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A COMPARATIVE STUDY ON THE DEVELOPMENT AND THE STRUCTURE OF SPERMS OF TRICHINELLA NATIVA, T. PSEUDOSPIRALIS AND T. SPIRALIS (NEMATODA)

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Abstract. The three *Trichinella* species, i.e., *T. nativa*, *T. spiralis* and *T. pseudospiralis* do not differ in their spermatogenesis. Mitotically dividing spermatogonia are connected by a bridge. Vacuoles originate from evaginations of the plasmalemma into the cytoplasm of primary spermatocytes. In secondary spermatocytes, these vacuoles communicate with the perinuclear region by means of the endoplasmic reticulum. Spermatids produced by the first and second meiotic division of secondary spermatocytes are joined by a cytoplasmic bridge. A dilation of vacuoles and cisternae brings about a separation of the group of four spermatids from the peripheral cytoplasm. In the testes of *T. spiralis*, a tubular sheath encircling sperms, and its evaginations forming tubules in the cytoplasm, have not been observed in testes of *T. nativa* and *T. pseudospiralis*. Sperms in the vas deferens of the individual species differ in the structure of the plasmalemma, in the number of mitochondria, and in the shape of membranous organelles of which those of *T. pseudospiralis* have a pore. Sperms in the uterus of the female differ from those in the vas deferens of the male in the structure of membranous organelles which contain a system of parallel, longitudinal microtubules running towards the pore, in addition to the original microfilaments. These differences might influence the functional state of the vas deferens during copulation, and a similar effect might be ascribed to the contact with a stimulating substance released from the uterus of the female during copulation, Therefore, we cannot ascribe a diagnostic importance to these signs.

Our present study on the development and the fine structure of sperms of *T. nativa*, *T. pseudospiralis* and *T. spiralis* has been made for the purpose of confirming that a) the three species do not copulate with each other as proved genetically by Britov and Boev (1975), Boev (1976), Komandarev et al. (1975), b) differences in the structure of the pseudobursa (Baruš et al. 1979, 1981, Hulínská and Shaikenov 1980) have an impact on the development and the structure of the sperm. As pointed out in a number of earlier studies (Foor et al. 1971, McLaren 1973, Burghardt and Foor 1975, Anya 1976), the spermatozoon of nematode species (*Dipetalonema viteae*, *Brugia pahangi*) undergoes a morphological and physiological change in the reproductive organ of the female brought about by the stimulating influence of a substance released in the uterus. It has further been proved that the secretion produced by the vas deferens of the male activates sperms of members of the genus *Ascaris*, and changes their structure (Foor and McMahon 1973). Owing to these facts established for other nematode species, and to a scarcity of studies on the spermiogenesis in the three *Trichinella* species (data by Slomiamy et al. 1981 concern only the spermatogenesis in testes of *T. spiralis*), we have studied in detail the spermatogenesis of the threee species under consideration, and the influence of both the vas deferens and the uterus on the development of sperms in the hope that an understanding of these processes might help to disclose reliable diagnostic signs upon which the individual members of the genus *Trichinella* could be distinguished. Anya (1976) has made a survey of studies on the spermatogenesis of various nematode species.

MATERIAL AND METHODS

Males of *T. nativa* and *T. spiralis* aged two days, and males of *T. pseudospiralis* aged two and seven days, were obtained from mice infected experimentally with these *Trichinella* species in the Zoological Institute, Academy of Sciences, Kazakh SSR. We had to use two and seven day-old males of *T. pseudospiralis*, because they need two days longer to complete their spermatogenesis than do males of *T. nativa* and *T. spiralis*. Apart from this difference in the age of males, experimental conditions were identical (infective larval dose, age and sex of the host, mode of isolation of the material, fixation, temperature). The material was fixed with 2.5 % paraformaldehyde and 4 % glutaraldehyde, buffered in 0.1 M cacodylate at pH 7.2 in a vacuum (for details see Hulínská and Shaikenov 1982). After a fixation with osmium, dehydration and centrifugation, using 20 males of each species, the material was embedded either in Epon or Araldite, cut with a Reichert ultramicrotome, stained with uranyl acetate and lead citrate, and inspected with a JEM 100 B.

