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Ultrastructural Studies on the Cercarial Integument of *Clonorchis sinensis* (Cobbold, 1875) Looss, 1907

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Introduction

Cercariae of *Clonorchis sinensis* were first observed in 1918 from a fresh water snail, *Parafossarulus manchouricus*, in Japan after the adult worm was discovered in 1877 at Okayama prefecture. The morphological features of the cercaria have been studied by several workers using light microscope such as Komiya and Tajimi (1940). Although the literature contains a reference to the ultrastructural observation of *C. sinensis*, the investigation has not been extended beyond adult integument by Inatomi *et al.* (1968). On the other hand, many fine structures on the cercariae except *C. sinensis* have been reported by many authors since Kruidenier and Vatter (1958) described briefly the cercarial integument with electron microscopy. More detailed descriptions about the cercaria have been made by a large number of researchers (Cardell and Philpott, 1960, on the muscle of *Himasthla quissetensis* ; Kruidenier and Vatter, 1960, on the muscle of *Schistosoma mansoni* and *Tetrapapillatrema concavocorpa* ; Cardell, 1962, on the body wall of *Himasthla quissetensis* ; Belton and Herris, 1967, on the cuticle of *Acanthatrium oregonense* ; Rees, 1967, on *Parorchis acanthus* ; Lumsden and Foor, 1968, on the muscle of *Heterobilharzia americana* ; Inatomi *et al.*, 1970, 1972, on the whole body of *Schistosoma japonicum* and *S. spindale* ; Tongu *et al.*, 1970, 1975, on the whole body of *Cercaria longissima* and penetration gland cells of *Metagonimus* ; Bibby and Rees, 1971, on the epidermis and associated struture of *Diplostomum phoxini* ; Morris, 1971, on the integument of *Schistosoma mansoni* ; Southgate, 1971, on the integument of *Notocotylus attenuatus* ; Hockley, 1972, on the integument of *Schistosoma mansoni* ; Hockley and McLaren, 1973, on the integumentary development of *Schistosoma mansoni* ; Powell, 1973, on the excretory bladder of *Schistosoma mansoni*). It may be concluded from the results of these investigations that the cercarial body integument is consisted of a large syncytium, i.e. the integument with sensory hairs and spines on its surface is connected to the epidermal cells having nuclei by narrow cytoplasmic tubules which are extended towards the parenchym through muscle layers. The details of cercarial integument, especially in *C. sinensis* are, however, not fully known. The present report describes the ultrastructural differences between body and tail integument of cercaria *C. sinensis* compairing with other species.

Materials and Methods

The fresh water snail, *Parafossarulus manchouricus* which is the first intermediate host of *Clonorchis sinensis*, was collected in the river of Naktong at Pusan in Korea. The cercariae obtained by dissection of the snails were immediatelly fixed in 1% cold gluteraldehyde solution with phosphate buffer at pH 7.4 for 1 or 2 hours and after rinse in buffer solution, followed by post-fixing in

1% osmium tetroxide solution with phosphate buffer at pH 7.4 for 2 hours.

The specimens were dehydrated in ethanol series by routine method and embedded in an epoxy resin mixture after passing through propylene oxide. The specimens were thin-sectioned with Porter-Blum ultramicrotome and stained by uranyl-acetate and lead citrate. Hitachi HS-8 electron microscope was used for observations.

Results

1. Body integument

Cercarial body was all shielded with a thin integument (I) (Figs. 9, 10, 12, 13, 14, 15, 16) which was much thinner than that of the adult worms. The integument (Fig. 13) of the anterior part near the oral sucker and sucker lumen was thinner than that of other parts of the body. The detailed structure of this integument, cytoplasmic covering without nucleus, showed a large syncytium structure, i.e. the integument which was bounded by a basement membrane from the fibrous layer (FL) (Fig. 16) consisting of fine fibril networks was connected to epidermal cells (EC) (Fig. 16) situated in a deep portion, having nucleus, by cytoplasmic tubules through the basement membrane, fibrous layer and muscle layers. Both outer and basal surface of the integument were limited by a plasma membrane about 80 Å thick. In the matrix of the integument, there were a few mitochondria and numerous secretion granules (Figs. 9, 10, 15, 16). The sucker part (Fig. 13), however, had fewer granules than other integument. These granules varying in size and electron-density seemed to be biconcave disc shape, and were classified into two groups in its density, i.e. homogenious dense granules and less-dense granules dotted with dense spots or covered with a dense part. Although the former were relatively located in the bottom half area near the basal plasma membrane (Figs. 10, 16), the latter were scattered somewhat parallel in the upper half near the integumentary surface and were roughly orientated with the long axis perpendicular to the outer plasma membrane (Figs. 10, 16). The outer plasma membrane of the integument was often covered by a surface coat (fuzz).

