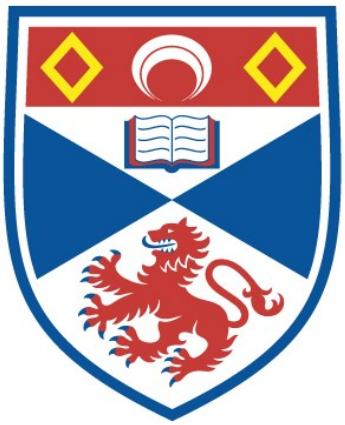
# STRUCTURE AND FUNCTION OF THE CEREBRAL ORGANS IN 'PARANEMERTES PEREGRINA', 'TETRASTEMMA CANDIDUM' AND 'AMPHIPORUS LACTIFLOREUS' (HOPLONEMERTEA : MONOSTILIFERA)

Helen M. Amerongen

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



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# Structure and function of the cerebral organs in *Paranemertes peregrina*, *Tetrastemma candidum* and *Amphiporus lactifloreus* (Hoplonemertea: Monostilifera)

by

Helen M. Amerongen

A thesis presented for the degree of Doctor of Philosophy at the University of St. Andrews

Gatty Marine Laboratory

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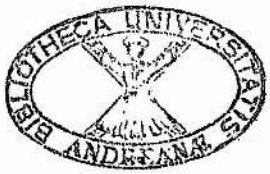



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## SUPERVISOR'S CERTIFICATE

I certify that Helen Amerongen has fulfilled the conditions laid down under Ordinance General No. 12, Resolution of the University Court 1967, No.1 of the University of St. Andrews and is accordingly qualified to submit this thesis for the degree of Doctor of Philosophy.

.....

## DECLARATION

I declare that the work reported in this thesis is my own and has not been submitted for any other degree.

.....

## CURRICULUM VITAE

I graduated from the University of Alberta with a Bachelor of Science degree in 1974 and a Master of Science degree in 1977. The work described in this thesis was carried out between January 1977 and June 1983.

## Acknowledgements

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## Abstract

The histology and ultrastructure of the cerebral organs have been studied in three species of monostiliferous hoplonemertean: *Paranemertes peregrina* Coe, *Amphiporus lactifloreus* (Johnston) and *Tetrastemma candidum* (O.F. Müller). The role of the cerebral organs in osmoregulation and behaviour has been investigated in *Paranemertes*. Based on the information obtained, it is concluded that the cerebral organs in these species are chemoreceptors.

The structure of the cerebral organs is essentially the same in the three species studied. The cerebral organs consist of two groups of sensory cells, two groups of gland cells, and two groups of endocytic/lysosomal cells (vesicular cells), as well as ciliated cells and support cells, surrounding a ciliated, blind-ending canal. The canal is functionally divided into two channels, designated the major and minor canals. According to the orientation of ciliary basal feet in cells of the canal epithelium, the minor canal is an incurrent channel, and the major canal is an excurrent channel. The organization of cell types with respect to the direction of flow in the canal is such that along the minor canal, Type A gland cell processes are upstream from Type 2 sensory cell dendrites, and Type 2 vesicular cells are downstream from the dendrites. Similarly, in the major canal, Type B gland cell processes are upstream, and Type 1 vesicular cells are downstream, from Type 1 sensory cell dendrites.

Based on this organization, and on the interpretation of cellular fine structure in the cerebral organs, it is proposed that the function of gland cells is to secrete a mucous coating over the sensory epithelium, and the function of vesicular cells is to remove this coating from the canal as the mucus is carried downstream from the dendrites by ciliary action.

In gland cells, the amount of secretion product present may be regulated by autophagic breakdown of secretion granules (crinophagy), according to a variable demand for secretion in the canal. Crinophagy contributes to the amount of vesicular material (degraded secretion product) present in the cerebral organs.

Although the dendrites are not innervated, dendrite sensitivity may be modulated by variation of the rate of flow through the canal, and the rate of mucous turnover across the two sensory epithelia. An efferent nerve fibre is present among the ciliated cells of the minor canal. The fibre is rare and its synapse has not been observed. It is thought that the fibre innervates a few cells which act as pacemakers, their cilia mechanically entraining the beat frequency of other cilia, thus determining the rate of flow through the canal.

There is no indication that vesicular material is disposed of outside the cerebral organs. In *Paranemertes* and *Amphiporus*, but not in *Tetrastemma*, the cephalic blood vessel lies adjacent to the posterior glandular part of the

cerebral organ, however, this association is not reflected in the internal structure of the cerebral organs. It is, therefore, unlikely that the cerebral organs in these species have an endocrine function.

The function of the cerebral organs in *Paranemertes* has been investigated by comparing the behaviour of intact worms with the behaviour of worms from which the cerebral organs have been surgically removed. Cerebral organ removal did not affect trail following behaviour, which is associated with homing, but it abolished the response of *Paranemertes* to prey trails. It is concluded that the cerebral organs of *Paranemertes* are chemoreceptors responsible for the detection of prey.

The behavioural physiology of *Paranemertes* has been investigated, using extracellular suction electrodes to record from the lateral nerve cords and the cerebral organ nerves. The results indicate that the cerebral organs are sensitive to prey extract and distilled water, but not to mechanical, thermal or photic stimuli.

The role of the cerebral organs of *Paranemertes* in salinity stress tolerance has been investigated by measuring the effect of cerebral organ removal on volume regulation, and by observing the effects of hypo-osmotic media on the cytology of the cerebral organs. Removal of the cerebral organs decreases volume regulatory capacity, however, a similar change is seen in sham-operated worms, indicating that the decreased capacity for regulation is due to the

operation itself and not to interference with a physiological role of the cerebral organs. Cytological changes caused by exposure to dilute sea water are similar to those seen in worms fixed in hypo-osmotic fixative. It is unlikely, therefore, that these represent a co-ordinated response of the organs to salinity stress. No exchange of material between the cerebral organs and the vascular system was observed. It is concluded that in *Paranemertes*, the cerebral organs are not involved in osmoregulation.

