Fine Structure of the Spermathecal Duct of the Cricket, *Gryllus bimaculatus*

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Abstract

The spermathecal duct of *Gryllus bimaculatus* is lined with a cuticular intima. Its wall consists of three cellular types: glandular cells, ductule carrying cells and epithelial cells. The glandular cells are absent from the proximal region near the junction of the duct with the genital chamber and from the distal region near the junction with the spermatheca. They are restricted to the median region where the spermathecal duct is highly convoluted. Each glandular cell has a cavity formed by an invagination of the apical cytoplasmic membrane. The cavity of the glandular cells and the lumen of the spermathecal duct are connected by a cuticular efferent ductule which is surrounded by a ductule carrying cell. The epithelial cells contain bundles of longitudinally arranged microtubules and large deposits of glycogen. In the epithelial cells, mitochondria are distributed mostly in the apical cytoplasmic zone. The concentration of mitochondria in the apical zone suggests their involvement in ion transport required for the regulation of the microenvironment within the lumen of the spermathecal duct. The muscle fibers surrounding the spermathecal duct are innervated by axons which contain electron-dense vesicles. The presence of glandular cells in the spermathecal duct is characteristic of *G. bimaculatus.*

Introduction

In many insects including Orthoptera, the sperm transfer from males to females is performed by the utilization of spermatophores [1, 2], which are formed by sperms and secretions of the male accessory reproductive glands [3]. In the cricket, after deposition of the spermatophore within the female genital chamber, the mixture of sperms and secretions of the male accessory glands is forced out from the spermatophore, and the sperms migrate through the spermathecal duct to the spermatheca which is a sperm reservoir [1]. The mechanism of the sperm migration to the spermatheca is still uncertain.

In *Rhodnius prolixus* [4], the secretions of the male accessory glands stimulate rhythmic contractions in the genital ducts of the female. The possibility that the secretions of the male cricket accessory glands regulate the mechanical activity of the muscles of the female spermathecal duct was reported recently [5]. The ultrastructure of the spermatheca, spermathecal duct and/or its associated glands of several orders of insects, including Blattariae [6, 7], Orthoptera [8], Hemiptera [9], Diptera [10-12], Coleop-

tera [13-15] and Hymenoptera [16], has been described. Nevertheless, there is still little information available about the fine structure of the cricket spermatheca and spermathecal duct. The present ultrastructural investigation on the spermathecal duct was therefore carried out to provide background information on the reproductive mechanism of the cricket, *Gryllus bimaculatus*.

Materials and Methods

Mated and newly emerged virgin females of the cricket, *Gryllus bimaculatus*, were obtained from the colony maintained at the Laboratory of Animal Physiology, Okayama University.

For light microscopy, the spermathecal ducts with spermathecae were fixed in Bouin's solution, dehydrated through an ethanol series and embedded in paraffin. Serial sections were made and stained by the Azan staining method.

For transmission electron microscopy, the spermathecal ducts were fixed with ice-cold 2.5% solution of glutaraldehyde in 0.1M phosphate buffer at pH 7.3 for 2-4 hours, washed in the same buffer for 2 hours, and post-fixed with ice-cold 1% osmium tetroxide in the same buffer for 2 hours. These specimens were dehydrated through an ethanol series and embedded in epoxy resin. The sections were doubly stained with uranyl acetate and lead citrate solution and examined with a Hitachi H-500 electron microscope.

For scanning electron microscopy, the specimens were dried in a CO_2 critical-point dryer after fixation and dehydration as for transmission electron microscopy, coated with gold-palladium, and examined with a Hitachi S-570.

Results

The spermatheca is an oval pouch about 1 mm long and 0.8 mm wide. It is connected to the genital chamber by the highly convoluted spermathecal duct (Fig. 1). The elongated spermathecal duct is about 20 mm in length. The spermatheca and spermathecal duct is surrounded by circularly arranged muscle fibers. Thin branches of nerves and tracheoles are attached to the muscle layer.

The spermathecal duct can be subdivided into three regions: the proximal region near the junction of the duct with the genital chamber, the median region, and the distal region near the junction with the spermatheca. The proximal region is composed of a single layer of slender epithelial cells with basally located elliptic nuclei. The duct in this region is about 80 to 90 μm in diameter with an inner lumen of about 6 to 8 μm in diameter. The cuticular intima is about 4 μm in thickness. The median region is highly convoluted, and constitutes most of the duct's length. The diameter of the duct is about 60 to 70 μm. The lumen of the median region is narrow (about 2 μm in diameter), and the cuticular intima is thin (about 2 μm in thickness). This region is composed of three cell types: (a) slender epithelial cells with basally located oval nuclei, (b) columnar cells with basally located large round nuclei, and (c) cells with small nuclei. The columnar cell has a large vacuole containing a stainable substance (Fig. 2). In

addition to these cells, apically recurved tubular structures, which extend from the cuticular intima into the wall of the duct, are present in the median region of the duct (Fig. 3). These tubular structures and the cells containing large vacuoles are peculiar to the median region. Near the junction to the spermatheca, the spermathecal duct becomes thicker (ca. 100 μm in diameter) and passes straight along the surface of the spermatheca (Fig. 1). The lumen of the duct is about 4 μm in diameter, and the cuticular intima is about 8 μm in thickness. In this region, the wall of duct consists of a single layer of slender epithelial cells with medially located elliptic nuclei.

