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Ultrastructural Study of the Tetrathyridium of *Mesocestoides corti* Hoeppli, 1925: Tegument and Parenchyma*

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Summary. The study of dividing and non-dividing tetrathyridia using electron microscopy shows that the mode of multiplication by antero-posterior fission of these larvae is due to a particular tissue which is called the 'apical massif'. The apical massif is a part of the tegumental syncytium. It is located at the top of the scolex. It represents a polynucleated cell mass which has cytomorphogenetic power. During asexual multiplication, it differentiates into tegumental syncytium, sub-tegumental muscles, glycogen-storing parenchyma cells, and other cell types. Parts of it remain undifferentiated. The hypothetic origin of the apical massif is discussed.

Longitudinal growth of the tetrathyridia occurs by invasion of migrating cells into the tegumental syncytium. These cells also originate from the apical massif. During asexual multiplication and longitudinal growth, filamentous microtriches are synthesized below the plasmalemma of the superficial cytoplasm of the tegumental syncytium. It is supposed that the blade-like microtriches derive from filamentous forms.

Introduction

The tetrathyridium of *Mesocestoides corti* is a cestode larva of special interest because of its ability to regenerate and multiply asexually (Specht and Voge, 1965; Hart, 1968). It divides by longitudinal antero-posterior fission (Hess, 1972; Novak, 1972) while all other multiplying cestode larvae proliferate by budding. The particular mode of asexual multiplication and the recent use of tetrathyridia for biochemical and pharmaceutical research call for exact information regarding the anatomy, histology, and fine structure of these larvae. This and following papers deal with the fine structure of the tetrathyridium of *Mesocestoides corti* and with the differentiation of its tissues during asexual multiplication and experimental regeneration.

* Part of the author's thesis

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Materials and Methods

Tetrathyridia of *Mesocestoides corti* originally isolated by Specht and Voge (1965) and cultivated in our laboratory in NMRI-mice were used in this study. Tissue descriptions were made on larvae with four suckers having terminated regeneration. Differentiation of the tissues was studied in tetrathyridia at various stages of asexual multiplication, and in regenerating, 2-sucker head fragments obtained by amputation and cultivated in NMRI-mice for 2 to 70 h. Scanning electron microscope (SEM) and transmission electron microscope (TEM) preparation techniques (glutaraldehyde/OsO_4-fixation) were those described by Hess and Guggenheim (1977). Glycogen was stained by the PAS-ATP-method (Periodic acid-Schiff-acide phosphotungstique) proposed by Thiery (1967). Ultrathin sections were studied on a Philips 201 EM. SEM observations were made on a Cambridge Mark 11A scanning electron microscope. For other autoradiographic experiments, tetrathyridia were incubated in NCTC 135 containing 1 μCi/ml of H^3-thymidine (specific activity 5 Ci/mM) at 37° C for 2 h, then transferred to NCTC 135 at 37° C and fixed 4 to 48 h later in Carnoy's fixative. Paraffin sections were treated by the stripping film method (Kodak AR 10) as described by Appleton (1972).

Results

The tetrathyridium of *Mesocestoides corti* is a flat, plerocercoid-like larva measuring up to 3 mm in length (Fig. 1). In the middle of the scolex (0.2 mm large) there is a dorsoventral depression or cleft that separates the left and right pairs of suckers. From this 'apical cleft' originates the longitudinal division of the larva. In dividing larvae a new apical cleft appears immediately after regeneration of the suckers in the middle of each of the scolices (Fig. 2). Behind the neck, the larval body enlarges up to 0.3 mm, and then tapers off posteriorly. Frequently there occur lateral outgrowths that represent remains of previous divisions (Hess, 1972; Novak, 1972).

Anatomically, the tetrathyridium consists of the tegument, the parenchyma, the suckers, the internal muscles, the excretory or osmoregulatory system, the nervous system, and the pool of germinative cells.

I. The Tegument

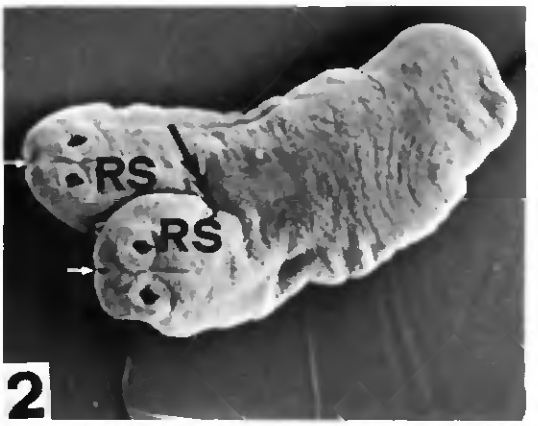
The tegument consists of the tegumental syncytium, the subtegumental muscle system, and the fibrous layer that separates the former (Fig. 3).

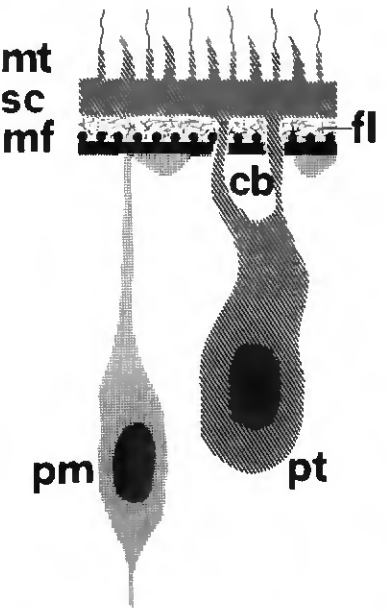
A. The Tegumental Syncytium

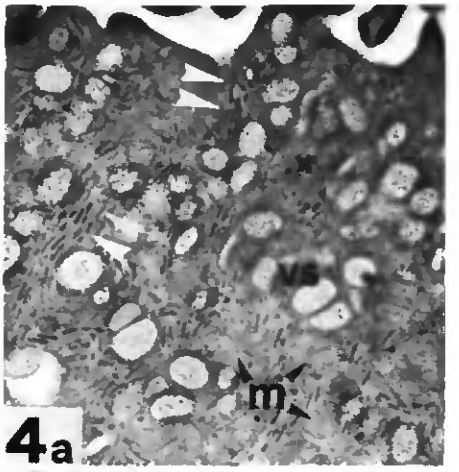
The tegumental syncytium is composed of non-nucleated superficial cytoplasm and perinuclear cell bodies lying in the cortical parenchyma.

a) The Superficial Cytoplasm

The superficial cytoplasm bears blade-like and filamentous microtriches (Hess and Guggenheim, 1977). It is of uniform structure on the whole body of the







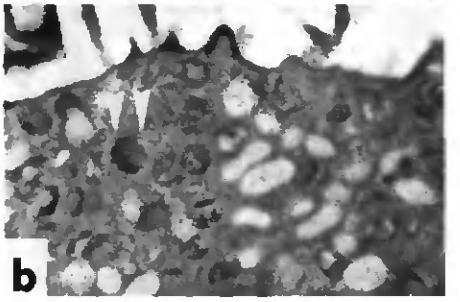


Fig. 1. Tetrathyridium of *Mesocestoides corti*. The cylindrical form of this larva is an artifact due to fixation for SEM. In vivo, this plerocercoid-like larva is flat. The left and right pairs of suckers (*S*) are separated by the apical cleft (*arrow*). ×670

Fig. 2. Tetrathyridium of *Mesocestoides corti*. Dividing larva observed by SEM. The division cleft (*double arrow*) divides the scolex into two parts. Each half of the scolex regenerates a pair of suckers (*RS*) and an apical cleft appears on each regenerated scolex (*arrows*). Subsequently, one or both scolices detach from the posterior part of the larva. ×355

Fig. 3. Tetrathyridium of *Mesocestoides corti*. Schematic representation of the tetrathyridial tegument. *mt* filamentous and blade-like microtriches; *sc* superficial cytoplasm; *cb* cytoplasmic bridges; *pt* perinuclear cell body of the tegumental syncytium; *mf* external circular and internal longitudinal muscle fibres; *pm* perinuclear cell body of the subtegumental muscle system; *fl* fibrous layer

Fig. 4. Tetrathyridium of *Mesocestoides corti*. The transverse section of the superficial cytoplasm of the tegumental syncytium shows vesicles containing a flocculate substance (*vs*), mitochondria (*m*), and disc-shaped bodies (*arrows*) cut transversally. **a** ×20,630 and sagitally, **b** ×20,630

tetrathyridium. No differences corresponding to the surface zones A, B, and C of the microthrix border have been observed. It measures 3 μm anteriorly and up to 6 μm on the posterior part of the larva. The superficial cytoplasm covering the suckers is only 0.6–0.8 μm thick and has no cytoplasmic bridges with the sucker tissue. Exteriorly, the superficial cytoplasm is limited by a unit-membrane. Internally, the superficial cytoplasm adjoins the fibrous layer. In the superficial cytoplasm the following organelles and inclusions occur (Fig. 4); mitochondria, vesicles containing a flocculate substance of low electron density, and disc-shaped bodies with a diameter of 0.25 μm. The latter are oriented perpendicularly to the tegumental surface except over the suckers where they are parallel. A few ribosomes occur near the internal border of the superficial cytoplasm. The cytoplasmic bridges connecting the superficial cytoplasm to the perinuclear cell bodies contain large electron-lucid spaces, ribosomes, microtubules, and some disc-shaped bodies.

b) The Perinuclear Cell Bodies

Whereas the superficial cytoplasm shows no structural differences related to the region of the larval body, the perinnclear cell bodies of the tegumental syncytium vary structurally along the body of the tetrathyridium. In the centre of the scolex between the suckers, and situated below the apical cleft, there is a voluminous reticulated cell mass which contains numerous nuclei (Fig. 5). This 'apical massif' is a part of the tegumental syncytium connected to the superficial cytoplasm by large cytoplasmic bridges. The nuclei of the apical massif (4×2 μm) are of irregular shape. Each contains a prominent, central nucleolus and little heterochromatin. Of particular interest is the occurrence of mitosis not observed in other parts of the tegument (Fig. 5, inset). The hyaloplasm of the apical massif shows high electron density which is typical of the whole tegumental syncytium. It contains a great number of free ribosomes and a well developed rough endoplasmic reticulum (RER), making it basophilic (Fig. 6). Numerous subsurface RER cisternae occur (Fig. 6, inset). Well delimited zones devoid of ribosomes contain Golgi complexes and probably smooth endoplasmic reticulum (SER). Small accumulations of alpha-glycogen may occur. Lipid droplets are rare.

Posterior to the apical massif, the perinuclear cell bodies of the tegumental syncytium are mono-nucleate (Fig. 7). Nuclei in mitotic division are not observed. The long nuclei contain few heterochromatin. The RER is less developed than in the apical massif and the cytoplasm never contains alpha-glycogen. Large vacuolar zones surround the numerous Golgi complexes (Fig. 7). In these zones small vesicles of the first order (∅ 0.06–0.2 μm), produced by the Golgi complexes contain an electron lucid substance. They fuse to form vesicles of the second order (∅ 0.3–3 μm) which contain the electron-lucid substance and membrane remnants of the fusing vesicles (Figs. 8 and 9). These quintalaminated aggregates may join together and form large crystal-like complexes (Fig. 10). Vesicles that contain only membraneous aggregates but no electron-lucid sub-

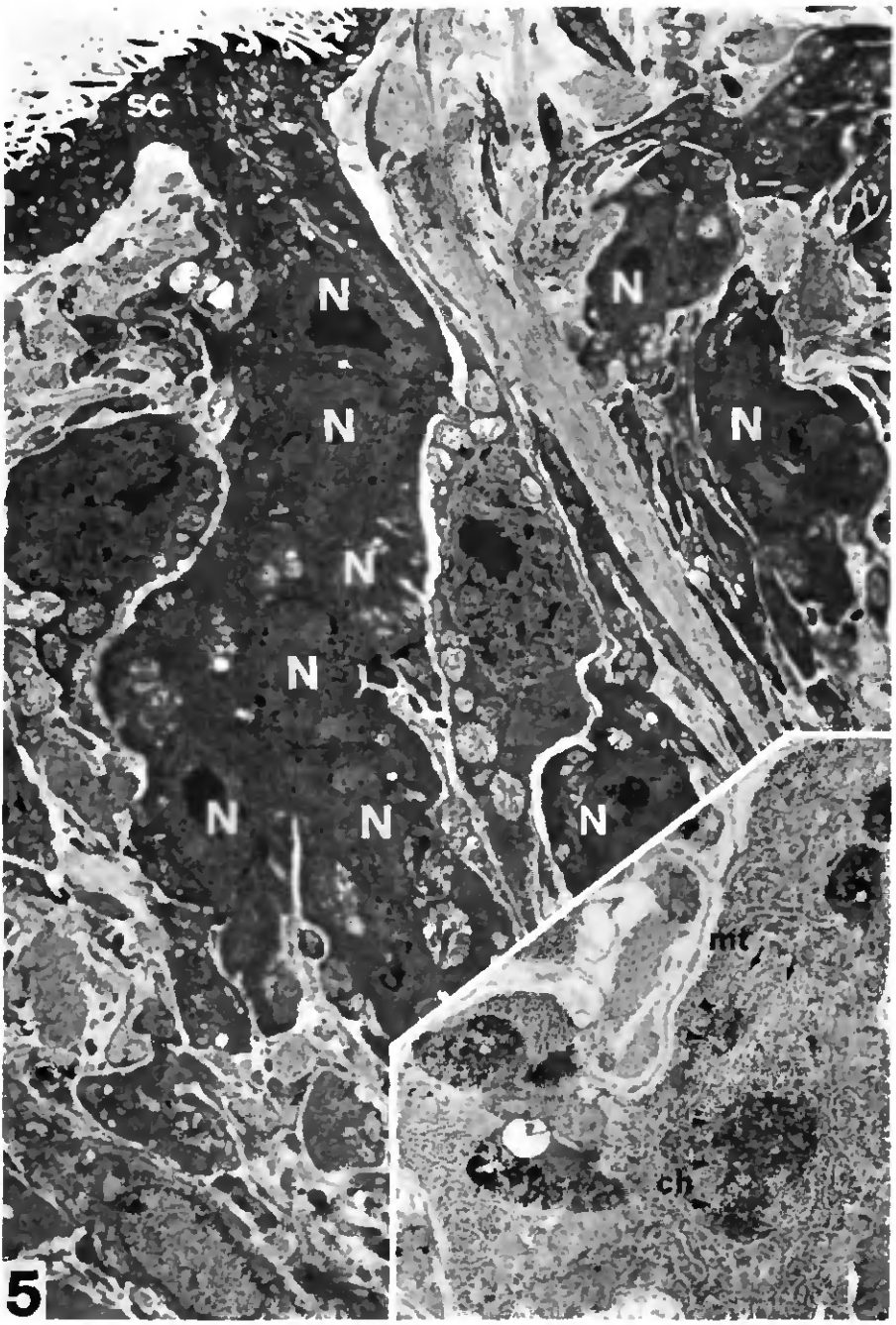


Fig. 5. Tetrathyridium of *Mesocestoides corti*. Transverse section of a part of the apical massif. This represents a reticulated, polynucleated cell mass in which nuclei (*N*) divide by mitosis (*inset*). As it is in cytoplasmic continuity with the superficial cytoplasm (*sc*) it represents a part of the tegumental syncytium. ×6,54 *s. Inset:* Part of the apical massif with nucleus in mitotic division; *ch* chromosomes, *mt* spindle microtubules. ×15,000

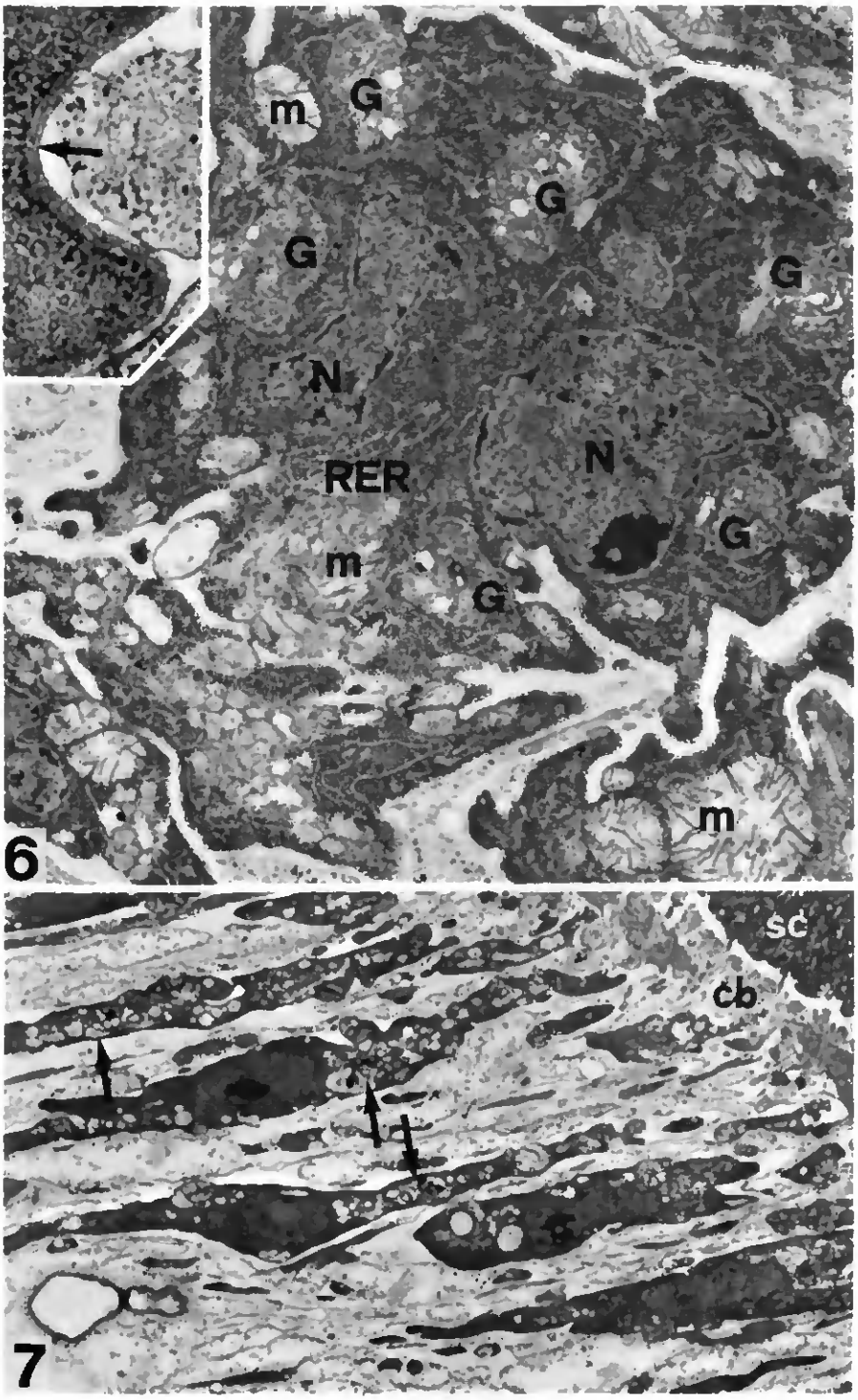


Fig. 6. Tetrathyridium of *Mesocestoides corti*. The cytoplasm of the apical massif contains numerous nuclei (*N*), a great number of free ribosomes, a well developed RER, numerous Golgi zones (*G*) and mitochondria (*m*). × 14,790. *Inset:* Subsurface cisternae of RER are characteristic for the apical massif and for the tegumental perinuclear cell bodies. × 54,000

Fig. 7. Tetrathyridium of *Mesocestoides corti*. Mononucleate perinuclear cell bodies of the tegumental syncytium posterior to the apical massif. Golgi zones are surrounded by vesicles (*arrows*); *sc* superficial cytoplasm; *cb* cytoplasmic bridges. × 4,815

stance are called third order vesicles. It may be supposed that they derive from second order vesicles that have secreted the electron-lucid substance into the intercellular space or even directly transferred into glycogen-storing parenchyma cells by a mechanism which remains enigmatic (Fig. 11).
The membrancous aggregates do not leave the cell body but remain in the third order vesicles, where they form electron-dense clumps (Figs. 8 and 12). The original crystal-like structure is often preserved, but homogenization also occurs. Acid phosphatases were not demonstrated in any type of vesicle. Third order vesicles are more numerous in tegumentary cell bodies of the posterior part of the tetrathyridia, where they may so densely fill some cell bodies that no first and second order vesicles or Golgi apparatus are found (Fig. 12).

