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The Fine Structure of the Hatched Oncospheres of *Hymenolepis nana*

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Introduction

The cellular organization of mature embryos of hymenolepidid cestodes has been the subject of several electron microscopic observations. Collin (1968, 1969) studied the hatched oncospheres of *Hymenolepis citelli* by electron microscopy, and Pence (1970) reported the fine structure of the mature eggs of *Hymenolepis diminuta*. Recently, Lethbridge and Gijsbers (1974) reported light and electron microscopic observations on hatched oncospheres of *H. diminuta*. They paid a special attention to the secretory function of the oncospheres. However, there have been no studies on the fine structure of the oncospheres of *Hymenolepis nana*.

Experiments dealing with the acquired resistance of mice to *H. nana* infection suggested that the oncosphere is a source of critical immunogens (Weinmann, 1970; Di Conza, 1969). Supporting evidence for this view was reported by Furukawa (1974) and Ito (1975). Both these authors demonstrated the *in vitro* activity of immune serum and some lymphoid cells from mice against artificially hatched and activated oncospheres of *H. nana*. However, the nature and localization of the immunogenic materials apparently present in the oncosphere are still obscure. Accordingly, we examined the fine structure of hatched and activated oncospheres of *H. nana* as a basis for the interpretation of future immunological studies.

Materials and Methods

Adult worms of *H. nana* were obtained from experimentally infected Swiss albino mice which were reared in our laboratory. Eggs were teased from gravid proglottids and stored at 4 C in 0.9% saline. Eggshells were removed by stirring the egg suspension with glass beads on a magnetic stirrer for 5 minutes. The artificial hatching of eggs was carried out by a method modified after Berntzen and Voge (1965) (Furukawa, 1974). Hatched, activated oncospheres were washed in saline and immediately fixed in 2% glutaraldehyde with 0.05 M Millonig's phosphate (pH 7.5) for an hour at 4 C. Postfixation was done in 1% osmium tetroxide buffered with Millonig's phosphate for an hour at 4 C. Oncospheres were dehydrated in a graded ethanol series and embedded in Epon 812 (Luft, 1961). Thin sections were cut with glass knives on an LKB Ultratome and stained in uranyl acetate and lead citrate (Reynolds, 1963). Sections were examined with an Hitachi HS-9 electron microscope.

Results

The terminology used here in describing

the oncosphere is after Collin (1968, 1969). The region of the oncosphere opposite the hook end is referred to as the anterior, and the hook end as posterior.

Photo. 1 shows a low magnification view of the whole oncosphere. The outer coats of the oncosphere consisted of a cytoplasmic layer, a basal lamina, and muscles. The oncospheral hooks and their associated musculature, the penetration gland cells, and several other undefined cells could be distinguished as the internal structure of the oncosphere.

The outer border of the oncosphere consisted of a cytoplasmic layer that had numerous cytoplasmic protrusions and which contained a few mitochondria (Photo. 1). The cytoplasmic outfoldings appeared to be increased in the anterior and posterior regions. Often the inner plasma membrane of the outer layer invaginated to form small fingerlike infoldings (Photo. 2). No nuclei were observed in the outer layer. The outer layer of cytoplasm rested on a basal lamina beneath which lay a layer of muscle cells. The outer layer and basal lamina almost entirely surrounded the oncosphere.

The muscle system of the oncosphere was complex but two groups of muscles, the somatic and hook muscles, could be distinguished (Photo. 2). The somatic muscles lay beneath the basal lamina and surrounded the oncosphere. Sets of hook muscles were closely associated with the six oncospheral hooks and occupied the bulk of the oncosphere. The hook muscle cells did not appear to terminate directly on the hooks; instead, they were attached to connective tissues surrounding the hook bases and hook collars (Photos. 2, 3, 5). Dense bodies formed by the aggregation of myofilaments indicated these sites of muscle attachment. The hook and somatic muscles consisted of nonstriated myofibers which contained thick and thin myofilaments extending in a plane parallel to the long axis. Glycogen granules were abundant in the peripheral cytoplasm of the muscle cells.

The hook of the oncosphere consisted of an outer layer, an electron-dense middle layer, and a central core made up of a laminated material (Photo. 4). A dense matrix collar, or a midpiece, surrounded the hooks and occurred between the outer layer and the basal lamina where the hooks protruded from the oncosphere (Photo. 3). This was continuous with the inner plasma membrane of the outer layer through a circular desmosome.

The penetration gland cells occupied the anterior half of the oncosphere and were filled with numerous dense granules (Photos. 1, 5, 6). These granules were surrounded by a limiting membrane and contained fine, granular materials of varying density in their matrices (0.21 to 0.26 μ in diameter). An oval nucleus, a few mitochondria, and Golgi complexes made up of small vesicles and flattened sacs, were also present in the gland cells (Photos. 5, 6). The lobular cytoplasm of the gland cells extended posteriorly and bifurcated into two or more branches, each of which further became attenuated to form narrow cytoplasmic projections (Photo. 7). Often four branches of the cytoplasm were found in a section (Photos. 8, 9). The cytoplasm of these projections was always filled with dense granules and clear vesicles which were morphologically similar to those observed in the perinuclear cytoplasm of the gland cells. Microtubules were situated at the periphery of the cytoplasm and as a single row in the attenuated portions of the gland cells. The apical cytoplasm of the gland cells and the tips of the microtubules protruded through the basal lamina at the posterior surface of the oncosphere (Photos. 9, 10, 11). Ofter large blebs of the cytoplasm containing dense granules protruded through the basal layer (Photos. 10, 11). Usually the outer layer of this portion was complicated by the occurrence of many cytoplasmic outfoldings, and the boundary between the outer layer and protruded cytoplasm of the gland cells was often hard to distinct. Possibly, this protruded cytoplasm was subsequently shed from the gland cells, and then released to

the outside of the oncosphere through the outer layer.

