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THE ACROSOME FORMATION OF THE ORIENTAL FRUIT FLY, *DACUS DORSALIS* HENDEL[1]

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ABSTRACT

W. Y. Lee, C. Y. Sun and W. S. Tsai (1975). *The acrosome formation of the oriental fruit fly* Dacus dorsalis *Hendel.* Bull. Inst. Zool., Academic Sinica **14**(1): 27-34. The acrosome formation of the oriental fruit fly *Dacus dorsalis* Hendel during spermiogenesis has been investigated by a transmission electron microscope. The proacrosomal granule is developed from the fusion of the vesicles of the Golgi complex in the late spermatocyte or the early spermatid. The acrosome is transformed from the proacrosomal granule by elongation. Most acrosomes in the oriental fruit fly are tapered structures, straight or slightly curved. A few with a ring structure on the tip of the spermatozoon can be found in the micro- graphs.

The spermatozoal structure and spermiogenesis of several insect species[5,6,8,11,23,26] have been described in many remarkable studies based on the observations with light microscopes. Since the advent of the electron microscope, many cell biologists have become interested in understanding the ultrastructure of insect spermatozoa and spermatogenesis[1,2,11,12,14~20,25~28]. Some of them have confined their attention to the formation and development of the acrosome during spermiogenesis[12~15,17,20]. The process of the differentiation to form the acrosome differs for the various insect species. However, the acrosome of all insect spermatozoa develops during spermatogenesis from the proacrosomal granule which can now be examined by an electron microscope. The proacrosomal granule is formed in every species of insect from the fusion of vesicles of the Golgi body.

In the present study, an electron microscope is used to observe the formation and development of the acrosome in the oriental fruit fly, *Dacus dorsalis* Hendel. Our present results will be helpful for a later study of the effect of radiation on the spermiogenesis in the same species of fly.

MATERIALS AND METHODS

Specimens of the oriental fruit fly *Dacus dorsalis* were raised in the laboratory. The testes were taken from the newly emerged flies and fixed in 6% glutaraldehyde in 0.1M cacodyate buffer, pH 7.2-7.4 for 3 hours in the cold. They were washed in 8% sucrose in 0.1M cacodylate, and then postfixed with a mixture of 2% osmium tetroxide in 0.1M cacodylate for 2 hours. Following the postfixation, tissues

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were dehydrated rapidly in a graded series of ethanol, beginning from 50% ethanol. They were then put through propylene oxide and embedded in Epon 812 or in the Spurr low viscosity medium. All these mixtures were polymerized at 60°C overnight.

Sections were studied with a Hitachi type 11-A electron microscope, and micrographs were taken at direct magnifications of 5000× to 10000×. The acceleration voltage of the electron beam was usually 50 KV or 75 KV.

OBSERVATIONS

In the oriental fruit fly, the Golgi material appears in the primary or the late spermatocyte. It consists of several parallel membranes and is associated with highly characteristic vacuoles that appear to be empty, and vesicles that usually contain some electron-dense materials. Usually the membranes of the Golgi material are almost straight or gently curved. In Fig. 1 the membranes of the Golgi material appear to be already fused together.

After the second meiotic division, the membranes of the Golgi material become cup-shaped and form a Golgi body (G) or Golgi complex (Fig. 2). On the outside surface, and filling the inside, there are both vacuoles (va) and vesicles (ve). Some of the vacuoles are rather bigger than others. At the same time, there is another element, a spherical granule appearing near the nucleus, between it and the Golgi complex. This spherical granule was called by Payne[17] as proacrosomal granule (pg), from which the acrosome is derived. As shown in Fig. 6, one may find a cup-shaped Golgi complex lying at the base of the nucleus and near the nebenkern (mit), which is formed through the coalescence of mitochondria. This structure usually appears in the early or the later stage of the spermatid. However, according to our observation, it might be a migration of the Golgi complex from the anterior end of the nucleus near the proacrosomal granule.

