ULTRASTRUCTURAL OBSERVATIONS ON THE SPERMATOGENESIS OF THE BRITTLE-STAR *AMPHIPHOLIS KOCHII* LÜTKEN (ECHINODERMATA: OPHIUROIDEA)

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With Text-figures 1–19

Introduction

In recent years, fine structural observations of the spermatogenesis of the echinoderms have been extensively made, and many attentions have been paid to the acrosome formation (Longo and Anderson, 1969; Dan and Shirakami, 1971; Atwood, 1974; Pladellorens and Subirana, 1975; Bickell *et al.*, 1980). All the foregoing observations on the acrosome structure agree in that the acrosome of the echinoderms is circular in shape. As to the beginning of the acrosome formation with an appearance of the proacrosomal vesicle, variation is found in the classes of the Echinodermata; in the holothuroid the proacrosomal vesicle is initially recognized in the spermatogonium (Atwood, 1974; Pladellorens and Subirana, 1975), in the crinoid it is in the spermatocyte (Bickell *et al.*, 1980), and in the echinoid and asteroid in the spermatid (Longo and Anderson, 1969; Dan and Shirakami, 1971). However, in the ophiuroid the beginning of the acrosome formation is unknown up to date.

The present paper deals with the ultrastructure of the male germ cells during spermatogenesis in the brittle-star *Amphipholis kochii* Lütken (Echinodermata: Ophiuroidea), with demonstrations that the acrosome formation is initiated in the spermatogonium and the resulted acrosome is not circular as usual but hat-shaped.

Materials and Methods

The brittle-star *Amphipholis kochii* used in the present study was collected at Abuta on the Pacific coast of south-western Hokkaido during the season from February to April. At each collection, about 10 male brittle-stars measuring more than 5 mm in disk diameter were captured. Our previous survey for the annual gonadal state of the present species has shown that all these animals are sexually mature (Iwata and Yamashita, 1982).

For transmission electron microscopic observations, the testes were prefixed with 5% glutaraldehyde in 75% sea water for 1 hour, and after several washings in 150% sea water, they were postfixed with 1% OsO_4 in 75% sea water for 30 min. The

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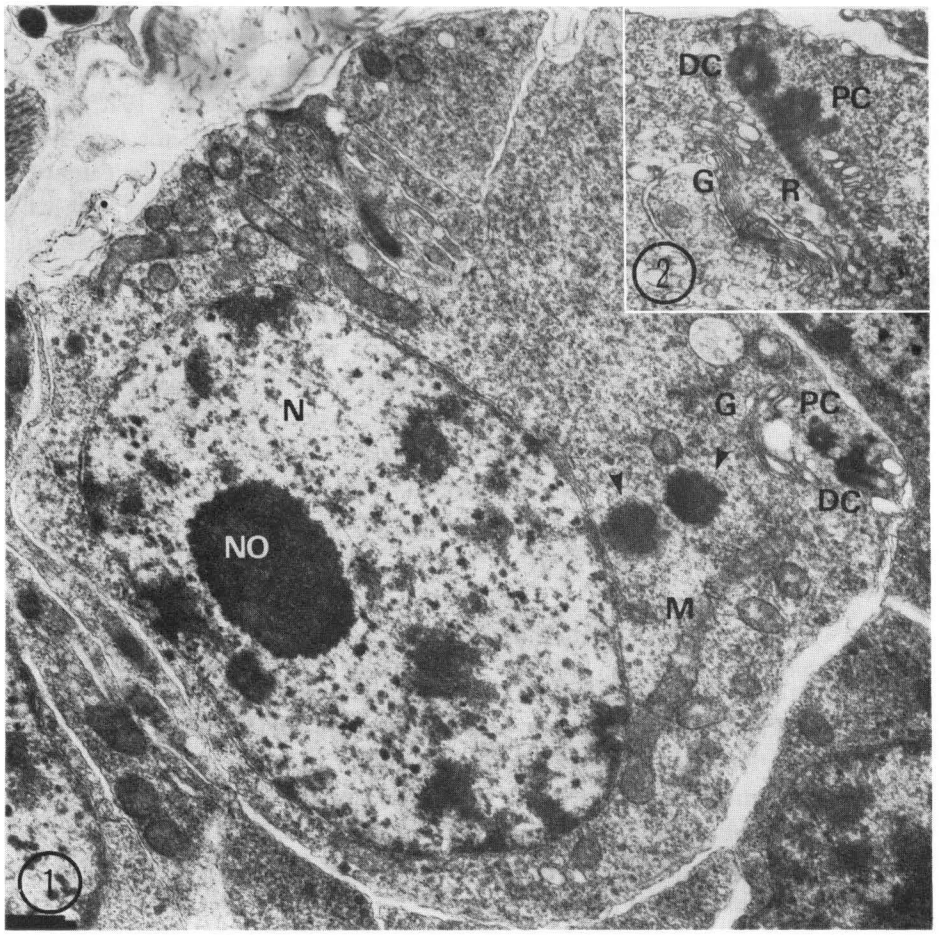
fixed testes were dehydrated in a graded acetone series and embedded in Epon 812 (Luft, 1961). Sections were cut with glass knives on a Porter-Blum MT-1 ultramicrotome. Thin sections were successively stained with 1% uranyl acetate for 15 min and with Reynolds' lead citrate solution for 3 min (Reynolds, 1963), and observed in a JEOL 100S electron microscope.

