STUDIES ON ECHINOCOCCOSIS XXI

ELECTRON MICROSCOPICAL OBSERVATIONS ON GENERAL STRUCTURE OF LARVAL TISSUE OF MULTILOCULAR ECHINOCOCCUS

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The characteristics of echinococcal tissue as determined by means of the light microscope have been described by numerous investigators. However, some uncertainties still exist concerning some of the structures observed. Recently, observations made by means of the electron microscope on the fine structure of the adult and larval *Echinococcus granulosus* (BATSCH, 1786) have been reported by MORSETH (1966, 1967[12,13]). Evidently the fine structure of the respective stages of *E. multilocularis* LEUCKART, 1863 has not been investigated.

The present paper reports observations on the structure of the larva of *E. multilocularis* made by means of the electron microscope.

MATERIALS AND METHODS

Fully developed larvae of *Echinococcus miltilocularis* LEUCKART, 1863 were obtained from the liver of experimentally infected cotton rats, *Sigmodon hispidus* SAY et ORD. The tissues were fixed in 6 per cent phosphate-buffered glutaraldehyde at a temperature of about 0°C, postfixed in 1 per cent osmic tetroxide (a modification of the double aldehyde-osmium fixation of SABATINI et al. (1963)), dehydrated in ethanol and propylene oxide, embedded in Epon 812, and sectioned by means of a Sovall MT-1 Porter-Blum ultramicrotome. The sections were mounted on copper grids and stained with saturated uranyl acetate in 50 per cent ethanol solution (WATSON, 1958), or in a combination of the latter and lead citrate (REYNOLDS, 1963) (10 minutes in uranyl acetate, followed by 10 minutes in lead acetate with brief washing in carbonate-free, glass-distilled water). The preparations were examined by means of the JEM-7 electron microscope. The thicker sections stained with toluidine blue were used to identify the area to be sectioned for electron microscopy. The paraffin sections prepared from the same materials were examined histochemically.

RESULTS

A Scolex

1 Tegument

As determined by means of light microscopy, the cuticle of cestodes is known to have

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three layers : a thin, external, cuticular layer; a thick internal, or main, cuticular layer; and a basement membrane, which is not a true constituent of the cuticle. The tegument of the protoscolex of the larval *Echinococcus* has been thought to be composed of the cuticle and subcuticular cells. We found in the larval echinococcus that the main cuticular layer consists of a superficial, syncytial cytoplasm (i. e., the distal cytoplasm) which is continuous with the tegumental cells (i. e., the perikarya or perinuclear cytoplasm) embedded in parenchyma (figs. 1～ 5), and we therefore apply to it the term "tegumental cytoplasm" previously proposed by MORSETH (1967)[12], instead of "distal cytoplasm of tegumental cells". The tegumental cytoplasm is thin on the suckers and rostellum, and it tends to increase in thickness posteriad from the anterior portion of the protoscolex. The outer surface of the tegumental cytoplasm has numerous projections, which are designated "microvilli" or "microtriches" by some authors. These projections are covered by a unit membrane (about 75 Å thick), MORSETH's[12] "external membrane", which is continuous over the outer surface of the tegumental cytoplasm. The projections on the anterior portion of the protoscolex, including the rostellum and suckers, are spine-like. The apical portion of the spinous projection is electron-opaque ; it has a longitudinally striated appearance, and is separated from the base part by a narrow, electron-lucid zone which is bisected or trisected by one or two electron-opaque, transverse lines. The projections on the posterior portion of the protoscolex are less well developed, and have a knob-like form. An electron-opaque, coronal area is present at the apex of the knob-like projections. Numerous vesicles and tubules were seen in the tegumental cytoplasm ; some contained granular and bacilliform, electron-opaque material. Mitochondria occur in considerable numbers in the basal region of the thin, tegumental cytoplasm at the anterior portion of the protoscolex. Such mitochondria, however, were rarely found in the posterior portion. These mitochondria have relatively few cristae in a rather electron-opaque matrix.

Numerous infoldings of the plasma membrane were seen on the outer and inner surfaces of the tegumental cytoplasm. Some of the infoldings were connected with solid or empty tubules in the tegumental cytoplasm. On the other hand, the plasma membrane on the basal surface of the tegumental cytoplasm is lined with a basement membrane composed of a compact, amorphous substance. Besides the infoldings mentioned above, indentations in the plasma- and basement membranes were found at regular intervals on the inner surface of the tegumental cytoplasm. In such indentations, the plasma membrane was associated with one of the electron-opaque patches as if they were the junction of half desmosomes, and it was connected also with the infoldings in such areas. Beneath the basement membrane is a broad zone of fibrous interstitium consisting of loose, reticular fibrils. This zone, with the basement membrane, separates the tegumental cytoplasm from the muscle fibers beneath. The zone of fibrous interstitium, together with the basement membrane in the strict sense, have been usually designated "basement membrane", as observed by means of the light microscope. The presence of fibrous interstitium in the intercellular spaces of the parenchyma also is confirmed by means of the electron microscope. The compact, stringy structure of fine fibrils (figs. 1 & 5) extended from the basement membrane of the tegumental cytoplasm across the zone of fibrous interstitium, reaching to the dense body on the projections of the muscle cells.

The cytoplasmic bridges extending from the perinuclear cytoplasm of the tegumental cells in the parenchyma are continuous with the tegumental cytoplasm. These bridges in the fibrous interstitium are surrounded also by a compact, amorphous substance connected with the basement membrane. The tegumental cell has a nucleus with a large nucleolus, and its perinuclear cytoplasm contains numerous ribosomes, mitochondria, Golgi complex, and vesicles. The vesicles mostly contain an electron-opaque material, and are usually concentrated near the Golgi complex. The vesicles showed the same structure as those in the cytoplasmic extensions and in the tegumental cytoplasm. The entire surface of the tegument is covered with a loosely reticulated, extracellular, fibrous substance, which is found to be PAS-positive by means of the light microscope.

2 Muscle cells and glycogen-containing cells in the parenchyma

The bundles of myofibrillar cytoplasm (figs. 1, 2 & 4) beneath the zone of fibrous interstitium, and which presumably correspond to the subcuticular muscle layer as seen by light microscopy, show a cytoplasmic continuity with the perinuclear cytoplasm of the muscle cells in the parenchyma. The perinuclear cytoplasm of individual muscle cells forms continuous, long projections consisting of amyofibrillar and myofibrillar cytoplasm, separately or together. Some of the projections are branched. The perinuclear cytoplasm contains numerous ribosomes, several mitochondria, and a Golgi complex. The amyofibrillar cytoplasm is filled with numerous, massive granules (900~1,400 Å in diameter) which are considered to be the alpha particles of glycogen.

The myofibrillar cytoplasm of muscle cell contains a number of myofibrils consisting of a thick myofilament (200~350 Å in diameter), and thin myofilaments (about 50 Å in diameter) (figs. 6, 7 & 18). The thick myofilament is irregularly surrounded by the thin ones, making up a bundle ranging from 700 to 800 Å in diameter. The number of thin myofilaments in such aggregations ranges from 9 to 16. A few sacs of agranular, sarcoplasmic reticulum, mitochondria, and a small quantity of beta glycogen particles (150~250 Å in diameter) occupy the peripheral sarcoplasm of the myofibrils. However, in the perinuclear cytoplasm of some muscle cells are seen bundles of myofibrils (figs. 7 & 21). The individual myofibers are separated by narrow, intercellular spaces filled with fine fibrils. The sarcolemma is indented in some places, and such indentations are found to be associated with considerable electron-opaque areas at the peripheral sarcoplasm (figs. 6 & 18).

In addition to the muscle cells mentioned above, the cells containing numerous alpha particles of glycogen in the perinuclear cytoplasm were found in the parenchyma (fig. 8). Those cells have also several projections which are filled up with alpha particles of glycogen. In the projections of those cells, however, myofibrils were not able to be found in the present photographs obtained. Therefore, we suggest that the glycogen-containing cells (glycogen storage cells) and the muscle cells are both present in the parenchyma, though the fine structure of the nucleus and mitochondria of the former have a strong resemblance to those of the latter.

In the cytoplasm containing alpha glycogen particles are found various inclusions composed of lipid, lamellae (presumably myelin), crystaloids, and/or fibrils. Such inclusions appear in various sizes, and they have no limiting membrane. In addition, large, spherical vacuoles are found in the cytoplasm containing alpha particles of glycogen; these are considered to be vestiges of calcareous corpuscles which were eliminated through the process of preparing the material. As far as we observed in the present investigation, we could not ascertain whether the glycogen-containing cytoplasm with the calcareous corpuscles and various inclusions belong to the muscle cell or to the glycogen-containing cell.

3 Excretory system

The parenchyma contains flame cells (figs. 9∼12) and excretory ducts (figs. 13 & 14). Cilia, numbering 32 to 54 according to the present electron micrographs, are present at one side of the flame cells. Each cilium consists of a pair of central filaments around which are arranged 9 pairs of peripheral filaments. The bundle of cilia is enveloped with a membraneous cytoplasm which extends from the perinuclear cytoplasm, and which is penetrated by many pores. Microvilli are present on the membraneous cytoplasm. Basal bodies and striped rootlets are found at the base of the bundles. A nucleus and perinuclear cytoplasm, somewhat resembling those of the tegumental cell, are present on the other side of the flame cell.

The wall of the excretory ducts is made up of membraneous extension of cytoplasm which resembles that of the tegument. Numerous knob-like projections occur on the inner surface of the wall. The nucleus and the perinuclear cytoplasm of the cells of the excretory duct are similar to those of the tegumental cell.

The excretory ducts are overlain by a thin layer of compact, amorphous substance (basement membrane), and are surrounded further by a layer which appears to have the same structure as the zone of fibrous interstitium beneath the tegumental cytoplasm.

4 Nervous system

The sensory endings appear as bulb-like, sacciform expansions in the tegumental cytoplasm of the suckers, rostellum, and adjoining areas (figs. 15, 16, 24 & 25). A cilium-like, terminal process (about 200 mμ in diameter) extends from the sacciform expansion beyond the tegumental surface. The expansion contains large, round mitochondria, microtubules and small vesicles (120∼300 Å in diameter). Septate desmosomes are seen between the membranes of the sacciform expansion and tegumental cytoplasm only in the area where the terminal processes leave the surface. The nerve fiber extends from the sacciform expansion including the microtubules, into the parenchyma.

The bundles of unmyelinated nerve fibers (axons) are visible in the parenchyma (fig. 17). The fibers contain round mitochondria and small vesicles. The latter are usually small (400∼500 Å in diameter), and round, and their contents are usually electron-transparent; however, a few of the small vesicles have electron-opaque contents. The few large vesicles seen (about 1,000 Å in diameter) contained electron-opaque material. Cell bodies continuous with the nerve fibers were occassionally seen. The perikaryon contains a rather featureless nucleus, a few small mitochondria, ribosomes and numerous small vesicles resembling those in the adjacent nerve fibers (fig. 19).

Many synaptic junctions, probably motor endings, were evident among the muscle cells (fig. 18). These neuromuscular junctions contain numerous, small vesicles (400∼500 Å in diameter). Part of the vesicles in the synaptic junctions contained electron-opaque granules.

The membrane of the synaptic junctions is uneven in density.

5 Sucker

The sucker is a distinct organ which is separated from the adjacent tissue by a well developed basement membrane (figs. 20~22). It is covered with tegumental cytoplasm containing comparatively numerous mitochondria, and it has spine-like projections on the outer surface. The sucker is made up largely of muscle cells. On the plasma membrane of muscle cells which are in contact with the basement membrane are found half desmosomes associated with electron-opaque areas (fig. 21). Bundles of nerve fibers were found to pass through the basement membrane among the muscle cells.

6 Hooklet

The hooklet consists of amorphous material and longitudinal fibers; it is covered by a thin, electron-opaque layer, and includes a pulp cavity which opens at the root (figs. 23~25). The pulp cavity is filled by tegumental cytoplasm containing numerous, dense bodies; the same tissue covers the root of the hooklet.

