Ultrastructure of the Gonadal Wall of Two Brittle-Stars, *Amphipholis kochii* and *Ophiura sarsii* (Echinodermata: Ophiuroidea)[1)]

By

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(*With 2 Text-figures*)

The fine structure of the gonadal wall of the echinoderms has been described in the following papers: Longo and Anderson, 1969, for the echinoids; Tangapregassom and Delavault, 1967, Bruslé, 1969, Walker, 1974, 1976, 1979 and 1980, for the asteroids; Atwood, 1973, Krishnan and Dale, 1975, for the holothuroids; Bickell *et al.*, 1980, for the crinoids. Up to date, however, there has been no published study concerned with the fine structure of the ophiuroid gonadal wall, although some light microscopic investigations have briefly treated it (Smith, 1940; Patent, 1976).

The present paper, therefore, describes the fine structure of the gonadal wall of the two brittle-stars, *Amphipholis kochii* Lütken and *Ophiura sarsii* Lütken. The paper also discusses a possible manner for nutrient transport to the gonads.

Materials and Methods

Samplings for *Amphipholis kochii* were made between the tidemarks on the coast of Abuta and those for *Ophiura sarsii* were made in Uchiura (Volcano) Bay at the depth of about 300 m.

The gonads of both species were severed from the disk, prefixed with 5% glutaraldehyde in 75% sea water, and preserved in the fixative until use. After several washings in 150% sea water, the prefixed gonads were post-fixed in 1% OsO_4 in 75% sea water for 30 min, dehydrated in acetone, and embedded in Epon 812 (Luft, 1961). Ultrathin sections were cut with glass knives on a Porter-Blum MT-1 ultramicrotome, stained with uranyl acetate and lead citrate (Reynolds, 1963), and examined in a JEOL JEM-100S electron microscope operated at 60 kV.

1) Contribution No. 28 from the Usujiri Fisheries Laboratory, Faculty of Fisheries, Hokkaido University.

Observations

I Gonadal wall of *Amphipholis kochii* Lütken

The gonad originates in a genital rachis, which is entirely enclosed by the genital coelomic sinus (GCS) and the genital hemal sinus, as is the case of other ophiuroids (Smith, 1940; Patent, 1976). The gonadal wall can be divided into two parts, an outer sac and an inner sac, in accordance with Walker's (1974) definition for the asteroid gonadal wall.

A *Outer sac*: The outer sac of the gonadal wall consists of three units: a visceral peritoneum, a connective tissue layer covered with basal laminae, and the outer epithelium of the GCS (Fig. 1A).

The visceral peritoneum possesses flagella which are scattered sparsely on its free outer surface (Fig. 1A). There are no collar-like projections around the flagellum (Fig. 1B). The cells of the visceral peritoneum are composed of an irregular ellipsoidal nucleus with a long axis parallel to the outer surface of the visceral peritoneum, and of a cytoplasm containing a small number of mitochondria, lipid bodies and free ribosomes. Nerve processes and longitudinal muscle fibers are observable in the visceral peritoneum (Fig. 1A).

The connective tissue layer is covered by a basal lamina measuring 50 nm in thickness (Fig. 1A) and we are able to see electron-lucent ground substances, collagen fibers and cells scattered freely in this layer (Fig. 1A).

The morphology of the cells that form the outer epithelium of the GCS is similar to that of the visceral peritoneum, except for the absence of the flagella and muscle fibers (Fig. 1A).

The GCS is narrow and the outer and inner epithelium are closely contiguous to each other at many places (Fig. 1A). There are no structural elements in the GCS.

B *Inner sac*: The inner sac is also composed of three units: the inner epithelium of the GCS, the genital hemal sinus covered with basal laminae, and a germinal epithelium (Fig. 1A).

