BULLETIN OF MARINE SCIENCE, 48(2): 296–307, 1991

ULTRASTRUCTURE OF CYTOPHORES IN SPERMATID MORULAE OF TWO *MICROPHTHALMUS* SPECIES (POLYCHAETA: HESIONIDAE)

Wilfried Westheide

ABSTRACT

Anuclear central cytoplasmic masses (=cytophores) in spermatid morulae of the two interstitial polychaetes *Microphthalmus carolinensis* and *M. listensis* attain their largest dimensions and greatest cytoplasmic variety during the process of nucleus shaping. Abundantly occurring organelles like "clouds" of mitochondria, extended ER cisternae, large vesicles and several structures of unknown nature, imply high biosynthetic activity, thus contradicting the view that the cytophores' most important function is synchronization of gamete development and uptake of superflous spermatid cytoplasm. The cytophores are highly species-specific, regarding structure and arrangement of organelles.

Anuclear masses of cytoplasm in the center of plasmodial clones of gametogenic stages are called cytophores (an older, less common, term is blastophore). Their cytological structure is poorly documented and their functional significance is scarcely considered. There are recent reviews on spermiogenesis that do not even mention the term, although cytophores are widely distributed in invertebrates. They especially occur during the development of male gametes in Platyhelminthes, some Nematoda, Bryozoa, Echiura (?), Pogonophora, Annelida, and rarely in Crustacea (Roosen-Runge, 1977; Jones and Gardiner, 1985; Gardiner and Jones, 1985; Jamieson, 1981; Sawada, 1984). In the Annelida, cytogenesis of sperm cells is well documented by TEM investigations (for references see Franzén and Rice, 1988; Jamieson, 1981) but only a few investigations refer to the ultrastructure of cytophores (for references see Jamieson, 1983; Sawada, 1984).

The most common functional aspect suggested is that of synchronizaton of male gamete development. Elimination of superfluous cytoplasm including various organelles is considered to be another important function of the cytophore (Stang-Voss, 1972; Kubo and Sawada, 1977; Troyer and Cameron, 1980). Martinucci et al. (1977) additionally assumed energy production inside the cytophore and synthesis of morphogenetic materials related to sperm differentiation. The present preliminary ultrastructural data of two *Microphthalmus* species support the assumption of the cytophores' important synthetic role during the late stages of spermiogenesis.

MATERIALS AND METHODS

The micrographs in this contribution were taken in the course of investigations on the reproductive organs of the interstitial meiofauna genus *Microphthalmus* (Westheide 1978, 1979). *Microphthalmus carolinensis* Westheide and Rieger, 1987, was collected on exposed coarse sand beaches of North Carolina. *M. listensis* Westheide, 1967, was found on a sand flat of the North Sea island Sylt. A series of specimens of both species was fixed after $MgCl_2$ narcotization in sucrose-picric acid-formaldehyde-glutaraldehyde with postfixation in osmium tetroxide (for details see Westheide and Rieger, 1987). Epon-araldite embedded specimens were sectioned with diamond knives and collected on bare grids. Examination occurred with a Zeiss EM 9S-2 (D. P. Costello Electron Microscope Laboratory, University of North Carolina, Chapel Hill), a Zeiss EM 10 (II. Zool. Inst., University of Göttingen) or a Zeiss EM 109 (Fachbereich Biologie/Chemie, University of Osnabrück).

RESULTS

Development of male germ cells of *M. carolinensis* and *M. listensis* takes place in the anterior part of the body (Westheide, 1967; Westheide and Rieger, 1987)

as in all *Microphthalmus* species. Germ cell clusters are embedded between the coelenchyme cells that fill the body cavity (Smith et al., 1986) and are progressively pushed aside in the course of spermatogenesis. The number of these clusters is relatively low in the few male segments of these small animals. Different stages of development from spermatogonia to bundles of completely differentiated spermatozoa occur at the same time, thus continuously providing fresh gametes for direct transmission of sperm. Individuals of the two species probably possess spermatogenic stages, sperm bundles and operating male copulatory organs over a period of several months.

Within the clusters of primary spermatocytes in *M. carolinensis* the cells are connected by prominent cytoplasmic bridges, but do not surround any pronounced cytophore-like cytoplasmic masses (Fig. 1A). The bridges have a width of circa 0.8 μm. They are characterized by scattered electron-dense particles and their plasma membrane is uniformly thickened by electron-dense material on the inner surface.

The first cytophores are found in the center of morulae of early spherical spermatids (Fig. 1B). They have an approximate diameter of 5 μm or more. Their cytoplasm is lightly granular and almost homogeneous with one or a few large non-membrane-bounded bodies and a few widely scattered mitochondria (Fig. 1B). In a series of 590 ultrathin sections through a morula of early spermatids, 507 nuclei were counted, suggesting eight spermatogonial generations (Rosen-Runge, 1977, 136).

During the process of nuclear shaping and elongation, spermatids are concentrated in one or two areas of the cytoplasmic mass. From here they grow out like tufts of hair on a skull. Whereas in the TEM-sections these cytophores may be of irregular, elongate shape, they usually appear spherical when they are squeezed out of the body of living animals. The volume of these late cytophores has increased considerably, reaching a diameter of about 25 μm. Their cytological structure has changed totally.

The anterior ends of the individual spermatids are intimately embedded into the marginal parts of the cytophore and densely packed in one or several rows (Figs. 2, 3, 4A). There is a more or less extended and somewhat subapical open area along the side of the spermatids that directly connects their cytoplasm with the cytophore without forming an exposed cytoplasmic bridge (Fig. 3A). Near this connection site a well developed active Golgi apparatus is situated in each of the spermatids (Figs. 2, 3B, 4B), which probably produces a prominent (diameter about 0.8 μm) non-membrane bounded (?) vesicle of homogeneous electron-transparent or moderately opaque appearance. It is suggested to be discharged into the cytophorous cytoplasm, where a similar structure can be found as an electron-dense body with homogeneous content.

