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FINE STRUCTURE OF THE DEFERENT DUCT AND THE SEMINAL VESICLE OF DIAPTOMUS CONEXUS (CRUSTACEA: COPEPODA)

M. Durfort,* M. G. Bozzo * & M. Poquet *

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RESUM

Ultrastructura del conducte deferent i de la vesícula seminal de Diaptomus conexus (Crustacea: Copepoda)

L'estudi ultrastructural de les cèl·lules que formen els tres trams anatòmics del conducte deferent demostra el seu caire secretor. I efectivament, les cèl·lules epitelials presenten un reticle endoplasmàtic rugós molt desenvolupat, nombrosos dictiosomes alhora que dues poblacions de mitocondris.

La riquesa d'estructures vesículo-membranoses minva a mesura que ens apropem a la vesícula seminal. Existeix, com és lògic, un paral·lelisme entre el desenvolupament de les estructures implicades en la síntesi i els grànuls de secreció formats per aquestes cèl·lules.

Els grànuls de secreció, uniformement densos als electrons, ocupen gran part de l'àrea apical de les cèl·lules i són molt més nombrosos en les cèl·lules dels trams proximal i mitjà que no en les del tram distal i en les de la vesícula seminal.

Els grànuls de secreció formats i lliurats per les cèl·lules epitelials del conducte deferent, juntament amb productes elaborats a nivell del testicle, són responsables de la formació de la cutícula, de la regió central i dels diversos materials que constitueixen la paret i el contingut dels espermatòfors.

INTRODUCTION

Spermatophores are a kind of «store-box» of spermatozoa and seminal liquids; their formation is a reproductive mechanism described in some invertebrate groups. The number of spermatophores lodged by each specimen on of the female genital pore during copulation amounts to one or two.

The formation of spermatophores and of copulatory mechanisms are therefore considered as preadaptive requirements for the transition from aquatic to terrestrial life (GHILAROV, 1956, 1959; SCHALLER, 1965, 1979; SHAROV, 1965).

* Departament de Morfologia Microscòpica. Facultat de Biologia. Universitat de Barcelona. 08028 Barcelona. Spain.

The presence of spermatophores has been described mainly in Arthropoda. A systematic, not structural, revision can be found in SHALLER (1979). Among the structural and ultrastructural studies carried out with Arthropoda we must mention LINLEY (1981), on Diptera, DALLAI (1975) on Collembola, and GADZAMA (1974) and HAPP *et al.* (1970) on coleoptera.

Crustacea Copepoda have been the most extensively studied arthropods; we must record the structural works carried out by BOULIGAND (1965), FAHRENBACH (1962), LEE (1972), RAYMONT *et al.* (1974), GHARAGOZLOV-VAN GINNEKEN (1978), POCHON-MASSON (1979), BLADES & YOUNGBLUTH (1981), all of them about free living copepoda, and also the papers by the group from Montpellier about parasitic copepoda on fisches: MANIER *et al.* (1977), COSTE *et al.* (1978), ROUSSET *et al.* (1978, 1983).

Although the presence of spermatophores has been described mainly in Arthropoda, it must be remembered that the first study about this organ was carried out by MILNE (1942) on Cephalopoda spermatophores. GOURBAULD & RENAUD-MORNANT (1982) have found spermatophores in a species of marine free living nematode, reproductive system unknown in this group.

In outline, the male reproductive system in copepoda can be composed of one or two testis, one or two deferent ducts and one or two seminal vesicles. In all cases, we find a deferent duct which is made up by a glandular epithelium with different features in each tract. Its cells are always responsible for the formation of secretion granules which are released into the duct in a merocrine way, and later they will be involved in the formation of the cuticular wall of spermatophores as well as the core of the spermatozoa-carrier box (RAYMONT *et al.*, 1974).

Secretions produced by the deferent duct have been extensively studied by BLADES & YOUNGBLUTH (1981), who succeeded in distinguishing twelve types of secretion granules in *Labidocera aestiva*: from those released in the front tract of the deferent duct to those which build up the mature spermatophore.

According to certain authors, the seminal vesicle would complete the cycle of secretion granules production, as it is described by ROUSSET & RAIBAUT (1983) in *Lerneutoma asellina* and by BLADES & YOUNGBLUTH (1981) in the species previously mentioned, *L. aestiva*. Other authors think, however, that it is simply a store.

MATERIAL AND METHODS

Specimens of *Diaptomus conexus* we studied come from Canadian lakes: Little Quill and Blaine. They were prepared for studying by transmission and scanning electron microscopes. They were first prefixed in glutaraldehyde-paraformaldehyde (3.5 %, pH 7.3) in phosphate Sörensen buffer or in sodium cacodylate, for two hours at 4 °C. Afterwards they were thoroughly washed in buffer and post fixed in osmium tetroxide (2 %) buffered in the same way. Some of the specimens stayed in the prefixative solution for 36 days, after which they were washed and post-fixed like the others. After their ultrastructural study we can affirm that fixation was correct.

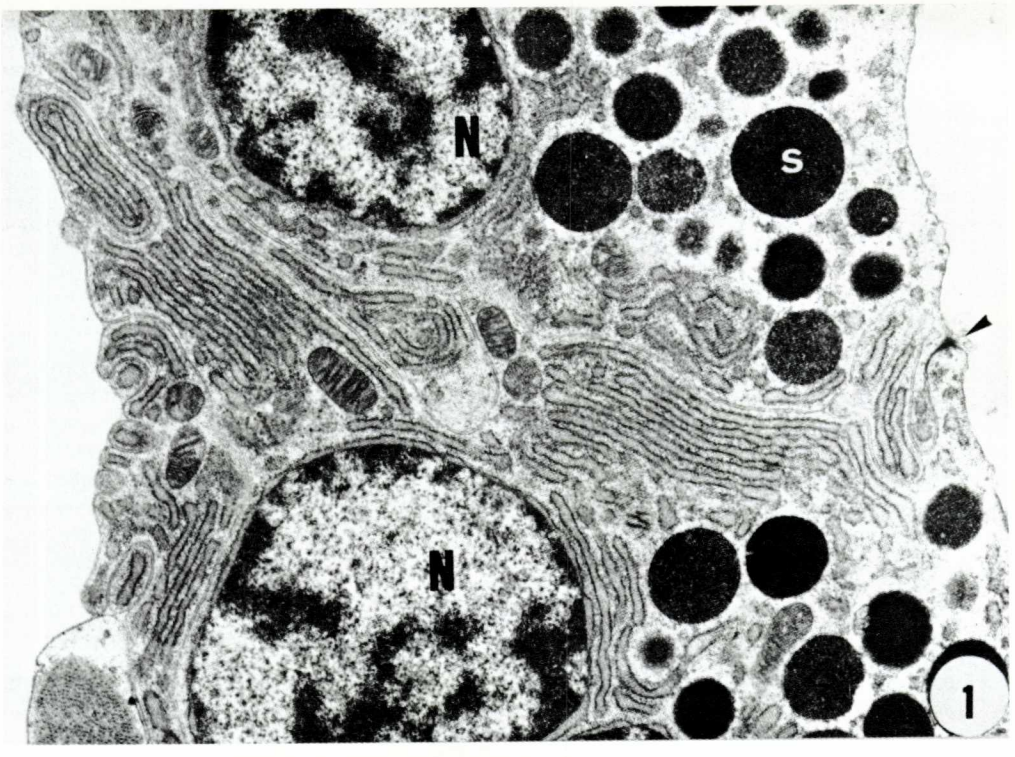
After careful dehydration by means of an ascendent gradation of acetones or alcohols, we continued with an inclusion of the specimens. We chose SPURR (1969) as the inclusion medium; being a low density resin, penetration through the cuticle of copepoda is relatively easy.

