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An Ultrastructural Study of the Spermatozoon of *Eudendrium ramosum*

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Summary. Spermatozoa of the colonial marine hydroid, *Eudendrium* were examined with the electron microscope. The spermatozoa of this species were found to differ greatly in structure from previously described spermatozoa in the phylum. The mature sperm are acrosomeless and retain a considerable amount of perinuclear cytoplasm following their maturation. The perinuclear cytoplasm contains numerous organelles: Golgi apparatus, endoplasmic reticulum, multivesicular bodies, ribosomes and membranous vesicles. The nucleus is elongate and cylindrical, rather than conical, in shape. The four wedge-shaped mitochondria which lie posterior to the nucleus form a fossa which contains proximal and distal centrioles. Centriolar satellites are associated with the distal centriole. The relatively short (15 μ) flagellum consists of two distinct segments: a proximal thick portion and a distal thin portion. The thick segment contains the typical 9+2 arrangement of tubules plus a variable number of peripheral, supernumerary tubules. The thin segment contains from one to eleven tubules.

The morphological differences between the spermatozoa of *Eudendrium* and those of closely related species are discussed with particular reference to sexual life history.

Key words: Spermatozoa — *Eudendrium* — Hydroidea — Coelenterata — Ultrastructure.

Introduction

The reports by Downing (1905) and Tannreuther (1909) are the first to include sketches of mature hydroid spermatozoa. According to Downing, the sperm head of *Hydra* is approximately 4.2 μ in length, with a conical nucleus that is capped anteriorly by an acrosome. He depicts a centrosome at the base of the nucleus in an extensive midpiece. One of the centrioles participates in the formation of the tail which is approximately 15 μ in length. Downing reported no *nebenkern* within these spermatozoa. Tannreuther's diagrams are much less imaginative and detailed. He shows a spermatozoon with a conical head, an ovoid midpiece and a tail which is 4 to 5 times the length of the head. None of the finer details (eg. acrosome, centrioles) are included in Tannreuther's description.

Tuzet (1929) was the next investigator to describe a mature hydroid spermatozoon. According to that author, the sperm of *Tubularia* has a 3.5 μ head and a

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13 μ tail. The sperm head (acrosome + nucleus + midpiece) is conical in shape. A minute "acrosome of Lenhossek" is located at the anterior pole of the nucleus. Contrary to Downing's observations, Tuzet observed four large spherical mitochondria lodged together at the base of the nucleus; these, together with two centrioles constitute the midpiece. The mature spermatozoon of *Hydra* has been described more recently by Brien and Reniers-Decoen (1950, 1951). These investigators suggest that the structure of the *Hydra* sperm conforms to the generalized plan of invertebrate sperm structure set forth by Tuzet (1950).

The application of electron microscopy to the study of male gametes has stimulated recent interest in the fine structure of gametes in the phylum Coelenterata. It was hoped that such studies would provide significant information on the relationship of gamete ultrastructure and phylogenetic position and that new data on the evolution of sexuality would become apparent from the study of the gametes in these primitive metazoa.

The study by Schincariol, Habowsky and Winner (1967) is the first publication to include an electron micrograph of a mature hydroid spermatozoon. According to their description, the sperm head of *Hydra fusca* is 3.7 μ in length and consists of a condensed, truncated nucleus and four mitochondria. The mitochondria are 0.7 μ in diameter and indent the base of the nucleus. The second micrograph of the *Hydra* spermatozoon was included by Weismann, Lentz and Barnett (1969) in their report on spermiogenesis in *Hydra littoralis*. Their observations corroborate those of Schincariol *et al.* in terms of overall structure.

The spermatozoon of *Pennaria tiarella*, a colonial marine hydroid, was the first to be described in detail (Summers, 1970a, 1970b). Its structure is somewhat similar to that of the spermatozoon of *Hydra* except that unique "anterior vesicles", resembling the proacrosomal vesicles in other species, are located at the apex of the sperm between the nucleus and the plasmalemma. Such vesicles have more recently been identified in the spermatozoa of two other colonial hydroids, *Campanularia* (Lunger, 1971) and *Tubularia* (Afzelius, 1971), in *Nausithoë*, a scyphomedusan (Afzelius and Franzén, 1971) and in *Bunodosoma*, an anthozoan (Dewel and Clark, 1971). Hanisch (1966, 1970) has reported on spermiogenesis in the colonial hydroid *Eudendrium*, but his reports do not include micrographs of the mature spermatozoa.

From these ultrastructural studies of sperm in the three classes of coelenterates and some additional corroborative studies (Stagni and Lucci, 1970a, 1970b) on *Hydra attenuata* some generalizations can be made. The coelenterate spermatozoon is composed of a conical head and a flagellum (approximately 3.5 μ and 30 μ, respectively). The head may (*Pennaria*, *Tubularia*, *Nausithoë*, *Bunodosoma*) or may not (*Hydra* spp.) contain vesicular, acrosome-like components anterior to the conical nucleus. Four spherical mitochondria lie at the base of the nucleus together with two (distal and proximal) centrioles and their associated satellite structures. Flagella have the characteristic 9 + 2 arrangement of fibrils.

The present report will describe, in detail, the morphology of the spermatozoon of the colonial hydroid, *Eudendrium*. From this study it will become apparent that the spermatozoon of this species differs considerably from the generalized coelenterate pattern which has emerged from previous studies and that such variations most likely reflect differences in sexual life history.

Materials and Methods

Colonies of *Eudendrium ramosum* were collected near Flatt's Bridge, Bermuda Islands and clusters of male gonophores were dissected from colonies maintained in the laboratory. Gonophores were fixed for 2 hours in 3% glutaraldehyde (Ladd Industries) in sterile (Millipore filtered) sea water at 4° C. Following fixation, the specimens were washed overnight in cold sterile sea water and then post-fixed in 1% osmium tetroxide (OsO_4) in sea water at 4° C. Subsequently, they were passed through graded alcohols at 4° C, propylene oxide and were embedded in Epon 812 according to a modification of Luft (1961). In order to ascertain the maturity of the spermatozoa, sperm were observed with the light microscope after they had been released naturally from the gonophores. These observations were in close agreement with the subsequent electron microscope findings.

Thin sections were cut with glass and diamond knives on a Porter-Blum MT-2 ultramicrotome and mounted on 75×300 mesh, 200 mesh or formvar coated, carbon stabilized 100 mesh grids. Thick sections (0.25–0.50 μ) for light microscopy were mounted on glass slides and stained with toluidine blue (pH 11.0). Thin sections were stained with saturated uranyl acetate and/or lead citrate (Reynolds, 1963) and examined with an EM 300 (Philips).

