3. Structural Changes during Spermatophore Formation of the *Nautilus pompilius*

by

Junzo TSUKAHARA[1)]

Abstract

Structural changes during the formation of spermatophore in the accessory gland of *Nautilus pompilius* from Fiji were studied.

Many clusters of spermatozoa are found in the aperture of the testis. However, these clusters are very scarcely observed in the proximal portion of vas deferens. Immature spermatophore is formed in the distal portion of vas deferens.

In the seminal vesicle of full grown male, irregularly coiled immature spermatophore is always found. Mature and tightly coiled spermatophore is stored in the spermatophore sac. As the maturation progresses, the wall of the spermatophore becomes thicker and more tough, and the crowd of the spermatid, "sperm rod", is packed more tightly.

The inner surfaces of reproductive organs other than testis are always lined with well developed microvilli.

Introduction

There have been only a few studies on the formation of spermatozoa in *Nautilus* (ARNOLD and WILLIAMS-ARNOLD, 1978, TSUKAHARA, 1985). Maturing spermatozoa are enclosed within the spermatophore in a accessory gland. So far as the writer is aware, however, there have been comparatively few investigations on the formation of spermatophore in *Nautilus*.

Histological observations during formation of spermatophore in the accessory gland were carried out by optical or electron microscopy in this study.

Materials and Methods

Five specimens of the male *Nautilus pompilius* captured off Suva in Fiji late

1) Department of Biology, Faculty of Science, Kagoshima University, Kagoshima 890, Japan.

in August or in September, 1986, were used for this study. The soft part of these specimens were dissected and testis and other reproductive organs were immediately fixed.

In the preparation of specimens for optical microscopy, small pieces of organs were fixed for 24 or 48 hr at room temperature with 5 % neutral formaldehyde in 90 % sea water. After dehydration they were embedded in paraffin, sectioned and stained with haematoxylin and eosin.

Scanning electron microscopic observation was undertaken with the specimens, that were fixed with 2 % glutaraldehyde in 90 % sea water adjusted at pH 7.4 with 0.05 M cacodylate buffer solution. The fixation was performed at room temperature for 2 hr. The specimens were then dehydrated and dried by critical dryer. After coated with gold by ion coater they were observed by S 450 scanning electron microscope (SEM).

For the preparation of the transmission electron microscopic specimen, samples were prefixed for 1 hr with 2 % glutaraldehyde in 90 % sea water and 0.05 M cacodylate buffer (pH 7.4) at room temperature. After rinsed three times with buffered sea water, post fixation was carried out for 1 hr with 1 % OsO_4 in buffered sea water at 0 °C. Thin sections of the specimen were stained with uranyl acetate and lead citrate. They were observed by H 600 transmission electron microscope (TEM).

Observations

The testis of the male *N. pompilius* is a large oval organ situated in the extreme posterior and upper part of the coelom (Fig. 1). A funnel shaped aperture of the testis opens closely to the entrance of the vas deferens. Accessory gland, which is formed around the convoluted vas deferens, lies at the right of the testis. Distal end of the vas deferens opens into the seminal vesicle, where coiled spermatophore are frequently found. From the aperture of the vesicle a short thick-walled tube leads forward to the spermatophore sac and the penis.

There are numerous seminiferous tubules in the testis. Many spermatids are making stepwise progress in their spermiogenesis within these tubules (Pl. 10, fig, A). A large number of the spermatids is crowded to make a mass without any observable intermediate cementing substance between the heads of them. The spermatids have still a small mass of cytoplasm beside a rod of nucleus (Pl. 10, fig. A : arrow).

The aperture of testis projects outward like a funnel (Pl. 10, fig. B). The inside wall of the aperture is constructed with epithelial cells having many microvilli with a length approximately 5 μm (Pl. 10, fig. C). On the bottom in the funnel, there are some clusters of crowded numerous spermatozoa (Pl. 10, fig. D).

