FINE STRUCTURE OF THE SPERMATOZOON OF *HYDROIDES HEXAGONUS* (ANNELIDA), WITH SPECIAL REFERENCE TO THE ACROSOMAL REGION

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ABSTRACT

This paper describes in some detail the structure of the acrosomal region of the spermatozoon of *Hydroides* as a basis for subsequent papers which will deal with the structural changes which this region undergoes during fertilization. The material was osmium-fixed and mild centrifugation was used to aggregate the spermatozoa from collection to final embedding. The studies concern also the acrosomal regions of frozen-thawed sperm prepared by a method which previously had yielded extracts with egg membrane lytic activity. The plasma membrane closely envelops four readily recognizable regions of the spermatozoon: acrosomal, nuclear, mitochondrial, and flagellar. The acrosome consists of an acrosomal vesicle which is bounded by a single *continuous* membrane, and its periphery is distinguishable into *inner, intermediate,* and *outer zones.* The inner and intermediate zones form a pocket into which the narrowed apex of the nucleus intrudes. Granular material adjoins the inner surface of the acrosomal membrane, and this material is characteristically different for each zone. Centrally, the acrosomal vesicle is spanned by an *acrosomal granule*: its base is at the inner zone and its apex at the outer zone. The apex of the acrosomal granule flares out and touches the acrosomal membrane over a limited area. In this limited area the adjoining granular material of the outer zone is lacking. The acrosomal membrane of the inner zone is invaginated into about fifteen short tubules. The acrosomal membrane of the outer zone is closely surrounded by the plasma membrane. At the apex of the acrosomal region a small *apical vesicle* is sandwiched between the plasma membrane and the acrosomal membrane. Numerous frozen-thawed specimens and occasional specimens not so treated show acrosomal regions at the apex of which there is a well defined opening or orifice. Around the rim or lip of this orifice plasma and acrosomal membranes may even be fused into a continuum. The evidence indicates that the apical vesicle and the parts of the plasma and acrosomal membranes which surround it constitute a lid, and the rim of this lid constitutes a natural "fracture line" or rim of dehiscence. Should fracture occur, the lid would be removed and the acrosomal vesicle would be open to the exterior.

INTRODUCTION

It has been known for some time that in a number of species the spermatozoon when treated with egg water or other suitable agents undergoes an acrosome reaction and produces an acrosome filament (13). It has been shown also that the spermatozoon produces the acrosome filament at fertilization and apparently the filament somehow delivers the stimulus which causes the egg to begin its fertilization reaction (8, 13). Recently Dan (14) has described the fine structure of the starfish

acrosome and of the acrosome filament produced following treatment with egg water. Otherwise little is known about the relationship of the filament to its structural antecedents in the unreacted acrosome. Moreover, there is no appreciable knowledge of the kind of relationship which the acrosome filament establishes with the egg plasma membrane, or of the role of this relationship in egg activation. Nor, finally, is much known about how the spermatozoon is incorporated into the egg, or, perhaps more precisely, how the two gamete cells become mutually incorporated to form a single fertilized cell.

The present series of papers will attempt to clarify some of the above points from studies on *Hydroides hexagonus*. This, the first paper, will describe the fine structure of the spermatozoon, but particularly the structure of the acrosomal region. The second paper (11) will deal with the changes which the acrosomal region undergoes in connection with passage through the vitelline membrane, and the third paper (10) will consider the manner in which the spermatozoon meets and unites with the egg proper.

MATERIALS AND METHODS

Spermatozoa of the marine annelid *Hydroides hexagonus* were obtained at Woods Hole, Massachusetts. Individual animals were caused to spawn into filtered sea water and the sperm was collected in a minimum amount of sea water as soon as shed. For each fixed preparation the sperm suspensions from many animals were pooled and then concentrated by very mild centrifugation with a hand centrifuge or with an electrical centrifuge. The latter was operated at nearly the same slow speed as the hand centrifuge by means of a Variac attachment, but, even so, many of the electrically centrifuged spermatozoa showed the acrosomal region partly detached from the nuclear region and the plasma membrane broken at the site of detachment. In some cases the freshly collected sperm was suspended in a 0.001 M solution of Versene in sea water. Since, however, no structural differences were observed between Versene-treated spermatozoa and those in sea water, no distinction between the two types of preparation is made in the account below.

The time interval from collection to fixation of the sperm ranged from a minimum of 10 minutes to about 30 minutes. Fixation was performed by mixing equal volumes of sperm suspension and 4 per cent osmium tetroxide in sea water; thus the final concentration of fixative was 2 per cent. After approximately ¾ hour in the fixative the material was washed successively in sea water, diluted sea water, and distilled water, and dehydrated in increasing concentrations of ethyl alcohol. The material was then infiltrated in three changes of a mixture of 85 per cent *n*-butyl and 15 per cent methyl methacrylate monomer containing 2 per cent Luperco as catalyst and polymerized in an oven at 63°C. Before each transfer the sperm was concentrated by very mild centrifugation.

Explanation of Figures

All figures are sections of spermatozoa of *Hydroides hexagonus*. All spermatozoa are from sea water, hand-centrifuged preparations, unless otherwise indicated.

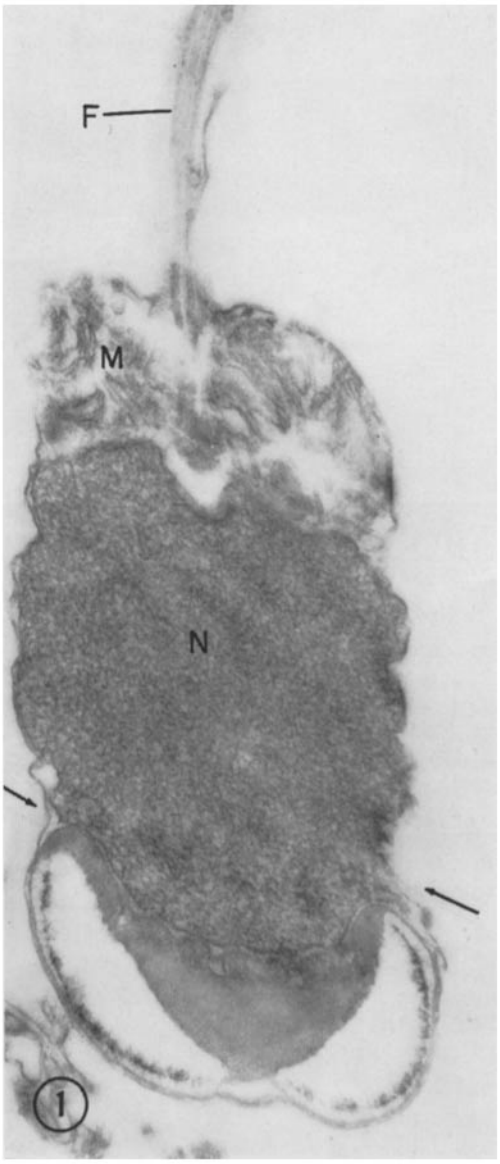
b, material lying between nuclear envelope and acrosomal membrane
c, cavity of acrosomal vesicle
g, acrosomal granule
i, inner zone of acrosomal membrane
ig, granular layer of inner zone
m, intermediate zone of acrosomal membrane
mg, granular layer of intermediate zone
n, nuclear envelope
o, outer zone of acrosomal membrane
og, granular layer of outer zone
p, plasma membrane
s, apical vesicle
t, tube-like invagination of inner zone of acrosomal membrane
D, distal centriole
F, flagellum or filament of flagellum
M, mitochondrion
N, nucleus
P, proximal centriole
T, ring of vesicles at base of flagellum
Y, Y-shaped attachment of filament to plasma membrane

FIGURE 1

Longitudinal section showing the four regions of the spermatozoon. Below a line joining the two arrows is the approximate extent of the acrosomal region. × 51,000.

Frozen-thawed sperm preparations which yielded egg membrane lysin, as reported earlier (9), also were fixed and sectioned in the manner described above. Although many spermatozoa were completely disintegrated by the freezing-thawing treatment, the acrosomal regions of others could still be recognized. Certain structural features seen in these regions help to clarify the relations seen in untreated spermatozoa prepared as described above.

The figures showing details of the mitochondrial and centriolar regions are of spermatozoa which were fixed while penetrating the vitelline membrane (11). It is not impossible, therefore, that the structures shown reflect some minor changes stemming from contact with the egg.



Sections were cut with a Porter-Blum microtome and spread by the method of Satir and Peachey (18); some effects of sectioning compression remained. The sections were stained with lead hydroxide by the method of Watson (19) as modified by Dalton and Zeigel (12), and examined with an RCA model EMU-3C electron microscope. All structures described were observed in serial sections of many specimens. The original magnifications of the micrographs ranged from 11,000 to 30,000; the final magnifications of the figures were obtained by photographic enlargement.

