8-1981

Ultrastructure of the Tegument of Intramolluscan Stages of Proterometra Edneyi Nomen Nudum

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1981

ULTRASTRUCTURE OF THE TEGUMENT OF INTRAMOLLUSCAN STAGES OF *PROTEROMETRA EDNEYI NOMEN NUDUM*

A Thesis

Presented to

the Faculty of the Department of Biology

Western Kentucky University

Bowling Green, Kentucky

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

by

Robin A. Munsey

August, 1981

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ULTRASTRUCTURE OF THE TEGUMENT OF INTRAMOLLUSCAN STAGES OF PROTEROMETRA EDNEYI NOMEN NUDUM

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ACKNOWLEDGEMENTS

I would like to express my sincere appreciation to the members of my committee, Dr. G.E. Dillard, Dr. E.J. Hoffman and Mr. J.R. McCurry, for their help and guidance throughout the course of this study. Particular thanks go to J.R. McCurry for his professional assistance with the electron microscopy and photography. I wish to express my sincerest gratitude to Dr. L.N. Gleason, Chairman of the Advisory Committee, for his assistance, direction, patience and especially for his encouragement and understanding.

Special thanks go to Mr. John Hanson, The Ohio State University, for his help with the scanning electron microscope, and to Dr. G.L. Uglem, University of Kentucky, for help in obtaining the material.

My very special thanks to David Hull for his photographic work and encouragement. And last but not least, thanks go to my family for their love, guidance and moral support.

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ULTRASTRUCTURE OF THE TEGUMENT OF INTRAMOLLUSCAN STAGES OF *PROTEROMETRA EDNEYI NOMEN NUDUM*

Robin A. Munsey August 1981 48 pages

Directed by: L.N. Gleason, G.E. Dillard, E.J. Hoffman and J.R. McCurry

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Larval stages of *Proterometra edneyi nomen nudum* were obtained from *Goniobasis laqueata* and prepared for transmission and scanning electron microscopy. The tegument of rediae, cercariae within rediae and mature cercariae were observed to see if any differences occurred in relation to environmental and functional factors. The tegument was found to be similar in organization to that previously reported for other trematodes. Rediae were found to have patterned, folded teguments with many small microvilli indicative of the function of this surface to absorb nutrients. Microvilli were also present on the tegument of the cercarial body within the rediae along with infoldings for increased surface area. Tegument of the mature cercarial body was smooth, reflecting the change in nutrient acquisition at maturity. Papillae, probably sensory in function, were present on the anterior portion

of the cercarial tail. As seen with transmission electron microscopy, the tegument in this area seemed more vesicular than in other areas. The tegument of the stem region was composed of many projections which would increase the surface area aiding in absorption of nutrients. This increased surface area could also be involved with respiratory exchange once outside of the snail. The tegument of the furcae was invaginated and the interior appeared more muscular, relating to their locomotive function. The tegumental differences observed in P. edneyi appeared to correspond to the environmental and functional states of the larval stages.

INTRODUCTION

In the phylum Platyhelminthes, there are three major parasitic groups (Whitfield 1979): monogenetic trematodes, digenetic trematodes and cestodes. Until the early 1960's, these parasites were referred to as the "armour plated parasites" because the outer covering was believed to be a non-living cuticle. The presence of a cuticle was used to explain how the worms could exist in the digestive tract of the host and be protected against pH changes, digestive enzymes and surfactants, such as bile salts. However, this did not explain how cestodes could obtain nutrients. These parasites have no mouth and have to absorb nutrients via this "impermeable" cuticle.

Threadgold (1963), while studying Fasciola hepatica, observed that the outer covering was actually a living layer of continuous cytoplasm, and he referred to it as a tegumental syncytium. Lee (1966) noted that in all three groups of Platyhelminthes there was a common organizational pattern for the tegumental syncytium. A single, unbroken plasma membrane (except for openings for sensory endings, mouth, genital apertures, excretory openings and gland ducts) rested on a continuous, non-nucleated layer of cytoplasm which was referred to as the distal cytoplasm or

external tegument. This cytoplasmic layer was separated from the muscle layer and the rest of the tissue by an internal plasma membrane and basal lamina. Nucleated portions of the distal cytoplasm were found below the muscle layer in areas referred to as tegumentary bodies, subtegument cells or cytons. These tegumentary bodies were connected to the distal cytoplasm by cytoplasmic connections which penetrated the muscle layers and basal lamina. Bogitsh (1968), Smith et al. (1969), and Wilson and Barnes (1974) noted that the tegumentary bodies were actively secreting substances that were transported through these cytoplasmic connections to the distal cytoplasm. Schmidt and Roberts (1977) and Whitfield (1979) stated that all three groups of Platyhelminthes have this basic organization, but there were differences between the groups, between genera, between species and even between areas of the same organism.

The purpose of this research was to determine if any surface or ultrastructural differences were present in the tegument of Proterometra edneyi nomen nudum Aliff 1973 (Trematoda:Azygiidae) in relation to environmental and functional factors.

As described by Aliff (1973), the adult of P. edneyi inhabited the esophagus of the fan-tail darter, Etheostoma flabellare. The larval stages were found around the rectal area of the aquatic snail, Goniobasis laqueata. The rediae,

the cystoforous, furcocercous cercariae within the rediae and the pre-emergence cercariae from the snail were used in this study.

MATERIALS AND METHODS

Rediae and cercariae of *P. edneyi* were obtained from *G. laqueata*. Six hundred snails were collected during the summer of 1980 from the south fork of Elkhorn Creek, Jessamine County, Kentucky. Snails were crushed in a vise and 30 of the 600 snails were found to be infected with *P. edneyi* larval stages. A total of 318 rediae and 239 cercariae were obtained with an average of 15.9 and 11.9 per snail, respectively, and prepared for transmission electron microscopy (TEM) and scanning electron microscopy (SEM).

Transmission Electron Microscopy

Specimens were fixed at 4 C in 5% glutaraldehyde-cacodylate buffer (0.1 M, pH 7.4) for 24 hours and post-fixed in 3% osmium tetroxide-cacodylate buffer (0.1 M, pH 7.4) for one hour. The samples were then dehydrated under vacuum through a graded series of ethanol with each increment being one hour. This sequence was followed by two rinses of 45 minutes in propylene oxide. Samples were then infiltrated with Epon 812 and placed in an oven at 70 C for 48 hours. Specimens were sectioned on a Reichert OM U-2 ultramicrotome with a DuPont diamond knife. Sections were obtained from the mid-region of the

rediae, from the mid-region of the intra-redial cercarial bodies, from the mid-region of the emerged cercarial bodies, between the papillae of the papillated region of the cercarial tail, from the cercarial tail stem region just anterior to the furcae and from the tail furcae. Sections were mounted on 200-mesh copper grids and stained with uranyl acetate for 3 hours and lead citrate for 5-6 minutes. They were then observed using a Zeiss EM 9S2 transmission electron microscope and representative photomicrographs taken.

Scanning Electron Microscopy

Specimens were fixed at 4 C in 5% glutaraldehyde-cacodylate buffer (0.1 M, pH 7.4) for 24 hours. They were dehydrated through a graded series of ethanol with each increment being 15 minutes and critical-point dried in a Samdri-790 Critical Point Dryer. Samples were then mounted on silver painted stubs, sputter coated with gold and examined with an Hitachi S-500 scanning electron microscope.

RESULTS

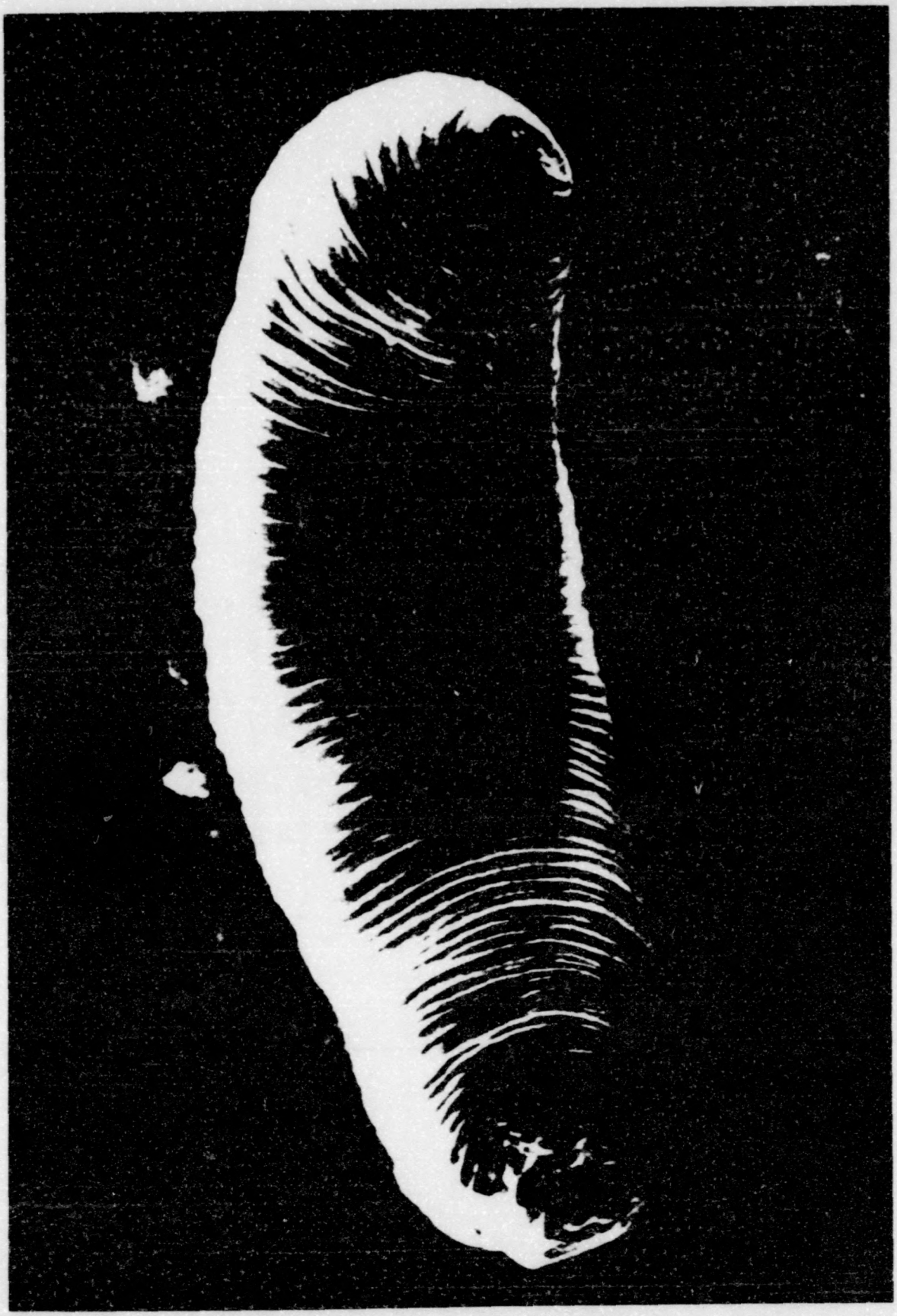
Rediae

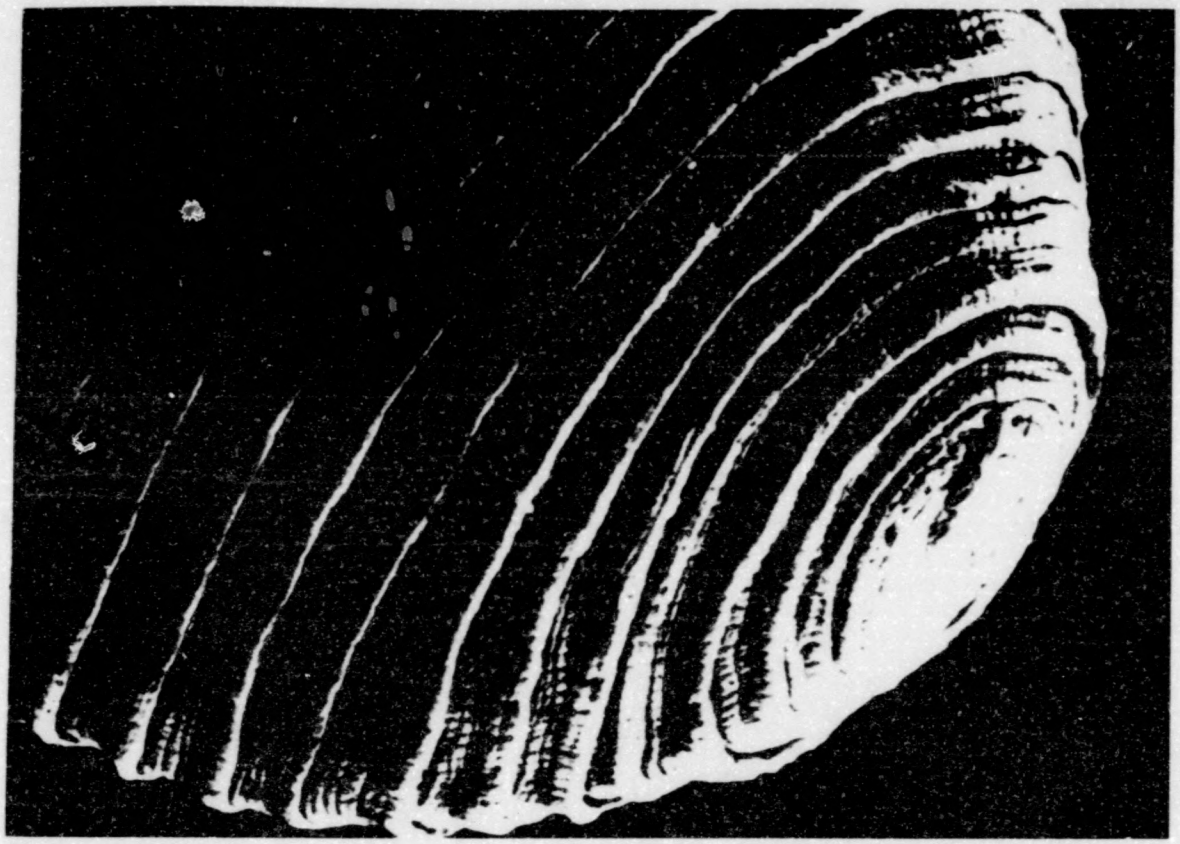
Externally, the tegument appeared as a series of rings around the rediae (Figure 1). The mouth appeared to be formed from infoldings of the tegument and had few papillae (Figure 2). The tegument of the posterior region resembled that of the remainder of the rediae. The rings continued from the region below the mouth to the posterior end, which bore no papillae (Figure 3). The rings were approximately 5 μ in diameter. The area between the rings consisted of numerous folded projections which were about 0.5-1.5 μ in width (Figure 4).

