HOKKAIDO UNIVERSITY

Title	STUDIES ON ECHINOCOCCOSIS XXIII : ELECTRON MICROSCOPICAL OBSERVATIONS ON HISTOGENESIS OF LARVAL ECHINOCOCCUS MULTILOCULARIS
Author(s)	SAKAMOTO, Tsukasa; SUGIMURA, Makoto
Citation	Japanese Journal of Veterinary Research, 18(3), 131-144
Issue Date	1970-09
DOI	https://doi.org/10.14943/jjvr.18.3.131
Doc URL	https://hdl.handle.net/2115/1963
Type	departmental bulletin paper
File Information	KJ00002369883.pdf

Hokkaido University Collection of Scholarly and Academic Papers : HUSCAP

Jap. J. vet. Res., **18**, 131-144 (1970)

STUDIES ON ECHINOCOCCOSIS XXIII

ELECTRON MICROSCOPICAL OBSERVATIONS ON HISTOGENESIS OF LARVAL *ECHINOCOCCUS MULTILOCULARIS*

Tsukasa SAKAMOTO & Makoto SUGIMURA*

Department of Parasitology
Faculty of Veterinary Medicine
Hokkaido University, Sapporo, Japan

(Received for publication, July 15, 1970)

Through the light- and electron-microscopical investigations on the histogenesis of larval *Echinococcus multilocularis* at 5~60 days after the inoculation, the following results were obtained.

The cyst wall at the initial unilocular stage, 5~7 days after the inoculation, is composed of 2 types of cells; the immature syncytial cells, and the light-stained undifferentiated cells with many polysomes. The latter show active mitosis. The wall of the multilocular cysts without a cuticular layer after 7~14 days comprises light- and dark-stained undifferentiated cells, and the syncytial cells. The dark-stained undifferentiated cells, which contain small mitochondria and polysomes, manifest active mitosis. The initial formation of the cuticular layer begins at the stage later than about 14 days. The cyst wall at this stage is constructed by the cells similar to those at the early stage, but many microvilli and terminal web-like structures are more distinctly demonstrable in the syncytial cytoplasm.

At the stage of brood capsule and protoscolex formations, new types of cells are confirmed to appear; the asteroid transforming cells, the muscle cells, the glycogen-storing cells, and the reticular interstitial cells. These cells can be distinguished from one another by the appearance of myofilaments, glycogen particles and mitochondria, respectively. The perikaryon of the syncytial cell removes to the inside of the germinal layer and is distant from its distal syncytial cytoplasm.

Brood capsule formation originates in the massive accumulation of the dark-stained undifferentiated cells proliferated. A lumen surrounded by the syncytial cells, which line the brood capsule and possess the microvilli, is established at the center of the cellular accumulation. At first, the anlage of protoscolex manifests also the massive accumulation of proliferated dark-stained undifferentiated cells on the brood capsule wall. The anlage protrudes gradually into the lumen of the brood capsule. The types of constituent cells of the brood capsule wall and protoscolex show a strong resemblance to those of the cyst wall.

INTRODUCTION

Up to this time, the light microscopical studies on histogenesis of larval

* Department of Veterinary Anatomy

multilocular echinococcus in experimental cases have been published by several investgators. However, in their descriptions of the organelles and differentiation of cells constructing hydatid tissue, there have been some confusions due to limited resolvability of light microscopy.

Concerning the fine structure of adult and larval *Echinococcus granulosus*, MORSETH (1966, '67[A, B]) reported some work. Recently, the present authors (1969) published the general ultrastructure of a completely developed larval *Echinococcus multilocularis*. They, however, have been able to find no papers dealing with the electron microscopical observations on histogenesis of larval cestodes.

The present paper is concerned with some electron microscopical observations on larval *Echinococcus multilocularis* for the period during the stage of the establishment of the unilocular cyst to the initial formation of the protoscolex, 5 to 60 days after the inoculation.

MATERIALS AND METHODS

Materials used were obtained from the liver of cotton rats infected experimentally with eggs of *Echinococcus multilocularis*. The animals were sacrified at desired intervals, 5 to 60 days after the inoculation. The tissue materials were fixed in ice-cold, phosphate-buffered 4% glutaraldehyde, and postfixed in 1% osmium tetroxide (the double aldehyde-osmium fixation). The materials were dehydrated in ethanol and propylene oxide, embedded in Epon 812, and sectioned by the ultramicrotome. The sections were mounted on nickel grids and stained with saturated uranyl acetate (WATSON, 1958), or a combination of it and lead citrate (REYNOLDS, 1963). The JEM-7 electron microscope was used for examination at magnifications of 2,000 to 20,000.

The thicker sections obtained from the same blocks were stained with toluidine blue and PAS, and examined under a light microscope for identification of the area of the electron microscopical preparations. On the other hand, the liver of each case was fixed with Carnoy solution or 10% formalin solution, and the sections were stained with hematoxylin-eosin and PAS. These preparations were used for light microscopic examination.

RESULTS

Each larval *Echinococcus* shows, successively, formations of a unilocular cyst, multilocular cyst, cuticular layer, and brood capsule and protoscolex. The development of echinococcal cysts in the same case is not always uniform; histological findings are very complicated because of the formation of new vesicles by means of successive exogeneous buddings, which take place in various degrees by the focus. Consequently, the echinococcal cysts at various stages were observed in the same specimen. In the present paper, the authors would like to classify the findings of echinococcal tissue in four developmental stages.

I Unilocular stage

This stage is light-microscopically recognizable at 1 to 7 days after the inoculation.

The cyst is small, about 15 to 70 μ in diameter, and spherical, and the wall appears to be established by monolayered cells with slightly basophilic cytoplasm and a spherical nucleus having a distinct nucleolus. The 5th and 7th day cases were examined electron-microscopically, and unilocular cysts were recognized in those cases. Among the cells of the cyst wall, two types are differentiable; A) the immature, syncytial, cyst-wall-forming cell (figs. 3 & 4), and B) the light-stained, undifferentiated cell (figs. 4~6). The syncytial cytoplasm of the immature, cyst-wall-forming cell, "the immature syncytial cytoplasm", is considerably electron-opaque because it contains numerous free ribosomes, as well as tubular smooth endoplasmic reticula and, at the outer zone, a number of small vacant vesicles. Mitochondria possess electron-opaque matrix and a few poor cristae. The outer surface of the immature, syncytial cytoplasm attaches directly to the host-cells and their degenerated materials. Usually, no microvilli are found on the surface of the immature, syncytial cytoplasm. The nucleus of the immature cyst-wall-forming cell contains a distinct nucleolus, and rather poor chromatins distributed homogeneously.

The light-stained, undifferentiated cell is roundish without cytoplasmic processes, and adheres to the inner surface of the immature, syncytial cytoplasm. The cytoplasm contains plentiful polysomes, a poor Golgi complex and some mitochondria with electron-lucid matrices and a few distinct cristae. The nucleus has a distinct, large nucleolus, and chromatins are scattered poorly mixed with a few, small, nodular accumulations of chromatins. Active mitosis is often demonstrable.

II Multilocular stage

In the 7th to 20th day cases, most larval *Echinococcus* manifest active multilocular vesiculation light-microscopically, but no formation of a cuticular layer is confirmable. The multilocular larval tissue is composed of an aggregation of several cysts, and is surrounded by connective tissue. The intercystic spaces are filled with infiltrated cells such as neutrophilic and eosinophilic leucocytes. Some cysts, however, are in contact with one another directly. The cyst wall is mostly established by so-called germinal cells appearing as a monolayer. Some cysts at the initial stage of multivesiculation are separated by a single or double-layered septa of these cells. This finding is also recognized electron-microscopically (fig. 15).

Together with 2 cell types found in the previous stage, electron-microscopically, another cell type is demonstrable in this stage; the undifferentiated cells on the inner surface of the syncytial cytoplasm are subdivided into two types, light- and dark-stained undifferentiated cells, respectively. The light-stained undifferentiated cell is similar to that in the previous stage. In comparison with the above type, however, the dark-stained undifferentiated cell (figs. 7~9) is smaller in size and slightly more electron-opaque because of an increase of polysomes in the cytoplasm, and the mitochondria are slightly smaller and denser. Mitosis is also confirmed frequently. A few microvilli begin to protrude from the outer surface of the syncytial cytoplasm of a few cysts. Sometimes, a massive appearance of alpha glycogen particles is demonstrable partially in the syncytial cytoplasm. The vesicles in the syncytial cytoplasm increase in size and number. The mitochondria are enlarged showing a matrix and cristae developed more densely and distinctly, than those in the earlier stage.

It is recognizable that the cell-body of some dark-stained undifferentiated cells is buried

partially or completely in the syncytial cytoplasm, participating in the formation of the cyst wall. And, at the portion which are in contact with each other, both the cells lose parts of their cell membranes. In some places of the syncytial cytoplasm, which forms the cyst wall, the outer ectoplasmic zone with numerous vesicles begins to be established incompletely. The inner zone contains the nuclei, large mitochondria and glycogen particles. In between the 2 zones, the terminal web-like structure consisting of loose filaments is recognized. Infoldings of the cell membrane are frequently observed to be invading irregularly in the inner zone of the syncytial cytoplasm.

III Stage of initial formation of the cuticular layer

Light-microscopically, the so-called cuticular or laminated layer is clearly positive for PAS-reaction in a part of the echinococcal cysts on the 14th day. This layer, electron-microscopically, is composed of extracellular, fine fibrous material, and appears in the interspace between the syncytial cytoplasm forming the cyst wall and the surrounding host-cells. The extracellular material is invaded by the microvilli of the syncytial cytoplasm (fig. 17). Some electron micrographs reveal the remaining tissue-cells, neutrophilic leucocytes (fig. 18) and their debris between the layer of extracellular material and the cyst wall. Most of the dark-stained undifferentiated cells and syncytial cells are similar to those of the early stage, although no light-stained undifferentiated cells are demonstrable. Some of the dark-stained undifferentiated cells possess the cytoplasm containing many polysomes and a considerable number of small mitochondria with a comparatively electron-opaque matrix and a few distinct cristae. Some of the cells are embedded in the syncytial cytoplasm of the cyst wall. Sometimes, the cytoplasm of the dark-stained undifferentiated cells (figs. 12 & 13) is partially continuous with the syncytial cytoplasm like that observed in the multilocular cysts without a cuticular layer.

In the 25th and 30th day cases, a large area of the liver is replaced by a number of spongy multilocular cysts with the cuticular layer showing various sizes and shapes. In almost all the cysts, the initial formation of the brood capsule is not yet confirmable. The ultrastructure of the cyst wall is basically similar to that mentioned above. The layer of extracellular material, the cuticular layer, covers the outer surface of the cyst wall, and becomes manifestly thicker with the lapse of time. The microvilli, which elongate from the outer surface of the syncytial cytoplasm, increase in number. In the syncytial cytoplasm of cysts on the 25th and 35th days, the stratified, terminal web-like structure composed of fine filaments is demonstrated distinctly in between the outer zone with many vesicles and the inner zone with mitochondria and glycogen particles.

IV Stage of formation of the brood capsule and protoscolex

In some cysts of the 25th and 30th day cases, the initial formation of brood capsules is recognized light-microscopically. Namely, a mass of germinal cells proliferated partially becomes a vesicular structure. The inner surface of the vesicle wall is lined with a thin, PAS-positive membrane. Light-microscopically, active formation of the brood capsule is observed in many cysts of the 35th to 45th day cases. An immature protoscolex, at first, is produced in many of the brood capsules. In the 50th and 55th day cases, each brood capsule contains a few protoscolices, with both mature and immature ones mixed together.

A Wall of cyst

In the cases on and after the 35th day, massive granules of alpha glycogen disappear rapidly from the syncytial cytoplasm with the development of the echinococcal tissue. The nuclei of syncytial cyst-wall-forming cells remove together with their surrounding cytoplasm from the inner surface of the cyst wall to the more inner side facing the cystic lumen (figs. 20 & 21); the cytoplasmic continuity is recognized between the perinuclear cytoplasm and the distal, syncytial cytoplasm. Otherwise, the cells (figs. 22~24) with several cytoplasmic projections containing many free ribosomes and polysomes appear in the inner side of the germinal layer. Sometimes, these cells contain alpha glycogen particles in their cytoplasm, and possess small and ovoid mitochondria with an electron-opaque matrix and a few cristae. These cells are designated as "asteroid, transforming cells" because of their aforementioned morphological characteristics. In addition, the cells (figs. 25~28) having many long cytoplasmic projections are found among the cells in the inner portion of the germinal layer. The cytoplasm of this cell has many large, longish-ovoid mitochondria with an electron-opaque matrix and a few distinct cristae, and often possess electron-opaque glycogen-like granules and a fibrous substance. The term "reticular interstitial cell" is used for these cells in this paper as a matter of convenience.

The muscle cells (figs. 32 & 33) with many projections, which contain myofibrils and beta glycogen particles or massive alpha glycogen particles, appear in the germinal layer. The muscle cells contain several small mitochondria with some distinct cristae and a comparatively electron-opaque matrix in the perinuclear cytoplasm.

Besides the above cells, cells (figs. 29~31), whose cytoplasm is filled with numerous massive alpha glycogen particles, are found in the inner side of the germinal layer. These cells contain also some small mitochondria with a comparatively electron-opaque matrix and distinct cristae in the perinuclear cytoplasm. Some of those cells with glycogen particles are recognized to fall into the lumen of the cyst. The term "glycogen-containing cell" was used for these cells in our previous paper (1969). To avoid confusion with the muscle cell and the syncytial, cyst-wall-forming cell with glycogen particles, the cells above-mentioned are designated as "glycogen-storing cells" in this paper.