Larger areas of the cytoplasm contain numerous narrow ER cisternae of more or less smooth character. They are usually arranged in large parallel stacks (Fig. 4A) or in concentric whorls (Fig. 2). The population of egg-shaped or almost spherical mitochondria (diameter about 400 nm) has dramatically increased. They are not evenly distributed in the cytophore but form dense aggregations with up to 8 per μm^2. Several of them appear inflated (Fig. 2) and may be in a process of degradation.

Golgi complexes were scarce inside the cytophore. One large apparatus (Fig. 3B) is closely situated to an irregular network of dark fibrous material. The nature of these fibroreticular bodies, some of which are surrounded by ER, is completely enigmatic (Figs. 2, 3B). Vacuoles with irregularly structured content may be autophagolysosomal compartments (Figs. 2A, 4D). Additional structures which have

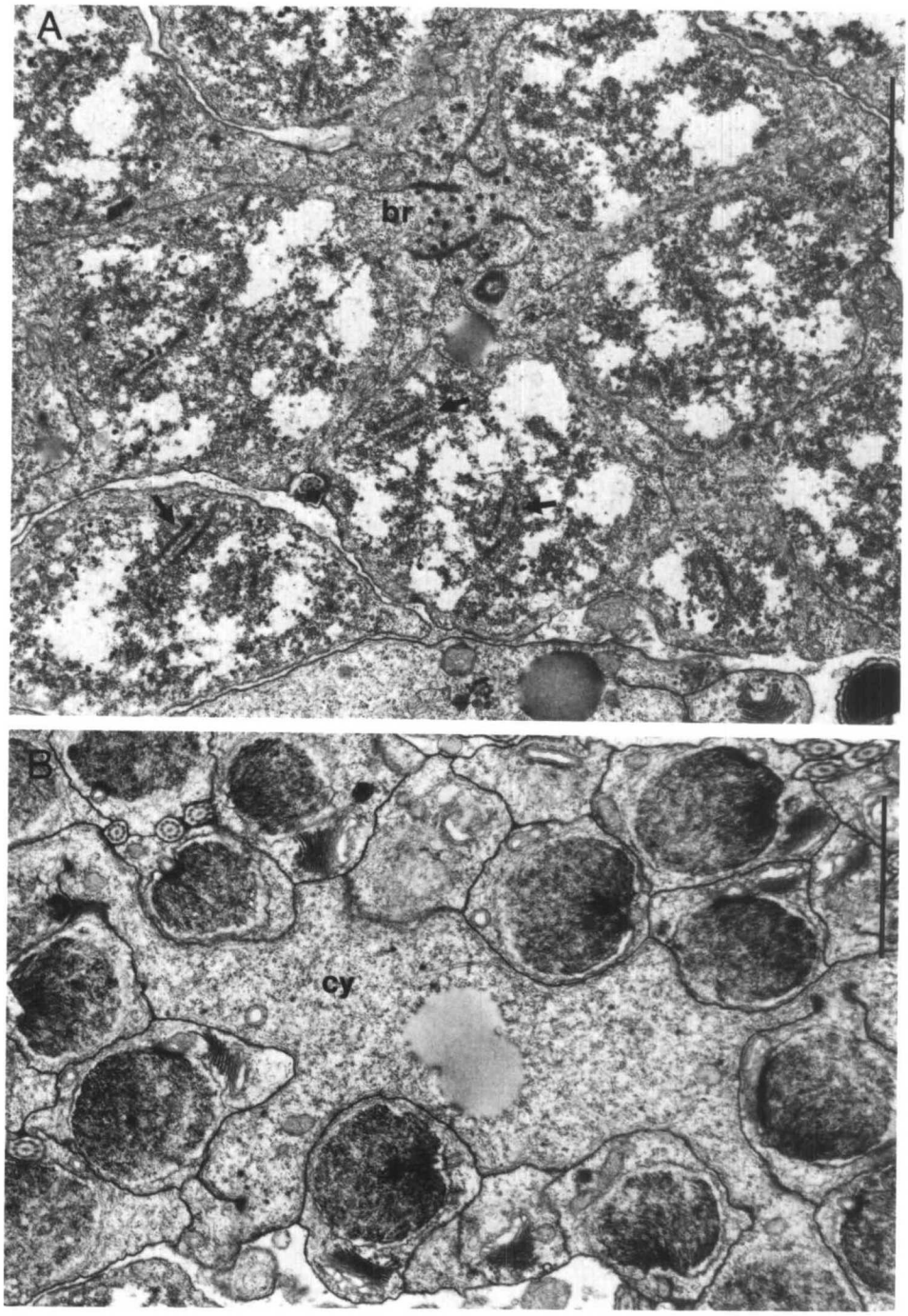

Figure 1. *Microphthalmus carolinensis.* A. Cluster of primary spermatocytes connected by cytoplasmic bridges (br). Arrows indicate synaptonemal complexes. B. Morula of early spermatids. cy = cytophore. Bars 2 μm.