B. The Sub-Tegumental Muscle System

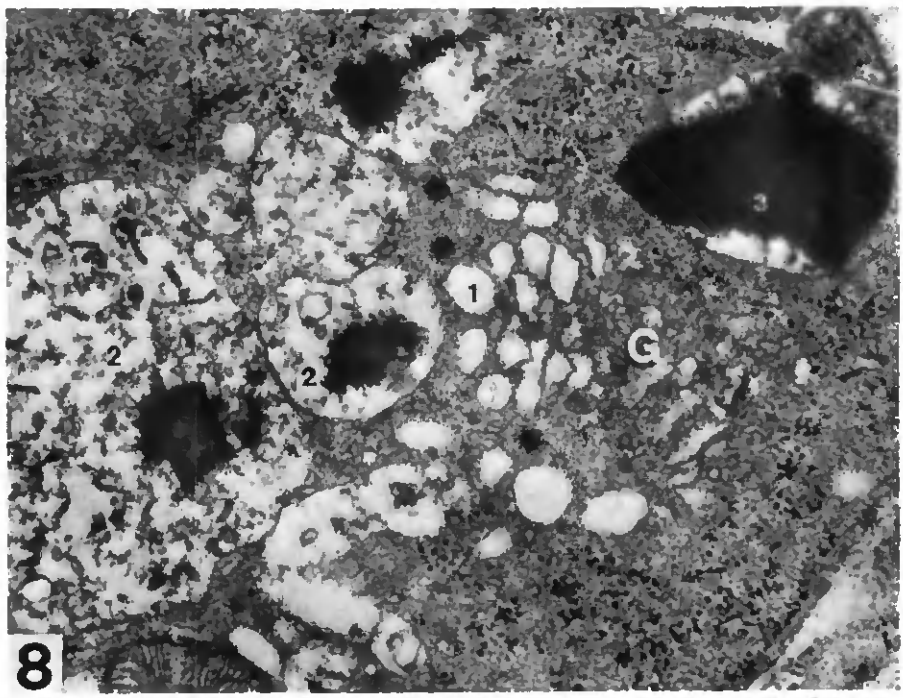
The sub-tegumental muscle system is a dense network consisting of external circular and internal longitudinal muscle fibres which lie close to the fibrous layer. The muscle cell bodies are situated in the cortical parenchyma (Fig. 13). They are distinguished from the perinuclear cell bodies of the tegumental syncytium by a less electron-dense hyaloplasm, a well developed cisternae forming RER, and the lack of Golgi apparatus and vacuolar zones. The muscle fibres are composed of myofibrils of 20 to 27 nm diameter (myosin) and 4 to 6.5 nm diameter (actin) (Fig. 14). Dense patches corresponding to the Z-lines of striated muscle are observed (Mac Rae, 1965). In some fibres microtubules of 23–30 nm diameter occur next to the myofibrils. Sarcoplasmic expansions of the fibres contain large mitochondria, ribosomes, and beta-glycogen (Figs. 15 and 16). These expansions are attached by tight or gap junctions to the perinuclear cell bodies of the tegumental syncytium. Axons containing dense-cored vesicles form neuromuscular junctions on the muscle fibres (Fig. 17).

II. The Parenchyma

The parenchyma of the tetrathyridium is separated into firm cortical and loose medullary parenchyma by the internal longitudinal muscles. Two cell types are attributed to this tissue: glycogen-storing cells and calcareous corpuscule cells.

A. The Glycogen-Storing Cell

The fully developed glycogen-storing cell is composed of a central part and of voluminous lobes which are attached to the former by narrow cytoplasmic bridges (Fig. 18). The central part of the cell includes the nucleus surrounded by a narrow zone of hyaloplasm containing ribosomes, some RER, and mitochondria; the lobes are filled with rosettes of alpha-glycogen and contain only small islets of organelle-possessing hyaloplasm (Fig. 27).



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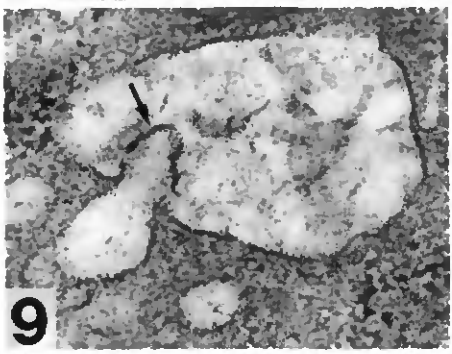

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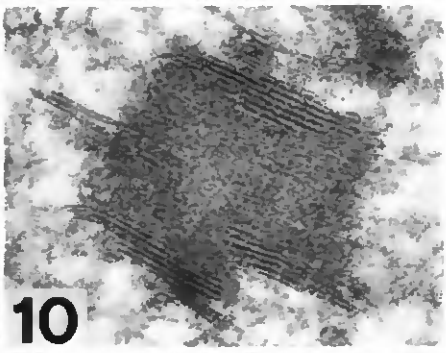

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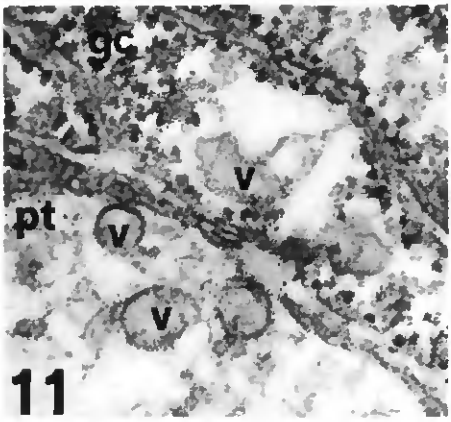

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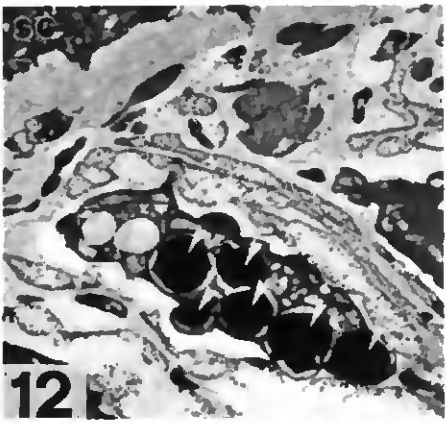

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B. The Calcareous Corpuscule Cell

In all tetrathyridia, calcareous corpuscule cells can be observed at various stages of development. They only accidentally show remnants of a concentric pattern when fixed by the glutaraldehyde-OsO_4-method used in this study. They are formed from cells of unknown origin that contain free ribosomes and numerous microtubules. In the centre of these cells, zones of granular cytoplasm appear in which precursors of the calcareous corpuscules are formed (Fig. 19). The calcareous corpuscule-precursors distinguish from lipid bodies by the lack of osmiophily. The precursor-forming cytoplasm is surrounded by a tubular labyrinth which opens into the intercellular space. During subsequent development the volume of the corpuscule precursors increases, and they fuse to form primary corpuscules, and then amalgamate to become secondary corpuscules (Fig. 20). The latter grow and fuse to form a single corpuscule of irregular shape. The granular corpuscule-synthesizing cytoplasm does not increase during the morphogenesis of the corpuscule. It forms a thin mantle surrounding the final corpuscule (Figs. 20 and 21), and in turn is surrounded by a lacuna which opens into the extracellular space. The lacuna represents the rest of the labyrinth of the primitive corpuscule forming cell. The corpuscule cell nucleus is located in a cytoplasmic expansion of the cell body. From each calcareous corpuscule cell originate long thin cytoplasmic filaments which extend into the intercellular space and attach to glycogen-storing cells by means of cell junctions. These cytoplasmic filaments contain ribosomes and mitochondria (Fig. 21).

III. Differentiation of the Tegument and the Glycogen-Storing Parenchyma Cells during Asexual Multiplication

Tissue formation during asexual multiplication is characterized by the growth of the apical massif followed by its longitudinal fission and differentiation

Figs. 8–12. Tetrathyridium of *Mesocestoides corti:* Tegumental perinuclear cell body

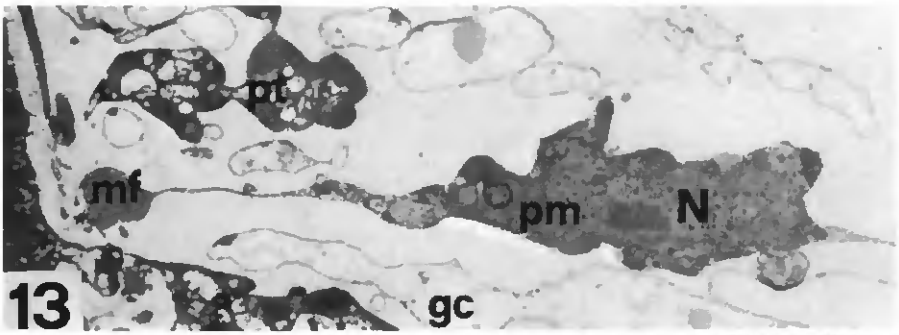
Fig. 8. Evolution of vesicles. Golgi apparatus (*G*) produce first order vesicles (*1*) which fuse to form second order vesicles (*2*). These contain the electron-lucid substance and aggregates of membranes of fused first order vesicles. Third order vesicles (*3*) contain membraneous aggregates but no more electron-lucid substance. × 40,700

Fig. 9. First order vesicle fusing with a second order vesicle. The unit membranes of the vesicles adjoin and form a quintalaminated aggregate (*arrow*). × 79,400

Fig. 10. Quintalaminated aggregates from large crystal-like membraneous complexes. × 118,000

Fig. 11. Small vesicles (*v*) or membrane fragments occur simultaneously in second order vesicles of tegumental cell bodies (*pt*) and in glycogen-storing cells (*gc*), when the two cell types are in close contact. It is uncertain whether or not these vesicles or fragments are directly or indirectly involved in the transport of the electron-lucid substance out of the tegumental cell body or if this observation needs another explanation. × 81,000

Fig. 12. Perinuclear tegumental cell body of the posterior part of the larva filled with third order vesicles (*arrows*). *sc* superficial cytoplasm. × 7,480



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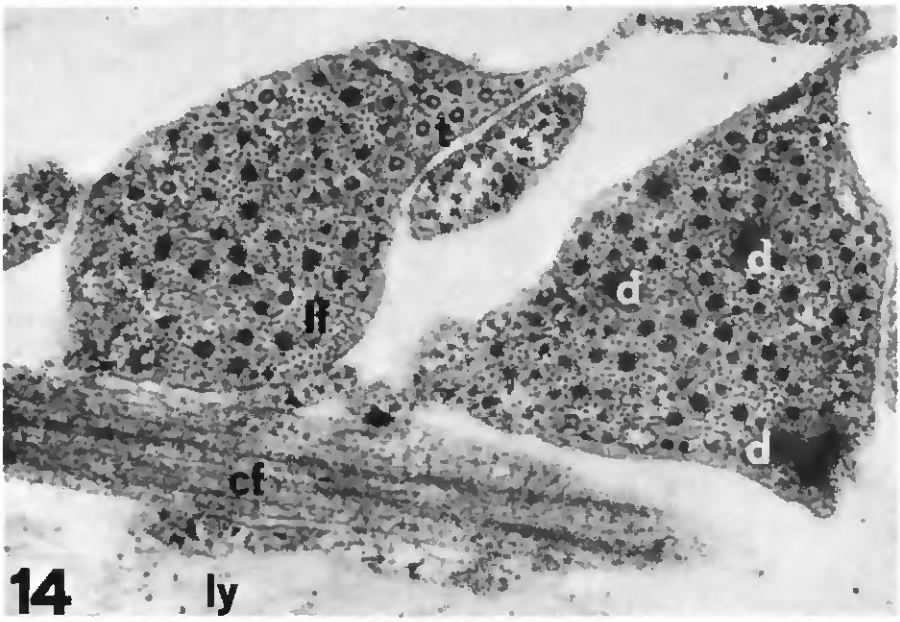

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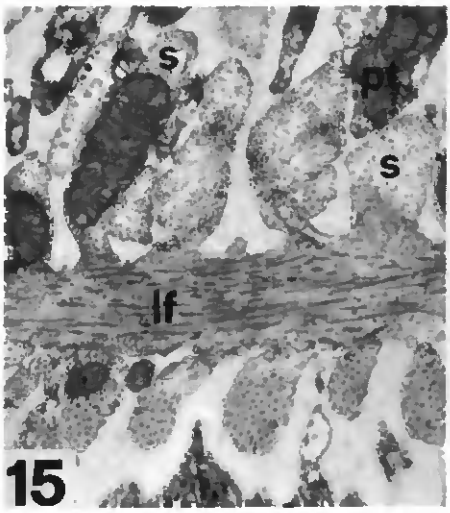

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(Fig. 22). During the growth-phase, the nuclei of the apical massif divide by mitosis and the cytoplasmic mass increases considerably. Few heterochromatic nuclei and well developed RER and Golgi zones indicate important synthetic activity. During the subsequent differentiation phase, vesicles comparable to the first order vesicles of the differentiated tegumental cell bodies appear in the Golgi zones, and glycogen rosettes accumulate in the cytoplasm. Simultaneously, plasmalemma formation takes place and the apical massif transforms into numerous mononucleate cell bodies which at first remain connected by cytoplasmic bridges (Fig. 23). These individual cell bodies differentiate into the perinuclear cell bodies of the tegumental syncytium (Figs. 24 and 26), glycogen-storing parenchyma cells (Figs. 24, 25, 27), the subtegumental muscle system (Fig. 28), and other cell types (sucker-blastema; Hess, in preparation). Some cells appear to remain undifferentiated. The future tegumental cells are recognized by their continuity with the superficial cytoplasm. The RER reduces and smaller mitochondria, smaller than those observed in the apical massif, develop. In the Golgi zones appear vesicles which are identical to the first and second order vesicles of the differenciated tegumental cells. The perinuclear cell bodies elongate and withdraw into the cortical parenchyma (Fig. 26).

The future parenchymal glycogen-storing cells originate from regions of the apical massif where initial deposition of alpha-glycogen took place. After formation of mononucleate cell bodies, large amounts of alpha-glycogen are accumulated in the peripheral cytoplasm of the young parenchymal cell (Fig. 25), a process that leads to the development of the lobes of the differentiated glycogen-storing parenchyma cells. The developed cell appears more than one hundred times larger than the original cell, and its lobes may reach from the cortical into the medullary parenchyma (Fig. 27).

Simultaneous with the differentiation of the tegumental syncytium and the glycogen-storing parenchymal cells, a third cell type, the sub-tegumental myoblast develops from the apical massif (Fig. 28). In these cells, the high electron density disappears and cisternae forming RER develops. Cytoplasmic extensions grow in which synthesis of contractile elements occurs. Myosin fibrils develop

Figs. 13–16. Tetrathyridium of *Mesocestoides corti*: Subtegumental muscle system

Fig. 13. The subtegumental muscle cell bodies (*pm*) located in the cortical parenchyma are connected to the muscle fibres (*mf*) by long cytoplasmic bridges. *gc* glycogen-storing cell; *pt* perinuclear cell body of tegumental syncytium. ×9,050

Fig. 14. Fine structure of the subtegumental muscle fibres. Thin myofibrils of actin surround thick myosin-myofibrils. The microtubule-like structures (*t*) represent developing myosin-myofibrils with electron transparent centres (see Fig. 28, inset). *cg* external circular fibre; *lf* internal longitudinal fibre; *ly* fibrous layer; *d* dense patches. ×98,500

Fig. 15. The sarcoplasmic expansions (*s*) of the muscle fibres contain mitochondria, ribosomes, and beta-glycogen. *pt* perinuclear cell body of the tegumental syncytium; *lf* longitudinal muscle fibre. ×20,000

Fig. 16. Specific staining for glycogen. The muscle fibres (*lf*) and sarcoplasmic expansions (*s*) contain granules of beta-glycogen (*small arrows*). In glycogen-storing cells (*gc*), the glycogen occurs predominantly in the alpha- or rosette-form (*large arrows*). ×46,000

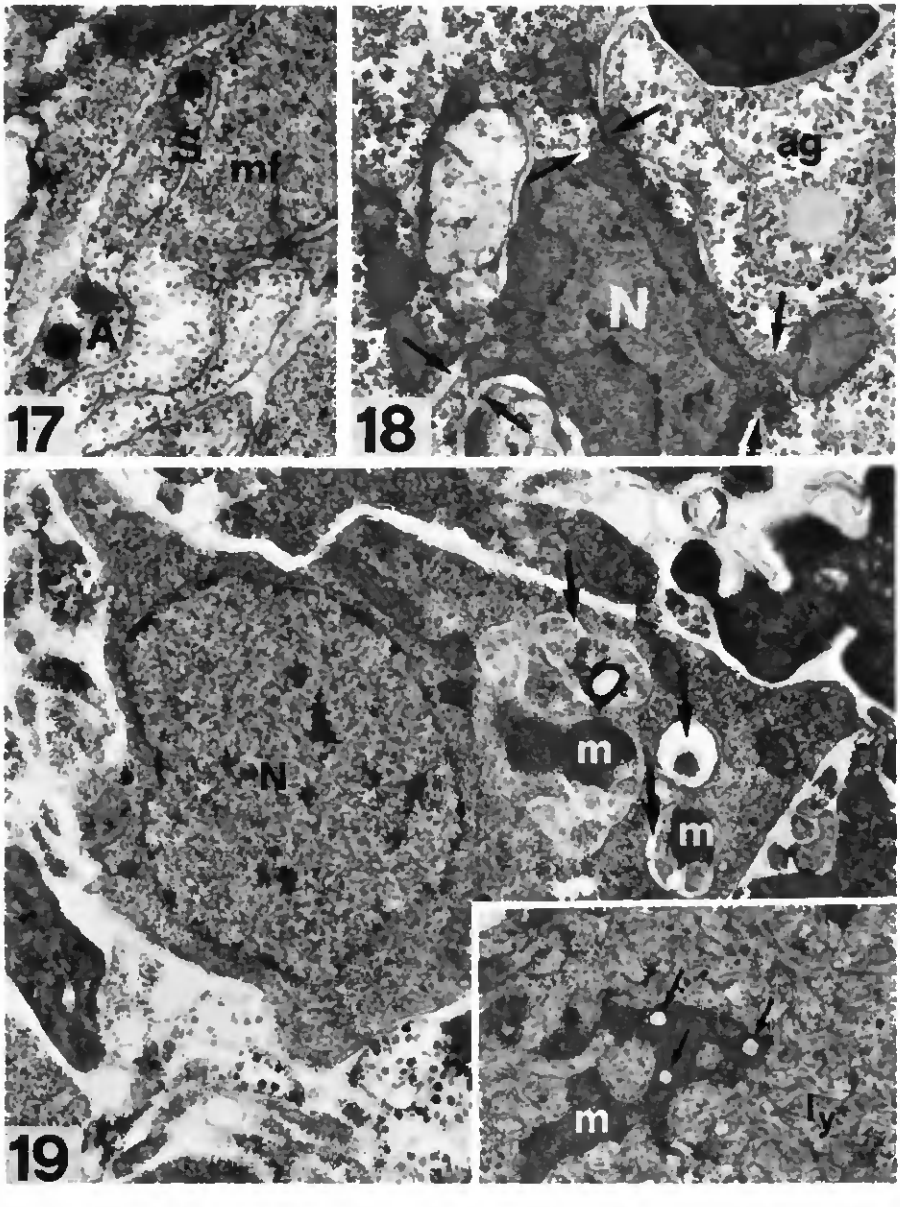
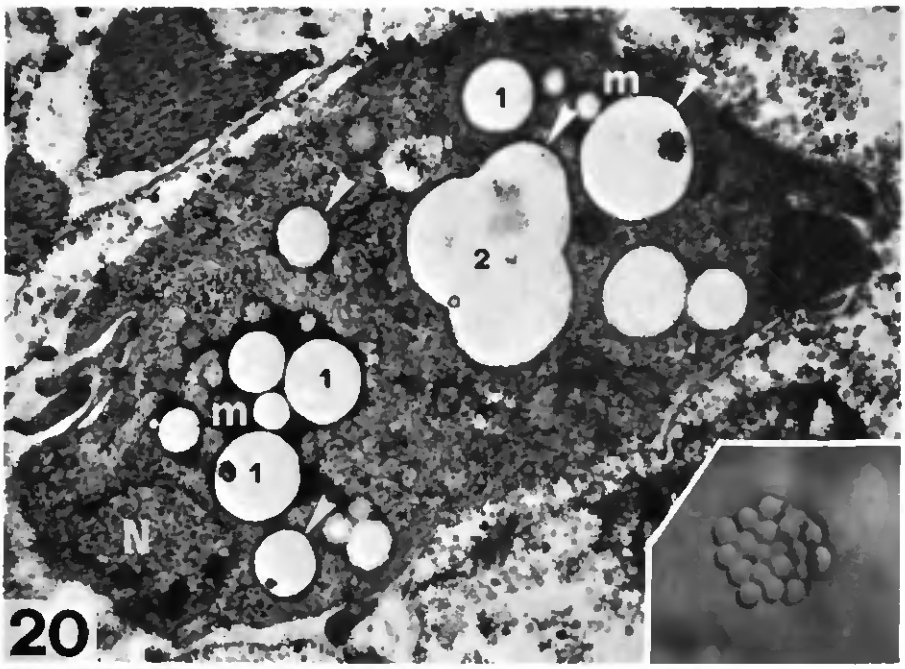


Fig. 17. Tetrathyridium of *Mesocestoides corti*. Axon (*A*) forming a neuromuscular junction (*S*) on a sub-tegumental muscle fibre (*mf*). × 44,500

Fig. 18. Tetrathyridium of *Mesocestoides corti*. Glycogen-storing parenchyma cell. Constrictions of the cell body (*arrows*) separate the central nucleate part (*N*) from the large glycogen-containing lobes. *ag* alpha-glycogen. × 15,300

Fig. 19. Tetrathyridium of *Mesocestoides corti*. Primitive calcareous corpuscule cell. Zones of electron-dense, granular cytoplasm (*m*) are surrounded by lacunae (*arrows*). × 17,950. *Inset:* In the electron-dense granulated cytoplasm appear the precursors of the calcareous corpuscules (*arrows*). The lacunae transform into a labyrinth (*ly*). × 18,670



