JACOB VAN DER LAND AND ARNE NØRREVANG

STRUCTURE AND RELATIONSHIPS OF *LAMELLIBRACHIA* (ANNELIDA, VESTIMENTIFERA)

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Synopsis

Lamellibrachia luymesi VAN DER LAND & NØRREVANG, 1975, is a large tube-dwelling marine worm. The only known specimen was collected in the Atlantic Ocean off Guyana at a depth of 500 m. A detailed description is given of the external morphology, the anatomy and the histology of this male specimen. Comparisons are made with the only known relative, *Lamellibrachia barhami* WEBB, 1969, occurring off the Pacific Coast of the United States. The two species of *Lamellibrachia* form a group of their own, the Vestimentifera, which is quite distinct from all other worms. They range among the most interesting recent discoveries of deep sea animals.

The body of the Vestimentifera consists of a muscular anterior part (vestimental region), provided with two lateral "wings", a very long tapering trunk and perhaps a small third region (opisthosoma). The anterior end carries two obturacula, which together form an operculum-like structure, and thousands of filiform tentacles (tentacular region). The regionation of the body cannot easily be compared with that of other animals. Perhaps the presence of two pairs of coelomoducts (the nephridia and the gonoducts) and an opisthosoma indicates that the ancestors of the Vestimentifera were segmented worms.

When in the tube the vestimental wings are folded over the dorsal side, thus forming a cavity, which is for the greater part lined with glandular epithelium. The function of the unique vestimental region remains obscure. The most remarkable aspect of the anatomy is the complete lack of an intestine, at least in the adult The question how these rather large animals can live without a gut remains unanswered. They share this unusual character with the Pogonophora, but these animals are extraordinarily thin.

The Vestimentifera and the Pogonophora have several other characters in common, but there are also many important differences, so that they cannot be regarded as directly related groups. The authors consider the Vestimentifera as well as the Pogonophora separate classes of the phylum Annelida. A broadening of the concept Annelida is probably the best solution to the systematic problems raised by the discovery of these two groups.

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Introduction

In this paper a detailed description is given of the holotype and only known (male) specimen of *Lamellibrachia luymesi* VAN DER LAND & NØRREVANG, 1975, a large tube-dwelling marine worm from the continental slope off Guyana. Not only the external morphology and gross anatomy are treated but histological descriptions of all organs and organ systems are given as well. Notes on ecology, functional aspects and life habits are added and relationships are being discussed.

History

In 1966 eight specimens of a large worm species were collected by the submersible "Deepstar 400" (U.S. Navy) at a depth of 1125 m in the North Pacific on the continental slope off California. The animals were studied thoroughly by WEBB, who published part of the results of his studies. In 1969(a) he described the main features of the animal including brief references to the internal organization. He named it *Lamellibrachia barhami* and decided to place it in a separate class of the phylum Pogonophora. Later WEBB published the results of a chemical analysis of the tube (1971) and detailed descriptions of the excretory system (1975) and the reproductive system (in press). Meanwhile the animal was also found in North Pacific waters off Oregon (WEBB, *in litt.*).

In 1970 VAN DER LAND secured one *Lamellibrachia* specimen from the Atlantic Ocean during the "Luymes" Guyana Shelf Epedition (Dutch CICAR-project no. 15). It was decided to use the specimen, which was in a reasonably good condition, for anatomical and histological study, not only because it belongs to another species but also because it apparently represents a further developmental stage, differing in several interesting details from WEBB's specimens. On the occasion of the symposium on the phylogeny and systematic position of the Pogonophora (Copenhagen, November 1973) we prepared a condensed report on the results of our studies (VAN DER LAND & NØRREVANG, 1975). In that paper we described the animal as a new species and expressed our opinion that *Lamellibrachia* should be placed in a separate class (Vestimentifera) of the phylum Annelida.

Material and methods

The tube with the male specimen was collected with an Agassiz trawl from silty bottom at a depth of 500 m on the continental slope off Guyana, 8°1′N 57°24′W ("Luymes" Guyana Shelf Exp. sta. 101 4th September 1970). The remnants of the holo-

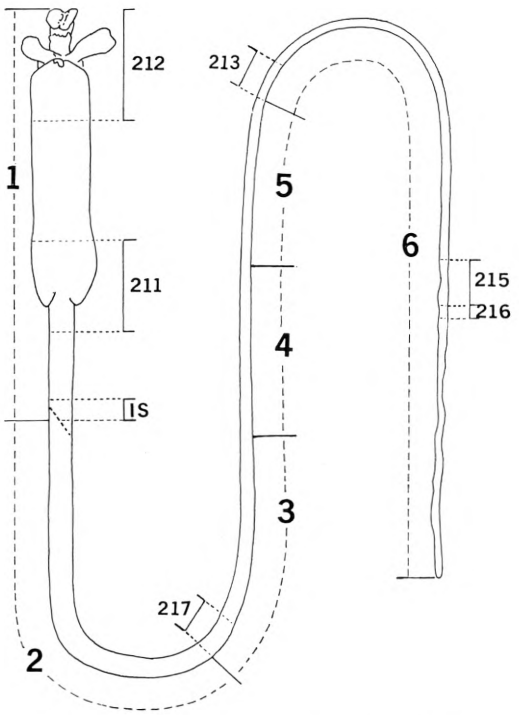

Fig. 1. Diagram illustrating the parts in which the animal was cut originally (nos. 1 to 6) as well as the parts that were serially sectioned (IS, 211, 212, 213, 215, 216, 217). Before sectioning several small fragments of the latter parts were embedded in plastic for thin sectioning or freeze dried for scanning electron microscopy.

type, the slide series and the reconstructed tube are kept in the Rijksmuseum van Natuurlijke Historie, Leiden (coll. no. 7222).

On deck the tube was cut into three pieces. The firm anterior part and the posterior part of the animal could easily be removed from the tube, but it was impossible to draw the thick but fragile middle portion (less than half of the animal) from the coiled middle part of the tube. Four colour transparancies of the living animal were made and the material was fixed in sea-water formalin. Later the rest of the animal could be brought out by cutting the middle part of the tube into small sections and by cutting these sections lengthwise as well. As a result this part broke into four pieces (Fig. 1). It goes without saying that the state of conservation of these pieces was not nearly so good as that of the anterior and posterior pieces.

After having made a number of colour and black-and-white photographs we selected several portions of the animal for serial sectioning (Fig. 1). For light microscopy the material, which had incidentally been transferred to 70 % alcohol, was embedded in Tissuemat through tetrahydrofuran. The serial sections were made with the microtome set at 8 μ. The greater part of the slides was stained with hematoxylin-eosin. Other staining methods applied to single slides include: 1) PAS (with and without

nuclear stain) for neutral mucopolysaccharides and mucoproteins. 2) PAS after treatment with diastase for glycogen. 3) PAS-Alcian Blue with nuclear stain for acid and neutral mucopolysaccharides. 4) Toluidine Blue, 1 % in distilled water resulting in metachromasia in acid mucopolysaccharides. 5) Ninhydrin-Schiff for proteins. 6) Einarsson's Gallocyanin for RNA. 7) Feulgen's reaction for DNA. 8) Oil Red 0 for lipids. 9) Gentiana Violet according to von Volkmann for lipofuscin.

Most series consist of cross sections. The obturacula and the tentacles were bent ventrally, so that the anterior series (no. 212) consists of horizontal sections of the obturacula, somewhat oblique cross sections of the anterior part of the vestimental region, and transitions in between (Plate 2:3). Series no. 215 consists of horizontal sections.

Small fragments of several parts of the body were embedded in Epon 812 following the normal procedure for electron microscopy. After reorientation small pieces were sectioned with glass knives on a Spencer rotary microtome or a Reichert Ultrotome 2. Some of the fragments were sectioned serially according to the method described by Nørrevang (1970a). The thin sections were studied with phase contrast microscopy, interference contrast microscopy, and (after staining with 0.05 % Toluidine Blue in 1 % borax) with normal light microscopy.

Some fragments of the vestimentum were prepared for electron microscopy. Small pieces of about 1.5×1.5×3 mm were gradually transferred to water, postfixed in 1 % aqueous osmium tetroxide for 30 minutes and embedded in Epon according to normal procedures. Sections of 50 to 100 nm were studied in the electron microscope after contrasting with uranyl acetate and lead citrate. The preservation was reasonably good, the procedures taken into account, and a few electron micrographs accompany this paper. Some pieces, *i.e.*, a slice of the vestimentum, a skin fragment from the trunk, a smeared mass of sperm from the coelom and a lobe of the trophosome, were studied in the scanning electron microscope after drying according to the method described by Nørrevang & Wingstrand (1970: p. 251).

Graphic reconstructions of the front end of the body, especially of the brain and the vascular system, were made according to the method described by Lison (1936). Wax models were prepared of the sinus valvatus and the brain.

Acknowledgements

The hydrographic vessel Hr. Ms. "Luymes" was made available by the Royal Dutch Navy to the Leiden museum for marine biological research. The Guyana Shelf Expedition was partly financed by the Nederlandse Organisatie voor Zuiver Wetenschappelijk Onderzoek ZWO (grant no. 76–13).

Thanks are due to several technicians and artists. Most of the microtechnical work, including serial sectioning and staining, was done by Miss Gerda Hvidmark and Mrs Else Schiøtt-Hansen (Copenhagen) and Mr Jack van Oyen (Leiden). The elaborate pencil drawings of Plates 1, 4 and 6 were executed by Mr Joop Wessendorp (Leiden).

The line drawings were made by Miss JULIE TESCH and Mrs ELISABETH BEYERHOLM (Copenhagen).

The text of the manuscript greatly benefited by the discussions we had with several attendants of the Pogonophora Symposium in Copenhagen in 1973. Our cordial thanks are due to our friend Prof. MICHAEL WEBB (University of Stellenbosch, South Africa) for valuable discussions and for generously putting at our disposal an unpublished manuscript, which resulted in considerable improvements of this paper.

External morphology and gross anatomy

Lamellibrachia luymesi is a large tube-dwelling animal. It is provided with tentacles and has a long tapering body, so it has the general appearance typical for most sedentary worms. The present specimen had a total length of about 555 mm (when still living) so this species, just like *L. barhami*, ranks among the largest tubicolous worms (Fig. 1).

The animal looks unsegmented, the three regions of the body not being separated by constrictions. Webb claims that in *L. barhami* a fourth posteriormost region (*opisthosoma*) can be recognized which is set off from the trunk by a constriction. In our specimen the hind end is missing so we can only describe three body regions:

1) The tentacular region, bearing the two obturacula and numerous tentacles;

2) The strongly muscular vestimental region, provided with two lateral wings, two dorsal ridges with ciliated grooves and a ventral ciliary field and containing the brain, the heart, the excretory organs and numerous glands;

3) The long cylindrical tapering trunk, containing the reproductive organs and the trophosomes.

Tentacular region

The greater part of the front end of the body is occupied by the bases of the two large stiff ivory-white obturacula (together called lophophoral organ by WEBB, 1969a), which are almost completely fused and point forwards, bending to the ventral side. They are nearly completely surrounded by a few thousands of tiny, filiform tentacles. These are arranged in several lateral concentrical semicircular series, each consisting of up to more than 50 tentacles. The cuticle of the proximal parts of all tentacles is fused. The lateral series meet ventrally, but on the dorsal side a narrow space is left open, in which the excretory pore is situated. In the live animal the distal parts of the tentacles undoubtedly stand out in the water, but when out of the water they form an irregular dark-red mass, lying against the obturacula.

The free tentacles are surrounded by a number of stiff lamellae. It is evident that each lamella is in fact a semicircular series of tentacles, which have completely fused by secreting a common thick cuticle. The outer tentacular lamellae of each side are largest and completely cover the smaller inner lamellae and the rest of the tentacles (Plate 1). The arrangement is the same as in the normal tentacles, which means that

the lamellae meet ventrally and leave an opening to the dorsal side. In the living animal they were red at their bases but for the greater part they were cream coloured. Apparently they do not contain as much blood as the normal tentacles.

The total length of the tentacular region from the collar to the tip of the obturacula is about 13 mm. The diameter of the two obturacula together is about 9 mm, which is somewhat more than the internal diameter of the anterior part of the tube.

The bases of the obturacula and the tentacles are deeply "rooted" in the muscular anterior end of the body (Plate 7:18) and all converge towards the brain, which lies ventrally in the anteriormost part of the vestimental region. The two excretory ducts run dorsad and close to the bases of the obturacula. They unite just before opening to the outside middorsally.

When the animal has retracted into the tube the opening is closed by the obturacula, acting as an operculum. The vestimental collar does not stand out then, but surrounds the basal portions of the tentacles (Webb, 1969a).

Vestimental region

In the live animal the length of the vestimental region, from the anterior collar to the ends of the vestimental wings, was aproximately 63 mm, which is about 11 % of the total length of the animal. With unfolded wings its width was 14 to 17 mm. Apart from the red blood vessels the general colour was pink in the central part, while the wings were yellowish for a large part, particularly in the posterior half on the ventral side.

The region (Plate 1) is characterized by the presence of two large lateral wings. It is not clearly delimited from the tentacular region and the trunk, of which it is a continuation. The nearly flat middorsal fleld (Plate 2:5) is bordered by longitudinal ridges along nearly its whole length. In each ridge (*lira vestimentalis*) lies a ciliated groove (*sulcus vestimentalis*). The rest of the dorsal surface has a rugose appearance. In the living specimen seven large longitudinal blood vessels could be seen shining through the skin: one in the middle (the dorsal vessel), which is still visible in the fixed specimen, two in each of the vestimental ridges (the blood lacunae) and one at the base of each wing (the vestimental vessels).

There is a midventral ciliary field, the rest of the ventral side being densely covered with papillae (papillate fields). At the anterior end there is a collar, consisting of two low, transverse folds (Plates 1:2, 2:3). In the middle the right fold ends in a small semicircular collar flap ("brain cover", Webb, 1969a), which is directed posteriorly. The thin borders of the wings show a tendency to curve towards the ventral side in the posterior half of the region (Plates 2:8; 4:12). A midventral blood vessel is visible through the skin, even in the fixed specimen. The two halves of the ventral nerve cord are visible as light lines bordering the ciliary field. Anteriorly they meet to enter the brain and close to the posterior end of the vestimental region they meet to form the single nerve cord of the trunk (Plate 2:8).

This description refers to the animal as it is outside the tube. When it is in the tube the wings cannot stand out. They are then folded over the middorsal field, meeting eachother and thus forming a cavity (vestimental cavity). Hence, the name of the region (WEBB, 1969a). WEBB's specimens were all in this state because they were fixed in the tube. When the living animal is taken out of the tube the wings spontaneously unfold.

Internally the greater part of the region is rather uniform (Fig. 2; Plates 3:10; 4:12). Most remarkable is the fact that the longitudinal muscles have expanded so enormously that they fill up by far the greater part of the region. The coelom is reduced to a narrow tube containing the dorsal blood vessel and lying approximately halfway between the dorsal and ventral sides. The ventral vessel lies in a strand of dorsoventral muscles (Plate 17:75). The wings are not very muscular; especially in the posterior half they are for the greater part filled with connective tissue (Plate 5:14). It is probably this tissue that causes the curling of the borders of the wing and the yellow colour. In this layer of connective tissue lie numerous pyriform glands, opening to the external sides of the wings (when folded). The epidermis of the dorsal side (facing the vestimental cavity) outside the ridges, which has a rugose appearance externally, solely consists of very high gland cells. The two halves of the intra-epidermal nerve cord each contain a tube filled with fluid (neurular tube).

The anteriormost portion of the vestimental region contains several vital organs. The dorsal blood vessel enters a muscular heart, from which two vessels go to the tentacles and one to each of the obturacula. Two vessels from the tentacles join the two vestimental vessels from the wings and open into a cavity (*sinus valvatus*) from which the ventral vessel originates. At a level between the dorsal and ventral vessels lies the pair of large excretory organs. The two halves of the ventral nerve cord unite and widen to form the brain. The two neurular tubes enter the brain separately and disappear in the brain as fine tubules. The folds of the collar contain numerous pyriform glands.

The two genital pores lie at the posterior ends of the two dorsal ridges. Approximately at that level the narrow perivascular coelom merges into the spacious coelom of the trunk and the retractor muscles appear as separate structures. The thick layer of longitudinal muscles is gradually reduced to normal proportions.

Trunk

The total length of the cylindrical trunk is approximately 480 mm, which is about 87 % of the total length of the animal but it should be remembered that our specimen is incomplete. The anterior part has a diameter of about 6 mm and it gradually tapers until it reaches a diameter of about 3 mm. The posterior part of the trunk (about 180 mm) does not taper and has alternating thin and more or less bulbous parts; its diameter varies from 1 to 3 mm.

The general colour of the anterior part was dark green with white organs (sperm sacs) and red blood vessels shining through here and there. We have not seen the

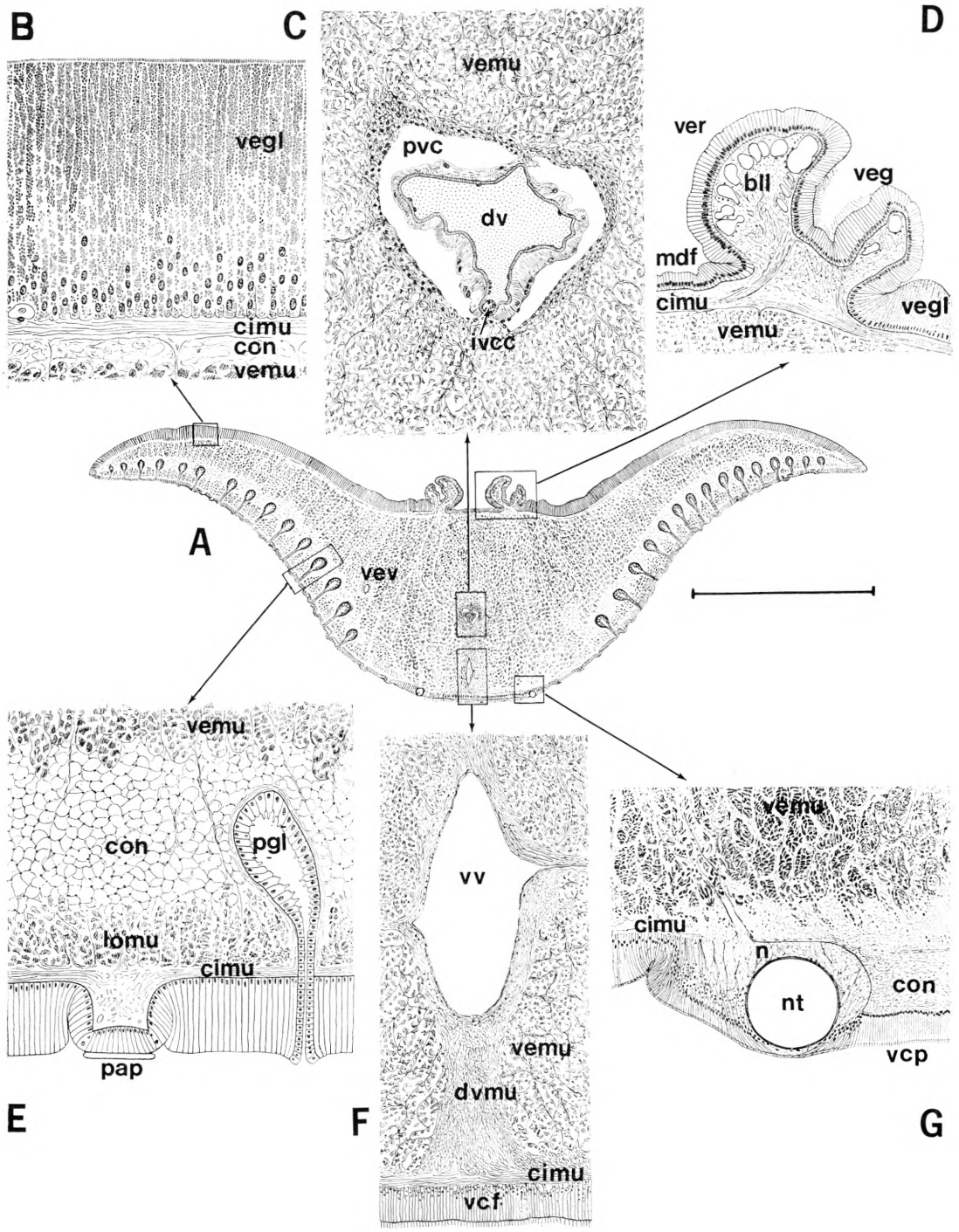

Fig. 2. Diagrammatic cross section of the anterior part of the vestimental region (A) and details of the same: vestimental glandular epithelium (B); dorsal vessel (C); vestimental ridge (D); "spring tissue", pyriform gland, and papilla with cuticular plaque (E); ventral vessel and ventral ciliary field (F); right half of the ventral nerve cord (G). The details are not enlarged to the same scale. bll = blood lacunae, cimu = circular muscles, con = connective tissue, dv = dorsal vessel, dvmu = dorsoventral muscles, ivcc = intravascular cell cord, lomu = longitudinal muscles, mdf = middorsal field, n = nerve cord, nt = neurular tube, pap = papilla with cuticular plaque, pgl = pyriform gland, pvc = perivascular coelom, vcf = ventral ciliary field, veg = vestimental groove, vegl = vestimental glandular epithelium, vemu = vestimental muscles, ver = vestimental ridge, vv = ventral vessel.

middle part of the living animal. In the posterior part the thickened portions were dark green and the thin ones pink.

In the anterior 70 mm or so the skin is rather thick and covered with numerous papillae (Plate 2:6). Just posterior to the vestimental region there are about 17 transverse grooves on the ventral side (Plate 2:7). In the middle part of the trunk, which is roughly the part lying in the loop of the tube (Plate 26:124), the skin is extremely thin and the number of papillae is very small. In the slender posterior part the skin is slightly thicker and the papillae are somewhat more numerous again (Plate 3:11).

We made only a restricted number of serial sections of the trunk (Fig. 1), so the internal anatomy is incompletely known.

Of the epidermal structures the papillae, either with a cuticular plaque or with the opening of a pyriform gland on top, are most conspicuous. Both types are present along the whole length of the trunk. In the area where the retractor muscles are situated (Fig. 7), the pyriform glands lie in between the muscles, but elsewhere they hang freely in the coelom. The ventral nerve cord is present along the whole trunk, but the neurular tube is present only in the anteriormost part, until about 30 mm posterior to the vestimental region. The layers of circular and longitudinal muscles are present everywhere. In addition to these layers there is a thick layer of longitudinal muscles which are tree-shaped or feather-like in cross section. These feathermuscles, which certainly act as retractors, are present in the anterior 30 to 40 mm of the trunk (Plates 3:9; 5:15). They are attached to the body wall and hang freely in the coelom.

In the anterior part of the trunk (Plates 3:9; 5:15) there is a continuous, irregularly lobed mass of parenchymatous tissue (*trophosoma*), filling a considerable part of the coelom. It is attached to the body wall by dorsal and ventral mesenteries. The dorsal blood vessel lies in the dorsal mesentery. The ventral blood vessel lies embedded in the trophosomal tissue but still it can be regarded as lying in the ventral mesentery. A pair of ciliated sperm ducts is also lying in the trophosome, close to the ventral blood vessel. The sperm ducts have no lateral openings to the coelom, but at regular intervals they communicate with eachother and with sperm sacs, which are present everywhere in the trophosome, constituting a considerable part of the total mass. Many thin, branched offshoots of the trophosome penetrate between the feather muscles and are attached to these.

In the rest of the trunk similar trophosomes alternate with areas where the coelom only contains free sperm (Plates 5:16; 6:17). We do not know how many trophosomes there are, but there must be several of them, probably more than ten. Of course their diameter gradually diminishes backwardly, but we have the impression that they also become shorter. Between and around the trophosomes there are enormous masses of sperm in the coelom, especially in the middle part of the trunk. Apparently a considerable sperm resorption takes place there.

Discussion

1) According to one of WEBB's figures (1969c: fig. 1D) the opisthosoma consists of seven segments, but his diagram is probably not based on actual observations. In fact the opisthosoma was only recognized by the presence of one or two constrictions (WEBB, 1969a: p. 37). The internal structure of this region remained underscribed so we cannot yet compare it with the segmented opisthosoma of the Pogonophora, nor can we make any sensible comment on it. It may not even be a separate body region.

2) The three functional body regions described above, the tentacular region, the vestimental region and the trunk, are not clearly delimited from eachother. The vestimental region is defined by the presence of the wings but internally it gradually merges into the two other regions. Consequently we cannot regard the regions as true segments. In fact the Vestimentifera must be called unsegmented animals as far as we now know, and as such only comparable to animals like Sipunculida and Echiurida. It is not impossible that the segmentation was secondarily lost as in these groups and that the two pairs of coelomoducts (nephridia and gonoducts) represent the last traces of an original segmentation. A comparison with primarily unsegmented coelomates like the Priapulida and the Nematoda is not very promising for several reasons (see other chapters).

3) It should be noticed that in other tube worms, particularly in sedentary polychaetes, there is a strong tendency towards specialization of distinct body regions. The presence of a head, a muscular "thorax" and an abdomen with reproductive organs is quite common.

Integument

In general the epidermis of *Lamellibrachia* can be described as a one-layered epithelium standing on a basement membrane, but there are considerable regional differences in the morphology of the epithelial cells. As an example it might be mentioned that the height of the cells varies from about 5 μm to about 300 μm.

On the greater part of the body the epidermis consists of unspecialized cells. They are not covered by a real cuticle but by microvilli enforced by a criss-cross layer of fibrils so they can be considered absorptive cells. In some areas there is a regular cuticle and there are also many small cuticular structures. The basement membrane is usually not very distinctive.

Ciliated cells are present in three different areas, *viz.*, (1) the ciliated tracts on the tentacles; (2) the ventral ciliary field of the vestimental region; (3) the vestimental grooves.

Many types of secretory cells are present in the epidermis, part of them being arranged in special glands: the pyriform glands and the vestimental glandular epithelium (see p. 76).

In principle the nervous system of *Lamellibrachia* is intra-epidermal, so the nerve cells must also be considered specialized epidermal cells. Other cells also have a

function in the nervous system, *e.g.*, the sensory cells, the supporting cells, and the cells forming the neurular tube (see p. 16 and p. 47).

A curious aspect of the Vestimentifera is that the basal parts of the epidermal cells in certain areas have a muscular function (see p. 67).

Special epidermal organs include the pyriform glands (see p. 17), the papillae (see p. 19), both very numerous and occurring all over the body, and the ventral ciliary field (see p. 81). Structures of unknown function are the distinct transverse grooves on the dorsal side of the trunk, just posterior to the vestimental region(Plate 2:7). There are about 17 of these shallow grooves, which, as far as we could ascertain, are not accompanied by any special epidermal cells or by certain internal structures. These grooves are not present in *L. barhami* (WEBB, *in litt.*). The latter species also has a characteristic epidermal feature, *viz.*, along the ventral side of the margin of the anterior part of each vestimental wing there is "a series of more or less equally spaced darkish lines, giving this part the appearance of a "zip" ". In *L. luymesi* there is no trace of such an ornamentation.

Unspecialized cells

Scattered in most regions of the body and occurring in larger patches on the ventral side of the vestimental wings we find rather unspecialized cells which appear to have an absorptive function. Micrographs of these cells are given on Plates 8:20 and 21; 11: 36, 38, 39.

The nuclei are ovoid, measuring about 4 μm by 2.5 μm, and they are usually placed in the middle of the cells or slightly displaced towards their bases. The lower two thirds of each cell are usually filled with PAS-positive granules which do not stain with Ninhydrin-Schiff. They react moderately with Alcian Blue. Most of the granules are 1 to 1.5 μm in diameter but there seems to be a size gradient within each cell, the larger granules being found basally. The apical 15 μm or so are light in the light microscope. About 4 μm below the surface a terminal web can be discerned as a faint line in thin sections, particularly when phasecontrast is applied (Plate 11:39). In some areas the amount of PAS-positive material is considerably larger than elsewhere and the granules extend into the apical parts of the cells. Whether this reflects a difference in function of different parts of the epithelium or functional stages in cell development could not be determined.

A faint line delimits these unspecialized cells towards the outside. It is slightly metachromatic with Toluidine Blue and also stains faintly with PAS. As seen in the light microscope this layer would be called a cuticle. However with the electron microscope the apical surface of these cells can be seen to be differentiated into numerous microvilli (Fig. 3; Plate 11:40). Each microvillus is 0.6 to 0.7 μm long and has a diameter of about 50 nm. The tips of the microvilli seem to be bent, running tangentially for a short distance. Between the microvilli there sometimes appear to be deep funnels, but these may represent artefacts. The microvilli may increase the surface area of the epithelium with as much as 100 to 200 $^0/_0$.

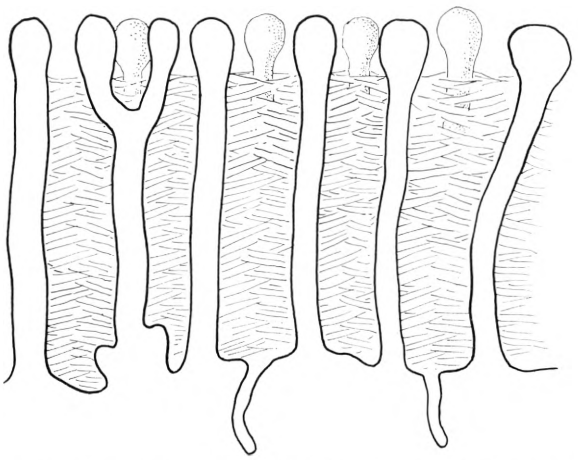

Fig. 3. Diagrammatic cross section of the pseudo-cuticle. For the greater part of their length the microvilli, which sometimes digitate, are embedded in an extra-cellular matrix consisting of criss-cross layers of fibrils.

Between the microvilli there is an extracellular matrix with fibrils arranged in a crosswise pattern between the microvilli. The significance of this matrix in relation to the cuticle as found in other parts of the body is discussed on p. 21.

Ciliated cells

The cilia occurring on the middle portions of the tentacles (Fig. 19; Plate 24:111, 113) are very long. They are arranged in tracts, two or three cells wide, on two opposite "corners" of each tentacle (these are more or less square in cross-section). They only occur in parts of the tentacles where the cuticle is still rather thick, so they are set in a groove. The ciliated cells appear dark in the sections but otherwise they seem to be rather undifferentiated.

The cells of the ventral ciliary field (Plate 8:24) are rather uniform. All are very high (about 150 μm) and very slender (width 3 to 5 μm). In most of them the cytoplasm is clear but in some there are varying amounts of PAS-positive granules. Some of them even attain the appearance of glandular cells. The cilia are relatively short (10 to 15 μm) and there are up to 50 or 100 cilia per cell. The rootlets are very long and extend almost 50 μm down into the cytoplasm, where they can be seen as a faint striation. The nuclei are located randomly in the basal part of the cells.

In the vestimental grooves the cilia (Plate 25:123) are about 50 μm long, somewhat shorter distally in the groove. Here, as in the ventral ciliary field, the ciliary rootlets extend about 25 to 50 μm into the cells. There seems to be some turnover of the ciliary cells as pycnotic nuclei are seen in cells that seem to be in the process of being extruded from the epithelium.

Secretory cells

Many types of secretory cells are present in the epidermis. On the dorsal sides of the vestimental wings there is a large glandular complex consisting of extremely tall cells (up to 300 μm high) packed with secretory granules from base to top (see p. 77). Similar but very much lower cells can be found scattered in the ventral vestimental epithelium. Other special glands are the pyriform glands (see p. 17).

There is always a ring of secretory cells around the cuticular plaques on the epidermal papillae (see p. 20). Other secretory cells with different types of secretion are found scattered in the epidermis, *e.g.*, around the openings of the pyriform glands. A conspicuous field of scattered gland cells is present in the anterior part of the ventral ciliary field and just in front of this field (see p. 81).

Nerve cells and sensory cells

Certain epithelial cells are differentiated into nerve cells. They are concentrated in the brain region and along the ventral nervecord. As such they are being treated separately in the chapter on the nervous system.

Especially in the tentacular region long nerve tracts can be seen extending from the brain. There are very few nuclei in these nerve tracts and accordingly we must assume that the nerve fibres are very long. They always lie at the base of the epithelium, except where there is a basal muscular region (see p. 67). In that case the nerve fibres pass outside the muscular region immediately under the layer of nuclei.

Distinct sensory organs have not been found in *Lamellibrachia*. There are, however, cells and cell clusters scattered in the epidermis which may have a sensory function (Plate 8:22). Mostly such cells were identified on account of their close connection with nerve fibres of the intraepithelial nerve net and their distinct morphology, by which they stand out against the normal epithelial cells. Although the nerve supply to the tentacles might suggest the presence of sensory organs or cells, none could be identified as such with certainty.

Epithelial muscle cells

In the tentacles and the obturacula the base of all or part of the epidermal cells is drawn out into long straight processes containing muscle filaments. Accordingly these cells may be characterized as epithelio-muscular cells. They are described in some detail on p. 67.

Cuticle and cuticular plaques

The greater part of the body is covered by a pseudo-cuticle, consisting of criss-cross layers of parallel fibres penetrated by microvilli, as described on p. 14. In annelids and pogonophores (Gupta & Little, 1970; own observations) it has been shown at the electron-microscopical level that a brushborder with extracellular matrix as described above, may merge into a regular cuticle. In fact this is intimated in *Lamellibrachia* in the brain region where the faint line over the unspecialized (absorp-

tive) cells and the vestimental gland cells gradually becomes thicker over the brain. Here a regular cuticle is present (Plate 19:81, 84). Parts of the tentacles and the obturacula are also covered by a cuticle, ranging in thickness from an almost invisible line to about 15 μm (Plate 22). The cuticle is secreted from the underlying epidermal cells and it shows a faint striation parallel to the epidermal surface. It reacts strongly with PAS, orthochromatically with Toluidine Blue and faintly with Ninhydrin Schiff. On the papillae, treated below, there are discs of cuticle, secreted and supported by a pad of specialized epithelial cells (Plate 10:31–33).

On the trunk curious cuticular structures were noted. Most of them have a central depression from which irregular plates protrude obliquely outwards. Thus the whole structure shows some resemblance to the petals of a flower, at least under the light microscope. A scanning electron micrograph is given on Plate 9:29, which shows that the complex actually has a more crystalline, shaly structure. They were found only in a restricted area just in the middle of the trunk, where the skin is very thin. They are rather numerous all around the trunk. They occur in different sizes and all have different shapes, so they make the impression of being abnormalities, although they might have a function in fixing the animal in the tube. There are no accompanying internal structures as far as we could ascertain.

Basement membrane

The basement membrane can only be distinguished in a few areas. This is obviously due to the fact that the base of the epithelium is so intimately interwoven with the underlying muscular layer that the membrane is indistinguishable in the histological sections. Only in the tentacles and the hind part of the trunk (Plate 8:21), where the body wall is extremely thin, can it be seen as a faint line between epithelium and muscle layer. It is slightly metachromatic and stains with PAS as is normal for basement membranes. As is described in detail on p. 72 it contains blood vessels between its two laminae.

Pyriform glands and cysts

Multicellular, pyriform glands are present along practically the whole length of the body from the collar to the end of the trunk. They are absent only in the tentacular region and on the dorsal side of the vestimental region. Thus they are present in every part of the body which is in contact with the tube. They are deeply insunk in the body. In the vestimental region they are embedded in the muscular tissue or the connective tissue (Fig. 2E; Plates 3:10; 4:12; 5:13, 14). In the anterior part of the trunk they lie between the feather muscles (Fig. 7), and in the rest of the trunk they hang freely in the body cavity (Plates 5:16; 6:17; 12:44, 47). They are extremely numerous in the outer or posterior side of the collar and in the vestimental wings (Plate 12:44). Elsewhere they are more scattered.

They consist of a glandular sac and a neck (Plate 12). The neck is a narrow tube connecting the sac with the exterior. It is lined with an epithelium of almost cuboidal

cells (8 to 10 μm high), with a very dense nucleus and light cytoplasm. Only towards the lumen does the cytoplasm stain slightly with Toluidine Blue and densely with PAS. A very faint line indicates the presence of a thin "cuticular" lining. The neck cells abut sharply upon the fully differentiated glandular cells.

Apparently there are two different types of pyriform glands, but transitions between both types are found occasionally. In one of them all cells of the glandular sac are fairly normal secretory cells with basal nuclei and various amounts of secretory material in their luminal parts. This material stains with PAS and Alcian Blue and reacts metachromatically with Toluidine Blue, but more so when it is lying in the lumen of the glandular sac. This fact suggests that the mucopolysaccharides are more acidic when extruded. Such glandular sacs can be seen in photograph Plate 12:44.

In other glands most of the cells belong to a totally different type (Plate 12:41, 46). Here many of the nuclei lie in the apical parts of the cells and the secretion is not given off to the lumen of the glandular sac but to peculiar intercellular spaces. Thus secretory products can be seen as flat plates, one edge of which is in contact with the lumen while the opposite edge seems to reach down to the basement membrane. These plates are metachromatic with Toluidine Blue and stain with Alcian Blue (Plate 12:41). It is evident that the plates are liberated into the lumen of the glandular sac eventually. Here the secretion becomes very similar to that of the other type of pyriform glands. The precursors of the secretion present in the base of the cells are orthrochomatic and stain with PAS.

In paraffin sections there are often very large intercellular spaces between the glandular cells of the last mentioned type, so that they seem to stand in tufts (Plate 12:46). No doubt this is an artifact caused by the preparation of the sections.

In the anterior part of the vestimental region the pyriform glands in the outer parts of the wings belong to the last mentioned type, while in the central part they belong to the normal type, first mentioned. Between these extremes there are glands with various ratios of both cell types.

The pyriform glands usually open to the outside on top of a small elevation very similar to the papillae carrying cuticular plaques (Plate 9:26, 27). The pores are surrounded by unicellular glands (Plate 12:41, 47). On the outside of the wall of the glandular neck there may be a layer of extremely thin cells between the cells of the neck and the surrounding epidermis.

Cyst-like structures were found in the vestimental region, particularly in the collar region, in close proximity of the multicellular glands, and in the posterior part of the trunk apposed to these glands (Plate 12:45). They consist of an extracellular mass, which may be of nearly the same size as the pyriform glands to which they are attached. The smaller cysts lie in spaces between the connective tissue cells of the vestimental collar. The larger ones are surrounded by a sheath of modified cells. This is always the case in the cysts in the posterior part of the trunk.

The matrix of the cysts is rather homogenous, but in some sections it appears to have been broken up into concentrical sheets. It stains rather heavily with haemat-

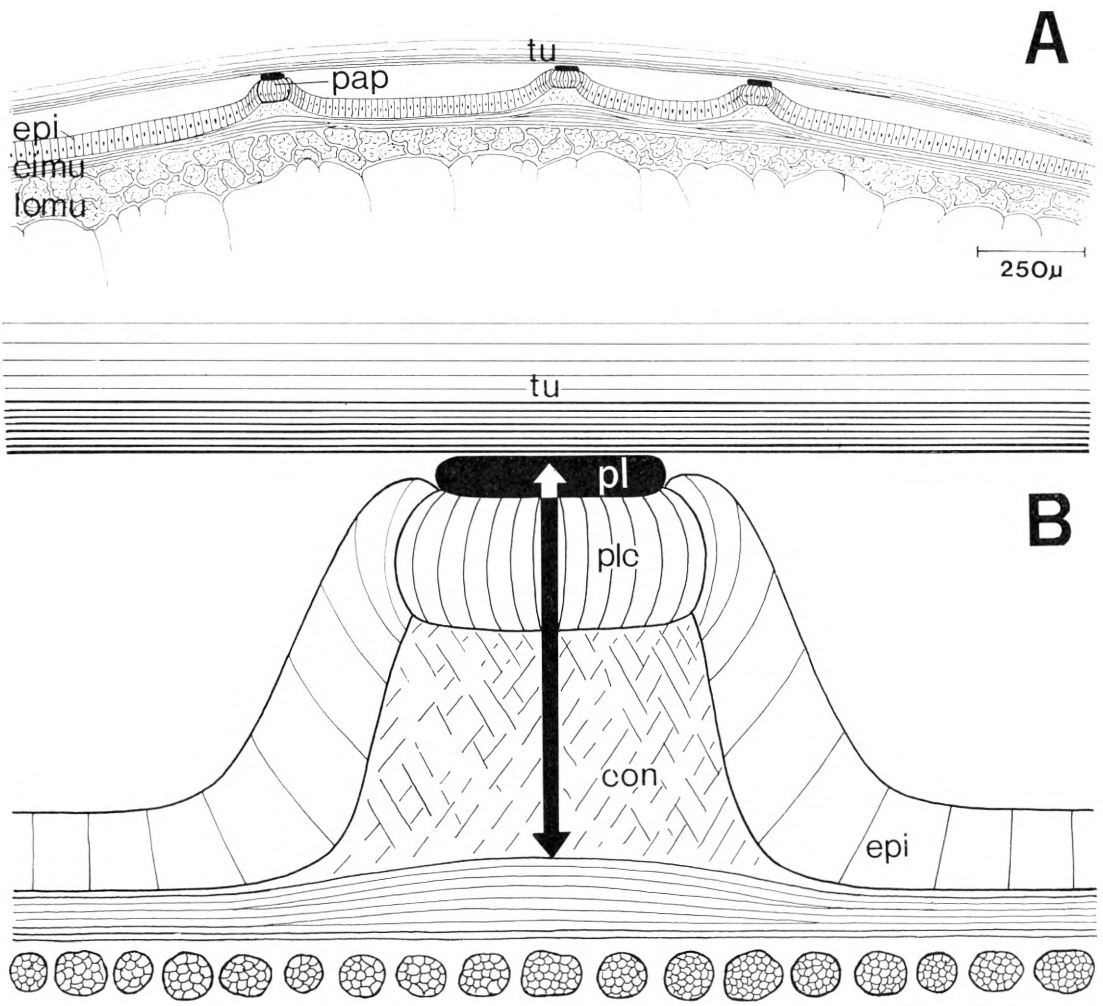

Fig. 4. Diagrams illustrating some functional aspects of the papillae with cuticular plaques. The papillae keep the body wall free from the wall of the tube when the circular muscles are contracted. At the same time they press the cuticular plaques against the wall of the tube by means of the cushion of connective tissue in the papillae. cimu = circular muscles, con = connective tissue, epi = epidermis, lomu = longitudinal muscles, pap = papilla, pl = cuticular plaque, plc = modified epidermal cells forming cuticular plaque, tu = tube.

oxylin and very heavily with stains for mucopolysaccharides, both acid and neutral. The PAS-positive material is usually found peripherally in the matrix of the cyst.

Most probably these cysts represent stores of waste products. At least we have no reason to suppose that they could be produced as a reaction to the presence of parasites.

Papillae

The greater part of the body is more or less densely beset with minute papillae, just visible to the naked eye. These papillae are only absent in the tentacular region,

including the anterior surface of the collar, the ventral ciliary field and the dorsal surface of the vestimentum. This means that they are present on all parts of the body which are normally in close contact with the inner wall of the tube. They are most numerous on the vestimentum (Plate 2:8) and the anterior part of the trunk (Plate 2:6–8), where they are very densely set. They are much less numerous on the thin-walled middle part of the trunk, but they are rather densely set again on the posterior part of the trunk (Plate 3:11).

There are two different types of papillae which are not restricted to certain regions but occur side by side everywhere. Most numerous are the papillae with a circular cuticular plaque on top (Plates 9:26–28; 10:30–33). On top of the papillae of the second type there is a pore of a pyriform gland (Plates 9:26, 27; 12:41, 47). Some histological details of the latter were given already on p. 17. The following description only refers to the first type.

These papillae are rather complex structures. The epidermis, as well as the body wall musculature and connective tissue are involved in their formation. The circular muscle layer normally lies immediately below the epidermal basement membrane. Under the papillae, however, there is a cone of connective tissue cells interspersed with scattered muscle cells lying outside the circular muscle layer. The latter is usually somewhat thickened here. The height of the cone of connective tissue varies considerably (Plate 10:31–33). On top of this cone there is a circular cluster of modified epidermal cells. The central cells are tallest so that the cluster forms part of a hemisphere lying in a cup of connective tissue. Large ovoid nuclei are located at the base of the cells and faint, dark lines extend through the light cytoplasm from the base to the apical surface. A narrow portion of the apical cytoplasm stains slightly with Alcian Blue. On top of the cluster there is a cuticular plaque, which fits accurately as a lid on a pot. It is up to 6 μm thick and reacts strongly with PAS and orthochromatically with Toluidine Blue. Thus its histochemistry is comparable to that of the cuticle of the brain region, the tentacles, and the obturacula.

The normal epidermal cells extend uphill the cone of connective tissue towards the cuticular plaque and its supporting cell cluster. These epidermal cells, or at least those close to the central cluster, are usually modified into secretory cells with strongly PAS-positive granules lying in the apical parts of the cells (Plate 10:31–32).

There appear to be regional differences in the height of the papillae. Mostly the cuticular plaque raises up to about 25 μm above the surface of the skin, but along the borders of the vestimental wings the cuticular plaques only just protrude above the level of the surface (Plate 9:28). It must be mentioned, however, that the wings are curled ventrally and the papillae may well be considerably higher when the wings are being closed over the dorsal side.

As illustrated in the diagrams of Fig. 4, the cuticular plaques situated on top of the papillae can be regarded as the points of contact between body and inner wall of the tube. Contraction of the circular muscle layer will press the cone of connective tissue outwards, and consequently the cuticular plaque as well. At the same time it

will draw the epidermis between the papillae away from the wall of the tube. Thus the animal will on one hand stick firmly to the inside of the tube, while on the other hand the epidermis is being separated from the tube by a narrow space, the width of which corresponds to approximately the height of the papillae. Since the majority of the epidermal cells are absorptive (see p. 14), nutritional material present between the epidermis and the wall of the tube can be absorbed. This may well be of the greatest importance for the feeding of the animal.

Keeping the body wall free from the tube is certainly not the only function of these papillae. The cuticular plaques undoubtedly facilitate gliding movements of the animal up and down the tube. Moreover they may also contribute to spread the secretion products of the pyriform glands evenly.

Discussion

1) The epidermis is in principle a one-layered epithelium, as it is in many other groups, including all socalled protostomians.

2) There are some specializations in the epidermis associated with the tubiculous life habits of the animal. Thus sensory cells are few and difficult to define and visualize. A similar lack of special sensory organs is found in sedentary animals belonging to different phyla, hydroids, ectoprocts, phoronids, annelids, pterobranchs and ascidians.

3) A thin cuticle is present in most areas of the body surface. It can easily be seen with the light microscope in the front part of the vestimental region, in the obturacula and in the tentacles. With the electron microscope it can also be shown to be present in the absorptive epithelium. This means, however that the term cuticle has to be defined on the ultrastructural level (Nørrevang, in prep.). The epidermal surface is covered with microvilli, which may digitate distally, and between the lower parts of the microvilli there is an extracellular matrix, which may be granular or fibrillar. Most often the fibrils are arranged in an elaborate and specific structural pattern. Cuticles of a similar structure have so far been found only in sipunculids (Moritz & Storch, 1970), annelids (Storch & Welsch, 1970) and pogonophores (Gupta & Little, 1970) and perhaps in certain nematodes (Gupta & Little, 1975: p. 51).

4) Pyriform glands like those occurring in *Lamellibrachia* are found also in pogonophores. The structure and function of the pogonophore pyriform glands has been discussed by Ivanov (1963: p. 38), Webb (1971), and on the electron microscopical level by Gupta & Little (1975) and E. Southward (1975a). In these animals the glands obviously secrete tube material, which is known to consist at least partly of chitin (Foucart, *et al.*, 1965). Gupta & Little (*l.c.*) got the impression that the substances secreted at the bottom of the glands are different from the secretions added more distally. Here attention may be drawn to the differentiated staining in some of the glands in *Lamellibrachia* (see p. 18).

Unfortunately little is known about the structure of pyriform glands in other annelids. Several types of unicellular and multicellular pyriform glands have been

found in polychaetes, but most of them were never described histologically. Several of them must also be involved in the production of tube material. In *Polydora* there are two very large multicellular pyriform glands in each of a certain number of segments in the midbody. We made serial sections of these glands and found that probably all gland cells open to the outside separately. The neck of these pyriform glands consists of a bundle of numerous ductules. Consequently the glandular sack has no lumen.

Multicellular glands with a lumen do occur in polychaetes, but we could not find examples of exactly the same type as the ones occurring in Vestimentifera and Pogonophora. Some have in principle the same structure *e.g.*, some of the light organs of *Chaetopterus*, which are tubular glands (Trojan, 1913). The light organs of *Odontosyllis* are pyriform but they may have a somewhat different structure (Dahlgren, 1916).

Multicellular pyriform glands also occur in several oligochaetes (Stephenson, 1930; Brinkhurst & Jamieson, 1971), *e.g.*, the setal and prostate-like glands. There are several types, but as far as we could find in the literature the epithelium of these glands always consists of normal epithelial cells with insunk gland cells in between.