In the later stage of the spermatid, the spherical proacrosomal granule is surrounded by a membrane (mb), as shown in Fig. 3. The Golgi complex disappears at this time.

Slightly later, as shown in Fig. 4, the spherical proacrosomal granule changes its shape to become an elongated horn. This structure we term the acrosome, instead of the proacrosomal granule. However, a spherical electron-dense structure appears on the top of the acrosome. We suspect that it is the residue of the spherical proacrosomal granule.

The acrosome is more developed in the later stage of the spermatid. It is more elongated and becomes a tapered structure, and also appears more condensed and osmiophilic, as shown in Fig. 5.

Acrosomes in the immature spermatozoa are shown in Figs. 7, 8 and 9. The nucleus of the spermatozoon undergoes an extensive elongation process, and the acrosome also undergoes elongation during spermiogenesis. The acrosomes

Explanation of Illustrations

Fig. 1. Spermatocyte with the Golgi Complex, Golgi membrane (G), the vacuoles (Va) and the vesicles (Ve). 17500×, 50 kV.

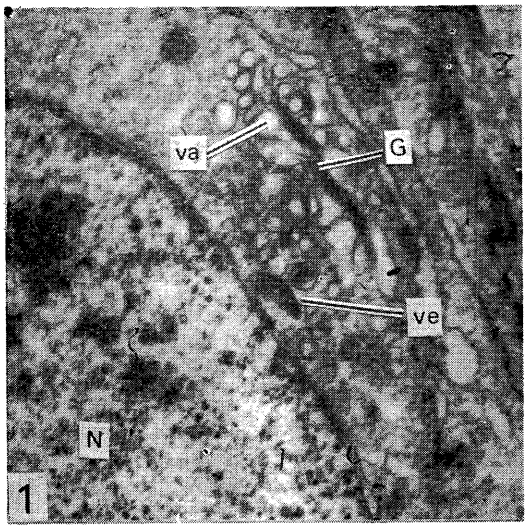
Fig. 2. Spermatid with cup-shaped Golgi complex (G). The vacuole (Va) appears much larger and the vesicles fuse to form a large spherical proacrosomal granule (Pg) near the nucleus (N). 17500×, 50 kV.

Fig. 3. The late spermatid, showing the proacrosomal granule (Pg) connected to the anterior edge of the nucleus (N). The Golgi complex disappeared, Membrane (Mb). 16000×, 75 kV.

Fig. 4. The late spermatid, the proacrosomal granule elongated to form a horn-shaped acrosome (A). 15000×, 50 kV.

Fig. 5. The late spermatid, the acrosome condense. 25000×, 50 kV.

