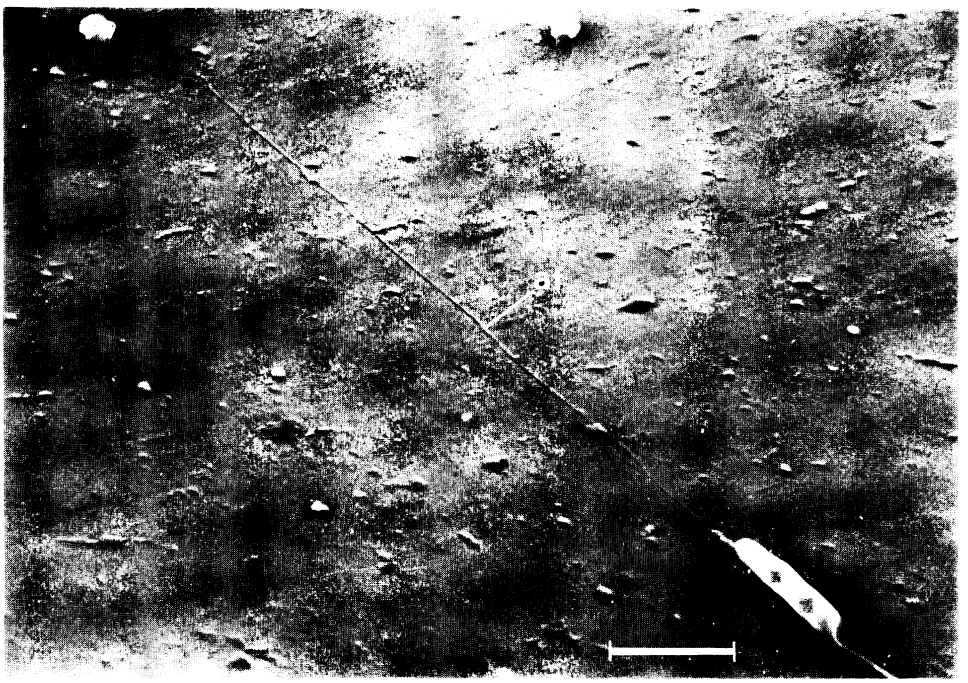


Fig. 9. SEM of the ejaculated spermatozoon discharging a long acrosomal filament (af). A clump of material seen at the tip of the acrosomal filament may be due to contamination during the preparation. (Bar=5 μm).

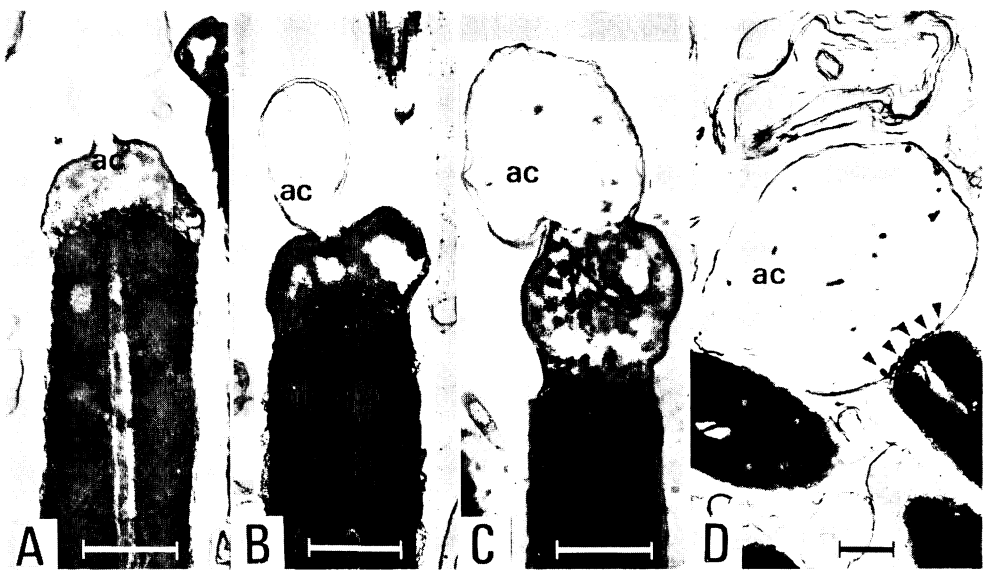


Fig. 10. Successive stages of unusual expansion of the acrosomal vesicle (ac) observed in the testicular spermatozoa. Note particulate materials attaching to the posterior membrane of the acrosomal vesicle in D (arrowheads). (A～D: bar=0.5 μm)

