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Histology and Biology of the Larval Stages of *Leucochloridium* Carus, 1835 (Trematoda, Digenea) as Revealed by Light and Electron Microscopy

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Ultrastructural studies of the metacercarial cyst and the coloured broodsacs of *Leucochloridium variae* and *L. holostomum* recovered from succineid pulmonates are presented. The metacercarial cyst wall is composed of three layers, designated outer (more or less lamellated with electron dense granules), middle and inner layer (both fibrous mucoid). A description of the cyst layers is given. Cyst development and functional biology of the metacercaria are briefly commented upon.

Electron micrographs of the anucleate distal cytoplasm of the broodsac tegument show that the whole surface is provided with microvilli embedded within a tegumental surface coat, and rests on a prominent distal layer of interstitial material. The brood chamber in contrast is provided with a nucleated cellular lining, but rests similarly on a prominent layer of granular-fibrous interstitial material. The tegument and the epithelial lining are described.

The perinuclear parts of the tegument and pigment cells were located inside the outer circular and the inner longitudinal muscle bundles. Multinucleated pigment cells were found and the pigment types that were observed are described. One of the geometrically organized membranous structures observed is suggested to have a photoreceptive function. Problems concerning sporocyst metabolism and biology are discussed.

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INTRODUCTION

Species of the genus *Leucochloridium* Carus, 1835 (sensu Bakke 1980) are unique in that the mother sporocyst develops coloured broodsacs, which react to external light stimuli with regular muscular activity (Mönnig 1922, Halik 1931, Hecker & Thomas 1963, Lewis 1977). The broodsacs are permanently connected to the central ramifying, cercariae-producing part by narrow tubes (see Bakke 1980). This type of sporocyst morphology and behaviour presents a number of biological problems since there are no reports of any sense organs, nervous, excretory or circulatory systems. Close similarities in adult morphology and ecology have resulted in considerable systematical confusion, and species identification based on the sporocyst pigments seems at the present stage essential to alleviate this situation (Bakke op. cit.). There have been relatively few studies on the histochemistry (Žďárská & Soboleva 1981) and histology or ultrastructure (i.e. light microscopy (LM): Heckert (1889), Magath (1920), Mönnig (1922), Kagan (1952), Nacheva et al. (1981); transmission electron microscopy (TEM): Storch & Welsch (1970), Graeber & Storch (1979), Bakke (1979)) of leucochloridiid sporocysts, although such studies could provide much useful information.

Encysting trematodes normally encyst on the surface of different objects or in the muscles, skin or viscera of a second intermediate host. Earlier ultrastructural studies on encysting cercariae indicate considerable variation in the formation, fine structure and histochemistry of the cyst layers, reflecting diverse evolutionary adaptations (see Stein & Lumsden 1971a). *Leucochloridium* species are unusual in that the cercariae encyst within their own previous larval stage, predominantly the sporocyst broodsacs. Knowledge of *Leucochloridium* cyst construction and development may have special significance for studies on cyst function as these cysts are not exposed to the environmental forces acting on free-encysting species. In addition, this information may also be of taxonomic interest since there are microscopical subgeneric differences in cyst appearance within *Leucochloridium* (see

Bakke 1980). Similar specific differences in cyst structure have also been reported within other genera (see Stein and Lumsden 1971b).

MATERIAL AND METHODS

The larval specimens of *Leucochloridium (Leucochloridium) variae* McIntosh, 1932 (see Bakke 1978, 1982) were recovered from naturally infected *Succinea ovalis* Say collected south of Platte River close to Louisville State Park (c. 41°N, 96°W), Nebraska, USA, in 1980 and *Succinea pfeifferi* Rossmässler collected in the Agdenes area (63°35'N, 9°45'E), Trøndelag, Norway, in June 1978.

The larval stages of *Leucochloridium (Neoleucochloridium) holostomum* (Rudolphi, 1819) (see Pojmanska 1975) were obtained from the same naturally infected *S. pfeifferi* in the Agdenes area in June 1978 (this represents the first record of *L. holostomum* in Norway).

After removal of the shell, the snail digestive gland and haemocoel were teased apart in 0.8% phosphate buffered (0.135 M; pH 7.4) NaCl to release the sporocyst. Both the sporocyst and metacercariae (released after puncture of the broodsac wall) were examined alive.

Metacercariae and pieces of broodsac of *L. holostomum* were fixed in 2,5% glutaraldehyde in phosphate buffer (0.135 M; pH 7.4) for 3 hours, and washed twice in buffer before postfixation for 2 hours in 2% osmium tetroxide in the same phosphate buffer. The material was then washed twice in phosphate buffer, dehydrated through a graded series of ethanols and transferred to propylene oxide before embedding in low viscosity Spurr resin which was polymerized at 70°C for 12 hours. The selected tissues of the larval stages of *L. variae* were processed in one of two ways. They were either fixed for 3 hours in 2.5% glutaraldehyde in phosphate buffer (0.135 M; pH 7.4) with or without 4% sucrose and then osmicated as described above, or they were fixed for 3 hours in 2.5% glutaraldehyde in cacodylate buffer (0.1 M; pH 7.4) containing 0.05% $CaCl_2$ and postfixed for 2 hours in 1% osmium tetroxide in the same buffer, but with added 7.5% sucrose. After fixation the dehydration-embedding procedures were as described above. All fixations were carried out at room temperature (c. 20°C).

Blocks were trimmed on a LKB pyramitome. Thin sections were cut with glass knives on a LKB ultratome, collected on formvar-coated copper grids, stained with a saturated solution of uranyl acetate in 50% ethanol and poststained with lead citrate in 0.1 N NaOH. Sectioned material was examined with a Siemens Elmiskop IA with magnifications between 2000 x and 40000x at 80 kV. Thick sections were also cut of the Spurr-embedded material, stained with toluidine blue and studied by light microscopy.

RESULTS

Metacercariae

The metacercariae were floating free inside the broodsac lumen, more or less influenced by the broodsac movements. The developing metacercarial cysts are thin and transparent whereas the older ones are opaque, thicker and brownish coloured in transmitted light (Fig. 1). Old *L. holostomum* cysts differ from those of *L. variae* in that they are transparent ventrally, but coloured dorsally. The pliable cyst wall is connected by fibres to the mobile metacercaria, which implies that the cyst partly changes form according to the metacercarial movements inside. The cyst wall is perforated by an anterior channel from the oral sucker and has a cyst plug located at the ventral sucker. A canal to the exterior from the excretory pore was not observed.

The cyst wall of both species is dorsally composed of three layers, here designated the inner, middle and outer layers (Fig. 2). The outer layer, which is not enclosed by a membrane, has a lamellated coarsely fibrous matrix and stains strongly with toluidine blue (Figs. 1, 2). This layer contains large electron dense granular inclusions (0.35 µm in diameter) randomly embedded within the matrix (Fig. 3). The size of the granules increased towards the periphery of the outer cyst wall layer, and their shape was round to hexagonal with a more dense outer layer and central core. The outer layer of the cyst, which is sometimes covered by an external amorphous cyst coat, is plastic and can easily be torn and removed, to reveal a highly viscous material which forms the inner hyaline capsule (Fig. 2). This capsule consists of two layers, the middle and the inner layer as seen in toluidine blue stained specimens. Both layers are slightly fibrous and the radially directed «fibres» in these layers tend to coalesce and cluster when fixed.

