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MALE CYPRIS METAMORPHOSIS AND A NEW MALE LARVAL FORM, THE TRICHOGON, IN THE PARASITIC BARNACLE *SACCULINA CARCINI* (CRUSTACEA: CIRRIPEDIA: RHIZOCEPHALA)

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Virgin externae of the parasitic barnacle *Sacculina carcini* Thompson were exposed to settlement of male cyprids. The events from settlement around the mantle aperture to arrival of the male cypris cells into the receptacle of the externa were studied by transmission and scanning electron microscopy. A small, hitherto unknown larva, the trichogon, escapes from the cyprid within *ca.* 20 min after settlement. A thin cuticle armed with long spines is preformed beneath the carapace of the male cyprid, and after metamorphosis this cuticle encloses the free trichogon. The trichogon is up to 220 μm long, unsegmented, has a variable amoeboid shape and a very simple structure. It includes parts of the cypris epidermis and two other types of cypris cells, but it has no appendages, muscles, sense organs or nervous tissue. The trichogon migrates through the mantle cavity of the externa and arrives at the entrance to the receptacle duct within 2 h after settlement. During the ensuing migration through the receptacle duct, the trichogon loses its spine-armed cuticle. Once inside the receptacle, the trichogon cells and the female cells of the receptacle are in direct contact, with no intervening cuticle. The implanted trichogon is regarded as a very specialized dwarf male. The formation of the trichogon from male cyprids, and of the kentrogon from female cyprids has many similarities, and the trichogon and the kentrogon are regarded as homologous instars. A trichogon is present in the Sacculinidae, the Lernaeodiscidae and most probably also in the Peltogastridae; i.e. in the same families where a kentrogon has been demonstrated to accomplish invasion of the decapod host.

Introduction

Most rhizocephalan species belong to the subgroup Kentrogonida, and all that have been investigated to date have separate sexes (Ichikawa & Yanagimachi 1958, 1960; Yanagimachi 1961; Lützen 1984; Høeg & Ritchie 1985). About a century ago Delage (1884) described what is now known to be the female part of the life cycle. A female cyprid settles on the prospective host (a decapod crustacean) and metamorphoses into a small larva, the kentrogon, which pierces the host cuticle with a stylet and injects the primordial parasite. After injection the primordium develops endoparasitically until it finally emerges on the surface of the host as the reproductive body, the externa.

It was originally believed that the externa was a self-fertilizing hermaphrodite. However, it had long been observed that cypris larvae settle around the mantle aperture of the juvenile externa. Delage (1884) regarded these cyprids as males, and with astonishing foresight he suspected them to have an important role in the life cycle. However, Smith (1906) and later authors dismissed the male cyprids as being without any function at all. Reinhard (1942) believed that the male cyprids of *Peltogaster paguri* fertilized a part of the first brood of the hermaphroditic externa, but Ichikawa & Yanagimachi (1958, 1960) were the first to show that the virgin externa is wholly female.

The rhizocephalan life cycle as presently known may be exemplified by *Sacculina carcini*: (1) male and female cyprids can be distinguished before settlement by size (Høeg 1984) and by differences in anatomy (Walker 1985); (2) male cyprids settle only on recently emerged externae, while female cyprids settle only on prospective hosts, which they infect by means of the kentrogon stage (Høeg 1984); (3) the recently emerged externa is a virgin female and cannot grow to the adult size and reproduce unless a male cyprid settles on it and implants cells into the male cell receptacles, formerly believed to be the testes (Lützen 1984). The externa itself cannot produce spermatozoa.

The metamorphosis of the female cyprid has been studied in a number of species by using light or electron microscopy (Veillet 1964; Høeg 1985*a*). Most recent studies on the role of

male cyprids, however, have focused on the male cells after their arrival into the receptacle and upon their subsequent effect on growth and reproduction of the externa (Ichikawa & Yanagimachi 1958, 1960; Høeg & Ritchie 1985). Almost nothing is known about the derivation of the implanted male cells in the cyprid, the details of male cypris metamorphosis and the migration of the cypris cells through the mantle cavity of the externa. This lack of knowledge makes comparison with the events in females impossible. The present study describes the events between male cypris settlement and arrival of the male cells into the receptacle in the rhizocephalan *Sacculina carcini*, a parasite of the shore crab *Carcinus maenas*. The male cyprid is shown to metamorphose into a hitherto unknown larva, the trichogon, which migrates through the mantle cavity and into the receptacle of the female externa.

Materials and methods

Shore crabs, *Carcinus maenas* (L.), parasitized by *Sacculina carcini* Thompson, were collected near the Station Biologique de Roscoff, Brittany, France, during two periods: 13–29 August 1982, and 24 May to 16 June 1983. Maintenance of crabs, collection and culture of parasite larvae, isolation of virgin parasite externae, and settling experiments with male cyprids were as in Høeg (1984). The frequency of recently emerged, virgin externae was at least an order of magnitude higher in May–June 1983 than in August 1982. Crabs hosting virgin externae were exposed to male cyprids for periods of between 15 min and several hours. If male cyprids had settled, the externa was either immediately fixed, or the crab with the parasite was kept isolated for periods of 15 min to several days before fixation. Male cypris settlement was observed on 30 virgin externae. To supplement these an additional 18 externae (externa width 3.5–6.0 mm, $\bar{x} = 4.2$ mm) which carried settled male cyprids were collected in the field. Free-swimming cyprids were fixed from the broods at the time of the settlement experiments. The fixative used was a mixture of paraformaldehyde (20 g l^{-1}) and glutaraldehyde (25 g l^{-1}) in a 0.1 M sodium-cacodylate buffer (pH = 7.4) and contained 65 ml of seawater and 3.4 g of sucrose per 100 ml final solution. After 1–3 h fixation the specimens were transferred to the same buffer (pH = 7.4, 3.4 g sucrose per 100 ml final solution) and stored at 4 °C for several weeks. After final dissection the specimens were postfixed in osmium tetroxide (10 g l^{-1}) (same buffer) and dehydrated. Specimens for scanning electron microscopy (SEM) were dehydrated through acetone and critical-point dried from liquid CO_2, coated with gold and examined in a Cambridge Stereoscan. For transmission electron microscopy (TEM), the specimens were dehydrated through ethanol and propylene oxide, and embedded in Epon. Cyprids fixed when free-swimming were cut into complete ultrathin section series with a diamond knife on a Reichert OMU3 microtome. Virgin externae and externae with settled males were initially cut at 2 μm and stained with Toluidine Blue, followed by ultrathin section series, when settled cyprids, trichogons, or receptacles were reached. Staining and examination of the ultrathin sections were as in Høeg (1985*a*).

The male cyprid

The cyprids of *Sacculina carcini* resemble those described by Høeg (1985*a*) from *Lernaeodiscus porcellanae*; the description below only deals with structures important to metamorphosis. Male cyprids (270–320 μm carapace length) are larger than females (230–270 μm) (Høeg 1984).

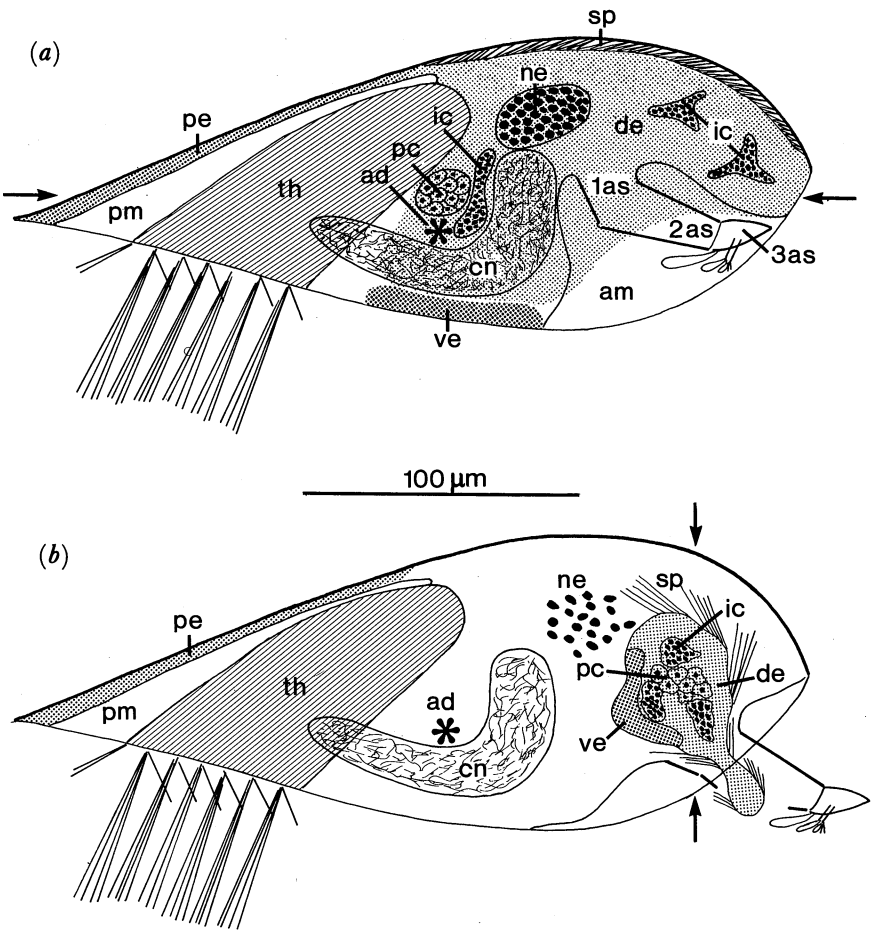


FIGURE 1. Diagrams of free-swimming male cyprid (*a*) and settled male cyprid enclosing a completed trichogon (*b*). In (*a*) the distribution of the three types of epidermis is shown as differently dotted areas; the extension of the mid-dorsal field of epicuticular spines beneath the carapace is indicated by (sp). Horizontal arrows show the level of section of figure 12. In (*b*) the trichogon has formed by closure of the dorsolateral epidermis (de) and the ventral epidermis (ve) as a sac incorporating the inclusion cells (ic) and the postganglion cells (pc); the epicuticle with spines (sp) accompanies the epidermis and surrounds the entire trichogon, which escapes through a rift in the cuticle of the second segment (2 as) of a cypris antennule; the posterior epidermis (pe) and the thorax (th) remains in the spent cyprid; the pigment of the nauplius eye (ne) is always expelled during metamorphosis (see text) and is absent from the spent cyprid; vertical arrows indicate the level of section in figure 19.

The body is enclosed by the carapace cuticle, *ca.* 0.7 µm thick (figure 2, plate 1). The thin, ventral cuticle is invaginated as the anterior and posterior mantle cavities. The anterior cavity accommodates a pair of four-segmented antennules. There are clear-cut differences in antennular structure between male and female cyprids (Walker 1985). The posterior mantle cavity is occupied by the thorax and vestigial abdomen (figure 1*a*). A more detailed transmission electron microscope study of the *S. carcini* cyprid appeared in Mourlan *et al.* (1985).

(*a*) *Epidermis*

Three types of epidermis were discerned in male cyprids. In addition, single epidermal gland cells are situated throughout the epidermis. Each gland empties through a pore in the carapace cuticle.

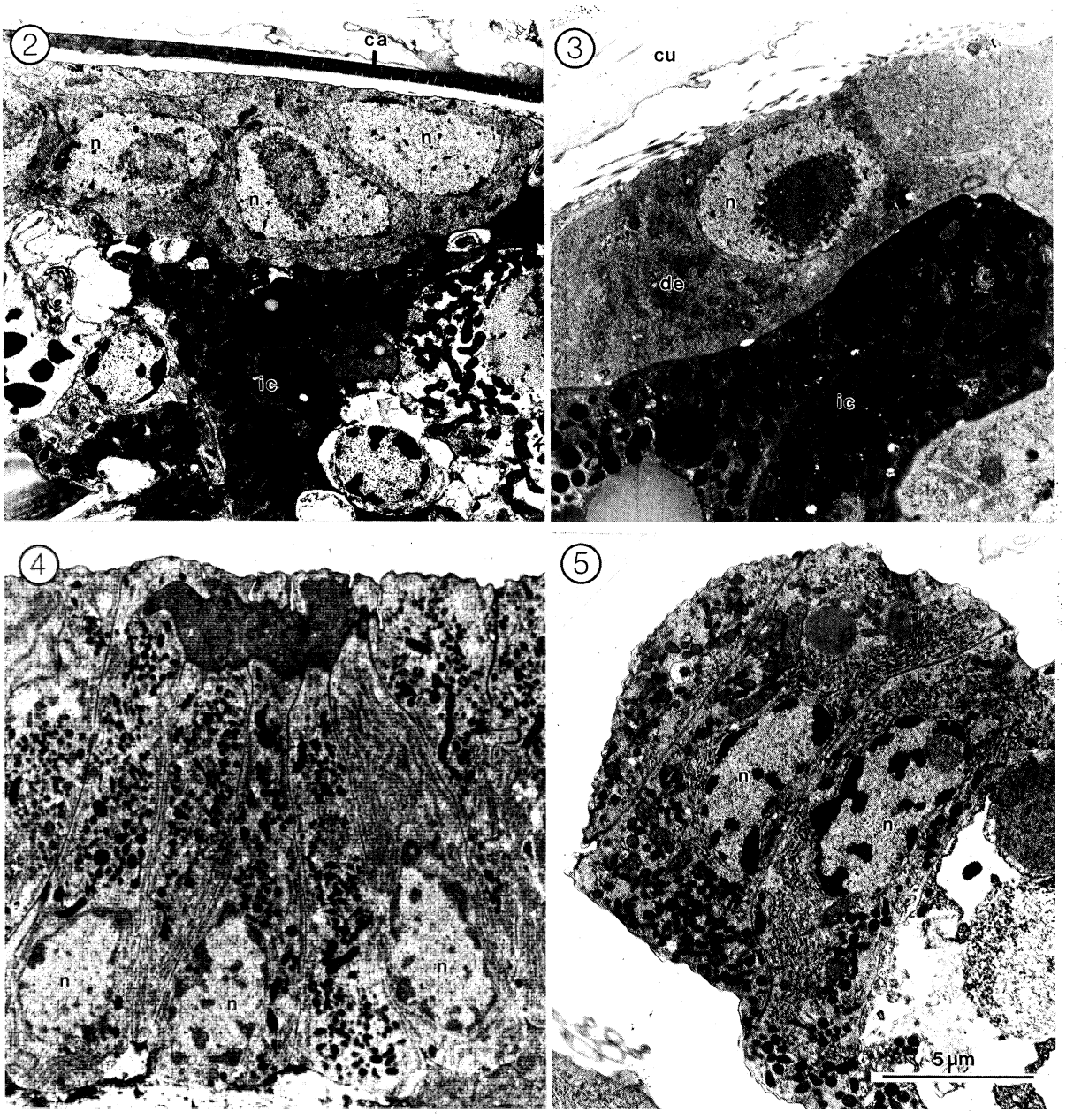


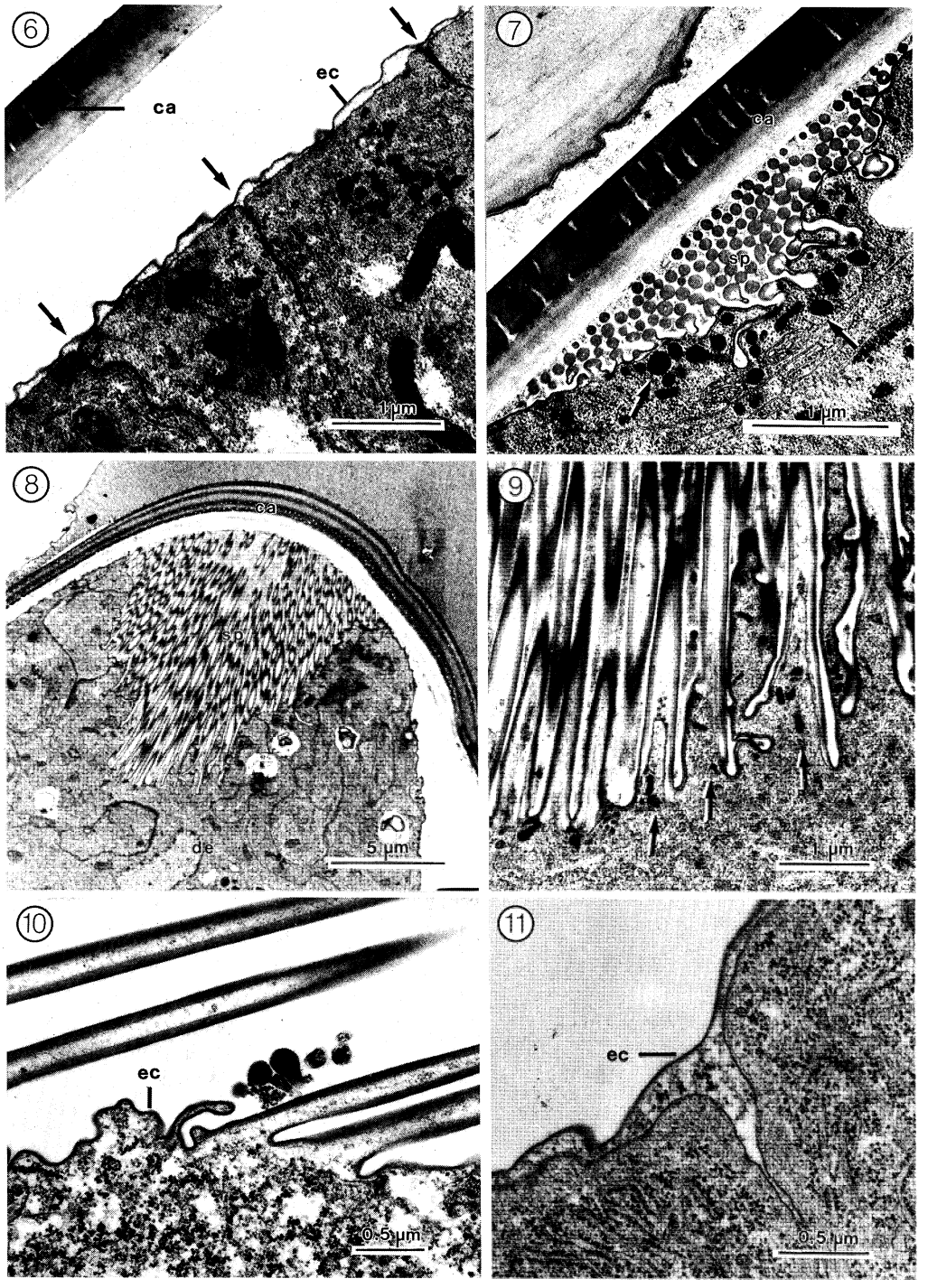
FIGURE 2. Cyprid. Dorsolateral epidemis cells are beneath the carapace (ca). An inclusion cell (ic) is seen immediately below the epidermis. TEM. Scale as in figure 5.

FIGURE 3. Free trichogon. Note the dorsolateral epidermis (de) and inclusion cell (ic); compare with figure 2. Cuticle at upper left is from the mantle cavity of the externa. TEM. Scale as in figure 5.

FIGURE 4. Cyprid, showing the ventral epidermis beneath the central nervous system. Ventral side is up. Epidermis and epicuticle have withdrawn from the cypris cuticle situated beyond the top of the figure. TEM. Scale as in figure 5.

FIGURE 5. Free trichogon, showing ventral epidermis cells. Epicuticular spines are absent above the cells; compare with figure 4. TEM.

(*Facing p.* 50)



FIGURES 6–11. For description see opposite.

Description of plate 2

Figure 6. Cyprid, showing epidermis and epicuticle (ec) beneath the carapace (ca); the epicuticle is clearly continuous above the boundaries between adjacent epidermis cells (arrows). TEM.

Figure 7. Cyprid, showing a small lateral spine field beneath the carapace; small, solid spines are seen in cross section, small electron-dense granules can be seen in dorsolateral epidermis cell (arrows). TEM.

Figure 8. Cyprid. Horizontal section through the anterior end, showing mid-dorsal spinefield (sp) between carapace (ca) and dorsolateral epidermis (de). TEM.

Figure 9. Detail of figure 8. Cytoplasm extensions protude into the hollow bases of the spines (arrows). TEM.

Figure 10. Epicuticular spines (sp) on the surface of a free trichogon; compare with figure 8. TEM.

Figure 11. Rectangle in figure 18. The epicuticle on the free trichogon (ec) is continuous over the boundary between adjacent epidermis cells; compare with figure 6. TEM.

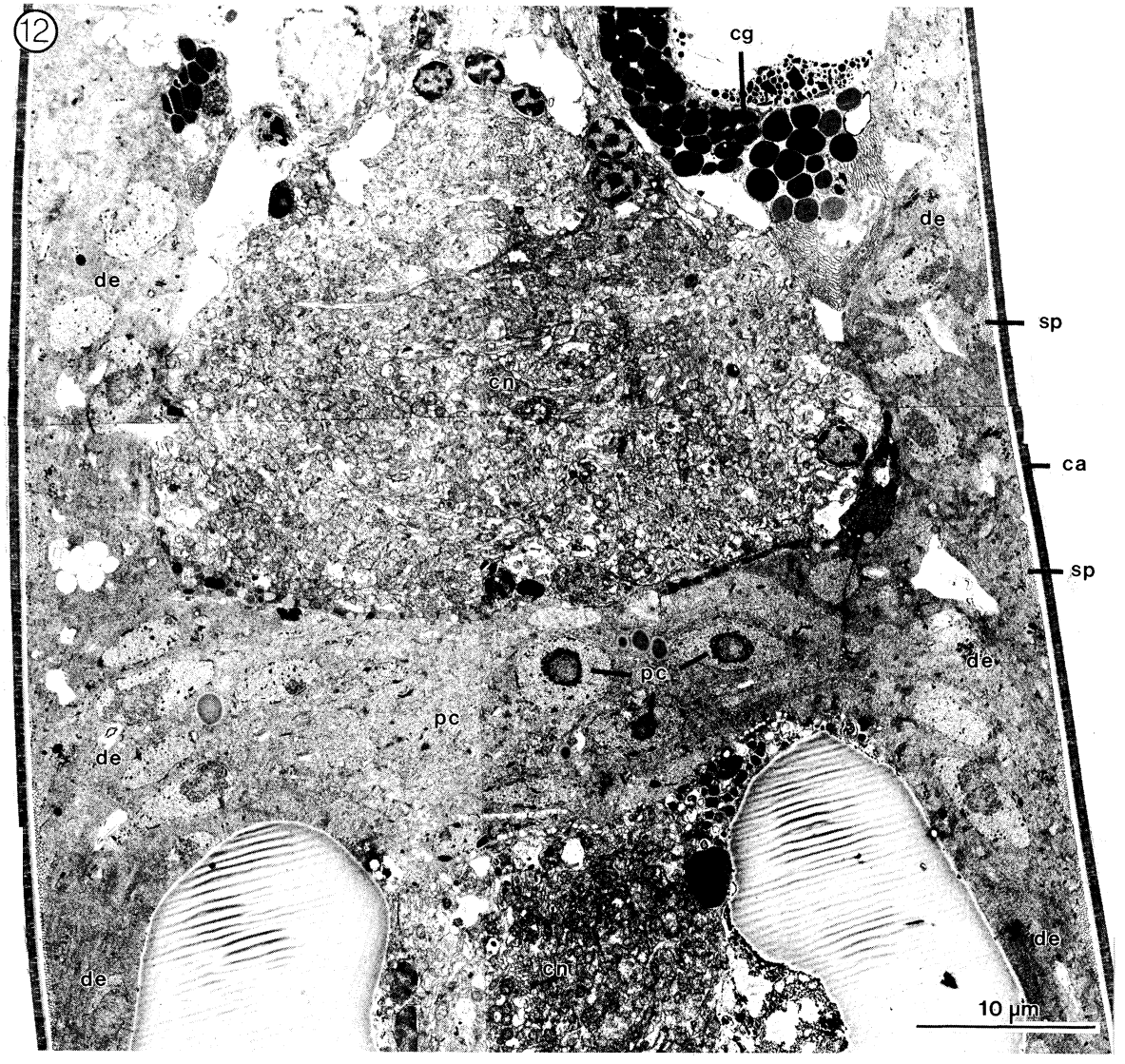


FIGURE 12. For description see opposite.

Description of plate 3

Figure 12. Cyprid, showing a horizontal section through body midregion at a level indicated by the arrows in figure 1*a*; the anterior end is up. Postganglion cells (pc) have contact with the dorsolateral epidermis (de) on each side of the body; note the several small spine fields (sp) beneath the carapace; the anterior lobe of the central nervous system (cn) is at the top of the figure, the posterior lobe at the bottom. TEM mosaic.

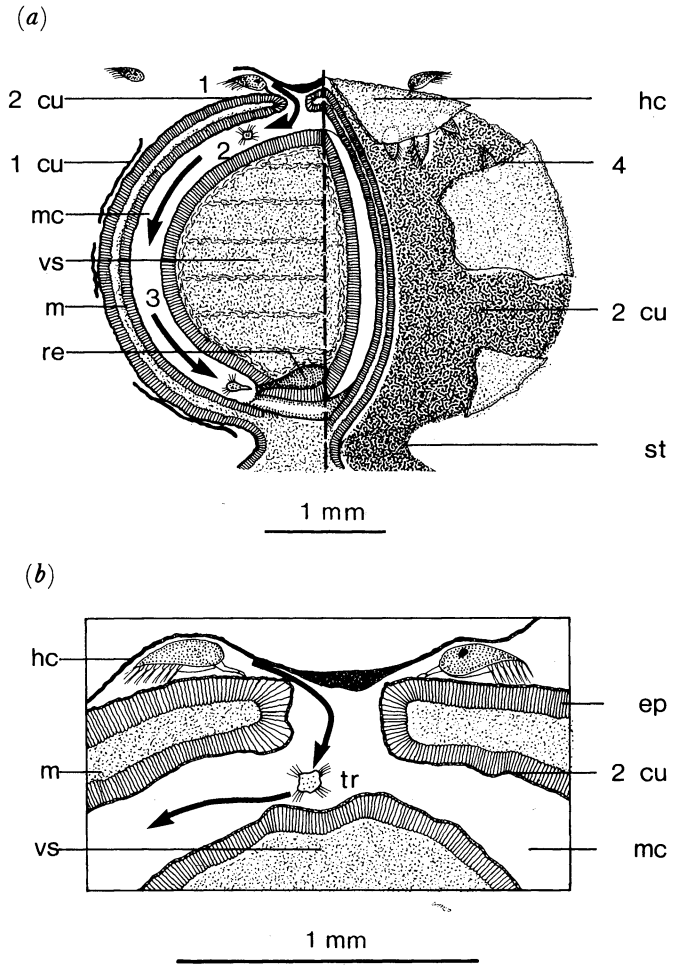


FIGURE 13. Diagram of male cypris settlement and metamorphosis on a virgin externa; see table 1 for comparisons. (*a*) (1) Male cyprids settle beneath the hood (hc) of the remaining 1st externa cuticle, which covers the mantle aperture; each settled cyprid metamorphoses into a trichogon, which migrates (2) through the mantle aperture and mantle cavity (mc), until it reaches the entrance to one of the receptacle ducts (3) and begins to penetrate into the receptacle (re); a few cyprids (4) have settled beneath small pieces of 1st externa cuticle (1cu) elsewhere on the externa, and such incorrectly settled cyprids never metamorphose into trichogons. (*b*) Diagram through mantle aperture in (*a*); two settled cyprids are covered by the hood (hc) of the 1st externa cuticle above the mantle aperture; a trichogon has already escaped into the mantle cavity from the cyprid at left; the presence of the naupliar eye in the right cyprid reveals that it has not yet metamorphosed.