In leeches (Mann, 1961) there are numerous pyriform glands in the body wall, but apparently they are all unicellular. The salivary glands are also unicellular pyriform glands with long ductules. All other glands in these animals, *e.g.*, the tubular glands, are also unicellular.

We may conclude that pyriform glands of many different types are common in annelids. However, glands of exactly the same type as in *Lamellibrachia* are hitherto only known with certainty from Pogonophora. According to Gupta & Little (1975: p. 49) the pyriform glands of Pogonophora can be compared with the setal sacs of annelids.

5) Papillae with cuticular plaques or openings of pyriform glands are also found in Pogonophora, but in these animals the papillae are more elaborate structures than in *Lamellibrachia*. In both groups they may have similar functions, since the cuticular plaques, situated on top of the papillae, are in contact with the inner surface of the tube in pogonophores also. The function of the connective tissue cushions in the papillae of *Lamellibrachia* was discussed on p. 20. The inner structure of the papillae of pogonophores seems to be different and usually rather intricate (Ivanov, 1963: p. 45). There may be coelomic spaces in the papillae, which is never the case in *Lamellibrachia*.

Cuticular structures in polychaetes which could be compared with the cuticular plaques of *Lamellibrachia* from a morphological but not from a functional point of view, are the many different kinds of structures on the proboscis in several families.

Coelom

For several scientists the word coelom has a definite meaning and a theoretical background, which is usually different in different countries and phylogenetical schools. Therefore we want to stress that its use in this paper has a minimum of theoretical implications; we just prefer it to a more general term like body cavity for practical reasons.

As far as we can see there is only a single coelom in our specimen because all coelomic cavities are probably connected with eachother. However, the various sections of the coelom certainly do not form a functional unit. Everywhere the coelom is bordered by a layer of cells but this "peritoneum" has an epithelial character in restricted areas only (see p. 25). The coelomic fluid contains a limited number of coelomocytes (see p. 25) and enormous numbers of spermatozoa.

Coelomic cavities

The morphology of the coelom (Fig. 5) is one of the most peculiar aspects of *Lamellibrachia*. The coelom can be called "normal" in the anterior part of the trunk only (Plate 3:9; 5:15). Here we see a spacious coelom divided into two lateral halves by the dorsal and ventral mesenteries and the trophosome. In this area the greater part of the coelom is occupied by the feather muscles, hanging freely into the body cavity. The central organs and the body wall are not only connected by the two mesenteries but also by numerous strings of trophosomal tissue running to the feather muscles (see p. 27). Because of this there are in fact practically no large open spaces through which the coelomic fluid can move freely. The large cavity visible in Plate 5:15 is an artifact. There are only narrow spaces on either side of the ventral mesentery and around the dorsal blood vessel. Probably peristaltic movements play a minor role in the anteriormost portion of the trunk.

In the greater part of the trunk, roughly 3/4 of its total length, the situation is different. The feather muscles are lacking here, so the inner surface of the body wall is

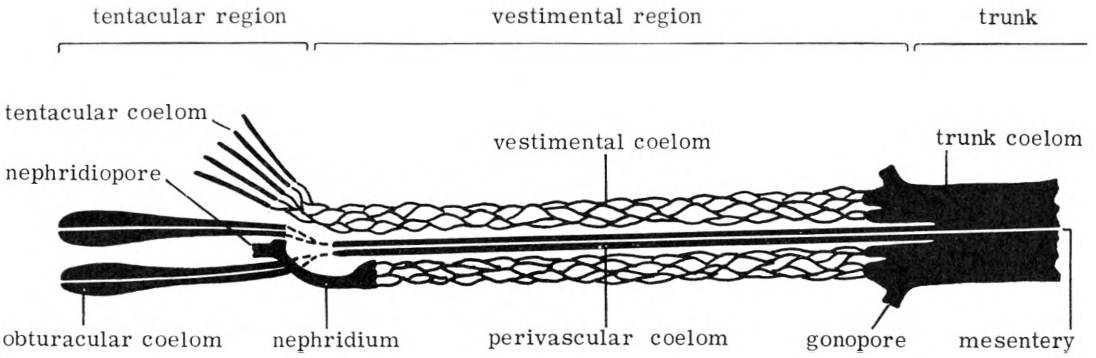

Fig. 5. Diagram of coelomic spaces and coelomoducts in the anterior part of the body.

smooth, apart from the pyriform glands hanging in the coelom (Plate 12:44). The coelom is divided into two halves only in the very small areas where trophosomes are attached to the dorsal and ventral mesenteries (Plate 6), so the two halves are practically completely fused. This is probably due to a process of degeneration (see p. 30), since WEBB's observations suggest that the two halves of the coelom are completely separated by mesenteries in younger specimens (WEBB, in press). Neither he nor we could find any trace of dissepiments.

In this part of the trunk (Plate 5:16) the coelom contains the trophosomes with a number of associated organs but also very large open spaces, particularly between successive trophosomes. It is evident that the coelomic fluid can move much more freely here than in the anterior part of the trunk, which undoubtedly facilitates peristaltic movements.

The anterior part of the trunk coelom penetrates into the posteriormost portion of the vestimental region (Plate 5:14), roughly to a level just anterior to the gonopores. In the rest of this region there are no large coelomic spaces. The vestimental musculature does not form a solid tissue. The muscle cells are all situated in narrow spaces filled with fluid (Plate 17:74, 75) and we believe that these spaces are derived from the coelom, although it is impossible to proove that they are still continuous with the general body cavity. This is certainly the case with the perivascular coelomic spaces (Plates 15:60; 17:75), which run from the trunk coelom through the whole length of the vestimental region to the heart. They form a cavity in which the dorsal vessel can freely make peristaltic movements and this is their most obvious function. Throughout its whole length the dorsal vessel is attached by "mesenteries" to the dorsal and ventral walls of the perivascular coelom. At the posterior end of this coelom there is a sphincter (Plate 15:59), which means that it can be shut off from the trunk coelom and function independently.

There are no visible spaces passing through the heart (Plate 16:67), but we must assume that they are present because the two obturacular coelomic spaces originate from the anterior part of the heart. Hence it appears that the heart of *Lamellibrachia* is in principle a pericoelomic sphincter, just like the sphincter at the posterior end of the perivascular coelom. Functionally it undoubtedly acts as a sphincter for the coelomic fluid as well as for the blood, which is a quite unusual situation.

Just anterior to the heart the dorsal blood vessel is split up into four vessels, the two obturacular vessels and the two tentacular arteries. The latter are surrounded directly by the tissue, but the former are lying in narrow, tubular coelomic cavities, just like the dorsal blood vessel (Plate 22:99–101). They are attached to the walls of these cavities by strings of tissue which probably do not form continuous "mesenteries". These obturacular coelomic cavities run backwards ventral to the heart (Plates 18:81; 16:67), then penetrate the brain close together (Fig. 15; Plate 19:81) and each enters an obturaculum, in which they widen considerably (Plate 22:99–101). See also p. 65.

Each tentacle has a coelomic cavity along its whole length (Plates 23, 24). The

afferent and efferent tentacular blood vessels run through this cavity and are attached to its wall (see the more elaborate description on p. 71). The tentacular coelomic cavities all disappear in the bases of the tentacles (Plate 23:110). We believe that they are continuous with the coelomic spaces in the vestimental muscular tissue but again this cannot be proved because the spaces are usually narrower than the thickness of the serial sections.

Coelomic epithelia

Ontogenetically all cells bordering the general body cavity must be regarded as being derived from the coelomic walls. Thus we must assume that the cells standing on the inside of the basement membrane of the body wall are in fact coelomic cells. However, they are modified into muscle cells (see p. 31). Likewise the blood vessels are in principle situated between the laminae of the basement membrane and accordingly the cells on the outside of the vessels are also derived from the wall of the coelom. The same applies to the cells on either side of the median mesentery. Only these have retained their epithelial character. The trophosomic tissue can also be considered to consist of a strongly modified coelomic epithelium.

The coelomic cells, whether epithelial, muscular or differentiated otherwise, join in the secretion of one lamina of the basement membranes. Where two coelomic epithelia meet "back to back" they secrete a common basement membrane and form a mesentery.

Coelomocytes

Scattered in the coelom there are varying numbers of coelomocytes, roughly belonging to two size categories. Both of them are spherical and have dense nuclei displaced to one side of the cells. The cytoplasm is eosinophilic and stains with PAS also. As mentioned the two categories of coelomocytes can be distinguished only by difference in size, the larger type measuring about 5 μm with a nucleus of about 3 μm, while the smaller type measures about 3 μm and has a nucleus of approximately 2 μm.

Both types occur in all parts of the coelom. The smaller type seems to outnumber the larger one slightly. In the hind part of the trunk the coelomocytes are much more numerous than in the anterior portions. They seem to be engaged mainly in sperm resorption. Coelomocytes of both types may gather in large irregular assemblies, which are most often apposed to the body wall, the mesenteries or the trophosomal lobes, but they may also be found free in the coelom. Mostly only mature sperm is resorbed, the spermatozoa being engulfed from one end. During this process the spermatozoon is being curled up in the cytoplasm of the coelomocyte. Occasionally the coelomocytes were seen to attain an amoeboid appearance engulfing larger portions of a spermatozoon at a time.

Sometimes multinuclear coelomocytes were observed, but they were not very common.

Discussion

1) In *Lamellibrachia* the normal pattern of coeloms of most, if not all, bilateral coelomate animals is evident in the trunk only. Here two spaceous lateral coeloms meet in the midline of the body, their epithelia forming a mesentery in which the main dorsal and ventral vessels are situated. In our specimen this mesentery is incomplete, but we have to regard this as a derived condition. In WEBB's specimens the mesenteries are complete throughout the whole length of the trunk (WEBB, in press and *in litt.*). Our specimen seems to be much closer to spawning than any of the males studied by WEBB, so the conditions met with in our specimen may be the result of degeneration.

The bilaterality of the coelom can be seen also in the vestimental and tentacular regions. The main dorsal and ventral vessels still represent interlamellar spaces in the mesentery here.

2) We have been unable to detect any transverse subdivision of the coelom. Our interpretation leads to the conclusion that each of the lateral coeloms is continuous from the tip of the obturacula to the hind end of the trunk.

However, there are openings from the coeloms to the exterior on two levels, *viz.*, the two gonopores in the hind end of the vestimentum and the single excretory pore at the front end of the body. We should like to know if both types represent real coelomoducts and, if so, if they represent the coelomopores of two separate segments. Since we have no other indications of the possible original segmentation of the body in *Lamellibrachia* we have to leave this problem open to further research and discussion.

3) The transformation of coelomic epithelium into muscle cells can be seen in many animal groups. Perhaps the muscles in the interior of the acorn (proboscis) of the Enteropneusta represents one of the best examples. Here the coelom is almost completely obliterated by muscle cells, except in primitive representatives like *Protoglossus* (*e.g.*, HYMAN, 1959: p. 100).

Similar conditions can be found among the Annelida. MICHAELSEN (1925) described the pregenital segments of *Lampodrilus vermivorus, i.e.*, nearly one third of the body length, to be almost completely occluded by muscles derived from the splanchnic coelomic epithelium. He described these segments as constituting a "rein muskulöses, sehr dickwandiges Organ" in which a coelomic space is left open only around the ventral nerve cord and the median blood vessels. The segmentation is almost indiscernible in this region. *Lampodrilus* is probably a carnivore and the muscularization of the anterior part of the body is apparently related to the way of feeding.

4) We believe that in the vestimental region the coelom is not only represented by the perivascular coelom but also by the lumina of all small muscular "nests" (described on p. 34). The two coelomic systems are completely separate from the posterior part of the vestimental region to the obturacula and the tentacles (diagram of Fig. 5). As far as we know the presence of two separate coelomic systems in the anterior part of the body is a unique feature of the Vestimentifera.

Trophosomes

Morphology

Trophosomes (from Greek *trophos* = nurse) are irregular masses of parenchymatous tissue in the trunk. In *L. barhami* there seems to be an uninterrupted trophosome along the whole length of the trunk (Webb, in press), but in our specimen there are several separate trophosomes. Their exact size and distribution are unknown because we made only a relatively small number of serial sections (see Fig. 1).

The trophosomes consist of large lobes (Plates 6; 13:50), which usually have a quite irregular shape. Each lobe is built up of a number of lobules, the structure of which is described in the next section. Numerous blood vessels are present in the lobes and on their surfaces (Plate 13:51). The trophosomal vascular system is described in some detail on p. 42.

The anterior trophosome seems to be the longest one. Probably it is attached to the body wall by mesenteries along its whole length. The two sperm ducts and numerous sperm sacs are embedded in the trophosomal tissue, particularly on the ventral side (Plates 3:9; 5:15). A peculiarity of the anterior trophosome is that numerous tiny, branched offshoots of the trophosomal lobes, carrying small blood vessels, run to the feather muscles to which they are attached (see also Plate 13:48, 49, 51).

We do not know how far the anterior trophosome runs backwards. We can only say that there is no trophosome present at a distance of about 140 mm from the anterior end (slide series no. 217; see Fig. 1). In the rest of the trunk trophosomes alternate with areas where the coelom only contains free sperm (Plates 5:16; 6:17). It is evident that the trophosomal tissue must have developed from the coelomic epithelium.

The posterior trophosomes differ from the first trophosome in the following aspects:

1) There are no offshoots to the musculature of the body wall (but there are no feather muscles either);

2) There are not only sperm sacs but also tubular branched testes, which are attached to the outside of the trophosomal lobes and opening into the coelom;

3) The sperm ducts are not only open at their posterior ends but they open to the coelom anteriorly as well; moreover there are several lateral openings to the coelom;

4) The trophosomes are attached to the dorsal mesentery over very short distances only;

5) The ventral blood vessel is split up into longitudinal canals which open to the coelom anteriorly as well as posteriorly; hence, it does not function as a blood vessel anymore in the posterior and greater part of the trunk.

We do not know how many trophosomes there are, but there must be several of them, probably more than ten. Of course their diameter gradually diminishes backwardly. We have the impression that they also become shorter. Between and around the trophosomes there are enormous masses of sperm in the coelom, particularly in the middle region of the trunk.

Histology

Each of the trophosomal lobes (Fig. 6) consists of a mass of cells belonging to at least two different types and of blood vessels that lie on the outside of the lobes and from which capillaries penetrate into the tissue. The two types of cells are described below as pigment cells and basophilic cells.

The pigment cells (Plate 14:52–54) are about 15 μm in cross section and the rather small nucleus, measuring about 4 μm in diameter, is usually displaced to one side. The cells look empty except for a varying number of pigment granules and some small, irregular lumps of cytoplasm staining with PAS. The PAS-reaction does not disappear after treatment with diastase. In some cells the granules are so small that they are hardly discernible, even with the highest magnification. It appears that the granules in each cell grow in size as well as in number and thus some cells may be regarded as being older than others.

Within the lobules the pigment cells lie in clusters or in strands. Each strand often consists of cells of roughly the same age as defined by number and size of the granules (Plate 14:54). The older clusters are most often found at the periphery of the lobules. This is particularly clear in the lobules that extend between the feather muscles (Plate 13:49). A process of degradation is an alternative explanation of the pattern of cell morphology.

The smallest granules usually lie in the scanty cytoplasm. The largest granules

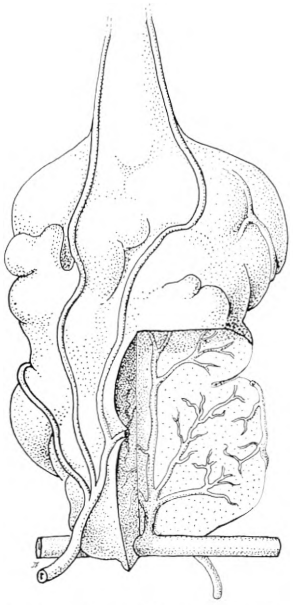

Fig. 6. Diagram of a trophosomal lobe showing disposition of blood vessels on the outside sending capillaries into the lobules, and in the centre of the lobe, sending capillaries outwards.

measure about 3 μm in diameter and occasionally they are even larger. These larger granules are mostly found in the seemingly empty parts of the cells.

The pigment granules are dark brown or blackish and can be seen as a dark hue on the trophosomes (Plate 13:50). In the middle of the trunk the dark areas can easily be seen through the skin. The granules seem to be darker after staining with haematoxylin-eosin, but after treatment with acid such as prescribed for the Feulgen and PAS stainings they appear lighter olive-brown. It can then be seen that most of them have a diffractive body in their centre.

The granules do not stain with PAS. The smallest granules stain slightly with Oil Red 0 and all granules react strongly to the VOLKMANN reaction for lipoproteins.

Scattered between the normal granules one may find some larger composite granules, obviously consisting of several pigment granules contained in a common vacuole.

We may safely conclude that the pigment cells contain stored waste products.

The basophilic cells are usually located centrally in the lobules of the trophosomes (Plate 14:52). They are characterized by a nucleus of irregular shape and different density, usually measuring about 4 to 5 μm in diameter. The cells are filled with vacuoles which in each individual cell belong to one size category. In what appear to be the younger cells the vacuoles are rather small, having a diameter of less than 2 μm. In older cells they may become much larger, measuring up to 6 by 8 μm, and attain an ovoid shape.

The vacuoles stain with EINARSSON's Gallocyanin and accordingly they may be supposed to contain RNA. The smaller vacuoles stain more densely than the larger ones, which is possibly due to the fact that the contents of RNA does not increase during the growth of the vacuole. Within the vacuoles the material staining with Gallocyanin is concentrated in minute granules, measuring about 0.3 μm in diameter, but this may well be an artifact. A slight metachromasia can be observed with Toluidine Blue in the younger vacuoles, while in the larger vacuoles a similar reaction may be masked by larger amounts of orthochromatic material.

The number of vacuoles (granules) in each cell seems to be constant throughout its lifetime. This can be concluded from the fact that the older cells are considerably larger than the younger ones. In fact the size of the cells seems to be proportional to the size of the containing granules.

During their growth the granules seem to stain increasingly densely with PAS. This reaction may be due to the larger size of the granules, but it does indicate an accumulation of PAS-positive material. The PAS staining does not diminish after treatment with diastase. The vacuoles also react to stains for lipofuscins, but this staining does *not* increase with the increasing size of the cells. The granules in the vacuoles stain slightly with FEULGEN, so a certain amount of DNA may be present. The staining is most pronounced in the smallest granules and disappears almost completely in the largest ones.

The younger and older basophilic cells are disposed in patches within the

lobules. As described on p. 42 the afferent blood vessels are often located centrally in the lobules. The youngest basophilic cells are commonly found in close contact with these vessels (Plate 14:52), while the older cells are located more peripherally. Sometimes the pattern is less pronounced. *E.g.*, in the strings of tissue extending between the feather muscles younger and older basophilic cells are disposed randomly.

We may conclude that the basophilic cells are storage cells. They contain considerable amounts of RNA in their cytoplasm, which indicates that they may synthesize proteins. They store PAS-positive material and their protein contents are also high.

Sometimes the trophosomal lobules are devoid of blood vessels. In these lobules the basophilic cells tend to be disposed at the periphery while the pigments cells are located centrally.

Discussion

1) In WEBB's specimens of *L. barhami* there is a continuous trophosome along the whole length of the body (WEBB, in press and *in litt.*). Undoubtedly the condition in our specimen of *L. luymesi* is the result of degenerative processes. Apparently WEBB's specimens represent younger stages, the testes being relatively poorly developed. Our specimen seems to be in an advanced stage of sperm production and the nutrients stored in the trophosome(s) have been utilized to produce the enormous amount of sperm.

2) The trophosomal cells seem to store reserve nutrients as well as excretory products, so the function of the trophosomes may be compared to that of a liver. Comparable organs in annelids also become loaded with waste products and Dales (1967: p. 104) introduced the term "kidneys of accumulation" to describe this.

Obviously the absorbing function of the endodermal intestinal cells has been taken over by the epidermal cells. The trophosomal cells may well be the site of metabolism of the nutrients taken up by the epidermis.

In a sence the trophosomes may be temporary structures, growing in a pregenital periode and degenerating during the production of germ cells.

3) Because of the presence of a mesentery in *Lamellibrachia* we know where we have to look for a gut. Not a trace of it was found. Apparently the intestine has been reduced completely. Whether the entoderm has disappeared entirely during evolution or is still present in early stages of ontogenesis remains unknown, but we believe that the adult only consists of ectoderm and mesoderm. We suppose that the trophosomes are completely mesodermal, being derived from the splanchnic leaf of the coelomic epithelium. A sign of its mesodermal derivation is the fact that trophosomal cells often line the outside of blood vessels.

4) Tissues similar to the trophosomes occur in oligochaetes as well as in polychaetes, where they are called chloragogen tissue, and in hirudineans, where they are named bothryoidal tissue. These tissues are always derived from the coelomic epithelium, thus being of mesodermal origin.

Ivanov (1963) described a spongy tissue in the trunk of pogonophores, "obviously representing a modification of the peritoneum" (*l. c.* p. 65).

Chloragogue cells were also described from sipunculids (Stephen & Edmonds, 1972).

In phoronids the coelom of the posterior part of the metasoma is also occupied by cell masses which are very similar to the trophosomes of *Lamellibrachia*. They also seem to be derived from the coelomic epithelium. Because they contain fat droplets they are called "tissu adipeux" (Dawydoff & Grassé, 1959: p. 1026) and in English literature they are indicated as "vasoperitoneal tissue" (Hyman, 1959: p. 248).

In mermitids (Nematoda) the intestine has no lumen and is transformed into a cell mass called trophosome (Hyman, 1951: p. 274). From the descriptions it is evident that the cells of these "trophosomes" are derived from the endodermal intestinal cells. Hence the trophosomes of mermitids seem to be similar in structure and function to the trophosomes of *Lamellibrachia*, but the derivation is different.

Musculature

Morphology

Muscle cells are present all over the body. Both smooth and striated muscle fibres occur. It is not our intention to describe all types of muscles in detail. The musculature of several organs is described elsewhere. *E.g.*, the muscles connected with the vascular system are described in the next chapter and the obturacular musculature is described in the chapter on the obturacula (p. 64). Here we confine ourselves to describing some of the more important parts of the muscular system.

The body wall musculature consists of two layers over the greater part of the body, an outer layer of circular muscles and an inner layer of longitudinal muscles (diagram Fig. 4). Both layers are practically uninterrupted along the whole length of the trunk. The inner layer is mostly considerably thicker than the outer layer. Both are much thicker in the anterior part of the trunk (Plates 5:15; 14:56) than in the middle and posterior parts (Plates 5:16; 8:21; 10:33). In the greater part of the trunk the inner surface of the body wall, *i.e.*, the layer of longitudinal muscles, is smooth (Plate 12:44), but in the anterior 35 mm or so there is a third layer of specialized longitudinal muscles with an intricate structure, *viz.*, the feather muscles. These muscles consist of bundles of fibres standing on a acellular lamella which is tree-shaped in cross-section and which is attached to the layer of normal longitudinal muscles (Fig. 7). Particularly in the anterior 25 mm of this region the feather muscles form a very thick layer occupying by far the greater part of the coelom (Plates 3:9; 5:15). The lamellae are tallest close to the ventral mesentery and gradually diminish in size towards the dorsal mesentery. Consequently by far the greater part of the muscle fibres is located in the ventral half of the body. The ventral muscles also reach farther backwards. In the posterior sections of series IS (Fig. 1) all feather muscles are already very low and restricted to approximately the ventral quarter of the trunk.

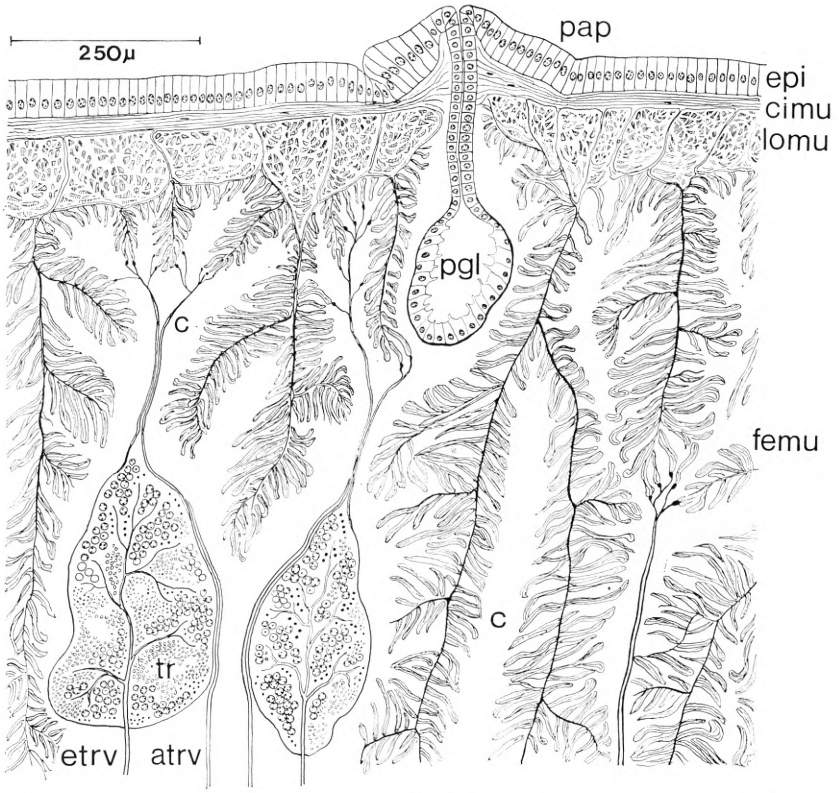

Fig. 7. Semi-diagrammatic drawing of a fragment of the body wall in the anterior part of the trunk, showing the three muscle layers, as well as some trophosomal lobes and a pyriform gland. atrv = afferent trophosomal vessel, c = coelom, cimu = circular muscles, epi = epidermis, etrv = efferent trophosomal vessel, femu = feather muscles, lomu = longitudinal muscles, pap = papilla, pgl = pyriform gland, tr = trophosomal lobe.

Anteriorly the feather muscles are attached to the vestimental muscles in the anterior most part of the trunk ceolom (Plates 5:14; 20:90). Extensions of the trophosome are attached to the feather muscles as described before on p. 27 (Fig. 7).

In the vestimental region the circular muscle layer in general remains of a modest thickness. Only over the ventral ciliary field it is unusually well developed (diagram Fig. 20; Plates 17:75; 20:90). The layer is weakest on the ventral sides of the vestimental wings, where the fibres and small fibre bundles are separated by connective tissue cells (Plate 11:38). In the posterior part of the vestimental region the layer of normal longitudinal muscles of the trunk gradually becomes thicker particularly on the lateral sides (Plates 14:56; 5:14). Further anteriorly this layer occupies nearly the whole ventral half of the body (Plate 20:90) and in front of the anterior end of the trunk coelom also the region dorsal to the perivascular coelom (Plate 4:12). In the anterior half of the vestimental region the longitudinal musculature has extended into the greater part of the wings as well (Plates 3:10; 5:13). Scattered bundles are present in the connective tissue of the wings.

The anterior extremity of the body is also nearly completely filled with muscles, but the orientation of the bundles becomes quite irregular here (Plate 7:18), particularly around the various organs in the area.

There is a strong dorsoventral musculature in the region between the perivascular coelom and the ventral body wall (Fig. 2F; Plate 17:75). The ventral vessel is completely surrounded by the dorsoventral muscles. In the posterior part of the vestimental region these muscles gradually pass into the ventral mesentery of the trunk coelom (Plate 20:90).

The layer of circular muscles is absent only in the tentacles and the obturacula. Here this layer is replaced functionally by the muscle fibres in the epidermal epithelium (see p. 67 and p. 72). In these organs the longitudinal muscles are normal.

Below the papillae there is a concentration of muscle fibres, obviously derived from the circular muscles of the body wall. These fibres are interwoven in a criss-cross pattern among the connective tissue cells supporting the papillae (Fig. 4B; Plate 10:31; see also p. 20). The musculature of the vestimental ridges is apparently also derived from the circular musculature (Fig. 2D; Plate 25).

Finally we just mention some special muscles described elsewhere: the obturacular muscle (Fig. 18, Plate 19:82; see p. 65), the heart (Plates 7:18; 16:67; see p. 41) and the sphincter of the posterior end of the perivascular coelom (Plate 15:59; see p. 24).

Histology

The circular musculature of the body wall lies as a practically complete lamina below the epidermis. As was noted earlier (p. 17) the basement membrane is usually very indistinct, but it appears to lie between the epidermis and the circular muscles. The muscle fibres are smooth and can be followed over considerable distances. The nuclei are oblong, 8 μm by 3 μm, lying with their long axis along the muscle fibres.

Histologically and cytologically the normal longitudinal muscles and the feather muscles are smooth and practically identical. They differ mainly in the shape of the basement membrane on which the individual cells are standing. It can only be shown in some places that the very complicated pattern of acellular membranes present in the thicker muscle layers (Plate 14:55–56), is continuous with the basement membrane underlying the epidermis. It shows all peculiarities of a basement membrane (see p. 17), both in structure and in staining properties.

The feather muscles have only been examined in cross sections. The muscle cells have attained the shape of lamellae closely set on extensions of the basement membrane. In general the cells are about 1 μm thick, 50 to 100 μm high and 500 to 1000 μm long. Larger and smaller cells occur side by side (Plate 13:48–49). The length of the cells has been estimated from the ratio of nuclei in proportion to the number of muscle cells present in the cross sections. There is one nucleus per one to two hundred muscle cells. The sections are 4 μm thick and the nuclei measure about 3 μm. Usually the nuclei were found somewhere at the outer edge of the lamellae in some normal cyto-

plasm (Plate 14:57). In a few cases the nucleus was found on one side of the lamella close to the basement membrane.

The ordinary longitudinal muscles and the longitudinal muscles of the vestimentum in principal have the same structure (Plate 14:55–56). All cells stand on a basement membrane, but here the basement membrane usually has a cylindrical shape, the muscle cells being attached to the inner wall of the cylinders. The cells are not lamellar but have more or less irregular profiles in cross sections.

The histology and cytology of the musculature of several organs are being treated in the chapters dedicated to these organs.

Discussion

1) The normal disposition of the body wall musculature in *Lamellibrachia* (as illustrated in diagram Fig. 4A) is not unusual and can in fact be compared with the situation in most other coelomate groups.

2) The feather muscles are highly specialized muscles, probably acting as retractors. However, they cannot be called unique since quite similar retractor muscles occur in Phoronida and in several polychaetes. In general, longitudinal muscles attain their greatest development in tube dwellers.

Claparède (1873: p. 62) already described in some detail a similar development of the longitudinal musculature in the anterior part of the body of serpulid polychaetes (*Myxicola* and *Protula*). In these animals the longitudinal musculature of the body wall also consists of an outer layer of normal muscles and a thicker inner layer of feather muscles, the "muscles longitudinaux à section pennée" in Claparède's terminology.

In Phoronida the longitudinal muscle fibres of the middle part of the trunk are aggregated into bundles which may become quite high. Although they are always of a much simpler structure than the feather muscles of *Lamellibrachia* they often have a feather-like appearance and are indicated by terms like "faisceaux musculaires d'aspect penniforme" (Emig, 1971: p. 541) and "bandes musculaires longitudinales pennées" (Dawydoff & Grassé, 1959: p. 1019). However, the basic structure of these muscle bundles seems to be completely different from the structure of the feather muscles of *Lamellibrachia*. According to Silén (1952) the core of the "feathers" is formed by the elongate basal parts of the muscle fibres and not by a fold of the basal membrane (see also Hyman, 1959: p. 237). We may conclude that the feather muscles of Phoronida are not homologous to those of *Lamellibrachia*.

3) A striking aspect of the anatomy of *Lamellibrachia* is the fact that the coelom in the anterior part of the body is nearly completely obliterated by muscular tissue. Although this situation does occur in other animals, including annelids, as was discussed before (see p. 26), it has undoubtedly arisen independently in Vestimentifera. It is not easy to understand the function of the very strong vestimental musculature. It can certainly act only very slowly because the coelomic fluid cannot move rapidly through the narrow spaces in the area. Consequently the vestimental muscles are no good

retractors. Rapid retraction can better be accomplished by the feather muscles. In fact we could only speculate on a possible function during locomotion (see p. 87).

4) Epithelial muscle cells are present at least in the tentacles and the obturacula (see description on p. 67 and p. 72). Hitherto it was generally assumed that such cells were confined to the lower Metazoa. However, recently (Boilly-Marer, 1972) they have also been described in annelids (Nereidae).

5) The main muscles of the vestimentum and the trunk are undoubtedly of mesodermal origin. Generally, mesodermal muscle cells are derived ontogenetically from the coelomic epithelium. This origin is evident indeed in the case of the feather muscles. The subepidermal basement membrane subdivides, sending out branching lamellae into the coelom, to which the feather muscle cells are attached. Moreover these are attached to the trophosome, which must also be a coelomic epithelium in origin. It is a curious fact that different coelomic epithelia remain connected with eachother here. A similar situation occurs in the tentacles (p. 72).

6) In the musculature of the vestimentum the situation is far more complicated. Nevertheless, we think that the separate "nests" of muscle cells (Plate 14:55) are in fact subdivisions of the coelom in which the muscle cells are standing on the surrounding basement membrane, which extends into the coelom. If this explanation be accepted it is easier to understand the structure and function of the nephridia (see p. 55). In fact for the explanation offered there the presence of coelomic spaces among the muscle cells is a prerequisite.

Vascular System

Morphology and circulation

The blood vascular system is one of the most important organ systems of *Lamellibrachia*. Conspicuous vessels are present throughout the whole body and together they contain much blood. When our specimen was wounded when taken out of its tube, it lost a considerable amount of blood.

In principle *Lamellibrachia* has a closed vascular system, although it should be noted that in some parts of the body the blood vessels form a system of lacunae and that in our (degenerating) specimen the main ventral vessel is broken up posteriorly and consequently is open to the coelom.

All main blood vessels are indicated in the diagram of Fig. 8. The two main median vessels extend throughout the whole length of the body and both are situated in the mesentery. The dorsal vessel (*vas dorsale*) has a definite wall along its whole length, which is strongly muscular, particularly in the anterior half of the body. Close to the anterior end of the body it ends in the heart. The ventral vessel (*vas ventrale*) seems to possess no wall of its own, the limiting membrane being the basement membrane of the surrounding muscle cells. Apparently the blood is transported through the ventral vessel in a more or less passive way.

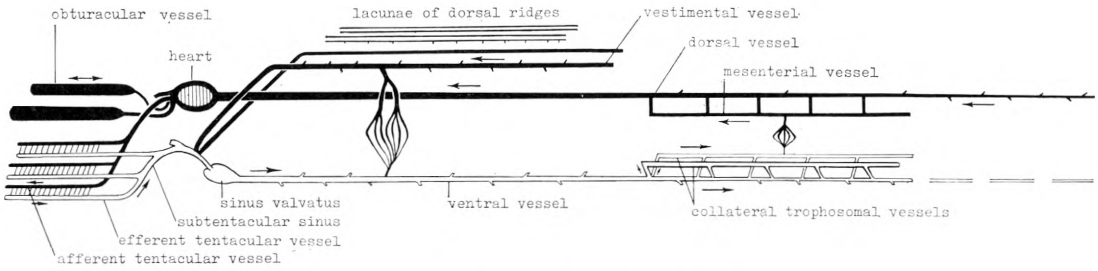

Fig. 8. Diagram of the vascular system.

In the trunk a minor vessel, the mesenterial vessel (*vas mesenterialis*), is found in the mesentery, ventral to the dorsal vessel to which it is connected at regular intervals. Numerous smaller vessels run from the trophosomes to the mesenterial vessel. In the ventral part of the anterior trophosome there are two conspicuous vessels running parallel to the main ventral vessel. We called them collateral trophosomal vessels (*vasa trophosomales collaterales*). From these vessels numerous small vessels penetrate the trophosome in all directions. The microcirculation in the trophosome is described in some detail on p. 42 (see also Plate 13: 51; Figs. 6, 7 and 12).

This description refers to the front part of the trunk. In the rest of the trunk the situation is much different, but since we are convinced that this is due to the fact that the trophosome is split up into several separate trophosomes we shall not describe it.

In the vestimental region and particularly at the front end of the body conditions are much more complicated (Plate 4; Figs. 8, 9 and 10). The body cavity is wholly obliterated by muscle tissue and only a narrow space, the perivascular coelom, is left open around the dorsal vessel, which lies in the mesentery of that coelom (Plate 15: 60; Fig. 2C). Approximately at the border between the trunk and the vestimental region strong circular muscles are present both on the wall of the dorsal vessel and around the perivascular coelom (Plate 15: 59). They may act as a sphincter.

It is remarkable that we could not find any smaller vessels leaving the dorsal vessel in the vestimental region. In the heart region the perivascular coelom seems to disappear.

Just anterior to the heart the dorsal vessel splits up into four major vessels, the left and right obturacular vessels and the left and right afferent tentacular vessels. The obturacular vessels are surrounded by narrow coelomic channels, which we think are continuations of the perivascular coelom. They run close to the midline and after making a loop and penetrating the brain, they enter the obturacula. In these organs they seem to end blindly. The tentacular vessels split up in an irregular, intricate way into a great number of small vessels, each of which enters a tentacle. The efferent tentacular vessels collect into spacious subtentacular sinuses, which fuse to form the left and right lateroventral vessels. After each of them has received one of the vestimental vessels they join in the midline ventral to the heart to form the ventral vessel. Close to the front end of the ventral vessel there is an intricate organ, the *sinus valvatus*, the

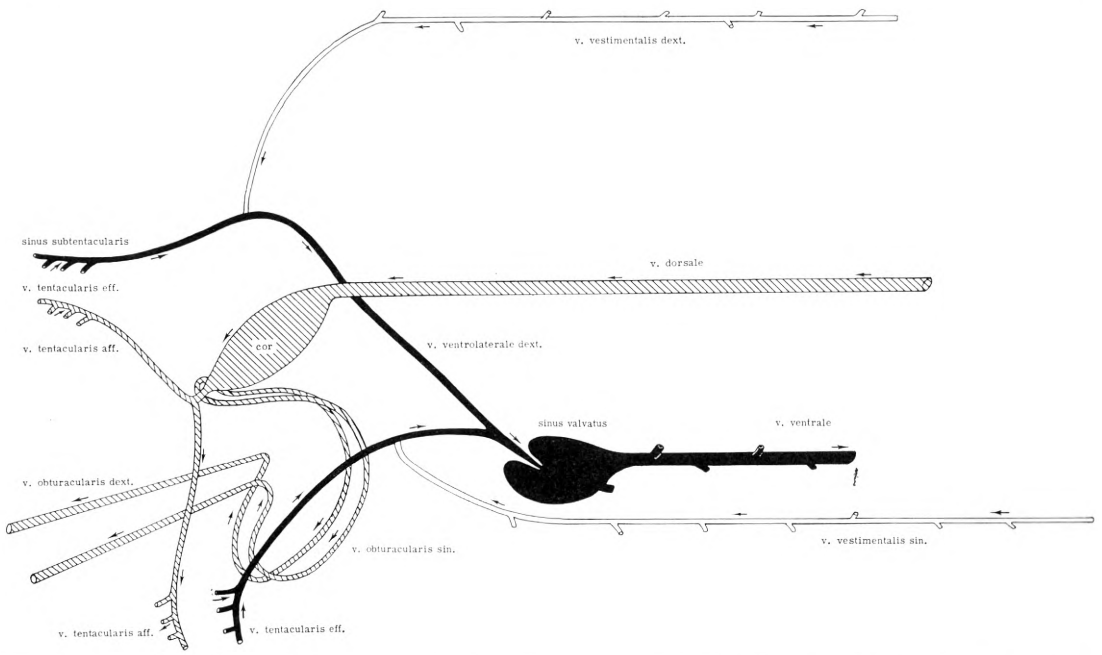

Fig. 9. Diagram of the vascular system in the tentacular region and the anterior part of the vestimental region.

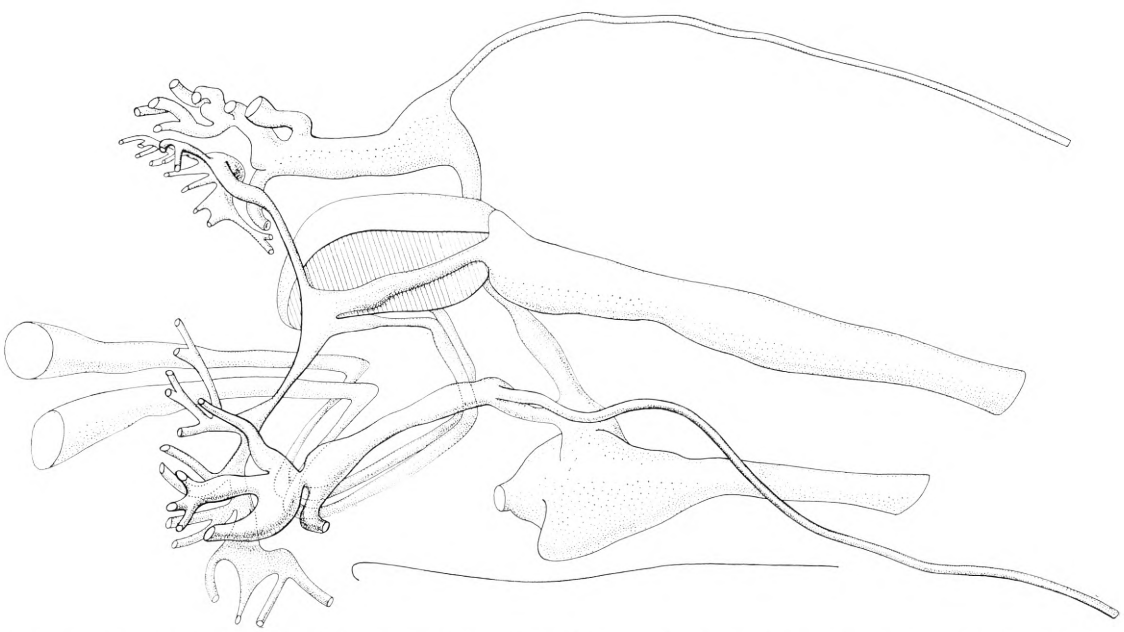

Fig. 10. Reconstruction (Lison method) of the main blood vessels in the tentacular region and the anterior part of the vestimental region in dorso-lateral, frontal view. The names of the various vessels are given in fig. 9.

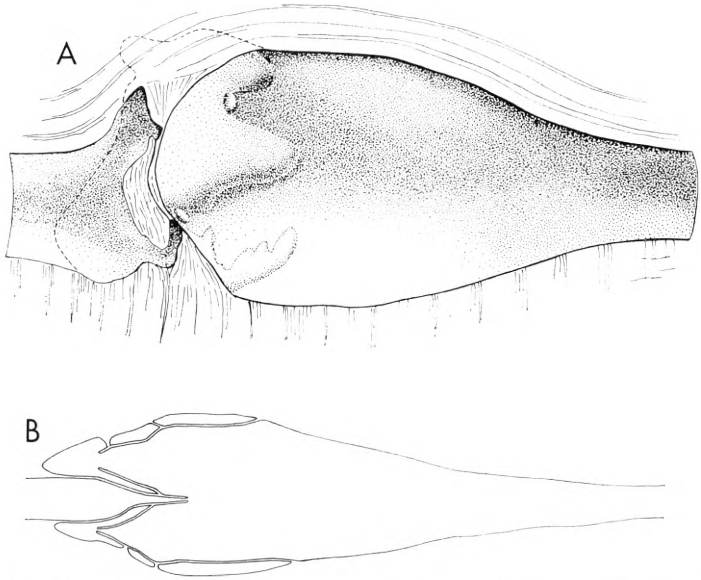

Fig. 11. Reconstruction of the right half of the *sinus valvatus* (A) and diagrammatic horizontal section of the same (B).

structure of which is illustrated in Fig. 11. Apparently it is a valve system which only allows a blood flow in a posterior direction. Unlike the dorsal vessel the ventral vessel is in close contact with the tissue of the vestimental region. Many small vessels enter it along its whole length.

In the vestimental region there are many sinuses and vessels the exact connections of which we have not been able to trace in detail. Most prominent are the sinuses in the vestimental ridges (see p. 76) and the subepidermal sinuses, which are most conspicuous under the papillate fields on the outer surface of the vestimental wings (Plates 4; 16: 68, 70). In the base of each wing there is a conspicuous longitudinal vessel with muscular walls, running along the whole length of the region. Anteriorly they enter the ventrolateral vessels.

As to the direction of the blood stream in the various vessels and sinuses (diagram Fig. 8) we only have a few indications.

In front of the heart and the *sinus valvatus* we can be reasonably sure of the flow directions. The blood is pumped into the obturacula and the tentacles by the activity of the heart. From the obturacula the blood can only return the same way, driven by contractions of the muscle fibres in the mesenchyme of these organs. We may assume that in the tentacles the blood passes from one side of each tentacle to the other through the small vessels at the base of the epidermis. In the pinnulate parts of the tentacles a blind ending branch extends into each pinnule, so there will be a directed flow from afferent to efferent vessels, the ebb-and-flow system of the pinnules being superposed on it.

The structure of the *sinus valvatus* (Fig. 11) prohibits a forward blood flow in the

ventral vessel. Consequently we must assume that the subtentacular sinuses are drained by the ventrolateral vessels. From the structure of the *sinus valvatus* and by analogy to other annelids it seems evident that the blood normally flows in a forward direction in the dorsal vessel and backwards in the ventral vessel. In all other vessels described we cannot be definite about the direction of the blood flow. Probably there is an ebb-and-flow system in which waves may travel in both directions in the greater part of the vascular system, as is the case in most annelids (DALES, 1967: p. 80). Consequently the arrows in Fig. 8 only indicate what we think to be the normal flow direction, but we do not want to suggest that the blood could not flow in an opposite direction at times.

We assume that the vestimental region receives blood from the ventral vessel and that it is drained by the two vestimental vessels. The system of lacunae in the dorsal ridges has no clear connections with the rest of the vascular system. Here and there small vessels can be seen running downwards. Perhaps the lacunar system is filled with blood through these vessels by means of pressure of the vestimental musculature and emptied through the same vessels, which means that we have an ebb-and-flow system here again. The reason for this supposition is that the lacunar system gradually becomes smaller anteriorly and posteriorly and that afferent and efferent vessels could be expected to be present at the anterior and posterior ends of the ridges in the case of a longitudinal blood flow through the ridges.

Histology

Dorsal vessel

The histology of the walls of the dorsal vessel shows little variation along its whole length. It is unlike all other blood vessels in *Lamellibrachia*. There is a distinct endothelium of which mostly only the nuclei can be distinguished in the sections. These are very flat and closely applied to the inside of the elastica and oriented longitudinally in the vessel. They measure about 10 by 3 μm and are less than one μm thick. In a few sections tangential views of part of the wall of the vessel gave an opportunity to study the structure of the endothelium in some detail (Plate 15:62–63). Each endothelial cell has a central cytoplasmic mass, about 10 μm broad and a little longer. Processes, about 35 μm long and a few μm broad, run in a longitudinal direction, while shorter processes extend to either side. The endothelial cells are in contact with eachother by means of these processes. Hence they form a network on the inside of the elastica (Plate 15:62). Up to 25 $^0/_0$ of the endothelial cells have larger and lighter nuclei. Perhaps these cells can be considered intermediates between normal endothelial cells and the cells of the intravascular cord (see p. 40). The endothelial cells are extremely thin, so their cytoplasm could not be studied in detail.

The acellular elastica is up to about 3 μm thick and consists of two approximately equally thick layers, separated by a thin dense line. There are minor regional differences in structure. The described pattern applies to the vestimental region. In sections stained with Toluidine Blue the inner layer is metachromatic, while the outer

layer is orthochromatic. Both layers stain deeply red with PAS (Plate 15:60). In certain sections the inner layer can be seen to be composed of thin longitudinally oriented fibrils, about six on every 10 μm. These fibrils are in fact closely set ridges, as can be seen in certain oblique sections (Plate 15:63), and they stain selectively with PAS and hematoxylin. The outer layer appears to consists of a homogenous mass. The elastica may be regarded as a specialized basement membrane.

On the outside of the elastica there is a muscular layer, the fibres of which are striated (Plate 15:61). Each muscle cell consists of a nucleus surrounded by normal cytoplasm and a fibriller part, which is in intimate contact with the elastica. Each fibre is about 2 μm broad and about 5 μm high. Its length could not be determined. The nuclei are rather large, measuring about 5 μm in diameter. In the posterior part of the trunk no muscle fibres could be discerned on the outside of the elastica, the outer layer of which is thickened considerably in this area (Plates 15:58; 16:64). Here the elastica is separated from the coelom by a layer of irregular, unspecialized cells. They constitute a proof that the muscle cells are in fact modified coelomic epithelial cells (see p. 25).

In the region of the gonopores (Plate 15:59) the narrow coelom dorsal to the vessel is partly filled with mesenchymatous cells and the perivascular coelom is surrounded by a sphincter.

Intravascular cell cord

The intravascular cell cord probably consists of specialized endothelial cells, although their appearance is totally different from the normal endothelial cells. Unlike the latter they contain abundant cytoplasm so they protrude into the lumen of the vessel. Their nuclei are much lighter than those of the endothelial cells and they are more rounded. Their cytoplasm is extremely light and stains with none of the techniques used, except for some few PAS-positive granules.

