1967

Spermiogenesis in Aedes aegypti (L.)

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Received February 12, 1967

Spermiogenesis (Wilson 1925) describes those transformations in which spermatids differentiate into spermatozoa. The term is synonymous with spermateleosis. Spermiogenesis is a necessary and interesting part of spermatogenesis: male gametes must pass from one parent to another, propel themselves into a suitable environment (the spermathecae), and later penetrate and fertilize female gametes. Such physiological versatility requires cells greatly modified from undifferentiated spermatids. Male germ tissue thus provides an abundant source of cells undergoing extensive and striking differentiation. Cellular organelles in particular may be observed with unusual clarity, and perhaps for this reason spermiogenesis has been exhaustively investigated with both the light and electron microscopes in many animal species. Fawcett (1958) presented an extensive review of the literature emphasizing mammalian spermiogenesis and sperm morphology. Edwards (1960) very briefly summarized work with the electron microscope on insect spermiogenesis while Franzen (1956) dealt with much of the literature on invertebrates other than insects.

Despite the abundance of reports on spermiogenesis in other forms the Diptera as a whole remain relatively ignored. Specifically, sperm differentiation and morphology has been investigated in the brachiceran species *Drosophila melanogaster* (Cooper 1950, Yasuzumi et al. 1958) and *D. virilis* (Yasuzumi et al. 1958, Clayton 1962), and in the nematoceran *Sciara coprophila* (Doyle 1933). The present paper describes spermiogenesis and sperm morphology in a nematoceran insect, *Aedes aegypti* (L.).

**Methods and material**

Observations were made on both living and fixed male germ cells of the Bangkok strain of *Ae. aegypti* obtained by standard rearing methods. Spermiogenesis was primarily studied with the phase contrast microscope on fresh, living cells in saline (Ephrussi and Beadle 1936) or 10–15% solutions of bovine serum albumin (Cohn fraction V: California Biochemical Corp.) in saline. The latter, while more difficult to use, had the advantage of maintaining cells for several hours in apparently intact morphological form while cell integrity lasted no longer than 20 minutes in saline alone. Spermiogenesis was found to occur in great abundance in pupae and young virgin adult males. Testes were extirpated in saline on a microslide. Extraneous tissues were removed with fine forceps and filter paper, fresh mounting medium was added, and the preparation was then coverslipped. Excess medium was again removed with filter paper, resulting in the coverslip gently squashing the testes thus exposing the cells within to observation. The coverslip was then sealed with fingernail polish. Mitochondria and organelles of mitochondrial origin were demonstrated by vital staining with .005% Janus Green B in saline; positive results were judged by the criteria of Showacre (1953). Vital staining with .01 to .005% neutral red chloride in saline was employed in efforts to demonstrate dictyosomal (Golgi) material, and especially the pro-acrosomal granule (Clayton et al. 1958).

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1 This research was sponsored by N.I.H. Grant GM 06021 and by Development Award K-3-GM-21529 to the second author. Scientific Article Number A1179, Contribution Number 3665 of the Maryland Agricultural Experiment Station.

2 Part of a thesis submitted by the first author to the Graduate School of the University of Maryland, College Park, in partial fulfillment of the requirements for the degree of Master of Science, December 1963. Present address: U.S. Naval Medical Research Unit 3 Field Facility, Addis Ababa, Ethiopia. APO New York, N.Y. 09319.
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Smears of testes fixed in methyl alcohol and stained with Giemsa were found useful. Modified Carnoy fixation (Breland 1961) and orcein staining (French et al. 1962) was used in examination of nuclei. For cytochemical procedures, extensive use was made of 10% formalin in saline or modified Carnoy fixed smears and whole mounts of pupal and adult testes, reproductive system, and gut. To these tissues were applied either the Feulgen nuclear reaction (for DNA) and fast green (Pearse 1961), the periodic acid-Schiff reaction of McManus for polysaccharides (Pearse 1961), or the triple stain of Himes and Moriber (1956) for simultaneous staining of DNA, polysaccharides, and protein. The methyl green-pyronin technique of Kay (1953) was employed for localization of deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). Photomicrographs of living cells were obtained at an initial magnification of 1455 diameters with Polaroid Land Film, type 57.