The proximal portion of the vas deferens is narrow and exceedingly thin

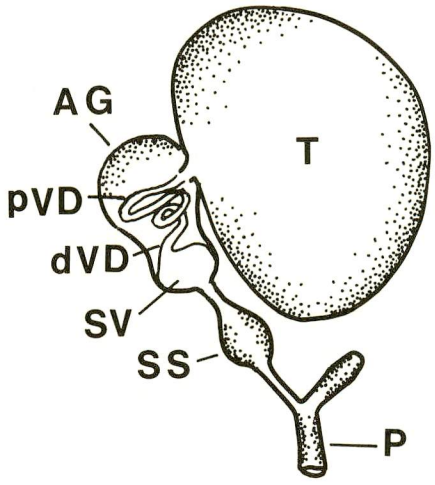


Fig. 1. Male reproductive organs viewed from above and front.
AG : Accessory Gland
dVD : distal Vas Deferens
P : Penis
pVD : proximal Vas Deferens
SS : Spermatophore Sac
SV : Seminal Vesicle
T : Testis

wall of a single flat layer of epithelium (Pl. 9, fig. A). There are many microvilli covered on the surface of the inner epithelial cells of the wall (arrow). More distal portion of the vas deferens has a thicker wall of undulated arrangement of epithelial cells (Pl. 9, fig. B). It is very scarce that the clusters of spermatozoa in the proximal vas deferens are found out. This scarcity of detection of spermatozoa suggests that the transference of the clusters may be rather fast. Immature spermatophore, simple and elongated tube, containing numberless spermatid is formed in the distal portion of vas deferens. Each spermatophore is approximately 800 μm in diameter.

The irregularly and loosely coiled spermatophore is always found in the seminal vesicle of full grown male (Pl. 11, fig. A). The distal end of the vas deferens opens into the tough-walled seminal vesicle. The outer thin wall of the spermatophore is slightly eosinophilic and less than 10 μm in thickness (Pl. 9, fig. D : arrow). On the other hand inner wall consists of eosinophobic loose substance. These different substances forming outer or inner wall may be secreted by inner epithelial cells of the vas deferens and seminal vesicle. Great number of spermatozoa are arranged to attach their head in the same direction and turn into tubular arrangement, "sperm rod" (Pl. 9, figs. C, D ; Pl. 11, fig. B). There is no stainable substance on the space between the sperm rods. The nucleus of spermatid is reached approximately 37 μm in length. The inside wall of the

seminal vesicle is covered with undulated epithelium and those cell surfaces have many tuft of microvilli (Pl. 11, figs. C, D). The average length of microvilli is approximately 8 μm.

In the spermatophore sac of full grown male, a coiled spermatophore is found several times. Outer tough-wall of it is approximately 25 μm in thickness and made of eosinophilic tight substance (Pl. 9, figs. E, F ; Pl. 12, fig. A). Inner wall of it, however, is more loose and medial eosinophilic. The same substance fills the space between sperm rods (Pl. 9, fig. F). Numerous tightly packed sperm rods are observed in full grown spermatophore (Pl. 9, figs. E, F ; Pl. 12, fig. B). High magnification SEM photograph shows that the sperm head is covered with many fluffy or filamentous substance (Pl. 13, fig. A : arrow). From the TEM observation it is suggested that sperm heads may be packed each other with cementing substance, which is medial osmiophilic amorphous material (Pl. 12, fig. C).

Near the posterior end of the penis there are many stripe-like rows of microvilli on the inside of the wall (Pl. 12, fig. D). Their length is reached approximately 30 μm.

Acknowledgments

The writer wishes to express his appreciation to Professor Shozo HAYASAKA of Kagoshima University for his kind criticism and improvement of the manuscript. The writer also wishes to express his deep gratitude to Dr. Akihiko SHINOMIYA, Dr. Kazushige TANABE, Dr. Uday RAJ and other members of the research project on *Nautilus* for their help in preparation of the specimens.

References

ARNOLD, J. M. and WILLIAMS-ARNOLD, L. D., 1978 : Spermiogenesis of *Nautilus pompilius*. 1. General survey. *J. Exp. Zool.*, 205, 13-26.

TSUKAHARA, J., 1985 : Histological and histochemical studies of gonads of *Nautilus pompilius* from Fiji. *Kagoshima Univ. Res. Center S. Pac., Occasional Papers*, 4, 50-60.

Plates 9-13

Explanation of Plate 9

Fig. A. Proximal vas deferens (pVD) in the accessory gland.
Arrow : numerous microvilli projected from the inner surface of the epithelial cells. ×80.

