ROLE OF THE GAMETE MEMBRANES IN FERTILIZATION IN *SACCOGLOSSUS KOWALEVSKII* (ENTEROPNEUSTA)

I. The Acrosomal Region and Its Changes in Early Stages of Fertilization

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ABSTRACT

Previous electron microscope studies of sperm-egg association in the annelid *Hydroides* revealed novel aspects with respect to the acrosomal region. To determine whether these aspects were unique, a comparable study was made of a species belonging to a widely separated phylum, Hemichordata. Osmium tetroxide–fixed polyspermic material of the enteropneust, *Saccoglossus*, was used. The acrosomal region includes the membrane-bounded *acrosome*, with its large *acrosomal granule* and shallow adnuclear *invagination*, and the *periacrosomal material* which surrounds the acrosome except at the apex; here, the acrosomal membrane lies very close to the enclosing sperm plasma membrane. After reaching the egg envelope, the spermatozoon is activated and undergoes a series of changes: the apex dehisces and around the resulting orifice the acrosomal and sperm plasma membranes form a continuous mosaic membrane. The acrosomal granule disappears. Within 7 seconds the invagination becomes the *acrosomal tubule*, spans the egg envelopes, and meets the egg plasma membrane. The rest of the acrosomal vesicle everts. The *periacrosomal mass* changes profoundly: part becomes a *fibrous core* (possibly equivalent to a perforatorium); part remains as a *peripheral ring*. The basic pattern of structure and sperm-egg association in *Saccoglossus* is the same as in *Hydroides*. Previous evidence from four other phyla as interpreted here also indicates conformity to this pattern. The major role of the acrosome is apparently to deliver the sperm plasma membrane to the egg plasma membrane.

INTRODUCTION

Until recently the role of the acrosomal structures in fertilization was largely unknown. A study of the fine structure of fertilization in the annelid *Hydroides hexagonus* (16, 18, 19, 27) revealed aspects which were entirely novel. Events were described which failed to support certain commonly held assumptions. The question arose as to whether these events were peculiar to *Hydroides*, and it seemed desirable to obtain comparable information about other species. For the present investigation a hemichordate was selected, representing not only another species but a widely separated phylum.

A major obstacle in fine structure studies of the events of sperm-egg association lies in the difficulty of finding a thin section containing the uniting gametes and, in addition, of finding them in an orientation favorable for interpretation. This difficulty was overcome, as in *Hydroides*, by using eggs deliberately made polyspermic. It is, of

course, of paramount importance to know whether such material can accurately reflect normal events. In *Saccoglossus* extensive and detailed studies on living material had already been made (24) and it had been concluded that, with the exception of size and duration of the fertilization cone, there were no discernible differences between monospermic and polyspermic material with respect to the early events of sperm-egg association.

Some clue to the extent to which the present sections of *Saccoglossus* reflect situations obtaining in life can be had by comparing these sections with serial photomicrographs of fertilization in living gametes (24).

The present paper is the first of two dealing with the fine structure of fertilization in *Saccoglossus*. A brief abstract has appeared elsewhere (20).

MATERIALS AND METHODS

Gametes of the enteropneust, *Saccoglossus kowalevskii*, were collected from animals found near Woods Hole, Massachusetts. The general methods of handling have been described elsewhere (13). Eggs were obtained by induced spawning (28), and sperm was obtained by induced spawning and by biopsy. The sperm was suspended in filtered sea water or in a 0.001 M solution of Versene in filtered sea water buffered to pH 8.2 (58). After collection, the eggs were washed several times in filtered sea water.

Polyspermy was induced by preinsemination treatment of the eggs for 3 minutes with a solution of ½ per cent nicotine in sea water, after which they were washed several times in fresh sea water. The degree of polyspermy was varied by varying the concentration of the inseminating sperm suspension. Insemination was effected by adding eggs to the sperm suspension.

Fixations were made at various times ranging from three seconds to several minutes after insemination; equal volumes of inseminated eggs were mixed with 4 per cent osmium tetroxide in sea water to give a final concentration of fixative of approximately 2 per cent. After about ¾ to 1 hour in the fixative, the eggs were transferred to 50 per cent ethyl alcohol and dehydrated through a series of increasing concentrations to absolute alcohol. Part of the fixed material was continued for embedding in a mixture of 85 per cent butyl: 15 per cent methyl methacrylate containing 2 per cent Luperco as a catalyst, and polymerized in an oven at 63°C; the remainder was prepared for Epon embedding according to the method described by Luft (44).

Sperm suspensions were fixed by the same methods used for eggs, except that they were concentrated by very low speed centrifugation at each step.

Sections were cut with a Porter-Blum microtome and a Dupont diamond knife. Methacrylate sections were spread by the method of Satir and Peachey (54). Sections were stained with lead hydroxide by the method of Watson as modified by Dalton and Zeigel (30), as well as by the method of Millonig (47) and of Karnovsky (42). Sections were examined with an RCA model EMU-3C electron microscope. Although some of the sections shown are not from serial sections, all of the structures described have been studied in serial sections. In the present study, all figures are of methacrylate sections unless otherwise noted. 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

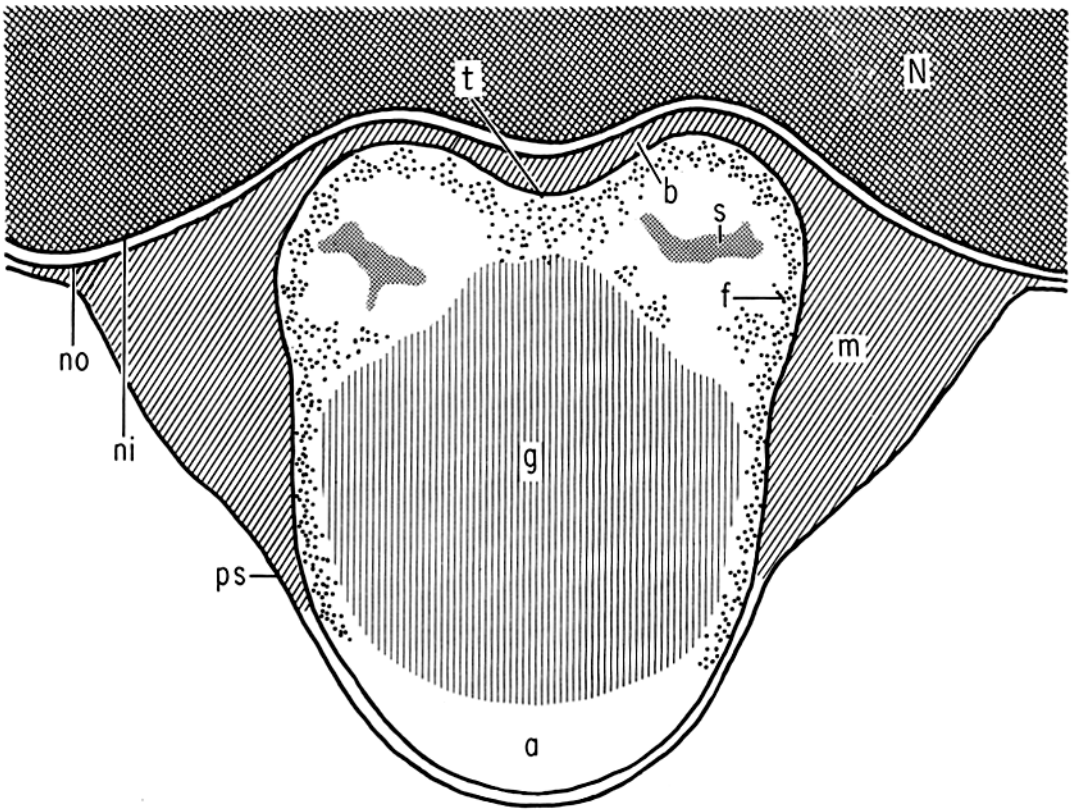
A. Spermatozoon before Fertilization

The living spermatozoon as seen in suspensions in sea water has a simple flagellum and a nearly spherical nucleus. A flat middle piece or mitochondrial region is closely applied to the nucleus, and at the opposite end of the nucleus the acrosomal region is notable only as a very small projection. Sections show that one continuous plasma membrane encloses all four regions. This hemichordate spermatozoon resembles those of certain echinoderms (25) more closely than it resembles that of the annelid *Hydroides hexagonus* (22).

Structure of Acrosomal Region

A diagram of this region is shown in Fig. 1, and sections are shown in Figs. 2 to 5. The region has the same appearance in certain spermatozoa from inseminated preparations as in specimens from sea water preparations. The external boundary is the *plasma membrane*, the inner boundary the *nuclear envelope*. Between these lie the membrane-bounded *acrosome* in an axial position, and the *periacrosomal mass* which surrounds the acrosome except at the apex. Proximally, the region intrudes into a shallow depression in the nucleus and distally, it protrudes beyond the spherical nuclear outline.

1. PERIACROSOMAL MASS AND CAVITY: A periacrosomal mass of dense and apparently finely granular material surrounds all but the apical part of the acrosome and occupies all but the apical part of the periacrosomal cavity. This material forms a thin sheet outside the base or adnuclear part of the acrosome and continues as a thicker ring around the sides.