Observations

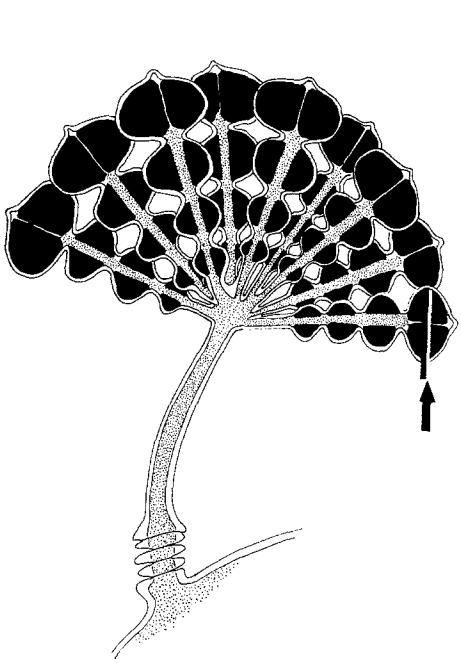
I. Sexual Life History

The sexual individuals of *Eudendrium* are radically different from the medusoid sexual individuals of most other hydroids. In this species the gonozooids are "degenerate hydranths" (Hyman, 1940) which form styloid gonophores. These gonophores are purely sexual in function and lack the tentacles and open hypostome for feeding. Each male forms 10–20 fingerlike styloid gonophores (Fig. 1) each of which bears from 3–5 swellings. Each of the proximal swellings contains a concentration of gametogenic cells which are sandwiched between ectoderm and endoderm. The mature male gametes are found within the most distal swelling. These may also be recognized by their large size, translucence and the absence of an endodermal core. Each of the swellings is covered by an ectoderm which is composed of two cell types: fixed epithelial cells and cnidocytes with nematocysts (Fig. 2). The epithelial cells do not contain muscular elements (myonemes) and the male gametes are not expelled by contraction; rather the gametes are shed when the wall of the terminal swelling ruptures and sloughs away. The spermatozoa thus released enter the female gonophore by passing through its epidermal epithelium, apparently via a small opening or micropyle (unpublished observations). Fertilization is therefore internal in this species. Gametes within the next swelling of the male gonophore then complete their maturation and are released in a similar manner. For additional information on the morphology of the female sexual individuals and embryonic development in *Eudendrium* the reader is referred to the definitive study of Mergner (1957).

II. Light Microscopic Observations

Mature *Eudendrium* spermatozoa were observed within the most distal swellings of the individual styloid gonophores. From cross sections (Fig. 2) it is evident that the spermatozoa are arranged in whorls within the mature swellings. It is also apparent that all of the male gametes within a single swelling are in the same stage of maturation.

Fig. 3 is a drawing from the light microscope of an unstained, living *Eudendrium* spermatozoon which was released normally from colonies which were main-



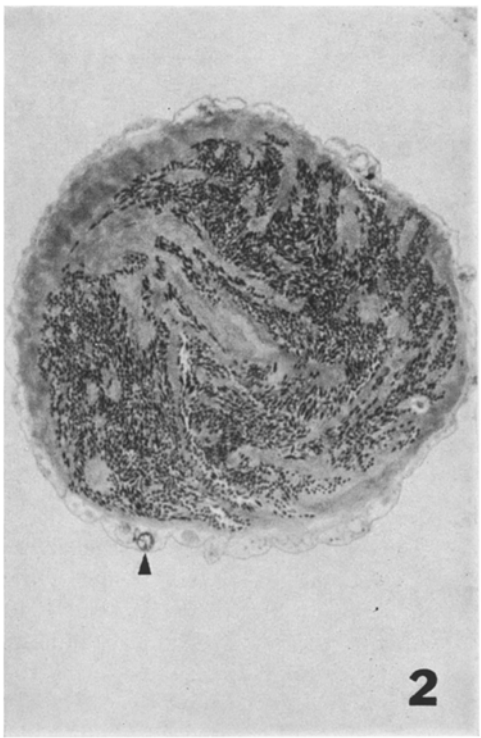
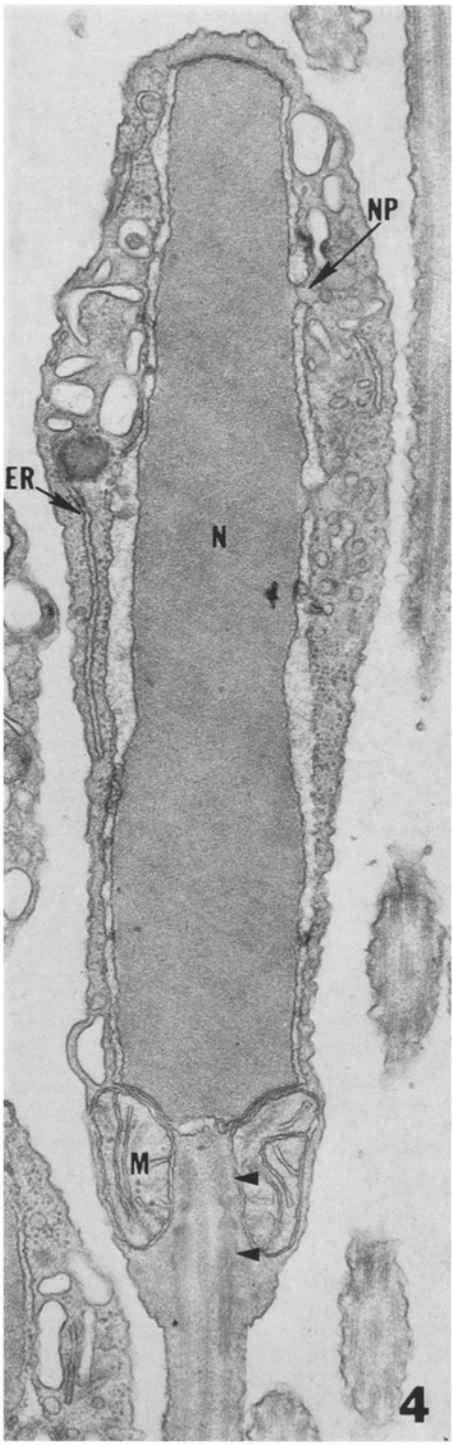


Fig. 1. Schematic representation of the gonozooid of *Eudendrium*. Earlier stages of spermatogenesis are sequentially arranged in the more proximal swellings. Mature sperm are found in the most distal swellings. The arrow indicates the plane of section shown in Fig. 2

Fig. 2. Thick section (0.5 μ) of a distal swelling which contains mature spermatozoa. Sperm heads are arranged in whorls within the swelling. Lightly staining areas are bundles of sperm tails. The swelling is surrounded by a simple epithelium. The arrowhead indicates a nematocyst within the epithelium. Toluidine blue stain

Fig. 3. Drawing of a living spermatozoon as seen with the light microscope

tained in culture. The sperm consists of an elongated, asymmetrical head which is 4.5 μ in length and a comparatively short, bipartite tail which is approximately 15 μ in length. Within the lateral bulge on the head are two or three highly refractile spheres. At the base of the head a cluster of mitochondria can be observed. The rather unusual appearance of this spermatozoon when viewed with the light microscope prompted the ultrastructural observations which are reported below.



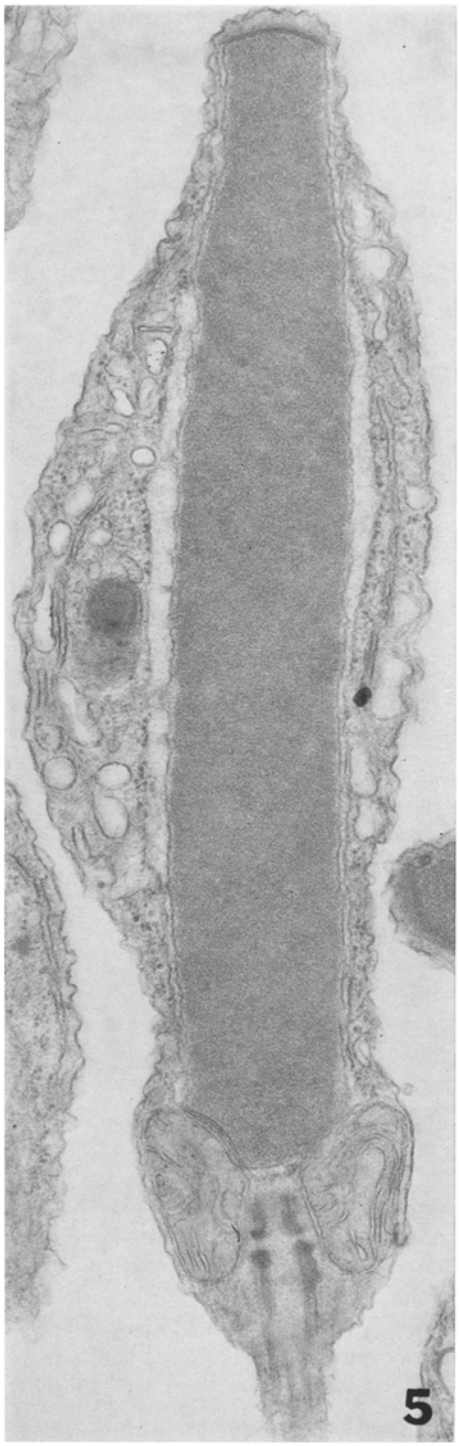


Fig. 4. A sagittal section of the spermatozoon of *Eudendrium* depicting the nucleus (*N*), mitochondria (*M*), endoplasmic reticulum (*ER*), nuclear pore (*NP*) and centrioles (arrowheads). Lead citrate stain only. ×32600