Semi-thin sections were stained according to conventional methods: *a*) methylene blue 1 % with borax 1 %; *b*) fuchsine-methylene blue; *c*) Shiff reagent technique.

Ultra-thin sections were obtained with an Ultracut OMU and subjected to double staining with uranyl acetate and lead ci-

FIG. 1. Tight junction (arrow) between two cells of the proximal deferent duct. of *Diaptomus conexus* Large nuclei (N) with lax chromatin, abundant granular endoplasmic reticulum and a great number of secretion granules (s). 15.000 x.
Zona de contacte (fletxa) entre dues cèl·lules del conducte deferent proximal de *Diaptomus conexus*. Nuclis grossos (N) amb cromatina laxa, abundant reticle endoplasmàtic rugós i nombrosos gràuls de secreció (s). 15.000 x.

FIG. 2. Detail of the basal zone of a cell of the proximal deferent duct. Notice the magnificent Golgi complex (G) which delimits a zone with highly granulous electron dense material. Detail of the basal muscular bundles in cross section. 45.000 x.
Detall de la part basal d'una cèl·lula del conducte deferent proximal. Observeu el magnífic complex de Golgi (G) que delimita una àrea de material altament granulós i dens als electrons. Detall d'un dels feixos musculars basals, en tall ransversal (FM). 45 000 x.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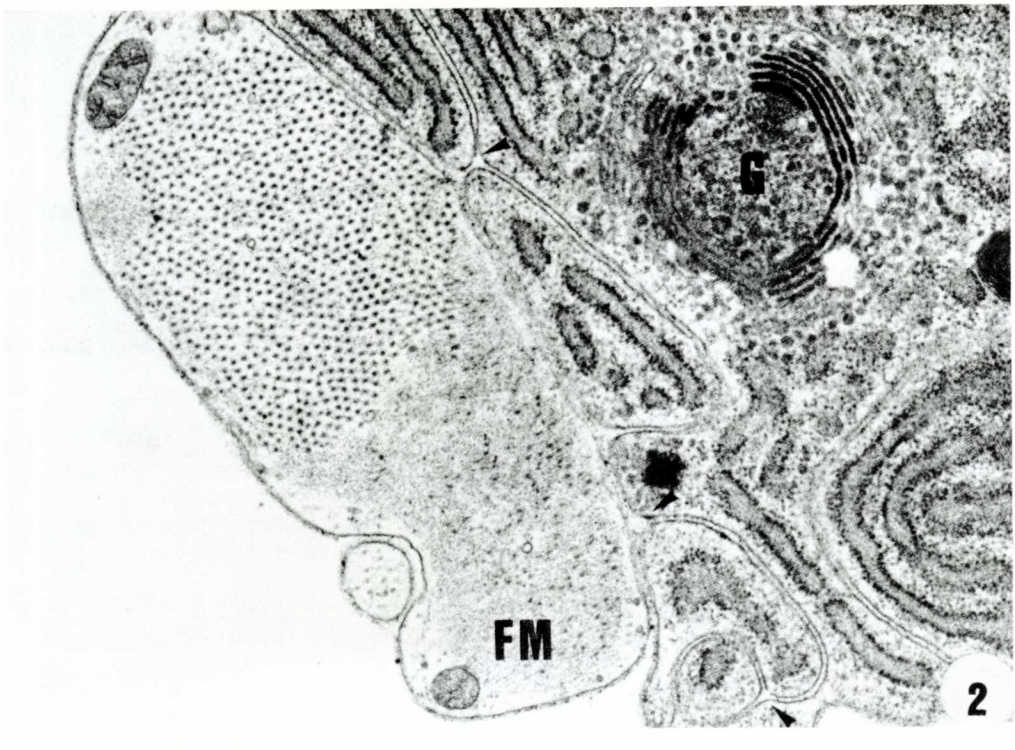
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trate. They were observed with a Phillips 200 transmission electron microscope from the Electron Microscopy Service of the University of Barcelona.

Some ultra-thin sections were stained with phosphotungstic acid (RAMBOURG, 1967) and others with silver protein (THIÉRY, 1967) in order to reveal the mucopolysaccharidic nature of the secretion granules.

Samples to be studied by scanning electron microscopy were subjected to careful dehydration by an ascendent gradation of acetones and immediately treated with amyl acetate before reaching the critical point.

Shadowing was carried out by covering the material with a gold layer and allowing this metal to evaporate during three minutes. This was done according to the «sputtering» method, using an E-5000 Polaron; in this way we could obtain a gold layer 300 Å thick.

OBSERVATIONS

Deferent duct

In *Diaptomus conexus* we find a deferent duct which is paired; we can distinguish three tracts in it: the testicular or proximal one, the median one and the distal one, or I, II and III according to various authors' nomenclature.

Proximal tract. This zone of the duct is the nearest to the testicle; in part it runs parallel to the gland, then turns and runs upwards. This zone is placed between the testicle and the mid-gut. In this tract of the duct the wall is made up by a mucosa (epithelium and basement membrane) and some bands of striated muscular fibres arranged periodically.

Mucosa. This is made up by a layer of cylindrical epithelial cells. Between these cells, on their apical zone, we can observe fight junctions. Along their lateral membranes can also be found some tight junctions, as well as interdigitations (Fig. 1). Sometimes we can observe canaliculi. On the apical zone the membrane emits a few short microvilli, covered by a well developed glucocalix.

The basal membrane is highly plicated and deep invaginations almost reach the nucleus. These invaginations are mainly occupied by endoplasmic reticulum vesicles which are often arranged concentrically.

Near the circular muscular band, the basement membrane often emits a greater number of invaginations than in the zones where there is no contact (fig. 2). This fact can be considered as a feature proper to this tract of the deferent duct.

Nucleus. There is only one nucleus; it is spherical and occupies the central basal zone of the cell. It is very bulky, measuring from 15 to 20 μm. There is a well developed perimembranous space, occupied by an uniformly electron dense material which has the same texture as that which fills the enlarged ergastoplasmic vesicles.