Fine Structure

Glandular Cells: Each glandular cell has a large central cavity (described above as a vacuole). The central cavity is formed by an invagination of the cytoplasmic membrane and is lined with many microvilli (Fig. 4). The cavity of each glandular cell contains the end apparatus which consists of a loosely packed, finely filamentous material (Fig. 5), the so-called 'felt-work' [17].

In the glandular cells of newly emerged virgin females (2 or 3 days old), the microvilli fill the narrow cavity and are in close contact with the felt-work. A small number of electron-dense, membrane-bound granules are scattered in the cytoplasm surrounding the cavity, but no secretory substance is visible within the cavity (Fig. 6). In older, mated females, the secretory granules within the cytoplasm increase in size and number, and a small amount of secretory substance is present between the microvilli within the cavity (Fig. 4, 5). In addition to these granules, large spherical, electron-dense vesicles and irregularly shaped, moderately electron-dense vesicles are found throughout the cytoplasm (Fig. 8). As the secretory cycle advances, the microvilli lining the cavity become more loosely arranged and separated by the secretory substance which is electron-dense and finely granular (Fig. 7).

In newly emerged females, some flattened cisternae of rough endoplasmic reticulum are sparsely scattered in the peripheral region of the cytoplasm of the glandular cells, while in older mated females, the cisternae of rough endoplasmic reticulum are dilated and mostly occupy the cytoplasm (Fig. 9). Mitochondria, randomly arranged microtubules, several Golgi bodies, and glycogen deposits are found in the glandular cells of both mated and newly emerged females.

The glandular cells were not found in the wall of spermatheca.

Epithelial Cells: The basal cytoplasmic membranes of the epithelial cells are infolded. The intercellular spaces, which invade deeply between the the adjacent epithelial cells (Fig. 11), contain the same material that forms the basement membrane. The lateral membrane facing these spaces possesses a dense area associated with microtubules. The apical half of lateral cytoplasmic membranes of adjacent cells are extensively interdigitated, and adjacent cells are linked by a septate junction (Fig. 13). The apical region of epithelial cells shows a highly irregular outline due to the numerous complicated invaginations of the cytoplasmic membranes (Fig. 10). The invaginated membranes surround narrow projections of the innermost layer of the cuticular intima, and

possess dense areas associated with tufts of microtubules (Fig. 12, 13).

A small amount of rough endoplasmic reticulum is sparsely scattered in the cytoplasm. Golgi bodies are present but not prominent. Mitochondria, although present throughout the cell, are distributed mostly in the apical cytoplasmic zone (Fig. 10). The concentration of mitochondria in the apical zone is particularly prominent in the epithelial cells which compose the distal and proximal region of the spermathecal duct, but this phenomenon is not prominent in the epithelial cells composing the median region of the duct. Glycogen deposits are present in the basal zone of the epithelial cells. In old, mated females, large glycogen areas are especially noticeable in the epithelial cells at the proximal region of the spermathecal duct (Fig. 10), while, in newly emerged, virgin females, they are prominent in the cells composing the distal region of the duct. The apical cytoplasmic protuberances of the epithelial cells often contain glycogen granules (Fig. 10).

Efferent Ductules and Ductule Carrying Cells: The secretory substance released into the central cavity of each glandular cell is conveyed to the lumen of the spermathecal duct through an efferent ductule (described above as a tubular structure). The tip of the ductule within the cavity is surrounded by a loosely-knit felt-work (Fig. 5). The efferent ductule approaches the cuticular intima of the spermathecal duct passing tortuously through a ductule carrying cell (described above as a cell with a small uncleus) (Fig. 14). Although the ductule through the cuticular intima of the spermathecal duct is not accompanied by a ductule carrying cell, it maintains its structural identity (Fig. 15). The lumen of the efferent ductule lined by the cuticular intima is about 0.2 to 0.3 μm in diameter.

