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ULTRASTRUCTURE OF THE CYST OF HYMENOLEPIS DIMINUTA LARVAE

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Dedicated to the memory of Academician K. I. Skryabin on the occasion of the centenary of his birthday

Abstract. Electron microscopical studies of the cyst of 1-month-old *H. diminuta* larvae revealed considerable thickening of the superficial syncytium of tegument and formation of a large number of cavities and canals. The cytoplasm of the tegument is filled with microtubules produced by cytons of parenchymal layer which are analogs of tonofibrils. These peculiarities, together with numerous processes of the external fibrous layer, are regarded as adaptative to the changes of the hydrostatic pressure of the hemocoel of the intermediate host. The superficial syncytium is covered with microvilli and keeps polycellular cytoplasmatic bonds with cytons, which ensures energetic and plastic requirements for the stabilization of the hypertrophied syncytium and its physiological regeneration. Accumulations of fibres identical with those of the connective tissue were found in the cytons of the tegument. The cyst parenchyma consists mostly of cells with widened canals of granular endoplasmic reticulum associated with the microfibrils and production of fibrous filaments. The internal fibrous layer is produced by typical fibroblasts. The tegument of the cercomer is thinner and is considered to be less differentiated.

In a majority of the studied cysticercoids the external surface of the cyst is represented by an electron-dense layer. There are differences only in the fine structure of the material from which it consists (Baron 1971, Rees 1973, Caley 1974, 1976, Gabrion and Gabrion 1976, Krasnoshchekov and Nikishin 1977). According to the data available, in *Hymenolepis diminuta* this structure has an organisation characteristic for the external syncytium (Ubelaker et al. 1970), but the authors studied young, 7-day-old larvae with a not fully formed cyst. This is indicated by the absence of hair-like processes typical of this species in the external layer (Voge 1960). The cyst tegument of *Hymenolepis microstoma* immediately after invagination has a structure similar to that described for *Hymenolepis diminuta*, but one month after invagination its cytoplasm is filled with dense, rod-shaped bodies (Caley 1974). Considering that the surface parts of the cyst play an important role in the adaptation of the larva for the survival in the intermediate host body and in the process of infection of definitive hosts, we have studied the ultrastructure of mature larvae of *Hymenolepis diminuta*. It was found that its structure, particularly that of the superficial syncytium, differs substantially from the structure described in the literature. We have therefore considered it expedient to supplement the available data on the ultrastructure of protective envelopes of mature cysticercoids of *Hymenolepis diminuta*.

MATERIAL AND METHODS

The experimental part of the studies was performed in the Department of Parasitology of the Polish Academy of Sciences. Mature, fully formed 1-month-old larvae of *H. diminuta* were obtained by infection of *Tenebrio molitor* L. The hosts were kept at temperatures of 20—22 °C. The invagination of larvae occurred on days 9—10 after infection. The fixation was carried out in 2 % glutaraldehyde on cacodylate buffer (pH 7.4) and postfixation in 1 % solution of osmium tetroxide. After dehydration

through graded alcohol series the larvae were embedded in EPON-812. The sections were prepared with Ultratome KV, contrasted by a saturated solution of uranyl acetate and lead citrate after Reinolds, and examined in a UEMV-100 K microscope.

A total of 5 larvae were studied in detail.

RESULTS

The cyst of *H. diminuta* has a thick layer of cytoplasmic syncytium of heterogeneous structure on the outside. Its surface is covered with microvilli measuring about 1 μm in length and 0.1 μm in thickness which are characterized by a marked polymorphism. The microvilli are usually cylindrical, with finely granular matrix, but they may be also branched and often with widened base. In the layer of microvilli bordered by a cytoplasmic membrane there are also dome-shaped evaginations of the cytoplasm of superficial syncytium filled with contents analogous to the matrix of microvilli. The evaginations of cytoplasm do not extend behind the layer of microvilli, they rarely reach the middle of microvilli layer. In this case there are 2—3 microvilli on their surface. These not fully formed evaginations of the cytoplasm, as well as the widened bases of individual microvilli, suggest that they are formed by filling of one or several neighbouring microvilli with a cytoplasmic material. On the upper border of microvilli there is a friable granular substance, fibrils, cellular detritus and host cells at various stages of destruction (Plate I, Fig. 1). Apical parts of microvilli are sunk in their cytoplasm, while the external cytoplasmic membrane of host cells is usually invisible on the side of microvilli.

The thickness of the superficial syncytium without the layer of microvilli is very variable being at most 10 μm. It can be separated into three zones. The superficial zone, 1.5—2 μm thick, is characterized by an increased density compared to lower layers. It consists of a densely pressed, finely granular material and longitudinal fibres (Plate I, Fig. 1).