RESULTS

Sperms of a two day-old male of *Trichinella nativa* and *T. spiralis*, and a two and seven day-old male of *T. pseudospiralis*, fill the entire seminal vesical and the vas deferens. In equally old females sperms are present in the receptaculum seminis, and that in its anterior portion. Faint differences distinguished later in the structure of sperms are still indistinct. There are two compact areas, one a concentration of cells (spermatogonia and growing spermatocytes) located peripherally in the distal end of testes of *T. nativa* (Plate I, Fig. 1) and *T. pseudospiralis* (Plate I, Fig. 2), one a concentration of spermatids and spermatozoa located centrally in the middle of the testes. There might, evidently, be differences in the arrangement of cells in the testis, e.g., the peripherally located spermatogenic area might encircle spermatids and spermatozoa, or spermatogonia might be concentrated at one side of the testis while the opposite side is occupied by spermatids and spermatozoa as, e.g., in the testis of *T. spiralis* (Plate I, Figs. 4. 5). Spermatogonia (A) are large, elongate cells (average size $2.5 \times 6\,\mu m$). They have an elongate nucleus and a prominent, spherical nucleolus. The electron dense cytoplasm contains free ribosomes and small mitochondria. Below the plasmalemma, there are vesicles of the Golgi apparatus and a few, short cisternae of the granular endoplasmic reticulum. Spermatogonia having divided mitotically are joined to the daughter cell by a bridge. Early, primary spermatocytes (B) are larger in size, irregularly elongate, with an increased number of mitochondria in the cytoplasm (Plate I, Fig. 3). The nucleus contains less chromatin, the nucleolus is more dispersed than in the spermatogonia. The peripheral plasmalemma is folded and forms evaginations and outfolds, and from these originate vacuoles in the peripheral cytoplasm of older spermatocytes (Plate I, Fig. 3). The area ringing the nuclear material of an older spermatocyte (C) is less electron dense, the transparent, perinuclear area contains mitochondria. Cisternae of the endoplasmic reticulum produce a complex of elongate, long structures encircling the electron lucid nuclear area, and communicating with vacuoles and vesicles of the Golgi apparatus (Plate I, Fig. 3). A connection has been established between meiotically dividing, secondary spermatocytes (D) and future spermatids (E). The latter contain long, electron dense mitochondria around chromosomes which produce centres of chromatin condensation (Plate II, Fig. 2). The more electron lucid central area among future spermatids contains vacuoles, a complex of transverse cisternae of the endoplasmic reticulum, and ribosomes. This central area (H) (Plate II, Fig. 1) comprizing the peripheral cytoplasm is connected with a group of four spermatids. Their separation from the peripheral cytoplasm is brought about by a dilation of cisternae and vacuoles joined

with the plasmalemma at the site of their connection with the peripheral cytoplasm which is elimited as a residual body (F) (Plate I, Figs. 3, 4). The residual body containing vacuoles, cisternae of the endoplasmic reticulum and vesicles of the Golgi apparatus breaks down inside large vacuoles in the testis (Plate I, Fig. 4). Early spermatids are small, oval or irregularly shaped, electron lucid cells, with mitochondria surrounding the nuclear area (Plate II, Figs. 1, 3). Spermatids are bound by a multiplasmic membrane during their spermiogenesis (Plate II, Fig. 6). Their centriole is composed of nine individual microtubules located in the vicinity of mitochondria (Plate II, Fig. 5). In the maturing spermatozoon, the number of vesicles connected with cisternae of the Golgi apparatus increases, and so does the agranular, endoplasmic reticulum and vesicles inside cisternae. Spermatozoa (G) are larger than spermatids, chromatin condenses in a spherical body which lacks a nuclear membrane (Plate III, Fig. 2). Spermatozoa pass into the dilated tube of the seminal vesicle the walls of which are made up of elongate cells. The electron dense cytoplasm of these cells is filled with a granular endoplasmic reticulum and vesicles of the Golgi apparatus. Below the seminal vesicle, in close vicinity of the cloaca, lies the vas deferens. In *T. nativa*, it contains active, secretory cells which produce granules. The cells are cylindrical, their elongate nucleus lies at the base. They contain irregularly dispersed chromatin and possess an oval, electron dense nucleolus (Plate IV, Fig. 3). The cytoplasm is filled with a well-developed, granular, endoplasmic reticulum and an increased number of ribosomes (Plate III, Fig. 1). Less electron dense areas of the cytoplasm contain vesicles of the Golgi apparatus and small mitochondria. Of the granules (1—1.5 μm in diameter) accumulated in the base of cells, some are present in the electron lucid part of the cytoplasm which produces outfoldings into the lumen of the vas deferens (Plate III, Fig. 2). The presence of connecting, desmosomal complexes in closely adjacent unit membranes of cells underlines the outlines of neighbouring cells. The electron lucid cytoplasm without ribosomes underlying the plasmalemma contains numerous vesicles of the Golgi apparatus (Plate IV, Fig. 3). Some of these vesicles are filled with an electron dense substance, others with small granules. Secretory cells produce plasmic outfoldings into the lumen. They are filled with a homogeneous, electron lucid substance (Plate III, Fig. 1). Anastomosing outfoldings sometimes dilate, sometimes attenuate in the lumen (Plate III. Fig. 3). Sperms in the lumen possess numerous spherical, membranous organelles (Plate III, Fig. 2). A spherical, electron dense body produced by chromatin condensation is ringed by 19—20 mitochondria which have a very dense matrix (Plate III, Fig. 3). The shape of sperms is irregular. The multiple plasmic membrane of adjacent sperms is thickened by a denser, homogeneous substance. Inside the sperm are spherical, membranous organelles but no tubular structures from which organelles originate. A section through the membrane specialization of a sperm shows four microfilaments each running to the opposite pole of the organelle. None of these organelles open with a pore into the plasmalemma (Plate II, Fig. 2). The vas deferens of *T. pseudospiralis* is similar to that of the other two *Trichinella* species in that it contains cylindrical, secretory cells joined by desmosomal complexes (Plate III, Fig. 4). Extensive, electron lucid areas with numerous vesicles and granules are seen in larger cells. Granules occur also in the lumen of the vas deferens among the individual sperms, and are lined with a transparent substance and membranes. In the basal part of the cell, the joining desmosomal complex passes into long, cytoplasmic outfoldings (Plate IV, Fig. 1). Numerous shorter villi-like, cytoplasmic outfoldings protrude into the lumen but never far enough to either separate or line individual sperms. The plasmalemma of cells is thin, folded, without a homogeneous substance between adjacent membranes of neighbouring cells (Plate III, Fig. 5). The sperm is more regular, circular in its

