FOLIA PARASITOLOGICA (PRAHA) 27: 237—242, 1980.

THE FINE STRUCTURE OF LARVAE OF TAENIA HYDATIGENA AGED SEVEN AND TEN DAYS

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Abstract. Studies on the fine structure of larval *T. hydatigena* during their initial phase of development have shown that the structure of their differentiating tegument is dissimilar to that of the adult cestode. It differs from it both in the number and shape of organelles (microvilli, rod-shaped bodies, invaginations of the basal membrane, mitochondria) present at the early ontogenesis of the bladder. Typical of the initial phase of the production of rod-shaped bodies in sub-tegumental syncytial cells is their limited appearance in the distal cytoplasm and the character of microvilli lacking both an electron-dense layer below the plasma membrane and distal points. At 10 days, during the further differentiation of the perinuclear cytoplasm, the number of rod-shaped bodies and invaginations of the basal membrane in the tegument increases, and more vesicles and cisternae of the Golgi apparatus appear in the cells. The layer of circular muscles and fibrils has not yet started to differentiate in the subtegument. It is dependent for its differentiation on the secretory activity of cells with dilated cisternae of the endoplasmic reticulum. The scolex anlage and the zone of growth of the bladder differentiate from proliferating germ cells. Glycogen-depositing cells are present at this stage of development. Later these cells change into glycogen-containing structures.

The present paper is a sequence of an earlier study on the histochemistry of enzymes of *Cysticercus tenuicollis* (Hulínská 1979), and the fine structure of developmental types designated as I, II and III. Our material for the present study was obtained from baby pigs at days 7 and 10 p. i. The study has been intended to complement our knowledge of the fine structure of 13 and 16 days old larvae of *Taenia hydatigena* with that obtained for 7 and 10 day-old larvae. So far, knowledge of the early developmental phase and of cell differentiation has been made available for the larva of *Echinococcus granulosus* (Sakamoto and Sugimura 1970), and for the cysticercoid of *Hymenolepis citelli* (Collin 1970).

MATERIALS AND METHODS

Larvae were obtained from experimentally infected baby pigs at days 7 and 10 p.i. They looked like either elongate or spherical bladders (Plate I, Figs. 1—3). Larvae of types I and II were recovered from migratory canals in the liver, those of type III from the surface of the liver. Measurements and data on the morphology of *C. tenuicollis* were essentially similar to those given in an earlier paper (Hulínská 1979). Pieces of the infested liver and free bladders were fixed in cacodylate-buffered glutaraldehyde, postfixed with a buffered solution of 1 % OsO_4, dehydrated in a graded aceton series and embedded in Vestopal. Sections were stained with uranyl, acetate followed by lead citrate, and examined with the JEM 100 B.

RESULTS

The 7 day-old larva (type I) was an elongate bladder covered with a thin tegument which was underlain by two types of cells, i. e., those forming the tegument and those forming muscle fibres. Protoplasmic strands were seen to transverse the bladder cavity. The tegument (thickness 1—2.3 μm) was composed of a distal cytoplasm and a border of microvilli differentiating from extensions of the cytoplasmic surface. The structure of the base of the microvillus was identical to that of the superficial layer of the distal cytoplasm. The surface of the microvilli was covered with a double membrane, but an inner membrane and an electron-dense area below it described for microtriches were still absent. The microvillous base contained microtubules running in parallel direction to the longitudinal axis of the microvillus (Plate I, Fig. 4). There were no microtubules in its apical part. Deep invaginations into the basal part of the cytoplasm were produced by the differentiating basal membrane. They were funnel-shaped with a vacuole, with a large, empty, central region at one side and a large mitochondrion at the other side (Plate I, Figs. 5, 6). Their pattern of distribution in the basal layer of the tegument was regular throughout the entire bladder wall. Rod-shaped bodies occurred in larger numbers in the central part of the cytoplasm than in its basal and topical parts. Ovoid (lamellated) bodies were not observed in the distal cytoplasm. Below the tegument, there were a few muscle-and connective tissue fibrils. Extensions of syncytial, subtegumental cells at the site of their junction with extensions of the distal cytoplasm were of a structure similar to that of the basal part of the tegument. They contained vacuoles, mitochondria and an occasional rod-shaped body (Plate I, Fig. 7). The electron-dense cytoplasm of large, syncytial, subtegumental cells forming the bladder (Plate II, reconstruction) contained several free ribosomes, small, electron-dense mitochondria, and a few vacuoles and cisternae of the Golgi complex. The endoplasmic reticulum was ill-developed. Typical of these cells were numerous, interconnected, plasmic extensions forming plasmic bridges. The Golgi complex consisted of a small number of flat cisternae. In their vicinity we observed a small number of membrane-bounded vacuoles (1—3) with electron-dense contents resembling a just differentiating rodshaped body (Plate III, Fig. 2). The second type of cells observed in the bladder were electron-dense cells which contained 'an increased number of mitochondria and cisternae of the endoplasmic reticulum. The dilated lumen of the endoplasmic reticulum was filled with an electron-lucid substance. A nucleolus was clearly visible in the large ovoid nucleus. Extensions emerging from these cells reached spaces between the individual muscle fibrils. Undifferentiated germ cells distributed among subtegumental, syncytial cells, were present in the opposite pole of the bladder, evidently the future scolex pole. These cells were smaller in size, their shape was spherical and they did not possess a cytoplasmic extension (Plate III, Fig. 1). They came in two different structures. Some were small and heavily electron-dense, some large and electron-lucid. A thin, cytoplasmic band surrounding the large nucleus of electron-dense cells contained polysomes and small mitochondria (Plate III, Fig. 3). The cytoplasm of larger cells contained ribosomes and larger mitochondria. Below the syncytial, cellular layer there were spaces bounded either by a membrane or by protoplasmic strands (Plate III, Fig. 4). Sometimes, there were present only irregularly shaped and intercommunicating structures filled with an electron-dense substance which resembled cisternae.