The tegument, when viewed with the TEM, was 0.5-1.0 μ in width and had many microvilli on the surface. The distal cytoplasm appeared vesicular and the basal lamina was conspicuous. No mitochondria or other cellular organelles were observed in the distal cytoplasm (Figures 5 and 6).

The few cytoplasmic connections present appeared to be oriented toward the interior of the rediae (Figures 7 and 8). Some of the cytoplasmic connections contained beta granules (Figure 5) and lipid bodies (Figure 7). Cytons, ranging from 6-10 μ in diameter, were vesicular and contained large nuclei, 2-6 μ in diameter. The entire redial wall was

Figure 1. SEM photomicrograph of the redia showing the tegumental surface as a series of concentric rings. The mouth, at the anterior end, is on the left. X 175.





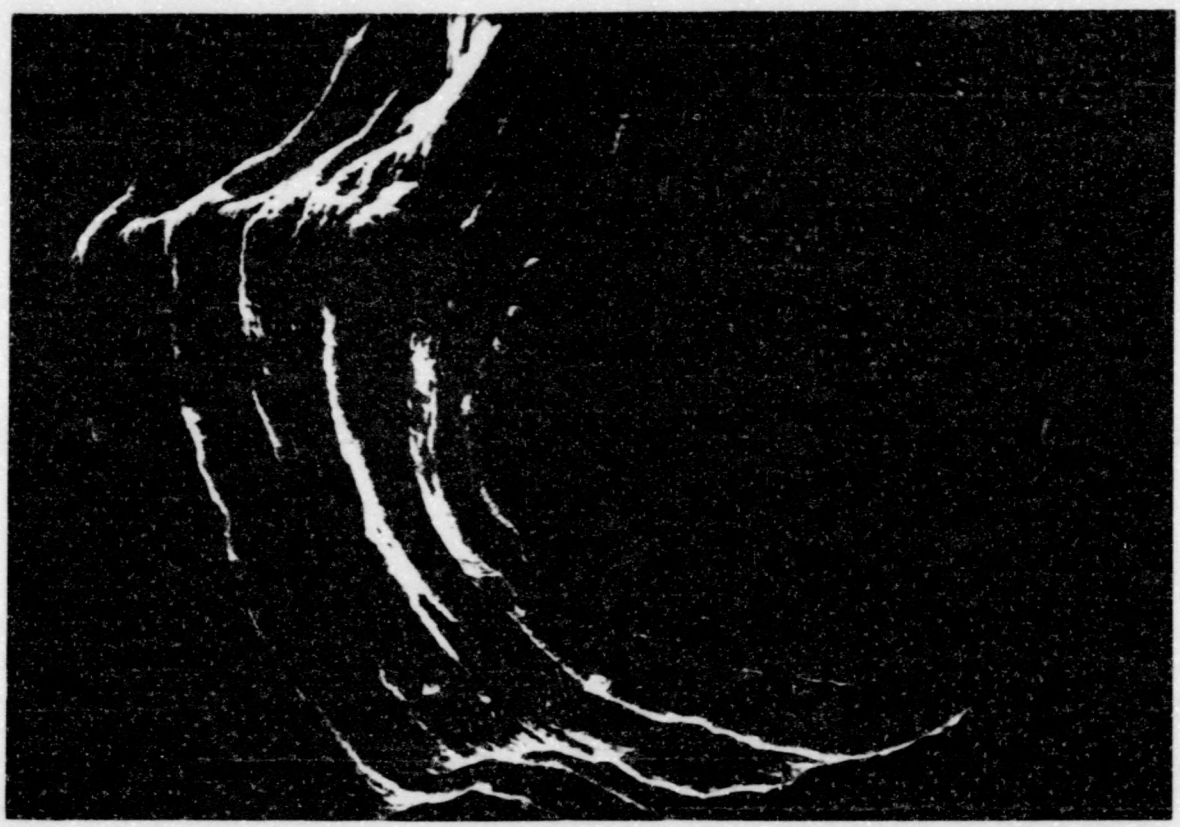


Figure 2. SEM photomicrograph of the redial mouth which is formed from infoldings of the tegument. Few papillae are present in this region. X 1000.

Figure 3. SEM photomicrograph of the posterior end of the redia. Note the continuing series of concentric rings and the lack of papillae. X 2000.

Figure 4. SEM photomicrograph of the redial tegumental surface showing one large concentric ring. Note the numerous folded projections on either side of the rings. X 8250.

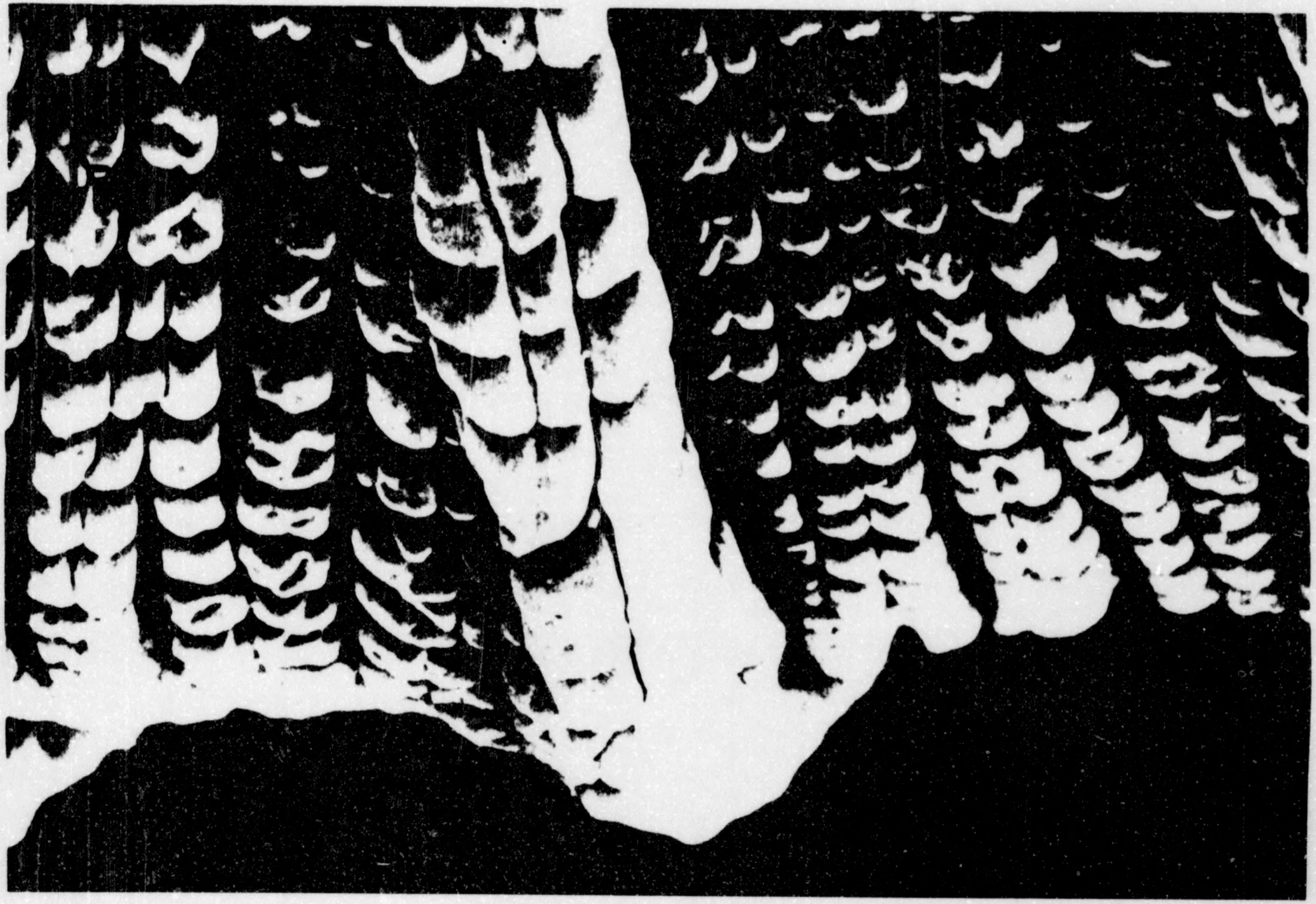
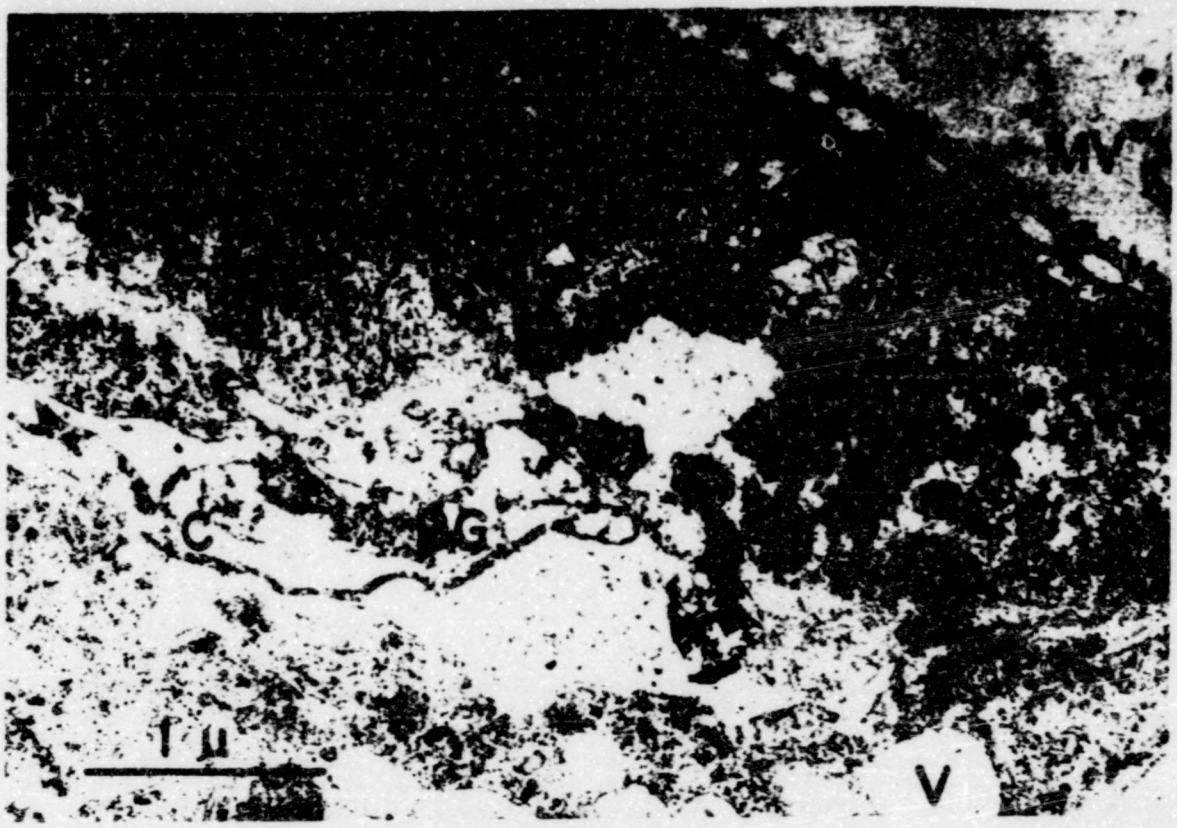


Figure 5. TEM photomicrograph of a cross section of the redia near the surface showing microvilli (MV) on the surface of the tegument (T). Note the cytoplasmic connections (C) containing beta granules (BG). Also present are vesicles (V) and longitudinal muscles (LM). X 29300.

Figure 6. TEM photomicrograph of a cross section of the redia near the surface showing the numerous microvilli (MV). Note the conspicuous basal lamina (BL). Also shown are cytoplasmic connections (C), longitudinal muscles (LM) and vesicles (V). X 24800.


MV
C
G
1 µ
V


MV
V

Figure 7. TEM photomicrograph of a cross section of the redia near the interior showing subtegumentary cells (SC) with nuclei (N). Note that the cytoplasmic connections (C) appear to be oriented toward the intraredial wall (IR). Also present are lipid bodies (LB). X 6800.

Figure 8. TEM photomicrograph of a cross section of the redia near the interior showing subtegumentary cells (SC) with large nuclei (N). Cytoplasmic connections (C) are present and oriented toward the intraredial wall (IR). X 8300.


2 μ


2 μ

18-22 μ in width (Figure 9). A composite drawing of the redial wall, from the tegument to the intra-redial wall, is shown in Figure 10.

Cercariae

The cercaria of P. edneyi consisted of a body and a tail. The tail was divided into three regions: a papillated region, which was the anterior end closest to the body; a stem, posterior to the papillated region; and the furcae or tail forks (Figure 11).

Intra-Redial Cercarial Bodies

The cercarial bodies appeared convoluted in SEM photomicrographs (Figure 12).

External to the primitive epithelium was a surface coat (glycocalyx) as observed in TEM sections. This substance was composed of a diffuse, fibrous-like material (Figures 13 and 14).

A primitive epithelium surrounded the tegument as observed in TEM photomicrographs (Figures 13 and 14). This primitive epithelium had few nuclei and no partitioning membranes. The areas where nuclei were present were thicker than areas without nuclei.

The tegument was 0.5-1.0 μ in width, and numerous microvilli were present on the invaginated surface (Figures 13 and 14). A few nuclei were present in the distal cytoplasm, along with vesicles, dense granules and some mitochondria. The basal lamina was less conspicuous than

Figure 9. TEM photomicrograph of a cross section of the redia. The tegument (T), in the upper right hand corner, and the intra-redial wall (IR) are separated by intercellular spaces, subtegumentary cells (SC) and cytoplasmic connections (C). Also note the lipid bodies (LB) and vesicles (V). X 8800.

LB
V
R
2 μ

Figure 10. A composite drawing of the redia. Note the microvilli (MV), tegument (T), vesicles (V), basal lamina (BL), circular muscles (CM), longitudinal muscles (LM), sub-tegumentary cells (SC), cytoplasmic connections (C), parenchymal cells (P) and intra-redial wall (IR).