Abbreviations:

AR;	acrosome	N;	nucleus
C;	centriole	NO;	nucleolus
DC;	distal centriole	NV;	nuclear vacuole
DJ;	desmosome-like junction	P;	periacrosomal material
ER;	endoplasmic reticulum	PC;	proximal centriole
G;	Golgi apparatus	R;	rootlet
M;	mitochondrion		

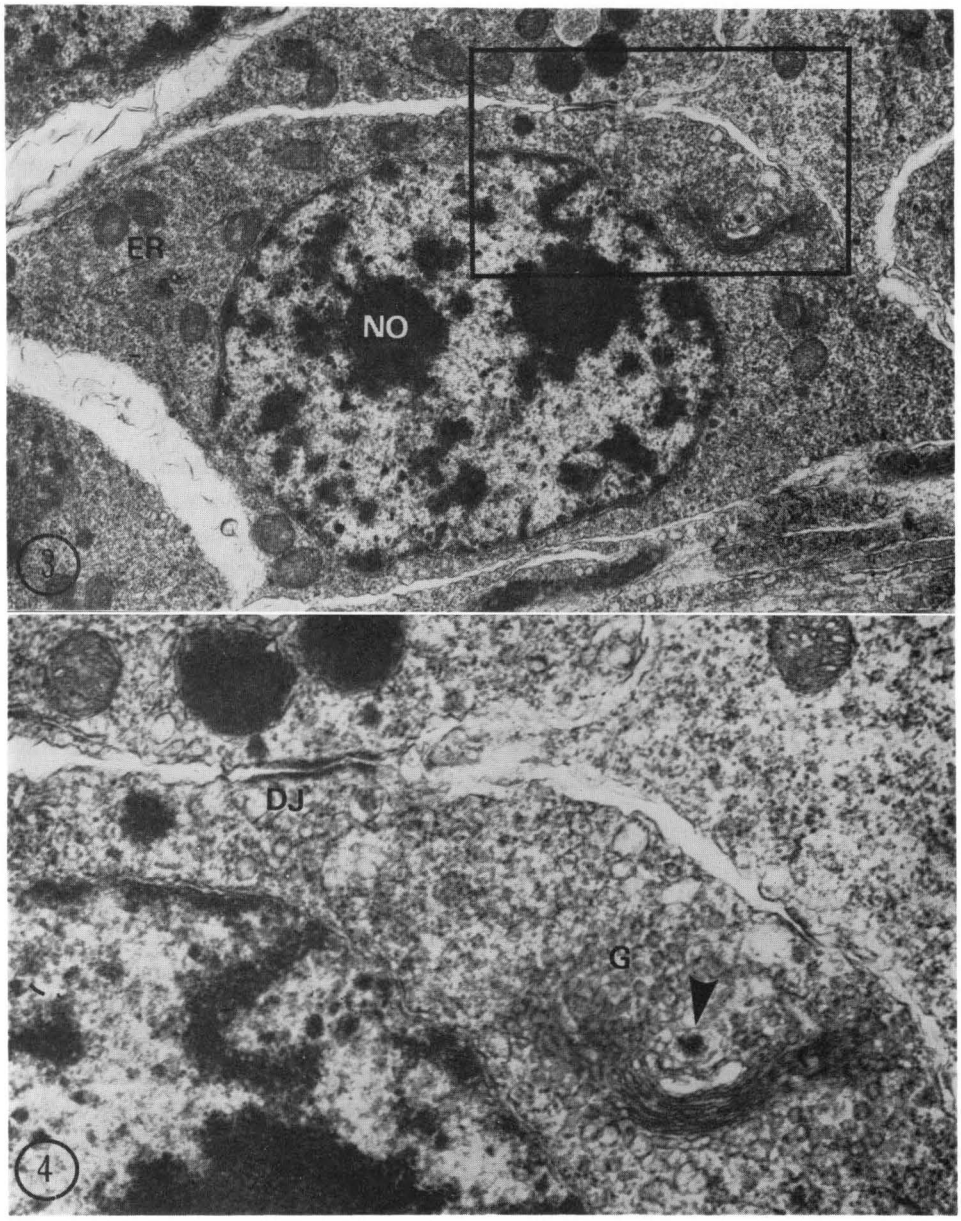


Figs. 1–2. 1. Electron micrograph of the spermatogonium. Arrowheads indicate the electron-dense materials either near or in contact with the nuclear memebrane. ×15,000; 2. Peripheral cytoplasm of the spermatogonium, showing the rootlet associated with the distal centriole. ×20,000.

Results

Spermatogonium (Figs. 1–4).