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# I. Introduction

## A. Nemertean Biology and Classification

The nemerteans are defined as acoelomate, bilaterally symmetrical animals with a complete digestive tract, a circulatory system, and an eversible proboscis enclosed in a cavity, the rhynchocoel, which lies dorsal to the digestive tract (Hyman, 1951; Gibson, 1972). Most are marine, living in sheltered spots under rocks, in crevices, among algal holdfasts, and burrowing in sand or mud. Almost all are carnivores and catch prey with the proboscis. By far the majority of nemerteans are free-living, but there is one group commensal in bivalves, and a few parasitic species are known.

Nemerteans are most closely related to the flatworms, and share in common with this group the possession of a ciliated epidermis, a protonephridial excretory system, and the lack of a coelom, the space between gut and body wall being filled with parenchyma. They differ from the flatworms in a number of features, many of which are considered advanced. These include the possession of a closed circulatory system, a complete digestive tract, a proboscis for capturing prey, and a more complex and centralised nervous system (Hyman, 1951). It has been suggested that nemerteans are on the direct line of evolution giving rise to the vertebrates (Jensen, 1960, 1963; Willmer, 1970), but the evidence presented to support this view is unconvincing

(Gibson, 1972). It is generally agreed that the nemerteans are an offshoot of the flatworms, more advanced than flatworms, but not on the direct line giving rise to the coelomates.

A summary of the classification of nemerteans based on Coe (1943) and Gibson (1972) is given in Table I, including the taxonomic position of genera principally discussed in this thesis.

**B. Structure of the cerebral organs**

The cerebral organs are complex neuroglandular organs characteristic of nemerteans and found in most members of the phylum. They are paired structures situated in the head before, adjacent to, or behind the brain. They are composed of nervous and glandular elements, surrounding a ciliated pit in simple forms, but more commonly forming a sac surrounding a ciliated blind-ending canal, the cephalic canal. The canal opens to the exterior on the head, in the cephalic slits or cephalic grooves; where these structures are absent the canal opens by a simple pore. The main nervous elements consist of what appear to be sensory neurons having dendrites with a free surface on the canal, and extending axons into the neuropil of the cerebral ganglia. The glandular elements consist of populations of pyriform exocrine gland cells which extend processes to the canal lumen. In addition to these gland cells, a group of vacuolated cells (=vesicular cells, also sometimes referred

to as gland cells, eg. Ferraris, 1979a), situated near the blind end of the canal has been described in some species (Dewoletzky, 1887; Ling, 1969a, b, 1970; Ferraris, 1979a). In some groups, the cerebral organs are situated in close association with the cerebral ganglia, or the vascular system, or both. This association with the vascular system is given considerable importance in interpretations of cerebral organ function.

The structure and position of the cerebral organs varies a great deal within the phylum. The simplest form of the organs is found in the Order Palaeonemertea. In more primitive representatives of this order the nervous system is superficial, situated just beneath the epidermis, external to the body wall musculature. In these species the cerebral organs are simple epidermal invaginations on the sides of the head surrounding a ciliated pit, with the sensory cell dendrites forming the base of the pit and the gland cells extending to the surface in its walls (eg. *Tubulanus*) (Bürger, 1895). At this level of complexity the cerebral organs are very similar to the ciliated pits of some rhabdocoel flatworms and may be homologous with them (Kepner and Taliaferro, 1912; Kepner and Cash, 1915). The cerebral organs are lacking in *Cephalothrix*, a feature considered primitive by Iwata (1960). However, if the cerebral organs are derived from ciliated pits as mentioned above, or from triclad auricular grooves as suggested by Gibson (1972), then the presence of the cerebral organs must

be considered primitive. More developed examples of cerebral organs also occur in palaeonemerteans, for example in *Hubrechtella* they are fully invaginated sacs which are closely associated with the cerebral ganglia and the cephalic blood vessel, and in this respect approach the condition found in heteronemerteans (Gibson, 1979a, b).

In the Order Heteronemertea the cerebral organs are large, complex structures fused with the dorsal cerebral ganglia and usually protruding into the cephalic blood lacunae. There is extensive knowledge of both histology and fine structure of the cerebral organs in *Lineus ruber*, thanks mainly to the work of Ling (1969a, b, 1970). In this species the canal extends inward from the cephalic slits and undergoes three right-angled bends within the cerebral organ proper, then ends blindly in an area of vesicular cells. There are two populations of gland cells, both producing neutral mucoprotein which is secreted into the canal at the first and second bends. The canal is divided into an incurrent and an excurrent channel by a septum formed of specialized cilia. In addition to bipolar sensory cells, the nervous elements include neurosecretory cells (=ganglion cells of Montgomery, 1897), which surround the exit of the cerebral organ nerve into the dorsal cerebral ganglion. Although it seems likely that these would be physiologically connected to the bipolar cells, so far no synapses or junctions of any kind have been identified. The cerebral organs are considered to have reached their peak of

development in the heteronemerteans (Scharrer, 1941; Gibson, 1972) because of their large size, intimacy of association with the vascular and nervous systems, and complexity of the nervous elements.

There is considerable diversity in the structure of the cerebral organs among the Hoplonemertea, and within this order they are lacking in some groups. In monostiliferous hoplonemerteans the cerebral organs are fully invaginated sacs of variable size, usually situated anterior to the brain. Neurosecretory cells are lacking in the cerebral organs of this group (Lechenault, 1963; Ferraris, 1978, 1979b). The gland cells show the same staining affinities as those in *Lineus*, and are also divided into two populations, both secreting into the canal lumen. Large vesicular cells are present in the posterior part of the organs near the blind end of the canal (Ling 1969a; Ferraris, 1979a). The degree of association with the central nervous and vascular systems varies; in some, the organs are remote from the cerebral ganglia but adjacent to the cephalic blood vessels, while in others the organs are juxtaposed to the cerebral ganglia but not closely associated with the vascular system. In none of the monostiliferans is the association with either the nervous or the vascular system as intimate as in the lineid heteronemerteans.

