m
n
sm
er
ps
pc
pa
0.2 μ
16 R

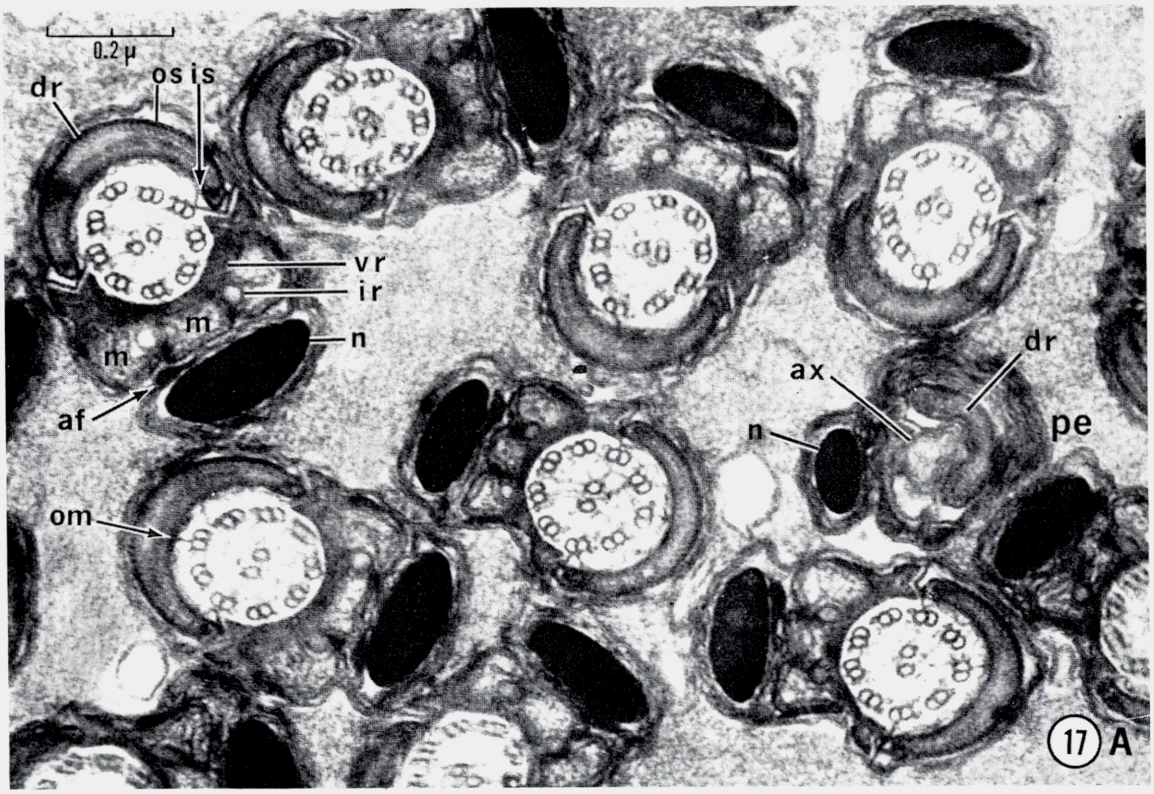

0.2 μ
dr
os
is
vr
ir
m
n
m
af
om
ax
dr
n
pe
17 A

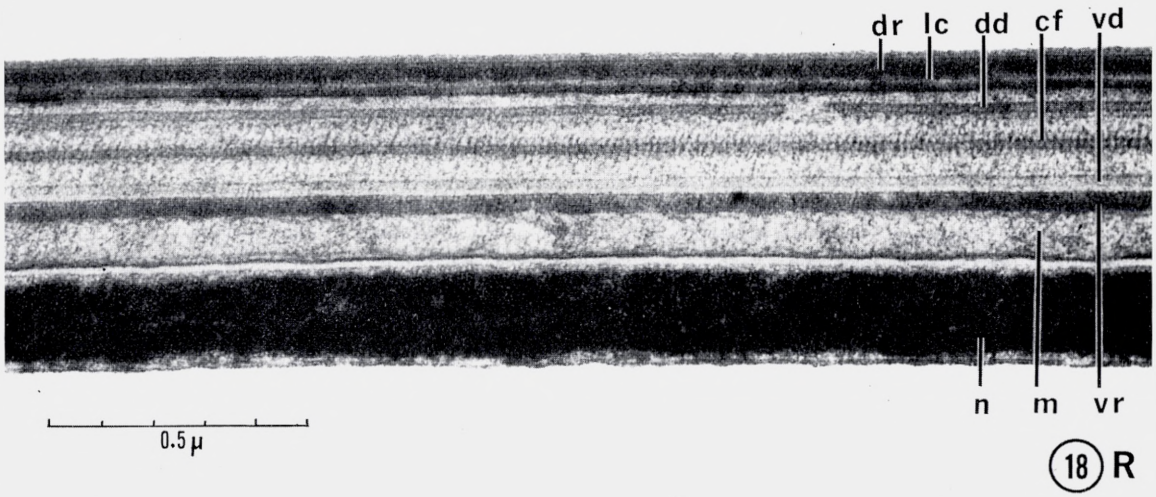
PLATE 6

Fig. 18. *Raillietiella*. Seminal vesicle. Median section of body.

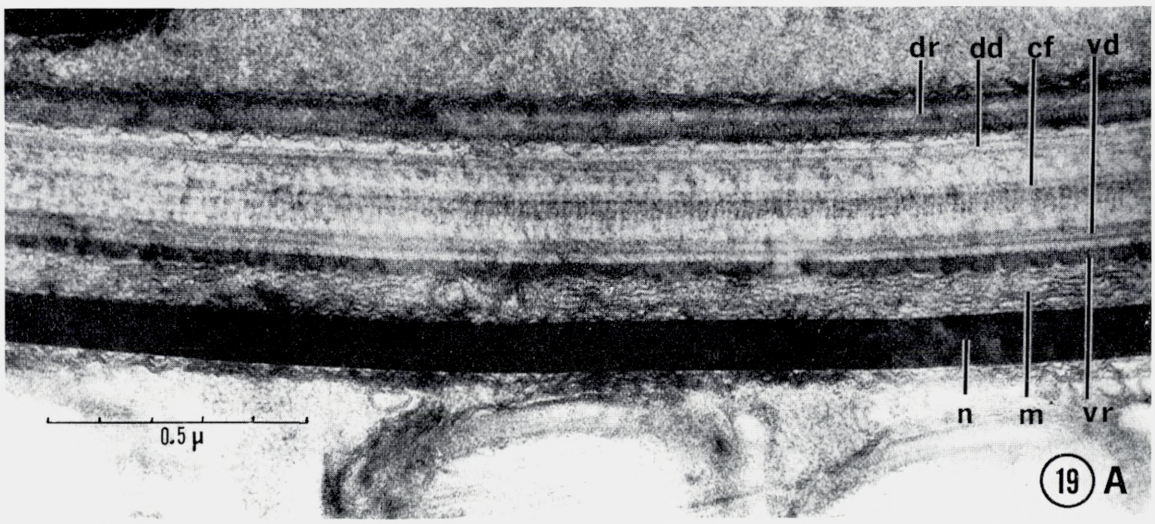
Fig. 19. *Argulus*. Receptaculum seminis. Median section of body.

Fig. 20. *Raillietiella*. Seminal vesicle. Median section of transitional region between body and pseudoacrosome. For comparison with *Argulus* see Plate 22: 111, which shows an almost mature spermatid.

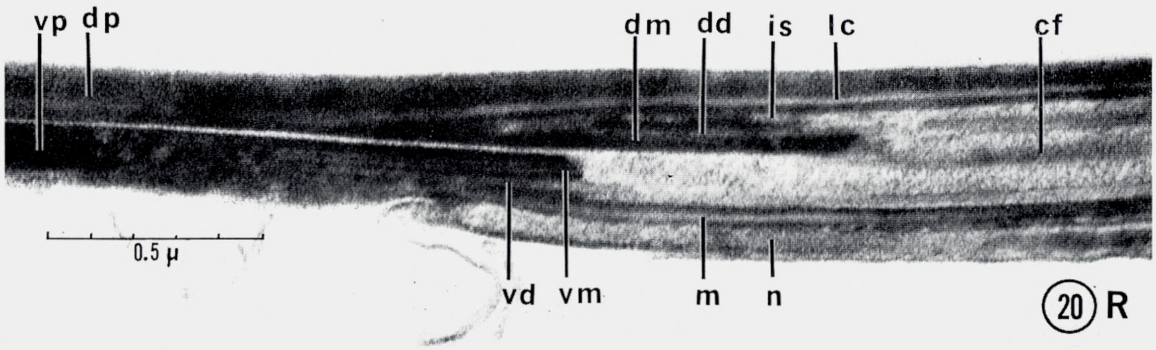
cf = central filaments of axonema, dd = dorsal doublets of axonema and centriole, dm = dorsal extension of pseudoacrosomal granular matter including filaments 9, 1, and 2 of centriole, dp = dorsal rod of pseudoacrosome, dr = dorsal ribbon, is = inner membranous sac of dorsal ribbon, lc = light core of dorsal ribbon, m = mitochondria, n = nucleus, vd = ventral doublets of axonema and centriole, vm = ventral extension of pseudoacrosomal granular matter, including ventral filaments of centriole, vr = ventral ribbon, vp = ventral rod of pseudoacrosome.


dr
lc
dd
cf
vd
n
m
vr
0.5 μ

18 R


dr
dd
cf
vd
n
m
vr
0.5 μ

19 A


vp
dp
dm
dd
is
lc
cf
0.5 μ
vd
vm
m
n

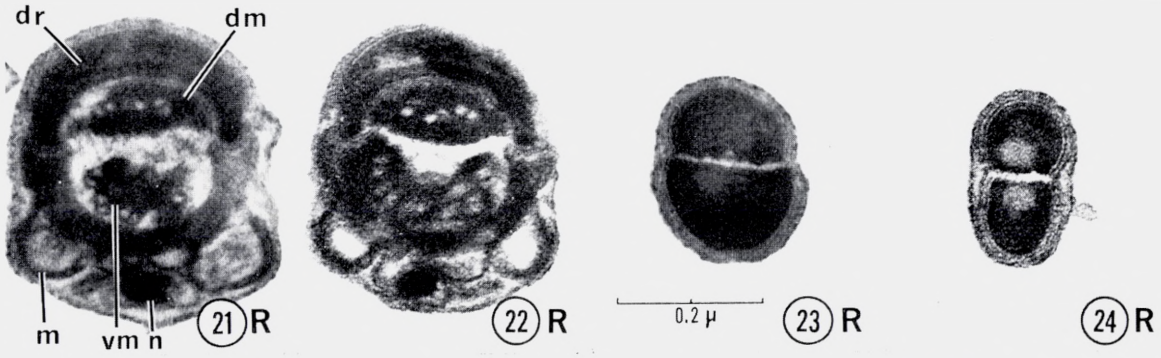
20 R

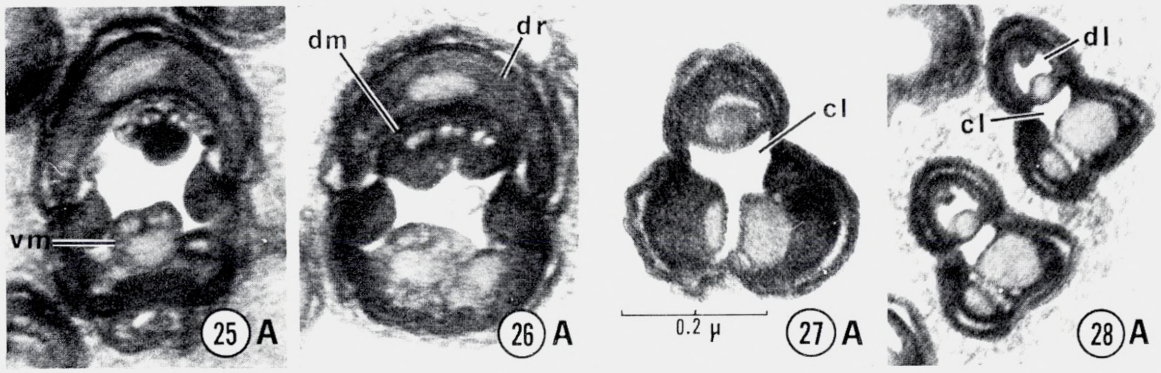
Plate 7

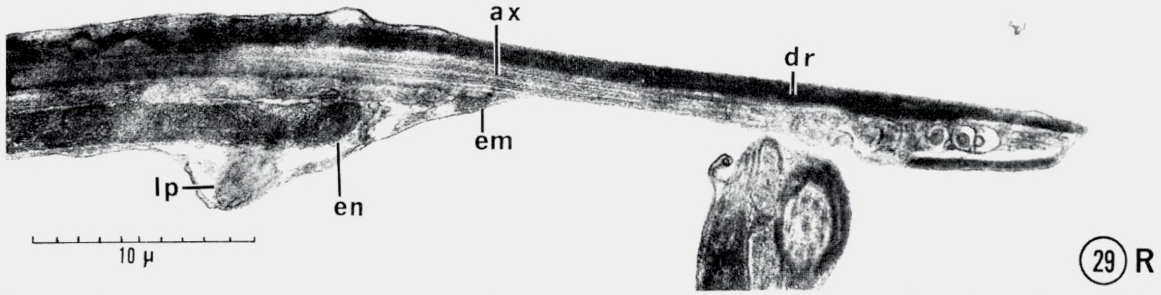
The acrosomes and the posterior ends of the spermatozoa. Magnification is the same in Figs. 21–28 and 31.