On and after the 35th day, the fibrous interstitium consisting of loosely reticulated, fine filaments appears in the interspaces among the constituent cells of the germinal layer.

B Brood capsule and protoscolex

In the 35th and 55th day cases, electron-microscopically, the initial formation of the brood capsule begins with the apparance of an accumulation of the dark-stained undifferentiated cells proliferating partially on the inner side of the germinal layer (figs. 34 & 35). At a central portion of the cellular accumulation, cells similar to the syncytial, cyst-wall-forming cells appear, and a lumen, which is lined with the cells having microvilli, is established. These cells lining the lumen (figs. 36 & 37) become syncytial, and their nuclei possess a large distinct nucleolus, and, within the cytoplasm, contain a large number of ribosomes and many longish-ellipsoid mitochondria with electron-opaque matrix and distinct cristae. Numerous vesicles with or without small electron-opaque granules are seen in the syncytial cytoplasm lining the lumen of the brood capsule. Thereafter, the lumen is enlarged in capacity with the lapse of time. The muscle cells and glycogen-storing cells

also appear in the outer side of the wall of the initial brood capsule, where a small number of dark-stained undifferentiated cells are recognizable.

The dark-stained undifferentiated cells of the brood capsule-wall, on the other hand, proliferate locally as mound-like accumulations covered with the syncytial cytoplasm. Then, the cellular accumulations protrude successively into the lumen of the brood capsule showing an ellipsoid-form; this structure is an initial protoscolex (figs. 40~42). Many asteroid transforming cells with several projections, and the muscle cells with many elongated projections are formed in the cellular accumulation, and the cells with numerous glycogen particles are found simultaneously. The syncytial cytoplasm covering the immature protoscolex manifests gradual thickening, and its characteristics are identical with those of the tegumental cytoplasm (fig. 43). Namely, the microvilli of the syncytial, tegumental cytoplasm of the immature protoscolex become the microtriches, of which the extremities manifest the spinous, electron-opaque, striated apical part. Many vesicles with electron-opaque granules, dense bodies, appear in the syncytial cytoplasm. The fibrous interstitium composed of loose reticular filaments are recognized in the interspaces among the cells establishing the immature protoscolex, and the basement membrane composed of a compact amorphous substance lines the plasma membrane on the basal surface of the tegumental cytoplasm.

Appearance of the cells of the nervous system and the flame cells could not be found in the echinococcal tissue at this stage. Also, some findings of the initial formation of the calcareous corpuscles and the wall of the excretory duct were demonstrable in the germinal layer at the latter stage. Their development, however, was not yet been examined fully by electron microscopy. Therefore, in the near future, we shall deal with the histogenesis of the structures above-mentioned.

Discussion

As to the histogenesis of larval *Echinococcus multilocularis*, Rausch (1954) reported the light microscopical observations on *Microtus pennsylvanicus*, Mankau (1955, '56, '57) in laboratory mice, Ohbayashi (1960) in voles *Microtus montebelli montebelli*, cotton rats *Sigmodon hispidus*, mice (strains dba, CF #1 and C57BL/6), and Webster & Cameron (1961) in cotton rats, gerbils, white mice, white rats and muskrats. Concerning the susceptibility of cotton mice *Peromyscus gassypinus*, field mice *P. polinotus*, deer mice *P. maniculatus* and cotton rats, Sadun et al. (1957) stated that cotton rats were highly susceptible to infection of *E. multilocularis*. They, however, did not carry out a histological description. Up to the present, there have been no electron microscopical observations on the histogenesis of larval *Echinococcus*, as far as we know.

Rausch (1954) stated that the larvae at 26 hours after inoculation consisted of a spherical mass of cells with prominent, dark-staining nuclei, and pale, acidophilic cytoplasm. Ohbayashi (1960) observed that the parasite composed of a thin "larval membrane" and a mass of germinal cells. He described that the

larval membrane showed a granular or indistinct appearance and disappeared at the time of multilocular vesiculation. In the present experiment, we could not obtain the electron microscopical findings of the parasites at a stage of the initial vesicle after the migration of hatched oncospheres. On the other hand, pertaining to the ultrastructure of the eggs of *Taenia taeniaeformis* and *Hymenolepis citelli*, respectively, NIELAND (1968) and COLLIN (1968, '69) stated that the hatched oncospheres without an oncospheral membrane were surrounded by an outer coat consisting of a cytoplasmic layer (cytoplasmic material), basal lamina and muscle. We also observed the same findings on the electron micrographs of oncospheres of *E. multilocularis* (unpublished). These electron micrographs revealed that the cytoplasmic layer was essentially analogous to our "syncytial cytoplasm forming the cyst wall", in the present paper. We would like to investigate the change of the cellular constituents of the oncosphere developing to an echinococcal cyst, in the near future.

RAUSCH (1954) reported that the larvae at 79 hours were seen as spherical to irregularly shaped vesicles which comprised flat cells. And, OHBAYASHI (1960) described that the unilocular vesiculation occurred in 3-day cases of *Microtus*, cotton rats and C57BL/6 mice and in 4-day cases of dba and CF#1 mice. He stated that the cyst wall was made up of a thin germinal cell layer alone at the stage between the disappearance of the larval membrane and the formation of the cuticular layer. On the other hand, we (1969) pointed out that the term "germinal cell" was applied to any cells of the germinal layer, but, in a strict sense, this term had to be applied to undifferentiated cells. In the present paper, it was confirmed that the cellular organization of the unilocular cyst at the 5th and 7th days established by two kinds of cells as follows:—

The cyst wall is made up of 1) the immature syncytial cells, which show characteristics partially resembling the tegumental cell, and 2) the light-stained undifferentiated cells with many polysomes, showing active mitosis on the inner surface of the cyst wall. The former is the immature cyst-wall-covering cell (immature cyst-wall-forming cell; syncytial cell of the cyst wall), and the latter is the light-stained undifferentiated cell (germinal cell in the strict sense).

RAUSCH (1954), in the vole at 212 hours after the infection, observed that a large proportion of cysts contained secondary vesicles which have originated by endogenous budding, and secondary vesicles were being produced also by exogenous budding. And, MANKAU (1957) found the multilocular vesiculation by endogenous budding in 10-day cases of mice. OHBAYASHI (1960), however, described that the 5-day cases of cotton rats, 10-day *Microtus*, 7-day dba, 10-day CF#1 and 7-day C57BL/6 indicated initial multilocular vesiculation, respectively, which progressed by means of exogenous budding. RAUSCH & JENTOFT (1957)

FIGURE 1 *Differentiation of cell elements of larval Echinococcus multilocularis*

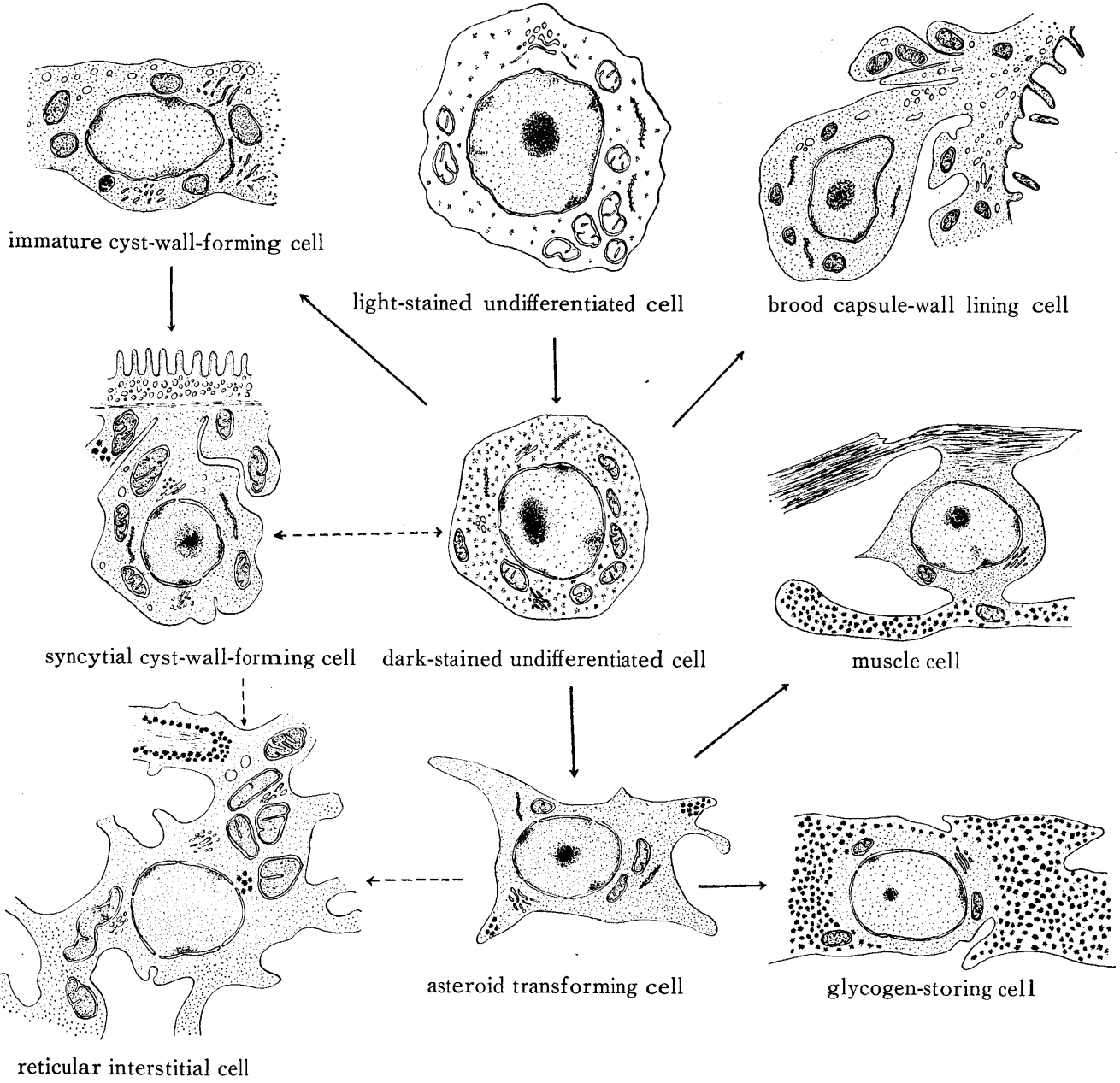
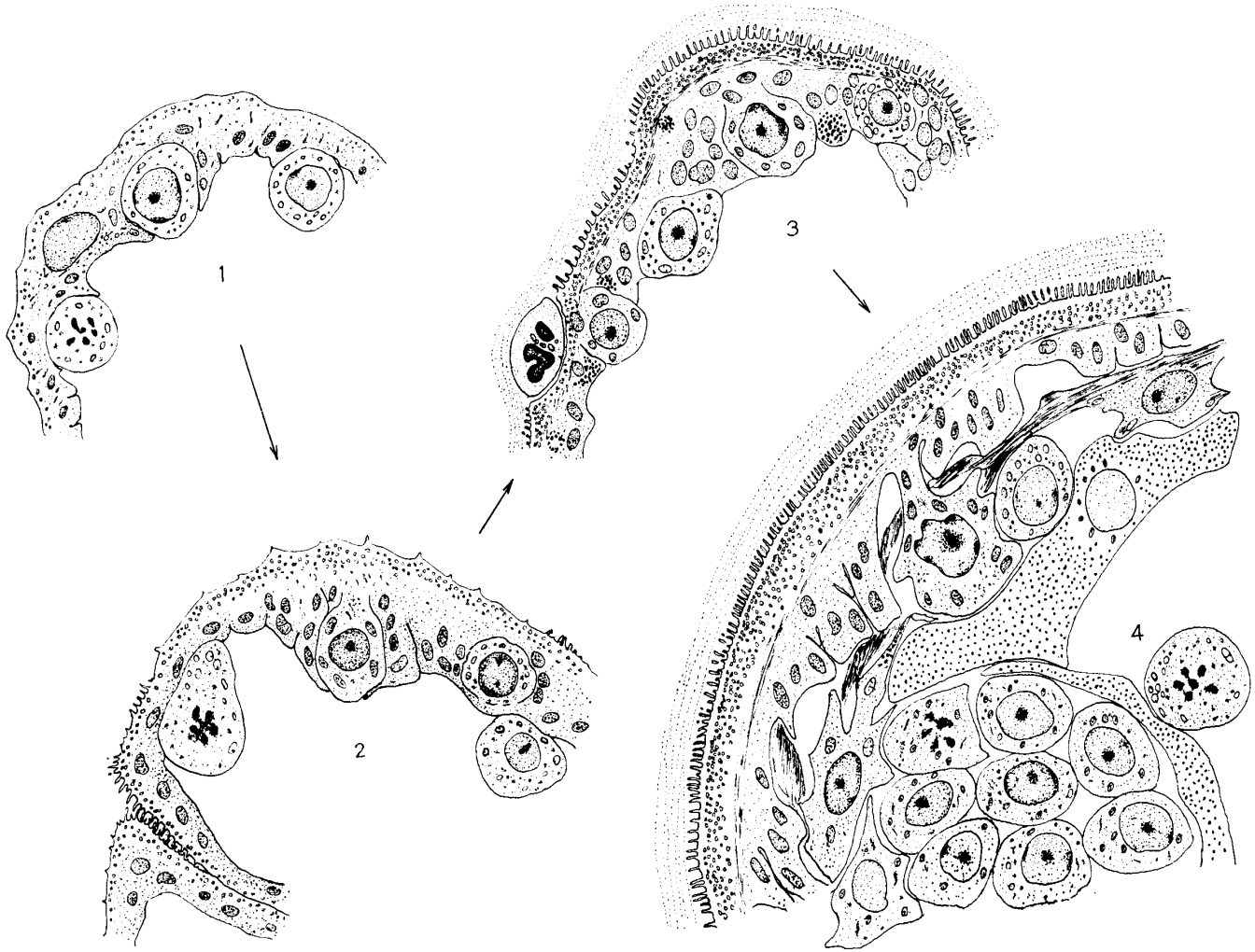


FIGURE 2 *Development of cyst-wall of larval Echinococcus multilocularis*



1: Unilocular stage
2: Initial stage of multivesiculation
3: Stage of initial formation of cuticular layer
4: Stage of initial formation of brood capsule

and YAMASHITA et al. (1962) also observed in vitro the exogenous multivesiculation of the cysts collected from experimentally infected rodents, and of the cysts originated from vesiculated protoscolices. In the present experiment, the multilocular vesiculation was demonstrable light-microscopically on and after the 7th day. In addition to the exogenous multivesiculation, light- and electron-microscopical examinations revealed that some of the initially multivesiculated cysts were divided with septa consisting of a single or two adjoining syncytial cytoplasms with or without a cuticular layer and without the interposition of the cellular elements of host (Figs. 15 & 16). The above findings seem to suggest the possibility of multivesiculation by means of a division from the cellular septa.