The central zone of cytoplasmic syncytium, representing on the average a half of its thickness (about 5 μm), has a spongiform appearance. It is most developed on lateral surfaces of the cysticercoid, tapering towards posterior pole and disappearing in the tegument of first parts of the cercomer. This zone consists of a dense mass of chaotically distributed microtubules measuring 20 nm in diameter and finely granular material (Plate I, Fig. 3). Among them is a system of canals and cavities of various size with a friable, slightly osmophilic finely granular material. The border of the cavities is not visible in some places. Large cavities are situated near the superficial concentration of the matrix of syncytium, small cavities are situated more deeply. In the central zone, there are some fields with well regulated organisation of accumulated microtubules in form of parallel or concentric rows, often with alternating layers of microtubules and bladders of equal size representing obviously transverse sections of microtubules (Plate I, Fig. 3).

The inner zone of superficial syncytium is penetrated with processes of the external fibrous layer. They reach the middle part of the tegument, where they lose among the microtubules (Plate I, Fig. 4). The cytoplasm of syncytium in this zone contains a friable matrix and a large number of microtubules running along the processes of the fibrous layer. There are also bundles of microtubules oriented at various angles to the processes. The inner surface of syncytium is bordered by a cytoplasmic membrane separated from the lower-lying fibrous layer by an electron-light space spreading also on the base of fibroblastic processes. No cellular organelles except microtubules in the superficial syncytium were found. Pseudomyelinic bodies localized mostly in the middle field were revealed in the inclusions (Plate I, Fig. 3).

The external fibrous layer consists of longitudinal bundles of microfibrils, among which are few inclusions of thin cytoplasmic processes. Cellular bodies were not found. In the central portion of the layer the fibrillar material is more friable containing circularly distributed bundles of muscular and fibrous filaments. Close to the inner surface of the fibrous layer lies a thin layer of longitudinal muscular fibres. The fibrous layer in posterior pole passes to tail process, tapering and losing in its basal portions.

The processes sunk into the inner zone of superficial syncytium measure 0.4—0.5 μm in diameter and consist of a basic substance identical with the fibrous layer in the remaining part and more dense fibrills measuring up to 50 μm in thickness. The basic substance is well developed near the base of processes. In the external parts, the fibrils are freely distributed in the matrix of the inner zone of superficial syncytium. In its central zone, they attenuate and can not be identified anymore (Plate I, Fig. 4)..

The parenchymatous layer is 7—8 nm thick and consists of two types of cells: cytons of the tegument and cells with widened canals of granular endoplasmic reticulum, which occur approximately in the ratio of 1 : 4. The cytons are elongated or polygonal, usually binuclear, united by wide cytoplasmic bonds with the syncytium (Plate II, Fig. 1). The nuclei are oval, 2.0—2.5×1.2—2.0 μm, dense, with a thin layer of chromatin, adjacent to internal membrane of karyolemma and pronounced nucleoli, often with denticulate margins. Small bundles (3—4 pieces) of granular oval incisions occur in the karyoplasm. The cytoplasm of tegument cells is of mozaic appearance: dense fields with a large number of ribosomes alternate with friable ones containing microtubules identical with those occurring in the surface of syncytium. The dense fields are always visible near the nucleus and on the periphery of cells. The microtubules are often arranged in parallel rows between which are spaces filled with a light matrix (Plate II, Fig. 3). There constantly occur cytoplasmic bonds between the internal zone of superficial syncytium and cytons of tegument containing numerous microtubules passing from the cellular cytoplasm into external cytoplasmic layer (Plate II, Fig. 4). Some mitochondria are small, oval, with dense matrix and light crysts and occur only in the bodies of cells. Golgi complex has not been found. Individual cells contain a large number of pseudomyelinic bodies. Fields extending up to 1/3 of section through the cell and densely filled with fibrous material identical with fibrous filaments are sometimes visible in the cytoplasm of cytons (Plate II, Fig. 2).

The cells with widened canals of granular endoplasmic reticulum are larger and of irregular form. Among them prevail cells with large cavities the size of which reaches up to 3.5×4.0 μm. There are 2—3 of these cavities in one cell. Other cells contain numerous cavities measuring up to 1.0—1.2×0.5—0.75 μm. These cells contain both widened and ordinary canals of the granular endoplasmic reticulum. The nuclei are oval, of moderate density, measuring 2×2.5 μm, sometimes with a small nucleolus. In the cells with large cavities the nuclei are smaller, angular and of pycnotic appearance. The internal membrane of the karyolemma is thickened, the external one is covered with a large number of ribosomes. The space between them is widened up to 20 nm (Plate III, Fig. 1). The cavities of endoplasmic reticulum contain granular material of moderate density, bordered with a membrane which is not visible in some places and is covered with ribosomes on the outside.