Fig. 21. *Raillietiella*. Cross section of transitional region between body and pseudoacrosome.
Fig. 22. *Raillietiella*. Cross section a little in front of the level of Fig. 21.
Fig. 23. *Raillietiella*. Cross section of basal part of pseudoacrosome.
Fig. 24. *Raillietiella*. Cross section of terminal region of pseudoacrosome.
Fig. 25. *Argulus*. Cross section of transitional region between body and pseudoacrosome.
Fig. 26. *Argulus*. Cross section a little in front of the level of Fig. 25.
Fig. 27. *Argulus*. Cross section of basal part of the pseudoacrosome.
Fig. 28. *Argulus*. Cross section of the terminal part of pseudoacrosomes.
Fig. 29. *Raillietiella*. Median section of posterior end of body.
Fig. 30. *Argulus*. Median section of posterior end of body.
Fig. 31. *Argulus*. Cross section of posterior end, at the level of the letters dr and n in Fig. 30.

ax = axonema, cl = central lumen of pseudoacrosome in *Argulus*, dl = dorsal lumen of pseudoacrosome in *Argulus*, dm = dorsal extension of pseudoacrosomal granular matter, including doublets 9, 1 and 2 in both species, dr = dorsal ribbon, em = end of mitochondria, en = end of nucleus, lp = lump of vesicular plasm near posterior end in *Raillietiella*, m = mitochondria, n = nucleus, vm = ventral extension of the pseudoacrosomal granular matter, including ventral centriole doublets: nos. 3–8 in *Raillietiella*, nos. 5 and 6 in *Argulus*.


dr
dm
m
vm
n
21 R
22 R
0.2 μ
23 R
24 R


vm
25 A
dm
dr
26 A
0.2 μ
27 A
dl
cl
28 A


ax
dr
em
lp
en
10 μ
29 R

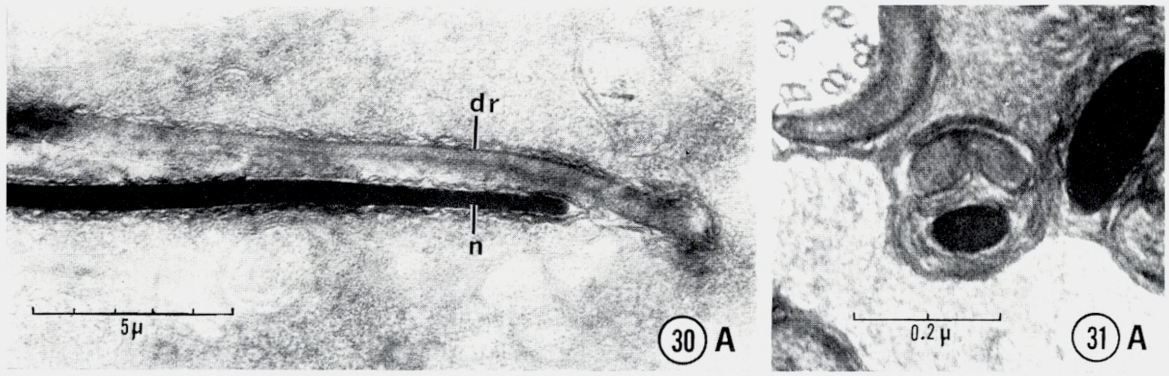

dr
n
5μ
30 A
0.2 μ
31 A

Plate 8

Development of the centriole complex.

Fig. 32. *Raillietiella*. Cyst in 2nd meiotic division. Chromosomes (ch) are visible in one cell, a centriole (black arrow) in the other. The centriole with two dark fibers is enlarged in the inset.

Fig. 33. *Raillietiella*. Cross section of centriole of early spermatid 1.

Fig. 34. *Raillietiella*. Cross section of centriole of spermatid 2. With three large dark fibers under the membranous sac (os).

Fig. 35. *Argulus*. Two centrioles, each with two dark fibers and a flat sac of endoplasmic reticulum, from a spermatocyte 2.

Fig. 36. *Argulus*. Spermatocyte 2 with two centrioles, one of which (dark short arrow) is shown enlarged in the inset. The darkened flat sac of endoplasmic reticulum and the two large dark fibers are distinct.

Fig. 37. *Argulus*. Spermatid 2. Cross section of centriole with membranous sac (os) and three large dark fibers.

Fig. 38. *Raillietiella*. Two centrioles from spermatocyte 1, one with a slight condensation at the end, suggestive of an apical membrane.

Fig. 39. *Raillietiella*. 2nd meiotic cell. Centriole with fairly distinct membrane-like condensations across the edges of the upper end.

Fig. 40. *Raillietiella*. Spermatid stage 2. Apical membrane and granulosome rather distinct.

Fig. 41. *Raillietiella*. Late spermatid 2. Distinct granulosome (g) attached to nucleus (n), apical membrane (am) and membranous sac (os) fully developed, the latter covering dark fibers.

Fig. 42. *Argulus*. Spermatid 3. Cross section of centriole. The outer membranous sac and three dark fibers distinct.

Fig. 43. *Argulus*. Similar stage and section as Fig. 42.

Fig. 44. *Argulus*. Spermatid, early stage 2. Membranous sac and its connection with the smooth endoplasmic reticulum distinct.

Fig. 45. *Argulus*. Late spermatid 2. Granulosome and apical membrane distinct.

am = apical membrane, an = annulus-like thickening of cell membrane in the bottom of the flagellar pit, ch = chromosomes, g = granulosome, m = mitochondria, n = nucleus, os = flat membranous sac, the prospective outer sac of the dorsal ribbon.

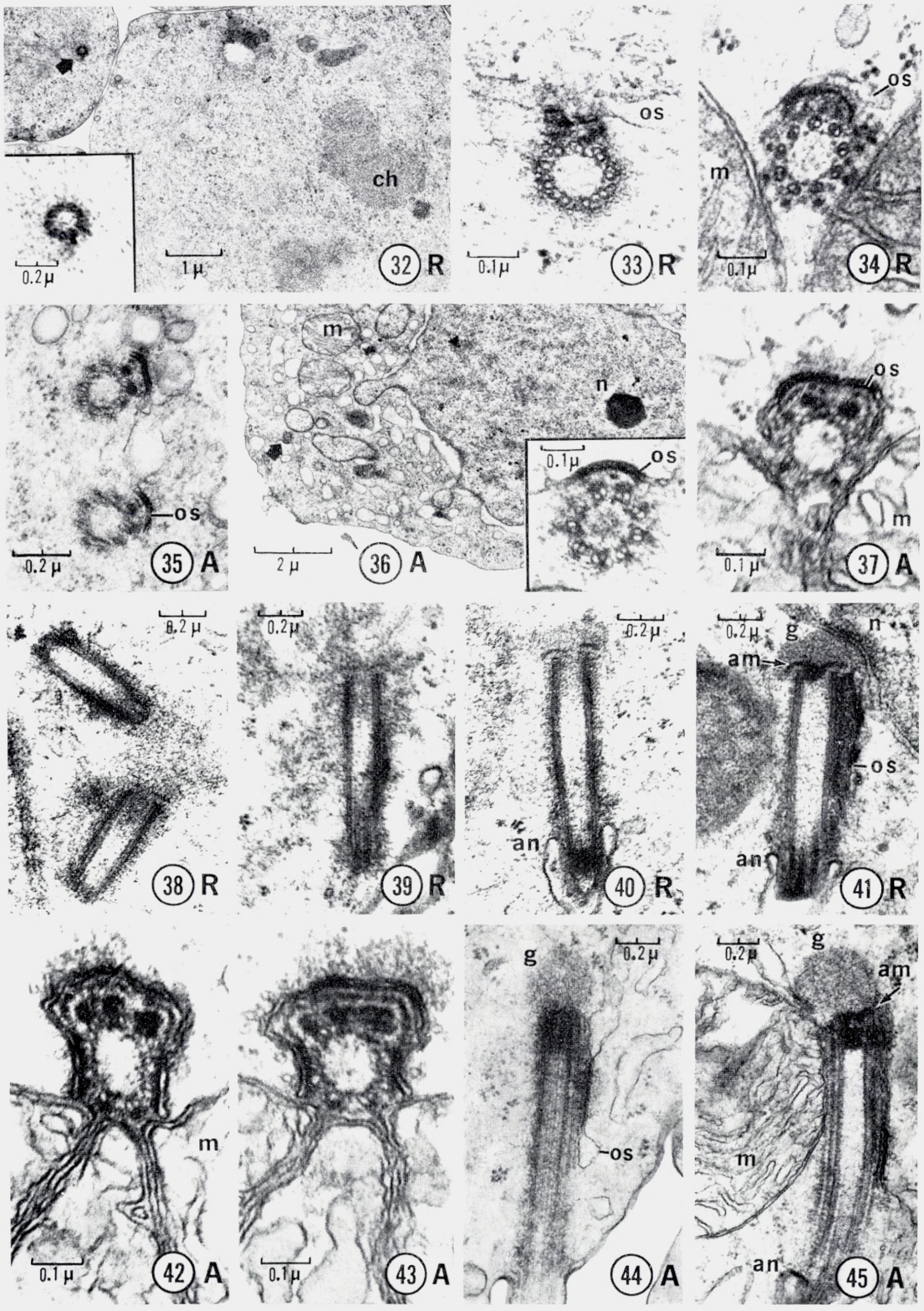

ch
0.2μ
1μ
32 R
os
0.1μ
33 R
os
m
0.1μ
34 R
os
0.2μ
35 A
m
n
os
2μ
36 A
os
m
0.1μ
37 A
0.2μ
38 R
0.2μ
39 R
0.2μ
an
40 R
0.2μ
g
n
am
os
an
41 R
m
0.1μ
42 A
0.1μ
43 A
g
0.2μ
os
44 A
0.2μ
g
am
m
an
45 A

PLATE 9

Early development of mitochondria.

Fig. 46. *Raillietiella*. Spermatocyte 1 with long, tubular mitochondria.

Fig. 47. *Raillietiella*. Spermatid 1–2. Picture suggestive of fusion of motochondria: the inner membranes of the presumed partners are separate, the outer membrane is common.

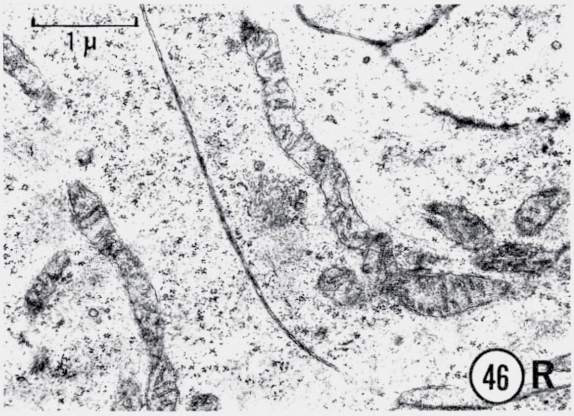
Fig. 48. *Raillietiella*. Spermatid 1. The group of spherical mitochondria.

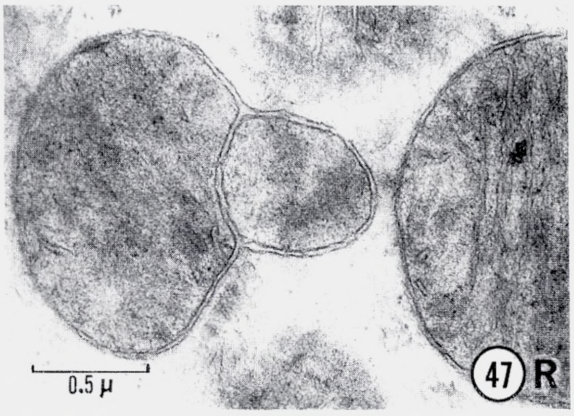
Fig. 49. *Argulus*. Spermatid 1. The group of mitochondria before they fuse to three.

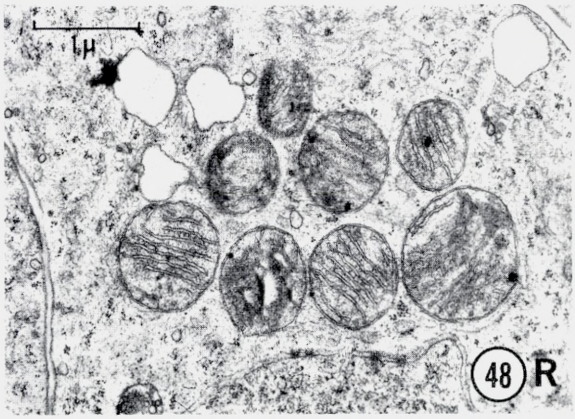
Fig. 50. *Raillietiella*. Spermatid 2. With the three spherical mitochondria. Dark, probably cementing substance is seen in the interspaces between the mitochondria.

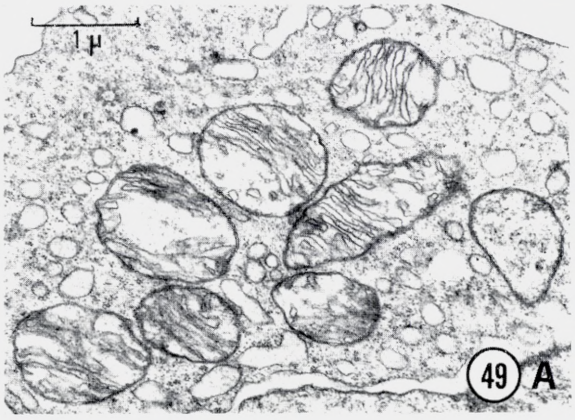
Fig. 51. *Argulus*. Spermatid 2. The three spherical mitochondria appear almost identical to those of *Raillietiella* in Fig. 50.

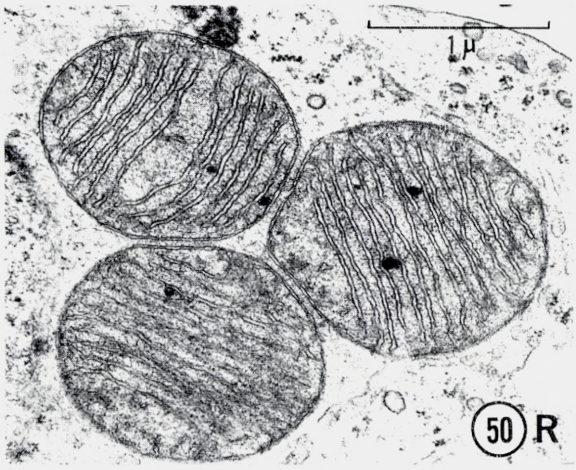
Continued in Plate 10.


1 μ
46 R


0.5 μ
47 R


1 μ
48 R


1 μ
49 A


1 μ
50 R

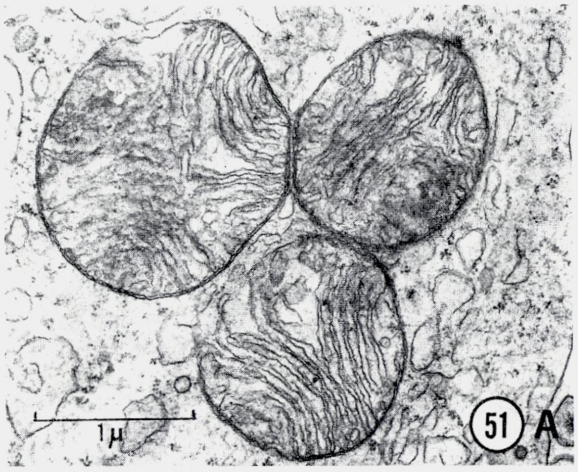

1 μ
51 A

PLATE 10

Fig. 52. *Raillietiella*. Spermatid 3. The three mitochondria have formed a common body, the "Nebenkern", and the axonema, covered by a dorsal ribbon, is in contact with all three.

