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CHANGES IN THE SPERMATOZOON DURING FERTILIZATION IN *HYDROIDES HEXAGONUS* (ANNELIDA)

II. Incorporation with the Egg

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ABSTRACT

This, the last of a series of three papers, deals with the final events which lead to the incorporation of the spermatozoon with the egg. The material used consisted of moderately polyspermic eggs of *Hydroides hexagonus*, osmium-fixed at various times up to five minutes after insemination. The first direct contact of sperm head with egg proper is by means of the acrosomal tubules. These deeply indent the egg plasma membrane, and consequently at the apex of the sperm head the surfaces of the two gametes become interdigitated. But at first the sperm and egg plasma membranes maintain their identity and a cross-section through the region of interdigitation shows these two membranes as a number of sets of two closely concentric rings. The egg plasma membrane rises to form a cone which starts to project into the hole which the spermatozoon earlier had produced in the vitelline membrane by means of lysis. But the cone does not literally engulf the sperm head. Instead, where they come into contact, sperm plasma membrane and egg plasma membrane fuse to form one continuous membranous sheet. At this juncture the two gametes have in effect become mutually incorporated and have formed a single fertilized cell with one continuous bounding membrane. At this time, at least, the membrane is a mosaic of mostly egg plasma membrane and a patch of sperm plasma membrane. The evidence indicates that the fusion of the two membranes results from vesiculation of the sperm and egg plasma membranes in the region at which they come to adjoin. Once this fusion of membranes is accomplished, the egg cytoplasm intrudes between the now common membrane and the internal sperm structures, such as the nucleus, and even extends into the flagellum; finally these sperm structures come to lie in the main body of the egg. The vesiculation suggested above appears possibly to resemble pinocytosis, with the difference that the vesicles are formed from the plasma membranes of *two* cells. At no time, however, is the sperm as a whole engulfed and brought to the interior of the egg within a large vesicle.

INTRODUCTION

Little is known about how the actual meeting of spermatozoon and egg is effected. In some species light microscope studies of living material (reviewed in 1) show that a fertilization cone rises around the acrosomal filament (tubule) once the filament has made contact with the egg plasma membrane. In several species at least, it is known that the sperm head may complete its passage into

the egg before the cone has receded. Therefore, as has been shown (1), subsequent recession of the cone cannot be responsible for the movement inward of the spermatozoon. There are no fine structure studies which deal with the gametes at this crucial time. As will be described below, in *Hydroides hexagonus* the time and location as well as the details of the actual incorporation of the spermatozoon are quite different from what light microscope studies might lead one to suppose.

In *Hydroides*, before it enters the cytoplasm of the egg the spermatozoon passes through the thick vitelline membrane, which it modifies in several ways. One of the effects is that a hole slightly larger than the spermatozoon is formed in the membrane by means of lysin from the spermatozoon (2, 4, 5). The spermatozoon, too, becomes modified and its acrosomal region changes drastically. One of the results is that as the sperm head nears the egg plasma membrane its advance element is a tuft of acrosomal tubules whose *outer* surfaces were once the *inner* surface of the acrosomal membrane. This surface formerly faced into and was part of the basal wall of an originally closed acrosomal vesicle (3), but now the vesicle has everted and the acrosomal membrane has been inserted, as in a mosaic, to become the apical part of the sperm plasma membrane. The subsequent activities of the acrosomal tubules and of the surface areas of the egg proper, as the spermatozoon incorporates with the egg, will be examined in the present paper.

MATERIALS AND METHODS

The materials used and the method of preparation were the same as those described in the preceding paper (6). Fixations were made at various intervals between 9 seconds and 5 minutes 10 seconds after insemination. There was some correlation between the stage of sperm head penetration and the time elapsed from insemination to fixation, although materials fixed after longer intervals contained a number of different stages. From observation of living material it has been found that entry into the egg proper is usually completed in about 3 to 5 minutes. The material used was moderately polyspermic but from observation of living specimens it seems likely that the basic pattern of the events reported here would be much the same in monospermic eggs. Most of the sections shown were selected from serial sections of the specimen being described.

OBSERVATIONS

The series of events described in this paper is shown diagrammatically in Fig. 1.

1. *Establishment of Contact between Acrosomal Tubules and Egg Plasma Membrane*

Spermatozoa which have nearly reached the egg proper are shown in Figs. 2 and 3. The major acrosomal changes have been accomplished, and the somewhat convoluted and/or branching acrosomal tubules project in front of the advancing nucleus. Eventually these tubules encounter the

Explanation of Figures

All sections are approximately longitudinal with respect to the spermatozoon. Since the sperm head is approximately circular in cross-section, the peripheral longitudinal sections are narrower than the central ones; however, the extreme divergences in length or width between certain specimens at similar stages are a reflection of compression during sectioning.

a, that part of sperm plasma membrane which was formerly acrosomal membrane
b, ill defined material which in earlier stages lies between nuclear envelope and acrosomal membrane at base of acrosome
mg, granules, or remnant, of material of intermediate zone of acrosome
n, nuclear envelope
pe, plasma membrane of egg
ps, plasma membrane of spermatozoon
t, acrosomal tubule
v, microvillus of egg
F, fertilization cone
IBL, inner border layer of vitelline membrane
M, mitochondrion
ML, middle layer of vitelline membrane
N, nucleus
OBL, outer border layer of vitelline membrane
Y, yolk granule in egg cytoplasm

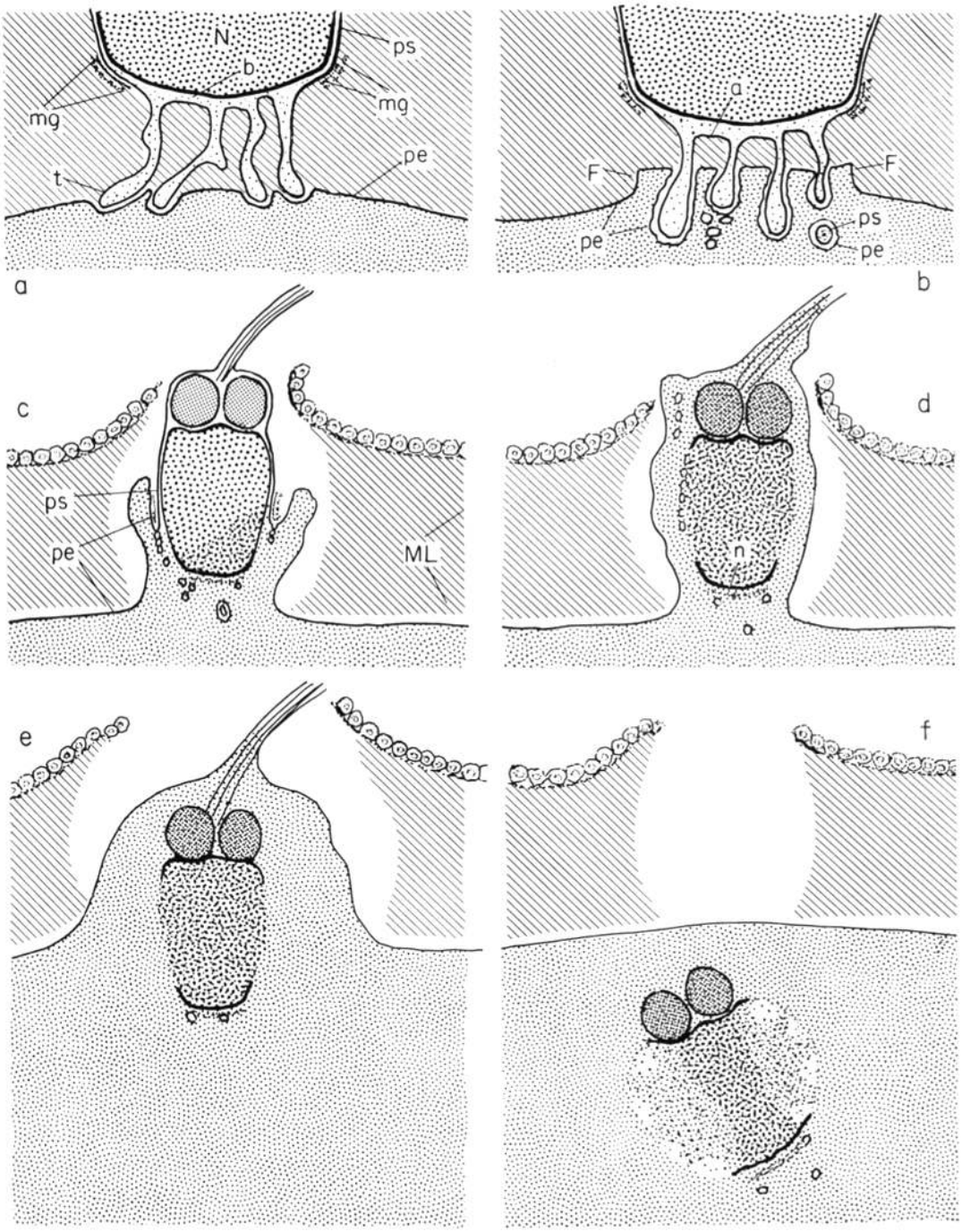


FIGURE 1

Diagrams of stages in fusion and incorporation of spermatozoon and egg. *a*, *b*, acrosomal tubules of sperm head indent egg but egg plasma membrane still intervenes between egg cytoplasm and sperm plasma membrane (of tubules); *c*, fusion of egg plasma membrane with sperm plasma membrane; vesiculation near presumed site of fusion; acrosomal tubules no longer visible, as such; egg cytoplasm now in direct contact with apical part of nucleus; *d*, within their common plasma membrane egg cytoplasm surrounds sperm structures, but fused gametes still have profile of both egg and spermatozoon; *e*, *f*, sperm structures move more deeply into egg cytoplasm; mitochondrial and apical parts of nuclear envelope remain still visible but peripheral part of nucleus becomes diffuse; fertilization cone recedes.

plasma membrane of the egg. In this species microvilli which are true extensions of the egg proper project into the vitelline membrane, and their distribution is such that, considering the area occupied by a typical tuft of acrosomal tubules, the approaching spermatozoon would probably encounter from one to five microvilli. Nevertheless, the most frequently observed area of extended contact with the egg plasma membrane is at or between the bases of the microvilli rather than above their outer extremities (Fig. 4). Typically, a tuft consisting of a number of lengthened and distended acrosomal tubules comes to be closely applied to the plasma membrane of the egg.