Fig. 5. Longitudinal section similar to Fig. 4 but stained with uranyl acetate and lead citrate. ×34400

III. Ultrastructural Observations

For the purposes of this description the spermatozoon will be divided into nuclear, mitochondrial and tail regions.

Nuclear Region. One of the unusual features of the *Eudendrium* spermatozoon is that it possesses a substantial amount of cytoplasm which is not shed or sloughed during spermiogenesis (Figs. 4, 5). The perinuclear cytoplasm contains free ribosomes (Figs. 4, 5), endoplasmic reticulum (Figs. 4, 12), Golgi apparatus (Fig. 7), lipid-like inclusions (Fig. 8), multivesicular bodies (Fig. 9) and large numbers of membranous vesicles (Fig. 6). There are no organelles (acrosomal bodies) between the plasmalemma and the anterior portion of the nuclear envelope. Endoplasmic reticulum is continuous with the outer leaflet of the nuclear envelope (Fig. 11). The uneven distribution of cytoplasm around the nucleus is responsible for the asymmetry of the sperm head (Figs. 5, 12).

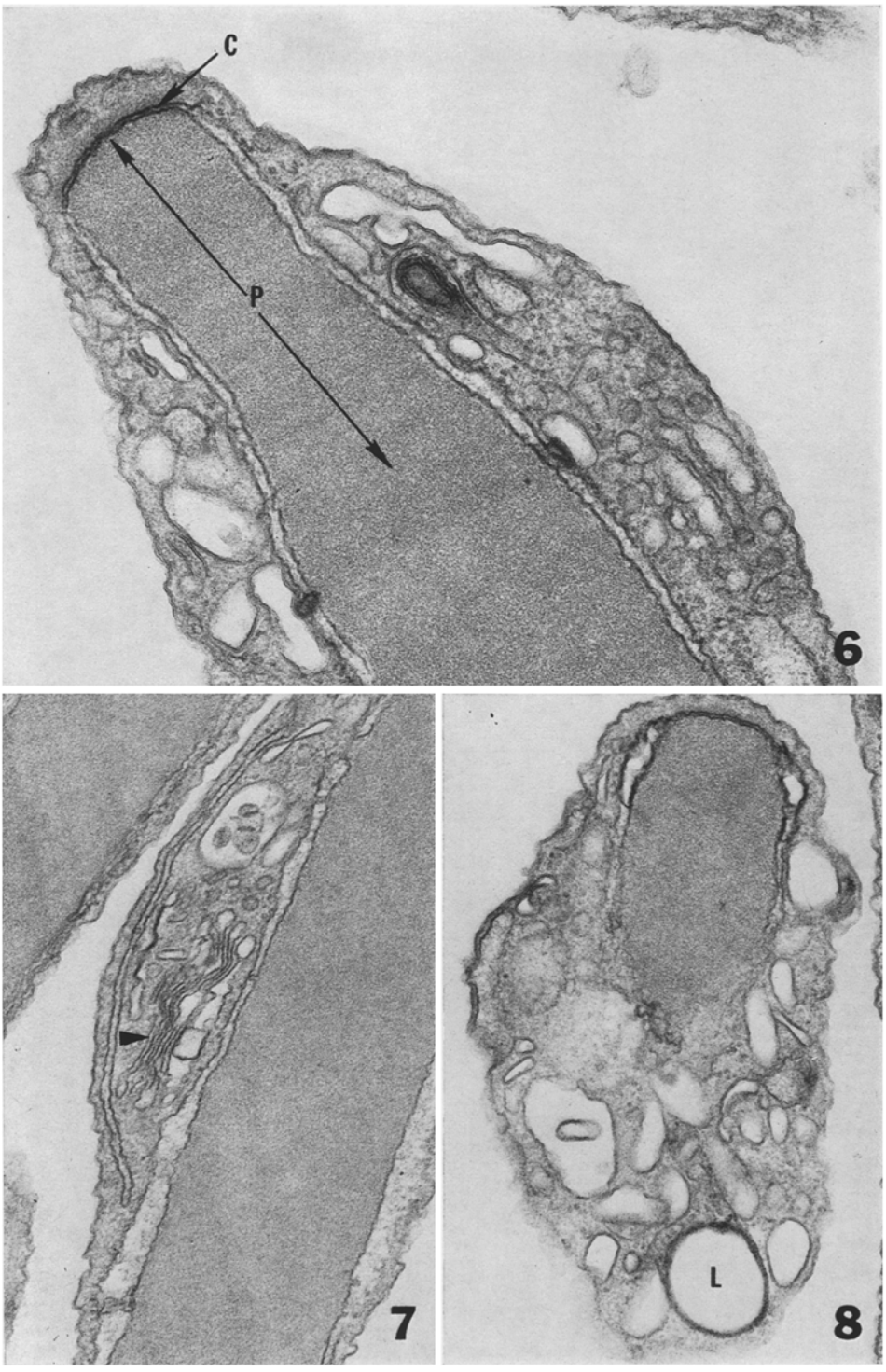
A plasmalemma surrounds the entire spermatozoon and a finely filamentous coat is observed on the plasmalemma of the flagellum (Fig. 19). These filaments are 40 Å in diameter and up to 500 mμ in length. Similar filaments were observed within the vesicles of the perinuclear cytoplasm (Fig. 19).

The nucleus of the *Eudendrium* spermatozoon appears cylindrical in longitudinal sections with a base to apex length of 3.7 μ. In cross section, the nucleus appears circular (Fig. 12) and varies between 0.4 μ and 0.6 μ in diameter, depending on the level of section, since the nucleus narrows slightly in a postero-anterior direction. The nucleus is covered entirely by a nuclear envelope, the lamellae of which are 70 Å in thickness, in contrast to the 80 Å thickness of the plasmalemma. Ribosomes adhere to the outer membrane of the nuclear envelope on its lateral aspect (Figs. 9, 10). The nuclear envelope may serve as a perinuclear cistern of endoplasmic reticulum because it is dilated to 60–80 mμ and filled with finely granular material. Prominent nuclear pores which are 700 Å in diameter and closed by diaphragmata are also present within the lateral portion of the nuclear envelope (Figs. 4, 10, 13). Ribosomes and pores are not present at the anterior and posterior extremities of the nuclear envelope. The slightly convex anterior pole of the nucleus is covered by a tightly adherent nuclear envelope (Figs. 4, 5, 6), the two membranes of which are unusually dense in their appearance. Four mitochondria lie the at posterior pole of the nucleus and where they indent the nucleus the nuclear envelope is also tightly adherent (Figs. 4, 9). The two laminae of the envelope are separated by 50–60 Å at these indentations. Where the proximal centriole approaches the basal portion of the nuclear envelope the membranes are separated by approximately 400 Å.