Photo. 12 and 13 show the processes of other types of gland cells that contained dense granules that were smaller (75 to 100 nm in diameter) than those of the penetration gland. Most of these processes lay near and between the muscle cells. Clear vesicles of varying sizes were also present along with these dense granules in the cytoplasm. The nucleus and perinuclear cytoplasm of these projections remain to be identified.

Discussion

The present study revealed that the cellular constituents of the oncospheres of *Hymenolepis nana* were similar to those of *H. citelli* (Collin, 1968, 1969) and *H. diminuta* (Pence, 1970; Lethbridge and Gijsbers, 1974). The basic structures also resembled those of *Taenia taeniaeformis* (Nieland, 1968) and *Dipylidium caninum* (Pence, 1967).

The outer layer of the oncosphere was of syncytial cytoplasm with numerous outfoldings and it rested on the basal lamina. No nuclei were observed in the outer layer. The nuclei were possibly situated beneath the basal lamina and connected to the perinuclear cytoplasm of the outer layer by protoplasmic strands. This has been suggested by Rybicka (1973) for the embryonic epithelium of *H. diminuta*. In this respect, the structure of the outer layer in cyclophyllidean oncospheres is basically the same as that observed at other developmental stages in cestodes (Lee, 1966). However, the surface of the outer layer of the oncosphere did not form the regular brushborder of microvilli as observed in several species of larval and adult cestodes (Lumsden, 1975). Collin (1970) demonstrated that microvillar projections were formed on the surface of the 3-day postembryonic stage of *H. citelli*.

Hatched and activated oncospheres are characterized by sequential movement of their hooks and changing body shape. Lethbridge (1971) reported detailed observations on the hook movements of *H. diminuta* and showed that the hooks exhibited a highly coordinated "breaststroke" action. Heath (1971) pointed out that the oncospheres of several taeniid cestodes were able to distort into almost any shape during penetration and migration in their intermediate hosts. It is possible that a modulated action of the somatic musculature may be responsible for the distortion of the body shape. Collin (1969) expected the presence of some sort of nervous system in *H. citelli* oncospheres. In *H. nana* oncospheres, the hooks and associated muscle system were well developed and their ultrastructure appeared to be quite similar to those of other cyclophyllidean oncospheres so far studied. In addition, the present study revealed that fine projections of granular cells were distributed near and between the muscle cells. These cells contained membrane-bound, dense granules which were smaller than those of the penetration gland cells, and they also contained numerous small clear vesicles in their cytoplasm. These may represent some sort of conductive elements as suggested by Collin (1969) for *H. citelli* oncospheres. However, the present study failed to demonstrate any particular connections that would suggest reciprocal actions between the muscle cells and the cytoplasmic projections. The function of these glandular cells is therefore still highly speculative.

The penetration gland cells of *H. nana* oncospheres consisted of an enlarged basal area, situated in the anterior half of the body, and narrow cytoplasmic processes extending beyond the basal lamina at the posterior surface of the oncosphere. A comparable structure has been observed in several cyclophyllidean oncospheres (Collin, 1968, 1969; Pence, 1967, 1970; Nieland, 1968; Lethbridge and Gijsbers, 1974). They described the attenuated portions of the gland cells as gland ducts. This term may not be accurate from the view point of histology, at least in *H. nana* oncospheres, since these cytoplasmic projections appeared to be a part of the penetration gland cells.

The cytoplasm of the attenuated portions was apparently continuous with the basal portion of the gland cells and, therefore, the dense granules observed in the cytoplasmic projections had never been "secreted" from the penetration glands. Rather, the present study revealed that the apical cytoplasm of the attenuated portions of the gland cells was directly protruded through the basal lamina. These observations suggested that the penetration glands of *H. nana* oncospheres are unicellular with no gland duct, and that they release their secretory materials as in apocrine secretion where a part of the apical cytoplasm is lost along with the secretory materials (Bloom and Faucett, 1968). Prominent microtubules in the peripheries of the attenuated portions of the gland cells may have a supportive function as well as assisting the movement of gland cell cytoplasm. A more definitive study is needed to determine the way in which the blebs of the gland cell cytoplasm are released through the outer layer to the outside of the body. A different type of secretory function was suggested by Lethbridge and Gijsbers (1974) who showed that expulsion of the gland materials in *H. diminuta* oncospheres involved a type of merocrine secretion.

The dense secretory granules of the penetration gland cells in the *H. nana* oncosphere showed some variations in size and electron density. Collin (1969) reported that, in *H. citelli*, two types of membrane-bound granules could be distinguished on the basis of their relative electron opacity. However, it remains to be determined whether or not these morphological differences reflect differences in chemical composition of the secretory materials. Lethbridge and Gijsbers (1974) reported two types of secretory granules with transitional form in *H. diminuta*, and suggested that the morphological changes may be associated with the maturation of the secretory materials. The structural and liquefaction changes of secretory granules preceding secretion were noted in *D. caninum* (Pence, 1967), in *T. taeniaeformis* (nieland, 1968) and in *H. diminuta* (Pence, 1970). These changes were not observed in the present study.

Recent histochemical and morphological observation support the view that the contents of the penetration glands are secreted during penetration of the hatched oncospheres through the tissues of their host. However, there are different opinions as to the function of these secretory granules. Several authors (Silverman and Maneely, 1955 ; Banerjee and Singh, 1969 a, b ; Heath, 1971 ; Lethbridge, 1971) proposed that the granules may contain cytolytic enzymes which attack the host tissues and thus facilitate the migration of the oncospheres. However, Barker (1970) suggested that the secretory materials, rather than being lytic, may have a lubricant function and may also protect the oncospheres from the digestive action of the pancreatic and intestinal juices of the host. Further studies are needed to characterize the function of secretory materials in the initial phase of the infective process.