Minute spines (S) (Figs. 9, 10, 13) measured about 1.5 μ in length, and presented a crystalloid lattice-like structure were located sparsely on the integument. In the anterior region, especially in the part opposed oral sucker lumen there were many spines gathered in group (Fig. 13). The rootlets of the spines reached the basal plasma membrane (Figs. 9, 10). The spine part extending out of the integument was covered with the same plasma membrane (Fig. 9) that covered the outer surface of the integment. Six short and seven long cilia so-called sensory hairs (SH) (Fig. 12) were observed on each lateral surface of the integument. These organs consisted of cilia process arising from a bulb (SB) (Fig. 12) which was embedded within the integument. The sensory bulb attaching to the integument by desmosomes contained mitochondria and several small vesicles. A thin fibrous layer (Fig. 16) situated between integument and muscle layers was composed of a fine fibril network made from thin filaments. Each filament was approximately 80 Å in diameter. The body muscle layers under the fibrous layer were composed of circular, longitudinal, diagonal and dorso-ventral muscles. These all belonged to the somatic muscle cell which have two kinds of myofilaments, thick ones, about 250 Å in diameter, and thin ones, about 50 Å in diameter. Each thick myofilament was surrounded by about 8 to 12 thin myofilaments arranged hexagonally. A large number of mitochondria and glycogen particles were visible in the peripheral parts of the muscle cells. The epidermal cells (Fig. 16) were situated under the muscle layers, and connected to the integument by narrow cytoplasmic tubules through fibrous layer and muscle layers. These cells including the nuclei were distributed Golgi complex, endoplasmic reticula, mitochondria, glycogen particles and dense granules of various sizes in the cytoplasm.

2. Tail integument

The integument (Figs. 5, 7, 8, 11, 14) of the cercarial tail was a large cell as in the case of body integument. It, however, differed from the body integument in the absence of cytoplasmic tubules, epidermal cells, spines and less-dense granules dotted with dense spots or covered with a dense part. The integument limited by plasma membrane from a thin fibrous layer was of approximetely 80 Å in thickness, and contained a few mitochondria and one kind of secretion granules, i.e. homogenious dense granules, in the matrix. The basal plasma membrane showed irregular infoldings into the matrix of fibrous layer. Although sensory hairs (Fig. 8) were located on the tail integumentary surface, it was diffiicult to identify their numbers in this present study. The spine and surface coat were unable to observe anywhere. The tail had two large fins (DF, VF) (Figs. 3, 4, 8, 11) infolding from the integument. Dorsal ones (DF) (Fig. 3) were extended from the tail end to the posterior two third, ventral ones (VF) (Fig. 3) from the tail end to the posterior third. Furthermore several fin-like structures (arrows) (Figs. 1, 2) were observed near the anterior end of the tail in a cross-section. These structures were shorter than fins in a cross-section, and had a basal plasma membrane of the integument infolded into the matrix of the fin-like structures (Fig. 5). Both fin and integument were linked together by common matrix (Figs. 3, 4, 8, 11). The fibrous layer was composed of a fine fibril networks of thin filament of about 80 Å in diameter. The space in the fibrous layer (arrow) (Figs. 8, 11) was often observed between basal integument and muscle. The muscle layer beneath the fibrous layer were consisted of the outer circular and inner longitudinal layer. In cross-section the inner longitudinal muscle cells except for the tail end were arranged into four groups (Figs. 1, 2, 3), each including 7 or 8 muscle cells. A striated muscle cell was consisted of contractile (C) (Fig. 5) and non-contractile portion (NC) (Fig. 5). The myofilaments, i.e. contractile portion were located in the outer half of the muscle cell in cross-section, salcoplasm, i.e. non-contractile portion including a nucleus, mitochondria and a lot of glycogen particles in the inner half of the muscle cell. The contractile portion was constructed of both thick and thin myofilament. In a cross-section the thick myofilaments appeared like microtubules and the thin ones like the small spots (Fig. 6). Each thick one was regularly surrounded by 8 to 12 thin ones. And also, A, I, H bands and Z-discs were observed on the myofilaments (Fig. 7). The diameter of tubular thick myofilament was 250 Å and that of the thin one 50 Å.