In some cells of the intravascular cell cord a faint but distinct striation could be observed in their basal parts, particularly in thin sections. This suggests that the basal cell membrane may be folded. In these cells the nuclei are situated approximately in the centre of the cells.

A considerable number of cells can be described as intermediates between ordinary endothelial cells and cells of the intravascular cord. The nucleus may be of intermediate morphology and the amount of cytoplasm may also be intermediate. Other abnormal cells are the small cells which are often present between the bases of the cord cells (Plate 16:64, 66). They stain darker with hematoxylin-eosin and slightly red with PAS. They may be cells with a special function but they may as well represent initial stages in the formation of cord cells.

The intravascular bodies, in fact only enlargements of the intravascular cell cord, show a considerable variation in shape and size. It may suffice to describe the cellular structure of one particular body. It is attached to the ventral wall of the vessel along a zone with a width of approximately 60 μm. At the attachment the cells are of

the darker type described above. The nuclei lie rather close so that the cells look almost cuboidal. The cell mass bulges into the lumen of the vessel (as in Plate 16:65). The greatest dimensions of the cell mass described here are in the order of 300 by 400 by 500 μm. There is a central cavity. The dark cells are found all around the periphery and there are also strands of dark cells extending towards the central cavity. The cells of these strands attain a long and slender shape (Plate 16:64).Most cells of the intravascular body are of the light type. In these cells droplets or granules occur which measure up to 4 μm in diameter but most of them are of minute size. They stain with eosin and very heavily with PAS suggesting that they contain neutral mucopolysaccharides and/or mucoproteins. Occasionally small extracellular spaces are filled with PAS-positive material. These spaces may communicate with the lumen of the vessel. Since droplets of similar material may be found on the surface of the cell mass, this material may actually be discharged into the blood stream. The central cavity is filled with material that stains like blood, but less intensively (Plate 16:65). Occasionally Alcian Blue-staining can be observed in the intercellular spaces. Typical endothelial cells can be seen covering the surface of the cell body (Plate 16:66).

We may conclude that the intravascular bodies consist of light, vacuolated and/or glandular cells. They seem to develop from endothelial cells since intermediate cells are common. Intercellular spaces and sometimes a larger central cavity contain a fluid with mucopolysaccharides and/or mucoproteins. Strongly PAS-positive droplets are discharged into the blood.

Heart

In the heart region the dorsal vessel is totally collapsed (Plate 7). The elastica is thrown into folds, suggesting that the lumen of the vessel can widen to a considerable diameter (Plate 16:67). Endothelial cells can be seen. The perivascular coelom has disappeared or is at best represented by small subdivided spaces immediately adjoining the elastica. As might be expected there are no muscle cells lining the outside of the elastica, the contractions being performed by the very strong outer layer of muscles.

The core of the heart, about 200 μm in diameter, lying around the collapsed vessel consists of spongy tissue. Around this core lie numerous concentrical sheets of muscle cells, interspersed with occasional lamellae consisting of flat coelomic pouches with weakly developed longitudinal muscle fibres. The lamellae look very much like extremely flattened "nests" of vestimental muscles (see p. 34).

Ventral vessel

It is rather difficult to describe the histology of the ventral vessel. In fact it is just a large fissure, either in the ventral mesentery (Plate 16:69) or in the vestimental musculature (Plates 16:70; 17:75). Along the vessel the muscle fibres mainly run in a dorso-ventral direction (Plate 17:75). In the vestimental region these muscle cells may be rather long and their nuclei measure up to 10 to 12 μm by about 2 μm. In the trunk the layer of dorso-ventrally orientated muscle cells is much thinner (Plate 16:69). By

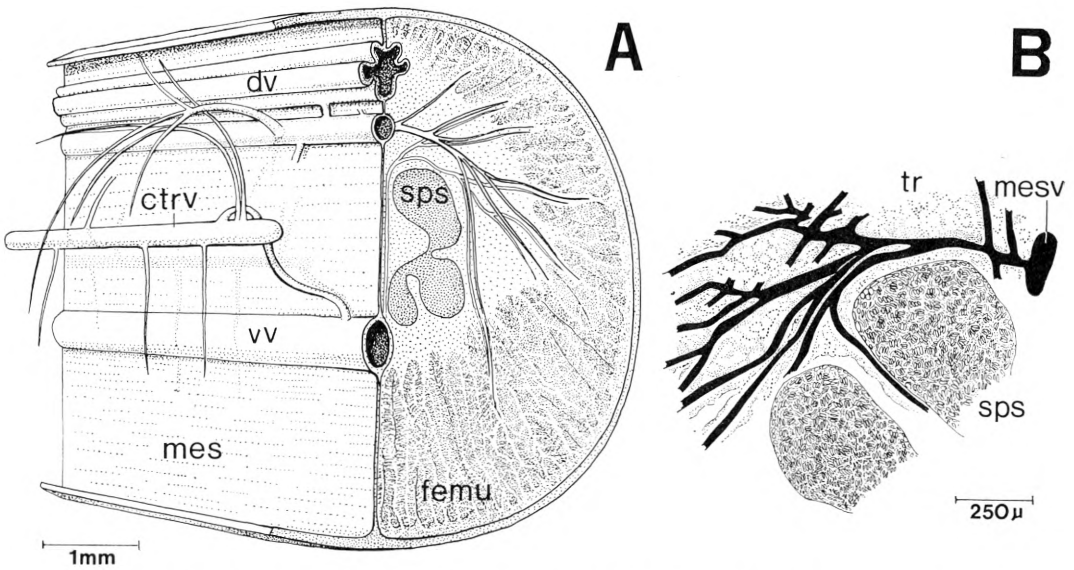

Fig. 12A. Diagram of the vascular system in the anterior part of the trunk (in latero-posterior view).
Fig. 12B. Detail of the trophosome illustrating the intricate vascularisation in this area. ctrv = collateral trophosomal vessel, dv = dorsal vessel, femu = feather muscles, mes = mesentery, mesv = mesenterial vessel, sps = sperm sac, tr = trophosomal tissue, vv = ventral vessel.

a layer of rather loose connective tissue it is separated from a second layer of muscle cells, in which the fibres run more or less at random.

Unlike the dorsal vessel the ventral vessel does not have a distinct endothelium. However, nuclei lying on the luminal side of the innermost muscle fibres have exactly the same appearance as the nuclei of the endothelial cells of the dorsal vessel. Apparently at least an incomplete endothelium is present. In the trunk the nuclei lie closer together than in the vestimental region, indicating that the endothelium is better developed here. On a scanning electron micrograph not reproduced here, the inner wall of the ventral vessel in the vestimental region looks spongy.

In the trunk a coelomic epithelium, although not very distinct, seems to cover the outer muscle layer (Plate 16:69).

Smaller vessels

The walls of the vessels leading to the dorsal vessel evidently still consist of three elements. There is an endothelium which is very similar to that of the dorsal vessel. It is not quite clear if there is a distinct elastica, but in several sections a seemingly acellular layer delimits the endothelium from an outer layer consisting of closely set cells (Plate 17:71).

The vascular system of the anterior trophosome was studied in some detail (Figs. 6, 7 and 12). The collateral trophosomal vessels, as well as the vessel connecting them to the ventral vessel have muscular walls not unlike the wall of the ventral vessel,

but of course considerably thinner. The blood reaches the trophosomal lobes by means of small vessels lying on the outer surface of the lobules (Plate 14:53). Their walls are extremely thin, often less than 1 μm. The capillaries run between the trophosomal cells and can only be seen as intercellular sinuses, at least when they contain blood (Plate 14:52). The walls of the blood vessels draining the trophosomal lobes are rather thick. There may be endothelial cells similar in structure to those described before. In some sections an outer layer could be distinguished. It consists of cells which are somewhat lighter than the endothelial cells but which have approximately the same dimensions.

Blood

In the live animal the blood was red. Nothing is known about the chemical nature of the blood pigment. Since the blood does not contain free cells we way conclude that it is dissolved in the plasma. In the microscopical sections the blood stains moderately with PAS and Ninhydrin-Schiff, indicating the presence of neutral mucopolysaccharides and/or mucoproteins. Some staining is observed after treatment with Gentiana Violet, indicating that lipofuscins may also be present.

Discussion

1) In general the vascular system of *Lamellibrachia* can easily be compared with the systems occurring in other annelids, both in structure and function. In fact its basic pattern of median dorsal and ventral vessels with opposite flow directions is found in most protostomians. As in many other annelids the main propulsion is due to the muscular dorsal vessel. In front of the heart the vascularization is very similar to that in sedentary polychaetes, especially those possessing an elaborate tentacular apparatus. As discussed on p. 75 these similarities even apply to minute details.

2) In other annelids the heart can only be described as a strongly muscular structure surrounding a blood vessel. It does not have a definite position. It may be lacking altogether, there may be a single heart associated with the dorsal vessel, and there may be several hearts, either around the dorsal vessel or around a number of other vessels, usually commissural vessels. However, as far as we know it can always be described as a strongly developed part of the musculature of the wall of the vessel. This is probably not the case in *Lamellibrachia*. We believe that both the vessel and the perivascular coelom penetrate the heart. Consequently the heart muscle should have derived from the musculature surrounding the coelom, *i.e.*, from the vestimental muscles or (in principle) from the body wall musculature. Provided our theory is correct this situation is quite unusual.

3) The fact that blood vessels are present within an epidermal tissue, *viz.*, the brain, is also quite unusual. Of course the blood cannot pass through the basement membrane. Consequently the brain vessels must be outfoldings of the epidermal basement membrane between brain cells and supporting cells.

4) According to HANSON (1949), who reviewed the literature on the blood systems of Oligochaeta and Polychaeta, an *endothelium* may be present in the blood vessels of these groups. However, the endothelial cells never constitute a complete cellular lining as in vertebrates. The endothelial cells have a branched structure and are only connected by their processes. This is exactly the condition found in *Lamellibrachia*. Morphological details of the cells, including the presence of fine ridges, are also comparable.

5) Structures which are similar or at least comparable to the intravascular cell cord of Vestimentifera occur in many oligochaetes, polychaetes and pogonophores. They are often called *corpus cardiacum*, heart body, or cardiac body, but these names are not very appropriate. Mostly these organs have nothing to do with the heart, nor can they be described as bodies. For these reasons we prefer to speak of an intravascular cell cord (*cordilla intravascularis*), which is enlarged in places to form intravascular bodies (*corpus intravascularis*).

The structure and function of the intravascular cords and bodies varies a great deal in the different groups (ASHWORTH, 1904; KENNEDY and DALES, 1958; ROMIEU, 1923; STEPHENSON, 1930). In Oligochaeta the intravascular cord generally has the form of a rod of cells, attached to the floor of the dorsal vessel and extending through a number of segments in the anterior part of the body (STEPHENSON, 1930). The cells may be aggregated into masses of various forms, most frequently appearing as an interrupted cord from segment vii to segment xv. In Oligochaeta its function is unknown. In Polychaeta the intravascular bodies may be haematopoeetic or similar in structure and function to chloragogen cells (EISIG, 1887; DALES and PELL, 1970) or they may act as valves governing the rate and direction of the blood flow (ASHWORTH, 1904).

The cells of the intravascular bodies in polychaetas (DALES and PELL, 1970) and pogonophores (GUPTA and LITTLE, 1975) have been shown to possess a basal lamina of their own, surrounding the intire cell. Typical endothelial cells may be present on the surface of the cell bodies in *Lamellibrachia*. This has also been described for *Arenicola* (ASHWORTH, 1904).

The intravascular cell cord is such a peculiar structure, that it could hardly have developed independently in the different groups mentioned above. This is one of the reasons why we consider these groups to belong to the same phylum.

6) Ontogenetically the blood spaces must be regarded as remnants of the blastocoel. Indeed the blood vessels are always found within basement membranes, *i.e.*, between the basement laminae of two apposed epithelia (or derivatives of epithelia). This is particularly evident in the case of the dorsal vessel. It lies in the mesentery and is bounded by extracellular material secreted by the adjoining cells, whether these are epithelial or muscular. In the obturacula the vessels lie between the two layers of the "mesentery" (Plate 22:100). It is evident that the blood spaces are situated between the basement laminae underlying the coelomic epithelia, which stand "back to back". These vessels also have an endothelial lining, as can be seen in the figure mentioned. In the tentacles the afferent and efferent vessels are both situated in the basement

membrane between the epidermis and the coelomic epithelium, bulging out into the coelom (Plates 23:104; 24:113). The lining of the ventral vessel is so thin that the presence of an extra-cellular membrane can only be deduced from its presence in a minor vessel seen on Plate 16:69. This vessel lies in the ventral mesentery and the serial sections show that it is connected with the ventral vessel, which is visible in the upper part of the same photograph.

7) The presence of a red blood pigment indicates that Vestimentifera, as well as Pogonophora and many other annelids and representatives of several other phyla (Manwell, *et al.*, 1966), make use of a specific oxygen carrier. Probably this fact must be considered in connection with the tube-dwelling habits of *Lamellibrachia*. Under normal conditions only the obturacula and the tentacles project from the tube (see Webb, 1969a: p. 18). The greater part of the body is practically always protected by the thick tube and consequently respiration can only take place through the surfaces of the obturacula and tentacles. It is not unlikely that an oxygen carrier is a necessity under these circumstances, at least for an animal of this size.

8) Some types of blood vessels in *Lamellibrachia* are blind ending. The most conspicuous ones are the obturacular blood vessels, but the small vessels in the pinnules are also blind ending. Blind ending blood vessels are quite common in sabellid and serpulid polychaetes (Dales, 1967: p. 83), but they also occur in several other families. Dales (1967: p. 79) considers them to be "perhaps the most interesting peculiarity of the annelid vascular system".

Nervous system

Morphology

The nervous system consists of a completely intra-epidermal central nervous system and of nerve fibres which partly penetrate into certain internal organs. Our techniques were not very appropriate for the study of delicate nerve fibres. Consequently we have to confine ourselves to a description of the central nervous system and some of the more conspicuous nerve fibres, which are mostly intra-epidermal.

The central nervous system consists of a midventral nerve cord, which runs through the epidermis along the whole length of the body, and a brain, which lies at the front end of the body at the base of the obturacula and the tentacles (see diagram Fig. 13). The nerve cord shows two conspicuous peculiarities. In the first place it is double from a short distance posterior to the brain to the end of the vestimental region, the two halves running on either side of the ventral ciliary field (Plates 1:2; 2:8; 4:12; 20:90). In the second place there are two fluid filled tubes lying in the nervous tissue (Fig. 2G; Plates 19:85; 20:88). They originate in the brain and unite at the posterior end of the vestimental region to form a single tube (Figs. 13 and 14), which runs through the ventral nerve cord of the trunk for its first 30 mm (its posterior end is to be seen in slide series IS). Down the trunk the nerve cord gradually diminishes in size, although in cross sections of the posterior part of the trunk it is still a prominent

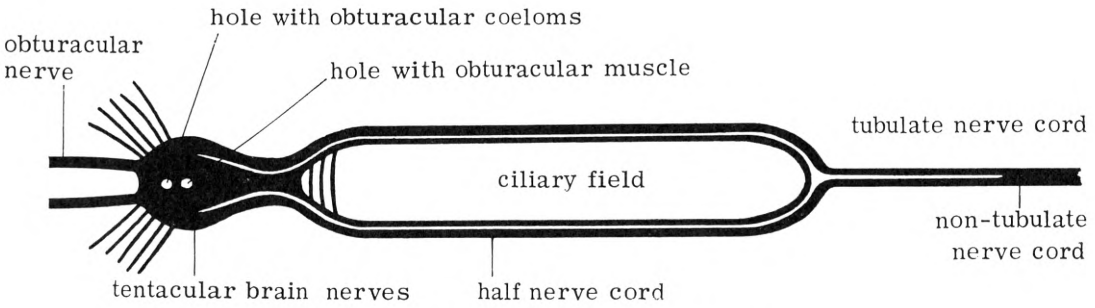

Fig. 13. Diagram of the central nervous system.

structure, bulging out of the epidermis, which has become rather thin there (Plate 20:89).

Along the greater part of their length the diameter of the neurular tubes remains rather constant, being about 100 to 150 μm. However, they are not straight and regular everywhere. To demonstrate this we give a reconstruction of the tubes (Fig. 14) in the area where they unite to form the single tube of the trunk. Some irregularities are due to contractions during fixation, but this certainly does not apply to some of the pockets in the figure nor to small side branches that are present here and there. Some of the latter are rather long and end as very thin tubules close to the surface of the epithelium. The posterior end of the tube in the trunk also lies very close to the body surface. We could not observe any direct connection to the outside.

In the area of the ventral ciliary field there is a rather dense nerve net between the two halves of the ventral nerve cord.

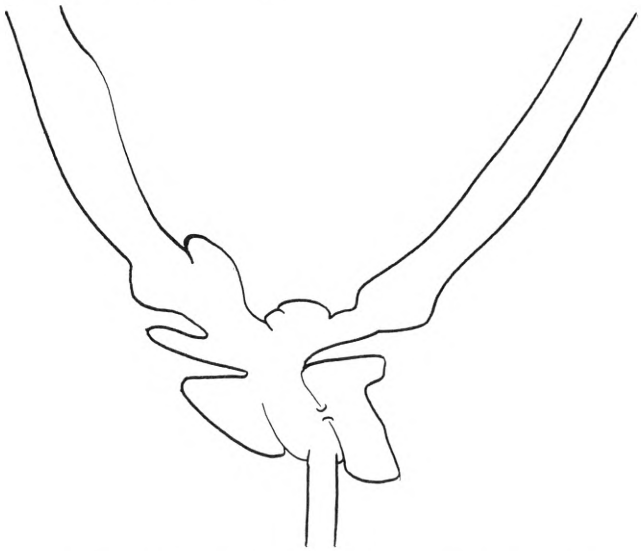

Fig. 14. Reconstruction of the neurular tubes in the area where the two anterior tubes join to form the single tube of the trunk.

Close to the front end of the body the two halves of the nerve cord unite and gradually enlarge to form the brain (Fig. 15; Plate 19:84). The two neurular tubes converge towards the brain and meanwhile the neural tissue lying on the adaxial sides of the tubes almost disappears. In the area just posterior to the brain the two halves of the nerve cord are connected by numerous fibres at the base of the epithelium (Fig. 13; Plate 19:83). Here both neurular tubes dive deeper into the epithelium, to come very close to the surface again in the posterior part of the brain. In this area both tubes become very narrow in two or three places. One of them even becomes so narrow that it cannot be recognized in the sections anymore. In the brain area both tubes have one or two side branches leading to the surface. Another irregularity is the presence of a short third tube running parallel to one of the main tubes. It is identical to the other tubes but has a length of only about 0.7 mm. All three tubes gradually become very narrow anteriorly, until they cannot be recognized in the sections anymore, which happens very close to the level of the obturacular muscle. We consider the third tube as an abnormality, mainly because it is present only on one side.

The ventral side of the brain is covered by a thick cuticular plate. Dorsally there are two prominent lateral lobes. There is a hole in the middle of the brain through which the two obturacular bloodvessels with their surrounding coelomic canals pass to the bases of the obturacula and the obturacular muscle runs to the ventral cuticular plate (Plates 18:80; 19:82). The morphology of the anterior half of the brain, in front of the hole, is dominated by the presence of the numerous tentacular nerves (Fig. 15; Plate 19:81) and the two very large obturacular nerves at the front end.

Histology

The ventral nerve cord

The walls of the neurular tubes (Fig. 2G; Plates 19:85; 20:88) are about 2 μm thick and appear very dense and stainable with PAS, eosin and orthochromatically with Toluidine Blue. We may conclude that the wall contains considerable amounts of mucoproteins. In some sections it can be seen to consist of concentrical fibres or lamellae. There are nuclei within the sheath and occasionally a nucleus can be seen on the inside of the wall. The derivation of the cells forming the wall is not quite clear, but from the proximal wall of the tube fibres run to the basement membrane of the epidermis (Plate 19:85). Consequently they belong to the same category as the supporting cells to be described below and we may conclude that the sheath of the neurular tube is formed by specialized epithelial cells. This is also indicated by their histochemical properties. Ontogenetically the neurular tube could have arisen either as an intercellular space or as an invagination of the epidermis. The neurular tube system contains a fluid which stains lightly with eosin and very slightly with Alcian Blue. The coagulated fluid is visible in Plate 20:88, ruffled during sectioning.

The morphology of the nerve cord proper is strongly influenced by the presence of the relatively large neurular tube. The normal epidermal cells, whether absorptive or glandular, climb over the bulging tube to cover it on the outside. Only the sub-

nuclear parts of these cells are lenghtened, so that the nuclei are found distal to the neurular tube. Apical to the nuclei most of the cells contain small granules that stain with PAS. On the outside of the tube the apical parts of the epithelial cells become very low (Plate 20:88). The subnuclear parts of these cells are drawn out into thin but dense filaments, which extend down to the basement membrane. Often several of these cells join to form bundles and occasionally low placed nuclei can be seen in such bundles.

The neural tissue is embedded between the bundles of supporting cells. The basal parts of the nerve cord mainly contain longitudinal nerves, the nuclei of the nerve fibres being more or less concentrated in the outer part of the nerve cord (Plate 19:85). At least two types of nerve cells are present *viz.*, one with large and light nuclei, best visible in Plate 19:85, and another type with smaller and denser nuclei (Plate 20:88). Particularly the cell bodies with the light nuclei often form clusters.

Usually the "cuticle" is slightly thickened over the neurular tubes.

In the hind end of the trunk the nerve cord is still only about 100 μm broad and about 30 μm high (Plate 20:89). There are few supporting cells, the epidermal cells with granules covering the nerve bundles in the anterior part of the body, are lacking here, and very few nuclei of nerve cells are present. This indicates that the nervous tissue mainly consists of nerve fibres and contains very few ganglionic cells.

The brain

Histologically the brain is also a continuation of the nerve cord. As the whole brain complex grows in height and width the supporting fibres become increasingly longer and nuclei are more often accompanying the strands of supporting cells. The supporting fibres attain the shape of an irregular network when part of them become laterally orientated (Plate 19:82).

The very thick cuticle covering the ventral side of the brain is secreted by an epithelium-like layer of cells in which the nuclei are so closely packed that they form several rows (Plate 19:81). Below these nuclei thin supporting fibres extend towards the basement membrane, However, part of these outer nuclei may belong to nerve cells, the fibrous parts of which extend between the supporting fibres.

Most ganglionic cells are situated further away from the body surface. The nuclei of these cells form large masses (Plate 19:82). They are rather large and accompanied by scant cytoplasm only. In several places a lumen can be seen in the centre of the nests of ganglionic cell bodies. Such a lumen, which may be present in all groups of cells, usually is surrounded by a conspicuous membrane which can in no way be distinguished from the cuticle.

The basement membrane delimiting the brain from the surrounding muscular tissue is continuous with the basement membrane of the epidermis of the vestimental region, the obturacula and the tentacles, which proves that the brain is also completely intra-epidermal.

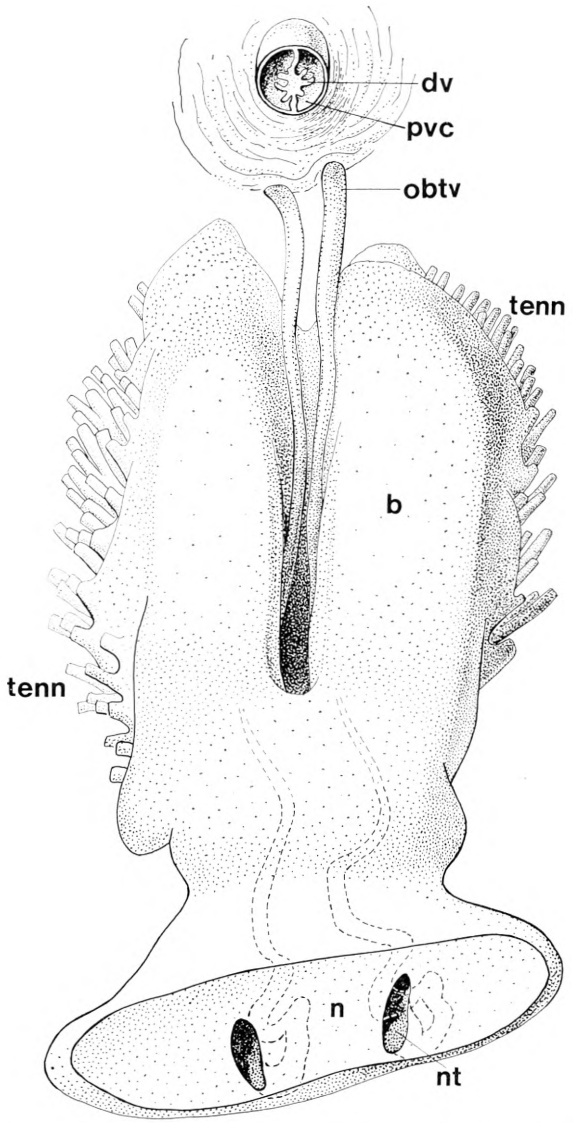

Fig. 15. Reconstruction (Lison method) of the brain in postero-dorsal view. b = brain, dv = dorsal vessel, n = nerve cord, nt = neurular tube, obtv = obturacular blood vessel, pvc = perivascular coelom, tenn = tentacular nerves.

Discussion

1) As far as we know *Lamellibrachia* is one of the largest animals in which the central nervous system seems to be wholly intra-epidermal. In Enteropneusta the nervous system is also intra-epidermal (Silén, 1950), but it has been shown by special techniques for neural elements and by electron microscopy that nerve fibres pass through the epidermal basement membrane (Dilly, 1969), at least in some species.

This is not the case in Pogonophora. GUPTA and LITTLE (1975: p. 55) concluded from an electron miscrocopical study of these animals that no nerve fibres pass through the epidermal basement membrane into the interior. We tried to stain sections of *Lamellibrachia* with the Bodian technique for nervous tissue, but without success. Therefore we could not prove with certainty that nerves pass the basement membrane, although a study of normally stained sections gave us the impression that this is the case (Plate 8:22). We must assume that the vestimental muscles and the feather muscles are innervated and we cannot see how signals could reach these muscles directly through the basement membrane as in Pogonophora.

2) At the front end of the body the double nerve cord fuses and at the same time swells to what may be called the brain. The obturacular coeloms and blood vessels, as well as the obturacular muscle traverse the middle of the brain, which consequently must be considered to be ring-shaped.

Probably this ring-shaped brain can best be compared with three different elements occurring in annelids, *viz.*, the dorsal brain or supraoesophageal ganglion, the oesophagial commissures and the suboesophageal ganglion. The anterior half of the brain is a lobed mass receiving numerous large nerves from the obturacula and the tentacles. This part is indeed comparable to the supraoesophageal ganglion of most annelids, which probably also mainly acts as a receptor and correlator of impulses from palps, antennae, eyes, tentacles and other frontal organs, and not as a co-ordinator of the nervous activities of the body (DALES, 1967: p. 133).

Among annelids there are several deviations from the normal situation. In hirudineans the suboesophageal ganglion is very large relative to the supraoesophagial one, and both are concentrations of segmental ganglia. In most myzostomids the ganglia of the ventral nerve cord are concentrated into a single elliptical mass, while the oesophagial commissures and the supraoesophagial ganglion are only weakly developed (PRENANT, 1959: p. 740). In Pogonophora the brain, *i.e.* the largest concentration of nervous elements, also has a ventral position, while the commissures and the dorsal ganglion are only represented by the "nerve ring of the protosoma" (IVANOV, 1963: p. 50; Fig. 51). The tentacular nerves arise from this ring. The anterior part of the central nervous system of sipunculids is of the normal annelid type (ÅKESSON, 1958: p. 70; HYMAN, 1959: p. 642; RICE, 1973: p. 14). However, in echiurids the three elements form a very wide ring without distinct swellings, which as a whole is considered to be the brain (BOCK, 1942: p. 47; DAWYDOFF, 1959: p. 863).

We may conclude that in Annelida *s.l.* the pre-oral brain often has a dorsal position, but that the "brain function" may be taken over by more posterior elements of the central nervous system, as is the case in *Lamellibrachia*. In our opinion the fact that the original dorsal ganglion has moved to the ventral side of the body is of lesser importance. Of course this remarkable phenomenon is related to the unique structure of the anterior part of the body and particularly to the absence of a mouth.

3) Within the brain mass globular spaces are found, which are filled with substances very similar to the cuticle. This situation can only be understood when we

assume that part of the epidermal cells were pushed away from the surface of the very thick epithelium. They retained their epidermic properties and secreted a cuticle of their own around enclosed spaces. In this view the cell bodies of the ganglionic cells of the brain are still situated in principle in the apical part of the epidermis, even if they have moved far into the brain tissue.

4) Along the greater part of the length of the vestimental region the ventral nerve cord is double, the two halves lying far apart, but in the rest of the body there is a single nerve cord. It is non-ganlionated, the nuclei of the nerve cells being distributed along its whole length. As far as we are aware this situation is unique but we do not want to attach too much importance to it.

Unpaired and non-ganglionated nerve cords are quite common. In the Annelida *s.l.* they do not only occur in aberrant groups like Pogonophora, Echiurida and Sipunculida, but also in polychaetes, *viz.* in *Arenicola* (Ashworth, 1904: p. 48). However, in these groups at least the anterior portion of the central nervous system is always double. With this in mind it is easier to understand the situation in *Lamellibrachia*. The double part is just unusually long.

Perhaps a comparison of the double part of the ventral nerve cord with the "nerve plate" in the anterior part of the trunk in Pogonophora is also justified, particularly because both apparently developed in connection with the ventral ciliary field. The sides of the "nerve plate" are formed by thicker bundles of nerve fibres (Ivanov, 1963: p. 54), which could be considered as the ventral nerve cord proper. The two separated halves of the nerve cord of *Lamellibrachia* have exactly the same position relative to the ciliary field.

5) In many animals, including most annelids, rapid contractions of the longitudinal muscles are controlled by giant fibres. They are well developed in tubeworms such as sabellids and serpulids (Dales, 1967: p. 111). In *Lamellibrachia* we could not find a trace of particularly large fibres.

6) The neurular tubes of the Vestimentifera are unique organs. Tube-like structures do occur in the nervous system of some other animals, but these can certainly not be considered homologous to the tubes of *Lamellibrachia*.

In some Enteropneusta a short tube is present in the collar region. However, this tube is lined with cells that have retained their epithelial character and secrete mucopolysaccharides into the lumen of the tube. Sometimes the tube is split up into a number of small separate holes. The nerve tubes of enteropneusts can well be compared with the groups of brain cells arranged around small holes, which have also retained their epithelial character but not with the neurular tubes.

In several echiurids there is a narrow canal in the centre of the fibrillar mass of the ventral nerve cord. It sometimes extends into the oesophageal commissures (Dawydoff, 1959: p. 865; fig. 683). It seems to be surrounded by a regular epithelium, but its precise structure and its function are unknown to us.

The cerebral tube of sipunculids is just a connection to the sea water in those species in which the brain has sunk away in a posterior direction from the anterior

body surface (Åkesson, 1958: p. 74; Hyman, 1959: p. 647). The ocular tubes, also occurring in several sipunculids, are tubular epidermal invaginations in the anterior part of the brain. They may contain refractive bodies and are usually considered to be eyes (Åkesson, 1958: p. 75; Hyman, 1959: p. 649). They are entirely different from the neurular tubes of *Lamellibrachia*.

The same holds for the cerebral organs of Nemertini, which are paired epidermal canals connected with the brain (Gibson, 1972: p. 64). They have a quite variable morphology, but they are always ciliated and always open to the exterior.

7) On p. 81 we describe how the neurular tubes could aid in forming a cavity in which the cilia of the ciliary field can move freely. However we do not suggest that keeping the ciliary field free from the inner wall of the tube is the only possible function of the neurular tubes. This does not explain why they are so closely connected with the nervous system and are also present in the trunk (In *L. barhami* the neurular tube seems to run much farther backwards than in *L. luymesi;* Webb, *in litt.*). It may well have a function in detecting external mechanical stimuli, particularly pressure of the body against the wall of the tube.

Excretory system

Morphology of the nephridia

A detailed description of the nephridia of *Lamellibrachia barhami* was given by Webb (1975). In general we can confirm the results of his studies and therefore we confine ourselves to some of the most important points.

The two nephridia are situated dorsal to the brain (Fig. 18). Each consists of two totally different parts, *viz.*, an excretory tree and an excretory duct (Fig. 16). The excretory trees consist of numerous fine tubules and form a single median mass, measuring somewhat more than 1 by 1 by 0.6 mm and lying closely applied to the dorsal surface of the brain and in part between the two lobes of the brain (Plate 18:80). The "stem" of an excretory tree enters the spacious excretory duct (Plate 18:79). This duct, which has a quite irregular shape, with several lobes, first runs backwards over a distance of nearly two mm. Then it bends sharply and runs in an anterior direction median and ventral to the first part. The excretory duct has a diameter varying between 200 and 400 μm in this region. After passing ventro-lateral to the heart (Plate 7:18) it bends to the median line, its diameter diminishing to about 100 μm, and joins the other duct to form a median common excretory duct (Plate 18:78). The nephropore has a terminal position dorsal to the bases of the obturacula. We can confirm Webb's observation that there is a concentration of circular muscle fibres around the distal parts of the excretory ducts and the proximal part of the common excretory duct.

The obturacular coelom with the obturacular blood vessels passes through the middle of the excretory trees before penetrating the brain (Fig. 16B). However, there is no connection between these vessels and the nephridia. The blood supply of the nephridia could not be studied in any detail. Webb (1975: p. 105) could give some

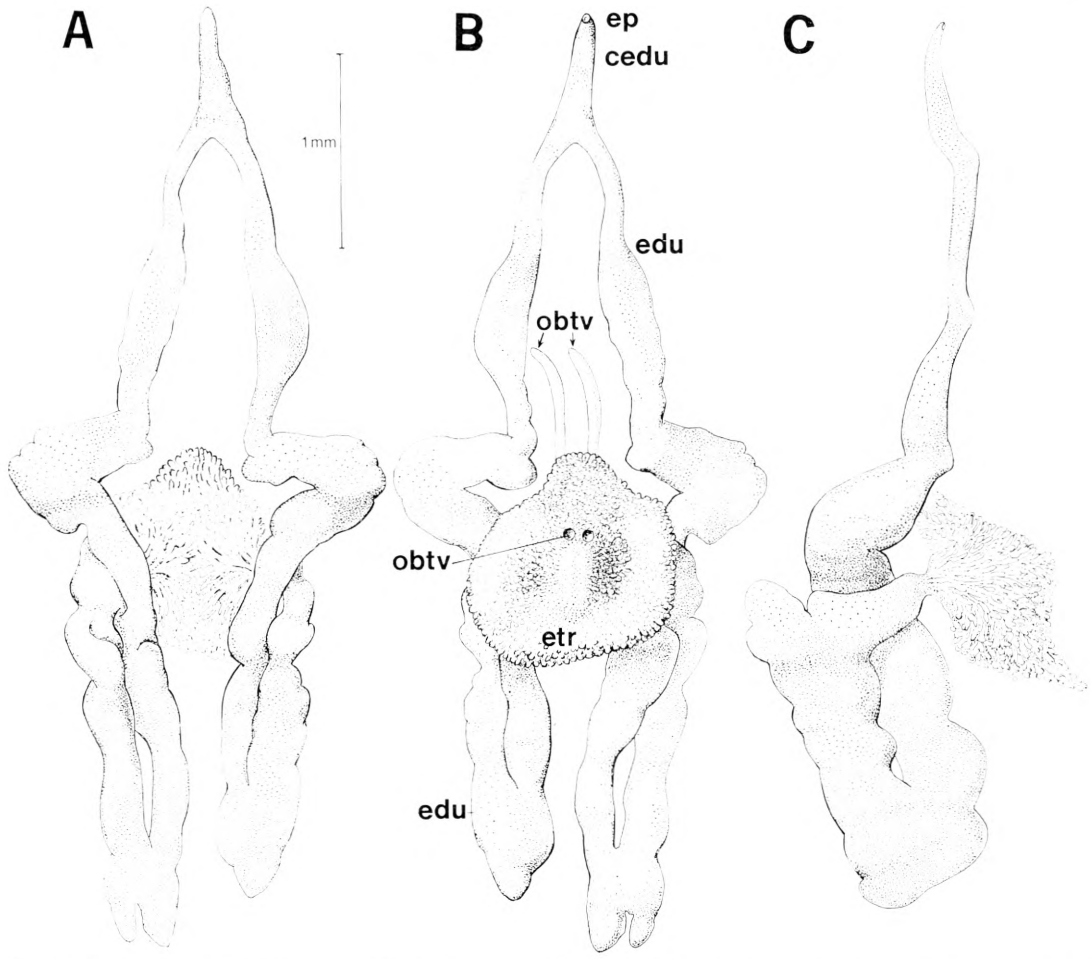

Fig. 16. Reconstruction of the nephridia in dorsal (A), ventral (B), and lateral (C) views. The course of the obturacular blood vessels, which on their way from the heart to the brain, penetrate the excretory tree, is indicated in fig. B. cedu = common excretory duct, edu = excretory duct, ep = excretory pore, etr = excretory tree, obtv = obturacular vessel.

information on this point because in one of his specimens some vessels in the area happened to be filled with blood. According to him one or two vessels originating from the point where the two ventro-lateral vessels meet to form the ventral vessel, run to the excretory tree. We found evidence of a vessel going from one of the ventro-lateral vessels, very close to the point where they join, to the excretory tree. In the bifurcation two vessels originate which almost certainly go to the excretory ducts.

Histology of the nephridia

Excretory tree

The tubules of the excretory tree vary considerably in diameter, from a few μm to about 20 μm where they enter the excretory duct (Plate 18:77, 80). The distal branches of the tree seem not to possess a distinct wall of their own, although some of them seem to be lined with a very thin epithelium, the nuclei of which are very flat and ovoid. So they show some resemblance to endothelial cells. In the larger tubules the wall consists of cells which are up to 2 μm high. The lumina of the tubules contain very long cilia, which are usually clogged to a central, indistinct mass. The length of the cilia could not be determined with certainty, but in some places it exceeds 75 μm. According to Webb (1975: p. 109) there were no cilia in the narrowest tubules in *L. barhami;* he suggests that these tubules might be intracellular.

Excretory duct

The transition from the excretory tubules to the excretory duct is quite abrupt (Plate 18:77, 79). In the duct the cells are set in long ridges which in cross sections appear as tufts (Plate 18:79). Accordingly the morphology of the cells varies considerably. Some cells are rather low and have rounded light nuclei, while those of the ridges are long and slender and have oblong, very dense nuclei. The low cells in the grooves between the ridges contain few scattered PAS-positive granules, while the apical parts of the ridge cells are literally studded with PAS-positive material.

There is a distinct basement membrane underlying the duct epithelium. It does not evaginate into the core of the ridges. At the base of the epithelium there may be large, empty spaces (Plate 18:79). It is not wholly clear whether these spaces are intra- or extra-cellular.

The epithelium is ciliated, but we could not determine with certainty if cilia are present on all cells. The long cilia stand out into the lumen of the duct which contains a slightly Alcian Blue-positive fluid and patches of PAS-positive material. Towards the confluence of the two ducts the height of the epithelium gradually diminishes. The wall of the common excretory duct has the same structure, but the ridges have become very low (Plate 18:78). This is in contrast to the situation in *L. barhami.* In that species the common excretory duct has a cuticular lining and consequently it must be considered an epidermal structure (Webb, 1975: p. 106; Figs. 6–8). In *L. luymesi* there is only an infolding of the epidermis forming the nephropore, but this does not go further inwards.

Other excretory organs

Throughout the whole length of the trunk the trophosomes contain enormous amounts of pigment granules. As was stated before (p. 28) we believe that the pigment cells contain stored waste products. Just like similar tissues in other animals the trophosomal tissue almost certainly is the site of several metabolic processes and as

such is a producer of waste products. Part of these are stored as inactive granules and part are presumably liberated into the blood or the coelomic fluid.

As described before (p. 18) cyst-like structures were found attached to the pyriform glands in certain areas (Plate 12:45) and the suggestion was made that they contain waste products, too. The pyriform glands could also act as excretory organs by secreting waste products with the tube material, an interesting suggestion put forward by GUPTA & LITTLE (1975: p. 47) in their description of tube formation in Pogonophora.

Discussion

1) Nephridia are often coelomoducts, *i.e.*, ducts leading from the coelom to the outside. The nephridia of *Lamellibrachia* can be called coelomoducts provided we assume that the tubules of the excretory tree are open to the coelom. The tubules and the coelomic spaces with which they should be connected are so thin that we cannot really prove that the coelomic fluid can enter the tubules. It looks as if the excretory tree is lying in solid tissue (Plate 18:76), but we believe that the vestimental musculature is derived from the coelomic epithelium and that the coelom in the anterior part of the body is split up into numerous fine channels. For this reason we assume that the excretory trees are multiple nephridiostomes draining the coelomic network of the area dorsal to the brain. There is no evidence that the excretory tubules are connected with blood vessels, which would be an alternative possibility of taking up waste products. The latter is undoubtedly the case with the excretory ducts.

2) Functionally the nephridia could be interpreted as follows. The tubules of the excretory tree drain the coelomic spaces between the muscle cells. By ciliary action the fluid is transported through the branched system towards the excretory ducts. In these ducts additional waste products are added to the fluid by secretion. At the same time water and perhaps also useful chemicals are resorbed by the epithelium. The empty spaces at the base of the epithelium may be an indication of fluid resorption from the lumen of the excretory ducts. The presence of PAS-positive granules in the epithelial cells may indicate resorption as well as secretion, but the latter is most likely to occur. The condensed fluid with excretory products is discharged through the excretory pore at the front end of the body, which means that it is discharged into the sea water outside the tube. The excretory ducts can be closed by the ring muscles around their distal parts and the proximal part of the common duct. This is a necessity when the animal is withdrawn in its tube.

3) Morphologically as well as physiologically the nephridia of the Vestimentifera are very similar to those of certain annelids. In annelids there are usually several pairs of nephridia, often one pair per segment, but in tube dwelling annelids there is a tendency to reduce the number of nephridia to a few pairs or even to a single pair (*e.g.*, in cirratulids, terebellids and sabellids; MEYER, 1887: p. 596; DALES, 1967: p. 32), situated close to the anterior end. In serpulids there is a single excretory pore just as in *Lamellibrachia*. The nephridia of Pogonophora were described by IVANOV (1963: p. 66) as "a pair of protosomal coelomoducts" and "epithelial ciliated canals". However,

this does not apply to *Siboglinum fiordicum* in which the excretory organs are in fact protonephidia belonging to a modified solenocyte type to be described (NØRREVANG, in prep.). In annelids protonephridia are common, sometimes occurring together with metanephidia in the same individual.

4) The excretory tree was called excretory gland by WEBB (1975) because he thinks the tubules are closed. We cannot deny this on the basis of our observations, but we are unable to see how this gland could work. In the first place the blood supply does not seem to be very important and in the second place the character of the ciliated epithelium does not suggest active secretion.

5) WEBB (1975) speculated on the fact that the excretory gland might link the two excretory ducts, because in this way the excretory gland could be considered as a homologue of the anastomosing duct of pogonophores. Indeed the two excretory trees (in our view) form a single mass so it is not easy to be decisive on this point.

6) The epidermal character of the common excretory duct in *L. barhami* may well represent a specific difference with *L. luymesi*.

Reproductive system

A detailed description of the reproductive system of *L. barhami* is given by WEBB (in press). However, the anatomy of our specimen differs considerably from the anatomy of WEBB's specimens and for this reason we present the results of our studies in some detail too. As mentioned before (p. 30) we think that our male specimen represents a further developmental stage and that this might explain most of the differences, although specific differences may be involved as well.

Morphology of the male reproductive system

When we consider each separate sperm producing unit a testis, our specimen has a great number of testes. Each testis is a branching complex of tubules (*tubuli testiculares*), lying closely applied to one or more trophosomal lobes (see diagram of Plate 6). The testes often lie over the outside of the trophosomal lobes so that they are partly exposed to the coelom, but the tubules may also creep between the lobes, as is the case with the tubules figured on Plate 20:87. Each testis has one pore opening to the coelom. This testicular pore may have a basal position, when the testis is more or less tree shaped, but it has a central position when the tubules run in different directions. The testes open to the coelom often in the close vicinity of a lateral pore in one of the sperm ducts. Although we have not seen sperm passing from a testis into a sperm duct in any of the sections, this situation might suggest that the sperm is preferably brought into the safety of the sperm duct and the sperm sacs immediately. We must admit however, that considerable amounts of maturing sperm are floating free in the coelomic fluid.

It is very difficult to count the intricate testes, but there are several of them on each of the trophosomes, even on the smallest ones, in the posterior part of the trunk.

Just to give an idea of how numerous they are we mention that in any cross section of the posterior part of the trunk in the order of 15 to 20 sections of testicular tubules may be found, belonging to about four different testes. In the anterior half of the trunk the number of cross sections of testicular tubules may be more than 40, representing about ten different testes. Therefore we estimate that there are several hundreds of them. As far as we know they are only lacking in the first trophosome, but we sectioned only part of it.

In each trophosome there are two longitudinal sperm ducts. Considering the fact that there are only two sperm ducts in WEBB's material of *L. barhami* (seminal channels in his terminology) we must assume that there were originally also only two sperm ducts in *L. luymesi* and that the situation found in our specimen was caused by degenerative processes (see discussion on p. 30). The sperm ducts are situated in the ventral part of the trophosomal complex (Plate 20:86), close to the ventral vessel. In the sections they can always be identified easily by the presence of a dorsal ciliated ridge, mushroom-shaped in cross section. Nearly always there is an open space between the ridge and the surrounding mass of sperm, when present (Plate 21:91). The sperm ducts of the posterior trophosomes all have a posterior and an anterior opening to the coelom, due to degeneration. In some places the wall between the sperm duct and the coelom has disappeared. Consequently only a ciliated ridge running over the outside of the trophosome is left, and even the ridge has disappeared sometimes. Cross sections of such areas show only one sperm duct. At regular intervals the sperm ducts communicate with eachother. The openings between the ducts vary considerably in size. In the anterior trophosome they may be up to 600 μm long. The anteriormost connection is so wide and so long (about 4 mm) that we can speak of a common sperm duct there (Plate 21:91). In *L. barhami* there is no common sperm duct, the anterior portions of the ducts being widely separated by trophosomal tissue (WEBB, in press).

Throughout the whole length of the trunk there are openings from the sperm ducts to the sperm sacs. Another type of openings in the walls of the sperm ducts, the lateral openings to the coelom, are restricted to the areas where testes occur. As mentioned before these pores often lie close to testicular pores.

To get some quantitative impression of the occurrence of the various types of openings in the sperm ducts we measured and counted them in an area with a length of 3.5 mm in the posterior part of the trunk (series no. 213). Here the right sperm duct was complete, but the left duct had no wall over a distance of 2.2 mm. In this area it could partly be recognized as a furrow in the trophosome and for a still greater part by the presence of the ciliated ridge on the outside of the trophosome. The latter was only lacking over a distance of about 1 mm. There were two openings between the two ducts. In the complete right duct there were seven openings leading to sperm sacs and also seven openings to the coelom, five of which were situated close to a testicular pore.

In the anterior parts of the sperm ducts the connections are rather numerous. In the anteriormost 8 mm (posterior to the common sperm duct) there were twelve smaller or larger openings between the two ducts.

The sperm sacs (*vesiculae seminales*) make up a considerable proportion of the volume of the trunk. They are thin-walled sacs solely acting as sperm reservoirs. They are least conspicuous in the posterior part of the trunk, where they partly contain developing sperm (Plate 5:16) or where the sperm is less closely packed. Moreover, they are branching through the whole trophosome. In sections of the anterior part of the trunk they appear as large black spots (Plate 5:14, 15), because there they are very densely packed with numerous bundles of mature sperm. To the naked eye the sperm sacs as well as the sperm ducts have a white colour (Plates 2:4; 3:9; 13:50). Not all sperm sacs have a direct connection with the sperm ducts. Most often they are arranged in bundles and have a common duct leading to one of the sperm ducts. Apparently they originated as branched tubules, derived from the wall of the sperm duct.

Not all sperm is stored in the sperm sacs. A considerable part is floating free in the coelomic fluid, not only during maturation (Plate 21:95) but also as full grown bundles of mature sperm (Plate 21:94). Particularly the middle region of the trunk contains an enormous amount of free floating sperm. In the greater part of the trunk the sperm ducts probably do not play an important role any more in the transportation of sperm. We assume that the sperm will be transported here by means of peristaltic movements of the body wall rather than by means of ciliary action in the narrow sperm ducts. However, it is evident that all sperm must pass through the anterior sperm ducts to reach the outside.

In the anterior part of the common sperm duct the ciliated ridges broaden to form lateral ciliated cushions in the area where two ducts lead to the body wall (Plate 21:92). These two ducts (seminal ducts in WEBB's terminology), which are ciliated all around (Plate 21:96), open to the outside in the posterior ends of the vestimental grooves (to be described later; p. 76).

