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Ultrastructural Observations on Spermatogenesis within Testes in *Haemaphysalis longicornis* (Acari: Ixodidae)

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A rnale tick gives elongated spermatids to a female, that is, spermatogenesis within testes completes itself until elongated spermatids. And then elongated spermatids develop into mature sperms after they enter into the female genital tract via the spermatophore. In the present study, ultrastructural observations of spermatogenic cells were made at each stage of spermatogenesis in male *Haemaphysalis longicornis*. Spermatogenic cells from spermatogonia to primary spermatocytes at the middle of their great growth phase were found in testes of unfed males. Subsequently, testes of *H. longicornis* are developed by feeding at their adult stage. The testes, or spermatogenic cells, were developed gradually until the 5-day feeding period was complete (the complete feeding stage). Spermatogenic cells at all stages from spermatogonia to elongated spermatids were contained in the testes of completely fed males. Spermatogenic cells were packed in cysts. Elongated spermatids which had been completed at the posterior ends of testes were made spermiation with the breakdown of the cyst cells. The spermatids were carried out of the testes through the central lumen. The cyst cells formed the lumen, and had some microvilli on their free surface.

INTRODUCTION

One of the common characteristics of tick reproduction is that. a male transfers elongated spermatids (prospermia) to a female. Spermatogenesis within the testes completes itself until elongated spermatids, and then these spermatids undergo 'spermateleosis', or the process of elongated spermatids becoming mature sperm after they enter into the female genital tract via the spermatophore. Ticks are often classified into Argasidae, Prostriata (Ixodidae: *Ixodes),* and Metastriata (Ixodidae except for *Ixodes),* and their reproductive system and the structure of the genital organs are different. Newly emerged adult males of Argasidae and Prostriata are able to copulate, and this shows that their testes are developed by feeding at the nymphal stage. Most Metastriata males, whereas, require feeding at the adult stage in order to copulate.

Tick spermatogenesis and the structure of testes had been studied in various species, and these were collected under some reviews by Balashov (1972), Oliver (1982), and Sonenshine (199 1). Some detailed studies observed the structure of elongated spermatids, their cellular processes, and spermateleosis in the female genital tract. In the present study, the development of testes during feeding, spermatogenic cells at each stage of spermatogenesis within testes, and cyst cells were observed with a transmission electron microscope in adult *Haemaphysalis longicornis* Neumann.

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MATERIALS AND METHODS

Nymphal *Haemaphysalis longicornis* used in the present study were collected by dragging on pastures in Kuju Highland, Oita Prefecture. They were fed on ears of laboratory rabbits. Unfed adult males were obtained from engorged nymphs in the laboratory. Males fed for 3 and 5 days were prepared.

Testes of all the above-mentioned males (unfed, 3-, and 5-day fed) were removed. All the testes were fixed with cold 3% glutaraldehyde in sodium cacodylate buffer (pH 7.2), postfixed with 1% OsO_4 in the same buffer, dehydrated in an ethanol series, and embedded in epoxy resin. Semi-thin sections (about 1.5 μm) were cut on a Porter-Blum MT-l, using a glass knife, and stained with toluidine blue for light microscopic observations. Thin sections (about 60nm) were cut on the same microtome and doubly stained with uranyl and lead acetate before examination in an Hitachi H-600A electron microscope.

RESULTS

Male *Haemaphysalis longicornis* had a paired tubular testes (Fig. 1). These joined to the vas deferens at their narrow anterior ends, and their posterior ends were connected to each other by a thin strand of tissue. The spermatogenic cells were packed