A more advanced stage of development was indicated by the fine structure of spherical bladders (type II). These differed slightly from those of the foregoing stage in an increased number of microvilli and in the structure of the distal cytoplasm which, apart from rod-shaped bodies, contained long invaginations of the basal membrane broadening towards its surface and sometimes dilating distally in a terminal vacuole (Plate IV, Figs. 1, 2). There was an increase both in the number of longitudinal muscle fibrils below the tegument and in extensions of the distal cytoplasma joining extensions ef syncytial, subtegumental cells. Cellular extensions contained both vacuoles filled with an electron-dense substance, and rod-shaped bodies. An ununiformness in the density of the cytoplasm was due to a larger concentration of Golgi cisternae and an increased quantity of bodies and vesicles in their vicinity (Plate V, reconstruction). Syncytial, subtegumental cells were intermixed with cells in which cisternae of the endoplasmic reticulum were dilated. Extensions of these cells were not communicating with the distal cytoplasm, but with muscle fibres. Glycogen-containing extensions were present among subtegumental cells. The extensions belonged to cells located in the inner layer of the bladder wall. The nucleus of these cells was large and electron-lucid, the perinuclear region was surrounded by an electron-dense cytoplasmic area (Plate VI, Fig. 1). The electron-dense cytoplasm contained ribosomes and small mitochondria, cisternae of the smooth endoplasmic reticulum were less numerous. The cytoplasm continued into extensions of the electron-lucid cytoplasm which contained glycogen. The inner part of larger bladders (type III) was filled with large, glycogen-containing structures, which were interspersed with cells of the differentiating parenchyma. A typical feature was the presence of a larger number of mitochondria (Plate VI, Fig. 2).

In bladders of type III recovered from the surface of the liver at day 10 p. i., microvilli were longer and more densely distributed, and the number of rod-shaped bodies was increased. Variations in the shape of the bodies were caused by a different quantity of secretion in them. Their occasional ovoid shape without a bounding-membrane was apparently due to the fact that their electron-dense contents had been released to the distal cytoplasm. The lumen of several invaginations in the vicinity of rod-shaped bodies was electron-lucid (Plate IV, Fig. 3), in others it was electron-dense and dilated (Plate IV, Fig. 4). The longitudinal muscle layer of the bladder was underlain by a continuous cytoplasmic layer (perinuclear cytoplasm) which contained large, ovoid nuclei with a conspicuous nucleolus (Plate VI, Fig. 3). In the cytoplasm there were mitochondria, vesicles, vacuoles and an increased number of rod-shaped bodies in their vicinity. The endoplasmic reticulum was thickened. Extensions of electron-dense cells with dilated cisternae (spherical in section) were filled with a secretory substance and communicated with longitudinal muscle fibres by means of long, thin extensions. The scolex anlage differentiated in the scolex pole. A further differentiation occurred both in the tegument and the subtegument. The perinuclear cytoplasm consisted of characteristic cells with ovoid nuclei, intercommunicating lumina of cisternae of the endoplasmic reticulum were filled with a secretory substance, in their vicinity there was an increased quantity both of cisternae of the Golgi complex and of rod-shaped bodies. Most ribosomes were bounded to cisternal membranes (Plate VI, Fig. 4). Electron-dense cells associated with the production of muscle- and connective tissue fibres contained either dilated cisternae of the endoplasmic reticulum which were filled with a secretory substance, or narrow cisternae with an empty lumen. Having regard to the presence of numerous, new elements in the scolex anlage which were absent in the bladder, its morphogenesis will be the subject of a separate study.

DISCUSSION

A typical feature of the tegument of differentiating cysticerci and cysticercoid was the transformation of the superficial syncytium in a homogeneous cytoplasm covered with microtriches (Sakamoto and Sugimura 1970, Baron 1971, Collin 1970, Caley 1974). In a 7 day-old *C. tenuicollis*, we found cytoplasmic bridges (communications) between the distal cytoplasm and syncytial, subtegumental cells. The structure of microvilli on the cytoplasmic surface was similar to that of microvilli described by Collin (1970) for the cysticercoid of *Hymenolepis citelli*, and by Caley (1974), for postembryonic stages of *H. microstoma*. The basal part of the cytoplasm contained invaginations of the basal membrane which elongated. In our early developmental stages, we did not find "microcanals" observed by Threadgold (1965) and Morris and Finnegan (1968). The layer of circular muscle fibres was not differentiated in our 7 day-old larva. The number of fibrils increased later as a result of the release of a cellular secretion from dilated cisternae, particularly at the time of the differentiation of the scolex anlage. Typical of syncytial, subtegumental cells were numerous branched extensions communicating both with each other and with the distal cytoplasm by means of cytoplasmic bridges. Bråten (1968 a, b), Morseth (1967), Featherston (1972) observed similar cytoplasmic bridges forming a connection between the distal and the perinuclear cytoplasm.