Histology of the male reproductive system

Testes

Apparently the testicular tubules are rather delicate organs for which the fixation procedures were not quite adequate. The cells of the epithelium look irregular and are considerably vacuolated (Plate 20:87), which is probably an artifact.

As explained above the testicular tubules share at least part of their walls with the trophosomal lobes. Here the epithelium is often very thin, in the order of 1 to 2 μm. The cells are flat and cover considerable areas. In some places the basement membrane is only visible with the highest magnifications. Trophosomal cells are standing on the other side of this basement membrane.

In the normal parts of the tubules the epithelium is usually about 15 μm thick. The cells are almost cuboidal and their nuclei are rounded and about 5 μm in diameter. Some nuclei are larger than the others, up to 10 μm in diameter. It is not unlikely that they belong to the initial stages in spermatogenesis. It is evident that at least part of the walls of the testicular tubules represents the germinal epithelium.

Occasionally blood vessels were noted in the basement membrane of the tubules. Spermatozoa in different early stages of spermatogenesis are usually present in the lumen (Plate 20:87).

Sperm ducts

The fine structure of the sperm ducts in the posterior part of the trunk could not easily be studied because of inadequate fixation. In general the epithelium consists of cuboidal cells, 10 to 15 μm high. The nuclei are rounded and about 3 to 4 μm in diameter. They are moderately dense. Finer details of the cytoplasm are not preserved. Part of the epithelium shares its basement membrane with the wall of the ventral vessel (Plate 20:86), although in some areas the sperm duct seems to be separated from the vessel by muscular elements. The rest of the ducts is probably always embedded in trophosomal tissue, although this may be present only as a thin layer in places where the ducts bulge out into the coelom. The connections with the coelom cannot easily be interpreted from a histological point of view. Probably this is not only due to bad fixation but also to degenerative processes which obscure the situation.

The ciliated ridge (*costa ciliata*) has a quite variable shape is cross section, but it always has a relatively narrow stem. The core of the ridge consists of a lamellar extension of the basement membrane, in which a blood vessel can often be seen. The ridge cells are very tall, up to 30 μm. Their nuclei are darker than those of the normal epithelial cells of the sperm ducts. They are situated in the basal parts of the cells (Plate 21:91). All cells of the "hood" carry long cilia, but not those of the "stem".

In the anterior part of the common sperm duct the ciliated ridges are flattened and broadened, covering most of the lateral sides of the duct. Here the ciliated cells become very tall and slender, measuring 30 to 60 μm in height and only 2 to 3 μm in diameter (Plate 21:92). The nuclei are invariably situated in the basal parts. The apical parts of the cells stain with eosin and many of them contain PAS-positive granules. Each cell carries many cilia, which may be up to 25 μm long, although most of them are about 15 μm long. In the blind ending part of the common sperm duct in front of the place where the two ducts run to the gonopores, the epithelium is slightly folded. The cells are much lighter here, and they are lower and less slender. Very little detail is seen in the cytoplasm. The ciliated areas are here lying on cushions of mesenchymatous tissue with some blood lacunae. The basement membrane, to be identified by its staining with PAS, lies below this cushion, so the tissue apparently belongs to the epithelium of the sperm duct.

In the ducts leading to the body surface (Plate 21:92, 96) the epithelium is very uniform. The cells are about 30 to 35 μm high and each of them is provided with cilia, which have a length of 20 to 25 μm. The nuclei are situated in the basal half of the cells; around and below the nuclei there are numerous PAS-positive granules. The cytoplasm of the apical half of the cells is striated, suggesting the presence of numerous ciliary rootlets which extend almost down to the level of the nuclei. There is a strong, PAS-positive basement membrane. Between the ciliated epithelium and the

basement membrane there is a number of cells with tangentially orientated nuclei. Some of these cells seem to be muscular, others are vacuolated. The vacuolated cells seem to be concentrated approximately halfway between the common sperm duct and the gonopore. The inner parts of the ducts show transitions to the epithelium of the ciliated areas of the common sperm duct. Here the epithelium is thrown into folds (Plate 21:92). Close to the gonopore the epithelium shows transitions to the ciliated epithelium of the vestimental grooves (see p. 78).

Sperm sacs

The walls of the sperm sacs are very thin even though they consist of the sperm sac epithelium, the basement membrane and the coelomic epithelium covering the sperm sacs. The epithelium covering the inside of the sacs is never more than 2 or 3 μm high. When fully extended the walls of the sperm sacs can be seen only as thin lines, even with high magnifications. Very often the basement membrane is studded with blood vessels. In our specimens all sperm sacs were filled with sperm. In earlier stages, when they are still empty or contain little sperm, the walls are likely to be much thicker.

Spermatogenesis

The first stages of spermatogenesis could not be studied properly in the sections. The first stages to be observed consist of clumps of spermatids, which are about 10 μm long and about 4 μm broad (Plate 20:87). Their cytoplasm stains rather dark and slightly metachromatic with Toluidine Blue. The nuclei are moderately dense and about 3 μm in diameter.

In the following stage the cells have attained the shape of drops, the pointed ends of which meet centrally in the cell mass (see Plate 21:95 in which several of the following stages are represented as well). Next, the cells dispose of most of their cytoplasm which is secreted centripetally to form a central acellular mass (*cytophorus*), the staining properties of which are identical with those of the cytoplasm of the earlier stages. At the same time the nuclei become darker. The cells remain connected to the cytophore by thin stalks.

The nuclei seem to be completely devoid of cytoplasm now and gradually they elongate until a final length of about 25 μm is attained. At this final stage the nuclei have a diameter of only about 1 μm. When the nuclei have reached a length of about 8 μm they are spindle shaped and a flagellum arises at their peripheral ends. Soon after that, when having reached a length of approximately 10 μm, the nuclei begin to twist around their longitudinal axis. Finally the spermatozoa are about 100 μm long, 25 μm of which represent the twisted nucleus (see scanning electron micrograph Plate 21:94). The twisted part of the sperm stains heavily with Feulgen.

The mature spermatozoa are invariably arranged in bundles. The number of spermatozoa per bundle is in the order of 100. Theoretically one could expect this number to be 128 when each bundle originates from one cytophore, which is probably

the case. These bundles have a diameter of 10 to 15 μm in their middle and they taper towards the anterior and posterior ends. Even in the duct leading to the gonopore the bundles can still be recognized (Plate 21:96), although they tend to become looser. Separate spermatozoa were also observed in these ducts. Apparently the spermatozoa become free when they are discharged.

Sperm resorption

A considerable proportion of the sperm lies free in the coelom. In many sections sperm in the coelom seems to be disintegrating, being engulfed and digested by coelomocytes (see also p. 25). We got the impression that mostly only mature sperm is attacked by coelomocytes, while the earlier stages are less apt to be resorbed. Bundles of disintegrating sperm are commonly attached to the trophosome and the ventral mesentery (Plate 20:86). Apparently the sperm is not only resorbed by coelomocytes but also by special cells of the coelomic epithelium.

Morphology of the female reproductive system

Webb (in press) prepared a detailed description of the female reproductive system of *L. barhami*, including many interesting observations. He kindly put his manuscript at our disposal, thus enabling us to mention here those details that we think to be most relevant for discussions on the comparative anatomy of the Vestimentifera and to prepare a simple diagram of the female system (Fig. 17C).

The ovaries are long tubular organs, attached to the ventral vessel and the mesentery dorsal to that vessel. The right ovary is very short and obviously non-functional, while the left ovary is very long, extending from the anterior part of the trunk over one third to half of its total length. Germinal epithelia are present in the ventral walls of both ovaries, over most of their length. In some places there are perforations in the mesentery which connect the two ovaries. In the right ovary great numbers of eggs are being produced. They are round and ultimately measure up to 100 μm in diameter. The ovary is basically tubular but depending on the number of eggs present it may widen considerably to form egg sacs.

The eggs are transported anteriorly through a ciliated oviduct, which runs along nearly the whole length of the ovary and which is attached to its outer wall. At regular intervals the ovary and the oviduct are connected by perforations in their common wall. The functional oviduct opens into a spacious ovisac situated close to the anterior end of the trunk coelom. The ovisac is connected with the gonopore by a short duct. Both ovisac and duct are non-ciliated. The non-functional oviduct, which opens directly to the outside, is without cilia.

The female gonopores have the same position as those of the male, but they are lying in a short groove without cilia.

As far as we know the female reproductive organs are closed, *i.e.*, there are no connections with the coelom.

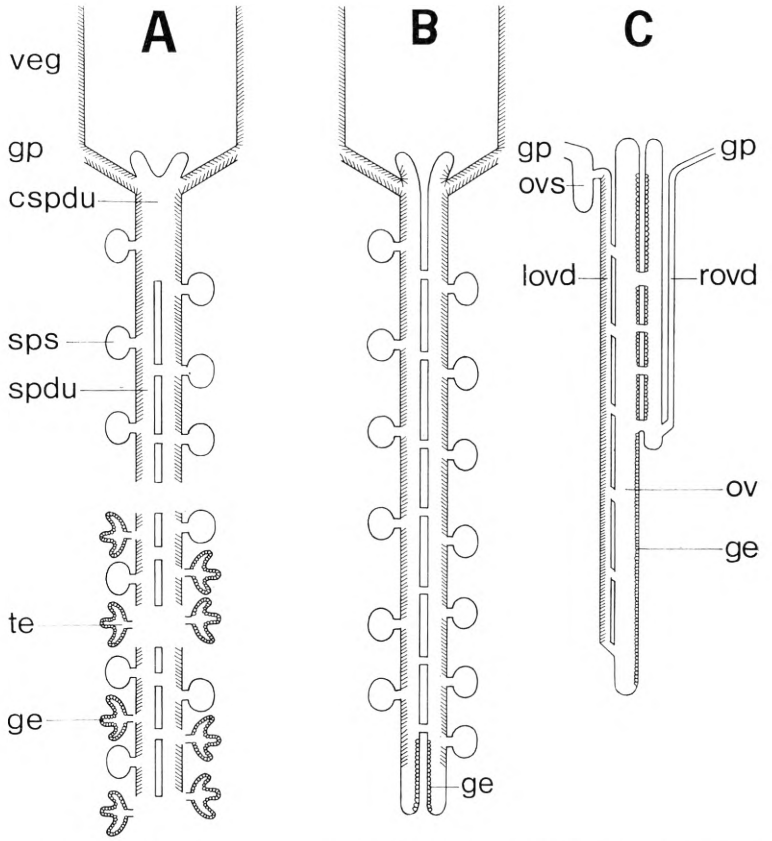

Fig. 17. Diagrams of the reproductive systems of the male *L. luymesi* (A), the male *L. barhami* (B), and the female *L. barhami* (C). Figs B and C are based on the descriptions by Webb (in press). cspdu = common sperm duct ge = germinal epithelium, gp = gonopore, lovd = left oviduct (functional), rovd = right oviduct (non-functional), ov = ovarium, ovs = ovisac, spdu = sperm duct, sps = sperm sac, te = testis, veg = vestimental groove.

Discussion

1) From the above description we could conclude that the sperm is produced by the coelomic epithelium, which is locally differentiated into tubular structures with an inner germinal epithelium, and that the sperm is transported to the outside by means of a single pair of coelomoducts. Basically this is a rather normal situation occurring in many animals, including annelids. The most unusual aspect is the fact that the sperm does not (or not only) enter the sperm duct by the original "nephrostome" (a funnel-like structure in many animals), but by lateral openings which often lie close to testicular pores. Perhaps this can be explained as an adaptation to the extreme length of the sperm producing area, which has to be "drained" by a single pair of gonoducts. At a later stage (as represented by our specimen; see diagram of Fig. 17A) the gonoducts are in fact considerably shortened (by degeneration) and the sperm is transported mainly through the coelom. However, it can be imagined that it is far more efficient

to transport the sperm through the very long sperm ducts at an earlier stage (as represented by WEBB's specimens), when the coelom is still filled with trophosomic tissue for a large part and the sperm is only produced in the posteriormost portion of the trunk.

However, we have to consider a different explanation of the whole system. WEBB's observations (WEBB, in press) suggest that there are no separate testes but that the germinal epithelium is located in the posterior parts of the sperm ducts, *viz.*, in the last 6 to 7 mm of each sperm duct (diagram 17B). It is unlikely that the two species differ so fundamentally in the structure of the reproductive organs. Therefore we have to try to bridge the gap between the two descriptions by explaining the differences as the result of developmental processes. We could imagine that the testes evolve as pockets in the lateral walls of the sperm ducts and that they gradually grow into the branched structures to be observed in our specimen. The connections between the sperm ducts and the testes should have been broken in the course of this development. This could explain why many of the testicular pores are still situated close to openings in the sperm ducts. It is evident that we have to accept a completely different basic structure of the male reproductive organs when this theory might prove to be correct. They must then be described as closed tubular structures which originally had no connection with the coelom. The situation in the female also fits best in the last mentioned theory.

2) In many animals of many different phyla the sperm cells are arranged in clusters in which the cell divisions are synchronized during the early stages of sperm development. However, the arrangement of maturing spermatids around a common acellular mass, the socalled cytophore (or blastophore), as described above for *Lamellibrachia*, is restricted to a few groups. As far as we are aware they are only known from some flatworms, Annelida, including oligochaetes, hirudineans, polychaetes, and pogonophorans, and from ectoproct bryozoans (Phylactolaemata).

GUPTA & LITTLE (1975: p. 58) consider it a character of systematic significance.

3) The mature spermatozoa are very similar to the spermatozoa of *Siboglinum* (Pogonophora) as described by FRANZÉN (1973). In the spermatozoon of *Siboglinum* an acrosomal region can be distinguished, followed by a nuclear region in which the nucleus is accompanied by a spirally wound mitochondrion, and, posteriorly, a tail region. In *Lamellibrachia*, likewise, there is no separate midpiece, and the nucleus is spirally wound, a fact that can easily be seen in Feulgen-stained sections as well as in the scanning electron micrographs (Plate 21:94). The length of acrosomal, nuclear and tail regions in *Lamellibrachia* are, respectively, 15, 25, and 60 μm.

We know that the enormous variation of spermatozoan structures in the whole animal kingdom has a great deal of functional aspects. Consequently it is not easy to use similarities in the structure of spermatozoa in systematic discussions. Nevertheless we want to stress the high degree of similarity between the spermatozoa of *Siboglinum* and *Lamellibrachia*, at the same time admitting that a similar regionation occurs in the sperm of certain polychaetes (FRANZÉN, 1956).

4) The filiform sperm may indicate that they are not simply discharged into the sea water, because in that case one would rather expect the sperm to be of the primitive type with the round head. Other aspects of the reproductive behaviour are discussed on p. 90.

5) The absence of a common sperm duct in the anterior part of the trunk in *L. barhami* may well be a specific character of this species.

Obturacula

Morphology

The obturacula are a pair of anterior terminal organs which are fused by the cuticle along the greater part of their length and implanted on the front end of the body. Together they form a trumpet-shaped structure. Anteriorly it is nearly circular with recurved edges (Plates 1:1–2; 2:3). Tapering towards the low and very narrow base (Fig. 18; Plate 7:18) it is gradually compressed laterally. In the fixed specimen its total length from the anterior tip to the brain is approximately 15 mm. At the anterior end it is about 10 mm high and about 8 mm wide. This relatively large anterior surface is due to the outfolded anterior edges of the obturacula and to the fact that the anterior halves move away from eachother. In the middle they are only 1 mm thick.

The conspicuous, ivory white structure makes a rigid impression, but in the living animal it is undoubtedly somewhat flexible. It is evidently a protective device, serving to close the entrance of the tube when the animal has retracted. The internal diameter of the front end of the tube is only about 8 mm, which means that the edges of the obturacula must be pressed against the wall when the animal retracts into the tube.

Functionally the two obturacula undoubtedly form a single unit and they are fused for a considerable part of their length, but anatomically the two organs are completely separate. A double cuticle (Plate 22:103) is present between them down to their bases on the anterior surface of the brain (Plate 7:18). It is quite remarkable that the cuticle is not only responsible for the formation of a single "operculum" out of two organs, but that it also serves to attach these organs to the body (Fig. 18). There is only a small, narrow slit at the base of each obturaculum by which the inner tissue is connected to the body. In fact this slit is so narrow (only about 100 μ wide) that only the obturacular nerve and the obturacular blood vessel with its surrounding coelomic channel can pass through and these elements can hardly serve as an attachment to the body proper. Nevertheless the obturacula are firmly attached to the body, in the first place because their cuticles are attached to the cuticles of the surrounding series of tentacles (Plate 23:108) and in the second place because their cuticles are attached to a thick cuticular plate (Fig. 18; Plate 19:81). This cuticular plate covers part of the brain and could be considered to have protective function, but is is more likely that it serves as an attachment structure for the obturacula. A single cuticular ridge extending over the ventral surfaces of the obturacula is also attached to this plate. A strong

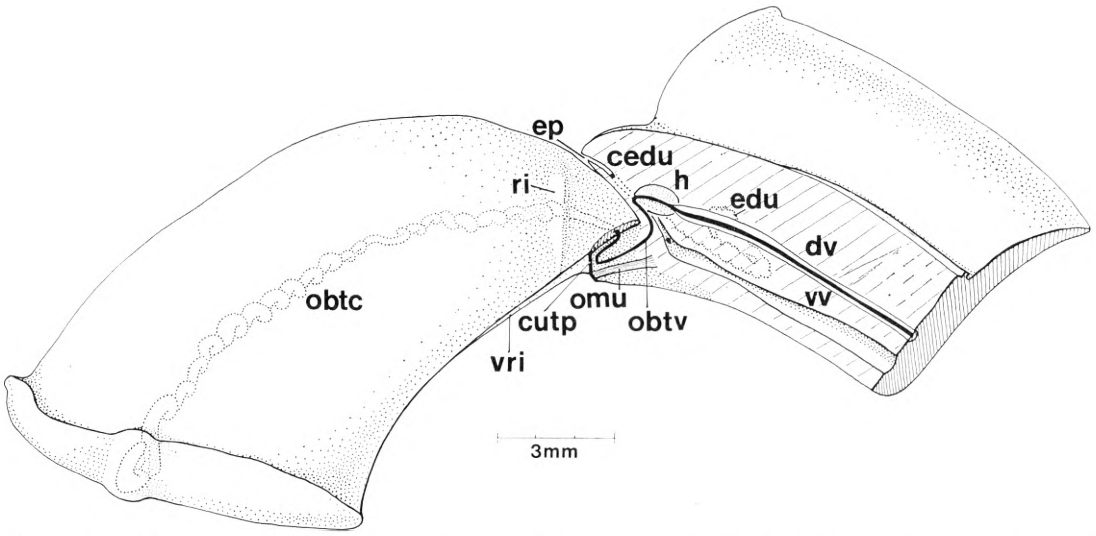

Fig. 18. The left obturaculum and a medial section of the front part of the body to show its connections to the body. The tentacles are not shown (compare Plate 2:3). cedu = common excretory duct, cutp = cuticular plate, dv = dorsal vessel, edu = excretory duct, ep = excretory pore, h = heart, obtc = obturacular coelom, obtv = obturacular vessel, omu = obturacular muscle, ri = lateral ridge on the basal part of the obturaculum, vri = medioventral cuticular ridge, common to both obturacula, vv = ventral vessel.

muscle, which runs through the central hole in the brain (Plate 19:82) is attached to the posterior end of the cuticular plate. The only function of this muscle that we can imagine is that it serves to move the obturacula and for this reason we called it obturacular muscle. We suppose that it can force the obturacula in a ventral direction, *i.e.*, in the position which it had in our specimen when fixed (Plate 2:3).

The obturacula are filled with parenchymatous tissue, consisting of connective tissue cells, muscle cells and a very extensive intercellular matrix. The basal portions of the obturacula are rather massive. They are penetrated by the obturacular coeloms, which roughly run in a longitudinal direction. Each of these coelomic channels is divided into two lateral halves by a mesentery-like structure in which the obturacular blood vessel is situated (Plate 22:99–101). The obturacular coelom originates from the anterior part of the heart, directly turning backwards and then in a ventral direction, through the hole in the brain, towards the body wall close to the cuticular plate (Fig. 18; Plate 19:81). Near the base of the obturaculum it bends again in a dorsal direction and then enters the obturaculum approximately in the middle of the basal slit. This intricate course can also be traced in Fig. 10. Of course it is highly dependent on the relative position of the different organs involved.

The obturacular coeloms are only narrow channels at first, but in the basal parts of the obturacula they gradually widen (Plate 23:108), soon to become very wide and coiled (Fig. 18; Plate 22:99–101). This area is even visible from the outside because it is bulging out medially (Plate 2:3).

In each of the obturacula there is only one major blood vessel, lying in the mesentery of the obturacular coelom along its whole length. Distally this vessel, which was empty in our specimen, gradually enlarges and ends blindly close to the edge. We could not find minor blood vessels branching off to the paranchyma, but it is evident that there must at least be some blood supply. When fully extended the obturacular blood vessels may occupy the greater part of the obturacular coeloms. Then they contain a considerable amount of blood.

The anterior edges of the obturacula, which turn backwards, become very thin and flexible. The internal tissue is very loose here and shows large cavities in places (Plate 22:97) and the cuticle gradually becomes very thin, particularly on the median sides of the organs.

On the adaxial side the basal part of each obturaculum is provided with a conspicuous dorsoventral ridge. This ridge is situated approximately at the level where the tentacles of each series fuse completely (Fig. 18; Plate 18:78).

Histology

Epidermis and cuticle

In comparison with the epidermis of the rest of the body the epidermal epithelium of the obturacula is modified in several respects. First of all, there is a very thick cuticle covering the organs completely, with the exception of the terminal edges. At the base of the organs the cuticle is about 5 μm thick, but already 2 mm away from the base the cuticle has reached a thickness of about 15 μm. About 7 mm away from the base the two organs separate. Just below this level, the cuticles can be seen to be interconnected by irregular cuticular bridges. Here the cuticle is thickest, measuring up to 70 μm. Towards the distal edges it gradually becomes thinner again. The outer cuticle of each obturaculum is fused with the cuticles of the inner tentacles, which are approximately equally thick, although there is a considerable variation in the thickness of the cuticles in this area (Plates 7:18; 23:108).

The cuticle is not uniform in appearance. There are minor irregularities which will not be treated in detail. A general trait seems to be a striation parallel to the surface. It is especially conspicuous in the area where the cuticles of the two organs are fused. Here about 75 bands can be discerned. Elsewhere the banding is more irregular, but wherever it was possible to count the number of bands there were about 70 of them, except close to the distal edges. Perhaps these bands reflect some physiological periodicity, which might be corresponding with a periodicity in the environment.

In those areas which are in contact with the tentacles there are many blebs in the cuticle, the significance of which is unknown. In the fused cuticles there are incarvings, in which processes of the underlying epidermal cells are extending. They might represent stretch receptors. The incarvings are particularly large in the incurved parts close to the tip of the obturacula and they contain specialized cells (Plate 22:102). Probably the cuticle is adapted here to being curved in the way shown in Plate 22:97, the cuticle being able to extend and be compressed.

The epidermis of the obturacula is one-layered (Plate 8:19). The nuclei are situated in the middle portion of the cells and form a row parallel to the surface of the epithelium. Its height varies from about 20 μm at the base of the obturacula to about 75 μm on the medial surfaces halfway to the distal edges. One type of cell is prevailing. It is only a few μm thick and reaches from the basement membrane to the cuticle. Its nucleus is spindle shaped, about 10 μm long and 1 to 2 μm thick. The cytoplasm is differentiated into a distal granulated region and a proximal muscular region. In fact practically all epidermal cells make the impression of being epithelial muscle cells. The granules of the outer parts of the cells stain orthochromatically with Toluidine Blue and deeply red with PAS. The staining properties of the granules are thus identical with those of the cuticle, so they may be regarded as precursors of the cuticle. Actual release of material from the epidermal cells to the cuticle could not be seen in the sections.

The muscular portions of the epidermal cells are orientated at a right angle to the muscle fibres inside the basement membrane (see below).

At regular intervals strands of fibres occur in the epithelium just below the nuclear region (Plate 8:19). These fibre bundles run among the epithelial cells for long distances. They may extend from the base of the obturacula to the distal edges, but this could not be ascertained. Occasionally a nucleus was seen in these fibre bundles. Evidently they constitute part of the intraepidermal nerve net. One would expect these nerve fibres to be located close to the basement membrane, as it is the case elsewhere, but obviously the presence of muscular elements caused a shift of the nerve bundles.

In the basal parts of the obturacula there are many vacuole-like cavities in the epithelium (Plate 22:103). Between two vacuoles the epidermal cells are very narrow, widening again above and below them. It is not impossible that these cavities are artifacts. Similar cavities occur elsewhere in the epithelium of the obturacula, but they are less numerous there. It may be noted that the cavities in the cuticle, mentioned before, are always found in contact with vacuoles in the epidermis. The cavities in the incurved apical part of the obturaculum always originate between the epidermal cells (Plate 22:102). A short neck is followed by a widening with a diameter of up to 30 μm. Very small cells, 3 to 4 μm in diameter, with a comparitively large nucleus can be seen in these cavities. Their cytoplasm stains slightly with PAS and eosin. They seem to be amoeboid and they may belong to a macrophage system. They are also numerous in the cavities in the distal edge of the obturaculum.

The basement membrane of the epidermis is very indistinct. This may be due to the fact that muscle and connective tissue fibres in the parenchyma are inserted on the basement membrane. The muscle fibres of the epidermal cells seem to be continuous with subepidermal fibre strands in the obturaculum, as if they are crossing the basement membrane (Plate 8:19). This curious fact can only be explained after a study of the ultrastructure of the epithelium.

Connective tissue

The greater part of the parenchyma of the obturacula consists of connective tissue, the bulk of which consists of an intercellular substance, which stains rather heavily with Alcian Blue and metachromatically with Toluidine Blue. This indicates that the intercellular matrix contains large amounts of acid mucopolysaccharides, while a slight staining with PAS indicates that neutral mucopolysaccharides and/or mucoproteins are also present. The amount of intercellular substance relative to the number of cells gradually increases from base to tip (compare Plates 18:78; 22:97–98).

The connective tissue cells are very long and slender. Cells with a length of up to 250 μm could be measured. They may even extend from the inner to the outer epidermis, but this could not be established with certainty. Two or several cells may meet by stout processes or they may be apposed for several μm. Besides, the cells send out slender processes into the matrix. It could not be determined whether these processes, which may be less than 0.5 μm thick, end blindly or make contact with other cells. The nuclei are about 5 μm long and about 3 μm thick. They stain rather densely and are always located close to a very light "vacuole" in the widest part of the cell, the cell "body". The cytoplasm stains rather heavily with PAS, but only slightly with eosin and metachromatically with Toluidine Blue. In the long and slender polar processes, which constitute the greater part of the cells, very fine granules occur with a diameter of about 0.5 μm. They stain heavily with PAS and may be organized in long beaded strands in which the individual granules can only just be distinguished. Sometimes there is a large PAS-positive mass in the cell body. There is much PAS-positive material in the cells when the matrix reacts only slightly, and conversely. However, it could not be determined whether the PAS-positive substance is given off to or taken up from the matrix by the connective tissue cells.

Two or several connective tissue cells may be found in bundles, but most of the cells lie separately in the matrix. In part of the parenchyma the slender portions of the cells were wavy, indicating that they were not fully extended (Plate 23:106). Elsewhere they may be stretched (Plate 23:105). Close to the epidermis the regular parallel organization of the connective tissue cells, perpendicular to the surface, becomes less distinct. Here the cells are more irregular and their nuclei are less dense, approaching those of the muscle cells in density and form. It is quite possible that the muscle cells and the connective tissue cells are derived from the the same mesodermal cells and that intermediate types occur.

The parenchyma shows a different organization around the coelomic cavities (Plate 22:99–101). The cells have a more irregular shape and the processes tend to be more wavy as if they are not extended to their full length. The parenchyma is very loosely organized in certain areas. This is particularly the case in the areas around the coiled part of the obturacular coelom, but also in the recurved distal edge (Plate 22:97).

Muscle cells

There is a layer of longitudinal muscle cells beneath the basement membrane, which is rather thick on the outside of the obturacula and rather thin under the medial surfaces. The cells are about 2 μm thick along their whole length, which may exceed 250 μm. The cytoplasm stains rather heavily with eosin and slightly with PAS. The nucleus is flat, 7 μm by 5 μm, and closely applied to one side of the muscle fibre, which is of the smooth type.

The muscle fibres are organized in sheets perpendicular to the basement membrane. Between these sheets the connective tissue cells reach the basement membrane.

In the basal parts of the obturacula there is a layer of closely applied tubular spaces between the basement membrane and the longitudinal muscles (Plate 22:103). These tubes, which run forward on the median sides of the obturacula, perhaps posses a cellular lining. It is not clear whether they represent coelomic spaces or blood vessels or lacunae.

Coeloms

Each of the obturacular coeloms is lined with an extremely thin epithelium, which can only be distinguished in oblique sections. The nuclei are very flat, measuring about 8 μm by 6 μm by 1 μm. They lie so close that they suggest the presence of a complete epithelioid lining. No basement membrane can be discerned, not even in the mesentery where the two halves of the coelom meet. Nevertheless, there must be a basement membrane, because the elastica of the obturacular vessel, which is situated in the mesentery, is actually a very thick basement membrane. Around the blood vessels the coelomic epithelium is modified into a strong muscular layer. In general the contents of the obturacular coeloms are invisible in the sections. In certain places they contain some fibrous or fluffy substance, which could not be identified. It stains with PAS as well as with Alcian Blue, indicating a high content of mucopolysaccharides.

Blood vessels

The wall of the obturacular vessels is very strong. In the apical part of the obturaculum the elastica is about 5 μm thick. An endothelium is present. On the outside the coelomic epithelium is differentiated into a strong muscular layer. In the basal parts of the obturacula the elastica and the muscular layer are not so well developed.

Discussion

1) We did not follow Webb (1969a) in using the term lophophoral organ. In the first place it is not a single organ but a paired structure. Secondly it can in no way be compared with the lophophoral organs of Phoronida, which are accessory sexual glands of the males. In the third place the name is inappropriate because *Lamellibrachia* has no lophophore.

2) The pair of obturacula obviously acts as an operculum by which the tube can be closed. The strong obturacula can easily prevent the entrance of predators parti-

cularly because the edges must be pressed firmly against the wall of the tube when the animal retracts further down into the tube, as was the case in our specimen.

3) It is not easy to compare the obturacula with opercula of other tube worms from a morphological point of view. In serpulids one of the filaments of the tentacular crown has been transformed into an operculum. Because of their different structure and their completely separate and unique connection with the body we cannot consider the obturacula to be transformed tentacles.

In sabellariid polychaetes the prostomium as well as the first and second segments contribute to the formation of the operculum (DALES, 1952: p. 450). This type of operculum is comparable to the "operculum" of *Lamellibrachia* in that it is at least partly formed by the fusion of lateral elements and that it is basically a paired structure.

4) The function of the single, blind ending, coiled blood vessel, which may contain considerable amounts of blood, may be hydrostatic in the first place. If blood is pumped into the vessel and the coelomic fluid does not escape at the same time, the central part of the obturaculum will bulge out more and more. Consequently the two obturacula will be pressed away from eachother. Indeed the blood cannot press away the coelomic fluid because the heart functions as a sphincter for the blood vessels as well as for the coelomic channels. The wavy appearance of part of the connective tissue cells indicates that they can be stretched further, which means that the volume of the obturacula could increase somewhat.

It is not clear for which purpose the obturacula should be pressed away from eachother. When the animal is in its tube the obturacula can be pressed against the wall in this way, but it is unlikely that this is the primary function of the coiled coelom. In the first place the obturacula are already passively pressed against the wall of the tube during retraction, simply because of their size relative to the diameter of the tube. Secondly the blood circulation cannot be stopped during the long periods over which the tube is likely to be closed now and then. Later (p. 88) we shall speculate on the possible use of the pair of obturacula as an anchor. This function could indeed only be performed when the obturacula are standing out laterally.

In the operculum of the serpulid *Pomatoceros triqueter* there is also a wide, blind ending blood vessel, which is coiled in a spiral round a core of connective tissue (HANSON, 1950: p. 118). Similar vessels, though not always coiled, occur in the opercula of other serpulids.

5) From a comparative morphological point of view it is important to locate the prostomium. The presence of the numerous tentacles and particularly of the two terminal obturacula makes the situation on the front end of the body of *Lamellibrachia* highly obscure. When we consider the anterior part of the brain, to which the obturacula are attached, as being derived from the original dorsal ganglion, we should look for the prostomium (and for the original mouth) in the area of the ventral cuticular plate. In this area we could not find a single structure which could possibly represent the original prostomium.

Tentacles

Structure and arrangement

There are two types of tentacles, *viz.*, the normal filiform, free tentacles and the tentacles that are fused and strengthened to form the tentacular lamellae (Plate 1).

In the fixed specimen the normal tentacles form a relatively inconspicuous mass closely applied to the obturacula (in the live specimen this mass was dark red and in the water the tentacles stand out). In fact they are only visible when the tentacular lamellae are drawn aside.

The filiform tentacles are arranged in about twenty semicircular series on either side of the two obturacula. Ventrally these series overlap slightly, but dorsally they leave a narrow slit open, in which the nephropore is situated close to the base of the obturacula. In the different series the tentacles vary considerably in size, both in length and in diameter. Probably the different series are of different age. Each series consists of more than 50 tentacles and consequently there are at least 2.000 tentacles of the normal type.

At their bases all tentacles are fused by their cuticles into a single mass and those of the inner series are fused with the obturacula as well. Here most tentacles are roughly square in cross section and arranged in a regular pattern in which the series can easily be recognized (Plate 7). All elements are separated by a thick cuticle which sometimes has a rather irregular shape (Plate 23:108), even rather far down into the anterior part of the body. Only the tentacles of the same series fuse completely close to the brain (Plate 23:110). In their middle portions the tentacles of a series are still fused, but the series are free from eachother, although they remain closely applied. These middle portions are characterized by the presence of two opposed, longitudinal ciliary tracts on each tentacle (Plate 24:111, 113). The cuticle is still rather thick here, but the ciliated cells have no cuticle. Consequently the cilia are lying in furrows, situated at opposite corners of the still approximately square tentacles. The terminal portions of the tentacles are completely free. The cuticle is very thin and cilia are absent again. They are provided with two or three longitudinal rows of multicellular pinnules, which in the fixed specimen are very short, their length being only up to 70 μm (Plate 24:114–116).

The normal tentacles are covered by about six tentacular lamellae on either side. These lamellae, which were only faintly red in the living specimen, evidently represent series of fused tentacles with a very thick cuticle. Each lamella consists of more than 50 tentacles. They may be up to 12 mm long, but some are much shorter (see diagrammatic drawing of Plate 1). The tentacles forming the lamellae remain fused along their whole length. Their cuticle is always very thick (Plate 23:109) and there are neither ciliated furrows nor pinnules. In general they have a larger diameter but otherwise they have the same structure as the normal tentacles.

Along its whole length each tentacle whether free or fused contains a circular coelomic space. Its diameter seems to be constant along the whole length of the ten-

tacle, even in places where the height of the epithelial cells is very small (compare Plate 23:104, 109). Only the terminal portions of the tentacles are flexible enough to allow for expansion by means of hydrostatic pressure, making them stand out in the water. Probably the blood rather than the coelomic fluid plays a role here. The tentacles of the fixed specimen are contracted and possibly much shorter than they were originally. The coelomic spaces "disappear" exactly at the point where the tentacles of each series fuse completely (Plate 23:110). Probably they are continuous with the coelomic spaces among the vestimental musculature (see p. 25).

The afferent and efferent blood vessels are situated in the walls of the tentacular coeloms, probably always in the walls towards the adjoining tentacles of the same series. They stand out into the coelom, more or less, depending on the amount of blood they contain. They may occopy a considerable portion of the coelom (Plate 23:104). More often than not the two opposite blood vessels are connected by one or even more irregular strands of tissue, consisting of coelomic epithelium cells reaching across the coelomic space. In the fused basal portions of each series of tentacles the efferent vessels combine to form spacious subtentacular sinuses (Plate 23:110), which enter the body (Plate 23:108). In their turn the subtentacular sinuses of each half fuse to form a large sinus going towards the *sinus valvatus* (Figs. 8, 9, 10). The afferent vessels are much narrower. They originate from a vessel underlying the tentacles (Plate 23:108), which splits up in an intricate way (Figs. 10, 11) and ultimately forms the thousands of afferent tentacular vessels.

Each tentacle contains conspicous nerve fibres. These originate from a large nerve trunk which is present in the basal part of each tentacular series and which originates directly from the brain. There are more tentacular nerves leaving the brain than there are tentacular series. Consequently each series receives more than one nerve trunk.

Histology

Epidermis and cuticle

In cross sections the nuclei of the epidermal epithelium are arranged in a row parallel to the surface except at the corners where they are disposed more at random. The majority of the epidermal cells belongs to a generalized type, the apical part of which is slightly orthochromatic with Toluidine Blue and stains moderately with eosin and PAS. Below this apical area, *i.e.*, immediately above the nuclear layer, the cytoplasm stains more intensively with PAS. The significance of this reaction remains unknown. Minute dense granules may be seen in the apical cytoplasm. The basal part of many, if not all, epidermal cells contains muscle fibrils, so these cells may be called epithelio-muscular cells. They form the ring musculature of the tentacles (see diagram Fig. 19; Plate 23:107).

A few cells in each cross section have different staining properties. Their apical cytoplasm stains intensely blue with Toluidine Blue and also with PAS, the material obviously merging into the cuticle (Plate 23:109).

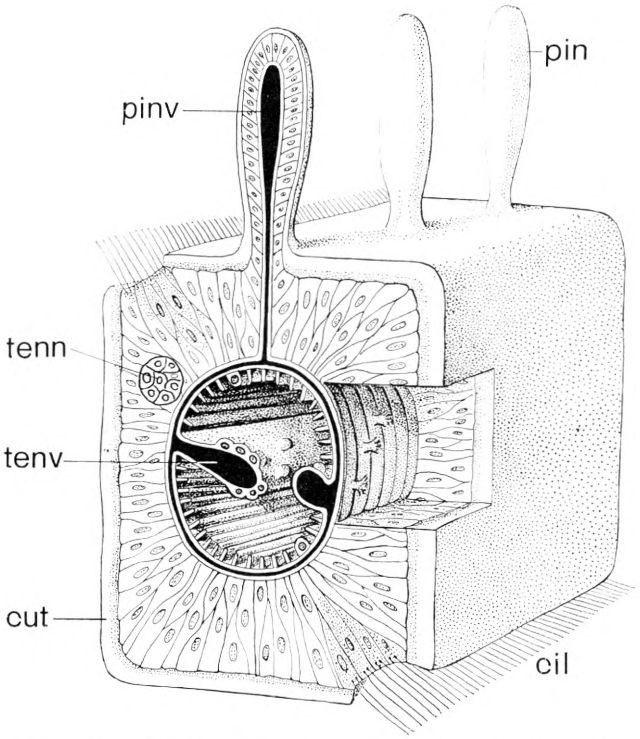

Fig. 19. Bloc diagram of a portion of a tentacle, showing pinnules and ciliated tracts (which in fact never occur on the same level), as well as the internal structure. cil = ciliated tract, cut = cuticle, pin = pinnule, pinv = pinnular blood space, tenn = tentacular nerve, tenv = tentacular blood vessel.

The ciliated cells in the middle portions of the tentacles lie in rows of two or three cells accompanied by occasional gland cells. They do not secrete cuticular material, so the cilia lie in a slit-like furrow in the cuticular lining.

Other cells have very dark nuclei, but otherwise they are similar to the normal epidermal cells. They may be sensory cells.

The size and the number of epidermal cells in a cross section vary according to the diameter of the tentacle. In the basal parts the epithelium is only about 10 μm high. In the middle parts, where the epidermis is thickest, its height is about 40 μm. Towards the free tips the height decreases again to about 10 μm.

In the cuticular lining, which is almost continuous, concentrical lines can be seen, particularly in the thick cuticle of the tentacular lamellae, just as in the obturacular cuticle (p. 66).

In most cross sections of the tentacles one or two nerve fibres can be seen in the epithelium, usually near the corners and in the apposed sides of the lamellae. In fact they run distal to the muscular basal parts of the cells, immediately below the nuclear level (Plate 23:107). The same situation exists in the obturacula (p. 67). The nerve

bundles are especially conspicuous in the basal parts of the tentacles (Plate 23:108, 110).

The basement membrane of the tentacular epithelium is rather strong, varying in thickness from less than 0.5 μm to 2 μm.

Coelomic epithelium

Most of the cells lining the coelom are epithelio-muscular cells. Their contractile parts are very long and slender constituting a longitudinal musculature (Plate 23:107) and the nuclei lie in small clumps of protoplasm protruding into the coelom. Another celltype lines the outer wall of the blood vessels, which are situated between the two lamina of the basement membrane between epidermal and coelomic epithelial cells. These cells are flat, with light nuclei. Only a few of them are spindle shaped and have a darker cytoplasm. The latter obviously are contractile.

Blood vessels

The two main vessels in each tentacle bulge out into the coelom. Mostly they are filled with blood, but some are empty and collapsed. In some sections there seem to be scattered cells lining the inside of the vascular wall, but the vascular wall is so thin that in cannot be determined with certainty whether there actually is an endothelial lining or not.

In certain tangential sections minute vessels can be seen to lie between the epidermal cells, above the muscular level. These capillaries can also be seen to be folds of the basement membrane. Apparently they carry blood from the afferent to the efferent vessel, through the epithelium. In the free parts of the tentacles they are particularly numerous. As many as 25 were counted along 100 μm of the length of a tentacle. Undoubtedly these capillaries provide a respiratory surface, as they are lying only 10 to 20 μm below the cuticle, which is very thin in the terminal portions of the tentacles. Certainly they also are responsible for the red colour.

Pinnules

The pinnules are multicellular (Plate 24:114–116). They contain a cylindrical extension of the intralaminar space of the basement membrane. Thus there is a blind ending blood sinus in the centre of each pinnule, lined by the outer lamina of the basement membrane and with four to six rows of cells on the outside. When the sinus is filled with blood the pinnules become club shaped (Plate 24:114). Apparently the blood can be pumped in and out of the sinuses, which are connected with the intraepidermal capillaries.

Discussion

1) It is remarkable that only a relatively small part of the total mass of the normal tentacles consists of elements exposed to the surrounding sea water. Indeed these parts seem to be adapted to an exchange of gasses, as is shown by the presence

of many capillaries, pinnules and a thin cuticle. The middle portions of the tentacles are partly fused with adjoining tentacles of the same series, their cuticles are very thick, and the water can penetrate into the slits between the series only by means of ciliary action.

2) A considerable number of the outer tentacles is fused to form the tentacular lamellae, which have a very thick cuticle and obviously serve a protective function. Fusion of tentacles into lamellae or other rigid structures is common in multitentaculate pogonophores and occurs in other groups as well. In phoronids the basal parts of the tentacles are partially fused over a longer or shorter distance, depending on the species (Dawydoff and Grassé 1959: p. 1015; Emig, 1971: p. 508; Hyman, 1959: p. 233). However, as far as we are aware a further specialization as protective organs is unknown in other groups.

3) In principle the pinnules are of the same type as in many polychaetes, being multicellular. They are quite unlike those occurring in Pogonophora. The pinnules of the latter group are not only unicellular, but they are also provided with unique afferent and efferent blood vessels derived from a single blood space penetrating into the pinnule cell (Nørrevang, 1965).

4) The vascular system of each tentacle consists of longitudinal afferent and efferent vessels connected by semi-circular, intraepidermal capillaries. A similar system occurs in pogonophores (Nørrevang, 1965) and in polychaetes. It was observed by Øelund (*in litt.*) in the terebellid *Thelopus*, but we do not yet know if this can be considered the normal vascular system of annelid tentacles in general. Other systems certainly occur.

Vestimentum

Morphology

A general description of the vestimental region, particularly of its external morphology, was given before (p. 9). In this chapter we just present some details of the vestimentum proper. The vestimentum is not a well defined part of the region. For convenience we use this term to denote the specialized part of the vestimental region, not belonging to the normal organ systems such as the vascular system, the nervous system, and the excretory system. Consequently the vestimentum includes the two wings and the vestimental ridges. The vestimentum is not a definite organ with one special function. Undoubtedly it has more than one function, but these are not yet clearly understood.

The structure of the vestimentum is illustrated in the diagram of Plate 4. A complete cross-section is given on Plate 3:10 and cross-sections of the wings on Plate 5:13 and 14. Several details are illustrated in the composite diagram of Fig. 2.

The wings (vestimental folds in Webb's terminology) stand out spontaneously when the animal is taken out of its tube and parts of the margins curl ventrally. The interior of the wings mainly consists of a spongy tissue which we call "spring tissue",

particularly in the posterior half of the vestimentum (Plates 4:12; 5:14). Anteriorly the vestimental musculature extends into the wings (Fig. 2A; Plates 3:10; 5:13). Here the spring tissue is mainly restricted to a narrow strip around the pyriform glands (Fig. 2E). The yellow colour of the wings in the posterior half of the vestimentum in the living animal is probably due to the presence of the spring tissue.

The wings receive blood directly from the ventral vessel. Subepidermal sinuses are common under the papillate field (Plate 16:68, 70). The region is not drained by the dorsal vessel but by special vessels, which ultimately enter into the two vestimental vessels. The vestimental vessels run anteriorly to join the subtentacular sinuses and enter the sinus valvatus (Figs. 9–10).

The papillate fields, extending from the nerve cord to the edges of the wings, are the least specialized areas. They are characterized by the presence of numerous papillae with cuticular plaques and pyriform glands, structures which are also common on the trunk. Apart from the papillae these ventral fields make a smooth impression (Plate 2:8). Webb (1969a) called the papillate fields "honeycomb-patterned superficial layer". When in the tube the papillate fields are pressed against the wall of the tube. They must play an important part in its construction.

The dorsal surface of the wings is covered by a very thick epithelium, the glandular epithelium, which only occurs on this part of the body. Webb indicated the areas as "corky lining" because of their appearance (1969a) and nutritive epithelium (in press) because in his opinion it serves to feed the eggs in the vestimental cavity when this acts as a brood chamber (see p. 90). The glandular epithelium is not smooth but thrown into numerous folds (Plates 1:1; 2:4, 5; 4:12). A deep longitudinal groove running the whole length of each field marks the place where the wing is folded.

The two vestimental ridges run along nearly the whole length of the vestimentum, bordering the middorsal field and the glandular fields. Anteriorly they gradually become narrower and lower moving away from eachother (Plate 1:1). Posteriorly they end at the gonopores (Plate 2:4). Here they do not move away from eachother as in *L. barhami* (Webb, 1969a: fig. 9B). The ridges are wavy along their whole length (Plate 2:5), which may indicate that the vestimentum was contracted in the fixed specimen. Each vestimental ridge (*lira vestimentalis*) contains a ciliated groove (*sulcus vestimentalis*). This groove lies in the middle of the ridge along the greater part of its length (Plate 2:4, 5), so the two halves of the ridge are of approximately the same size. Locally there may be differences due to the presence of different amounts of blood in the blood sinuses. However, in the anterior part of the ridge the groove gradually moves to the abaxial side (Plate 25:117, 121) and becomes shallower at the same time (Plate 25:119). The groove disappears totally close to the anterior end of the ridge. Within the ridges, below the basement membrane of the epithelium, there is a core of connective tissue with blood lacunae (Plate 25). The lacunae are quite irregular and they contained much blood throughout the greater part of the length of the ridges, at least in the living specimen, as is evident from the colour photographs made on board.

As the lacunae are mostly located distally in the ridges they may cause a considerable swelling of the ridges when filled with blood and close the grooves to a certain extent. When they are empty the grooves probably are wide open. There are muscular strands between the connective tissue cells. They may also have something to do with movements of the ridges. The ring musculature of the body wall passes below the ridges (Plate 25:117). From this muscle layer a few strands run to the bottom of the grooves where they are attached to the basement membrane (Fig. 2D).

According to WEBB (in press) vestimental ridges are lacking in the female, as far as we know the only sign of external sexual dimorphism. In one female he observed a very narrow middorsal field.

Histology

The papillate fields

The epithelium of the papillate fields is rather uniform except for the large number of papillae. The height of the epithelium between the papillae varies between 25 and 60 μm. The surface area of each cell is 15 to 25 μm^2, *i.e.*, approximately equal to the surface area of the glandular cells on the dorsal sides of the wings. The epithelium is smooth in the areas close to the ventral nerve cord and folded on the outer parts. The latter is undoubtedly due to the ventral curling of the wings.

Two cell types are common, *viz.*, a light one and a dark one. The light cells are most numerous. The darker cells are only common in the lateral regions. The light cells (Plate 8:20, 22, 23) are identical to the absorptive cells occurring all over the body, which were described before (p. 14). The less common dark cells (Plate 8:23, 25) contain granules and in some sections fibrous components seem to traverse these cells from the surface to the basement membrane. Very dense fibres extend from the basement membrane far into the epidermis where they seem to fade out. The nature of these fibres could not be established. Similar cells were observed in the middorsal field and on the outer sides of the vestimental ridges (p. 79).