The width of the middle cyst layer is highly variable even in the same specimen, and it sometimes appears to be absent (or atrophied?). The inner layer which can be subdivided into a basal lightly staining part and a peripheral heavily staining part (Fig. 2), rests directly on the metacercaria with the «fibres» connected to tegumental elevations. This suggests that the inner layers of

the cyst is produced by the tegument itself. LM observations on the tegument in the early stages of metacercarial development, particularly at the posterior of the metacercaria, reveal outgrowths on the tegument of various shapes and sizes. These outgrowths appear to disintegrate and release their contents into the inner cyst wall (Figs. 4a, b). By TEM, cystogenous glands were observed with electron dense granules (Fig. 5). No granules were observed by TEM in the inner or middle cyst layer.

The metacercarial tegument conformed in structural organization to that generally found in trematodes, i.e. an anucleate distal cytoplasmic layer, resting on a basal lamina, and connected by cytoplasmic processes to an inner perinuclear cytoplasm.

Broodsacs

Young broodsacs without coloured bands were capable of movement, but in older broodsacs only the coloured distal end was able to perform the different types of muscular contractions (i.e. peristaltic, pendular and rhythmic). The movements also occurred and started *in vitro,* independently of the snail, but were dependent on temperature and light intensity. The broodsacs also reacted to mechanical stimuli. The pulsating power was observed to be diminished in old sacs, which appeared flabby and often bent.

Figures 6 and 7 demonstrate that the surface of the broodsac was thrown up into microvillus-like outgrowths (measuring up to c. 1 μm) and cytoplasmic projections which were embedded within a less electron dense matrix which formed the surface coat. The microvilli may appear both as ridge-like folds and digitiform structures. The outer surface membrane was found in some cases to form crypts which may indicate pinocytotic activity. The electron dense cytoplasm of the anucleate distal part of the tegument contained irregularly shaped bodies of varying density and size (up to ca. 0.35 μm) (Fig. 6a) and vesicles which emptied into the tegumental surface coat (Fig. 7). This tegumental secretion seems to be localized to the distal end of the broodsac. No Golgi, mitochondria, GER, or free ribosomes were observed, which indicates a sparse tegumental metabolic activity. There is a prominent basal lamina of medium density (thickness varying, up to 0.25 μm), resting on a well developed layer of interstitial material, through which cytoplasmic processes from the distal cytoplasm go to the perinuclear parts of the tegument. Pits in the internal plasma membrane extend into the distal cytoplasm forming deep lacunae with the same density as the basal lamina. The ends of these pits were occasionally observed to be swollen and were electron lucent (Figs. 6a, b).

The distal layer of interstitial material merges with that surrounding the muscles, filling up most of the intercellular spaces. The well-developed outer circular and inner longitudinal muscle bundles are of the smooth unstriated type, containing thick and thin myofibrils and beta-glycogen. In *L. holostomum,* the longitudinal muscles were observed to be aggregated in separate bundles surrounded by interstitial material. The longitudinal protuberant coloured stripes in part 3 in this species (see Bakke 1980) represent accumulations of circular muscle bundles and pigment cell processes.

The brood chamber has an electron dense nucleate epithelial lining provided with cytoplasmic folds and lamellae protruding into the sac lumen (Figs. 8, 9). The indistinct and shadowy outline of some projections suggests that they are flat, ribbon-like folds. This cellular lining, bounded by frequently observed desmosomes (Fig. 17b), rests on a well developed layer of granular-fibrous interstitial material. There was no direct evidence of a continuity between the epithelial cells and the cells inside the interstitial layer, although cytoplasmic processes were frequently observed penetrating deep into this matrix. The frequency of the lamellae increased towards the distal end with the increased number of invaginations and folds of the epithelial lining. The epithelial cells contained more or less spherical secretory bodies of various sizes, but of the same density. Observations suggest that the lamellae may play a part in the observed secretion of the bodies, although no epithelial rupture was observed. Between the lamellae electron dense spherical corpuscles (up to c. 2 μm) could occasionally be seen caught in *L. holostomum.*

The nucleus of the cells in the lining had a regular envelope, with chromatin appearing as dense patches often condensed near the periphery of the nucleus (in *L. holostomum* a prominent nucleolus was frequently observed). The cytoplasm of the lining contains a well-developed GER and numerous free ribosomes. Relatively large invaginations of the lining into the interstitial material filled with vesicles were frequently observed distally in the broodsacs. Two types of inclusions were observed in this epithelium in *L. variae.* One represents lanceolate lamellated bodies, as typically seen in Fig. 9, the others are irregular shaped bodies, often triangulate, with an amorphous granular matrix coar-

ser than that of the spherical secretory bodies (Fig. 10).

The pigments which contribute to the banded appearance of the broodsacs were distributed intracellularly within so-called pigment cells, which have membranal contact with the tegumental cytoplasmic processes and muscle cells, but never with the distal cytoplasm of the tegument itself. The perinuclear part of the pigment cells was situated below the muscular layers, against the inner layer of interstitial material, and was found to represent a syncytium distally in *L. variae* broodsacs (Fig. 11). These cytoplasmic parts were especially rich in developing (?) pigments of different sizes, free ribosomes, GER, mitochondria and electron-lucent vacuoles (Fig. 11). The pigments observed had variable electron density and did not appear to be secreted, but occurred single or aggregated in large numbers particularly in the pigment cell processes between and outside the muscle layers, often forming tubercles containing clusters of pigment granules. These parts of the pigment cells contained mitochondria which were frequently large (up to c. 0.9 μm), and also numerous beta-glycogen-like granules as judged from their size (Fig. 12). No microtubules were observed in the pigment cells.

No main differences in the appearance of the pigment granules were observed between *L. holostomum* and *L. variae,* although the range in granule types appeared larger in the latter, with very variable size, contents and shape (Figs. 12—14). The size was up to c. 5 μm, the matrix varied from granular homogeneous with varying density (Fig. 12) to heterogeneous due to spherical dense patches (Fig. 13a) or light regular (Fig. 13b) or irregular (Fig. 13c), all inclusions appearing in varying numbers and sizes. The shape of the pigment granules as seen in sections varied from the normally approximately round types (Fig. 12), to those which were irregular in shape, of which the main types observed are shown in Figs. 14a—e. In some cases the pigments could appear as apparently non-membrane bound aggregations of heteromorphic bodies of different electron densities within a granular matrix which was poorly separated from the surrounding cytoplasm.

Figures 15 to 17 show four types of different geometric cell inclusions only observed in the *L. variae* broodsacs. In Fig. 15 a lamellated type occurring in the distal cytoplasm of the tegument can be seen; this type of inclusion often encircled an electron denser center. This structure was relatively frequently observed. Its appearance often differed slightly, but it was always approximately spherical. The second type was a crystal-like structure seen individually or accumulated into larger clumps within the distal tegument as shown in Fig. 16 or occasionally also in the epithelial lining. Two other inclusions were observed within the epithelial lining, one membranous oval (Fig. 17a) and one spherical containing parallel undulating membranes (Fig. 17b). An appendage which appears to contain microtubules, seems to pass out from the latter type of inclusion body. No pigments or cilia were found associated with the structures.

