Japanese Journal of Ichthyology
Vol. 28, No. 2 1981

魚類学雑誌
28巻2号 1981年

The Ultrastructure of Spermatozoa with a Note on the Formation of the Acrosomal Filament in the Lamprey, *Lampetra japonica*

Haruo Jaana and Tadashi S. Yamamoto

(Received January 19, 1981)

Abstract The mature spermatozoon of *Lampetra japonica* is about 130 μm long. The head is rod-shaped, measuring about 8 μm long and 1 μm wide. It bears a membrane-bounded acrosomal vesicle at its anterior end. The anterior surface of the nucleus in the head forms a depression, where a subacrosomal ring is found. The bottom of the depression leads to the nuclear canal. The central fiber, which starts from the posterior surface of the acrosomal vesicle, perforates the subacrosomal ring and runs the entire length of the nuclear canal. The elongated nucleus forms a pouch laterally near the posterior end of the head, where two cylindrical centrioles are visible; the axes of both centrioles coincide with a long axis of the spermatozoon. The tail flagellum shows the 9+9+2 structure known in that of animals with internal fertilization. There is no strictly defined middle piece, but several mitochondria are detected near the centrioles or in regions far from the sperm head.

An acrosomal filament is formed in some spermatozoa when they are subjected to stimulation at the time of fixation: the central fiber is discharged from the nuclear canal and pushes against the posterior membrane of the acrosomal vesicle; this induces the fusion of the anterior membrane of the acrosomal vesicle with the plasma membrane. The acrosomal filament thus formed consists of the central fiber and its surrounding membrane. No changes in the morphology of the subacrosomal ring are detected during the filament formation. We conclude that the acrosome reaction closely resembles that reported in certain molluscs and annelids which have an axial rod in the head.

In 1952 Dan demonstrated that spermatozoa of the sea urchin undergo an acrosome reaction in the presence of homologous egg water and show a particular morphological change. Since that time, the importance of this reaction in the process of fertilization has been reported not only in marine invertebrates (Dan, 1956; Colwin and Colwin, 1961, 1963; Franklin, 1970; etc.) but also in vertebrates (Fawcett, 1975): in most animals, the spermatozoon must first undergo the acrosome reaction in response to the egg environment; otherwise, it would not be capable of penetrating the egg. On the occasion of this reaction some proteolytic enzymes are released from the acrosome, which attack the egg envelope in order to assist penetration.

The structural change of the sperm head following the acrosome reaction divides roughly into two types: (1) in a variety of invertebrate spermatozoa, the acrosome reaction involves the formation of one long filament or several small ones at the anterior end of the head (Colwin and Colwin, 1961, 1963; Takashima and Takashima, 1963; Dan et al., 1964; Niijima and Dan, 1965b); (2) no filament formation, however, occurs in spermatozoa of such vertebrates as amphibians and mammals (Pikó and Tyler, 1964; Yanagimachi and Noda, 1970; Raisman and Cabada, 1977). Although the spermatozoa of bony fish normally bear no acrosomes and therefore do not show acrosome reaction at the time of fertilization (Iwamatsu and Ohta, 1978), particular cases of type (1) in vertebrates have been reported in cyclostomes (Ballowitz, 1905; Kille, 1960) and ganoid fishes (Dettlaff and Ginsburg, 1963); their spermatozoa possess an acrosome at the anterior end of the head and form a long filament after the acrosome reaction.

A morphological change of sperm head at fertilization of lampreys has been observed at light (Kille, 1960) as well as electron microscopic levels (Afzelius and Murray, 1957; Follenius, 1965; Stanley, 1967; Nicander and Sjödén, 1971). Since the formation of a long filament following

the acrosome reaction has not been reported in other vertebrate spermatozoa, observations on the fine structure of lamprey spermatozoa and the process of the filament formation should be useful as an aid to understanding the significance of the acrosomal filament in the penetration of the spermatozoon into the egg. For this purpose the present study was carried out.

Materials and methods

Adult males of the river lamprey, *Lampetra japonica*, were captured in June, 1979, in Barato, suburb of Sapporo, and raised without feeding in a tank containing well water (at about 22°C). In these aerated conditions, they matured and ejaculated semen within ten days. The semen and the testes from these individuals were used in this study.