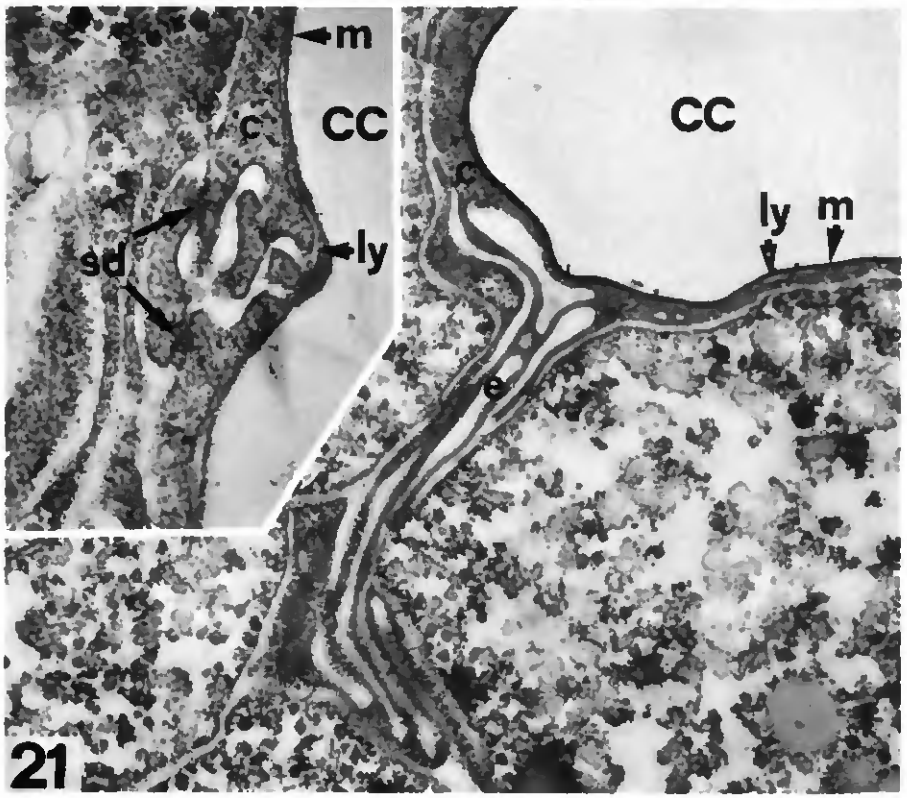


Fig. 20. Tetrathyridium of *Mesocestoides corti*. Calcareous corpuscule precursors enclosed in the electron-dense granular cytoplasm (*m*, *arrows*) fuse to form primary (*1*) and secondary (*2*) corpuscules. ×17,590 seen by light-microscopy

Fig. 21. Tetrathyridium of *Mesocestoides corti*. Final calcareous corpuscule (*cc*) enclosed in an electron-dense mantle (*m*) formed by the electron-dense cytoplasm. The mantle is surrounded by a lacuna (*ly*) which leads to the periphery of the cell (*c*). The lacuna is separated from the intercellular space by septate desmosomes (*sd*). Long cytoplasmic filaments (*e*) extend in the intercellular space. ×25,100. *Inset:* ×44,950

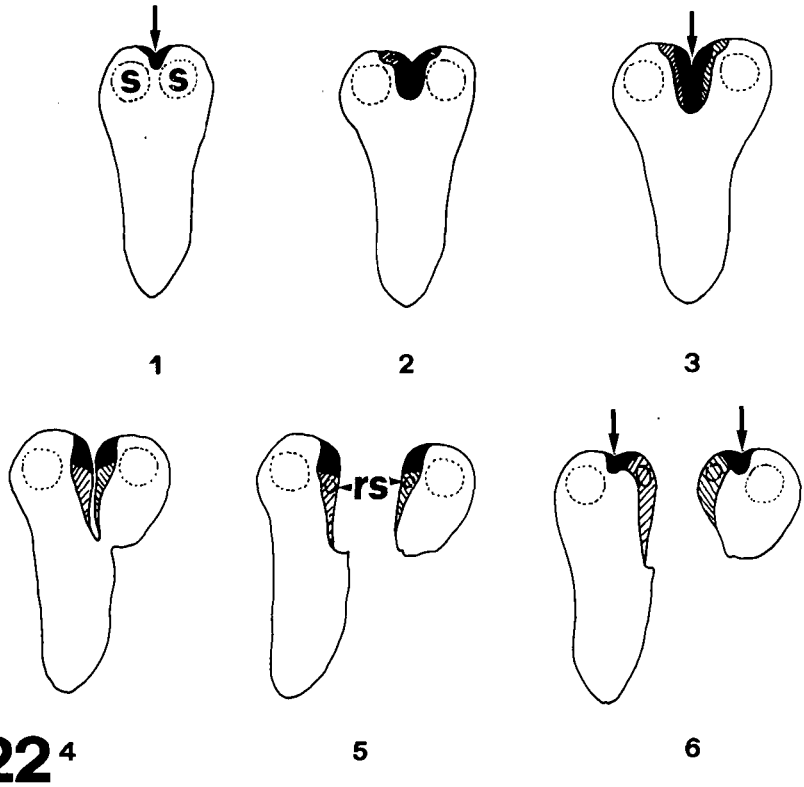
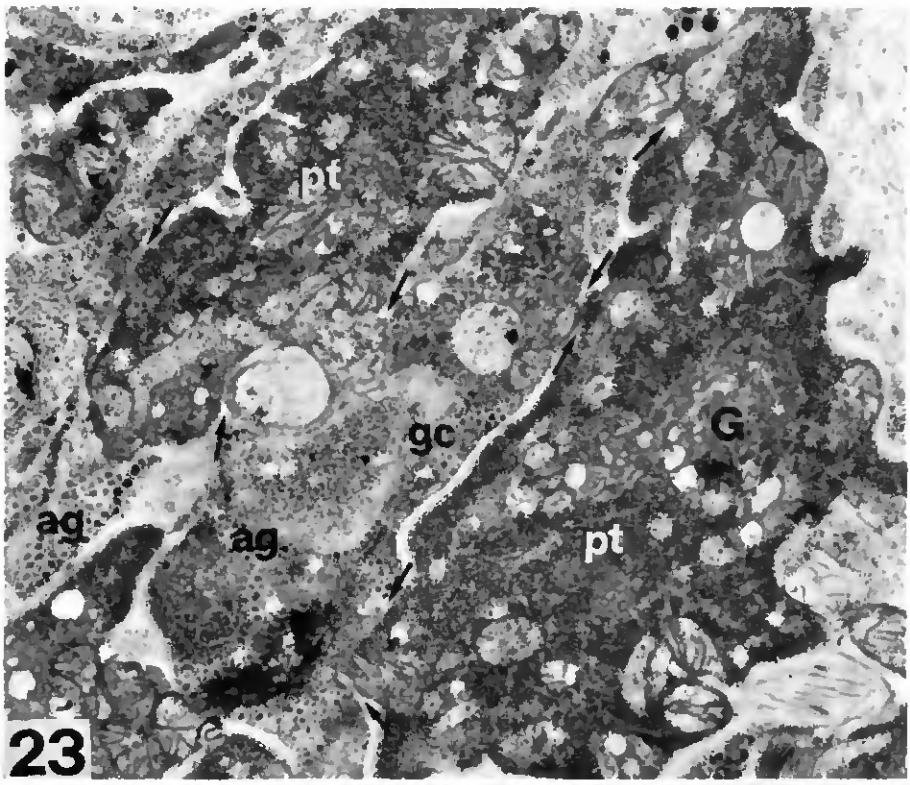


Fig. 22. Tetrathyridium of *Mesocestoides corti:* Asexual multiplication. *1* In the nondividing larva, the apical massif (*black zone*) is restricted to a small area between the suckers (*S*). *Arrow:* apical cleft. (see also Figs. 1 and 2). *2* During the predivision phase the apical massif increases and its lateral areas (*hatched zones*) differentiate into parenchymal cells and tegument. *3* The apical cleft progresses posteriorly, splitting the scolex into two parts (*arrow*) during the growth and lateral differentiation of the apical massif. *4* Division stops in the middle of the larval body. A tegumental constriction preceeds the separation of a half-scolex. Differentiation of the tegument and parenchyma continues (*hatches zones*) but the apical massif remains undifferentiated in the future apical centres of the two scoleces (*black zones*). *5* One, or both, of the scolices separates from the posterior part of the larva. The 'anlage' of a new pair of suckers (*rs*) forms in each scolex. *6* Final regeneration and achievement of symmetry. The apical cleft develops (*arrows*) in each regenerating scolex

Fig. 23. Tetrathyridium of *Mesocestoides corti.* Differentiation of the apical massif during asexual multiplication. Rosettes of alpha-glycogen (*ag*) accumulate in the apical massif and plasmalemma formation takes place to separate the future glycogen-storing parenchyma cells (*gc*) and tegumental syncytium cell bodies (*pt*) (*arrows*). In the latter, first order vesicles appear around the Golgi zones (*G*). ×16,520

Fig. 24. Tetrathyridium of *Mesocestoides corti.* Late phase of differentiation of tegumental syncytium and glycogen-storing parenchyma cells. A perinuclear cell body of the tegumental syncytium (*pt*) is still in cytoplasmic continuity with a glycogen-storing parenchyma cell (*gc*). Separation of the two cell types is imminent (*arrows*). ×12,200

Fig. 25. Tetrathyridium of *Mesocestoides corti.* Young glycogen-storing parenchyma cell during accumulation of alpha-glycogen (*ag*), in the peripheral lobes. ×20,000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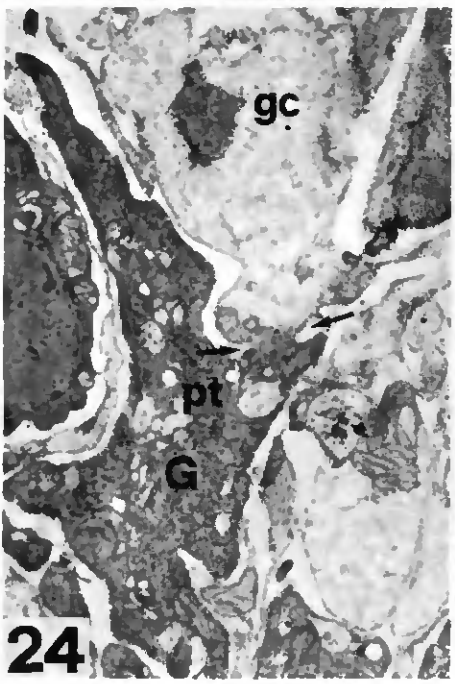
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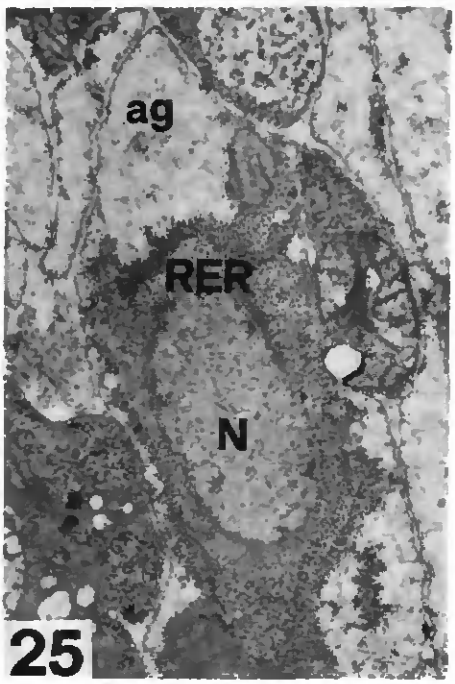
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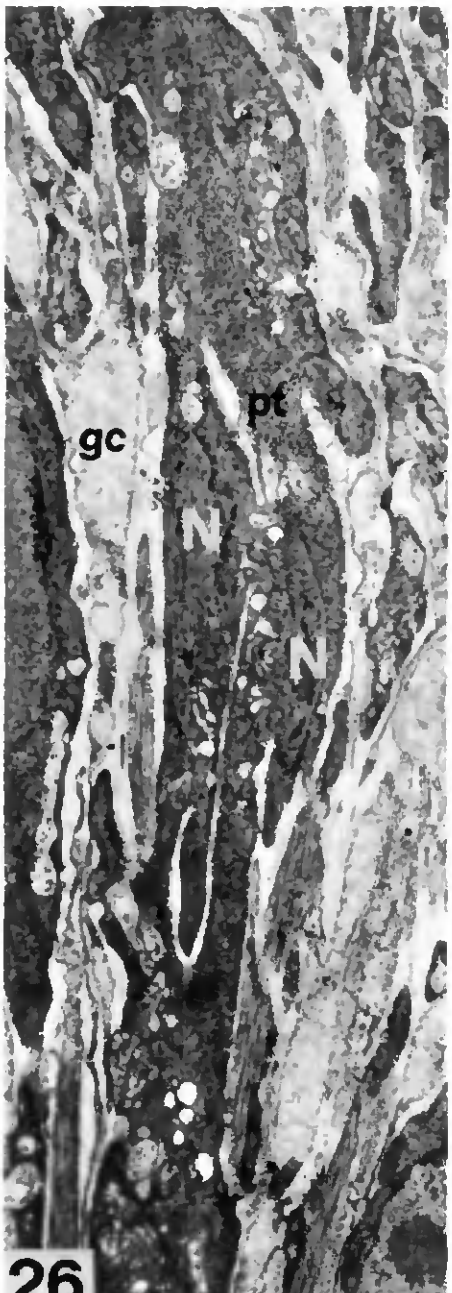

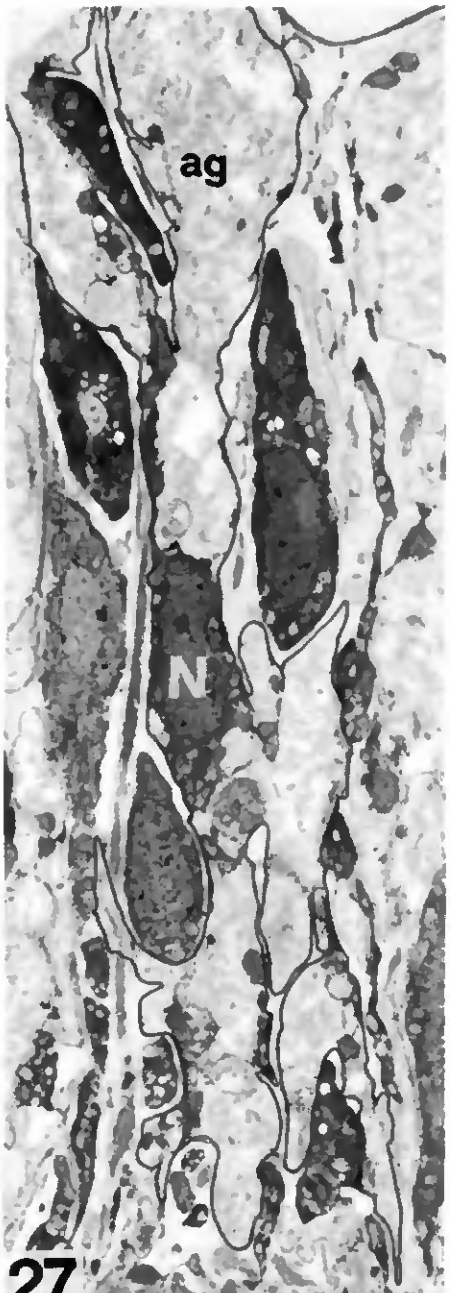

Fig. 26. Tetrathyridium of *Mesocestoides corti*. Differentiation of the tegumental syncytium. The polynuclear perinuclear cell bodies (*pt*), having separated from the glycogen-storing cells (*gc*), transform into mono-nucleate cell bodies (*N*: nuclei). ×11,500

Fig. 27. Tetrathyridium of *Mesocestoides corti*. Final differentiation of the parenchymal glycogen-storing cell. The cell accumulates large amounts of alpha-glycogen (*ag*) in the peripheral lobes. ×5,770

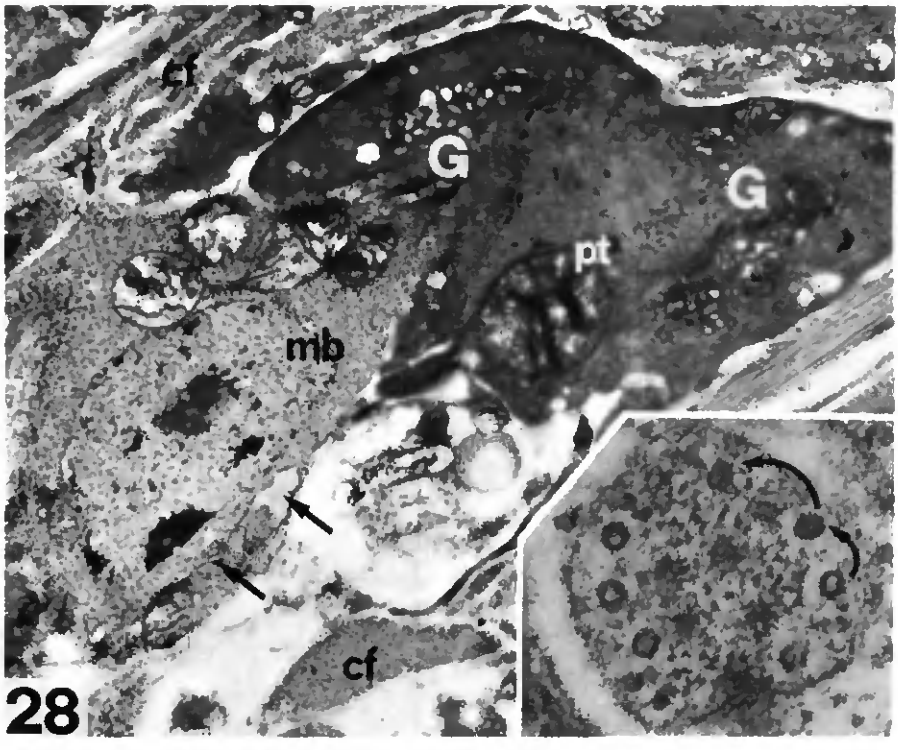

Fig. 28. Tetrathyridium of *Mesocestoides corti*. Differentiation of a sub-tegumental myoblast from the apical massif. The future cell body of the tegumental syncytium (*pt*) and the future myoblast (*mb*) are still in cytoplasmic continuity but differ structurally. In the less electron-dense future myoblast cysternae-forming RER develops (*arrows*). Golgi apparatus are restricted to the perinuclear cell bodies of the tegumental syncytium (*G, pt*). Myosin fibrils develop from myofibres with electron-transparent centres (*inset: arrows*) located in the cytoplasmic extensions which project from the myoblast (*cf*). × 11,380. *Inset* × 153,000

progressively from myofilaments with electron transparent centres (∅ 23–30 nm) (Fig. 28, inset) and become surrounded by actin myofibrils.

IV. Longitudinal Tegumental Growth of the Tetrathyridia

Tetrathyridia of *Mesocestoides corti* grow continuously, even when not in division. The length of the larvae remains constant by periodic autotomy of the posterior part (Hess, 1972; Novak, 1972). Longitudinal growth depends on growth of the tegument. In tetrathyridia, mitosis does not occur in the tegumental cell bodies, but may be observed in the germinal cells and in the apical massif. The location of the apical massif between the suckers excludes its direct contribution to the longitudinal growth of the tegumental syncytium of the posterior parts of the body. Consequently, it may be assumed that tegumental growth occurs by invasion of particular cells into the tegumental syncytium as observed by Wikgren and Knuts (1970) in plerocercoids of *Diphyllobothrium dendriticum*.