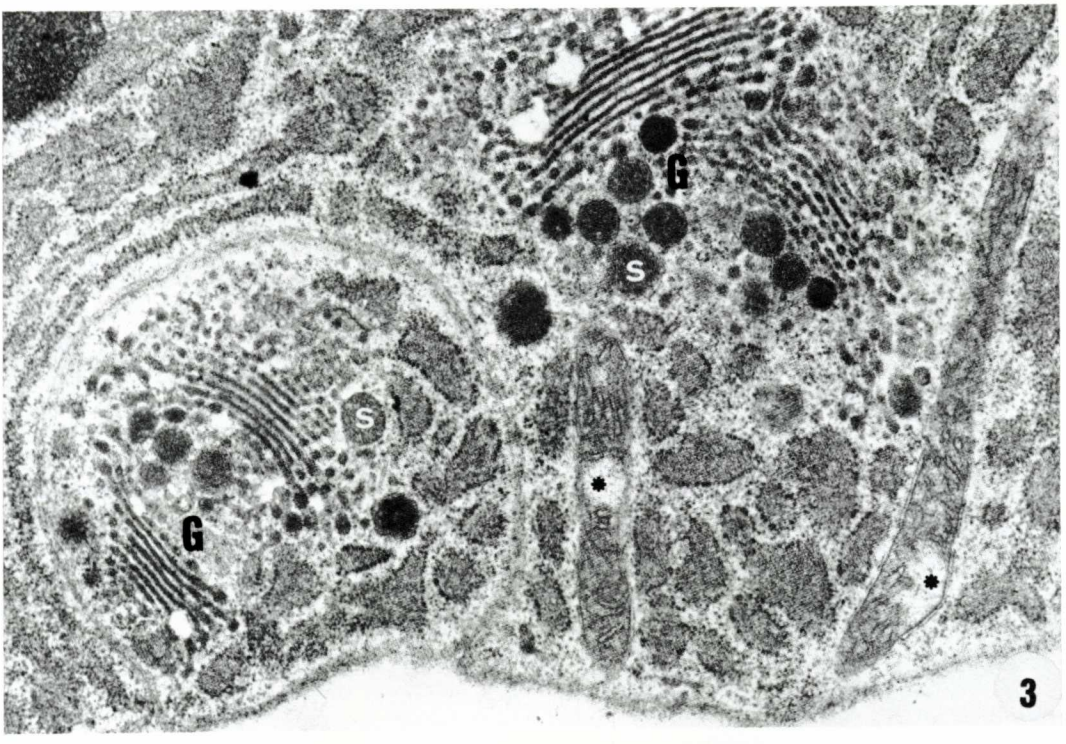
Chromatin is found mainly in big granules attached to the inner nuclear membrane, although in the central nucleoplasm some accumules of different morphology stand out from others, depending on the direction in which the material has been cut.

There is a nucleolus measuring 2 μm in diameter, usually placed eccentrically. It shows an interesting evolution during cellular cycle, although it is very difficult to distinguish between its two components.

Golgi complex. In the highest activity stage this appears very developed, with various dictyosomes measuring from 8 to 10 μm; they can be found between the nuclear zone and the apical pole of the cell, in a number between 8 and 10. Each dictyosome is made up by 6 to 12 cisternae and in the maturation zone many secretion granules of different diameter can be observed.

We would mention that the median and proximal tracts of the deferent duct are those in which the number of dictyosomes is larger. They can easily be made visible by using the conventional staining techniques: using uranyl acetate and lead citrate.

Ergastoplasm. This is highly developed and occupies most of the cellular space. Its vesicles are often placed parallel to the cellular membrane while they completely surround the nucleus (figs. 1, 4). In the zones where interdigitations are ob-



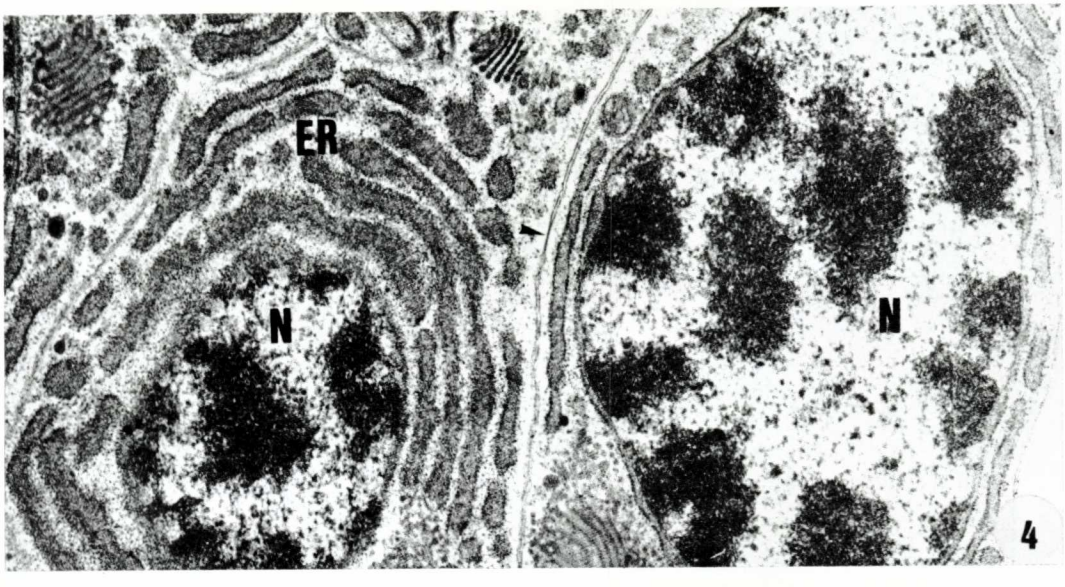


FIG. 3. Notice the great development of the Golgi complex, proper to the proximal deferent duct, as well as a great number of secretion granules (s). Two well developed mitochondria can also be observed (*). 45.000 x.
Observeu el gran desenvolupament del complex de Golgi, propi d'aquest tram del conducte deferent proximal, així com nombrosos grànuls de secreció (s). En aquesta imatge es veuen dos magnífics mitocondris (*). 45.000 x.

FIG. 4. A well developed tubuliform ergastoplasm (ER) surrounds the nuclei (N). 40.000 x.
Un ergastoplasma tubuliforme ben desenvolupat envolta els nuclis. 40.000 x.

served, the ergastoplasm appears in a concentric display (fig. 13).

We can often notice fusion processes between expansions of granular endoplasmic reticulum vesicles and vesicles released by the Golgi complex from the maturation zone. During the most important stage of secretion, vesicles appear very enlarged, measuring 8 μm on average; they show uniformly electron dense contents.

The ribosomal fraction is very important in this tract of the deferent duct. A great number of Palade granules are scattered between ergastoplasmic saccules and Golgi complexes.

Microtubules. Fine microtubule bundles can be found between endoplasmic reticulum vesicles, as well as in the vicinity of secretion granules. We suppose they are labile, since it has been impossible to localize microtubular structures in the specimens which stayed in glutaraldehyde-paraformaldehyde for 36 days.