At day 10 p.i., an increase in the volume of the perinuclear cytoplasm shortened the distance between individual subtegumental cells until it formed a continuous layer around their nuclei. In an earlier study on 13 and 16 day-old larvae of *Taenia hydatigena* (Hulínská 1978) these cells, which had a more advanced endoplasmic reticulum, were designated as type B.

In larvae at their initial stage of development, we distinguished among subtegumental cells two types of germ cells, i.e., smaller, electron-dense cells, and larger, electron-lucid cells. The former were involved in the formation of the scolex anlage of a 10 day-old larva. Sakamoto and Sugimura (1970) observed similar cells in a differentiating echinococcus, Collin (1970) found them at the time of the differentiation of the cysticercoid. Proliferating germ cells observed in a 13 day-old larva of *T. hydatigena* (type A — Hulínská 1978) played evidently an important role in the later differentiation of the proliferating zone of the bladder (neck) region, and in the rostellar region of the scolex.

In our opinion, cells with dilated cisternae of the endoplasmic reticulum, present in the earliest larval stages, were of importance in the production of muscle-and connective tissue fibers supporting the bladder wall. The cells were found in increased numbers in the circular muscle fibers and connective tissue fibers of the scolex anlage. Cells of this type called type C (Hulínská 1978) were observed in 13 and 16 day-old larvae. Caley (1974) found similar cells with dilated cisternae of the endoplasmic reticulum in a csyticercoid and referred to them as to myofibroblasts. Sagamoto and Sugimura (1970) used the term "reticular interstitial cells" for similar cells in their observation. Ubelaker (1970) suggested that the basal membrane was formed by these cells. Cells from a deeper layer of the bladder wall (type II) with large extensions filled with glycogen could be found only at the very beginning of the parenchymal differentiation. A nucleus was absent in these large, glycogen-containing structures recovered from a 10 day-old larva. We have used in the present study the term "glycogen-containing cells" suggested by Sakamoto and Sugimura (1970) for similar cells in a differentiating echinococcus. Typical of these cells was a narrow, electron-dense, cytoplasmic band surrounding the ovoid nucleus. Evidence was provided that these cells did not synthetize protein because their Golgi system was ill-developed and they did not have a rough endoplasmic reticulum. On the other hand, the synthesis of glycogen was suggested by the presence of a smooth endoplasmic reticulum and a lareg quantity of mitochondria. The distribution of glycogen was studied by Read and Simmons (1963). Cheng and Dyckman (1964), and Lumsden (1966) suggested that glycogen was produced mainly by, and stored in cells of the "medullary parenchyma" of a growing *Hymenolepis*. Drochmans (1962) studying the morphology of glycogen granules disclosed their presence in cells with an ill-developed Golgi complex. These cells contained a large quantity of small, ovoid mitochondria. In support of suggestions by Lumsden et al. (1974) was our observation of the initial development of microtriches appearing first in the form of microvilli without distal points and containing a limited number of rod-shaped bodies which had just started to originate in the distal cytoplasm from subtegumental, syncytial cells.

ТОНКАЯ СТРУКТУРА ЛИЧИНОЧНОЙ СТАДИИ ЦЕСТОДЫ *TAENIA HYDATIGENA* В ВОЗРАСТЕ 7 И 10 ДНЕЙ

Д. Гулинска

Резюме. Исследование тонкой структуры личиночной стадии *T. hydatigena* в ранней фазе развития показало, что структура дифференцирующегося тегумента отличается от структуры тегумента взрослых цестод. Обнаружены разницы в количестве и форме органелл (микроворсинок, палочковидных телей, инвагинаций базальной мембраны и митохондрий), находящиеся в связи с ранним онтогенезом пузыря. Для ранней фазы образования палочковидных телец в недифференцированных синцитиальных клетках типично наличие небольшого количества телец в дистальной цитоплазме и характер микроворсинок, у которых отсутствуют электронно плотный слой под плазматической мембраной и дистальные острия. В течение последующей дифференциации субтегументальной перинуклеарной цитоплазмы на 10-й день развития увеличивается содержание телец и длинных инвагинаций мембраны в тегументе и повышается количество везикул и цистерн аппарата Гольджи. В начале развития еще не дифференцируется слой состоящий из кольцевых мышц и фибрилл. Образование этого слоя связано с секреторной активностью клеток с расширенными цистер нами эндоплазматического ретикулума. Из пролиферирующих зародышевых клеток дифференцируется зачаток сколекса и область роста пузыря. Только в начале развития встречаются клетки, депонирующие гликоген, из которых в течение дальнейшего развития возникают гликогенные образования.

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Received 30 August 1979.

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EXPLANATIONS

A — microvilli, B — distal cytoplasm, C — rod-shaped bodies, D — invaginations of the basal membrane, E — muscle fibrils, F — cytoplasmic connections (bridges), G — mitochondria, H — host cells, Ch — syncytial, subtegumental cells, I — nucleus, J — dilated cisternae of the endoplasmic reticulum, K — cells with dilated cisternae of the endoplasmic reticulum, L — electron-dense germ cells, M — electron-lucid germ cells, N — protoplasmic strands, O — nucleus of the original cell, P — glycogen-containing cell, Q — glycogen structures, R — glycogen-containing extensions, S — perinuclear cytoplasm, T — Golgi apparatus, U — vacuoles, V — vesicles.

FOLIA PARASITOLOGICA (PRAHA) 27: 242, 1980.