When larvae were cultivated in a culture medium containing H^3-thymidine, nuclei of the apical massif and germinative cells incorporated the radioactive

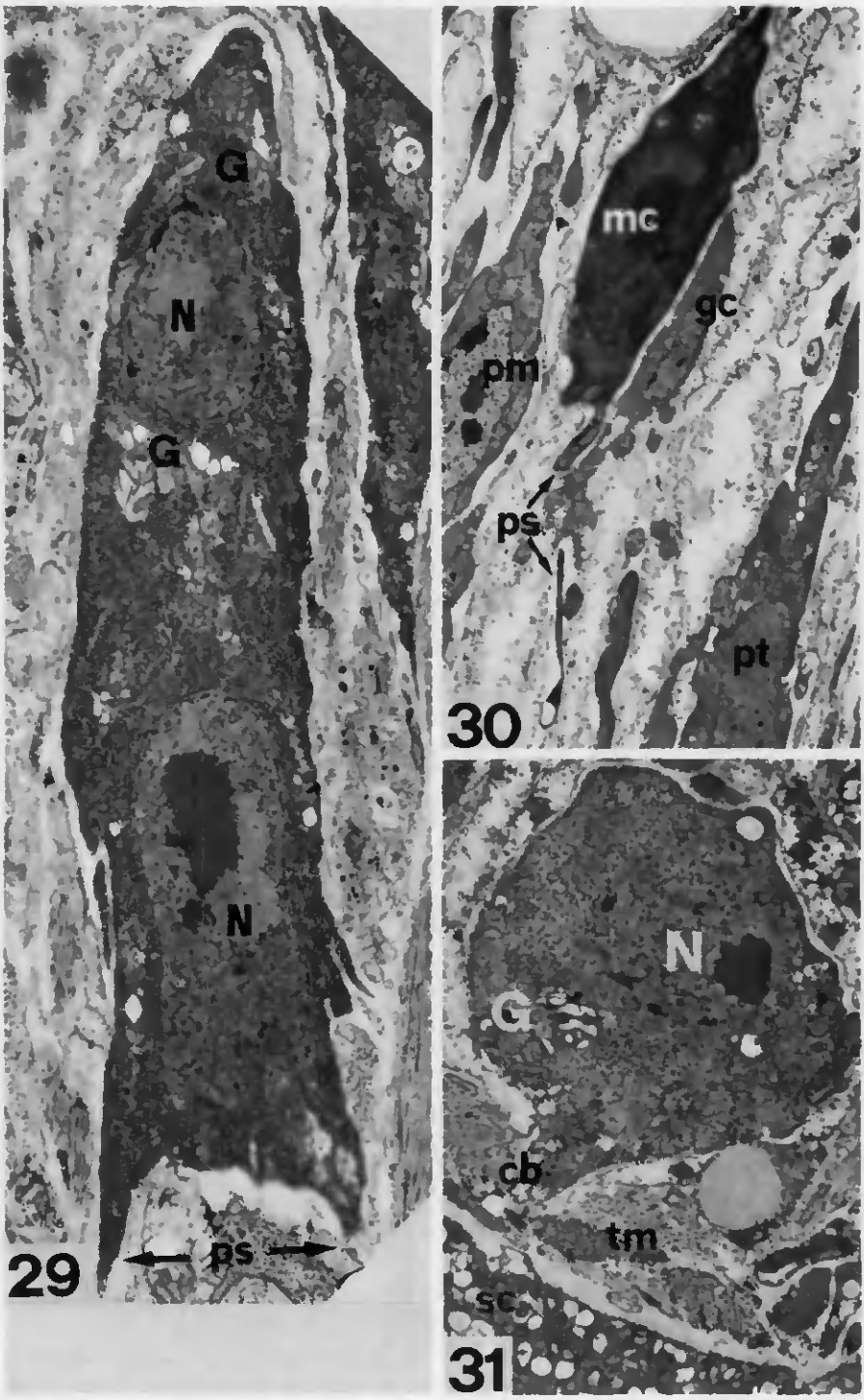

Fig. 29. Tetrathyridium of *Mesocestoides corti*. Migrating binuclear cell with Golgi apparatus (*G*) and well developed RER; *ps:* pseudopods. × 13,700

Fig. 30. Tetrathyridium of *Mesocestoides corti*. Migrating cell (*mc*) near the tegument. *ps:* pseudopod; *pt:* perinuclear cell body of the tegumental syncytium; *pm:* cell body of the sub-tegumental muscle system; *gc:* glycogen-storing parenchyma cell. × 7,300

Fig. 31. Tetrathyridium of *Mesocestoides corti*. Migrating cell after integration into the tegumental syncytium. The Golgi apparatus (*G*) is not yet surrounded by vesicles. *cb*: cytoplasmic bridges; *sc:* superficial cytoplasm; *tm:* sub-tegumental muscles. × 12,200

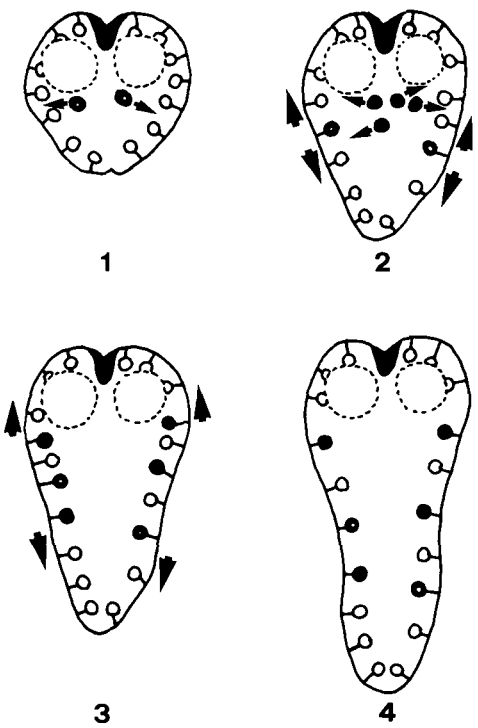

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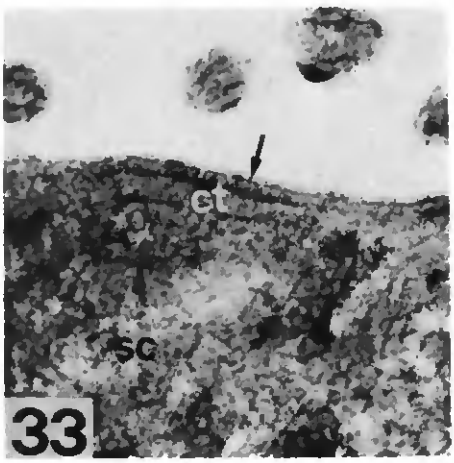
Fig. 32. Tetrathyridium of *Mesocestoides corti*, longitudinal growth *1–4*. Schematic representation of tegumental growth by invasion and integration of undifferentiated cells. *Black with clear centre:* first generation of migrating cells. *Black:* second generation of migrating cells. *Small arrows:* direction of migration and integration. *Large arrows:* resulting growth of superficial cytoplasm

compound. As early as 24 h later, marked cells were found integrated into the tegument. It appears that these cells had migrated to the tegument. Such migrating cells, identified by TEM in the parenchyma, are characterized by their electron-dense hyaloplasm, well developed RER, large mitochondria, several Golgi apparatus, and long pseudopods (Figs. 29 and 30). Close to the apical massif, such cells are often binucleate. When the migrating cells integrate into the tegumental syncytium (Fig. 31), Golgi apparatus become more numerous, and first, second, and third order vesicles develop. The functionally integrated cells produce structural proteins of the tegumental syncytium resulting in the growth of the superficial cytoplasm (Fig. 32). Migrating cells transforming into sub-tegumental myoblasts have not been observed. Functional circular sub-tegumental muscle fibres may synthesize myofibrils and divide longitudinally, thus increasing the number of muscle fibres simultaneously with the longitudinal growth of the tegumental syncytium.

V. Morphogenesis of Microtriches

Filamentous and blade-like microtriches occur on the tegument of the tetrathyridia (Hess and Guggenheim, 1977). Their morphogenesis is observed during asexual multiplication and longitudinal growth.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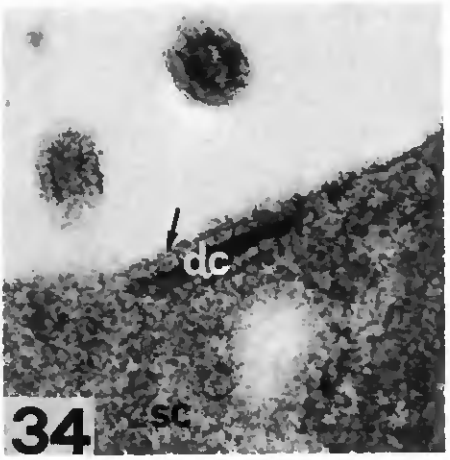
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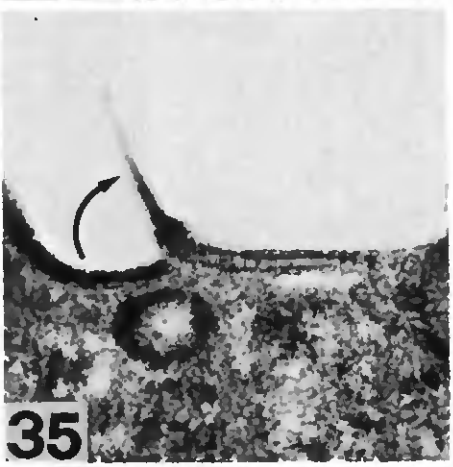
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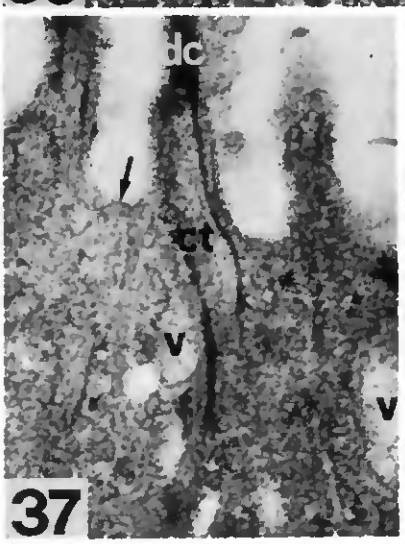
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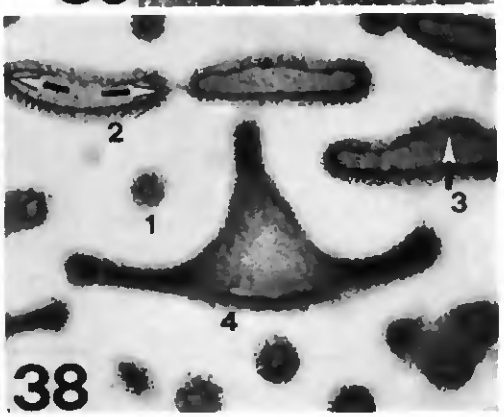
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A. Filamentous Microtriches

The dense cord and the central tube of this type of microtriches are synthesized in the superficial cytoplasm and are joined to the plasmalemma (Figs. 33 and 34). The shafts are always directed posteriorly. The organelles which participate in the synthesis of the microtriches are undetermined because of the absence of ultrastructural modifications in the microthrix-forming cytoplasm and in the neighbouring perinuclear cell bodies. When synthesis is terminated, the shaft becomes surrounded by the tegumental plasmalemma, becomes erect, and takes a position which is perpendicular to the tegumental surface (Fig. 35). Subsequently the base begins to emerge, and the central tube moves to the same position as the shaft, i.e. perpendicular to the tegument surface (Fig. 36). During the emergence of the microthrix, vesicles adhere to the synthesized elements and fuse with the tegumental plasmalemma (Fig. 37).

B. Blade-like Microtriches

Intrategumental stages of this type were not found in the superficial cytoplasm, but erected forms intermediate between filamentous and blade-like microtriches are observed. These represent enlarged filamentous microtriches with the large axis oriented perpendicularly to the long axis of the larva. The width and length of their posteriorly directed support vary (Fig. 38).

Discussion

In cestodes asexual multiplication is uncommon. It is restricted to a few cyclophyllidean larvae belonging to different families. The best known examples

Figs. 33–37. Tetrathyridium of *Mesocestoides corti:* Morphogenesis of filamentous microtriches

Fig. 33. Synthesis of the central tube (*ct*) of the base adjoining the plasmalemma (*arrow*) of the superficial tegumental cytoplasm. × 117,500

Fig. 34. Synthesis of the dense core (*dc*) of the shaft which adheres to the plasmalemma (*arrow*) of the superficial tegumental cytoplasm (*sc*). × 117,500

Fig. 35. Erection of the shaft. × 62,930

Fig. 36. Base following the movement of the shaft. × 70,000

Fig. 37. Outgrowth of the base. Plasmalemma-formation by vesicles (*v*), adjoined to the central tube, which subsequently fuse with the superficial unit membrane (*arrow*). × 82,700

Fig. 38. Tetrathyridium of *Mesocestoides corti.* Hypothetical sequence of morphogenesis of blade-like microtriches. The filamentous microthrix (*1*) enlarges laterally (*2*) and forms a posteriorly directed support (*3*). *4:* blade-like microthrix. *1–4* are sections of the base. × 62,270

are the cysticercoids of *Hymenolepis prolifer* and *Hymenolepis pistillum*, the cysticerci of *Taenia crassiceps*, the coenuri of *Multiceps serialis* and *Multiceps multiceps*, and the hydatids of *Echinococcus granulosus* and *Echinococcus multilocularis*. All these larvae multiply by budding. The tetrathyridium of *Mesocestoides corti*, on the other hand, is a plerocercoid-like larva that divides by antero-posterior fission. Using TEM, a tissue was discovered that is related functionally with this mode of multiplication. This tissue, named the 'apical massif', is an inwards directed protuberance of the tegumental syncytium, and is situated between the suckers of the larva. It is distinguished from the posterior mononuclear cell bodies of the tegumental syncytium by the presence of dividing nuclei and its morphocytogenetic power. The apical location of this tissue explains the mode of multiplication of the tetrathyridia. The apical cleft, visible in all larvae as a small dorsoventral depression between the suckers, advances posteriorly after cytoplasmic growth of the apical massif and nuclear divisions. This cleft splits the apical massif into two parts, producing two half-scoleces which each lacks a pair of suckers. The fission stops at the posterior end of the apical massif. Subsequently, one or both scolices detach; if not, multiheaded larvae are produced. Tegumental syncytium, subtegumental muscles, and glycogen-storing parenchyma cells differentiate from the apical massif during the regeneration phase. A part of the anlagen of the suckers also derives from this tissue (Hess, in preparation). The apical massif does not differentiate in the apical centre of each regenerating scolex, and thus preserves its capacity of cytomorphogenesis. The importance of germinative cells which also occur in the tetrathyridia is only partially known (Hess, in preparation); they transform into myoblasts of the internal muscle system and possibly other cell types, but they never form tegumental syncytium or sub-tegumental muscles. On the other hand they are also found in the anlagen of the suckers. The interactions, if any, between the apical massif and the pool of germinative cells, are unknown.

The apical massif is also the source of migrating cells which produce longitudinal tegumental growth by invasion of the tegumental syncytium. These cells, which have a fine structure identical to that of the apical massif, cannot be confused with germinative cells as the latter have no or few RER and one or no Golgi apparatus (Hess, in preparation). The longitudinal growth of the tegument by invasion of cells was first observed in plerocercoids of *Diphyllobothrium dendriticum* by Wikgren and Knuts (1970). Integration of free cells into the cyst wall, which is homologous to tegumental growth, also occurs during morphogenesis in hydatid cysts (Sakamoto and Sugimura, 1970).

The ontogenetic origin of the apical massif is a matter of conjecture. One could speculate that it derives from the binucleate centre observed in preoncospheres of various cestodes (Mokhtar-Maamouri, 1976; Ogren, 1956, 1957, 1958; Rybycka, 1964). Rybycka (1973) showed by TEM that this primitive syncytium ('the binucleate epithelial cell') is the origin of the tegument of the oncosphere and consequently of the larval and adult tegument.

The large number of free ribosomes and the well developed RER in the apical massif indicate active synthesis of proteins, which is related to the formation of new structures. In the differentiated perinuclear cell bodies, the fine structure is modified; free ribosomes are still frequent but RER is decreased, and the

activity of Golgi apparatus leads to an accumulation of three types of vesicles in the cytoplasm. The Golgi-, or first order, vesicles fuse to form second order vesicles which contain the synthesized substance and fragments of the vesicular membranes. It is evident that the third order vesicles, containing membraneous aggregates, are formed from second-order vesicles which have eliminated their electron-lucid content.

The tegumental cell bodies seem to lack an enzyme system to eliminate the third order vesicles, which accumulate and may obstruct the cytoplasm and lead to the disparition of Golgi apparatus and first and second order vesicles. This may be considered as an ageing process. The increased number of such 'old' cell bodies in the posterior part of the tetrathyridia is due to the addition of new tegumental cells in the more anterior parts of the larvae during longitudinal growth. The accumulation of 'old' cell bodies possibly exhibits an induction, leading to the separation of the posterior part of the body by transverse fission.

The fine structure of the perinuclear cell bodies has also been described for other cestodes. Threadgold (1962) found well developed Golgi apparatus, 'protein cristalloids', and 'fatty inclusions' in the tegumental cell bodies of *Dipylidium caninum*. In adult *Proteocephalus pollanicoli*, the same author (Threadgold, 1965) noted 'phospholipid bodies'. Baron (1968) found 'whorls of membranes' in the tegumental perinuclear cell bodies of *Cysticercus longicollis*. It is assumed that these inclusions are identical to the membraneous aggregates of the second and third order vesicles described here.

As a consequence of the asexual multiplication and the longitudinal growth of the tegument, synthesis of new microtriches occurs. The filamentous microtriches are of intrategumental formation. This observation is supported by Lumsden (1974) who described intrategumental synthesis of microtriches in *Spirometra mansonoides*. Intrategumental stages of blade-like microtriches are not observed, but intermediate forms between filamentous and blade-like microtriches are, however, found. Thus, the blade-like microtriches may develop from filamentous forms.

The structure of the sub-tegumental muscles of the tetrathyridium is comparable to that of other cestodes (Lumsden and Byam, 1967; Slais et al., 1972). The sub-tegumental myocytes are distinguished from other cell types by the absence of Golgi apparatus, the lack of glycogen deposits in the perinuclear cytoplasm, and the presence of cysternae forming RER. During asexual multiplication, the tegumental myoblasts derive from the apical massif. Myosin myofilaments develop from microtubule-like structures (myofilaments with electron-transparent centres). Such developing myofibrils observed here are also found in functional muscles. As no migrating tegumental cells differentiate into sub-tegumental myoblasts, the sub-tegumental muscles may follow the growth of the tegumental syncytium by the synthesis of new contractile elements and the subsequent longitudinal division of functional fibres.

The deposit of alpha-glycogen in the apical massif and the subsequent separation of these deposits from the tegumental syncytium leads to the formation of the glycogen-storing cell during asexual multiplication. During the first stages of development, tegumental cell bodies and glycogen-storing cells remain in

cytoplasmic continuity. This relationship of glycogen-storing cells and tegumental cell bodies accounts for contradictory descriptions of the tegumental cell bodies. Beguin (1966) showed that tegumental cell bodies contain alpha-glycogen while other authors (for example Baron, 1968) describe these cells without glycogen deposits.

In the mature glycogen-storing cell, constrictions of the plasmalemma subdivide the glycogen-storing cell into a central nucleated part and voluminous glycogen-containing lobes. That these lobes may separate from the central nucleated portion and become anucleate glycogen-sacs cannot be excluded. Such dedifferentiation processes have been observed during experimental regeneration of the tetrathyridia (Hess, in preparation).

Nieland and von Brand (1969) and Swiderski et al. (1970) demonstrated that the calcareous corpuscules of cestodes are intracellular formations. Observations here indicate that undifferentiated cells containing a zone of electron-dense, granular cytoplasm produce small corpuscule-precursors which fuse to form a unique corpuscule. The labyrinth surrounding the corpuscule-forming cytoplasm, also observed by Nieland and von Brand (1969), establishes a contact of the granular corpuscule-forming cytoplasm with the interstitial liquid. The septate desmosomes occurring at the orifice of the labyrinth may be temporary formations.

The cytoplasmic filaments originating from the calcareous corpuscule cell body considerably increase the cellular surface. This may represent an adaptation of an absorbing cell. Under experimental conditions, these filaments are capable of phagocytosis (Hess, in preparation), thus supporting the theory that calcareous corpuscules serve to stock metabolic end-products which cannot be eliminated from the body. As phosphatases have been demonstrated in the calcareous corpuscules of various cestodes (for review see Smyth, 1969) it is probable that the calcareous corpuscules represent formations comparable to the residual bodies of the lysosomal system.

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References

Appleton, T.C.: Stripping film autoradiography. In: Autoradiography for biologists, P.B. Gahan, ed., London and New York: Academic Press 1972

Baron, P.J.: On the histology and ultrastructure of *Cysticercus longicollis*, the cysticercus of *Taenia crassiceps* Zeder, 1800 (Cestoda: Cyclophyllidea). Parasitology **58**, 497–513 (1968)

Beguin, F.: Etude au microscope électronique de la cuticule et de ses structures associées chez quelques Cestodes. Essai d'histologie comparée. Z. Zellforsch. **2**, 30–46 (1966)

Hart, J.: Regeneration of tetrathyridia of *Mesocestoides* (Cestoda: Cyclophyllidea) in vivo and in vitro. J. Parasitol. **54**, 950–956 (1968)

Hess, E.: Contribution à la biologie larvaire de *Mesocestoides corti* Hoeppli, 1925 (Cestoda, Cyclophyllidea). Note préliminaire. Rev. Suisse Zool. **79**, 1031–1037 (1972)

Hess, E., Guggenheim, R.: A study of the microtriches and sensory processes of the tetrathyridium

of *Mesocestoides corti* Hoeppli, 1925, by transmission and scanning electron microscopy. Z. Parasitenkd. **53**, 189–199 (1977)
Lumsden, R.D.: Brush border development in the tegument of the tapeworm *Spirometra mansonoides*. J. Parasitol. **60**, 209–226 (1974)
Lumsden, R.D., Byam, J.: The ultrastructure of cestode muscle. J. Parasitol. **53**, 326–342 (1967)
Mac Rae, E.: The fine structure of muscle in marine Turbellarioans. Z. Zellforsch. **68**, 348–362 (1965)
Mokhtar-Maamouri, F.: Etude ultrastructurale de la gamétogenèse et des premiers stades du développement de deux Cestodes Tetraphyllidea. Thèse, Académie de Montpellier 1976
Nieland, M.L., Brand, T. von: Electron microscopy of cestode calcareous corpuscle formation. Exp. Parasitol. **24**, 279–289 (1969)
Novak, M.: Quantitative studies on the growth and multiplication of tetrathyridia of *Mesocestroides corti* Hoeppli, 1925 (Cestoda: Cyclophyllidea) in rodents. Can. J. Zool. **50**, 1189–1196 (1972)
Ogren, R.E.: Development and morphology of the oncosphere of *Mesocestoides corti*, a tapeworm of mammals. J. Parasitol. **42**, 414–428 (1956)
Ogren, R.E.: Morphology and development of oncospheres of the cestode *Oochoristica symmetrica* Baylis. J. Parasitol. **43**, 505–520 (1957)
Ogren, R.E.: The hexacanth embryo of a dilepidid tapeworm. 1. The development of hooks and contractile parenchyma. J. Parasitol. **44**, 477–483 (1958)
Rybicka, K.: Embryonic development in *Moniezia expansa* Rudolphi, 1810 (Cyclophyllidea, Anoplocephalidae). Acta Parasiol. Pol. **12**, 313–330 (1964)
Rybicka, K.: Ultrastructure of the embryonic syncytial epithelium in a cestode *Hymenolepis diminuta*. Parasitology **66**, 8–18 (1973)
Sakamoto, F., Sugimura, M.: Studies on echinococcosis. XXIII. Electron microscopical observations on histogenesis of larval *Echinococcus multilocularis*. Jpn. J. Vet. Res. **18**, 131–144 (1970)
Slais, J., Serbus, C., Schramlova, J.: The fine structure of the contractile elements in the bladder wall of *Cysticercus bovis*. Folia Parasitol. (Praha) **19**, 165–167 (1972)
Smyth, J.D.: The physiology of cestodes. Edinburgh: Oliver and Boyd 1969
Specht, D., Voge, M.: Asexual multiplication of *Mesocestoides tetrathyridia* in laboratory animals. J. Parasitol. **51**, 268–272 (1965)
Swiderski, Z., Huggel, H., Schoenenberger, N.: Electron microscopy of calcareous corpuscle formation and their ultrastructure in the cestode *Inermicapsifer madagascariensis*. 7ème Congrès International de Microscopie Electronique, Grenoble 1970
Thiery, J.P.: Mise en évidence des polysaccharides sur coupes fines en microscopie électronique. J. Microsc. **6**, 987–1018 (1967)
Threadgold, L.T.: An electron microscope study of the tegument and associated structures of *Dipylidium caninum*. Q. J. Microsc. Sci. **103**, 135–140 (1962)
Threadgold, L.T.: An electron microscope study of the tegument and associated structures of *Proteocephalus pollanicoli*. Parasitology **55**, 467–472 (1965)
Wikgren, B.J., Knuts, G.M.: Growth of subtegumental tissue in cestodes by cell migration. Acta Acad. Abor. B **30**, 16 (1970)

Received April 17, 1979

Z. Parasitenk. 53, 189–199 (1977)

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A Study of the Microtriches and Sensory Processes of the Tetrathyridium of *Mesocestoides corti* Hoeppli, 1925, by Transmission and Scanning Electron Microscopy*

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Summary. Two types of microtriches occur on the tegument of the tetrathyridium of *Mesocestoides corti* as shown by scanning and transmission electron microscopy. One is filamentous and the other large and blade-like. The large form has a complex structure with a posteriorly directed support, so that its transverse section is T-shaped. The distribution of the two types on the body surface of the larva is not homogeneous. On the anterior part of the larva, including the suckers (Zone A), both are present. In the region behind the suckers (Zone B), only blade-like microtriches occur and on the posterior extremity (Zone C) only filamentous forms are seen. Between the Zones B and C there is an intermediate region with both types present. The authors suppose that the blade-like microtriches play an important role in tissue penetration, while the filamentous forms are involved in food uptake. Two types of sensory processes are identifiable. One is club-shaped, the other is a very long filamentous process.