shape, it has less membranous organelles and mitochondria (13) than the foregoing species. The plasmalemma is underlaid by tubular structures of which some collapse and enclose cisternae of the agranular, endoplasmic reticulum (Plate IV, Fig. 2). Other, differentiated organelles contain 3—4 microfilaments under the multiple membrane, which communicates with the plasmalemma by means of a short, electron dense canal with an opening pore (Plate IV, Fig. 4). In *T. spiralis*, the formation of tubules in the sperm starts already in the testis; in the seminal vesicle, these tubules form concave structures of which several fuse and enclose vesicles. Tubules originate from evaginations of the plasmalemma of the sperm. Their multiple plasmic membrane contains a homogeneous, faintly fibrillar substance. Several evaginations anastomose, others appear as concave tubules in the cytoplasm, others fuse and enclose part of the cytoplasm (Plate II, Fig. 6). A comparison of sperms of the two *Trichinella* species, from the vas deferens of the male and the seminal sac of the female, shows that the structure of membranous organelles differs even in individuals of the same species. Membranous organelles of the sperm in the reproductive organ of the female contain 8—9 microtubules (Plate IV, Fig. 6) running horizontally in direction of the connecting canal which opens in a pore into the plasmalemma of the sperm. In contrast, organelles in sperms located in the female sexual organ differ in shape: they are oval in *T. nativa*, irregular in *T. pseudospiralis*, sac-shaped and largest in *T. spiralis*. Membranous organelles of the sperm in the male sexual organ of *T. nativa* arealways spherical. During the fertilization of oocytes, vesicles (vacuoles) are released from membranous organelles (Plate IV, Fig. 5) which are now small, spherical, and without transverse microtubules. In sections there are 3—4 microfilaments below the pore similar to those in organelles of sperms of the male reproductive organ.