G. I. Pozniak: Russian-English Dictionary of Helminthology and Plant Nematology.

Commonwealth Institute of Helminthology Technical Communication No. 49, Commonwealth Agricultural Bureaux, Farnham Royal 1979, X + 108 pp.

At the present period of rapid development of science and necessary translation literature the need for specialized dictionaries becomes increasingly urgent. The reviewed publication is one of such dictionaries. Following a brief introduction and guide to users, abbreviations used and Cyrillic alphabet and transliteration there are 100 pages of terms containing over 6000 entries. At the end of the book very useful is a list of current abbreviations, primarily of various Soviet Institutes and research centres. Also a list of useful dictionaries and most important references are appended.

Besides the fields mentioned in the title the terms cover general parasitology, partly also human medicine and veterinary science and ecology. Also names of all anthelminthics of Russian origin have been included. The entries are arranged according to the Cyrillic alphabet and are composed primarily of nouns and adjectives. With each entry a translation or the English equivalent is given. In some cases definitions and explanations are also provided, mainly for terms which lack English equivalents, used in Russian in a sense different from that in English or with different meanings in different fields.

The terms have been selected very appropriately and their treatment is of high standard. Despite this some inaccuracies accidentally occurred in the text which I should like to point out. There are misspellings (the correct term on p. 15 should read gel'mintotsenoz, on p. 16 generatsiya gamogeneticheskaya) or grammatical genus of noun and adjective does not coincide (the Latin names on p. 5 should read *Wardium arcticum*, on p. 9 colliculus genitalis, on p. 95 *Cysticercus parenchymatosus*). Ectoparasites and endoparasites given as equivalents of terms for ectogel'minty and endogel'minty do not seem to be suitable (p. 98). The English definition provided for some terms is sometimes questionable (e.g. ochag antropurgicheskiy on p. 56). In this case I do not consider correct the synonymy of antropurgicheskiy to be sinantropnyi. However, these inaccuracies do not in any way debase the value of the book.

On the contrary, the appearance of this dictionary should be highly appreciated. It covers very aptly the term spectrum of relevant fields andi ncludes even terms difficult to find in other types of dictionaries, e.g. terms no more used in the Soviet helminthological literature today. Very valuable are also translations and explanations of different abbreviations. The entire text reflects the beneficial influence of cooperation with the Soviet specialists in the preparation of this dictionary. Its appearance will be undoubtedly welcomed by a wide community of translators, abstractors and scientists consulting Russian helminthological literature.

Dr. V. Černý, C.Sc.