OBSERVATIONS

In the spermatozoon of *Hydroides hexagonus* the plasma membrane closely envelops four readily distinguishable regions. The acrosomal and nuclear regions make up the bullet-shaped head, behind which follow the middle piece or mitochondrial region and the flagellum (Fig. 1).

The Acrosomal Region

A diagram of this region is shown in Fig. 2. A large acrosomal vesicle fits against the narrowed apical end of the nucleus in such a way that the nucleus seems to indent the vesicle (Figs. 3, 14, and 20). The structure of the vesicle is such that its bounding membrane together with all the specially differentiated regions within it appear to comprise a single unit. Three fairly distinct zones of the periphery may be distinguished: the *inner zone* which lies against the more or less flattened apex of the nucleus, the *intermediate zone* which encircles the sides of the narrowed apical part of the nucleus, and the dome-shaped *outer zone* which is curved away from the nucleus and is closely invested by the plasma membrane of the spermatozoon. Each zone consists of its respective zone of the acrosomal membrane together with characteristic granular material which lines the membrane. With regard to the membrane, it should be stressed that *these zones are merely adjacent areas of one single continuous structure, the acrosomal membrane.*

The Inner Zone: In this zone the acrosomal membrane is invaginated into about fifteen short tubules which extend in a direction approximately parallel to the long axis of the sperm head (Figs. 3, 14, and 20). They are more or less evenly spaced when seen in cross or oblique sections

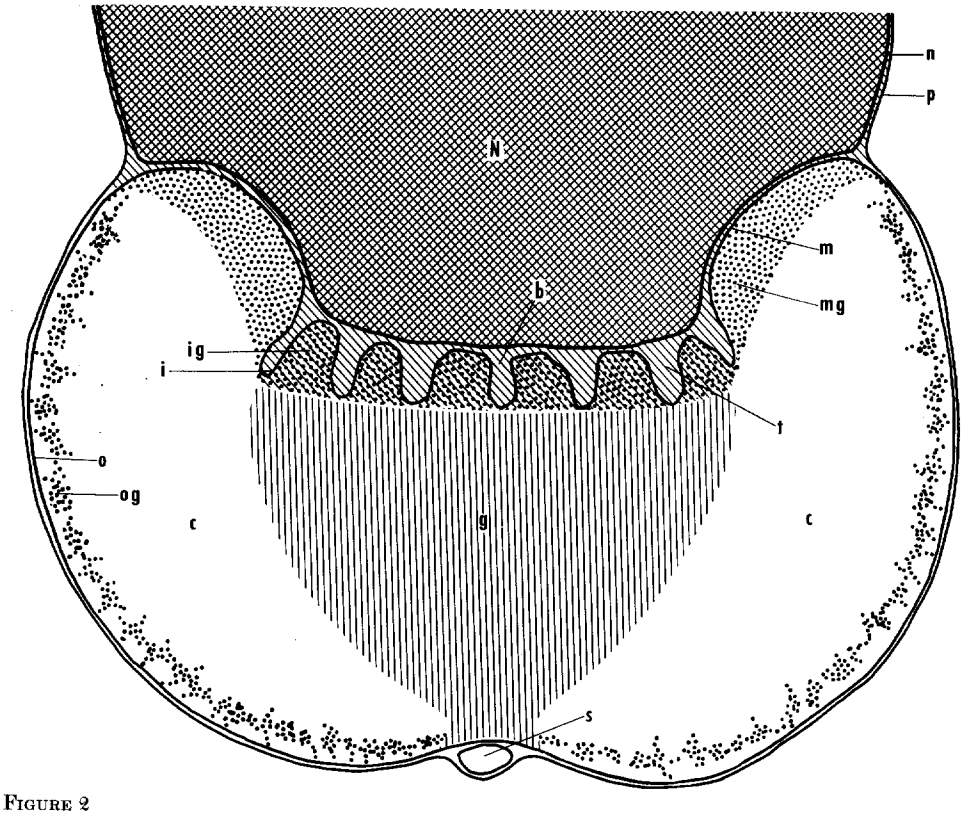


FIGURE 2

Diagram of median longitudinal section through acrosomal region.

FIGURES 3 TO 7

Longitudinal sections through the acrosomal region.

FIGURE 3

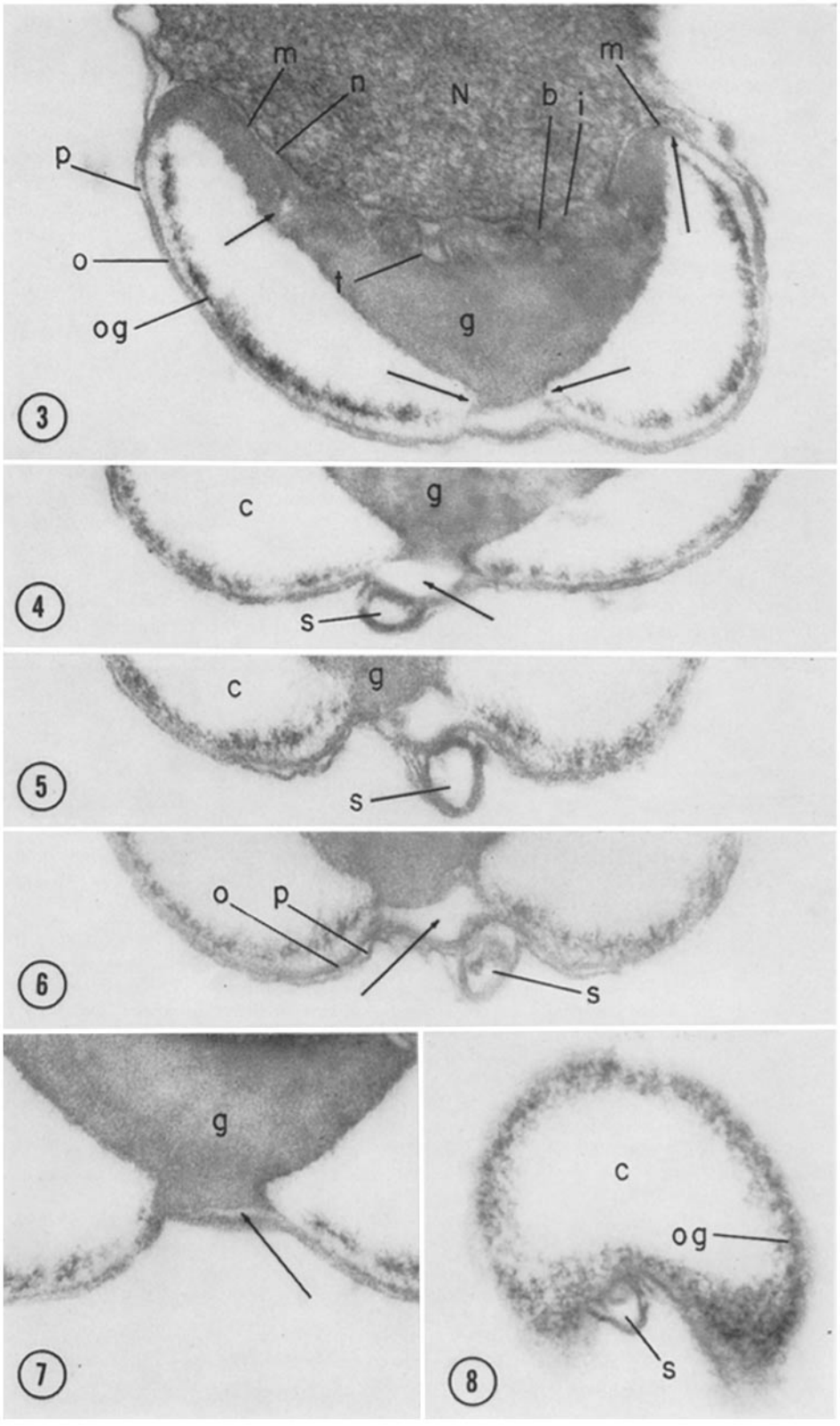
One continuous acrosomal membrane encloses the acrosomal vesicle. The outer zone of the acrosomal membrane (*o*) is enclosed by the plasma membrane (*p*); the intermediate (*m*) and inner (*i*) zones adjoin the nuclear envelope (*n*). Inner zone granular material surrounds the invaginated tubules (*t*) of the acrosomal membrane. The tubules are partly occupied by material (*b*) which also extends to the nuclear envelope. The apical part of the acrosomal granule (*g*) is flared (bottom arrows) and the flared perimeter is in contact with the outer zone of the acrosomal membrane. The granules of the outer zone (*og*) touch the acrosomal granule only outside the perimeter of the flare. Right arrow points to junction between intermediate (*m*) and outer zones. Left arrow points to junction between intermediate and inner zones. × 84,000.

