ACROSOME FORMATION IN THE HOUSE CRICKET

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ABSTRACT

Acrosome formation during spermatogenesis in the house cricket was studied with the electron microscope. In the early spermatid there is a single Golgi body, called the acroblast, which is cup-shaped, the walls being composed of a number of parallel membranes. A pro-acrosomal granule then appears within the acroblast. Next, the granule migrates to the nucleus, where it becomes attached. The acroblast then migrates away from the attached granule and is eventually sloughed off. In the first stage of acrosome differentiation the granule assumes the shape of a blunt cone and its base invaginates deeply so that it becomes hollowed. Within the space created by the invagination a new structure forms which, from the first, has the shape of a hollow cone. The two cones constitute the mature acrosome. Both have a biconvex cross-section.

INTRODUCTION

The anterior end of the nucleus of a flagellated animal sperm is capped by a distinct organelle, the acrosome, which varies considerably in shape and size, depending on the species, from minute granules as in sea urchins (1) to fibrils of great length as in *Gerris*, the pond skater (2). The acrosome has no obvious homologue in the spermatocytes which give rise to spermatozoa, but it was known fairly early that the precursor of the acrosome, the pro-acrosomal granule, develops from another cell inclusion, the acroblast, which is Golgi-like in nature. By 1920 Bowen (3) had demonstrated that the acroblast is not simply homologous with the Golgi material of the spermatocyte, but is in fact derived from this material. Of acrosome differentiation itself little was known, most authors treating it as a more or less simple change in shape of the pro-acrosomal granule as the spermatid matured. In *Gerris*, which is especially favorable material, Pollister (2) figured complicated changes in the acrosome as it developed, with the appearance of at least two substances in the pro-acrosomal granule which go on to form a bipartite acrosome, one substance forming a sheath which encloses a core formed from the other substance.

Several electron microscope studies (4, 5) of acrosome development have been published recently which have given new information on the mode of formation of the pro-acrosomal granule and its attachment to the nucleus. None of these studies report much real change in the granule during its differentiation after it is attached to the nucleus.

The following work is a description of the electron microscopy of acrosome formation in the house cricket, *Acheta domestica*. It illustrates details of the deposition of the pro-acrosomal granule on the nucleus and demonstrates that an entirely new structural part of the acrosome arises in the later stages of differentiation.

MATERIALS AND METHODS

Specimens of the house cricket, *Acheta domestica*, were purchased from a dealer and cultured in the laboratory.

Good fixation of the spermatogenous cells was achieved with a mixture of 2 per cent osmium

tetroxide in 12 per cent Dextran (Mann Research Laboratories, Inc., New York; clinical grade A, mol wt 200,000 to 275,000), buffered to pH 7.2 with veronal buffer and used ice cold. Small pieces of the testes of mature crickets or late nymphs were fixed in the above mixture for ½ to 1 hour. Following this fixation the testes were postfixed (6) for about 12 hours in an ice cold solution of 10 per cent formalin (methanol free; Heyden Newport Chemical Corp., New York) buffered to pH 7.2 with sodium bicarbonate.

Following postfixation, tissues were dehydrated rapidly in a graded series of ethanol solutions beginning with 70 per cent ethanol. They were then embedded in a mixture of 4 per cent methyl and 96 per cent *n*-butyl methacrylate to which 0.2 per cent uranyl nitrate was added to minimize "explosion" (7). The methacrylate was polymerized at 45°C.

Sections were cut with a Porter-Blum microtome and mounted on grids previously coated with a carbon film (8). They were then stained from 1 to 2 hours with lead hydroxide (9).

Sections were studied with an RCA EMU-3E electron microscope and micrographs were taken at direct magnifications up to × 32,000. The beam potential was 100 kv.

OBSERVATIONS

In the cricket the Golgi material of primary spermatocytes consists of small elements scattered throughout the cytoplasm and termed dictyosomes by earlier workers (3). The structure of each dictyosome is typical for Golgi material; it consists of several pairs of parallel membranes, which are associated with highly characteristic vacuoles that appear to be empty, and vesicles that contain some electron-dense material (Fig. 1). The pairs of membranes of the dictyosomes are usually fairly straight or gently curved and are closed at each end, as shown in Fig. 2, but a single membrane may also be folded several times in serpentine fashion. Occasionally, continuity of the parallel membranes with the membrane of a vacuole occurs, as indicated in Fig. 2.

Though no accurate count of the dictyosomes in a single spermatocyte was made, there appear, from observations with the light microscope, to be at least a dozen or perhaps two or three times that number.

After the second meiotic division the dictyosomes fuse to form a single Golgi body, or acroblast, as it was termed earlier (3). As shown in Fig. 3, the acroblast is cup-shaped, with fairly thick walls that consist of six to ten regularly spaced membranes (Fig. 4). Along the outside border and filling the inside, there are both vacuoles and vesicles which are similar in size and appearance to those associated with the dictyosomes of primary spermatocytes.

Typically the acroblast and the nebenkern (which is formed through the coalesence of mitochondria) lie fairly close together near the base of the nucleus in the early spermatid (3, 10). Several bodies which are electron-dense and have a membranous internal structure also lie near the acroblast (Fig. 3). Although these are of unknown origin, they may be the so-called Y granules reported by earlier workers (10–12).

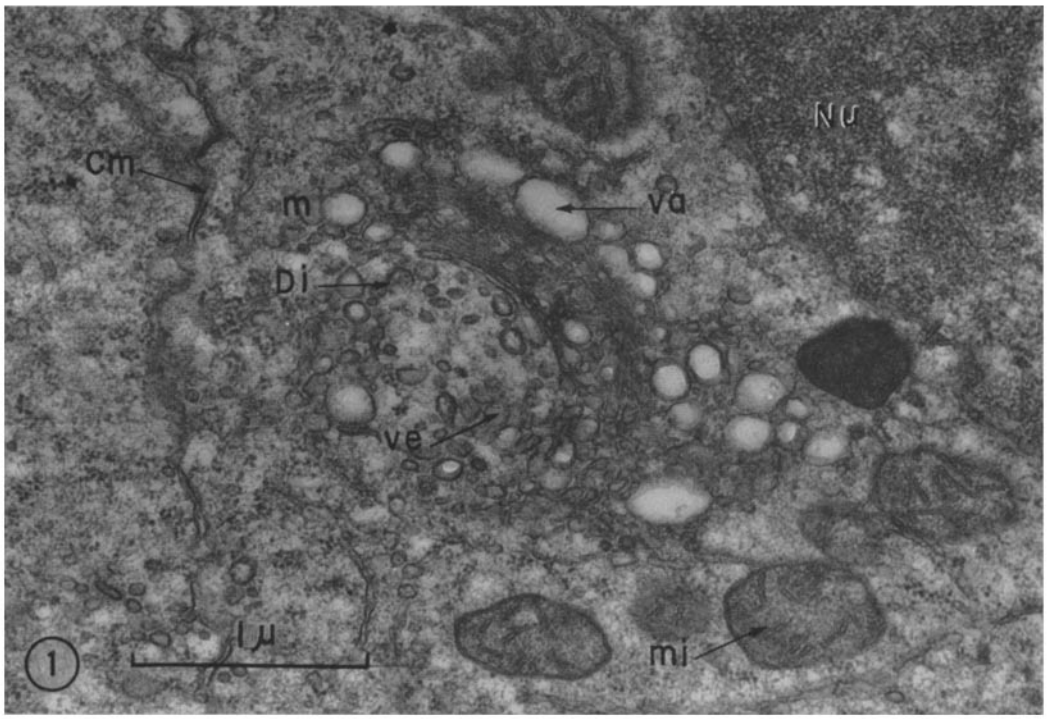
The acroblasts of later spermatids (Fig. 5) contain another element: a homogeneous spheroid called the pro-acrosomal granule, since at least part of the acrosome is derived from it. The pro-acrosomal granule is bounded by a membrane that appears single in Fig. 5, but in the thinner

FIGURE 1

A low power micrograph showing a part of a primary spermatocyte. A dictyosome (*Di*) appears in the center. Its structure consists of paired membranes (*m*), and associated with it are empty vacuoles (*va*) and vesicles (*ve*). A few mitochondria (*mi*), cut in transverse section, lie near the dictyosome. A small segment of the nucleus (*Nu*) appears in the upper right, and the cell membrane (*Cm*) appears at the left of the dictyosome. × 30,000.