RAUSCH (1954) and MANKAU (1957) found the cuticular layer (subgerminal membrane) at 20 days after the inoculation, and OHBAYASHI (1960) demonstrated the cuticular layer in a 19-day case of the cotton rat, 26-day *Microtus* and C57BL/6, and 30-day dba and CF#1, respectively. In the present paper, light- and electron-microscopically, a layer of extracellular substance (the cuticular or laminated layer) was confirmed in some of the 14-day cases.

As for the mechanism of cuticular layer formation, OHBAYASHI (1960) stated that the germinal cells were capable of producing the substance which composed the cuticular layer. SCHWABE (1959) described the interesting presumption that the laminated membrane was produced only when metabolic products of the germinal cells were precipitated in the presence of an antibody. YAMASHITA et al. (1962), however, observed the thickening of the cuticular layer of vesiculated protoscolices cultured in vitro, and they found that the thickening of the layer of the vesiculated protoscolices cultured together with HeLa cells was more remarkable than the protoscolices alone. In the present observations, no relationship could be ascertained between the quantity of the electron-opaque substance in the vesicles of the syncytial cytoplasm and the appearance of the extracellular substance. The electron-opaque substance in the vesicles of the syncytial cytoplasm of the germinal layer was less in quantity than that in the tegumental cytoplasm of the protoscolex. In some electron micrographs, the formation of the cuticular layer is recognized outside neutrophilic leucocytes, host-tissue cells and their debris which is located in contact with the outer surface of the syncytial cytoplasm (fig. 18). The findings mentioned above seem to show that the cuticular layer is formed not only by the metabolic substance of the echinococcal tissue, because the cuticular layer should not be located in the host-tissue outside the cyst, when it is formed only as a product of the larval tissue. Further observations are expected for a solution of the problem of why the syncytial cytoplasm of the brood capsule and the tegumental cytoplasm of the protoscolex never form the layer of extracellular substance, although the two

show an ultrastructure resembling the syncytial cytoplasm of the germinal layer.

With the lapse of time, at about the stage of the 14th to 30th day, the nuclei of the syncytial cells of the cyst wall protrude interiorly together with the perikarya from the syncytial cytoplasm, because they remove to the inner side of germinal layer. Otherwise, no mitotic division of the syncytial cells of the cyst wall was confirmable as far as the present experiment was concerned. The cells, which were very similar to the dark-stained undifferentiated cell, were partially continuous with the surrounding syncytial cytoplasm of the cyst wall (figs. 12～14). Many infoldings of the cell membrane were seen on the inside of the syncytial cytoplasm. Judging from the above findings, it may be presumed that some of the dark-stained undifferentiated cells, which manifest active proliferation, join with the syncytial cytoplasm in connection with the development of the cyst wall. It, however, is possible that the immature syncytial cells are able to be dedifferentiated. Through the examination of secondary echinococcosis, YAMASHITA et al. (1960) clarified that the protoscolices injected intraperitoneally showed cystic metamorphosis. YAMASHITA et al. (1962) and SAKAMOTO et al. (1965) confirmed that the subcuticular cell (tegumental cell) of the vesiculated protoscolices incubated in vitro metamorphosed to asteroid-shaped cells (designated presumably as cyst-wall-forming cells and undifferentiated cells). From these facts, it seems to suggest the possibility that the dedifferentiation of syncytial cells in the cyst wall may also take place during the process of histogenesis. Contrary to the above, there always exists the possibility, too, that undifferentiated cells which remain in the tissue of protoscolices can proliferate and differentiate. At present, we have no data concerning the histogenetical process of syncytial cytoplasm. From the findings obtained, however, it is considered that the joining of dark-stained undifferentiated cells in the development of syncytial cells is most pausible.

The structure, which showed a resemblance to the terminal web in the intestinal epithelial cell of mammals, was demonstrated in the syncytial cytoplasm of the cyst wall from around the time of the initial formation of the cuticular layer. This structure is considered to play a physiological role in dividing the outer absorbing portion of cytoplasm without mitochondria from the inner proper portion of cytoplasm, or to act as a cytoskeleton, because its ultrastructure is analogous to that of the terminal web in the intestinal epithelial cell.

The temporary appearance of alpha glycogen particles was recognized in the syncytial cytoplsam of cases during the 15th to 30th days. It is the most reasonable conclusion that the above phenomenon may be attributable to the temporary metabolic change of these cells, judging from the following fact. The glycogen particles of the syncytial cells disappear at a stage later than the above,

when the appearance of other cells with glycogen such as the glycogen-storing cells and muscle cells can be confirmed. On the other hand, it is also possible that the immature syncytial cells, in which nutrients are accumulated, are transformed into other glycogen-storing cells parallel with differentiation of the cells.

In cases later than the 35th day, muscle cells with many projections, which contain myofibrils and beta glycogen particles, or massive alpha glycogen particles, are demonstrable in the cyst wall, electron-microscopically. At the same time, the glycogen-storing cells, which are filled with numerous masses of glycogen particles, are recognized in the same area. Otherwise, the glycogen-storing cells are found among the dark-stained undifferentiated cells proliferating actively in the initial formation of brood capsule. These glycogen-storing cells are thought to play a role in storing the nutrition. These muscle cells and glycogen-storing cells manifestly resemble the asteroid transforming cells in the fact of the existence in them of glycogen particles and mitochondria with electron-opaque matrix and distinct cristae.

On and after the 35th day, the asteroid transforming cells with several cytoplasmic projections appear in the inner side of the germinal layer, and in the cellular accumulation of the initial brood capsule and protoscolex. The cell is analogous to the dark-stained undifferentiated cell because of the existence of polysomes, numerous ribosomes and small ovoid mitochondria with an electron-opaque matrix and distinct cristae, although glycogen particles and the asteroid shape are lacking in the latter. Consequently, it is considered that the cell differentiates from the dark-stained undifferentiated cell.

The reticular intersitital cells with several long cytoplasmic projections, which contain many electron-opaque glycogen-like massive granules and, occasionally, a fibrous crystalloid substance, are found among the cells in the cyst wall. The cells are considered to originate from the asteroid transforming cell or syncytial, cyst-wall-forming cell, because the cell manifests the intermediate characteristics between the syncytial cell and asteroid transforming cell, namely the possession of many ribosome, glycogen particles and large, longish-ellipsoid mitochondria with an electron-opaque matrix. It may still leave room for the possibility that a horizontal section of the perinulear cytoplasm of the tegumental cell may show the appearance of the reticular interstitial cell. At present, it is not clear whether this cell transforms into the glycogen-storing cell and muscle cell or not. Clarification of this problem is left for future study. At any rate, the cellular construction of the cyst wall seems to be completed at about 35 days after the infection.

RAUSCH (1954) detected a few protoscolices in *Microtus pennsylvanicus* at an early developmental stage, after 45 days. MANKAU (1957), however, observed

the formation of brood capsule and protoscolices in mice after a period of months. OHBAYASHI (1960) concluded that the days required for brood capsule and mature protoscolex formations, respectively, were 20 and about 53～60 in the cotton rat, 44 and 53～60 in the *Microtus*, 49 and 60～90 in the dba-mouse, and 90 and 150 in the CF♯1-mouse. WEBSTER & CAMERON (1961) demonstrated rudimentary and mature protoscolices in the cotton rat after 4 and 7～8 weeks, respectively, and they found cysts filled with protoscolices in DBA/1-J mice after 37 weeks. In the present work, light-microscopically, the initial brood capsule and protoscolex formations were seen in some of the 25- and most of the 35-day cases, respectively. Electron-microscopically, however, the brood capsule and protoscolex were found in the cases after 35 days, since the visual field is limited in narrow areas.

A massive accumulation of dark-stained undifferentiated cells showing active mitosis are noted in the cases at the initial formation of brood capsules. Thereafter, the lumen lined with the syncytial cells is formed in the center of the accumulation. These findings strongly suggest the possibility that the syncytial cells, which line the brood capsule, differentiate from the dark-stained undifferentiated cells.

The syncytial cytoplasm of the immature protoscolex is continuous from that on the inner surface of brood capsule. Moreover, the syncytial cytoplasm of the protoscolex, with the lapse of time, shows the characteristics of tegumental cytoplasm because of the appearance of an electron-opaque apical spine on the extremity of the microvilli (subsequently, microtriches) and the increase of the electron-opaque substance in the vesicles of the cytoplasm. Therefore, it goes without saying that the tegumental cells originate from the syncytial cells which line the wall of the brood capsule.

ACKNOWLEDGMENTS

The authors wish to express their cordial thanks to Prof. J. YAMASHITA and Dr. M. OHBAYASHI of this department for their kind advice in this study and review.

REFFERENCES

1) COLLIN, W. K. (1968): *J. Parasit.*, **54**, 74
2) COLLIN, W. K. (1969): *Ibid.*, **55**, 149
3) MANKAU, S. K. (1955): *Ibid.*, **41**, Suppl., 29
4) MANKAU, S. K. (1956): *Am. J. trop. Med. Hyg.*, **5**, 872
5) MANKAU, S. K. (1957): *J. Parasit.*, **43**, 153
6) MORSETH, D. J. (1963): *Expl Parasit.*, **16**, 207
7) MORSETH, D. J. (1966): *J. Parasit.*, **52**, 1074
8) MORSETH, D. J. (1967): *Ibid.*, **53**, 312
9) MORSETH, D. J. (1967): *Ibid.*, **53**, 492

10) NIELAND, M. L. (1968): *Ibid.*, **54**, 957
11) RAUSCH, R. (1954): *J infc. Dis.*, **94**, 178
12) RAUSCH, R. & JENTOFT, V. L. (1957): *J. Parasit.*, **43**, 1
13) OHBAYASHI, M. (1960): *Jap. J. vet. Res.*, **8**, 134
14) SADUN, E. H., ALLAIN, N. D. S. & KING, N. M. (1957): *J. infc. Dis.*, 100, 273
15) SAKAMOTO, T. & SUGIMURA, M. (1969): *Jap. J. vet. Res.*, **17**, 67
16) SAKAMOTO, T., YAMASHITA, J., OHBAYASHI, M. & ORIHARA, M. (1965): *Ibid.*, **13**, 128
17) SCHWABE, C. W. (1959): *Am. J. trop. Med. Hyg.*, **8**, 20
18) WEBSTER, G. A. & CAMERON, T. W. M. (1961): *Can. J. Zool.*, **39**, 877
19) YAMASHITA, J., OHBAYASHI, M. & SAKAMOTO, T. (1960): *Jap. J. vet. Ret.*, **8**, 315
20) YAMASHITA, J., OHBAYASHI, M., SAKAMOTO, T. & ORIHARA, M. (1962): *Ibid.*, **10**, 85