For transmission electron microscopy (TEM), small pieces of the testes were fixed in 5% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4, for 2 hours at 0～4°C. After rinsing in the same buffer, they were postfixed in 1% osmium tetroxide buffered with cacodylate for 1 hour at 0～4°C. The specimens were then dehydrated through an acetone series and embedded in Epon

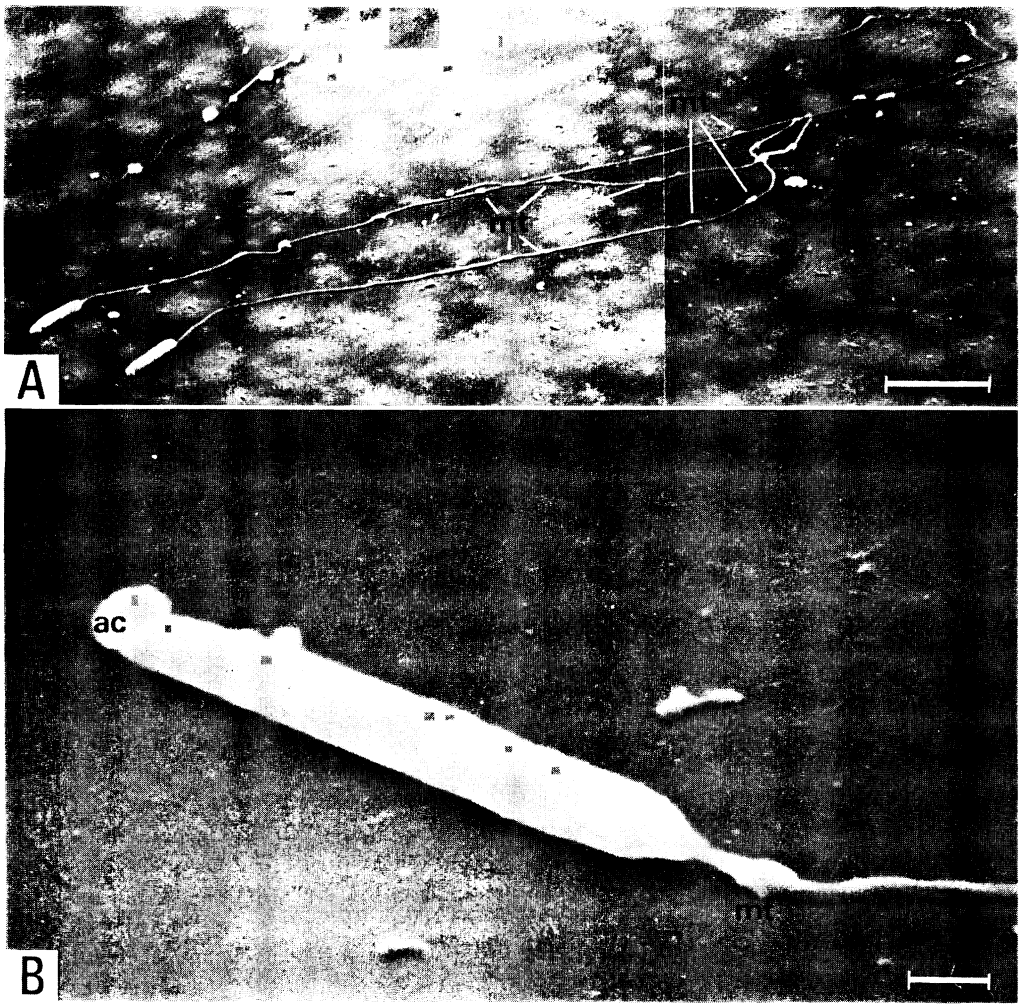


Fig. 1. SEM of the ejaculated spermatozoa. In A, some regions containing mitochondria (mt) are visible along the tail. There is no strictly defined middle piece. The high magnification of the sperm head is shown in B. Note the acrosome (ac) at the anterior end of the head. (A: bar=10 μm; B: bar=1 μm).

812. Sections were cut on a Porter-Blum MT-1 ultramicrotome and stained with uranyl acetate and lead citrate. They were examined in a JEOL JEM-100S electron microscope.