B Brood capsule

Fundamentally, the inner surface of the wall of the brood capsule is lined by a thin syncytial cytoplasm (figs. 1 & 26~29) which is continuous with the tegumental cytoplasm of the protoscolex. The brood capsules fill compactly the fully developed echinococcal cysts, and their walls are adherent to one another. Thus, both surfaces of the walls of the brood capsules appear to be covered by syncytial cytoplasms strongly resembling that of the tegumental cell. The syncytial cytoplasm has microvilli on its inner surface, and contains vesicles larger than those of the tegumental cytoplasm. The perikarya of the syncytial cytoplasm, muscle cells, glycogen-containing cells, excretory ducts and spherular vacuoles regarded as vestiges of calcareous corpuscles are present along the inner side of the syncytial cytoplasm. In the interstices among the above-mentioned elements is a fibrous interstitium resembling that observed beneath the tegumental cytoplasm. The muscle cells, glycogen-containing cell, excretory ducts, and spherular vacuoles are morphologically identical with those of the protoscolex. The content of the cavities of the brood capsules includes numerous projections desquamated from their walls and from the tegument of the protoscolices, along with various electron-opaque fibrils and granules. These structures are all PAS-positive when viewed by means of the light microscope. Degenerative changes, such as transformation of the nuclei and desquamation of the projections and portions of the syncytial cytoplasm, were observed in some brood capsules in fully developed larvae.

C Cyst wall

It is readily discerned by means of the light microscope that the wall of the fully developed multilocular cyst is comprised of an outer, laminated, cuticular layer lacking nuclei, and an inner germinal layer. By means of the electron microscope, the cuticular layer (figs. 30~33) is found to consist of fine, fibrous, extracellular materials uniformly distributed. The outer side of the germinal layer is covered by syncytial cytoplasm, and the outer surface of the latter is attached to the cuticular layer by means of a number of microvilli (about 1,100~1,500 Å in diameter), which extend into the cuticular layer. Compared with the syncytial cytoplasm lining the wall of the brood capsule, that of the germinal layer is very thick, possesses better developed microvilli, and contains numerous small vesicles and many large mitochondria. The inner side of the germinal layer in the fully developed larva is continuous, without a line of demarcation, with the thin, syncytial cytoplasm lining the wall of the brood capsule. The distal, syncytial cytoplasm of the germinal layer is continuous with perikarya resembling those of the tegumental cell. The outer surface of the syncytial cytoplasm, with microvilli, is covered with a unit membrane (45~60 Å thick), similar to that of the tegumental cytoplasm. Muscle cells, glycogen-containing cells and excretory ducts are present in the interstices between the syncytial cytoplasms of both germinal layer and brood capsule. Vestiges of calcareous corpuscles are seen in the cytoplasm containing alpha glycogen particles. No essential differences between the components of the germinal layer and the wall of the brood capsule were found in the present study.

D Calcareous corpuscles

The few calcareous corpuscles remaining in the material studied exhibited a laminated structure; they were composed of fine granules and an amorphous substance (fig. 34). Calcium (by KOSSA's method and sodium alizarin sulfonate staining), polysaccharides (by MCMANUS' PAS method), acid polysaccharides (by toluidine blue staining in pH 7.0 and pH 2.5), protein (by ninhydrine SCHIFF and alloxane SCHIFF methods) and lipid (by Sudan black B and Sudan III stainings) were found histochemically in calcareous corpuscles.

DISCUSSION

The covering of cestodes has been considered by some authors to consist of three layers: a thin, exterior, cuticular layer; a thick, homogeneous cuticular layer; and a basement membrane. The cuticular layer has been usually regarded as being as substance secreted by long-necked, subcuticular cells in the parenchyma. Recent investigations by means of electron microscopy have shown that the cuticle is a syncytial, distal cytoplasm joined by cytoplasmic extensions to the subcuticular, tegumental cells lying in the parenchyma. ROTHMAN (1959) suggested the term "tegument", instead of "cuticle", because the former does not connote a non-living tissue.

The thin, external, cuticular layer described by means of the light microscope has been found to consist of projections of the tegumental cytoplasm. These projections were termed "microvilli" by READ (1955) and by KENT (1957), because they resembled the microvilli found on the intestinal mucosa of vertebrate animals. On the other hand, the projections were designated "microtriches" by ROTHMAN (1959, 1960, 1963), because several workers before 1900 had described and illustrated minute "fibrils" on the tegumental surface. This term was used also by THREADGOLD (1962, 1965), RACE et al. (1965), and BRÅTEN (1968). ROTHMAN (1963) stated that the solid, distal portion of the microthrix may serve as an additional means for resisting the current of the intestinal content of the host, but presumably does not have an absorptive function, whereas the proximal portion is medullated and capable of absorption. We consider that the term "microthrix" is appropriate for the projections of tegumental cytoplasm.

In contrast to the above, the medullated projections of syncytial cytoplasms covering the wall of the brood capsule and the germinal layer have no electron-opaque apical portions such as are found in the microvilli of the intestinal epithelium of vertebrates. Therefore, we consider that the term "microvilli" conveys the concept of the physiological function of these projections.

COUTELEN (1927), in *E. granulosus*, and VOGEL (1957), in *E. granulosus* and *E. multilocularis*, observed minute spines on the surface of the strobila by means of the light microscope. MORSETH (1966, 1967[12]) stated that the tegument of the strobila and the protoscolex of the larval *E. granulosus* consisted of a cytoplasmic modification (distal cytoplasm) of tegumental cells lying in the parenchyma, and that its surface was covered by projections. We have confirmed that the tegument of the protoscolex of the larval *E. multilocularis* is morphologically similar to the tegument of *E. granulosus* as described by MORSETH[12]. The projections on the anterior portion of the protoscolex, including the rostellum and suckers, are well developed, and of long, spinous form. These projections are covered by a unit membrane (plasma membrane) which is continuous with that of the outer surface of the tegumental cytoplasm. The electron-opaque, apical part of the spinous projections having longitudinal striations is separated from the basal part by a narrow, electron-lucid zone which bisected or trisected by one or two electron-opaque lines. These findings are similar to those of MORSETH (1967)[12], in regard to the scolex of *E. granulosus*. Numerous vesicles and tubules which are empty, or which contain bacilliform, electron-opaque material, considered to be analogous to MORSETH's[12] "dense bodies", are seen in the tegumental cytoplasm.

The infoldings of plasma membrane are found on both the outer and inner surfaces of the tegumental cytoplasm. These were observed also by THREADGOLD (1965), in *Proteocephalus pollanicoli* GRESSON, 1952. In addition, the distinct, shallow indentations of the basal plasma membrane occur at regular intervals, and the plasma membrane in those indentations are associated with electron-opaque areas in the tegumental cytoplasm, resembling half desmosomes. BRÅTEN (1968), in a study of *Diphyllobothrium latum* (LINNAEUS, 1758), stated that the basal plasma membrane folded at intervals into the tegumental cytoplasm, but no amorphous zone of greater density was present within the folds.

The pore canal and the pinocytotic vesicles observed in the tegument of other adult taeniid cestodes were not found in the photographs prepared for the present study. The absence of the pore canal in the tegument of the protoscolex of *E. multilocularis* agrees with MORSETH's (1967)[12] findings in *E. granulosus*.

The zone described by means of the light microscope as the basal membrane (MONNÉ, 1959), or the basement membrane (WISNIEWSKI, 1930), was called the basement membrane by RACE et al. (1965). THREADGOLD (1965) applied the term "basement membrane" only to the electron-opaque zone underlying the basal plasma membrane. On the other hand, BRÅTEN (1968) reported that the basal membrane, as seen with the light microscope, was the basement layer, which was divided into several zones; a plasma membrane; an amorphous zone of greater density set off by an electron-opaque zone; and a relatively thick region made up of a fibrous material. MORSETH (1966) stated that a less specific term should be used until the question concerning the specific germ layer of the tegument of cestodes is resolved. He applied the term "fibrous zone". THREADGOLD (1963) recognized the same structure in the epidermis of *Fasciola hepatica* LINNAEUS, 1758, and called it "interstitial material". We consider that the terms "basement membrane" and "fibrous interstitium" should be used for the thin, electron-opaque layer underlying the basal plasma membrane of the tegumental cytoplasm, and for the zone composed of loosely arranged, fine fibrils, respectively. The stringy structures connecting between the basement membrane and the electron-opaque areas of projections of the muscle cells are seen in the zone of fibrous interstitium. The cytoplasmic extensions of the tegumental cells in the zone of fibrous interstitium are surrounded by the compact, amorphous substance which extends from the basement membrane. From the findings described above, the stringy structure and basement membrane appears to serve as a bond between the tegumental cytoplasm and the parenchyma.

The perikaryon of the tegumental cell, as in other cestodes, occurs among the muscle cells and the glycogen-containing cells in the parenchyma. The vesicles which contain mostly electron-opaque material tend to gather close to the Golgi complex of the tegumental cell. Similar findings were reported by MORSETH (1967)[12] in *E. granulosus*. These vesicles were also identified in other areas of the perinuclear cytoplasm and in the cytoplasmic extensions. These findings seem to indicate that the vesicles pass from the Golgi complex into the tegumental cytoplasm.

A zone with the same structure as the fibrous interstitium is seen in the extracellular spaces such as those surrounding the excretory system in the protoscolex, as well as in the intercellular spaces between the syncytial cytoplasms of the adjoining brood capsules and between the syncytial cytoplasms of the brood capsules and the germinal layer. On the other hand, the zone surrounding the suckers is composed of an amorphous substance as the basement membrane of the tegument. Half desmosomes are visible on the plasma membrane of muscle cells in contact with the aforementioned zone. We consider, therefore, that the term "basement membrane" should be applied to the zone apart from the fibrous interstitium described above.

We confirmed that the muscle bundles under the tegumental cytoplasm correspond to the subcuticular muscles described by means of the light microscope, and consist of myofibrillar projections extending from muscle cells into the parenchyma. The projecting cytoplasm of the individual muscle cells lying in the parenchyma consist of two portions: amyofibrillar and myofibrillar. These findings agree with those of LUMSDEN (1967), pertaining to adult tetraphyllean, trypanorhynchan, and cyclophillidean cestodes. The electron-opaque glycogen particles in the amyofibrillar portion reveal the same structure as described by LUMSDEN (1965, 1966, 1967) in some adult cyclophyllidean and trypanorhynchan cestodes. LUMSDEN & BRYAM (1967) stated that the contractile portion of the muscle cell consists of a single, elongate myofiber, which may be branched in some areas, especially in the transverse layer. The present electron micrographs show that the perinuclear cytoplasm possesses some processes which extend and branch to the amyofibrillar or to the myofibrillar portions of the cells. In some muscle cells, however, bundles of myofilaments are present also in the perinuclear cytoplasm. The number of thin myofilaments surrounding the single thick myofilament ranges from nine to 16.

Besides the aforementioned muscle cells, the cells which contain numerous alpha particles of glycogen in the cytoplasm including the perinuclear cytoplasm, are recognized in the parenchyma. No myofibril, however, is found in the projections of those cells. Therefore, it is supposed that the glycogen-containing cells (glycogen storage cells) are present in the parenchyma.

The glycogen-containing cytoplasm contains vacuoles from which calcareous corpuscles have disappeared through the process of preparing the material, and various inclusions which consist of lipid, lamellae, crystaloids, and/or filaments, respectively. As far as we observed in the present investigation, we could not ascertain whether the glycogen-containing cytoplasms with the calcareous corpuscles and various inclusions belong to the muscle cell or to the glycogen-containing cell. Calcium, polysaccharides, acid polysaccharides, protein and lipid were found histochemically in calcareous corpuscles. Their existence in the same area of cytoplasm would seem to indicate that some of these inclusions evolve into calcareous corpuscles. Detailed observations on calcareous corpuscles could not be made in the present material, because they were destroyed in the process of preparation. The genesis of calcareous corpuscles should be investigated in larvae in different stages of development by other methods.