Introduction

The extensive literature dealing with the structure and physiology of the cestode tegument has been reviewed by Lee (1972) and Lumsden (1975). Transmission electron microscopy (TEM) studies have contributed to this field in the past, though recently the use of the scanning electron microscope (SEM) has provided another means for investigating the tegument (Berger and Mettrick, 1972; Ubelaker et al., 1973; Andersen, 1975; Yamane, 1975). Our knowledge of the tegument is far from complete, the published results often being fragmentary; however, these techniques have contributed greatly to our understanding.

Microtrichial polymorphism among larval and adult pseudophyllidean and cyclophyllidean tapeworms has been reported by Andersen (1975), Berger and Mettrick (1971), Blitz and Smyth (1973), Bråten (1968), Featherston (1972), Grammeltvedt (1973), Race (1966) and Yamane (1968, 1975).

This study has been undertaken to determine the relationship between microtrichial polymorphism and body region in a species not yet examined by TEM and SEM before.

* Part of thesis of the first author.

Materials and Methods

Tetrathyridia of *Mesocestoides corti* originally isolated by Specht and Voge (1965) from *Sceloporus occidentalis bisertatus* in California were maintained in our laboratory in the peritoneal cavities of NMRI-mice. The larvae were washed in worm NCTC 135 and fixed in 2% glutaraldehyde buffered by 0.1 M Na-cacodylate, pH 7.2, at 4° C for 16 h, theo rinsed in 0.16 M Na-cacodylate, pH 7.2, at 4° C for 1 day and postfixed in 1% OsO_4 buffered by 0.1 Na-cacodylate, pH 7.2, at room temperature for 90 min. After a short wash in buffer, the larvae were dehydrated in acetone, and embedded in SPURR, propylene oxide being used as an intermediate solvent. Sections made on a SORVALL-ultramicrotome equipped with a diamond knife were mounted on copper grids and double stained with uranyl acetate and lead citrate. The sections were studied on a Philips 201 EM. For SEM, the same fixations as above were used. The wet specimens were quickly submersed in 30% acetone, dehydrated in an ascending series of acetone up to 100% (steps 10%) and finally dried by the critical-point method using CO_2 (Cohen, 1974). For critical-point drying we used a Bomar SPC-1500. After drying, the objects were mounted on specimen stubs, surrounded with silver paint and coated with a thin layer of carbon-gold in a Leyhold-Heraeus EPA 100 evaporator. A Cambridge Mark IIA scanning electron microscope was used to examine the specimens at accelerating voltages of 10 keV under different angles.

Results

1. The Microtriches

Two types of microtriches are found on the body surface of tetrathyridia (Fig. 1). They differ in size, form and ultrastructure but both are composed of a base and a shaft. An apparently well-developed glycocalix or other secretions cover the microtriches.

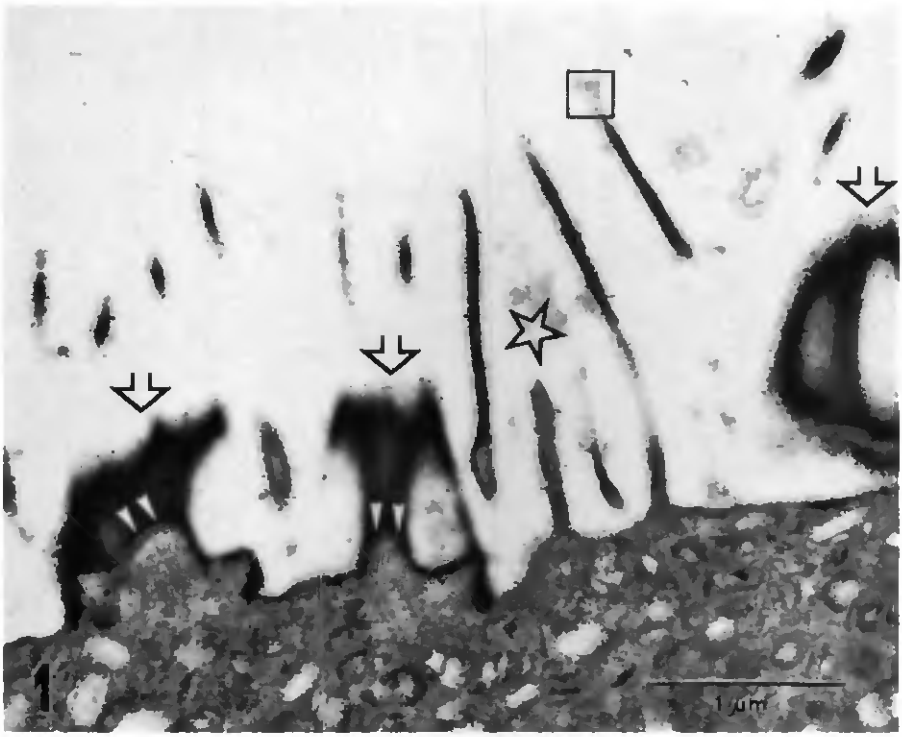


Fig. 1. Tetrathyridium of *M. corti*. Transverse section of the tegument showing two types of microtriches. *Asterisk*: filamentous microtriches; *arrows*: blade-like microtriches; *double arrows*: multilaminated baseplate; *square*: glycocalix. 26,250×

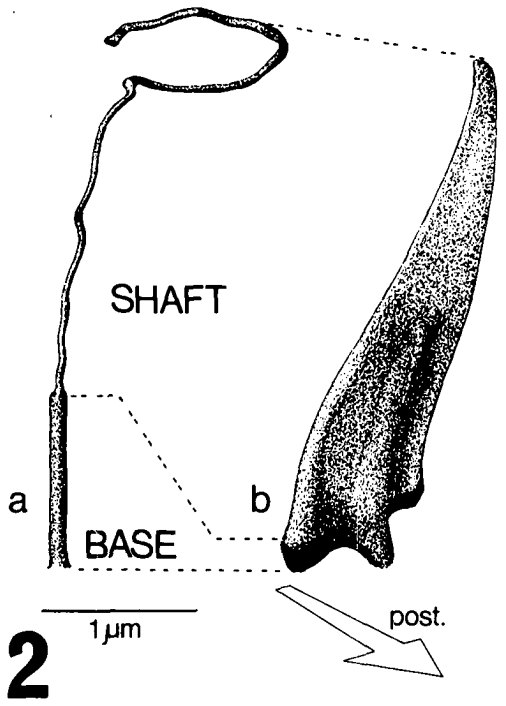


Fig. 2. Tetrathyridium of *M. corti*. Reconstruction of the two forms of microtriches. *a*: filamentous type, *b*: blade-like type, *post.* indicates the antero-posterior axis of the body. Approx. 24,000×

The first microthrix (filamentous type) is cylindrical (Fig. 2a). The length of the base varies from 0.5 to 1.3 µm. The bases of the microtriches of the scolex region are generally shorter than those of the posterior part of the larvae. In transverse sections (Fig. 3a), which have a diameter of 0.08–0.09 µm, the base is surrounded by a trilaminar unit membrane, 60–90 Å thick. A central tube, 0.06 µm in diameter, is seen in the base. It is closed distally. This tube is joined to the unit membrane by means of radially arranged elements (Fig. 3a, arrows). In the centre of the microthrix, few parallel microfilaments are present. They are attached to the upper end of the central tube. The electron dense distal part or shaft (Figs. 2a and 3b) measures from 1.1 µm (posterior part of the larvae) to 7 µm (anterior part of the larvae) in length and from 0.04 to 0.06 µm in diameter. The centre is an electron-dense cord, 0.02 µm in diameter and surrounded by a granular cytoplasm. It is separated from the central tube of the base by a transverse layer of cytoplasm. A unit membrane forms the outer limit.

The second type (blade-like type) is a large microthrix with a transversely orientated spatulate blade and with a posteriorly directed support (Fig. 2b). The tip is slightly bent anteriorly. The base, having a T-shaped cross-section (Fig. 4a), measures 0.9 µm in width and is 0.3 to 0.5 µm high. The outer limit is a unit membrane, reinforced by several dense layers. This reinforcement is more important on the anterior face. Numerous parallel microtubules are seen in the central part of the base. The multilaminate baseplate (Fig. 1, double arrows) separating base from shaft is 0.04 µm thick and is composed of five layers. These laminae are not continuous with the dense layers reinforcing the base. The 1.5–2.5 µm high shaft

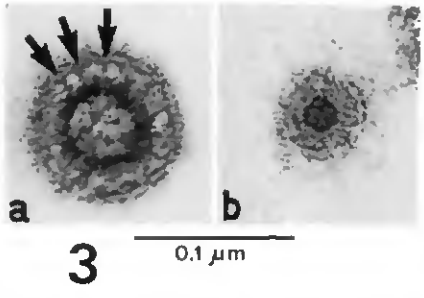


Fig. 3a and b. Tetrathyridium of *M. corti*. Transverse sections of the base (**a**) and the shaft (**b**) of the filamentous microthrix. The arrows point to the radially arranged elements joining the unit membrane to the central tube. 210,000×

(Figs. 2b and 4b) is surrounded by a 90 Å thick unit membrane enclosing a 100 Å thick, finely granulated layer. The medulla is composed of numerous thick-walled parallel microtubules, each with a diameter of about 100 Å.

The body surface of the tetrathyridium is divisible into three distinct zones (Fig. 5).

Zone A. This zone comprises the anterior extremity of the larva and the suckers. The posterior limit is a curved line following the posterior and lateral borders of the suckers. In Zone A both types of microtriches are present. On SEM photographs of Zone A only the shafts of the very long (up to 7 μm) filamentous microtriches are seen (Fig. 6a). They cover and hide the tips of the blade-like microtriches as shown on transverse sections of the tegument (Fig. 6b). The bases of the filamentous microtriches are only 0.5 μm high in this region, the shafts measure up to 7 μm. The shafts of the filamentous microtriches in the sucker cavities are slightly thinner than those from the sucker borders or from the rest of Zone A. The blade-like microtriches are about 2.5 μm high.

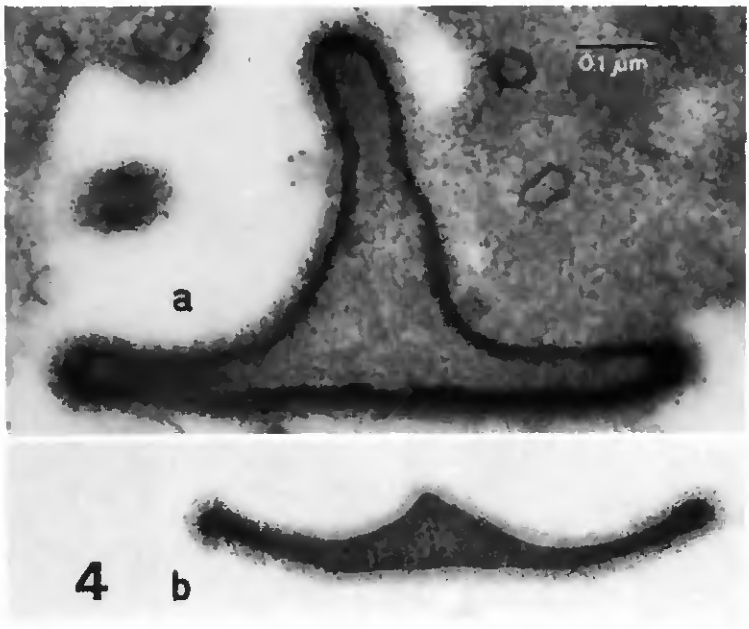


Fig. 4a and b. Tetrathyridium of *M. corti*. Transverse sections of the base (**a**) and the shaft (**b**) of the blade-like microthrix. 100,000×

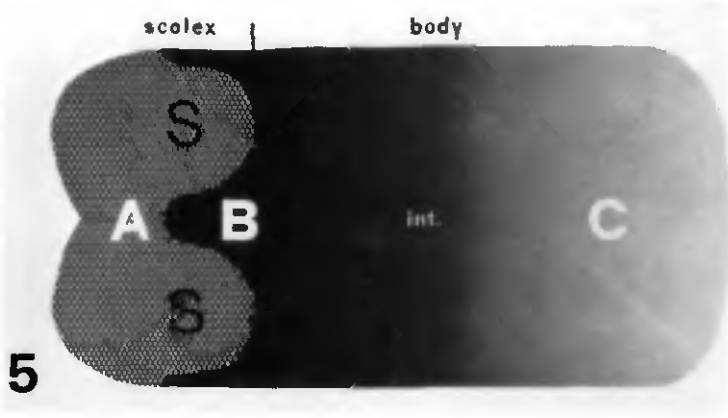


Fig. 5. Tetrathyridium of *M. corti*. Schematic representation of the body zones (explanation in the text). *S*: suckers; *int.*: intermediate region

Zone B. The border between Zone A and B is distinct and its position has been mentioned above. Posteriorly, Zone B merges imperceptibly into Zone C. In the anterior part of Zone B the blade-like microtriches are very numerous, with about four elements per μm^2 (Fig. 7). No filamentous microtriches are seen. They reappear in the intermediate region between Zone B and C (Fig. 8a), and become more

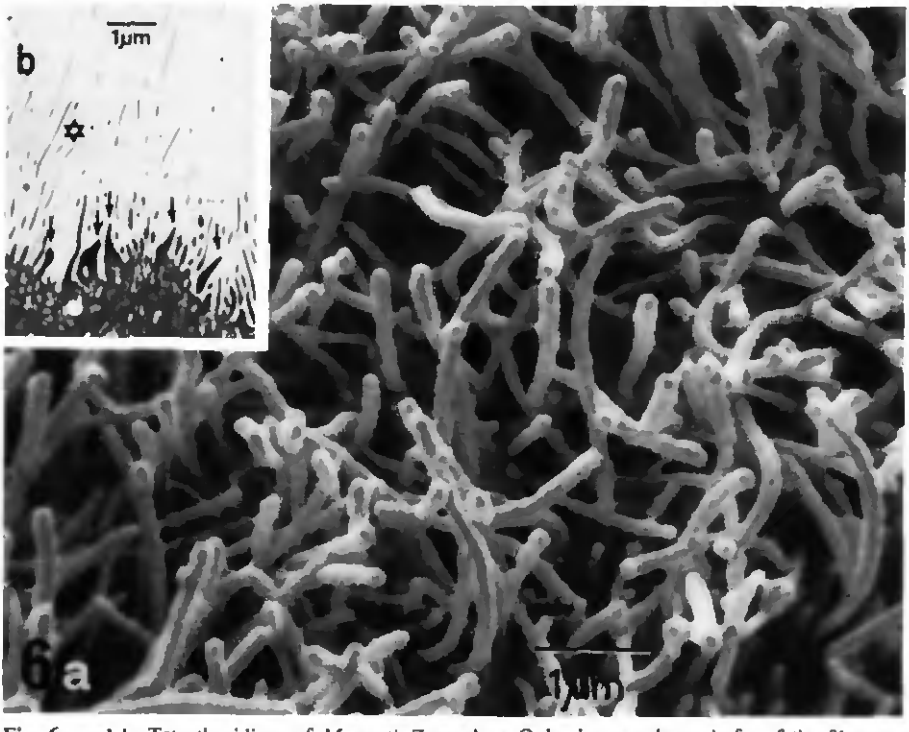


Fig. 6a and b. Tetrathyridium of *M. corti*. Zone A. **a** Only the very long shafts of the filamentous microtriches are seen by SEM (15,000×). **b** Sections show that both types of microtriches are present (6,500×). *Arrows:* blade-like microtriches; *asterisk:* the very long shafts of the filamentous microtriches

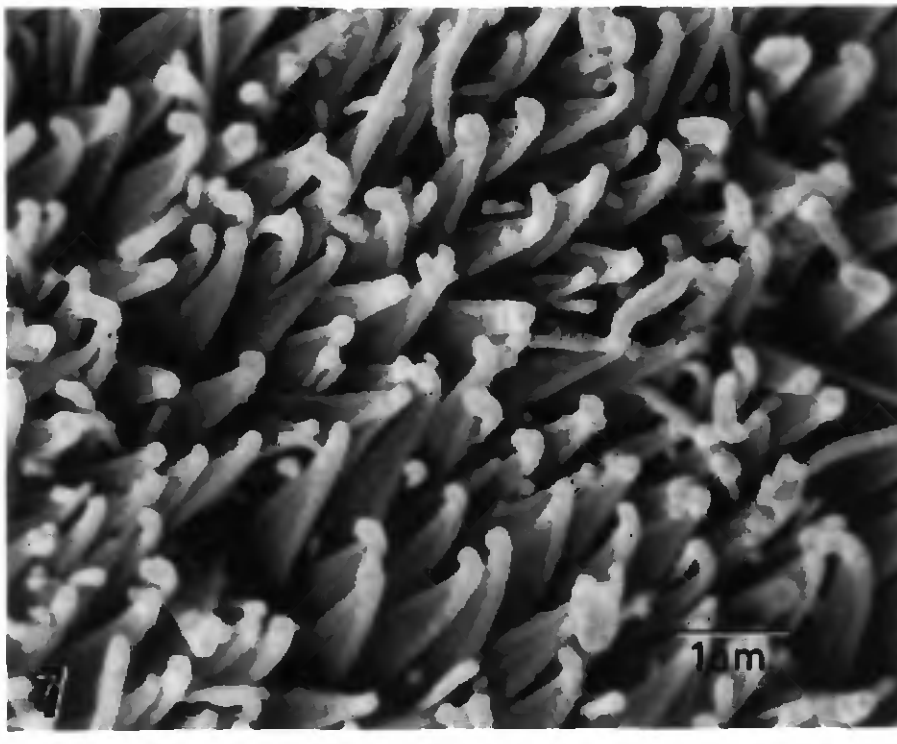


Fig. 7. Tetrathyridium of *M. corti*. Zone B. SEM-picture: Only blade-like microtriches are present. 15,000×

numerous posteriorly, whereas the number of blade-like microtriches/μm^2 decreases. In the intermediate region, the filamentous microtriches are short and the blade-like microtriches are often small and mis-shapen. The latter seem to be arranged in rows in both Zones A and B and in the intermediate region (Fig. 8b).

Zone C. Zone C is at the posterior extremity of the larva. In this zone only filamentous microtriches are present (Fig. 9a and b). Their base is 0.8–1.3 μm and the shaft about 2 μm long. The shafts in this zone are slightly thinner than those of the microtriches of Zone A.

2. Sensory Processes

Two types of sensory processes were detected on the scolex by SEM (Fig. 10).

The first is club-shaped, measuring 1.5–2 μm in length. Its terminal bulb measures 0.7 μm and the stalk 0.2 μm in diameter.