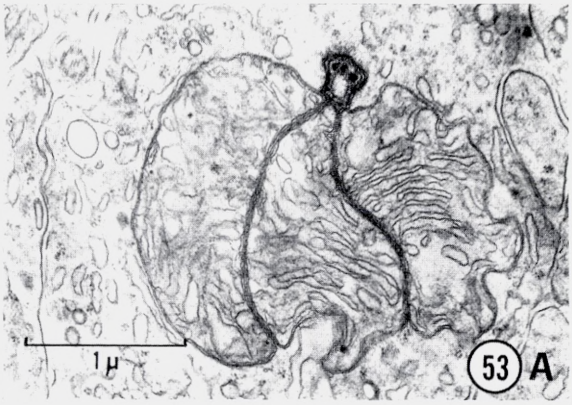
Fig. 53. *Argulus*. Spermatid 3. The contour of the mitochondria in the "Nebenkern" appears more irregular than in Raillietiella, but this may be an artifact. The centriole, with outer membranous sac and three dark fibers, is in contact with all three mitochondria.

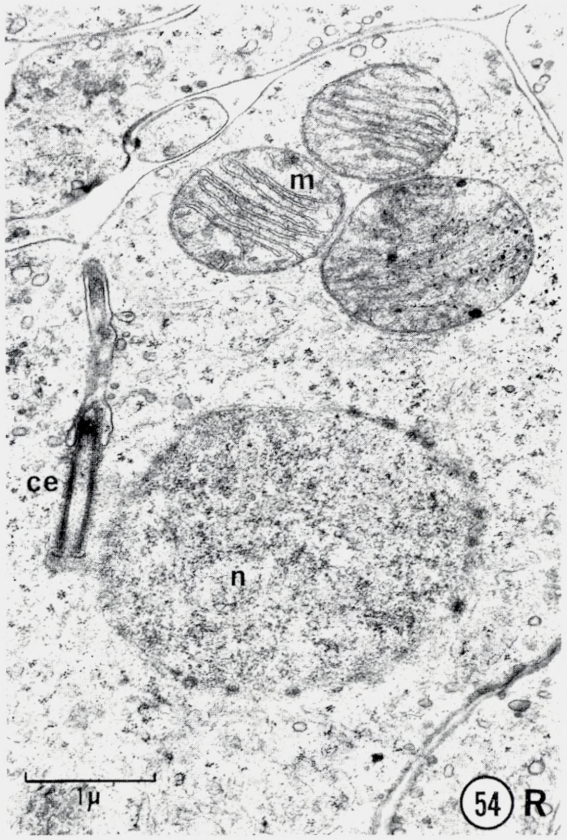
Fig. 54. *Raillietiella*. Early spermatid 2. The three mitochondria, the centriole and the nucleus have not yet established the contact typical of later stages. The centriole is attached to the bottom of a very deep flagellar pit.

Fig. 55. *Argulus*. Spermatid 2. Still no definite contact between nucleus, mitochondria and centriole. The latter has developed an axonema.

ce = centriole, m = mitochondria, n = nucleus.


1μ
52 R


1μ
53 A


m
ce
n
1μ
54 R

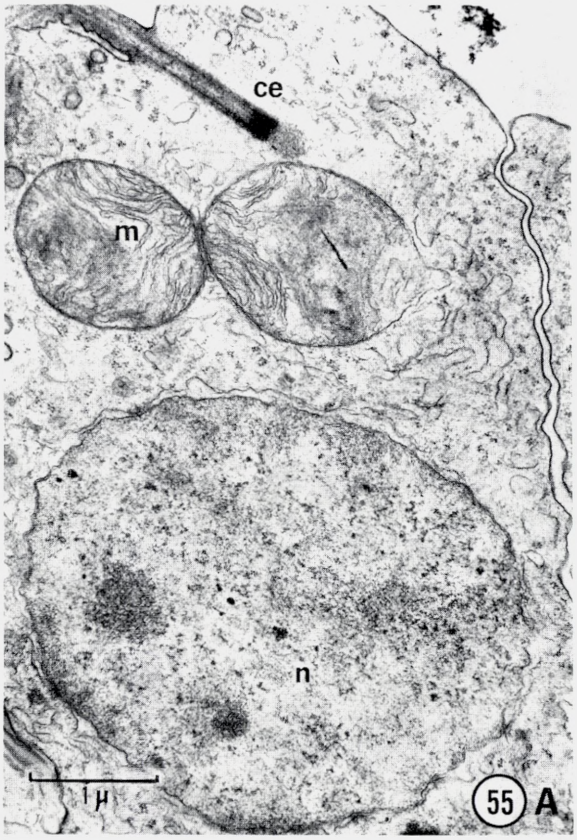

ce
m
n
1μ
55 A

Plate 11

Development of the flagellum in *Raillietiella*.

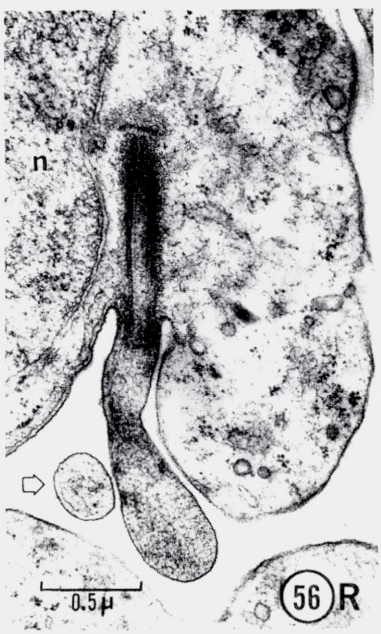
Fig. 56. *Raillietiella*. Spermatid 1. Centriole and first flagellar rudiment without axonema. The white arrow shows a cross section of another flagellar rudiment, in which no axonema is present.

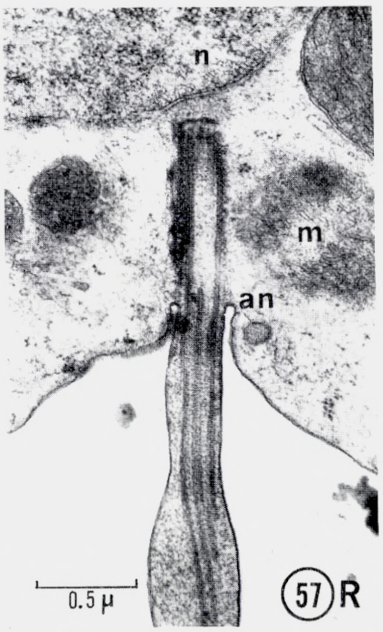
Fig. 57. *Raillietiella*. Spermatid 2. The granulosome is attached to the nucleus, and the centriole has developed an axonema in the flagellum.

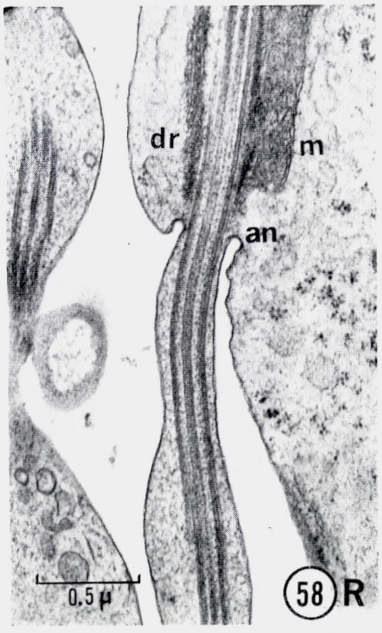
Fig. 58. *Raillietiella*. Spermatid 4 or 5. Part of the axonema is included in the cell body proper, and the free flagellum remains short. The posterior tongues of the mitochondria (m) and the dorsal ribbon (dr) grow posteriorly so they remain in contact with the annulus-like bottom of the flagellar pit (an).

Fig. 59. *Raillietiella*. Late spermatid 2. The relations of flagellum, flagellar pit, centriole, apical membrane, granulosome, mitochondria (m) and nucleus are shown. Compare text figures 3 and 11A for explanations.

an = annulus-like thickening of cell membrane in the bottom of the flagellar pit, dr = dorsal ribbon, m = mitochondria, n = nucleus, np = nuclear pores.


n
0.5 μ
56 R


n
m
an
0.5 μ
57 R


dr
m
an
0.5 μ
58 R

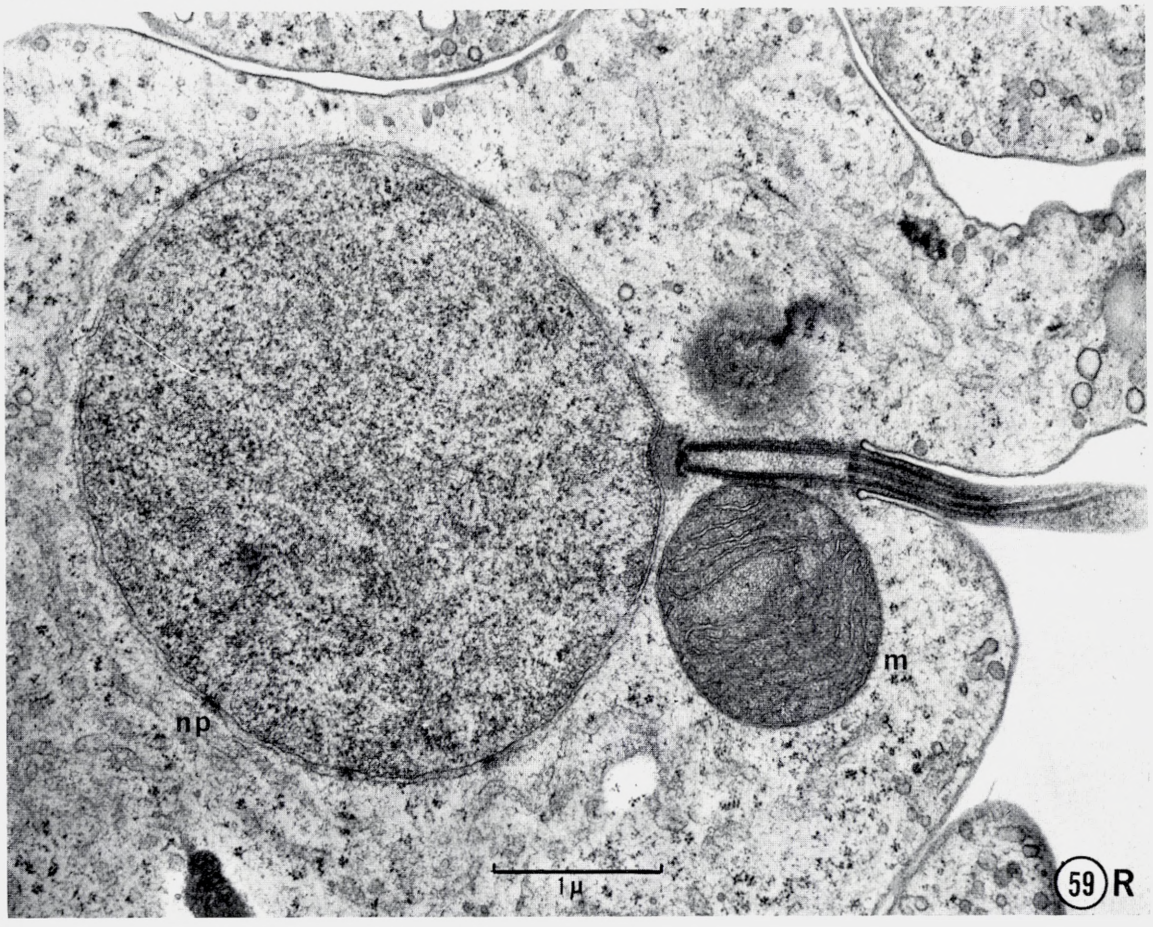

m
np
1 μ
59 R

Plate 12

Dislocation of the centriole complex.

Fig. 60. *Raillietiella*. Early spermatid 4. The point of contact of the centriole complex has moved over to the side of the nucleus, and the apical membrane (am) is detached from the end of the centriole (ce). Inset shows magnified centriolar region.

Fig. 61. *Raillietiella*. Late spermatid 4. Dislocation of the centriolar complex to the anterior end of the nucleus nearly completed. Inset shows magnified centriolar region.

Fig. 62. *Argulus*. Early spermatid 4. Similar to corresponding stage of *Raillietiella* (Fig. 60) but the PGM tends to form a long rod (pm) in front of the aperture of the centriole.

Fig. 63. *Argulus*. Spermatid 4, a little more advanced than fig. 62.

am = apical membrane, ce = centriole, dr = dorsal ribbon, g = granulosome, m = mitochondria, n = nucleus, nk = mitochondrial "Nebenkern", pm = pseudoacrosomal granular matter (PGM), vm = less compact granular matter in front of mitochondria, later refound as "ventral extension of PGM including the ventral filaments of the centriole" (See text fig. 11 and 12).

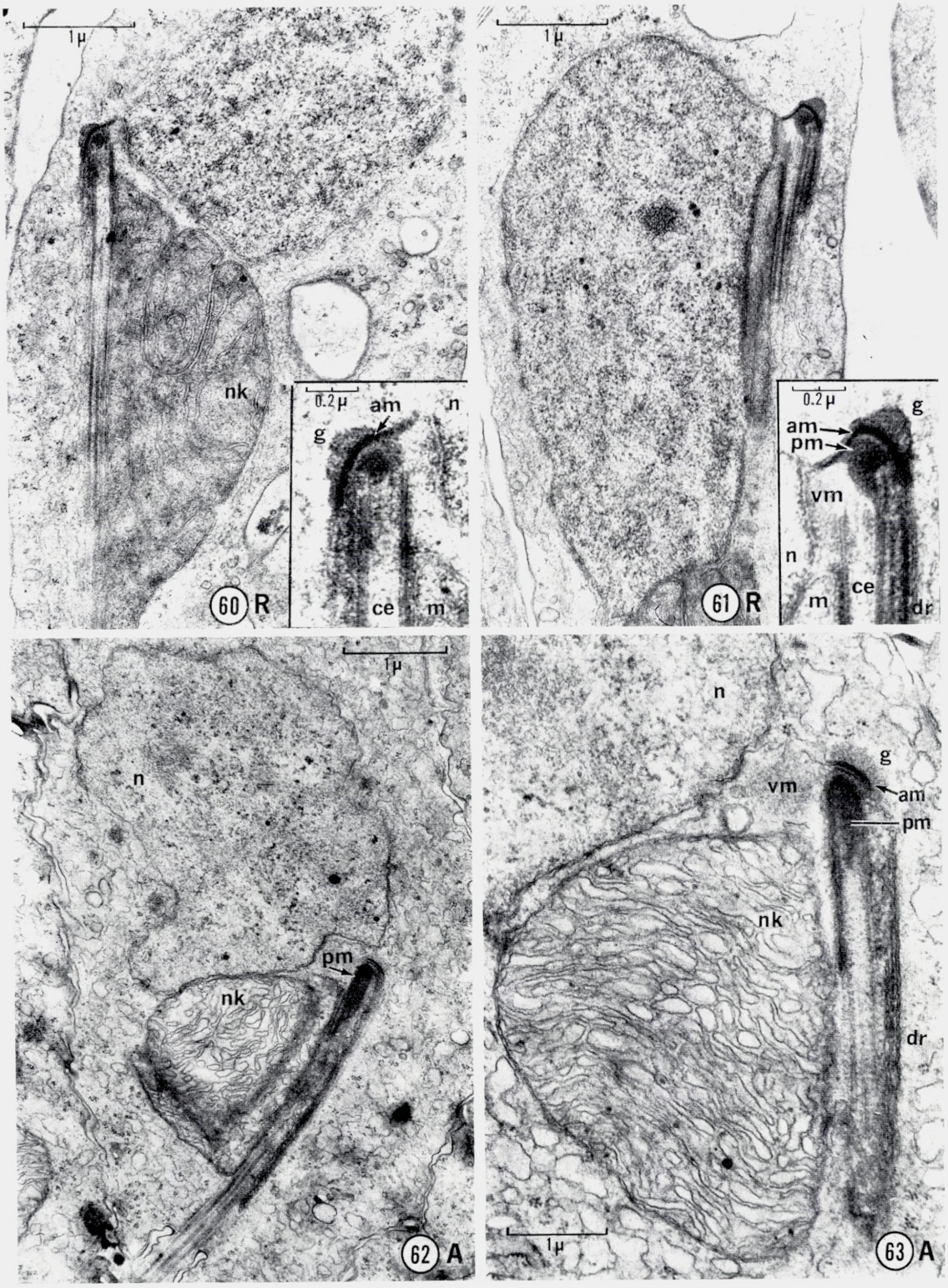

1μ
nk
0.2μ
am
n
g
ce
m
60 R
1μ
0.2μ
g
am
pm
vm
n
m
ce
dr
61 R
1μ
n
pm
nk
62 A
n
g
vm
am
pm
nk
dr
1μ
63 A