The flame cells with cilia showing the pattern 9+2, and the excretory ducts consisting of cytoplasmic modifications similar to those in the tegumental cytoplasm are essentially the same as those described by MORSETH (1967)[12] in *E. granulosus*. Similar findings have been obtained in *Multiceps serialis* (GERVAIS, 1847) and *Hymenolepis diminuta* (RUDOLPHI, 1819) by RACE et al. (1965) and LUMSDEN (1965), respectively.

MORSETH (1967)[13] did not observe sensory endings in sections of protoscolices of *E. granulosus*. However, we found considerable numbers of sensory endings in the relatively thin tegumental cytoplasm of the suckers and rostellum. Motor endings (neuromuscular junctions) containing numerous, small vesicles were identified among the muscle cells. The membrane of these junctions is thickened opposite to the muscle cells. The sensory endings, nerve fibers, and motor endings show essentially the same structure as do those in the adult *E. granulosus* as described by MORSETH (1967)[13], and in the cercaria of *Fasciola hepatica*, as described by DIXON & MERCER (1965).

We found that the hooklets are surrounded by tegumental cytoplasm which penetrated into the hollow root of the hooklet. These findings indicate that the tegumental cytoplasm has an important role in the formation of hooklets. This opinion was stated also by RACE et al. (1965), who observed a vacuolated, cuticular membrane surrounding the root of the hooklet in the larval *Multiceps serialis*.

It has been considered previously, on the basis of light microscopy, that only the inner surface of the wall of the brood capsule was lined by a thin cuticule extending from that of the protoscolex. MORSETH (1967)[12] also reported that the wall of the brood capsule of *E. granulosus* is a highly vacuolated layer with nuclei at irregular intervals. We also confirmed that the inner surface of the wall of the brood capsule of *E. multilocularis* is covered essentially by the syncytial cytoplasm similar to that of the tegument. The syncytial cytoplasm has numerous vacuoles and microvilli. The vesicles are large and more vacuous than are those of the tegumental cytoplasm. The perikarya of the syncytial cytoplasm, muscle cells, glycogen-containing cells, excretory ducts, and fibrous interstitium are found along the syncytial cytoplasm. The walls of the brood capsules, which occur in compact masses in the fully developed larvae of *E. multilocularis*, adhere to one another. The above-mentioned elements are present in the interspaces between the walls of adjoining brood capsules. Some degenerative changes, including transformation of nuclei and desquamation of microvilli and the syncytial cytoplasm, are often observed in brood capsules of fully developed, echinococcal tissue. Consequently, the completion of protoscolices and the attainment of infectivity is evidently followed by degeneration of some of the cells in the walls of the brood capsules.

Evidence obtained by light microscopy indicates that the wall of the fully developed cyst is comprised of the outer, laminated, cuticular layer lacking cells, and of the inner germinal layer. MORSETH (1967)[12] determined by means of the electron microscope, that the outer, laminated layer of *E. granulosus* is made up of layers of particulate material in a back ground of fine, irregularly arranged fibers. In contrast to MORSETH's[12] findings, we recognized no laminated structure, but the cuticular layer of the cyst of *E. multilocularis* consisted of fine, extracellular, fibrous material distributed uniformly.

The outer surface of the germinal layer of the cyst of *E. multilocularis* is overlain by the distal, syncytial cytoplasm, which is attached to the cuticular layer by means of microvilli extending into the extracellular materials (cuticular layer). On the inner side of the germinal layer are many components: perikarya of syncytial cytoplasm, resembling those of tegumental cells; muscle cells; glycogen-containing cells; excretory ducts; vestiges of calcareous corpuscles; and fibrous interstitium. These components are continuous, without a line of demarcation, with those of the adjoining wall of the brood capsule. In contrast with the syncytial cytoplasm of the brood capsule, that of the germinal layer is very thick and has many well developed microvilli; it also contains numerous mitochondria of various sizes and shapes. The differences between the two types of syncytial cytoplasm are perhaps attributable to differences in physiological function, e. g., absorption of nutrient and secretion or excretion. Except for the differences in the syncytial cytoplasm, no essential differences in the components of the germinal layer and brood capsule could be confirmed in the present study.

We recognized that the tegument, wall of the excretory duct, and coverings of the wall of the brood capsule and germinal layer in the larval *E. multilocularis* consist essentially of syncytial cytoplasm which extends from cells having similar cyto-morphological characteristics. These cells contain many vesicles, with or without electron-opaque material, in the distal, syncytial cytoplasm and in proximity to the Golgi complex of the perinuclear cytoplasm. On the other hand, the muscle cells in the parenchyma and suckers of protoscolex, brood capsules, and germinal layer appear uniform; the muscle cell comprises the perinuclear cytoplasm and the amyofibrillar and myofibrillar portions of the projections which are filled mainly by ribosomes, glycogen particles, and myofilaments. Except for special cells, such as those of the nervous system, glycogen-containing cells and the flame cells, it is concluded that the cells in the tissue of *E. multilocularis* comprise two cell-types, as mentioned above. However, we presume that undifferentiated cells other than the above-mentioned may be found in the tissue of the larval *Echinococcus*. For example, the term "germinal cell" is applied to any cells observed by means of the light microscope in the germinal layer, but, in the strict sense, this term should be applied to undifferentiated cell.

SUMMARY

The general fine structure were studied on fully developed larval *Echinococcus multilocularis* in cotton rats by use of electron microscope.

The tegument of protoscolex consists essentially of the distal, syncytial cytoplasm (tegumental cytoplasm) of tegumental cell in the parenchyma. The surface of tegument is covered with spinous projections, on the rostellum and suckers, and with knob-like projections, on the posterior part of protoscolex. The tegumental cytoplasm contains vesicles and tubules with or without electron-opaque material. The cytoplasmic continuity is recognized between the tegumental cytoplasm and the perinuclear cytoplasm of tegumental cell. The basement membrane is found beneath the basal plasma membrane of tegumental cytoplasm. The zone of fibrous interstitium underlaying the basement membrane is continuous with the extracellular interspace in the parenchyma.

The muscle cells in the parenchyma and sucker of protoscolex, brood capsule and germinal layer comprise the perinuclear cytoplasm with ribosomes and the projections which consist of the amyofibrillar portion with glycogen particles and myofibrillar one with myofibrils. The cells containing numerous alpha particles of glycogen in the perinuclear cytoplasm and the projections, are found in the above-mentioned places. The sucker established by a mass of muscles and is surrounded with a basement membrane.

In the tegumental cytoplasm of rostellum and suckers, the sensory endings are confirmed and show the bulb-like expansion with a cilium-like terminal process. Unmyelinated nerve fibers and those synaptic junctions are recognized among the muscle cells in the parenchyma and the sucker. The nerve fiber contains mitochondria and vesicles, and the synaptic junction has many vesicles accumulating on one side of it.

The flame cell in the parenchyma has a bundle of cilia characterized by the general pattern (9+2) and the bundle is enveloped with the extension of membraneous cytoplasm. The wall of the excretory duct is made up of the syncytial cytoplasm showing the same character as the tegumental cell.

The inner surface of the wall of the brood capsule is covered with the syncytial cytoplasm possessing the villiform projections (microvilli). The perikarya of the syncytial cytoplasm, muscle cells, glycogen-containing cells, and excretory ducts occur along the outer side of the syncytial cytoplasm. The interspace among the above elements is filled with fibrous interstitial material (fibrous interstitium).

The cuticular layer of the cyst wall is composed of extracellular, fine, fibrous material which distributes quite equally without lamination. The outer surface

of the germinal layer is overlain with the distal, syncytial cytoplasm resembling tegumental cytoplasm. The distal, syncytial cytoplasm of the germinal layer containing a number of mitochondria attaches to the cuticular layer, and is ornamented with a number of well developed microvilli which invade into the cuticular layer. The perikarya of the syncytial cytoplasm, muscle cells, glycogen-containing cells, excretory ducts and fibrous interstitium are recognized along the inner side of the distal, syncytial cytoplasm. Those components are continuous, without a line of distinction, to those of the adjoining brood capsule wall.

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EXPLANATION OF PLATES

All scales printed in the figures are shown at 1 μ.

PLATE I

Fig. 1 The cellular arrangement of the tegument in posterior portion of protoscolex and the wall of brood capsule

The cytoplasmic bridge (Cb) extended from the perinuclear cytoplasm (Pc) of tegumental cell in the parenchyma is continuous with the distal, syncytial cytoplasm (tegumental cytoplasm; Tc). The surface of tegument is covered with a number of small knob-like projections (microtriches; Mt). The tegumental cytoplasm contains many vesicles (V) and tubules with or without electron-opaque material. The basal plasma membrane of tegumental cytoplasm is underlain with the basement membrane (Bm). The zone of fibrous interstitium (Fiz) occurs beneath the basement membrane, and the same structure (Fi) is seen in the extracellular space of parenchyma also. The shallow indentations (arrows) of plasma membrane with the basement membrane appear at regular intervals on the inner surface of tegumental cytoplasm. The bundles of myofibrillar projections (Mf) occur beneath the fibrous, interstitial zone. The stringy structures (double arrows) connected with electron-opaque areas of myofibrillar projections are seen in the zone of fibrous interstitium. The surface of two adjoining brood capsule walls is overlain with syncytial cytoplasm (Sc), but the desquamation of syncytial cytoplasm is seen on the underlying brood capsule wall. The perikaryon (P) of the syncytial cytoplasm, excretory duct (Ed) and fibrous interstitium are recognized along the inner side of the desquamated syncytial cytoplasm.

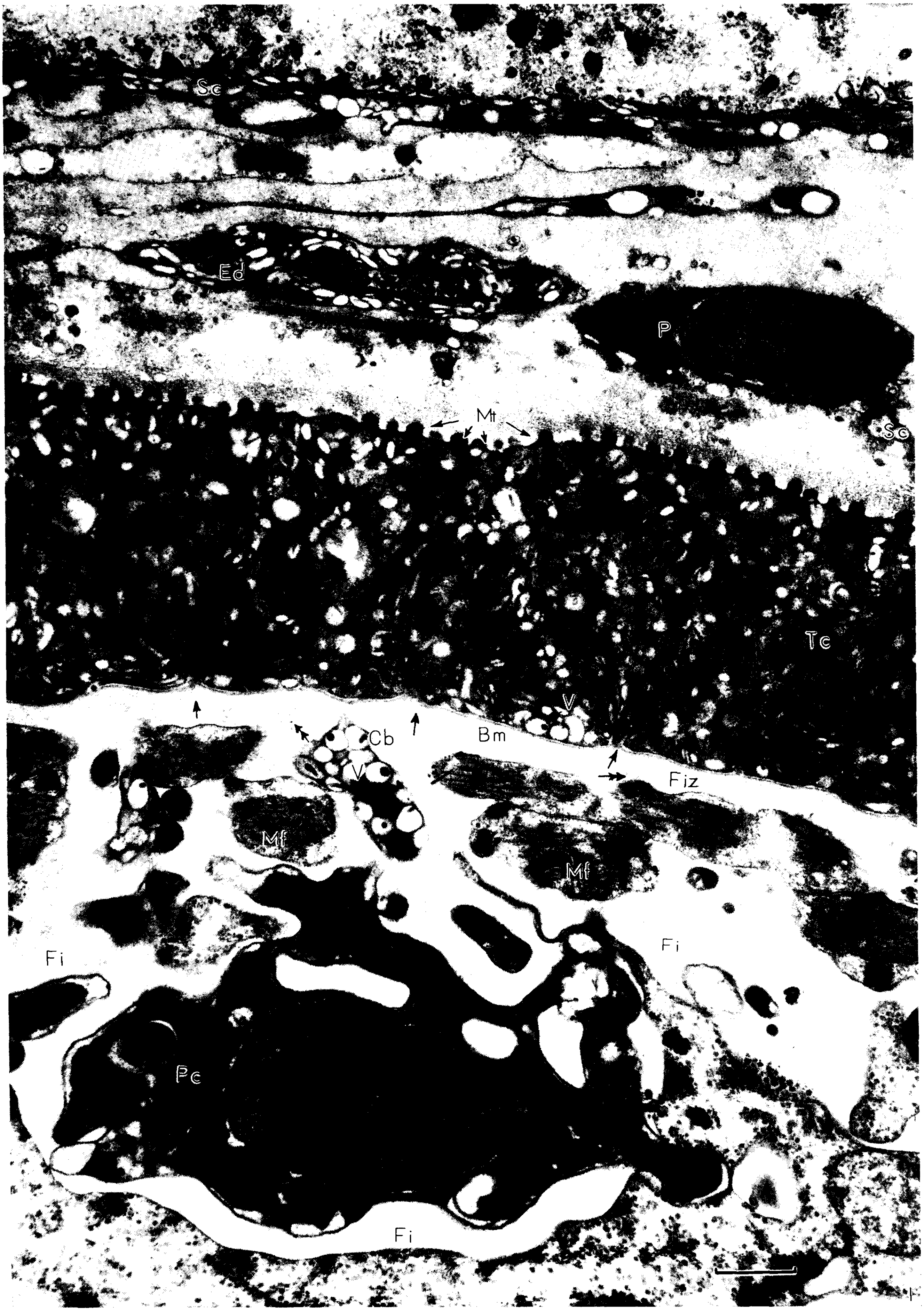

Sc
Ed
P
Mt
Sc
Tc
V
Cb
Bm
V
Fiz
Mf
Mf
Fi
Fi
Pc
Fi

Plate II

Fig. 2 The cytoplasmic continuity (Cb) is recognized between the tegumental cytoplasm (Tc) and the perinuclear cytoplasm (Pc) of tegumental cell. The surface of the tegument is covered with electron-opaque, extracellular substance (PAS-positive substance; Ps).