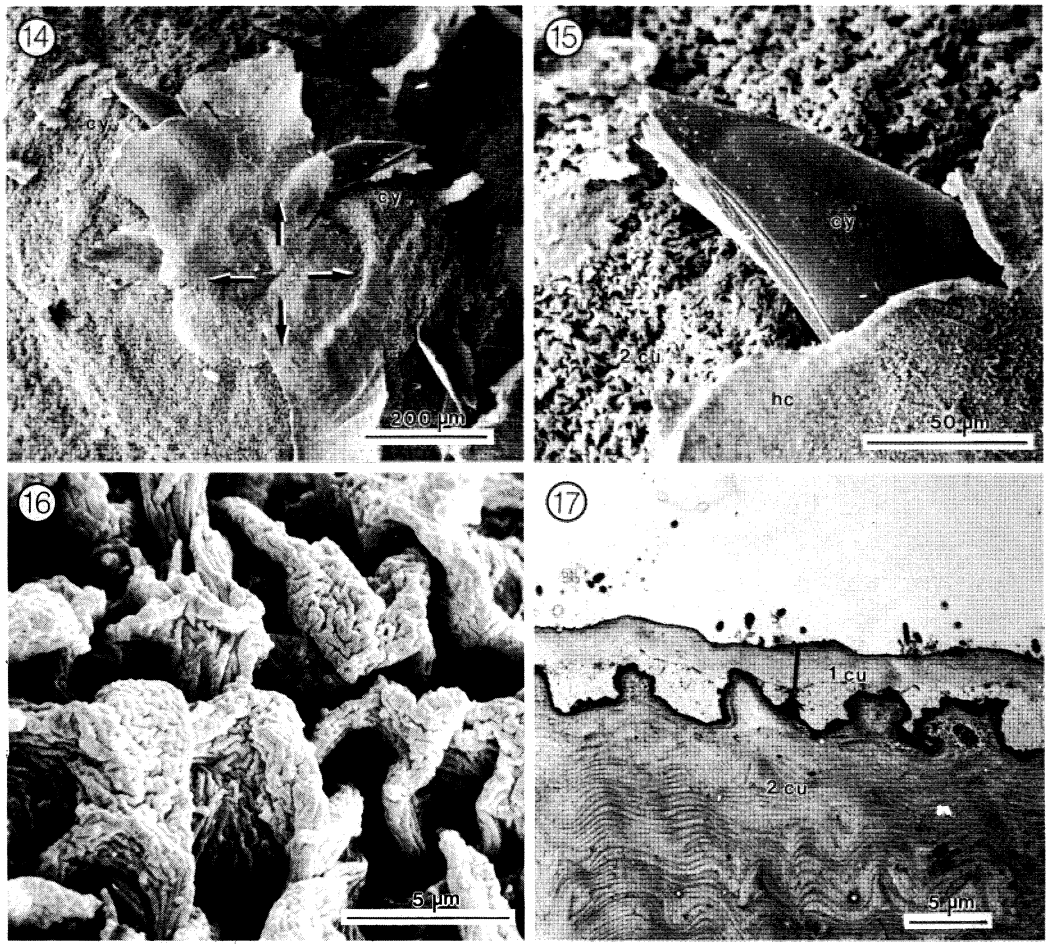


FIGURE 14. Apical end of an externa, showing two settled male cyprids (cy) partly covered by the hood cuticle; the outline of the mantle aperture beneath the hood is faintly visible (arrows); a cyprid at the mid-left periphery of the hood has fallen off but has left an impression. SEM.

FIGURE 15. Detail of figure 14; the posterior end of a settled cyprid protudes beyond the hood cuticle (hc); the 2nd externa cuticle (2 cu) has a much rougher surface than the hood of 1st externa cuticle. SEM.

FIGURE 16. Excresences on the surface of the 2nd externa cuticle. SEM.

FIGURE 17. Smooth 1st externa cuticle (1 cu) above the rough-surfaced and much thicker 2nd externa cuticle (2 cu). TEM.

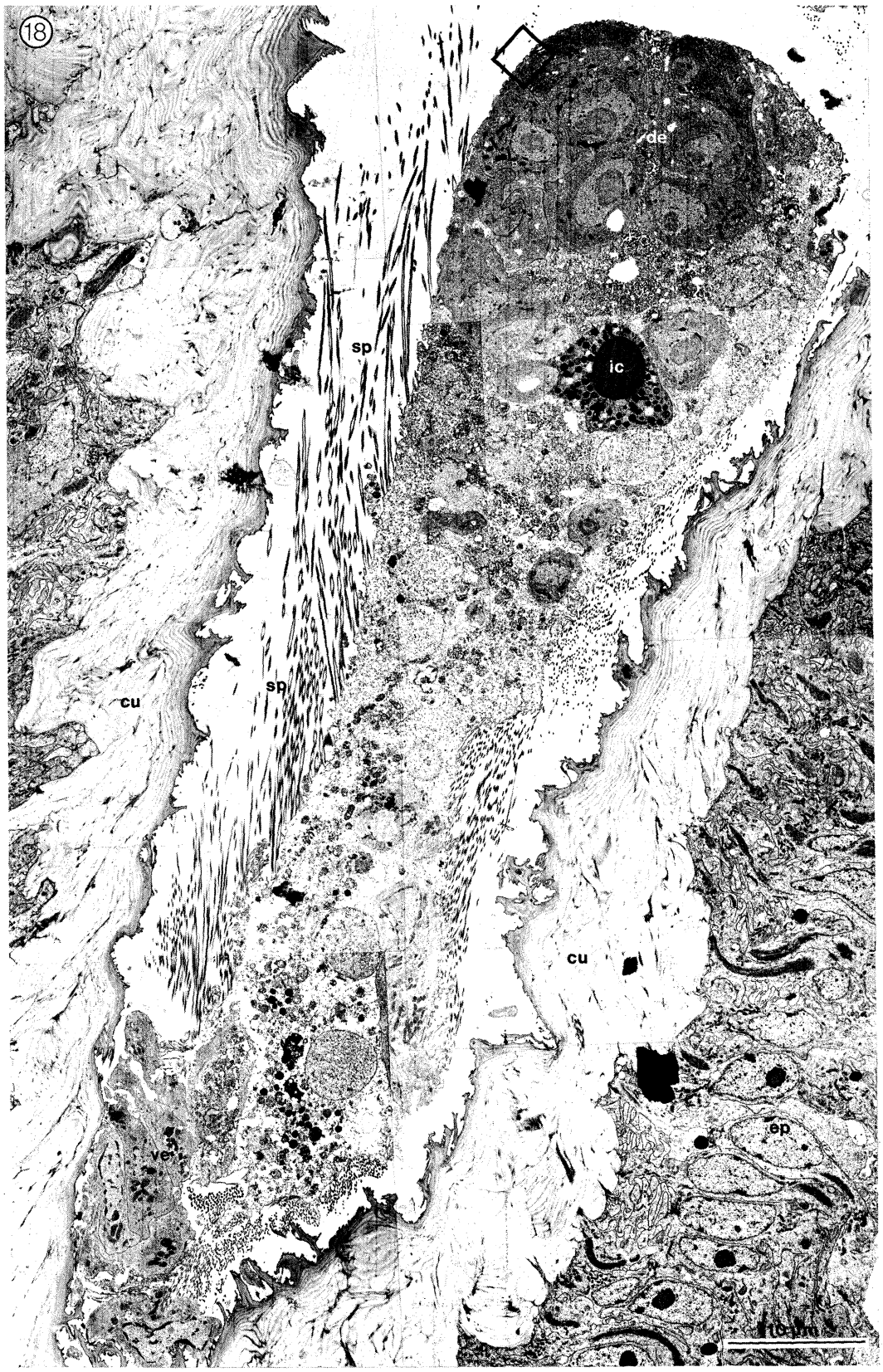


FIGURE 18. For description see opposite.

The first type of epidermis, the dorsolateral epidermis, occurs in the anterior part of the body, i.e. in front of the thorax (figure 1*a*). It varies in thickness from 2.5–12 μm but is usually *ca.* 5 μm. The dorsolateral epidermis cells are cuboid, with a large nucleus (*ca.* 5 μm in diameter), which occupies most of the cell volume and contains a single large nucleolus (figure 2, plate 1). The cytoplasm contains rough endoplasmic reticulum and in some cells also small, electron-dense granules, which tend to be located near the apical plasma membrane (figure 7, plate 2). Most anteriorly the dorsolateral epidermis only extends about two thirds of the distance towards the ventral flange of the carapace, but in the middle of the body it is continuous with a second kind of epidermis, the ventral epidermis, which lines the thin cuticle below the central nervous system (figure 1). The ventral epidermis is columnar and up to 10 μm high (figure 4, plate 1). The cells do not usually have a single large nucleolus, but have the electron-dense material of the nucleus arranged peripherally along the nuclear envelope. The cytoplasm contains a large number of small, electron-dense granules.

The third kind of epidermis lines the posterior mantle walls and is very thin with long slender nuclei. Unlike the dorsolateral and ventral epidermis, this posterior epidermis is not involved in metamorphosis.

No basal lamina is present beneath the epidermis anywhere in the cyprid.

(*b*) *The spiny epicuticle*

The cuticle of the carapace consists of an outer electron-dense exocuticle that contains numerous pore canals, and an inner and much lighter-staining endocuticle without pore canals (figure 7, plate 2). Even before settlement the dorsolateral and the ventral epidermis (see above) have secreted a new, post-ecdysial cuticle beneath the carapace. This cuticle is only up to 17 nm thick and is therefore considered as an epicuticle. It is either closely applied to the apical cell surface or separated by a narrow space. Its nature as a cuticle layer is demonstrated by its armament of spines (see below) and by its continuity over boundaries between adjacent epidermis cells (figure 6, plate 2). In the midregion of the cyprid the epicuticle is continuous all around the ventral side below the central nervous system. More anteriorly, the epicuticular layer ends blindly with the epidermis in the ventral part of the mantle walls. The posterior epidermis does not secrete an epicuticle below the carapace. The epicuticular layer therefore ends blindly where the dorsolateral epidermis is replaced by the posterior epidermis (figure 1*a*).

The epicuticle beneath the carapace is armed with long, narrow spines. These spines are always concentrated in special areas, spine fields, which are of very variable size. In most places the epicuticle and epidermis are only separated from the carapace by a narrow space. In spine fields, however, the epidermis and epicuticle have withdrawn up to 2 μm from the carapace, and the closely spaced spines project obliquely and semiparallel into the space between epicuticle and carapace and reach the underside of the latter (figures 7 and 8, plate 2). A long and continuous spine field extends along the dorsal midline from the anteriormost end to a point above the central nervous system. The total length of this mid-dorsal field is *ca.* 100 μm, and

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Description of plate 5

Figure 18. Trichogon in the entrance to a receptacle duct; the trichogon is migrating through the duct towards the bottom of the figure; epicuticular spines are absent from the posterior end of the trichogon; large spines (sp) from the mid-dorsal spine field in the cyprid extend along the left side of the trichogon; note also dorsolateral epidermis (de), ventral epidermis (ve) and inclusion cells (ic) in the trichogon; sculptured cuticle (cu) and columnar epithelium of the receptacle duct are shown (rectangle at top in figure 11). TEM mosaic.

all of its 40 μm long spines extend in an anterior direction (figures 1*a* and 8). The remaining spine fields are lateral and only up to 3 μm wide (figure 7). No spine fields are seen in connection with the ventral epidermis, although the epicuticle is present.

The diameter of the epicuticular spines decreases from 100–500 nm basally to less than 30 nm at the tip. The spines of the mid-dorsal field are large and hollow for the greater part of their length (figures 8 and 9, plate 2), whereas the spines of the remaining fields are much smaller, and hollow only at the base (figure 7). All spines are continuous with the general epicuticle and are without any special articulation. Villus-like projections of the epidermal plasma membrane extend into the base of the spines, but no microfilaments or microtubules are associated with them (figure 9).

At muscle attachments there is no separation of the epidermis from the carapace cuticle and no epicuticle is secreted.

(*c*) *The inclusion cells*

A number of these unusual cells lie here and there in the body. Some of them lie directly beneath the epidermis (figure 2), whereas others lie deeper in the tissues, and a few are always found anterior to the postganglion cells (see below; figure 1*a*). The inclusion cells resemble melanophores in having a variable shape with one or several slender extensions. Almost the entire cell volume is filled with numerous small, round or ovoid inclusions. These are usually very electron-dense, but in some cells many of the inclusions stain lightly or not at all. The electron-dense inclusions resemble pigment, but they are smaller and of less angular shape than the pigment granules of the naupliar eye (figures 23 and 24, plate 8).

(*d*) *The postganglion cells*

The central nervous system lies in a ventral position in the middle of the body between the two mantle cavities (figure 1*a* and figure 12, plate 3). It is divided into an anterior and more dorsal lobe, which is broadly connected to a posterior and more ventral lobe. The pigmented naupliar eye lies on top of the anterior lobe, and the carapace adductor muscle connects the two sides of the carapace directly above the connection between the two ganglion masses.

Just behind the anterior ganglion and just above the adductor muscle lies a group of 20–30 cells, which extends as a transverse cell bridge connecting the dorsolateral epidermis on both sides of the body (figures 1*a* and 12). In size and morphology of the nucleus, these postganglion cells resemble the dorsolateral epidermis. The postganglion cells, however, never contain the electron-dense inclusions seen in some of the epidermis cells, and they have very little endoplasmic reticulum. Just outside the postganglion cells the usually cuboid cells of the dorsolateral epidermis are distinctly columnar, and their nuclei lie in a basal position. Because there is no basal membrane it is sometimes difficult to classify a particular cell as either postganglion or epidermis, except where the cell can be seen to extend out to the cuticle. The embryology of the postganglion cells is unknown, but because they are separated from the cuticle by ordinary epidermis it is likely that they are of non-epidermal origin. It is believed that they are included in the trichogon (see below) and develop into spermatozoa inside the receptacle of the externa.

Settlement of the male cyprid

When emerging on the ventral side of the abdomen of the host crab, the virgin externa is 2–3 mm wide, globular, and covered with a very smooth, 1–2 μm thick cuticle. Because there is no mantle aperture at this stage, the cuticle is continuous over the apical end of the externa. When the externa has grown to a width of 3–5 mm, the original cuticle begins to peel off in variably sized, irregular pieces, exposing the second externa cuticle (figure 13*a*, facing plate 4). The second externa cuticle is up to 20 μm thick and has a heavily sculptured surface (figures 16 and 17, plate 4). The mantle aperture has developed at the apical end as an irregularly shaped opening, and the second externa cuticle is continuous through the aperture with a smooth cuticle lining the mantle cavity (figure 13*b*).

At the time of cypris settlement a fairly large piece of the original cuticle always remains at the apical end of the externa and covers the aperture as a hood (figures 13 and 14, plate 4). Hence there is access to the aperture only beneath the free rim of this hood, where numerous passages between the hood and the second cuticle layer connect the aperture with the exterior (figure 13*b*).

Externae without a mantle aperture never carry attached male cyprids in the field, so the parasite apparently does not attract males at this early stage. In the laboratory, settlement of males occurred only on externae with a mantle aperture and with an externa width of 2.9–5.3 mm ($\bar{x} = 3.9$, $n = 30$). One externa measured 2.7 mm just after emergence in the laboratory. After 18 days it had attained a width of 4.2 mm and was invaded by males.

Upon contact with a suitable virgin externa the male cyprids initially walk around on their antennules until they reach the apical end. Here they crawl beneath the hood of unshed cuticle covering the mantle aperture (figures 13 and 14) apparently trying to penetrate as far as possible before attaching themselves permanently. In the final position the front end of the cyprid points towards the aperture, both antennules are outstretched, and the 3rd segments are attached close together to the externa by cement secretion. Some cyprids succeed in crawling completely beneath the hood, whereas in others the posterior half of the body still protudes beyond the rim of the hood (figure 15, plate 4). Usually the cyprid ends up on its side and may produce a bulge in the hood cuticle. The entire process of crawling beneath the hood and attaching by cementation usually lasts less than 20 min, including the time needed for the free-swimming cyprid to find the virgin externa in a 2 l experimental vessel. Delage (1884, figures 48–51) illustrated the settled males beautifully and correctly attributed the presence of the hood to an incomplete moult of the externa. However, Delage incorrectly stated that the mantle aperture is entirely blocked by the hood cuticle, although his figure 51 actually shows a narrow communication in front of a settled cyprid.

In my experiments, 120 cyprids settled around the mantle apertures of 30 externae (mean number per externa = 4.0, range 1–20). An additional 21 cyprids had settled elsewhere on the externae beneath other pieces of remaining original cuticle (figures 4 and 13*a*). These peripherally settled cyprids were also present on 3 out of 18 cyprid-carrying externae collected in the field. Such incorrectly settled cyprids do not metamorphose, and whereas the cyprids around the mantle aperture lose the naupliar eye in trichogon formation, the eye pigment remains visible in the peripherally settled cyprids for several days until they die and decay.

After settlement the male cyprid metamorphoses into a hitherto unrecognized stage, the trichogon. The trichogon enters the mantle cavity through one of the narrow passages around

the perimeter of the mantle aperture (figure 13*b*), travels through the cavity and enters a male cell receptacle (figures 13*a* and 27). The morphology of the free trichogon is discussed first so that the details of its formation can be more easily understood.

The morphology of the free trichogon

The trichogon is unsegmented and has no appendages (figures 18, plate 5, and 27). When travelling through the mantle cavity it is up to 220 μm long and has a vermiform shape, tapering towards the anterior end. When escaping from the cyprid, however, and when passing through the receptacle duct, the trichogon can change its shape almost like an amoeba or a slime mould (see below).

The entire trichogon is surrounded by a 15 nm thick and spiny epicuticle identical to that beneath the carapace of the unmetamorphosed male cyprid (figures 10 and 11, plate 2). In the trichogon the cuticle is often separated by a narrow space from the apical cell surface (figure 11). The distribution of spines in the trichogon reflects the spine fields of the cyprid. The large-diameter spines (up to 40 μm long) of the cyprid's dorsolateral field are easily recognized in the trichogon, extending from the posterior end and up along one of the sides (figure 18). The small spines of the cyprid's numerous lateral spine fields are unevenly distributed in bundles on the general surface of the trichogon. All spines on the trichogon point obliquely backwards with regard to the direction of migration. The name 'trichogon' was constructed as a parallel to the female 'kentrogon', and refers to the spiny or hairy surface (Greek: trichos = hair, gonos = larva).

Three, and probably four, cell types are recognized in the trichogon, namely: the dorsolateral and ventral epidermis, the inclusion cells, and the postganglion cells (figure 1*b*). Five to ten cells resemble the inclusion cells of the cyprid, except that they lack the cell extensions seen in the latter larva (figure 3). Usually they contain electron-dense granules, but as in the cyprid, some or all of the inclusions may be light staining. The inclusion cells are irregularly distributed, most lying in the posterior centre of the trichogon body (figure 18 and figure 22, plate 7).

A number of cells whose nuclei contain peripheral electron-dense material lie in one or two disc-shaped groups at the surface of the larva (figure 22). They contain numerous small, electron-dense granules, and epicuticular spines are never present above these cells. These characteristics show that they derive from the ventral epidermis of the cyprid (figures 4 and 5).

Most of the trichogon body consists of cells with a morphology similar to the dorsolateral epidermis of the cyprid; i.e. with large round nuclei having a single nucleolus (figures 3 and 22). These cells do not form as regular an epithelium as in the cyprid. Moreover, it seems that

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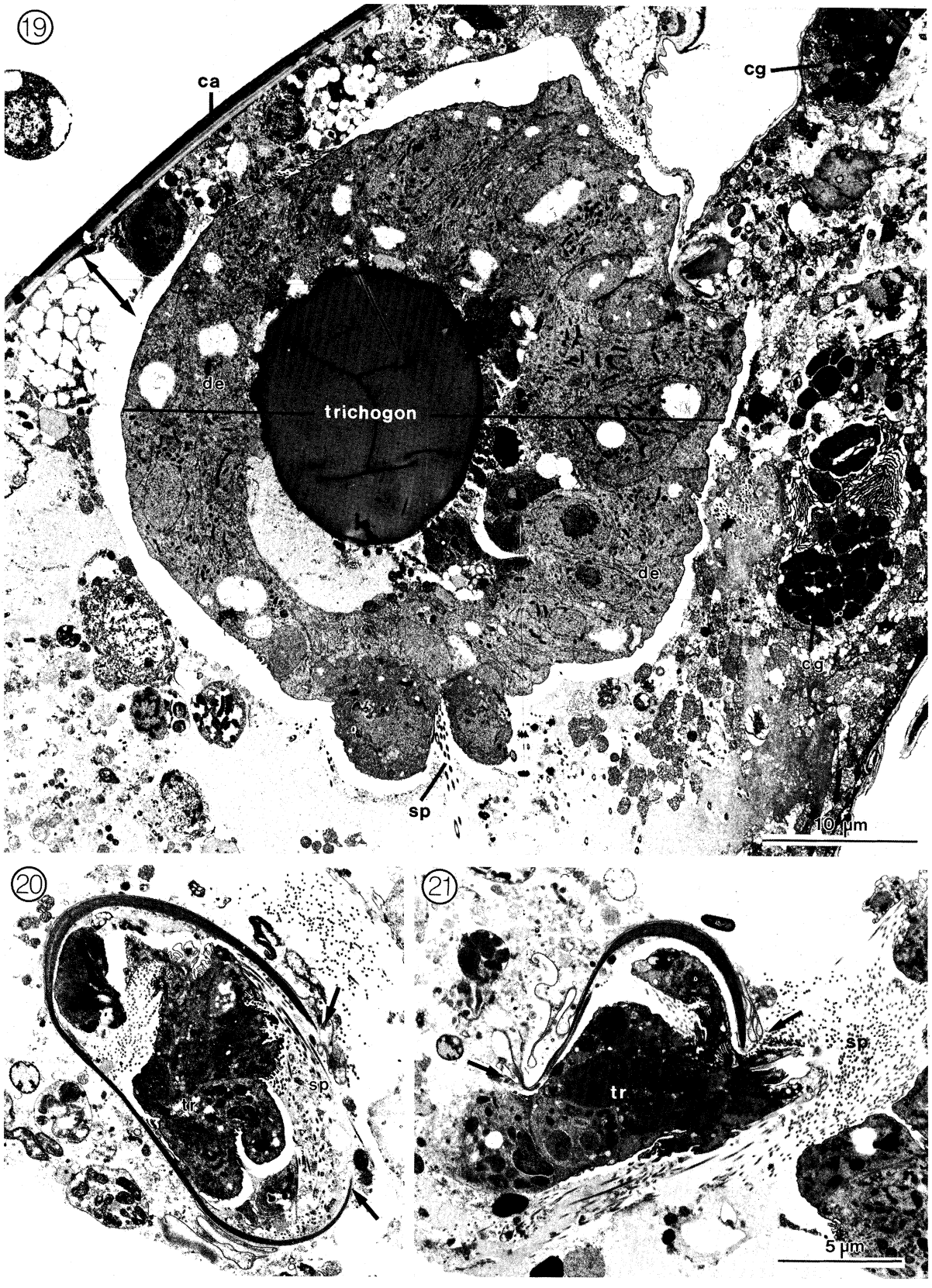
Description of plate 6

Figure 19. Cyprid less than 20 min after settlement, enclosing a completed trichogon. Cross section through the anterior end at a level indicated by the arrows in figure 1*b*. The cypris epidermis has withdrawn from the carapace (double-headed arrow) and closed as the trichogon sac; tissue outside the trichogon, e.g. the cement glands (cg), is disintegrating; the large vacuole in the trichogon is probably an artefact. TEM mosaic.

Figure 20. Antennule of a settled cyprid. Cross section through the 2nd segment of the specimen in figure 19. Trichogon cells (tr) are covered by the spiny epicuticle (sp) within the antennule, whose cuticle has split open (arrows). TEM.

Figure 21. More distal cross section of 2nd antennular segment in figure 20. The edges of the rift in the antennular cuticle are widely separated (arrows), and the trichogon is escaping from the antennule. TEM.



FIGURES 19–21. For description see opposite.

(*Facing p.* 54)

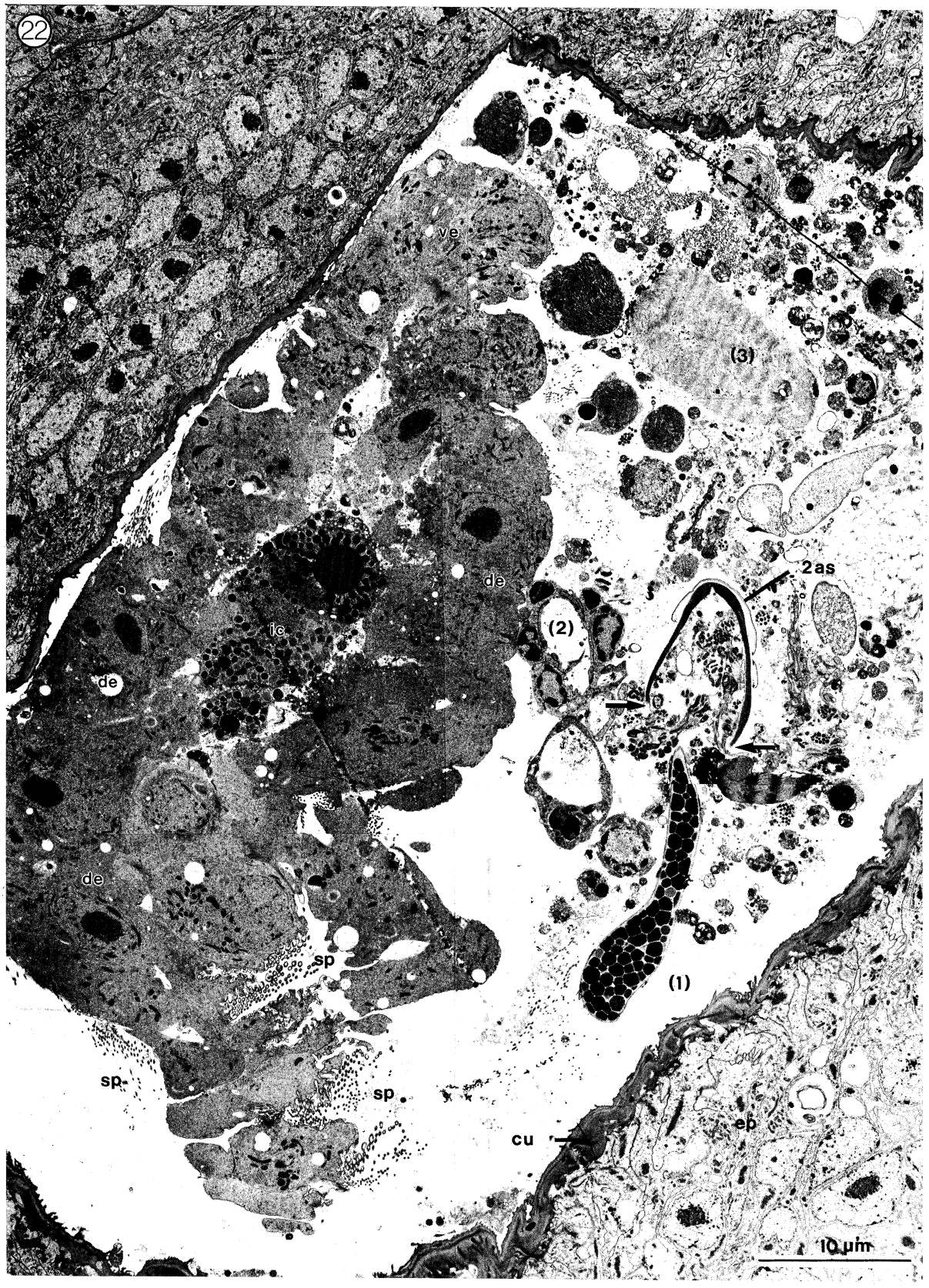


FIGURE 22. For description see opposite.