Among the polystiliferous hoplonemerteans, the cerebral organs are lacking in the Pelagica (Coe, 1927a, b), but well developed in the Reptantia. In the former group all sense

organs are reduced or absent, including the cerebral organs. This is correlated with the relatively unchanging bathypelagic habitat that members of this group inhabit. In the Reptantia the cerebral organs are very large and complex. The cerebral organ canal bifurcates within the organ, extending one branch into a sensory glandular region and one into a sac, which in some forms also has an area of sensory epithelium (Punnett, 1903; Stiasny-Wijnhoff, 1926). In *Curranemertes natans* the canal in the sac is greatly contorted and bifurcates a second time near its blind end, giving rise to a secondary sac (Kirsteuer, 1973). Vesicular cells have not been described in the Reptantia, but in the illustrations of *Curranemertes* cerebral organs (Kirsteuer, 1973) an area similar to the (Type 2) vesicular cell area in the species of this study is shown in his Figure 13.

The cerebral organs, as well as other sense organs, are reduced or absent in the Order Bdellonemertea (Jackson, 1935), a feature correlated with the commensal habit of members in this group.

In parasitic and commensal forms in all groups the cerebral organs are often lacking, for example in the monostiliferans, *Carcinonemertes*, parasitic on the eggs of crabs (Humes, 1942), and *Gononemertes australiensis*, which lives commensally in the ascidian *Pyura* (Gibson, 1974). Some anomalies exist, in which the glandular (eg. *Carinoma armandi*) (Bürger, 1895) or the nervous (eg. *Gononemertes parasita*) (Brinkman, 1927) elements are absent. Recently a

new hoplonemertean species, *Divanella evelinae*, has been described (Gibson, 1973) in which the cerebral organs are absent, and are perhaps replaced by a large neuroglandular complex which opens to the surface in a number of places and is also apparently sensory. Unfortunately, very little is known of the natural history of most nemerteans, and therefore it is not possible to correlate structural differences with environmental or behavioural variables in most cases.

**C. Function of the cerebral organs**

The function of the cerebral organs has not been clearly established in any species. Quatrefages (1846) suggested that they might have an auditory function, because of an apparent similarity between the cerebral organs and the "auditory organs" (=statocysts) of molluscs, but probably mistook the secretion mass for an otolith. A respiratory function was proposed by Hubrecht (1875, 1880). According to his view, the cerebral organ canal served to decrease the distance between the CNS and the exterior, and this feature, combined with the presence of haemoglobin in cells of the CNS, allowed more efficient gas exchange. McIntosh (1873-74) proposed an excretory function, based on the appearance of the gland cells, having first overlooked the connection between the cerebral organs and the CNS, but later rejected this idea in favour of a sensory function (McIntosh, 1876). Dewoletzky (1887) and Bürger (1895)

suggested that they may be chemoreceptors detecting water quality, because of a striking sensitivity of some nemerteans to changes in such things as carbon dioxide concentration in the surrounding medium. The cerebral organs have been presumed chemoreceptors involved in prey detection, based on the observation of an intensified ciliary beating in the canal in the presence of food (Reisinger, 1926), and an increased sensitivity to food following their regeneration in *Prostoma* (Hoplonemertea) (Kipke, 1932).

Scharrer (1941) postulated a neurosecretory function, largely based on the close association of the cerebral organs with the vascular system which is evident in some species, particularly in the Lineidae (Heteronemertea), and the common embryological origin of the neuroglandular tissue and the CNS. She felt that the cerebral organs might represent a stage in the evolution of neurosecretory cells where gland cells could be transformed into nerve cells and could shift from an exocrine to an endocrine function. However, she overlooked the fact that in all cerebral organs the gland cells are exocrine, regardless of the degree of association with the central nervous and vascular systems, and that the gland cells do not produce axons and may not receive innervation. Although neurosecretory cells have since been identified in the cerebral organs of lineids (Lechenault, 1962; Servettaz and Gontcharoff, 1973, 1974, 1976a, b; Korn, 1974; Moretto *et al*, 1975; Ferraris, 1978,

1979a), these are distinct from the gland cells, and they direct their axons to a neurohaemal area in the cerebral ganglia.

From the results of recent experimental work, a possible role of the cerebral organs in salinity stress tolerance has been suggested (Lechenault, 1965; Ling, 1970; Ferraris, 1979a, c). Ling (1970), in *Lineus ruber*, and Ferraris (1979a), in *Lineus socialis* and *Amphiporus lactifloreus*, reported changes in the cytology of cerebral organ gland cells during hypo- and hyperosmotic stress. Ling (1970) claimed a relationship between the secretion of gland cells into the canal, and uptake of mucoprotein from the canal into vesicular cells together with accumulation of acid mucopolysaccharide in these cells in *Lineus*. Ling (1970) and Ferraris (1979a) also observed histological profiles which suggested transfer of acid mucopolysaccharide from vesicular cells to basal cells and out of the cerebral organ to the vascular system, during exposure of the worms to hypo-osmotic stress. They suggested that acid mucopolysaccharide released from the cerebral organs might be an endocrine substance with an active role in osmoregulation. The salinities used (50% and 150% sea water) are extreme, however, and in worms subjected to such a stress some cytological change should be expected due to the direct effect of reduced or increased osmotic pressure on cellular structure. Ling (1970) and Ferraris (1979a) did not consider the possibility that they might be observing direct

osmotic effects on the cytology of gland and vesicular cells.

Lechenault (1965) studied weight changes in two species of lineids subjected to hypo-osmotic sea water, and found that intact worms, which gained weight in dilute sea water, were able to reduce their weight back to normal over a 72 hour period. Using ablation and grafting techniques, he demonstrated that worms lacking both the cerebral ganglia and the cerebral organs were incapable of this volume regulation, but that if either the cerebral organs or the cerebral ganglia were present, volume regulation occurred. His results support a neurosecretory role of the cerebral organs, but contra-indicate a non-nervous endocrine role.

