STUDIES ON THE LARVAL STRUCTURE AND METAMORPHOSIS OF *BALANUS BALANOIDES* (L.)

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Balanus balanoides (L.) has seven planktonic larval stages. The first six are nauplius larvae while the seventh is the cypris larva. The cypris larva is specially adapted to locate a suitable place for settlement.

The structure of the nauplius larva is basically similar to that of the nauplii of other crustacean groups. During successive nauplius stages, however, the simplicity of its anatomy is progressively obscured by the development of the cypris organ systems. All the organ systems do not differentiate simultaneously, but development is closely related to the time at which the organ must start to function. The three pairs of nauplius appendages, antennules, antennae and mandibles, are used in locomotion and the latter two pairs are also used in feeding. The six pairs of cypris thoracic swimming appendages, and the first and second maxillae with their associated ganglia and muscles, develop from groups of ectoteloblasts and mesoteloblasts in the ventral thoracic region of the nauplius. The compound eyes develop as outgrowths of the lateral lobes of the brain. The paired cement glands develop pre-orally. The end sacs of the adult maxillary glands develop as cavities in the somites of the second maxillary segment. The cypris antennules develop within the nauplius antennules but differentiation of their intrinsic musculature is delayed until after the nauplius-cypris moult. The various muscles of the cypris carapace are fully formed by the time of the nauplius-cypris moult.

During, and after, the moult, a number of morphological and histological changes occur. The antennae and mandibles regress, the intrinsic musculature and cement ducts of the antennules complete their development. At the same time *all* the nauplius muscles and the antennal glands histolyse. Until these changes are completed the cypris larva is probably unable to settle, and thus to initiate the changes leading to the completion of metamorphosis. Rudimentary adult mandibles, and first and second maxillae are incorporated into the oral cone. After the moult the digestive region of the nauplius mid-gut epithelium and other epithelial cells are sloughed off into the gut lumen and digested together with the remains of the food ingested by the nauplius. The oesophagus and hind gut are now closed and the cypris larva does not feed. The adult digestive glands develop at the junction of the oesophagus and mid-gut. In the cypris the nauplius frontal filaments are associated with the compound eyes and connected to the brain via the optic ganglia. The median eye is apparently unchanged. Paired antennular ganglia are present. Those neurons, which innervated nauplius structures which have histolysed, also degenerate. The nauplius antennal glands degenerate at the nauplius-cypris moult; the maxillary glands are probably the functional organs of ionic regulation in the cypris as well as in the adult. The conspicuous multinucleate oil cells of the cypris are probably a food reserve. The paired masses of yellow cells in the carapace, originate in the antennae of the nauplius and migrate into the carapace after the moult.

During the 24 h between settlement and the moult to the young adult, all the cypris muscles histolyse. The muscles break up spontaneously into short fragments which are then ingested by phagocytic haemocytes. There is widespread histolysis of neurons in the nervous system and further cells are sloughed from the gut epithelium. The adult mantle muscles, which are recognizable in the free swimming cypris larva, complete their differentiation, and in the few hours preceding the cypris-adult moult the adult thoracic muscles develop. The nervous system assumes its adult form and adult neurons differentiate from cells which had previously lain dormant in the nervous system. The compound eyes, frontal filaments and optic ganglia degenerate, but the median eye persists apparently unchanged. The yellow cells disperse, but their function is unknown. The cement glands persist in the adult, but the adult gland cells differentiate from cells aroung the collecting duct of the larval gland while the larval cement gland cells histolyse. The median eye persists, but in the newly moulted adult the three components separate giving rise to the three adult photoreceptors: a pair of pigmented ocelli and a median unpigmented photoreceptor. Shortly after the moult the young adult resumes feeding.

This study has shown that metamorphosis in *Balanus balanoides* must be thought of in terms of the change from the nauplius through the cypris to the young adult and not just as the changes taking place between settlement and ecdysis to the young adult.

1. Introduction

Balanus balanoides (L.) (Cirripedia, Thoracica) has seven planktonic larval stages. The first six are nauplius larvae while the seventh is the cypris larva—specially adapted to locate a suitable place for settlement and the completion of metamorphosis to the attached adult stage. Although a great deal of research has been carried out on cirripedes as fouling organisms, our knowledge of the precise anatomical changes that take place during the metamorphosis, from the larval stages to the adult, is still incomplete. There is an extensive literature on the morphology of the nauplius larva and several accounts of cirripede 'metamorphosis' have been published (Darwin 1851; Hoffendahl 1904; Doochin 1951; Bernard & Lane 1962; Kaufmann

1965). Most authors consider 'metamorphosis' to be the change from cypris to adult; however, I hope to show in this paper that the process of metamorphosis in cirripedes can more usefully be considered as comprising the change from stage 6 nauplius larva through the cypris larva to the adult. A detailed study of the anatomical changes that occur during metamorphosis in *B. balanoides* is of interest for several reasons. First as an aid to the interpretation of adult anatomy and to provide a basis for comparative studies on metamorphosis in the other sub-orders of the Cirripedia (Acrothoracica, Rhizocephala and Ascothoracica). Secondly, to provide the basic knowledge of morphological changes which is essential to more detailed studies on particular aspects of metamorphosis and settlement. Finally, to compare with the metamorphoses of other organisms to see whether the problems common to metamorphosis are solved in a similar manner.

2. Materials and methods

Nauplius and cypris larvae of *B. balanoides* were collected from the Menai Straits in April and early May, fixed in Bouin's fluid preheated to 60 °C (Wilson 1932) and staged by size according to Crisp (1962). Stage 6 nauplii were subdivided into three groups according to the degree of pigmentation of the developing compound eyes of the cypris larva:

(*a*) Stage 6 (1)—unpigmented.

(*b*) Stage 6 (2)—red-brown pigmentation.

(*c*) Stage 6 (3)—black pigmentation.

Living Stage 6 (3) nauplius larvae moulted in the laboratory to give cypris larvae and these were fixed at intervals during the hours immediately following ecdysis.

A number of cypris larvae settled on pitted slates in the laboratory and these were sampled at intervals between the time of attachment and ecdysis which occurs about 24 h later (Crisp & Stubbings 1957). A further series was sampled at daily intervals during the first 10 days growth after metamorphosis. All samples were fixed in preheated Bouin's fluid.

Several specimens from each sample were dehydrated in ethyl alcohol, cleared in cellosolve and embedded in Ester Wax 1960 (Steedman 1960). Longitudinal, transverse and frontal sections were cut at a thickness of 4 μm. The sections were stained with Masson's trichrome stain using either Hansen's iron trioxyhaematin or Heidenhain's iron haematoxylin (Pantin 1959).

It would perhaps have been preferable to use larvae reared in culture in order to be able to estimate precisely the points in the larval sequence at which particular organ systems appear. It seemed more important to the author, however, to describe the sequence of events rather than to be able to estimate the exact moment at which a particular organ system appears, or the relative times taken to pass through the different stages in metamorphosis, and for this purpose samples taken in the field were adequate.

Observations on fixed and sectioned material were supplemented by observations on whole living larvae, and by phase-contrast examination of blood smears from free-swimming and attached cypris larvae.

3. The anatomy of the nauplius larva

Darwin (1851, 1854) described the external morphology, and as much of the internal anatomy as could be discerned from whole specimens, of Stages 1 and 2 larvae of *Scalpellum vulgare* (*S. scalpellum* (L.)), *Ibla quadrivalvis* Cuv. and *Balanus balanoides* (L.). His errors of interpretation

were gradually corrected and further anatomical details described by subsequent workers, notably Münter & Buchholz (1869), von Willemoes-Suhm (1876) and Hoek (1876), but it was not until the classic work of Groom (1894) that there was a satisfactory account of the internal anatomy of cirripede nauplius larvae. Groom reviewed the early literature on cirripede embryology and larval development and gave a very thorough and accurate account of the anatomy and histology of Stages 1 and 2 nauplius larvae of *Balanus perforatus* Bruguiére, *Chthamalus stellatus* (Poli), *Lepas anatifera* (L.), *L. pectinata* Spengler and *Conchoderma virgatum* (Spengler). Unfortunately he did not publish his studies on the later stages of larval development.

The following account of the anatomy and histology of the nauplius larva of *B. balanoides* confirms and extends Groom's observations.

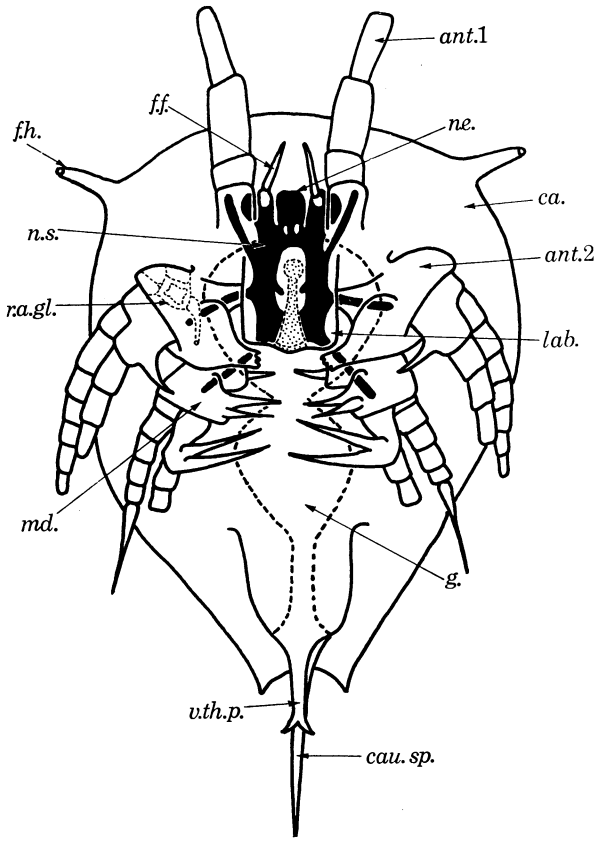


FIGURE 1. Diagram to illustrate the major features of the anatomy of the nauplius larva of *B. balanoides* as seen from the ventral surface. For list of abbreviations see p. 280.

The newly hatched nauplius larva of *B. balanoides* in British waters average 340 μm in total length (carapace plus caudal spine). They moult five times attaining an average length of 1150 μm in the sixth and final nauplius stage (Crisp 1962). The main features of the nauplius structure are retained until the nauplius-cypris moult, but in stages 4 to 6 the simplicity of the nauplius anatomy is progressively obscured by the development of the rudiments of the cypris appendages and organs.

(*a*) *External features*

The external features (figure 1) of the cirripede nauplius larva have been described by numerous workers so they will be mentioned only briefly here.

The dorsal body cuticle is expanded to form a shield-shaped carapace with a pair of prominent frontal horns which are characteristic of all known cirripede nauplii (figures 1 to 5).

Ventrally there are three pairs of jointed appendages: the uniramous, four-segmented antennules, the biramous antennae, and the biramous mandibles. The details of their setation and external morphology have been described by Bassindale (1936) and Crisp (1962) and will not be considered further here. The prominent labrum lies between the protopodites of the antennae and overhangs the mouth anteriorly (figures 1 and 6). A pair of unjointed filiform appendages are found between the antennules and anterior to the labrum on the ventral surface of the larva—these are the frontal filaments (figures 1 and 6).

Posterior to the appendages lies the thoracic region of the nauplius which becomes relatively larger at each moult in order to accommodate the developing thorax and limbs of the cypris stage (figure 6). The thorax tapers to a bifurcate ventral process (figures 1 and 3). The anus is situated between the ventral thoracic process and the caudal spine which projects posteriorly from beneath the posterior border of the carapace.

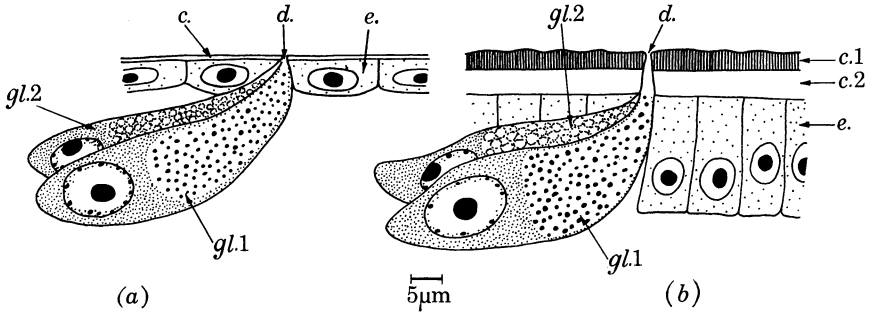


FIGURE 2. Diagram to compare the structure of the integument of the nauplius larva with that of the cypris larva. (*a*) The integument of the nauplius larva. (*b*) The integument of the cypris larva. The two-celled epidermal glands open through ducts (*d*) to the exterior. The secretion in one cell of the gland (*gl.* 1) stains black with iron haematoxylin, that in the second cell (*gl.* 2) stains pale green with light green.

(*b*) *Integument*

Following Richards (1951) the 'integument' is here understood to include both the cuticle and the underlying epidermis with its basement membrane. Over most of the body surface the cuticle is approximately 1 μm thick increasing to 3 μm in the region of the mouth. The epidermal cells are flattened or cubical. There are regularly arranged epidermal glands opening at intervals through the carapace cuticle. Their structure is seen most clearly at this stage (figure 2*a*) and is the same as that of the cypris epidermal glands (figure 2*b*). Each gland consists of two glandular cells approximately 60 μm long opening through a single cuticular pore and with nuclei 12 to 15 μm in diameter. The secretory granules within the two cells have markedly different affinities for stains: the secretion in one cell of the pair stains black with Heidenhain's iron haematoxylin while that in the second cell stains pale green with light green.

A pair of very much larger, but histologically similar, epidermal glands open through pores situated at the tip of each frontal horn. In the Stage 6 nauplius each frontal horn gland consists of a pair of spindle-shaped cells about 130 μm long with nuclei about 15 μm in diameter. All these epidermal glands bear a superficial resemblance, both in their structure and in their reaction to staining with iron haematoxylin and light green, to the luminescent glands in the

copepod *Metridia lucens* Boeck described by Clarke, Conover, David & Nicol (1962). However, there is no record of luminescence in cirripede nauplii and their function is unknown.

Anteriorly an unpaired, unicellular epidermal gland opens through a pore in the mid-dorsal line (figure 6) (Kauri 1962). The secretion in this cell stains pale green with light green but its function is unknown.

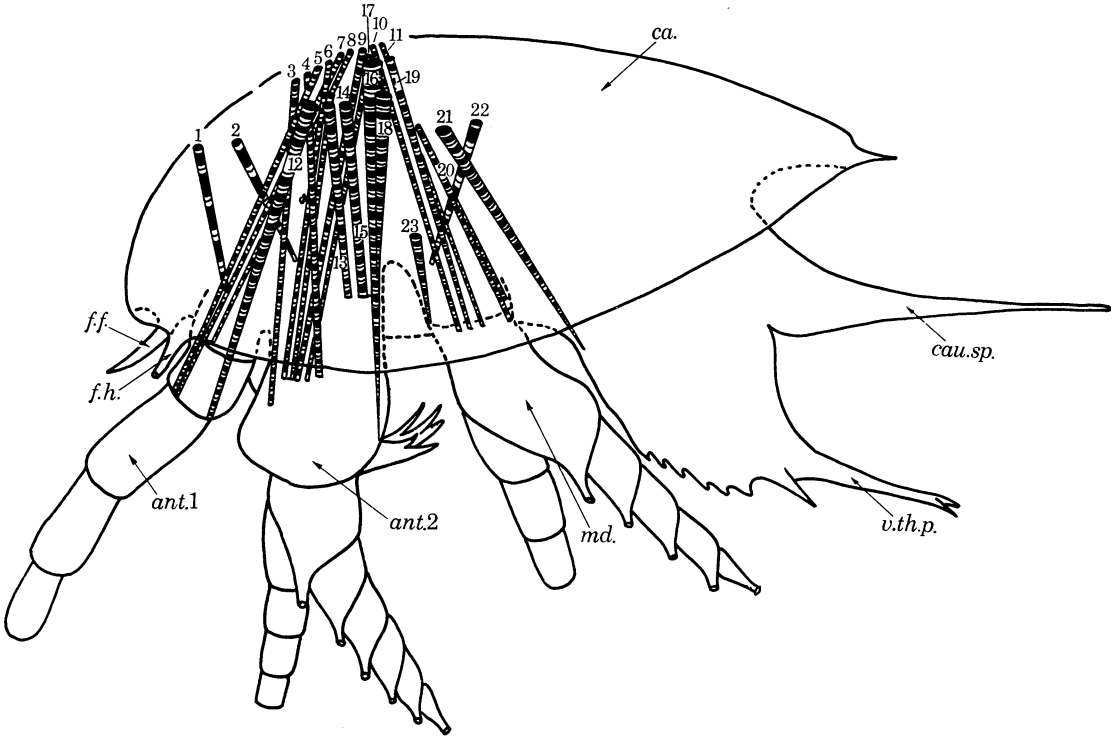


FIGURE 3. Diagram to illustrate the disposition of the dorsal extrinsic limb muscles of the nauplius larva of *B. balanoides*. Reconstructed from whole mounts and serial sections. The muscles are numbered 1 to 23 to simplify description in the text.

(*c*) *Musculature*

The extrinsic limb muscles and other muscles in the main body region of the nauplius larva are illustrated in figures 3 to 5. The disposition of the muscles was studied to determine which, if any, were retained in the cypris larva and the adult. The precise manner in which each muscle is attached to the integument was not investigated.

Two types of striated muscle were found: first, muscles with a sarcomere length of 1.5 to 2 μm with well marked A bands (type A), and secondly muscles with a sarcomere length of 5 to 9 μm and less distinct A bands (type B) (figure 19, plate 58). Most of the extrinsic and intrinsic limb muscles are of type A. The dorsal extrinsic limb muscles arise from the dorsal region of the carapace (figures 3 and 4) which is probably held taut by the hydrostatic pressure of the body fluids. The ventral extrinsic limb muscles are attached to the ventral endoskeleton (figure 5) which seems to be held in position by muscles of type B. The latter muscles are so placed as to act as braces tying the endosternites to one another and to the exoskeleton (figure 5). The endosternites are tied dorsally to the integument by the paired muscles 2 and 22. Ventrally they are tied to the protopodites of the limbs by the paired muscles 26, 28 and 37 (figure 5). The two arms of the anterior endosternites are joined to the exoskeleton directly, and to each other by a single transverse muscle (24) passing behind the oesophagus. The posterior endosternite

is joined directly to the exoskeleton at the base of the mandible. In addition, there is a pair of endoskeletal rods attached to the exoskeleton between the bases of the antennae and mandibles which is tied to the posterior endosternite by the paired muscle 35 and to the arms of the anterior endosternite by the paired muscle 30 (figure 5).

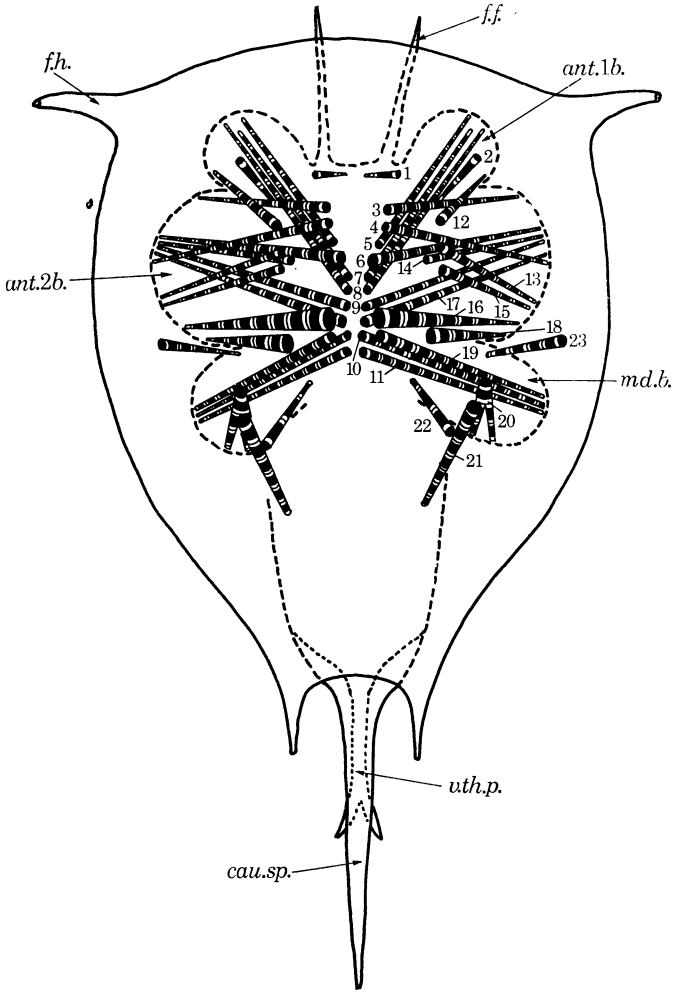


FIGURE 4. Diagram to illustrate the disposition of the dorsal extrinsic limb muscles in the nauplius larva of *B. balanoides*, viewed from the dorsal aspect. Reconstructed from whole mounts and sections. The muscles are numbered to simplify description in the text.

The two types of muscle described above probably correspond to the fast and slow crustacean muscle fibres described by Fahrenbach (1967). He found that fast fibres were characterized, under the light microscope, by having a short sarcomere length (2.5 μm in the appendicular muscles of *Balanus cariosus*) while slow fibres were characterized by having a long sarcomere length (8 μm in the scutal depressor muscles of *B. cariosus*). In the fast fibres seen under the electron microscope, each thick myofilament was surrounded by six thin filaments while in the slow fibres each thick myofilament was surrounded by approximately twelve thin filaments. Similar differences were found between fast and slow fibres in other crustaceans. In the accessory flexor muscle of the meropodite of the walking leg of *Cancer magister* these morphological differences were correlated with the physiological properties of the two types of muscle. The fibres with

the short sarcomere length are characterized by the rapid development of tension in response to a brief stimulus and rapid relaxation as a result of sustained depolarization. The fibres with the long sarcomere length, however, maintain contractions over long periods of time during sustained depolarization. It would seem that the type A muscles seen in the nauplius larva are fast muscles while the type B muscles are slow. The latter muscles probably fall into the category of slow muscles described by Fahrenbach as 'postural'.

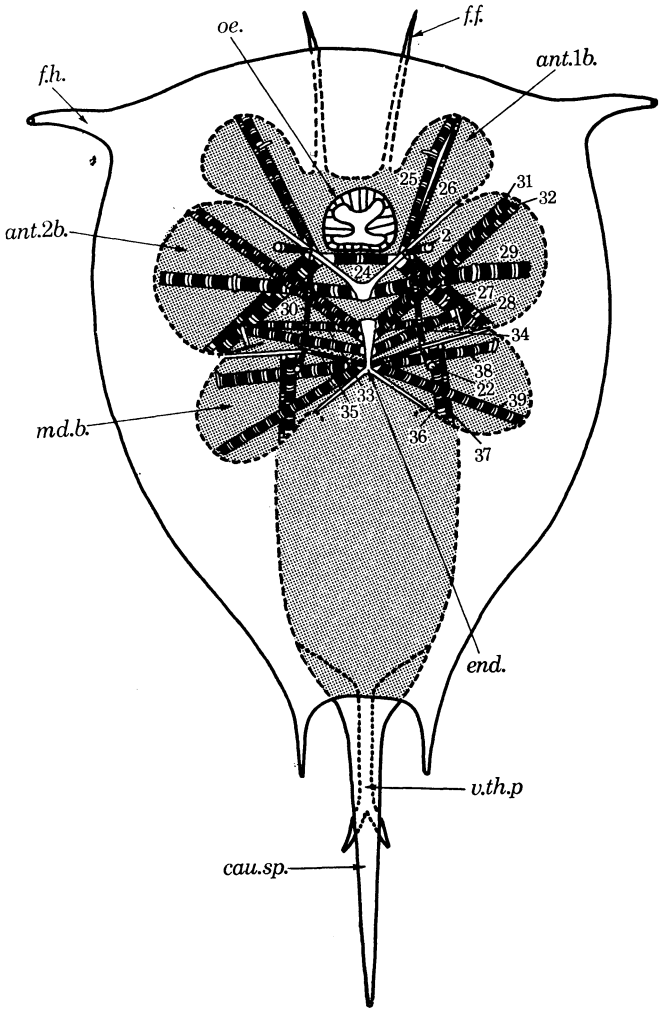


FIGURE 5. Diagram to illustrate the disposition of the ventral extrinsic limb muscles in the nauplius larva of *B. balanoides*, viewed from the dorsal aspect with the overlying structures omitted. Reconstructed from whole mounts and sections. The muscles have been numbered 24 to 39 to simplify description in the text.

There are twenty-three pairs of muscles attached to the dorsal region of the carapace (figures 3, 4). Of these, three pairs are of type B: first a pair joining the dorsal and ventral regions of the carapace and passing between the nauplius eye and the protocerebrum (1), secondly a pair passing to the anterior ventral endosternite (2), and thirdly a pair passing to the posterior ventral endosternite (22). Of the remaining twenty pairs, which are of type A, nineteen pairs are attached at various points in the protopodites of the limbs while the twentieth pair is attached to the integument just anterior to the developing first pair of maxillae (21) (figure 3). The nineteen pairs going to the protopodites are distributed as follows: four pairs

to the antennules (5, 7, 8 and 12), ten pairs to the antennae (3, 4, 6, 9, 13, 14, 15, 16, 17 and 18) and five pairs to the mandibles (10, 11, 19, 20 and 23) (figures 3, 4).

The ventral extrinsic limb muscles arise from the endosternites: one pair (25) runs anteriorly to the antennules. There are six pairs of muscles (27, 29, 31, 32, 33 and 34) passing into the antennae to be attached in the protopodite (figure 5), and three pairs (36, 38 and 39) passing into the mandible again to be attached in the protopodite.

(*d*) *Nervous system and sense organs*

The anatomy of the nervous system in the first five nauplius stages is shown diagrammatically in figures 1 and 15. In the absence of information on the embryological origin of the different regions of the nervous system it was decided to use nomenclature based on anatomical considerations alone. The pre-oral brain receives the four short nerves from the median nauplius eye antero-dorsally (Kauri 1962) and has two lateral lobes, in each of which a spherical sinus is situated ventrally at the base of the frontal filament (figures 20 and 21, plate 58). The antennular nerves arise ventro-laterally from the posterior region of the brain. The circumoesophageal connectives join the brain to the first pair of post-oral ganglia, the antennal ganglia. Shortly before these connectives enter the antennal ganglia the labral nerves arise ventrally. The paired antennal ganglia are joined by a transverse commissure and give rise to the antennal nerves laterally. Longitudinal connectives join the antennal ganglia to the second pair of post-oral ganglia, the mandibular ganglia. These, too, are joined by a transverse commissure and give rise to the nerves to the mandibles.

Bullock & Horridge (1965) attempted to standardize the nomenclature of the anterior ganglia of the crustacean nervous system. They based their definitions on commonly accepted usage of the terms rather than on phylogenetic or embryological considerations of homology. In their scheme the 'protocerebrum' was defined as the anterior region of the brain receiving nerves from the eyes and frontal organs, the 'deutocerebrum' as the region of the endings of the antennular sensory nerves, the 'tritocerebrum' as the 'ventral, caudal, or inferior part of the brain which gives rise to nerves to the labrum, the stomatogastric system to the alimentary canal and a post-oral commissure' and which receives sensory nerves from the antennae. On the basis of their definitions the pre-oral brain of the cirripede nauplius would consist of protocerebrum + deutocerebrum while the post-oral antennal ganglia would be the tritocerebrum. The mandibular ganglia would be the same.

The structure of the nauplius eye was well described by Kauri (1962) in Stages 5 and 6 nauplius and cypris larvae of *B. balanoides* and *B. crenatus*. There are two pigment cells. These are arranged in the centre of the nauplius eye so as to form three pigment cups: one ventral and two dorso-lateral (figure 22, plate 58). All three pigment cups contain four retinula cells, each of which forms a rhabdomere along the side of the cell adjacent to another retinula cell. In each dorso-lateral component there is also a fifth cell, smaller than the retinula cells and apparently without a rhabdomere. In each component of the eye the apposed rhabdomeres form the rhabdome (figure 22, plate 58).

In the ventral component, all four retinula cells send processes to the median ventral region of the brain via a median ventral nerve. In each lateral component, however, the two dorsal retinula cells send processes via a median dorsal nerve to the brain, while the two ventral retinula cells and the fifth cell send processes via a lateral nerve to the brain.

The sinus at the base of each frontal filament (figures 20 and 21, plate 58), seems to contain

coagulated material, which stains with light green. The lumen of the filament contains longitudinal fibres which may arise, at least in part, from the material in the sinus, but which probably also includes two or more nerve fibres arising from the lateral lobes of the brain (figure 21, plate 58). Kauri (1966) investigated the frontal filaments and their associated structures, in a number of cirripede larvae, and concluded that they are 'sensory papilla X organs'. The present author considers, however, that the frontal filaments may be sensory organs, but that there is insufficient evidence for their suggested neurosecretory role and their precise function is unknown.

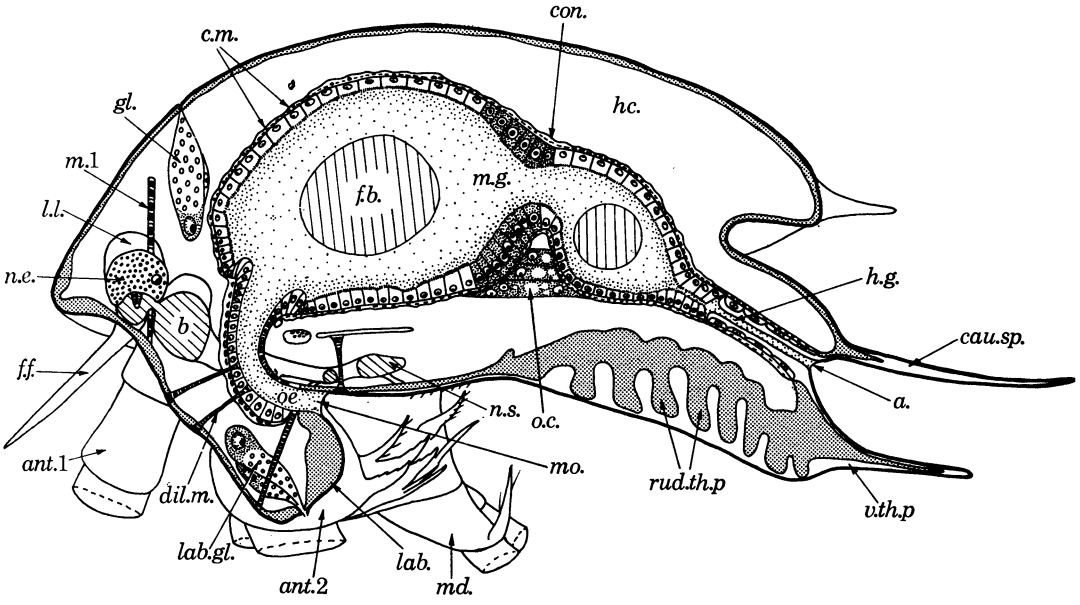


FIGURE 6. Diagram to illustrate the anatomy of a Stage 5 nauplius larva of *B. balanoides* as seen in sagittal section. To simplify this figure most of the muscles have been omitted.

(*e*) *Alimentary canal*

The mouth lies beneath the labrum (figure 6). The oesophagus, which is lined with cuticle continuous with that covering the body surface, passes anteriorly for a short distance and then dorsally to enter the mid-gut. The wall of the oesophagus consists of a single layer of cubical epithelial cells lying on a basement membrane. Radial dilator muscles are attached to the cuticle lining the oesophagus and run to the labral cuticle and the ventral internal skeleton. There is also a layer of striated circular muscles surrounding the oesophagus.

The mid-gut is divided into two regions by a constriction which may reflect a functional division. The region of the mid-gut anterior to the constriction can be further subdivided into two sections characterized by the appearance of the gut epithelium (figure 6). In the anterior section the gut epithelial cells are 8 to 10 μm high with nuclei 5 μm in diameter and frequently a striated border up to 2 μm in depth. In the posterior section the gut epithelial cells are strongly basiphil and increase from a height of 15 μm in Stage 2 to 30 μm in Stage 6 with a corresponding increase in nuclear diameter from 5 to 10 μm. These cells also have a striated border about 2 μm high and there are small droplets in the cytoplasm in the distal region of the cells. These cells are very similar to those of the adult digestive gland and may have the same function. They disappear during development, just before the digestive gland first appears (see § 5*f*).

In Stage 1 and early Stage 2 nauplius larvae, vacuoles containing large irregular granules are present in the epithelial cells of the mid-gut. These granules are the last remnants of the yolk (Groom 1894).

In the region of the mid-gut behind the constriction, the epithelial cells average 7 μm in height with nuclei 5 μm in diameter and a striated border 1 μm deep. The hind-gut, like the oesophagus, is lined with cuticle and the anus is between the caudal spine and the ventral thoracic process. There is an outer layer of circular muscle over the whole of the mid-gut which apparently consists of unstriated muscle fibres.

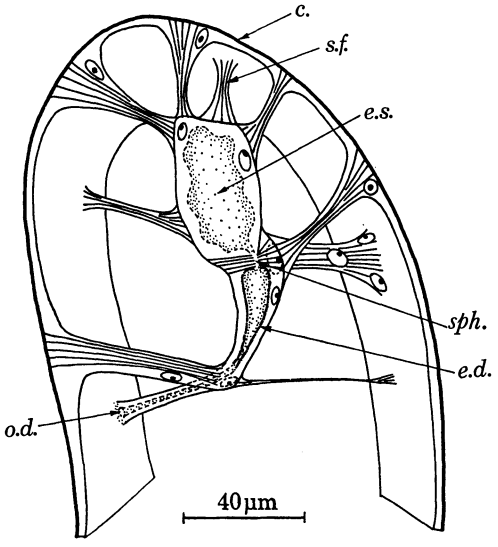


FIGURE 7. Diagram to illustrate the structure of the antennal gland in the nauplius larva of *B. balanoides*.

At the tip of the labrum is found the pore or pores of the labral glands (figure 6) (= the 'axial' gland of Groom 1894). The histological appearance of these glands is similar to that of the adult labial glands (Walley 1967). In the Stage 2 nauplius larva there is a single gland consisting of four glandular cells opening through a common duct. By Stage 6 there are three glands. Each gland cell contains secretion granules staining with light green. The secretion produced by the glands may bind food particles together before these are ingested.

