

Electron Microscopic Study on the Spermatogenesis of Chimaera, *Chimaera phantasma**¹

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Comparing with the fine structure of spermatogenic cell of selachians and other vertebrates, that of the chimaera was observed with light and electron microscopes. The origin of chimaera testis differed essentially from that of selachians. However the chimaera had many resemblances to the selachians in the formation of the spermatozoon, except for the following two points; first, the X organ and the axial midpiece rod of the neck were amorphous and, second, there was only one accessory on the tail flagellum.

With the advancement of the study on selachians, various special characteristics on the function and structure of organs and tissues have come to be understood. Studies on chimaeras belonging to the cartilaginous fishes, however, are scarce and it is very interesting whether the spermatogenesis are common to chimaeras or not like above-mentioned characteristics. The arrears, especially, in the study on the ultrastructure are conspicuous. Some light microscopic observations on the spermatogenesis of selachians have been already reported (Matthews 1950; Chen et al. 1973) and there was little difference in the spermatogenesis between selachians and chimaeras except the origin of testis. As the observations on the spermatogenesis of selachians using the electron microscope have increased (Stanley, 1964, 1966, 1971a, 1971b; Sandborn, 1970; Mattei, 1978; Hara, 1978; Tanaka et al., 1978), the structure of a spermatozoon and details of the spermatogenesis have been made clearer. These studies pointed out that the structure of spermatozoon and spermatogenesis in selachians can be distinguished from those of other vertebrates

such as teleosts, reptiles, birds and mammals. Furthermore, it was confirmed that the structure and developmental process of a spermatogenic cell of selachians resembled other vertebrates more than teleosts although teleosts have a closer affinity in classification (Tanaka et al., 1978). Following these findings, chimaeras being closest in affinity to selachians are compared with selachians and other vertebrates instead of teleosts in this study.

The authors secured some fresh specimens of the testes of chimaeras, *Chimaera phantasma*, and observed these ultrastructure by light and electron microscopes. In this paper, the details of the spermatogenesis of this species are described.

Materials and Methods

Most specimens used in this study were caught by bottom long line for tilefishes, *Branchistegus* spp., at 200-300m depth in the coastal waters of Nagasaki, while some were caught by the deep trawl net off Shizuoka. Fishermen usually release these fish after capture because the their market

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value in Nagasaki is not so high. Sometimes, however, this fish is sold at the market for at low price. The rearing of this fish for an extended period was so difficult because healthy specimens could not be secured from the catch of bottom long line. The hooked fish when pulled up rapidly from about two hundred meters depth, do not live long. It was more difficult to get the fresh materials in summer, since the fish was alive only for a few hours after capture.

In May 1980 among chimaeras caught by a small bottom long line boat at Mogi, suburb of Nagasaki City, three individuals were landed alive. These live specimens with the hook not removed were transported to the laboratory of the Faculty of Fisheries where they could be fortunately kept alive for a few days in each tank (51 x 36 cm, 31 cm depth). After completing the preparations of pre-fixing agent for electron microscopic samples, the testes were immediately picked out from live specimens and were fixed for examination. These adult specimens were 560-605 mm in body length (see Malagrino et al., 1977). The pre-fixation was made with a mixed solution of Karnovsky's glutaraldehyde-formaldehyde and Millonig's buffer. The post-fixation was made with a mixed solution of 2% osmic acid and Millonig's buffer. Fixed materials were dehydrated by ethanol, embedded in epon by means of Luft after transposition into propylene oxide and were made into blocks. These blocks were sliced into sections by LKB-V type and Poter- II type ultramicrotomes and stained by toluidine blue for light microscopic observation. The region for the electron microscopic viewing was decided by observation and after trimming, the sections were sliced by a diamond knife. The specimens were stained twice by uranyl acetate for 20 min and lead citrate for 3 min at normal room temperature. Finally, the cells were observed and photographed by an electron microscope (JEM-100CX). Using light microscope, the specimens, picked out from the testes fixed by formalin and stained twice by eosin and haematoxylin, were also observed.

Observations and Discussions

1. Light Microscopic Observation

The spermatogenesis of chimaera resembled that of selachians. The origin of the testis, however, differed essentially from that of selachians. In selachians the testis is formed at the anterior tip of epigonal organ on each side and at maturity the testis occupies the whole part of epigonal organ. On the other hand, chimaeras do not have the epigonal organ. The internal tissues of the gonad examined showed that of the testis at the juvenile stage. The testis increases in its weight in proportion to their growth like many other vertebrates, and its weight increases rapidly just before sexual maturity (Malagrino et al., 1981).

The testis of this species examined was of seminiferous follicles type. Spermatogonia appeared in the basement membrane of the follicle and thrust the seminiferous epithelium up to the lumen (Fig. 1). Spermatogonia divided repeatedly, transformed and filled up the follicle (Fig. 2). Spermatogonia in the same follicle developed in the same stage and about 64 spermatozoa were formed in a clump. Each clump turned the head to the basement membrane of the follicle, the tail flagellum to the lumen and then afterwards a sperm ball was formed (Fig. 3). Most clumps consisted of 64 spermatozoa while some had as few as 56. Each follicle had about 250 clumps and produced about 16,000 spermatozoa. Stanley (1966) reported the same observation that most clumps consisted of 64 spermatozoa in selachians. For spotted dogfish, *Scyliorhinus caniculus*, a follicle produced about 32,000 spermatozoa and about 16,000 in skate, *Torpedo marmorata* (Stanley, 1966). The chimaera produced almost the same amount of spermatozoa from a follicle as selachians. Spermatogenic cells, mantled and developing in the follicle, transferred from the abdominal side to the dorsal side of the testis. A follicle in contact with a spermiduct, transferred the spermatozoa to the duct. As a result of the transfer, the follicle is emptied and degenerated (Fig. 3 and 4).

2. Electron Microscopic Observation

2-1. Spermiocytogenesis

The nucleus of the spermatogenic cell at spermatogenesis was covered by the thick cytoplasm containing considerable amount of mitochondria (Fig. 5) and chromosomes in the nucleus were observed clearly during the nuclear division (Fig. 6). It seems that divided spermatogenic cells connect with each other by the intercellular bridge and develop in the same state as in selachians and mammals (Fig. 7). It seems that this intercellular bridge exists for a long time from spermatogenesis to just before the completion of the spermatozoon through spermatohistogenesis. Similar to selachians during the division, a large irregular nucleus and a sertoli cell consisting of thin cytoplasm in a spermatogenic cell were observed (Fig. 8).

2-2. Spermiohistogenesis

The spermatogenic cell changes to the spermiohistogenesis stage through the division of spermatogonia and the appearance of the Golgi apparatus in the cytoplasm (Fig. 9).

DEVELOPMENT OF ACROSOMAL VESICLE AND FORMATION OF HEAD CAP

After the Golgi apparatus appeared in the cytoplasm of the spermatogenic cell, the circular acrosomal vesicle appeared nearby this apparatus (Fig. 10). In nucleus, nuclear granules gathered in the acrosomal vesicle side and the electron density of a part of the nuclear membrane became higher than the other part (Fig. 11). Mitochondria dispersed in cytoplasm concentrated near the acrosomal vesicle. With the acrosom-nuclear adhesion, high electron density and uniform belt shape layer were formed in its adhesive part. This layer expands over the nuclear membrane. The nucleus adhered firmly to the acrosomal vesicle and became hollow by the pressure from the acrosomal vesicle at the adhesive part (Fig. 11-13). Moreover, granules were observed in the acrosomal vesicle in chimaera, although absent on squalen shark, *Centrophorus atromarginatus* (Tanaka et al., 1978) (Fig. 11-13). The layer (Fig. 11), expanding in the belt shape of the adhesive part between nucleus and acrosomal

vesicle, became the head cap with the rapid increase of the nuclear granules and then covered the anterior of the nucleus (Figs. 13 and 25-a). Before and after the appearance of the acrosomal vesicle, centrioles appeared in the cytoplasm near the opposite pole (Fig. 10). Two centrioles formed at a right angle to each other (Fig. 14); The one near to the nucleus is called the proximal centriole and the other farther is called the distal centriole. The centrioles showed a general structure composed of nine triplet microtubules and each triplet was inclined about 30° to its transverse section.