DISCUSSION

In an electron microscopic study on the spermatogenesis of *T. spiralis*, Slomiamy et al. (1981) observed that germ cells proliferate peripherally in the testis, and that spermateleosis occurs in the centre (lumen) of the testis. A similar observation was made by Neill and Wright (1973) for *Capillaria hepatica*. We found either that spermatogonia and spermatocytes occurred at one side of the testis, and spermatids and spermatozoa at the other side, or that spermatozoa inside the testis were encircled by spermatogonia and spermatocytes. Our description of the microscopic structure of spermatogonia and spermatocytes of *T. nativa* and *T. pseudospiralis* was not different from that given by Slomiamy et al. (1981) for *T. spiralis*. However, we disclosed an additional feature: peripheral vacuoles were produced from evaginations of the plasmalemma into the cytoplasm of primary spermatocytes. In secondary spermatocytes, after a breakdown of the nuclear membrane, vacuoles communicated with the perinuclear area by means of elongate cisternae of the endoplasmic reticulum. At the time of a meiotic division of secondary spermatocytes, a communication of future spermatids with the peripheral cytoplasm of the spermatocyte was established by bridges reported also by Slomiamy et al. (1981) for *T. spiralis*. Below the plasmalemma, the bridge contained vacuoles, vesicles of the Golgi apparatus, a small number of mitochondria, elongate cisternae of the endoplasmic reticulum running parallelly to the mid-portion of the bridge, and membranes similar to the microtubules described by West (1978) for *Hydra hymanae*, and regarded as remnants of a dividing spindle apparatus. We observed a dilation of vacuoles, vesicles and cisternae of the endoplasmic reticulum at the site of the bridge, and the origin of a system of cavities below the attenuated plasmalemma of the bridge after a re-arrangement of mitochondria close to the site of a chromatin condensation of the spermatid. After a separation of spermatids from the bridge, it was eliminated as a "residual body" together with remnants of the peripheral cytoplasm. This mode of elimination of the cytoplasm of sperms was described by Goldstein and Triantaphyllou (1980) for *Meloidogyne haple*. A separation of the cytoplasmic lobe from a group of spermatids adjacent to the site of the intercellular bridge was described by Neill and Wright (1973) for *Capillaria hepatica*. These authors observed tubules in the early spermatid; these appeared to collapse upon themselves forming concave vesicles which, in their turn, appeared to fuse with adjacent units to give double membrane loops enclosing a portion of the cytoplasm. Early spermatids and spermatozoa in the testes of *T. nativa* and *T. pseudospiralis* had no tubules from which membranous organelles could originate. On the other hand, signs of a starting tubule formation was observed in sperms of *T. spiralis* at a time at which it was still in the testis. Tubule formation started as evaginations of the plasmalemma which were ununiform in length and shape; some were branching, most of the tubules terminated in a vesicle. The description of the origin of membranous organelles from collapsing tubules in sperms of *T. spiralis* given by Slomiamy et al. (1981) did not differ from that given by Neill and Wright (1973). A number of authors suggested that vesicles of the Golgi apparatus and granules of the terminal sac of cisternae of the endoplasmic reticulum participated in the production of membranous organelles (Lee 1971, Beams and Sekhon 1972, McLaren 1973), the function of which was not understood. Goldstein and Triantaphyllou (1980) put forth an association of tubules with the motility system. We observed these differences in the structure of membranous organelles in mature sperms from the vas deferens of the male and the receptaculum seminis of the female: Organelles of sperms in the female reproductive organ contained a system of parallel, longitudinal microtubules running in direction of the pore, in addition to the four original microfilaments. While the oocyte was fertilized, these organelles released vesicles (Hulínská and Shaikenov 1982). Sperms from the vas deferens of *T. nativa* and *T. pseudospiralis* differed in the structure of the plasmalemma in both the shape and the size of membranous organelles, and in the presence of a pore connecting these organelles with thin plasmalemma (*T. pseudospiralis*). Differences in the structure of sperms recovered from the vas deferens of the male and the uterus of the female, and demonstrated in these *Trichinella* species confirmed that Burghardt and Foor (1975) were right in assuming that the uterus produced a stimulating substance which changed the structure of the sperm.

РАЗВИТИЕ И СТРУКТУРА СПЕРМАТОЗОИДОВ *TRICHINELLA NATIVA*, *T. PSEUDOSPIRALIS* И *T. SPIRALIS* (NEMATODA)

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Резюме. Различия между *T. nativa*, *T. spiralis* и *T. pseudospiralis* не проявляются во время сперматогенеза. При митотическом делении сперматогонии соединены с помощью мостика. Вакуоли возникают из выпячиваний плазмалеммы в цитоплазму первичных сперматоцитов. Во вторичных сперматоцитах эти вакуоли соединены с перинуклеарной областей при помощи эндоплазматического ретикулюма. Сперматиды, возникающие при первом и втором мейотических делениях вторичных сперматоцитов, соединены с помощью цитоплазматического мостика. Расширением вакуолей и цистерн отделяется от периферической цитоплазмы группа четырех сперматид. Трубчатая оболочка, окружающая спермы в семенниках *T. spiralis* и ее выпячивания, образующие трубочки в цитоплазме, не наблюдались в семенниках видов *T. nativa* и *T. pseudospiralis*. Спермы в vas deferens отдельных видов отличаются по структуре плазмалеммы, количеству митохондрий и форме мембранных органелл. Мембранные органеллы вида *T. pseudospiralis* имеют отверстия. Спермы

в матке самки отличаются от сперм в vas deferens самца по структуре мембранных органелл, содержащих, кроме первоначальных микрофиламентов, систему параллельных продольных микротрубочек, направленных к отверстию. Эти различия могут оказывать влияние на функциональное состояние vas deferens при копуляции. Однако подобное влияние может оказывать также контакт со стимулирующим веществом, выделяемым из матки самки в течение копуляции. Следовательно эти различия нельзя считать видоспецифичными характерами.