The space between nuclear envelopes is often united through narrow anastomoses with widened cavities of the granular endoplasmic reticulum. The cellular cytoplasm does not differ in the density from the karyoplasm. It contains large spherical mitochondria with a dense matrix and canals of ergastoplasm with polyribosomes. The amount of cytoplasm and cellular organelles decreases with increasing size of cavities.

The cellular cytoplasm with widened canals of granular endoplasmic reticulum often contains myofibrils elongating into muscular fibres of the external fibrous layer (Plate III,

Fig. 2). In addition to them there is a fibrillar material analogous to the filaments of the fibrous layer (Plate III, Fig. 3). It was also observed that the content of widened cisterns of the endoplasmic reticulum was transformed into fibrous filaments secreting into intercellular space.

The internal fibrous layer measures 8—9 μm on the lateral surface of the cyst. It consists of bundles of fibrous filaments orientiny in various directions. Among them are isolated bipolar fibroblastic cells (1.5×0.5 μm) with a dense oval nucleus (0.75× ×0.35 μm) and a small amount of dense cytoplasm on poles of cells (Plate III, Fig. 4). These cells are more numerous in the posterior pole of the cyst wall where they differ in larger size and more abundant cytoplasm with well visible ergastoplasm. In addition to fibroblastic cells, the inner fields of the fibrous layer contain excretory cells and ducts localized also more often in posterior pole of the larva.

The innermost layer of the cyst is 1.8—2 μm thick and consists of compact myelin-like membranes (Plate III, Fig. 4). A small quantity of concentrated cytoplasm between cytoplasmic membranes is preserved only near the cells of this layer. The cells resemble fibrocyte cells, but differ from them in larger size (5×1 μm), in the nuclei (2.5×1.0 μm), dense karyoplasm, and marked accumulation of chromatin. The cytoplasm is dense and rich in ribosomes. The cellular elements are localized in the external compact portion of the layer. The internal portions of the layer, adjacent to the neck, are fibrillar and form a friable reticulum filling the cyst cavity of the larva.

The data on the ultrastructure of individual elements of the cyst wall and their relationship are summarized in the scheme (Plate IV).

The surface of the tegument of cercomer is covered with microvilli identical with those described on the cyst surface. The superficial syncytium is divided into two layers: external, which is dense and 0.5—0.75 μm thick, and internal, 1—1.5 μm thick, containing numerous light vesicles and mitochondria with a dense matrix (Plate I, Fig. 2). The tegument is situated on the basal plate with longitudinal muscular fibres on the inner side. Cells of the tegument are bound by 0.5 μm wide cytoplasmic bounds with superficial syncytium. Cells of muscular type are localized deeper. These cells are characterized by a spherical nucleus (1.0—1.1×0.8—0.9 μm) with nucleolus measuring up to 0.4 μm, accumulation of matrix of nucleus near the inner membrane of the karyolemma, dense exoplasm with developed granular endoplasmic reticulum and wide layer of light exoplasm containing a small number of small, dense mitochondria. Small groups of cells of a similar structure are visible in the central fields of the cyst, but they differ in smaller size and more compact cytoplasm. These cells are considered as less differentiated.

Cellular elements of the cercomer are friable, in the central fields there are large cavities containing a light, finely granular material and a small number of small polygonal cells with long cytoplasmic processes and pycnotic nuclei.

DISCUSSION

Characteristic for the tegument of cysticercoids is the transformation of the superficial syncytium into a dense homogeneous layer with a loss of microvilli and cytoplasmic bonds between cytons and superficial cytoplasm due to the development of a large fibrous layer of the cyst (Baron 1971, Rees 1973, Caley 1974, 1976, Gabrion and Gabrion 1976). In contrast to this obviously most widely distributed type of cysticercoids the external syncytium of mature *H. diminuta* larvae keeps a structure similar to the typical tegument of cestodes: there are microvilli on its surface, the external syncytium keeps a common cytoplasmic nature and bonds between cytons and cyto-