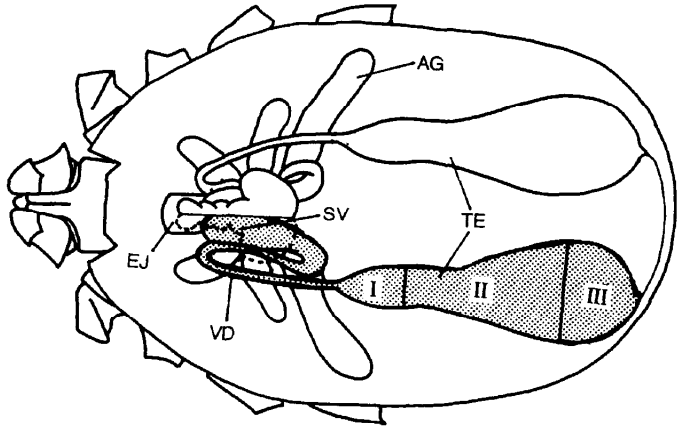

Fig. 1. The diagram of the dorsal view of genital glands in an adult male. The relative size of testes (TE) is almost same as that of a feeding male. A paired testes are connected by a strand of the connective tissue at their posterior ends. The vas deferens (VD) forms the seminal vesicle (SV) after it turns twice, and the seminal vesicle opens to the dorsal surface of the ejaculatory duct (EJ). Spermatogenic cells in the area III are at more advanced stage than those in the area I. AG, male accessory genital glands.

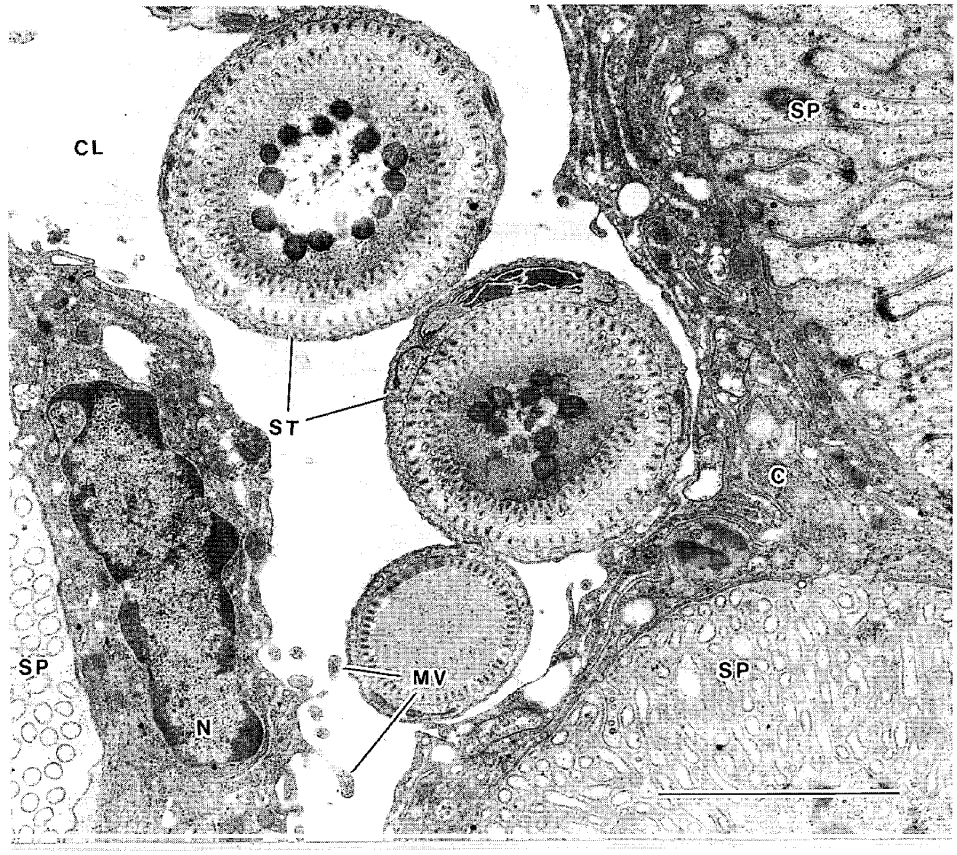

Fig. 2. Electron micrograph of the cyst cells (C) facing on the central lumen (CL). These cells have a small number of microvilli (MV) on their free surface. Completed elongated spermatids (ST) are found in the lumen. Note spermatogenic cells (SP) in three cysts are at, different stages. N, nucleus. Bar = 3 μm.

in cysts composed of **cyst** cells (Fig. 2). Testes which were aggregations of cysts were covered with the loose connective tissue. The central lumen as a passage of elongated spermatids to the vas deferens extended the length of the testes, that is, cysts were arranged radially around the lumen on cross sections of the testes. The central lumen was formed by cyst cells, and cyst cells facing on the lumen had microvilli. Spermatogenic cells contained in the posterior region of testes were at a more advanced stage of spermatogenesis than those in the anterior region.

