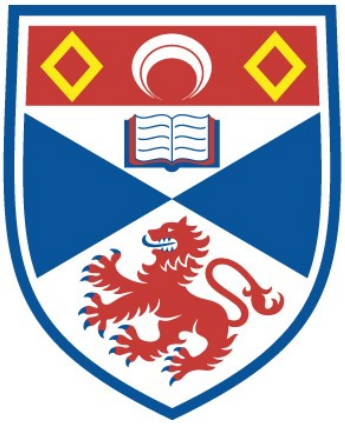
LIGHT AND ELECTRON MICROSCOPICAL STUDIES ON THE TROCHOPHORE LARVA OF HARMOTHOE IMBRICATA, POLYNOID POLYCHAETE

Patricia L. Holborow

A Thesis Submitted for the Degree of PhD
at the
University of St Andrews



1972

Light and Electron Microscopical Studies on the Trochophore Larva of *Harmothoë imbricata* (Polynoid Polychaete).

By

Patricia L. Holborow
Gatty Marine Laboratory,
University of St. Andrews.

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A thesis submitted for the degree of

Doctor of Philosophy.

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Supervisor's Certificate

I certify that Patricia Holborow has fulfilled the conditions laid down under Ordinance Number 16 of the University Court, St. Andrews, and is accordingly qualified to submit this thesis for the degree of Doctor of Philosophy.

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Declaration

I declare that the work recorded in this thesis is my own and has not previously been submitted for any other degree. The work was begun in 1966.

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Curriculum vitae

Educated at the Otago Girls' High School, Dunedin, New Zealand, between 1956 and 1960. Obtained School Certificate in 1958, accredited University Entrance 1959. Attended the University of Otago, Dunedin, New Zealand between 1961 and 1964. Awarded the Womens' Scholarship for 1964.

Subjects studied at University: First year: Chemistry I, Physics I, Mathematics I and Zoology I: Second year: Zoology II and Physiology II: Third year: Zoology III and Physiology III: Fourth year: Zoology Honours. Biochemistry was also read without view to examination.

Graduated 1964, B.Sc. Hons., II.(1).

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SUMMARY

The trochophore larva of Harmothoë imbricata was examined by both light and electron microscopy. Light microscopy of living trochophores was limited to a study of the action of cilia and muscles, and observations on trochophores at various stages from the egg to the fully formed trochophore. The observations on the developing trochophore revealed the presence of apparently uniquely acting cilia. Fixed, sectioned trochophores were examined under the light microscope as an adjunct to electron microscopy to give the gross anatomy.

Scanning electron microscopy was used to determine the arrangement of cilia and glands on the surface of the trochophore. This demonstrated that previous views, at least on the apical array of cilia, were erroneous, and that there is considerable asymmetry in the positions of both cilia and gland pores.

Transmission electron microscopy of the fully formed trochophore afforded the first information at an ultrastructural level on all the organ systems of a trochophore, and revealed the presence of previously unknown structures such as modified cilia and a probable photoreceptor which is additional to the pigmented eyespot.

Certain general issues of contention in electron-microscopical work were able to be resolved in the course of this work. This included the finding of neuro-ciliary synapses, and certain significant

features in the development of trochophores from the egg to the fully formed trochophore.

The suitability of this animal for experimental investigations became clear as the fine structural analysis proceeded. Accordingly, preliminary studies were made on pepsin digestion of thin sections and the testing of drugs on whole animals before fixation.

INTRODUCTION

The trochophore larvae of Polychaetes have been a source of study and speculation since the middle of the last century. The resulting abundant literature falls into two main groups. One group of workers concentrated on cell cleavage and followed the development stages from the egg to the trochophore. This study was pioneered by E.B. Wilson (1892), who recorded the cell lineage of Nereis and developed a notation system by which the origin and history of each cell could be known. Other embryologists repeated this operation, among whom were Treadwell (1901) studying Podarke, Child (1900), working on Arenicola, Mead (1897) on Amphitrite and Clymenella.

The second group described the trochophore sometimes along with an account of the reproduction of the adult worms and sometimes with descriptions of later larval stages. Many of the descriptions are only of the appearance of the whole larvae, and some of the descriptions, particularly those of the larva of Harmothoë imbricata, appear to be erroneous. Details of this are discussed later. Thorson (1946), reviewing the literature in relation to the larval fauna of the Danish Øresund Sound, drew attention to confusion, inaccuracies and the imcompleteness in our knowledge of Polychaete larvae. This is still the situation, but much valuable, detailed work has been reported.

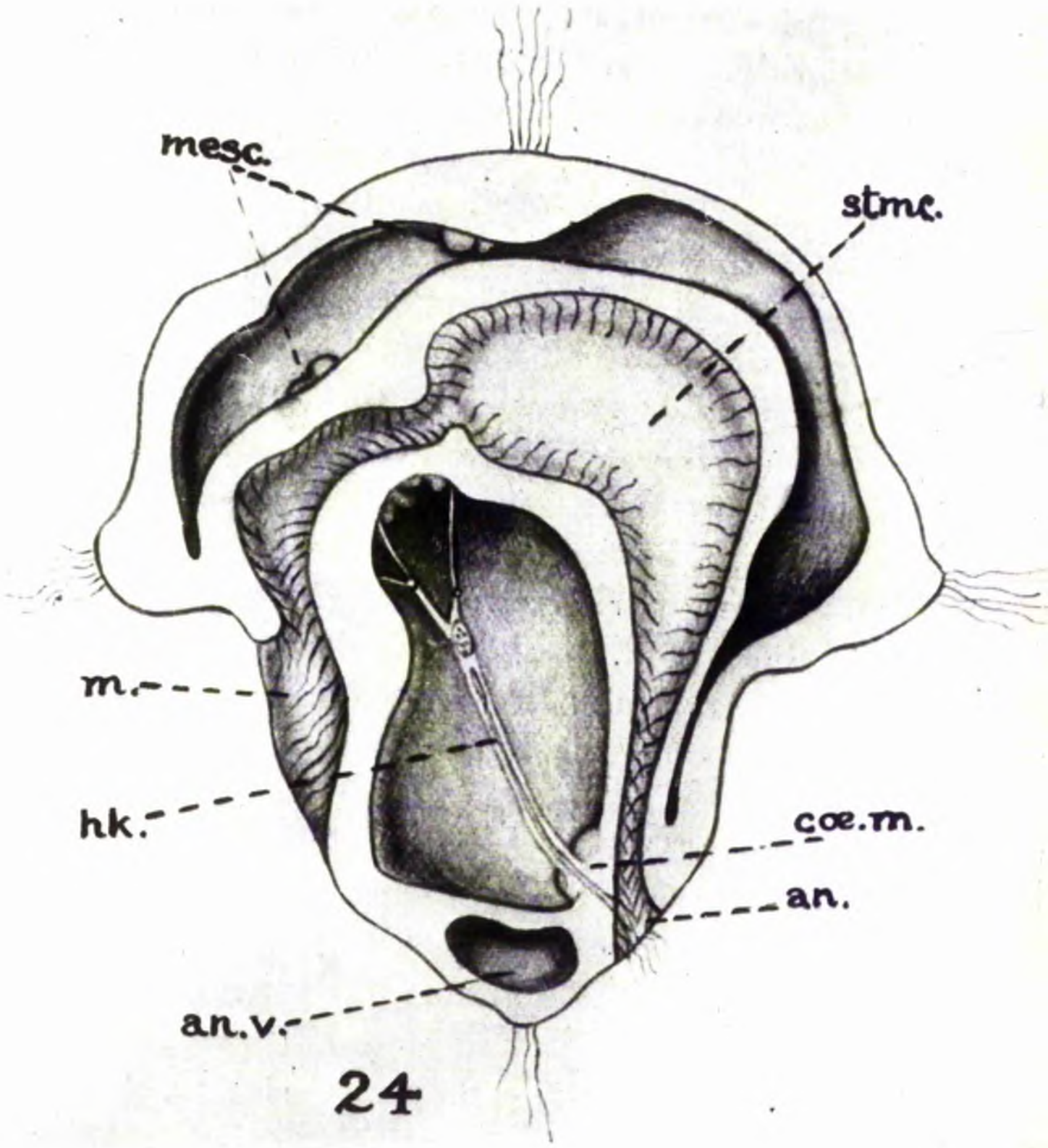
Hatschek (1878) developed the trochophore theory, that the trochophore recapitulated the larva of the ancestor of most of the bilateral phyla.

In 1886 Hatschek pointed out the difference between scattered cells of mesenchyme and definitive cells of the mesoblast bands, but observed that mesenchyme cells originated from the mesoblast bands. Many other workers followed with similar observations, including Fraipont (1887) and Wilson(1890). On the other hand, cell lineage workers (mentioned above) distinguished between coelomesoblast and larval mesenchyme, and around the turn of the century there was much discussion surrounding the ontogeny and phylogenetic significance of the developing mesoderm. However, most of the work was in the form of a general description. Häcker (1896) classified different larval stages into trochophore, metatrochophore (I and II) and nectochaeta, and adopted technical terms for some of the larval organs. Claparède (1863) and Gravely (1909 a and b) added to these terms by naming more tracts of cilia. D.P. Wilson (1928a, 1928b, 1929, 1932a, 1933, 1936a, 1936b) described trochophores from several families, and also cut sections of the mitraria larva of Owenia fusiformis. Among other accounts in varying degrees of detail were those of Agassiz (1866), Day (1934), Enders (1909), Flatterly (1923), de Saint-Joseph (1887), Salensky (1882, 1883), Segrove (1940), and more recently, Cazaux (1971).

Although considerable variety of trochophore forms are evident from this work, there has arisen a concept of a typical trochophore based on that of a Eupomatus (Shearer, 1911; see Fig. (i)). The apical tuft is regarded as a characteristic (Hyman, 1951) and by comparison with the pilidium and veliger larvae, a primitive feature of

Fig. (i).

Diagram of a trochophore by Shearer (1911). This is the trochophore of Eupomatus: coe. m.coelomesoblast, an. anus, an.v.anal vesicle, mesc. mesencheyme or ectomesoblast, hk, head kidney, m. mouth, stmc. stomach.



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trochophore larvae. Previous workers on Harmothoë imbricata (Sars, 1845; McIntosh, 1900 and Rasmussen, 1956) also depict an apical tuft, which although a feature of trochophore being incubated under the elytra of the parent worm, is not present in the fully formed trochophores. The pattern of cilia around the apex of the Harmothoë trochophore is reminiscent of the ring of cilia found in Nepthys and Glycera (Fuchs, 1911), but general text accounts of trochophores fail to include mention of apical cilia other than the central tuft even when describing variations in trochophore external anatomy.

Other findings of general relevance provided by the electron microscope are the size and relationship of ciliary groups, lengths and numbers of cilia, arrangement of superficial glands, and all of the details of the structure of the internal organ systems. Segrove (1940) in his work on Pomatoceros gave some data on the lengths of cilia, and Gravely (1909a and b), studying trochophore of various species, also gave lengths but added a note that the difficulties in measuring were great and the measurements were simply for comparative purposes. Where sections have been cut of trochophores (Segrove, 1940; Wilson, 1932b and others) the overall anatomy has been clearly described, but only with the electron microscope could the numbers and layout of nerves, the structure of the protonephridium, eyes, muscles and digestive system, etc. be known.

It is evident that a number of misconceptions on trochophore structure have arisen because of the limitations of the light microscope, and sometimes the fixative used caused mistaken identification of structures and confusion of some structures with others. Åkesson (1967)

points out that Kleinenberg (1886) and Meyer (1901) probably mistook nephridial tubes for nerves, but Åkesson has not yet used the electron microscope for his material, Several authors, among them Shearer (1911) and Segrove (1940) refer to a "head kidney", anal vesicles and statocysts. The "head kidney"is equivalent to the protonephridium. The latter two structures are not present in the Harmothoë trochophore. However, the supplementary unpigmented photoreceptor of the adult Harmothoë and other polychaetes appears as an empty vesicle when fixed in Bouin (Golding, personal communication), and as this structure is present in the trochophore it could represent the so called vesicles or statocysts of earlier authors. Certain patches of lipid in epidermal cells also appear like empty vesicles under certain conditions of fixation.

The previous studies on the trochophore of Harmothoë imbricata by Sars (1845) and McIntosh (1900), and on Polynoid larvae more generally by Leschke (1903), Nolte (1936), Pettibone (1953) and Rasmussen (1956) afford little information, several contradictions and a number of errors. The problem of apical tufts has already been mentioned with regard to the lack of recognition that other arrangements exist. Except for Gravely (1909a), authors describing Polynoids have failed to note the apical circlet. So deep is the conception of the standard form that Daly (1972), having observed the apical tuft of the embryo being incubated, could not arrive at the conclusion that this structure might only be temporary. With reference to the account of the fully formed trochophore given by Holborow, Laverack and Barber (1969) Daly stated

"It is not yet possible to relate this pattern of definitive larval cilia to the changing pattern which has been observed during early development, especially in the apical region".

The next most recent accounts, those of Rasmussen (1956) and Korn (1958) are full of the misconceptions which arise due to the limitations of the light microscope. For example Rasmussen (1956) states that the eyes have lenses (not evident in electron microscopy), that the prototroch has two rows of cilia (in fact the effective number of rows is four, borne by five rows of cells), that there is no anal opening, and that the oesophagus is thick-walled (an appearance probably produced by the longitudinal and horizontal oriented roots of the dense lining of cilia).

As this study is primarily an electron microscopical one it is not surprising that various parts of the work bear more relevance to questions arising in this field than to previous work on trochophores. This is particularly true of various findings in the study of the developing trochophore from which problems currently discussed in the literature were able to be elucidated. Three points were outstanding in this respect. The first of these relates to the matter of pore complexes of nuclei , a problem summed up by Weston Ackerman, Greider and Nikolewski (1972), who observed that it is not known whether pore complexes change with age. The second issue under discussion is whether or not the numbers of microtubules and ribosomes in nerves varies with age (Peters and Vaughn, 1967; Schmitt and Samson, 1968; Tennyson, 1970; and Lyser 1971). The third subject is the growth of cilia which, in spite

of a number of studies, is not yet fully known. Sotelo and Trujillo-Cénoz (1958) described ciliary filaments in the chick as developing from chains of vesicles. Roth and Shigenaka (1964) summarized ciliary formation in protozoa as aggregation of protein molecules into filaments and arrangement of filaments. Satir (1965), in a review, gave an even more general summary of the process which has remained incompletely described.

Aspects of the electron microscopy of the fully formed trochophore also proved significantly relevant to electron microscopical work in other animals.

First: Records of neurociliary synapses are very few (Dilly, 1972; Horridge and Mackay, 1964), and according to Horridge (1968) there are no known examples of transmission of a wave between ciliated cells that is achieved by nerves.

Secondly: Two types of photoreceptor, one with receptor membranes derived from cilia and one with microvilli providing the receptor membrane surface, have so far been reported in only a few invertebrates (Barber, Evans and Land, 1967; Barber and Land, 1967; Boyle, 1969; Golding, personal communication). In the past, such findings contributed to discussion arising from the now untenable phylogenetic division of animals according to eye types (Eakin, 1963). The interest is now in relation to possible function suggested partially from behavioural evidence (Dorsett and Hyde, 1968), from ablation experiments (Crisp, 1972) and from electrophysiology (Gorman and McReynolds, 1969). The evidence suggests that ciliary photoreceptors monitor an "off" response to light and mediate shadow reflexes, whereas microvillous photoreceptors monitor an "on" response.

Thirdly: Cilia throughout the animal kingdom typically have a 9+2 configuration, and considerable attention has been focussed on cilia which deviate from this configuration by Afzelius (1963), Horridge (1964), Pitelka (1963), Satir (1962), among others. Cilia with additional filaments to the normal axoneme were reported for the first time from the present work in a paper given in 1969 to the Fourth European Marine Biology Symposium (Holborow, 1971). Similar cilia have been reported by Ling (1970). In each of these independent studies, an assumption was reached that the cilia function in a manner unique for cilia, as a valve.

In general the electron microscopy of other organ systems provided interesting comparisons with the fine structure of similar systems in other animals, without having the same significance as the above for current discussions.

Although the trochophore is very small, it proved quite difficult to achieve good fixation. A wide variety of fixatives, fixation times and buffers were used, and the animals were embedded in araldite or epon. There are abundant reports of specificity of reaction of single types of cell to different fixatives (Millonig, 1961; Rosenbluth, 1963; Tormey, 1964; Baker, 1965; and many others). However, reports comparing the reaction of several cell types in any series of experiments are lacking. In the trochophore it was found that adjacent cells of different types may not be equally well fixed with any one fixative. Also nuclei and endoplasmic reticulum, by which one theoretically judges fixation success (Pease 1960), have a different appearance in cells

of different organ types. The same holds for microvilli. It is therefore evident that the small size of the trochophore and the variety of its organ systems and cell types (some in the process of differentiating) make it particularly useful for experiments in fixation.

For the same reasons, of small size and varied tissue types, the trochophore is a good vehicle for a comparative examination of the reaction to drugs of organelles in specialised and unspecialised cells.

Until recently drugs were used in ultrastructural studies in order to determine a certain property of the organ under study. For example, Behnke and Forer (1967) used colchicine to establish the difference between microtubules of various organelles, and this chemical has also been widely used in studies on the division of cells by Pickett-Heaps (1967), Borisy and Taylor (1967), Marsland (1968). Caffeine has been used in much the same way, having been used to inhibit cytokinesis in plant cells (López-Sáez, Risueño, and Giménez-Martín, 1966; Pickett-Heaps, 1969). Another way of tackling the question has been to look at the effects of drugs on organ systems where a reaction to that substance is already known to exist. Conney (1967) reviewed over 100 substances known to be metabolised more rapidly after exposure of the organism to that substance and a number of workers, among them Jones and Fawcett (1966), Rubin, Hutterer and Lieber (1968), have studied the effect of one or a few of these known substances on liver ultrastructure.

More general exploratory investigations are now being reported such as the effect of chloramphenicol on insect flight muscle compared with its effect in man (Smith, Smith, and Yunis, 1970) and the effect

of inhalational anaesthetics on the heliozoan Actinosphaerium (Allison, Hulands, Nunn, Kitching and Macdonald, 1970). The ultrastructure of different organs of many types are remarkably similar throughout the animal kingdom and it is therefore not unreasonable to look for effects of drugs on fine structure in simple organisms before testing more complex organisms.

The drugs chosen for the study with the trochophore were caffeine, phenobarbitone, bendrofluazide and ethanoestradiol. They were chosen because they are all freely available and the first two are known to have an effect on intracellular organelles or cellular function. With all these drugs it was hoped to determine which if any of the different cell types are effected and in what way. With phenobarbitone there was also the opportunity to compare the trochophore cells having a pattern of endoplasmic reticulum similar to vertebrate liver cells with those cells.

Pepsin digestion has not been widely used in electron microscopy although it has proved valuable in distinguishing differences between similar intracellular organelles (Behnke and Forer, 1967). In the trochophore this method was used to confirm suspected differences between the additional microtubules and the filaments of the axoneme of the gullet-valve cilia.

The drug testing and pepsin digestion experiments are dealt with briefly as they are not intended to be more than investigations into areas which could prove valuable for further study. The major part of the work is the anatomical analysis.

THE LIVING TROCHOPHORE AND THE ANATOMY OF THE FULLY FORMED TROCHOPHORE

MATERIALS AND METHODS

I. Collection and Maintenance of Animals and Light Microscopy

Adult specimens of Harmothoë imbricata were collected from the sea in St. Andrews Bay and occasionally at Kingsbarns during March and April. Adults were kept individually, in non-aerated containers in which sea water was changed frequently, usually daily. The animals were fed every week on pieces of Buccinum and thrived. Some adults of opposite sexes were kept in pairs until they mated so that developing trochophores of precisely known age could be examined.

After release, the trochophores were collected and transferred to clean glass jars and were fed on a culture of Nitzchia every three days. Live trochophores were observed and photographed using Nomarski phase contrast on a Zeiss microscope. Measurements of the rate of beat of cilia were made with a Strobosun (1203B Dawe Instruments) Stroboscope. The trochophores were held in nylon mesh.

A number of tests of a preliminary nature were carried out on living trochophores by infiltrating the nerve transmitters 5-hydroxy-tryptamine, acetylcholine, nor-adrenaline and glycine in the concentration of 10^{-5} g/ml under the coverslip and observing changes in the rate of beat of cili

II. Preparation of Material for Transmission Electron Microscopy

1. Fixation

Three basic fixatives were used: osmium, glutaraldehyde and acrolein. These were used with various buffering systems

and at different osmolarities. With the latter two fixatives, post-fixation with osmium in the buffer appropriate to the particular trial was carried out. The recipes are summarized below. Buffer solutions were always adjusted to pH 7.4 using a pH meter. The osmolarities given were calculated. Triple strength sea water was made by gently evaporating to $\frac{1}{3}$ volume filtered sea water.

Some osmolarities were measured on an osmometer. The reading is given in brackets after the calculated value.

A. Osmium

A.1 (i) 0.2 ml. 2% osmium tetroxide solution and 0.2 ml. animals in sea water. Dilution is by half. This was the most widely used fixative and the standard method for developing trochophores and drug-tested adult trochophores. It is the fixation used in the illustrations unless otherwise stated. Fixation times were 10, 20, 30 minutes and 1 hour. (ii) Some tests were made using less osmium to give less dilution, i.e. 0.4 ml. of animals in sea water, 0.2 ml. of osmium.

A.2 0.4 ml. 2% osmium, 0.2 ml. animals in sea water, 0.2 ml. triple strength sea water. Osmium at 1%, overall solution strength slightly above that of normal sea water.

A.3 Osmium with cacodylate. 0.1 ml. of 0.2M sodium cacodylate buffer solution was mixed with 0.3 ml. of triple strength sea water, 0.2 ml. of animals in sea water and 0.6 ml. of 2% osmium. Overall osmium strength 1%. Osmolarity of the solution, 949 milliosmoles.

A.4 Osmium with veronal. Sodium veronal (barbital) and sodium acetate were made up into solution according to Palade (1952) and brought to pH 7.4 with 0.1N hydrochloric acid. 0.1 ml. of this veronal buffer was mixed with 0.1 ml. of triple strength sea water, 0.2 ml. of animals in sea water and 0.4 ml. of osmium tetroxide. Osmolarity, 700 milliosmoles.

B. Glutaraldehyde.

B.1. Glutaraldehyde and cacodylate buffer.

(i) 4 ml. of 25% glutaraldehyde was mixed with 50 ml. of 0.2M sodium cacodylate solution, and 5g. sucrose, giving an osmolarity of 855 (793) milliosmoles. One part of this mixture was added to one part of animals in sea water, giving an osmolarity of 927 milliosmoles and a glutaraldehyde concentration of approximately 1%.

(ii) 3 ml. of 25% glutaraldehyde was mixed with 22 ml. of 0.2M sodium cacodylate solution and 1.7 ml. of triple strength sea water, ($850_{\Lambda}^{(805)}$ milliosmoles). One part of this mixture was added to one part of animals in sea water. Osmolarity, $925^{(931)}$ milliosmoles, 1.4% glutaraldehyde. In order to obtain a higher concentration of glutaraldehyde, animals in sea water were centrifuged gently for 2 minutes in the clinical centrifuge and 1 ml. of animals in sea water was added to 20 ml. of cacodylate glutaraldehyde. The concentration of glutaraldehyde was then 2.7% and the osmolarity $857_{\Lambda}^{(850)}$ milliosmoles.

The specimens were washed in a buffered wash made with 15.5 parts of cacodylate buffer to 4.5 parts of triple strength sea water, or 8.5g. of sucrose added to 50 ml. of cacodylate buffer ($900_{\Lambda}^{(939)}$ milliosmoles).

Following at least half an hour and occasionally up to 20 hours of wash, post fixation was by cacodylate-osmium. Usually 2% osmium was mixed in equal parts with cacodylate buffer. In some cases one part of buffer wash was mixed with one part of triple strength sea water and two parts of 2% osmium tetroxide, giving an osmolarity of 960 milliosmoles.

B.2. Glutaraldehyde with veronal buffer.

8 ml. of 25% glutaraldehyde were added to 42 ml. of stock veronal buffer (see A.4). 5 ml. of this were mixed with 5 ml. of sea water and animals. This gives 2% glutaraldehyde and an osmolarity of 890 (899) milliosmoles.

The veronal wash was increased in osmolarity with 1.975 g. of sucrose per 25 ml. of buffer. Post fixation was with equal quantities of 2% osmium tetroxide and buffer wash.

C. Acrolein

C.1. 1 ml. of 100% acrolein was mixed with 1 ml. of 0.2 M cacodylate buffer and 8 ml. of animals in sea water, giving 10% acrolein. Fixation was for one hour. The same proportions of buffer and animals were used with 10% acrolein giving a 1% solution.

C.2. 1 ml. of 10% acrolein was mixed with 1 ml. of animals and 1 ml. of 0.2M cacodylate buffer. Both were washed in a cacodylate buffer wash and post fixed in cacodylate osmium, with two parts of osmium tetroxide mixed with one part of triple strength sea water and one part of cacodylate buffer. Fixation was for one hour.

2. Dehydration and embedding.

Ethanol dehydration was used with the first fixations done with 50:50 OsO_4 and animals in sea water. The osmium was washed out with distilled water, and graded ethanols added, up to absolute ethanol. Some specimens were prestained with phosphotungstic acid in absolute

alcohol in the cold for 1 hour and rinsed in absolute ethanol before final dehydration in acetone or propylene oxide (Gray, 1959). Pre-staining was also done with uranyl acetate, and neutral lead citrate in 70% acetone (Giménez-Martín, Risueño and Lopéz-Sáez, 1967). Most of the material, however, was not prestained and most dehydration was through graded acetone solutions, with three changes of absolute acetone and a calcium chip in the last solution.

Araldite or epon (1:1) mixed 50:50 with absolute acetone were then added and left overnight. Most of the Araldite or epon remaining was scooped off the following morning. Fresh Araldite or epon were added and after one hour the specimens were transferred to further fresh Araldite or epon in shallow plastic containers. These were placed in the oven at 37°C for at least thirty six hours and often three days.

3. Cutting and Staining

The specimens were examined under the microscope in order to select a known orientation for cutting. In most cases the eyes and shape of the trochophore made this possible, although some fixations blackened the trochophores to such a degree that the eyes became invisible. In some cases the orientation was partly lost during mounting and cutting a face on the block because a small change in angle has a great effect when the specimen is so small. During cutting at least one section was taken for toluidine blue staining and light microscopy for every grid that was made.

The sections were collected on unfilmed grids and stained for three minutes in lead citrate followed by three minutes in uranyl

They were washed for 15 sec. in distilled water
acetate (Venable and Coggeshall, 1965), at room temperature. In hot conditions, the staining dish was chilled in the deep freeze in order to slow down evaporation and prevent crystal formation on the grids. The staining times had to be increased to six minutes in a cooled dish. The specimens were viewed on an AEI EM 6B electron microscope, operating at 60 Kv.

III. Preparation of Material for Scanning Electron Microscopy

Two completely different procedures were employed to prepare material for the scanning electron microscope. Some specimens which had been fixed in 1% osmium in sea water (the standard method, A.1) were partially dehydrated in graded ethanols. They were taken down to water and a small drop of water containing some animals was put into an aluminium foil 'boat' which was then immersed in a bath of "Arcton 12" (dichlorodifluoromethane I.C.I. Ltd.). The Arcton was cooled with liquid nitrogen and kept just above freezing at -155°C. The frozen water drops were transferred to a larger boat, chilled with liquid nitrogen and placed on a cold probe inside a vacuum coating unit. The probe was maintained at -25°C. The specimens were freeze-dried and coated with carbon before removing from the vacuum. Following this they were given a further coating of approx. 200Å of carbon followed by approx. 300Å of gold (Barber and Boyde, 1968; Boyde, 1967).

The specimens were examined on a Cambridge Scientific Instruments "Stereoscan" scanning electron microscope operating at 10Kv and stereoscopic pair images (for 10° tilt) were recorded.

A further set of specimens was prepared by critical point drying, according to Anderson (1951). They were dehydrated in graded

acetone solutions to absolute acetone and transferred to nylon mesh bags especially made from samples of mesh supplied by Henry Simon Ltd. The bag containing trochophores was suspended in amyl acetate solution and placed in the cavity in the screw of the critical point dryer. The amyl acetate was displaced by carbon dioxide under pressure. The dry trochophores were tipped onto a stub freshly coated with a sellotape solution in chloroform. They were taken to the Zoology Department of the University of Edinburgh on stubs in a miniature desiccator box made for the purpose. The stubs and trochophores were coated with gold palladium in a vacuum coating unit, and viewed in a Cambridge Scientific Instruments "Stereoscan" scanning electron microscope.

RESULTS

I. Light Microscopy

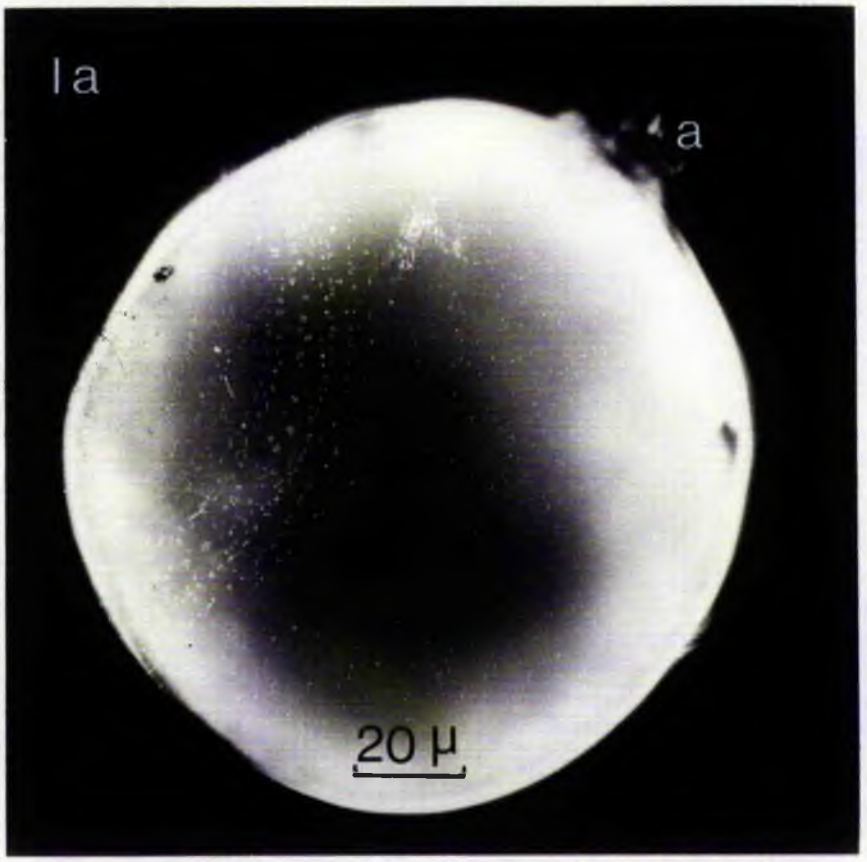
1. The Developing Embryo.

The eggs of Harmothoë imbricata are retained under the elytra of the adult worm for fourteen days, during which time the trochophore develops all its organ systems. The eggs are spherical, 120μ in diameter. Although they are opaque, early cell divisions may be seen, up to the 8-cell stage. Gastrulation takes place on the third day. Cilia first appear between the fourth and sixth day, at the apex. These cilia grow to 30μ long and form a tuft which is shed before the trochophore is released. The cilia oscillate in unison at a slow rate and with a long interval between the forward and backward stroke such that each stroke appears to be active (Figs. 1 and 3). The tips of the cilia adhere to each other and to other trochophores or foreign matter if that happens to be near them. When the tips of the cilia are attached to a firm object, the body of the trochophore is rotated one way and then back. The rate of rotation varies, and Fig. 2 is a histogram of times for ten oscillations measured from 41 animals. The variation in rate of the beat of the tuft does not appear to depend on age, as members of the same brood in the same microscopical sample, i.e. subject to the same temperature and oxygen content in the water, show wide variations in rate.

On the 7th day the prototroch begins to grow, on the 8th day the neurotroch appears and on the 9th day the akrotroch and cilia which will form the apical ring can be seen. From the time they are visible with the light microscope, the cilia may be seen beating, although several days elapse before they grow to their full length. Except for the

Fig. 1.

7-day-old trochophores seen with the light microscope. The dividing cells are no longer distinguishable. The apical cilia, a, lie towards the camera in the upper view and away ffom the camera on the lower view. Prototroch cilia, p, are just visible.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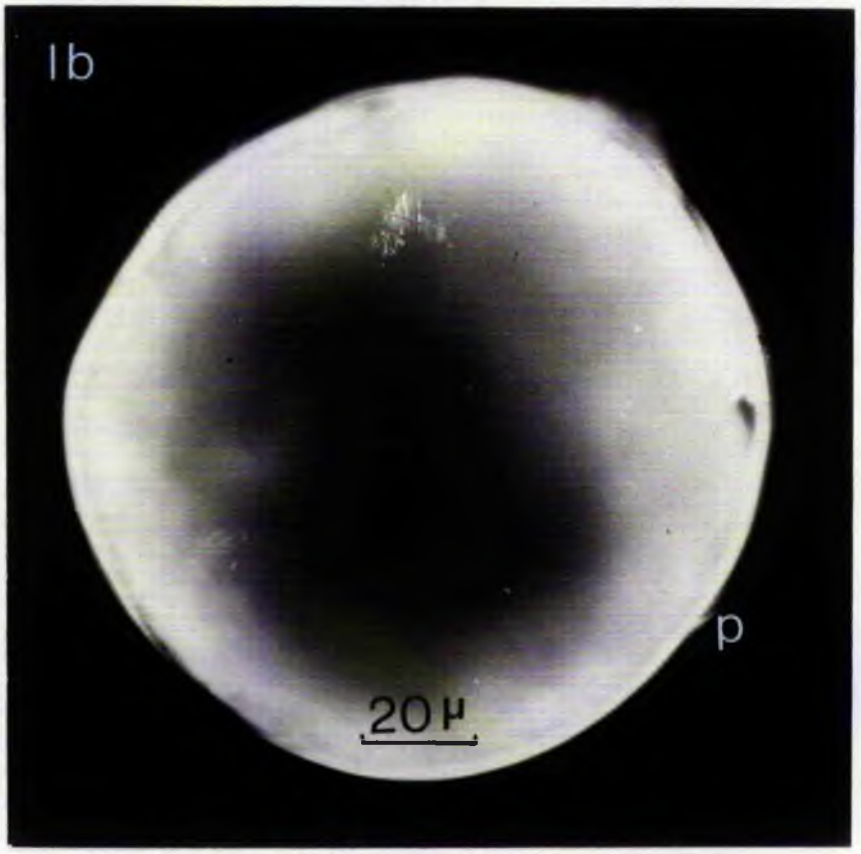
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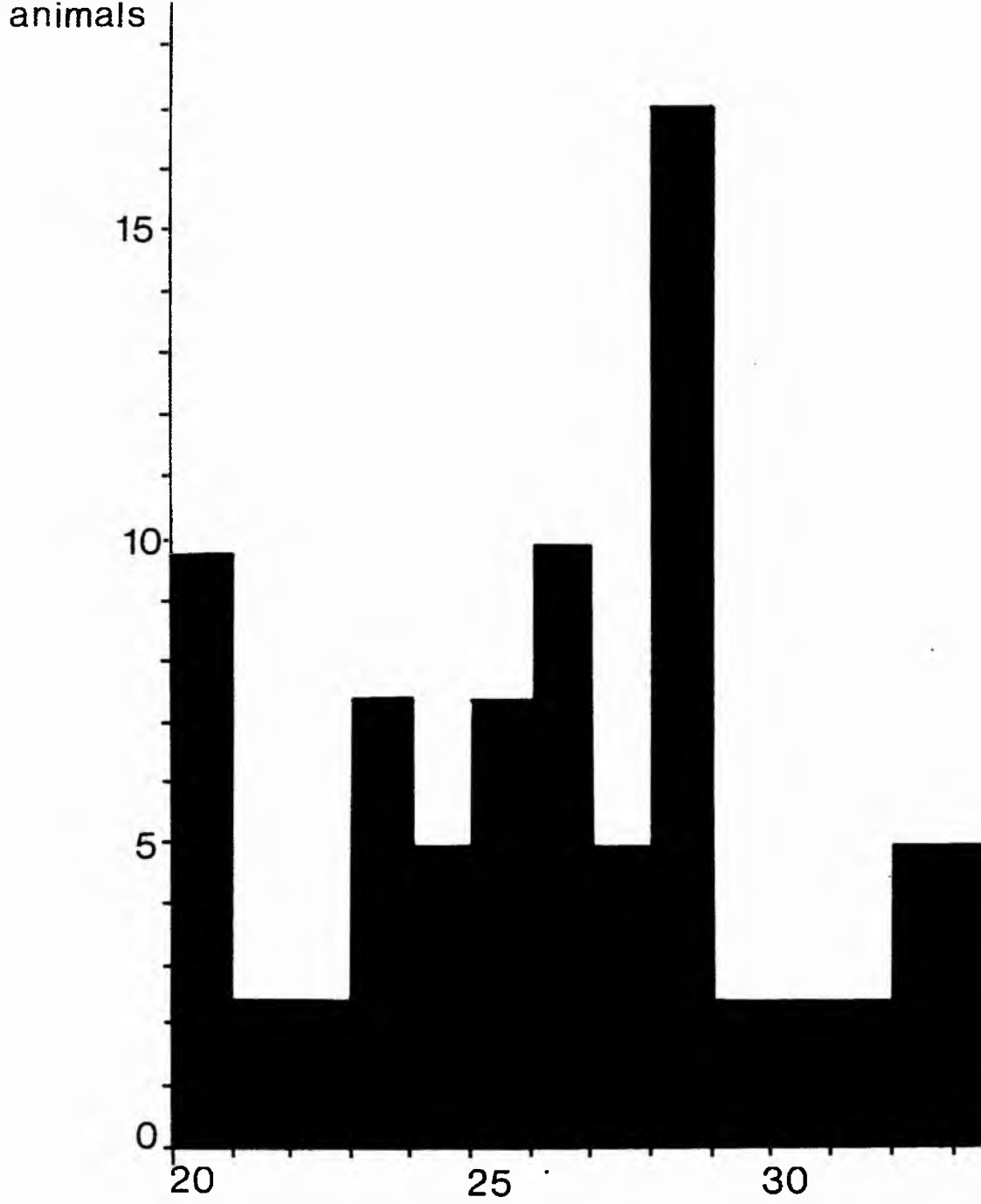
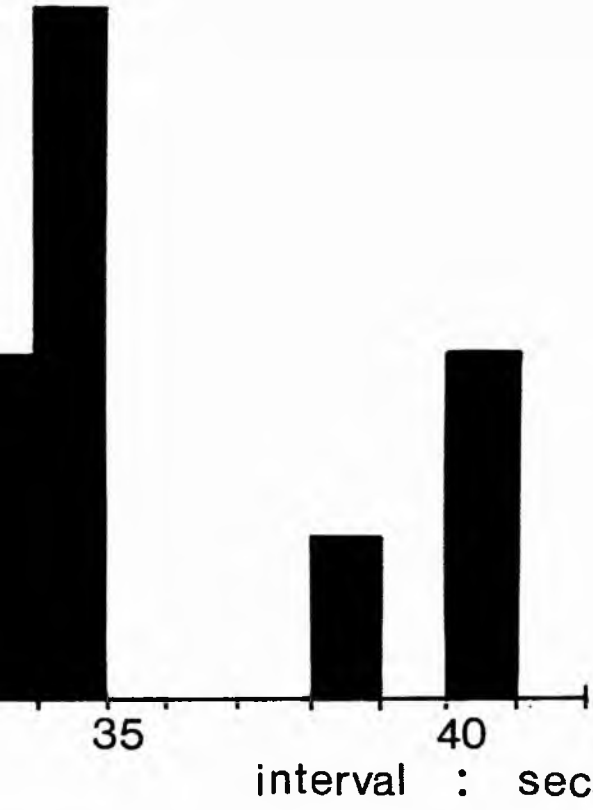
20 µ

Fig. 2.

Histogram showing the distribution of animals with different intervals between the forward and backward strokes of the apical cilia. The percentage of animals in each column is shown on the vertical axis and the interval between forward and backward strokes is given on the horizontal axis. The numbers are the actual measurement of times for 10 oscillations, so that 1/10 of these times represents roughly the intervals between strokes, the duration of the stroke taken as negligible relative to the interval. Measurements were obtained by averaging several readings for each of the total of 41 animals.


animals
15
10
5
0
20
25
30


35
40
interval : secs.

first apical cilia, the cilia grow inside a protective, translucent envelope which disappears on the 9th to 10th day. Figs. 3a and b show two nine-day-old trochophores. The prototroch cilia can be seen inside the protective envelope of one of the trochophores, and the apical tuft can be seen in its end-of-stroke positions. The eyespot appears as a crescent on the 8th day, an arch on the 9th day, then as it grows it assumes a kidney shape. (Fig. 4).

The trochophore show almost no increase in size until the 8th day, then growth proceeds steadily. At the 8th day, the trochophores measure 130μ long and 120μ in diameter. They increase 10μ in length per day and 7μ in breadth until at the time of release they measure on average 190μ long and 160μ broad. These events are summarized in the diagram Fig. 4.

2. The Fully Developed Trochophore

Fig. 5. shows a fully developed trochophore photographed by flash. The view is lateral but from the upper hemisphere such that the various tracts of cilia and the eyes may be seen, but the true shape is not apparent. The characteristic shape of these trochophores with prominent upper lip, large rounded lower hemisphere and smaller more conical upper hemisphere, slightly flattened at the top, is shown in Fig. 6. In some specimens food boluses could be observed rotating in the part of the stomach in the upper hemisphere, and defecation was observed on several occasions. Frequently the irritation of the light and the coverslip caused contraction of the muscles which can extensively deform the shape of the trochophore. The elasticity of the body contents appeared to be the restoring force. Fig. 6. is a diagram showing the direction of movement

Fig. 3.

A pair of 9-day-old trochophores. The apical tuft is in two positions (marked in ink). The prototroch, p, can be seen inside the envelope surrounding the trochophore on the right.

3a

3 b

Fig. 4.

This series of diagrams summarises the stages of development of the trochophore during the 14 days of incubation. (Not to scale)

Day 1. The egg divides once.

Day 2. Second and third divisions.

Day 3. Division proceeds rapidly until a mass of 64 cells are seen. Gastrulation takes place at about this stage.

Day 4. The egg is opaque and individual cells can no longer be seen. The apical tuft appears.

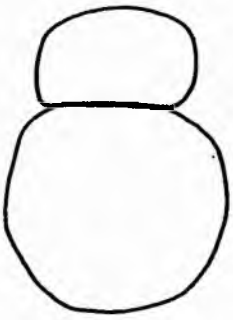
Day 5-6 (not illustrated). Little change is seen, except for lengthening of apical cilia.

Day 7. The prototroch appears.

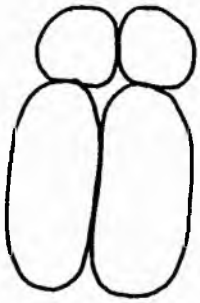
Day 8. Neurotroch, prototroch, eye as thin crescent.

Day 9. Neurotroch and prototroch develop. Eye becomes arch, thickens slightly.

Day 10. Apical tufts appear. Lengthening of cilia and thickening of the pigment cup of the eye are the only changes externally visible until the trochophore is ready for release.



1



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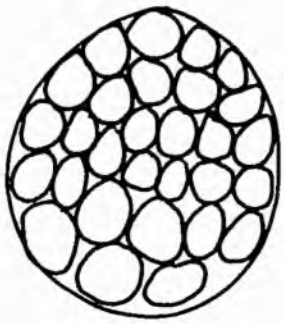

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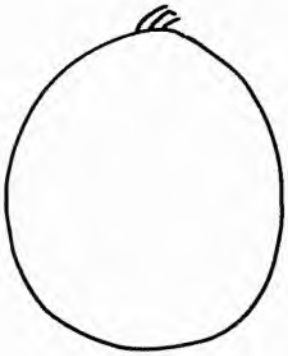
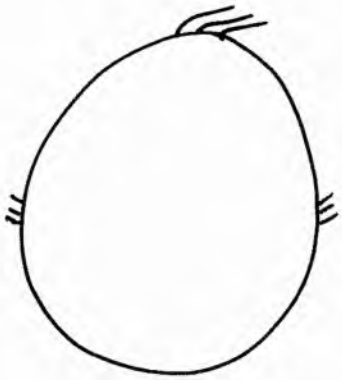
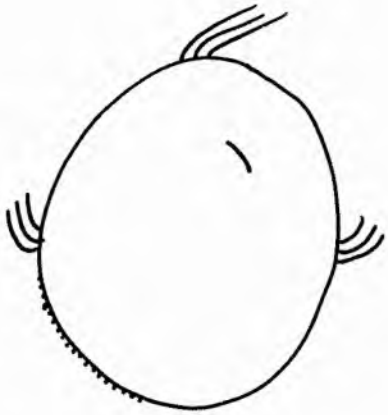
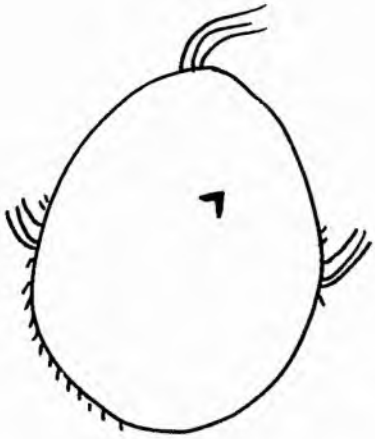






4 - - - - - - - 7

8



9

10 - - - - - 14

Fig. 5.

This shows a fully developed trochophore as seen with the light microscope. Both eyes, e, are visible as the view is from the upper hemisphere. The mouth, m, is in the lower hemisphere. The prototroch, akrotroch and apical cilia can be seen as well as areas of pigmentation.

5

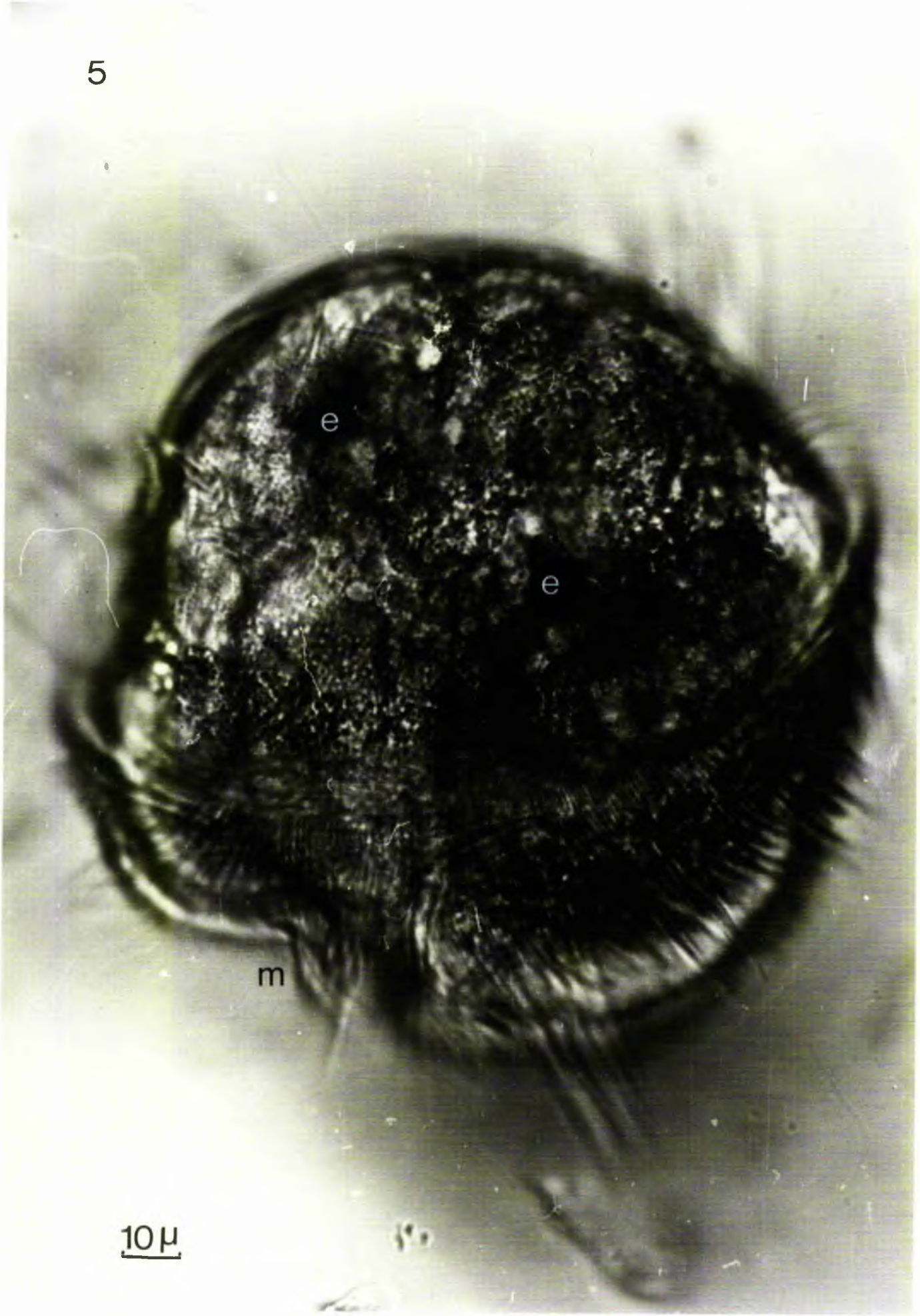
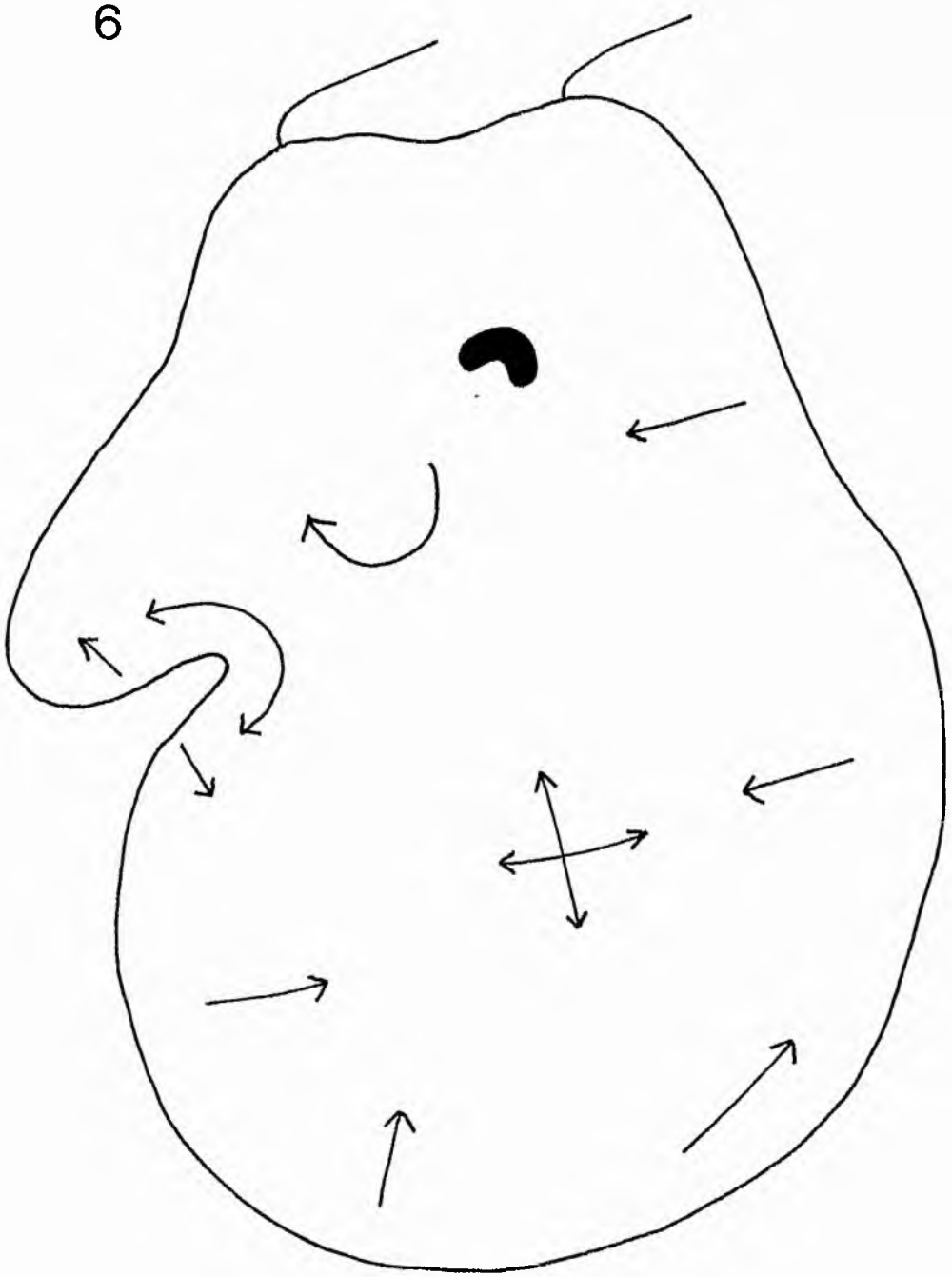


Fig. 6.

Diagram to illustrate activity of the muscles of the trochophore. The arrows indicate direction in which contraction took place; double headed arrows show where shortening occurred between the arrow heads. (Not to scale)

6



of parts of the body when different muscles contracted.

Propulsion movement of the trochophore takes place by beating of the prototroch cilia. The rate of beat of the prototroch cilia, of 41 animals measured with a stroboscope ranged from 350 beats per minute to 925 beats per minute with an average of 700 beats per minute. Fig. 7 is a histogram showing the proportion of animals in each range of rate of prototroch beating. The median lies between 700 and 800.

Variability in rate and direction of swimming may be affected by the akrotroch (rate 400 beats per minute) and also perhaps by the apical cilia, which beat intermittently with the active stroke towards the mouth. The neurotroch cilia are short and beat away from the mouth at a rate of 1500 beats per minute, but the current they set up appeared to be compensated for by inward beating of mouth and lip cilia. Fig. 8 is a diagram showing the direction of beat of the various tracts of cilia, and also the current set up around the mouth. In the preliminary experiments with nerve transmitters, 5-hydroxy-tryptamine (10^{-5} g/ml) and noradrenalin (10^{-5} g/ml) increased the rate of beating of all the tracts of cilia, while glycine (10^{-5} g/ml) and acetylcholine (10^{-5} g/ml) decreased the rate of beating of all tracts of cilia. The continuous beating of the neurotroch became intermittent, with short bursts of beating interspersed by pauses after the addition of acetylcholine (10^{-5} g/ml) and glycine (10^{-5} g/ml). This effect could be reversed by the addition of 5-hydroxytryptamine (10^{-5} g/ml) or noradrenaline (10^{-5} g/ml). Further, the neurotroch cilia were shown in the course of these experiments to form two panels, an anterior section (nearer the mouth) and a posterior section. The cilia in the anterior group ceased beating before the cilia in the posterior group.

Fig. 7.

Histogram showing range of rates of beat of trochophore cilia and proportion of animals with the various rates. The vertical axis gives the percentage of animals and the horizontal axis gives the cycles per minute of the long prototroch cilia. (41 animals in sample).

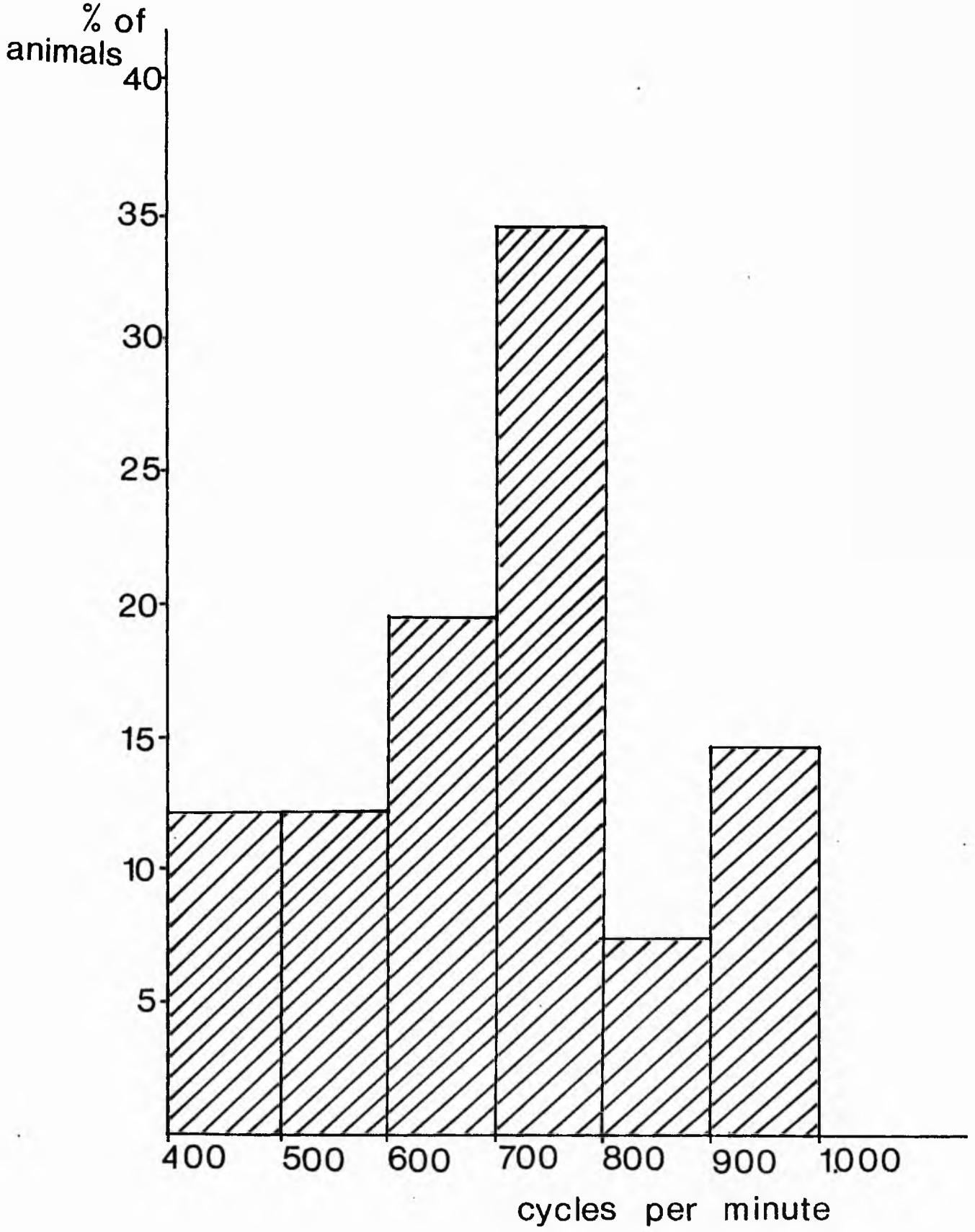
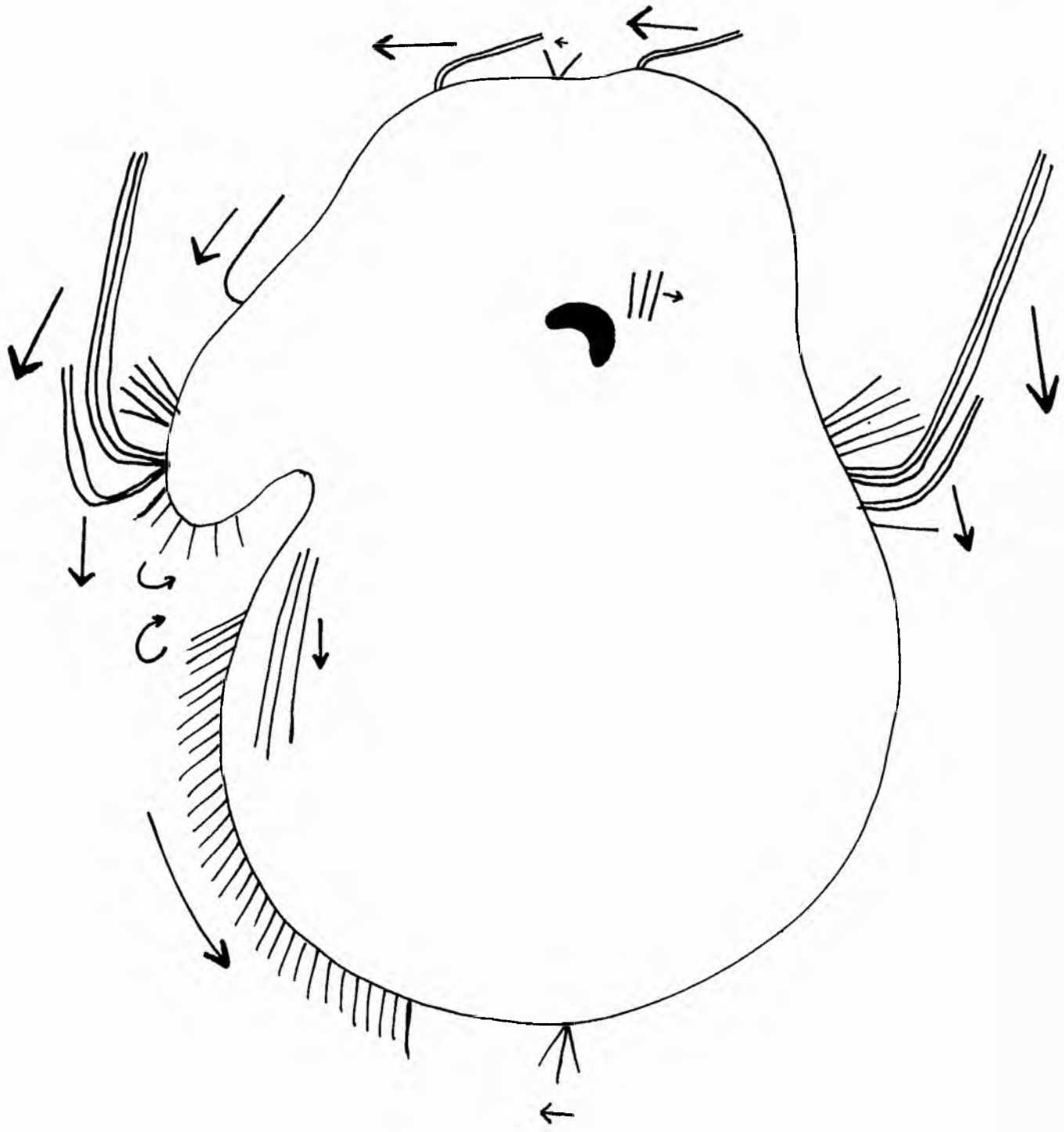

% of animals
40
35
30
25
20
15
10
5
400
500
600
700
800
900
1000
cycles per minute

Fig. 8.

Diagram showing the direction of beat of the cilia of the trochophore. (Not to scale)



II. Scanning Electron Microscopy

The scanning electron microscope permits accurate measurement of the tracts and positions of cilia and other projecting surface organelles. The trochophore possesses all the usual tracts of cilia, but some of these are asymmetrically placed and of atypical arrangement.

Figs. 9 (a and b) are left hand and right hand side views of the trochophore showing the apical cilia, akrotroch, prototroch, neurotroch, anal tuft and lateral tufts. The long cilia lateral to the mouth are evident in the left hand view and are not found on the right hand side.

The small lateral tufts of cilia in the middle of the upper hemisphere are cilia which occur near the eye, itself not visible in scanning electron microscope pictures.

The projecting mouths of gland pores are an outstanding feature in the upper hemisphere. A large pair lie near the eye on either side and two smaller openings lie more posteriorly and nearer the prototroch. A set of four small projections just above the right hand end of the akrotroch are gland pores which are asymmetrically placed. There is no counterpart on the left hand side of the akrotroch. Figs. 10 (a and b) show details of the two types of gland pore.

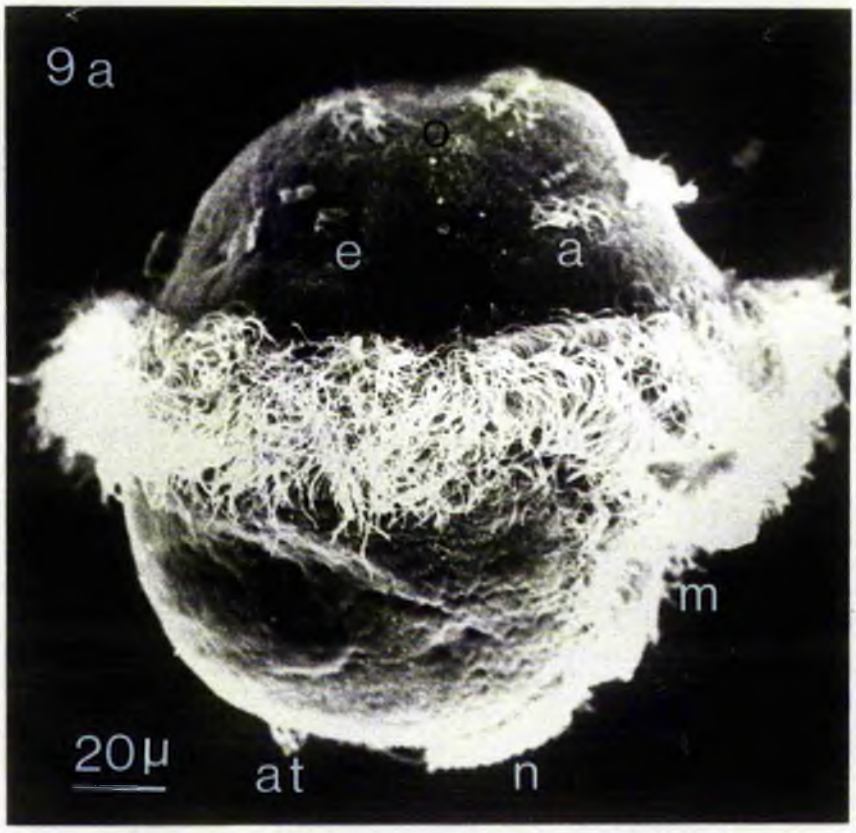
The akrotroch is an asymmetrical structure. It lies on the ventral side only, half way between the apical and prototroch cilia, and extends one quarter of the way around the circumference of the animal at this point, a distance of some 40–48μ. The eye tufts, which

Fig. 9(a)

Scanning electron microscope view of the right side of a trochophore. Note lateral opening, o, of apical cilia, akrotroch, a, with a set of 4 gland pores above the first line of akrotroch cilia, cilia, e, beside the eye and a pair of gland pores above these. A single pore may also be seen above the prototroch in a mid lateral position. The mouth, m, and neurotroch, n, and anal tuft, at, lie in the hyposphere.

Fig. 9(b)

Left side of the trochophore. The apical cilia run forwards and the gap between the lines on this side is anterio-lateral. The arrangement of glands can be seen to be different from those on the right. Two pairs of pores can be seen mid-laterally on the anterior side of the episphere and a single pore lies just above the prototroch towards the dorsal side. The anal tuft is barely seen as it lies to the right. Note the long cilia, l, on the left side of the mouth.


9a
o
e
a
m
20µ
at
n

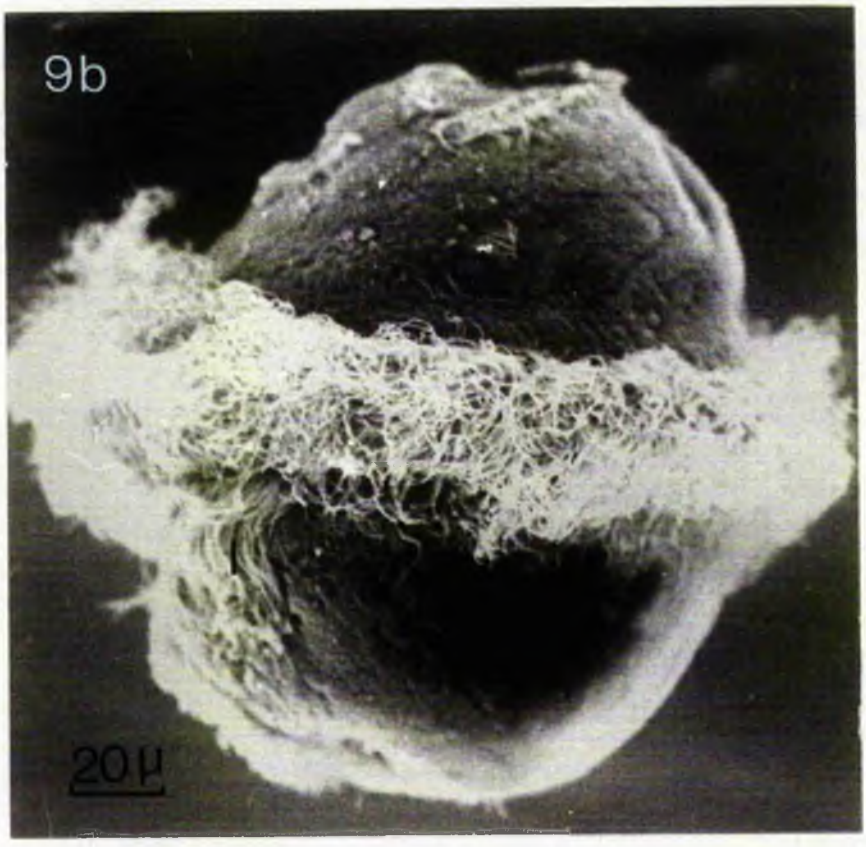
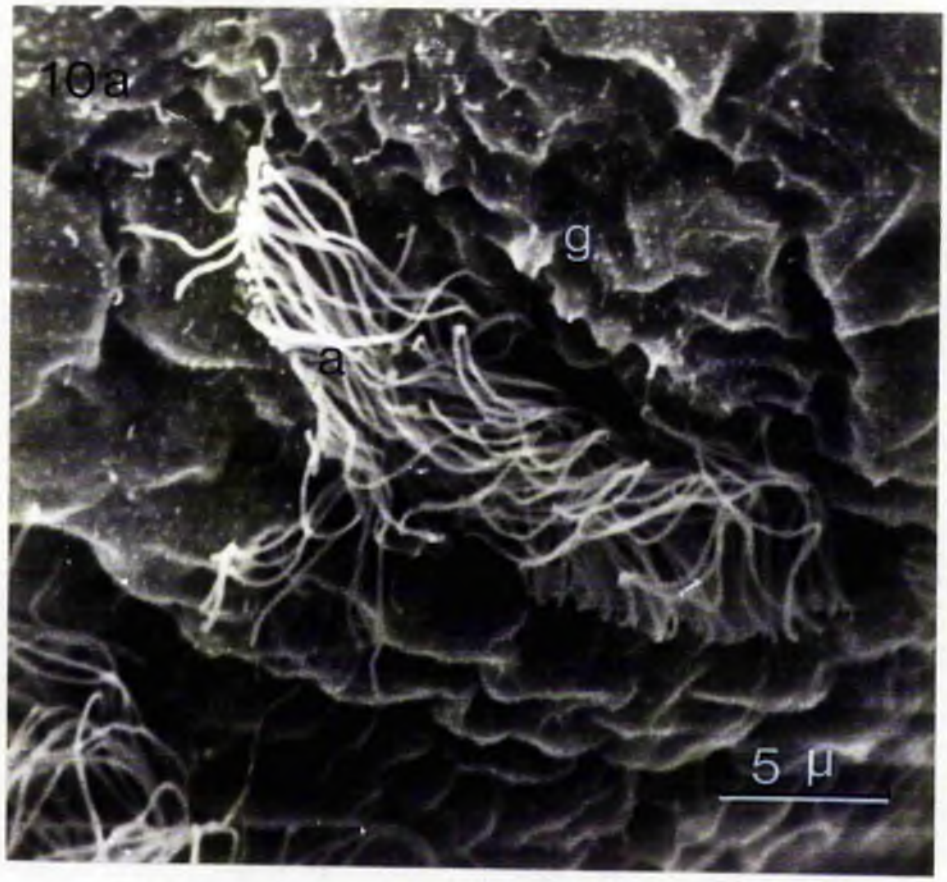

9b
20µ

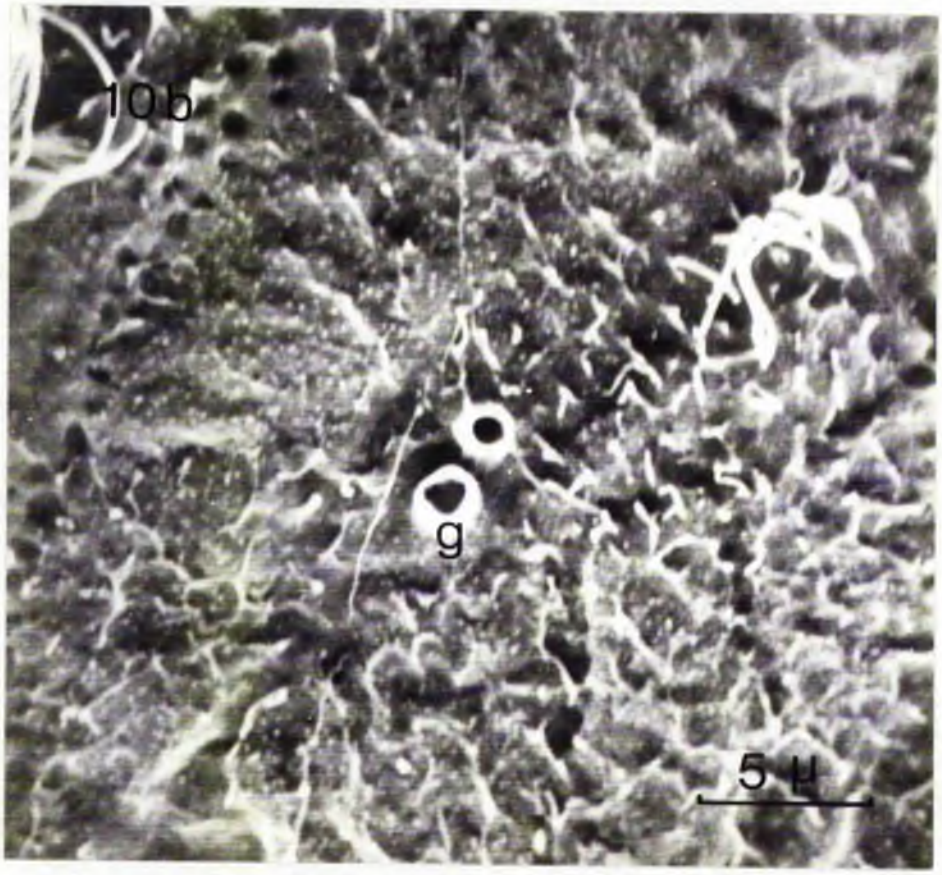
Fig. 10 (a)

Set of 4 gland pores, g, lying above the first line of akrotroch cilia, a, on the right side of the trochophore. These are the openings of type I glands described later.

Fig. 10(b)

Gland pores, g, situated mid-way up the episphere, above and behind the eye. These are the pores of type III glands.


10a
g
a
5 µ


10b
g
5 µ

are at the same level, may be remnants of a complete akrotroch. The akrotroch that remains consists of four lines of cilia 8μ to 10μ long separated by spaces of 2μ to 7μ.

The apical cilia lie in an array around the apex, in five more or less straight lines, (Fig. 11, and see Holborow, Laverack and Barber, 1969). In the centre of the apical region are a few short cilia. The lines and their arrangement are remarkably consistent. Three longer lines of cilia lie towards the right. These are 23μ long anteriorly, 22μ long projecting posteriorly and 25μ long on the posterior side. The latter two meet at right angles. The anterior, 23μ long line has a curve ventrally as though made up of two lines, perhaps from two cells. For most of its length it is parallel to the akrotroch. The 25μ long line is very slightly convex, curving up into the apical area. Two 13μ long lines complete the apical figure. These lie on the left hand side with a 60° angle and a gap of 5μ between them. The posterior one is separated from the long posterior line by a 2μ gap, and the anterior is separated from the long anterior line by an 8μ gap. On the other side of the long anterior line is a 10μ gap. A few cilia project from the most apical point. All the cilia are 8μ long.

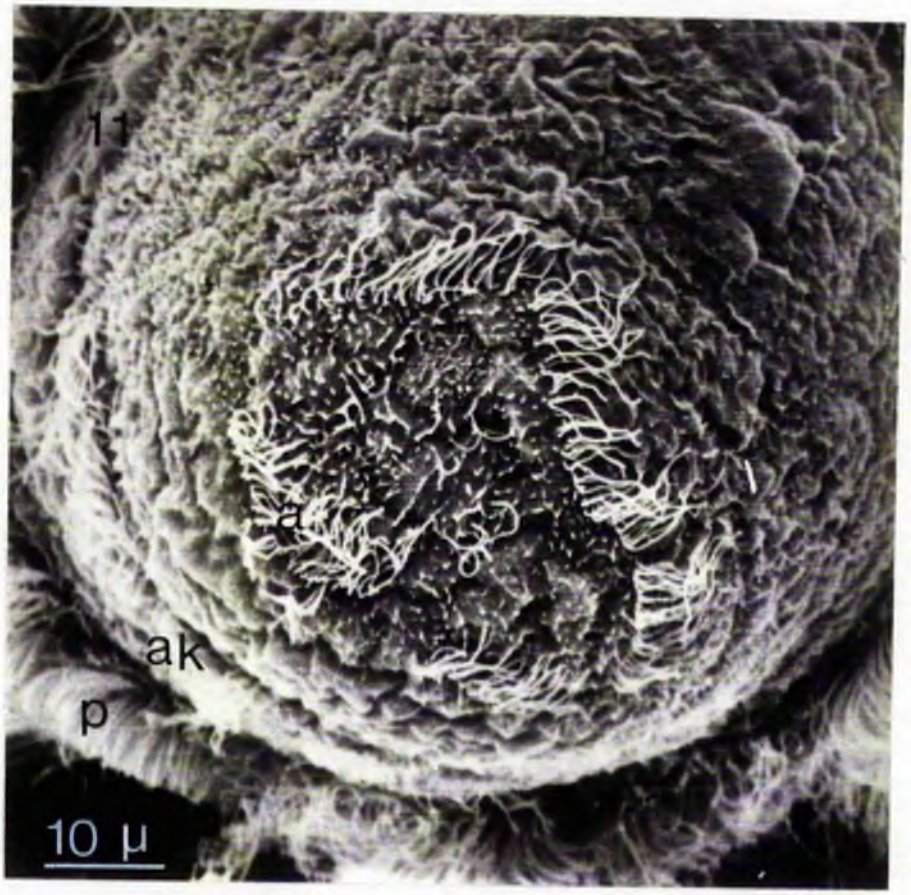
In some scanning electron micrographs (Fig. 12), the prototroch can be seen to be made up of at least three lines of cilia. The uppermost cilia are 20μ to 30μ long and, by their position, are apparently coordinated in activity with lower cilia which are 14μ and 6μ long. Beneath these, cilia continuous with the upper lip cilia (and therefore not metatroch cilia) appear, by their arrangement and position, to

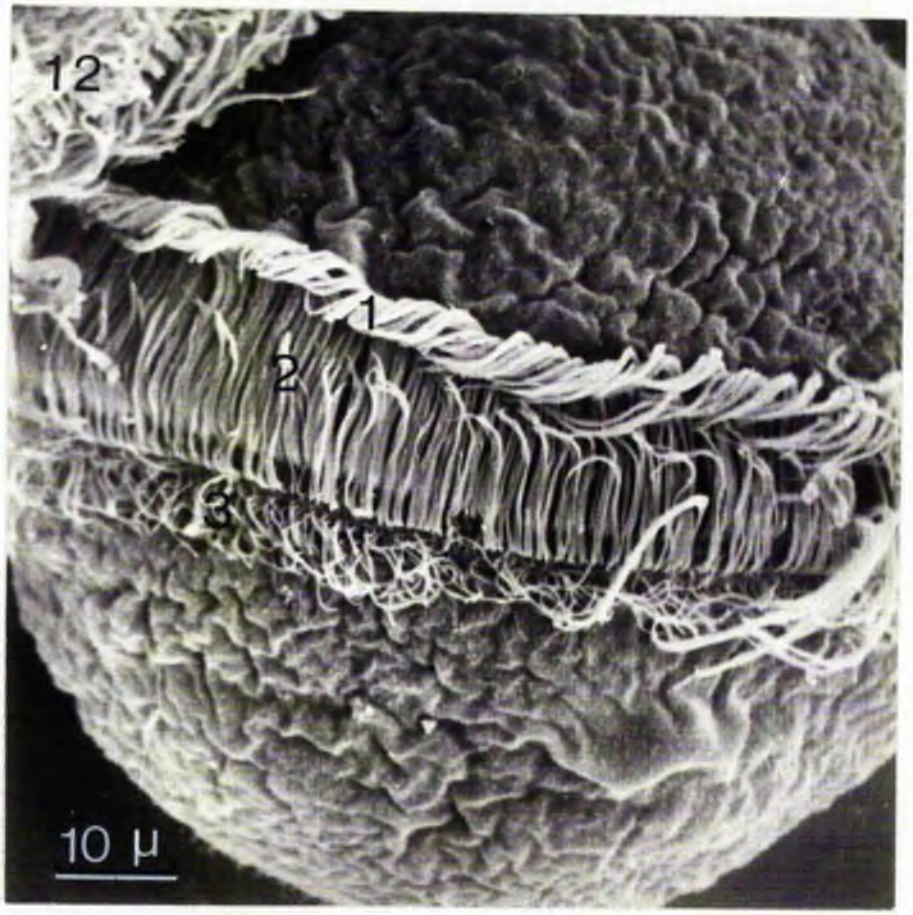
Fig. 11.

