

# THE ACROSOME REACTION IN *MYTILUS EDULIS*

## I. Fine Structure of the Intact Acrosome

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### ABSTRACT

The intact acrosome of the *Mytilus edulis* spermatozoon consists of a conical vesicle, the basal side of which is deeply invaginated so that the whole vesicle forms a sheath around a very slender axial rod, about  $2.7 \mu$  long, inserted in a tube passing through the nucleus. The annular base of the acrosomal vesicle is filled with a homogeneous substance; the outer wall of the vesicle is lined with a somewhat irregular layer of a particulate substance interspersed with very fine tubular elements, and its lumen is nearly filled by a strand of material which extends from the inner tip of the invagination to the apex of the acrosome. The lumen of the invagination appears empty except for the rod and a delicate sleeve-like structure which surrounds it. The plasma membrane of the sperm cell lies in immediate contact with the acrosomal membrane over its whole outer surface. In its general organization, this molluscan acrosome shows a rather close homology with that of the annelid *Hydroides*.

Although for some time it has been evident that spermatozoan acrosomes undergo a change at the egg surface, their minute size and the rapidity of the change make it impossible to follow in detail the course of events in living spermatozoa, and necessitate recourse to the cumbersome procedure of stopping the reaction by fixation at short intervals and searching for appropriate sections under the electron microscope.

The first such study, carried out by Colwin and Colwin (2-4) with the gametes of *Hydroides hexagonus*, has provided a dynamic picture of the way in which the acrosome of this species opens, releasing a vitelline coat lysin, everts and sends out tubular extensions of the acrosomal membrane which stimulate the egg to form a fertilization cone, and finally mediates the incorporation of the sperm cell with the egg through fusion of their respective plasma membranes.

Investigation into the details of the acrosome reaction in sea urchin and starfish spermatozoa (6-9) reveals a different series of events. In both these echinoderm groups, precursor material contained in the intact acrosome is organized into a rod which elongates within a membranous covering, formed at the time of reaction, to give rise to a single acrosomal process.

Earlier studies using chiefly light microscopy (5, 10, 14) have demonstrated that the unusually large acrosome of the *Mytilus edulis* spermatozoon also reacts under appropriate conditions to form a single slender process and release a vitelline coat lysin. A preliminary study of the fine structure of this acrosome (5) suggested that it is different in several essentials from those of *Hydroides* (2) and the echinoderms, but fundamentally similar to the acrosomes of the oyster (11) and the bivalve mollusc *Barnea* (13). The present paper will de-

scribe in detail the intact acrosome of the *Mytilus* spermatozoon; the series of changes by which the acrosomal process is formed will be reported in the following paper (12).

#### MATERIALS AND METHODS

Specimens of *Mytilus edulis* collected near the Misaki Marine Biological Station or in Tokyo Bay were induced to spawn by electrical stimulation (10). Sea water suspensions of spermatozoa were fixed for 1 hour with 1 per cent  $\text{OsO}_4$  in sea water, embedded in a 4:6 mixture of styrene and butyl methacrylate, sectioned with a Porter-Blum microtome,<sup>1</sup> and observed with a JEM T-5 electron microscope.

#### RESULTS

##### *Phase Contrast Observation of Living Spermatozoa*

The head of the *Mytilus* spermatozoon has a length of about  $7\ \mu$ , half of which consists of the tapering, conical acrosome. As seen with the oil immersion objective (10), the basal part of this organelle contains a highly refringent substance located in a ring lying closely adjacent to the anterior surface of the nucleus. Through the center of this ring an axial structure extends to the tip of the acrosome. With close observation it is also possible to see a slender, threadlike structure passing through the center of the nucleus between the acrosome and the point of insertion of the flagellum.

##### *Electron Microscopy of Sectioned Spermatozoa*

The acrosome of the *Mytilus* spermatozoon consists essentially of a large, conical acrosomal vesicle<sup>2</sup> containing several substances characteristically disposed within its membrane (Fig. 1, *b*). Its posterior side is invaginated to form a lumen within which is a slender axial rod (Fig. 1, *a*).

The rod appears in most cases to be solid (Figs. 2, 5, and 6), and has a diameter of about  $50\ \text{m}\mu$ . After fixation with osmium tetroxide its

<sup>1</sup> The Porter-Blum microtome used in this study was purchased with part of a Rockefeller Foundation grant-in-aid to Tokyo Metropolitan University, administered by Professor Katsuma Dan.