2. *Indentation of Egg by Acrosomal Tubules*

The egg plasma membrane next becomes deeply indented by the acrosomal tubules (Figs. 5 to 10). A low fertilization cone usually delineates the general area over which the indentation is taking place. A cross-section through an invading tubule and the correspondingly depressed area of the egg plasma membrane shows two closely adjacent concentric rings. These *appear* to lie within the egg cytoplasm although in fact the outer ring is actually the egg plasma membrane, while the inner ring is the sperm plasma membrane, which is still literally *outside* the egg cytoplasm. In the section shown in Fig. 5 several small circular vesicles are seen immediately below the deepest part of one indentation. Their possible significance will be discussed later.

The morphological relationship between the penetrating acrosomal tubules and the indented egg is not always as clear as in the preceding cases. The tubules may become much convoluted, as may also any microvilli that are involved; thus in sections such as the one shown in Fig. 11 one cannot be sure which part is spermatozoon and which is egg. Moreover, the appearance of the tubular contents in some instances makes it difficult to distinguish the tubule from the adjacent egg cytoplasm.

3. *Incorporation of Sperm Structures*

(*a*) *Fusion of Sperm and Egg Plasma Membranes:* After the acrosomal tubules have deeply indented the egg plasma membrane, the enlarging fertilization cone of the egg comes to project into the hole which the spermatozoon earlier had produced in the vitelline membrane by means of lysis (Fig. 13). But the cone does not literally engulf the sperm head. Close inspection of the specimen shown in Figs. 16 and 17 shows that the egg plasma membrane is simply continuous with the plasma membrane of the spermatozoon. There is no point at which a line of fusion or junction between them can be distinguished. However, if it be assumed that the part which lies close against the sperm nucleus is the sperm plasma membrane and that the part which encloses the egg cytoplasm is the egg plasma membrane, then the area of junction between them must lie in the very limited area of membrane between the tips of the guide lines *pe* and *ps* in these figures. Near the presumed area of junction lies a remnant of acrosomal material which remains with the

FIGURE 2

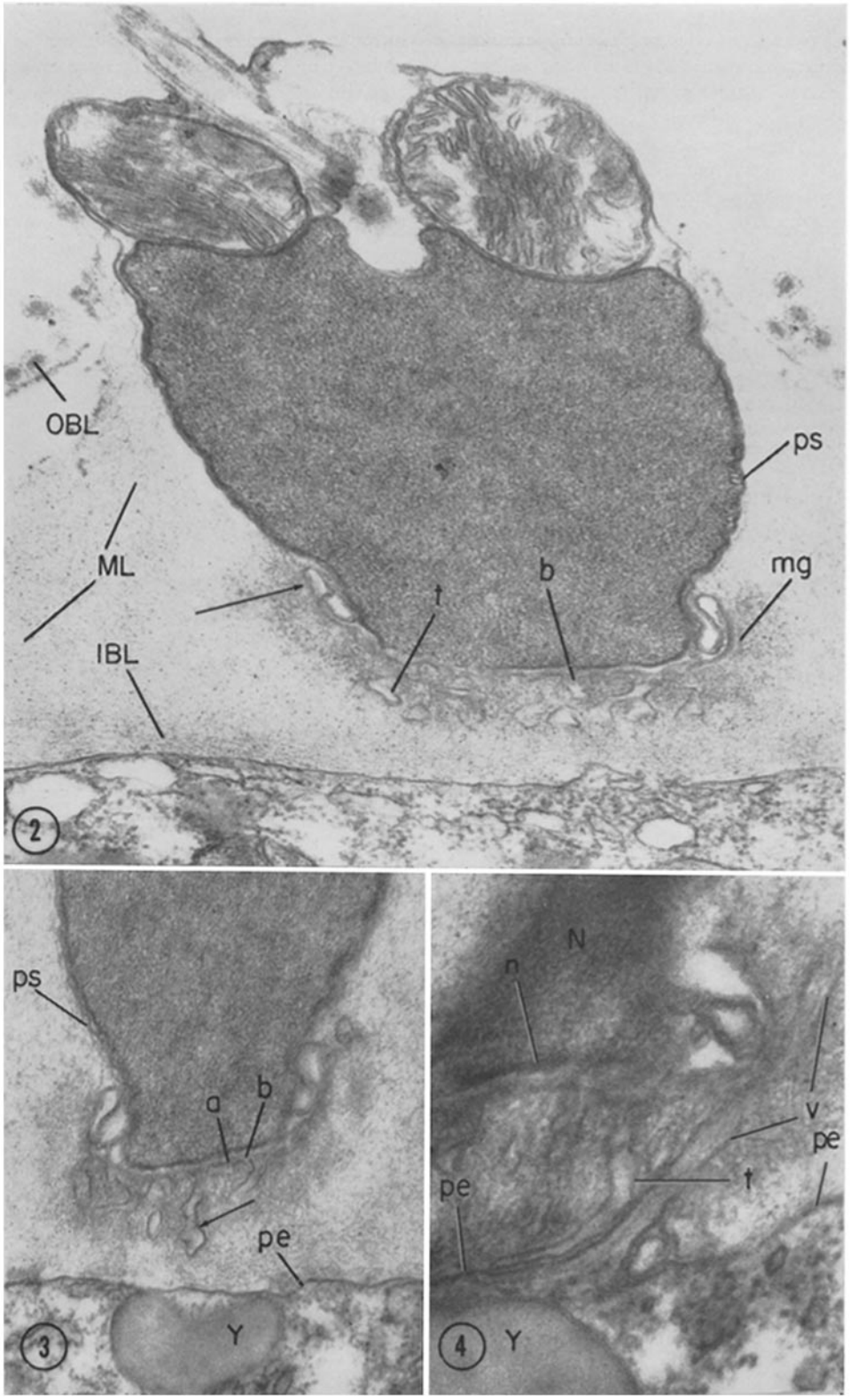
Sperm head which has been drawn far into vitelline membrane by eversion of acrosome. Acrosomal tubules approach egg plasma membrane. Arrow: folded part of sperm plasma membrane marks site of earlier end-to-end junction of acrosomal and plasma membranes to become one continuum. Dense row of intermediate zone granules (*mg*) of everted acrosome will later persist as remnant identifying this area. × 74,000.

FIGURE 3

Apical region of sperm head has nearly reached egg plasma membrane. Arrow: enlarging, slightly convoluted acrosomal tubule. Membrane of this tubule, formerly *inside* acrosome, now is apical part of sperm plasma membrane. × 65,000.

FIGURE 4

Apical region of spermatozoon which is near egg. Acrosomal tubules are in contact with egg plasma membrane near base of microvillus. × 113,000.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sperm plasma membrane. The appearance of this remnant in earlier stages may be seen in Figs. 2, 6, and 8. But the salient feature of the section shown in Fig. 16 is the row of small rounded vesicles lying in the cytoplasm just beneath the presumed zone of junction on the left side of the section. Similar groups of vesicles are shown in the specimen in Figs. 12 to 15. In Fig. 15, above the arrow, is a configuration which suggests that one of these vesicles is open to the outside, just at the junction of the two membranes. In Fig. 12 a row of *contiguous* vesicles leads into the cytoplasm from the presumed junction. It should be noted that in Figs. 12 and 16 the lower vesicles in the row lie beside an apparently intact part of the nuclear envelope. In a few specimens the presumed zone of junction was at a higher level with reference to the sperm head than in the figures shown here, and in some of these a more extensive area of vesiculation was seen. In other specimens the presumed zone of junction, as seen in longitudinal sections, was at quite different levels on opposite sides of the sperm head.

In the specimens shown in Figs. 13 and 15 to 17 the acrosomal tubules cannot be seen, with the possible exception of one small concentric configuration in Fig. 17. Instead, clusters of small rounded vesicles lie in the cytoplasm outside the apical part of the nuclear envelope. In this region, in Fig. 16, a line of moderately dense material presumably represents material which in earlier stages is seen between the nuclear envelope and the base of the acrosome (Figs. 3, 6, and 11).

Gradually (Figs. 18 to 21) the egg cytoplasm with its distinguishing RNP particles comes to surround the sperm nucleus. Neither the egg plasma membrane nor the sperm plasma membrane intervenes between them. Then the mitochondria of the middle piece and at least the proximal parts of the filaments of the flagellum, too, come thus to lie directly within the egg cytoplasm. Except for the outer part of the flagellum, which has not been studied, it is clear from serial sections that *a single continuous plasma membrane now encloses the merged egg cytoplasm and sperm structures.* But the merged area still protrudes beyond the general profile of the egg, and in this protrusion, or cone, only a thin layer of egg cytoplasm surrounds the sperm structures. Indeed, at the stages shown here and especially in Figs. 19 and 20, the cone still bears essentially the shape of the spermatozoon. Later, more of the egg cytoplasm enters the cone (Fig. 24). Next, the sperm structures come to lie within the main body of the egg. Finally the cone retreats.