Summary

Electron microscopic observations were made on the hatched and activated oncospheres of *Hymenolepis nana*. The outer surface of the oncosphere was composed of a cytoplasmic layer with numerous outfoldings and rested on the basal lamina. The somatic musculature lay beneath the basal lamina and covered the oncosphere. A number of hook muscles were distributed among the six oncospheral hooks and they were attached to the connective tissue surrounding the bases and collars of the hooks. The hooks were composed of three layers. The penetration gland cells consisted of an enlarged basal area situated in the anterior half of the oncosphere, and narrow cytoplasmic projections extending beyond the basal lamina at the posterior surface of the body. These cells were filled with numerous membrane-bound secretory granules. Microtubules were situated in the peripheral cytoplasm of the gland. Apical cytoplasm

of the gland cells protruded through the basal lamina and blebs of cytoplasm containing secretory granules were found between the basal lamina and the outer layer. The fine processes of the glandular cells contained small cored and clear vesicles and these were found near and between the muscle cells.

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小形条虫六鉤幼虫の微細構造

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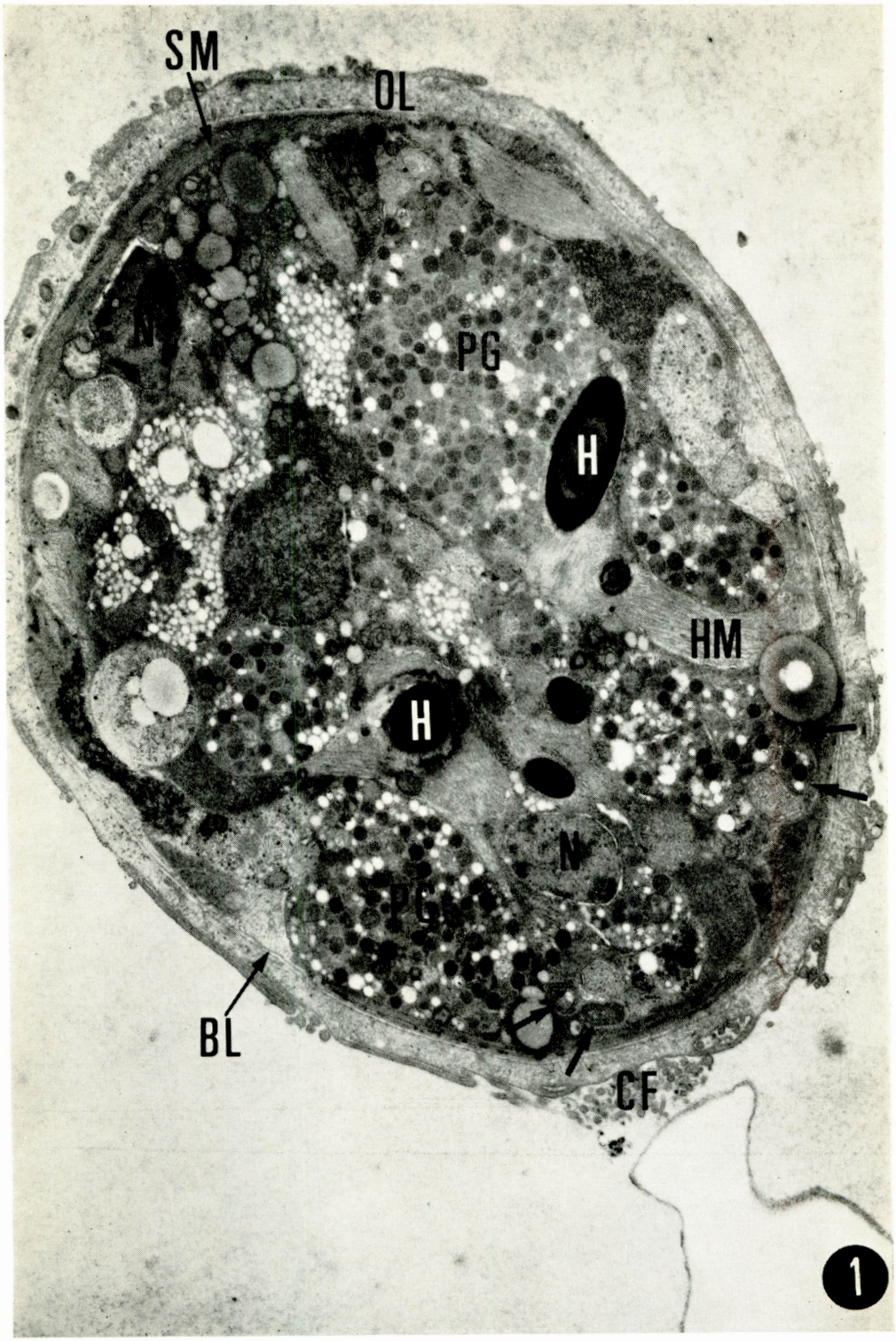
小形条虫六鉤幼虫の微細構造を電子顕微鏡により観察した．六鉤幼虫の最外層は一層の細胞質からなり，表面には繊毛状の突起が多数見られる．つぎに基底膜をはさんで一層の筋組織があり，これらは幼虫全体を包んでいる．鉤は三層構造を示し，一群の筋組織が附随している．筋線維の構造は高等動物の横紋筋に類似するが横紋は形成しない．侵入腺は体前半にあり，その細胞質中には球形の，限界膜を有する電子密度の高い顆粒が多数含まれる．この細胞本体より後方に向つて数本の細胞質突起が派生し，これらはさらに細管となつて体後方の基底膜外に開く．