**Observations**

I. **Observations on living cells**

1. **Cytological description of spermatogonia and spermatocytes**

   Cells in the anteriormost portion of the testes of pupae and adults are spermatogonia (Fig. 1). Living mature spermatogonia are round cells, about 12 to 15 μ in diameter, each with a nucleus which fills almost the entire cell. The nucleolus of each cell appears as a granular, usually spherical mass located centrally or slightly acentrically within the nucleus. The mitochondria of spermatogonia, when vitally stained with Janus green, appear typically as small, randomly dispersed, blue-green granules throughout the cytoplasm. Examination of fixed, orcein-stained squashes and living cells disclosed that spermatogonial divisions occurred infrequently and in relatively few numbers of about 5 to 20 cells at a time exclusively in young pupae. In contrast, the meiotic divisions occurred in very great numbers throughout the pupal period.

   In nearly pupal life most spermatogonia enlarge to become primary spermatocytes (Fig. 2). The mature spermatocyte is spherical and is 15 to 20 μ in diameter. The nucleus may be either round or triangular and occupies less of the cell than that of a spermatogonial nucleus. The chromosomes of the pre-meiotic primary spermatocyte are visible in the living cell as very thin, chromomere bearing, precisely paired filaments closely approximating the nuclear membrane. A large granular, spherical nucleolus is present in each nucleus. In anticipation of meiosis and spindle formation, the once granular mitochondria progressively form threadlike filaments and cluster with increasing density around the nucleus. The nucleus and cell as a whole become triangular as meiotic prophase advances. At metaphase I, the spermatocyte may be triangular or oval in shape, and the nucleolus and nuclear membrane disappear (Fig. 5). In anaphase I, the spermatocyte elongates and the chromosomes migrate to each pole. The spindle fibers and centrioles are normally obscured by mitochondria. However, as the preparation ages, mitochondria become displaced thereby rendering the extensive, three dimensional spindle visible. The meiotic chromosomes of living cells do not seem to be significantly different in appearance from those prepared with the orcein method of Breland (1961) and French *et al.* (1962). Meiosis has been described with much detail in *Ae. aegypti* (Akstein 1962, Krafsur 1964, Mescher and Rai 1966) and will not be noted here,
Figs. 1—6. Some cytoplasmic aspects of spermatogenesis in *Aedes aegypti* (L.). Scale equals 10 microns. 1, spermatogonia from the anterior portion of a pupal testis. 2, pre-pachytene primary spermatocytes. 3, pre-pachytene primary spermatocytes. Note the presence of concentric, coalescing membranes surrounding the nuclei (arrow). The granules are mitochondria. Cells are mounted in 12% bovine serum albumin-saline. 4, prepachytene primary spermatocytes. Note the perinuclear clusters of mitochondria and the conspicuous nucleoli. 5, a spermatocyte in metaphase I. Observe the localization of granular and filamentous mitochondria about the spindle (arrow) and the absence of a nuclear membrane. 6, two coalesced prophase II cells. The filamentous mitochondria (arrow) appear to delimit the spindles. 

nucleoli-like bodies and is surrounded by concentric membranes. The groups of cells obviously resulted from two conjoined secondary spermatocytes. 9, two spermatids in "stage 1" of spermiogenesis joined together by a cytoplasmic bridge. Arrow points to the mitochondrial nebenkern. 10, three "stage 1" spermatids. The axial filament is not yet present. Note the apparent presence of two nucleoli in one of the spermatids (arrow). 11, three teardrop-shaped spermatids in "stage 3" of spermiogenesis. 12, a spindle-shaped "stage 4" spermatid in which the nucleus has folded back into the cell body. Note the elongating nebenkern and axial filament. The arrow points to the developing juxtanuclear bodies, between which originates the axial filament. No nucleoli are observable. 13, a "stage 6" spermatid. The arrow points to the knob-like origin of the two nebenkern derivatives. The nucleus has begun to condense. 14, 15, and 16, "Stage 7" spermatids. The arrow in figure 14 shows the prominent juxtanuclear bodies. Note the kinoplasmic droplets on the flagella in figures 15 and 16. 17, 18, and 19, "Stage 8" spermatids. Note the progressive elongation of the nucleus and apparent loss of the juxtanuclear bodies. The arrow in figures 18 points to the neck region where the juxtanuclear bodies have apparently become drawn out.