FIGURES 4 TO 7

Tip of arrow in Figs. 4 and 6 lies in region of separation of the flared apex of the acrosomal granule from the acrosomal membrane. If the section is more peripheral, this separation is almost absent (arrow, Fig. 7). The apical vesicle (*s*) is sandwiched between plasma and acrosomal membranes. Magnifications: Fig. 4, × 67,000; Fig. 5, × 91,000; Fig. 6, × 84,000; Fig. 7, × 91,000.

FIGURE 8

Approximately oblique section through the apical vesicle. × 62,000.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(Fig. 10), and are well shown in surviving acrosomal vesicles of frozen-thawed sperm (Figs. 15 to 19). These invaginations contain a moderately opaque material which also seems to form an ill defined layer between the acrosomal membrane and the nuclear envelope at the base of the acrosome. On the inner or cavity side of the acrosomal membrane, a finely granular material of moderate opacity fills the region between the tubular invaginations (Figs. 15 and 16).

The Intermediate Zone: Applied to the intermediate zone of the acrosomal membrane is a thick finely granular layer of material which is moderately electron opaque (Figs. 3, 14, 17 to 20). This layer thins gradually as it approaches the outer zone. In the transitional area between the intermediate and inner zones the layer appears in some sections to remain thick but in others to thin abruptly. Presumably the latter condition is seen when the section happens to pass through a peripherally placed invaginated tubule of the inner zone.

The Outer Zone: The transition from intermediate to outer zone is clearly marked by the characteristic appearance of the material applied to the acrosomal membrane (Figs. 3, 10 to 14, 17 to 20). In the outer zone small irregularly shaped masses of dense granular material form a thin layer which is much less compact but more electron opaque than in the intermediate zone. The sperm plasma membrane invests the acrosomal membrane so closely that in some specimens it is difficult to see them as two entities. In frozen-thawed material the two membranes are partly separated and both can readily be distinguished (Fig. 17).

The Acrosomal Granule: This is a relatively large structure which occupies a considerable part of the cavity of the vesicle (Figs. 3, 14, 17 to 20). It is approximately hemispherical in outline; its broad, flat base is closely applied to the basal end of the acrosomal vesicle. There it adjoins both the granular material and the blind ends of the invaginated tubules of the inner zone. Generally, the demarcation between the granule and the inner zone is easily seen. The apical end of the granule does not taper off to complete the hemi-

FIGURES 9 TO 13

Sections at various levels of the acrosomal region.

FIGURE 9

Oblique section through the nucleus and basal part of acrosomal vesicle. Arrow points to junction between intermediate (*m*) and outer (*o*) zones of the acrosomal membrane. Several of the invaginated tubules (*t*) are seen in oblique section. Note structure of the granular layer (*og*) which is applied to the outer zone membrane. × 84,000.

FIGURE 10

An approximate cross-section at the level of the inner zone. Some of the invaginated tubules (*t*) of the inner zone membrane have been cut in cross-section and are more or less round in outline, while others have been cut in various degrees of obliqueness. Granular material lies in these tubules as well as between them. × 79,000.

FIGURE 11

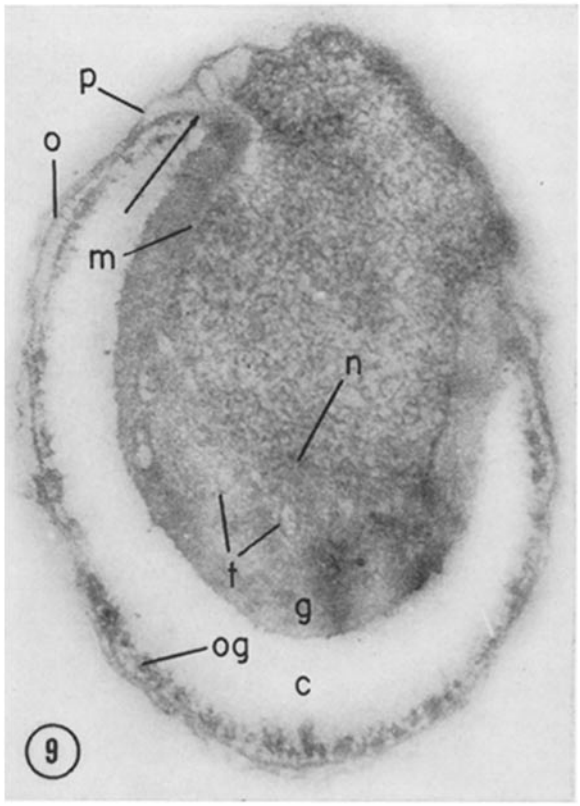
Oblique section through the region of the acrosomal granule approximately where it begins to flare out. × 47,000.

FIGURE 12

Section showing the indented part of the granular layer (*og*) of the outer zone attached to the flared part of the acrosomal granule (*g*). × 58,000.

FIGURE 13

Section at the level of attachment of the granular layer (*og*) of the outer zone to the flared region of the acrosomal granule (*g*). × 71,000.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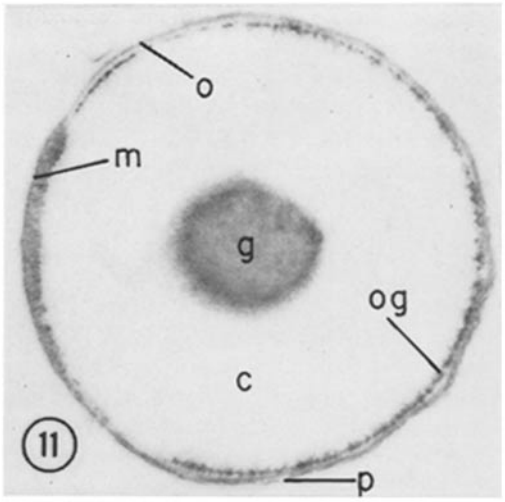
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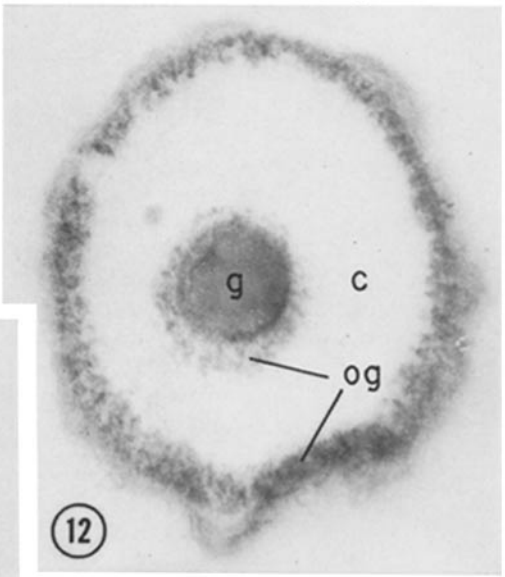
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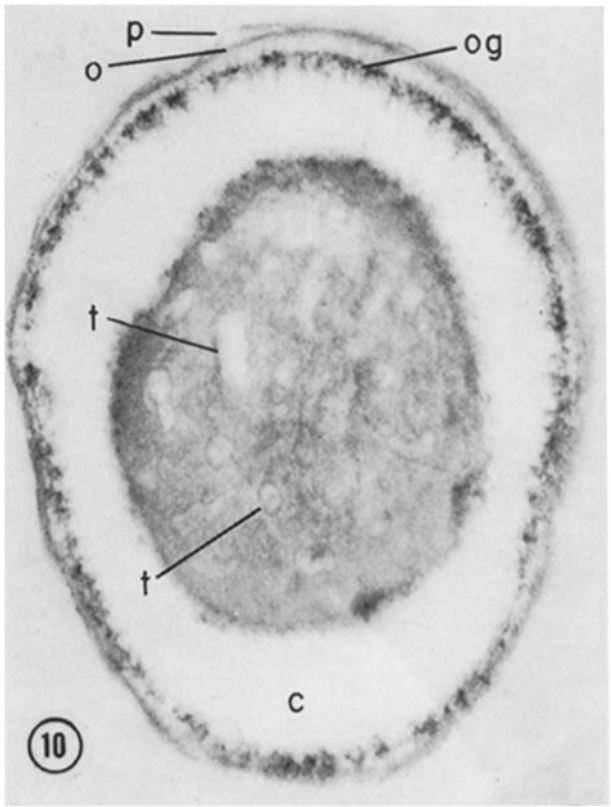
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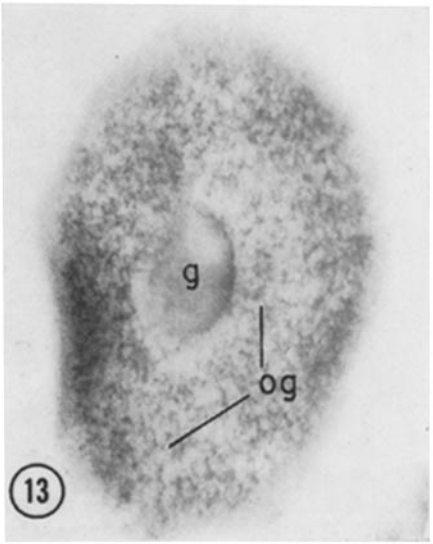
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spherical outline. Instead it flares out and meets the acrosomal membrane at the tip of the sperm head. Here, then, the thin granular layer of the outer zone is lacking from the circular area occupied by the apex of the granule (Figs. 3 to 7, 12, 13). The granule is not surrounded by a membrane although its outline is fairly well defined. It is a moderately opaque body which in at least some sections gives the impression of being formed of subunits.