Mitochondria. We have not found a great number of these organelles. Two different populations can be observed: in one of them mitochondria show an elliptical shape and measure from 6 to 8 μm in diameter; in the other they are very elongated, measure about 8 to 20 μm in length and their number is smaller. In these enlarged mitochondria, cristae are placed perpendicularly to the main axis, whereas in elliptical mitochondria they are parallel or perpendicular to the main axis.

These organelles are uniformly distributed, although they are often more abundant in the apical pole of the cell.

Lysosomes. Structures similar to residual vesicles, filled with mielinic formations, are sometimes found in the basal pole of these secretory epitelial cells (fig. 17). We would mention that they may have something to do with crinophagia processes, which have been recorded only in the testicular and median tracts of the deferent duct.

Secretion granules. In the first tract cells of the deferent duct, two populations of secretion granules stand out: minute dense granules measuring 5 μm in diameter, which are found in the maturation side of dictyosomes; and large granules, measuring about 20 μm in diameter which are mainly found in the apical pole of the cells and are very electron dense. The first are surrounded by a membrane; both types of granules are positive to phosphotungstic acid technique as well as to Thièry's technique. In serial sections different compactation degrees can be observed.

The last tract is the zone where a greater amount of granules can be noticed in the inmost epithelial cells.

In the third tract of the deferent duct the mucosa epithelium becomes a double layer. The first layer close to the lumen is made up of flattened cells with a greater number of microvilli than in the preceding tracts, and with a cytoplasm almost obliterated by secretion granules.

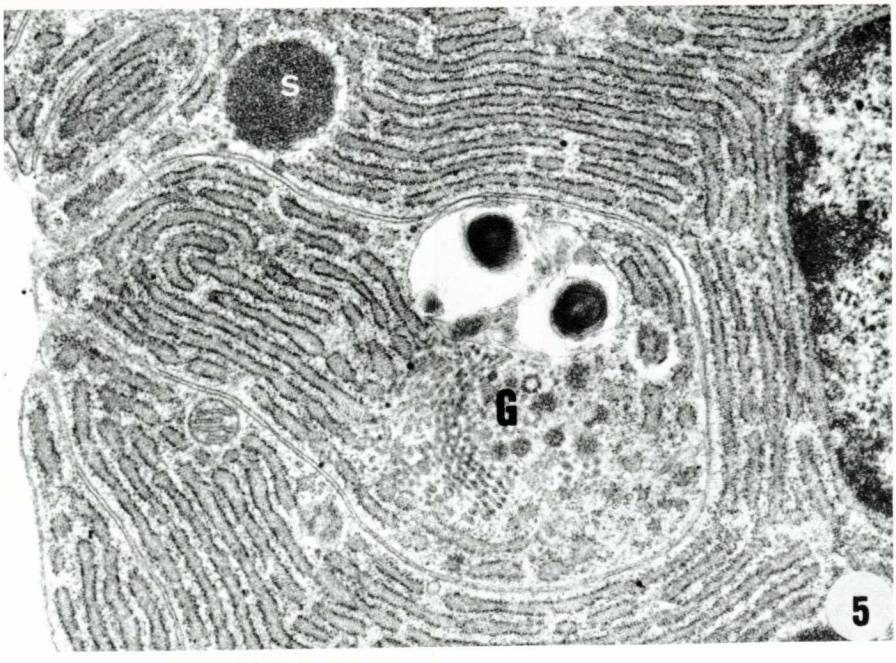
The furthest layer from the lumen is made up of cells which show the same features we have described for the first tract. We noticed in them a great number of interdigitations and basal membrane invaginations, as well as a tendency for dictyosomes to be placed in the apical pole. An important decrease of secretion granules with respect to the cells previous tracts is another feature proper to these basal cells.

Seminal vesicle. This measures 50-60 μm in diameter and is limited by a layer of

FIG. 5. Basal zone of the median deferent duct. Notice that granular endoplasmic reticulum shows an important development too. Tigth junctions are found between the cells.
Zona basal del tram mitjà del conducte deferent. Observeu com el reticle endoplasmàtic rugós presenta igualment un considerable desenvolupament. Hom veu contactes íntims entre les cèl·lules.

FIG. 6. Detail of a secretion granule (s) growing from a material produced by the Golgi complex. 45.000 x.
Detall d'un gràmul de secreció en formació (s) a partir de material procedent del complex de Golgi. 45.000 x.

FIG. 7. Apical zone of a secretory cell of the median deferent duct of *Diaptomus conexus*, full of secretion granules (s) which arise from the Golgi complex. 45.000 x.
Zona apical d'una cèl·lula secretora del tram mitjà del conducte deferent de *Diaptomus conexus*, carregada de gràmuls de secreció d'origen golgià (s). 45.000 x.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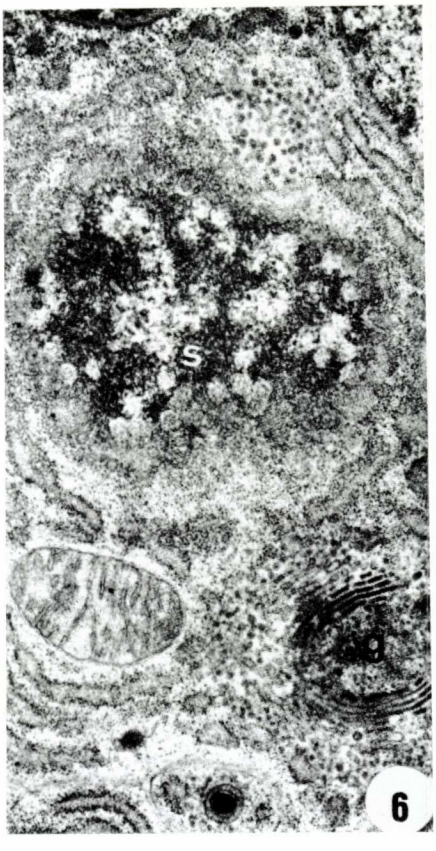
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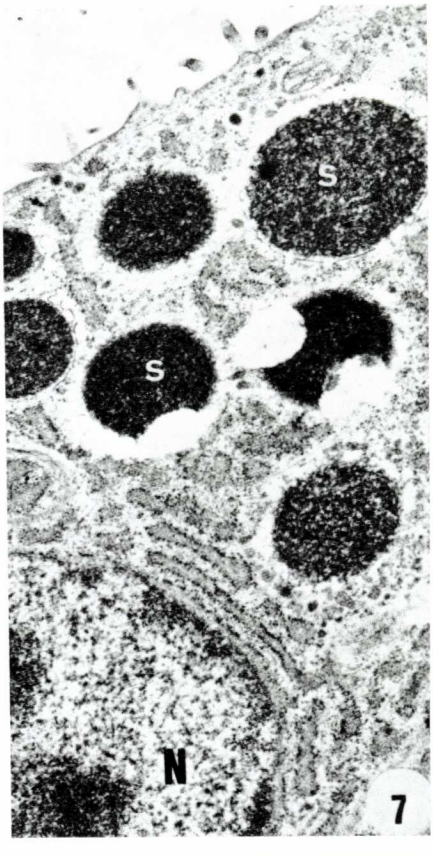
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very plastic cells, joined to each other by the same contacts we described for the preceding tracts.