Apex of the trochophore. Note the 5 lines of the apical cilia, a, with some centrally placed cilia. The akrotroch, ak, runs part of the way round the ventral half of the episphere. Parts of the prototroch, p, are just visible.

Fig. 12.

Prototroch. Three groups of cilia may be seen. The upper lines are hidden by the long cilia, 1. Two sets of shorter cilia lie below these, one set, 2, co-ordinated with the main locomotor cilia, the other, 3, acting independently.


11
a
ak
p
10 µ


12
1
2
3
10 µ

beat independently of the cilia above. In most scanning micrographs (Fig. 9 a and b), the prototroch is in disarray. The full complexity of the arrangement of the prototroch cilia could be observed only with the transmission electron microscope.

The neurotroch is a panel of short cilia running from the mouth of the anus (Fig. 13). It is 20μ to 25μ broad at the top, 17μ broad at the base and 30μ to 35μ long. Beyond the anus and set slightly to the right hand side is a tuft of small cilia (Fig. 14). The pattern of cilia in the neurotroch in no way suggests the division observed in activity in the living animal. The lie of the cilia indicates that the metachronal wave is laeoplectic according to the terminology of Knight - Jones (1954), but with a delay in starting of the following row, which means that orthoplectic or diaplectic designation cannot be given. The mouth (Fig. 13) is 35μ long and 15μ to 17μ broad. The metachronal wave of the beating of the cilia can be seen to be at an angle to the lip and a whirlpool effect is sometimes seen, although the cilia beat inwards. This is because the metachronism is not symplectic and has a lag in sequence. The set of long cilia on the left hand side of the mouth are most evident in Fig 14.

III. Transmission Electron Microscopy.

1. Overall Anatomy.

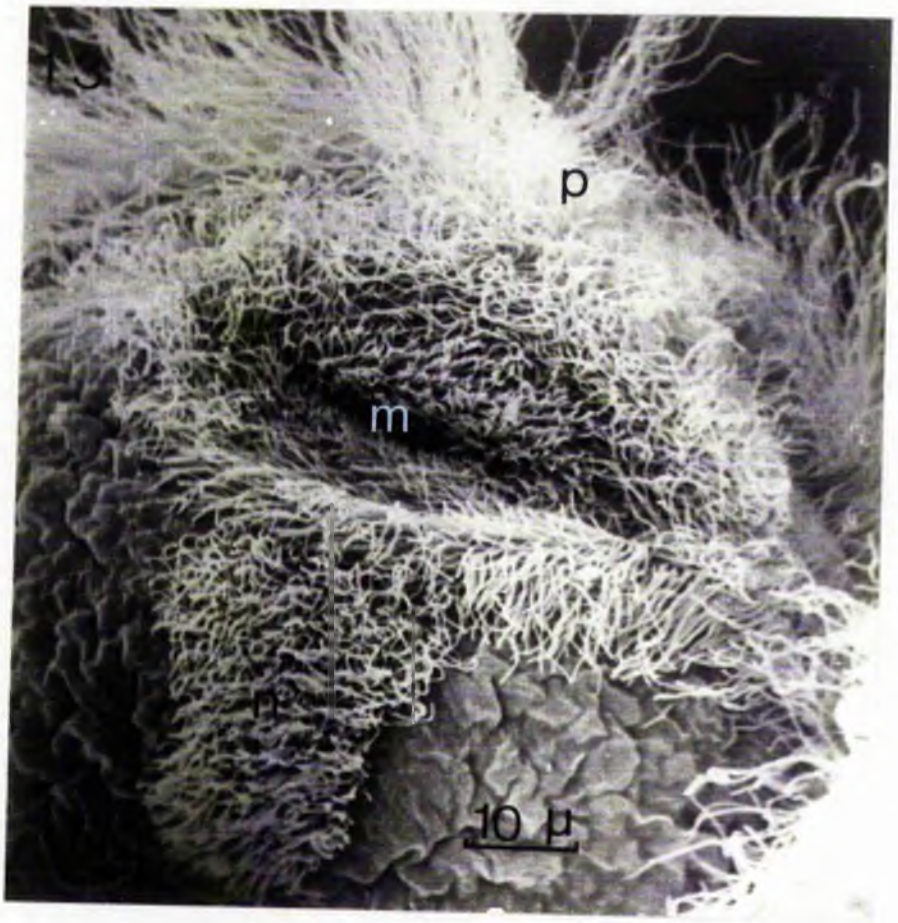
Figure 15, is a diagrammatic representation of a mid-saggital section of the trochophore showing basic features of the anatomy. This perspective represents the overall view obtainable by low-power electron microscopy and light microscopy and is intended

Fig. 13.

Scanning electron micrograph of the mouth, m, and neurotroch, n. The prototroch, p, is above.

Fig. 14.

Scanning electron micrograph of the hyposphere of the trochophore showing mouth, m, neurotroch, n, anal tuft, a, and long cilia, l, on the left side of the mouth.


13
p
m
n
10 µ

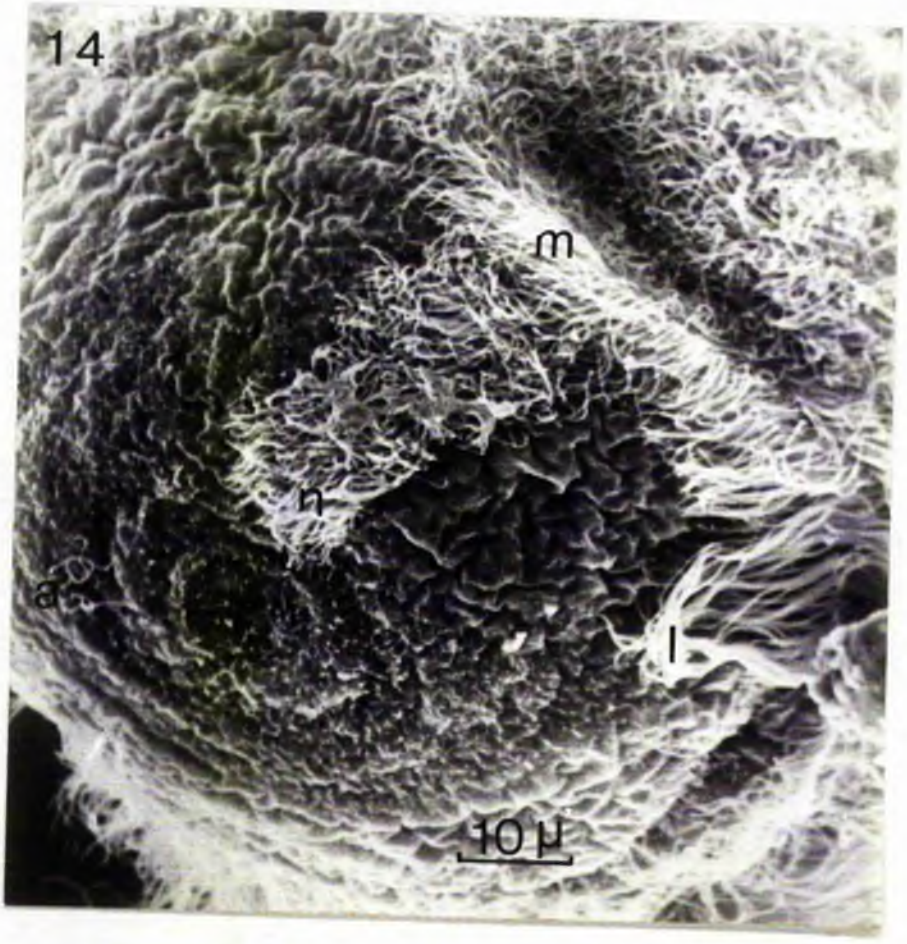
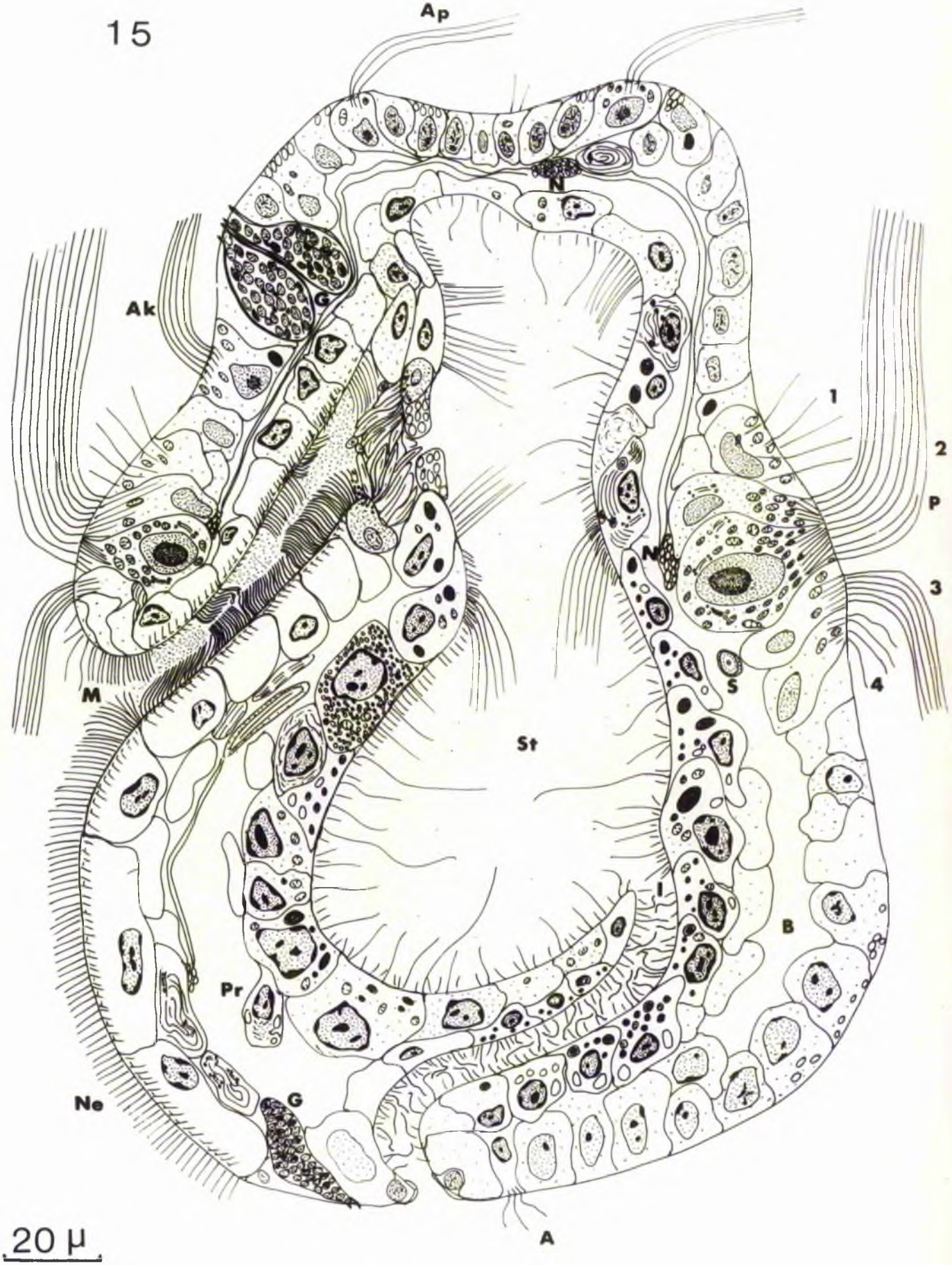

14
m
n
a
l
10 µ

Fig. 15.

Diagrammatic representation of a saggital section of the mature trochophore at the time of release from the parent worm. A, anal tuft; Ak, akrotroch; Ap, apical cilia; B, blastocoel; G, glands; P, prototroch with 4 sets of cilia, 1, 2, 3, 4, on 5 cells; Pr, protonephridium; S, solenocyte; St, stomach.

15



20 µ

to show only the features that can be seen by this means and the relative positions of the various organs. The gullet-stomach-intestine complex forms the dominating element. The prototroch cells bearing the long prototroch cilia are relatively large, twice as big as any other cells. Nerve cells are indistinguishable from the undifferentiated ectodermal cells and axons can barely be discerned. Muscles are also difficult to identify. The solenocyte and protonephridial duct are more easily seen because of their position in the blastocoel.

Details of the arrangements of each of the organ systems are given in the following sections dealing with each organ system in turn.

2. Surface Cilia (i) Apical cilia.

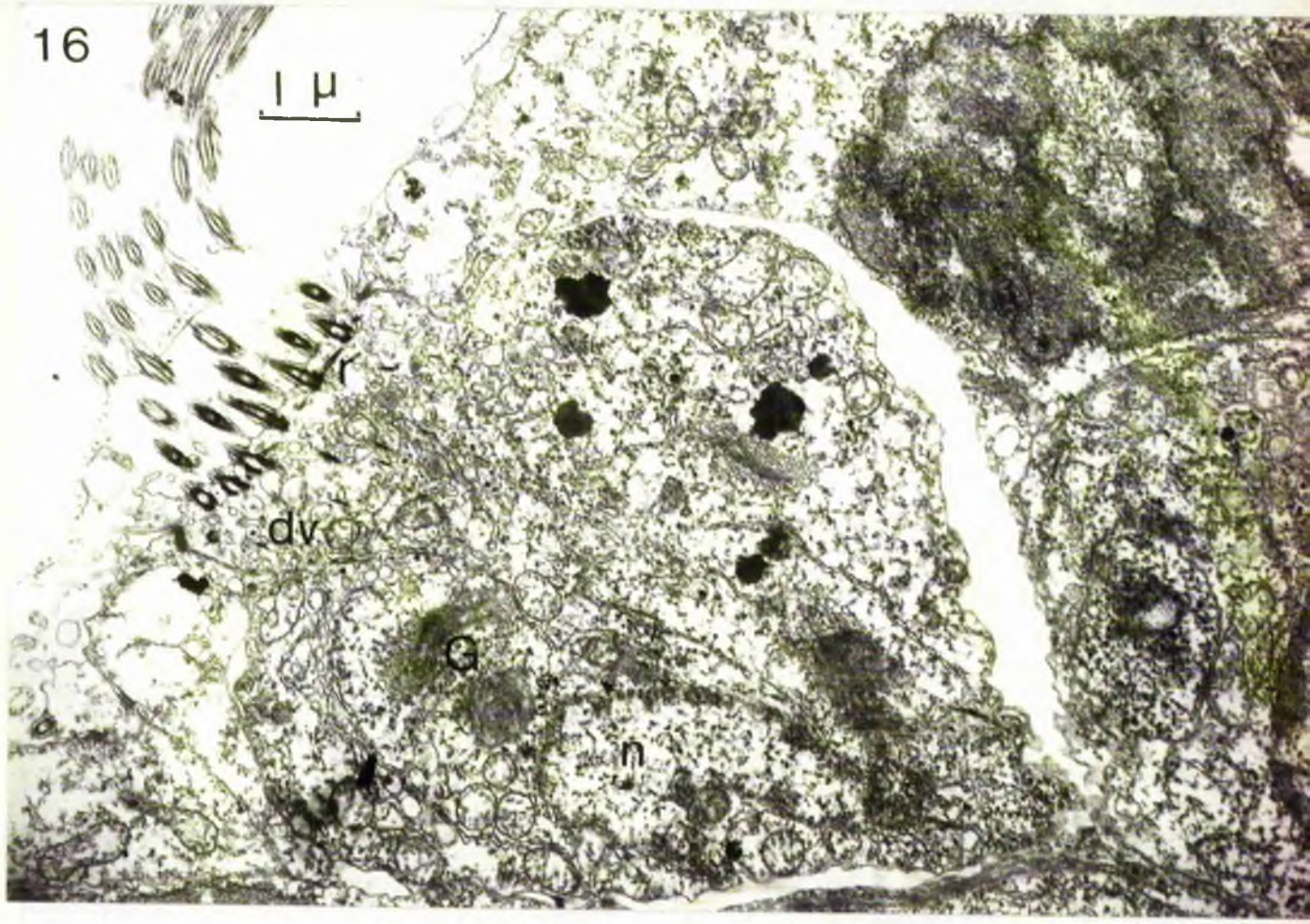
The surface ciliated cells are discussed in order from the apex down. The apical cilia are derived from eight cells, possibly more. These cells bear the cilia in rows of 4 to 6 and the cilia form groups of 5 to 12 (~~Fig. 16 and~~ see Holborow, 1971). The basal feet are uniformly oriented with a small lateral root on the opposite side of the basal body (Fig. 16). The cilia are 0.1μ apart but set in an alternating pattern so that the lateral root does not touch another basal body. There is a long, thin longitudinal root (Fig. 17). The cells are 2-3μ broad at the top, expand to 8μ broad and taper off in a long thin process which extends into the blastcoel before joining up with other axonal processes. The nucleus is situated just below the large bulge and usually has a well developed nucleolus. Golgi bodies are a prominent feature of these cells and occur in the broad area of the cell. The Golgi figures are

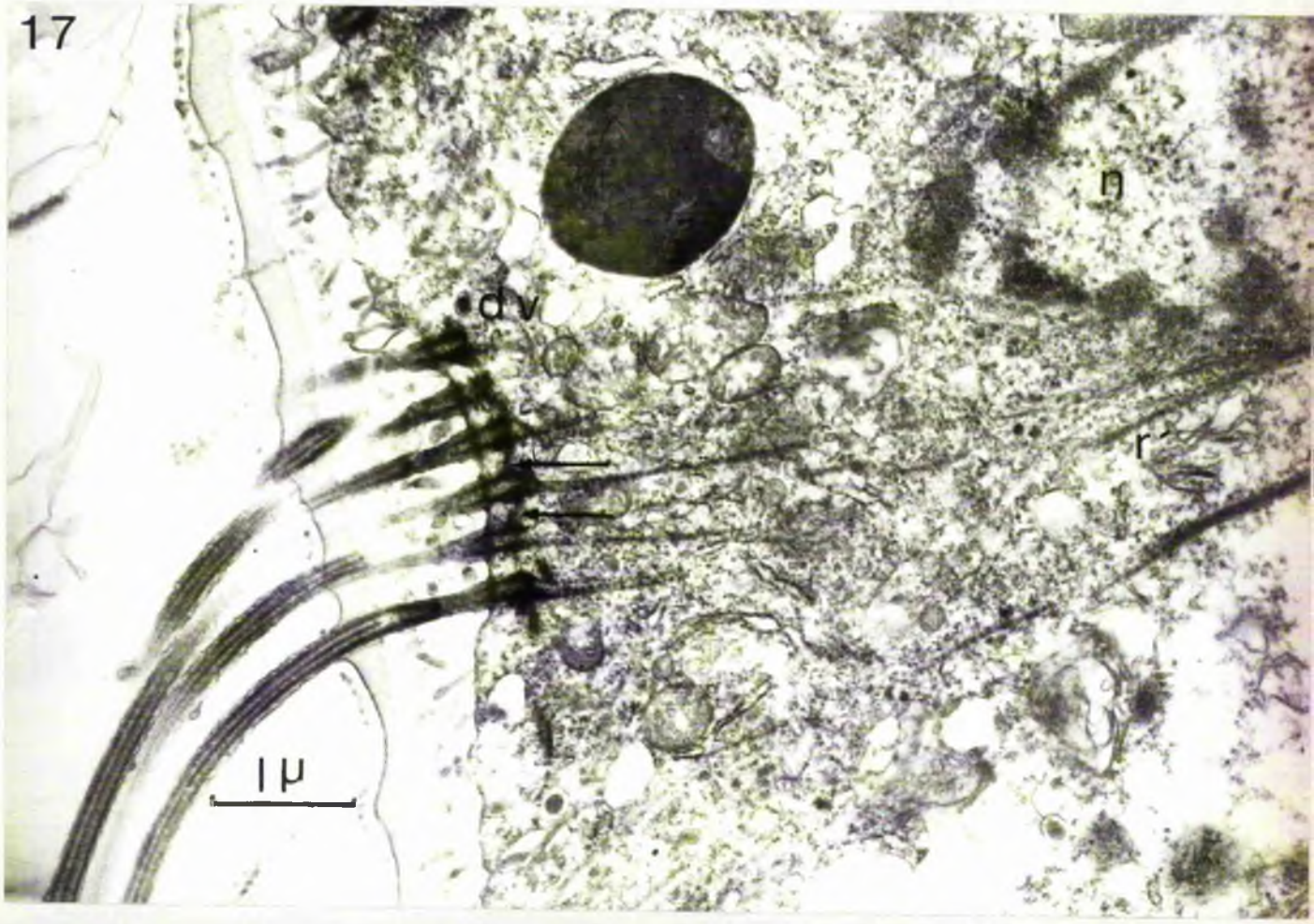
Fig. 16.

Apical cilia. Note tapering tail of the cell bearing the cilia, well developed Golgi figures, G, dense-cored vesicles, dv, lateral roots of the cilia, r, nucleus, n.

Fig. 17.

Apical cilia. Note oriented basal feet, arrows, longitudinal roots, r, extending to the nucleus, n, dense-cored vesicles, dv. Cacodylate glutaraldehyde B. tid.


16
1 µ
r
dv
G
n


17
n
dv
r
1 µ

large and appear to produce many 500–700Å vesicles. Occasional dense cored vesicles 1300Å across are also found above the nucleus. Rough, and smooth-surfaced endoplasmic reticulum is scattered between the Golgi (Figures), usually in the form of a pair of membranes roughly parallel to the cell membrane, with no two pairs closer than 0.5μ. The endoplasmic reticulum tends to concentrate in the tapering portion of the cell. Mitochondria are abundant throughout the cell. Remnants of yolk granules are sometimes found and vacuoles containing dense material or a few clear vesicles (although not resembling multivesicular bodies) are scattered among the ciliary roots in the peripheral portion of the cell. The akrotroch cells are identical in form to the apical cells, and the cilia also form into clumps.

(ii) The Prototroch

The prototroch band which circles the equatorial plane of the larva is made up of 5 rows of cells. The uppermost row bears short, widely spaced cilia in 5 alternating lines (Fig. 18). These cilia have a pair of rootlets projecting at an angle of 45° into the cell, and at right angles to each other. They beat downwards as do all the prototroch cilia, but frequently have a stiff appearance. The middle two rows of cells are the largest cells in the whole trochophore and bear the main prototroch cilia. The bases of the cilia occupy the whole of the distal portion of the cell, which is only 3–4μ wide at the desmosome junctions beneath the cuticle. The effect of this is that the cilia from both cells emerge as a continuous set in the longitudinal direction and can form up into aggregations based on this.

Fig. 18.

Prototroch, slightly oblique longitudinal section. The four sets of cilia and five cells of the prototroch are evident.

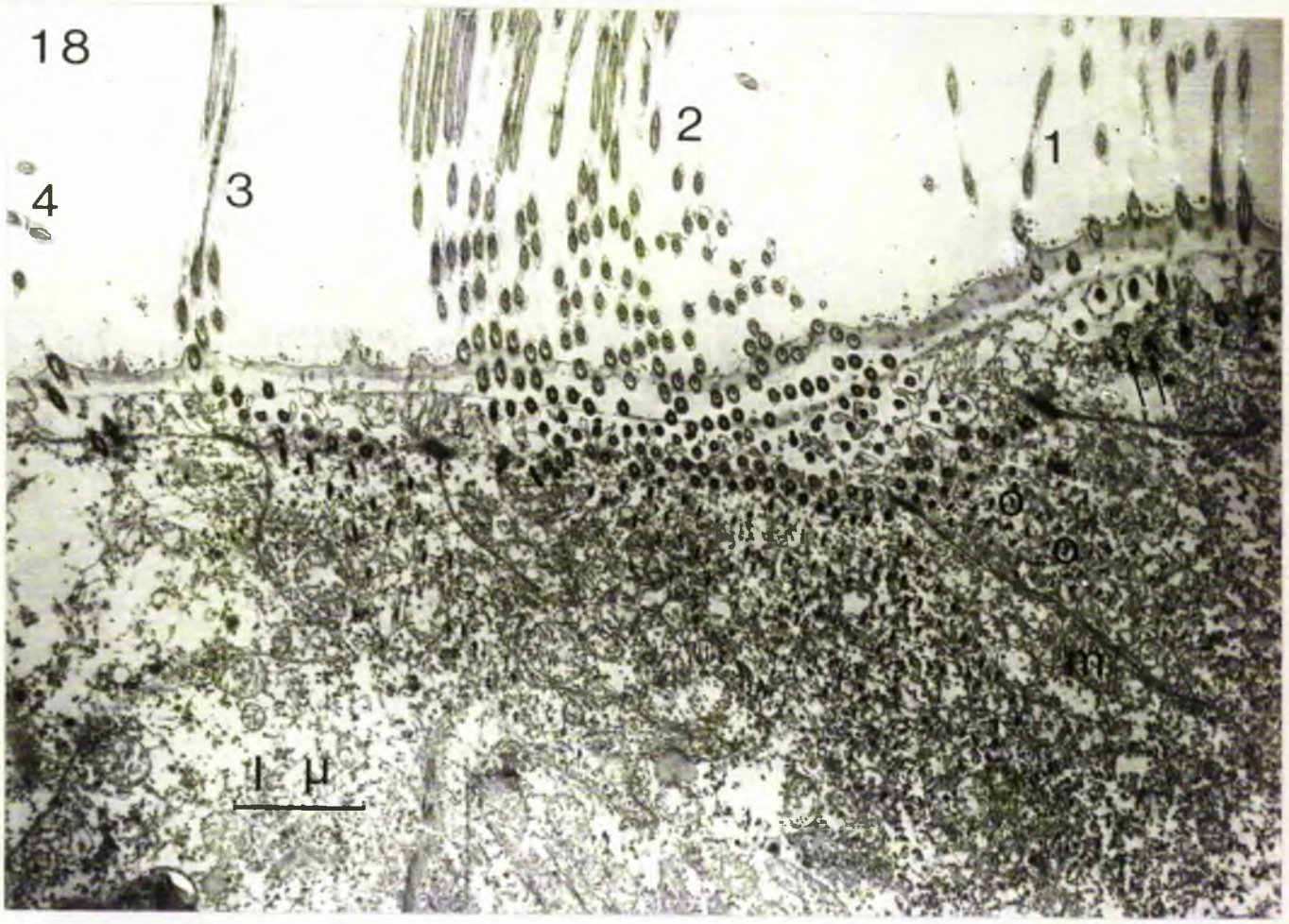
1. Short cilia on the upper side of the prototroch; notice basal foot and oblique roots on the third and fourth basal bodies in the cell, arrowed.
2. Main locomotor band from two cells. Basal feet are directed towards the base of the picture.
3. Group of cilia below main cilia.
4. Ungrouped cilia at base of prototroch.

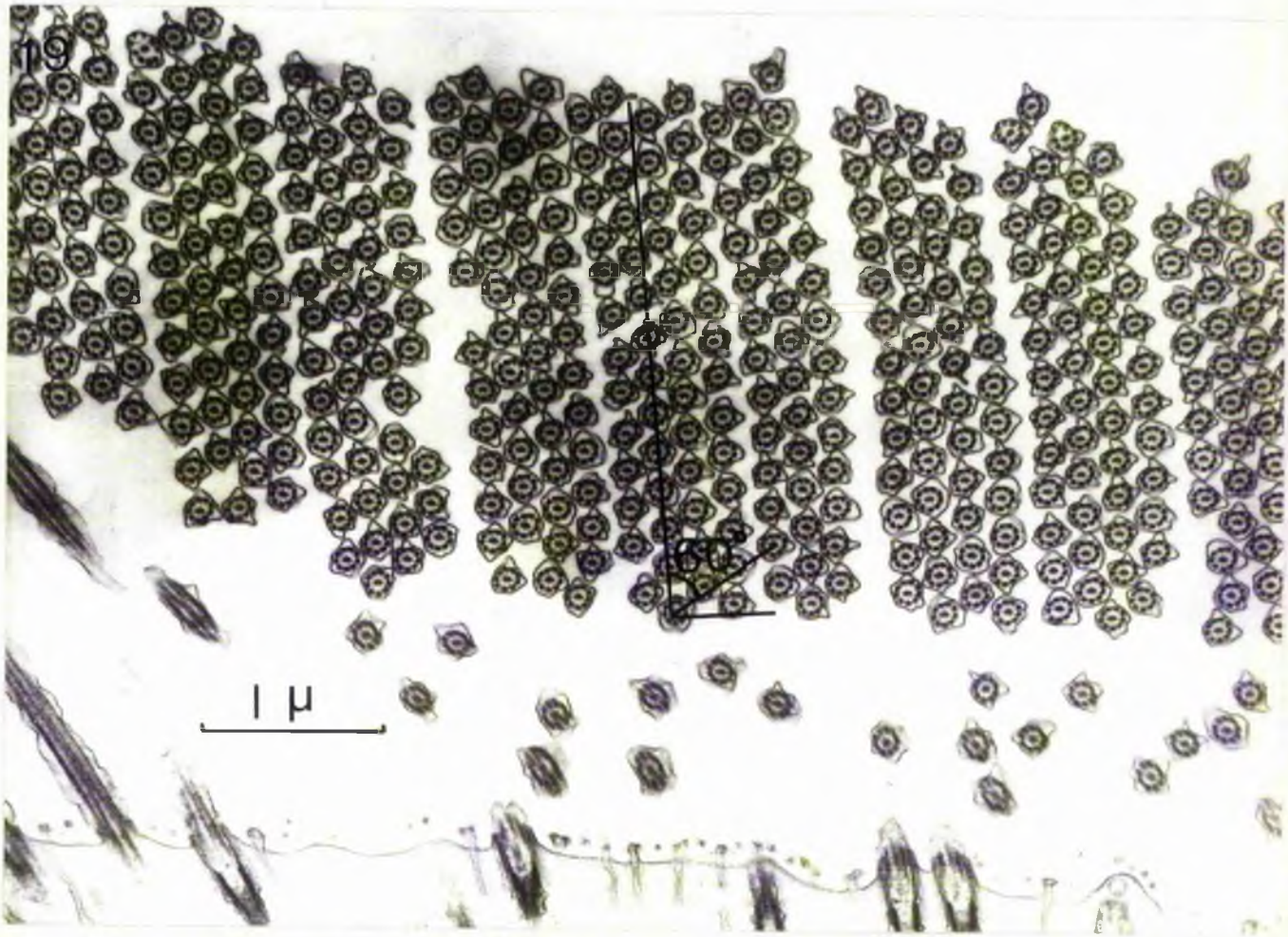
Mitochondria, m, are prominent, particularly along the adjacent membranes of thw two central cells.

Roots cut in T.S. ringed with O.

Fig. 19.

Groupings of main locomotor cilia of the prototroch. All the grouped cilia here are supplied by 2 cells which contribute half of each group. Note oriented central filaments.


18
4
3
2
1
1 μ


1 μ

An average of 44 cilia make up the aggregation (Fig. 19), with 10 to 15 cilia in the long axis of the clump, 3, 4 or 5 cilia in the short axis. The membranes of the cilia are not attached to each other although the cilia are packed in the most compact arrangement, in lines at 45° to 60° to the long axis of the clump. Each of the pair of cells bears half of some 16 bundles, approximately 350 cilia in all. The basal bodies in both cells point in the same direction, although the orientation is not precise, but the rootlets project into the cells in opposite directions (Fig. 20), the ones nearest to the central desmosome being at an angle of 50° to each other, the ones near the opposite desmosome being at an angle of 90°. The roots vary in thickness and in transverse section have a dumb-bell or flattened 's' shape (see Fig. 18). In longitudinal section (Fig. 21) the variation in thickness shows up as longitudinal differences in density of the root, and longitudinal filaments are visible. Striations with 6 bands, periodicity 600Å are evident and the many mitochondria in this part of the cell are closely packed such that they are frequently in contact with the roots. When this occurs, the mitochondria are found to have their long axes parallel with the roots, but the cristae were not seen associating with the dark band of the striations as in Branchiostoma (Olsson, 1962).

The distal adjoining membranes of the large cells are also heavily supplied with mitochondria (Fig. 18), and in fact, the high density of mitochondria in the distal half of these cells is one of their outstanding features. The nucleus is also distinctive, pear-shaped and large compared with others in the trochophore, being 6μ

Fig. 20.

The prototroch cut in longitudinal section showing the group of long cilia arising from the 2 central cells. Note desmosomes, d, longitudinal roots, r, projecting in opposite directions in the two cells and basal feet, arrows, oriented in the same direction in both cells.

20

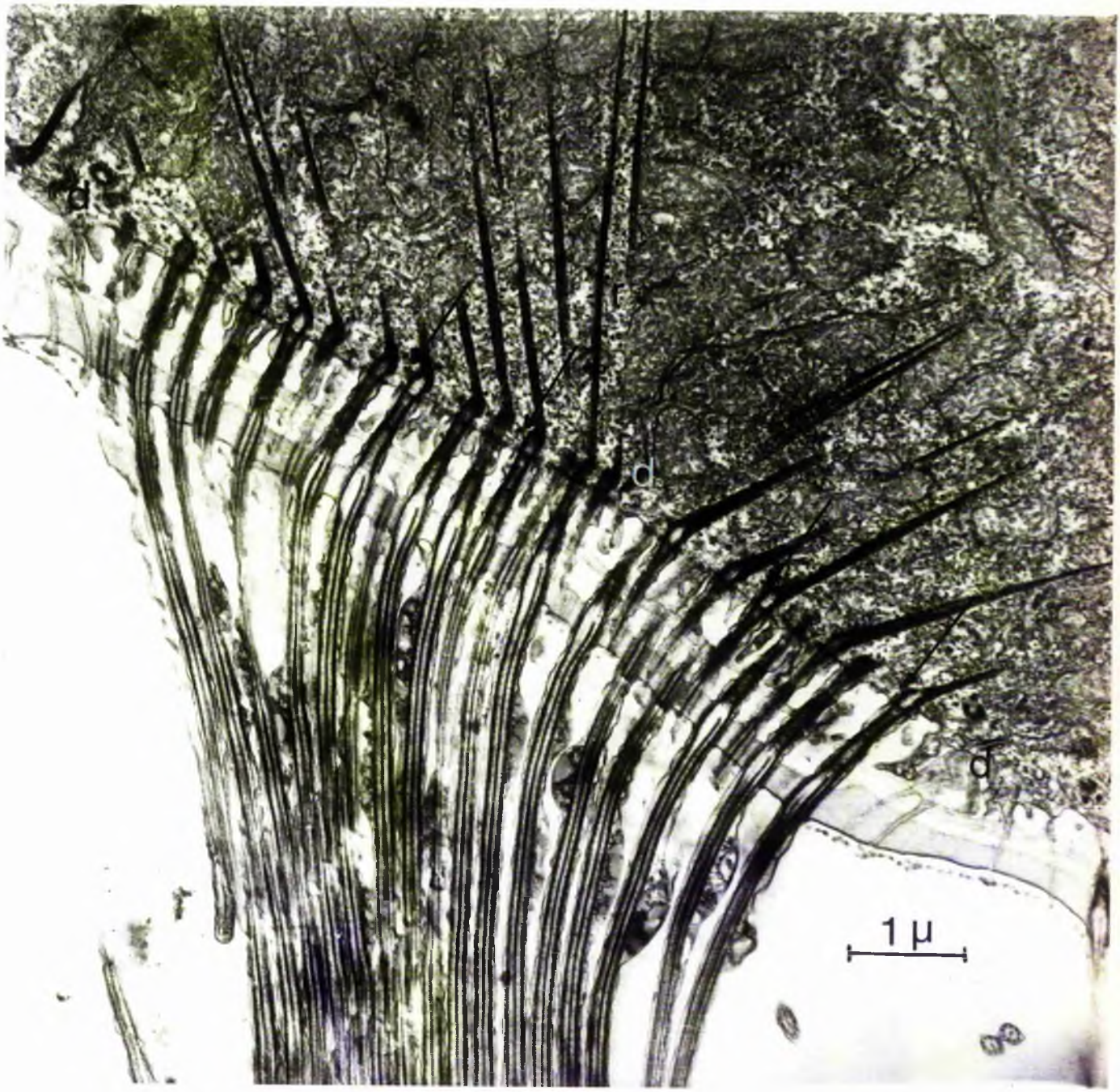


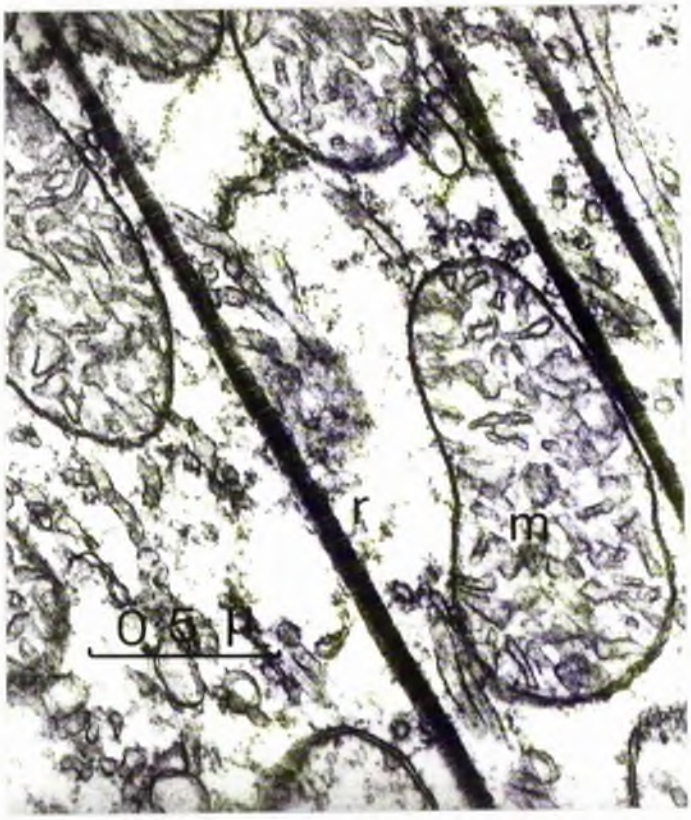
Fig. 21.

Prototroch longitudinal roots, r, and their relationship to the mitochondria, m.

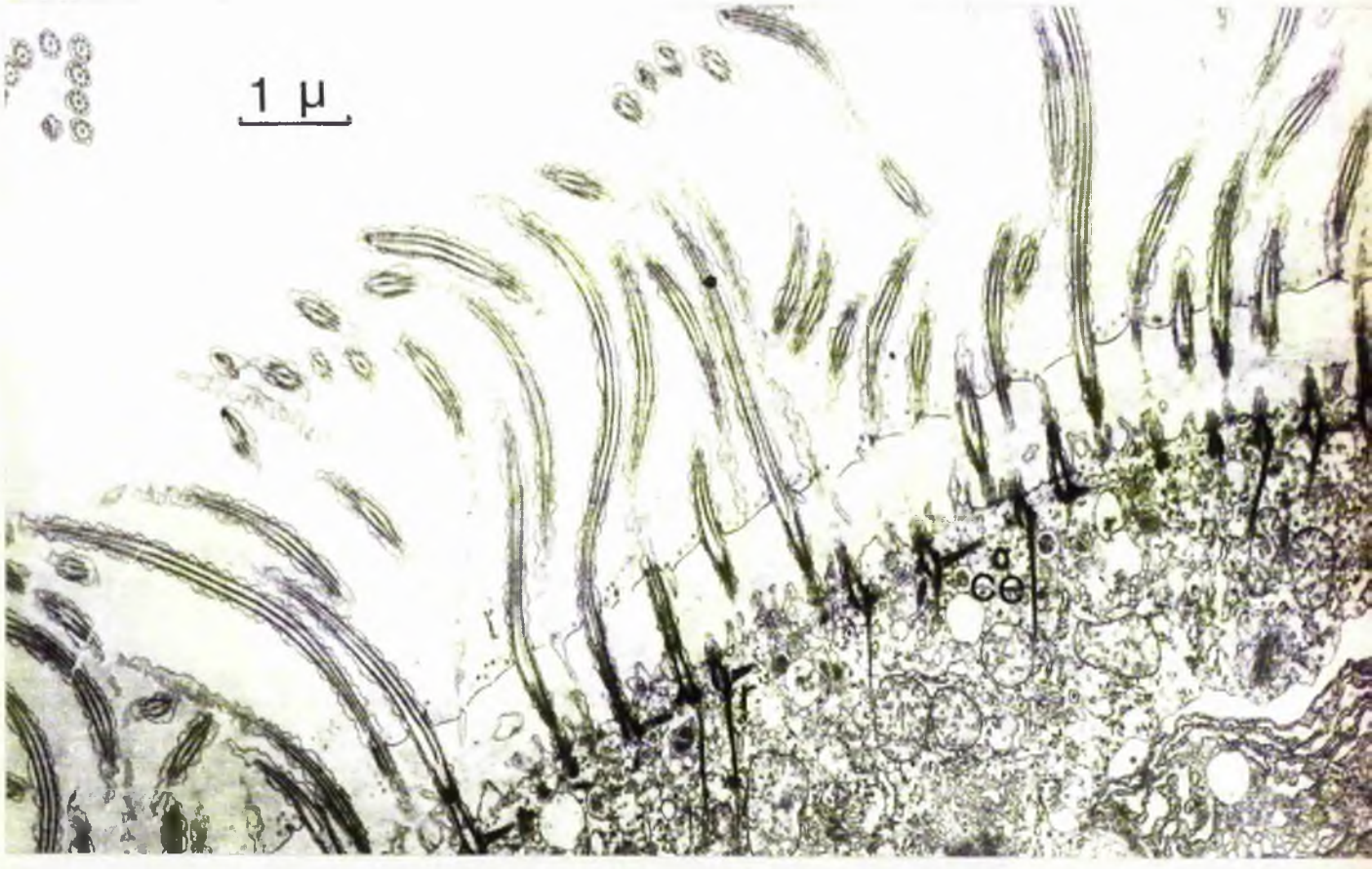
Fig. 22.

The neurotroch. Note longitudinal and lateral roots, r, oriented basal feet opposite the lateral roots, and centriole, ce.

21



22



broad and 11μ long. The matrix is pale, more relatively uniformly granular than in other nuclei , and there is a large, dense nucleolus. Several Golgi bodies lie near the nucleus, on the upper distal side. The nucleus is situated on the proximal side of the cell, broadest side innermost.

The 6μ and 14μ cilia of the lower prototroch cells are in clusters of 15 to 20 and beneath these is another row of cells bearing cilia which are not co-ordinated in activity with the above prototroch cilia. The latter cilia are continuous with the cilia of the upper lip. In line with this band of cilia there is a set of long cilia on the left hand side of the mouth. These cilia increase in length with age.

(iii) The Neurotroch

The neurotroch cilia are 4-5μ long and have a short thick lateral root opposite the basal foot, and a long thin root at an angle of 120° to the lateral root (Fig.22). Centrioles at right angles to the basal body are occasionally found. The neurotroch is only one cell wide at the base and two cells long. Large glandular cells, described below, indent the neurotroch cells to give them an hour-glass appearance, except that the proximal edges are very flattened, forming extended processes. One of these processes runs along beneath the glandular cell on the distal side of the blastocoel, and the other enters an axon bundle. This ciliated cell therefore bears resemblance to a bipolar neurone. The cilia beat towards the anus.

Anal tuft The last surface bundle of cilia is a collection of about 15 cilia in 3 to 5 groups, 16μ beyond the end of the neurotroch, slightly to the right of the anus.

3. The Nervous System

(i) General structure

Toluidine blue preparations of sectioned trochophores for the light microscope did not reveal any information on the nervous system (Fig. 23). The electron microscope, however, showed that the nervous system is fairly extensive, although not of the form that might have been expected from light microscope studies of other trochophores. Most of the apparently undifferentiated cells in the epidermis at the apex of the trochophore are nerve cells or sensory cells contributing to an axon mass of some 100 to 150 axons and dendrites (Figs. 24 and 25). From this axon mass, circumoesophageal connectives run down to join with a sub-prototroch axon mass of some 30 to 50 axons running circularly (Fig. 26). There are five axons associated with the eye and some of these supply the ciliated cells beside the eye. From the eye an axon runs down on the ectodermal side of the blastocoel to join with the sub-prototroch nerves. A group of some twenty axons extends into the posterior portion of the trochophore and is found adjacent to the base of terminal neurotroch cells which themselves contribute axons.

(ii) Types of nerve

There appear to be three types of axons. Most of the nerves

Fig. 23.

Light micrograph of a trochophore, frontal, longitudinal section, showing apical cilia, a; blastocoel, b; glands, g; gullet, o; neurotroch, n; and prototroch, p.

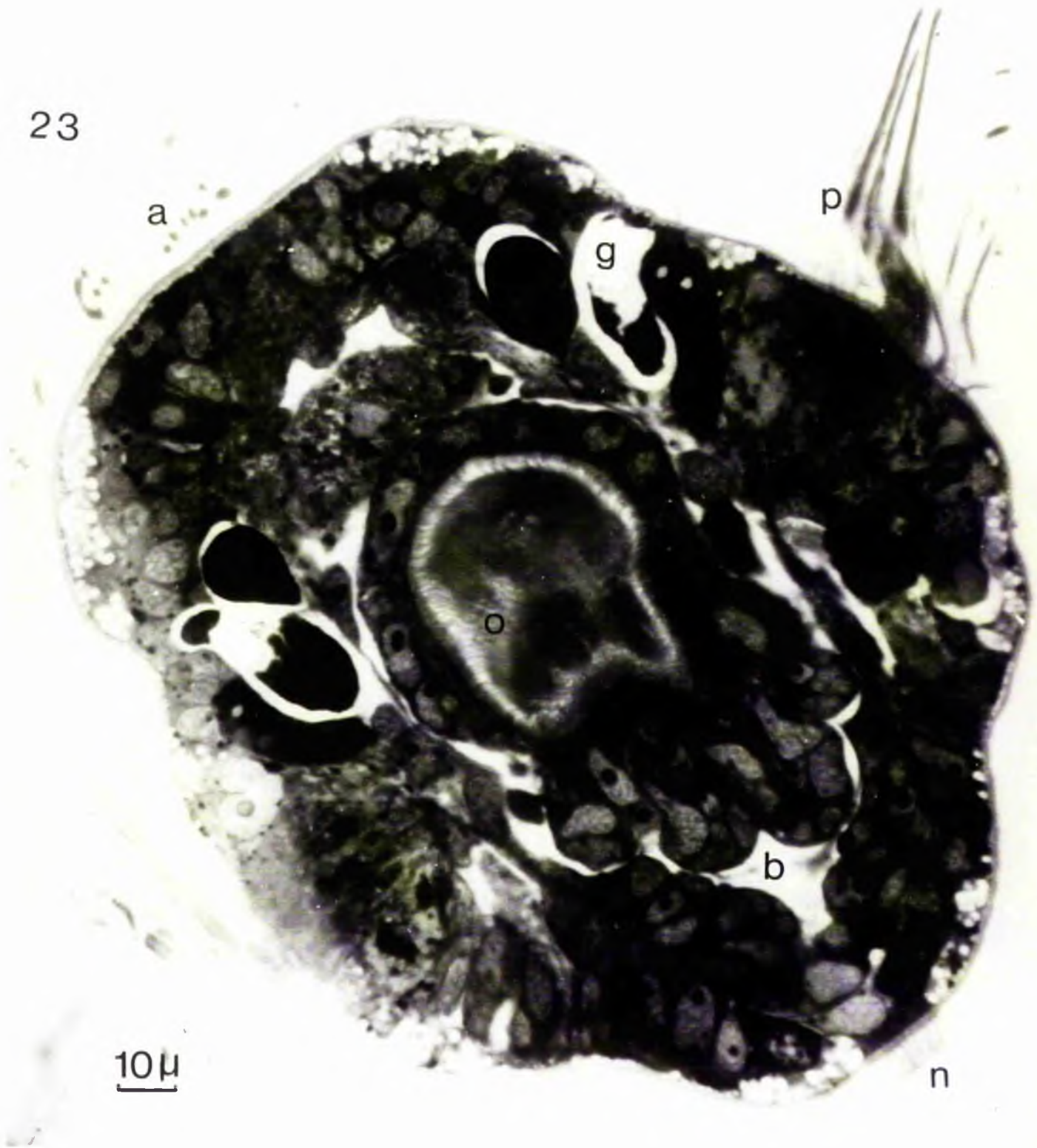

23
a
p
g
o
b
10µ
n

Fig. 24.

Apical cells, many of which are presumptive nerve cells. 20 minutes osmium with sea water A1.(ii).

24

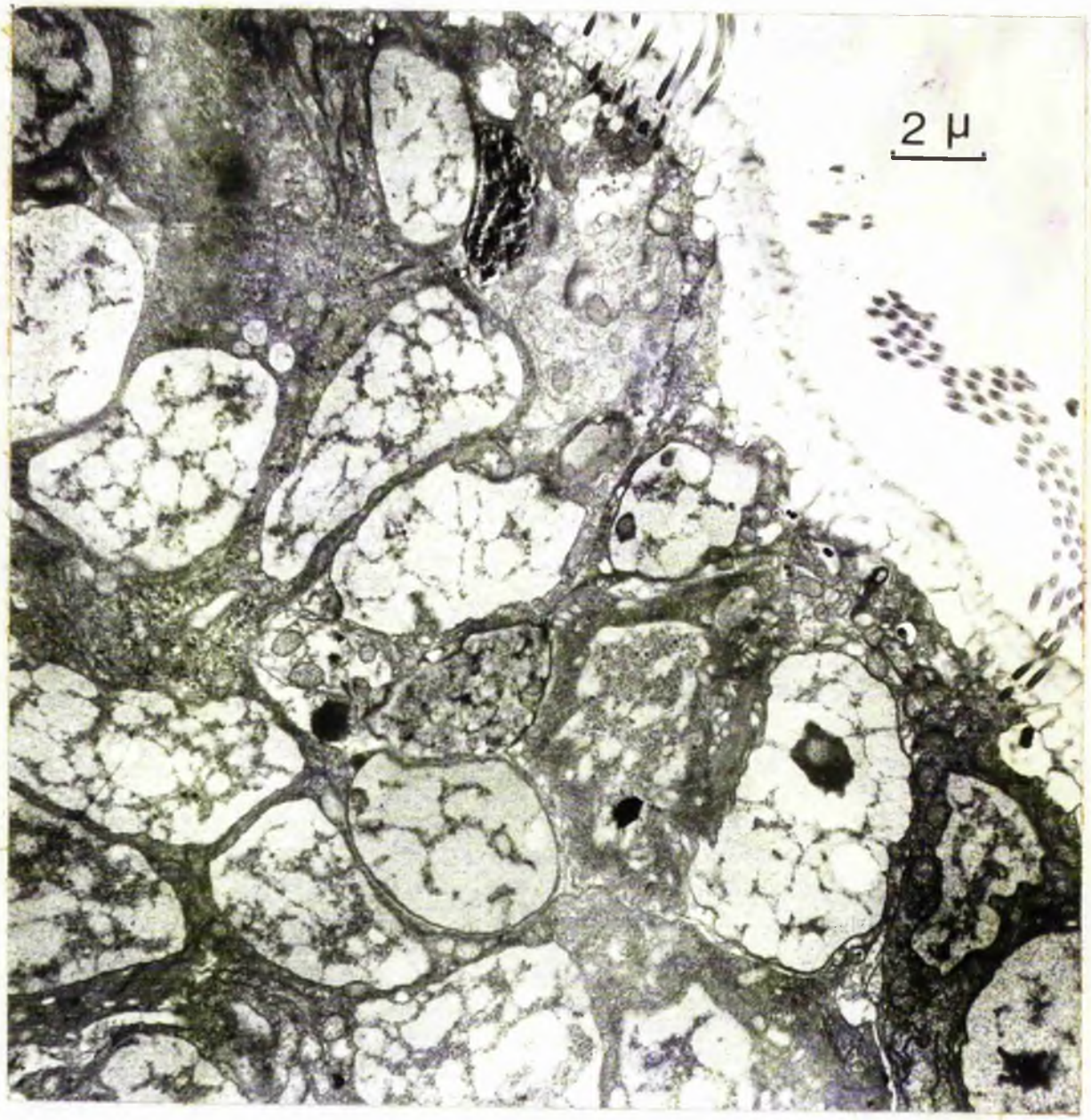


Fig. 25.

Part of the apical axon mass, showing axon-axon synapses, s, and neurosecretory cells, N.

25

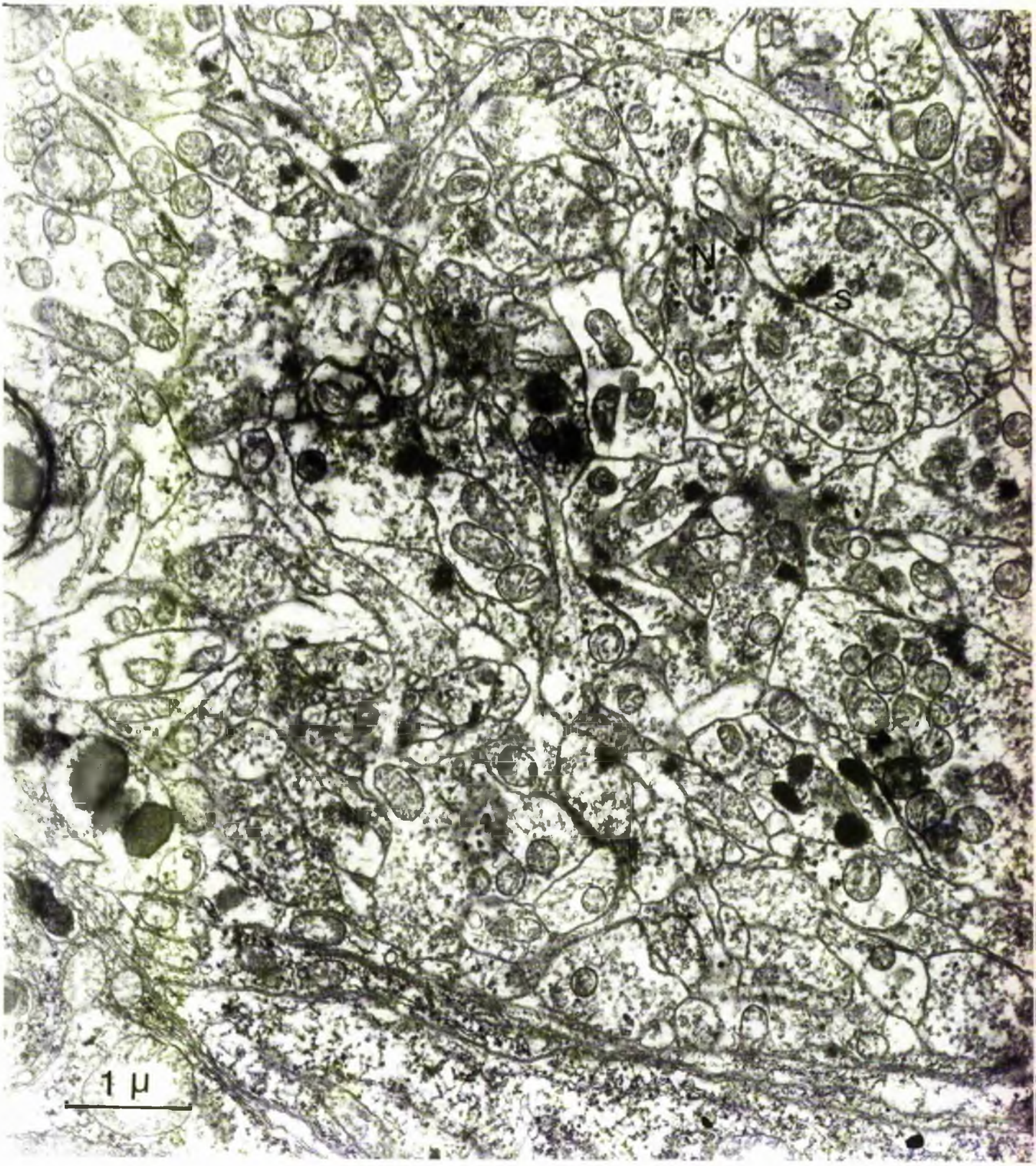


Fig. 26.

General view showing circumoesophageal commissure, C, and sub-prototroch nerves, S. The gullet is lower right. Note muscle, m, between gullet cells and blastocoel, b.

26

contain clear vesicles that are 400Å in diameter and of the type generally considered to be cholinergic. These axons are 0.1μ to 1μ in diameter. Some five percent of nerves contain dense-cored vesicles of the order of 1000Å along with the 400Å clear vesicles (Fig. 25). In the apical nerve bundles and in the circumoesophageal commissures the nerves containing dense-cored vesicles have such a concentration of these vesicles that they give a definite appearance of being neurosecretory nerves. These axons are up to 3μ in diameter. In the prototroch nerve, the axons with dense-cored vesicles have these as a minor addition to the predominance of clear vesicles, giving the impression that the function of the dense-cored vesicles in this instance is to enhance or inhibit the effect of the clear vesicles.

(iii) Synapses

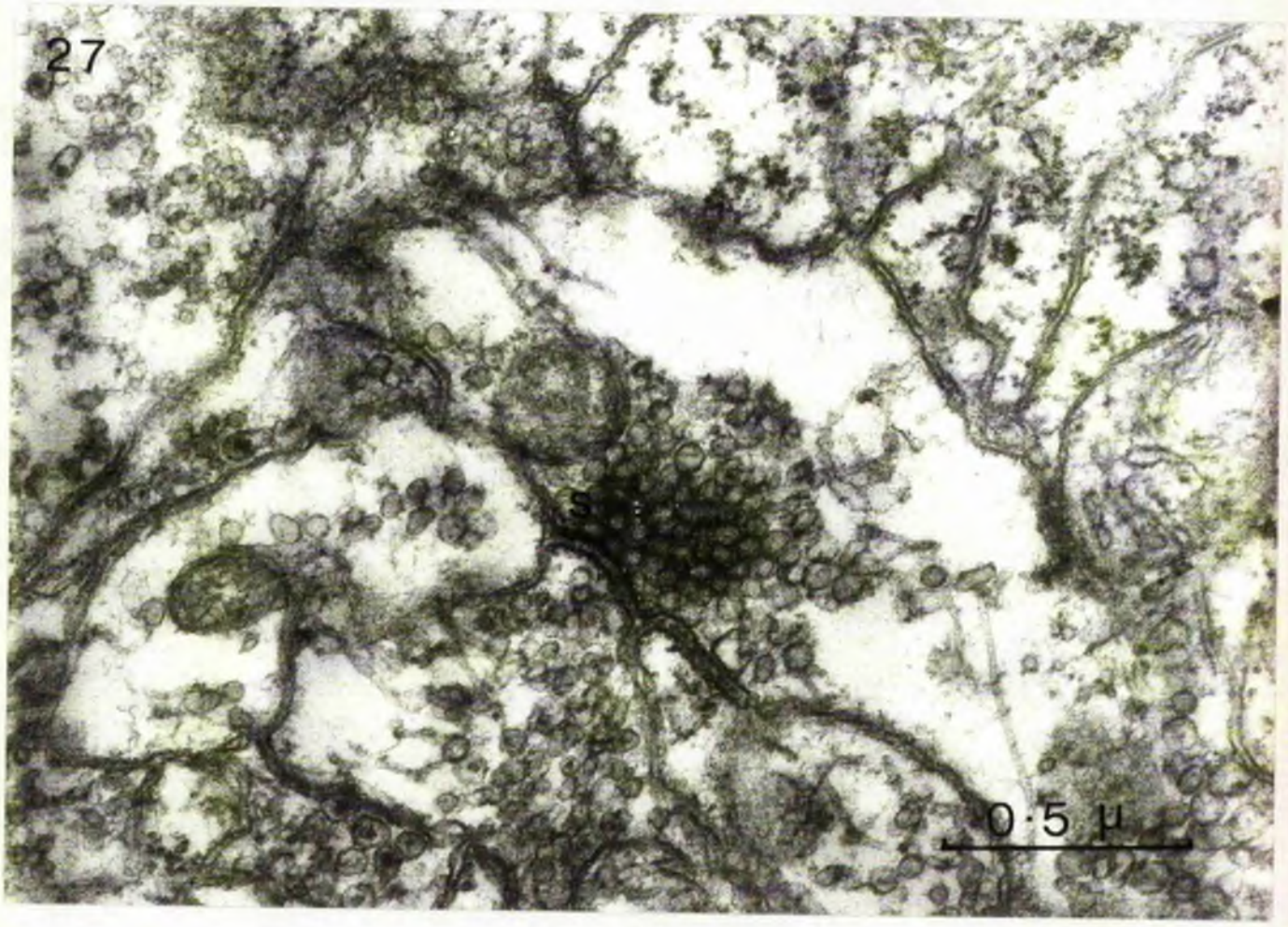
Axon-to-axon synapses occur roughly in the ratio of one to every four axons as in Fig. 25, where among 105 nerves there are 20 synapses and 5 groupings of vesicles such as occur at synapses. The groupings have been cut above their area of contact with the membrane. Fig. 27. shows a typical synapse with the collection of vesicles on the membrane, a 180μ cleft with a granular line at its centre, membrane thickenir and some evidence of a presynaptic bar. Fig. 28 shows an axon-to-axon synapse in the sub-prototroch nerve with similar characteristics to the one just described but with the difference that this nerve contains both dense-cored and clear vesicles.

Fig. 27.

Axon to axon synapse, s, showing clear vesicles clustered at the synapse, membrane thickenings, dense material particularly concentrated centrally in the cleft.

Fig. 28.

Axon to axon synapse. The presynaptic nerve contains clear vesicles, v, and dense-cored vesicles, v'. Prototroch cell, p.


27
S
0·5 μ

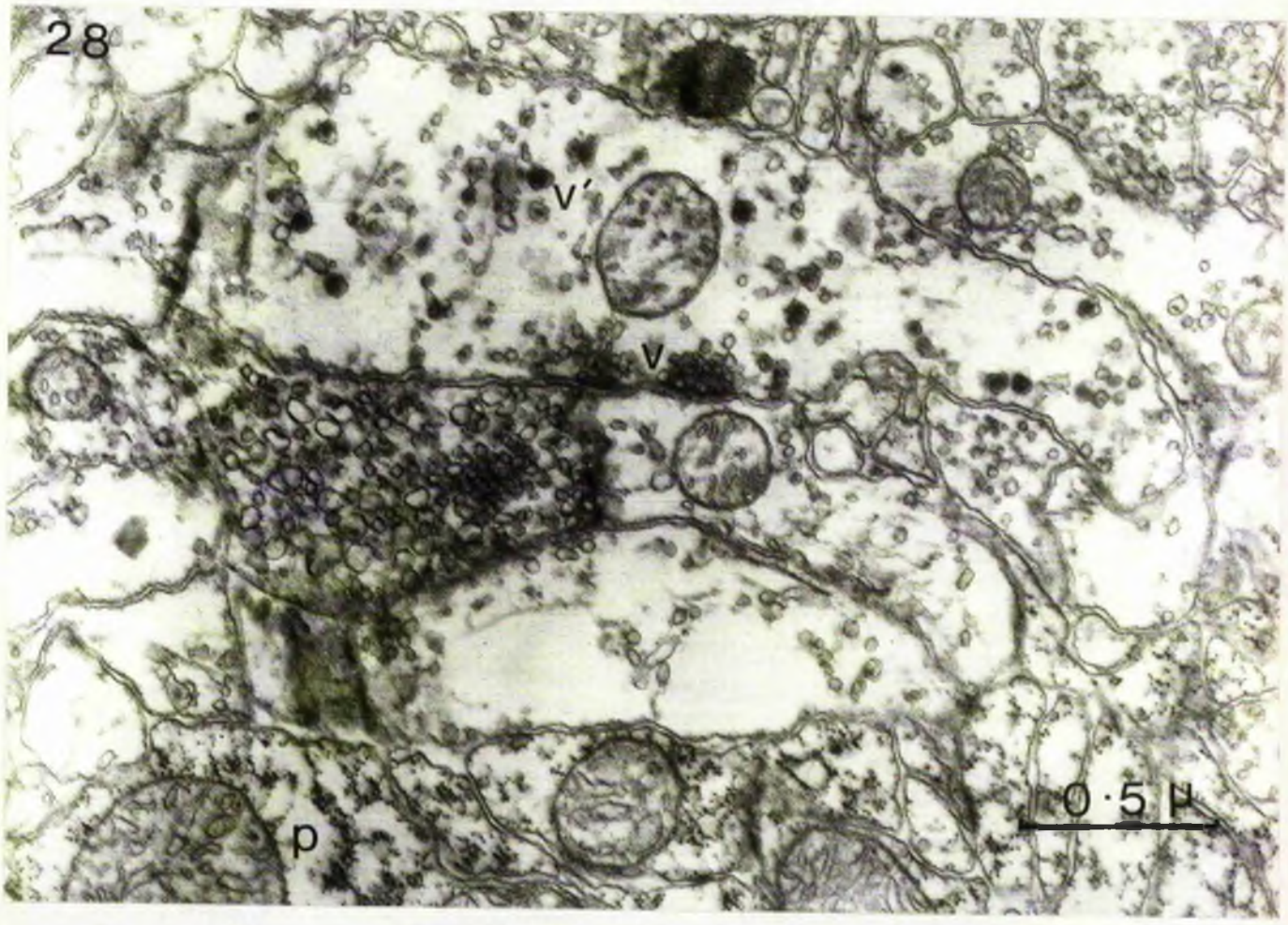

28
V'
V
P
0·5 μ

Fig.29.

Low power view of the prototroch, p, and the sub-prototroch nerve, n, to show the size of the neurociliary synapse relative to the size of the prototroch cell, and its position. The synapse is in the square which is shown at greater magnification in the inset.

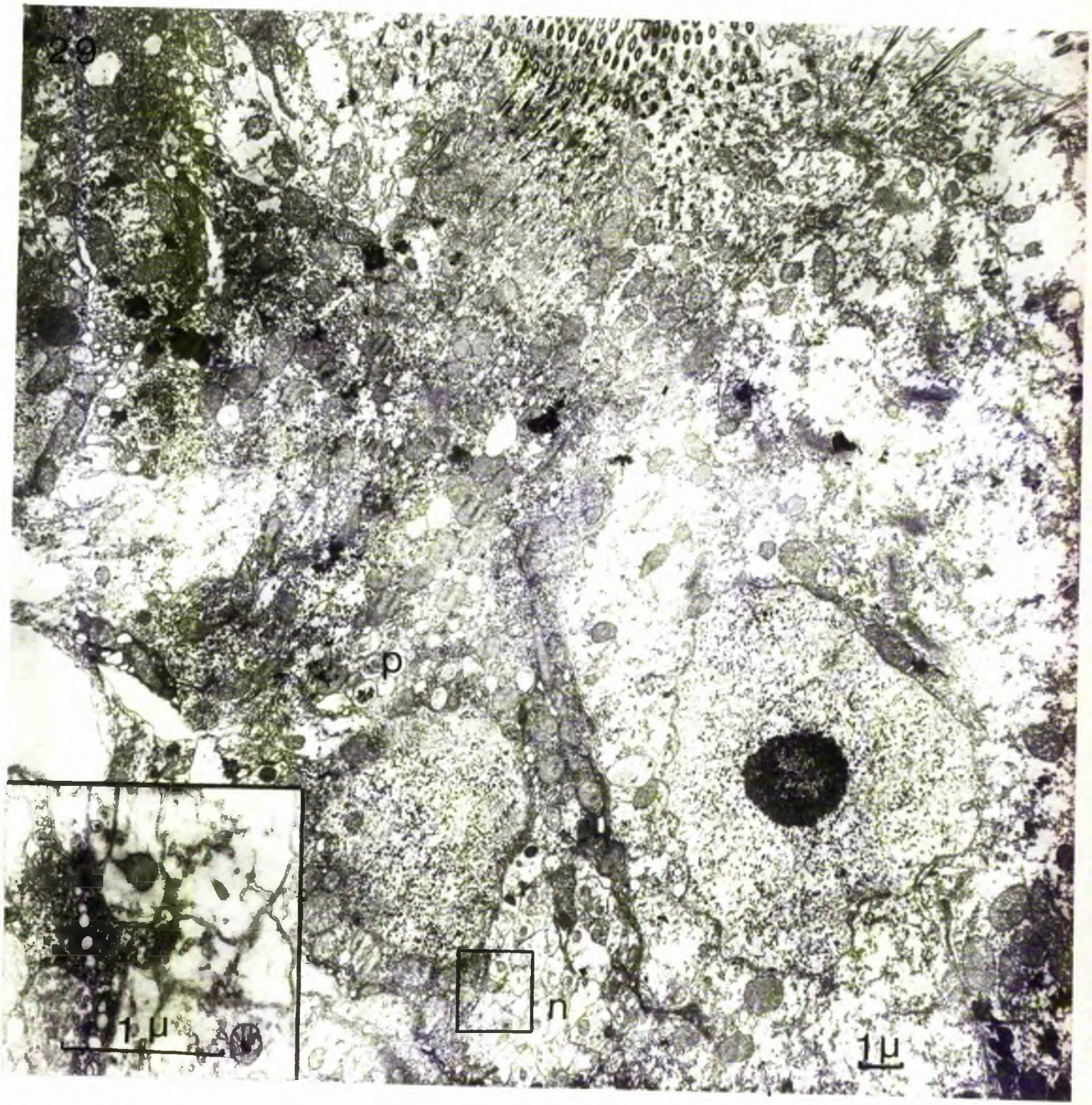
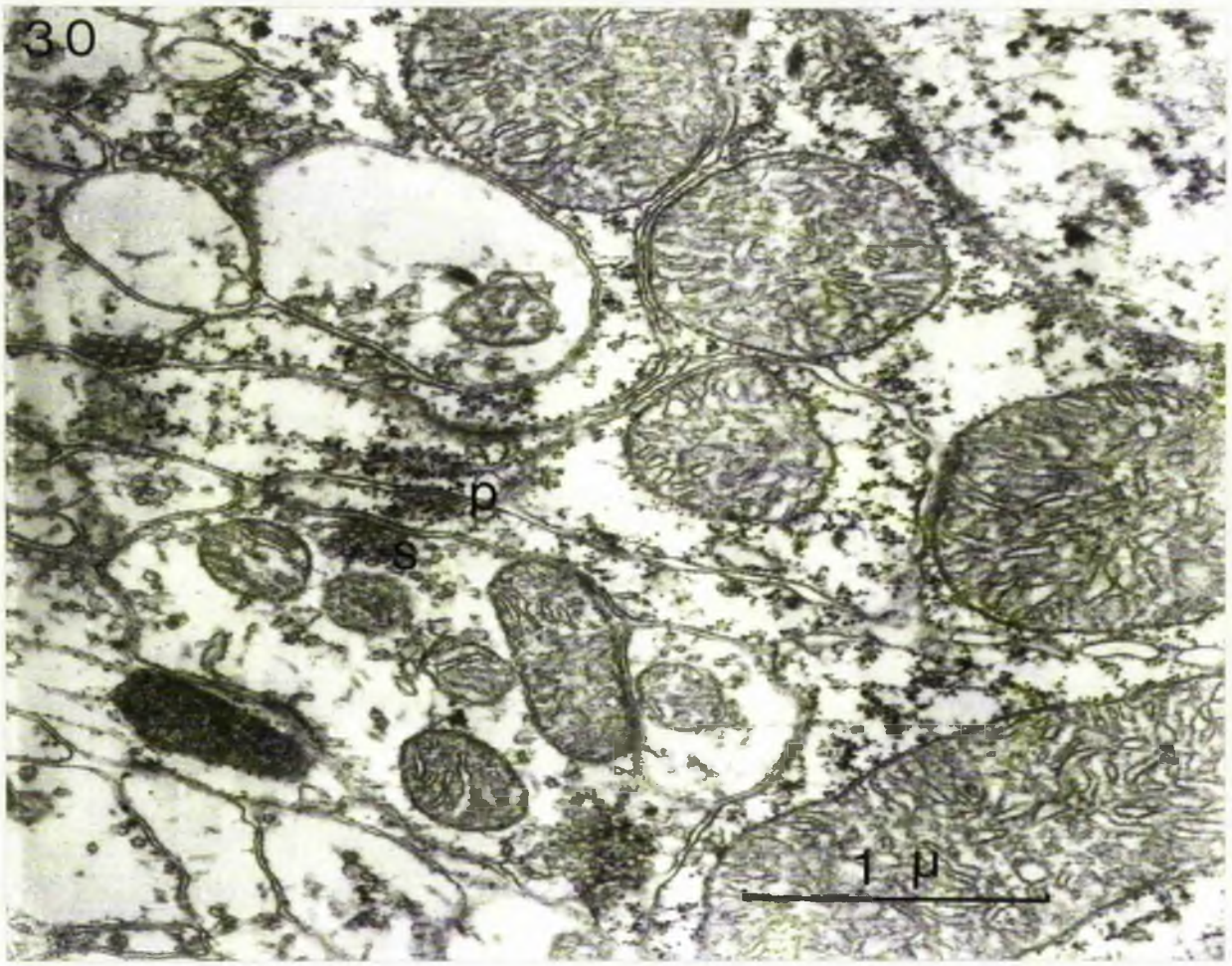

29
p
n
1 μ
1μ

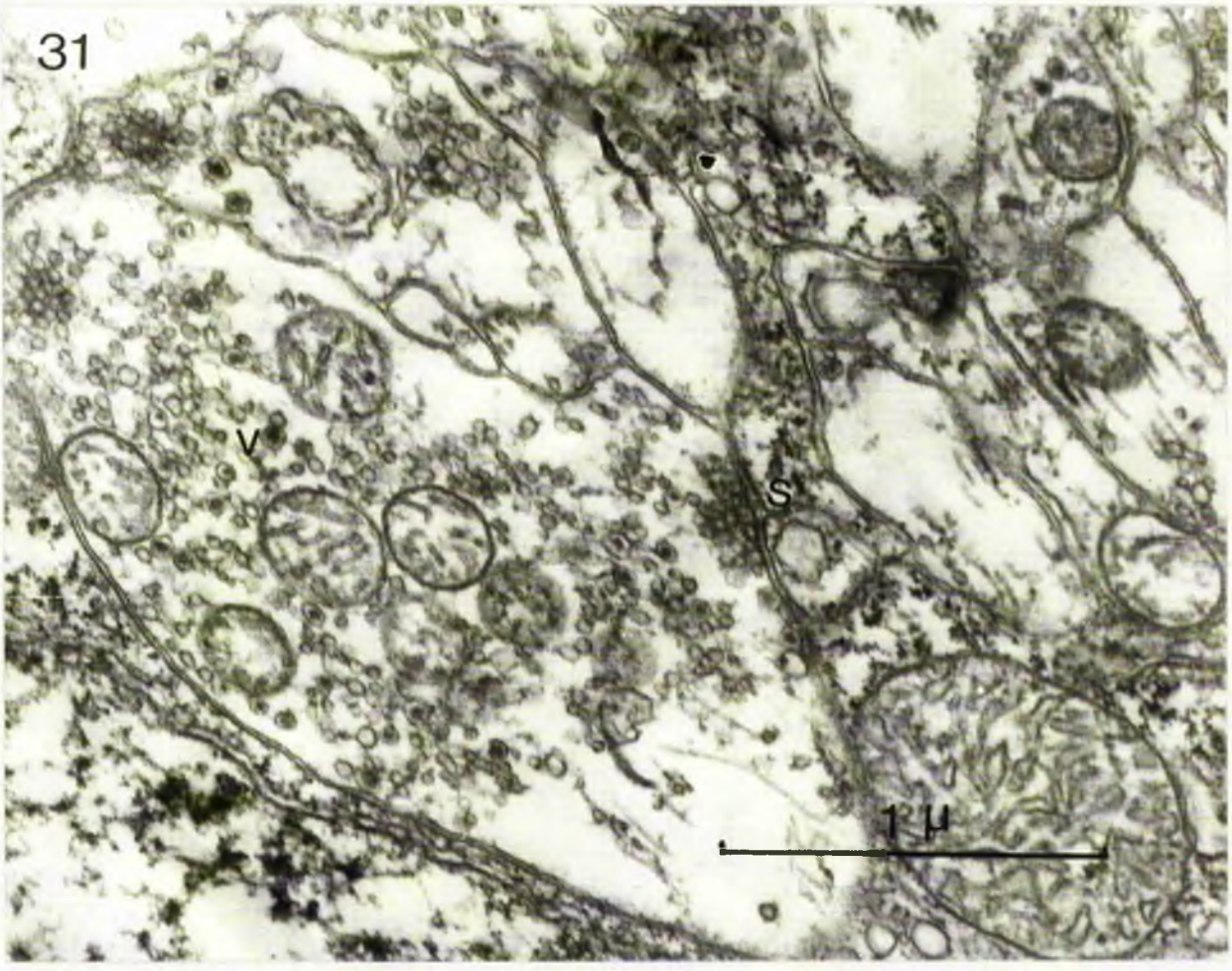
Fig. 30.

Neurociliary synapse, s, onto prototroch cell, p. Note the large mitochondria of prototroch cell and abundant ribosomes, in contrast with the small mitochondria and lack of ribosomes in the nerve.

Fig. 31.

Neurociliary synapse, s, onto prototroch cell. Note the mitochondria and ribosomes as above, also dense-cored vesicles, v.


30
p
s
1 μ


31
v
s
1 μ

Neurociliary synapses are a feature of this animal, and Fig. 29 shows the prototroch and part of its axon mass at a point where there is a synapse onto the prototroch cell. Figs. 30 and 31 show synapses from two types of neurones onto the prototroch cell, the pre-synaptic neurone in Fig. 31 having both dense-cored and clear vesicles. Synapses were not found onto the neurotroch or apical cells, although nerve fibres were found adjacent to these cells.

4. Surface Opening Glands.

There are three types of surface-opening gland in this trochophore. The glands which form the set of 4 above the akrotroch are illustrated in the transmission electron micrograph (Fig. 32) and referred to as type I. These glands have a thick, fibrous, investing sheath which is continuous with the array of electron-dense microvilli which support the pore. The pore of these glands is 0.3μ in diameter at its opening; the microvilli are 700Å in diameter, 0.7μ long and are 3 deep around the pore opening (Fig. 33). The neck of the gland itself is 0.8μ across to the outside of the sheath and is packed with uniform, membrane-bound granules of uneven density. The granules are 0.3μ wide, 0.4μ long and have a layered appearance with three dark bands on each side. These glands are thin and tapering.