Spermatogonia and early primary spermatocytes were contained in the anterior part of the testes (Fig. 1, area I) in feeding males (Fig. 3). These cells were small (about 6 μm in diameter), and had a large nucleus. The polysomes and mitochondria were found in their cytoplasm, and intercellular bridges had already formed. Subsequently, the great growth phase of primary spermatocytes began, and these cells increased in size gradually (about up to 30 μm in diameter) during this phase (Fig. 4a, b). These cells occupied the

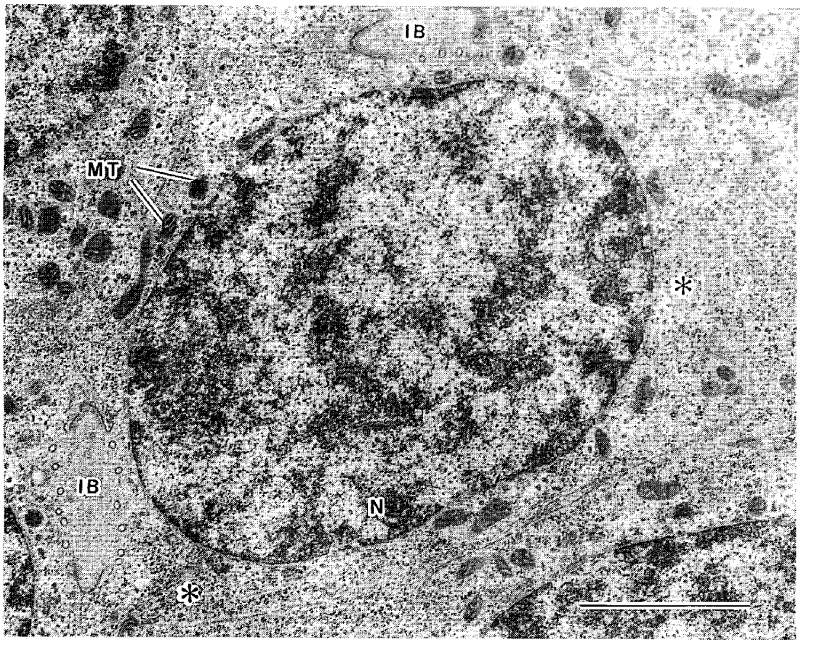

Fig. 3. Electron micrograph of the early primary spermatocytes. The cells are relatively small (approximately 6 μm in diameter), and have a large nucleus (N). A large number of polysomes (*) are found in lheir cytoplasm. Intercellular bridges (IB) have already formed. MT, mitochondrion. Bar = 2 μm.

middle large part of the testes (Fig. 1, area Ⅱ). The polysomes disappeared and the Golgi complex emerged in the primary spermatocytes after the beginning of this phase (Fig. 4c). The subplasmalemmal cisternae which would become cellular processes on the surface of sperms began to be formed by the membrane originated from the Golgi complex. When the great growth phase completed and the middle-sized subplasmalemmal cisternae were formed, the size of the cells reduced (about 16 μm in diameter) (Fig. 1, area II). Early spermatids which had formed the largest subplasmalemmal cisternae were found immediately after this reduction in size (Fig. 5). Thereafter, the relocation of the nucleus and subplasmalemmal cisternae to opposite poles of the cells occurred, and at this time an electron dense acrosome emerged between the nucleus and plasma membrane. The relocated subplasmalemmal cisternae began to be enveloped by the cytoplasm synchronized with a fusion of the membrane at the base of the subplasmalemmal cisternae (Fig. 6a). The subplasmalemmal cisternae had become tubular, and the electron dense fibrillar materials were found at their base where a fusion of the membrane occurred. As the result of the envelopment, the cisternal cavity was completed (Fig. 6b). The reduction in size also occurred between Fig. 6a and 6b. The intercellular bridges which had been found from the primary spermatocytes in the testes of an adult male disappeared after the beginning of the formation of the cisternal cavity. These cells in