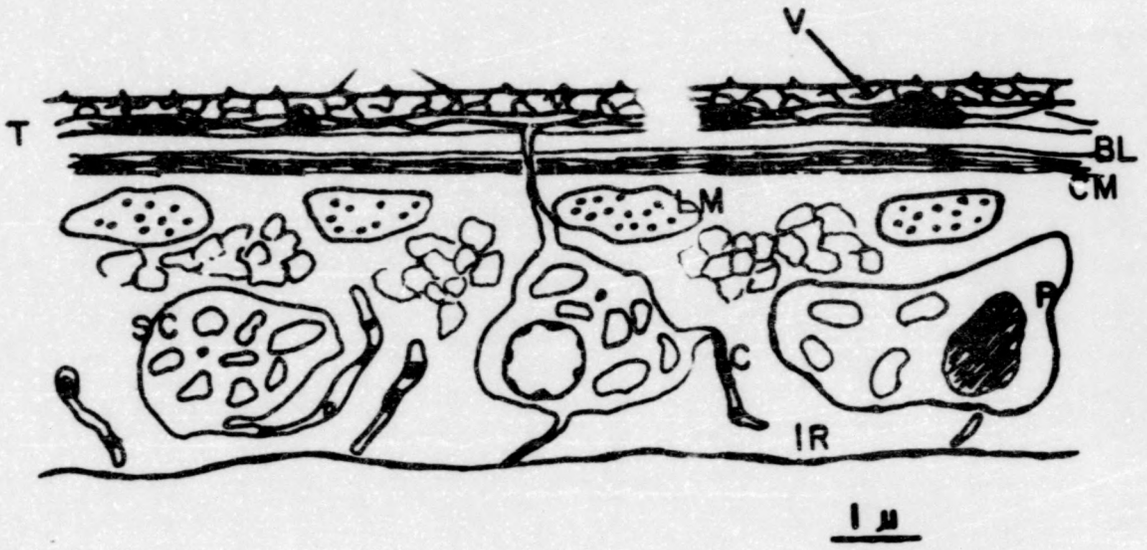

V
T
BL
CM
LM
P
C
IR
1 µ

Figure 11. SEM photomicrograph of the cercaria, the body being at the lower right. The rest of the cercaria consists of the tail which is divided into three regions. The papillated region is connected with the body. The stem region follows with the furcae (forked tail) being at the posterior end. X 175.

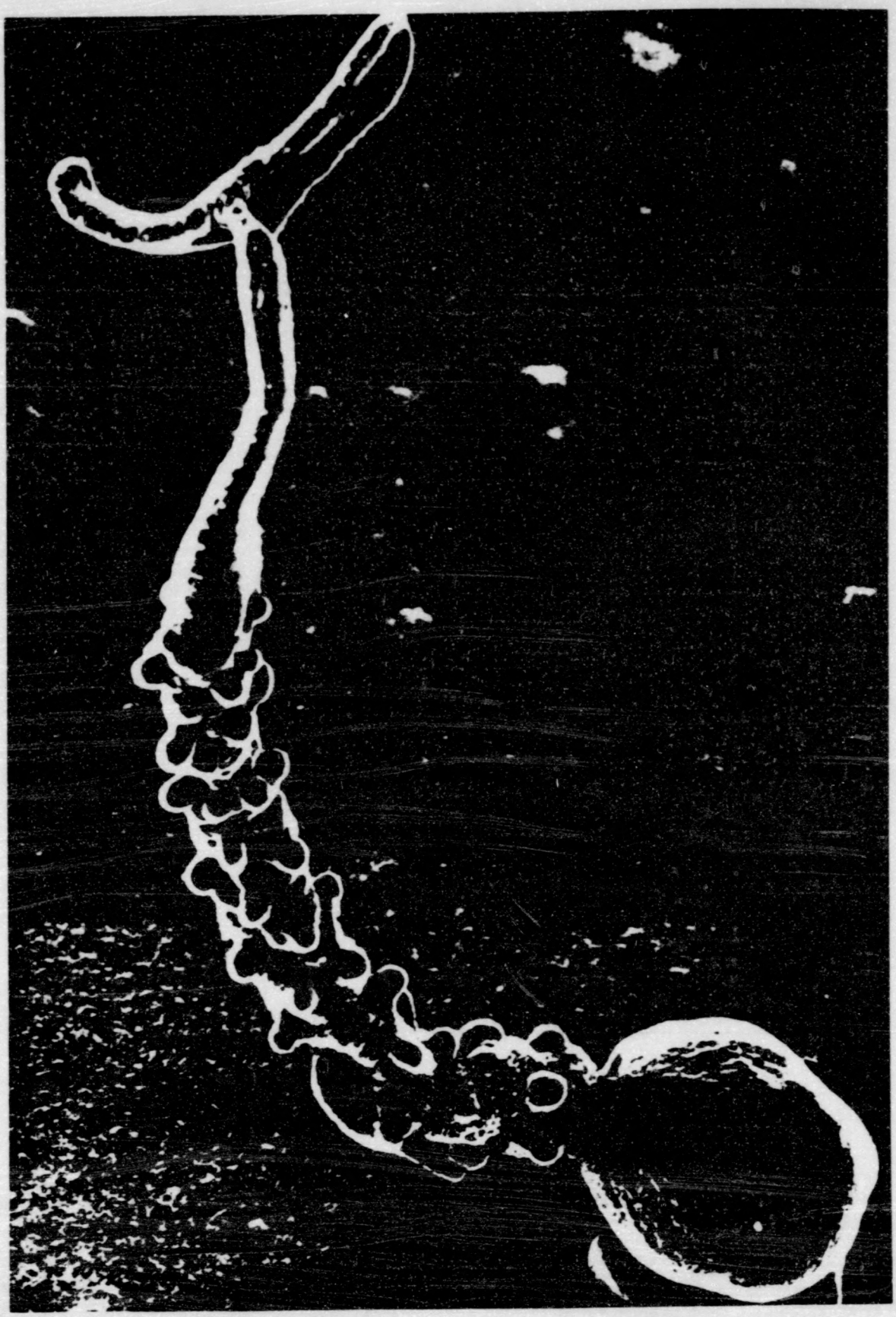


Figure 12. SEM photomicrograph of an immature cercarial body. Note the convoluted tegument of this stage. X 450.

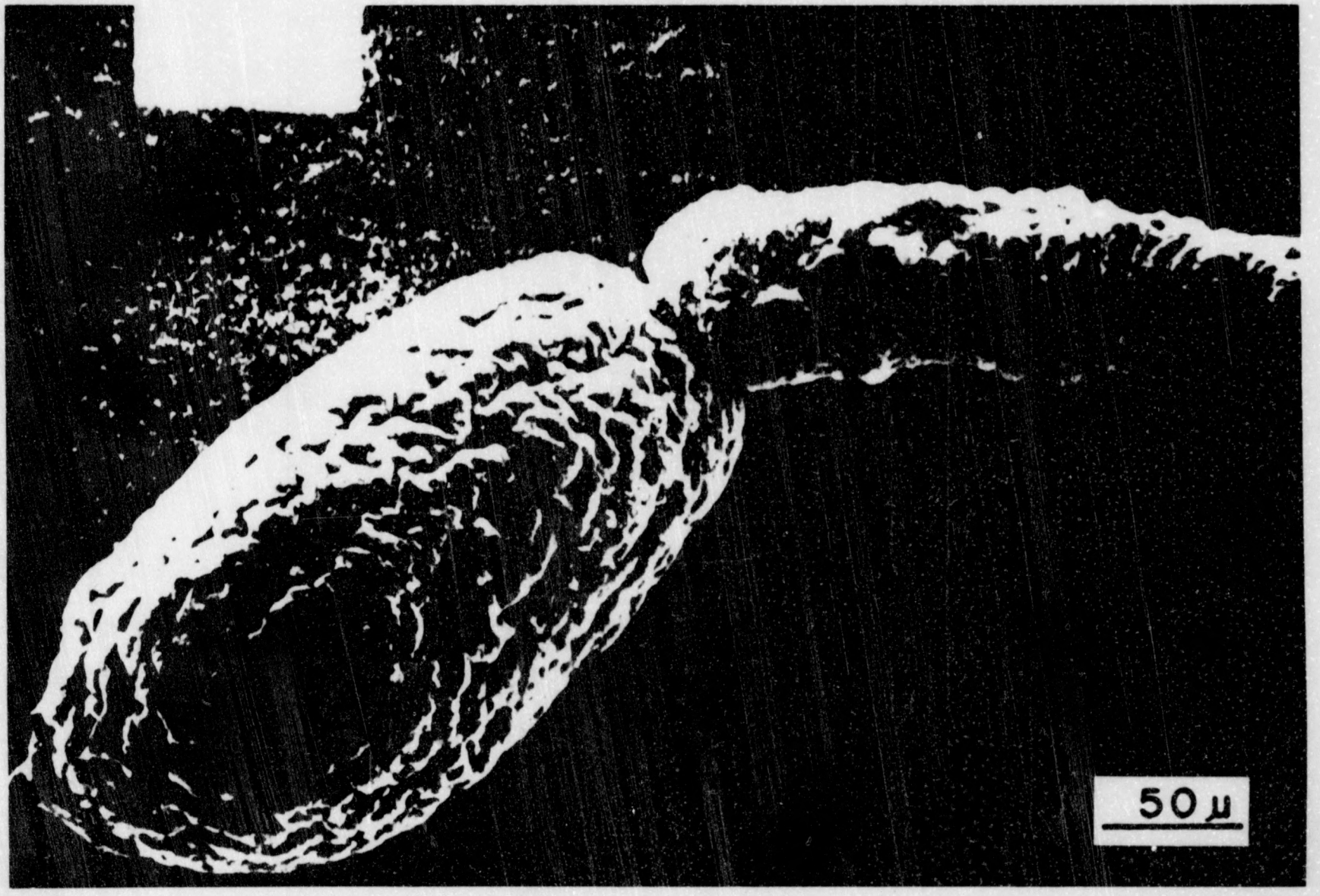
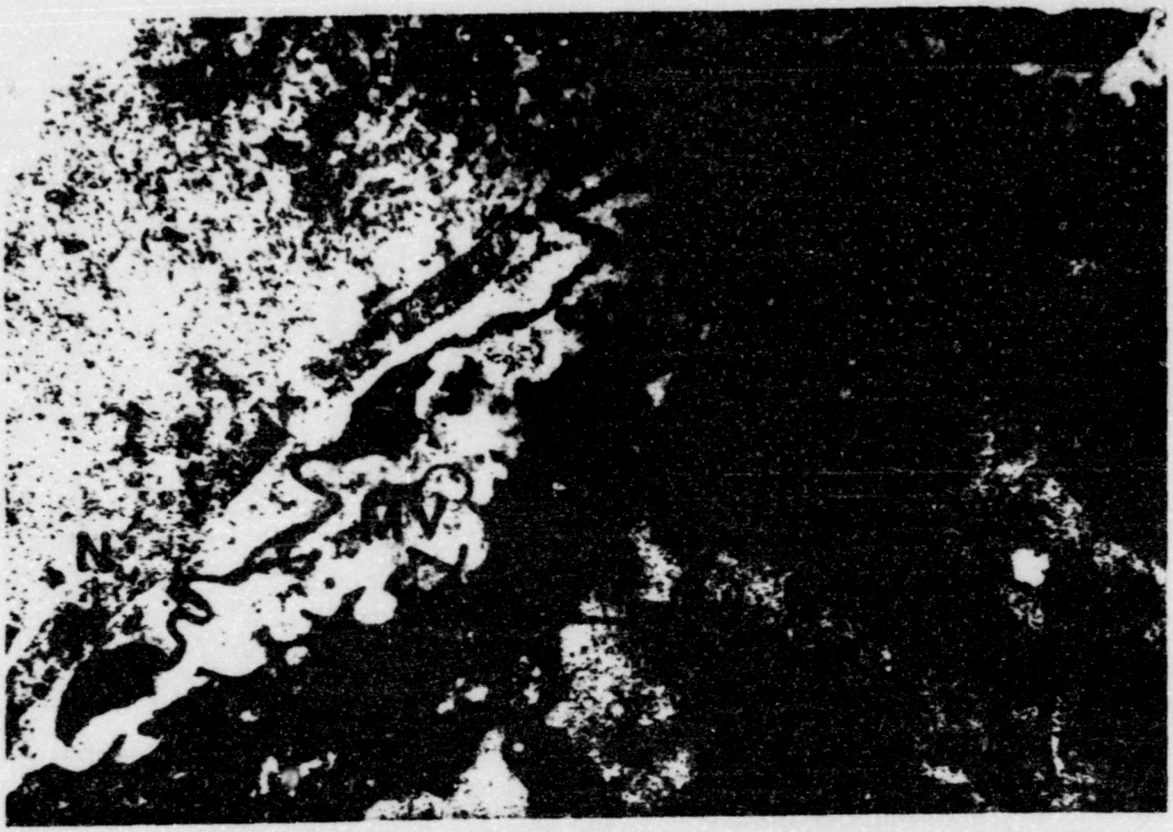
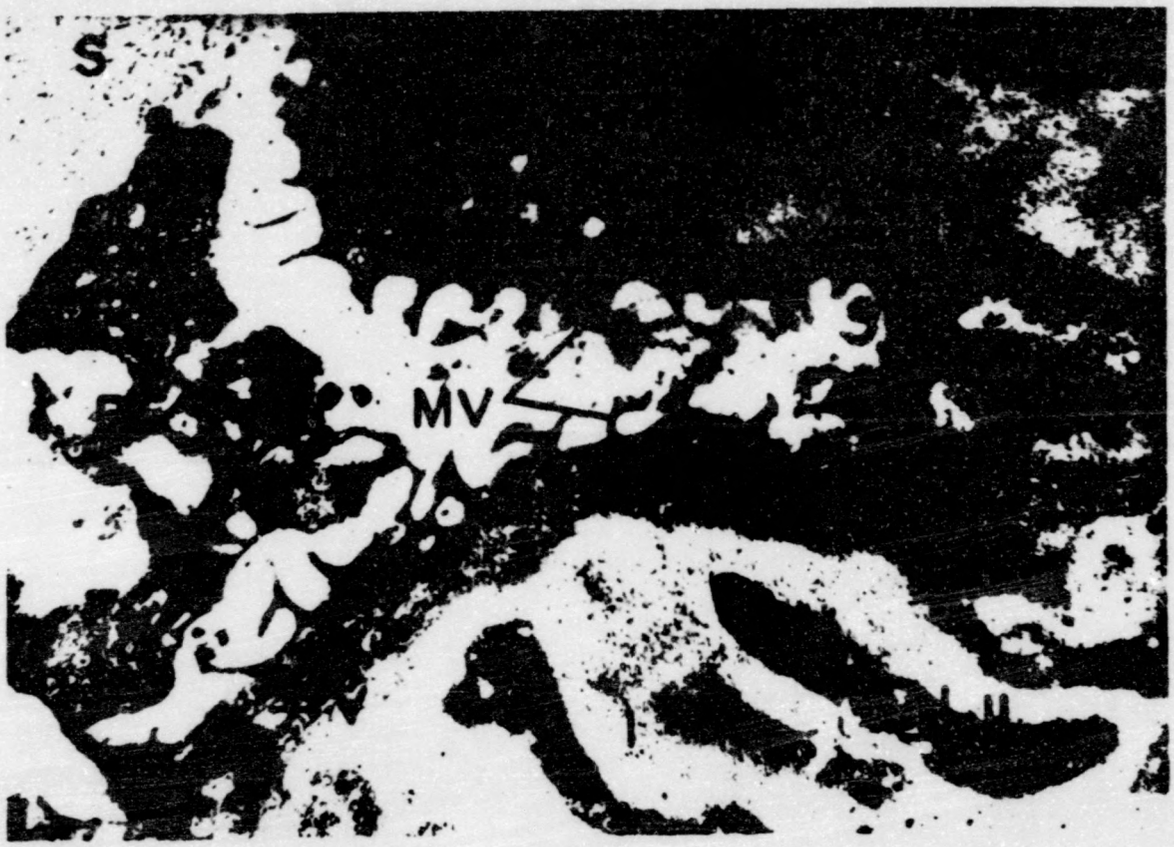

50μ

Figure 13. TEM photomicrograph of a cross section of an intra-redial cercarial body. Note the surface coat (S) and primitive epithelium (PE) with nuclei (N). Also present are numerous microvilli (MV) on the tegumental (T) surface. A nucleus (N) and dense bodies (DB) are present within the tegument and a cytoplasmic connection (C) is seen fusing with the basal lamina (BL). X 24800.