Because of epithelial cells' plasticity, their height is variable. Their basal membrane shows a great number of folds, which sometimes remind us of renal podocytes, although here they are not lying on any other structure.

On the apical membrane we find a greater number of microvilli, and they are slenderer than the ones in the earlier tracts. Many endocytosis are also observed (figs. 7, 17).

The cells are mononucleated, like those of the earlier tracts, and show an important decrease in endoplasmic reticulum and dictyosomes. They still show some secretion granules in their apical pole, but they are smaller and less dense than those in the tracts observed earlier.

Some circular striated muscular bands are placed periodically joined to the secretory epithelium basement membrane. The fine structure of this muscular fibre is the one proper to Arthropoda. This muscular fibre is more developed in the first and second tracts of the deferent duct than in the third. In the testicular tract we can notice a greater number of basal plasmalemma invaginations along the contact zone with the muscular bundle (fig. 2) than in the same zone of the median tract (figs. 17, 18).

DISCUSSION AND CONCLUSIONS

Taking into consideration the direction in which ultrathin sections have been cut, together with ultrastructural features described for secretory epithelial cells, we can say that sometimes it becomes extremely difficult to recognize the site we are observing and to distinguish whether it is the testicular or the median tract; in this case, the observation of spermatophore formation degree can be useful.

The fact that epithelial cells along the deferent duct are mainly secretory was already mentioned by HEBERER (1932) and is manifested by a great development of ergastoplasm and Golgi complex, as well as by the presence of secretion granules, as was already noticed by RAYMONT *et al.* (1974) using an electron microscope. Secretory features found in the three tracts of the deferent duct correspond in outline to those found by RAYMONT *et al.* (1970) in *Calanus finmarchicus* and to those described in an harpaticoid species: *Tisbe holoturiae*, by POCHON-MASSON & GHARAGOZLOU-VAN GINNEKEN (1975) and GHARAGOZLOU-VAN GINNEKEN & POCHON-MASSON (1978, 1979), among others.

As distinct from *Tisbe holoturiae*, secretion granules found in *Diaptomus conexus* do not arise solely from fusion of Golgian vesicles, but also from coalescence of ergastoplasmic vesicles.

After shadowing we did not find any cytochemical differences among granules formed in each of the three tracts of the deferent duct in *D. conexus*. Although in the spermatophore we found important morphological differences among the materials which build up the core (fig. 16), we have never noticed the diversity of granules described by GHARAGOZLOU-VAN GINNEKEN & POCHON-MASSON (1979) in spermatophores of *T. holoturiae*.

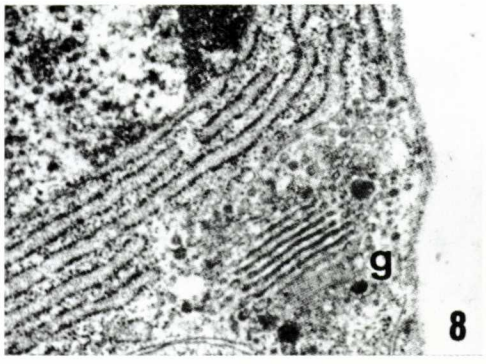
We agree with RAYMONT *et al.* (1974) in considering the testis to be made up of germinal cells and abortive cells. These last cells are involved in the formation of some of the materials which will make up the spermatophora capsule.

The seminal vesicle in *Diaptomus conexus*, as distinct from what happens in *Labidocera aestiva* (BLADES & YOUNGBLUTH, 1981) and in the species of Chondracanthida studied by ROUSSET & RAIBAUT (1983),

FIG. 8 to 11. Various aspects displayed by the Golgi complex (g) and its relation to the granular endoplasmic reticulum (er). 45.000 x.
Diversos aspectes adoptats pel complex de Golgi (g) i la seva relació amb el reticle endoplasmàtic rugós (er). 45.000 x.

FIG. 12 and 13. Arrangements shown by the endoplasmic reticulum (er) in cells of the median deferent duct. 45.000 x.
Disposicions adoptades pel reticle endoplasmàtic (er) en les cèl·lules del tram mitjà del conducte deferent. 45.000 x.