FORMATION OF HEAD

After the acrosome-nuclear adhesion, nuclear granules began to develop completely to metamorphosis (Fig. 11). By the time the head cap covered half of the nucleus, the nuclear granules increased in density, and the electron density became high (Fig. 15). Developed spot shaped nuclear granules began to show the needle shape (Fig. 16-18) while the circular nucleus transformed to a long and slender shape. The needle shaped granules were compressed lengthways and the high electron density head was completed before long (Fig. 24 and 25-b)

FORMATION OF NECK

At the same time the nuclear granules in spermatogenesis developed in its spotted state, an organ originating in the proximal centriole appeared (Fig. 19). This organ is the same organ as one observed in squalen shark and is called the X organ (Fig. 19). After the appearance of the X organ, another structure along side and adhering to it was observed. This becomes the central pole of the neck of spermatozoon and is called the axial midpiece rod (Fig. 19). These two structures were also observed in selachians. The X organ is amorphous and the axial midpiece rod is structural as described by Tanaka et al. (1978). This structure was formed like a pile of thin disk and its longitudinal section showed the stripe-pattern of light and shade. In chimaeras, both structures of the X organ and the axial midpiece rod were amorphous and of

medium electron density (Fig. 19). It was difficult to distinguish these two organs in chimaera because the axial midpiece rod was not structural and different from that of selachians. The X organ existed only at the earlier stages of the metamorphosis of the spermatogenic cell like selachians and it seemed that the structure as shown in Fig. 20-22 surrounded the X organ at this stage. The X organ and the structure surrounding it disappeared after the change in the position of the axial midpiece rod (Fig. 23). It was recognized that the axial midpiece rod was an organ closely connected to both the proximal centriole and the distal centriole, and that they became the neck after making an inroad into the head (Fig. 25-c). The distal centriole was located at the end of the neck and the tail lengthened from there (Fig. 24, 25-e and h). About the time when the neck was completed, all mitochondria in the cytoplasm concentrated and arranged in a single layer around the neck (Fig. 25-d, e, f and g). The neck was shorter and slender than the head (Fig. 24 and 25-g). Among the species of selachians, length and thickness of neck have a little difference. For example, on the narrow-headed seven gill shark, *Heptranchias perlo*, the neck is very slender and the thickening around the mitochondria is almost the same in size as that of the head (Fig. 28 and 29). Moreover, the structure of the blue shark, *Prionace glauca*, is the same as that of the narrow-headed seven gill shark. As described by Tanaka et al. (1978), the thickness of the neck of squalen shark, *Centrophorus atromarginatus*, however, is almost the same as that of the head like the deep-sea luminous shark, *Etmopterus lucifer* (Fig. 32). These various necks play equally some important roles in the movement of

spermatozoon. It was considered that the neck of chimaera was also flexible and elastic, and contributed greatly to the ability of the spermatozoon's tail flagellum movement. Moreover, like selachians, the neck was the most distinctive characteristic.

APPEARANCE OF ANNULUS

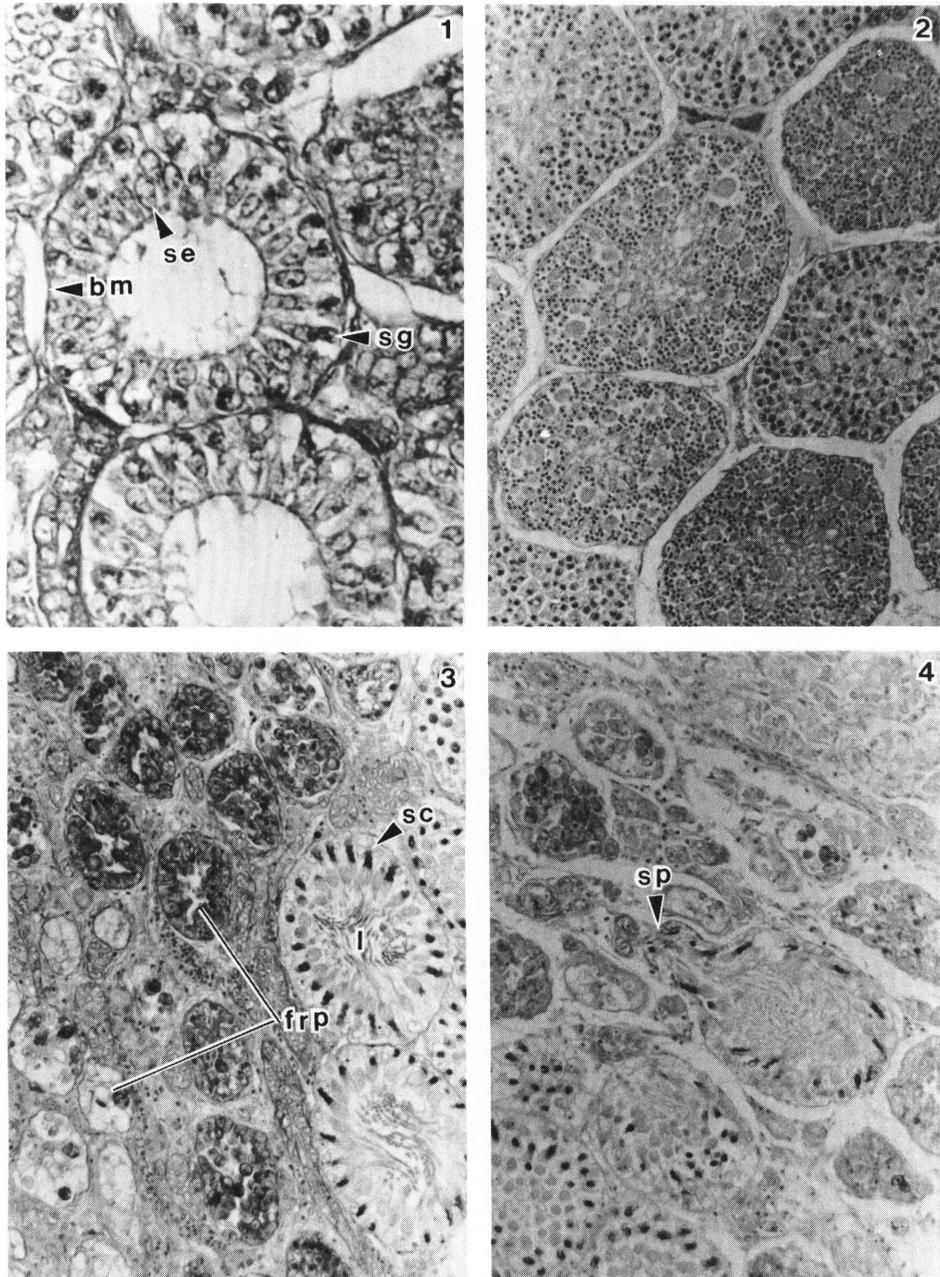
From the observations made to date on the spermatogenesis of selachians, it has been recognized that the annulus appears at the considerably developed stage of metamorphosis in the spermatogenic cell. It was found from this observation, however, that the annulus appeared at an earlier stage in chimaera, i. e. it had already appeared along the cell membrane at the earlier stage of spermiogenesis when the centriole appeared (Fig. 10). There were small ridges on the surface of annulus as shown in Fig. 10 and these small ridges disappeared when the annulus was completed. The annulus was observed at the boundary between neck and tail flagellum (Fig. 24, 25-e and h). Since the annulus was located at the end of the neck, it could be said that the annulus prevented the mitochondria arranged around the neck from moving toward the tail flagellum (Fig. 24-i).

FORMATION OF TAIL FLAGELLUM

AT the stage of the appearance of the centriole in the cytoplasm, the tail flagellum developing from the distal centriole appeared earlier than the appearance and formation of the neck. It projected out of cell and grew longer (Fig. 10). The tail flagellum of the chimaera showed a general structure similar to that of the selachians. The villus and flagellum were the same; a pair of axial filaments on the center and nine pairs of them around

Explanations of Figures

af-axial filament, av-acrosomal vesicle, amr-axial midpiece rod, an-annulus, bm-basal membrane, c-cytoplasm, ce-centriole, cf-outer columnar fiber, ch-chromation, cm-cell membrane, dc-distal centriole, frp-follicle in involution period G-Golgi apparatus, h-head, ib-intercellular bridge, l-lumen, m-mitochondria, n-nucleus, ng-nuclear granules, ns-nuclear sheath, pc-proximal centriole, sc-sperm clump, se-seminal epithelium, sg-spermatogonium, so-sertoli cell, sp-spermiduct, spc-spermatogenic cell, tf-tail flagellum, x-X organ.



- Fig. 1. The seminiferous follicles of *Chimaera phantasma* at early stage at x 370; Spermatogonia developed from the basal membrane appear.
- Fig. 2. The spermatocytes formed by multiple divisions of spermatogonia and filled in follicle at x 90.
- Fig. 3. A section through late follicles in the dorsal region of the testis at x 90; Sperm clumps consisted of completed spermatozoon and follicle in involution period are shown.
- Fig. 4. A section through late follicles in the near region with in Fig. 3 at x 90; A follicle is releasing its spermatozoon into the spermiduct.