(*f*) *Excretory system*

In the adult the organs responsible for excretion and ionic regulation are probably the paired maxillary glands but these glands are not present in the nauplius larva except in a rudimentary state (see § 5 *g*). During this investigation antennal glands were found in the cirripede nauplius larva for the first time, although Grobben described antennal glands in copepod nauplii as early as 1881. They are thought to be the organs responsible for ionic-regulation and excretion in the nauplius.

Antennal glands were found in all the nauplius stages (figure 7; figures 23 and 24, plate 58) in the protopodites of the antennae. Each gland consists of an end sac and an excretory duct which opens to the exterior on the posterior face of the limb. The end sac is a flattened sac, about 60 μm long by 25 μm wide, suspended in the haemocoel by nine bundles of fibres. The wall of the sac consists of a network of fibres to the inner surface of which are attached about six epithelial cells. These cells stain lightly and have numerous granules in the cytoplasm

(figure 23, plate 58); they are very similar to the cells lining the end sac of the maxillary gland of the adult.

At the junction between the end sac and the excretory duct there are three cells with intracellular fibrils similar to those described by Cannon (1925, 1926) in the antennal glands of ostracods and the maxillary glands of *Chirocephalus diaphanus* Prevost. These cells are so arranged that the fibrils form a triangle and Cannon supposed that they acted as a sphincter muscle around the duct between the end sac and the excretory duct. In spite of a very careful search, no duct between the end sac and the excretory duct in the cirripede nauplius antennal gland, was observed, but it seems probable that it does run through the centre of the triangle of fibrils.

The excretory duct (figure 24, plate 58) can be subdivided into two regions. The proximal region, next to the end sac, drains into the distal region which opens to the exterior through a small pore on the posterior surface of the protopodite. The proximal region is funnel-shaped, tapering from a diameter of about 15 to 5 μm with an irregular intracellular duct (figure 7; figure 23, plate 58). The cytoplasm of the duct cell has a very homogeneous appearance and stains pale green with light green; the cytoplasm bordering the lumen of the duct stains more deeply than the rest. The distal region of the excretory duct tapers from about 5 to 3 μm in diameter and its narrow intracellular duct is lined by an extremely delicate layer of cuticle continuous with that covering the body surface (figure 7; figure 24, plate 58).

(*g*) *Oil cells*

Lying in the mid-gut constriction and in a band encircling the middle region of the mid-gut are found cells which are characteristically elongated with a finely granular basiphil cytoplasm, one or more cytoplasmic vacuoles and one or more (up to 8) nuclei (figure 6; figures 25 and 26, plate 58). Cells of this type are present in the mid-gut constriction of the newly hatched larva and they increase in size and number as the larva grows. They can be seen to be multinucleate by the time the nauplius reaches Stage 4 or Stage 5. These are the cells which develop into the oil cells of the cypris larva (see § 5*j*).

Description of plate 58

Figure 19. Two types of striated muscle in the nauplius larva. *A*, type A with a sarcomere length of 1·5 to 2 μm; *B*, type B with a sarcomere length of 5 to 9 μm.

Figure 20. Section through the brain of a nauplius larva showing the median eye and the sinuses in the brain at the bases of the frontal filaments. *e.*, median nauplius eye; *f.f.*, frontal filament; *s.*, sinus.

Figure 21. Section through the base of a single frontal filament. *a.*, nerve fibres passing from the brain to the filament; *f.f.*, frontal filament; *fi.*, fibres within the filament; *s.*, sinus at the base of the filament.

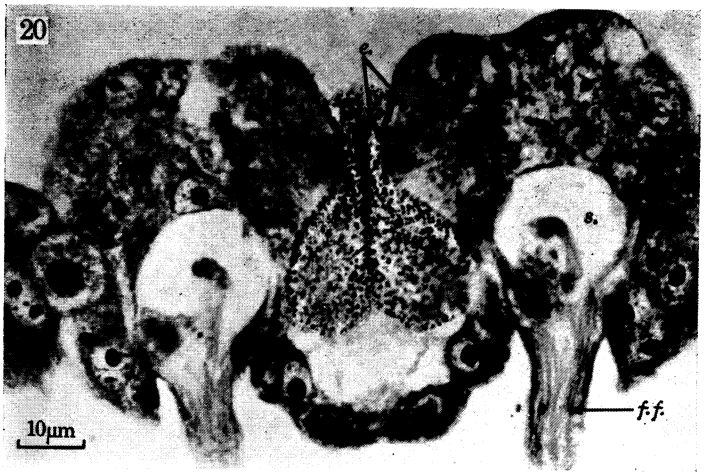
Figure 22. Transverse section through the median eye showing the two pigment cells and the three components of the eye, two dorso-lateral and one ventral. *d.c.* 1 and *d.c.* 2, dorso-lateral components; *p.c.*, pigment cells; *ret.*, retinula cell; *rh.*, rhabdome; *v.c.*, ventral component.

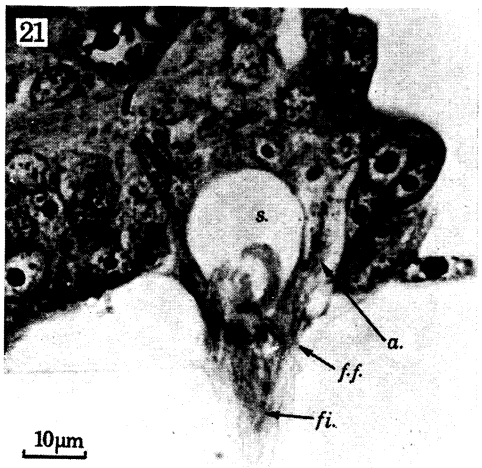
Figure 23. Section through the end sac and part of the excretory duct of the antennal gland. *d.p.*, proximal region of the excretory duct; *e.s.*, end sac; *f.*, intracellular fibrils; *s.f.*, suspensory fibres; *sph.*, probable position of sphincter between the end sac and excretory duct.

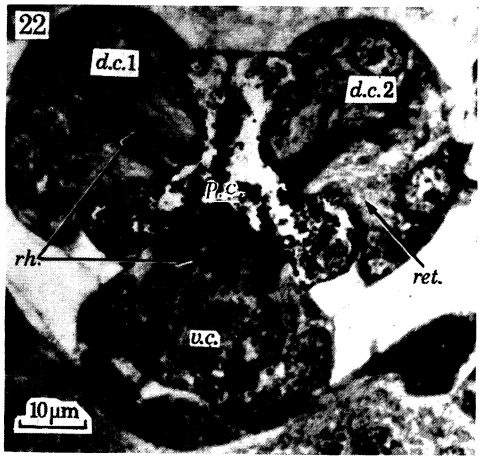
Figure 24. Section through the excretory duct of the antennal gland. *d.d.*, distal region of the duct; *d.p.*, proximal region of duct; *p.*, excretory pore.

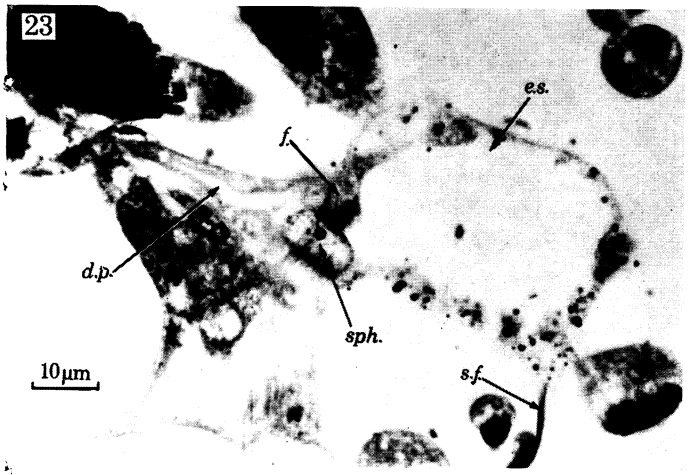
Figure 25. Frontal section through the oil cells lying in the mid-gut constriction of a Stage 5 nauplius larva. *m.g.a.*, anterior region mid-gut; *m.g.p.*, posterior region of mid-gut; *o.c.*, oil cells.

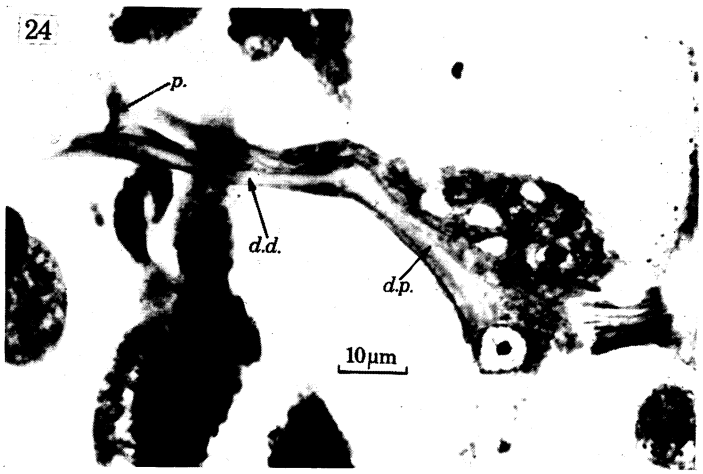
Figure 26. Frontal section through a single oil cell lying in the mid-gut constriction of an early stage 6 nauplius larva. *nu.*, oil cell nuclei; *vac.*, oil vacuole.

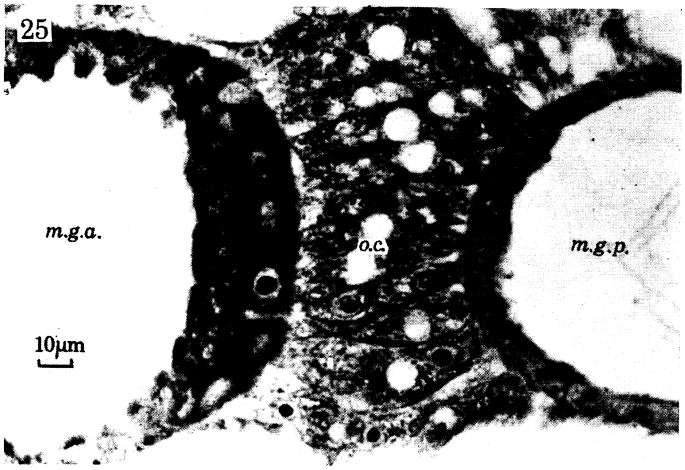


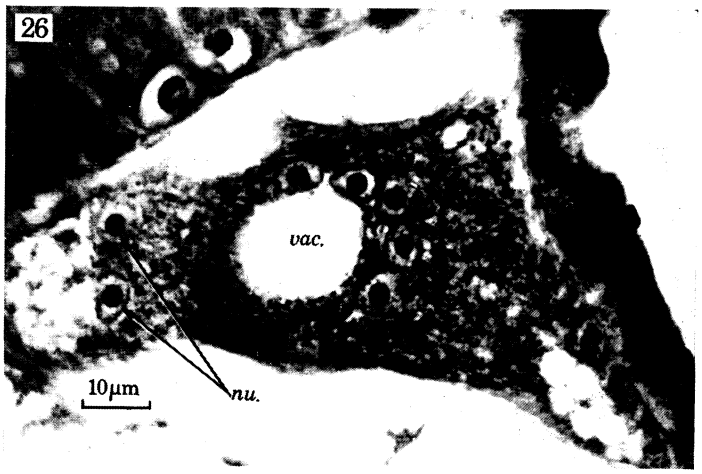












FIGURES 19 TO 26. For legends see facing page.

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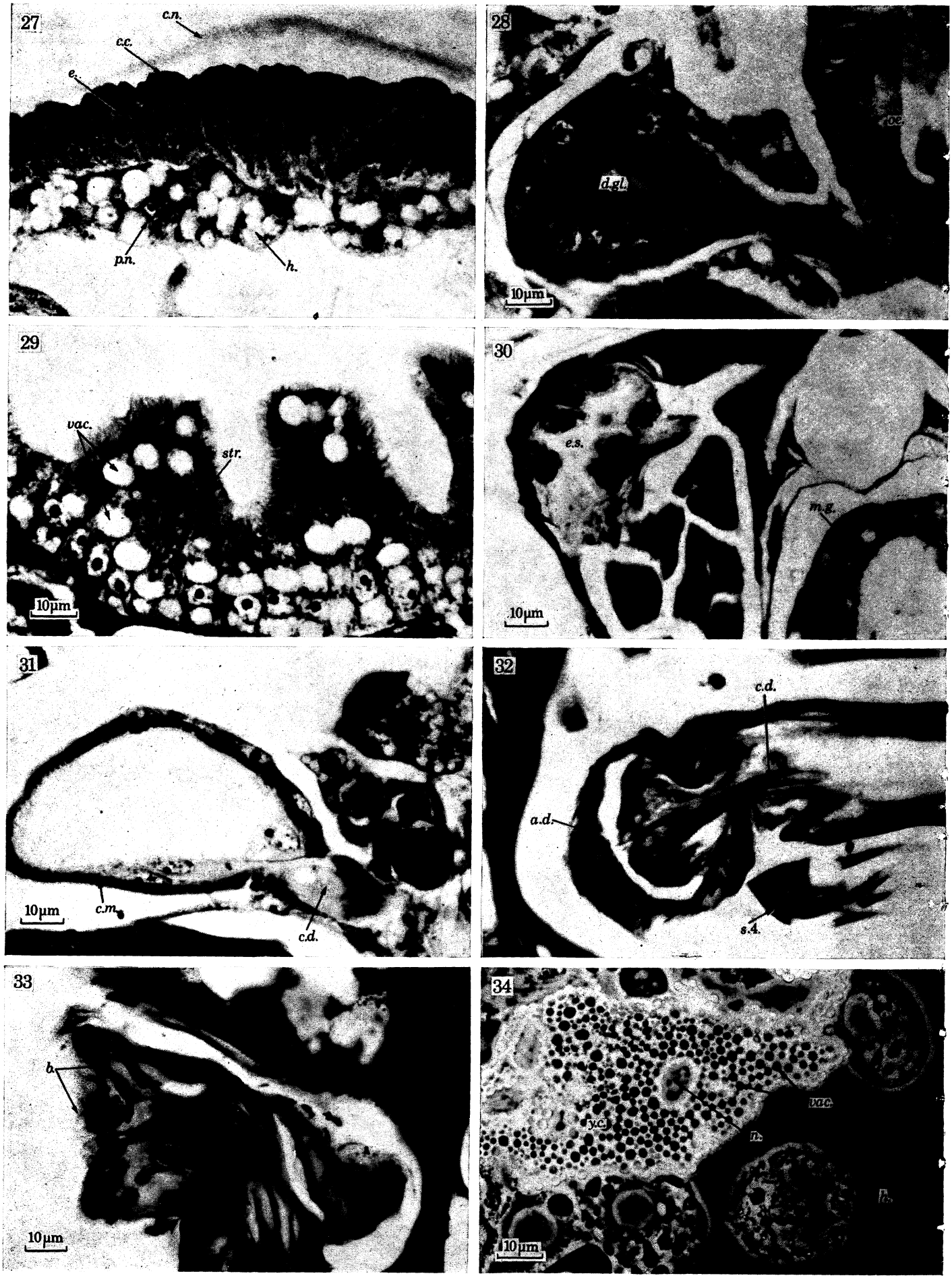

FIGURES 27 TO 34. For legends see facing page.

(*h*) *Haemocytes*

The nauplius larva has no heart; presumably the blood circulates in the haemocoel as a result of muscular activity. Haemocytes are found scattered through the haemocoel, and often applied to the under surface of the epidermis or in contact with the developing oil cells in the mid-gut constriction. In sections the haemocytes appear as large irregular cells with one, or perhaps more, nuclei and a number of cytoplasmic vacuoles (figure 27, plate 59). The vacuoles often appear to be empty, suggesting that their contents are water or alcohol soluble and have been lost in preparation. The number of haemocytes increases considerably in the later nauplius stage, but whether they arise by cell division at a specific site within the nauplius or whether they arise by the division of circulating haemocytes, is not known. Cell division in haemocytes was not observed. A prophase nucleus was found in one haemocyte (figure 27, plate 59), but it is possible that it would have given rise to an extra nucleus within the haemocyte rather than an extra haemocyte. The functions of these cells are described in §§ 5 *d* and 5 *k*.

4. The anatomy of the cypris larva

Darwin (1851, 1854) described the cypris larvae of *L. australis* Darwin and other cirripedes and remarked on the great morphological similarity between the larvae of different species. Claus (1869) described the anatomy of the cypris larvae of *L. pectinata*, *L. fascicularis* and *Conchoderma virgatum*. Like Darwin he worked from whole mounts and dissections but added considerably to Darwin's observations. Apart from work by Hoek (1884) on *L. australis* and Hoffendahl (1904) on *Poecilasma aurantium* Darwin, little advance in our knowledge of the anatomy of the cypris larva was made until the work of Doochin (1951) on *B. improvisus* and *B. amphitrite niveus*. The following description of the anatomy of the cypris larva of *B. balanoides* confirms and extends many of Doochin's observations, and also corrects some of the mistakes in the functional interpretation of structure made by Doochin and other authors.

The cypris larva of *B. balanoides* is approximately 1100 μm long and 500 to 600 μm dorso-ventrally. In the first few hours following the nauplius–cypris moult certain morphological

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Description of plate 59

Figure 27. Section through the integument of a late Stage 6 nauplius larva showing the haemocytes beneath the epidermis. *c.c.*, pre-ecdysial carapace cuticle of cypris larva; *c.n.*, carapace cuticle of nauplius larva; *e.*, epidermis; *h.*, haemocyte; *p.n.*, haemocyte nucleus in prophase. Scale as on figure 28.

Figure 28. Section through one of the paired rudimentary digestive glands in the cypris larva. *d.gl.*, digestive gland; *oe.*, oesophagus.

Figure 29. Section through the wall of the mid-gut of the cypris larva. *str.*, striated border; *vac.*, vacuoles.

Figure 30. Section through the antero-lateral region of the thorax of the cypris larva showing the end sac of the maxillary gland. *e.s.*, end sac; *m.g.*, mid-gut.

Figure 31. Longitudinal section through the muscular sac of the cypris cement gland system. *c.d.*, collecting duct; *c.m.*, layer of circular muscle.

Figure 32. Longitudinal section through the distal region of the cypris antennule showing the cement duct *a.d.*, adhesive disk; *c.d.*, cement duct; *s.* 4, fourth segment of the antennule. Scale as on figure 33.

Figure 33. Section through the adhesive disk showing the terminal branches of the cement duct. *b.*, branches of the cement duct.

Figure 34. Living haemocytes and a yellow cell from a cypris larva as seen under phase contrast illumination. The irregular outline of the yellow cells is a result of damage to the limiting membrane of the cell. *h.*, haemocyte; *n.*, nucleus of the yellow cell; *vac.*, vacuoles; *y.c.*, yellow cell.

changes take place which will be described in the section on the development of the cypris larva. This account of the structure of the cypris larva is concerned with the larva after these postecdysial changes have been completed. Such a larva, if presented with a suitable surface, will settle and complete its metamorphosis.

(*a*) *External features*

Some features of the anatomy of the cypris larva are illustrated diagrammatically in figure 8.

The whole animal is enclosed in a bivalved carapace, the two valves of which can be drawn together by a ventral adductor muscle (figures 8, 9 and 12). The anterior and posterior mantle cavities are effectively separated by the oral cone. The anterior mantle cavity encloses the antennules (figures 8, 9 and 12) and the compound eye–frontal filament complexes which are contained in dorso-lateral pockets (figures 10 and 11). The posterior mantle cavity surrounds the thorax which bears six pairs of biramous swimming appendages (figures 8, 9 and 12).

The antennules arise dorso-laterally in the anterior mantle cavity. At the base of each antennule the cuticle is thickened on either side forming an antero-posterior 'rod'. The antennules have four segments (figure 8). The basal segment is directed posteriorly and articulates with the forwardly directed second segment to form an 'elbow' which is accommodated in posterior pockets of the anterior mantle cavity. The long second segment tapers distally to its point of articulation with the third segment. The third segment is approximately bell-shaped with the closed mouth of the bell forming an adhesive disk (figure 8; figure 33, plate 59). This adhesive disk enables the cypris larva to cling to any type of surface—even glass. The cuticle on the adhesive surface has a felt of short (2 to 3 μm) hair-like processes (= microtrichia of Richards 1951) which were figured by Nilsson-Cantell (1921) in the cypris larva of *Scalpellum ventricoseum* Hoek and *Tetraclita divisa*. The ducts from the cement glands open through pores on the adhesive disk (see § 4*g*). The short fourth segment, which bears a number of long setae, arises laterally from the third segment. The structure of the third segment of the antennule has recently been described in considerable detail by Nott & Foster (1969) and the adhesive surface by Nott (1969).

The oral cone of the cypris bears the rudimentary adult mouthparts: paired mandibles, first and second maxillae. At this stage both first and second maxillae have epidermal glands of a type similar to those seen in the adult labium. The glands of the first maxillae are lost before the final ecdysis to the adult and the significance of their presence at this stage is not known. The epidermal glands in the second maxillae persist in the adult as the first of the labial glands.

There are six pairs of thoracic appendages each consisting of a small basal segment bearing two rami each with two segments. The details of setation are not considered here. On each side of the anus at the posterior end of the thorax are found the uniramous two-segmented caudal appendages (figure 9).

(*b*) *Integument*

The appearance of the carapace integument in the fully developed cypris larva, as seen in section, is shown diagrammatically in figure 2*b*. The cuticle is about 7 μm thick; an outer layer 3 μm thick (pre-ecdysial cuticle) which stains heavily with iron haematoxylin, and an inner layer 4 μm thick which stains with light green (postecdysial cuticle) (figure 2*b*). Beneath this thick layer of cuticle the epidermis consists of tightly packed columnar cells. Epidermal glands exactly the same as those found in the nauplius larva (see § 3*b*), open through pores scattered over the surface of the carapace. The cuticle covering the thorax and appendages

and lining the mantle cavities is about 1 μm thick and has a weak affinity for light green except in the region of muscle attachments, where there is often an outer layer which stains heavily with iron haematoxylin. The surface of the carapace cuticle is irregularly sculptured (figured 2*b*). In lightly stained preparations the pre-ecdysial cuticle is seen to be striated (figure 53, plate 62). These striations may indicate the positions of pore canals. The postecdysial cuticle shows no striations.

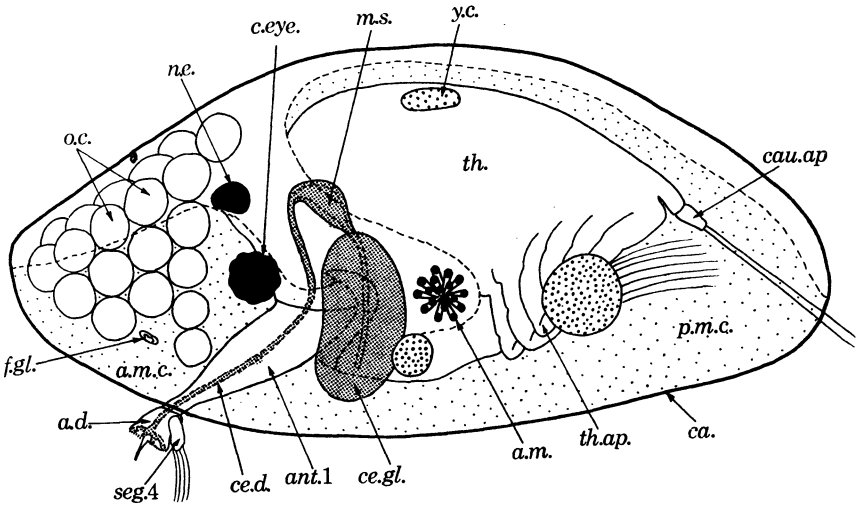

FIGURE 8. Diagram to illustrate the main features of the anatomy of the cypris which can be seen in the living specimen. To simplify the diagram the muscles have been omitted.

The cypris frontal glands open through a pair of complex cuticular pores in a ventro-lateral position towards the anterior end of the carapace (figure 8). Darwin (1854) described these pores in the cypris larva of *B. balanoides* and other cirripedes and interpreted the sac-like glands associated with them as 'acoustic organs'.

(*c*) *Musculature*

The extrinsic limb musculature in the thorax and the musculature of the carapace are illustrated diagrammatically in figure 9.

There are ten pairs of extrinsic muscles associated with the antennules. Two pairs of muscles (1 and 2), which arise in the anterior region of the carapace, are attached to the integument in the region of the inner cuticular rod at the base of each antennule. One pair of muscles (6), arising antero-dorsally from the carapace, is attached to the integument at the anterior end of the outer cuticular rod. A fourth pair of muscles (3), arising antero-dorsally, is attached proximally in the second antennular segment. The remaining six pairs arise dorso-laterally in the posterior half of the carapace (8, 9, 10, 11, 12 and 13). Of these the first two pairs (8 and 9) are attached to the inwardly projecting fold of cuticle between the first and second segments of the antennule and on the median side. The third pair (10) of muscles is attached to the integument at the posterior end of the outer cuticular rod at the base of each antennule. The remaining three pairs (10, 11 and 12) are attached dorsally to the posterior pockets of the anterior mantle cavity which accommodate the 'elbows' of the antennules.

Two pairs of muscles (4 and 5) arise on either side of the mid-dorsal line in the anterior region of the carapace and are attached antero-dorsally in the thorax on an endo-skeletal bar or plate which in turn gives rise to paired short muscles (14) attached to the lateral wall of

the thorax. A pair of slender muscles (7) arises on either side of the mid-dorsal line and runs ventralwards, but extensive search has not revealed its precise point of attachment. The last remaining pair of carapace muscles (16) arises ventro-laterally in the posterior region of the carapace and runs anteriorly to attach on either side of the oral cone.

The transverse adductor muscle (15) is attached ventro-laterally on the carapace (figures 8 and 9). It consists of two cones of muscle joined by a tendon which passes above the circum-oesophageal connectives and posterior to the oesophagus (figure 12).

The intrinsic musculature of the antennules is complex and is not considered here.

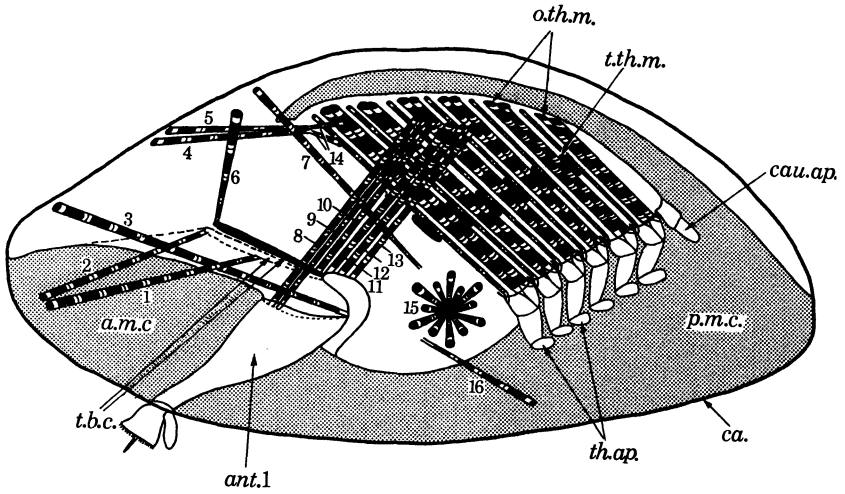

FIGURE 9. Diagram to illustrate the disposition of the muscles of the cypris larva in the carapace and thorax. The carapace muscles have been numbered, 1 to 16, to simplify the description in the text.

In the thorax there are six paired muscle blocks running to the bases of the appendages (figure 9). Each muscle block consists of three muscles: a slender anterior muscle attached proximally to the anterior face of the basal segment of the limb, a stouter muscle which is attached distally to the posterior face of the basal limb segment by a tendon, and the third muscle which is attached proximally to the posterior face of the same limb segment. Six transverse strands of muscle link the two sides of the thorax and run between the gut and the thoracic ganglia (figure 9).

There are two very small muscles associated with each compound eye (figure 10). They arise just above the point where the optic nerve enters the eye; one passes anteriorly in an oblique direction above the frontal filament to be attached to the antero-median face of the compound eye while the second passes laterally to be attached to the posterior face of the eye. These muscles are presumably responsible for the quivering movements of the eyes seen in the living cypris larva.

The rudiments of the adult opercular retractors (figure 11) and adductor scutorum can be seen even in the free-swimming cypris larva.

(*d*) *Nervous system and sense organs*

If the central nervous system of the cypris larva is compared with that of the nauplius it is found that flexure has occurred resulting in a shift of the long axis of the thoracic ganglion relative to the long axis of the brain (figures 12 and 14). The median eye of the nauplius larva is retained. A dorsal view of the central nervous system is illustrated in figure 15.

The lateral lobes of the brain are connected by the optic tracts to the optic ganglia, which lie just anterior to the compound eye—frontal filament complexes (figures 10 and 15). The optic ganglia in their turn are connected to the compound eyes and frontal filaments by the short optic nerves. The antennular nerves leave the posterior region of the brain ventro-laterally and at first pass dorsally before curving ventrally into the antennules where they enter the antennular ganglia (figure 15). Posteriorly the brain is joined to the thoracic ganglion by the circum-oesophageal connectives. The thoracic ganglion gives rise to six pairs of nerves innervating the musculature associated with the swimming appendages.

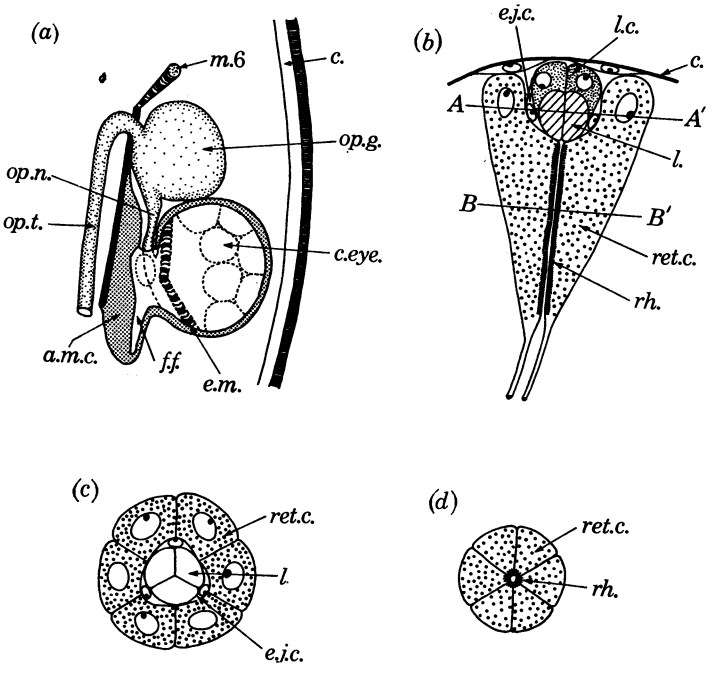

FIGURE 10. Diagrams to show the relationship between the optic ganglion and the compound eye, and the structure of a single ommatidium in the compound eye of the cypris larva. (*a*) Optic ganglion and compound eye. (*b*) Longitudinal section through a single ommatidium. (*c*) Transverse section *AA'* through a single ommatidium. (*d*) Transverse section *BB'* through a single ommatidium.

As in the nauplius larva the brain is connected to the median eye by four short nerves (figure 15). The structure of the median eye is apparently the same as in the nauplius larva (see § 3*d*).

The frontal filaments are found attached to the median faces of the compound eyes. Each compound eye—frontal filament complex is situated in a dorso-lateral pocket of the anterior mantle cavity (figures 10 and 11), attached by a short 'stalk' through which passes the optic nerve from the compound eye and frontal filament to the optic ganglion. Each compound eye (figures 10 and 11) has 10 to 12 ommatidia of the eucone type. Each ommatidium has a spherical lens, about 8 μm in diameter, secreted in three sectors by three cells which are apparently modified epidermal cells. Surrounding the lens secreting cells is an envelope of three flattened cells apparently similar to cells thought by Debaisieux (1944) to be modified epidermal cells. In describing the ommatidia of *Artemia salina* L. he called these cells 'épidermiques juxta-cristallines'. Below the lens and in contact with it is the cylindrical rhabdome formed by the apposition of six rhabdomeres formed by the six retinula cells. The pigment granules are contained in the cytoplasm of the retinula cells as in *Artemia* (Debaisieux 1944). Processes from

the retinula cells seem to form the optic nerve. The outer surface of the eye is covered with a thin layer of cuticle below which is a layer of flattened epidermal cells. Of the crustacean eyes described by Debaisieux (1944) the compound eye of the cypris larva resembles that of *Artemia* most closely.

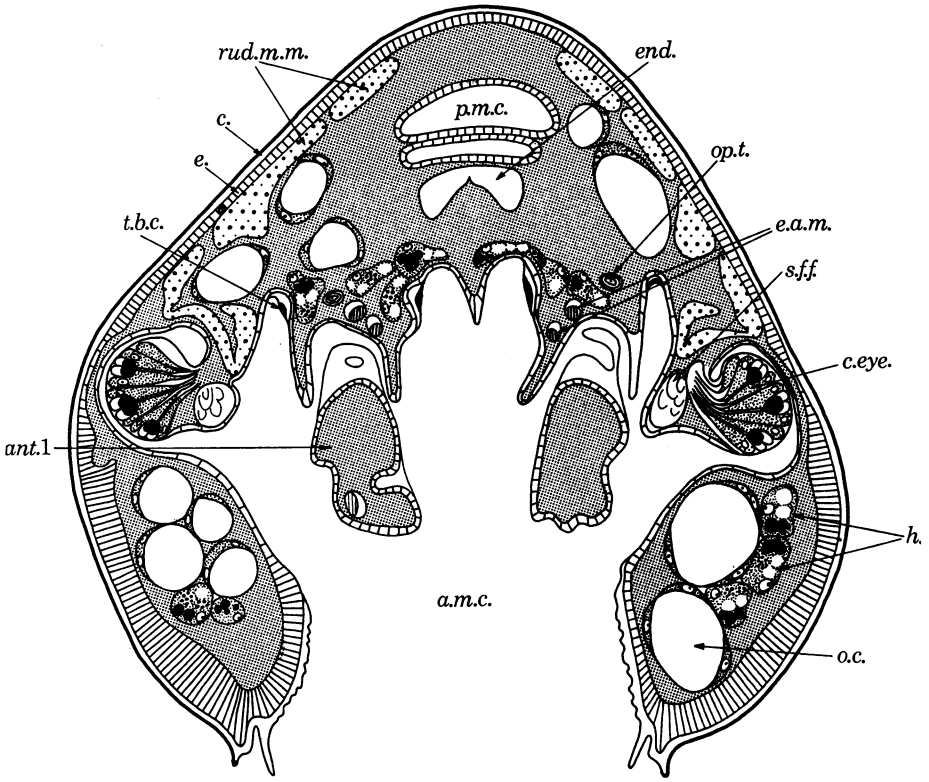

FIGURE 11. Diagram to show the structure of the cypris larva of *B. balanoides* as seen in transverse section in the region of the compound eyes.