7, two cells in very late telophase II. The arrow shows the cleavage furrow. Note that the mitochondria are in the form of thick rods. One small nucleolus is present in each nucleus.

8, a group of four cells in very late telophase II. Each nucleus contains from two to four
Two dense nuclei rapidly form at telophase and both the nucleolus and nuclear membrane reappear in each cell. Cytokinesis soon follows. Two secondary spermatocytes are formed as a result of this division.

The secondary spermatocyte is difficult to differentiate clearly from the pre-pachytye primary spermatocyte. It seems that the secondary spermatocyte exists in interkinesis and early prophase for only a short period of time. Mitochondria tend to remain clumped in two masses at right angles to each other, a condition established in cytokinesis of the first meiotic division. In the second spermatocyte division mitochondria at first consist largely to threadlike filaments of varying lengths stretching from pole to pole. These thicken considerably as telophase II approaches. In telophase II a nuclear membrane is quickly formed and the nucleolus appears soon afterwards in each cell. While karyokinesis is complete with the formation of two haploid nuclei, cytokinesis is not. The two products of the second meiotic division remain connected by a rather broad cytoplasmic bridge in which the now greatly thickened, rod-like mitochondria are pinched by the cleavage furrow (Fig. 7). The newly formed nuclei are found at the distal ends of the microtubrial mass. The mitochondria condense and apparently fuse as cytokinesis progresses. With the conclosure of cytokinesis mitochondria have in most cells formed an optically single mass termed the *nebenkern* (Fig. 10).

Vital neutral red staining occurred as a function of time in only a small proportion of spermatogonia and spermatocytes. Such staining characteristically disclosed an orange fluid filled vacuole containing one to three red granules. Vital neutral red staining of spermatids occurred only rarely, and no proacrosomal granule could be demonstrated.

2. Spermiogenesis

Clearly spermiogenesis is a continuous process, but for purely descriptive purposes it is advantageous to arbitrarily divide the process into a series of stages. In this paper, a spermatid is characterized as passing through eight stages in attaining the status of a mature spermatozoon.