(*b*) *Nuclear Envelope:* In regions where the sperm plasma membrane closely adjoins the nuclear envelope it is often difficult if not impossible to discriminate between the two, even in stages in which the nucleus has not yet entered the egg cytoplasm (Figs. 2 and 3). So, too, in entering spermatozoa (Figs. 12 to 17), where the sperm plasma membrane adjoins its nucleus, it cannot always be established with certainty whether the nuclear envelope is present or not. However, in regions where the egg cytoplasm adjoins the sperm nucleus, both early and late stages show the following features (Figs. 21 to 24). The nuclear envelope stains heavily and is clearly visible on the apical face of the nucleus. It is visible, too, in the mito-

FIGURES 5 TO 7

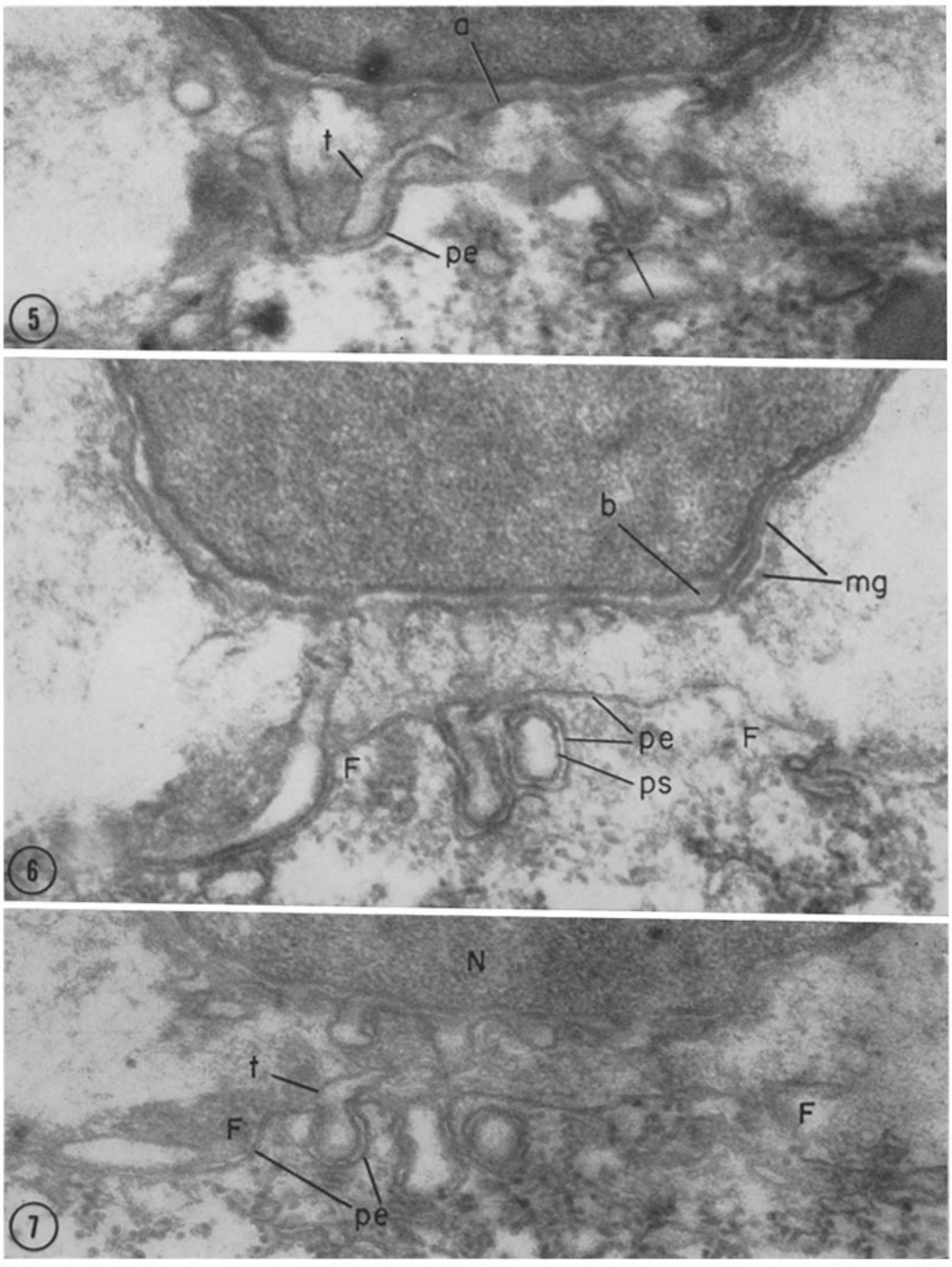
Serial sections 1, 3, and 4, respectively, through area in which acrosomal tubules have indented plasma membrane of egg. × 108,000.

FIGURE 5

One long acrosomal tubule (*t*) extends from its base near nucleus to area in which it seems to indent egg. Arrow: group of small vesicles lying beneath deepest point of indentation of an invading acrosomal tubule.

FIGURES 6 AND 7

Tangential sections through invading acrosomal tubules and the correspondingly depressed area of the egg show characteristic configurations consisting of two concentric rings. A very low fertilization cone (between *F* and *F*) rising from egg delineates general area in which contact between egg and sperm plasma membranes takes place.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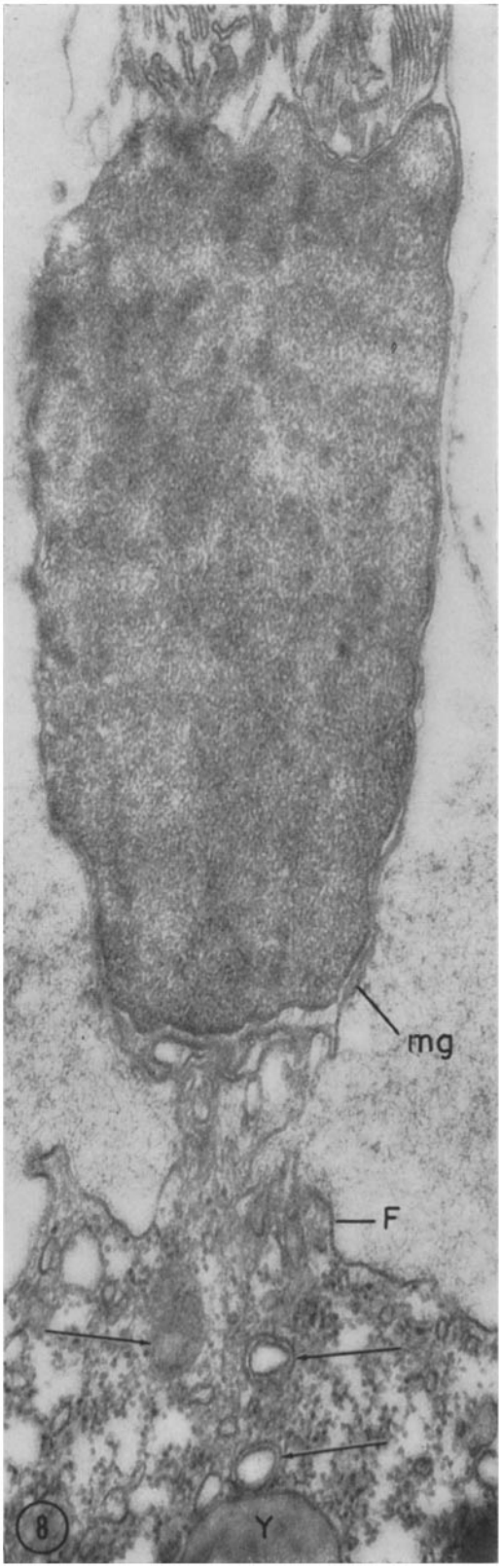
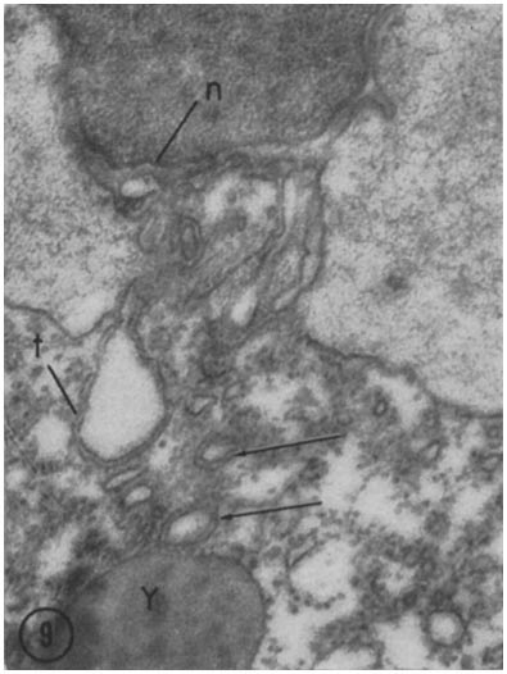




FIGURES 8 AND 9

A specimen showing deeper penetration of egg than shown in previous figures. *Y* indicates a yolk granule. Arrows indicate typical concentric figures of which inner ring is sperm plasma membrane of acrosomal tubule and outer ring is indented egg plasma membrane.