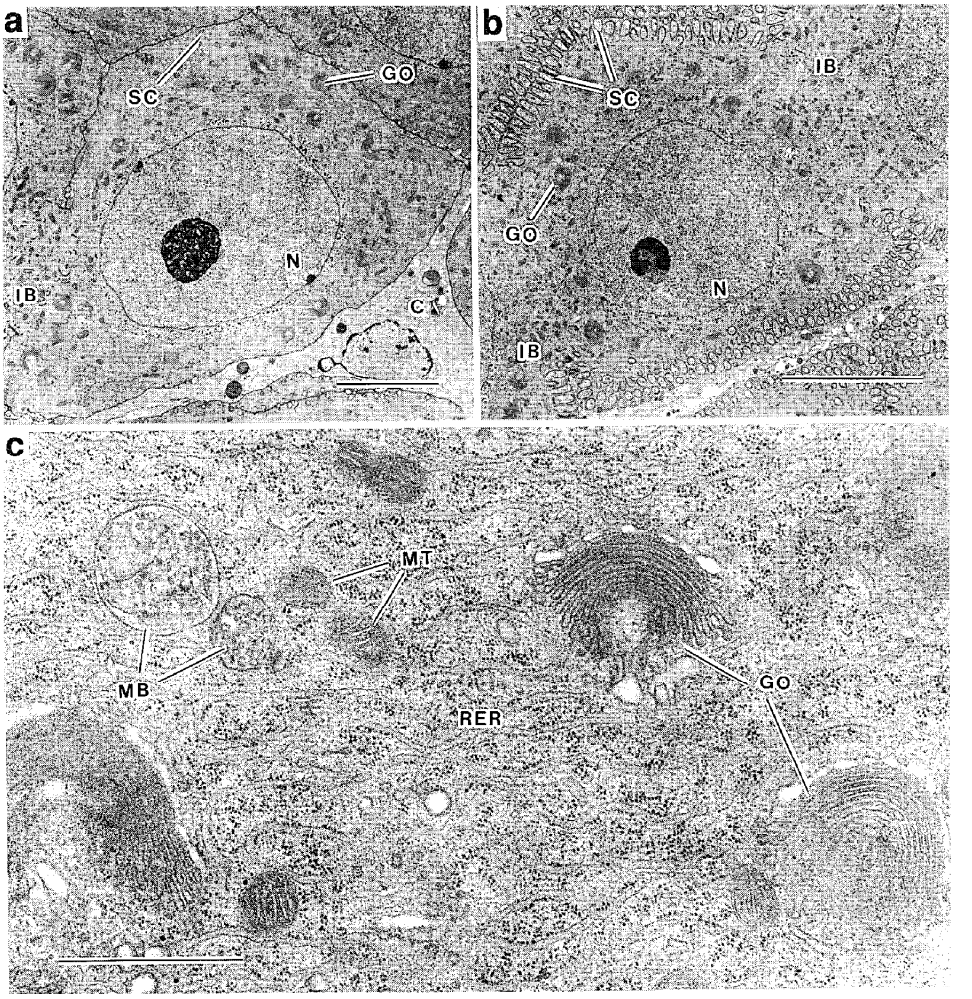

Fig. 4. Electron micrographs of primary spermatocytes during their great growth phase. The contrast between the nucleus (N) and cytoplasm is flat. **a:** At the middle of the great growth phase. The cell has enlarged (approximately $25\mu m$ in diameter). The small subplasmalemmal cisternae (SC) are added beneath the plasma membrane. The intercellular bridge (IB) has expanded. **b:** The largest primary spermatocyte (approximately $30\mu m$ in diameter) at the end of the great growth phase. The larger subplasmalemmal cisternae and intercellular bridge are found. c: The cytoplasm of the cells at the great growth phase. Note the remarkable Golgi complex (GO). C, cyst cell; MB, multivesicular body; MT, mitochondrion; RER, rough endoplasmic reticulum. Bars = (a) $5\mu m$, (b) $10\mu m$, (c) $1\mu m$.