The spermatogonium, 7 μm, in diameter, has a large oval nucleus of about 5.5 μm along the greater axis and 4 μm along the shorter (Figs. 1 and 3). One or two



Figs. 3–4. 3. The spermatogonium. Two nucleoli are seen in the nucleus. ×12,500; 4. High magnification of the region outlined in Fig. 3. Arrowhead shows the proacrosomal vesicle formed by Golgi apparatus. ×30,000.

nucleoli are usually found in the nucleus (Figs. 1 and 3). The cytoplasm contains ovoid or tubular mitochondria, Golgi apparatus and two centrioles proximal or distal in situation. The mitochondria scattered in the cytoplasm show no particular distribution (Figs. 1 and 3). The Golgi apparatus is usually located near the centrioles (Figs. 1 and 2). The proacrosomal vesicle makes its appearance in the spermatogonial stage. The Golgi apparatus found near the proacrosomal vesicle seems to be a contributor for the vesicle formation (Fig. 4). The proacrosomal vesicle is represented as a membrane-bound coarse accumulation of fine granules. The distal and proximal centrioles are perpendicular to each other, and both of them are located in the peripheral cytoplasm (Figs. 1 and 2). A striated fibrous rootlet extending inward from the distal centriole is observable (Fig. 2). Besides these organella, well-developed endoplasmic reticula, numerous free ribosomes and occasional multivesicular bodies are also found (Figs. 1 and 3). The spermatogonium also contains electron dense materials present either near or in contact with the nuclear membrane (Fig. 1). These materials seem to be similar to the amorphous materials described in the echinoid germ-line cells (Houk and Hinegardner, 1981). Desmosome-like junctions joint the spermatogonia (Fig. 4).

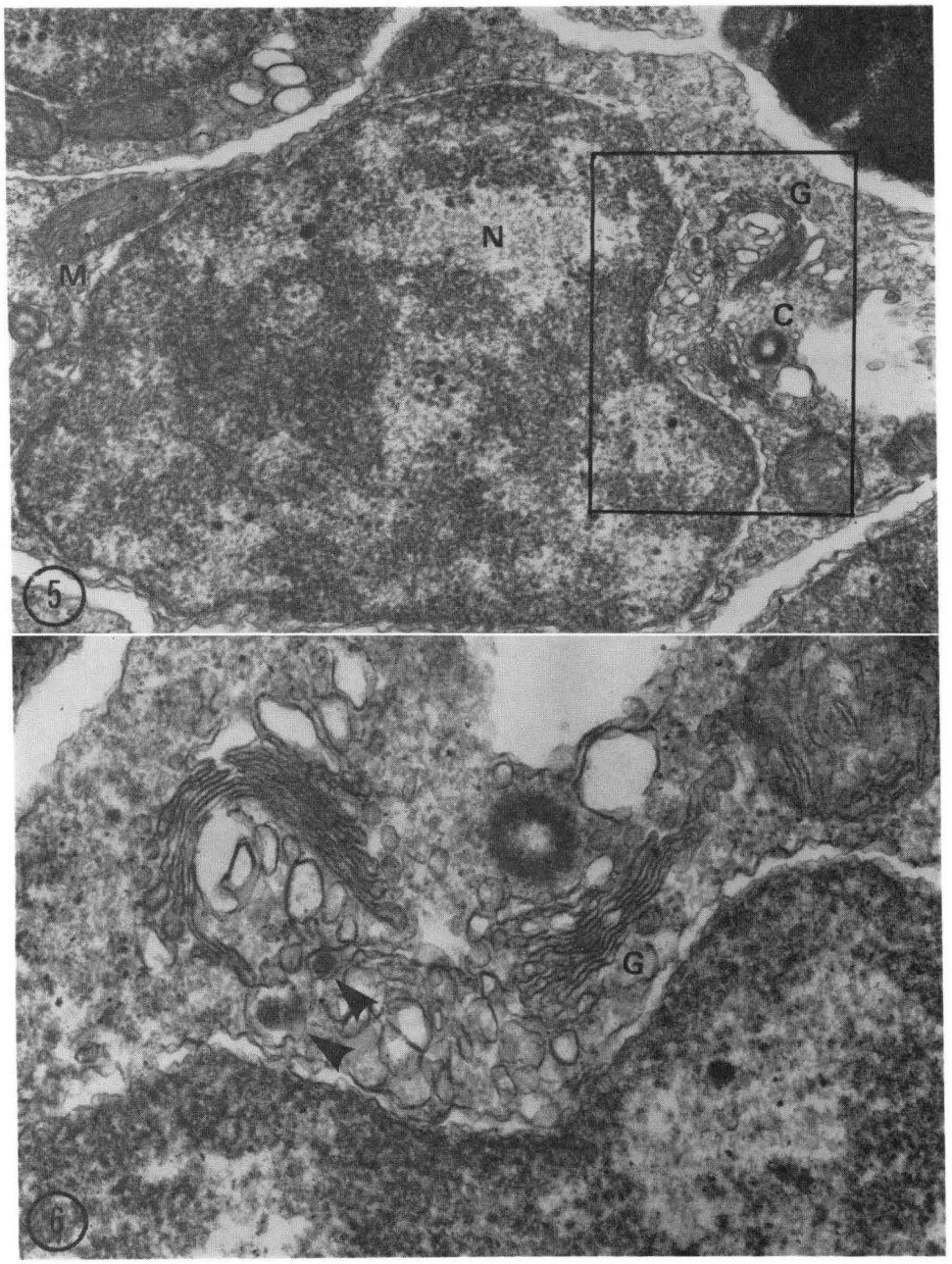
Spermatocyte (Figs. 5 and 6).