FIGURE 2

A dictyosome at a higher magnification than in the preceding figure. The paired membranes (*m*) are regularly spaced. They are mainly rather straight, but at *a* they are folded several times. At *b* one of the membranes is in continuity with the membrane enclosing a vacuole (*va*). × 110,000.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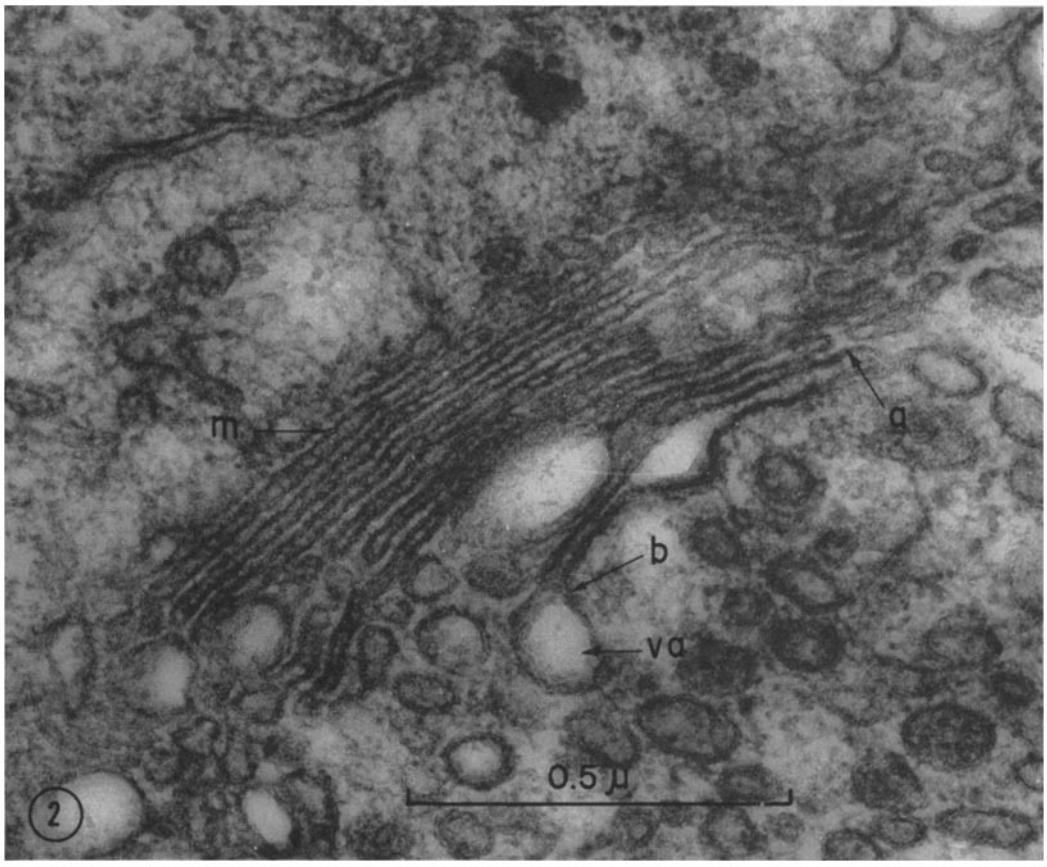
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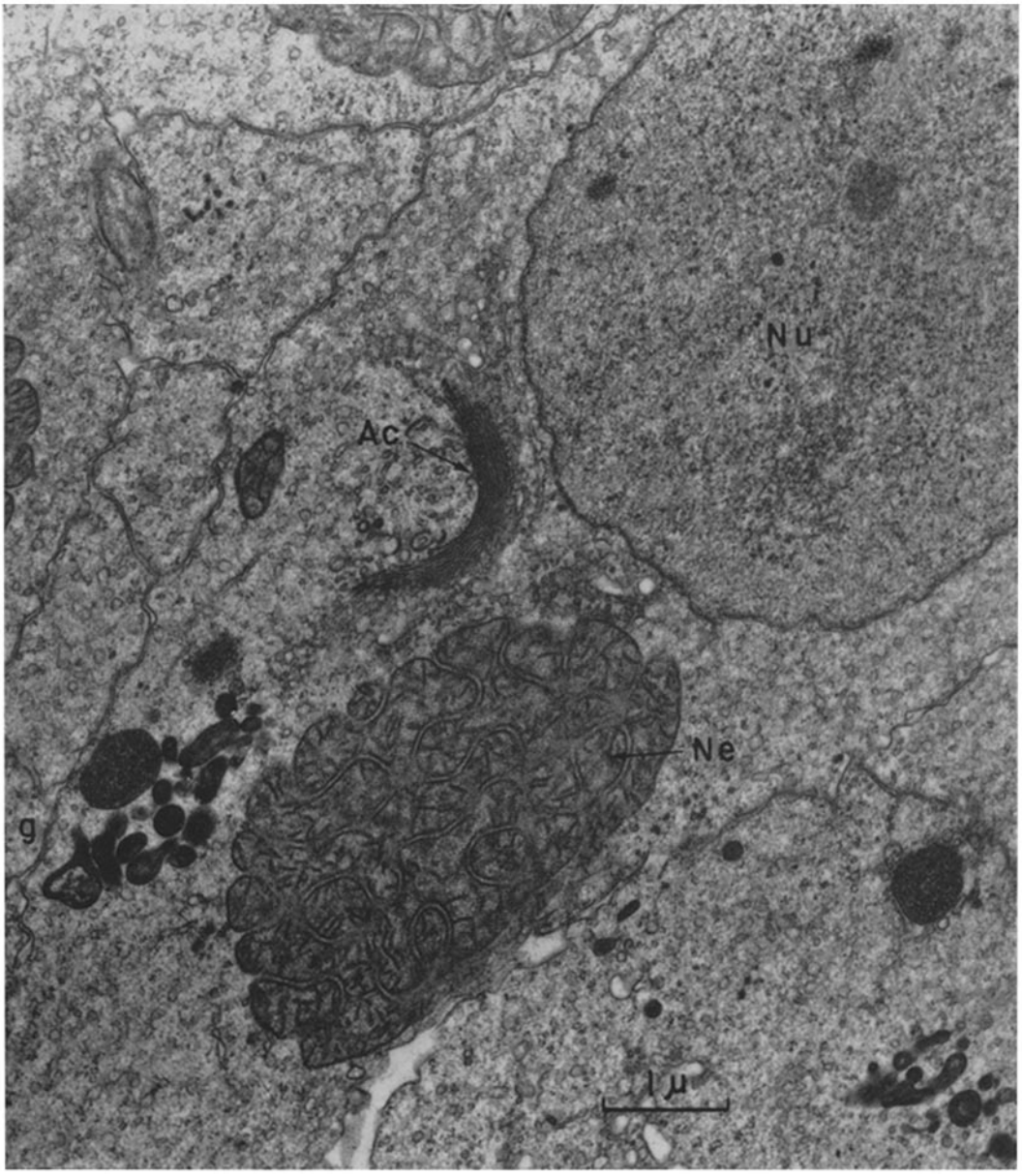


FIGURE 3

A survey micrograph showing some of the elements of an early spermatid. The cup-shaped acroblast (*Ac*) lies at the base of the nucleus (*Nu*) and near the nebenkern (*Ne*), which is formed from mitochondria. Also shown here is a cluster of dense granules (*g*) of unknown origin. × 15,000.

section of Fig. 8 it is seen to be resolved into two elements. The granule is confined to the vesicular region of the acroblast and has no continuity with the walls. From other studies (3, 4) it seems probable that the granule grows within the acroblast, but an unequivocal seriation of growth stages was not obtained in the present work. The proacrosomal granule reaches maximum size while still within the acroblast, which has in the meantime, through migration and rotation, come to lie

with its open side facing the nucleus close to the developing axial filament. Then the pro-acrosomal granule leaves the confines of the acroblast, migrates to the nucleus, and becomes attached to it. Fig. 6 shows a granule which has just begun the migration.

Fig. 7 shows a granule midway between the acroblast (not in the figure) and the nucleus. The surface facing the nucleus is flattened and a membrane is in contact with it there. This membrane, which will be called the interstitial membrane, is distinctive in that it is single (*i.e.* unpaired), is fairly thick, and has a fuzzy profile. It is attached to the granule centrally and its ends are free.

In the granule of Fig. 8, which is quite close to the nucleus, the interstitial membrane is aligned more or less parallel to the nuclear membrane. In Fig. 9, the interstitial membrane is nearly in contact with the nuclear membrane in the fairly small area indicated on the figure where they are separated by a constant distance. One end of the interstitial membrane is free and curls away from the nucleus; the other seems to merge with the granule membrane.

A granule that is fully attached to the nucleus appears in Fig. 10. The interstitial membrane is interposed between the nucleus and the granule and is separated from each of them by a constant distance of about 200 A. It covers a surface area of the nucleus considerably larger than the area of the granule that is adjacent to the nucleus.

A rearrangement of the parts of the spermatid takes place during the period of attachment of the granule to the nucleus. The entire cell elongates, as do the nebenkern and axial filament. The acroblast membranes migrate posteriorly from the site of attachment of the granule, where they are eventually sloughed off. The granule is attached initially to the posterior part of the nucleus, but comes to lie on the anterior part in the later spermatids.

Following attachment, the granule flattens, as shown in Fig. 11, and its substance appears divided into two parts, one being dense and occupying the basal periphery of the granule, the other being less dense and occupying the central portion. Adjacent to the nucleus, there is a slight invagination of the granule near its center. At this point the interstitial membrane is discontinuous.

Later, as shown in Fig. 12, the granule has the shape of a cone, with the height about equal to the width of the base. An electron-dense band of material occupies the basal half of the cone. The apical half contains a band of lower density. There is an invagination of the base of the granule to the extent of about half its height.

Several other features of the spermatid are clear in Fig. 12. The nucleus is elongate and the granule is situated at its anterior end. The complex of blepharoplast, axial filament, and so-called basophilic centriole extends up one side. Within the nucleus there is a thin shell of material of high density.

Further details of the invagination of the granule are shown in Fig. 13. The interstitial membrane comes up to, but does not enter, the invagination (also apparent in Fig. 12). The invagination contains amorphous material of high density which, even though it is not ordered, seems not likely to be an artifact because of the constancy of its appearance.