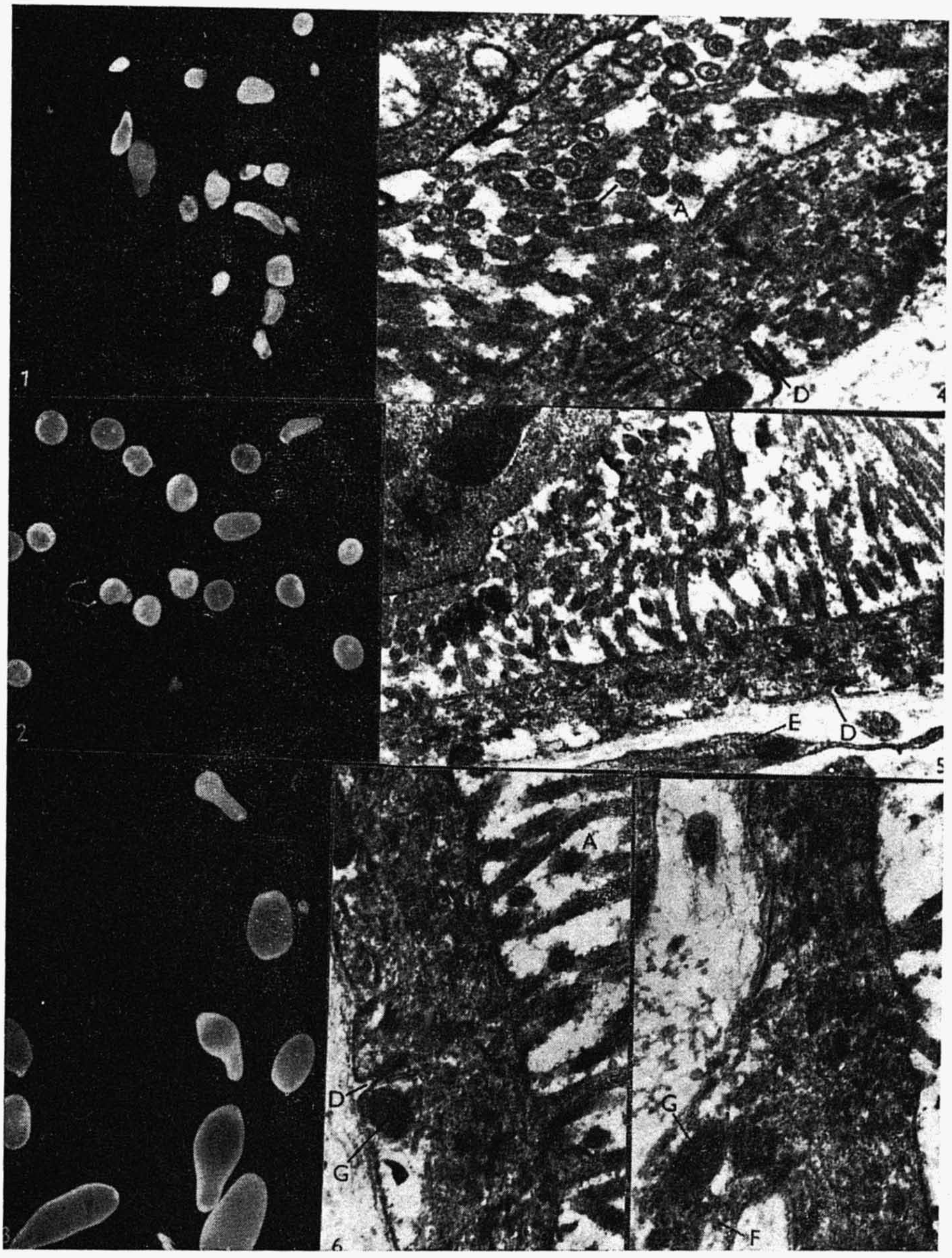


Fig. 1. Larvae of *Taenia hydatigena* (type I) recovered at day 7 p.i. from migratory canals in the liver (× 84). **Fig. 2.** Larvae (type II) from migratory canals under the liver surface at day 7 p.i. (× 84). **Fig. 3.** Larvae (type III) from the surface of the liver at day 10 p.i. (× 104). **Fig. 4.** Bladder tegument of a 7 day-old larva of *T. hydatigena*. Cross section showing radial arrangement of microtubules in mid-microvillus. Rod-shaped bodies present in the distal cytoplasm, the basal part contains invaginations of the basal membrane (× 20 000). **Fig. 5.** Microvilli in contact with the host cell differentiate from extensions of the distal cytoplasm. The thin membrane at the base is underlain by innumerous muscle- and connective tissue fibrils (× 12 400). **Fig. 6.