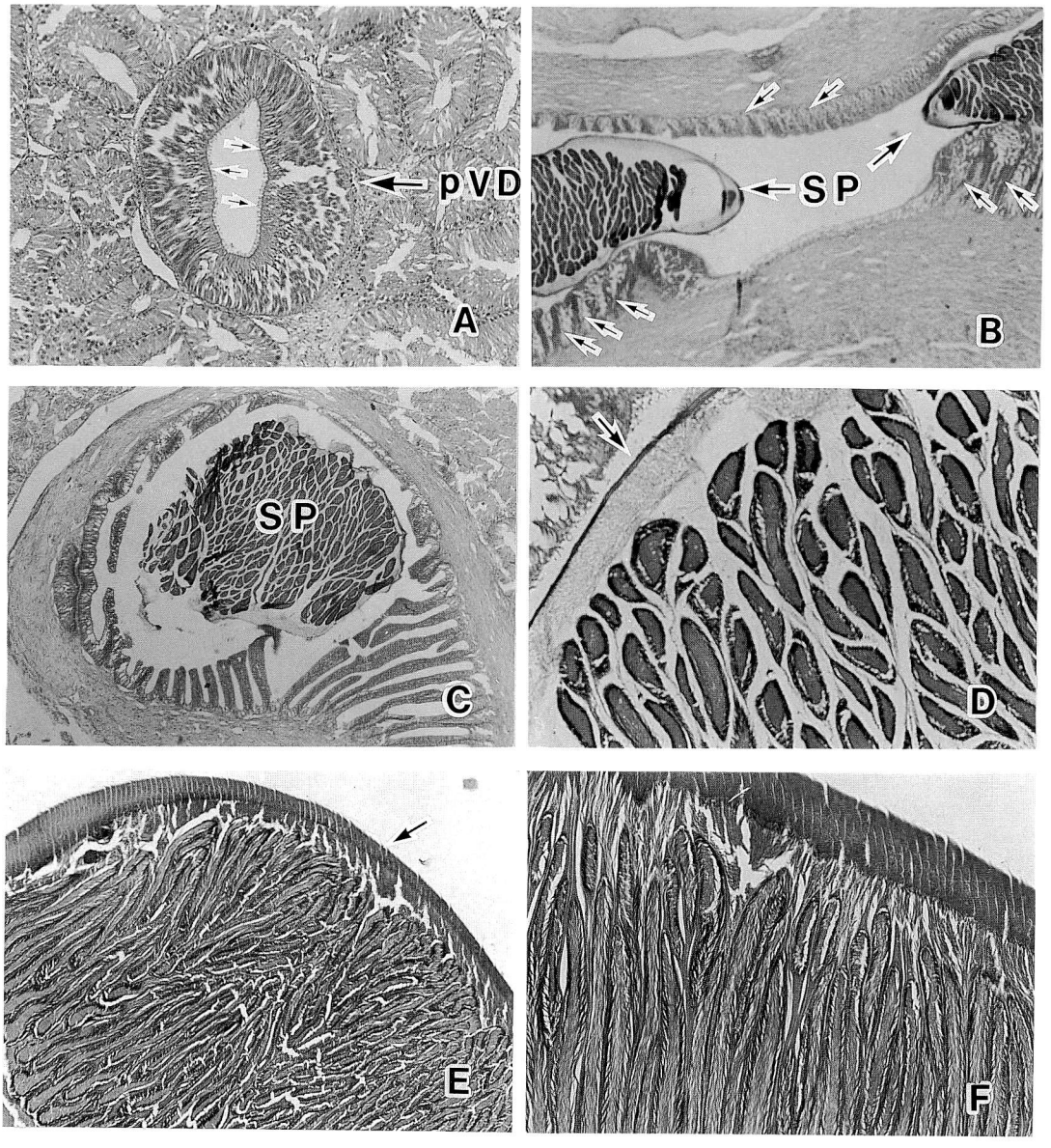
Fig. B. Longitudinal section of the distal vas deferens:
Arrow : undulated inner epithelium. SP : Spermatophore ×30.

Fig. C. Immature spermatophore (SP) in the seminal vesicle. ×30.

Fig. D. High magnification of the immature spermatophore.
Arrow : outer wall of the spermatophore. ×140.

Fig. E. Mature spermatophore.
Arrow : outer wall of the spermatophore. ×60.