The intima of the ductule consists of (i) a superficial electron-dense region, (ii) an underlying homogeneous layer of moderate electron density, and (iii) a fibrous region lying immediately beneath the cytoplasmic membrane of the ductule carrying cell (Fig. 16). The superficial region consists of a pair of dense layers: the one closest to the lumen is about 10 nm thick, and the other is thinner, about 7 nm in thickness. The electron-transparent zone between the two electron-dense layers is about 7 nm. The homogeneous underlying layer is about 20-30 nm in thickness. The fibrous zone is not uniform in thickness. The outer surface of this zone is extremely irregular, and often extends into the cytoplasm of the ductule carrying cell. Occasionally, the irregularly shaped deposits of electron-dense substance are present in this extension of the fibrous zone (Fig. 14). The fibrous zone becomes very thin or disappears at the region where the ductule invades the glandular cell, and changes into a felt-work structure within the cavity of the glandular cell (Fig. 5).

The infolded cytoplasmic membrane of the ductule carrying cell encloses the wall of the efferent ductule (Fig. 14). The adjoining cytoplasmic membranes of the glandular and ductule carrying cell are linked by a septate junction (Fig. 5). The cell organella of these cells are poorly developed. Microtubules, small mitochondria, scattered profiles of rough endoplasmic reticulum are dispersed throughout the cytoplasm.

Occasionally, small Golgi bodies are observed.

Muscles and Axons: The muscle wall enveloping the spermathecal duct consists of two or three layers of insect visceral muscle. Axons containing electron-dense vesicles (ca. 100 nm in diameter) approach the muscle fibers and make neuromuscular junctions with them. In the axon terminals, both large dense and small clear vesicles (ca. 50 nm in diameter) are present (Fig. 17).

Discussion

The spermathecal duct of the cricket, *G. bimaculatus*, is characterized by the presence of glandular cells. In the cockroach, *Periplaneta americana* [6], and the fruit fly, *Drosophila melanogaster* [12], glandular cells are present only in the wall of spermatheca. In the mosquito, *Aedes aegypti* [10, 11], glandular cells are present in both the spermatheca and spermathecal duct, but their distribution in the spermathecal duct is restricted to a particular region near the junction of the duct with the spermatheca. In contrast, in *G. bimaculatus*, the glandular cells were found only in the median region where the spermathecal duct is highly convoluted, and not found either near the junction with the genital chamber or near the junction with the spermatheca. They were also absent from the wall of the spermatheca.

The general form of the glandular cells in *G. bimaculatus* is similar to that in other insects [6-8, 10-13, 15], i. e., the glandular cell has a central cavity formed by invagination of its apical cytoplasmic membrane, and releases its product there. The secretory substance is exported to the lumen of the organ through the cuticle-lined efferent ductule. Thus, the secretory structure in the spermathecal duct of *G. bimaculatus*, like that of other insects, falls into the Class 3 category in the scheme devised by Noriot and Quennedey [18] for insect epidermal glands.

The tip of the efferent ductule within the cavity of the glandular cell was surrounded by a loosely-knit felt-work. This structure appears to be of general occurrence within the cavities of the glandular cells in the spermathecal duct and spermatheca [6-8, 10-12, 15]. Conti *et al.* [15] demonstrated in *Dytiscus marginalis* that the felt-work structure contains resilin and neutral polysaccharides. The fine structure of the intima of the efferent ductule is different from that of the efferent ductule of *P. americana* [6]. The intima of the efferent ductule of *P. americana* consists of two distinct regions: an inner dense region and an underlying region of lower density where both longitudinal and radial periodicity are observed. In contrast, the efferent ductule intima in *G. bimaculatus* could be subdivided into three regions, and the periodicity was not observed in any region.

In *P. americana*, Gupta and Smith [6] observed the aggregation of moderately electron-dense vesicles in the glandular cell cytoplasm surrounding the central cavity. They suggested that the secretory vesicles gradually loose their initial density and increase in size prior to their release by a 'blebbing off' of pieces of cytoplasm. In *G. bimaculatus*, however, the aggregation of small electron-dense granules around the cav-

ity was observed, but that of the moderately electron-dense vesicles was not. Probably the contents of the small electron-dense granules are released directly into the cavity. The relationship of the electron-dense granules and moderately electron-dense vesicles was not clarified in the present study. Ahmed and Gillot [19] demonstrated in *Melanoplus sanguinipes* that the secretory substance of the glandular cells in the spermatheca contains protein and acidic mucopolysaccharide.

The aggregation of mitochondria within the apical cytoplasm of epithelial cells has been observed in the spermathecae of several species of insects [6, 8, 9], and it is presumed that the aggregation of mitochondria concerns the ionic regulation necessary to the maintenance of viable sperms [9]. In *G. bimaculatus*, the polarized distribution of mitochondria was prominent in the epithelial cells of the proximal region near the junction of the duct with the genital chamber and in those of the distal region near the junction with the spermatheca, but not so in the median region of the spermathecal duct. This fact suggests that the epithelial cells of the proximal and distal region of the duct are functionally specialized for the regulation of the microenvironment within the lumen of the spermathecal duct.