The perinuclear parts of the tegument and the subtegumental cells were difficult to distinguish from each other, due to their size and numerous interdigitations of cytoplasmic processes. Some of the nucleated cells, often aggregated close to and surrounded by invaginations of the brood chamber epithelium, apparently had a storage function, as the whole cytoplasm was filled up with spherical globules. Processes from these cells were seen going into the muscular areas, and may represent the globule containing cells demonstrated in Fig. 14e. The alpha (rosette) type of glycogen was frequently observed within such parenchymal cells containing accumulation of globules as shown in the figure.

DISCUSSION

Cyst

Kagan (1951), Storch & Welsch (1970) and Lewis (1974a, 1977) described the *Leucochloridium* cyst as composed of two layers, in contrast to the present three as seen in stained material (Fig. 2, see Ždárská 1981). This cyst was believed by Heckert (1889) to originate from a double cercarial ecdysis: the cuticula remained with the larvae, forming a protective covering with fluid between the wall and the worm. This was an idea already proposed by Moulinié (1856) — as he named the cercaria *Cerc. exfoliata* indicating an exfoliation of the cuticula — and Wagener (1857). Mönnig (1922), Wesenberg-Lund (1931) and Hohorst (1937) accepted this explanation and stated that the mucoid substance was chemically related to the cuticula. The ultrastructure of the cyst layers (Figs. 2, 3) and modern knowledge of the living tegument (see Figs. 4, 5), in addition to observations on another *Leucochloridium* species (Bakke 1979), have demonstrated that the cyst wall is not produced as result of an ecdysis, but is apparently formed by precursor materials synthesized by cercarial subtegumental gland cells. The gland cells observed in

the encysted metacercaria (Fig. 5) may have functions other than cyst formation (see Strong & Cable 1972), they may for example be enzymic in nature (see Davies 1979). They seem, however, to start to function before reaching the final host (Fig. 5, see also Žďárská & Soboleva 1981). A histochemical comparative study of the gland cells and cyst wall would be indicative in this respect. Beside the secretional activity shown in Figs. 4, 5 only material from the excretory pore has been seen (by light microscopy) to leave the parasite and become entrapped within the cyst.

Von Siebold (1853), Heckert (1889) and Kagan (1951) found no trace of an excretory canal penetrating the cyst in leucochloridiids, as in the present results (in contrast to Zeller (1874), Mönnig (1922), Hohorst (1937), and Pojmanska (1972)), and Heckert (op. cit.) stressed that the excretory products were accumulated within the «inner cyst» causing its lack of transparency. Material deriving from the excretory pore as seen by light microscopy in *L. variae,* did apparently not represent the outher granular layer causing the lack of transparency (colour). These granules (see Storch & Welsch 1970) — «blasenförmigen Körpern»), found by Žďárská (1981) to be calcium particles, are supposed to be derived from tegumental secretions. Kagan (1951) believed that the sporocyst wall secreted parts of the metacercarial cyst. The present TEM examination of the sporocyst complex gave no clear evidence to support such a hypothesis. However, the lanceolate lamellated bodies seen in the broodsac lining may contribute to the formation of the outer cyst wall which appears lamellated as seen by LM (see Dixon 1968). Histochemical studies on the early cyst formation are essential to an understanding of the development of the cyst layers (see Žďárská 1981).

Lewis (1974b, 1977) suggested that the cyst was an adaptation to prevent water loss. Snail tentacles may rupture, leaving the sporocyst free on the ground, as was observed in terraria. Although the hygroscopic cyst could be a mechanism of increasing the survival time of the infective stage, this transmission mechanism seems infrequent, only occurring by accident (cf. Wesenberg-Lund (1931), Lewis (1974a)). The possibility that the cyst (and broodsac) function as a protection against host digestive enzymes or the physical process of trituration can not be excluded, but has so far not been proved. Differences in cyst digestability depending on parasite species are found (Lewis 1977).

Byrd (1940) suggested that *Leucochloridium* cysts provided an environment for the metacercaria and a source of food during its resting period. The sucking in and out of the oral mouth capsule was believed to represent the tugging of the envelope substance. The present light and TEM studies show that this is incorrect. The oral sucker plug (mouth capsule) ruptures, thus making a channel with direct contact between the caecae and the brood chamber fluid.

The cyst of free encysting species of trematodes (see Dixon & Mercer 1964, Dixon 1975) appears to be more compact and complex in composition than those of *L. variae* and *L. holostomum* which encyst within the sporocyst. No examinations have been made on «cyst» ultrastructure of *Urotocus* Looss and *Urogonimus* Monticelli in the same family, but macroscopically their «cysts» appear simpler, thinner and more rigid (Timon-David 1957, Schmidt 1965, Lewis 1974b). As the branching sporocyst of these genera, whose metacercariae infect the same bird groups, is uncoloured, with no muscular sacs and tubes, the cyst differences between these species and *Leucochloridium* may be related to these sporocyst differences. This may indicate that a primary function of the cyst is to give mechanical protection to the spined metacercariae (Bakke unpubl.) or to the internal broodsac wall, during the broodsac's movements and rhythmic pulsations. In addition, the pliable cyst may lighten the transport of developing and moving cercariae from the central sporocyst through the narrow tubes to the broodsacs (see Wesenberg-Lund 1931, p. 114).

According to Žďárská & Soboleva (1981) and Žďárská (1981) the *L. paradoxum* cyst is not a cyst, but an hypertrophied glycocalyx and a part of the tegument. This implies that the *L. paradoxum* metacercaria are non-encysted.

Metabolism

Nutrition for the developing cercariae must be derived from the fluid within the sporocyst lumen. Judging from the distribution of the surface cytoplasmic extensions (the frequency of which increases towards the central sporocyst) and from the microvilli which cover the sporocyst complex (Bakke 1979), nutrients are absorbed through the wall of the whole sporocyst complex from the surrounding snail tissue and haemocoel in contrast to Mönnig's (1922) statement. Tegumental pinocytotic acitvity may also occur at different sites along the sporocyst complex (Bakke op.cit.). The circulation of nutrients may be facilitated by means of sporocyst

movement. The nutritional flow within the cavity of the sporocyst complex could, however, be reduced or stopped by the frequently observed encysted metacercariae «trapped» within tubes and branches of different developmental stages (see Lewis 1974a).

The food remnants from the metacercariae observed leaving the oral plug seem to be trapped within the brood chamber, as are, presumably, the end products of metabolism. A chemical analysis of the fluids would be informative in this respect. No transport mechanisms of substances were ultrastructurally observed from the brood chamber through the epithelial lining. In addition, no excretory system was observed in the broodsacs in the present investigations (see also Heckert 1889, Mönnig, 1922, and Popiel & James 1978).

The rupture of storage cells, releasing the contents within the sac lumen as described by Heckert (1889), Mönnig (1922 — «auflösen der Protoplasm») and Kagan (1952), was not observed. The extensive development of free ribosomes and GER (see Storch & Welsch 1970), lamellae and secretory bodies in the epithelial lining (Figs. 8, 9), is morphologically indicative of a system specialized for the synthesis and export of secretory material, which may represent nutrition for cercarial development. This lining was not seen to have membranous contact with other cell types, but it is not quite clear whether the cavities filled with interstitial material occupy an extra- or an intra-cellular position (see Mönnig 1922, Threadgold & Gallagher 1966). The well developed interstitial material is thought to be a sort of skeletal system determining the shape of the body resisting the higher turgidity within the chamber (especially at sac contractions) as seen when the wall is punctured (see Ingram & Hewitt 1943, Threadgold & Gallagher op. cit.).