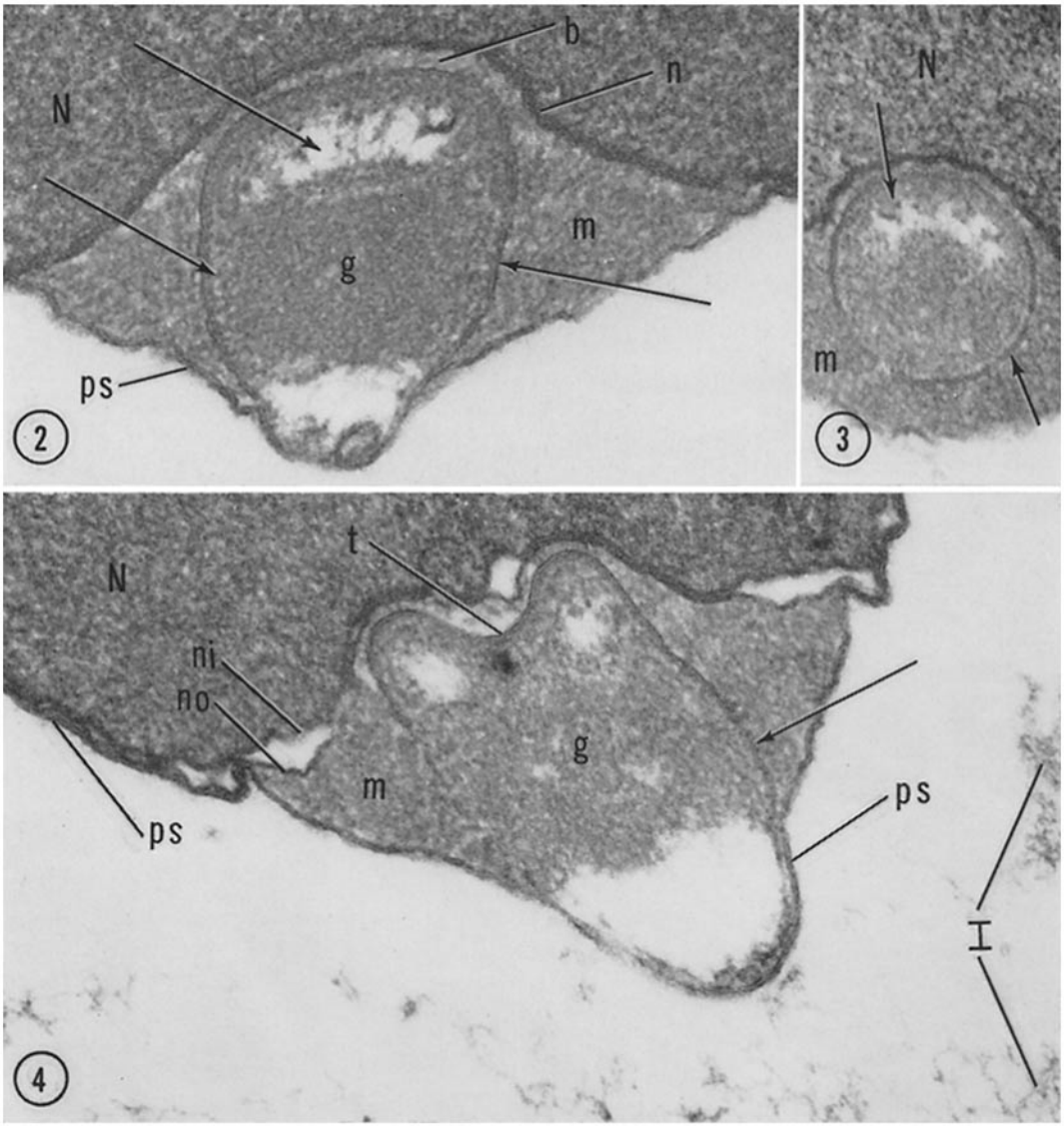


Explanation of Figures

Unless otherwise indicated, all sections are approximately longitudinal with respect to the spermatozoon.

a, apical space in acrosome
b, adnuclear sheet of periacrosomal mass or material
c, fibrous core of acrosomal tubule
f, layer of finely granular material in acrosome
g, acrosomal granule
m, periacrosomal mass or material
M, mitochondrion
n, nuclear envelope
ni, inner membrane of nuclear envelope
no, outer membrane of nuclear envelope
N, nucleus
pe, egg plasma membrane
ps, sperm plasma membrane
pv, perivitelline space
s, stellate body in acrosome
t, invagination of acrosomal membrane at adnuclear end, or acrosomal tubule
v, microvillus
I, outer egg envelope
II, inner egg envelope

FIGURE 1 Diagram of median longitudinal section of unactivated acrosomal region. Except apically, where the acrosomal and sperm plasma membranes are almost contiguous, periacrosomal material forms a sheet (*b*) between nucleus and acrosome, and elsewhere (*m*) is confined by sperm plasma membrane (*ps*), outer membrane of nuclear envelope (*no*), and acrosomal membrane. Within the acrosome an apical space (*a*) separates the acrosomal granule (*g*) from the acrosomal membrane; except apically, this membrane is lined by finely granular material (*f*). A ring-shaped space containing stellate bodies (*s*) lies within the base of the acrosome. A single shallow invagination (*t*) indents the adnuclear end of the acrosome.



FIGURES 2 to 4 Apical parts of unactivated spermatozoa from inseminated preparations.

FIGURE 2 Acrosomal region is closely applied to nucleus, indenting it axially but not laterally. Section shows lateral part of adnuclear end of acrosome, with periphery of ring-shaped space (tip of upper arrow), but axial part of apex, with apical space beyond acrosomal granule (*g*). Between nucleus and acrosome lies adnuclear sheet (*b*) of periacrosomal material (*m*) which elsewhere is more extensive. Lower left arrow: finely granular layer. Right arrow: membrane of acrosomal vesicle. × 115,000.

FIGURE 3 Oblique horizontal section through adnuclear end of acrosome, showing part of ring-shaped space. Upper arrow: stellate body in the ring-shaped space. Lower arrow: acrosomal membrane. × 95,000.

FIGURE 4 Median section showing invagination (*t*) of adnuclear end of acrosome. In some regions, outer and inner membranes (*no*, *ni*) of nuclear envelope are separated. Periacrosomal material (*m*) is confined by sperm plasma membrane (*ps*), acrosomal membrane (arrow), and nuclear envelope. Spermatozoon is barely attached to outside of egg envelope (*I*). × 108,000.

2. APEX: In this region, only, the acrosomal membrane lies very close to the plasma membrane. In some specimens both membranes are unwrinkled (Fig. 5), and in others both are wrinkled. Most frequently, however, the plasma membrane is unwrinkled and the acrosomal membrane wrinkled or even, apparently, vesiculated (Figs. 2 and 4).

3. ACROSOME: The acrosomal membrane is the wall of the *acrosomal vesicle*. This vesicle is nearly filled by a large dense *acrosomal granule* and lined, except at the apex, by a thin *finely granular layer* of material (Figs. 2 and 7). Where this layer is absent, an *apical space* separates the acrosomal granule from the acrosomal membrane (Figs. 2, 4, 5, and 7). Where the layer is present, it adjoins the granule except that a *ring-shaped space* (Figs. 2 to 7) separates them around the sides of the adnuclear end of the vesicle; about a dozen irregularly *stellate bodies* are arranged in a circle within this space. The center of the adnuclear end of the vesicle (*i.e.* the base of the vesicle) is indented by *a single shallow invagination* (Figs. 4 and 5). Only the acrosomal vesicle with its contents can properly be called "the acrosome" (18).

4. MEMBRANES: The plasma membrane, the acrosomal membrane, and the two membranes which compose the nuclear envelope are tripartite or unit membranes, in the sense of Robertson (53). The two membranes of the nuclear envelope generally lie close together but sometimes are separated. When they happen to be separated along the border of the acrosomal region, the membrane relationships of this region are made quite clear (Figs. 4 and 5).

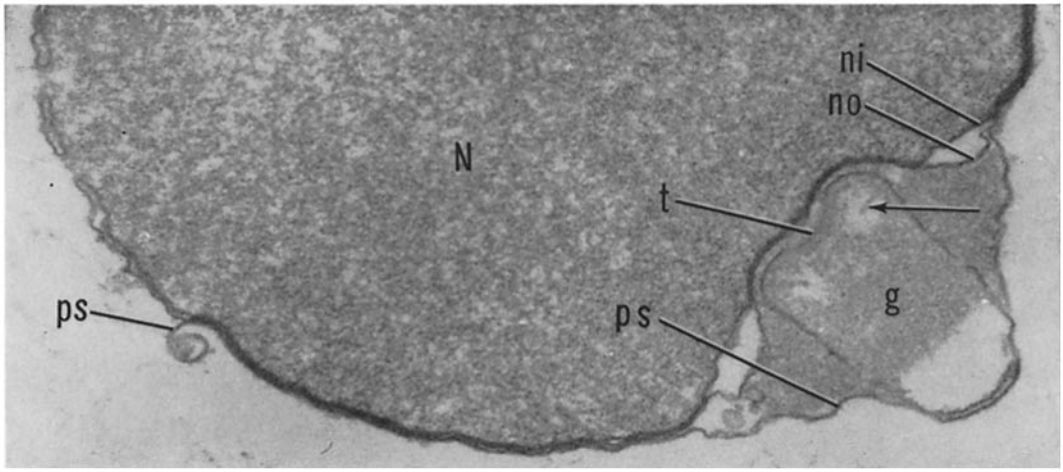


FIGURE 5 Unactivated spermatozoon, barely attached to egg envelope; acrosomal region shown in nearly median section. At adnuclear end of acrosome is invagination (*t*). At apex, beyond apical space, unwrinkled part of acrosomal membrane is nearly contiguous with sperm plasma membrane (*ps*). Tip of arrow points to stellate body in section of ring-shaped space within base of acrosome. At left of figure, sperm plasma membrane is curled back, but outer and inner membranes of nuclear envelope remain. Epon embedding. × 61,000.

B. Periphery of Unfertilized Egg

In ripe eggs which have been shed, the plasma membrane is surrounded by two envelopes which are loosely felt-like, as seen in sections (Fig. 6). The inner, denser, envelope is the functional "vitelline membrane" (23). Microvilli, which are extensions of the plasma membrane, cross a shallow perivitelline space and touch, *but do not project into* the inner envelope. An approaching spermatozoon, then, must penetrate the two barrier envelopes before it can meet the egg plasma membrane. The combined thickness of the two envelopes is about twice the length of the arriving sperm head, as can be seen in photomicrographs of living specimens (24); the reduced relative thickness of envelopes in some sections is

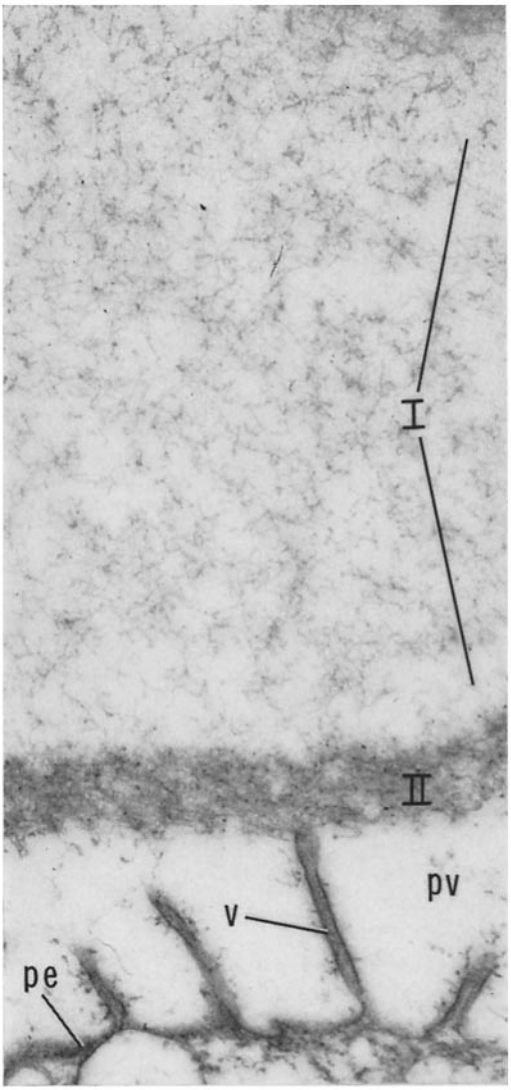


FIGURE 6 Ripe unfertilized egg; portion of periphery showing felt-like structure of egg envelopes (*I, II*) covering egg plasma membrane (*pe*). Microvilli (*v*) are in perivitelline space (*pv*) and touch but *do not enter* inner egg envelope. × 50,000.

attributed to differential shrinkage during preparation.