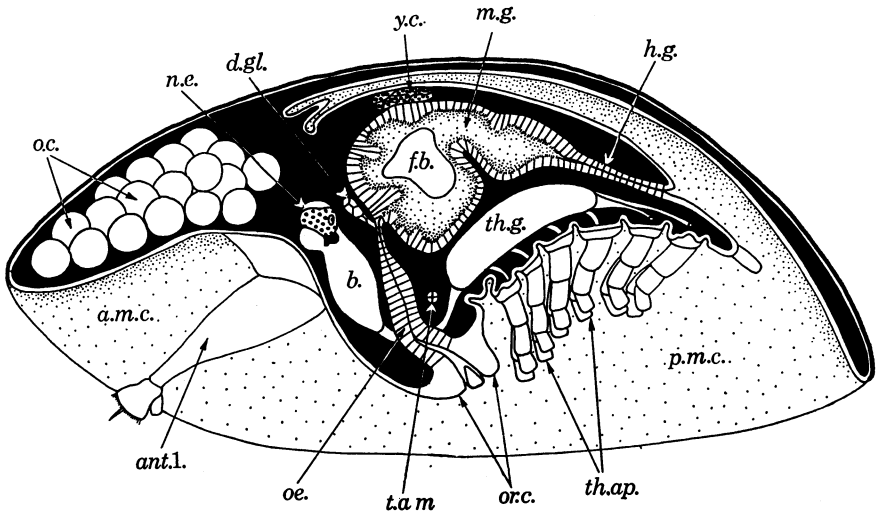

FIGURE 12. Diagram to illustrate the anatomy of a cypris larva of *B. balanoides* as seen in sagittal section. To simplify this figure most of the muscles have been omitted.

(*e*) *Alimentary canal*

Although the rudiments of the adult mouthparts are present by this time, the lumen of the oesophagus is closed (figure 12) and obviously functionless. The rudimentary adult digestive glands lie on either side of the oesophagus (figure 28, plate 59) and join the alimentary canal at the junction of oesophagus and mid-gut. The digestive glands are the 'caeca' described by Darwin (1851) and the 'oesophageal' glands described by Doochin (1951). The wall of the mid-gut consists of a single layer of columnar cells ranging in height from 8 to 30 μm with conspicuous vacuoles in the cytoplasm (figure 29, plate 59) and basal nuclei 5 μm in diameter. The free surface of these cells often has a striated border 3 μm in depth. The lumen of the hind-gut is closed. In the lumen of the mid-gut there is usually a mass of material which is the residue of food ingested by the Stage 6 nauplius larva and may also include cells sloughed from the gut epithelium shortly after the nauplius–cypris moult (see § 5*f*).

From the condition of the gut and the observed behaviour of the cypris larva it can be stated quite categorically that the cypris larva of *B. balanoides* does not feed. Darwin (1851) came to this conclusion which was re-asserted by Doochin (1951) in his study of *B. amphitrite niveus*, although he did not put forward any further evidence on this point. Bernard & Lane (1962), in their study of *B. amphitrite niveus*, found 'food material' in the gut and observed evidence of 'active secretion' in the gut epithelium. They also observed the adult mouthparts and concluded from all these observations that the larvae were feeding actively. The present study has shown, however, that the mouthparts are rudimentary, that the 'food material' in the mid-gut was not freshly ingested and that the secretory activity that they observed in the gut epithelium, was probably concerned with the digestion of food ingested by the Stage 6 larva and of cells sloughed from the gut epithelium. Bernard & Lane (1962) further observed 'ciliated cells' in the 'glandular labyrinth'. Although it is difficult to interpret the precise nature of the 'glandular labyrinth' there seems little doubt that the 'ciliated' cells observed were in fact, gut epithelial cells with a striated border.

(*f*) *Excretory system*

The maxillary glands, which persist into the adult stage, can be recognized in sections (figure 30, plate 59) because of the characteristic appearance of the cells lining the end sacs. The two end sacs are found antero-ventrally in the thorax. The excretory ducts pass anteriorly and ventrally to open on the posterior face of the second maxillae. It seems likely that these glands are functional at this stage. The nauplius antennal glands are lost at the nauplius–cypris moult (see § 5*g*).

(*g*) *Cement glands*

The cement glands can be seen as a pair of large kidney-shaped bodies in the carapace just posterior to the compound eyes (figure 8). Each cement gland consists of a compact mass of elongated secretory cells, each of which communicates with a collecting duct running in a dorsal direction along the median face of the gland (figures 8 and 13*a*). Each glandular cell is about 70 μm in length with a nucleus 8 to 10 μm in diameter. The cytoplasm of the cell contains vacuoles full of secretory material which appears as a reticulum with granules after fixation. The collecting duct joins an oval sac (figure 8) the wall of which has an outer layer of circular muscle (figure 31, plate 59). This sac tapers to join the cement duct which enters the base of the antennule and runs along the length of the antennule (figure 32, plate 59) to the

adhesive disk on the third segment where it branches (figure 33, plate 59) opening through a number of pores on the adhesive surface. The cement duct appears to be lined with a very thin layer of cuticle.

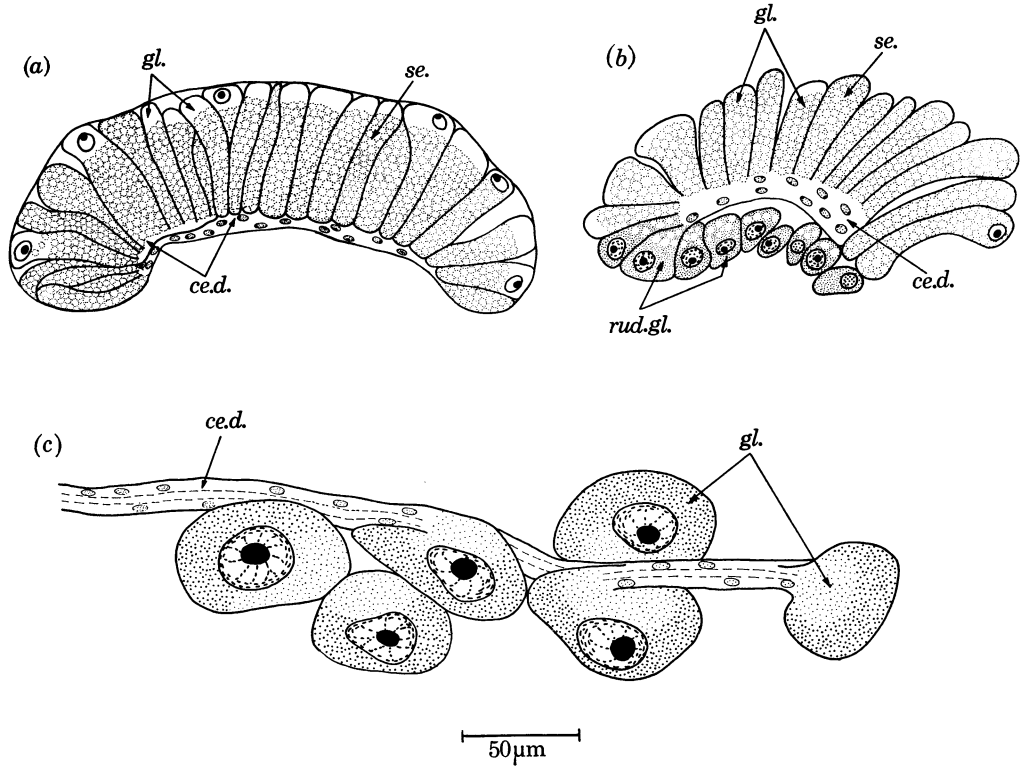

FIGURE 13. Diagram to illustrate changes in the structure of the cement glands of *B. balanoides* as the cypris larva completes its metamorphosis to the adult. (*a*) Cement gland as seen in transverse section of a cypris larva. (*b*) Cement gland as seen in transverse section of an attached cypris larva. (*c*) Part of the cement gland in an adult barnacle approximately ten weeks after metamorphosis.

Darwin (1851) traced the cement ducts from the adhesive disks to 'two long, rather thick, gut-formed masses', in the carapace. He concluded that these 'gut-formed masses' served a dual purpose—as cement glands and rudimentary ovaries. These bodies probably correspond to the cement glands described in this paper but there is no evidence to support the theory that they give rise to ovarian tissue. Doochin (1951) described the cement glands as a 'pulpy mass' occurring within the antennules and interpreted Darwin's cement glands as 'shell' glands homologous with the 'shell' glands of the Branchiopoda. Bernard & Lane (1962) confirmed Darwin's indentification of the cement glands but failed to detect the ducts or their openings in the antennules and concluded that the cement was extruded through pores at the base of the antennules to travel down the outside of the antennules. The present study has confirmed Darwin's original observations but not his assertion that the ovarian tissue arises from the larval cement glands (see § 7*h*).

(*h*) *Yellow cells*

In living cypris larvae a pair of very conspicuous yellow, roughly spherical, masses can be seen in the posterior region of the carapace on either side of the thorax (Walley 1964). Sometimes a pair of smaller yellow bodies can be seen in the carapace just behind the adductor muscle.

There is also a median, unpaired, yellow mass in the thorax above the gut (figures 8 and 12). Each of these yellow bodies is a compact mass of yellow cells. The examination of fresh tissue smears from living cypris larvae, by phase contrast, shows that the cytoplasm of these cells is full of spherical vacuoles about 2 μm in diameter (figure 34, plate 59) containing a pale yellow material which is fluorescent in ultraviolet light. Each yellow cell is about 20 μm in diameter with a nucleus 5 μm in diameter. As well as the small cytoplasmic vacuoles there is usually a larger vacuole, 7 to 8 μm in diameter, containing a deep yellow material which is not fluorescent in ultraviolet light, and a group of small dark brown granules which are also not fluorescent in ultraviolet light. If these yellow cells are allowed to dry up, the contents of the small vacuoles form bright yellow, needle-shaped crystals which are fluorescent in ultraviolet light.

(*i*) *Oil cells*

Oil globules are a conspicuous feature of living cypris larvae (figures 8, 11 and 12). Each globule is a spherical multinucleate cell. Most of the volume of the cell is taken up by a large vacuole containing oil while the cytoplasm, with up to eight nuclei, is displaced to the periphery of the cell (figure 11). The oil is probably a food reserve providing an energy source during this non-feeding phase in the life-history. The cells slowly diminish in size and number, finally disappearing during the first few days after the cypris-adult moult.

(*j*) *Haemocytes*

As in the nauplius larva there are numerous vacuolate haemocytes in the haemocoel. The probable functions of these cells will be discussed in § 5*d* and § 5*k*.

5. Development of the cypris larva

There have been few accounts of the development of the cypris larva. Krohn (1860) described the development of the compound eyes and thoracic limbs of the cypris within the last nauplius stage of two unidentified species of cirripedes, while Claus (1869) described the fate of the nauplius appendages in the cypris larvae of several *Lepas* spp. Chun (1896) also described in detail the development of the cypris thoracic appendages and antennules and the formation of the oral cone, by the separation of the maxillary rudiments from the thoracic limbs, in several species of the Lepadidae. Apart from these early accounts few authors have described the full larval development of any cirripede except for taxonomic purposes in which case the emphasis is on external morphology not internal structure. Kühnert (1934) described the larval development and metamorphosis of *Alcippe lampas* including details of the internal anatomy from histological sections. Batham (1945, 1946) described the larval development of *Ibla idiotica* and *Pollicipes spinosus*, while Kaufmann (1965) described the larval development and metamorphosis of *Scalpellum scalpellum*. In none of these accounts was the internal anatomy or histology studied in detail.

Although superficially the nauplius larva and the cypris larva appear to be very different (figures 1 and 8), the transformation from one to the other is simple and easy to understand (figure 14). Development of the cypris organ systems does not proceed synchronously: as one would expect, the time at which the differentiation of a particular organ system is completed, is closely related to the time at which the particular system is required to function.

(*a*) *External features*

In the nauplius larva the development of the cypris first becomes apparent in the differential growth of the ventral thoracic region of the nauplius. As the nauplius stages succeed one another, the ventral thoracic region becomes relatively larger and the cypris limb rudiments can be seen below the cuticle. It is not until Stage 6 that further changes become obvious in the living nauplius. During this stage, pigment granules are deposited in the developing compound eyes, so that the nauplius appears to have three eyes, and the yellow cells become conspicuous in the antennae.

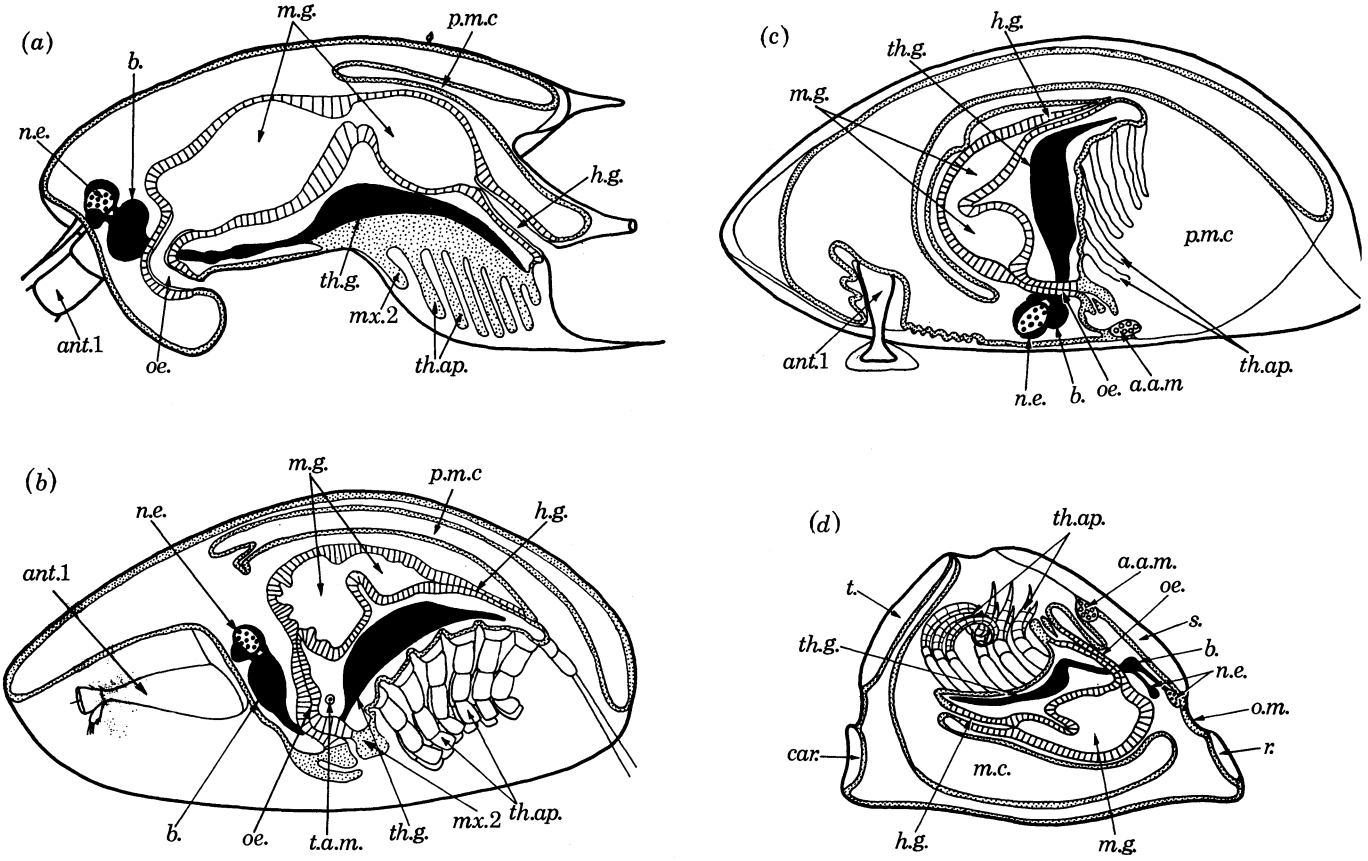

FIGURE 14. Diagram to illustrate changes in the orientation of the nervous system, alimentary canal, and other structures during metamorphosis in *B. balanoides*. (*a*) Stage 6 nauplius larva. (*b*) Cypris larva. (*c*) Settled cypris larva. (*d*) Young adult.

Immediately after the moult and before the two valves of the carapace are drawn together ventrally, the young cypris larva retains several nauplius features. Although the antennules have the cypris segmentation, the antennae and mandibles briefly retain their biramous structure as was noted by Claus (1869) and Kaufmann (1965) in *Scalpellum*. As the postecdysial changes proceed the antennae and mandibles regress, the antennules are withdrawn into the anterior mantle cavity, the oral cone with the rudiments of the adult mouthparts and the thorax with six pairs of swimming appendages, are withdrawn into the posterior mantle cavity.

(*b*) *Integument*

Changes before the nauplius–cypris moult. In Stage 6 (1), before secretion of the cypris cuticle begins, the epidermis separates from the nauplius cuticle and withdraws from the caudal spine, the ventral thoracic process and the frontal horns. Then in Stage 6 (2) or early in Stage 6 (3), the epidermis invaginates posteriorly in a position dorsal and lateral to the gut forming the posterior mantle cavity (figure 35, plate 60), while anteriorly it invaginates around the developing compound eyes to form the dorso-lateral pockets of the anterior mantle cavity which accommodate the compound eyes in the mature cypris larva.

Secretion of the cypris cuticle begins in Stage 6 (3). In preparations stained with Masson's trichrome stain, using iron haematoxylin, the cuticle covering the thorax and the developing anterior and posterior mantle cavities is stained pale green while the future carapace valves are stained black (figure 27, plate 59). The transition between the two types of cuticle is found at the cuticular ridges which mark the ventral limit of the carapace valves (figure 11).

Changes after the nauplius–cypris moult. At ecdysis the cuticle (pre-ecdysial cuticle) covering the carapace valves is about 3 μm thick and stains heavily with iron haematoxylin. The cuticle lining the mantle cavities and covering the thorax is about 1 μm thick and stains pale green. An inner layer of cuticle (postecdysial cuticle), 4 μm thick, which also stains pale green, is deposited below the pre-ecdysial cuticle of the carapace valves, after ecdysis.

The frontal horn glands of the nauplius are retained as the frontal glands of the cypris (figure 36, plate 60). The scattered epidermal glands of the nauplius are retained, apparently unchanged, in the cypris larva (figure 2).

(*c*) *Appendages*

(i) *Antennules*

Changes before the nauplius–cypris moult. Each of the four segments of the cypris antennule are formed within the corresponding segment of the nauplius antennule (Nott & Foster 1969). The nauplius antennular muscles remain active until the moult. There are no myofibrils formed in the cypris antennular myoblasts before the moult. The antennules are covered with a thin layer of cuticle while around the base of each antennule the cuticle is folded.

Changes after the nauplius–cypris moult. During the hours following ecdysis the antennular cuticle becomes differentially thickened, the nauplius antennular muscles histolyse and the cypris antennular muscles complete their development (see § 5*d*). At the same time the cement ducts are formed and the antennular ganglia migrate into the proximal region of the antennules. Because of this state of incomplete differentiation of the antennules it seems probable that the cypris larva is unable to test the substratum and settle during this period. The folded cuticle around the base of each antennule expands to form the pockets of the anterior mantle cavity around the 'elbows' of the antennules.

(ii) *Antennae*

Changes before the nauplius–cypris moult. The antennae show little change in external structure before ecdysis. A very thin layer of cuticle without setae is deposited below the nauplius cuticle. A mass of yellow cells is found in the protopodite at the base of the two rami in each antenna.

Changes after the nauplius–cypris moult. The antennae retain their biramous structure briefly after ecdysis, but during the first few hours after ecdysis they regress and no trace of them is retained in the cypris larva or the adult, unless they are represented by the palps on the labrum.

As the antennae regress the yellow cells migrate from the protopodites of the antennae into the carapace and then posteriorly to their final positions in the mature cypris larva (figures 39, 40 and 41, plate 60).

At the same time the tissues of the antennae—muscles, antennal glands, epidermis—histolyse and the fragments pass into the haemocoel where they are ingested by the phagocytic haemocytes and possibly by the migrating yellow cells as well.

(iii) *Mandibles*

Changes before the nauplius–cypris moult. The mandibles, like the antennae, show no marked changes in external structure before ecdysis. A thin layer of cuticle is deposited below the nauplius cuticle.

Changes after the nauplius–cypris moult. The mandibles also retain their biramous structure for a short time after ecdysis but like the antennae their existence is transient and they shrink as tissue histolysis proceeds. The rudiments of the adult mandible develop from cells on the median side of the base of the nauplius mandible.

(iv) *Postmandibular appendages*

Changes before the nauplius–cypris moult. The development of the postmandibular segments in *Ibla quadrivalvis* Cuv. was described by Anderson (1965). The sequence of events in *B. balanoides* seems to be similar although it was not studied in such detail.

The postmandibular appendages—first and second maxillae, six pairs of thoracic limbs—develop in the ventral thoracic region below the hind gut and the posterior region of the mid-gut. In the Stage 1 nauplius larva the arrangement of the cells in this region is obscure because of the invaginated ventral thoracic spine. At the moult to the Stage 2 nauplius, which occurs within an hour or so of hatching, this spine evaginates and the arrangement of the cells becomes clearer. A small number of the ectodermal cells forming the ventral wall of the thorax are enlarged (= ecto-teloblasts of Anderson 1965). In the haemocoel but in contact with the ventral ectoderm is a pair of mesodermal cell masses (= mesoteloblasts of Anderson 1965). Unfortunately Stage 3 nauplius larvae were not available. By Stage 4 the paired simple rudiments of the two pairs of maxillae and the six pairs of thoracic appendages are clearly defined. The second maxillae and the six pairs of thoracic limbs form an unbroken antero-posterior sequence, but

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Description of plate 60

Figure 35. Median longitudinal section through a Stage 6 (3) nauplius larva to show the developing cypris structure. *ant.* 1, antennule. *b.*, brain; *c.c.*, cypris carapace cuticle; *m* 4, 5 and 14, cypris muscles; *m.g.a.*, anterior region of mid-gut; *m.g.p.*, posterior region of the mid-gut; *n.c.*, nauplius carapace cuticle; *o.c.*, oil cells; *p.m.c.*, posterior mantle cavity of cypris; *t.g.*, cypris thoracic ganglion; *t.l.*, cypris thoracic limbs.

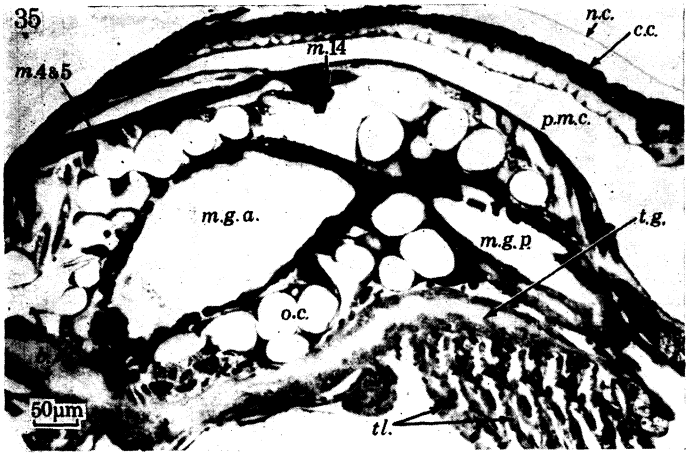
Figure 36. Section through the developing cypris frontal glands. *c.c.*, cypris carapace cuticle; *d.*, duct of nauplius frontal glands; *f.gl.*, frontal gland; *f.h.*, frontal horn; *n.c.*, nauplius carapace cuticle.

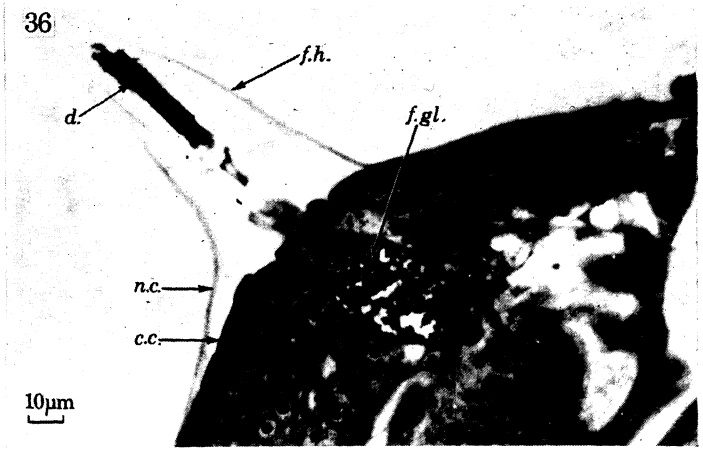
Figure 37. Median longitudinal section through a cypris larva to show, by comparison with figure 35, the changes which take place at the nauplius—cypris moult. Key to the lettering as for figure 35.

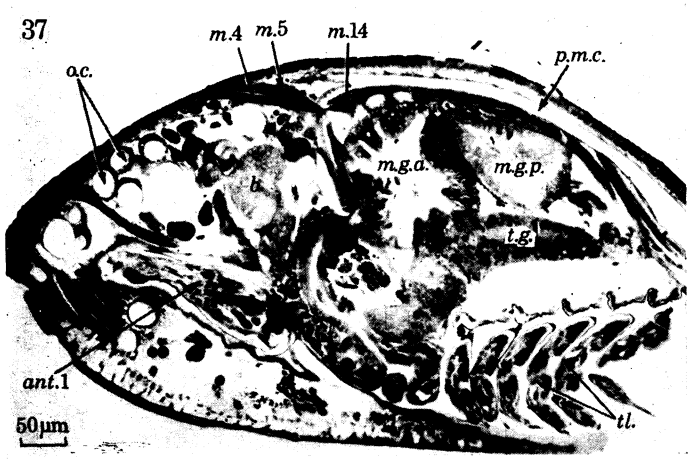
Figure 38. Living haemocytes from a cypris larva as seen under phase contrast illumination. *h.*, haemocyte; *n.*, nucleus; *vac.*, vacuoles containing ingested tissue fragments.

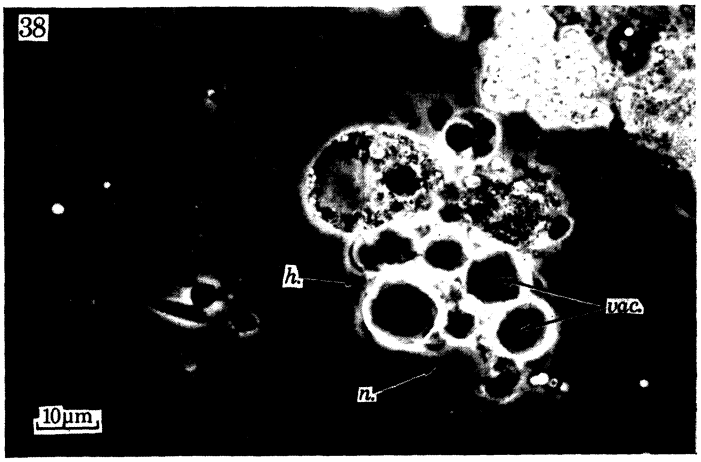
Figures 39 to 41. A sequence of photographs to show the migration of the yellow cells from the antennae into the carapace after the nauplius–cypris moult. *c.e.*, compound eye; *o.c.*, oil cells; *y.c.*, yellow cells.

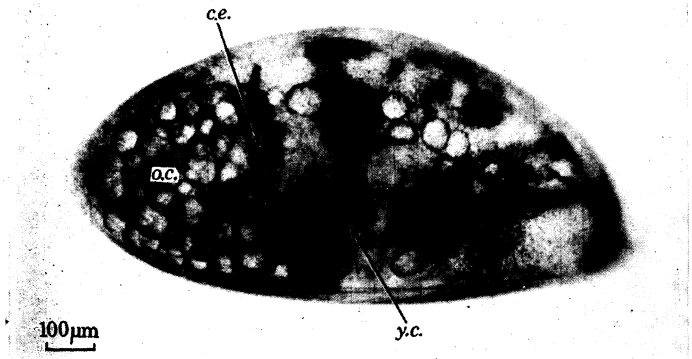
Figure 42. Longitudinal section through the lateral region of the thorax in a Stage 6 (3) nauplius to show the groups of three extrinsic limb muscles associated with each limb. *m.*, muscles.

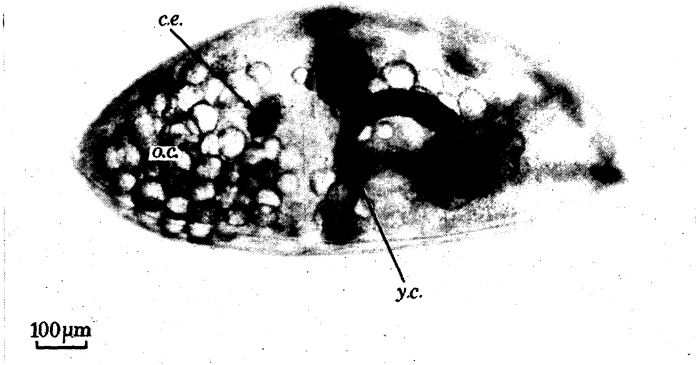


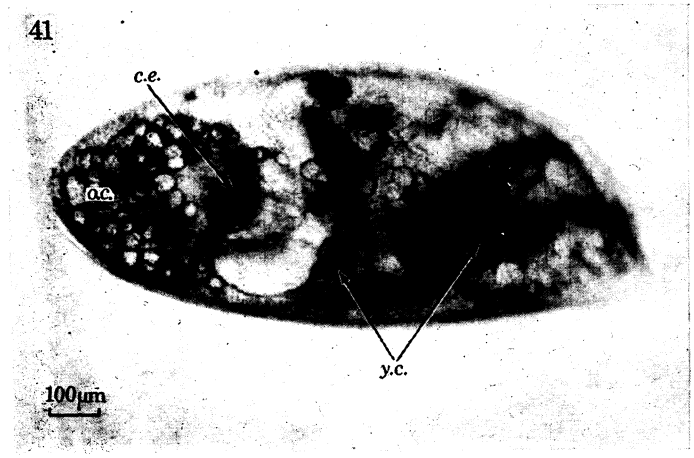


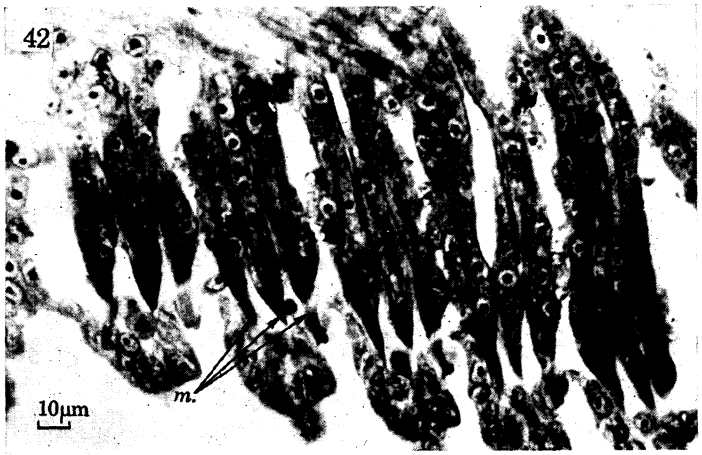












FIGURES 35 TO 42. For legends see facing page.