The cellular components of the inner epithelium of the GCS resemble those of the visceral peritoneum. However, the muscle fibers run circularly, unlike the longitudinal fibers in the visceral peritoneum (Figs. 1A and C). The muscle fiber

Abbreviations

AC, amoeboid cell
BL, basal lamina
CF, collagen fiber
CM, circular muscle fiber
CTL, connective tissue layer
F, flagellum
GCS, genital coelomic sinus
GE, germinal epithelium
GHS, genital hemal sinus
HC, hemal cell
IE, inner epithelium of GCS
IS, inner sac
LM, longitudinal muscle fiber
N, nucleus
NE, nerve process
OE, outer epithelium of GCS
OS, outer sac
VP, visceral peritoneum

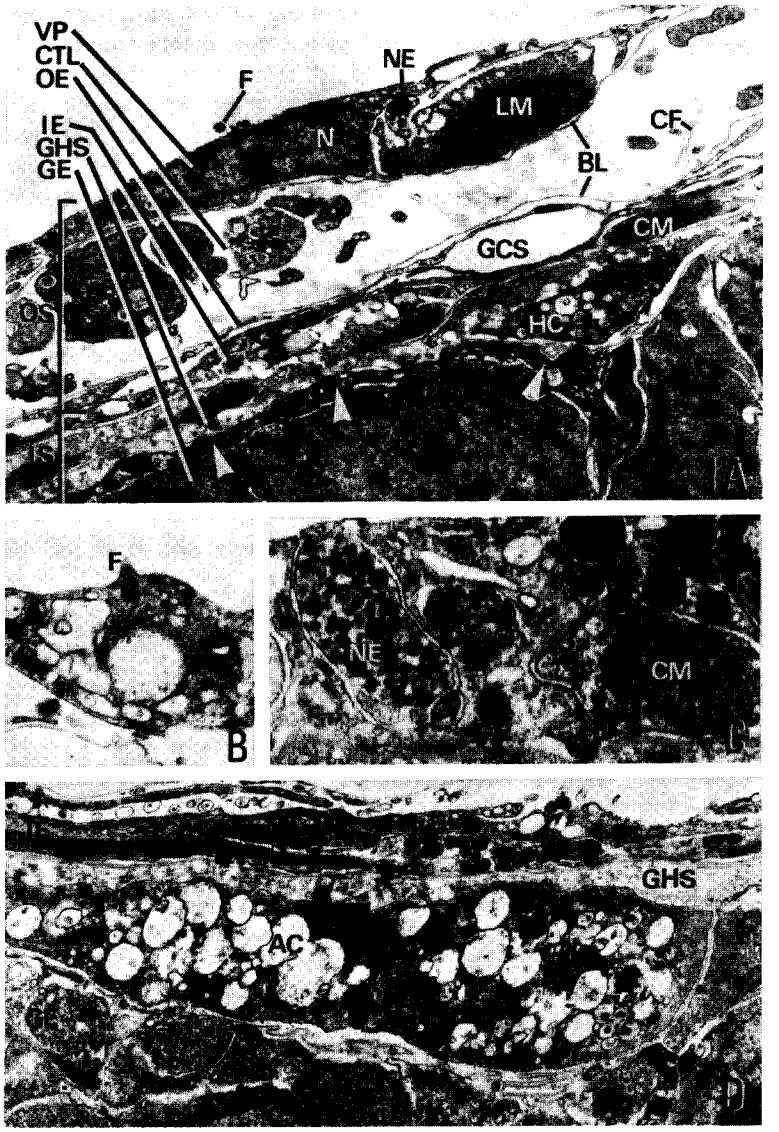

Fig. 1. Electron micrograph of the gonadal wall of *Amphipholis kochii*. A: Transverse section of the gonadal wall. Arrowheads show the cytoplasm of the amoeboid cell in close contact with the genital hemal sinus. The asterisk indicates the cytoplasm of the cell scattered freely in the connective tissue layer. × 6,200. B: The visceral peritoneum, showing the flagellated cell without collar-like projections. ×8,300. C: Longitudinal section of the inner epithelium of the genital coelomic sinus, showing the nerve process and muscle fiber. × 16,600. D: The amoeboid cell which comes in contact with the genital hemal sinus. × 6,640.

consists of thick and thin myofilaments measuring 30 nm and 8 nm in diameter respectively (Fig. 1C). Nerve processes are also found in the inner epithelium of the GCS. They contain membrane-bound neurosecretory granules measuring 60 nm to 130 nm in diameter (Fig. 1C).