The second, filamentous type is a 0.2 μm thick process, up to 20 μm long. From measurements and sections examined by TEM these structures are identifiable as sensory endings of the platyhelminth type (Figs. 11 and 12). The proximal bulb is embedded in the distal cytoplasm of the tegument and attached to it by a septate desmosome forming a circle (Figs. 11 and 12, arrows). The bulb contains an apical electron-dense ring surrounding the root of the distal process, vesicles, mitochondria

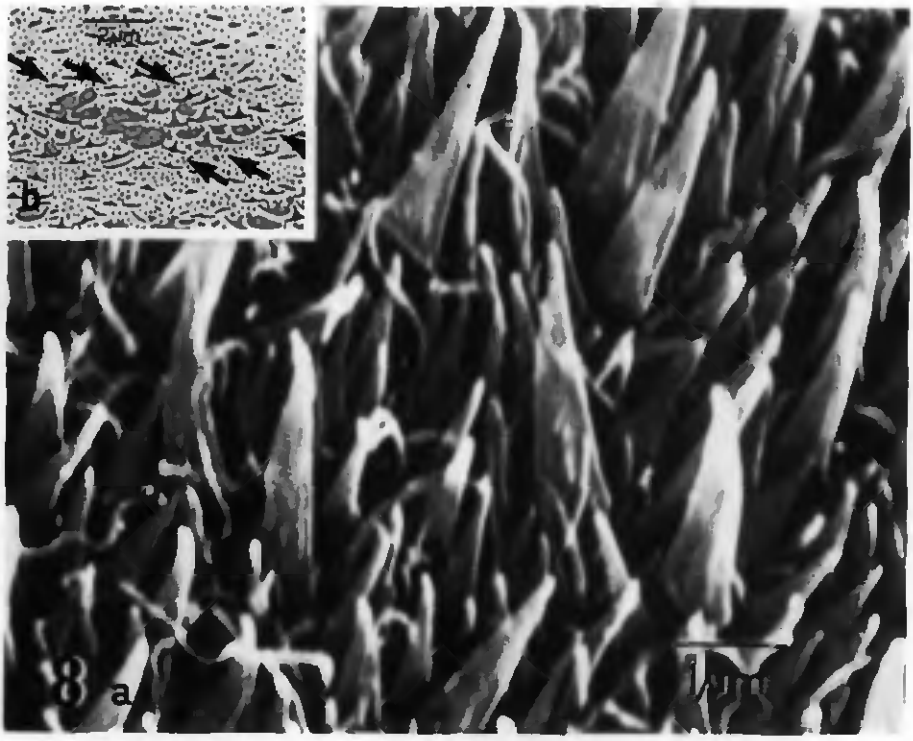


Fig. 8a and b. Tetrathyridium of *M. corti*. Intermediate region between the Zones B and C. **a** By SEM blade-like microtriches and short filamentous microtriches are seen to occur together (15,000×). **b** On tangential sections of the body surface the blade-like microtriches seem to be arranged in rows (*arrows*) 4500×

and microtubules. The distal process contains parallel microtubules. These microtubules traverse the terminal bulb of the club-shaped process and are attached on the unit membrane.

Discussion

Microtrichial polymorphism has been reported by Bråten (1968), Yamane (1968, 1975), Grammeltvedt (1973), and Andersen (1975) for several species of *Diphyllobothrium*, two species of *Diplogonoporus* and *Spirometra erinacei*, Berger and Mettrick (1971) for three species of *Hymenolepis*, Race (1966) and Featherston (1972) for two species of *Taenia* and Blitz and Smyth (1973) for *Raillietina cesticillus*. Rifkin et al. (1970) described tegumentary hooks and microvilli on the tegument of *Tylocephalum* metacestodes.

In most cases comparisons are made between different species or larval stages are compared with adults but reports on the surface topography of the different body regions of the same stage of a species are often fragmentary or absent. It is also necessary to complete SEM-studies by TEM because long filamentous microtriches might conceal short blade-like forms, as has been described above for tetrathyridia.

The good preservation of the microtriches by the critical point drying method for SEM and the comparison of SEM photographs with sections examined by TEM

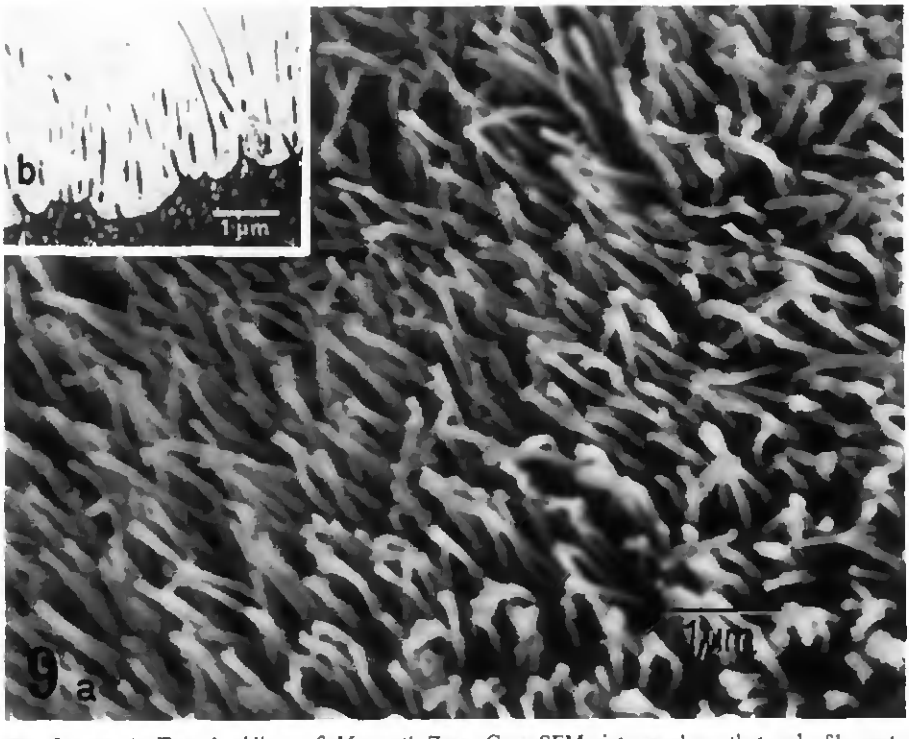
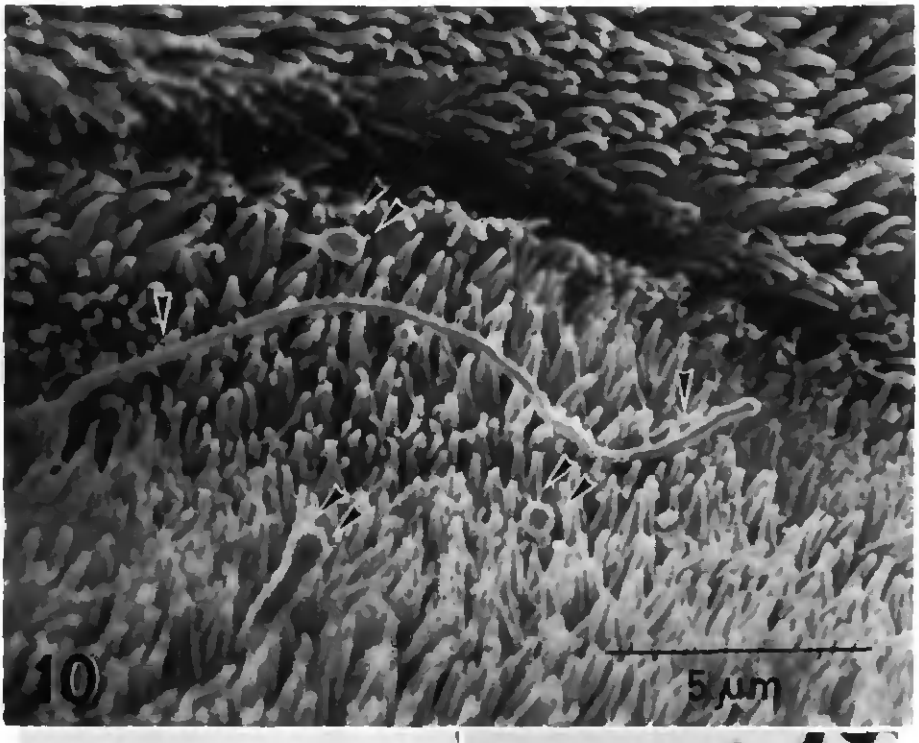


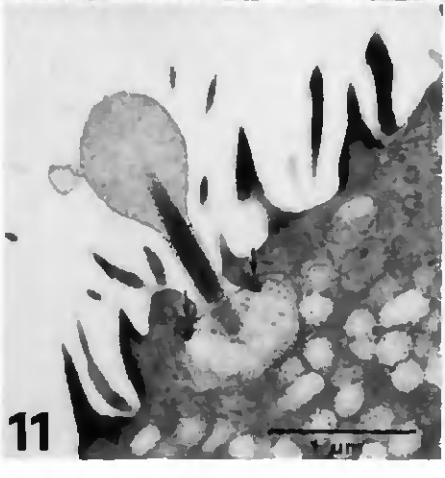
Fig. 9a and b. Tetrathyridium of *M. corti*. Zone C. **a** SEM-pictures show that only filamentous microtriches occur on the posterior part of the larva (15,000×). **b** Sections show that no blade-like microtriches occur in this body region. 8,500×

allows us to state that there are two distinct forms of microtriches in tetrathyridia of *M. corti*: The well-known filamentous microthrix and the blade-like microthrix never described before. From the changes in relative abundance of the two types of microtriches along the body of the larvae it could be suggested that only a single microthrix type is present whose morphology changes slightly along the body. However, intermediate forms are not found, where the two types occur together in Zone A on the scolex and their form and ultrastructure is different. A typical multilaminate baseplate as found in the blade-like microthrix is absent in the filamentous type.

The large microthrix, described in detail by Jha and Smyth (1969) in *Echinococcus granulosus*, seems to be an intermediate type between the filamentous and the blade-like microtriches described here. This microthrix has a polyhedral transverse section. A posteriorly directed support is not mentioned.

A retrospective examination of results and TEM photographs published by various authors, at the time, unaware of the existence of a microtrichial polymorphism, reveals that large, possibly blade-like microtriches occur in many species on the scolex and neck region of adult and larval cestodes. We think that also the tegumentary hooks described by Rifkin et al. (1970) for metacestodes of *Tylocepholum* are homologous to the blade-like microtriches described here. Filamentous microtriches seem to be typical for the body regions posterior to the neck and for the rostellum. This distribution of large and filamentous microtriches corresponds to the distribution found on tetrathyridia.





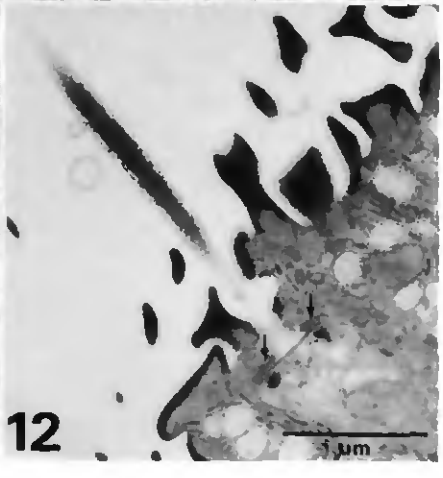


Fig. 10. Tetrathyridium of *M. corti*. Two types of sensory endings are seen by SEM on the body surface of the scolex (Zone B). *Simple arrow:* filamentous sensory process; *double arrow:* club-shaped sensory processes. 7,000×

Fig. 11. Tetrathyridium of *M. corti*. Transverse section of the tegument showing the club-shaped sensory process and the proximal bulb. The arrows point to the septate desmosome. 19,000×

Fig. 12. Tetrathyridium of *M. corti*. Transverse section of the tegument showing the filamentous sensory process and the proximal bulb. The arrows point to the septate desmosome. 19,000×

The greatest similarity of the surface topography exists between tetrathyridia and plerocercoids of *Diphyllobothrium*.

It is evident that we cannot propose a general pattern of the distribution of the two microthrix forms for cestodes other than the tetrathyridium of *M. corti*, based solely on the data available from published results; nevertheless both a fundamental microtrichial dimorphism and a distribution of the two types resembling that described here for the tetrathyridium of *M. corti* seem to exist for other cestodes too. However, only further comparative studies by TEM and SEM can confirm this hypothesis.

On SEM and TEM pictures the blade-like microtriches have always the same form and are never distorted. The bases of the filamentous forms are straight while their shafts are more or less randomly directed. This suggests that the blade-like microtriches and the bases of the filamentous forms are rigid while the shafts of the latter are flexible. This hypothesis is in accordance with our ultrastructural observations. The shaft of the blade-like microthrix is made up by parallel thick-walled microtubules and in the base of the filamentous microthrix we observed a central tube joined to the unit membrane. Such structures are probably rigid. This might be important from the functional point of view. Most workers attribute an absorptive function to the microtriches although the exact mechanism of food uptake is not known (Review by Lumsden, 1975). The presence of two distinct types, however, suggests that different functions correspond to the two forms.

We think that the rigid blade-like microtriches covering the anterior part of the larvae are specialized forms for locomotion. Tetrathyridia traverse all tissues very rapidly, although specialized histolytic glands or structures similar to the tegumental vesicles described by Kwa (1972) in the scolex of *Spirometra erinacei* have never been found. It is possible that the larvae tear the tissues by means of the suckers and penetrate into the wound, the presence of the posteriorly directed rigid blades of the blade-like microtriches preventing a retreat.

The filamentous microtriches are probably involved in nutrition. They are found on the whole body surface except the relatively narrow Zone B. The rigid bases prevent the shafts being squeezed between the blade-like microtriches. It is also possible that the bases are mobile as suggested by Rothmann (1963). It must be noted that the filamentous microtriches of Zone A and C differ slightly by their dimensions. This possibly indicates different functions of the filamentous microtriches in the two zones.

The sensory processes observed correspond to the basic pattern of platyhelminth sensory endings. The club-shaped termination, however, to our knowledge, has not yet been described from cestodes and it might represent an osmoreceptor. The second, filamentous type is known from sections but has never been shown on SEM pictures. The extreme length of the process is somewhat surprising.

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References

Andersen, K.: Comparison of surface topography of three species of *Diphyllobothrium* (Cestoda, Pseudophyllidea) by scanning electron microscopy. Int. J. Parasit. **5**, 293–300 (1975)

Berger, J., Mettrick, D.F.: Microtrichial polymorphism among hymenolepid tapeworms as seen by scanning electron microscopy. Trans. Amer. Micr. Soc. **90**, 393–403 (1971)

Blitz, N.M., Smyth, J.D.: Tegumental ultrastructure of *Raillietina cesticillus* during the larval-adult transformation, with emphasis on the rostellum. Int. J. Parasit. **3**, 561–570 (1973)
Bråten, T.: The fine structure of the tegument of *Diphyllobothrium latum* (L.). Z. Parasitenk. **30**, 104–112 (1968)
Cohen, L.A.: Critical point drying in principles and techniques of scanning electron microscopy. M.A. Hayat, ed., Vol. 1, pp. 44–112. London: Van Nostrand Reinhold Company 1974
Featherston, D.W.: *Taenia hydatigena*. IV. Ultrastructure study of the tegument. Z. Parasitenk. **38**, 214–332 (1972)
Grammeltvedt, A.E.: Differentiation of the tegument and associated structures in *Diphyllobothrium dendriticum* Nitsch (1824) (Cestoda: Pseudophyllidea). An electron microscopical study. Int. J. Parasit. **3**, 321–327 (1973)
Jha, R.K., Smyth, J.D.: Ultrastructure of microtriches in *Echinococcus granulosus*. Exp. Parasit. **25**, 232–244 (1969)
Kwa, B.H.: Studies on the sparganum of *Spirometra erinacei*. III. The fine structure of the tegument in the scolex. Int. J. Parasit. **2**, 35–43 (1972)
Lee, D.L.: The structure of the helminth cuticle. Advanc. Parasit. **10**, 347–379 (1972)
Lumsden, R.D.: Surface ultrastructure and cytochemistry of parasitic helminths. Exp. Parasit. **37**, 267–339 (1975)
Race, G.J., Larsh, J.E., Esch, G.W., Martin, J.H.: A study of the adult stage of *Taenia multiceps* (*Multiceps serialis*) by electron microscopy. J. Elisha Mitchell Sci. Soc. **82**, 44–57 (1966)
Rifkin, E., Cheng, T.C., Hohl, H.R.: The fine structure of the tegument of *Tylocephalum* metacestodes: With emphasis on a new type of microvilli. J. Morph. **130**, 11–24 (1970)
Rothman, A.: Electron microscopy studies of tapeworms: The surface structures of *Hymenolepis diminuta* (Rudolphi, 1819) Blanchard, 1891. Trans. Amer. Micr. Soc. **82**, 22–30 (1963)
Specht, D., Voge, M.: Asexual multiplication of *Mesocestoides* tetrathyridia in laboratory animals. J. Parasit. **51**, 268–272 (1965)
Ubelaker, J.E., Allison, V.F., Specian, R.D.: Surface topography of *Hymenolepis diminuta* by scanning electron microscopy. J. Parasit. **59**, 667–671 (1973)
Yamane, Y.: On the fine structure of *Diphyllobothrium erinacei* with special reference to the tegument. Yonago Acta med. **12**, 169–181 (1968)
Yamane, Y., Maejima, J., Yazaki, S.: Scanning electron microscopic observations of the tegumental structure of diphyllobothriid cestodes. Yonago Acta med. **19**, 197–206 (1975)

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Ultrastructural Study of the Tetrathyridium of *Mesocestoides corti* Hoeppli, 1925 (Cestoda): The Regeneration of 2 - Sucker Head Fragments *

by

E. HESS

With 15 figures

ABSTRACT

Two-sucker head fragments of *M. corti* tetrathyridia regenerate the missing parts of their body. Primary wound closure takes place by muscle contraction, by the formation of a cicatrization syncytium from cell bodies of the tegumental syncytium, and by the strong adhesion among the lobes of glycogen-storing parenchyma cells. Definitive wound healing is however not achieved. The tail fragment bearing the wound is discarded. Degenerating cell fragments are phagocytized by calcareous corpuscule cells. Germinative cells and dedifferentiated glycogen-storing cells represent the main pool of cells needed for the progressive phase of regeneration. Parts of the tegument participate in the formation of the suckers.

INTRODUCTION

HART (1968) described the regeneration of head fragments from tetrathyridia of *Mesocestoides corti in vivo* and *in vitro*. He found that fragments with at least 1 sucker successfully regenerated the lacking organs and developed into normal tetrathyridia capable of asexual multiplication. Experimentally produced fragments without suckers survived for a short time much in the same way as spontaneously detached tail fragments (HESS 1972), but were unable to regenerate. HESS & GUGGENHEIM (1977) and HESS (1980, 1981) studied the fine structure of tetrathyridia of *M. corti* and the morphogensis of the tegument, the parenchyma, the internal muscles and the suckers during asexual mul-

* Part of the author's thesis.

tiplication. In this study, the reaction of tetrathyridia to injury and the reparative regeneration of 2-sucker head fragments is described.

MATERIALS AND METHODS

Tetrathyridia of *Mesocestoides corti* originally isolated by SPECHT & VOGE (1965) and cultivated in our laboratory in NMRI-mice were used for this study. Tetrathyridia bearing four suckers were cut transversally behind the scolex in order to obtain 4-sucker head fragments which were cut sagitally to produce 2-sucker head fragments. The working solution used was warm NTCT 135 containing antibiotics (VOGE & COULOMBE 1966). Immediately after amputation, the 2-sucker head fragments were injected into the peritoneal cavity of NMRI-mice. Six hours, 15 hours, 3, 4, 5, 6, 7 and 10 days after injection, the regenerating fragments were collected from their hosts and fixed in 2% glutaraldehyde buffered by 0,1 M Na-cacodylate, pH 7, 2, at 4° C for 1 day and post-fixed in 1% OsO_4 buffered by 0,1 M Na-cacodylate, pH 7, 2, at room temperature for 90 minutes, dehydrated and embedded. The sections were studied on a Philips 201 EM.

RESULTS

The shock

The immediate reaction of the 2-sucker head fragments to amputation is a violent muscular contraction lasting for 5 to 15 minutes (Fig. 1). After relaxation, the fragments show normal mobility. The muscles of the wound region remain however contracted, thus holding the wound closed. The suckers of the fragments move convulsively and are projected as the longitudinal parenchymal muscles (sucker retractors) are cut.

The fragments 6 hours after amputation

Morphology: The 2-sucker head fragments are of oval or longish shape. The suckers are still evaginated. The wound surface is reduced to a small slit covered by a cap with hyaline appearance *in vivo* (Fig. 1).

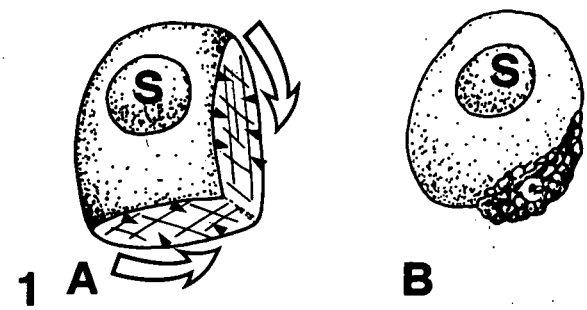

FIG. 1.

Tetrathyridium of *M. corti:* Schematic representation of the shock contraction of the 2-sucker head fragments. A: before; B: after contraction; Arrows: direction of contraction; S: sucker; c: cap of expulsed cells.

Fine structure

The tegumental syncytium: The tegumental syncytium opposite to the wound has a normal fine structure but the polynuclear cell mass of the apical massif which has been sectioned during amputation has disappeared. Towards the wound the superficial layer becomes thinner, the space between the microtriches is larger than normal, and the disc-shaped bodies are in a parallel position to the surface (normal position is perpendicular): the superficial cytoplasm seems to be stretched.

The tegument of the wound lips is relatively thick. This is due to the lasting contraction of the subtegumental muscles in the wound region. The superficial cytoplasm of this zone shows signs of degeneration. The zone of normal cytoplasm passes imperceptibly into a strongly vacuolated region which bears microvilli and finally into a layer of homogeneous granular appearance (Fig. 2). The homogeneous zone represents the lips of the wound. The degeneration of the tegumental superficial cytoplasm takes place in zones which have lost close contact with the perinuclear cell bodies. Some of the perinuclear cell bodies of the tegumental syncytium around the wound region fuse and form bi- and multinucleated cell masses (Fig. 3). In these cell bodies the large nuclei have a central nucleolus each, and the RER and the Golgi apparatus are well developed. The cytoplasm still contains vesicles filled with cristal-like membraneous aggregates which are typical for the perinuclear cell bodies of the normal tegumental syncytium (HESS, 1980).

In zones where the subtegumental muscle layer and the tegumental syncytium have been separated mechanically during amputation, the original structure of the latter is modified. In this case, the perinuclear cell bodies fuse with the superficial cytoplasm to form a unique nucleated layer (Fig. 4).