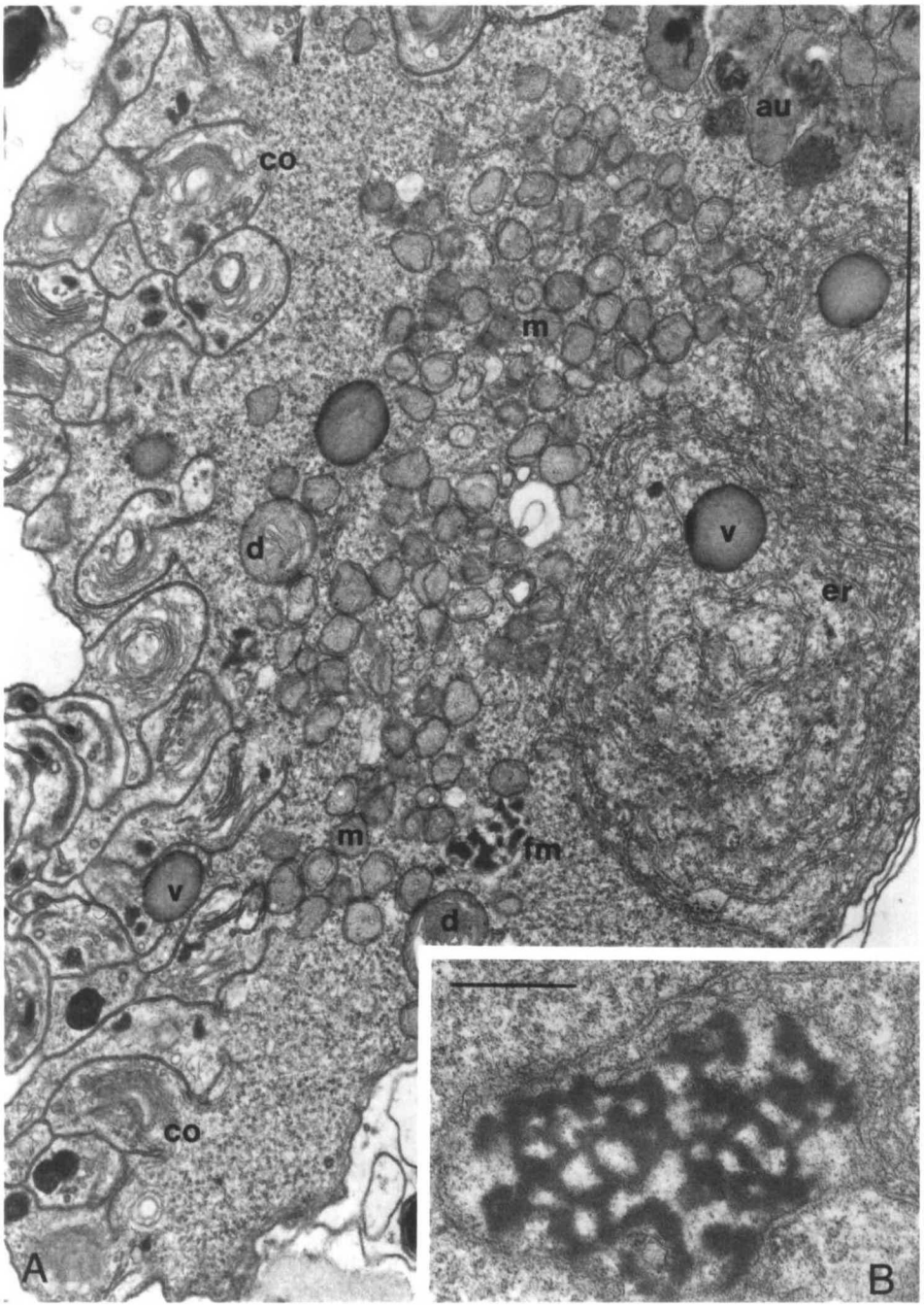

Figure 2. *Microphthalmus carolinensis.* A. Cytophore with "cloud" of mitochondria (m) and whorl of endoplasmic reticulum (er). Anterior parts of spermatids intimately embedded at one side; with extended cytoplasmic connections (co). d = inflated, degenerating (?) mitochondrion; fm = fibroreticular material; v = vesicle and its supposed pathway from formation site in the anterior part of spermatids (left side) to center of cytophore; au = putative autophagolysosomal compartments. B. Fibroreticular material surrounded by ER cisternae. Bar in A 2 μm, in B 0.5 μm.