EXPLANATION OF PLATES

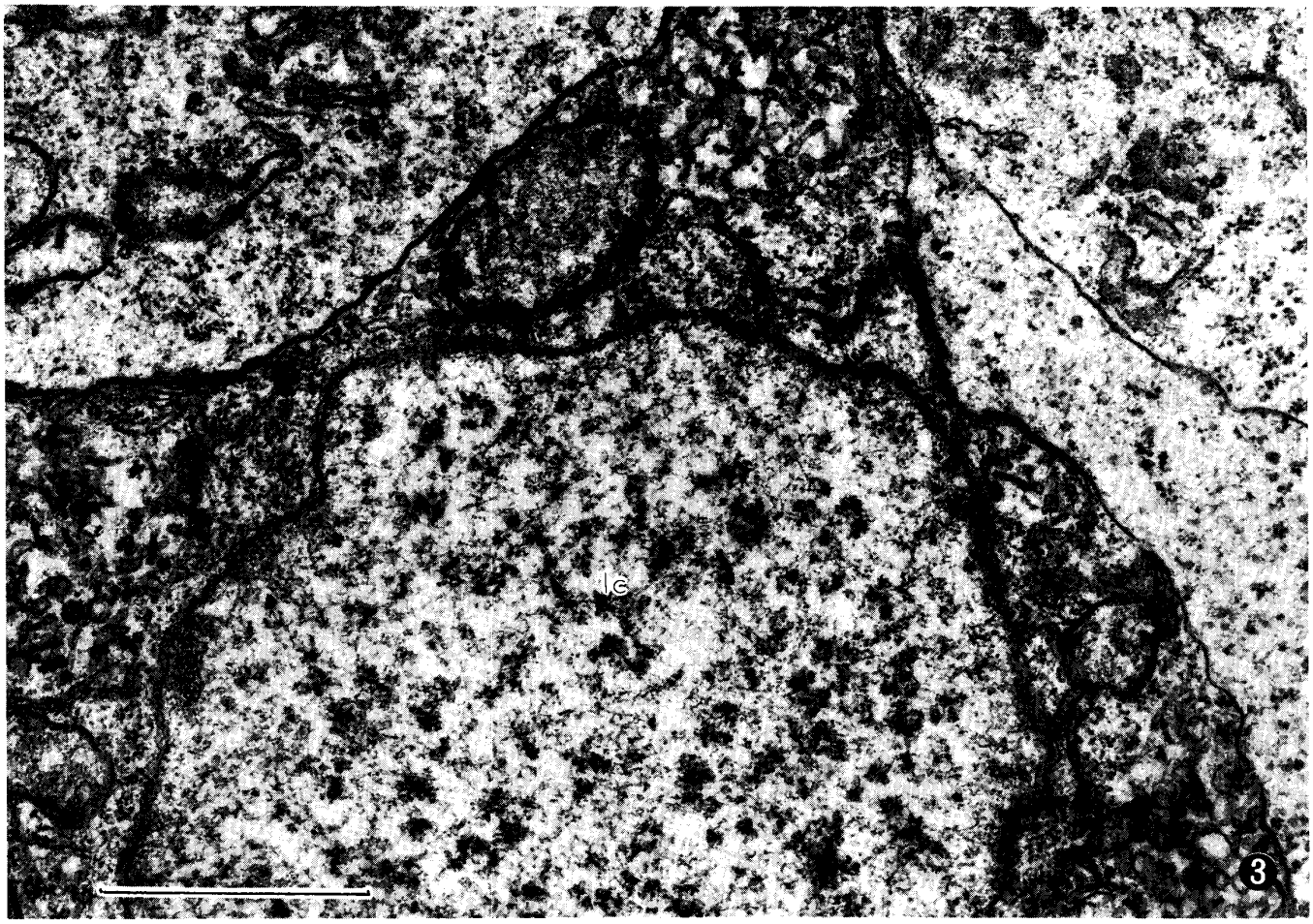
All scales printed in the figures are shown at 1 μ.

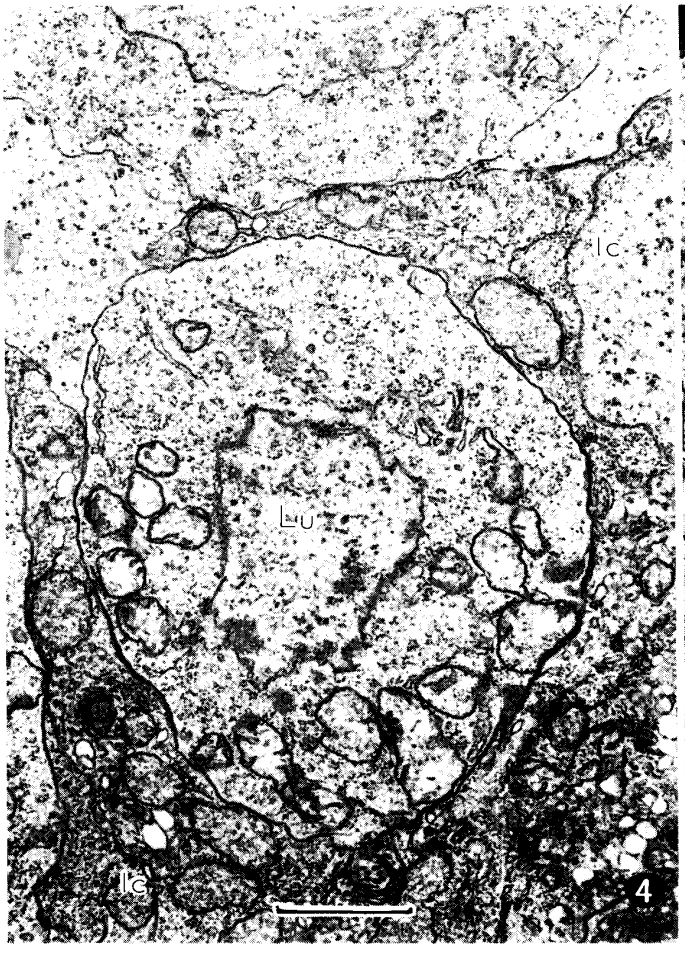
PLATE I

Fig. 3 Immature cyst-wall-forming cell (Ic) with many vesicles 7th day

Fig. 4 Immature cyst-wall-forming cell (Ic) and light-stained undifferentiated cells (Lu) 7th day

Fig. 5 Light-stained undifferentiated cell (Lu) 7th day


IC
3


IC
LU
IC
4

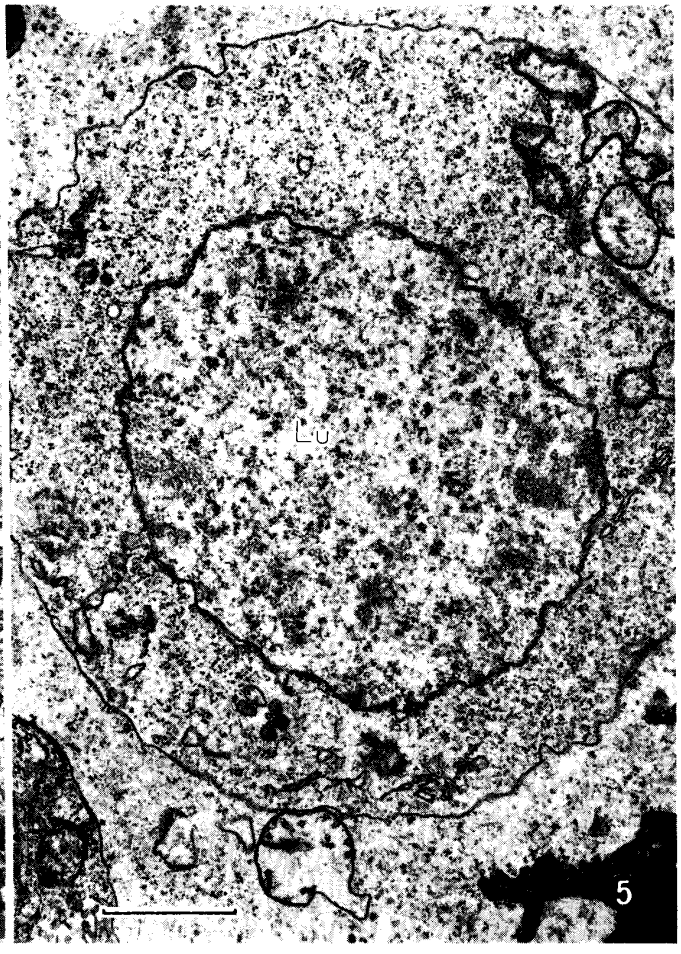
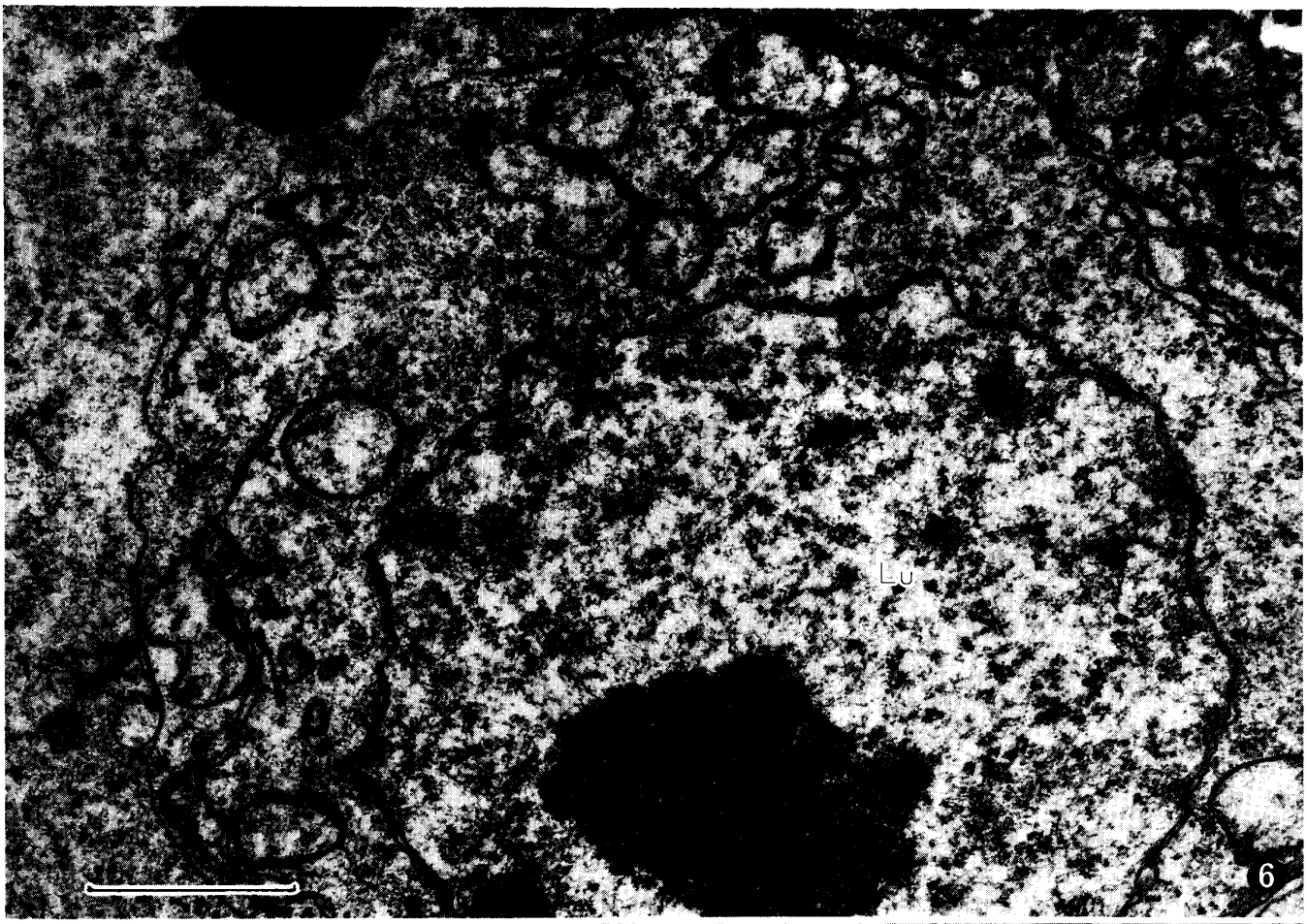

LU
5

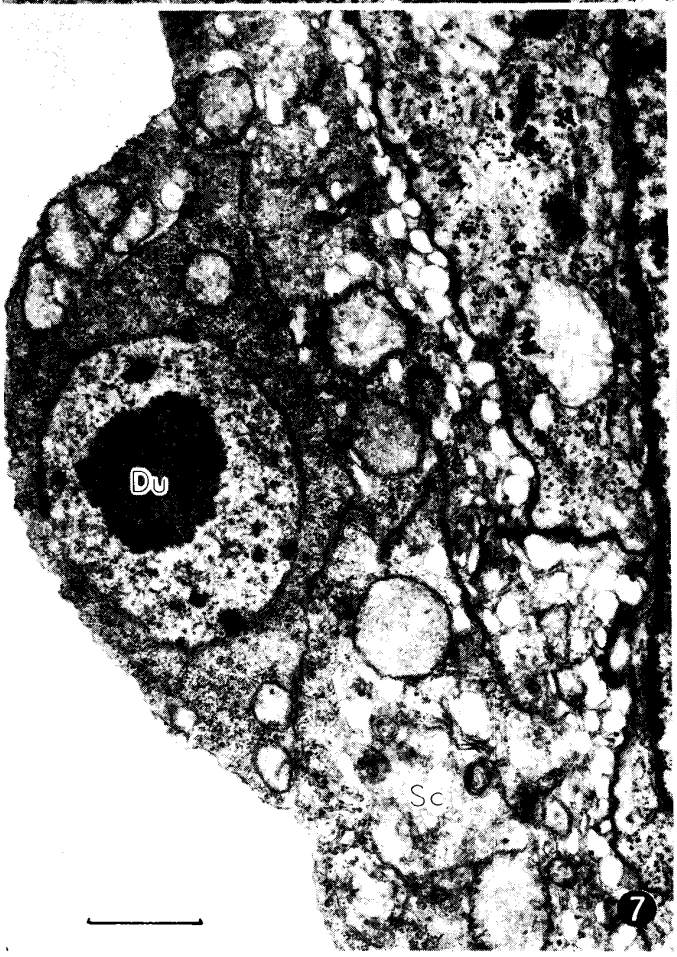
PLATE II

Fig. 6 Light-stained undifferentiated cell (Lu) with polysomes 7th day

Fig. 7 Dark-stained undifferentiated cell (Du) and syncytial cytoplasm (Sc) without cuticular layer 14th day