Fig. F. High magnification of the mature spermatophore. ×160.


pVD
A
SP
B
SP
C
D
E
F

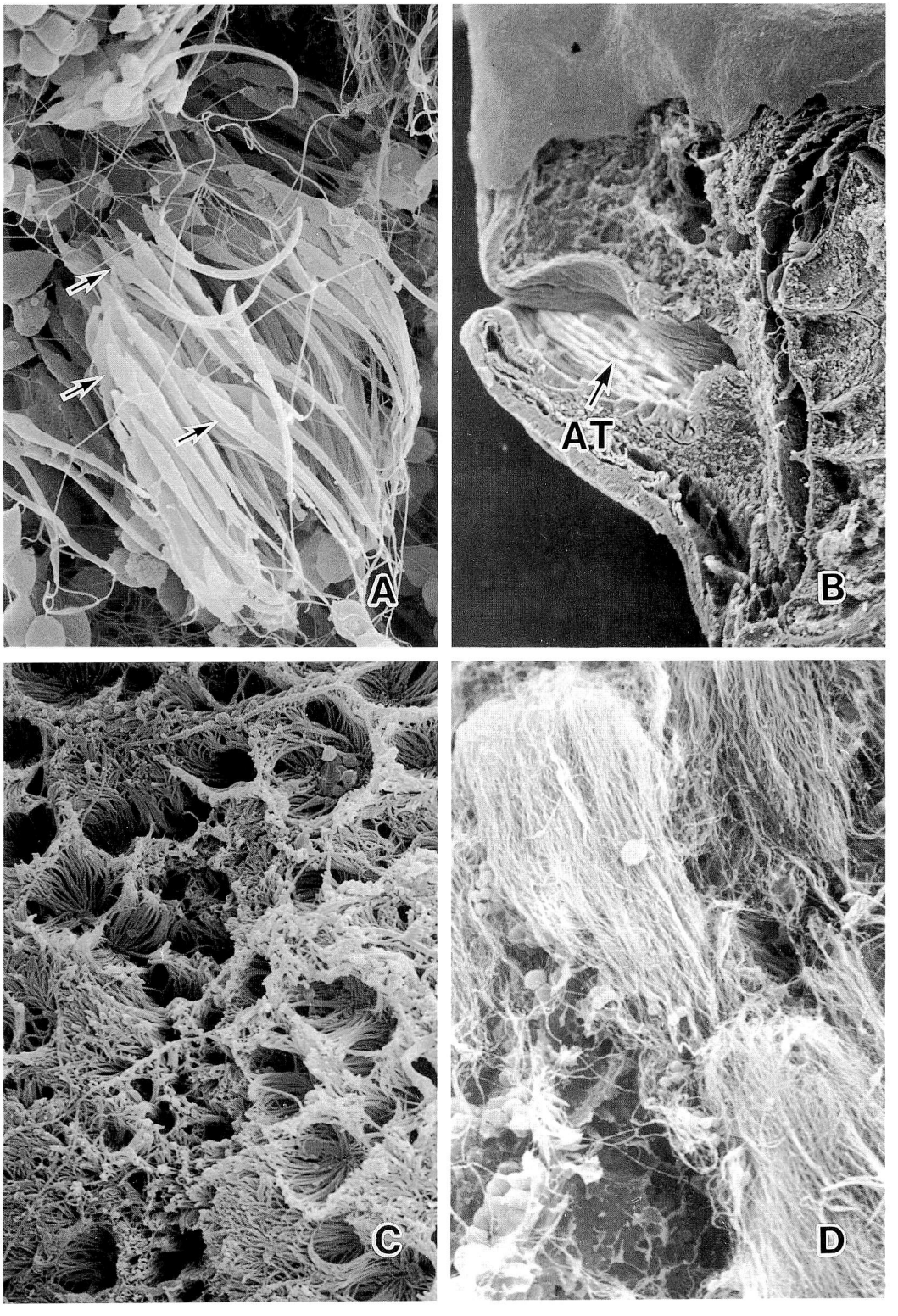
Explanation of Plate 10

Fig. A. Immature spermatozoa in the seminiferous tubule of the testis.
Arrow : a small mass of cytoplasm beside a rod of nucleus. ×1600.

Fig. B. Aperture of the testis (AT). ×36.

Fig. C. Numerous microvilli projected from the inner epithelial cells of the aperture of the testis. ×1700.

Fig. D. Clusters of the crowded numerous spermatozoa. ×180.