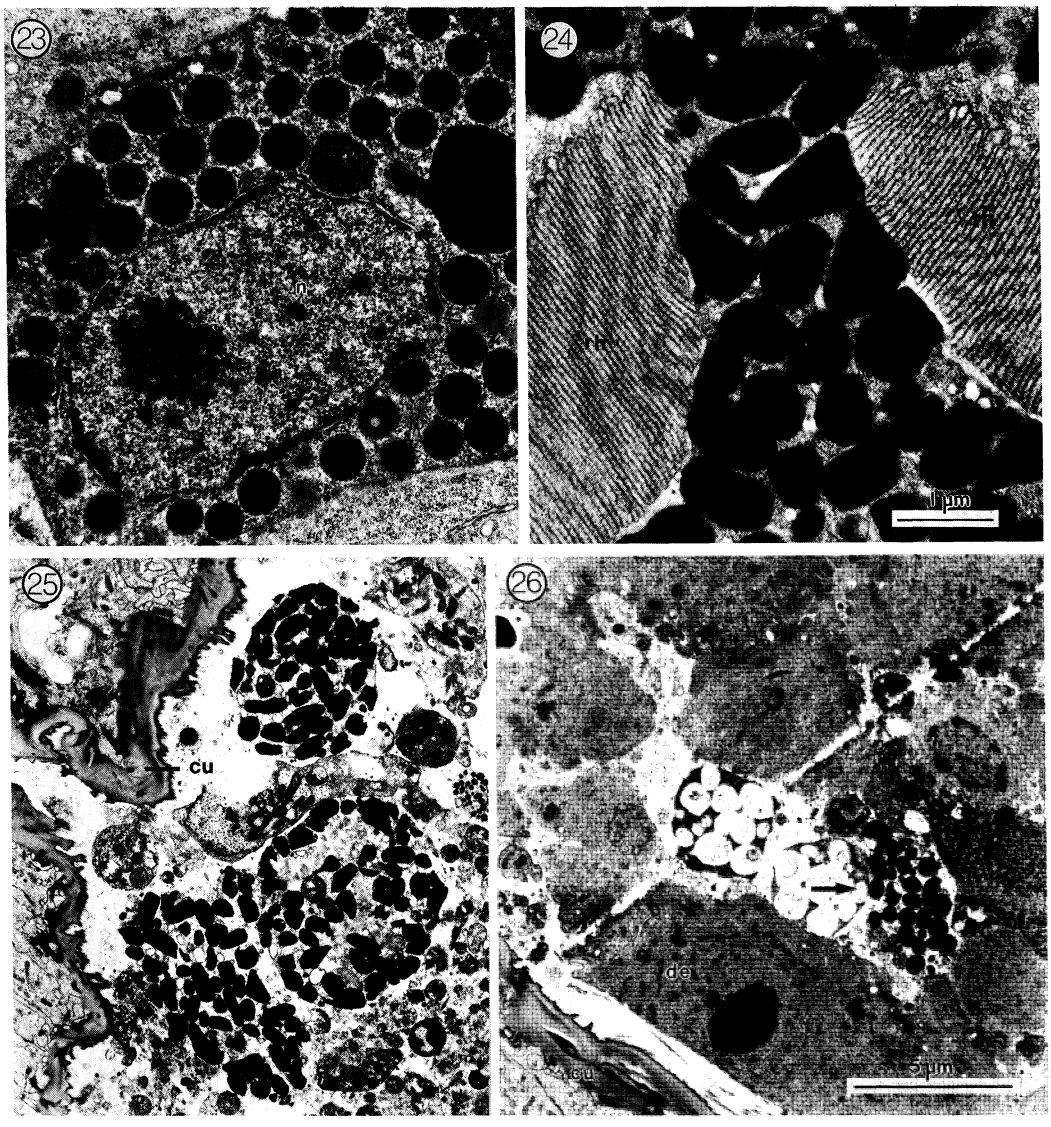


FIGURE 23. Inclusion cell of an implanted trichogon; large rectangle in figure 34. Scale as in figure 24. TEM.

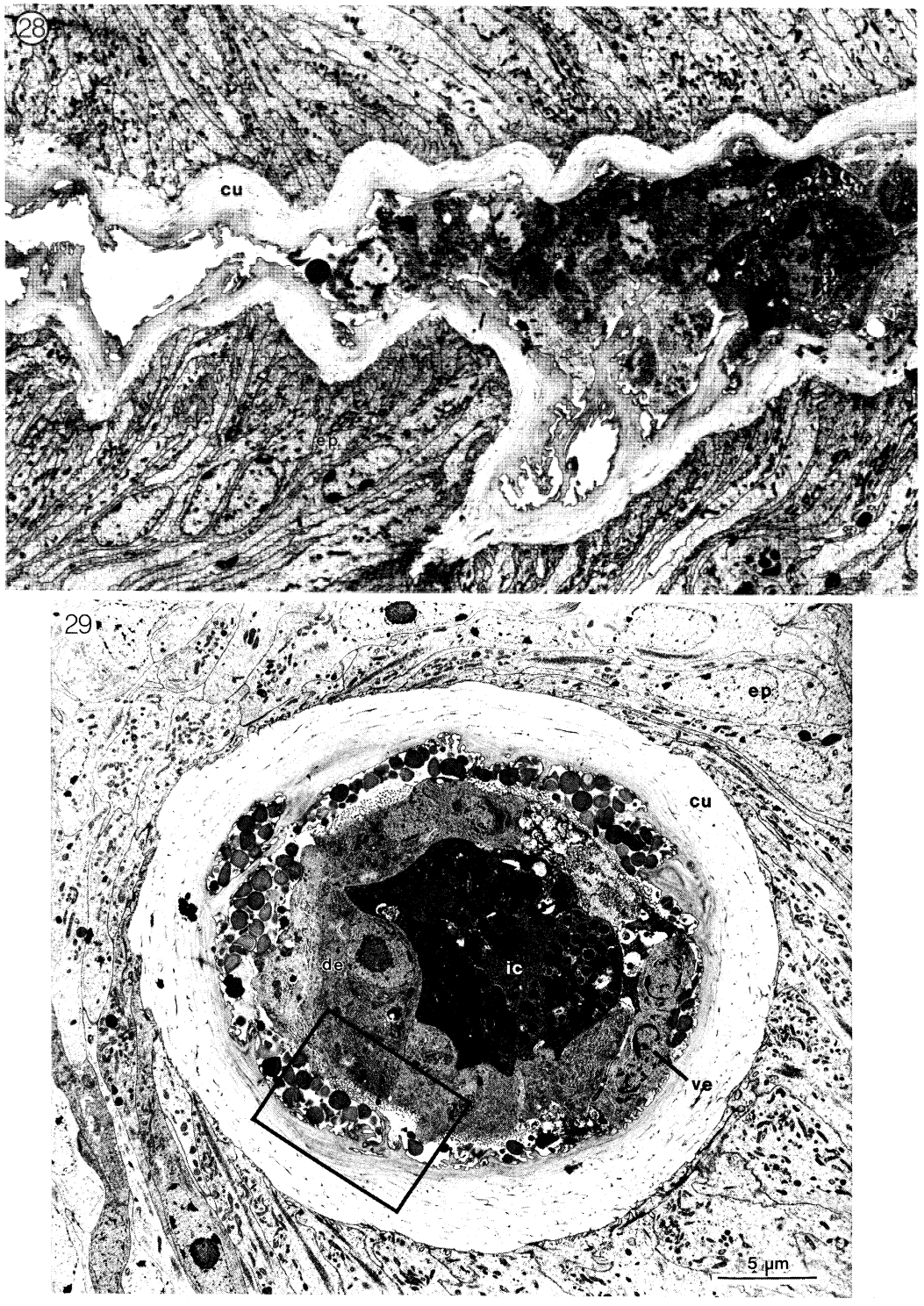
FIGURE 24. Cyprid, showing irregularly shaped pigment granules of the naupliar eye; (rh) rhabdome. TEM.

FIGURE 25. Naupliar eye pigment expelled into the mantle cavity of the externa by a metamorphosing cyprid or an early trichogon (see text); the cuticle of the mantle cavity is at left. Scale as in figure 26. TEM.

FIGURE 26. Naupliar eye pigment (arrow) lying free within a lacuna of an early trichogon; the lacuna communicates with the exterior at left; the cuticle of the mantle cavity is shown at lower left. The diagonal ridge is an artefact of sectioning. TEM.

DESCRIPTION OF PLATE 7

FIGURE 22. Early trichogon less than 20 min after cypris settlement, lying within the mantle aperture of the externa. At mid-right is shown the split 2nd segment (arrows) of the antennule through which the trichogon escaped (compare with figures 20 and 21); disintegrating cypris tissue, expelled through the antennule after trichogon escape, includes a large antennular gland (1), the epithelium of the muscular sac of the cement gland (2) and unidentified muscles (3); within the trichogon are seen dorsolateral epidermis (de), ventral epidermis (ve) and an inclusion cell (ic); the mantle cavity epithelium and cuticle are at top and lower right. TEM mosaic.



FIGURES 28 AND 29. For description see opposite.

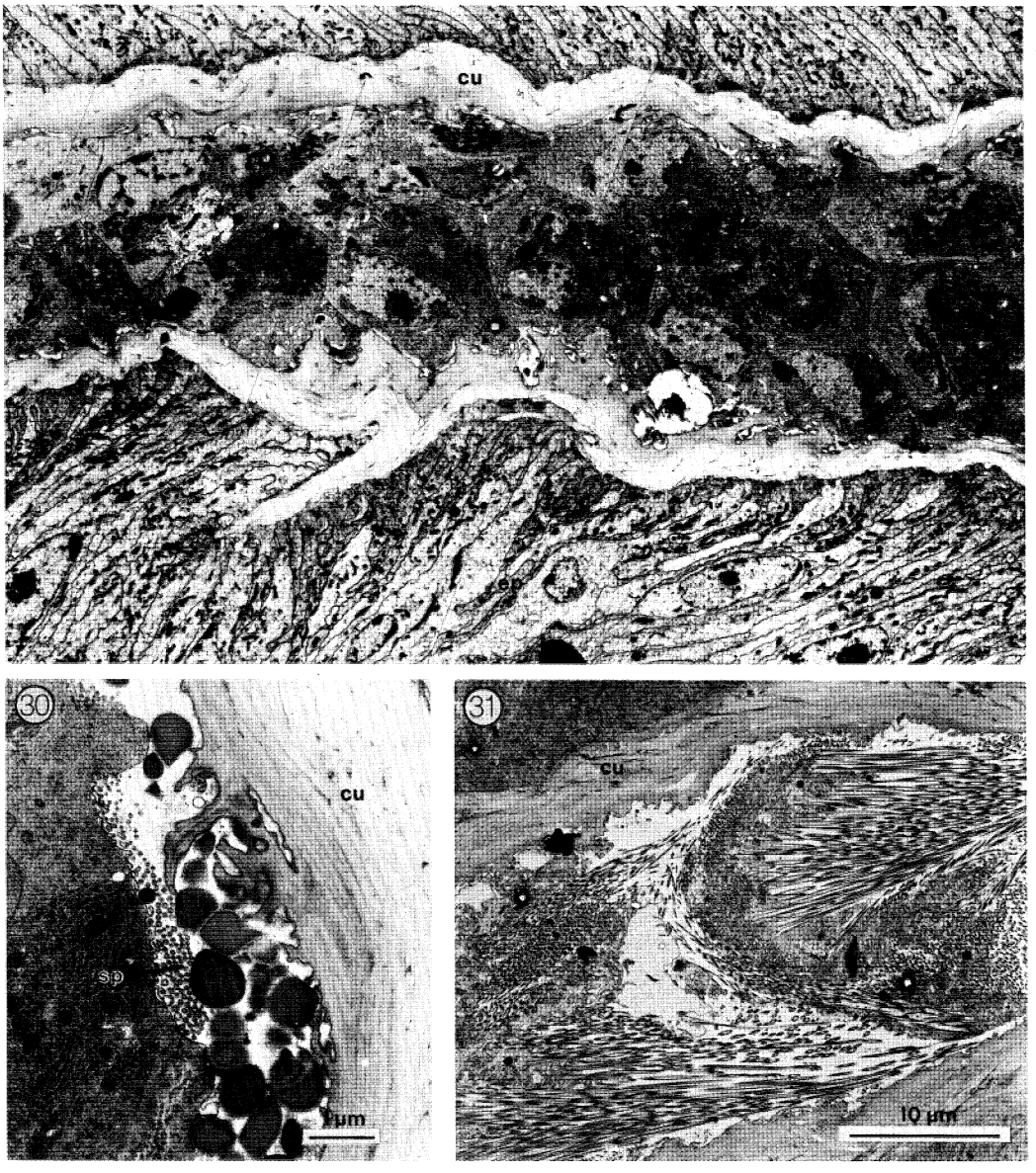


FIGURE 30. Rectangle in figure 29; spiny epicuticle still surrounds the trichogon in the distal part of the duct; granules between the trichogon and the duct cuticle may originate from the receptacle. TEM.

FIGURE 31. Epicuticle with spines shed by trichogon in the distal part of receptacle duct. TEM.

DESCRIPTION OF PLATE 9

FIGURE 28. Longitudinal section of a trichogon migrating (towards left) in the receptacle duct, *ca.* 1.5 h after cypris settlement; the part of the trichogon shown here has lost its epicuticle; the epithelium (ep) of the duct is highly columnar, with slender nuclei. Two page TEM mosaic. Scale as in figure 29.

FIGURE 29. Cross section of a trichogon in the distal part of the receptacle duct, still surrounded by the spiny epicuticle; inside the trichogon are seen the dorsolateral epidermis (de), ventral epidermis (ve) and inclusion cell (ic). TEM.

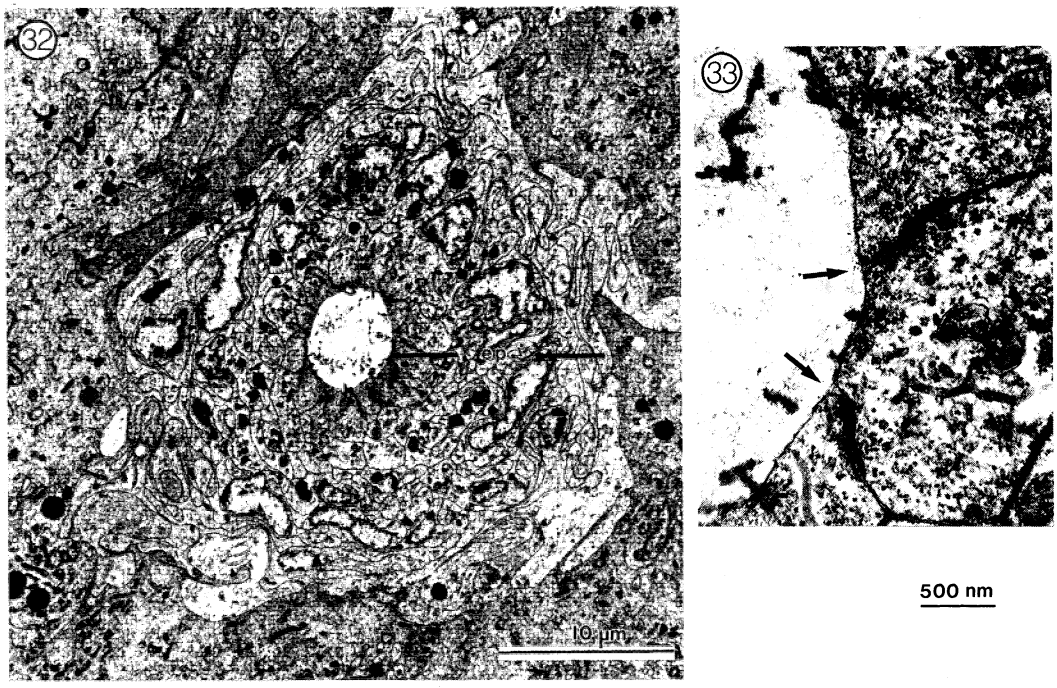


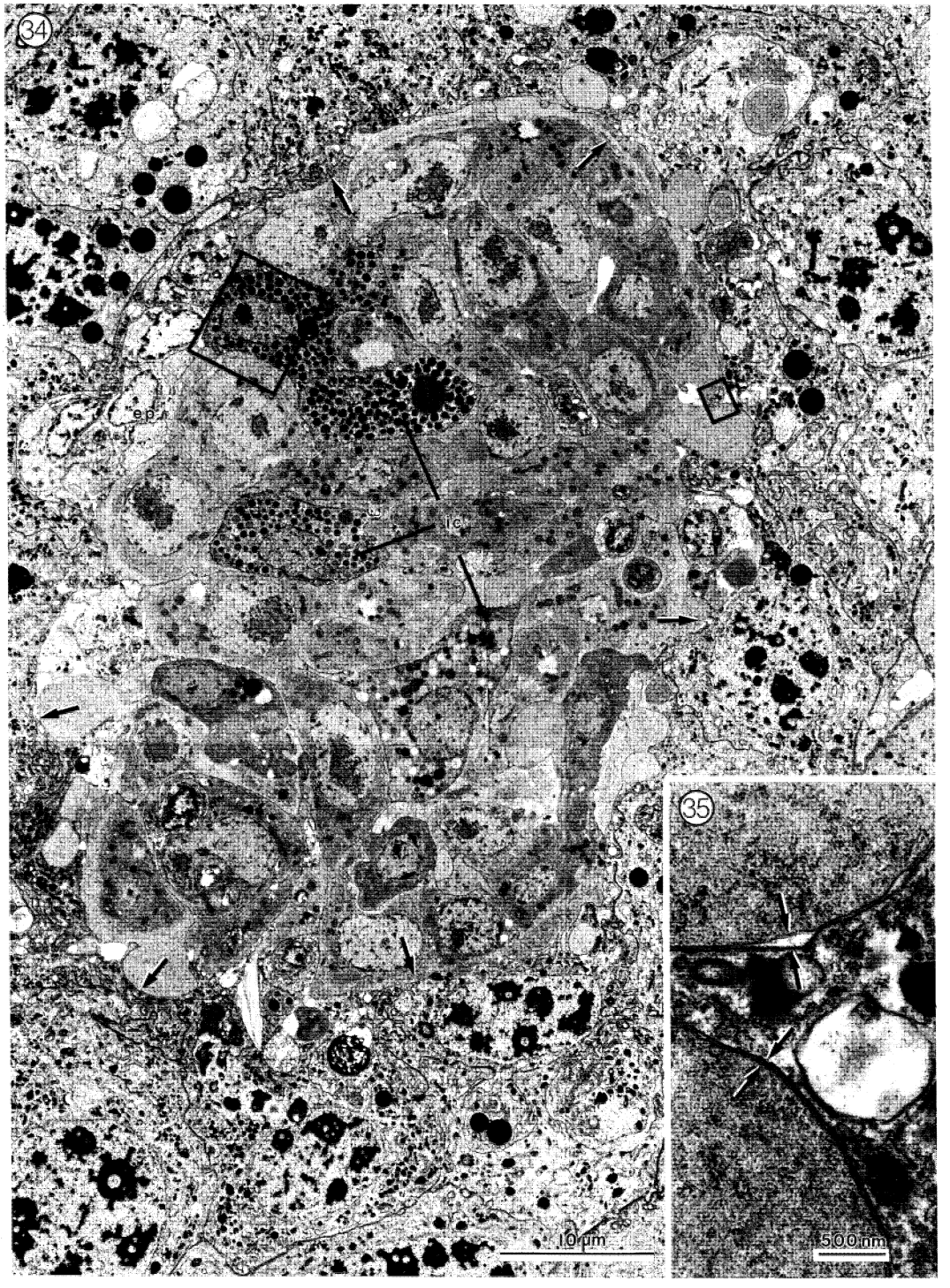
FIGURE 32. Cross section through the most proximal, cuticle-free end of the terminal canal; note the interdigitating epithelium with lobulate nuclei, compare with figure 34, which shows the same region after trichogon implantation. TEM.

FIGURE 33. Detail of figure 32. No cuticle covers the epithelium in the most proximal part of the terminal canal; the junction between epithelial cells is arrowed. TEM.

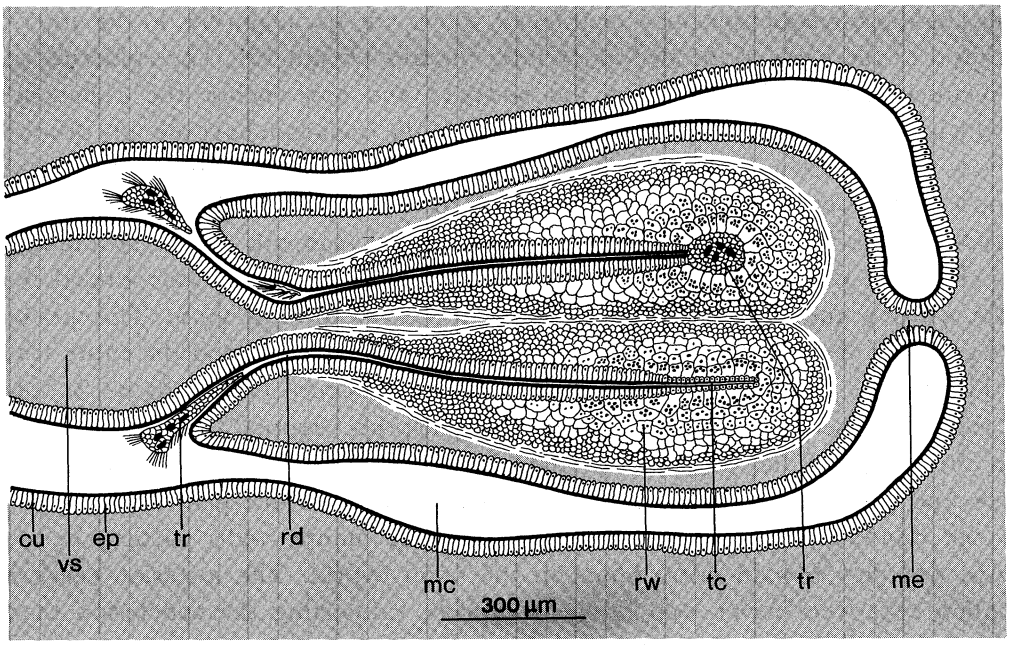
DESCRIPTION OF PLATE 12

FIGURE 34. The same region as in figure 32, after implantation of a trichogon in the receptacle, less than 15 h after cypris settlement. Arrows denote the distinct border between the small trichogon cells and the large vacuole-containing cells of the receptacle wall; displaced epithelium (ep) of the obliterated terminal canal is at upper left; several inclusion cells are denoted by ic (see figure 23); the remaining trichogon cells may include both dorsolateral epidermis and postganglion cells. TEM mosaic.

FIGURE 35. Small rectangle in figure 34. There is no cuticle at the border between trichogon cells (left) and cells of the receptacle wall (right). TEM.



FIGURES 34 AND 35. For description see opposite.



E 27. Diagrammatical cross section through the basal part of a juvenile externa, showing the two receptacles and the receptacle ducts (rd) leading into the mantle cavity (mc) on each side of the mesentery (me). One receptacle (bottom) is still virgin, because there is no trichogon in the terminal canal (tc), but a trichogon (tr) is just entering the duct. The receptacle at the top has already received a trichogon, which has obliterated the narrow terminal canal and lies in direct contact with the large cells of the receptacle wall (rw). The lost cuticle of the implanted trichogon lies in the distal part of the receptacle duct and prevents the later-arriving trichogon from entering the receptacle.

most of the trichogon cells must be considerably shuffled around during the form changes associated with escape from the cyprid and ensuing migration (see below). Some cells in the centre of the trichogon resemble the dorsolateral epidermis, but because they are separated from the cuticle by other cells and never contain electron-dense granules in the cytoplasm, they more likely derive from the postganglion cells of the cyprid. In the cyprid the close connection between the postganglion cells and the epidermis supports the conclusion that the former are also included in the trichogon (figure 12).

The unicellular epidermal glands, muscles, and nerve cells are not present in the trichogon.

The metamorphosis of the cyprid into the trichogon

Among a large number of cyprids with post-settlement ages less than 20 min, about equal numbers were either empty carapaces from which the trichogon had already escaped or cyprids that had not yet begun trichogon formation. None of the cyprids were at an intermediate stage of trichogon formation, but a single settled cyprid contained a fully formed trichogon within the cypris carapace (figure 1*b* and figure 19, plate 6). These results show that the formation of the trichogon does not begin until after settlement, is very fast, and that the trichogon has quite often escaped within a few minutes after cypris settlement. The actual escape was observed alive in one cyprid (less than 20 min after settlement). This cyprid looked empty except for a small red-brown body, the trichogon, in the anterior end of the body. Suddenly, within a couple of seconds, the trichogon escaped from the cyprid into the mantle aperture and disappeared from view.

In the single pre-release specimen mentioned above, the trichogon lies in the anterior end of the cyprid at the bases of the two antennules (figures 1*b* and 19). The epidermis and the spiny epicuticle have withdrawn from the carapace and now enclose the trichogon body, within which inclusion cells are seen. All tissues outside the trichogon have already disintegrated to a degree almost beyond recognition, but the cement glands can still be identified. The trichogon is in the act of escaping through one of the cypris antennules, since a slender part of the trichogon lies within the first and second antennular segments (figure 1*b* and figure 20, plate 6). Like the remainder of the trichogon, this body extension is surrounded by spiny epicuticle, which is absent from the antennules of the free-swimming cyprid. The cuticle of the second segment of the antennule concerned has split longitudinally, and the entire trichogon escapes like a slime mould through the very narrow rift (figure 1*b* and figure 21, plate 6). Similar rifts are present in the antennules of empty cyprids (figure 22).

When the trichogon has escaped, the dorsolateral and the ventral epidermis, the spiny epicuticle, and the pigment of the nauplius eye are absent from the spent cyprid (figure 1*b*). Neither the thorax nor the epidermis of the posterior mantle walls participate in metamorphosis, but they remain fairly intact in the spent cyprid. The inclusion cells and the postganglion cells were never seen in spent cyprids, supporting the conclusion that these elements are included in the trichogon.

TABLE 1. METAMORPHOSIS IN MALES OF *SACCULINA CARCINI*

time after cyprid settlement	events in metamorphosis
0 min	settlement of a male cyprid at the mantle aperture of a virgin externa
10 min	closure of spiny epicuticle and anterior epidermis into the trichogon incorporating the inclusion cells and (?) the postganglion cells
20 min	escape of the trichogon through one of the cypris antennules
20–25 min	expulsion of tissue fragments from the spent cyprid; expulsion of naupliar eye pigment from the free trichogon or the spent cyprid
20–40 min	migration of the trichogon through the mantle cavity
40 min	penetration of the trichogon into the receptacle duct
40+min	shedding of the spiny epicuticle in the distal part of the receptacle duct
ca. 10 h	arrival of the trichogon into the terminal canal of the receptacle
ca. 5+days	spermiogenesis proceeds in the implanted trichogon

Besides the trichogon itself, a considerable amount of tissue is ejected pell-mell from the cyprid during metamorphosis. Inside the mantle aperture near the antennule of an empty cyprid were seen the pigment granules of the naupliar eye (figure 25, plate 8), the muscular sac of the cement gland, a large unicellular antennular gland, pieces of muscle and their carapace attachments, and various unidentifiable tissue (figure 22).

Recently escaped trichogons were found both beneath the cuticular hood and within the mantle aperture itself, close to the cyprids whence they originated. These early trichogons have a more irregular shape than the vermiform larvae travelling through the mantle cavity (figure 22). One early trichogon within the mantle aperture contained a number of very electron-dense granules, situated in a lacuna of the body that communicated with the exterior (figure 26, plate 8). These granules were eye pigment, and, unlike the inclusion cells which were also present, they were not enclosed within a cell. However, later stages of the trichogon definitely do not contain eye pigment but only the inclusion cells (figures 18, 23, 24 and

figure 34, plate 12). Because, moreover, eye pigment could be seen lying free within the mantle aperture (figure 25), it appears that the pigment at least sometimes escapes within the trichogon but is quickly expelled from it. There is hardly time to break it down inside the trichogon within the short life span of this larva.