<sup>2</sup> This term is used, following the Colwins (2), to denote the vesicle which contains the major part of the acrosomal substance, but without the implication that structures or materials found outside its membrane are excluded from the acrosomal apparatus (see Discussion).

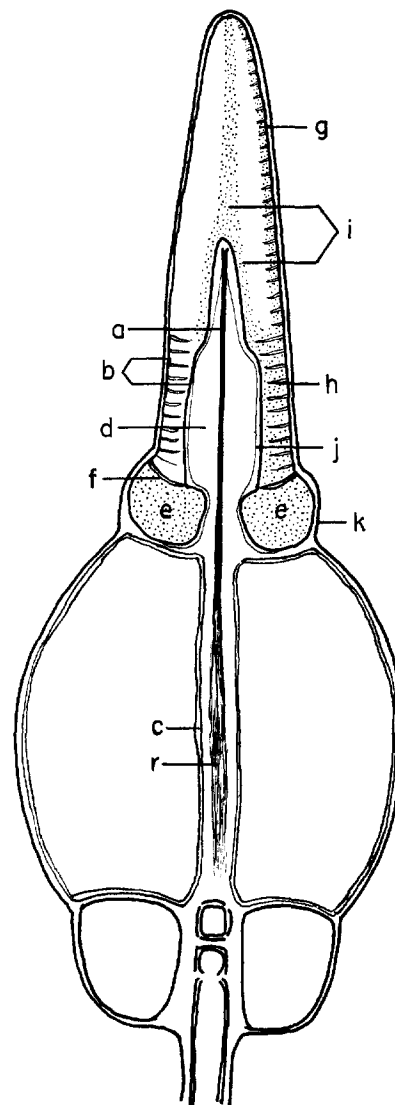


FIGURE 1 Diagrammatic longitudinal section of *Mytilus* spermatozoon, showing details of intact acrosome. *a*, axial rod; *b*, acrosomal membrane; *c*, nuclear envelope; *d*, lumen formed by invagination of acrosomal membrane; *e*, basal ring material; *f*, partition bounding basal ring; *g*, *h*, material lining acrosomal membrane; *i*, axial strand; *j*, membranoid sleeve surrounding axial rod; *k*, plasma membrane; *r*, unoriented rod precursor material.

substance gives no indication of longitudinal components, and it has no bounding membrane. The basal end of the rod is inserted in a tubular passage through the center of the nucleus, about  $100\ \text{m}\mu$  in diameter and bounded by the nuclear envelope

(Figs. 1, *c*; 2, 5, and 6). The posterior part of this passage is filled with vaguely filamentous material, which is organized anteriorly into the compact rod about  $0.5 \mu$  below the surface of the nucleus (Fig. 6).

The rod extends beyond the anterior face of the nucleus into a deep invagination in the posterior side of the acrosomal vesicle (Fig. 1, *d*). The annular base of the vesicle encircling the mouth of the invagination contains a conspicuous mass of homogeneously particulate substance (in Figs. 1 and 4), obviously the refringent material observed here with the phase contrast microscope. The distal boundary of this basal ring is formed by a thin partition (Figs. 1, *f*; 5 and 6), which becomes more evident after the acrosome reacts.

The outer wall of the acrosomal vesicle (*b* in Figs. 1, 3, and 4) is internally lined with a thick layer of particulate material (*g* in Figs. 1, 2, and 3) interspersed with extremely tenuous paired lines which are believed to represent tubular elements. The part of this layer (*h* in Figs. 1, 2, and 4) adjoining the basal ring has a thickness of about  $200 m\mu$ , and fills the space between the outer side of the acrosomal membrane and the wall of the invagination. Where the lumen of the invagination narrows, this layer becomes abruptly thinner (*ca.*  $100 m\mu$ ); this dimension is maintained in the apical two-thirds of the vesicle. The tubular elements characteristic of this layer are 10 to  $15 m\mu$  in diameter, and are arranged perpendicular to the acrosomal membrane. They are barely detectable in most sections of intact acrosomes (Figs. 2 and 6) except in the thick-walled part of the lining layer (Fig. 4), but become more evident as the material around them disperses (see Nijima and Dan, 12, Figs. 2, 3, and 4).