plasmic layer are developed. However, during the process of differentiation it acquires characters differing from those of the tegument of larval or adult forms. Its thickness increases many times: the superficial syncytium is filled with a large number of microtubules; there arises a system of canals and cavities filled with a liquid; the cytoplasm is penetrated by numerous fibrils of processes of the external fibrous layer running in radial direction from inner to outer surface. The mentioned peculiarities of the structure of cyst tegument seem to serve to adaptation to the existence in the body cavity of intermediate host and compensation of changes of hydrostatic pressure of the hemocoel. These functions are realized in a different way in larvae of different cestode species. In *Tetracantha octacanta* by the formation of an external cyst (Rees 1973), in *Aploparaksis furcigera*, as well as obviously in other cysticercoids of the diplocyst type, by the formation of an external envelope from the tail appendage (Gulyaev 1977). According to obtained data, in *H. diminuta* this function is realized by the hypertrophy of superficial syncytium and formation of cavities inside it and peculiar framework of fibrous filaments and microtobules. In such case the latter play a supporting role and they are homologous with tonofibrils of the epithelium of mammals. They are formed in the cells of tegument and are released into the superficial syncytium through cytoplasmic bonds. In the same way the matrix is enlarged. Another possibility is that it is formed by destruction of microtubules in the superficial fields of syncytium.

Complexes of microtubules were found also in the tegument of adult cestodes where they seem to participate in osmoregulation (Threadgold and Read 1970, Threadgold and Arme 1974). However, their number is much higher in the syncytium of mature larvae and they were not found to be accumulated in specialized structures in form of perpendicular rows. Microtubules were described also in cytoplasmic processes between superficial syncytium and cytons of the tegument and they are regarded as a system of transport between the surface of tegument and bodies of its cells (Threadgold 1962). A small number of microtubules were also observed in superficial syncytium, where they are more often regarded as invagination of basal cytoplasmic membrane (Threadgold 1965, Morris and Finnegan 1969). Nevertheless, formation of microtubules in such a large quantity in the cytoplasm of cytons of tegument with the release into the superficial syncytium and their accumulation in the syncytium have not been observed previously.

The structure of the tegument of cercomer is typical of the larval tegument of cestodes. It is less differentiated as opposed to the tegument of the cyst, which is indicated by the presence of mitochondria in the superficial syncytium, absence of large cavities and accumulation of microtubules. No formation of microtubules in the cytons of cercomer and their secretion into the cytoplasm of syncytium was observed. In the cercomer base there are all stages of the transition from the tegument of cercomer to the tegument of cyst.

In addition to cytons of the tegument the parenchymatous layer of *H. diminuta* cyst contains cells with widened canals of granular endoplasmic reticulum. These cells have a double function: they are bodies of muscular cells and produce fibres of interstitial tissue of external fibrous and intermediate layer. This enables to determine these cells as myofibroblastic elements, which supports the earlier assumptions (Caley 1974, 1976). Analogous cells were found by us in the cercomer where they have been reported by other authors in the larvae of the same cestode species (Ubelaker et al. 1970). However, the cells with widened canals of granular endoplasmic reticulum situated in the cercomer were not found to produce fibrous material. In the tail appendage there was only a small amount of this material and it is represented here by a basal plate. The cells of this type localized in the cercomer differentiate into muscular cells, but it is possible that they participate in the formation of the basal membrane as in the case

with these cells localized in the cyst (Ubelaker et al. 1970). It may be supposed that the widening of canals of granular endoplasmic reticulum occurs when the secretion of fibrous filaments into the intercellular space is decreased, obviously due to the development of sclerosis of the cyst wall. The secretion is deposited in the granular endoplasmic reticulum with progressing widening of canals which leads to atrophic changes of myofibroblasts. The internal fibrous layer is produced by typical fibroblasts. The finding of fibroblastic filaments in the cytons of tegument is due to the involution and replacement with connective tissue.
participance in the production of connective tissue, but further studies are necessary to confirm this hypothesis.

The cyst tegument of mature larvae of *H. diminuta* keeps a structure corresponding to active absorption surface, but due to a thick internal fibrous and pseudomyelin layer the penetration ability of the cyst wall for the absorbed substances is very small (Krasnoshchekov 1975). The microvilli, which are reduced in larvae of other cestode species, are preserved on the surface of *H. diminuta* cyst. This seems to ensure energetic and plastic requirements for the stabilization of the hypertrophied superficial syncytium.