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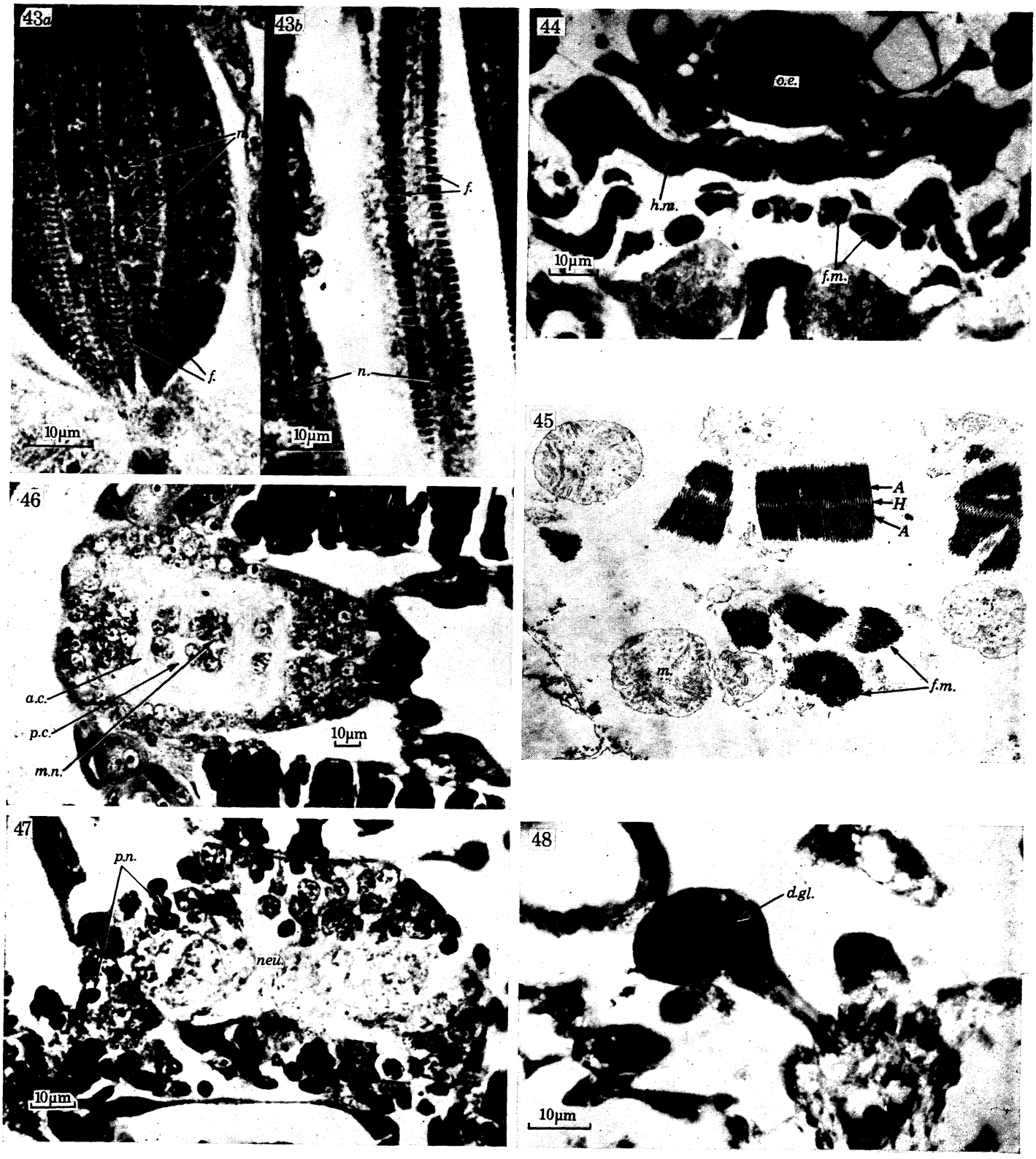

FIGURES 43 TO 48. For legends see facing page.

the rudiments of the first maxillae appear as lateral outgrowths, immediately behind the points of attachment of muscle pair number 21. The ectoderm along the mid-line is several cells in depth, and on either side are bands of mesodermal cells. The developing segments are delimited by shallow transverse grooves in the mesoderm. Ventral extensions of the mesoderm form the core of each limb rudiment. Both ectodermal and mesodermal cells were dividing rapidly in this stage and in the succeeding Stage 5 nauplius larva. In Stage 5 larvae the developing thoracic ganglia were linked anteriorly to the mandibular ganglia by two bands of cells, presumably the developing connectives. By Stage 6 (1) the thoracic limb rudiments have become biramous but the rudiments of the second maxillae remain simple. During Stage 6 the thoracic ganglia complete their differentiation (see § 5*e*), and the extrinsic and intrinsic limb muscles develop (see § 5*d*). The somites of the second maxillary segment each develop a cavity which becomes the end sac of the maxillary gland (see § 5*g*).

Changes after the nauplius–cypris moult. During or shortly after the moult, the thorax is drawn up into the posterior mantle cavity and the six pairs of swimming appendages start to function.

As the biramous antennae and mandibles regress, the adult oral cone is formed. The rudimentary adult mandibles develop and the rudimentary first and second maxillae become incorporated into the oral cone so that a distinct break appears, in the previously continuous series of appendages (Chun 1896), separating the second maxillae and the first pair of thoracic swimming appendages (figure 14).

(*d*) *Musculature*

Changes before the nauplius–cypris moult. The muscles of the cypris larva do not all develop simultaneously. The carapace muscles and the thoracic muscles are fully developed when the moult takes place, but the intrinsic muscles of the antennules do not complete their differentiation until after the moult. The paired blocks of mesodermal cells, which develop into the extrinsic muscles of the thoracic limbs, are clearly defined in Stage 6 (1). As the epidermis invaginates dorsally to form the posterior mantle cavity and cuticle deposition begins, the muscle rudiments extend dorsally on either side of the gut and become attached to the dorsal thoracic integument. Ventrally they are attached in the basal segments of the thoracic appendages (see § 4*e*).

Description of plate 61

Figures 43*a*, *b*. Sections through the extrinsic thoracic limb muscles of the cypris before (*a*) and after (*b*) the nauplius–cypris moult. *f.*, striated fibrils; *n.*, nuclei.

Figure 44. Transverse section through a cypris larva, shortly after the nauplius–cypris moult, to show histolysis of the ventral extrinsic muscles of the nauplius. *f.m.*, fragments of histolysed muscle; *h.m.*, histolysing muscle *oe.*, oesophagus.

Figure 45. Electron micrograph showing fragments of histolysed muscle in the cypris larva shortly after the nauplius–cypris moult. *A.*, A bands in a single sarcomere cut longitudinally; *f.m.*, fragments cut obliquely and transversely; *H.*, H zone crossing the A band; *m.*, mitochondrion. Final magnification × 10,940.

Figure 46. Oblique section through part of the thoracic ganglion in the Stage 6 (3) nauplius larva. *a.c.*, anterior commissure; *m.n.*, median nerve; *p.c.*, posterior commissure.

Figure 47. Transverse section through the brain of the cypris larva immediately after the nauplius–cypris moult. *neu.*, neuropile; *p.n.*, pycnotic nuclei.

Figure 48. Transverse section through the cypris larva shortly after the nauplius–cypris moult to show a very early stage in the development of the digestive gland. *d.gl.*, digestive gland.

Figure 45 is reproduced by kind permission of Dr J. A. Nott of the N.E.R.C. Unit of Marine Invertebrate Biology at the Marine Science Laboratories, U.C.N.W.

The origin of the myoblasts giving rise to the carapace muscles and antennular muscles is obscure. They could perhaps arise by the aggregation of wandering myoblasts or by proliferation from localized areas of the epidermis. However, no centres of cellular proliferation either giving rise to wandering cells or to epidermal ingrowths, were seen; this presumably indicates an inadequacy in the sampling since there must be a phase of cell division giving rise to the myoblasts which can be seen in Stage 6 (3). In the free-swimming cypris larva certain of the adult mantle muscles appear to arise by proliferation from the epidermis: this may also be the case in the Stage 6 nauplius. Clearly this needs further investigation.

Whatever their origin, the muscle rudiments are syncytial and the fibrils are deposited peripherally. The striations are most clearly seen in the extrinsic muscles of the thoracic limbs (figure 43, plate 61). In Stage 6 (3) these muscle fibrils show alternate light (0.5 μm) and dark (1.0 μm) bands—apparently the I and A bands. It is not until the muscles start to function in the newly moulted cypris larva (figure 43*b*, plate 61) that the H zone is seen to cross the A band. The Z membrane has not been seen in these muscles, presumably because it is near the limits of resolution of the light microscope.

Although the myoblasts are in position in the antennules by the end of Stage 6 (3) no muscle fibrils are deposited before ecdysis.

All the nauplius muscles remain fully functional until the moult to the cypris larva.

The carapace adductor muscle develops in a position immediately posterior to the ventral attachments of the paired muscles numbered 21 in the nauplius larva, but apparently anterior to the developing first and second maxillae.

Changes after the nauplius–cypris moult. From a comparison of the approximate lengths of the major cypris muscles before and after ecdysis, it is possible to suggest some of the mechanisms involved in the morphological changes.

During the nauplius–cypris moult the lateral parts of the carapace are drawn ventralwards and together, forming the valves of the cypris carapace. This is brought about by the shortening of the cypris adductor muscle (15). The distance between its points of attachment in both nauplius and cypris is 450 μm, but whereas in the former it is not straight, in the latter it is pulled taut between the two valves of the carapace and must have decreased in length considerably.

Another striking change that takes place at this moult is in the position of the extrinsic thoracic limb muscles relative to the gut. In the Stage 6 nauplius these muscles differentiate on either side of the hind gut and the posterior half of the mid-gut. In the cypris, however, the *whole* of the gut, from the junction of the oesophagus and mid-gut to the anus, lies between the extrinsic limb muscles in the thorax. This change in position is apparently brought about by the shortening of the two pairs of thoracic retractor muscles (4 and 5). In the nauplius, before ecdysis, they are 330 μm long, but in the free-swimming cypris only 135 μm long (figures 35 and 37, plate 60). Thus it seems that during or after ecdysis the thoracic retractors shorten, drawing the thorax and its musculature forward on either side of the gut, possibly rotating around the tendon of the adductor muscle. As a result the gut becomes compressed in an antero-posterior direction (figure 12) and the oil cells move from the region of the mid-gut constriction to the anterior region of the carapace, where they are found in the mature cypris larva.

As the two valves of the carapace are drawn together the anterior and posterior mantle cavities are formed. The antennules are apparently drawn up into the anterior mantle cavity

by the shortening of muscles 7–12. In the Stage 6 (3) nauplius they are approximately 430 μm long. In the cypris larva they are approximately 220 μm long.

Thus the morphological changes which occur at this moult are apparently brought about by the shortening of certain muscles, in some cases to less than half their length before ecdysis. This raises the question as to whether the shortening mechanism is a 'conventional' muscular contraction or a different type of contraction. Examination of the muscles at different stages in the shortening, using an electron microscope, should yield an answer to this question.

The remaining carapace muscles presumably play their part in establishing the cypris structure but their roles are probably ancillary to those of the muscles described above.

After the moult, when the shape of the cypris antennule has been established, the cuticle becomes thickened differentially and the muscle fibrils are laid down in the myoblasts.

Immediately after the moult *all* the nauplius muscles, except perhaps the circular muscles of the gut, break down. They appear to undergo a process of spontaneous fragmentation similar to that described by Crossley (1965) in certain of the larval abdominal muscles of *Calliphora erythrocephala* during the pupal phase of muscle histolysis. In sections of fixed material the muscles appear to be contorted (figure 44, plate 61) and they then break up into fragments. Crossley reported that in *Calliphora* the striations were lost before the muscles fragmented; however, this is not the case in *Balanus* cypris larvae. Most of the muscle fragments show well marked striations, although within a fragment there seems to be transverse fragmentation of the fibrils. These muscle fragments stain intensely with iron haematoxylin and Xylidene ponceau. Under the electron microscope it appears that breakage in the fibrils occurs between adjacent sarcomeres, in the region of the I bands and the resulting fragments retain the typical arrangement of myofilaments seen in the intact muscle (figure 45, plate 61). The fragments are ingested by the numerous vacuolate haemocytes and as intracellular digestion proceeds the striations disappear until they are no longer recognizable as having been derived from muscle. In this whole process there is no evidence of invasion of the muscles by haemocytes—histolyis appears to be spontaneous, though presumably under hormonal control.

(*e*) *Nervous system and sense organs*

The change from nauplius to cypris involves, first, the development of the nervous centres associated with the specialized cypris structures—antennules, compound eyes, and thoracic limbs—and, secondly, a change in the relative position of the different components (figures 14 and 15).

The presumptive postmandibular ganglia are first recognizable in Stage 5, differentiation into neurons and axons occurs in Stage 6 (1). At the latter stage they form a continuous series of paired ganglia joined longitudinally by connectives and connected anteriorly with the mandibular ganglia. The members of each pair of ganglia are joined transversely by anterior and posterior commissures (figure 46, plate 61). The anterior commissures always have a slightly larger diameter than the posterior and the anterior commissure, joining the ganglia of the first maxillary segment, is particularly large. The commissures are joined longitudinally by a median nerve (figure 46). After the nauplius–cypris moult it is not easy to distinguish between the individual thoracic ganglia because of antero-posterior compression. Ventrally paired nerves pass to the paired thoracic swimming appendages.

There is no change, visible with the light microscope, in the nauplius eye before or after the nauplius–cypris moult.

During Stages 4 and 5, cell division laterally in the lateral lobes of the brain produces the rudiments of the compound eyes. During Stage 6, these cells differentiate into the retinula cells (see § 4*d*) and possibly into lens-secreting cells. The latter cells may, however, arise from the epidermal cells which invaginate to surround the developing compound eyes, early in Stage 6. The spherical lenses are secreted in three sectors, each sector being secreted by a single cell. As development of the compound eyes proceeds pigment is deposited in the retinula cells, red at first and then turning through brown to black.

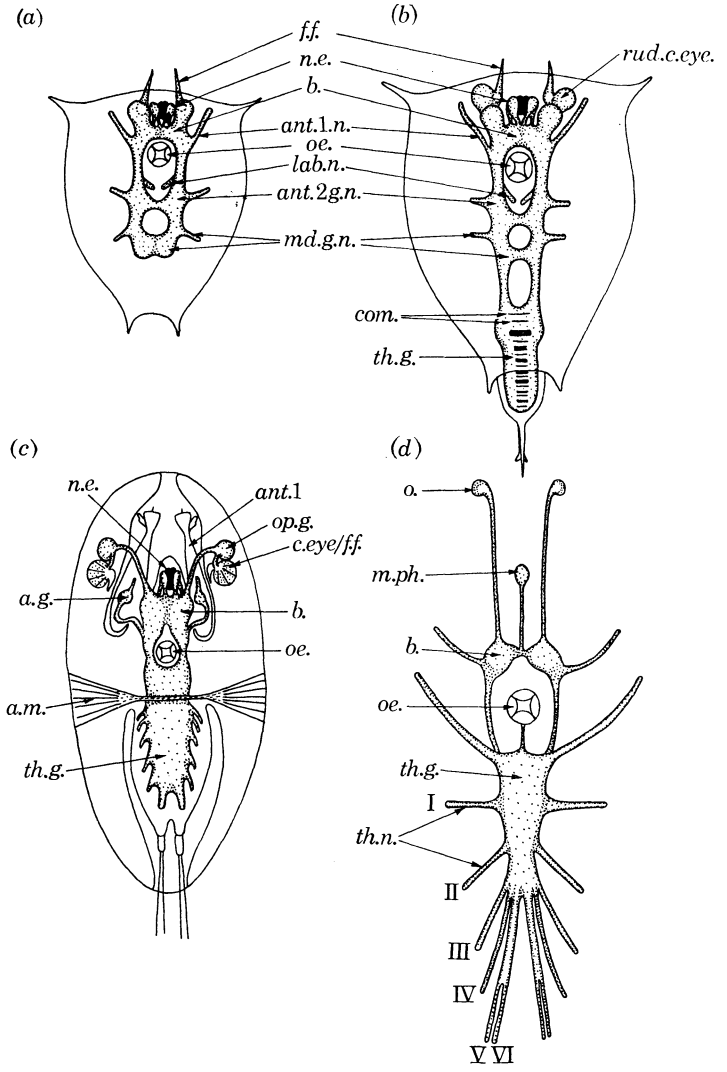

FIGURE 15. Diagram to illustrate changes in the central nervous system during metamorphosis in *B. balanoides*. (*a*) Central nervous system of a Stage 2 nauplius larva. (*b*) Central nervous system of a late Stage 6 nauplius larva. (*c*) Central nervous system of the cypris larva. (*d*) Central nervous system of the adult.

The frontal filaments become separated from the brain and attached to the median side of the compound eyes (figures 10 and 11). The ventral epidermis of the nauplius invaginates dorsally to surround the compound eyes and the frontal filaments almost completely, except for the region carrying the short optic nerve joining the compound eye and frontal filament to the optic ganglion. The optic nerves develop by the elongation of the tracts joining the compound eye–frontal filament complexes to the lateral lobes of the brain. The optic nerves probably

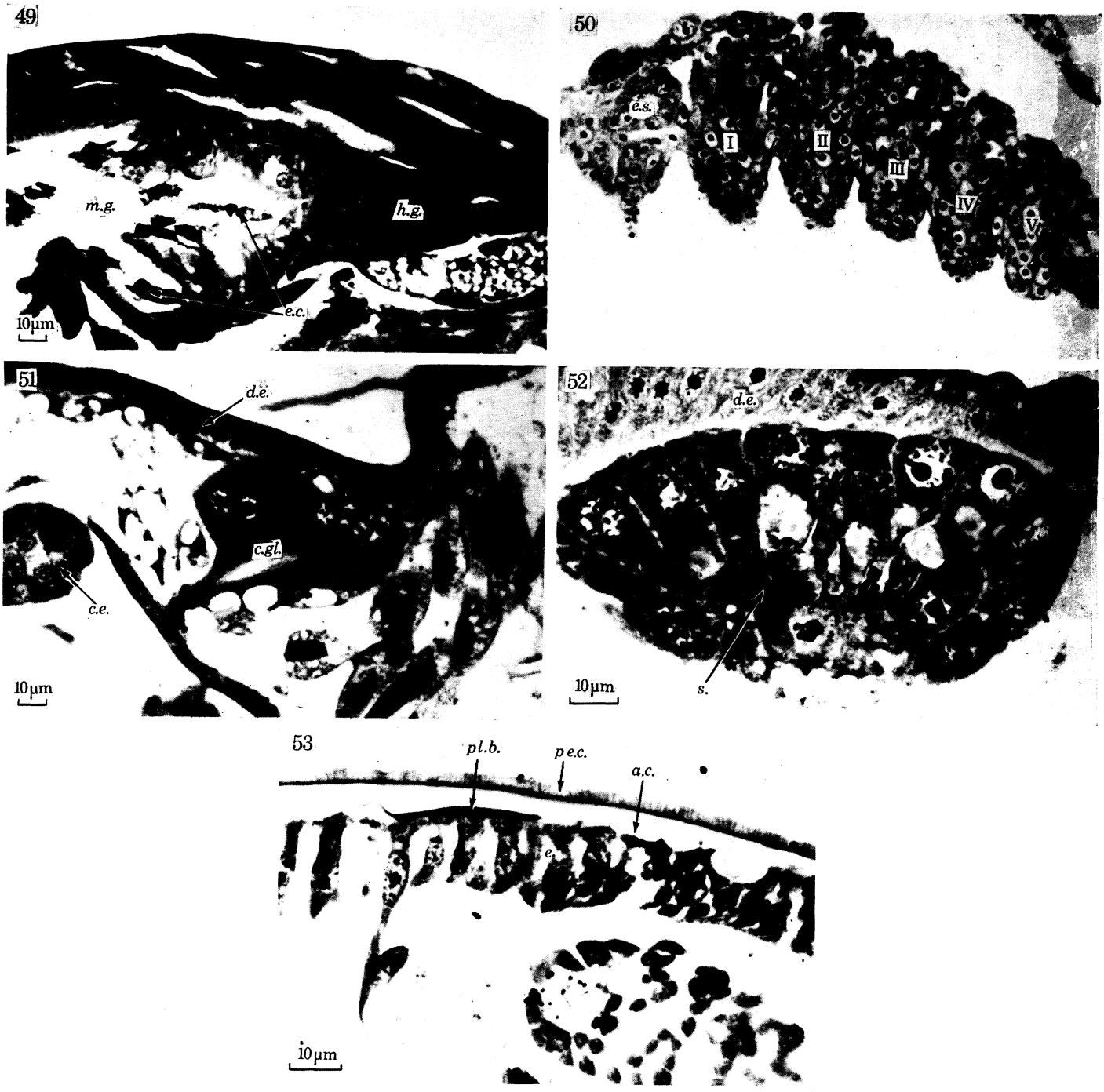

FIGURE 49. Longitudinal section through the mid-gut and hind gut of the cypris larva to show the extrusion of redundant cells into the gut lumen. *e.c.*, extruded epithelial cells; *m.g.*, mid-gut; *h.g.*, hind gut.

FIGURE 50. Longitudinal section through the developing thoracic region in the Stage 6 (1) nauplius larva to show the end sac of one maxillary gland developing in the somite of the second maxillary segment. I–V, Somites of the first to fifth thoracic segments; *e.s.*, developing end sac of maxillary gland. Scale as on figure 49.

FIGURE 51. Transverse section through the lateral region of an early Stage 6 nauplius larva to show a developing cement gland. *c.e.*, developing compound eye; *c.gl.*, cement gland; *d.e.*, dorsal epidermis.

FIGURE 52. Transverse section through a developing cement gland in a Stage 6 (3) nauplius larva. *d.e.*, dorsal epidermis; *s.*, droplets of secretion in the gland cells.

FIGURE 53. Transverse section through the integument of a settled cypris larva shortly before the cypris–adult moult. *a.c.*, cuticle of developing adult; *e.*, epidermis; *pe.c.*, pre-ecdysial cuticle; *pl. b.*, boundary of developing shell plates.

(*Facing p.* 265)

contain processes from the retinula cells and nerve fibres from the frontal filaments. The lateral lobes of the brain become separated from the main part of the brain, by the elongation of the optic tracts, to become the optic ganglia of the cypris larva.

The precise origin of the antennular ganglia is not known; it is probable that they arise by the elongation of the antennular tracts in the brain carrying newly differentiated neurons to a peripheral position, thus forming the ganglia. These antennular ganglia innervate the muscles and special sense organs of the antennules.

After the nauplius–cypris moult, a number of pycnotic nuclei are seen in the central nervous system (figure 47, plate 61). These are probably the nuclei of degenerating neurons which formerly innervated the nauplius muscles and other redundant structures.

(*f*) *Alimentary canal*

The gut retains its typical nauplius structure until the nauplius to cypris moult.

As a result of the morphological changes which occur at this moult, the gut becomes compressed in an antero-posterior direction and changes its position relative to the thoracic musculature (see § 5*d*). As the biramous nauplius antennae and mandibles regress the rudiments of the adult mouthparts appear on the oral cone.

The lumina of both the oesophagus and the hind gut are closed (figure 12). The oesophagus passes through a very narrow bridge of tissue into the thorax where it joins the mid-gut. During the postecdysial changes the digestive glands appear on either side of the oesophagus (figure 48, plate 61) and are well grown by the time the typical cypris structure is established (figure 28, plate 59).

The epithelium lining the mid-gut in the cypris larva varies in height from 15 to 30 μm. The cells are frequently vacuolated and have a high brush border (figure 29, plate 59). The glandular cells of region 2 of the anterior part of the nauplius mid-gut degenerate and are shed into the lumen of the gut together with other apparently redundant cells of the gut epithelium (figure 49, plate 62). These extruded cells are probably digested by the remaining epithelial cells together with the remnants of food ingested by the Stage 6 nauplius larva. The residual 'food body' remains in the gut until after the cypris–adult moult. When feeding is resumed this residue is voided.

(*g*) *Excretory system*

The end sacs of the adult maxillary glands appear as cavities in the paired somites of the second maxillary segment in Stage 6 (1) (figure 50, plate 62). At the nauplius–cypris moult the glands are apparently fully formed and the secretory ducts can be traced to their apertures on the oral cone. Since the nauplius antennal glands degenerate towards the end of the last nauplius stage, it seems likely that the maxillary glands are the functional organs of excretion and ionic regulation in the cypris larva as well as in the adult.

(*h*) *Cement glands*

The rudiments of the cement glands arise pre-orally from the ventral epidermis lying laterally to the rudiments of the compound eyes in the Stage 5 nauplius larva. At this stage they consist of two groups of epidermal cells which have become columnar. As development proceeds the paired groups of columnar cells sink below the epidermis and then move dorsalwards, eventually coming to lie beneath the dorsal epidermis of the carapace (figure 51, plate 62). At the same

time the number and size of the cells in each group increases. Secretion droplets first appear in the cells in Stage 6 (3) (figure 52, plate 62).

The muscular sacs, and cement ducts in the antennules do not complete their differentiation until after the nauplius cypris moult.

(*i*) *Yellow cells*

Before the nauplius–cypris moult the yellow cells are located in the antennae where they can be seen clearly both in the living larvae and in sections.

After ecdysis, as the antennae regress and all the nauplius muscles histolyse, the yellow cells move from the antennae to the posterior region of the carapace (figures 39, 40 and 41, plate 60). The group of yellow cells in the thorax appears as a compact mass above the gut in the Stage 6 larva. The yellow cells, migrating from the antennae, are associated with fragments of histolysed tissue but it is not clear, from sections, whether these are lying between the cells or are contained in intracellular vacuoles.

As in the case of the myoblasts, the origin of these cells is obscure, although it seems likely that they arise by the transformation of haemocytes. The author stated in an earlier paper (Walley 1964) that these cells were important phagocytes, ingesting and digesting fragments of histolysed tissue, but further work has shown that this is not so; they may be phagocytic but this is not their primary function. Their precise role in metamorphosis has still to be discovered.

(*j*) *Oil cells*

The precursors of the oil cells are present in the newly hatched nauplius. They are elongated cells, characteristically with a finely granular basiphil cytoplasm, encircling the gut in the region of the mid-gut constriction (figure 25, plate 58; figure 6). By stage 5 they are clearly recognizable as developing oil cells—each cell is multinucleate and has one or more vacuoles (figure 26, plate 58) but only one of these vacuoles increases in size until by late Stage 6 each cell consists of a large spherical vacuole with only a thin envelope of cytoplasm containing 7 or 8 nuclei (figure 11).

Most of these cells are found in the region of the mid-gut constriction until ecdysis, when they move forwards to the anterior region of the carapace, where they are found in the cypris. This change in position is probably passive, the oil cells being carried forward as the body fluids are redistributed during and immediately after ecdysis.

(*k*) *Haemocytes*

A few vacuolate haemocytes have been recognized in Stage 2, but they become more numerous in successive nauplius stages. During Stage 6 they are very abundant and appear to congregate around or close to organs carrying out rapid synthesis, for example beneath the epidermis (figure 27, plate 59), surrounding differentiating muscles and clustered around the developing oil cells. This suggests that they may play a part in transporting the raw materials for syntheses.

After ecdysis, as muscle histolysis proceeds, they become phagocytic and ingest and digest fragments of muscle (figure 38, plate 60).

6. Morphological and histological changes after settlement

The behaviour of the cypris larva before settlement has been well described by a number of workers (Doochin 1951; Knight-Jones & Crisp 1953; Crisp & Stubbings 1957). After a short exploratory phase, during which the cypris larva tests the substratum with its antennules,

it becomes attached by the adhesive disks on the third segment of its antennules and then is securely fixed by the secretion produced in the cement glands and poured out on to the substratum via ducts in the antennules. Crisp & Stubbings (1957) observed that in *B. balanoides* approximately 24 h elapsed between attachment and the moult to the pin-head sized barnacle and their observation was confirmed in the present study. During this period the cypris larva lies quiescent and apparently inactive, it is, however, a period of intense morphogenetic activity.

It is this change from free-swimming cypris larva to attached adult which has been regarded as 'the metamorphosis of the barnacle' by most recent authors (Runnström 1925; Doochin 1951; Bernard & Lane 1962). Darwin (1851) and Hoffendahl (1904) described the morphological changes that take place during this period in *L. australis* and *Poecilasma aurantium* respectively. Doochin (1951) described the general morphological changes after settlement in *B. improvisus* and *B. amphitrite niveus*. His account is valuable because it emphasizes the similarity between the different species of *Balanus* and gives a clear guide to the morphology of the metamorphosing cypris larva, although his functional interpretations are sometimes incorrect. Tissue histolysis after settlement was observed by Darwin (1854), Bernard & Lane (1962) and Walley (1964). All those structures which are peculiar to the cypris or nauplius stages and which are not retained in the adult, histolyse, and the fragments are ingested by phagocytic haemocytes. While histolysis of redundant organs proceeds, the development of the organs of the young adult is completed.

(*a*) *External features*

The general morphological changes after settlement, in *B. balanoides* are illustrated diagrammatically in figure 14. The attached cypris larva is adpressed to the substratum ventrally and becomes slightly flattened. As the internal changes proceed, the yellow cells disperse (Walley 1964) and the larva becomes progressively more opaque. The tips of the antennules are cemented to the substratum and the slightly shrunken and distorted shafts of the antennules lie perpendicularly to the substratum. The most striking change is in the position of the thorax and limbs. The long axis of the thorax turns through approximately 90° in the vertical plane. This movement includes the nervous system and brings the nauplius eye close to the ventral surface of the cypris (figure 14). The limbs straighten out and lie perpendicularly to the ventral surface of the thorax and the mouth and oral cone move posteriorly. The mantle cavity now extends from the dorsal region of the carapace almost to the ventral surface, surrounding the thorax on all sides and leaving only a small connecting region between the future prosoma and the future mantle tissue (figure 14). Already it is possible to discern the adult organization.

(*b*) *Integument*

Shortly after attachment the epidermis separates from the cuticle. Folds appear along some of the lines which demarcate the future opercular valves and wall plates, and in areas where considerable expansion will take place at ecdysis, such as the region of the future rostrum. As secretion of the new layer of cuticle proceeds, connective tissue fibres are laid down, linking the shell to the cuticle lining the mantle cavity of the adult (figure 17). If the integument is examined 17 to 18 h after attachment, there is a new layer of cuticle 1 to 5 μm thick over the whole surface of the epidermis (figure 53, plate 62) and it is this layer of cuticle which is moulded at ecdysis to form the basal membrane, wall plates and operculum of the young barnacle. In addition much of the inner, postecdysial, layer of the cypris carapace cuticle has been reabsorbed. Certain parts of the new layer of cuticle stain differently from the rest; while most

of the cuticle is stained pale green, there are certain well defined bands which stain black with iron haematoxylin, or, in the absence of iron haematoxylin, red with Xylidene ponceau (figure 53). From a reconstruction in three dimensions it was found that these differentially stained bands represented the limits of the developing shell plates. The picture obtained by such a reconstruction (figure 16) agrees very well with that obtained by direct observation of the living cypris larva (Runströmm 1925) although only four wall plates were found in contrast to the five apparently observed by Runströmm. There was no sign of the carino-laterals which appear several days after ecdysis, on day 4 in *B. improvisus* (Costlow 1956), after intial calcification has taken place.

All the epidermal glands degenerate and histolyse. There are no glands of this type in the mantle or shell epidermis of the adult, although there are scattered unicellular glands (Thomas 1944).

At each of the six larval ecdyses the whole cuticle is shed. In the adult, however, while the cuticle covering the thorax and lining the mantle cavity is shed at ecdysis, the cuticle constituting the wall plates and opercular valves is heavily calcified and is never shed at ecdysis. In *B. balanoides* the cuticle forming the base of the animal, which is attached to the substratum, is uncalcified as are also the flexible opercular membrane and the cuticle joining the various shell plates to each other and to the base. The manner in which the shell of the adult barnacle grows has been studied in *B. improvisus* by Costlow (1956), and in several other species by Bocquet-Védrine (1963, 1964, 1965, 1966). Costlow & Bookhout (1953, 1956) studied the relationship between shell growth and the moulting cycle in *B. improvisus* and *B. amphitrite niveus* respectively. They measured the daily increment in the area of the basal membrane and related this to the time of moulting measured in days. This method revealed no correlation between time of moulting and increase in shell size and they concluded that shell growth was 'continuous but erratic' and 'not associated with the 2–3 day intermoult period of the body exoskeleton'. In his study of shell growth in *B. improvisus*, Costlow (1956) found no evidence of cyclical secretory activity in the cells responsible for secreting the shell components, which could be related to the moulting cycle. Bocquet-Védrine, however, studied species with a simpler shell structure than *B. improvisus*—viz. *Chthamalus stellatus* (Poli) (1963), *Elminius modestus* (1964, 1965), *Acasta spongites* Darwin (1966)—and made a comparison between the stages in the moulting cycle (Drach 1939) and the stages of secretion of the shell. She concluded that 'La sécrétion des pièces de la muraille et de l'opercule est discontinue et résulte d'une activitée cyclique de l'épithélium qui s'identifie rigoureusement avec l'activité de l'épithélium de la masse viscérale, édifiant un squelette temporaire.' If this is so, it indicates that, although the whole epidermis continues to respond to the presumed cyclical moulting hormone system, the nature of the response, when compared with the situation in the larval stages, has been modified in those areas of the epidermis producing the calcareous adult shell. This regional modification of the epidermis presumably takes place in the period between settlement and the moult to the young adult. It is possible that it is accompanied by ultrastructural changes that might be revealed with the electron microscope.

(*c*) *Musculature*

It seems likely that the change in position of the thorax after settlement is brought about by the contraction of specific muscles but they have not been identified.

During the 24 h between settlement and ecdysis *all* the cypris muscles, with the possible exception of the circular muscles of the gut, histolyse. The process is not synchronous throughout

the cypris larva; it seems that the carapace muscles degenerate very shortly after attachment, while those in the thorax may not do so until some hours later. As in the histolysis of the nauplius muscles, in the newly moulted cypris larva, degeneration seems to occur by spontaneous fragmentation, releasing pieces of muscle into the haemolymph which are then ingested by the phagocytic haemocytes.

The musculature of the adult *B. balanoides* was well described by Gutmann (1960) and will not be considered in any detail here. The positions of the developing median scutal depressor muscles, the lateral scutal depressor muscles, the median tergal depressor muscles and the adductor muscle are indicated in figure 17. The rudiments of these muscles are all recognizable in the free-swimming cypris larva (figure 11). Figure 17 also illustrates the position of the small muscle fibres which may be important in establishing the conical shape of the young adult and which are thought to become the 'fixation fibres' of the adult.

In the past there has been some discussion on the origin of the adult adductor muscle. Doochin (1951) concluded that the larval adductor persisted in the adult. This conclusion is implied by Bernard & Lane (1962) in the statement 'the conic mass of adductor muscles shrinks to nearly cylindrical form and is displaced toward the ventral surface'. This is, however, a surprising conclusion when one considers that it necessitates the migration of the muscle from a position ventral to the gut, past the oesophagus to a position dorsal to the gut—as Hessler (1964) comments 'Clearly this is topographically impossible'. In fact there is no evidence to suggest that it does happen. The larval adductor muscle histolyses and the adult adductor muscle develops as an entirely new structure in a pre-oral position.

The muscles of the adult prosoma, thorax, and limbs, develop during the 4 to 6 h immediately preceding the moult from the cypris larva to the young adult.

(*d*) *Nervous system and sense organs*

As a result of the rotation of the thorax within the carapace, the nervous system comes to lie in the position shown in figure 14, bringing the brain and nauplius eye into a 'ventral' position. As histolysis proceeds, numerous pycnotic nuclei appear in the brain and thoracic ganglia. These nuclei probably belong to those neurons which innervated the cypris musculature, compound eyes and other specialized organs, and which degenerate when these structures histolyse. As these neurons degenerate they are replaced by others which will innervate the developing adult musculature and sense organs. It is not clear whether these adult neurons are differentiated from cells arising by the division of undifferentiated cells in the ganglia, or whether they arise from cells lying dormant in the ganglia from the Stage 6 nauplius. Since no sign of cell division was seen in the ganglia at this time, the second explanation seems the more likely. According to this theory the larval nervous system, developed in the embryo and extended during the later nauplius stages, carries within itself a complement of undifferentiated cells which will become adult neurons at the appropriate time. A similar process is seen during metamorphosis in the Lepidoptera (Nordlander 1967) but the adult neurons are said to arise from 'ganglion mother cells' present in the larval nervous sytem.

The antennular and optic ganglia, the compound eyes and frontal filaments, all degenerate. The lenses from the compound eyes pass into the haemocoel where they are ingested by the phagocytic haemocytes, while most of the pigment granules are extruded and lost at ecdysis.