FIGURE 8

Shows sperm head in vitelline membrane. Left arrow: grazing section of wall of indenting tubule. ×77,000.

FIGURE 9

Serial section adjacent to that shown in Fig. 8; arrows point to same structures indicated by correspondingly placed arrows in Fig. 8. × 83,000.

chondrial region, although it does not appear to be as thick a structure there. But in most of the large intervening region of the periphery it cannot clearly be seen and the impression is that it is no longer present at all sites here. However, many small rounded vesicles lie very close to the nuclear material in at least some sections of this region (Figs. 21, 22, and especially 23).

(*c*) *Acrosomal Remnant:* It has been shown elsewhere (6) that during its passage through the vitelline membrane the everting acrosomal vesicle releases much fine granular material. Although most of this disappears, a thin sheet and a few small masses of granules sometimes remain close to the acrosomal membrane and later serve to identify that part of the sperm plasma membrane at which the former acrosomal membrane had joined it (Figs. 2, 6, and 8). As shown above (Figs. 12, 14, 16, and 17), this remnant may still be present and thus serve to identify the sperm

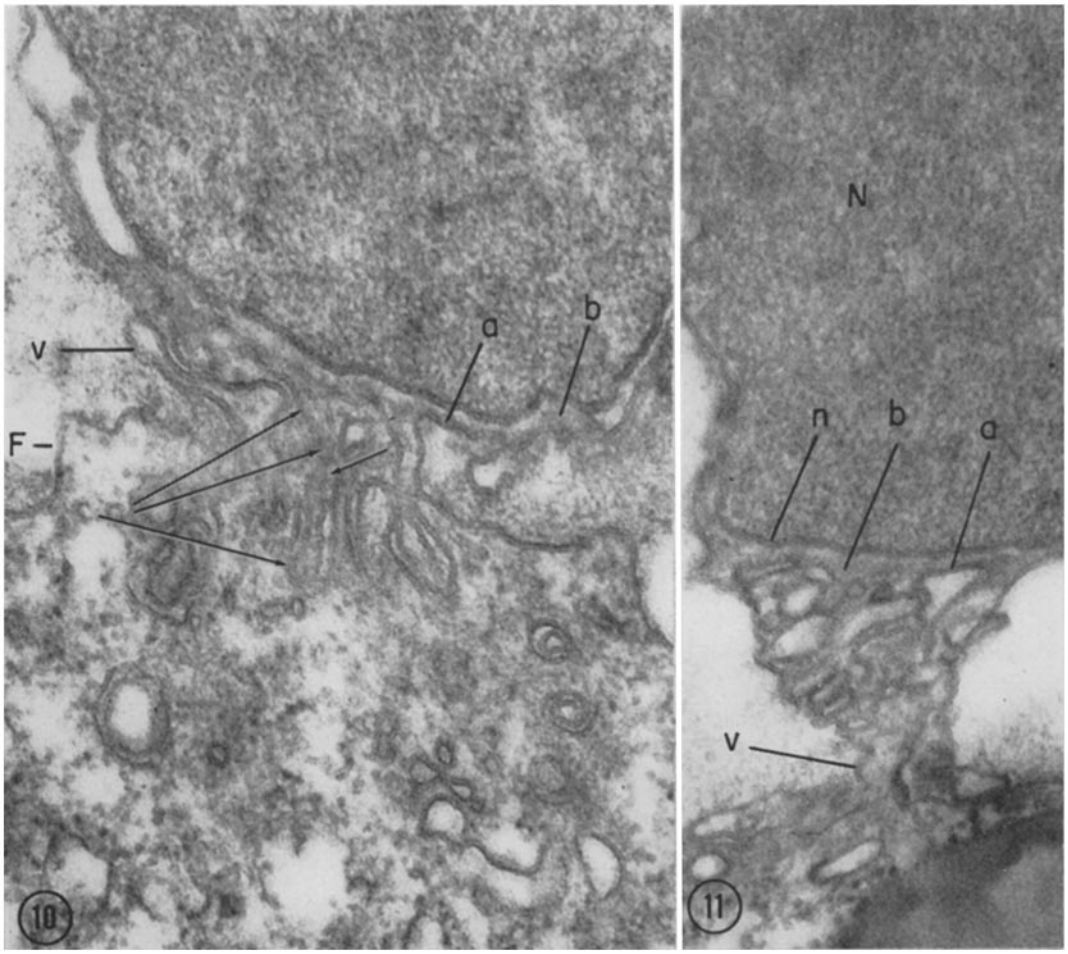


FIGURE 10

Acrosomal tubules of sperm head indenting egg. Arrows: acrosomal tubule can be traced from origin near apex of nucleus to indented area in plasma membrane of egg. Note typical concentric figures and well defined fertilization cone. Large projection (*v*) is possibly a microvillus. × 109,000.

FIGURE 11

Unusually narrow area of engagement. Convoluted, possibly vesiculated acrosomal tubules and convoluted microvillus not distinguishable from each other in some areas. × 108,000.

plasma membrane, even after the egg and sperm plasma membranes have fused. Thus far it has not been identified in subsequent stages.

4. *Sperm Structures after Incorporation*

Fig. 25 shows a section of a spermatozoon which presumably was beginning to undergo the changes which would lead to formation of the male pronucleus. Although the material of the nucleus has dispersed peripherally, the apical and mitochondrial ends of the nuclear envelope appear to have remained intact. The mitochondria, too, have maintained the same relative position as previously. The line of faintly staining material which formerly lay between the base of the acrosome and the nuclear envelope can still be seen in a position close to the apical part of the nuclear envelope. It has much the same appearance as in the previous stage (Figs. 20 to 22 and 24). A few small rounded vesicles are still associated with it.

DISCUSSION

1. *Fusion of Sperm and Egg Plasma Membranes: Incorporation of Sperm Structures*

Following interdigitation of the acrosomal tubules with the egg plasma membrane, the latter starts to rise around the sperm head, and the sperm and egg plasma membranes are then seen to be a continuum. An acrosomal remnant identifies the sperm plasma membrane (6) where the latter meets the egg plasma membrane. There is evidence that this continuum arises by fusion and that the fusion is accomplished by vesiculation in the region where the two membranes adjoin. At least this is a possible interpretation of such a section as the one shown in Fig. 12; here one vesicle is in contact with the membrane at the presumed line of junction and a line of other vesicles leads from the first one into the cytoplasm. There is also evidence that vesiculation between the two membranes occurs around the apex of the sperm head. This is a possible interpretation of the more or less linear arrangement of the small vesicles lying below an acrosomal tubule which has indented the egg plasma membrane of the specimen shown in Fig. 5. Extensive vesiculation between acrosomal tubules and indented egg plasma membrane could account for the presence of clusters of vesicles in the region of the sperm apex at the time when the acrosomal tubules are no longer seen. The nuclear envelope, too, seems to undergo vesiculation (Figs. 21 and 23). It is not likely, however, that this envelope is the source of the vesicles discussed above, because in some instances this envelope appears to be still intact in regions where these vesicles are found (Figs. 12 and 16, at arrow). It is suggested that progressive vesiculation begins in the apical region and extends for at least a short distance up the sperm head, and that this vesiculation results in the fusion of egg and sperm plasma membranes.

No matter how the fusion is accomplished, it is clear that by a rather early stage (Fig. 16) *the egg plasma membrane and the sperm plasma membrane become one continuous mosaic membrane, and that the two formerly separate cells then constitute a single cell.*

FIGURES 12 TO 15

From serial sections of a sperm head in early stage of fusion with egg. Figs. 12 and 14 are from section 1 and Figs. 13 and 15 are from sections 5 and 7, respectively.