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Explanation of lettering in plates and figures

A — spermatogonia, B — early, primary spermatocyte, C — older, primary spermatocyte, D — secondary spermatocyte, E — spermatids 1, 2, 3, F — residual body, G — spermatozoa, H — connecting bridge, I — vacuoles, J — centriole, K — chromatin condensation, L — microfilament, M — mitochondria, N — nucleus, O — membranous organelles, P — plasmalemma, Q — outfoldings of secondary cells, R — ribosomes, S — cisternae of the endoplasmic reticulum, T —vesicles of the Golgi apparatus, U — connecting desmosomal complex, V — granules, X — pore, Y — tubules.

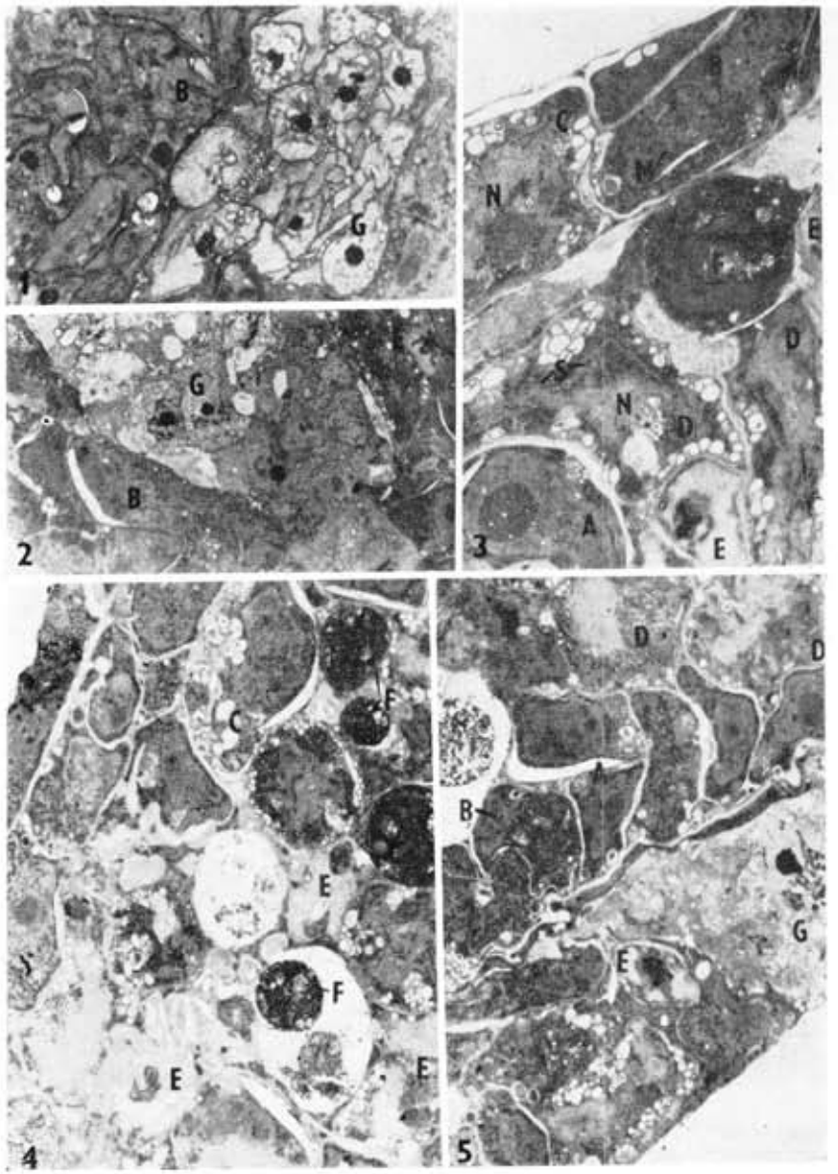

Fig. 1. *Trichinella nativa*, distal end of the testis with peripherally organized spermatocytes encircling spermatids and spermatozoa (× 5,280). **Fig. 2.** *Trichinella pseudospiralis*, spermatozoa (G) in mid-testis surrounded by spermatocytes (B) (× 4,290). **Fig. 3.** Spermatogonia (A) of *T. nativa* have a spherical nucleolus in the nucleus. Vesicles of the Golgi apparatus occur in a narrow strip of cytoplasm. Primary spermatocytes (B) contain an increased number of mitochondria. In an old spermatocyte (C) with a diffusive nucleus, vacuoles and vesicles ring transparent areas in the cytoplasm. A secondary spermatocyte (D) containing an increased number of long cisternae of the endoplasmic reticulum around the nuclear region. Spermatids (E) contain dense mitchondria organized centrally around a chromatin condensation. (× 7,200). **Fig. 4.** Section through the testis of *T. spiralis* showing spermatocytes (B), (C), spermatids (E) and a residual body (F) (× 6,200). **Fig. 5.** Testis of *T. spiralis* with spermatogonia (A) and spermatocytes on the one side, spermatids (E) and spermatozoa (G) on the other side (× 6,200).