Fig. 14 shows a later spermatid, and there is no separation of the granule material into dense and less dense components. The extent of the invagination is about three-fourths the height of the granule, and at its anterior end there is a tapered structure of indistinct outline. The amorphous material seen in the preceding figure is not present. In this or in any of the succeeding micrographs it is not obvious where the interstitial membrane is. The nucleus is filled with fine fibrils at this time.

Slightly later, as shown in Fig. 15, the tapered structure within the invagination has developed into a distinctly pointed structure with a hollow interior. In this longitudinal section it seems to consist of two concave arms of high density, but these are really sections through the walls of a hollow cone, as may be determined from the transverse section of the later stage in Fig. 19. In the ensuing description this structure will be called the inner cone.

The invagination is complete in Fig. 16; its extent is about nine-tenths the height of the granule. The inner and outer boundaries are separated by about 400 to 500 A except at the base, where the separation is greater. The inner cone is nearly as long as the invagination, and its walls are thicker and more distinctly outlined than in earlier stages. The material of the nucleus at this stage is in the form of thick fibers.

An acrosome near the end of spermiogenesis is shown in Fig. 17. At the base of the invagination the granule has pinched in toward the center and

nearly sealed the opening. The inner cone is thicker than in the previous figure.

The acrosome of a mature spermatozoon is shown in Fig. 18. It is a bipartite structure consisting of concentric hollow cones. The outer cone completely encloses the space of the invagination. Its boundaries are demarcated by membranes. The base is solid and bulges up past the lower extremities of the inner cone. The inner cone is a well developed structure which extends the length of the space created by the invagination. It is sharply outlined though no membrane bounds it. In longitudinal profile the inner cone describes a graceful curve.

A transverse section through the base of an acrosome of a mature sperm is shown in Fig. 19. Starting from the outside and progressing inward toward the center, one encounters the cell membrane, then two more membranes which are the outer and inner membranes of the outer cone, then some diffuse material. Next there is the inner cone, which seems to have a somewhat lower relative electron density here than it had in the preceding micrographs. This condition has been observed occasionally in both longitudinal and transverse sections, and its cause is unknown. Finally, in the center of the acrosome there is some of the dense material at the base of the outer cone. From this transverse section it is apparent that both the outer and inner cones are flattened and have a biconvex cross-section.

A brief qualitative study of the staining reactions of the developing acrosome was done with the light microscope, using fast green at low pH for protein amino groups (13) and the PAS reaction for polysaccharides on tissues fixed by freeze-substitution (14). The parts of the acrosome are too small for reliable measurements of concentration of stains or even for detailed visual localization, but several facts did emerge from the study.

The distribution of fast green stain in the pro-acrosomal granule is uniform until the stage shown in Fig. 11, when a higher concentration appears in a band at the base of the granule and a lower concentration at the apex. With the PAS reaction the granule also shows uniform staining until the stage shown in Fig. 11, when the PAS stain seems to be localized in the basal half; none is visible in the apical half.

DISCUSSION

The chief interest of the foregoing observations lies in the information they provide on the mode of acrosome formation after the pro-acrosomal granule is attached to the nucleus; the inner cone is formed as a structure distinctly different from the granule.

In acrosome formation, the postattachment events in the cricket are strikingly different from those in mammals, as described by Burgos and Fawcett for the cat (4) and man (5). In the latter organisms the acrosome is derived wholly from the pro-acrosomal granule, and the postattachment stages consist essentially of a remodeling of the granule—to form a solid cone in cat sperm, and a thin shell covering the anterior part of the head in human sperm. Thus in each case the acrosome has only one component.

These differences in the mode of acrosome formation between the cricket and mammals should not be taken as contradictory, but rather as evidence that there are two types of acrosome: a simple type having one component, and a compound type having more than one.

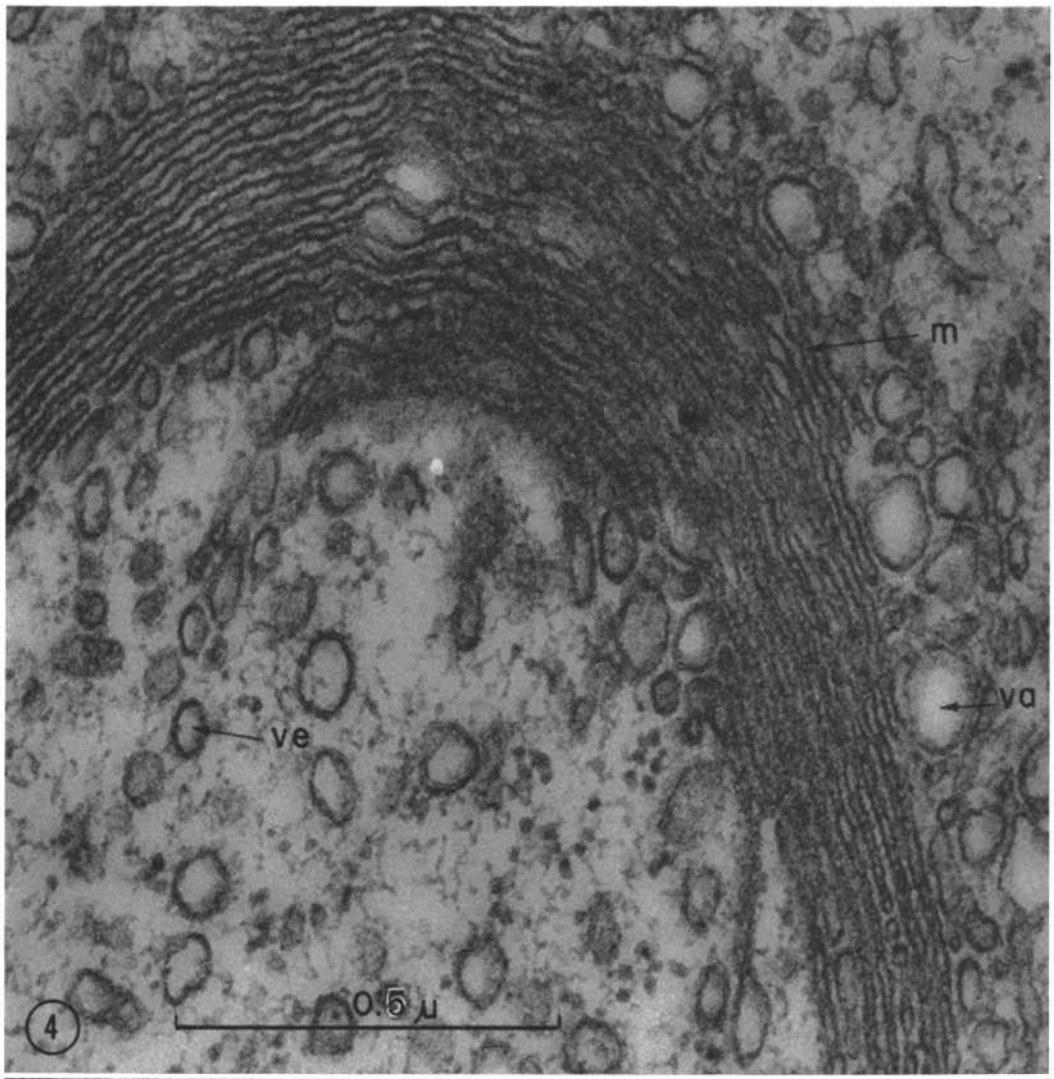
There is evidence which suggests that the compound type is of widespread occurrence. Thus, using the electron microscope, Dan (15) found that in mature starfish sperm the acrosomes have the form of an inverted cone embedded in the sperm head. Within the acrosome there are three

FIGURE 4

An acroblast at higher magnification. Its walls consist of regularly spaced membranes (*m*), and there are vacuoles (*va*) and vesicles (*ve*) associated with it. × 100,000.

FIGURE 5

A slightly later spermatid than in the preceding figure. The acroblast (*Ac*) is sectioned near the open end in a plane perpendicular to the plane of sectioning of the acroblasts in Figs. 3 and 4. Within the acroblast is the pro-acrosomal granule (*Gr*). Parts of the nucleus (*Nu*) and the nebenkern (*Ne*) are also shown. × 32,000.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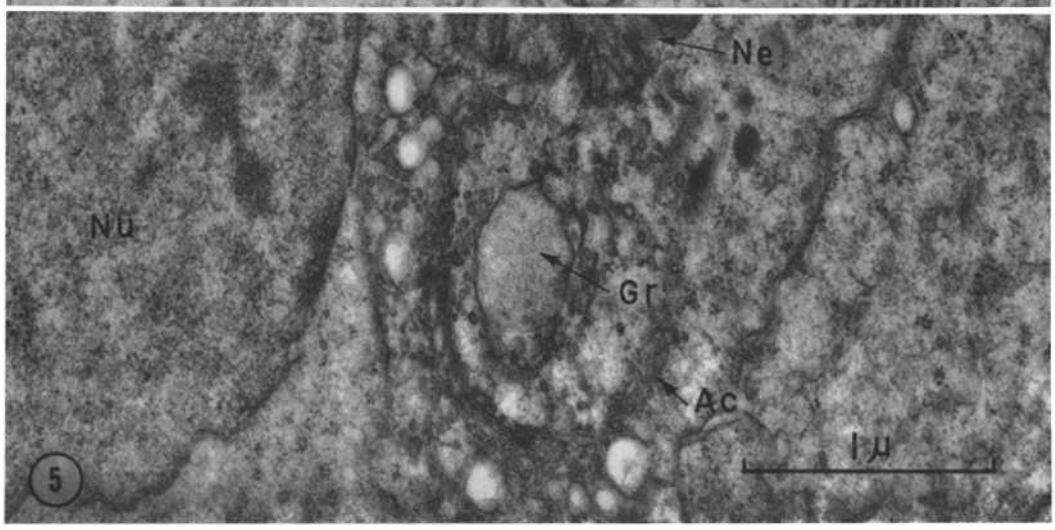
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distinct regions which differ in electron density. With the light microscope and the techniques of cytochemistry, Moriber (16) found that the pro-acrosomal granule of *Gerris* is divided into PAS-positive and -negative regions, and that in the bipartite acrosome that develops from it (2) the sheath is PAS-positive and the core negative.