In the metabolism of flukes the interrelationships of parenchymal cells are important (Threadgold & Gallagher 1966). In *Leucochloridium* it seems that the well developed system of pigment cells, frequently observed adjacent to tegumental bridges and muscle aggregations, fulfill this parenchymal function, and may represent a transport system for metabolites. The pigment cells are also most extensively developed in the distal pulsating end, where they apparently form a syncytium (Fig. 11). (Mönnig (1922) recorded a «Myoblastensyncytium» in the wall). The tegumental secretion not earlier observed (Fig. 7), may be trapped within or represent the tegumental surface coat (not mentioned by Storch & Welsch 1970 — see Fig. 2), which may be a possible protection against mechanical wearing and snail haemocytes. (Old sacs may remain intact for some time in dead snails where putrefaction has started). The lacunae extending into the tegument from the basal lamina may provide for the movement of substances into the tegumentary cytoplasm from the interstitial material through the basal lamina by pinching off the electron lucent globules at the ends to form vesicles (see Fig. 6b).

Light sensitivity

It is extremely rare to find «eyes» in sporocysts (Thomas 1883, Crandall 1960, Basch & Sturroch 1969, see also Faust 1918, Pond & Cable 1966). Nevertheless, the possibility still exists that photoreceptors are developed to some degree in many trematodes but have not been recognized because pigment cells are absent. Phototactic behaviour has for example been reported both with or without records of non-pigmented eyespots (Isseroff & Cable 1968). The physiological and biological/ecological significance of the older broodsacs' rhythmic pulsations in *Leucochloridium* and its dependence on light intensity, spectral range, temperature, humidity and the diurnal periodicity of snail behaviour (Mönnig 1922, Halik 1931, Wesenberg-Lund 1931, Kagan 1952, Hecker & Thomas 1963, Lewis 1972, 1977, Bakke unpublished) is not known, although an effect in the transmissional ecology is postulated (v. Siebold 1853, Zeller 1874, Wesenberg-Lund 1931, Halik 1931, Wunder 1932, Kagan 1952, Hecker & Thomas 1963). The pendular and peristaltic broodsac movements may be of importance in the localization and penetration into the snail tentacles. The broodsac turgidity in «mature» sacs is apparently important for all the movements which also occur independently of the snail, but the details of the behavioural coordination concerning the triggers, transmission and the synchronisation are unknown.

The behaviour is of the orthokinetic type, where intensity and spectral range (see Halik 1931, Lewis 1972, 1977) and humidity and temperature (see Wesenberg-Lund 1931) initiate locomotion and control the frequency of rhythmic movements. No nerve system has been identified (Heckert 1889, Mönnig 1922, Storch & Welsch 1970), and it should also be stressed that the pulsation frequency may differ between broodsacs of the same sporocyst (see Mönnig 1922). Photosensitivity is, however, no assurance that morphologically distinguishable photoreceptors

are present (see Steven 1963 — on the dermal light sense). Nevertheless, the obvious light sensitivity will require a receptor and a mediating system, to bridge and control the photoreception act and the resulting motor response of the effector. The stimulus for muscular activity has been suggested to be dependent on the pigments (Mönnig 1922, Wesenberg-Lund 1931). The present observations on the pigment cells give no support to such a hypothesis. However, as the pulsations are limited to the coloured cap of the broodsac, a correlation between the action spectra (see Halik 1931) and spectral absorption properties of the pigments (see Nadakal 1960a) may provide evidence for an eventual connection. In spite of the possibility of a dermal light sense or that the smooth muscles themselves may be sensitive to light (myogenic as proposed by Kagan 1951) (or a direct action on a still undiscovered type of nervous system), a search was undertaken for a localized, morphologically recognizable photoreceptor. This search was based on a common feature of photoreceptors, namely extensive surfaces (see Moody 1964) upon which a photopigment presumably is spread, although extreme diversity in «eyes» is generally known (Arvy 1975). (SEM studies of the sporocyst microtopography (Bakke unpublished) have not revealed morphologically any surface structure for sense perception). Of the geometrically arranged structures observed (see Figs. 15—17), only three had the elaboration of membranes which may indicate a photoreceptive function (see Eakin 1963, 1965). Of these Fig. 17b shows a promising structure with undulating membranes and an electron dense appendage with longitudinal striations (filaments or microtubules?) leading from it. (Fig. 17a may be the same structure cut in a different plane of section). No cilia were observed in connection with these structures (see Wilson 1970, Lyons 1972, Brooker 1972, Short & Gagné 1975). Speculations on the function of these intracellular structures seem useless until more details are known.

Pigments

Leucochloridium sporocysts are unique due to the extreme development of pigment, whose colour and distribution are taxonomically important (see Bakke 1980). Comparative histology revealed by LM has been used in leucochloridiid taxonomy on the larval stages (Nacheva et al. 1981). In the present TEM investigations no significant differences were seen between the *L. variae* and *L. holostomum* pigments. The intracellular pigments occurred not only between the muscles as described by Heckert (1889) and Mönnig (1922) (see Žďárská & Soboleva 1981), but also within aggregations of pigment cell processes outside the muscle layers. Microtubules — suggesting a transport of the pigment from the inner perinuclear cytoplasm (see Fig. 11) to the outer cell parts — were not observed in association with pigment cells. (Storch and Welsch (1970) recorded, however, microtubules in «plasma-processes» within the wall). The colour (produced only in the presence of light (see Mönnig 1922, Hescheler 1922, Wesenberg-Lund 1931)) intensity is dependent on the frequency of pigments and the thickness of aggregations, which in turn is dependent on age (see Pojmanska 1969). The chemical nature, origin and nutritional aspects of pigmentation are unknown (see Nadakal 1960b, Žďárská & Soboleva 1981); however, the genetical basis of green and brown broodsacs is proved by double infections (see Bakke 1980). The pigment cells may have a central functional importance in sporocyst physiology, as it is suggested for the pulsations (Lewis 1977), and transmissional ecology (Ulmer 1971, Holmes & Bethel 1972).