Fig. 8 Dark-stained undifferentiated cell (Du) and syncytial cytoplasm (Sc) with alpha glycogen particles (arrows) 14th day


Lu
6


Du
Sc
7

Sc
Du
Sc
8

PLATE III

Fig. 9 Dark-stained undifferentiated cell (Du) showing mitosis, and two syncytial cytoplasms (Sc) of adjoining cyst walls

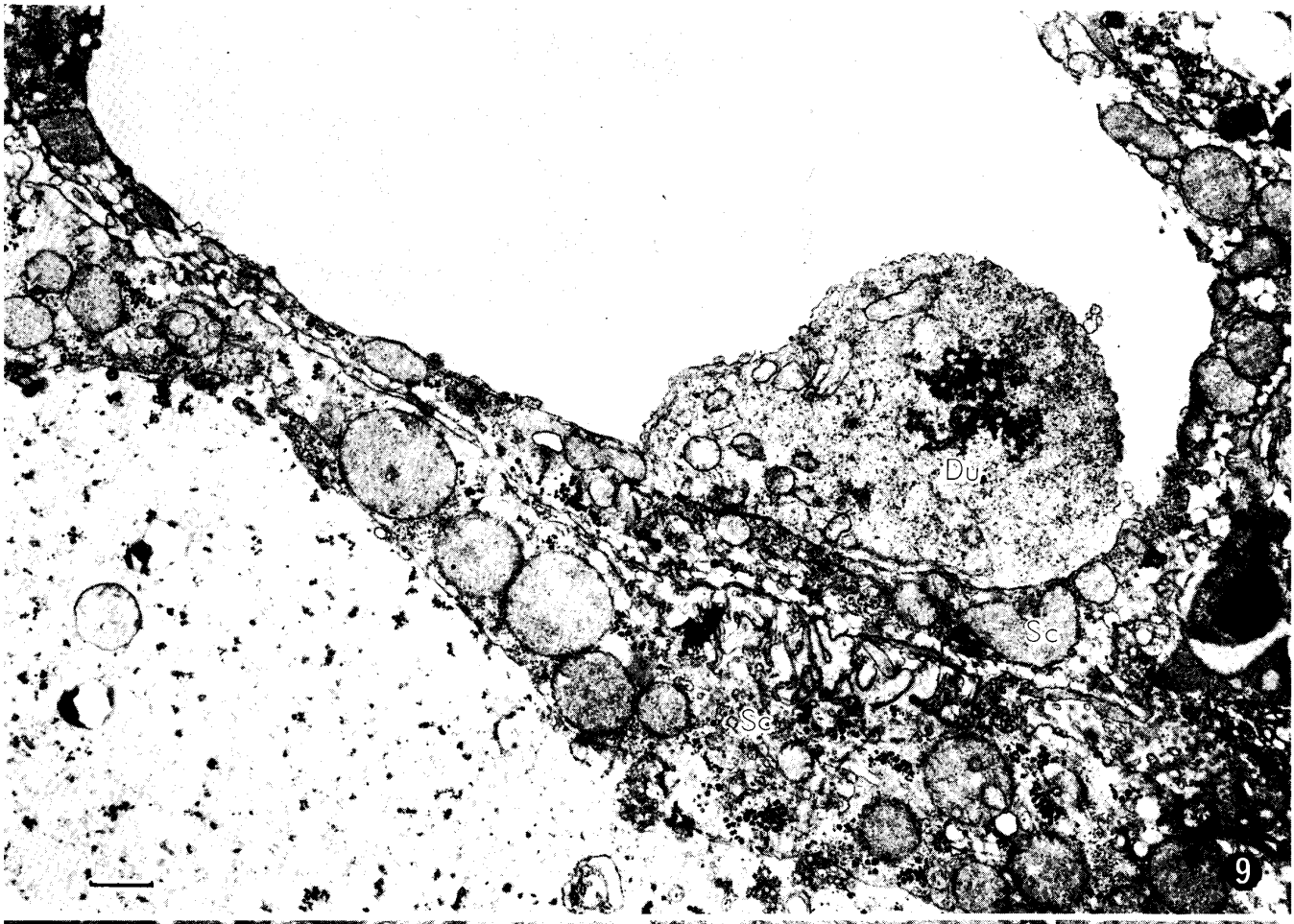
The interposition of the cuticular layer and host-cell are not found between two syncytial cytoplasms. 14th day

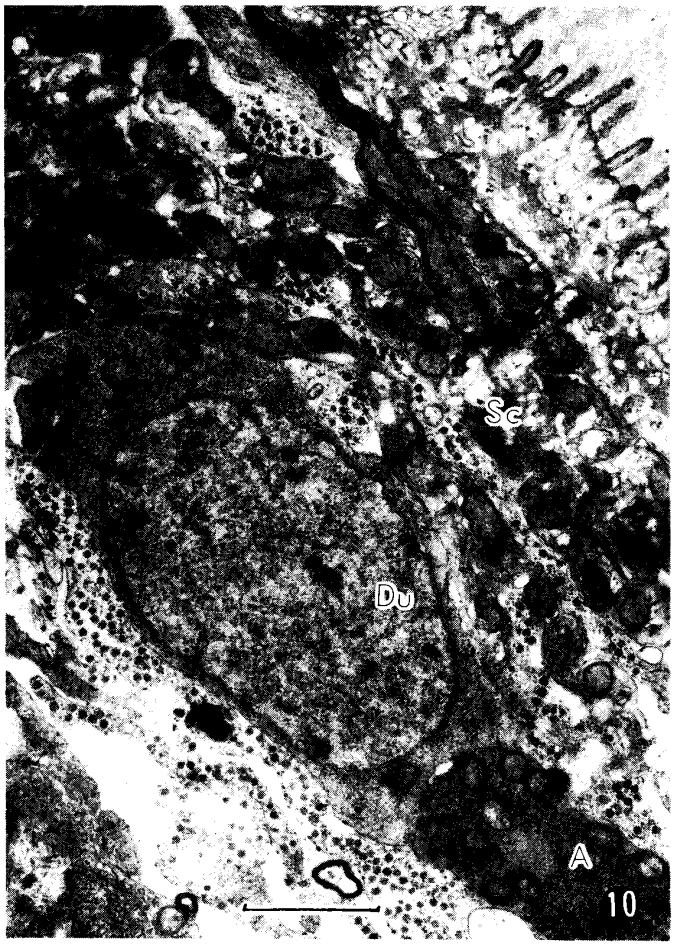
Fig. 10 Dark-stained undifferentiated cell (Du), asteroid transforming cell (A) and syncytial cytoplasm (Sc) of the cyst wall

The syncytial cytoplasm possesses microvilli and many mitochondria of various shapes. 36th day

Fig. 11 Syncytial cyst-wall-forming cell (Cw)

The perinuclear cytoplasm of the cell is incompletely divided from the syncytial cytoplasm surrounding it by fragmental, cell-membrane-like structures. 14th day


Du
Sc
Sc
9


Sc
Du
A
10

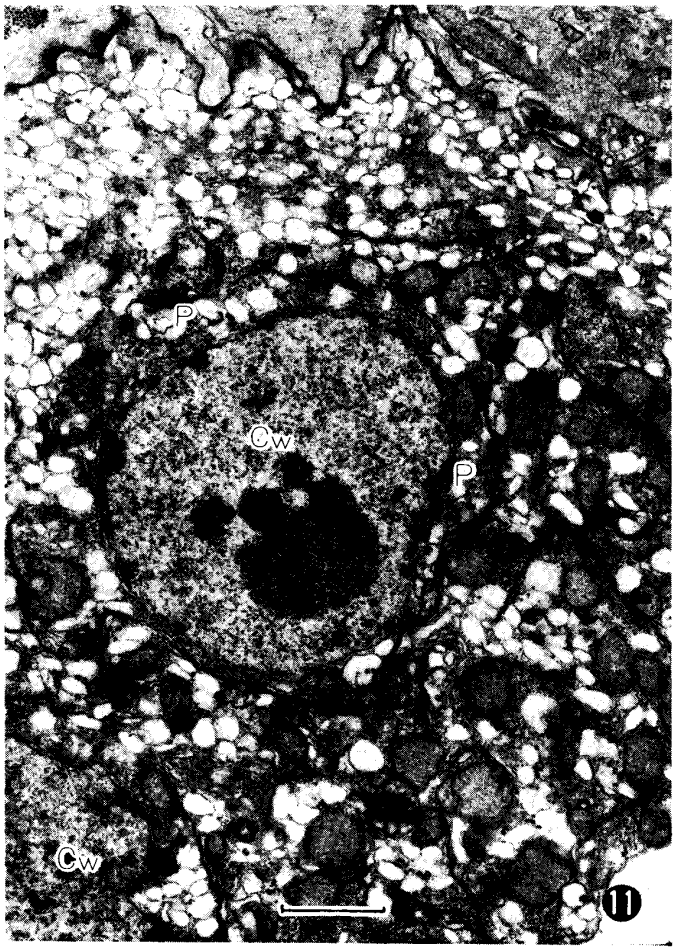

P
Cw
P
Cw
11

PLATE IV

Fig. 12 Dark-stained undifferentiated cell (Du) in the syncytial cytoplasm (Sc) of the cyst wall and cuticular layer (Cu)

The cytoplasm of the dark-stained undifferentiated cell with small mitochondria is partially continuous (arrow) with the syncytial cytoplasm containing large mitochondria. 14th day

Fig. 13 Cytoplasm of the dark-stained undifferentiated cell (Du) with polysomes in the syncytial cytoplasm (Sc) of the cyst wall

A partial continuity is seen between both cytoplasms. 14th day

Fig. 14 Syncytial cyst-wall-forming cell (Cw) and cuticular layer (Cu)

The perinuclear cytoplasm (P) with small mitochondria is partially divided by a cell-membrane-like structure from the surrounding syncytial cytoplasm (Sc) with large mitochondria. 14th day