Plate 13

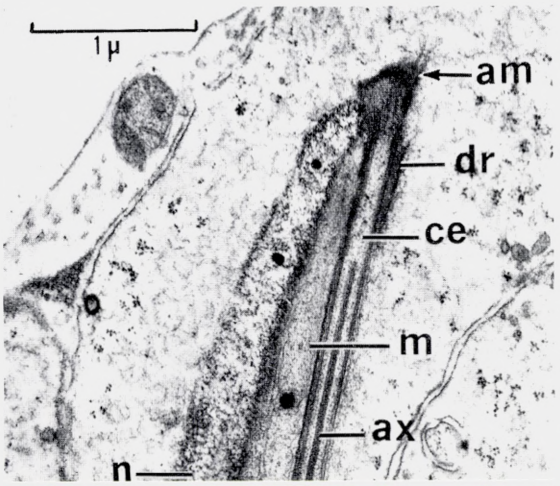
Development of anterior end in spermatid 5.

Fig. 64. *Raillietiella*. Median section of spermatid 5, showing the parallel development of anterior nuclear tongue (n), anterior mitochondrial tongues (m), centriole (ce) and axonema (ax). The pseudoacrosome is still little advanced.

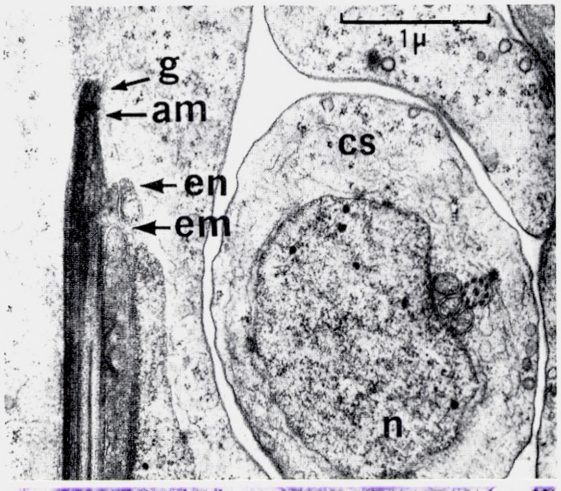
Fig. 65. *Raillietiella*. Spermatid 5, somewhat more advanced. The pseudoacrosome is growing out, so the apical membrane (am) and granulosome (g) are distinctly separated from the end of nucleus (en) and mitochondria (em). The cross section (cs) shows nucleus, the three anterior mitochondrial rods, axonema and dorsal ribbon.

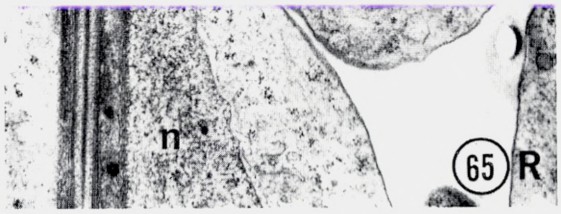
Fig. 66. *Argulus*. Spermatid 5. The pseudoacrosome is far developed (pa), but no anterior nuclear tongue is formed. Instead a true acrosome (av) and acrosome filament (af) is present.

af = acrosome filament, am = apical membrane, av = acrosome vesicle, ax = axonema, ce = centriole, cs = cross section of spermatid, dr = dorsal ribbon, em = end of mitochondria, en = end of nucleus, g = granulosome, m = mitochondria, n = nucleus, pa = pseudoacrosome.

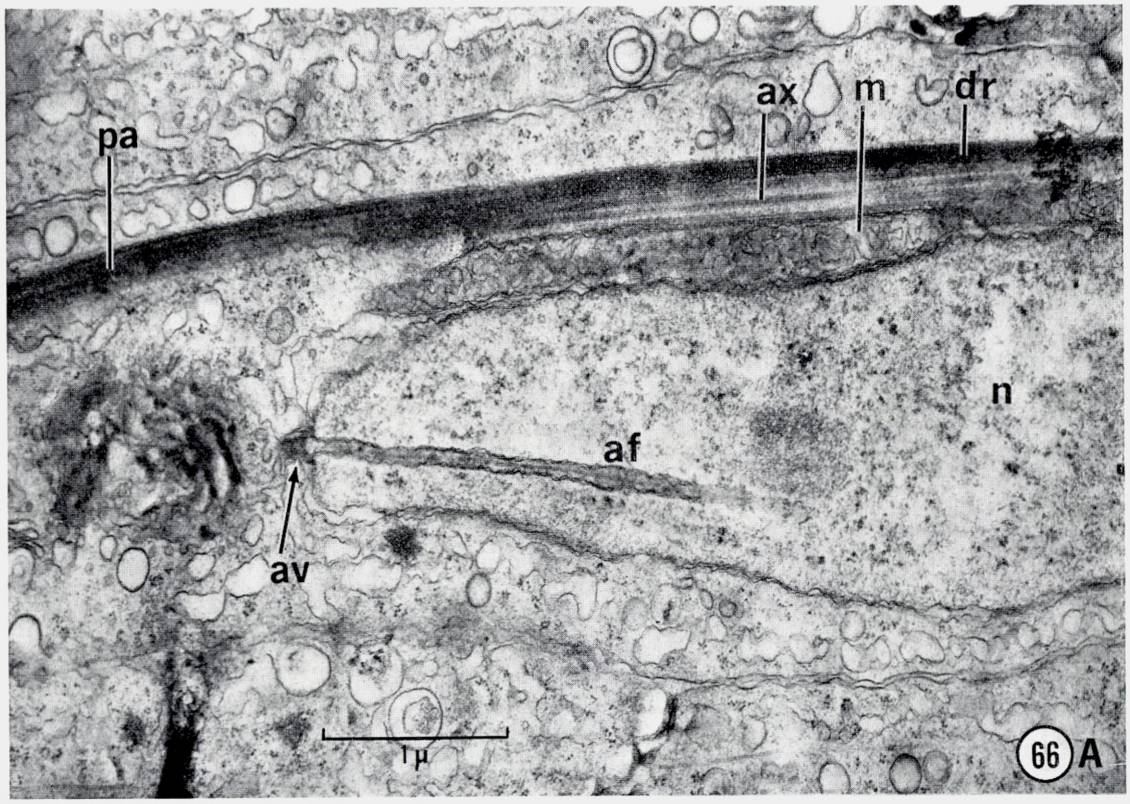


64 R





65 R



66 A

PLATE 14

Development of the true acrosome in *Argulus*.

Fig. 67. *Argulus*. Spermatid 3–4. Anterior pole of nucleus (n) and the large Golgi complex (go). A dense, post-acrosomal granule covered by a flat acrosomal vesicle is attached to the nuclear membrane (arrow).

Fig. 68. *Argulus*. Early spermatid 4. Large Golgi complexes (go) surround the developing acrosome, which is attached to the nuclear membrane.

Fig. 69. *Argulus*. Early spermatid 4. Magnification of acrosome vesicle (av) and postacrosomal granule (gr) at the same stage of development as in Fig. 68.

Fig. 70. *Argulus*. Spermatid 4. The post-acrosomal granule has grown out to form an acrosome filament (af) inside a tube formed by invagination of nuclear membranes.

Fig. 71. *Argulus*. Spermatid 6–7. Slightly oblique median section of the anterior end of nucleus (n), showing acrosome vesicle (av) and acrosome filament (af) in relation to the empty anterior end of the median mitochondrion (m), the axonema (ax) and the dorsal ribbon (dr). Compare Plate 13: 66 and text figure 18.

af = acrosome filament, av = acrosome vesicle, ax = axonema, dr = dorsal ribbon, gr = postacrosomal granule, m = mitochondria, n = nucleus.

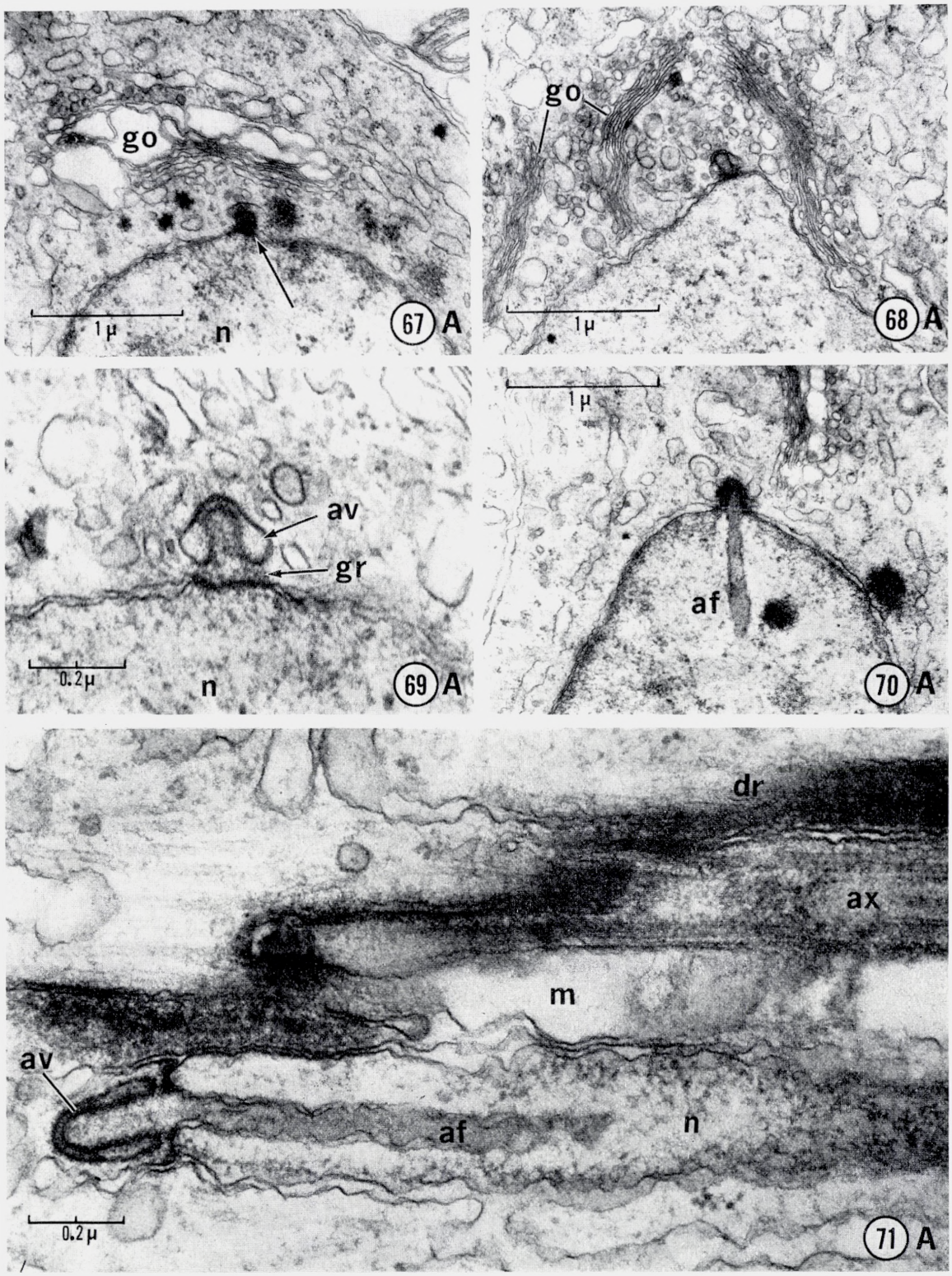

go
1μ
n
67 A
go
1μ
68 A
av
gr
0.2μ
n
69 A
1μ
af
70 A
dr
ax
m
av
af
n
0.2μ
71 A

Plate 15

Transformations of the "Nebenkern" in stages 4 and 5.

Fig. 72. *Raillietiella*. Late stage 4. The anterior tongues of the mitochondria (at) are distinct along the surface of the nucleus.

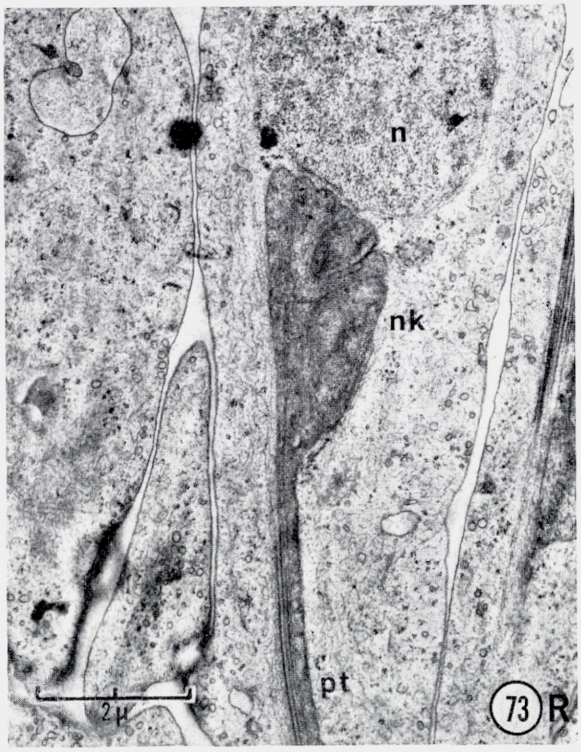
Fig. 73. *Raillietiella*. Late stage 4. The posterior tongues (pt) of the mitochondria along the axonema are distinct.