The lumen enclosed by the invaginated acrosomal membrane has a characteristic shape, narrow where it passes through the basal ring, widest where it is surrounded by the thick lining layer, and again narrowing to fit closely around the apical part of the axial rod. Except for the rod in its center and a membranelike structure which will be described below, this lumen appears to be empty; *i.e.*, it contains no other electron-scattering material which is preserved with osmium tetroxide.

From the level where the layer lining the outer wall of the acrosomal vesicle becomes thinner, another layer, also about  $100 \mu$  thick and composed of homogeneously particulate material (Fig. 1, *i*), is applied to the inner surface of the

invaginated membrane, extending distally beyond the invagination as an axial strand which nearly fills the apical part of the vesicle (Fig. 3). The surface of this mass is more condensed than its interior, but it lacks a bounding membrane.

A delicate membranoid structure (Figs. 1, *j*; 2, 5, and 6), always less conspicuous than the adjacent acrosomal membrane, extends over the posterior face of the basal ring and through the opening in its center, to form a loose sleeve around the rod where it passes through the otherwise empty-appearing wide part of the vesicular invagination.

The plasma membrane of the sperm cell (Fig. 1, *k*) closely invests the acrosomal membrane, extends around the nucleus and five mitochondrial spherules of the midpiece, and continues posteriorly as the outermost covering of the flagellum.

## DISCUSSION

Even if the strikingly more generous dimensions of the *Mytilus* acrosome are disregarded, its general construction bears little resemblance to that of either the sea urchin (1, 8) or the starfish (6, 9) acrosome. With the *Hydroïdes* acrosome (2), on the other hand, it has several characteristics in common. The most obvious of these is the presence of a large acrosomal vesicle, defined by a conspicuous membrane, within which a number of evidently different substances are arranged in a constant pattern. In both cases the acrosomal membrane is invaginated at the side apposed to the nucleus, much of its internal extent is lined by a layer of fuzzy-appearing material, and externally it is covered only by the plasma membrane.

Sections through the apex of the *Mytilus* acrosome (Fig. 3) fail to reveal the presence of any special structure similar to the apical vesicle of the *Hydroïdes* spermatozoon which could be regarded as a possible candidate for the role of trigger. It may be significant, however, that in both acrosomes the mass of presumably lytic material located in the lumen of the acrosomal vesicle ("acrosomal granule" in *Hydroïdes*, "axial strand" *Mytilus*) is found in close contact with the inside of the acrosomal membrane in the apical region where a trigger mechanism might be expected to operate (2, 4, 12).

These spermatozoan representatives of two different phyla show a close similarity, then, with respect to the general aspects of their acrosomal vesicles: the position of each in relation to the

nucleus and plasma membrane, its content of various substances in definite positions within a conspicuous membranous envelope, and the fact that it is invaginated from the side facing the nucleus. In *Mytilus*, however, another essential part of the acrosomal apparatus is the axial rod which lies within this invagination, and consequently outside the vesicle proper. Moreover, the less definitely organized mass of material continuous with the base of the rod in the nuclear

passage, the membranoid structure surrounding the rod, and the fluid (?) contents of the vesicular invagination are also extravesicular. The demonstrable part played by these structures and substances in the formation of the acrosomal process (12) makes it necessary to regard them as primary components of the acrosomal apparatus, even though they are located outside the acrosomal vesicle.