For scanning electron microscopy (SEM), the ejaculated spermatozoa were smeared on cover slips previously coated with poly-L-lysine and fixed in the same manner as described for TEM. They were dehydrated in ethanol and critical-point dried in carbon dioxide. After being coated with a thin layer of gold, the spermatozoa were examined in a JEOL JSM-T20 scanning electron microscope.

Results

The entire length of the spermatozoon is about 130 μm. Its head is rod-shaped and measures about 8 μm in length and about 1 μm in width as observed by SEM (Fig. 1). The anterior one-eighth of the head appears to be separated from the rest by a distinct constriction. Thus, the spermatozoon appears as if it bears a globule at its anterior end. The posterior portion of the head is slender in shape and directly followed by a flagellum measuring about 122 μm in length. No distinct middle piece is observed behind the head.

Longitudinal sections of the sperm head reveal that the anterior globule corresponds to the acrosome, which consists of a membrane-bounded vesicle with flocculent contents having moderate electron density. In some cases, the contents occupy nearly the entire lumen of the vesicle (Fig. 2A), while in others only a small number of particles, probably composed of the same materials, are found to be attached to the inner surface of the membrane of the acrosomal vesicle (Fig. 2B).

Behind the acrosomal vesicle there is an electron dense ring structure, the subacrosomal ring, the side view of which is conical in shape (Fig. 2). Posterior to the ring, the nucleus occupies the bulk of the sperm head. The anterior surface of the nucleus forms a funnel-shaped depression which fits in the posterior end of the subacrosomal ring. The bottom of the depression leads to a narrow blind sac or nuclear canal which is lined with an invaginated nuclear envelope (Fig. 3). A long central fiber, starting from the posterior surface of the acrosomal vesicle and perforating the subacrosomal ring, is found in the canal lumen. It spirals loosely in the mid

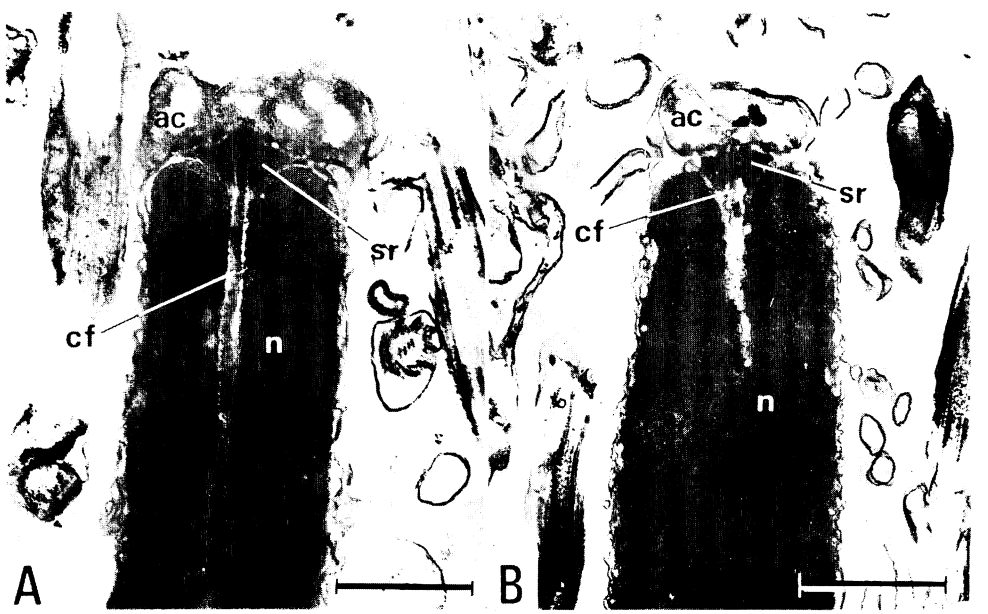
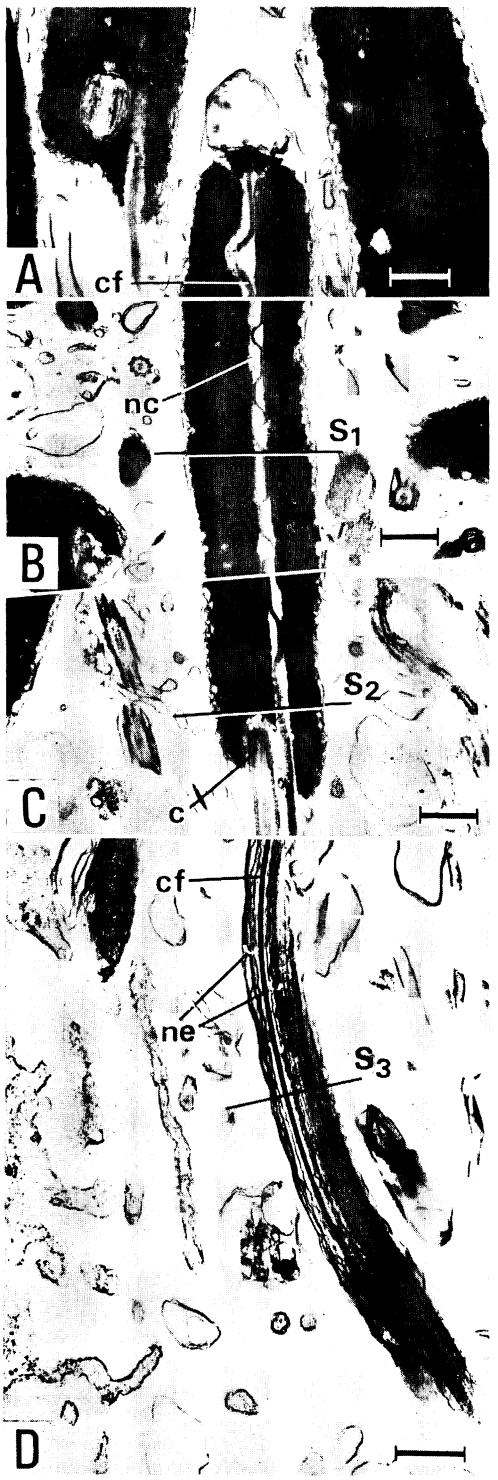