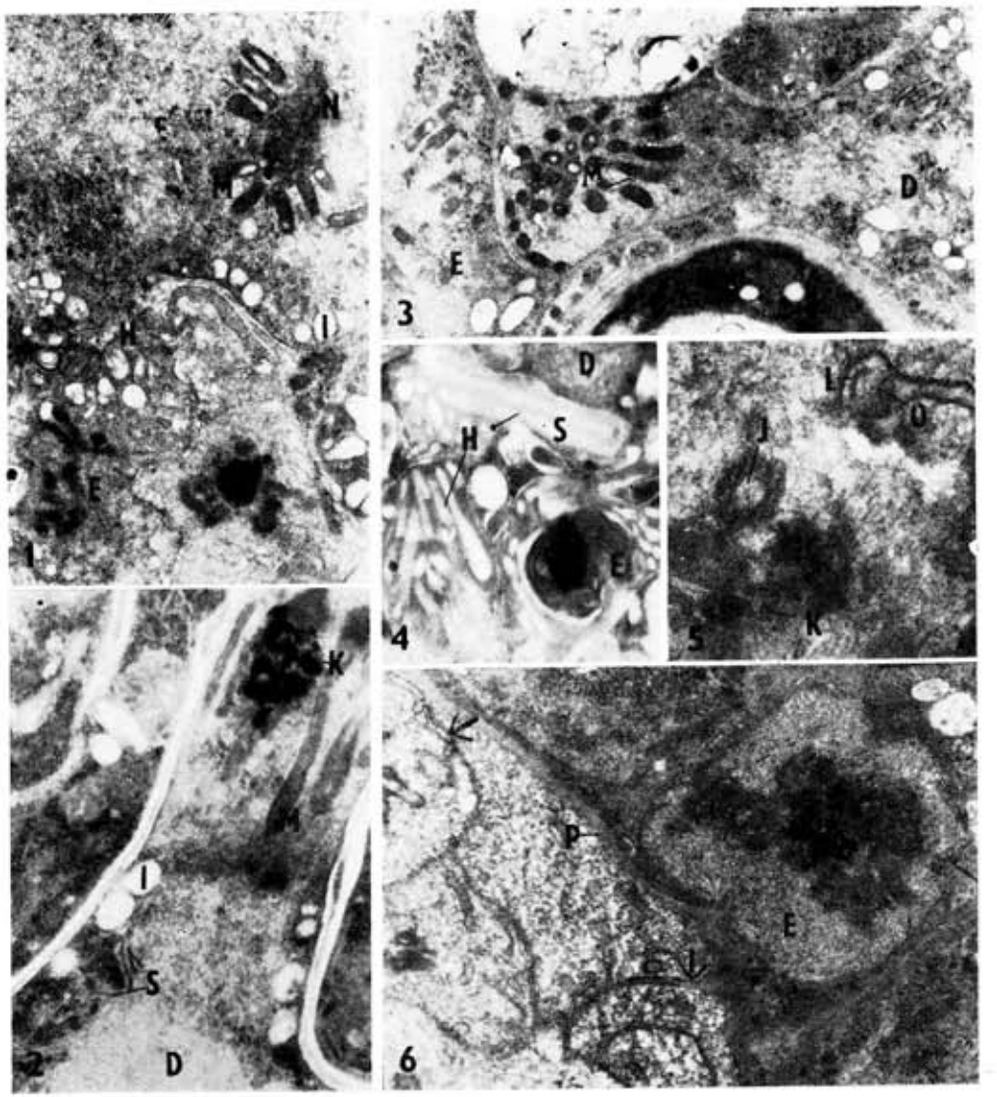

Fig. 1. Spermatids of *T. pseudospiralis* joined by a bridge (H) with the peripheral cytoplasm of a secondary spermatocyte (× 9,470). **Fig. 2.** A differentiating spermatid (E) of *T. nativa*, containing long mitochondria arranged around a condensation of chromatin in the nuclear region. The spermatid is joined to a secondary spermatocyte (D) whose peripheral cytoplasm contains vacuoles (× 10,580). **Fig. 3.** Joining of two spermatids (E) of *T. spiralis* with the vacuolated cytoplasm of a secondary spermatocyte (D) (× 9,200). **Fig. 4.** Dilation of vacuoles and cisternae of the endoplasmic reticulum in the bridge connecting spermatids of *T. nativa* (× 10,500). **Fig. 5.** Centriole formed by 9 microtubules in the nuclear area of a spermatid of *T. nativa*. An originating membranous organelle with 4 microfilaments (× 21,200). **Fig. 6.** Testis of *T. spiralis* with spermatids (E) covered with a multiple, plasmic membrane. Note evaginations of the plasmic membrane (arrow) in spermatozoa. Several anastomosing evaginations produce tubules in the cytoplasm (× 11,200).