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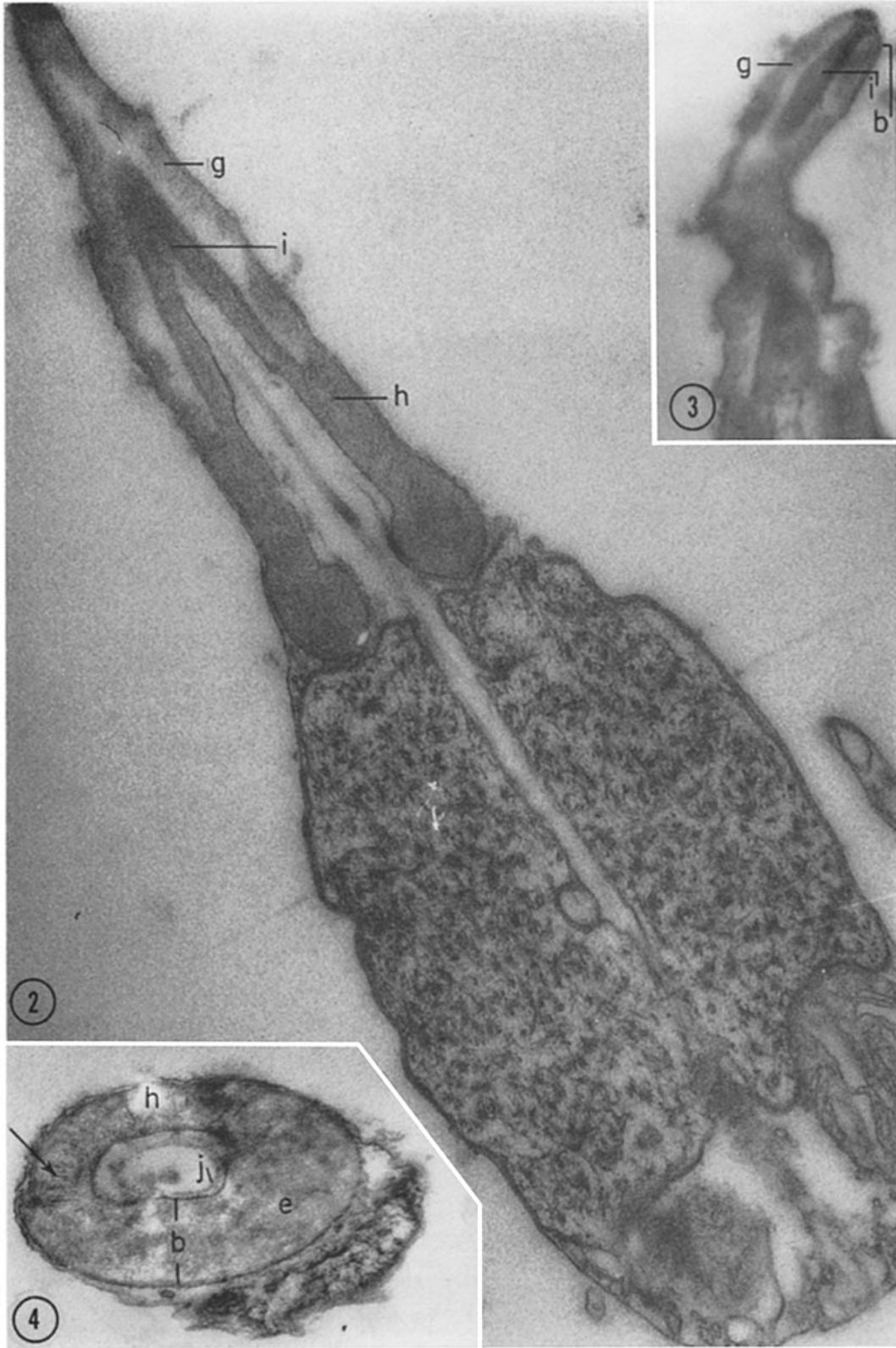
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FIGURE 2 Longitudinal section of *Mytilus* spermatozoon with intact acrosome. (Axis of acrosome departs from plane of section distally. *g*, *h*, material lining acrosomal membrane; *i*, axial strand.  $\times$  ca. 37,500.

FIGURE 3 Longitudinal section through apex of acrosome. Central strand of particulate material (*i*) extends to apex, ends in contact with inside of acrosomal membrane (*b*). *g*, material lining acrosomal membrane.  $\times$  ca. 37,500.

FIGURE 4 Diagonal section through base of acrosome. Microtubules characteristic of layer lining acrosomal vesicle appear at upper left (arrow); lower right side of section passes through basal ring (*e*) and cuts nucleus tangentially. *b*, acrosomal membrane; *h*, material lining acrosomal membrane; *j*, membranoid sleeve surrounding axial rod.  $\times$  ca. 44,500.