Fig. 3 Spinous projections (microtriches) on the outer surface of anterior portion of protoscolex
The projections are covered with a unit membrane. Apical part (A) of the projections is separated from the basal part by a narrow, electron-lucid zone bi- or trisected by one or two electron-opaque, transversal lines (arrows). The tubules (Tu) are seen in the tegumental cytoplasm.

Fig. 4 The indentation (arrow) of plasma membrane with the basement membrane are seen on the inner surface of tegumental cytoplasm (Tc). Beneath the basement membrane, there is a zone of fibrous interstitium (Fiz) which consists of loose reticular fibrils. The electron-opaque, extracellular substance (Ps) occurs on the surface of tegument.

Fig. 5 The plasma membrane in the indentation of the inner surface of tegumental cytoplasm is associated with the electron-opaque area as if they are the junction of half desmosome (arrow). The junction is continuous with stringy structure (Ss) consisting of fine fibrils in the zone of fibrous interstitium (Fiz).

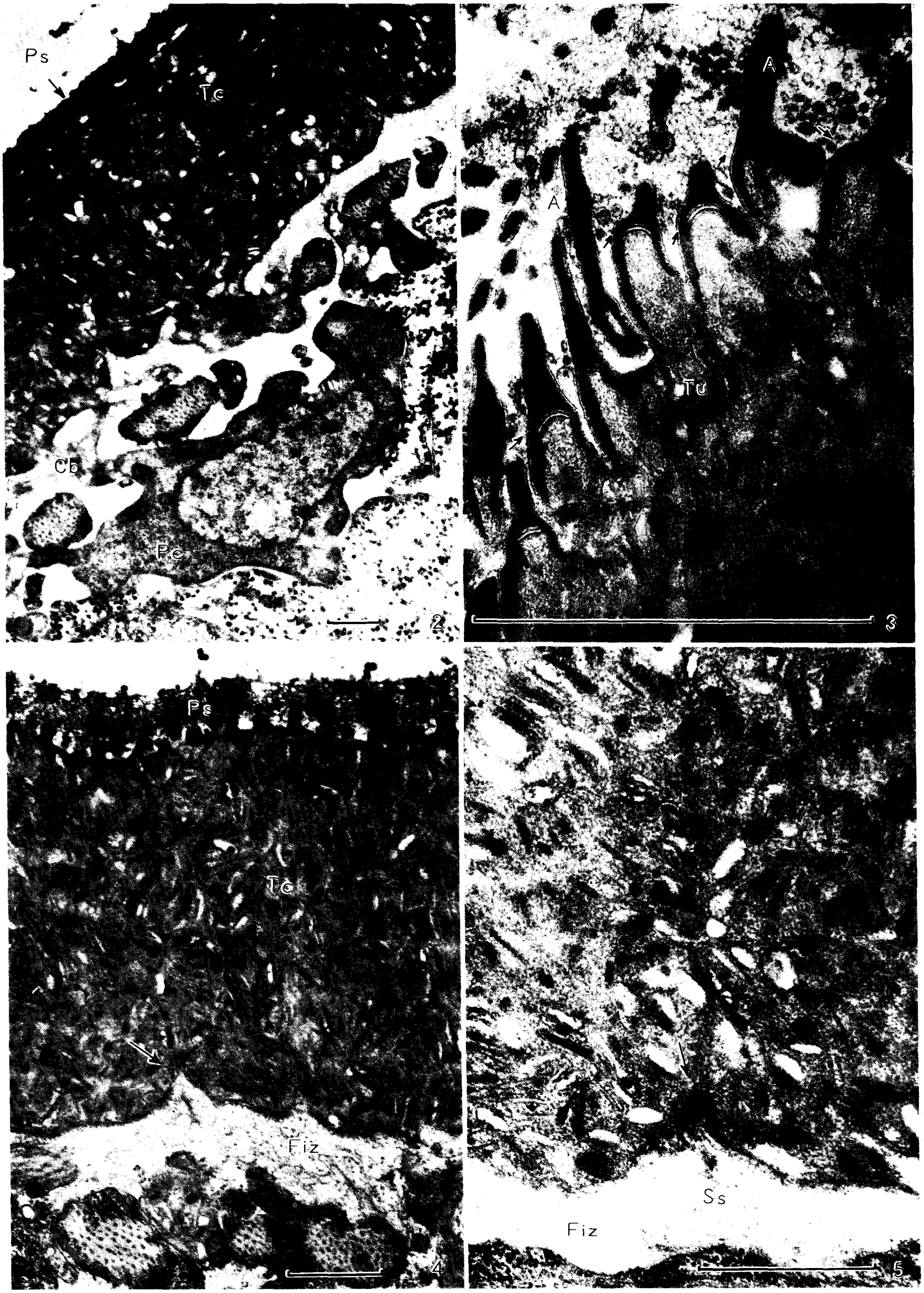
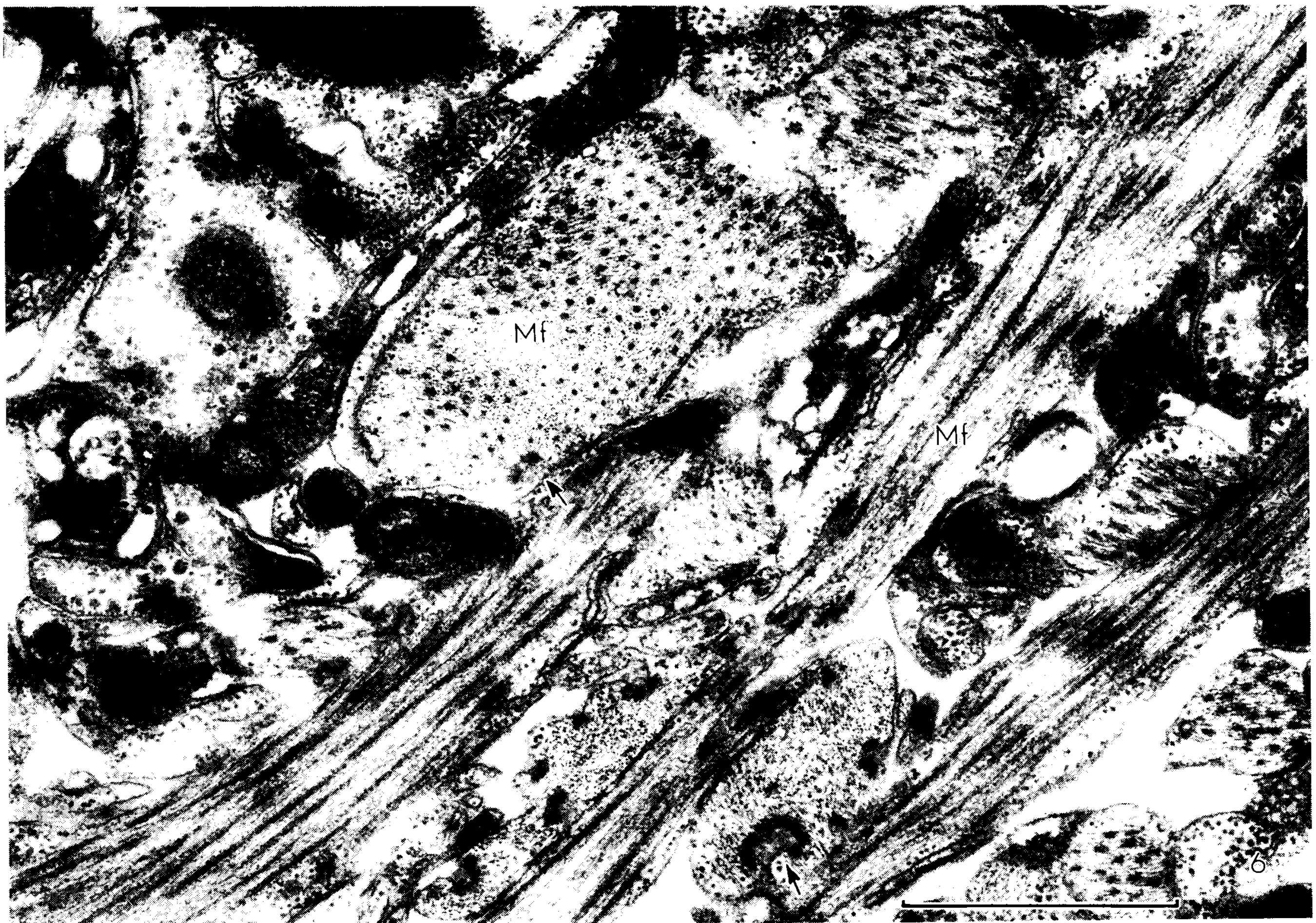

Ps
Tc
Cb
Pc
2
A
A
Tu
3
Ps
Tc
Fiz
4
Ss
Fiz
5

PLATE III

Fig. 6 Muscle cells in the parenchyma of protoscolex
The myofibrillar projections (Mf) of muscle cells contain a number of myofilaments which consist of a central thick myofilament and some ten surrounding thin ones. The shallow indentations (arrows) of sarcolemma are recognized to be associated with electron-opaque areas at the peripheral sarcoplasm.

Fig. 7 The perinuclear cytoplasm of muscle cells in the parenchyma of protoscolex comprises the perinuclear cytoplasm with ribosomes and a few mitochondria. Some of muscle cells, however, have the bundles of myofibrils in the perinuclear cytoplasm also (arrow).

Fig. 8 The cells containing many massive granules of alpha particles of glycogen in the perinuclear cytoplasm (glycogen-containing cell; Gc) are seen in the parenchyma of protoscolex.


Mf
Mf
6


7


Gc
8

PLATE IV

Fig. 9 The longitudinal section of a flame cell (Fc) in the parenchyma of protoscolex
The bundle of cilia (C) is enveloped with the membraneous cytoplasm (Ce) extending from the perinuclear cytoplasm.

Fig. 10 The cross section of flame cell and excretory duct (Ed) in the parenchyma of protoscolex
Most of the cilia are characterized by the general pattern (9+2) and the bundle of cilia is enveloped with a cytoplasmic wall (Ce) possessing microvilli (Mv).

Fig. 11 Enlargement of the flame cell in figure 9
The bundle of cilia is enveloped with cytoplasmic extension (Ce). A small excretory duct (Ed) is recognized near the apical part of the bundle of cilia.