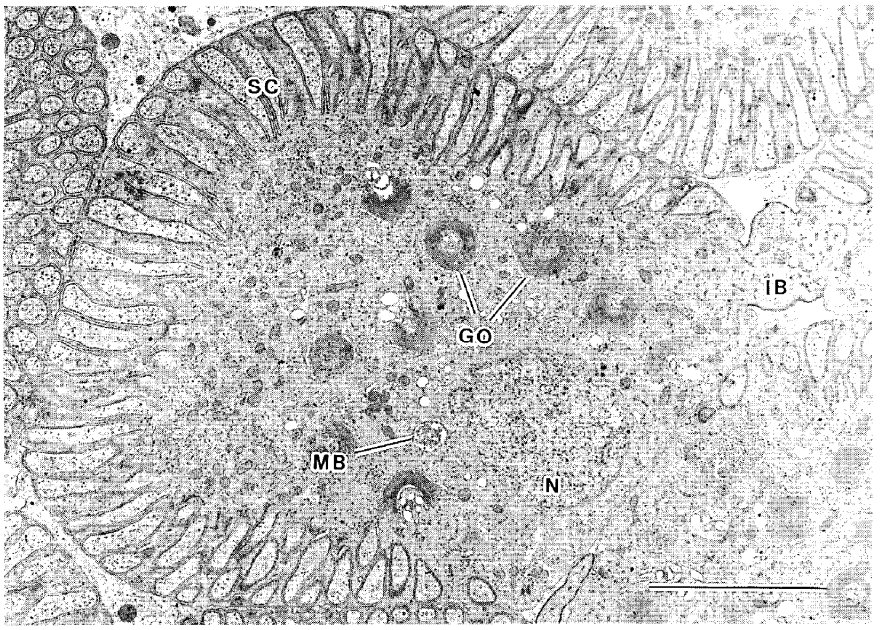

Fig. 5. Electron micrograph of spermatids just after their maturation division. The size of the cells have reduced (approximately 16 μm in diameter). The largest subplasmalemmal cisternae (SC) and remarkable Golgi complex (GO) are found. The intercellular bridge (IB) has become narrow again, -MB, multivesicular body; N, nucleus. Bar = 5 μm.

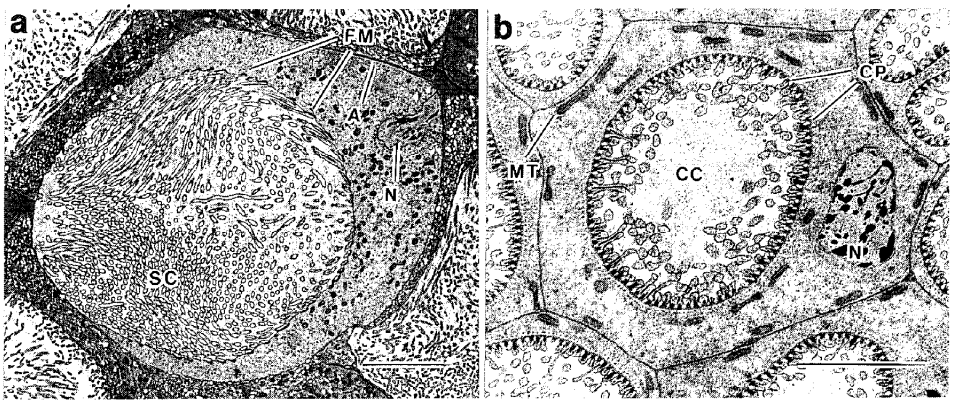

Fig. 6. Electron micrographs of spermatids during the formation of the cisternal cavity (CC). **a:** The subplasmalemmal cisternae (SC), which has become tubular after the relocation of the nucleus (N) and subplasmalemmal cisternae to opposite poles occurred, are being enveloped by the cytoplasm. Electron dense fibrillar materials (FM) are found at the base of the subplasmalemmal cisternae. The acrosome (A) emerged between the nucleus and plasma membrane. **b:** The formation of the cisternal cavity is completed, and the subplasmalemmal cisternae are changing into the cellular processes (CP). Note the tubular mitochondria (MT). Bars = (a) 5 μm, (b) 2 μm.