Glycogen deposits have been noted in the spermathecal epithelial cells of *Tenebrio molitor* [14], *P. americana* [6], and *M. sanguinipes* [8]. In *G. bimaculatus*, they were noticed in the epithelial cells at the proximal region of the spermathecal duct in old mated females, whereas in newly emerged females they were prominent in the cells composing the distal region of the duct near the spermatheca. This phenomenon may result from the difference in the degree of cellular maturation. Glycogen deposits are frequently found in developing insect epithelia [20].

Axons containing both large electron-dense and small clear vesicles were widely distributed among the muscle fibers of the spermathecal duct. The identification of the neurons and of their neurotransmitters requires further physiological and immunohistochemical study.

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Explanation of figures

Fig. 1. Spermathecal duct (SD) and spermatheca (ST). The spermathecal duct is thick in the proximal region near the junction with the spermatheca (arrow). × 60. GC, spermathecal pouch of the genital chamber.

Fig.2,3. Oblique sections through the median region of the spermathecal duct of a mated female. Azan. 2: A large vacuole containing a stainable substance (arrow). × 440. 3: A tubular structure which extends from the cuticular intima (CU) into the wall of the spermathecal duct (arrow). × 440. MU, muscle.

Fig. 4. A longitudinal section through a glandular cell of a spermathecal duct of a mated female. The cavity of the glandular cell is filled with many microvilli (MV). × 4,000. CU, cuticular intima; DC, ductule carrying cell; EP, epithelial cell; MU, muscle; N, nucleus; SP, sperm.

Fig. 5. An enlarged view of Fig. 4 showing the end apparatus within the cavity. Closely packed microvilli (MV) abut the felt-work (FW). × 25,000. DC, ductule carrying cell; ED, efferent ductule; SJ, septate junction; SS, secretory substance; SG, secretory granule.

Fig.6,7. Sections through a central cavity of a glandular cell. 6: A newly emerged, virgin female. Microvilli (MV) are in close contact with the felt-work (FW). No secretory substance is visible between the microvilli. × 15,000. 7: A mated female. Microvilli (MV) are separated from each other by the secretory substance (SS), which fills the cavity. × 15,000. SG, secretory granule.

Fig.8,9. Glandular cells of a spermathecal duct in a mated female. 8: Large spherical, electron-dense vesicles (DV) and rather irregularly shaped, moderately electron-dense vesicles (MV) are visible, in addition to small secretory granules (SG). × 9,000. 9: Dilated cisternae of rough endoplasmic reticulum (RER), a Golgi body (G), and glycogen granules (GL) are visible. × 12,000.

Fig. 10. A longitudinal section through epithelial cells in the proximal region of a spermathecal duct of a mated female. Large glycogen areas (GL) are visible. The arrow points to the apical cytoplasm containing glycogen granules. × 4,000. CU, cuticular intima; N, nucleus.

Fig. 11. A longitudinal section through the basal portion of epithelial cells in the distal region of a spermathecal duct of a mated female. An intercellular space (arrows) invades deeply. × 25,000. BM, basement membrane; GL, glycogen; MT, microtubule.

Fig. 12. An oblique section through the apical portion of an epithelial cell in the proximal region of a spermathecal duct of a mated female. Microtubules surround cuticular processes (arrows). Note the presence of many mitochondria (M). × 14,000. CU, cuticular intima.

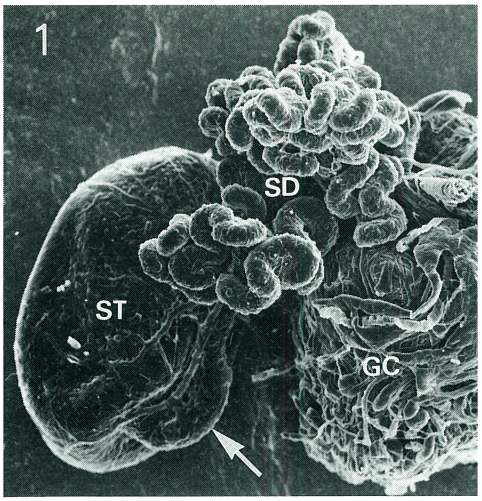
Fig. 13. A longitudinal section through the apical portion of epithelial cells in the median region of the spermathecal duct of a newly emerged, virgin female. The cytoplasmic membrane possesses a dense area associated with tufts of microtubules (MT). × 26,000. SJ, septate junction.

Fig. 14. A ductule carrying cell of a mated female. Electron-dense deposits (arrows) are present in the cuticular intima (CU) of the efferent ductule (ED). × 14,000. EP, epithelial cell; N, nucleus.

Fig. 15. A longitudinal section of an efferent ductule (ED) in the cuticular intima of a spermathecal duct of a newly emerged virgin female. × 9,300. CU, cuticular intima.