FIGURES 12 AND 14

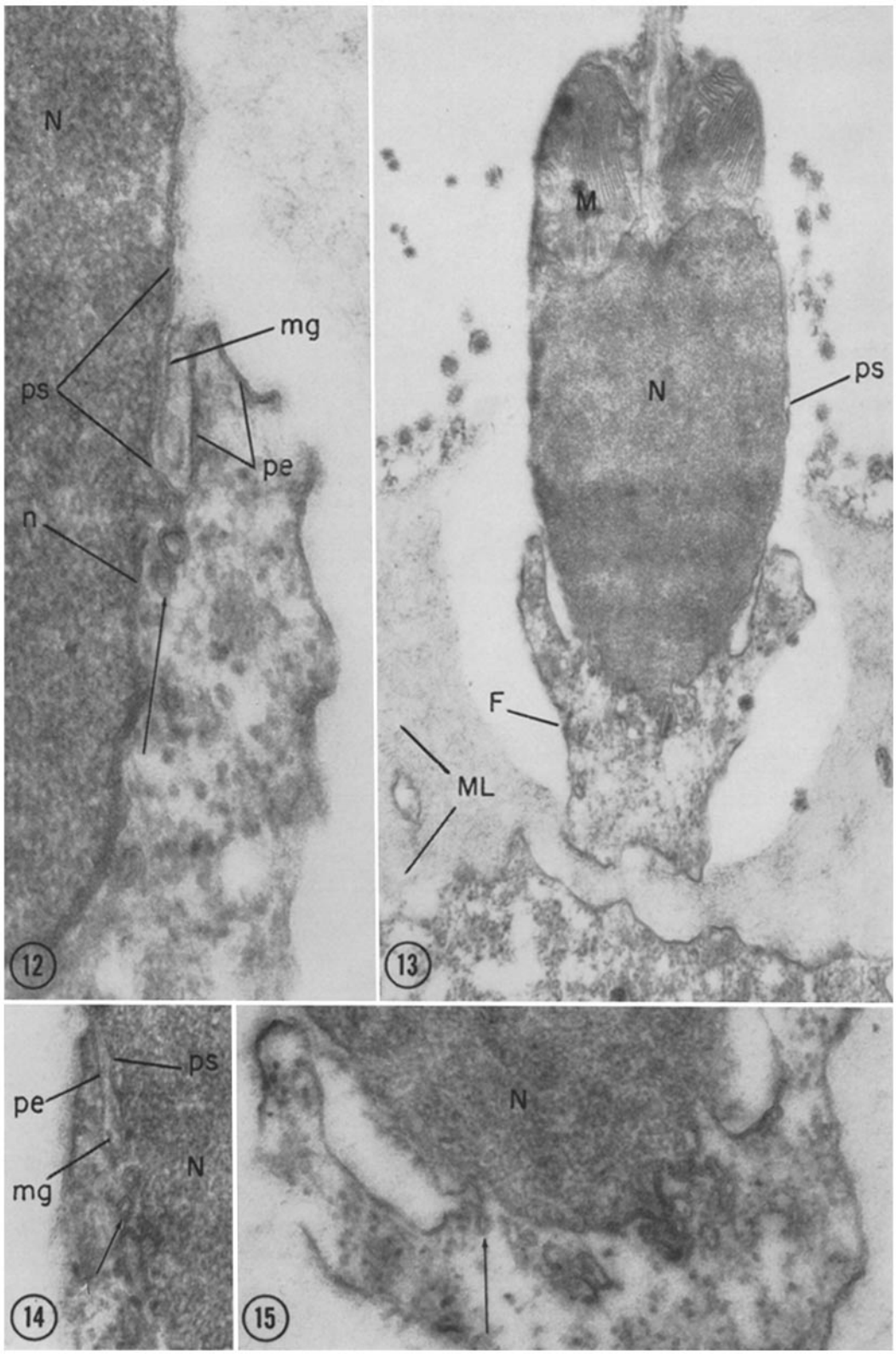
Details of right and left sides of section, showing region of junction between sperm plasma membrane and egg plasma membrane. Arrows: line of small round vesicles in egg cytoplasm just beneath region of junction. Fig. 12 × 147,000; Fig. 14 × 102,000.

FIGURE 13

Shows position of this specimen in cone. × 58,000.

FIGURE 15

Detail of area near apex of sperm nucleus. Note groups of small vesicles near presumed area of junction between sperm and egg. Arrow: vesicle seems to be forming at region of junction. No acrosomal tubules remain visible. × 108,000.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Within this continuous membrane the egg cytoplasm then comes to surround the remaining sperm structures. But the now single cell still has the profile of both gametes, as shown in Fig. 20. In this specimen the egg cytoplasm had reached at least part way into the flagellum. If seen in living material, with the light microscope, specimens such as this might be interpreted erroneously as showing an intact spermatozoon which had not yet been incorporated with the egg. Eventually the profile becomes that of the egg alone. It is not known, however, whether the sperm plasma membrane is finally removed in some way or whether, instead, it remains as part of the plasma membrane of the fertilized egg.

It may be of interest to compare the events of fertilization as seen in *Hydroides* with Tyler's (9) "specific pinocytosis as a possible sperm-engulfing process." Tyler's scheme essentially seems to be one of progressive envelopment of the spermatozoon *as a unit* by egg plasma membrane. The stimulation postulated for this is a fertilizin-antifertilizin reaction between the plasma membranes of the spermatozoon and egg. As the initial fertilizin-antifertilizin reaction occurs between the tip of the spermatozoon and the egg plasma membrane, this somehow brings into contact adjacent regions of the plasma membranes of the two cells; eventually the spermatozoon is encompassed by egg plasma membrane which is bound to sperm plasma membrane by fertilizin-antifertilizin molecules. This structure, essentially a huge vesicle containing the spermatozoon, then recedes into the main region of the egg cytoplasm and is pinched off.

In Tyler's scheme the egg plasma membrane is responsible for moving the sperm head through the vitelline membrane. But in *Hydroides* eversion of the acrosome appears to move the sperm head into the vitelline membrane (6), and *only then* is functional contact between sperm and egg plasma membranes established. Furthermore, in *Hydroides* no large vesicle ever encloses the sperm head. Instead, the sperm and egg plasma membranes fuse to form one continuous membrane and thereby the spermatozoon incorporates with the egg. This fusion takes place while the sperm nucleus is still located in the region of the vitelline membrane. Only later, and by means still unknown, do the already incorporated sperm structures come to lie within the main body of the egg. The vesiculation process which appears to mediate the fusion of the sperm and egg plasma membranes does seem to resemble pinocytosis. However, pinocytotic vesicles are derived from the membrane of a single cell (8), whereas the vesicles described here appear to be derived from the membranes of *two* cells. If, nevertheless, this vesiculation be termed pinocytosis, then this pinocytosis is certainly different in time and detail from that postulated by Tyler.

2. *The Acrosomal Tubule in Egg Activation*

(*a*) In an earlier paper (4) it was pointed out that in *Saccoglossus* and *Hydroides* there was some evidence that the "acrosome filament" was tubular. This has been completely confirmed for *Hydroides* (6). In the same paper (4, p. 499) it was stated that "if the filament is tubular the possibility

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FIGURES 16 AND 17

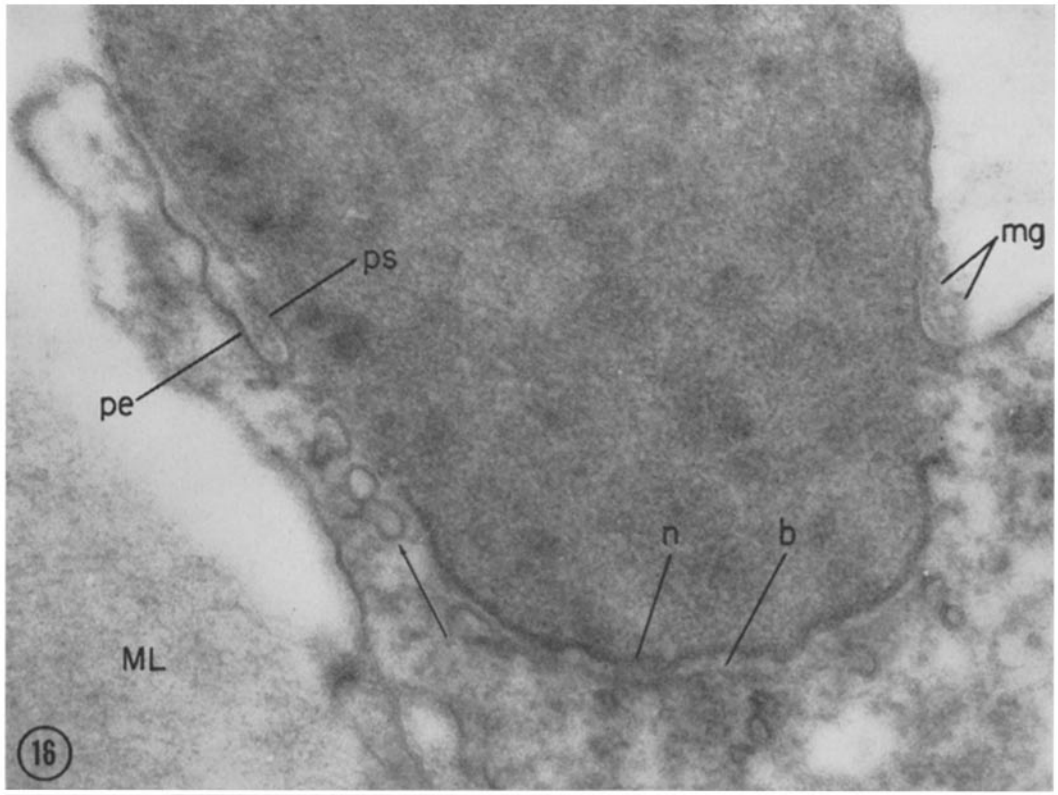
Sections 1 and 6, respectively, of a spermatozoon entering egg cytoplasm. About the same stage as shown in Fig. 13. Note continuity of egg plasma membrane and sperm plasma membrane. Arrows: groups of vesicles below area of junction of the two plasma membranes. Remnants of acrosomal material (mg) typically lie near sperm plasma membrane. × 108,000.

FIGURE 16

A nearly median section. Note intact part of nuclear envelope in apical region. Faint line below nucleus is material which in earlier stages lay between nuclear envelope and base of acrosome.