The Cavity of the Acrosomal Vesicle: In most of the specimens studied the acrosomal vesicle appeared to contain a large ring-shaped cavity which was completely spanned at its center by the acrosomal granule (Figs. 3, 11). Occasionally, however, such a cavity was virtually non-existent (Fig. 14). Since both conditions of cavity size may be found in the same preparation and, indeed, in contiguous specimens, it is assumed that variation in degree of distention of the vesicle may exist in living specimens at the time of fixation.

Relationships at the Apex of the Sperm Head: The structure of this region deserves special consideration, for, as will be reported in succeeding papers (10, 11), it is this region which makes initial contact with the vitelline membrane and which undergoes considerable change at that time. In many sections, what might be considered to be the morphological tip of the sperm head, that is, the region with the flared part of the acrosomal granule, appears to be indented like a dimple (Figs. 3, 7). A frequent observation is that in the indented region the acrosomal membrane is detached from the tip of the acrosomal granule, so that a disc-shaped space exists between these two structures. Around the perimeter of this disc, however, the granule and the membrane remain attached to each other. It may be mentioned again that the thin layer of granules, which elsewhere adjoins the outer zone of the membrane, stops just outside the margin of this perimeter (Figs. 3, 12, 13).

A small vesicle occupies a position just apical to the above mentioned disc-shaped area (Figs. 4 to 6, 8). This apical vesicle is sandwiched between the acrosomal membrane and the plasma membrane. The vesicle rarely occupies more than two adjacent longitudinal sections, although the disc-shaped area extends through at least four such serial sections.

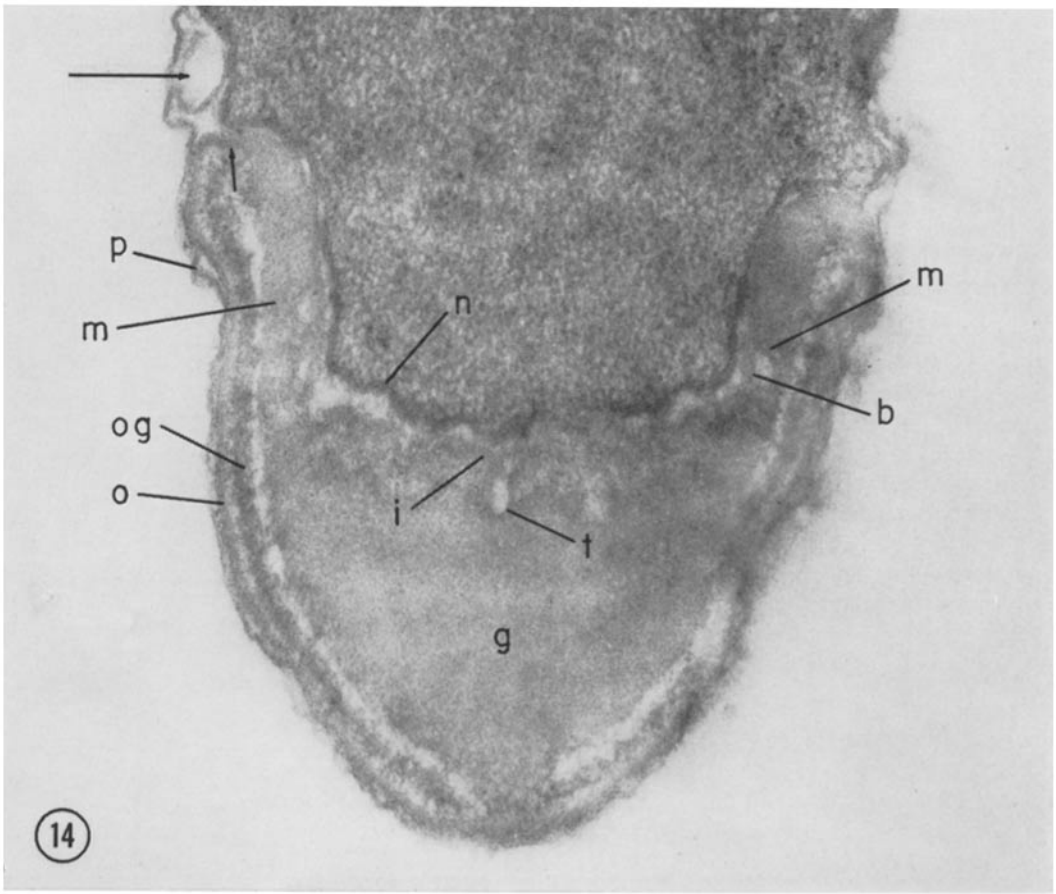
Sections of the electrically centrifuged preparations contain an occasional specimen in which the tip of the acrosomal region has broken open and formed a circular orifice. In such a specimen the edge of the plasma membrane appears to be fused to the edge of the acrosomal membrane so that the two membranes form a *continuum* which constitutes the rim or lip of the opening. The apical vesicle, which in unruptured specimens is sandwiched between these two membranes, may remain attached to some point on the rim (Fig. 20). The acrosomal granule is detached from its apical connections. In sections of the frozen-

FIGURE 14

Longitudinal section through the head of an unattached spermatozoon in an inseminated culture. The components of the acrosome are arranged as if they constituted a head cap. The outer zone is in close contact both with the intermediate zone and with the acrosomal granule; numerous fine strands connect the latter structures with the outer zone. The tubular invaginations (*t*) of the inner zone membrane are apparent. In all respects this acrosomal region seems to be similar to that of most other spermatozoa in sea water except that the acrosomal vesicle is not distended. The marked difference in thickness and organization of the granular material of the intermediate and outer zones of the acrosomal membrane is seen at their junction (lower arrow). Upper arrow points to a vesicle between the plasma membrane and the nuclear envelope. (For *m* on left side, read *mg*.) × 116,000.

FIGURES 15 AND 16

Serial sections through the inner zone of a frozen-thawed spermatozoon. Fig. 15 shows the left part of one section and Fig. 16 the right part of the adjacent section. The tubular invaginations (*t*) of the inner zone of the membrane (*i*) show clearly. At (*b*) in Fig. 16, note granular material *within* one of the tubules; the arrow points to granular material *between* tubules. × 244,000.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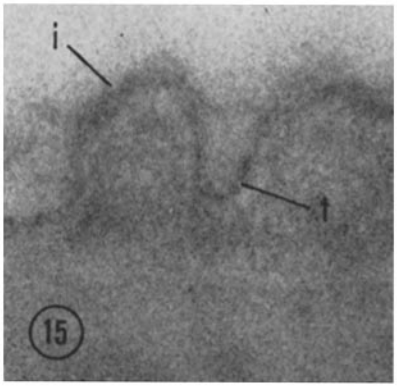
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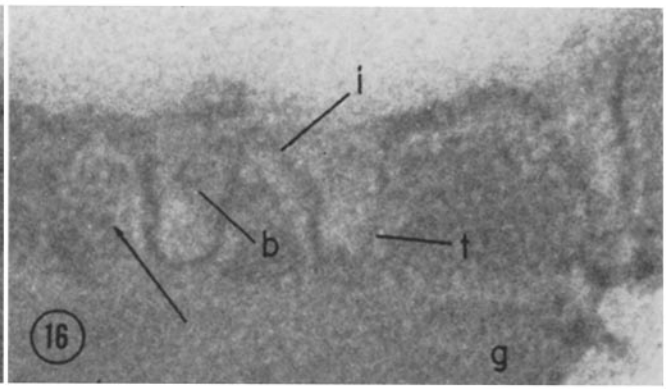
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thawed sperm many ruptured acrosomal vesicles are found. In these, too, the rupture is confined to the apical region, and plasma and acrosomal membranes appear to be fused around the perimeter of the opening (Figs. 17 to 19).