The penetration of the trichogon into the receptacle duct

The trichogons arrive at the entrance to the receptacle ducts less than 2 h after cypris settlement, and one larva had even travelled the 2–4 mm through the mantle cavity within 40 min of cypris settlement. The trichogon does not follow a special path on its migration through the mantle cavity. On live externae, trichogons could be detected through the transparent mantle as reddish bodies. They were seen to be shuffled rapidly around within the mantle cavity by peristalsis of the mantle musculature to an extent that would seemingly obliterate any movements by the trichogons themselves. Isolated trichogons inspected alive in the light microscope showed no movements of their own and did not react if fixative was added. On sections, no secretion products gluing the trichogon to the cuticle of the mantle cavity were seen.

The two, up to 1 mm long, sac-like receptacles lie adjacent in the basal part of the visceral sac near the stalk (figure 13*a*). Distally, each receptacle tapers into a narrow duct that opens into the mantle cavity, one on each side of the mesentery (figure 27). The 25 μm high, columnar duct epithelium is continuous with the epithelium of the general mantle cavity. The duct is lined with a *ca.* 3 μm thick cuticle, whose surface is irregularly sculptured (figure 18 and figure 30, plate 10). The lumen of the duct is no more than 1 μm wide and in places it has almost collapsed. The ducts are surrounded by muscle layers.

Two or more trichogons could often be seen at the entrance to the same duct (figure 27). While entering the duct, the bulk of the trichogon body lies in the wider funnel-shaped entrance, while a single, very slender body extension protudes far into the duct proper (figures 18 and 27). The cells of this extension are often compressed into narrow shapes. In the distal part of the duct the trichogon cells remain enclosed by the epicuticle, whose spines point backwards towards the duct entrance (figures 29, plate 9, and 30). The trichogon body farther into the duct has lost the epicuticle and here the trichogon cells lie almost single file in the narrow tube (figure 28, plate 9). The anteriormost cells in the duct resemble the ventral epidermis cells (figure 28, left part).

When the entire trichogon has entered the duct it is extremely vermiform and without a cuticle. The spiny epicuticle (and a few cells) has been sloughed off. It was probably torn apart by the rough duct-cuticle, and now lies as a disordered mass in the distal part of the duct (figure 27 and figure 31, plate 10).

The arrival of the trichogon into the receptacle

Migration through the receptacle duct and shedding of the spiny epicuticle is probably slow, because trichogon cells were not seen inside the receptacle until *ca.* 10 h after cypris settlement. However, only a few specimens were examined in the 2–10 h time range.

The trichogon cells eventually reach the proximal blind end of the receptacle duct. The final *ca.* 100 μm of the duct, the terminal canal, has a distinct morphology. The cuticle is only

1 μm, and the epithelium only 10 μm, thick. The epithelium is cuboid with oval nuclei, as opposed to the high and columnar epithelium with slender nuclei in the rest of the canal (figure 27). The final few micrometres of the terminal canal have no cuticle at all, and here the epithelium cells interdigitate and have lobulate nuclei (figures 32 and 33, plate 11).

When the vermiform trichogon reaches the cuticle-free part of the terminal canal it contracts into an ovoid shape 40–60 μm across (figures 27 and 34). This disrupts the 5 μm wide terminal canal, and its epithelium comes to lie as isolated patches of cells at the periphery of the trichogon cells (figure 34). The cells of the receptacle wall immediately beyond the disrupted canal epithelium are large and ovoid, 20–50 μm across. They have many densely staining vacuoles in the cytoplasm and densely staining bodies in their large, irregularly shaped nuclei (figure 34). In most places the trichogon cells lie directly appressed to these large cells, and the border between the male and female cells is very distinct (figure 34, arrows). No cuticle separates the male (trichogon) and the female (receptacle) cells (figure 35, plate 12).

The trichogon cells in the receptacle are tightly packed with few intercellular spaces. The majority of them are of the dorsolateral epidermis and the postganglion cell type, but the inclusion cells are also present and as a rule lie more or less in the centre (figure 34). Although the ventral epidermis cells were seen in the duct they could not be identified with certainty inside the receptacle. It is possible that they are torn apart during passage of the duct, where they lie anteriormost in the vermiform larva. Only a single trichogon enters the centre of each receptacle. In many externae additional trichogons were seen at the duct openings or within the distal duct part of receptacles, which already contained implanted trichogon cells (figure 27). However, such late-arriving larvae are effectively blocked from entering further by the 'corking effect' of the lost epicuticle of the first-arriving trichogon.

After implantation, the trichogon cells begin to rearrange themselves, and after 5–6 days spermiogenesis begins as evidenced by an increasing number of mitoses in cells looking like dorsolateral epidermis or postganglion cells. The morphological similarity between these two cell types makes it difficult to ascertain which of them develops into spermatozoa. It is most likely, however, that only the postganglion cells are actual germ cells. If this is true they must be regarded as a primordial testes in the unmetamorphosed cyprid. The inclusion cells do not seem to take part in spermiogenesis. A detailed study of the implanted trichogon, its spermiogenesis, and interaction with the female externa is now in progress.

Discussion

It is now evident that the material implanted by male *Sacculina carcini* cyprids into the virgin externa is a true instar, the trichogon, because it consists of several cell types including epidermis and is surrounded by a cuticle. The trichogon is the only truly 'amoeboid' larva described from the Crustacea. It was perhaps already illustrated in *Drepanorchis neglecta* by Smith (1906, plate 6), which shows a mass of 'embryonic cells' in the front end of a settled cyprid in the process of escaping through an antennule. Although Smith erroneously stated that the cells were not enveloped by cuticle and had no function, his plate 6, figure 12 actually shows a tight cluster of spines at the tip of the cells in the antennule, which could well be the trichogon spines described here. In a brief note Veillet (1985) described male cypris metamorphosis in three rhizocephalans including *S. carcini*, and his results are in general agreement with the present paper. Veillet, however, does not furnish any micrographs or

detailed illustrations, and his very rough sketch of the *S. carcini* trichogon is wanting in showing a uniform coverage of small spines with no signs of spine fields or distinction between large and small spines.

(*a*) *The formation and structure of the trichogon*

Although the trichogon contains no muscles, comparison with metamorphosis in females nevertheless makes it likely that muscles are responsible for its formation. The trichogon is formed after cypris settlement by a rather sudden closure of the anterior epidermis and spiny epicuticle into a sac. In female cyprids the similar closure of epidermis into the kentrogon body is effected by contraction of the ex- and intrinsic antennular muscles (Høeg 1985*a*). Most, but not all, of these muscles are excluded from the kentrogon during the process, whereas in trichogon formation all the involved muscles are seemingly excluded. Another mechanism in trichogon formation could be contractile filaments (actin–myosin) within ordinary epidermis cells. Such filaments are known to be active in metamorphosis in some invertebrate larvae (Cloney 1978) but were not identified in the present study.

Naupliar eye pigment is not included or is at least very quickly eliminated from the trichogon. Durant & Veillet (1972) and Veillet (1985) incorrectly stated that the eye pigment enters the receptacle of *S. carcini*. Most probably they saw the eye pigment disappear from the cyprid and, by using only the light microscope, confused it with the somewhat similar looking inclusion cells, which do enter the receptacle.

The formation of the trichogon must be regarded as a moult, because the cypris epidermis separates from the carapace and secretes a new cuticle. The cyprid–trichogon moult, however, is very specialized because: (1) the new cuticle consists only of an epicuticular layer; (2) the endocuticle of the old layer (the carapace) is not reabsorbed; (3) the moult involves only the anterior part of the body; and (4) most non-epidermal elements are excluded from the new instar (the trichogon). In all these respects trichogon formation and kentrogon formation are similar (Delage 1884; Høeg 1985*a*). The immediate decay of cypris tissues not included in the trichogon is also similar to kentrogon formation. The trichogon needs to reabsorb neither the carapace endocuticle nor the lost tissues, because its lifetime as a free larva is short (1–2 h), and once inside the receptacle it is probably nourished by the female cells.

The electron-dense granules seen in the cypris epidermis could possibly be epicuticular material. If this is the case, however, secretory activity has ceased before settlement, and no cuticle is secreted by the free trichogon.

The swift formation of the trichogon is only possible because the spiny epicuticle is preformed in the free-swimming cyprid. The rapid metamorphosis and migration through the mantle cavity may have evolved through competition between individual males, because only the first-arriving trichogon at any receptacle will take part in reproduction.

Because the epidermis of the trichogon seems somewhat loosely arranged, one function of the delicate epicuticle must be to hold the larva together as it makes its drastic changes of shape during escape and migration. The cuticular spines most probably play an important role during migration. They may prevent the larva from being flushed backwards in the mantle cavity by peristalsis, thus effecting a net motion towards the base of the externa where the openings of the receptacles are situated (figure 13*a*). The absence of muscles or contractile epidermal filaments within the trichogon itself makes it less likely that it is self-mobile. Because trichogons were in fact observed to be shuffled around by mantle peristalsis, it is possible that the entire 'migration' of the trichogon is effected by the mantle musculature and by the muscles

of the receptacle duct. This, however, does not explain how the trichogon 'locates' the small entrance to the receptacle duct in the absence of apparent sense organs or self-mobility.

(*b*) *The implanted trichogon*

The receptacle of *S. carcini* is a specialized tube-like invagination of the general mantle cavity epithelium, which is surrounded by the subepidermal cells of the receptacle wall. Durant & Veillet (1972) described the juvenile *S. carcini* receptacle by using paraffin sections, and their results agree with the present study except for failing to identify the special morphology of the terminal canal. Ichikawa & Yanagimachi (1960), however, described an almost solid receptacle with no central canal in juvenile *S. senta* externae. They maintained that the male cypris cells entered by dissolving the central female cells. A somewhat similar mechanism was described by Høeg (1982) in *Clistosaccus paguri*.

Because the entire trichogon, including some male somatic cells (epidermis, inclusion cells), is implanted, the receptacle houses not just male gonia or a male gonad, but an entire, highly reduced, male organism. I therefore consider the implanted trichogon as an extremely reduced dwarf male. Dwarf or complemental males are known from many cirripedes and are interestingly discussed by Klepal (1985). Nowhere, however, is the male and its final residence site so specialized as in kentrogonid rhizocephalans. The only parallel within the Crustacea seems to be the Copepod family Xenocoelomidae, whose life cycle shows several similarities to the Rhizocephala (Bresciani & Lützen 1974). In *S. carcini* an externa will mature and reproduce even if only one receptacle receives a trichogon. Normally, however, an externa houses two different males, and this could have important consequences for the fertilization and resulting sex frequency of the broods (see Høeg 1984; Walker 1985).

In the preceding text the trichogon has been called a larva. It is perhaps an academic question whether the trichogon instar ceases with the shedding of the spiny epicuticle or whether it continues to exist inside the receptacle itself. The two stages are fairly continuous and not separated by a metamorphosis. Hence it is perhaps more appropriate to characterize the free trichogon as a juvenile male rather than a larva, because the implanted stage is considered as a dwarf male (i.e. a reduced adult). Moreover, in other cirripedes, stages following cypris metamorphosis are generally considered to be juveniles rather than larvae.

(*c*) *Comparison of the trichogon and the kentrogon*

The male trichogon is mobile and short-lived (a few hours), lacks appendages, and secretes no cuticle after its escape. The female kentrogon is stationary, longer-lived (a few days), retains the cypris antennules, and secretes additional protective cuticle (table 2). The kentrogon has a more complex structure than the trichogon because it includes several muscles and parts of the cement glands (Høeg 1985*a*). In addition, the kentrogon pierces the host cuticle by an injection stylet, whereas the trichogon does not penetrate any cuticle layers on its way to the receptacle. The only rhizocephalan in which penetration has been shown to occur in males is *Clistosaccus paguri*, although a similar mechanism will probably be found in other aberrant rhizocephalans (Høeg 1985*b*). Despite these differences it is possible on several grounds to regard the trichogon and the kentrogon as homologous instars: (1) their formation from the cyprid is quite similar, being largely an incomplete moult, in which the anterior epidermis closes around other elements; (2) both are cuticle-covered stages, which are intercalated between the cypris metamorphosis and the stages devoid of cuticle: in males being the cells

TABLE 2. COMPARISON OF THE TRICHOGON AND THE KENTROGON

trichogon	kentrogon
instar following male cyprid	instar following female cyprid
formed by incomplete moult of anterior cypris epidermis	formed by incomplete moult of anterior cypris epidermis
mobile, and of variable shape	stationary
unsegmented	unsegmented
no appendages	antennules
enclosed by epicuticle with spines	enclosed by smooth cuticle
no cuticle secretion after liberation from cyprid	secretes protective cuticle after liberation from cyprid
no muscles	includes antennular muscles
no glands	includes parts of cypris cement gland
no sense organs	no sense organs
no penetration of cuticles	penetrates host cuticle with stylet
implanted in receptacle as sperm producing dwarf male devoid of cuticle	infects host by injection of one or several cells devoid of cuticle

inside the receptacle, in females the cell (or cells) injected into the host by the kentrogon (Høeg 1985*a*).

(*d*) *Comparison with males in other rhizocephalans*

Male cyprids of *Lernaeodiscus porcellanae* (Lernaeodiscidae) have an epicuticle with spinefields below the carapace, and after settlement they transform into a spine-covered trichogon that escapes through an antennule just as in *S. carcini* (J. T. Høeg, unpublished observations). I have not studied male cypris settlement in species of the Peltogastridae, but for comparison some large and presumably male cyprids of *Peltogaster paguri* were sectioned (Høeg & Lützen 1985). Like *S. carcini* and *L. porcellanae* these cyprids had an epicuticle with spines below the carapace, strongly indicating that a trichogon is formed after settlement. The uncharacterized clumps of male cypris cells depicted in paraffin sections from the mantle cavities of *Peltogaster paguri*, *Peltogasterella gracilis* and *Sacculina senta* by Ichikawa & Yanagimachi (1958, 1960) are most probably trichogons. The melanin granules among the male-cypris cells of *P. gracilis* could well correspond to the inclusion cells of the *S. carcini* trichogon.

In conclusion, a trichogon has now been observed, or its presence strongly indicated, in representatives of all three families of the Kentrogonida (*sensu stricto*), namely the Peltogastridae (*Peltogaster paguri*). the Lernaeodiscidae (*Lernaeodiscus porcellanae*) and the Sacculinidae (*S. carcini*). The presence of a trichogon in the very same families, where a kentrogon has been demonstrated to accomplish invasion of the host, indirectly supports the suggestion that the two types of larvae are homologous. In two peltogastrids (*Peltogasterella sulcata* and *P. gracilis*), Veillet (1985) observed that male cyprids metamorphose into a larva largely comparable to the *S. carcini* trichogon, except for lacking cuticular spines. Because however, a spine-covered trichogon is present in another peltogastrid (*Peltogaster paguri*), the absence of spines in the *Peltogasterella* 'trichogon' is probably an advanced (apomorphic) trait, whereas a spine-covered trichogon is primitive (plesiomorphic) in the Kentrogonida.

It seems that the trichogons always escape from the male cyprid through one of the antennules. Escape through the antennules is reported from *Peltogaster* (Reinhard 1942), *Peltogasterella* (Ichikawa & Yanagimachi 1958), *Lernaeodiscus* (J. T. Høeg unpublished results) and *Sacculina* (Veillet 1985; this paper). Veillet (1960) claimed that the 'male cyprid cells' escape between the antennules in *Septosaccus*, but recent observations by J. Lützen (personal communication) demonstrate antennular escape in this genus also. In contrast, the kentrogon

uses the 2nd and 3rd antennular segments for attachment to the host, and it always escapes from the female cyprid by rupturing the thin ventral cuticle between the antennules (Høeg 1985*a*).

Nothing comparable to the kentrogon or the trichogon is known from any other cirripedes, nor indeed from any other crustaceans. However, cryptogonochorism (the presence of extremely reduced dwarf males residing within special receptacles of females) is also known from the copepod family Xenocoelomidae (Bresciani & Lützen 1974). In *Aphanodomus* the exuvium of a male copepodite stage is left behind in the atrium of the female, whereas the male larva is later found in the receptaculum masculinum as an extremely simple, unsegmented stage without appendages. The undescribed migration from atrium to receptaculum in *Aphanodomus* could well turn out to be accomplished by a larva analogous to the rhizocephalan trichogon.

A kentrogon is maintained to be absent in some aberrant rhizocephalans, the Akentrogonida. These comprise the barnacle-infesting Chthamalophilidae, and a few other genera of uncertain position (Bocquet-Vedrine 1961, Bocquet-Vedrine & Bourdon 1984). Moreover, the Chthamalophilidae were originally described as self-fertilizing hermaphrodites without males (and hence without a trichogon). However, the absence of a kentrogon in the Akentrogonida, although highly probable, still rests on indirect evidence, because cypris settlement and host infection has never been observed in any of the species.

The alleged absence of a kentrogon and some other important traits have been used as arguments that the Akentrogonida are the most primitive of extant rhizocephalans. A detailed study of the evolution of the kentrogon and the trichogon, and of cypris settlement and metamorphosis in the Akentrogonida, is therefore of the utmost importance for any comprehensive treatment on evolution and phylogeny of the Rhizocephala.

The financial support of the Carlsberg Foundation, the University of Copenhagen and the Danish Natural Science Research Council (grant number 81-3408) is gratefully acknowledged. I am much indebted to the Director and staff of the Station Biologique de Roscoff, France for their hospitality and assistance. Mrs E. Schiøtt Hansen and Mr J. Fuglsang assisted with great skill in TEM and SEM preparations, and Mrs B. Beyerholm and Miss R. Bjørklund are acknowledged for drafting the artwork. The Institute of Historical Geology and Paleontology (University of Copenhagen) kindly provided SEM facilities. Dr M. Grygier (the Smithsonian Institution, U.S.A.), Dr G. Walker (Marine Science Laboratories, U.K.) and Professor D. T. Anderson, F.R.S. (University of Sydney, Australia) improved upon previous versions of the manuscript and should receive my warmest thanks. I am likewise indebted to Dr P. Duckert, Assistant Professor J. Lützen, Dr J. Mossin, Dr B. Westergaard, and to my wife Dr M. Holst-Knudsen for their interest, help and encouragement throughout this study.

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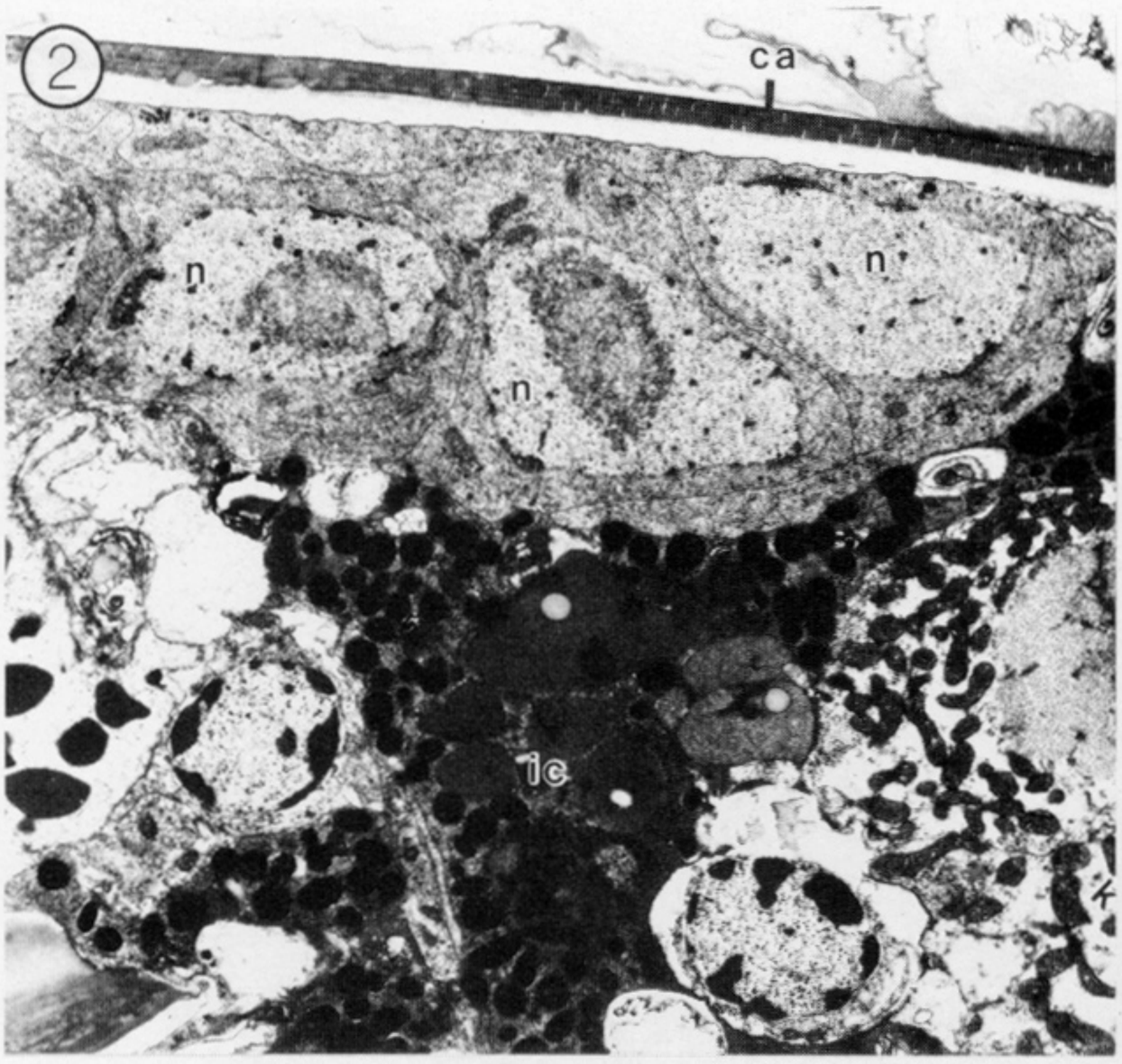
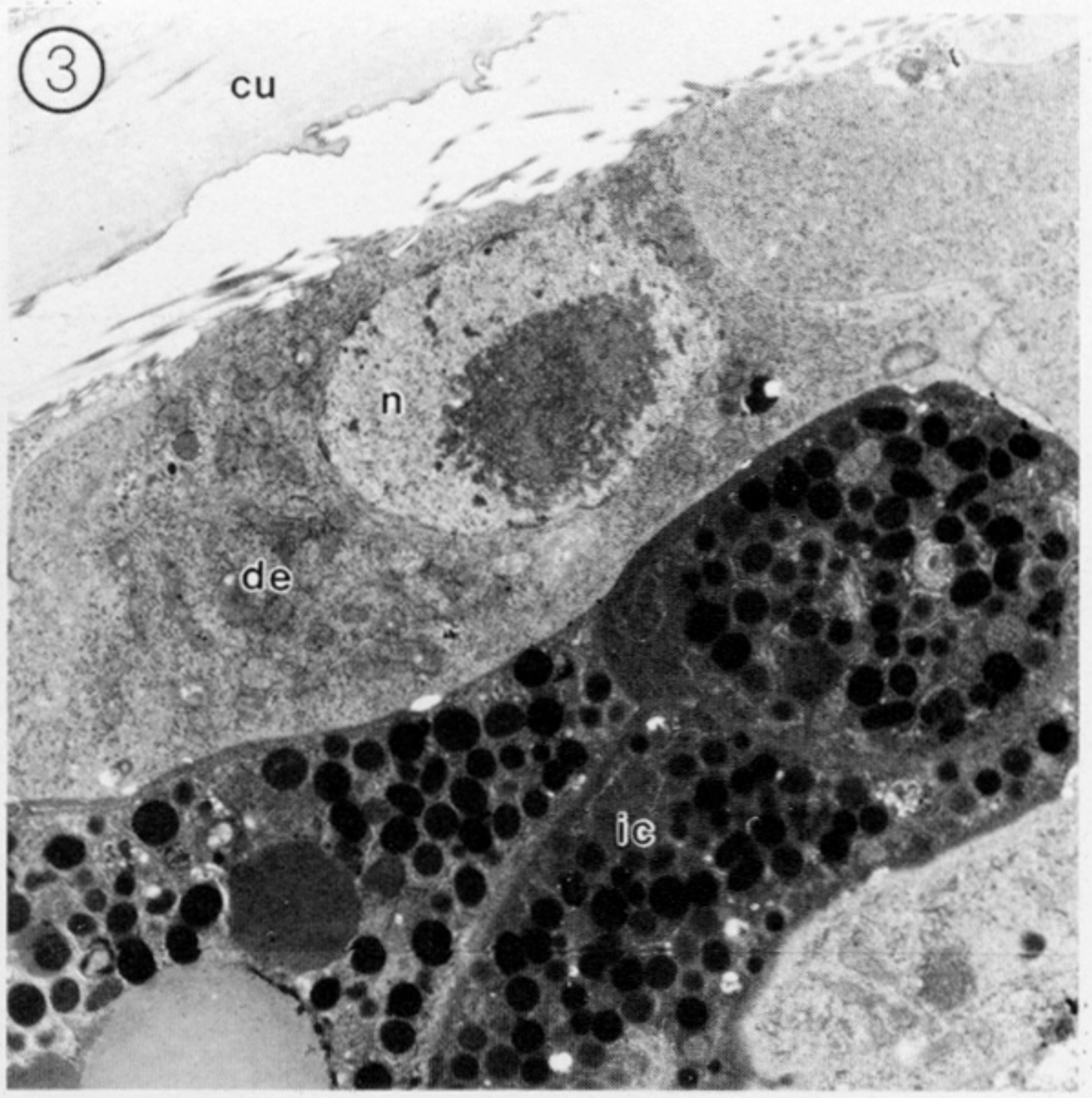
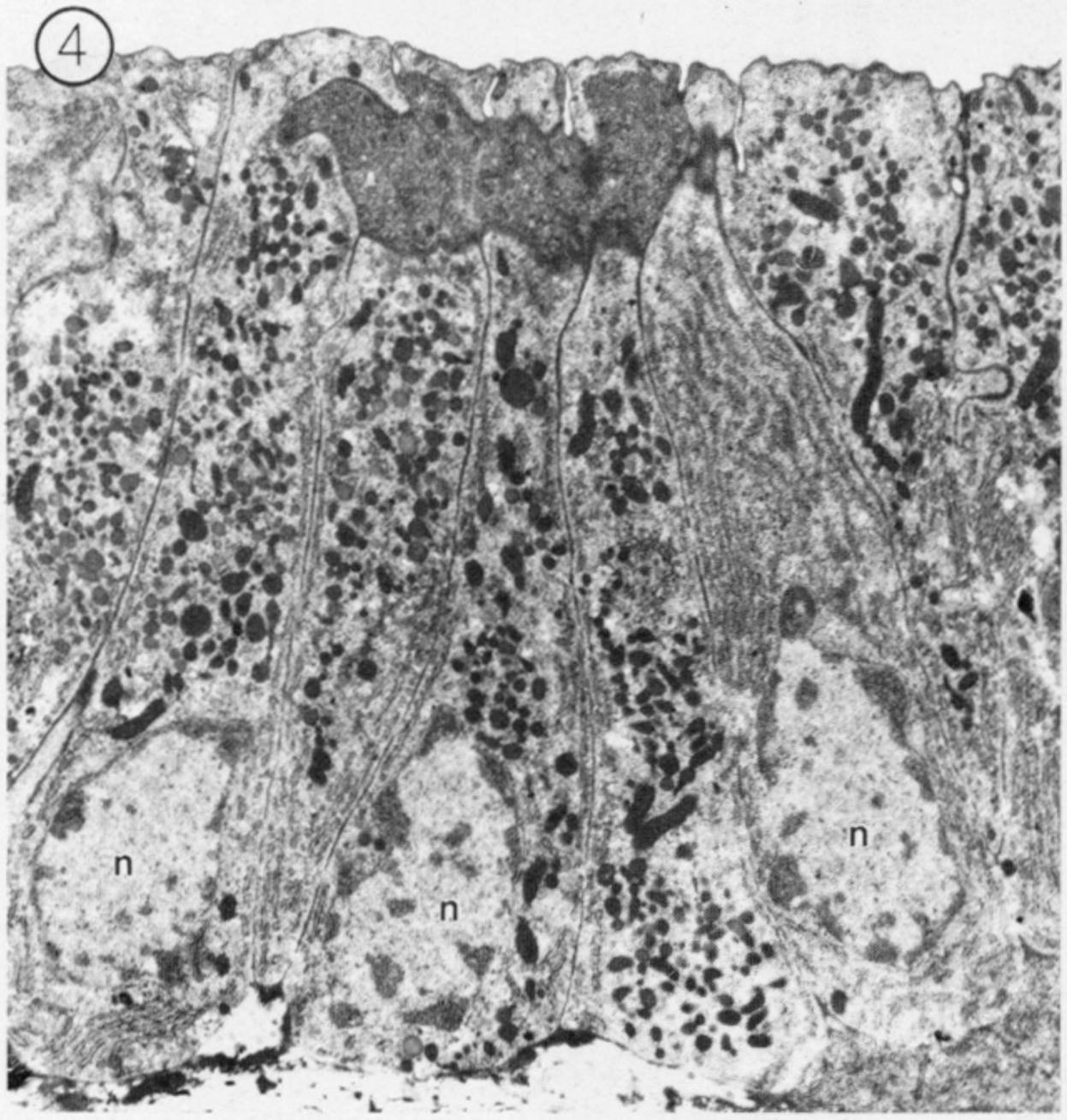
Abbreviations used on the figures

ad	carapace adductor muscle
1 as	1st antennular segment
2 as	2nd antennular segment
3 as	3rd antennular segment
4 as	4th antennular segment
am	anterior mantle cavity
ca	carapace
cc	cement canal
cg	cement gland
cn	central nervous system
cu	cuticle
1 cu	1st cuticle layer of externa
2 cu	2nd cuticle layer of externa
cy	cyprid
de	dorsolateral epidermis
ec	epicuticle
ep	epithelium/epidermis
hc	hood cuticle (of 1st externa cuticle) above mantle aperture
ic	inclusion cell
m	mantle
mc	mantle cavity
me	mesentery suspending visceral sac in mantle cavity
n	nucleus
ne	naupliar eye (in cyprid)
pc	postganglion cells
pe	posterior epidermis
rd	receptacle duct
re	receptacle
rh	rhabdome of naupliar eye (in cyprid)
rw	receptacle wall
sp	spines on epicuticle
st	stalk of externa
tc	terminal canal of receptacle duct
th	thorax
tr	trichogon
ve	ventral epidermis
vs	visceral sac