FIGURE 17

Section in more lateral position. To left of nuclear apex, note one concentric ring figure reminiscent of indenting acrosomal tubule.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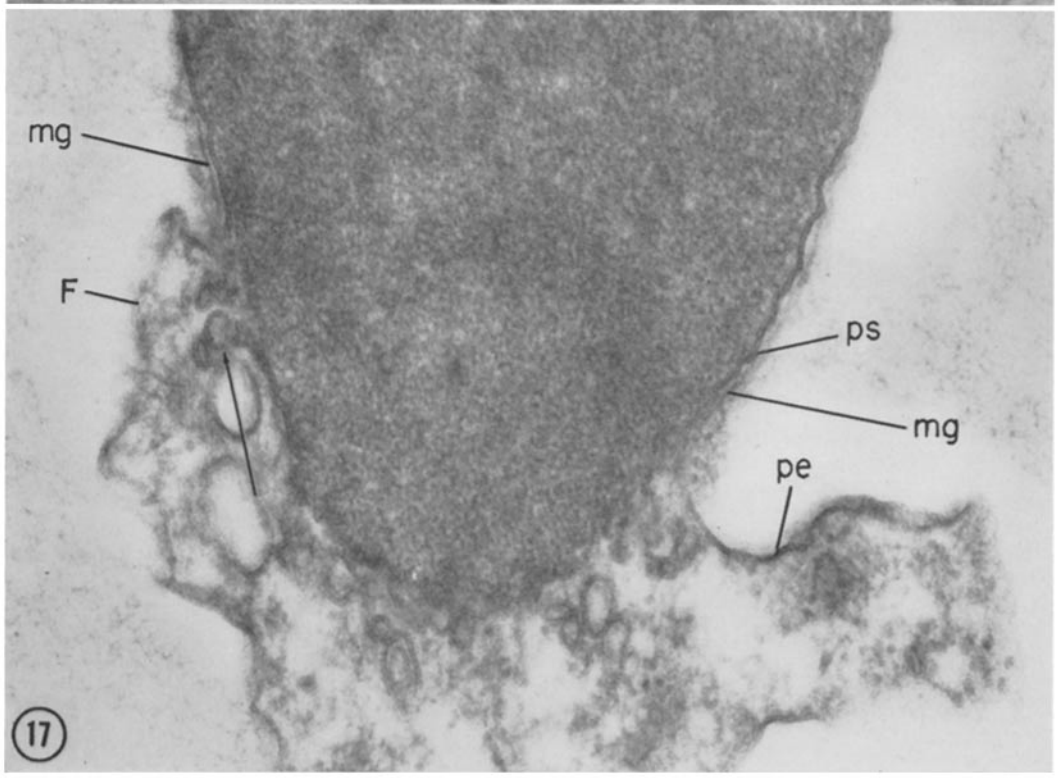
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exists that the tube conveys some substance to the egg. Bowen was unaware . . . of the acrosome filament . . . yet his suggestion that the spermatozoon might discharge some enzyme-like substance into the egg cytoplasm to initiate the fertilization reaction now merits serious reconsideration." The present observations in *Hydroides* suggest that the whole tuft of acrosomal tubules becomes incorporated into the egg. While these observations cannot decide the validity or otherwise of Bowen's concept, the tuft of acrosomal tubules certainly could be a vehicle for transporting such an initiating substance. In any case, the contents of these tubules sooner or later mingle with the egg cytoplasm.

In the starfish, Dan (7) argues that since pricking with a glass needle is an effective method for activating unfertilized echinoderm eggs, "there seems to be no *a priori* necessity for postulating an activating enzyme in addition to the pricking

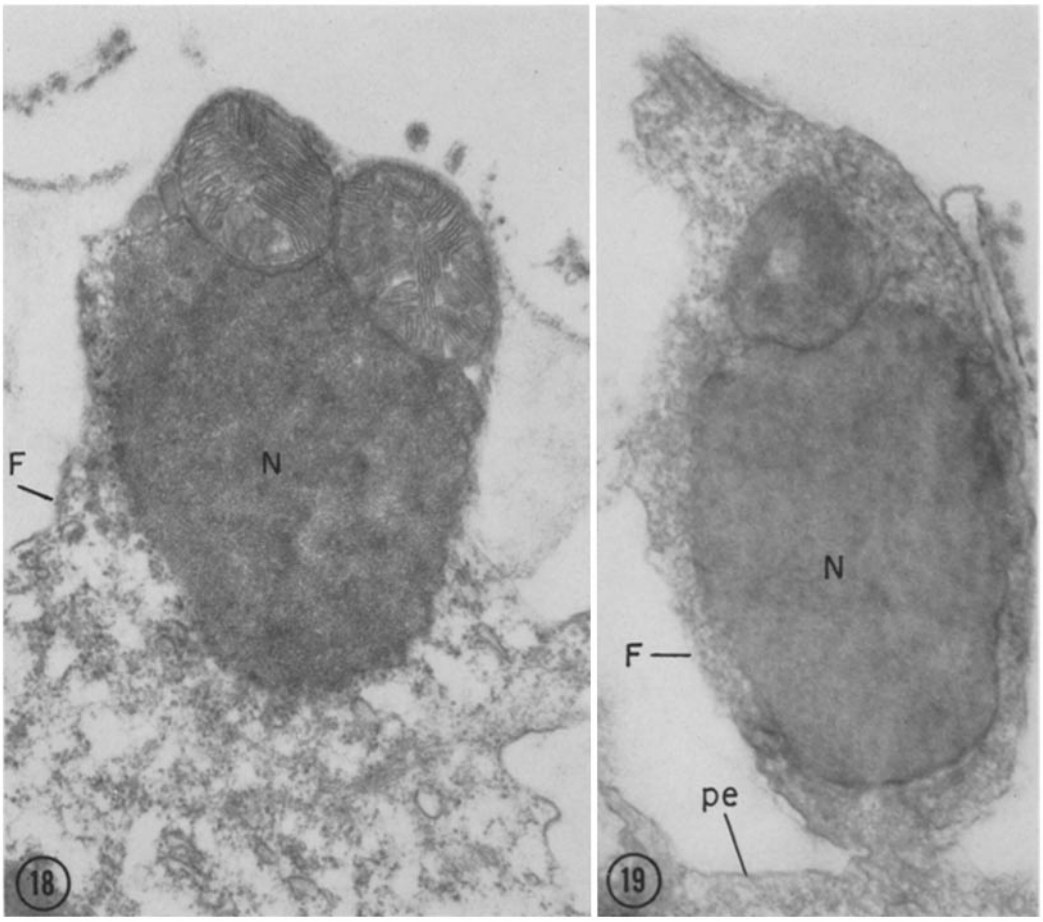


FIGURE 18

Within now continuous egg-sperm plasma membrane, thin layer of egg cytoplasm surrounds sperm nucleus and mitochondria. × 54,000.

FIGURE 19

Specimen in slightly later stage than in Fig. 18; more of egg cytoplasm lies between sperm structures and single continuous egg-sperm plasma membrane. Note egg cytoplasm in flagellum. × 53,000.

stimulus delivered by the filament" (p. 26). In *Hydroides* the acrosomal tubules certainly do not appear to deliver a "prick" to the egg in the sense that a glass needle may be supposed to prick an egg. Since the starfish filament studied, which was formed in egg water, is interpreted to be not tubular but fibrous, Dan considers that the physical mechanism for the injection of an activating