Figure 14. TEM photomicrograph of a cross section of an intra-redial cercarial body. Note the surface coat (S) and primitive epithelium (PE). Also present are numerous microvilli (MV) on the tegumental (T) surface and vesicles (V) within the tegument. A cytoplasmic connection (C) is also noted. X 24800.


N
MV


S
S
MV

that observed in the rediae. Numerous cytoplasmic connections joined with the distal cytoplasm (Figures 13 and 14). Circular and longitudinal muscles, intercellular spaces and cytons were present internal to the basal lamina (Figure 15).

Emerged Cercarial Bodies

As seen in SEM photomicrographs, the body portion of the emerged cercaria (Figure 16) was smoother than when within the redia (Figure 12).

There was no apparent surface coat or primitive epithelium surrounding the tegument of the cercarial body. The tegument was 1.0-2.0 μ in width with fewer microvilli on the surface and no nuclei in the distal cytoplasm. However, in the distal cytoplasm, numerous lipid bodies and mitochondria were present. The basal lamina, as in the developing cercarial body, was not very conspicuous (Figures 17 and 18). Few cytoplasmic connections between the distal cytoplasm and cytons were observed (Figure 19). Composite drawings of teguments of the intra-redial cercarial body and emerged cercarial body are shown in Figures 20 and 21, respectively.

Papillated Region

As observed with the SEM, the tegument of this area had many large papillae with cavity pits scattered over and between them. Tubercles were also present on and around the papillae (Figure 22).

Figure 15. TEM photomicrograph of a cross section of an intra-redial cercarial body. A primitive epithelium (PE) is external to the tegument (T). Also note the intercellular spaces (I), circular muscles (CM), longitudinal muscles (LM) and subtegumentary cells (SC) which are internal to the tegument. X 8300.

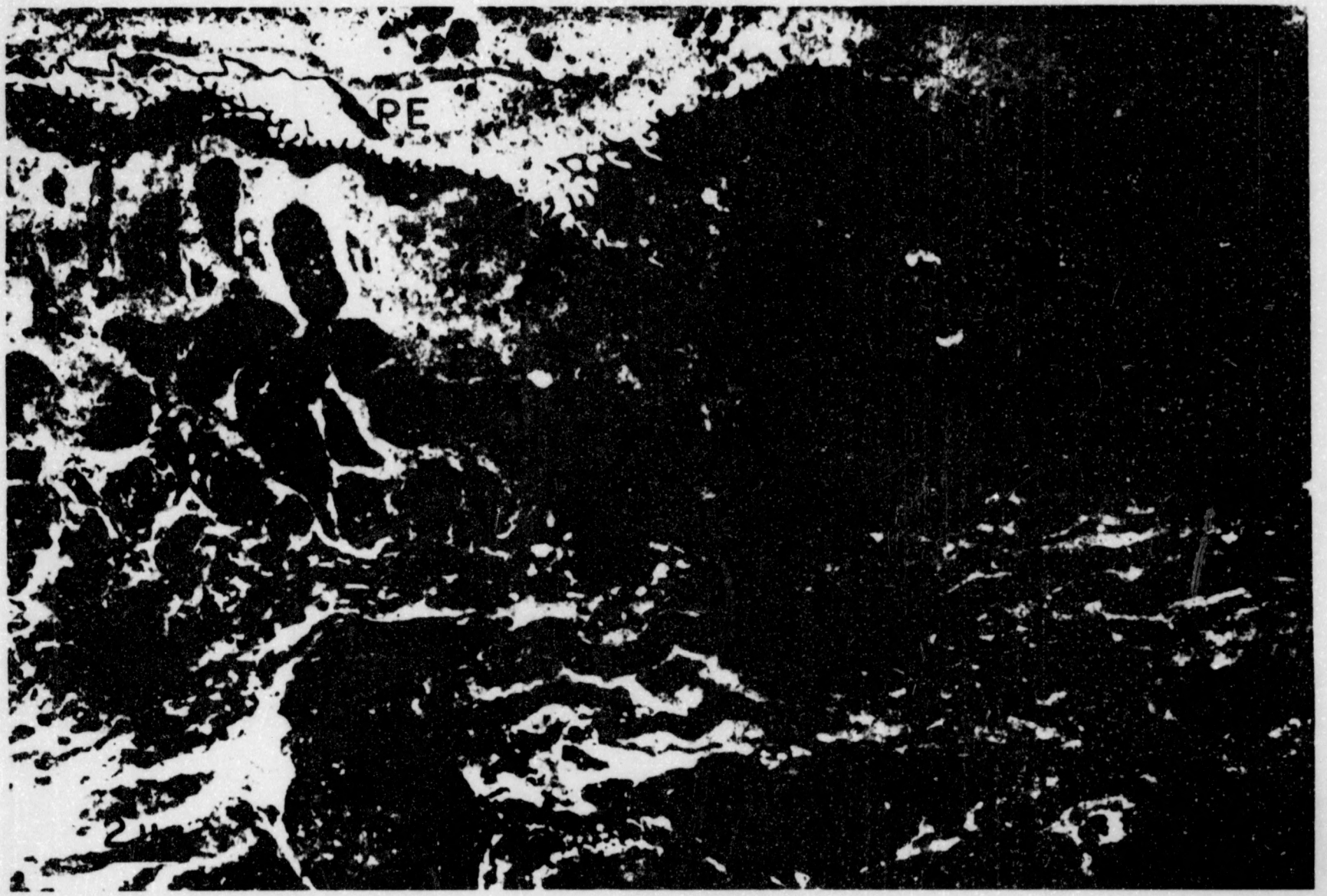

PE

Figure 16. SEM photomicrograph of a mature cercarial body showing a relatively smooth surface. Also note the ventral sucker, located on the left half of the body, which will attach to the esophagus of E. flabellare, and the oral sucker, located on the right half of the body, which will acquire the nutrients. X 550.

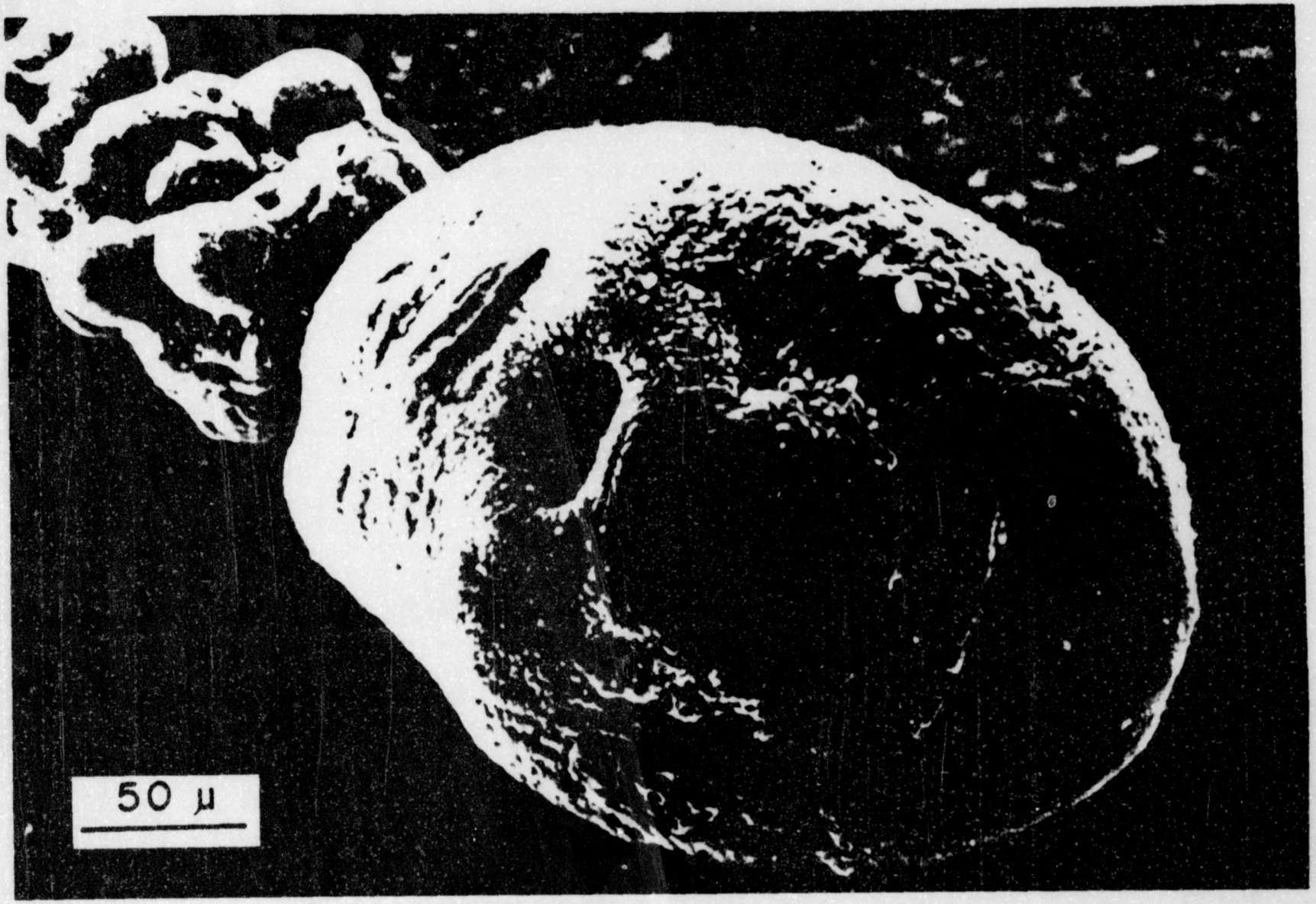

50 µ

Figure 17. TEM photomicrograph of a cross section of a mature cercarial body. Note mitochondria (M) within the tegument (T) and a connecting cytoplasmic connection (C). X 24800.

Figure 18. TEM photomicrograph of a cross section of a mature cercarial body. Note the mitochondria (M) and lipid bodies (LB) present in the tegument with a few scattered cytoplasmic connections (C). Also shown are the internal plasma membrane (IPM) and basal lamina (BL). X 27000.

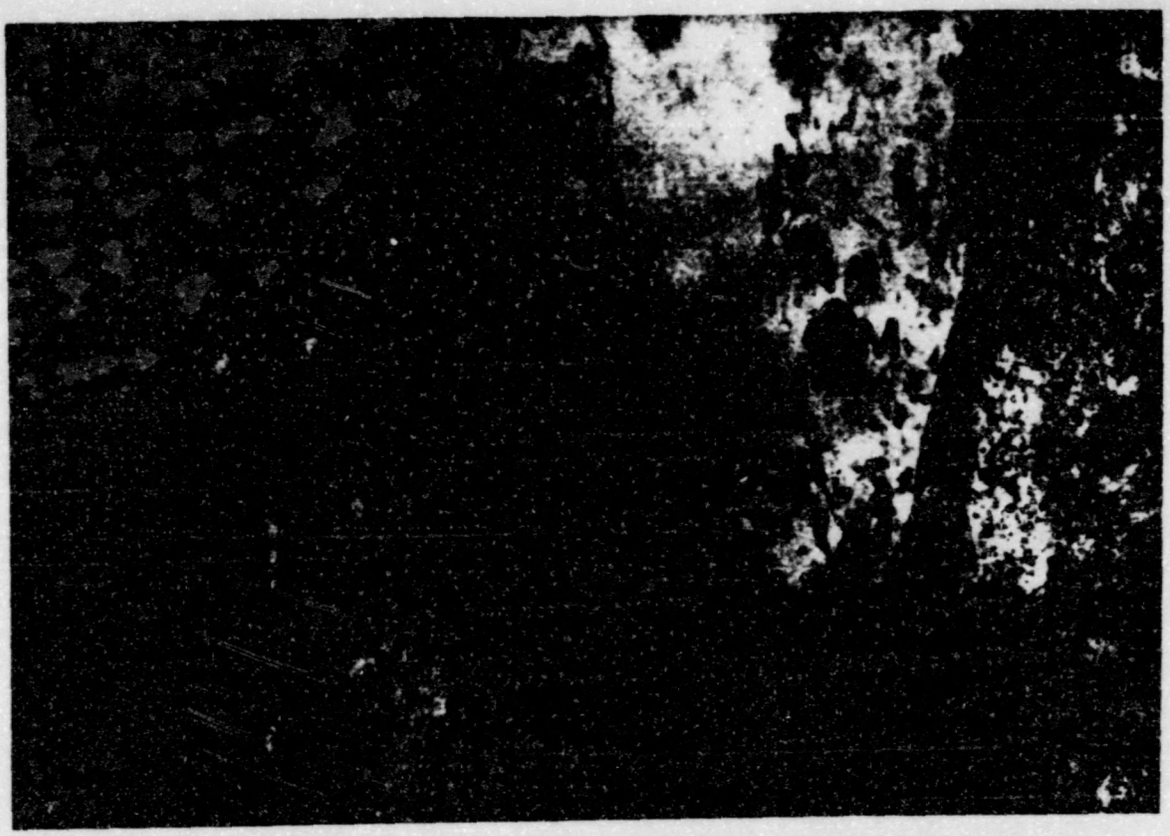
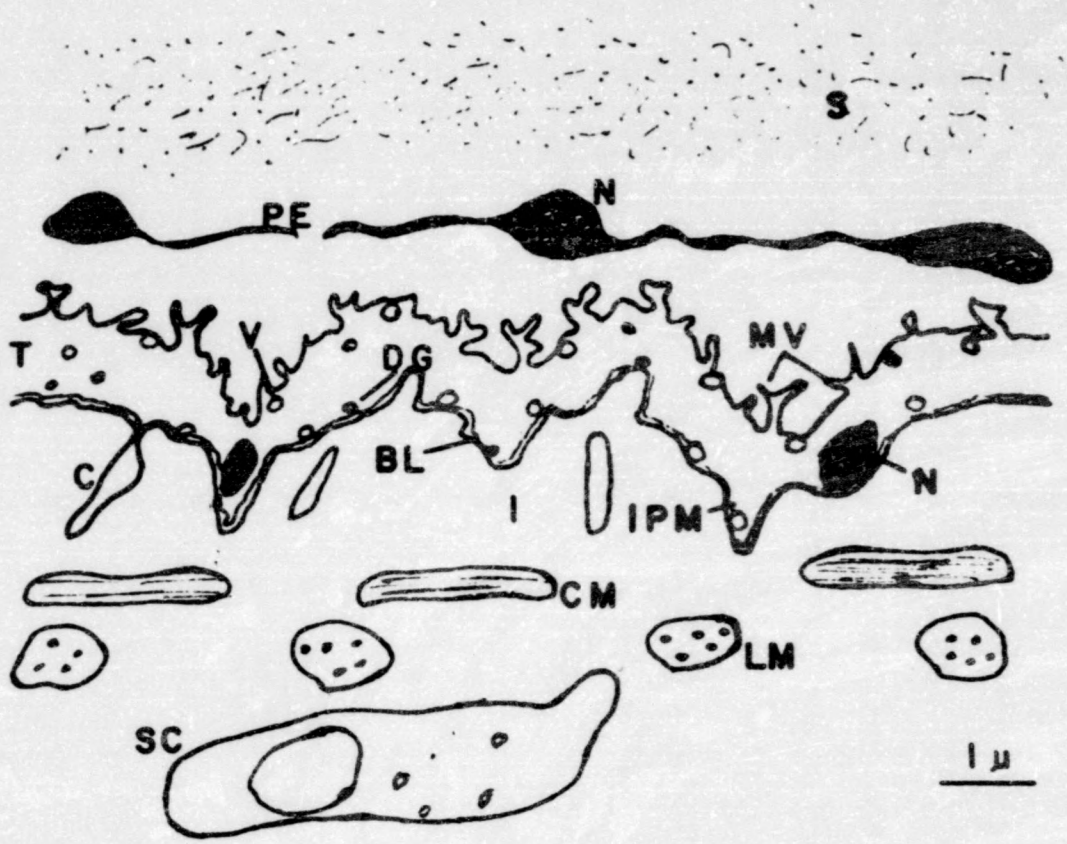