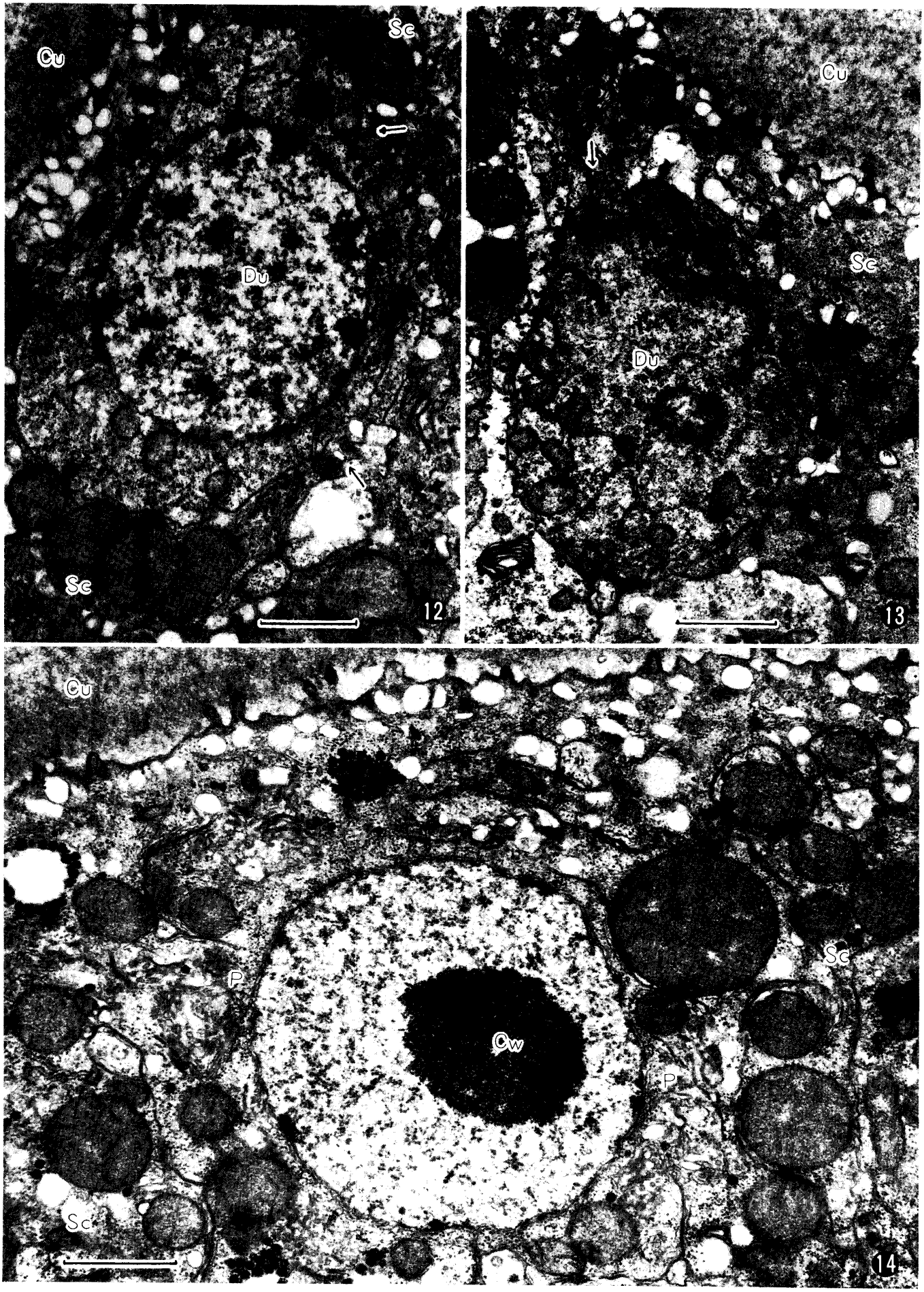

Cu
Sc
Du
Sc
12
Cu
Sc
Du
13
Cu
P
Cw
P
Sc
Sc
14

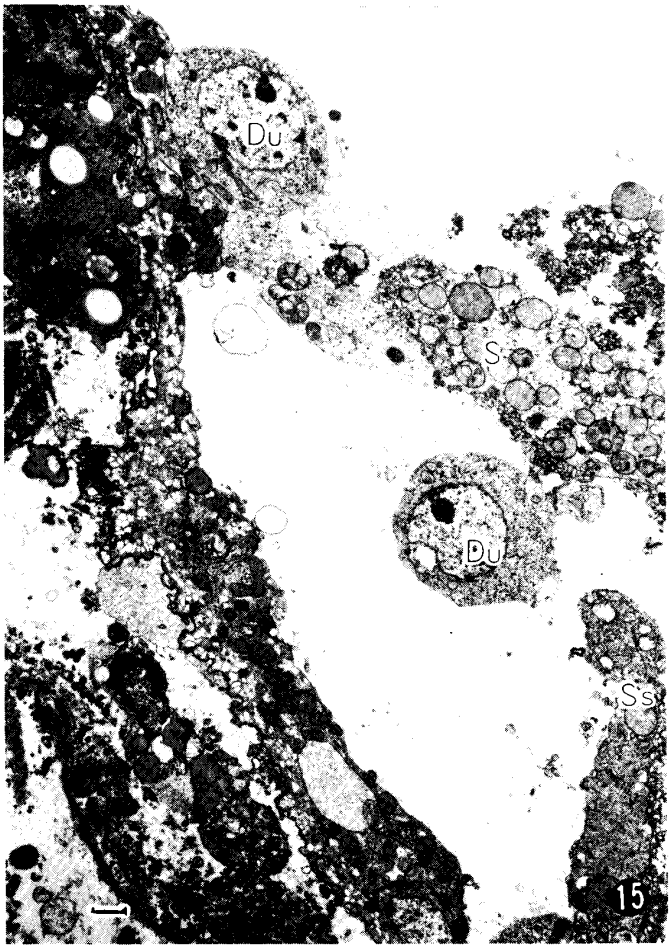
PLATE V

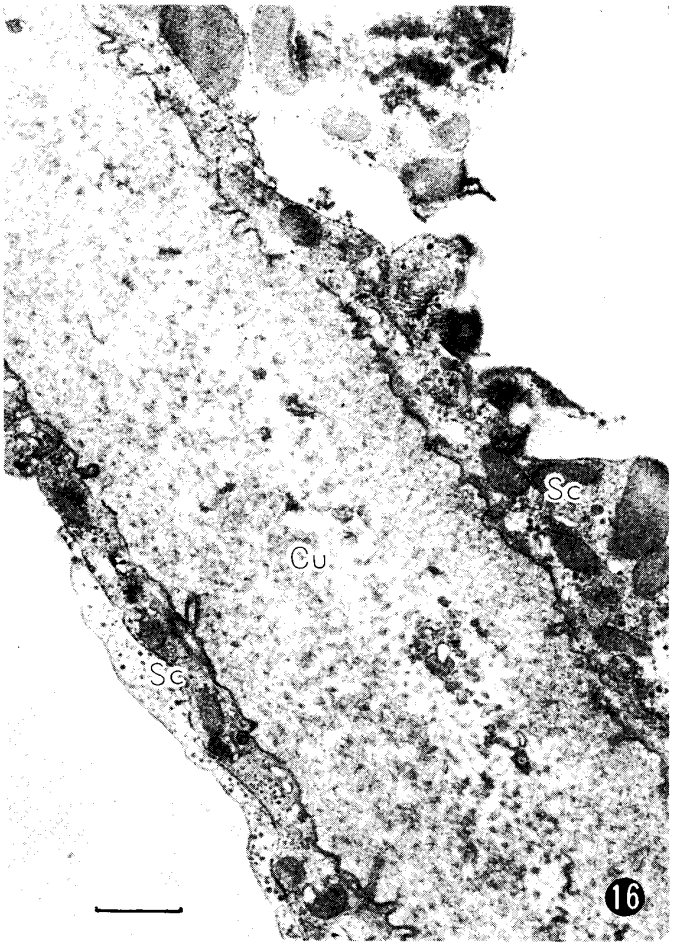
Fig. 15 Cyst at initial stage of multivesiculation
The cyst is separated by septa consisting of single (S) and double layered (Ss) syncytial cytoplasms, and a few dark-stained undifferentiated cells (Du) adhere to the septa. 14th day

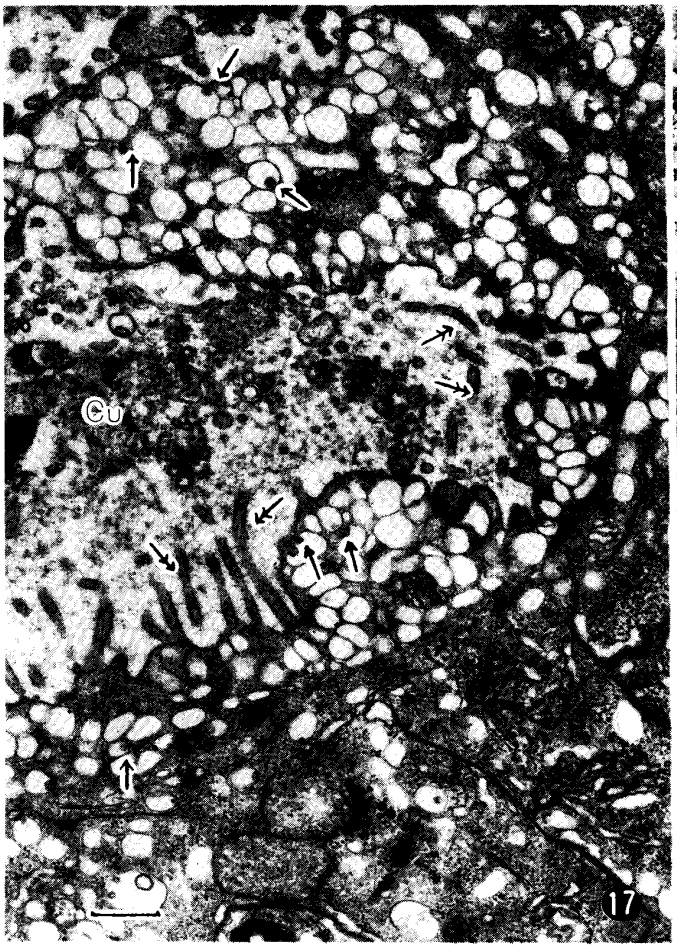
Fig. 16 Two adjoining syncytial cytoplasms (Sc) with a cuticular layer (Cu) without the interposition of the host-cell 36th day

Fig. 17 Initial formation of the cuticular layer (Cu) and microvilli (double arrows) of the syncytial cytoplasm (Sc) with vesicles containing a dense-body (arrows) 14th day

Fig. 18 Neutrophilic leucocyte (N) covered with a cuticular layer (Cu), and syncytial cytoplasm (Sc) with numerous glycogen particles 14th day


Du
S
Du
Ss
15


Sc
Cu
Sc
16


Cu
17

Sc
Cu
N
18

PLATE VI

Fig. 19 Initial cuticular laryer (Cu) and cyst-wall-forming cell (Cw) with glycogen particles in its cytoplasm 14th day

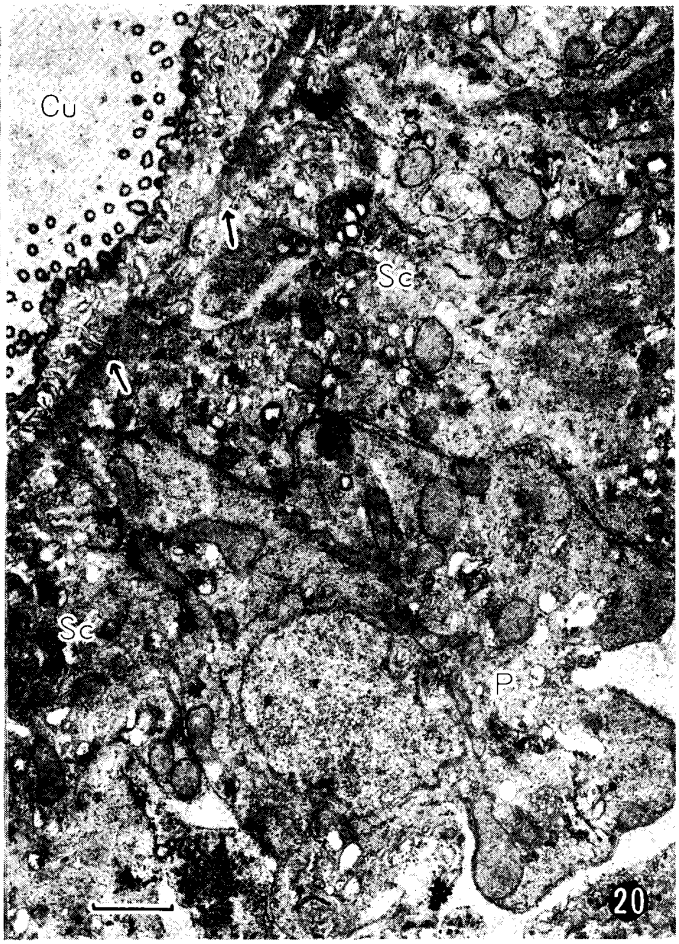
Fig. 20 Cyst-wall-forming cell and cuticular layer (Cu)

A terminal web-like structure (arrows) is seen between the outer zone with numerous vesicles and the inner one with mitochondria. The perikaryon (P) of the cell is partially separated from its syncytial cytoplasm (Sc), and removes to the inside of cyst wall. 36th day

Fig. 21 The perikaryon (P) of the cyst-wall-forming cell is separated from its distal, syncytial cytoplasm (Sc)

The cytoplasmic continuity (Cb) is seen between the perinuclear cytoplasm and the distal one. The perinuclear cytoplasm contains a considerable number of polysomes and a distinct Golgi complex. 55th day

Cu
Cw
19


Cu
Sc
Sc
P
20

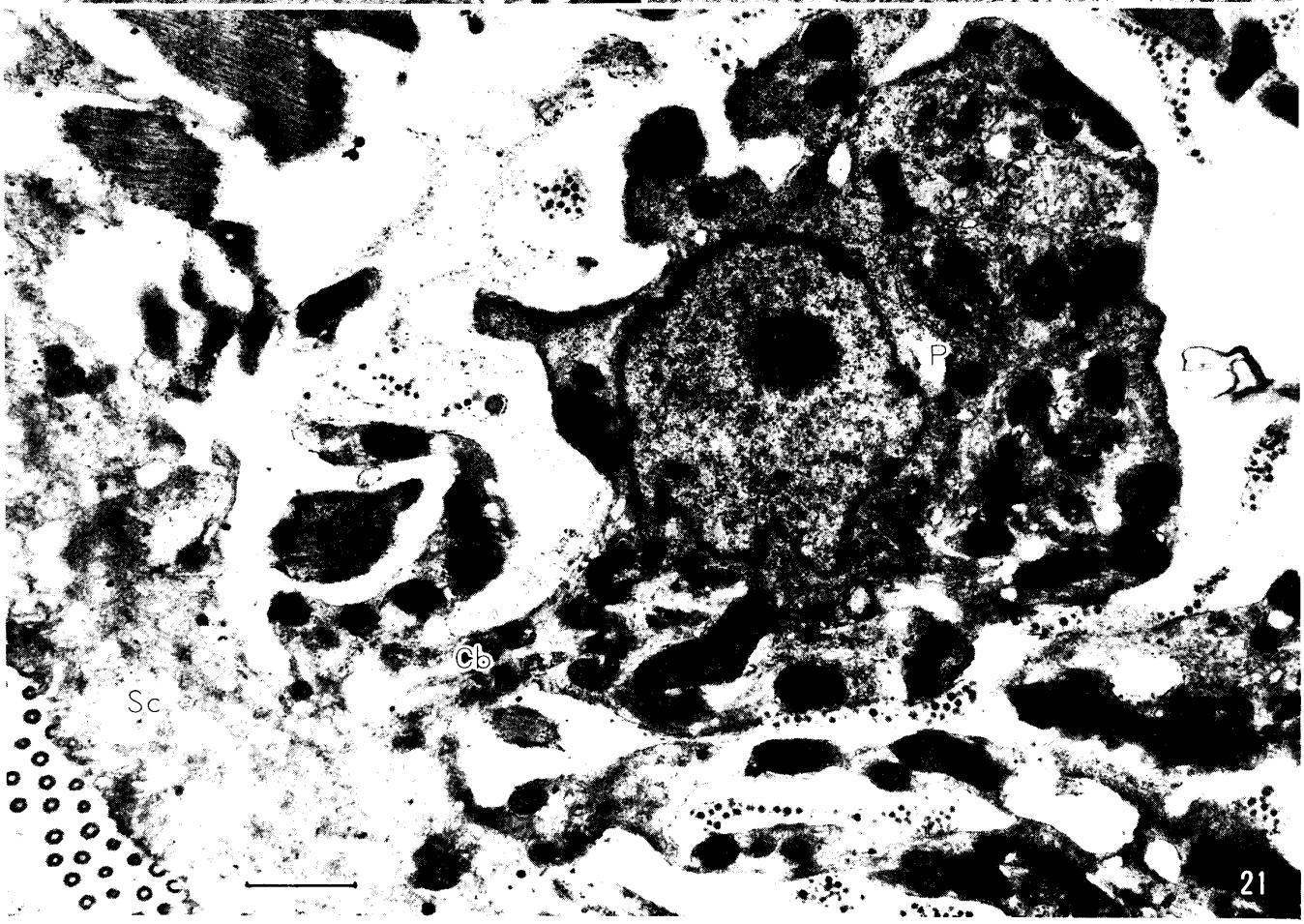

P
Cb
Sc
21

PLATE VII

Figs. 22 & 23 Asteroid transforming cell (A) in the cuticular layer of cyst wall 36th day