Fig. 6. Anterior region of the *Eudendrium* spermatozoon in longitudinal section. Note that the nucleus narrows anteriorly (*P*) and is capped by a dense, tightly adherent portion of the nuclear envelope (*C*). Lead citrate. ×55100

Fig. 7. Longitudinal section showing Golgi apparatus (arrowhead) within perinuclear cytoplasm. Lead citrate. ×46200

Fig. 8. Oblique section of the anterior region which illustrates a lipid-like inclusion (*L*). Lead citrate. ×41000



Figs. 6—8

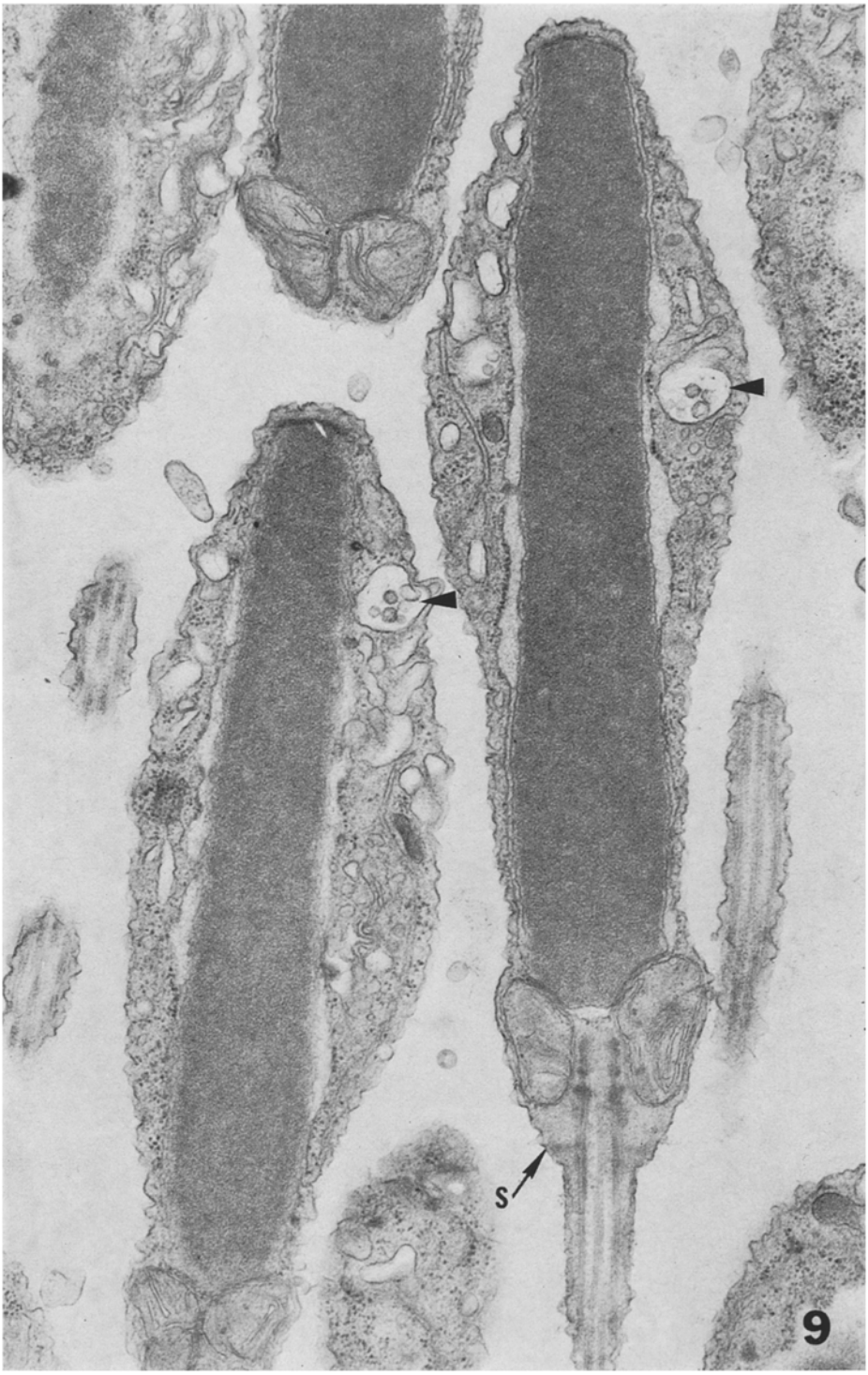


Fig. 9. Two spermatozoa in sagittal section. Note the prominent multivesicular bodies (arrowheads) and the centriolar satellites (*S*). Uranyl acetate-lead citrate. ×31800

Fig. 10. Longitudinal section of the nuclear region showing a nuclear pore (*NP*) and the ribosomes which adhere to the outer membrane of nuclear envelope (arrowheads). Lead citrate. ×59800