of the central fiber does the subacrosomal ring show any morphological change: it maintains its previous position, i. e. in the funnel-shaped depression on the anterior surface of the nucleus. As the central fiber is discharged, the nuclear canal disappears from the nucleus (Fig. 12). A trace of the canal is, however, observable as an area meager in chromatin.

Discussion

The spermatozoon of various animals is known to be equipped for the task of penetration into the egg by the possession of specialized head structures — the acrosomal vesicle and periacrosomal or subacrosomal elements. The spermatozoon of *Lampetra japonica* also possesses a vesicular acrosome at the anterior end of the head. The present observation reveals that different structural formations of the acrosomal vesicle occurred even in contiguous specimens. According to Nicander and Sjödén (1971), different procedures of fixation cause variable pictures of the acrosome; they proved that the contents of the acrosomal vesicle in *L. fluviatilis* fixed in osmium tetroxide can be distinguished from those fixed in glutaraldehyde. Since, in the present study, the fixation of materials for TEM was performed invariably in glutaraldehyde and osmium tetroxide, the difference in the acrosomal structure might represent a variation in the susceptibility of spermatozoa to stimulation. A marked heterogeneity in fine structure of the acrosomal contents has been reported in the spermatozoon of starfish (Hagiwara et al., 1967), molluscs (Niijima and Dan, 1965a, b; Lewis et al., 1980) and annelids (Sawada et al., 1975). In these cases, however, such heterogeneity reflects the existence of two kinds of materials within the acrosomal vesicle.