Figure 19. TEM photomicrograph of a cross section of a mature cercarial body showing scattered cytoplasmic connections (C). Also note the mitochondria (M) and lipid bodies (LB) within the tegument (T). X 10200.

Figure 20. Composite drawing of the intra-redial cercarial body. Note the surface coat (S), primitive epithelium (PE), nuclei (N), microvilli (MV), tegument (T), vesicles (V), dense granules (DG), internal plasma membrane (IPM), basal lamina (BL), cytoplasmic connections (C), intercellular spaces (I), circular muscles (CM), longitudinal muscles (LM) and subtegumentary cells (SC).

Figure 21. Composite drawing of the mature cercarial body. Note the tegument (T), mitochondria (M), lipid bodies (LB), internal plasma membrane (IPM), basal lamina (BL), intercellular spaces (I), circular muscles (CM), longitudinal muscles (LM), cytoplasmic connections (C) and subtegumentary cells (SC).


S
PE
N
T
V
DG
MV
C
BL
IPM
N
CM
LM
SC
1 µ

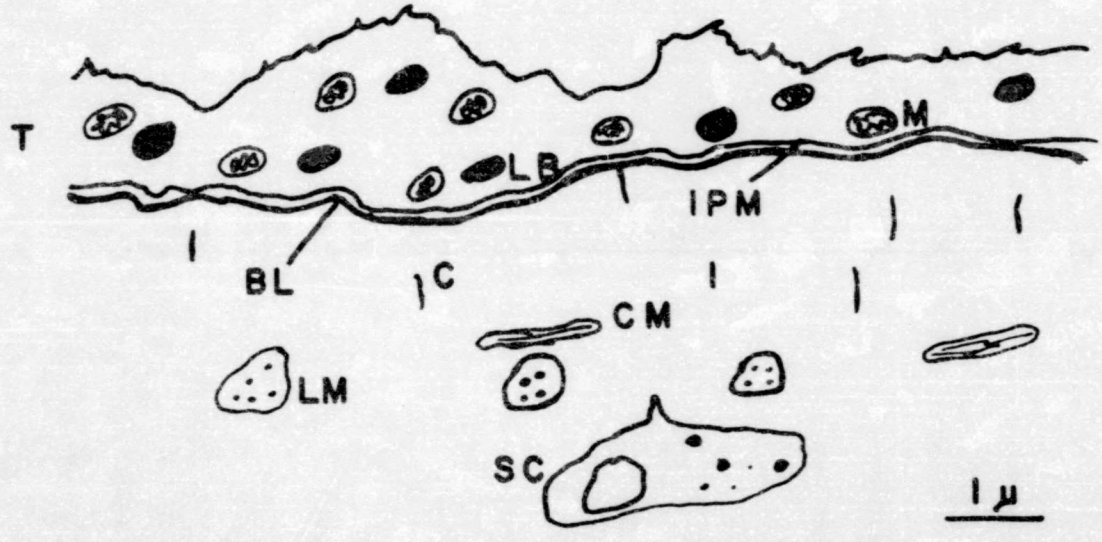
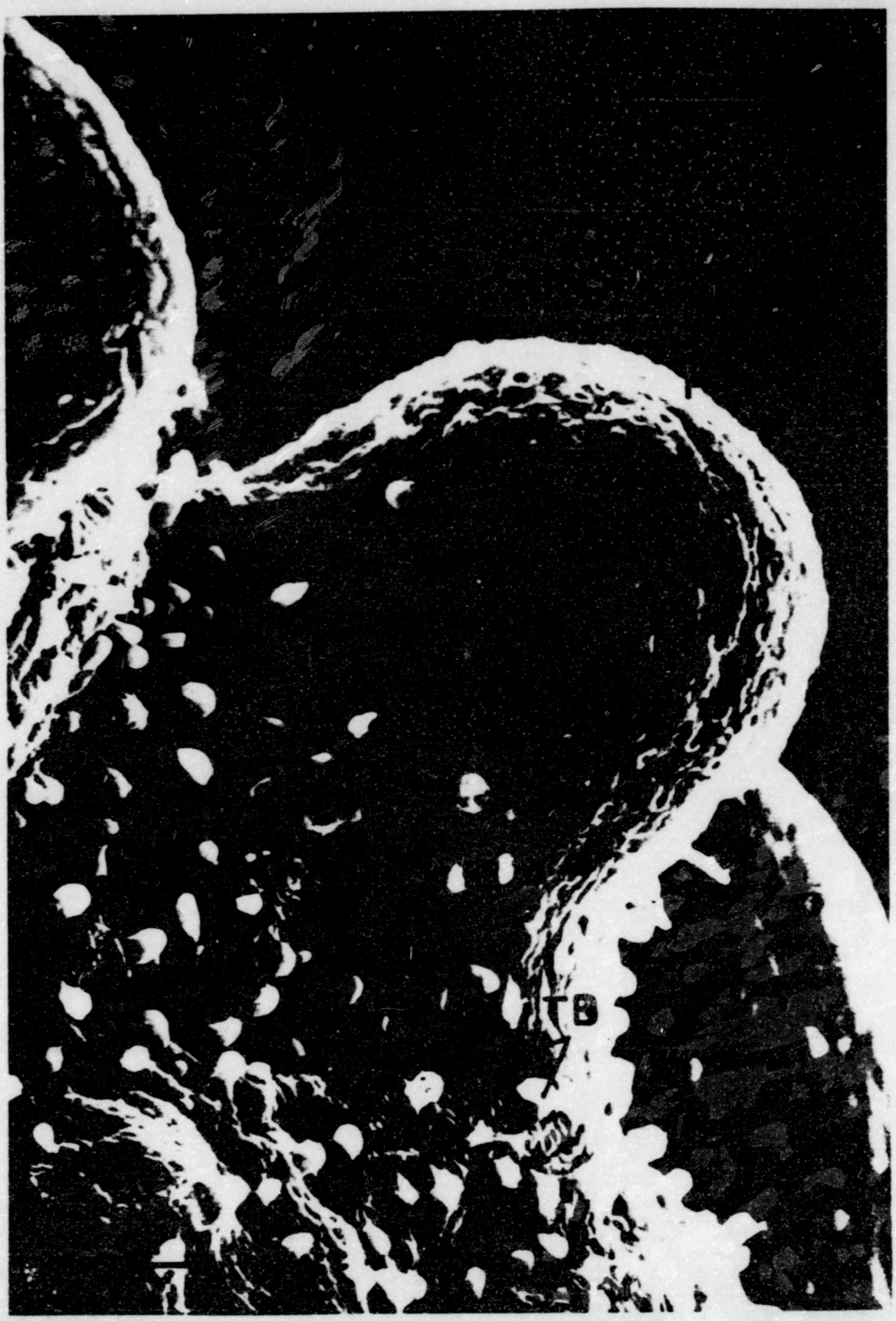

T
M
LB
IPM
BL
C
CM
LM
SC
1 µ

Figure 22. SEM photomicrograph of the papillated region of the tail. Note the large papillae (PA) with scattered cavity pits (CA) and tubercles (TB). X 3750.


TB

Areas between the papillae were observed with the TEM. In this region the tegument was 1.5-2.0 μ in width. The distal cytoplasm was denser than in the body and more vesicular and invaginated (Figure 23). The internal plasma membrane and basal lamina were not as conspicuous as in the rediae (Figure 24). Cytoplasmic connections, cytons, parenchymal cells and circular and longitudinal muscles were present internal to the basal lamina (Figures 24 and 25).

Stem Region

Tubercles were also present in this region, but only along the lateral midline (Figure 26). The remainder of the tegumental surface was formed by many projections of the tegument (Figure 27).

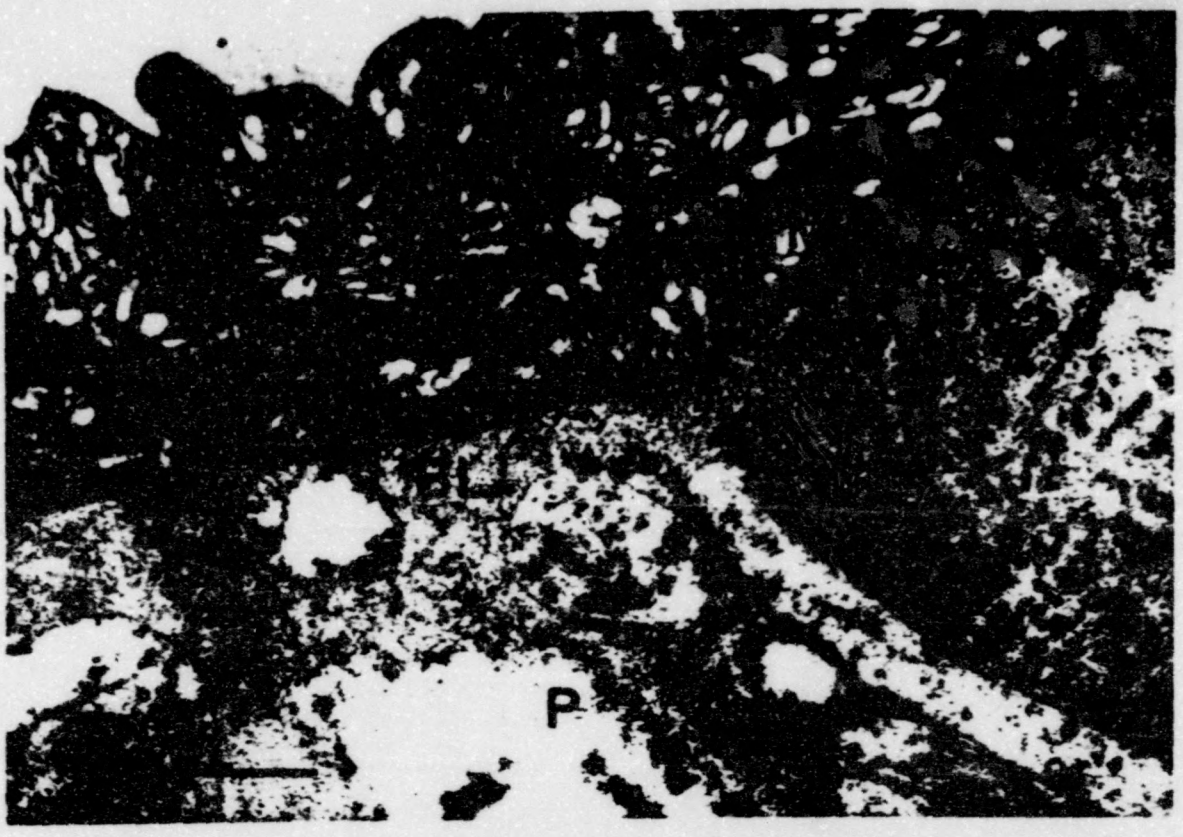
As observed in TEM photomicrographs of the stem region near the furcae, the tegument was approximately 1.0 μ thick (Figure 28). The distal cytoplasm contained mitochondria and T1 and T2 secretory bodies. An internal plasma membrane, a basal lamina and large intercellular spaces were also present. Cytoplasmic connections containing beta granules and lipid bodies were present in the surrounding tissue. Longitudinal muscles and furcae muscles were also present below the basal lamina (Figures 28 and 29).

Furcae

A SEM photomicrograph of the furcae is shown in Figure 30. The tegument of the furcae, as observed in TEM,

Figure 24. TEM photomicrograph of a cross section of the area between the papillae from the papillated region of the tail. Note the numerous vesicles (V) present in the tegument (T). Also shown are the internal plasma membrane (IPM) and basal lamina (BL). Interior to the tegument are longitudinal muscles (LM) and parenchymal cells (P). X 24800.

Figure 25. TEM photomicrograph of a cross section of the area between the papillae from the papillated region of the tail. Note the circular muscles (CM) and longitudinal muscles (LM) to the interior of the tegument (T). Also shown are cytoplasmic connections (C). X 24800.