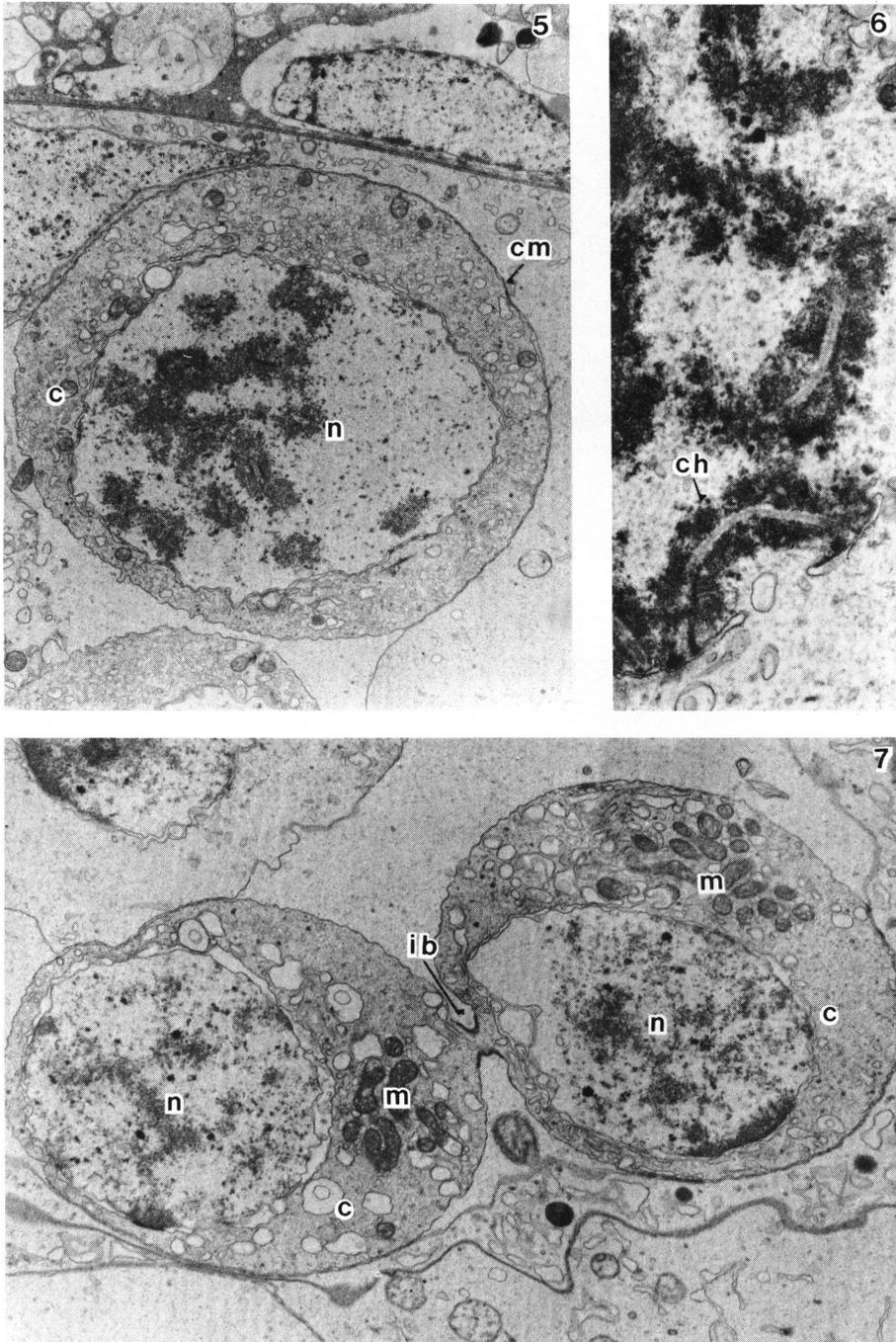


Fig. 5. The spermatogenic cell in the period of spermiocytogenesis at x 6,200;

Fig. 6. A section through a nucleus at x 22,000; Chromatins are observed clearly in the nucleus during the nuclear division.

Fig. 7. A section through the spermatogenic cells connected with the intercellular bridge each other at x 9,500; The intercellular bridge plays the important role for the development on the same state.

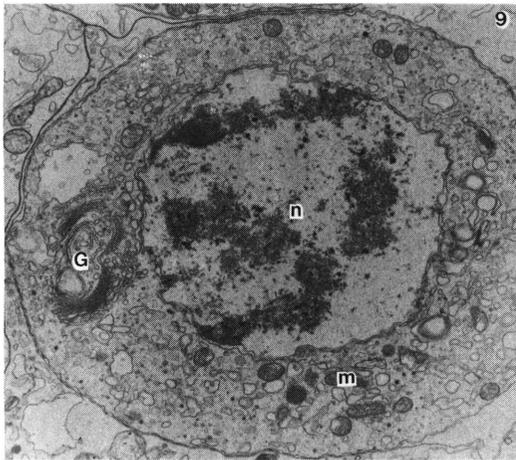
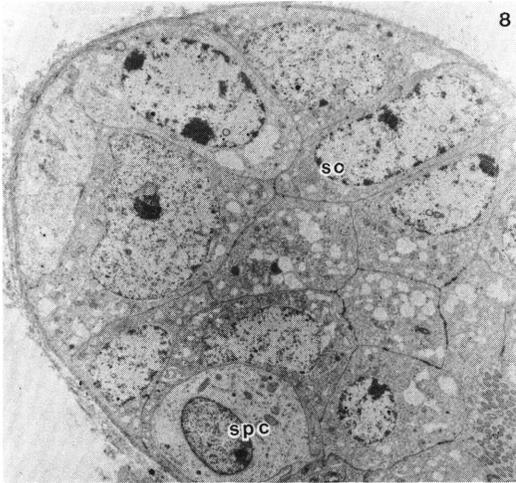


Fig. 8. Spermatogenic cell and sertoli cell at x 2,700; A large irregular nucleus and sertoli cell consisting of thin cytoplasm are observed during division.

Fig. 9. A section through a spermatogenic cell of later stage than that in Fig. 8 at x 2,300, Golgi apparatus appears in cytoplasm and many mitochondrias are seen.