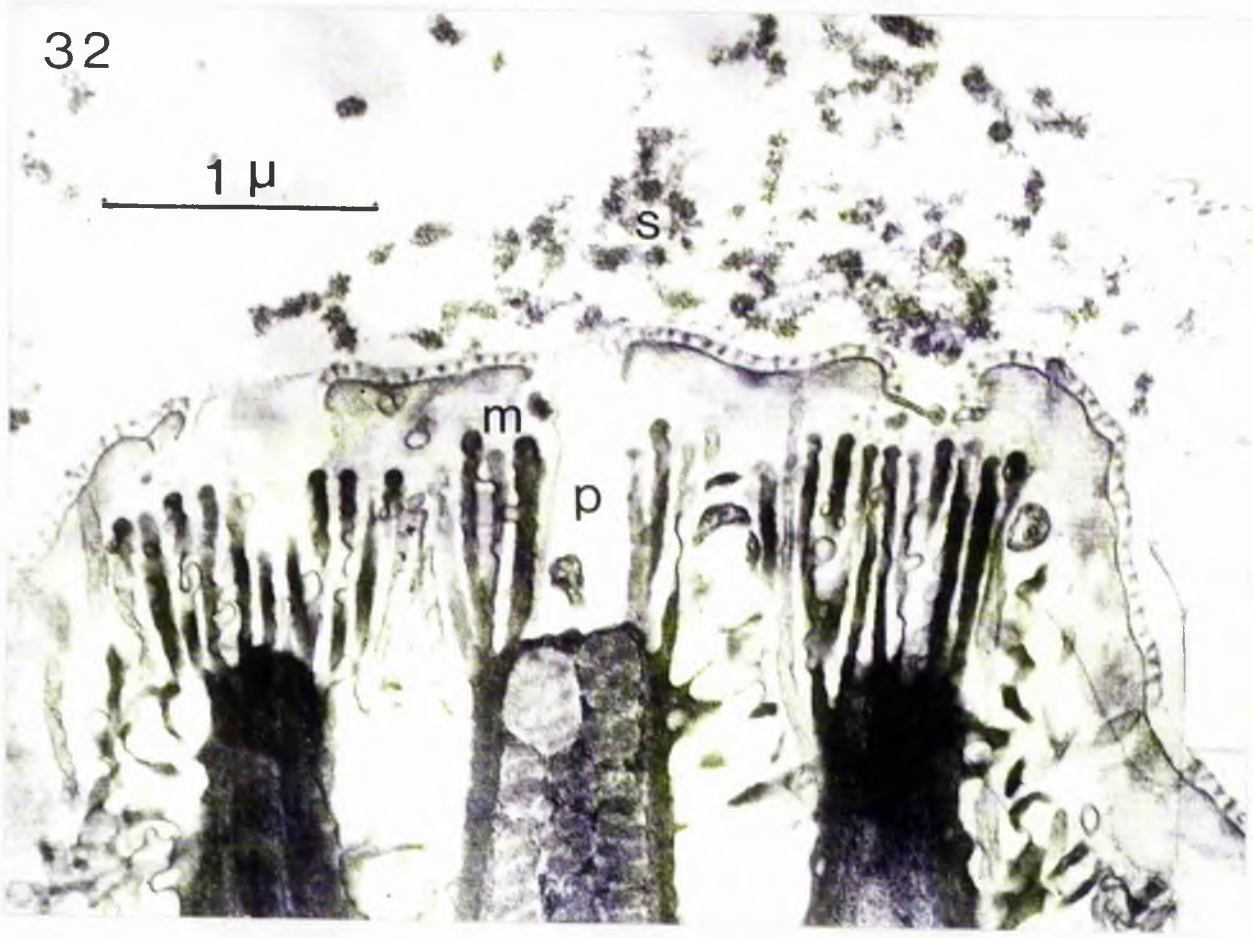
The glands which occur as pairs on each side of the trochophore, type II (Fig. 34), have a thin, not very electron-dense sheath and a single row of 1μ long microvilli at the pore opening. The sheath is composed of longitudinal, uniformly spaced microtubules 0.07μ apart (Fig. 35). The pore is about 0.7μ in diameter at the opening. In these glands there

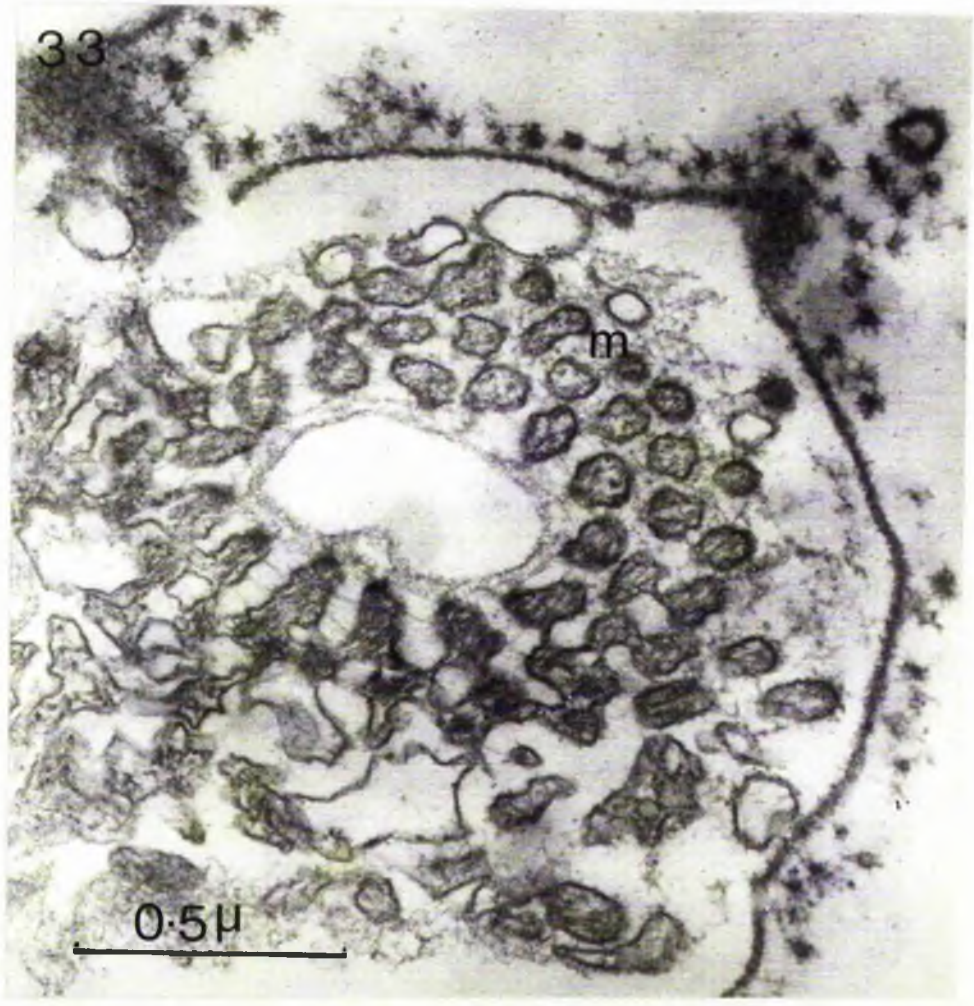
Fig. 32.

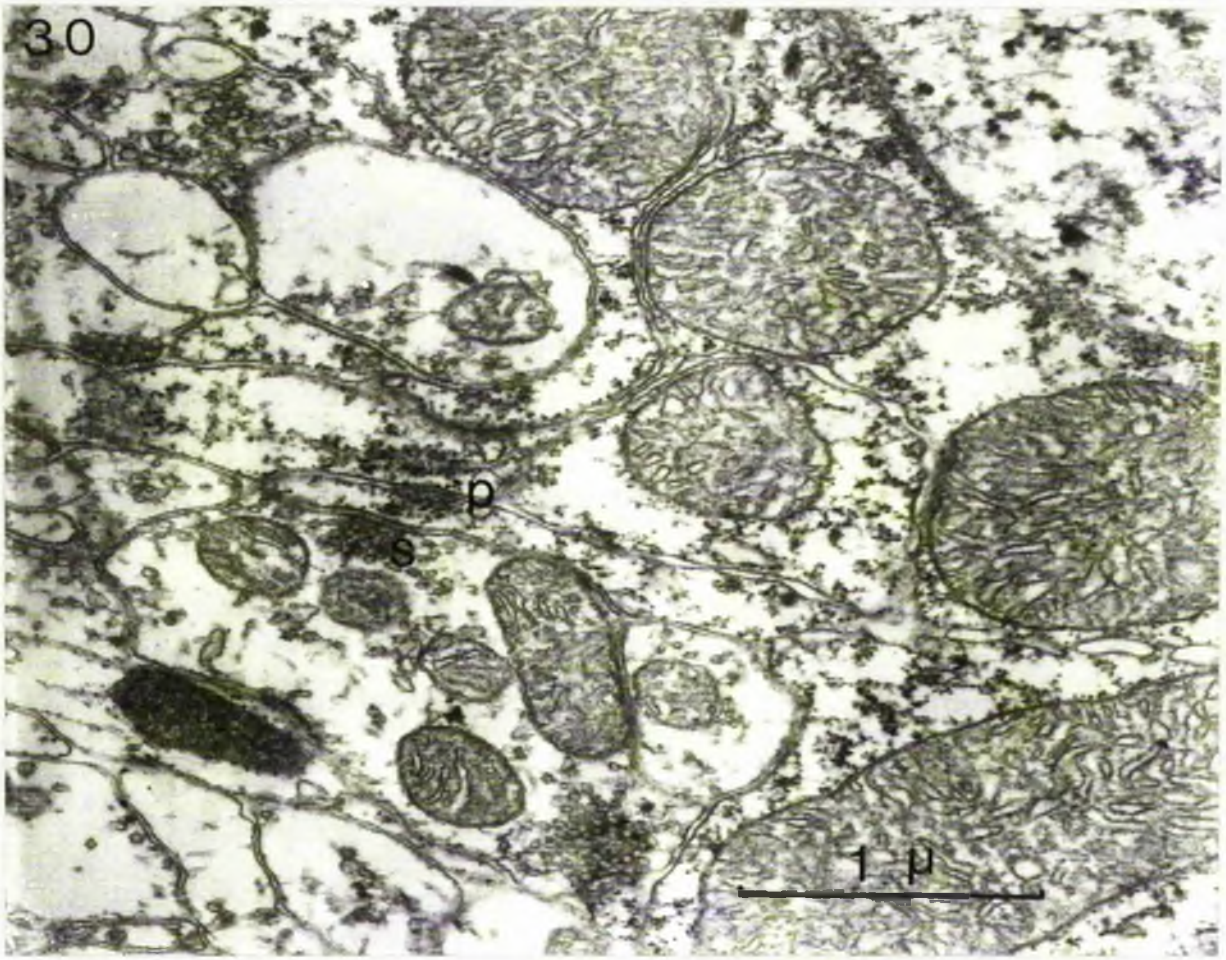
Gland type I. Note released secretion material, s, microvilli, m, supporting pore, p.

Fig. 33.

Pore of gland cut in transverse section, showing microvilli, m. Note fine filaments connecting obliquely sectioned microvilli.


32
1 μ
s
m
p


33
m
0·5μ


30
p
s
1 μ

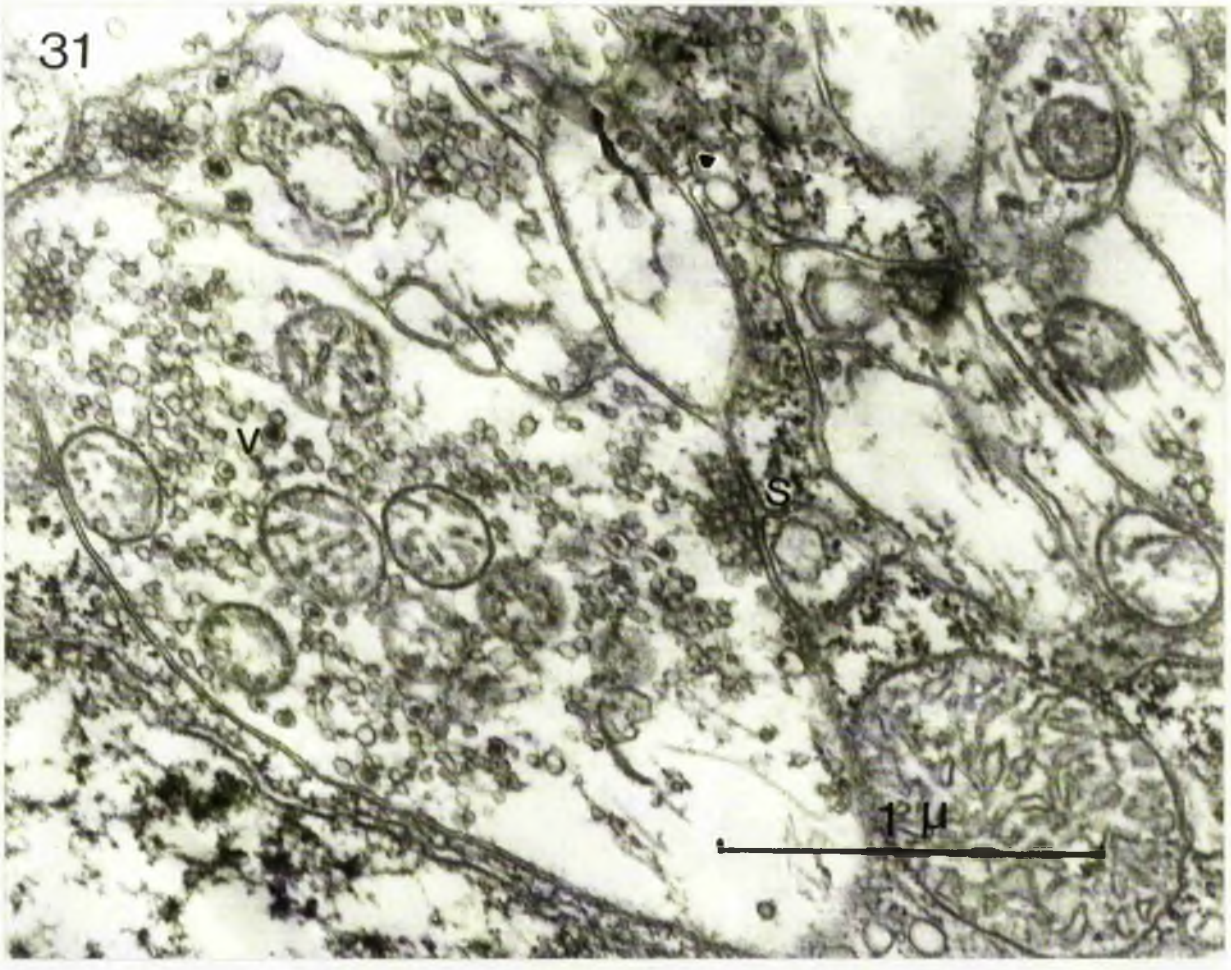
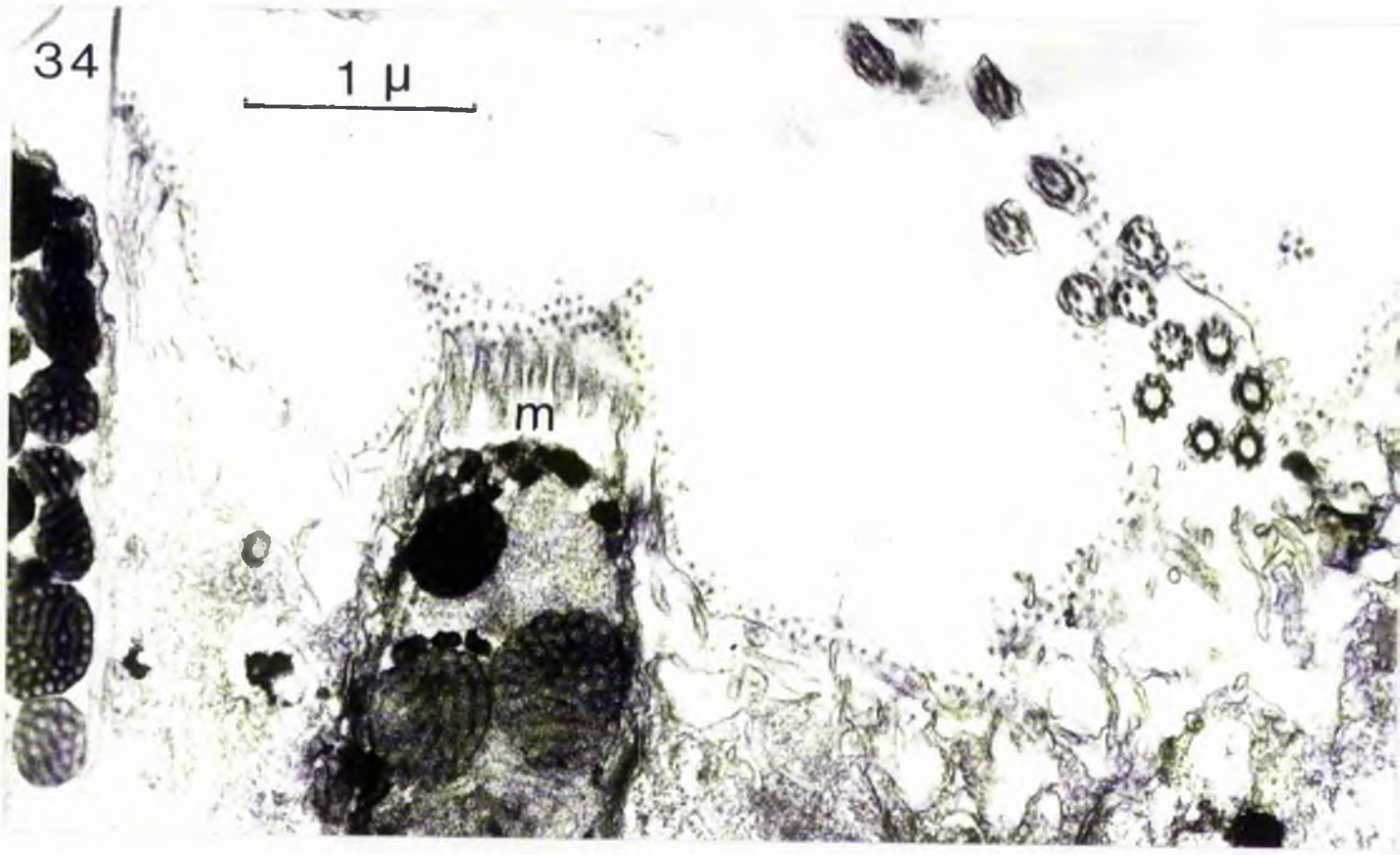

31
v
s
1 μ

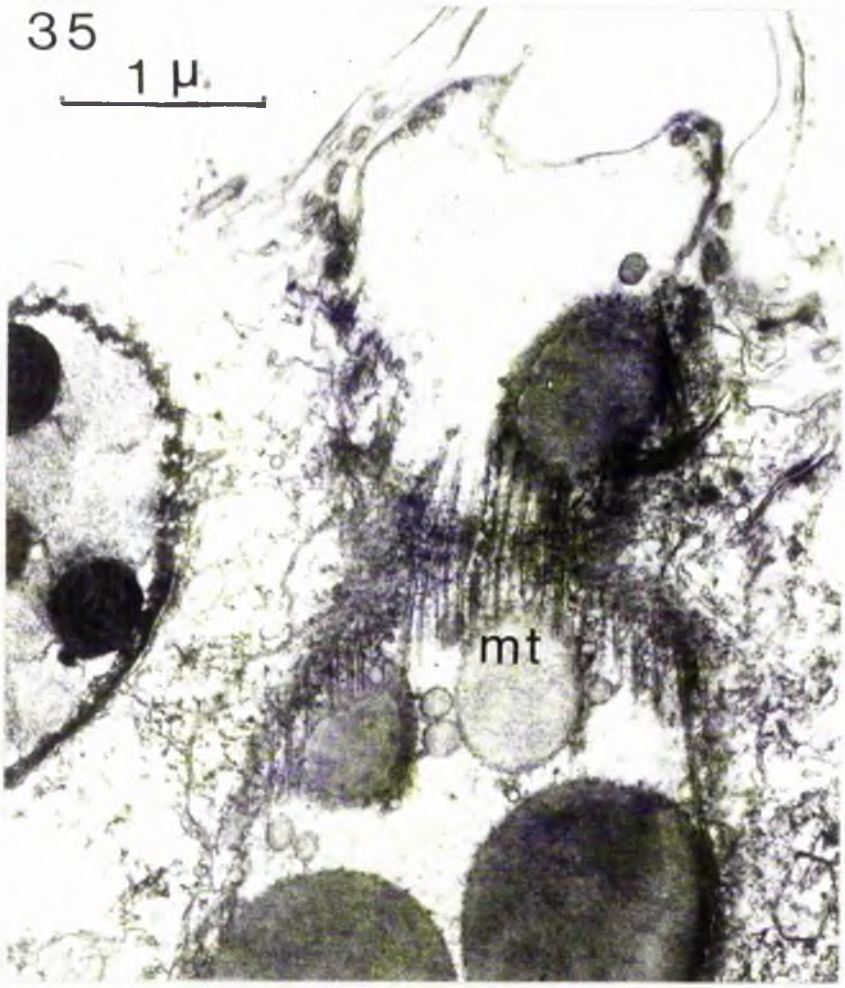
Fig. 34.

Gland type II. There is a single row of microvilli, m, at the pore.

Fig. 35.

Gland showing microtubule sheath, mt.


34
1 μ
m


35
1 μ
mt

appear to be three types of secretion droplet, all membrane-bound. One set of droplets are uniformly electron-dense oval-shaped, 0.2μ long. They tend to be distributed near the periphery. The second set have a characteristically compartmentalised appearance, as if composed of coiled tubules 0.05μ in diameter, and are of varying electron-density. These granules are mostly spherical, 0.6μ in diameter, and are the most abundant in the glands. Finally, large, lightly electron-dense granules fill the rest of the space. These granules are up to 1μ long, 0.7μ wide, and their shape conforms with the space available. All these granules are manufactured in the one cell, each gland having its own cell source. These glands are pear-shaped.

Finally, there is a gland containing only large, uniformly pale, granulated droplets and with a single row of microvilli at the pore (Fig. 36). The pore is 0.9μ wide, 1.7μ wide at the level of the microvilli. The droplets are 2.5μ wide, membrane-bound. There is a very thin sheath around the gland. These glands may be an older form of the previously described Type II gland.

The contents of all three types of gland are synthesized on rough-surfaced endoplasmic reticulum with widely-spaced cisternae (Fig. 37, 38). Golgi bodies feature prominently, with some dense vesicles (Fig. 37). Mitochondria occur in spaces between arrays of endoplasmic reticulum while the cell is actively producing secretion material. Later the cell may be entirely filled with secretion droplets with a region at the base containing the nucleus and a few of the normal cell components. In some instances (Fig. 39) mitochondria remain distributed around the periphery of the secretion material up to the

Fig. 36.

Gland type III, or possibly an older version of type II. There is a single row of microvilli at the pore, p. Microtubules, t, can be seen at the base of the gland. The large mass of secretion materials, probably supplies an adjacent gland.

36

Fig. 37.

Developing gland showing granular endoplasmic reticulum, er, and Golgi apparatus, G. Arrow points to a Golgi vesicle full of granular material. Double-headed arrows point to granules enlarged by coalescence. Cacodylate glutaraldehyde fixation.

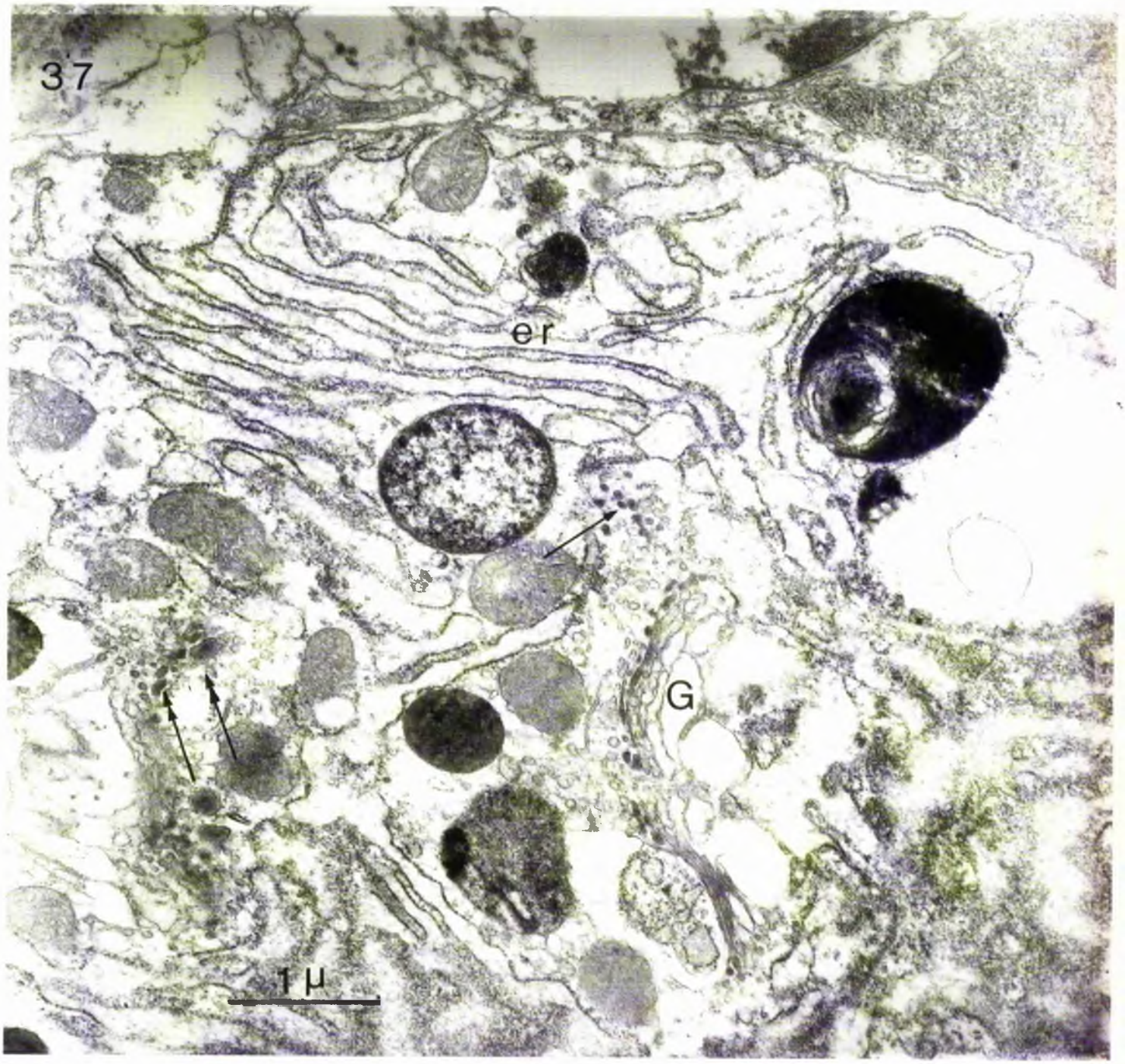

37
er
G
1 µ

Fig. 38.

Gland endoplasmic reticulum fixed in veronal glutaraldehyde. The granular endoplasmic reticulum cisternae are filled with lightly electron-dense contents. The mitochondria, m, have a denser matrix than seen with osmium, and have rounded cisternae. Other cell inclusions in this micrograph are nucleus, n, showing coarser condensation of chromatin than with osmium, lipid droplets, l, and secretion granules, s.

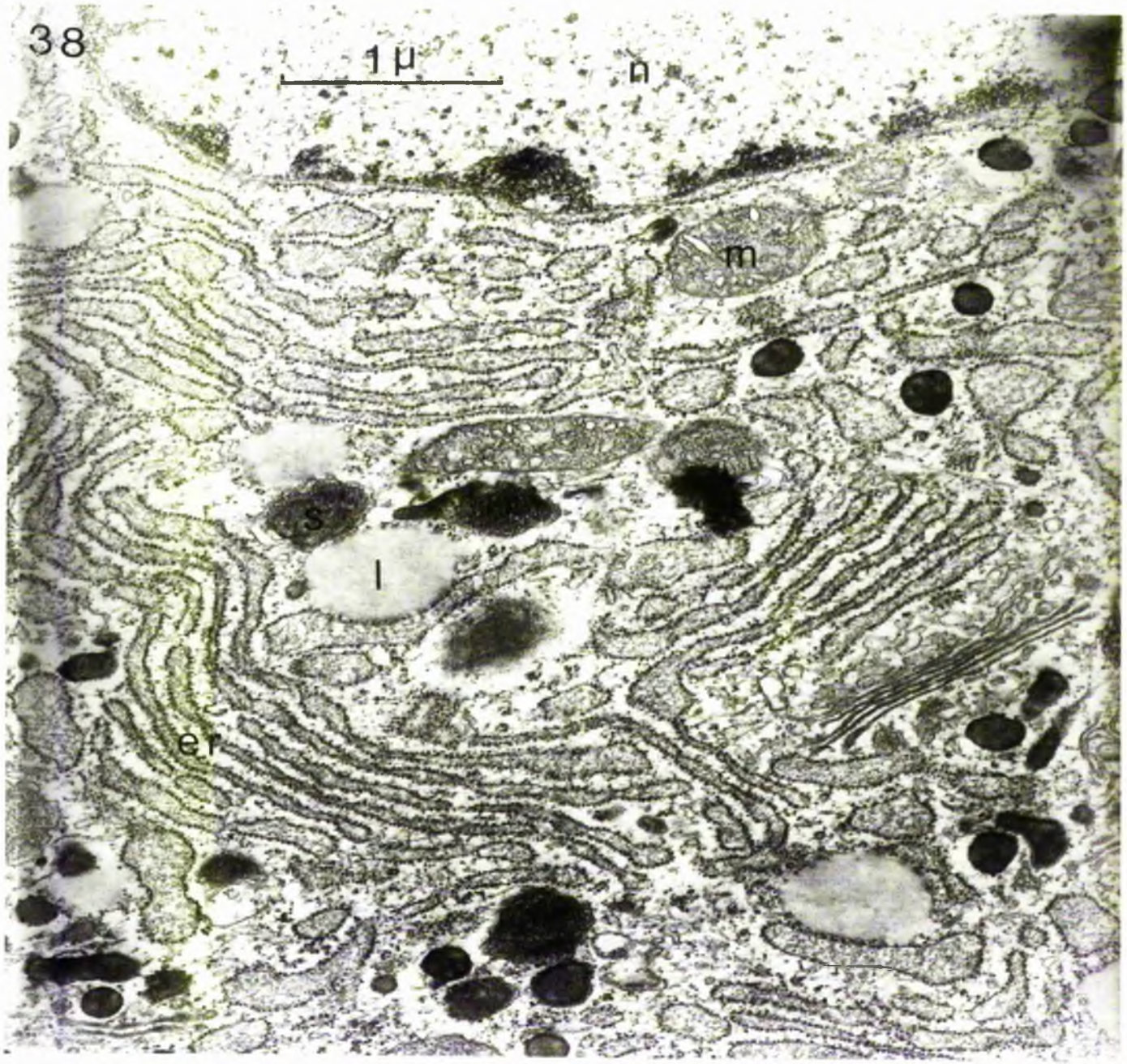
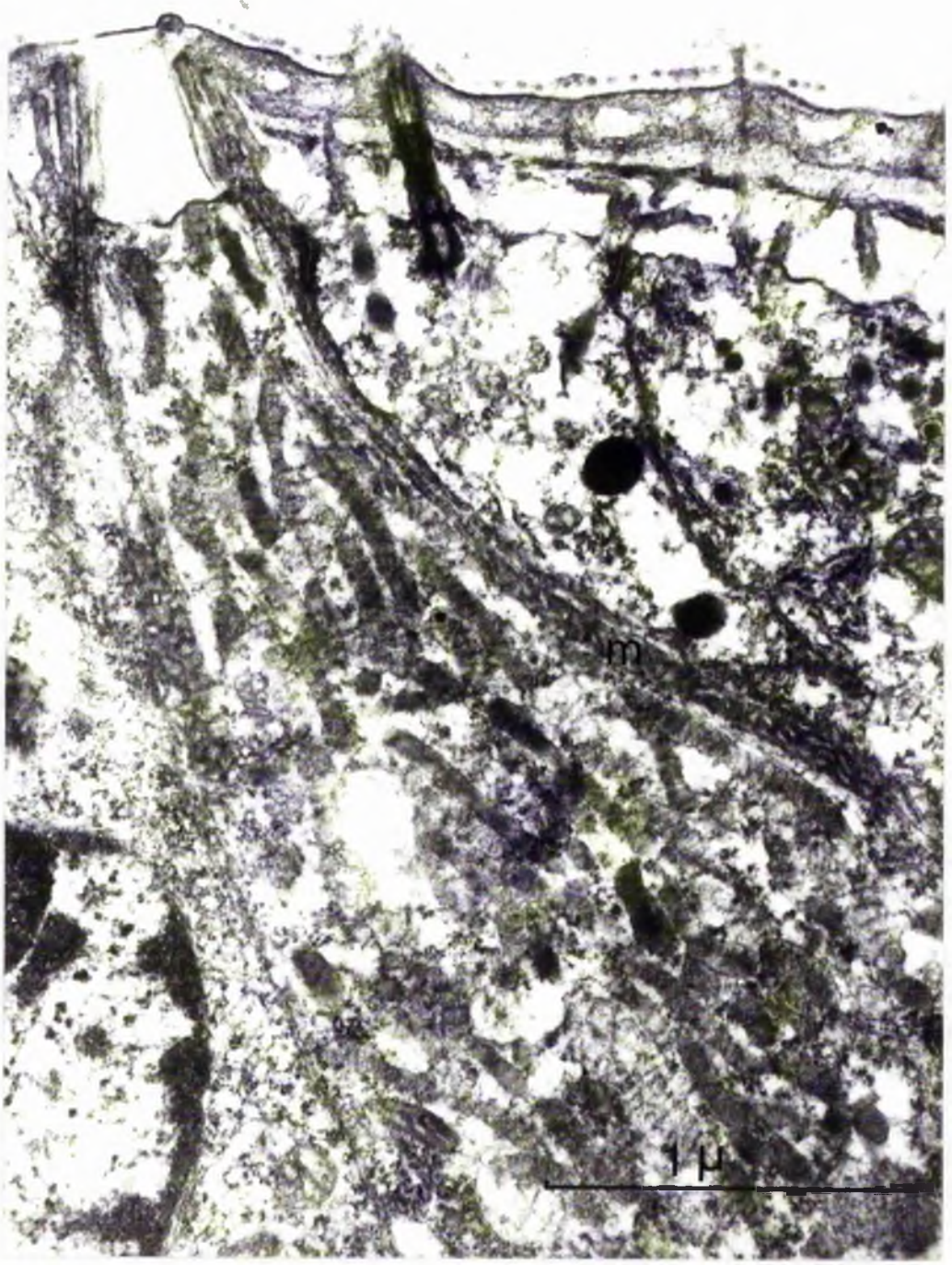

38
1 µ
n
m
s
l
er

Fig. 39.

Gland to the side of the anus showing mitochondria, m, lying at the periphery just under the sheath of the gland. The elongated form of the mitochondria is not commonly found, compare mitochondria in the adjacent cell. The electron-dense matrix and form of the cristae in the mitochondria occurs with the fixation, Acrolein, C.2.

39



level of the pore, but more usually there is nothing but secretion material at this level, Glands in which secretion material is building up are 20μ long, 10μ broad. The release of secretory material appears to take place by fusion of the membrane of each droplet with the containing cell membrane. Subsequent bursting of the droplet allows the secretory product to escape and stream through the pore while the cell membrane remains intact, (Fig. 32).

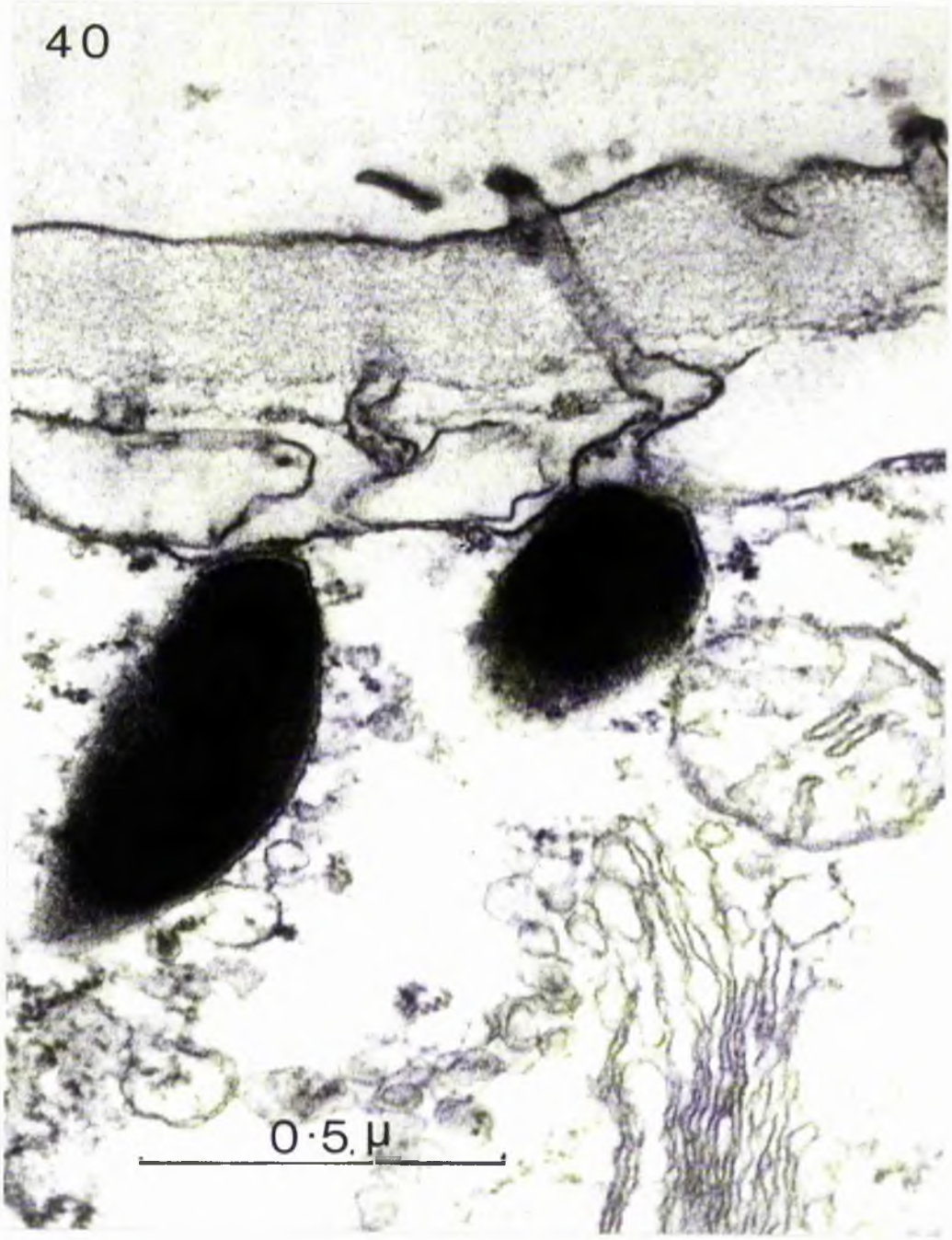
5. The Cuticle.

The cuticle is on average 0.5μ thick. There are three dense bands (see Figs. 18,19,20). The most peripheral is simply a strongly electron-dense line 100Å thick, directly overlying the main part of the cuticle which is a uniformly, moderately electron-dense layer 0.3μ to 0.4μ thick. Beneath this there is a 0.1μ space and a finely fibrous layer 0.1μ thick. There is then usually a small, variable, probably artefactual space between this layer and the cell membrane. Dense granules lie over the surface of the cuticle. These granules are 200Å in diameter, but irregular in profile probably owing to the network of 40Å fibres radiating from them and forming interconnections.

Microvilli, 0.05μ thick, 0.7μ to 1μ long, perforate the cuticle at varying intervals and terminate with a cap (see Fig. 40). They are particularly abundant in the anal region and among the cilia on the upper side of the prototroch where they are 0.1μ apart. In apical cells the microvilli are 0.4μ apart, and in cells lateral to

Fig. 40.

Cuticle with microvillus projecting through. Note cap on the microvillus. The secretion droplet below the microvillous may be released through it, and may aid the process of respiration, the function suggested for microvilli here.


40
0·5 μ

the glandular cells, 1.5 to 2μ apart. On the basis of the necessity of the trochophore to respire through the cuticle and the distribution of microvilli with greater density over the cells of higher metabolic rate, the microvilli appear to function as aids to respiration. Formation of the cuticle is independent of the presence of microvilli (see development of cuticle).

The cuticle appears fibrous and shrunken with cacodylate glutaraldehyde, lacks the dense line and is only 0.1μ thick. With veronal glutaraldehyde the cuticle also appears to be badly fixed, but less so than with cacodylate glutaraldehyde. It is 0.25μ thick, has the electron-dense line and peripheral granules. All osmium fixatives appear better but with triple strength sea water and osmium, superficial granules are lost or patchy.

6. Digestive System.

The trochophore has a gullet, stomach, and intestine (Fig. 15) and feeds on Coscinodiscus asteromphalus and other organisms. In general very little food was seen in the digestive tract, as section cutting was easier when there was nothing in the stomach and freshly released trochophores were normally used before food had been ingested.

(i) Gullet

The gullet lumen is 10μ to 12μ by 6μ, and up to 80μ long to its furthest edge which is above the opening into the stomach (Fig. 15). Some 96 cells form the gullet, the lumen of which is surrounded by 12 cells at any point in transverse section. The nuclei

lie at the base of the cells and take up about two thirds of the cell. Mitochondria, and other usual cell organelles are present in the distal third, along with ciliary roots. Dense-cored vesicles 1000-1500Å in diameter lie among the basal bodies and roots.

From the mouth opening throughout its entire length, the gullet is lined with cilia. These cilia have basal bodies oriented in the same direction, towards the stomach, and lateral roots which lie parallel to the line of the gullet (Fig. 41). The cilia are 0.2μ apart such that the lateral roots pass the basal body of adjacent cilia. A thin longitudinal root projects at an obtuse angle to the lateral root back into the cell. Both lateral and longitudinal roots have striations with 600Å periodicity.

Cilia are known to beat towards the basal foot (Gibbons, 1961) and the gullet cilia of the trochophore therefore all beat inwards towards the stomach. The pattern of cilia in the gullet is often in the form of groups of approximately 120 (Fig. 41). This number may be the complement of cilia from one cell as the gullet cells average 9μ wide. This assessment does not hold for cells of the upper lip end of the gullet which are long and narrow at the periphery (Fig. 42). Here the cells are 1.7μ wide and bear only a single line of cilia. A single basal body can be seen in each cell at the edge of the gullet. In this figure the pattern of cilia most commonly found is seen, that is with all the cilia on one side of the gullet in one phase of beat, while those on the other are in a different phase.

Fig. 41.

Gullet cilia showing oriented roots, r, and grouping of the cilia, c. "Valve cilia", v c, can be seen on the top right side. Circular and longitudinal muscles, m, lie directly outside the gullet cells and axons, n, are also evident.

41

(ii) Gullet-stomach opening.

At the opening of the gullet to the stomach is a group of some 100 enlarged cilia overlying an area of thin tissue in the stomach wall (Fig. 43). These cilia consist of a single axoneme plus between 1 and 33 randomly spaced single filaments all surrounded by an expanded membrane enclosing a space of up to 1μ in diameter (Fig. 44). The cilia are short and terminate more abruptly than ordinary cilia in the gullet. They have a normal basal body (Fig. 45) with a basal foot and a rootlet. The axoneme has a closely applied membrane for a distance of 0.5μ as it passes through the cuticle of the gullet wall. Distal to the cuticle the space enclosed by the membrane increases to accommodate the additional filaments, which are thought to bud off from the axoneme.

While the ordinary gullet cilia are closely grouped, their membranes are separate. By contrast the membranes of the large cilia are fused to each other by a moderately electron-dense matrix which imposes a constant intercellular space of approximately 160Å between the individual component cilia (Fig. 44). The axoneme is always situated to the side of the expanded membrane that lies nearest the mouth, but it is not attached to the membrane. Lines drawn through the central pair of filaments are on the whole parallel, and the basal feet are uniformly oriented in the same direction as those of the ordinary cilia. These modified cilia therefore also beat towards the stomach and as their membranes are fused they probably act as a functional whole and beat synchronously. Slow beats of twenty-five

Fig. 42.

Upper lip and part of the gullet opening. Note that the cilia on the left side of the gullet, l, are cut transversely while the others are cut longitudinally. The narrow necks, n, of 4 cells on the upper lip can be seen in the centre of the picture. (Veronal osmium. A 4.)

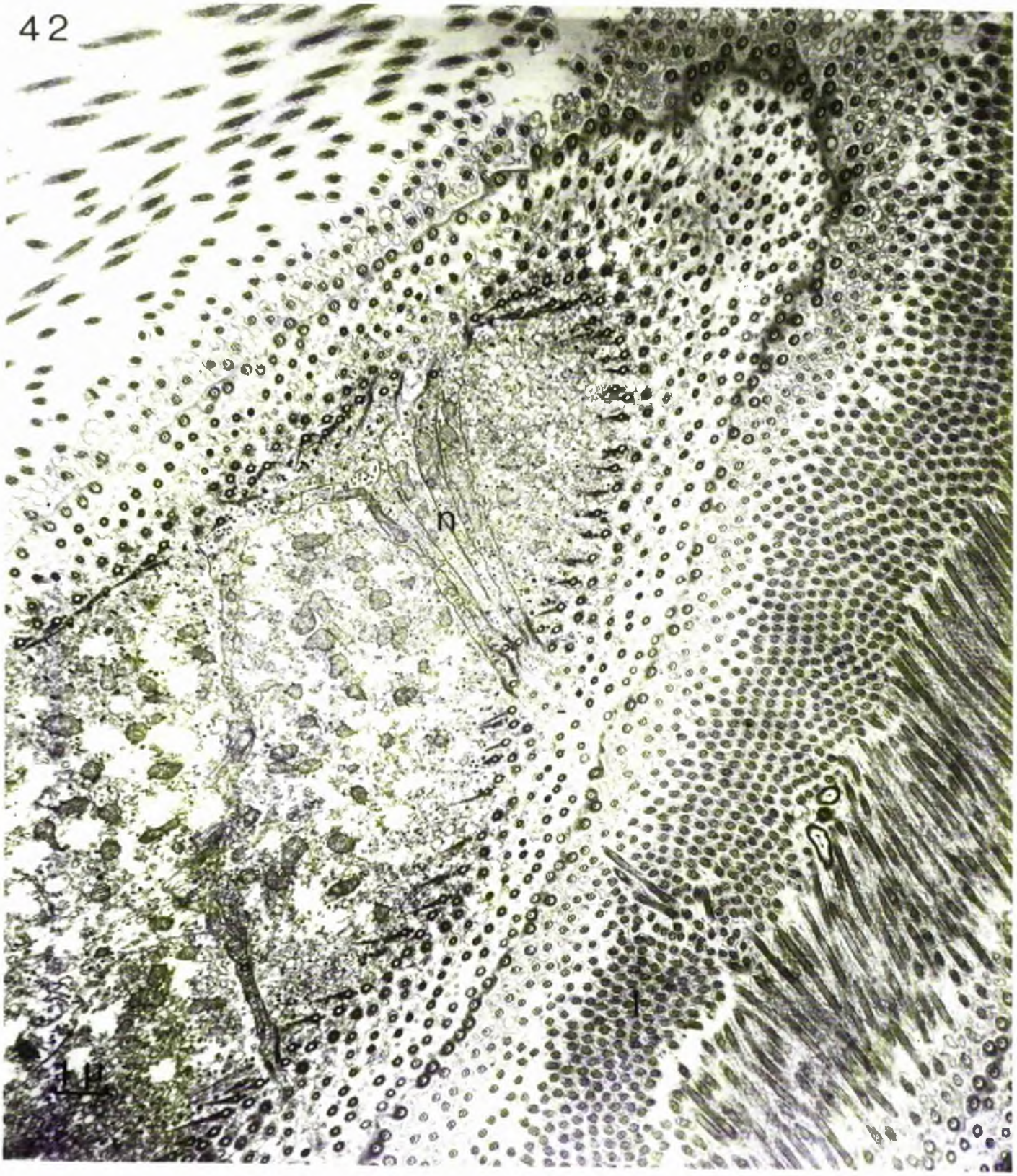

42
n
1 µ

Fig. 43.

Junction of gullet, g, with the stomach, s, just below the opening to show valve cilia, borne by a cell, c, containing dense-cored vesicles. This cell projects into a patch of stomach wall consisting of collections of empty-looking vesicles, e.

43

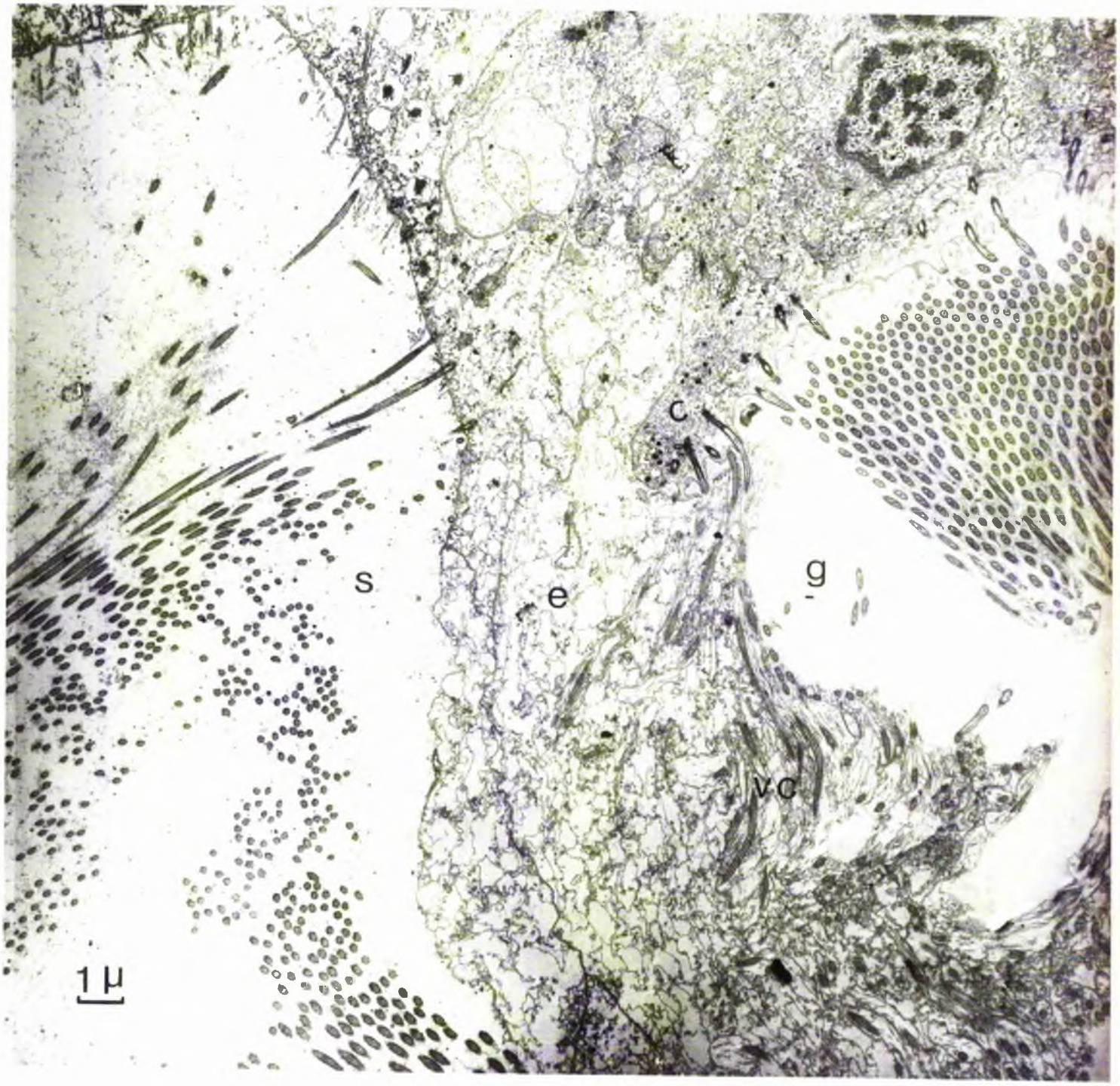


Fig. 44.

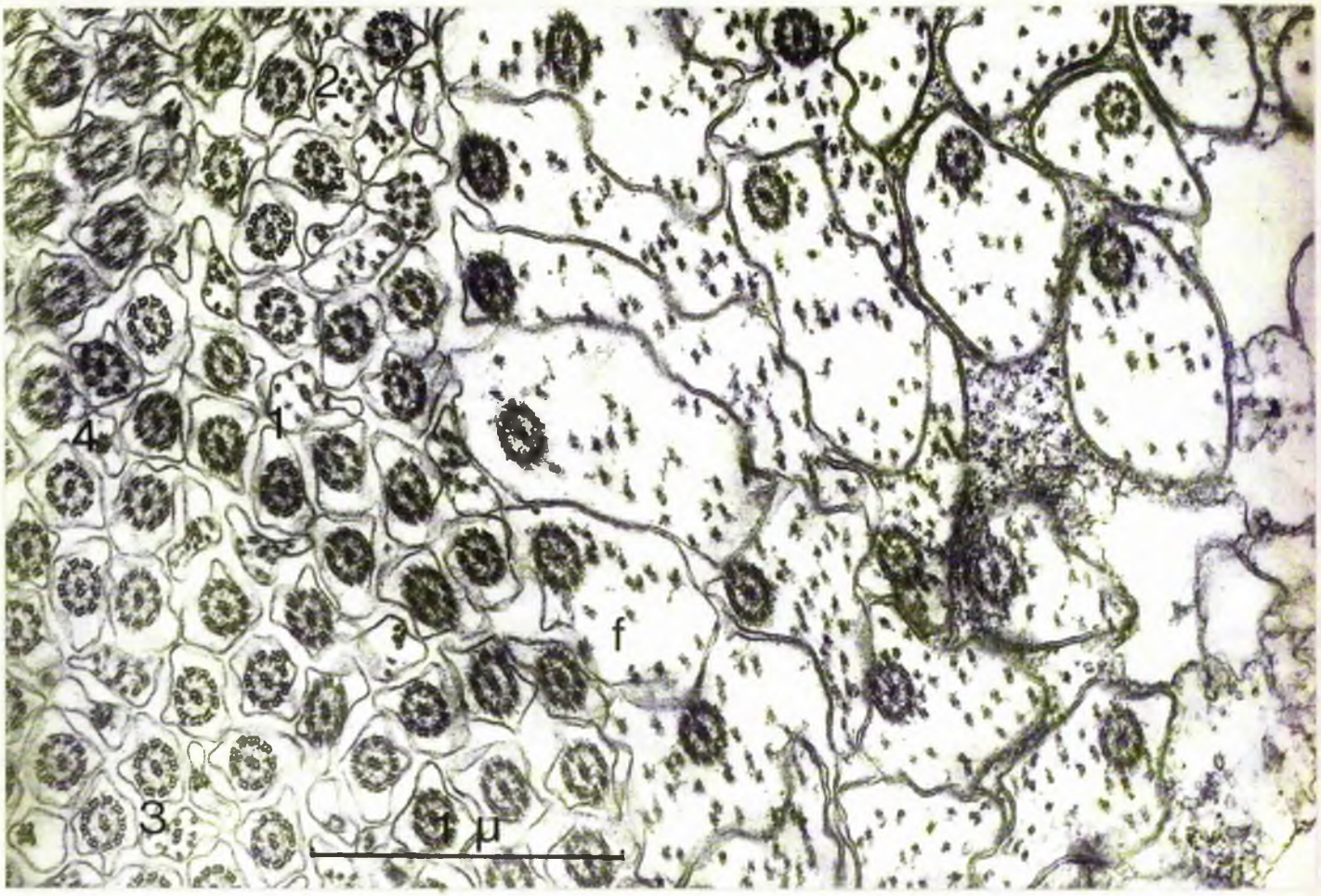
Gullet cilia. Among the ordinary cilia on the left of the micrograph, the pattern of termination can be seen.
1 is a cilium with 6 single peripheral filaments, 2 doublets and the central pair.
2. has 9 singlets peripherally and the central pair.
3. has the central pair, and 7 single peripheral filaments.
4. has the central pair only.

The valve cilia on the right are cut at an angle to the transverse section so that the outlines of the filaments, f, are not regular, but they may be seen to be microtubules. (Cacodylate osmium, 4.3)

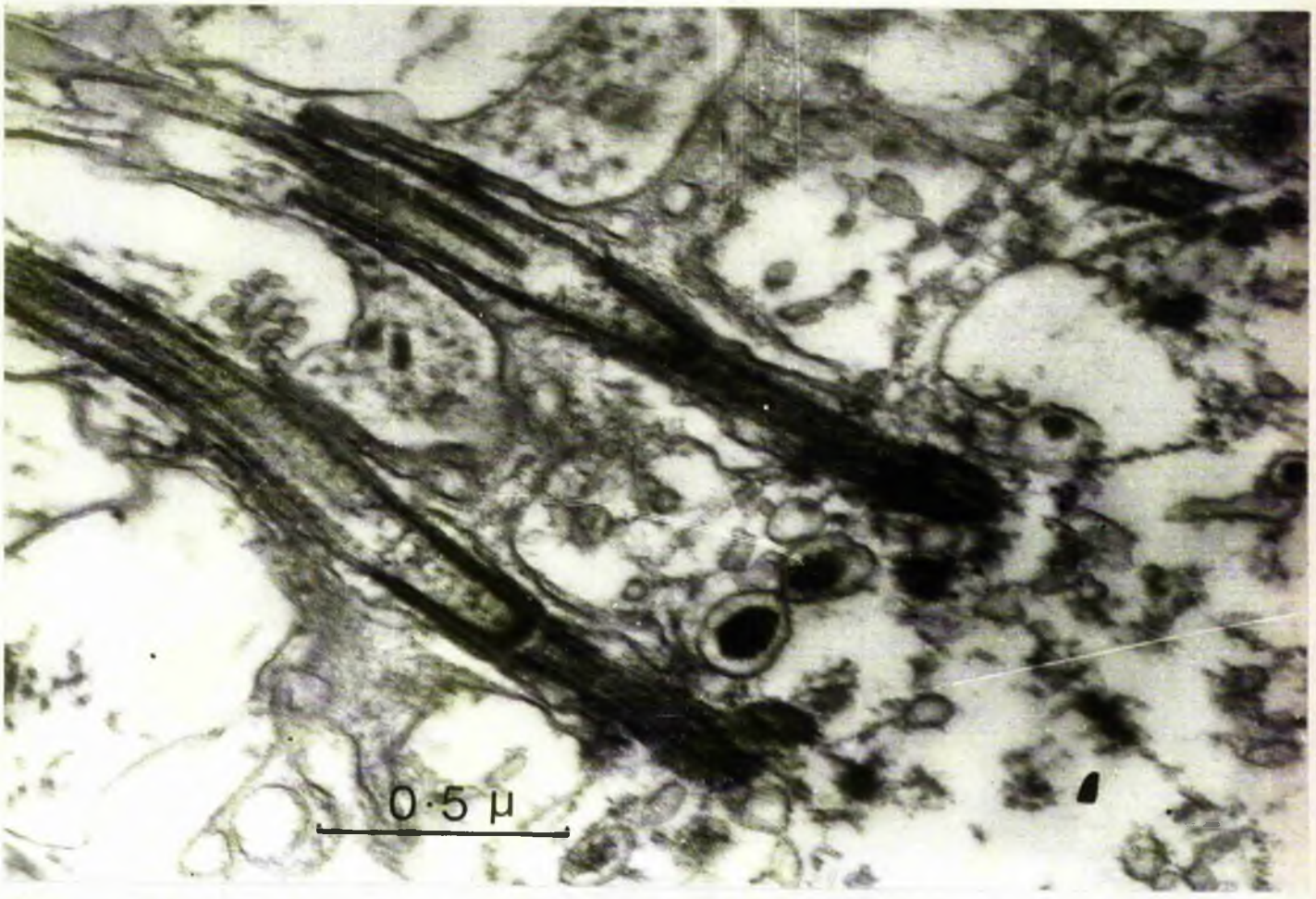
Fig. 45.

Longitudinal section of the base of the gullet valve cilia. Note dense-cored vesicles, absence of roots.

44



45



movements every thirty seconds observed in the gullet of living trochophores may represent the beating of these cilia.

Only two or three cells bear these cilia. These cells project into an area of stomach wall some 10μ across consisting of irregular, empty-looking vacuoles with a few normal cell components at the periphery (Fig. 43). This area originates from two cells and encircles the opening between the gullet and the stomach.

From the position, arrangement and structure of the expanded cilia it is apparent that a valve action is possible. As the stomach fills, the vacuous area of stomach will undergo the most distortion. This could directly affect the expanded cilia or the cells bearing them by a stretching deformation of the membranes. A beat of the mass of valve cilia then closes off the opening of the gullet to the stomach. Clearly only a small area of stomach wall could have thin tissue. This limits the number of cells which can bear the "valve" cilia. The expanded form of the cilia represents an economy in mass of ciliary projection relative to cell surface.

The expanded "valve" cilia of the gullet showed distinctive reactions to the different fixatives, and were apparently fixed best with 2% osmium mixed 50-50 with sea water (Fig. 44). The outline of the cilia was reasonably regular, joining with membranes of other cilia by a uniform, moderately electron-dense matrix. The additional

filaments were on the whole distinct and uniformly distributed. Osmium with triple strength sea water caused shrinkage of the gullet cilia. Their outline was irregular and they were no longer joined. The additional filaments were unevenly distributed. Veronal glutaraldehyde also caused shrinkage and collapse of these "valve" gullet cilia, and the additional filaments were not apparent. Instead a uniform matrix filled the space of the cilium (Fig. 46). The matrix of the axonemes was also thicker. Acrolein (1% with cacodylate) caused expansion of the membrane of the "valve" gullet cilia and granulation of the additional filaments (Fig. 47). Many of the gullet cilia with simple 9 + 2 configuration had expanded membranes and this also sometimes occurred with osmium 50-50 with sea water, usually when the osmium had been added drop by drop to the animals in sea water, rather than rapidly in a previously measured amount.

(iii) The stomach

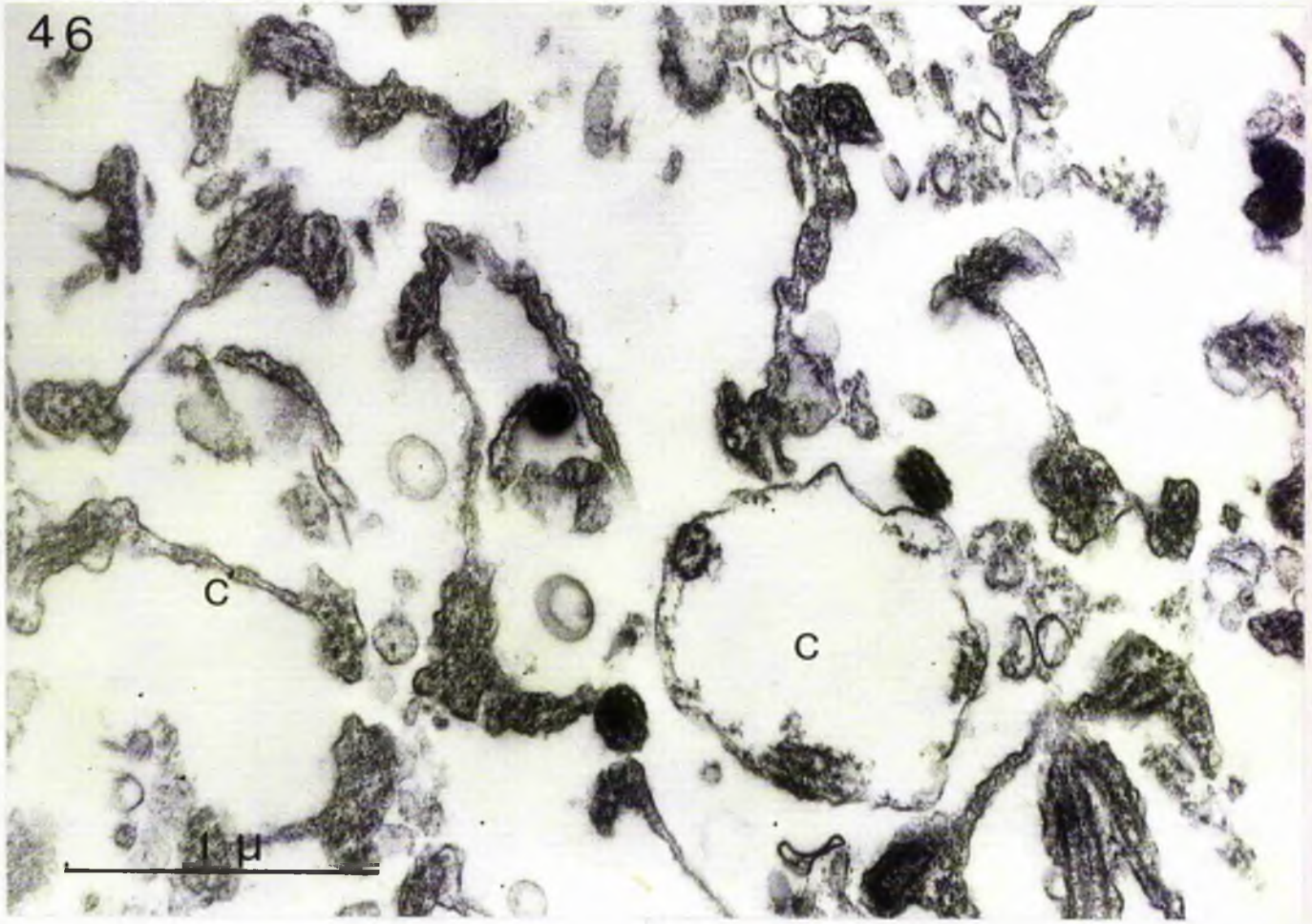
The stomach is a large cavity lined with cilia and microvilli and divided into an upper and lower region separated by a 5μ wide channel. There are three basic cell types in the stomach wall, secretory cells, absorptive cells and cells concerned with moving the food about. The first cell type is filled with rough-surfaced endoplasmic reticulum, found in some instances in closely packed parallel lines and in others in more vesicular form. The two arrangements are sometimes found adjacent (Fig. 48). The fixation in this figure is with glutaraldehyde, which is superior in fixation of all forms of endoplasmic reticulum. Acrolein produced evenly-spaced cisternae but the

Fig. 46.

Gullet valve cilia, veronal glutaraldehyde fixation. The additional ciliary filaments have condensed into granules. The cilium central in the micrograph, c, is expanded and the axoneme has doubled back on itself, the granules are distributed on the periphery; other cilia, ć, have collapsed.

Fig. 47.

Gullet cilia, acrolein fixation. The gullet valve cilia, c, have more expanded membranes than seen with osmium fixation. The additional filaments are absent and the space within the membrane is filled with a granular matrix. Some normal gullet cilia, c′, also have expanded membranes.


46
C
C
1 μ

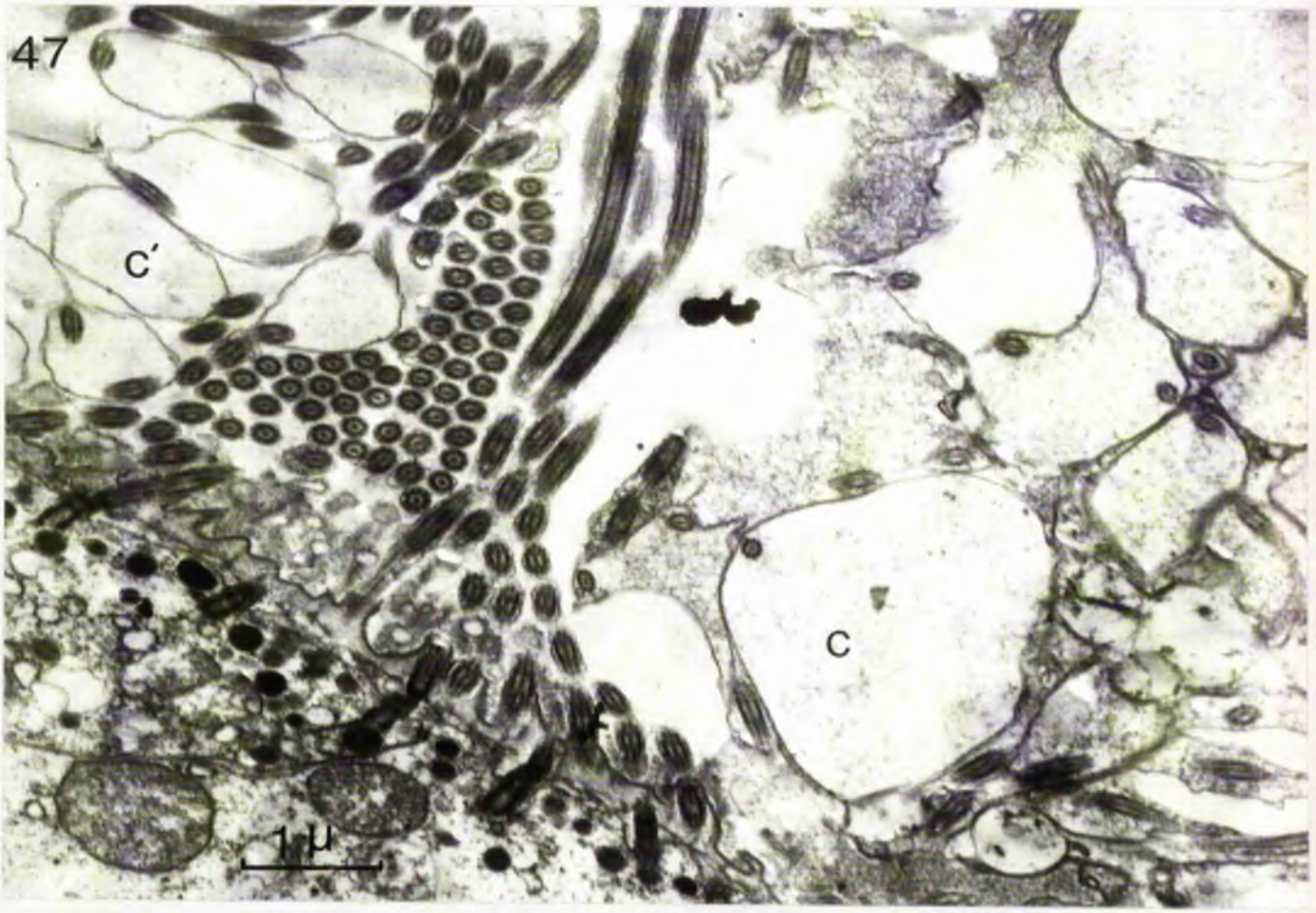
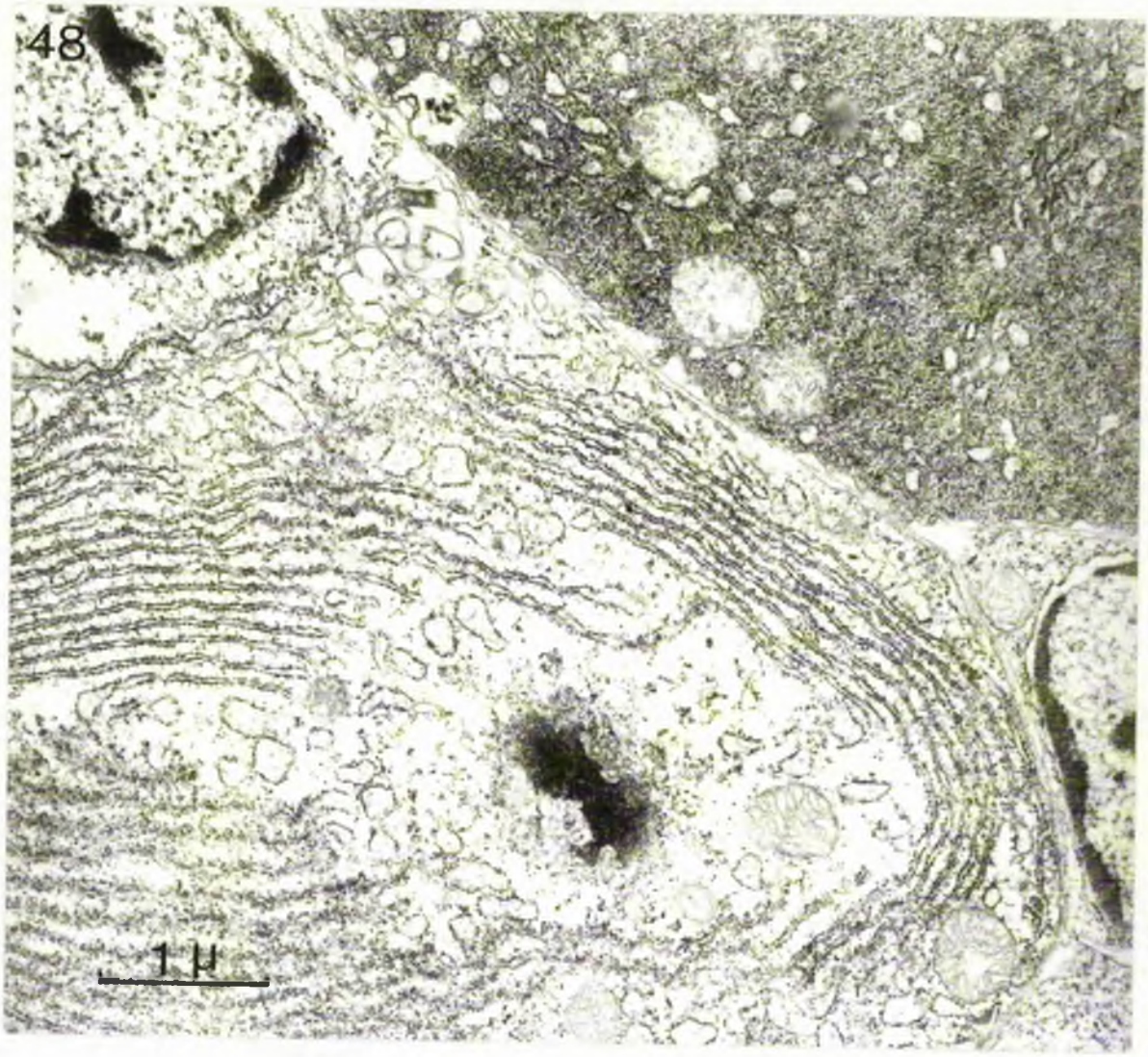

47
C'
C
1 μ

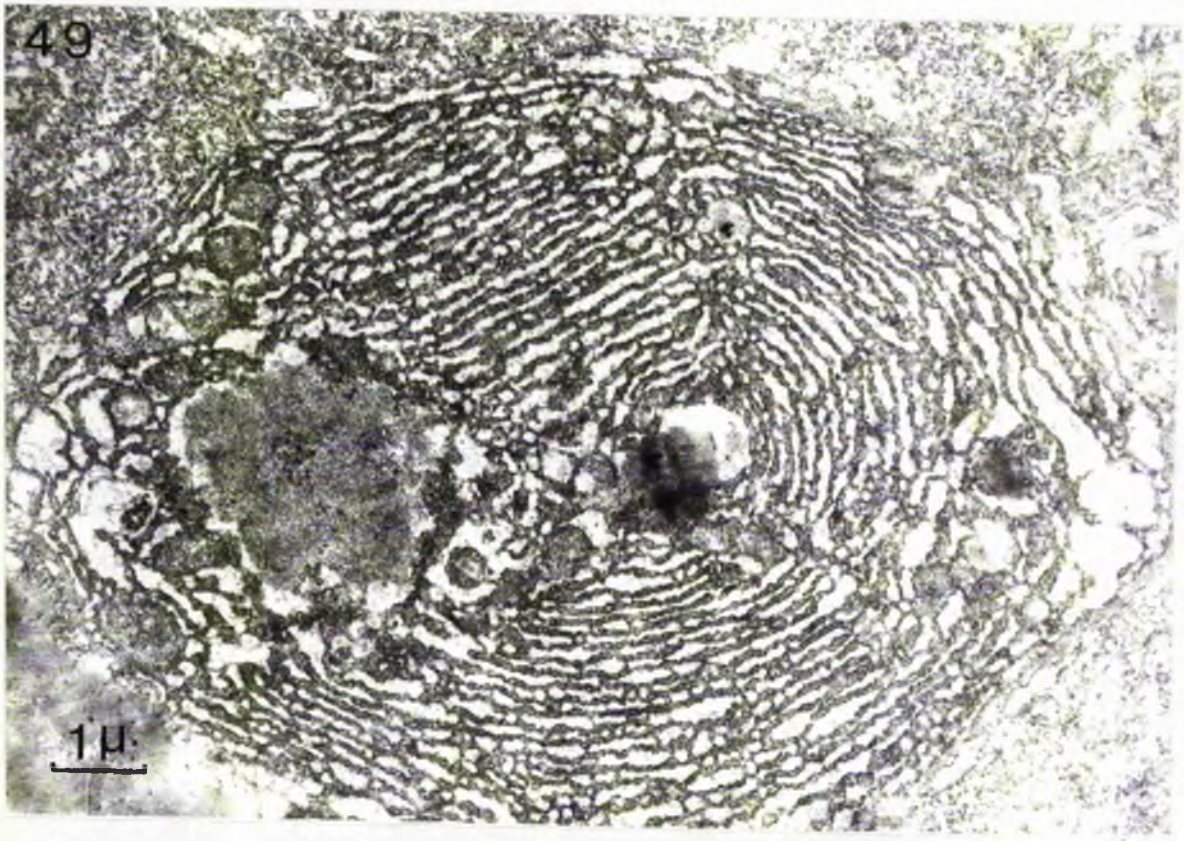
Fig. 48.

Stomach endoplasmic reticulum fixed with veronal glutaraldehyde. Note the narrow, regular cisternae. The outer surfaces bearing the ribosomes, are more widely spaced. This is the characteristic arrangement in protein-secreting cells.

Fig. 49.

When fixed with acrolein, the stomach endoplasmic reticulum becomes uneven, the cisternae are wider and the ribosome-bearing side appears electron-dense and uniformly granular.


48
1 μ


49
1 μ

membranes were not smooth, giving a shrunken appearance to the structure (Fig. 49). With osmium fixation the endoplasmic reticulum in one of the cells tended to become vesicularised and in the other the membranes were not apparent. Membrane-bound secretory droplets with a two or three layered form are found in spaces between the endoplasmic reticulum formations. (See Holborow, 1971).