Discussion

Cercarial integument has been observed previously in *Notocotylus attenuatus* (Southgate, 1971), *Himasthla quissetensis* (Cardell, 1962), *Cerithidea californica* (Bils and Martin, 1966), *Schistosoma mansoni* (Morris, 1971; Hockley, 1972; Hockley and McLaren, 1973), *Diplostomum phoxini* (Bibby and Rees, 1971), *Cercaria longissima* (Tongu *et al.*, 1970), *Schistosoma japonicum* (Inatomi *et al.*, 1970) and *Schistosoma spindale* (Inatomi *et al.*, 1972) by electron microscopy since Threadgold (1963) provided an ultrastructure of trematode integument. While these observations were not exttended to *Clonorchis sinensis* cercaria. According to Hockley (1972), young cercariae were covered with a thin nucleated primitive epithelium which was lost when the true integument appeared beneath it. And the integument of matured cercaria was at first simillar to the primitive epithelium in that it was a nucleated. In the present study the ultrastructural features had closely simillar morphology as in the integument of other cercariae. In other words the integument of the body in *C. sinensis* cercaria was composed of a large syncytium. The cytoplasmic tubules were extended from the integument of the body, but were absent from the tail integument. It is probably correct to state the tail integument need not have the epidermal cells.

Because cercarial tail is detached from the body in a short time after the cercaria emerged, and tail integument is connected to the body integument each other. It has been especially noteworthy that the integument contains many granules by several authors, e.g. Bibby and Rees (1971), Hockley (1972, 1973), Morris (1971), Bils and Martin (1966), Belton and Harris (1967), Southgate (1971), Harris *et al.* (1974), and Inatomi *et al.* (1970, 1972). Among them Morris (1971), Hockley and McLaren (1973) and Inatomi *et al.* (1970) have reported that the integument of cercariae includs two types of secretion granules in its shape so-called spherical and elongate body.

Furthermore Bibby and Rees (1971) have mentioned two types of secretion granules in its shape from metacercaria. As concerns the secretion granules, Harris *et al.* (1974) has suggested that one kind of secretion granule was located in the integument of metacercaria and adult of *Leucochloridiomorpha constantiae*, namely spherical of elongate bodies profiles all represent sections at different angles through bodies each of which is a membrane-bounded, biconcave disc. On the basis of the available evidence in the present investigation, it can safely be said that one kind of secretion body in the shape is present. Cercariae of various kinds are covered with a surface coat of fibrous materials on the outer plasma membrane. The chemical nature of this coat have been mentioned as a glycocalyx by Stein and Lumsden (1973) and Harris *et al.* (1974). Kruidenier and Vatter (1958) suggested that the surface coat of *S. mansoni* cercariae was produced by post-acetabular glands. Hockley (1972) also indicated the contents of the ducts were continuous with the surface coat in *S. mansoni*. But he suggested that the surface coat of *S. mansoni* cercariae may be formed by dense glanules which appear to originate from Golgi complexes in the subtegumental cells. In the cercaria of *Diplostomum phoxini*, Bibby and Rees (1971) reported that the dense granules scattered irregularly in the matrix below are probably on their way out to replace those at the periphery which may discharge their contents on the surface. In the present study on *C. sinensis* cercariae there is no direct evidence linking the secretion granules to the surface coat, although the granules distributed within the matrix became closely associated with the outer plasma membrane. The presence of apparently striated muscle and smooth muscle has been commented on by several workers in the past (Cardell and Philpott, 1960; Lumsden and Foor, 1968; Inatomi *et al.*, 1970, 1972; Tongu *et al.*, 1970). *C. sinensis* cercaria have longitudinal muscle consisting of striated muscle fibers as in the case of other cercarial tail. The rapid, vibratory movement of the cercarial tail contrasts markedly with the slower, undulatory movements characteristic of the forebody. The presence of first contracting muscles has enabled certain trematode cercariae to display dramatic differences in speed and rate of contraction of the tail and body muscles. As concerns the tail fins, Komiya and Tajimi (1940) has reported that the cercaria of *C. sinensis* had two tail fins by light microscope. In the present study several fin-like structures were observed near the anterior end of the tail besides two large fins. It was, however, difficult to identify whether these stractures are small fins or folds of the integument.