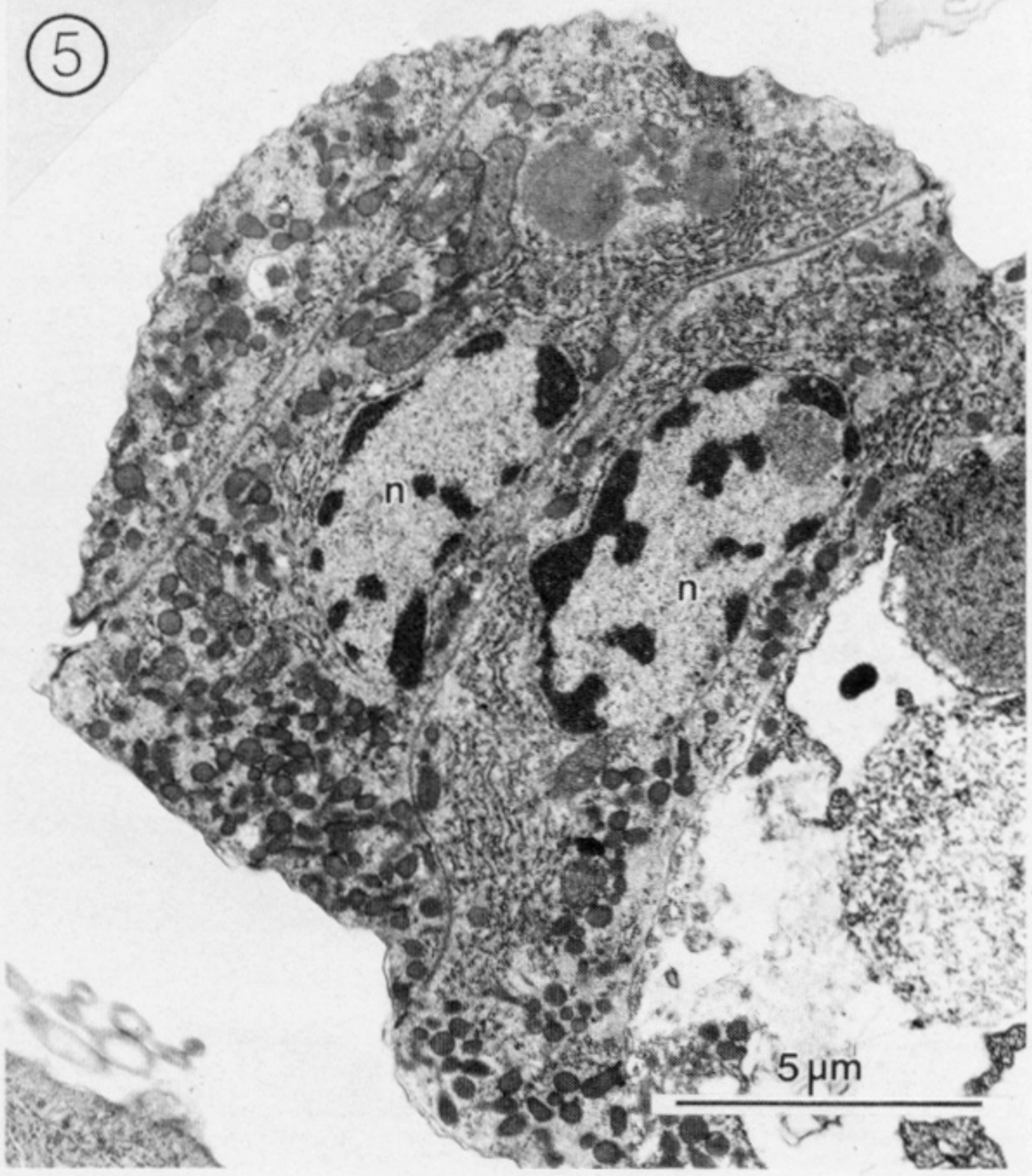
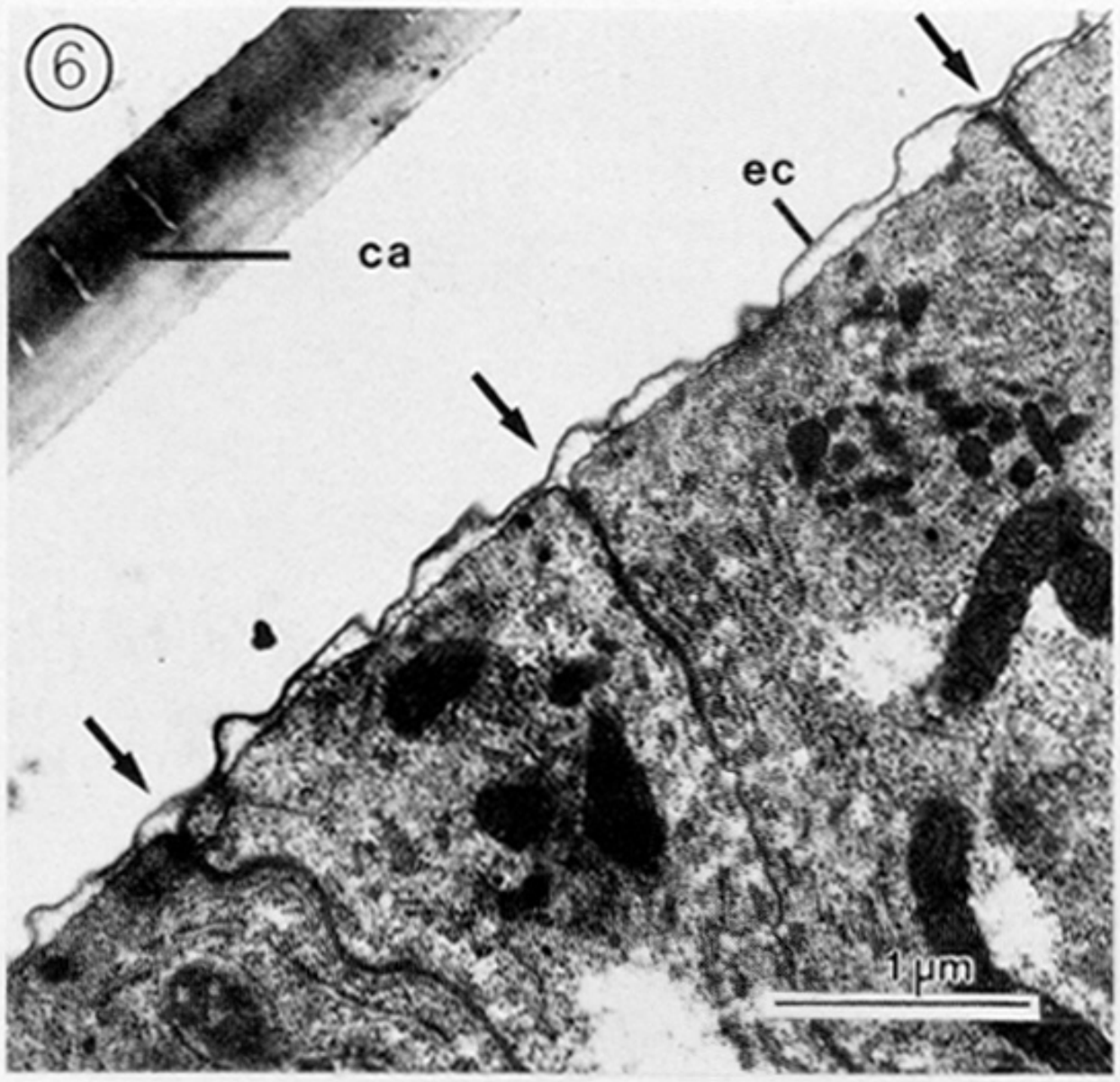


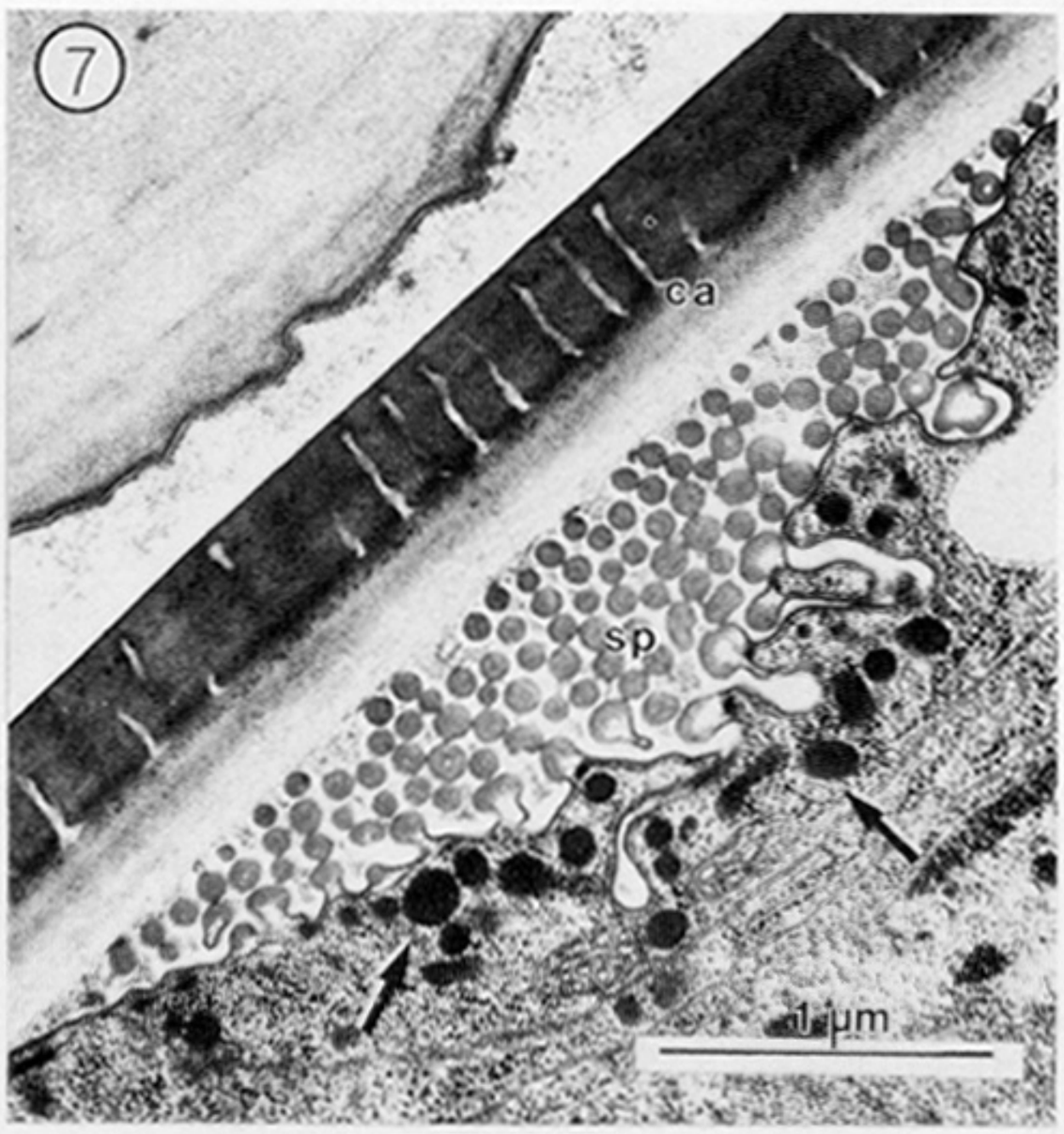
FIGURE 2. Cyprid. Dorsolateral epidemis cells are beneath the carapace (ca). An inclusion cell (ic) is seen immediately below the epidermis. TEM. Scale as in figure 5.

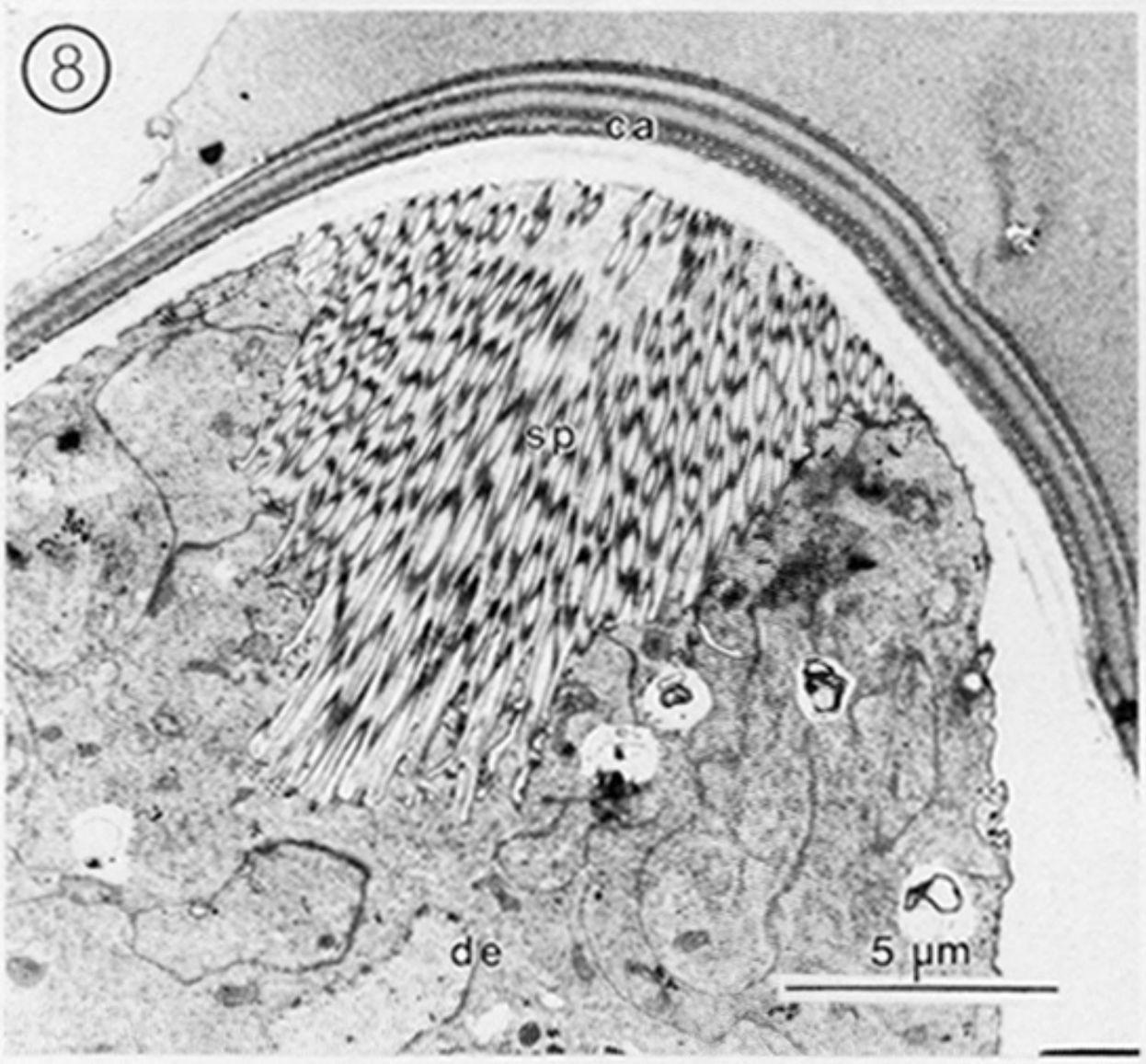
FIGURE 3. Free trichogon. Note the dorsolateral epidermis (de) and inclusion cell (ic); compare with figure 2. Cuticle at upper left is from the mantle cavity of the externa. TEM. Scale as in figure 5.

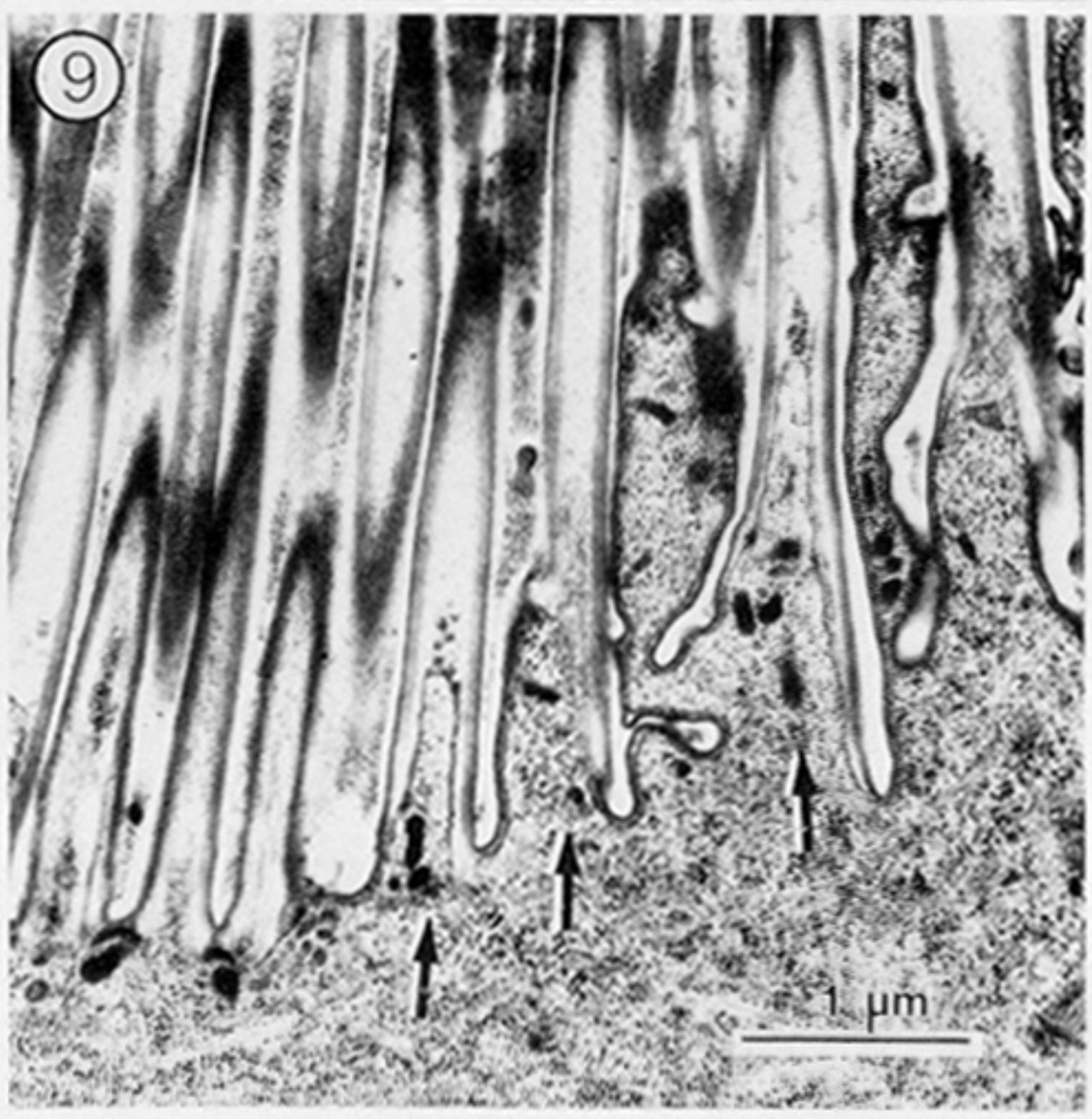
FIGURE 4. Cyprid, showing the ventral epidermis beneath the central nervous system. Ventral side is up. Epidermis and epicuticle have withdrawn from the cypris cuticle situated beyond the top of the figure. TEM. Scale as in figure 5.

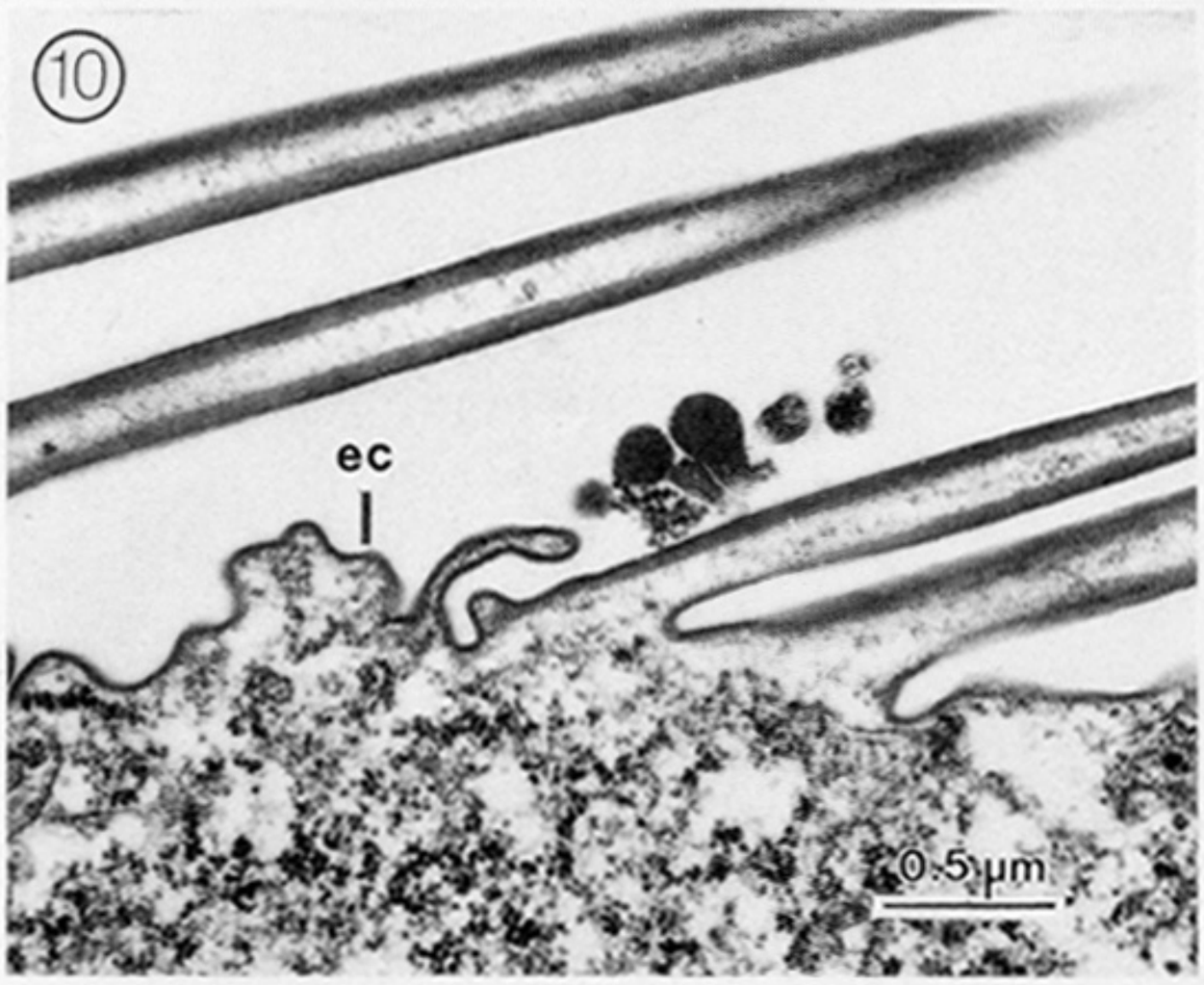
FIGURE 5. Free trichogon, showing ventral epidermis cells. Epicuticular spines are absent above the cells; compare with figure 4. TEM.