Fig. 74. *Argulus*. Late stage 4. Anterior tongues of the mitochondria are thicker than in *Raillietiella* and less sharply demarcated from the "Nebenkern" (nk).

Fig. 75. *Argulus*. Spermatid 5 with a fairly long pseudoacrosome (pa). The nucleus extends also behind the "Nebenkern" (nk) with a posterior nuclear tongue (pn).

at = anterior tongues of mitochondria, n = nucleus, nk = mitochondrial "Nebenkern", pa = pseudoacrosome, pn = posterior nuclear tongue, pt = posterior mitochondrial tongues.


at
n
nk
2 μ
72 R


n
nk
pt
2 μ
73 R


2 μ
n
at
nk
74 A

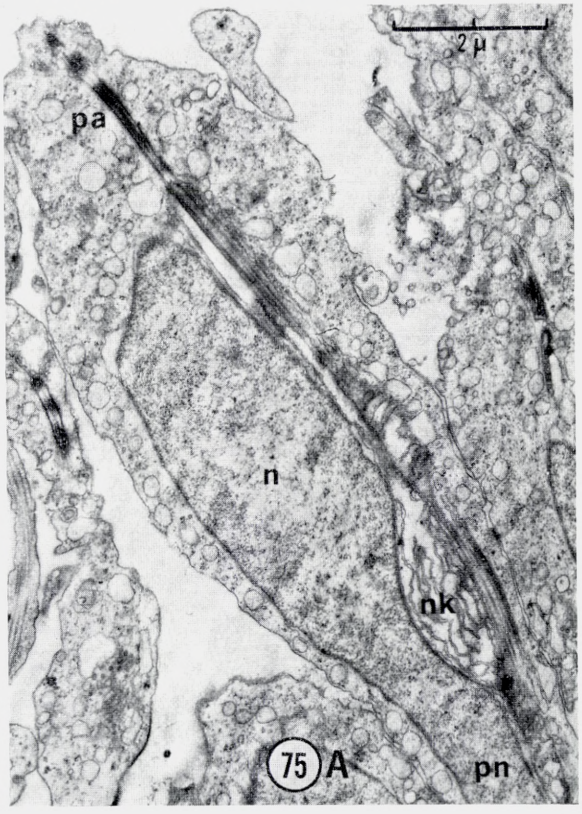

2 μ
pa
n
nk
75 A
pn

Plate 16

Development of the body, particularly dorsal and ventral ribbons.

Fig. 76. *Raillietiella*. Spermatid 4–5. Cross section behind the "Nebenkern". The granular matter (cr) of dorsal ribbon is located between membranous sacs (os and is) formed by the endoplasmic reticulum. The dark outer filaments 4–7 (df) are believed to be included in the ventral ribbon in later stages.

Fig. 77. *Argulus*. Spermatid 5. Cross section corresponding to that in Fig. 76, but no outer dark filaments are present.

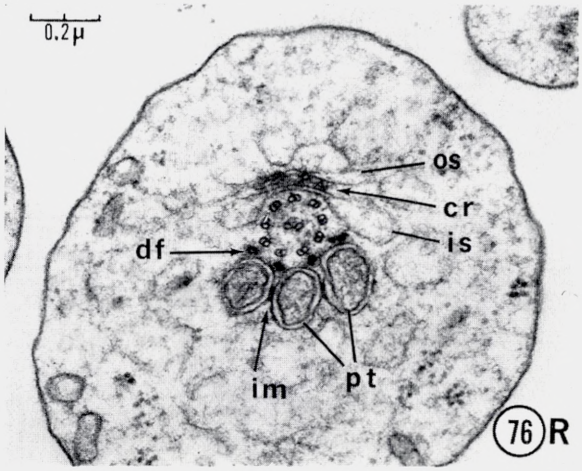
Fig. 78. *Argulus*. Cross section through mitochondrial "Nebenkern" (nk) in spermatid 4. The location of the granular matter between the inner and outer sacs of the dorsal ribbon is distinct.

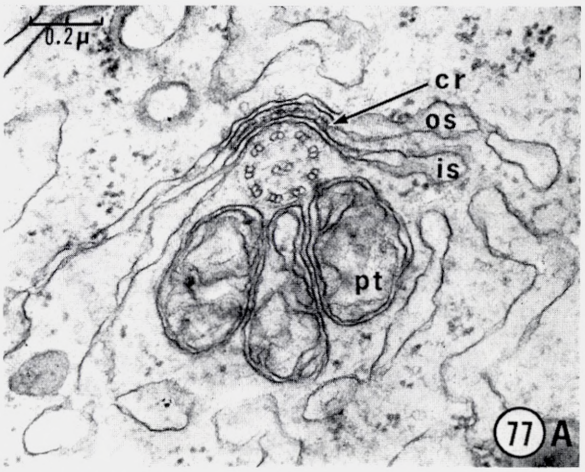
Fig. 79. *Argulus*. Late spermatid 5. The acrosome filament in the tube formed by the nuclear membranes is visible within the anterior nuclear tongue (nt).

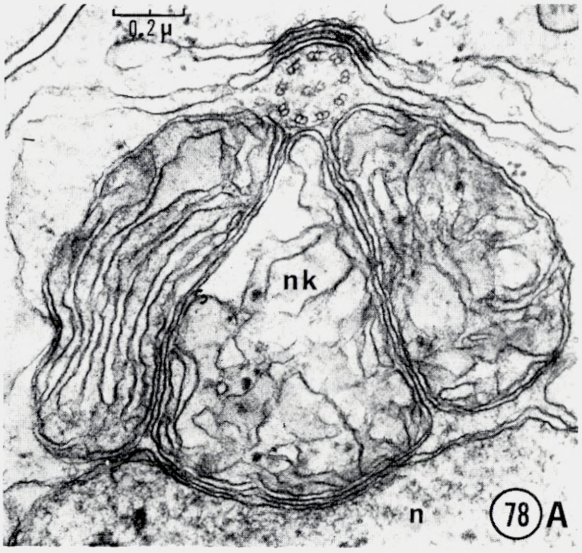
Fig. 80. *Raillietiella*. Spermatid 6. The two sacs of the dorsal ribbon are still continous with the endoplasmic reticulum (os and is). The ventral ribbon (vr) is being formed and has a membrane-like contour. nuclear condensation has lead to the formation of large chromatin clumps (nc).

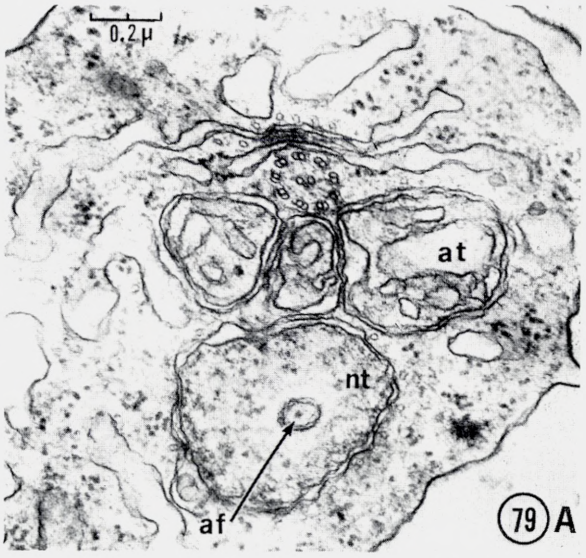
Fig. 81. *Argulus*. Spermatid 6–7. Section and stage corresponding to those in Fig. 80. Section goes through the anterior end of the body, where the median mitochondrion lacks inner membrane. Nuclear condensation has started as seen by the chromatin lamellae (nc) attached to the tube of the acrosome filament (af). Ventral ribbon is little developed: a membrane-like contour is seen below the ventral doublets of the axonema (vr).

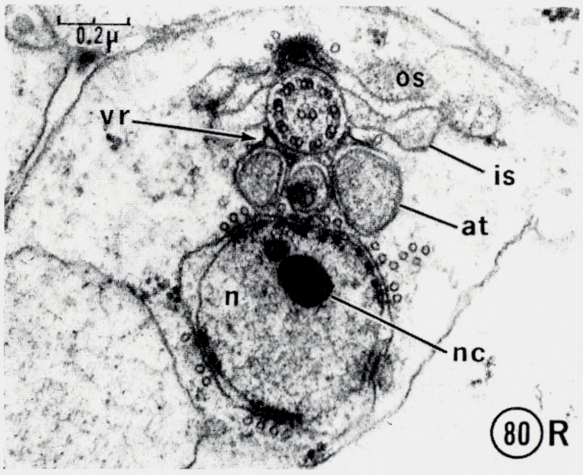
af = acrosome filament, at = anterior mitochondrial tongues, er = smooth endoplasmic reticulum, df = dark outer fibres of axonema, is = inner membranous sac of dorsal ribbon, im = intermitochondrial (cementing?) matter, n = nucleus, nc = cromatin condensations in nucleus, nk = mitochondrial "Nebenkern", nm = row of dark bodies, probable precursor of the prospective secondary nuclear membranes, os = outer membranous sac of dorsal ribbon, pt = posterior tongues of mitochondria, vr = ventral ribbon.


0.2μ
os
cr
is
df
im
pt
76 R


0.2μ
cr
os
is
pt
77 A


0.2μ
nk
n
78 A


0.2μ
at
nt
af
79 A


0.2μ
os
vr
is
at
n
nc
80 R

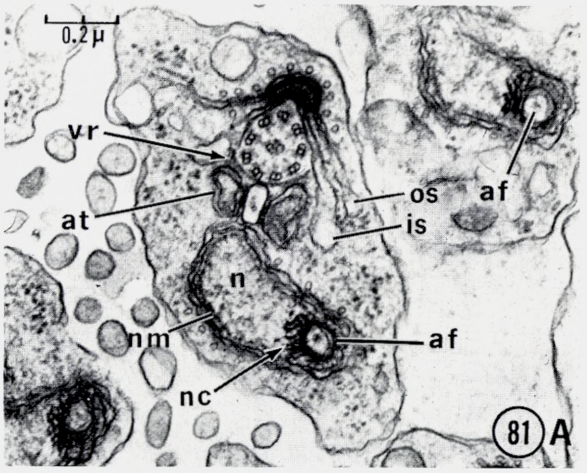

0.2μ
vr
os
af
at
is
n
nm
af
nc
81 A

Plate 17

Late development of the body in stages 6–7, particularly nuclear condensation.

Fig. 82. *Raillietiella*. Spermatid 6. Median section of body. Large chromatin clumps (nc) are formed in the nucleus.

Fig. 83. *Raillietiella*. Spermatid 7. Cross section of nearly mature spermatid. The chromatin clumps in the nucleus have begun to fuse (nc). Numerous microtubules surround dorsal ribbon and mitochondrial rods.

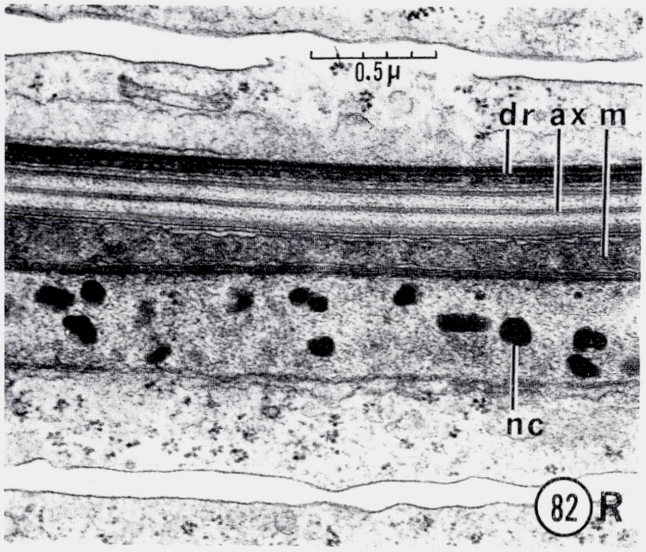
Fig. 84. *Argulus*. Spermatid 6–7. Ventral ribbon (vr) contains two dark dots, probably corresponding to outer dark fibers 5 and 6. Nucleus asymmetrical, with growing chromatin rod (nc) along axonemal filament tube (af), and indications of a secondary membrane (sm).

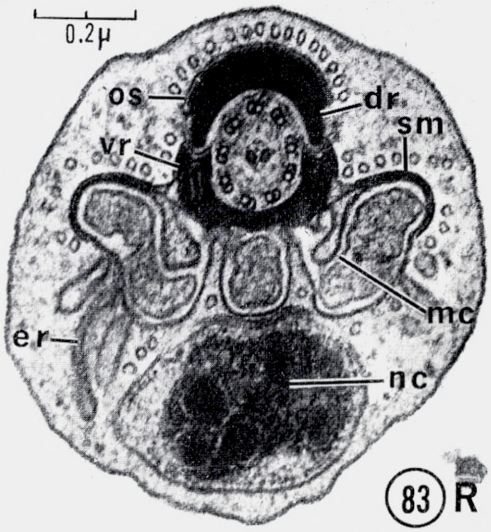
Fig. 85. *Argulus*. Spermatid 7. Sacs of the dorsal ribbon have lost connection with endoplasmic reticulum. The ventral ribbon has two dark centres in the intermitochondrial space. The chromatin rod now has distinct dorsal and ventral walls (nc).

Fig. 86. *Argulus*. Spermatid 7. Asymmetrical, fully condensed nucleus. Tube of acrosome filament collapsed and probably present between primary and secondary nuclear membranes (af). Large lateral organelles (lo). Oblique membrane of doublet 1(om) is formed. Intermitochondrial rods still closely related to ventral ribbon (ir).

Fig. 87. *Argulus*. Late spermatid 7. Cross section far anteriorly, where the dorsal ribbon is very high. Remnant of lateral organelle (lo). The intermitochondrial rods (ir) have migrated down in the interspaces between the mitochondria.

af = acrosomal filament or its tube, ax = axonema, dr = dorsal ribbon, er = smooth endoplasmic reticulum, ir = intermitochondrial rods, is = inner membranous sac of dorsal ribbon, lo = lateral organelles, m = mitochondria, mc = mitochondrial cristae, nc = nucleus, particularly chromatin condensations, om = oblique membrane between axonemal doublet 1 and dorsal ribbon, os = outer membranous sac of dorsal ribbon, sm = probable formation of secondary nuclear membranes, vr = ventral ribbon.