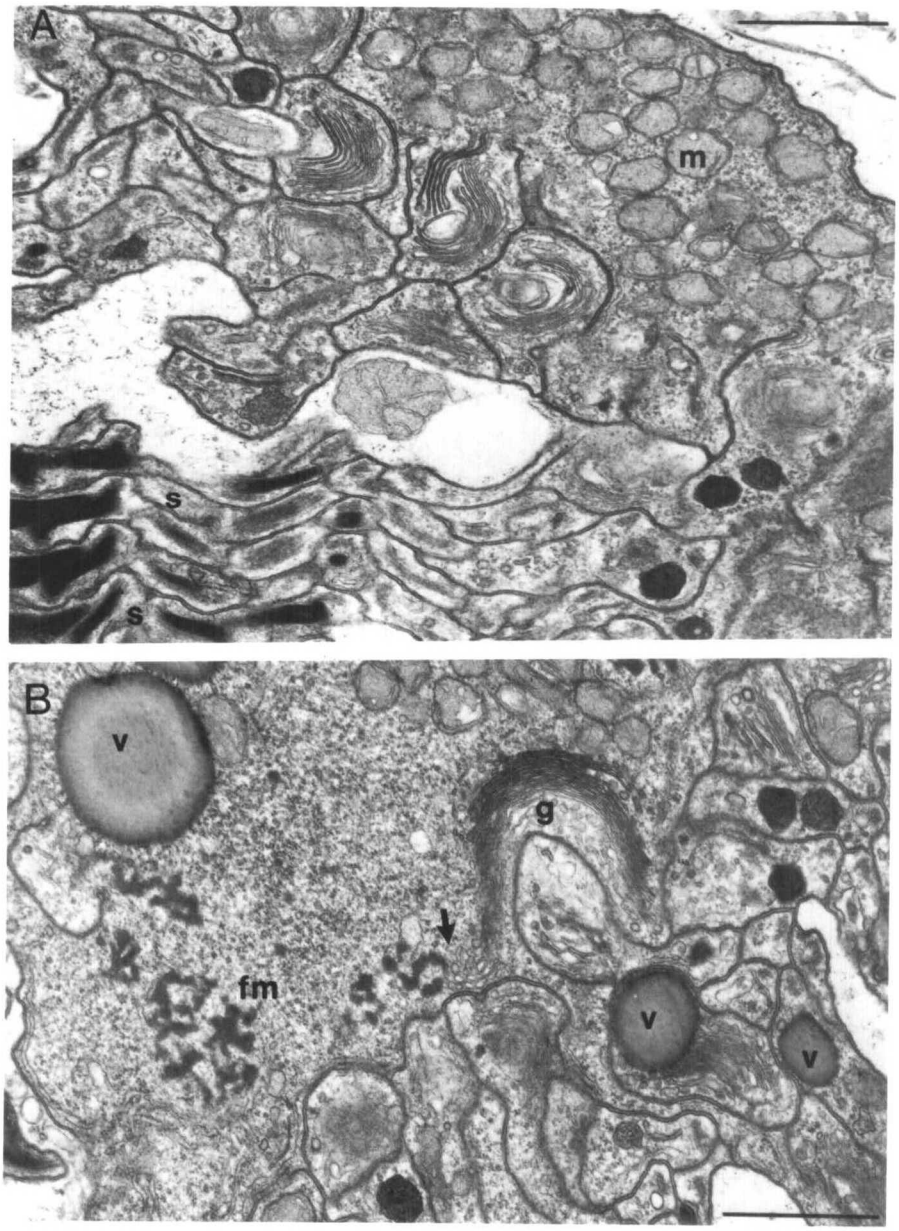

Figure 3. *Microphthalmus carolinensis*. A. Part of cytophore with "cloud" of mitochondria (m) and detaching spermatozoa (s). B. Part of cytophore with condensing vesicle (v) in the anterior part of spermatids (right) and within the cytophorous cytoplasm (left). Fibroreticular material (fm) in close association to Golgi complex (g) (arrow). Bars 1 μm.

been found in the *M. carolinensis* cytophore are extended fields of small regular vesicles (Fig. 4C).

Residual bodies occur around the spermatogenic morulae (Fig. 5). Their contents are completely homogeneous except for various myelinated structures. They may be the residues of degenerated cytophores after the spermatozoa detached.

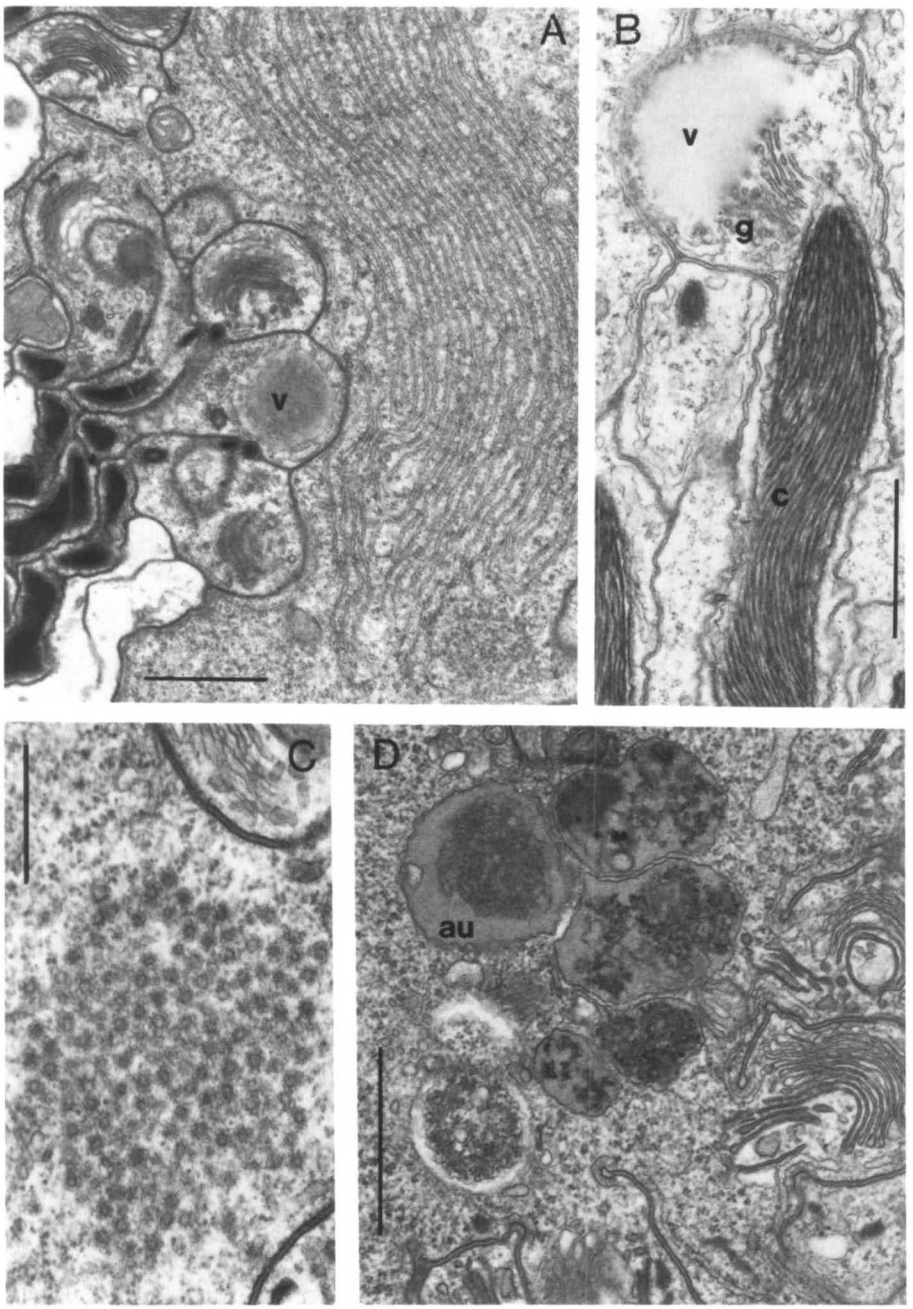

Figure 4. *Microphthalmus carolinensis.* A. Parallel arrays of endoplasmic reticulum in cytophorous cytoplasm. B. Anterior part of spermatid with strands of chromatin (c), Golgi apparatus and vesicle (v) in statu nascendi. C. Field of small vesicles in cytophorous cytoplasm. D. Concentration of putative autophagolysosomal compartments (au) in cytophorous cytoplasm. Bars in A, B, D 1 μm, in C 0.5 μm.