A
AT
B
C
D

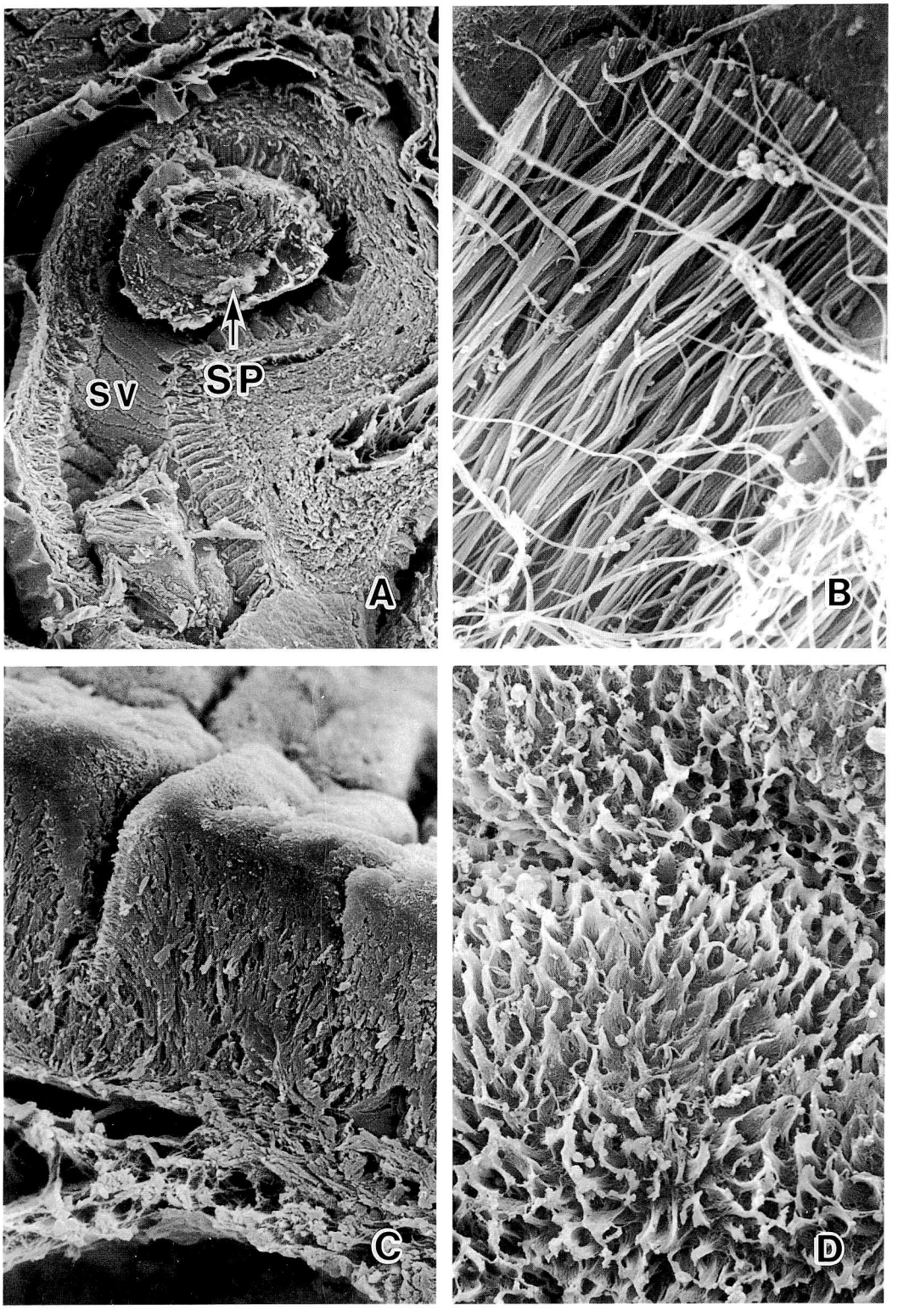
Explanation of Plate 11

Fig. A. Spermatophore (SP) in the seminal vesicle (SV). ×40.

Fig. B. A cluster of mature spermatozoa, "sperm rod", in the spermatophore. ×2300.

Fig. C. Undulated inner epithelium of the seminal vesicle. ×460.

Fig. D. Numerous tufts on the surface of epithelial cells of the seminal vesicle. ×950.