FIG. 14 and 15. Detail of secretion granules (s) in this median tract. In micrograph number 14 it can be seen that the granule is surrounded by a membrane. 45.000 x.
Detall de gràmuls de secreció (s) en aquest tram mitjà. Es pot observar a la fig. 14 com el gràmul és envoltat per una membrana. 45.000 x.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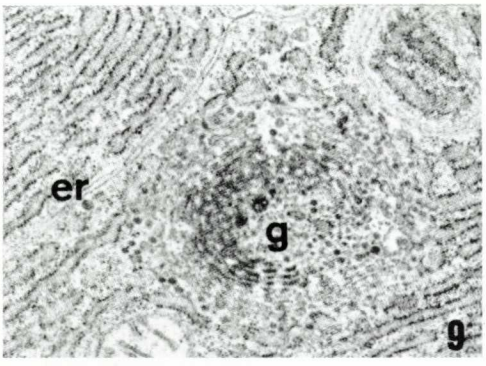
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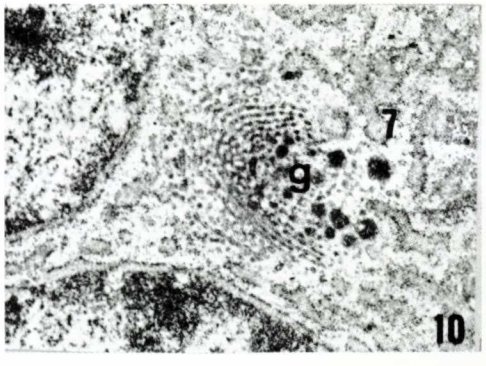
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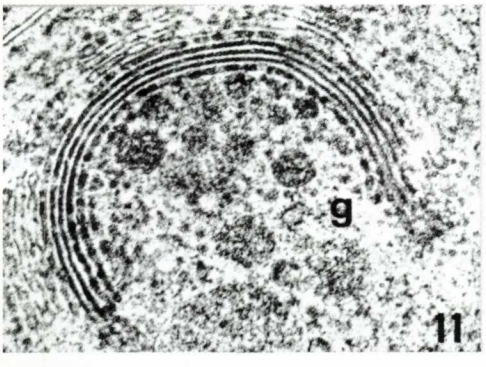
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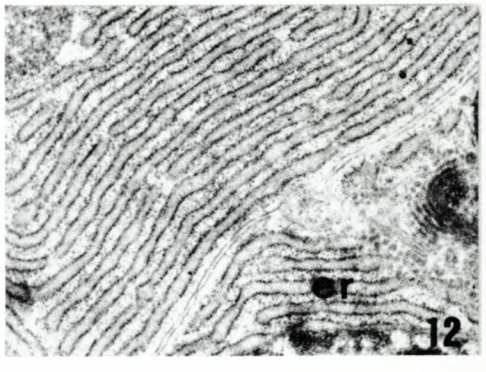
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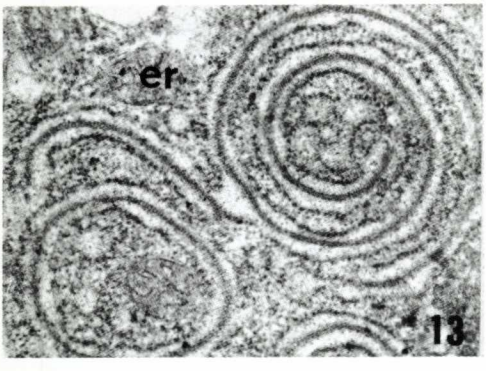
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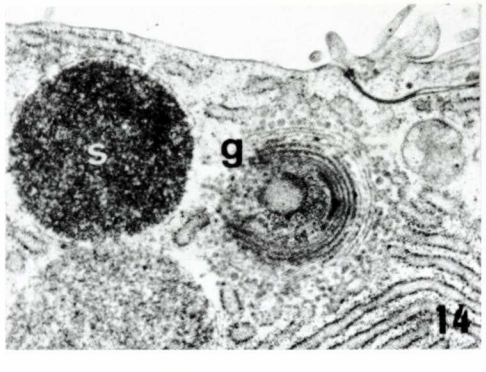
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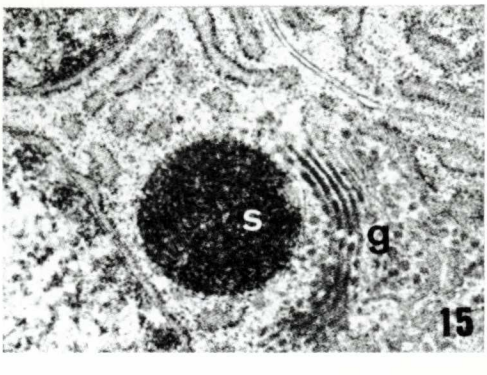
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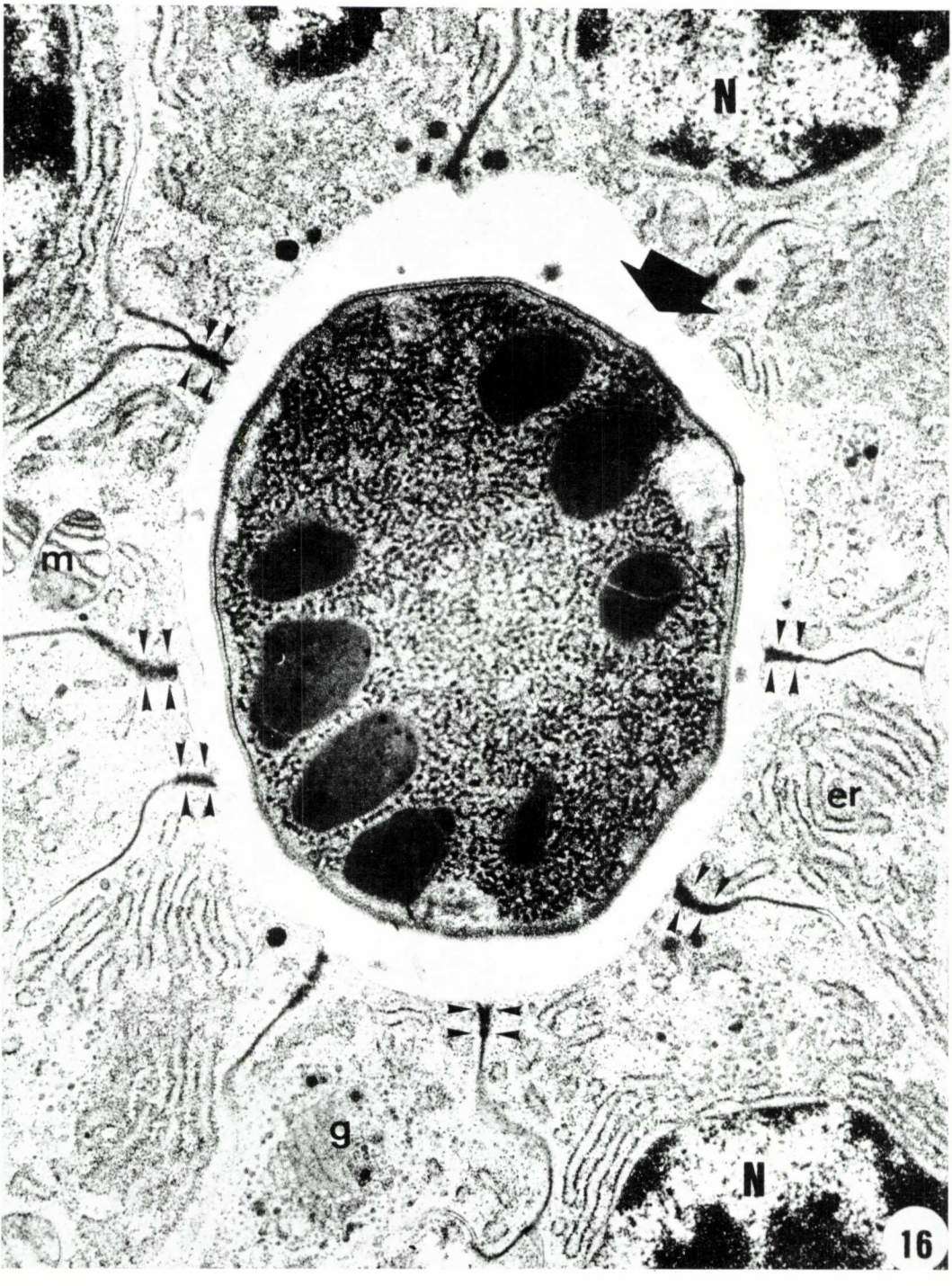

FIG. 16. Detail of the deferent duct in its median tract: the apical zone of the cells. Notice the tight junctions (arrows). The lumen is occupied by the growing spermatophore. 35.000 x.
Detall del conducte deferent, en el tram mitjà, corresponent a la part apical de les cèl·lules. Observeu els contactes (fletxes). La llum del conducte és ocupada per l'espermatòfor en formació. 35.000 x.

