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Title FINE STRUCTURE AND HISTOCHEMISTRY OF THE CUTICLE OF THE CERCARIA OF ACANTHATRIUM OREGONENSE (MACY)

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The cuticle of the cercaria of Acanthatrium oregonense was studied by means of electron microscopy and histochemistry. The integument of this cercaria has a definite ultrastructure which varies in different regions of the body. The body cuticle is a syncytium containing vesicles and mitochondria. It is bounded externally by a double surface membrane and internally by a basement membrane. The vesicles were grouped morphologically into three types, two with fibrillar contents and the third containing homogeneous electron-dense material.

The sucker is a modification of the body cuticle and contains vesicles of only one of the three types and no mitochondria. The tail cuticle has the same three layers (surface membrane, cytoplasmic layer, basement membrane) as the body cuticle, but no vesicles are present. Instead there are small elongate cavities along the

periphery of the cuticle.

Chemically the body cuticle and sucker may be differentiated from the tail cuticle by the presence of acid mucopolysaccharides. The subcuticular layer of the body has a concentration of glycogen. The differences in chemistry and morphology of the body and tail cuticle may be related to the temporary nature of the tail.

FINE STRUCTURE AND HISTOCHEMISTRY OF THE CUTICLE OF THE CERCARIA OF ACANTHATRIUM OREGONENSE (MACY)

by

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FINE STRUCTURE AND HISTOCHEMISTRY OF THE CUTICLE OF THE CERCARIA OF *ACANTHATRIUM OREGONENSE* (MACY)

INTRODUCTION

Only recent investigations of cercariae have emphasized their functional morphology and physiology rather than their place as intermediate stages of trematode life cycles. During its brief existence the cercaria must be effectively adapted to three environments (first intermediate host, aquatic environment, and second intermediate or definitive host) each imposing its own physiological demands.

The integument of trematodes has been of interest as one of the adaptations to an endoparasitic environment. Early morphological studies of trematode integument were limited to consideration of the adult stage. Odlaug's review of the early work of light microscopists reveals that the cuticle of adult trematodes was generally characterized as a homogeneous structureless mass (1948). Various theories arose concerning the origin of the helminth cuticle and whether it was a modification of the epithelium or a secretion. Odlaug (*loc. cit.*) described fibrous processes of the parenchymal cells passing between the muscles of the body wall and making contact with the granular cuticle.

The development of the electron microscope has permitted a more detailed characterization of the cuticle. Senft, Philpott and

Pelofsky (1961) described the integument of Schistosoma mansoni as "a vast spongy layer" and "an acellular amorphous covering" with possible connections to the submuscular layer. The work of Threadgold (1963) on Fasciola hepatica showed that the integument is cellular and consists of an internal nucleated and an external syncytial layer connected by protoplasmic strands. These findings were confirmed by Burton's work on the frog lung-fluke, Haematoloechus medioplexus (1964).

Kruidenier and Vatter (1960) published the first observations on the ultrastructure of the cercariae of Schistosoma mansoni and Tetrapapillatrema concavocorpa, but they limited their discussion to the musculature. Cardell and Philpott (1960) described mitochondria-like osmiophilic bodies especially dense in the region adjacent to the basement membrane in the tail of the cercaria of Himasthla quissetensis. Working with the same cercaria Cardell (1962) described a second type of osmiophilic body present in the body cuticle along with the mitochondrial type previously observed in the tail cuticle.

The only work to focus specifically on the integument of cercariae is a study of the histogenesis of trematode cuticle from the sporocyst stage to the adult trematode (Bils and Martin, 1966). Observations made by means of the electron microscope were correlated with histochemical tests. The integument of the cercaria

was demonstrated to consist of a cytoplasmic layer containing mitochondria and numerous dense globules that show a positive periodic acid-Schiff (PAS) reaction. This cytoplasmic layer is bounded by a basement membrane and by a surface membrane which may be quite irregular.

Axmann (1947) did the first studies localizing glycogen deposits in various larval stages and the adults of three schistosomes. Her work has been continued in investigations of the histochemistry of larval trematodes and their effects on the pathology of the molluscan intermediate hosts (Cheng, 1963a, b; Cheng and Snyder, 1962, 1963; Porter, 1966). In these studies glycogen was very diffuse if present in the sporocysts, but in the rediae appeared to be concentrated in the body wall and oral sucker. Traces of glycogen were present in developing cercariae; the staining became quite intense by the time the cercariae emerged.

More detailed histochemical studies have been carried out on the cuticle of adult intestinal helminths including flukes, tapeworms, nematodes and Acanthocephala (Monné, 1959). Monné found a non-acid mucopolysaccharide in the main cuticular layer of Fasciola hepatica which he concluded was converted to the acid mucopolysaccharide found on the surface of the cuticle. Lal and Shrivastava (1960) got a positive PAS reaction in the cuticle of adult Fasciola indica and suggested that this might be due to

glycoproteins. Björkman, Thorsell and Liernert (1963) using Fasciola hepatica concluded that the cuticle has an outer layer of mucopolysaccharides or mucoproteins and an inner portion containing glycogen granules. Autoradiography used in conjunction with electron microscopy has demonstrated that areas of glycogen deposits, as shown to occur histochemically in the subcuticular region in Haematoloechus medioplexus, are sites of glycogen synthesis (Burton, 1962).

The objective in initiating this study was to provide more detailed information about the structure of the cercaria with special emphasis placed on the cuticle. The cercariae used in this study were identified by the characteristic flexing and extension of the body, bringing the acetabulum into apposition with the oral sucker, which has been described for the cercariae of Acanthatrium oregonense (Burns, 1961a, b). The adult of A. oregonense is an intestinal parasite of the bat first described by Macy (1939). The life cycle was worked out by Knight and Pratt (1955) who first described this virgulate xiphidiocercaria. A discussion of the formation and function of mucoids in virgulate cercariae has been done by Kruidenier (1951).

MATERIALS AND METHODS

Naturally infected snails, Oxytrema siliqua (Gould) were collected from Oak Creek one-half mile north of the Oak Creek research laboratories, Benton County, Oregon, during January and May 1966. Freely shed cercariae were obtained by keeping the snails in stream water at room temperature for 24 hours. The cercariae thus obtained were fixed in preparation for ultrastructural and histochemical study.

Electron Microscopy

Cercariae were fixed in 3% glutaraldehyde buffered with phosphate to pH 6.0 or 7.4 for one hour at room temperature, washed in phosphate buffer of the same pH and post-fixed in 1% osmium tetroxide (Palade, 1952). The organisms were then dehydrated through a graded series of alcohols and flat embedded in Araldite polymerized at 60° C. for 12 hours (Luft, 1961). Individual animals were reembedded on Araldite blocks.

Thin sections were cut with a glass knife on a Porter-Bloom microtome, mounted on parlodion-coated grids, and stained with a saturated aqueous solution of uranyl acetate (Marinozzi and Gautier, 1962) followed by lead citrate (Reynolds, 1963). Sections were examined with the RCA EMU-2D electron microscope. Initial

magnifications of the micrographs ranged from 1200X to 13,200X, and the micrographs were photographically enlarged.

Histochemistry

Specimens embedded in Araldite as previously described were sectioned at two microns on a Porter-Bloom microtome using glass knives and stained with Richardson's stain, a combination of methylene blue and Azur II (Richardson, Jarrett and Finke, 1960). The sections were used to orient structures seen in the electron micrographs.