P

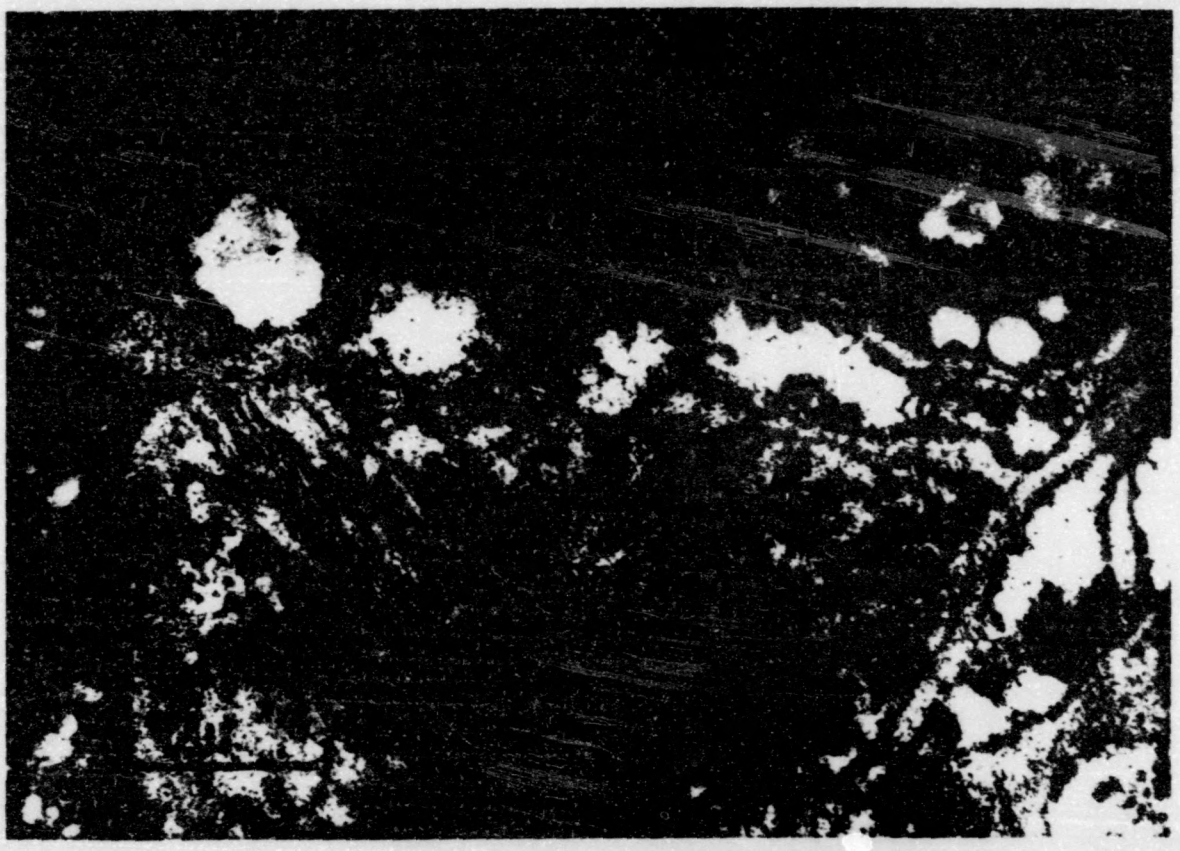


Figure 26. SEM photomicrograph of the cercarial tail showing the three regions. The papillated region is at the top followed by the stem region, ending with the furcae (forked tail). Note the tubercles along the lateral midline of the stem region. X 400.

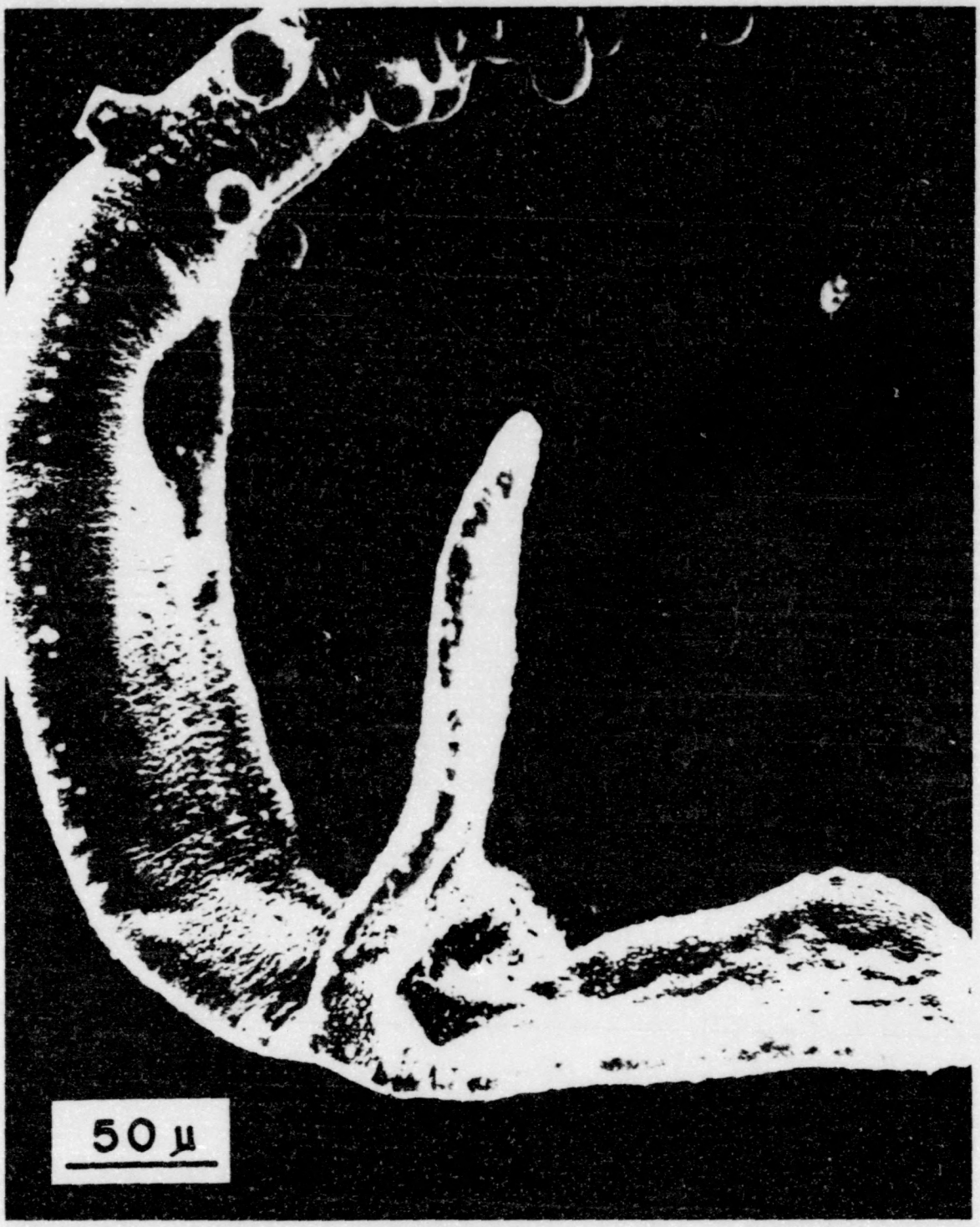

50 μ

Figure 27. SEM photomicrograph of the cercarial stem region. The surface is formed by many projections of the tegument with tubercles arising from the midline. X 5000.

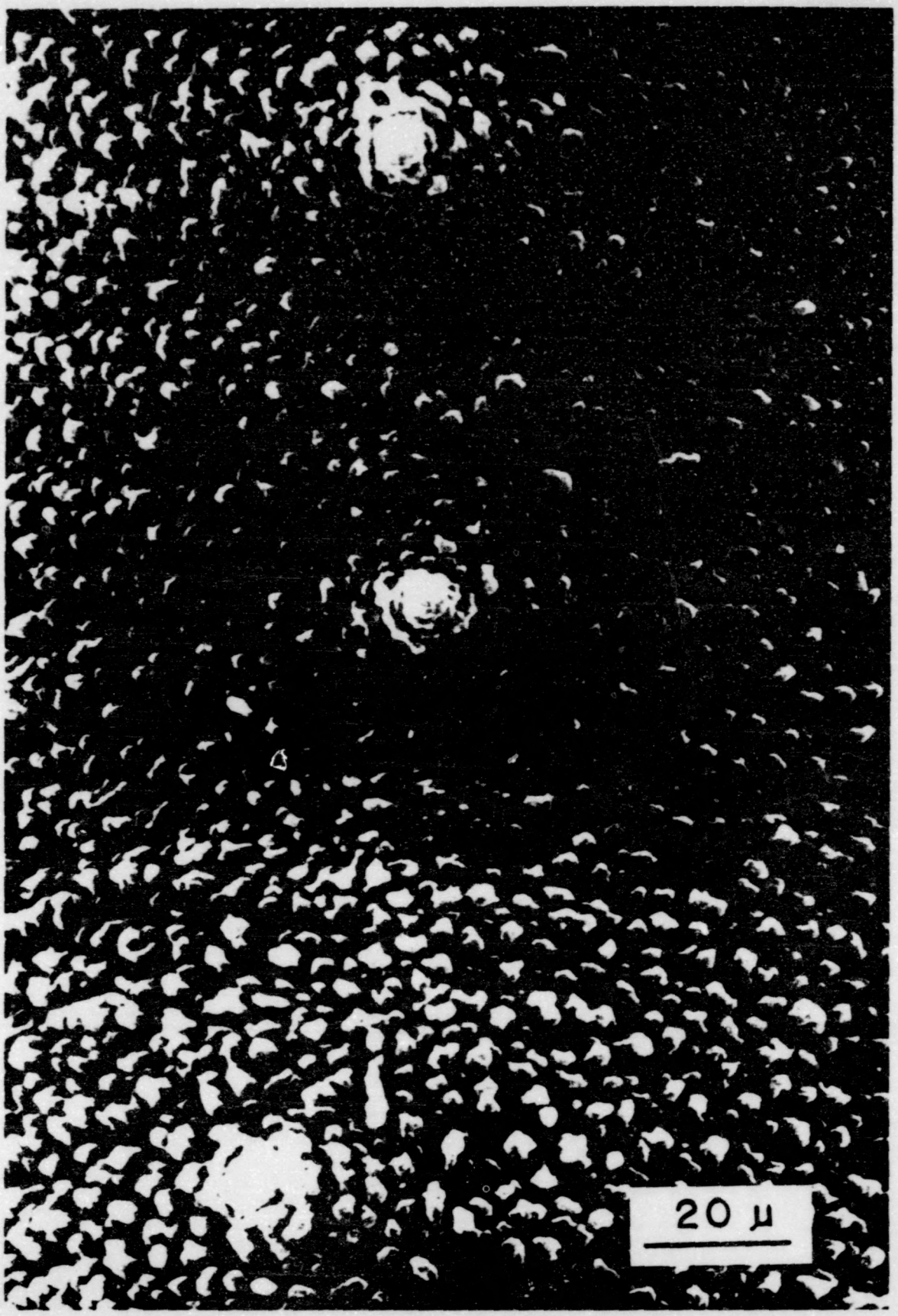
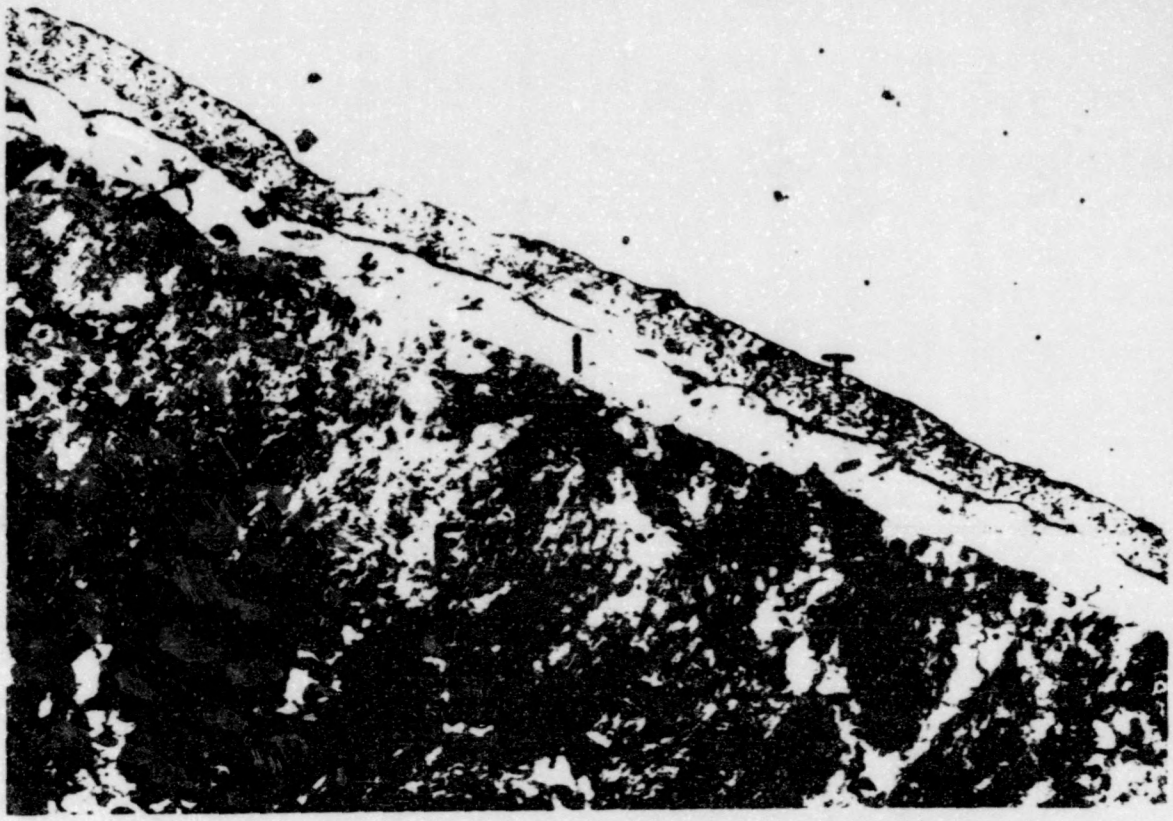

20 μ

Figure 28. TEM photomicrograph of a cross section of the cercarial stem region near the furcae. Note the intercellular space (I), furcal muscles (FM) and longitudinal muscles (LM) interior to the tegument (T). X 7800.