The validity of grouping acrosomes into two general types depends in part on the definition of the term "acrosome." Originally this term (see the discussion in Wilson, 17) was coined to name the apical structure of sperm. The term is used in this sense in the present paper. Recently, however, several workers (18, 19) have adopted a new definition which assumes that the acrosome is entirely PAS-positive and develops solely from the pro-acrosomal granule. These assumptions were derived from work on mammals, which seem to have a simplified type of acrosome structure. They would not, for example, fit the case of *Gerris*, where the pro-acrosomal granule itself is differentiated into PAS-positive and -negative regions. Further, the assumption that the acrosome is always formed from the pro-acrosomal granule alone is not supported by the manner of development of the inner cone in cricket sperm.

Certain aspects of the differentiation of the acrosome in crickets are noteworthy since they do not occur in the organisms that have been studied so far with the electron microscope, or they differ in some important details.

One difference is seen in regard to the membrane surrounding the pro-acrosomal granule while it is in the acroblast. In the cricket the granule has a thin membrane closely applied to its surface. In mammals, *e.g.* the cat (4), the granule is contained within one of the vesicles of the acroblast, which is somewhat larger than the granule, so that a clear space surrounds the granule and no membrane bounds its surface. The similar fates of the granule membrane of the cricket sperm and the vesicle membrane of the cat sperm (*i.e.*, they both enclose the mature acrosome) suggest that the two are analogous structures. Perhaps in the cricket the granule is contained within one of the acroblast vesicles but fills it completely.

The interstitial membrane, which seems to play a role in the process of attachment of the granule to the nucleus, and possibly in the invagination also, has no counterpart in other organisms that have been studied. Burgos and Fawcett (4) clearly show that in the cat the acrosome membrane is in direct contact with the nuclear membrane; no third membrane is interposed between them.

The origin of the interstitial membrane poses an interesting question. This membrane might arise from the wall of the acroblast, break loose from the rest of the wall, and become associated with the granule. However, the unique morphology of this membrane indicates that such an origin is less probable than the interstitial membrane's arising *de novo* at the time the granule leaves the acroblast. The latter concept is supported by the observation that the interstitial membrane seems to be capable of growth independent of the acroblast. It increases in extent long after the acroblast has migrated from the vicinity (compare the membranes in Figs. 7 and 12).

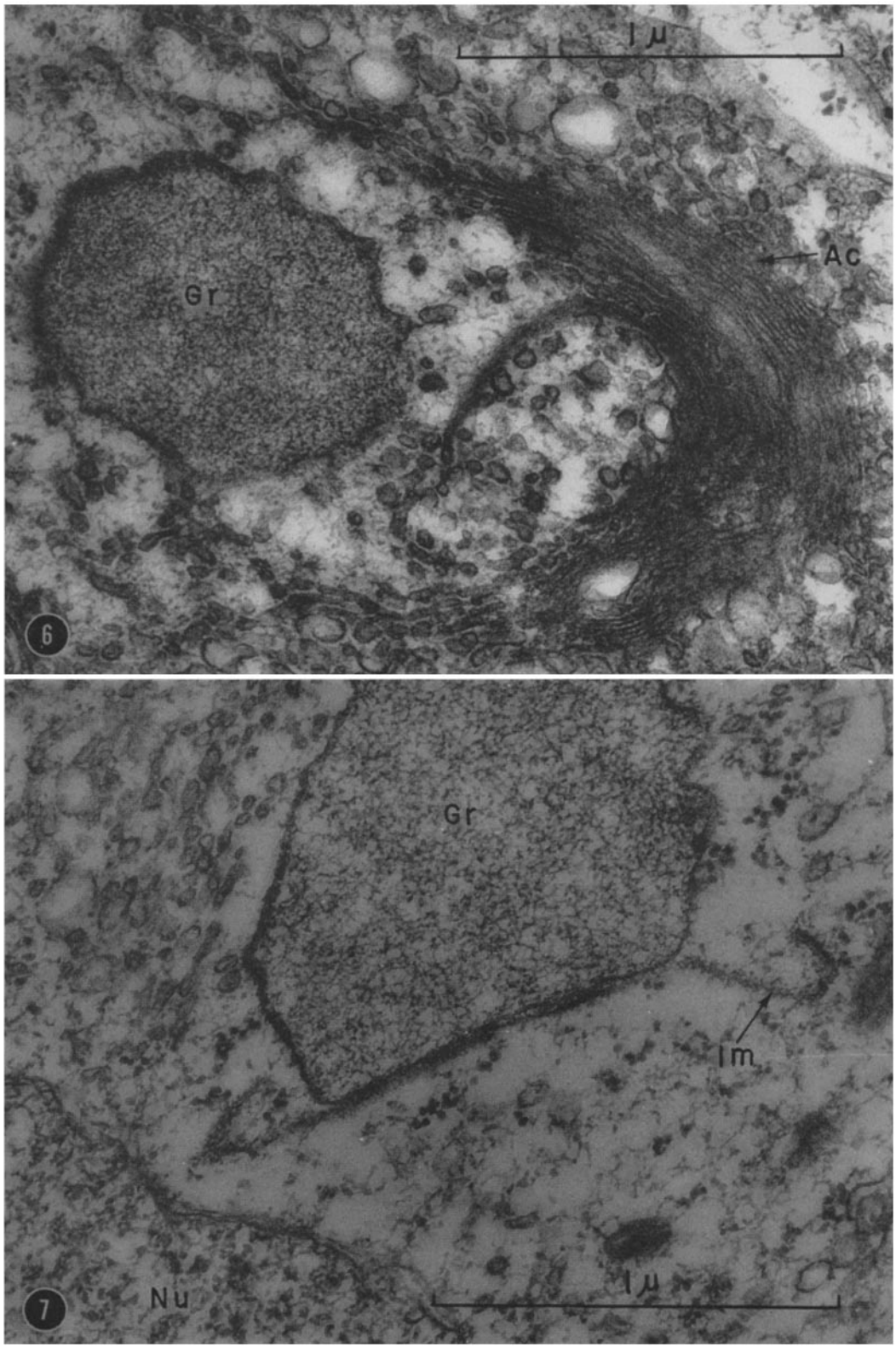
The development of the electron-dense band in the attached granule has not been reported in electron microscope studies of other organisms. The heterogeneous PAS staining of the granule at this time suggests that the same phenomenon that occurs here also occurs in *Gerris* (16), one difference being, however, that in *Gerris* the granule becomes heterogeneous while it is still associated with the acroblast; in the cricket this

FIGURE 6

A micrograph showing the acroblast (*Ac*) and a pro-acrosomal granule (*Gr*) which has just begun its migration to the nucleus. × 58,000.

FIGURE 7

A granule (*Gr*) midway in its migration to the nucleus. The interstitial membrane (*Im*) is closely associated with the granule at its flattened surface. A part of the nucleus (*Nu*) appears at the lower left of the figure. × 62,000.


1 μ
Gr
Ac
6
Gr
Im
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occurs after the acroblast has migrated away from the granule.

The most striking event in acrosome differentiation in the cricket is the formation of the inner cone. The immediate precursor of this structure seems to be the amorphous material that appears within the early invagination as shown in Fig. 13; at least, this is suggested by the fact that at a slightly later stage (Fig. 14) the rudiments of the inner cone are clearly identifiable and the amorphous material is no longer in evidence. One circumstance suggests that at least some of the material of the inner cone could come from the granule. During the invagination of the granule its volume is decreased. There is not a corresponding increase in the concentration of the material of the granule, as judged subjectively from the apparent lack of an increase in its electron density. This implies that some material leaves the granule, possibly being "squeezed out" in a sense; this material could very well enter the invagination to become incorporated into the inner cone. It is not possible at present to determine the amount of material in the inner cone and compare it with the material that might be "squeezed out" of the granule, but a comparison of Figs. 16 and 17 indicates that the inner cone continues to increase in mass after the granule ceases its invagination. This suggests that all the inner cone material does not come from the granule, even though some of it may.