In summary, although it is generally accepted that the cerebral organs are sensory structures, simply on the basis of their neuroanatomy, the modal sensitivity of the receptors, the role of the cerebral organs in behaviour, and the function of the gland and vesicular cells with respect to the nervous elements are not known. Moreover, the functional relationship of the cerebral organs with the vascular system is not understood. There is some evidence that the cerebral organs play a role in osmoregulation, but this interpretation does not assign a function to the sensory elements. Also, the evidence of Lechenault (1965) suggests a neurosecretory role, and can only apply to heteronemerteans, since in hoplonemerteans neurosecretory cells are lacking (Lechenault, 1963; Ferraris, 1978, 1979a).

The evidence of Ling (1970) and Ferraris (1979a) suggests an endocrine role of the cerebral organs in osmoregulation, but comes from histological studies of osmotic effects which do not consider the relative contribution of "non-physiological" osmotic changes to the cytology of gland and vesicular cells.

**D. Objectives**

The cerebral organs are complex structures characteristic of the nemerteans. In spite of the fact that a relatively large amount of the literature on nemerteans has been devoted to these structures, their function has not been satisfactorily established in any species. The objective of this study has been to explore the structure and function of the cerebral organs in some hoplonemerteans of the Sub-order Monostilifera. The histology and fine structure of the cerebral organs is described in *Paranemertes peregrina* Coe, *Tetrastemma candidum* (O.F. Müller) and *Amphiporus lactifloreus* (Johnston). The role of the cerebral organs in the behaviour of *Paranemertes* has been investigated by comparing the behaviour of worms with and without cerebral organs. In addition, preliminary results have been obtained from electrophysiological recordings of activity in the lateral nerve cords and cerebral organ nerves. The role of the cerebral organs in salinity stress tolerance has been examined in *Paranemertes* by observing the effects of cerebral organ removal on volume

regulatory capacity, and by observing the cytological changes occurring in the cerebral organs as a result of exposure to hypo-osmotic media.

It is concluded that the cerebral organs in the three species studied are chemoreceptors in which the function of gland cells is to secrete a mucous coating over the dendrites, and the function of the vesicular cells is to remove this coating material, thereby allowing a constant turnover of the material across the sensory epithelium. It is thought that in the hoplonemerteans studied, it is unlikely that the cerebral organs have an endocrine function.

## II. Material and Methods

### A. Animals

*Paranemertes peregrina*, hereafter referred to as *Paranemertes*, was collected from the mudflat at Snug Harbor, San Juan Island, U.S.A., or at the head of Bamfield Inlet, Bamfield, Canada. The worms come out to feed at low tide and can be picked off the surface of the mud. Only worms from Snug Harbor were used in anatomical studies, but worms from both populations were used in behavioural studies.

*Tetrastemma candidum* was collected from *Fucus vesiculosus* or *Ascophyllum* sp. from the East Rocks, St. Andrews, Fife, using the method described by Kirsteuer (1967). Seaweed was brought back to the laboratory and put into glass aquaria which were then filled with sea water and allowed to stand for one to two days. Under these circumstances the sea water becomes deoxygenated and the worms come to the surface, where they can be collected with a glass pipette.

*Tetrastemma melanocephalum* (Johnston) was collected from approximately mid-tide level of the Eden Estuary, St. Andrews. Shovelfuls of the top 1 cm of mud were sieved, giving a mass of worm tubes, clam shells and debris; this material was collected in buckets and brought back to the laboratory, where it was put into glass aquaria and covered with seawater. After a day or two the worms emerge from the debris and can be collected from the surface of the

seawater. Of the two species of *Tetrastemma*, the study focused mainly on *T. candidum*, and except where indicated, all figures are from this species. Because the structure of the cerebral organs in the two species was the same, the results are presented together.

*Amphiporus lactifloreus*, hereafter referred to as *Amphiporus*, was collected from under rocks at the mid-tide level, in surge channels east of the town of St. Andrews and from the Castle Rocks, St. Andrews.

Worms were kept in running sea water for up to three weeks prior to use, and were not fed, except where specified.

**B. Structure of the cerebral organs**

**1) Electron microscopy**

Prior to fixation the worms were anaesthetized in equal parts of isotonic $MgCl_2$ and sea water for one to two hours. Anaesthetised worms were decapitated, and the heads were fixed in 2.5% glutaraldehyde in 0.4M phosphate buffer (pH 7.6) and 0.14M NaCl (Dunlap, 1966, Cloney and Florey, 1968). The heads of *Paranemertes* and *Amphiporus* were bisected while in glutaraldehyde to allow full penetration of the fixative. *Tetrastemma* heads are very small and required no further dissection. The tissue was post-fixed in 2% $OsO_4$ in the same buffer or in 1% sodium bicarbonate buffer. This fixative gives good cytological preservation in many marine

invertebrates. Although it is specifically designed for invertebrates from Puget Sound (salinity=32 ppt), it also gave good results with the species from St. Andrews Bay where the salinity is 34 ppt (Laverack and Blackler, 1974), therefore the osmolarity was not adjusted for *Tetrastemma* or *Amphiporus*. As an alternative, some tissue was fixed in 2.5% glutaraldehyde in 0.2 M cacodylate buffer, and post-fixed in $OsO_4$ in the same buffer.

All tissue was dehydrated in an ethanol series and embedded in Spurr's or Epon epoxy resins. Thin (silver-grey) sections were cut on a Porter-Blum MT2 ultramicrotome, mounted on uncoated copper grids and stained in uranyl acetate and lead citrate (Reynolds, 1963, or Venable and Coggeshall, 1965). Sections were examined with a Phillips EM201 or an AEI EM 6B at 60 kv.

**2) Light microscopy**

One micron sections of glutaraldehyde-osmium fixed tissue embedded in plastic were cut on a Porter-Blum MT1 ultramicrotome and stained with Richardson's stain (Richardson *et al*, 1960). Richardson's is a mixture of two metachromatic stains, Methylene Blue and Azure II, that colours acid mucosubtances pink and the rest of the tissue in shades of blue. Some tissue was fixed in sea water Bouin, dehydrated in a methanol-xylene series and embedded in paraffin. Seven micron sections were cut, mounted on glass slides and stained with Masson's Trichrome (Humason, 1967).