The primary and secondary spermatocytes are not distinguishable in feature probably because none of the morphological characters differ from each other. The spermatocyte measures about 5 μm in diameter. The nucleus being about 3.5 μm is more condensed than that in the spermatogonium, but the density and distribution of the mitochondria, Golgi apparatus, centrioles and rootlet are similar to those in the spermatogonium (Fig. 5). Several proacrosomal vesicles are found near Golgi apparatus (Fig. 6). The electron-dense materials found either near or in contact with the nuclear membrane are not detected in this stage. The formation of a flagellum extending from the distal centriole begins in the spermatocytic stage. The desmosome-like junctions found between the spermatogonia are still observable during this stage.

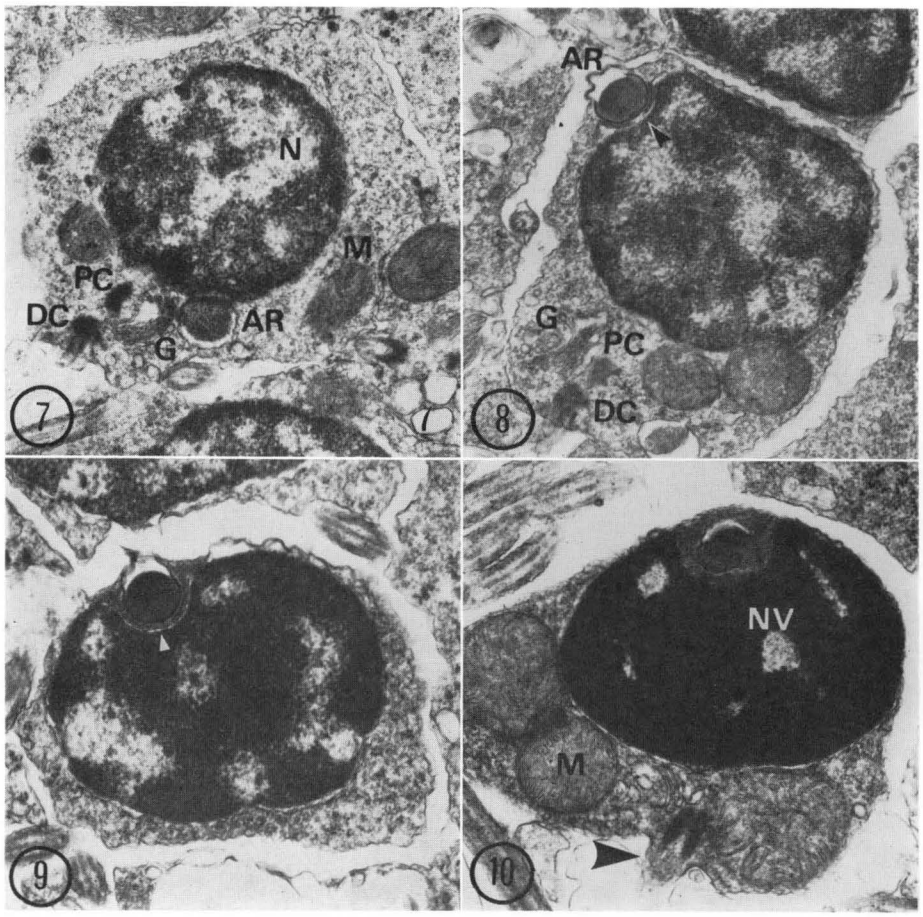
Spermatid (Figs. 7–16).

The early spermatid, about 3 μm in diameter, has a circular nucleus measuring about 2.5 μm in diameter (Figs. 7 and 8). The chromatin granules embedded in homogeneous matrix are aggregated heterogeneously. During spermiogenesis, condensation of the chromatin is accelerated, leaving several nuclear vacuoles, and the circular nucleus becomes ellipsoidal in shape (Figs. 7–10). The cytoplasm is confined to the posterior region of the cell, where the mitochondria reduce in number and gather to form a single doughnut-shaped mitochondrion in the mature spermatozoon (Figs. 10 and 18). The developed endoplasmic reticula found during the previous stages are much reduced in this stage.

The proximal and distal centrioles remain perpendicular to each other in the early spermatid as during the previous stages (Figs. 7 and 8), but in the mature spermatozoon the axis of the proximal centriole lies at an angle of about 30° from