No structure homologous with the inner cone has been described in mammals. However, Burgos and Fawcett (20) described in toad sperm a hook-shaped, electron-dense structure which resembles a section of the inner cone. They have also reported that this hook-shaped structure originates in about the same place as the inner cone of the cricket sperm. This fact, together with the similarity in general appearance, suggests that the hooked structure of toad sperm and the inner cone of the cricket sperm may be homologous. There are enough differences between them, however, to make a conclusion on this point uncertain. The hooked structure of the toad sperm is not completely enclosed by the head cap, whereas the inner cone of the cricket sperm is enclosed by the pro-acrosomal granule; the morphology of the hooked structure is similar to, but certainly not identical with, that of the inner cone.

It should be noted that Burgos and Fawcett do not consider the hooked structure of toad sperm to be acrosomal in nature, but rather, using the criteria and terminology of Leblond and Clermont (18) for the apical structures of sperm, they call it a perforatorium. This classifying of the apical structures of sperm into non-acrosomal components, using criteria that seem to fit certain mammals only, seems unwarranted.

There is good evidence that acrosomes of echinoderm and molluscan sperm may possess two distinct activities (21, 22). They extrude a filament which attaches the sperm to the egg, and they appear to contain lysins which act to break down egg membranes. Electron microscope studies of these sperm (15) have shown the acrosomes to be of the compound type, *i.e.* having two or more distinct components, which suggests that morphological subdivisions reflect a corresponding division of substances and functions within the acrosome.

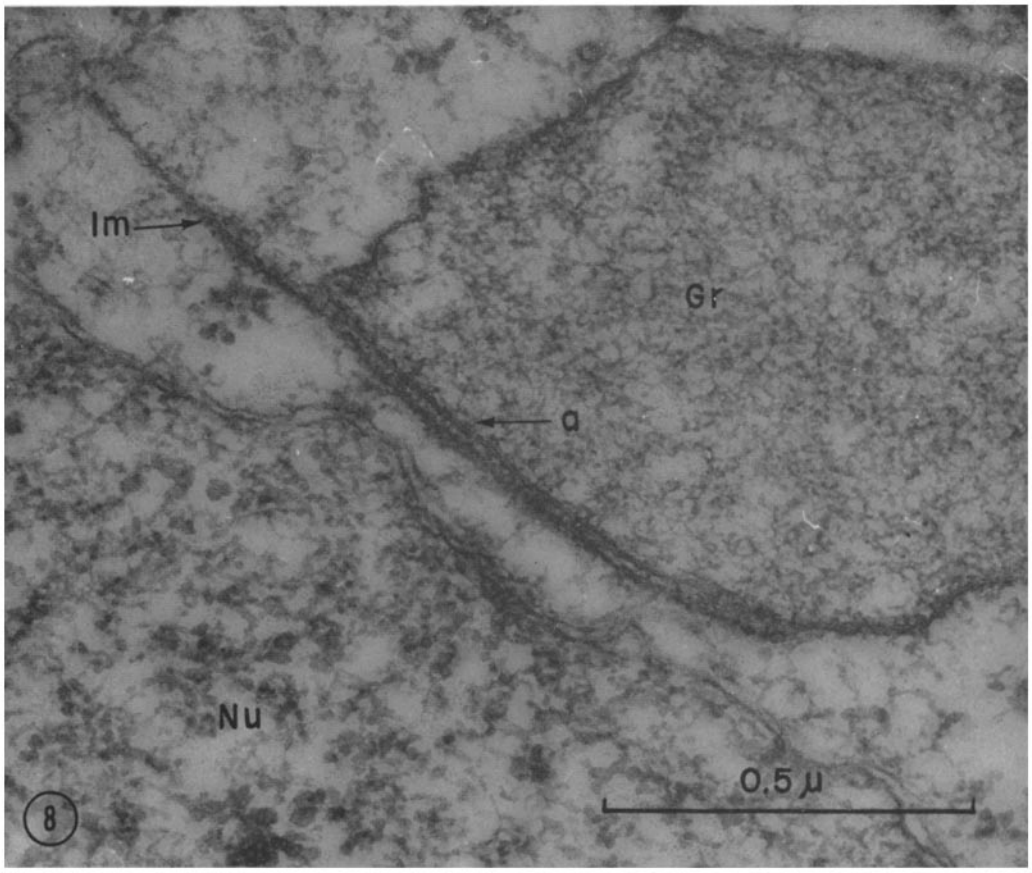
The extensive study by Colwin and Colwin (23, 24) of the spermatozoon of the annelid *Hydroides* showed that the acrosome is of the compound type and that during fertilization it acts to attach the sperm to the egg with a number of finger-like projections. Colwin and Colwin further observed that a granule within the acrosome disappears early in fertilization, and they suggest that this granule may be the site of membrane lysins.

FIGURE 8

A granule (*Gr*) which is close to the nucleus (*Nu*), with the interstitial membrane (*Im*) between them. At *a* the doubleness of the granule membrane is apparent. × 95,000.

FIGURE 9

A granule (*Gr*) which has become attached to the nucleus (*Nu*) in the region at *a*. The interstitial membrane (*Im*) is interposed between the granule and the nucleus. × 100,000.


Im
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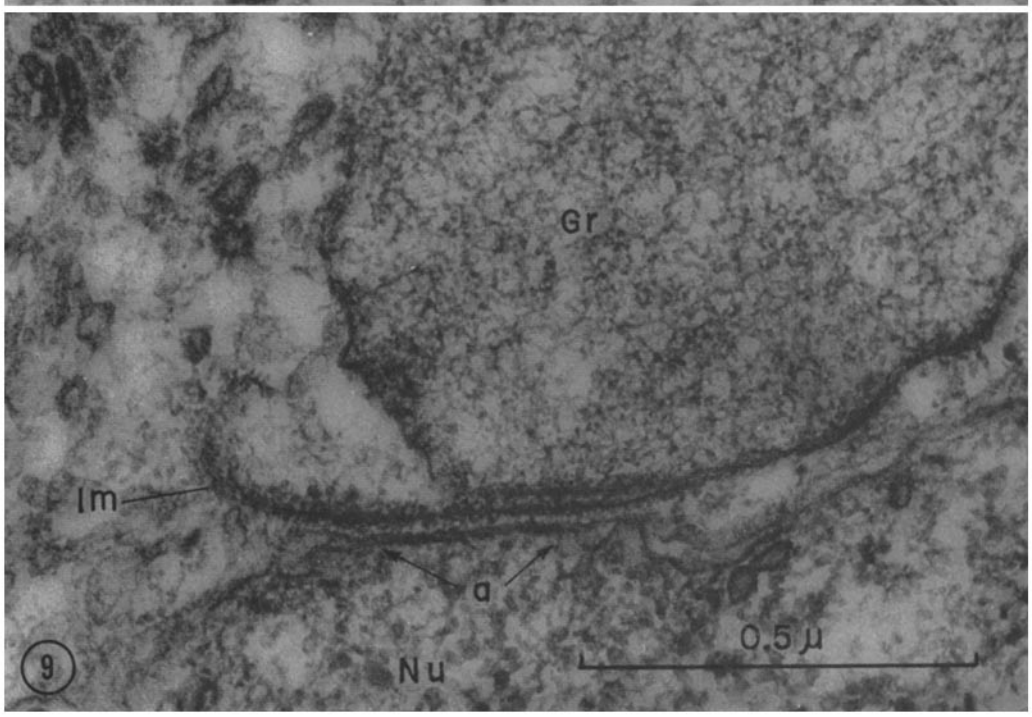
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Neither the inner nor the outer cone in the cricket acrosome can be interpreted as a preformed filament. Thus, as Dan (21) suggests for molluscs and echinoderms, if a filament is formed by the acrosome it must be formed at the time of extrusion. Just how the parts of the cricket acrosome might function in such a process, or where membrane lysins might be localized, must be the subject of future work.