Summary

Ultrastructural features of cercarial integument and associated tissues of *Clonorchis sinensis* cercariae were described by electron microscopy. The body integument without nucleus was composed of a large syncytium, i.e. the integument was connected to the epidermal cells with nucleus, and contained a lot of secretion granules of biconcave disc shape and mitochondria. These granules of varying size and electron-density were devided into two groups in its electron-density, homogenious dense granule and less-dense granule dotted with dense spots or covered with a dense part. Minute spines were distributed on the surface of the

body integument. Sensary organs, sensary hairs of six pairs of short and seven pairs of long, were laid on each lateral side of the body integument. The fibrous layer consisting of fine filaments were situated beneath the basal plasma membrane of the integument. The body muscle belonging to somatic muscle cells under the fibrous layer was composed of circular, longitudinal, diagonal and dorsoventral layer. The epidermal cells having nuclei, mitochondria, many secretion granules and Golgi complexes were situated under the muscle layer, and connected with the integument by narrow cytoplasmic tubules.

The tail integument was simillar to that of the body. But it differed from the body integument in the absence of epidermal cells, spines, and less-dense granules dotted with dense spots or covered with a dense part in the integumentary matrix. The muscle layers of the tail were composed of circular smooth and longitudinal striated muscle cells showing A, I, H bands and Z discs. In a cross-section the longitudinal striated muscles except for the tail end were arranged into four groups, each including seven or eight muscle cells. The tail had two large fins on both ventral and lateral side. The integment and the fin were linked together by common natrix.

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肝吸虫セルカリアの体表微細構造

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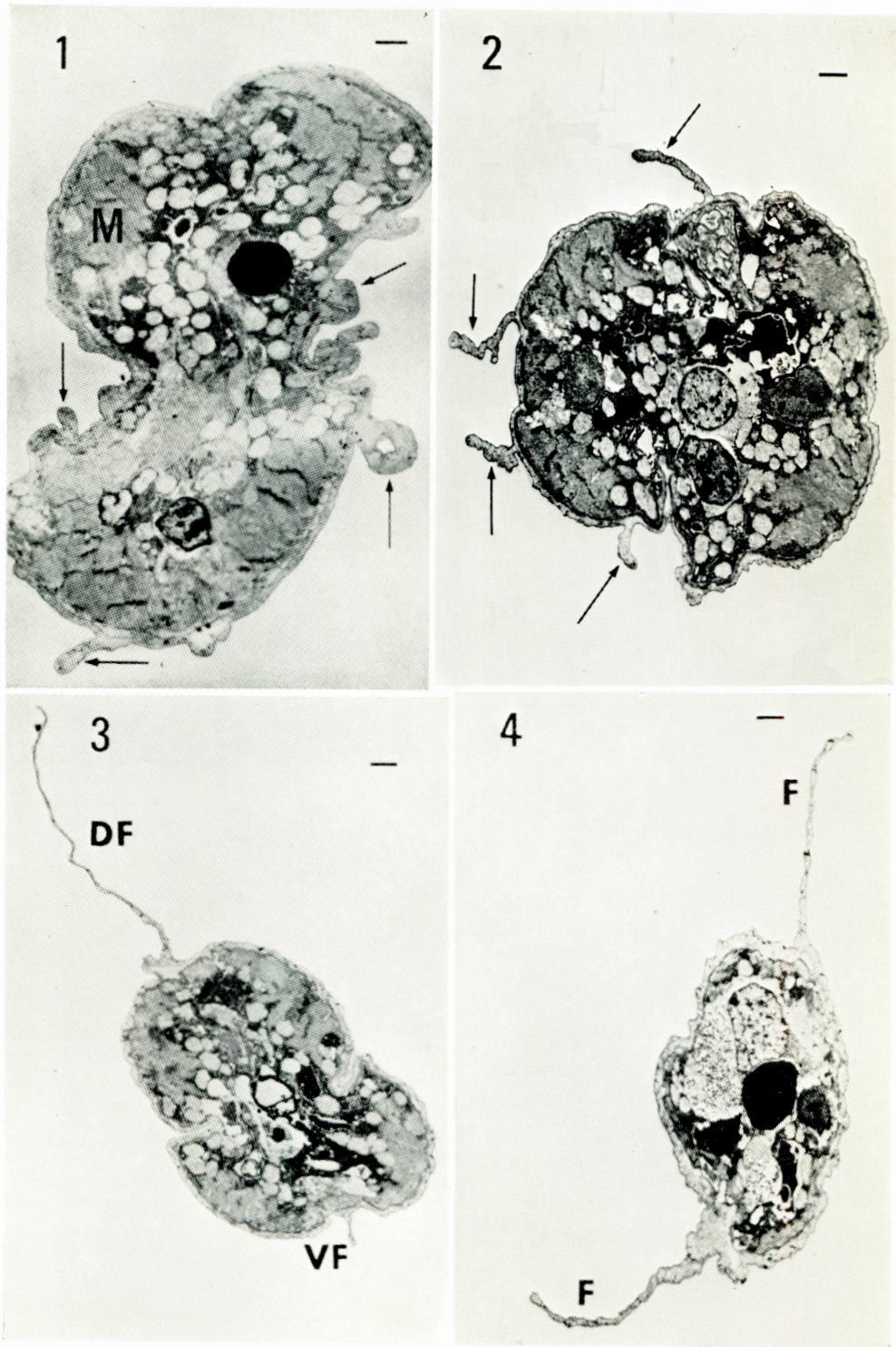
韓国の洛東江で採集したマメタニシから自然遊出させた肝吸虫のセルカリアを用いて，体表構造を電子顕微鏡を用いて観察した．