Serial sections from tissue fixed in Bouin, embedded in wax and cut at 7 μm were used initially for their ease of preparation. However, much better fixation and resolution was obtained with tissue fixed in glutaraldehyde-osmium, embedded in plastic and cut at 1 μm. Although much more time consuming, this method was used mainly. The greater resolution was helpful in investigating critical points such as the possibility of exchange of material between the cerebral organs and the vascular system, and the effect of osmotic change on fixation quality. One micron sections were also cut from blocks destined for electron microscopy just before or just after thin sectioning and were used for orientation. Sections were examined with a Zeiss Universal Ultraphot photomicroscope using Nomarski optics. All light micrographs are from one micron sections.

**C. Acid phosphatase localization**

The method of Brunck and Ericsson (1972) was applied in an attempt to identify acid phophatase activity in lysosomes of gland cells and vesicular cells in the cerebral organs, but it was not successful. The commercially available B-glycerophosphate substrate is from vertebrate tissue, and incubations were therefore carried out at 37°C and at pH 7.2. It is possible that these parameters are not suitable for nemertean tissue.

D. Calibration

Measurements of structures visible in the light microscope were made using an ocular micrometer calibrated with a stage micrometer.

All measurements of ultrastructural features were made on printed micrographs. These were calibrated using micrographs of catalase crystals taken at the same electron microscope magnification, and printed at the same enlarger magnification as micrographs of structures to be measured. Catalase is an organic crystal with a constant period of 8.75 nm.

E. Operations

The cerebral organs were removed from *Paranemertes* anaesthetized in equal parts of sea water and isotonic $MgCl_2$. With the ventral side of the worm down, a small cut was made in either side of the head midway between the anterior and posterior cephalic grooves. The cut was spread open with forceps thus exposing the cerebral organ, which was then removed with a fine hooked-tip dissecting pin. The success of the operations was verified histologically. Six operated worms were fixed in Bouin's, embedded in paraffin, sectioned, and stained with Masson's Trichrome (Humason, 1967). Examination of these showed five with complete ablations and one in which the cerebral organ of one side was incompletely removed but the cerebral organ nerve was cut. Since operated worms were used in experiments lasting

up to six days, the time course of regeneration was followed over a one week period. Six worms fixed 3, 5 and 7 days after the operation were prepared for histology as described above and examined for signs of regeneration. The cerebral organs had not regenerated after one week.

Sham operations were done by making a cut on either side of the head behind the posterior cephalic groove adjacent to the cerebral ganglia and teasing the underlying muscle tissue with a dissecting pin, or by removing the tip of the snout just in front of the anterior cephalic grooves. The extent of surface damaged is estimated to be the same in all these operations. However, the sham operations do not damage the CNS and therefore are probably less severe than the operation which removes the cerebral organs.

Within twelve hours of any of the operations the cuts were healed and the worms were capable of proboscis eversion. Experiments were begun 24 hours or more after the operation.

**F. Mudflats**

Two artificial mudflats were set up in the laboratory in sea tables 60 cm X 130 cm. Surface mud collected at low tide from a *Paranemertes* collecting site was distributed in the sea tables. The central drain was raised by inserting a piece of plexiglass tubing which extended 20 cm above the mud, and sea water was allowed to flow continuously to simulate high tides. To simulate low tides, the sea water

supply was turned off, and the tubing was set ajar in the drain, allowing the water to run out slowly. Low tides were scheduled once or twice a day, at times corresponding approximately to actual low tides at Snug Harbor or Bamfield, as it was found that more worms were active at these times. Each mudflat contained 10 to 12 worms, which were replaced with fresh ones every five or six days. The stimuli will be described with the results. The Binomial Test was used to evaluate the statistical significance of the results.

**G. Electrophysiology**

Recordings were obtained using suction electrodes with tip diameters of 20 $\mu$m or greater. Signals were amplified using an a.c. preamplifier with a half amplitude bandpass of 80 to 1000 hz, and were recorded on a Gould Brush 220 chart recorder. All dissections were performed on worms anaesthetized in equal parts of 0.36 M $MgCl_2$ and sea water. The dissections are described with the results.

**H. Volume regulation**

The range of salinities occurring at Snug Harbor was determined using a refractometer to measure the salinity of puddles on the mudflat at low tide during extremes of weather. On a very hot day in July 1980, at midday, after the tide had been out for several hours, the salinity measured in four samples was 32 ppt, 33 ppt, 33 ppt and 36

ppt (the normal concentration of sea water at Snug Harbor is 32 ppt). On a rainy day in June 1981, after two hours of drizzling rain during low tide, the salinity measured in four samples was 30 ppt, 31 ppt, 26 ppt and 5 ppt (the very dilute sample was obtained from the crevice of a small rock). It is possible that *Paranemertes* is exposed little if at all to extremes of salinity. Roe (1976) reported that fewer worms appear on the mudflat on hot days, and those that appear do so for shorter periods. She observed the greatest densities of worms during low tides at night. Worms generally do not come out in the rain, and return to their burrows if it begins raining while they are on the mud surface. Thus the salinities used in this study (75% and 50% sea water) are probably extreme, but were chosen so that a comparison with earlier literature (Ling, 1970; Ferraris, 1979a) would be possible.

Worms with and without cerebral organs, as well as sham-operated worms, were tested for their ability to regulate volume in dilute sea water, using weight as an indication of volume. Solutions of 50% and 75% sea water were prepared by dilution of filtered sea water with distilled water. Worms were kept individually in covered glass finger bowls containing 200 ml of the experimental sea water maintained at 10 to 12°C. Wet weights were obtained as follows: using fine, hooked-tip forceps, worms were removed, shaken once, and weighed on a Mettler top loading balance. The weight was recorded to the nearest milligram. To

determine the repeatability of weights, six worms from 100% sea water were weighed five times each at 30 second intervals. Identity of weights for each worm was highly significant ($P<.005$).