Relation of Acrosomal Membrane to Nuclear Envelope: As seen in specimens from hand-centrifuged preparations, the inner and intermediate zones of the acrosomal membrane closely adjoin the neighboring regions of the nuclear envelope (Figs. 3 and 14), but the same structures appear to be partly separated in many of the electrically centrifuged specimens (Fig. 20) and in all the frozen-thawed ones (Figs. 17 to 19). It appears that the separation can take place in any part of the involved area, but even when the nucleus is severely disrupted, in frozen-thawed material, complete separation is seldom seen. Whether widely separated or closely applied, the nuclear envelope and acrosomal membrane sometimes show intervening material. Some of this is the previously mentioned ill defined layer which lies between the nucleus and the base of the acrosome. Similar material is often observed in sperm heads fixed during egg penetration (10, 11). Apparently, too, there are pulled-out portions of the membranes of both nucleus and acrosome.

Nuclear, Mitochondrial, and Flagellar Regions

The Nuclear Region: The nucleus constitutes the largest part of the sperm head. The apical part of the nucleus is a well defined region of diminished cross-section around which fit the inner and intermediate zones of the acrosomal membrane (Figs. 1, 3, 9, and 14). The two membranes which make up the nuclear envelope are not often seen as separate entities, but the presence of both is probably reflected in the greater density and thickness of this envelope as compared with the acrosomal or plasma membranes (Figs. 14 and 22). Not infrequently one or more vesicles may lie between the nuclear envelope and the plasma membrane. They are found especially in the region at which the nucleus begins to taper. There is not, however, any specific vesicular structure which rings the nucleus at this point.

Mitochondria: Four somewhat egg-shaped mitochondria are arranged in a ring around the proximal end of the flagellum. Each lies partly in an indentation at the base of the nucleus (Figs. 21 and 22).

Centrioles: The centriolar apparatus lies partly in a pit or depression of the basal end of the nucleus. It consists of two short cylindrical components which presumably correspond to what in other spermatozoa are termed proximal and distal centrioles. Usually the long axis of the distal centriole is parallel to the long axis of the sperm head, and the long axis of the proximal centriole is oblique to the latter axis (Fig. 22). However, both centrioles occasionally have their long axes in almost the same straight line (Fig. 23). Views of the proximal centriole as sectioned in various planes may be seen in Figs. 22, 24, and 25; clearly, a denser peripheral region surrounds a less dense central region. In the distal centriole, too, the center is less dense than the periphery.

Flagellum: Cross-sections of the flagellum show the usual arrangement of filaments in a flagellum

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FIGURES 17 AND 18

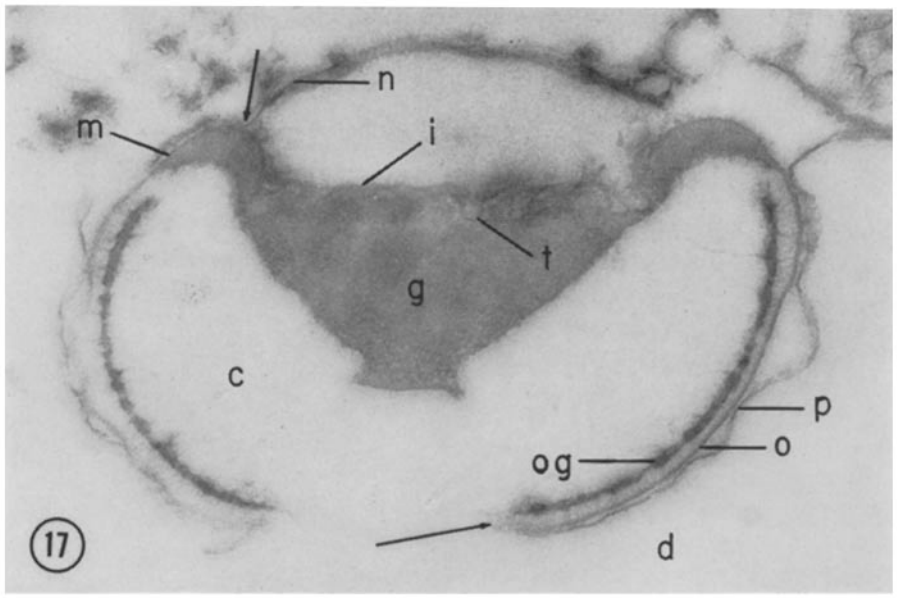
Longitudinal sections through the acrosomal regions of frozen-thawed spermatozoa.

FIGURE 17

On the left side a part of the nuclear envelope (*n*) has remained firmly attached to the acrosomal vesicle in the region of the intermediate zone (upper arrow). The vesicle is open at its apex, and plasma membrane and outer zone membrane appear fused at the lips of the orifice (lower arrow). The flared region of the acrosomal granule is not in contact with the granular layer of the outer zone (*og*). × 72,000.

FIGURE 18

A part of the nuclear envelope is associated with the intermediate zone at both the left and the right side of this figure. The plasma membrane and outer zone membrane are fused at the lip of the orifice (arrow). One of the tubules (*t*) of the inner zone membrane (*i*) has been cut obliquely; the ones to the left and right of it have been cut nearly longitudinally. × 72,000.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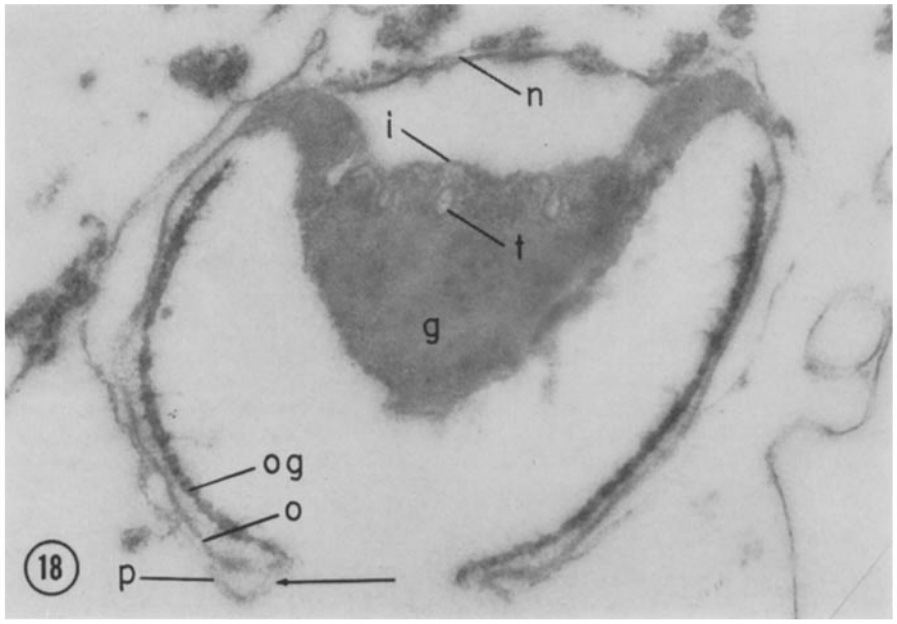
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(2, 15). Two central filaments are surrounded by a peripheral group of nine doublets (Fig. 27). As the filaments span the interval from the distal centriole to the base of the flagellum (Figs. 21 and 23), they first appear not to be associated with any other structures. But then, more distally, the doublets of the peripheral filaments make structural associations with the plasma membrane of the flagellum by means of Y-shaped connections (Figs. 28 to 33). These connections are arranged in such a way that the two arms of the Y attach to the membrane and the single arm to the outer aspect of the doublet. In the section shown in Fig. 23 dense strands are associated with the filaments in the region at which the flagellum emerges from the body of the spermatozoon. Possibly these strands are the Y connections as seen in a different sectional view. It has not been determined how far along the flagellum the zone of these Y connections extends.

Numerous small vesicles form an irregular ring just under the plasma membrane in the region where the latter turns outward to become the membrane of the flagellum (Figs. 21 to 24, 32, and 33). These vesicles usually are carried with the plasma membrane if the latter becomes elevated from the mitochondria (Figs. 23 and 24). Their origin and function are unknown but they were found in all spermatozoa, including those fixed while penetrating eggs.

It is not uncommon to find small vesicles along the length of the flagellum whether or not the spermatozoon is associated with an egg (Fig. 26).

DISCUSSION

The acrosomal region of *Hydroides*, with its large granule within the acrosomal vesicle, in over-all respects bears a surprising likeness to the one so exquisitely described by Burgos and Fawcett (6) in the developing spermatid of the cat. In later stages of development in the cat, the vesicle collapses over the nucleus to form a head cap. Since the development of the spermatozoon of *Hydroides* has not been studied, it is not known whether the collapsed vesicle stage, equivalent to the head cap, occurs in this species. Almost all the sperm heads examined for the present study showed distended acrosomal vesicles and not head caps. Nevertheless, the existence of occasional exceptions (Fig. 14) does suggest that a head cap stage may occur.