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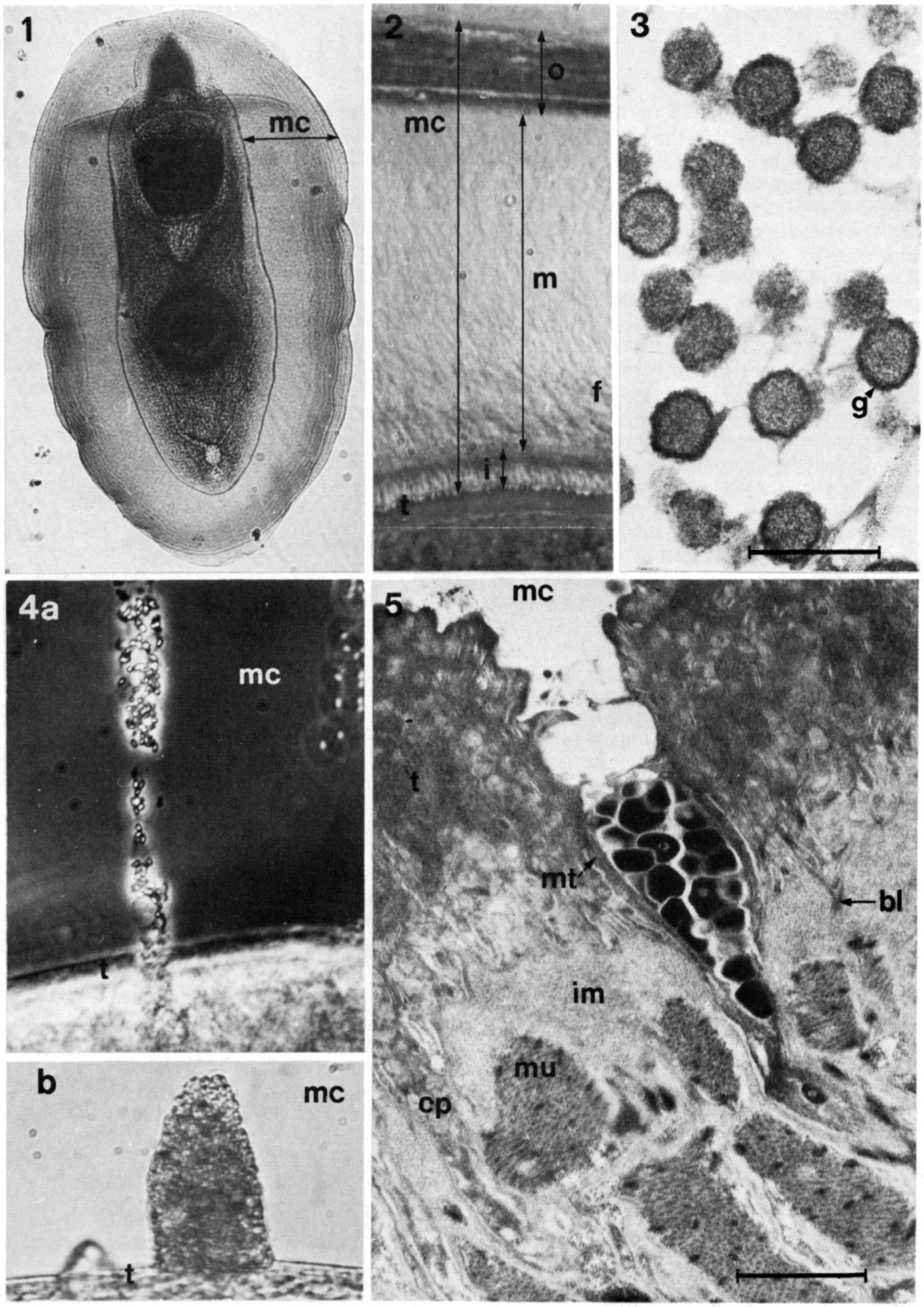
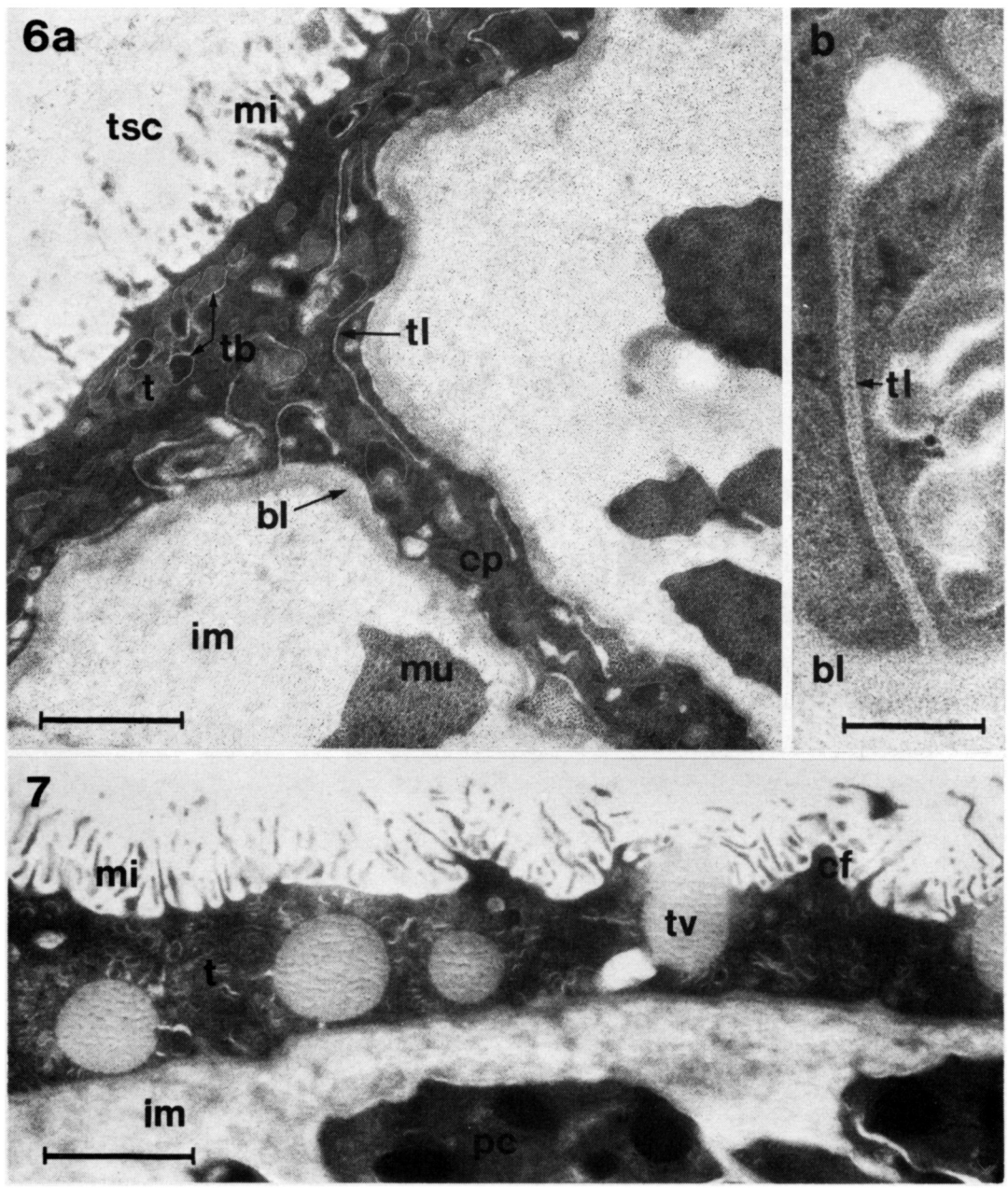


Figs. 1—18. All phase contrast (Figs. 1, 2, 4) and transmission electron microscope (Figs. 3, 5—17) micrographs of the metacercariae (Plate 1) and sporocyst broodsacs (Plates 2—7) are of *Leucochloridium variae*, except Figs. 2, 3 which are of *L. holostomum*. (For abbreviations used in Figures 1—18 see p. 55).

Fig. 1. A living metacercaria within the cyst as seen under cover glass pressure. Fig. 2. A toluidine blue stained dorsal section of the entire cyst wall. Fig. 3. The ultrastructure of the electron-dense granules from the distal part of the outer cyst layer (scale bar 0.5 μm). Figs. 4a, b. Secretions into the cyst from the living metacercaria tegument (a) and accumulated secretions as seen on the tegument (b). Fig. 5. Gland duct opening on metacercaria tegument containing secretory droplets and lined with microtubules (scale bar 1 μm).