In the genital hemal sinus, we are able to recognize a large number of fine fibrous and granular materials, which are probably nutrients, a few collagen fibers and occasional hemal cells. The large cytoplasm of the hemal cells possesses abundant nutritive bodies (Fig. 1A).

The germinal epithlium comprises the gametogenic cells and the somatic cells known as the amoeboid cells (Iwata and Yamashita, 1982). The amoeboid cells are usually found among the gametogenic cells or in the central portion of the gonad and form a large assembly there. Occasionally we are able to see them make close contact with the genital hemal sinus (Figs. 1A and D). The phagocytic ability of the amoeboid cells has already been demonstrated in our previous paper (Yamashita and Iwata, 1983).

II Gonadal wall of *Ophiura sarsii* Lütken

The gonadal wall is separable into an outer and inner sac, as is the case of *A. kochii* (Fig. 2A).

A *Outer sac*: The outer sac consists of a visceral peritoneum, a connective tissue layer covered with basal laminae, and the outer epithelium of the GCS (Fig. 2A).

The nucleus of the visceral peritoneal cell is irregular ellipsoidal and contains a thick mass of heterochromatins (Fig. 2A). The flagella are scattered sparsely on the free surface of the visceral peritoneum as in *A. kochii* (Fig. 2A). The flagellated cells, however, possess collar-like projections around the flagellum (Fig. 2B), unlike *A. kochii* (cf. Fig. 1B). These flagellated cells have been called "flagellated-collar cells" by Walker (1979). Nerve processes and longitudinal muscle fibers are slso present in the visceral peritioneum (Fig. 2C).

The connective tissue layer is mainly composed of electron-lucent ground substances, collagen fibers and cells scattered sparsely (Fig. 2A).

The outer epithelium of the GCS is similar to the visceral peritoneum. However, the flagella and muscle fibers are absent in this layer, although the nerve processes are present (Fig. 2A).

The GCS is relatively broader than that of *A. kochii* (Fig. 2A).

B *Inner sac*: The inner sac consists of the inner epithelium of the GCS, a genital hemal sinus covered with basal laminae, and a germinal epithelium (Fig. 2A).

The structure of the inner epithelium of the GCS resembles that of the visceral peritoneum. Circular muscle fibers are present (Fig. 2D), in place of the longitudinal muscle fibers found in the visceral peritoneum (Fig. 2C).

Abundant nutrients and hemal cells are observable in the genital hemal sinus (Fig. 2A). The hemal cells possess a large cytoplasm that includes many nutritive bodies (Fig. 2A). The nucleus of the hemal cell is ovoid and is provided with a