いずれも細胞質の内面には微小管が配列されている．時にこの開口部より，分泌顆粒を含んだ侵入腺細胞の一部が突出するのが認められる．この他に，侵入腺の分泌顆粒より小形で限界膜に包まれた電子密度の高い顆粒を含む腺細胞が存在する．この腺細胞は侵入腺に比べて小さく，筋線維に接してみられるのが特徴である．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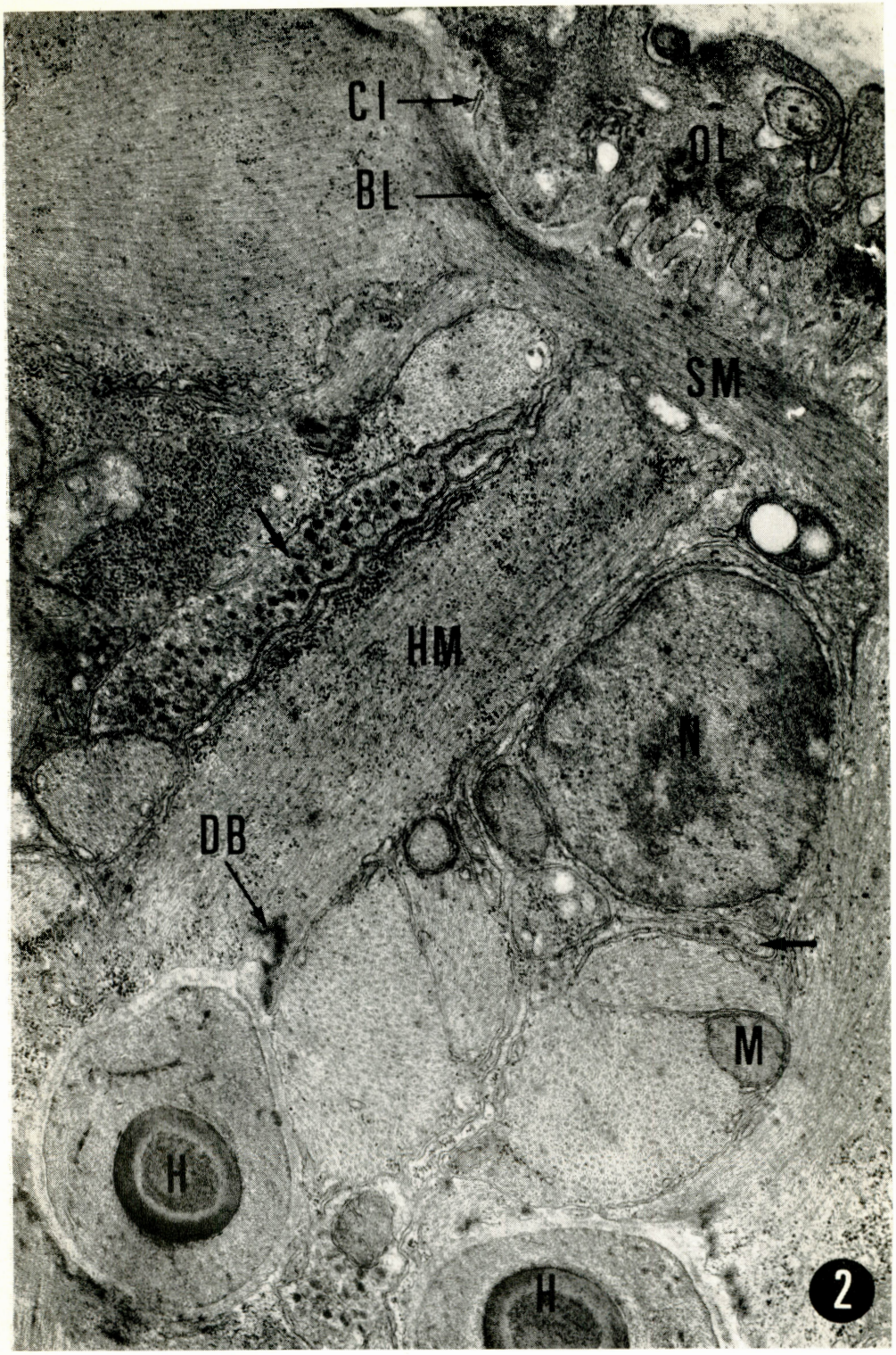
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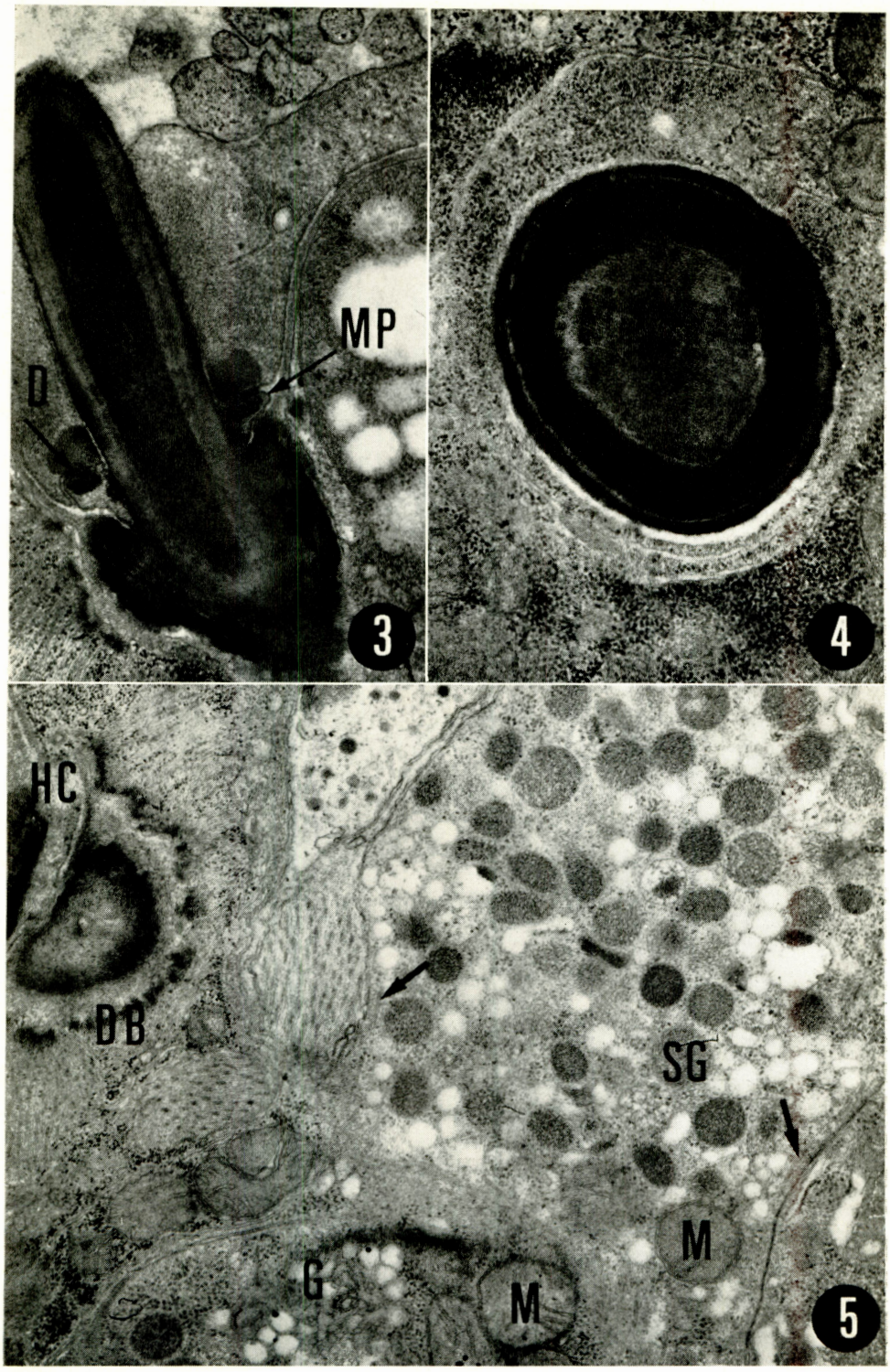
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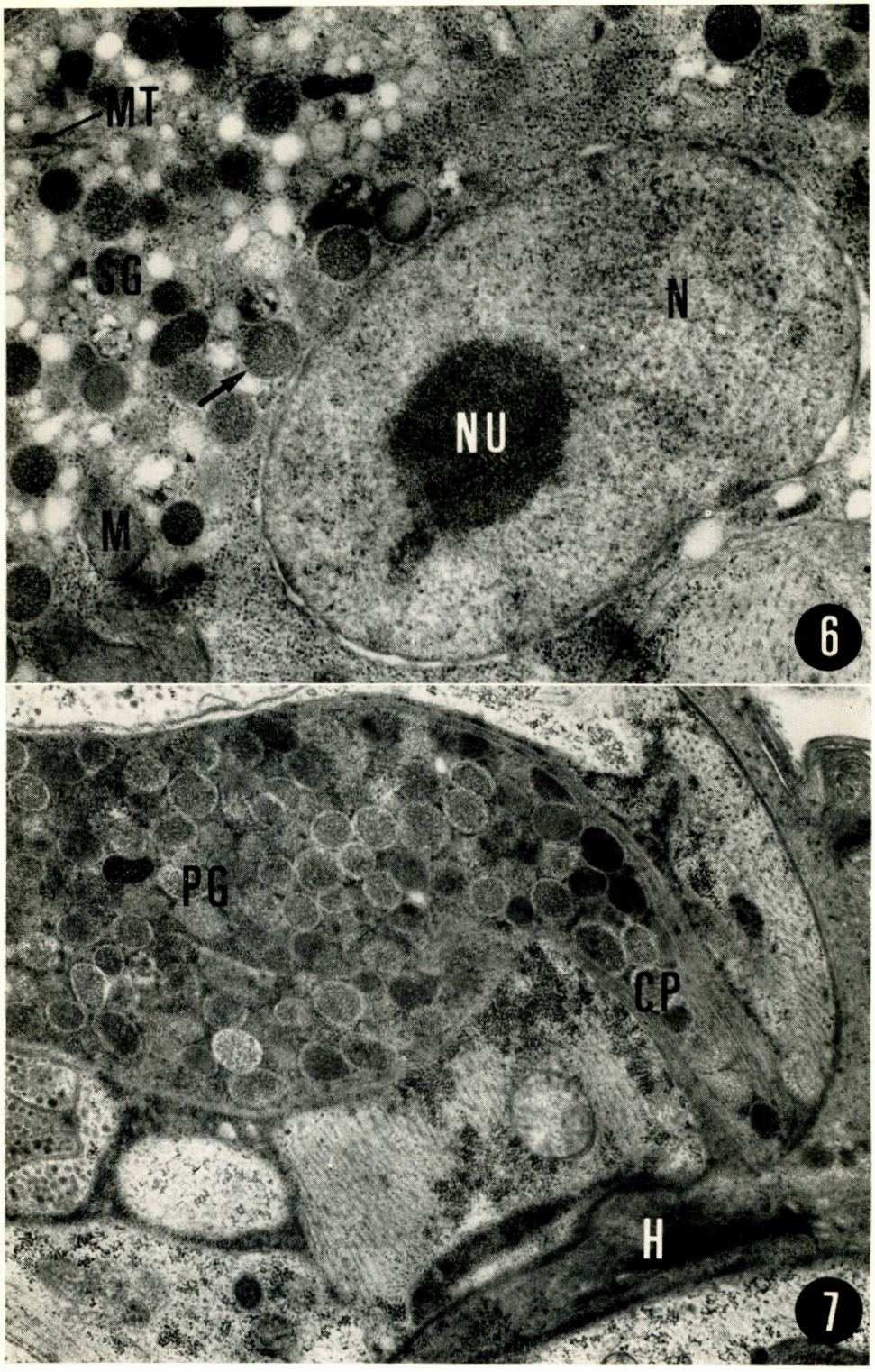
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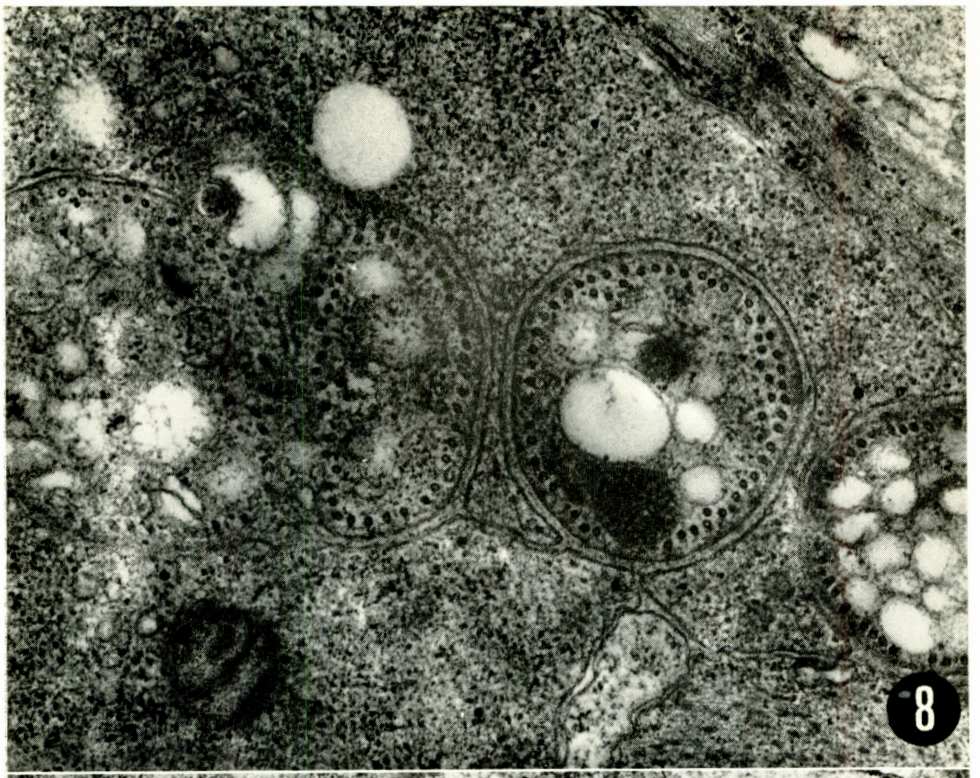