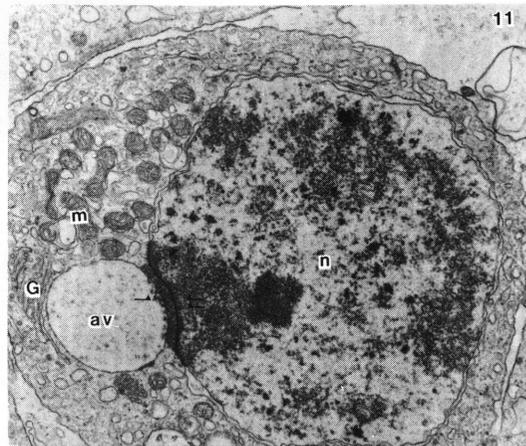
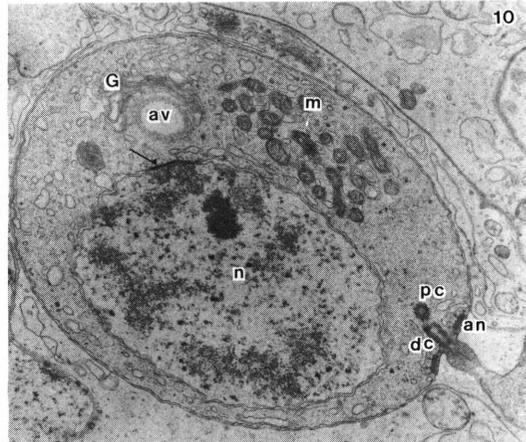
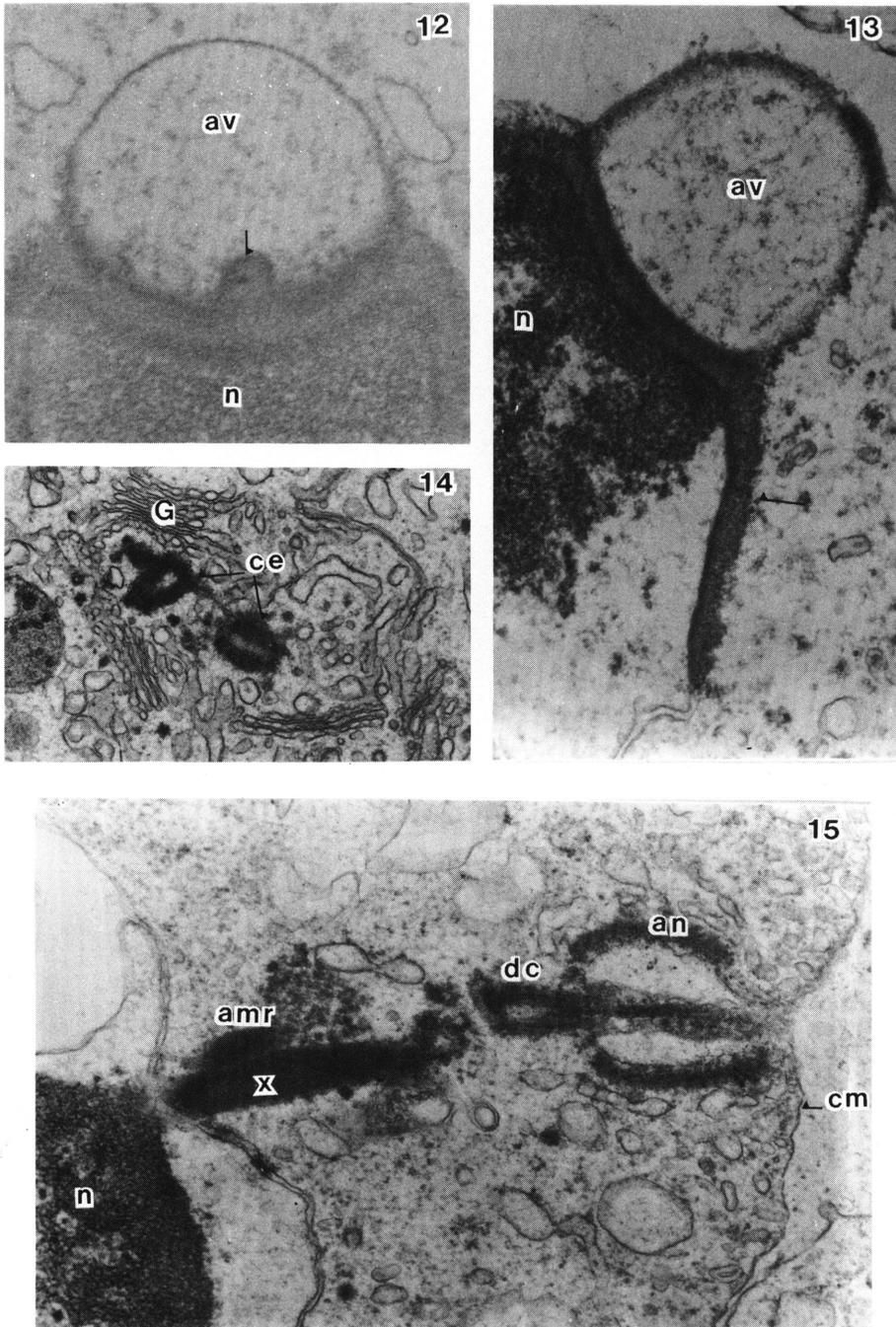


Fig. 10. A section through a spermatogenic cell at x 9,300; Acrosomal vesicle and centrioles appear in the cytoplasm.

Fig. 11. A section through a spermatogenic cell at x 10,500; Acrosomal vesicle adheres to nucleus and nuclear chromatin forms a coarse network (\rightleftharpoons) attached to the acrosom-nuclear adhesion. Granules (\leftarrow) are also seen in acrosomal vesicle.



- Fig. 12. A section through the acrosom-nuclear adhesion at x 26,500; Accumulation of glanules (←) is shown on adhesion site.
- Fig. 13. A section through the spermatogenic cell at x 30,000; Fibrous nuclear sheath (←) covers anterior tip of head.
- Fig. 14. A section through the two centrioles at x 24,500; These centrioles form at a right angle to each other.
- Fig. 15. A section through the axial midpiece rod, X organ, centriole and annulus at x 24,500; Nuclear glanules increase in density and head cap covers half of the nucleus.

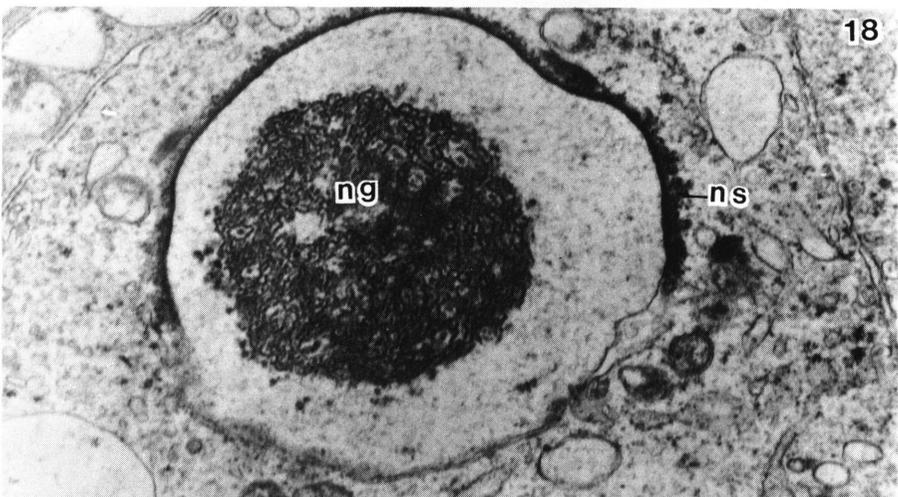
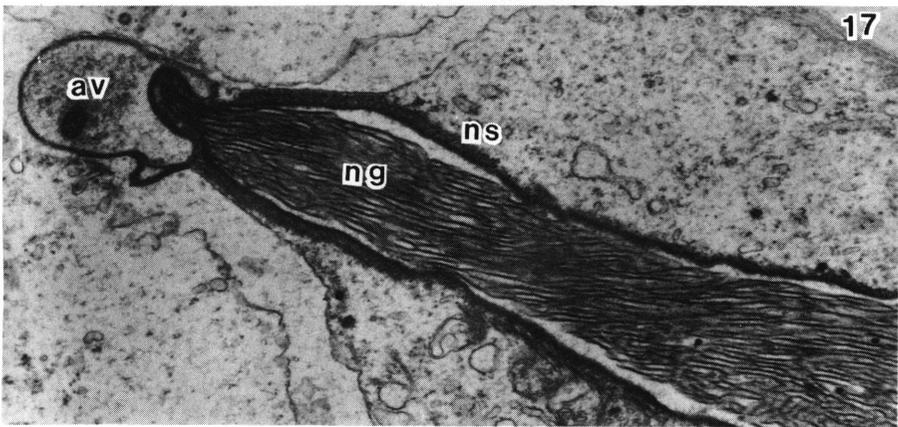
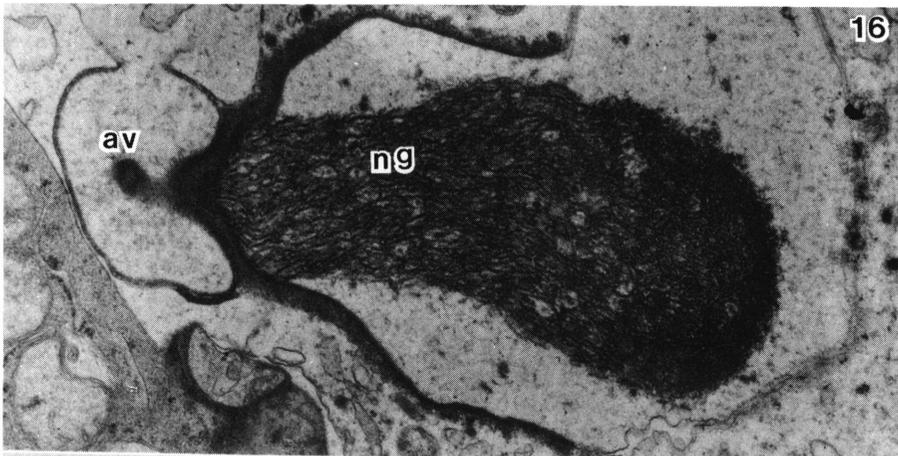


Fig. 16. A section through the acrosomal vesicle and nucleus at x 20,000; Nuclear granules begin to show the needle shape.

Fig. 17. A section through the acrosomal vesicle and nucleus of later stage than that of Fig. 16 at x 14,000; Nucleus and nuclear granules elongate and nuclear sheath develops.