Fig. 16. A longitudinal section of an efferent ductule of a mated female. The intima of the ductule consists of a pair of superficial electron-dense regions (D1, D2), a homogeneous layer (D3) and a fibrous region (D4). × 98,000. DC, ductule carrying cell; SS, secretory substance.

Fig. 17. An axon terminal (AX) forming a neuromuscular junction with a muscle fiber (MF) which envelops the spermathecal duct. Large dense (large arrow) and small clear (small arrow) vesicles are present in the axon terminal. × 26,000.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SD
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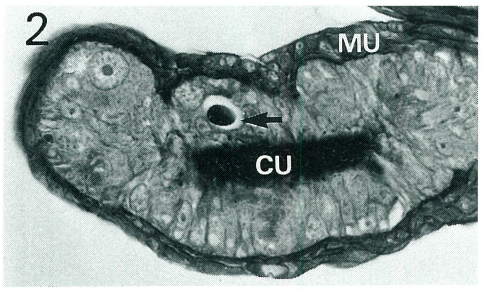
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MU
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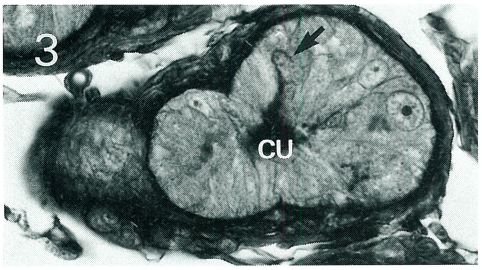
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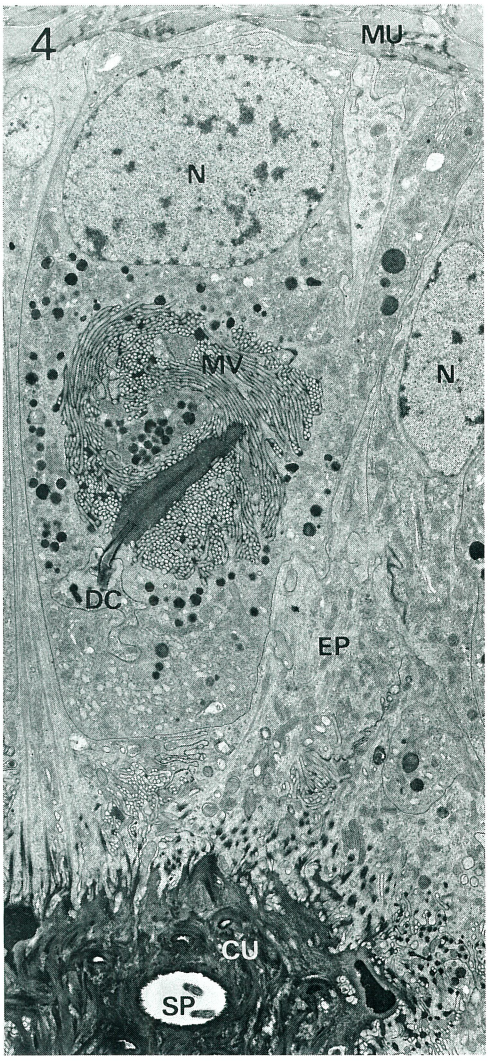
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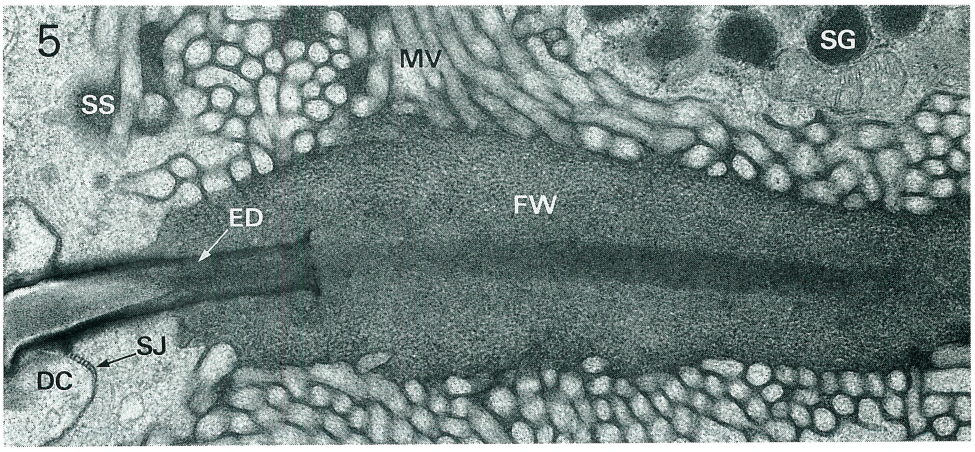
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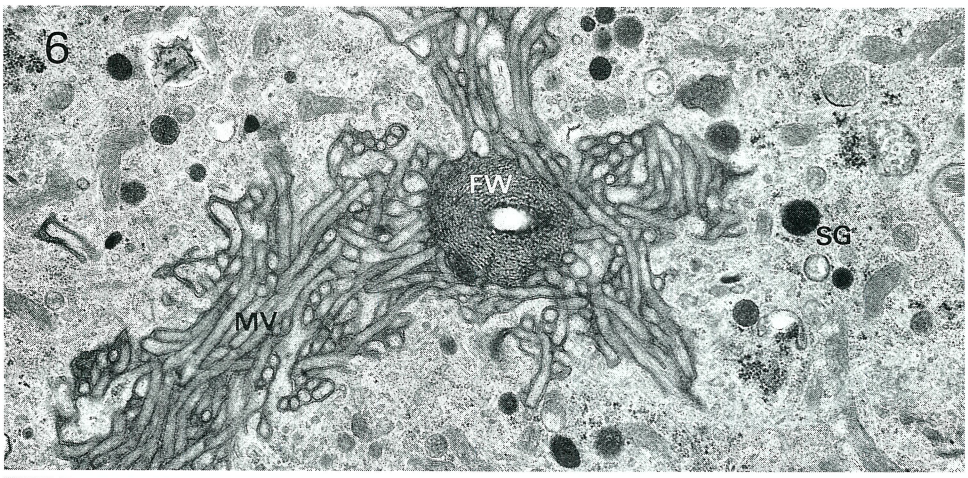