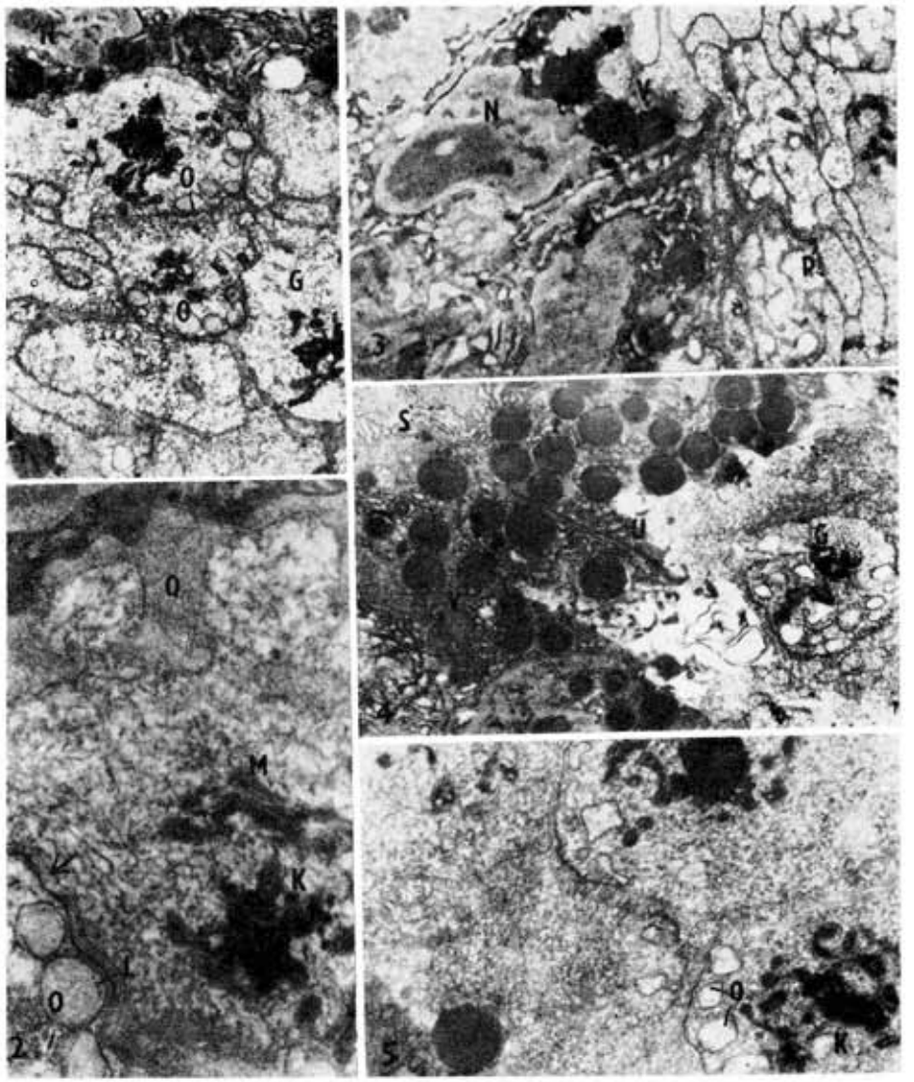

Fig. 1. Vas deferens of *T. nativa*. The secretory cell has a large, electron lucid nucleus, little chromatin and a dense nucleolus, a well-developed endoplasmic reticulum and granules in the cytoplasm. Secretory cells form long outfoldings among spermatozoa. Spermatozoa contain large, spherical, membranous organelles and a plasmalemma thickened with a homogeneous substance (×8,800). **Fig. 2.** Spherical, membranous organelles with four microfilaments below the multiple, plasmic membrane (arrow) which contains a homogeneous substance identical to that in the outfoldings (Q) (× 16,320). **Fig. 3.** Cells of the vas deferens of *T. nativa* containing a large nucleus, a nucleolus, and a well-developed endoplasmic reticulum. Vesicles of the Golgi apparatus in the cytoplasm. Numerous outfoldings of cells into the lumen (× 9,800). **Fig. 4.** Vas deferens of *T. pseudospiralis* containing numerous granules sending short, villi-like processes into the lumen. Note the desmosomal complex connecting the cells. The plasmalemma of spermatozoa is thin, neither the homogeneous substance nor outfoldings among spermatozoa are visible (×10,000). **Fig. 5.** Spermatozoa of *T. pseudospiralis* with a thin plasmalemma underlaid by irregularly shaped, membranous organelles (× 13,500).