Fig. 12 Enlargement of the flame cell in figure 9
Basal bodies (B) and striped rootlets (R) occur at the base of bundling cilia (C).

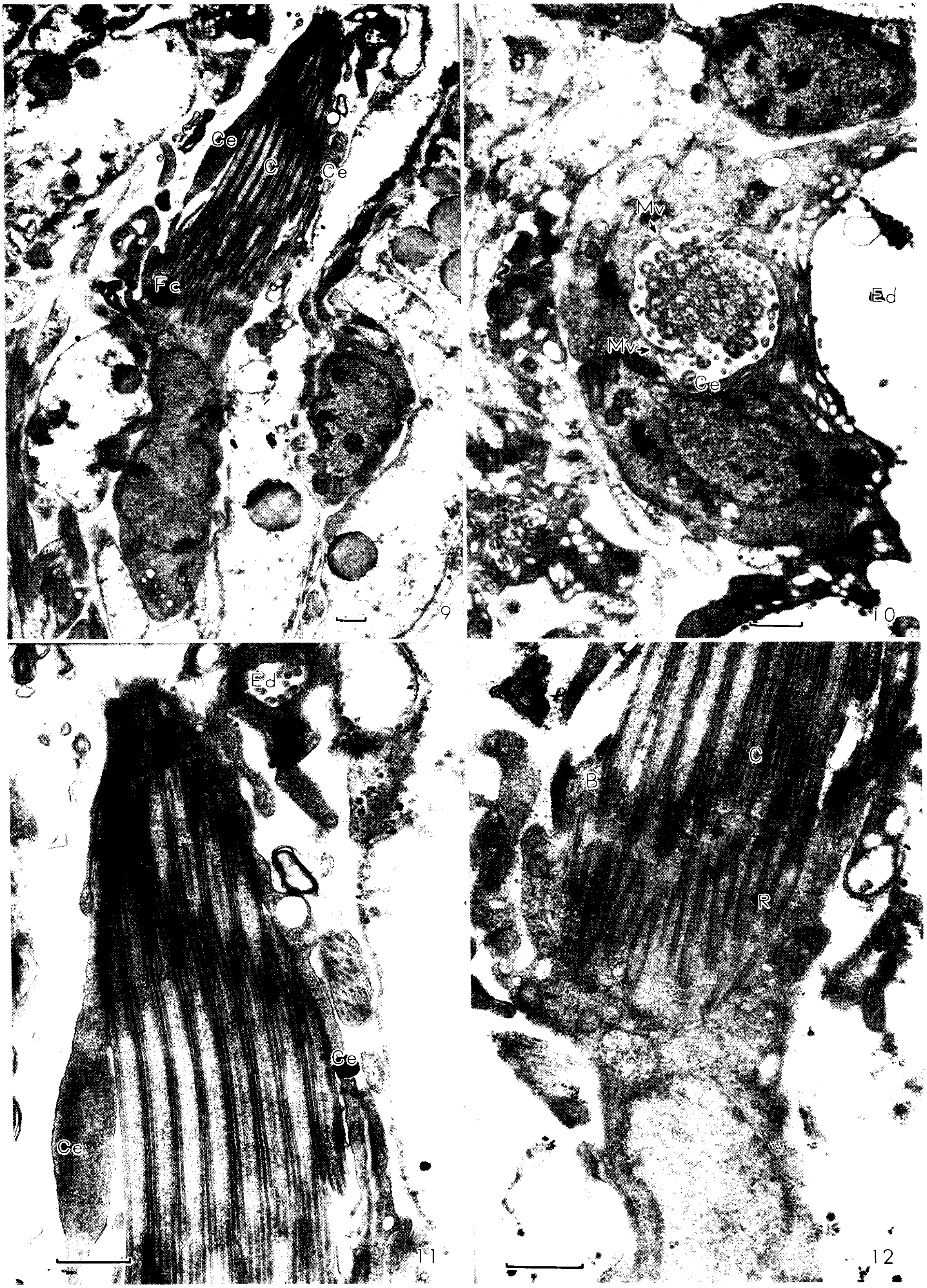

Ce
C
Ce
Fc
9
Mv
Mv
Ce
Ed
10
Ed
Ce
Ce
11
B
C
R
12

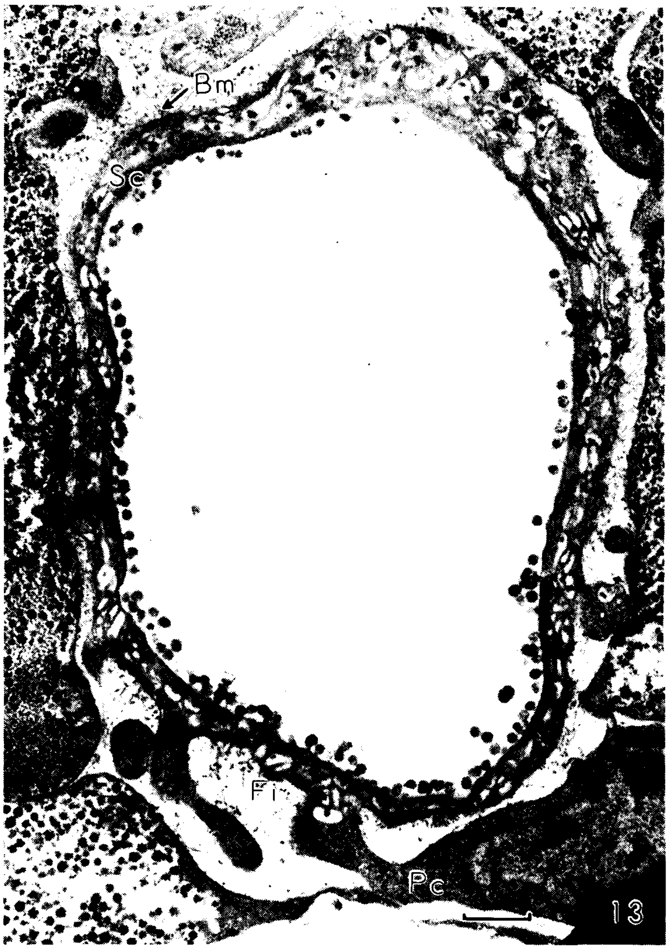
Plate V

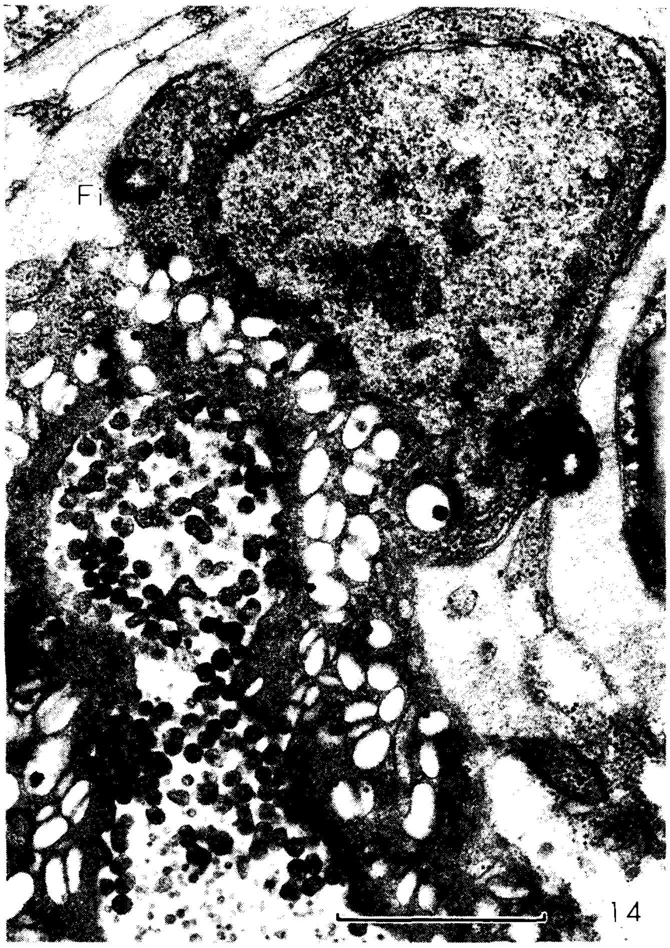
Fig. 13 The excretory duct in the parenchyma of protoscolex
The wall of excretory duct is constructed of distal, syncytial cytoplasm (Sc) which is continuous with the perinuclear cytoplasm (Pc) of the cell showing similar characteristics to those of tegumental cell. The excretory duct is surrounded with basement membrane (Bm) and fibrous interstitium (Fi).

Fig. 14 The excretory duct in germinal layer shows the same structure as that in protoscolex. The excretory duct is surrounded with only fibrous interstitium (Fi).

Fig. 15 The sensory ending in the tegumental cytoplasm
The cilium-like terminal process elongates from the sacciform expansion. The expansion contains large mitochondria (M) and small vesicles. Septate desmosomes (Sd) connect the unit membrane of the expansion with the adjoining plasma membrane of tegumental cytoplasm.

Fig. 16 The sensory ending in the tegumental cytoplasm near hooklet (H)
The microtubules (Tu) and a mitochondrion (M) are contained in the expansion. Septate desmosome (arrow) is recognized on the cell membrane of the expansion.