Fig. 2. Longitudinal sections through the anterior portion of the testicular sperm head. The acrosomal vesicle (ac) contains a large amount of flocculent materials in A but possesses a small number of particles in B. Note the central fiber (cf) starting from the posterior surface of the acrosomal vesicle. sr, subacrosomal ring; n, nucleus. (A, B: bar=0.5 μm)



region of the nucleus, where it is round in cross section (about 40 nm in diameter) (Fig. 4A). In the posterior region of the sperm head, however, it runs rather straight and is ellipsoidal in section (about 70 and 30 nm in long and short axes) (Fig. 4B, C). The central fiber extends posteriorly to about 3～4 μm in length from the posterior end of the nucleus containing chromatin (Figs. 3D, 5) and terminates at the blind end of the nuclear canal, where the nucleus contains no distinct chromatin (Figs. 3D, 4C). Since the distribution of chromatin in the nucleus is restricted anteriorly, the acrosome and the nuclear region containing chromatin may be observed by SEM as the sperm head. If the posterior region of the nucleus is also defined as the head of the spermatozoon, the length of the sperm head is about 12 μm, which is 1.5 times larger than that measured by SEM.

At the posterior portion near the chromatin-containing region, the elongated nucleus forms a pouch laterally which holds two cylindrical centrioles (Fig. 6). The axes of these centrioles run parallel with each other and coincide with the long axis of the spermatozoon. Each centriole shows a typical triplet configuration in transverse sections (Fig. 6B). The tail flagellum of the spermatozoon originates from one of these centrioles. From these observations, we can illustrate diagrammatically the fine structure of the lamprey sperm head as shown in Fig. 7.