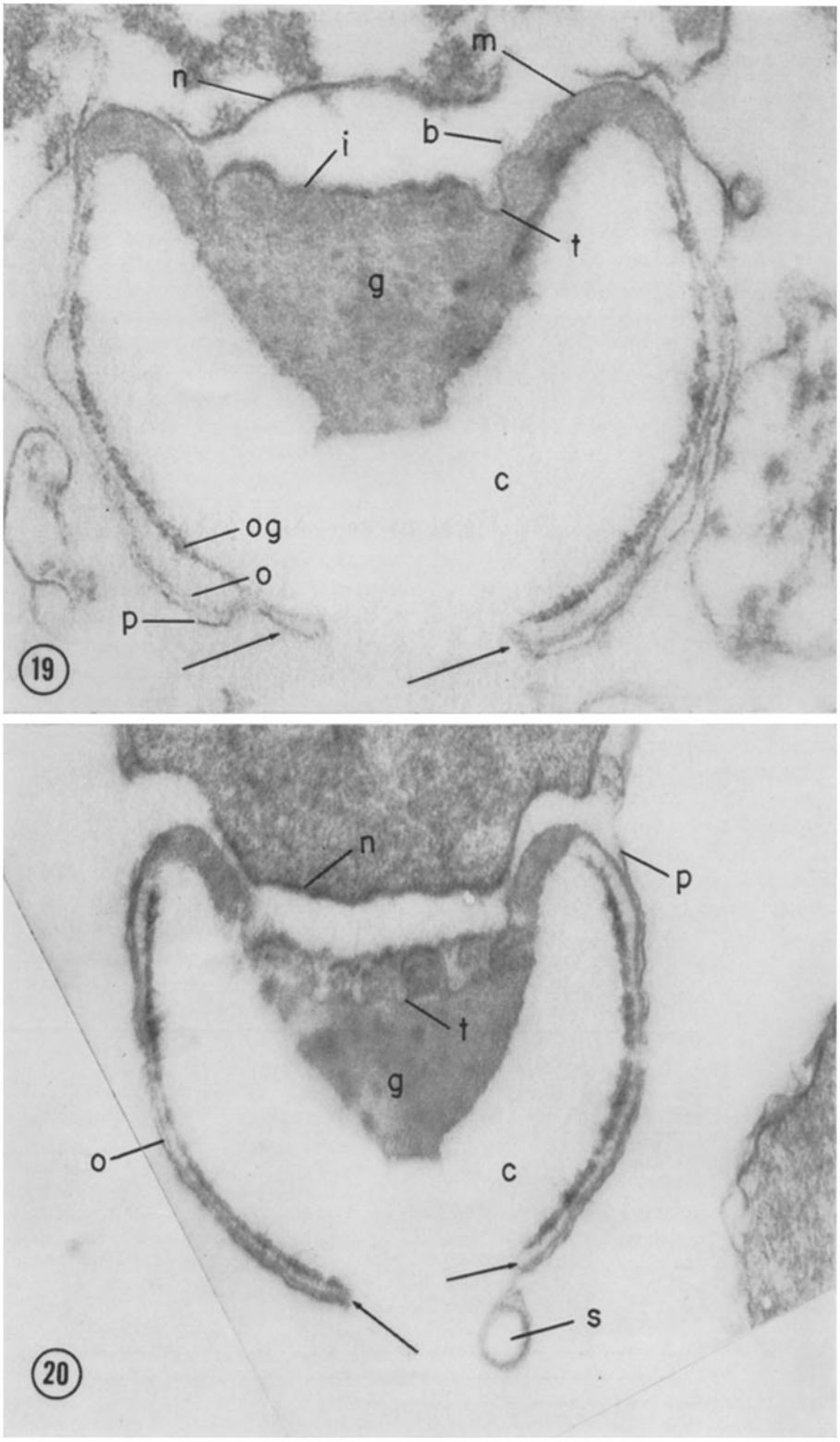
All the sperm heads described here were from preparations in which the spermatozoa had been suspended in sea water for a period of at least 10 to 30 minutes. It may be mentioned that the fertilizing capacity of *Hydroides* sperm increases up to a half-hour after the sperm has been in sea water (17); indeed, it is difficult to fertilize eggs with freshly suspended sperm. One might conjecture that the change necessary to cause physiological ripening of the spermatozoon in *Hydroides* would be distention of the vesicle after the spermatozoon is shed into sea water. The exceptional undistended condition described above might characterize spermatozoa physiologically still unripe. The physiological ripening in *Hydroides* may be the counterpart of changes leading to "capacitation" in the sperm of some mammals.

FIGURE 19

Longitudinal section through the acrosomal region of a frozen-thawed spermatozoon. The vesicle is open at its apex and the plasma membrane and outer zone membrane are fused (right arrow). The left arrow points to a region where plasma membrane and outer zone membrane between them form a space which might contain the apical vesicle in other sections. × 91,000.

FIGURE 20

Longitudinal section through the acrosomal region of a spermatozoon from an electrically centrifuged preparation. The acrosomal vesicle is open at its apex and the plasma membrane and outer zone membrane appear to be fused around the orifice (left and right arrows). An apical vesicle (*s*) appears to be sandwiched between plasma membrane and outer zone membrane in an arrangement of these membranes similar to that pointed to by the left arrow of Fig. 19. × 66,000.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In these the spermatozoa must reside for a time within the female genital tract before they are capable of fertilizing eggs. It has been inferred that during this period the spermatozoa themselves undergo certain changes (3, 7). Austin and Bishop (4) report that in several rodents the acrosome becomes modified and subsequently becomes detached. These changes in the acrosome are considered to constitute "capacitation."

Terminology: Many years ago Bowen (5) wrote (p. 349) that "no structure in the spermatid has been subject to more contradictions of description and homology than the acrosome" and that "the term 'Akrosoma' . . . first applied by Lenhossék . . . to the little granule within the vesicle . . . has been applied so widely to the material as a whole which forms the apical body [that there is] little reason for attempting to restrict the word to Lenhossék's original meaning."

It is proposed that the term *acrosome* be used to refer to the acrosomal membrane and all elements enclosed therein. In the case of *Hydroides* the acrosome would include the three recognizable zones of the acrosomal vesicle, the granular material associated with these zones, the cavity of the vesicle, and the acrosomal granule. In the case of the cat (6), for example, the term acrosome would include the membrane (inner and outer layer) which constitutes the head cap, and the "acrosome," which in its earlier developmental stages is termed the "acrosomal granule." The expression "acrosomal region," at least as applied to *Hydroides*, has been used in the present paper to include the acrosome, the apical vesicle, and that part of the plasma membrane which is associated with them.

Other Species: Dan (14) has recently described the fine structure of the starfish acrosome. Most of the acrosome lies in a depression of the apex of the nucleus, whereas in *Hydroides* and in the cat the acrosome externally invests the nuclear apex. Perhaps because of this positional difference it is difficult to recognize such structural homologies as may exist. It may be, however, that the "top-shaped mass of homogeneously electron dense material . . . lacking a bounding membrane" which occurs in the center of the starfish acrosomal region might represent the acrosomal granule as found in *Hydroides*. Moreover, the several zones of various density which lie peripheral to the top-shaped mass might be counterparts of the several granular layers applied to the three zones of the acrosomal membrane in *Hydroides*.

Dan reports that there is a firm attachment between the nuclear membrane and the basal plate of the acrosome filament which forms following treatment of the spermatozoon with egg water. She considers that there is evidence that the membrane of the basal plate and the nuclear membrane "unite at the periphery of the plate to make up the membrane covering the rest of the nucleus." She postulates that an especially close connection might form between acrosome and nuclear surface during spermatogenesis, and that the acrosomal vesicle might displace the outer layer of the nuclear envelope so that the later differentiation of the acrosome might take place in direct contact with the inner layer of the nuclear envelope.