C. Sperm-Egg Association

ATTACHMENT: Sections of inseminated eggs show some spermatozoa in which the apex of the sperm plasma membrane is barely attached to the outer egg envelope (Figs. 4 and 5), and others in which the apex is in fairly extensive contact with the envelope (Fig. 7). These are considered to represent the very early stages of sperm-egg association; the appearance of the acrosomal region is still the same as in unattached spermatozoa.

CHANGES IN ACROSOMAL REGION (FIG. 8): Very soon after attachment, the sperm head extends a long slender apical projection through the two egg envelopes. In living material viewed under the light microscope, this projection has a thread-like appearance (22, 24). Dan (32) introduced the term "acrosome filament" for this kind of structure, but electron microscopy has shown it to be tubular in *Saccoglossus* (22), and hence the term *acrosomal tubule* will be used here. Extension of this tubule through the egg envelopes is related to salient changes in the acrosomal region. These may be summarized as follows. 1. The apex undergoes dehiscence. 2. The acrosomal granule is released. 3. The basal invagination of the acrosomal membrane deepens and becomes the acrosomal tubule. 4. The acrosomal vesicle is everted. 5. The periacrosomal mass changes in appearance and distribution.

1. DEHISCENCE (FIGS. 9 TO 12): The sperm plasma and acrosomal membranes open apically and thus the interior of the acrosomal vesicle is exposed to the outside. Around the rim of the resulting orifice these two membranes are found to be joined, constituting a single continuum, that is, after dehiscence the acrosomal membrane is a mosaically inserted part of the sperm plasma membrane. Dehiscence and the joining of the two membranes seem to be related events, but it is not known whether they are causally related. After the apex is open, a few small vesicles or curled fragments of membrane lie in the egg envelope; these are not attached to the rim (Figs. 9 and 10).

2. ACROSOMAL GRANULE (FIGS. 9 TO 12): As a result of dehiscence the acrosomal granule is exposed and comes in contact with the egg envelope. The granule soon disintegrates and disappears.

3. ACROSOMAL TUBULE: At the adnuclear end of the now open acrosomal vesicle, the shallow invagination of the acrosomal membrane deepens (Figs. 9, 10, and 12) and soon lengthens into a long, slender acrosomal tubule (Figs. 13 and 15). Within 7 seconds the tubule is approximately

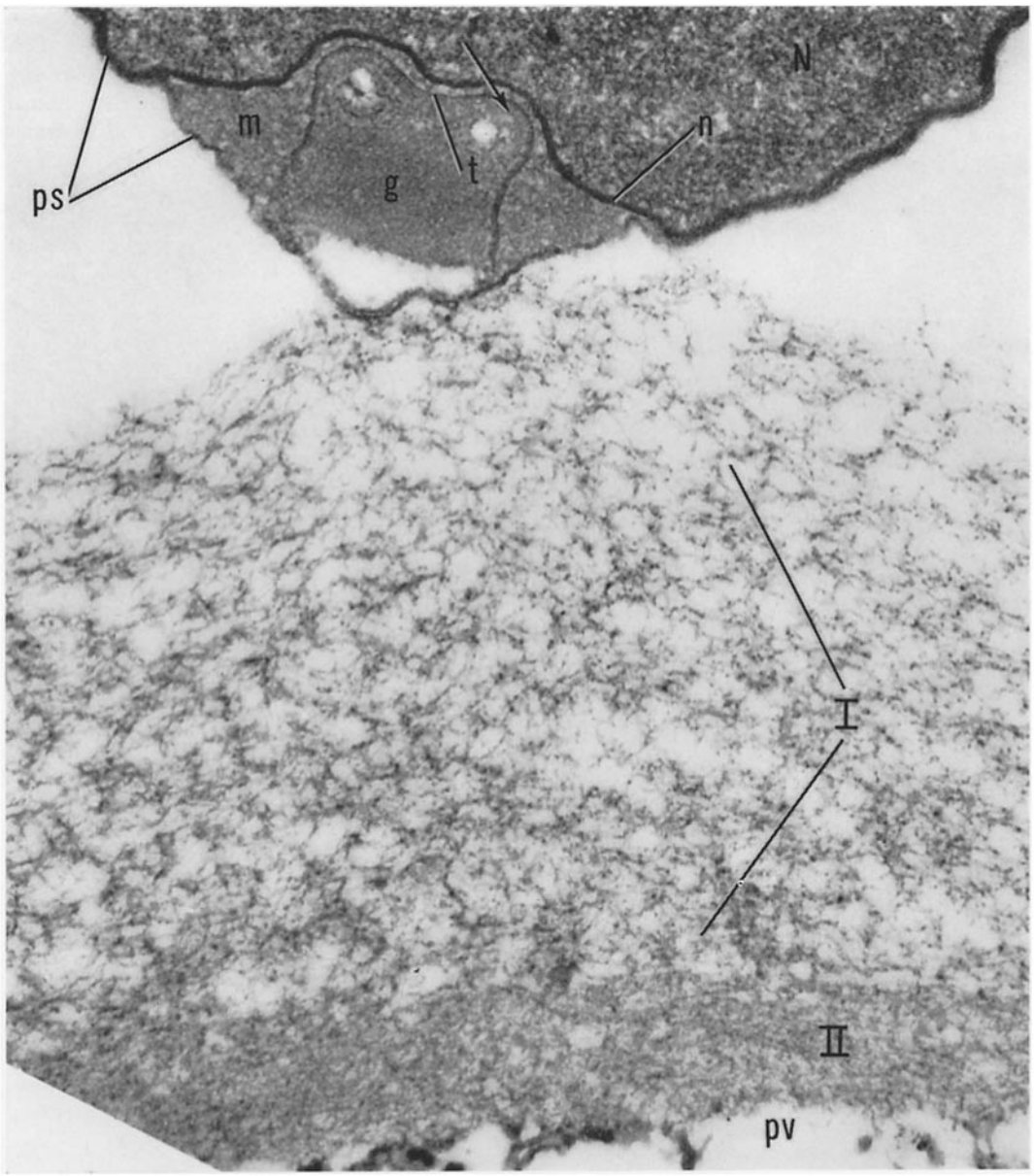


FIGURE 7 Apical region of spermatozoon attached to egg envelope, but showing no signs of activation. Note extensive area of contact between sperm plasma membrane and egg envelope. Arrow: finely granular layer lines acrosomal membrane. × 77,000.

twice as long as the sperm nucleus. Initially, only a small portion of the acrosomal membrane moves away from the periacrosomal mass and for some time the width of the long tubule remains approximately the width of this very limited area. Since the acrosomal membrane is now an inserted part of the sperm plasma membrane, this tubule is, in fact, the apical portion of the sperm plasma membrane.

4. EVERSION OF ACROSOMAL VESICLE (FIGS. 8 AND 14 TO 17): Next, the rest of the acrosomal membrane moves away from the periacrosomal mass. It everts completely and is added to the tubule as a second, proximal, seg-

ment. This eversion is simply an unfolding of the already continuous membrane. As a consequence of eversion, the plasma membrane of the entire proximal part of the acrosomal region is added as a third, and basal, segment of the tubule, and the periacrosomal cavity in effect becomes the cavity of the base of the tubule. For a while, the everted membrane or acrosomal vesicle remains as a groove in the wall of the tubule, and this permits the three segments to be identified (Fig. 16). Later, the groove smooths out and the segmental origins of the tubule are no longer discernible.

When the acrosomal vesicle everts, its finely granular lining and the stellate bodies from the adnuclear end move, or are turned out, against the material of the egg envelope (Figs. 15 and 16). Eventually they disappear.

5. PERIACROSOMAL MASS: The periacrosomal cavity is at all times continuous with the cavity of the acrosomal tubule but, as indicated above, the spatial relations of the two cavities gradually change. The change is closely correlated with the history of the periacrosomal mass which may be described as follows.

(a) *Presence of vesicles and loss of electron opacity.* The mass remains virtually unchanged in aspect until after the tubule completes its initial elongation. Then, small membrane-bounded vesicles appear, especially in the axial region, and from then on, until long after eversion, vesicles of various

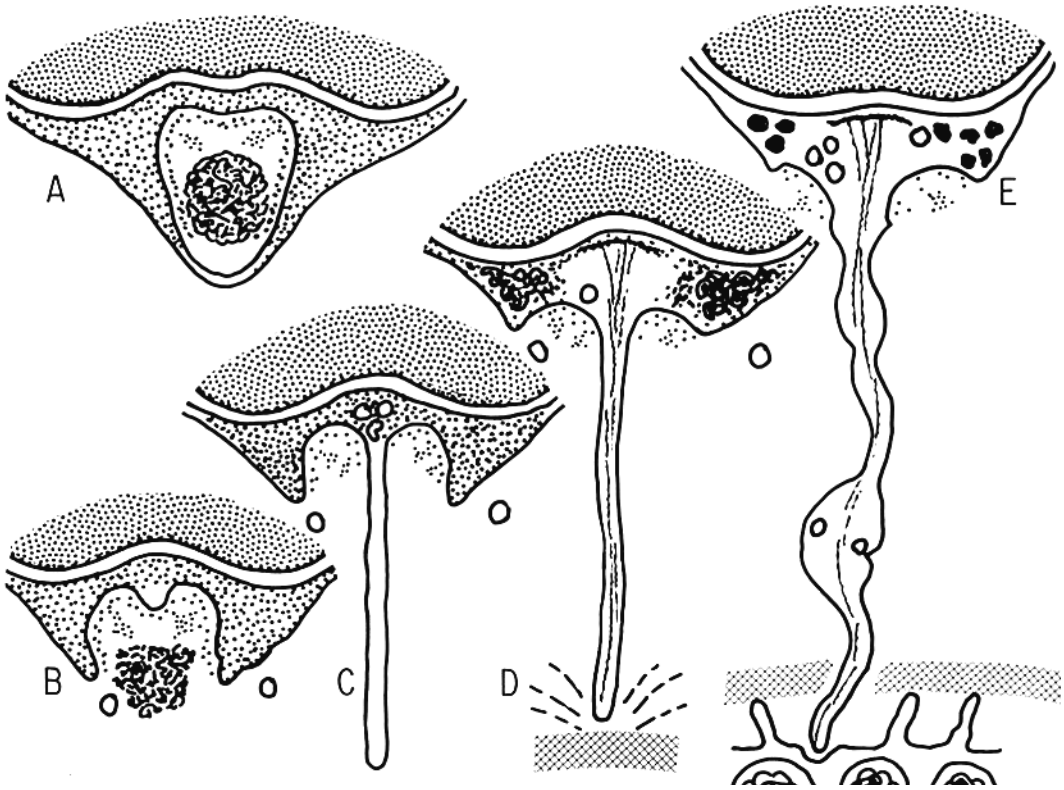


FIGURE 8 Diagrams of acrosomal region at successive stages at beginning of fertilization. *A*, unactivated; *B* to *E*, activated. *B*, soon after dehiscence: acrosomal and sperm plasma membranes are continuous; acrosomal granule exposed; invagination of acrosomal membrane at adnuclear end of acrosome has begun to deepen. *C*, acrosomal tubule undergoes initial rapid lengthening. *D*, early stage of eversion of acrosomal vesicle; acrosomal tubule has penetrated outer egg envelope; note fibrous core and other changed parts of periacrosomal material. *E*, acrosomal tubule, having penetrated inner egg envelope, has met egg plasma membrane; eversion is almost complete. (Not all figures are drawn to same scale.)