Although no strictly defined middle piece is recognized by SEM, TEM detected several mitochondria of the spermatozoon. They are round in shape and have cristae in their internal lumen (Fig. 8). In some spermatozoa, these mitochondria are found in the vicinity of the proximal region of the tail flagellum (Fig. 8A),

Fig. 3. Longitudinal sections of the testicular sperm head showing nuclear canal (nc). The canal contains the central fiber (cf). Lines S_1–S_3 indicate corresponding levels of sections illustrated in Fig. 4. c, centriole; ne, nuclear envelope. (A～D: bar=0.5 μm)

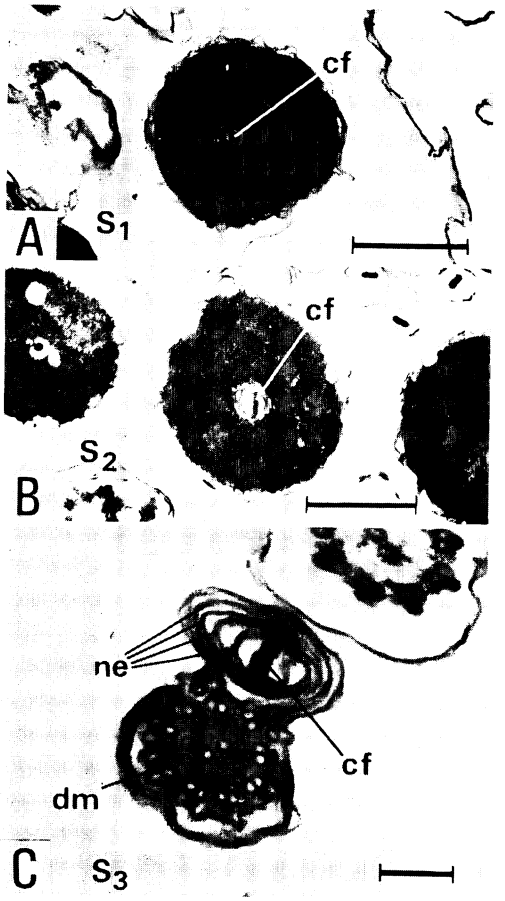


Fig. 4. Transverse sections through the middle (A) and posterior (B) regions of the head and through the proximal region of the tail (C) of the testicular spermatozoa. The cross section of the central fiber is round in the middle region of the head but is ellipsoidal in the posterior region of the head and in the proximal tail. In C, the flagellar components of the tail are seen embedded in a moderately dense matrix (dm). The levels of sections ($S_1 \sim S_3$) are shown in Fig. 3. cf, central fiber; ne, nuclear envelope. (A, B: bar=0.5 μm; C: bar=0.1 μm)

but in others their locus is distant from the posterior end of the sperm head (Fig. 8B, C). The reason why the distribution of mitochondria varies in each spermatozoon is obscure.

The main components of the tail flagellum are nine pairs of fused peripheral microtubules (doublets) and two separated central microtubules. Furthermore, it contains nine dense

Fig. 5. Longitudinal section through the posterior region of the testicular sperm head. Note the central fiber (cf) extending beyond the posterior level (arrowheads) of the nuclear region containing chromatin. (Bar=1 μm)

fibers which are found in contact with the outer surface of doublets (Figs. 6B, 8C), thus showing a 9+9+2 structure. All of these components are embedded in a moderately dense matrix at the level of the proximal tail (Fig. 4C). In the tail region, however, the matrix is electron transparent around the components (Fig. 8C).

Spermatozoa with an unusual long filament at the tip of their head were frequently observed by SEM (Fig. 9). The filament is 30~50 μm in length and about 0.1 μm in width. Since these values are apparently smaller than those of the tail flagellum, the filament is clearly distinguished from the tail. It is clearly the acrosomal filament. It appears that the formation of the acrosomal filament is induced by the stimulation of immersion in fixatives. In order to observe such formation of the acrosomal filament, TEM was also applied in the testicular spermatozoa.