To determine whether *Paranemertes* is capable of volume regulation, intact worms were kept in 75% and 50% sea water, and the changes in their weights were followed over a 60 hour period. A control group of intact worms was placed in 100% sea water and weight changes in this group were measured at the same intervals and over the same time period as those in the experimental groups. Ten worms, chosen randomly with respect to size, were used in each group. The worms were weighed after 1, 3.5, 6, 12, 24, 36 and 60 hours.

As there appeared to be a difference in the response of intact and operated worms, a second experiment was performed using sham-operated worms in addition to worms with cerebral organs removed and intact worms. In this experiment six groups of ten worms each were used as follows: 1) intact worms in 75% sea water, 2) intact worms in 100% sea water, 3) worms without cerebral organs in 75% sea water, 4) worms without cerebral organs in 100% sea water, 5) sham-operated worms in 75% sea water, and 6) sham-operated worms in 100% sea water. Worms were weighed after 1, 4, 7, 10, 24, 36 and 48 hours.

## I. The effect of hypo-osmotic stress on cerebral organ cytology

### 1) Effect of hypo-osmotic fixative on cerebral organ cytology

To determine the cytological effect of osmotic damage on the cerebral organs, worms from normal sea water were fixed in phosphate buffered glutaraldehyde of reduced osmolarity. Since the fixative kills the tissue on contact, any difference in cytology, as compared to normally fixed tissue, is likely to be the result of osmotic damage during fixation, rather than of a physiological response of the tissue. Fixatives with osmolarities 50% (480 milliosmoles, =mosm) and 75% (730 mosm) of normal were prepared by modifying the concentration of buffer and salt.

### 2) Effect of 50% and 75% sea water on cerebral organ cytology

To determine the cytological effect of salinity stress on the cerebral organs, *Paranemertes* subjected to 75% and 50% sea water for 1, 6 and 24 hours were fixed in phosphate buffered glutaraldehyde with equivalently reduced osmolarity.

None of the worms were anaesthetized prior to fixation. Following at least 24 hours in glutaraldehyde, all tissue was postfixed in 2% $OsO_4$ in 0.2M phosphate buffer. No attempt was made to modify the osmolarity of the secondary

fixative, since osmium tetroxide is thought to destroy the osmotic activity of cells (Wood & Luft, 1965; Bone & Denton, 1971). Tissue was dehydrated in an ethanol - propylene oxide series and embedded in Epon epoxy resin. Sections approximately 1 μm thick were cut with a Porter Blum MT2 ultramicrotome, mounted on glass slides and stained with Richardson's stain (Richardson *et al*, 1960).

## III. Structure of the cerebral organs

### A. Results

#### Position of the cerebral organs and shape of the canal

In *Paranemertes* the cerebral organs (Fig. 1) are located adjacent to the cephalic blood vessel, well anterior to the cerebral ganglia, to which they are connected by the cerebral organ nerve. The cerebral organs of *Amphiporus* (Fig. 2) are in a similar position with respect to the central nervous and vascular systems, except that in the latter species, the blood vessel passes the organ dorsomedially while in the former it passes the organ medially or ventromedially. In *Tetrastemma* (Fig. 3) the cerebral organs are closely associated with the cerebral ganglia but not with the vascular system. Along their postero-medial surface the cerebral organs are directly apposed to the dorsal cerebral ganglia from which they are separated by a thin connective tissue sheath interrupted at the point of entry of the cerebral organ nerve. The cephalic blood vessel in this species forms a simple loop in the head which passes on the medial side of the ocelli, and of the anterior part of the cerebral organ. It bends medially anterior to the cerebral ganglia, and therefore has no contact with the posterior glandular part of the cerebral organ.

Each cerebral organ communicates with the external medium via a ciliated canal, the cephalic canal, which opens on the ventrolateral surface in the anterior cephalic groove. In *Paranemertes* the canal gives off a small posteriorly directed branch just inside the cerebral organ proper, bends posteriad at an angle of 90° or greater and ends blindly in the posterior part of the cerebral organ (Fig. 1). Near the blind end the canal bifurcates, giving off a very short medial branch and a longer lateral branch. The shape of the canal in *Amphiporus* is virtually the same, except that the anterior branch is usually missing or is very small (Fig. 2). In *Tetrastemma* the canal extends postero-medially without any marked bends, and there is no branching of the anterior part of the canal (Fig. 3). In all species, the shape of the canal varies with the extent of contraction of the worm.

**Histology of the cerebral organs (Figs. 1-12)**

*1) The canal epithelium;*

In the three species studied, the anterior and medial quarter of the canal epithelium consists of ciliated cells (and of the processes of Type A gland cells). The facing epithelium is differentiated into Type 1 vesicular cells in the anterior part of the cerebral organ (surrounding the small posteriorly directed branch of the canal in *Paranemertes*). Along the remainder of the canal up to the point of bifurcation, there is a sensory epithelium,

consisting of Type 1 sensory cell dendrites associated with ciliated support cells (Figs. 1,2,3). Type 2 sensory cell dendrites are situated on or just anterior to the medial fork, Type B gland cell processes are situated on the lateral fork, and Type 2 vesicular cells are in between.

The canal appears to be divided into two parallel channels throughout. The cells separating the antero-medial ciliated cell epithelium from the vesicular/sensory epithelium bear long cilia with well-developed striated rootlets, and appear to form a barrier between the two channels of the canal (Fig. 4A). In a short segment of the canal near the blind end, cells in the same position (separating major and minor canal epithelia) produce a septum formed of cilia and microvilli, which completely separates the two channels (Fig. 4B). Using the terminology of Ling (1969a), who described a comparable division of the canal in the cerebral organs of *Lineus ruber*, the smaller channel will be referred to as the minor canal and the larger channel as the major canal. Cells forming the septum near the blind end of the canal will be referred to as lappet cells.