Even as this extensive degeneration occurs the adult central nervous system (figure 15) is built up. The brain becomes reduced in size relative to the rest of the nervous system—

presumably because of the loss of the neurons connected with the complex extrinsic and intrinsic musculature of the antennules, with the special sense organs of the antennules, and with the compound eyes and frontal filaments. The rhabdomes of the median nauplius eye become indistinct and are apparently lost but the adult photoreceptors (Gwilliam 1965), which are derived from the nauplius eye (see § 7*c*), have been shown to have rhabdomeres when examined with the electron microscope (Fahrenbach 1965). Whether the adult rhabdomeres are new structures or derived from those in the larval nauplius eye is not known.

(*e*) *Alimentary canal*

After settlement further cells are sloughed from the gut epithelium and form a deeply staining mass in the lumen of the anterior region of the mid-gut. The epithelium in this region of the gut consists of vacuolate cells similar to those seen in the free-swimming cypris larva, while the epithelium in the posterior region of the mid-gut consists of a uniform cubical–columnar epithelium without vacuoles in the cells.

During the period between settlement and ecdysis, the lumen reappears in the oesophagus and hind-gut, and appears, for the first time, in the digestive gland. By the time ecdysis takes place, the typical U-shape of the adult gut has been established (figure 14).

(*f*) *Excretory system*

The maxillary glands show no visible changes and are probably fully functional throughout this period.

In the adult cirripede there is a mass of cells, lying in the haemocoel above each digestive gland, which are histologically identical with those lining the end sac of the maxillary glands and the end sac of the nauplius antennal glands. These probably correspond to the 'organe céphalique clos' of Bruntz (1902). A careful search has so far failed to reveal any ducts to the exterior from these cell masses. They first appear close to the digestive glands prior to the cypris–adult moult. Nothing is known about the function of these cells.

(*g*) *Cement glands*

It seems likely that the secretion of the cement at settlement is under nervous control. Presumably the stimuli inducing the cypris larva to settle also trigger the release of the cement. From a study of the anatomy of the cementing apparatus it seems most likely that the gland cells release their secretion into the muscular sacs via the collecting ducts. The cement would then be expelled through the ducts in the antennules by the peristaltic contractions of the muscular sacs.

After settlement the cypris cement gland cells do not degenerate but the muscular sacs break down and as the tissues withdraw from the antennules the cuticular lining of the cement duct remains. Adult-type cement gland cells appear in a cluster around the collecting duct of the cypris cement gland (figure 13). Presumably new cement ducts, opening through pores in the newly secreted basal membrane, are formed, but so far these ducts and their pores have not been found.

(*h*) *Yellow cells*

Shortly after settlement the compact masses of yellow cells break down and the individual cells disperse in the haemocoel. They are no longer thought to be the primary phagocytes

(see § 5*i*), but their movement is so precisely integrated with the other internal changes that one must conclude that they play an important but unknown role in this final phase of metamorphosis.

(*i*) *Haemocytes*

As histolysis proceeds the haemocytes enter their second phagocytic phase, engulfing fragments of muscles and other tissues. The engulfed fragments are apparently digested in the intracellular vacuoles. At ecdysis the haemocytes still contain numerous partially digested fragments.

7. Morphological and histological changes during and immediately after the moult to the young adult

The morphological changes which take place at this moult are illustrated in figures 14 and 17. The establishment of the truncated cone shape of the shell is brought about by muscular activity leading to the redistribution of body fluids. If an attached cypris larva, which has started the changes involved in the completion of metamorphosis, is detached from the substratum, it will moult and assume the shape of the normal young adult while freely floating at the surface of the water or lying at the bottom of the dish. During a normal cypris–adult

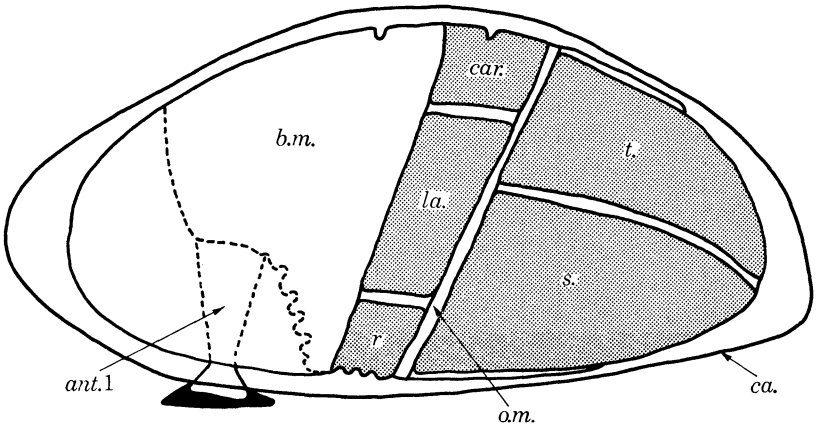

FIGURE 16. Diagram to illustrate the positions of the shell components in the adult cuticle as it is deposited in the attached cypris larva.

moult, it seems that contraction of the tergal depressor muscles and the lateral scutal depressor muscles brings about a redistribution of the body fluids, and as a result folded areas of cuticle expand. In the region of the future rostrum, this results in the operculum moving upwards, away from the substratum, and towards its final position. As a result of this movement, the body of the barnacle rotates through a further 90° coming to rest suspended in the mantle cavity from the scutal plates, with the ventral surface of the thorax uppermost. The mantle cavity of the adult corresponds to the posterior mantle cavity of the cypris larva (figure 17) while the anterior mantle cavity together with the anterior region of the carapace valves are incorporated into the basal membrane of the adult. The angle between the wall plates and the basal membrane is probably established by the contraction of the muscle-like enlargements of the fixation fibres which tie the wall plates to the basal membrane (Gutmann 1960) (figure 17). The cuticle lining the mantle cavity is kept in position by numerous connective tissue fibres linking it to the opercular valves and wall plates, and by a number of short muscles linking

it to the basal membrane (Gutmann 1960). At ecdysis the whole of the cuticle covering the cypris larva is shed, except that covering the antennules. The remnants of the antennules are retained beneath the basal membrane of the adult (Darwin 1851, 1854). Most of the pigment granules from the compound eyes are shed with the cuticle.

The newly moulted pin-head sized barnacle is covered with a layer of flexible uncalcified cuticle. No calcification occurs until the adult form of the young cirripede is established. At first there are only four wall plates but the two carino-laterals appear several days after ecdysis. The six pairs of biramous swimming appendages of the cypris larva, become the six pairs of biramous feeding appendages of the adult.

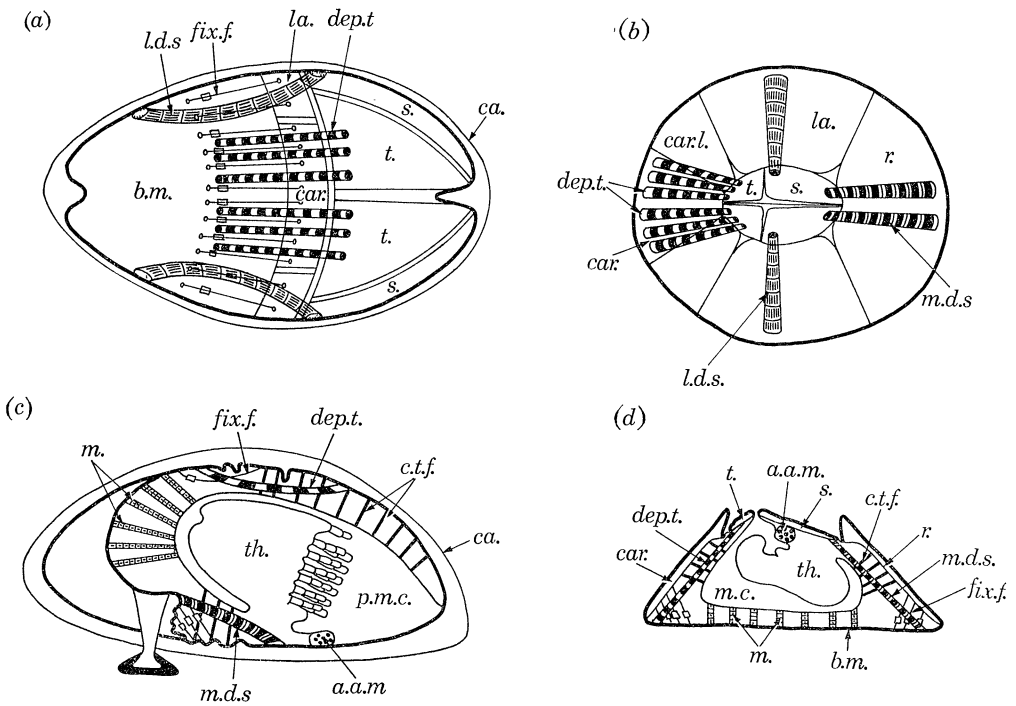

FIGURE 17. Diagram to illustrate the development of the adult mantle muscles after settlement in *B. balanoides*. (*a*) Adult mantle muscles in an attached cypris larva viewed from above. (*b*) Adult mantle muscles viewed from above. (*c*) Adult mantle muscles as seen in a sagittal section through an attached cypris larva; (*d*) Adult mantle muscles as seen in a sagittal section through an adult.

The account given here of the cypris–adult moult confirms that given by earlier workers, particularly Doochin (1951), but differs fundamentally from that given by Bernard & Lane (1962). They described a postecdysial stage, the 'decorticated settler'. This stage had a number of unusual features: it was described as 'a gelatinous parenchyma; with a number of organelles embedded in the parenchyma; and three distinctive organs henceforth referred to as "cylindrical cells", "glandular labyrinth" and "gut primordium"'. It is very difficult to correlate this description with the results obtained in the present study or in any previous study. Perhaps the most surprising feature of this 'decorticated settler' stage, was the reported absence of cuticle covering the epidermis. Doochin (1951) describing metamorphosis in the same species (*B. amphitrite niveus*) gives no evidence to suggest the existence of such a stage in the development and there is no evidence of any stage devoid of a layer of cuticle in *B. balanoides*.

(*a*) *Integument*

At ecdysis the cuticle covering the future basal area and the wall plates is 1 to 2 μm thick while that covering the thorax and lining the mantle cavity is < 0.5 μm thick. The edges of the shell compartments are delimited in stained sections, by the differential staining of the cuticle (figure 53, plate 62; figure 16). After the moult further cuticle matrix is deposited beneath the pre-ecdysial cuticle of the opercular valves and wall plates and this is later hardened by the deposition of calcium salts.

(*b*) *Musculature*

The musculature of the adult has been described in detail by Gutmann (1960). Histolysis of the cypris musculature is complete by the time ecdysis take place but the vacuolate haemocytes still contain fragments of muscle in intracellular vacuoles. By the time the cypris–adult moult takes place the adult musculature is fully formed. The mantle muscles are probably particularly important in establishing the shape of the shell of the young adult.

(*c*) *Nervous system and sense organs*

The structure of the adult nervous system is illustrated in figure 14 and 15. Darwin's (1854) description of the nervous system of *Balanus* spp. has been revised recently by Gwilliam (1965). He found that there were three photoreceptors in a number of *Balanus* spp. that he examined, these consisted of a pair of pigmented ocelli and a median unpaired and unpigmented photoreceptor organ. He found that the lateral ocellar axons entered the supra-oesophageal ganglion independently of the axons from the median photoreceptor. He stated: 'While it appears that the detailed structure of the three compartment of the larval median eye does not coincide with that of the presumed separate components of the adult photoreceptors, the mere existence of three distinct compartments in the nauplius eye indicates a possible developmental source of the three adult photoreceptors.' And this is indeed the case. Thus the ventral component of the nauplius eye is retained as the median photoreceptor (Darwin's 'ophththalmic ganglion'), while the two dorsal components become separated from each other and from the brain (supra-oesophageal ganglion) during the 24 h following ecdysis, to form the lateral ocelli of the adult barnacle. The photoreceptive properties of all three organs, in the adult barnacle, have been demonstrated by Gwilliam (1963, 1965) while their ultrastructure has been described by Fahrenbach (1965).

(*d*) *Alimentary canal*

The structure of the alimentary canal, of the pin-head sized barnacle (figure 14), is in all respects similar to that of the mature adult. The oral cone bears the now functional mandibles, maxillae and labial palps. The oesophagus and hind gut are lined with cuticle continuous with that covering the body surface. The U-shaped mid-gut is lined with a cubical-columnar epithelium, the cells of which frequently have a striated border.

Feeding is resumed in the young adult and the digestive glands show histological evidence of secretory activity. The gut increases in size rapidly during the first 10 days of growth and numerous mitoses can be seen in the epithelium. The epidermal glands of the second maxillae are retained as the adult labial glands, which open on the labial palps (Walley 1967).

(*e*) *Excretory system*

The morphology and histology of the maxillary glands of the adult *B. balanoides* have been described by Nilsonn-Cantell (1921). The maxillary glands are thought to be the organs of

excretion and ionic regulation in the adult barnacle. The maxillary glands of the pin-head sized barnacle are in all respects, except size, the same as those of the mature adult, consisting of an end sac and an excretory canal opening on the labium.

(*f*) *Cement glands*

In the adult the cells of the cement gland lie in the connective tissue between the mantle cavity and the basal membrane. The gland consists of a number of large glandular cells each with an irregular vacuole communicating with a system of intracellular ducts opening through the basal membrane. The precise disposition and structure of these cement gland ducts is not known in *B. balanoides* but has been described in *Elminius modestus* by Bocquet-Védrine (1965). The chemical nature of the secretion produced by these glands is not known. The gland cells seem to arise around the collecting duct of the cypris cement gland before ecdysis (figure 13). After ecdysis the cement cells of the cypris cement gland break down and the newly formed adult gland cells become separated—apparently by the elongation of the cells forming the collecting ducts.

(*g*) *Yellow cells and haemocytes*

Immediately after ecdysis the haemocytes can be seen in the mantle tissue spaces, and contain fragments of histolysed intracellular vacuoles. The yellow cells can be seen particularly in the regions underlying the shell plates. One week later these haemocytes and yellow cells can no longer be clearly distinguished, but the spaces between the organs are filled with undifferentiated tissue in which are well defined blood lacunae. The origin of this tissue is not known. It may arise by the transformation of the haemocytes, but far more detailed study will be required to elucidate this point. The evidence regarding the transformation of circulating haemocytes into other tissues in insects has been reviewed by Jones (1962), who concluded that there is no definite evidence that such haemocytes are the exclusive source of the various different types of connective tissue cell.

(*h*) *Reproductive system*

Several workers (Darwin 1851, 1854; Doochin 1951; Barnard & Lane 1962) have described the rudiments of the reproductive system, particularly the ovaries, as being present in the cypris larva. However, no trace of ovarian tubules or testis follicles were ever seen at any stage in the development of *B. balanoides* until the rudiments of the reproductive system appear 3 to 5 weeks after metamorphosis (Walley 1965).

8. Discussion

The present study has confirmed that the adult shell and mantle tissue develop from the pre-oral region and the carapace of the nauplius larva. The pre-oral origin of the cement glands, in the nauplius larva, provides further evidence in support of this interpretation of adult structure. The adult adductor scutorum develops independently of the cypris carapace adductor muscle; it develops pre-orally after the histolysis of the larval muscle. Hessler (1964) correctly states that the larval adductor is postoral in position and considers it to be post-maxillary. He supposes, correctly, that the adductor muscle develops behind the paired muscles 21, but allocates this pair of muscles to the first maxillary (maxillulary) segment. The first maxillae, however, do not develop until the later nauplius stages (figure 18) and the muscles associated with the adult first maxillae do not develop until the late cypris stage, whereas

the paired muscles 21 are present in all the nauplius stages and, apparently, even before the delimitation of the first maxillary segment. Thus there seems no reason to allocate these muscles (21) to the first maxillary segment and it seems that the cypris carapace adductor muscle should be considered to develop between the mandibular and first maxillary segments, rather than in a postmaxillulary position as suggested by Hessler (1964).

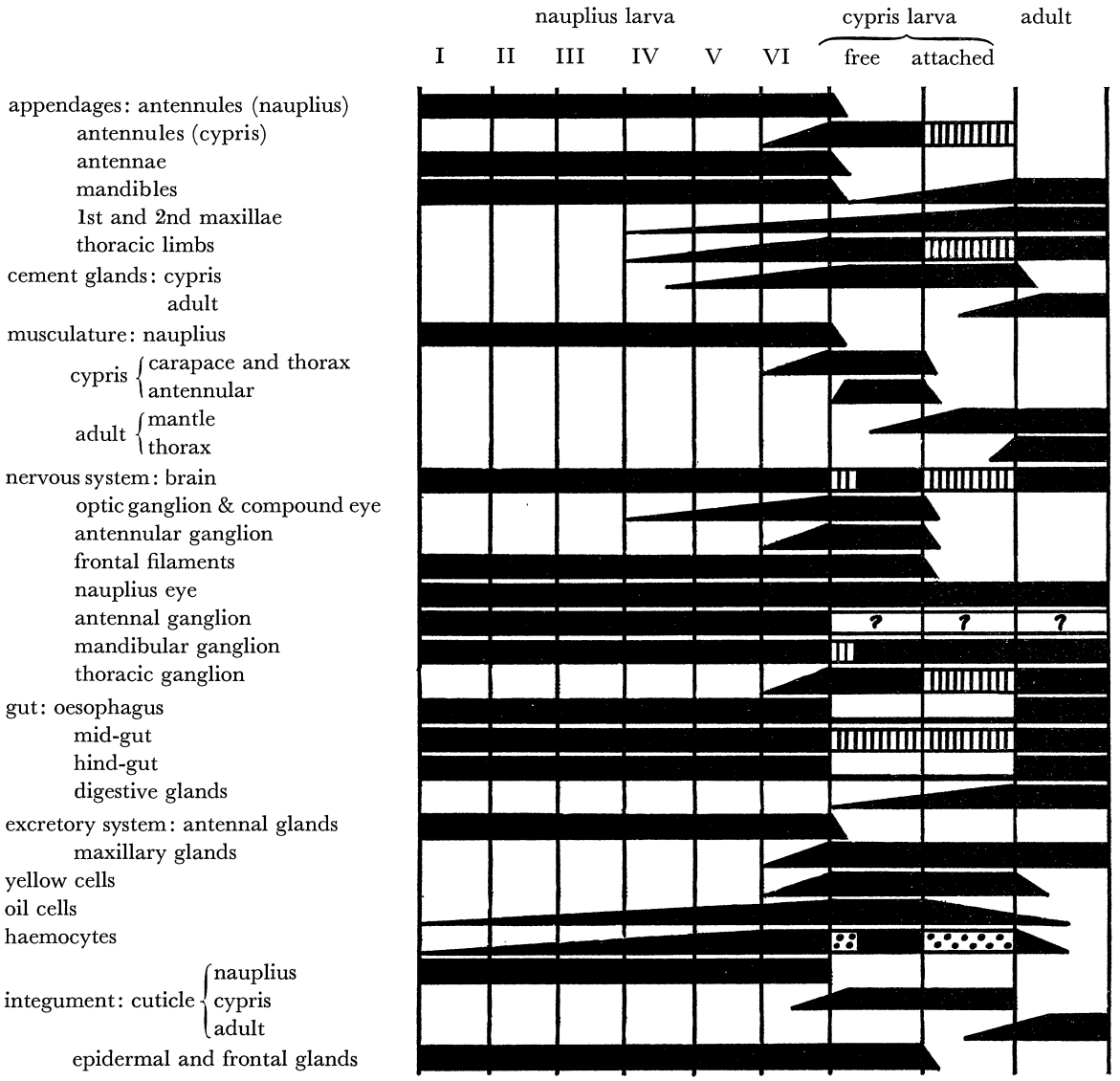

FIGURE 18. Table summarizing the anatomical changes that take place during the larval development and metamorphosis of *B. balanoides*. The development, persistence and/or loss of each organ system and set of appendages is represented by the solid black areas. ▥ indicates histolysis, ⚄ indicates a phase of phagocytic activity, [?] indicates that the fate of that structure is not known.

The development of the postmandibular segments in *B. balanoides* seems to be essentially similar to their development in *I. quadrivalvis* (Anderson 1965). The sequence of events in *Ibla* is considerably speeded up, however, as it is lecithotrophic and passes through its whole larval development, from hatching to cypris, in 6 to 7 days as compared with about 4 weeks in *B. balanoides*. Anderson reported that in *Ibla* the maxillary segments formed 'as a continuation

of the nauplius sequence' of segment formation. In *B. balanoides*, however, the second maxillary segment arises from the same group of mesoteloblasts and ectoteloblasts as the six thoracic segments. The origin of the first maxillary segment is not easy to determine; it may, as Anderson suggests, arise as a continuation of nauplius segment formation or it may have the same origin as the remaining postmandibular segments. This point needs further investigation.

Until the present time most studies on cirripede 'metamorphosis' have been concerned only with the change from cypris larva to adult perhaps as a result of concentration on the marine fouling aspect of cirripede settlement and development. Figure 18 summarizes the anatomical changes that take place during the larval development of *B. balanoides*. It is clear that the development of adult structures begins even in the later nauplius stages, but that the greatest rate of change is during the last nauplius stage, through the cypris larva to the young adult. Thus I prefer to consider cirripede metamorphosis as comprising the two-stage process, nauplius to cypris and cypris to adult.

From studies on adult cirripedes Newman (1967) suggested that the four- and six-plated forms have arisen from ancestral eight-plated forms by 'concrescence and/or loss of certain plates'. The differential staining of the plate borders provides a technique for studying the course of development of the shell plates in other cirripedes. Comparison of these very early stages in shell plate development in the different forms, using this technique, might help to decide between these alternatives.

The cypris larva is specially adapted to recognize and settle on surfaces suited to the requirements of the adult cirripede: 'its whole organization is apparently adapted for the one great end of finding a proper site for its attachment and final metamorphosis' (Darwin 1851). The most obvious adaptations for this function are the special sense organs—the complex sensory apparatus of the antennules (Nott & Foster 1969) and the paired compound eyes—the well-developed swimming appendages and the adhesive apparatus consisting of cement glands and adhesive disks of the antennules. Bayne (1965) emphasized the combination of efficient locomotory systems—the velum and foot—and special sense organs—eyespots, statocysts, apical and abdominal sense organs—in the pediveliger larva of *Mytilus edulis*. 'This is the stage at which the larva becomes sensitive to certain features of the environment that may stimulate attachment and the adoption of the benthic habit' (Bayne 1965). In both the *Balanus* cypris larva and the *Mytilus* pediveliger the development of specialized organs is accompaned by behavioural changes essential for the successful transition from the planktonic to the benthic environment, and once this transition has occurred these organs are lost or modified.

Darwin (1851) described the cypris larva as a 'locomotive pupa' by analogy with the non-feeding pupal stage of insects. This analogy is strengthened by the observation of tissue histolysis during metamorphosis (Darwin 1854; Bernard & Lane 1962) and of the ingestion and digestion of histolysed tissue fragments by phagocytic cells (Walley 1964). During metamorphosis in *B. balanoides*, and in other groups as well, the development of a particular organ system is initiated well before it is required to function (figure 18) so that the transition from one stage to the next can be achieved as smoothly as possible. In some cases the changes are so extreme that development of the structures belonging to the next stage cannot proceed while the organism is still feeding and moving about normally. In such cases the organism enters a state of apparently suspended animation, becoming immobile and apparently dormant within a protective outer casing. But within, there is intense morphogenetic activity as old structures are broken down and new ones differentiate. Such a stage is illustrated by the pupae of the

Diptera, Lepidoptera and other groups of insects, and to a limited extent by the cypris larva of the cirripedes in the period between settlement and the moult to the young adult. In all these cases, and in the metamorphosis of the Amphibia (Metchnikoff 1892), of *Ostrea edulis* (Cole 1938) and probably in many other animals, the redundant structures histolyse and the resultant fragments are ingested and digested by phagocytic cells. In certain polychaete larvae (Wilson 1932) and nemertine larvae (Cantell 1966), the unwanted tissues are sloughed and then eaten by the newly metamorphosed animal and presumably digested in the normal way. In both these cases these are mechanisms for the rapid disposal of redundant tissues and the re-utilization of the materials contained in these tissues for the rapid growth of new organs. It has been shown that the completion of the metamorphic change can be delayed until the right conditions for the succeeding phase are encountered (Thorson 1950; Wilson 1952; Bayne 1965). The experiments of Crisp & Meadows (1963) suggest that this may also be the case in cirripedes.

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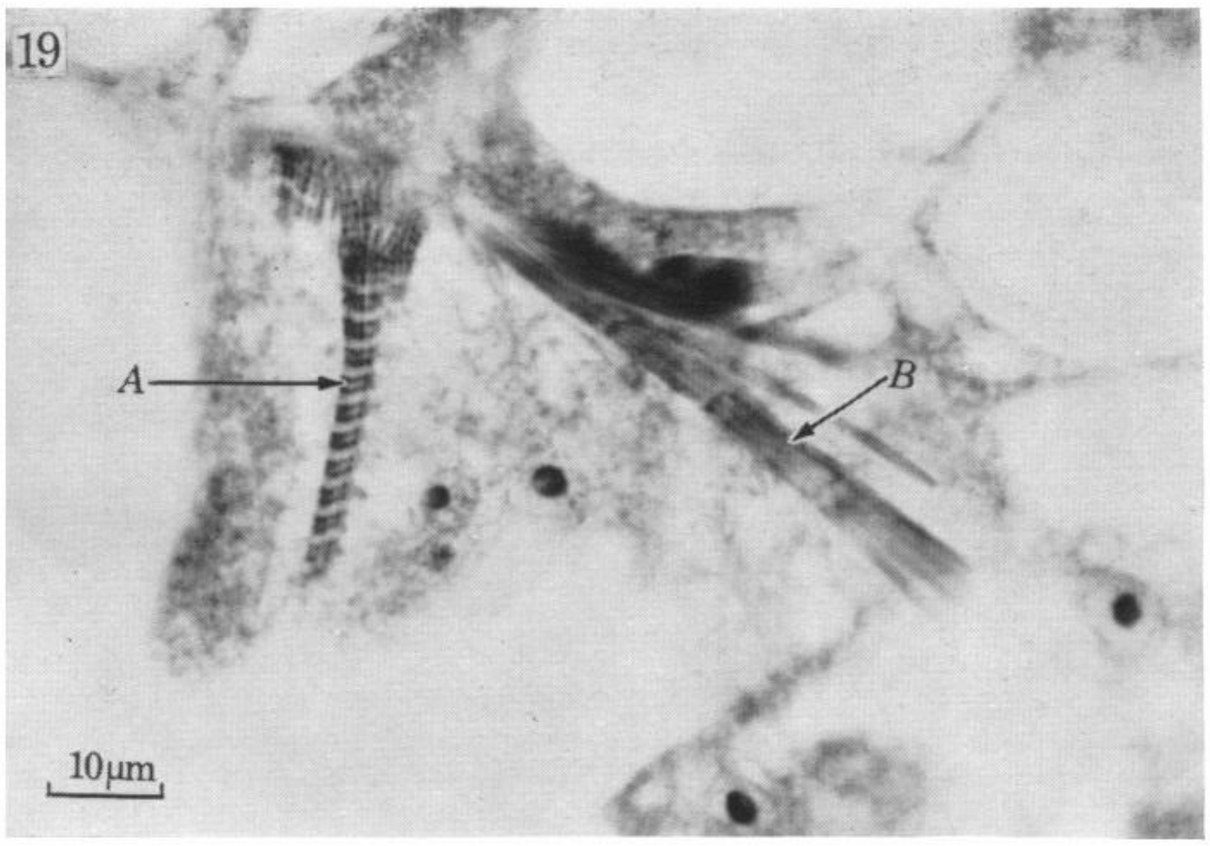
KEY TO ABBREVIATIONS ON FIGURES 1 TO 17 (Not on the plates)