We observed two types of spermatozoa, whose

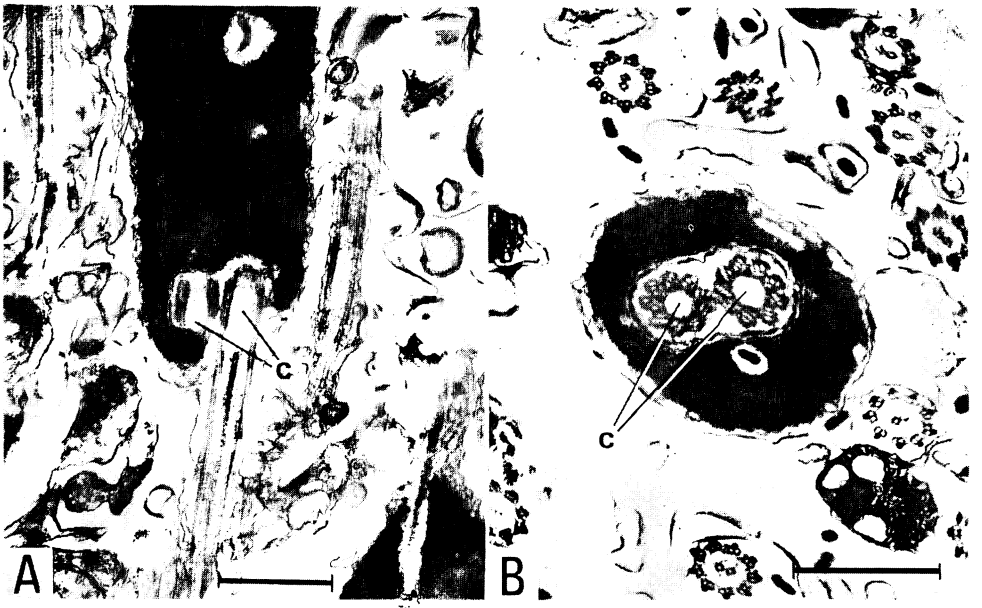
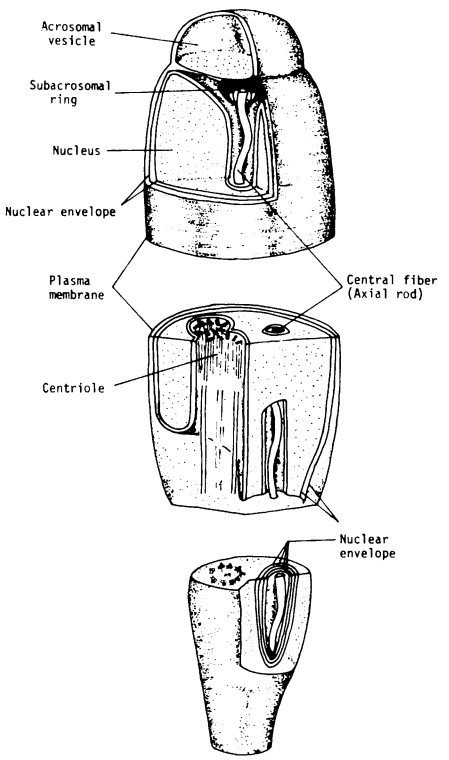


Fig. 6. Longitudinal (A) and transverse (B) sections through the posterior region of the testicular sperm head showing the centrioles (c). The parallel arrangement of two centrioles is clear. Note the typical triplet configuration of the centriole. (A, B: bar=0.5 μm)



acrosomes undergo different processes of reaction to the stimulation. One is shown in Fig. 10. The acrosomal vesicles of these spermatozoa expand in response to the treatment for TEM. With the expansion, the flocculent materials in the vesicle disperse throughout the lumen. Noticeably, a small number of particulate materials attach to the posterior membrane of the acrosomal vesicle (Fig. 10D). The subacrosomal region shows no change during such expansion. In the other type of spermatozoa, we can follow the process of the formation of the acrosomal filament (Fig. 11). At the first step, the anterior end of the central fiber pushes against the posterior membrane of the acrosomal vesicle which comes into contact with the anterior membrane of the vesicle (Fig. 11A). Then the anterior membrane of the acrosomal vesicle appears to fuse with the plasma membrane at the

Fig. 7. Diagrammatic illustration of the fine structure of the sperm head.