Fig. 24 Asteroid transforming cell (A) with a few glycogen particles in the immature protoscolex 55th day

Fig. 25 Reticular interstitial cell (R) having reticular long projections in the cellular layer of cyst wall 36th day

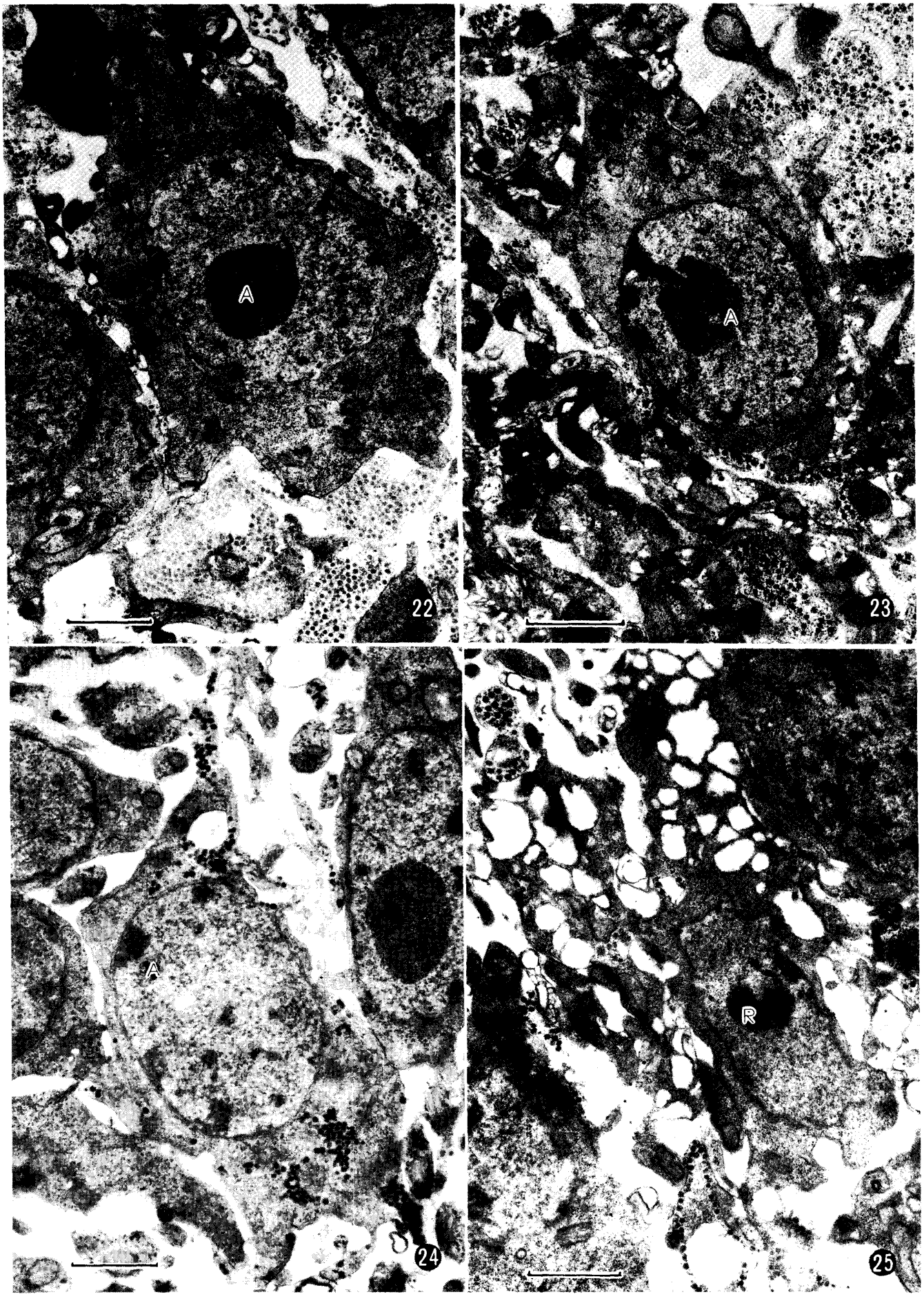
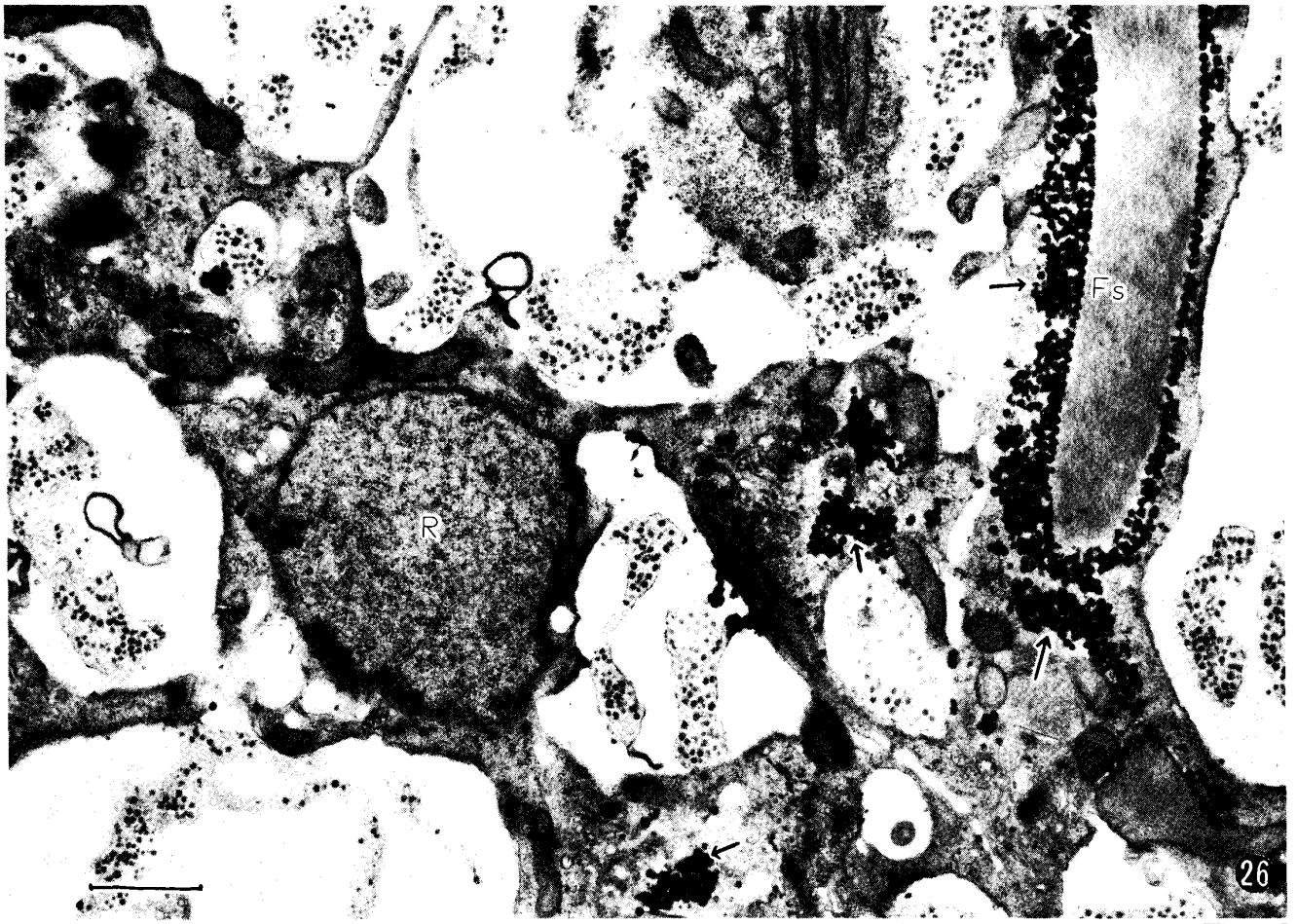

A
22
A
23
A
24
R
25

PLATE VIII

Fig. 26 Reticular interstitial cell (R) having projections with electron-opaque glycogen-like granules (arrows) and a fibrous substance (Fs) 55th day

Figs. 27 & 28 Reticular interstitial cell (R) with long reticular projections 55th day


R
Fs
26

R
27

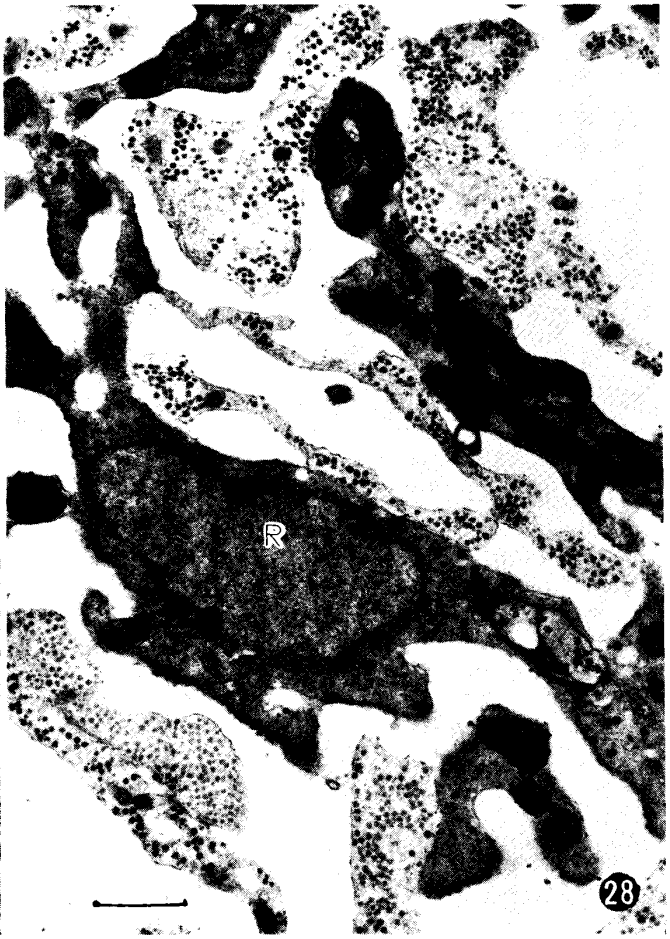

R
28

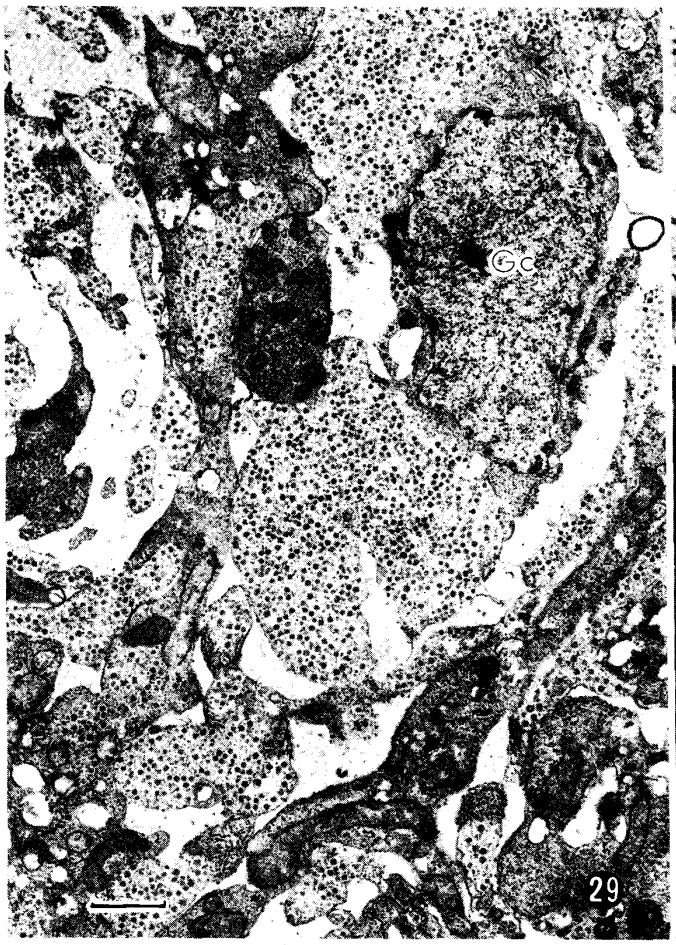
PLATE IX

Fig. 29 Glycogen-storing cells (Gc) with numerous alpha glycogen particles in their cytoplasm 36th day