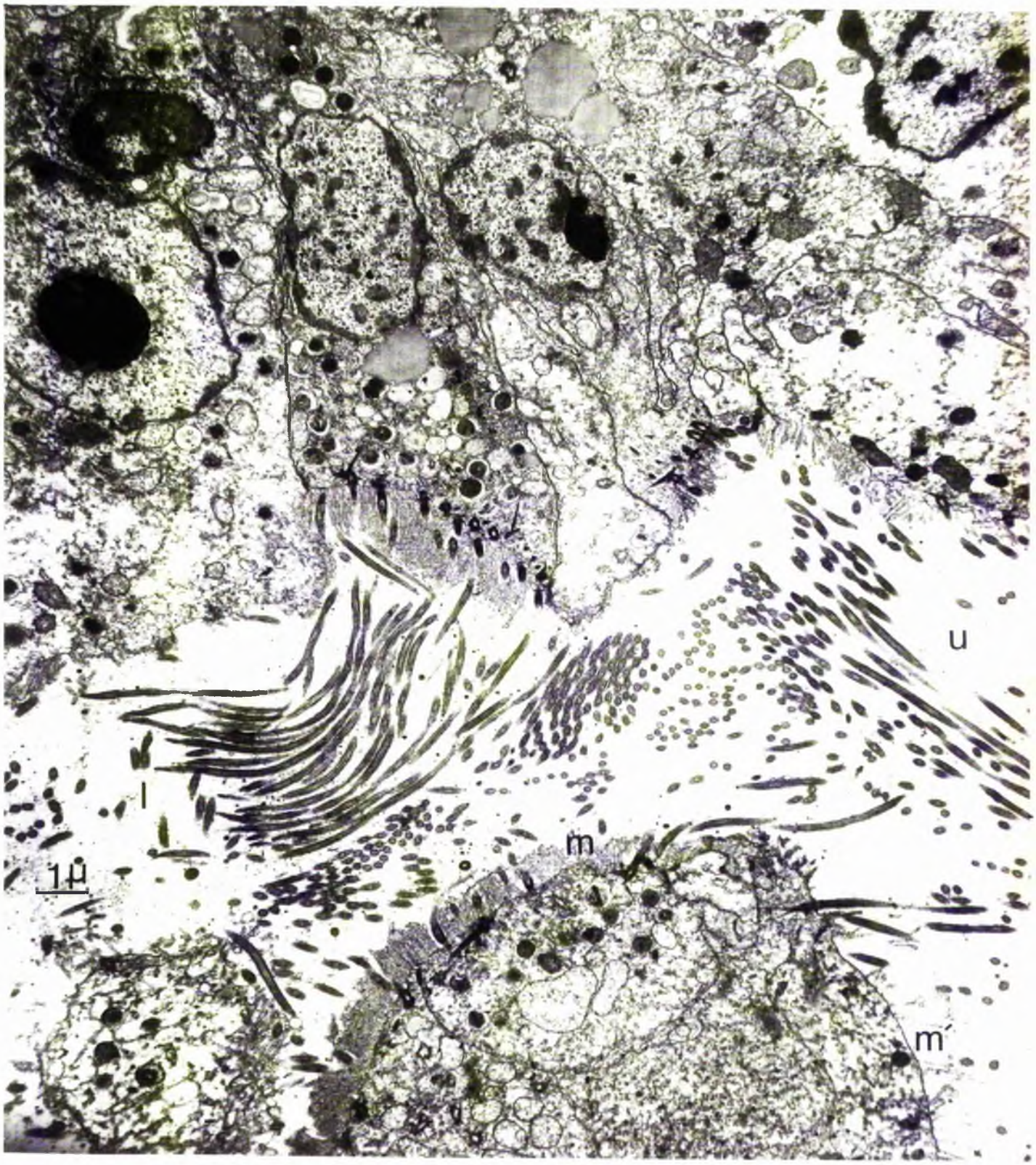
The endoplasmic reticulum of stomach cells should be compared with that of ectodermal glands (Figs. 37, 38). In the ectodermal glands, the cisternae of the endoplasmic reticulum are widely spaced and filled with a moderately electron-dense matrix (although there is some variation in the matrix according to fixation, as shown). The endoplasmic reticulum of stomach cells has narrow, empty cisternae and the ribosome studded side of the membranes have a wider space between them, the reverse of the situation in ectodermal glands. The pattern seen in stomach cells is typical of liver or pancreas (Fawcett, 1966) whereas that in ectodermal glands is more typical of cells such as thyroid.

The absorptive cells have a surface layer of 1μ long microvilli and occasional cilia. In some areas of the stomach, the microvilli are sparse and project in different directions, whereas in other areas the microvilli form a dense array (Fig. 50). These cells frequently contain uniformly pale droplets, presumably lipid, ranging between 0.6 to 2.0μ in diameter, and dark, spherical, membrane-bound

Fig. 50.

Opening between upper, u, and lower, l, regions of the stomach. Note areas of dense microvilli, m, and sparse microvilli, m′. In these areas the cilia are also dense and sparse respectively.

50



granules 0.4μ in diameter. They also retain yolk longer than other cells. The nucleus tends to lie on the distal side of the cell, and mitochondria are distributed evenly throughout the cell.

The channel between the upper and lower sections of the stomach (Fig. 50) is lined with cells bearing many cilia as well as a dense array of microvilli. The cilia have a lateral and longitudinal root and probably move the food between the two stomach regions. The cells contain lipid and dark granules and are probably absorptive as well as serving a food-moving function. Cells more clearly primarily concerned with food moving are found in either side of the gullet-stomach opening (Fig. 43), and have sparse microvilli.

(iv) The Intestine.

The intestine is 1μ to 5μ in diameter, and in longitudinal sections appears as a crescent beneath the stomach. The cells are small, 5μ wide, with nuclei at the base, and have an inner border of cilia and microvilli (Fig. 51). The microvilli in this figure have the shrunken, and on the right side, fusing form characteristic of unbuffered osmium fixation. With buffered osmium and glutaraldehyde the microvilli are well formed, with minor differences depending on the fixative. Veronal glutaraldehyde microvilli tend to be constricted at the base (Fig. 52); cacodylate glutaraldehyde microvilli have dense membranes (Fig. 53); acrolein produces even more dense membranes, of cilia as well as microvilli; cacodylate osmium microvilli are bulbous.

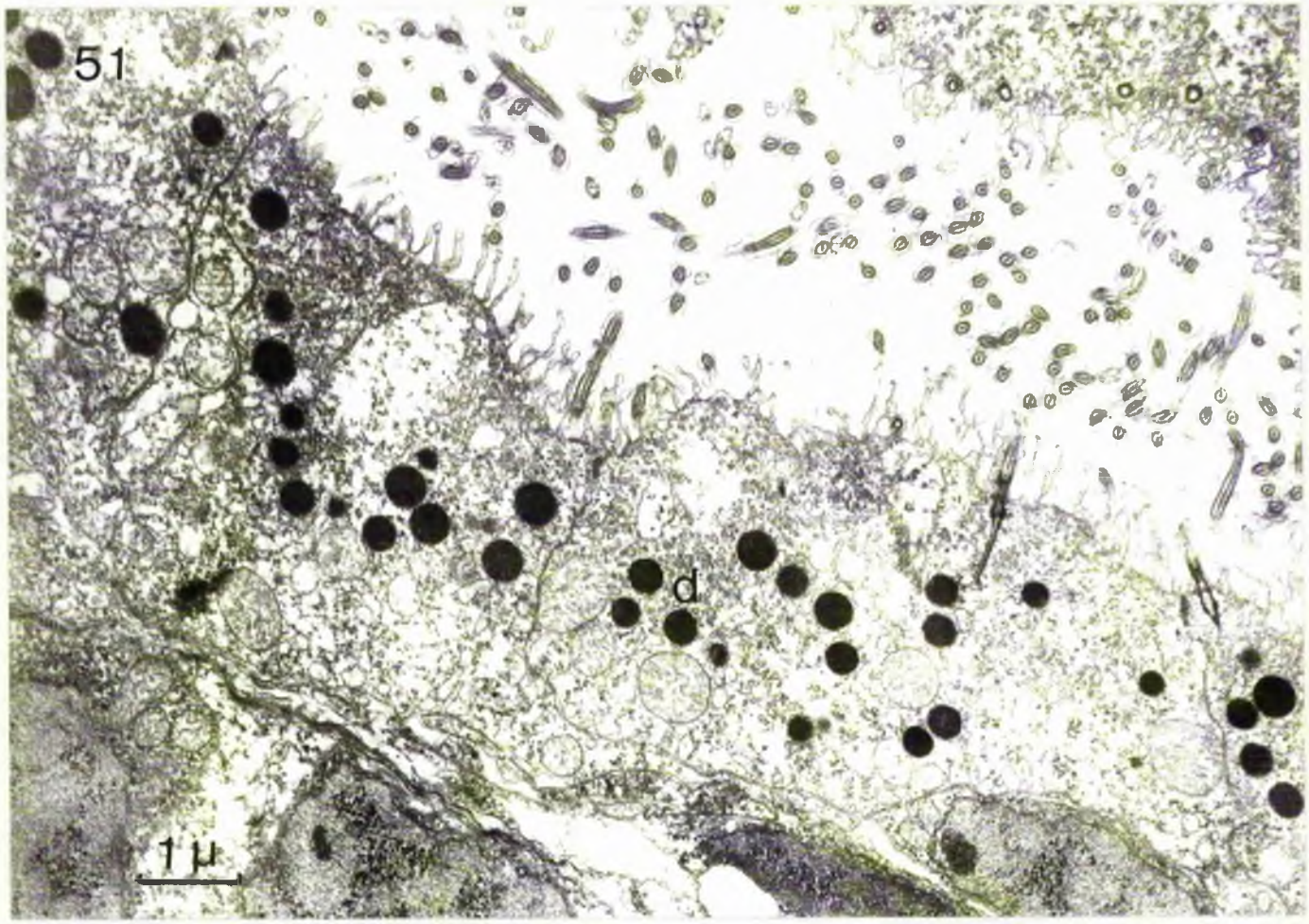
The cells are joined by septate desmosomes, with a macula adherens at the cell surface. Phagocytosis appears to take place in some parts of the intestine (Fig. 53). There are numerous 0.1μ vesicles

Fig. 51.

Intestine, standard osmium fixation. Note irregular shape of microvilli. Digestive granules, d, may be ingested fat in the form of triglycerides, or secretion products.

Fig. 52.

Intestinal microvilli fixed with veronal gluteraldehyde. Note slightly pinched bases.


51
d
1 µ

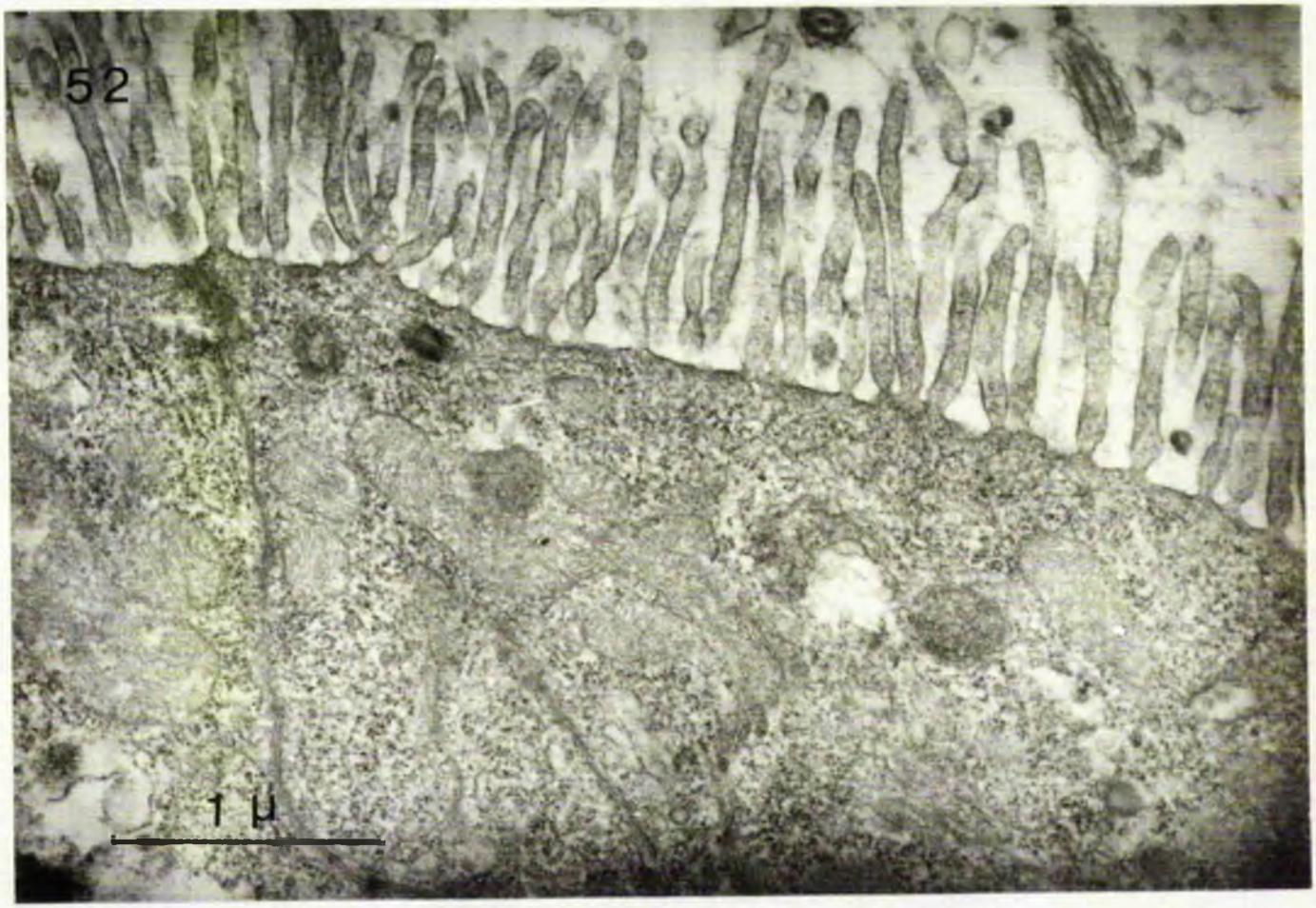
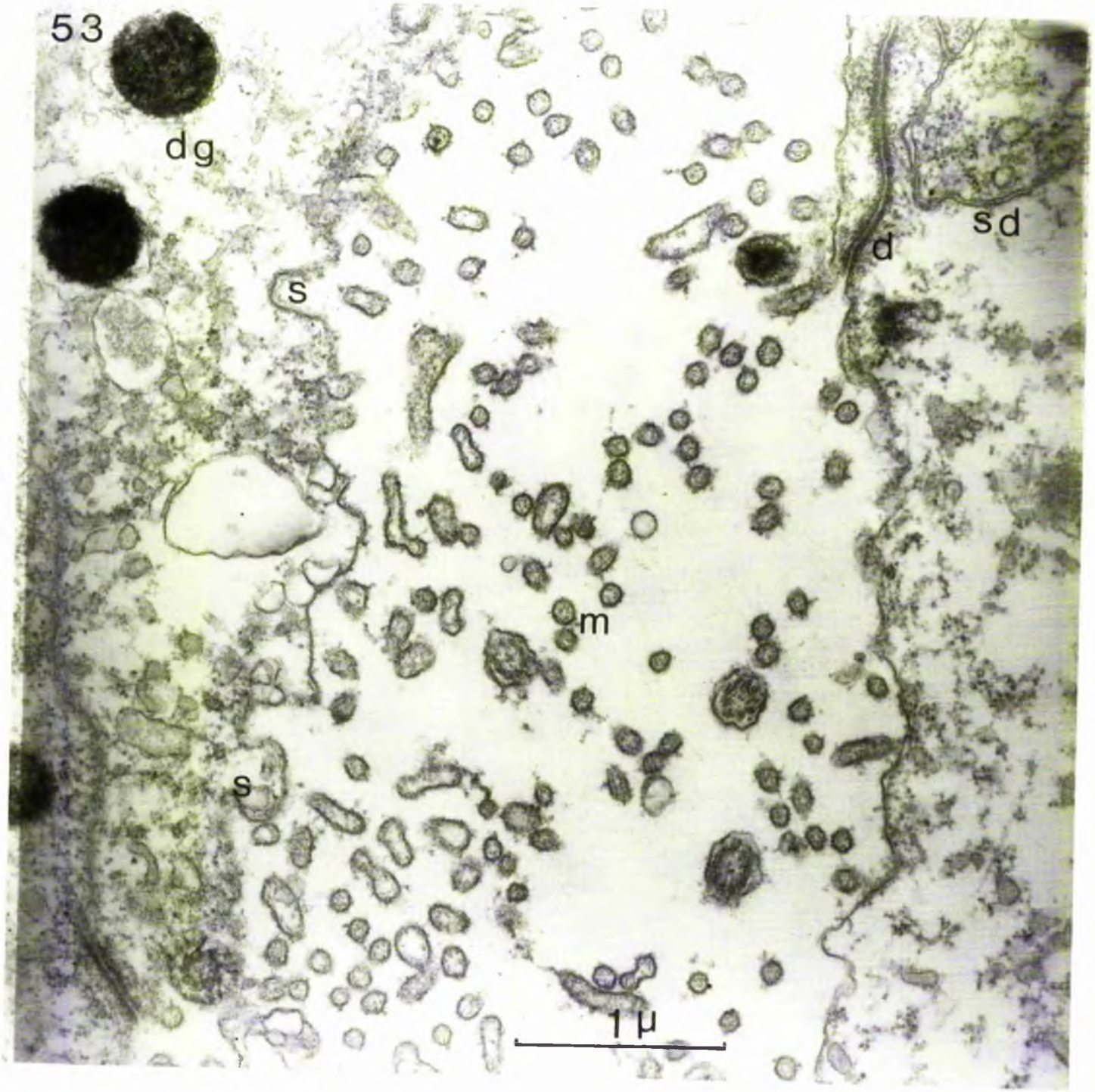

52
1 µ

Fig. 53.

Cacodylate glutaraldehyde microvilli, m, in the intestine. Note also digestive granule, dg, and desmosome, d. The septate part of the desmosome, sd, is deeper in the cell layer. Indentations and infoldings, s, of the cell surface, enclose a uniform, lightly electron-dense matrix similar to that of vacuoles in the cell, suggesting phagocytosis.


53
dg
s
d
sd
m
s
1μ

beneath the microvillous border, larger vacuoles and mitochondria. Most cells contain spherical dark granules, 0.5μ in diameter, and many contain lipid droplets of oval form up to 2.5μ long.

The anal opening is surrounded by muscle (Fig. 54). Cuticle infolds to a depth of some 7μ into the proctodoeum. Nearly three times the number of microvilli normally projecting through the cuticle, project through the proctodael cuticle.

7. The Muscular System.

The complexity of contractile activity of the living trochophore (described earlier) is reflected in terms of muscle tissue, although extensive areas of muscle are not found as the muscles are small.

Two circular muscles 0.5μ or 0.3μ in cross-section encircle the gullet some 3μ inside the mouth opening (See Fig. 41).Another circular muscle is found at the stomach end of the gullet. One, or perhaps more, muscles 0.6μ in diameter pass around the top of the gullet and are seen in some longitudinal sections only. (See Fig. 26). These muscles consist of a rather random-looking collection of thick and thin filaments, (Fig. 55), as do the muscles in the anal region. Similar muscle is found in the blastocoel near the solenocyte and protonephridial duct. (See Fig. 65).

Beneath the prototroch nerve mass, adjacent to the blastocoel, there is a circular muscle with an orderly arrangement of thick and thin myofilaments resembling the obliquely striated muscle of Glycera (Rosenbluth, 1968), and depicted in the diagram (Fig. 56). The

Fig. 54.

Edge of the anal opening showing proctodael infolding of cuticle with many microvilli, mv, sphincter muscle, m, tuft of anal cilia, a,.

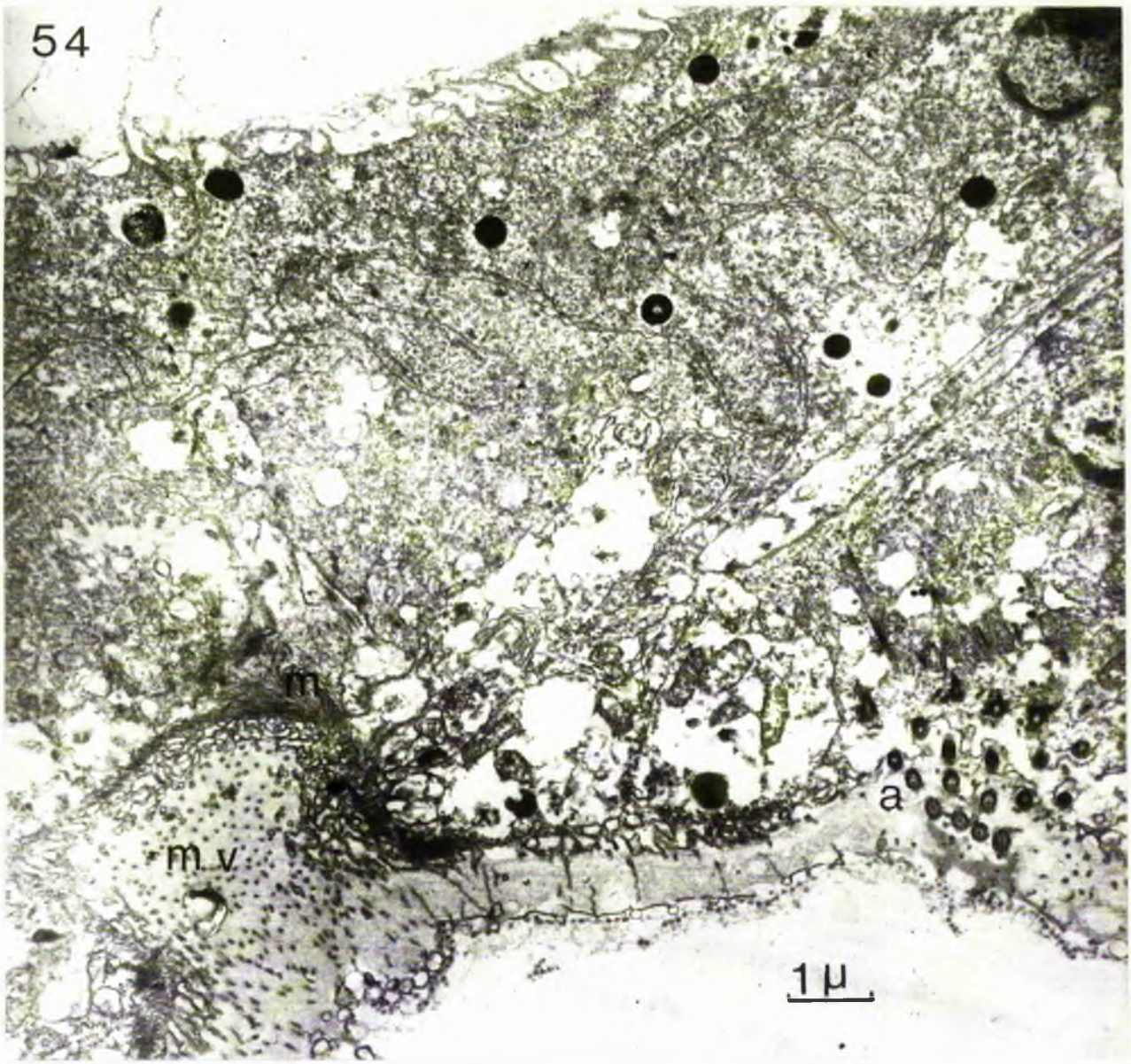

54
m
a
m v
1μ

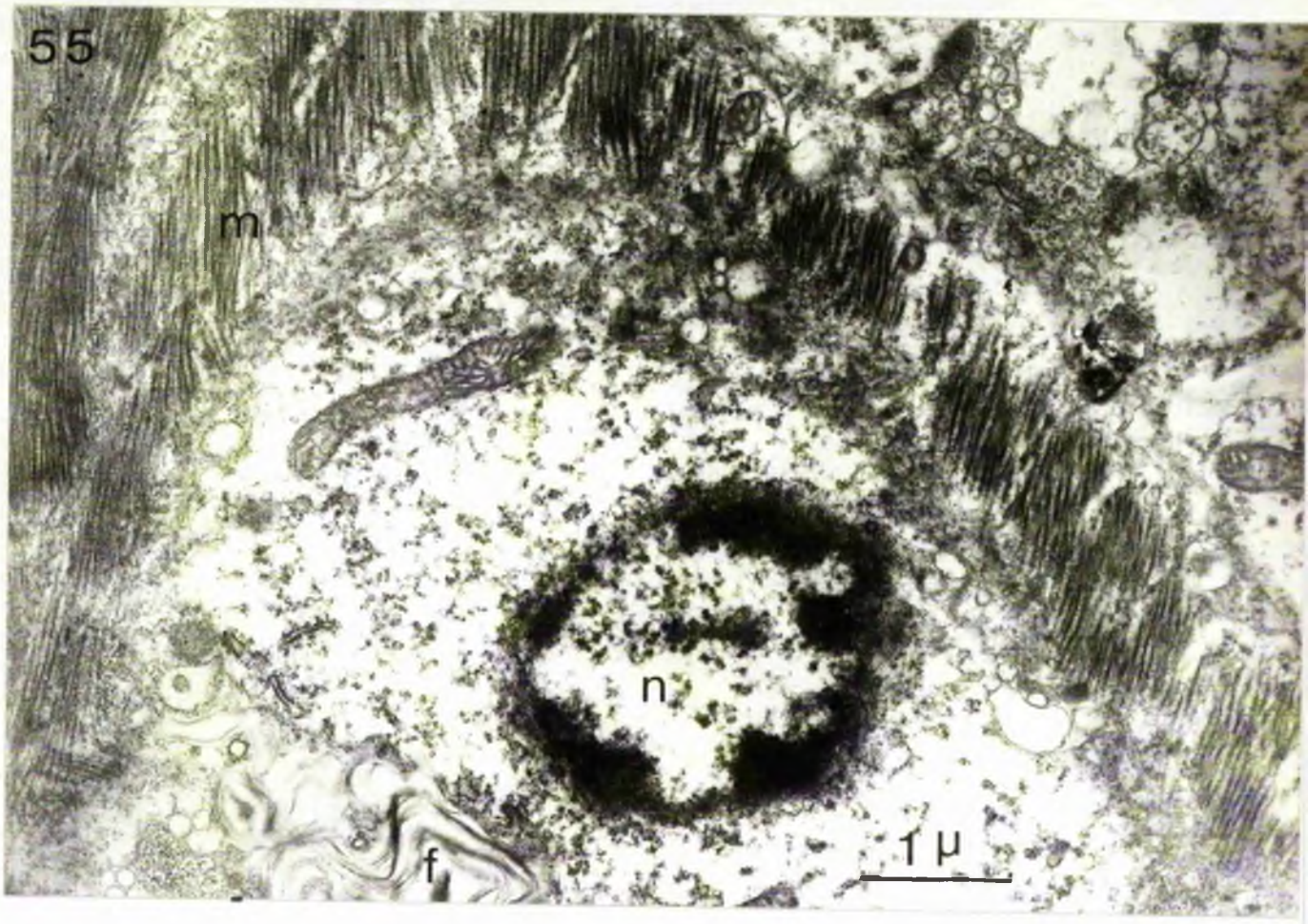
Fig. 55.

Trochophore muscle, m, sectioned longitudinally. Thin and thick filaments can be seen. This muscle forms part of a system that increases after hatching of the trochophore and may represent developing adult muscle. The central nucleus, n, undergoing autolysis and the large myelin figure, f, at the base of the picture is typical of ageing and degenerating cells. The section is of trochophore one week after hatching. Cacodylate glutaraldehyde B.i.(ii).

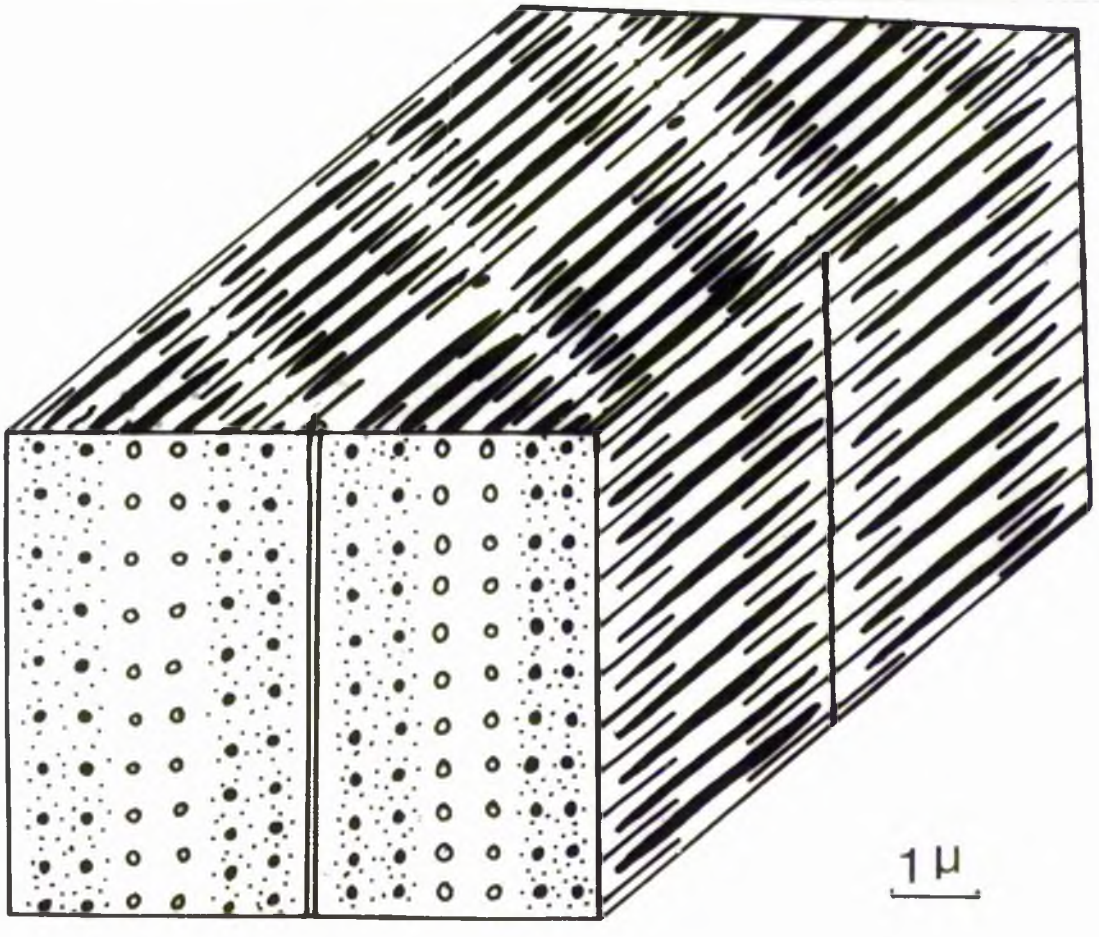
Fig. 56.

Three dimensional diagrammatic representation of the trochophore striated muscle, showing the arrangement of the thick and thin myofilaments.

55



56



muscle is 0.5μ to 0.8μ wide and 4.5μ long in transverse section. Each band is 0.5μ wide. Relatively large mitochondria, 1.6μ long, may be found between layers of the muscle. Perfect transverse sections of this muscle were difficult to obtain, which makes the thin myofilaments unclear, but some individual circlets around the thick myofilaments could be seen. The number of thin filaments per thick filament is 6 to 8, but the arrangement is not perfectly regular, as also found in earthworm striated muscle (Knapp and Mill, 1971). In most instances adjacent thick myofilaments shared the thin myofilaments between them, but a few separate circlets could be found such that two layers of thin myofilaments lay between the thick myofilaments. In this striated muscle, the thick myofilaments have an outer electron-dense ring and a less dense core inthe middle region of the filaments but the ends are solid (see Fig. 56).

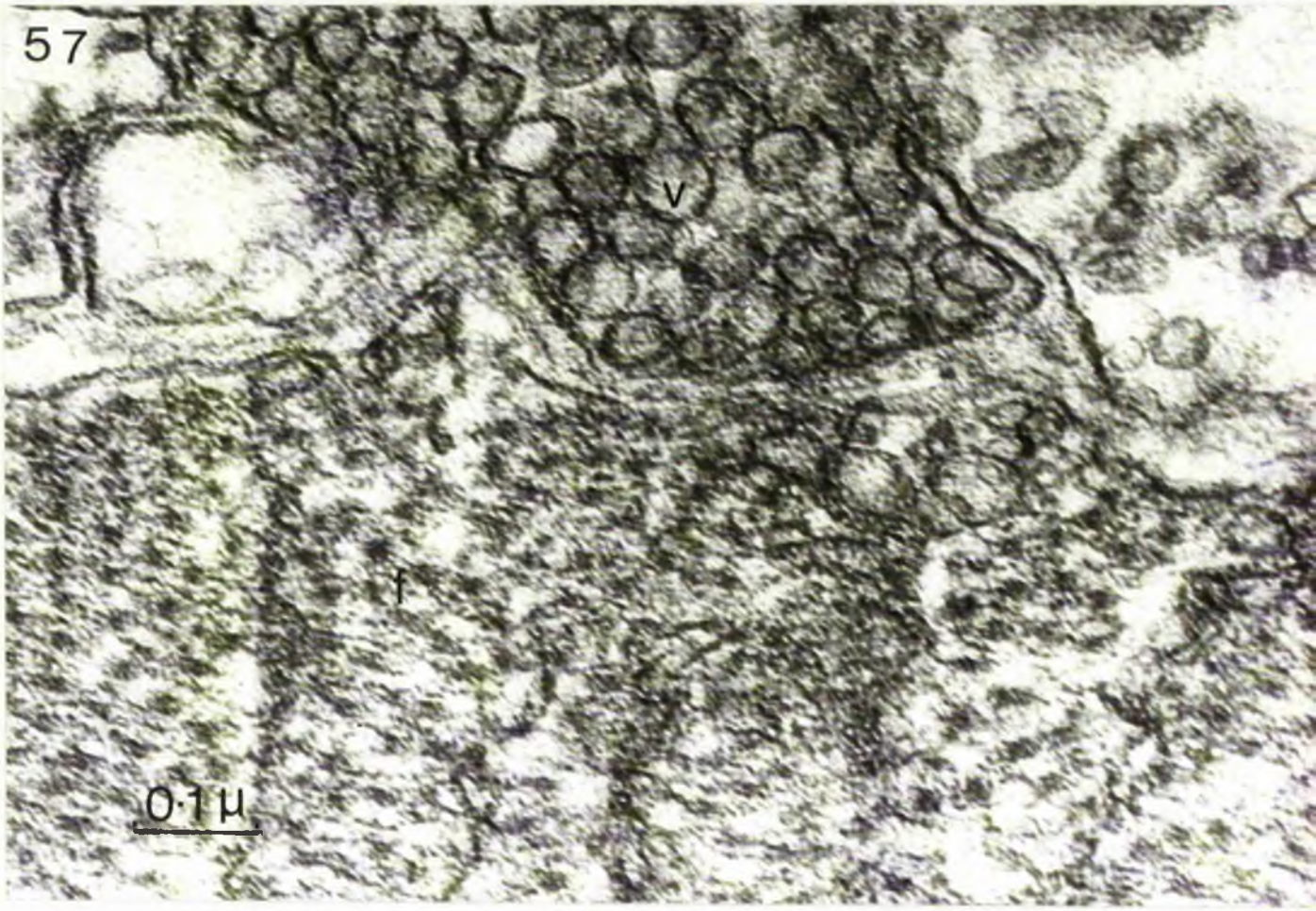
Neuromuscular junctions are found on this muscle (Fig. 57). The junctional gap is usually 300Å. Between the nerve and muscle membranes there is a somewhat diffuse central basement membrane which is less electron-dense than the surrounding cell membranes. This basement membrane continues around the muscle in the 500Å gap separating it from the other cells. The process of the nerve supplying the muscle is a "foot" 0.4μ in diameter filled with 500Å to 700Å vesicles. In some older trochophores a mesodermal growth (Fig. 58)

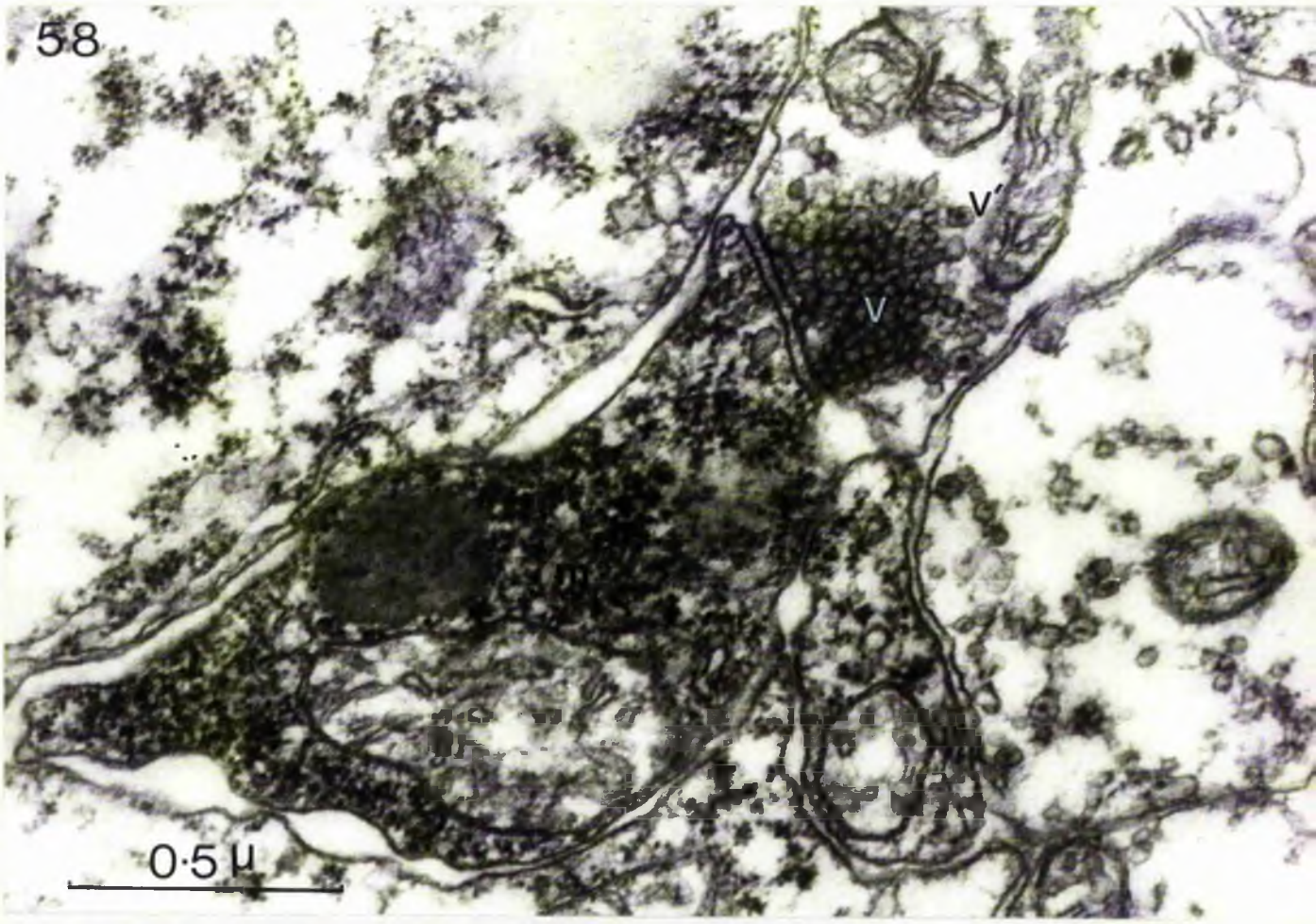
Fig. 57.

Neuro-muscular junction. The nerve terminal is packed with clear vesicles, v. The muscle fibres, f, are not cut in perfect transverse section, giving a fuzzy appearance, but fine and thick myofilaments can be distinguished.

Fig. 58.

Nerve terminal onto mesoderm, m, just below the prototroch. Note dense-cored vesicles, v', at periphery of clear vesicles, v, clustered on the membrane.


57
v
f
0·1 μ


58
v'
v
0·5 μ

appears in the form of long processes containing free ribosomes and mitochondria. Nerves develop collections of vesicles adjacent to these processes (Fig. 58), suggesting possible subsequent development of the processes into muscles.

8. The Protonephridium.

The larval protonephridium consists of two solenocytes which open into a duct composed of a single line of cells each of which add cilia to the duct. There are a pair of protonephridia situated on either side of the gullet and opening towards the base of the neurotroch. The structure of the protonephridium is summarised in the diagram (Fig. 59) in which some of the effects of different fixatives are also illustrated.

(i) Solenocyte

The solenocyte arises from a cell floating free in the blastocoel (Fig. 60), and consists of a flagellum contained in a tube, the lumen of which is 0.7 to 0.8μ in diameter. The tube is supported by 15 rods, and these are surrounded by a thin layer of cytoplasm giving an overall diameter to the outer extremity of 1.2 to 2.5μ (Fig. 61). The fixation in Fig. 61 is 1% unbuffered osmium with sea water. With this fixative the rods interconnect and are also in continuity with the outer cytoplasmic wall in a way suggesting that the separation is by means of large vacuoles. With buffered osmium the rods are still interconnected but their membrane is no longer continuous with that of the cytoplasmic wall. Osmium with triple strength sea water produces separate rods with

Fig. 59.

Diagrammatic representation of the protonephridium in longitudinal section on the right, transverse section on the left. s, solenocyte; pd, protonephridial duct; r, rods of the solenocyte; c, cilium of the solenocyte. Lower left is a diagram of the appearance of the solenocyte after standard osmium fixation. Above, central is a diagram of the solenocyte after acrolein or glutaraldehyde fixation. (Not to scale).

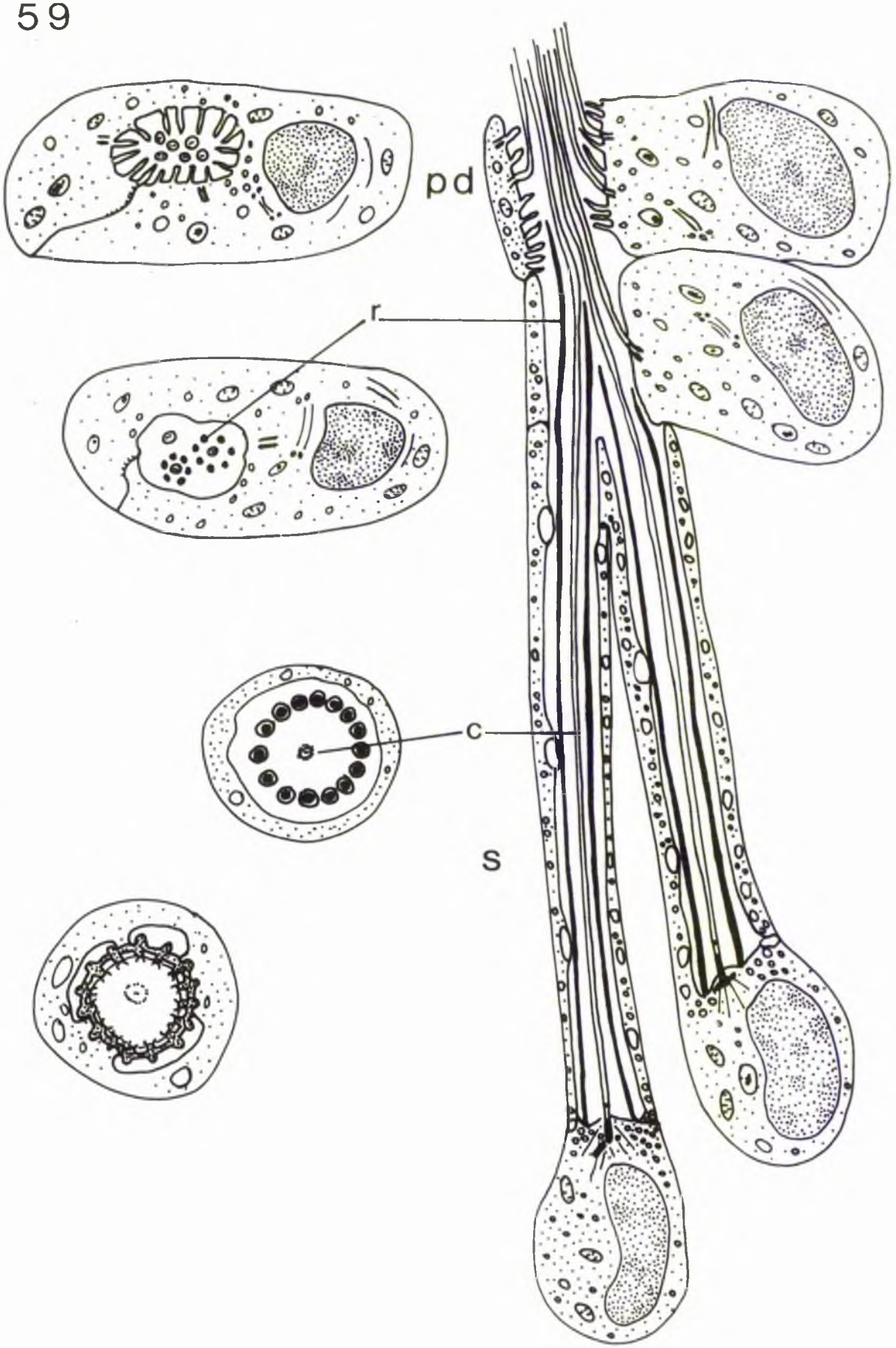

pd
r
c
s

Fig. 60.

Mid-longitudinal section of the base of the solenocyte showing the cell body with nucleus, vesicles, mitochondria, cytoplasmic extensions and rods forming the solenocyte tube, cilium central in the tube.

60

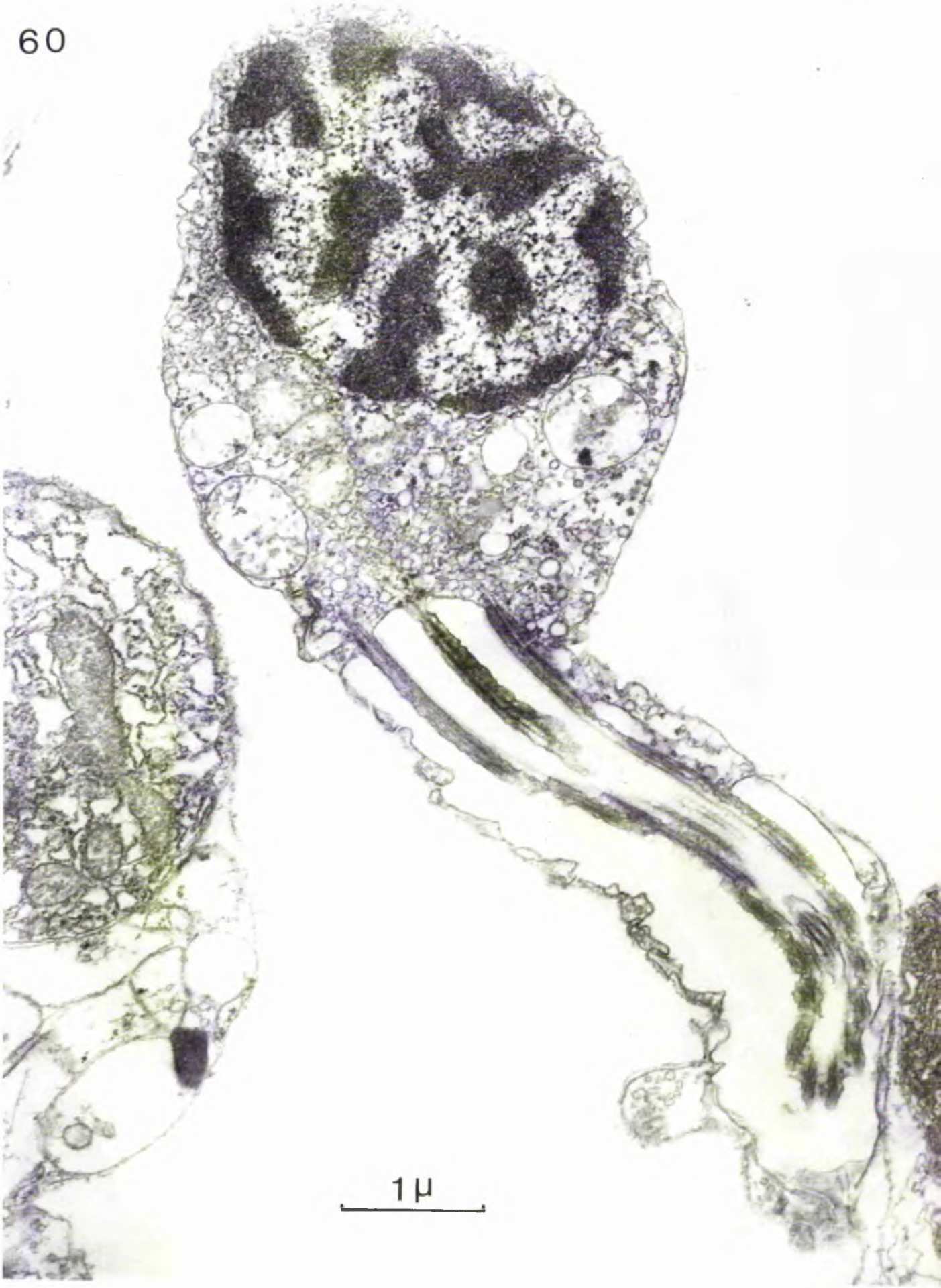


Fig. 61.

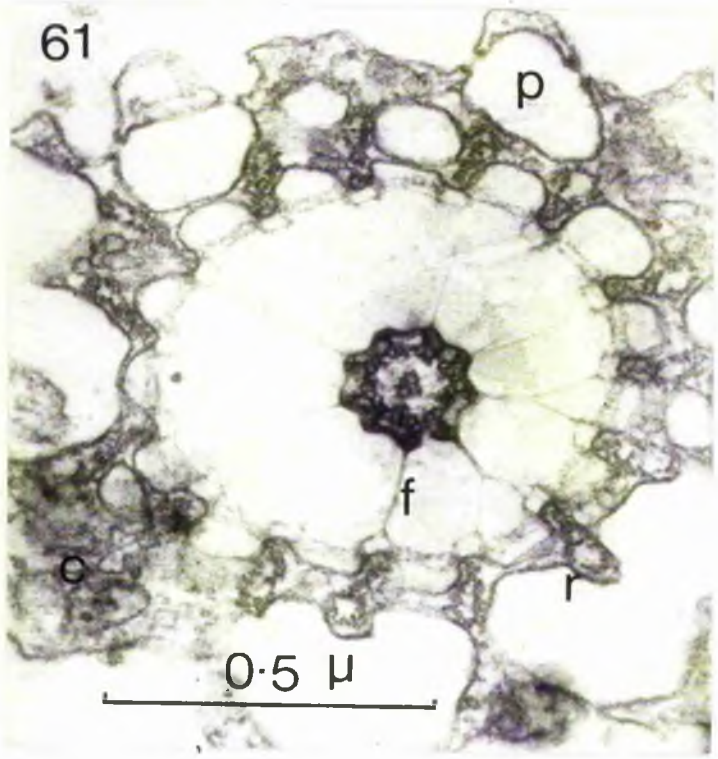
Transverse section of the solenocyte, standard osmium fixation. Note bridges between the rods, r, and the cytoplasm, c, and the pinocytotic fold, p. Filaments, f, at the top of the rods, joining the rods and joining the rods to the cilium are also evident.

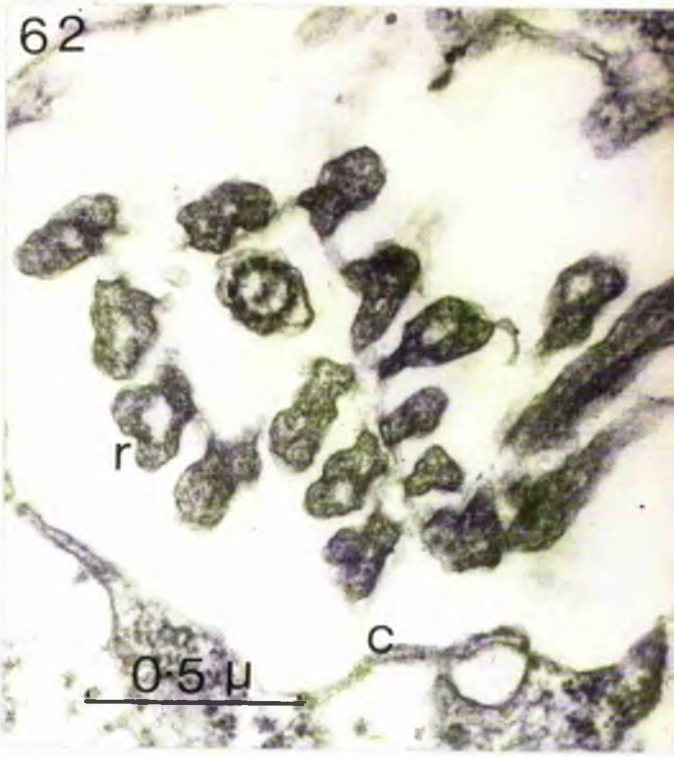
Fig. 62.

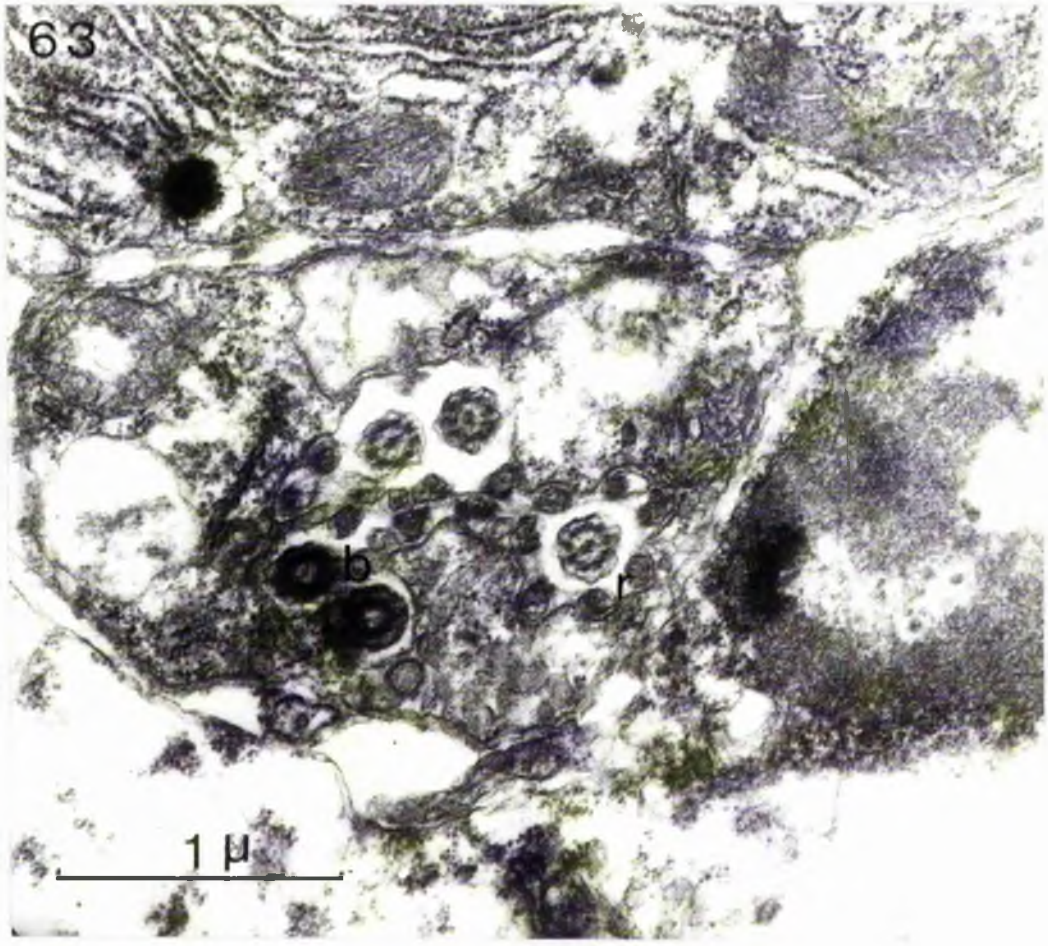
Transverse section of the solenocyte, osmium with triple strength sea water fixation. Note discrete membranes of the rods, r, but their uneven shape. The cytoplasmic wall, c, is narrow and well separated from the rods because the rods have collapsed in towards the cilium.

Fig. 63.

Protonephridial duct with terminating solenocytes, veronal glutaraldehyde fixation. Note rods of solenocyte, r, basal bodies of protonephridial duct cilia, b.


61
p
f
c
r
0·5 μ


62
r
c
0·5 μ


63
b
r
1 μ

an irregular outline and peripherally condensed granular contents (Fig. 62). Glutaraldehyde and acrolein fixation produce solenocytes with rods with separate membranes, bearing some resemblance to microvilli, although the glutaraldehyde rods are pear-shaped in cross-section and have an irregular outline. The lumen of the tube is collapsed with all but the standard osmium fixation, but the cytoplasmic layer shows more content and less vesicularisation. It is therefore difficult to evaluate the true structure of this organelle without evidence from other organ systems. It has already been shown that microvilli are poorly preserved by unbuffered osmium and well preserved by most of the other fixatives. The rods of the solenocyte may then be assumed to have independent membranes.

The structure of the solenocyte cell is simple. The nucleus is situated distal to the solenocyte process and the cytoplasm contains mitochondria, microtubules, a small amount of rough-surfaced endoplasmic reticulum, free ribosomes, and numerous vacuoles, increasing in number as the solenocyte projection is reached. The peripheral rods have a tapering process of tubules at their bases, extending 0.2μ into the cytoplasm beneath the solenocyte. Tubules also appear to be the main formation within them, with standard osmium fixation but other fixations give a granular matrix. The flagellum has a basal body with anchorage onto a cartwheel formation of basal feet, and the basal body lies 0.25μ above the point at which the flagellum leaves

the main body of cytoplasm. The movement of the flagellum is slight: it is clearly always found in the centre of the tube. Filamentous connections between the flagellum and the rods (Fig. 61) also suggest that there is little movement. The filaments are 40Å across and are infrequently seen bridging the flagellum with the rods, but always found at the tips of the rods where they form curving arches and interconnecting lines.

The peripheral cytoplasmic wall of the solenocyte is filled with vesicles and vacuoles. Some of the vacuoles appear to have been formed by thin folds of tissue which are occasionally found arched to enclose part of the blastocoel. This suggests that pinocytosis is taking place. Sometimes gaps in the cytoplasmic layer occur, such that the blastocoel becomes continuous with the space between the cytoplasmic layer and the rods.

(ii) Duct

The tip of the solenocyte tapers as it enters the main protonephridial duct, and up to 8 rods enter and terminate in the duct lumen (Figs. 59, 63). The cells of the duct each contribute cilia until there are about 20. Fig. 64 shows an early stage in the duct when there are 3 cilia and the basal bodies of 2 other cilia cut in transverse section. Fig. 65 shows a later stage, when there are 15 cilia and a basal body. The cilia have a basal foot and rootlet. The cilia are usually separate, but may occasionally be found closely adjacent such as to produce straight lines along adjoining membranes in hexagonal form. The membranes surrounding the cilia

Fig. 64.

Protonephridial duct in transverse section. Note 3 cilia, c; basal bodies, b; and septate desmosome, sd.

64

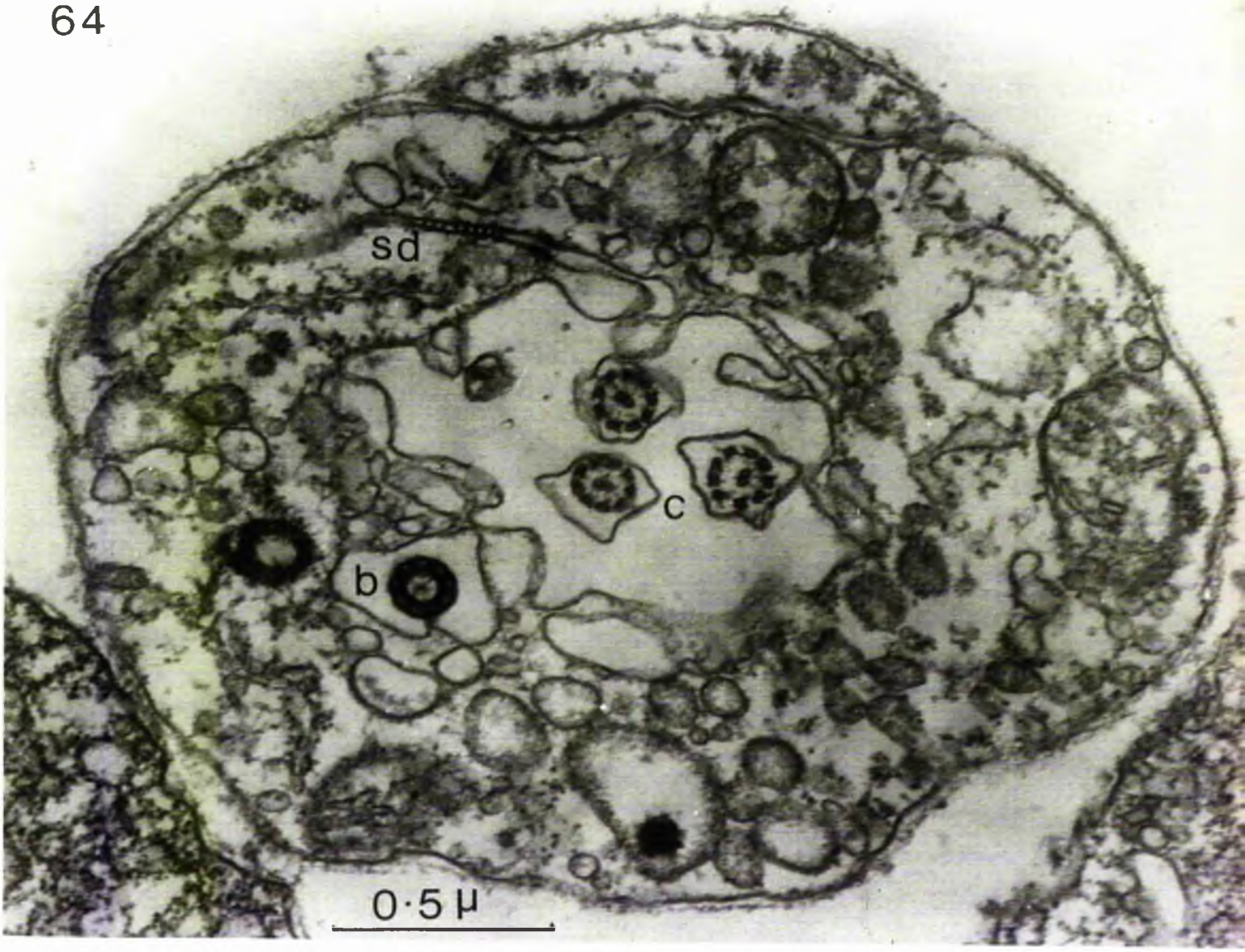
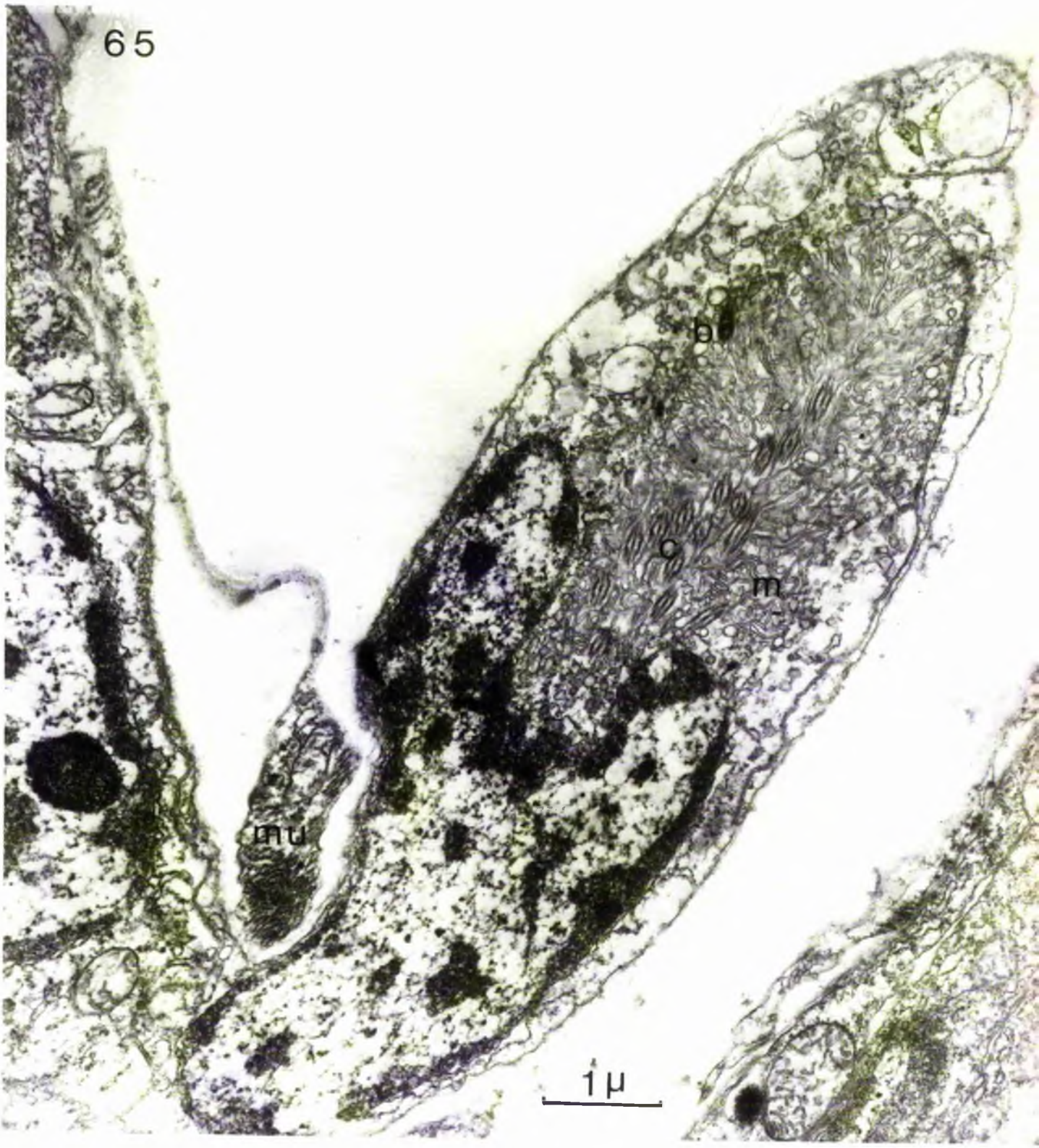


Fig. 65.

Protonephridium. Duct showing 15 cilia, c, and a basal body, b . The microvilli, m, surrounding the cilia have the form of tubules with the standard osmium fixation used here. Note the muscle strand, mu, on the left hand side.


65
b
c
m
mu
1µ

(Fig. 65) are the remains of microvilli, which take this form after 1% unbuffered osmium fixation. Although they differ from gut microvilli fixed the same way (Figs. 50,51) they are identical with eye microvilli, discussed in the next section. The lumen of the proto-nephridial duct is formed when each cell along its length makes two cytoplasmic extensions that join at the side opposite the nucleus with a septate desmosome (Fig. 64). This gives the appearance of an intracellular duct. The cells contain many vesicles, Golgi bodies, some small areas of endoplasmic reticulum, and large vacuoles containing granular material.

9. Visual systems

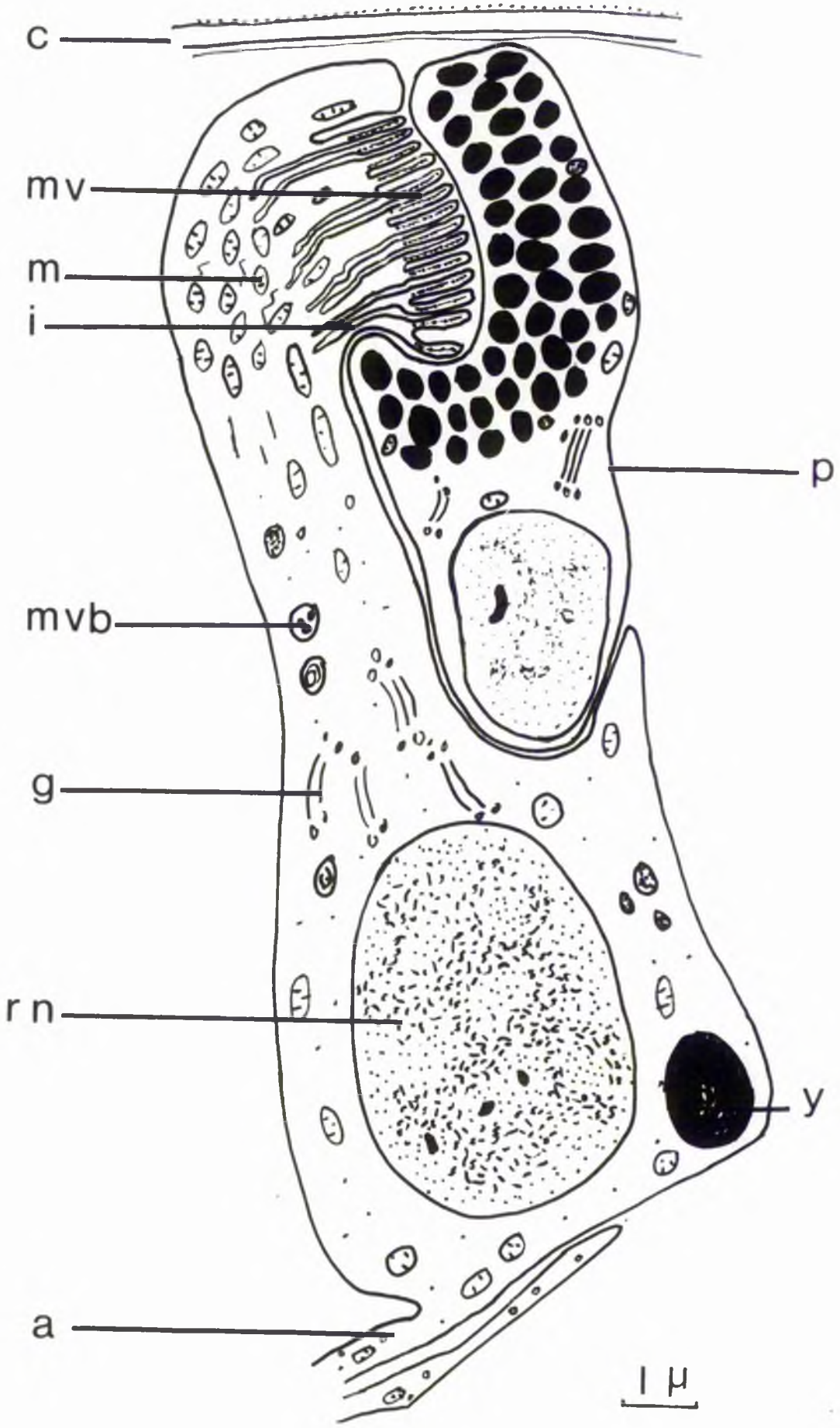
(i) Larval eyes.

A single eye is found slightly towards the dorsal side and nearly half way up the upper hemisphere on each side of the trochophore. The eye illustrated by the diagram (Fig. 66) is of inverse type, consisting of a pigment cup derived from a single cell, which encloses a rhabdomeric photoreceptor apparatus arising from one, or exceptionally two, cells. A group of about 180 cilia near to the eye beats away from the eye (See Holborow and Laverack, 1972).

The pigment cup is 3μ deep, 10μ long, 10μ wide, and made up of 0.5μ granules packed in rows, 3 to 5 in a row. The nucleus of the pigment cell lies directly beneath the pigment cup and is closely bounded by the cell membrane such that the layer of cytoplasm surrounding it is 0.2μ thick or less. The nucleus is 4μ wide at its broadest point

Fig. 66.

Diagram of the eye of the trochophore in longitudinal section. a, axon; c, cuticle; g, Golgi apparatus; i, invaginated membranes; m, mitochondria; mv, microvilli; mvb, multivesicular body; p, pigment cell; rn, receptor cell nucleus; y, yolk granule.


c
mv
m
i
p
mvb
g
rn
y
a
1 µ

(adjacent to the pigment cup) and 6μ long.

The receptor cell extends through the full depth of the ectoderm, 20μ, and sends off an axon along the ectodermal side of the blastocoel. This joins other nerve fibres running from the apex to the prototroch. The cell can be divided into three regions apart from the axon. First, just above the axon is the basal region, 8μ broad, almost entirely filled with the oval nucleus (7.5μ by 5.5μ). The cytoplasm around the nucleus contains the usual organelles except that the Golgi bodies tend to be found higher up in the middle region of the cell. This region is a narrow portion where the receptor cell passes over the edge of the pigment cup. Multivesicular bodies and myelin figures are the other features in this region (Fig. 67).

The upper region is again broad with half the breadth made up of receptor membranes, the other half containing mitochondria, free ribosomes and rough endoplasmic reticulum. The receptor apparatus is two-layered. The layer adjacent to the pigment cup consists of rows of microvilli, 0.1μ in diameter, 1.2μ long. Although closely packed, the membranes of the microvilli are not fused. They have an electron-dense core (Fig. 68). The inner layer of membranes appear to be invaginations of the cell surface and at their inner ends associate with endoplasmic reticulum (Fig. 69). They are usually aligned with the microvilli, straight and parallel, but may be found set at angles and curved. They occur as pairs of membranes

Fig. 67.

Larval eye. The receptor cell can be seen to extend from the cuticle to the blastocoel, b. The receptor nucleus, rn, lies at the base of the cell, with Golgi bodies G, myelin figures, f, and multivesicular bodies, v, above. The receptor membranes, r, project into the pigment cup, p. The pigment cell nucleus, pn, is partially enfolded by the receptor cell. Note cilia, c, which are part of a group of up to 180 cilia adjacent to the eye.

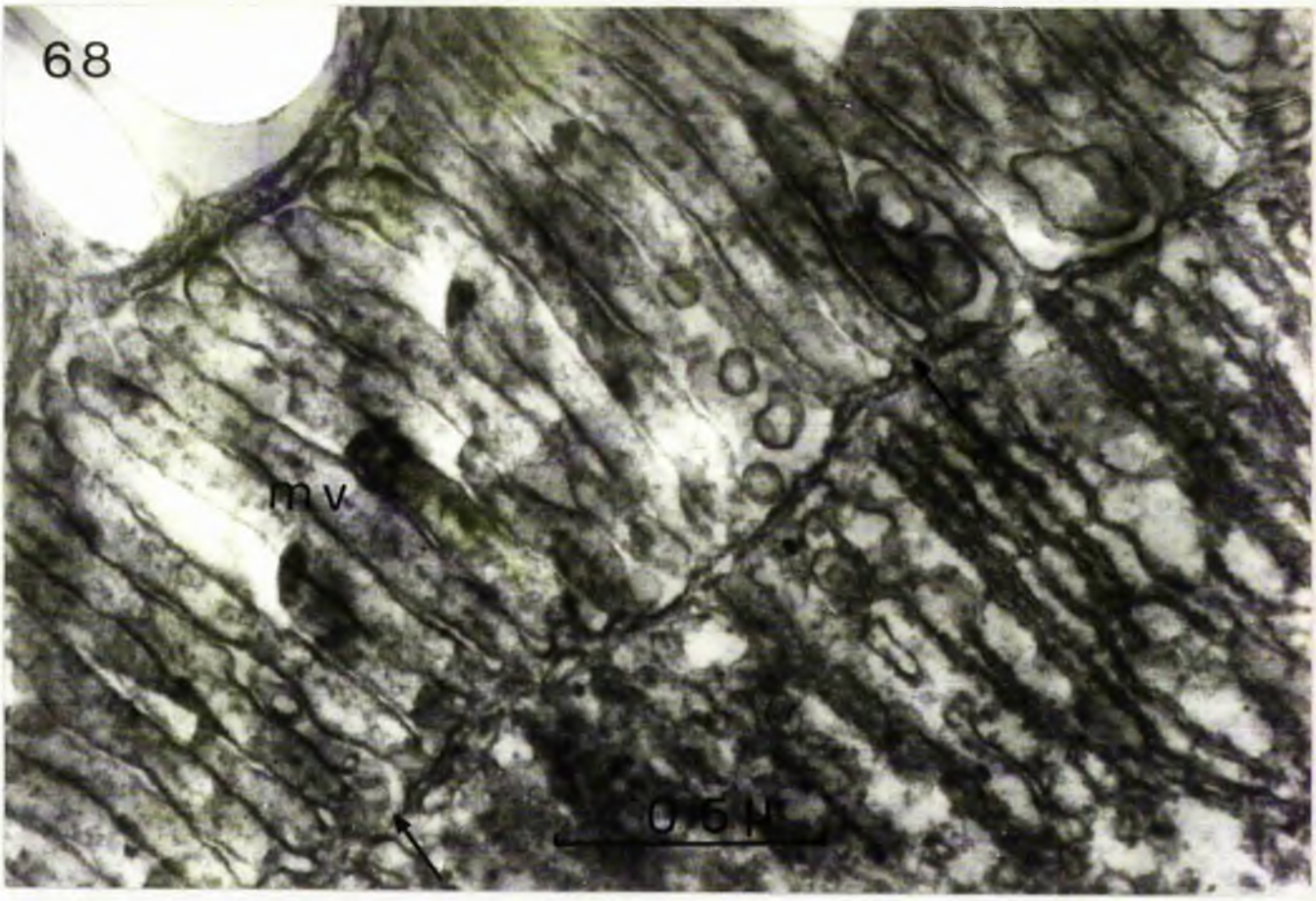

67
c
r
p
v
pn
G
f
rn
1μ
b

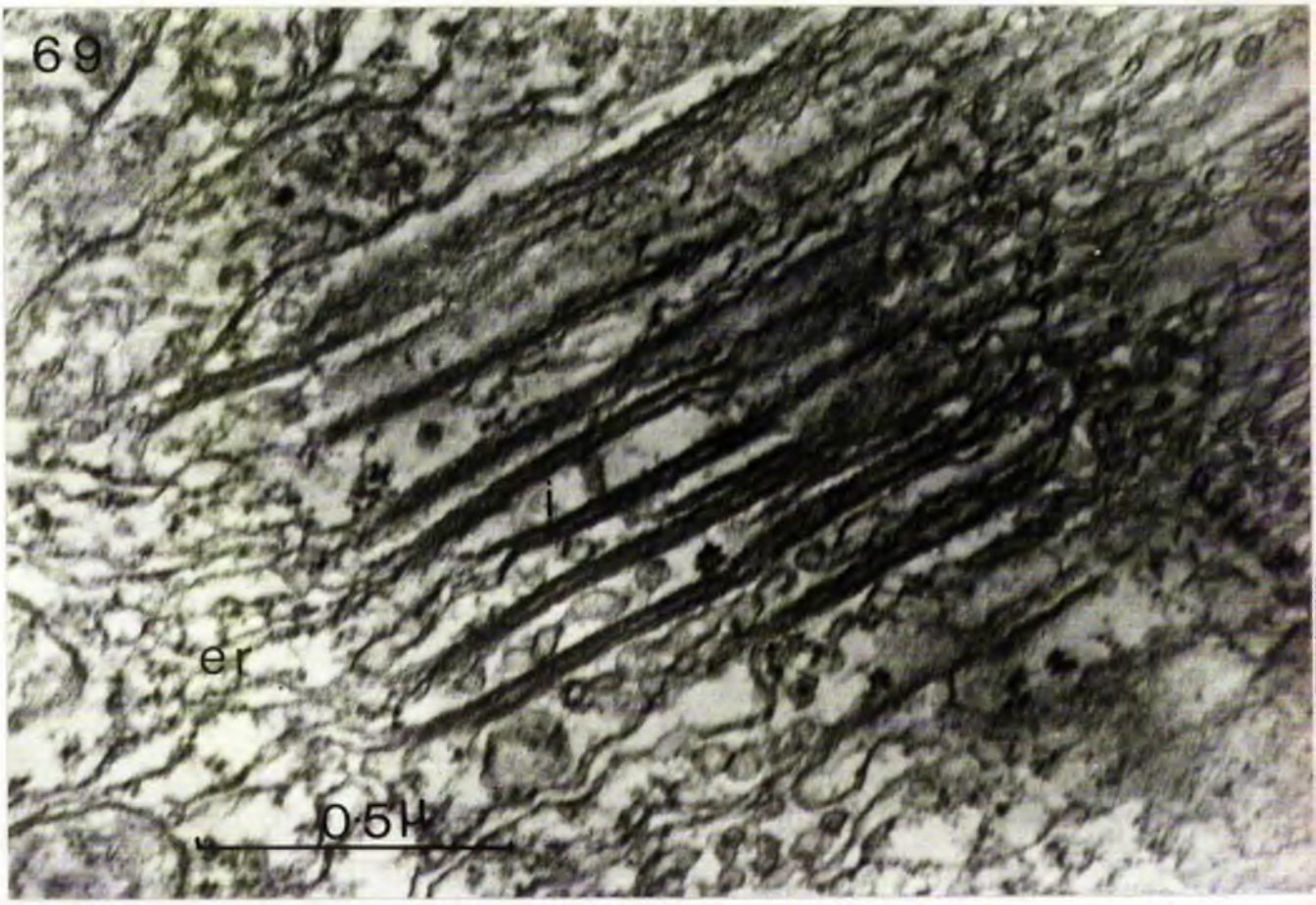
Fig. 68.

Microvilli of the eye, mv, showing continuity with the inner layer of membranes at the arrows. Note electron-dense core of the microvilli. Cacodylate glutaraldehyde fixation.

Fig. 69.

Invaginated membranes, i, from the base of the microvilli, here in the form of tubules after standard osmium fixation. The invaginated membranes are connected to granular endoplasmic reticulum, er.