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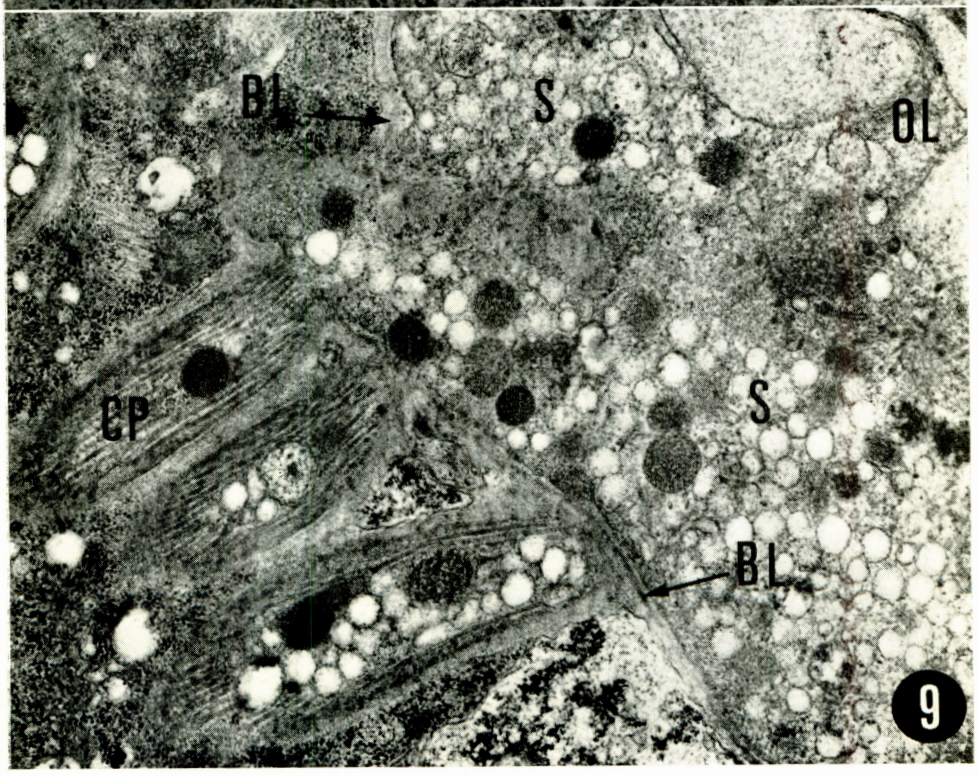
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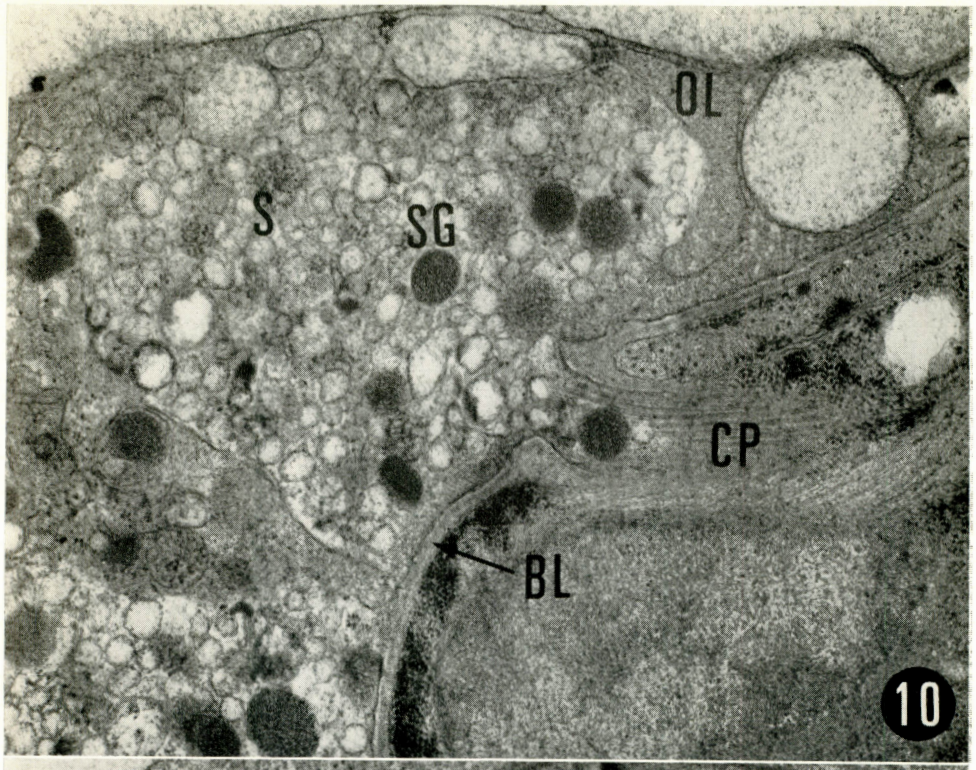
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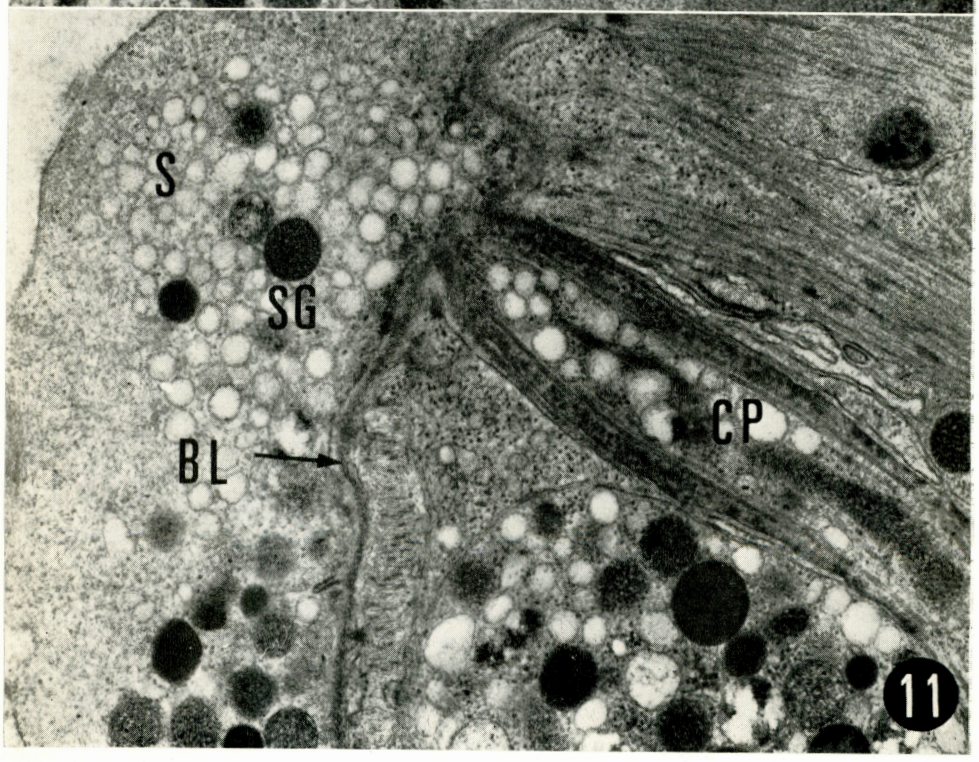
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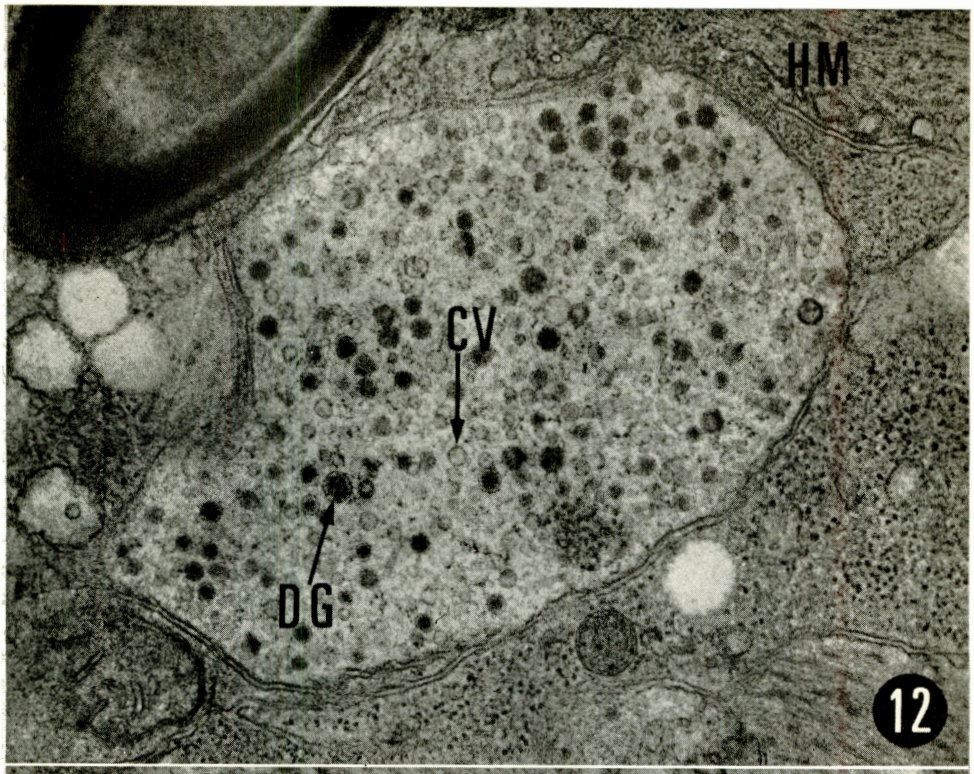
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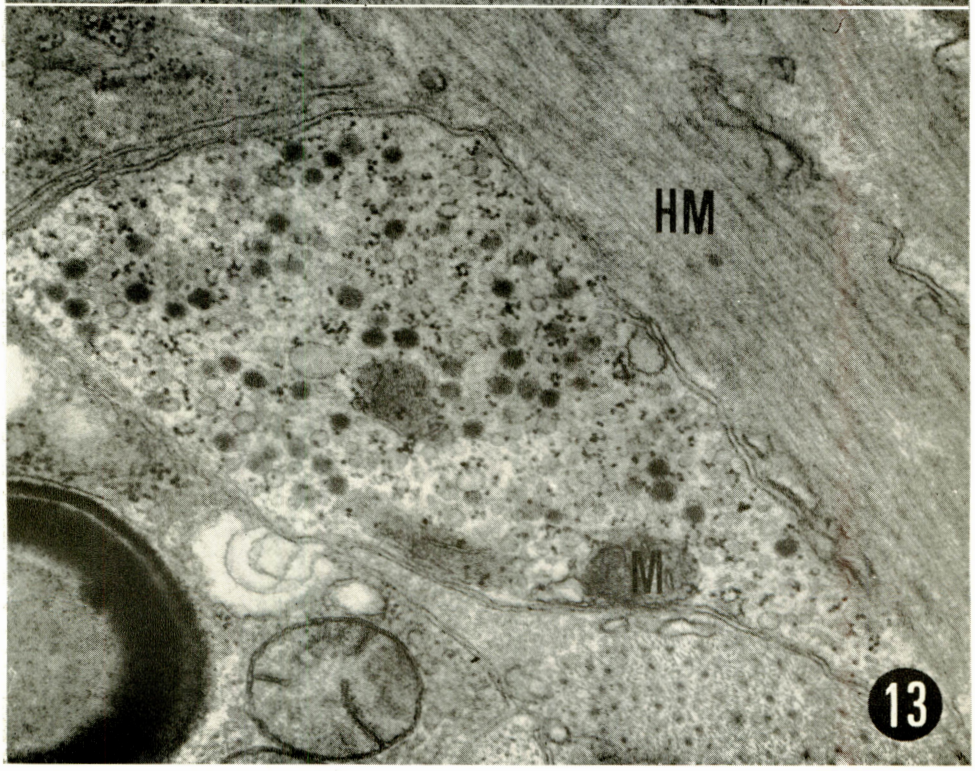
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Explanation of Photographs