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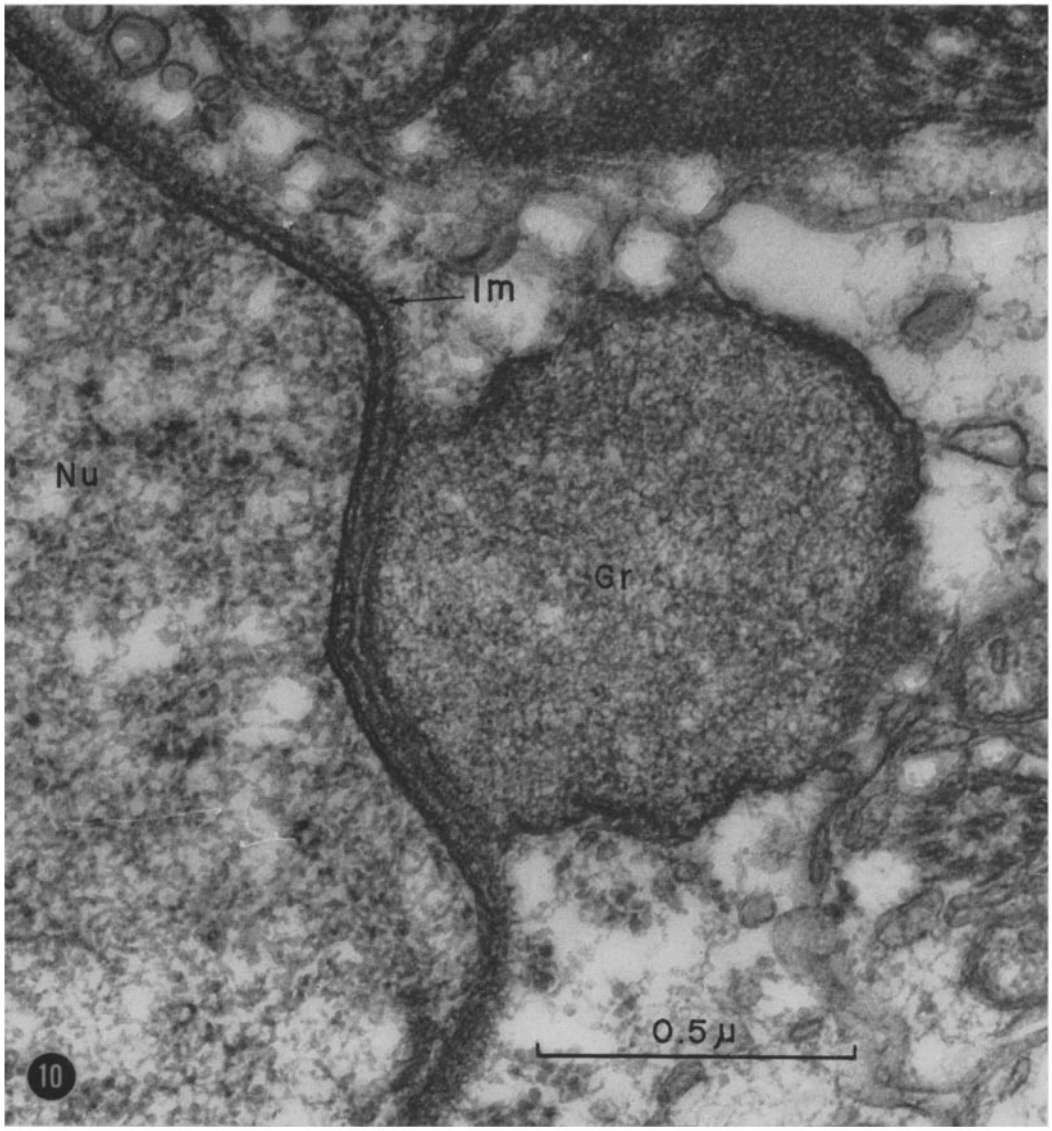
FIGURE 10

A granule (*Gr*) which is fully attached to the nucleus (*Nu*). The interstitial membrane (*Im*) covers a considerable extent of the nucleus. × 82,000.

FIGURE 11

A later stage than in Fig. 10; the granule (*Gr*) has become flattened. Its substance is now divided into dense (*a*) and less dense (*b*) regions. At (*c*) it has begun to invaginate, and at this point the interstitial membrane (*Im*) is discontinuous. × 92,000.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0.5 µ
10

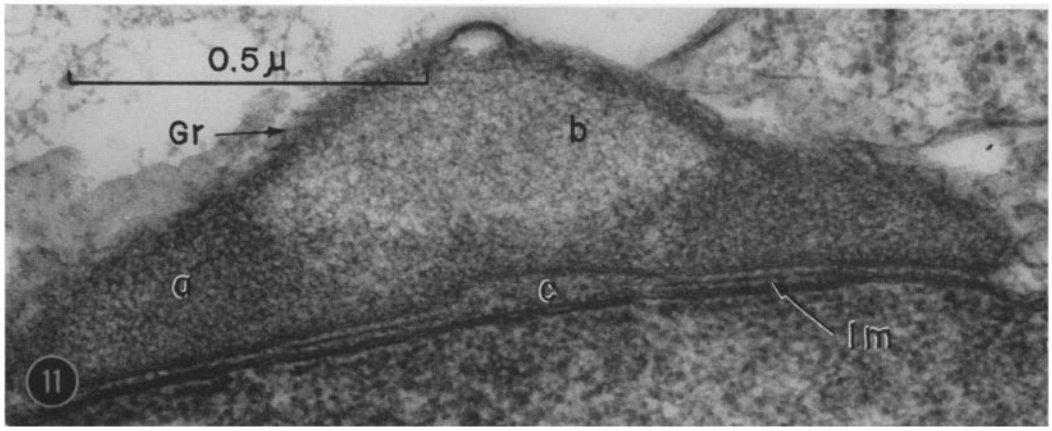
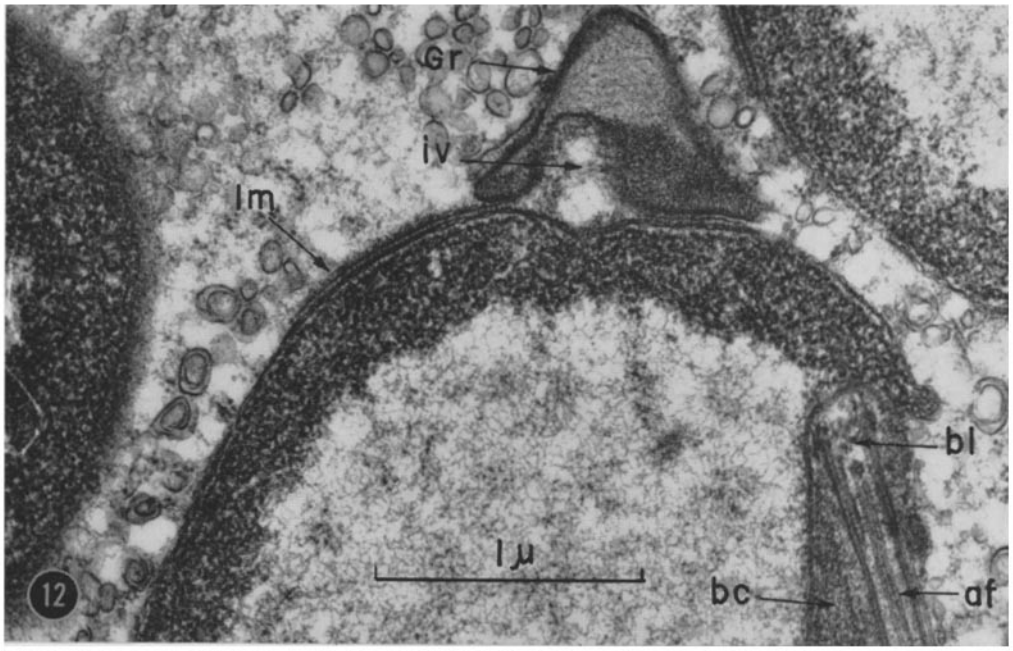

0.5 µ
Gr
b
a
c
Im
11

FIGURE 12

A cell in mid spermiogenesis. The granule (*Gr*) has the shape of a cone. The invagination (*iv*) is extensive. The basal half of the granule has higher density than the apical half. The interstitial membrane (*Im*) runs part way down the left side of the nucleus. At the right side of the nucleus is the blepharoplast (*bl*), axial filament (*af*), and the "basophilic centriole" (*bc*). × 33,000.

FIGURE 13

A slightly later stage than shown in Fig. 12. The invagination of the granule contains amorphous material (*am*). A small segment of the interstitial membrane (*Im*) is visible. It does not enter the invagination. × 135,000.