68
mv
0.5 μ


69
er
0.5μ

grouped into sets of two pairs with a more electron-dense matrix between them than normal cytoplasm. The sets of pairs are 160Å and 200Å apart and 700Å from the next set. The membranes of the pair are themselves about 100Å apart, but they flare out at a depth of 1 to 2μ in the cell into closed "extracellular" cisternae of the granular endoplasmic reticulum.

In figure 69 the membranes of the microvilli form fine tubules and vesicles 700Å in diameter. This is a characteristic artefact of osmium fixation and has been reported in the eyes of other annelids (Hermans and Cloney, 1966).

(ii) Supplementary visual organelle.

Among nerve cell bodies in the apex ectoderm is an organelle composed of cilia and microvilli (Fig. 70). This organelle has no pigment cup and is entirely different from the eyes of the trochophore and those of the adult Harmothoë, but it bears sufficient resemblance to photoreceptors in other annelids to be termed a visual organ.

Two cells produce and surround the organ. Fig. 71 shows a developmental stage in which a recently divided centriole and small rootlets at the base of the cilia are present. A section a little further through the same organelle, (Fig. 72), shows cilia and microvilli present together. The cilia divide, with some filaments passing into each arm (Fig. 73), and the microvilli increase in number. The rootlets of the cilia disappear but microtubules project in a star-like array from the basal body (See Holborow and Laverack,

Fig. 70.

Presumed photoreceptor derived from cilia, basal bodies marked, b, and microvilli, of which a small clump by the basal bodies is marked, m.

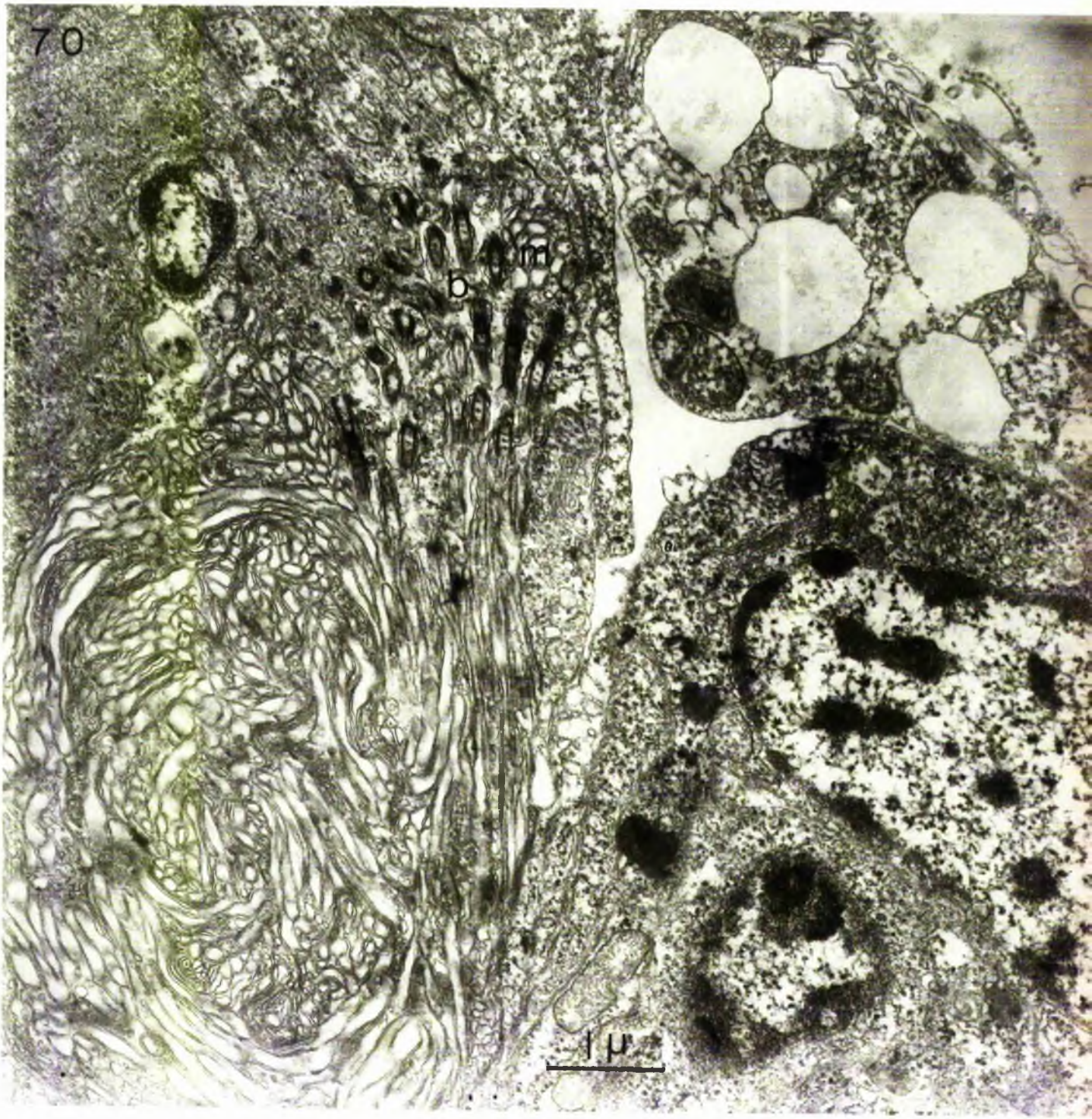

70
m
b
1 μ

Fig. 71.

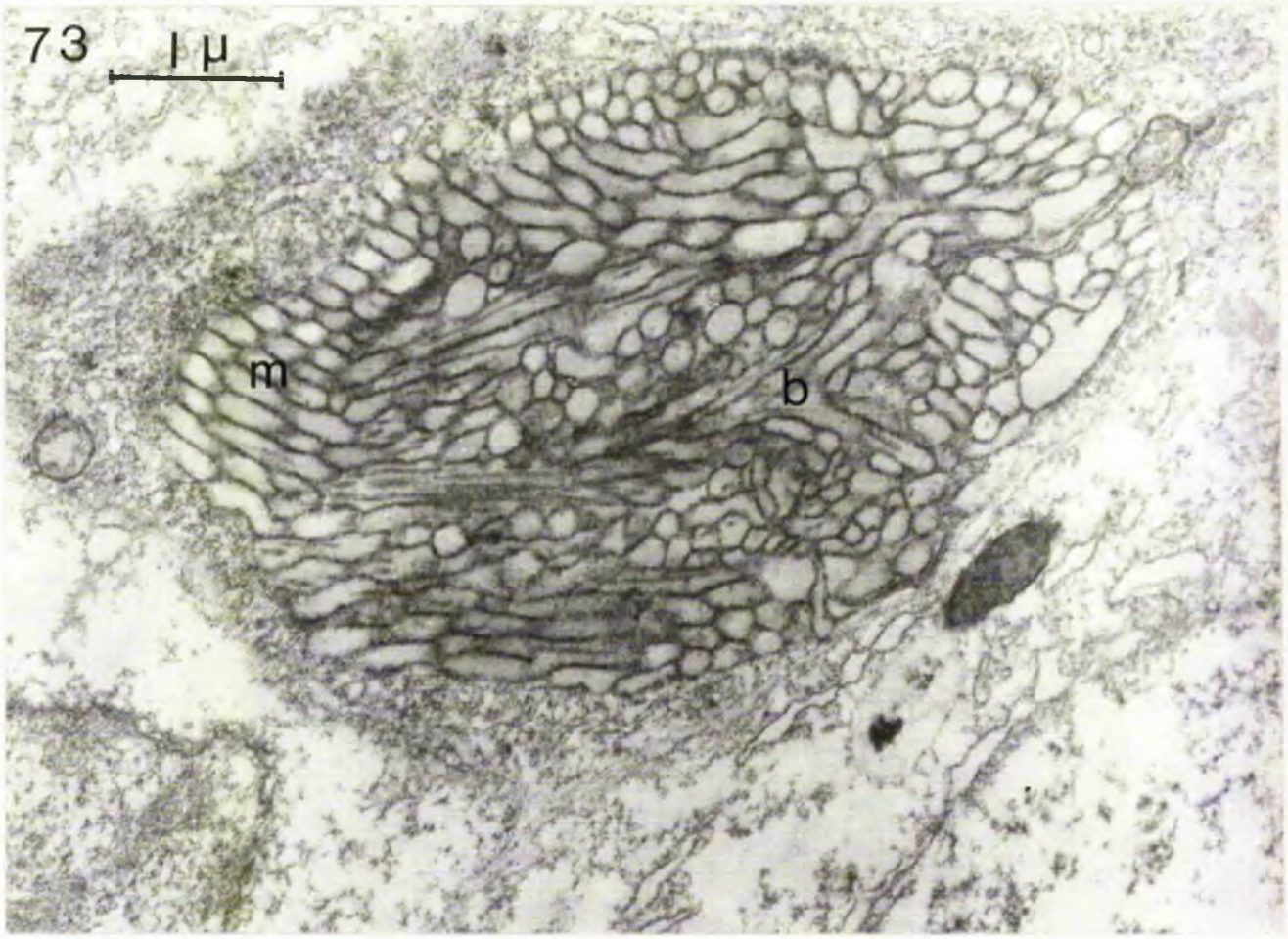
Developing organelle with centriole, c, and rootlet, r.

Fig. 72.

Developing organelle with microvilli on left hand side.

Fig. 73.

Section through the top of the ciliary visual organelle showing branching cilium, b, microvilli, m.


73
1 μ
m
b


71
1 μ
c
r


72
1 μ

1972) and at all stages may be found scattered in the cytoplasm of this part of the cell, gradually tending to become oriented with the cilia and the cell membrane. In the mature organelle roughly 30 cilia project from the narrow necks of two cells and coil together in a mass. The microvilli which project in from the sides of the cells when they enfold the mass in its early stages grow together with the cilia.

The mass of the fully developed organelle is an oval 7.5μ long and 5μ wide, with the bases of the cilia extending 1.5μ below the coiled mass and some 14 microvilli lying eccentrically on the outer side of the bases of the cilia (Fig. 70).

The cilia are distinctive. The pattern of filaments in the axoneme is normal but this breaks down in an unusual sequence (compare with Fig. 44). The central filaments are lost first followed by the termination of the peripheral pairs as pairs until the final filaments remaining are a single pair (Fig. 74). Few filaments extend more than 4μ above the basal body. The basal body differs from others in the trochophore. It extends 0.6μ below the basal plate in the form of slightly splaying filaments connected at the upper end by a 0.2μ long electron-dense thickening. The basal plate is a very dense bar 300Å thick and above it there are three centrally placed particles 200Å across and 0.1μ apart. (Fig. 75).

This description is of the organelle after osmium fixation.

Fig. 74.

Single pair of peripheral filaments as axoneme terminates.

Fig. 75.

Basal body of cilium, standard osmium fixation. Note breadth of the basal plate.

Fig. 76.

Basal body of cilium, acrolein fixation. Note granular matrix of cilium.

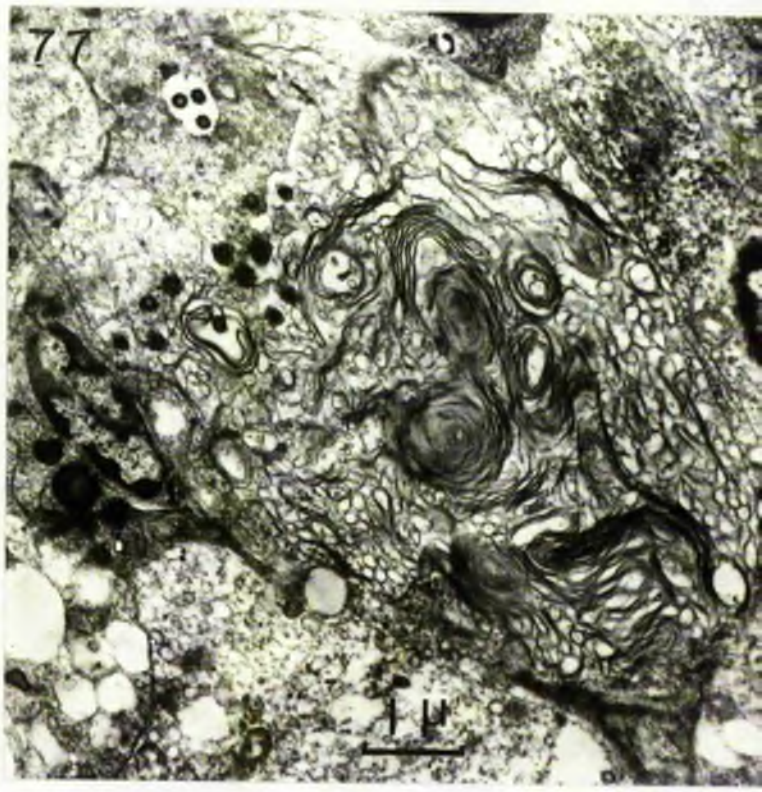
Fig. 77.

Presumed photoreceptor after acrolein fixation.

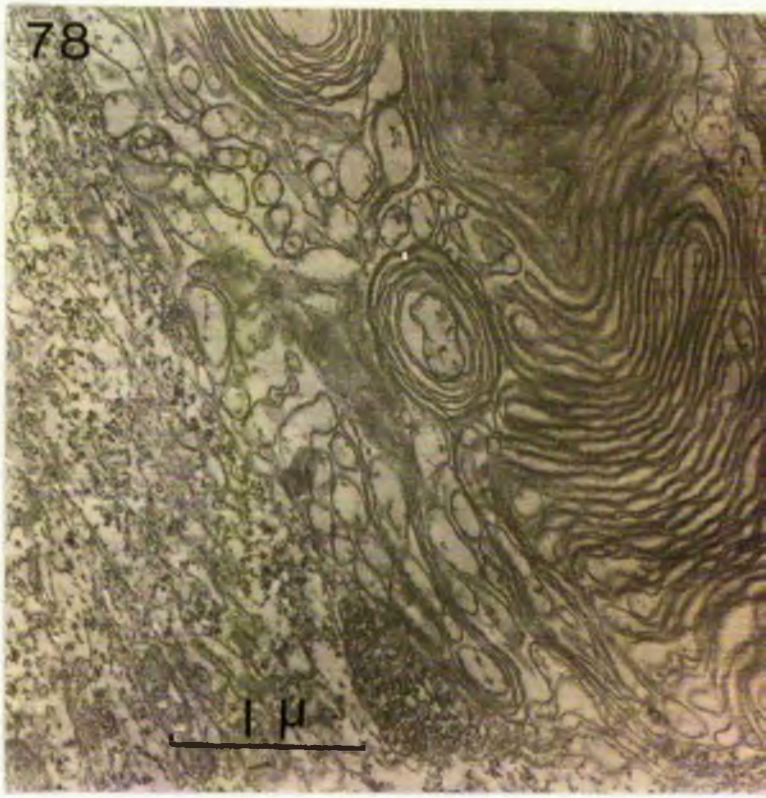
Fig. 78.

Presumed photoreceptor after glutaraldehyde fixation.


74
0.5 μ


77
1 μ


75
0.5 μ


78
1 μ


76
1 μ

With acrolein fixation the fibres of the cilium dissolve into scattered granules connected by fine filaments although the form of the basal body does not appear to be seriously affected (Fig. 76). The form of the whole organelle is different with both acrolein and glutaraldehyde fixations, which both produce whorled membranes as a predominant feature (Figs. 77 and 78).

10. The Adult Eye.

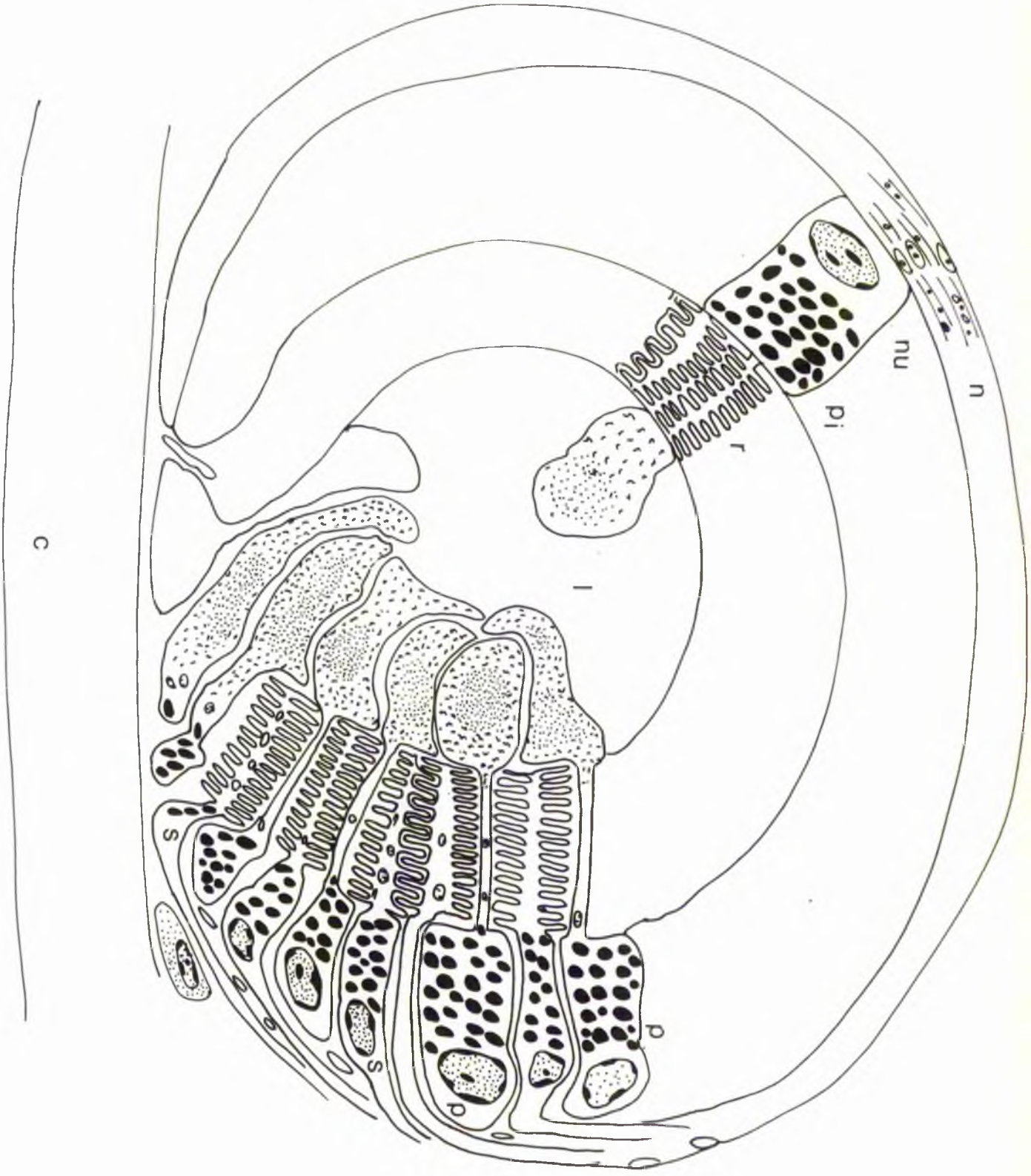
The adult eye was examined in order to compare it with the juvenile eye, and will be described briefly. The eye (Fig. 79) is 160μ by 100μ, almost equivalent in size to a trochophore. It is composed of a pigment cup, 7μ to 15μ thick, a 40μ thick receptor layer and a lens which is 60μ broad and 80μ deep. Outside the pigment cup are the receptor cells and their axons.

The receptor cells are up to 80μ long, not including the axon. The cell body containing the nucleus lies directly below the pigment layer and gives rise to a 0.4μ to 0.6μ diameter axon which runs around the eye cup, joining with other axons before entering the optic nerve. The axons contain small mitochondria, 600Å dense-cored and 300Å clear vesicles, lipid droplets, vacuoles and microtubules, and a variety of granules including oval, membrane-bound, electron-dense bodies between 0.2μ and 0.4μ in size. Between the axons are supporting cells containing 0.3μ wide bundles of collagen (Fig. 80).

The sensory cell is 1μ to 3μ wide as it passes through the pigment layer and contains, besides the normal cell organelles, pigment. Between the pigment cell and the lens the visual cell processes

Fig. 79.

Diagram showing basic structure of the eye of the adult Harmothoë imbricata. On the upper right the layers of the eye are depicted from the outside inwards; n, optic nerve; nu, nucleus of pigment cell; pi, pigment layer; r, receptor layer (microvilli); l, lens. Lower left shows the way in which the different systems inter-relate. The pigment cells, p, give rise to processes which connect with the lens. The receptor cells send an axon, s, into the optic nerve. They are pigmented and adjacent receptor cells have interdigitating microvilli in the receptor layer. (Not to scale)


c
s
l
r
pi
nu
n
p
d

are straight, 0.4μ to 1μ wide, although when cut in transverse section they may be s, x or y shaped. On each side these processes bear 2μ to 3μ long microvilli which interdigitate with microvilli from opposite processes in a not-strictly-alternate arrangement. The microvilli are straight and at right angles to the processes, which means also that they are at right angles to the incoming light. Whorls and vesicles in the microvilli are taken to be fixation artefacts. The microvilli are 700Å in diameter. Mitochondria and vesicularised smooth endoplasmic reticulum are the main constituents of the microvilli-bearing visual cell processes (Fig. 81). Infolded membranes are also present.

The pigment cells form an elliptical cup which completely surrounds the receptor layer and the lens, except for a 60μ wide aperture. The inner wall of the pigment cup is smooth. Each pigment cell has three distinct regions: an outer pigment-bearing region, 7μ to 15μ thick, where the nucleus is also situated, a thin process which passes between the receptor cell processes, and an expanded portion forming the lens (Fig. 81).

The outer pigment-bearing region of the cells is packed with 0.3μ to 0.6μ diameter granules, sometimes arranged in lines, particularly in the inner region above the nucleus. The nucleus lies on the proximal side of the cell and has a regular outline and oval form with the long axis tangential to the eye cup. The cells have an irregular outline but do not appear to project into the sensory cells. The pigment cell process in the receptor layer carries

Fig. 80.

Adult eye, n, optic nerves; r, receptor layer; p, pigment; c, collagen bundles.

80

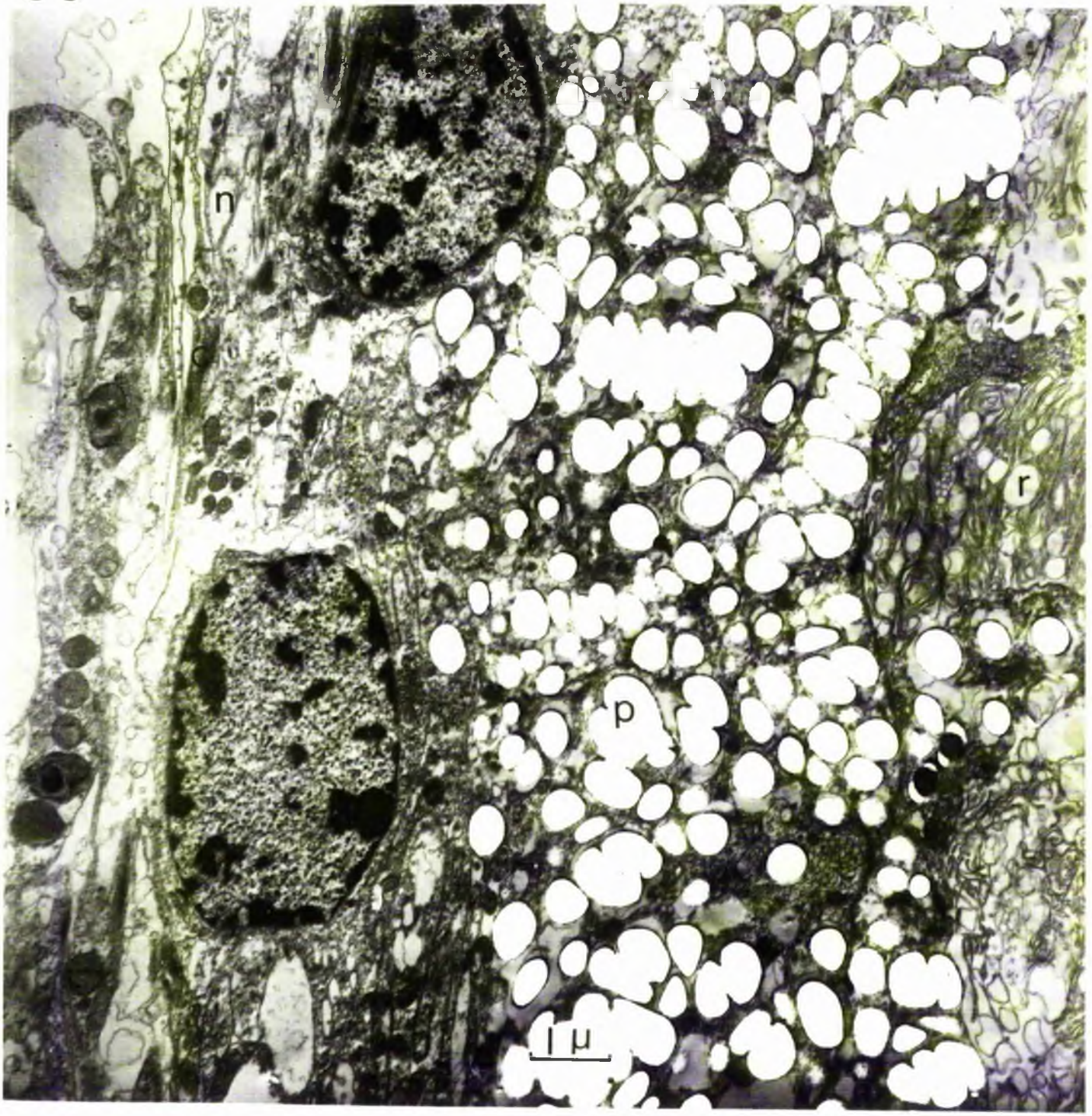
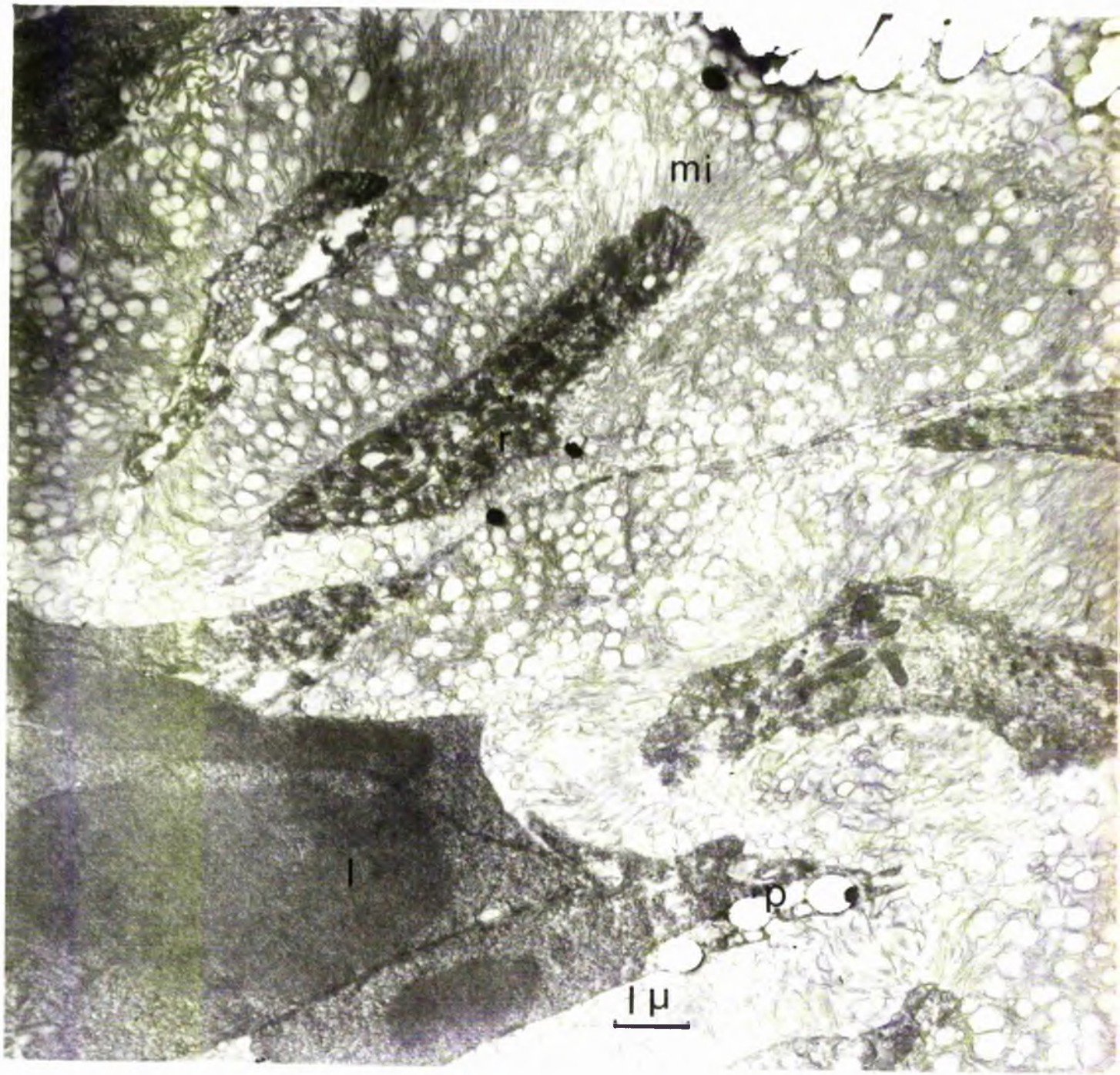


Fig. 81.

Adult eye showing receptor cell process, r, with microvilli, mi, forming receptor membranes, lens, l, and pigment cell process, p, connecting with the lens.


mi
r
l
p
1 µ

some pigment granules and some lens material into the receptor layer. Movement of pigment may be a feature of dark adaptation but has not been examined in this animal. These processes bear some short, dense microvilli, and contain granular cytoplasm, mitochondria and striated collagen filaments.

The lens is made up of a cluster of cell processes 1 μ to .4μ wide. Each process contains in the central region a dense array of double membranes formed into apparently empty tubules 200Å in diameter. (Fig. 81) In the periphery the tubules are more loosely arrayed, tangled and sinuous, making an area less electron-dense overall. (Fig. 81)

DISCUSSION

1. Observations on living trochophore.

(i) Embryonic stages.

The habit of Harmothoë imbricata of incubating the developing eggs under the elytra of the female worm has been reported from widespread geographic localities by a number of authors. Sars (1845) recorded spawning near Norway, and gave the duration of incubation (subsequently rarely measured) as two weeks. McIntosh (1900) reported brood protection by the St. Andrews (Scotland) Harmothoë, Izuka (1912) found similar incubation behaviour by this species near Japan. Saemundsson (1918) noted eggs under the elytra of specimens from Iceland, Rasmussen (1956) observed incubation among Harmothoë in Danish waters. Only one occurrence of absence of incubation has been reported for this species. Cazaux (1968) found that the larvae of Harmothoë at Arcachon, France, have a completely planktonic development. In view of the number of reports of the incubation habit that are available, surprizingly little is known of the development of the larvae. Daly (1972) gave the first day-by-day account of the changes in the external appearance of the larva during development as a means of determining when the female had spawned. The findings here are in close agreement with his.

The major disagreement in the literature is in the measurement of egg sizes. According to Sars (1845), the diameter of the egg is 50μ. McIntosh (1900) gives the diameter as 56μ to 76μ,

Pettibone (1953) states that the eggs are 136μ, Rasmussen (1956) finds them to be 150μ, Cazaux (1968) gives a measurement of 80μ and Daly (1972) 140μ to 160μ, although his figure scales are not in agreement, indicating a smaller size of some 110μ for the fertilized egg, 136μ for the length of 8 day stage, and 159μ for the 11-day stage. This agrees fairly well with the findings here.

The apical tuft of the embryonic trochophore is the most noteworthy feature because of the unusual mode of beating of the cilia and the contradiction between the apparent function of the tuft and the assumed function of apical cilia in trochophores.

Rasmussen (1956) states that there is an apical tuft but makes no observations on the mode of beating of the cilia. A mid-apical tuft of cilia is regarded as characteristic of trochophores. (Hyman, 1951, and others) and a general consensus has emerged that the apical tuft is sensory. Shearer (1911) depicts the apical tuft as arising from a sensory plate in Eupomatus; E.B. Wilson describes apical cilia as sensory in Nereis (1932a), Notomastus (1933) and Polydora (1928b); Meyer (1901), in a detailed account of the nervous system of Lopadorhynchus, showed nerve cell bodies lateral to the apex.

However, there seems to be no evidence that the apical cilia are sensory. No electrophysiology has been attempted and no nerves have been demonstrated emanating from the apical ciliated cells. The slowly oscillating apical tuft of the incubating Harmothoë trochophore attaches and rotates the trochophore. The rotations of

the body of the trochophore could circulate the fluid around it and help to clear away metabolic waste and bring fresh oxygen. These original apical cilia apparently serve a purely mechanical function related to the incubation habit of the worm.

The delay between the active stroke and "recovery" stroke of the incubation apical cilia, and the power of the "recovery" stroke causing opposite rotation of the trochophore, lead to the implication that there are in fact just active strokes here. This appears to be a unique mode of beating.

The apical cilia which subsequently develop grow in lines and may be sensory. A full discussion follows the electron microscopical account of their arrangement and structure.

(ii) The beating of cilia.

The reason for the irregularity in the beating patterns of both the long and the short apical cilia is problematical. Sleigh (1962) discusses the determination of the rate of beat of cilia on the basis of continuous beating, subject to the limitations of "rate of contraction or rate of excitation". Harris (1961) theorized that the duration of the effective stroke is proportional to the cilium length. Further, where the cilia are compounded together the effective stroke is inversely proportional to the number of component cilia while the recovery stroke is unaltered. The short apical cilia complete a single cycle of beat in less time than it takes the long cilia of the incubation period to complete one stroke in one direction. It may be said that the

long apical cilia, while acting together, are not functioning as compound cilia. Beyond that, both these sets of cilia exhibit beating patterns that are unlike any which have been previously discussed go any extent.

Intermittent cycles of beat such as shown by the short apical cilia have been recorded elsewhere (Santer and Laverack, 1971 and others), but there has been no explanation of the cause of the intervals.

Preliminary experiments on trochophores show that continuous beating of the neurotroch becomes intermittent with the application of acetylcholine and glycine and that cilia with intermittent cycles of beat, beat continuously after the application of noradrenalin and 5-hydroxytryptamine. Innervation to ciliated cells may control patterns of beating by release of these transmitters.

The measured rate of beat of cilia of the trochophores prototroch of between 6 and 15 strokes a second falls within the range of beat rate reported in other animals (see Sleigh, 1962 for review; Hakansson and Toremalm, 1965; Pontin, 1966).

2. Fixation

Most workers in electron microscopy try more than one fixative for their material, but usually settle for a single one without commenting on the different effects produced by the different fixatives. Ling (1969, 1970) illustrated two differing effects of fixation without commenting on these results although the structural

difference was considerable (see Discussion on gullet cilia).

The most commonly used fixative appears to be glutaraldehyde with cacodylate buffer according to Sabatini, Bensch and Barrnett (1963). Millonig's (1961) phosphate buffered osmium has been popular, and phosphate buffers are also frequently used with glutaraldehyde with Millonig's buffered osmium as a post fixation. In this work on trochophores, precipitation occurred with phosphate buffers made up to the required high osmolarity (higher than that required for vertebrate material) and sections were not cut of this material. Potassium permanganate has been used for fixation, and is particularly effective in fixing membranes, although not as good as osmium for overall fixation (see comparative study by Rosenbluth, 1963). Rarely, some additives have been used experimentally in fixation, such as a nitrogen mustard derivative (Williams and Luft, 1968). Formaldehyde has quite frequently been used with glutaraldehyde, according to the method by Karnovsky (1965), and veronal and collidine buffered glutaraldehyde have also been widely used. Holt and Hicks (1961a and b) studied formalin fixation and the use of veronal buffers with formalin, but formalin is rarely used in electron microscopy at the present time. Eakin and Westfall (1964) and Ling (1970) used 2% osmium with potassium dichromate in sea water (after the method of Dalton, 1955), but the effects were much as with osmium alone.

Imaizumi and Hama (1969) tried a triple fixative starting with phosphate buffered osmium, then glutaraldehyde then uranyl acetate. Recently, fixatives have been used in more extensive mixtures;

Tandler and MacCallum (1972) used a perfusate mixture of acrolein glutaraldehyde and formaldehyde buffered with chromate-dichromate. They also compared the effect of different fixatives on mucous droplets.

Many workers have increased the osmolarity of their solutions by the addition of sucrose, sodium chloride and sodium bicarbonate, but few have noted the osmolarity achieved. Sjöstrand (1956) stated that solutions should be isotonic, but Drochmans (1960) found 1% osmium teroxide in distilled water successful, and many workers using hypotonic fixative media, including that of Palade (1952), achieved reasonably satisfactory results.

Comparisons of types of fixation have not been widely made, and as they have usually been applied to a particular organ in a particular animal, they have not always elucidated a general situation. Claude (1961) looked at osmium fixations in acid and alkaline media, Schultz and Karlsson (1965) studied the effect of osmolarity, pH and fixative concentration; Baker (1965) looked at the effects of concentration, duration and temperature. Wood and Luft (1965) studied the influence of buffer systems on fixation with osmium tetroxide. Hayat and Giaquinta (1970) showed the value of rapid fixation and embedding. Yamamoto, Nakagawa and Nakagawa (1969) studied the effects of pH and buffers with formalin. Recently Peracchia and Mittler (1972b) have tried raising the pH and warming phosphate-buffered glutaraldehyde during the course of fixation; these workers have also investigated the use of glutaraldehyde with hydrogen peroxide (1972a). Törack (1965)

and Van Harreveld and Khattab (1969) fixed with hydroxyadipaldehyde, postfixing with osmium.

Comparisons of actual fixatives, as distinct from comparisons of one fixative with different conditions, have been made by Tormey (1964) who looked at the different effects of osmium and gluteraldehyde on ciliary membranes in the rabbit eye; Baker (1965) who compared formaldehyde and osmium in the rabbit pancreas, and Rosenbluth (1963) who examined the different effects of osmium and permanganate in the toad spinal ganglia. Other studies have followed these, on similar lines without clarifying the differences for a wide range of tissues.

This work with the trochophore showed that while each fixative had particular merits for certain types of tissue it compared unfavourably with the other fixatives in certain respects. Overall, 2% osmium tetroxide with 50% sea water gave the best general effect even though it was not superior to any of the other fixatives for any particular tissue. Once the defects of this fixative could be recognised, adequate interpretations of tissue structures and comparisons could be made between different age groups of animals. Increasing the osmolarity of the osmium proved of value in eliminating some of the undesirable effects.

The dramatic differences in reaction of invertebrate, particularly polychaete, eye membranes to different fixatives have led to some serious misinterpretations, such as that of Hermans and Cloney (1966), corrected after glutaraldehyde fixation was used by Hermans (1969).

Eakin and Westfall (1964) ignored the curious tubular and vesicular appearance of the eye membranes of the trochophore of Neanthes succinea and correctly interpreted them as microvilli, but mistakenly interpreted the infolded membranes as also being microvilli. Dorsett and Hyde (1968) noted whorled membranes among the microvilli in the eye of Nereis virens, but this is apparently a fixation artefact of glutaraldehyde. Heavily vesiculated cells such as those of Neanthes succinea (Eakin and Westfall, 1964), probably show this as an artefact of the osmium fixation. Differences in the appearance of nuclei and endoplasmic reticulum can be quite distinctive to the fixative used as well as a particular cell, and it is clearly of value to use a variety of fixation techniques before interpretations are made. It is also clear that comparisons must be made with other workers' findings with some caution, bearing in mind differences in fixation. Prestaining, while supposedly demonstrating some cellular structures better than post-staining, eg, synaptic vesicles (Gray, 1959), did not give as good contrast as the poststaining of Venable and Coggeshall (1965). A further disadvantage of prestaining was that the whole thickness of the section carried stain, preventing sharp focus.

3. Surface Anatomy and Surface Cilia

Without the scanning electron microscope, complete understanding of surface structure relationships is not possible. Asymmetry in trochophore has rarely received comment, and the expectation of symmetry has led to some misconceptions. Gravely (1909a),

Rasmussen (1956) and Korn (1958), possibly assuming symmetry, describe two tongues of cilia to either side of the mouth in the Polynoidae. The asymmetrical arrangement of gland openings and types of pore are another feature hitherto unexpected. Åkesson (1963) and Korn (1958) noticed ventral and ventro-lateral glandular areas in the episphere, but details of their arrangement and numbers remained obscure.

(i) Apical cilia.

A single apical tuft of cilia is regarded as characteristic of trochophores (Hyman, 1951), but apical rings have been depicted. Gravely (1909a) shows a neat apical ring of cilia on his diagram of a Polynoid trochophore, Korn (1958) recognises a ring with lateral gaps from his light microscope studies of Harmothoë imbricata, Fuchs (1911) depicts an apical ring with a dorsal gap on the trochophores of Nepthys and Glycera. In all these studies the apical circlet is shown as symmetrical, evenly curving lines, whereas in the Harmothoë trochophore studied here, the lines tend to be straight, of consistently different lengths and set at various angles.

The stiff cilia in the centre of the apical region are typical of ciliated sensory cells (Horridge, 1965; Cobb, 1968; Crisp, 1971; Santer and Laverack, 1971). The peripheral ring cilia may be sensory, or they may serve a purely mechanical function: they may create currents to establish directionality of incoming stimuli, steer the animal or simply protect the short apical cilia from mucus. Contamination with mucus would not be a problem in trochophores which

have a single tuft of long cilia, and this could be why a ring of apical cilia is not found in these cases.

The dense-cored and clear vesicles among the ciliary basal bodies and the upper part of the roots may be involved in initiating and stopping bursts. These vesicles are absent in cells bearing continuously beating cilia.

(ii) Prototroch cilia.

The main prototroch band is obviously the locomotory organ, but function of the lower and particularly the upper prototroch cilia is problematical. The lower cilia are not always in phase with the long cilia and may have a balancing role for straight swimming. The upper prototroch cilia are widely spaced and too short to be involved in locomotion. They may be sensory, particularly as they appear stiff in some instances. The prototroch band is not usually described in any great detail, but one of the authors who has shown separate parts in the prototroch is Segrove (1940), working on Pomatoceros. Segrove found that the Pomatoceros trochophore has a prototroch consisting of two layers of cilia with a feeding band below these and a post oral ring or metatroch. The cilia of the posterior ring are 40μ long and described as "much stouter and three times as long as those of the anterior ring". Segrove suspected that these cilia are compound structures and that they broke into their component parts when the animal was fixed.

Korn (1958) described the prototroch cilia of Harmothoë

imbricata as forming three groups and being 35μ long, 45-60μ long and 90-95μ long. He depicted two cells, with a vague suggestion of a third, bearing these cilia (Fig. ii). The two cells depicted by Korn both give rise to the longest cilia (40μ here). Korn's diagram lacks three of the cell groups that give rise to the cilia of the prototroch band, and one of the lines of cilia is also missing. (Fig. iii).

The longest cilia supplied by the two central cells form compound groups. The posterior set are also compound and appear to correspond to the short locomotor cilia forming the first row in Pomatoceros. Segrove thought it probable that the two sets of locomotor cilia arise from two rings of large prototrochal cells, but an electron microscopical study may reveal that these cells in fact contribute to the longest cilia as in Harmothoë. The anterior band of short non-compound cilia in Harmothoë has no counterpart in Pomatoceros, and there is no metatroch in Harmothoë, but the lower band in Harmothoë may correspond to the feeding band in Pomatoceros.

In Harmothoë osmium fixation preserves the compound structure of the prototroch cilia in some cases but not others. It is possible, therefore, that compounding or individual activity of the cilia are not necessarily caused by fixation, but each state may occur in the living animal. In a scanning microscope study on the ciliated protozoan Spirostomum, (Boyde and Barber, 1969), ciliary compound groups were

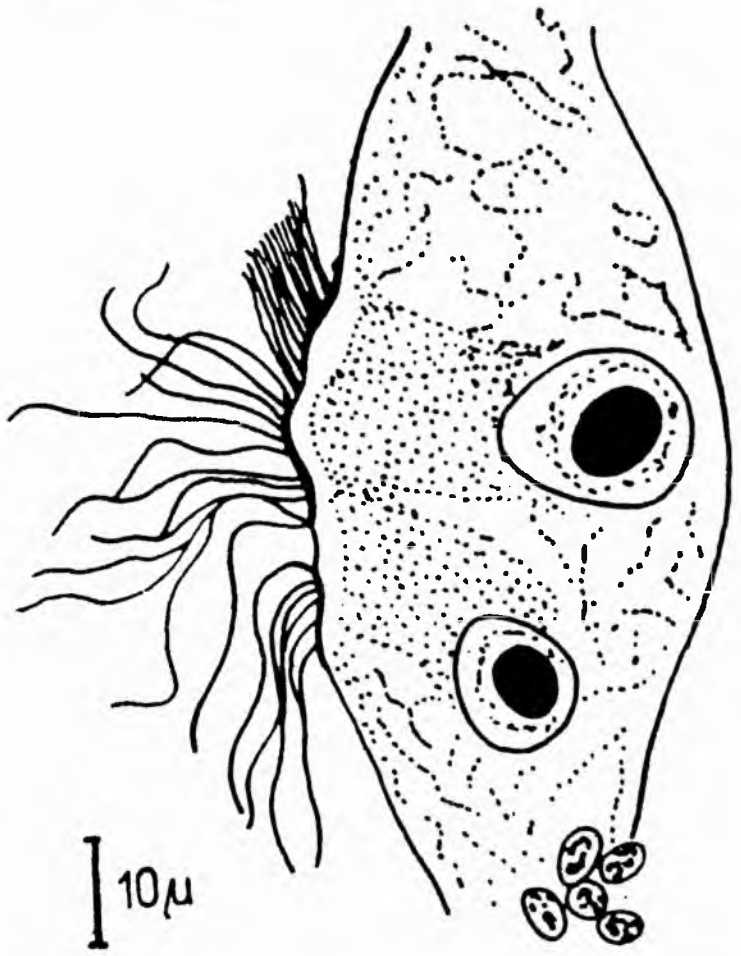
Fig. (ii)

Diagram of the prototroch by Korn (1958). Note that two cells form the basis of the prototroch with a suggestion of a third on the upper side. The relationship of the cilia to the cells is unclear, and the small cilia underlying the prototroch are not shown.

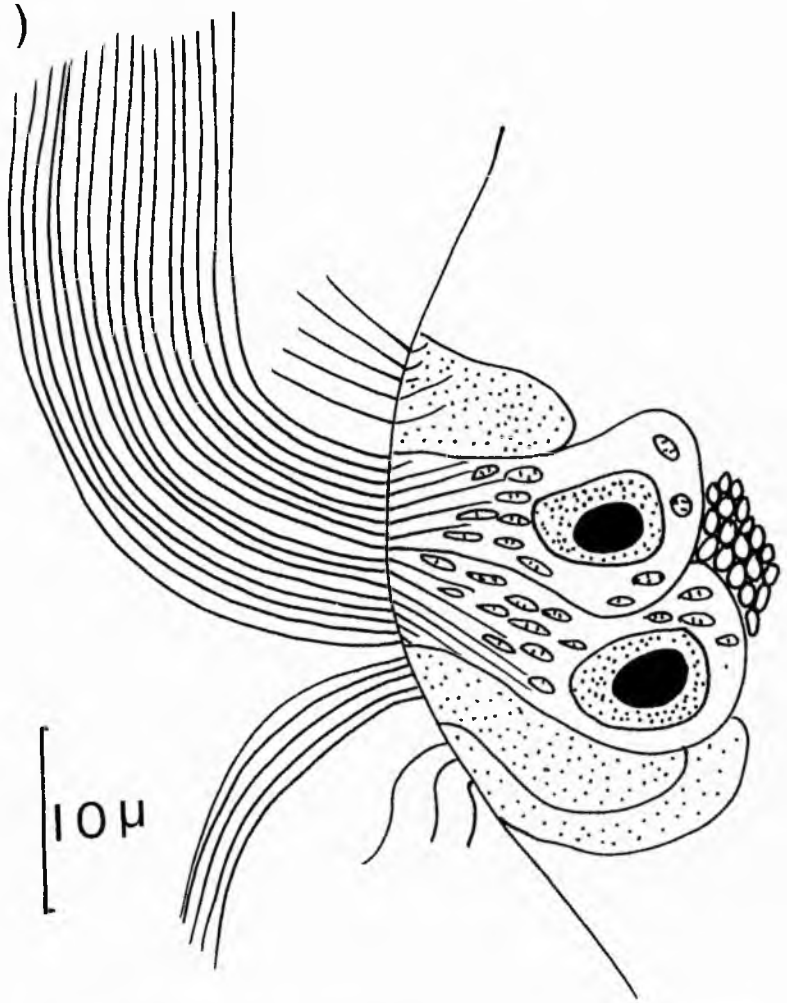
Fig. (iii).

Diagrammatic representation of the prototroch as determined with the electron microscope in this study. The two central cells give rise to the long cilia and the three other cells, one above and two below, each give rise to different sets of cilia. A group of axons runs circularly beneath the prototroch.

(ii)



(iii)



preserved in the fixed, freeze-dried material, but in the unfixed specimens the compound nature was lost. In Segrove's diagram of Pomatoceros, all the cilia are shown in the same phase of beat and it is our finding that when the cilia are in the same phase of beat they are compound. It seems possible that the trochophore may exhibit an "escape reaction" by simultaneous beating of compound cilia.

(iii) Synapses

There are few reports of neurociliary synapses (Horridge and MacKay, 1964; Dilly, 1972) and according to Horridge there are no known examples of transmission by nerves of a wave between ciliated cells. Two mechanisms for metachronal co-ordination have been widely discussed (for references see Sleigh, 1962; Horridge, 1968), and both may occur. These are (1) a neuroid transmission mechanism with stimuli being transmitted through the basal protoplasm and (2) mechanical interaction in which the movement of one cilium may influence the next by the viscous drag communicated through the surrounding water. Normal beat can be triggered by the movement of a neighbouring cilium, and where any cilium can beat spontaneously the fastest-beating cilium, usually the first in a row, becomes the pacemaker.

However, as Sleigh (1962) points out, the presence of a well-developed nervous system in metazoan animals allows a means of controlling the activity of cilia, and he cites various instances where excitatory and inhibitory control are found. Dilly (1972) demonstrated a possible inhibitory effect of nerves on the ciliary rate in Rhabdopleura,

and drew attention to the question of whether transmission is electrical or chemical or a combination of the two. Sleigh (1962) reviewed a number of experiments on the effect of chemicals and, in particular, nerve transmitters on cilia, and would appear to accept that nerve transmitters act directly on the cilia. Horridge (1968), on the other hand, regards membrane potential as the means by which the message crosses from synapses to the cilia.

By whatever means nerves influence cilia or ciliated cells, there is little doubt that they may effect some aspect of control. In the trochophore the diaplectic metachronism means that viscous interaction between beating cilia is not as great as in symplectic metachronal waves, but co-ordination may still take place by simple mechanical means as the cilia are closely arrayed. However, the occurrence of a simultaneous swimming stroke of the cilia is most satisfactorily explained by there being a neurally instigated response. Other aspects of the possible role of nerves in controlling cilia in the trochophore are discussed on page 56.

(iv) Ciliary Roots

Among other functions suggested for ciliary roots, that of anchorage is the most widely accepted (Sleigh, 1962). The difference in the roots of the prototroch and other cilia can be explained by the different stresses that are set up in the cells by the beating of the cilia. With the tendency of prototroch cilia to beat in a compound unit and the arrangement of roots in a splayed pattern, the stresses would be evenly distributed throughout the cell, and lateral stresses at the surface of

the cell reduced. The spacing of the neurotroch cilia and their individual beat requires these to bear a lateral root to take lateral stress, and possibly to help co-ordination of the metachronal wave. The very long, fine root seen on apical cilia may be partly responsible for conduction of the received impulse away from the cilium, a function suggested for the roots of other sensory cilia, e.g. locust scolopale cilium. That the rootlets may also be involved in the direction of products required for metabolism to the cilium is indicated by the tendency for mitochondria to lie closely adjacent to the roots. The high energy requirements of the prototroch cilia are marked by dense aggregations of mitochondria at their roots, in contrast with the relatively poorly supplied apical and neurotroch ciliary areas.

4. The Nervous System

The work of Meyer (1901) on the nervous system of Lopadorhynchus has been regarded as the definitive work on trochophore nervous systems, and has been used in many textbooks. Meyer depicted an arrangement of nerves consisting in three episphere nerve rings, a large prototroch nerve ring and a hyposphere nerve ring, with at least twelve longitudinal nerves in the episphere, somewhat less in the hyposphere. This arrangement has been compared to the turbellarian orthogonal nervous system in discussion on phylogenetic relationships. Åkesson (1967) reviews the work arising through acceptance of Meyer's description and gives a detailed comparison of the work of

Meyer and of

Kleinenberg (1886) who also worked on Lopadorhynchus. Åkesson then gives a fresh account of the nervous system of that trochophore, demonstrating the inaccuracy of the previous work. He suggests that Meyer and Kleinenberg may have described part of the nephridial system as nerves. The nervous system shown by Åkesson arises from six big multipolar cells (as also shown by Meyer and Kleinenberg) and a reticulum of numerous small multipolar cells. There is a prototroch nerve ring, a ring connecting the posterior fibres of the large cells in the episphere, circumoesophageal commissures and a ventral nerve cord. The adult brain rudiment is depicted ventrally.

The larval nervous system of Harmothoë imbricata has not been described previously, although Åkesson (1963) has described the morphogenesis of the head from paraffin sections. The relevant part of that study is the account of the older trochophore with a large portion of the apical region of the episphere filled with cells of the brain rudiment and a central neuropile. These elements are also present in the newly released trochophore, as described here. The number of axons in nerve tracts has never been counted previously, and suggests a degree of complexity previously not visualised. However, the arrangement of nerves in the Harmothoë trochophore is simpler than that described for Lopadorhynchus, as it lacks a reticular system, and has as the predominant element the apical nerve group. The prototroch nerve ring is a well recognised typical feature of trochophores,

and it is useful to have verification of at least one of its functions, innervation by synapses onto the prototroch cells.

5. Surface Opening Glands

Although the glands can be classified into at least two types on the basis of pore size, number of rows of microvilli, and on the appearance of the secretion material, in the conventional system of classification they fall into the same category. Ranvier (1887) set up a classification of glands into three types according to the mode of release of the secretory product. Palay (1958) summarized the classification system as follows: "Holocrine glands release whole cells as secretory substance, apocrine glands lose only the apical parts of the cells, and meocrine glands extrude only the special product". In fact, all the trochophore glands appear to be meocrine, with the release of each droplet taking place by fusion of the droplet membrane with the surface membrane of the cell while only the contents of the droplet are extruded through the pore. Occasionally the droplets seem to retain a membrane, but no breaks in the apical membrane of the cell were observed. Kim, Nasjleti and Hans (1972) observed membrane-bound droplets in the lumen of the rat sublingual gland, and on stimulation of the gland demonstrated total release of stored droplets leaving only a row of vesicles retaining the cytoplasm of the cell. Goblet cells also exhibit this type of release of mucus (Hollman, 1963; Neutra and Leblond, 1966),

and it is possible that under certain conditions the trochophore may release all the stored mucus in the same way. The release of secretion by coalescence of the limiting membrane of secretory droplets with the plasmalemma is characteristic of pancreatic and other cells (Ichikawa, 1965 and others), and this appears to be the usual situation in the trochophore.

The different appearance of the secretion material is a deceptive feature in distinguishing between glands, for smaller, darker, granules can be developmental stages leading to the large clear granules. Gupta and Little (1970) studying pogonophores found mucous gland cells with droplets of "spongy or honeycomb" appearance alongside droplets with dense homogeneous content, and suggested that the former were developmental stages of the latter. In the trochophore, droplets of honeycomb appearance are found alongside large, pale droplets at the pore opening in Type II glands. As in the pogonophore, the droplets of honeycomb appearance may be immature forms of the larger droplets. Although the largest droplets are pale, and not dark like those in the pogonophore (Gupta and Little, 1970), they resemble mucous droplets described elsewhere, particularly the mucoprotein of the goblet cell in the vertebrate intestinal epithelium (Ito and Winchester, 1963; Trier, 1963; Hollman, 1963).

The gland opening is similar to that found in the mucus-secreting glands of enteropneusts (Nørrevang, 1965) and of

goblet cells. Where there is only one ring of microvilli there is also a resemblance to choanocyte structure of sponges (Fjerdingstad, 1961). The trochophore glands are not surrounded by cells bearing microvilli; and in the trochophore the extension of cuticle gives additional support. The projection of the gland mouth so that the secretion is released above the level of the cuticle assists the spread of the secretion. Nørrevang (1965) observed that this may be a possible function for the gland "chimney" of the enteropneust buccal cavity mucous glands.

The arrangement and relationship of the rough endoplasmic reticulum and the Golgi apparatus is typical of many types of gland. There has been much debate over the role of the Golgi apparatus, reviewed by Palay (1958), and the evidence then as well as the growing body of information since indicates that the Golgi packages material produced on the endoplasmic reticulum. Tandler and MacCallum (1972) illustrate vesicles from the Golgi apparatus fusing with a condensing vacuole in a serous cell of the hedgehog submandibular gland.

The sheath around the mature gland resembles the theca of goblet cells, which Palay (1958) suggests is made up of compressed ergastoplasm. However, in the trochophore a tangential section indicated that the theca is at least in part composed of microtubules. Coleman (personal communication) has found glands with microtubular sheath in the leech, and Dorsett and Hyde (1970) report that the epidermal glands of Nereis are surrounded by an array of longitudinally oriented microtubules.

6. The Cuticle

In Grassé (1959) adult polychaete cuticle is said to be derived in part from mucous cells in the epidermis. The larval cuticle resembles the superficial zone of the cuticle of Syllis described by Boilly (1967) and the epicuticle of the adult Harmothoë (Lawry, 1967). In substance it is also like the epicuticle of Platyneris dumerilii (Brökelman and Fischer, 1966), although the form of the Platyneris cuticle differs in having deep folds. It also lacks superficial granules. It appears that growth of the cuticle takes place from cells immediately beneath. Clément (1969) suggests that pores through the rotifier cuticle are for secretion of cuticle through the external medium, but the rotifier cuticle has the osmophilic line on the inside and appears less dense superficially. There is therefore little reason to suppose that a similar process takes place in trochophores. The trochophore cuticle resembles more the cuticle of the pogonophore tentacular crown (Gupta and Little, 1969), the only differences being lack of the osmophilic line in the pogonophore and a thicker fibrous layer. Similar dense granules are on the surface of the pogonophore cuticle, and also microvilli with a dense cap.

Annelids respire through the body surface, and as the cuticle of the adult is thick and that of the trochophore and the epicuticle of the adult is of relatively impermeable appearance, it is

suggested that the microvilli perform a respiratory function. The increased numbers of microvilli in ciliated cells with high-energy requirements supports this view. However, their presence in high density at the anus could be accounted for if increased numbers of microvilli simply increase the flexibility of the cuticle. The suggestion of respiratory function still holds for this area as muscles are present just below the cuticle at this point. Respiratory function is therefore the most likely role of the microvilli. It is interesting to note that in certain insects (Noirot and Noirot-Timothée, 1969), there are no microvilli in the proctodael cuticle, but in regions of maximum permeability the dense, epicuticular zone is reduced or indented to sufficient depth to give the appearance of canals.

7. The Digestive System

Most authors on trochophores have recognised the presence of cilia in the digestive tract, and Rasmussen (1956) comments on two lengths of cilia in the stomach: "The stomach ciliation consisted of short fine cilia apart from one small part of the hindmost wall which has a bundle of long cilia". This agrees with findings here except that there was more than one area with long cilia. Rasmussen also stated that the oesophagus did not appear to open into the stomach and that the anus did not open at this stage. During this study living trochophores were frequently observed defecating and food

boluses could be seen turning in the stomach, indicating that Rasmussen's findings do not apply to Scottish Harmothoë trochophores.

Rasmussen (1956) described the oesophagus as a rather thick-walled duct. This appearance is probably given by the thick mass of cilia, or at least by the lateral ciliary roots, which, as they interdigitate, form quite a dense array. The gullet is, of course, also lined with cuticle, although this is thin.

(i) Gullet cilia

The cilia of the gullet with expanded membranes and additional fibres resemble cilia reported by Ling (1969, 1970), in the cephalic organs of the nemertine Lineus ruber. In his first account (1969), Ling described the cilia as having a dilated membrane and a cytoplasmic content of similar electron density to that of the cytoplasm of the cell body. He stated that three fixation methods had been used, but did not indicate which fixation was shown in the illustrations. From fixation studies conducted on trochophores it would appear that the glutaraldehyde fixation furnished Ling's illustration in this early work. In his 1970 reports Ling makes no particular comment on these specialized cilia, but his illustrations, obtained apparently (by our assumption) from the first of his two fixation methods, clearly show cilia with additional filaments. Ling observed in 1969 that there was cohesion between the membranes of the cilia and regarded this set of cilia as forming a septum between the

halves of the canal. He made no observations on the derivation or function of the additional filaments.

In the trochophore, the fusion of membranes is identical to that of the cilia of the nemertine, the central filaments are nearly always uniformly oriented, and the basal feet point in the same direction. The cilia thus beat in the same direction, and the fusion of membranes indicates simultaneous activity of the groups so that a valve action is possible.

The "thin" vesicular area of the stomach wall underlying the cilia is such that an increased pressure in the stomach would immediately be transmitted to the cilia. The resulting deformation of the cilia might stimulate their activity. An alternative mechanism could be a stretching effect on the cell bearing the cilia as these cells lie in the vesicular tissue. Vesicular cells in the nematode, superficially similar in appearance, are not associated with the expanded cilia and perform a phagocytotic function (Ling, 1970).

The development of the additional filaments of these expanded cilia appears to take place by the same mechanism as the development of accessory fibers in insect sperm as described by Cameron (1965) and Kiefer (1970). The process which these authors illustrate is one of budding from the b-subfibre extending the length of the subfibre so that the accessory fibre forms in a manner equivalent to rolling a sheet into a tube. The function of the fibres in the trochophore cilia

is probably to maintain the shape of the expanded membrane and give the whole cilium more overall rigidity. Many authors have suggested a skelet function for microtubules (Byers and Porter, 1964; Ledbetter and Porter, 1963; Newcomb, 1969; Porter, 1966; Tilney and Gibbons, 1969 and others). Porter (1966) and Fawcett (1966), Macgregor and Stebbings (1970) suggest that the microtubules form channels in which parts of the cytoplasm move. The base of the cilium is only as wide as the axoneme, ruling out a channel-forming function and leaving the more obvious skeletal function for microtubules of the trochophore expanded adult cilia. Whether these microtubules play a part in the movement of the cilia is unknown.

(ii) The Stomach

Although little is known of the detailed histology of the gut of the adult Harmothoë imbricata, some polychaetes have been studied (see Dales, 1967 for review) and in at least one, Clymenella torquata, the intestine is divisible histologically into four regions. The functions of the regions are not fully known, but from the structure two types of secretory cells are identified, cells which are secretory, have a brush border and so may also be absorptive, and purely absorptive cells. The stomach is frequently found to be a storage cavity, unciliated. In the Harmothoë trochophore; digestive processes take place mostly in the stomach, where specialized purely secretory cells are found. The dense granules in all the stomach cells are probably

digestive enzymes. The cilia stain more darkly than the gullet cilia and this different reaction could be the result either of a difference in vivo through adaptation of the stomach cilia to the environment of digestive fluid, or an effect during fixation caused by interaction of the digestive fluid with the fixative. The microvilli that are found in the stomach, and on some cells in the form of a brush border, indicate that absorption is taking place here too.

(iii) The Intestine

The intestinal cells with brush border, cilia and occasional secretory granules are clearly multi-functional, absorptive, food-moving and also contributing to digestive processes. The cells are all similar.

The vesicles and vacuoles are suggestive of pinocytotic and phagocytotic vacuoles. Many authors have previously thought that the uptake of lipid in the intestine was in the form of small particles by pinocytosis, and possibly also partly as soluble products of hydrolyses (Palay and Karlin, 1959; ~~Wotton, 1963;~~ Napolitano and Kleinerman, 1964; Strauss, 1964). However Porter (1969), discussing the independence of fat absorption and pinocytosis, concluded that absorption takes place through the microvilli. Where there are microvilli providing a large surface area this is the obvious pathway. The vesicles, then, may be related to retained membrane after the secretory granule is released, in the same way that small vesicles are involved in membrane retrieval in neuro-secretory axons (Smith, 1970). The vacuoles may contain an

incomplete collection of secretory material.

8. Muscular System.

The muscles of the trochophore, while considerably smaller, have the typical structure of invertebrate muscle (Hansen and Lowy, 1961). The striated muscle thick myofilaments with their dense outer ring are like the myofilaments of circular muscles of the earthworm (Mill and Knapp, 1970a). The thick myofilaments of the longitudinal muscles of the earthworm have a uniformly electron-dense appearance, while those of the circular muscles have an outer granular ring surrounding a lighter core of a dense granule in the centre. The dense granule is lacking in nearly all the thick myofilaments of the trochophore but may be seen in a few. The significance of these differences in thick myofilaments is unknown.

The structure of the neuromuscular junction of the trochophore is similar to that of the earthworm as described by Mill and Knapp, (1970b), except that the 300Å gap is more similar to the synaptic 400-500Å cleft of vertebrate twitch and slow muscle fibres (Birks, Huxley and Katz, 1960; Pilar and Hess, 1966), and the 500Å synaptic cleft of the Nematode Ascaris (Rosenbluth, 1965). The trochophore synaptic cleft contains a basement membrane as seen in the Lumbricus cleft, but the Lumbricus cleft is 850-1200Å. Among other invertebrates the synaptic cleft is the narrower, 50Å to 200Å across. (See review table, Mill and Knapp, 1970b). Basement membranes are present in vertebrate

synaptic clefts, and appear to occur less often in invertebrates.

Multiterminal and polyneuronal innervation has been indicated in adults of the polychaetes Nereis and Harmothoë (Dorsett, 1963, 1964; Horridge, 1959) but so far it has not been possible to determine whether this is the condition in the trochophore. Dual innervation appears to be the means by which fast and slow contractions of muscles are effected. (Dorsett, 1964; Horridge, 1959; Mill and Knapp, 1970). It seems unlikely that the trochophore muscles have more than one nerve ending, and variation in muscular activity would appear to be unnecessary where the muscles are apparently not contributing in any significant way to locomotion.

9. Protonephridium

The simple larval protonephridium, with one or two solenocytes opening into an intracellular duct, is of a form well known in the literature (see review, Goodrich, 1945). Electron microscopical studies on invertebrate excretory organs are still relatively few. Kümmel and Brandenburg (1961) compared various choanocytes and solenocytes using the electron microscope, but were more interested in gross comparison than the details of fine structure. All their illustrations are diagrams with apparent modifications of features, for example the rods are rather oddly portrayed as rectangles or triangles. They show 17 rods in the solenocyte of Glycera and 10 in that of Branchiostoma, whereas the Harmothoë trochophore always has 15.

Where the fine structure of rods of excretory organs are shown, they are dissimilar to those of the trochophore solenocyte. The tube of the flame cell of the rotifer (Mattern and Daniel, 1966) is supported by rods which bear striations like ciliary roots; that of the fish tapeworm *Diphyllobothrium latum* (von Bonsdorff and Telkkä, 1969) has two rows of rods, an inner row of nearly circular ones with a dense peripheral row of elongated oval rods with a dark membrane, clear space and electron-dense core. The granular tubular contents of the trochophore rods correspond more closely to choanocyte structures. The choanocyte of a sponge described by Fjerdingstad (1961), and the choanocyte of an enteropneust described by Nørrevang (1964), both consist of a flagellum surrounded by a collar of rods which Nørrevang calls microvilli. The membranes of the microvilli are separate in both types of choanocyte, whereas in the solenocyte the membranes are found to be continuous with the standard osmium fixation, separate with higher osmolarity osmium, glutaraldehyde and acrolein.

In the choanocyte and the trochophore solenocyte, the rods are linked by fine 40Å fibrils. The enteropneust choanocyte and the trochophore solenocyte both have projections of these microfibrils into the lumen of the tube. Nørrevang (1964) suggested that the substance of the microfibrils is mucus and that the inward projection indicates an inward sweep of water between the microvillous rods. As the trochophore rods are on the whole interlinked, flow between them cannot be great. Also,

filaments often link the central cilium with the rods. It is therefore unlikely that these filaments are mucus arranged under the influence of flow. However they bear some resemblance to the mucopolysaccharide on the surface of intestinal villi (Fawcett, 1966).

The thin tube of cytoplasm around the solenocyte, separated from the solenocyte by large vacuoles, appears to perform pinocytosis. Thin folds of tissue are occasionally found arched to enclose parts of the blastocoel.

The septate desmosomes which characterise the cell junctions on the lumen side of the protonephridium are a permeability barrier, a function first suggested for septate desmosomes by Wood (1959), and for the desmosomes in planarian protonephridium (Pederson, 1961). Since then, studies using the extracellular tracer ruthenium red indicate that the septa do not seem to be impermeable (Gilula and Satir, 1969; White and Walther, 1969; Leik and Kelly, 1970). More recently, Hand and Gobel (1972), using the extracellular tracers lanthanum oxide and ruthenium red, showed that septate junctions possess barrier properties, and suggested that as ruthenium red stains acid mucopolysacchrides, it may be staining components of the intercellular space rather than inertly filling a potential space in the lattice. Extracellular tracers have not been used in the trochophore, but septate junctions occur in positions where a permeability barrier would be required. It is notable that cell junctions at the periphery of the protonephridium are not of the septate type.

10. Visual systems

(i) Larval eye.

The larval eye of the trochophore of Harmothoë is fundamentally similar to the eye of the trochophore of Neanthes succinea, described by Eakin and Westfall (1964). Both trochophores possess a pigment cup containing receptor cell processes consisting of an outer layer of microvilli and an inner layer of membranes. Eakin and Westfall state that the structure in Neanthes consists of two layers of microvilli, the microvilli of the inner layer being narrower than the microvilli and in a parallel array. Their illustrations show that the inner layer of microvilli consists of groups of approximately four membranes running in different directions into the cell and interspersed with mitochondria and other cytoplasmic inclusions. These would therefore seem to be infolded membranes rather than microvilli. In some respects the membranes resemble the "furrows" of invaginated membrane at the base of the rhabdomeres in the Sapphirina nauplius retinular cell (Elofsson, 1969), the nauplius eye membranes differing in being more closely packed and penetrating deeper into the receptor cell. In the trochophore the membranes associate with endoplasmic reticulum and may function as channels to increase the efficiency of transport of visual pigment to the microvilli, or may even participate in the visual process.

Eakin and Westfall (1964) stated that the continuity of the inner and outer retinular layers is not easily demonstrated, and

this is also the case with the Harmothoë trochophore, although interconnections were able to be demonstrated in this study. The difficulty pointed out by Eakin and Westfall arises because the infolded membranes lie in groups with spaces between the groups, with no correlation to the regular size and spacing of the microvilli, so that folds of membrane come to lie horizontally beneath the bases of microvilli.

(ii) Supplementary visual organelle.

The unusual structure situated mid-apically is assumed to be a photoreceptor because it possesses a mass of compacted cilia and microvilli affording increased membrane surface area in a manner characteristic of visual organelles. Its situation and particular resemblance to previously described invertebrate eyes support this functional interpretation.

Dhainaut-Courtoise (1965) reported a photoreceptor of ciliary origin in the brain of Nereis pelagica, while the normal eyes are probably of rhabdomeric type as are those of Nereis virens (Dorsett and Hyde, 1968). Krasne and Lawrence (1966) showed that the polychaete Branchiomma vesiculosum has eyes of ciliary origin, although the structure bears little resemblance to the organelle in the trochophore. Marked similarity is seen, however, between this trochophore eye and the eye of the oligochaete, Lumbricus terrestris (Röhlich, Aros and Virágh, 1970) which contains both cilia and microvilli. Recently, a

"ball-like" mass of non-motile cilia has been described as a potential photoreceptor in the larva of the bryozoan Bugula neritina by Woollacott and Zimmer (1972). More significantly, Golding in a personal communication has shown that identical, although larger, structures to those of the trochophore occur in the brain of the adult Harmothoë imbricata.

Sensory cilia usually have a 9 + 0 configuration and have this form in the photoreceptors of a wide range of invertebrates: the mollusc Pleurobranchis pileus (Horridge, 1964) the mollusc Cardium edule (Barber and Wright, 1969), the polychaete Branchiomma vesiculosum (Krasne and and Lawrence, 1966), the oligochaete Lumbricus terrestris (Röhlich, Aros and Virágh, 1970), and others. But 9 + 2 sensory cilia occur in the supposed light-sensitive organelle of Littorina (Charles, 1966), Aplysia (Hughes, 1970), Onithochiton neglectus (Boyle, 1969) and Bugula neritina (Woollacott and Zimmer, 1972). Eakin and Brandenburger (unpublished, quoted by Eakin, 1972) found 9 + 2 sensory cilia in the eye of the hydromedusan Polyorchis penicillatus and although Eakin (1972) regards the 9 + 2 pattern as rare, a 9 + 0 configuration would not appear to be a necessary criterion for visual cilia.

The question of whether the eye membranes are of ciliary or microvillous origin became of considerable interest when Eakin suggested in 1963 that the annelid-arthropod-molluscan line of animals have the photoreceptor organ derived from microvilli, and the echinoderm-

coelenterate-vertebrate line of animals have photoreceptor membranes derived from cilia. Exceptions to this scheme have been found among the invertebrates and are reviewed by Eakin (1965), Krasne and Lawrence (1966) and Laverack (1968). Eakin makes no mention of his original scheme in his latest review (1972).

Where two types of photoreceptor occur in the one animal, as in the trochophore, the adult Harmothoë (Golding, personal communication), Pecten (Barber, Evans and Land, 1967), Onithochiton (Boyle, 1969), Nassarius (Crisp, 1972, and in a personal communication) difference of function of the two types of organelle is of more interest than implications for phylogeny.

Electrophysiological evidence from Pecten shows that the photoreceptors differ in function. Recordings from Pecten were first made by Hartline and Graham (1938). Land (1966) showed that the distal, ciliary photoreceptor gives an "off" response and that the proximal, microvillous photoreceptor gives an "on" response. Gorman and McReynolds (1969) and McReynolds and Gorman (1970a and b) obtained intracellular recordings showing that the distal "off" receptors give a hyperpolarizing response to light, whereas the proximal "on" receptors give a depolarizing response. Vertebrate photoreceptors, derived from cilia, are hyperpolarized by light (see Tomita, 1970 for review), and so are ciliary photoreceptors of some Ascidians (Gorman, McReynolds and Barnes, 1971)

but the latter authors have also found a hyperpolarizing microvillous photoreceptor, in Salpa democratica. In attempts to record from the trochophore, "off" responses were obtained in the region of the ciliary photoreceptor but the size of the animal prevented accurate localization of the organelle and success in repetitions of the experiment. The finding of a similar, larger organelle in the adult invites an electrophysiological investigation.

Because of the paucity of electrophysiological work in this area, discussion has tended to centre around behaviour. Barber and Land (1967), Land (1968) and Dorsett and Hyde (1968) point out that shadow reflexes and the habit of retreating into darkness for safety are characteristic of animals with ciliary photoreceptors, including among annelids and molluscs Branchiomma vesiculosum (Krasne and Lawrence, 1966), Pecten maximus (Land, 1966), Cardium edule (Barber and Land, 1967), and in addition to these Onithochiton neglectus (Boyle, 1969). Crisp (1971) described structures derived from whorled microvilli and cilia on the siphon tip of Nassarius reticulatus and in 1972 reported that removal of this structure interfered with the animal's response to shadows. A shadow response in a trochophore is hardly appropriate and the ciliary organelle in the trochophore may perform the function of reversing the photopositive response prior to settling.

In a personal communication, Dr. Golding of the University of Newcastle Zoology Department said that he is working on the hypothesis

that the ciliary photoreceptor in the brain of the adult Harmothoë imbricata may be connected with the neurosecretory cells and be associated with the reception of light for rhythms.

Shadow reflexes and rhythms are common features among invertebrates. Where ciliary photoreceptors occur in addition to rhabdomeric photoreceptors they are not always adjacent, particularly in annelids where ciliary photoreceptors appear to lie in the brain (Dhainaut-Courtoise, 1965; Golding, personal communication). So far there has been little effort to find more than one type of photoreceptor among invertebrates, and few electrophysiological studies. This is a field where further study is required to resolve the questions of function and why two types of photoreceptor should occur in one animal.

10. The Adult Eye.

Although metamorphosis was not observed, the relationship of the adult eye to the larval eye can be discussed in terms of straightforward morphology. The adult eye is so much larger than the larval eye that separate growth is logical, although with its rhabdomeric form, the larval eye could be accommodated into the adult eye. The main modification required would be a change in the shape of the sensory cell such, that the process bearing microvilli elongated, narrowed, and grew microvilli all around at right angles to the axis.

After a fairly long period of stability, it is unlikely that the larval receptor cell would undergo these changes. In fact, Rasmussen

(1956) clearly indicated that the larval eyes disappear during metamorphosis.

Electron microscopical studies on the eyes of polychaetes have been limited to a few families. The eyes of Nereids have been described by Eakin (1963), Fischer (1963), Eakin and Westfall (1964), Fischer and Bröckelmann (1966), Dhainaut-Courtoise (1965), Dorsett and Hyde (1968); the eyes of one Opheliid have been described by Hermans and Cloney (1966), Hermans (1969); those of a Glycerid by Manaranche (1968) and of Sabellids by Krasne and Lawrence (1966) and Kerréis (1966, 1968).

The photoreceptors so far described among adult polychaetes are varied in details such as lens structure, arrangement of microvilli, whether the receptor cells contain pigment or not, etc., but except for the ciliary photoreceptors of the Sabellids Branchiomma vesiculosum (Krasne and Lawrence, 1966) and Dasychone bombyx (Kerréis, 1968) and the receptor in the brain of Nereis pelagica (Dhainaut-Courtoise, 1965) they are fundamentally similar in form. In all there is a pigment cup surrounding receptor cell processes from which a dense array of microvilli project. The receptor cell bodies lie beneath or among the pigment cell bodies and the eyes are of converse type. The pigment cells have extensions through the layer of receptor processes and give rise to a lens or vitreous body. The eye of Armandia brevis (Hermans and Cloney, 1966), is simpler in form and of inverse type, resembling the larval

eye of the Harmothoë trochophore. Hermans and Cloney (1966) compare the Armandia eye with the developing eye in Neanthes succinea (Eakin and Westfall, 1964) on the basis of the simplicity of both eyes and the similarity of the diaphragm of Armandia to the vitreous body of Neanthes, although the Neanthes eye is of converse type.

In fact the substance of the diaphragm of Armandia is of different appearance to that of Neanthes, just as the lenses in other annelids have various internal structures. In Nereis vexillosa and Platynereis dumerilii the lens is found from granules 0.1μ to 0.2μ in diameter (Eakin, 1963, Fischer and Brökelmann, 1966). In Nereis virens the lens is formed from arrays of rods or filaments, 500Å in diameter, which run a parallel but convoluted course through the lens (Dorsett and Hyde, 1968). The sedentary polychaetes Branchiomma (Krasne and Lawrence, 1966) and Dasychone (Kerneis, 1968) have lenses composed of closely packed vesicles mostly 0.2μ in diameter but some up to 0.5μ, with contents of varying electron density. It is therefore not surprising that the lens in Harmothoë is entirely different from all of these, consisting of irregularly massed, 200Å tubules.

The presence of pigment granules in the receptor cells of Harmothoë resembles the condition in Platynereis. Although this is exceptional in the known polychaete eyes and in other annelids (Hansen, 1962, Röhlich and Török, 1964), the number of annelid eyes so far described is still relatively small. Pigment occurs in sensory cells

in other invertebrate eyes, notably in Peripatus (Eakin and Westfall, 1965).