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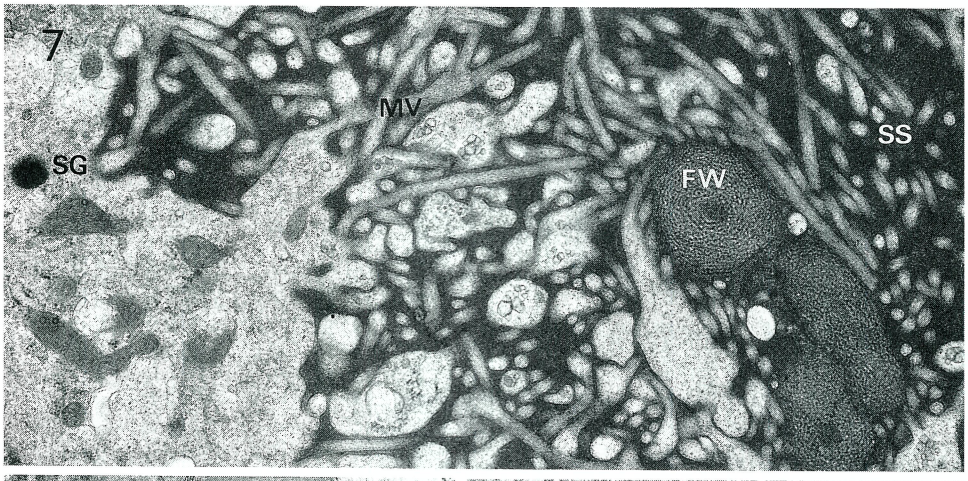
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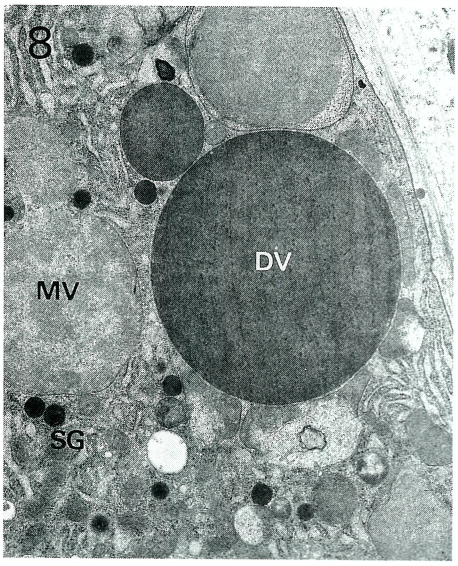
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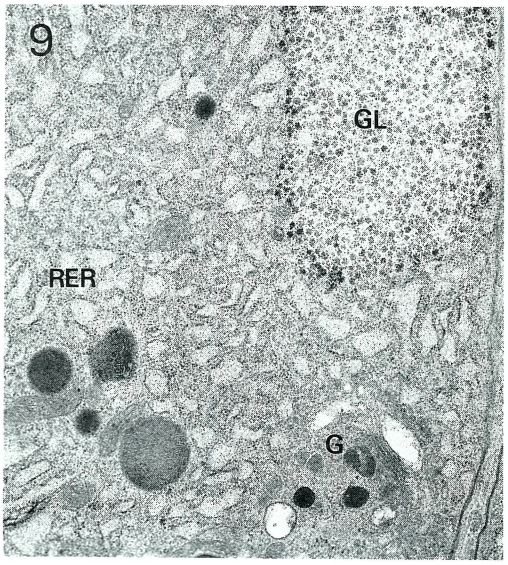
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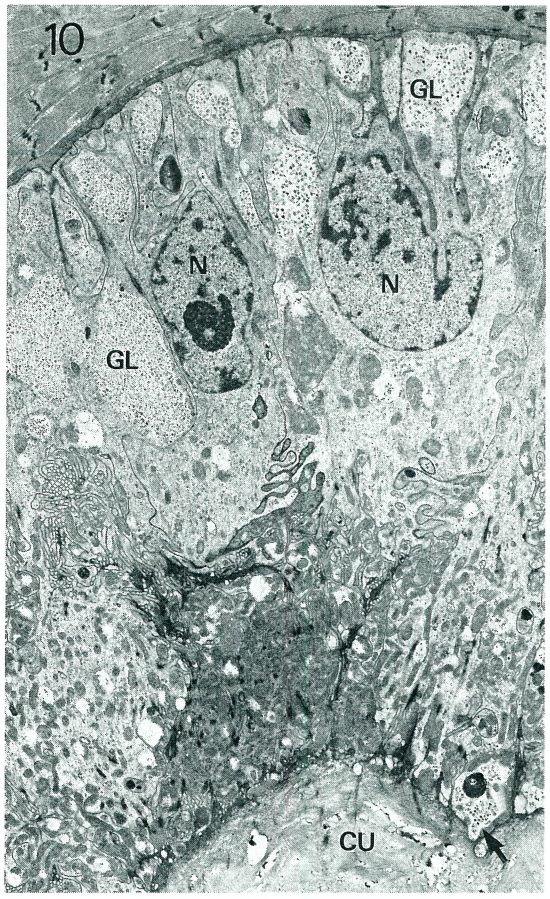
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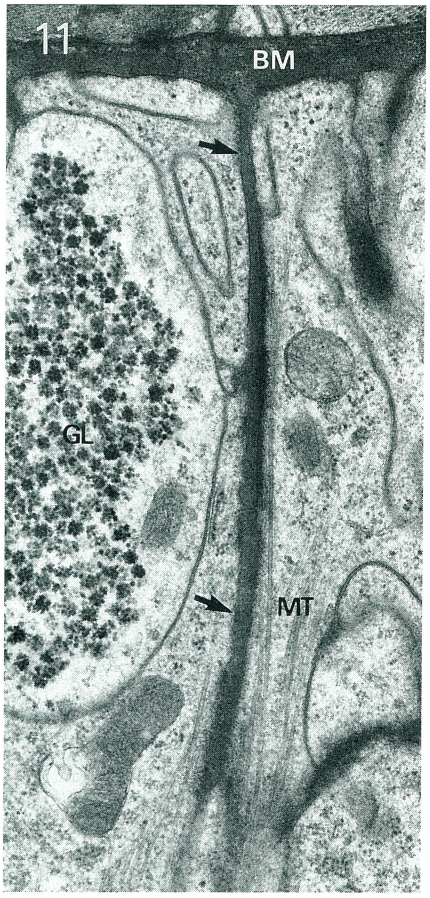
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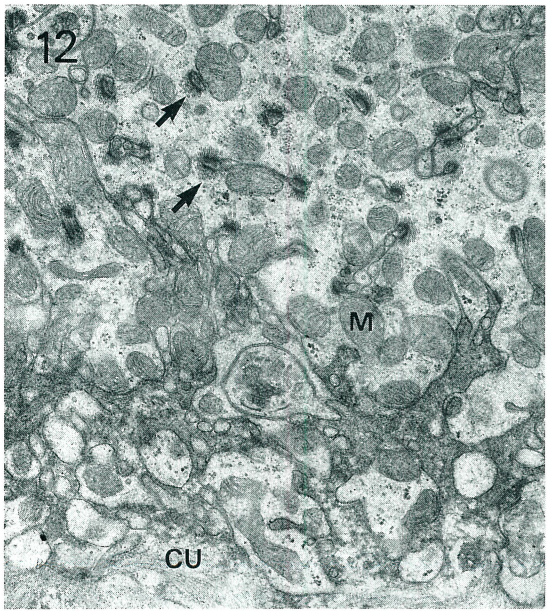