Another set of specimens was fixed for one hour at room temperature in 2% glutaraldehyde buffered with phosphate to pH 7.2 with sucrose added to a concentration of 1.5%. These animals were then washed in phosphate buffer of the same pH and treated with the periodic acid Schiff reaction (PAS) for polysaccharides following the Hotchkiss method outlined by Gurr (1960). Some of the specimens used as controls were treated with a 1% diastase solution for ten minutes for selective removal of glycogen (Gurr, 1960). The cercariae were then dehydrated through an alcohol series and embedded in Araldite as described above. Two micron sections were cut on a Porter-Bloom microtome and mounted on clean glass slides with Canada balsam.

Animals embedded in Araldite showed less compression when

sectioned at two microns than paraffin-embedded material sectioned at the same thickness. However, the contact with water necessitated in floating the Araldite sections off the glass knife and the moist heat used to expand the sections seemed to result in a leaching out of the Alcian blue stain. Paraffin-embedded material which had been fixed in hot Schaudinn's was therefore employed for the Alcian blue staining for mucopolysaccharides. High melting point paraffin (60°-62° C.) was used, and two-micron sections were obtained using a knife chilled by carbon dioxide vapors. These sections were mounted on albumenized slides, stained with Alcian blue, dehydrated, and mounted in Canada balsam (Barka and Anderson, 1963).

OBSERVATIONS

The cercaria of Acanthatrium oregonense moves actively and its size and shape may vary considerably with the degree of flexure of the body, which is small and may be oval or elongate in shape. The tail is usually contracted giving it an accordion-like appearance, but when extended it is thin and may be longer than the body. Cercariae kept in stender dishes were observed to spend most of their time creeping in a caterpillar-like fashion along the bottom of the dish. The majority of cercariae fixed were in a flexed position with the oral sucker apposing the acetabulum and with the tail contracted.

Electron Microscopy

Study of the electron micrographs of the cercaria of A. oregonense indicates that this animal possesses an integument of definite structure which varies in different regions of the body. A morphological description of the body integument will be presented first since basic structure can be most clearly demonstrated here. The cuticle can be divided into three regions: 1) a thin double outer surface membrane, 2) a wide cytoplasmic layer containing many vesicles and mitochondria, and 3) an inner basement membrane (Plate V). Beneath the basement membrane are two layers of

muscle, an outer circular and an inner longitudinal layer (Plate V). Interior to these layers of muscle are the parenchymal cells, irregular in outline and with large nuclei and mitochondria (Plates II and IV).

The mitochondria in the cytoplasmic layer are concentrated in the region proximal to the basement membrane (Plate V) and are small in comparison to the mitochondria found in the parenchyma. No cell boundaries are visible in the cytoplasmic layer although protoplasmic extensions may be seen to pass between the muscle fibers and connect with the parenchymal cells beneath (not illustrated).

The surface of the body cuticle is irregular and has spines which are homogeneous in appearance and bounded by the surface membrane (Plate IV). The matrix of the cuticle is finely granular and of medium electron density. The vesicles contained within it (Plates IV and V) may be divided into three types: 1) homogeneous electron dense, 2) finely fibrillar with electron opaque periphery, 3) condensed fibrillar with electron opaque periphery. The first type of vesicle may be irregularly shaped, sometimes also containing some finely fibrillar material. The latter two types of vesicles are round. The finely fibrillar vesicles appear to coalesce in some areas (Plate V). Separating the cytoplasmic layer of the cuticle from the muscle layers is a basement membrane of finely fibrillar material (Plate V).

The sucker is a large bilobed structure densely packed with vesicles similar to the finely fibrillar type found in the body cuticle (Plates VI and VII). Some of these vesicles may be seen in the buccal cavity. In the medial infolding of the sucker is a stylet which appears homogeneous in cross section (not illustrated).

The structure of the cuticle of the body and tail can best be compared and contrasted at the place where these two integumentary types join (Plate III). An accumulation of electron dense material is seen at this transition zone. In contrast to the body integument the tail cuticle is thinner, lacks the vesicles previously described for the body surface and possesses a matrix of lesser electron density (Plates II and III).

The tail is bounded by a double membrane similar to that found on the surface of the body (Plates VIII and IX). The surface of the distal portion of the tail is highly irregular and may be thrown into slender projections (Plate IX). The cytoplasmic layer is distinguished by small elongate cavities along the outer edge and small mitochondria adjacent to the basement membrane (Plate IX).

Large folds involving the cuticle, basement membrane and associated muscle layers are produced by spiraled contractions of the tail (Plates II and VIII). The muscles in the tail are more prominent, but can't be separated into the same distinct layers as the body wall musculature. The area beneath the muscles is

compactly filled with large mitochondria and unidentified electron-dense granules (Plates VIII and IX).

Histochemical Observations

The use of Richardson's stain resulted in a metachromatic reaction in the body cuticle and sucker (Plate I, fig. 1), indicating the presence of acid mucopolysaccharides or sphingolipids. The Alcian blue stain was positive in the sucker and body cuticle (Plate I, fig. 2), demonstrating the presence of acid mucopolysaccharides in these two areas.

With the PAS reaction there was intense staining in the subcuticular region of the body and a slightly positive reaction in the body and tail cuticle (Plate I, fig. 3 and 4). After treatment with diastase the subcuticular PAS-positive material disappeared, but the cuticle of both body and tail retained a slight background stain. This would indicate that the subcuticular PAS-positive material is glycogen and that there is present in the body and tail cuticle some type of non-specific aldehyde.

DISCUSSION

In contrast to the findings of other studies of trematodes (Odlaug, 1948; Senft, Philpott, and Pelofsky, 1961), the cuticle of Acanthatrium oregonense cercariae has a distinct and highly organized ultrastructure which may be divided into two morphological types (body and tail) according to the region of the cercaria on which it is found (Plate III). Both types of integument are characterized by a double surface membrane bounding a syncytial layer containing mitochondria and having cytoplasmic extensions uniting it with the parenchymal cells (Plate V). This outer cytoplasmic covering is not subdivided by cell membranes nor does it contain any nuclei, thus verifying its true syncytial nature. Similar protoplasmic extensions from the parenchymal cells have been shown to occur in other adult and larval trematodes (Threadgold, 1963; Burton, 1964; Bils and Martin, 1966) and in cestodes (Rothman, 1963; Rosario, 1962; Threadgold, 1962, 1965). Bils and Martin in their study of cercariae found the same three basic cuticular layers (surface membrane, cytoplasmic layer, basement membrane) that were observed in A. oregonense.

Axmann (1947), working with a furcocercous cercaria, found glycogen in the tail stem, but not in the distal forked portion of the tail. The highly folded tail cuticle of the A. oregonense cercaria

possesses no glycogen or acid mucopolysaccharide and has no distinctive structure other than small elongate cavities and a few small mitochondria (Plates VIII and IX). Cardell and Philpott (1960) found round cavities in the tail cuticle of the cercaria of Himasthla quissetensis and compared them with structures previously seen in the cuticle of an adult cestode by Read (1955). The round shape led Cardell and Philpott (1960) to conclude that the cavities did not form a continuous canal system as suggested by Read (1955). Although the cavities in the tail cuticle of A. oregonense were elongate, their continuity could not be traced, and in the opinion of this author no network of canals can be postulated at this time.