Photo. 1 Medial oblique section of whole oncosphere, showing outer layer (OL) with cytoplasmic foldings (CF), oncospheral hooks (H), associated hook muscles (HM), and penetration gland cells (PG) with their narrow cytoplasmic projections (arrows). A portion of somatic muscles (SM) beneath the basal lamina (BL) and nuclei (N) are also shown. × 9,000.

Photo. 2 Cross section of an oncosphere showing muscle system. Hook muscle (HM) is connected with the connective tissue surrounding the hook base (H). Dense bodies (DB) indicate the site of muscle attachment. The basal lamina (BL) separates the outer layer (OL) and somatic muscles (SM). Fine processes of granule-containing cells are seen between the hook muscles (arrows). N, Nucleus ; M, Mitochondria ; CI, Cytoplasmic infoldings. × 30,000.

Photo. 3 A dense matrix collar or midpiece (MP) occurs where the hook exits from the oncosphere. The midpiece is continuous with the inner plasma membrane of the outer layer by a circular desmosome (D). × 28,200.

Photo. 4 Cross section of an oncospheral hook which is composed of three layers. × 35,000.

Photo. 5 A portion of the penetration gland cell showing secretory granules (SG), mitochondria (M) and Golgi apparatus (G). Microtubules are situated at the periphery of the gland cell (arrows). The hook collar (HC) and associated musculature with prominent dense bodies (DB) are also shown. × 27,000.

Photo. 6 The nucleus (N) and a nucleolus (NU) of the penetration gland cell. The secretory granules are surrounded by a limiting membrane (arrow). SG, Secretory granules ; M, Mitochondria ; MT, Microtubules. × 27,000.

Photo. 7 A portion of the lobular cytoplasm of the penetration gland (PG). The gland cell becomes attenuated to form the cytoplasmic projection (CP). The tip of the cytoplasmic projection reaches the basal lamina near the hook region (H). × 21,900.

Photo. 8 Cross section of four branches of the penetration gland cell. Each branch is supported internally by a single row of microtubules. × 43,000.

Photo. 9 Four branches of the penetration gland cell (CP) open separately through the basal lamina (BL). A mass of secretory materials (S) is seen between the basal lamina and the outer layer (OL). × 22,500.

Photos. 10 and 11 Longitudinal section of the exits of the cytoplasmic projections of the penetration gland cells (CP). Blebs of penetration gland cytoplasm (S) containing secretory granules (SG) are protruded between the basal lamina (BL) and the outer layer (OL). Photo. 11, × 29,000 ; Photo. 12, × 35,000.

Photos. 12 and 13 Fine processes of other granular cells lie near and between the hook muscle cells (HM). Membrane-bound dense granules (DG), membranous clear vesicles (VC) and a few mitochondria (M) can be seen in the cytoplasm. Photo. 12, × 38,500 ; Photo. 13, × 46,000.