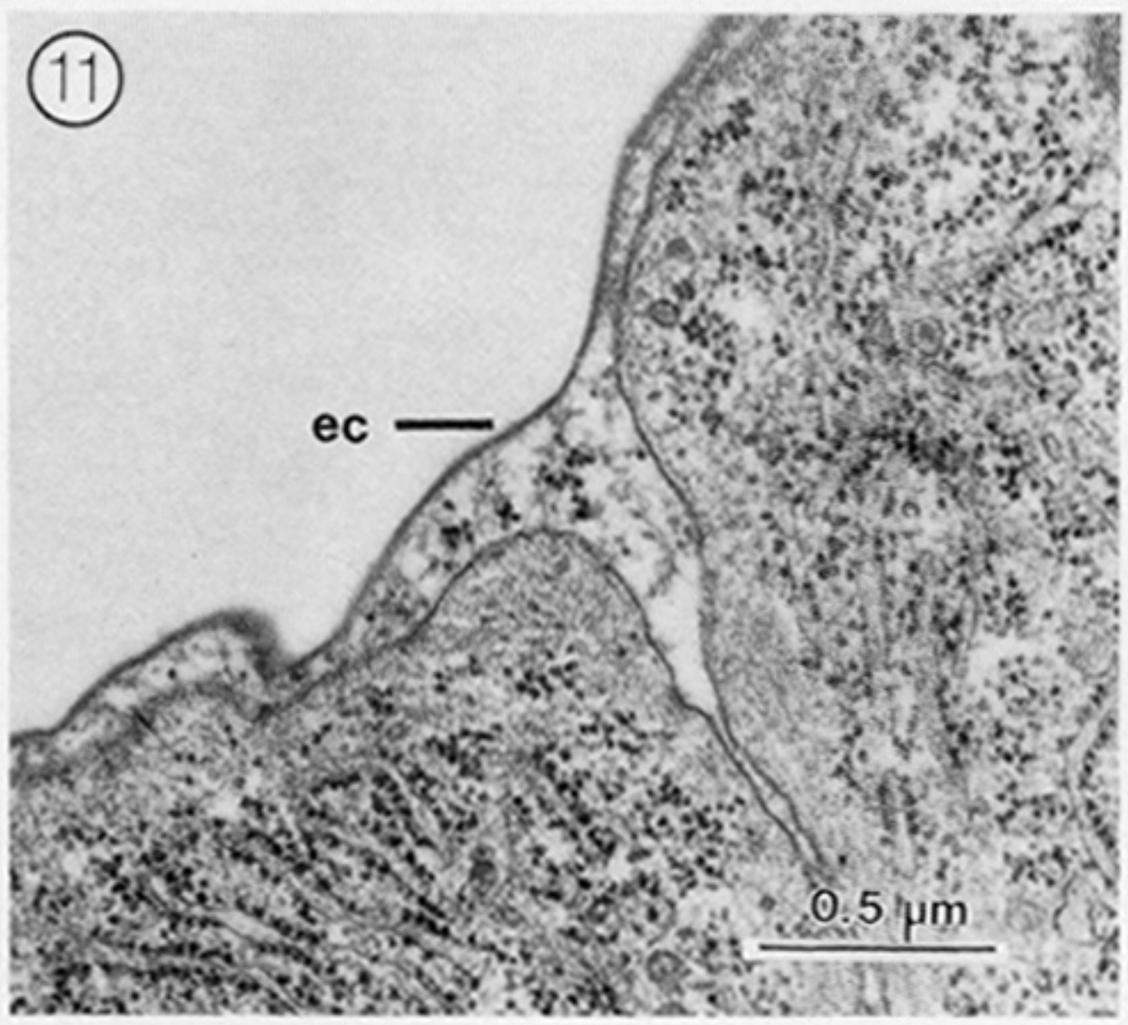












FIGURES 6–11. For description see opposite.

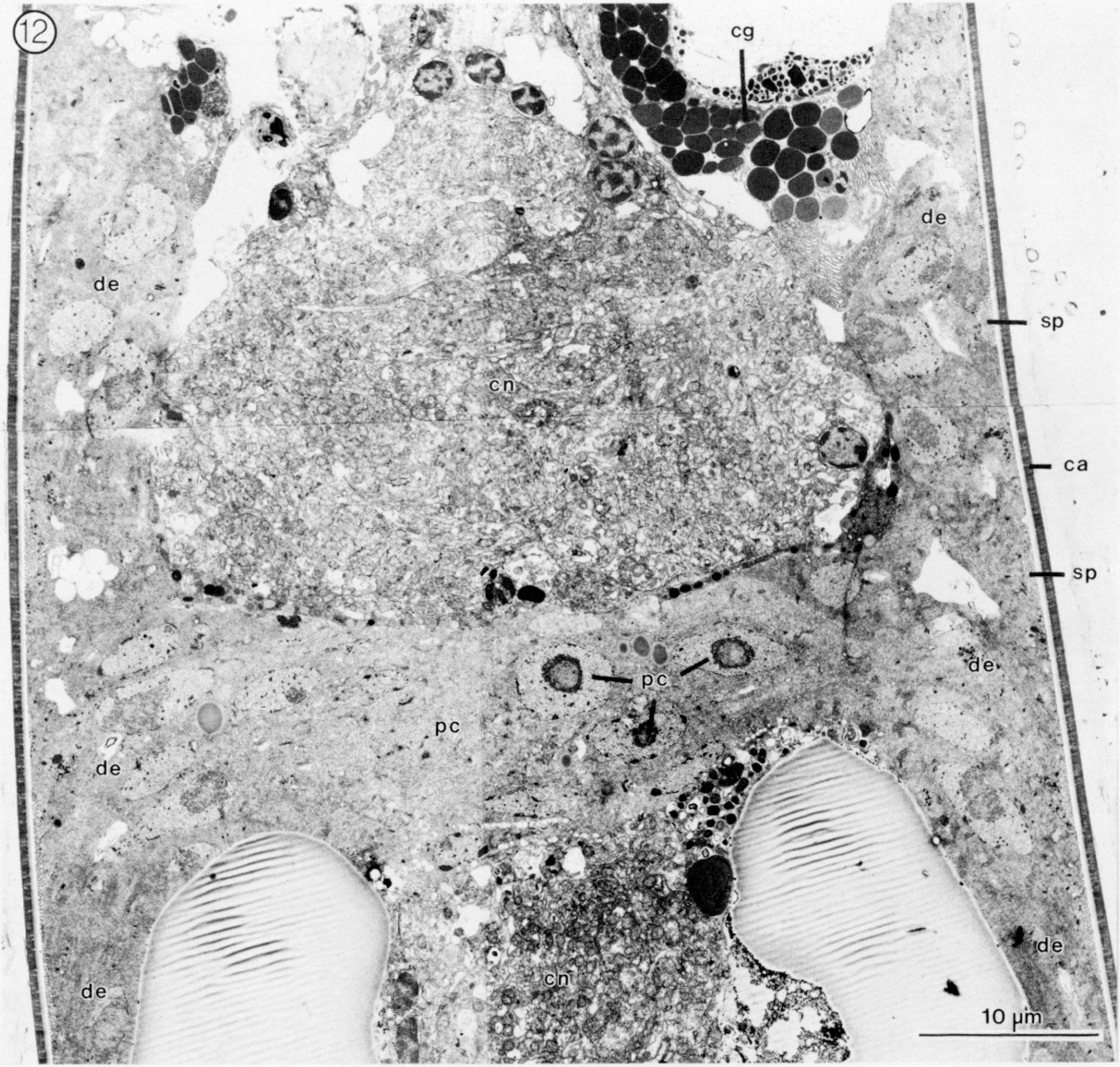
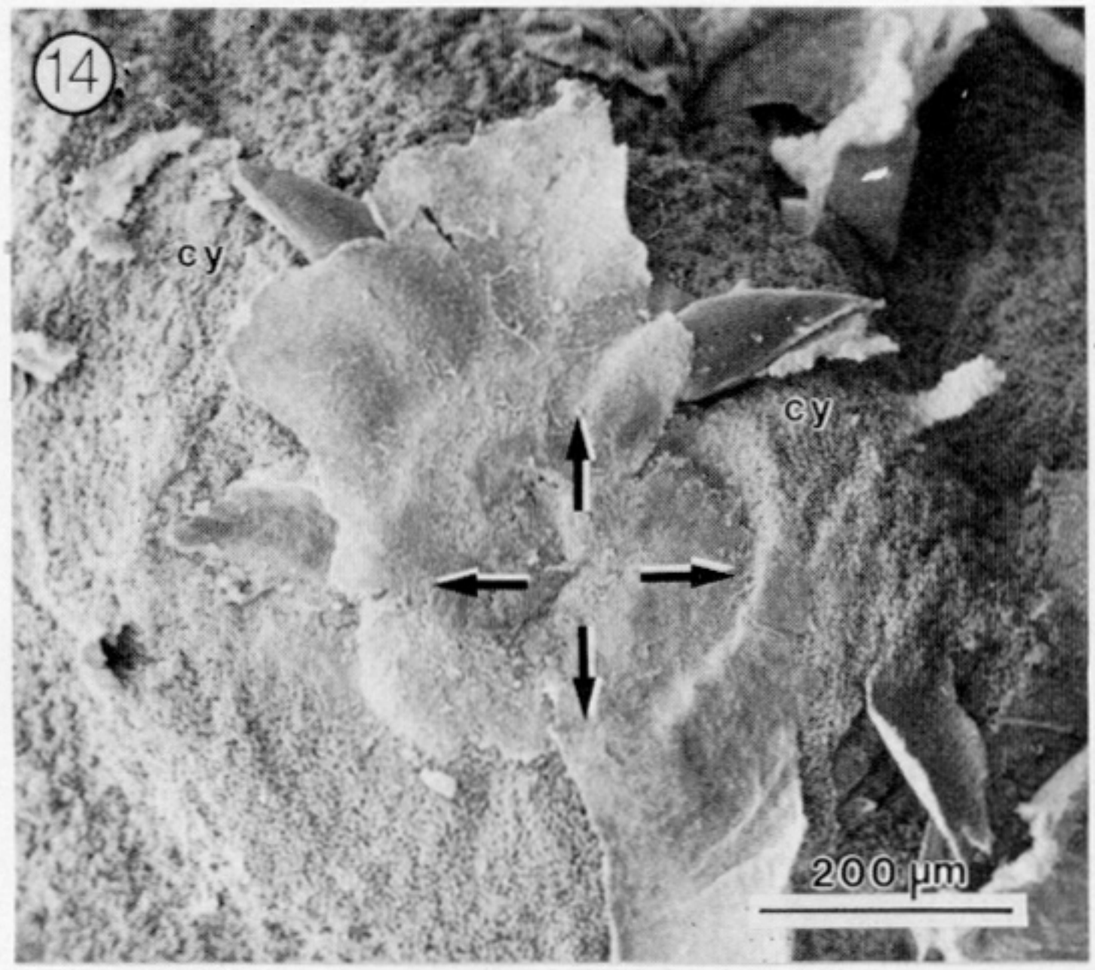
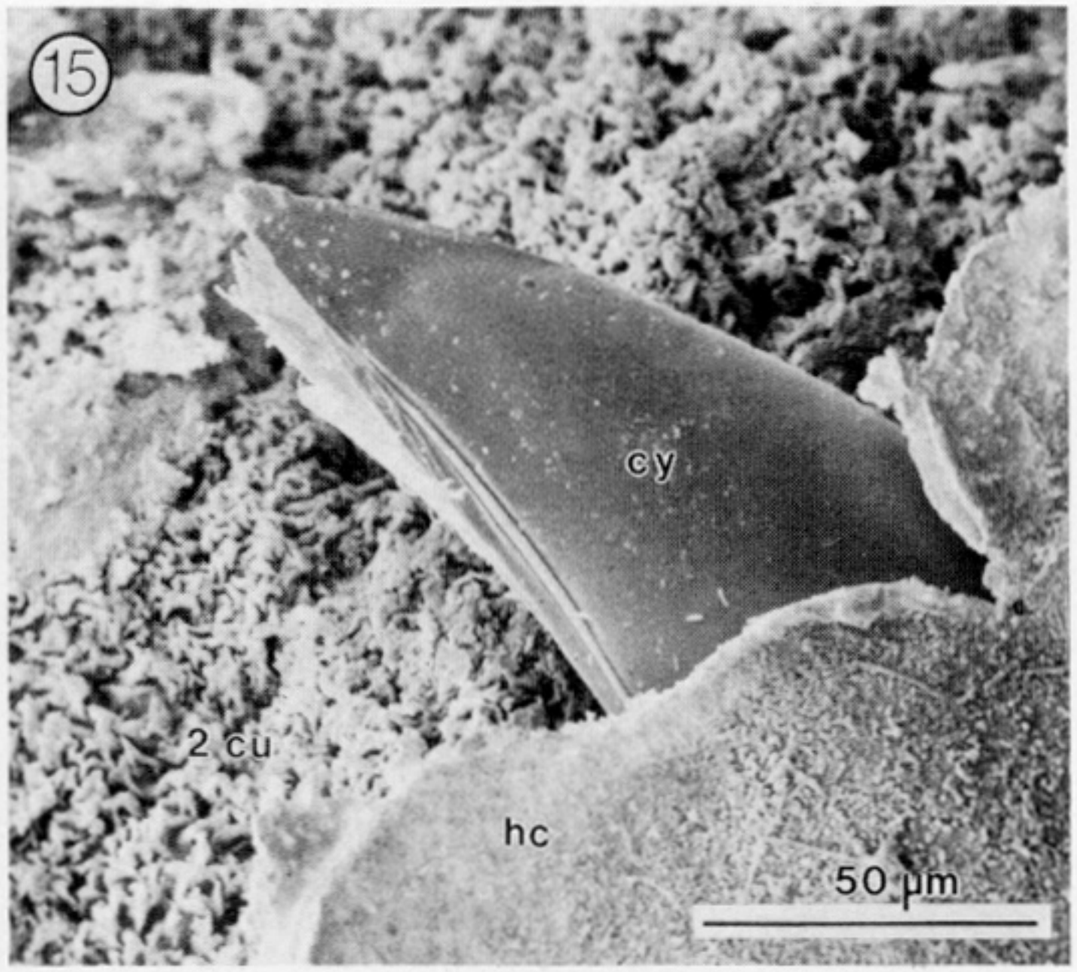
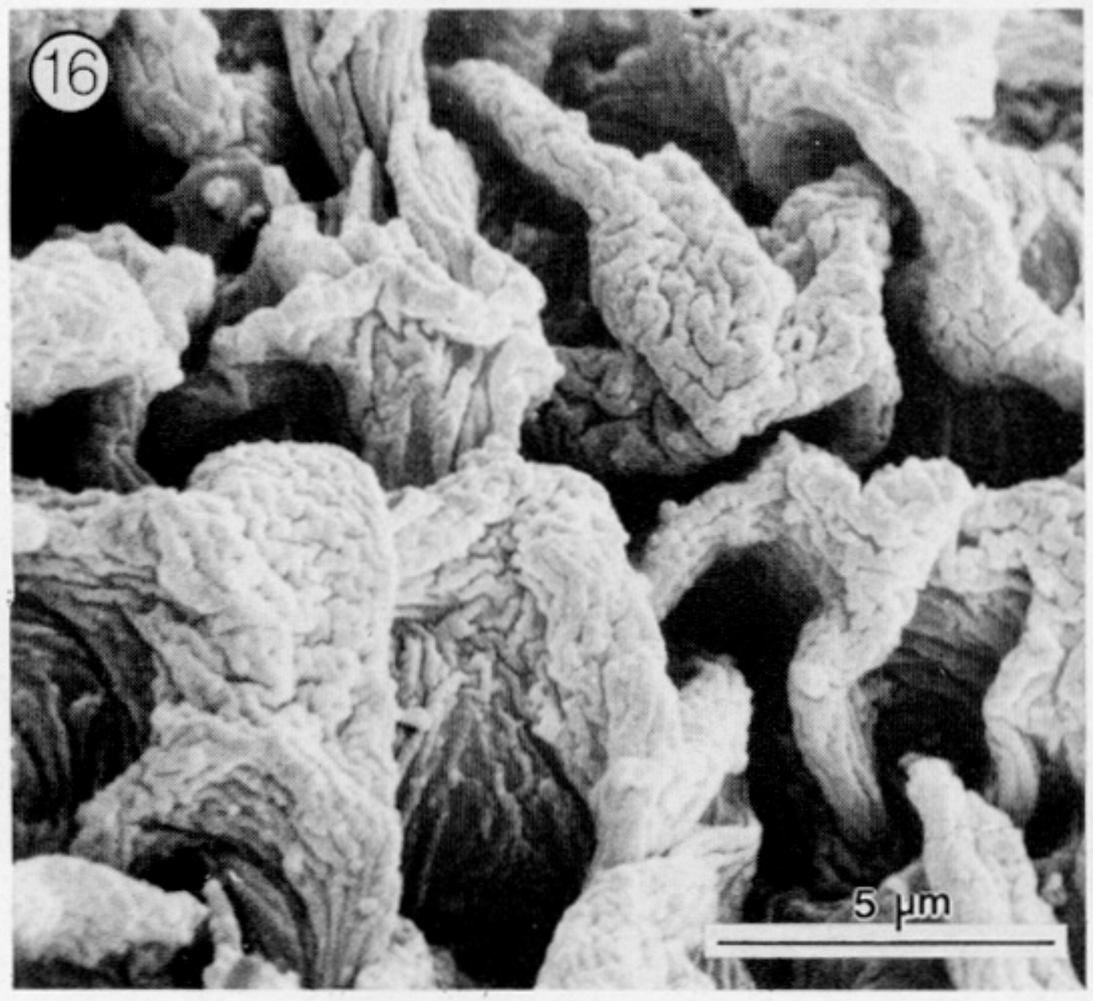


FIGURE 12. For description see opposite.







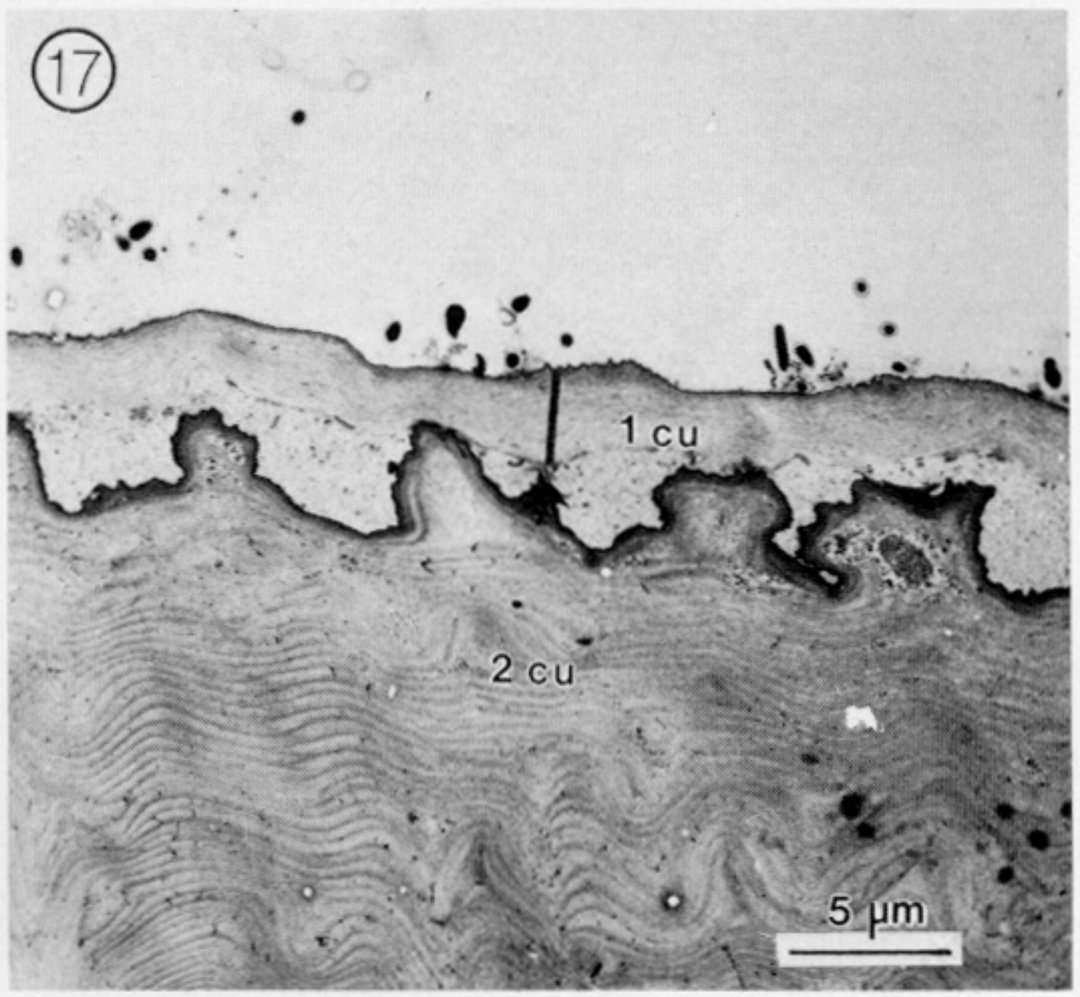


FIGURE 14. Apical end of an externa, showing two settled male cyprids (cy) partly covered by the hood cuticle; the outline of the mantle aperture beneath the hood is faintly visible (arrows); a cyprid at the mid-left periphery of the hood has fallen off but has left an impression. SEM.

FIGURE 15. Detail of figure 14; the posterior end of a settled cyprid protudes beyond the hood cuticle (hc); the 2nd externa cuticle (2 cu) has a much rougher surface than the hood of 1st externa cuticle. SEM.

FIGURE 16. Excresences on the surface of the 2nd externa cuticle. SEM.

FIGURE 17. Smooth 1st externa cuticle (1 cu) above the rough-surfaced and much thicker 2nd externa cuticle (2 cu). TEM.

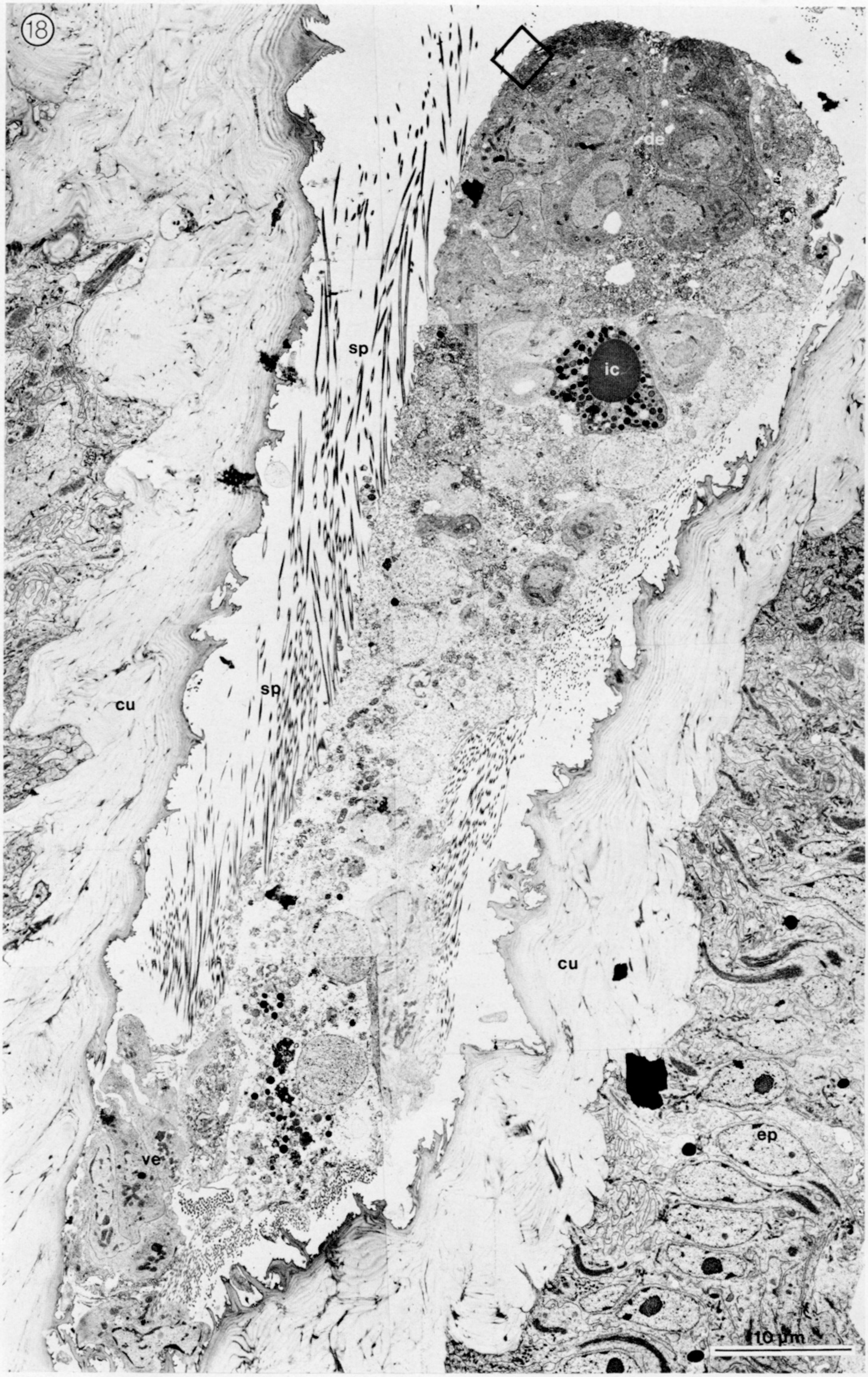
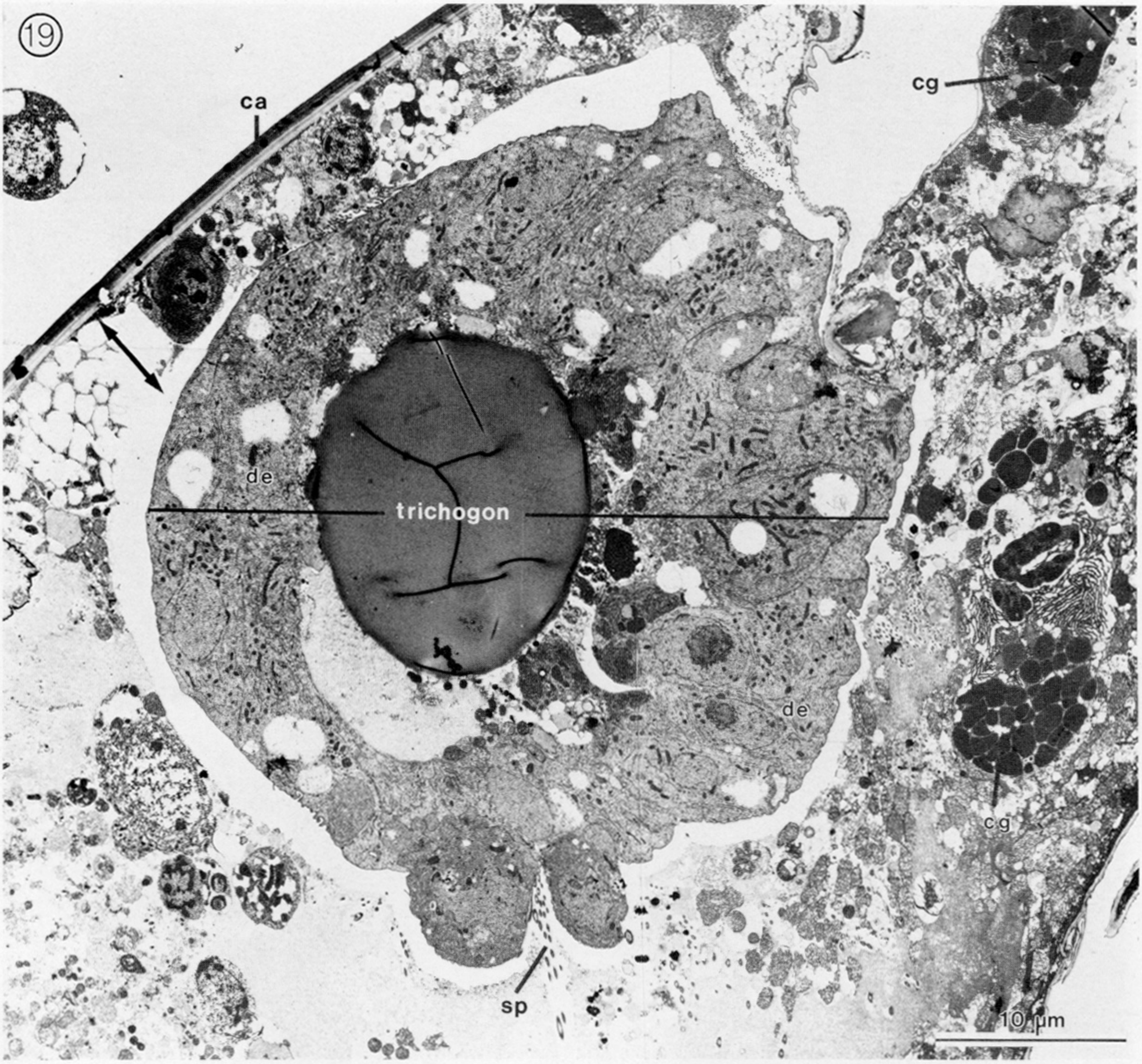
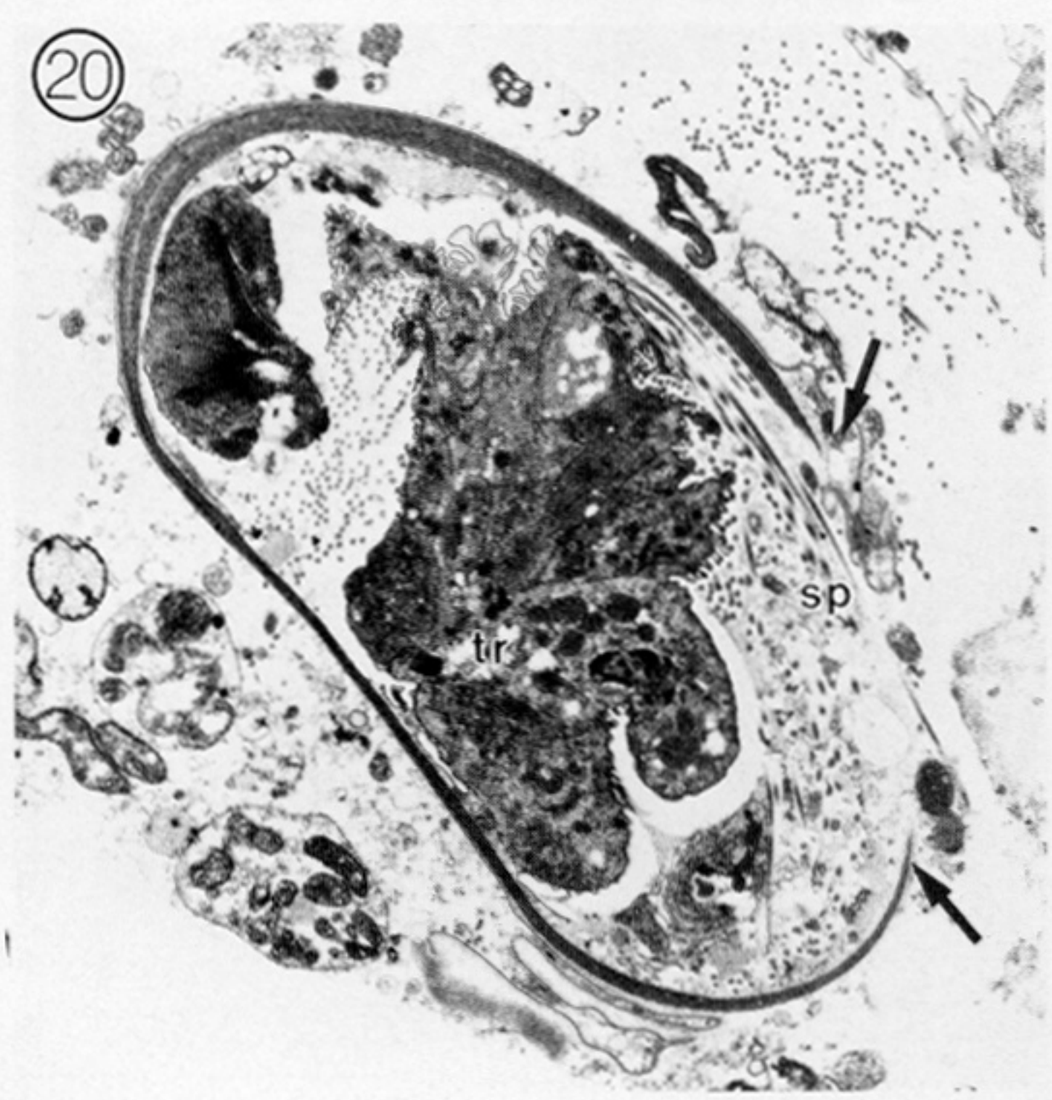
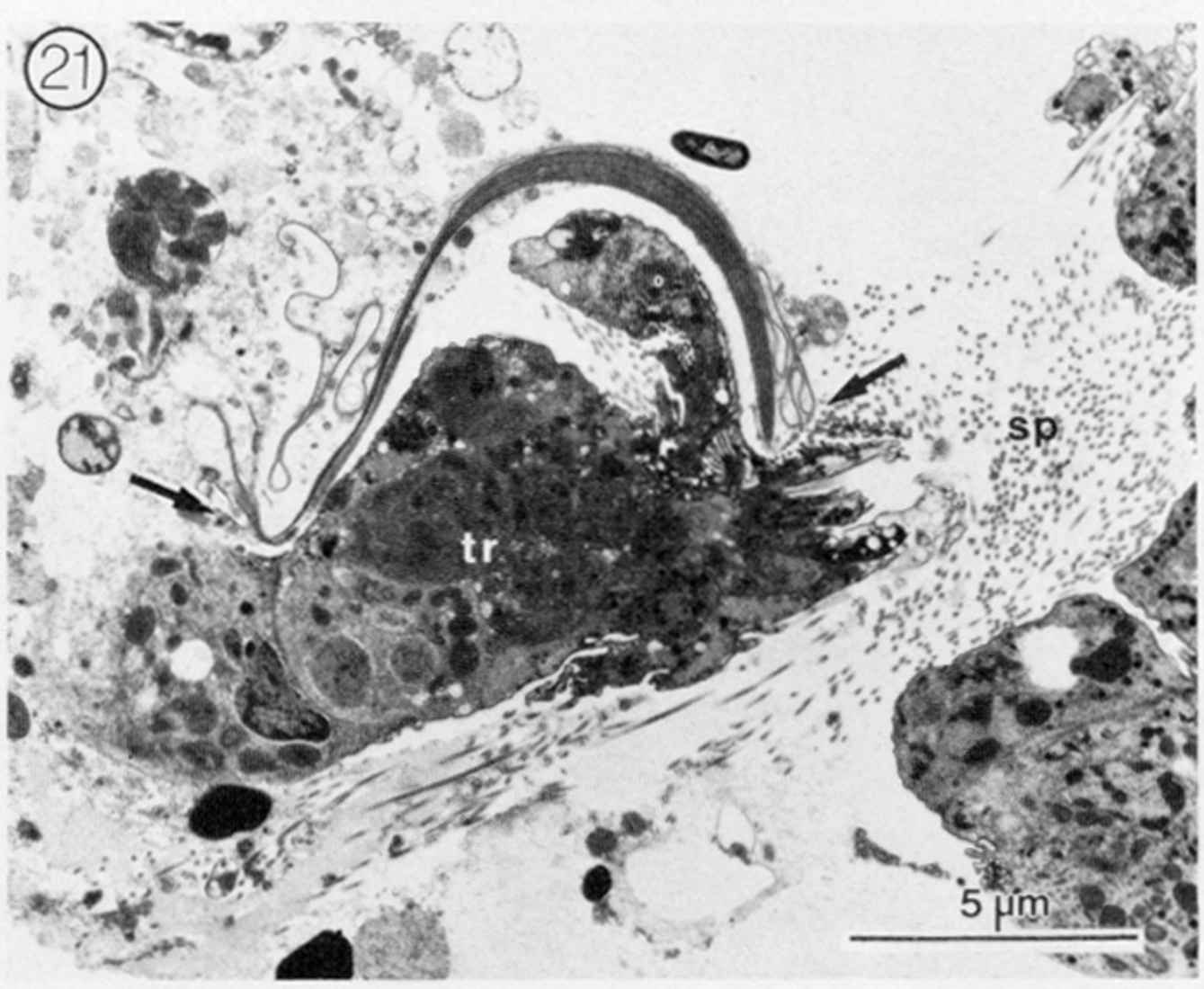