** Proximal to invaginations of the basal membrane are mitochondria and a vesicle (× 21 000). **Fig. 7.** An extension of the distal cytoplasm containing mitochondria, vesciles and ribosomes (× 21 000).

Reconstruction of a 7 day-old larva (type I) of *T. hydatigena*. In contact with the distal cytoplasm by means of cytoplasmic extensions are large, syncytial, subtegumental cells in which there are mitochondria, ribosomes, electron-dense vacuoles, small cisternae of the Golgi complex and an ill-developed endoplasmic reticulum. Cells with a more electron-dense cytoplasm and dilated cisternae of the endoplasmic reticulum are not connected with the distal cytoplasm. Host cells are present on the surface of the tegument (× 10 700).

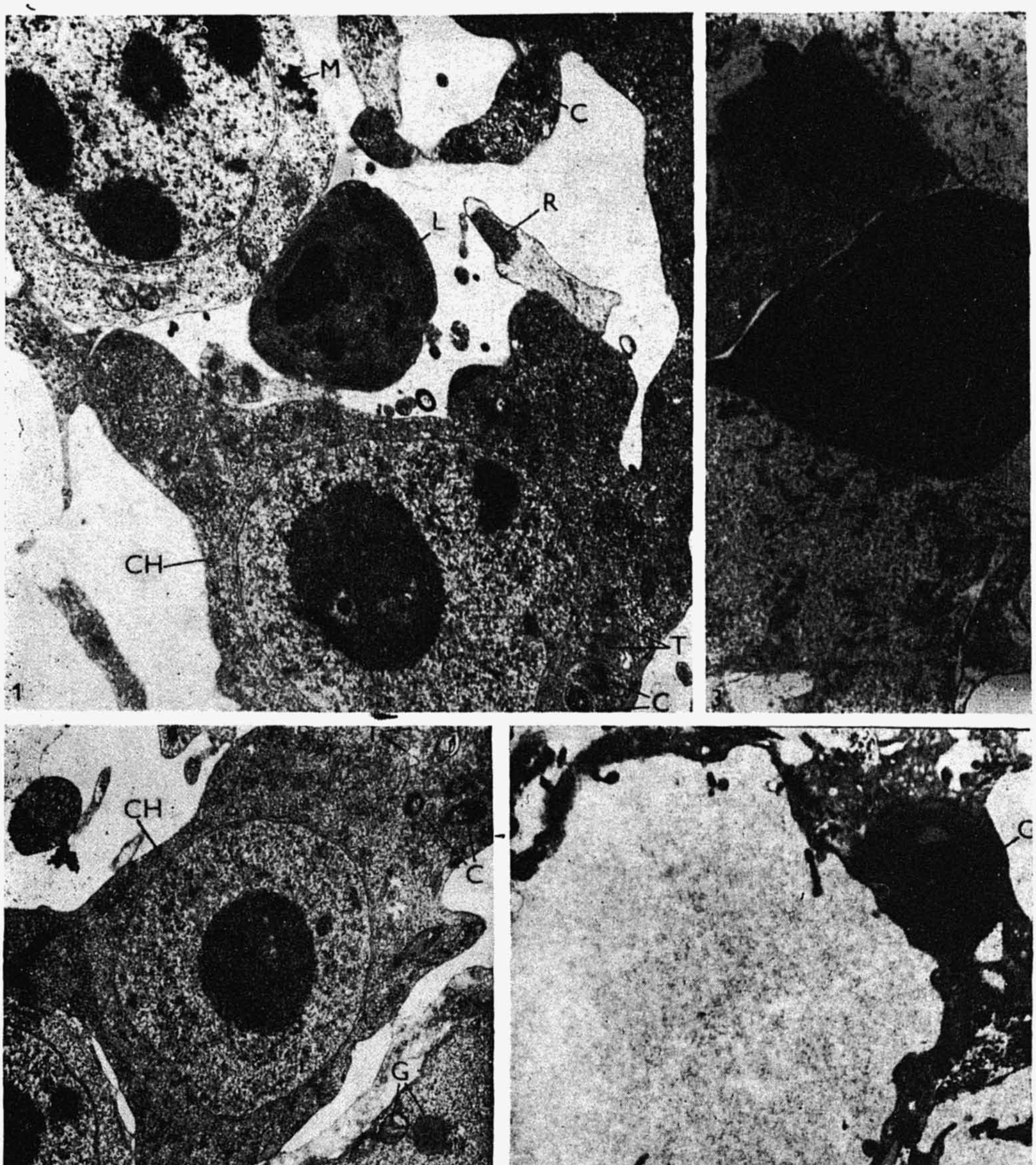
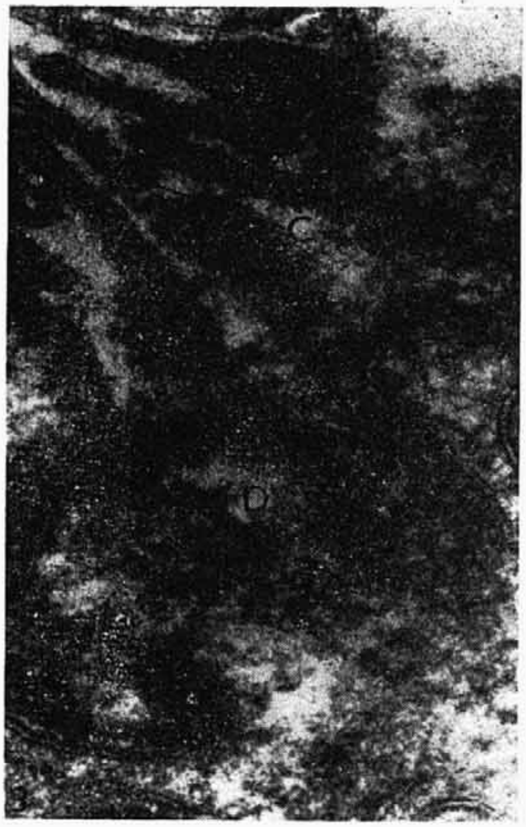