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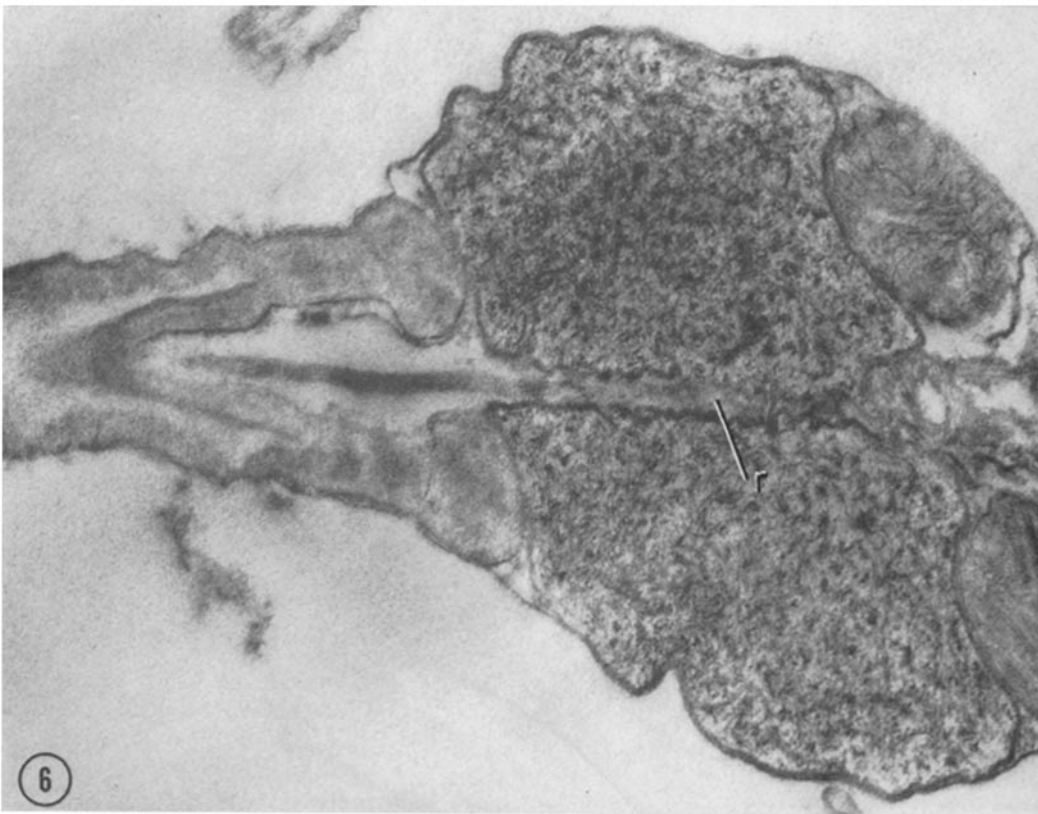
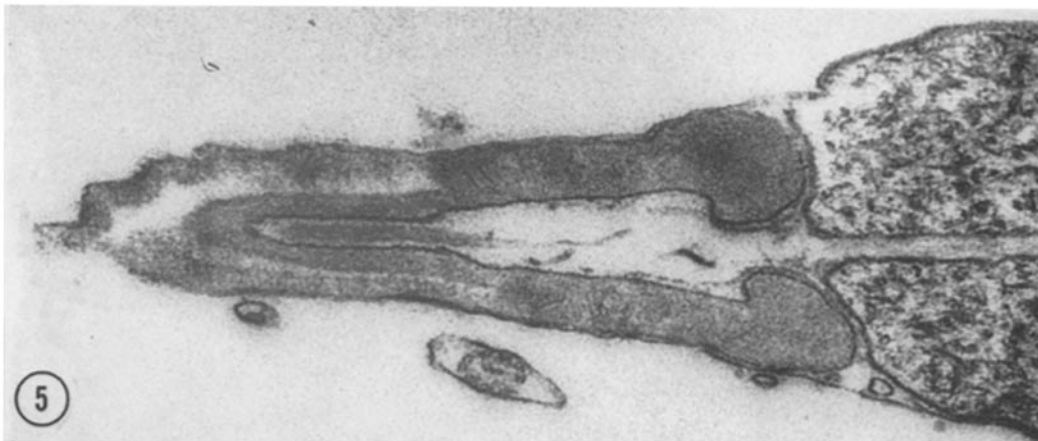


FIGURE 5 Longitudinal section, showing relation of axial rod to distal part of acrosomal membrane invagination.  $\times 40,000$ .

FIGURE 6 Longitudinal section to show tubular passage through center of nucleus, containing un-oriented material ( $\tau$ ) believed to be axial rod precursor.  $\times 40,000$ .