УЛЬТРАСТРУКТУРА ЦИСТЫ ЛИЧИНОК *HYMENOLEPIS DIMINUTA*

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Резюме. Электронномикроскопическое изучение цисты личинок *H. diminuta* в возрасте 1 месяца показало значительное утолщение поверхностного синцития тегумента с образованием большого количества полостей и каналов. Цитоплазма тегумента заполнена микротрубочками, продуцируемыми цитонами паренхиматозного слоя, являющимися аналогами тонофибрилл. Эти особенности, наряду с многочисленными отростками наружного фиброзного слоя, рассматриваются как адаптативные к изменениям гидростатического давления гемоцеля промежуточного хозяина. Поверхностный синцитий покрыт микроворсинками и сохраняет многочисленные цитоплазматические связи с цитонами, что обеспечивает энергетические и пластические потребности для стабилизации гипертрофированного синцития и его физиологическую регенерацию. В цитонах тегумента выявлены скопления волокон, сходных с соединительно-тканными. В паренхиме цисты преобладают клетки с расширенными канальцами ГЭС, связанные с миофибриллами и продукцией фиброзных волокон. Внутренний фиброзный слой продуцируется типичными фибробластами. Тегумент церкомера более тонкий и расценивается как менее дифференцированный.

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KEY TO LETTERING OF FIGURES

BL — basal lamina
CLL — cyst lining layer
Ct — cyton of tegument
Cv — cavity
Er — rough endoplasmic reticulum
F — fibroblast
f — fibres of collagen
HC — host cell
IFL — inner fibrous layer
Mb — microvilli
Mf — myofibre
Mt — mitochondrion
N — nucleus
Nl — nucleolus
OFL — outer fibrous layer
PFL — processes of outer fibrous layer
SC — superficial cytoplasmic layer of tegument