a. anus
a.a.m. adult adductor muscle
a.d. adhesive disk
a.m. cypris adductor muscle
a.m.c. anterior mantle cavity
*ant.*1 antennule
*ant.*1*b.* base of antennule
*ant.*1*g.* antennular ganglion
*ant.*1*n.* nerve to antennule
*ant.*2 antenna
*ant.*2*b.* base of antenna
*ant.*2*g.n.* antennal ganglion and nerve
b. brain
b.m. basal membrane
c. cuticle
*c.*1 pre-ecdysial cuticle
*c.*2 post-ecdysial cuticle
ca. carapace
car. carina
car.l. carino-lateral plate
cau.ap. caudal appendage
cau.sp. caudal spine
ce. cement
ce.d. cement duct
ce.gl. cement gland
c.eye compound eye
c.m. circular muscles around the gut.
com. commissures joining the paired thoracic ganglia
con. mid gut constriction
c.t.f. connective tissue fibres
d. duct
dep.t. tergal depressor muscle
d.gl. digestive gland
dil.m. oesophageal dilator muscle
e. epidermis
e.a.m. extrinsic antennular muscles
e.d. excretory duct
e.j.c. 'épidermique juxtacristalline' cell
e.m. eye muscle
end. endosternite
e.s end sac
f.b. food body
f.f. frontal filament
f.gl. frontal gland opening
f.h. frontal horn
fix.f. fixation fibre
g. gut
gl. gland cell
*gl.*1 and 2 epidermal gland cells
h. haemocyte
hc. haemocoel
h.g. hind gut
l. lens
la. lateral wall plate
lab. labrum
lab.gl. labral glands
lab.n. labral nerve
l.l. lateral lobe of brain
l.c. lens-secreting cell
l.d.s. lateral scutal depressor muscle
m. muscle
*m.*1 nauplius muscle number 1
*m.*6 cypris muscle number 6
m.c. adult mantle cavity
md. mandible
md.g.n. mandibular ganglion and nerve
md.b. base of mandible
m.d.s. median scutal depressor muscle
m.g. mid gut
mo. mouth
m.ph. median photoreceptor
m.s. muscular sac
*mx.*2 second maxilla
n.e. nauplius eye
n.s. nervous system
o. ocellus
o.c. oil cell
o.d. opening of excretory duct
oe. oesophagus
o.m. opercular membrane
op.g. optic ganglion
op.n. optic nerve
op.t. optic tract
or.c. oral cone
o.th.m. oblique thoracic muscle
p.m.c. posterior mantle cavity
r. rostrum
r.a.gl. right antennal gland
ret.c. retinula cell
rh. rhabdome
rud. c. eye. rudimentary compound eye
rud.gl. rudimentary adult cement gland
rud.m.m. rudimentary adult mantle muscles
rud.th.ap. rudimentary thoracic appendages
s. scutum
se. secretion
*seg.*4 fourth segment of antennule
s.f. suspensory fibres
s.f.f. sinus at base of frontal filament
sph. position of sphincter
t. tergum
t.a.m. tendon of cypris adductor muscle
t.b.c. thickened bar of cuticle at base of antennule
th. thorax
th.ap. thoracic appendages
th.g. thoracic ganglion
th.n. thoracic nerves innervating thoracic limbs I–VI
t.th.m. transverse thoracic muscles
v.th.p. ventral thoracic process
y.c. yellow cells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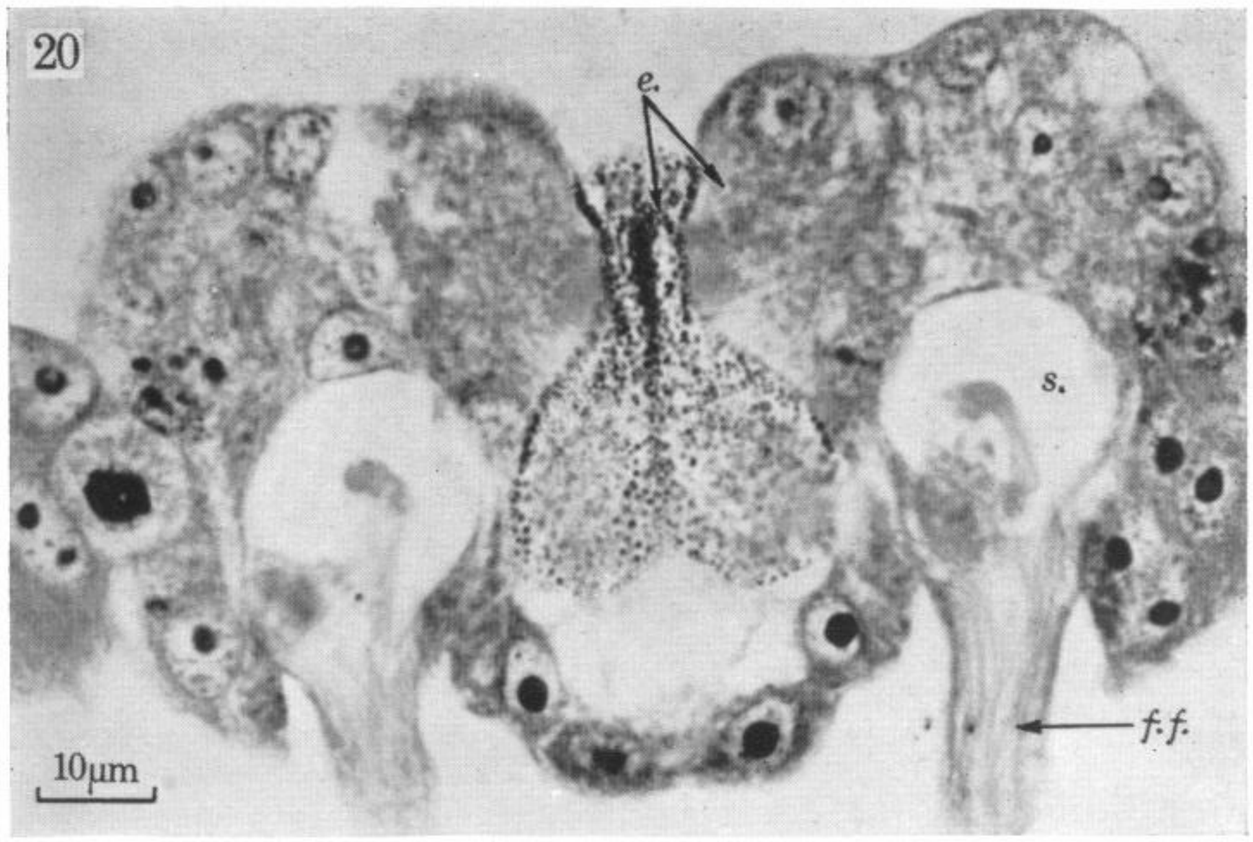
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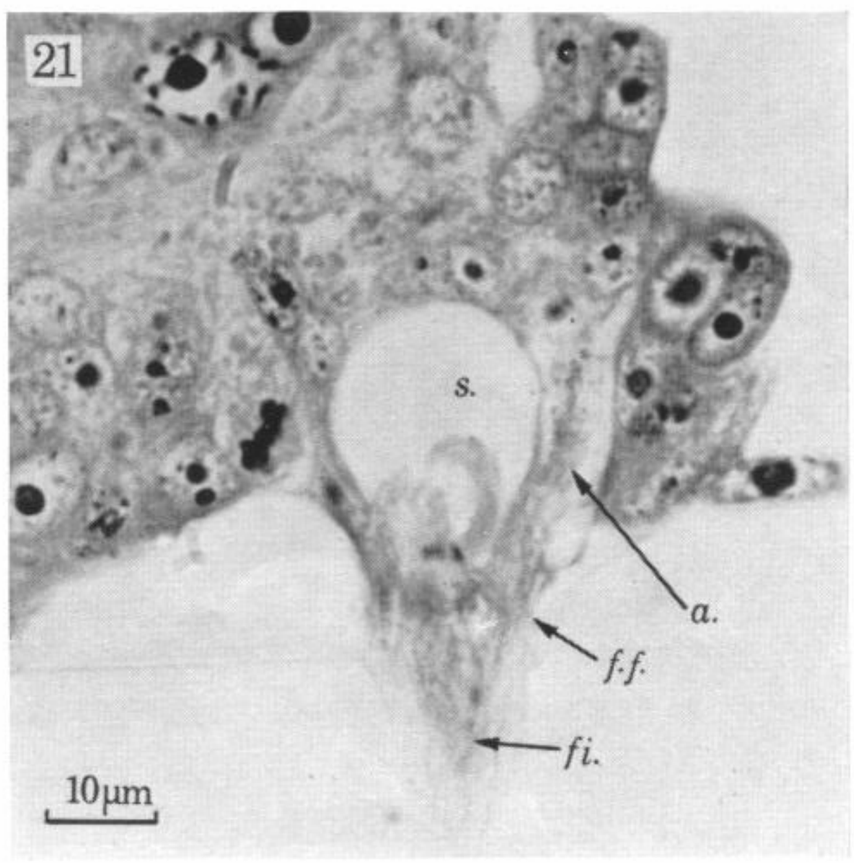
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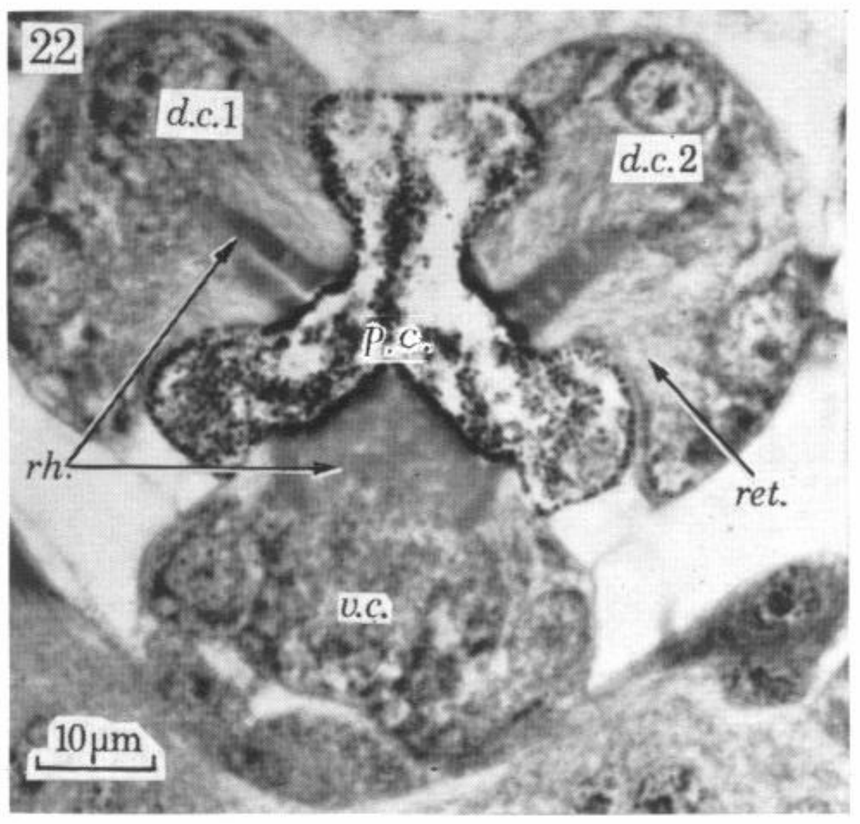
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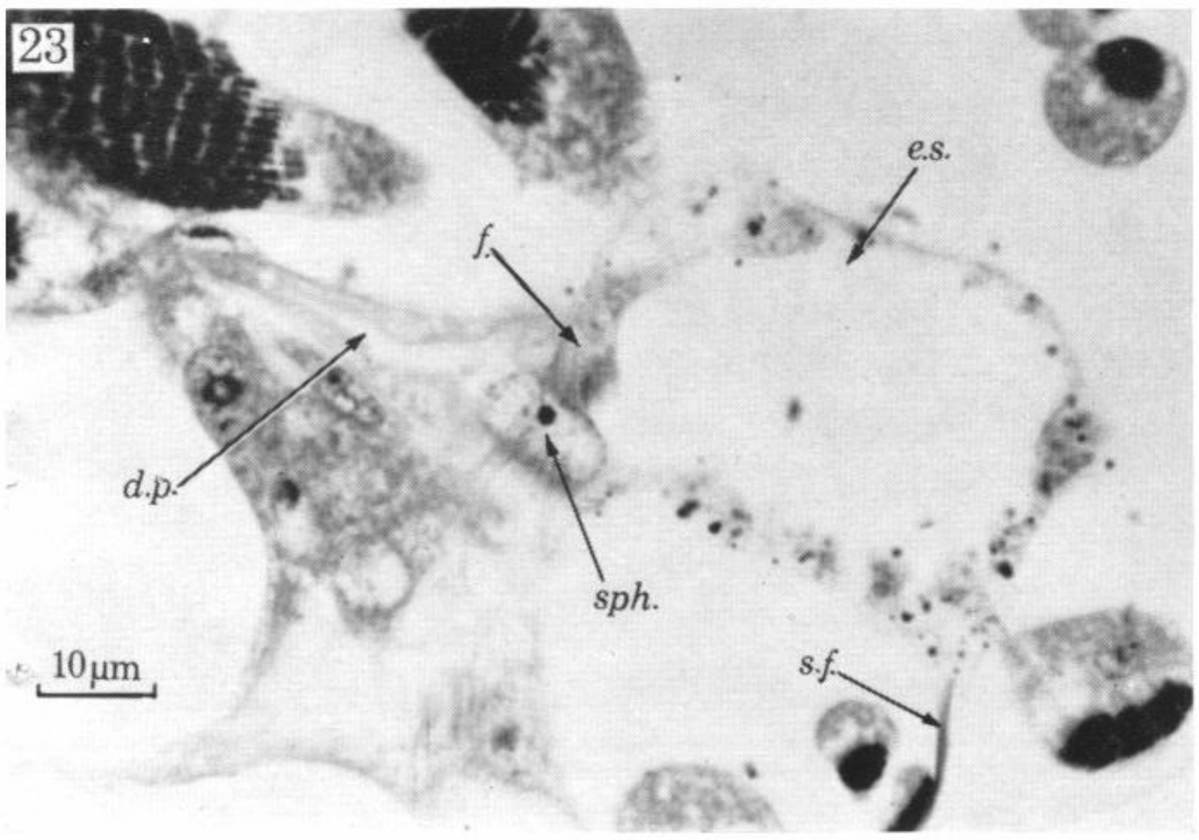
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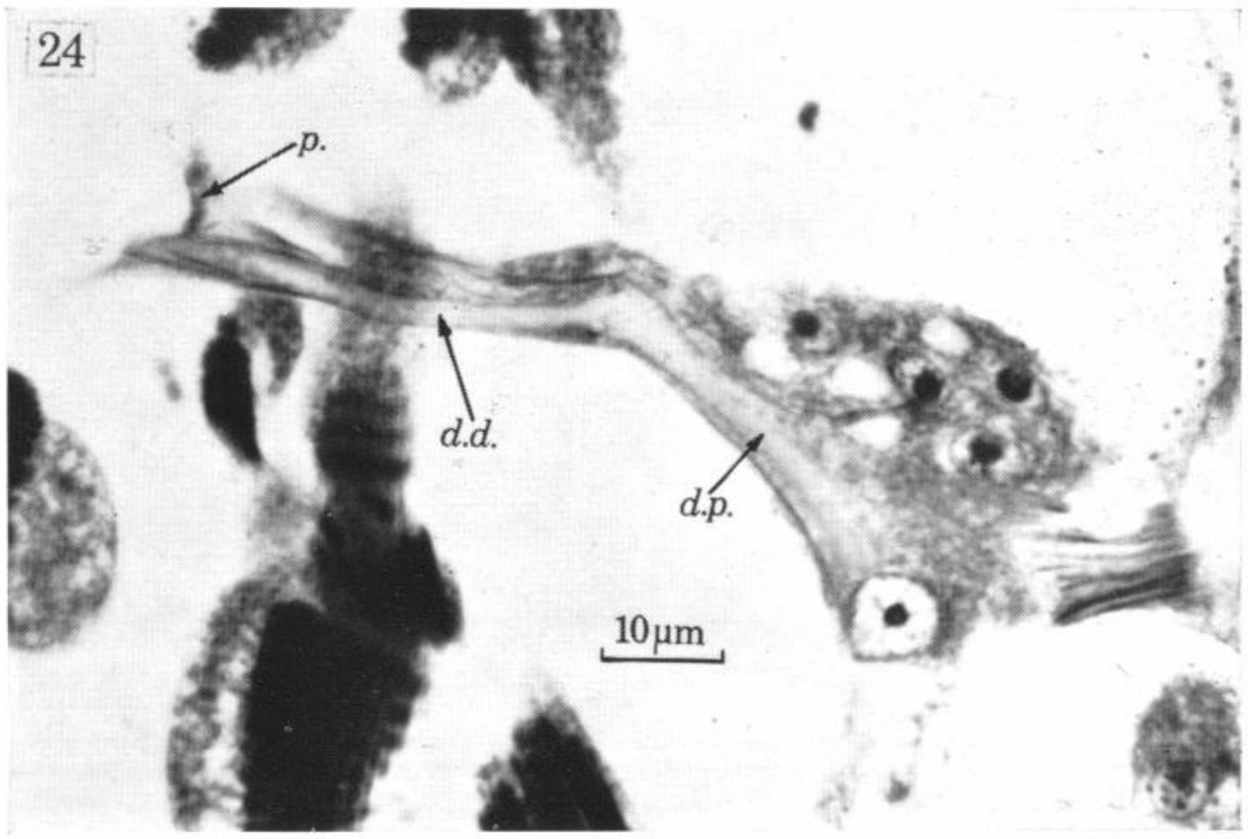
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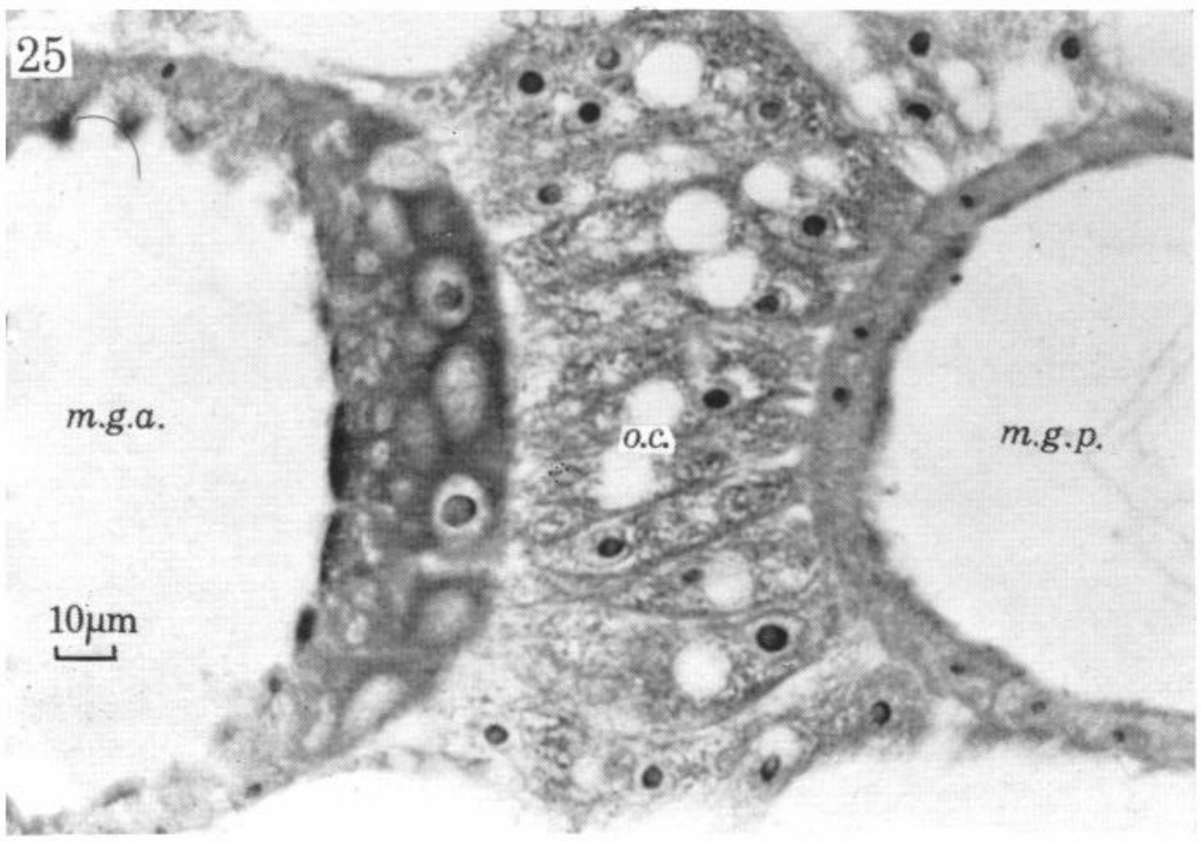
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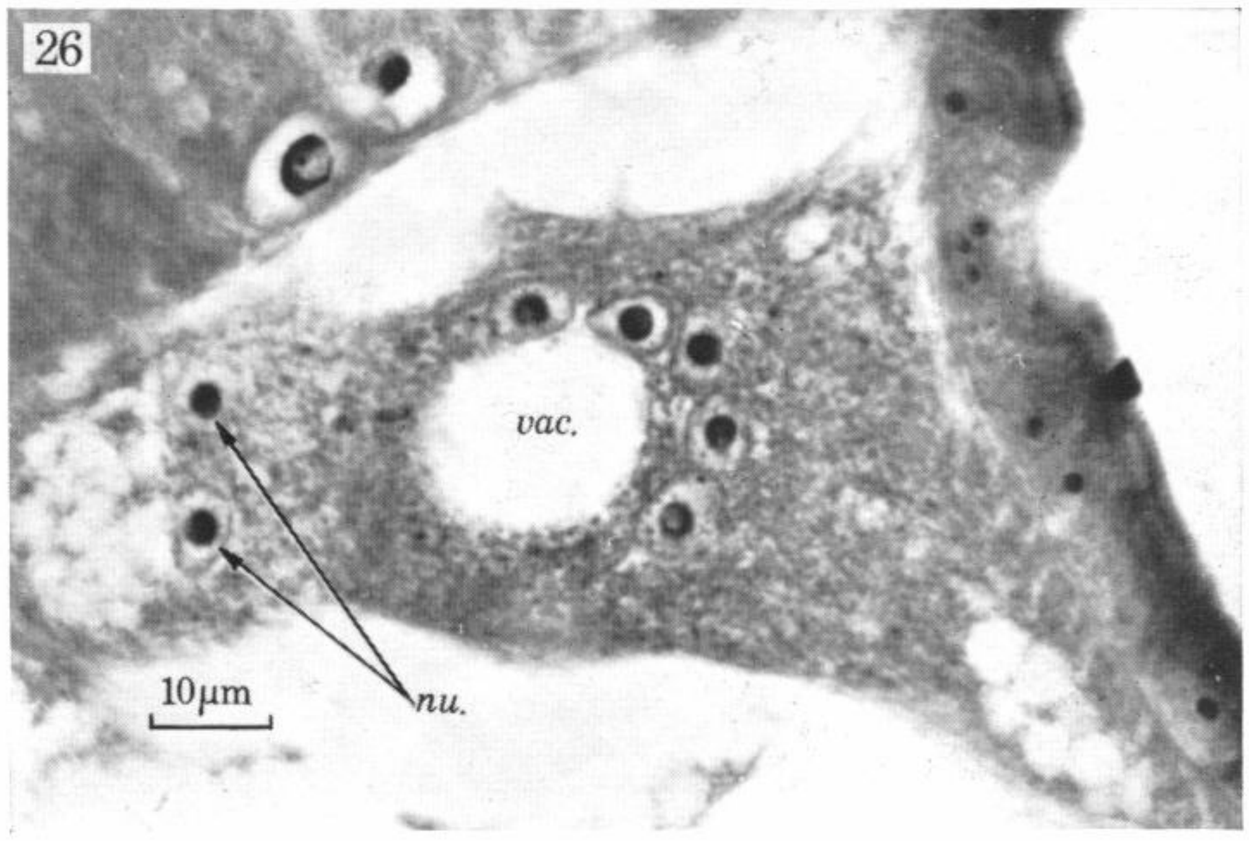
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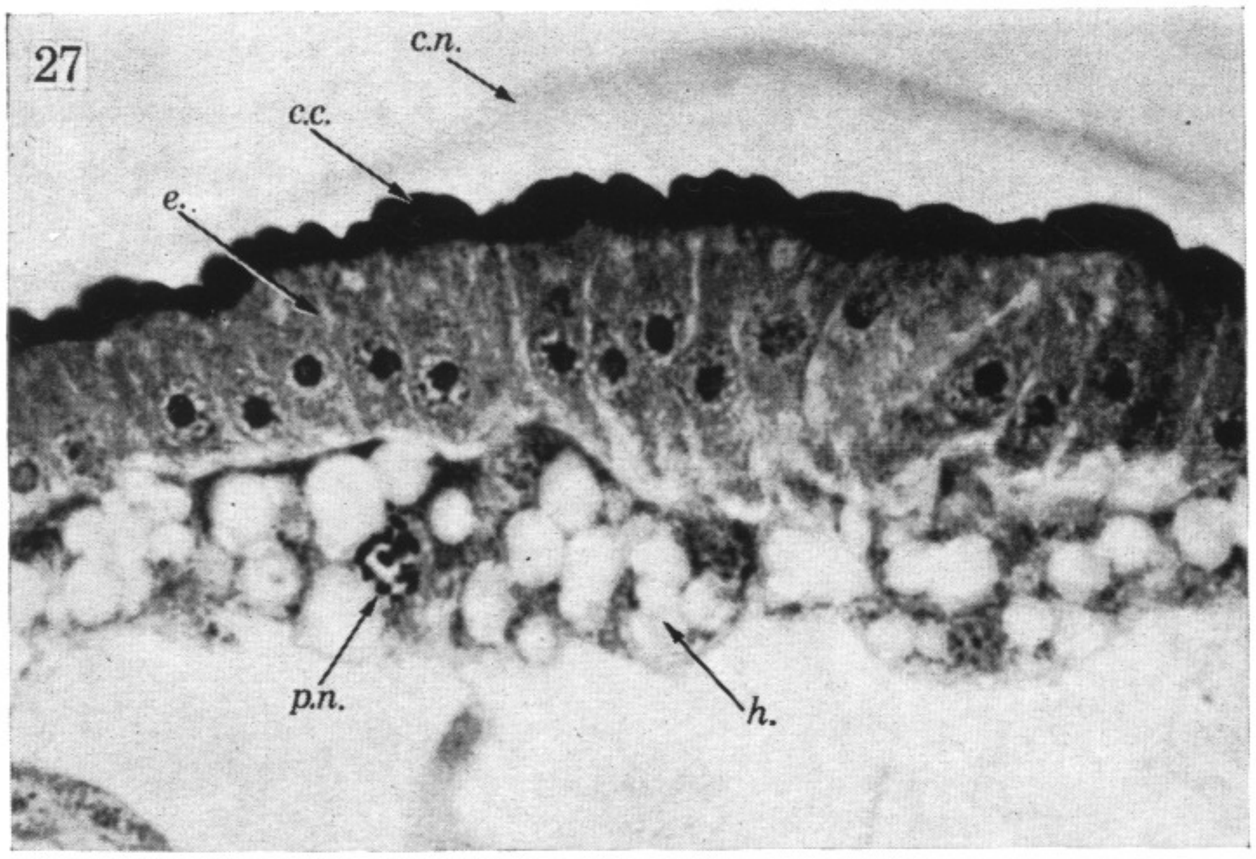

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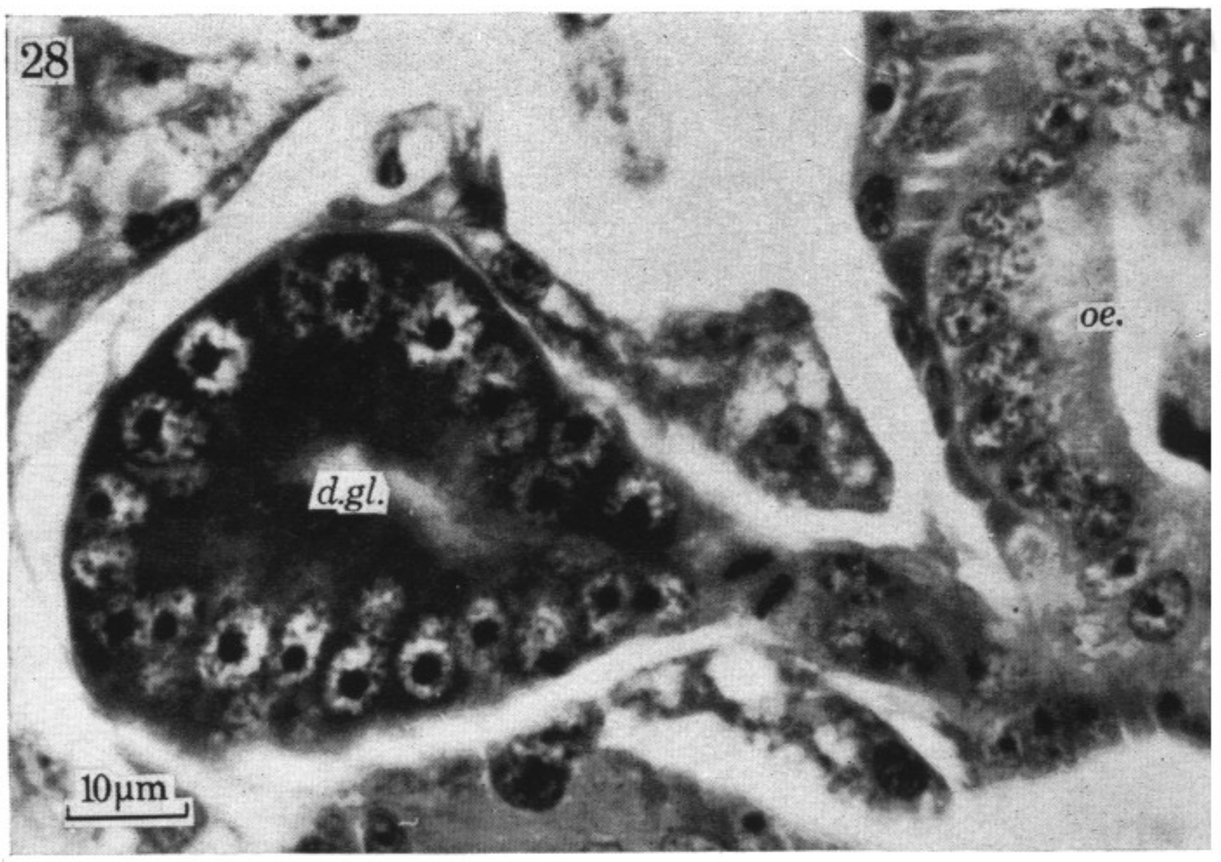

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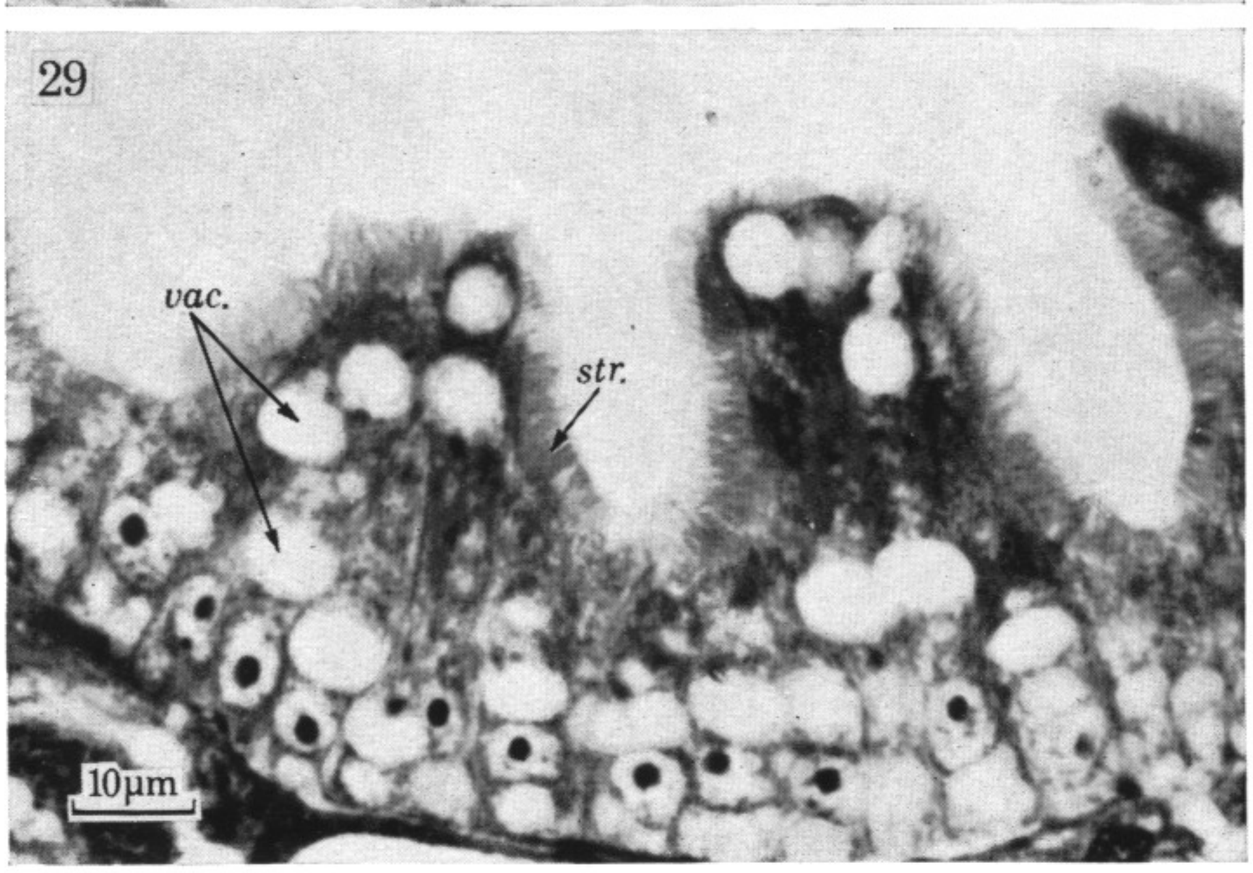

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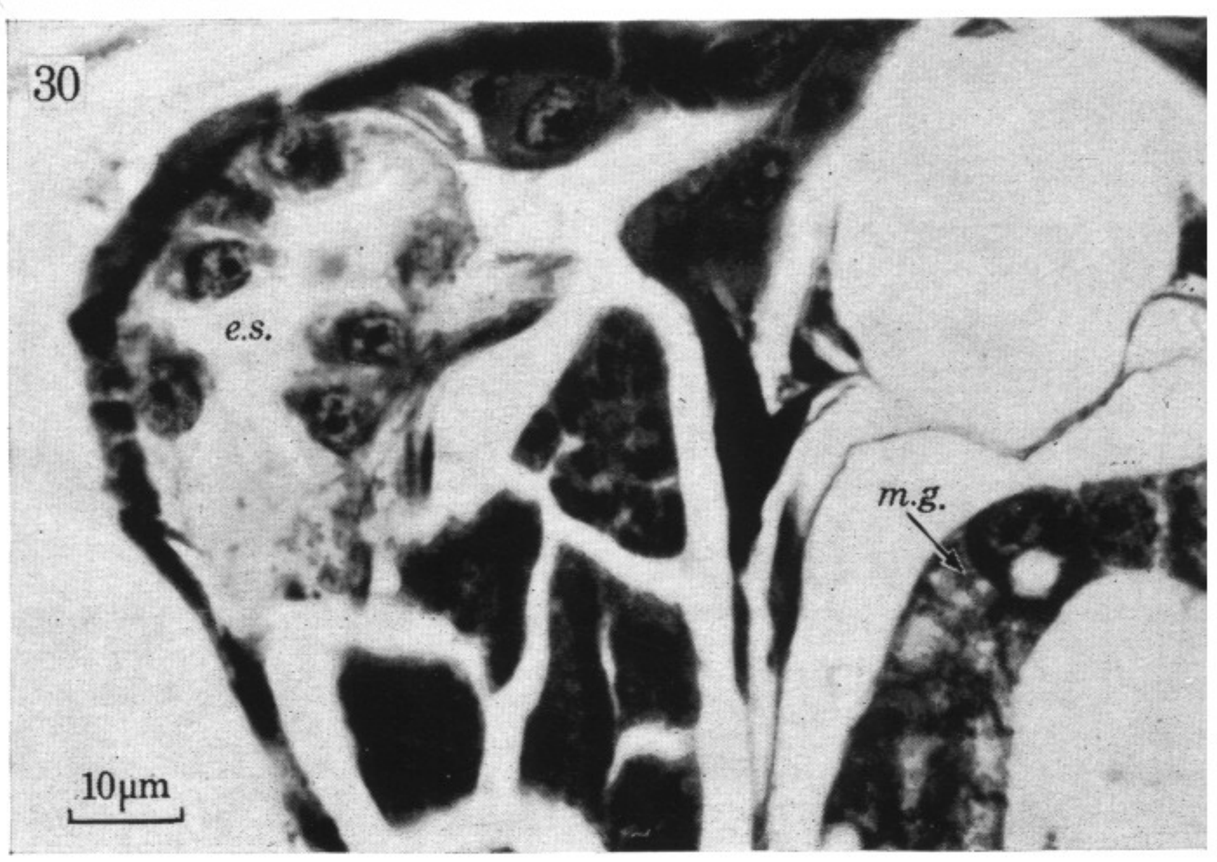

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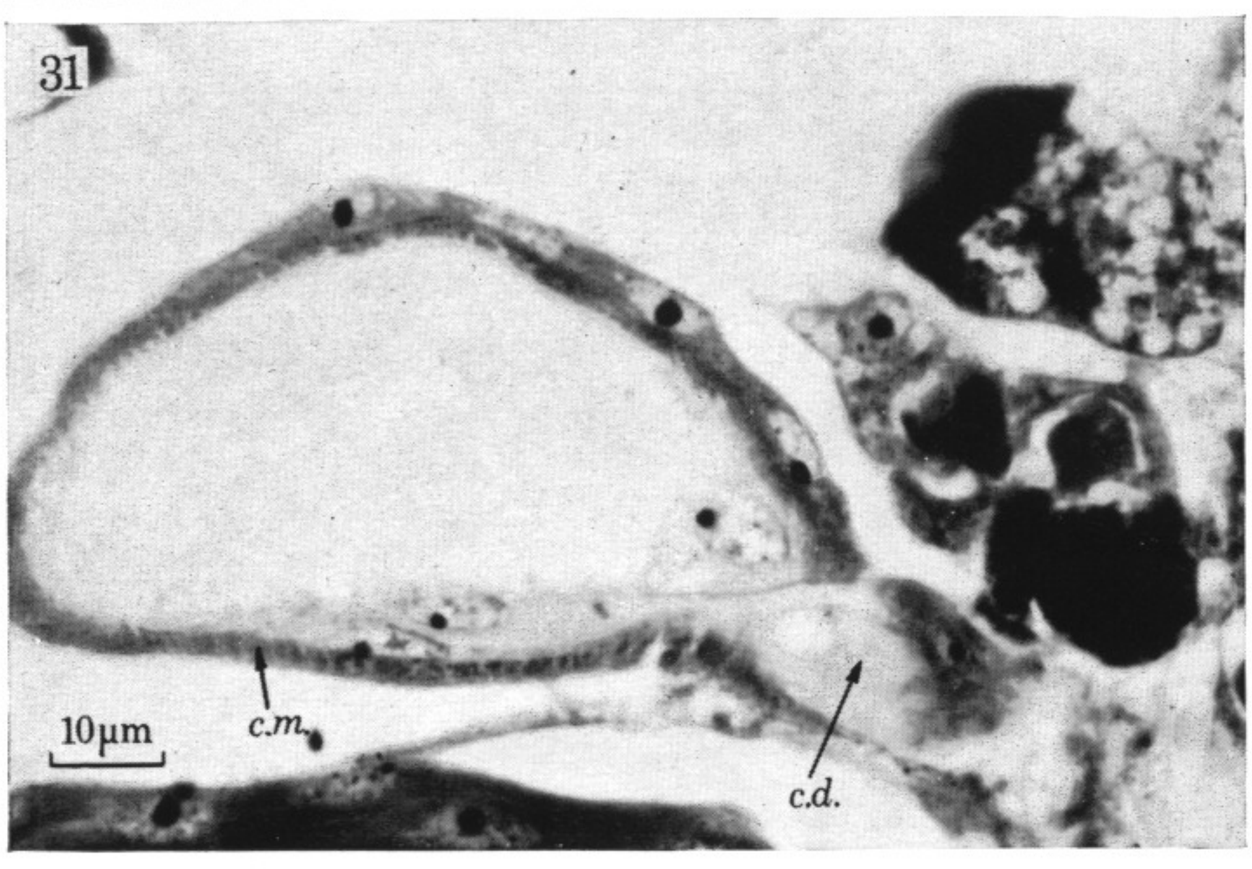