The glandular fields

The glandular epithelium consists of very tall cells (Fig. 2B; Plates 11:35; 25:120, 122). Its height varies considerably, but usually the cells are 200 to 300 μm high. There are several folds and grooves in the epithelium. Down in the grooves the cells are only 100 to 175 μm high. Those standing on either side curve towards the lumen of the groove. The width of the cells varies considerably too. In areas without folds or grooves there are approximately 100 nuclei per 1500 to 2000 μm^2. Thus each cell has a surface area of about 20 μm^2. This figure does not correspond with those obtained by the study of plastic sections. In these sections, in which the cell boundaries are conspicuous (Plate 11:35), the cells are 6 to 7 μm wide, which means that their surface area is about twice as large.

The nuclei are ovoid and measure about 4 by 6 μm. They are situated in the

lower parts of the epithelium, in a zone 15 to 50 μm above the basement membrane. Scattered nuclei may be found considerably higher in the epithelium.

Each cell is densely packed with eosinophilic granules, less than 2 μm in diameter. The granules stain orthochromatically with Toluidine Blue, are moderately PAS-positive, and strongly positive with Ninhydrin-Schiff. Alcian Blue stains a faint ring around most, if not all, of the granules. In the basal parts of the cells there is a multitude of strongly PAS-positive granules, less than 0.5 μm in diameter. Most of these are situated below the level of the nuclei, but some can be seen in the distal parts of the cells. Larger PAS-positive bodies, about 5 μm in diameter, found scattered in the epithelium, may be aggregations of smaller granules. They do not stand out out specifically in sections stained with Ninhydrin-Schiff or Alcian Blue.

The outer 3 to 4 μm of the cells contain less granules than the rest of the cells. In plastic sections (Plate 11:35) this region appears much lighter and a distinct line can be seen to divide the less dense portion of the cell from the basal part. Probably this line represents some sort of a terminal web.

We may conclude that the eosinophilic granules contain neutral mucopolysaccharides and considerable amounts of proteins, with α-amino acids. The larger bodies and the small granules mainly contain neutral mucopolysaccharides. The cells have all characteristics of glandular cells, but it must be admitted that the presence of a terminal web is more characteristic of absorptive cells.

Between the glandular cells there are extremely slender cells of a more general type. They have not been identified in the electron microscopical sections. In the light microscope they appear as thin, dense lines, only widened where the spindle-shaped nucleus is situated. Often some of these cells lie close together and in such cases the cell bodies can be seen to contain PAS-positive material.

The glandular epithelium covers an extensive area so we may conclude that a considerable amount of secretions can be released to the outside.

The spring tissue

The spring tissue (Plates 12:41; 17:73; 25:120) cannot easily be described in exact terms because the cells are vacuolated. A spring tissue cell has a diameter of 10 to 15 μm. Its nucleus is small, about 2 by 3 μm, and very dark. It is displaced towards one side of the cell. The cell body looks empty except for a few very small PAS-positive granules and some slightly basophilic coagulated material. Thus a big vacuole is the most prominent feature of the cell.

The vestimental ridges

The ciliated epithelium of the grooves is very susceptible to fixation procedures. The plastic sections (Plate 25:123) probably give the most realistic picture of the epithelium. The cells are very high (75 to 100 μm) and very slender (usually they have a diameter of only 2 to 3 μm). The nuclei measure about 3 by 6 μm and have a rather variable density, some being dark, others rather light. They lie basally in the cells and

are usually surrounded by PAS-positive granules. The apical surface of the cells stains heavily with basic stains. Obviously the staining denotes the ciliary basal bodies. Ciliary rootlets reach 20 to 30 μm down into the cells. In the bottom of the grooves the cilia may reach a length of about 50 μm, while the distal cilia are less than 10 μm long. Between the bases of the ciliated cells at the bottom of the grooves, another cell element is present, probably nerve cells. Some of the ciliated cells have darker nuclei. Some are even pycnotic and there are all stages of transition from normal to pycnotic nuclei. Occasionally cells with pycnotic nuclei seem to be detached from the basement membrane and lying wedged in between normal cells. These cells are possibly in the process of being extruded. These extruding cells are often full of small, densely staining granules.

The rest of the epithelium of the vestimental ridges is very different. The cells are also very tall, measuring about 90 μm in height and 4 to 5 μm in diameter. The ovoid nuclei are situated basally in the cells. The middle third of the cells is provided with a varying number of small and minute granules, staining densely with Toluidin Blue and PAS. On the median side of the ridges these granules are much more numerous and larger, filling a substantial part of the cells. In each cell a thin and very dense filament extends from the basement membrane to the apical part of the cell, where it seems to disappear. It is visible in plastic sections only and its function is obscure.

Discussion

1) In Enteropneusta the anterior part of the trunk is provided with a pair of winglike structures, the genital pleurae, at least in some of the larger species. They contain the gonads and certainly show only some superficial resemblance to the wings of *Lamellibrachia*. Perhaps a comparison with the thoracic membrane of serpulid polychaetes makes more sense. The thoracic membranes are winglike structures running along all segments of the thoracic part of the body of most serpulids (Meyer, 1888: p. 494). As in Vestimentifera they are continuous with the anterior collar and they contain connective tissue and muscles. However, as their name implies they are much thinner. They are provided with numerous blind ending blood vessels and certainly have another function than the wings of *Lamellibrachia*, but this does not mean that they could not be homologous.

2) The histochemical properties of the glandular epithelium give no definite clue to its function. The secretion could be enzymatic and serve to digest material present in the vestimental cavity, but the compound of mucopolysaccharides and proteins may as well have a completely different function. From this point of view it could even serve to nourish maturing eggs in the vestimental cavity. Webb (in press) suggests that the vestimental cavity might act as a brood chamber mainly because the cell contents look like yolk and because some eggs were found lying in secreted (?) material covering the epithelium. However, it is unlikely that both male and female possess a brood chamber.

3) The vestimental ridges are male organs, which are lacking in the female (WEBB, in press). It is evident that they serve to transport sperm to the anterior part of the vestimental region. In this way the sperm can be discharged into the sea water while the animal remains in its tube. Contraction of the muscles attached to the basal membrane of the bottom of the grooves combined with a swelling of the distal portions of the ridges by means of blood pressure, must result in the formation of a tube which is practically closed toward the vestimental cavity (Fig. 2D).

4) In our specimen the wings stand out, but when the animal is in the tube they are folded over the dorsal side where they touch eachother, thus enclosing a vestimental cavity (WEBB, 1969a). The vestimental wings are forced against the wall of the tube by means of the spring tissue, which in this way serves to keep the cavity open. The function of this cavity is still completely obscure. Theoretically food material could be digested in the cavity, but we have no indication that this actually happens. Moreover we are unable too see how the animal could bring material, *e.g.*, sediment, into the vestimental cavity. We can just state that the cavity is in open contact with the sea water by means of the furrow between the tentacles.

5) We called the spongy tissue in the wings "spring tissue" because it apparently not only supports but also tends to curl the wings. Circumstantial evidence is the fact that the wings curl especially in the posterior part of the vestimentum where the spring tissue is best developed and where muscles are practically non-existent (Plates 5:14; 25:120). Moreover it is highly unlikely that muscles could serve to keep the vestimental cavity open (item 4, above). Apparently movements of the anterior halves of the wings are accomplished by muscular action.

6) Tissues similar to the spring tissue are found in a number of invertebrate phyla. The most striking resemblance is found in the "odontophore cartilage" of the molluscs *Aplysia* and *Stagnicola* (PERSON & PHILPOTT, 1967). As far as can be judged from the micrographs there is a great similarity even in minute details. A less pronounced similarity is found *e.g.* in the endoskeletal complex in the tentacles of the polychaete *Endistylia* (PERSON & MATHEWS, 1967) and the "chordoid tissue" in the front end of the turbellarian *Nematoplana* (AX & AX, 1969).

PERSON & PHILPOTT (*l.c.*) have reviewed this type of tissue and call it cartilage. The definition of cartilage as present in vertebrates would have to be widened considerably when we should want to include the spring tissue of *Lamellibrachia*. The review stresses the supporting function of this type of tissue, but elasticity may also be a common feature.

7) The fact that the posterior parts of the vestimental ridges move away from eachother in *L. barhami* may represent a specific difference with *L. luymesi*.

Ventral ciliary field

Description

The ventral ciliary field can be considered a separate organ. It extends along the ventral side of the vestimental region from an area slightly posterior to the brain to the bifurcation of the ventral nerve cord near the posterior end of the vestimentum. Laterally the cilated epithelium is bordered by the two halves of the ventral nerve cord (Plate 1:2 and the diagram of Fig. 13).

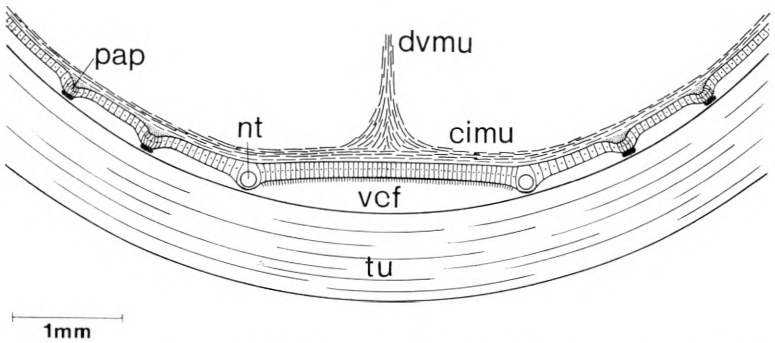

Fig. 20. Diagrammatic cross section of part of the ventral body wall in the vestimental region to illustrate a possible function of the ventral ciliary field. It can be imagined that a channel is formed by a combined action of the neurular tubes, the circular muscles, and the dorsoventral muscles. The cilia could maintain a water current in this channel. cimu = circular muscles, dvmu = dorsoventral muscles, nt = neurular tube, pap = papilla with cuticular plaque, tu = tube (thickness on scale), vcf = ventral ciliary field.

The general surface morphology of the field is shown in the scanning electron micrograph Plate 10:34. The ciliated cells were described on p. 15. The cilia are glued together by a hardened mucus in the fixed specimen (Plate 10:34) and therefore they are liable to be torn off easily. This is undoubtedly the reason why cilia are altogether absent in many sections and why Webb (1969a) did not describe the ciliary field in *L. barhami*.

In the anterior 2 to 2.5 mm of the field numerous glandular cells are found among the normal ciliated cells. Similar non-ciliated gland cells are especially numerous just in front of the ciliary field. Most probably they secrete the mucus which is present over the whole field. This is not unexpected because numerous mucus producing glands are usually present in the anterior part of ventral ciliary fields like this one, *e.g.*, on the ventral side of free-living flatworms.

The base of the ciliated epithelium is provided with an extensive nerve net, which originates from the two halves of the ventral nerve cord on either side of the ciliary field. This nerve net is particularly dense in the anteriormost portion of the field.

The circular muscle layer of the body wall is especially well developed under the ciliary field (Fig. 20). The epithelium itself stands on a layer of connective tissue. A distinct basement membrane could not be discerned.

Discussion

1) A ventral ciliary field is present in several groups, particularly in larval forms but also in many adults. A longitudinal ventral band of cilia occurs at least in the primary larvae of representatives of the Annelida including Pogonophora, Mollusca, Brachiopoda, Sipunculida, Entoprocta, and Enteropneusta (JÄGERSTEN, 1972: p. 244). The cilia are always rather short and usually shorter than those which serve for swimming. The ventral ciliary field primarily functions as an organ of locomotion (creeping organ). As a rule it occupies in the larvae the greater part of the distance between mouth and anus. In annelids it is often situated exactly in the suture which is formed when the blastopore is being closed. According to JÄGERSTEN's theory (1972: p. 171) the ventral ciliary field is an ancient adult character in annelids.

2) Possibly the whole ciliary field is kept free from the inner wall of the tube by the protruding neurular tubes, as illustrated in Fig. 20. The circular muscles are particularly strong under the ciliary field (Plate 20:90) and moreover there are strong median dorsoventral muscles in this area (Plate 17:75). If these muscles are retracted the ciliary field will be drawn inward and the neurular tubes will be pressed against the wall of the tube. A shallow cavity is formed then in which the cilia can move freely and possibly create a backwardly directed water current (see discussion on food uptake, p. 89).

Tube

Description

The tube is of quite irregular shape (Plate 26:124) no straight parts being longer than 30 mm. In the middle there is even a complete loop. The total length is 687 mm. The tube has an outer diameter of 10 mm at the anterior end and gradually tapers towards the posterior end, which has a diameter of 4.5 mm. Its anterior part is creamish white. Posteriorly the colour gradually becomes slightly brownish. There is no trace of discolouration as an indication that part of the tube has been situated under the surface of the sediment. The anterior part (Plate 26:125) is ringed, apparently caused by the fact that newly formed segments of the tube are continuous with the inner but not with the outer layers of the older parts. Posteriorly (Plate 26:126) the rings soon become irregular being nearly invisible in the second half of the tube (Plate 26:127), as if the outer surface gradually wears off. The thickness of the wall is rather uniform, about 1 mm, except near the posterior end, where it is only about 0.5 mm thick. The wall has a laminated structure, consisting of several layers. The outer layers are opaque and consist of very hard, somewhat fibrous material, not quite unlike wood at first sight. The inner layers are flexible and transparent and of brownish colour.

In general this description corresponds well with WEBB's description of the tubes of *L. barhami* (WEBB, 1969a). In his specimens the posterior ends have a diameter of 0.9 to 2.1 mm, but in our specimen the end makes the impression of being broken off, so originally a narrower posterior part may have been present. In *L. barhami* the anteriormost part of the tube is characterized by the presence of distinct

collars or "funnel rims" and black-brown markings, while in *L. luymesi* there are only inconspicuous rings (Plate 26:125) and no markings. These differences may be specific.

Position of the animal in the tube

When the animal was caught it was invisible in its tube. The obturacula must have been situated about 100 mm away from the entrance of the tube and the posterior end of the animal was situated about 40 mm away from the posterior extremity of the

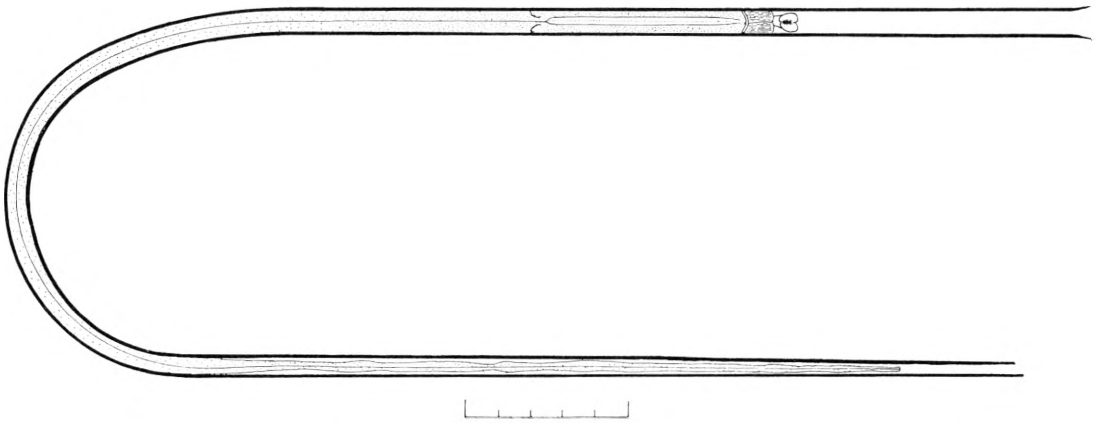

Fig. 21. Diagram to illustrate the position of the animal in the tube. The scale represents 50 mm.

tube (Fig. 21). The diameter of the two obturacula together is about 8 mm. This is also the approximate internal diameter of the tube at the level where the obturacula were situated. Although they are somewhat flexible it is unlikely that the animal could retract into the tube much further.

Probably at least the tentacular region normally protrudes from the tube, as can be concluded from the observations by Dr. Eric Barham, who saw *L. barhami* on the sea floor. He wrote: "When first seen their red lophophores were fully exposed but these were contracted rapidly in the manner of sabellid tube worms" (Webb, 1969a, p. 18).

On board, about 220 mm of the anterior part and about 230 mm of the posterior part of the tube of our specimen were cut off. The anterior and posterior portions of the animal could easily be drawn out of the cut off parts of the tube, but the rest of the animal could not be removed from the coiled middle part of the tube, not even after it had been cut into small sections (Fig. 1). The very thin body wall strongly adhered to the wall of the tube of which parts of the inner layer are still adhering to the animal.

Chemical composition

A chemical analysis of the tube of *L. barhami* was made by WEBB (1971). The results showed that the tube material (dry weight) is mainly composed of proteins (more than 36 %) and glucosamine (37 %). The presence of glucosamine is strong evidence that the tube partly consists of chitin, which is a polymere of N-acetyl-D-glucosamine. Additional chemical or physical methods are required to prove the presence of chitin (JEUNIAUX, 1963; RUDALL, 1955). Moreover we should like to know which type of chitin is present. Therefore we applied the X-ray diffraction technique to some ground tube material, without previous chemical treatment. The X-ray diffraction diagram showed a considerable number of lines of which the following could be measured well: 9.45, 6.97, 4.84, 4.51, 4.06, 3.34, and 3.25 Å. The results confirm that chitin is present and that it is closest to the alfa-type, particularly because of the lines 9.45 and 3.34. Part of the other reflexions could be ascribed to the presence of oriented proteins.

Tube construction

The tube is made up of several layers, so the tube material is undoubtedly secreted at intervals. As was suggested before (see p. 21) the material is almost certainly produced by the pyriform glands. These are present practically everywhere, so apparently the whole body is involved in the tube formation. Of course this does not mean that all parts of the body are equally active. The wall is relatively thin in the posterior and oldest part of the tube, so we must assume that tube formation is not very significant there. The production of tube material is most important at the anterior end. Here the tube has its greatest diameter and has a thick wall. The ringed appearance of the tube is probably caused by the fact that new parts are formed at intervals as suggested by WEBB (1971).

The irregular shape of the tube can be explained by assuming that the position of the tube on the bottom has changed very often but that the new parts have always been added in approximately the same direction relative to the sea floor.

The above is well in accordance with the fact that the pyriform glands are most numerous in the vestimental region and that they are particularly numerous on the posterior surface of the collar. We believe that the lengthening of the tube is one of the most important functions of the latter organ, if not the only function. Collar-like structures with a similar function are present in certain sedentary polychaetes, *e.g.*, in sabellids and serpulids.

Discussion

1) All evidence suggests that the tube of *Lamellibrachia* lies free on the substrate, which is quite unusual. The tubes of practically all tube worms are at least partially buried in sediments or attached to hard substrates. A famous exception is the polychaet *Hyalinoecia tubicola* (MÜLLER), which crawls over the bottom by means of its

anterior parapodia, dragging its tube (WIGLEY and EMERY, 1967). In principle *Lamellibrachia* could also move over the surface of the sediment, either actively or passively. In any case it seems to be capable of preventing coverage by the sediment.

2) The tube is not much longer than the animal itself. Just like polychaetes with relatively short tubes *Lamellibrachia* has no means to move efficiently up and down its tube. Animals which have long tubes (relative to their body length), such as pogonophores and several polychaetes, are equipped with a variety of organs which facilitate locomotion in the tube.

3) The tube formation in *Lamellibrachia* is not very peculiar. It can easily be compared with the procedures occurring in several pogonophores (WEBB, 1971). A comparison with sedentary polychaetes is not very useful. In this group a great diversity is found in the building apparatuses, in the histochemical nature of the glandular secretions and in the chemical composition of the tubes (DEFRETIN, 1971).

4) The tube is mainly composed of proteins and chitin. Tubes of many tube-dwelling polychaetes (DEFRETIN, 1971) and of pogonophores (WEBB, 1971) also contain considerable amounts of (tanned) proteins. Chitin has never been found in polychaete tubes so far (DEFRETIN, 1971). Of course only relatively few tubes of polychaetes have been analyzed properly, but even tubes which look most "chitinous", like those of *Hyalinoecia*, were shown not to contain chitin. We know that polychaetes do produce chitin (RUDALL, 1955; JEUNIAUX, 1963) since it could be shown to be present in the setae of several species. It is remarkable that these animals apparently do not make use of this capability in constructing their tubes, at least not very often. Chitin seems to be invariably present in pogonophore tubes (BRUNET and CARLISLE, 1958; SOUTHWARD and SOUTHWARD, 1966; WEBB, 1971).

5) As far as we know the chitin produced by polychaetes and pogonophores is always of the beta-type (BLACKWELL, et al., 1965). The chitin of *Lamellibrachia* looks more like alfa-chitin, the typical arthropod chitin, which is also common in molluscs. We do not know how significant this observation is but for the time being we cannot consider it to be very important. In the first place the types of chitin are not always clearly recognizable, so there may be more than the three types which are usually recognized. In the second place their distribution among the different groups is not yet sufficiently known. Thirdly, in certain groups the type of crystallization may be of no significance at all, since it is known that alfa-, beta-, as well as gamma-chitin may occur in one species (*Loligo*).

Ecology

Associates

One living specimen of a barnacle (*Verruca* spec.) was attached to the anterior part of the tube (Plate 26:124). Markings on this part of the tube show that at least five other specimens have been present earlier. At 160 to 170 mm from the entrance there were dead remnants of a small hydroid colony.

In the jar in which the specimen had originally been kept, five male copepods were found. They represent a new genus and a new species and have meanwhile been described by HUMES (1973) as *Tychidion guyanense*. They belong to the cyclopoid family Clausidiidae. The species of this family usually show some degree of host specificity, but the group occurs as commensals with a wide variety of hosts. Most species occur in the gill chambers of crustaceans, in lamellibranch shells or in polychaete tubes. The new species is quite different from all known species of the family. It cannot yet be determined to which of the other genera it is most closely related because females are lacking.

Copepods were also present between the tentacles, but they were only found in the series of cross-sections of the tentacular mass. Since complete specimens are lacking it is impossible to establish the identity of these animals. Probably they belong to another species than those found loose in the jar but even this is not beyond doubt. WEBB (1969a) also found quite large numbers of copepods between the tentacles of *L. barhami;* they have not yet been identified.

Aggregations

Observations on *L. barhami* suggest that this species may occur in clusters. The type series consists of eight specimens which were found together (WEBB, 1969a). The collector observed that "the tangle of tubes were entwined". The specimens found off Oregon were collected as a much larger aggregation. More than 20 tubes formed a dense, flattened mass, with the anterior openings of the tubes usually lying at the periphery (WEBB, *in litt.*). The tubes were all of approximately the same size and must have been together for quite a long time.

Habitat

The specimen was dredged from the silty bottom of the continental slope at a depth of about 500 m. The fact that the net was torn indicates that a hard object must have been present on the bottom, but we do not believe that rocks occur in the area. There are fossil coral reefs at a depth of approximately 100 m, but the slope is not very steep, so it is impossible that parts of these rocks were transported over the considerable distance to a depth of 500 m. Outcrops of basal rocks have been found nowhere in the whole area. *L. barhami* was also found on a silty slope (at a depth of 1125 m), but in that case large boulders and outcrops were present.

The temperature of the water near the bottom is unknown, but according to the data present in the CICAR Regional Data Center, National Oceanographic Data Center, Washington, the average temperature at a depth of 500 m in the area is about 7.5°C. There are 44 observations available, ranging from 6.6 to 8.7°C. The temperature at the locality where *L. barhami* was collected was 3°C.

Because the net of the Agassiz-trawl was severely damaged, only about 90 specimens of other animals were obtained. Hence we only have a meagre impression

of the composition of the fauna in the area. The small catch included: 1 hydroid colony, 8 colonies of a gorgonian (*Chrysogorgia elegans* (VERRILL)), 2 small anemones on the branches of *Chrysogorgia* colonies, 1 large galatheid (*Munidopsis* spec.), 16 small galatheids (*Uroptychus* spec.) on the branches of the *Chrysogorgia* colonies, 18 specimens of Decapoda Natantia, belonging to the families Crangonidae (*Prionocrangon* spec. and *Pontophilus* spec.), Nematocarcinidae (*Nematocarcinus* spec.), and Pandalidae (*Plesionika* spec.), 1 large lamellibranch shell, 1 small cephalopod, 2 small echinoids, 30 specimens of two species of ophiuroids, and 10 fishes, *viz.*, 1 large and 6 small macrurids, 1 congrid eel, 1 brotulid, and 1 myctophid. An earlier haul in the same area was even less succesful because the cod-end of the trawl had blocked the opening. This catch included: a few colonies of the gorgonian *Chrysogorgia elegans*, 1 sponge, 1 empty tube of a pogonophore, 3 galatheids, 10 to 15 amphipods, 1 prawn, 3 pycnogonids (*Colossendeis macerrima* WILSON, det. J. H. STOCK), 40 to 50 ophiuroids, 1 asteroid (*Pectinaster gracilis* VERRILL, det. J. H. C. WALENKAMP), 3 small holothurians, 4 stalked crinoids (*Rhizocrinus lofotensis* SARS), and 1 tunicate. Most important groups normally constituting the bathyal macrobenthos are thus represented. The apparent paucity of polychaetes and molluscs is remarkable. The dominance of decapod crustaceans and ophiuroids is not unusual.

Functions and life habits

Locomotion

Normally locomotion of tube worms is restricted to the larval phase and to movements inside the tube. However, in the case of *Lamellibrachia* we are tempted to speculate on the possibility of locomotion of the adult with its tube. Direct observations of the animals on the bottom as well as the facts that epizoa are present on the tube and that there are no signs of discolouration show that the tubes normally lie free on the bottom. Consequently the animals must at least have a means of preventing coverage by the sediment. The fact that the tube subsequently grows in different directions indicates that the position of the tube has changed in the course of time. Undoubtedly this is the reason why the tube is coiled or at least of a quite irregular shape.

We may conclude that the animals must move now and then. Of course this may happen in a passive way, *e.g.*, by bottom currents, but the animal is certainly not adapted to easy transport in this way. The only way in which the animal could move actively is by means of the anterior part of the body, which should then be outside the tube. It is quite unlikely that the animal can actively crawl over the bottom by ciliary action or by muscular movements. We can only imagine locomotion with the help of an anchor in the way in which several lamellibranchiate molluscs move. The vestimental region can undoubtedly be extended considerably. This could be done slowly with the help of its rather weak circular muscles and hydrostatic pressure, but certainly not with any force. The presence of "spring tissue" in the vestimentum obviously curling the wings ventrad (see p. 75), seems to exclude the possibility that the whole

vestimental region can be outside the tube. Probably the animal would be unable to fold the vestimental wings over the dorsal side again when wishing to retreat into the tube again. The obturacula could be imagined to form an anchor if thrust down into the sediments (as described on p. 70). Then contraction of the vestimental longitudinal muscles and the feather muscles would result in a forward movement. We would not like to suggest that *Lamellibrachia* is an active wanderer, but the occurrence of occasional movements could at least explain the presence of the strong vestimental musculature. The vestimental region can be shortened to a certain extent by its own musculature but can only be retracted by the feather muscles.

Of course the above holds good only for single specimens. When the animals occur in aggregations, as was observed for *L. barhami* (p. 86), they certainly are incapable of moving actively. The worms of the cluster received by WEBB must have remained together for most of their lives, as suggested by the fact that the narrow, older parts of the tubes of these animals were already intricately interwoven. In this case the animals can only be transported passively, *e.g.*, by means of strong currents, which are not uncommon indeed on the continental slope.

We suppose that the middle region of the body normally remains in approximately the same part of the tube. The anterior part of the body can be retracted further down the tube by means of the normal longitudinal muscles of the body wall. The feather muscles probably mainly serve to rapidly retract the tentacular region back into the tube in case of danger, as was observed by BARHAM (see p. 83). Further movements up and down the tube could be accomplished by peristaltic movements of the body wall musculature. However the absence of holdfasts and the fact that the muscles of the body wall are not very strong in the greater part of the trunk, indicate that these movements cannot be very important. Moreover they would be of no great use in the comparatively short tube.

Respiration and feeding

Dr. ERIC BARHAM, who first collected *L. barhami* (WEBB, 1969a), had an opportunity to observe the exposed "red lophophores". We also observed that the blood in our specimen had a red colour, thus containing an oxygen carrying pigment (p. 43). From our studies of the vascular system it is also evident that the tentacular vascularisation comprises a very important part of that system. We may conclude that respiration mainly takes place by means of the normal tentacles. The tentacular lamellae are probably not important because they do not contain so much blood and the cuticle is very thick in these organs. The structure and vascularisation of the obturacula indicate that they play no role at all in this respect, the distance from the blood to the surrounding sea water being far too great.

The ciliary bands on the middle portions of the tentacles may create a water current between the series of tentacles, but we do not know the direction of this current. Anyway, this ciliation cannot easily be compared with the large, continuous and con-

verging ciliary tracts of food-collecting lophophores as present in several other groups. Consequently we believe that the normal tentacles exclusively serve for respiration.

It is remarkable for a non-parasitic animal the size of *Lamellibrachia* not to possess a gut. In fact we know of no free-living animal of this size which is wholly dependant on nutrients taken up through the epidermis. Pogonophora are faced with the same problem but their dimensions are much smaller and, perhaps even more important, they are buried in the substratum. We now know (see *e.g.*, A. SOUTHWARD, 1975 and STEPHENS, 1975) that the concentrations of amino acids available for skin resorption in certain sediments is sufficiently high to account for the nourishment of pogonophores. Furthermore the tube has been shown to be permeable to such dissolved compounds. In *Lamellibrachia* the tube is certainly not permeable and it is not buried in the sediment either.

As discussed above (p. 87) it is highly improbable that more than the tentacular region and part of the vestimental region can be extended out of the tube. Consequently the uptake of nutritive material, if any, must take place through the skin inside the tube. As described before (p. 19, Fig. 4) there is a small free space between the tube and the skin over most of the body surface. This space is so narrow (50 to 100 μm) that only a very slow water current can pass through. Such a current can only be propelled by the ventral ciliary field and by peristaltic movements of the trunk. By analogy to ventral ciliary fields on other animals the cilia can be expected to beat backwards and thus create an ingoing water stream along the ventral side of the animal. Except for the posterior parts of the vestimental wings, which may press against the inside of the tube, no structures seem to be able to lead such a water current. Most probably the current will pass out of the tube at its hind end. The epidermis is well adapted for the absorption of substances with a low molecular weight (see p. 14).

It is highly unlikely that enough food material can be drawn into the tube by means of the current mentioned above, so we have to find at least an additional explanation for that. In this connection we have to take into account the vestimental cavity and particularly the vestimental glandular epithelium (see also p. 16 and p. 76). The type of secretion of these glandular areas is unknown, but it is not impossible that food particles are trapped and digested by the secreted material. Dissolved substances could then be absorbed by other parts of the epidermis. According to PÉQUIGNAT (1972: p. 39–40) this type of feeding, which he calls "surface macrophagy" is quite common among several kinds of animals living on the continental slope. In these animals the gut often does not play an important role.

In our earlier paper (VAN DER LAND & NØRREVANG, 1975: p. 97) we put forward an alternative or at least additional theory. Younger stages of *Lamellibrachia* are unknown but we have to account for the possibility that they still have a gut. If this be the case the animal is capable of storing food reserves in the trophosome when it can still feed in a normal way. Some of WEBB's specimens (1975b) were in a rather early stage of sexual maturity and indeed they contained much more trophosomal tissue along the whole length of the trunk than our specimen, which represents a later stage.

Apparently the food reserves as stored in the trophosome are being used for the production of egg cells and sperm. In this process not only the trophosome but also other organs degenerate. Several oligochaetes and polychaetes also stop feeding after reaching sexual maturity, using all their reserves for the production of gametes and often the instestinal tract degenerates to a considerable extent.

Excretion

Food can only be taken up very slowly and consequently the metabolic rate in *Lamellibrachia* must be very low. There is a correspondingly low production of waste products. We assume that a substantial part of the waste nitrogenous compounds can be eliminated through the skin in the form of ammonia. The vestimental region, in which a considerable part of the musculature is concentrated, is likely to have a comparatively high metabolism. The rather large nephridia probably mainly discharge waste products from this region. It is difficult to see how the waste products of the trunk are disposed of if not through the body wall. At least some of the waste products are stored in the pigment cells of the trophosome, which show some properties very similar to those of the chloragogenous tissue of oligochaetes (p. 29).

Reproduction

According to WEBB (1969a; in press) the size of the eggs of *L. barhami* is small. They have a diameter of only about 75 μm. A larva emerging from such a small egg is likely either to have a very short larval life or to possess some feeding organ. Apparently *Lamellibrachia* is a rare animal, the individuals or groups of individuals being widely separated, so the larval life cannot be very short. Dispersal can only take place by means of the larva and we would not at all be surprised if they would turn out to be planctotrophic.

WEBB (in press) suggested that the female keeps the shed eggs in the vestimental cavity, where fertilization and further development take place. We do not believe that the vestimental cavity with its glandular epithelium serves to incubate the embryos, mainly because in that case it would be difficult to explain the presence of exactly the same structures in the males.

The sperm is free, *i.e.*, there is no spermatophore as in Pogonophora. Admittedly the sperm occurs in bundles around a common cytophore, but in our specimen we found single spermatozoa in one of the gonoducts, so we assume that all spermatozoa will be free when discharged. Therefore fertilization probably takes place in the water.

As was discussed on p. 63 the structure of the spermatozoa is of an advanced type, which is not an indication of external fertilization, according to the theory of FRANZÉN (1956). The extremely large number of gametes which is being produced, also indicates that there is a very great initial loss of gametes. This would not be the case when there is an internal fertilization of a comparable type of fertilization. Moreover internal fertilization is mostly concomitant with either the occurrence of penis-like depository organs in the male or with the presence of spermatophores.

We caught only a single specimen but both samples of *L. barhami* received by WEBB consisted of a number of entangled specimens. Perhaps the animals are normally gregarious which would facilitate external fertilization particularly when the discharge of gametes is synchronized.

Systematics

The two species of Lamellibrachia

The general morphology of *L. luymesi* is well in accordance with the description of *L. barhami*, so we do not hesitate to place it in the same genus, but we do not consider these animals conspecific for several reasons.

The specimen from the Atlantic Ocean could be expected to belong to a different species for geographical reasons. Of course several benthic worms are widely distributed and sometimes they are true cosmopolites, but the vast majority of the species is restricted to certain areas. This is usually the case with bathyal animals (not with abyssal species). There are very few faunal connections between the Pacific and the tropical Atlantic Ocean. Suffice it to give one example to demonstrate this. Of the 351 species of sedentary polychaetes occurring along the Californian coast (HARTMAN, 1969) 265 are only known from the Pacific Ocean, 35 are cosmopolitan, 2 are also known from the South Atlantic, and 49 are also known from the North Atlantic. Of the last mentioned group most species occur only in northern cold waters and not a single one occurs in tropical waters. Of course the polychaetes of the tropical Atlantic Ocean are not nearly as well known as the polychaetes of California, but even with our present knowledge it is remarkable that these two areas only have a small number of species in common that are cosmopolitan or have a worldwide distribution in warm water.

The present specimen differs in several details from *L. barhami* as described by WEBB. Undoubtedly some of the differences are due to individual variation, to different state of conservation, and to differences in the developmental stage, but at least some are probably of systematic significance. When more material becomes available in the future we will know better where to look for systematic characters.

	L. luymesi	*L. barhami*
Total length	555 mm	369–426 mm
Maximum diameter of trunk	6 mm	4.5–4.7 mm
Trunk in % of the total length	86 %	89 %
Length of obturacula	13 mm	4.5–9.4 mm
Length of vestimental region	63 mm	25–35 mm
Length of tube	687 mm	599–724 mm
Maximum diameter of tube	10 mm	7–9 mm

Considering the fact that part of the trunk and part of the tube are apparently lacking, we may conclude that the specimen of *L. luymesi* is evidently larger than the largest specimen of *L. barhami,* even when we consider that the measurements of *L. luymesi* refer to the live specimen. Most striking is the much greater length of the vestimental region, especially because the specimens of *L. barhami* could not possibly contract more than our specimen, which had been removed from the tube and consequently could freely contract itself.

At present we can list the following morphological differences:

1) In *L. barhami* there are two pairs of tentacular lamellae. In *L. luymesi* there are about six pairs of these lamellae, and they do not end in short free tentacles as in *L. barhami.*

2) Along the ventral side of the margin of the anterior part of each vestimental wing of *L. barhami* there is a "series of more or less equally spaced darkish lines, giving this part the appearance of a zip" (WEBB, 1969a: p. 32). In *L. luymesi,* there is no trace of such an ornamentation.

3) In *L. barhami* the anterior and posterior parts of the vestimental ridges diverge, whereas this is only the case with the anterior parts of the ridges in *L. luymesi.*

4) The series of conspicuous transverse grooves on the dorsal side of the trunk just behind the vestimental region in *L. luymesi* is absent in *L. barhami* (WEBB, *in litt.*).

5) In *L. luymesi* the neurular tube is present only in the anterior part of the trunk (about 30 mm), while in *L. barhami* it seems to run much farther backwards (WEBB, *in litt.*).

6) In *L. barhami* the common excretory duct has a cuticular lining, which is lacking in *L. luymesi.* In the latter species the epithelium of the common excretory duct is a continuation of the epithelium of the excretory ducts.

7) In *L. luymesi* there is a common sperm duct, whereas in *L. barhami* the sperm ducts remain separate along their whole length.

8) In most tubiculous worms, including sedentary polychaetes and pogonophores, the structure of the tube is of systematic significance. This may also be the case in *Lamellibrachia.* The general shape is the same in both species, but distinct collars or "funnel rims" accompanied by black-brown markings on the anterior part of the tube of *L. barhami* are lacking on the tube of *L. luymesi.*

Only in the case of items (6) and (7) it is possible to discriminate between primitive and derived conditions. In both cases the more primitive condition occurs in *L. barhami.*

The name of the new species was derived from the name of the hydrographic vessel Hr. Ms. "Luymes".

We summarize this paragraph by giving differential diagnoses of the two species:

Lamellibrachia barhami Webb, 1969

The vestimental region is relatively short, representing 8 % of the total body length. There are two pairs of tentacular lamellae fringed with short free tips. Along the ventral side of the margins of the vestimental wings there is a series of dark lines. The trunk is not provided with distinct transverse grooves. The neurular tube runs along the greater part of the length of the trunk. The common excretory duct has a cuticular lining. There is no common sperm duct and the male gonopores are widely separated, and consequently the posterior parts of the vestimental ridges as well. The anterior part of the tube is provided with distinct collars, accompanied by black-brown markings.

Distribution: Pacific Ocean, continental slope off California and Oregon.

Lamellibrachia luymesi Van der Land & Nørrevang, 1975

The vestimental region is relatively long, representing about 11 % of the total body length. There are six pairs of tentacular lamellae which are not fringed with free tips. The ventral sides of the margins of the vestimental wings do not show any ornamentation. The dorsal side of the trunk is provided with a series of transverse grooves, just behind the vestimental region. The neurular tube of the trunk is present only in its anterior part. There is a real common excretory duct, its epithelium not being provided with a cuticular lining. The two sperm ducts join to form a common sperm duct in the vestimental region. The two male gonopores as well as the posterior parts of the vestimental ridges lie close together. The tube is rather smooth along its whole length and has no conspicuous collars.

Distribution: Atlantic Ocean, continental slope off Guyana.

Relationships

Our earlier paper on *Lamellibrachia* (VAN DER LAND & NØRREVANG, 1975) was particularly dedicated to a discussion of its systematic status. Moreover, several remarks on relationships were already made in most of the preceding chapters. Consequently we can confine ourselves here to giving a short summary of our conclusions and making some additional remarks.

We already stressed that we can only arrive at some tentative conclusions about the systematic status of the group, mainly because we do not yet know anything about the earlier developmental stages (we only know the size of the eggs). A developmental study is likely to give some more information on the general body plan, which is quite obscure in the adult. With this restriction in mind we came to the conclusion that the Vestimentifera can best be considered a separate class of the phylum Annelida. In our opinion our present knowledge does not permit an inclusion in the Pogonophora (which we also consider a class of the phylum Annelida), as was done by WEBB (1969a).

Regionation of the body

It was mentioned earlier that the fact that the body is regionated in *Lamellibrachia* is of little importance for phylogenetic considerations. Also the presence of a tentacular crown or mass in the front end of the animal is a widespread character. As discussed below the structure of the tentacular region as such may lend us some clues for our considerations.

An absolutely unique character is found in the general structure of the vestimental region. The whole body in this region is filled with muscles and connective tissue leaving space only for the vessels and the nephridia. The dorsad folding of the vestimental wings over the spacious vestimental cavity is unique and will be treated in detail below.

The trunk is of trivial structure, only the trophosomes are peculiar.

Webb (1969a) originally described an opisthosoma being a bulbous widening of the posterior end of the body demarcated from the rest of the trunk by a furrow. However, the interior structure of that region remains undescribed so we do not know whether it is comparable in structure to the segmented opisthosoma of Pogonophora or other annelids.

Thus we are left with an animal that may be either trimerous or polymerous. The embryology is unknown and, accordingly, we can comment next to nothing on the regionation as such. If *Lamellibrachia* is trimerous it should be compared with *e.g.* Hemichordata and Phoronida. In these groups, however, tentacles are present only in Pterobranchia and Phoronida. In Hemichordata the coelom regionation is very different from that of Vestimentifera. The acorn of enteropneusts and the cephalic shield of pterobranchs represent the prosoma, and in the latter group the tentacles are rooted in the collar which corresponds to the mesosoma. In Phoronida the tentacles are implanted upon a real lophophore and belong to the second segment, the epistome representing the prosoma (see *e.g.* Emig, 1971). Some scientists regard both epistome and lophophore as belonging to the prosoma. In that case only two body regions are recognizable in phoronids. If Vestimentifera are polymerous they should be compared with Annelida *sensu stricto* and with Pogonophora. In these groups the tentacles are usually rooted in the anteriormost body region and in pogonophores a very long and unsegmented trunk is separated from the tentacular region by a socalled mesosoma which is, however, very different from the vestimental region in *Lamellibrachia*. On the other hand we find a great variation within the annelids, so that it seems unjustified to compare with any specific family or order. The oligochaet *Lamprodrilus vermivorus* shows an obliteration of several pregenital segments by muscular tissue, but in details it is very different from conditions found in the vestimental region of *Lamellibrachia*. Furthermore, oligochaetes never possess an elaborate tentacular apparatus. Obviously, the Hirudinea must be left out of the present discussion. So we are left with the group Polychaeta. Tentacular crowns are present in many polychaete families and especially among Serpulidae and Sabellidae we find many similarities in the structure of the tentacular region. Details will be discussed below. Nephridia are found in the anterior-

most parts of the body in many polychaetes. In many sedentary polychaetes segments may fuse, the dissepiments being reduced, especially in the anterior part of the body, but we have been unable to find anything comparable to the vestimental region in *Lamellibrachia*, although (as mentioned on p. 79) the sides of the anteriormost segments may be drawn out into socalled thoracic membranes which fold dorsally.

Pogonophora show a regionation which is in some aspects similar to that of Vestimentifera. In some species the tentacles are set in a ring very close to the intraepidermal "brain" followed by a "frenular region" which is, however, provided with a spacious coelom. An incomplete (NØRREVANG, 1970) diaphragm delimits this region from the trunk proper ("gonadal region") which is occupied by the reproductive organs for most of its length. What corresponds to the frenular region is filled with muscular tissue in *Lamellibrachia* and forms the vestimental region. The male gonopores in Pogonophora are situated just behind the mentioned diaphragm, so their position corresponds to that found in Vestimentifera. The pogonophore female gonopores, however, are set far back on the trunk and the ovaries lie in front of these apertures.

Thus we must conclude that the regionation of the body in Vestimentifera is unique and no evident homologues are found neither in Polychaeta nor in Pogonophora.

Tentacular region

As is evident from the description on p. 71 the tentacles are set in an elaborate pattern, but they are not implanted on a distinct a lophophore. It was mentioned earlier (p. 75) that the pattern of the coelomic spaces and blood vessels in the tentacles of *Lamellibrachia* is identical to that found in Pogonophora and in some Polychaeta. However, in the pterobranch *Rhabdopleura* the tentacles show a similar pattern with a coelomic space in the centre and afferent and efferent vessels lying in opposite walls in the basement membrane, although no connective vessels were seen (pers. obs.). It obviously remains to be studied whether this pattern of tentacular vascularisation is a basic pattern to be found in all or most tentaculate animals.

Some of the tentacles of *Lamellibrachia* are provided with multicellular pinnules with a single blood space so that the blood must move by ebb and flow. Such pinnules are found only in Vestimentifera and Polychaeta.

The general pattern of vascularisation in the tentacular region of *Lamellibrachia* includes afferent tentacular vessels derived from the dorsal vessel and efferent tentacular vessels draining to the ventral vessel through subtentacular sinuses. Similar systems are found among sedentary, tentaculate polychaetes and – the similarity perhaps being somewhat less evident – in Phoronida (EMIG, 1971). The vascular system proximal to the tentacles in Pterobranchia is insufficiently known, so that no comparison can be made.

As mentioned on p. 43 neither heart nor *sinus valvatus* of *Lamellibrachia* has its counterpart in other groups, the heart being a sphincter around both dorsal vessel and

perivascular coelom. There are similar sphincters in Serpulidae, but they close only the dorsal vessel (HANSON, 1950).

As discussed on p. 70 the obturacula are unique structures, not finding their counterpart anywhere, except perhaps in the (single) operculum of Serpulidae as *e.g. Pomatoceros* and *Mercierella* which develop from one tentacle. The operculum in Sabellariidae (*e.g. Phragmatopoma*) develops as an outgrowth from two or more segments (DALES, 1952) and thus cannot be compared with the obturacula.

Vestimental region

The obliteration of the front part of the body with muscles finds its counterpart nowhere among annelids. On the ventral side there is a broad ciliary field bordered by the nerve cords. Such fields are so widespread that its mere presence is of no use in a phylogenetic discussion.

In Pogonophora, as in Vestimentifera, there is a nerve net at the base of the cells of the broad ciliary field which is bordered by rather thick nerve trunks (IVANOV, 1963, figs. 33 and 36). In archiannelids as *e.g. Protodrilus* (PIERANTONI, 1908) there is also a nerve trunk present on either side of a ventral ciliary band. The latter however, runs along the whole length of the body and it does not widen in any place to form a ciliary field. In "deuterostomia" the nerve tract is always single and median.

In fact such ventral ciliary fields are usually present at the site where the blastopore closes during embryogenesis. The central nervous system usually develops on either side in the lateral blastopore lips. In Vestimentifera and Pogonophora we have the only instances of a wide ciliary field persisting in the adult.

Trunk and opisthosome

The trunk is still more than the other regions characterized by the absence of a gut. In other animals this usually is the most dominant organ which affects the whole structure of the region. Apart from this the trunk has a quite normal anatomy with two lateral coelomic spaces meeting in the median plane to form a mesentery. Particularly in the early stages of sexual maturity as represented by the known specimens of *L. barhami*, the single trophosome is the dominant organ, forming the greater part of the volume of the trunk. Similar tissues are present in several other animal groups (p. 30), in which they may also be dominant structures. Later the trophosome is largely replaced by reproductive organs and the sexual products then form the greater part of the volume of the trunk. This is also a not unusual phenomenon in several other animal groups.

We have to conclude that the general structure of the trunk does not give any clue to the relationships of the Vestimentifera.

The *opisthosoma* (mentioned by WEBB, 1969a) may be of considerable systematic significance, as it proved to be in the Pogonophora, but its structure is as yet unknown and there is some doubt whether it really represents a separate body region or forms part of the trunk (p. 13).

Organ systems

Most of the relevant discussions were already presented in the preceding chapters. Here we just repeat some of the most important items.

Epidermis: A considerable part of the epidermis consists of absorptive epithelium and large areas may be capable of taking up dissolved food substances. However, in recent years it has become clear that many marine animals of several phyla possess absorptive epithelia. Only the structure of the cuticle over the absorptive cells may be considered of systematic significance. A quite similar structure occurs in polychaetes, sipunculids and pogonophores (p. 21).

Some epidermal structures, *viz.*, the cuticular plaques and the pyriform glands are only known from Pogonophora. The similarity between these structures and comparable structures in other groups, especially in other annelids, is at least less striking (see p. 21 and p. 22).

The coelom and the musculature give little evidence pertinent to a phylogenetic discussion. We just note that feather muscles of a similar structure seem to be present also in polychaetes and phoronids (p. 34).

Vascular system: Histologically this system is similar to that of other annelids, although an endothelium has also been found in Hemichordata. The most striking peculiarity is the presence of an intravascular cell cord and intravascular bodies. Similar structures are only found in oligochaetes, polychaetes and pogonophores.

The nephridia are metanephridia showing general similarities to those of several other groups, except for the fact that the nephrostome is split up into a multitude of narrow tubules, which is a peculiarity of *Lamellibrachia*. IVANOV (1963) described metanephridia in pogonophores, but after the discovery of solenocytes in *Siboglinum* this organ system is in need of reinvestigation (p. 55).