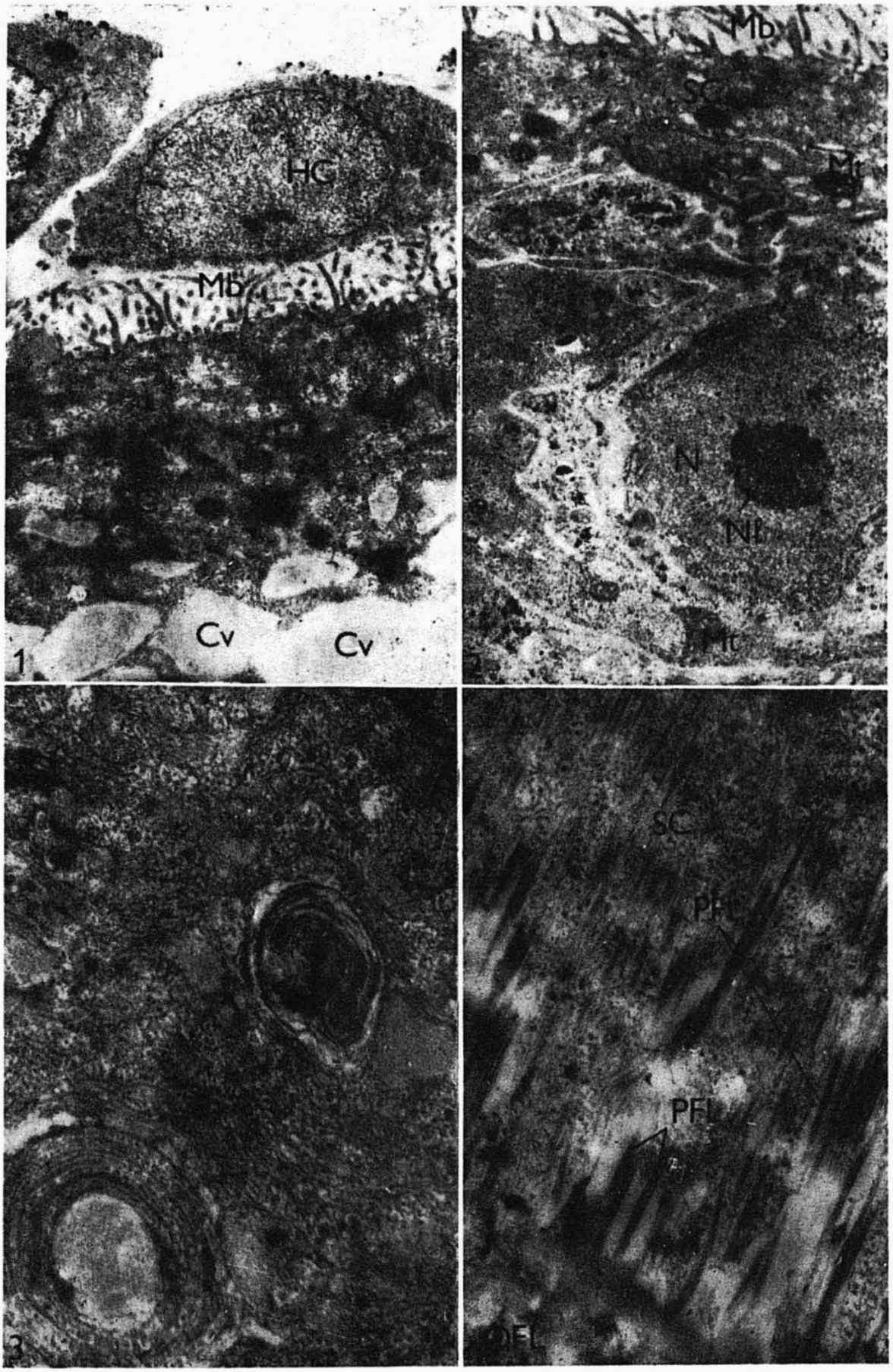

Fig. 1. Outer part of superficial cytoplasmic layer of cyst tegument in the region of cercomer. Host cells undergoing destruction visible on its surface (×6825). **Fig. 2.** Superficial cytoplasmic layer of cercomer tegument. The parenchymal cell of cercomer (×6825). **Fig. 3.** Microtubules and myelin-like body in middle zone of cyst tegument (×12 940). **Fig. 4.** Processes of outer fibrous layer (arrow) in inner zone of superficial cytoplasmic layer of cyst tegument (×7875).