It is of interest that the microvilli of the receptor structures in Peripatus are like those of Harmothoë in dimensions and "test-tube brush" arrangement. Eakin (1965) in his work on evolution of photoreceptors reviewed the arrangement of microvilli in various invertebrate rhabdomeric receptors. Leeches, arthropropods, cephalopods, Peripitas, and flatworms were said to have straight lateral villi, whereas in Nereids they were supposedly intertwined (see Eakin, 1965, for references). This scheme proved not as clear cut as then represented, as MacRae (1966) described the eye of the flatworm Notoplana acticola as having bent, tortuous microvilli, and the microvilli of Nereis virens are straight (Dorsett and Hyde, 1968). Microvilli in other annelids are also straight (Hermans, 1969). Eakin (1968, 1972) now stresses the variety of forms of extensions from both cilia and microvilli in invertebrate eyes.

No cilia, or remnants of cilia, were seen in the adult eye of Harmothoë, such as are seen in other polychaetes (Eakin, 1963; Fischer and Brökelmann, 1966; Dorsett and Hyde 1968), in Peripatus (Eakin and Westfall, 1965) and in other invertebrates (Boyle, 1969; Eakin and Brandenburger, 1967), but in general it is thought that these cilia or rudiments play no role in photoreception (see Hermans and Eakin, 1969, for discussion).

II. THE FINE STRUCTURE OF THE DEVELOPING TROCHOPHORE

Methods

Trochophores were extracted from beneath the elytra of incubating worms by means of a pipette and fixed for 30 minutes in a volume of 2% osmium tetroxide equal in volume to the trochophores in sea water. No buffers were used. After washing, the animals were dehydrated in graded acetone solutions and left overnight in a mixture of 100% acetone and epon. The following day they were transferred to fresh acetone and placed in the oven at 37°C for 24 to 72 hours. They were sectioned, mounted, stained and examined as described in Chapter I. Serial sections were not made specifically for light microscopy, but for each grid made, a group of sections were collected and placed sequentially on slides, making a fair approximation to serial sectioning.

The earliest stages were not examined using the electron microscope, but sections were cut for light microscopy.

Results

1. Gastrulation

The cell divisions in the Harmothoë trochophore result in macromeres which at the time of gastrulation are more than twice the size of the micromeres. The macromeres have more darkly staining contents than the micromeres because they contain more yolk granules. At the time of invagination to form the gastrula, the cells of the

future endoderm become pear-shaped with their nucleii at the inner, swollen end. At the same time the ectoderm grows around the narrow end of the pear-shaped cells, finally enclosing them. The invagination therefore appears to be by a combination of emboly and epiboly.

2. Cell Structure

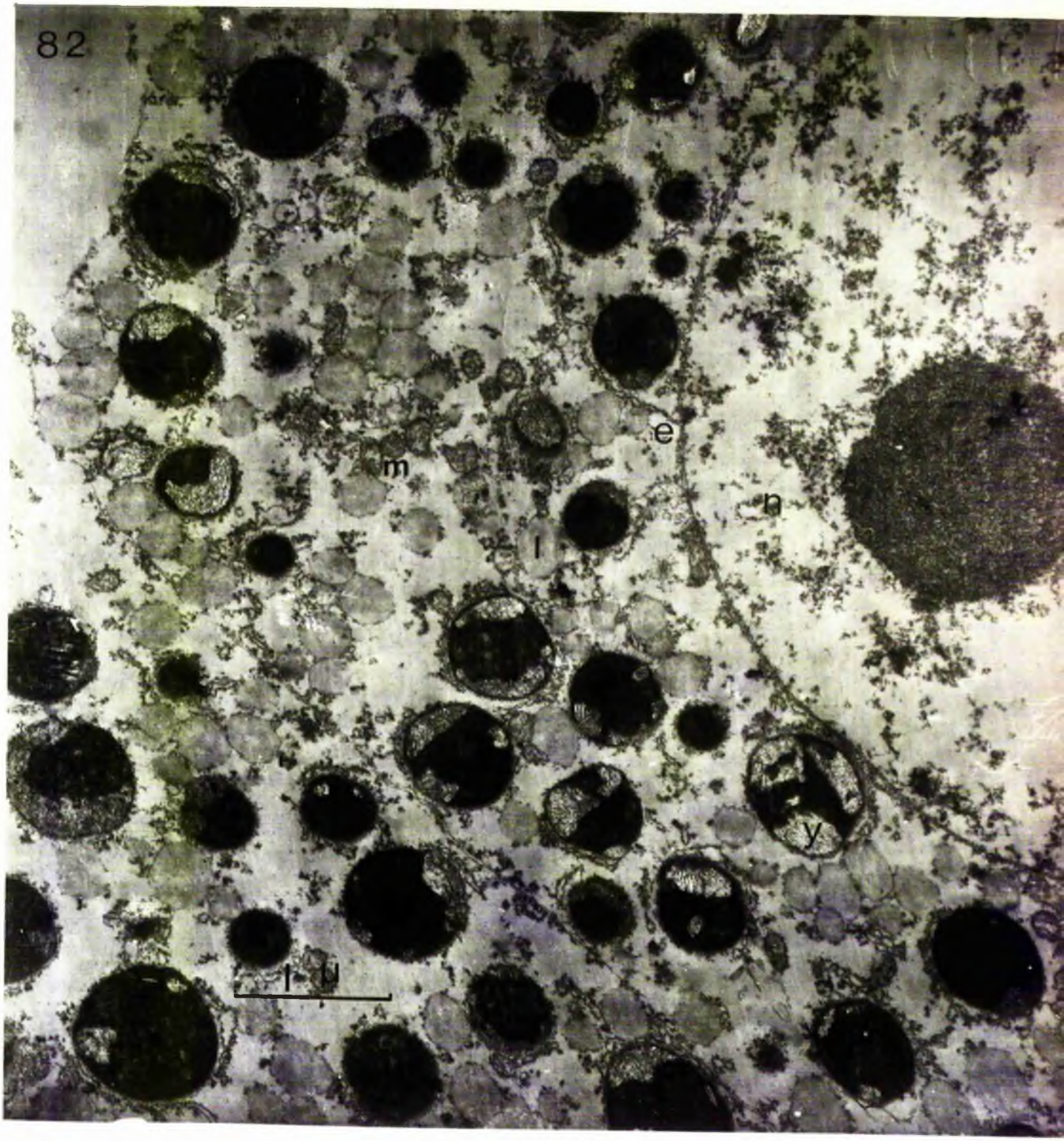
Electron microscopy reveals that the cells of the 5-day-old trochophore contain quantities of yolk and lipid granules, sparse cytoplasm, no Golgi bodies and very small mitochondria, 0.1μ compared with 1μ in the fully formed trochophore (Fig. 82). The lipid droplets are 0.1μ to 0.2μ across, and the yolk granules are up to 0.5μ across. Cells of the ectoderm are characteristically less yolky than those of the endoderm throughout development and lose their yolk by the 12th to the 14th day. Free swimming trochophores are frequently found with yolk still present in the endoderm. The small lipid granules of the developing trochophore are not found in the fully formed trochophore, although after feeding commences, lipid is found in large droplets in the intestinal cells.

Six-day old trochophores lack desmosomes, but the cells frequently appear to be joined by tight junctions. Peripheral cell membranes, i.e. adjoining the cuticle, are undifferentiated, and do not become distinct until the 7th day. Desmosomes appear on the 9th day.

Small strands of endoplasmic reticulum, some 0.4μ long, with a narrow, irregular cisternal space are found in the cytoplasm

Fig. 82.

Cell of a 5-day-old trochophore showing large amounts of lipid, l, yolk, y, nucleus, n, small strands of endoplasmic reticulum, e, and small mitochondria, m. Note the distribution of endoplasmic reticulum, in small projections all around the nuclear membrane and around the yolk granules.


82
e
m
n
l
y
1 µ

of more mature cells. These strands may be seen attached to and perhaps budding from the nuclear membrane, and partially surrounding the yolk granules, which are themselves membrane-bound (Fig. 82). Ribosomes are attached to these endoplasmic reticulum strands, but are more widely and irregularly spaced than as normally found on rough-surfaced endoplasmic reticulum. The micrograph, Fig. 82 is from a 5-day-old trochophore and the yolk granules can be seen to be losing their contents.

Dividing cells are in evidence and figure 83 shows a chromosome-bearing spindle with a divided centriole at the apex.

3. Nuclear Pores

The nuclei of recently divided cells have membranes characterised by distinct pore complexes (Fig. 84), which at a later stage disappear, leaving a simple nuclear membrane perforated by few pores. The pores are 0.1μ in diameter to the outer edge with an 500Å to 600Å pore and a central plug. A uniform basement membrane 0.1μ deep lies between them. High concentrations of free RNA particles lie outside the nuclei .

4. Development of Cilia.

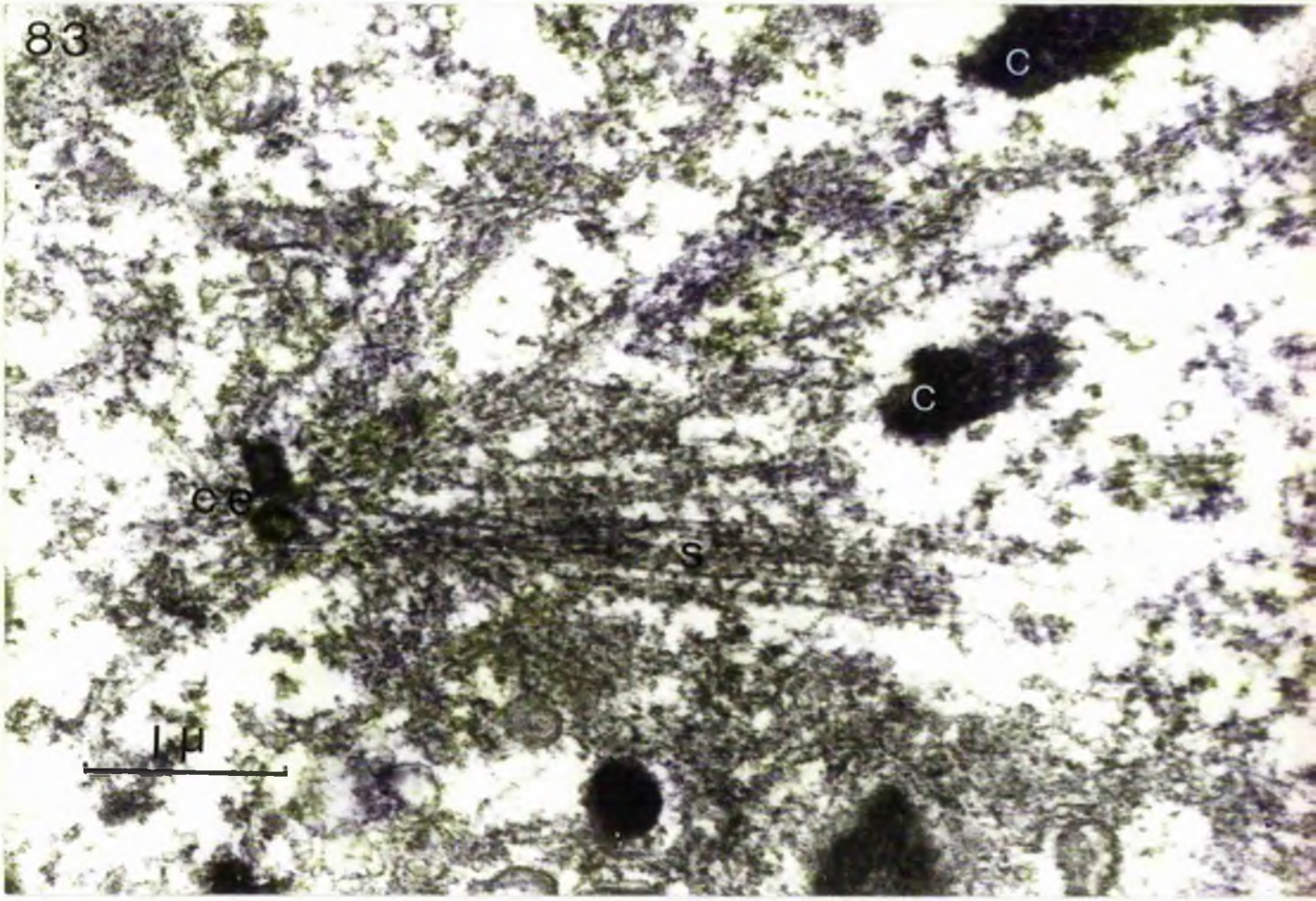
The formation of cilia in all cells which will bear cilia (including the gut and protonephridium), is preceded by development in the cells of oval, electron-dense bodies roughly 0.3μ broad to 0.6μ long. These bodies have a membrane derived from the Golgi apparatus and have a uniform, moderately fine granular matrix (Fig. 85). They are

Fig. 83.

Spindle, s, of mitotic cell. On the left side are the centrioles at right angles, ce, and on the right, chromosomes, c. 6-day-old trochophore.

Fig. 84.

Annulae, a, of nucleii of recently divided cells. 6-day-old trochophore.


83
C
C
ce
S
1μ

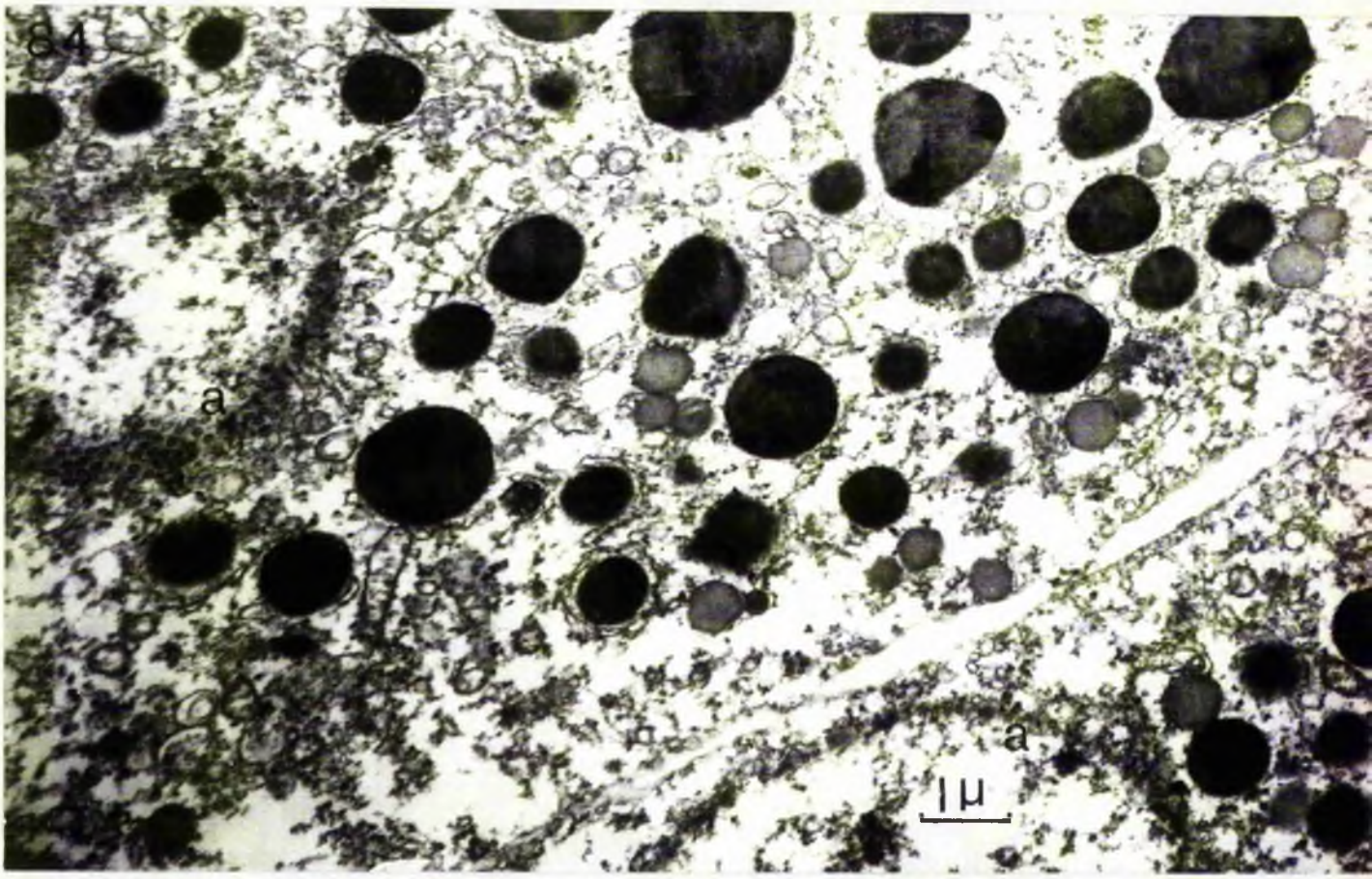
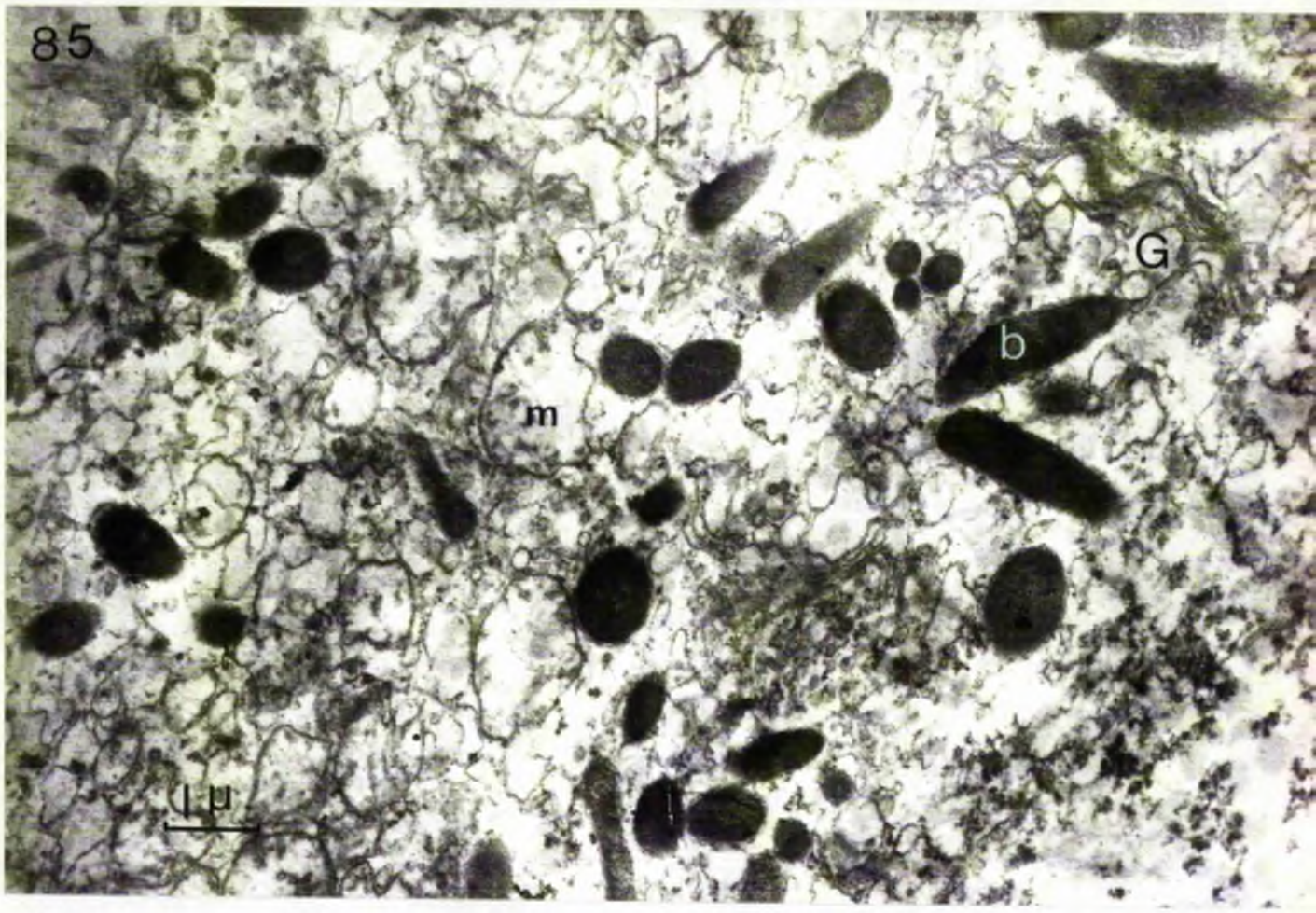

84
a
a
1μ

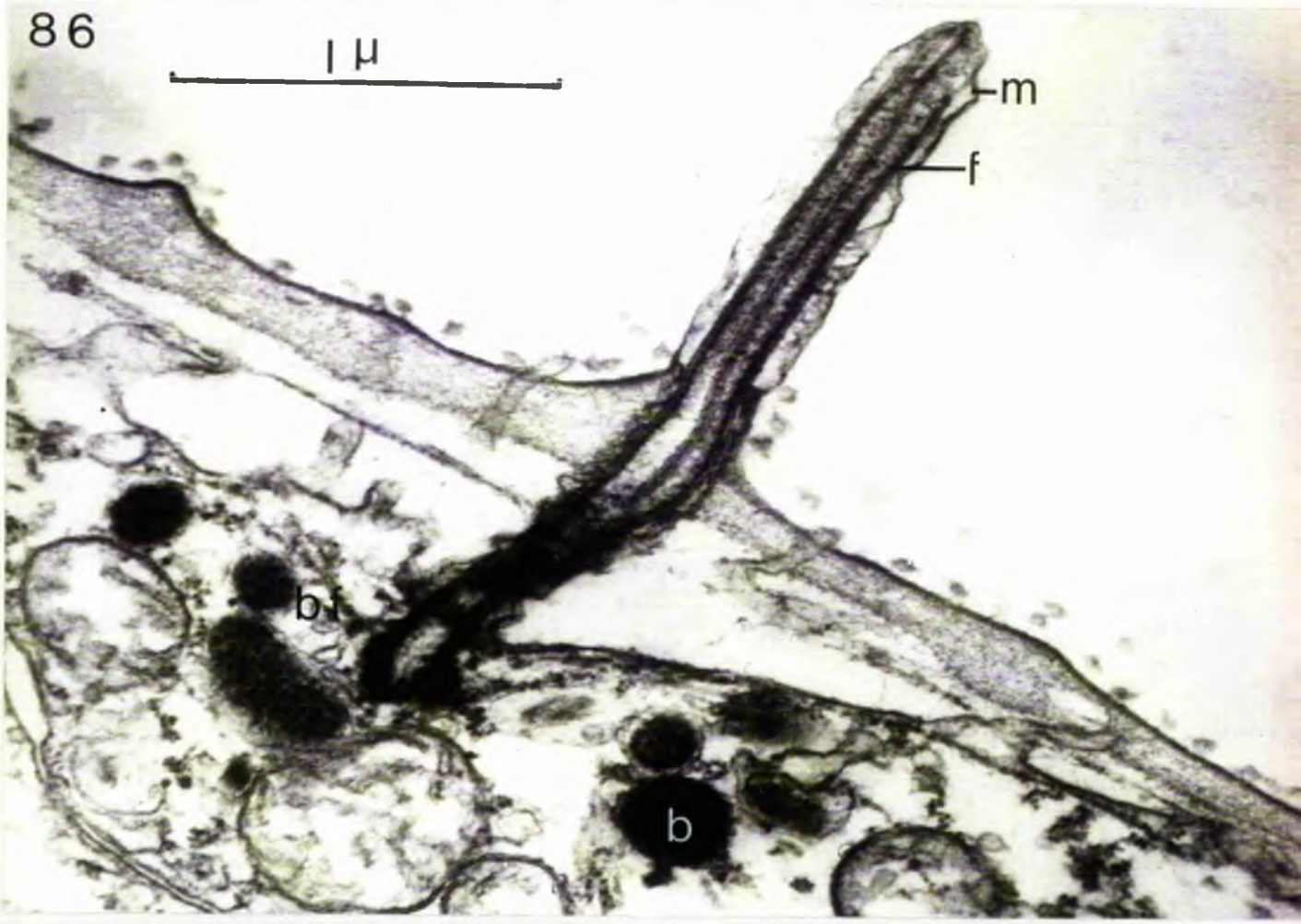
Fig. 85.

Ciliary generating bodies, b. Note Golgi membrane, G, attached to one of the bodies. The mitochondria, m, have indented, irregular membranes and few cristae at this stage. 5-day old trochophore.

Fig. 86.

Ciliary bud. The filaments, f, terminate in microtubules, m, which extend up to the membrane. The basal foot, bf, is attached to the basal body by a microtubule. The ciliary generating body, b, appears to have oozed a small portion of matrix (diacrine secretion). 6-day old trochophore.


85
G
b
m
1 μ


86
1 μ
m
f
b
b

not oriented in any particular plane at the time of formation. They do not transform into basal bodies and are not always closely associated with basal bodies when these appear, but as the growth of cilia proceeds, these granulated bodies gradually disappear. First the contents become less electron-dense, and finally the structure shrinks, finally leaving a small empty vesicle. The secretion of the substance of the ciliary generating bodies is illustrated in Fig. 86, and takes place by a process known as diacrine secretion (Kobyashi and Fujita, 1969), by which the substance diffuses through the membrane of the granule. The sequence of events in the development of the cilia is first, appearance of a centriole. This enlarges into a basal body, the root then begins to grow, and following that filaments grow upwards from the centriole. The cell membrane invaginates towards the centriole, attaches mid-way down the basal body and as the cilium grows it pushes the expanding cell membrane upwards. The central filaments precede the peripheral filaments (Fig. 86), which lag some 0.17μ behind. Fine strands connect the peripheral filaments with the cell membrane just below the apical point containing the tip of the central filaments. The bulge in the axoneme as the cilium passes through the cuticle in figure 86 is typical in the trochophore (see Fig. 20), and it is known from the position of the basal foot relative to this gap that the direction of beat is towards the widest gap between the central and peripheral filaments at this point. The small granule lateral to the basal body is thus the developing basal foot, attached to the basal body

only by microtubules at this stage. The thicker structure on the other side is a lateral root, apparently earlier in development than the basal foot. Mitochondria increase in numbers and size at the base of the growing cilia and are often closely associated with the basal bodies (Fig. 86).

The prototroch at 9 days is shown in Fig. 87. Ciliary generating bodies are aligned parallel to the line of growth of cilia and have moved up to the cuticle. Microtubules and multivesicular bodies are prominent at this stage in the distal part of the developing prototroch cells. They are not as evident in the prototroch of the fully formed trochophore.

The stomach opening appears on the 7th day to 8th day and growth of cilia in the stomach begins on about the 8th day. The gullet cilia with extra filaments form at the same time as ordinary gullet cilia, and develop the additional filaments as the axoneme grows. Ciliation of the intestine takes place later.

5. Development of the cuticle.

The cuticle is closely applied to the surface of the external cells, and until the 8th day lacks the superficial electron-dense line. There is no sub-cuticular fibrous layer, and the main body of the cuticle is not uniformly electron-dense as in the mature trochophore, but is made up of arching structures which grow to enclose areas of a different texture. Both stages are illustrated in figure 88. The superficial granules form an irregular "cloud" over the cuticle, and

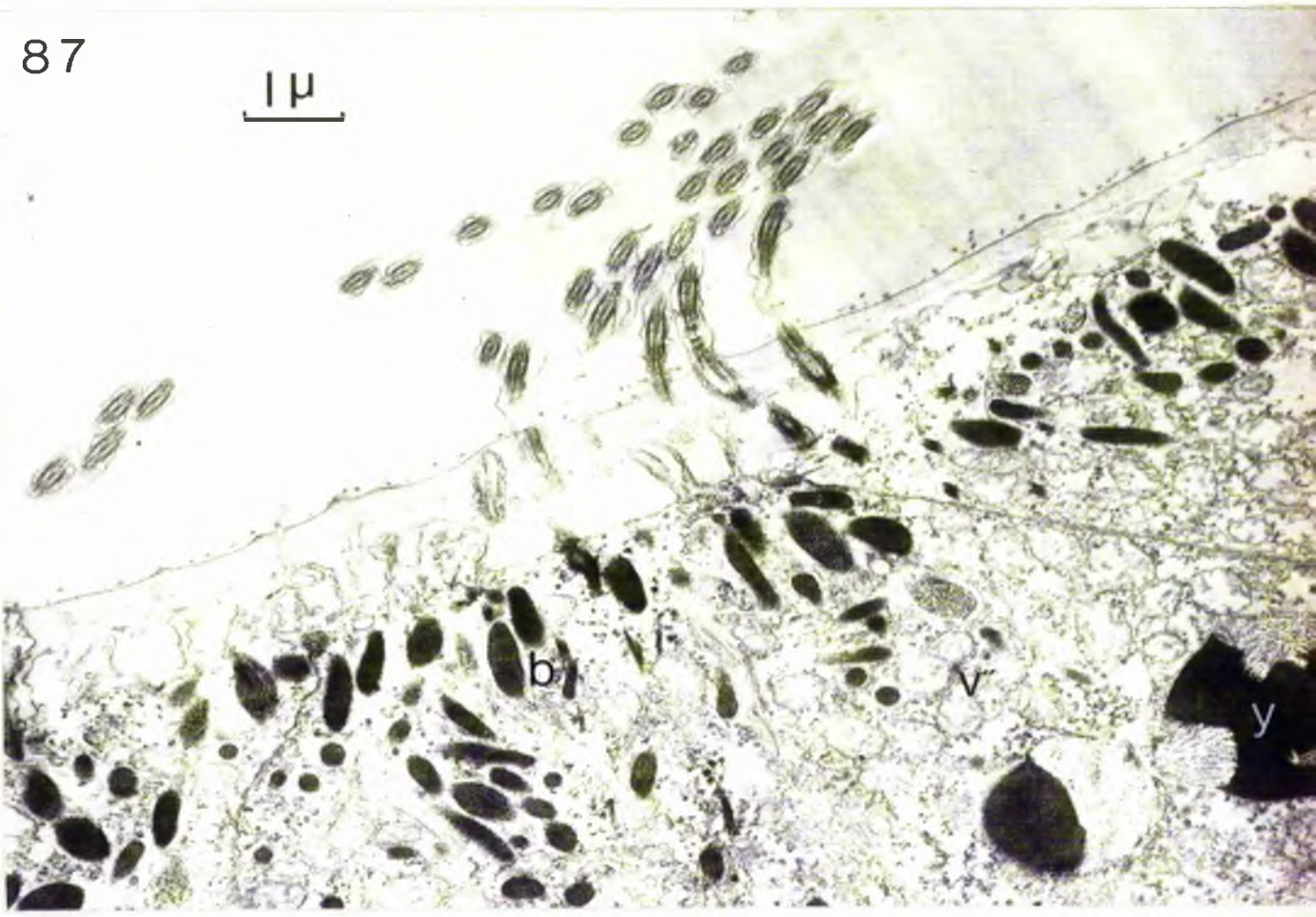
Fig. 87.

Prototroch at 9-day stage. b, ciliary generating body, y, yolk granule. Note absence of roots. v'' is a multivesicular body.

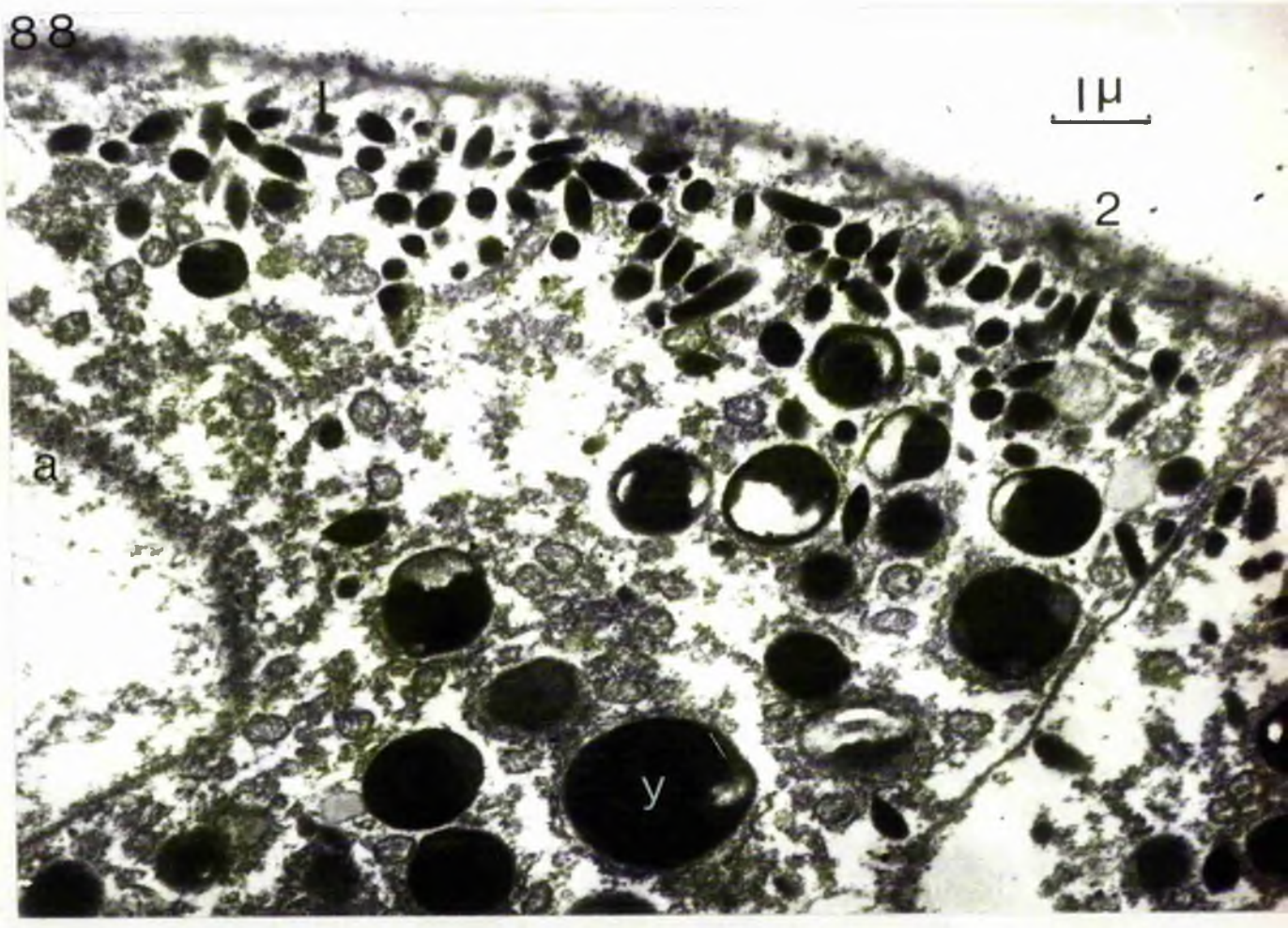
Fig. 88.

Development of cuticle. 1, extensions growing down to enclose spaces, 2; y, yolk granules; a, annulae of nucleus. 5-day old trochophore.

87



88



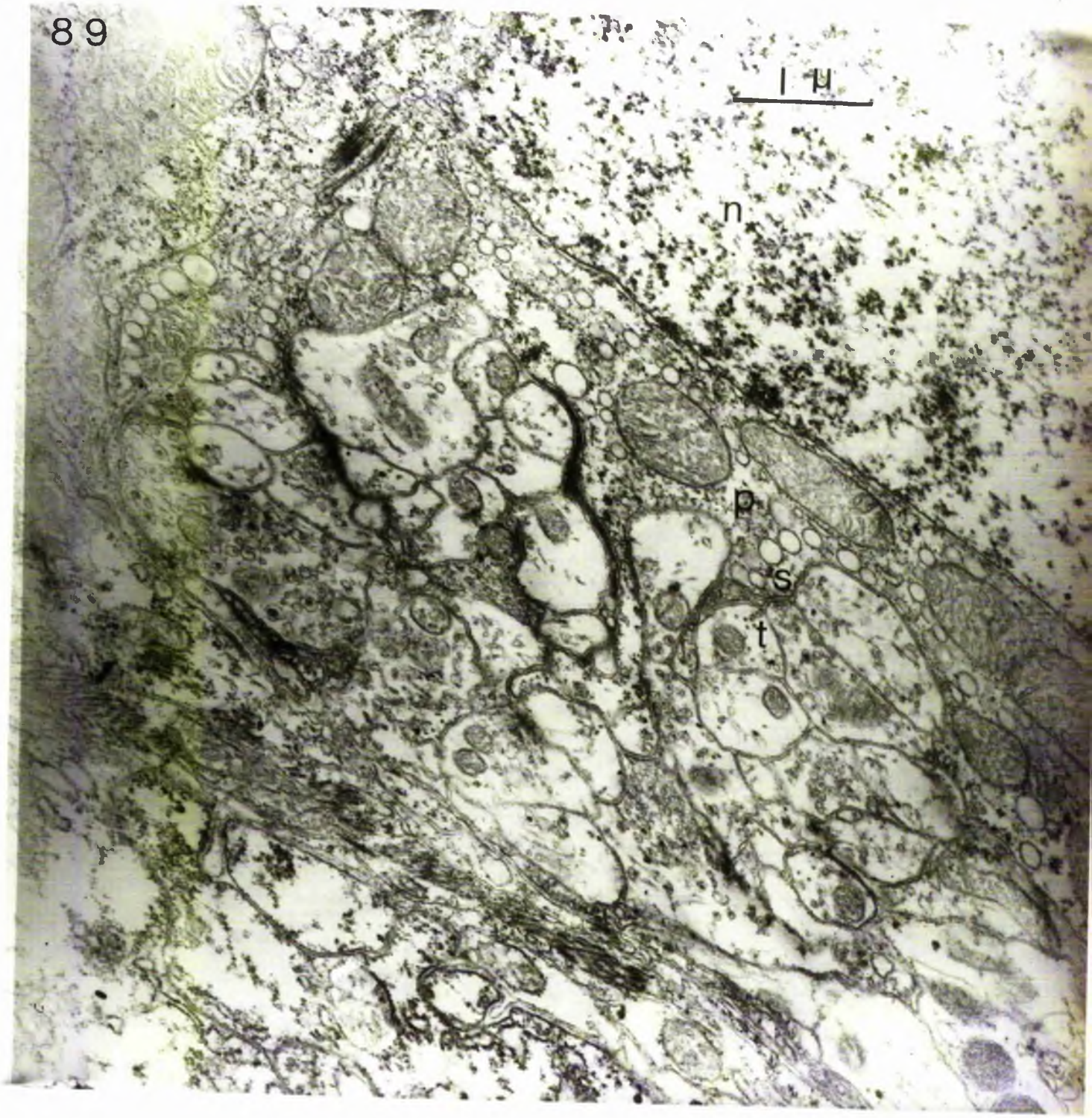
presumably as growth continues they become evenly stretched out over the surface, forming the line seen later. No interconnecting strands are seen at this stage, nor are microvilli yet present, except where apical cilia have begun to grow. Two days later, by the 8th day, the cuticle more resembles the mature form. The superficial electron-dense line has appeared but is not as dark and distinct as it will be later. The superficial granules are hollow and still not interconnected. The cell membrane is more distinct. Denser areas alternate with less dense areas in the main substance of the cuticle.

6. The developing nervous system

No nerves are seen up to the 6th day. At the time at which each of the main prototroch cells contribute 6 cilia to a group (instead of the later 44) only one relatively undifferentiated axon may be found beneath the prototroch cells. This is at the 8-9day stage. A short time later, the number of axons has increased and developed characteristic vesicles, both clear and dense-cored, although the contents of the dense-cored vesicles do not stain as darkly as later. These juvenile nerves differ from the nerves seen in the free-swimming trochophore in containing ribosomes and large numbers of microtubules (Fig. 89). Ribosomes are absent in the nerves of the free-swimming trochophore, and microtubules are few. At the earliest stage of differentiation of the nerves, the microtubules appear before synaptic vesicles.

Fig. 89.

Sub-prototroch nerves, 9-day trochophore. Note microtubules, t, neurociliary synapse, s, on the protroch cell, p. The prototroch nucleus, n, is above.


89
1 μ
n
p
s
t

Discussion

1. Gastrulation

The pattern of early development of the trochophore, while not studied in detail here, shows a basic similarity to that of other polychaetes. The pattern of gastrulation varies (E.B. Wilson, 1882), with embolic invagination taking place among those polychaetes where segmentation is equal, and epibolic invagination being more common in the greater number of cases in which segmentation is unequal. Hyman (1951) states that a typical invaginate gastrula arises partly by epiboly and partly by emboly. This is the situation for the Harmothoë trochophore, although epiboly is the predominent element in the gastrulation here.

2. Nuclear pores.

The nuclear pores of the Harmothoë trochophore in its juvenile stages are of particular interest, as they apparently disappear in the nucleus of more mature trochophores. Nuclear pores were first observed by Callan and Tomlin (1950) who examined the nuclear membrane of an amphibian oocyte by removing it and laying it upon a grid for subsequent electron microscopy. Among workers using sectioning for electron microscopy, Afzelius (1955) demonstrated details of the two-layered form of membrane with an "annular" and "discontinuous" layer in the sea urchin oocyte, these layers being homologous with the porous and continuous layers of Callan and Tomlin (1950). Since then,

nuclear pore complexes have been recognised in a wide range of animals and plants, and much discussion has taken place regarding their function. (Barnes and Davis, 1959; Moor and Mühlethaler, 1963; Weiner, Spio and Lowenstein, 1965; Franke, 1967; Frankeand Scheer, 1970a and b;and others). Afzelius (1955) suggested that the annuli indicate nuclei in an active phase. Grasso, Swift, Ackerman (1962), studying the development of erythrocytes in mammalian fœtal liver thought that a low number of pores indicated low metabolic activity. Weston, Ackerman, Greider, Nikolewski (1972) suggested that the vesicles of the nuclear membrane contribute to the Golgi complex; Ito and Lowenstein (1966) found that nuclear membrane permeability changes during development under the influence of a growth hormone. In general, there is agreement that pores are characteristic of active nuclei , and that they are fundamentally correlated with the efflux of RNA-containing particles. LaCour and Wells (1972) found that in particular types of cell the number of nuclear pores can increase with increased metabolic activity. They stated, however, that it is not known whether pores are sometimes short-lived.

The Harmothoë trochophore nuclear pores are within the range of dimensions found for other animals (see Wiener, Spio and Lowenstein, 1965), and have the plug characteristic of nuclear pores They are, however, transient, and are found only on nuclei of cells in the process of rapid division, and the nuclei of cells undergoing differentiation.

It is notable that reports of nuclear annuli are from work on oocytes or growing tissues, particularly fœtal tissues. It therefore may be the case that pores are present not just in highly metabolic cells but that the pores have a particular function in cells which have not yet fully differentiated, or which are dividing at a certain rate. In fact nuclear annuli of this type are not present in many highly active cells, such as the trochophore prototroch cells, and many other animal and plant cells with high metabolic activity as indicated by high concentrations of mitochondria. The annuli are present in cells in which the whole cell is in a state of change, either towards division, or further differentiation. Rapid transmission of nuclear DNA-stored information by free movement of RNA through the pores would be more important at such a time than simply where high metabolic activity is taking place.

3. Cilia

Developing cilia have been studied in two basic fields: spermiogenesis (Bawa, 1964; Kiefer, 1966, 1970; Reger, 1969; and many others), and the development and regeneration of cilia or flagella in ciliates or flagellates (Renaud and Swift, 1964; Roth and Shigenaka, 1964; Dingle and Fulton, 1966; Rosenbaum and Child, 1967; Allen, 1970 and others). Sotelo and Trujillo Cenóz (1958), examining the neural epithelium of the chick embryo, were some of the first workers to study the development of cilia, and Sorokin (1962) described the

formation of cilia by fibroblasts and smooth muscle cells. Recent regeneration work using the inhibitor of protein synthesis, cycloheximide, and the inhibitor of microtubules assembly, colchicine (Rosenbaum and Child, 1967; Rosenbaum and Carlson, 1969) has shown that protein precursors are formed and then passed to the tip of the flagellum, where they are assembled into filaments.

There have been no previous reports of the presence of granular, membrane-bound bodies such as were found in the trochophore as precursors to the formation of cilia or basal bodies, although Kiefer (1966) mentions mitochondria with "dense-rod-like inclusions" in flagellar formation in drosophila spermiogenesis, and other authors note collections of granules or amorphous dark bodies at the site of basal body or flagellar formation. The trochophore may have evolved a packaging system for collecting large quantities of ciliary precursor material as a means of ensuring rapid development of large numbers of cilia on one cell.

The actual appearance of the ciliary bud, with peripheral-fibre growth lagging behind central-fibre growth and preceding fine fibres attaching to the cell membrane, cannot be compared with the pattern in other animals as this aspect of ciliary growth has not previously been described.

In his review of ciliary morphogenesis, Satir (1965) summarises the process as requiring (1) synthesis of the centriole (2) filament

morphogenesis within the cytoplasmic matrix and (3) the involvement of smooth membrane-bound elements from the Golgi apparatus or the cell membrane. The empty vesicles referred to in (3) above in no way resemble the large electron-opaque ciliary generating bodies of the trochophore, but are involved in membrane formation. In the trochophore the cilium axoneme is not generated within the cytoplasmic matrix, but grows out into an invaginated pocket of cell membrane. Thus in several respects the generation of cilia in the trochophore differs from the patterns so far described in other animals, but as the accounts as yet are few, and do not give full details of the appearance of the growing cilia, it is highly probable that the development of cilia at least on other ciliated epithelia may be similar.

4. The Cuticle

The development of cuticle has not been documented in other annelids and no comparison can be made. It is, however, clear that the microtubules are not equivalent to the cuticle secreting pores of rotifers (Clément, 1969). The egg membrane apparently constitutes the fundamental simple cuticle, and this is modified during growth.

5. Nerve Structure

Various authors studying growing nerves have noticed changes in density of microtubules as nerves enlarge, but have not established whether the actual number of microtubules change. Peters and Vaughn (1967), Schmitt and Samson (1968), Tennyson (1970) and Lyser (1971) are among those who report a higher density of microtubules in small

nerves than in larger axons. In the trochophore it is clear that the density and actual number of microtubules decreases with age. Antigenic evidence suggests that microtubular protein may be an integral component of neuronal and synaptic vesicle membranes (Twomey and Samson, 1972). It is therefore possible that the microtubules of the trochophore may break down to provide the tubulin subunits to be incorporated into the membranes of synaptic vesicles.

While they exist, however, the microtubules may perform one of two functions, both widely reported in the literature. The role of microtubules in determing cell shape has often been documented (Ledbetter and Porter, 1963; Byers and Porter, 1964; McIntosh and Porter, 1967; Tilney and Gibbons, 1969 and others). Secondly, the function of microtubules in directing and maintaining the flow of cytoplasm and ribosomes has been widely recognised (Porter, 1966; Sabnis and Jacobs, 1967; Macgregor and Stebbings, 1970; and others). The high concentration of microtubules in the axon of the juvenile trochophore quite possibly serves both roles, supporting the growing axon and helping to direct the flow of ribosomes and protein percursors down the axon.

The presence of ribosomes in the developing nerves is not unique. Tennyson (1970), studying the structure of the axon and growth cone of the dorsal root neuroblast of the rabbit embryo, observed that ribosomes decrease with age, and that there are none in older axons,

as is the case with trochophore. In view of the transient ribosomes, and temporary high density of microtubules preceding the appearance of vesicles, it would seem that instead of vesicles and cytoplasmic matrix being manufactured in the cell body and growing out with the axon, manufacture takes place in the axon.

6. Other Structures

The presence of microtubules in the distal part of juvenile prototroch cells may indicate that there is more cytoplasmic movement at this stage. The presence of multivesicular bodies is problematical. While many authors have noted the presence of multivesicular bodies, (Barber and Dilly, 1969; Eakin and Westfall, 1964; MacRae, 1966, and many others), there has been little comment on their function. Bacetti and Bedini (1964) suggest that they may be involved in pinocytosis. Multivesicular bodies have been found in a wide variety of invertebrate eyes and sense organs, but there have been no previous reports of their disappearance with age. In the trochophore, where they are temporary, it is tempting to suggest that the multivesicular bodies contain either breakdown or precursor products that are involved in the differentiation of the cell. In particular, they may provide temporary storage for membrane remains of ciliary generating bodies.

Overall, the findings in the development of the trochophore throw much light on a wide variety of problems, and further studies on embryonic systems would seem to be a rewarding field.

III. EXPERIMENTS ON TROCHOPHORES

1. Pepsin Digestion of Sectioned Trochophores

Method

Trochophores were fixed and embedded in the usual way, and sections were cut and placed on grids. Some were stained with lead citrate and uranyl acetate as usual and examined in the electron microscope, while others were treated before being examined. The grids were prepared for pepsin digestion according to a modified method of Leduc and Bernhard (1960). They were floated for 10 to 20 minutes on 10% Hydrogen Peroxide, which oxidizes the reduced osmium fixative to a solution of osmium tetroxide. After rinsing in distilled water each grid was floated on a drop of 0.5% pepsin in 0.1N HCl for 10 minutes, 20 minutes and 30 minutes at 37°F in a moist chamber. They were then rinsed in distilled water, and stained with lead citrate and uranyl acetate in the usual manner. Controls were treated with hydrogen peroxide and left for 30 minutes on droplets of 0.1N HCl in the moist chamber.

Results

Cilia in the prototroch, the basal bodies, roots, basal feet, basal plates and all ciliary fibrils were digested partially or completely by 30 minute pepsin treatment. The cuticle and mitochondria remained undigested. Only the dark bands of the roots

were digested so that the striated effect remained. The longitudinal filaments of the roots were digested. The bases of the cilia were more readily digested than the tips, and the peripheral filaments were digested before the central pair. The a-fibre disappeared first, leaving the arms, (Fig. 90). On the whole, the b-fibre disappeared simultaneously with the central pair. In Fig. 90 it can be seen that in every cilium not all the peripheral filaments have been affected. The ones which remain are not identical in each cilium. Occasionally one of the central pair of filaments also disappears before the other.

The "valve" cilia of the gullet follow the same pattern for the axoneme, but the peripheral filaments remain after the axoneme has been completely digested. The membrane and binding material between the membranes of these cilia are never digested. The stomach cilia are more resistent to digestion than those of the gullet, but the intestinal cilia react identically to the ordinary gullet cilia and prototroch cilia. Microvilli never show any sign of being digested. The dark-staining granules at the base of the valve gullet cilia are digested.

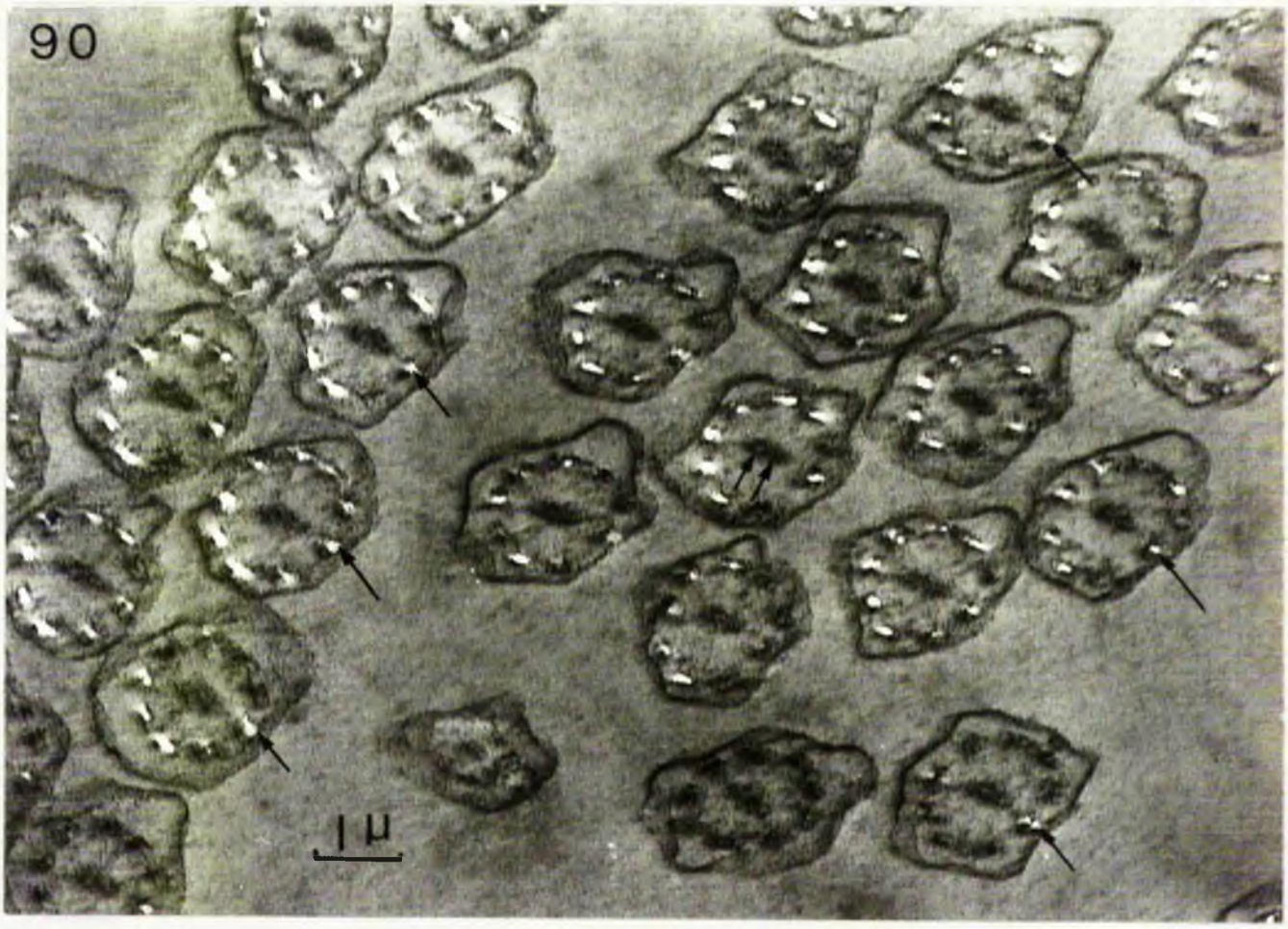
In all cells the yolk granules are digested away, but digestive granules remain (Fig. 91). Much nuclear material, and, where there is no nucleolus, the ribosomes in the nucleus and the cytoplasm appear also to be affected (Fig. 91).

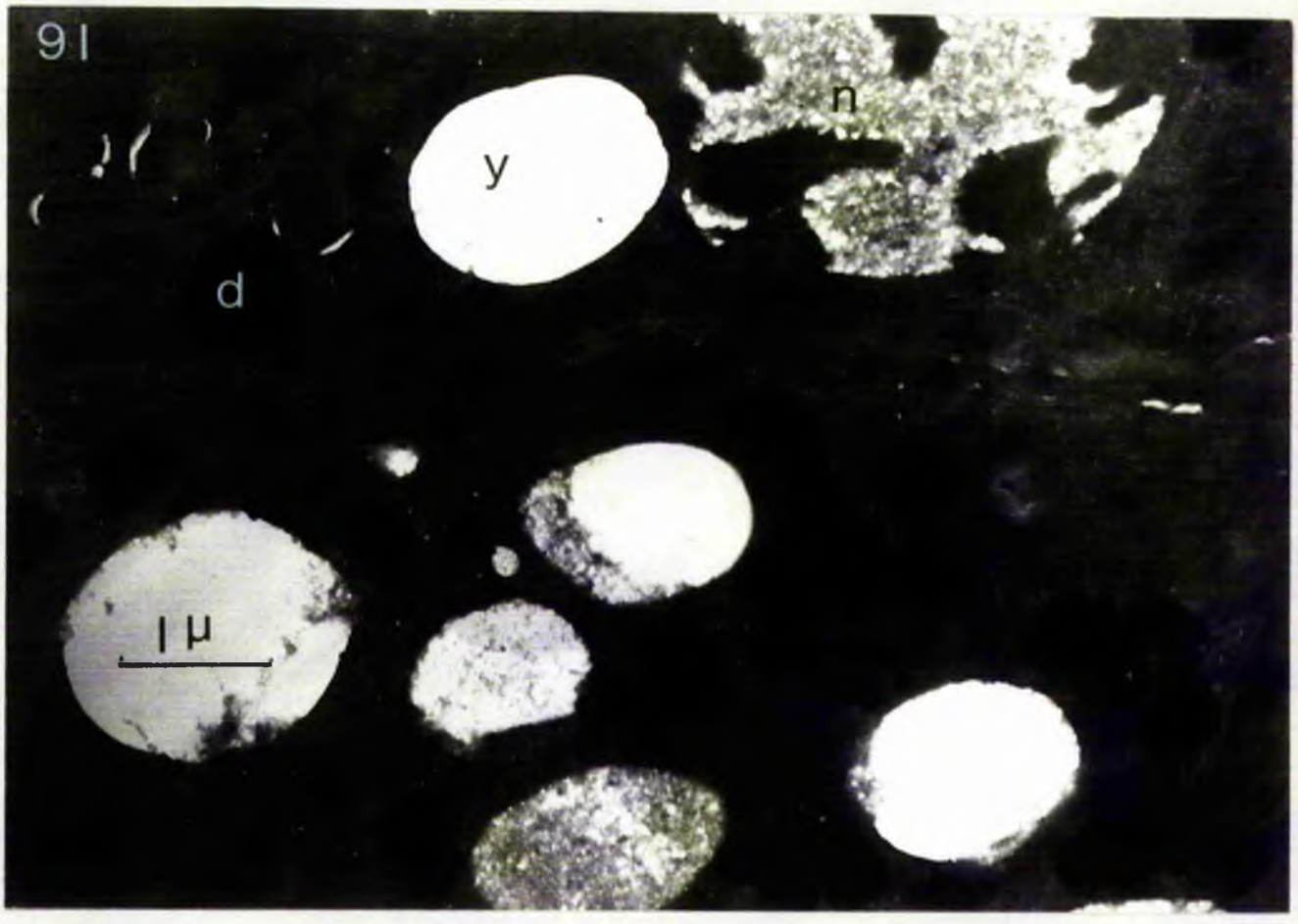
Fig. 90.

Pepsin digested cilia. Single arrows point to some of the a-fibres which have been digested out, leaving holes. The b-fibres remain. The pair of arrows in the centre point to the central pair of filaments which remain undigested.

Fig. 91.

Pepsin digested gut cells. Digestive granules, d, are undigested. The nucleus, n, is partially digested, showing the distribution of protein in it. The yolk granules, y, are partially or completely digested, possibly depending on the state of their contents before fixation. Some variation in density of yolk granules is commonly seen.


90
1μ


91
n
y
d
1μ

Short digestion removes only the superficial granules on the cuticle, but prolonged digestion strips both the granules and the electron-dense peripheral lines. All membraneous figures, e.g. myelin bodies and mitochondria, and the cell membrane are unaffected, although parts of the nuclear membrane are lost. The fine membranes connecting the central and peripheral ciliary fibrils are not digested. The controls were unaffected.

Discussion

Digestion experiments in electron microscopy are still relatively few. Leduc and Bernhard (1960) digested ultra-thin sections of the thymus and pancreas of the rat with desoxyribonuclease and pepsin; Bernhard and Leduc (1960) digested the pancreas of the rat with perchloric acid and ribonuclease; Leduc, Byczkowska-Smyk and Bernhard (1960) digested the pancreas of the rat with pepsin and trypsin; and Bernhard, Granboulan, Barski, and Tournier (1961) performed pepsin and trypsin digestion on a virus, Douglas, Riples and Ellis (1970) studied enzymatic digestion of desmosomes. Behnke and Forer (1967) pepsin digested (among other experimental procedures) various tissues containing microtubules. All of the early experiments were carried out without previous H_2O_2 treatment, and for periods from 4 to 24 hours. The pepsin digestion of the rat pancreas (Leduc, Byczkowska-Smyk and Bernhard, 1960) caused loss of mitochondria, glycogen and zymogen granules, in contrast with the

findings in the trochophore. Differences in fixation and embedding probably account for these differences, but if this is so, then comparisons with the material of Behnke and Forer (1967) are also difficult, as their material was glutaraldehyde fixed, while the trochophores were fixed with osmium only.

Behnke and Forer (1967), on the basis of colchicine treatment, pepsin digestion, negative staining and heat treatment classified microtubules into 4 classes: A tubules, B tubules, central tubules, and cytoplasmic tubules. They also decided that there was a gradient of sensitivity along the 9 + 2 tubules, either because of differences in age, or because of difference in total exposure to fixative because of accessory fibres, etc., blocking the penetration of fixative in the lower segment of the sperm they were using for their study. However, it is our finding that in a normal axoneme, without differential barriers to fixation along its length, the tip is less readily digested than the base. In this case it seems safe to assume that the growth of the cilium causes this difference in reaction. This may also account for the relatively higher stability of the additional filaments of the gullet cilia, which bud from the axoneme after it has formed. In fact these filaments should be equivalent to accessory tubules, but they are more stable than the central tubules, whereas Behnke and Forer class accessory and central tubules together. The matrix within the expanded membrane may

play a part in this difference, or it may be an effect of different fixation, as explained before.

2. Drug testing living trochophores for electron microscopy

Methods

Newly released trochophores were placed in solution of a drug in sea water, and some were fixed for sectioning the following day, some the following week, and some after two weeks.

The drugs were ordinary pharmacutical products, obtained from Boots the Chemists and were: 1. Caffeine 2. phenobarbitone 3. Ethanoloestradiol 4. Benzofluazide.

Each drug was dissolved in sea water to make doses equivalent to 100 times a normal dosage for humans and 10 times a normal dosage for humans. Trochophores were added to these solutions.

Fixation was with one portion of 2% osmium tetroxide to one portion of animals in sea water containing the drug, for ½ to 1 hour. The dehydration and embedding were as usual, with rapid reduction to 100% acetone, overnight in acetone with epon, and final transference to fresh epon for hardening the next day.

Results

1. Caffeine

Caffeine was the only drug which had an immediate visible effect on the trochophores: they stopped active swimming and sank to the bottom of the container. However, trochophores which had been

in 100 times normal dose for one week were still alive, and the electron-microscope picture of the animal looked much as normal for a trochophore which had been free swimming for one week. One outstanding feature was the presence of large numbers of microtubules in the nerves, a feature characteristic of the embryonic but not the fully developed trochophore.

2. The phenobarbitone-treated material was examined in some detail, as phenobarbitone has been recognised as a drug which increases endoplasmic reticulum formation in liver. Firstly, animals which had been in 100 times normal human dose for one day were examined. No change was observed in the fine structure. Secondly, animals which had been kept in 10 times normal human dose for one week were examined. A number of aberrant features were observed. The secreting cells of the stomach, which are characterised by a dense array of tubular or vesicular formations of endoplasmic reticulum, with peripheral areas of lamellar formations, remained much as normal, but other stomach and intestinal cells had shrunken mitochondria with shrivelled, wrinkled membranes. No increase in endoplasmic reticulum was observed in any of the cells. The microvilli were better preserved in the sromach and intestine than with standard unbuffered osmium fixation. The membranes of the stomach cilia were also more closely applied to these cilia.

In the prototroch cells, several changes had taken place. The mitochondria cristae appeared convoluted, even whorled in some cases, and multivesicular bodies appeared. More microvilli than normally seen projected through the cuticle. The nucleus occasionally contained odd inclusions which are characteristic of the adult nucleus. Large bodies (1.7μ long, 1.25μ broad) containing apparently crystalline material were also common in the prototroch and other cells.

There appeared to be a slight increase in the number of microtubules in the nerves, but this was not as dramatic as with caffeine.

3. Ethanoloestrodiol. Samples of trochophores left for 24 hours in the solution representing 100 times the normal human dose were sectioned. All the structures appeared normal and no unusual inclusions were found. The cellular structures, however, had the form seen with buffered osmium tetroxide, rather than the pattern commonly seen with the standard fixation of 1% osmium in sea water used here. In particular the granular endoplasmic reticulum was more evenly spaced and lay in a more regular pattern than usually seen with the standard fixation. It is probable that the increase in concentration of the solution by the drug caused this effect.

4. Benzofluazide. Trochophores which had been kept for one week in a sea-water solution containing 100 times the normal human dose were sectioned. In general the appearance did not deviate far from that normally seen, except in the nerves. A marked reduction in the number of vesicles was frequently seen and a small group of microtubules,

usually centrally placed, were then the major feature in the axon. In the sub-prototroch nerve apparently normal vesicles could be seen uniformly distributed in the axons, but at points where synaptic contacts might be expected, electron-dense masses (0.2μ long, 0.15μ broad) replaced the aggregations of vesicles normally found. It was possible to see vesicles in some of these masses, indicating that they may be dense aggregations of vesicles in which some vesicular membranes have broken down. If the process of accumulation of transmitter substance is not subject to feedback inhibition, then the accumulations found here could have resulted from a block of the mechanism of release.

Discussion

There is a growing interest in the effect of drugs on cell organelles and a general acceptance of invertebrate material and plants in this line of investigation. Allison and Nunn (1968) and Allison, Hulands, Nunn, Kitching and MacDonald (1970) worked on the effect of inhalation anaesthetics on the helizoan Actinosphaerium. Colchicine has been studied in relation to the division of various invertebrate eggs (Borisy and Taylor, 1967; Marsland, 1968; Marsland and Hecht, 1968), and the division of plants (Pickett-Heaps, 1967), and has been used in studies to differentiate microtubules (Behnke and Forer, 1967) and in other microtubule studies (Tilney, 1968). It has been widely examined, both biochemically and by electron microscopy, for effects on neurones (see Hansson and Sjöstrand, 1971, for references).

Chloramphenicol effects on mitochondria have been investigated in

plants (Smith-Johannsen and Gibbs, 1972), and its effects in man have been compared with those in insects (Smith, Smith and Yunis, 1970). The insect, with its large flight muscle mitochondria, was found to be a highly favourable vehicle for testing this drug. Chemicals known as inhibitors, 2-phenyethanol and puromycin, have been tested biochemically in several biological systems (Bostock, 1970; Dunn, Owen and Kemp, 1970).

Caffeine has been used to inhibit cytokinesis in plant cells (López-Sáez, Risueño and Giménez-Martín, 1966; Pickett-Heaps, 1969; and others), and to inhibit fertilization in Arbacia (Cheney and Lansing, 1955), but has not been tested for more general effects.

Drugs of specific action have been tested in systems where an anticipated effect can be examined, such as the effects of insulin, hydrocortisone and prolactin on mammary gland explants (Mills and Topper, 1970) and the effects of androgen on the adrenal (Nickerson, Skelton and Molteni, 1970). Relative to the number of studies on other drugs and organ systems, much work has been done on the effect on liver morphology by phenobarbital and other substances (Hutterer, Schaffner, Klion and Popper, 1968; Jones and Fawcett, 1966; Remmer and Merker, 1963; Rubin, Hutterer and Lieber, 1968; and many others). Other studies include the effect of thallium on nervous tissue (Hendelman, 1969) and the effect of vincristine sulfate on neurones (Schochet and Earle Lampert, 1968).

Except for the studies of Mills and Topper (1970) and Nickerson, Skelton and Molteni (1970), the work outlined above has not been closely related to the known pharmacological properties of the drugs. The choice of drugs for test on the trochophore was based on the general

availability and use of the drugs and their operation on cell activities of different organ systems. Caffeine and phenobarbitone have both been used previously in electron microscope studies (see above), but there are no reports on their effects on nerves although both are known to affect the nervous system, caffeine stimulating the brain and phenobarbitone inducing sleep. The increase in microtubules in nerves observed after caffeine treatment is therefore of particular interest and further investigations in other animals could prove rewarding.

Ethanoloestrodiol is a hormone simulating the effects of oestrogen. Any effect on the fine structure of cell systems in the trochophore would have provided an indication of more general action than normally expected from such a hormone, comparing for example with the results of studies on the effect of chloramphenicol on mitochondria (Smith, Smith and Yunis, 1970).

Bendrofluazide is a diuretic whose mode of action is not yet fully known, but which ultimately operates by inhibiting the uptake of water by tubule cells in the kidney. In the trochophore its effect on nerves is more major than on any other system and suggests two sites of influence at the subcellular level. The first is an increase in microtubules and decrease in vesicles, tubulin elements of whose structure are already known to be interchangeable (Twomey and Samson, 1972). The second is an increase in accumulation of presynaptic vesicles, suggesting blocking of the releasing mechanism.

The trochophore can be seen from these tests to provide a useful animal for preliminary investigations into the subcellular action of drugs in various organ systems.

Conclusions

Studies on polychaete larvae have in the past been generally restricted to light microscope descriptions, often including an account of metamorphosis, and much of the approach to larval investigations up to the present day has been limited by the history of investigations of this type. Some experimental work has been done on responses of larvae (not necessarily polychaete) to light and gravity (Knight-Jones and Quasim, 1955; Bayne, 1963; Crisp and Ghobashy, 1971, and others), but so far almost no experimental work has been done to test responses to chemicals or drugs, and there have been few measurements of the rate of beat of cilia or swimming of larvae, and none on polychaete larvae. Electron microscopical studies on larval structure may be said to be virtually non-existent, and scanning electron microscopy of larvae has never been undertaken before.

The information provided by the present studies opens wide fields for further investigation, not only applicable to the biology and structure of larvae, but to the overall methodology of electron microscopy, and to the growing concern over the effect of drugs on cell ultrastructure.

REFERENCES

Afzelius, B.A., 1955. The ultrastructure of the nuclear membrane of the sea urchin oocyte as studied with the electron microscope. Expl. Cell Res. 8, 147-158.

Afzelius, B.A., 1963. Cilia and flagella that do not conform to the 9+2 pattern. I. Aberrant members within normal populations. J. Ultrastruct. Res., 9, 381-392.

Agassiz, A., 1866. On the young stages of a few Annelids. Ann. Lyc. Nat. Hist., New York, 7, 303-343.

Åkesson, B., 1963. The comparative morphology and embryology of the head in scale worms (Aphroditidae, Polychaeta). Ark. Zool., 16, 125-163.

Åkesson, B., 1967. On the nervous system of the Lopadorhynchus larva (Polychaeta). Ark. Zool., 20, 55-78.

Alison, A.C. and Nunn, J.F., 1968. Effects of general anaesthetics on microtubules, Lancet, 2, 1326-1329.

Alison, A.C., Hulands, G.H., Nunn, J.F., Kitching, J.A. and Macdonald, A.C., 1970. The effect of inhalational anaesthetics on the microtubular system in Actinosphaerium nucleofilum. J. Cell Sci., 7, 483-499.

Anderson, D.T., 1959. The embryology of the Polychaete Scoloplosarmiger. Q. Jl. microsc. Sci., 100, 89-166.

Anderson, T.F., 1951. Techniques for the preservation of three-dimensional structure in preparing specimens for the electron microscope. Trans. N.Y. Acad. Sci. 13, 130-134.

Bacetti, B. and Bedini, C., 1964. Research on the structure and physiology of the eyes of a Lycosid spider. Archs. ital. Biol. 102, 97-122.

Baker, J.R., 1965. The fine structure produced in cells by fixatives. J. Microsc. Soc., 84, 115-131.

Barber, V.C. and BOYDE, A., 1968. Scanning electron microscope studies of cilia. Z. Zellforsch. mikrosk. Anat., 84, 269-284.

Barber, V.C. and Dilly, P.N., 1969. Some aspects of the fine structure of the statocysts of the molluscs Pecten and Pterotrachea. Z. Zellforsch. mikrosk. Anat., 94, 462-478.

Barber, V.C., Evans, E.M. and Land, M.F. 1967. The fine structure of the eye of the mollusc Pecten maximus. Z. Zellforsch. mikrosk. Anat., 76, 295-312.

Barber, V.C. and Land, M.F., 1967. Eye of the cockle Cardium edule: Anatomical and physiological investigations, Experientia, 23, 677-701.

Barber, V.C. and Wright, D.E., 1969. The fine structure of the sense organs of the cephalopod mollusc, Nautilus, Z. Zellforsch. mikrosk. Anat., 102, 293-312.

Barnes, B.G. and Davis, J.M., 1959. The structure of nuclear pores in mammalian tissue. J. Ultrastruct. Res., 3, 131-146.

Bawa, S.R., 1964. Electron microscope study of spermiogenesis in a fire-brat insect. Thermobia domestica Pack. I. Mature spermatozoan. J. Cell Biol., 23, 431-446.

Bayne, B.L. 1963. Responses of Mytilus edulis larvae to increases in hydrostatic pressure. Nature, Lond., 198, 406-7.

Behnke, O. and Forer, A., 1967. Evidence for four different classes of microtubules in individual cells. J. Cell Sci., 2, 169-192.

Bernhard, W. and Leduc, E., 1960. Essais de cytochemie ultrastructurale Action sur l'ergastoplasm. C.R. Acad. Sci. Paris, 250, 3411-3413.

Bernhard, W., Granboulan, N., Barski, G. & Tournier, P., 1961. Essais cytochemie ultrastructurale. Digestion de virus sur coupes ultrafines. C.R. Acad. Sci. Paris, 252, 202-204.

Birks, R., Huxley, H.E. and Katz, B., 1960. The fine structure of the neuromuscular junction of the frog. J. Physiol. Lond., 150, 134-144.

Boilly, B., 1967. Contribution a l'étude ultrastructurale de la cuticle épidermique et pharangienne chez une annélide polychète (Syllis Amica Quatrefages). J. Microscopie, 6, 469-484.

Von Bonsdorff, C.H. and Telkkä, A., 1969. The flagellar structure of the flame cell in fish tapeworm, Diphyllobothrium latum. Z. Zellforsch., mikrosk. Anat. 70, 169-170.

Borisy, G.G. and Taylor, E.W., 1967. The mechanism of action of colchicine. Colchicine binding to sea urchin eggs and the mitotic apparatus. J. Cell Biol., 34, 535-548.

Bostock, C.J., 1970. The effect of 2-phenyl ethanol on the DNA synthesis cycle of Schizosaccharomyces pombe. J. Cell Sci., 7, 523-530.

Boyde, A., 1967. A single-stage carbon-replica method and related techniques for the analysis of the electron microscope image. J. R. micros. Soc., 86, 359-370.

Boyde, A. and Barber, V.C., 1969. Freeze-drying methods for the scanning electron-microscopical study of the protozoan Spirostomum ambiguum and the statocyst of the cephalopod mollusc, Loligo vulgaris. J. Cell Sci., 4, 223-239.

Boyle, P.R., 1969. Fine structure of the eyes of Onithochiton neglectus. (Mollusca, Polyplacophora). Z. Zellforsch., mikrosk. Anat. 102, 313-332.

Brandt, K.A. and Apstein, C., 1938. Nordisches Plankton. Kiel u. Leipzig. Verlag von Lipsius und Fischer.

Brökelman, J. and Fischer, A. 1966. Uber die cuticula von Platynereis dumerilli (Polychaeta). Z. Zellforsch., mikrosk. Anat. 70, 131-135.

Byers, B. and Porter, K.R., 1964. Oriented microtubules in elongating cells of the developing lens rudiment after induction. Proc. Acad. Sci. USA., 52, 1091-1099.

Callan, H.G. and Tomlin, S.G., 1950. Experimental studies on amphibian oocyte nuclei . I. Investigation of the structure of the nuclear membrane by means of the electron microscope. Proc. R. Soc. B., 137, 366-378.

Cameron, M.L., 1965. Some details of ultrastructure in the development of flagellar fibers of the Tenebrio sperm. Can. J. Zool. 43, 1005-1009.

Cazaux, C., 1968. Etude morphologique du développement larvaire d'annélides polychètes (Bassin d'Arcachon). I. Aphroditidae, Chrysopelalidae. Archs. Zool. exp. gén., 109, 477-543.

Cazaux, C., 1971. Développement larvaire de Microspio mecznikowianus. Fourth European Marine Biology Symposium. (Ed. D.J. Crisp). Cambridge Univ. Press., 247-257.

Charles, G.H., 1966. Sense organs of the Mollusca. In: The physiology of the Mollusca, vol. 2. (Ed. K. Wilbur and C.M. Younge). New York-London Academic Press., 455-480.

Cheney, R.H. and Lansing, A.J., 1955. Caffeine inhibition of fertilization in Arbacia. Exp. Cell Res., 8, 173-180.

Child, C.M., 1900. The early development of Arenicola and Sternaspis. Arch . Entw Mech. Org., 9, 587-722.

Claparède, R.E., 1863. Beobachtungen uber Anatomie und Entwicklung. Leipzig: Engelmann.

Claude, A., 1961 . Problems of fixative for electron microscopy. Results of fixation with osmium tetroxide in acid and alkaline media. Path. Biol., Paris, 9, 933.

Clément, P., 1969. Premières observations sur l'ultrastructure comparée des téguments de rotifères. "Vie Milieu". SérieA: Biologie Marine, 20, 461-482.

Cobb, J.L.S., 1968. The fine structure of the pedicellariae of Echinus esculentus (L). II. The Sensory System. Jl. R. Micros. Soc., 88, 223-233.

Conney, A.H., 1967. Pharmacological implications of microsomal enzyme induction. Pharmac. Rev., 19, 317-366.

Crisp, D.J. and Ghobashy, A.F.A.A., 1971. Responses of the larvae of Diplosoma listerianum to light and gravity. Fourth European Marine Biology Symposium (Ed. D.J. Crisp). Cambridge:University Press, 443-465.

Crisp, Mary, 1971. Structure and abundance of receptors of the unspecialized external epithelium of Nassarius reticulatus (Gastropoda, Prosobranchia). J. mar. biol. Ass. U.K., 51, 865-890 .

Crisp, Mary, 1972. Photoreceptive function of an epithelial receptor in Nassarius reticulatus (Gastropoda, Prosobranchia). J. mar. biol. Ass. U.K., 52, 437-442.

Dales, R., Phillip, 1963. and 1967. Annelids. Hutchison University Library, London.

Daly , J.M. 1972. The maturation and breeding biology of Harmothoë imbricata (Polychaeta:Polynoidae). "Marine Biology" Internat. J. On life in Oceans and Coastal Waters, 12, 53-66.

Dalton, A.J., 1955. A chrome-osmium fixative for electron microscopy. Anat. Rec. , 121, 281.

Dawydoff, C., 1928. Traité d'embryologie comparée des invertébrés. Masson and Cie, Paris.

Dawydoff, C., 1959. Ontogenèse des annélides. In: Traité de Zoologie, Tome V. Masson and Cie, Paris.

Day, J.H., 1934. Development of Scolecolepis fuginolosus (Claparède). J. mar. biol. Ass., 19, 633-54.

Dhainaut-Courtoise, N., 1965. Sur la présence d'un organe photorécepteur dans le cerveau de Nereis pelagica L. (Annélide polychète). C.R. Acad. Sci. Paris, 261- 1085-1088.

Dilly, P.N., 1972. The structures of the tentacles of Rhabdopleura compacta (Hemichordata) with special reference to neurociliary contro Z. Zellforsch. mikrosk. Anat., 129, 20-39.

Dingle, A.D. and Fulton, C., 1966. The development of the flagellar apparatus of Naegleria. J. Cell Biol., 31, 43-54.

Dorsett, D.A., 1963. The motor axon terminations of annelids. Nature, Lond., 198, 406.

Dorsett, D.A., 1964. The sensory and motor innervation of Nereis. Proc. R. Soc. B., 159, 652-667.

Dorsett, D.A. and Hyde, R., 1968. The fine structure of the lens and photoreceptors of Nereis virens. Z. Zellforsch. mikrosk. Anat., 85, 243-255.

Dorsett, D.A. and Hyde, R., 1970. The epidermal glands of Nereis. Z. Zellforsch. mikrosk. Anat., 110, 219-230.

Drochmans, P., 1960. Electron microscope studies of epidermal melanocytes and the fine structure of melanin granules. J. biophys. biochem. Cytol., 8, 165-180.

Douglas, W.H.J., Riples, R.C. and Ellis, R.A., 1970. Enzymatic digestion of desmosome and hemidesmosome plaques performed on ultra-thin sections. J. Cell Biol., 44, 211-214.

Dunn, M.J., Owen, E. and Kemp, R.B., 1970. Studies on the mechanism underlying the inhibition by puromycin of cell aggregation in vitro. J. Cell Sci., 7, 557-573.

Eakin, R.M., 1963. Lines of evolution of photoreceptors. In: General Physiology of Cell Specialization (Ed. D. Mazia and A. Tyler). New York:McGraw-Hill, 393-425.