Figs. 6a, b. a: The syncytical broodsac tegument provided with microvilli and a cytoplasmic bridge which leads to the perinuclear cytoplasm (scale bar 1 μm). b: Detail of a tegumental lacuna (scale bar 0.5 μm). Fig. 7. Tegumental secretory vesicles (scale bar 1 μm).

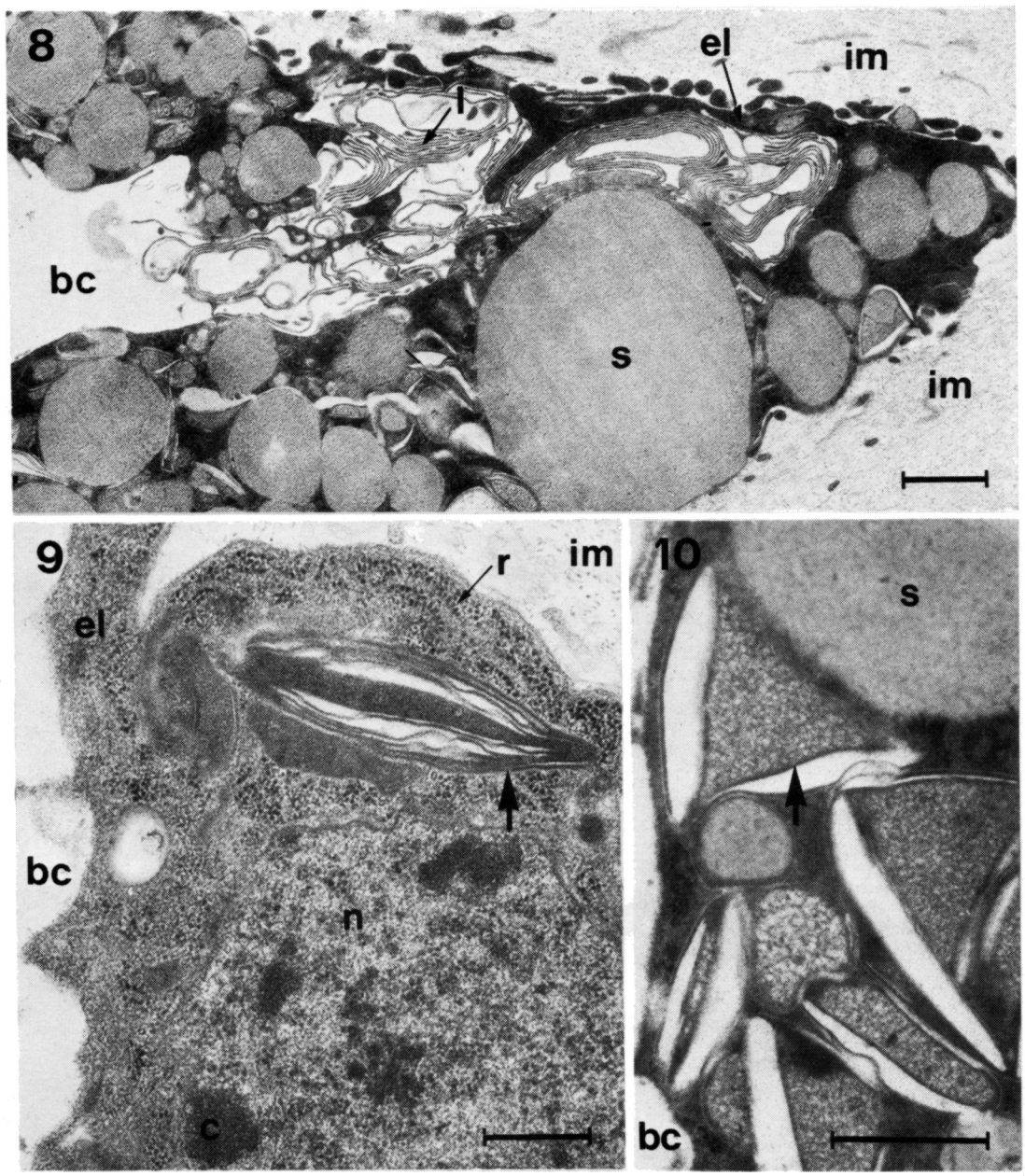


Fig. 8. The epithelial lining provided with secretory bodies and lamellae protruding into the brood chamber (scale bar 1 μm). Fig. 9. A typical lamellated inclusion (see arrow) and part of a nucleus within the epithelial lining (scale bar 0.5 μm). Fig. 10. Irregular shaped bodies often forming triangles (see arrow) within the epithelial lining (scale bare 0.5 μm).