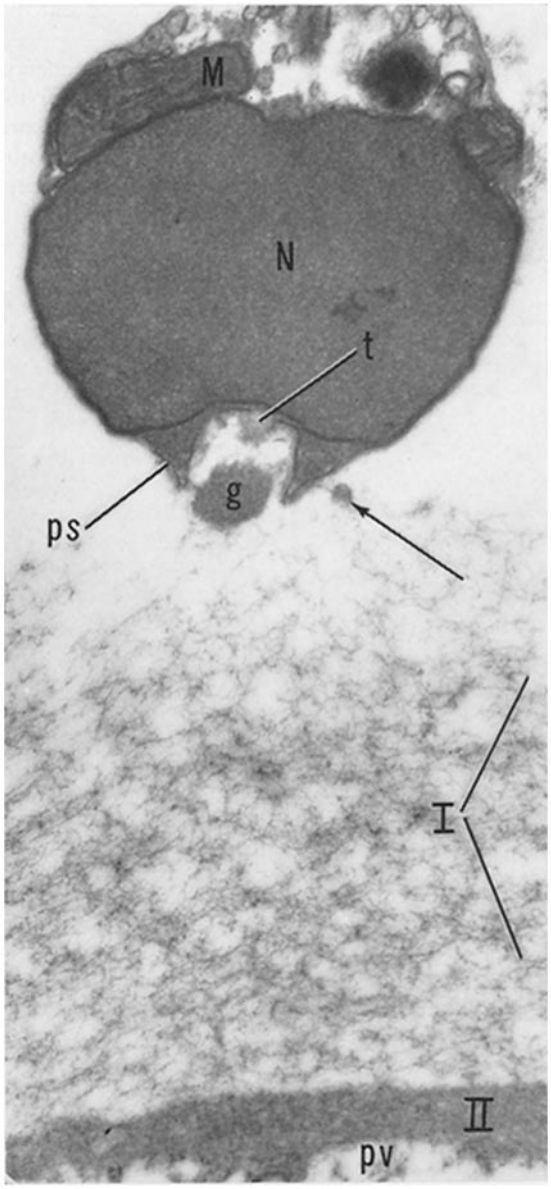


FIGURE 9 Sperm head at egg envelope, showing release of acrosomal granule through apex of open acrosomal region, shortly after dehiscence. Adnuclear invagination (*t*) of acrosomal membrane has begun to deepen. Arrow: curled fragment of membrane in egg envelope outside periacrosomal mass. × 42,000.

sizes are seen (Figs. 13, and 15 to 17). They are tightly packed at the start but soon become scattered. The region in which they are scattered has little or no electron opacity. This region gradually enlarges; that is, more and more of the periacrosomal mass loses electron opacity. Now, almost from the beginning the acrosomal tubule contains material which lacks electron opacity. Both confluent cavities, then, contain material which has this quality. After eversion, many vesicles occupy the newly added proximal parts of the tubule but a few are found also in the distal part. Some are closely applied to the membranous wall of the tubule.

b) *Adnuclear sheet and peripheral ring* (*Figs.* 14 *to* 17). Not all of the periacrosomal mass loses its electron opacity. One portion remains as a thin sheet adjoining the nuclear envelope. This sheet is circular in outline and centrally located. It does not quite reach the lateral margin of the

periacrosomal space. Another portion remains visible as a thicker ring of material lying just within the lateral margin of the periacrosomal cavity. At first, it retains the initial dense granular appearance of the periacrosomal mass, but later, it appears as a few irregularly rounded clumps of very homogeneous material of only medium electron opacity. This peripheral ring material remains recognizable throughout the subsequent history of the acrosomal tubule and will serve as a kind of landmark during later stages of fertilization.

(c) *Fibrous core* (*Figs.* 14 *to* 18). A few strands of moderately dense fibrous material form a delicate core which runs the entire length of the completed tubule. At the proximal end it occupies an axial position but distally its position is sometimes eccentric. The core meets and seems to be attached to the adnuclear sheet mentioned above (Figs. 16 and 17). Although the core is occasionally seen in earlier stages, it becomes well defined at about the beginning of eversion. Like the material of the peripheral ring, it remains visible during subsequent stages of fertilization.

SHAPE OF ACROSOMAL TUBULE: In living specimens the tubule seems straight or only slightly curved, but thin sections of spermatozoa in the egg envelopes show tubules which are usually bent, curved, or even coiled, and the general contour is different at different stages. Before eversion takes place the tubule is uniformly slender (Fig. 13) but the base widens as the acrosomal vesicle everts (Fig. 14); next, the everted vesicle is a constricted link between a slender part and a broad base (Fig. 16), and finally the broad base tapers directly into the slender part (Fig. 17). Meanwhile, as the periacrosomal mass loses electron opacity and the membrane-bounded vesicles become more apparent, the tubule in at least some cases is bizarre in outline, having many surface concavities. In later stages, portions of the tubule become distended and usually alternate with irregular lengths which are undistended (Figs. 16 and 19). This condition frequently obtains at about the time the egg becomes activated. Finally the tubule is again undistended.

POSITION OF ACROSOMAL TUBULE: Sometimes the tubule extends into the egg envelope along a path which lies at an approximately normal angle to the egg surface. Perhaps equally often the path lies at a different angle and is, therefore, longer. Specimens have even been found in which the tubule extended into the egg envelope in a direction away from the egg plasma membrane.

EFFECT OF SPERMATOZOON ON EGG ENVELOPES: It has been shown previously that a pit occurs in the outer envelope in the immediate vicinity of a penetrating spermatozoon (22, 24). In the present material a pit somewhat wider than the associated sperm head has been observed in the outer part of the outer envelope. In some cases, close to the tubule the material of this envelope looks more diffuse than elsewhere (Fig. 18); however, in certain regions it looks much denser. The impression is given that the tip of the tubule, or the side where there is a bend or a coil, may push against the envelope material and compress it (Figs. 15 and 19). In the inner of the two egg envelopes such an appearance of compression was not observed. Instead, the material of this envelope simply seemed to be missing wherever a tubule passed through it (Fig. 18).

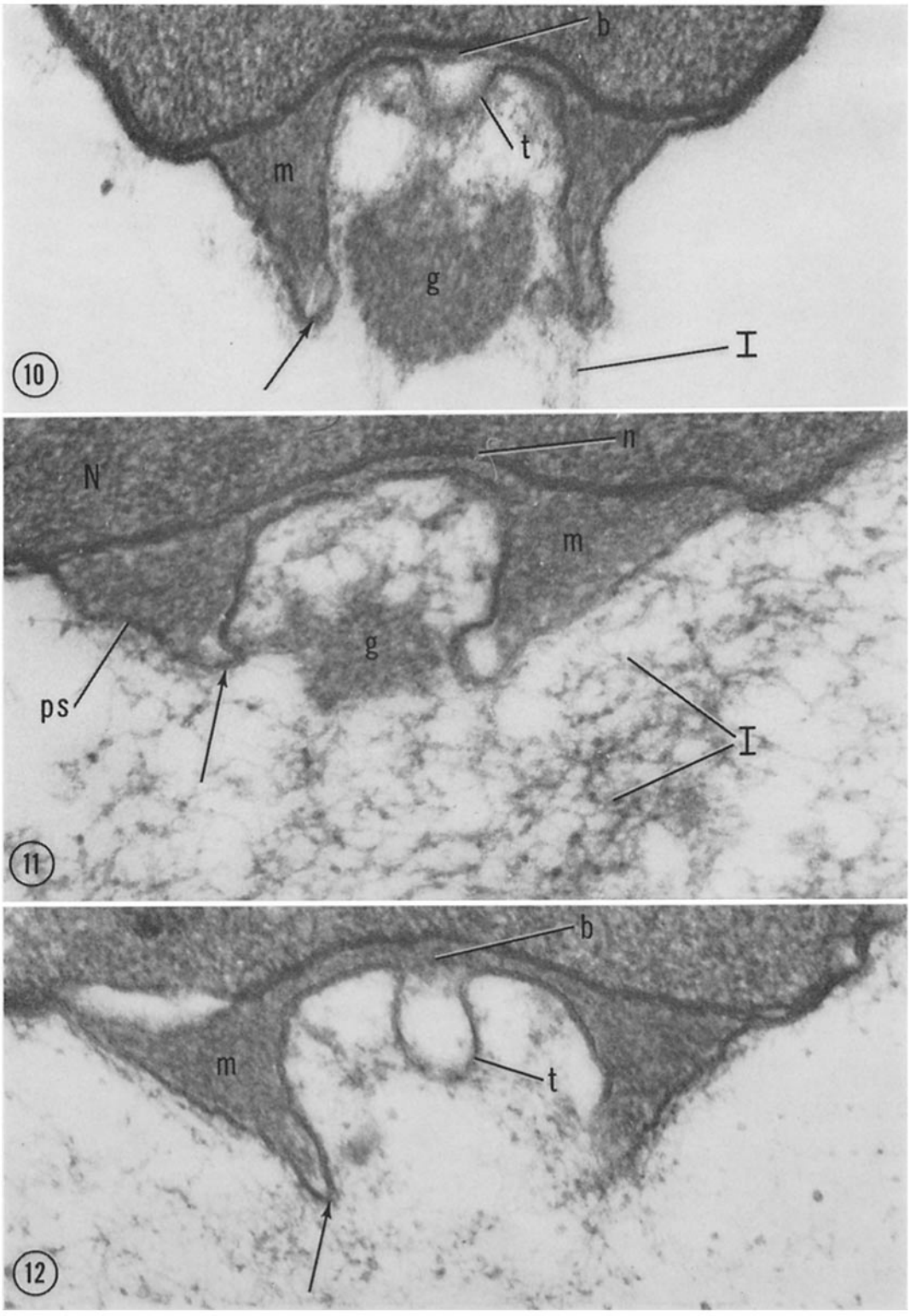
CONTACT OF TUBULE WITH EGG PLASMA MEMBRANE: An acrosomal tubule which is

FIGURES 10 to 12 Acrosomal region in early stages of sperm activation, shown at higher magnification than in Fig. 9. All arrows: approximate site of junction of plasma and acrosomal membranes: site is in rim of orifice resulting from dehiscence; acrosome membrane is now mosaically inserted part of sperm plasma membrane.