gr
iv
lm
bl
1 μ
bc
af
12

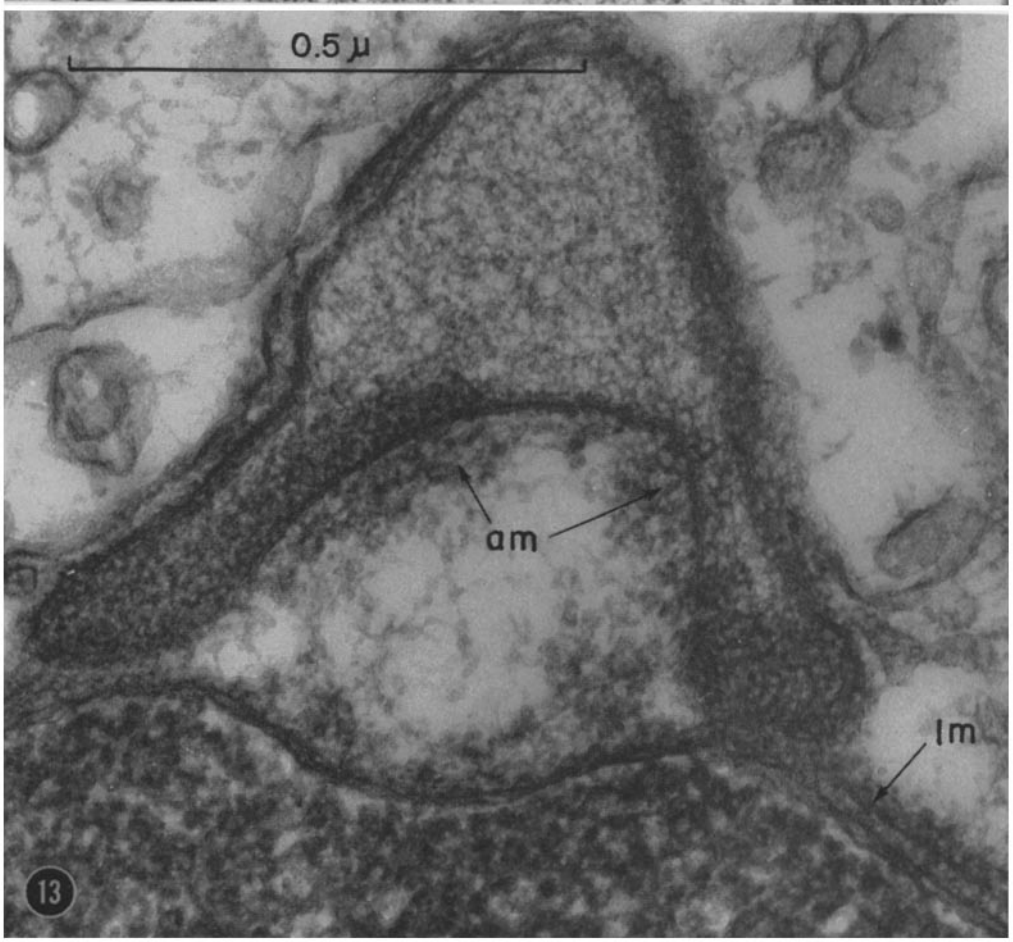
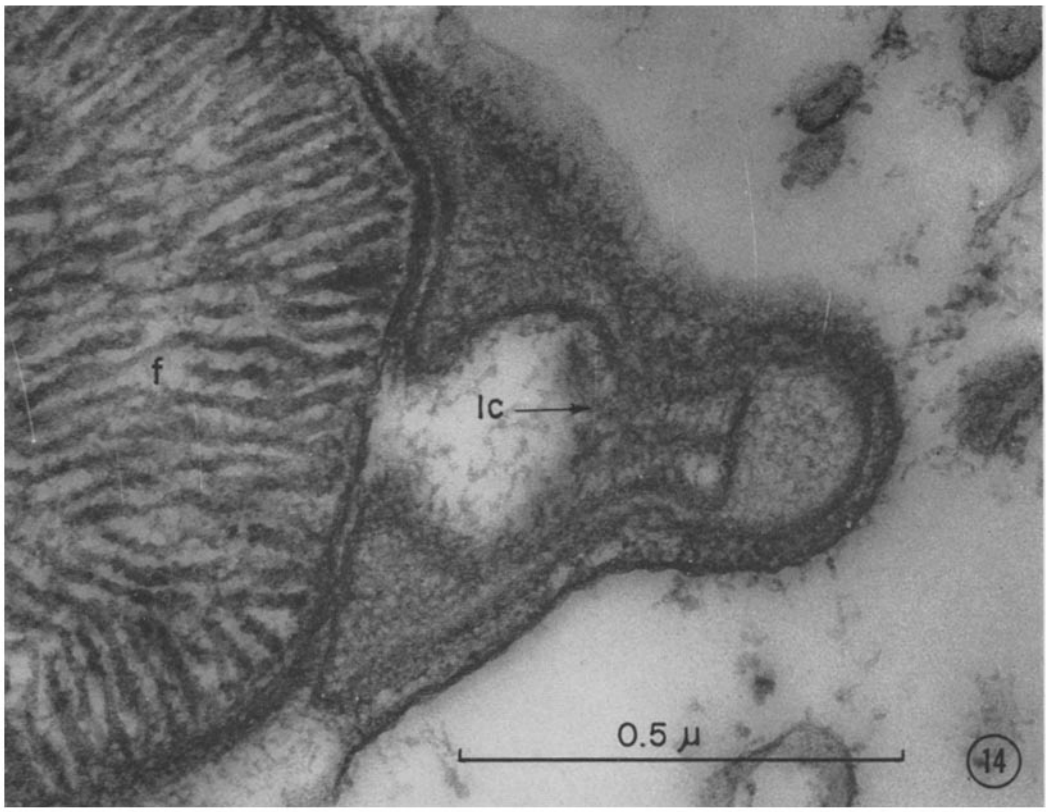

0.5 μ
am
lm
13

FIGURE 14

A later spermatid than in the preceding two micrographs. The rudimentary inner cone (*Ic*) is visible at the anterior end of the invagination. The nucleus contains fine fibers (*f*). × 115,000.

FIGURE 15

A spermatid in which the inner cone (*Ic*) is developed enough to be distinctly pointed and hollow. × 100,000.


f
lc
0.5 μ
14

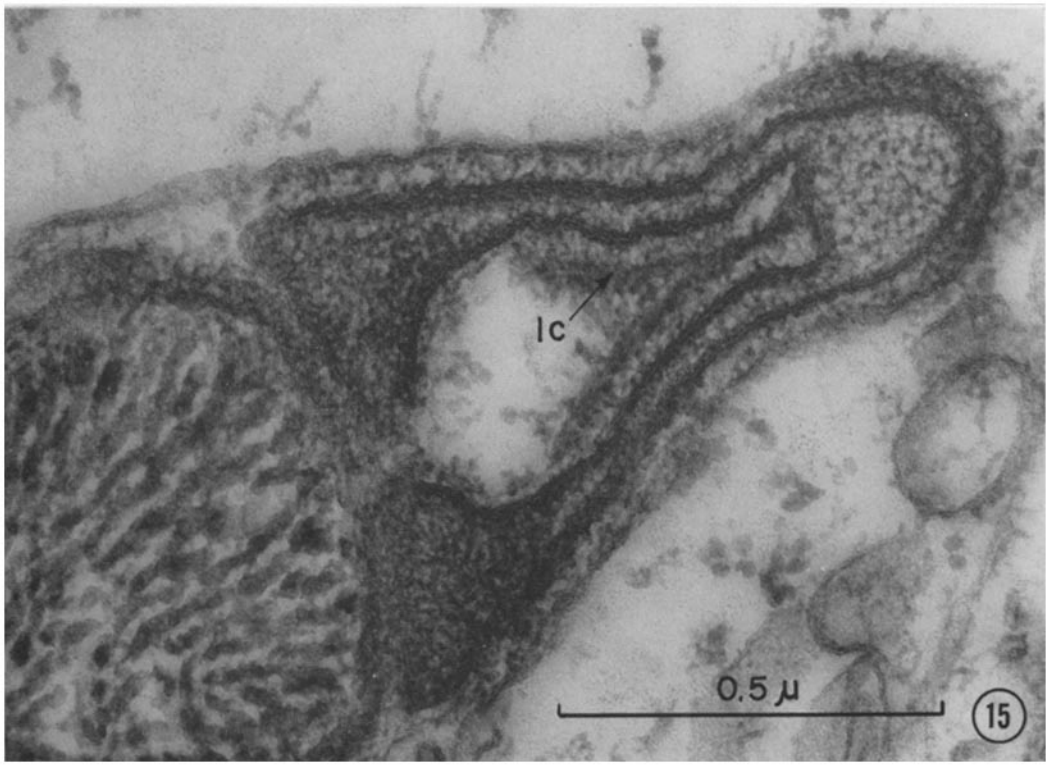
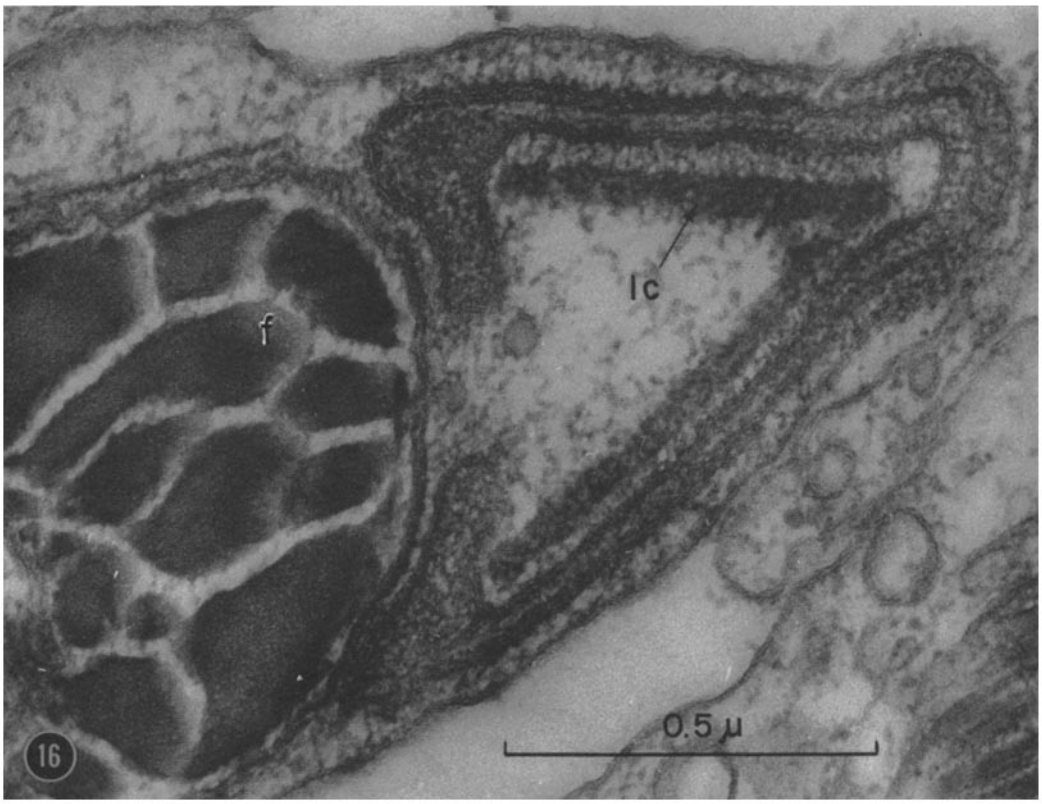

lc
0.5 μ
15

FIGURE 16

A spermatid in which the invagination of the granule is completed. The inner cone (*Ic*) is well developed. The nucleus contains thick fibers (*f*). × 96,000.