Nervous system: It was argued (p. 50) that in spite of the ventral position of the brain in *Lamellibrachia*, it is in fact ring-shaped. The same applies to Pogonophora in which the brain is also ventral. When the effects of the absence of a gut are taken into account it is easier to compare the structure of the central nervous system with that of other annelids (p. 50).

The reproductive system shows little specialization in the gonads and the duct systems. The position of the gonopores at the hind end of the vestimental region tells little because we cannot yet compare the general body plan with that of other animals. We can just state that the gonopores of males and females are situated in exactly the same place. In pogonophores the female pores have a much more posterior position. The sperm has a rather peculiar structure and shows great similarities to that of pogonophores and probably some polychaetes.

The phylum Annelida

The fact that we regard both the Vestimentifera and the Pogonophora as classes of the phylum Annelida necessitates a reconsideration of the concept of this phylum. At this moment we are unable to present an exact diagnosis. The old concept has never

been defined sharply and a thorough study of at least the literature on all groups involved would be necessary to arrive at an adequate circumscription.

In our opinion the concept of the group Annelida has to be widened to include the following classes: Polychaeta (including Archiennelida), Oligochaeta, Hirundinea, Myzostomida, Echiurida, Sipunculida, Pogonophora, and Vestimentifera.

The Archiannelida are usually maintained as a separate group for practical reasons but most specialists agree that this group consists of families that have little in common and that each of them is more closely related to different polychaete families (HERMANS, 1969; JOUIN, 2971; FAUCHALD, 1974).

Nowadays there is again a tendency to stress the relationships of the Sipunculida with the Annelida (ÅKESSON, 1958; RICE, 1973). The similarity of their cuticle to that of the Annelida could be an important argument in this case also.

Several specialists have agreed for a long time already that the echiurids are related to the polychaetes (BOCK, 1942; DAWYDOFF, 1959: p. 902) although there are still some who prefer to give them an independant position as a phylum between the Annelida and the Mollusca (STEPHEN & EDMONDS, 1972: p. 343), mainly for embryological reasons.

There is no doubt that the clearly segmented Annelida, *viz.*, Polychaeta, Oligochaeta and Hirudinea, are closely related. In the past there has been some controversy about the systematic status of the Myzostomida, but nowadays they are usually considered as annelids (PRENANT, 1959: p. 779) and often even as polychaetes.

Until quite recently most specialists considered the Pogonophora to be deuterostomes and consequently regarded them as being most closely related to Hemichordata. However, recent research on many aspects of their structure has led to an almost unanimous acceptance of a "protostomian" relationship, as was evident for the first time during the 1973 symposium on "The phylogeny and systematic position of Pogonophora" in Copenhagen (see proceedings in Zeitschr. zool. Syst. Evol-forsch. Sonderheft 1, 1975). This involved a considerable change in the nomenclature because the concepts dorsal and ventral had to be reversed. Of course not all specialists agree that the phylum Pogonophora should be included in the phylum Annelida.

One of the most important conclusions is that the Annelida *sensu lato* include groups of animals in which there is no segmentation or in which segmentation is obscure, as in sipunculids, echiurids and Vestimentifera, or in which only part of the body is segmented as in pogonophorans. Of course within the Annelida *sensu stricto* there are also groups in which segmentation is obscure (certain polychaetes, certain archiannelids, Myzostomida and Hirudinea) but nevertheless we have to get used to the idea of a wider concept. We prefer this to a situation in which several small groups are considered separate phyla, often on subjective grounds or for historical reasons. The rank of a phylum should only be given to really separate entities.

Rijksmuseum van Natuurlijke Historie
Raamsteeg 2
NL–2300 RA Leiden
the Netherlands

Institute of Comparative Anatomy
University of Copenhagen
Universitetsparken 15
DK–2100 Copenhagen Ø – Denmark

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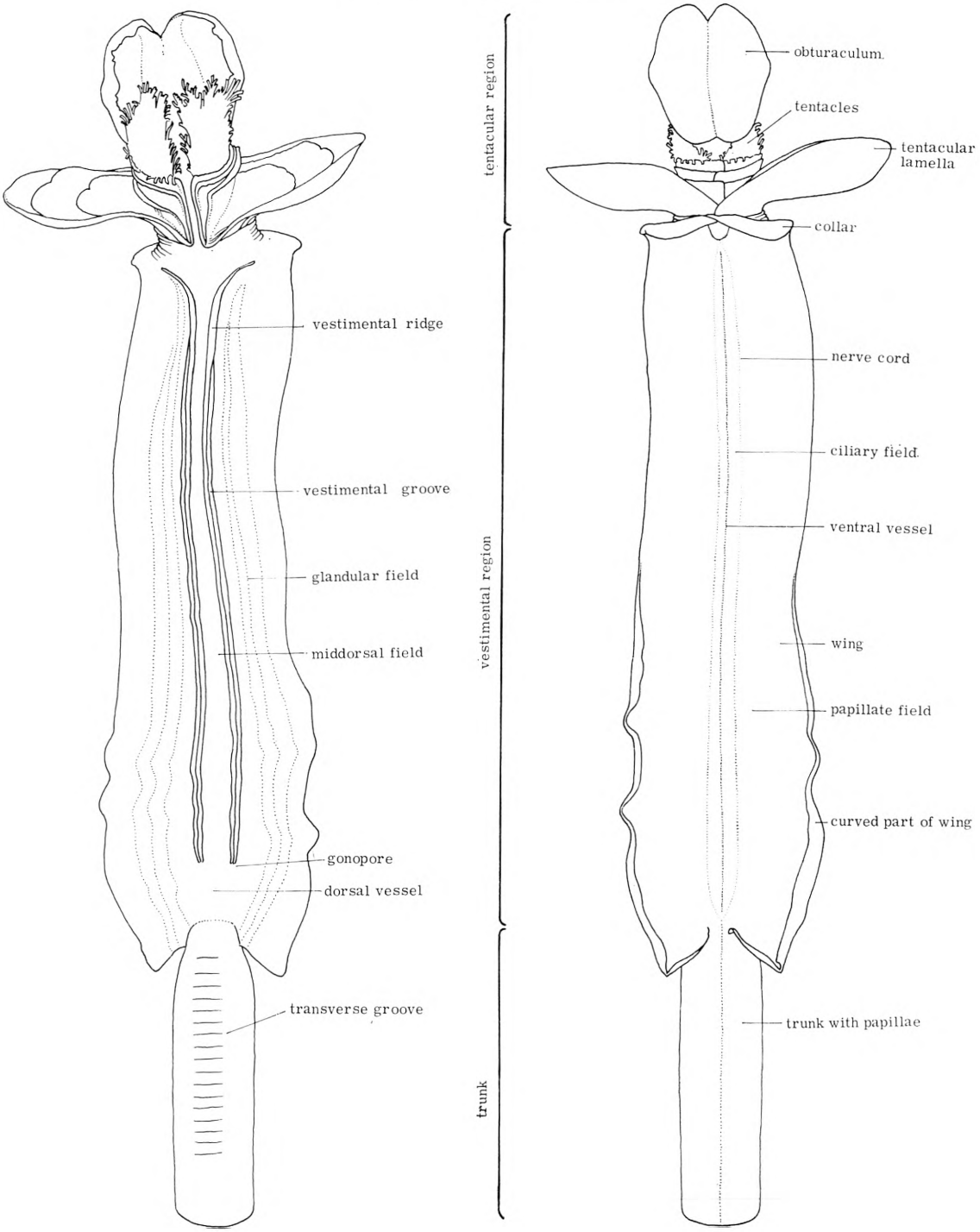
Færdig fra trykkeriet december 1977.

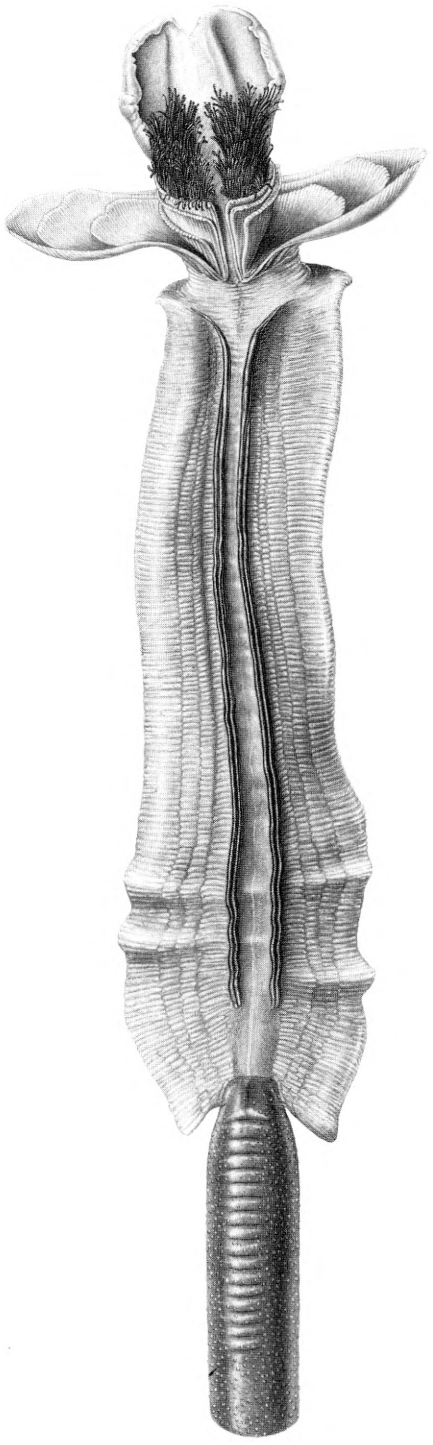
PLATES

PLATE 1

Fig. 1. Anterior part of the body in dorsal view. Pencil drawing made after colour slide of the live specimen and photographs of the fixed specimen. Tentacles diagrammatized.

Fig. 2. The same in ventral view.





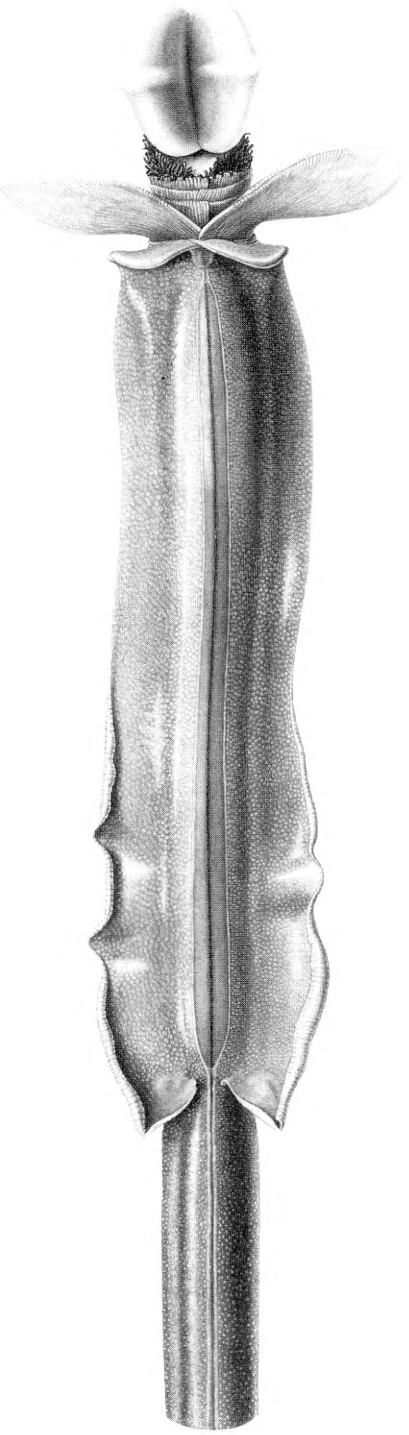


PLATE 2

Fig. 3. Tentacular region and anterior part of vestimental region in lateral view. The scale represents 5 mm.

Fig. 4. Detail of dorsal side of posterior end of vestimental region with wound in which the common sperm duct, spermsacs, and the dorsal blood vessel are visible. The scale represents 2 mm.

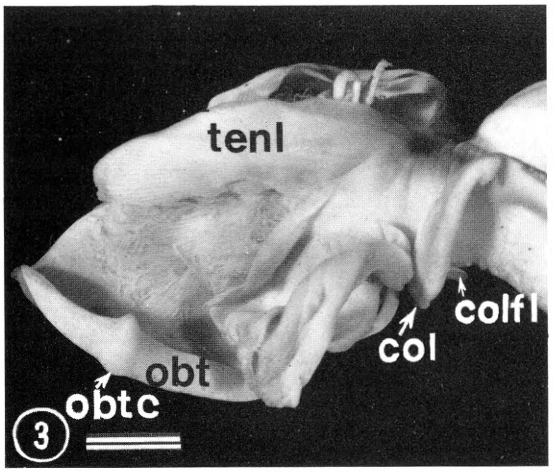
Fig. 5. Detail of the middorsal area in the posterior half of the vestimental region. The scale represents 1 mm.

Fig. 6. Detail of the ventral side of the anterior part of the trunk with numerous papillae and with the ventral nerve cord and the neurular tube shining through. The scale represents 1 mm.

Fig. 7. Posterior end of vestimental region and anterior part of trunk in dorsal view, showing most of the transverse grooves. The scale represents 2 mm.

Fig. 8. The same in ventral view. Same scale as in fig. 7.

col = collar, colfl = collar flap, dv = dorsal vessel, mdf = middorsal field, nt = neurular tube, obt = obturaculum, obtc = obturacular coelom, spdu = common sperm duct, sps = sperm sac, tenl = tentacular lamella, veg = vestimental groove, vgl = vestimental glandular epithelium, ver = vestimental ridge, vv = ventral vessel.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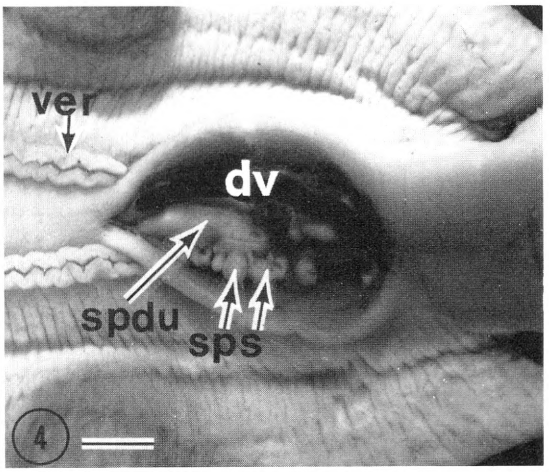
colfl
col
obt
obtc
3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dv
spdu
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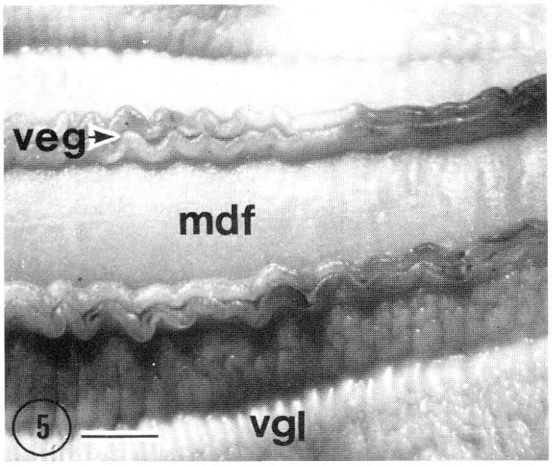
4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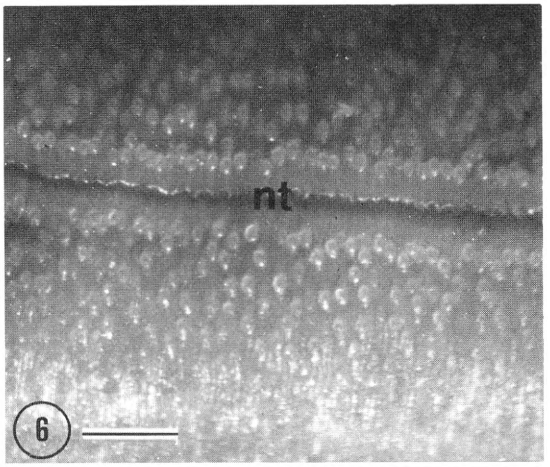
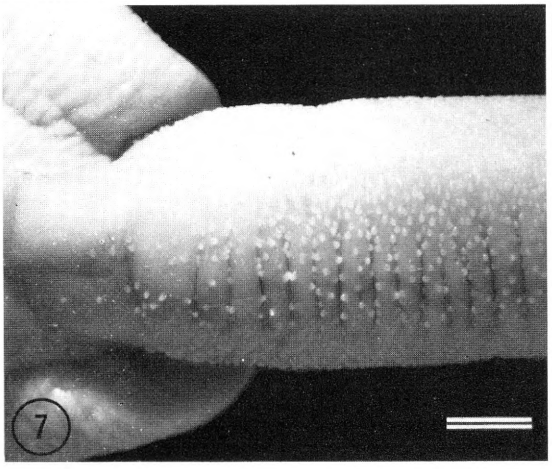
mdf
vgl
5


nt
6


7

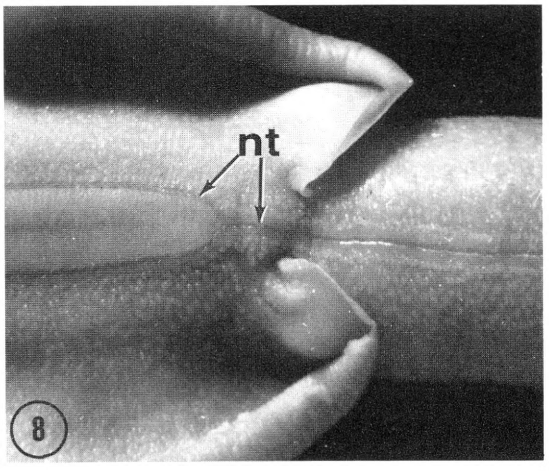

nt
8

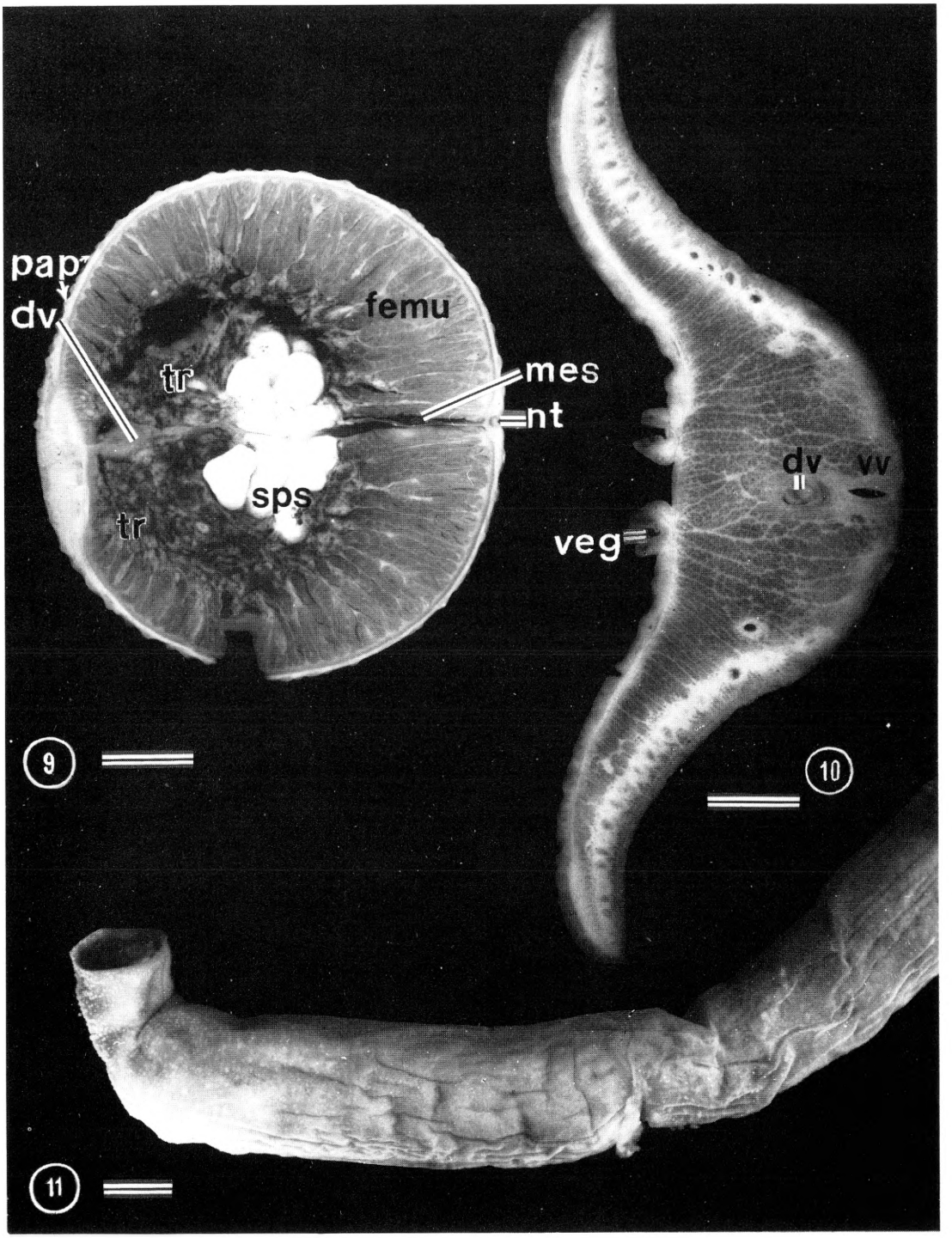
PLATE 3

Fig. 9. Macrophotograph of slice of anterior part of trunk. Note the strings of trophosomal tissue (with dark coloured blood vessels) penetrating between the feather muscles. Compare microphotograph Plate 5:15. The scale represents 1 mm.

Fig. 10. Macrophotograph of slice of anterior part of vestimental region. Compare with microphotograph Plate 5:13 and drawing of posterior part on Plate 4. In the anterior part of the region the wings are not curled, probably because they are more muscular and contain less connective tissue (white in the photograph). The scale represents 1 mm.

Fig. 11. Portion of posterior part of the trunk. The scale represents 2 mm.

dv = dorsal vessel, femu = feather muscles, mes = mesentery, nt = neurular tube, pap = papilla, sps = sperm sac, tr = trophosome, veg = vestimental groove, vv = ventral vessel.


pap
dv
femu
tr
mes
nt
sps
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vv
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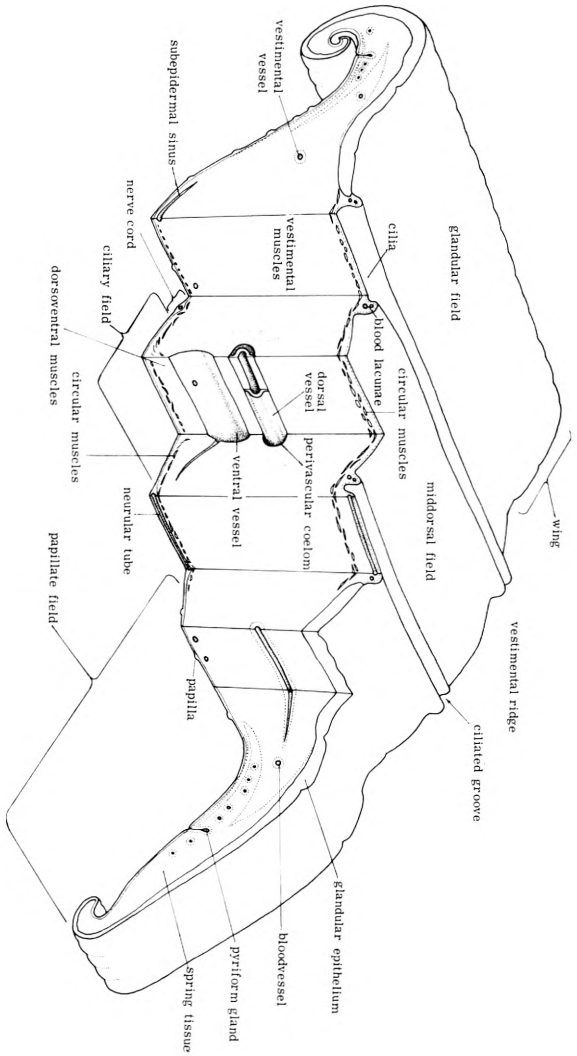
10
11

PLATE 4

Fig. 12. Bloc diagram of portion of posterior half of vestimental region. Compare with photograph Plate 3:10.



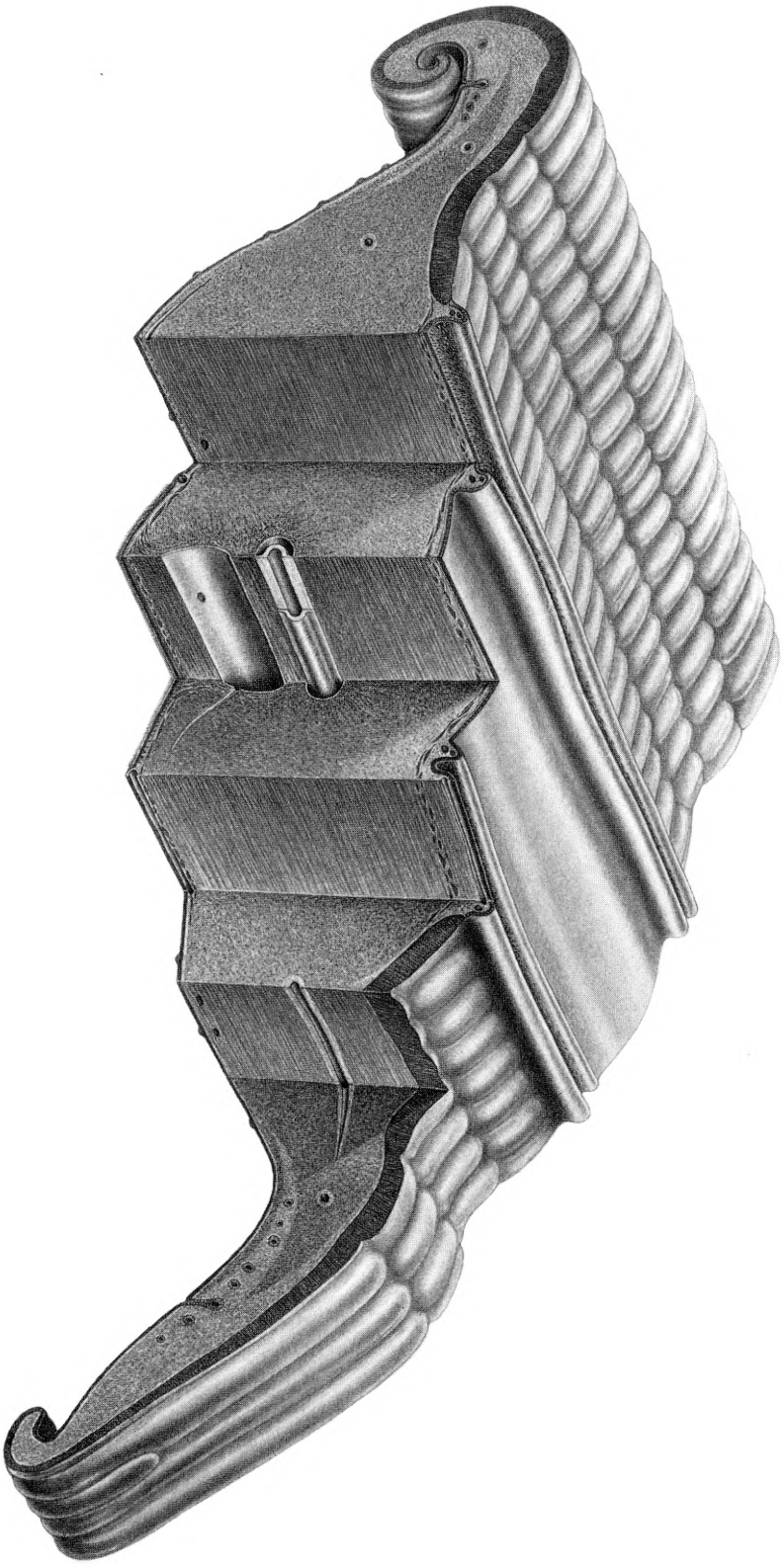


PLATE 5

Cross sections through different parts of the body.

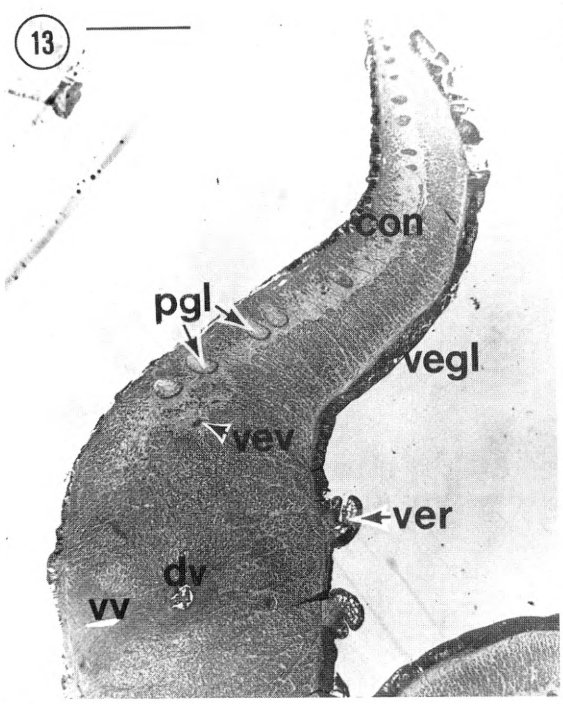
Fig. 13. Anterior part of vestimental region. The scale represents 1 mm.

Fig. 14. Posterior part of vestimental region. The scale represents 1 mm.

Fig. 15. Anterior part of trunk. The scale represents 1 mm.

Fig. 16. Posterior part of trunk. The scale represents 1 mm.

con = connective tissue, dv = dorsal vessel, femu = feather muscles, mes = mesentery, n = nerve cord, nt = neurular tube, pap = papillae, pgl = pyriform gland, spdu = sperm duct, sps = sperm sac, tet = testicular tubules, tr = trophosome, vegl = vestimental glandular epithelium, vemu = vestimental muscles, ver = vestimental ridge, vev = vestimental vessel, vv = ventral vessel.


13
con
pgl
vegl
vev
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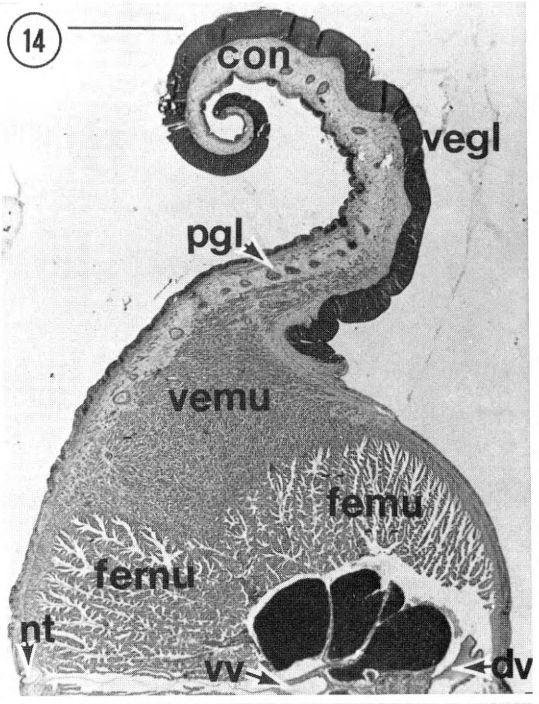
dv
vv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con
vegl
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vemu
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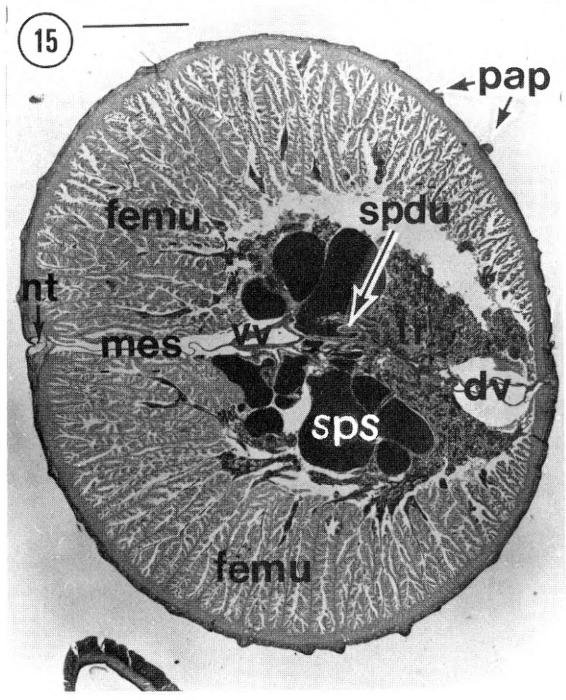
vv
dv


15
pap
femu
spdu
nt
mes
vv
tr
dv
sps
femu

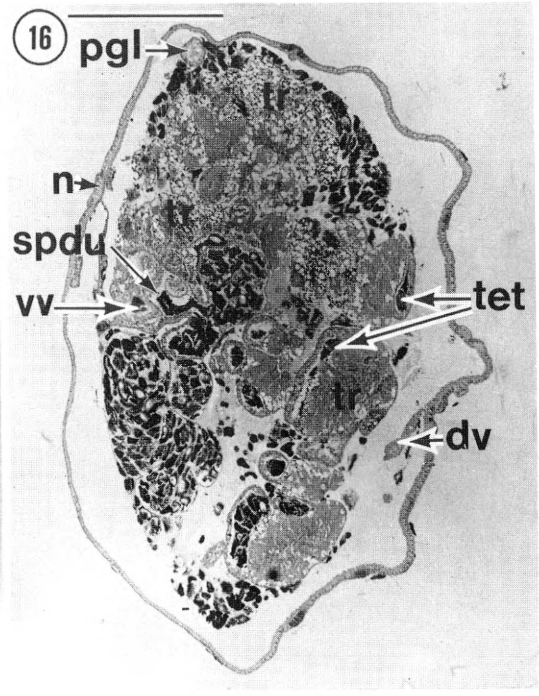
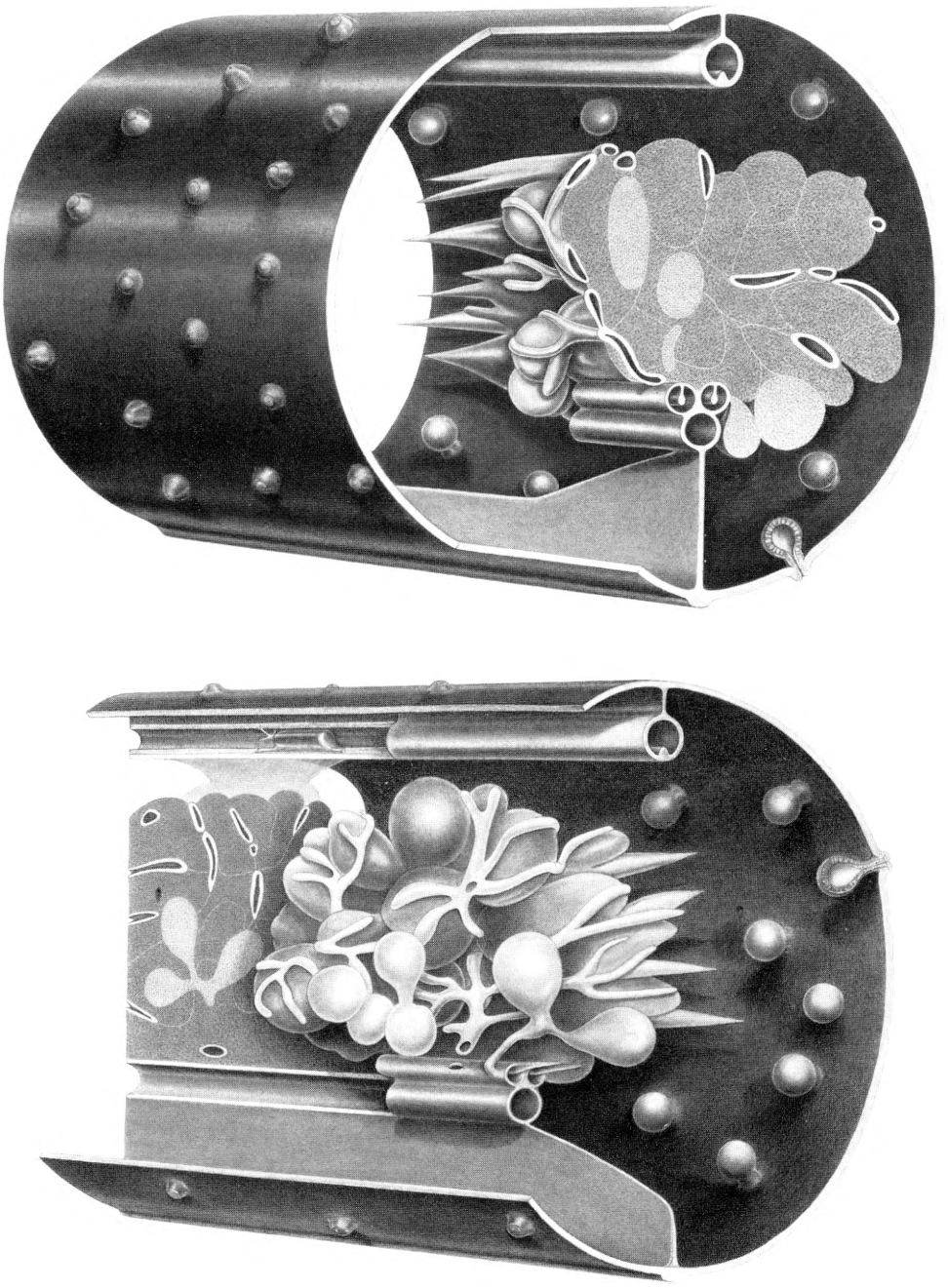

16
pgl
tr
n
tr
spdu
vv
tet
tr
dv

PLATE 6

Fig. 17. Bloc diagrams of portions of posterior part of the trunk, showing anterior (top) and posterior (bottom) part of a trophosome. Compare with photograph Plate 5:16.



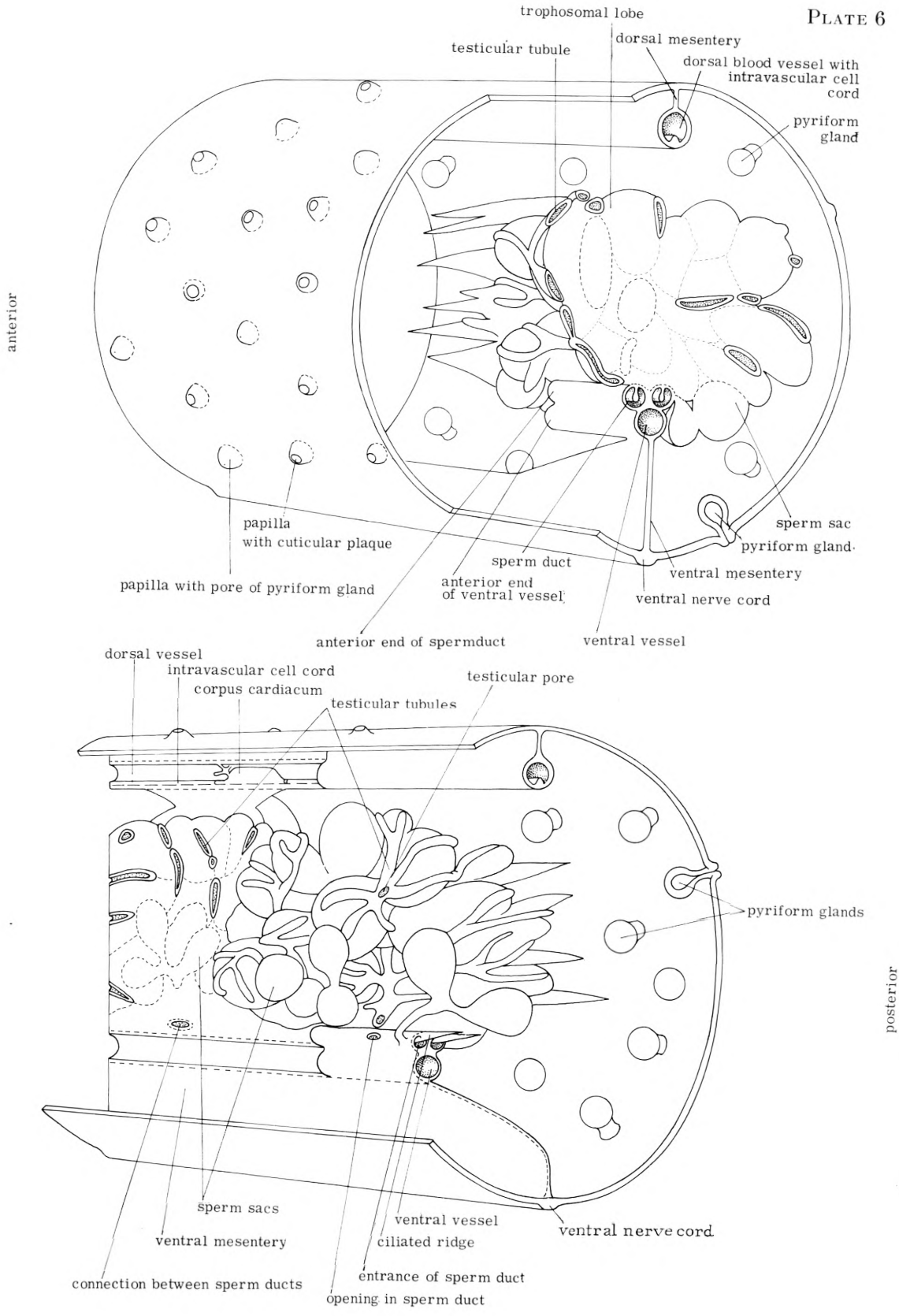

trophosomal lobe
testicular tubule
dorsal mesentery
dorsal blood vessel with intravascular cell cord
pyriform gland
anterior
papilla with cuticular plaque
papilla with pore of pyriform gland
anterior end of spermduct
anterior end of ventral vessel
sperm duct
ventral vessel
ventral nerve cord
ventral mesentery
sperm sac
pyriform gland
dorsal vessel
intravascular cell cord
corpus cardiacum
testicular tubules
testicular pore
pyriform glands
posterior
sperm sacs
ventral mesentery
connection between sperm ducts
opening in sperm duct
entrance of sperm duct
ciliated ridge
ventral vessel
ventral nerve cord

PLATE 7

Fig. 18. Oblique cross section through anteriormost part of vestimental region and base of tentacular region. The scale represents 1 mm.

edu = excretory duct, h = heart, obt = obturaculum, obtc = obturacular coelom, obtv = obturacular blood vessel, ssi = subtentacular sinus, ten = tentacle, tenl = tentacular lamella, tenn = tentacular nerves.

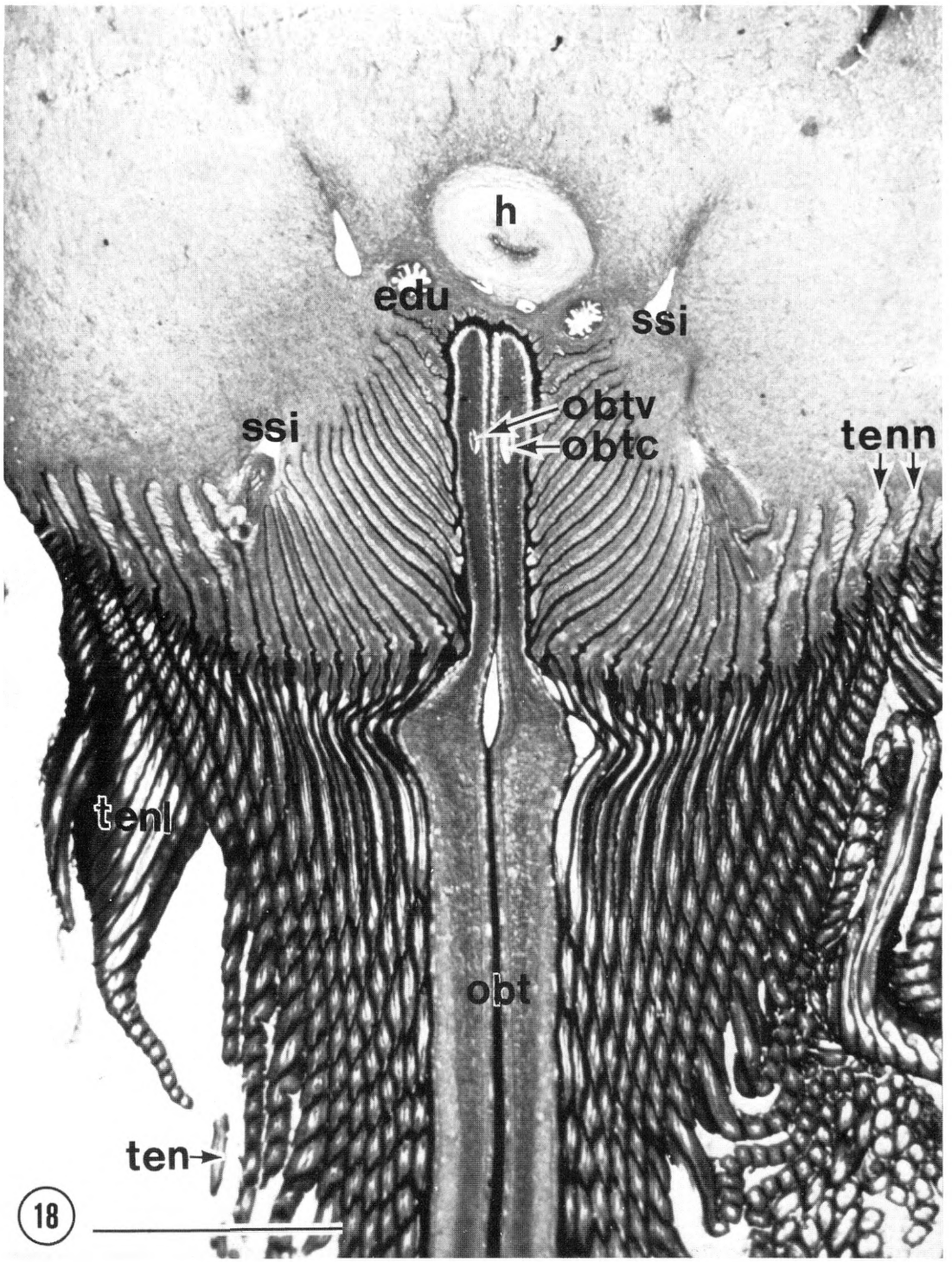

h
edu
ssi
ssi
obtv
obtc
tenn
tenl
obt
ten
18

PLATE 8

Details of epidermis. All scales represent 20 μm.

Fig. 19. Middle part of an obturaculum. The cuticle is extremely thick. The cytoplasm is differentiated into a granulated part and a muscular part, with the nucleus in between. Nerve fibres run between the cells.

Fig. 20. Unspecialized epithelium of the ventral side of a vestimental wing. The nuclei do not have a definite position. The apical parts of the cells usually contain granules. Vacuoles are common in the basal parts.

Fig. 21. Hind end of the trunk. The cells contain large vacuoles. The nuclei have very different positions.

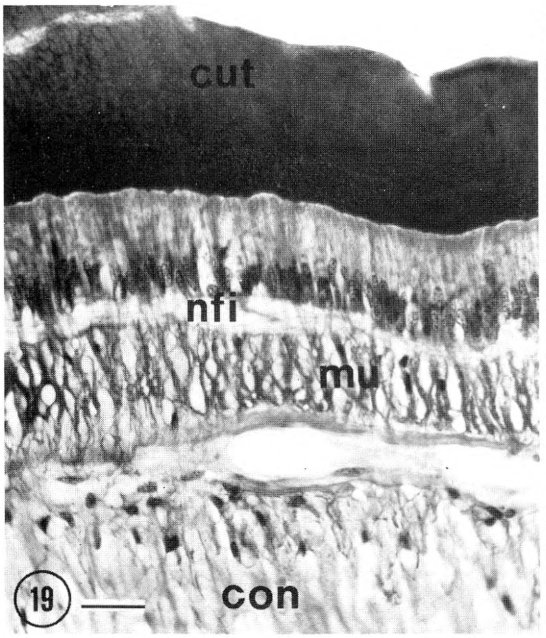
Fig. 22. Ventral side of vestimental wing. A group of cells which probably have a sensory function, among absorptive cells characterized by the presence of PAS-positive granules in the lower two thirds of the cells.

Fig. 23. Ventral side of the vestimental region close to the edge of a wing. Several dark cells with unknown function among the light, normal, absorptive cells.

Fig. 24. Ventral ciliary field. The very long rootlets of the cilia can be seen as a faint striation in the apical parts of the cells. The nuclei are randomly distributed in the basal halves of the cells. Most cells contain numerous PAS-positive granules.