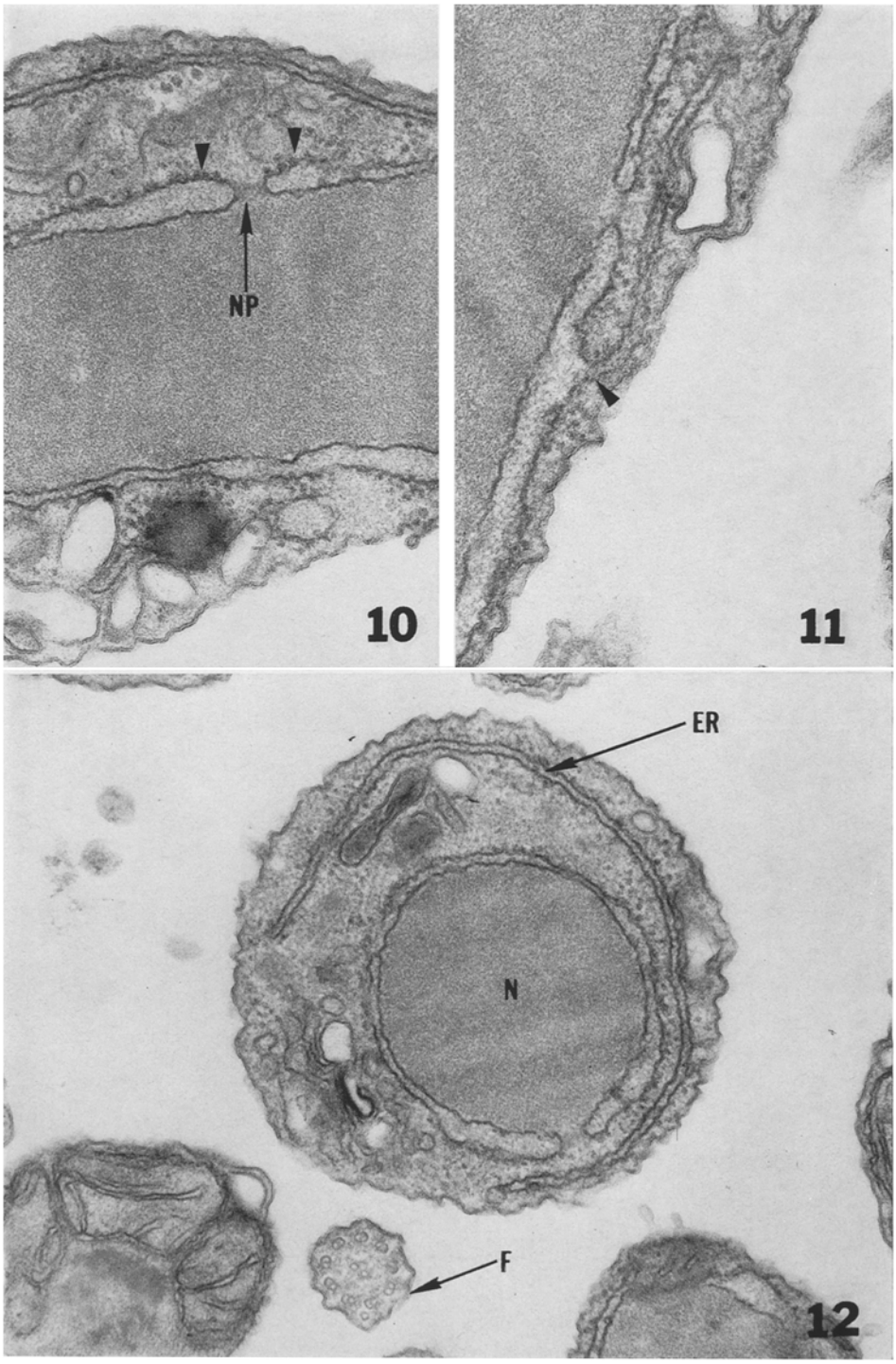
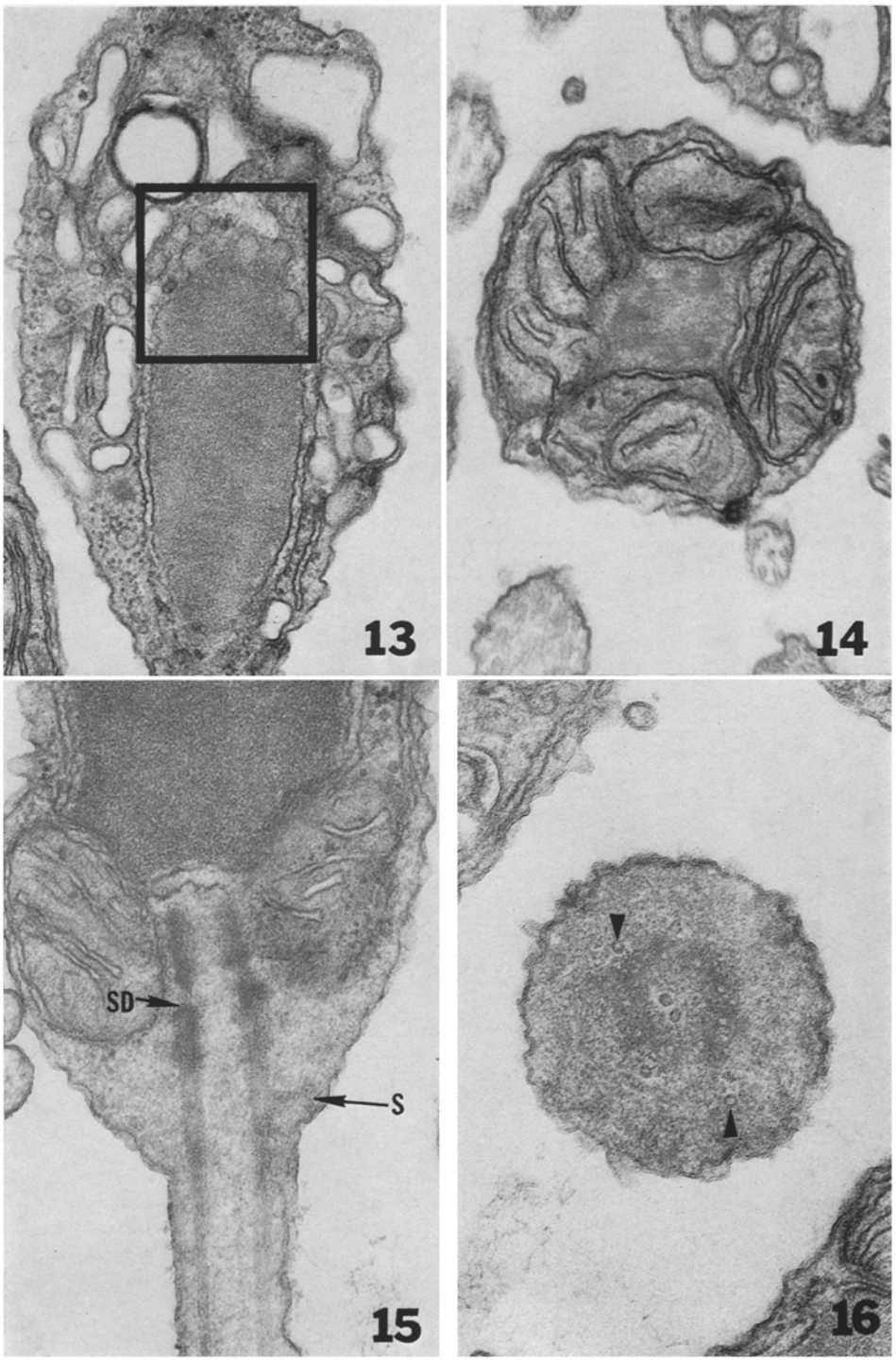


Fig. 11. Longitudinal section of the nuclear region which depicts the continuity between nuclear envelope and endoplasmic reticulum (arrowhead). Lead citrate. ×63800

Fig. 12. Transverse section through the nuclear region showing the asymmetrical placement of the nucleus (*N*) within the perinuclear cytoplasm. Endoplasmic reticulum is labelled (*ER*). A flagellum (*F*) is shown in transverse section to contain a peripheral, supernumerary microtubule. Lead citrate. ×48600



Figs. 13—16

The condensed chromatin of the *Eudendrium* sperm nucleus is composed of filaments which are 40–50 Å in diameter. It is devoid of the irregularities and nuclear vacuoles observed in sperm of many other vertebrate and invertebrate species.

Mitochondrial Region. Four mitochondria of similar size lie posterior to the sperm nucleus. In cross section the mitochondria are reniform (Fig. 14) and in longitudinal section they are wedge-shaped or triangular (Figs. 4, 5). The inner and outer mitochondrial membranes are separated by a space of 60 Å. The mitochondria contain a few cristae which range from 500 mμ–700 mμ in length and 150 Å–200 Å in width. The radially arranged mitochondria and the base of the nuclear envelope delimit a conical fossa which contains the two centrioles.

The centrioles are in line with one another and the axis of the tail and are separated by 400 Å. Each of the centrioles is cylindrical, 180 mμ in diameter, embedded in a dense matrix and contains nine sets of triplets (Fig. 16). Semi-lunar densities (possibly equivalent to the "desmosome-like" structures which are described, but not depicted, by Hanisch, 1966) cap the triplets of the distal and proximal centrioles where they abut each other (Figs. 15, 17). There does not appear to be a continuity of microtubules between the two centrioles although they are connected by substantial amounts of electron dense material. The proximal centriole has the typical 9 triplet +0 doublet structure and is considerably shorter (200 mμ vs. 450 mμ) than the distal centriole (Figs. 5, 17).

The distal centriole can be recognized in cross section by its 9 triplet +1 doublet microtubular structure (Fig. 16) or by the satellites associated with it (Figs. 9, 18). The beta fiber doublet of the tail extends well into the lumen of the distal centriole (Fig. 16). It is difficult to determine the exact length of the distal centriole in longitudinal sections because a clear junction between the centriole and the flagellum cannot be observed (i. e. the presence of a terminal plate). Therefore, measurements of the length of the distal centriole are based on the slight differences in density between the distal centriole and the flagellum.