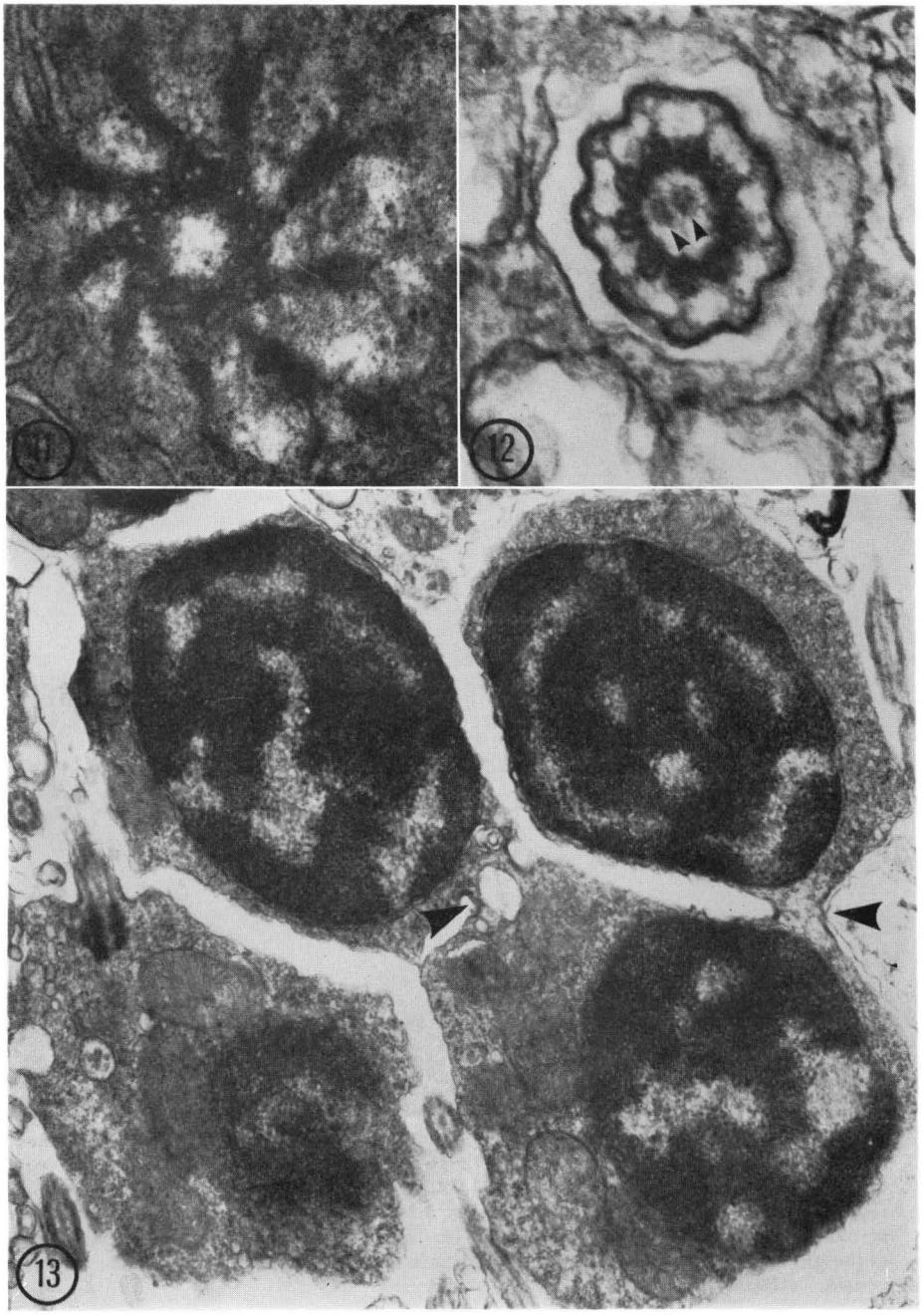
Figs. 5–6. 5. The spermatocyte, having more condensed nucleus than that of the spermatogonium. ×20,000; 6. High magnification of the region outlined in Fig. 5. Arrowheads indicate the proacrosomal vesicles. ×48,000.



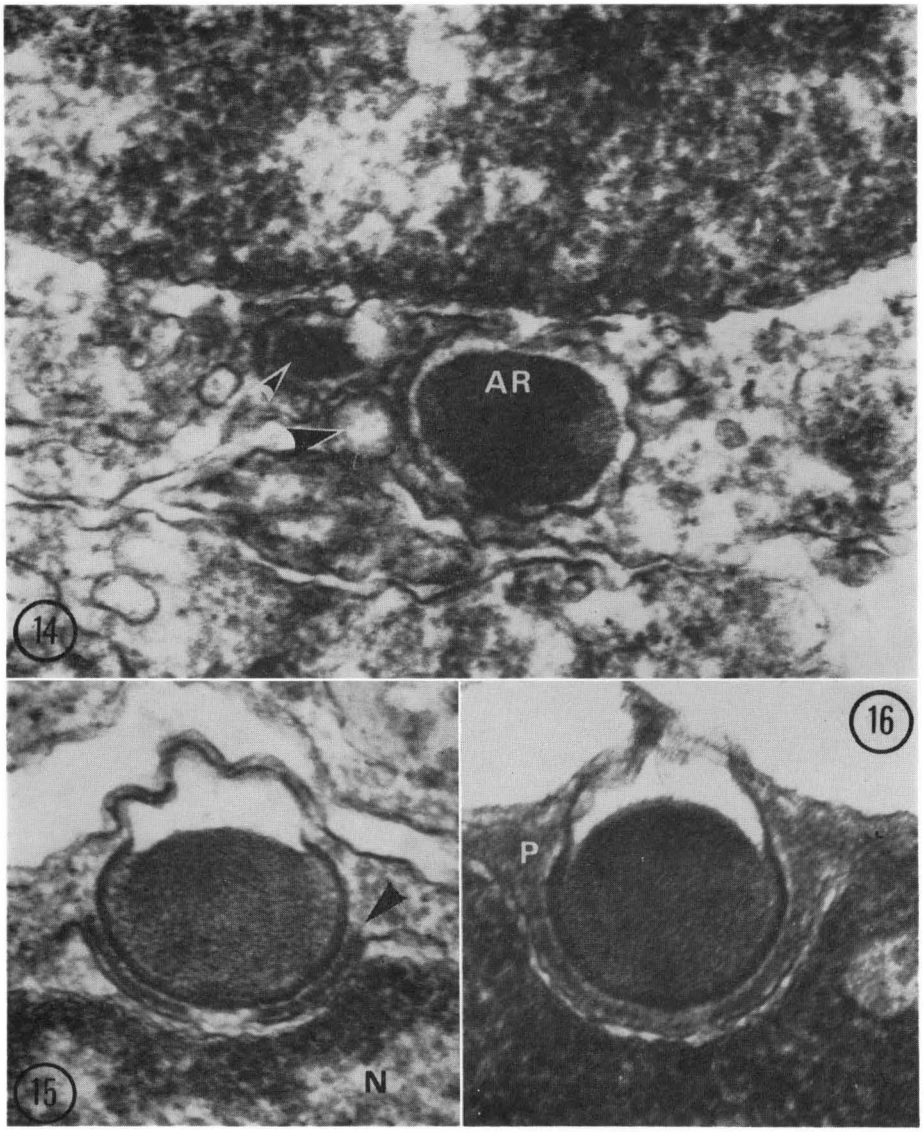
Figs. 7–10. 7. Electron micrograph of the early spermatid, showing a circular acrosome located at posterior portion of the cell and two centrioles which remain perpendicular to each other. ×16,000; 8. The spermatid more advanced than that in Fig. 7. The acrosome is transported to the anterior portion of the cell. A shallow indentation of the nucleus (arrowhead) is seen beneath the acrosome. ×15,000; 9. The spermatid more developed than that in Fig. 8, showing the deep cup-shaped acrosomal fossa (arrowhead) in which the acrosome is circular in shape. ×20,000; 10. The late spermatid. The cytoplasm is confined to the posterior portion of the cell. Note the acrosome different in its shape. The flagellum (arrow-head) extends at an angle of about 30° from the antero-posterior axis of the acrosome and nucleus. ×20,000.