Fig. 18. A transverse section through the nucleus of spermatogenic cell of almost same stage as in Fig. 16 at x 17,000;

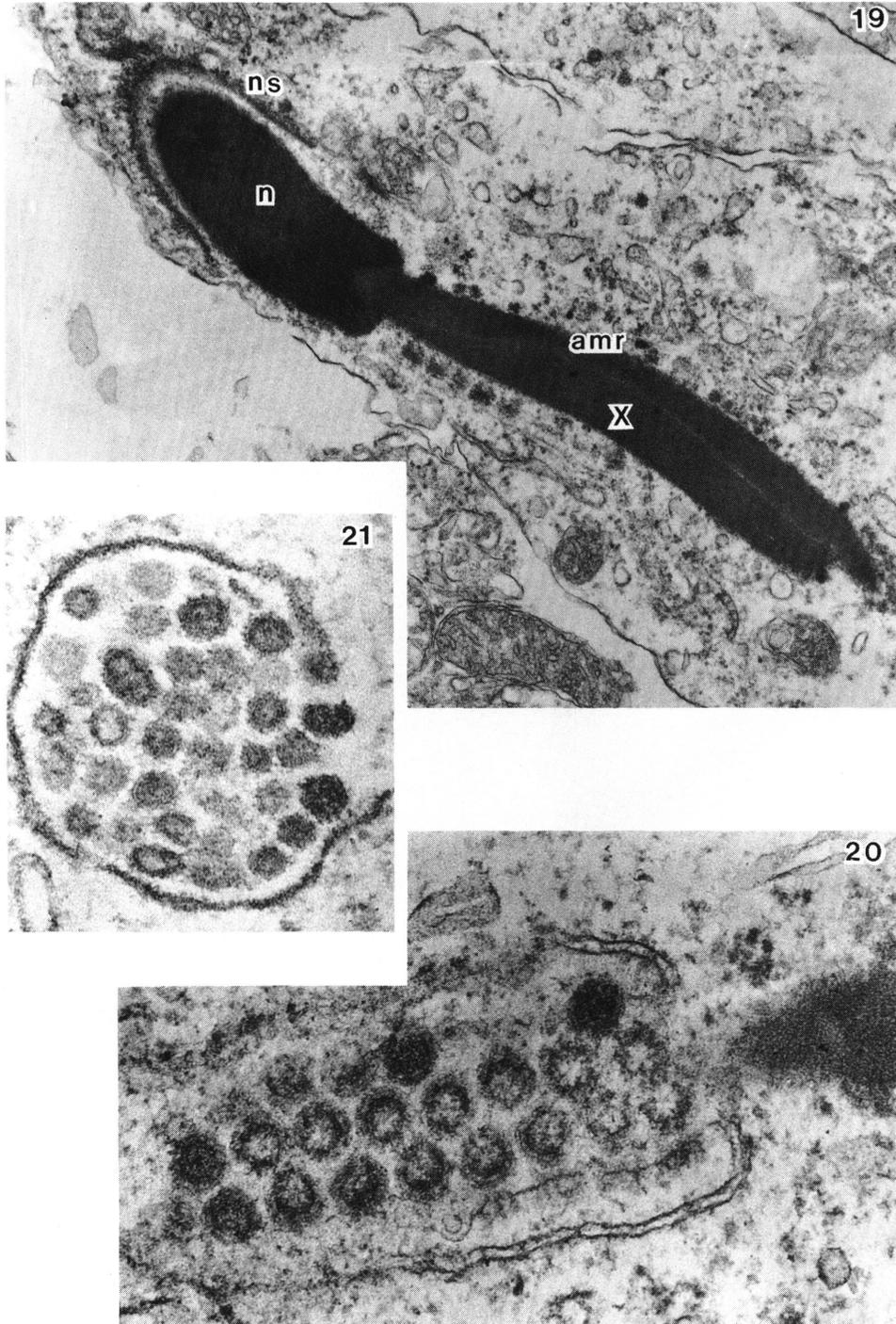


Fig. 19. A section through a portion of the axial midpiece rod and X organ at x 27,700; These two organs are twisted about each other.

Fig. 20. A section through the structure at the early stage of the neck development at x 51,500; This structure surrounds the X organ.

Fig. 21. A transverse section through the structure shown in Fig. 20 at x 98,000.

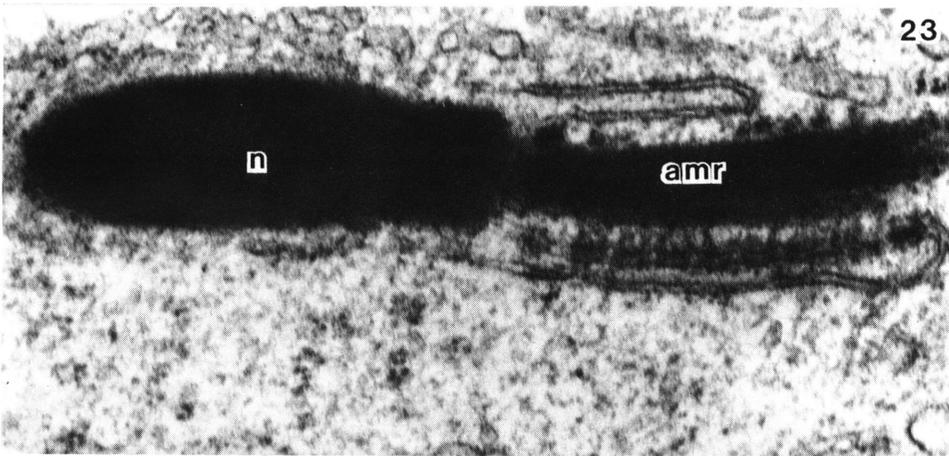


Fig. 22. A section through the structure shown in Fig. 20 and 21 at x 46,000; This structure disappears after the change in the position of the axial midpiece rod.

Fig. 23. A section through the axial midpiece rod at x 57,000; The neck is almost completed.

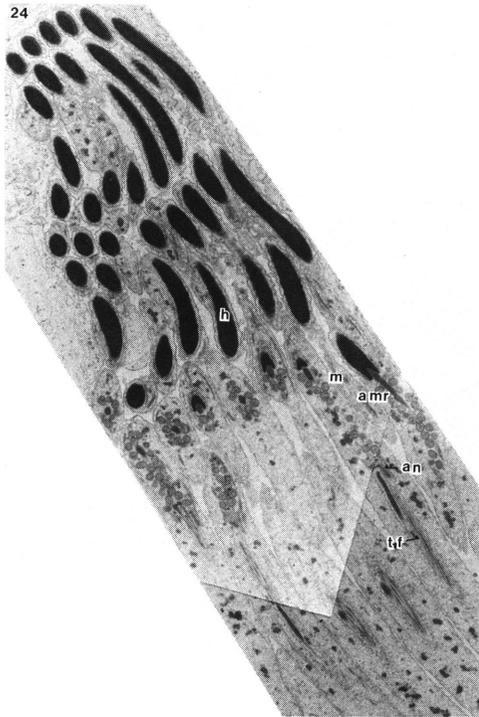


Fig. 24. Longitudinal section through a bundle of completed spermatozoa at $\times 5,000$; Annulus is observed at the boundary between neck and tail flagellum.

the center in transverse sections (Fig. 26 and 27). It was recognized from the longitudinal section (Fig. 24) and also from the transverse section (Fig. 27) that on this long tail flagellum a high electron density microtubule twisted about these axial filaments at the part near the neck and made only one accessory. In contrast to this, there are two accessories on selachians. For example, the squalen shark has a pair of accessories on both sides of the axial filament. Moreover, there are a few differences between the dimension and the structure of accessories among the species considered. Like selachians, the accessory is not present at the posterior part of tail flagellum, instead, only an axial filament is present.