A considerable amount of cytoplasm surrounds the distal centriole just caudal to the mitochondria (Figs. 4, 5). Embedded within this cytoplasm are from 1 to 4 microtubules which lie outside the centriolar matrix. These tubules were observed in longitudinal (Fig. 17) and transverse (Fig. 16) sections. The microtubules are aligned parallel to the distal centriole and enter the flagellum (Figs. 12, 17).

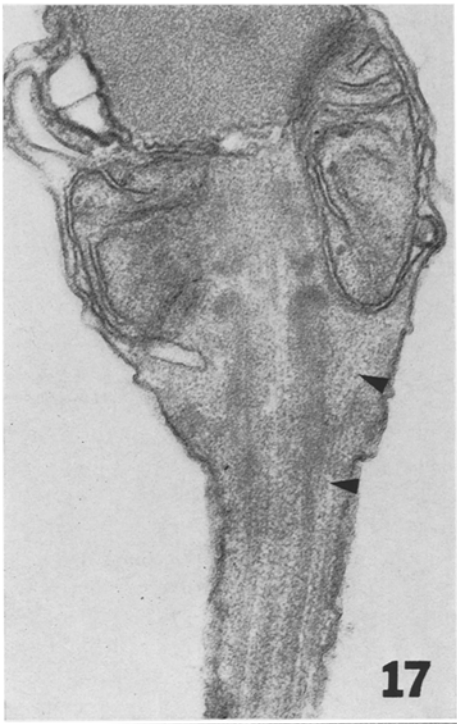
Centriolar satellites were observed, in longitudinal (Figs. 9, 15) and transverse (Fig. 18) sections, to emanate from the caudal third of the distal centriole and

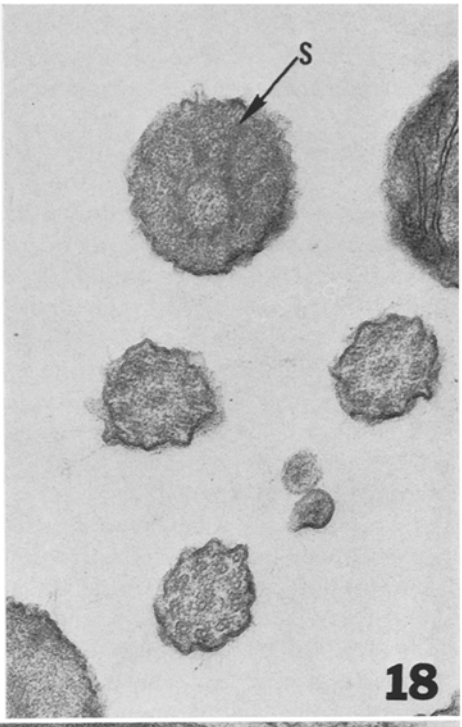
Fig. 13. Oblique section of the nuclear region. Tangential cuts of several nuclear pores are shown within the box. Lead citrate. × 41000

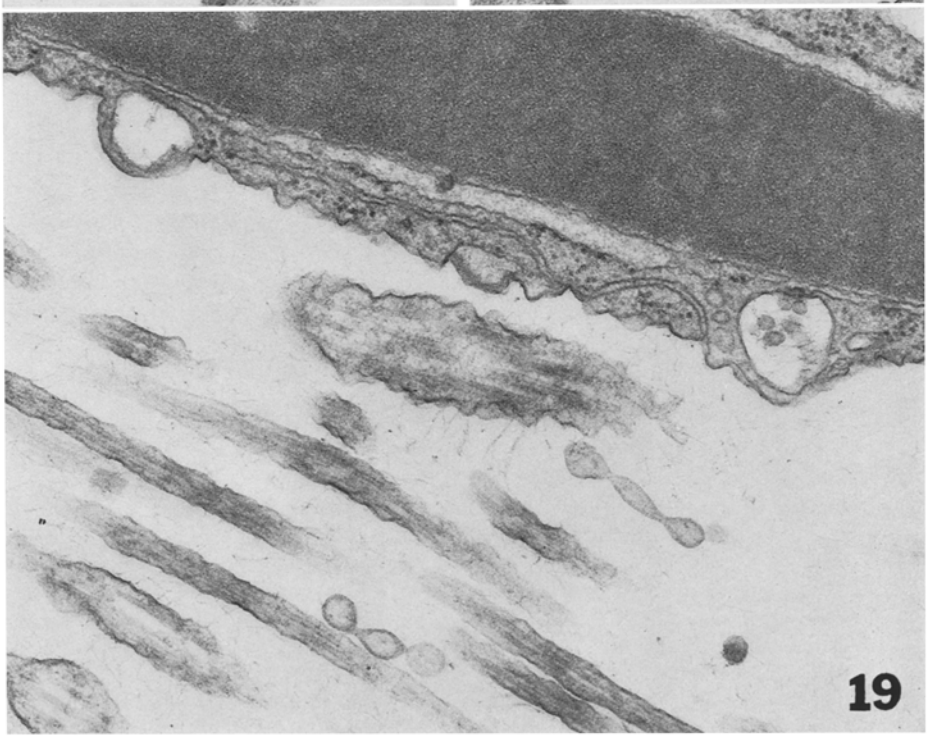
Fig. 14. Transverse section of the mitochondrial region depicting the four, reniform mitochondria. Lead citrate. × 54900

Fig. 15. Longitudinal section of the mitochondrial region showing centrioles capped by semilunar densities (*SD*). A centriolar satellite (*S*) is also labelled. Uranyl acetate-lead citrate. × 57700

Fig. 16. Transverse section of the distal centriole and pericentriolar cytoplasm showing the 9 triplet+1 doublet structure of the centriole. Supernumerary microtubules are indicated by the arrowheads. Lead citrate. × 100000







Figs. 17—19

extend radially to the plasmalemma. Even though it is likely that a satellite exists for each of the nine sets of triplets a maximum number of five satellites was observed in a single cross section. These observations are contrary to the findings of Hanisch (1966) who reported that no satellites are present after spermiogenesis. The satellites are considerably shorter than those observed in other species and are not in intimate contact with mitochondrial membranes. The satellites stain more densely with uranyl acetate than with lead citrate.

Tail Region. The flagellum of the mature *Eudendrium* spermatozoon consists of a proximal thick portion 8–9 μ in length and 200–250 mμ in diameter and a distal thin segment which is 6 μ–7 μ in length and tapers from 200 mμ–60 mμ in diameter. The transformation between thick and thin segments is rather abrupt (Fig. 3).

The flagella are unusual from an ultrastructural standpoint. Cross sections of the thick segments contain the 9+2 arrangement of microtubules and quite often from 1 to 3 supernumerary tubules which lie peripheral to the alpha doublets (Fig. 12). These additional microtubules are most commonly located between the plasmalemma and the number 7 or 8 alpha doublets although they are not confined to these locations. It is likely that the supernumerary flagellar microtubules are the more distal extensions of those observed within the cytoplasm surrounding the distal centriole. Many of the microtubules of the thick segment end abruptly and where they terminate, the thick segment becomes transformed into the thin segment. This region of transition has been observed in longitudinal section (Fig. 20).

The thin segments contain from one to eleven microtubules as seen in cross section (Fig. 21). It is evident that the central doublet of tubules does not extend intact to the end of the flagellum.

Discussion

The spermatozoon of *Eudendrium* bears little resemblance to the spermatozoa of *Hydra*, *Pennaria*, *Campanularia*, *Tubularia* and *Nausithoë* when viewed with the light microscope. The *Eudendrium* sperm head is asymmetrical and fusiform in shape, rather than conical, and is considerably longer than that of other coelenterate spermatozoa. The tails of *Eudendrium* spermatozoa are only 15 μ in length and consist of two distinct segments.