Fig. 6. The late spermatid, the Golgi complex (G) appears at the posterior of the nucleus (N) near the mitochrondrial derivatives (MIT). 17500×, 75 kV.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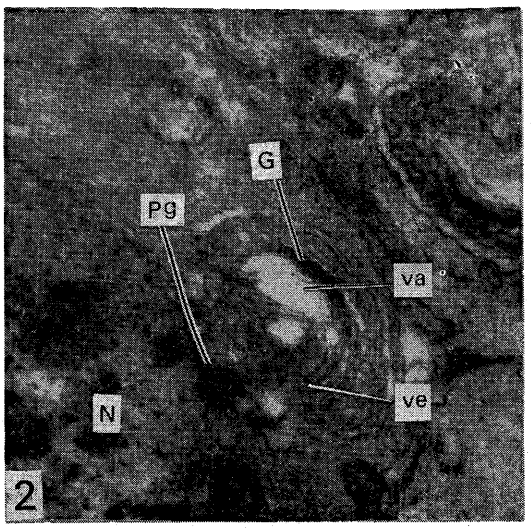
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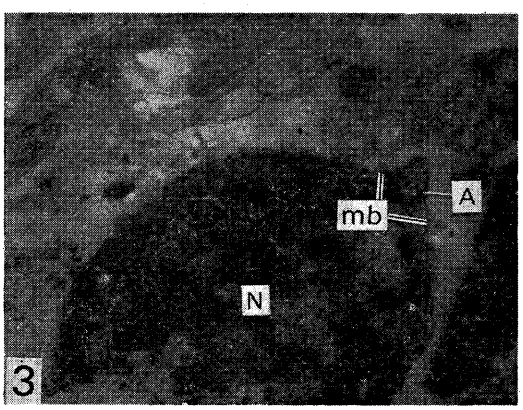
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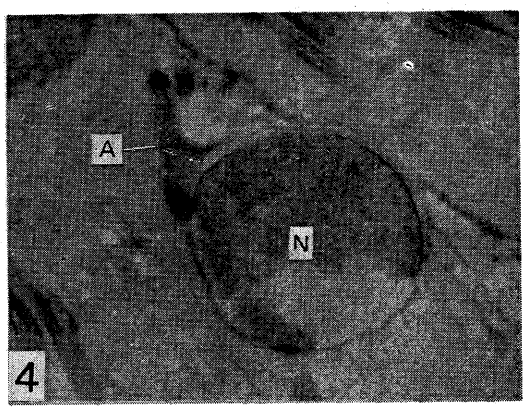
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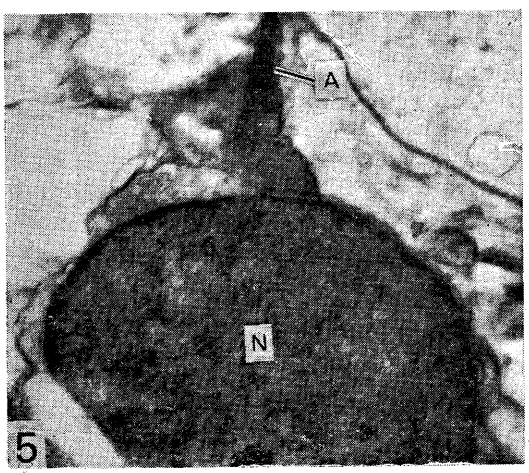
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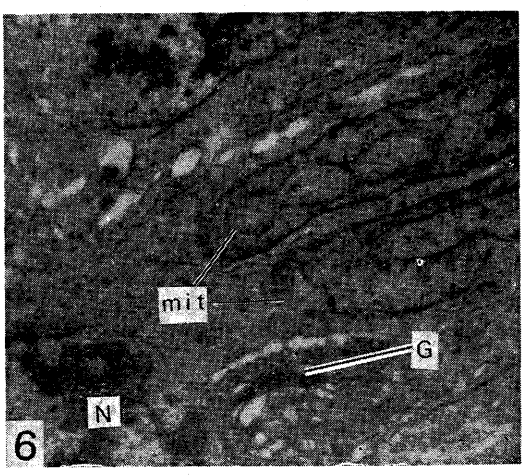
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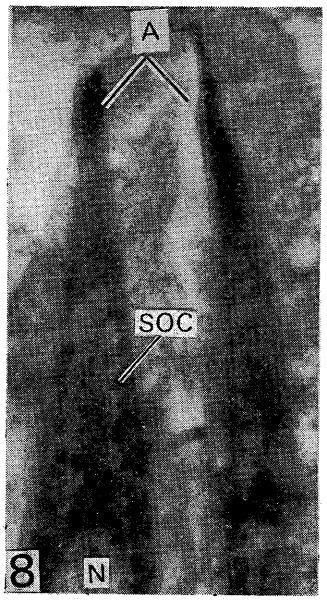