FIGURE 10 Mid-section, at about same stage as in Fig. 9. Between rim and acrosomal granule, at right, is curled fragment of membrane. Invagination (*t*) of adnuclear end of acrosomal membrane has begun to deepen. × 143,000.

FIGURE 11 Lateral section of nearly same stage as in Fig. 10. Edge of acrosomal granule is frayed. × 126,000.

FIGURE 12 Mid-section of specimen from which acrosomal granule had practically disappeared (verified in serial sections). Invagination of acrosomal membrane (*t*) is deeper than in Fig. 10. × 143,000.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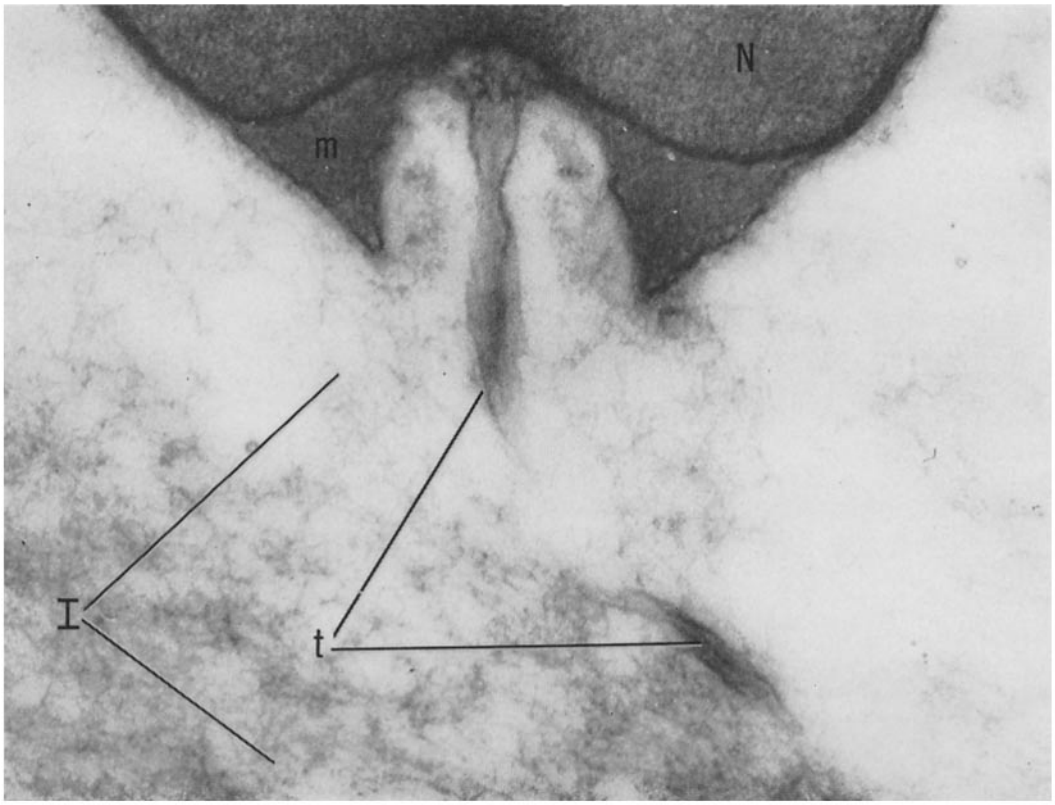


FIGURE 13 Stage of completion of initial elongation; invagination of adnuclear end of acrosomal membrane has become long, slender acrosomal tubule (*t*). Distal part of tubule not shown. Above base of tubule, small vesicles lie in axial part of periacrosomal mass. × 115,000.

pointed in a suitable direction and which successfully extends through both of the egg envelopes (Fig. 18) is in a position to reach the egg plasma membrane. If it does reach the egg plasma membrane, it is finally in a position to activate the egg. A spermatozoon with the tip of its tubule in contact with the egg plasma membrane is shown in Fig. 19. This specimen was fixed 7 seconds after insemination. When living material is examined, it is obvious that contact can be achieved in appreciably less than 7 seconds.

DISCUSSION

I. *Acrosomal Structure and Sperm Activation in Saccoglossus and Hydroides*

A. Acrosome and Acrosomal Region

The term *acrosome* is used here to mean the acrosomal vesicle plus all its contents, the wall of the vesicle being the acrosomal membrane and the principal contents being the acrosomal granule. This is in keeping with an earlier proposal of the authors (18).

It is now proposed that the expression *acrosomal region* also be added to the general terminology, to signify the acrosome plus all its ancillary structures including the overlying plasma membrane and any periacrosomal material. The use of "acrosomal region" together with "acrosome" permits a conceptual discrimination which contemporary observations of fine structure patently demand. Since the acrosomal region contains the acrosome, "acrosome" is more restrictive in sense and "acrosomal region" more inclusive.

B. Comparison of Acrosomal Regions of Saccoglossus and Hydroides

The annelid *Hydroides hexagonus* has been described in a study similar to the present one (18, 27) and can be compared in detail with *Saccoglossus*.

In both species a small amount of periacrosomal material lies between the acrosome and the nuclear envelope, but *Hydroides* lacks the extensive lateral periacrosomal material of *Saccoglossus*. This difference in distribution is correlated with a major difference in appearance. In *Saccoglossus*, the lateral periacrosomal material separates the plasma membrane from the sides of the acrosome, and the profile of the acrosomal region is much broader than that of the enclosed acrosome. In *Hydroides*, where the material is absent laterally does not extend to the apex; and the layer of finely granular material, which everywhere else lines the vesicle, is absent at the apex. The membrane is widely separated from the acrosomal granule in *Hydroides* but not in *Saccoglossus*. In *Saccoglossus* there is no clear counterpart of the small apical vesicle which is sandwiched between the acrosomal and plasma membranes in *Hydroides*, but, in a sense, in both species the apex is a specialized region. Within the acrosome itself the differences between the species are of degree rather than kind, and in both species *the basic structural pattern of the acrosome is the same.*

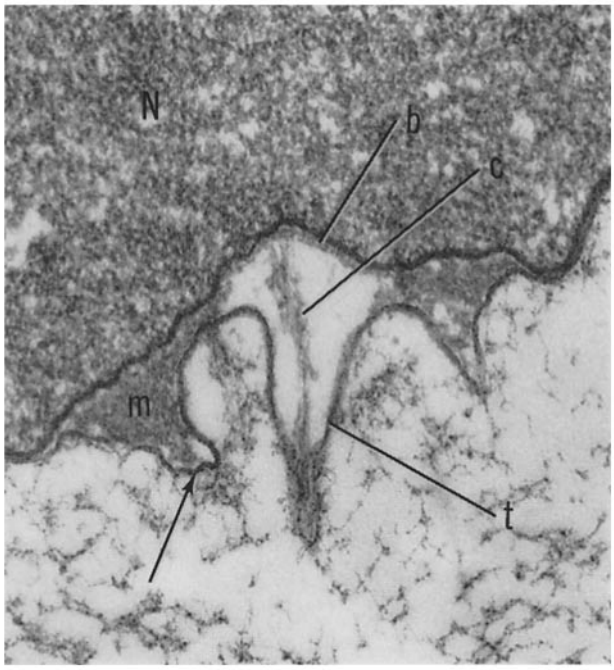


FIGURE 14 Shows basal part, only, of acrosomal tubule (*t*) in stage, following initial tubular elongation, when acrosomal vesicle begins eversion. Note that periacrosomal cavity is confluent with cavity of tubule. Fibrous core (*c*) extends into tubule from adnuclear sheet (*b*) of periacrosomal material. Laterally, periacrosomal material (*m*) still retains its initial granular appearance. Axially, everting acrosomal vesicle has separated from adnuclear sheet, and remaining contents of vesicle now meet material of egg envelope. Arrow: approximate site of junction of segments of now continuous mosaic sperm plasma membrane. × 71,000.

the plasma membrane is close to the sides of the acrosome, and the acrosomal region has almost the same profile as the enclosed acrosome. But in both species the acrosome is contained within the plasma membrane and the basic relation of acrosome to acrosomal region is the same.

In both species, the acrosome is a closed membrane-bounded vesicle whose wall, the acrosomal membrane, is invaginated at the adnuclear end and is almost contiguous with the sperm plasma membrane at the apex. The invagination is a single shallow one in *Saccoglossus*, whereas in *Hydroides* there are a number of invaginations and they are deep enough to be considered tubules. In both species, a large acrosomal granule is contained within the vesicle but

C. Sperm Activation

The many changes which the spermatozoon undergoes following its initial association with the egg have a parallel in the changes which the egg undergoes after it meets the spermatozoon. Just as the release of cortical granules or the elevation of a fertilization membrane are said to result from *activation of the egg*, so, too, dehiscence of the acrosomal region or the elongation of an acrosomal tubule could be said to result from *activation of the spermatozoon.* It is proposed, therefore, that the term "activation" be applied to

spermatozoa in a sense complementary to that of its use as applied to eggs.

In this sense the dehiscence and tubular elongation, etc., in *Saccoglossus*, for example, are results of sperm activation. And spermatozoa induced by various agents to undergo some or perhaps all of their early changes of fertilization (25, 36) are artificially activated or in some cases partially activated spermatozoa.

much as the concept of egg activation has facilitated analysis of the roles of the egg in the initial stages of fertilization.

D. Comparison of Sperm Activation in Saccoglossus and Hydroides

This comparison is shown in Fig. 20.