Fig. 30 Glycogen-storing cell (Gc) and dark-stained undifferentiated cell (Du)
The cytoplasm of the glycogen-storing cell is filled up with alpha glycogen particles. 55th day

Fig. 31 Free, glycogen-storing cell (Gc) in the lumen of the cyst and a mass of dark-stained undifferentiated cells proliferated at the initial stage of the brood capsule formation
The cytoplasmic projections with many glycogen particles cover the cellular mass and lie among the cells constructing it. 36th day

Fig. 32 Muscle cell (Mc) having projections with myofibrils (Mf) and glycogen particles (G) 55th day


Gc
29

Gc
Du
30

Gc
G
G
31

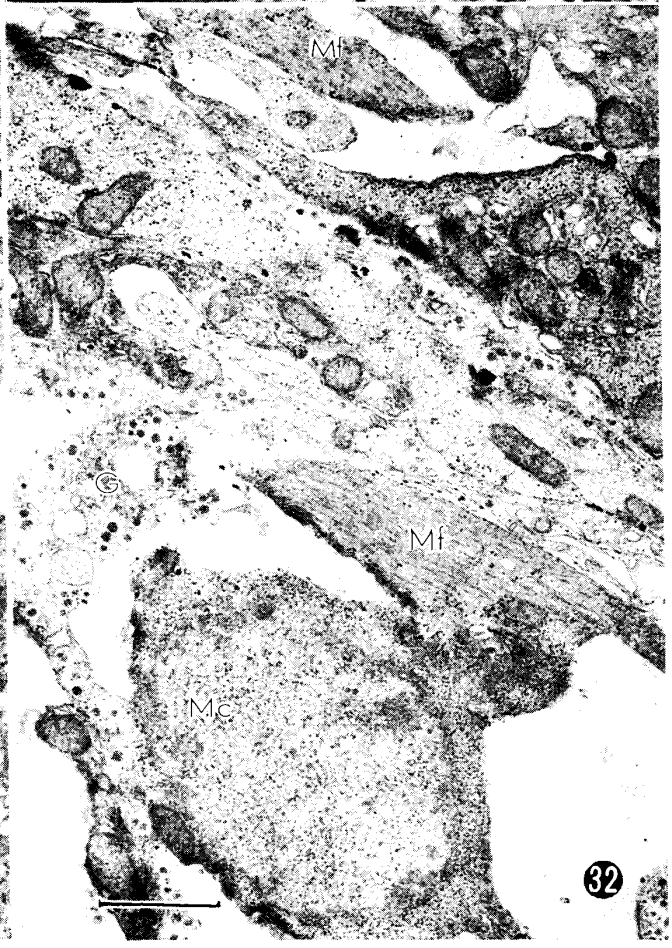

Mf
G
Mf
Mc
32

PLATE X

Fig. 33 Syncytial cytoplasm (Sc) with microvilli (Mi), cytoplasmic projections with glycogen particles and muscle cells (Mc) in the cellular layer of cyst wall

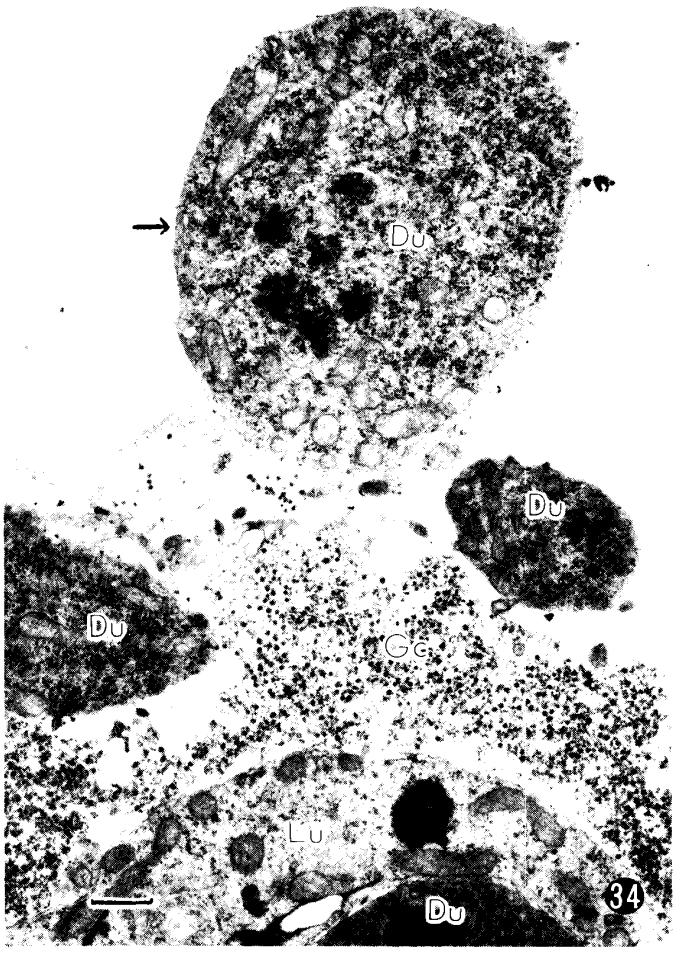
The muscle cell has long projections with myofibrils (Mf) and glycogen particles (arrow). 55th day

Fig. 34 Dark-stained undifferentiated cell (Du) and the cytoplasm of the glycogen-storing cell (Gc) in the cellular mass at the initial stage of brood casule fromation

A dark-stained undifferentiated cell shows mitosis (arrow). 55th day

Fig. 35 Light- (Lu) and dark- (Du) stained undifferentiated cells in the cellular mass at the stage of initial brood capsule formation 55th day

Mi
Sc
Mf
Mf
Mf
Mc
33


Du
Du
Du
Du
Gc
Lu
Du
34

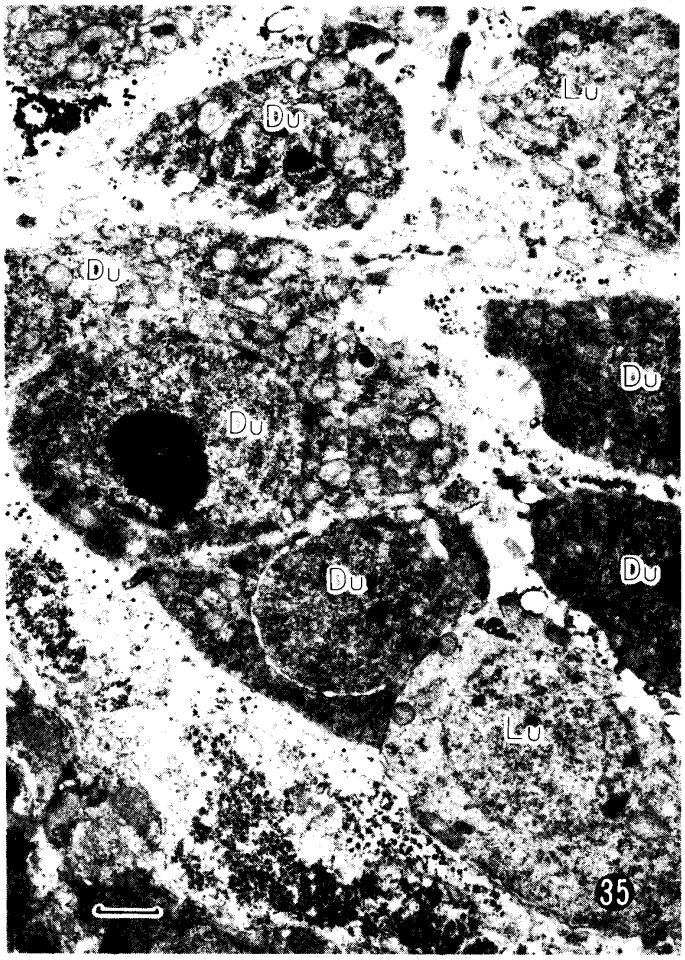

Lu
Du
Du
Du
Du
Du
Du
Lu
35

PLATE XI

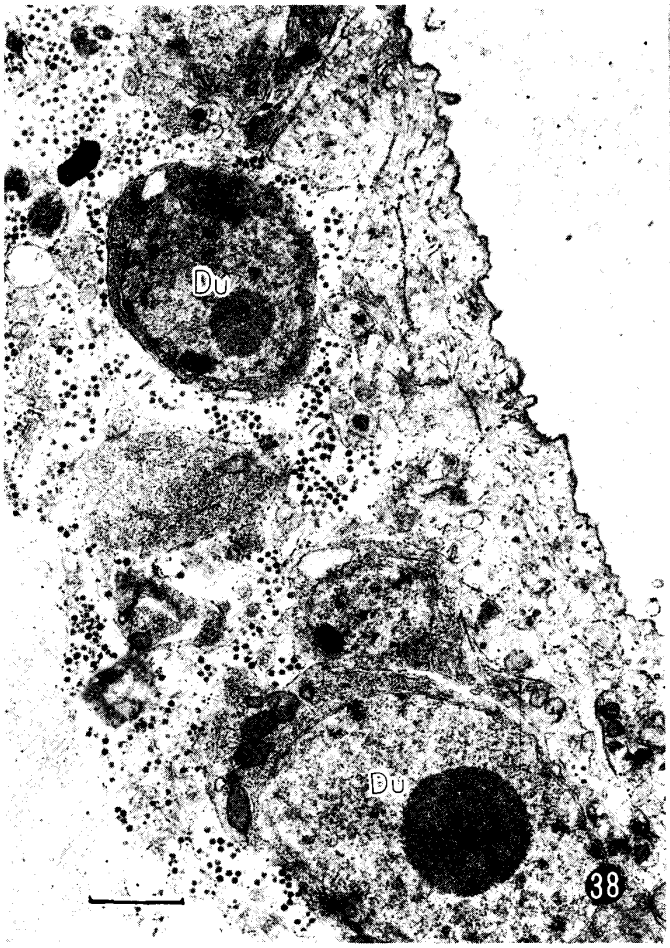
Figs. 36 & 37 Brood capsule-wall-lining cell (Bc) 36th day

Fig. 38 Dark-stained undifferentiated cells (Du) in the brood capsule-wall 36th day

Fig. 39 Muscle fibers (Mf) in the walls of the two adjoining brood capsules 36th day

Bc
36

Bc
37


Du
Du
38

Mf
Mf
39

PLATE XII

Fig. 40 Initial formation of the protoscolex

The cellular mass of dark-stained undifferentiated cells proliferated under the syncytial cytoplasm protrudes into the lumen of the brood capsule. 36th day

Fig. 41 The massive proliferation of dark-stained undifferentiated cells under the syncytial cytoplasm in the initial formation of the protoscolex

An arrow shows a mitosis of the cell. 36th day

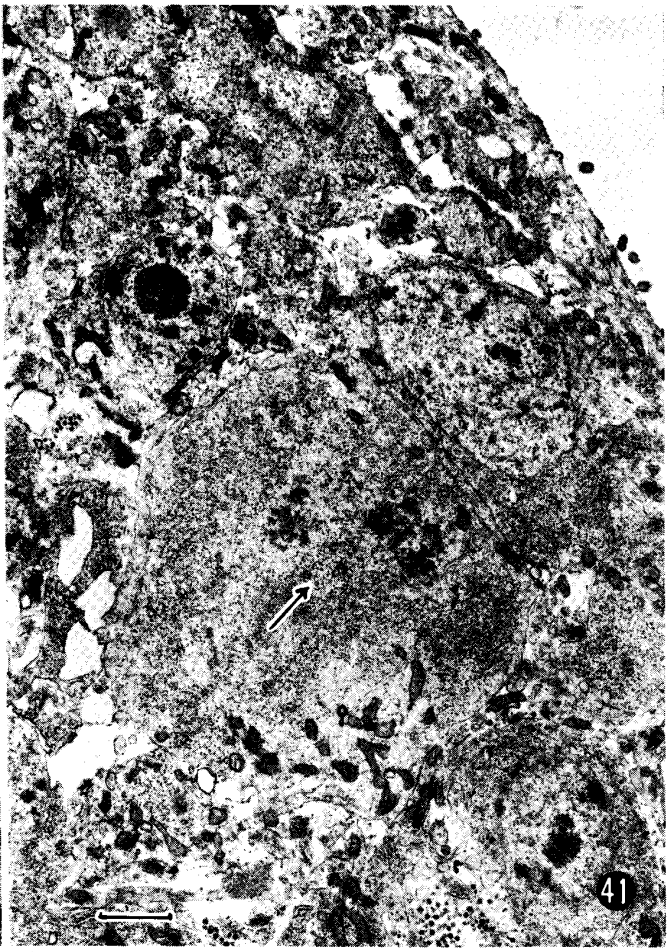
Fig. 42 Immature protoscolex

The tissue under the syncytial cytoplasm consists mainly of asteroid transforming cells. The projections of glycogen-storing cells and muscle cells, in which glycogen particles and myofibrils are contained, are seen among the cells. 55th day

Fig. 43 Protoscolex and brood capsule wall

The tegumental cytoplasm of the protoscolex possesses microtriches (arrows) and vesicles with a dense-body (double arrows). Muscle fibers (Mf) are seen under the tegumental cytoplasm. 55th day

Du
40


41

42

Mf
Tc
Mf
Mf
43