（1） 体部

体表は他種のセルカリアと同様に角皮層で被われている．この層は隔壁が無く合胞性で体外表の全体を被つている．この角皮層の底部からはところどころに細い管状の細胞質が筋層をつらぬいて深部の柔組織にまでのびて上皮細胞と連絡している．つまり角皮層と上皮細胞は1個の大きな多核合胞細胞を構成している．角皮内にはミトコンドリアや種々の形と電子密度を示す顆粒が多数存在するが，この顆粒は円盤状の形態を持つと考えられる．核は角皮層内には無く上皮細胞に位置している．角皮表面は電子密度のやや低い羽毛状物質で被われ，規則的に配列する多数の皮棘や感覚毛が体表面より突出しているのがみられる．感覚毛は短かいのが6本と長いのが7本それぞれの体側に生えていて，角皮内に根を持つ感覚細胞の中心より発する1本の繊毛であり，この感覚細胞の底部は神経線維と連絡している．皮棘の内部は高電子密度の結晶状の格子様構造を呈し，底部は角皮層の形質膜に接し自由表面への突出部は角皮の形質膜で覆われている．この角皮層の下には細線維構造よりなる薄い角皮下層がある．この下方に平滑筋よりなる筋層が位置しており輪走，縦走，斜走筋の順に層をなしている．