In contrast, the acrosomal membrane of *Hydroides* may readily be distinguished from the nuclear envelope, as seen in sections. From the frozen-thawed or electrically centrifuged material, moreover, it is clear that the two structures are

FIGURES 21 AND 22

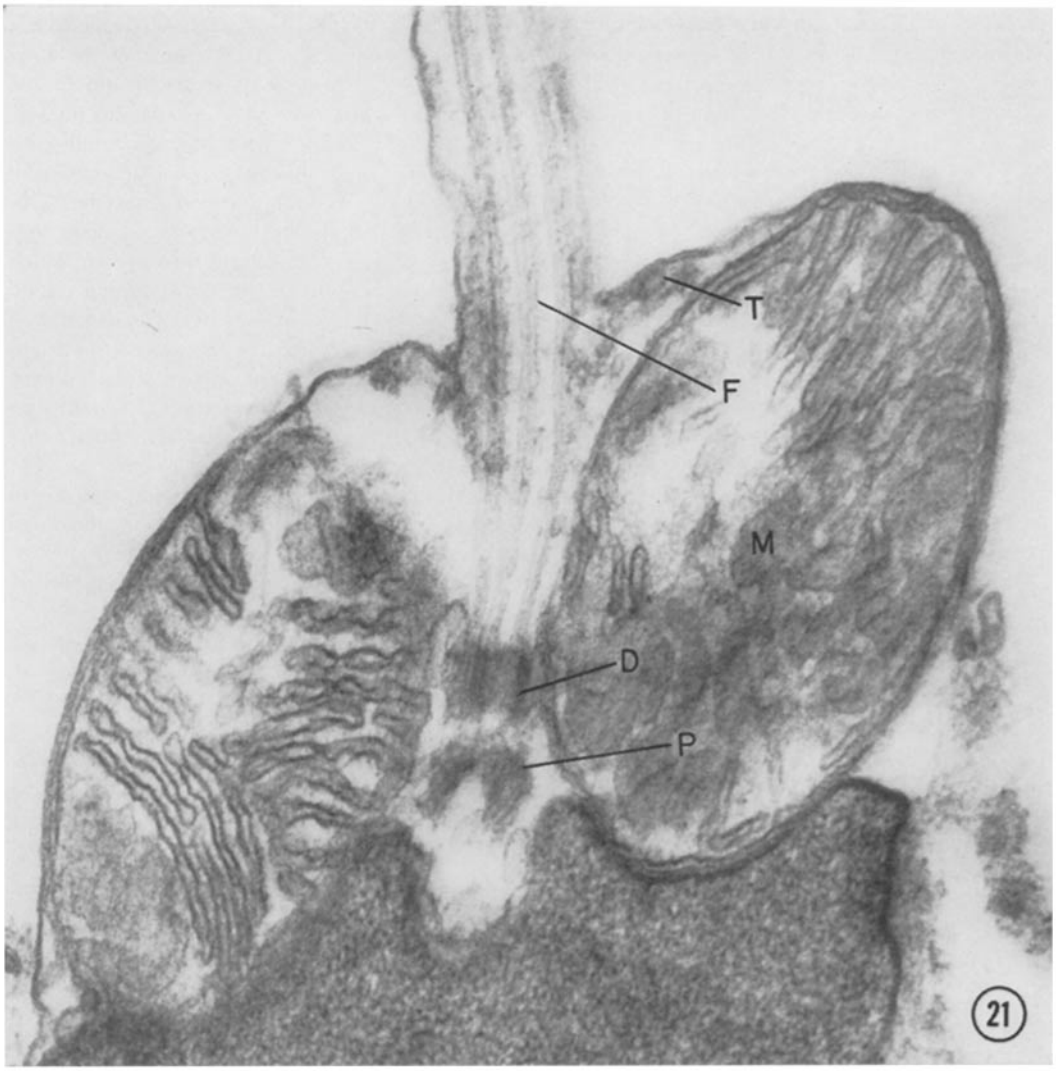
Longitudinal sections through the mitochondrial region of spermatozoa in process of egg penetration.

FIGURE 21

The filaments of the flagellum insert into the distal centriole, which has been cut through the wall of the cylinder. Strands extend between the distal and proximal centrioles. × 103,000.

FIGURE 22

Both proximal and distal centrioles are shown. This figure demonstrates the cylindrical form of the centrioles and shows that the central region of the cylinder is less dense than the peripheral region. × 92,000.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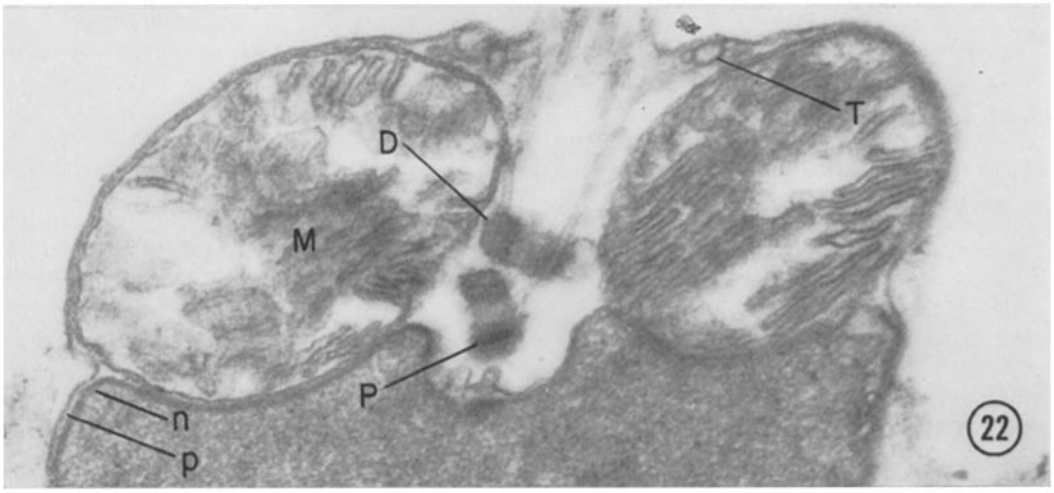
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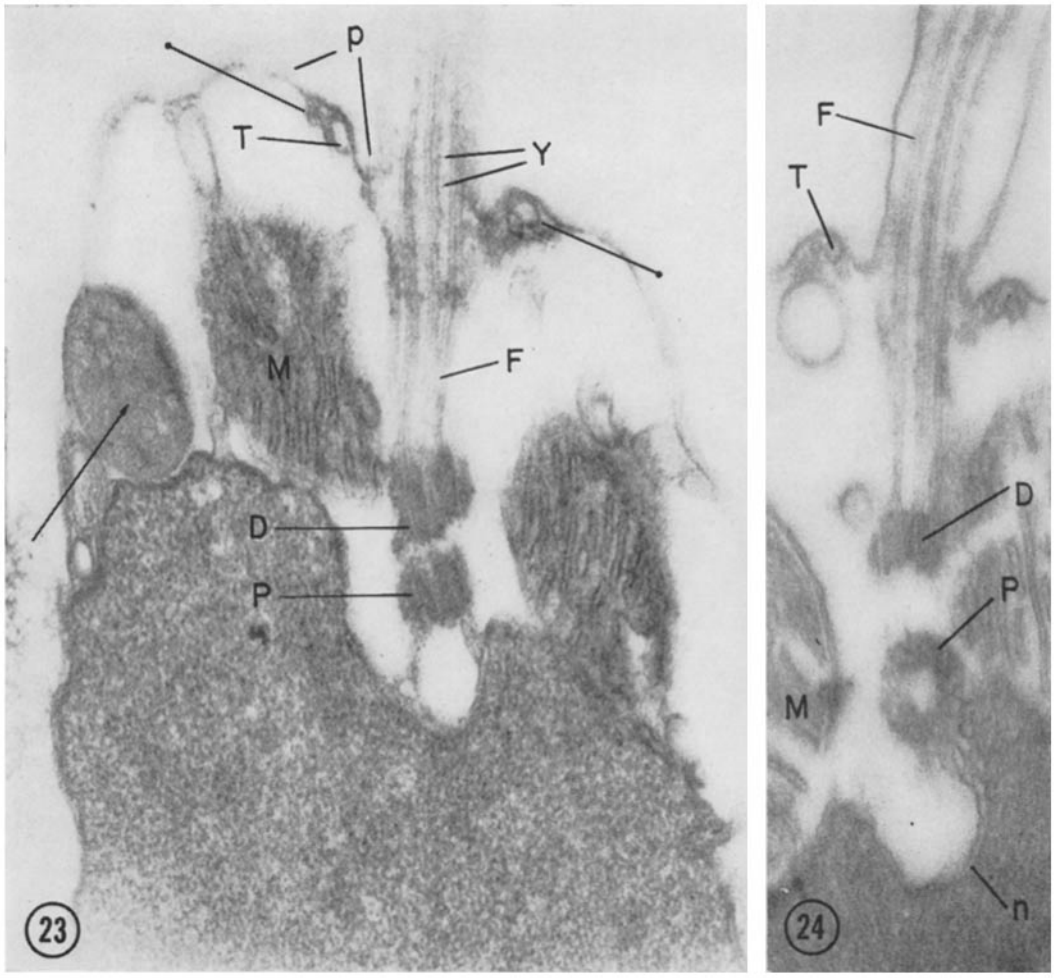

FIGURES 23 AND 24

Longitudinal sections through the mitochondrial region of spermatozoa in process of egg penetration.

FIGURE 23

The centrioles have been cut through the dense walls of the cylinders. Strands appear to extend between the distal (*D*) and proximal (*P*) centrioles. Strands also extend between the proximal centriole and the bottom of the centriolar pit. These strands may associate with the nuclear envelope, but this relationship is uncertain. The flagellar filaments (*F*) insert into the distal centriole. The short dark regions indicated by the letter *Y* may be a different sectional view of the Y-shaped attachments shown in Fig. 33. A ring of vesicles (*T*) appears in close contact with the plasma membrane (*p*) surrounding the base of the flagellum. A section in the plane joining the two dots in the upper part of this figure would give approximately the section shown in Fig. 33. Arrow: grazing section of mitochondrion. × 106,000.