that of the distal one (Fig. 17). The rootlet accompanied with the distal centriole disappears completely in the spermatid, and instead a centriolar satellite complex associates the distal centriole. In the centriolar satellite region, nine spoke-like satellites emanate from the dense matrix of the distal centriole and bifurcate into secondary sopkes (Fig. 11). In transverse section through a proximal tip of the central tubules of the flagellum, nine Y-shaped connectives are found between the flagellar membrane and the peripheral tubules of the flagellum (Fig. 12).

The desmosome-like junctions still remain in the early spermatid, but later they disappear and instead, intercellular bridges take a function to join the spermatids.



Figs. 11–13. 11. Cross section of the centriolar satellite region. Nine spoke-like satellite emanate from the dense matrix of the distal centriole and bifurcate into secondary spokes. ×97,500; 12. Cross section through the proximal tubules of the flagellum (arrowheads). Nine Y-shaped connectives are seen. ×100,000; 13. The spermatids jointed by intercellular bridges (arrowheads). ×20,000.



Figs. 14–16. 14. The cytoplasm of the early spermatid. The proacrosomal vesicle immediately before fusion to form the acrosome is seen (large arrowhead). Another proacrosomal vesicle is also indicated (small arrowhead). ×44,000; 15. High magnification of the acrosomal region of the spermatid in Fig. 8. Fibrous materials (arrowhead) are seen between the acrosomal vesicle and nucleus. ×66,000; 16. High magnification of the acrosomal region of the spermatid in Fig. 9. Electron-lucent space is seen at anterior portion of the acrosomal vesicle. ×80,000.

The intercellular bridges are short and cylindrical, being marked by an annular thickening on the cytoplasmic side of the plasma membrane (Fig. 13).

The proacrosomal vesicles fuse to form an acrosomal vesicle in the early spermatid (Fig. 14). At first, the acrosomal vesicle is found at a caudal part of the cell (Fig. 7), but during spermiogenesis it is transported to the anterior portion of the

cell (Fig. 8). During this movement, a shallow indentation of the nucleus, which becomes an acrosomal fossa in the further stage, is formed beneath the acrosomal vesicle (Fig. 8). Between the acrosome and nucleus fibrous materials are usually found (Figs. 8 and 15). These materials seem to be precursors of the periacrosomal material. The acrosomal vesicle contains electron-dense fine granules and the vesicle membrane bulges out at the anterior apex of the vesicle, producing an electron-lucent space between the membrane and the mass of granules (Figs. 8 and 15; 9 and 16). The acrosomal granule looks like denser at the apex of the vesicle, although condition of its density differs slightly in sections (Figs. 15 and 16). The vesicle membrane surrounding the basal half of the acrosome is coated with electron-dense material (Figs. 15 and 16). As spermiogenesis proceeds, the nucleus is indented at its apex to form a deep cup-shaped acrosomal fossa (Figs. 7–10).