Bm
Sc
Fi
Pc
13


Fi
14


Sd
Sd
M
15

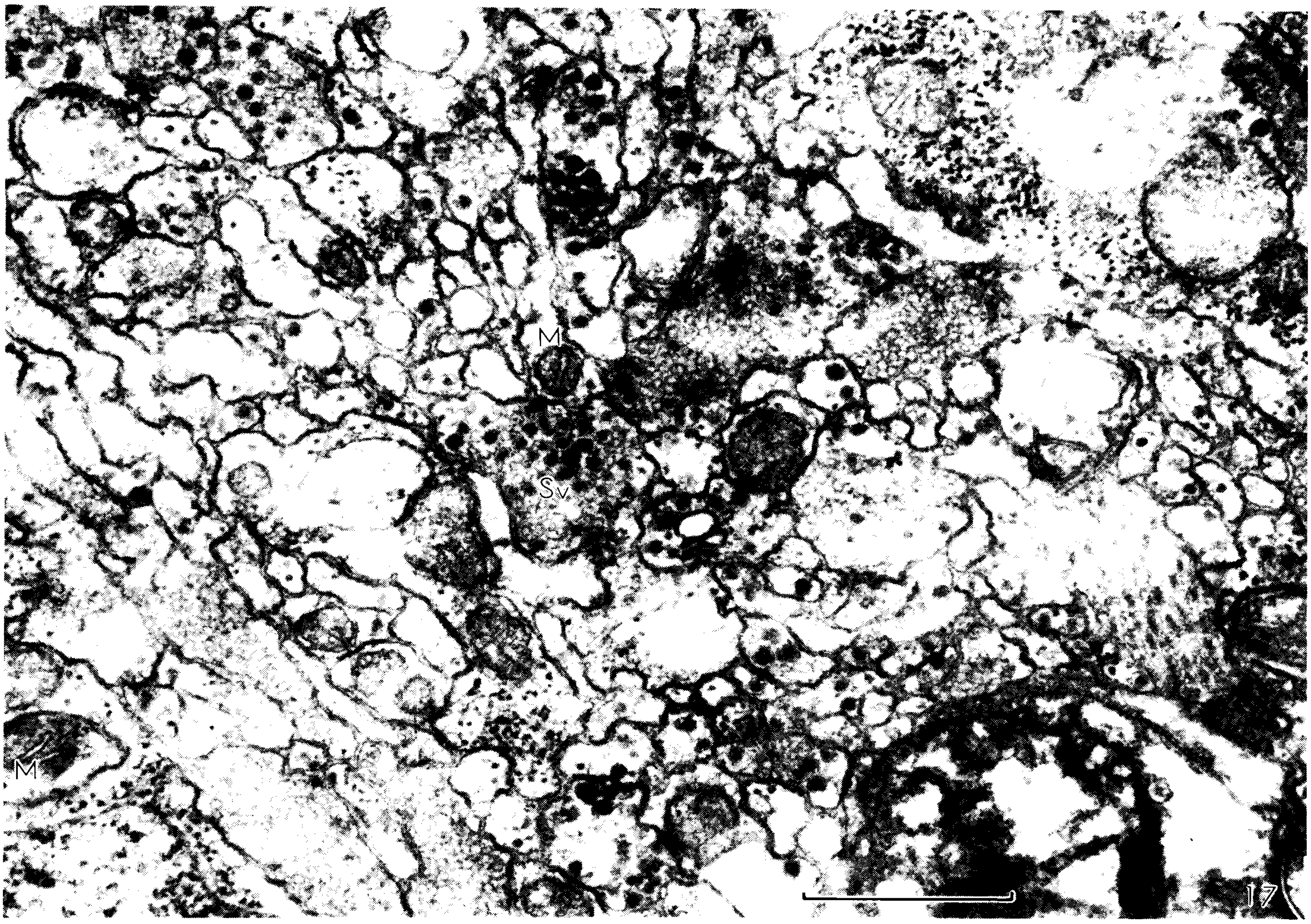

M
Tu
H
16

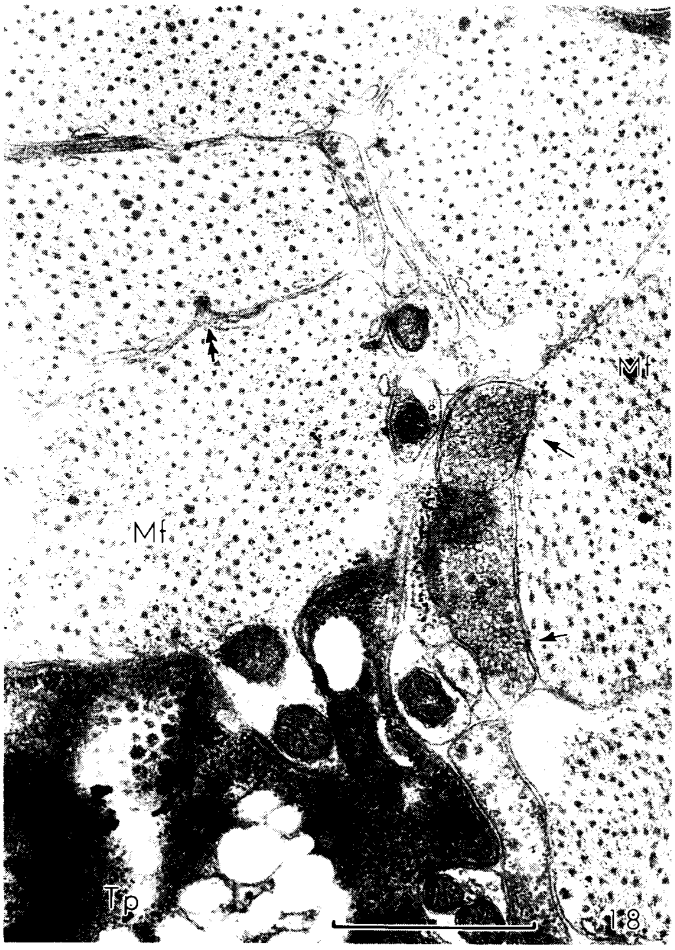
PLATE VI

Fig. 17 The bundles of nervous fibers and many synaptic vesicles (Sv) in the parenchyma of protoscolex
The nervous fibers and the synaptic junctions contain mitochondria (M) and many small vesicles with or without electron-opaque substance.

Fig. 18 Synaptic junction (presumed neuromuscular junction) containing large numbers of small clear vesicles and a few cored vesicles (synaptic vesicles) is seen among the myofibrillar projections of muscle cells (Mf) and tegumental cell (Tp) in the parenchyma of protoscolex. The plasma membrane of nerve (synaptic membrane) and muscle cell are closely adherent without intervention of a basement membrane. In these areas the opposing membranes are denser (arrows). The shallow indentation (double arrow) is seen in the myofibrillar projection.

Fig. 19 The cell body of nervous cell is found among the nervous fibers in the parenchyma of protoscolex. The perikaryon contains the rather featureless nucleus, a few mitochondria and many small vesicles (arrow) similar to those in adjacent nervous fibers.


M
Sv
M
17


Mf
Mf
Tp
18

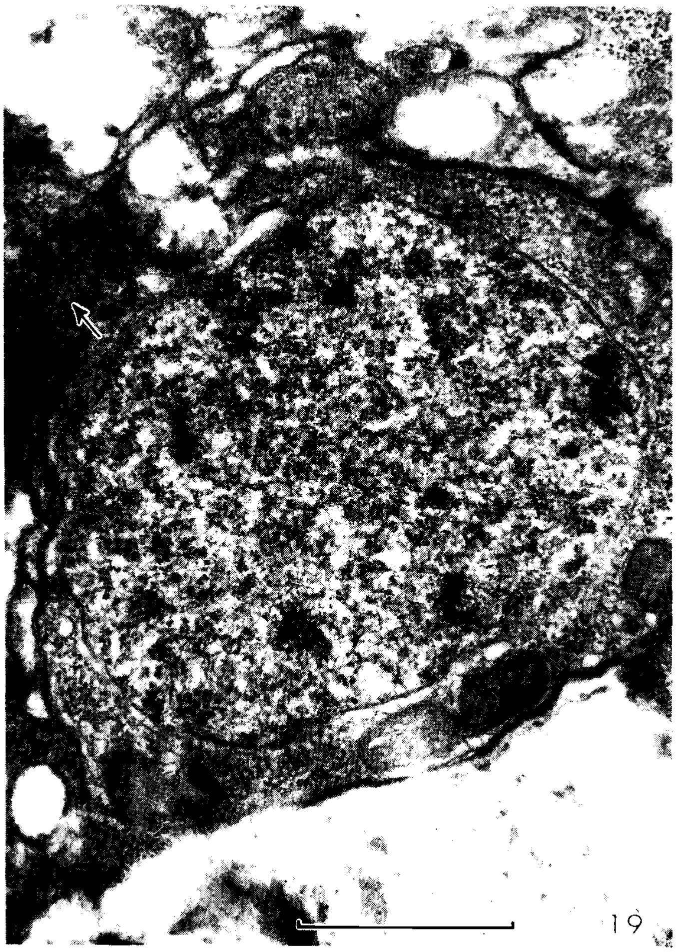
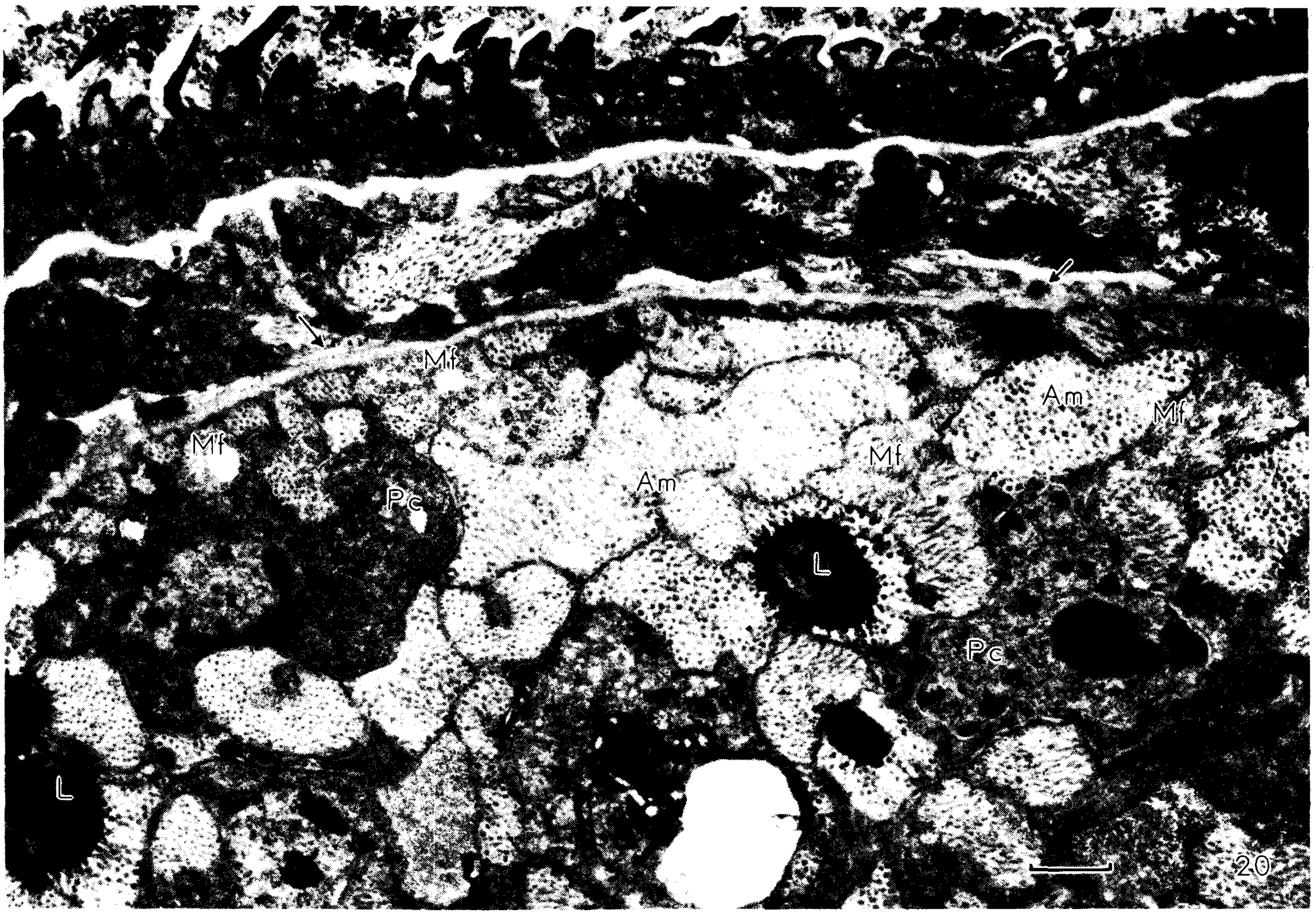

19

PLATE VII

Fig. 20 The sucker surrounded with a basement membrane (arrows) is compactly filled up with muscle cells which consist of perinuclear cytoplasm (Pc) with ribosomes, amyofibrillar projections (Am) with alpha particles of glycogen and inclusion (Lipid; L) and myofibrillar ones (Mf) with myofibrils and beta particles of glycogen.

Fig. 21 The sucker separated from neighbouring tissue with a basement membrane (Bm). Half desmosomes (arrows) associated with electron-opaque patches are seen on the plasma membrane of muscle cells which are in close contact with basement membrane. Muscle cell with myofibrillar projection (Mf) and tegumental cell containing vesicles (V) with or without dense body in perinuclear cytoplasm are seen in the parenchyma.

Fig. 22 The outer surface of sucker is covered with the tegumental cytoplasm (Tc) which possesses the well developed spinous projections. The inner surface of sucker filled with muscle cells is separated from adjacent tissue with basement membrane (arrows).