（2） 尾部

尾部の角皮も体部と同様の多核合胞細胞と考えられるが，上皮細胞や細管は認められなかつた．角皮層内には一様な電子密度の顆粒だけが少数存在するのみである．角皮表面に於ても感覚毛は存在するが皮棘，羽毛状物質等は認められない．尾部の角皮層には腹側と背側に大きなヒレが存在するのが特長である．これの基質は完全に角皮層と連絡しており角皮層が突出した様な構造を呈している．背側のものは尾端より $\frac{2}{3}$ 位いまでのびており，腹側のものは尾端より $\frac{1}{3}$ 位いまでの間に位置している．角皮層下にはやはり薄い角皮下層があつて更にこの下に平滑筋よりなる輪走筋が位置し，それの下に縦走筋が存在する．しかしこの縦走筋は他のものと異なり，A, I, M 帯とZ板が明瞭に認められる横紋筋より構成されている．しかし細線維をみると1本の太いフィラメントを取囲む細いフィラメントの数は8～12本で不規則である．1本の縦走筋と横断像でみると角皮層に近い外側にはフィラメントのある収縮する部分があり，内方は筋形質になつていてここに核，ミトコンドリア等細胞内器官が位置している．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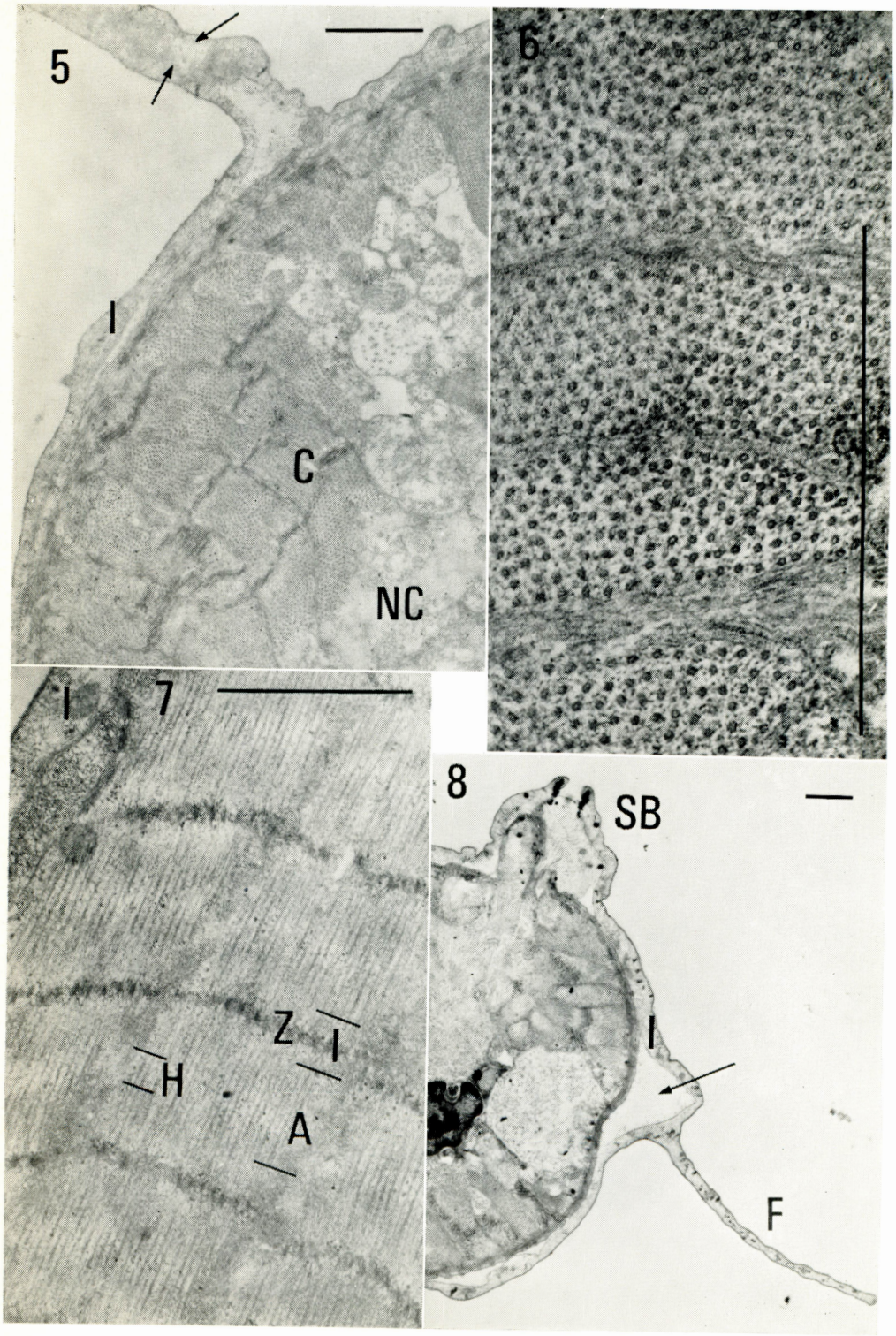