Eakin, R.M., 1965. Evolution of photoreceptors. Cold Spring Harb. Symp. quant. Biol., 30, 363-370.

Eakin, R.M., 1968. Evolution of photoreceptors. In: Evolutionary Biology, Vol. II (eds. T. Bobzhansky, M.K. Hecht, W.C. Steere), New York: Appleton-Century-Crofts.

Eakin, R.M., 1972. Structure of Invertebrate Photoreceptors. In: Handbook of Sensory Physiology. Vol. II. Photochemistry of Vision (Ed. H.J.A. Dartnall). Springer-Verlag. 625-684.

Eakin, R.M. and Westfall, J.A., 1964. Further observations on the fine structure of some invertebrate eyes. Z. Zellforsch. mikrosk. Anat., 62, 310-332.

Eakin, R.M. and Westfall, J.A., 1965. Fine structure of the eye of Peripatus (Onychophora). Z. Zellforsch. mikrosk. Anat., 68, 278-300.

Eakin, R.M. and Brandenburger, J.C., 1967. Differentiation in the eye of a pulmonate snail, Helix aspersa. J. Ultrastruct. Res., 18, 391-421.

Elofsson, R., 1969. The ultrastructure of the nauplius eye of Sapphirina (Crustacea:Copepoda). Z. Zellforsch. mikrosk. Anat., 100, 376-401.

Enders, H., 1909. Study of the life history and habits of Chaetopterus variopedatus. J. Morph., 20, 479-531.

Fawcett, D.W., 1966. An Atlas of fine structure: The Cell. Philadelphia and London: W.B. Saunders Company.

Fewkes, J.W., 1883. On the development of certain worm larvae. Bull. Mus. Comp. Zool. Harv., 11, 167-268.

Fischer, A., 1963. Über den Bau und die Hell-Dunkel-Adaptation der Augen des Polychäten. Platynereis dumerilii. Z. Zellforsch. mikrosk. Anat., 61, 338-353.

Fischer, A., and Brökelmann, J., 1966. Das Auge von Platynereis dumerilii (Polychaeta). Z. Zellforsch. mikrosk. Anat., 71, 217-244.

Fjerdingstad, E.J., 1961. The ultrastructure of choanocyte collars in Spongilla lacustris. Z. Zellforsch. mikrosk. Anat., 53, 645-657.

Flatterly, F.W., 1923. Polychaeta. Rep. Dove mar. Lab., 12, 98-112.

Fraipont, J., 1887. Le genre Polygordius. Fauna Flora Golf. Neapel, 14, Monographie.

Franke, W.W., 1967. Zur Feinstruktur isolierter Kernemembranen aus tierischen Zellen. Z. Zellforsch. mikrosk. Anat., 80, 585-593.

Franke, W.W. and Scheer, U., 1970a. The ultrastructure of the nuclear envelope of the amphibian oocyte. I. The mature oocyte. J. Ultrastruct. Res., 30, 288-316.

Franke, W.W. and Scheer, U., 1970b. The ultrastructure of the nuclear envelope of the amphibian oocyte. A reinvestigation. II. The immature oocyte and dynamic aspects. J. Ultrastruct. Res., 30, 317-321.

Fuchs, H.M., 1911. Note on the early larvae of Nepthys and Glycera. J. mar. biol. Ass. U.K., 9, 164-170.

Gibbons, I.R., 1961. The relationship between the fine structure and direction of beat in gill cilia of a lamellibranch mollusc. J. biophys. biochem. Cytol., 11, 179-205.

Gilula, N.B. and Satir, P., 1969. Ultrastructural and cytochemical observations on the septate junctions and the ciliated epithelium of the mussel gill. J. Cell Biol., 43, 43A.

Giménez-Martín, G., González-Fernandez, A., and Lopéz-Sáez, J.F., 1965. A new method of labelling cells. J. Cell Biol., 26, 305-309.

Giménez-Martín, G., Risueño, M.C. and Lopéz-Sáez, J.F., 1967. A simple staining technique for electron microscopy with lead uranyl acetate. Experientia, 23, 316-317.

Goodrich, E.S., 1945. The study of nephridia and genital ducts since 1895. Q. J. Micros. Sci., 86, 113-392.

Gorman, A.L.F., and McReynolds, J.S., 1969. Hyperpolarization and depolarizing receptor potential in the scallop eye. Science, N.Y. 165, 309-310.

Gorman, A.L.F., McReynolds, J.S., and Barnes, S.N., 1971. Photoreceptors in primitive chordates: fine structure, hyperpolarizing receptor potentials, and evolution. Science, N.Y. 172, 1052-1054.

Grassé, Pierre, P., 1959. Traité de Zoologie. Tome V. Masson et Cie.

Grasso, J.A. Swift, H. and Ackerman, G.A., 1962. Observations on the development of erythrocytes in mammalian fetal liver. J. Cell Biol., 14, 235-254.

Gravely, F.H., 1909a. Polychaete larvae. L.M.B.C. Mem. typ. Br. Mar. Pl. Anim., 19, 1-79.

Gravely, F.H., 1909b. Studies on Polychaete Larvae. Q. Jl. microsc. Sci., 211, 597-628.

Gray, E.G., 1959. Axosomatic and axodendrite synapses of the cerebral cortex: an electron microscope study. J. Anat., 93, 420-433.

Gupta, B.L. and Little, C., 1969. Studies on Pogonophora. 2. Ultrastructure of the tentacular crown of Siphonobranchia. J. mar. biol. Ass. U.K., 49, 717-741.

Gupta, B.L. and Little, C., 1970. Studies on Pogonophora. 4. Fine structure of the cuticle and epidermis. Tissue and Cell, 2, 637-696.

Häcker, V., 1896. Pelagische Polychatenlarven. Z. wiss. Zool., 62, 74-168.

Hakansson, C.H. and Toremalm, N.G., 1965. Studies on the physiology of the trachea. 1. Ciliary activity indirectly recorded by a new "light beam reflex" method. Ann. Otol. Rhinol. Lar. , 74, 954-969.

Hand, A.R. and Gobel, S., 1972. The structural organization of the septate and gap junctions of Hydra. J. Cell Biol., 52, 397-408.

Hansen, K., 1962. Electron mikroskopische Untersuchungen der Hirudineen-Augen. Zool. Beitr., 7, 83-128.

Hanson, J. and Lowy, J., 1961. The structure of the muscle fibres in the translucent part of the adductor of the oyster Crassostrea angulata. Proc. R. Soc. B., 154, 173-196.

Hansson, H. and Sjöstrand, J., 1971. Ultrastructural effects of colchicin on the hypoglossal and dorsal vagal neurones of the rabbit. Brain Res. Osaka, 35, 379-396.

Harris, J.E., 1961. The mechanics of ciliary movement. In: The cell and the organism. (Eds. T.A. Ramsay and V.B. Wigglesworth). Cambridge, Univ. Press., 22-36.

Hartline, H.K. and Graham, C.H., 1938. The discharge impulses in the optic nerve of Pecten in response to illumination of the eye. J. cell. comp. Physiol., 11, 465-477.

Hatschek, B., 1878. Studien uber Entwicklungsgeschichte der Anneliden. Ein Beitrag zur Morphologie der Bilaterien. Arb. Zool. Inst. Univ. Wien, 1, 1-128.

Hatschek, B., 1886. Studien uber Entwicklungsgeschichte der Anneliden. Entwicklung der trochophora von Eupomatus uncinatus. Arb. Zool. Inst. Univ Wien, 6, 121-148.

Hayat, M.A. and Giaquinta, R., 1970. Rapid fixation and embedding for electron microscopy. Tissue and Cell, 2, 191-195.

Hendelman, W., 1969. The effect of thallium on peripheral nervous tissue in culture: A light and electron microscope study. Anat. Rec., 163, 198-199.

Hermans, C.O., 1969. Fine structure of the segmental ocelli of Armandia brevis. (Polychaeta:Opheliidae). Z. Zellforsch., 96, 361-371.

Hermans, C.O. and Cloney, R.A., 1966. Fine structure of the prostomial eyes of Armandia brevis (Polychaeta:Opheliidae). Z.Zellforsc 72, 583-596.

Hermans, C.O. and Eakin, R.M., 1969. Fine structure of the cerebral ocelli of a Sipunculid, Phascolosoma agassizii, Z. Zellforsc 100, 325-339.

Holborow, P.L., 1971. The fine structure of the trochophore of Harmothoë imbricata. Fourth European Marine Biology Symposium (Ed. D.J. Crisp). Cambridge University Press., 237-246.

Holborow, P.L. and Laverack, M.S.L., 1972. Presumptive photoreceptor structures of the trochophore of Harmothoë imbricata. Mar. Behav. Physiol., 1, 139-156.

Holborow, P.L., Laverack, M.S.L. and Barber, V.C., 1969. Cilia and other surface structures of the trochophore of Harmothoë imbricata (Polychaeta). Z. Zellforsch., 98, 246-261.

Hollman, K.H., 1963. The fine structure of the goblet cells in the rat intestine. Ann. N.Y. Acad. Sci., 106, 545-554.

Holt, S.J. and Hicks, R.M., 1961a. Use of veronal buffers in formalin fixatives. Nature, 191, 832.

Holt, S.J. and Hicks, R.M., 1961b. Studies on formalin fixation for electron microscopy and cytochemical staining purpose. J. biophys. biochem. Cytol., 11, 31-45.

Horridge, G.A., 1959. Analysis of the rapid responses of Nereis and Harmothoe" (Annelida). Proc. R. Soc. B., 150, 245-262.

Horridge, G.A., 1964. Presumed photoreceptor cilia in a ctenophore. Q.Jl. Microsc. Sci., 105, 311-317.

Horridge, G.A., 1965. Non-motile sensory cilia and neuromuscular junctions in a ctenophore independent effector organ. Proc. R. Soc. B., 162, 333-350.

Horridge, G.A., 1968. Interneurons. London and San Francisco: Freeman and Co. Ltd.

Horridge, G.A. and Mackay, B., 1964. Neurociliary synapses in Pleurobrachi (Ctenophora). Q. Jl. MicroscSci., 105, 163-174.

Hughes, H.P.L., 1970. A light and electron microscope study of some Opisthobranch eyes. Z. Zellforsch., mikrosk. Anat. 106, 79-98.

Hutterer, F., Schaffner, F., Klion, F.M. and Popper, H., 1968. Hypertrophic, hypoactive smooth endoplasmic reticulum. A sensitive indicator of hepatotoxicity exemplified by Dieldrin. Science, N.Y. 161, 1017-1019.

Hyman, L.H., 1951. The Invertebrates. Platyhelminthes and Rhynchocoela. The acoelomate Bilateria. Vol. II. New York and London. McGraw-Hill.

Ichikawa, A., 1965. Fine structural changes in response to hormone stimulation of the perfused canine pancreas. J. Cell Biol., 24, 369-385.

Imaizumi, M. and Hama, K., 1969. An electron microscope study on the intestinal cells of the gizzard of the love bird (Uroloncha domestica). Z. Zellforsch., mikrosk. Anat. 97, 351-357.

Ito, S. and Lowenstein, W.R., 1966. Permeability of a nuclear membrane: changes during normal development and changes induced by growth hormone. Science, N.Y. 150, 909-910.

Ito, S. and Winchester, R.J., 1963. The fine structure of the gastric mucosa in the bat. J. Cell Biol., 16, 541-577.

Izuka, A., 1912. The errantiate Polychaeta of Japan. J. Coll. Sci. Imp. Univ. Tokyo, 30, 1-262.

Jones, A.L. and Fawcett, D.W., 1966. Hypertrophy of the agranular endoplasmic reticulum in hamster liver induced by phenobarbital (with a review on the functions of this organelle in liver). J. Histochem. Cytochem., 14, 215-232.

Karnovsky, M.J., 1965. A formaldehyde-gluteraldehyde fixative of high osmolarity for use in electron microscopy. J. Cell Biol., 27, 137A-138A.

Kerneis, A., 1960. Photorécepteurs due panache de Dasychone bombyx. C.R. Acad. Sci. Paris, 263, 653-656.

Kerneis, A., 1968. Nouvelles données histochimiques et ultrastructurales sur les photorécepteurs "branchiaux" de Dasychone bombyx (Dalyell) (Annélide:Polychète). Z. Zellforsch., mikrosk. Anat. 86, 280-292.

Kiefer, B.I., 1966. Ultrastructural abnormalities in developing of X/O Drosophila melanogaster. Genetics, 54, 1441-1452.

Kiefer, B.I., 1970. Developmental organization and degeneration of the Drosophila sperm flagellum. J. Cell Sci., 6, 177-194.

Kim, S.K., Nasjleti, C.E. and Hans, S.S., 1972. The secretion process in mucous and serous secretory cells of the rat sublingual gland. J. Ultrastruct. Res., 38, 371-389.

Kleinenberg, N., 1886. Die Entstehung des Annelids aus der larve von Lopadorhynchus. Z. Wiss. Zool., 44, 1-227.

Knapp, M.F. and Mill, P.J., 1971. The contractile mechanism in obliquely striated body wall muscle of the earthworm, Lumbricus terrestris. J. Cell Sci., 8, 413-425.

Knight-Jones, E.W., 1954. Relations between metachronism and the direction of ciliary beat in metazoa. Q. Jl microsc. Sci., 95, 503-521.

Knight-Jones, E.W. and Quasin, S.Z., 1955. Responses of some marine plankton animals to changes in hydrostatic pressure. Nature, Lond., 175, 941-942.

Kobyashi, S. and Fujita, T., 1969. Fine structure of mammalian and avian pancreatic islets with special reference to D. cells and nervous elements. Z. Zellforsch. mikrosk. Anat., 100, 340-363.

Korn, H., 1958. Vergleichend-embryologische Untersuchungen an Harmothoë Kinberg, 1857 (Polychaeta, Annelida). Organogenese und Neurosecretion. Z. Wiss. Zool., 161, 346-443.

Krasne, F.B. and Lawrence, P.A., 1966. Structure of the photoreceptors in the compound eyespot of Branchiomma vesiculosum. J. Cell Sci., 1, 239-248.

Kümmel, G. and Brandenburg, J., 1961. Die Reusengeibelzellen (Cyrtocyten). Z. Naturf., 16b, 692-697.

LaCour, L.F. and Wells, B., 1972. The nuclear pores of early meiotic prophase nucleii of plants. Z. Zellforsch. Mikrosk. Anat., 123, 178-194.

Land, M.F., 1966. Activity in theoptic nerve of Pecten maximus in response to changes in light intensity and to pattern and movement in the optical environment. J. Exp. Biol., 45, 83-99.

Land, M.F., 1968. Functional aspects of the optical and retinal organization of the mollusc eye. Symp. Zool. Soc. Lond., 23, 75-96.

Laverack, M.S., 1968. On the receptors of marine invertbrates. Oceangr. Mar. biol. Ann. Rev., 6, 249-324.

Lawry, J.V., 1967. Structure and function of the parapodial cirri of the Polynoid Polychaete, Harmothoë. Z. Zellforsch. mikrosk. Anat., 82, 345-361.

Ledbetter, M.C. and Porter, K.R., 1963. A "microtubule" in plant cell fine structure. J. Cell Biol., 19, 239-250.

Leduc, E. and Bernhard, W., 1960. Essais de cytochimie ultrastructurale. Action dur la chromatine. C.R. Acad. Sci. Paris, 250. 2948-2952.

Leduc, E., Byczkowska-Smyk, W. and Bernhard, W., (1960). Essais de cytochimie ultrastructurale. Digestion par la pepsine et la trypsine. C.R. Acad. Sci. Paris, 250, 4052-5054.

Leik, J. and Kelly, D.E., 1970. Septate junctions in the gastrodermal epithelium of Phialidium: a fine structural study utilizing ruthenium red. Tissue and Cell, 2, 435-441.

Leschke, M., 1903. Beiträge zur Kenntnis der pelagischen Polychaetenlarven der Kieler Föhrde. Wiss. Meeresunters. Abt. Kiel., 7, 113-134.

Ling, E.A., 1969. The structure and function of the cephalic organ of a nemertine, Lineus ruber. Tissue and Cell, 1, 503-524.

Ling, E.A., 1970. Further investigations on the structure and function of cephalic organs of a nemertine Lineus ruber. Tissue and Cell, 2, 569-588.

López-Sáez, J.F., Risueño, M.C. and Giménez-Martín, G., 1966. Inhibition of cytokinesis in plant cells. J. Ultrastruct. Res., 14, 85-94.

Lyser, K.M., 1971. Microtubules and filaments in developing axons and optic stalk cells. Tissue and Cell, 3, 394-404.

Macgregor, H.C. and Stebbings, H., 1970. A massive system of microtubules associated with cytoplasmic movement in telotrophic ovarioles. J. Cell Sci., 6, 431-449.

McIntosh, W.C., 1900. A monograph of the British Annelids. Part 11. Polychaeta. London: Ray Soc. Publs, 215–442.

McIntosh, J.R. and Porter, K.R., 1967. Microtubules in spermatids of the domestic fowl. J. Cell Biol. 35, 153–174.

MacRae, E.R., 1964. Observations on the fine structure of photoreceptor cells in the planarian Dugesia tigrina. J. Ultrastruct. Res., 10, 334–339.

MacRae, E.R., 1966. The fine structure of photoreceptors in a marine flatworm. Z. Zellforsch., mikrosk. Anat. 75, 469–483.

McReynolds, J.S. and Gorman, A.L.F., 1970a. Photoreceptor potentials of opposite polarity in the eye of the Scallop, Pecten irradians. J. gen. Physiol., 56, 376–391.

McReynolds, J.S. and Gorman, A.L.F., 1970b. Membrane conductances and spectr sensitivities of Pecten photoreceptors. J. gen. Physiol., 56, 392–406.

Manaranche, R., 1968. Sur la présence de cellules d'allure photoréceptrice dans le ganglion cérébroide de Glycera convoluta (Annélide polychète). J. Microscopie., 7, 44a.

Marsland, D.A., 1968. Cell division enhancement of the anti-mitotic effects of colchicine by low temperature and high pressure in the cleaving eggs of Lytechinus variegatus. Exp. Cell Res., 50, 369–376.

Marsland, D.A. and Hect, R., 1968. Cell division: combined anti-mitotic effects of colchicine and heavy water on first cleavage in the eggs of Arbacia punctilata. Exp. Cell Res., 51, 602–608.

Mattern, C.F.T. and Daniel, W.A., 1966. The flame cell of a rotifier. J. Cell Biol., 29, 552–554.

Mead, A.D., 1897. Embryology of Marine Annelids. J. Morph., 13, 227-326.

Meyer, E., 1901. Studien über der Körperbau der Anneliden. Mitt. zool. St n. Neapel., 14, 247-585.

Mill, P.J. and Knapp, M.F. 1970a. The fine structure of obliquely striated body wall muscles in the earthworm, Lumbricus terrestris. Linn. J. Cell Sci., 7, 233-261.

Mill, P.J. and Knapp, M.F. 1970b. Neuromuscular junctions in the body wall muscles of the earthworm, Lumbricus terrestris. Linn. J. Cell Sci., 7, 263-271.

Millonig, G.J., 1961. Advantages of a phosphate buffer for osmium tetroxide solutions in fixation. J. appl. Phys. , 32, 1637.

Mills, E.S.and Topper, Y.J., 1970. Effects of.Insulin, Hydrocortisone and Prolactin on mammary gland explants. J. Cell Biol., 44, 311-328.

Moor, H. and Mühlethaler, K., 1963. Fine structure in frozen etched yeast cells. J. Cell Biol., 17, 609-628.

Napolitano, L.M. and Kleinermann, J., 1964. Unit membranes and lipid absorption in the small intestine. J. Cell Biol., 23, 65A.

Neutra, M. and Leblond, C.P., 1966. Synthesis of the carbohydrate of mucus in the Golgi complex as shown by electron microscope radio-autography of goblet cells from rats injected with glucose-H3. J. Cell Biol., 30, 119-136.

Newcomb, E.H., 1969. Plant microtubules. A. Rev. Pl. Physiol., 20, 253-288.

Nickerson, P.A., Skelton, F.R. and Molteni, A. 1970. Observations of filaments in the adrenal of androgen treated rats. J. Cell Biol., 47, 277-280.

Noirot, Ch. and Noirot-Timothée, C., 1969. La cuticle proctodéal des insectes. 1. Ultrastructure comparée. Z.Zellforsch., mikrosk Anat. 101, 477-509.

Nolte, W., 1936. Annelidenlarven 1. In: Nordisches Plankton, (ed: K.A. Brandt and C. Apstein) Vol.10, Kiel u. Leipzig. Verlag von Lipsius und Fischer, 39-16

Nørrevang, A., 1964. Choanocytes in the skin of Harrimania kupfferi (Enteropneusta). Nature, Lond., 204, 398-399.

Nørrevang, A., 1965. On the mucous secretion from the proboscis in Harrimania kupfferi (Enteropneusta). Ann. N.Y. Acad. Sci., 118, 1052-69.

Olsson, R., 1962. The relation between ciliary rootlets and other cell structures. J. Cell Biol., 15, 596-599.

Palade, G.E., 1952. A study of fixation for electron microscopy. J. Exp. Med., 95, 285-297.

Palay, S.L., 1958. The morphology of secretion. In: Frontiers of Cytology. Yale Univ. Press.: New Haven, Conn., 305-342.

Palay, S.L. and Karlin, L.J., 1959. An electron microscope study of the intestinal villus. II. The Pathway of fat absorption. J. biophys. biochem. Cytol., 5, 373-380.

Pease, D.C., 1960. Histological techniques for electron microscopy. New York: Academic Press.

Pederson, K.J., 1961. Some observations on the fine structure of planarian protonephridia and gastrodermal phagocytes. Z. Zellforsch., mikrosk. Anat. 53, 609-628.

Peracchia, C. and Mittler, B.S., 1972. Fixation by means of gluteraldehyde-hydrogen peroxide reaction products. J. Cell Biol., 53, 234-238.

Peracchia, C. and Mittler, B.S., 1972. New gluteraldehyde fixation procedures. J. Ultrastruct. Res., 39, 57-64.

Peters, A. and Vaughn, J.E., 1967. Microtubules and filaments in the axons and astrocytes of early post-natal rat optic nerves. J. Cell Biol., 32, 113-119.

Pettibone, M., 1953. Some scale-bearing polychaetes of Puget Sound and adjacent waters. Univ. of Washington Press, Seattle, 1-89.

Pickett-Heaps, J.D., 1967. The effects of colchicine on the ultrastructure of dividing plant cells. Xylem wall differentiation and distribution of cytoplasmic microtubules. Devl Biol., 15, 206-236.

Pickett-Heaps, J.D., 1969. Preprophase microtubule bands in some abnormal mitotic cells of wheat. J. Cell Sci., 4, 397-420.

Pilar, G. and Hess, A., 1966. Differences in internal structure and nerve terminals of the slow and twitch muscle fibers in the cat superior oblique. Anat. rec., 154, 243-251.

Pitelka, D.R., 1963. Electron Microscopic Structure of Protozoa. New York: Pergamon Press, Inc.

Pontin, R.M., 1966. The osmoregulatory function of the vibratile flames and the contractile vesicle of Asplanchna (Rotifera). Comp. Biochem. Physiol., 17, 1111-1126.

Porter, K.R., 1966. Cytoplasmic microtubules and their functions. In: Principles of Biomolecular Organization (Ed. G.E.W. Wolstenholme and M. O'Connor), 308-356.

Porter, K.R., 1969. Independence of fat absorption and pinocytis. Fed. Proc., 28, 34-40.

Ranvier, L., 1887. Le méchanisme de la sécrétion. J. Micrograph., 11, 7-15.

Rasmussen, E., 1956. Faunistic and biological notes on marine invertebrates. III, The reproduction and larval development of some polychaetes from the Isefjord with some faunistic notes. Biol. Meddr , 23, 1-84.

Reger, J.F., 1969. A fine structure study on spermiogensis in the Arachnida, Leiobunum sp. (Phalangida: Harvestmen). J. Ultrastruct Res., 28, 422-434.

Remmer, H. and Merker, H.J., 1963. Drug induced changes in liver endoplasmic reticulum: Association with drug metabolizing enzymes. Science, N.Y. 142, 1657.

Remmer, H. and Merker, H.J., 1965. Effect of drugs on the formation of smooth endoplasmic reticulum and drug-metabolizing enzymes. Ann. N.Y. Acad. Sci., 123, 79-97.

Renaud, L.F. and Swift, H., 1964. The development of basal bodies and flagella in Allomycas arbusculus. J. Cell Biol., 23, 339-354.

Röhlich, P. and Török, L.J., 1964. Electronen mikroskopische Beobachtungen an den Schzellen des Blugtegels. Hirudo medicinalis L. Z. Zellforsch., mikrosk. Anat 63, 618-635.

Röhlich, P. Aros, B. and Virágh, S.Z., 1970. Fine structure of photoreceptor cells in the earthworm, Lumbricus terrestris. Z. Zellforsch., mikrosk. Anat. 104, 345-357.

Rosenbaum, J.L. and Child, F.M., 1967. Flagella regeneration in protozoan flagellates. J. Cell Biol., 34, 345-364.

Rosenbaum, J.L., and Carlson, K., 1969. Cilia regeneration in Tetrahymena and its inhibition by colchicine. J. Cell Biol., 40, 415-425.

Rosenluth, J. 1963. Contrast between osmium-fixed and permanganate-fixed toad spinal ganglia. J. Cell Biol., 16, 143-157.

Rosanbluth, J., 1965. Ultrastructure of somatic muscle cells in Ascaris lumbricoides. II. Intermuscular junctions, neuromuscular junctions and glycogen stores. J. Cell Biol., 26, 579-591.

Rosenbluth, J., 1968. Obliquely striated muscle. iv. Sarcoplasmic reticulum, contractile apparatus, and endomysium of the body muscle of a Polychaete, Glycera, in relation to its speed. J. Cell Biol., 36, 245-259.

Roth, L.E. and Shigenaka, Y., 1964. The structure and formation of cilia and filaments in rumen protozoan. J. Cell Biol., 20, 249-270.

Rubin, E., Hutterer, F. and Lieber, C.S., 1968. Ethanol increases hepatic smooth endoplasmic reticulum and drug metabolizing enzymes. Science, N.Y. 159, 1469-1470.

Sabatini, D.D., Bensch, K. and Barrnett, R.J., 1963. Cytochemistry and electron microscopy. The preservation of cellular ultrastructure and enzymatic activity by aldehyde fixation. J. Cell Biol., 17, 19-58.

Sabnis, D.D. and Jacobs, W.P., 1967. Cytoplasmic streaming in the coenocytit marine alga Caulepa prolifera. J. Cell Sci., 2, 465-472.

Saint-Joseph, B. de, 1887. Les Annélides Polychaètes des côtes de Dinard. Annls. Sci. nat. Zool., 7, ser. 1, 127-270.

Salensky, W., 1882. Études sur le développement des annélides. 1. Psymobranchus. 2. Nereis. Archs Biol., 3, 345-378.

Salensky, W., 1883. Études sur le développement des Annélides. 3. Pileolaria, Aricia, Terebella. Archs Biol., 4, 143-261.

Santer, R.M. and Laverack, M.S., 1971. Sensory innervation of the tentacles of the Polychaete Sabella pavonina. Z. Zellforsch. mikrosk. Anat., 122, 160-171.

Sars, M., 1845. On the development of the Annelids. Ann. Mag. nat. Hist., 16, 183-188.

Satir, P., 1962. On the evolutionary stability of the 9+2 pattern. J. Cell Biol., 12, 181-184.

Satir, P., 1965. Structure and function in cilia and flagella-facts and problems. Protoplasmalogia, 3:1,1-52.

Schmitt, E. and Samson, F.E., 1968. Neuronal fibrous proteins. Neurosciences. Res. Prog. Bull., 6, 113-219.

Schochet, S.S., Lampert, P.W. and Earle, K.M., 1968. Neuronal changes induced by intrathecal vincristine sulfate. J. Neuropath. exp. Neurol., 27, 645-658.

Schultz, R.L. and Karlsson, U., 1965. Fixation of the central nervous system for electron microscopy by aldehyde perfusion. II. Effect of osmolarity, pH of perfusate and fixative concentration. J. Ultrastruct Res., 12, 187-206.

Segrove, F., 1940. The development of the Serpulid Pomatoceros triqueter L. Q. Jl. Micros Sci., 82, 467-540.

Shearer, C., 1911. On the development and structure of the trochophore of Hydroides uncinatus (Eupomatus). Q. Jl. Micros Sci., 13, 543-590.

Sjöstrand, F.S., 1956. The ultrastructure of cells as revealed by the electron microscope. Int. Rev. Cytol., 5, 455-533.

Sleigh, M.A., 1962. The biology of cilia and flagella. Oxford: Pergamon Press.

Smith, U., 1970. The origin of small vesicles in neurosecretory axons. Tissue and Cell, 2, 427-433.

Smith, U., Smith, D.S. and Yunis, A.A., 1970. Chloramphenicol-related changes in mitochondria ultrastructure. J. Cell Sci., 7, 501-521.

Smith-Johannsen, H. and Gibbs, S.P., 1972. Effects of chloramphenicol on chloroplast and mitochondrial ultrastructure in Ochromonas danica. J. Cell Biol., 52, 598-614.

Sorokin, S., 1962. Centrioles and the formation of rudimentary cilia by fibroblasts and smooth muscle cells. J. Cell Biol., 15, 363-377.

Sotelo, J.R. and Trujillo-Cénoz, O., 1958. Electron Microscopic study on the development of ciliary components of the neural epithelium of the chick embryo. Z. Zellforsch. mikrosk. Anat., 49, 1-12.

Strauss, E.W., 1964. Fat absorption from oil-in-water emulsions by sacs of everted intestine from golden hamster in vitro. J. Cell Biol., 20, 175-197.

Saemundsson, B., 1918. Bidrag til Kundskaben om Islands polychaete Børsteorme (Annulata Polychaete Islandiae). Vidensk. Meddr dansk. Naturh. Foren., 69, 163-241.

Tandler, B. and MacCallum, D.K., 1972. Ultrastructure and histochemistry of the submandibular gland of the European hedgehog. Erinaceous europaeus. L. J. Ultrastruct. Res., 39, 186-204.

Tennyson, V.M., 1970. The fine structure of the axon and growth cone of the dorsal root neuroblast of the rabbit embryo. J. Cell Biol., 44, 62-79.

Thorson, G., 1946. Reproduction and larval development of Danish marine bottom Invertebrates. Meddr Danm. fisk. -og Havunders. Ser. Plankton., 4, 1-523.

Tilney, L.G., 1968. Studies on the microtubules in Heliozoa. IV. The effect of colchicine on the formation and maintenance of the axopoda of Actinosphaerium nucleofilum (Barnett). J. Cell Sci., 3, 549-562.

Tilney, L.G. and Gibbons, J.R., 1969. Microtubules in the formation and development of the primary mesenchyme in Arabacia punctulata. II. An experimental analysis of the role of these elements in the development and maintenance of cell shape. J. Cell Biol., 41, 227-250.

Tomita, T., 1970. Electrical activity of vertebrate photoreceptors. Q. Rev. Biophys., 3, 179-222.

Torack, R.M., 1965. The extracellular space of rat brain following perfusion fixation with glutaraldehyde and hydroxyadipaldehyde. Z. Zellforsch., mikrosk. Anat. 66, 352-364.

Torack, R.M., 1966. The penetration of thorotrast into brain spaces following osmium, glutaraldehyde and hydroxyadipaldehyde fixation. J. Ultrastruct. Res., 14, 590-601.

Tormey, J. McD. 1964. Differences in membrane configuration between osmium tetroxide-fixed and glutaraldehyde-fixed ciliary epithelium. J. Cell Biol., 23, 658-664.

Treadwell, A. 1901. The cytogeny of Podarke obscura verrill. J. Morph., 17, 399-486.

Trier, J.S., 1963. Studies on small intestine crypt Epithelium. 1. The fine structure of the crypt epithelium of the proximal small intestine of fasting humans. J. Cell Biol., 18, 599-620.

Twomey, S.L. and Samson, F.E., 1972. Tubulin antigenicity in brain particulates. Brain Res., Osaka, 37, 101-108.

Van Harreveld, A. and Khattab, F.I., 1969. Changes in extracellular space of the mouse cerebral cortex during hydroxyadipaldehyde fixation and osmium tetroxide fixation. J. Cell Sci., 4, 437-453.

Venable, J.H. and Coggeshall, R., 1965. A simplified lead citrate stain for use in electron microscopy. J. Cell Biol., 25, 407-408.

Weston, J.C., Ackerman, G., Grieder, M.H. and Nikolewski, R.F., 1972. Nuclear membrane contributions to the Golgi complex. Z. Zellforsch., mikrosk. Anat 123, 153-160.

White, R.H. and Walther, J.B., 1969. The leech photoreceptor cell: Ultrastructure of clefts connecting the phaosome with extracellular space demonstrated by lanthanum deposition. Z. Zellforsch. mikrosk. Anat., 95, 102-108.

Wiener, J., Spio, D. and Lowenstein, W.R., 1965. Ultrastructure and permeability of nuclear membranes. J. Cell Biol., 27, 107-117.

Williams, N.E. and Luft, J.H., 1968. Use of a nitrogen mustard derivative in fixation for electron microscopy. J. Ultrastruct. Res., 25, 271-292.

Wilson, D.P., 1928a. The post-larval development of Lomia medusa. J. mar. biol. Ass. U.K., 15, 129-146.

Wilson, D.P., 1928b. The larvae of Polydora ciliata and Polydora hoplura. Ibid., 15, 567-603.

Wilson, D.P., 1929. The larvae of the British Sabellarians. Ibid., 16, 221-268.

Wilson, D.P., 1932a. The development of Nereis pelagica. Ibid., 18, 203-217.

Wilson, D.P., 1932b. On the mitraria-larva of Owenia fusiformis. Phil. Trans. R. Soc. B, 221, 231-334.

Wilson, D.P., 1933. The larval stages of Notomastus latericeus. J. mar. Biol. Ass. U.K., 18, 511-518.

Wilson, D.P., 1936a. The development of the Sabellid Branchiomma vesiculosum. Q. Jl Microsc. Sci., 78, 543-603.

Wilson, D.P., 1936b. The development of Amphitrite tentaculata. J. mar. Biol. Ass. U.K., 20, 567-579.

Wilson, E.B., 1882. Observations on the early development stages of some polychaetous Annelids. Stud. Biol. Lab. Johns Hopkins Univ. 2, 271-299.

Wilson, E.B., 1890. The origin of mesoblast bands in Annelids. J. Morph., 4, 205-219.

Wilson, E.B., 1892. The cell-lineage of Nereis. J. Morph., 6, 361-480.

Wood, R.L., 1959. Intercellular attachment in the epithelium of Hydra, as revealed by electron microscopy. J. biophys. biochem Cytol., 6, 343-352.

Wood, R.L. and Luft, J.H., 1965. The influence of buffer systems on fixation with osmium tetroxide. J. Ultrastruct. Res., 12, 22-45.

Woollacott, R.M. and Zimmer, K.L., 1972. Fine structure of a potential photoreceptor organ in the larva of Bugula neritina (Bryozoa). Z. Zellforsch. mikrosk. Anat., 123, 458-469.

Yamamoto, I., Nakagawa, T. and Nakagawa, S., 1969. Comparative study of buffered, neutralized and unbuffered formalin with respect to preservation of ultrastructure. J. Electron Microsc. Chiba Cy 18, 308-311.

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Presumptive Photoreceptor Structures of the Trochophore of *Harmothoë imbricata* (Polychaeta)

P. L. HOLBOROW and M. S. LAVERACK

Department of Natural History and Gatty Marine Laboratory
University of St. Andrews, St. Andrews, Scotland

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The behaviour of the *Harmothoë* trochophore changes with age, the larva being phototropic initially and later photonegative.

The trochophore possesses two ocelli midway between the prototroch and the apex in a mid-lateral position. They appear first at the eighth day of development and grow to be kidney-shaped structures. There is a pigment cup derived from a single cell that encloses a rhabdomeric type of photoreceptor apparatus that is also derived from a single (or rarely two) cell.

In the late trochophore (14 days old) an organ of different origin and formation but of presumed photoreceptor type begins to develop among nerve cell bodies below the apex of the animal. This structure consists of an array of membranes developed from both cilia and microvilli. The cilia are of 9 + 2 configuration.

INTRODUCTION

Although the number of electron microscope studies of Annelid eyes steadily grows, there are as yet relatively few on the eyes of Polychaetes (Dhainaut-Courtois, 1965; Dorsett and Hyde, 1968; Eakin and Westfall, 1964; Fischer and Brökelmann, 1966; Hermans and Cloney, 1966; Hermans, 1969; Kernéis, 1966, 1968; Lawrence and Krasne, 1965; Krasne and Lawrence, 1966). Of these, only Eakin and Westfall describe a trochophore eye, that of *Neanthes succinea*. Fischer and Brökelmann describe the eye of the juvenile worm of *Platynereis dumerilii* but not that of the trochophore. The other work is on adults.

Although the reports are few, they illustrate a variety of photoreceptor structures, especially the nature of photoreceptor membranes, some of which are of ciliary origin whilst others are derived from microvilli. Annelids with ciliary photoreceptors are often treated as exceptions to the general rule that rhabdomeric eyes are characteristic of coelenterates, annelids and arthropods.

The present study is of some significance in this discussion as it gives an account of presumed photoreceptors of both types in one animal. There is also behavioural evidence that suggests difference of function of the two types of photoreceptor, a feature of considerable importance in explaining exceptions to the scheme of lineage in basic photoreceptor organization, proposed by Eakin in 1963.

Some attention is given to variations in structure due to different methods of fixation, as a further contribution to discussions on this subject in connection with eyes (Hermans, 1969; Hughes, 1970; Kabuta, Tominaga and Kuwabara, 1968; Röhlich, 1966; Tormey, 1964).

MATERIAL AND METHODS

Adult specimens of *Harmothoë imbricata* were collected in St. Andrews Bay (Scotland) and maintained in the laboratory in individual containers. The water was changed daily and the animals were fed on small portions of fresh *Buccinum*. The temperature at which they were kept averaged +10°C. Animals were found with developing eggs at various stages under their elytra. Some males were kept in the same container as females and these animals coupled and mated. The females brooded the fertilized eggs under their elytra for about fourteen days. Samples of these embryos were examined every second day during development. Fixations were made every two days by measuring one volume of animals in sea water into a container and adding an equal volume of chilled 2% osmium tetroxide. Fixation was carried out in the cold for ½ hour. Fully developed trochophores were fixed in this manner and also by a variety of other methods which were found to be less satisfactory. Glutaraldehyde buffered to pH 7.4 with cacodylate, and 1% acrolein also buffered with cacodylate, each followed by a buffered wash and ½–1 hour post fixation with cacodylate buffered osmium gave some interesting results which are illustrated.

The specimens were dehydrated in acetone and embedded in Epon. Sections were cut on an LKB ultramicrotome, picked up on unfilmed grids, and stained for 3 minutes in lead citrate followed by 3 minutes in 2% uranyl acetate (Venable and Coggeshall, 1965). The sections were examined in an AEI EM6B electron miscroscope.

Live animals were examined and photographed using Nomarski phase contrast.

For scanning electron microscopy, osmium fixed specimens were dehydrated in alcohol to 100%, transferred to amylacetate and dried in a critical Point Drier using the principle of Anderson (1951). They were coated with gold/palladium in the Zoology Department of the University of Edinburgh and viewed with the Cambridge Scientific Instruments "Stereoscan" scanning electron microscope of the Department of Engineering, University of Edinburgh.

RESULTS

A. The Larval Eye

The two larval eyes are situated on the sides of the upper part of the trochophore about 15μ above the top of the prototroch. The eye first appears on the eighth day of development while the embryonic trochophores are still attached together in a mass under the elytra of the parent worm. The eye starts as a thin crescent then appears as an arch (ninth day, Figure 1) and by the fourteenth day is fully developed (Figure 2) with a pigment cup that faces forwards and upwards.

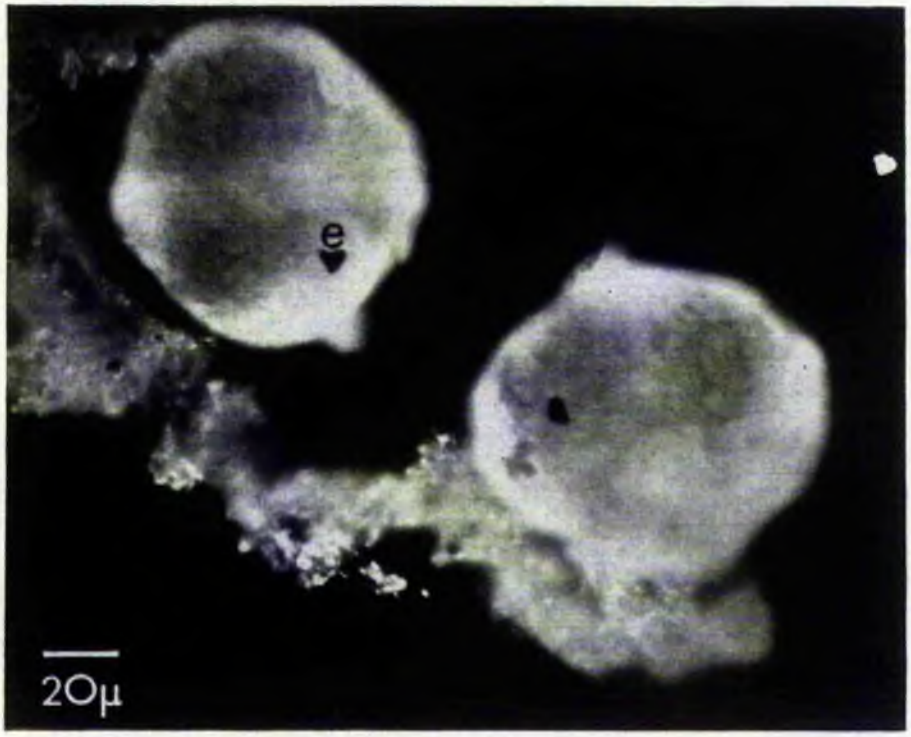


FIGURE 1 Nine-day-old trochophore with "arched" eye (e).

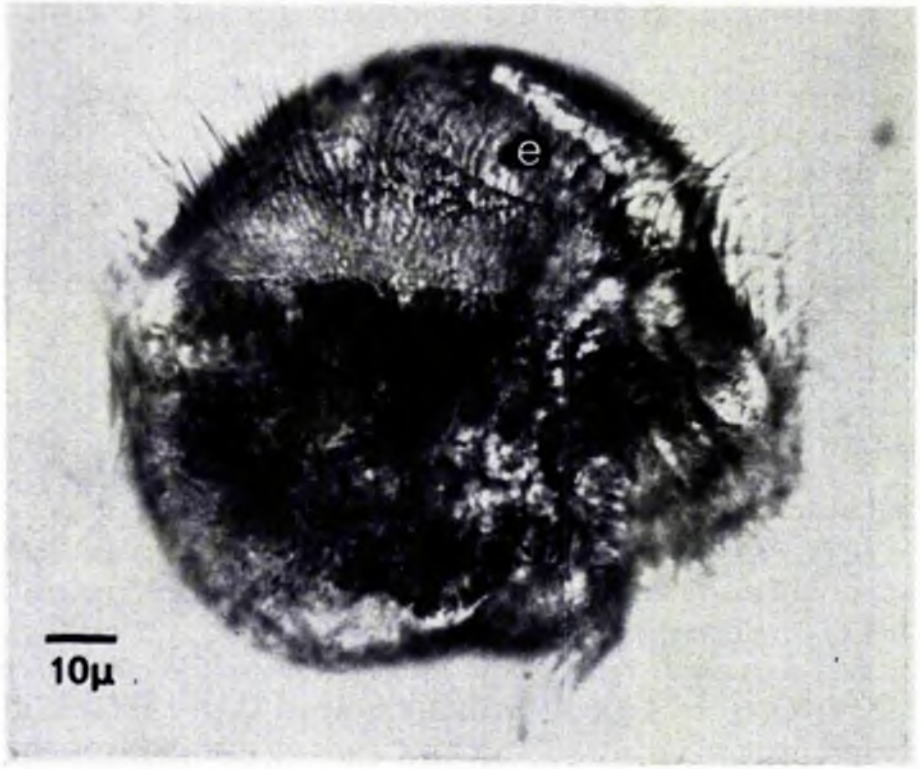


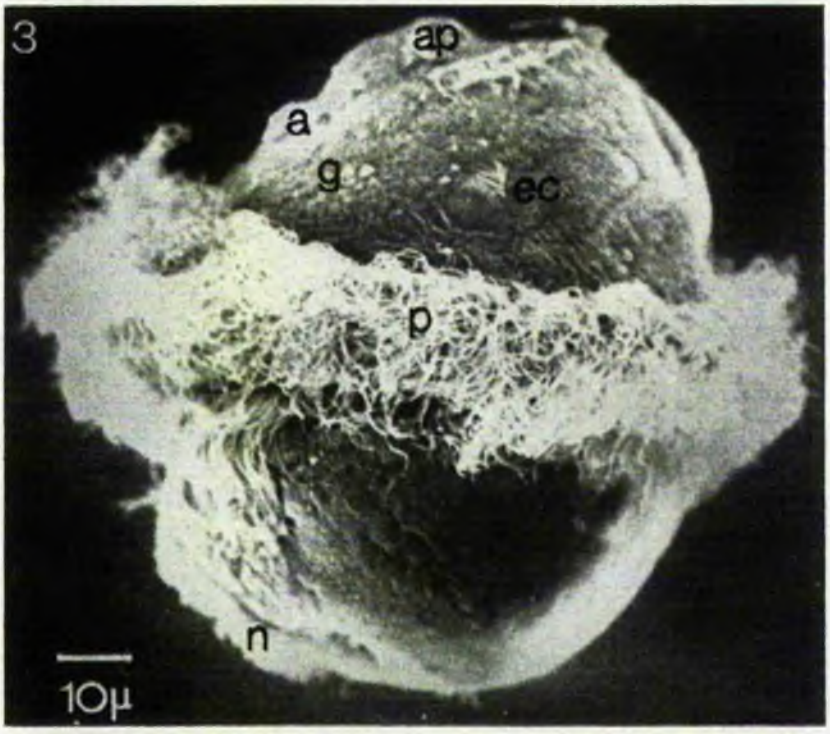
FIGURE 2 Fifteen-day-old trochophore with large eye (e).

Glands and cilia lie close to the eye (Figures 3 and 4). Although in the scanning electron micrograph there seem to be only a few cilia, these are in fact clusters of cilia, with up to approximately 180 cilia present in each collection (Figure 5). The cilia close to the trochophore eye have a basal foot and a short lateral root, 0.2μ long, curving under the basal body of the next adjacent cilium, and a fine longitudinal root extending 1.2μ into the cell. The cilia are uniformly arranged with central pairs of filaments and basal feet that point away from the eye (Figure 5). The trochophore cilia therefore probably all beat away from the eye towards the back of the trochophore.

The eye itself is of inverted type (Figure 6). It consists of a pigment cup produced by a single cell and either one or two photoreceptor cells. A cilium was observed extending from a receptor cell of the larval eye during development but it was in no way connected with the membranes forming the retina.

The pigment cup The pigment cup is 2–3μ deep and made up of granules 0.5μ in diameter packed in rows 3–5 to a row. The granules are membrane bound and interspersed with some clusters of ribosomes, some rough endoplasmic reticulum, clear vesicles (700 nm) and a few mitochondria and golgi figures. The pigment layer extends to 0.5μ below the cuticle and is bounded all round by a layer of cytoplasm containing the above-mentioned inclusions.

Immature pigment granules contain a uniform grey granular matrix. The nucleus of the pigment cells lies directly beneath the eye cup and is closely



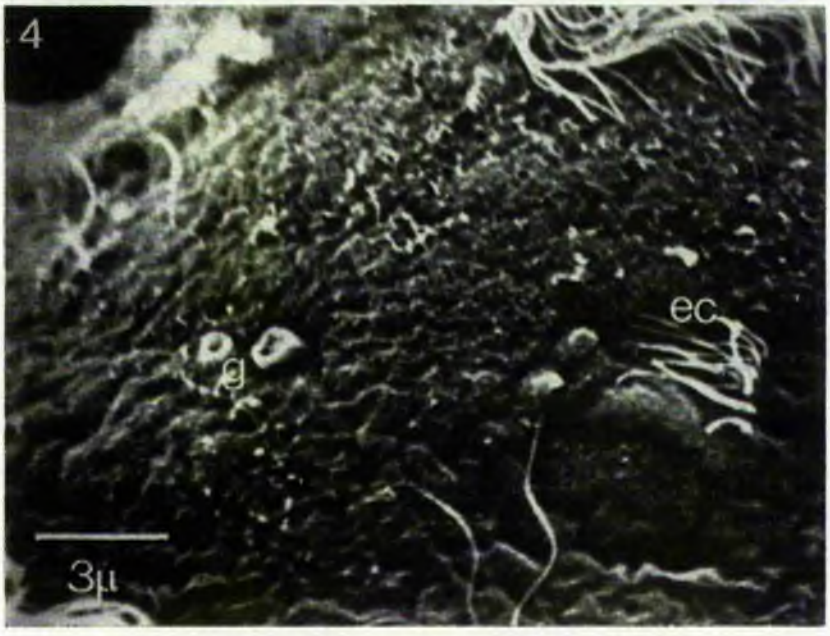


FIGURE 3 Scanning electron micrograph showing the side of a trochophore. Note prototroch (p), neurotroch (n), akrotroch (a), apical (ap) and eye (ec) cilia. Pores of glands (g) may also be seen.

FIGURE 4 Scanning electron micrograph of the eye region of the trochophore. Gland pores (g) lie on the anterior side of the eye and the cilia (ec) lie behind.

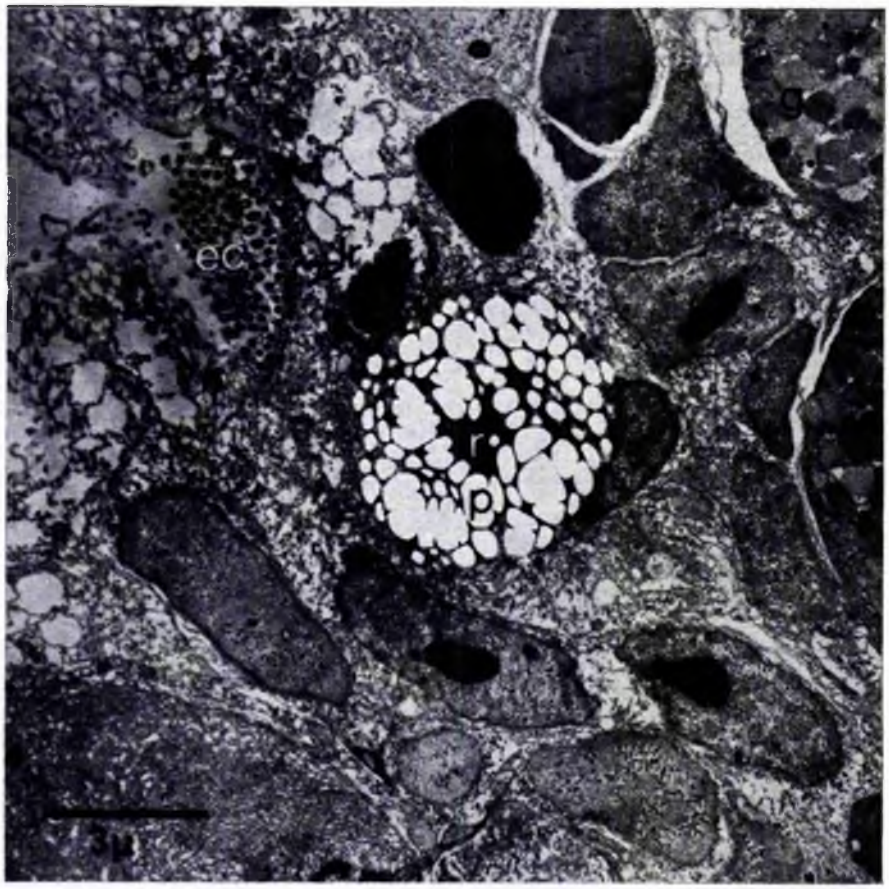


FIGURE 5 Transverse section through the upper part of the trochophore showing the gland (g), pigment cup of the eye (p), some of the receptor apparatus (r), and the eye cilia (ec). Note lateral roots (lr) which are opposite the basal feet.

bounded by the cell membrane such that the layer of cytoplasm surrounding it is 0.2μ thick and sometimes less. The nucleus is slightly broader at the top than at the base, 4μ wide at its broadest point and 6μ long.

The receptor cell The receptor cell occupies the full depth of the ectoderm, about 15–20μ (see Figure 7), and gives rise to an axon that extends along the extoderm side of the blastocoel. This axon enters a bundle of other nerve fibres that pass from the apex to the prototroch.

The cell can be divided into three regions apart from the axon. First, the basal region; this is the broadest part of the cell, 8μ across, and is almost entirely filled by the nucleus, which is squat and oval, 7.5 × 5.5μ. The cytoplasm around the nucleus contains mitochondria, ribosomes, rough-surfaced endoplasmic reticulum, lipid droplets and yolk granules if these are still present. This region of the cell partly enfolds the pigment-cell nucleus lying below the pigment cup. At the level of the pigment-cell nucleus, the receptor cell narrows to a 2μ diameter neck. In this area are golgi bodies, myelin figures,

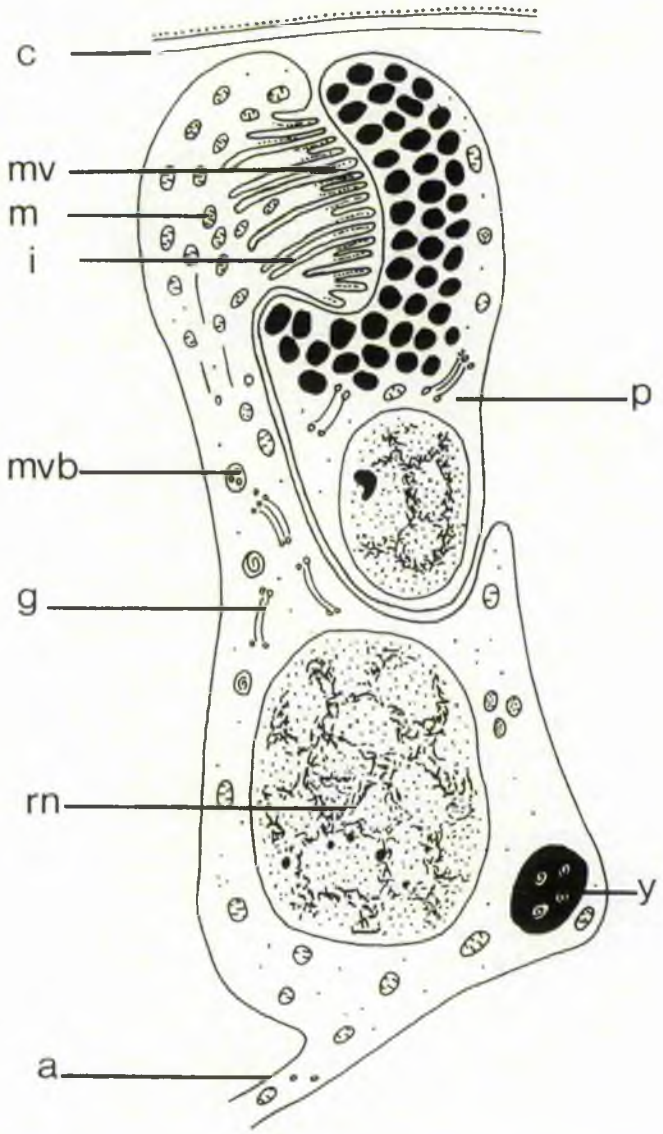


FIGURE 6 Diagrammatic representation of a longitudinal section through the pigmented larval eye; a, axon; c, cuticle; mvb, multivesticular body; g, Golgi body; i, invaginated membranes; m, mitochondria; mv, microvilli; rn, nucleus of photoreceptor; p, pigment cell; y, yolk droplet.

endoplasmic reticulum, ribosomes and small vesicles, possibly originating from the golgi apparatus. There are also multivesicular bodies of problematic function but also found in a number of other invertebrate eyes (Fahrenbach 1964; Eakin and Westfall, 1964; MacRae, 1966; and others).

The upper region of the cell is again broad although at this point half of the breadth is made up of receptor membranes. These fill the pigment cup and lie at right angles to the incoming light. Adjacent to the photosensitive apparatus

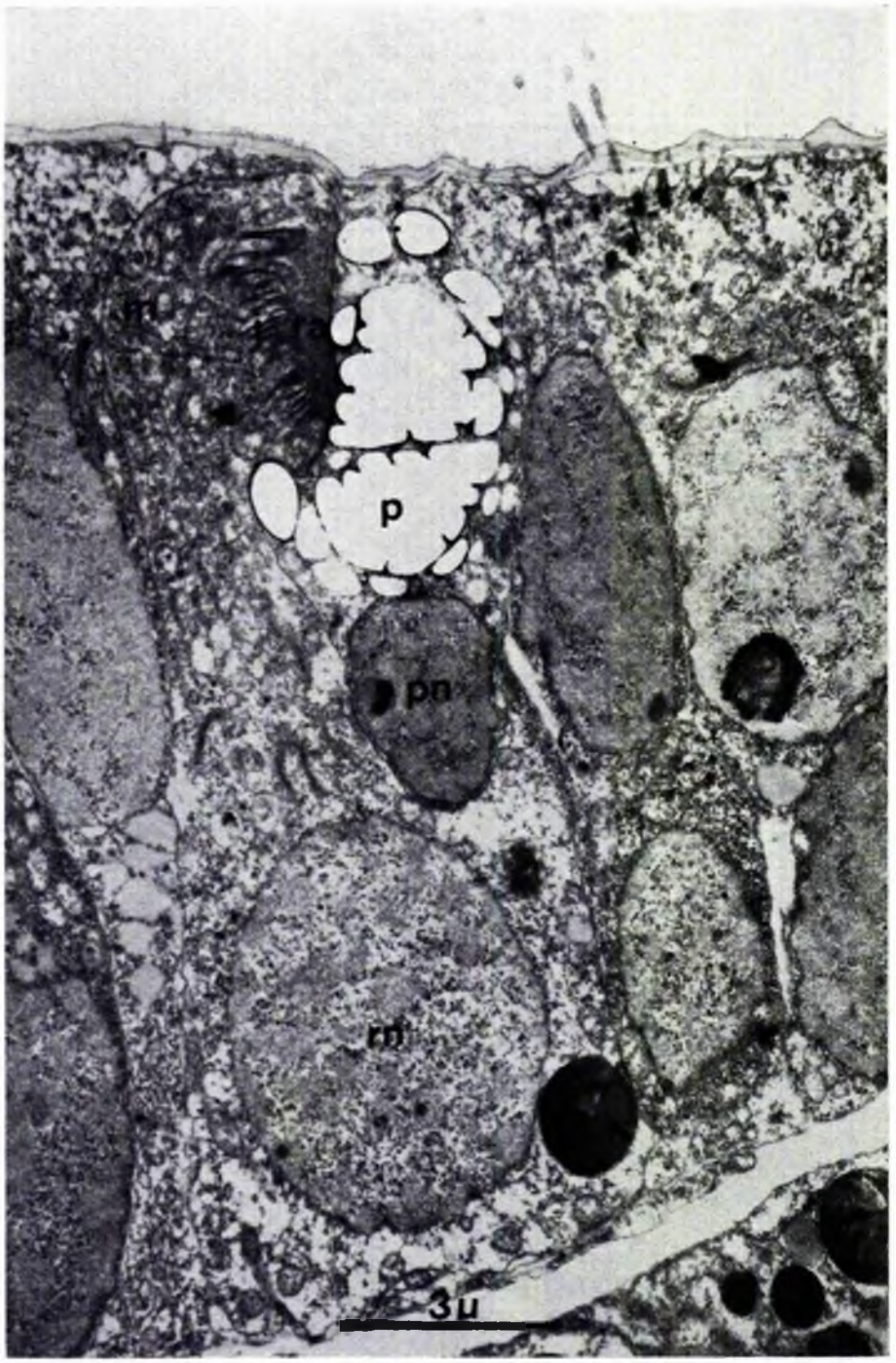


FIGURE 7 Sagittal cut through the eye of the trochopore. Pigment cup (p), pigment cell nucleus (pn), receptor cell nucleus (rn), invaginated membrane (i), receptor apparatus (ra), mitochondria (m).

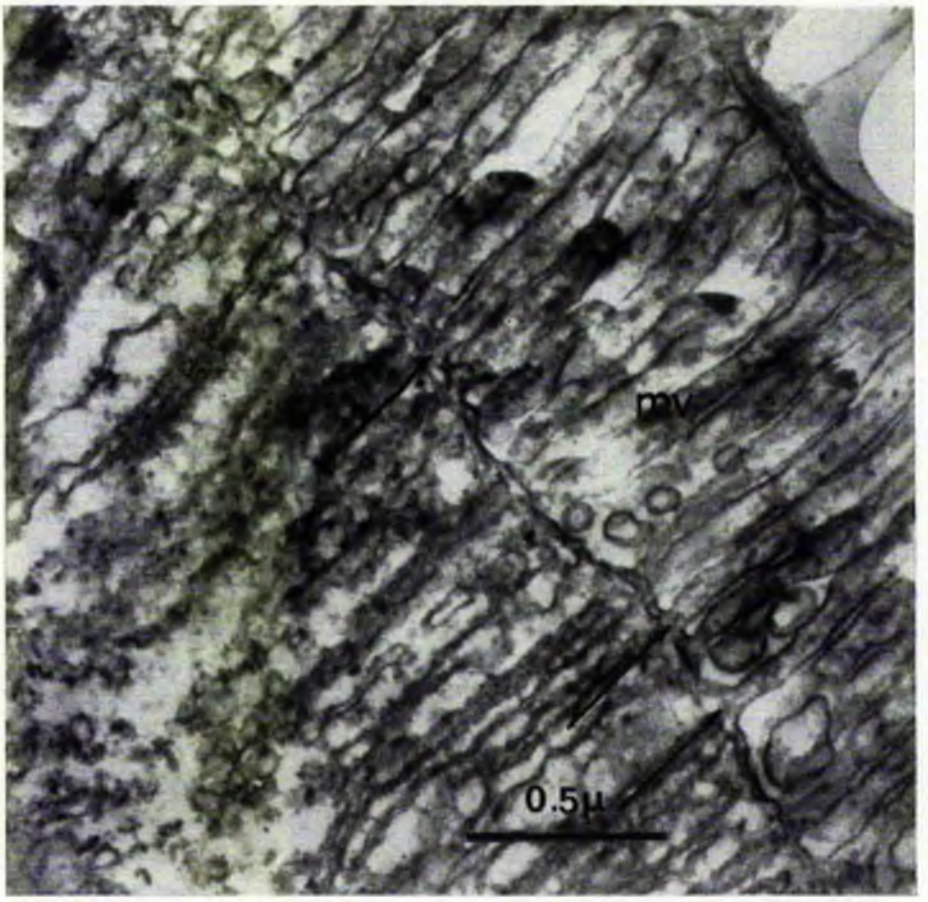


FIGURE 8 The receptor apparatus of the trochophore eye showing the microvilli (mv) and invaginated membranes. Arrows indicate connection between the two layers.

is a dense concentration of mitochondria, ribosomes and rough endoplasmic reticulum.

The photoreceptor apparatus is in two layers (Figures 7, 8 and 9). The layer which abuts the pigment cup consists of rows of microvilli 0.1μ in diameter and 1.2μ long (Figure 8). They have a trilaminar unit membrane. Although they are closely packed, the membranes do not fuse to form a five-layered structure as found in rhabdomeric eyes, as illustrated by Boyle (1969). The microvilli have an electron-dense core. Direct osmium fixation usually causes these microvilli to acquire the appearance of tubules of varying diameter and vesicles 100 Å to 200 Å in diameter (Figure 9).

The inner layers of membranes appear to be invaginations of the cell surface and at their inner ends associate with endoplasmic reticulum (Figure 9). They are usually aligned with the microvilli, straight and parallel, but they may be found set at angles and curved (Figure 7). They occur as pairs of membranes grouped into sets of two pairs with a more electron dense matrix

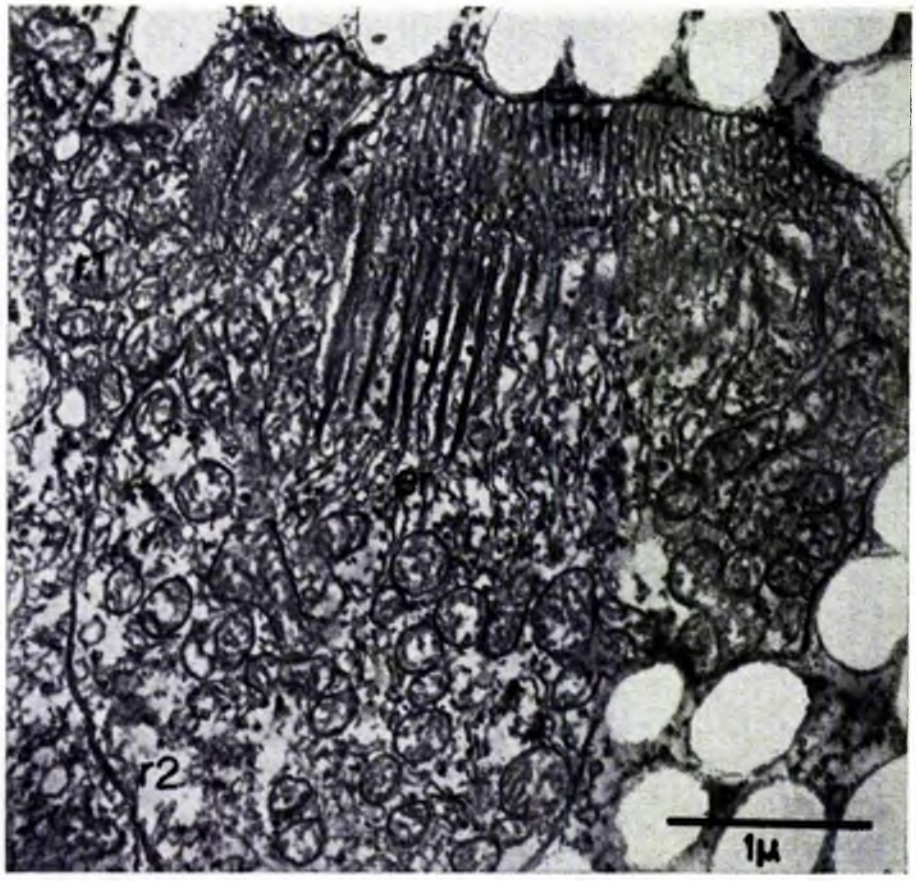


FIGURE 9 Two receptor cells (r1, r2) contribute to this eye with a desmosome (d) between them. The microvillous layer (mv) is in the form of tubules. The invaginated membranes (i) are confluent with rough endoplasmic reticulum (er).

between them than the normal cytoplasm (Figure 8). Where groupings of more than four occur (Figure 7) the cytoplasm between the groups is especially "thin" and the impression is one of shrinkage, that is, an artefactual grouping. The normal condition would appear to be a separation of two sets of pairs by a distance of 160 Å to 200 Å, and between groups by a distance of 660 Å (Figure 9). The membranes of the pair are themselves about 100 Å apart, very similar to the outer membrane of mitochondria, but they flare out at a depth of 1 to 2μ into the cell into closed "extracellular" cisternae of the granular endoplasmic reticulum. The continuity of the microvilli and invaginated membranes is not easily demonstrated because of the complexity of three dimensional projections of inward and outward folding of surface membrane. In Figure 8, however, the core of some microvilli may be seen to be continuous with the dense layer between the invaginated membranes.

A bundle of six axons occurs central to the visual cell. They are 0.5μ to 1μ in diameter, contain microtubules, mitochondria, clear vesicles, and some

possess dense cored vesicles. Thickened membranes and a collection of vesicles against the membrane of one axon indicated possible synaptic sites. The exact origin and destination of the axons is unknown.

B. The Central Visual Organelle

Among nerve cell bodies below the apex of the trochophore is an organelle composed of cilia and microvilli. Although this structure differs from the eyes of the trochophore and those of the adult *Harmothoë*, it bears sufficient resemblance to photoreceptors in other annelids to be termed a visual organelle. It has some features similar to those of a photoreceptor in the brain of *Nereis pelagica* (Dhainaut-Courtois, 1965) and also those of the recently described *Lumbricus* eye (Röhlich, Aros and Virágh, 1970).

The organelle is derived from one cell or more frequently from two cells and it is partly surrounded by extension of these cells but also by supporting cells. Some sections of early stages show the structure surrounded entirely by a parent cell.

In the presumptive sensory cells two centrioles may occur at right angles, and there are small rootlets at the base of the cilia. Both cilia and microvilli are present together. The cilia grow upwards and divide, with some filaments passing into each arm (Figure 10). At this time microvilli are more abundant. The rootlets of the cilia are later lost and no horizontal centrioles are ever found in older structures. Basal feet also are found only in young stages. Microtubules project in a star-like array from the basal body and at all stages these may be found randomly scattered in the cytoplasm of this part of the cell although at later stages they tend to be orientated roughly parallel with the bases of the cilia and the cell membrane (Figure 11).

Up to 30 cilia contribute to the mature organelle as illustrated in Figure 11. They project from narrow necks of two adjacent cells and coil together into a mass. Microvilli grow outwards into the space surrounded by the cells when they enfold the mass in its early stages. They are occasionally found broken into vesicles and fine tubules as tends to happen with annelid visual microvilli subjected to direct osmium fixation. Using acrolein and glutaraldehyde as fixatives, whorled membranes are the predominant feature (Figures 12 and 13).

The cilia are unlike other cilia found in the trochophore, particularly in the basal region (Figure 14). The basal bodies are 0.6μ long below the basal plate and tend to look a little splayed. The basal plate is a very dark band 300 Å thick; below it is a 0.2μ long, less dense bar and above it are three centrally placed moderately electron dense patches 200 Å across and 0.1μ apart.

The cilia have a 9 + 2 pattern of filaments at the base. Distal to this the appearance is of 9 + 0. The ciliary filaments disappear within 4μ above the basal bodies. The central pair disappear first then the peripheral fibres remain

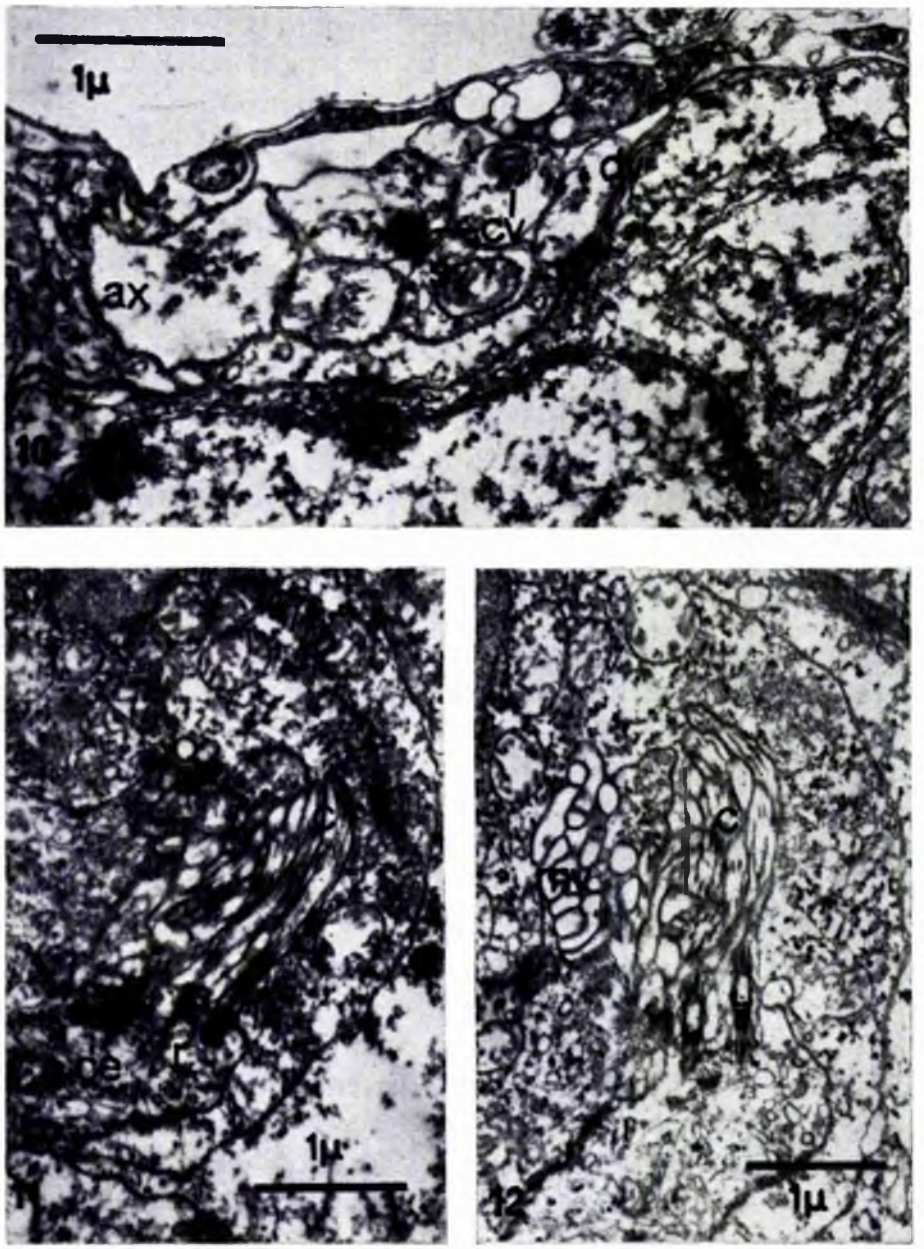


FIGURE 10 Axon bundle beneath the eye with synapse (s) between two axons, dense cored vesicles (dv) and clear vesicles (cv).

FIGURES 11, 12 Diagrammatic representation of two sections from the same area of the mid-apical, unpigmented organelle at an early stage of development. Two centrioles (ce) at right angles to each other occur at this stage and not in later stages. Rootlets (r) and cilia (c) are present.

as pairs until they terminate, which they do at different levels (Figure 15). This differs from cilia termination as normally seen in the trochophore (Holborow unpublished) and from that described by Satir (1965) for lamellibranchs in which the central pair is lost first, and the peripheral fibres become singlets

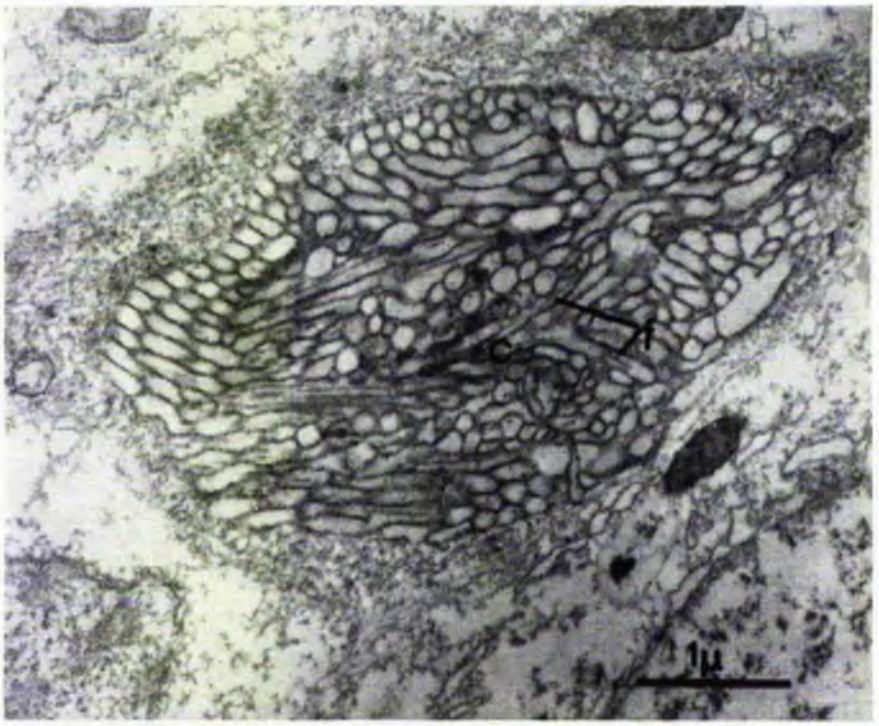


FIGURE 13 Transverse section of the organelle showing a divided cilium (c) with filaments (f) passing into each arm.

then terminate. With acrolein fixation there tends to be less coherence of the fibres of the cilia of this organelle, and above the basal body they may disappear entirely or a few may remain as short, irregular lengths (Figure 16).

DISCUSSION

A definitive characteristic of eyes, whether vertebrate or invertebrate, is the presence of an enlarged surface area produced by microvilli or membranes derived in association with membranes of cilia. In 1963 Eakin set out an hypothesis that among annelids, arthropods and molluscs the photoreceptor is of rhabdomeric type, derived from microvilli, and that the echinoderm–coelenterate–vertebrate line of animals have photoreceptor membranes derived from cilia. A number of exceptions, anticipated by Eakin (1965), have been found among the invertebrates and are reviewed by Krasne and Lawrence (1966), and Laverack (1968). There is now increasing discussion on whether or not a ciliary photoreceptor functions differently from one derived from microvilli. Electrophysiological investigation of the ciliary based photoreceptors of *Pecten* (Hartline and Graham, 1938; Land, 1966) and of *Cardium* (Barber and Land, 1967) showed that these photoreceptors give a primary "off" response. Dorsett and Hyde (1968) and Barber and Wright (1969) both

FIGURE 14 Diagrammatic representation of an oblique section of the basal region of the organelle showing 9 + 2 and 9 + 0 configurations of cilia (arrows). The basal bodies (bb) of the cilia have microtubules (mt) radiating from them.

compare the shadow reflexes of these lamellibranch molluscs with that of *Branchionma* and correlate the ciliary photoreceptors with "off" responses and reaction to moving shadows. Boyle (1969) remarked on the shadow reflex of *Onithochiton*, a response which could be mediated with the ciliary lamellate bodies accessory to the rhabdomeric eye. Hughes (1970) suggested that the ciliated cells in *Aplysia* eyes may also have this specialized function.

A considerable effort was made to examine the trochophore by electrophysiology. On two occasions only a microelectrode in the midapical region recorded a single primary "off" response. While it is tempting to accept this finding as a valid recording from a visual organelle, it was not possible to determine whether it was in fact made in the ciliary organelle described here. However, the behaviour patterns of the trochophore indicate that it is possible that the two types of visual organelle monitor different responses towards light. In the first few days after release, the trochophore swims towards the light. This reaction lessens, parallel with the development of the apical organ-

FIGURE 15 Mature organelle in longitudinal section. The cilia (c) have no roots; mv, microvilli. Osmium fixation.

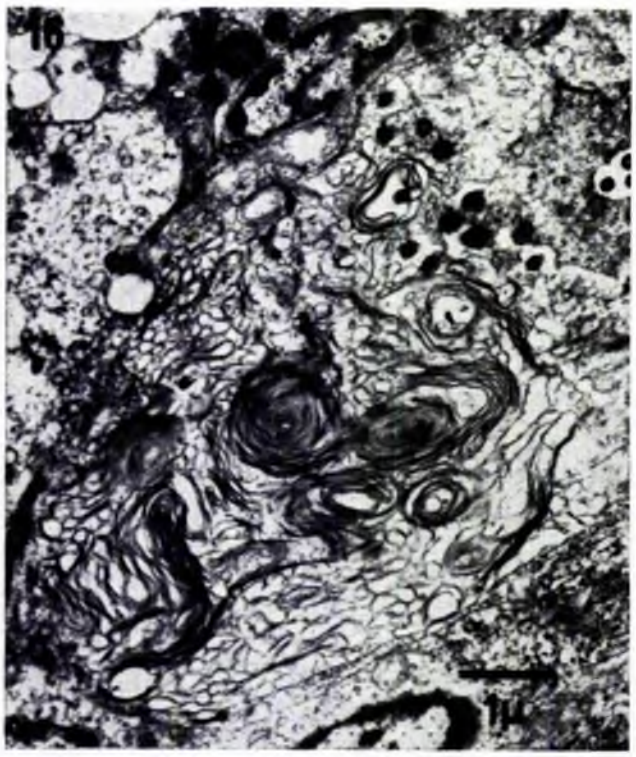


FIGURE 16 Unpigmented central organelle fixed in acrolein.

elle, and is finally reversed. On the evidence presented here it is suggested that the rhabdomeric organelle monitors the positive light response and the ciliary one the negative response.