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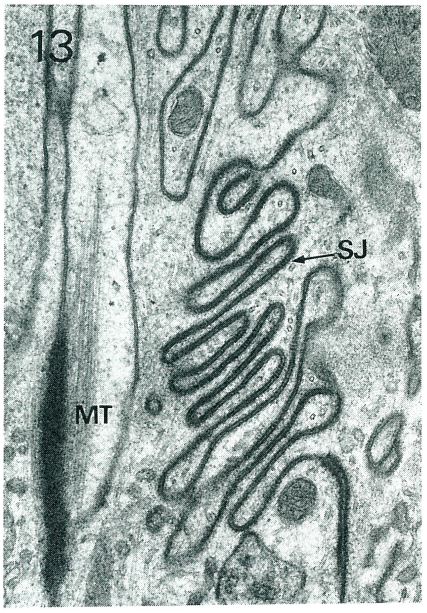
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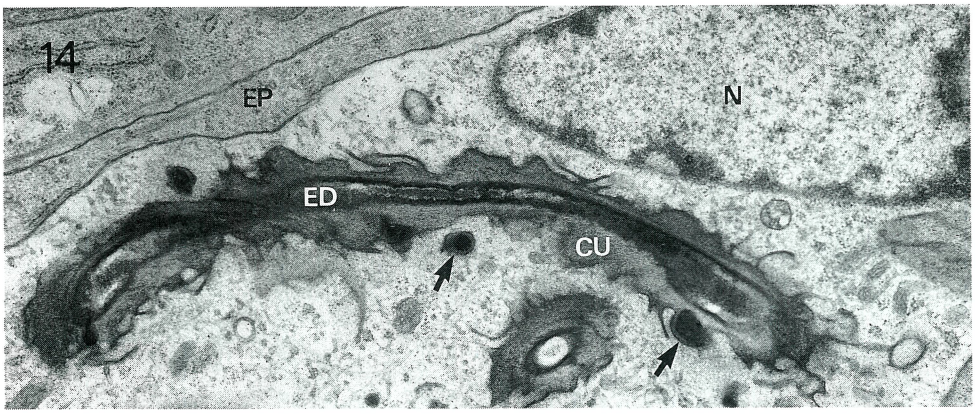
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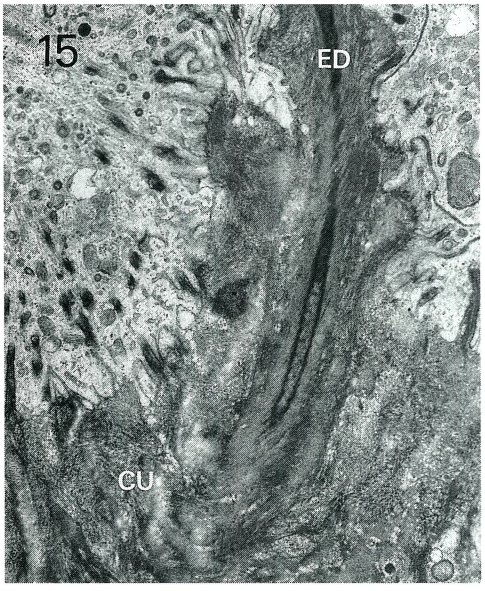
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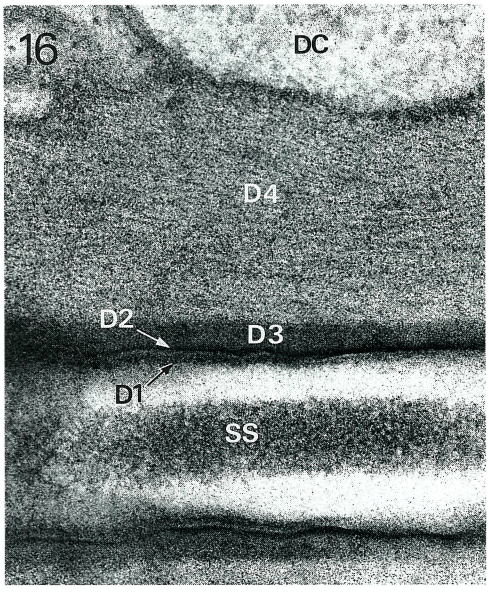
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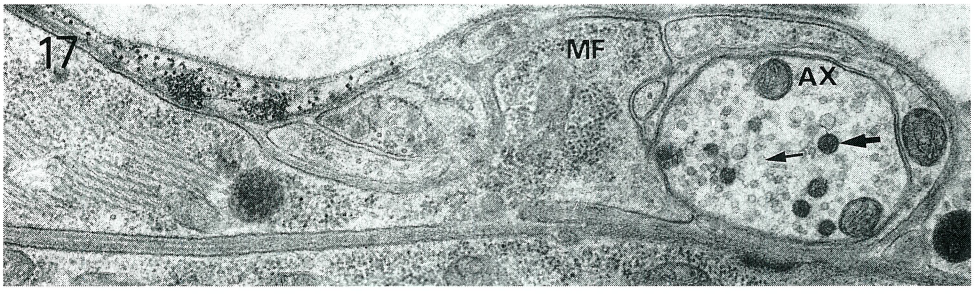
SS


17
MF
AX

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