Mitochondria and vesicles have often been described in the outer syncytial layer of adult and larval cestodes (Read, 1955; Rosario, 1962; Rothman, 1963; Threadgold, 1962, 1965; Race et al., 1965), in the cuticle of adult trematodes (Threadgold, 1963; Burton, 1964) and in cercariae (Cardell and Philpott, 1960; Cardell, 1962; Bils and Martin, 1966). In the cercaria of A. oregonense the structure of the body cuticle is contrasted to that of the tail by the presence of vesicles of three structural types (Plate V). None of the vesicles, vacuoles or granules described by the above authors for the cuticle of parasitic helminths has the fibrillar nature characteristic of the vesicles of the body cuticle of the cercaria of

A. oregonense. The occurrence of finely fibrillar material within vesicles of the homogeneous electron-dense type suggests that there is a sequence in the development of the vesicles, with the finely fibrillar-filled vesicles as the intermediate stage and the condensed fibrillar type as a final stage. Vesicles of the finely fibrillar variety were the only ones observed to coalesce.

Threadgold (1963) in a study of adult Fasciola hepatica distinguished between membrane-limited vesicles at the outer edge of the thick cuticle and membranous vacuoles arising from the basement membrane. He described the vacuoles as filled with homogeneous material of low electron density or granular material and suggested that the pinocytotic vesicles might signify a secretory or excretory activity such as the secretion of mucus. However, he found no mucous layer coating the cuticle. Burton (1964) described membrane-bounded ovoid bodies in the cuticle of the adult lung-fluke Haematoloechus medioplexus which appeared to contribute their contents to the cuticular matrix. In addition he observed a second type of vesicle and numerous mitochondria.

Ovoid osmiophilic bodies have been observed in the cuticle of cercariae, especially at the anterior end of the animal, but were absent in the tail cuticle (Cardell, 1962). These resembled the mitochondria described by Read (1955) in tapeworm cuticle.

Although spines were shown in the electron micrographs of

A. oregonense (Plate IV), none was of the magnitude described in observations with the light microscope (Knight and Pratt, 1955; Burns, 1961a). Bils and Martin (1966) described certain segments of the basement membrane of the cercarial integument being involved with spine formation. This was not shown in A. oregonense.

It has been suggested that the irregularities of the cuticular surface of trematodes and the microthriches described for cestode cuticle (Read, 1955; Rosario, 1962; Rothman, 1963; Threadgold, 1962, 1965; Race et al., 1965), although morphologically dissimilar, serve a similar function, possibly providing more absorptive area. This would be of greater significance to the adult cestode, which lacks a mouth and must absorb nutrients through the cuticle, than to the adult trematode. However, the cercarial stage of a trematode doesn't actively ingest food and must therefore absorb nutrients or rely on stored energy.

Bils and Martin (1966) described numerous small dense globules in the cuticle that were PAS-positive whereas Axmann (1947) found that the greatest concentration of glycogen was in the subepithelial tissues of the cercarial and miracidial stages. Björkman, Thorsell, and Lienert (1963) in their study of adult Fasciola hepatica discovered glycogen of a granular form in the cuticle, and Hedrick and Daugherty (1957) localized glycogen in the subcuticular region of cestodes.

The glycogen concentration is progressively increased from the primary sporocyst stage, reaching a maximum level at the time of cercarial release (Axmann, 1947). An interesting scheme has been proposed in regard to glycogen deposits in various larval stages of a trematode (Cheng, 1963a, b; Cheng and Snyder, 1962, 1963). In studies of larval stages within the snail hepatopancreas it was found that no PAS-positive granules were present in daughter sporocysts or unemerged cercaria in areas where the host glycogen was still high. As the larval trematodes developed the amount of glycogen they contained increased, being deposited in the body, but not in the tail. This was concurrent with a glycogen depletion within the cells of the hepatopancreas. This would indicate that the parasite is able to utilize the host glycogen, but probably does so by some method other than direct absorption of the large glycogen molecules through both the sporocyst and cercarial walls.

The fact that no PAS-positive material was found along the inner surfaces of the wall of daughter sporocysts eliminated the possibility of active transport of glycogen from the host across the cercarial wall. Cheng and Snyder (loc. cit.) postulated that a glycogen-digesting enzyme secreted by the parasite breaks down host glycogen to simple sugars which can be absorbed by the sporocyst, which lacks a mouth. Simple sugars can then be resynthesized as glycogen within the body of the cercaria. This was later

substantiated by Cheng and Snyder (1963) when they observed glucose deposits adhering to the outer surface of the sporocysts. They found no such glucose deposits in cercariae, indicating that immediate resynthesis must take place. Since the sporocysts are in direct contact with the host glycogen, it is not necessary to resynthesize it to glycogen for storage.

Burton's work (1962) with radioglucose uptake in the lung-fluke Haematoloechus medioplexus confirmed the theory that glucose can be absorbed through the cuticle of the adult and then resynthesized as glycogen. Electron-dense granules were observed in the parenchyma of the cercariae of A. oregonense (Plate VIII) similar to the beta glycogen granules described by Lumsden (1965) in cestode cuticle. However, the position of these granules didn't correspond to the distribution of glycogen as shown by the PAS reaction, since the granules occurred with equal density in the parenchyma of the body and tail.

Glycogen is a common energy reserve, and especially large amounts are found in parasitic worms (Axmann, 1947). It is interesting to note that an intense PAS reaction was present in the subcuticular region of the body, but absent in the tail of A. oregonense (Plate I, fig. 3). The tail is a temporary structure and doesn't become a part of the adult trematode, detaching instead when the cercaria enters the next host. Therefore, as pointed out

by Cheng (1963a), it doesn't require a stored carbohydrate. Published micrographs of cercariae show that, like A. oregonense, they possess a high concentration of mitochondria in the tail which could supply ATP for energy utilization by the rapidly contracting muscles (Cardell and Philpott, 1960). The fact that miracidia have a high concentration of glycogen may also be related to energy utilization since these free-living ciliated stages are active swimmers (Axmann, 1947).

Bils and Martin (1966) observed PAS-negative bacilliform rod material of the same nature as described by Cardell (1962) from the cystogenous glands. Cystogenous gland material was not seen in this study, but could perhaps occur later in development. In A. oregonense the transformation of the cercaria to the encysted metacercaria does not occur until after metamorphosis of the second intermediate host.

Morphologically speaking, the sucker of A. oregonense seems to represent a modification of the body cuticle previously mentioned (Plates VI and VII). The only published electron micrographs of cercariae with the sucker (Cardell, 1962) show a heavy muscularized structure with a thin layer of cuticle lining the orifice. No such morphology was seen in the sucker of A. oregonense, but the general outline corresponds to previous studies made using the light microscope and supra-vital dyes (Knight and Pratt, 1955;

Burns, 1961a).

It was not possible to verify the presence of the virgula described by Kruidenier (1951). According to him the virgula appears in mature cercariae prior to their emergence from rediae and becomes modified, almost to the point of disappearing after the cercariae emerge. He postulated that the virgula is a reservoir for secretions from paired unicellular glands that arise posterior to the oral sucker. He furthermore suggested that mucin discharged from the virgula supplements the weak oral sucker in attachment, acts as a lubricant during pre- and post-emergent migrations of the cercariae in intermediate hosts, and serves as a protective coating from secretions of their own penetration glands or from the host tissue (Stirewalt and Kruidenier, 1961). The study of A. oregonense offered no support for Kruidenier's statement (loc. cit.) that the oral suckers of virgulate cercariae have tissues in a "comparatively disorganized state."