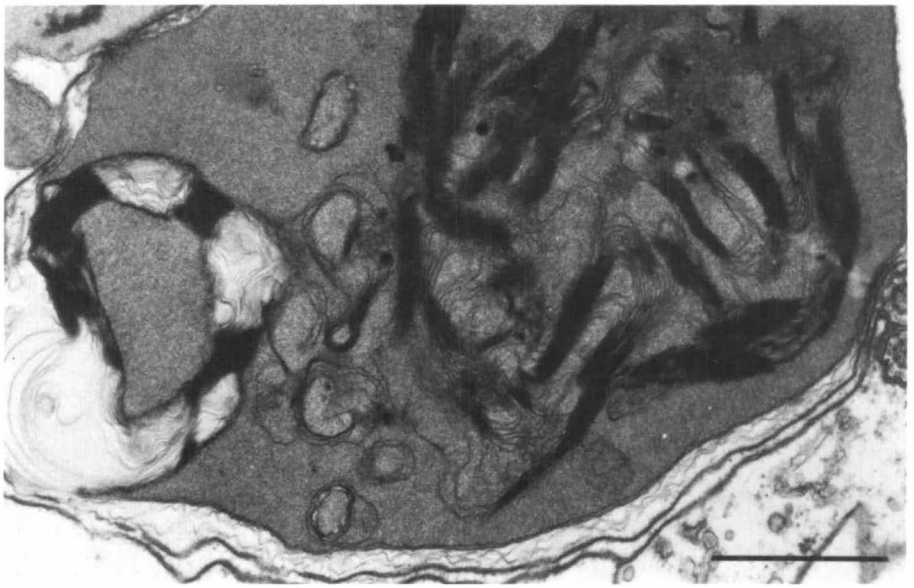

Figure 5. *Microphthalmus carolinensis.* Residual body of cytophore. Bar 1 μm.

Cytophores in the spherical morulae of elongating spermatids of *M. listensis* have a diameter of about 20 μm. Their cytological structure is distinctly different from the *M. carolinensis* cytophore (Fig. 6). A ribosome-rich cytoplasm is present throughout the cytophore and interspersed with an irregular system of narrow sER cisternae. Numerous small elongated mitochondria (diameter 200 nm) are scattered throughout the cytophore. Besides a variety of different vesicles including putative autophagosomes, large vesicles (lipid droplets?) with a diameter of about 1.3 μm represent the most obvious structures. Their content appears to be extracted by fixation except for a grey margin. Some of them are grouped closely together or are fused. The production site of these vesicles is the anterior end of the spermatids where they are found close to an active Golgi complex.

Small dark particles are considered to be glycogen. They occur individually, in smaller groups or in large conspicuous clusters. Many of them intimately surround the large vesicles (Figs. 6, 7). Individual particles or small groups are also found within the spermatids (Fig. 7A).

Within profiles of the ER, one can find peculiar structures consisting of central dark granular material regularly surrounded by smaller granular particles (Fig. 6, inset).

Discussion

Despite general congruence of the cytophore structure in the two species investigated, details are so different that species identification and determination by means of a single micrograph of a cytophore can be easily done. This also holds true when cytophores of other species, e.g., *M. arenarius,* are considered. These distinct differences were unexpected especially because *M. listensis* and *M. carolinensis* are eidonomically very similar, but they corroborate results of earlier ultrastructural investigations on photoreceptors (Pietsch and Westheide, 1985), sperm, genital glands (Westheide and Rieger, 1987), and chaetae (Specht and Westheide, 1988) in the *Microphthalmus-listensis*-group.