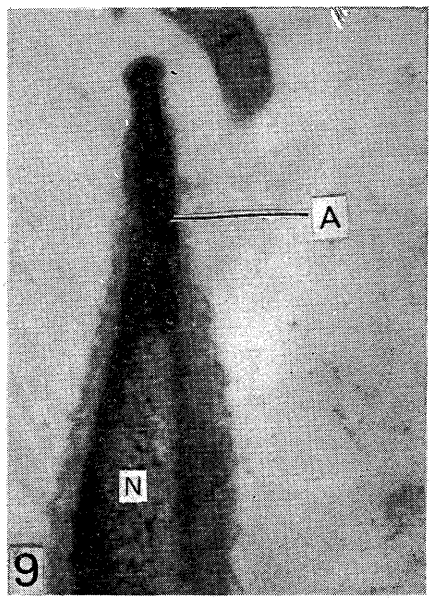

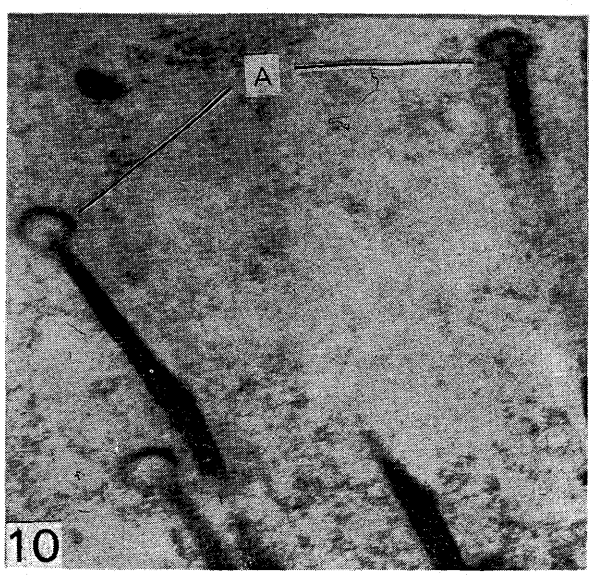

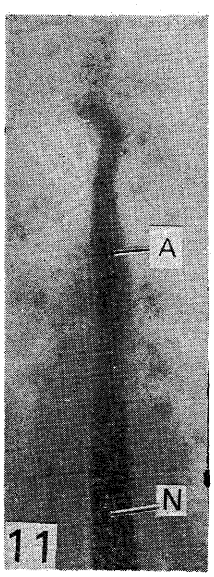

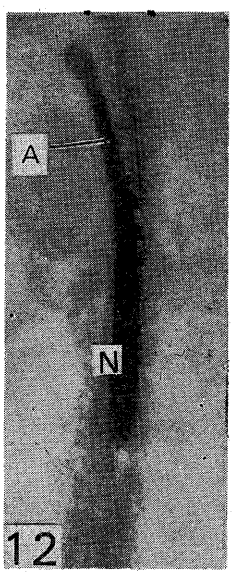

Fig. 7–8. Immature spermatozoa, the acrosome (A) is shown as a tapered structure protruding into on the nucleus (N). Socket (Scc). 27500×, 15000×, 50 kV.

Fig. 9. Immature spermatozoon, the acrosome (A) is shown as a tapered structure. A knob-shaped structure surrounded by membrane occurs on the tip of the acrosome. 25000×, 50 kV.

Fig. 10. The mature spermatozoa, a ring structure on the top of the spermatozoa. 40000×, 50 kV.

Fig. 11. The mature spermatozoa, a hook-like structure of the acrosome (A) protrudes into the nucleus (N). 35000×, 50 kV.

Fig. 12. The mature spermatozoa, the acrosome with a knob-like structure on its tip. 45000×, 50 kV.

shown in Figs. 7 and 9 appear as tapered structures at the anterior end of the nucleus. They are straight or slightly curved in shape, and the acrosome protrudes into the nucleus. Fig. 8 shows that the tip of the acrosome has the shape of a knob surrounded by the membrane, and acrosomal socket is located between the acrosome and the nucleus.