All histochemical studies previously made on trematode cuticle have been done on the integument of adults (Monné, 1959; Björkman, Thorsell and Lienert, 1963). Monné postulated that the main cuticular layer produces neutral mucopolysaccharides which are converted to acid mucopolysaccharides and secreted onto the surface of the worm. In a study of adult Fasciola hepatica Björkman, Thorsell and Lienert (loc. cit.) showed that the cuticle

was proteinaceous with a mucopolysaccharide or mucoprotein rim. Threadgold (1963) correlated his observations of the ultrastructure of Fasciola hepatica with the histochemical observations of Björkman, Thorsell, and Lienert. He assumed that the electron-dense zone of small vesicles and pinocytotic invaginations corresponded to their external mucoprotein or mucopolysaccharide rim, and that the relatively large vacuoles elsewhere in the cuticle represented non-glycogen polysaccharides.

Many mucopolysaccharides and mucoids show a high resistance to digestion by proteolytic enzymes (Meyer, 1955), and a parasitic helminth possessing a cuticle impregnated with these substances would be protected from digestion by the host. The acid mucopolysaccharide lining of the vertebrate intestine may serve to protect it from harmful enzymes secreted by intestinal parasites.

Acid mucopolysaccharides in the cercaria of A. oregonense are found in the sucker and body cuticle, but not in the tail (Plate I, fig. 1 and 2). Recalling the biology of this animal, the body is the only portion of the cercaria that continues to develop in the second intermediate host as the metacercaria, while the tail is lost. Thus the tail of the cercaria, after it emerges from the redia, is exposed only to the enzymes produced by the snail hepatopancreas and only for a brief period, while the body is

also exposed to the enzymes produced by the second intermediate host until a cyst wall is formed.

SUMMARY

The integument of the cercaria of Acanthatrium oregonense is protoplasmic and has a definite ultrastructure which varies in different regions of the body. The external layer of the cuticle is syncytial and without nuclei, but contains mitochondria and numerous vesicles. It is bounded by an outer double surface membrane and an inner basement membrane. The internal region of the integument is cellular, contains mitochondria and nuclei, and connects to the external layer by protoplasmic extensions between the intervening layers of muscle.

The vesicles of the external layer of integument are of three types as determined by their contents. These vesicles are present in the sucker and body cuticle, but are absent in the tail cuticle. Two of the three types of vesicles contain fibrillar material, while the third is characterized by a homogeneous electron-dense substance. Acid mucopolysaccharides are present in the sucker and body cuticle, and glycogen occurs in the subcuticular layer of the body.

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APPENDIX

Plate I

Fig. 1. Cercaria stained with Richardson's stain showing metachromatic reaction in sucker and body cuticle. 560X

Fig. 2. Cercaria stained with Alcian blue. Note positive reaction in the sucker and body cuticle. 560X

Fig. 3. Cercaria stained with PAS. Dense staining occurs in the subcuticular layer. Staining in the cuticle is less intense. 560X

Fig. 4. Enlargement of fig. 3. 1,250X

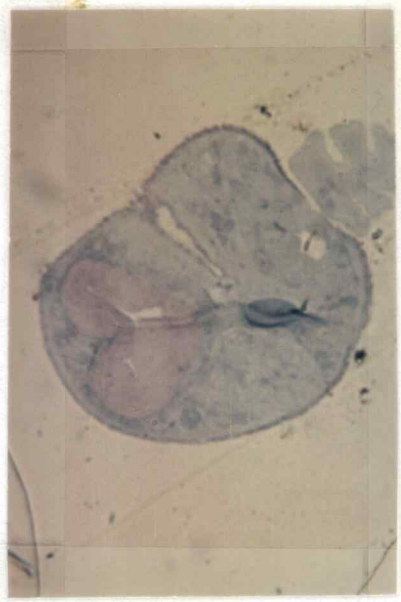


Fig. 1

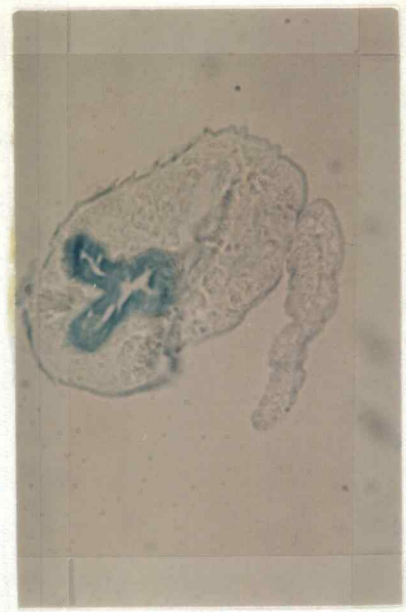


Fig. 2

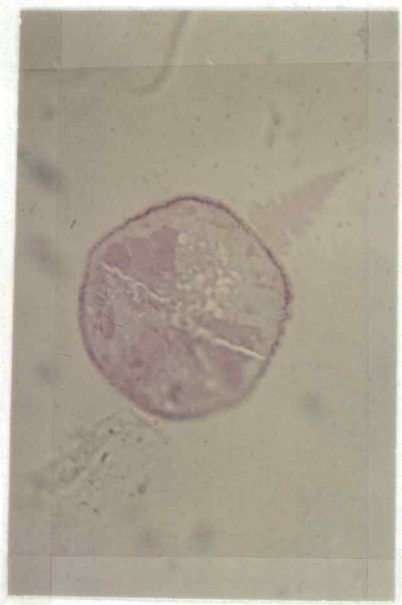


Fig. 3

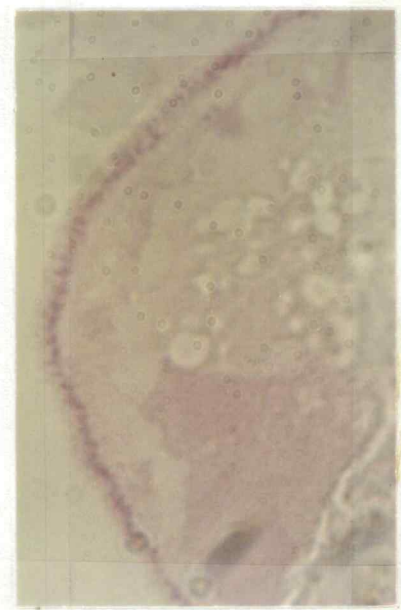


Fig. 4

Plate II

Low power electron micrograph showing attachment of tail to posterior portion of the body. The tail is characterized by numerous deep folds. Note that the tail is attached to the body by three thin masses of tissue. Parenchymal cell nuclei (N) can be seen below the cuticle. Tissue was fixed in 3% glutaraladehyde and post-fixed in 1% osmium tetroxide at pH 7.4. The stain was uranyl acetate followed by lead citrate. (4,600X)