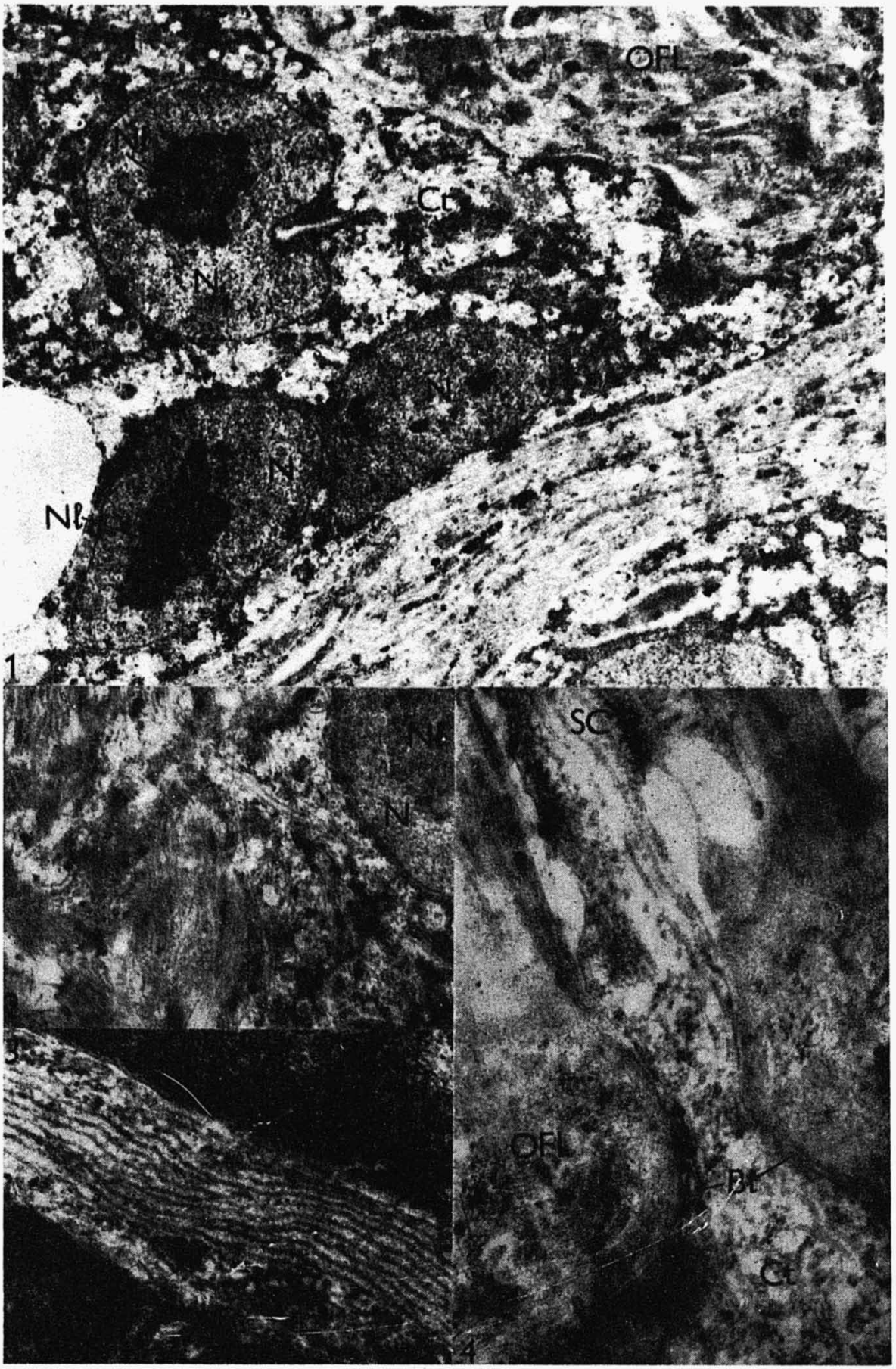

Fig. 1. Cyton of cyst tegument with three nuclei (×6825). **Fig. 2.** Part of cyton cytoplasm is filled with microtubules and collagen-like fibres (×7875). **Fig. 3.** Part of cyton cytoplasm containing parallel rows of microtubules (×12 940). **Fig. 4.** Cytoplasmic connection between cyton and superficial cytoplasm of tegument (×12 940).

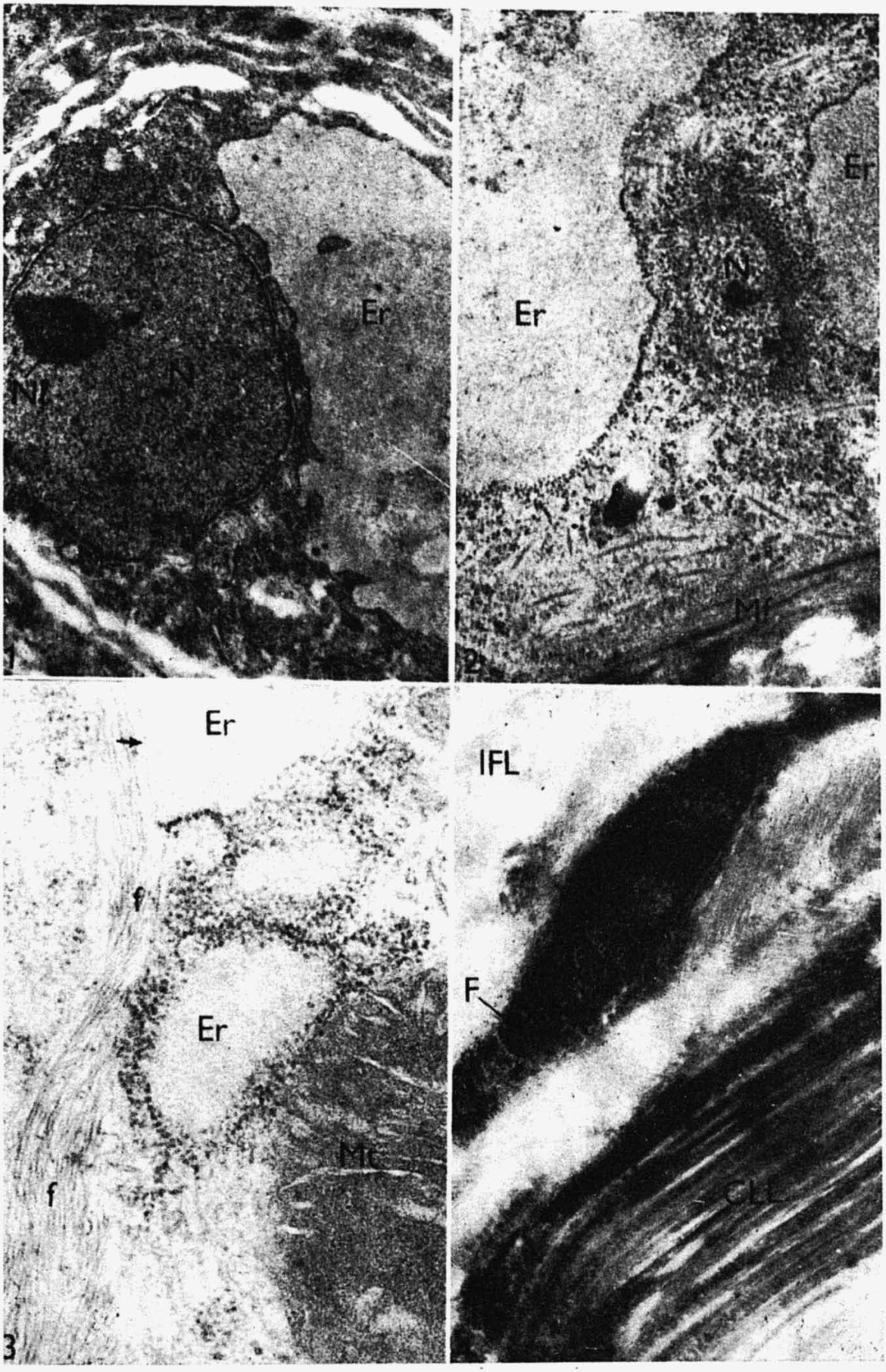

Fig. 1. Part cell with dilated rough endoplasmic reticulum (×12 940). **Fig. 2.** Part of cytoplasm of cell with dilated rough endoplasmic reticulum showing myofibres (×12 940). **Fig. 3.** Binding of collagen-like fibres with the contents of canals of widened granular endoplasmic reticulum (×17 250). **Fig. 4.** Inner fibrous layer and cyst lining layer (×68 25).