Fig. 1. The cytoplasm of smaller, electron-dense germ cells distributed among syncytial, subtegumental cells contains polysomes, the cytoplasm of larger, electron-lucid germ cells contains ribosomes and larger mitochondria. Germ cells are ovoid. Cisternae of the endoplasmi reticulum are absent in their cytoplasm and they do not produce cytoplasmic extensions. Vacuoles filled with an electron-dense substance can be seen in the vicinity of cisternae of the Golgi system in syncytial, subtegumental cells (× 17 000). **Fig. 2.** Electron-dense vacuoles close to the Golgi apparatus of syncytial, subtegumental cells are originating, rod-shaped bodies (× 16 200). **Fig. 3.** Germ cells among extensions of syncytial. subtegumental cells (× 21 400). **Fig. 4.** Inside the bladder, protoplasmic strands are lining cisternae filled with a granular substance. A wider, protoplasmic strand contains an electron-dense, small nucleus and a nucleolus common to several cisternae (× 10 700).



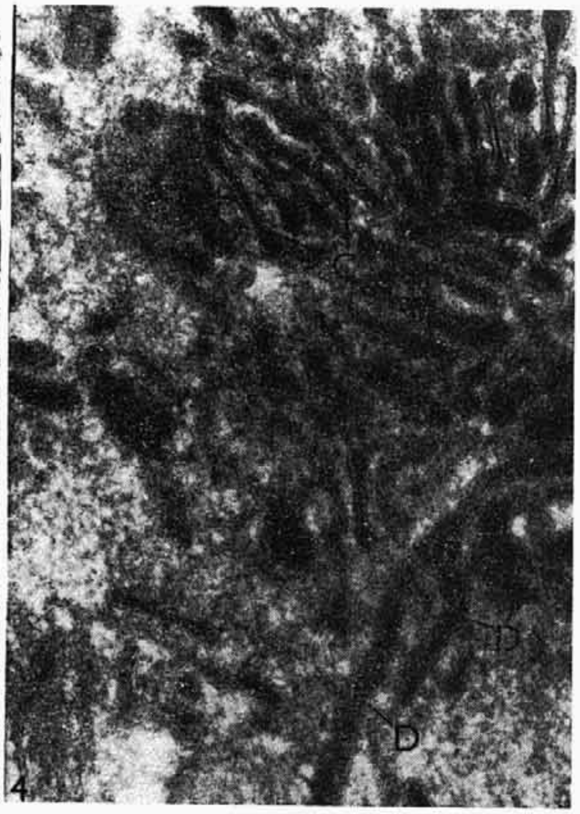
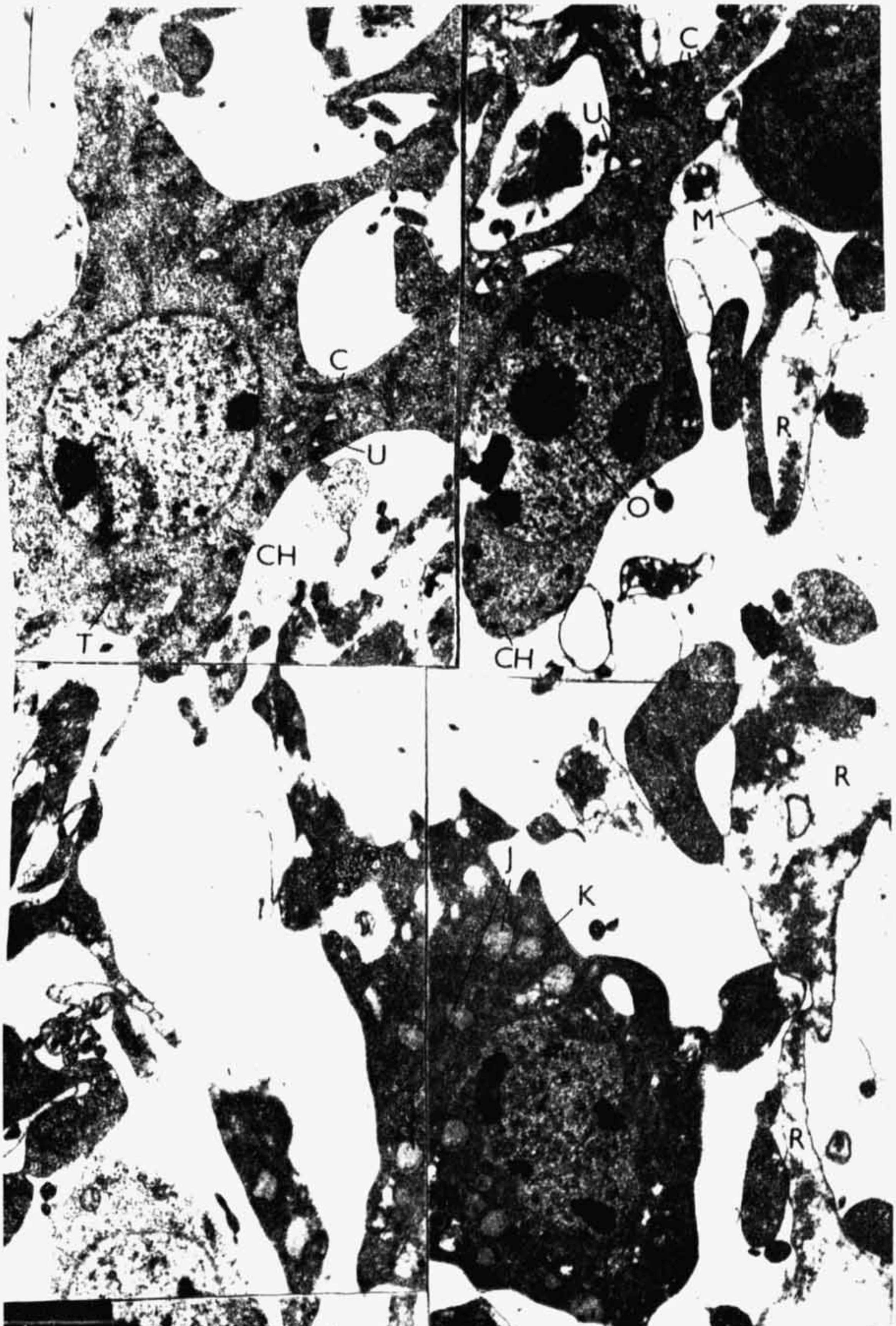
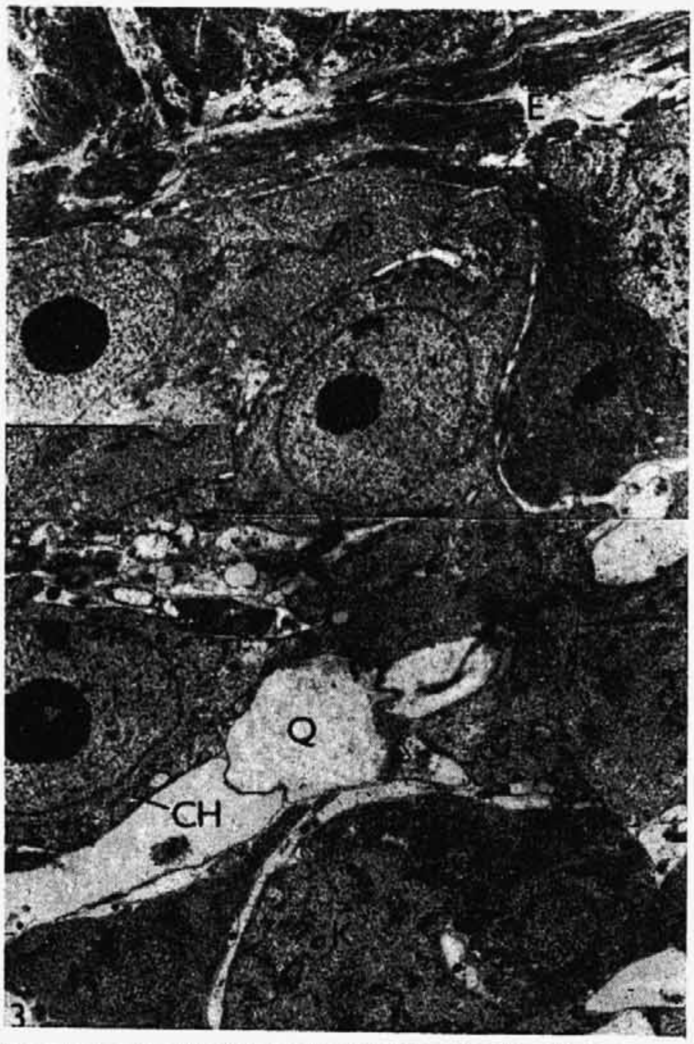
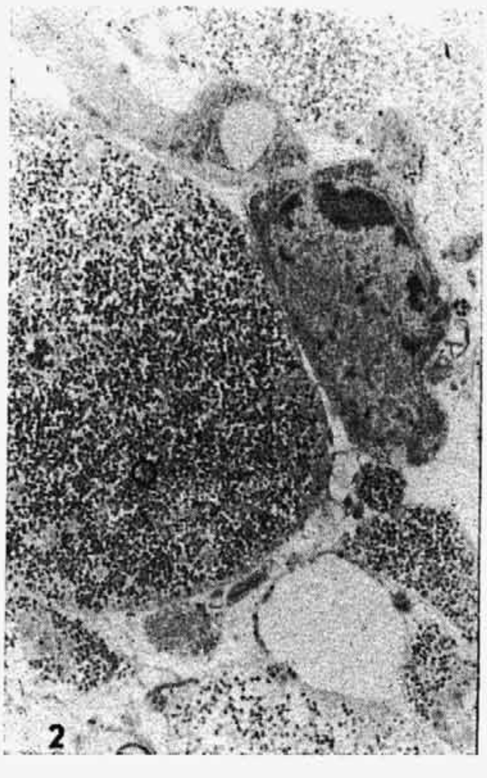