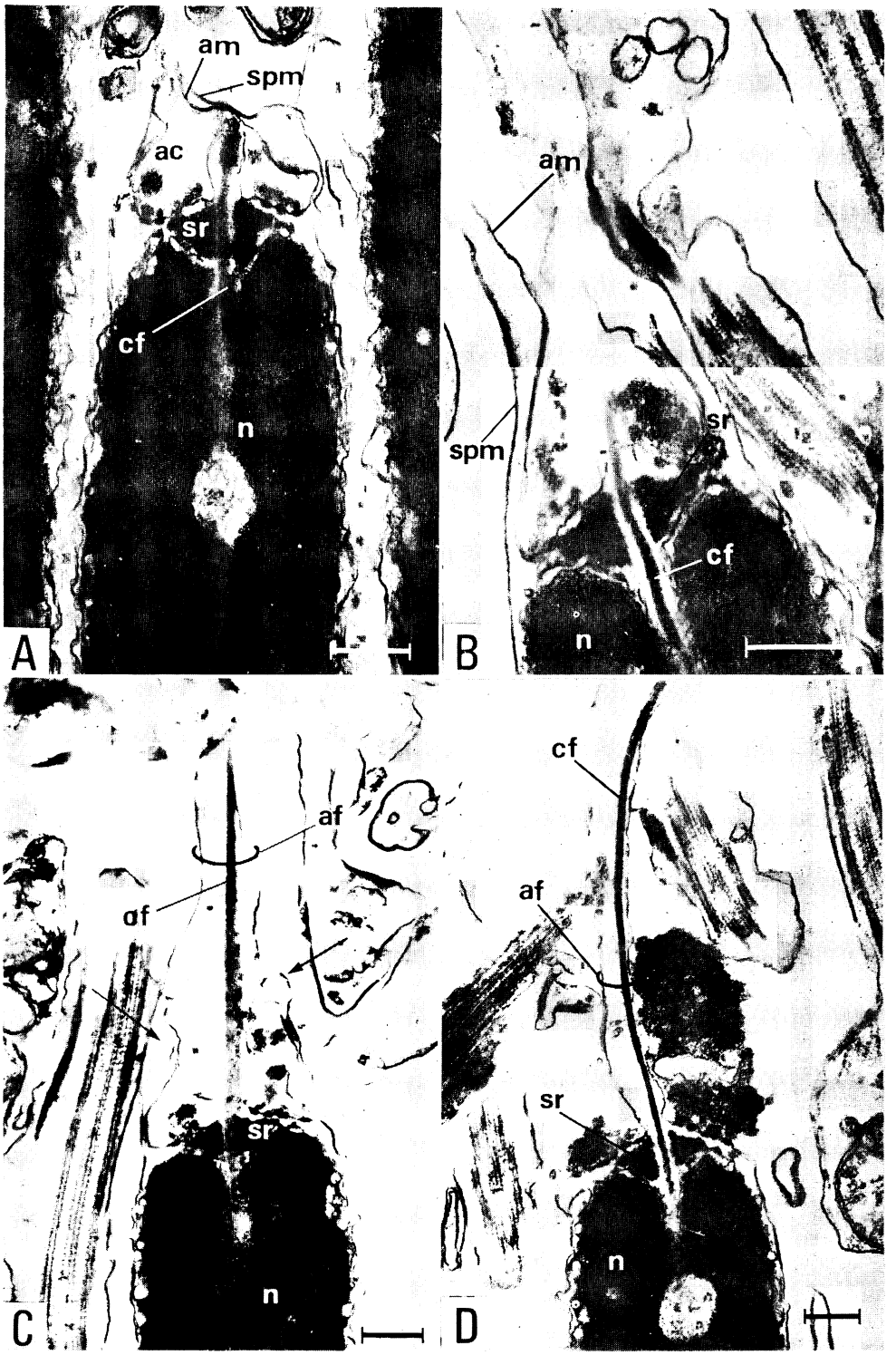
The electron dense subacrosomal ring was not a membrane-limited structure and was situated in a funnel-shaped depression formed by invagination of the nucleus. Although a similar electron dense ring structure behind the acrosome has been observed in other lamprey species (Follenius, 1965; Stanley, 1967; Nicander and Sjödén, 1971), no conclusive evidence has been obtained as to the physiological role of this structure. In the sperm head of a polychaete worm, *Nereis japonica*, Takashima and Takashima (1963) described a similar structure and called it a basal ring. These authors surmised that the ring acts as a fixing apparatus of the axial rod during the acrosome reaction.

In *Lampetra japonica*, at the posterior level of the chromatin-containing region, the nucleus forms a pouch where two parallel centrioles were found. The same situation has been also reported in *L. planeri* (Follenius, 1965; Stanley, 1967). This parallel orientation of centrioles is not, however, unique to lamprey spermatozoa, as Amemiya et al. (1980) have obtained a similar picture in spermatozoa of the echinothurid sea urchin.

In the present study, no strictly defined middle piece was observed by SEM. Several mitochondria were, however, detected in the vicinity of the proximal region of the flagellum or in the region far from the posterior region of the sperm head. Stanley (1967) observed that neither neck nor middle piece is not clearly defined in the spermatozoa of *Lampetra planeri*, while several elongated mitochondria are arranged longitudinally along the tail.

According to Fawcett (1970) and Yasuzumi (1974), the locomotor apparatus of the tail flagellum shows an uncomplicated structure in relatively primitive animals with external fertilization; it consists of nine pairs of doublets and two central microtubules (9+2). In animals with internal fertilization, however, the locomotor apparatus possesses an additional outer row of electron dense fibers in the periphery of the doublets, thus showing a 9+9+2 structure. In *Lampetra japonica* as well as in *L. planeri* (Follenius, 1965; Stanley, 1967), although fertilization takes place in external surroundings, the tail flagellum shows the 9+9+2 structure. This structure of the flagellum resembles that found in some insects (André, 1961) and reptiles (Austin, 1965), but is slightly different from the sperm tail of birds (Furieri, 1963) and mammals (Fawcett, 1975), where the outer dense fibers are larger in diameter of cross sections and less closely apposed to the doublets.