The subtegumental and parenchymal muscles: Cut muscle fibres of both the subtegumental and the parenchymal muscle systems decompose. In the perinuclear cell bodies of intact muscle cells an increased activity of the RER including formation of cisternae occurs. These cisternae release their content into the interstitial space (Fig. 5). The composition and function of the secretion is unknown. At the same time myoblasts are found in the parenchyma. They have long cytoplasmic processes in which myofibrils are synthesized as during asexual multiplication (HESS 1980).

Glycogen-storing parenchyma cells and calcareous corpuscule cells: A great number of glycogen-storing parenchyma cells and calcareous corpuscule cells have been expulsed during the shock contraction. After relaxation of the muscles, the remaining parenchymal glycogen-storing cells which are joined together by gap junctions form a loose network. The striking feature of the parenchyma of 6 hour old fragments was the presence of large interstitial spaces probably due to increased osmolality (Figs. 3-8). Glycogen-storing lobes of parenchyma cells encapsulate or envelop decomposing cell fragments (i.e. muscle fibres) but they do not phagocytize them. The phagocytosis of decomposing fragments takes place by calcareous corpuscule cells. These cells have long cytoplasmic filaments or lamellae (ruffle membranes ?) which pick decomposing fragments out from the envelopes formed by the glycogen-storing parenchyma cells and phagocytize them (Figs. 6, 7, 8). The phagosomes advance in the cytoplasmic filaments towards the calcareous corpuscule and are incorporated in the latter. During the migration of the phgaosome, homogeneization of the content occurs. In the cytoplasm of phagocytizing calcareous corpuscule cells one finds vesicles with electron-dense granules, possibly primary lysosomes (Fig. 7). These vesicles seem to fuse with the phagosomes which migrate towards the calcareous corpuscule (Fig. 8).

Some of the parenchymal glycogen-storing cells undergo dedifferentiation. In such cells plasmalemma formation takes place between the ribosome-containing perinuclear cytoplasm and the glycogen-storing lobes (Fig. 9). Subsequently, the glycogen-storing lobes detach from the nucleated cell body. Numerous free lobes accumulate in the interstitial space adhering together by gap-junctions (Figs. 10, 14). The dedifferentiated cells are similar to germinative cells. They have a large nucleus and their cytoplasm contains only free ribosomes and some small mitochondria (Fig. 10).

Germinative cells: The mitotic activity of the germinative cells does not seem to be increased; no quantitative analysis has however been undertaken. Many of the germinative cells differenciate into muscle cells. Dark cells which form components of the osmoregulatory or excretory system (protonephridia, canal cells) probably also derive from germinative cells.

The cap: The wound surface is covered by a cap composed of different types of cells and cell fragments, mainly glycogen-containing lobes and muslces fibres (Fig. 11). Some of the cells remain alive but seem to dedifferentiate, most of them vacuolize and decompose as do the cell fragments.

The fragments 15 hours after amputation

The morphology of 15 hour old fragments is the same as 6 hour ones.

Fine structure

Tegumental syncytium: In the perinuclear cell bodies of the whole larva an accumulation of lipid droplets is observed. In the zones opposite the wound, no other modifications are observed. In the wound region, the formation of a syncytial cell mass from tegumental perinuclear cell bodies continues. We call it the cicatrization syncytium (Fig. 12). It remains in cytoplasmic continuity with the tegumental syncytium. Its cytoplasm contains a great number of free ribosomes, mitochondria and well developed Golgi apparatus and SER. The RER seems to decrease and the electron-dense vesicles with membrane aggregates which are normally found in the tegumental syncytium have disappeared. Each nucleus contains a large central nucleolus and the heterochromatine is finely scattered throughout the nucleoplasm. Mitotic divisions are not observed. The cicatrization syncytium forms a network which separates the parenchyma from the cap. This wound closure is however incomplete and the cicatrization syncytium never transforms into normal tegument.

Parenchyma: In the wound region, the glycogen-storing parenchyma cells divide into a large number of fragments. The gap junctions which held these small glycogen-storing lobes together are so numerous that they are arranged side by side (Fig. 14). It is possible that the fragmentation of the glycogen-storing lobes is related to the dedifferentiation of the glycogen-storing parenchyma cells.

The fragments 3 to 10 days after amputation

Three days after amputation most of the 2-sucker head fragments have begun the progressive phase of regeneration (definition see page 939). Large individual differences concerning the speed of regeneration are observed. Some fragments develop sucker anlagen after 3 days, while others show first signs of differentiation only after 10 days.

In the anterior part of the fragments a blastema is formed by undifferentiated cells. It is impossible to decide whether the blastema cells derive from the apical massif or from the pool of germinative cells.

Tegument: The apical massif which had disappeared during the first days after amputation reappears when the sucker anlagen are formed. It is situated between the original and the regenerating suckers. Its firm structure is the same as in non-dividing tetrathyridia (HESS 1980). Some days after amputation, the 2-sucker head fragments begin to grow. The tegumental growth takes place by the integration of migrating cells into the tegumental syncytium behind the suckers. This mode of tegumental growth has also been observed in normally developing tetrathyridia (HESS 1980).

The cicatrization syncytium which is formed in the wound region by the tegumental syncytium closes the wound incompletely. It never transforms into normal tegument with microtriches, thus it is unable to restore the body surface of the animal. The reconstitution of the tegument takes place indirectly. When the fragments have achieved a certain length or degree of development, the posterior part of the animal is eliminated by transversal fission. This is a normal way used by the tetrathyridia to reduce their body length and to eliminate the aged posterior part of their body (HESS 1972, 1980). After transversal fission, the superficial cytoplasm of the tegumental syncytium invaginates and fuses with the main excretory canals thus forming the terminal vesicle(s). In this way, normal reconstitution of the body surface is achieved.

The suckers: The sucker anlagen are visible by light microscopy at the earliest 3 days after the operation. In the electron microscope different cell types composing the sucker blastema are distinguishable.

1. The first differentiated cells which appear are fibroblasts with very long pseudopods. They form a cup the rim of which touches the fibrous layer of the tegument (Fig. 13). This cup separates the sucker anlage from the surrounding scolex blastema. The fibrous layer of the sucker is secreted when the differentiation of the myoblasts is advanced.

2. Myoblasts with long cytoplasmic expansions differentiate in the blastema (Fig. 15). They develop RER-cisternae and form the muscle fibres of the perpendicular, transversal lower and longitudinal lower muscle systems. The transversal and the longitudinal upper sucker muscles represent transformed circular and longitudinal subtegumental muscles (Fig. 13) (see HESS, 1981).

3. Cells which accumulate alpha-glycogen are scattered throughout the anlagen. They grow and develop into glycogen-storing sucker cells (Fig. 15).

4. The cell bodies of the tegumental syncytium are slightly electron-denser than the cells named above and their cytoplasm contains well developed RER and Golgi apparatus (Fig. 15). During the sucker morphogenesis, the cytoplasmic bridges between the superficial cytoplasm and the perinuclear cell bodies disappear and the latter fuse to form the interstitial syncytium.

5. Neurons seem to differentiate late. Their development has not been studied in detail.

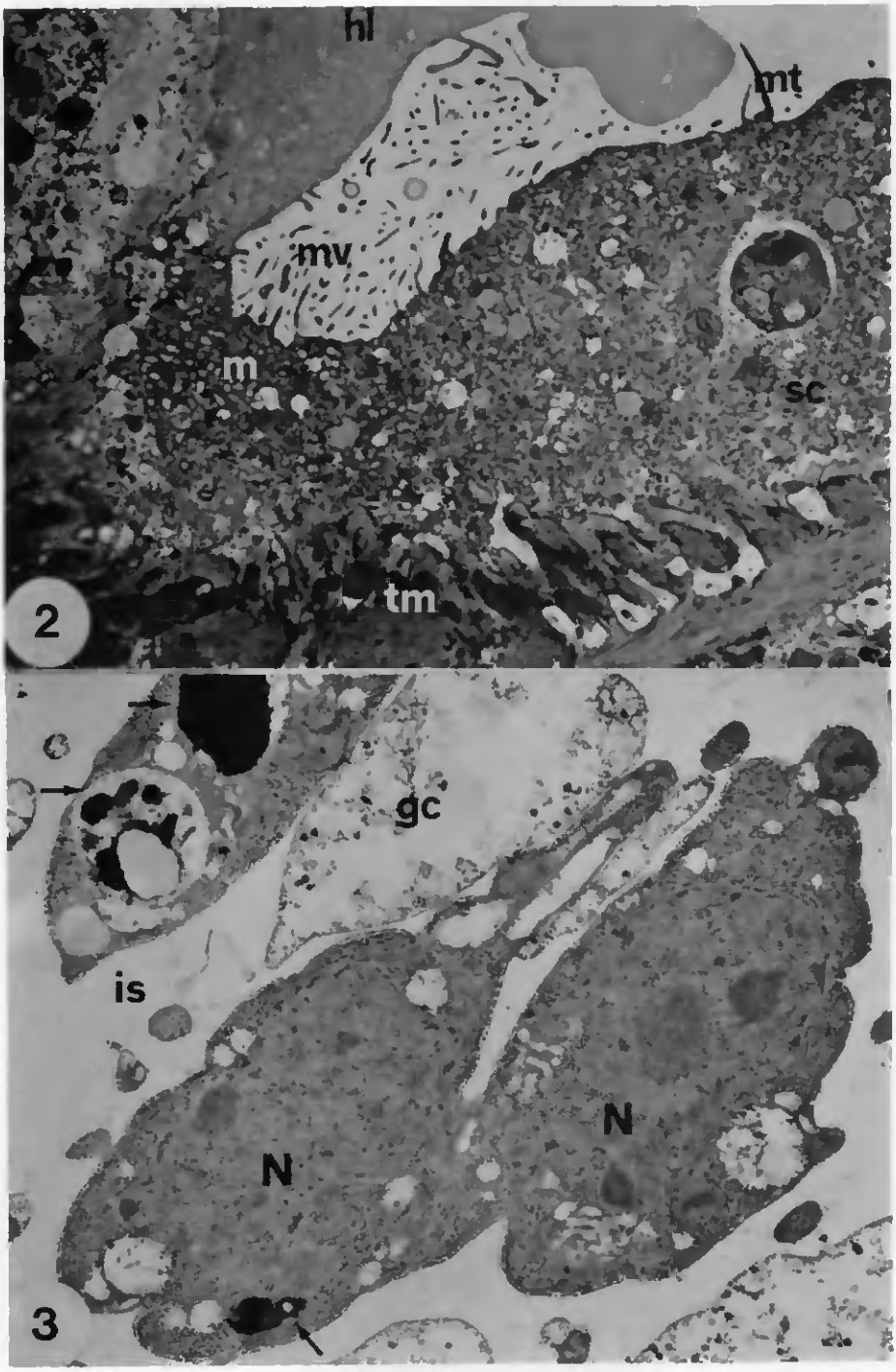
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FIG. 2.

Tetrathyridium of *M. corti*: Section of the tegumental wound lip. Between the superficial cytoplasm of relatively normal appearance (sc) and the degenerated homogeneous layer (hl) extends a zone of vacuolated microvilli-bearing cytoplasm (m). mv:microvilli; tm: subtegumental muscles. 5770×

FIG. 3.

Tetrathyridium of *M. corti*: Binucleated cell bodies of the tegumental syncytium near the wound lip. N: nuclei; arrows: vesicles containing cristal-like inclusions; gc: glycogen-storing parenchyma cell; is: interstitial space. 16,870×

FIG. 4.

Tetrathyridium of *M. corti:* Transverse section of the tegumental syncytium near the wound lip having lost the original organization. The perinuclear cell bodies (N: nuclei) have fused with the superficial cytoplasm (sc). is: interstitial space. 5780×.

FIG. 5.

Tetrathyridium of *M. corti*: Cell body of a myocyte with well developed RER cisternae (c); is: interstitial space. 26,200×. Inset: Secretion of the content of a RER cisterna (c) into the interstitial space. 56,680×.

FIG. 6.

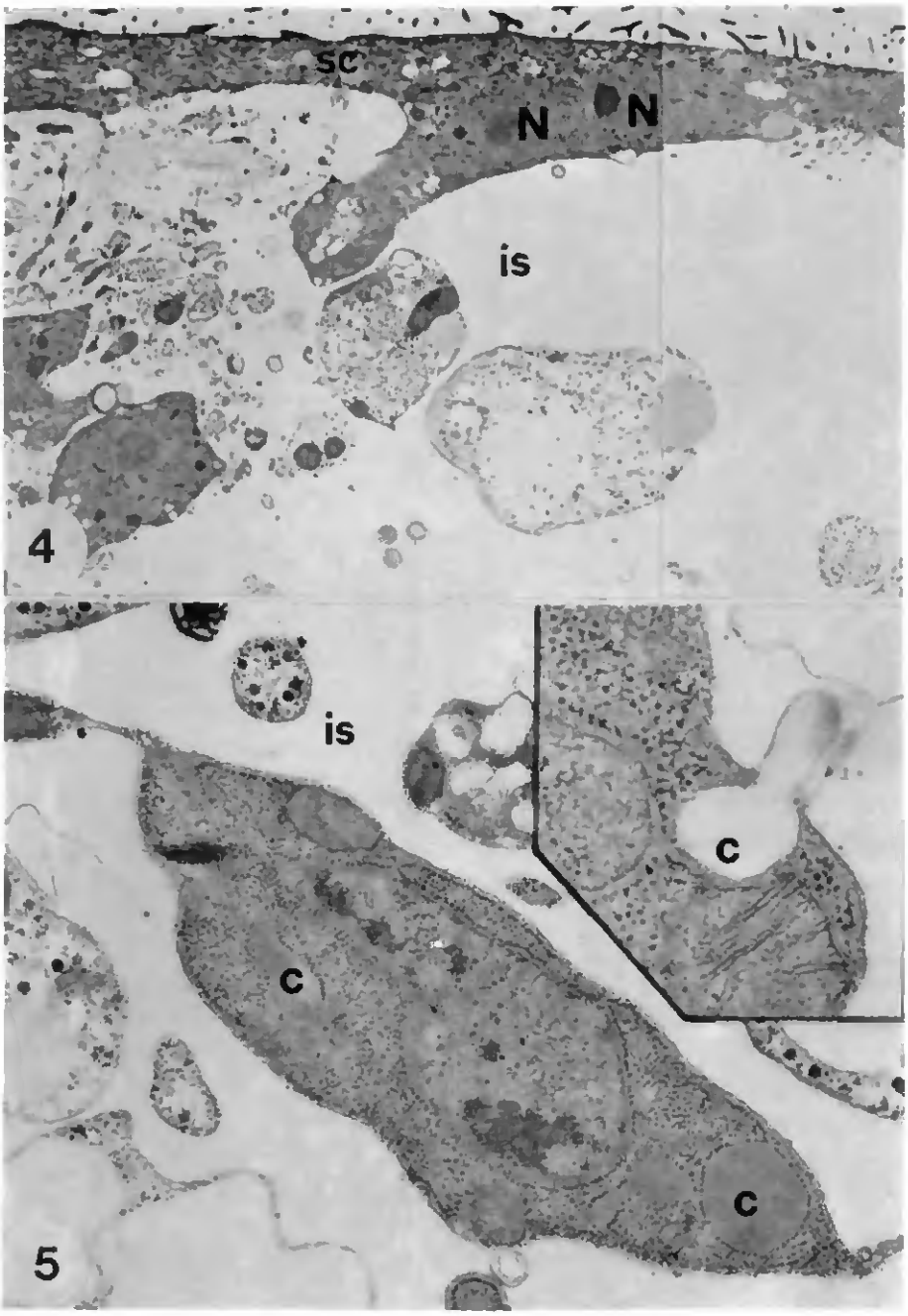
Tetrathyridium of *M. corti*: Cytoplasmic filaments or lamellae (arrows) of a calcareous corpuscule cell isolate and phagocytize a degenerating muscle fragment (df) from its envelope formed by a glycogenstoring parenchyma cell (gc). 18,670×.

FIG. 7.

Tetrathyridium of *M. corti*: Phagosome (ps) enclosed in a cytoplasmic expansion of a calcareous corpuscule cell; cc: calcareous corpuscule; arrows: primary lysosome-like vesicle. 26,870×.

FIG. 8.

Tetrathyridium of *M. corti*: Vacuole (ps) containing electron-dense granules (arrow) (autophagic vesicle ?) situated near the calcareous corpuscule (cc). 26,870×.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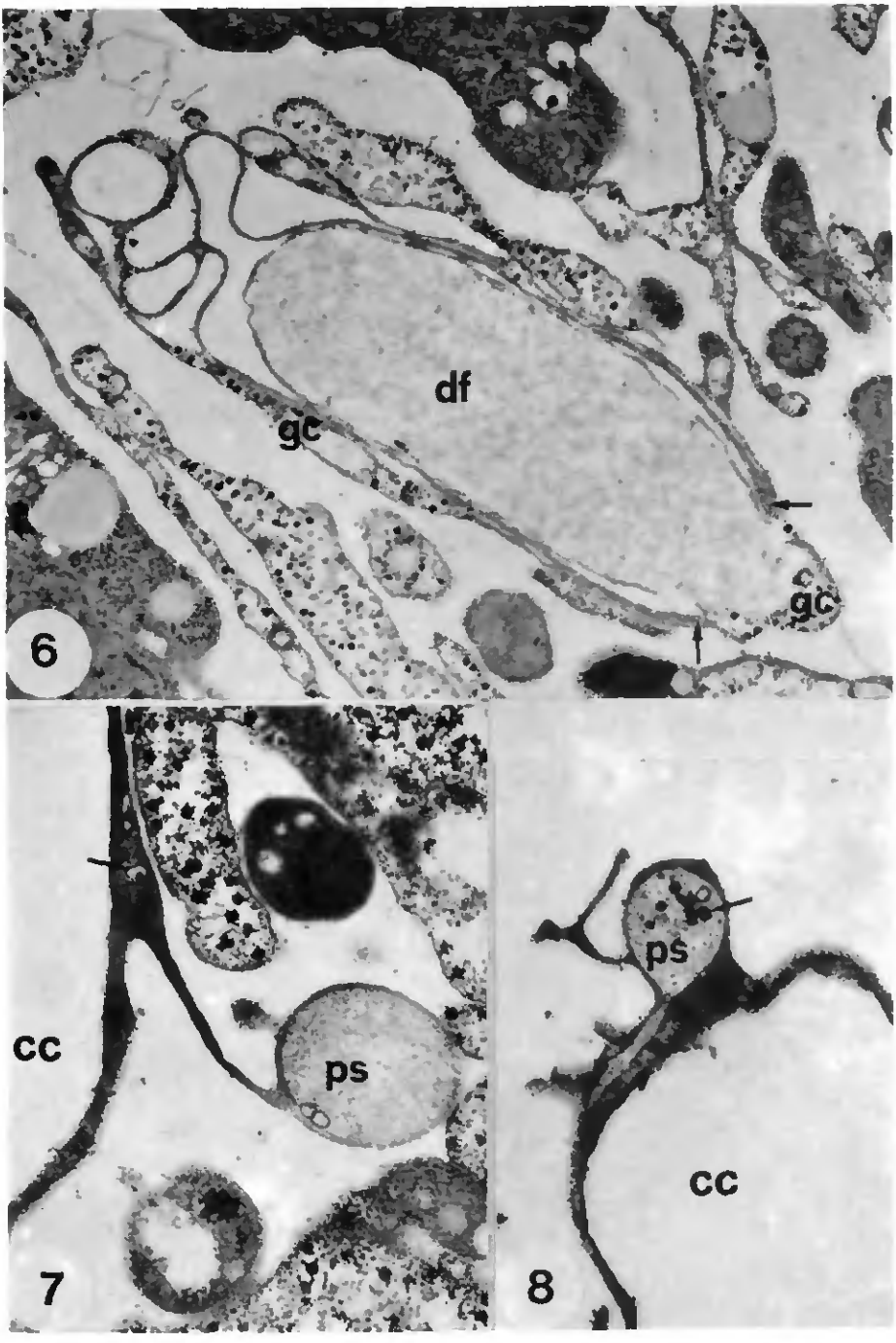
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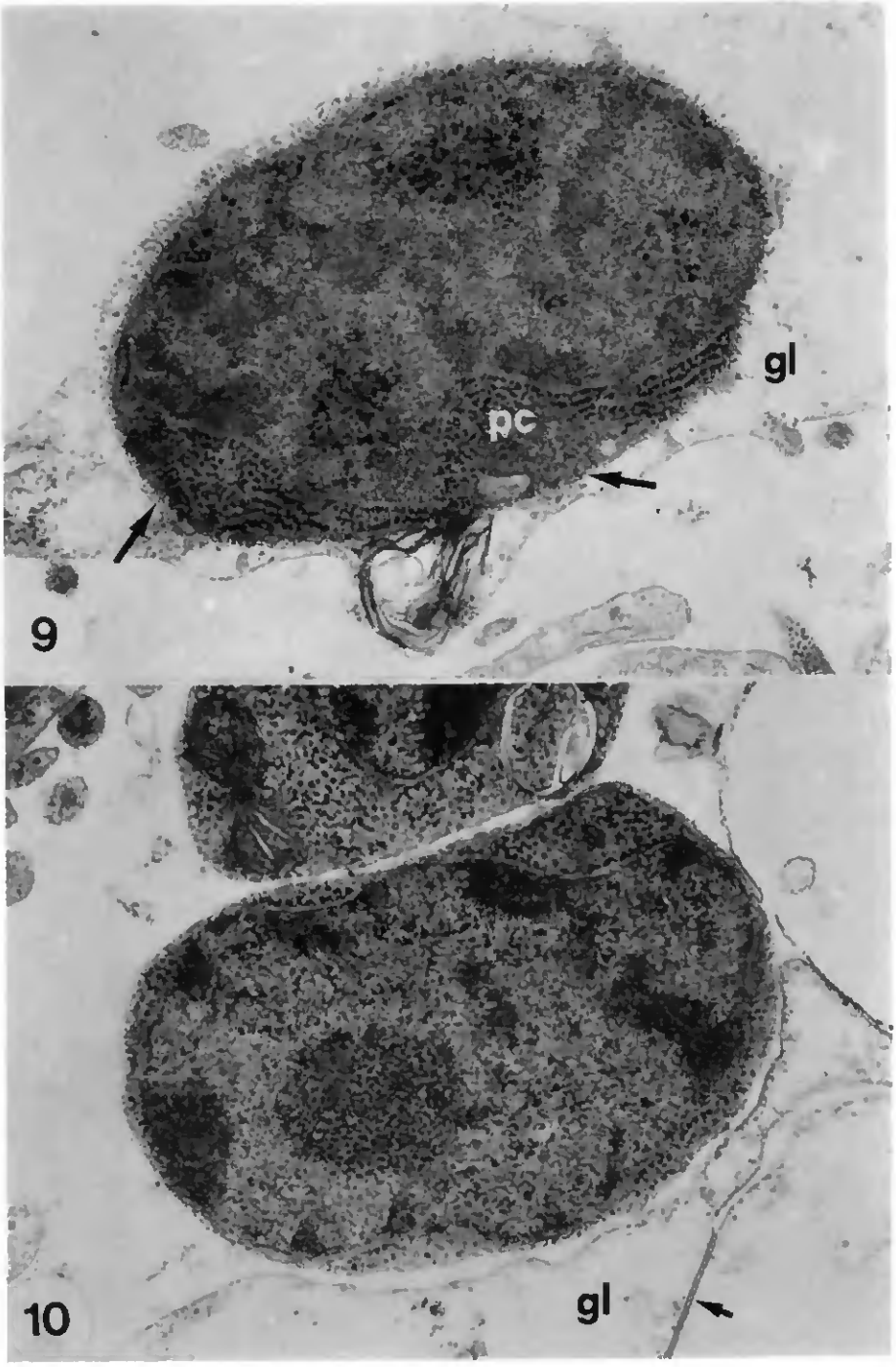
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FIG. 9.