The electron microscope reveals additional differences which are not apparent at the light microscopic level. The fine structural morphology of the *Eudendrium*

Fig. 17. Longitudinal section of the mitochondrial region. Supernumerary microtubules are located in the pericentriolar cytoplasm and in the flagellum (arrowheads). Lead citrate. × 59400

Fig. 18. Transverse section of the distal centriole showing centriolar satellites (*S*). Lead citrate. × 68600

Fig. 19. Longitudinal section of several flagella demonstrating the filamentous coating which covers the flagellar membrane. Similar filaments may be seen within the adjacent multivesicular body. Also note the longitudinal sections of thin segments of flagella. A pair of tubules can be observed within each. Uranyl acetate-lead citrate. × 48200

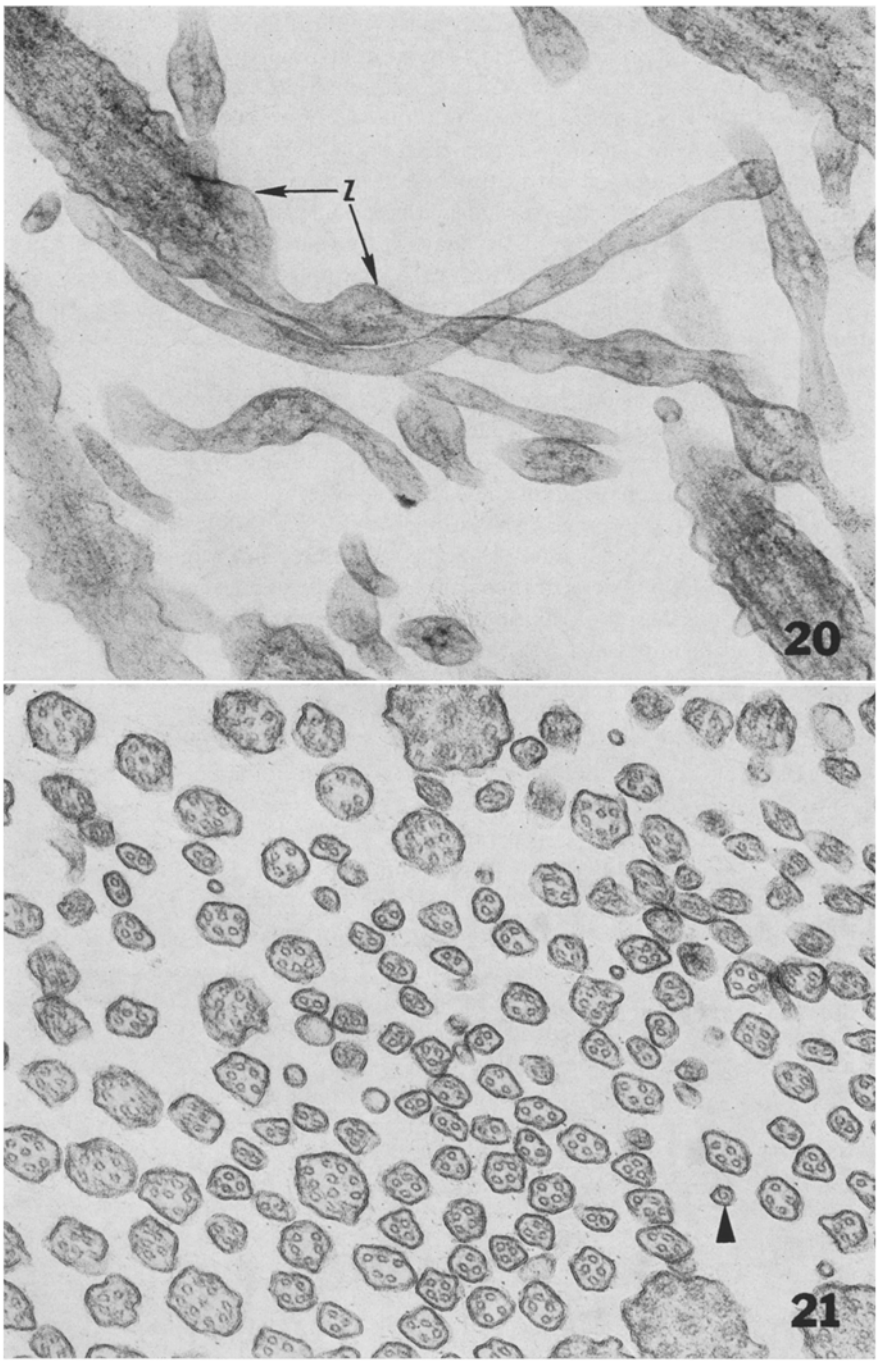


Fig. 20. Longitudinal section of a flagellum which shows the zone of transition between thick and thin segments (*Z*). Lead citrate. × 39600

Fig. 21. Thin segments of flagella in transverse section. A thin segment which contains one tubule is indicated by the arrowhead (lower right). Lead citrate. × 57500

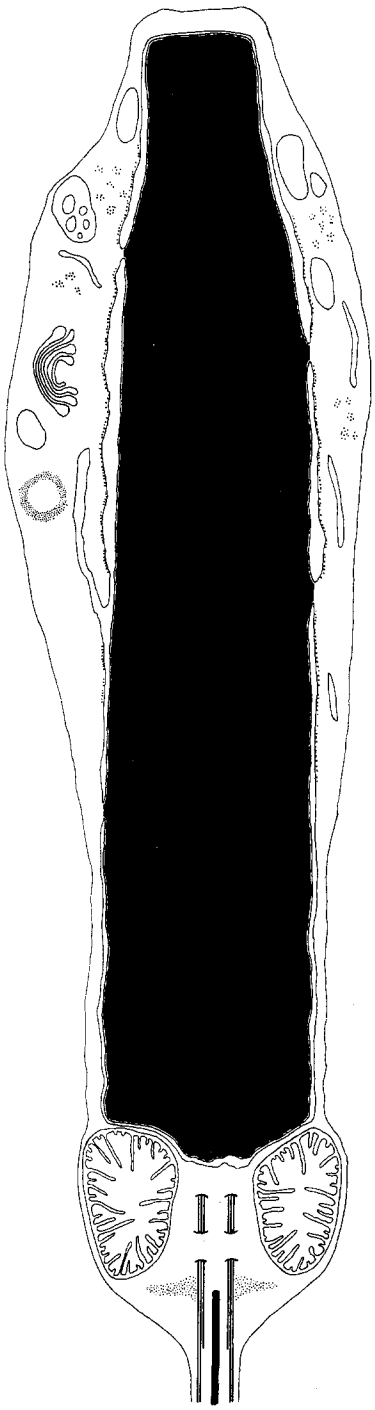


Fig. 22. Schematic representation of the composite ultrastructural features of the spermatozoon of *Eudendrium*

spermatozoon (summarized in Fig. 22) will be compared to that of the previously described coelenterate spermatozoa on a regional basis.

Acrosomal Region. The spermatozoa of coelenterates, with the exceptions of *Hydra* and *Eudendrium* are characterized by the presence of a number (from 2–40) of anteriorly placed vesicles which lie between the anterior pole of the nucleus and the plasmalemma. These vesicles have been compared to the proacrosomal vesicles of other species (Summers, 1970b; Lunger, 1971; Afzelius and Franzen, 1971; Afzelius, 1971). Although this aggregate of vesicles bears little resemblance to the acrosomal complexes of vertebrate and other invertebrate species (see reviews by Dan, 1967; Franklin, 1970), there is some preliminary evidence to suggest that such vesicles may function as an acrosome during fertilization in *Campanularia* (O'Rand, 1971).