Figure 29. TEM photomicrograph of a cross section of the cercarial stem region near the furcae. Note T1 secretory bodies (T1) and T2 secretory bodies (T2) within the tegument (T). Also shown are the internal plasma membrane (IPM) and basal lamina (BL) with an adjacent intercellular space (I). Furcal muscles (FM) and longitudinal muscles (LM) are present along with cytoplasmic connections (C) containing beta granules (BG). X 24800.


I

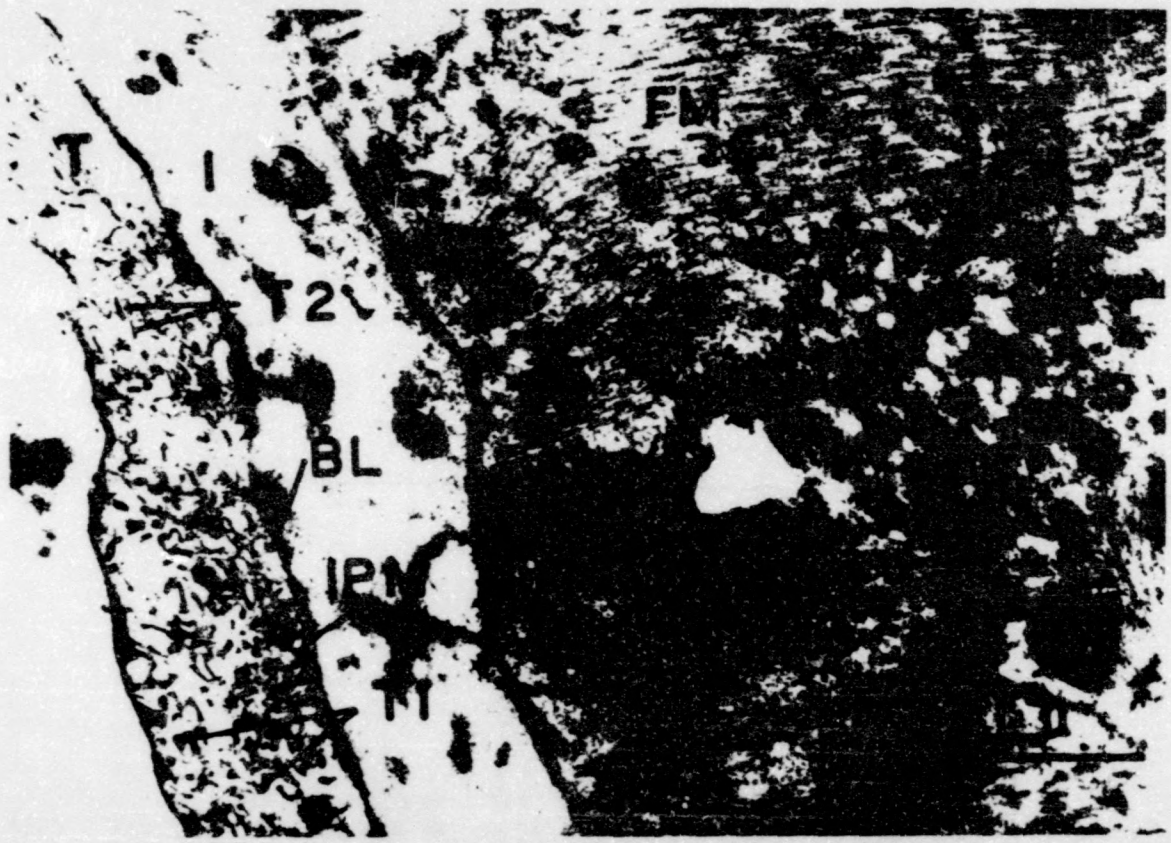
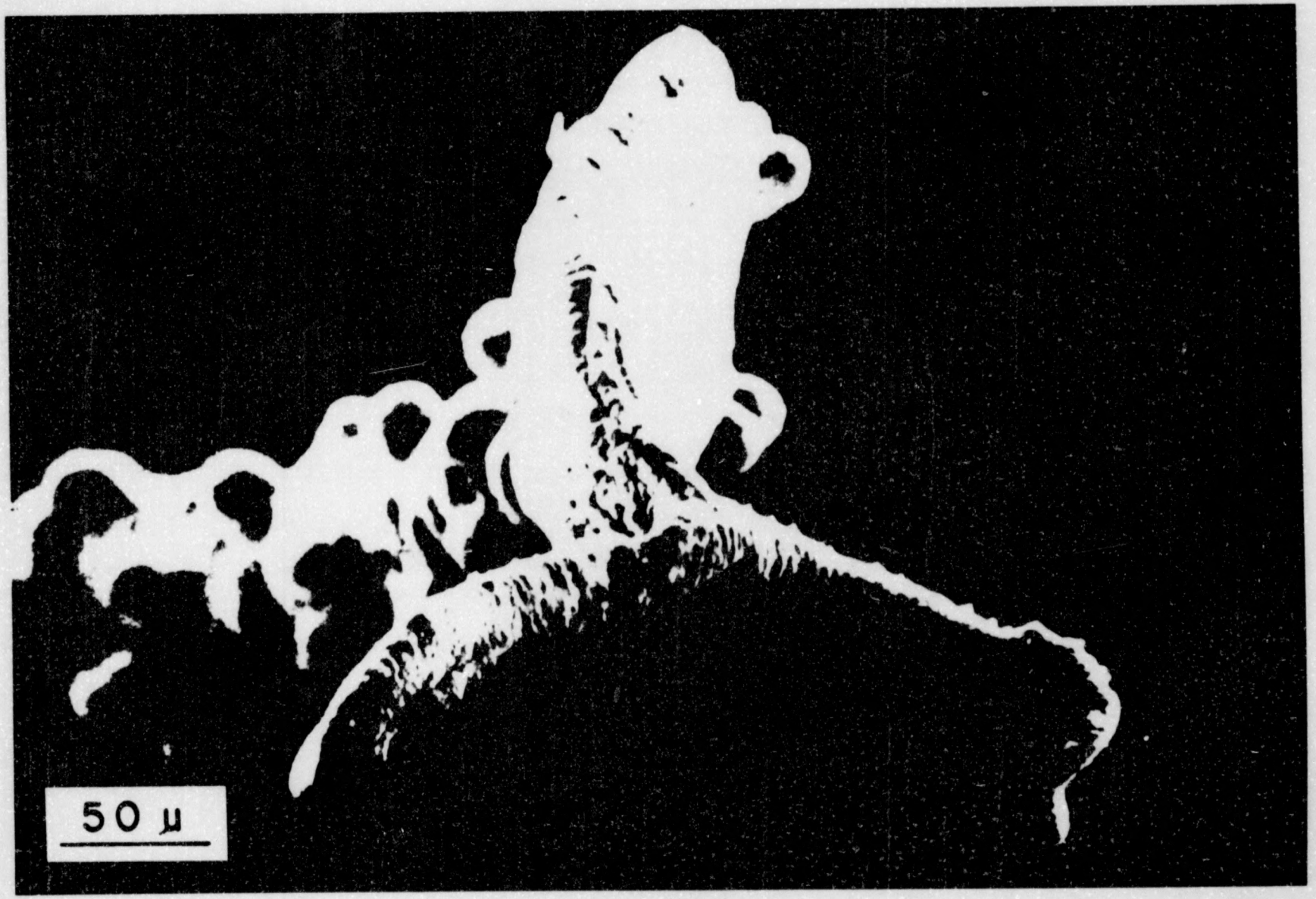

FM
I
T2
BL
IPM

Figure 30. SEM photomicrograph of the cercarial furcal region. X 500.


50 µ

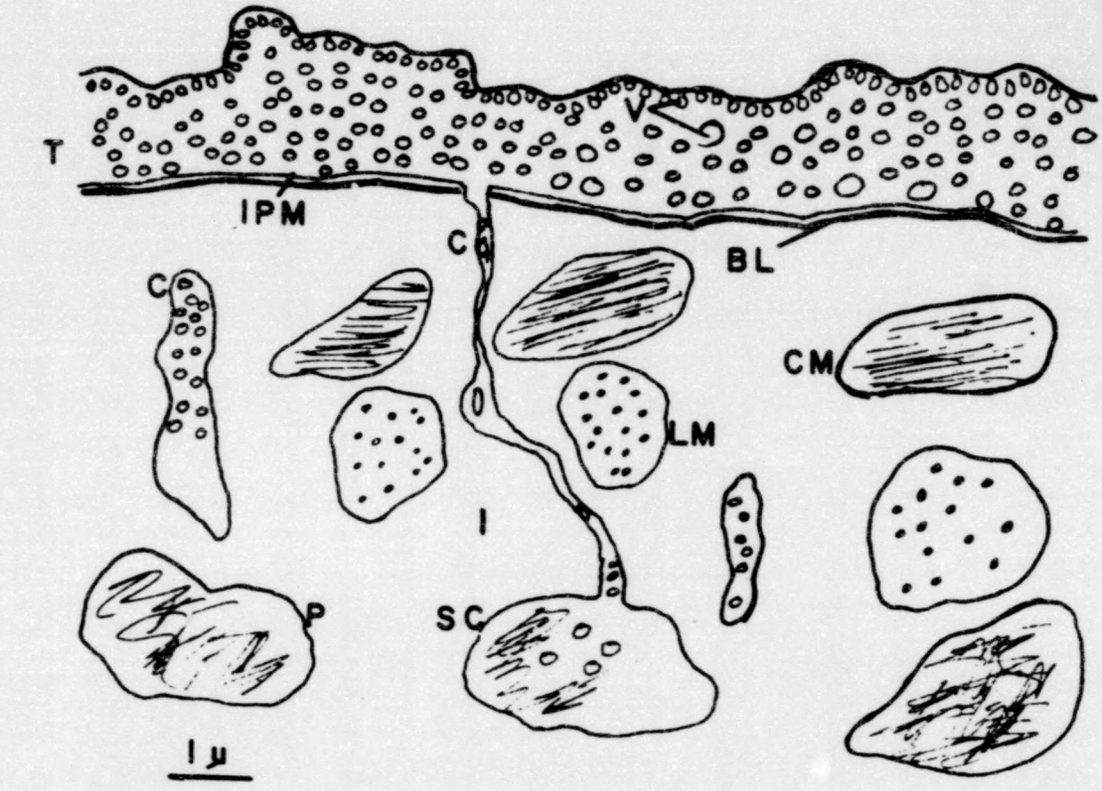
was 2.0-3.0 μ in width, invaginated and vesicular. No mitochondria or other cellular organelles were observed in the distal cytoplasm. The internal plasma membrane and basal lamina were not conspicuous. The area below the tegument was composed predominantly of muscle layers and intercellular spaces with few cytoplasmic connections (Figure 31). The different compositions of the tegument in the papillated, stem and furcal regions of the tail are shown in composite drawings in Figures 32, 33 and 34, respectively.

Figure 31. TEM photomicrograph of a cross section of the cercarial furcal region. Note the numerous vesicles (V) present in the tegument (T) and the invaginations of the tegument (IT). Also shown interior to the tegument, are intercellular spaces (I) and circular muscles (CM). X 33800.

1 μ

Figure 32. Composite drawing of the area between the papillae from the cercarial papillated region. Note the tegument (T), vesicles (V), internal plasma membrane (IPM), basal lamina (BL), cytoplasmic connections (C), intercellular spaces (I), circular muscles (CM), longitudinal muscles (LM), subtegumentary cells (SC) and parenchymal cells (P).

Figure 33. Composite drawing of the cercarial stem region. Note the tegument (T), T1 secretory bodies (T1), T2 secretory bodies (T2), internal plasma membrane (IPM), basal lamina (BL), intercellular spaces (I), longitudinal muscles (LM), circular muscles (CM), furcal muscles (FM), cytoplasmic connections (C) and lipid bodies (LB).


T
V
IPM
C
BL
C
CM
LM
I
P
SC
1 μ

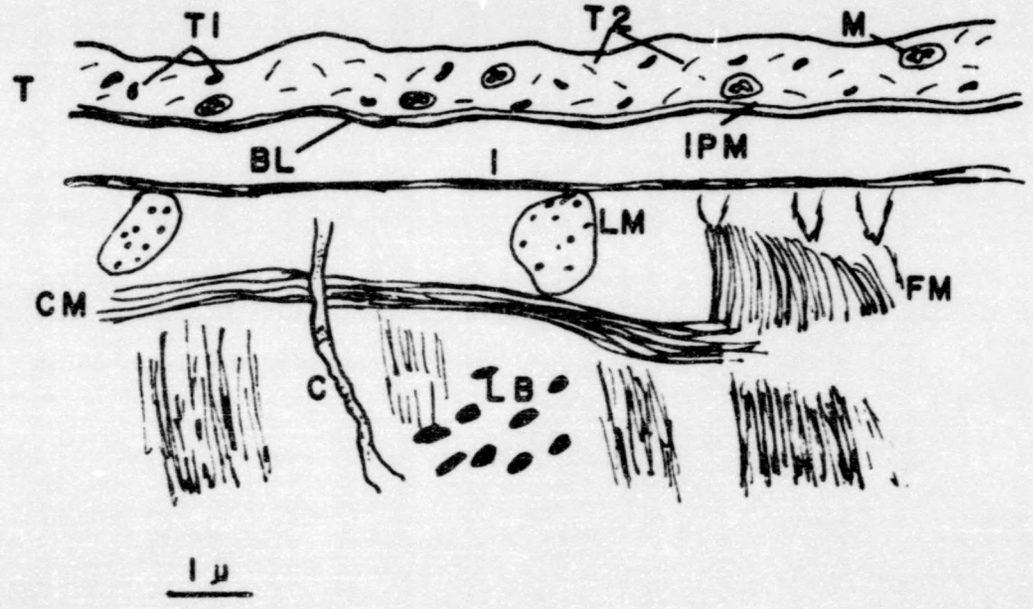
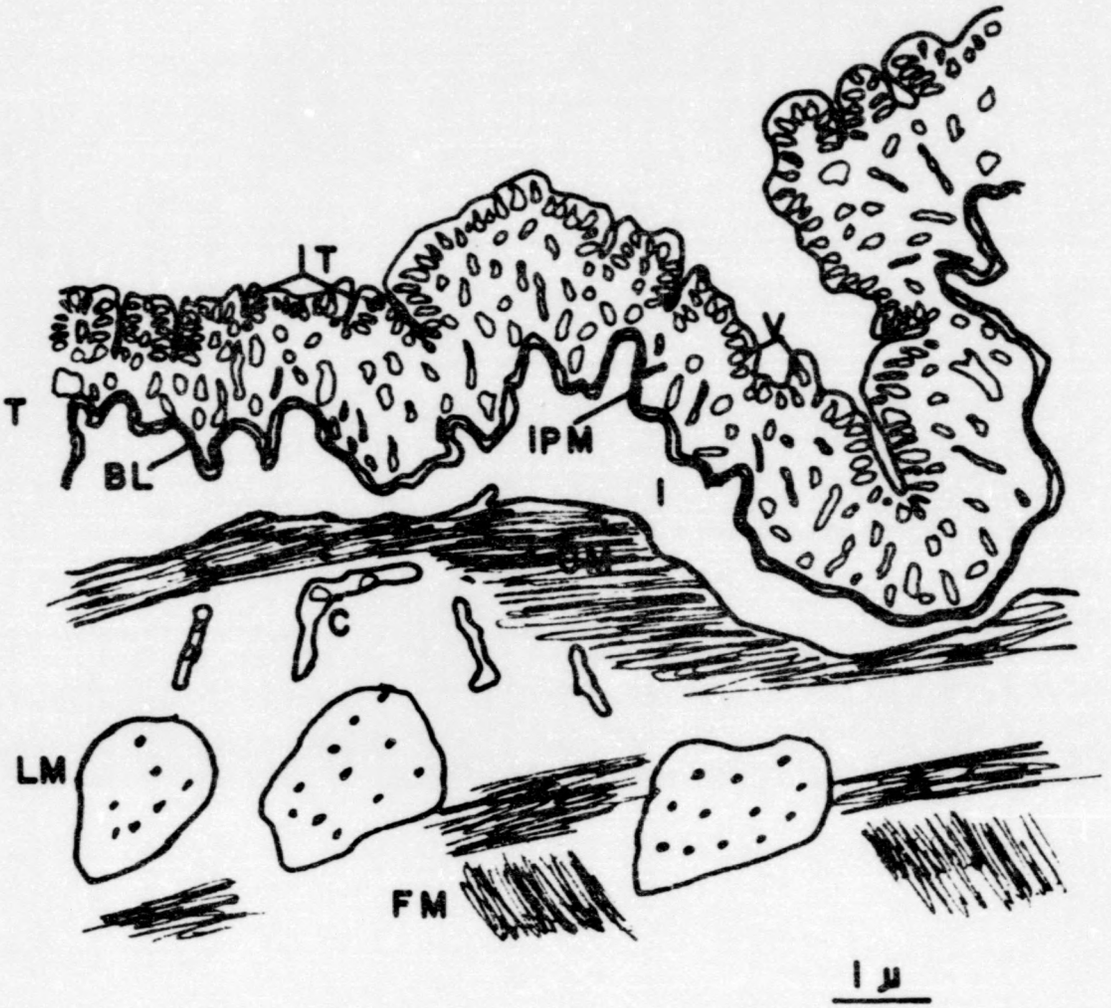