FIGURE 18. For description see opposite.







FIGURES 19–21. For description see opposite.

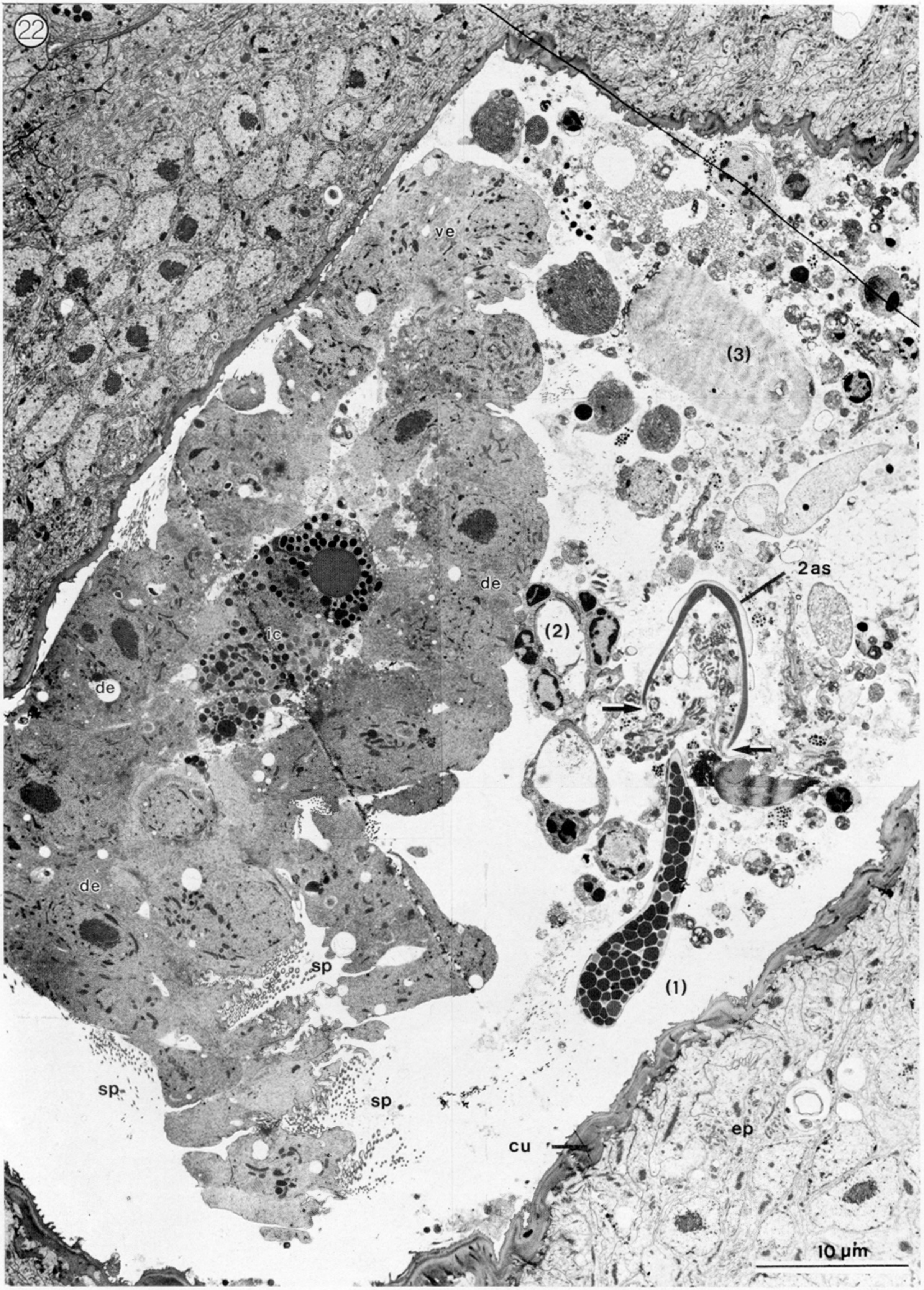
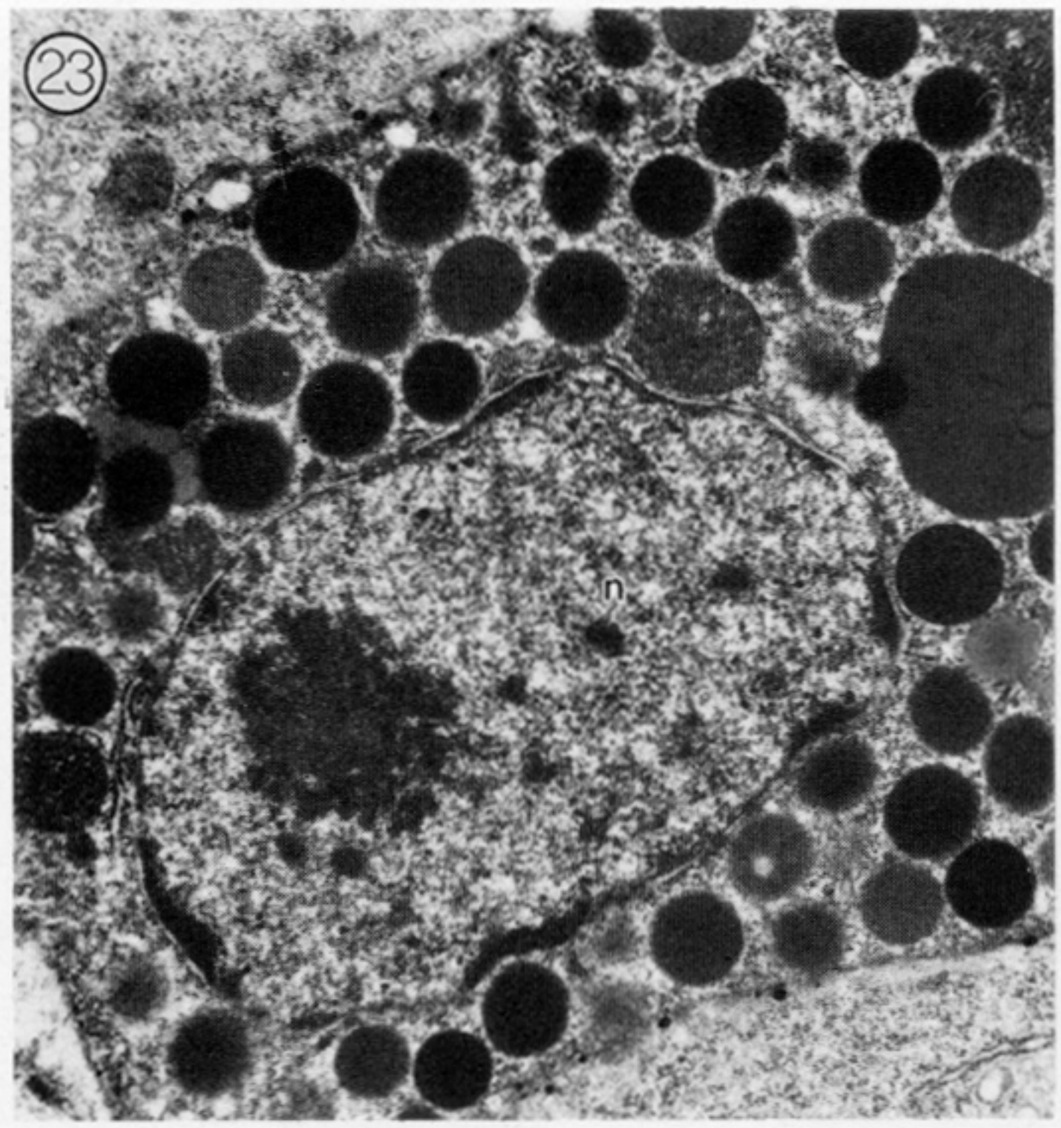
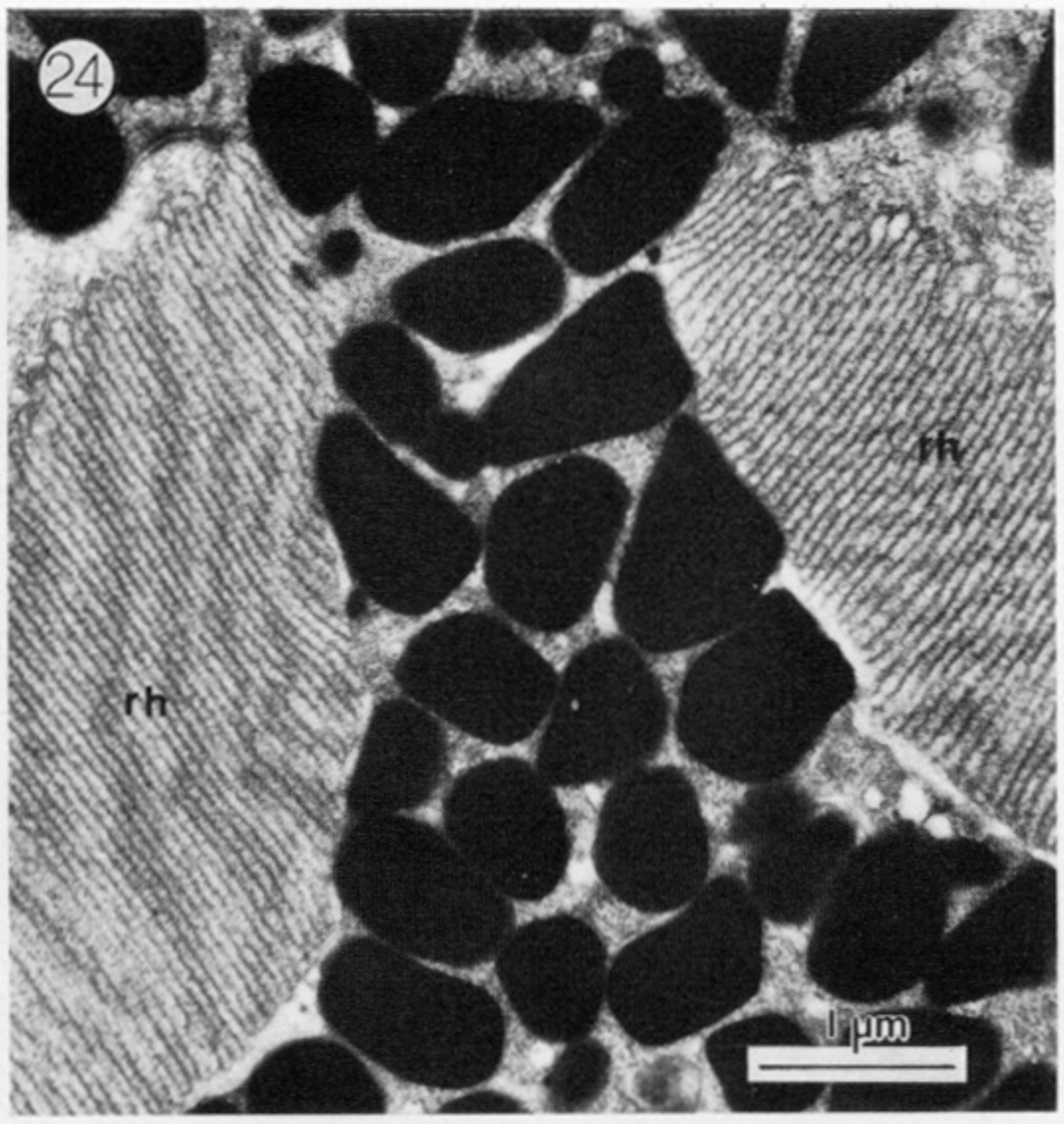
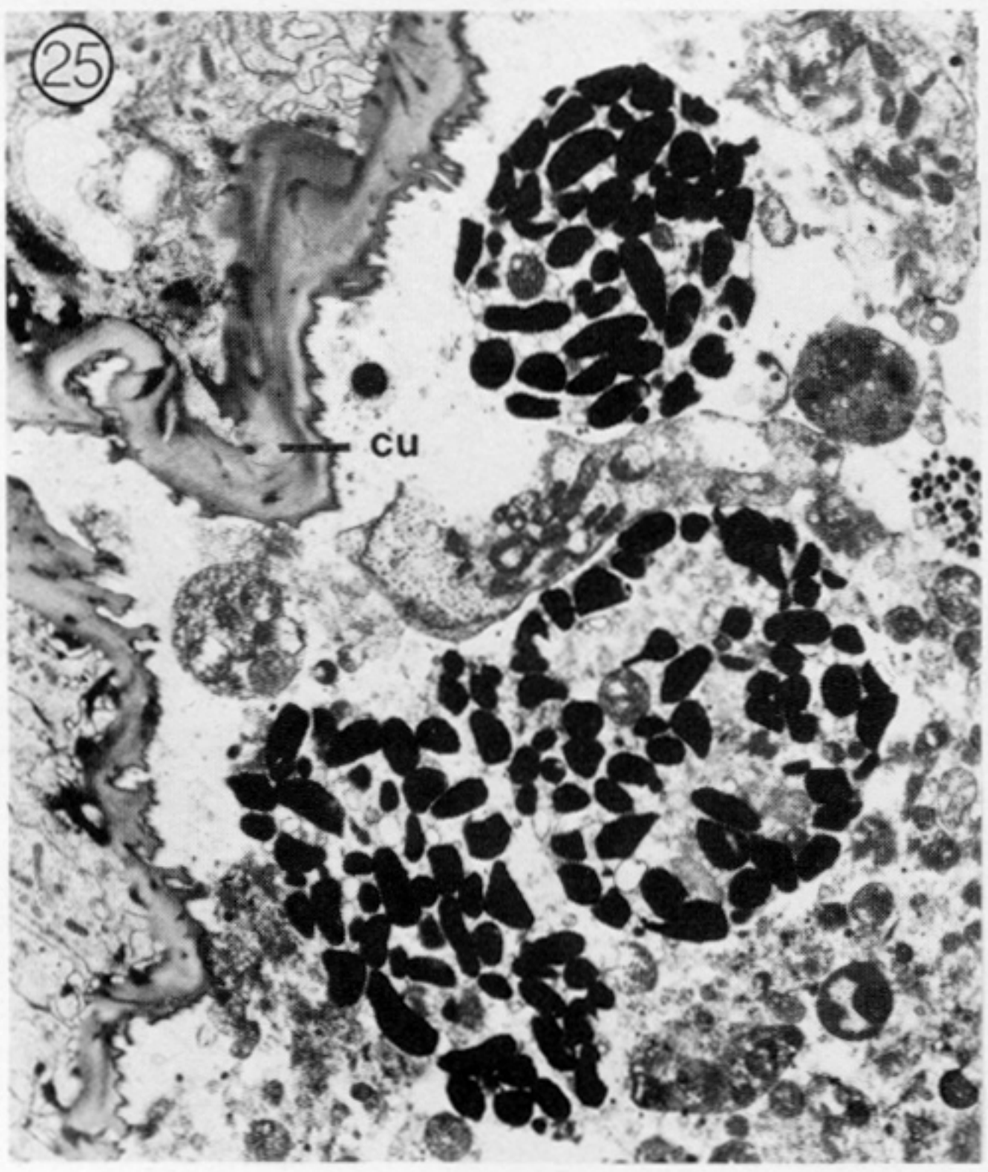


FIGURE 22. For description see opposite.







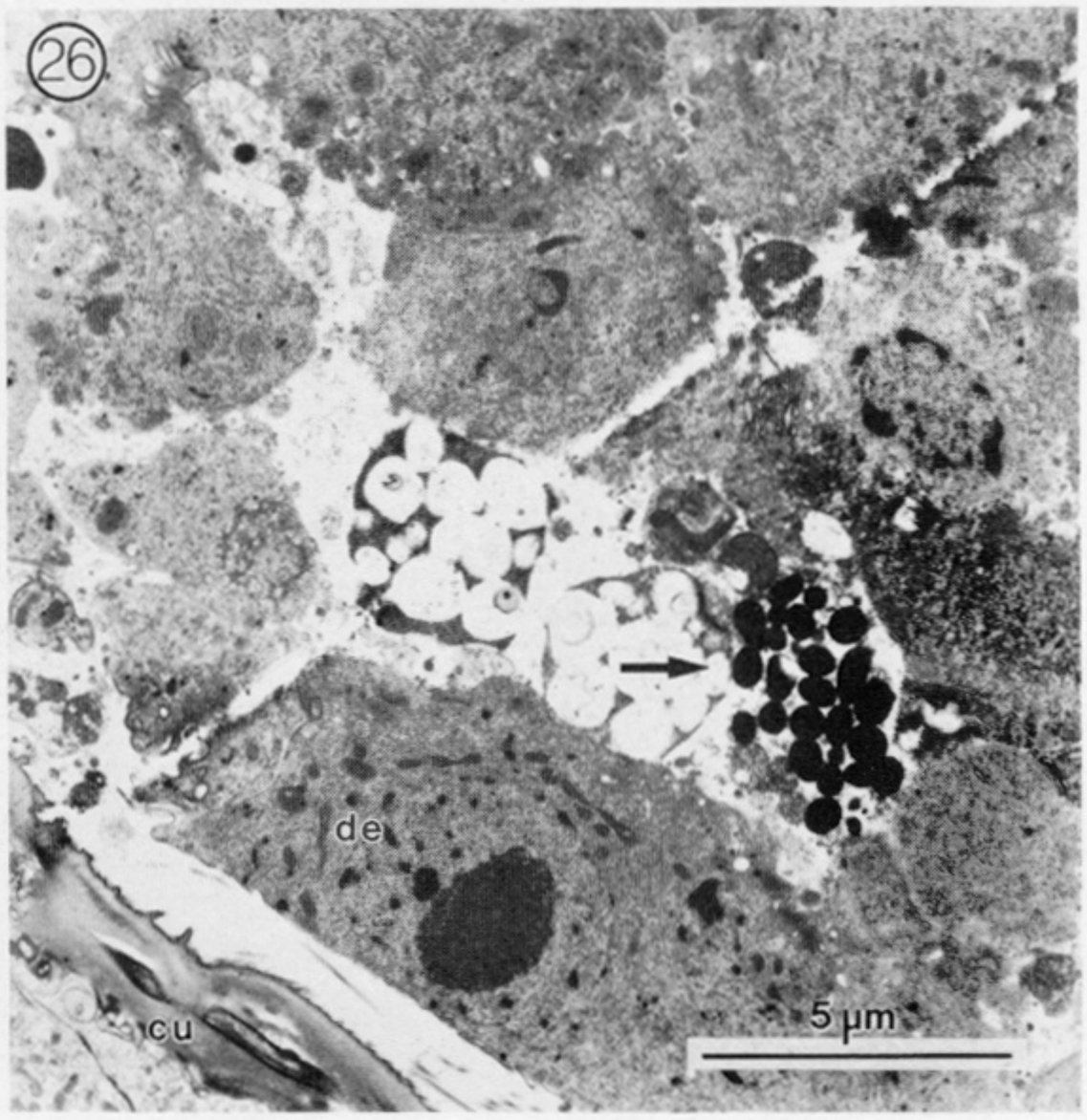
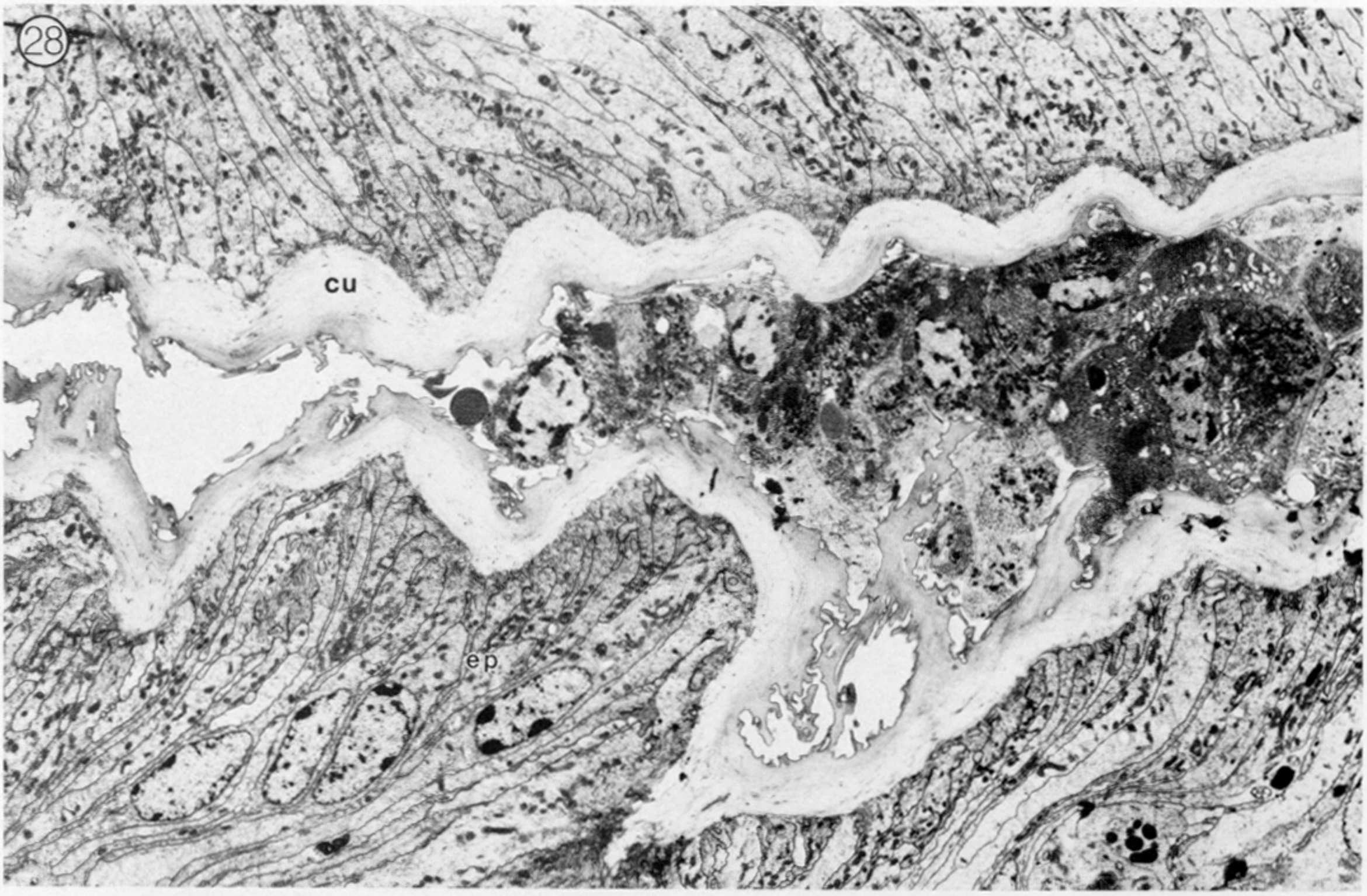