The general structure of the spermatozoon in *Lampetra japonica* is thus essentially the same as that previously reported in *L. planeri* and *L. fluviatilis* by Follenius (1965), Stanley (1967) and Nicander and Sjödén (1971).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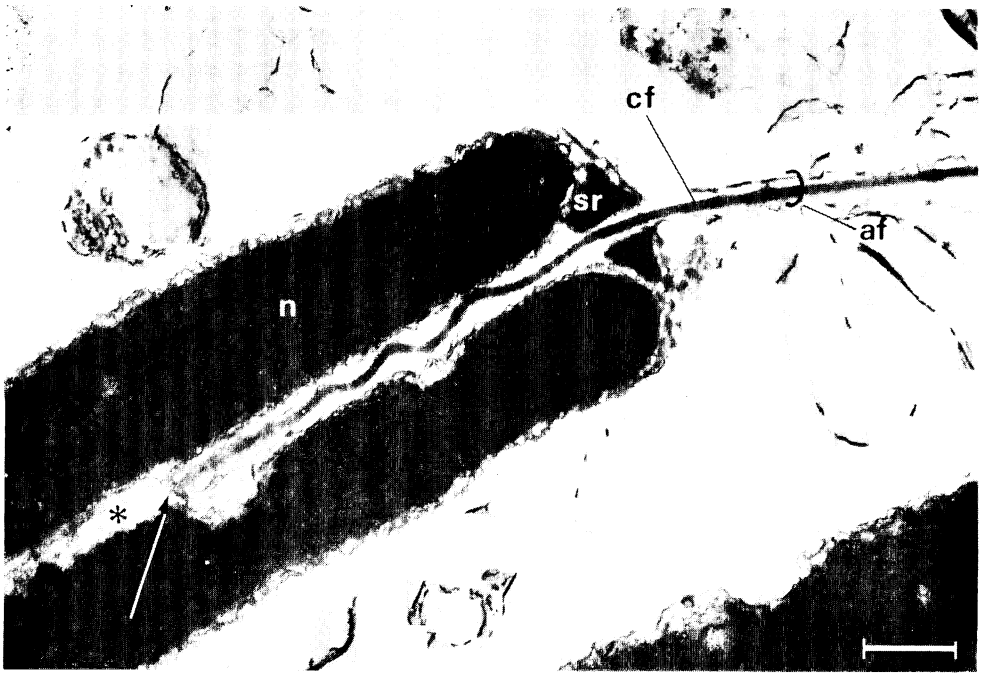

Fig. 12. Longitudinal section through the anterior region of the testicular sperm head discharging the central fiber (cf). The nuclear envelope can be seen immediately behind the posterior end of the central fiber (arrow). Note the previous nuclear canal (asterisk) which is an area meager in chromatin. af, acrosomal filament; n, nucleus; sr, subacrosomal ring. (bar=0.25 μm)

Since the egg envelope of the lamprey does not have a micropylar apparatus (Yamamoto, 1955), the fertilizing spermatozoon must dissolve the envelope in order to enter the ooplasm. The acrosome in most animals examined is known to release materials which are factors responsible for dissolving the egg envelope at the time of acrosome reaction (Colwin and Colwin, 1960; Elinson, 1974; Green and Summers, 1980; etc.). During the process of fertilization in lampreys, this reaction may occur in the vicinity or at the surface of the jelly mass covering the animal pole of the egg (Yamamoto, 1956; Kille, 1960), In addition, on contact with fixatives, the lamprey spermatozoon seems to undergo the acrosome reaction even in the testis. A long filament of the head pictured by Ballowitz (1905) in lamprey spermatozoa may be a product of the acrosome reaction which occurred at the time of fixation. It may therefore be concluded that the lamprey spermatozoon is a system highly susceptible to stimulus, as already suggested by Kille (1960).