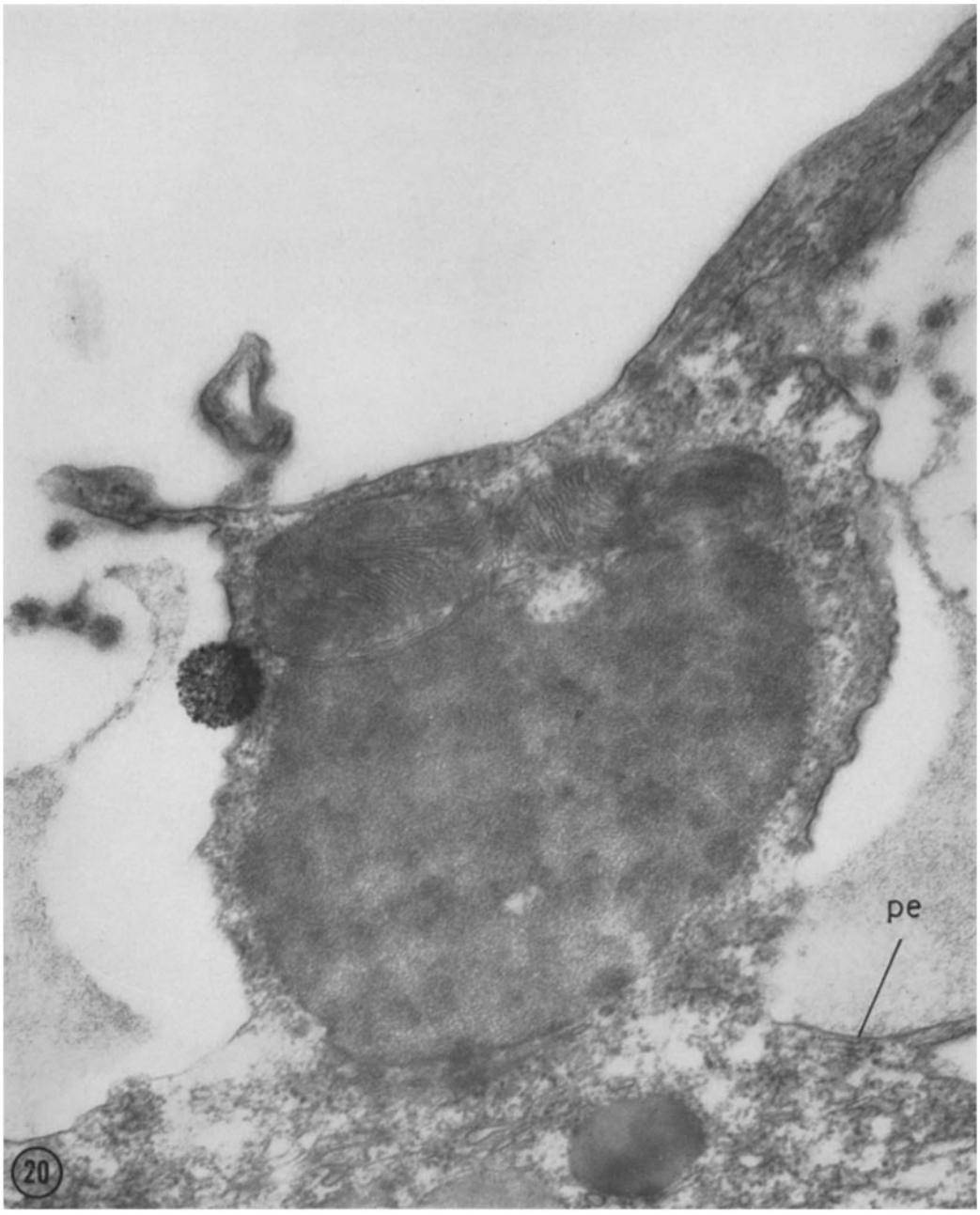
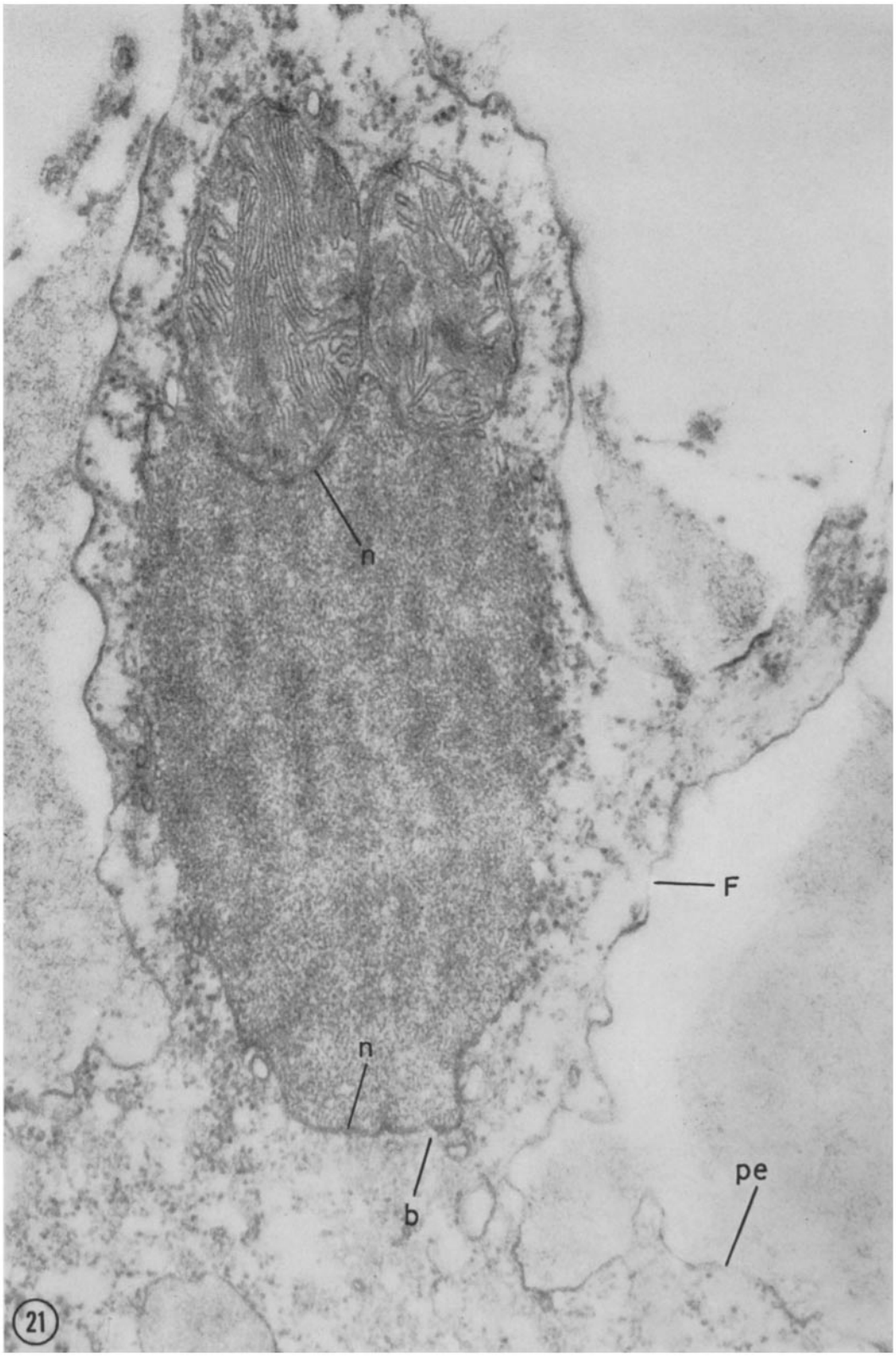


FIGURE 20

Specimen with continuous egg-sperm plasma membrane. Egg cytoplasm can be seen among filaments of projecting flagellum. × 65,000.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substance is not present. In addition, she suggests that even if the filament were tubular, the tremendous capillary resistance inside the starfish filament (diameter 0.1 μ) would preclude it as a pathway for injecting any substance into the egg. The latter consideration may be a cogent argument against the existence of an activating substance, but only if it is supposed that an activating substance is *injected*. It could just as easily be supposed that some constituent of the filament itself, once inside the egg cytoplasm (by vesiculation?), could be an egg-activating substance. The point to be stressed at this time is that the evidence is not available to make a final judgment. It is certainly possible that egg activation does not fall into a single pattern for all species.

(*b*) It was stated previously (4) that the early events of sperm entry may be considered to fall into two phases, at least in *Saccoglossus* and several other species as well as in *Hydroides*. In the *first phase* the spermatozoon arrives at the vitelline membrane or other coverings which serve as a

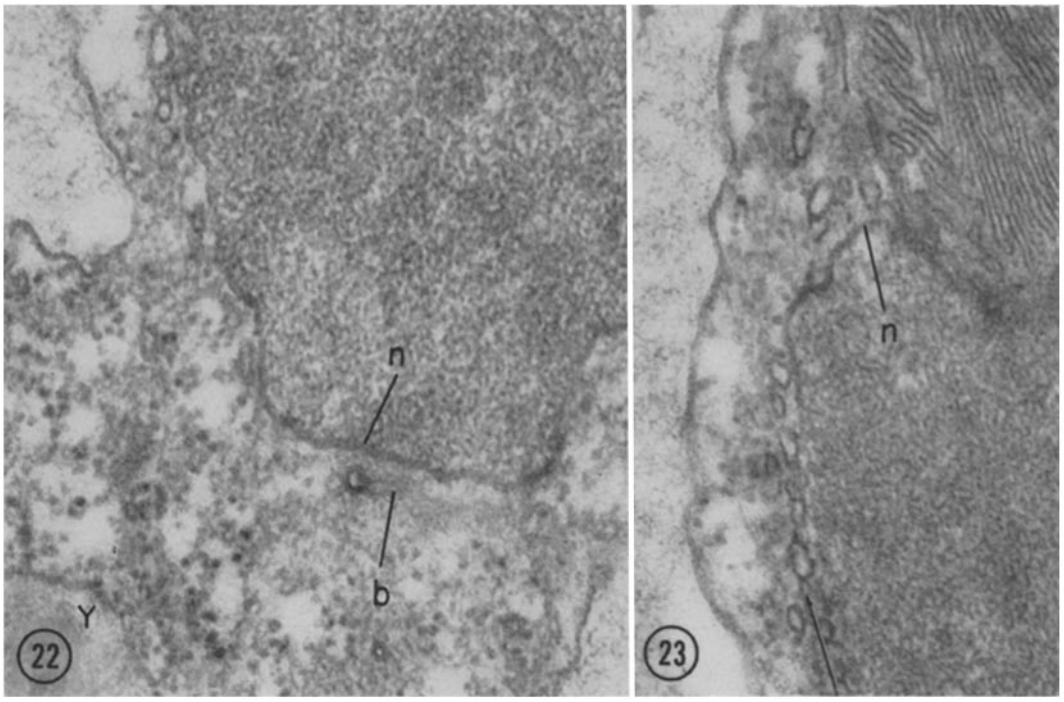

FIGURE 22

Same specimen as shown in Fig. 21, and nearly adjacent section. Nuclear envelope still intact in apical region. × 113,000.

FIGURE 23

Another nearly adjacent section of specimen shown in Figs. 21 and 22. Nuclear envelope still visible at mitochondrial end of nucleus, but many small vesicles (arrow) and no nuclear envelope occupy peripheral area of junction between sperm nucleus and egg cytoplasm. ×108,000.