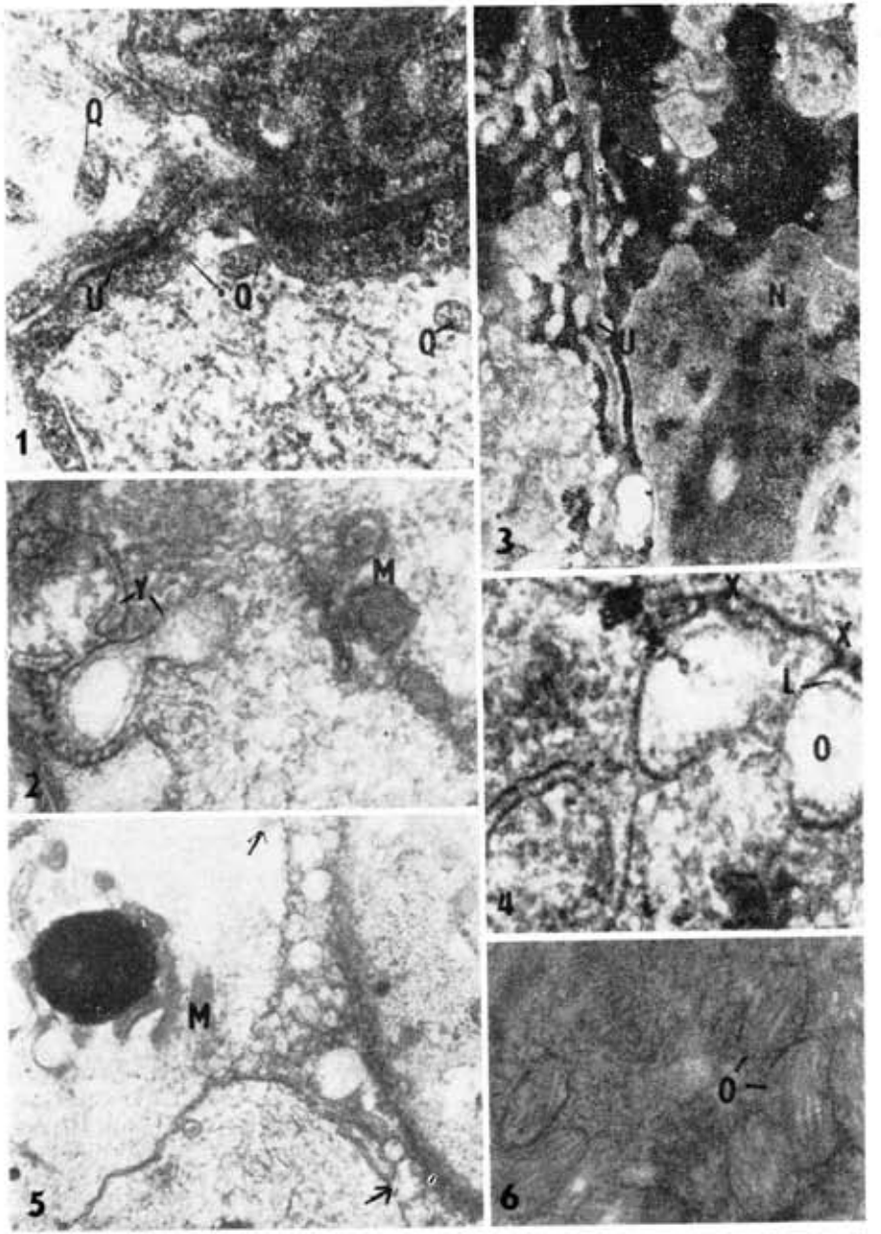

Fig. 1. Desmosomal complex of secretory cells in the vas deferens of *T. pseudospiralis* passing into plasmic outfoldings which protrude into the lumen (× 24,200). **Fig. 2.** Formation of membranous organelles from tubular structures in *T. pseudospiralis* (× 21,300). **Fig. 3.** Desmosomal complex in the vas deference. Vacuoles and vesicles of the Golgi apparatus containing a substance which is less electron dense than that in the granules are present below the plasmalemma (× 17,200). **Fig. 4.** Membranous organelles in sperms of *T. pseudospiralis* are connected with a canal opening through a pore into the plasmalemma (× 48,000). **Fig. 5.** Spermatozoa in the receptaculum seminis of *T. spiralis* in the vicinity of an oocyte. A secretion is released into the spaces between the individual plasmalemmas, membranous organelles are smaller in size (arrow) (× 12,200). **Fig. 6.** Membranous organelles in sperms of *T. pseudospiralis* recovered from the uterus contain a system of micro-