The “stage 1” spermatid is a round cell containing a large spherical nucleus and a smaller, phase-dark, broadly pyriform or round nebenkern (Fig. 20A). Within the nucleus lies a nucleolus and one or two nucleoli-like inclusions: the latter appear as the preparation ages beyond 5 to 10 minutes (Figs. 10, 11, 20A). Pyronin Y staining (specific for RNA) shows however, that spermatid nuclei probably contain only one nucleolus. Initiation of spermatid development begins in “stage 2” of spermiogenesis (Fig. 20B). During this period the nebenkern apparently divides into two subequal halves. Concurrently, a fine fiber, the axial filament, appears. This may be observed to lie between the two nebenkern spheres. The point of origin of the axial filament is an extremely small body which lies on the nuclear membrane. “Stage 3” is characterized by initial elongation of the spermatid distal to the nucleus (Figs. 11, 20C). The nebenkern now assumes a
Fig. 20. Semi-diagrammatic illustration of spermiogenesis in Aedes aegypti (L.). A, the “stage 1” spermatid. Illustrated are the nucleus (n), nucleolus (nuc), membranes of the endoplasmic reticulum (mem), neutral red staining vacuole and granule (nr), and mitochondrial nebenkern (neb). B, a “stage 2” spermatid illustrating the axial filament (af). C, a “stage 3” spermatid. D, “stage 4” in spermiogenesis. An apical cytoplasmic filament (ap) and axial filament (af) are shown. E, a “stage 4” spermatid as it usually appears in saline illustrating a small juxtanuclear body (jxb), neutral red staining complex (nr), and two cytoplasmic swellings or droplets (cd). F, “stage 5” in spermiogenesis. Labels show the anterior lobes of the nebenkern filaments (nl), nebenkern filament (nf), cytoplasm (cy), and posterior portion of the descending nebenkern (neb). G, a “stage 5” spermatid in the form it often assumes in saline. Cell presents least possible surface area; inclusion of albumin in saline eliminates this configuration. The nucleus (n), nebenkern filaments (nfs), and nebenkern (neb) are labeled. H, anterior appearance of a “stage 6” spermatid illustrating the developing juxtanuclear body (jxb). I, terminal portion of the flagellum of a “stage 6” spermatid showing the fully extended nebenkern (neb) and axial filament (af). J, the “stage 7” spermatid. The marginal cytoplasm (cy) surrounding the nucleus and the conspicuous juxtanuclear bodies (jxb) are shown. K, a spermatid in “stage 8” of spermiogenesis. Illustrated are the nucleus (n), cytoplasmic droplets (cd) and axial filaments (af). L, the mature spermatozoon showing the nucleus (n), which constitutes the head piece, the slightly forked or bifurcated posterior of the nucleus (ft), and the flagellum (fl).

triangular shape and together with the axial filament begins to move distally from the still round nucleus. The axial filament seems always to slightly exceed the nebenkern in length, penetrating the cell wall and distending it. Fragmentation of the protruding axial filament may become apparent in saline after about 10
minutes exposure (Figs. 20D, 20G). The “stage 4” spermatid may be identified by its spindle shape (Figs. 12, 20D). The nucleus becomes an oblong ovoid structure. The nebenkern continues to elongate leaving behind it two phase-dark ribbons of Janus green-staining mitochondrial material. A “stage 5” spermatid (Fig. 20F) is recognized by its developing flagellum bearing numerous cytoplasmic swellings along its length (kinoplasmic droplets). Two terminal swellings of the nebenkern are found in the distal portion of the flagellum. The mitochondrial ribbons described in “stage 4” condense into two round parallel filaments between which lies the thinner axial filament. “Stage 6” of spermiogenesis is characterized by development of the head piece. The “stage 6” spermatid is identified by its extensive flagellum and a spindle shaped nucleus (Figs. 13, 20H). The nucleolus has by this stage been lost to view. The amount of cytoplasm surrounding the nucleus progressively decreases as the flagellum increases in length. The nucleus condenses in breadth, elongates, and becomes increasingly tapered. A phase-dark juxtanuclear body becomes increasingly evident at the point where the axial filament and nuclear membrane meet (Figs. 13, 20H).