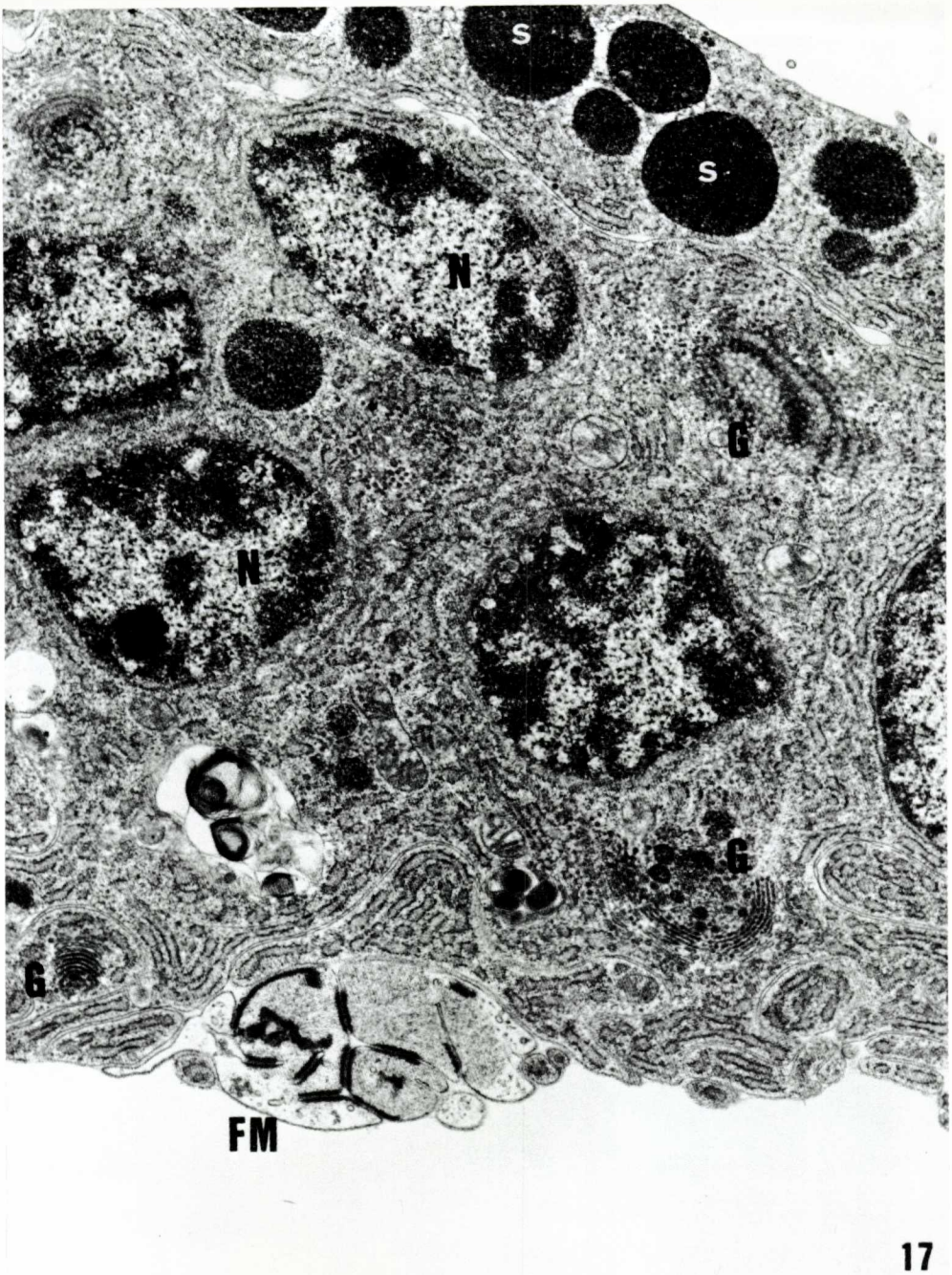

FIG. 17. In the terminal median zone of the deferent duct the wall has two or three layers, but we find the structural features to be the same as in the cells of the previous tract. A lysosome with mielinic structures within it can be seen in the basal zone. A muscular fibre can also be observed in cross section (FM). 23.000 x.

En la part mitjana terminal la paret del conducte deferent és bi- o triseriada, però les característiques ultrastructurals són les mateixes que en les cèl·lules del tram anterior. En la part basal es pot veure un lisosoma amb formacions mielíniques al seu interior. En aquesta imatge es pot veure una fibra muscular tallada transversalment (FM). 23.000 x.