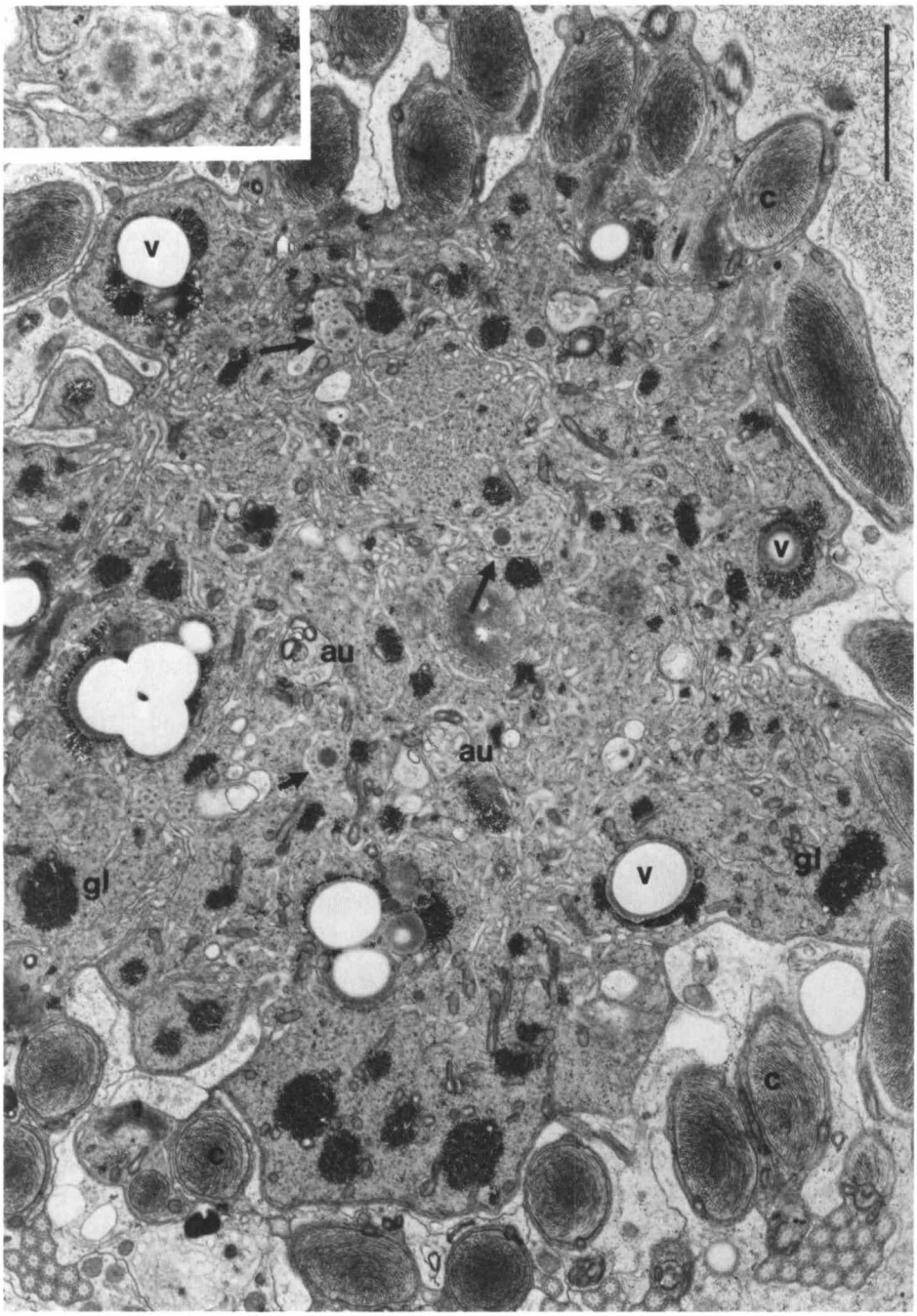

Figure 6. *Microphthalmus listensis*. Morula of elongating spermatids (c) with central cytophore. Note single and concentrated particles of putative glycogen (gl), large vesicles (v), numerous mitochondria, and extended system of sER cisternae. Arrows point to dark granular material regularly surrounded by smaller granular particles (see also inset). Bar 3 μm.

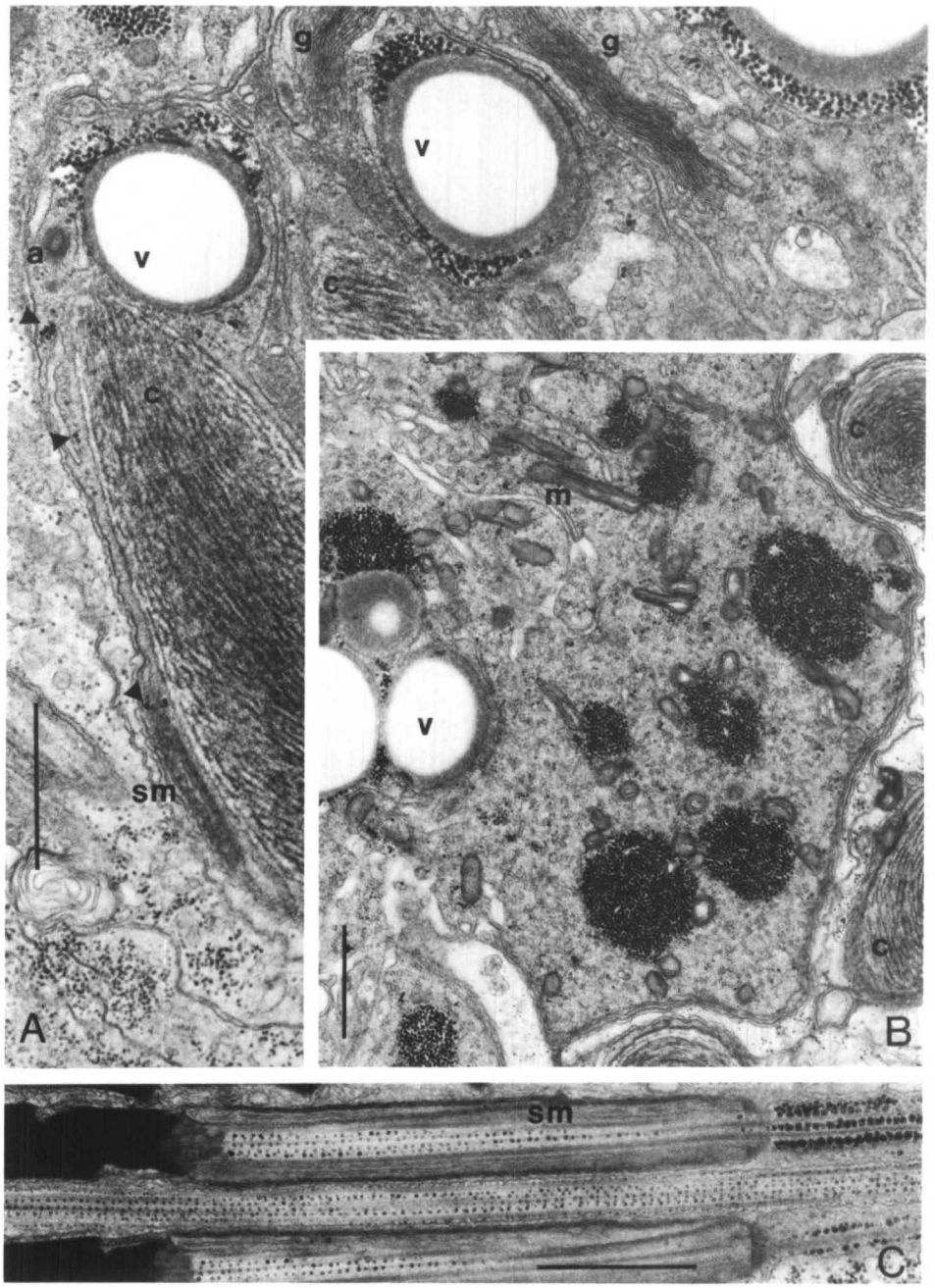

Figure 7. *Microphthalmus listensis.* A. Anterior part of elongating spermatids with chromatin strands of nuclei (c), Golgi apparatus (g) and condensing vesicles (v). Glycogen (?) particles around vesicle and scattered in the peripheral cytoplasm of spermatids (arrow heads). B. Marginal area of cytophore with clusters of glycogen (?) particles, mitochondria (m), large vesicles (v) and sER cisternae. C. Mature spermatozoa. Middle piece between nucleus (left side) and tail region. Note electron-dense glycogen (?) particles around the axoneme. a = acrosome; sm = mitochondria of spermatids or spermatozoa. Bars 1 μm.