Fig. 25. Ventral side of vestimental wing.

cimu = circular muscles, con = connective tissue, cut = cuticle, gr = granulated part of cells, lomu = longitudinal muscles, mu = muscular part of cell, nfi = nerve fibres, sen = sensory cells [?].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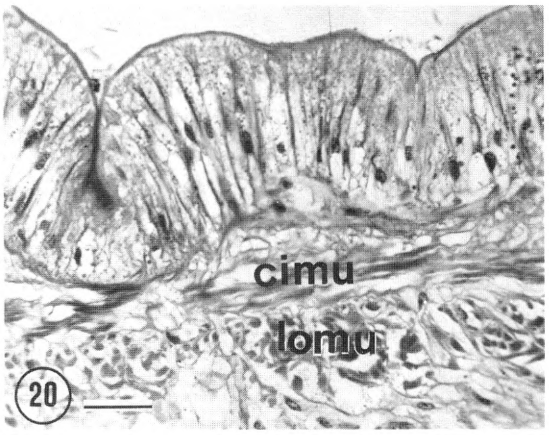
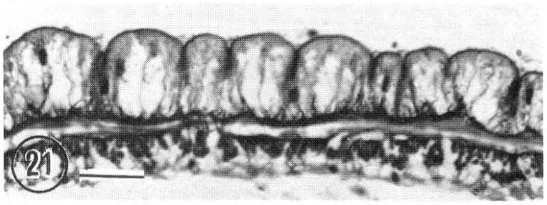
nfi
mu
con
19


cimu
lomu
20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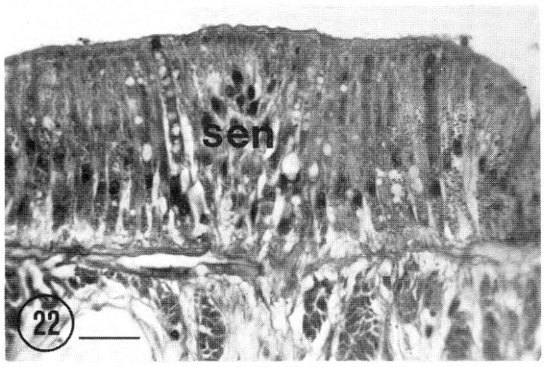
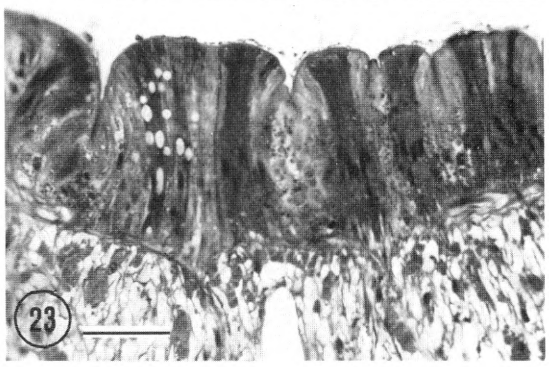

sen
22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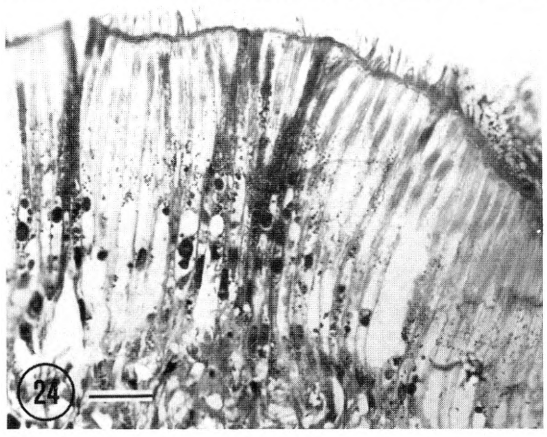
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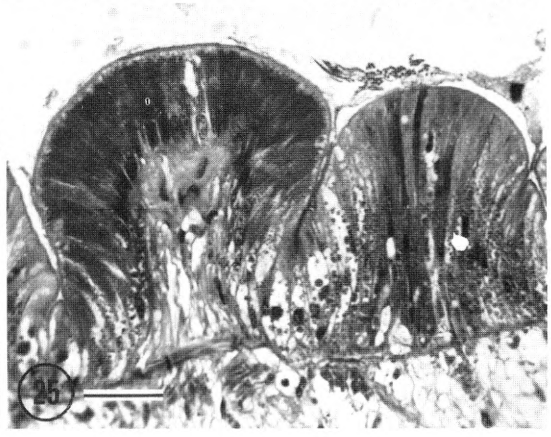
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PLATE 9

Scanning electron micrographs of surface structures.

Fig. 26. One papilla with cuticular plaque and one with opening of pyriform gland. The cushion-like arrangement of the epidermal cells, many of which are visible individually, is probably an artifact.

Fig. 27. Four papillae with cuticular plaques and one with the pore of a pyriform gland. The latter type is usually somewhat smaller than the other but of approximately the same height.

Fig. 28. Papillae with cuticular plaques from an area where the body was apparently pressed against the wall of the tube.

Fig. 29. Irregular cuticular structure from the middle region of the trunk. Note the shaly structure.

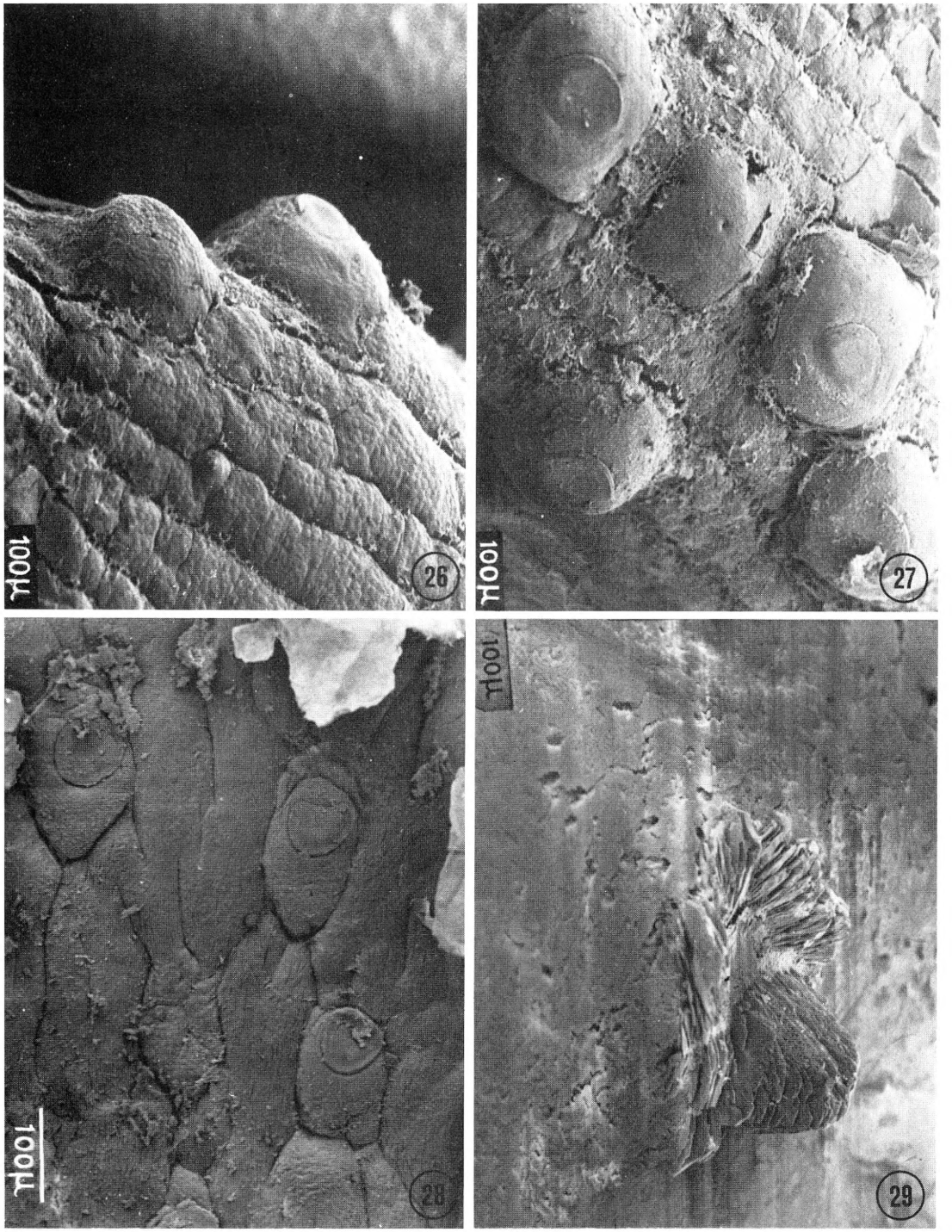

100μ
26
100μ
27
100μ
28
100μ
29

PLATE 10

Fig. 30. Papilla with very thick cuticular plaque. Scanning electron micrograph. The scale represents 100 μm.

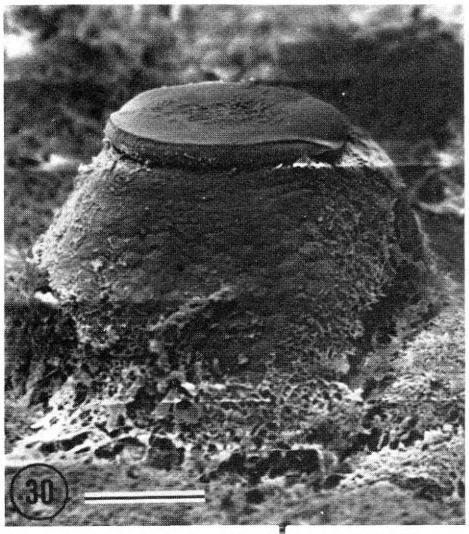
Fig. 31. Papilla with cuticular plaque from the vestimental region. The core of the papilla is filled with connective tissue and muscle fibres. The modified epidermal cells underlying the plaque apparently secrete the latter. The other epidermal cells are filled with strongly PAS-positive granules and apparently have a secretory function. The scale represents 100 μm.

Fig. 32. Papilla with cuticular plaque from the ventral side of a curled vestimental wing. In this situation the papilla hardly protrudes above the surface. Note the large ovoid nuclei of the specialized epidermal cells underlying the plaque and the strings of secretory products in the apical parts of the cells. The scale represents 50 μm.

Fig. 33. Papilla with cuticular plaque from the posterior part of the trunk. The cushion of connective tissue is very small and secretory cells are lacking around the plaque. The scale represents 20 μm.

Fig. 34. Portion of the ventral ciliary field. Scanning electron micrograph. The cilia are glued together by mucus. The scale represents 200 μm.

cimu = circular muscles, con = connective tissue, lomu = longitudinal muscles, pl = cuticular plaque, plc = modified epidermal cells forming cuticular plaque, sec = secretory epidermal cells.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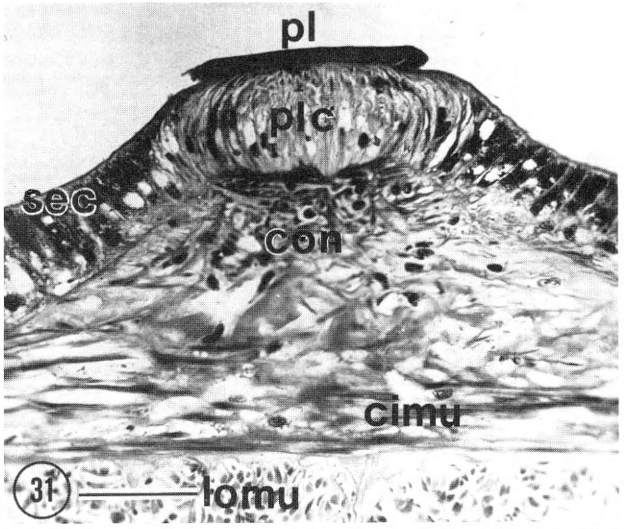
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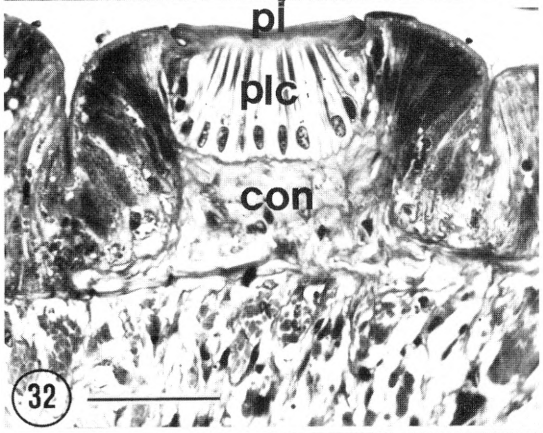
sec
con
cimu
31
lomu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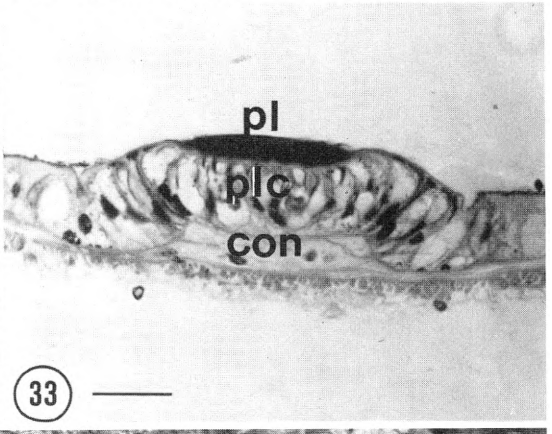
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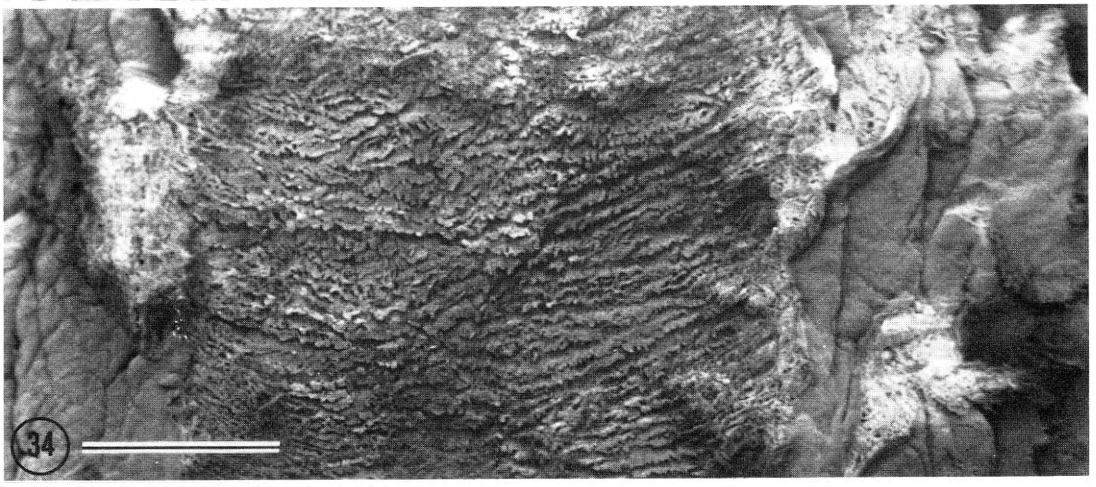

34

PLATE 11

Fig. 35. Detail of vestimental glandular epithelium. The very tall cells are densely packed with eosinophilic granules. Thin section. The scale represents 20 μm.

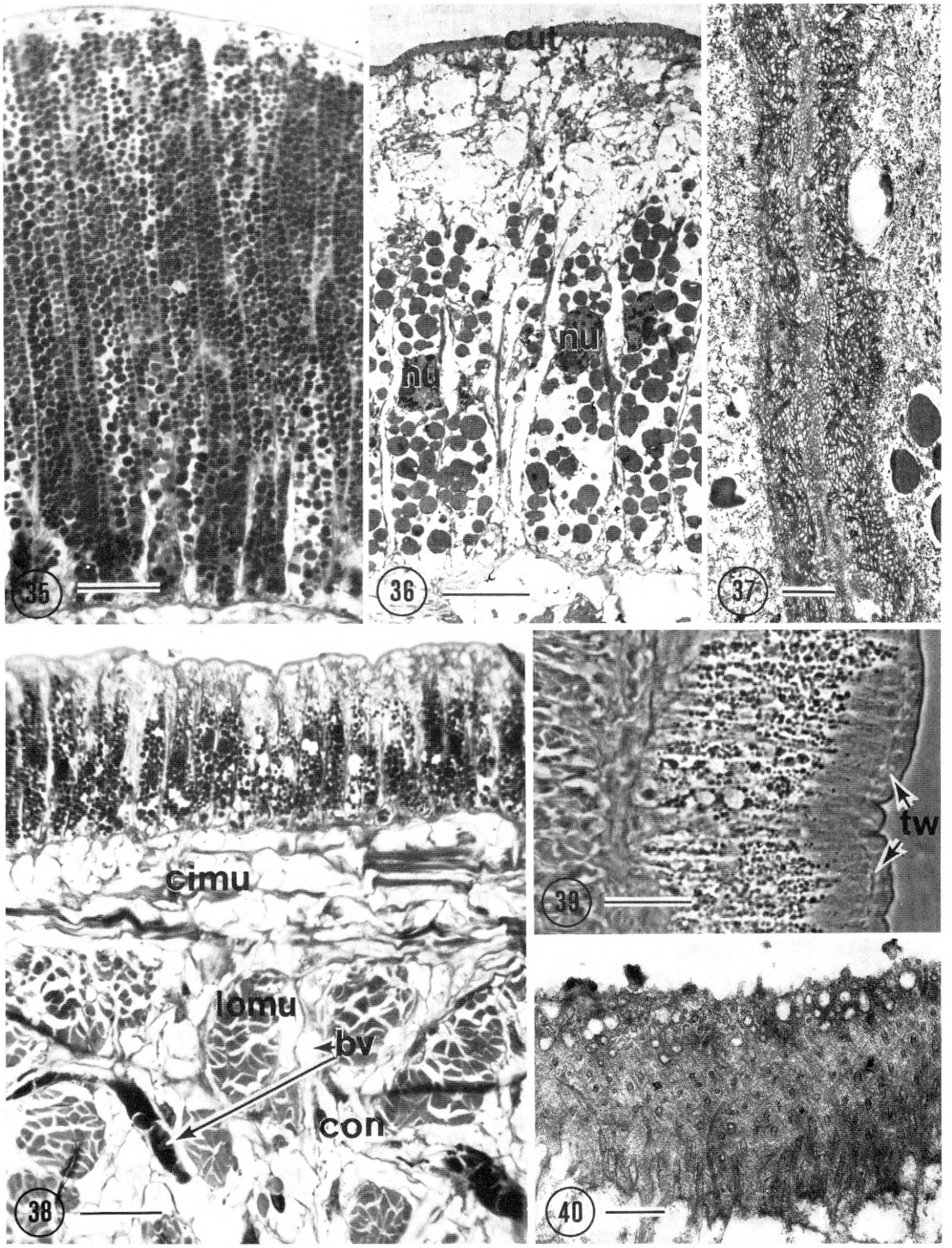
Fig. 36. Absorptive cells of ventral epithelium of vestimental wing. Electron micrograph. The nuclei are situated slightly below the middle of the cells. The lower two thirds of the cells contain numerous granules. The scale represents 10 μm.

Fig. 37. Tangential section of a fold of the "cuticle". Electron micrograph. The numerous holes in the fibrillar extra-cellular matrix represent the microvilli. Compare with Fig. 40 below. The scale represents 2 μm.

Fig. 38. Absorptive epithelium of ventral side of a vestimental wing, with underlying connective tissue, in which circular and longitudinal muscles and blood vessels can be seen. The scale represents 20 μm.

Fig. 39. Absorptive epithelium of vestimentum. Phase contrast micrograph in which the terminal web is visible as a faint line through the apical parts of the cells. The scale represents 20 μm.

Fig. 40. "Cuticle" of absorptive cell of vestimental wing. Electron micrograph showing microvilli which are embedded in an extra-cellular matrix consisting of criss-cross layers of fibrils. The scale represents 1 μm. bv = blood vessel, cimu = circular muscles, con = connective tissue, cut = cuticle, lomu = longitudinal muscle, nu = nucleus, tw = terminal web.


cut
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cimu
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PLATE 12

Pyriform glands.

Fig. 41. Gland embedded in connective tissue of vestimental wing. Its pore is situated on top of a papilla. The epithelium of the neck contains very dense nuclei. In the epithelium of the glandular sac most of the nuclei are displaced towards the apical parts of the cells and the secretion is given off into spaces between the cells (note the dark lines). Thin section. The scale represents 20 μm.

Fig. 42. Closely set glands embedded in the muscular tissue of the collar. Cross sections at various levels are represented. The scale represents 100 μm.

Fig. 43. Cross section of a gland embedded in connective tissue. Most of the nuclei are situated in the basal parts of the secretory cells and the secretion is given off from the apical parts. The scale represents 20 μm.

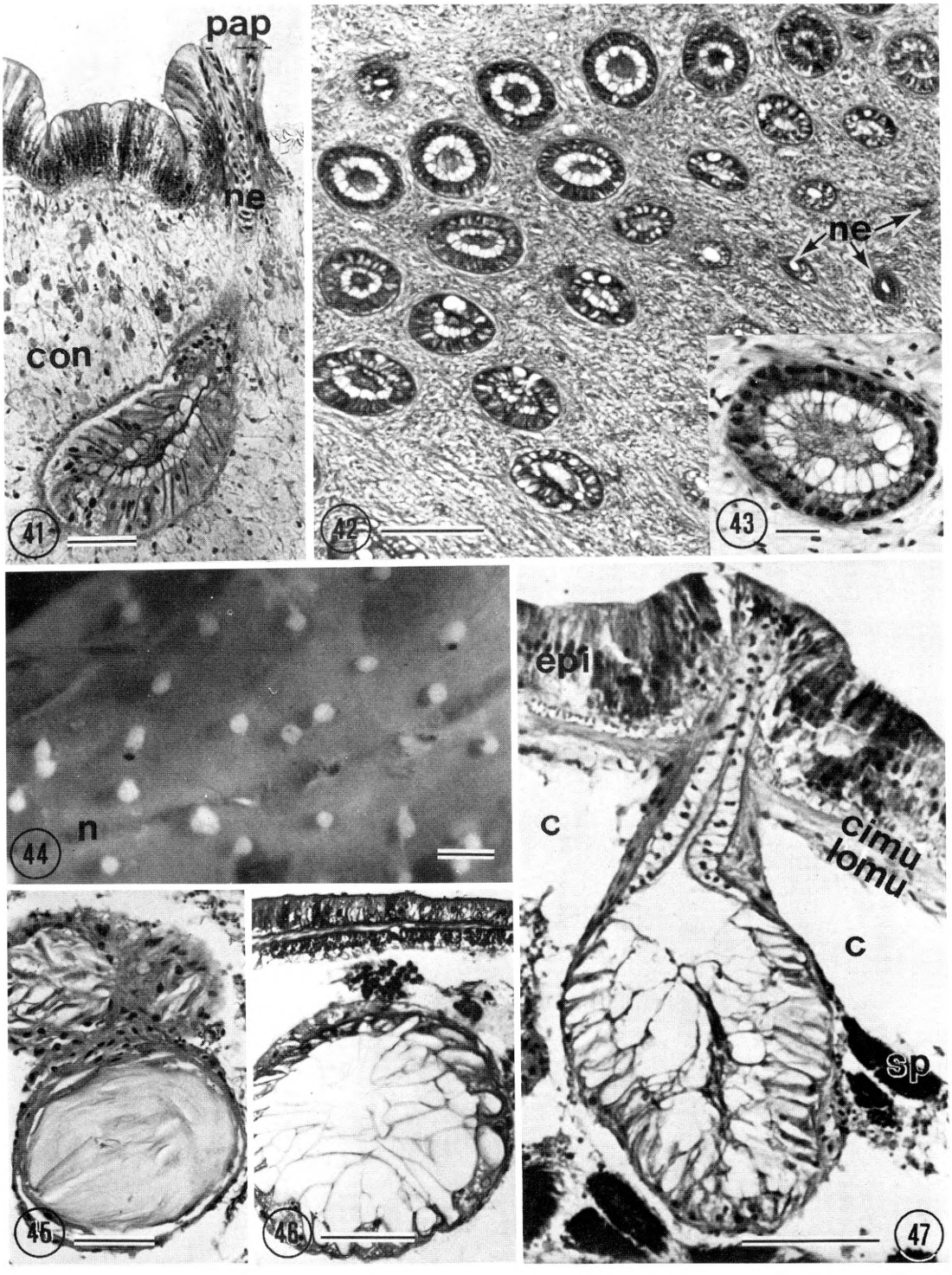
Fig. 44. Macrophotograph of the inner side of the skin of the middle part of the trunk showing several glands hanging freely in the coelom. The ventral nerve cord can be seen as a dark line. The scale represents 500 μm.

Fig. 45. Gland from posterior part of the trunk with cyst attached to it. The scale represents 50 μm.

Fig. 46. Gland from posterior part of the trunk. Thin section. The scale represents 50 μm.

Fig. 47. Gland from posterior part of the trunk. The pore is situated on top of a low papilla. The cuboidal epithelium of the neck has a distinct cuticular lining and is sharply set off from the secretory epithelium. The scale represents 100 μm.

c = coelom, cimu = circular muscles, con = connective tissue, epi = epidermis, lomu = longitudinal muscles, n = nerve cord, ne = neck, pap = papilla, sp = sperm.


pap
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41
42
ne
43
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44
epi
c
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lomu
c
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46
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PLATE 13

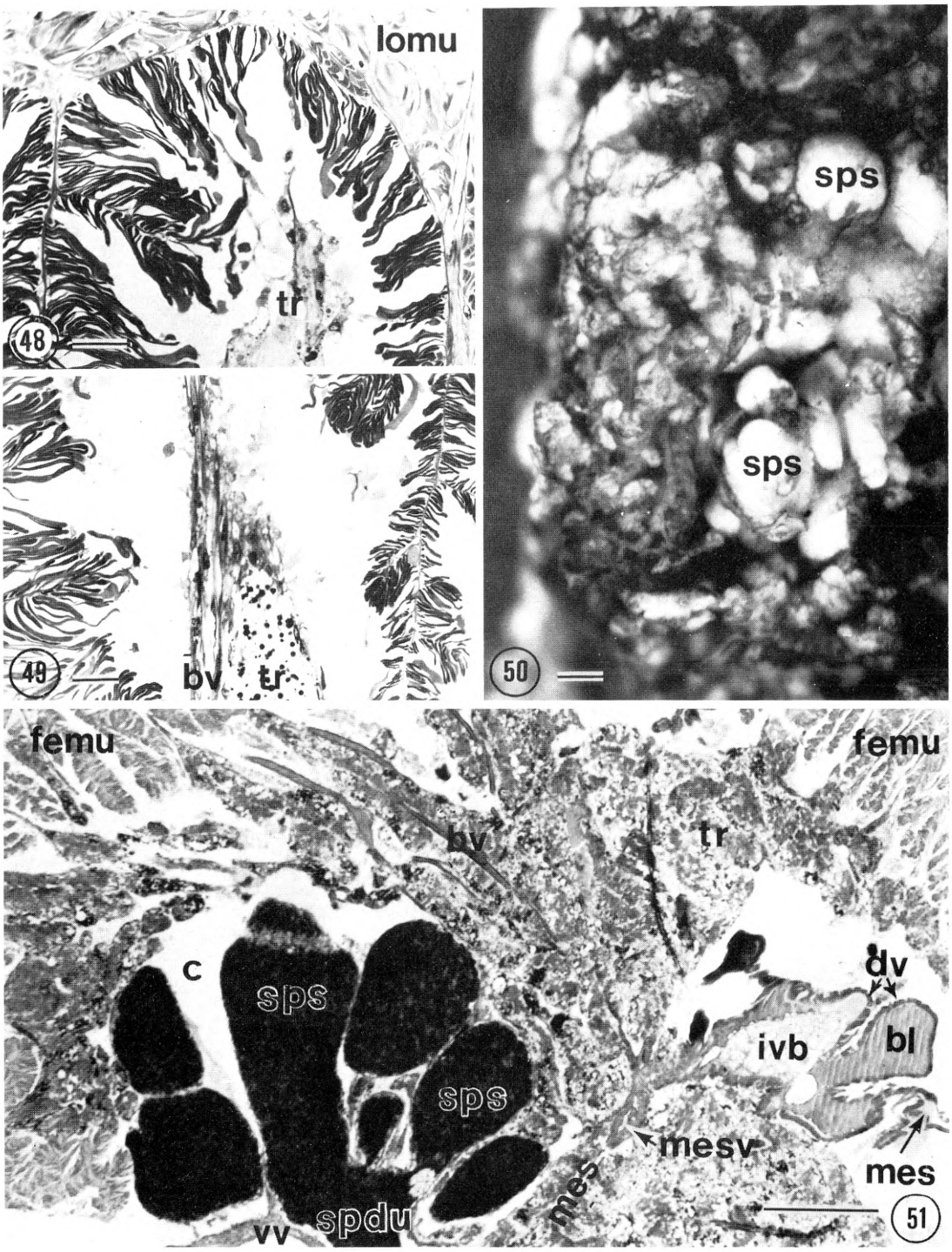
Fig. 48. Feather muscles in the anterior part of the trunk, with root-like extensions of the trophosome attached to some of the muscle cells. Thin section. The scale represents 20 μm.

Fig. 49. Feather muscles and trophosomal lobe with small blood vessels. Thin section. The scale represents 20 μm.

Fig. 50. Trophosome from the middle part of the trunk, with several (white) sperm sacs embedded in it. Macrophotograph. The scale represents 500 μm.

Fig. 51. Detail of cross section of anterior part of the trunk, showing the intricate structure of the trophosome with trophosomal blood vessels and extensions to the feather muscles. Other details include the dorsal blood vessel filled with blood and with an intravascular body, the mesenterial blood vessel, part of the ventral blood vessel, the left sperm duct communicating with the right one and with sperm sacs. The scale represents 500 μm.

bl = blood, bv = blood vessel, c = coelom, dv = dorsal vessel, femu = feather muscle, ivb = intravascular body, lomu = normal longitudinal muscles of the body wall, mes = mesentery, mesv = mesenterial vessel, spdu = sperm duct, sps = sperm sac, tr = trophosome, vv = ventral blood vessel.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bv
tr
49
sps
sps
50
femu
bv
tr
femu
c
sps
sps
dv
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bl
mesv
mes
mes
vv
spdu
51

PLATE 14

Fig. 52. Detail of trophosomal lobe, with central blood vessel surrounded by cells containing basophilic vacuoles and peripheral cells containing pigment granules. The youngest basophilic cells are closest to the blood vessel, while the oldest cells (with the largest vacuoles) are situated more peripherally. The scale represents 50 μm.

Fig. 53. Detail of trophosomal lobe in which the different types of cells are less regularly distributed. The scale represents 50 μm.

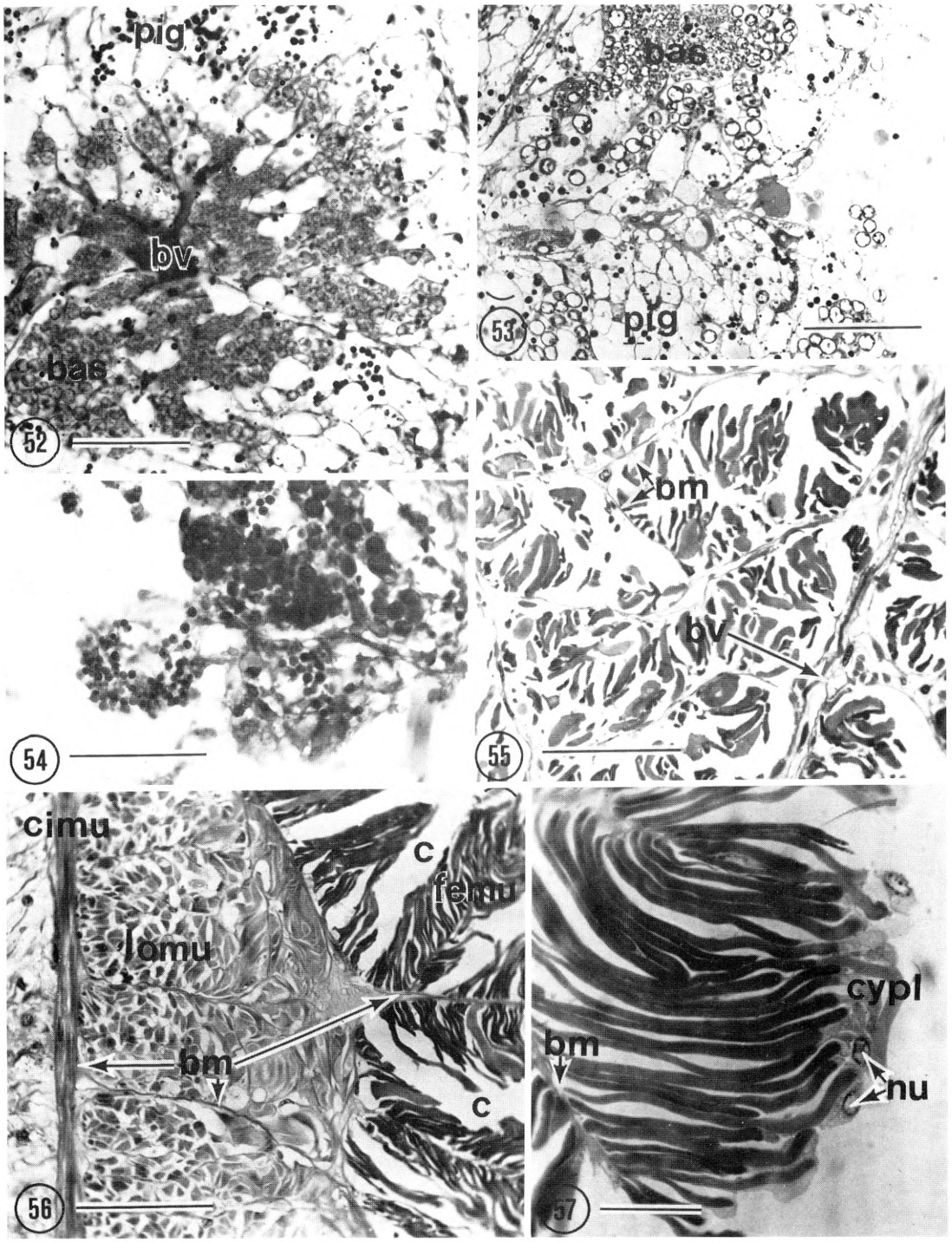
Fig. 54. Trophosomal lobe with clusters of pigment cells of different age, as defined by the size of the granules. Thin section. The scale represents 50 μm.

Fig. 55. Detail of longitudinal muscles of the vestimental region. The basement membrane forms cylinders in which the muscle cells are hanging. Thin section. The scale represents 50 μm.

Fig. 56. Feather muscles, normal longitudinal muscles, and circular muscles of the body wall in the posterior part of the vestimental region. Thin section. The scale represents 50 μm.

Fig. 57. Detail of feather muscles, showing normal cytoplasm near the outer edge of the muscle cells, in which nuclei can be seen. Thin section. The scale represents 20 μm.

bas = basophilic cells, bm = basement membrane, bv = blood vessel, c = coelom, cimu = circular muscles, cypl = cytoplasm of muscle cell, femu = feather muscle, lomu = longitudinal muscles of the body wall, nu = nucleus, pig = cells with pigment granules.


pig
bv
bas
52
bas
pig
53
54
bm
bv
55
cimu
c
femu
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bm
c
56
cypl
bm
nu
57

PLATE 15

The dorsal blood vessel.

Fig. 58. Empty vessel in the posterior part of the trunk, hanging freely in the coelom. It is attached to the body wall by the mesentery, but not to the trophosome. The vessel is surrounded by coelomic epithelium, but not by muscles as in the next figures. The scale represents 20 μm.

Fig. 59. Empty vessel in the posterior part of the vestimental region at the posterior end of the perivascular coelom. The coelomic cavity is surrounded by a sphincter. The wall of the vessel consists of muscle cells for the greater part, but on the dorsal side there are numerous very small cells instead of muscle cells. Dorsal to the vessel the perivascular coelom is partly filled with mesenchymatous tissue. The scale represents 200 μm.

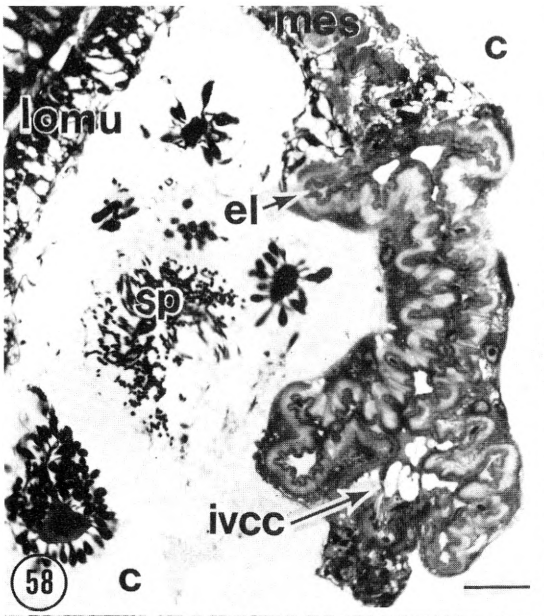
Fig. 60. Vessel in the vestimental region filled with blood. The scale represents 100 μm.

Fig. 61. Section in which the elastica was cut obliquely, showing the striation of the inner layer. The scale represents 20 μm.

Fig. 62. Oblique section of the wall of the vessel, showing the structure of the endothelium. The scale represents 50 μm.

Fig. 63. Oblique section of the wall of the vessel, showing that the striation of the inner layer of the elastica is caused by the presence of closely set ridges. The scale represents 20 μm.

bl = blood, c = coelom, el = elastica, ivcc = intravascular cell cord, lomu = longitudinal muscles, mes = mesentery, mu = muscle cells of the wall of the vessel, pvc = perivascular coelom, sp = sperm, sph = sphincter.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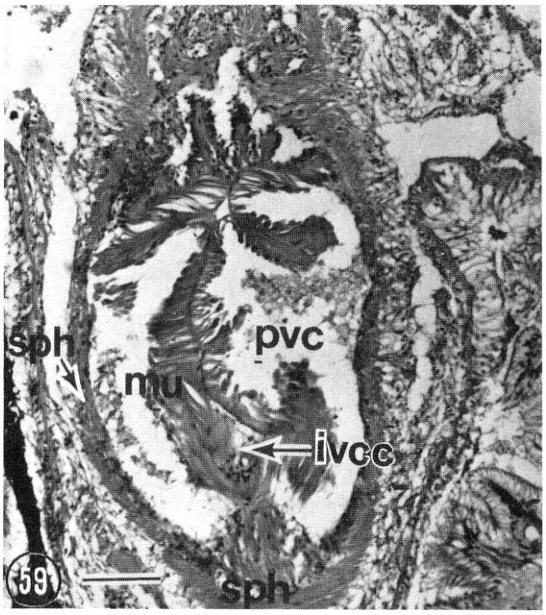
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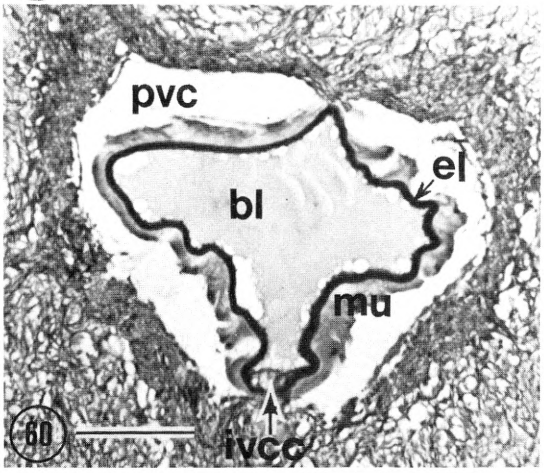
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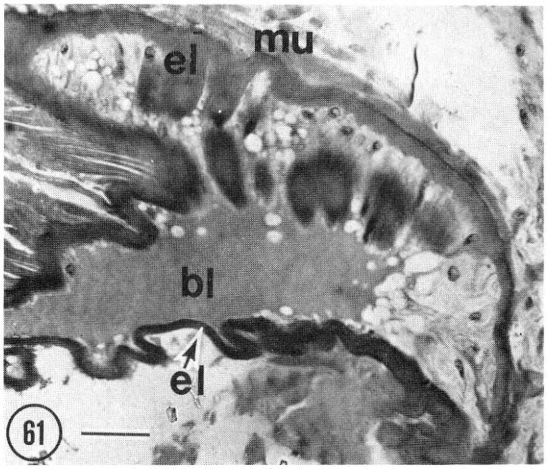
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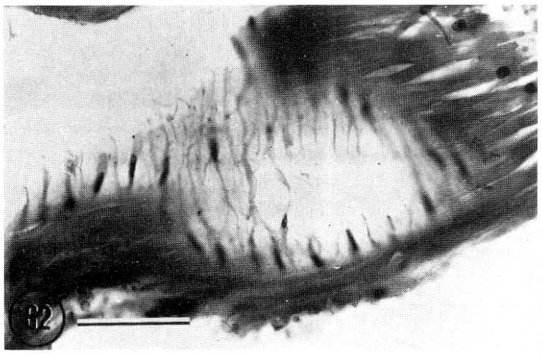
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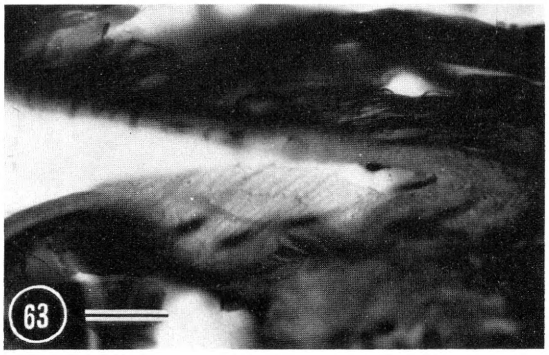

63

PLATE 16

Fig. 64. Dorsal blood vessel in the posterior part of the trunk, nearly completely blocked by an intravascular body. The cells of the intravascular body look particularly empty in this section and only very few nuclei can be seen (compare next figure). Some strands of dark cells are present. The scale represents 100 μm.

Fig. 65. Dorsal vessel partly filled with blood and with a rather large intravascular body. The latter has a particularly large intercellular cavity filled with coagulated, blood-like fluid. The cells are relatively small (compare fig. 64) and not much vacuolated. The scale represents 100 μm.

Fig. 66. Detail of intravascular body with endothelial cells covering its surface. The scale represents 20 μm.

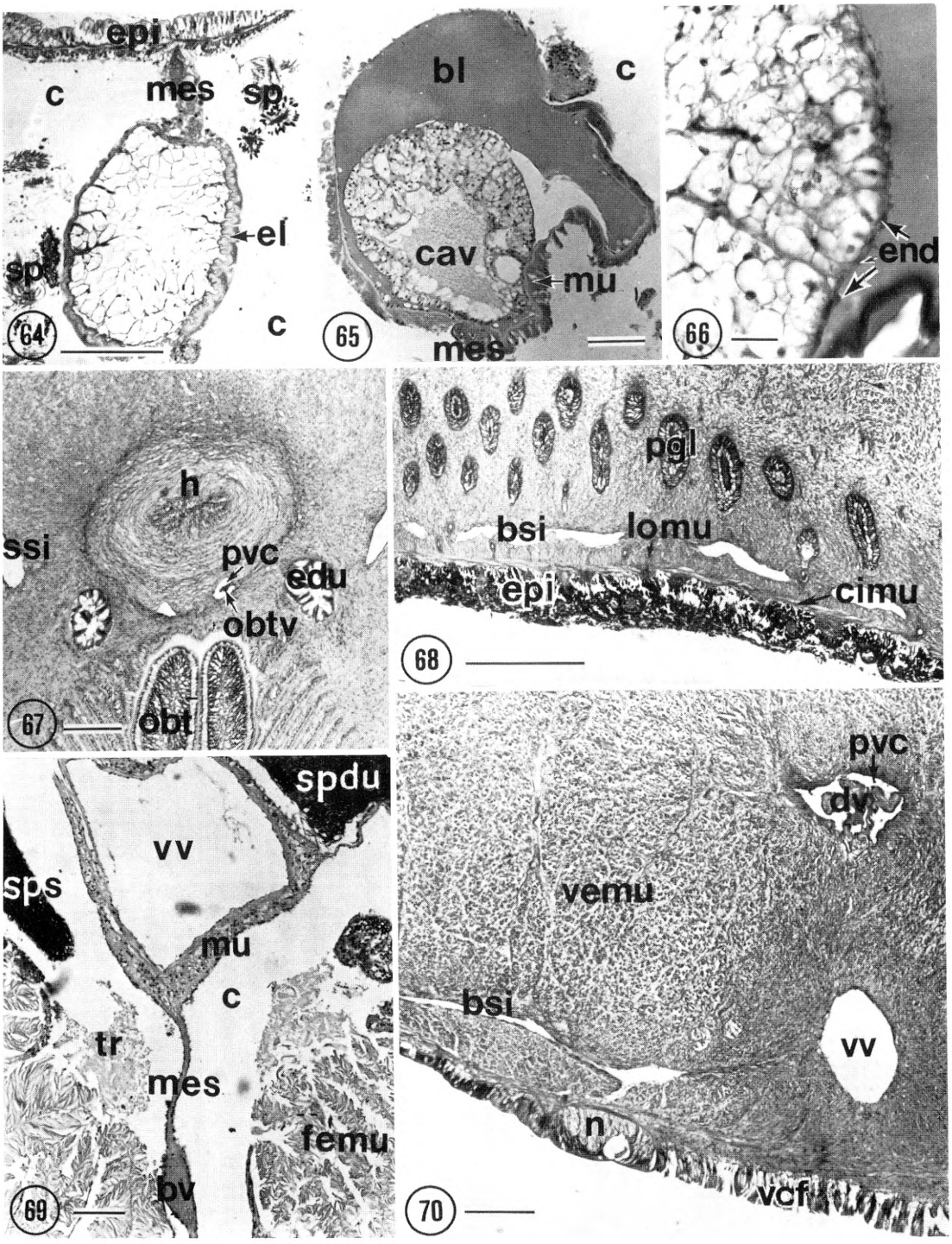
Fig. 67. Cross section through the heart, embedded in the muscular tissue of the front end of the body. Compare with Plate 7 for better orientation. The scale represents 200 μm.

Fig. 68. Subdermal blood sinuses and pyriform glands on the ventral side of a vestimental wing. The scale represents 500 μm.

Fig. 69. Ventral vessel, and blood vessel lying in the mesentery and running from the ventral vessel to the ventral body wall. The musculature of the wall of the blood vessel is poorly developed, when compared with the dorsal vessel in the same region (Plate 15:59). The scale represents 100 μm.

Fig. 70. Part of a cross section through the vestimental region, showing the dorsal and the ventral vessel (both empty) and subdermal blood sinuses leading from the ventral vessel to a vestimental wing. The scale represents 200 μm.

bl = blood, bsi = blood sinus, bv = blood vessel, c = coelom, cav = intercellular cavity in intravascular body, cimu = circular muscles, dv = dorsal vessel, edu = excretory duct, end = endothelium, el = elastica, epi = epidermis, femu = feather muscles, h = heart, lomu = longitudinal muscles, mes = mesentery, mu = muscles of the wall of the blood vessel, n = ventral nerve cord, obt = obturaculum, obtv = obturacular blood vessel, pgl = pyriform gland, pvc = perivascular coelom, sp = sperm, spdu = sperm duct, sps = sperm sac, ssi = subtentacular sinus, tr = trophosome, vcf = ventral ciliary field, vemu = vestimental muscles, vv = ventral vessel.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pgl
bsi
lomu
epi
cimu
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spdu
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mu
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tr
mes
femu
pv
69
pvc
dv
vemu
bsi
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70

PLATE 17

Details of various blood vessels.

Fig. 71. Longitudinal section of a trophosomal blood vessel. The scale represents 20 μm.

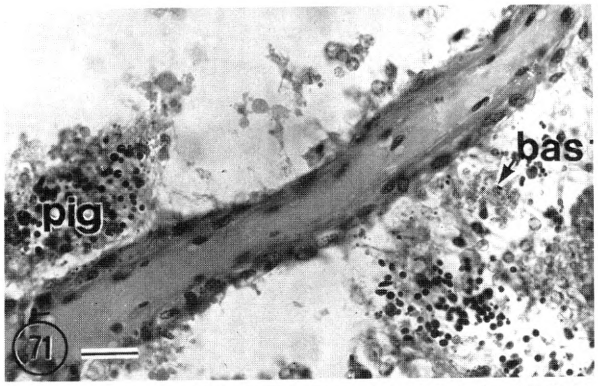
Fig. 72. Oblique section of a small vessel in the spring tissue of a vestimental wing, close to a longitudinal muscle. The scale represents 20 μm.

Fig. 73. Small blood vessel running underneath the glandular epithelium in the connective tissue of a vestimental wing. The scale represents 20 μm.

Fig. 74. Small blood vessels in the longitudinal musculature of the posterior part of the vestimental region, running in the basement membrane of the muscle cells. The scale represents 20 μm.