0.5μ
dr
ax
m
nc
82 R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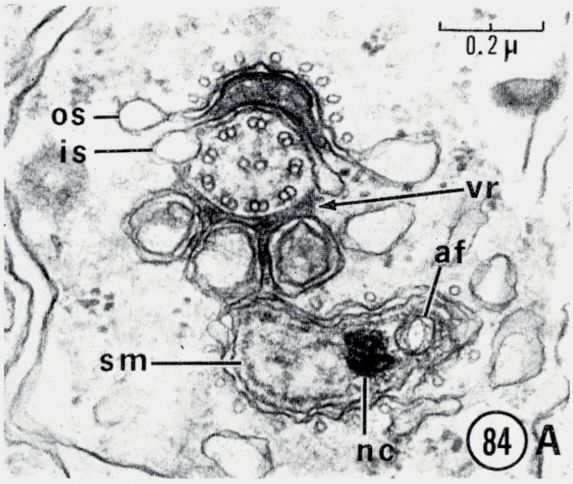
nc
83 R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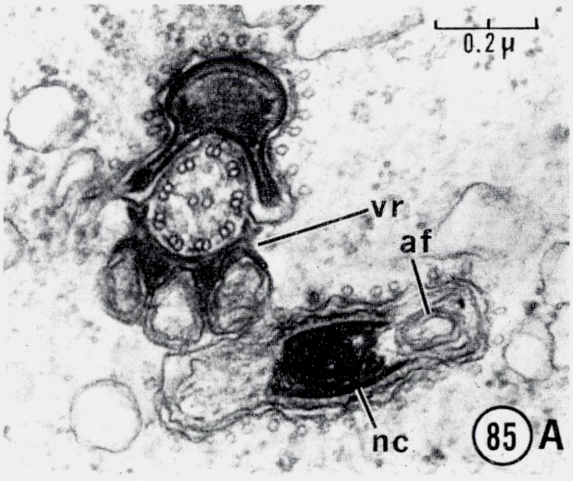
os
is
vr
af
sm
nc
84 A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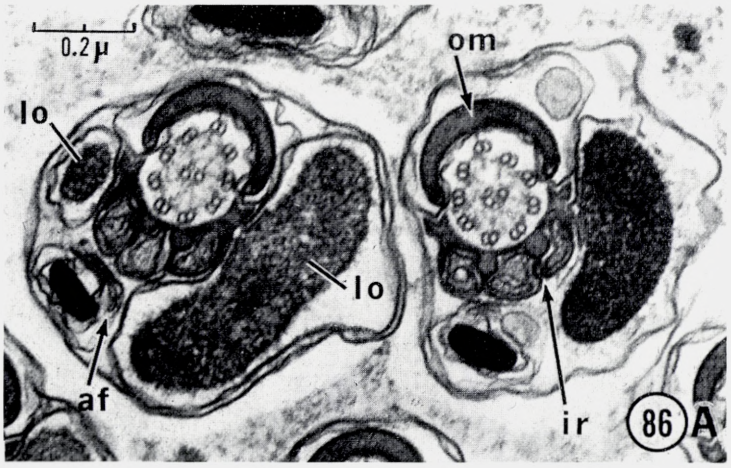
vr
af
nc
85 A


0.2μ
om
lo
lo
af
ir
86 A

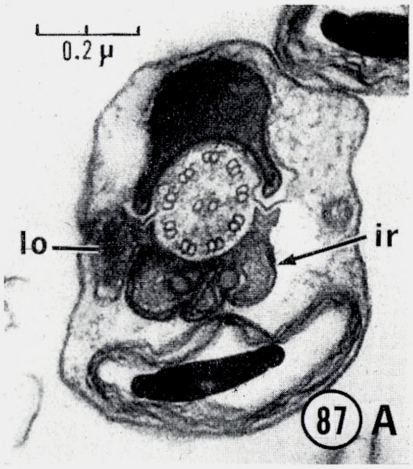

0.2μ
lo
ir
87 A

PLATE 18

Fig. 88. *Argulus*. Spermatid 7. Cross section of body showing lateral organelles (lo), the one on the same side as the nucleus little developed, and the row of flat vesicles (rv). The nucleus is still strongly asymmetrical.

Fig. 89. *Argulus*. Spermatid 7. Paramedian section through the row of flat vesicles (rv). The half-circular thickenings of the ventral ribbon (vr) are distinct.

Fig. 90. *Argulus*. Spermatid 7, somewhat younger than the one in Fig. 89. The ventral ribbon consists of separate half-rings (vr).

Fig. 91. *Raillietiella*. Spermatid, late stage 4. Abnormal specimen with two centrioles (ce) and axonemata. Dorsal ribbon (dr), apical membrane (am), pseudoacrosomal granular matter (pm) developed in connection with each centriole.

Fig. 92. *Argulus*. Spermatid 5. The figure shows that the flat sac of smooth endoplasmic reticulum, which forms the outer sac of the dorsal ribbon (os), is closely related to the Golgi apparatus (go). Many vesicles fill the space between typical Golgi lamellae and the sac, connected with the ribbon.

am = apical membrane, ax = axonema, ce = centriole, dr = dorsal ribbon, er = smooth endoplasmic reticulum, go = Golgi apparatus, is = inner membranous sac of dorsal ribbon, lo = lateral organnelles, m = mitochondria, n = nucleus, os = outer membranous sac of dorsal ribbon, pm = pseudoacrosomal granular matter, rv = row of flat vesicles, vr = ventral ribbon, w = wing-like posterior continuation of ventral sheath of the pseudoacrosome, on each side of the centriole.

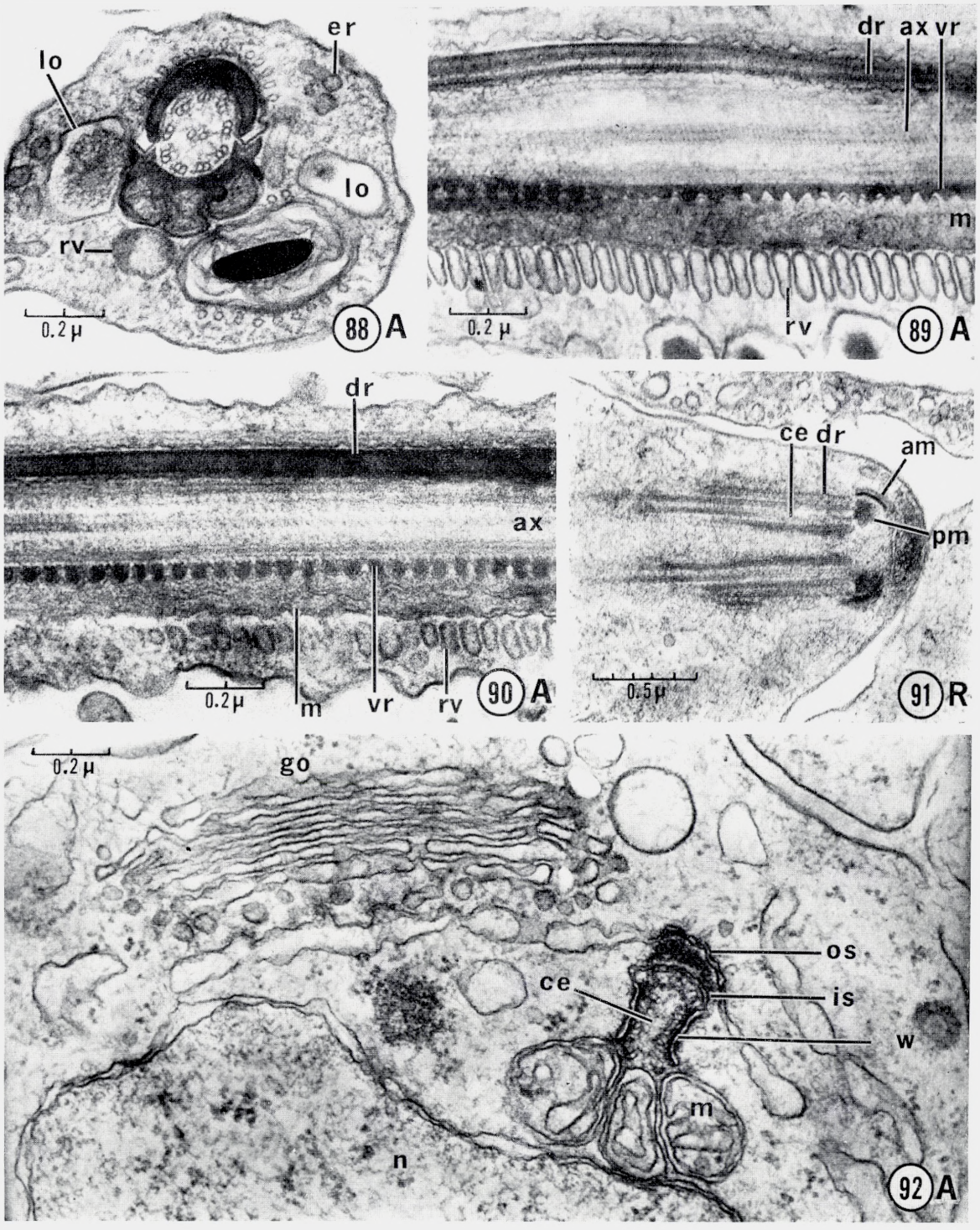

er
lo
lo
rv
0.2 μ
88 A
dr ax vr
m
rv
0.2 μ
89 A
dr
ax
0.2 μ
m
vr
rv
90 A
ce dr
am
pm
0.5 μ
91 R
0.2 μ
go
os
ce
is
w
m
n
92 A

Plate 19

Cross section of early pseudoacrosome rudiment in *Argulus*. Compare text figure 16.

Fig. 93. *Argulus*. Spermatid 4–5. Cross section of posterior part of centriole (ce), showing the three anterior mitochondrial tongues, the nucleus, and the two membranous sacs of the dorsal ribbon with granular substance (cr) between.

Fig. 94. *Argulus*. Spermatid 4–5. Cross section through the anterior, laterally compressed end of the centriole (ce), which is covered on each side by the wing-like posterior extensions of the ventral sheath of the pseudoacrosome (w). The median mitochondrion lacks inner membrane in this terminal region. The sac forming the wings is continous with the inner sac of the dorsal ribbon (is).

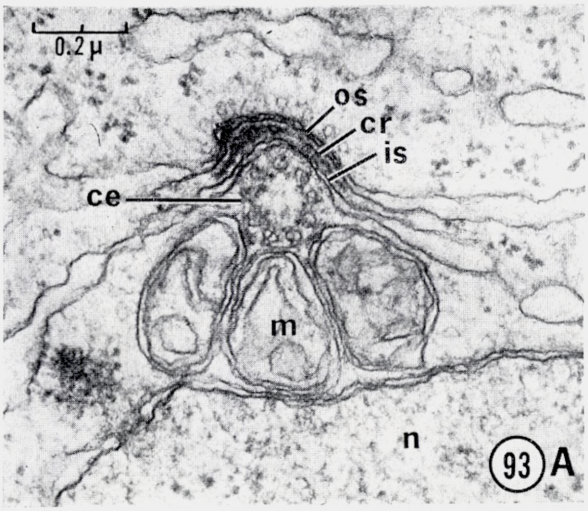
Fig. 95. *Argulus*. Spermatid 4–5. Cross section in front of the mitochondria, through pseudoacrosomal granular matter which forms horizontal lamellae (la) and a more diffuse cloud (vm) down to the wall of the nucleus (n). The lateral plates (w) cover the lamellae from the sides. No inner sac of the dorsal ribbon is present at this level. The sacs forming the lateral wings (w) fuse with the outer sac of the dorsal ribbon (thick arrow).

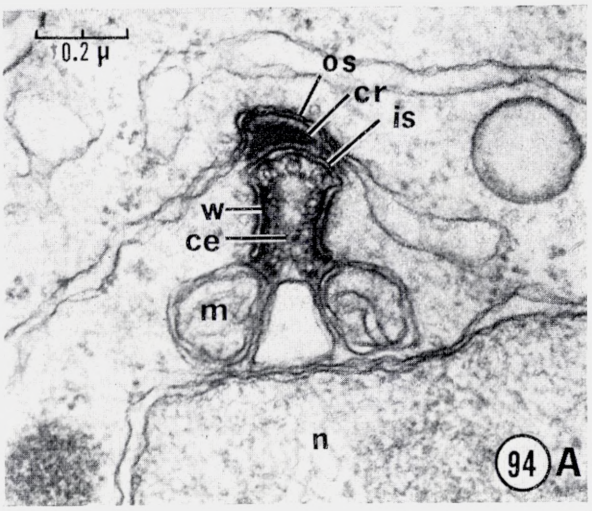
Fig. 96. *Argulus*. Spermatid 4–5. Cross section just behind the apical membrane. The thick arrow indicates continuity of the outer sac of the dorsal ribbon with the sac covering the lateral wings, to form the common membranous sac of the pseudoacrosome (ps).

Fig. 97. *Argulus*. Spermatid 4–5. Cross section through the posterior part of the apical membrane (am), showing the undivided state of the dark pseudoacrosomal matter (pm) at this level.

Fig. 98. *Argulus*. Spermatid 4–5. Cross section near the top of the pseudoacrosome. The lateral plates (w) are almost continuous with the apical membrane, which later forms the ventral sheath.

am = apical membrane, ce = centriole, cr = granular core of dorsal ribbon, g = granulosome, is = inner membranous sac of dorsal ribbon, la = lamellae formed by the PGM, m = mitochondria, n = nucleus, os = outer membranous sac of the dorsal ribbon, pm = pseudoacrosomal granular matter (PGM), ps = pseudoacrosomal membranous sac, vm = ventral, less condensed position of PGM, in contact with nucleus, w = lateral plates; later, when the ventral sheath has been formed, they occur as wing-like extensions of its posterior end.