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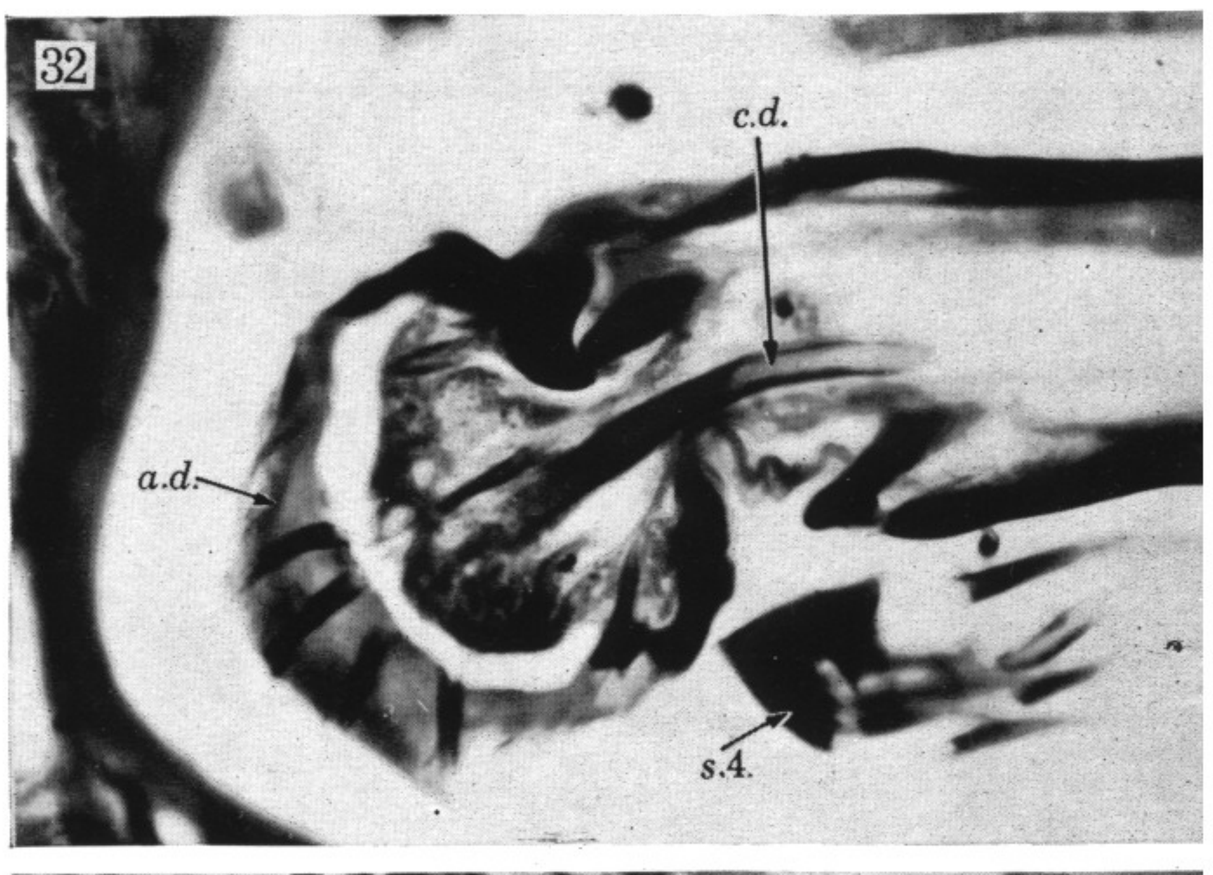

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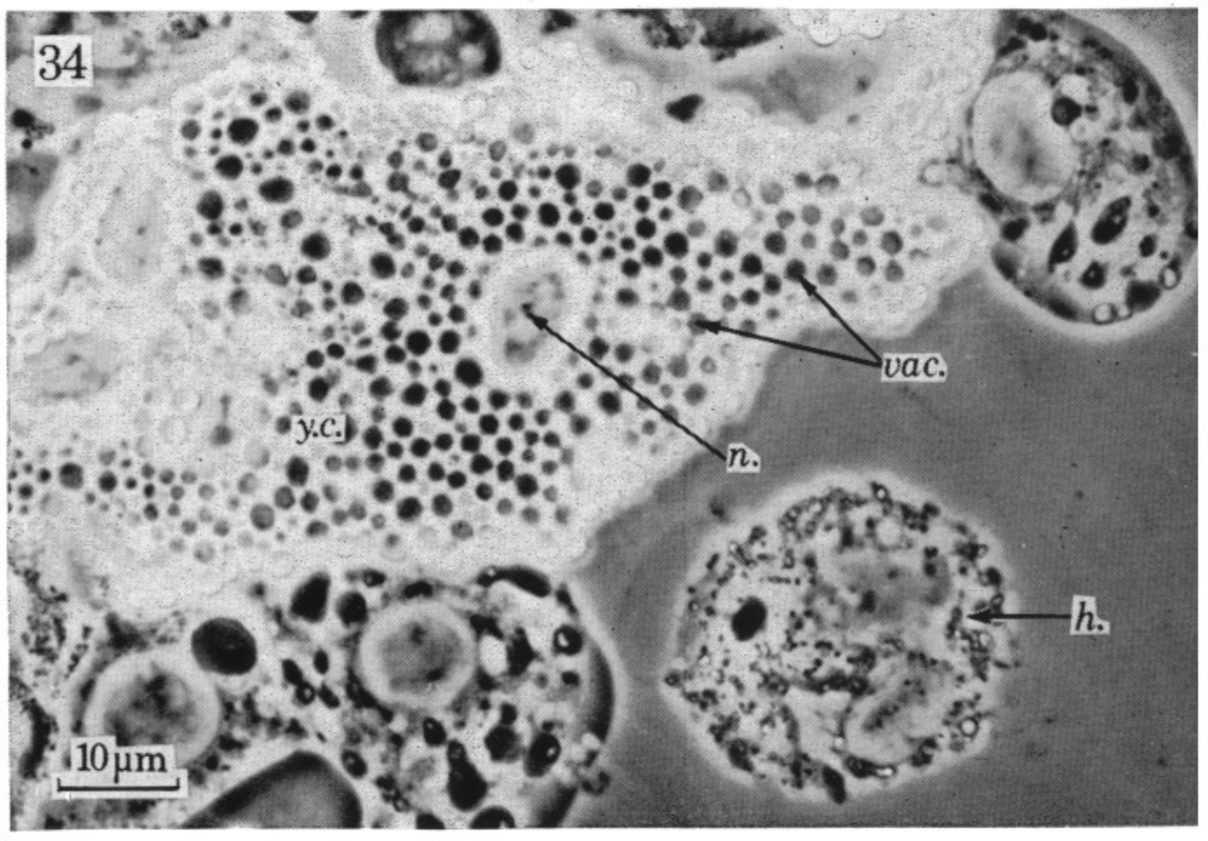

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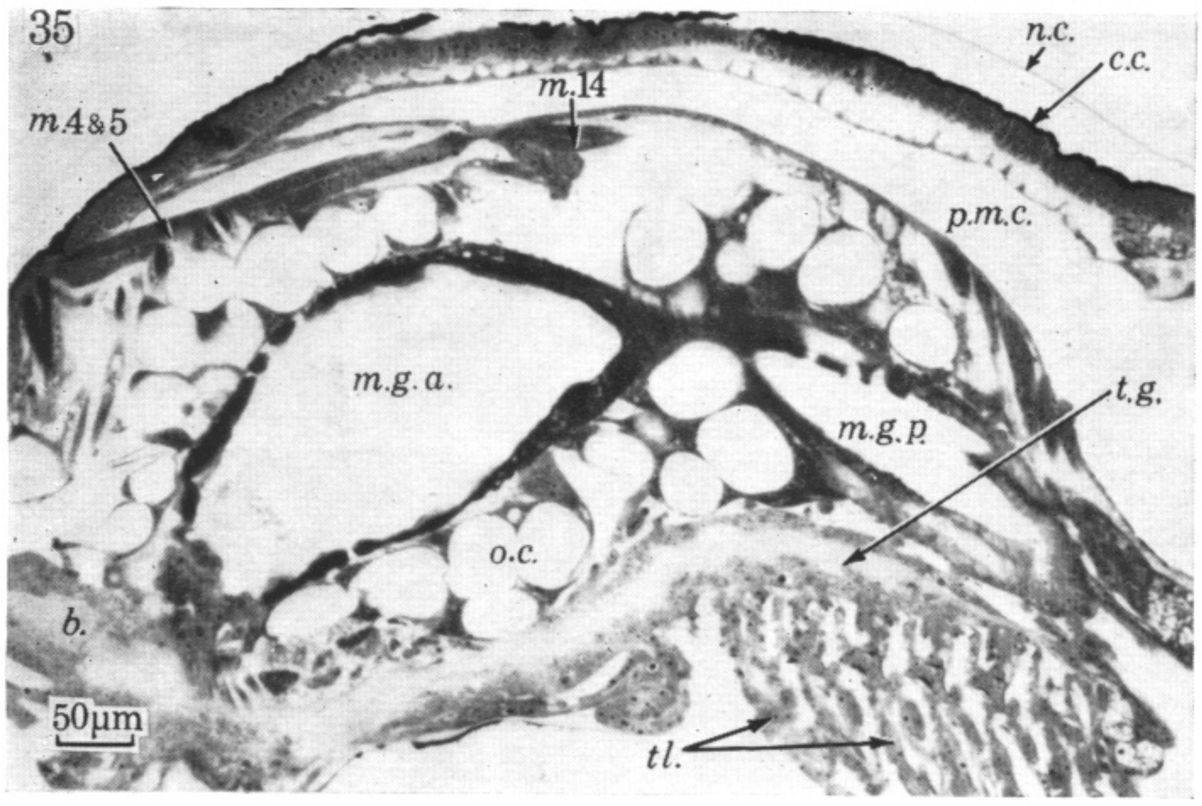

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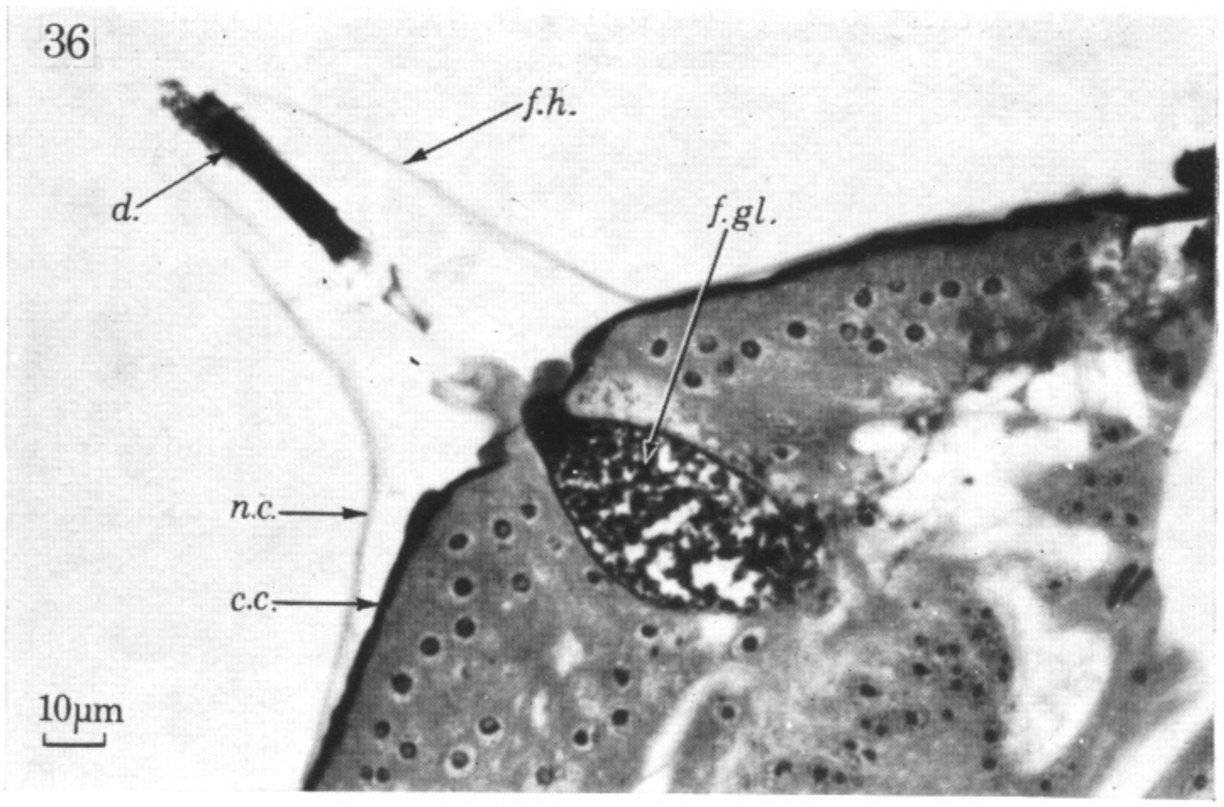

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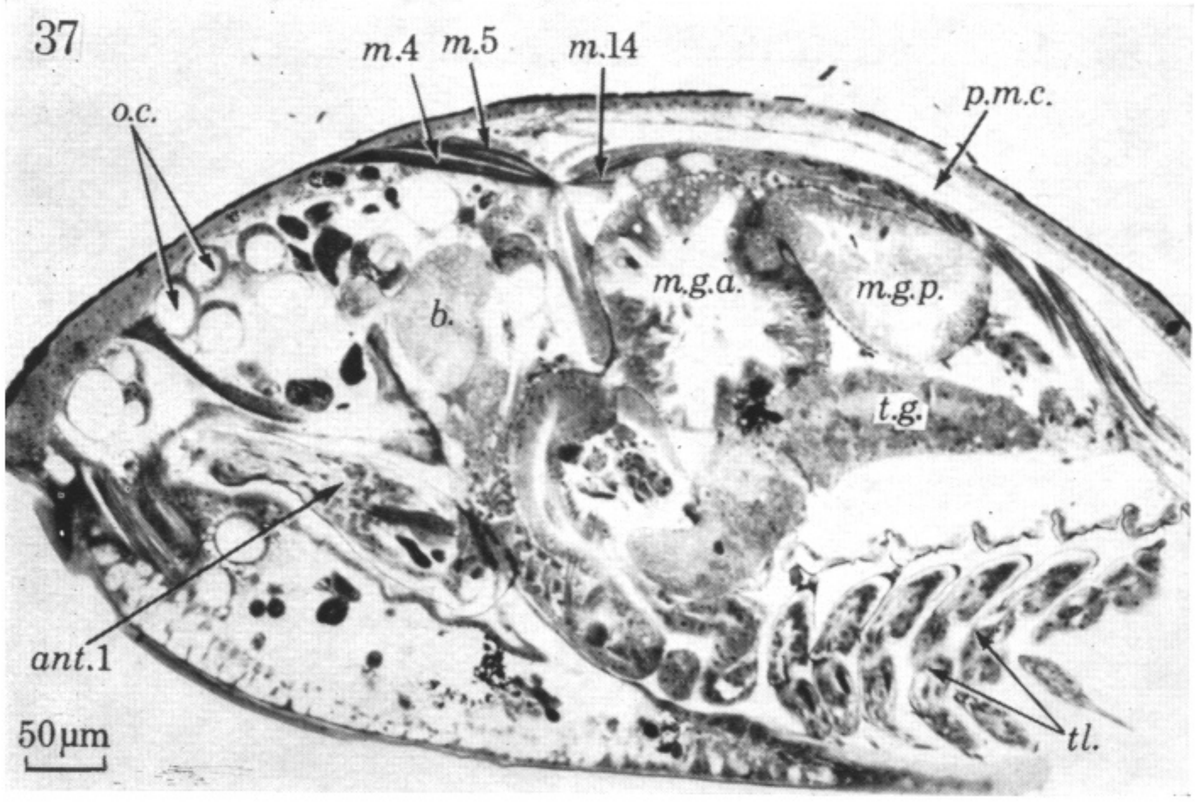

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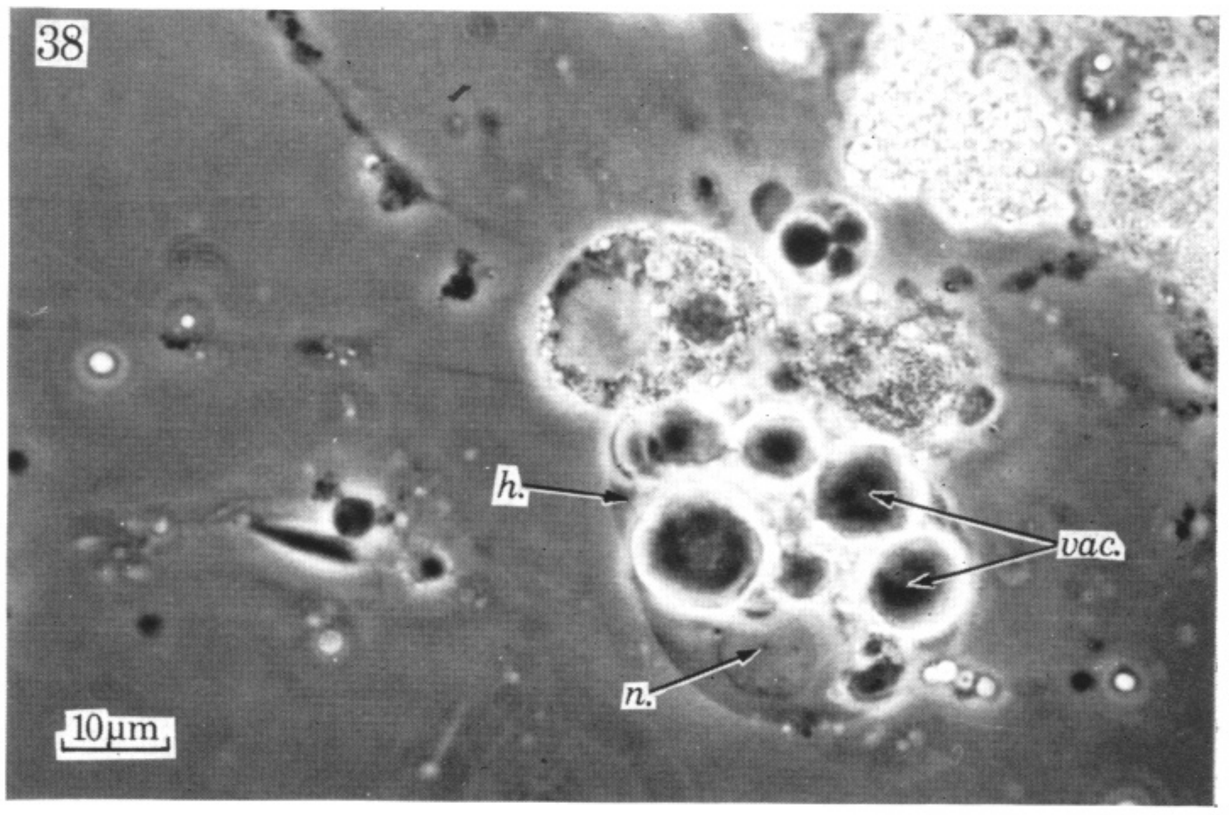

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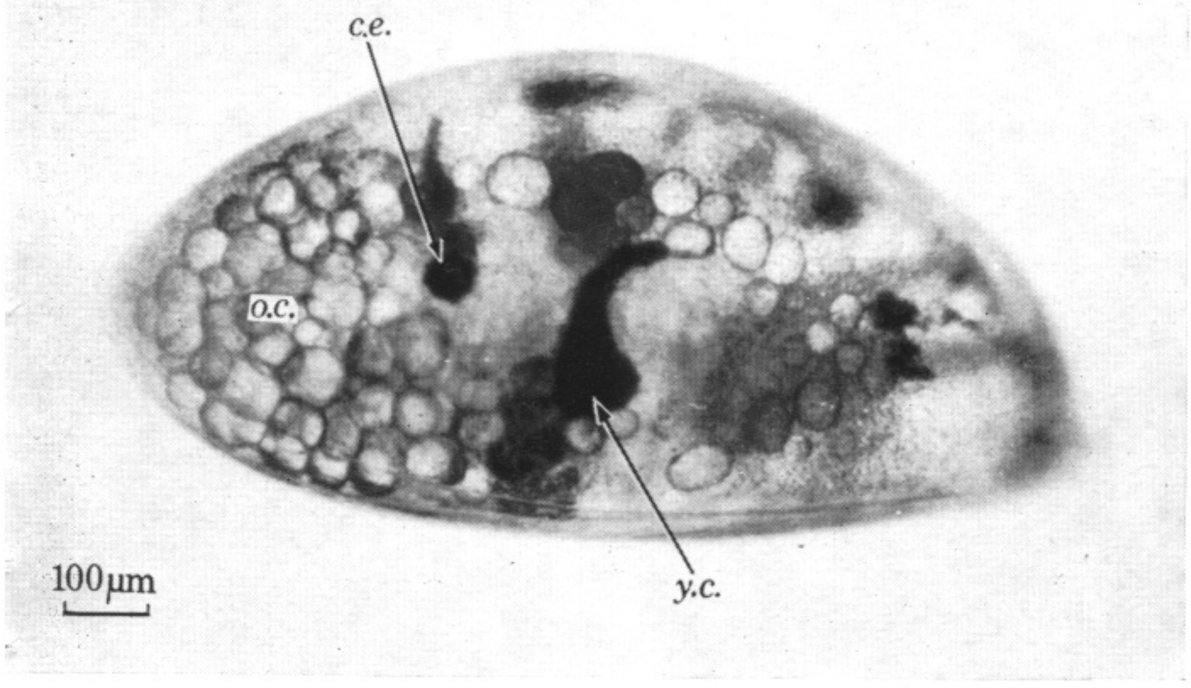

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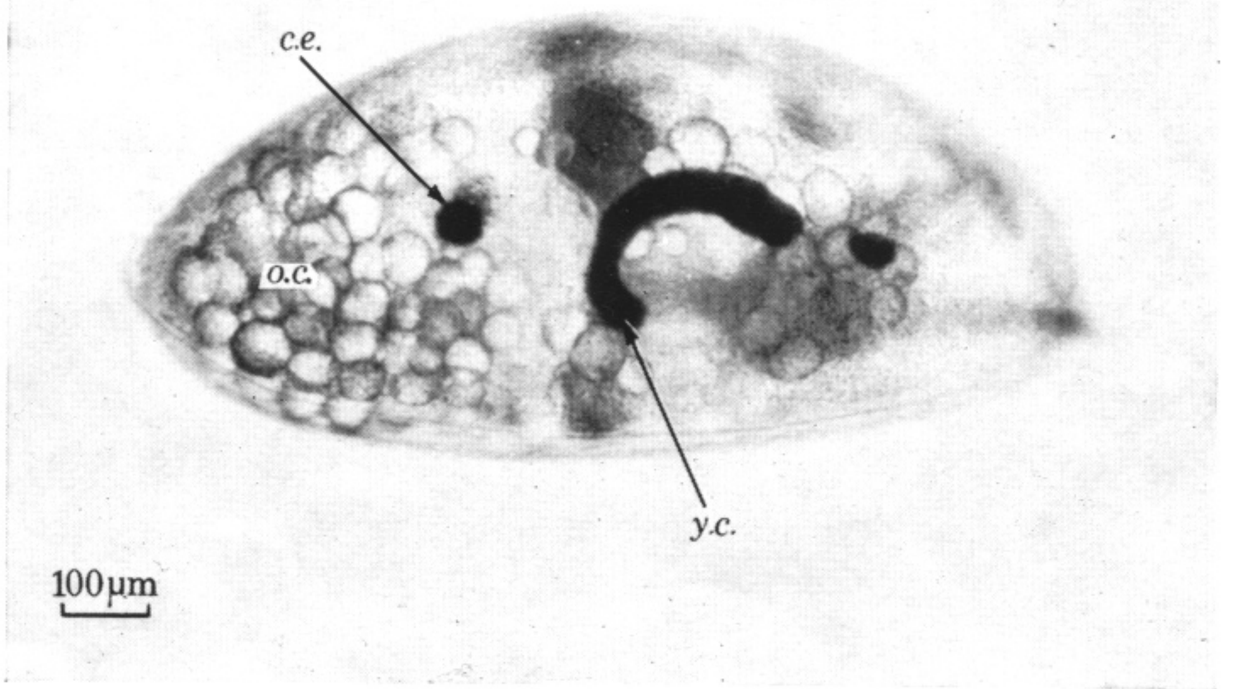

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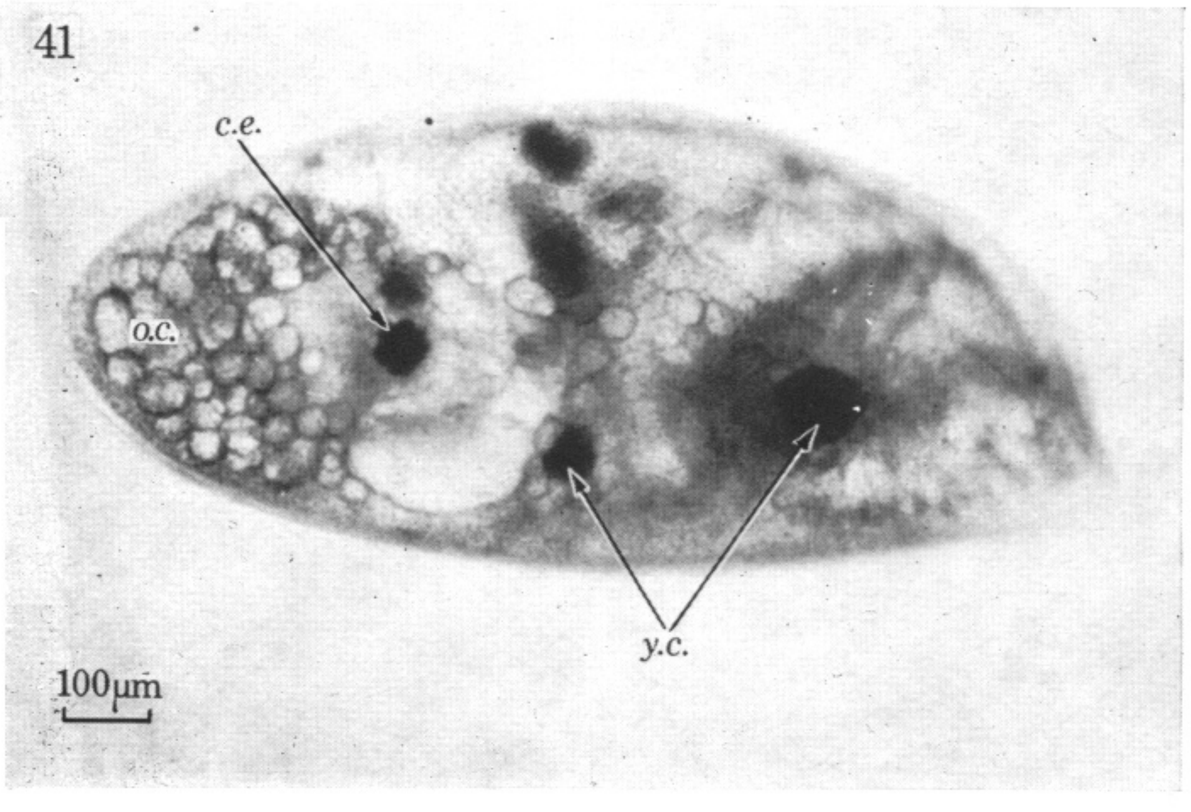

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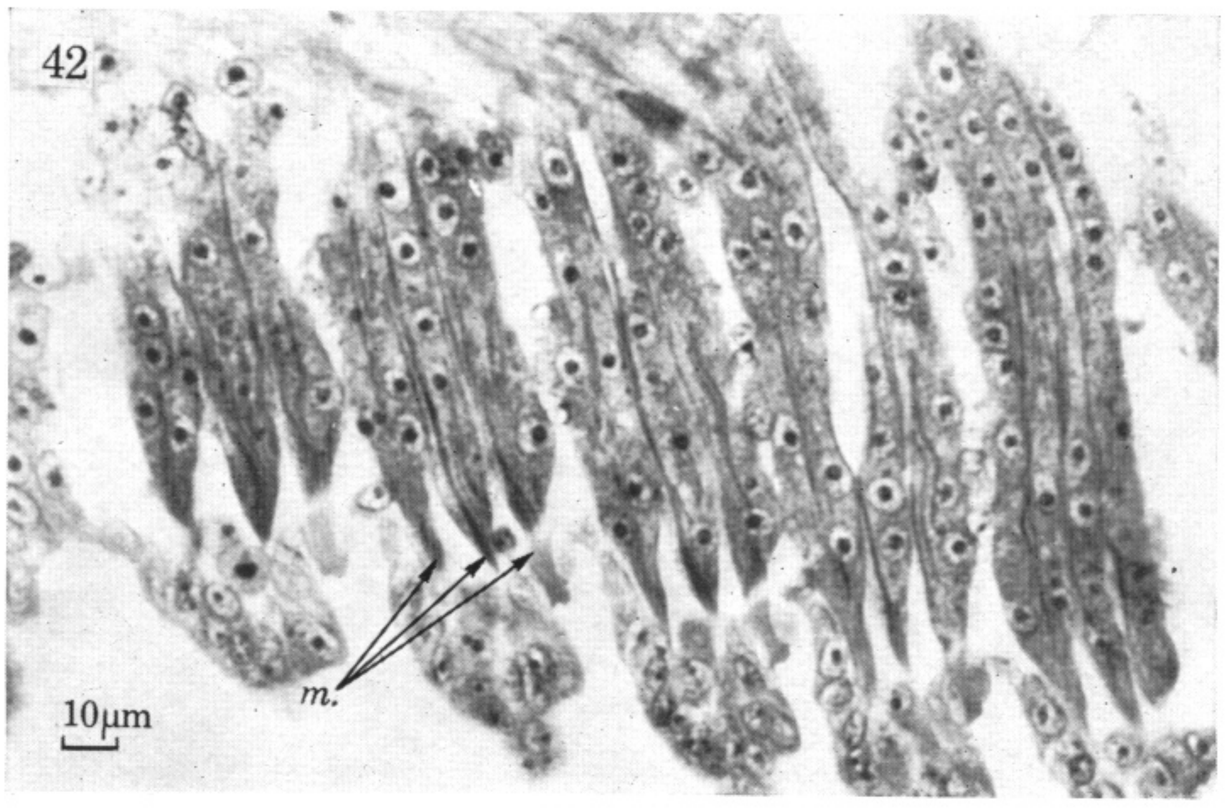

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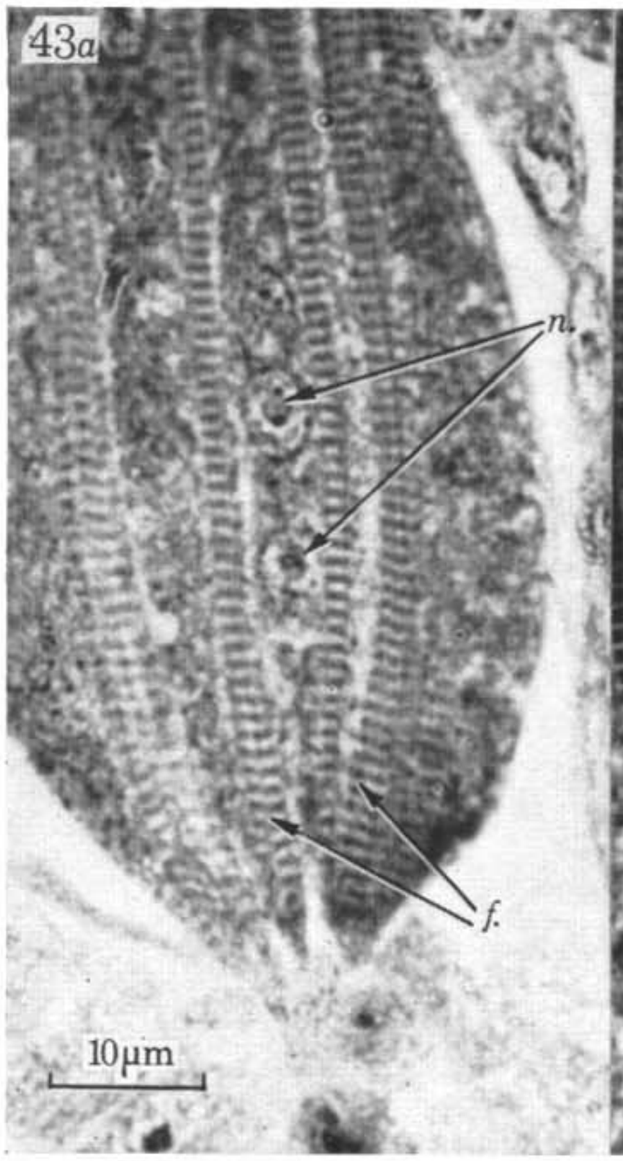

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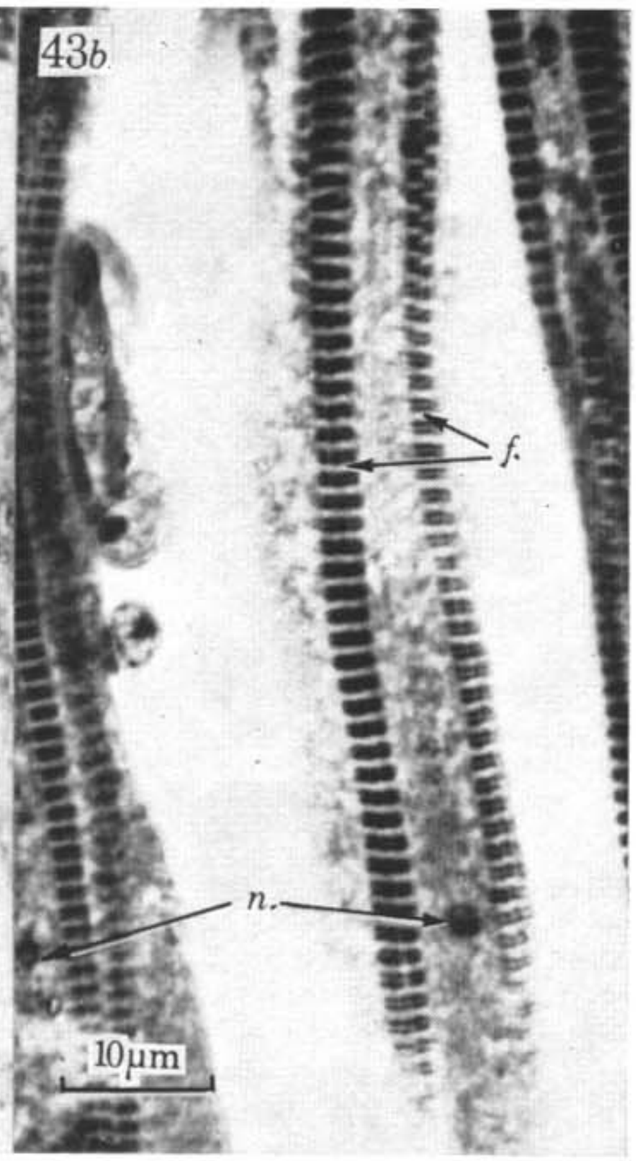