*2) Sensory cells;*

Type 1 sensory cells constitute the main nervous component of the cerebral organ. Their cell bodies form the bulk of the cerebral organ in the mid-region (Figs. 5A,6,7B). The dendrites of these cells extend to the canal lumen among ciliated support cells, comprising a large

sensory epithelium which lines much of the major canal. Type 2 sensory cell dendrites form a discrete patch of the minor canal epithelium near the blind end of the canal (Fig. 7B), and their cell bodies are situated posterior to the blind end, adjacent to the connective tissue capsule of the cerebral organ (Figs. 5B,6,7A,8A). Type 1 and Type 2 sensory cell bodies are similar in morphology, except that the latter have nuclei which are slightly larger, and have less clearly demarcated areas of hetero- and euchromatin. Both types of sensory cell are bipolar. The axons of Type 1 and 2 sensory cells together form the cerebral organ nerve. Type 2 sensory cells have not been described previously and were overlooked in *Amphiporus* by Ling (1969b) and Ferraris (1979a).

*3) Gland cells;*

The cerebral organs contain two populations of gland cells, distinguishable by their position, location of secretory processes, staining affinity and secretory granule morphology (EM). The smaller group, referred to as Type A gland cells, is located on the dorsal, medial and ventral surface of the organ in the mid-region (Figs. 5A,9A,10A). The larger group (Type B) comprises several large lobes surrounding, and posterior to the blind end of the canal (Figs. 7B,8A,8B,9B). Both groups are clearly exocrine, extending processes containing secretory granules to the canal lumen. Type A gland cell processes open into the minor canal among the ciliated cells opposite the main sensory

epithelium (Figs. 6,9A,10A). Type B processes open into the major canal near the blind end (Figs. 6,7B,9B,10B). Type A secretory granules stain dark blue and Type B granules stain dark blue to blue-green. The amount of secretion product within gland cells is variable (Fig. 8B). Gland cells filled with granules may also contain large globules formed by the coalescence of some of the granules (Figs. 5,10A). Additionally, both Type A and B gland cells sometimes contain one or a few vacuoles with heterogeneous contents (Figs 5B,7B,8B) which stain pink with Richardson's stain. The material in these vacuoles is identical to that in Type 2 vesicular cells (described below) and will be referred to as Type 2 vesicular material. The secretion product of gland cells has been characterised in *Lineus*, *Cerebratulus* and *Amphiporus* as a neutral mucoprotein (Ling, 1969a, b; Bianchi *et al*, 1972).

*4) Vesicular cells;*

Type 1 vesicular cells are tall columnar ciliated cells with basally located nuclei (Figs. 6,11,12). The apical cytoplasm of these cells tends to be very lightly stained and the apical border, defined by the row of striated rootlets, is very irregular. Except for the ciliary structures, inclusions in the apical region cannot be resolved with the light microscope. The Type 1 vesicular material visible at this level of resolution consists of vesicles of variable size and some large vacuoles of irregular shape with heterogeneous, dense contents. Some

vesicles are clear, others are stained various shades of blue. The material in vacuoles is stained blue, purple or pink with Richardson's. The amount of vesicular material in Type 1 vesicular cells varies from one individual to another and among the three species. In *Tetrastemma* and *Amphiporus* there is a greater number of large vesicles with heterogeneous contents, while in *Paranemertes*, this latter type of vesicle is relatively uncommon, but the vesicles with clear or uniformly dense contents are generally more numerous.

Type 1 vesicular cells, which are present in all three species studied, have not been described previously, having apparently been overlooked in *Amphiporus* by Ling (1969b) and Ferraris (1979a), although Ferraris mentioned "ciliated cells lining the canal up to the second bend (which) contain sparsely distributed granules" of variable staining affinity (Ferraris, 1979a, p. 437) and these may be the same cell type.

Type 2 vesicular cells comprise a small number of very large cells having a free surface on the minor canal at the blind end, and immediately posterior to these, a few large cells which appear to be subepithelial (Figs. 4B,5B,7A,8C,10B). Occasionally, an isolated Type 2 vesicular cell is observed among Type A or Type B gland cells in areas remote from the blind end of the canal. As mentioned above, Type 2 vesicular material is sometimes seen within or among gland cells (Figs. 5B,7B,8B). It is also present in isolated

clumps which appear to be extracellular, among sensory cells (Fig. 8A). In histological preparations, Type 2 vesicular material is resolved as large spherical vacuoles with heterogeneous contents staining blue to pink with Richardson's. Type 2 vesicular material differs from Type 1 in that it stains predominantly pink rather than blue, the vacuoles containing it are larger, and the vacuoles tend to be spherical rather than irregular in shape. As with Type 1 vesicular material, the amount present varies from one individual to another, and is generally greater in *Amphiporus* and *Tetrastemma* than in *Paranemertes*.

*5) Subepithelial support cells;*

Subepithelial support cells (Fig. 8A) are multipolar cells which extend processes among gland cells, Type 2 vesicular cells, sensory cells, and axons in the cerebral organ nerve. Subepithelial support cells almost certainly correspond to the basal cells described by Ling (1970) and Ferraris (1979a) in the cerebral organs of *Lineus* and *Amphiporus*. However, in this study these cells were not found to be specifically associated with vesicular cells or vesicular material.

**Fine structure of the cerebral organs (Figs. 13-43)**

*1) Orientation of basal feet in cells of the canal epithelium;*

In all cells in the cerebral organ bearing cilia, the ciliary basal bodies have two structures projecting from

them laterally (Fig. 13). A short, uniformly electron dense hooked structure projects from one side, and a conical structure with pronounced striations projects from the opposite side. In oblique sections, it has been determined that the hooked structure consistently corresponds in position to the 5 and 6 microtubule pairs of the ciliary axoneme (Fig. 13), and it is therefore considered to be the basal foot, as defined by Gibbons (1961). The structure opposite is termed the accessory striated rootlet. In ciliated cells, ciliated support cells and Type 1 vesicular cells, which together form most of the canal epithelium, there is great consistency in the orientation of basal feet. In all three species, in cells lining the minor canal (ciliated cells), the basal feet are on the side of the basal body nearest the blind end. In cells lining the major canal (ciliated support cells and Type 1 vesicular cells), the basal feet are on the side of the basal body nearest the open end of the canal (Fig. 18). In ciliated cells separating the major and minor canals, and in lappet cells, the basal feet are oriented in the plane of cross section through the canal (Figs. 14B,15).