The process of the formation of the acrosomal filament induced at the time of fixation is, in principle, quite similar to that observed in other invertebrate spermatozoa (annelids: Colwin and Colwin, 1961; Takashima and Takashima, 1963; molluscs: Niijima and Dan, 1965b; Lewis et al., 1980; echinoderms: Dan and Hagiwara, 1967; hemichordates: Colwin and Colwin, 1963); It is summarized as follows. (1) The occurrence of fusion between the sperm plasma membrane

Fig. 11. Longitudinal sections through the testicular sperm head showing successive stages of the filament formation. The posterior membrane of the acrosomal vesicle is pushed anteriorly by the central fiber (cf) in A. The fusion of the sperm plasma membrane (spm) with the anterior membrane (am) of the acrosomal vesicle is obvious in B. The sperm plasma membrane is seen in a form of a collar (arrows) around the proximal region of the filament in C. The acrosomal filament (af) consists of the central fiber and its surrounding membrane as seen in D. ac, acrosome; n, nucleus; sr, subacrosomal ring. (A~D: bar=0.25 μm)

and the membrane of the acrosomal vesicle, (2) the release of contents from the acrosomal vesicle, and (3) the eversion of the posterior membrane of the acrosomal vesicle.

In some testicular spermatozoa, a marked expansion of the acrosomal vesicle was noticed. In this case, no trace of the discharge of central fiber from the nuclear canal was detected. These observations may be explained as follows: The formation of the acrosomal filament is normally initiated by a slight expansion of the acrosomal vesicle and follows the discharge of the central fiber leading to membrane fusion at the apex of the sperm head. As the result of the formation of an opening on the membrane of the acrosomal vesicle, the expansion is ceased. When the discharge of the central fiber does not occur, no opening is formed on the membrane of the acrosomal vesicle; thus the expansion of the acrosomal vesicle continues.

As for the mechanism of the discharge of the central fiber from the nuclear canal, no conclusive evidence was obtained in the present study. With the discharge of the fiber, however, the nuclear canal disappears, leaving merely a trace behind. Takashima and Takashima (1963) suggested that in *Nereis japonica* the wrinkling and shrinkage of the nuclear envelope covering the internal surface of the nuclear indentation observed in the sperm head of the polychaete worm act as a trigger for the discharge of the acrosomal filament. The capability of the nuclear canal for discharging the central fiber might be correlated with physiological maturity in the lamprey spermatozoa.

The acrosomal filament of the lamprey exceeded 50 μm in length. Since the surface of the filament was covered with the previous acrosomal and plasma membranes, there should occur a considerable increase in the surface area of the spermatozoon after the acrosome reaction. Two different ways have been postulated which account for the increase in the surface area; one is the stretching of the preexisting plasma membrane (Nicander and Sjödén, 1971) and the other is the synthesis of new membrane (Dan and Hagiwara, 1967). The marked expansion of the acrosomal vesicle in some testicular spermatozoa may indicate that the plasma membrane has a remarkable stretching property at the apex of the sperm head.

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カワヤツメ精子の微細構造と先体糸形成

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精子頭部は棒状で，先端に先体胞，次にリング状構造物があり，その後方で核表面は漏斗状にくぼむ．くぼみ底部は核内を縦走する盲管に続く．盲管内には繊維状桿がらせんを巻いて走る．長く伸びた核の表面はその後端近くで側面に盲のうをつくり，ここに平行に並ぶ2個の中心粒を含む．鞭毛は 9+9+2 の構造を示す．中片は明瞭でなく，ミトコンドリアは鞭毛に沿って少数みられる．

固定時に一部の精子は先体糸を放出する．その形成で，繊維状桿が先体胞の後膜を押し上げ，先体胞の前膜と細胞膜が融合する．先体糸は繊維状桿をこれら膜が囲む構造物である．先体糸形成の様式は，繊維状桿を頭部にもつ軟体．環形動物精子の場合に類似する．

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