The anatomical difference between types of eyes may be real, but some descriptions indicate that fixation artefacts are drastic in occurrence and frequency. For example, many of the annelid eyes so far described show curiosities in the form of microvilli; e.g. large vacuoles in *Nereis virens* (Dorsett and Hyde, 1968), or breakdown of the microvilli into an irregular cluster of vacuoles and tubules (Eakin and Westfall, 1964). Eakin and Westfall recognized the structures as microvilli and made no comment on the distortion. As an adjunct to this it may be noted that the large numbers of vesicles in the receptor cell of the trochophore, *Neanthes succinea*, are very like fragments of endoplasmic reticulum which assume this form under certain conditions of fixation. Hughes (1970) illustrates similar effects to those in *Armandia* described previously on microvilli in *Aplysia*.

Fixation effects on membranes have long been under discussion. Tormey (1964) compared osmium and gluteraldehyde fixation of ciliary epithelia in the eye of the rabbit and concluded that glutaraldehyde is superior in preventing breakdown of continuous membrane surfaces. Röhlich (1966) showed that OsO_4 fixation of degenerating and regenerating planarian retinal clubs

resulted in serried vesicles and anastomosing tubules, whereas with gluteraldehyde there is a regular array of microvilli. Röhlich (1967) working on *Daphnia* found that exposure to light causes breakdown of the microvilli and further, that planarians in prolonged darkness suffered breakdown of microvilli into anastomosing tubules or rows of vesicles. The appearance of the degenerated planarian eyes is characteristic of the effects of osmium fixation, and this finding is disputed in detail by Kabuta, Tominaga and Kuwabara (1968).

The invaginated membranes produced by light exposure of *Daphnia* (Röhlich 1967) could be analogous to the trochophore invaginated membranes but no investigation has yet been made of light and dark adapted trochophore eyes. The invaginated cell membranes are similar in some respect to "furrows" of invaginated membrane at the base of the rhabdomere in the *Sapphirina* nauplius retinular cell (Elofsson, 1969), although the nauplius eye membranes are more closely packed and penetrate deeper into the cell. Eakin and Westfall (1964) found a similar feature in the eye of the trochophore of *Neanthes succinea*, but described it as a layer in which the microvilli are narrower than the outer layer of microvilli and in a parallel array. This is a curious interpretation of the evidence of the pictures, which show an outer layer of microvilli broken up into vesicles of various sizes and an inner layer of groups of approximately four membranes running in different directions into the cell, and interspersed with mitochondria and other cytoplasmic inclusions. The membranes are to all appearances intracellular and the same as the *Harmothoë* trochophore invaginated membranes.

Different fixation techniques have such different effects on visual epithelia that critical trials appear to be required with more than one fixation, in invertebrates at least, before definitive descriptions may be made.

Acknowledgements

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References

Anderson, T. F. (1951). Techniques for the preservation of three dimensional structure in preparing specimens for the electron microscope. *Trans. N.Y. Acad. Sci.* **13**, 130–134.

Barber, V. C. and Land, M. F. (1967). Eye of the cockle, *Cardium edule*: Anatomical and physiological investigations. *Experientia* **23**, 677–701.

Barber, V. C. and Wright, D. E. (1969). The fine structure of the eye and optic tentacle of the mollusc *Cardium edule*. *J. Ultrastruct. Res.* **26**, 515–528.

Boyle, P. R. (1969). Fine structure of the eyes of *Onithochiton neglectus* (Mollusca: Polyplacophora). *Z. Zellforsch.* **102,** 313–332.
Dhainaut-Courtois, N. (1965). Sur la présence d'un organe photorécepteur dans le cerveau de *Nereis pelagica* L. (Annélide polychaète). *C.R. Acad. Sci. Paris* **261,** 1085–1088.
Dorsett, D. A. and Hyde, R. (1968). The fine structure of the lens and photoreceptors of *Nereis virens*. *Z. Zellforsch.* **85,** 243–255.
Eakin, R. M. (1963). Lines of evolution of photoreceptors. In: *General physiology of cell specialisation* (ed. D. Mazia and A. Tyler). McGraw-Hill Book Co, New York pp. 393–425.
Eakin, R. M. (1965). Evolution of photoreceptors. *Cold Spr. Harb. Symp. Quant. Biol.* **30,** 363–370.
Eakin, R. M. and Westfall, J. A. (1964). Further observations on the fine structure of some invertebrate eyes. *Z. Zellforsch.* **62,** 310–332.
Elofsson, R. (1969). The ultrastructure of the nauplius eye of *Sapphirina* (Crustacea: Copepoda). *Z. Zellforsch.* **100,** 376–401.
Fahrenbach, W. H. (1969). The fine structure of a nauplius eye. *Z. Zellforsch.* **62,** 182–197.
Fischer, A. and Brökelmann, J. (1966). Das Auge von *Platynereis dumerilii*. *Z. Zellforsch.* **71,** 217–244.
Hartline, H. K. and Graham, C. H. (1938). The discharge of impulses in the optic nerve of *Pecten* in response to illumination of the eye. *J. Cell. Comp. Physiol.* **11,** 465–477.
Hermans, C. O. (1969). Fine structure of the segmental ocelli of *Armandia brevis* (Polychaeta: Opheliidae). *Z. Zellforsch.* **96,** 361–371.
Hermans, C. O. and Cloney, R. A. (1966). Fine structure of the prostomial eyes of *Armandia brevis* (Polychaeta: Opheliidae). *Z. Zellforsch.* **72,** 583–596.
Hughes, H. P. J. (1970). A light and electron microscope study of some Opisthobranch eyes. *Z. Zellforsch.* **106,** 79–98.
Kabuta, H., Tominaga, Y. and Kuwabara, M. (1968). The rhabdomeric microvilli of several Arthropod compound eyes kept in darkness. *Z. Zellforsch.* **85,** 78–88.
Kernéis, A. (1966). Photorécepteurs de panache de *Dasychone bombyx*. *C.R. Acad. Sci. Paris* **263,** 653–656.
Kernéis, A. (1968). Nouvelles données histochimiques et ultrastructurales sur les photorécepteurs "branchiaux" de *Dasychone bombyx* (Dagell) (Annélide Polychete). *Z. Zellforsch.* **86,** 280–292.
Krasne, F. B. and Lawrence, P. A. (1966). Structure of the photoreceptors in the compound eyespots of *Branchiomma vesiculosum*. *J. Cell. Sci.* **1,** 239–248.
Land, M. F. (1966). Activity in the optic nerve of *Pecten maximus* in response to changes in light intensity and to pattern and movement in the optical environment. *J. exp. Biol.* **45,** 83–99.
Laverack, M. S. (1968). On the receptors of marine invertebrates. *Oceanogr. Mar. Biol. Ann. Rev.* **6,** 249–324.
Lawrence, P. A. and Krasne, F. B. (1965). Annelid ciliary photoreceptors. *Science* **148,** 965–966.
MacRae, E. K. (1966). The fine structure of photoreceptors in a marine flatworm. *Z. Zellforsch.* **75,** 469–484.
Röhlich, P. (1966). Sensitivity of regenerating and degenerating planarian photoreceptors to osmium fixation. *Z. Zellforsch.* **73,** 165–173.
Röhlich, P. (1967). Fine structural changes induced in photoreceptors by light and prolonged darkness. *Symp. Neurobiol. Inverts.* 95–109.
Röhlich, P., Aros, B. and Virágh, Sz. (1970). Fine structure of photoreceptor cells in the earthworm *Lumbricus terrestris*. *Z. Zellforsch.* **104,** 345–357.
Satir, P. (1965). Studies on cilia. II. Examination of the distal region of the ciliary shaft and the role of the filaments in motility. *J. Cell. Biol.* **26,** 805–834.
Tormey, J. McD. (1964). Differences in membrane configuration between osmium tetroxide-fixed and glutaraldehyde-fixed ciliary epithelium. *J. Cell. Biol.* **23,** 658–664.
Venable, J. H. and Coggeshall, R. (1965). A simplified lead citrate stain for use in electron microscopy. *J. Cell. Biol.* **25,** 407–408.

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THE FINE STRUCTURE OF THE TROCHOPHORE OF *HARMOTHOE IMBRICATA*

P. L. HOLBOROW

Gatty Marine Laboratory, University of St Andrews, Scotland

INTRODUCTION

The annelid trochophore has been studied with the light microscope since the middle of the last century and the overall anatomy is reasonably well known. Most workers described external features and development and Thorson (1946) reviews much of this work. E. B. Wilson (1892) gave an account of the cell lineage of *Nereis* and several other workers made similar studies on other species. D. P. Wilson (1932) sectioned the mitraria larva of *Owenia fusiformis* for the light microscope and Segrove (1940) cut the *Pomatoceros* trochophore, but certain of the internal organ systems were not seen in detail.

Electron microscopical study has been sadly neglected, the only work so far reported being a note on the eye of the trochophore of *Neanthes succinea* by Eakin & Westfall (1964).

This investigation is an overall examination with the electron microscope of the trochophore of *Harmothoë imbricata* (Polynoid polychaete) and some comments will be made on comparative features and apparent functions of parts.

METHOD

The trochophores were collected as they were released from under the elytra of adult worms brought into the laboratory. The larvae were fixed in a mixture of one volume of sea water containing the trochophores and an equal volume of osmium tetroxide at 4 °C for half to one hour. The embedding medium was Araldite.

Two separate methods were used for preparing material for the scanning electron microscope. Dr Vernon Barber of Bristol prepared several samples of specimens by freeze drying, followed by carbon and gold coating (see Holborow, Laverack & Barber, 1969, for details of this method and further references).

A second set of specimens was prepared in St Andrews by critical point drying. The animals were fixed in the usual way, washed, and placed in nylon mesh bags. They were passed through graded alcohols to absolute

alcohol and transferred to amyl acetate which is miscible with absolute alcohol and with liquid carbon dioxide. The bag of specimens was then quickly placed in a chamber and the amyl acetate was washed off with a flow of liquid carbon dioxide. When all the amyl acetate was removed, the chamber was sealed and the pressure was raised by heating the chamber until the critical pressure was reached at which the liquid and gas phases of carbon dioxide occur simultaneously. This avoids surface tension effects which may cause breaking or sticking together of the cilia. This 'critical point drying' procedure was first described by Anderson (1951). The dry specimens were tapped from the bag on to stubs painted with sellotape solution and coated under vacuum with gold palladium. They were viewed in a Cambridge Stereoscan scanning electron microscope.

RESULTS AND DISCUSSION

The features of the living animal seen under the light microscope consist of apical 'tufts', a 'tuft' between the apex and mouth, prototroch, neurotroch and two somewhat kidney-shaped eyes, set slightly back, with some cilia on the surface nearby. The shape of this trochophore is characteristic of polynoid trochophores. The apex is more conical than the posterior pole, with a slight apical dip, and there is a prominent upper lip. In the living animal ciliary movement in the gut can be observed but the internal organs cannot be seen with any clarity.

The overall scanning electron microscope view (Plate 1 *a*, *b*) shows with greater clarity the same external features seen with the light microscope. In addition, the protuberances of gland openings may be seen. There are two pairs of large openings on each side, two smaller single ones more posteriorly and nearer the prototroch. A set of four small projections at the right-hand end of the akrotroch, just above the first cilia, may also be gland pores. These are the only gland pores which are asymmetrically placed.

Further asymmetry occurs in the arrangement of apical cilia (Plate 2 *a*). The cilia lie in five lines around the apex in a formation which more resembles a trapezium than a ring. Three longer lines of cilia lie towards the right. These are approximately 23, 22 and 25 μm long. There is usually a distinct right angle on the dorsal side between the 22 and 25 μm lines. To the left are two 13 μm long lines of cilia with an angle of about 60° between them. The dorsal lines of cilia are close together with gaps of about 2 μm, but to the left and the ventral side there are gaps of 5, 8 and 10 μm between the lines of cilia. All but one of the lines are reasonably straight. It is not known whether this consistently irregular formation is character-

istic of this species or whether it occurs among other trochophores with apical rings of cilia. Gravely (1909) shows a neat apical ring on his diagram of a polynoid trochophore but Fuchs (1911) shows a gap in the apical ring of the trochophores of *Nepthys* and *Glycera*. This suggests that some irregularity may be present, but a scanning microscope study is needed before the precise arrangement of these cilia can be known.

With both the light and scanning microscope the cilia are very often found pointing away from the mouth. The reasons for this and their organization are problematical.

The cilia are 8 μm long. A few cilia of the same length occur centrally. The transmission electron micrograph (Plate 2*b*) shows that the cilia are 5–8 in a group and have oriented basal bodies with a lateral rootlet opposite the basal body, that is, parallel to the body surface, and a long root penetrating centrally. The cells have long thin extensions towards the blastocoel, but it is not known whether these connect directly with prototroch cells. The nerve bundle at the apex contains over 100 axons and some of these may derive from apical cells, although the extensions seen often bypass the bundle.

The akrotroch consists of four lines, 8–10 μm long, of 10 μm long cilia, separated by spaces of 2–7 μm. It runs about one-quarter of the way around the upper ventral side of the animal.

The organization of the prototroch, also, is not as straightforward as previously thought. Four or five rows of cells make up the prototroch band, all the cells being joined distally by desmosomes. The upper row bears short, widely spaced single cilia, the middle two rows bear the long cilia, the fourth and fifth rows bear the shorter cilia. Transmission electron micrographs of longitudinal sections show the two central cells bearing a continuous line of cilia. The basal bodies in both cells are uniformly oriented but the rootlets project into the cells in opposite directions. In certain animals the main, 20 μm long cilia are found to be uniformly oriented and are grouped into bundles of some 40–50 cilia. Each bundle is shared between two of the long, narrow main cells of the prototroch. Each of these cells bears up to 16 half bundles, making *c.* 350 in all. The smaller cilia (14 and 6 μm long) may also form bundles of 5–15 cilia grouped together. In none of these groups is there any connection between the membranes of the cilia. As the formation of cilia into groups occurs only occasionally and simultaneously with uniform orientation of direction of all the prototroch cilia around the animal, it is possible that this is a reaction mediated by nerves. For figures and further discussion of this, see Holborrow *et al.* (1969).

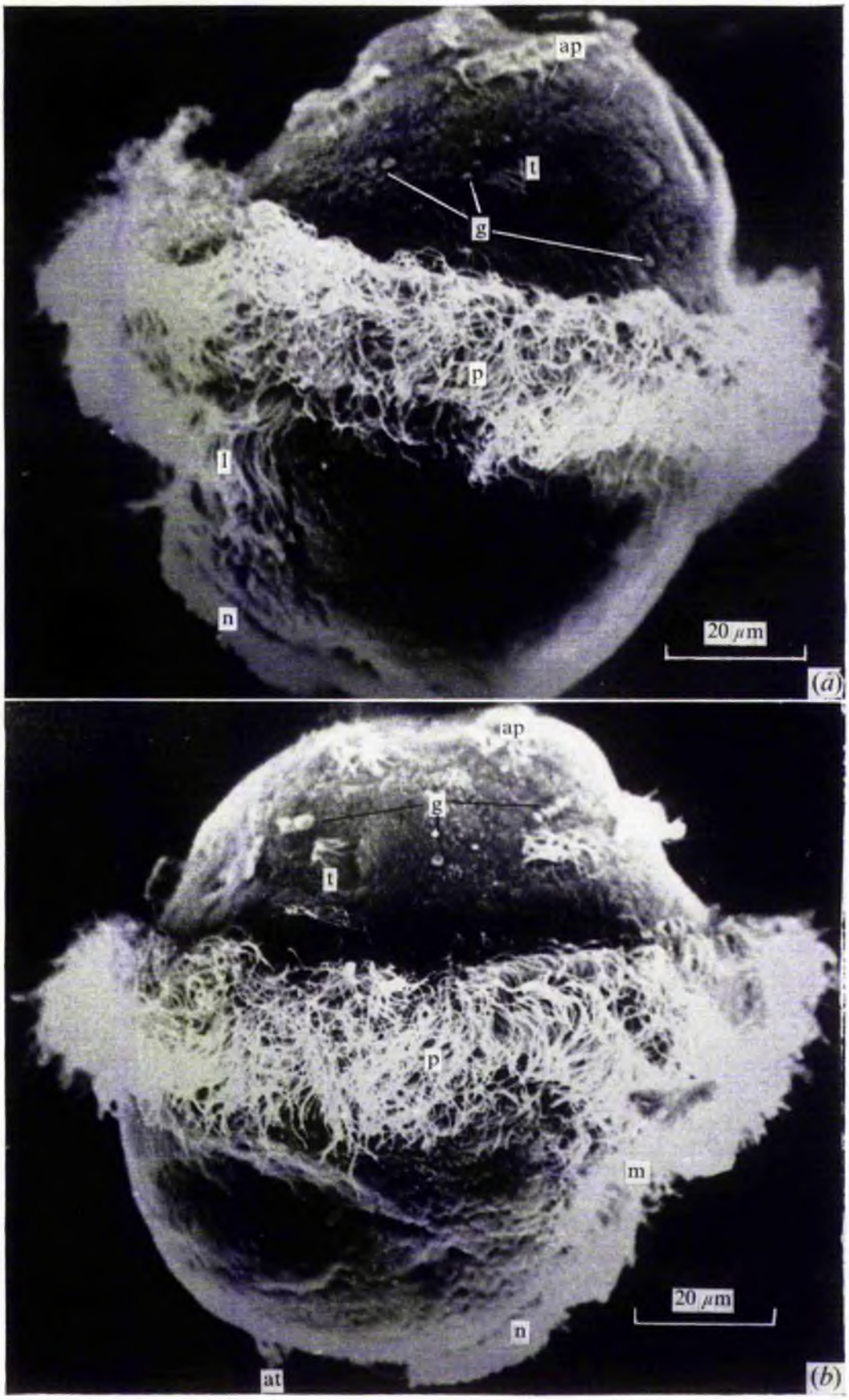
The neurotroch runs from the mouth to the anus and is a broad, tapering

band of 5 μm long cilia with lateral and longitudinal rootlets like those of the apical cilia. The basal bodies are uniformly oriented pointing away from the mouth and the neurotroch cilia therefore beat towards the anus. A tuft of 3–5 cilia lies some 16 μm beyond the end of neurotroch slightly to the right of the anus (Plate 1 *a*). The mouth is relatively large, being about 40 μm long and 20 μm wide. A group of long cilia occurs on the left side only of the mouth (Plate 1 *a*, *b*). Gravely (1909) describes two tongues of cilia, to either side of the mouth in the Polynoidae, but may be assuming a symmetry which is certainly not the rule with these trochophores.

In a panorama of about one-third of the animal (Plate 3) the prototroch, neurotroch, gullet and blastocoel are the dominating features. The prototroch cells are densely packed with mitochondria and ribosomes, particularly at the periphery, and have a large, distinctive nucleus and nucleolus. To either side of the neurotroch there are cells packed with unstained, membrane-bound vesicles 0.6–0.9 μm long and 0.5–0.8 μm broad. These may be lipid. Similar cells are found just beneath the cuticle in various other regions. Beneath these are a pair of active glandular cells with widely spaced cisternae of granular endoplasmic reticulum, large golgis, some mitochondria and islands of secretion droplets. These do not open to the exterior and could be the site of hormone secretion to control growth and metamorphosis. The neurotroch cells are roughly oblong with tapering proximal extensions going under the glandular cells towards two or three small axons. No synapses have yet been found in this region.

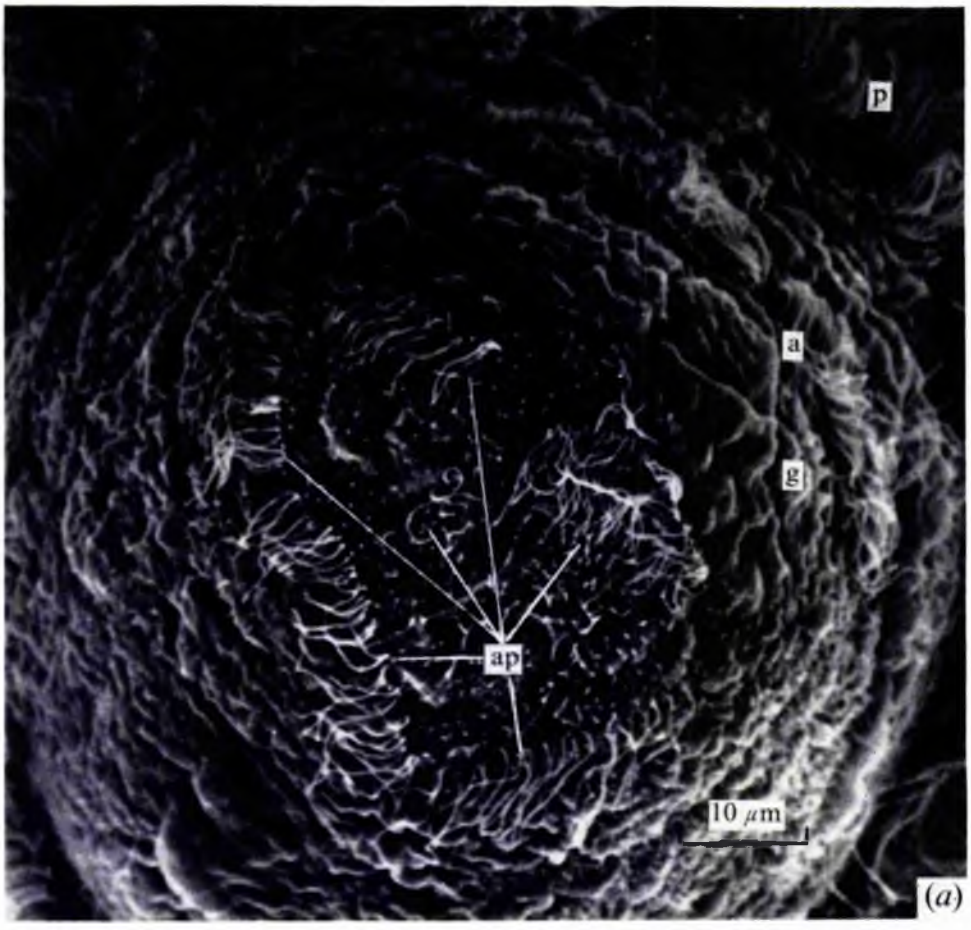
The blastocoel contains some recently divided cells and in section the area of the cytoplasm is small relative to the nucleus. One in Plate 3 appears to arise from the wall of the gullet and is one of the larval mesoderm cells which produces musculature of the gullet. At least one of the other cells floating in the blastocoel gives rise to a solenocyte, part of the larval protonephridium.

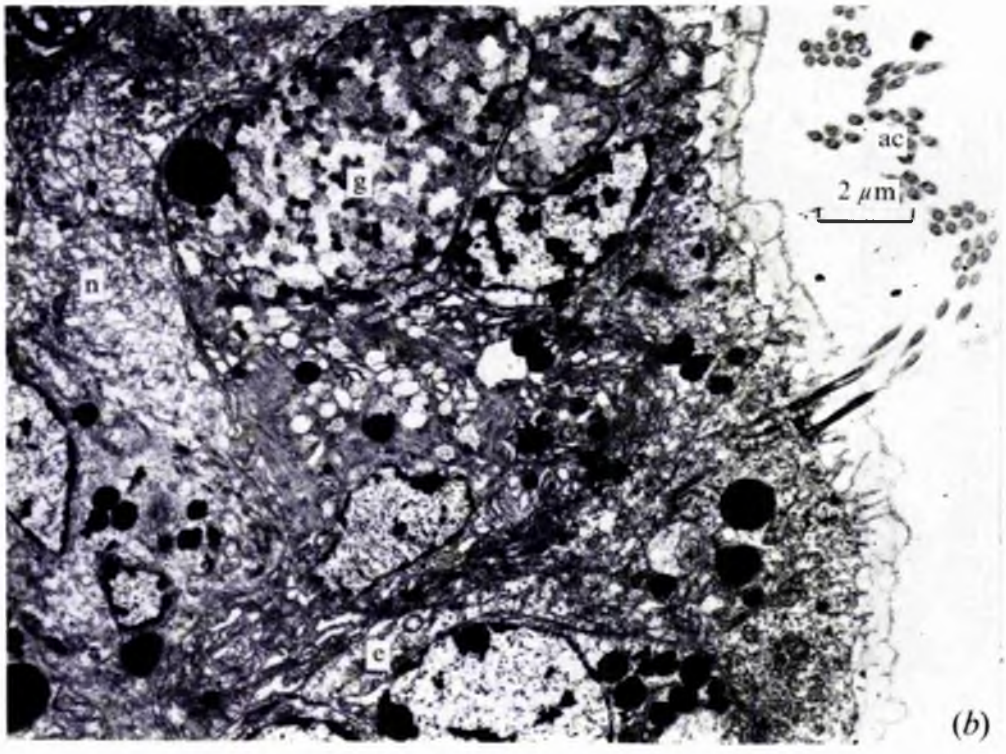
The gullet (Plate 4 *a*) is densely lined with cilia with oriented basal bodies indicating that the direction of beat of all the cilia is towards the stomach. There are two rootlets for each cilium, one parallel with the cuticle, the other projecting centrally into the cells. The stomach is lined with cilia and spaced, 1 μm long microvilli, some of which are clavate. There are two or three populations of cells making up the stomach wall. The majority bear only a few cilia and a moderately dense carpet of microvilli, some bear tufts of long cilia (Plate 4 *a*) and some are glandular and have a dense array of microvilli at the surface with perhaps one cilium. There is a small area of stomach wall where the tissue is highly vacuolated. This lies between the stomach and gullet, near the opening of the gullet to the stomach. A cluster



Holborow: PLATE 1

Scanning electron micrographs of a trochophore. (*a*) Left-hand side view. (*b*) Right-hand side view. ap, apical cilia; t, small lateral tufts of cilia, probably those associated with the eyes; g, projecting pores of glands; p, prototroch; l, tongue of long cilia on the left-hand side of the mouth; m, position of mouth; n, neurotroch; at, anal tuft to the right posterior of the anus.

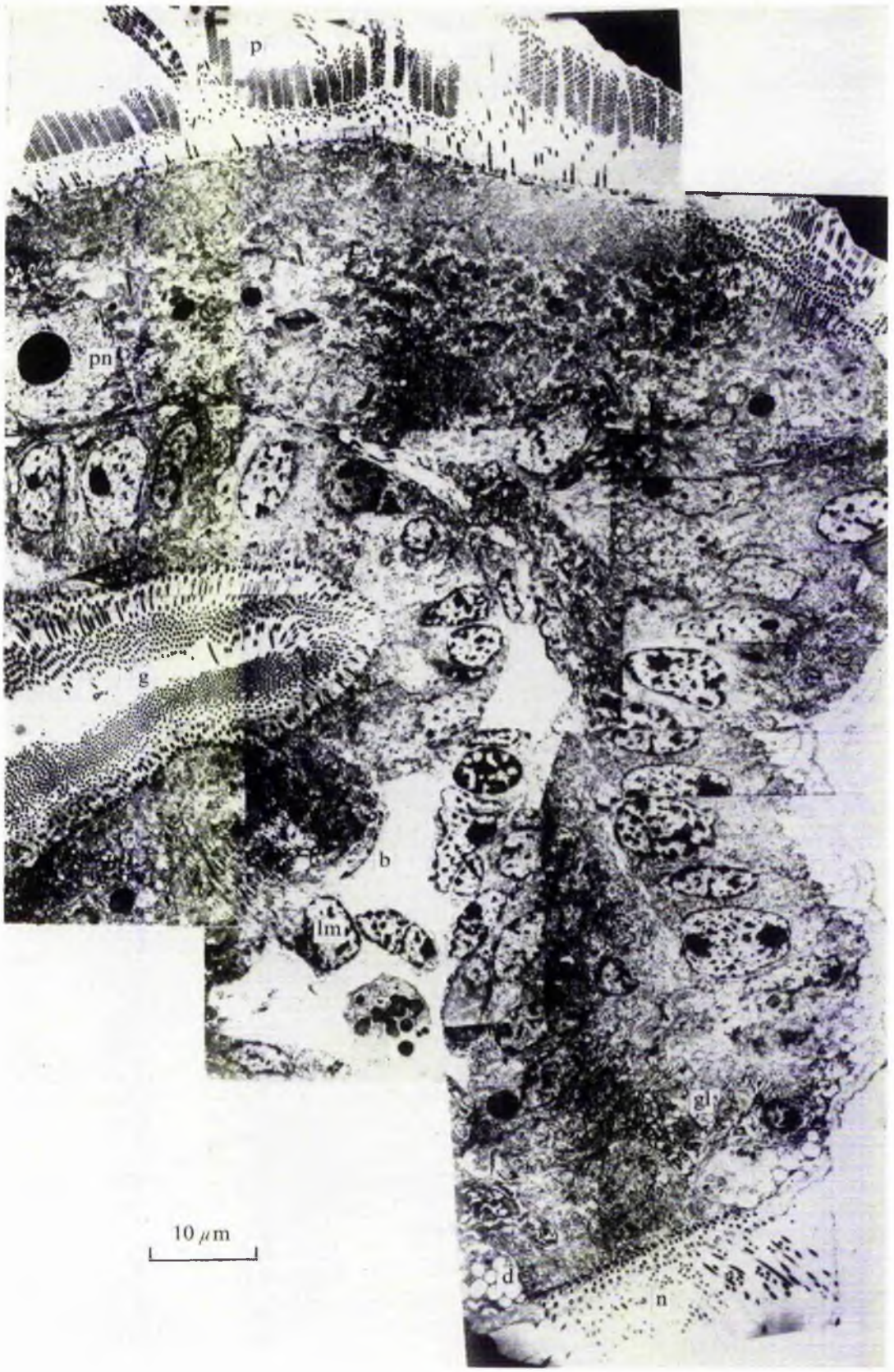




Holborow: PLATE 2

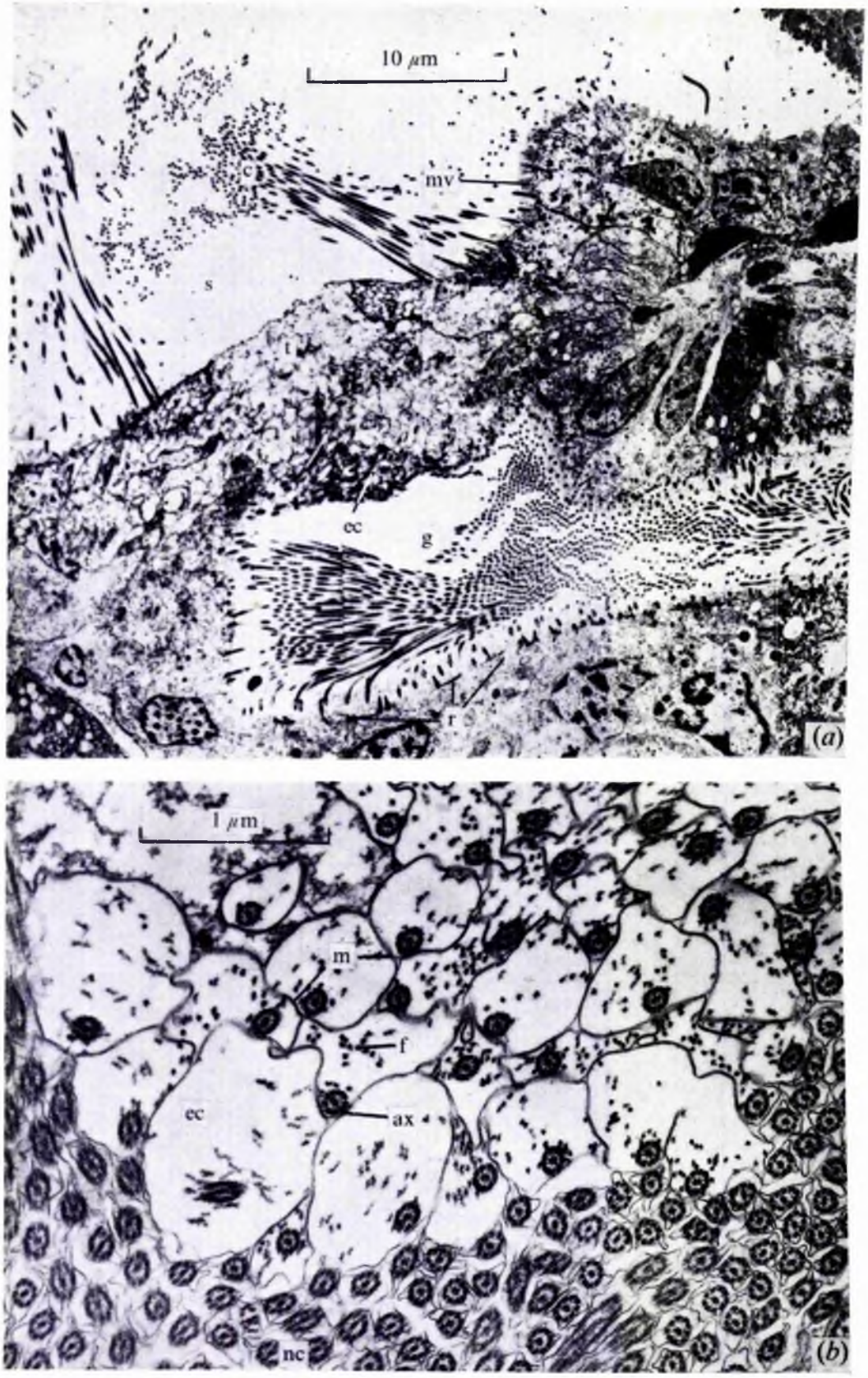
(*a*) Scanning electron micrograph of the upper hemisphere. ap, apical cilia; g, set of four gland pores at right-hand end of akrotroch; a, akrotroch; p, prototroch.

(*b*) Transmission electron micrograph of apical cells. ac, apical cilia; e, tapering extension of apical cell; g, glandular tissue; n, bundle of nerves.



Holborow: PLATE 3

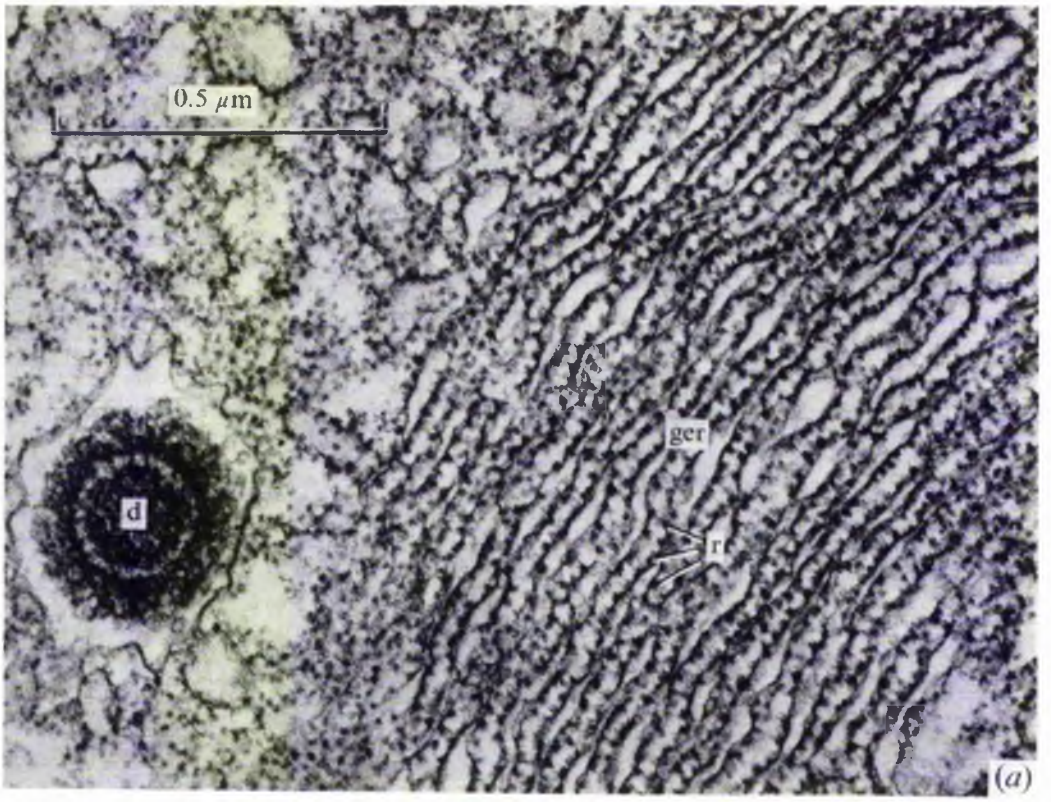
Panorama to show overall organization. p, prototroch cilia in orderly aggregations; pn, prototroch nucleus; b, blastocoel; g, gullet lined with cilia; lm, larval mesoderm cell; gl, glandular cell; n, neurotroch cilia; d, group of droplets on either side of neurotroch.

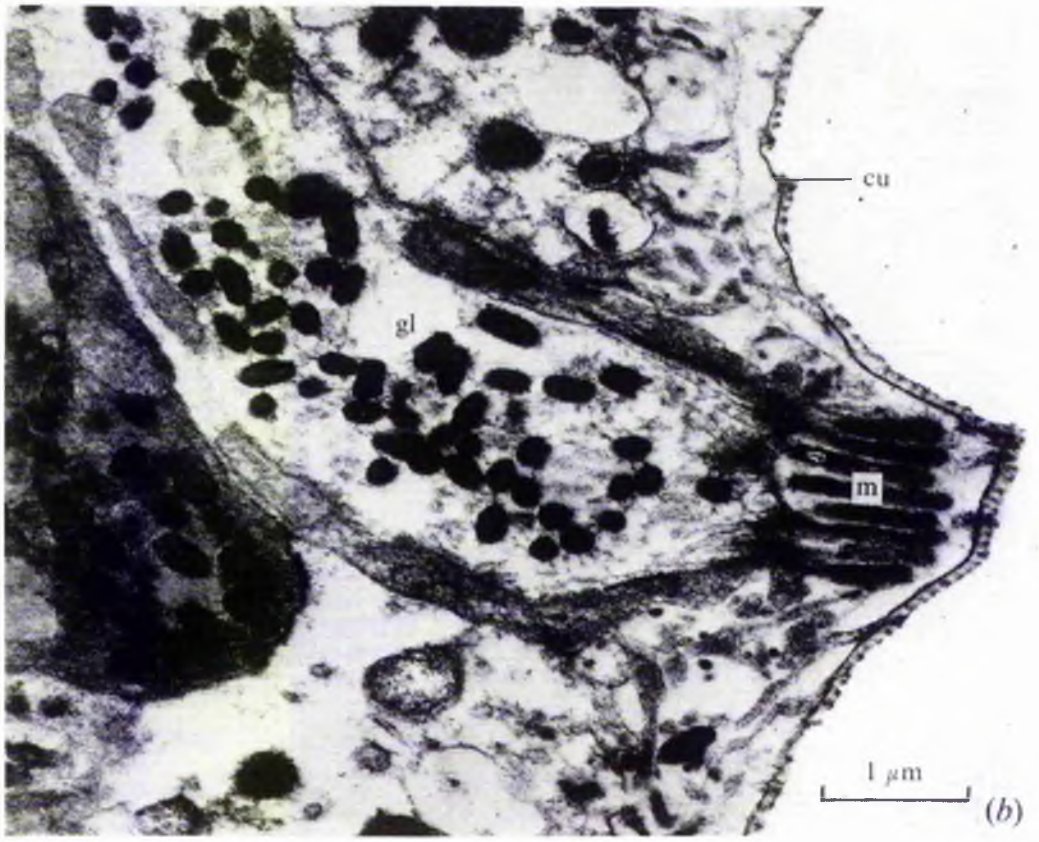


Holborow: PLATE 4

(*a*) Panorama of gullet (g) and stomach (s). t, area of thin tissue in stomach wall; c, cilia; mv, microvilli; ec, expanded cilia of gullet; r, oriented rootlets of normal gullet cilia.

(*b*) Expanded cilia of the gullet (ec); ax, axoneme with normal 9+2 configuration; f, extra filaments; m, fused membranes; nc, normal cilia.

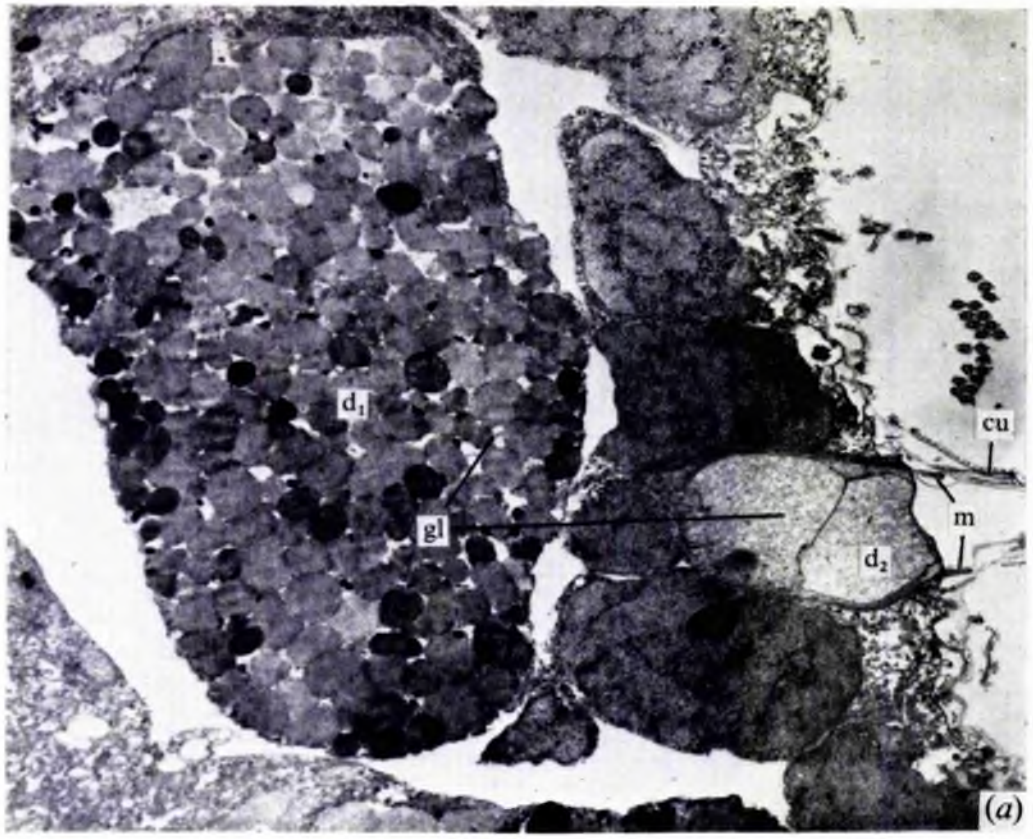


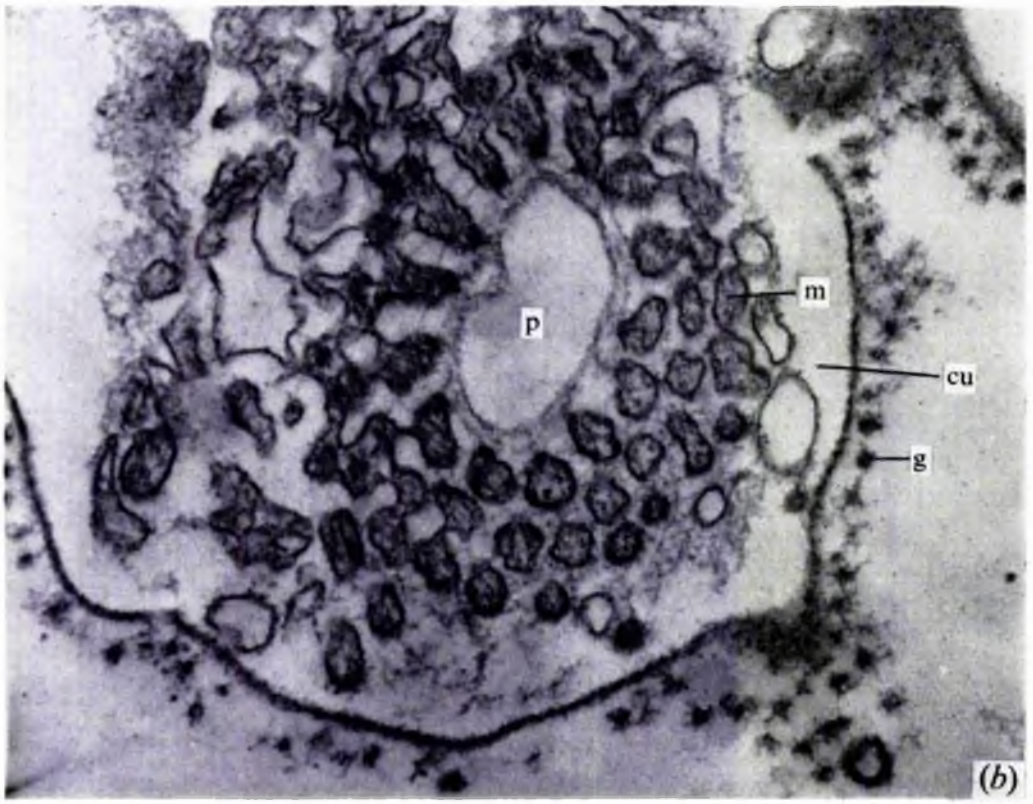


Holborow: PLATE 5

(*a*) A stomach secretory cell. ger, granular endoplasmic reticulum; r, ribosomes; d, secretion droplet.

(*b*) Superficial secretory cell (gl); m, microvilli supporting pore; cu, cuticle.

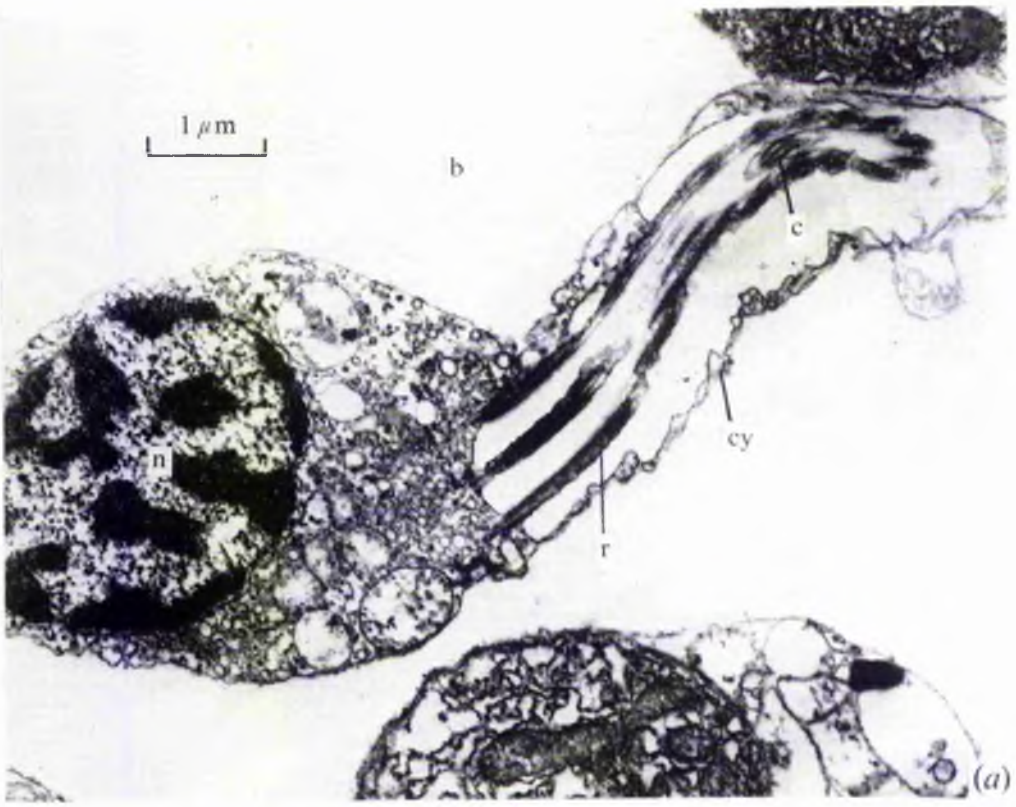


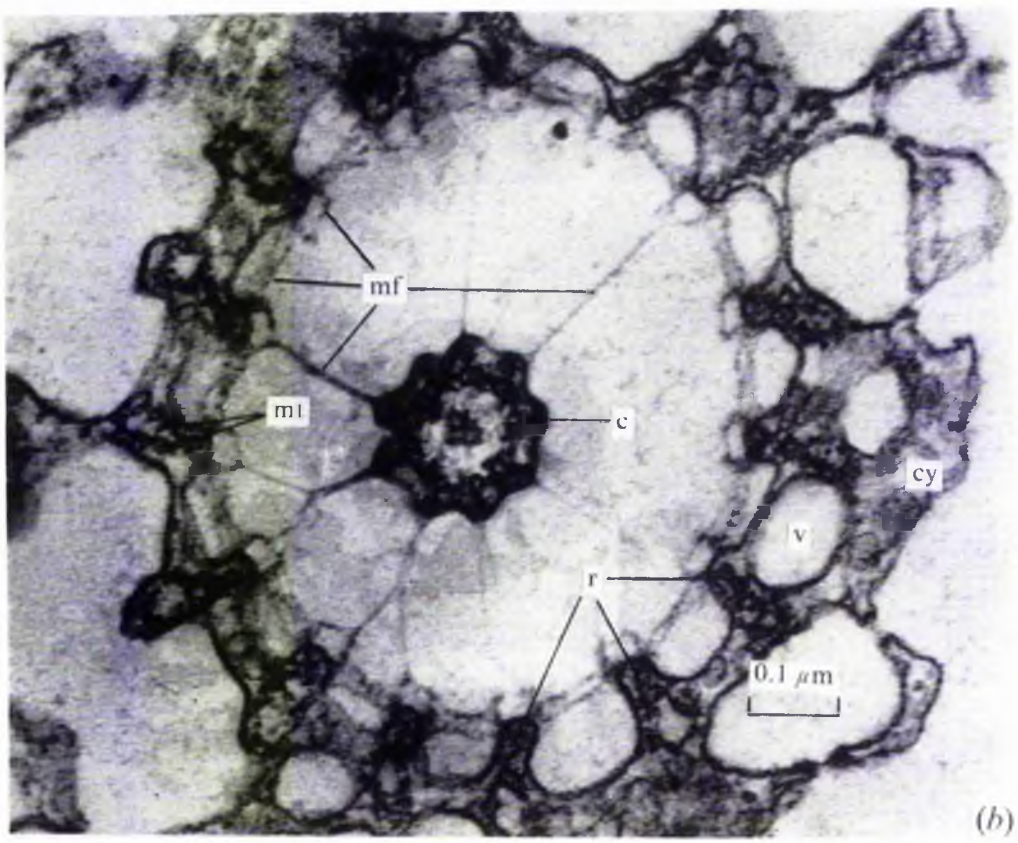


Holborow: PLATE 6

(*a*) Another type of superficial secretory cell (gl). d_1, immature droplets; d_2, mature droplets about to be released; m, microvilli supporting pore; cu, cuticle extending pore.

(*b*) Transverse section of pore of superficial gland. p, pore; m, microvilli; cu, cuticle; g, granules on external side of cuticle.

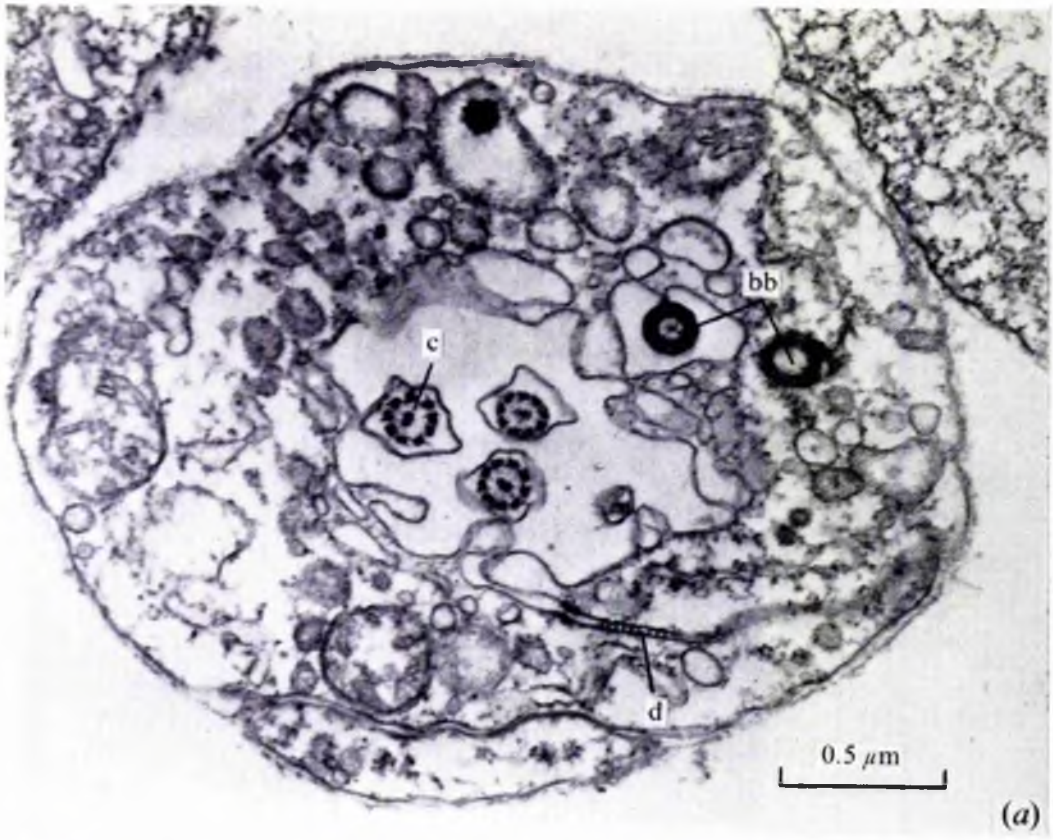


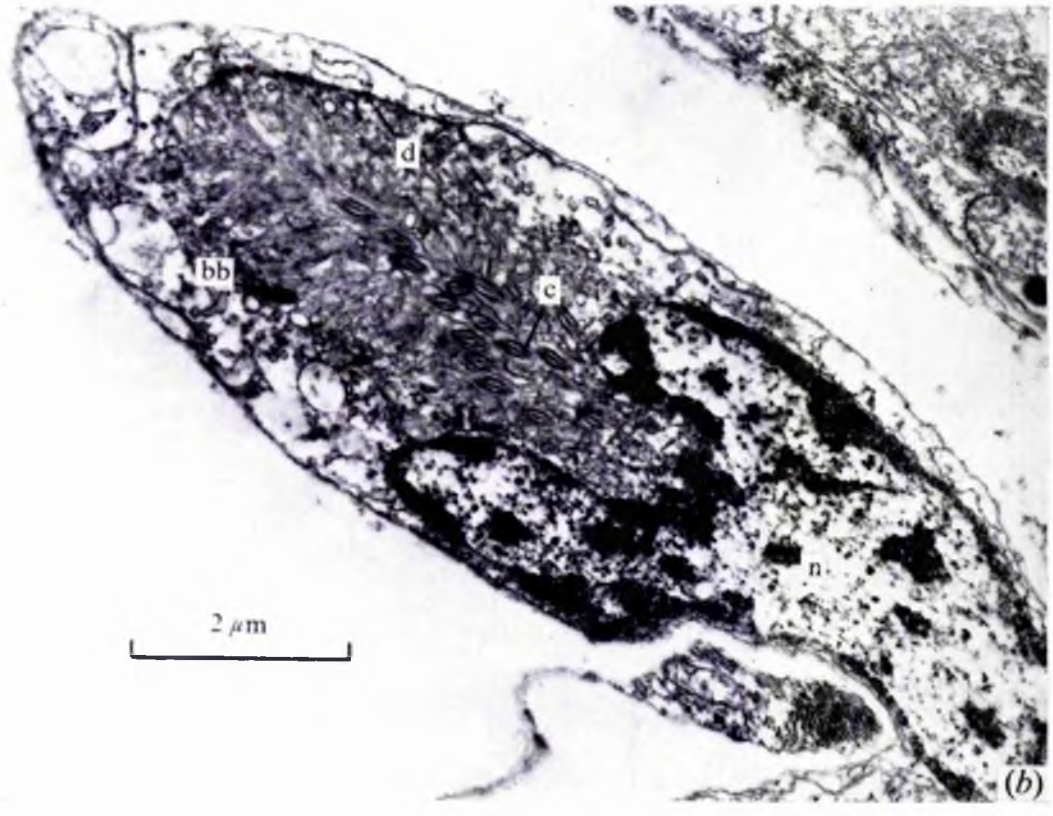


Holborow: PLATE 7

(*a*) Longitudinal section of part of solenocyte. b, blastocoel; c, cilium; r, rods of solenocyte tube; cy, cytoplasm surrounding tube; n, nucleus.

(*b*) Transverse section of solenocyte. c, cilium; mf, microfilaments; r, rods; mt, microtubules in rods; v, vacuoles; cy, cytoplasm.

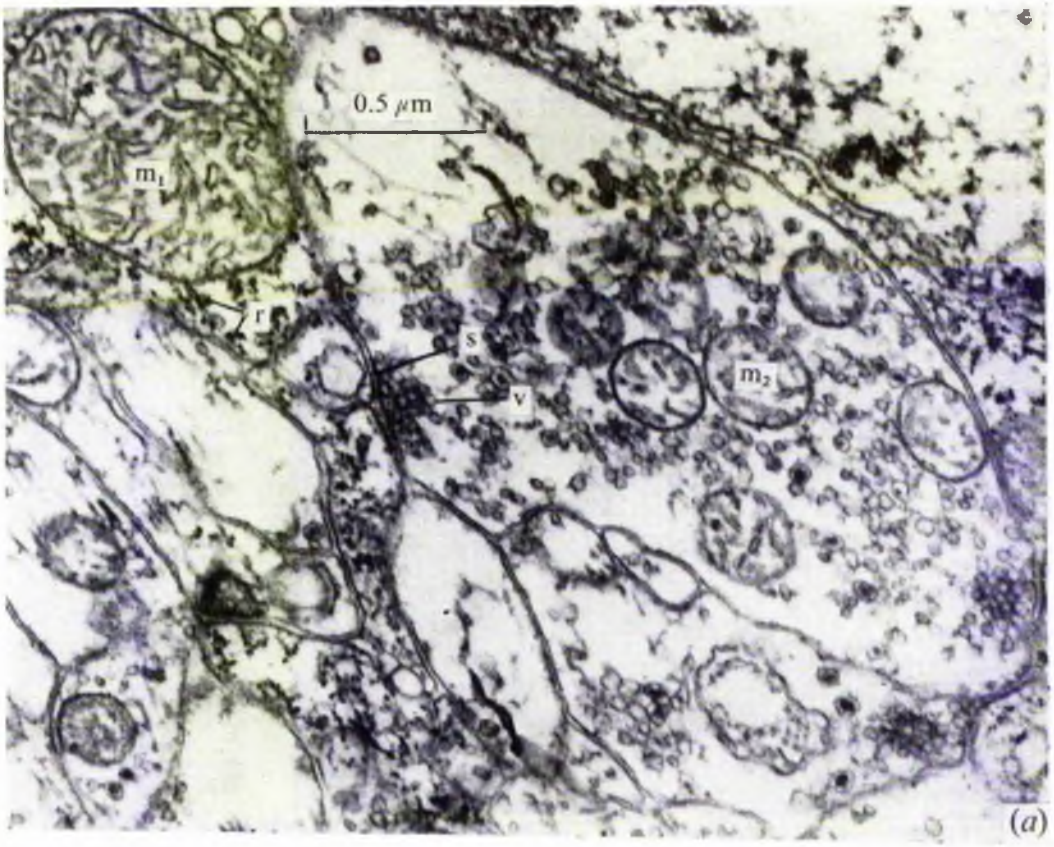


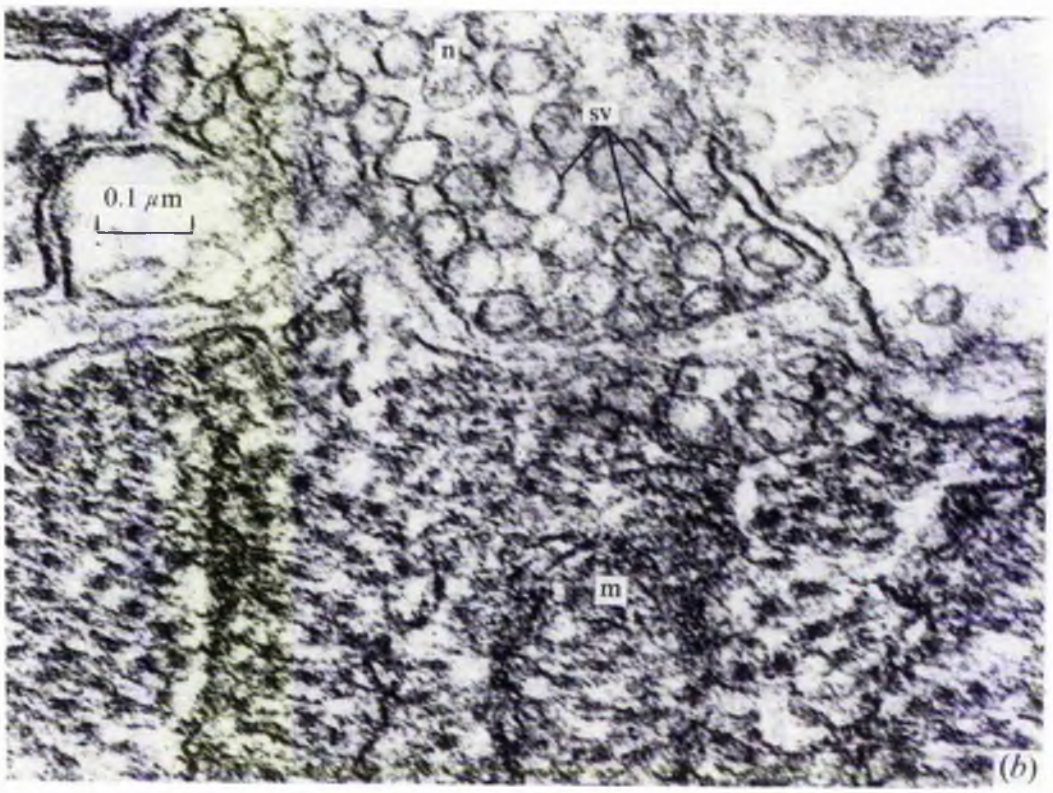


Holborow: PLATE 8

(*a*) Transverse section of duct of protonephridium. c, cilium; bb, basal bodies; d, septate desmosome.

(*b*) Transverse section of a lower portion of the protonephridial duct. c, cilium; bb, basal body; d, desmosome; n, nucleus.

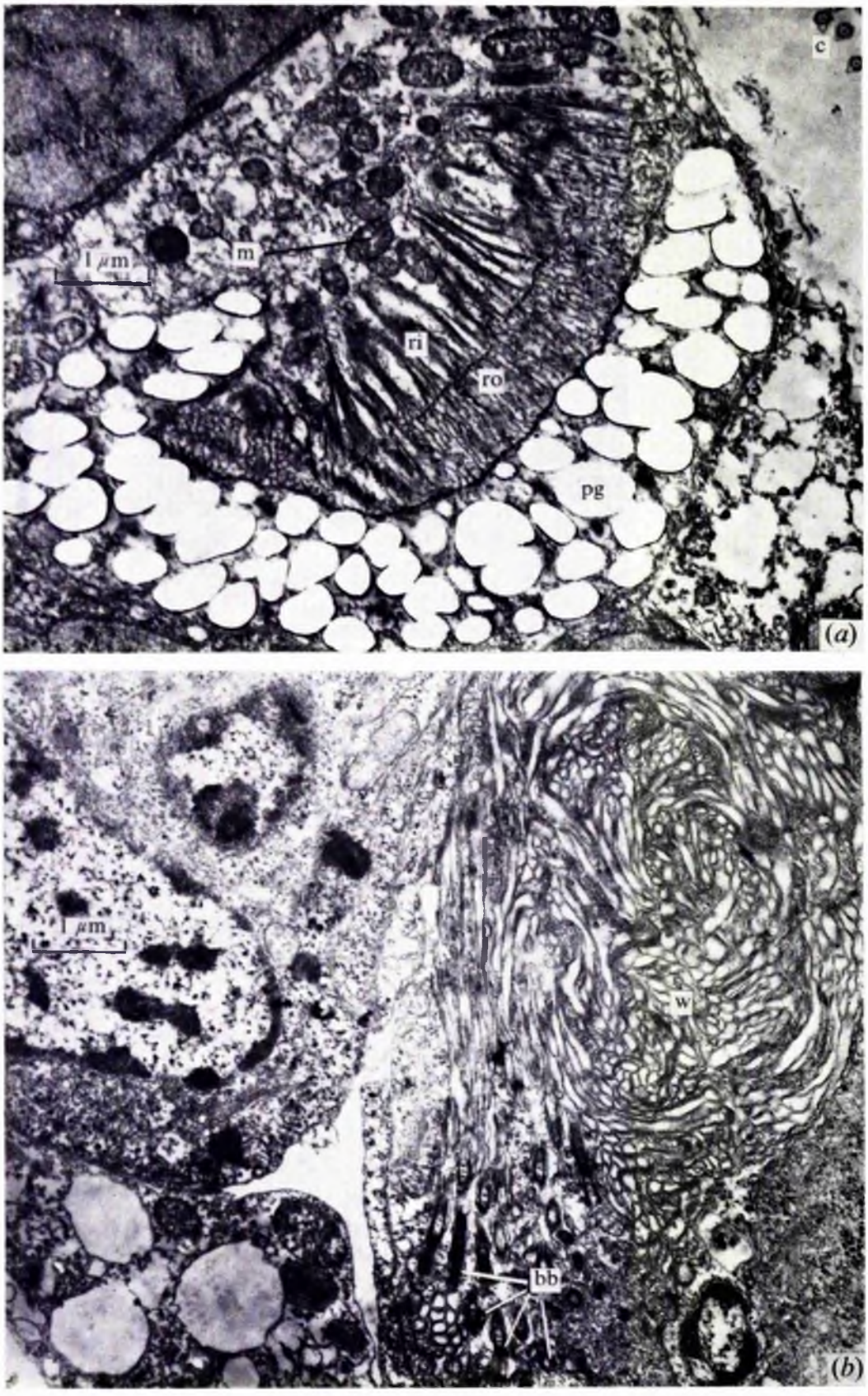




Holborow: PLATE 9

(*a*) Neurociliary synapse (s). v, synaptic vesicles; m_1, mitochondrion of prototroch cell; r, ribosomes (in prototroch cell but not nerve cells); m_2, mitochondrion of nerve cell.

(*b*) Neuromuscular junction. n, nerve; m, muscle; sv, synaptic vesicles.



Holborow: PLATE 10

(*a*) Larval eye. pg, pigment granules of pigment cup; ri, inner layer of retina; ro, outer layer of retina; m, mitochondria of light sensitive cell; c, cilia.

(*b*) Problematic body, possibly developing adult eye. w, whorl of membranes; bb, basal bodies of cilia from which the whorl of membranes arise.

of unusual cilia in the gullet overlie this tissue and project into the opening of the gullet to the stomach.

In structure these cilia are unique, each having an expanded membrane and extra filaments (Plate 4*b*). The membranes are fused by a uniform electron-dense substance similar to that between ciliary membranes in the flame cell of the fish tapeworm *Diphyllobothrium latum* (von Bonsdorff & Telkkä, 1966). The central filaments normally have the same orientation, as do the basal bodies. This indicates that the cilia beat in the same direction (Gibbons, 1961). As the membranes are fused it can be assumed that the cilia beat in unison. The function would appear to be a valve action, with the thin tissue of the stomach transmitting stomach pressure to the unusual cilia and the cells bearing them. The group of enlarged cilia could then move into and close the opening between stomach and gullet.

The secreting cells of the stomach are typical of exocrine secreting tissue, with close-packed cisternae of granular endoplasmic reticulum (Plate 5*a*) (Fawcett, 1966). The droplets of secretion are, however, unusual and distinctive, having an internally layered structure (Plate 5*a*). The only similar droplets to these are mineralized granules in the Malpighian tubules of *Rhodnius* (Wigglesworth & Perry, 1967) but the trochophore granules are only 0.3 μm across whereas the *Rhodnius* granules are between 0.8 and 2 μm across.

The glands opening on to the surface are quite different from stomach gland cells in internal structure and in the type of secretion produced (Plates 5*b*, 6*a*). There appears to be more than one type of externally opening gland but the basic type of secretion is probably mucus. All the glands open on to the surface by a projecting pore supported by one ring of 1 μm long microvilli in some cases or by three rings in others (Plate 6*b*). The cuticle is also pushed upwards to the mouth of the pore and projects beyond the microvilli to further raise the height of the opening. The opening is 0.6 μm in diameter and the whole projection is 1.7 μm across, 2–3 μm in height. Nørrevang (1965) describes microvilli-surrounded gland openings in an enteropneust and suggests that the function of the microvilli is to ensure a free release of mucus by raising the outlet above the body surface.

The larval protonephridium consists of one or two solenocytes which open into an intracellular duct, the cells of which add cilia to the duct. Such protonephridia are well known in the literature (Goodrich, 1945). The solenocyte arises from a cell floating free in the blastocoel (Plate 7*a*) and consists of a cilium contained in a tube, the lumen of which is 0.7–0.8 μm in diameter. The tube is supported by 15 interconnecting rods (Plate 7*b*). The rods appear to be composed of clusters of microtubules, unlike those

in the flame cell of a rotifer, which bear striations like ciliary roots (Mattern & Daniel, 1966). Kümmel & Brandenburg (1961) studied with the electron microscope and compared various choanocytes and solenocytes, but were more interested in a gross comparison than the details of fine structure. Their diagram of the solenocyte of *Glycera* shows 17 rods in the wall, but even the gross structure of the rods appears to have been modified for the diagram.

Certain features of the fine structure of choanocytes do correspond with features of the solenocyte. The choanocyte of a sponge described by Fjerdingstad (1961) and the choanocyte of an enteropneust described by Nørrevang (1964) both consist of a flagellum surrounded by a collar of rods which Nørrevang calls microvilli. The membranes of the microvilli are separate in both types of choanocyte, whereas in the solenocyte the membrane of the rods is often found to be continuous (Plate 7*b*). In the choanocytes and trochophore solenocyte the rods are linked by fine fibrils, some 40 Å across. The enteropneust choanocyte and the trochophore solenocyte both have projections of these microfibrils into the lumen of the tube. Nørrevang suggested that the substance of the microfibrils is mucus and that the inward projection indicates an inward sweep of water between the rods. As the trochophore rods are on the whole interlinked, flow between them would not be great. Also, the filaments often link the central cilium with the rods. It is therefore unlikely that these filaments are mucus arranged under the influence of flow. They bear some resemblance to the mucopolysaccharide filaments on the surface of intestinal villi (Fawcett, 1966).

Around the outside of the tube of the solenocyte is a thin layer of cytoplasm separated from the rods by large vacuoles. Some of these vacuoles appear to have been formed by thin folds of tissue which are occasionally found arched to enclose part of the blastocoel. This suggests that pinocytosis is taking place.

The tip of the solenocyte tapers as it enters the main protonephridial duct and up to eight of the rods enter and terminate in the duct lumen. The cells of the duct each contribute cilia until there are about 20. Plate 8*a* shows an early stage in the duct with three cilia and the basal bodies of two other cilia cut in transverse section. Plate 8*b* is a later stage in the duct showing 15 cilia and a basal body. Internal septate desmosomes are a feature of the duct cells (Plate 8*a*). Septate desmosomes have been reported in a planarian protonephridium (Pederson, 1961), and are thought to form a diffusion barrier.

The nervous system is quite complex. A group of some 100–150 axons

occurs near the apex and radial nerves run on the ectoderm side of the blastocoel. Up to 50 axons run circularly under the prototroch and neuro-ciliary synapses have been found to the main prototroch cells (Plate 9*a*). A muscle patch occurs beneath these and there are neuromuscular junctions (Plate 9*b*).

The larval eye consists of a cup of pigment and a two-layered retina of microvilli, the first layer spaced by 700 Å, the second layer, nearest the pigment, spaced by 1000 Å (Plate 10*a*). It is difficult to ascertain whether the second layer is continuous with the first but it is possible that it is produced by branching of the microvilli. The eye is similar to that of the *Neanthes succinea* trochophore with which Eakin & Westfall (1964) experienced the same difficulty.

One further structure warrants comment. This is a problematical body of whorled membranes apparently derived from cilia (Plate 10*b*). Apart from the presence of a central pair of filaments in the cilia, this structure bears some resemblance to the receptor region of the eye of the Mollusc, *Cardium* (Barber & Wright, 1969). The cilia branch and coil, rapidly losing their filaments, and the resulting array of membranes are seen longitudinally and transversely cut in different areas in the same section. This body is thus distinctly different in formation from the ciliary-derived lamellate bodies in the eye of a chiton (Boyle, 1969) and the eye of a ctenophore (Horridge, 1964). The neatly whorled membranes in the *Pecten* eye (Barber, Evans & Land, 1967), and the stacks of membranes in *Branchioma* (Krasne & Lawrence, 1966) also appear to have originated in a different manner. In a comparative chart of the derivation of membranes from cilia in eyes by Eakin (1965), there is no indication of branching cilia. This structure represents a further category in ciliary-derived membranes, but whether it becomes the eye has yet to be determined.

Although there are no cilia in the fully developed adult eye, this does not rule out the possibility that it may be derived from this structure. The cilia appear in only a small part of the mass of membranes and diminish in frequency as the size of the mass increases.

Eakin (1963) places annelids in a group of animals with rhabdomeric-type eyes derived from microvilli, by contrast with animals with eyes derived from cilia. Dorsett & Hyde (1968) support this view in a study of the eye of *Nereis virens*. An exception is a report of cilia in the eyes of *Branchioma* (Lawrence & Krasne, 1965; Krasne & Lawrence, 1966). In *Harmothoë*, no cilia have yet been found in the adult eye, but the present work reports that an eye-like structure of ciliary origin is in the process of development in the larva.

SUMMARY

Light microscopy of living and sectioned trochophores gives a general but limited view of the anatomy. The scanning electron microscope reveals the arrangements of cilia and gland openings and a certain amount of asymmetry is found. The apical region has five lines of cilia arranged in a roughly trapezoid form. Four short lines of cilia make an akrotroch running one-quarter of the way around the ventral side of the animal. The prototroch cilia are in three rows around the girth of the trochophore. The neurotroch is a broad band of short cilia running from the mouth and terminating in front of the anus. A small patch of cilia lies to the right on the other side of the anus and a tongue of long cilia is found at the left of the mouth.

The transmission electron microscope study completes the interpretation of external organization and the fine structure of all the internal organ systems is described. The functional and comparative significance of some of the findings is discussed.

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REFERENCES

ANDERSON, T. F. (1951). Techniques for the preservation of three-dimensional structure in preparing specimens for the electron microscope. *Trans. N.Y. Acad. Sci.* **13**, 130–4.

BARBER, V. C., EVANS, E. M. & LAND, M. F. (1967). The fine structure of the eye of the mollusc *Pecten maximus*. *Z. Zellforsch. mikrosk. Anat.* **76**, 295–312.

BARBER, V. C. & WRIGHT, O. E. (1969). The fine structure of the eye and optic tentacle of the mollusc *Cardium edule*. *J. Ultrastruct. Res.* **26**, 515–28.

BONSDORFF, C.-H. V. & TELKKÄ, A. (1966). The flagellar structure of the flame cell in fish tapeworm (*Diphyllobothrium latum*). *Z. Zellforsch. mikrosk. Anat.* **70**, 169–79.

BOYLE, P. R. (1969). Fine structure of the eyes of *Onithochiton neglectus* (Mollusca: Polyplacophora). *Z. Zellforsch. mikrosk. Anat.* **102**, 313–32.

EAKIN, R. M. (1963). Lines of evolution of photoreceptors. In *General Physiology of Cell Specialization*, pp. 393–425 (ed. D. Mazia & A. Tyler). New York: McGraw-Hill.

EAKIN, R. M. (1965). Evolution of photoreceptors. *Cold Spring Harb. Symp. quant. Biol.* **30**, 363–70.

EAKIN, R. M. & WESTFALL, J. A. (1964). Further observations on the fine structure of some invertebrate eyes. *Z. Zellforsch. mikrosk. Anat.* **62**, 310–32.

DORSETT, D. A. & HYDE, R. (1968). The fine structure of the lens and photoreceptors of *Nereis virens*. *Z. Zellforsch. mikrosk. Anat.* **85**, 243–55.

FAWCETT, D. W. (1966). *An Atlas of Fine Structure. The Cell.* Philadelphia and London: W. B. Saunders Company.

FJERDINGSTAD, E. J. (1961). Choanocyte collars in *Spongilla lacustris* (L.). *Z. Zellforsch. mikrosk. Anat.* **53**, 645–57.

FUCHS, H. M. (1911). Note on the early larvae of *Nepthys* and *Glycera*. *J. mar. biol. Ass. U.K.* **9**, 164–70.

GIBBONS, I. R. (1961). The relationship between the fine structure and the direction of beat in gill cilia of a lamellibranch mollusc. *J. biophys. biochem. Cytol.* **11**, 179–205.

GOODRICH, E. S. (1945). The study of Nephridia and genital ducts since 1895. *Q. Jl microsc. Sci.* **86**, 113–392.

GRAVELY, F. H. (1909). Polychaete larvae. *L.M.B.C. Mem. typ. Br. mar. Pl. Anim.* **19**, 1–79.

HOLBOROW, P. L., LAVERACK, M. S. L. & BARBER, V. C. (1969). Cilia and other surface structures of the trochophore of *Harmothoë imbricata*. *Z. Zellforsch. mikrosk. Anat.* **98**, 246–61.

HORRIDGE, G. A. (1964). Presumed photoreceptor cilia in ctenophores. *Q. Jl microsc. Sci.* **105**, 311–17.

KRASNE, F. B. & LAWRENCE, P. A. (1966). Structure of the photoreceptors in the compound eyespots of *Branchiomma vesiculosum*. *J. Cell Sci.* **1**, 239–48.

KUMMEL, V. G. & BRANDENBURG, J. (1961). Die Reusengeibelzellen (Crytocyten). *Z. Naturf.* **166**, 692–7.

LAWRENCE, P. A. & KRASNE, F. B. (1965). Annelid photoreceptors. *Science, N.Y.* **148**, 965–6.

MATTERN, C. F. T. & DANIEL, W. A. (1966). The flame cell of a rotifer. *J. Cell. Biol.* **29**, 552–4.

NØRREVANG, A. (1964). Choanocytes in the skin of *Harrimania kupfferi* (Enteropneusta). *Nature, Lond.* **204**, 398–9.

NØRREVANG, A. (1965). On the mucous secretion from the proboscis in *Harrimania kupfferi* (Enteropneusta). *Ann. N.Y. Acad. Sci.* **118**, 1052–69.

PEDERSEN, K. J. (1961). Some observations on the fine structure of planarian protonephridia and gastrodermal phagocytes. *Z. Zellforsch. mikrosk. Acad.* **53**, 609–28.

SEGROVE, F. (1940). The development of the Serpulid *Pomatoceros triqueter*, L. *Q. Jl microsc. Sci.* **82**, 467–540.

THORSON, G. (1946). Reproduction and larval development of Danish marine bottom invertebrates. *Meddr. Kommn Danm. Fisk. og Havunders.* Ser. Plankton. **4**, 1–523.

WIGGLESWORTH, V. B. & PERRY, M. (1967). In *The Ultrastructure of the Animal Cell*, p. 159 (ed. L. T. Threadgold). London and Oxford: Pergamon Press.

WILSON, D. P. (1932). On the Mitraria larva of *Owenia fusiformis*. *Phil. Trans. R. Soc. Ser.* B **211**, 231–334.

WILSON, E. B. (1892). The cell lineage of *Nereis*. *J. Morph.* **6**, 361–480.