SV
SP
A
B
C
D

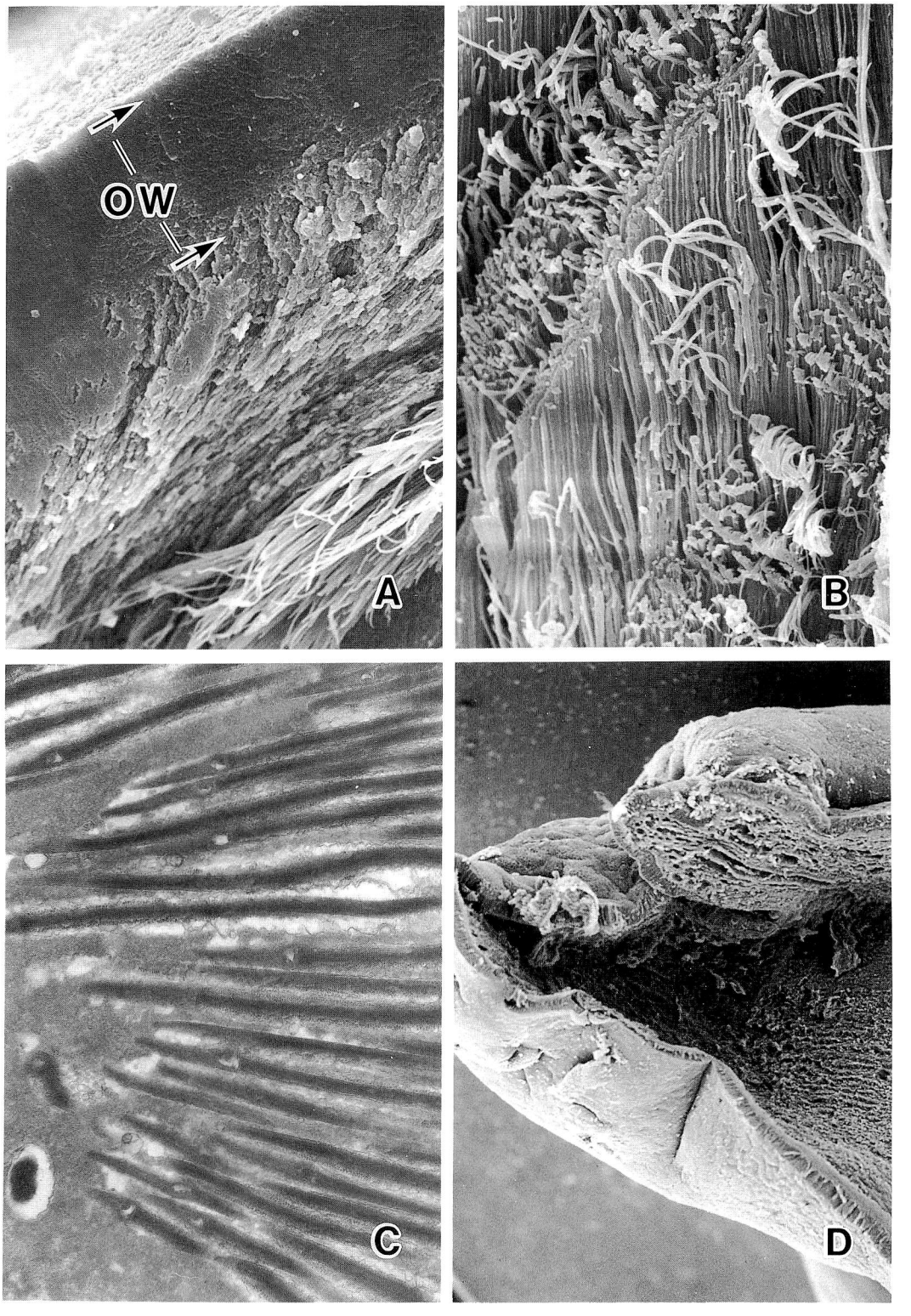
Explanation of Plate 12

Fig. A. Cross section of the wall of the mature spermatophore.
OW : outer wall (from arrow to arrow). ×900.

Fig. B. "Sperm rod" in the mature spermatophore. ×2000.

Fig. C. Transmission electron microscopic photograph of the sperm head in the mature spermatophore. ×16000.

Fig. D. Distal end of the penis. Many stripe-like rows of microvilli on the inner surface of the penis. ×38.


OW
A
B
C
D

Explanation of Plate 13

Fig. A. High magnification photograph of the head of spermatozoon (arrow) in the mature spermatophore. ×6000.


A