ON THE SERTOLI CELL

Observing the clump of completed spermatozoa and the basement membrane of the seminiferous

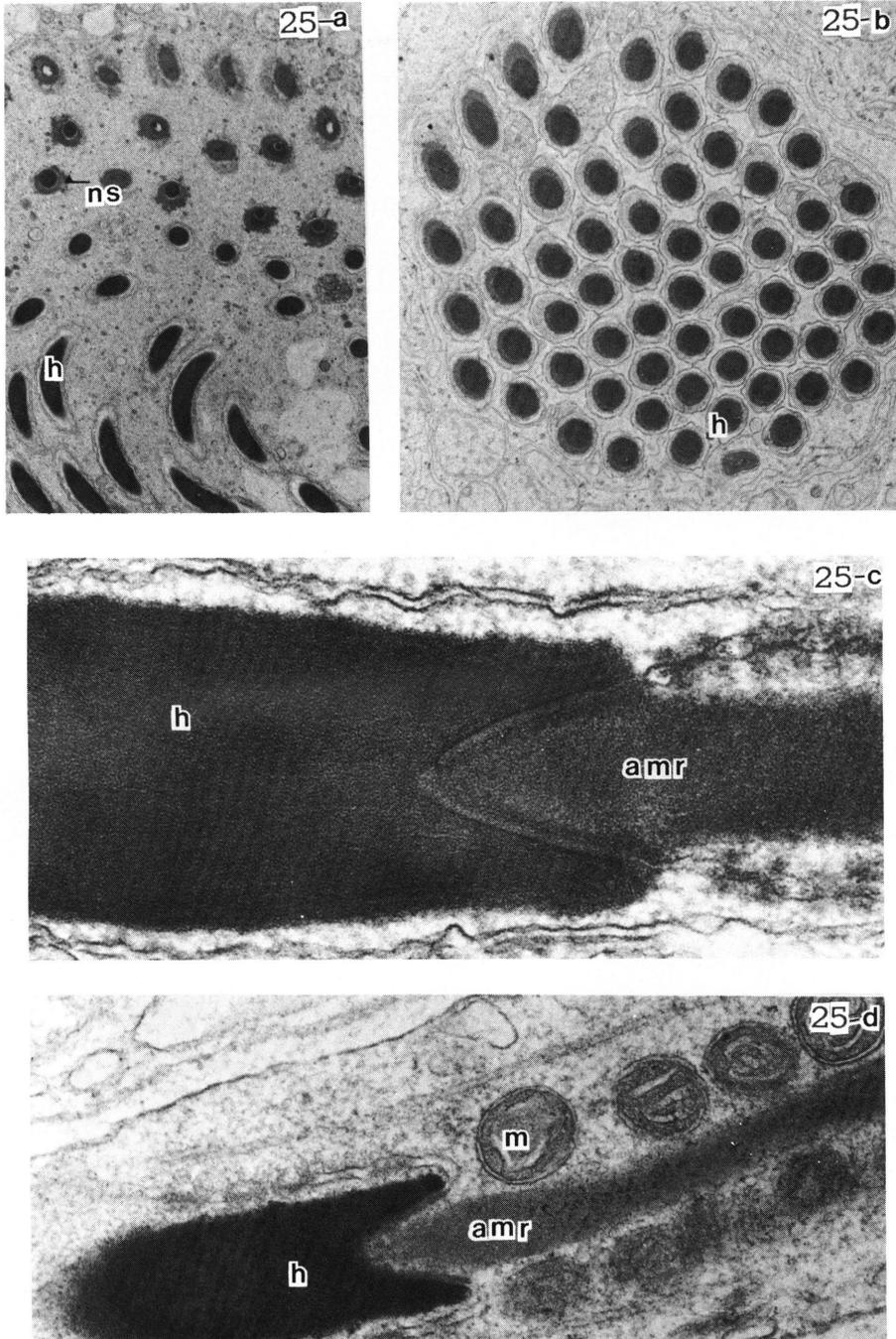
follicle, there was a large indeterminate nucleus (Fig. 33) just above the clump. A membrane forms a line of demarcation between the bordering clumps. It was conjectured from these observations that some sertoli cells stand in a row along the basement membrane. Sertoli cell might be connected with each other and a tight junction might exist. Depending on the mammals, it is usually for certain that the tight junction exist. This junction requires further study.

3. Scanning Electron Microscopic Observation

The general method for understanding the details of structure of spermatozoa is to observe the thin sections with the transmission electron microscope, although it is difficult to understand the structure of the whole spermatozoon with this method. Then, the spermatozoa were observed with the scanning electron microscope. As shown in Fig. 35, a spermatozoon consisted of a little twisted head, a little thicker neck than that of head and a long tail flagellum. The length of each part was quite long compared with that of mammals and teleosts.

The head tapered to a point at the anterior end and its thickness was uniform at the middle and posterior parts (Fig. 35 and 25-g). The neck was surrounded by mitochondria and was thicker than head. Consequently, the thickness of the neck, observed with the scanning electron microscope, differed from that observed with the transmission electron microscope (Fig. 24). The tail flagellum was slightly slender and longer than the head and was twisted round at the end. From the observations on the thin sections of testis, it seemed that the whole spermatozoon was considerably twisted. The spermatozoon was, however, twisted two or three times loosely at the head part. In the case of smooth dogfish, *Mustelus griseus*, the head was twisted seven or eight times and the tail flagellum was also twisted more than that. Fixation or the other preparation may lead to some questions about the structure of the spermatozoon of chimaera.

Spermatozoa intertwined with each other in the



- Fig. 25-a. A transverse section through the anterior region of head at x 12,500; Head is covered with the nuclear sheath.
- Fig. 25-b. A transverse section of the heads almost completed at x 10,500; Nuclear granules are compressed lengthways and electron density of head is so high.
- Fig. 25-c. A longitudinal section through the head-midpiece junction area at x 80,000; Axial midpiece rod becomes the neck after making an inroad into the head.
- Fig. 25-d. A longitudinal section through the completed head and neck at x 43,000; All mitochondria is arranged in a single layer around the neck.

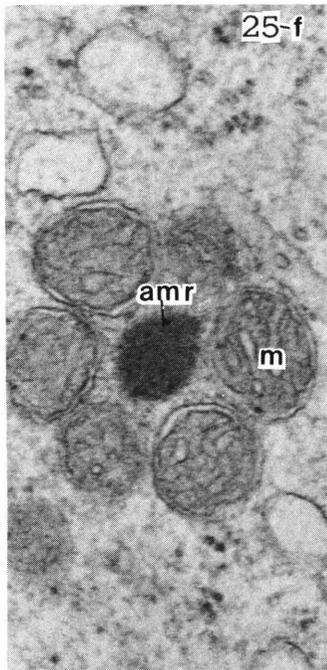
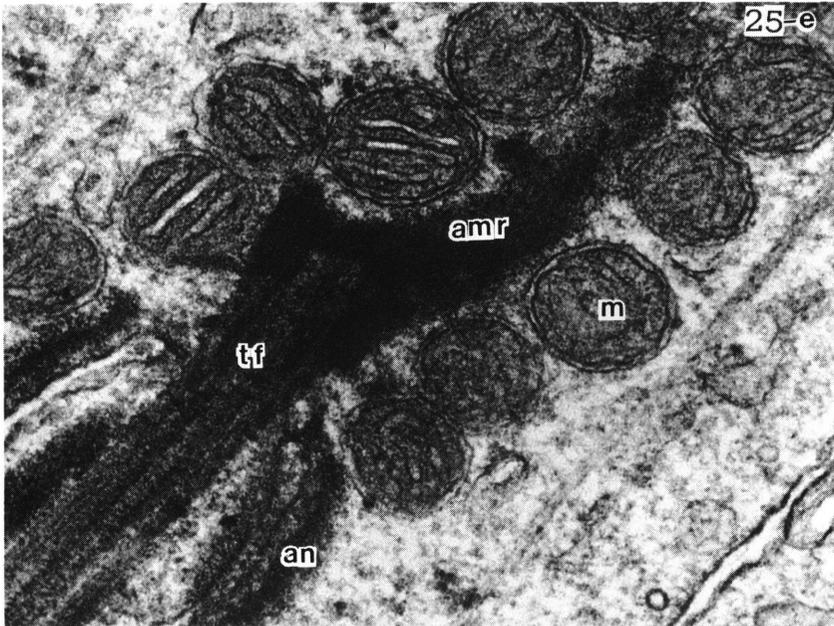


Fig. 25-e. A longitudinal section through the completed neck-tail junction area at x 60,000; Mitochondria surround the axial midpiece rod and the annulus is observed at this junction area.

Fig. 25-f. A transverse section of the neck at x 27,000; Axial midpiece rod in surrounded by mitochondria.