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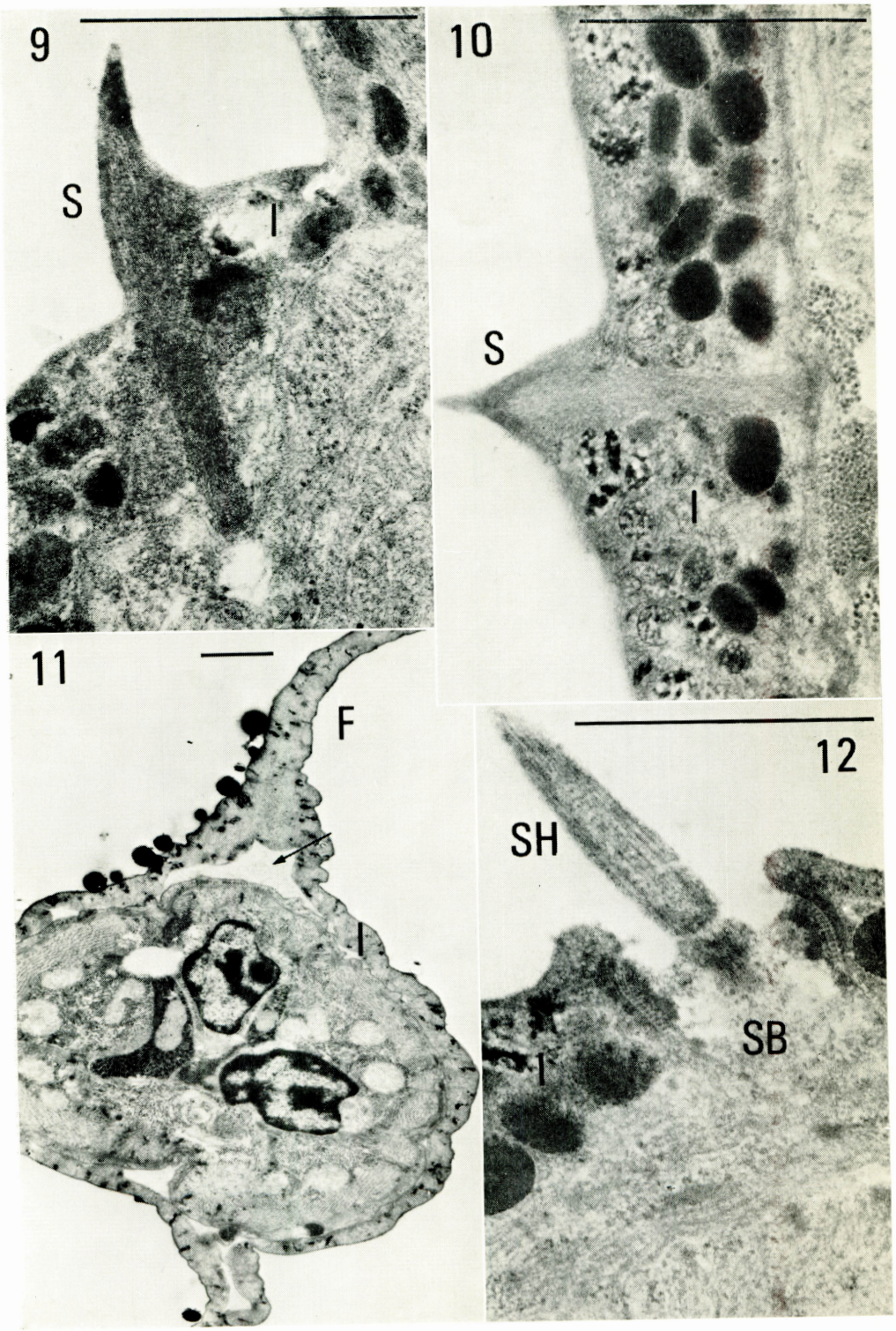
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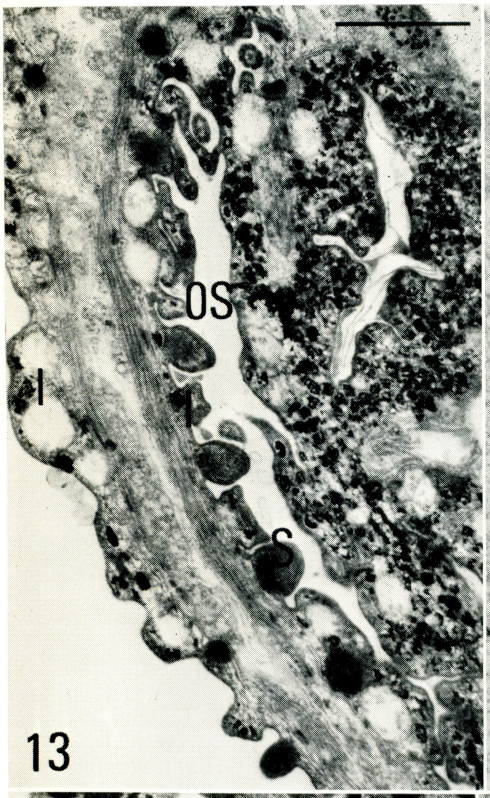
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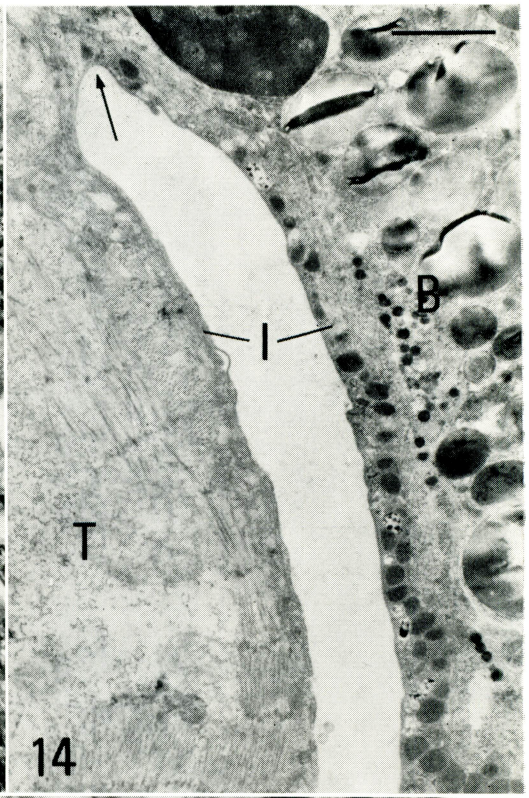
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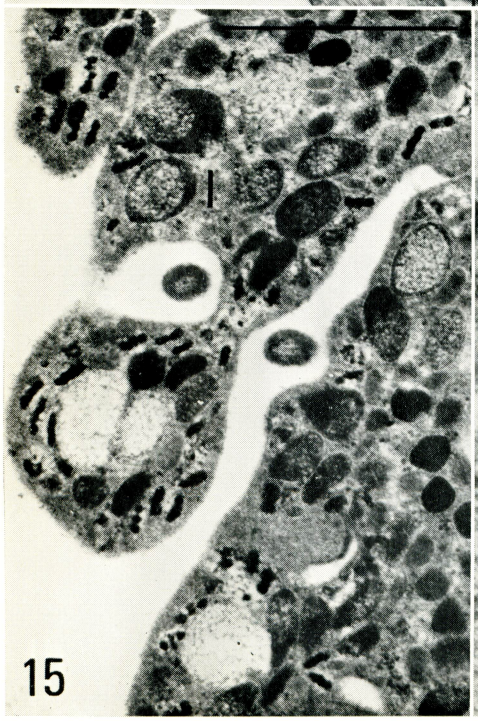
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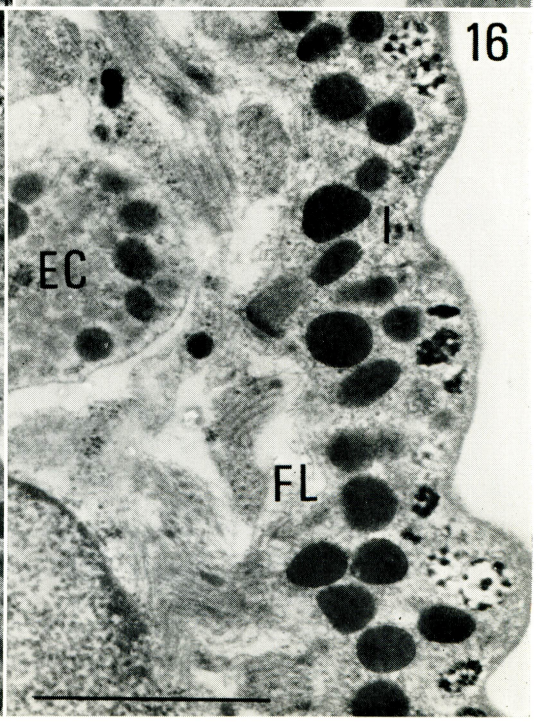
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Explanation of Figures