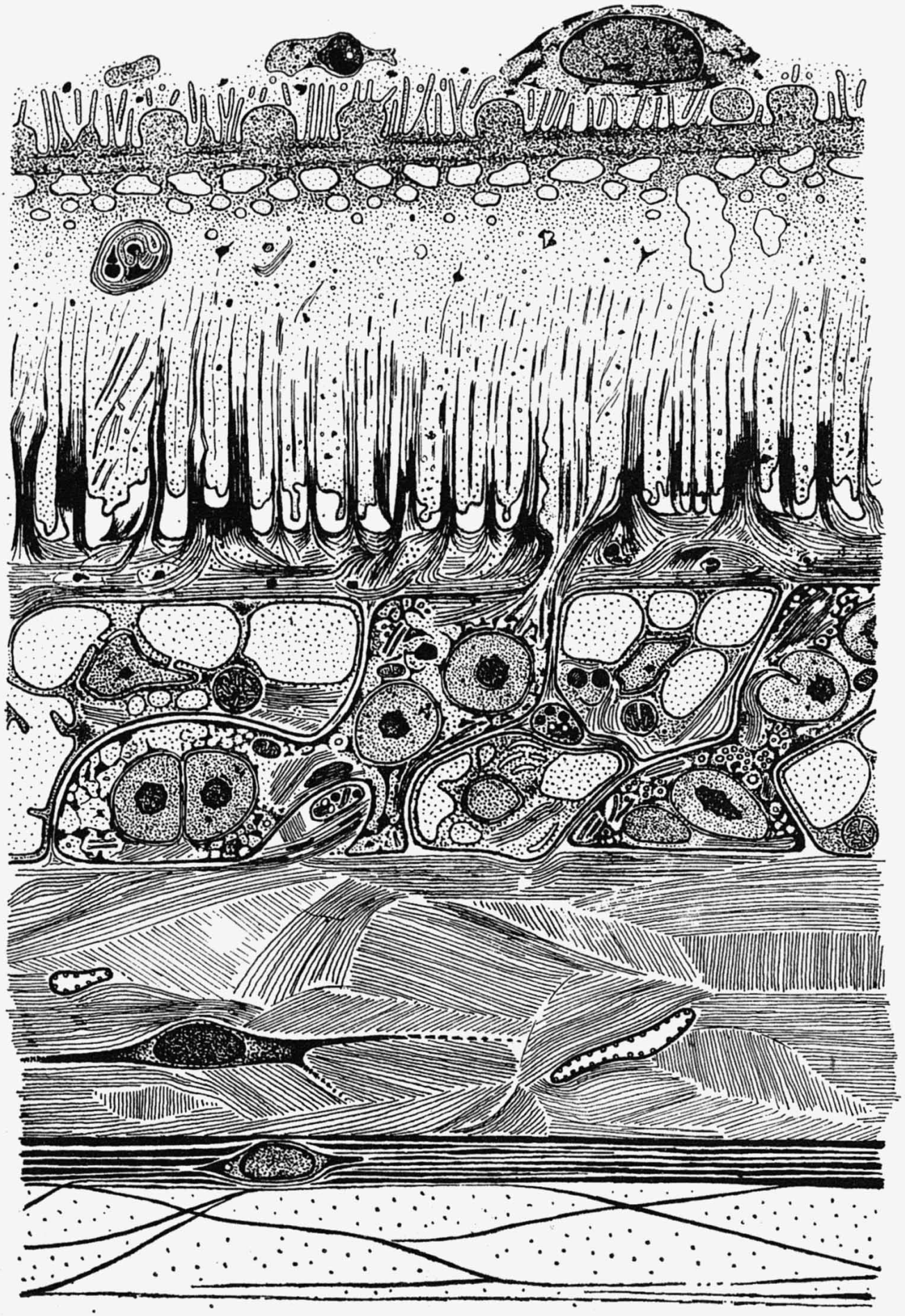

Fig. 1. A diagrammatic representation of cyst ultrastructure of *H. diminuta* cysticercoid.