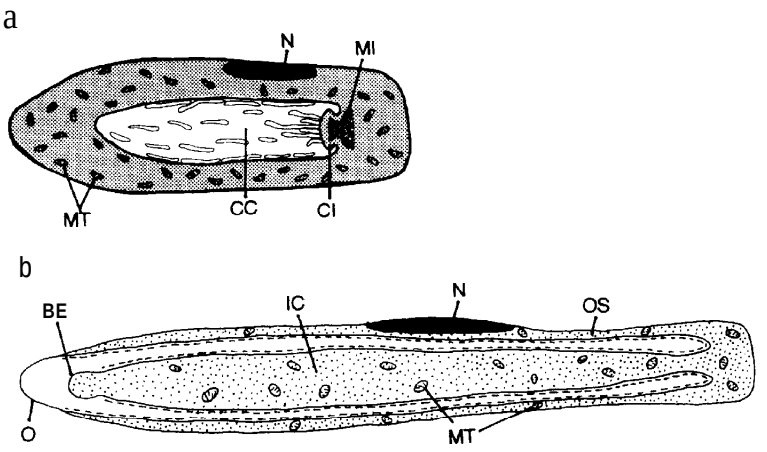

Fig. 7. Diagrams of the elongating (a) and completely elongated (b) spermatids. **a:** The cisternal tube (CI) is invaginated into the cisternal cavity (CC) after the beginning of the elongation. The nucleus (N) became tubular. The bundle of microtubules (MI) are found at the base of the cisternal tube. **b:** The invagination of the cisternal tube is finished, and the inner cord (IC) are completed. The inner cord is surrounded by a thin outer sheath (OS). Cellular processes (broken line) are arranged on the external surface of the inner cord and the inner surface of the outer sheath. The bulbous expansion (BE) is found at the tip of the inner cord. The nucleus is situated in the outer sheath. MT, mitochondron; 0, operculum.

which the cisternal cavity had been formed began to elongate, and then the invagination of the cisternal tube (the formation of the inner cord) occurred (Fig. 7a). The elongated spermatids consisting of the outer sheath and inner cord were completed after the elongation of the cells and formation of the inner cord (Fig. 7b).

In spite of their feeding stage, the spermatogenic cells which were at the same stages and same volume as those of unfed males were continually found in the anterior region of all the testes. In the testes at each feeding stage, the most advanced spermatogenic cells at the posterior ends of the testes were as follows; slightly enlarged primary spermatocytes (unfed) (Fig. 4a), and early spermatids just after the maturation division (3-day fed) (Fig. 5).

DISCUSSION

Tick testes are paired tubular organs. It is known that these are fused posteriorly (Argasidae), broadly joined (most Prostriata), or connected only by an extremely thin filamentous strand of tissue (Metastriata) (Oliver, 1982). As in other ticks (Oliver, 1982), groups of spermatogenic cells are arranged in cysts. The development of all cells within a particular cyst is synchronous, and the number of spermatogenic cells appears to be