0.2 μ
os
cr
is
ce
m
n
93 A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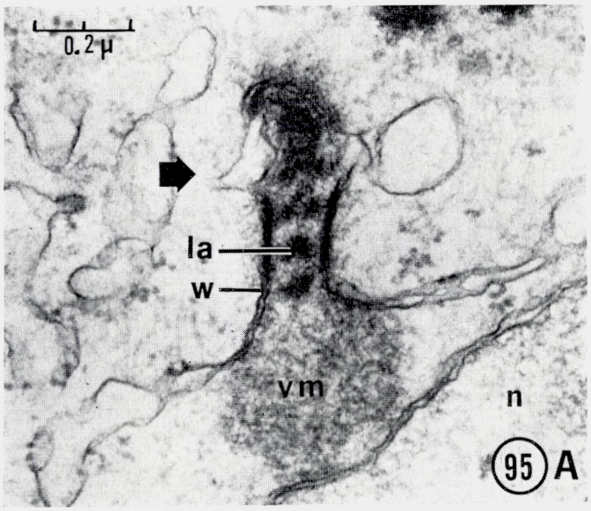
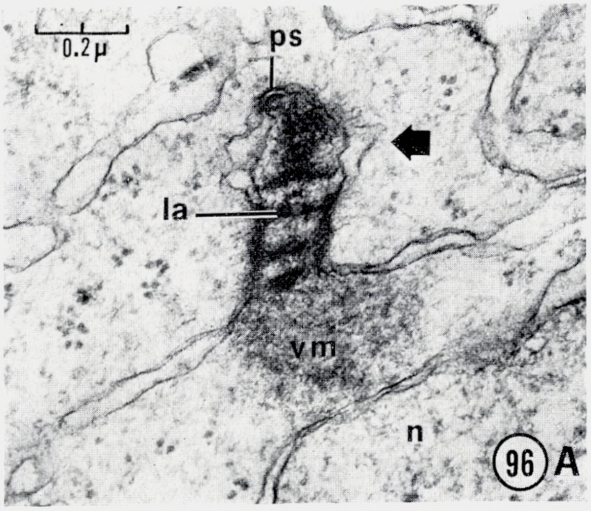
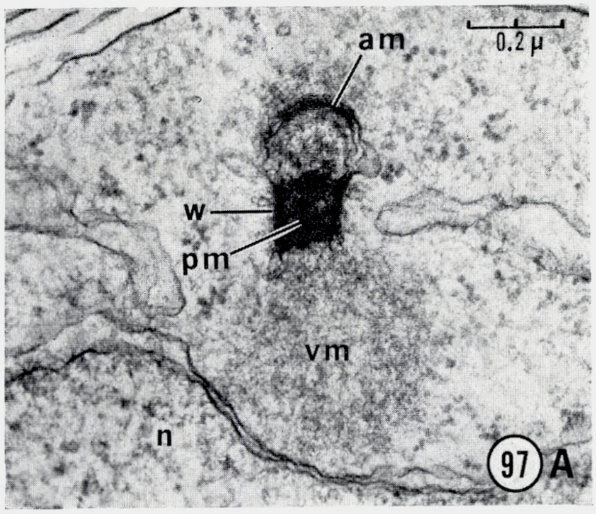
os
cr
is
w
ce
m
n
94 A


0.2 μ
la
w
vm
n
95 A


0.2 μ
ps
la
vm
n
96 A


0.2 μ
am
w
pm
vm
n
97 A

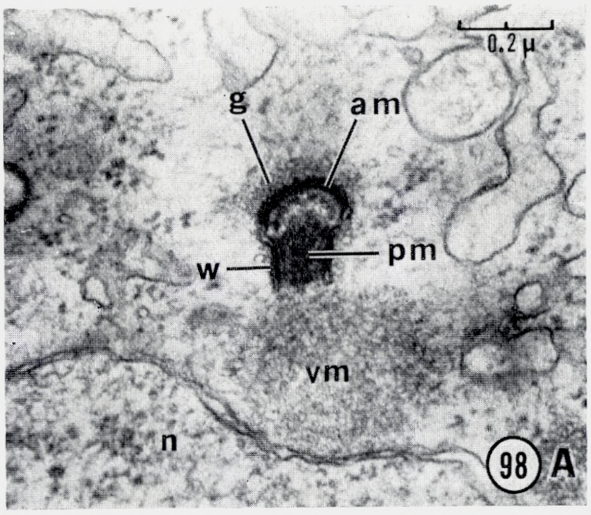

0.2 μ
g
am
w
pm
vm
n
98 A

Plate 20

Early development of the pseudoacrosome.

Fig. 99. *Raillietiella*. Spermatid 5–6. Cross section through base of early pseudoacrosome, with a large ventral sheath (vs), a small dorsal sheath (ds), and PGM partly shaped as a tube (pm).

Fig. 100. *Raillietiella*. Spermatid 5–6. Cross section near end of young pseudoacrosome with tube-shaped PGM.

Fig. 101. *Raillietiella*. Spermatid 6. Cross section near end of pseudoacrosome. Somewhat more advanced than in Fig. 100. The PGM forms a median lemella (pm), attached to the dorsal sheath.

Fig. 102. *Argulus*. Spermatid, late stage 5. Cross section of pseudoacrosome and Sertoli cell. Dorsal and ventral sheaths are like those of *Raillietiella* and are surrounded by a membranous sac (ps). The spermatid is separated from the Sertoli cell by a narrow intercellular space (is). The Sertoli cell has large mitoehondria (sm) and granular endoplasmic reticulum (gr).

Fig. 103. *Argulus*. Spermatid, late stage 5. More advanced than in Fig. 102. Distinct horizontal lamellae (la) are formed within the ventral sheath, but a median lamella (ml), attached to the dorsal sheath (ds), is very like the one in *Raillietiella*.

Fig. 104. *Raillietiella*. Spermatid, late stage 5. Horizontal section through a very early pseudoacrosome, protruding very little above the ends of the mitochondria (m). The lateral plates or wing-like extensions of the ventral sheath (w) are distinct.

Fig. 105. *Argulus*. Spermatid stage 4. Median section of the rudiment of the pseudoacrosome, showing the membranous sac covering the apical membrane (am), and the plug-like development of the PGM (pm), with a tendency to form lamellae to the right.

am = apical membrane, ax = axonema, ds = dorsal sheath, g = granulosome, gr = endoplasmic reticulum with ribosomes in the Sertoli cell, is = intercellular space between spermatid and Sertoli cell, la = horizontal lamellae of PGM, m = mitochondria, ml = median lamella of PGM, n = nucleus, nk = mitochondrial "Nebenkern", pm = pseudoacrosomal granular matter (PGM), ps = membranous sac around pseudoacrosome, sm = mitochondria of Sertoli cell, vm = ventral, less compact portion of PGM, in contact with the nucleus, vs = ventral sheath of pseudoacrosome, w = lateral plates, later posterior wing-like extensions of the ventral sheath of the pseudoacrosome.

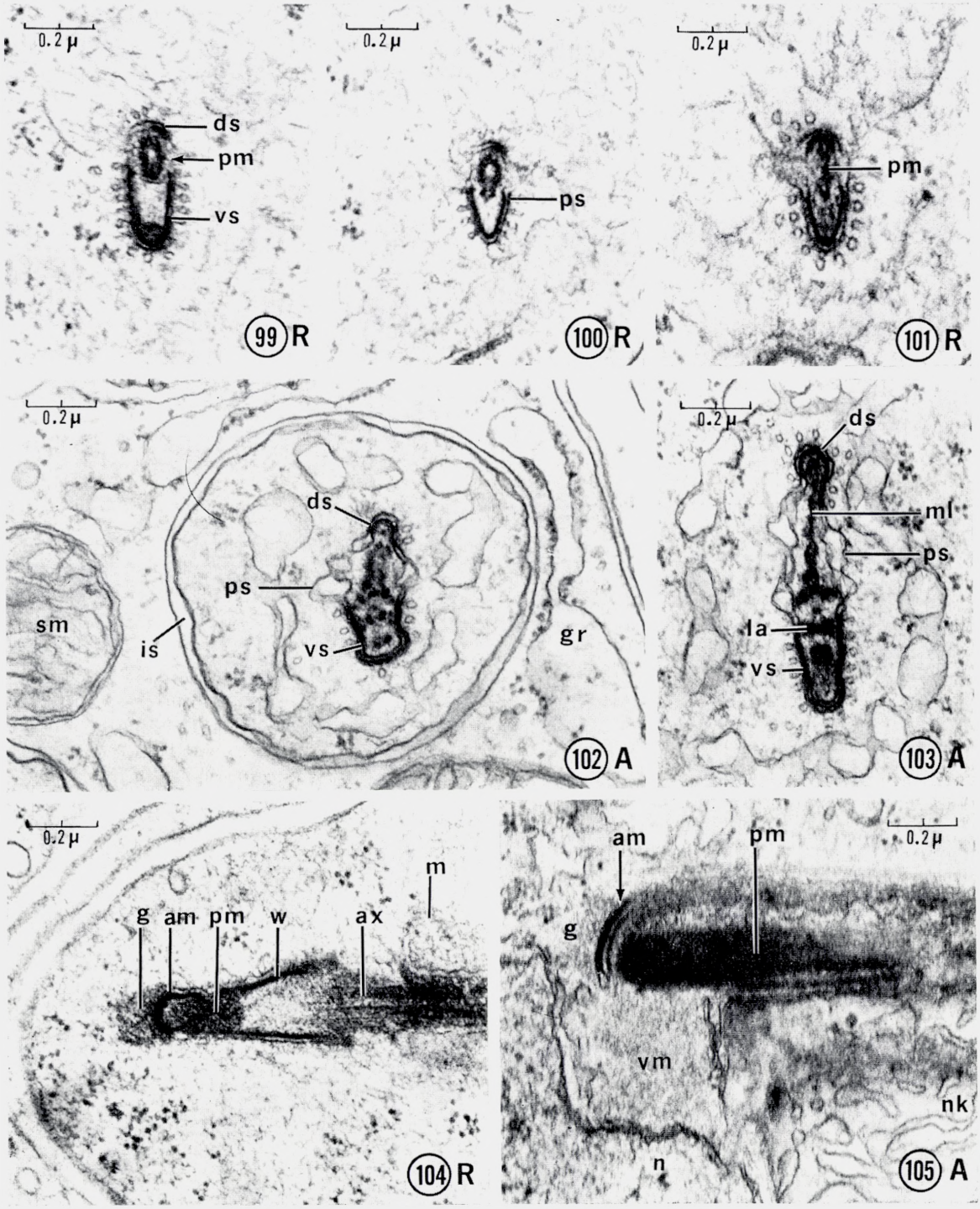

0.2 μ
ds
pm
vs
99 R
0.2 μ
ps
100 R
0.2 μ
pm
101 R
0.2 μ
ds
ps
sm
is
vs
gr
102 A
0.2 μ
ds
ml
ps
la
vs
103 A
0.2 μ
m
g
am
pm
w
ax
104 R
am
pm
0.2 μ
g
vm
nk
n
105 A

PLATE 21

Final development of the pseudoacrosomes.

Fig. 106. *Raillietiella*. Spermatid 6. Cross sections of pseudoacrosomes and of a single granulosome (g). The ventral and dorsal sheaths (vs and ds) begin to fill with PGM. A distinct membranous sac (ps) surrounds the entire pseudoacrosome.

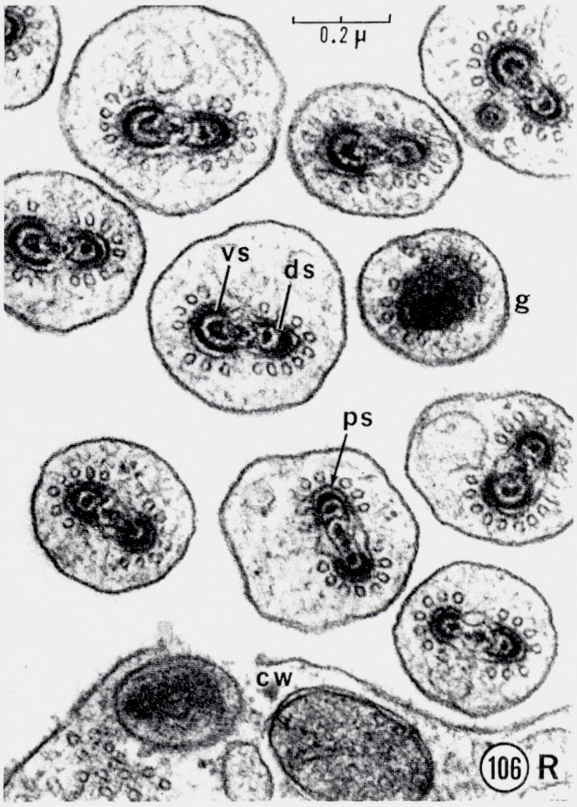
Fig. 107. *Raillietiella*. Spermatid 7. Cross sections of pseudoacrosomes, in which dorsal and ventral rods (dp and vp) have been formed by filling of dorsal and ventral sheaths with PGM. A cleft begins to appear between the rods. The membranous sac around the pseudoacrosomes is distinct and is still connected with vesicles of endoplasmic reticulum in the plasm (thick arrow). Numerous microtubulus surround the growing pseudoacrosome.

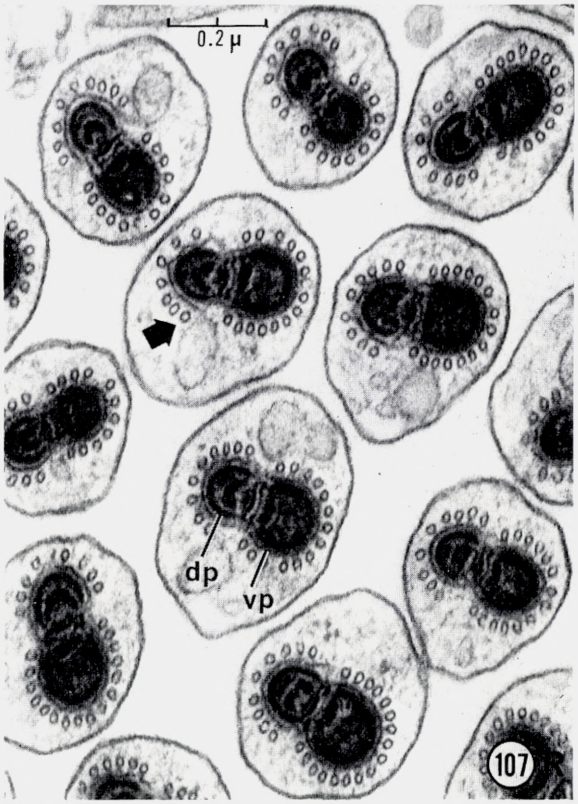
Fig. 108. *Argulus*. Spermatid, late stage 6. Cross section of pseudoacrosomal rudiment, still almost symmetrical. Corresponds to the developmental stage in *Raillietiella*, shown in Fig. 106.

Fig. 109. *Argulus*. Spermatid, late stage 6. Cross section of pseudoacrosome, in which an asymmetry begins to develop in the contents of the ventral sheath (vs).

Fig. 110. *Argulus*. Spermatid 7. Nearly mature pseudoacrosome in cross section. The contents of the ventral sheath have developed the asymmetry typical of the mature stage.

cw = wall of spermatocyst with inclusion body and mitochondrion, dp = dorsal rod of pseudoacrosome, ds = dorsal sheath, g = granulosome, ps = pseudoacrosomal membranous sac, vp = ventral rod of pseudoacrosome, vs = ventral sheath of pseudoacrosome.