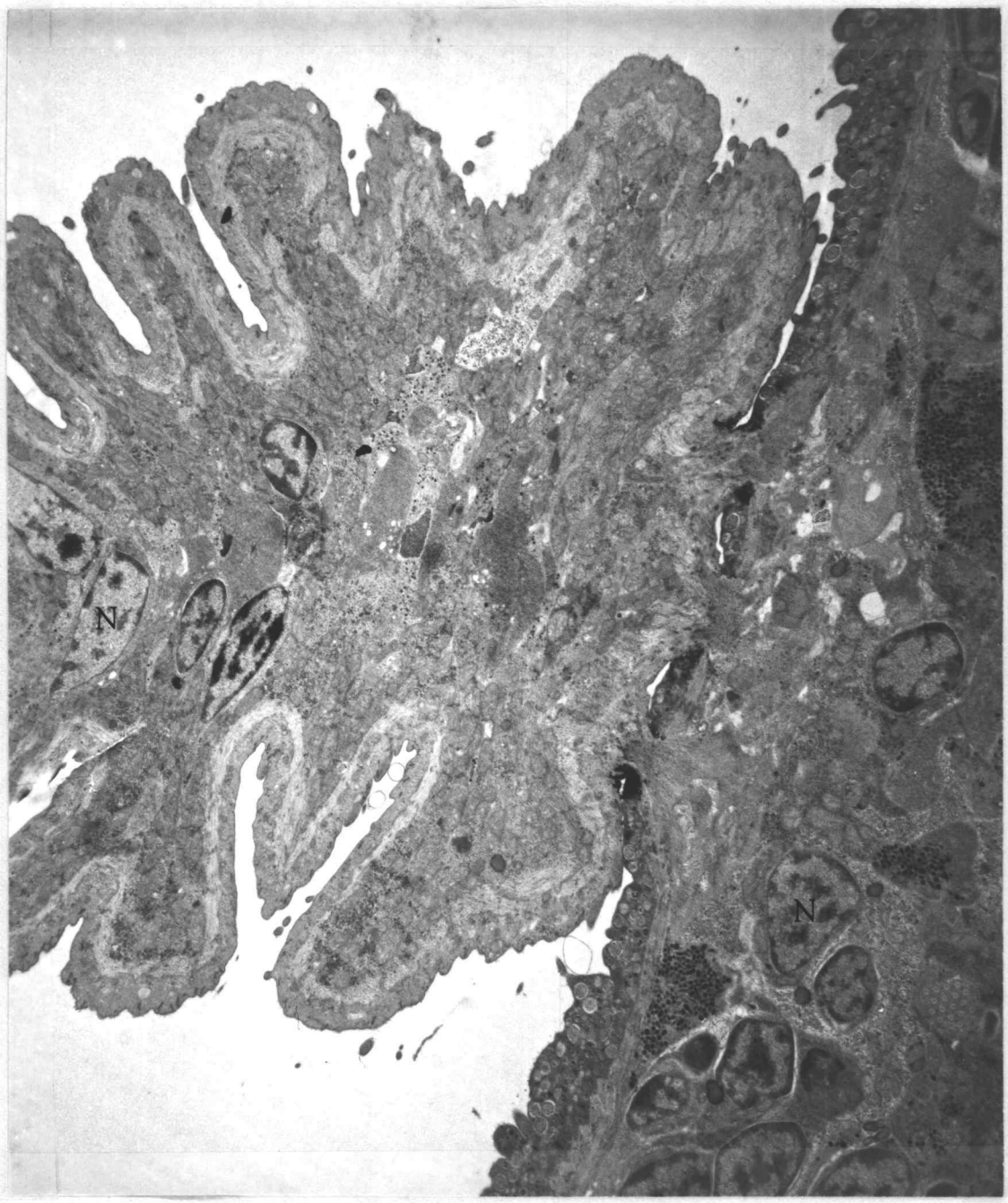

N
N

Plate III

An enlargement of the transition zone between body and tail cuticle. The cuticle of the body (Cb) is highly vesicular whereas the cuticle of the tail (Ct) is rather homogeneous. The deep groove located at the junction of tail and body shows an accumulation of electron-dense material. Muscle layers (MU) can be seen underlying the cuticle in both regions. Numerous mitochondria (M) are visible in the tail. (31,800X)

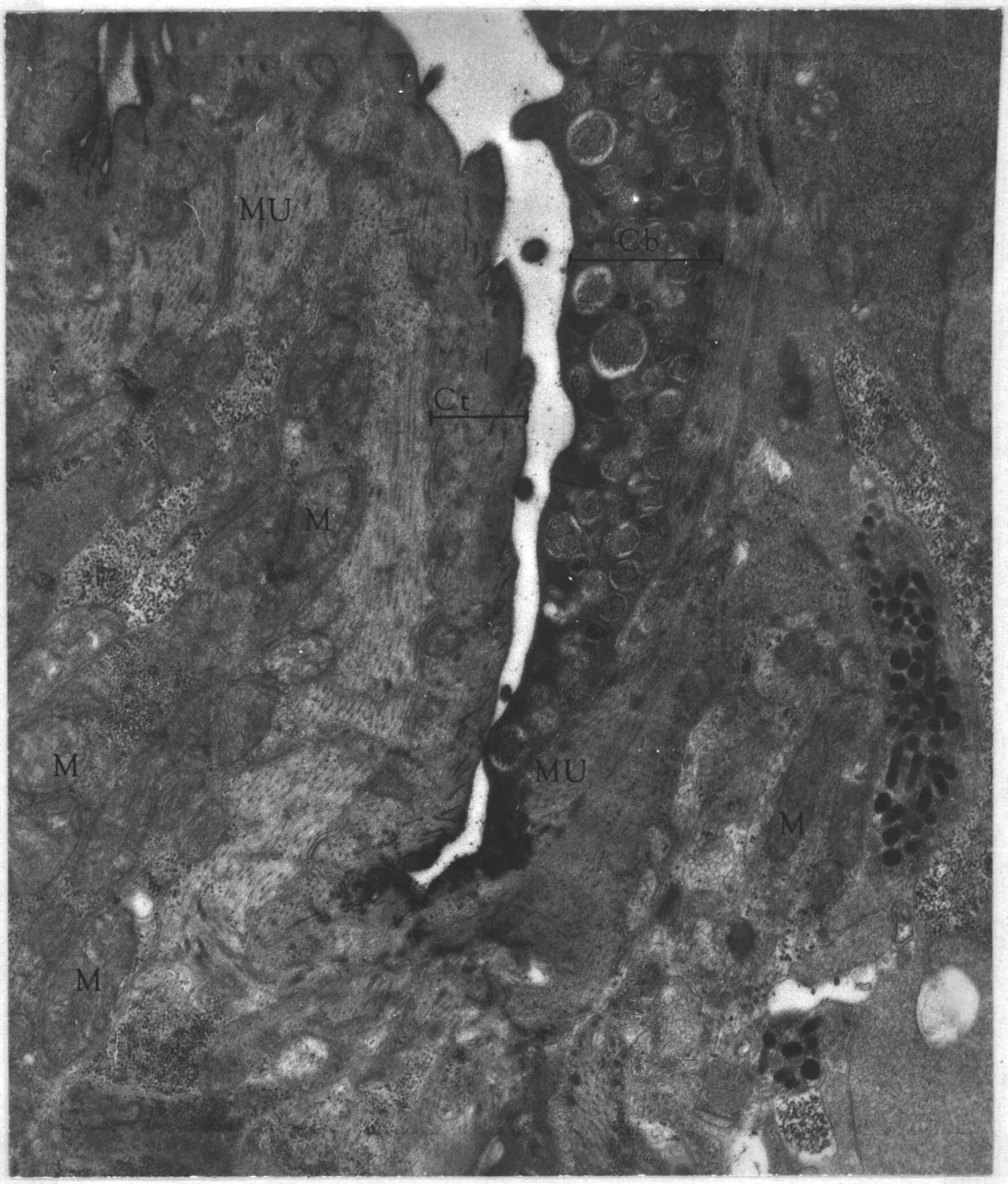

MU
Cb
Ct
M
M
MU
M
M

Plate IV

The body cuticle is composed of numerous vesicles of different types. Note the spines (S) projecting through the cuticular surface. A muscle layer (MU) may be recognized below the cuticle. Mitochondria (M) are visible interior to the muscle layer.
(34,500X)