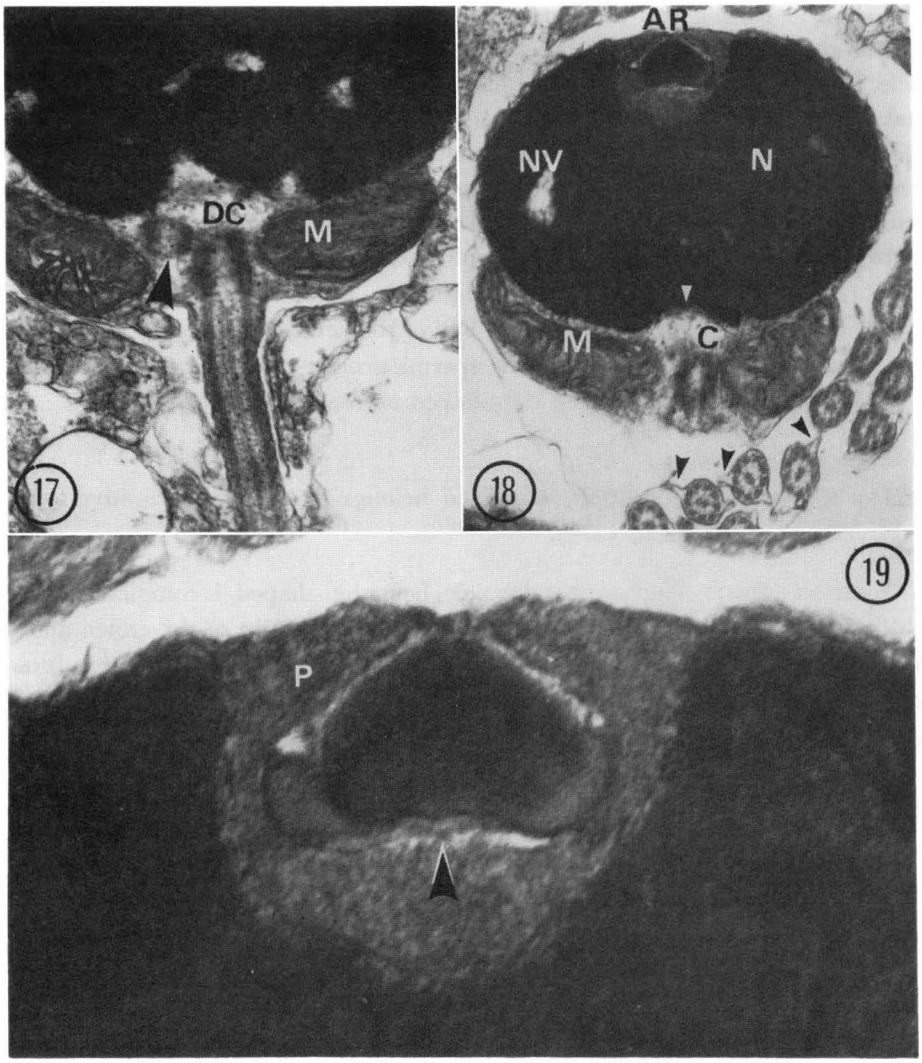
Spermatozoon (Figs. 17–19).

The spermatozoon of *Amphipholis kochii* belongs to a typical primitive type defined by Franzen (Franzen, 1970).

Acrosomal region: The acrosomal vesicle, being hat-shaped, is completely bounded by a limiting membrane and measures 0.35 μm at the height of the crown and 0.55 μm in breadth at the brim (Fig. 18). It is composed of fine granular substances and their condensation is denser at the crown than at the brim (Figs. 18 and 19). The electron-lucent space of the acrosome in the mature spermatozoon becomes much thinner than that in the spermatid (Figs. 15, 16 and 19). In the basal area of the acrosome, electron-lucent disk is not observable in the present species, whereas it is present in *Ophiopholis aculeata, Asterias forbesi* and *Ctenodiscus crispatus* (Summers *et al.*, 1975). The limiting membrane of the acrosome at the brim is coated with electron-dense material (Fig. 19). The periacrosomal material originated from the fibrous materials in the spermatid is made of fine fibrous materials. A part of them is specialized into a fibrous tuft beneath the acrosome, where the acrosome is slightly indented upward and electron-lucent space is formed (Fig. 19). This specialization of the periacrosomal material at the base of the acrosome has been reported in other echinoderms (Hagiwara *et al.*, 1967; Mabuchi and Mabuchi, 1973; Tilney *et al.*, 1973; Summers *et al.*, 1975; Hylander and Summers, 1975; Colwin *et al.*, 1975).

Nuclear and mitochondrial region: The nucleus measures about 1.5 μm along the antero-posterior axis and 2.3 μm in width, being highly condensed with several nuclear vacuoles (Fig. 18). As well as the anterior pole of the nucleus, the posterior one is slightly indented to form a centriolar fossa (Fig. 18). The single doughnut-shaped mitochondrion, about 0.8 μm in height and 1.8 μm in width, has a central cavity which houses the centrioles (Figs. 17 and 18).

Flagellum: The flagellum being about 90 μm in length and 0.3 μm in diameter is composed of ordinary 9+2 structure (Figs. 12 and 18). It extends posteriorly



Figs. 17–19. 17. Caudal region of the mature spermatozoon, showing the proximal centriole (arrowhead) lying at an angle of about 30° from the axis of the distal centriole. ×30,000; 18. Longitudinal section of the mature spermatozoon and transverse section of the flagella. White arrowhead shows the centriolar fossa. The flagellar membrane is projected laterally (black arrowheads). ×25,000; 19. High magnification of the acrosomal region of the spermatozoon in Fig. 18. The full-formed acrosome is hat-shaped and condensed at the crown. The membrane of the acrosomal vesicle is more electron-dense at the brim than at the crown. Note the periacrosomal material organized as a fibrous tuft beneath the base of the acrosomal vesicle (arrowhead). ×100,000.

at an angle of about 30° from the antero-posterior axis of the acrosome and nucleus (Fig. 10). The flagellar membrane frequently expands laterally and these lateral expansions are roughly alighned with the central tubules in transverse section (Fig. 18). These lateral expansions were also found in other brittle-star *Ophiocoma echinata* and many fishes (Hylander and Summers, 1975; Afzelius, 1978).