FIGURE 21

Specimen at stage similar to that shown in Fig. 20. Note single egg-sperm plasma membrane. Apical and mitochondrial parts of sperm nuclear envelope still visible and apparently intact. × 78,000.

barrier to the egg; the acrosome filament (tubule) spans the barrier and establishes the first contact between the sperm head and egg plasma membrane. The egg begins to react almost at once. In the *second phase*, the "filament" and its connected sperm head move through the vitelline membrane or other barriers and into the egg. Even though both phases involve passage through the same barrier, the first phase is accomplished in a matter of seconds, whereas the second phase usually re-

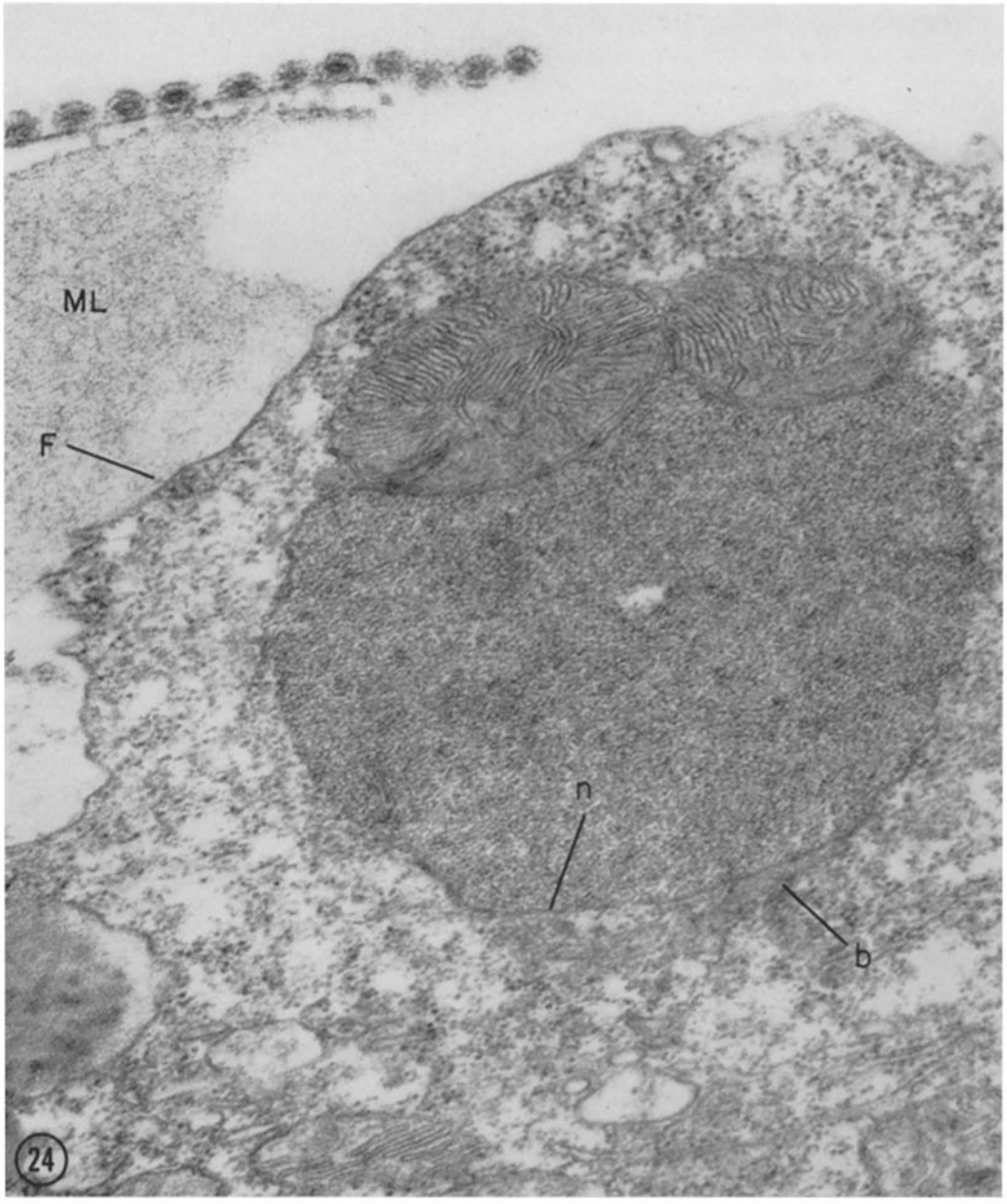

FIGURE 24

Shortly before sperm structures move deeper into body of egg cytoplasm. Later stage than Figs. 21 to 23. Nuclear envelope still intact in apical and mitochondrial regions. × 84,000.

quires several minutes. It was considered that the second phase was accomplished as the sperm lytic agent brought about local erosion of the vitelline membrane. With regard to the first phase it was stated that "it is not known by what means the acrosome filament initially spans the barriers . . . it is simply not clear in what way, if any, the suggested local erosion of the membrane would participate in the filament's initial rapid spanning of the barriers." Since the first phase occurs within a matter of seconds, it seemed unlikely that such a formidable barrier as is presented by the vitelline membrane of *Hydroides* could be penetrated so rapidly by lytic action on the part of the spermatozoon. However, recent evidence for *Hydroides* clearly establishes this as a fact (6), since preparations fixed only 9 seconds after insemination show that almost all the depth of the vitelline membrane has been lysed away and that the tuft of acrosomal tubules is almost in contact with the egg plasma membrane. In *Hydroides*, then, the initial rapid spanning of the vitelline membrane by the acro-

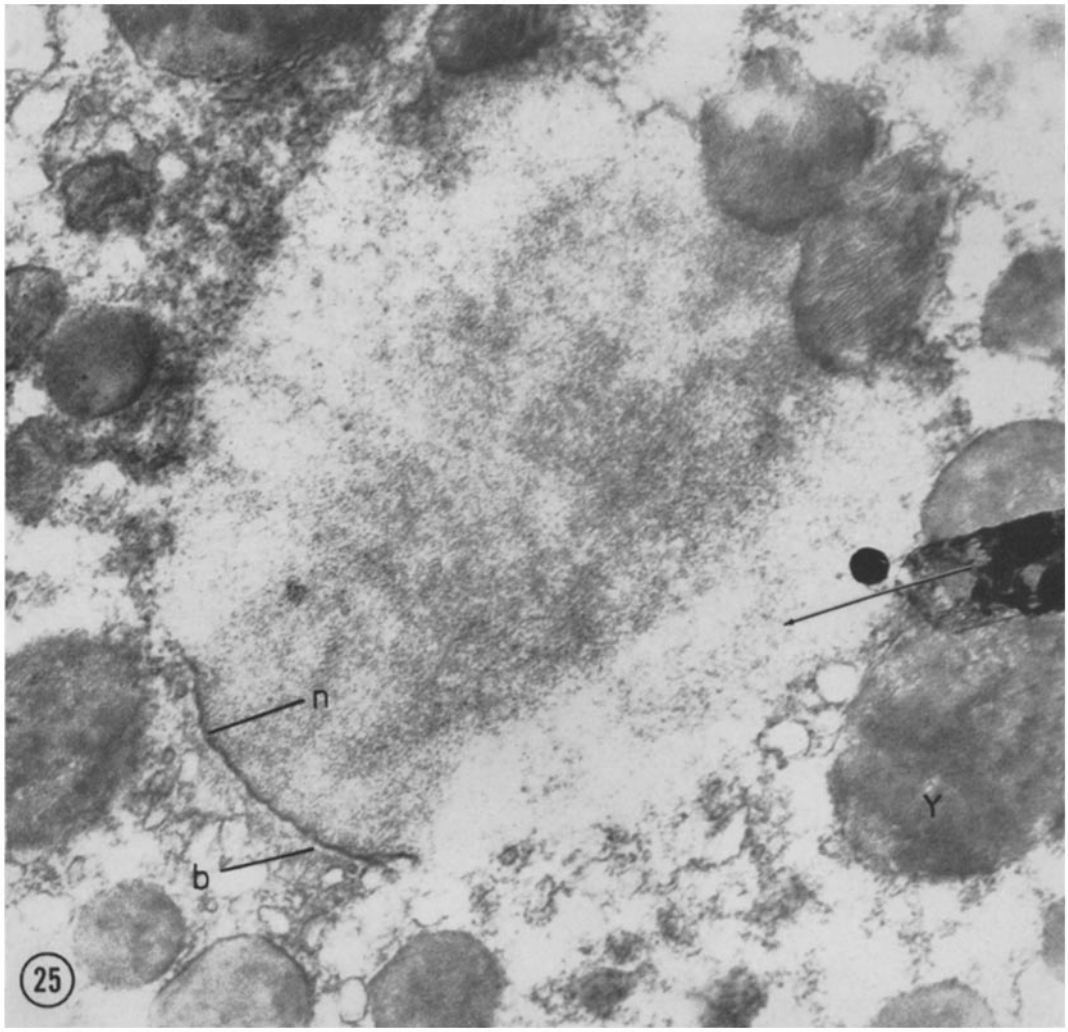

FIGURE 25

Sperm structures lie well within egg cytoplasm. Nuclear envelope and material from between nucleus and base of acrosome still visible near apex of spermatozoon, and sperm mitochondria still easily recognizable although laterally (at arrow) the nucleus has become diffuse. ×54,000.

somal tubules during the first phase *is* made possible by the lytic action of the spermatozoon.

The longer duration of the second phase now seems referable not to the period required for lysis of the vitelline membrane, but, rather, to the following events which succeed the initial contact of acrosomal tubules and egg plasma membrane: the interdigitation of filament and egg plasma membrane, the formation of the fertilization cone, and the events involved in the mutual incorporation of spermatozoon and egg.

In view of the observations in *Hydroides*, it seems not unlikely that in other species, too, the rapid spanning of the vitelline membrane or other barriers by the acrosomal tubule during the first phase may be due to lytic activity on the part of the spermatozoon. However, in the starfish, Dan (7) considers that there is no place for a lysin in the series of events making up the fertilization process, and believes that it is safe to assume that the filament is able to pierce the barrier "with the push derived from the chemical change which causes its formation." Resolution of the apparent differences between these species may come as further studies are made.

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