The “stage 7” spermatid is spear-shaped (Figs. 14, 15, 16, 20J). Little cytoplasm remains about the flat, tapered nucleus and the striking juxtanuclear body now appears as a phase-dark, conspicuous collar between the head piece and flagellum. Together they vary 15-20μ in length. The juxtanuclear body reaches maximum development (about 1.4×1.4μ) in “stage 7” and at this point its discretely double morphology becomes evident, appearing as two laterally adjacent bodies separated by only the axial filament. Destruction of cells in preparations made hypotonic by addition of small amounts of distilled water demonstrates that the juxtanuclear bodies are remarkably plastic in response to osmotic shock. While mitochondria, mitochondrial derivatives, and all other cell inclusions immediately rupture, the juxtanuclear bodies swell as much as ten times their original size. Upon addition of saline they rapidly revert to their normal size. A further property of the juxtanuclear bodies is that unlike mitochondria and mitochondrial derivatives, they lightly but irreversibly stain with Janus green. They are thus not of mitochondrial origin. The “stage 8” spermatid presents a striking appearance (Figs. 17, 18, 19, 20K). The flagellum has by now assumed its final length (about 200μ) and few to many small kinoplasmic swellings remain. The mitochondrial filaments may remain parallel or develop a loosely helical relationship throughout the flagellum. The fusiform structure of the “stage 7” nucleus becomes greatly exaggerated throughout “stage 8”; it now appears as a very long, (35–40μ) broad (2–3μ) and flat structure, surrounded by only a narrow margin of cytoplasm. Though broad, the nucleus is dorsoventrally very thin since it is of low optical density. The manner in which “stage 8” nuclei condense to form the head piece of the mature spermatozoon is not clear in living cells. The once prominent juxtanuclear bodies apparently elongate and are then lost to view as the flagellum reduces in diameter and becomes increasingly optically dense. “Stage 8” closes
The mature spermatozoon is thread-like, with a phase-dark, dense, filiform nucleus averaging 38–40 μ. The flagellum is continuous with the head piece, but optically less dense. Unlike differentiating spermatids, mature spermatozoa remain intact in diluted saline or in distilled water for periods exceeding 10 minutes. No intracellular detail can be observed in mature spermatozoa at 1455X with phase contrast microscopy.

Careful examination of living cells with the phase-contrast microscope revealed the presence of phase-dark, roughly concentric, coalescing lines surrounding the nuclei of spermatogonia and spermatocytes. The lines surround the nebenkern and nucleus of spermatids in the first three "stages" of spermiogenesis (Fig. 3). Vertical focusing demonstrates them to have depth. They are thus three dimensional and may be considered as membranes. The membranes increase in quantity and in optical density as cells "age" in saline or albumin saline. The membranes of spermatogonia and spermatocytes are of a more extensive nature than those of spermatids, and further, become covered with mitochondrial granules on their outer surfaces. Structures of similar morphology and behavior have been described in male germ cells of Drosophila and guinea pigs (Fawcett and Ito 1958, Ito 1960).

II. Observations on fixed and stained cells

Two interesting questions were raised in the study of living cells. First, why the apparent lack of dictyosomal (Golgi) material, acroblast, and a typical apical acrosome? Second, what are the nature of the juxtanuclear bodies? Cytochemical methods were used in efforts to resolve these questions. The Feulgen nucleal reaction as a test for deoxyribonucleoprotein (DNA) indicated that only nuclei contained this material. The juxtanuclear bodies were negative. The entire head piece of the mature spermatozoon was positive; no other structure on the nucleus was detected. The periodic acid-Schiff reaction was used to demonstrate cell organelles containing polysaccharides (Pearse 1961). The reaction is considered to be positive for dictyosomes, acroblast, and acrosome of both insect and mammalian male germ cells by numerous workers (Moriber 1956, Bawa 1960, Kaye 1962, Clermont et al. 1955, Cylaton et al. 1958). Male germ cells of Ae. aegypti failed however, to give a positive test. On the other hand, strong PAS reactions were simultaneously obtained in other components of the reproductive and digestive systems. The triple cytochemical procedure of Himes and Moriber (1956) utilizes the azure-A-Schiff reaction for DNA; the PAS reaction for polysaccharides; and naphthol yellow S staining for protein. The results are summarized in Table 1 and agree with those obtained with the Feulgen nucleal and PAS reactions used separately. Thus nuclei of germ cells stained blue with azure-A-Schiff; cytoplasm and flagella stained yellow.

Methyl green-pyronin Y staining according to the procedure of Kay (1953) gave excellent color contrast but a high degree of cellular distortion. Nucleoli and cytoplasm of germ cells stained red with pyronin indicating these contained RNA
while head pieces of mature spermatozoa, nuclei and chromatin stained green indicating DNA. Some detail in the anterior region of “stage 7” spermatids was observed: the weakly RNA positive juxtanuclear bodies were found to be clearly double with the azial filament separating them and originating from a very small granule within a cleft in the posterior of the nucleus.