Fig. 25-g. A transverse section of the completed neck at x 95,000;

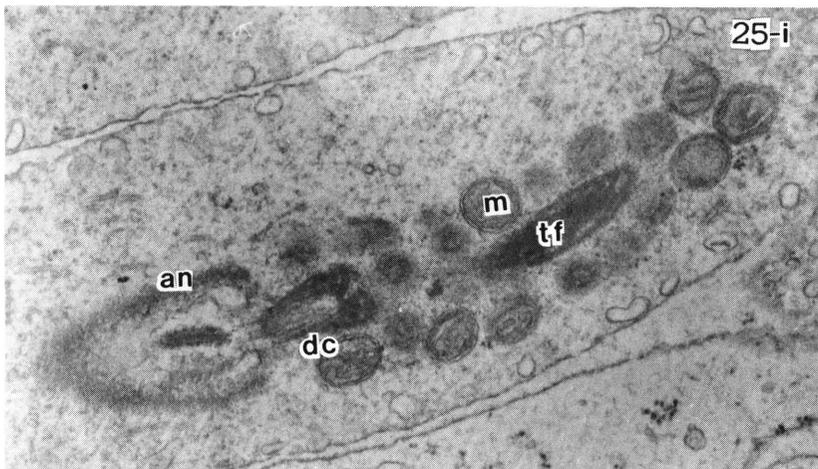
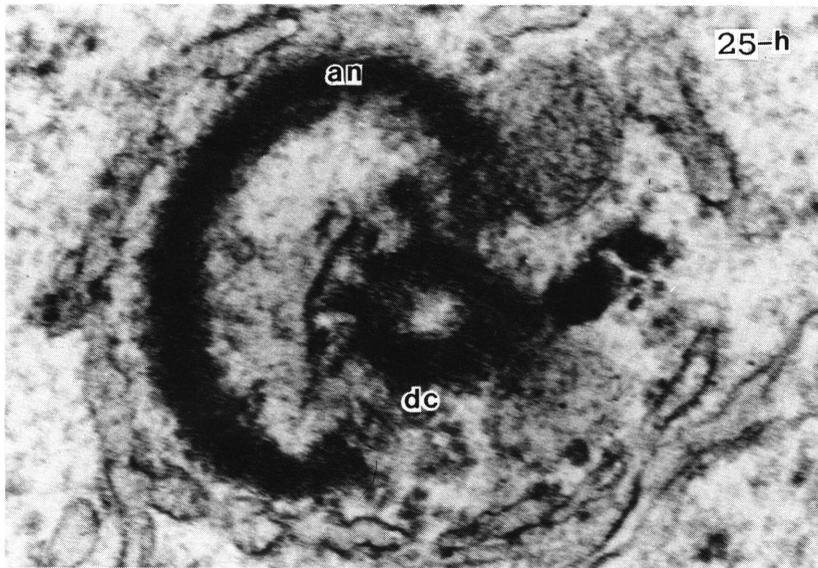


Fig. 25-h. A transverse section through the annulus and distal centriole at x 72,000;
Fig. 25-i. A longitudinal section through the annulus at x 24,000

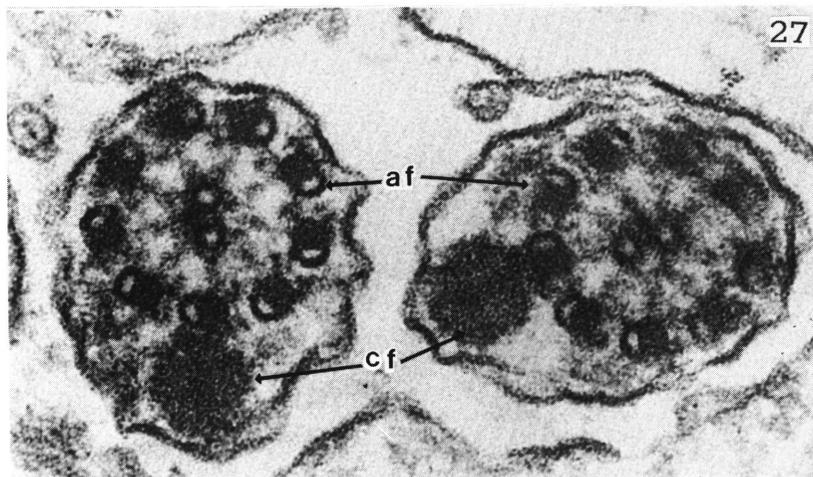
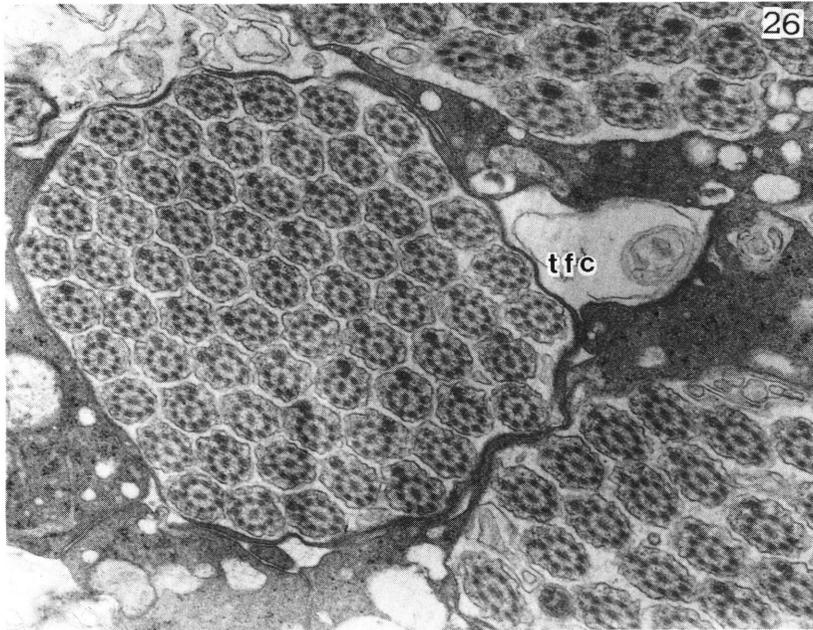
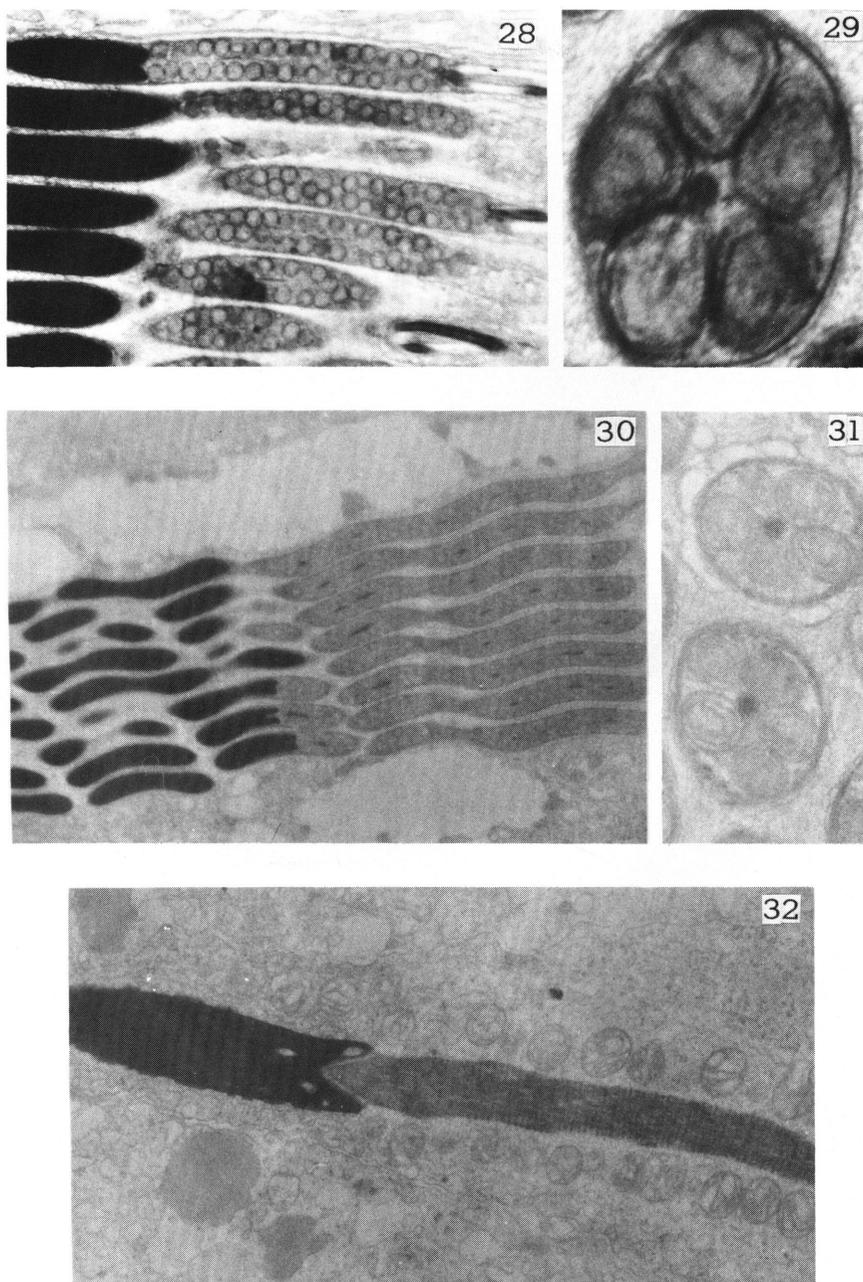


Fig. 26. A transverse section through the tail of sperm clump at x 19,000; A pair of axial filament on center and nine pairs of that around the center are seen.