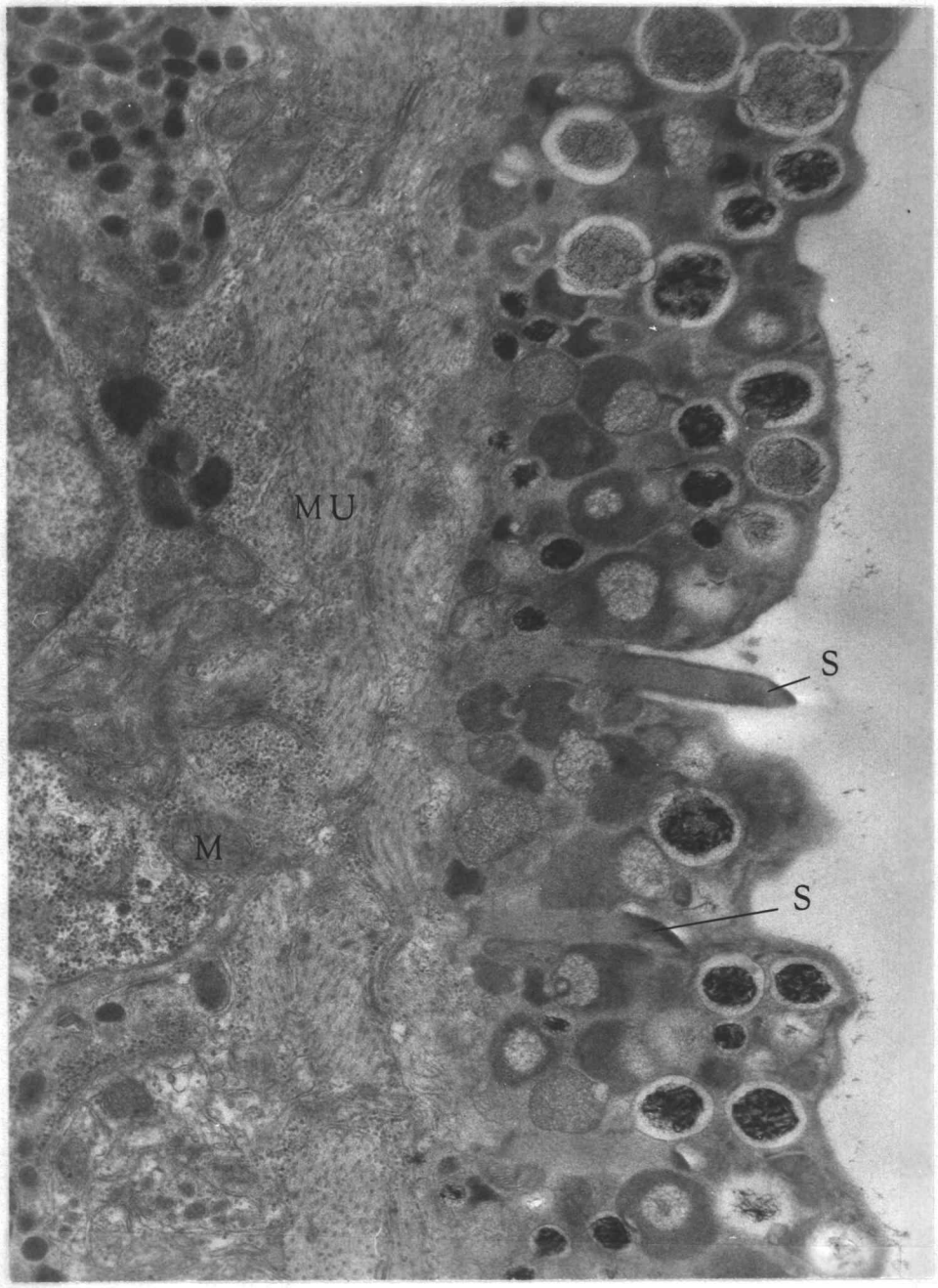

MU
S
M
S

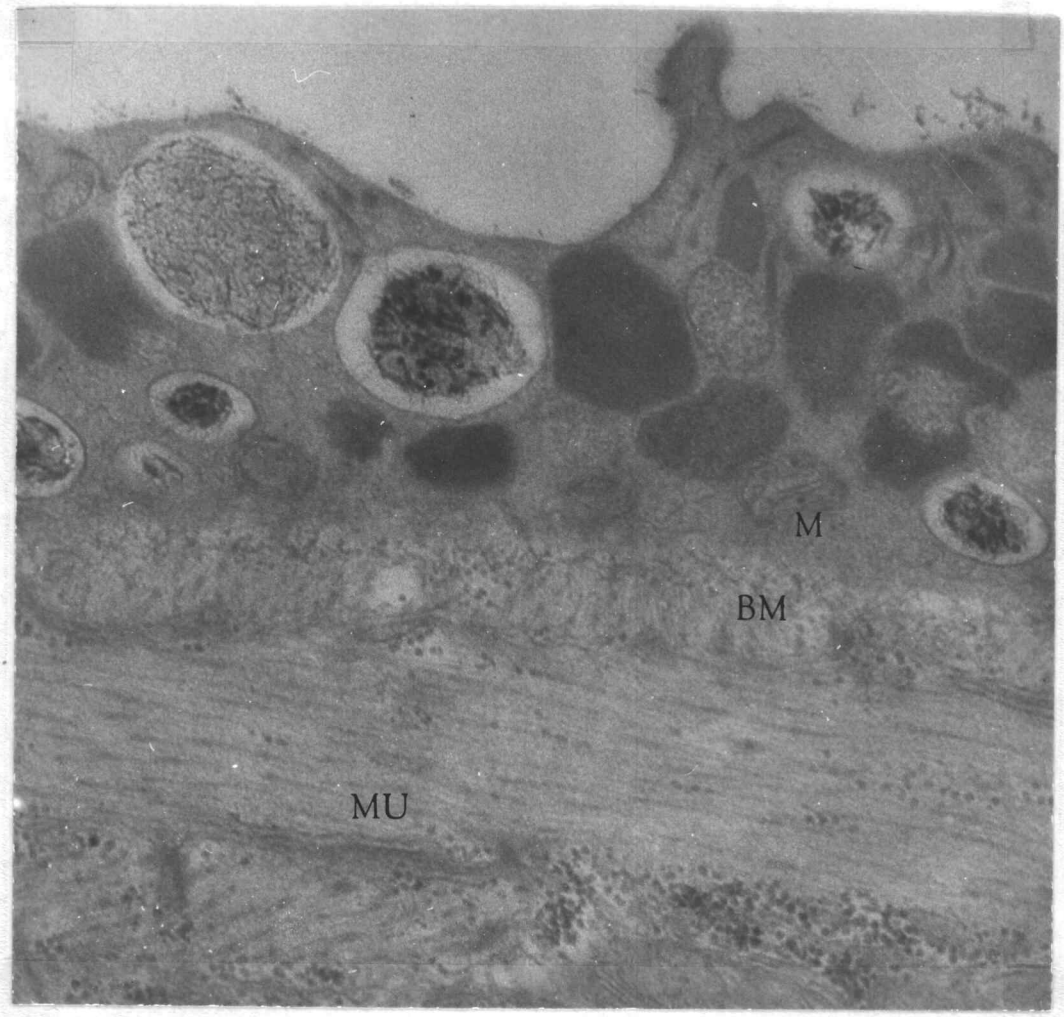
Plate V

Top. High power electron micrograph showing the three layers of the body cuticle:

1) a thin outer surface membrane
2) a thick cytoplasmic layer with mitochondria (M) and three distinct types of vesicles
3) an inner basement membrane (BM)

Note the layer of muscle (MU) beneath the basement membrane. (61,600X)

Bottom. Surface of body cuticle is highly irregular. Vesicles appear to be coalescing (arrow). Two distinct layers of muscle (MU) are seen below a basement membrane (BM). (31,000X)