Mf
Mf
Mf
Mf
Am
Am
Pc
Pc
L
L
20


V
Bm
Mf
21

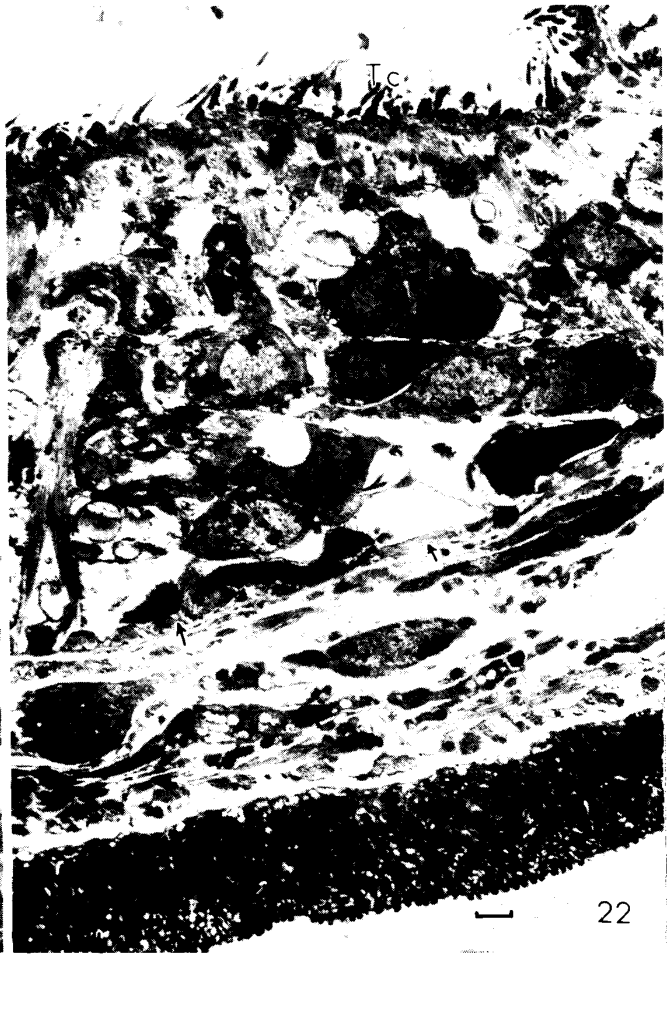

Tc
22

Plate VIII

Fig. 23 The longitudinal section of hooklet (H)
The hooklet has a pulp cavity which contains tegumental cytoplasm with numerous dense bodies.

Fig. 24 The longitudinal section of hooklet (H)
A sensory ending (Se) occur in the tegumental cytoplasm near hooklet.

Fig. 25 The cross section of hooklet (H)
A sensory ending (Se) is seen in the tegumental cytoplasm near the hooklet. The infolding (arrow) of plasma membrane connected with empty tubule (Tu) is recognized on the inner surface of tegumental cytoplasm.

23

24

25

PLATE IX

Fig. 26 In this micrograph, the walls of three brood capsules are seen to adhere each other. The perikaryon (Pc) of syncytial cytoplasm is recognized in the interspace between the syncytial cytoplasms (arrows). The cavities of brood capsules contain numerous projections desquamated from the walls of brood capsules and the tegument of protoscolices, electron-opaque fibrils and granules.

Fig. 27 The wall of brood capsule is overlain with syncytial cytoplasm possessing microvilli (Mv). The perikaryon (Pc) and myofibrillar projections of muscle cell are seen between the syncytial cytoplasms.

Fig. 28 The walls of brood capsules
The perikaryon (Pc) of a muscle cell is seen between the syncytial cytoplasms (Sc) which contain many vesicles with dense bodies.

Fig. 29 The degenerated perikaryon (Pc) of muscle cell and desquamation of microvilli of syncytial cytoplasms (Sc) of brood capsules

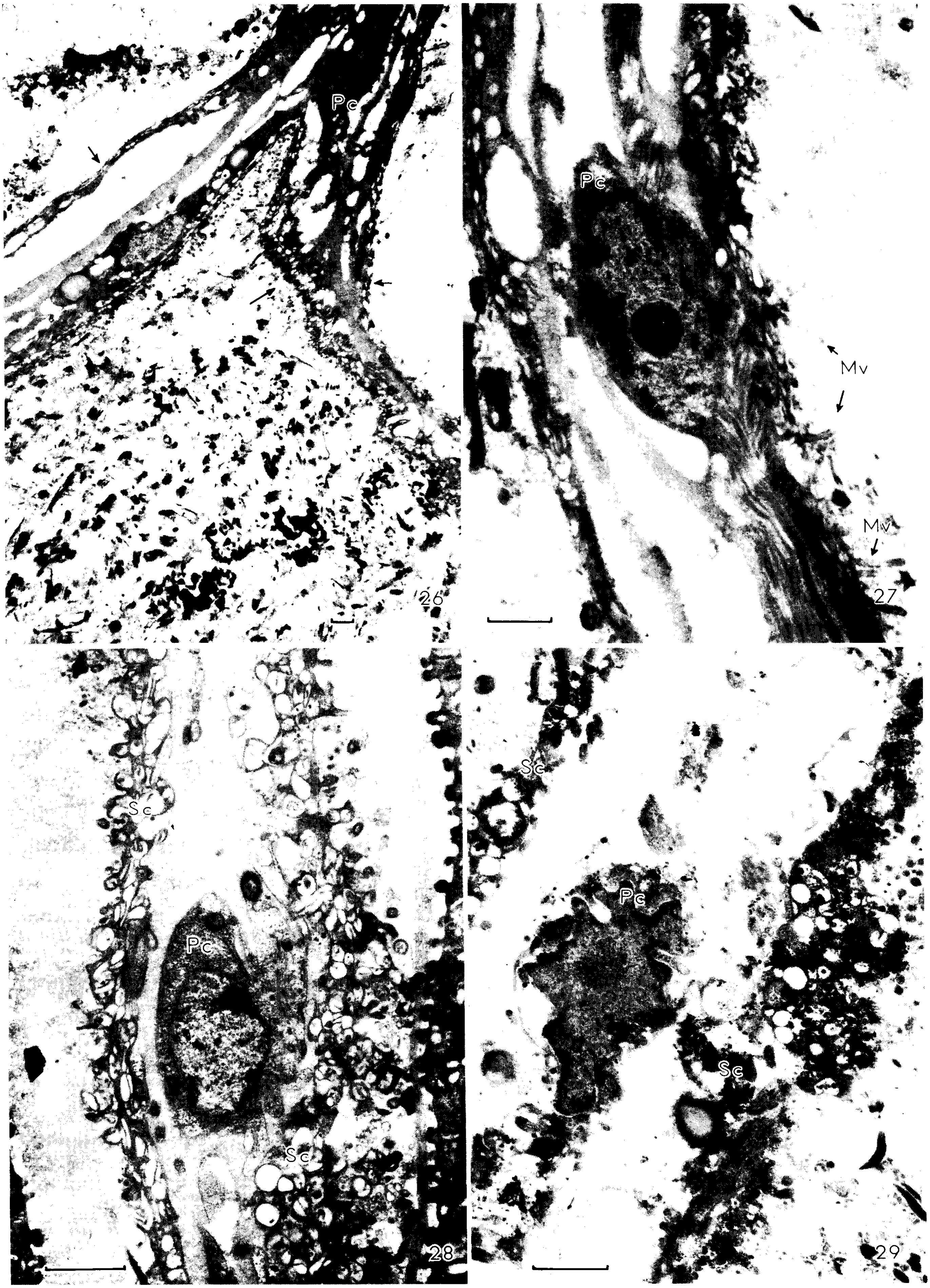
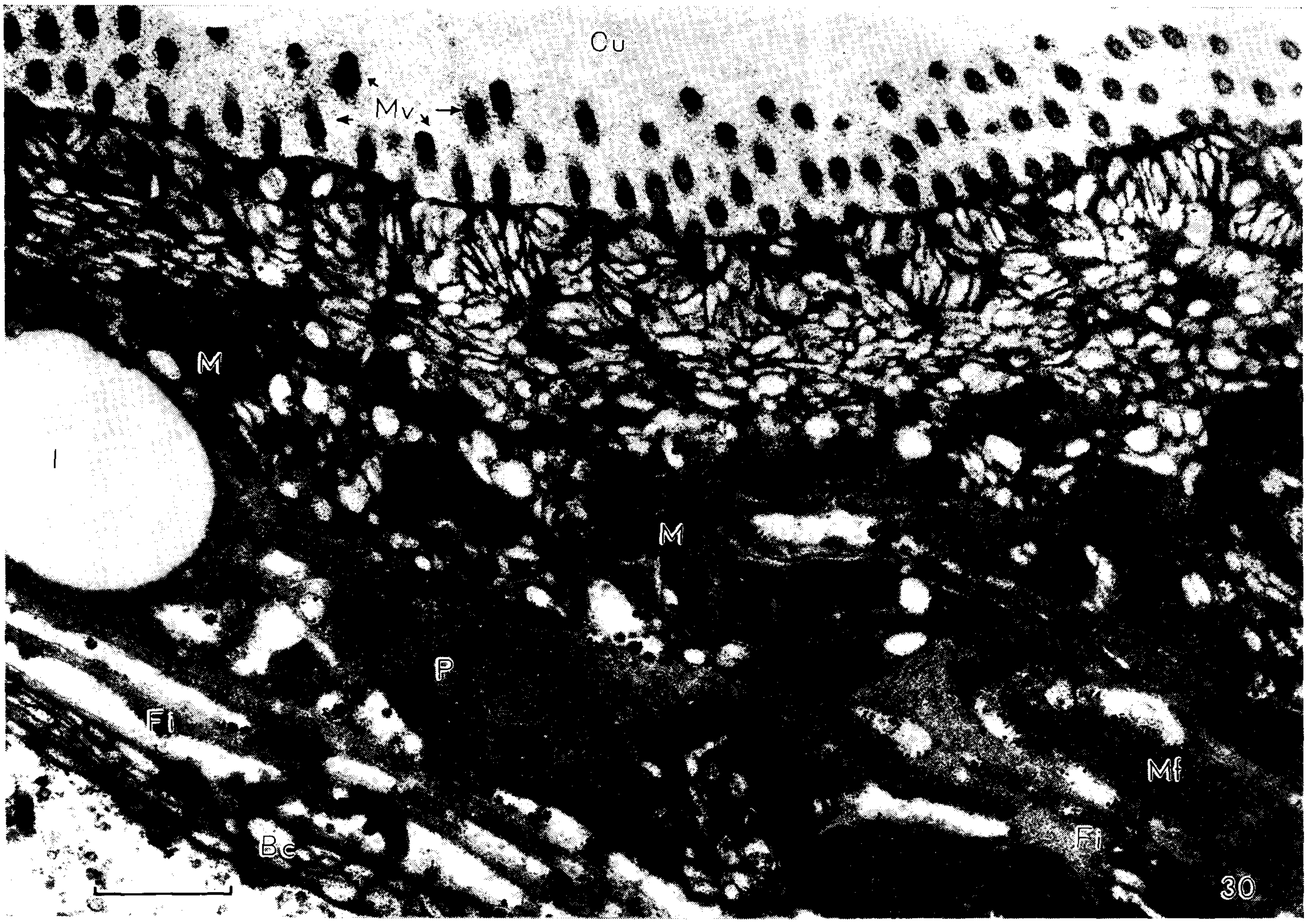

Pc
26
Pc
Mv
Mv
27
Sc
Pc
Sc
28
Sc
Pc
Sc
29

PLATE X

Fig. 30 The outer layer (cuticular layer; Cu) of cyst wall is composed of extracellular fibrous material distributing equally. The outer surface of germinal layer covered with the thick distal, syncytial cytoplasm possessing a number of well-developed microvilli (Mv) and mitochondria (M). The inner side of germinal layer is lined with the wall of brood capsule (Bc). The perikarya of syncytial cytoplasm, muscle fibers (Mf), inclusion (I) and fibrous interstitium (Fi) occur between two syncytial cytoplasms.

Fig. 31 Along the inner side of syncytial cytoplasm (Sc) of germinal layer, there are many muscle fibers (Mf) surrounding fibrous interstitium (Fi). Many microvilli (Mv) penetrate into the cuticular layer (Cu). The inner surface of germinal layer is lined with brood capsule wall (Bc).