Discussion

The acrosome formation is generally initiated in the stage of spermatid (Welsch and Storch, 1976). In the echinoderms, however, this fashion is recognizable in the echinoids and asteroids, but the holothuroids and crinoids come off this rule; the proacrosomal vesicles are found in the spermatogonium in the holothuroids and in the spermatocyte in the crinoids (Atwood, 1974; Pladellorens and Subirana, 1975; Bickell *et al.*, 1980). The present study demonstrated that the acrosome formation of the ophiuroid *Amphipholis kochii* was also uncommon and nearly same to the case of the holothuroids.

The presence of proacrosomal vesicles in the spermatogonia has been recognized in the mussel *Mytilus edulis* with a suggestion that the male germ cells of *Mytilus* have failed to evolve the necessary machinery for the rapid production of acrosomal materials at a specific later period of spermatogenesis, on account of the absence of a well-developed endoplasmic reticula in the spermatid (Longo and Dornfeld, 1967). The present study also showed the absence of a developed endoplasmic reticula in the spermatid of *A. kochii*. The same property suggested in the germ cells of *Mytilus* may occur in the present species.

Another account for the precocious production of the proacrosomal vesicle seems to be possible. It is concerned with the short duration of spermiogenetic stage insufficient to produce enough amount of the acrosomal materials. Our unpublished data proved that the duration of spermiogenetic stage of *A. kochii* at 14°C was 2 days, in spite of the fact that the echinoid which has an ordinary pattern of the acrosome formation took 4 days at the same temperature (Holland and Giese, 1965). The precocious production of the proacrosomal vesicle may be indispensable in the present species, because of the rapid metamorphosis of the spermatid to spermatozoon. In any case, the most important thing accompanied with such a precocious production of the proacrosomal vesicles is concerned with the request of the germ cells that the proacrosomal vesicles in the spermatogonium or spermatocyte must be equally distributed among the spermatid during cell division. The mechanism for the distribution of the proacrosomal vesicles among the animals studied is fairly uncertain, and remains to be resolved.

As reviewed by Bickell *et al.* (1980), at least three characteristics of the acrosome formation are present in all echinoderms: 1) the acrosome is derived from Golgi apparatus; 2) the acrosome is formed at caudal end of the cell; 3) the vesicle membrane of the acrosome develops a region of more osmiophilic characteristics. These three characteristics are also recognized during the ophiuroid spermatogenesis in the present study, suggesting that these characteristics are very basic during the echinoderm spermatogenesis.

Concerning the transportation of the acrosome from caudal end to anterior pole of the spermatid, on the basis of the present observations, it is reasonable to assume that the transportation is caused by the rotation of the nucleus accompanying the acrosome at an angle of nearly 180° while the cytoplasm remains stationary or, on

the contrary, by the rotation of the cytoplasm while the nucleus and acrosome remain. The same mechanism has suggested already for other echinoderms (Krishnan and Dale, 1975; Bickell *et al.*, 1980), but it is quite uncertain how the rotation is achieved. We could not observe force-generating or -mediating organella such as microtubules or microfilaments during the rotation of the present species. The lack of these organella has been reported by the above-cited authors, thus further detailed observations should be necessary for this problem.

To date, the acrosome of all echinoderms studied until now including three ophiuroid species is represented as a circle in shape (Summers *et al.*, 1975; Hylander and Summers, 1975; Fontain and Lambert, 1976), with the exception of one holothuroid *Cucumaria pseudocurata,* which has an elongated dorso-ventrally compressed spermatozoon and very irregular shaped acrosome (Atwood, 1975). The hat-shaped acrosome of *A. kochii* is truely very remarkable as compared with other members of the echinoderms. In the fact that the early acrosome of the present species was circular, one assumption may be allowed to state that the shape of the mature acrosome is secondarily modified from the basic circular shape.

Summary

The present paper deals with the ultrastructural changes of the male germ cells during spermatogenesis of the brittle-star *Amphipholis kochii* (Echinodermata: Ophiuroidea), with special reference to the acrosome formation. The proacrosomal vesicle produced by Golgi apparatus is initially recognizable in the spermatogonial stage. A circular acrosome originated from the proacrosomal vesicles is formed at caudal end of the spermatid, and it is transported to anterior pole of the cell during spermiogenesis. The spermatozoon is composed of the hat-shaped acrosome, ellipsoidal nucleus provided with a deep cup-shaped acrosomal fossa and shallow centriolar fossa, doughnut-shaped mitochondrion, and flagellum. The present species has remarkable characteristics in that the acrosome formation is initiated in the spermatogonium and that the acrosome of the mature spermatozoon is uncommon in shape among those of other echinoderms.

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