M
BM
MU

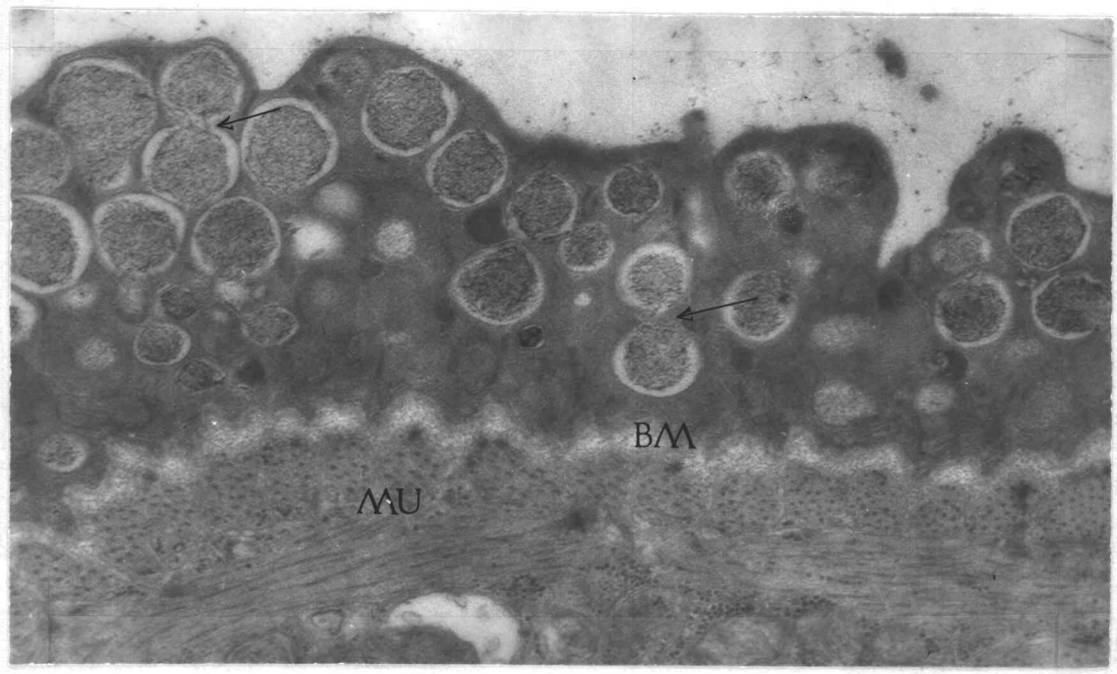

BM
MU

Plate VI

Low magnification micrograph of the anterior region of the body showing a portion of the large bilobed oral sucker which is densely packed with vesicles. A few of these vesicles (arrow) are seen within the buccal cavity. The medial infolding of the sucker indicates the region of the stylet. (5,900X)

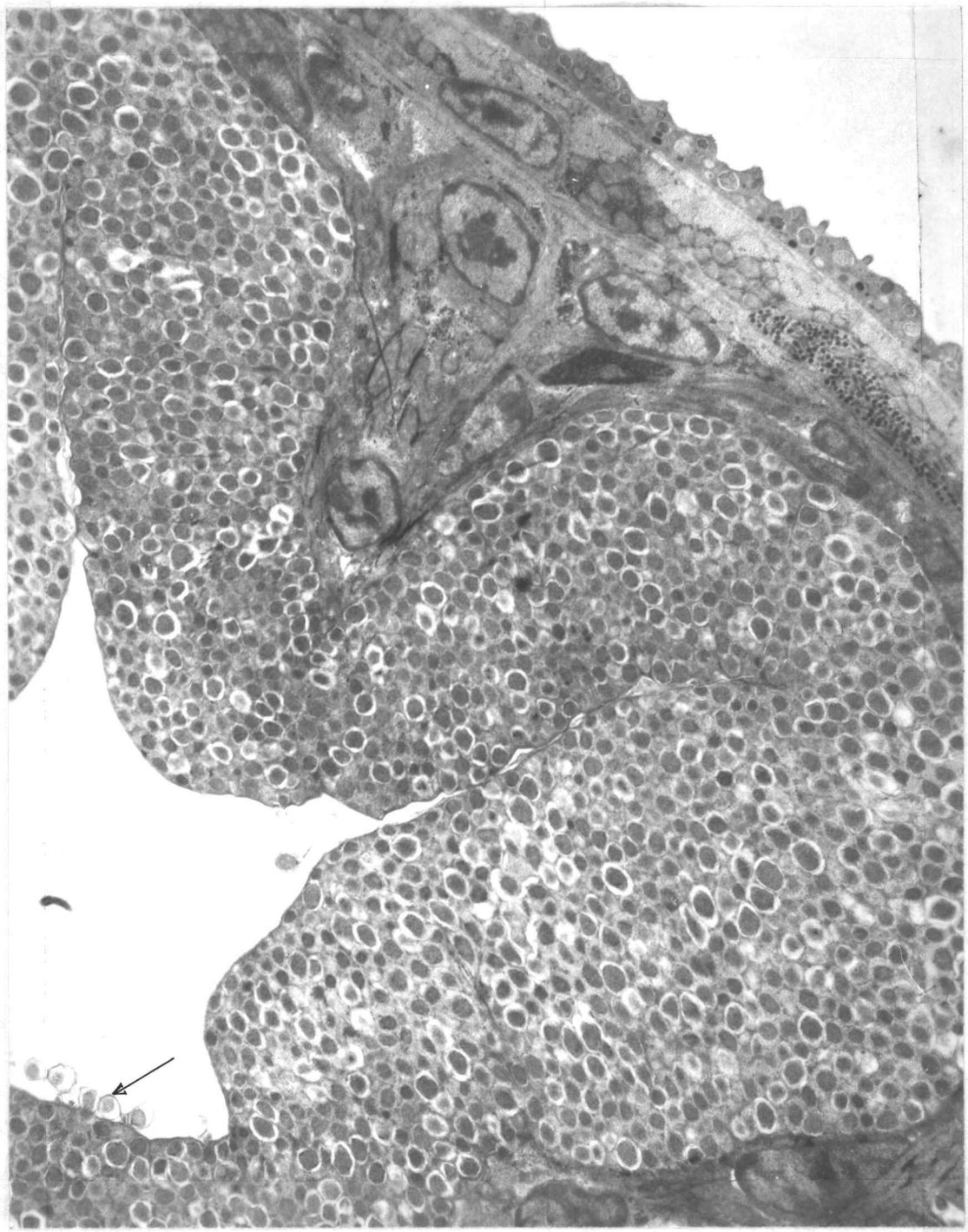


Plate VII

The fibrillar nature of the vesicles is shown in this high power micrograph of the sucker. (61,000X)