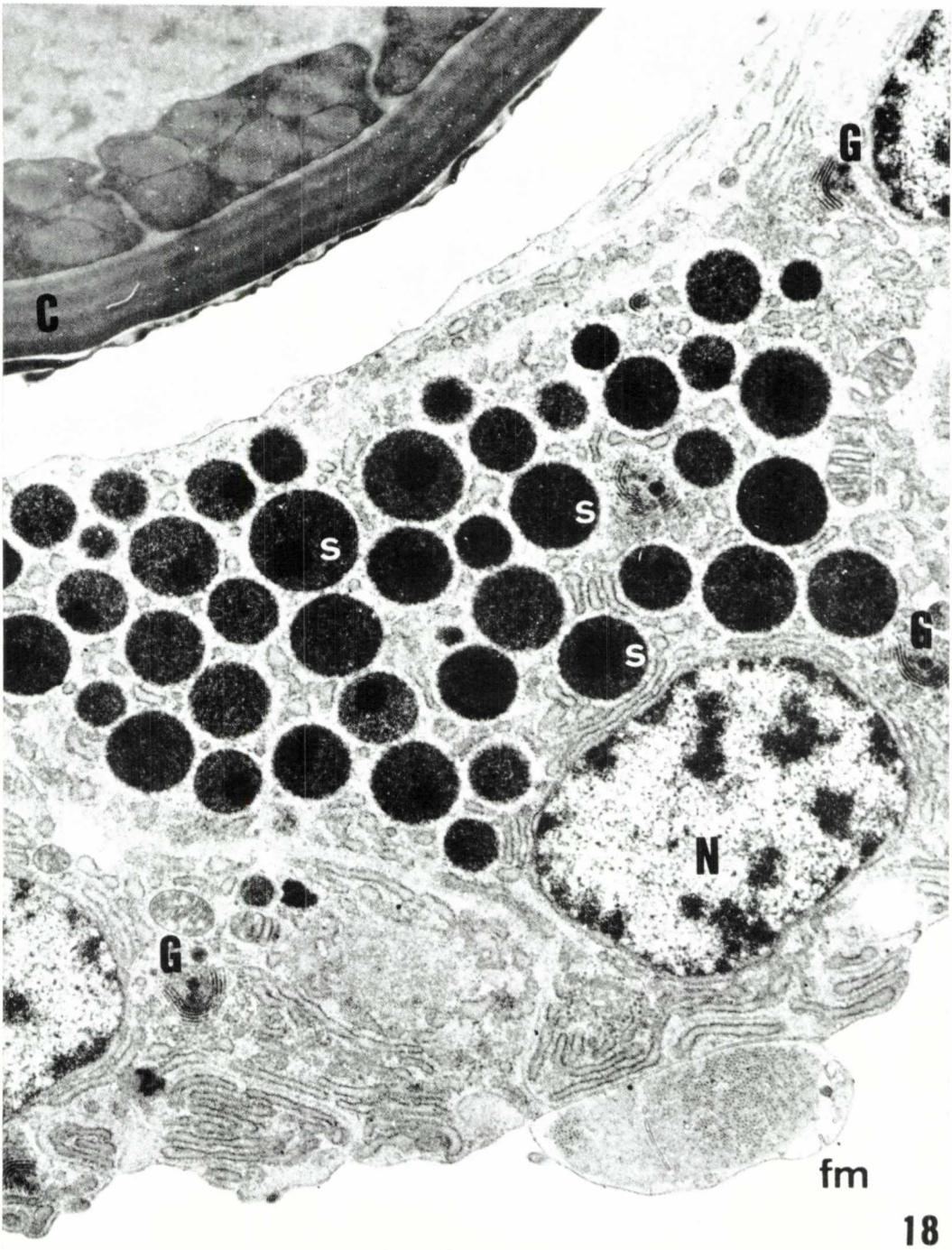

FIG. 18. Last tract of the distal deferent duct, although its ultrastructural features are still secretory, with a great profusion of secretion granules (S). The wall of the spermatophore (notice the striated cuticle, C) indicates to us which section of the deferent tract we are observing. 30.000 x.
Tram final del conducte deferent distal, si bé les característiques ultrastructurals continuen essent igualment secretores, amb una gran profusió de grànuls de secreció (s). La paret de l'espermatòfor (observeu la cutícula estriada, C) ens indica en quin tram del conducte deferent ens trobem. 30.000 x.

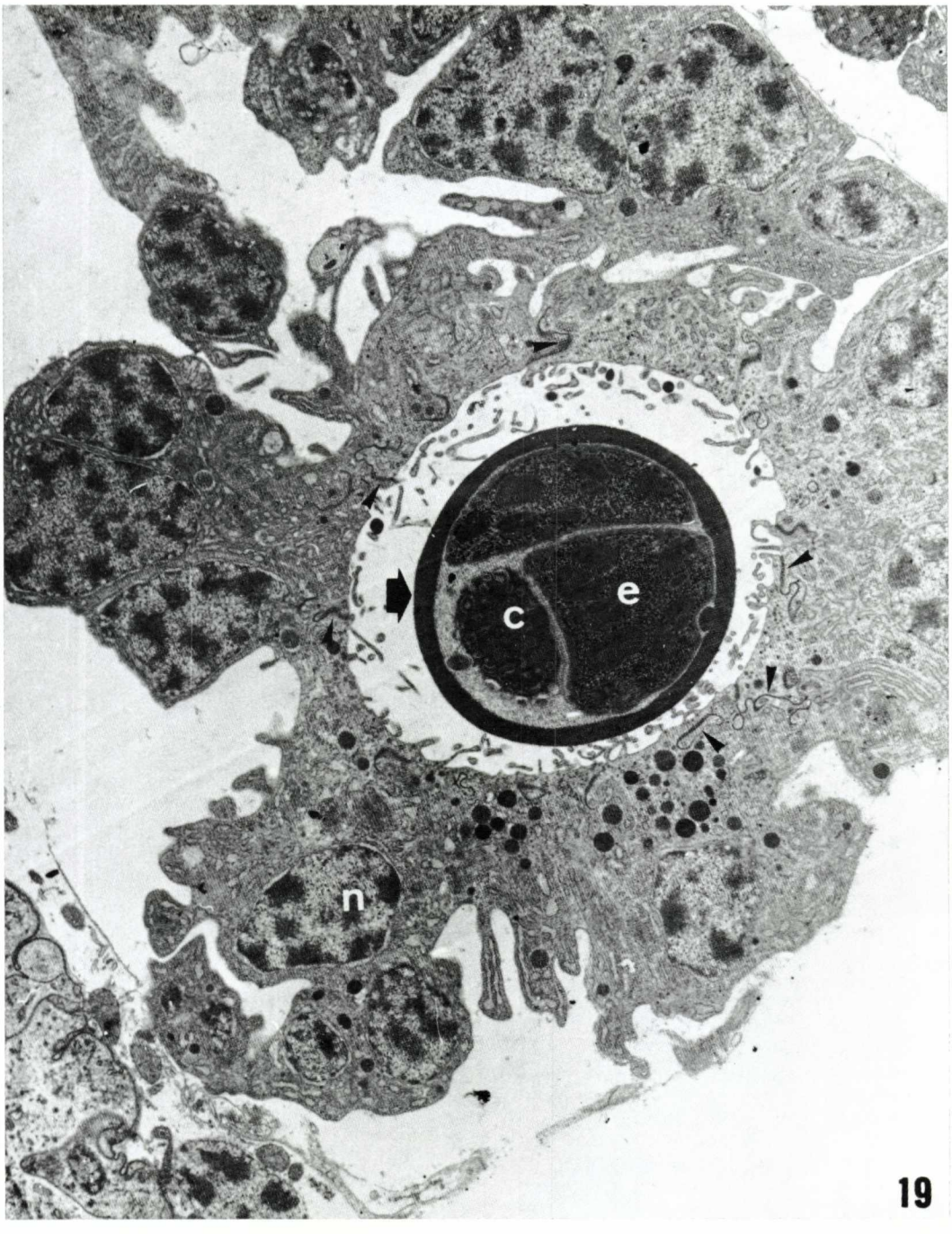

FIG. 19. Detail of the seminal vesicle. Notice the irregularity in the basal zone of the cells, as well as the tight junctions (arrows) and the abundance of microvilli. In the lumen we can see a well developed spermatophore in cross section, with its nucleus or core, which in this case is eccentric and with two accumules of spermatozoa. 8.000 x.

Detall de la vesícula seminal. Es pot observar la irregularitat de la part basal de le cèl·lules, així com els contactes cel·lulars (fletxes) i la riquesa de microvil·lis. En la llum de la vesícula hom veu un tall transversal de l'espermatòfor ben format, amb el seu nucli, en aquest cas excèntric i amb dues masses d'espermatozoides (e). 8.000 x.

does not play any important role in the production of secretory granules.

This is confirmed by an important decrease of vesicular-membranous systems, as well as by the fact that the spermatophore found in the median tract of the deferent duct appears completely formed.

Some other cytochemical techniques remain which have still not been put into practice in order to distinguish accurately the various granules we found in spermatophores. We could also apply autoradiography techniques and, at the same time, it would be interesting to check if the spermatophore has any incidence in vitellogenesis in *Diaptomus conexus*. This influence has only been proved in *Bladera fusca* by BROUSE-GAURY & GOUDEY-PERRIÈRE (1983), who came to the conclusion that the spermatophore, which is found in the genital pouch of females, regulates ovaric physiology.

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