Modified Carnoy fixation (Breland 1961) with orcein staining (French et al. 1962) was especially suitable for detailed examination of spermatid nuclei due to an almost complete lack of distortion and shrinkage. In many cells, especially in older preparations, cytoplasm became very lightly stained and could be observed with phase contrast optics. “Stage 1–4” nuclei are round with granular chromatin; those of “stage 5” more condensed and “egg-shaped”. Maximum nuclear density and heaviest staining is reached in the now “bullet” shaped “stage 6” spermatid.

Table 1. Results obtained with the cytochemical procedure of Himes and Moriber (1956) applied to tissues of pupal and young adult male *Aedes aegypti* (L.)*

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Naphthol yellow S (protein)</th>
<th>PAS (carbohydrates)</th>
<th>Azure-A-Schiff** (DNA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Midgut and hindgut</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Malphigian tubules</td>
<td>3</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Germ cells</td>
<td>3</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Fat body</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Seminal vesicles</td>
<td>3</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Rectal papillae</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Accessory glands</td>
<td>3</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Vas efferens and deferens</td>
<td>3</td>
<td>1</td>
<td>3</td>
</tr>
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</table>

* 3, indicates strong staining reaction; 2, moderate; 1, weak; and O, no staining reaction.
** Only nuclei stained.

Throughout “stage 7” nuclei become increasingly fusiform, tapered, and less dense; the posterior margins of such nuclei are slightly convex. Nuclei of “stage 8” spermatids appear as very thin, broad structures, light in staining. The manner in which the broad and flat “stage 8” nucleus transforms into the filiform head piece is difficult to observe: it would seem at first to involve lateral condensation. However, critical examination reveals the presence of a posterior median cleft in nuclei extending longitudinally beyond 1 μ of both these and those of mature spermatozoa; further, the lateral margins appear optically denser throughout much of the nuclear length although it is conceivable that the latter may be the result of birefringence. It is therefore possible that final nuclear differentiation may take place by coiling of the lateral margins inward to the longitudinal axis.

Discussion

Mitochondrial behavior in the spermatogenesis of *Ae. aegypti* seems much like that described in *Gerris* (Moriber 1956) and *Drosophila* (Cooper 1950, Clayton 1962, Yasuzumi *et al.* 1958) while unlike that in the more closely related *Sciara*. In the
latter, mitochondria form progressively larger spheres in the course of meiosis, and these persist through spermiogenesis and in the mature gamete (Doyle 1933). Thus, spermatozoa of Sciara conform to the "primitive" type of metazoan sperm found in many marine invertebrates (Franzen 1956).

The ultimate form the mitochondrial filaments take in the mature spermatozoon of Ae. aegypti may only be speculated on in studies with the light microscope. Phase contrast observations on differentiating spermatids indicate that the two filaments remain separate and parallel throughout the length of the flagellum with only occasional gyres. This is similar to D. melanogaster (Yasuzumi et al. 1958). In D. virilis and Gelastorrhinus, however, the nebenkern forms helical sheaths of mitochondrial material about the axial filament of mature spermatozoa (Yasuzumi et al. 1958). In vertebrate spermatozoa the nebenkern forms a sheath about the axial filament in the "middle piece" of the flagellum (Fawcett 1958).