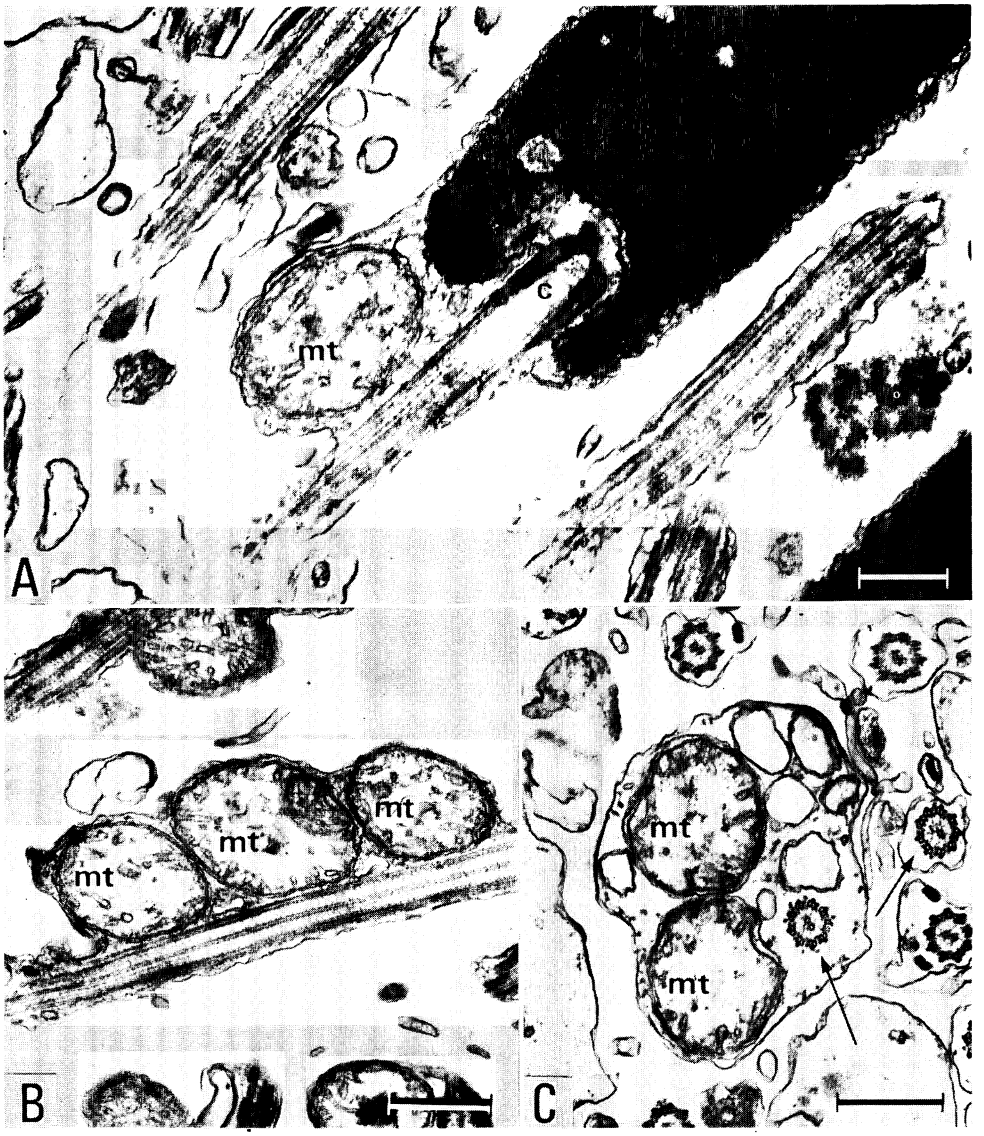


Fig. 8. Sections through the regions containing mitochondria of the testicular spermatozoa. Several mitochondria (mt) can be seen in the vicinity of the proximal region of the tail flagellum in A but are found in the region far from the posterior end of the sperm head in B and C. Note the 9+9+2 structure of the tail (arrows). c, centriole. (A: bar=0.25 μm; B, C: bar=0.5 μm).

apex of the sperm head, thus the inner surface of the acrosomal vesicle is exposed outward (Fig. 11B). When the central fiber is discharged from the nuclear canal, the spermatozoon has a conspicuous collar of membrane around the proximal region of the fiber (Fig. 11C). As the discharge of the central fiber goes further, however, such a collar of membrane becomes invisible (Fig. 11D). The central fiber is covered with the membrane which previously formed the acrosomal vesicle and the outer surface of the spermatozoon (sperm plasma membrane). There are electron dense materials on the outer surface of the filament, and these may be the residue of materials which have been contained in the acrosomal vesicle. At no stage of the discharge