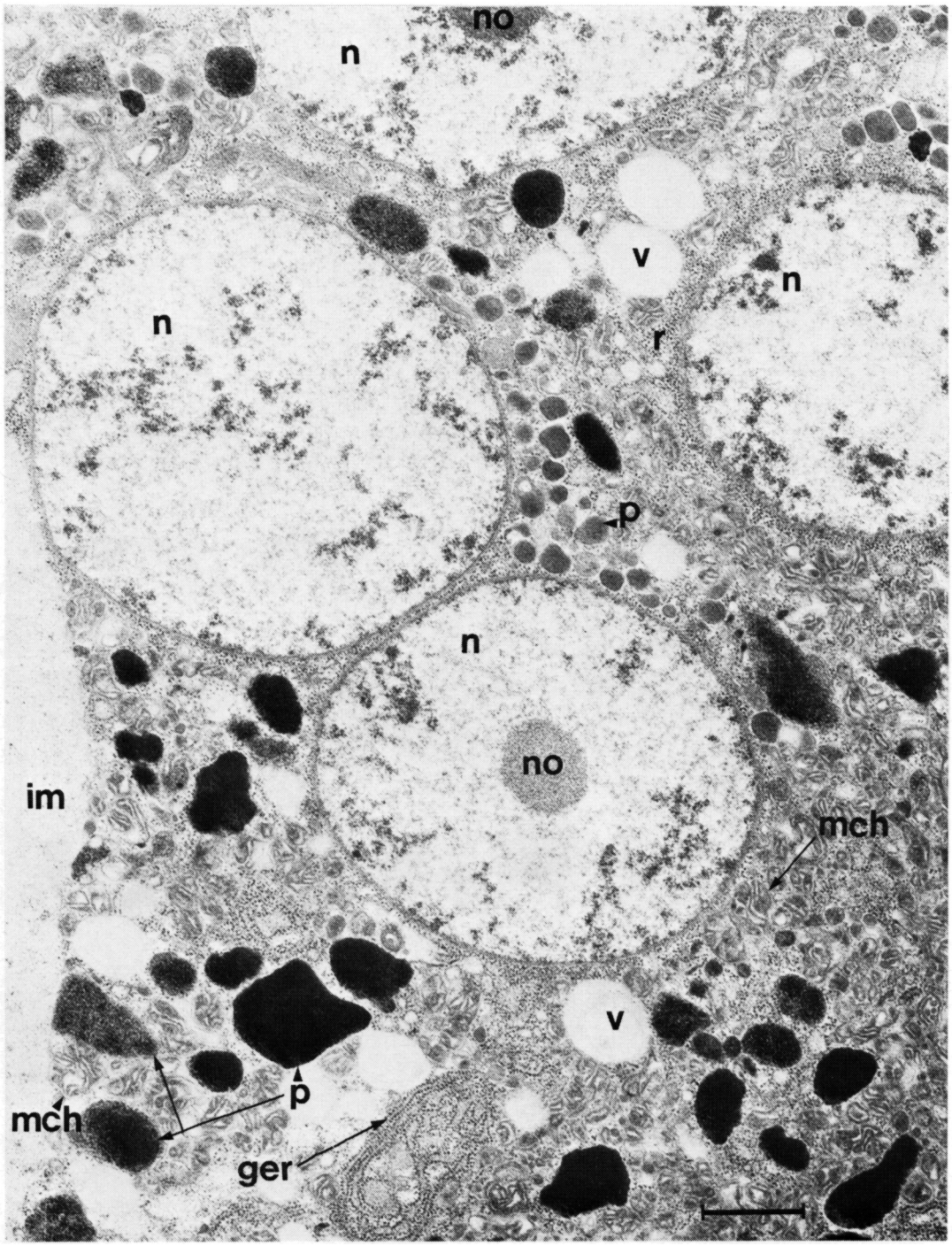


Fig. 11. The perinuclear part of a pigment cell showing a highly metabolically active cytoplasm (scale bar 1 μm).

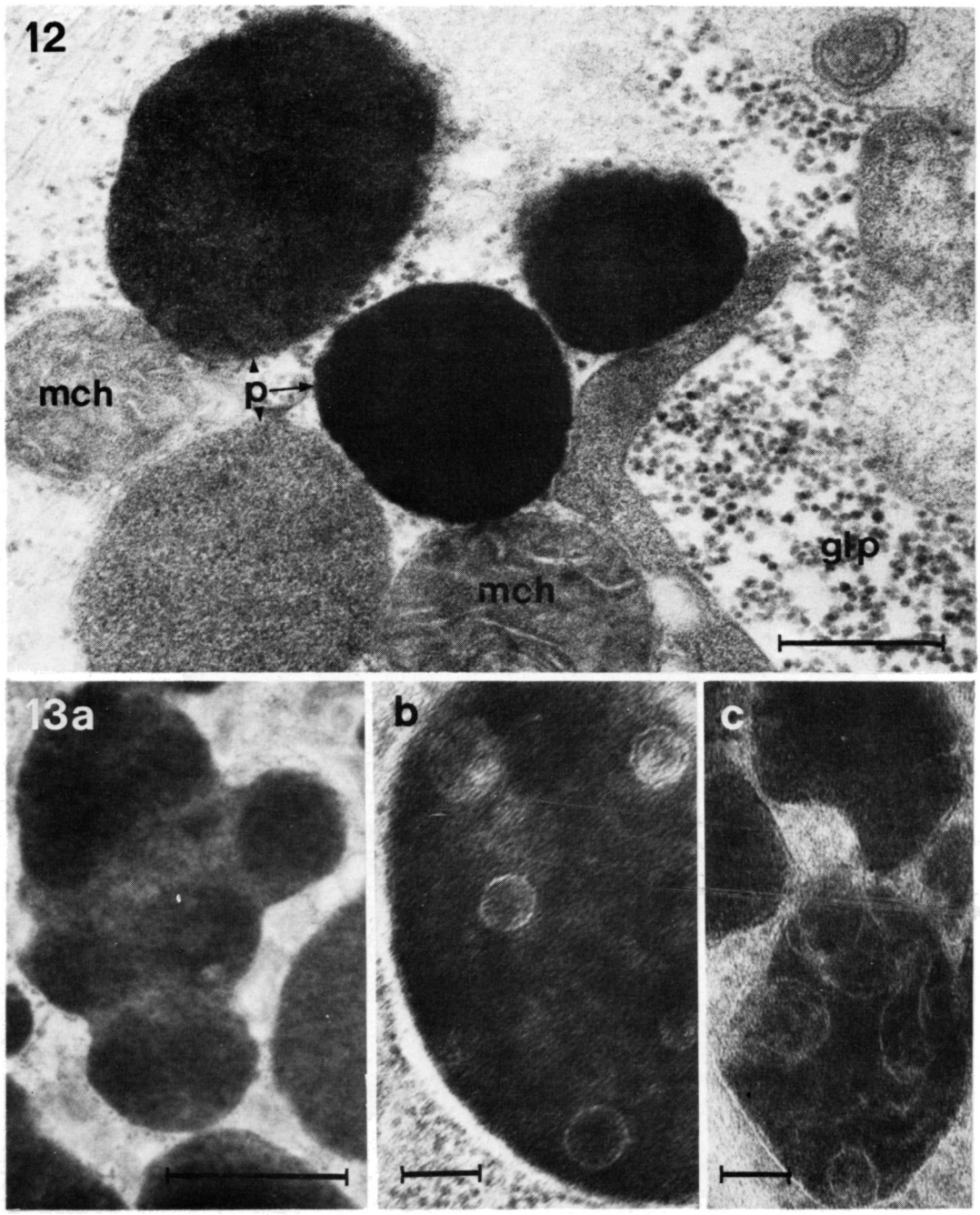
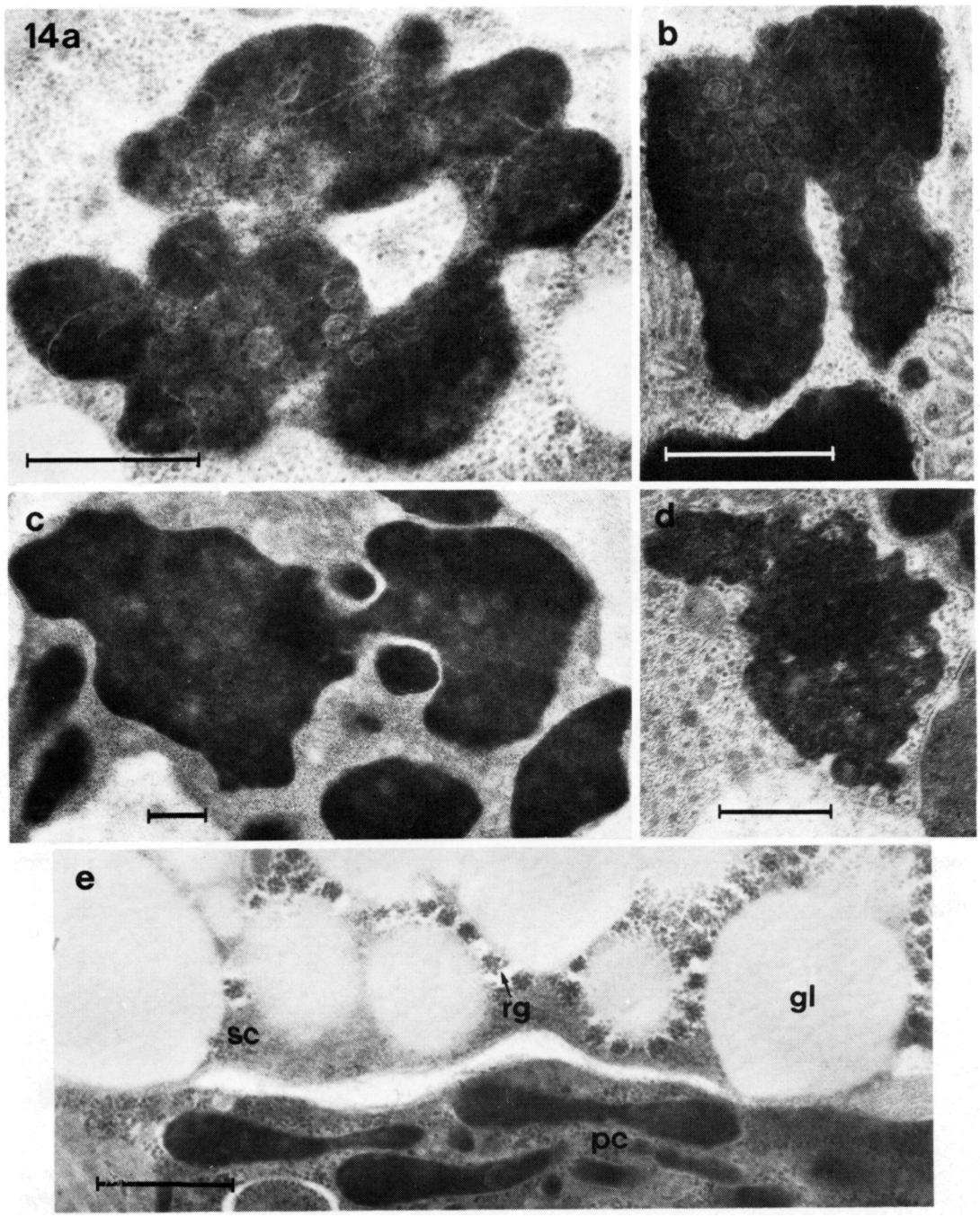