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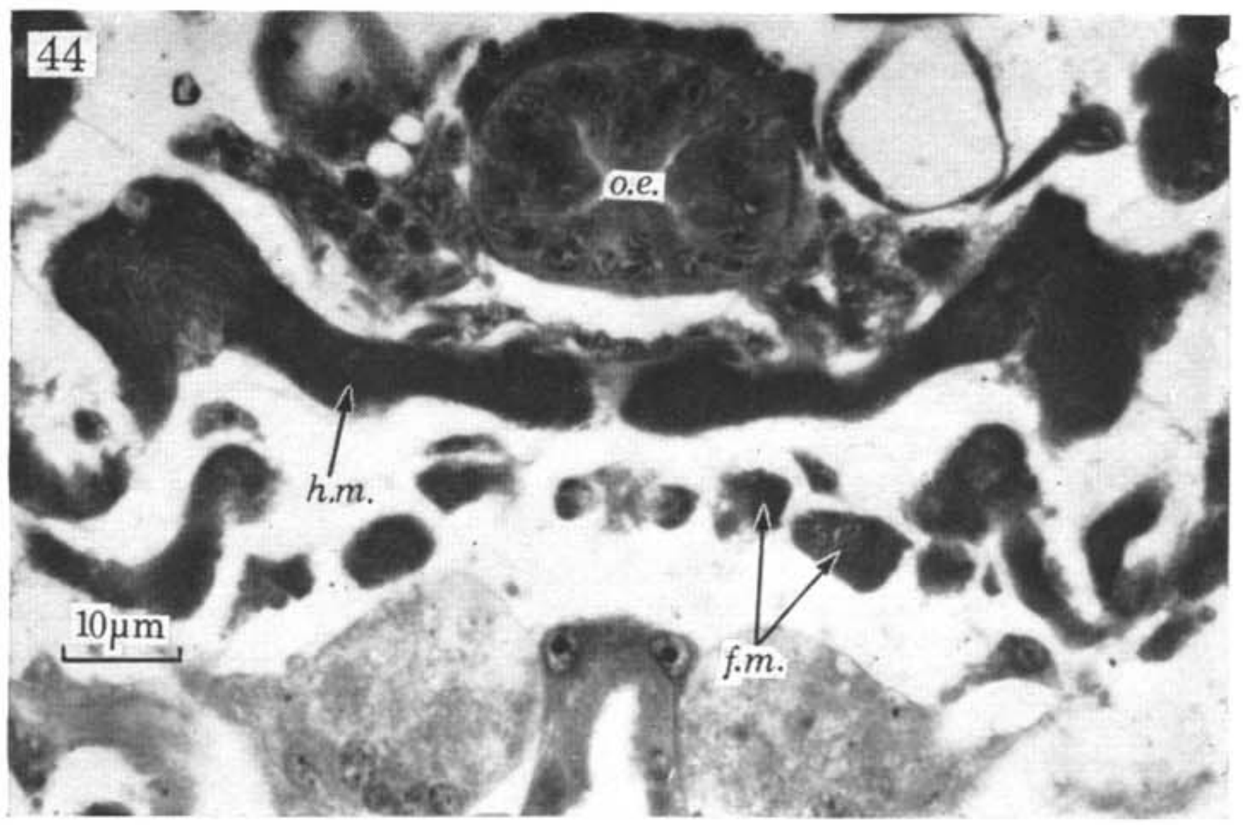

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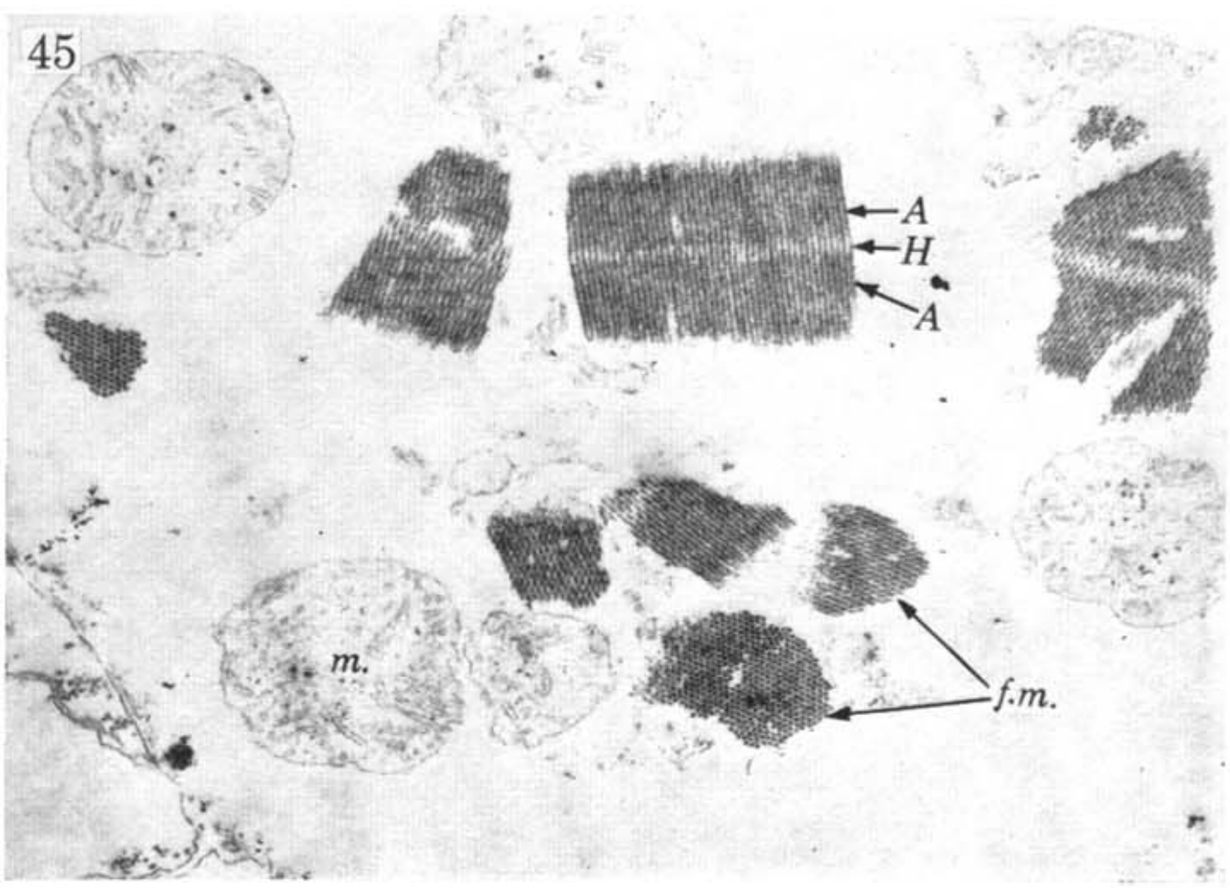

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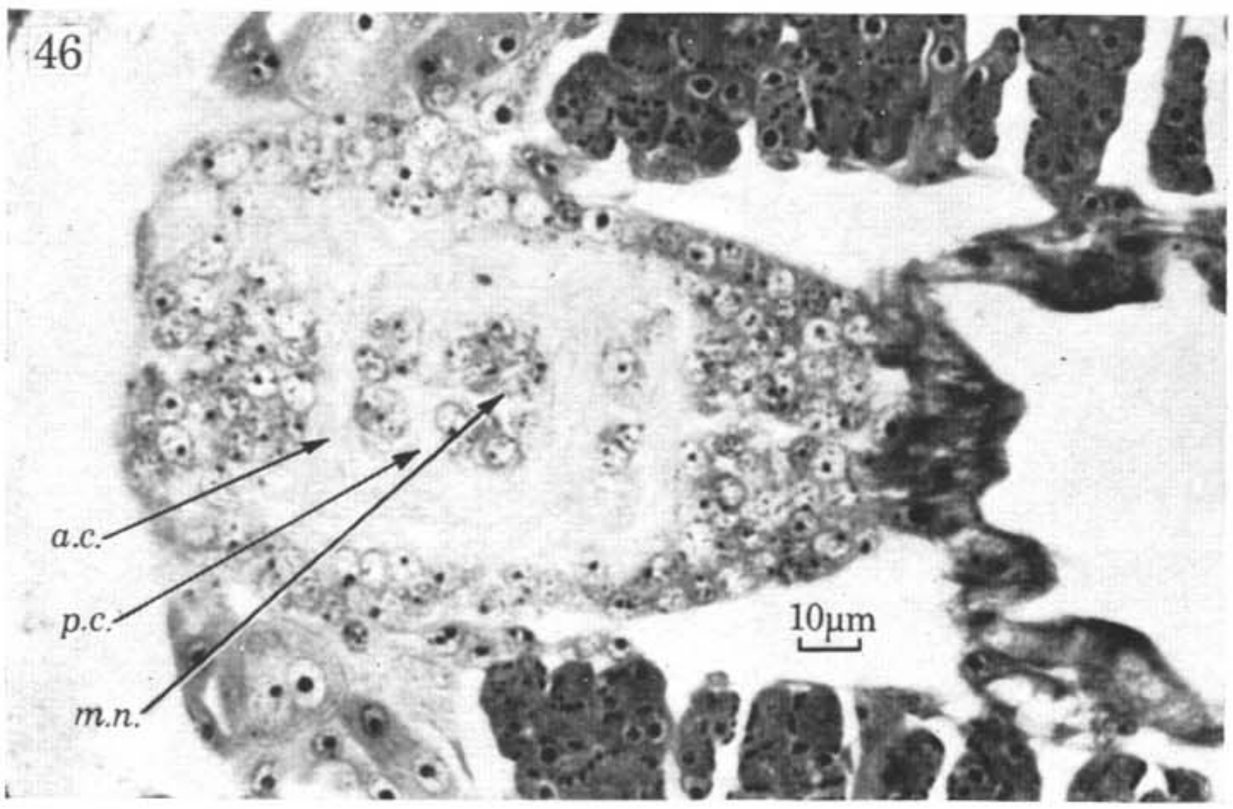

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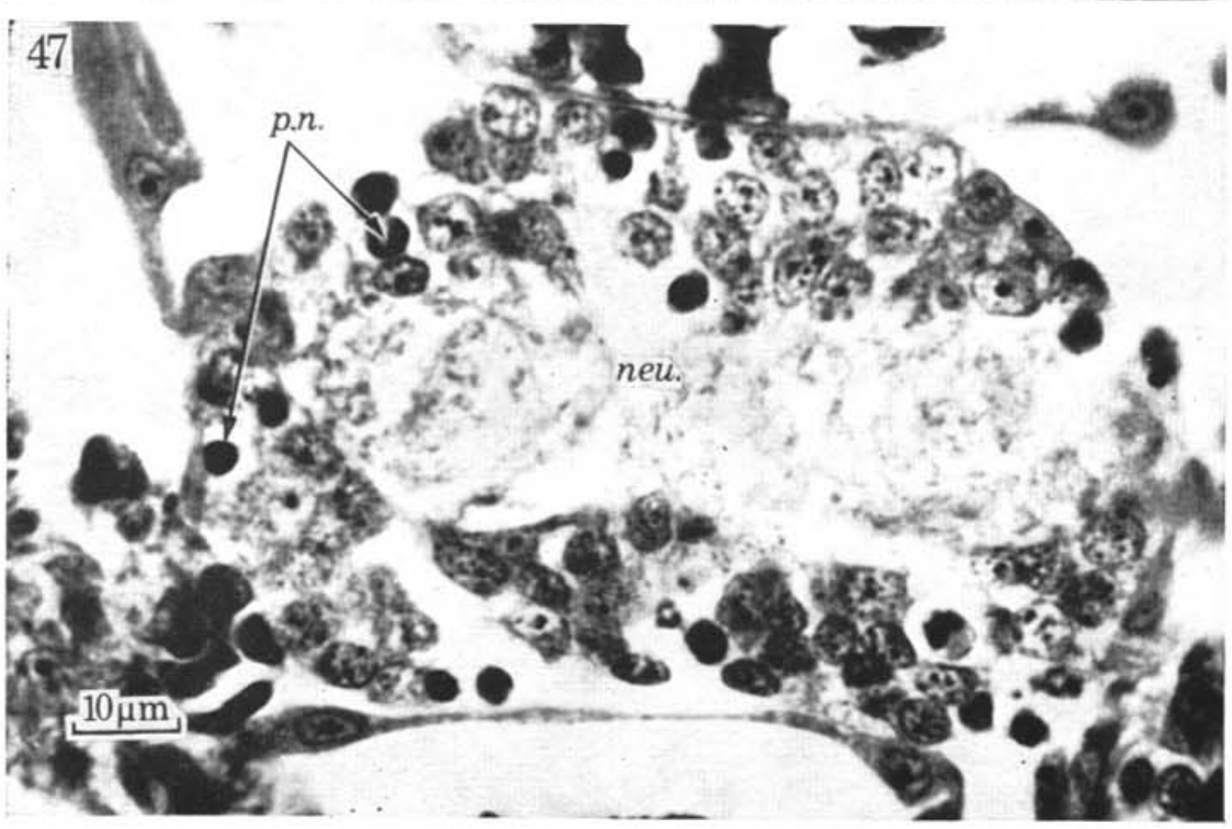

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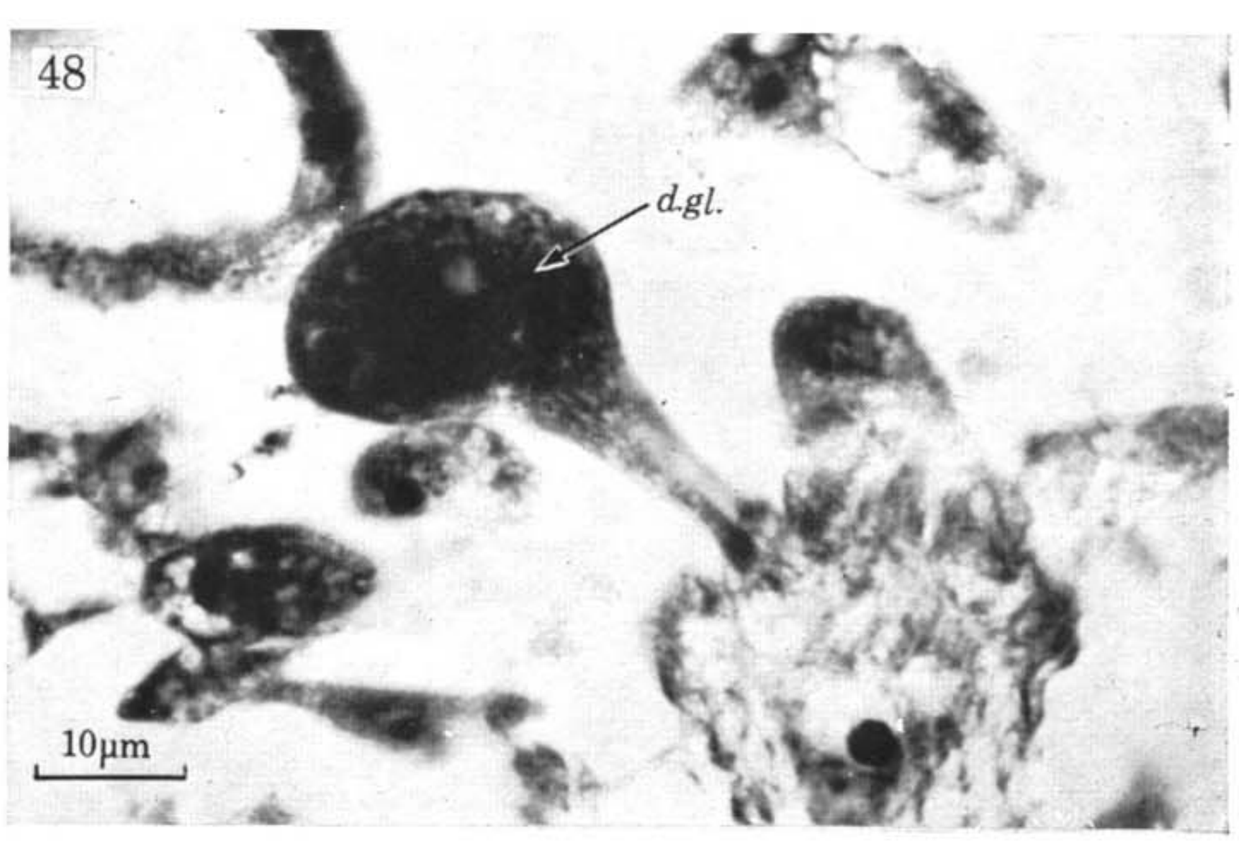

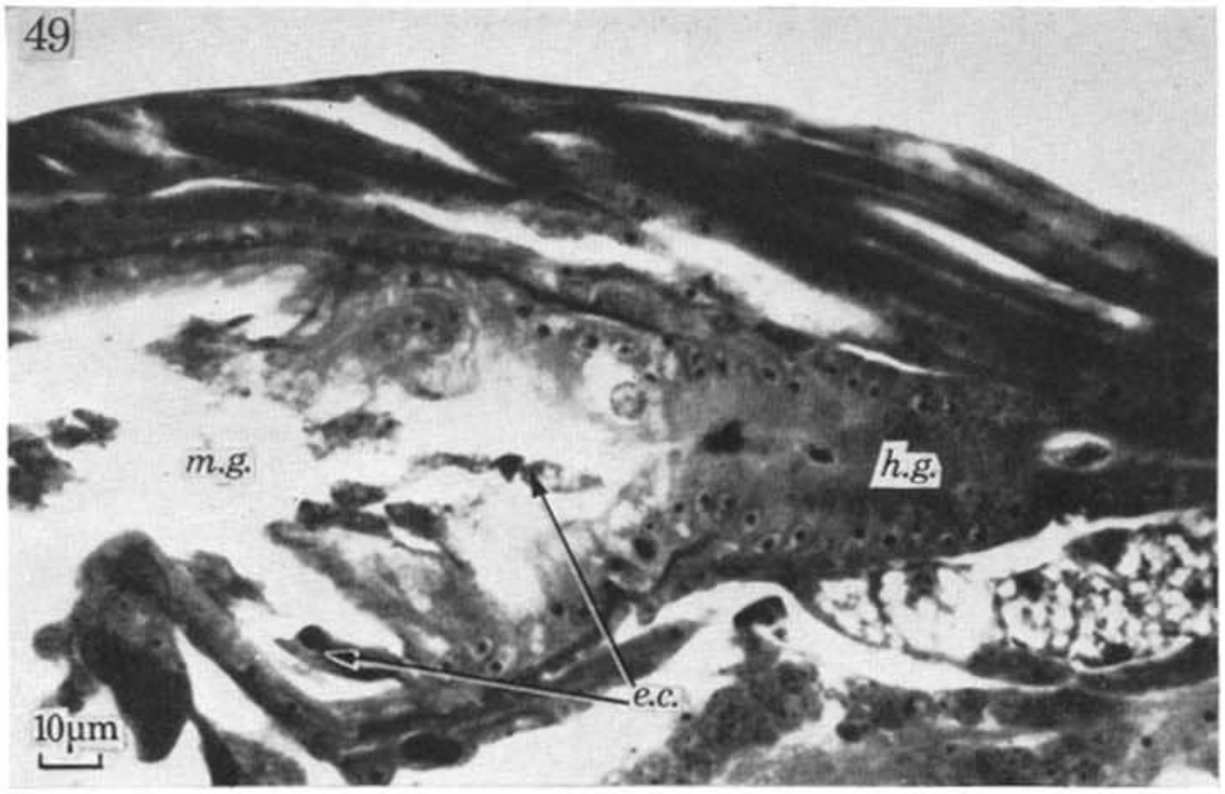

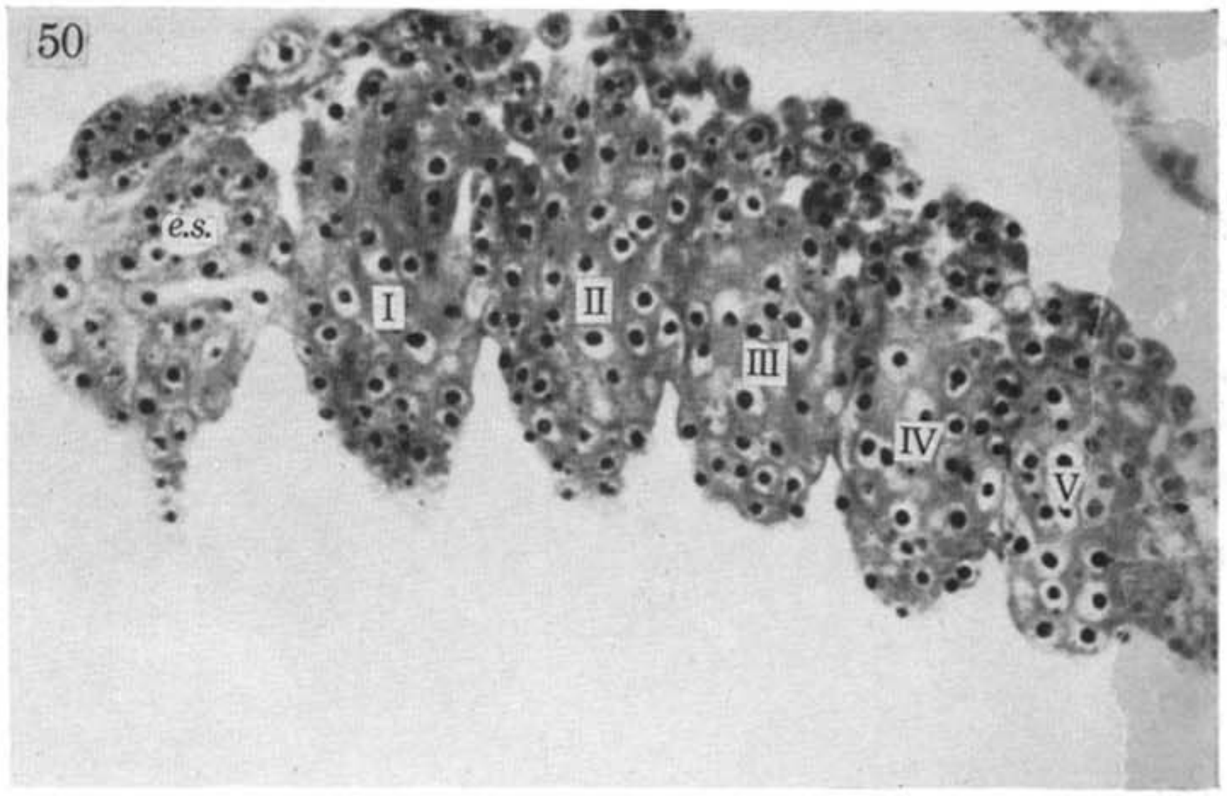

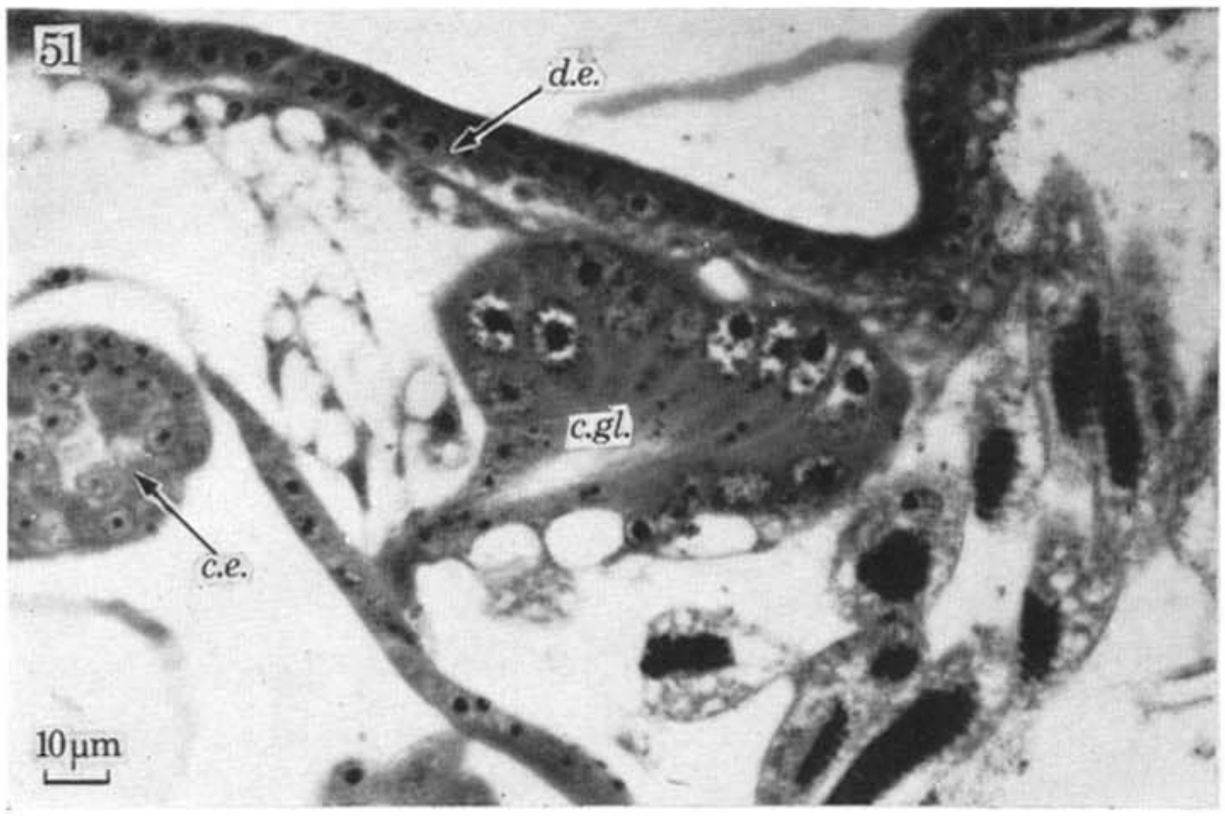

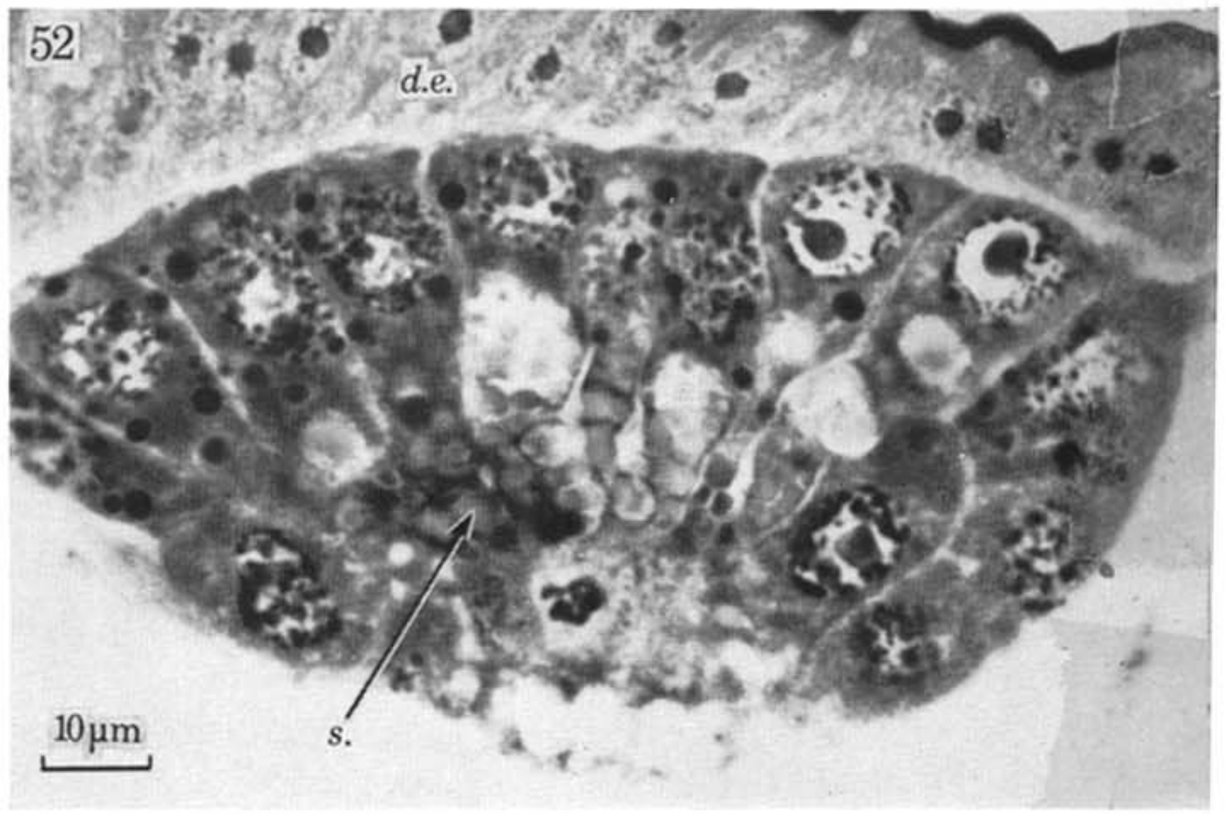

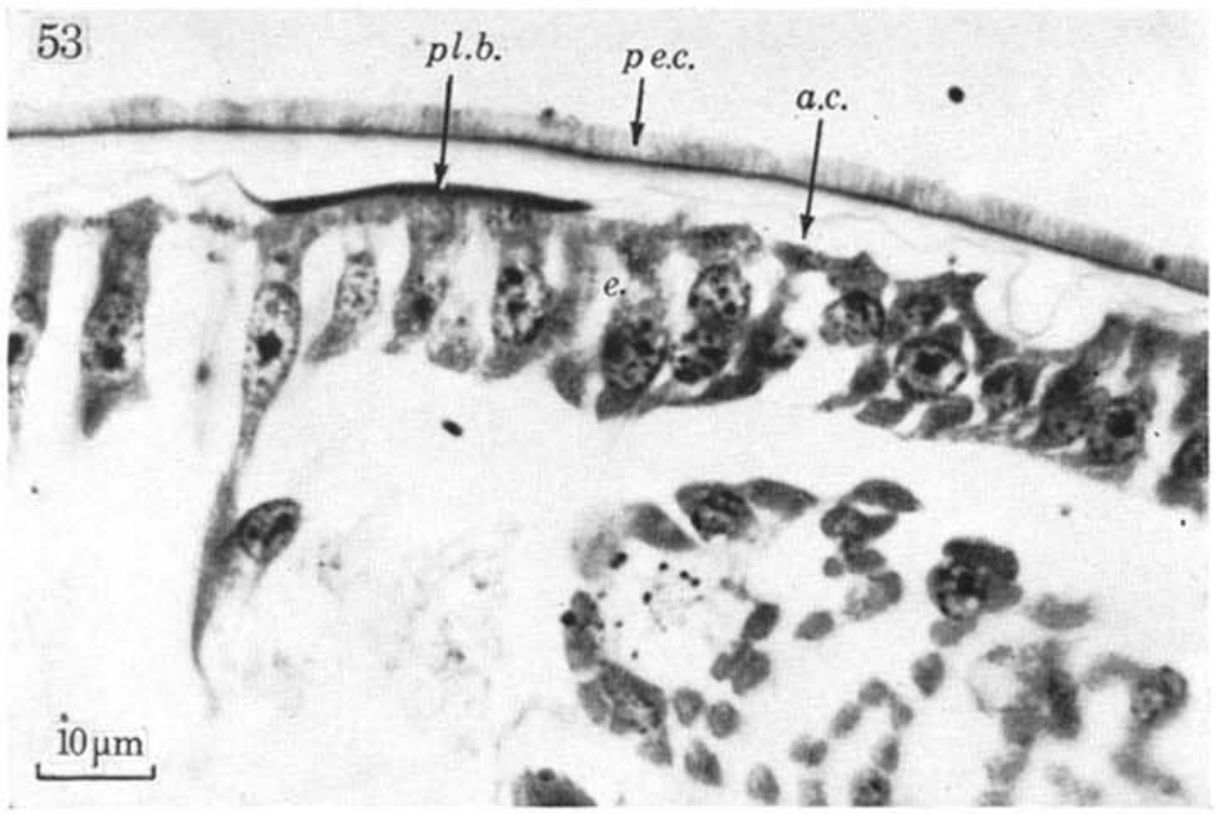

FIGURE 49. Longitudinal section through the mid-gut and hind gut of the cypris larva to show the extrusion of redundant cells into the gut lumen. *e.c.*, extruded epithelial cells; *m.g.*, mid-gut; *h.g.*, hind gut.

FIGURE 50. Longitudinal section through the developing thoracic region in the Stage 6 (1) nauplius larva to show the end sac of one maxillary gland developing in the somite of the second maxillary segment. I–V, Somites of the first to fifth thoracic segments; *e.s.*, developing end sac of maxillary gland. Scale as on figure 49.

FIGURE 51. Transverse section through the lateral region of an early Stage 6 nauplius larva to show a developing cement gland. *c.e.*, developing compound eye; *c.gl.*, cement gland; *d.e.*, dorsal epidermis.

FIGURE 52. Transverse section through a developing cement gland in a Stage 6 (3) nauplius larva. *d.e.*, dorsal epidermis; *s.*, droplets of secretion in the gland cells.

FIGURE 53. Transverse section through the integument of a settled cypris larva shortly before the cypris–adult moult. *a.c.*, cuticle of developing adult; *e.*, epidermis; *pe.c.*, pre-ecdysial cuticle; *pl. b.*, boundary of developing shell plates.