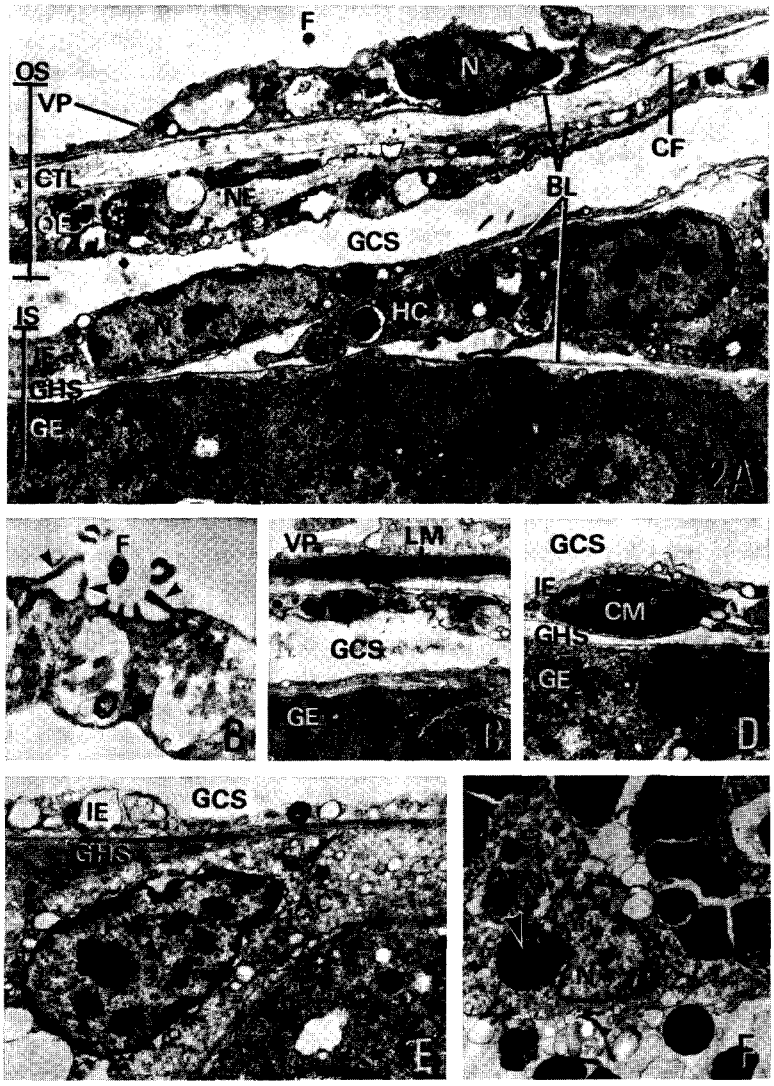

Fig. 2. Electron micrograph of the gonadal wall of *Ophiura sarsii*. A: Transverse section of the gonadal wall. × 5,000. B: The flagellated-collar cell on the visceral peritoneum. Note the collar-like projections (arrowheads) around the flagellum. × 8,300. C: Longitudinal section of the gonadal wall, showing the longitudinal muscle fiber in the visceral peritoneum. × 5,000. D: Longitudinal section of the inner sac, showing the circular muscle fiber in the inner epithelium of the genital coelomic sinus. × 5,000. E: The amoeboid cell is in close contact with the genital hemal sinus. × 5,000. F: The amoeboid cell found among the gametogenic (spermatogenic) cells. Note that the spermatozoon (arrowhead) is ingested by the amoeboid cell with phagocytic action. ×2,500.

thick mass of heterochromatins located mainly along the nuclear membrane (Fig. 2A).

The germinal epithelium comprises the gametogenic cells and the amoeboid cells. The amoeboid cells are sometimes in close contact with the genital hemal sinus (Fig. 2E). The phagocytic ability of the amoeboid cells in *O. sarsii* is also recognizable (Fig. 2F).

Discussion

The present observations show that the fine structure of the gonadal wall of the two brittle-stars, *Amphipholis kochii* and *Ophiura sarsii*, is similar, except only for the morphology of the flagellated cells present in the visceral peritoneum. The facts observed in the present study therefore seem to be applicable to the general feature of the gonadal wall of the ophiuroids.

To date, there have been no published fine structural observations on the gonadal wall of the ophiuroids, although Davis treated it briefly. According to Davis' unpublished data cited by Atwood (1973), the gonadal walls of the ophiuroids and the asteroids are similar, but the muscle fibers in the outer epithelium of the GCS are lacking in the ophiuroids while being present in the asteroids. The present study confirms these findings. It also describes the differing cellular character of the gonadal wall of the ophiuroids and the asteroids: the flagella found on the visceral peritoneum of the former are much smaller in number than those of the latter; the muscle fibers in the inner epithelium of the GCS are circular in the former, but they are longitudinal in the latter (Tangapregassom and Delavault, 1967; Bruslé, 1969; Walker, 1974, 1976 and 1979).