An extensive cytophore is not present before spermatids arise out of the meiotic divisions. The cytophore attains its largest dimension and greatest cytoplasmic structural variety during the process of nuclear shaping. It is characterised by organelles involved in biosynthesis, which abundantly occur throughout its entire cytoplasm. It is likely that the majority of these organelles did not pass from the spermatid cytoplasm into the cytophore. This holds true for the mitochondria, the genesis of most of them must have taken place within the cytophore.

These facts clearly suggest that in the *Microphthalmus* species the cytophore's most important function is synthesis. Several of the structures are very similar to what have been described for the biosynthetically very active cytoplasm of vitellogenic oocytes (Eckelbarger, 1984a), e.g., the stacks and whorls of ER, the mitochondrial "clouds," the fibroreticular material in *M. carolinensis* that somewhat resembles the fibrogranular nuage. Martinucci et al. (1977) suggest that the materials synthesized in cytophores of lumbricids migrate into the elongating sperm cells and are involved in their morphogenesis or represent energy-rich substances. Indeed, in the cytophore of *M. listensis* there are prominent concentrations of dark particles assumed to be glycogen, which may pass into the spermatids and later on fill up the tail region of the spermatozoa. Unfortunately, such particle concentrations have not been observed so far in the cytophores of *M. carolinensis,* although sperm tails in this species contain similar particles (Westheide and Rieger, 1987, fig. 8). It cannot be excluded that materials produced are also exported from the plasmodial complex and are involved in processes other than spermiogenesis. The micrographs available do not show structural indications for such exocytotic processes, but in *M. listensis* large vesicles similar to those in the cytophore occur in the coelenchymal cells around the spermatid morulae (Fig. 6). Other supposed functions such as intake of superfluous cytoplasm including organelles of the spermatids and synchronization of germ cell development (Stang-Voss, 1972; Troyer and Cameron, 1980; Sawada, 1984) are assumed to be directly or indirectly connected with formation and existence of a cytophore but do not represent its sole function. This also holds true for the supposed ability of the cytophore to self-destruct and to form a residual body after the spermatozoa have detached (Martinucci et al., 1977; Troyer and Cameron, 1980).

The idea of a synthetic function of the cytophorous organelles not mainly involved in the differentiation of spermatids is supported by the fact that, in a series of polychaete taxa spermiogenesis takes place without the occurrence of cytophores. Sawada (1984) listed 14 species from 7 families (Polynoidae, Nereidae, Glyceridae, Syllidae, Spionidae, Sabellidae, Serpulidae) in which cytophores do not exist. Also the dorvilleid taxa *Ophryotrocha* (Berutti et al., 1978) and *Apodotrocha progenerans* (own observation), and the sabellariid *Phragmatopoma lapidosa* (Eckelbarger, 1984b) do not possess cytophores.

The small hesionid, *Hesionides arenaria,* demonstrates that spermiogenesis can take place with (see *Microphthalmus*) and without cytophores within the same family. In this species spermatids develop in tetrads (Westheide, 1967; 1984), as, e.g., in *Harmothoë imbricata* (Daly, 1974), *Phragmatopoma lapidosa* (Eckelbarger, 1984b) and various nereid species (Hauenschild and Fischer, 19689; Sawada, 1984). The four *Hesionides* spermatids are connected by cytoplasmic bridges that become more extensive and finally lead to the formation of a common central mass in the course of sperm detachment. Except for vesicle fields at the level of the cytoplasmic connections (Westheide, 1984) this cytoplasm is homogeneous and does not possess organelles observed in the true cytophores of *Microphthalmus* (Westheide, 1984).

Rice (1981) found eight spermatids connected by a common cytoplasmic bridge in *Polydora* (Spionidae). Recently a type of spermiogenesis has been detected in *Pisione remota,* which in annelids shows similarities only to Myzostomida (Jägersten, 1939). The extremely aberrant aflagellate spermatozoa of this pisionid (Westheide, 1988) develop within a spermatocyst consisting of several cyst cells which envelop a cluster of about 20 spermatids (Westheide, in prep.).

For the time being it remains unexplained how spermatids in these species are able to develop without the supposed synthetic or nutritive activities of a cytophore. The adaptive significance of the different types of spermiogenesis also cannot yet be understood. There is little or no evidence for correlations between habitat, lebensform-type or body size and the different types of spermiogenesis. Spermatid tetrads, for instance, occur in meiofaunal as well as in macrofaunal species which live in a wide spectrum of different habitats. There is no correlation with reproductive biology. Species with spermatid tetrads as well as those with cytophores may display external fertilization and direct sperm transfer, respectively. No correlation can be seen with sperm types. For instance, both *Microphthalmus* and *Hesionides* have highly modified spermatozoa. It is possible, however, that spermatocyst formation in *Pisione remota* may be connected to the extremely aberrant aflagellate sperm.

Acknowledgments

Skillful technical assistance of M. Olomski, M. Biedermann and W. Mangerich is greatly acknowledged. Thanks are due to Prof. Dr. S. A. Rice, Tampa, for critically reading the manuscript. I am also grateful to Prof. R. M. Rieger for hospitality and providing laboratory facilities in the University of North Carolina, Chapel Hill.

Literature Cited

Berruti, G., M. Ferraguti and C. Lora Lamia Donin. 1978. The aflagellate spermatozoon of *Ophryotrocha*: a line of evolution of fertilization among polychaetes. Gamete Res. 1: 287–292.