0.2 μ
vs
ds
g
ps
cw
106 R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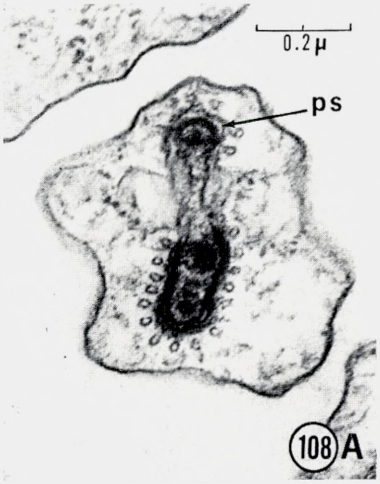
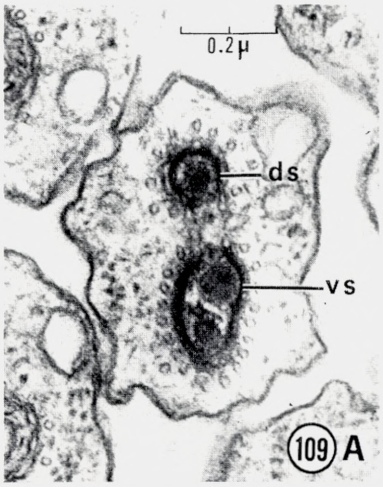
dp
vp
107


0.2 μ
ps
108 A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ds
vs
109 A

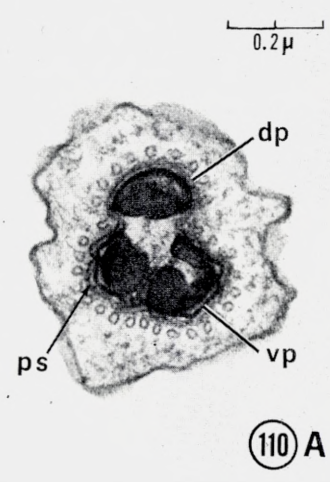

0.2 μ
dp
ps
vp
110 A

Plate 22

Development of transitional region between body and pseudoacrosome.

Fig. 111. *Argulus*. Spermatid 7. Median section of nearly mature spermatid. Body to the left, pseudoacrosome with dorsal rod (do) and ventral rod (vp) to the right. The relations of the dorsal ribbon (dr), inner sac of the dorsal ribbon (is), dorsal doublets (dd) and dorsal extension of PGM (dm) are particularly distinct. Compare text figure 13 D.

Fig. 112. *Argulus*. Spermatid 7. Cross section through centriole. A vestigial true acrosome is possibly present below (a). The centriole doublets 9, 1, and 2 have converged and are embedded in a dorsal portion of PGM (dd). Ventrally, the doublets 5 and 6 are in the same way embedded in a ventral portion of PGM (vd).

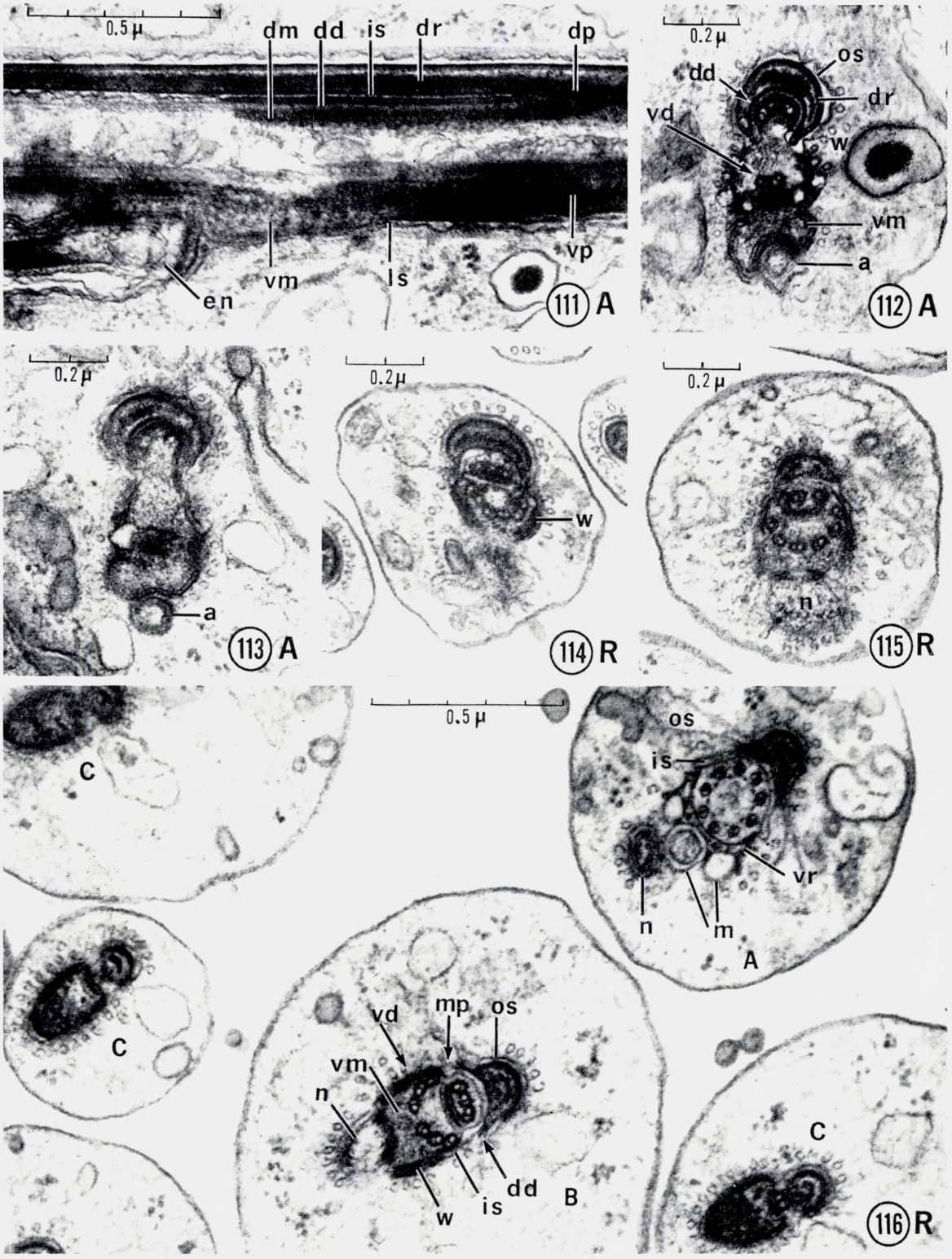
Fig. 113. *Argulus*. Spermatid 7, a little younger than in Fig. 112, with a distinct acrosome (a).

Fig. 114. *Raillietiella*. Cross section of transitional region. All six ventral doublets of the centriole visible (no. 3–8). For explanations see Fig. 116.

Fig. 115. *Raillietiella*. Spermatid 6–7. Cross section of transitional region. For explanation see Fig. 116.

Fig. 116. *Raillietiella*. Spermatid 6–7. Cross sections through posterior part of centriole (A), through anterior part of centriole (B), and through pseudoacrosomes (C). The constant pattern of the centriolar doublets in this region is evidenced by this figure, Figs. 114–115, and Plate 7: 21–22. The three dorsal doublets (dd) fuse with PGM and are connected with the dorsal rod of the pseudoacrosome, the six ventral doublets (vd) connect with the ventral rod in a similar way. The posterior, wing-like extensions of the ventral sheath (w) are distinct in this figure and in Figs. 112 and 114. The ventral ribbon (vr) in Fig. A appears to contain dark rods, perhaps the outer dark filaments 4–7.

a = acrosome, dd = dorsal doublets of centriole, dm = dorsal portion of PGM with doublets 9, 1 and 2. dp = dorsal rod of pseudoacrosome, dr = dorsal ribbon, en = anterior end of nucleus, is = inner membranous sac of dorsal ribbon, ls = posterior limit of membranous sac on the ventral side of the pseudoacrosome, m = mitochondria, mp = membrane-like condensation in the PGM (see text Fig 12), n = nucleus, os = outer membranous sac of dorsal ribbon, vd = ventral doublets of centriole, vr = ventral ribbon, vp = ventral rod of pseudoacrosome, w = wing-like posterior extensions of the ventral sheath of the pseudoacrosome (to the left and below the w).


0.5 μ
dm
dd
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dr
dp
en
vm
ls
vp
111 A
0.2 μ
os
dd
dr
vd
w
vm
a
112 A
0.2 μ
a
113 A
0.2 μ
w
114 R
0.2 μ
n
115 R
C
0.5 μ
os
is
vr
n
m
A
C
vd
mp
os
vm
n
dd
w
is
B
C
116 R

PLATE 23

Morphology of the top of the growing pseudoacrosome.

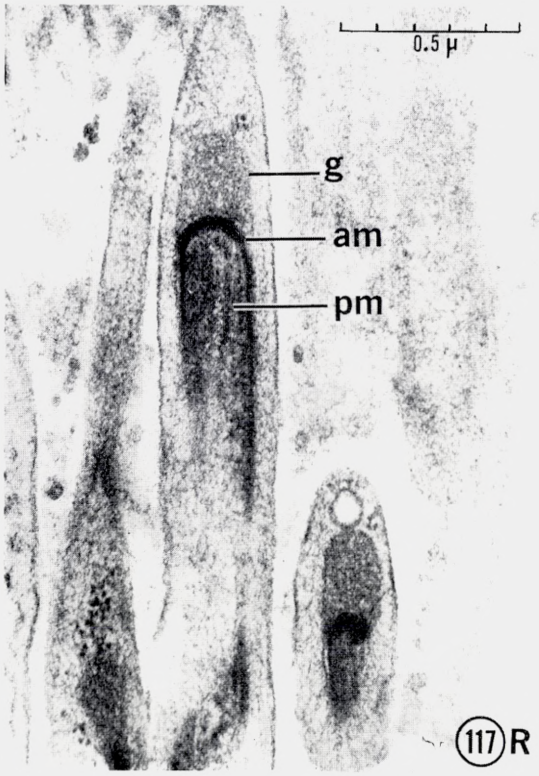
Fig. 117. *Raillietiella*. Spermatid 6. Note the large granulosomes (g) attached to the apical membranes (am).

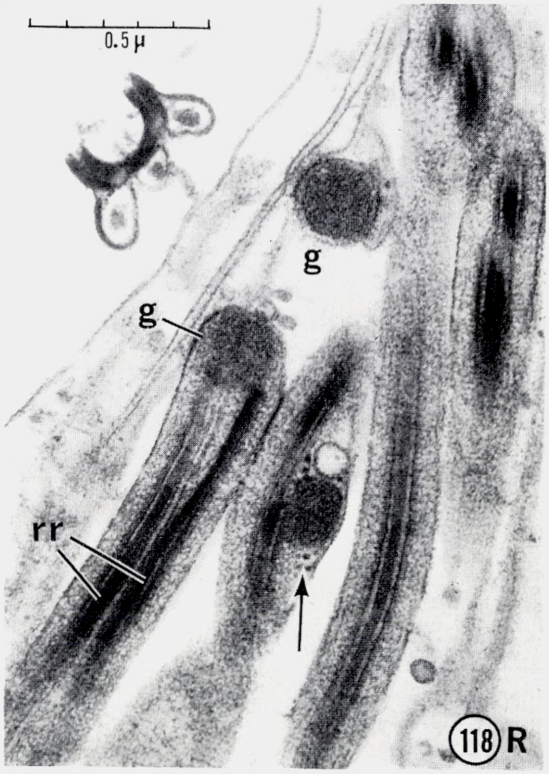
Fig. 118. *Raillietiella*. Spermatid 7. Several pseudoacrosomes with well-filled dorsal and ventral rods (rr) are longitudinally or obliquely cut. The granulosomes (g) are either on the top of the structure or, in one case, by-passed by one rod (arrow). Note also the wall of the cyst in the upper left part of the figure. The growing pseudoacrosomes are not surrounded by Sertoli cells or other plasm.

Fig. 119. *Argulus*. Spermatid 6. The top of the pseudoacrosome with apical membrane (am) and granulosome (g), the latter less distinct than in *Raillietiella*. The apical membrane is directly continous with the ventral sheath (vs), less so with the dorsal sheath (ds).

Fig. 120. *Argulus*. Spermatid 6. Longitudinal section through the end of the pseudoacrosome, showing particularly the differentiation of the PGM (pm) into longitucinal lamellae (la).

am = apical membrane, ds = dorsal sheath of pseudoacrosome, g = granulosome, la = horizontal lamellae of the PGM, pm = pseudoacrosomal granular matter (PGM), rr = rods of the pseudoacrosome, vs = ventral sheath of the pseudoacrosome.


0.5 μ
g
am
pm
117 R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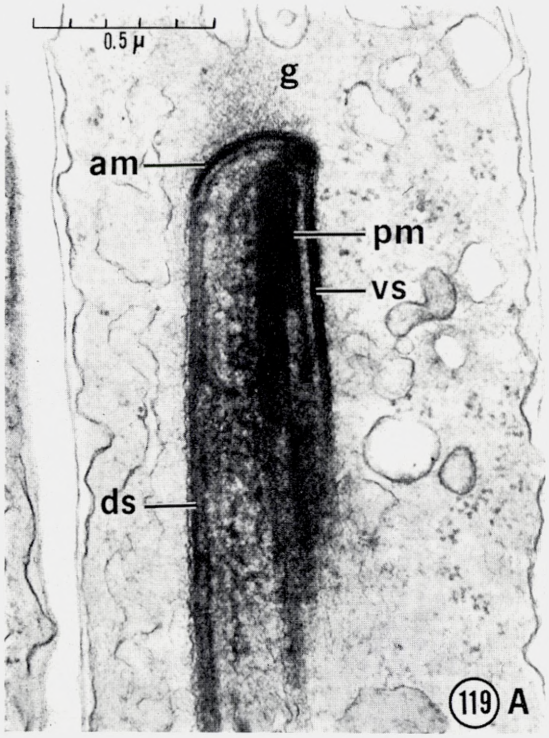
g
g
rr
118 R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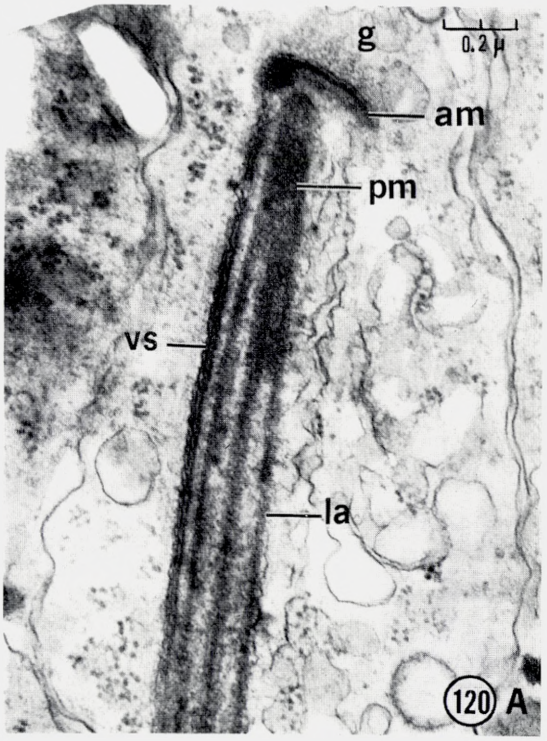
g
am
pm
vs
ds
119 A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am
pm
vs
la
120 A