FIGURE 17

A late spermatid. At *a* the base of the granule has begun to pinch in and seal off the invagination. There is a solid shell (*s*) of material in the nucleus. × 105,000.


lc
f
0.5 μ
16

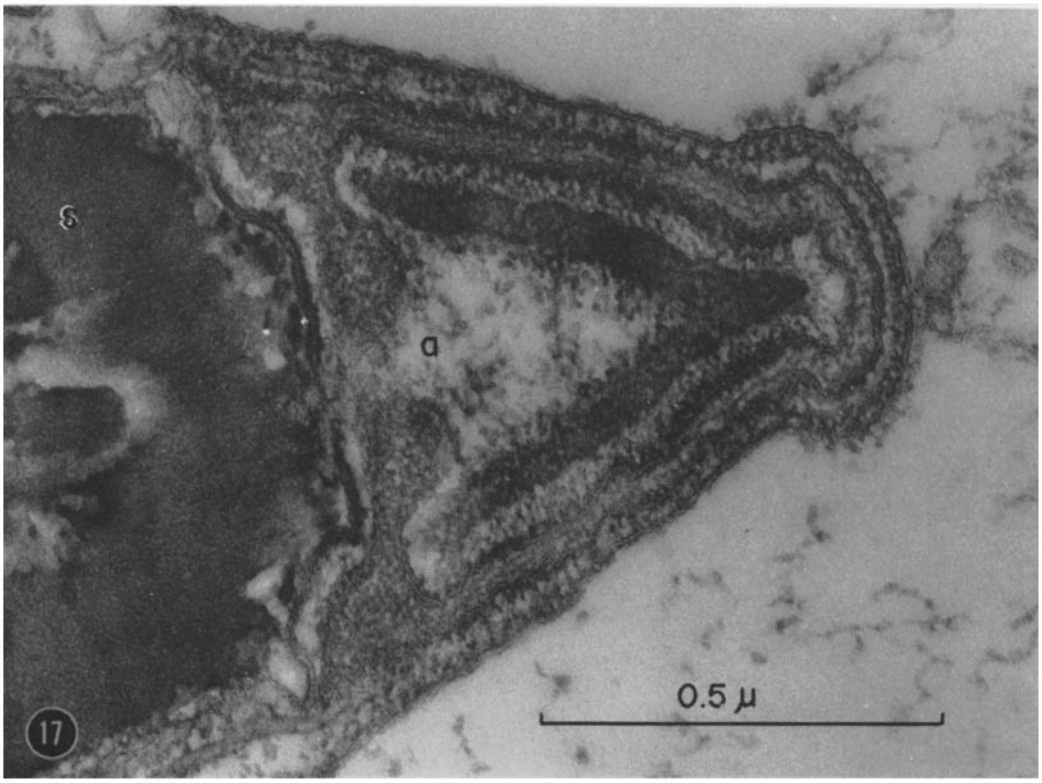
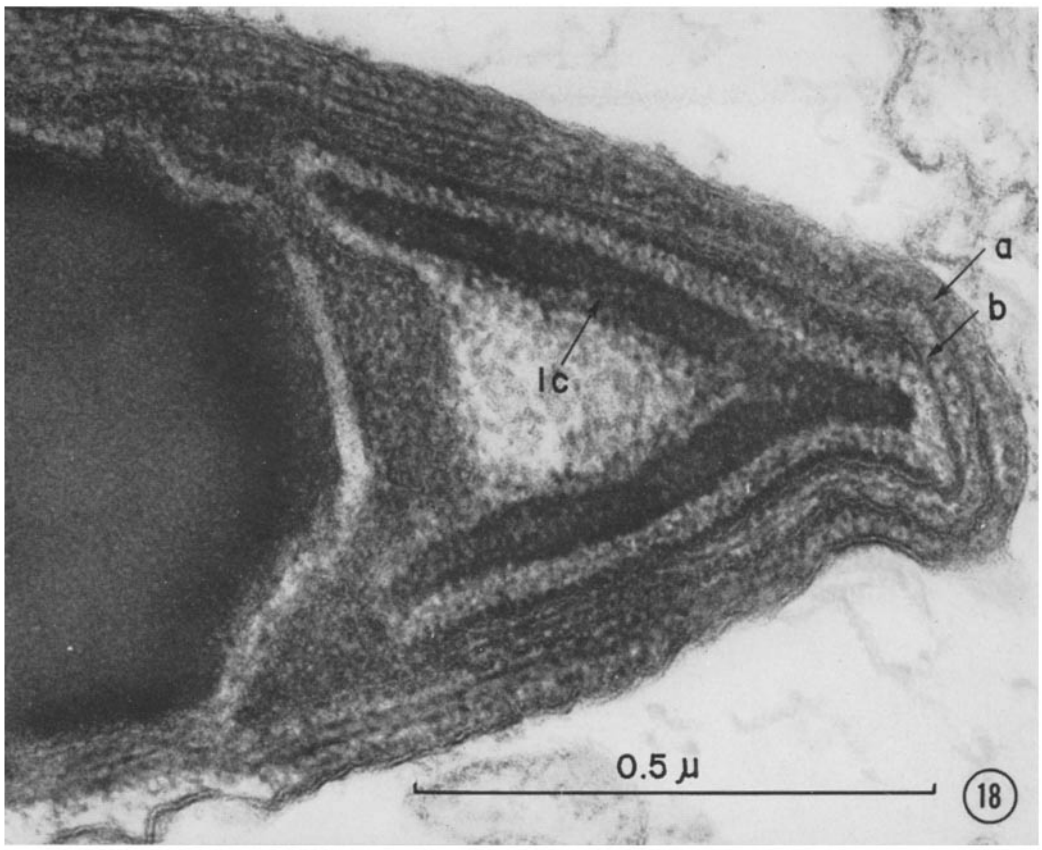

s
a
0.5 μ
17

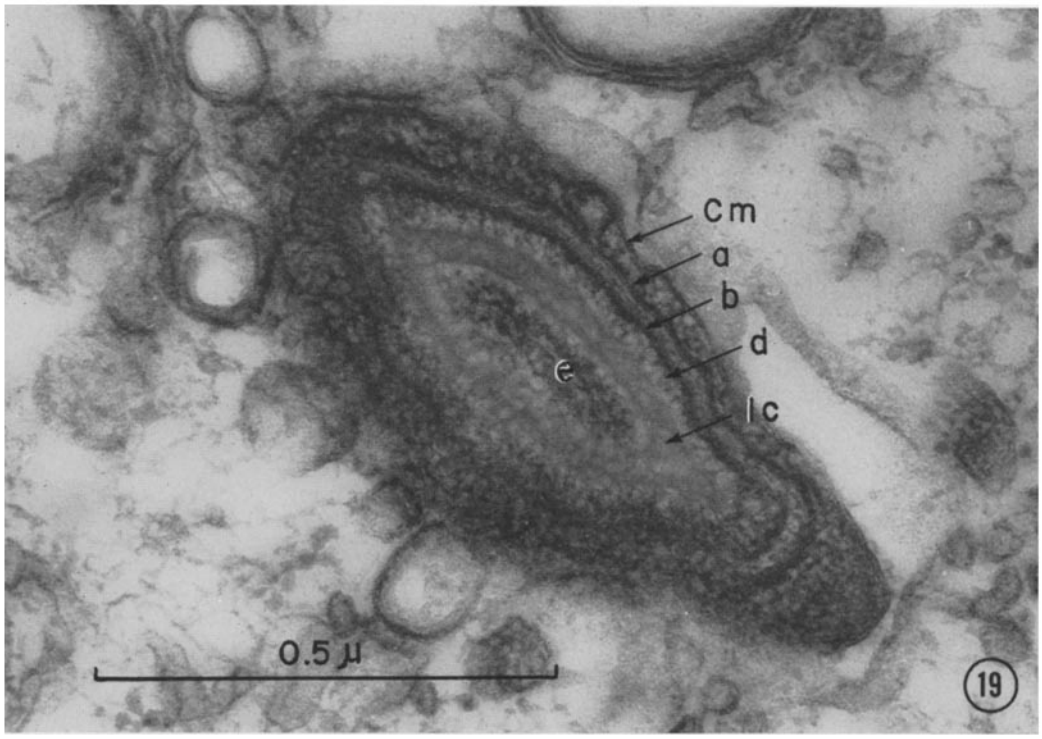
FIGURE 18

The acrosome of a mature spermatozoon. It is bounded by the outer member of the granule membrane (*a*). The inner member (*b*) of the granule membrane lines the space of the invagination, which is now completely sealed at the base. The inner cone (*Ic*) is nearly as long as the invagination. × 135,000.

FIGURE 19

A transverse section through the base of a mature acrosome. The elements visible are: the cell membrane (*Cm*), the outer member of the granule membrane (*a*), the inner member of the granule membrane (*b*), diffuse material (*d*), the inner cone (*Ic*), and outer cone material at the base of the granule (*e*). × 120,000.


a
b
lc
0.5 μ
18


Cm
a
b
d
e
lc
0.5 μ
19