At the end of spermiogenesis, three types of acrosomes are found in the micrographs. The first type is shown in Fig. 10, in which the acrosome forms a ring structure at the proximal end of the sperm. Fig. 11 shows an acrosome shaped like a hook, and in Fig. 12 the third type of acrosome is rod-like shape with a knob at the end, and surrounded by a membrane.

DISCUSSION

The acrosome is essentially a membrane-bound granule packed with proteinous materials. This acrosome formation process takes place in in all spermatozoa of the many kinds of insects which have been observed with electron microscopes, except in two caddis fly species, *Polcentropus* and *Hydropsycha*, which do not have the acrosome[20]. The acrosome is believed to be consist of a substance for penetrating the egg, thereby facilitating the process of fertilization. Phillips[21] stated that the acrosomes are a specialized type of secretion granules of lysosome. They contain hydrolytic enzymes when they are mature.

The formation of acrosome generally occurs throughout spermiogenesis, beginning with the appearance of the spherical proacrosomal granule near the cup-shaped Golgi complex, between the Golgi and the nucleus. In most species of animals been described, the disposition of the proacrosomal granules is in the concave side of the Golgi complex, for exsmple, in the house cricket *Acheta* studied by Keys[16], the fungus gnat *Sciara* and the stink bug *Euchistus* studied by Phillips[20], and *Amphibian anoure* studied by Suadis[24]. In the case of the spur throated grasshopper *Concephalus*, the proacrosome granule is located on the convex side of the Golgi complex, on the side of the Golgi facing away from the nucleus[20], In the present study, the proacrosomal granule of the oriental fruit fly appears on the concave side of the Golgi complex, as in most insects.

In the present study, there are found vacuoles and vesicles associated with the Golgi membrane in the primary or the later stage of spermatocyte (Fig. 1). In the primary spermatid, a spherical proacrosomal granule appears near the nucleus, between it and the Golgi complex (Fig. 2). Burges and Fawcett[7] studied the spermatid differentiation in the toad *Bufo arenarum* Hensel and reported that the granules which arise within the vesicles of the Golgi complex fuse to form a single acrosomal granule. Payne[17] studied the formation of the pro-acrosomal granule in the toad bug *Gelastocoris occlatus* in detail with the light microscope and the electron microscope. He indicated that the proacrosomal granule originates in the Golgi vesicles and is formed by the fusion of a large oval-shaped acrosomal mass in the early spermatid. Hoage and Kessel[15] investigated the spermiogenesis of the drone honey bee *Apis mellifera* L. and found that numerous vesicles containing granular material fused together to form a larger vesicle with a granule. When the spermatid is mature, this granule migrates to the anterior region of the nucleus.

The formation of the proacrosomal granule is also evident in the oriental fruit fly. The small granules may be found within each vesicle of the Golgi complex, appearing as the electron dense material in the micrographs. (Fig. 1). Many vesicles are fused to each other, and several granules may then be found close together, so as to have the appearence of cluster of granules or one large granule.

There are various ways of transformation from the proacrosomal granule to the acrosome in insect species. In the toad bug *Gelostocoris oculatus*[17], the proacrosomal granule at the anterior end of the nucleus becomes lodged in

an indentation into the nucleus and subsequently elongates longitudinally to form a tapering cylinder, and the Golgi complex migrates to the tail position of the late spermatid. The process of acrosome formation in the fungus gnat *Sciara coprophila*[18], and also in the drone honey bee[15], is similar.

In the house cricket *Acheta domestica*[16], the proacrosomal granule grows within the acroblast (the Golgi complex) and migrates away from the acroblast to the nucleus, and then is transformed to become an acrosome with a bipartite structure consisting of concentric hollow cones. The acrosomal formation of the oriental fruit fly, shown in Fig. 13, is different from that of the toad bug and the fungus gnat, and also different from that of the house cricket. Its proacrosomal granule migrates to the anterior portion of the nucleus, and gradually develops the tapered structure of the acrosome. This process of development of the acrosome resembles the cases of house fly[14] and rabbit[22].