DEHISCENCE: In both species, after meeting the egg envelope the apex of the acrosomal region

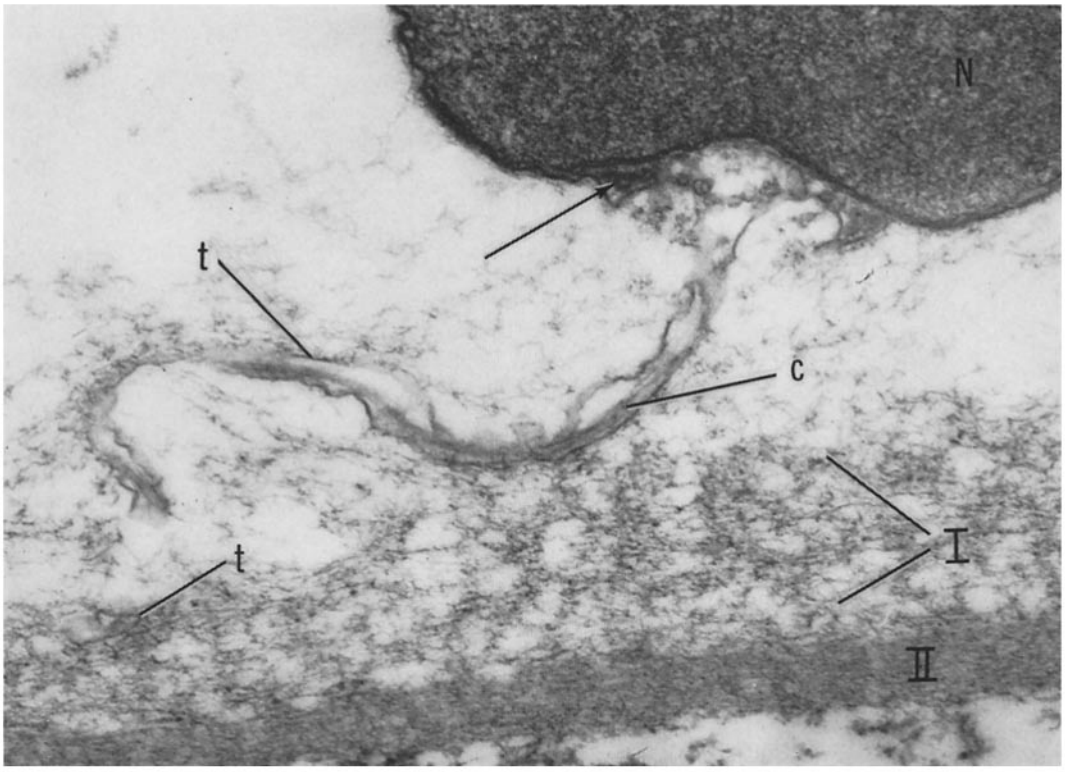


FIGURE 15 Slightly later stage of eversion than in Fig. 14. Note granular acrosomal contents *outside* concave profile of everting acrosomal vesicle. Part of acrosomal tubule (*t*) shown does not include apical portion. Tubule, containing fibrous core (*c*), is curved and in some regions slightly distended. Near confluence of tubular and periacrosomal cavities lie small vesicles of periacrosomal material. Arrow: clumps of periacrosomal material (peripheral ring). × 70,000.

Dan's invaluable contribution of the concept of the *acrosome reaction* (33) has drawn attention to the occurrence of major change in the acrosomal region after stimulation. Long ago, Lillie suggested that the spermatozoon as well as the egg becomes fertilized. The proposed concept of sperm activation offers a framework within which the changing acrosomal region can be related to its precise roles in the initial stages of fertilization,

opens and forms a circular orifice which exposes the interior of the acrosomal vesicle to the outside. In *Hydroides* (18, 27), the evidence suggests that there is a fixed site of dehiscence, a rim around which the opening regularly occurs, and that a small "lid" composed of the *apical vesicle* sandwiched between pieces of plasma and acrosomal membrane regularly is removed. In *Saccoglossus*, as seen thus far, dehiscence is always at the apex;

it is suggested that the unattached vesicles or fragments of membrane, which regularly are found nearby in the egg envelope after dehiscence, are cast-off parts of the plasma and acrosomal membranes of the apex. In both species, then, *a structurally specialized apical region responds to the activation stimulus in much the same way: by dehiscence.*

MOSAIC NATURE OF SPERM PLASMA MEMBRANE: In both species, the acrosomal and plasma membranes regularly form a continuum at the orifice created by dehiscence (18, 27). It is not known how the junction is accomplished, but in both species *after dehiscence the sperm plasma membrane is a mosaic, and the inserted piece is the former acrosomal membrane.*

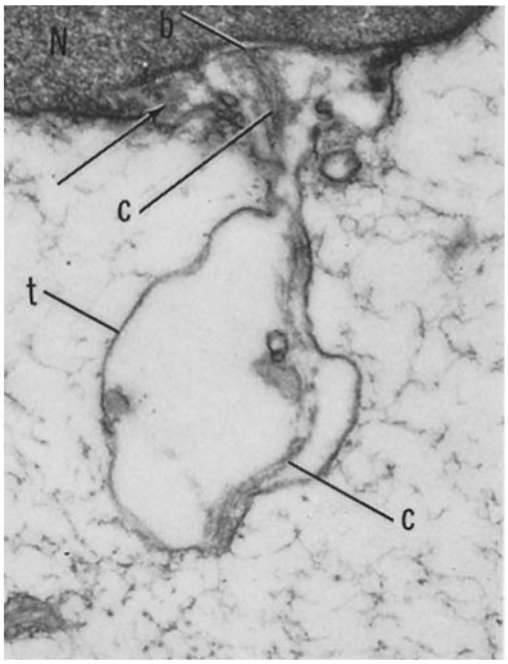


FIGURE 16 Later stage of eversion than in Fig. 15. Distal part of acrosomal tubule not shown. Periacrosomal cavity (tip of arrow) now linked to cavity of acrosomal tubule (*t*) by narrow neck formed by acrosomal vesicle which is almost fully everted. In axial region, fibrous core (*c*), which extends from adnuclear sheet (*b*), appears also in tubule. Part of tubule shows much distended condition. × 54,000.

ACROSOMAL GRANULE: This granule is released in both species. In *Hydroides*, there is strong evidence that the granule of the individual spermatozoon is responsible for the lytic removal of egg envelope material, with the result that a pathway is formed permitting the acrosomal tubules to reach the egg plasma membrane (17, 26, 27). In *Saccoglossus*, evidence for a lytic role of the granule is not so well established. However, a pit does form in the outer part of the outer egg envelope, a pit visible in living material (22, 24); and where the inner envelope is breached by the tubule, the material of this envelope appears to be missing (Fig. 18). It would seem that in *Saccoglossus* a possible role of the disappearing acrosomal granule might be the lysis of at least some part of the egg envelopes. In any event, in both species *the released acrosomal granule rapidly disappears.*

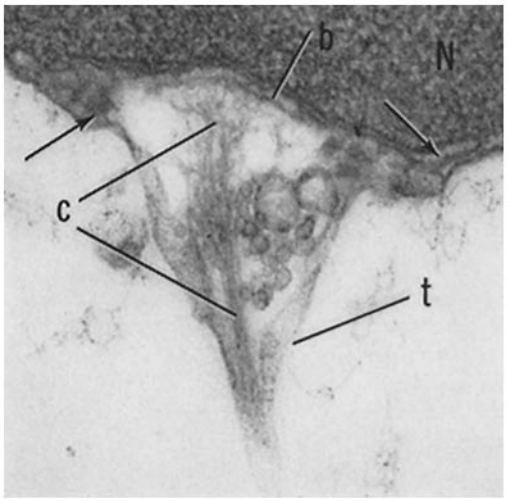


FIGURE 17 Specimen with completely everted acrosomal membrane, and having acrosomal region which indents nucleus less deeply than in earlier stages. Entire periacrosomal cavity now part of cavity of acrosomal tubule (*t*). Large and small vesicles lie in cavity. This median section shows fibrous core (*c*) extending from adnuclear sheet. Arrow at left: material of peripheral ring. Arrow at right: tip lies in space between two membranes of nuclear envelope. Only proximal portion of tubule is shown. × 72,000.

ACROSOMAL TUBULES: The invagination of the adnuclear end of the acrosomal (now sperm plasma) membrane deepens rapidly and becomes the first part of the spermatozoon to encounter the egg plasma membrane. In *Saccoglossus* a single tubule is formed, in *Hydroides* a number of tubules, but *the basic phenomenon of elongation occurs in both species.*

Several aspects of tubular elongation deserve consideration, although the mechanism of this elongation is really not known. (a) *Elongation occurs very rapidly*, requiring less than 9 seconds in either species. This suggests that an osmotic mechanism may be involved. In both species the contents of the acrosomal tubule are not acrosomal but periacrosomal in origin. A depolymerization of periacrosomal material would lead to an increase of osmotic pressure within the tubule(s). The periacrosomal material certainly does undergo some sort of change in *Saccoglossus*, but such a change is less evident in *Hydroides*. Possibly there is a causal relation between the amount of periacrosomal transformation and the volume of the elongated tubule. This volume *seems* much greater in *Saccoglossus*, but the matter has not been studied in detail. (b) *Increase in length greatly exceeds increase in width.* Why is the growth of the tubule(s) essentially unidirectional? The elongation of a fibrous core might cause a tubule to extend in this way; however, it is not known that the fibers in *Saccoglossus* actually do lengthen the tubule, and in *Hydroides* fibrillar material of this sort has not yet been observed. Again, an *in situ* reaction between the sperm plasma membrane and the egg envelope might cause unidirectional lengthening of a tubule, but in *Saccoglossus*, at least, this possibility seems unlikely because artificially activated spermatozoa, also, produce a long thin acrosomal process (22). (c) *Not the entire acrosomal membrane, but only a very limited region of the adnuclear part of this membrane, undergoes the initial great tubular lengthening.* Does this region of the membrane have special properties not possessed by adjacent parts of the same membrane? Or does the periacrosomal material beneath this region differ from the other periacrosomal material? These questions have not been answered. (d) In any event, *the tubular surface greatly increases.* Does this increase reflect extensibility or some other property of the existing membrane? Manton and Friedmann (45) describe a possibly commensurate plastic deformation in the plasma membrane of