Cu
Mv
M
I
M
P
Fi
Mf
Bc
Fi
30

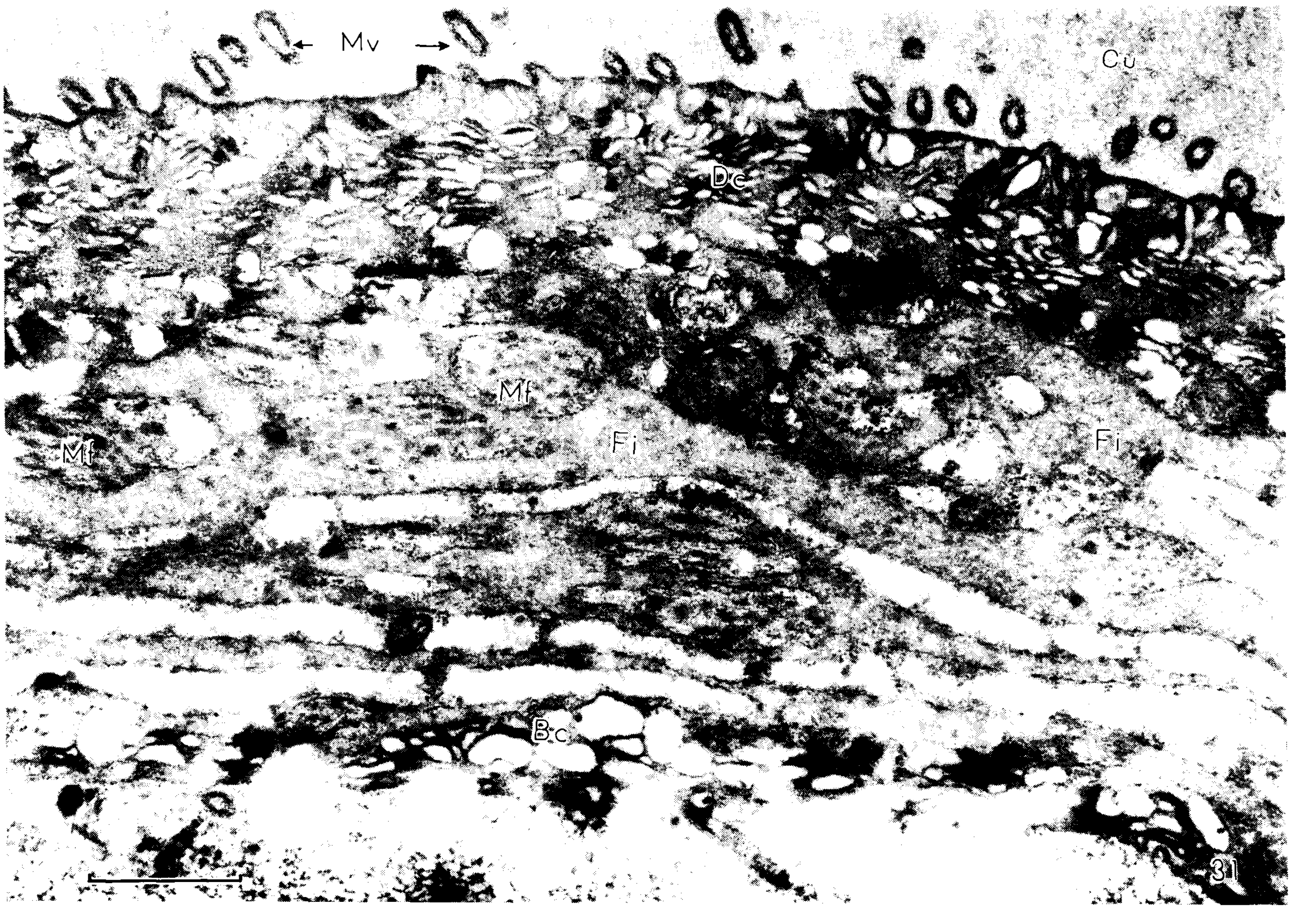
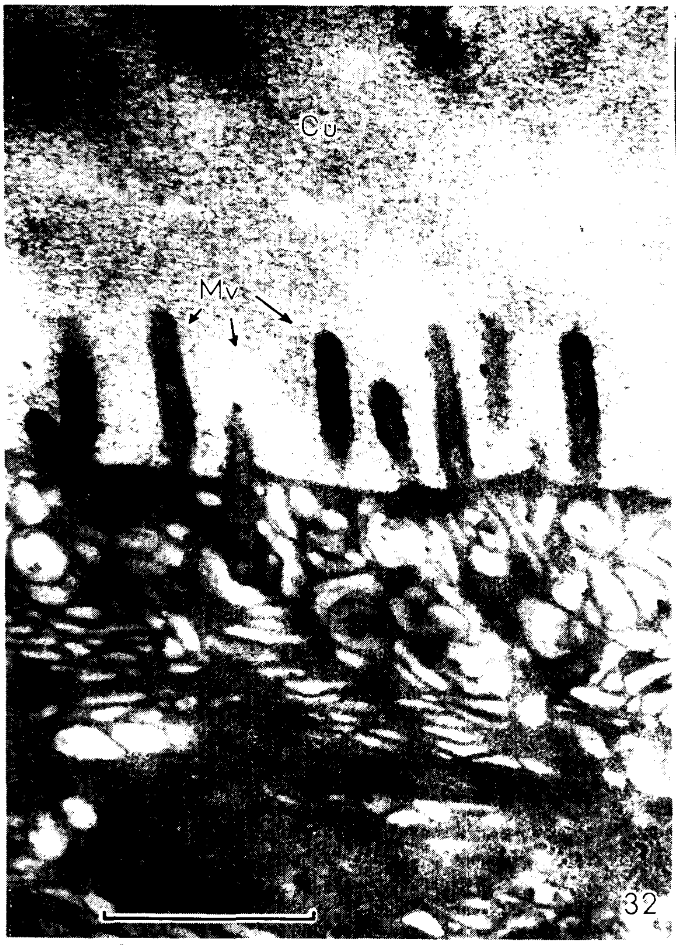

Mv
Cu
Dc
Mf
Mf
Fi
Fi
Bc
31

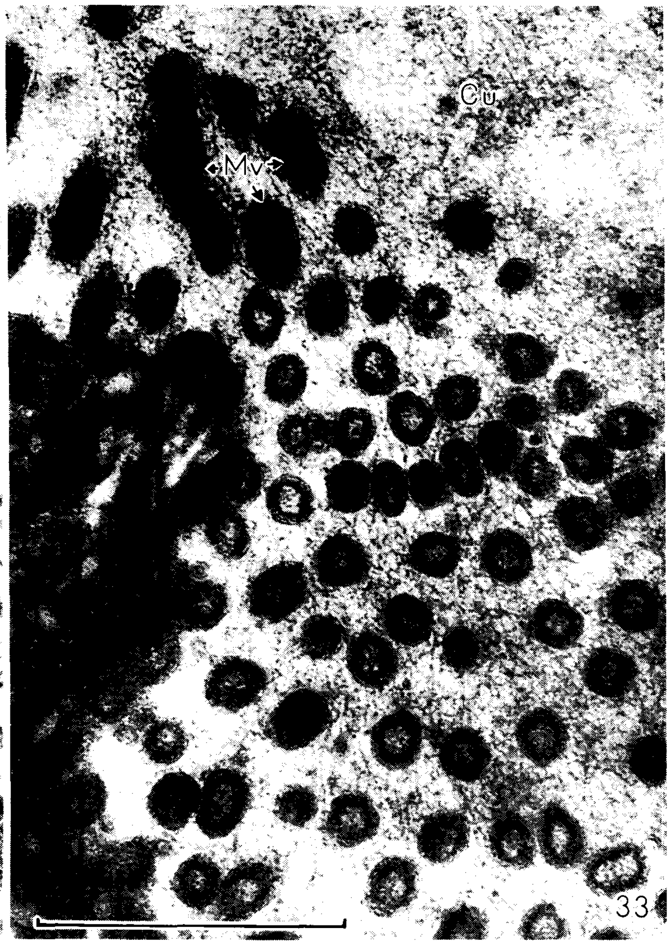
Plate XI

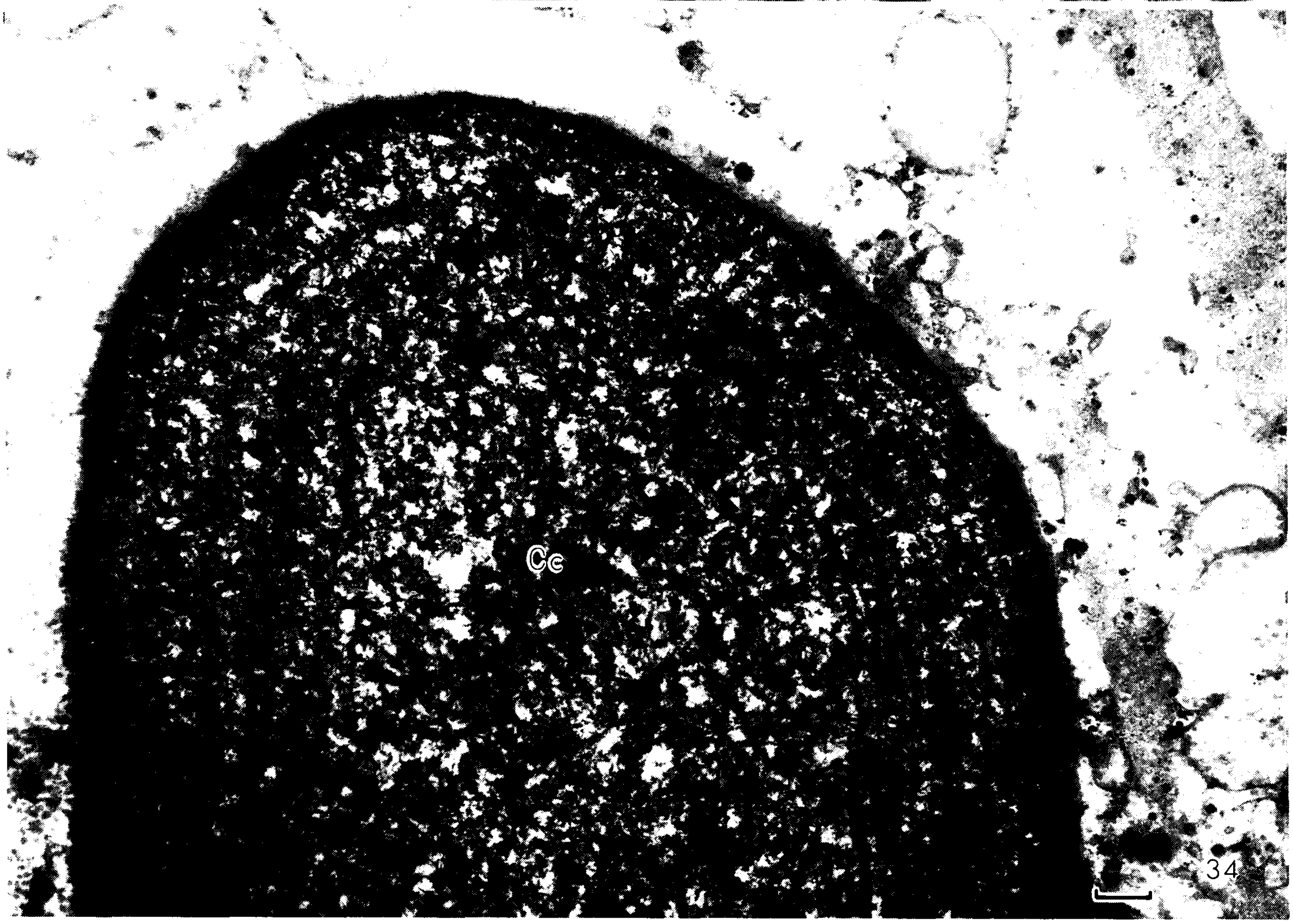
Fig. 32 The longitudinal section of microvilli (Mv) of germinal layer The microvilli extend into the cuticular layer (Cu).

Fig. 33 The cross section of microvilli (Mv) which extending into the cuticular layer (Cu)

Fig. 34 Calcareous corpuscle (Cc) is surrounded with the cytoplasm containing alpha particles of glycogen.


Cu
Mv
32


Cu
Mv
33


Ce
34