Tetrathyridium of *M. corti*: Dedifferentiating parenchymal glycogen-storing cell. Plasmalemma formation (arrows) takes place between the perinuclear cytoplasm (pc) and the glycogen-storing lobes (gl). 43,490×.

FIG. 10.

Tetrathyridium of *M. corti*: The dedifferentiated glycogen-storing cell has a fine structure similar to a germinative cell; gl: glycogen-storing lobe; arrow: gap-junction. 43,490×.

FIG. 11.

Tetrathyridium of *M. corti*:
Section of the cap with expulsed cells and cell fragments. 5660×.

FIG. 12.

Tetrathyridium of *M. corti*: Part of the cicatrization syncytium. N: nuclei; l: lipid droplets; arrows: zones of Golgi-apparatus and/or SER. 8180×.

FIG. 13.

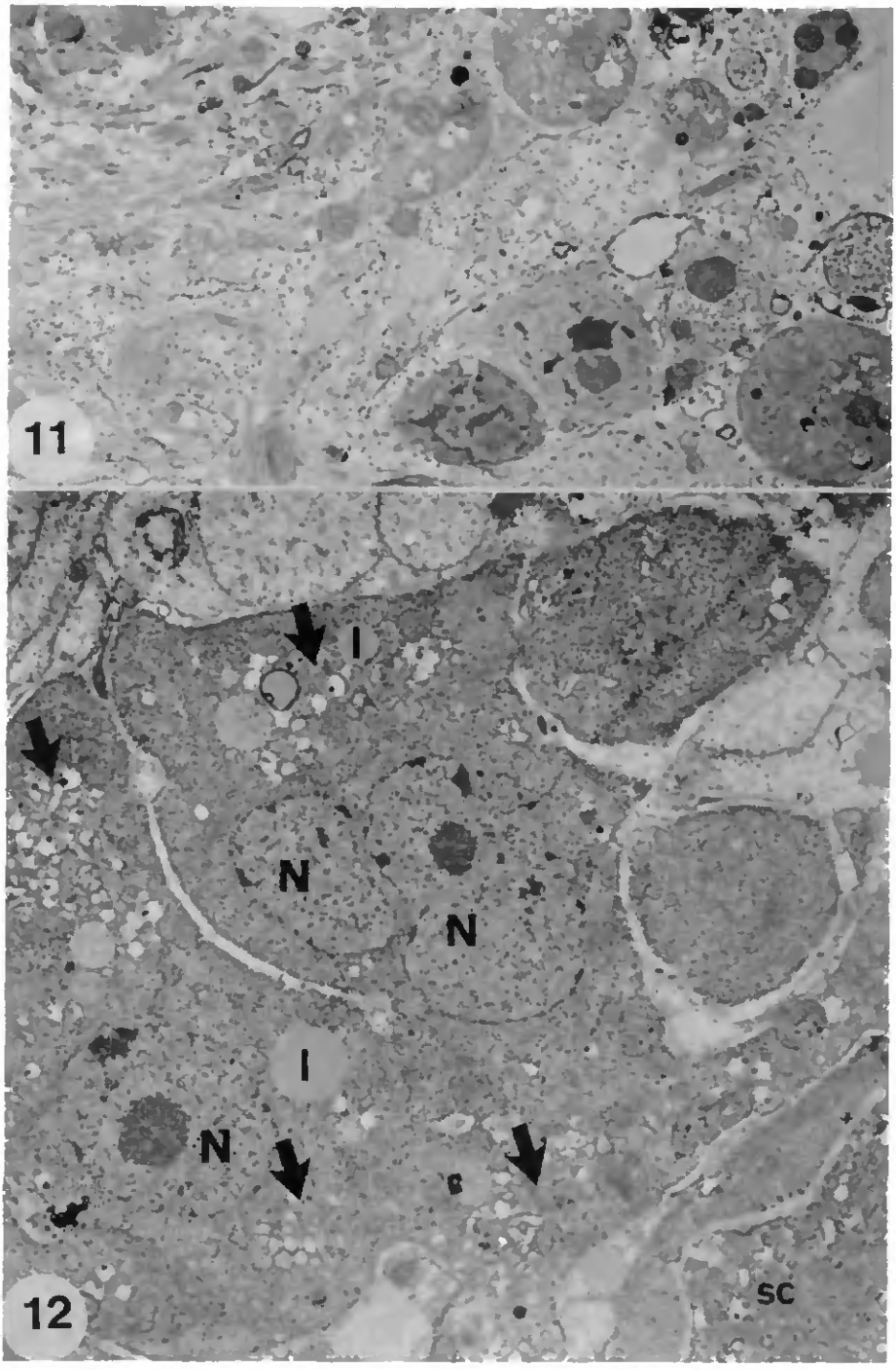
Tetrathyridium of *M. corti*: Sucker anlage of a 3 day old fragment. Fibroblasts (fb, arrows) separate the sucker anlagen (S) from the parenchymal part (P) of the scolex blastema. The sucker anlage contains undifferentiated cells (uc) and precursors of the interstitial syncytium (is) which are transformed perinuclear cell bodies of the tegumental syncytium detaching from the superficial cytoplasm (sc); asterisk: subtegumental muscles transforming into the transversal upper muscle system of the sucker. 4225×.

FIG. 14.

Tetrathyridium of *M. corti*: Glycogen-storing lobes of the wound region adhering together by numerous gap-junctions (arrows). 38,020×.

FIG. 15.

Tetrathyridium of *M. corti*: Section of a differentiating sucker anlage with undifferentiated cells (uc), myoblasts (mb), and glycogen-storing cells (asterisk). The cell bodies of the developing interstitial syncytium (is) may still have cytoplasmic continuity (arrows) with the superficial cytoplasm of the tegumental syncytium (sc). u: upper transversal and longitudinal sucker muscles deriving from the subtegumental muscles. 5535×.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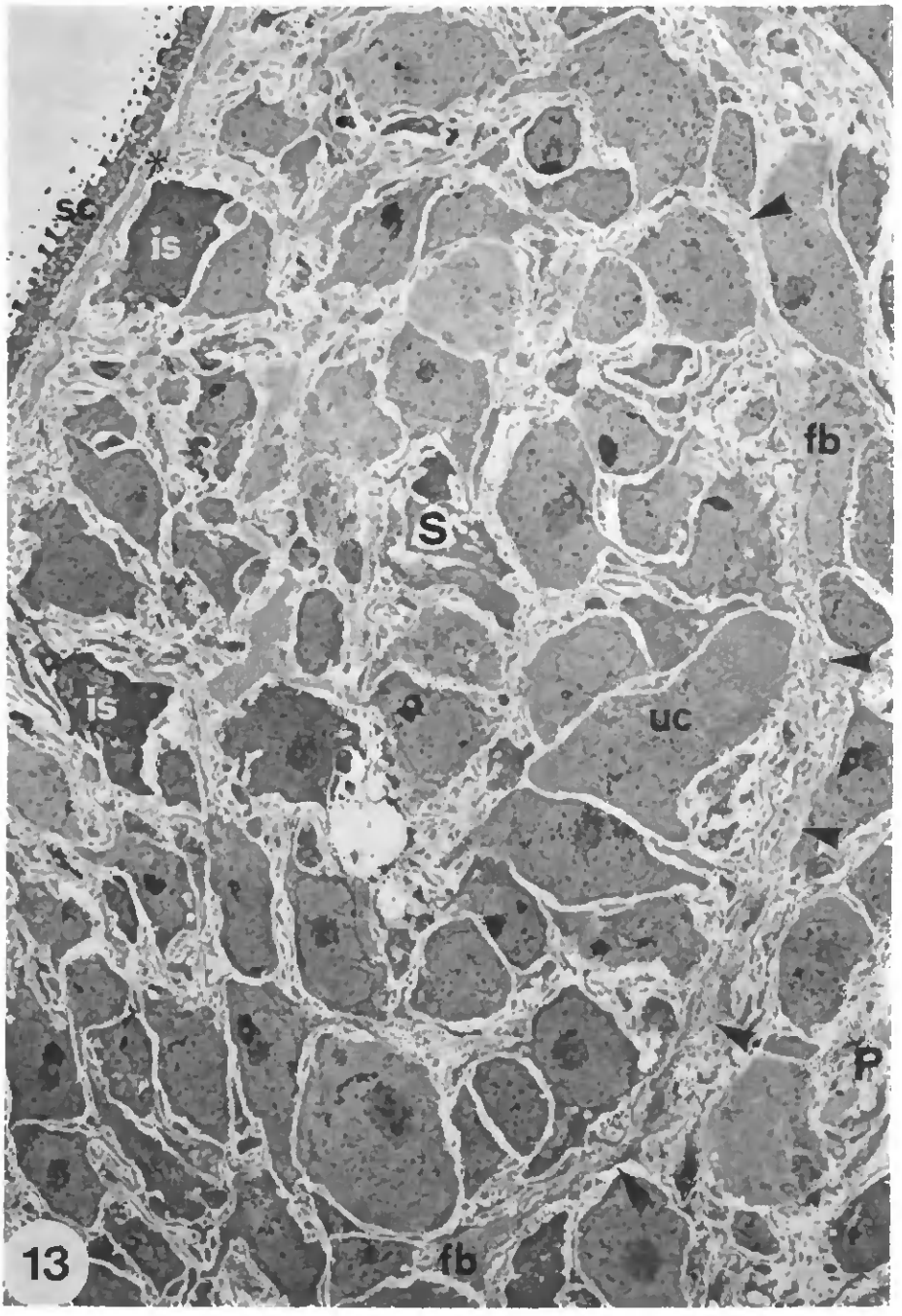
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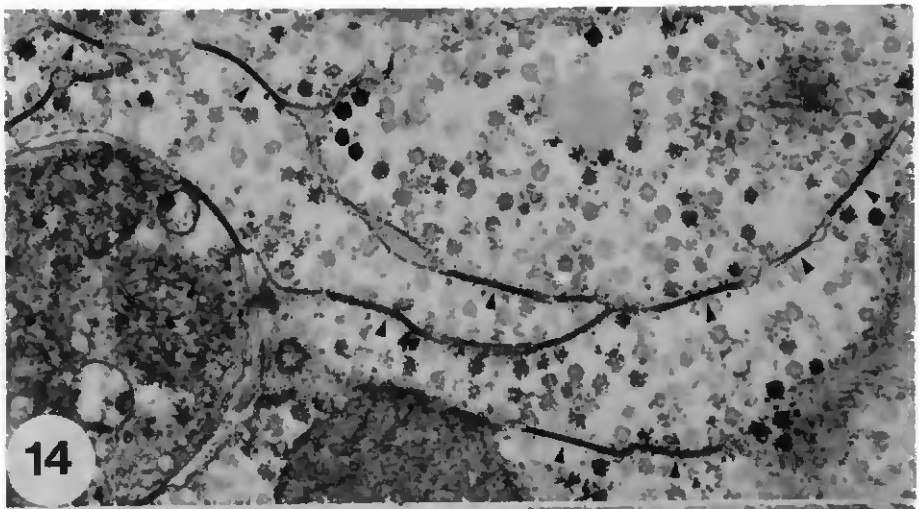
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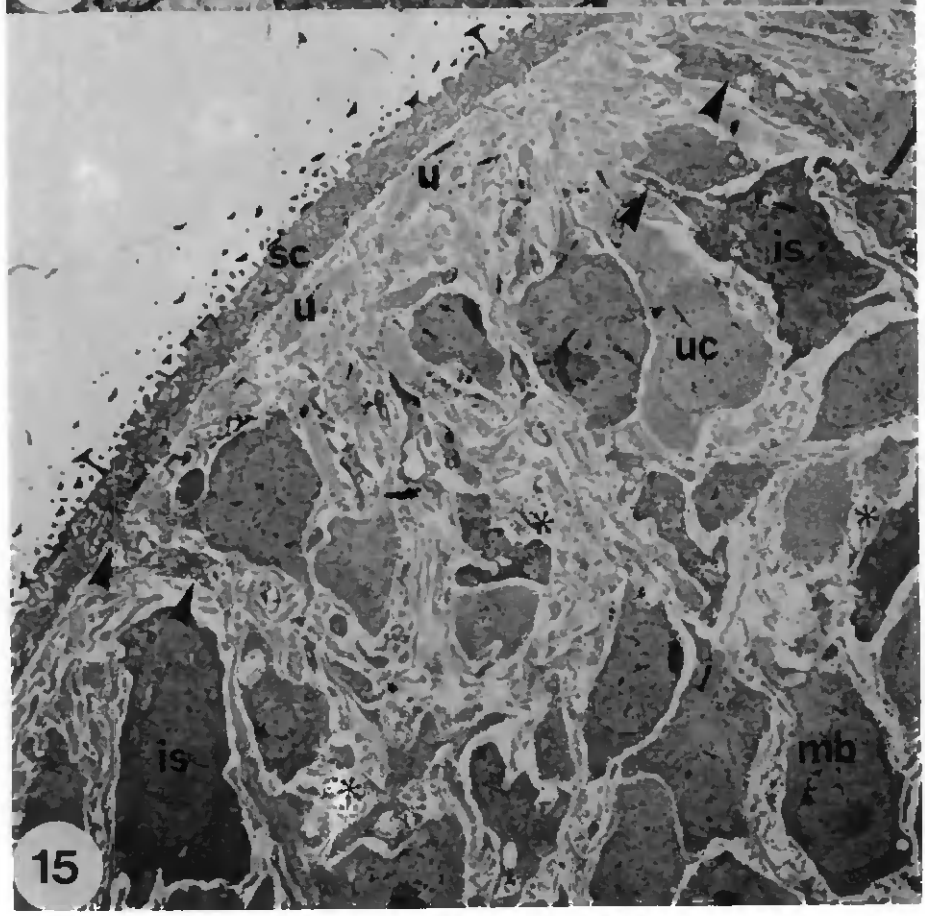
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DISCUSSION AND CONCLUSIONS

According to NEEDHAM (1952) every process of reparative regeneration is divided into a regressive phase and a progressive phase. The regressive phase is induced by the trauma and comprises i) wound closure, ii) destruction of injured cells, iii) defense against infection and toxic substances, iv) predetermination of the cells needed for the progressive phase. The progressive phase comprises i) the formation, ii) the growth, and iii) the differentiation of the blastema. In this study, NEEDHAM's theory is confirmed about regenerating 2 sucker head fragments of tetrathyridia of *M. corti.*

The initial wound closure takes place by the shock contraction of all muscle systems which draw the tegumental wound edges together. The shock contraction also expulses a great number of parenchymal cells. thus reducing the volume of the parenchyma. Subsequently, the muscles relax except the subtegumental muscles of the wound region. Thus the body surface increases and the superficial cytoplasm of the tegumental syncytium is stretched, which is conspicuous by the position of the disc shaped bodies parallel to the larval surface. The tegumental extension seems to be a passive one, possibly due to an increase of the osmotic pressure of the interstitial fluid. This would also explain the loose structure of the parenchyma.

A second mechanism of wound closure is the formation of the cicatrization syncytium between the cap of expulsed cells and the parenchyma. It has to be pointed out that the cicatrization syncytium is a part of the tegumental syncytium. The failure of the cicatrization syncytium to transform into normal tegument with microtriches underlines the antero-posterior determination of the tegumental syncytium (HESS 1980). The definite healing of the wound is achieved indirectly by discarding the posterior part of the body including the wound. The elimination of a tail fragment occurs periodically in *M. corti* tetrathyridia which have to be considered as continuously growing metacestodes.

The glycogen-storing parenchyma cells have a triple function during reparative regeneration. By dedifferentiation, they produce cells which are cytologically identical to germinative cells and which are supposed to have high histogenetic potency. Thus they contribute also to the progressive phase of regeneration. In the wound region, the glycogen-storing lobes of parenchyma cells divide into numerous small fragments adhering together by a great number of gap junctions. This reaction which is probably related to dedifferentiation could work against stress in the wound region and protect the parenchyma against external influences. Glycogen-storing parenchyma cells also envelop degenerating cell fragments thus isolating them from the uninjured tissue.

The final elimination of cell fragments occurs by calcareous corpuscule cells. The capacity of phagocytosis of these cells has never been described before. This observation may be important as the role of the calcareous corpuscule cells is still a matter of conjecture (for review see SMYTH 1969). The experimental model used here could contribute towards the examination of the functions of the calcareous corpuscule cells or at least to studying exhaustively one of their capacities. At any rate our observations seem to support the hypothesis that calcareous corpuscules have to be considered as a kind of residual body.

The anterior inviginated part of the tegumental syncytium, called the apical massif (HESS 1980), is histogenetically the most active part of the tegumental syncytium during asexual multiplication. It differentiates into tegumental syncytium, subtegumental muscles, glycogen-storing parenchyma cells and parts of the suckers. During reparative regeneration the apical massif disappears histologically. It is not clear if it transforms entirely into tegument or if it contributes also to the formation of the scolex blastema

from which the suckers derive. It is however evident that parts of the suckers, i.e. the interstitial syncytium and the upper sucker muscles derive directly from the tegumental syncytium and the subtegumental muscles, thus they are indirect products of the apical massif. This observation confirms the description of the morphogenesis of the suckers during asexual multiplication (HESS 1981). After the formation of the sucker anlagen, the apical massif reappears. Histologically it is probable that, from this moment on, it forms new superficial cytoplasm and differentiates into subtegumental muscles and parenchymal glycogen-storing cells as during asexual multiplication (HESS 1980).

In conclusion, the ability to reparative regeneration is well developed in *M. corti* tetrathyridia and closely related to its ability of asexual multiplication as seen by the reaction of the tissues.

The fact that only scolex-bearing fragments regenerate a complete tetrathyridium is probably a consequence of the anatomy of these animals. The main nervous ganglia as well as the apical massif is situated near the suckers (HART 1968). Thus each single sucker head fragment contains a part of the central nervous system which is known to play the most important role for all processes of typical regeneration.

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BIBLIOGRAPHY

HART, J. 1968. Regeneration of Tetrathyridia of *Mesocestoides* (Cestoda: Cyclophyllidea) *in vivo* and *in vitro*. *J. Parasit.* 54: 950-956.

HESS, E. 1972. Contribution à la biologie larvaire de *Mesocestoides corti* Hoeppli, 1925 (Cestoda, Cyclophyllidea). Note préliminaire. *Revue suisse Zool.* 79: 1031-1037.

— 1980. Ultrastructural Study of the Tetrathyridium of *Mesocestoides corti* Hoeppli, 1925: Tegument and Parenchyma. *Z. Parasitenkd.* 61: 135-159.

— 1981. Ultrastructural Study of the Tetrathyridium of *Mesocestoides corti* Hoeppli, 1925: Pool of Germinative Cells and Suckers. *Revue suisse Zool.* 88: 661-674.

HESS, E. and R. GUGGENHEIM. 1977. A Study of the Microtriches and Sensory Processes of the Tetrathyridium of *Mesocestoides corti* Hoeppli, 1925, by Transmission and Scanning Electron Microscopy. *Z. Parasitenkd.* 53: 189-199.

NEEDHAM, A. 1952. Regeneration and wound-healing. *London, Mathuen.* 269 pp.

SMYTH, J. D. 1969. The Physiology of Cestodes. *Oliver and Boyd, Edinburgh.* 279 pp.

SPECHT, D. and M. VOGE. 1965. Asexual multiplication of *Mesocestoides Tetrathyridia* in laboratory animals. *J. Parasit.* 51: 268-272.

VOGE, M. and L. S. COULOMBE. 1966. Growth and asexual multiplication *in vitro* of *Mesocestoides Tetrathyridia*. *Am. J. trop. Med. Hyg.* 15: 902-907.

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