Figs. 1, 2 Cross-section of the tail near the anterior end. Longitudinal muscles (M) are arranged into four groups. Small fin-like structures (arrows) are seen on the surface.

Fig. 3 Cross-section of the middle portion of the tail having a dorsal (DF) and a ventral fin (VF).

Fig. 4 Cross-section of the posterior portion of the tail showing both ventral and dorsal fin.

Fig. 5 Cross-section of the tail striated muscle showing contractile (C) and non-contractile (NC) portion. I : integument

Fig. 6 Cross-section of the contractile portion of tail striated muscle. Tubular thick myofilament is surrounded by 8 to 12 thin myofilament.

Fig. 7 Longitudinal section of contractile portion of tail striated muscle with A, I, H bands and Z disc. I : integument

Fig. 8 Cross-section of the tail through a fin and sensory bulb. There is a space (arrow) between integument (I) and circular muscle.

Figs. 9, 10 Longitudinal section through a spine (S) presented a crystalloid lattice-like structure. I : integument

Fig. 11 Cross-section of the tail through the fins (F) with a space (arrow) between integument and circular muscle. Tail integument contains a few dense secretion granules.

Fig. 12 Semilongitudinal section of the sensory hair (SH) and bulb (SB).

Fig. 13 Showing the integument (I) near the oral sucker (OS). Many spines (S) are gathered in group.

Fig. 14 Longitudinal section of the body and tail integument (I). Both of them are linked togather by common matrix (arrow).

Figs. 15, 16 Showing body integument contained secretion granules of varing size and electron density. Epidermal cell (EC) is located under the fibrous layer (FL) and muscle layer.

(Scale is one micron in each figure)