The ovum of *Eudendrium* is completely contained within the tightly adherent ectodermal epithelium of the female gonophore. The ovum is fertilized and develops to the planula stage within the gonophore (Hyman, 1940; Mergner, 1957). Although the mode of fertilization in *Eudendrium* was unknown to earlier investigators (Hargitt, 1918), recent observations indicate that fertilization occurs through a micropyle in the epithelium of the gonophore. Several investigators (Dan, 1967; Colwin and Colwin, 1967; Austin, 1968; Nakano, 1969) suggest that the acrosomeless condition in teleost sperm is somehow related to the presence of a micropyle in the chorion of the teleost ovum. Longo and Anderson (1970) present evidence in other species to indicate that acrosome-bearing sperm may also fertilize their respective ova through a micropyle. In any case, the spermatozoon of *Eudendrium* is acrosomeless and this may be related to the presence of a micropyle on the surface of the ovum. The reported absence of an acrosome in *Hydra* sperm (Schincariol *et al.*, 1967; Weismann *et al.*, 1969; Stagni and Lucci, 1970a, 1970b) may also be related to the presence of an indentation in the chorion at the site where sperm contact is made (A. L. Burnett, unpublished observations).

Nuclear Region. Most vertebrate and invertebrate spermatozoa (including most of the coelenterate spermatozoa) are devoid of cytoplasmic elements except for those functionally concerned with motility and egg entry. Excess cytoplasm is generally shed during the final stages of spermiogenesis as a residual cytoplasmic droplet. However, mature *Eudendrium* spermatozoa are unusual in that a large amount of perinuclear cytoplasm is retained. The persistence of this cytoplasm and the variety of organelles within it (rough endoplasmic reticulum, ribosomes, Golgi apparatus, lipid-like droplets and nuclear pores) are characteristic of a metabolically active cell which suggests that this spermatozoon may endure a long period of free life. In addition, the terminal swellings of the gonophore lack a gastrodermal core and it is likely that stored products (eg. lipid droplets) are necessary for the subsistence of spermatids during their maturation. An alternative explanation of the presence of cytoplasm in these spermatozoa can be drawn from the observations of Lunger (1971) who has demonstrated that a substantial amount of cytoplasm persists in the released spermatozoa of *Campanularia*. It is possible that final maturation of these sperm (involving the loss of residual cytoplasm) occurs after they have reached their destination.

The conical type of nucleus which is evident in *Hydra*, *Campanularia*, *Nausithoë* and *Tubularia* sperm is also characteristic of the spermatozoa of many other in-

vertebrates (eg. Afzelius, 1955; Colwin and Colwin, 1961; Pasteels and de Harven, 1962). The conical configuration evidently arises during nuclear morphogenesis without the presence of a microtubular manchette (Longo and Anderson, 1969; Weismann *et al.*, 1969). However, Dan (1970) suggests that a microtubular manchette or network is generally involved in the formation of sperm with elongate nuclei such as that reported here for *Eudendrium* (eg. the earthworm, Anderson, Weismann and Ellis, 1967; the chicken, McIntosh and Porter, 1967). Such a cytoskeleton does not appear during formation of the elongate nucleus in *Eudendrium* sperm (Hanisch, 1966; 1970). This lends support to the recent conclusion of Fawcett, Anderson and Phillips (1971) that the microtubular manchette is not necessary for the morphogenesis of an elongate sperm nucleus.

The nuclei of many vertebrate and invertebrate sperm (including the coelenterates) contain vacuoles. Nuclear vacuoles are consistently unbounded by membranes and have been termed accidents in chromatin condensation by Fawcett (1958). The absence of such vacuoles in *Eudendrium* sperm suggests basic differences in the process of chromatin condensation.

Mitochondrial Region. In many of the invertebrate spermatozoa, the mitochondrial regions are characterized by the presence of four mitochondria, two or more centrioles and their associated satellite structures (Galtsoff and Philpott, 1960; Colwin and Colwin, 1961; Pasteels and de Harven, 1962; Summers, 1970b). Although the spermatozoan mitochondria of *Eudendrium* are wedge-shaped and those of other hydroids spherical, all 5 species display a radial arrangement of the mitochondria.

In all coelenterate sperm, at least two centrioles lie within the fossa which is bounded by the four mitochondria. The centrioles are in-line with one another (and with the axis of the tail) in *Eudendrium* and *Tubularia* (Afzelius, 1971). In *Pennaria* (Summers, 1970b), *Hydra* (Burnett, Davis and Ruffing, 1966), *Campanularia* (Lunger, 1971) and *Nausithoë* (Afzelius and Franzén, 1971) the proximal centriole lies at an angle of 45°–90° to the distal centriole (and the axis of the tail). The significance of these differences in centriolar alignment is presently unknown. The distal centriole of the *Eudendrium* sperm is somewhat unusual in that it contains a central pair of tubules. These are most likely in continuity with the beta pair of the flagellum. The centriolar satellites which are observed in the mature spermatozoa of *Eudendrium* were not noted by Hanisch (1966). This is due to the fact that satellites are not readily discernible when osmic acid is utilized as a primary fixative (see Summers, 1970b). Centriolar satellites have been identified in the spermatozoa of 26 species of vertebrates and invertebrates and the structure and significance of this interesting complex is presented elsewhere in detail (Summers, 1972).

Tail Region. The spermatozoa of all coelenterates thus far described possess a typical flagellum containing the 9+2 arrangement of fibrils. However, the flagellum of the *Eudendrium* sperm deviates significantly from the 9+2 pattern. The flagellum originates at the distal centriole as a 9+2 flagellum plus a variable number of peripheral, supernumerary microtubules. Tubules are eliminated from the axoneme in a distal direction. It is possible that this deletion proceeds in an orderly or sequential manner although such a sequence could not be determined with the methods employed in this study. It is unlikely that the supernumerary

microtubules function as motile elements of the sperm tail; it is more probable that they serve as skeletal elements which impart rigidity to the tail region. It should be noted that the two-tubule configuration is very frequently observed in transverse sections of flagellar bundles. Thus it is most likely that the "thin segment" of the flagellum contains only two microtubules.

Phylogenetic Considerations. Spermatozoa have been found to be widely variant in structure. This was obvious even in the light microscopic studies in the early part of this century as is seen in Wilson (1925). It has been difficult to relate the structure of spermatozoa to taxonomic position for, as was stated by Hadzi (1963), "The form, the structure and spermatogenesis are so widely different not only when we compare animal groups but also within the frame of one and the same systematic unit; we are therefore unable to establish on this basis a clear idea of the phylogeny of animals . . ." Fawcett (1970) has also suggested that ". . . the morphology of sperm correlates better with the nature of the environment in which fertilization takes place than it does with systematic characters or phylogenetic rank."

Nevertheless a general plan for the morphology of spermatozoa of marine invertebrates with external fertilization has emerged from a multitude of light and electron microscopic studies (see review by Franklin, 1970). The spermatozoa of coelenterates generally correspond to this pattern. However, the spermatozoon of *Eudendrium* represents a significant variation. Accordingly one would expect to find variations in sexual life history and mode of fertilization in *Eudendrium* to account for the variations observed in the morphology of the spermatozoon. Indeed, such differences are present. The reproductive individuals of *Eudendrium* are hydranth derived styloid gonophores, rather than the medusoid type gonophores which characterize most other genera of hydroids. The sperm of *Eudendrium* are released passively as contrasted with the active muscular expulsion of sperm by gonomedusae. Finally, the spermatozoa of *Eudendrium* gain access to the ovum through a micropyle in the ectoderm which surrounds it.

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