TI
T2
M
T
BL
I
IPM
LM
CM
FM
C
LB
1 μ

Figure 34. Composite drawing of the cercarial furcal region. Note the tegument (T), invaginations of the tegument (IT), internal plasma membrane (IPM), basal lamina (BL), cytoplasmic connections (C), circular muscles (CM), longitudinal muscles (LM) and furcal muscles (FM).


T
T
BL
IPM
I
C
LM
FM

DISCUSSION

Through the observations of SEM and TEM photomicrographs, it was found that differences did occur in the tegumental area of P. edneyi in relation to environmental and functional factors. Not only were differences observed between the rediae, cercariae within the rediae and mature cercariae, but also between areas of the same cercariae as reported by Schmidt and Roberts (1977) and Whitfield (1979).

The redial tegument of P. edneyi was a series of rings which were in a patterned, folded arrangement as opposed to the unorganized arrangement observed in other redial teguments such as those of Parorchis acanthus (Rees 1971) and Cryptocotyl lingua (Irwin et al. 1978).

An important factor in this particular redia is that as it matures, the functional mouth, pharynx and gut become non-functional (Aliff 1973). Thus, the only means for this germ sac to acquire nutrients necessary for the production of cercariae is via the tegument. The numerous folds of the tegument would increase the surface area and possibly compensate for the lack of a digestive tract, enabling the redia to acquire more nutrients from its molluscan host (Rees 1971, Irwin et al. 1978). The

numerous microvilli observed on the surface of the tegument would also increase the surface area of the redia.

The mouth of the P. edneyi redia was formed by infoldings of the tegument as in C. lingua (Irwin et al. 1978). However, fewer papillae were observed around the mouth of the P. edneyi redia than for C. lingua. The lack of papillae may be related to the loss of a functional mouth.

Vesicles present in the distal cytoplasm could also aid in the absorption of nutrients. Reader (1972) placed horseradish peroxidase tracer on the outside of the redia of Sphaeridiotrema globulus. This substance was too large to go through the wall but was observed within vesicles present in the distal cytoplasm, cytons and parenchymal cells. Reader (1972) concluded that the horseradish peroxidase was being taken through the tegument via the vesicles. Therefore, these vesicles may have an important function in the acquisition of nutrients from the snail.

No mitochondria or other organelles were observed in the distal cytoplasm of the tegument of the redia. These results support those of Rees (1971), who worked with P. acanthus redia. Rees (1971) observed a conspicuous basal lamina beneath the tegument of the redia. This was also observed in the redia of P. edneyi.

Beta granules were present in some of the cytoplasmic connections. These granules are usually associated with high energy requirements (Gress and Lumsden 1976).

Therefore, the tegumental area with which these cytoplasmic connections were associated may have a high metabolic activity.

While studying Schistosoma mansoni, Wilson and Barnes (1974) observed that cytoplasmic connections from the sporocyst connected to developing cercariae and postulated that there was direct transport of nutrients from the sporocyst to the developing cercariae. In the rediae of P. edneyi, the cytoplasmic connections appeared to be oriented more toward the interior, possibly connecting with the developing cercariae and providing directly the necessary nutrients.

A surface coat (glycocalyx) was observed external to the primitive epithelium of the intra-redial cercarial body. It appeared as a diffuse, fibrous-like material which Hockley (1972) and Gress and Lumsden (1976) observed surrounding the adults of S. mansoni. The surface coat of Fasciola hepatica was found to be composed of glycoprotein and sialic acid (Threadgold 1976) and could be sloughed off in response to the host's immune system and rejuvenated by secretory bodies produced by the cytons (Hanna 1980). The glycocalyx of S. mansoni provided mechanical protection from surface damage and also regulated permeability (Hockley 1972). Hockley (1972) also stated that the glycocalyx was probably formed from dense bodies which appeared to originate in Golgi complexes in the cytons.

The glycocalyx around developing cercariae of P. edneyi may have been formed by the redia since it was external to the primitive epithelium and did not appear to be associated with the mature tegumental wall.

The primitive epithelium surrounding the tegument had few nuclei and no partitioning membranes, thus forming a syncytium. This was also observed by Hockley (1972) who found the primitive epithelium of the cercariae to persist until the embryo was a multicellular mass and had begun to form the true tegument. Once the true tegument had begun to form beneath the primitive epithelium, the latter would begin to slough off. This appeared to be the case in the developing cercariae of P. edneyi.

The function of the primitive epithelium is believed to be protection until the true tegument has been formed. The temporary larval covering may be necessary to allow for growth of the embryo while the true tegument will not need to increase much in size if it is not formed until the embryo is well developed (Hockley 1972).

Since the intra-redial cercarial bodies probably do not have a functional mouth and gut when immature and developing, they must acquire their nutrients from the rediae via the tegumental wall (Rees 1971). The numerous convolutions and microvilli present on the tegumental surface would increase the surface area for acquisition of more nutrients.

Vesicles were present in the distal cytoplasm of the developing cercarial bodies and probably indicate the active acquisition of nutrients as in the redial stage (Reader 1972). The fact that nutrients are first absorbed by the redia and transported throughout the body and into the developing cercariae may account for the apparent absence of mitochondria from the distal cytoplasm of the cercariae (Rees 1971).

Cytons usually have an abundance of granular endoplasmic reticulum, Golgi bodies and mitochondria and are actively secreting substances (Threadgold 1967, Bogitsh 1968, Smith et al. 1969, Wilson and Barnes 1974). The substances are then transported to the tegument through the cytoplasmic connections. The numerous cytoplasmic connections observed in the developing cercarial bodies of P. edneyi were probably transporting substances from the cytons to the tegument. These substances may have been contributing to the construction of the tegument or transportation of enzymes for the breakdown of nutrients.

A glycocalyx was not present surrounding the mature cercarial body. However, Threadgold (1976) stated that the morphology and histochemistry of the glycocalyx of F. hepatica varied depending on the environment immediately prior to fixation and also on post fixation treatment. Also, conventional electron microscope fixation appeared to preserve only about one-half the total thickness of the

glycocalyx. In the case of P. edneyi, TEM fixation did not appear to have preserved any of the glycocalyx of the mature cercarial body if present.

The reduction of microvilli on the tegumental surface of the mature cercarial body and the relatively smoother surface probably was a reflection of the change in method of nutrient acquisition. The mouth and gut were more developed and functional and the cercariae would soon emerge from the snail to be relocated in the definitive host where nutrients would be acquired through the oral sucker.

The absence of nuclei in the distal cytoplasm is a common feature of all trematodes examined to date by TEM, but the functional advantage is unknown (Hockley 1972). From other observations, it appears that once the nuclei become pycnotic and disappear, the cytons become continuous with the tegument.

Tegument and cyton contact of S. mansoni was described by Hockley (1972) as being processes from subtegumentary cells migrating out and becoming continuous with the tegument while the nucleated parts of the cell remained deep within the tissue. Bils and Martin (1966) showed that the tegument of Acanthoparyphium spinulosum cercariae was at first simply an outer nucleated layer. Eventually the nuclei came to lie internal to the basal lamina of the tegument, though still in cytoplasmic connections with the

tegument, thus forming the cytons. It appeared in the case of P. edneyi cercariae that nuclei in the distal cytoplasm became pycnotic and disappeared. The subtegumentary cells were already present, perhaps as cystogenous or parenchymal cells, and the processes of these cells then migrated out to connect with the tegument, forming the typical organization of a trematode tegument.

Mitochondria were more prevalent in the distal cytoplasm of the mature cercarial body of P. edneyi than in the developing cercarial body, perhaps indicating an active transport system while within the snail. Uglem (1980) reported that the cercarial bodies of P. macrostoma were capable of glucose transport while the tails were not. Smith et al. (1969) reported numerous, small mitochondria in the distal cytoplasm of S. mansoni cercarial bodies and stated they might be useful in maintaining an osmotic barrier against the upcoming hypertonic aquatic environment.

The few cytoplasmic connections observed in the mature cercarial body were usually not connected to the tegument or cytons and appeared to be degenerating. Hockley's (1972) observations were similar for the cercarial bodies of S. mansoni, and he concluded that once products were discharged via cytoplasmic connections into the distal cytoplasm from a cyton, the cytoplasmic connections degenerated. Once the cercarial bodies entered the definitive host, the cytoplasmic connections would reform

and resume the function of transporting substances to the tegument for its continuous formation.

The tail regions were divided into papillated, stem and furcal regions. There was a cavity present in the papillated region in which the cercarial body withdraws prior to emergence from the snail. Once out of the snail and in the water, this free-swimming stage has a brief existence and must be ingested by the definitive host during this time in order to complete the life cycle. Therefore, the cercarial tail serves as a "bait" for the fan-tail darter by swimming to the surface of the water and sinking to the bottom in a regular pattern (Prior and Uglem 1979). Once cercariae are ingested by the definitive host, there must be an immediate release of the cercarial body from the cavity. This must occur in order for the adult to inhabit its appropriate site, the esophagus of E. flabellare. If there is not an immediate release, the body would be digested along with the tail.

The papillated region of the tail was composed of numerous papillae, cavity pits and tubercles which were probably sensory in function (Schmidt and Roberts 1977). These structures may serve as the mechanism which causes the release of the cercarial body from the cavity. These sensory organs may also function as stimulating devices when the cercariae hit bottom stimulating the upward swimming.

The distal cytoplasm in the papillated region appeared thicker and denser, possibly for protection, since the cercarial body was contained within this region. More cytoplasmic connections were observed in this region than the other two regions. These were probably related to the transportation of material substances from cytons to the distal cytoplasm for formation of the protective tegument which outside environmental conditions may destroy. The tegumental surface was invaginated which would increase the surface area aiding in the absorption of nutrients while the cercariae were inside the snail. The vesicles present in the distal cytoplasm may result from the absorption of nutrients when inside the molluscan host or may transport materials to aid in protection (Reader 1972).

The stem region also contained a few tubercles which were probably sensory in function. The remainder of the tegumental surface was formed by many projections which would increase the surface area. Nutrients could be absorbed through the tegument of the tail while within the rediae. However, in the free-swimming state, the increased surface area may possibly be concerned with respiratory exchange since the tail requires considerable energy during swimming (Rees 1971). The stem region connects with the furcae, which are the actual swimming apparatuses, and may transfer required energy to them.

Mitochondria were present in the distal cytoplasm of the stem region and may indicate some type of active transport. There were also secretory bodies present which Threadgold (1967) labeled T1 and T2 secretory bodies; T1 were small, ovoid bodies with granular contents, and T2 were biconcave, disk-like bodies with dense contents. The tegumental cytons of adult F. hepatica were of two types; one (Type 1) produced the T1 secretory bodies and the other (Type 2) produced the T2 secretory bodies (Threadgold 1967). These secretory bodies were transported from the cytons to the distal cytoplasm. Two possible functions for these secretory bodies have been postulated. 1) Their function is to combine with substances absorbed (either selectively or because the fluke cannot prevent their entry) through the tegument. Such combinations could serve as an extension of the general protective function of the tegument or as a preliminary part of the process of assimilation and digestion. 2) They are excretory substances. However, this possibility is remote due to their mode of origin and the presence of an excretory system in this animal (Threadgold 1967). The presence of beta granules in the distal cytoplasm was probably indicative of a high metabolic area as in the redia (Reader 1972).

The tegument of the furcae was thick, invaginated and vesicular. The thickness and vesicular nature may aid in the protection of this region as it does in the papillated

region (Reader 1972). Invaginations of the tegumental surface increase the surface area and may be concerned, as in the stem region, with respiratory exchange (Rees 1971). The subtegumentary area, which was composed primarily of muscle layers, would have a high energy requirement and metabolic rate because of its function. No mitochondria or other cellular organelles were observed in the distal cytoplasm indicating the tegument of the furcae was not involved with any active or passive transport system.

In conclusion, differences were observed in the tegumental area of the different intramolluscan larval stages of P. edneyi in relation to functional and environmental factors. Studies should be conducted on the tegument of adult P. edneyi to compare the tegument of this stage in its environment with the tegument of the larval stages reported herein. The functional relationship of the tegumental structures in all stages needs to be investigated.

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