Of particular interest was the failure to observe any indication of an acrosome or its formation in germ cells of Ae. aegypti. The acrosome of most flagellate spermatozoa is derived from a Golgi-like structure, the acroblast, which in turn arises from fusion of Golgi elements (dictyosomes) in the spermatocyte or spermatid (Bowen 1924, Doyle, 1933, Cooper 1950, Franzen 1965, Fawcett 1957, 1958, Moriber 1956, Bawa 1960, Kay 1962). These structures have been reported to be quite visible in living cells under phase contrast (Franzen 1956, Moriber 1956, Fawcett and Ito 1958, Clayton et al. 1958, Bawa 1960, Clayton 1962), and their apparent absence in Aedes supports the view that an acrosome is not elaborated in this species. Cytochemical tests specific for DNA, RNA, polysaccharides and protein were attempted in efforts to better characterize substructures observed in living cells. Staining for DNA (Feulgen, azure-A-Schiff, methyl green) indicated that the head piece of late spermatids and spermatozoa, exclusive of the juxtanuclear bodies, was composed of deoxyribonucleoprotein. The possibility remains that the juxtanuclear bodies might be the acrosome of Ae. aegypti: indeed, a postnuclear acrosome was described in the thysanuran insects Lepisma and Thermobia with phase contrast and electron microscopy (Bawa 1960, 1964). This was shown not to be so in Ae. aegypti. In male germ cells, the PAS reaction is considered specific only for Golgi material and acrosome (Moriber 1956, Clayton et al. 1958, Kaye 1962, Bawa 1960); the absence of PAS staining in testicular cells of Aedes despite strong, simultaneous staining in other tissues thus argues against the presence of an acrosome or its precursors in this insect.

Of what significance the absence of an acrosome in Ae. aegypti may have remains to be seen, since it has been shown that this organelle plays an important role in egg fertilization in various echinoderms, molluscs, and coelenterates (Colwin and Colwin 1964). Among the insects, however, acrosomeless sperm are not unique; they have been reported to occur in a beetle, Ptinus (Jacob 1959) and in certain scale insects (Hughes-Schrader 1946, Robison 1966).

The nature and fate of the postnuclear bodies is unclear in Aedes. On the
basis of their origin and lack of PAS staining, it is suggested that they are not a postnuclear arcosome, nor derived from mitochondria, but are analogous to the "juxtanuclear bodies" described by Sotelo and Trujillo-Cenoz (1958) in the grasshopper Laplatacris dispar. Sotelo et al. suggest the juxtanuclear bodies are probably formed under centriolar activity but could find no function for them. In Aedes the juxtanuclear bodies can be seen to be drawn out in length before they are lost to view and are presumably incorporated into the flagellum of the spermatozoon.

The present study described coalescing, lamellar membranes in sex cells of Aedes. These structures were observed with phase microscopy in guinea pig and Drosophila germ cells by Fawcett and Ito (1958) and Ito (1960). Studies with the electron microscope by these authors confirmed their hypothesis that the concentric lamellar systems were pairs of membranes identical with those of the cisternae of the endoplasmic reticulum.

Dissimilarities exist in regard to the presence of an acrosome and juxtanuclear body, and in nebenkern formation among sperm of Drosophila, Aedes, and the closely related Sciara. Other differences may well exist. It may therefore be of phylogenetic significance to compare sperm morphology among the Culicidae and other Diptera with the electron microscope. Indeed, a study with the electron microscope may well resolve the questions of final maturation of the head piece, the nature of the juxtanuclear bodies, and the apparent absence of an acrosome and its precursors.

**Summary**

1. Spermiogenesis was studied in living and fixed cells from pupal and young adult testes of Aedes aegypti (L.).
2. Fusion of thickened, rod-like mitochondria in cytokinesis of the second meiotic division results in nebenkern formation. Very early in spermiogenesis an axial filament makes its appearance, extending from the nuclear membrane to bisect the nebenkern. In the course of differentiation the axial filament and nebenkern extend distally from the nucleus to result in flagellum formation.
3. Condensation and elongation describe nuclear differentiation. Midway in spermiogenesis two juxtanuclear bodies of uncertain origin become strikingly apparent in the head piece but are lost to view in final maturation.
4. No dictyosomes, acroblast or acrosome were observed. Further, germ cells were periodic acid-Schiff negative. Thus no acrosome develops in spermiogenesis.
5. The fully differentiated spermatozoon is a thread-like cell consisting of (a) a needle-like nucleus and (b) a flagellum of two mitochondrial threads and a central axial filament.

**Acknowledgments**

We thank Dr. Charles L. Graham for helpful suggestions concerning the manuscript and Mrs Helen J. Krafsur for typing and editing.
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Cytologia 32, 1967