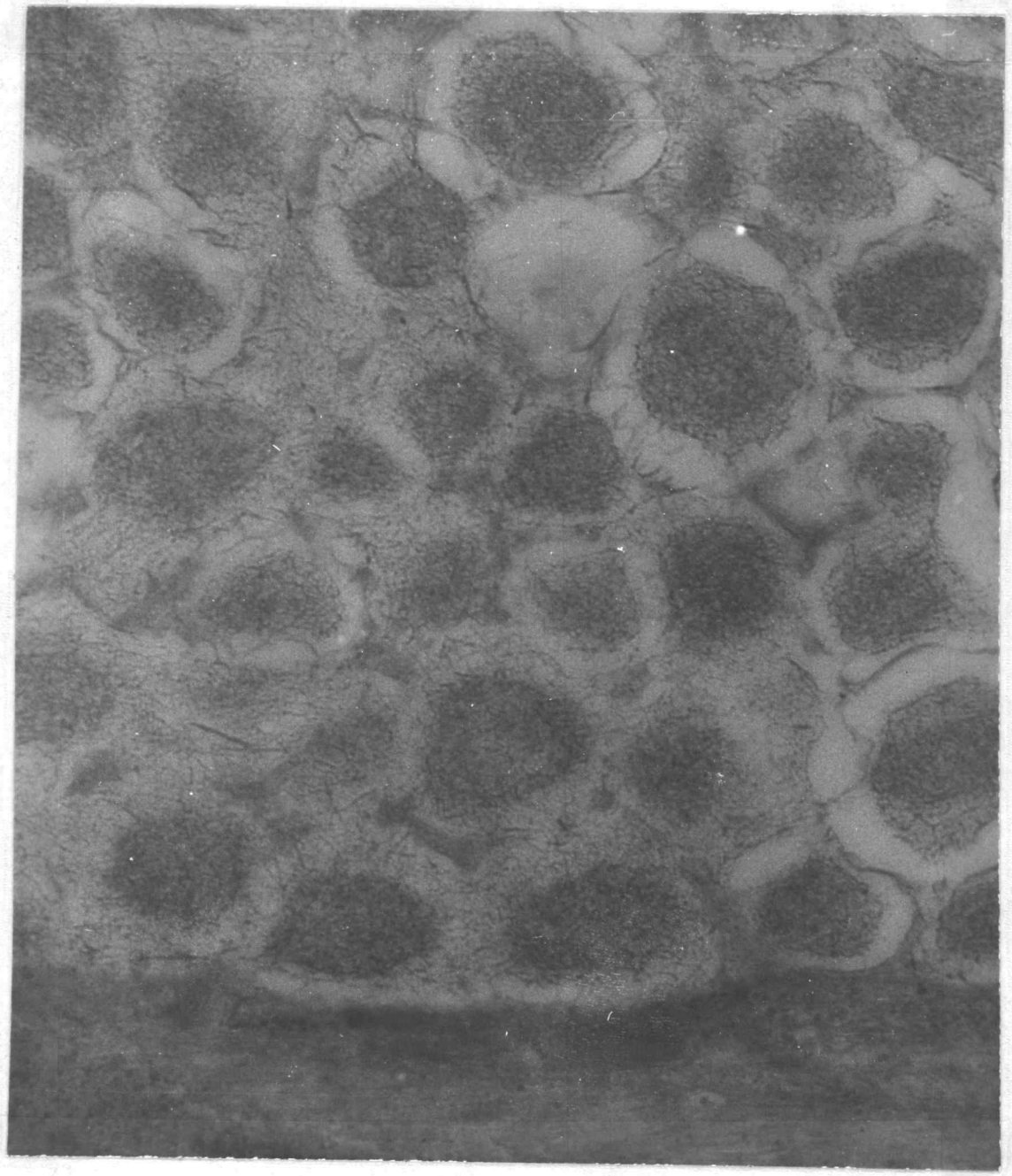


Plate VIII

A large fold in the tail at high magnification showing the homogeneous nature of the cuticle. Note cavities at outer edge of cuticle (arrow). Mitochondria (M) and unidentified electron-dense granules (G) are packed tightly into area beneath muscle layers (MU). (38,500X)

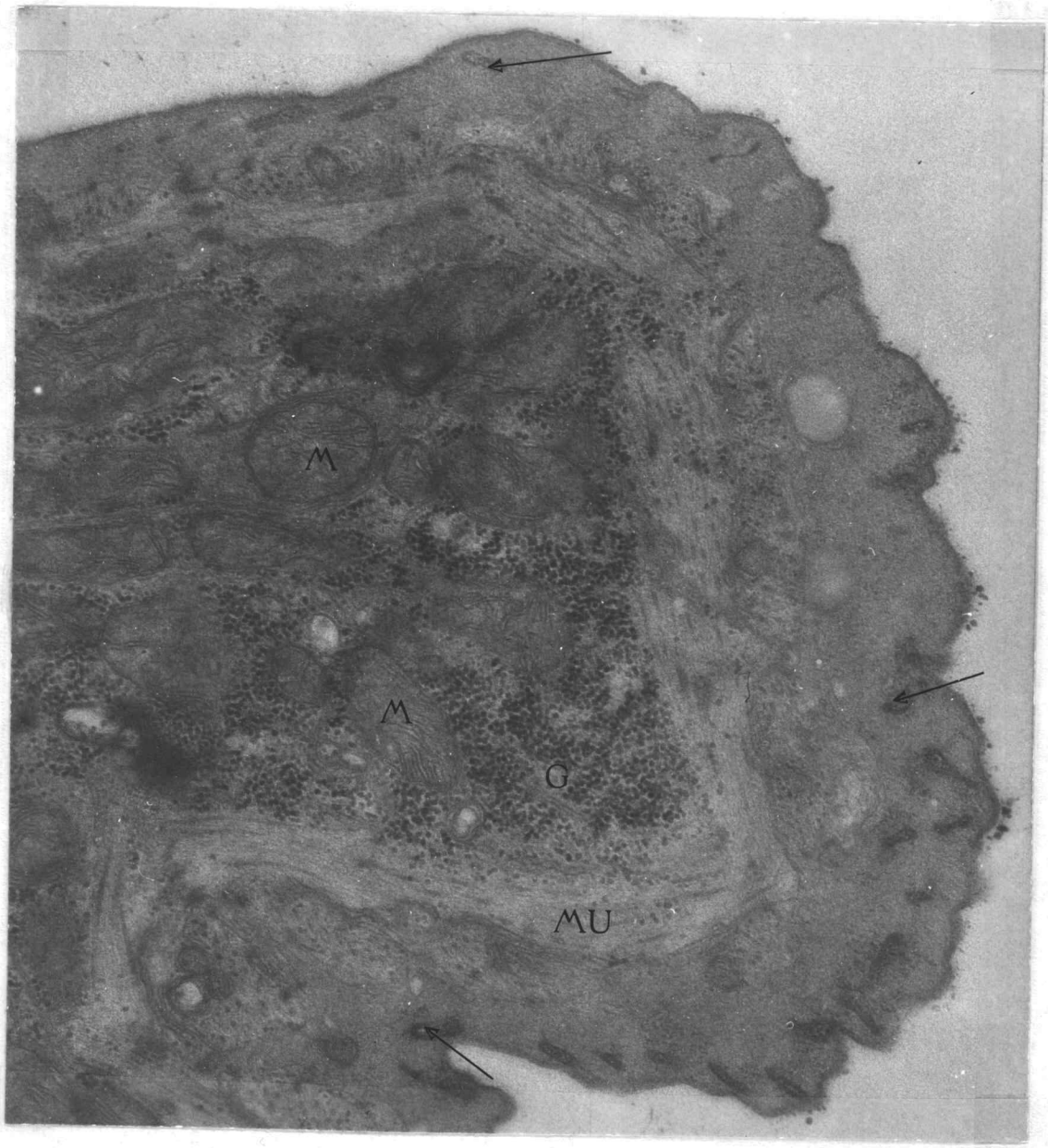

M
M
G
MU

Plate IX

The distal portion of the tail is characterized by numerous projections of the cuticle. A double membrane may be observed on the outer surface of the cuticle. Many elongated cavities (arrow) are shown within the homogeneous matrix of the cuticle. (42,800X)