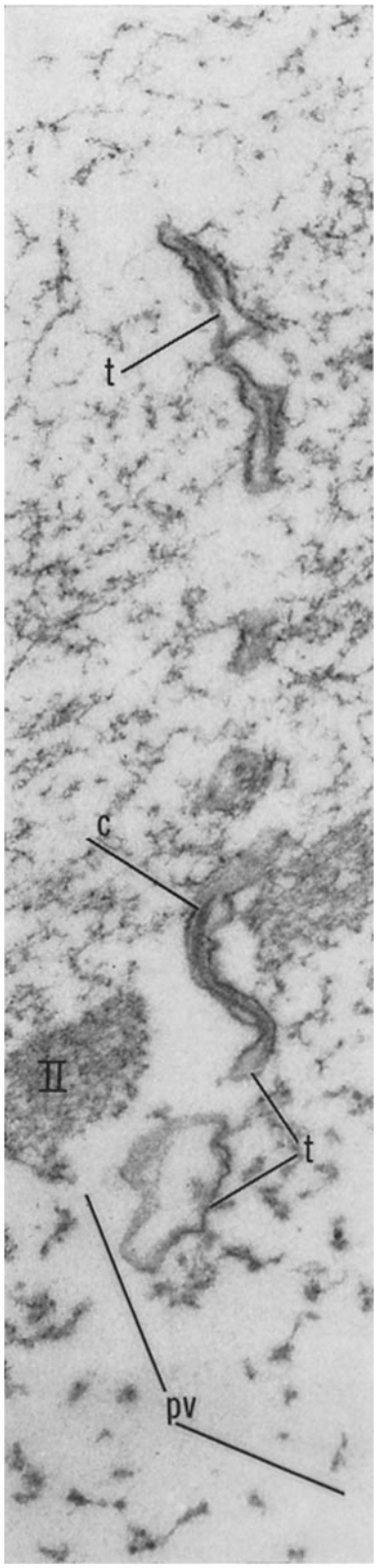


FIGURE 18 Portions of a tubule (*t*) which has spanned egg envelopes. Undistended portions lie in envelopes, and slightly distended portion lies in perivitelline space (*pv*). Material of inner egg envelope (*II*) is absent near tubule; material of outer envelope looks slightly less dense near tubule than elsewhere. Note fibrous core (*c*) in tubule. × 57,000.

certain algae and favor the concept of liquid flow rather than that of stretching. Could slippage of adjoining parts of the acrosomal membrane account for this surface increase? Or, does new membrane rapidly form? It seems possible that small, probably molecular, additions might be inserted among the original structural elements of the membrane while the latter is being subjected to expansive forces within the tubule. A potential source of such material might be the periacrosomal mass. The actual factors in this initial great surface increase are not yet known. But after the tubule has already lengthened there is evidence to indicate that new membrane may be added. Thus, in *Saccoglossus*, membrane-bounded vesicles later are seen in the periacrosomal cavity and some of these are in positions which suggest that they might join the membrane of the tubule and in this way become inserted mosaic portions. A few small vesicles occur within the tubules of *Hydroides* also, and for this species, too, this source of added membrane is postulated.

EVERSION: Initial tubular elongation is followed by complete eversion of the acrosomal membrane. In *Saccoglossus*, eversion is not extensive until after the tubule has lengthened considerably, whereas in *Hydroides*, eversion and lengthening occur simultaneously, but in neither species do the two events at first seem to be mutually dependent ones. In *Saccoglossus*, though, the tubule gains additional basal segments as a consequence of eversion.

It is not known what causes eversion, but in *Hydroides* some kind of reaction seems to take place between the egg envelope and the granular lining of the everting membrane, and in *Saccoglossus*, too, granular material is turned out against the egg envelope; possibly in both species the granular lining in some way assists in eversion during sperm-egg association.

In *Hydroides*, eversion almost certainly serves to draw the sperm nucleus through the egg envelope, whereas in *Saccoglossus*, it seems simply to lengthen and widen the base of the acrosomal tubule; but in both species, different as they are in detail, a salient result of eversion-with-elongation is that the outer surface of the apical part of the sperm plasma membrane is a surface which formerly faced into the cavity of the acrosome. This surface, the one which will establish contact with the egg plasma membrane, is a very recently exposed surface.

PERIACROSOMAL MASS: It is suggested that in *Saccoglossus* a portion of this extensive material becomes transformed into the delicate fibrous aggregations which constitute the core of the acrosomal tubule. The mechanism might involve a polymerization of the type described for fibrinogen-fibrin transformation (40). *Hydroides* apparently has no fibers in its tubules, and the two species differ in this respect. It is suggested that in *Saccoglossus* another portion of the periacrosomal mass gives rise to the vesicles which appear in the periacrosomal space, and that still another part in some way increases the volume of the tubular contents (other than core). In *Hydroides*, similar functions of the periacrosomal material are not precluded; certainly the material is in the acrosomal tubules before as well as after activation, but in *Hydroides* one might expect a less extensive performance because there is so little of the material.

At least one part of the periacrosomal material has the same fate in *Saccoglossus* (29) as in *Hydroides* (19, 27); it enters the egg cytoplasm in advance of the sperm nucleus.

E. Conclusion

In both *Saccoglossus* and *Hydroides*, sperm activation during fertilization results in dehiscence with its attendant membrane fusion, release of the acrosomal granule, elongation of the acrosomal tubule(s), and eversion of the acrosomal membrane (Fig. 20). Although some activities of the periacrosomal mass appear to be unique to *Saccoglossus*, others clearly occur in both species. In sum, the basic structure of the acrosomal region is essentially the same in both species, and the basic events of sperm activation also are essentially the same. Since species belonging to such different phyla are, in these respects, so similar, one might conjecture that this pattern of structure and events would obtain in other phyla as well. And since the events are correlated so closely with the structure of the region, one might conjecture, too, that even fugitive knowledge of either the structure or the events in another species could be extrapolated reliably to indicate the general pattern in that species. On the basis of these conjectures, the pertinent findings of other investigators in other phyla are summarized in the section below.

II. *Acrosomal Structure and Sperm Activation in Other Phyla*

The following material is considered in the context of and with the use of the terminology of the present paper. The interpretations are those of the present authors and not necessarily those of the original investigators.

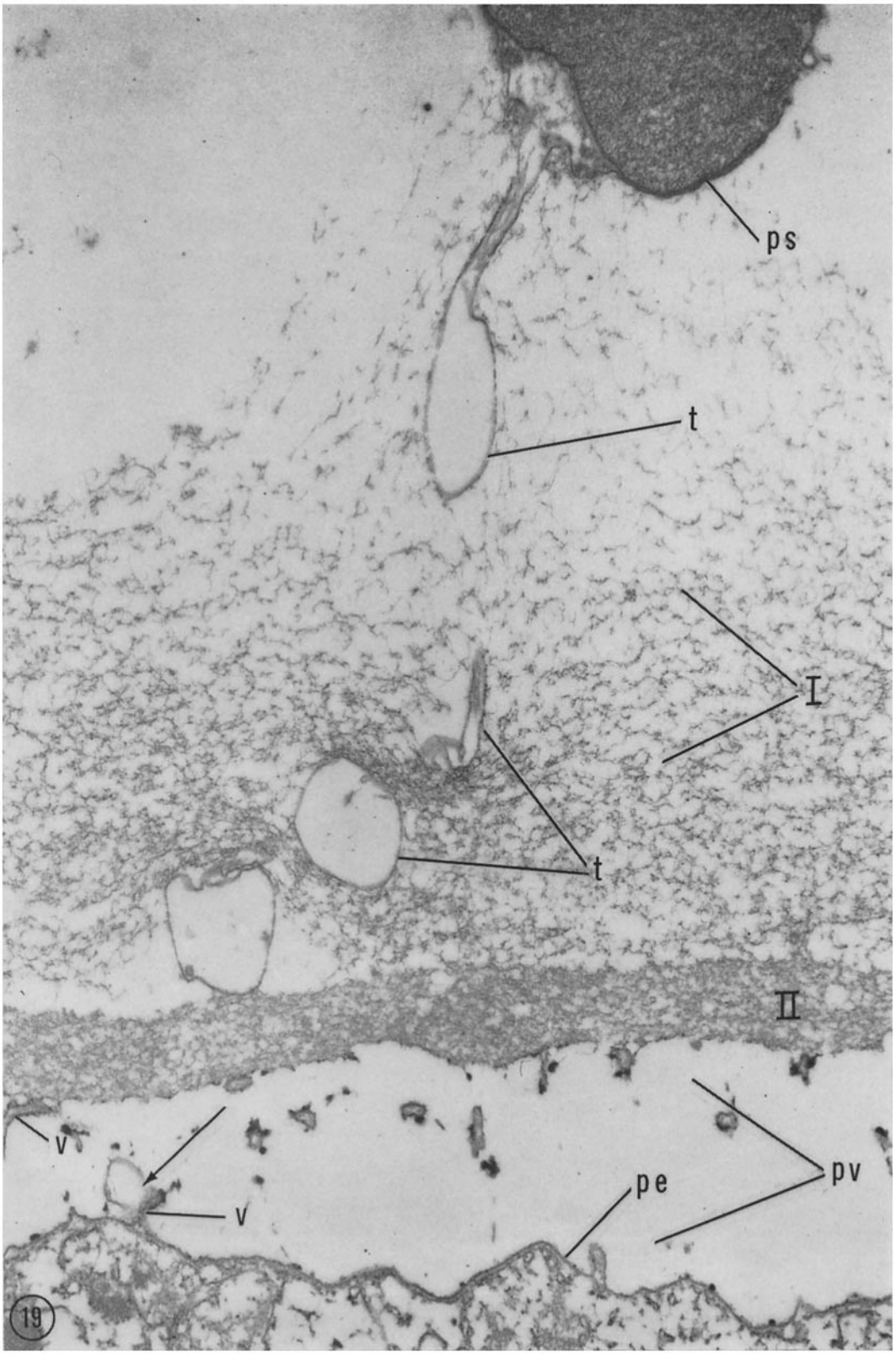
MOLLUSCS: From structural evidence (33, 39) there is what can be called an acrosomal region containing both a periacrosomal mass and a membrane-bounded acrosome. The periacrosomal material occupies the center of the adnuclear region and extends into a deep invagination of the base of the acrosome. Within the acrosome, dense material suggests an acrosomal granule. The region is known to change following activation (33, 36) and in early stages of fertilization (46), and can be said to produce an acrosomal tubule containing a fibrous core. There is lysis of the egg envelope (8, 56), and the acrosome is the suggested lytic source (57, 59). An acrosomal granule or its equivalent may be expected to be released. When put together, the clues from a number of species suggest that molluscs probably do follow the *Hydroides-Saccoglossus* pattern.

ARTHROPODS: In two species of crayfish (49, 50, 60) and in the house cricket (43) the adnuclear end of the acrosomal vesicle becomes deeply indented during development. There is what can be called periacrosomal material, and most of this lies in the acrosomal invagination. The acrosomal vesicle of the crayfishes contains dense but fibrous, and sometimes "tubular," material which might be equivalent to an acrosomal granule, and in the house cricket an acrosomal granule forms. Thus, there is some structural evidence of the *Hydroides-Saccoglossus* pattern in arthropods. Extrapolating, it is suggested that at sperm activation the adnuclear invagination of the acrosomal vesicle may lengthen and that the contents of the acrosome may be released and bring about lysis of egg envelope material.