Fig. 12. Spherical and homogeneous pigments of different electron density as seen in section (scale bar 0.5 μm). Figs. 13a—c. Heterogeneous pigments demonstrating the typical different types of inclusions: electron dense (a), electron light regular (b) and irregular (c) (scale bars: 1 μm (a), 0.1 μm (b, c)).



Figs. 14a—e. The other main types of pigments outlines as seen in sections. e: also shows the globule-like inclusions in another cell (see text) (scale bars 0.5 µm).

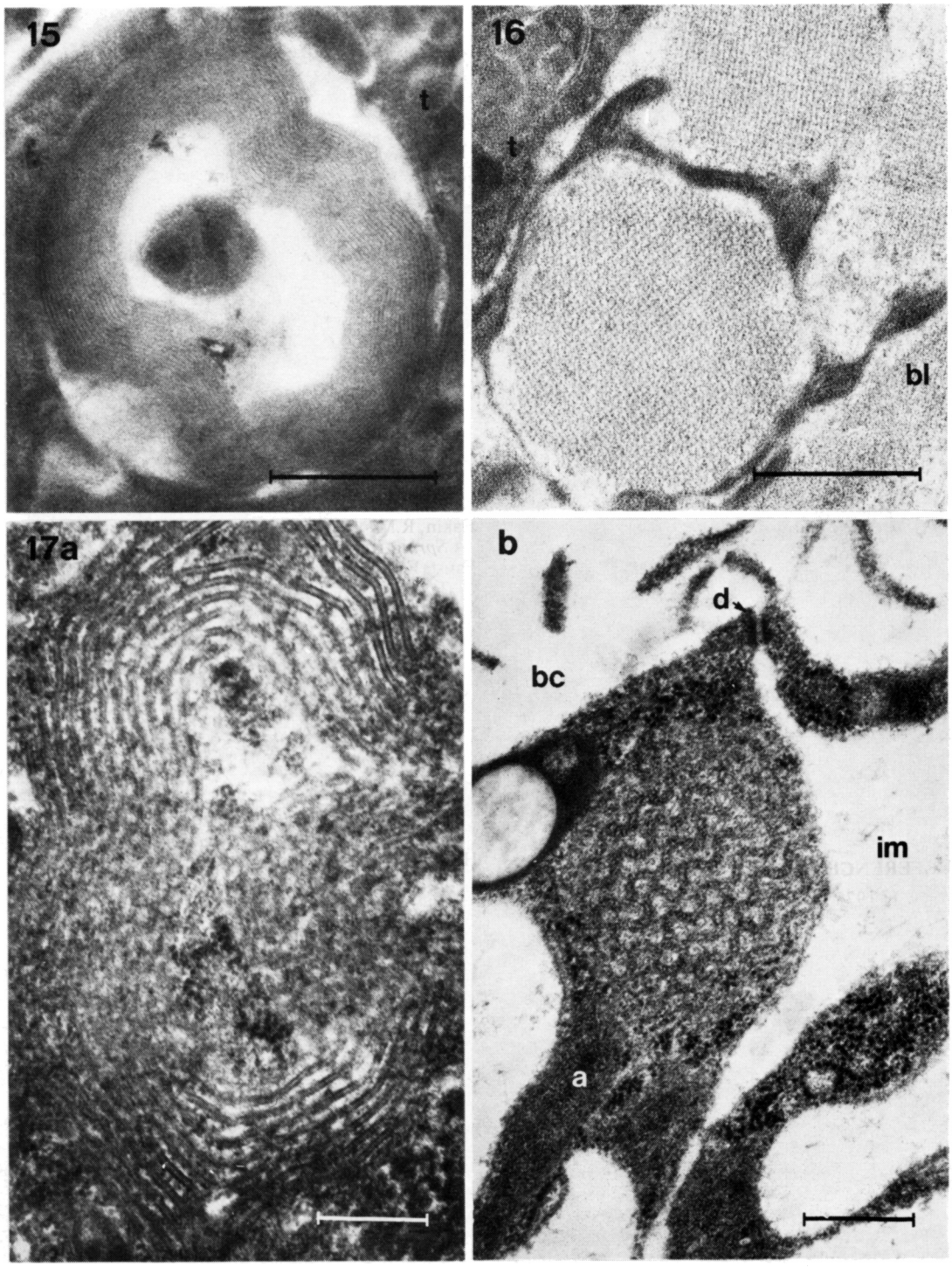


Fig. 15. Lamellated tegumental inclusion (scale bar 0.25 μm). Fig. 16. Crystallized tegumental inclusions (scale bar 0.5 μm). Figs. 17a, b. Membranous structures as observed within the epithelial lining (scale bars 1.0 μm).

Abbreviations used in the figures:

a	appendage
bc	brood chamber
bl	basal lamina
c	chromatin
cf	cytoplasmic fold
cp	cytoplasmic process
d	desmosome
f	fibres
el	epithelial lining
g	electron-dense granule
ger	granular endoplasmic reticulum
gl	globules
glp	glycogen particles
i	inner cyst layer
im	interstitial material
l	lamellae
m	middle cyst layer
mc	metacercarial cyst
mch	mitochondrion
mi	microvilli
mt	microtubuli
mu	muscle fibres
n	nucleus
no	nucleolus
o	outer cyst layer
p	pigment
pc	pigment cell
r	ribosomes
rg	rosette glycogen
s	secretory body
sc	storage cell
t	distal part of tegument
tb	tegumental bodies
tl	tegumental lacunae
tsc	tegumental surface coat
tv	tegumental vesicle
v	vacuoles

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