Daly, J. M. 1974. Gametogenesis in *Harmothoë imbricata* (Polychaeta: Polynoidae). Mar. Biol. 25: 35–40.

Eckelbarger, K. J. 1984a. Comparative aspects of oogenesis in polychaetes. Pages 123–148 *in* A. Fischer and H.-D. Pfannenstiel, eds. Polychaete reproduction. Fortschr. Zool. 29.

———. 1984b. Ultrastructure of spermatogenesis in the reef-building polychaete *Phragmatopoma lapidosa* (Sabellariidae) with special reference to acrosome morphogenesis. J. Ultrastruct. Res. 89: 146–164.

Franzén, A. and S. A. Rice. 1988. Spermatogenesis, male gametes and gamete interactions. Pages 309–333 *in* W. Westheide and C. O. Hermans, eds. The ultrastructure of Polychaeta. Microfauna Marina 4.

Gardiner, S. L. and M. L. Jones. 1985. Ultrastructure of spermiogenesis in the vestimentiferan tube worm *Riftia pachyptila* (Pogonophora:Obturata). Trans. Am. Microsc. Soc. 104: 19–44.

Hauenschild, C. and A. Fischer. 1969. *Platynereis dumerilii.* Mikroskopische Anatomie, Fortpflanzung, Entwicklung. Großes Zool. Prakt. 10b. Gustav Fischer, Stuttgart. 55 pp.

Jägersten, G. 1939. Über die Morphologie und Physiologie des Geschlechtsapparates und den Kopulationsmechanismus der Myzostomiden. Zool. Bidrag Upps. 18: 165–242.

Jamieson, B. G. M. 1981. The ultrastructure of the Oligochaeta. Academic Press, London. 462 pp.

———. 1983. Spermiogenesis in the oligochaetoid polychaete *Questa* (Annelida, Questidae). Zool. Scr. 12: 179–186.

Jones, M. L. and S. L. Gardiner. 1985. Light and scanning electron microscopic studies of spermatogenesis in the vestimentiferan tube worm *Riftia pachyptila* (Pogonophora:Obturata). Trans. Am. Microsc. Soc. 104: 1–18.

Kubo, M. and N. Sawada. 1977. Electron microscopic study on sperm differentiation in *Perinereis brevicirris* (Polychaeta). Cell Struct. Funct. 2: 135–144.

Martinucci, G. B., B. Felluga and S. Carli. 1977. Development and degeneration of cytophorus during spermiohistogenesis in *Eisenia foetida* (Sav.). Boll. di Zool. 44: 383–398.

Pietsch, A. and W. Westheide. 1985. Ultrastructural investigations of presumed photoreceptors as a means of discrimination and identification of closely related species of the genus *Microphthalmus*. Zoomorphology 105: 265–276.

Rice, S. A. 1981. Spermatogenesis and spermultrastructure in three species of *Polydora* and *Streblospio benedicti* (Polychaeta: Spionidae). Zoomorphology 97: 1–16.

Roosen-Runge, E. C. 1977. The process of spermatogenesis in animals. Cambridge University Press, Cambridge. 214 pp.

Sawada, N. 1984. Electron microscopical studies on spermatogenesis in polychaetes. Pages 99–114 *in* A. Fischer and H.-D. Pfannenstiel, eds. Polychaete reproduction. Fortschr. Zool. 29.

Smith, P. R., J. Lombardi and R. M. Rieger. 1986. Ultrastructure of the body cavity lining in a secondary acoelomate, *Microphthalmus* cf. *listensis* Westheide (Polychaeta: Hesionidae). J. Morphol. 188: 257–271.

Specht, A. and W. Westheide. 1988. Intra- and interspecific ultrastructural character variation: the chaetation of the *Microphthalmus-listensis*-group (Polychaeta, Hesionidae). Zoomorphology 107: 371–376.

Stang-Voss, C. 1972. Ultrastrukturen der zellulären Autophagie. Elektronenmikroskopische Beobachtungen an Spermatiden von *Eisenia foetida* (Annelidae) während der cytoplasmatischen Reduktionsphase. Z. Zellforsch. 127: 580–590.

Troyer, D. and M. L. Cameron. 1980. Spermiogenesis in lumbricid earthworms revisited. I. Function and fate of centrioles, fusion of organelles and organelle movement. Biol. Cell. 37: 279–286.

Westheide, W. 1967. Monographie der Gattungen *Hesionides* Friedrich and *Microphthalmus* Mecznikow (Polychaeta). Ein Beitrag zur Organisation und Biologie psammobionter Polychaeten. Z. Morph. Tiere 61: 1–159.

———. 1978. Ultrastructure of the genital organs in interstitial polychaetes I. Structure, development and function of the copulatory stylets in *Microphthalmus* cf. *listensis*. Zoomorphologie 91: 101–118.

———. 1979. Unusual granules in the ejaculatory duct of a *Microphthalmus* species (Polychaeta, Annelida). Cell Tissue Res. 197: 262–270.

———. 1984. Genesis and structure of the modified spermatozoon in the interstitial polychaete *Hesionides arenaria* (Annelida). Biol. Cell 50: 53–66.

———. 1988. The ultrastructure of the spermatozoon in *Pisione remota* (Annelida: Polychaeta) and its transformation in the receptaculum seminis. J. Submicrosc. Cytol. Pathol. 20: 169–178.

——— and R. M. Rieger. 1987. Systematics of the *Microphthalmus-listensis*-species-group (Polychaeta: Hesionidae): facts and concepts for reconstruction of phylogeny and speciation. Z. Zool. Syst. Evolut.-forsch. 25: 12–39.

DATE ACCEPTED: March 20, 1990.

ADDRESS: *Spezielle Zoologie, Fachbereich Biologie/Chemie, Universität Osnabrück, Postfach 4469, D-4500 Osnabrück, FRG.*