FIGURE 24

The proximal centriole (*P*) has been cut approximately in cross-section and the less dense central region shows clearly. × 79,000.

capable of separation. Indeed, they have been found to be separated at many if not all of the loci at which they are ordinarily contiguous. These observations suggest that the very intimate developmental relationship postulated for the starfish does not obtain in *Hydroides*. On the other hand, total separation of the acrosomal membrane from the nuclear envelope was rarely observed in *Hydroides*. Possibly the ill defined material which lies between these two structures normally serves as some sort of attachment between them. The *apparently* pulled-out parts of the contiguous membranes certainly suggest *some* degree of close attachment between the two. Whatever its means may be, the attachment is sufficiently firm so that in the normal course of events the nucleus and acrosome remain together despite the considerable changes which the acrosomal region undergoes during egg penetration (10, 11). The real nature of the supposed attachment simply is not entirely clear.

Dan (13) also has described the acrosomal region of *Mytilus*. On the basis of her sketch (her Fig. 13), based on unpublished electron micrographs, this acrosome, too, may be homologized with that of *Hydroides*. The "outer sheath," that is, the part of the acrosomal membrane in contact with the plasma membrane, might be the equivalent of the outer zone of the acrosomal membrane in *Hydroides*, the "central tube" might be the equivalent of the inner zone, and the part associated with the nuclear region might be the equivalent of the intermediate zone. At the apex, between the outer and central tubes, is a knob-like granule which might correspond to the acrosomal granule in *Hydroides*. One might even speculate that the single "central tube" is the equivalent of a single one of the tubular invaginations of the inner zone of the acrosomal membrane in *Hydroides*. Similarly, the acrosomal region of the oyster (16) may conform to this pattern.

Afzelius (1) has reported electron microscopic studies of the acrosomal region of four species of sea urchin. In each a granule, presumably the acrosomal granule, is found.

Although, in the foregoing, an attempt is made to homologize the structure of the acrosome in several species of widely diverse phyla, no final judgment on this matter can be made until electron microscopic studies of the development of the acrosome of these and other species are available.

Invaginations of the Acrosomal Membrane: In *Hydroides* it has been shown that the inner zone of the acrosomal membrane possesses about fifteen or so tubular invaginations which project toward the basal part of the acrosomal granule. In subsequent papers on sperm entry (10, 11) it will be shown that these tubules play a surprising and remarkable role in the association of spermatozoon with egg proper.

The Sperm Apex: As compared with the rest of the spermatozoon, the tip of the sperm head appears to be particularly fragile and subject to rupture. This is shown occasionally in "untreated" spermatozoa (Fig. 20) and by numerous spermatozoa in frozen-thawed preparations (Fig. 19). Serial sections of ruptured acrosomal regions clearly show that the rupture is along a circular line confined to the apex. It is suggested that the tip of the sperm head possesses a natural "fracture line" which under appropriate circumstances constitutes a rim of dehiscence.

The plasma membrane and the outer zone of the acrosomal membrane lie very close together in the circular area over which they meet when they sandwich the apical vesicle. It is a striking fact that in both "untreated" and frozen-thawed preparations, *when the tip of the spermatozoon ruptures along its natural fracture line the edges of these two membranes remain together as if fused to form the rim or lip of the new orifice*. It is suggested that they may be fused even before dehiscence occurs.

The significance of the existence of the natural fracture line and of its associated circular area around which the plasma and acrosomal membranes meet and form a continuum will become apparent when the entry of the spermatozoon into the vitelline membrane is described in a subsequent paper (11).

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FIGURES 25 TO 33

Sections through spermatozoa in process of egg penetration.

FIGURE 25

An oblique section through the proximal centriole (*P*). × 107,000.

FIGURES 26 AND 27

Longitudinal and cross-sections, respectively, through the flagellum.

FIGURE 26

Several vesicles (arrow) of unknown significance are present. × 91,000.

FIGURE 27

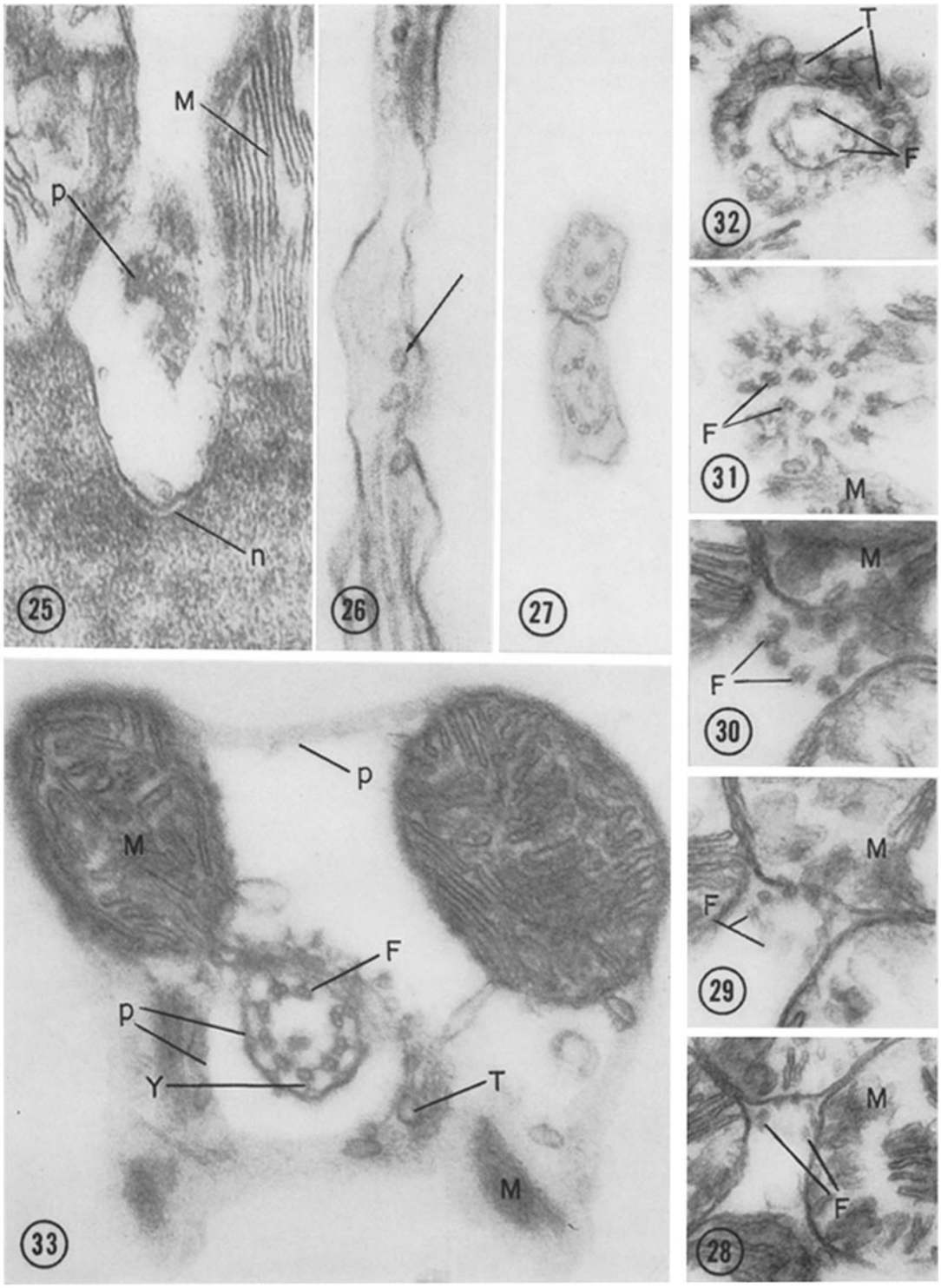
Cross-section through two flagella. There is a peripheral group of nine doublets of filaments and a central group of apparently two single filaments. × 93,000.

FIGURES 28 TO 32

Approximate cross-sections of one spermatozoon (serial sections 1, 2, 4, 5, and 6, respectively). As the sections proceed out toward the base of the flagellum (Fig. 32), the flagellar filaments appear to become associated with the Y-shaped structural elements seen in Fig. 33. × 67,000.

FIGURE 33

An approximate cross-section through the base of the flagellum. A section in the plane joining the two dots in the upper part of Fig. 23 would give approximately the section shown in this figure. Structural elements in the form of Y-shaped strands connect the peripheral doublets of filaments to the plasma membrane (*p*). The ring of vesicles (*T*) appears closely associated with the plasma membrane (*p*) at the base of the flagellum. × 92,000.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