Fig. 27. Enlarged photograph of Fig. 26 at x 120,000.



- Fig. 28. A longitudinal section through the head-neck junction area of *Prionace glauca* at x 10,000; The neck is very slender and thickening around the mitochondria is almost same in size as that of the neck.
- Fig. 29. A transverse section through the neck of *Prionace glauca* at x 59,000;
- Fig. 30. A longitudinal section through the head-neck junction area of *Heptranchias perlo* at x 3,200;
- Fig. 31. A transverse section through the neck of *Heptranchias perlo* at x 7,300;
- Fig. 32. A longitudinal section through the head-neck junction of *Etmopterus lucifer* at x 13,000; The thickness of the neck is almost same as that of the head.

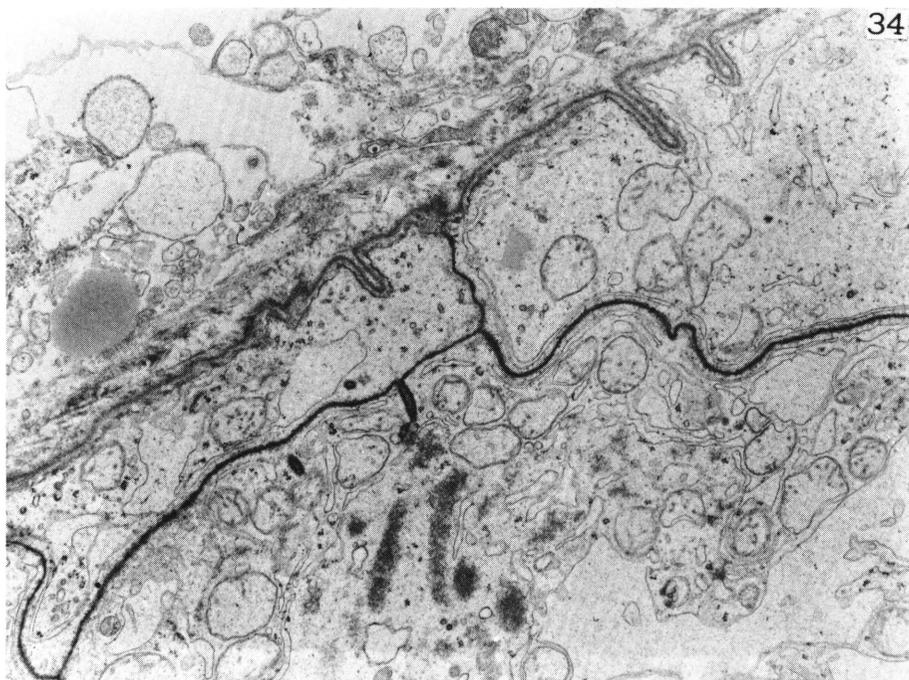
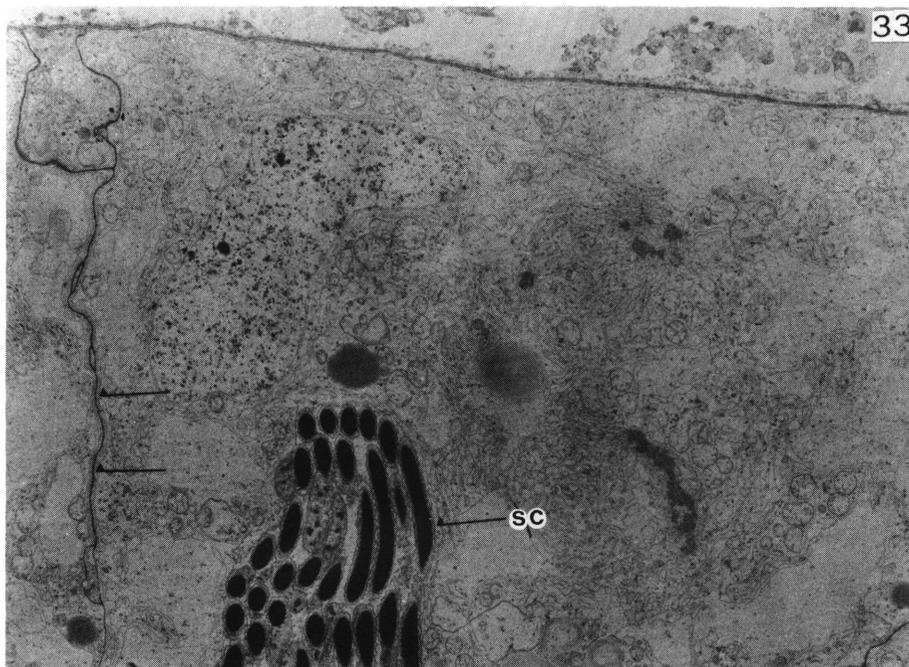


Fig. 33. A section of the basal membrane of seminiferous follicle at x 3,800; the heads of the clump point towards the basal membrane. There is a large indeterminate nucleus just above the clump and cysto membrane (\Leftarrow).

Fig. 34. Enlarged photograph of Fig. 33 at x 10,500;

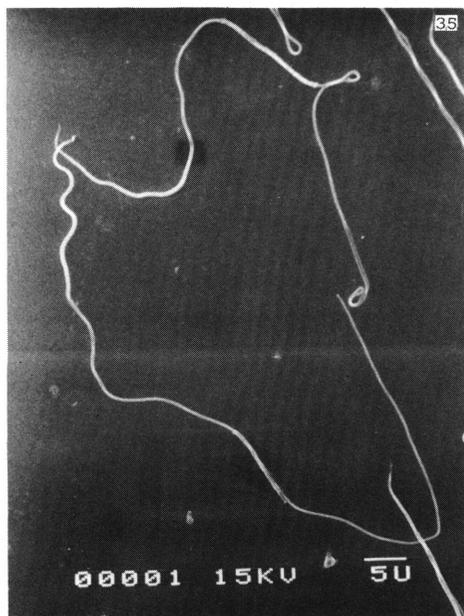


Fig. 35. Scanning electron microscopic photograph of the whole spermatozoon of *Chimaera phantasma*. The spermatozoon is twisted loosely at the head part.

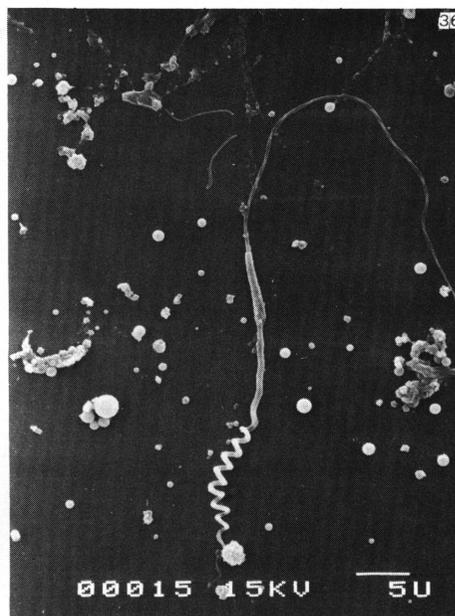


Fig. 36. Scanning electron microscopic photograph of the whole spermatozoon of *Mustelus griseus*. The head is twisted so hardly.

testis make up a clump. Seminal fluid from vesicula seminalis was observed with spermatozoa in clumps was not observed. It would be interesting to find out where these clumps break.

In conclusion, it is considered that chimaeras differ morphologically from selachians as observed in the fine structure on spermatogenesis using transmission and scanning electron microscopes. There is a difference in the structure of the neck between chimaeras and selachians. Furthermore, there is only one accessory on the tail flagellum of chimaeras.

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ギンザメ *Chimaera phantasma* の精子形成について

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ギンザメの精細胞の微細構造について、板鰓類並びに他の脊椎動物のそれと比較しながら、電子顕微鏡的観察を行った。精巢の起源は板鰓類のそれとは全く異なる。精子の形態は次の二点を除いて板鰓類とは多くの類似点を有する。すなわち、その相違点とは、頸部の X organ と axial midpiece rod のいずれもが無構造であること、および尾部のアクセサリーが一つしかないことである。