ECHINODERMS: In sea urchins (1–3, 35) and starfishes (34, 35) the acrosomal granule can be said to be membrane-bounded. If there is an adnuclear invagination of the acrosomal vesicle before activation, it is probably a shallow one, much as in *Saccoglossus*. In sea urchins, material which can be called the periacrosomal mass is chiefly concentrated at the adnuclear end of the acrosomal region, as in *Hydroides*. Starfishes have a more extensive periacrosomal mass and it is more widely distributed within the acrosomal region, as in *Saccoglossus*. As Dan (31–33) first demonstrated, sea urchin and starfish spermatozoa can be artificially activated and will produce a slender apical projection. Sections of activated specimens (2, 35, 55) can be taken to indicate that the acrosomal membrane invaginates and elongates into a single acrosomal tubule. Sea urchins (2), and starfishes and holothurians (14, 25) produce the acrosomal projection also at the beginning of sperm-egg association. In sum, there in strong evidence that echinoderms exhibit the *Hydroides-Saccoglossus* pattern of acrosomal structure and sperm activation.

VERTEBRATES: In species such as the toad (11), chicken (51), cat (10), and man (37, 41), an acrosomal vesicle develops which contains a dense acrosomal granule. When the head cap forms, in effect the adnuclear end of this vesicle becomes invaginated. There is "periacrosomal material" between the nuclear envelope and acrosomal membrane. In various rodents, Austin and Bishop (6) reported finding, in tubal fluid, or in the cumulus or the zona, spermatozoa in which the acrosome was modified and/or lost; in some it was said to be loosened or partially detached and the perforatorium exposed. Moricard (48) noted acrosomal change or disappearance in spermatozoa which had passed through the zona. Could the loss of the acrosome be only an apparent loss? If one compared an intact acrosomal region of *Saccoglossus* or *Hydroides* with a fully activated region, without knowing about

FIGURE 19 Egg and activated spermatozoon, showing *initial contact between plasma membranes* of the two gametes. Following earlier contact with egg envelope, spermatozoon became activated and extended acrosomal tubule (*t*) through egg envelopes (*I, II*) and into perivitelline space (*pv*) where (below arrow) sperm plasma membrane at tip of acrosomal tubule is now in contact with egg plasma membrane at base of microvillus (*v*). Specimen fixed 7 seconds after insemination. × 37,000.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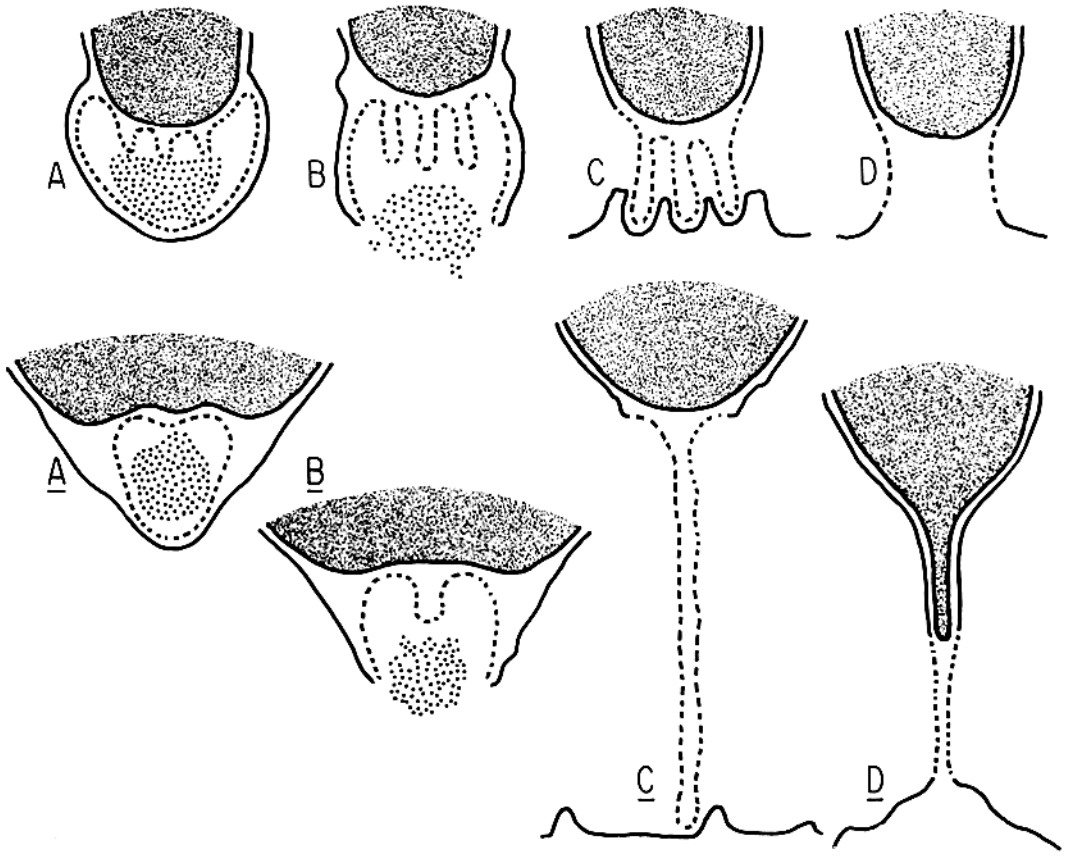

FIGURE 20 Diagram of *Hydroides-Saccoglossus* pattern of sperm-egg association. *A* to *D: Hydroides*; *A* to *D: Saccoglossus*. Non-membranous egg envelope not shown. *A*, *A:* acrosomal region before meeting egg envelope. *B*, *B:* at egg envelope, dehiscence occurs, followed by eversion of acrosomal vesicle and dissipation of acrosomal granule. Lengthening acrosomal tubule(s) present(s) newly exposed surface which was formerly (in *A*,*A*) inner surface of acrosomal vesicle. *C*, *C:* sperm plasma membrane (wall of acrosomal tubule(s)) makes its first contact with egg plasma membrane. *D*, *D:* early zygote; continuous plasma membrane established by fusion of gamete plasma membranes. Broken line in *A*, *A* represents acrosomal membrane, which later is part of sperm plasma membrane, shown in *B*, *B* and *C*, *C*, and later still is part of zygote membrane, shown in *D*, *D*. The periacrosomal cavity, rather than the cavity of the acrosome, becomes continuous with the egg cytoplasm.

the intermediate stages, one might easily conclude that the acrosome had been lost. The application of enzymes to egg envelopes by the individual spermatozoon has been postulated for some time (4, 7), although the enzymes themselves have not yet been localized in the spermatozoon. It seems justified to conclude, then, that the *Hydroides-Saccoglossus* pattern does occur also in the phylum Chordata. There are, however, forms such as the guppy (38, 52) which have acrosomeless spermatozoa, and their existence implies an exception to this pattern.

CONCLUSION: There are more similarities to the *Hydroides-Saccoglossus* pattern of acrosomal structure and sperm activation than descriptions in other contexts at first suggest. Apparently, this pattern does reflect the basic pattern in four other phyla besides the Annelida and Hemichordata. A more detailed consideration of the evidence for this is presented elsewhere (21). But even though the pattern is evident in at least six phyla, a broad generalization regarding its occurrence will not be justified until studies are available of the fine structure of sperm-egg association in more species.

III. *Periacrosomal Material and Perforatorium*

The material within the acrosomal region but *not* within the acrosome, is called here the periacrosomal mass or material. Its amount and distribution differ from species to species, but in most species at least some of it lies between the nuclear envelope and the adnuclear end of the acrosome. In sea urchins it extends into an apical indentation or cave of the nuclear envelope and is said to be fibrous (1, 35). It fills the invaginated tubules of the acrosomal membrane of *Hydroides*. In molluscs (33, 36, 39) there is what can be called a very deep indentation of the acrosomal membrane, and even before activation the periacrosomal material within this invagination appears to contain fibrous elements. In the house cricket, dense periacrosomal material, which similarly occupies a deep indentation of the acrosomal vesicle, is called the "inner cone" by Kaye (43), and Kaye considers this "inner cone" to be a possible homologue of the perforatorium of the toad (11).

The word "perforatorium" in the older literature, as used by Mèves (46), for example, does not always permit discrimination between the perforatorium and the acrosome, let alone the acrosomal region. In the present context, the perforatorium must be regarded as consisting of periacrosomal material. In the toad the perforatorium is dense, fibrous, and hook-shaped and it lies between the nuclear envelope and the adnuclear part of the acrosome (11). In the chicken, Nagano (51) interprets as a perforatorium a dense elongate (and perhaps fibrous) structure extending from a cave in the nuclear envelope to an invagination of the acrosome. A perforatorium has been described for many mammalian species (5); in the rat it has been interpreted to be a thickening or outgrowth of the nuclear envelope (12), but Burgos and Fawcett (11) do not consider this interpretation an established fact. The shape of the perforatorium is bizarre in some vertebrates; in mammals especially, fine structure studies of the perforatorium and the region it occupies are much needed.

The concept of a thread-like process extending from the apex of the spermatozoon as a consequence of activation has been implicit in the expression "acrosome filament." Early electron micrographs of whole mounts of "acrosome filaments" (32, 33, 36) gave clues to the presence of fibrous material within a membrane,—in the present context: a fibrous core within the acrosomal tubule. Dan describes a fibrous shaft in the acrosomal process of thin-sectioned starfish and sea urchin spermatozoa (34, 35). She suggests that the fibers arise by polymerization, but her scheme postulates different precursor elements than would seem to be involved in *Saccoglossus*. In any case, the core found *after* activation is longer than the entire acrosomal region *before* activation, and it is safe to assume that *some* transformation of *some* material produces this core.

There is, then, present in many species *before* activation a sometimes fibrous periacrosomal material, and in many other species a fibrous structure is produced *as a consequence of* activation. It is suggested that the two types of structure may be equivalent; that is, the core of the elongated acrosomal tubule may be equivalent to the perforatorium or the "inner cone." But until the function of these various structures is known in relation to fertilization, it is not possible to draw definitive conclusions about their structural homologies. To classify "the acrosome" into "simple" and "compound" types (43) at this time seems premature.

IV. *Role of Acrosome*

It has been a recurrent view that the acrosome might activate the egg (9), and the discovery of the "acrosome filament" (31, 32) led to the supposition that this projection might deliver the stimulus (24, 32, 33). In living specimens of certain species, it appears that the acrosomal process is the first part of the spermatozoon to reach the living egg surface and that it does somehow stimultate the egg (14, 15, 25). But in *Saccoglossus* this newly lengthened tubule is not an intact acrosome and embodies only a part (the reversed membrane) of the original acrosome. The major contents of the acrosome are dissipated while the tubule is traversing the egg envelopes, and the major role of the acrosome is *not* to activate the egg but to convey the sperm plasma membrane to the egg plasma membrane, as a consequence of which the egg subsequently is activated. The acrosome of *Hydroides* plays a similar role (27). The history of the acrosomal tubule after it meets the egg plasma membrane is discussed in the next paper (29).

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