species specific (Khalil, 1969, 1970). Cyst cells were also called 'nutritive cells' (Reger, 1961) or 'interstitial cells' (Raikhel, 1983), and the only cyst cells facing on the central lumen may have microvilli. In ticks the spermatogenic cells are arranged in the reverse order usually found in most tubular testes of invertebrates (Oliver, 1982), that is, spermatogenic cells found in the posterior region are at a more advanced stage of spermatogenesis than those in the anterior region connecting to the vas deferens. Strongly stained primary spermatocytes in the anterior part of the testes in other ticks were also found in *H.* longicornis. As remarkable polysomes are found in their cytoplasm, it is suggested that the structural protein synthesis in these cells are active. According to the detailed study on stages of the maturation division in tick testes (Oliver and Brinton, 1972), it is certain that in most species, if not all, diplonema is the first recognizable meiotic stage apparent at the end of the great growth phase of primary spermatocytes. The characteristic chromosomes at metaphase or anaphase were found at the end of the great growth phase in *H. longicornis*. As the reduction of cell size was observed immediately after this time, we thought that the maturation division occurred. The second reduction of cell size was also observed during the formation of the cisternal cavity in ***H. longicornis***. However, it is described that the reduction in size at each succeeding division is present but less pronounced (Oliver, 1972). Oliver (1982) also indicated that the spermatogenic cell of which the nucleus was located centrally and the periphery remains surrounded by cup-shaped subplasmalemmal cisternae was the early spermatid. So we think that this stage is similar to that of ***H.*** longicornis as stated in Results. Furthermore, it is described that the growth and differentiation of the spermatid into the elongate cell is not a growth in mass but rather a continual change in shape, and the inner cord continually growth at the expense of the outer sheath which become relatively thinner (Casteel, 1917). In the present study, the second reduction in size may be reduction in area on sections with the change in shape. As ***H. longicornis*** is similar to other ticks on changes of spermatogenic cells, we adopted the stages of Oliver and Brinton (1972).

Some detailed studies on tick spermatogenesis particularly observed the structure of elongated spermatids, cellular processes, and the specialization of sperm membrane (El Said and Swiderski, 1980; Oliver and Stone, 1983; Wüest et *al.*, 1978). The subplasmalemmal cisternae were also called 'subsurface cisternae' (Reger, 1962) or 'cortical alveoli' (Raikhel, 1983). Although origin of the subplasmalemmal cisternae is uncertain, the endoplasmic reticulum (Reger, 1961, 1962, 1963), Golgi complex (Reger, 1974) and plasma membrane (Oliver and Brinton, 1972; Suleiman and Brown, 1978) have been suggested as origins. In *H. longicornis*, the timing of Golgi complex emerging and disappearing synchronized with the timing of the addition of subplasmalemmal cisternae being initiated and completed, respectively. Therefore we support that subplasmalemmal cisternae originate from Golgi complex. The subplasmalemmal cisternae change into the cellular processes with a formation of the cisternal cavity by a fusion of membrane (Reger, 1962). Subsequently the cisternal tube which would become an inner cord surrounded by an outer sheath invaginates into the cisternal cavity. There are many descriptions on the cellular processes which are also called motile processes. In early spermatids, electron dense fibrillar materials appear at the base of cellular processes with a formation of cellular processes. These have been also called 'fibrillar-granular like

materials' (Reger, 1974) or 'bundles of microfibrils' (El Shoura, 1986), and are similar to those identified by El Said et *al.* (1981) and Wüest et *al.* (1978). It is suggested that a contractile system is involved in sperm movements (Rothchild, 1961). So it is generally known that these fibrillar structures participate in the movement. However, in the recent study (Witalinski and Dallai, 1994), it is also described that the actin concerned with motility are found in the cytoplasm of sperm. As mentioned above, it has been discussed that the cellular processes take part only in the sperm movements. But tick spermatogenic cells undergo remarkable morphological changes, that is, the great elongation from rounded spermatids to elongated spermatids and spermateleosis in the female genital tract. It is supposed that these fibrillar materials found in the cellular processes take part in their formation of the morphology. It has been described that the modes of tick sperm movements are gliding, rotational, bending, rolling, and wavy movements (Feldman-Muhsam and Filshie, 1974; Oliver and Brinton, 1972; Wüest *et al.*, 1978), and that of *H. longicornis* is gliding (Kakuda, personal communication).

Furthermore, the presence of mitochondria was observed on the sperm motility. Since a relatively high degree of cellular differentiation occurs in the spermatids, one expects relatively high metabolism and consequently large numbers of mitochondria for oxidative metabolism (Reger, 1961). Casteel (1917) explained that mitochondria are situated at the base of the motile processes (cellular processes) in tick spermatozoa, since large number of mitochondria are always found at the neck region, or the level of origin of motile filaments, in typical flagellate spermatozoa.

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