Fig. 1. Microvilli of a bladder (type II) on the surface of the distal cytoplasm which is traversed by long invaginations of the basal membrane. The structure of invaginations is similar to that of the plasmic membrane. Sometimes. invaginations terminate in a vacuole (× 70 000).
Fig. 2. Rod-shaped bodies filled with an electron-dense secretion. Bodies which are not covered with a double membrane are ovoid in shape. The structure of the long invagination is similar to that of the wall of rod-shaped bodies. A vesicle of pinocytotic origin in the top right corner (× 70 000).
Fig. 3. The cytoplasm of the tegument of a bladder (type III) contains an increased number of rod-shaped bodies and long invaginations. The structure of both organelles is dependent on the amount of secretion inside them. When filled to the rim, they are elongate bottle-shaped. The double membrame grows thinner at the broadest side and the electron-dense substance is released to the distal cytoplasm (× 113 000). **Fig. 4.** Organisation of rod-shaped bodies and invaginations of the basal membrane in the superficial layer of the distal cytoplasm. The lumen of invaginations is dilated and filled with an electron-dense substance (× 70 000).



Reconstruction of the subtegumental area of a bladder (type II) showing syncytial, subtegumental cells with long extensions. An occasional, ovoid cell without cytoplasmic extensions is a germ cell of the bigger type. There are glycogen-depositing, cytoplasmic extensions and cells with a large quantity of ribosomes and dilated cisternae of the endoplasmic reticulum. The cytoplasm of the syncytial, subtegumental cell contains electron-dense vacuoles close to the Golgi system, and rod-shaped bodies (× 15 000).





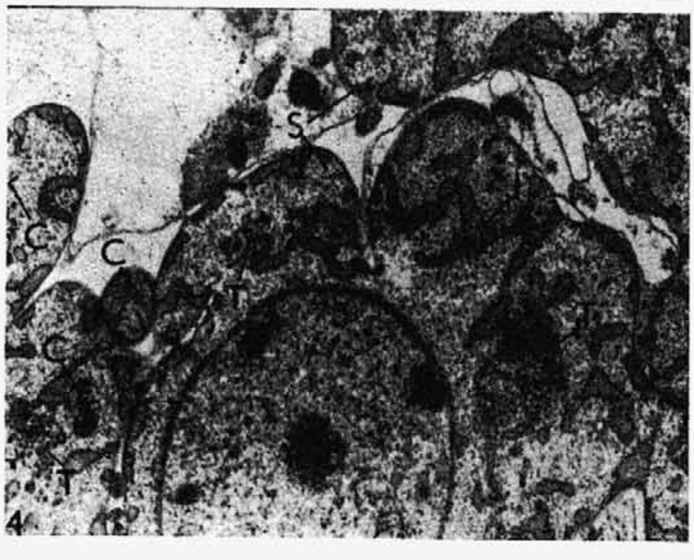


Fig. 1. A glycogen-containing cell from the inner part of the wall of a bladder (type II). The narrow strip of electron-dense cytoplasm in the perinuclear region contains ribosomes, mitochondria, the endoplasmic reticulum is smooth. The strip passes into pockets in the electron-lucid cytoplasm in which glycogen is deposited (× 8 500). **Fig. 2.** Glycogen-depositing extentions are bigger in the next developmental phase (type III). They are bounded by the original cellular membrane and in some sites by a narrow cytoplasmic strand in which nuclei are mostly absent. Cells of the differentiating parenchyma can be seen among these structures × 14 200). **Fig. 3.** The subtegumental perinuclear cytoplasm of a bladder of type III. The electron-dense nucleolus of a large, ovoid nucleus is spherical. The cytoplasm contains an increased number of mitochondria and Golgi cisternae, and an enlarged endoplasmic reticulum. Extensions of more electron-dense cells are communicating with muscle fibres. In sections through these extensions, dilated cisternae of the endoplasmic reticulum appear to be spherical (× 6 800). **Fig. 4.** Perinuclear cytoplasm of subtegumental cells of the scolex anlage of a 10 day-old larva (× 10 000).