Fig. 75. Part of a cross section through the vestimental region, showing dorsal and ventral vessels. The dorsal vessel contains a large intravascular body. The ventral vessel lies in a bundle of dorsoventral muscles interspersed with connective tissue, running from the dorsal vessel to the ventral body wall, where it fans out. Thin section. The scale represents 250 μm.

bas = basophilic cells, bl = blood, cimu = circular muscles, con = connective tissue, dv = dorsal vessel, dvmu = dorsoventral muscles, femu = feather muscles, ivb = intravascular body, lomu = longitudinal muscle, pig = pigment cells, vcf = ventral ciliary field, vegl = vestimental glandular epithelium, vemu = vestimental muscles, vv = ventral vessel.


pig
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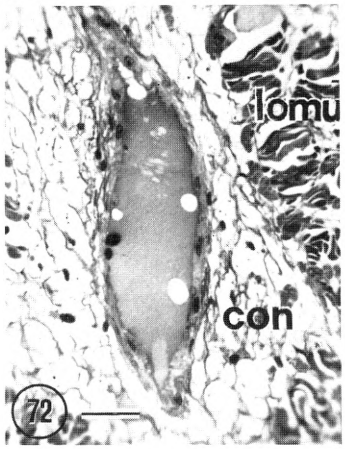
71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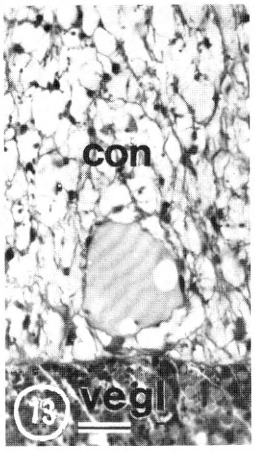
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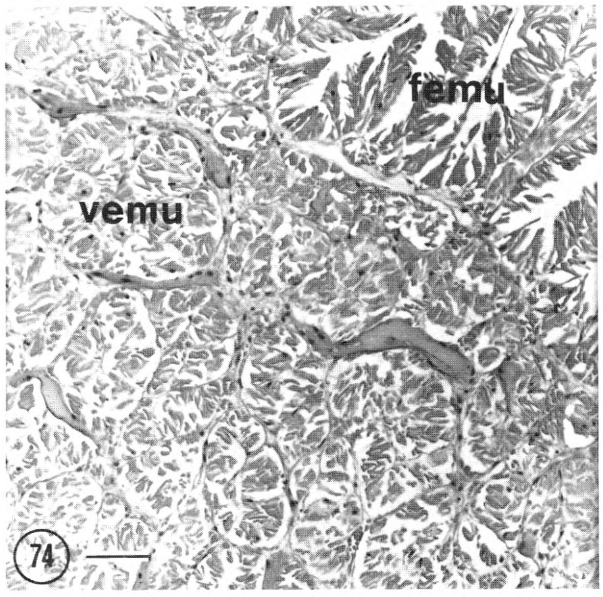
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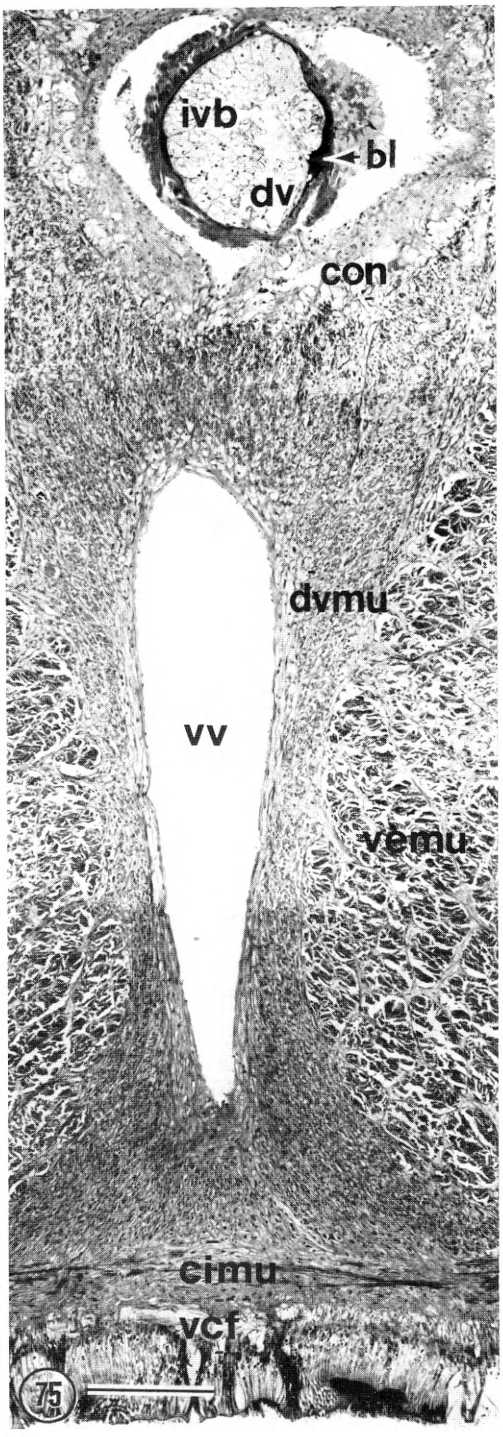
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bl
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cimu
vcf
75

PLATE 18

Excretory system.

Fig. 76. Detail of excretory tree. The scale represents 20 μm.

Fig. 77. Detail of excretory duct. The scale represents 20 μm.

Fig. 78. Cross section through common excretory duct just before it opens to the outside. The scale represents 100 μm.

Fig. 79. Tubules entering an excretory duct. The scale represents 100 μm.

Fig. 80. Cross section through brain and excretory system at level where excretory tubules enter the excretory ducts. The scale represents 200 μm.

b = brain, cedu = common excretory duct, cil = cilia, cut = cuticle, dv = dorsal vessel, edu = excretory duct, etr = excretory tree, etu = excretory tubule, obt = obturaculum, ri = ridge, tenc = tentacular coelom, vemu = vestimental muscles, vlv = ventrolateral vessel.

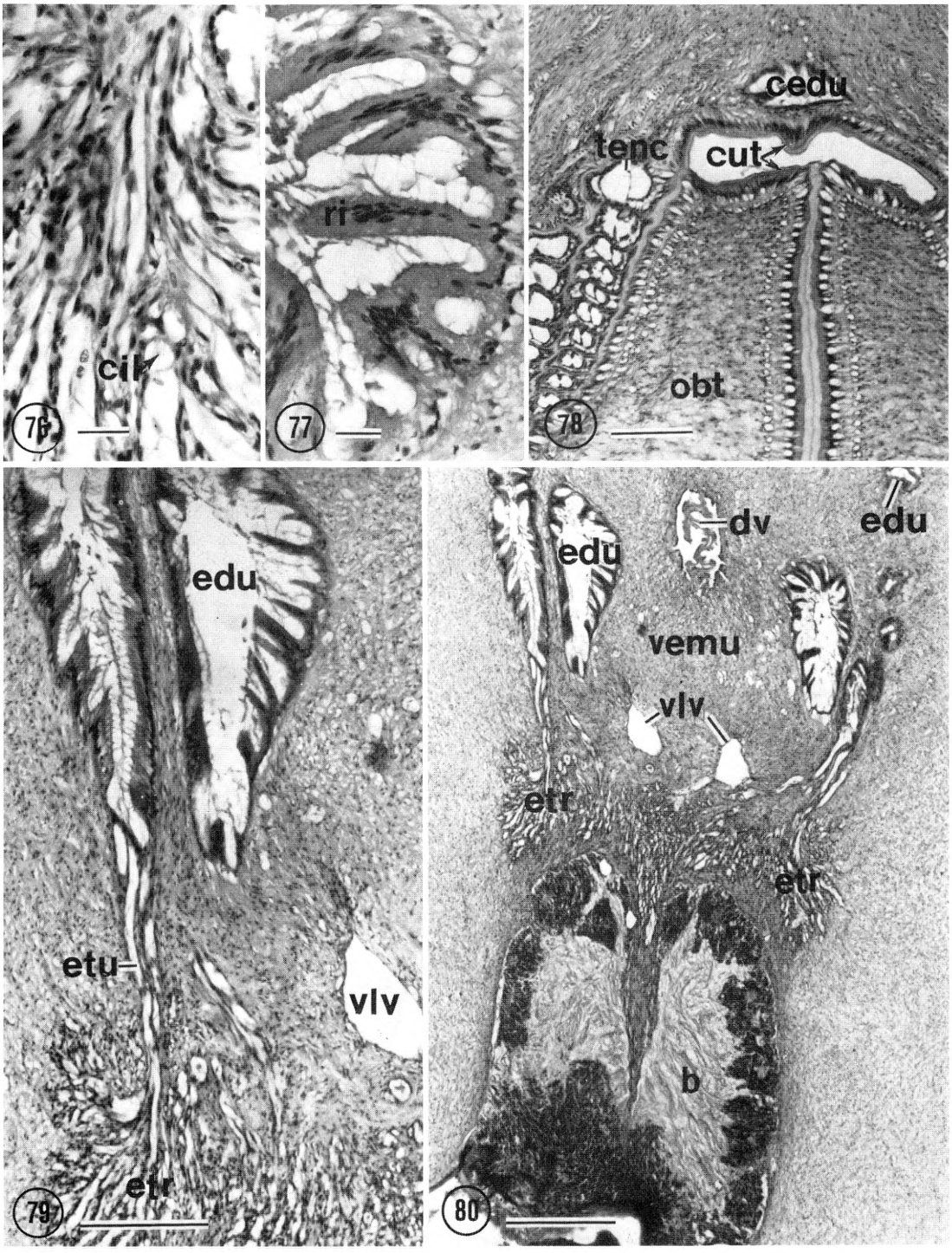
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PLATE 19

Nervous system.

Fig. 81. Cross section through the anteriormost part of the brain. The ganglionic cells lying at the bases of the obturacula can be seen in the middle. Groups of ganglionic cells are also present at the bases of the tentacles. The irregular black structure at the bottom is the thick cuticular plate which extends from the obturacula over the ventral surface of the brain. The scale represents 200 μm.

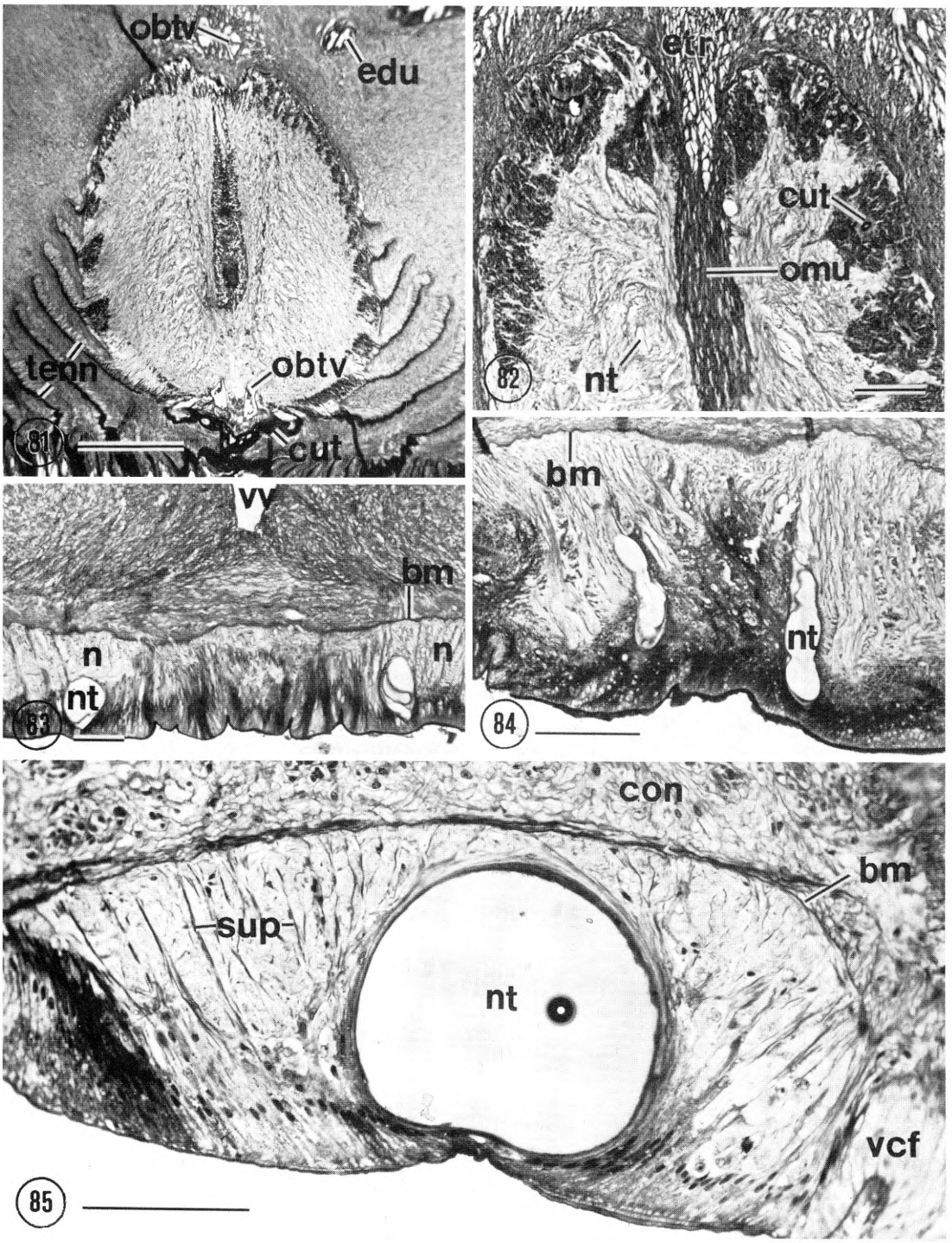
Fig. 82. Cross section through the posterior part of the brain where it is penetrated by the obturacular muscle. The neurular tubes lie in the middle of the two masses of nerve fibres. A lumen surrounded by a cuticle is present in some of the groups of ganglionic cells. The scale represents 100 μm.

Fig. 83. Ventral nerve cord, somewhat posterior to the brain. The two halves are still lying close together and are connected by numerous nerve fibres. The scale represents 200 μm.

Fig. 84. Ventral nerve cord, still closer to the brain. The two neurular tubes dive deeper into the nervous tissue at this level. The scale represents 200 μm.

Fig. 85. Thin section of half of the ventral nerve cord in the region of the ciliary field. A few cells of the latter are visible in the right corner. The scale represents 100 μm.

bm = basement membrane, con = connective tissue, cut = cuticle, edu = excretory duct, etr = excretory tree, n = nerve cord, nt = neurular tube, omu = obturacular muscle, obtv = obturacular vessel, sup = subnuclear part of supporting cells, tenn = tentacular nerve, vcf = ventral ciliary field, vv = ventral vessel.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edu
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81
cut
etr
cut
omu
82
nt
vv
bm
n
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nt
83
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nt
84
con
bm
sup
nt
vcf
85

PLATE 20

Reproductive system.

Fig. 86. Sperm ducts and ventral vessel in the posterior part of the trunk. Sperm is present in these three ducts as well as in the coelom. Clusters of degenerating sperm are attached to the mesentery and the trophosome. The scale represents 100 μm.

Fig. 87. Thin section of testicular tubules with developing spermatozoa, between two trophosomal lobes. The scale represents 20 μm.

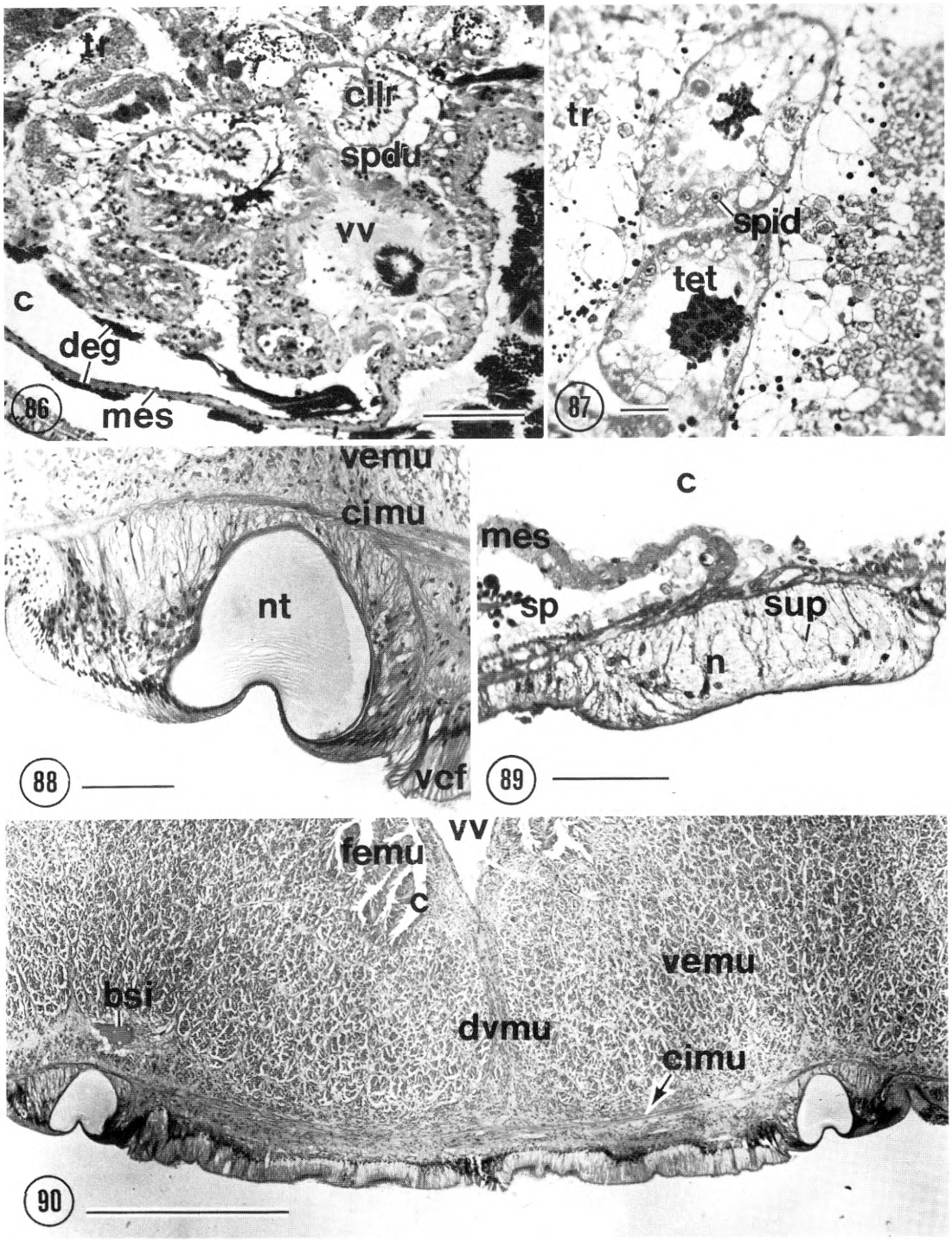
Nervous system.

Fig. 88. Half of the ventral nerve cord in the posterior part of the vestimental region. Part of the ventral ciliary field is visible in the right corner. The scale represents 100 μm.

Fig. 89. Thin section of the ventral nerve cord in the posterior part of the trunk. The scale represents 50 μm.

Fig. 90. Ventral ciliary field and ventral nerve cord in the posterior part of the vestimental region. The scale represents 500 μm.

bsi = blood sinus, c = coelom, cilr = ciliated ridge, cimu = circular muscles, deg = degenerating sperm, dvmu = dorsoventral muscles, femu = feather muscles, mes = mesentery, n = nerve cord, nt = neurular tube, sp = sperm, spdu = sperm duct, spid = spermatid[?], sup = supporting cells, tet = testicular tubule, tr = trophosome, vcf = ventral ciliary field, vemu = vestimental muscles, vv = ventral vessel.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90

PLATE 21

Reproductive system.

Fig. 91. The ciliated ridges of the common sperm duct in the posterior part of the vestimental region. The scale represents 100 μm.

Fig. 92. The completely ciliated part of one of the ducts connecting the sperm duct with the gonopore. The scale represents 100 μm.

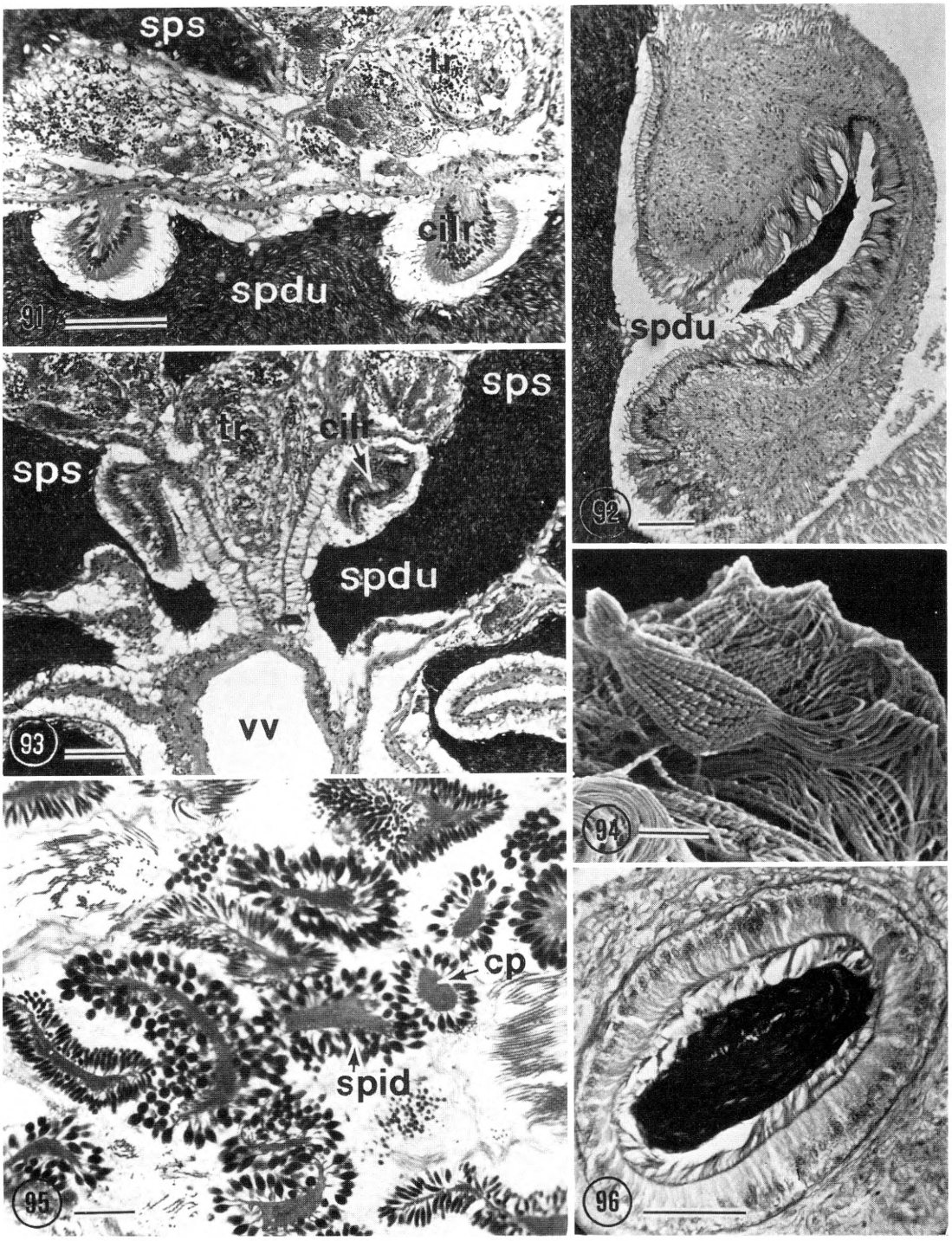
Fig. 93. Ventral vessel and sperm ducts in the anterior part of the trunk. Each sperm duct communicates with a sperm sac. The scale represents 100 μm.

Fig. 94. Scanning electron micrograph of sperm taken from the coelom. The anterior regions, probably acrosomes, are closely applied. The spirally wound median region corresponds to the nucleus, probably in connection with the presence of mitochondria. There is no mid piece, and the tails are seen to the right. The scale represents 20 μm.

Fig. 95. Several stages of spermatogenesis in the coelom of the posterior part of the trunk. Thin section. The scale represents 20 μm.

Fig. 96. Cross section of completely ciliated sperm duct close to the gonopore filled with sperm. The basal nuclei of the epithelial cells are surrounded by numerous PAS-positive granules and the apical parts of the cells are filled with rootlets of the cilia. The scale represents 100 μm.

cilr = ciliated ridge, cp = cytophore, spdu = sperm duct, spid = spermatid with oval nucleus, sps = sperm sac, tr = trophosome, vv = ventral vessel.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96

PLATE 22

Details of horizontal sections of the obturacula

Fig. 97. A curved tip, where the epidermis as well as the cuticle become very thin. Note the cavities in the cuticle, which are shown better in Fig. 102. The scale represents 100 μm.

Fig. 98. In the middle region the cuticle is extremely thick. Left and right obturacula are still fused. The scale represents 200 μm.

Fig. 99. In the anterior half the coiled obturacular coelom widens considerably. The scale represents 500 μm.

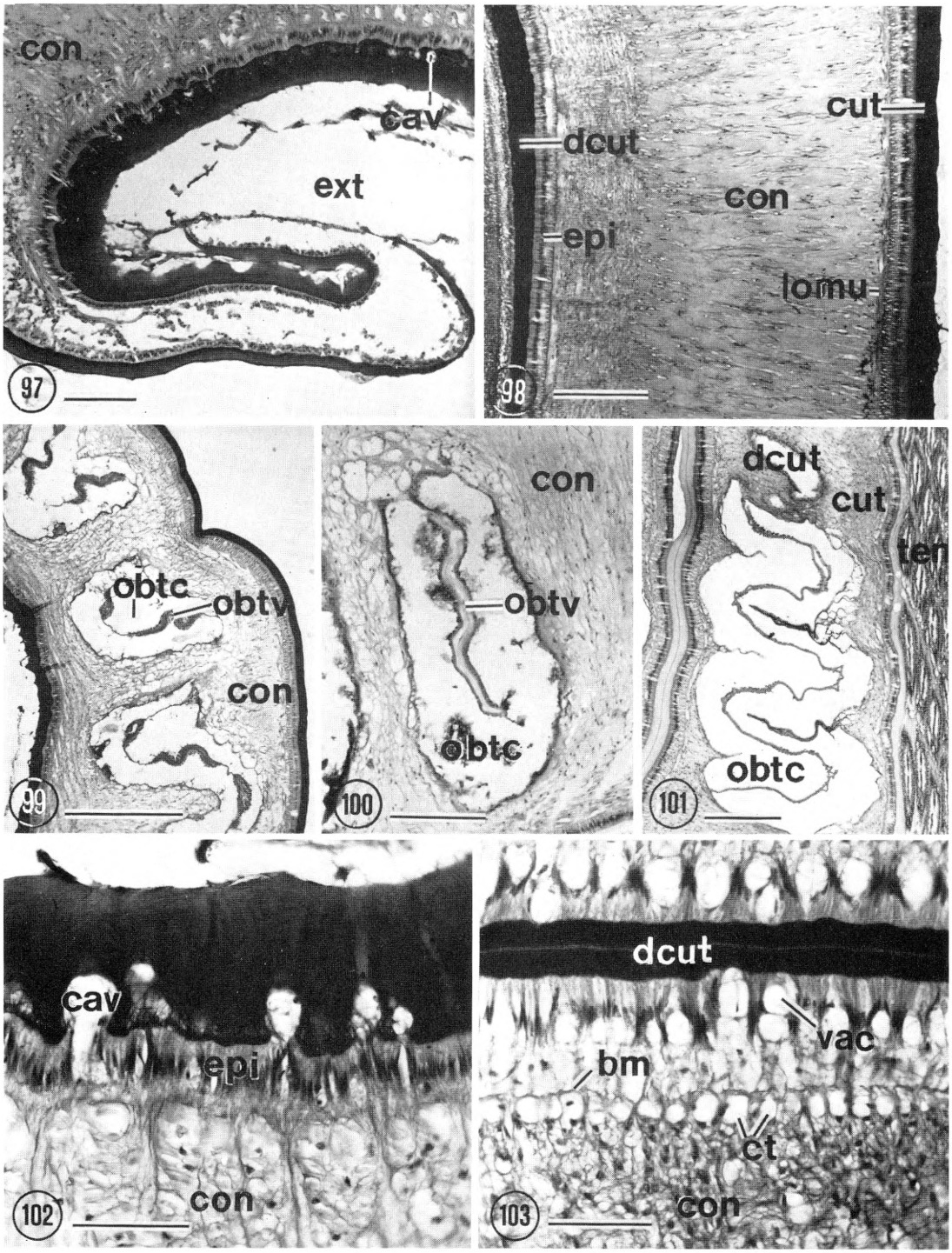
Fig. 100. Detail of obturacular coelom with obturacular blood vessel. The scale represents 200 μm.

Fig. 101. The same. The scale represents 500 μm.

Fig. 102. Cuticle and epidermis close to the tip of the organ (compare Fig. 97). Several cavities are present and in most of them nuclei of specialized cells can be seen. The scale represents 50 μm.

Fig. 103. Epidermis and double cuticle of the basal part. Numerous large vacuoles or vacuole-like spaces are present in or between the epidermal cells. Coelomic(?) tubes are present just below the basement membrane. The scale represents 50 μm.

bm = basement membrane, cav = cavity, con = connective tissue, ct = coelomic(?) tubes, cut = cuticle, dcut = double cuticle, epi = epidermis, ext = exterior, lomu = longitudinal muscles, nu = nucleus, obtc = obturacular coelom, obtv = obturacular blood vessel, ten = tentacles, vac = vacuoles (or vacuole-like cavities).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ten
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103

PLATE 23

Details of tentacles and obturacula.

Fig. 104. Cross section of a number of fused tentacles. The scale represents 100 μm.

Fig. 105. Connective tissue of an obturaculum with cells in extended condition. The scale represents 50 μ.

Fig. 106. The same with coiled cells. The scale represents 50 μm.

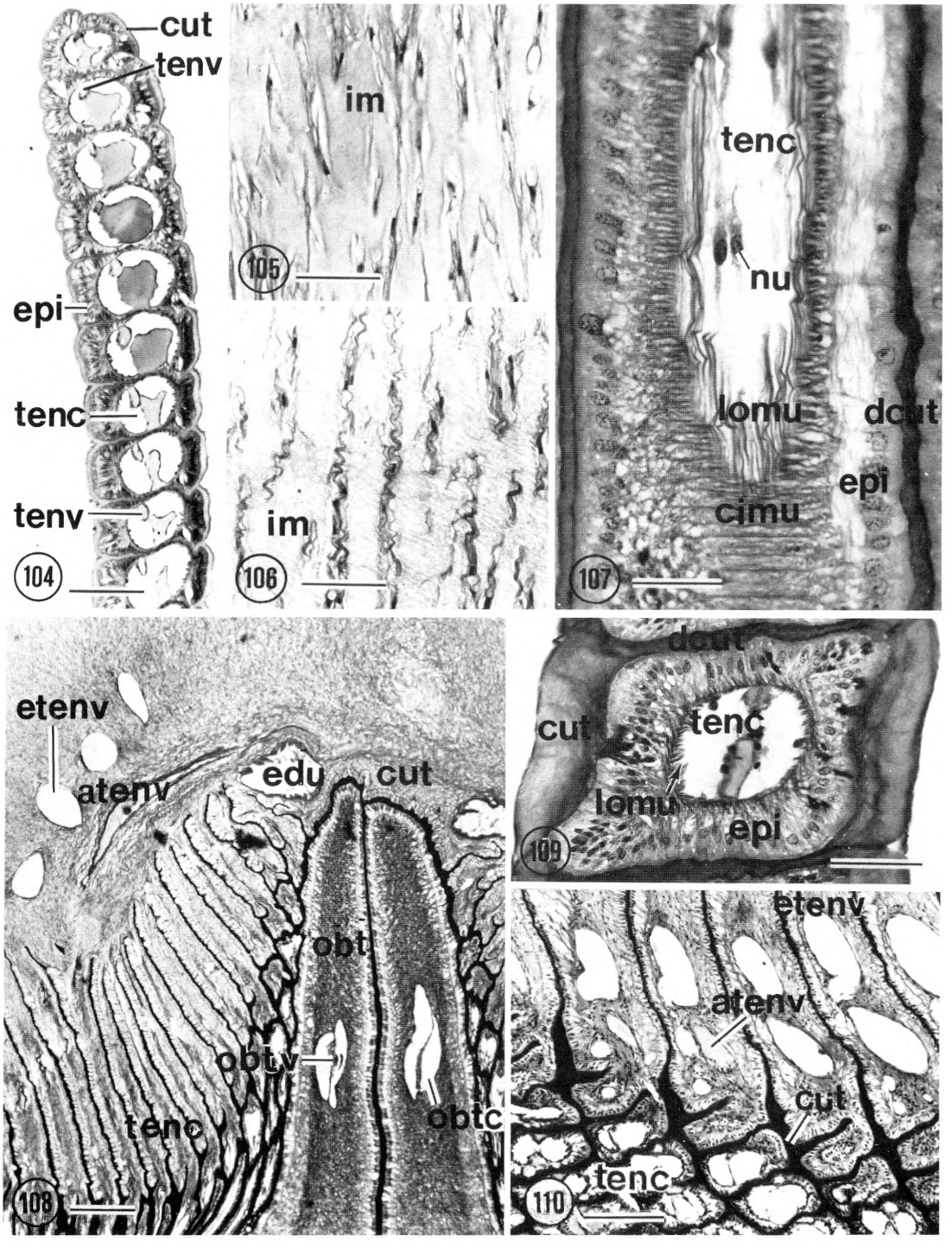
Fig. 107. Tangential longitudinal section of a tentacle. The scale represents 50 μm.

Fig. 108. Tangential section of the bases of the two obturacula and part of the tentacles. The black lines all represent the cuticle, which has penetrated deeply into the tissue of the anterior part of the body. The scale represents 200 μm.

Fig. 109. Cross section of one element of a tentacular lamella. Thin section. The scale represents 50 μm.

Fig. 110. Tangential section of the bases of a number of tentacles. When the tentacles of one series fuse completely the tentacular coeloms disappear and the efferent blood vessels widen considerably. The scale represents 100 μm.

atenv = afferent tentacular blood vessel, cimu = circular muscle fibres, cut = cuticle, dcut = double cuticle, edu = excretory duct, epi = epidermis, etenv = efferent tentacular blood vessel, im = inter-cellular matrix, lomu = longitudinal muscle fibres, nu = nucleus, obt = obturaculum, obtc = obturacular coelom, obtv = obturacular blood vessel, tenc = tentacular coelom, tenv = tentacular blood vessels (afferent or efferent).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edu
cut
obt
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obtc
108
dcut
cut
tenc
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epi
109
etenv
atenv
cut
tenc
110

PLATE 24

Details of the tentacles.

Fig. 111. Cross section of a number of tentacles in the area where they are still fused all around. There are only narrow spaces between the first and second series from the left. In each of the tentacles of the left series there are two groups of ciliated cells, standing out by their darker cytoplasm (compare Fig. 113). The scale represents 100 μm.

Fig. 112. Cross section of two tentacles on the same level. The scale represents 100 μm.

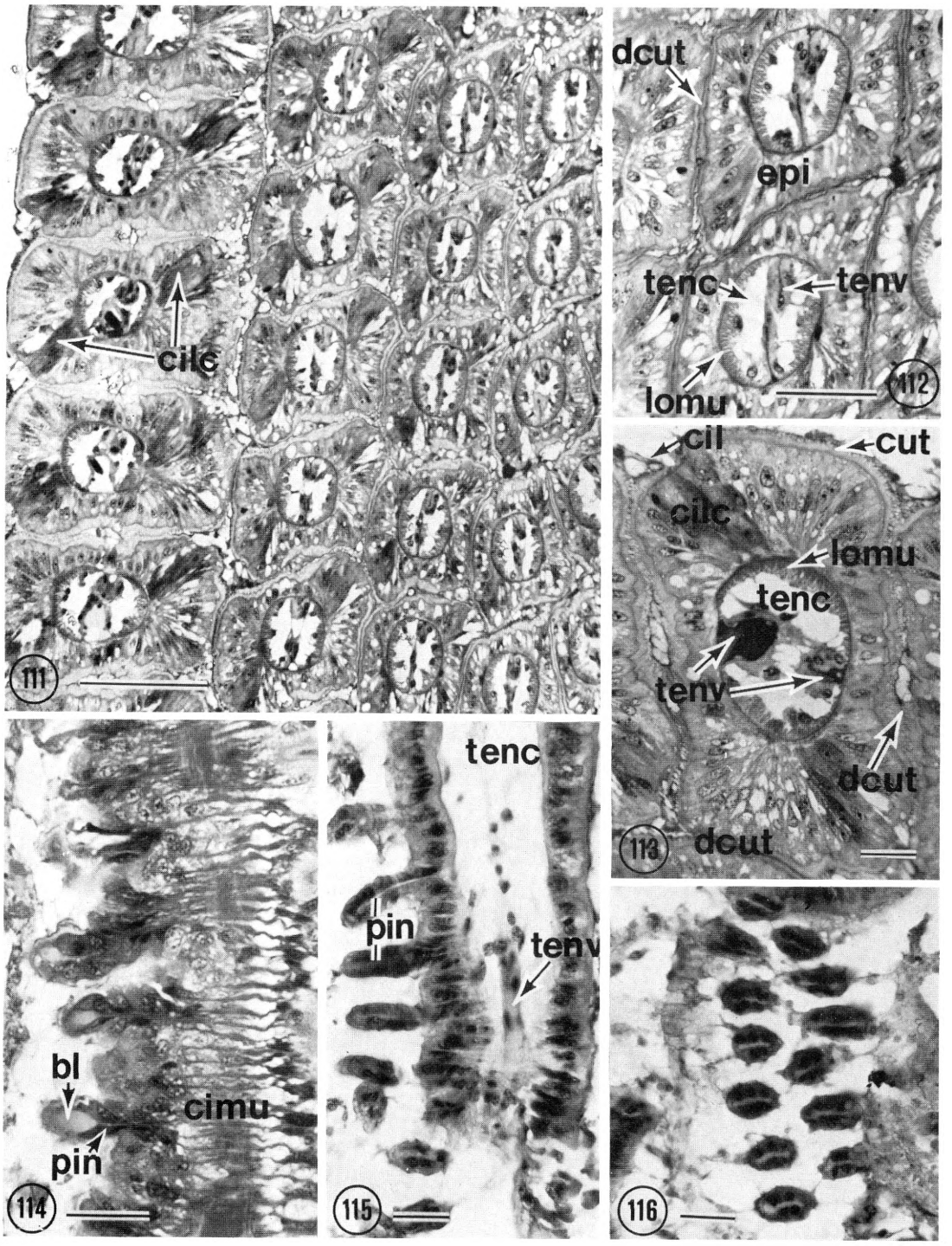
Fig. 113. Cross section of the middle of a tentacle with a ciliated groove. Thin section. The scale represents 20 μm.

Fig. 114. Longitudinal section of the epidermis of the distal part of a tentacle with pinnules. The scale represents 20 μm.

Fig. 115. Longitudinal section (slightly oblique) of the distal part of a tentacle with pinnules. The scale represents 20 μm.

Fig. 116. Cross section of a group of pinnules, arranged in two rows. The light area in the centre of each pinnule represents the blood sinus. The scale represents 20 μm.

bl = blood, cil = cilia, cilc = ciliated cells, cimu = circular muscle fibres, cut = cuticle, dcut = double cuticle, epi = epidermis, lomu = longitudinal muscle fibres, pin = pinnule, tenc = tentacular coelom, tenv = tentacular blood vessel (afferent or efferent).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bl
cimu
pin
114
tenc
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115
116

PLATE 25

Details of the vestimentum.

Fig. 117. The vestimental ridges on the anterior part of the vestimental region in the area where they lie closest together and where the vestimental grooves move to the abaxial sides of the ridges. The scale represents 200 μm.

Fig. 118. Cross section of the left vestimental ridge. In the abaxial half there are two separate groups of blood sinuses, one empty and one filled with blood. In the adaxial half there are only a few very small, empty sinuses. The scale represents 200 μm.

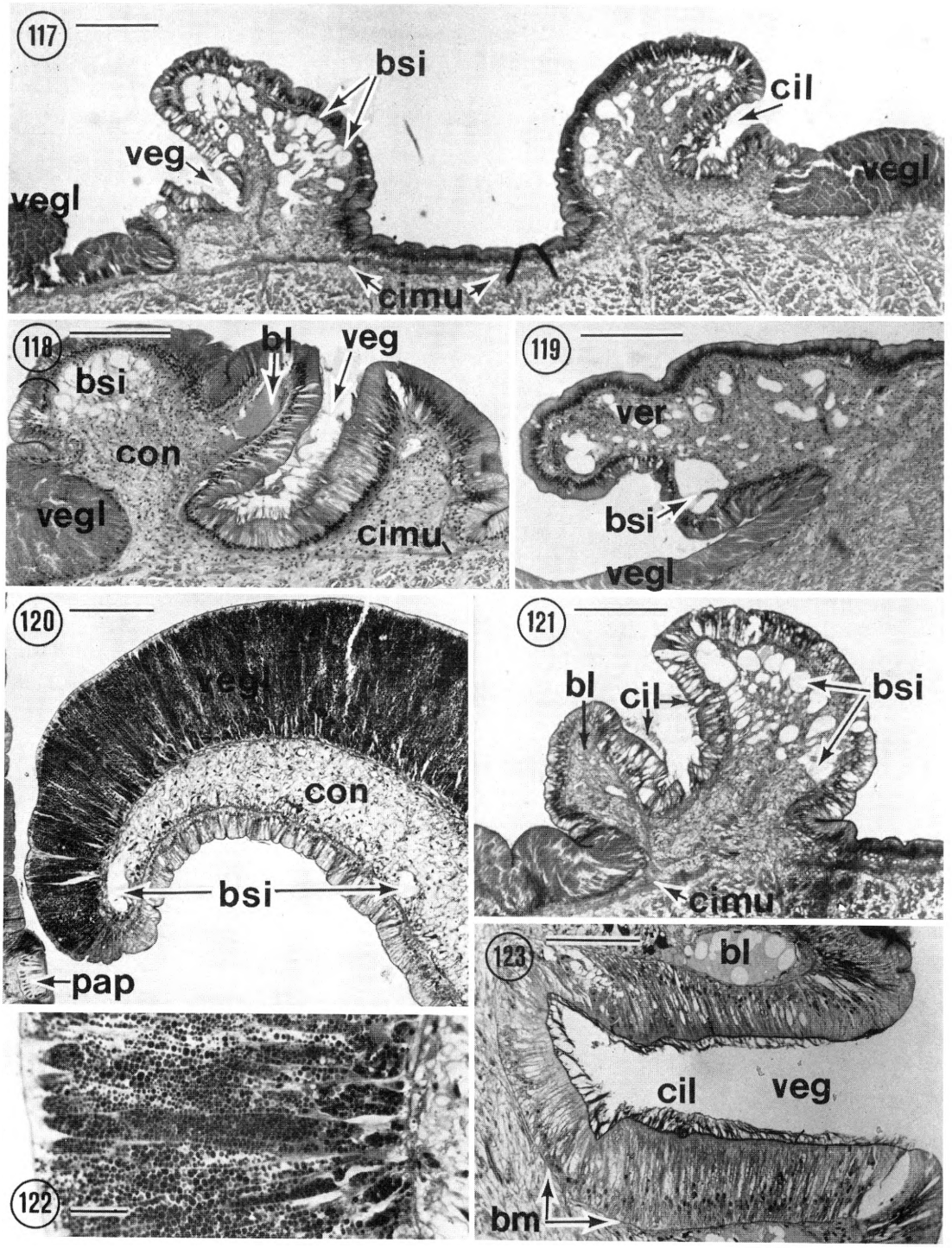
Fig. 119. Cross section of the left vestimental ridge close to the anterior end of the ciliated groove. The scale represents 200 μm.

Fig. 120. Cross section of the curved border of a vestimental wing. Thin section. The scale represents 100 μm.

Fig. 121. Cross section of the left vestimental ridge in the anterior part of the vestimental region. Contrary to the situation in Fig. 118 above there are numerous blood sinuses in the adaxial half and very few in the abaxial half of the ridge. The scale represents 200 μm.

Fig. 122. Detail of the vestimental glandular epithelium. Thin section. The scale represents 20 μm.

Fig. 123. Thin section of the ciliated epithelium of a vestimental groove. The scale represents 100 μm. bl = blood, bm = basement membrane, bsi = blood sinus, cil = cilia, cimu = circular muscles, con = connective tissue, pap = papilla, veg = vestimental groove, vegl = vestimental glandular epithelium, ver = vestimental ridge.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vegl
120
vegl
con
bsi
pap
121
bl
cil
bsi
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122
123
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cil
veg
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PLATE 26

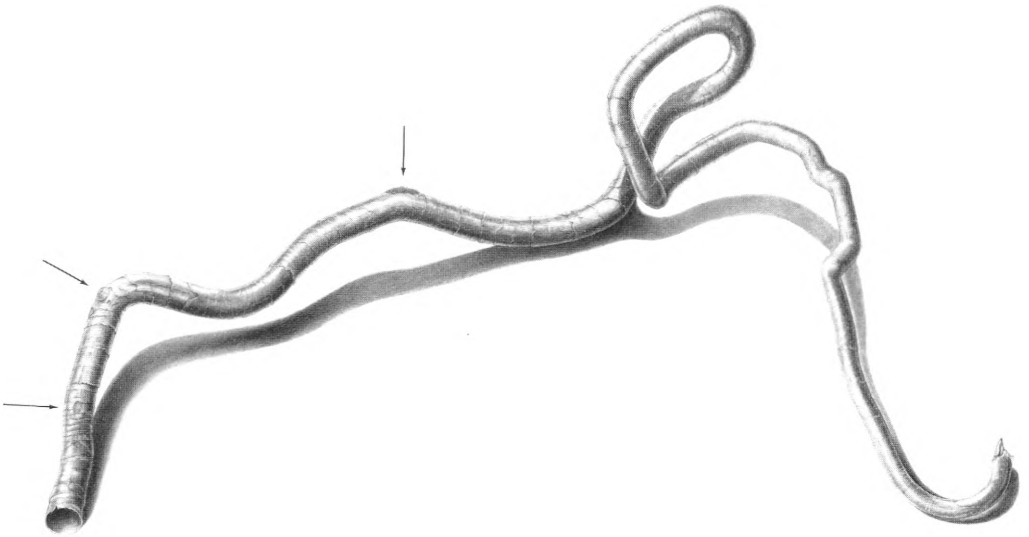
The tube. The scale represents 2 mm.

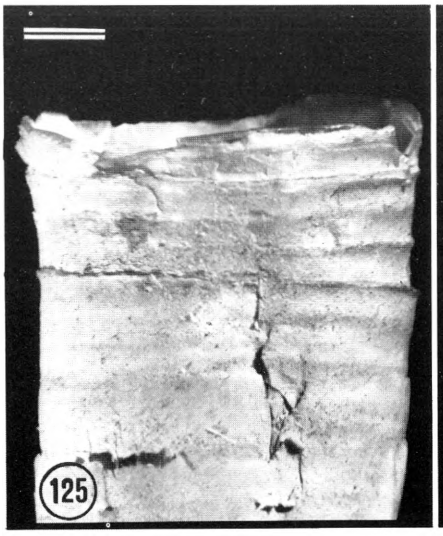
Fig. 124. Pencil drawing of the tube, after photograph of the complete tube taken on board the ship and the dry fragments of the tube. Dead remnants of a colony of hydroids, a barnacle (*Verruca* spec.) and markings of other specimens are present on the tube.

Fig. 125. The ringed anterior part of the dry tube.

Fig. 126. In the middle part of the tube the rings become quite irregular. The outer layers of the tube consist of a whitish, opaque, fibrous material.

Fig. 127. Posteriorly the tube becomes smooth and the rings are practically invisible here.

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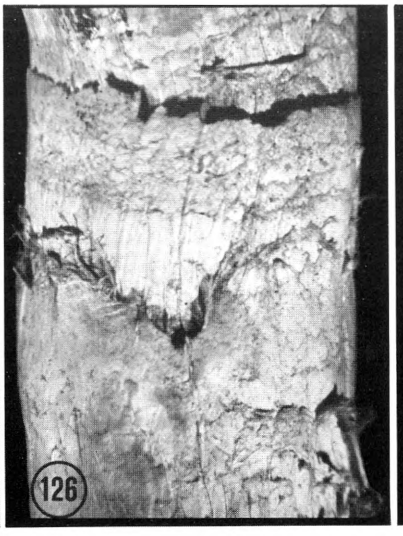
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127

Det Kongelige Danske Videnskabernes Selskab

Biologiske Skrifter

Biol. Skr. Dan. Vid. Selsk.

kr.

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