In the echinoids, the nutrients are believed to be reserved mainly in the gonads (Lasker and Giese, 1954; Giese *et al.*, 1959; Pearse and Giese, 1966). The particular nutrient transport for gametogenesis is therefore not necessary for this echinoderm. On the other hand, it has been suggested that the nutrients are mainly reserved in the pyrolic caeca for the asteroids (Farmanfarmaian *et al.*, 1958; Anderson, 1966; Giese, 1966; Mauzey, 1966; Rao, 1966; Kim, 1968; Crump, 1971; Nimitz, 1971 and 1976; Ferguson 1975a and b; Jangoux and Van Impe, 1977; Barker, 1979; Harrold and Pearse, 1980) and in the stomach for the holothuroids (Farmanfarmaian, 1963). In these echinoderms the nutrients for gametogenesis must therefore be transported from the reservoir to the gonad. In the ophiuroids, however, we are uncertain where the nutrients are mainly reserved. Schechter and Lucero (1968) have reported though that the cytoplasm of the stomach cells of the ophiuroid *Ophiuroiderma panamensis* is heavily laden with lipid bodies and represents a very abundant nutritive storehouse. Moreover, my unpublished data proves that the stomach wall of *A. kochii* becomes thinner as the gametogenesis proceeds. Judging from these observations, it is likely that in the ophiuroids the nutrients are reserved mainly in the stomach and transported to the gonad during the process of gametogenesis.

As for the route for the nutrient transport from the reservoir to the gonads, it has been thought that in the asteroids the coelomic fluid and hemal sinus are the route for transport (Ferguson, 1964a and b; Walker, 1979; Broertjes *et al.*, 1980a and b). In the ophiuroids, our previous observation that thymidine injected intracoelomically was absorbed into the gonad within a short time (Yamashita and Iwata, 1983) suggests that nutrients such as thymidine are transported from the coelomic fluid to the gonad through the gonadal wall. On the other hand, the present study shows that abundant nutritive materials and hemal cells containing many nutritive bodies are found in the genital hemal sinus, suggesting the nutritive transport to the gonad through the genital hemal sinus. These findings allow us to suggest that the coelomic fluid and/or hemal sinus is a route for nutritive transport to the gonad in the ophiuroids, as in the case of the asteroids.

With regard to the problem of how the nutrients transported to the gonads are distributed over the germinal epithelium, the somatic cells found in the germinal epithelium have been noticed by several authors, and it has been suggested that these somatic cells serve as a vehicle for distribution of the nutrients over the germinal epithelium (Walker, 1979 and 1980; Bickell *et al.*, 1980). Our previous (Yamashita and Iwata, 1983) and present observations that the somatic cells in the ophiuroid gonads (the amoeboid cells) have a phagocytic role also suggest a qualification of these cells as a vehicle of nutritive distribution. Moreover, the present finding of a close contact of the amoeboid cell with the genital hemal sinus through which the nutrients may be transported proves that these cells play an important role in the distribution of the nutrients over the germinal epithelium.

In conclusion, we may summarize the nutrient transport for gametogenesis of the ophiuroids as follows: the nutrients mainly reserved in the stomach wall are transported to the gonad through the coelomic fluid and/or hemal sinus and are distributed over the germinal epithelium by the amoeboid cells scattered over the germinal epithelium.

Summary

This paper deals with the ultrastructure of the gonadal wall of the brittle-stars, *Amphipholis kochii* Lütken and *Ophiura sarsii* Lütken. The gonadal walls of the two species are very similar to each other, except for the morphology of the flagellated cells present in the visceral peritoneum. The basic structure of the gonadal wall of the ophiuroids is more similar to that of the asteroids than that of other echinoderms, except for a density of the flagella on the visceral peritoneum and for the arrangement of the muscle fibers. It is suggested that nutrients reserved in the stomach wall are transported to the gonad through the coelomic fluid and/or hemal sinus and that the transported nutrients are distributed over the germinal epithelium by the amoeboid cells.

The author wishes to express his gratitude to Prof. F. Iwata, Hokkaido University, for his guidance and critical reading of the manuscript. Thanks are also due to Prof. T.

Igarashi, Director of the Usujiri Fisheries Laboratory, Hokkaido University, for providing laboratory facilities and to Mr. Y. Arashida for his help in collecting the samples of *Ophiura sarsii*.

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