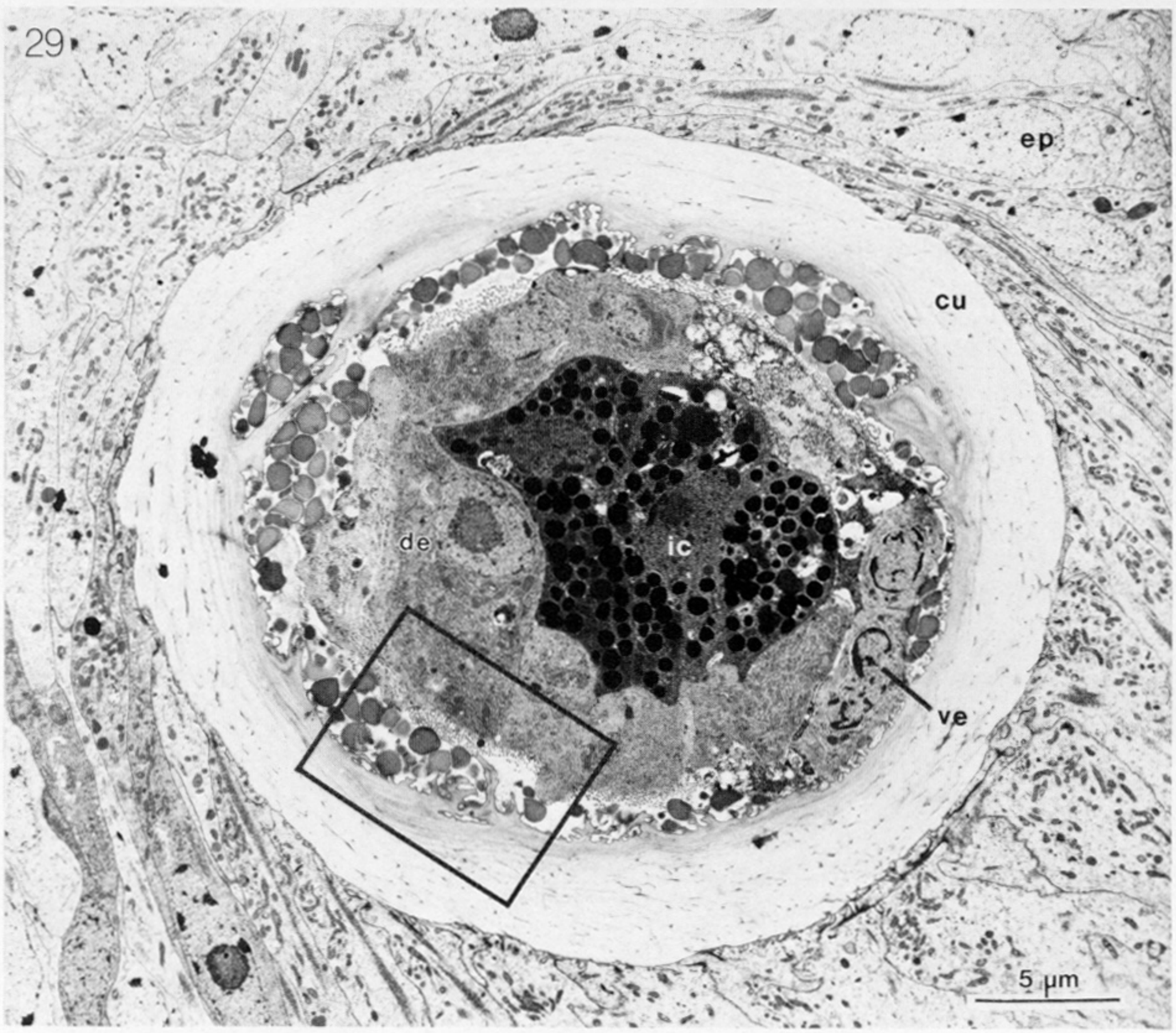
FIGURE 23. Inclusion cell of an implanted trichogon; large rectangle in figure 34. Scale as in figure 24. TEM.

FIGURE 24. Cyprid, showing irregularly shaped pigment granules of the naupliar eye; (rh) rhabdome. TEM.

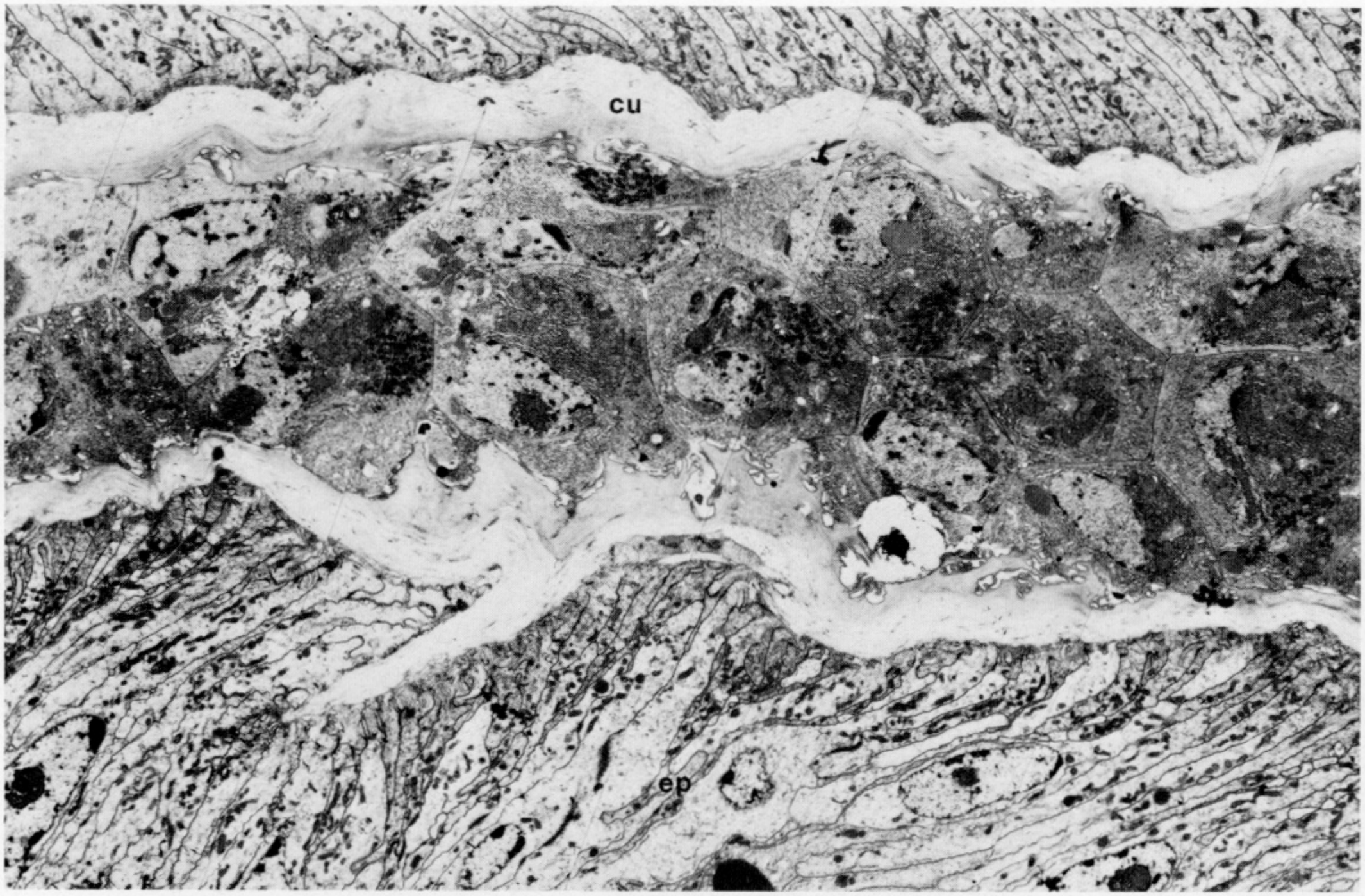
FIGURE 25. Naupliar eye pigment expelled into the mantle cavity of the externa by a metamorphosing cyprid or an early trichogon (see text); the cuticle of the mantle cavity is at left. Scale as in figure 26. TEM.

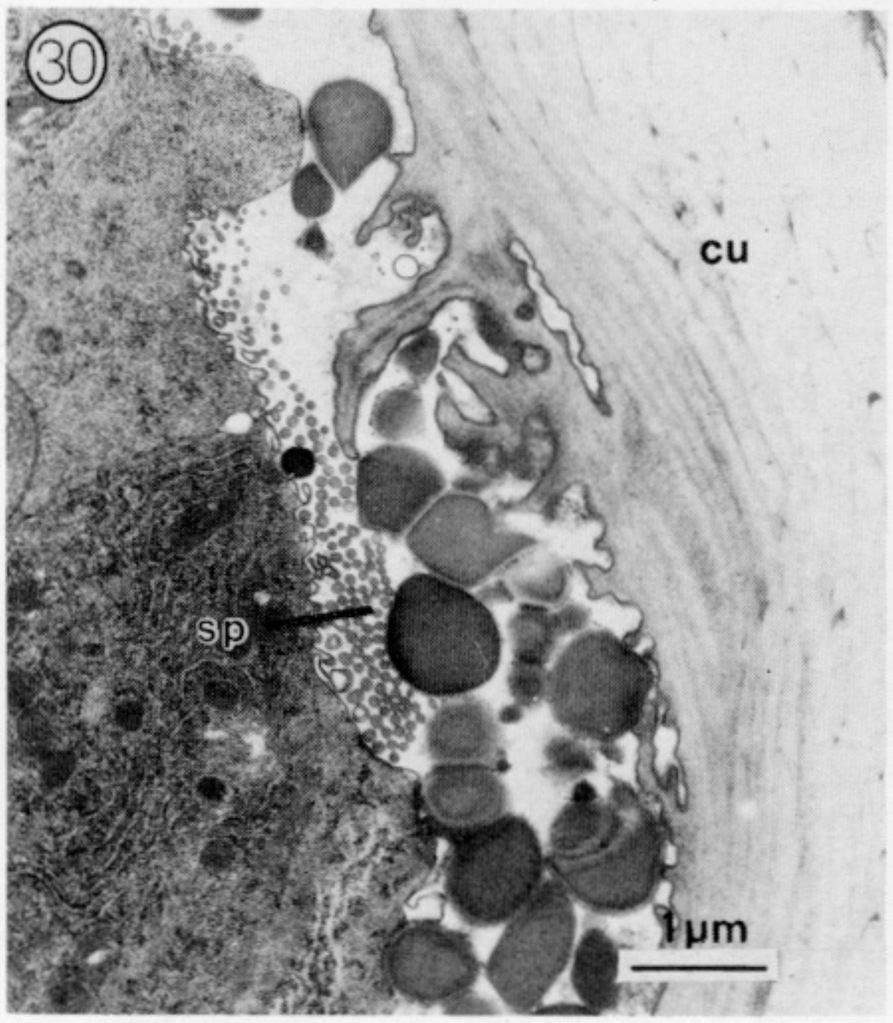
FIGURE 26. Naupliar eye pigment (arrow) lying free within a lacuna of an early trichogon; the lacuna communicates with the exterior at left; the cuticle of the mantle cavity is shown at lower left. The diagonal ridge is an artefact of sectioning. TEM.





FIGURES 28 AND 29. For description see opposite.





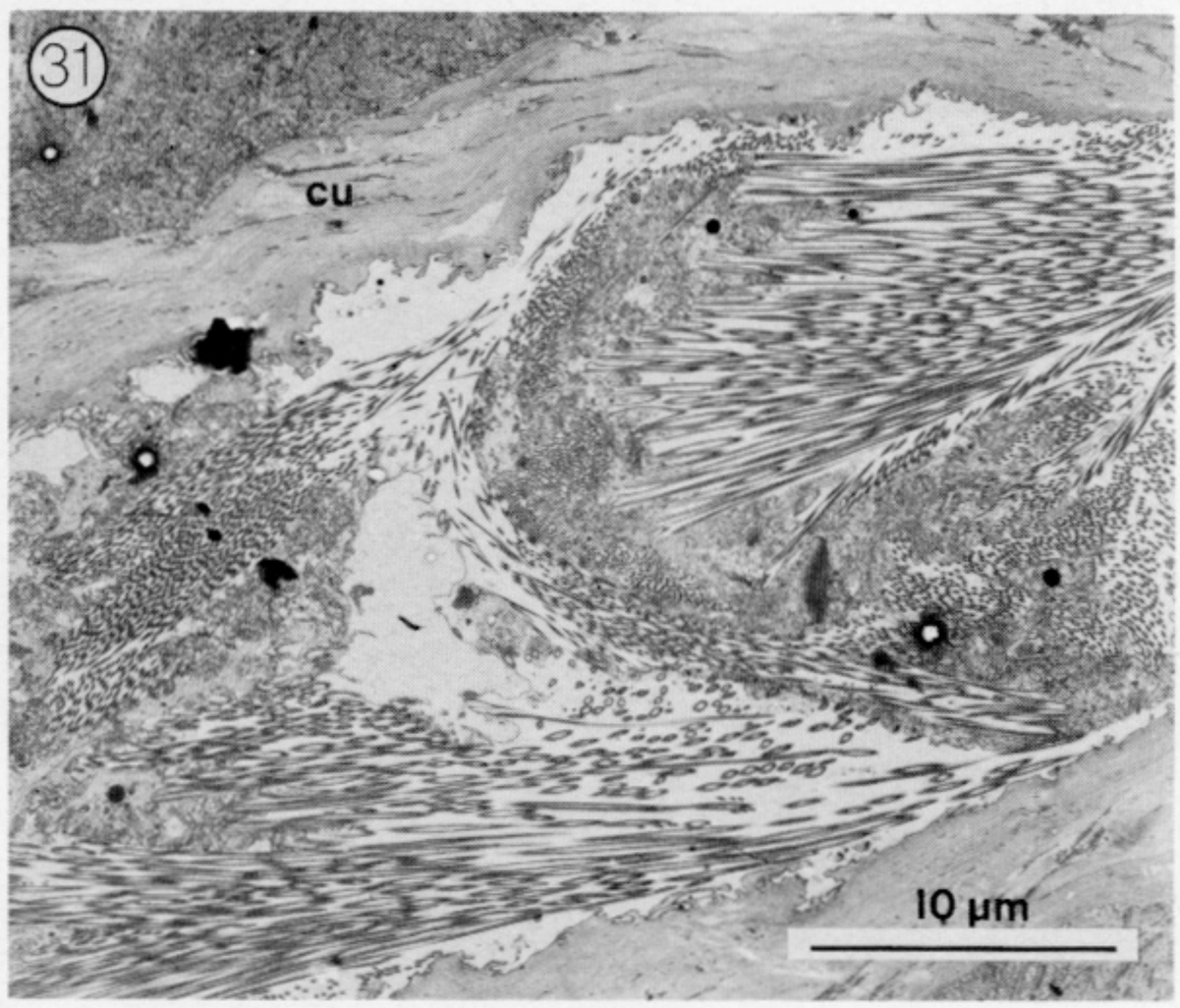
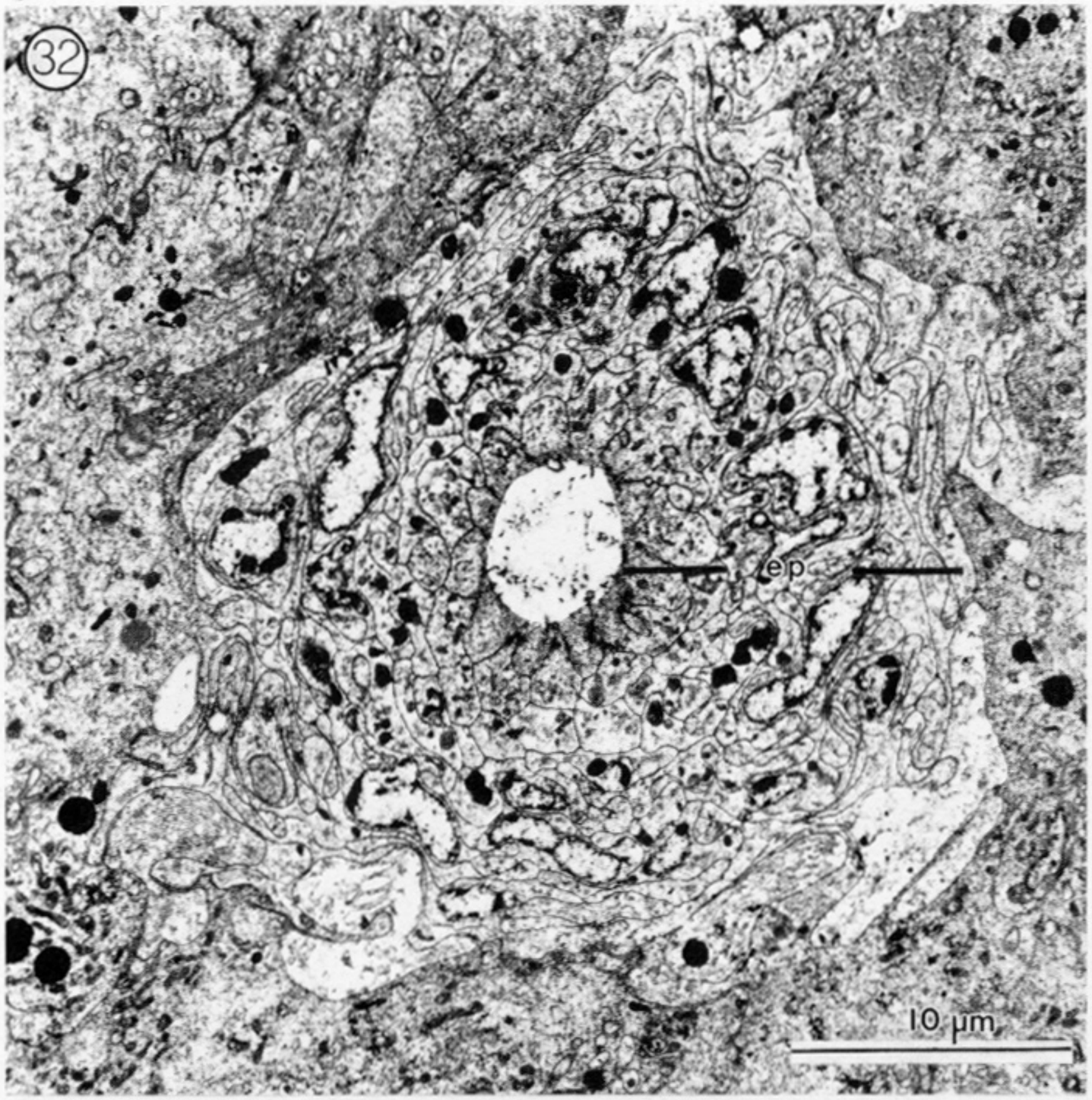


FIGURE 30. Rectangle in figure 29; spiny epicuticle still surrounds the trichogon in the distal part of the duct; granules between the trichogon and the duct cuticle may originate from the receptacle. TEM.

FIGURE 31. Epicuticle with spines shed by trichogon in the distal part of receptacle duct. TEM.



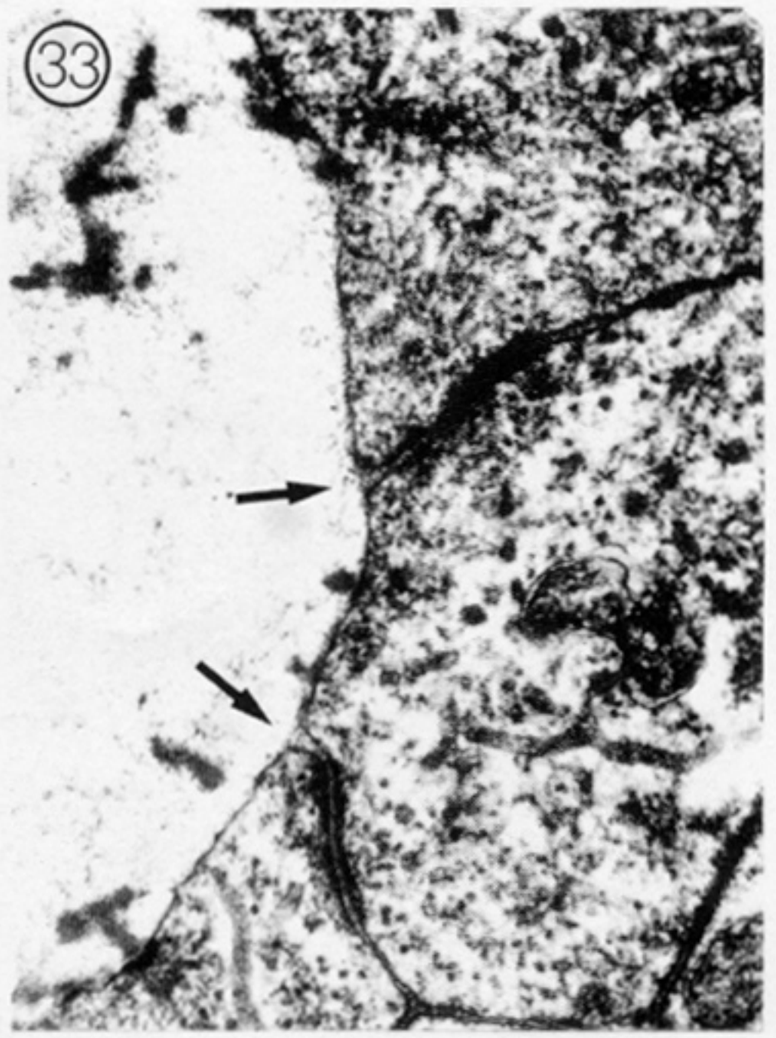
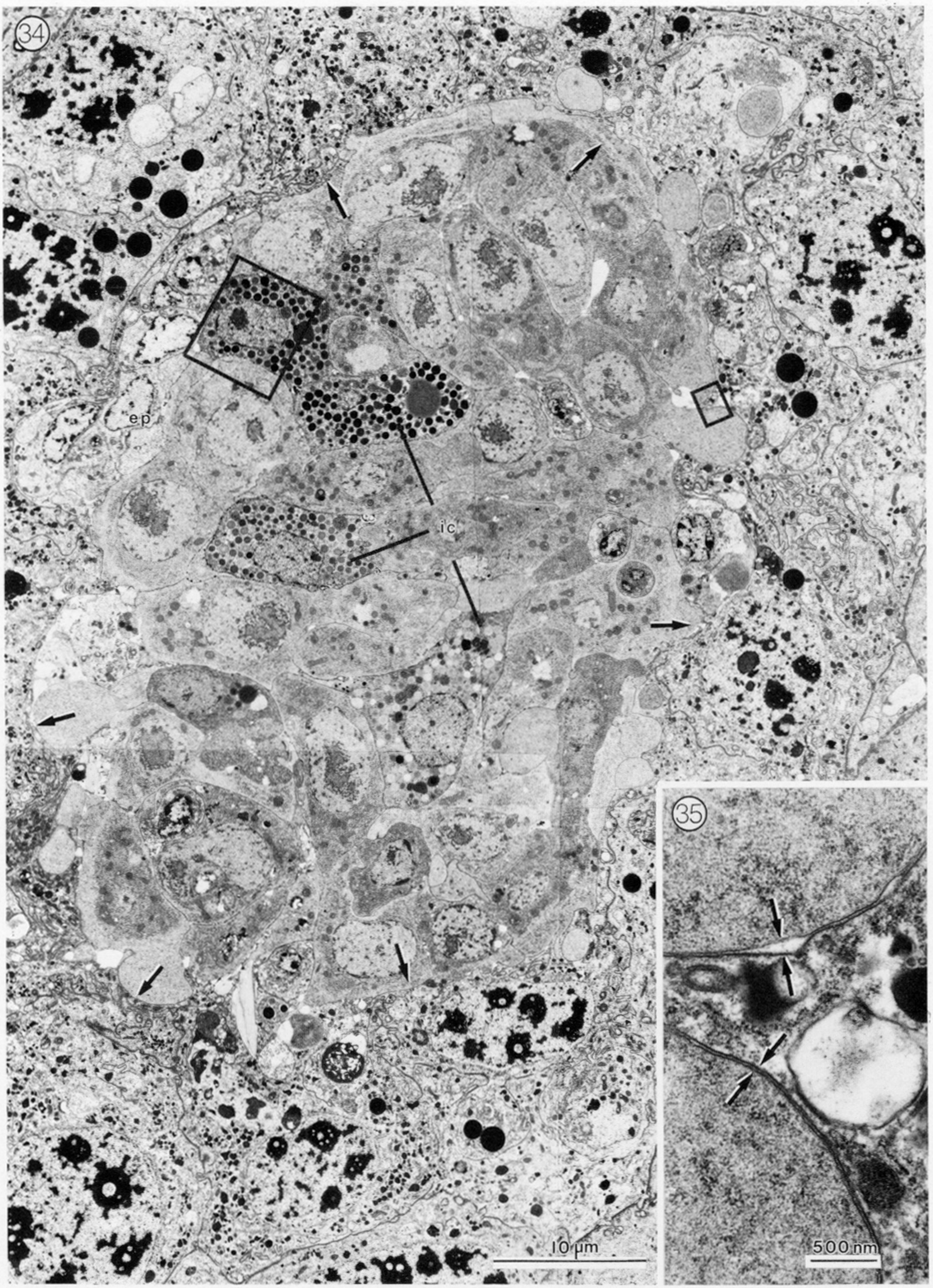


FIGURE 32. Cross section through the most proximal, cuticle-free end of the terminal canal; note the interdigitating epithelium with lobulate nuclei, compare with figure 34, which shows the same region after trichogon implantation. TEM.

FIGURE 33. Detail of figure 32. No cuticle covers the epithelium in the most proximal part of the terminal canal; the junction between epithelial cells is arrowed. TEM.



FIGURES 34 AND 35. For description see opposite.