The shape, size and internal substructure of fully formed acrosomes show wide variation in different species. The toad bug[17] and the water strider[27] have a large acrosome, and the fungus gnat[19] and also the house fly[14] have a small one. In the water strider[27] and the starfish *Mytilus*, there is a cavity between the acrosome and the nucleus[9]. The acrosome of the oriental fruit fly connects to the nucleus by insertion. This is also observed in the fungus gnat[19] and the house fly[14]. Some insects show a definite substructure in the acrosome. For example, the acrosome of the caddis fly[20] consists of a thin rim of material around the anterior edge of nucleus. In house cricket[16], cave cricket[25], American cockroach[11] and some of Auchenorhyncha insects[12], the acrosome has an obvious delineation between an inner and outer cone. But the acrosome of the oriental fruit fly has a different substructure In the fully mature spermatozoa, it is a solid structure in the shape of a tapered as cylindrical rod, resembling the acrosome of the house fly[14] or of *Drosophila melanogaster*[26]. According to the phylogenetic aspects of the morphology of spermatozoa and spermiogenesis[13], the morphology of the fully formed acrosome of the

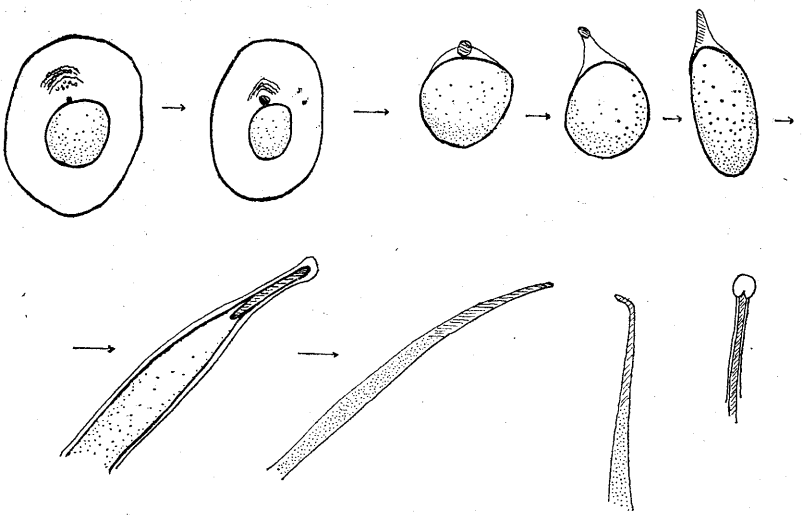

Fig. 13. The development of the acrosome during spermiogenesis in the oriental fruit fly (*Dacus dorsalis* Hendel)

oriental fruit fly should belong to the primary type, being similar to *Spirorbis pagenstecheri* of polycheater Annelida. A review of the literature shows that a ring structure on the tip of the acrosome is very rare, but we do not attempt to discuss this question here.

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東方果實蠅精子穿孔器（Acrosome）的形成研究

李文蓉　孫敬宇　蔡文珊

本研究爲利用超薄切片法電子顯微鏡觀察東方果實蠅的精子穿孔器 (Acrosome) 在精子 (Spermatozoon) 由精子細胞 (Spermatid) 轉變過程中的發生與成熟經過，在精母細胞後期高爾基體 (Golgi body) 附近有許多空泡 (Vacuoles) 和囊泡 (Vesicles)，囊泡結合而成一個原穿孔器顆粒體 (Proacrosomal granule)，此粒體與精核 (Nucleus) 連接，拉長而變成爲穿孔器。東方果實蠅精子的穿孔器有尖塔狀，長棒狀，垂直或頂端微彎曲，更有一些穿孔器在精蟲頂端具環狀構造。