*2) Ciliated cells and lappet cells;*

Ciliated cells (Figs. 14,16) form the epithelium of the minor canal along most of its length. Each cell has 12 to 18 cilia distributed evenly over the apical surface of the cell. A long stout striated rootlet is directed downwards from the proximal end of each basal body towards the

nucleus. The dark cross-banding material of each rootlet extends beyond the edge of the rootlet into the cytoplasm and is continuous with the cross-banding material of adjacent rootlets. Towards the base of the rootlets the bundles of longitudinal filaments split and join with filaments of adjacent rootlets. Thus within each ciliated cell there is an anastomosing complex of striated rootlets forming a cone-shaped network with the tip of the cone ending very near the nucleus.

The ciliated cells contain a very large number of small mitochondria. These are distributed throughout the cell and are particularly numerous in the apical cytoplasm associated with the striated rootlet network and in the basal cytoplasm in the perinuclear region. In this latter region, large areas of the cytoplasm are filled with pools of glycogen, and large numbers of mitochondria are associated with these pools. A number of large clear vesicles and a few smaller dense bodies are present in the apical and middle regions of the cytoplasm. In cells separating the major and minor canals, numerous small clear vesicles are present in the apical cytoplasm. Clumps of free ribosomes and a few cisternae of rough endoplasmic reticulum are present in the nuclear region. The Golgi complex is small and is rarely seen, which suggests that there may be only one per cell.

Along the full length of the canal, barrier-forming ciliated cells, which separate the major and minor canals, are specialized in having a more strongly developed striated

rootlet complex than other ciliated cells (Fig. 14). Also, the cytoplasm has a slightly different appearance than that in adjacent cells, being less finely granular. Lappet cells (Fig. 15), which form a septum dividing the canal lumen, do not have well-developed striated rootlet complexes. The septum formed by these cells consists of a mass of very long cilia and microvilli which extend across the canal lumen and interdigitate extensively with those from the opposite side. In *Amphiporus*, lappet cells have a relatively dark cytoplasm and are therefore quite conspicuous.

*3) Nervous elements among ciliated cells;*

Among the ciliated cells, near the base of the epithelium, there are a few small bundles of nerve fibres which are distinct from the sensory elements of the cerebral organ. The fibres contain small vesicles with dense contents, mitochondria and microtubules (Fig. 16). Their cell bodies have not been observed, and are probably located outside the cerebral organs. The fibres are present in all three species studied, but they are very rare, and their synapse has not been identified.

*4) Ciliated support cells;*

Ciliated support cells are associated with the dendrites of Type 1 sensory cells, and together these form a large segment of the major canal epithelium (Figs. 17,18,19,20). Each support cell has 6 to 14 cilia, distributed evenly over the apical surface, as well as a few microvilli. From the basal body of each cilium there

projects a basal foot, an accessory striated rootlet and a striated rootlet. Unlike the striated rootlets in ciliated cells of the minor canal, in ciliated support cells the rootlets are discrete, adjacent rootlets anastomosing little if at all. The support cell cytoplasm is filled with bundles of microfilaments oriented mainly in the plane of cross section through the canal (Fig. 17). These attach to striated rootlets and to the cell membrane at desmosomes. The cells contain a moderate number of mitochondria, and small pools of glycogen. As in ciliated cells, the rough endoplasmic reticulum and Golgi complex are poorly developed. A few dense bodies, which are probably secondary lysosomes or residual bodies, are present in the mid-region in most of the cells. The material in these dense bodies (Fig. 21) sometimes resembles Type 1 vesicular material, but there is a very small amount of this material in support cells relative to the amount in Type 1 vesicular cells, where it is abundant and characteristic. Near the apical surface the support cells are attached to Type 1 sensory cell dendrites by desmosomes (Zonula adherens) and septate junctions. A few small clear vesicles are present in the apical cytoplasm. Coated vesicles and pits have not been observed in these cells.

*5) Sensory cells;*

The dendrites of Type 1 sensory cells extend to the lumen of the major canal among the ciliated support cells, with which they are associated in a honeycomb pattern (Figs.

17,18,19,20,21). The dendrites are slender processes with relatively clear cytoplasm. They are unciliated. The free surface bears microvilli which extend out over the support cells and among the support cell cilia, forming an elaborate microvillous network (Fig. 19). A few coated pits and vesicles are present in the dendrites at and just below the apical surface. Many uncoated clear vesicles of variable size are present throughout the cytoplasm of the dendrites. The coated vesicles occasionally have small dense cores. Numerous microtubules are present in the cytoplasm. These extend to the apical tip of the dendrites but not into the microvilli, and are often closely associated with coated and uncoated vesicles. Very long tubular mitochondria are present throughout the dendrites except in the apical region. A few dendrites have been traced in serial sections to their somata (Figs. 21,22), which collectively form the bulk of the cerebral organ in the mid-region. In each soma, the nucleus is centrally placed and is usually surrounded by little cytoplasm (Figs. 21B,22A), except in cells where the endoplasmic reticulum and Golgi complex are greatly amplified (Fig. 22,23), as described below.

The cytoplasm contains rough endoplasmic reticulum and a Golgi complex of variable morphology. In some cells the reticulum is moderately developed; many free ribosomes are present, and there is a small amount of smooth endoplasmic reticulum containing light granular material. In these cells, the Golgi complex is well developed and consists of

many tightly packed, large, flat cisternae (Fig. 22B). In other cells the endoplasmic reticulum is smooth surfaced, the cisternae are very swollen and have formed into vesicles, and few free ribosomes are present in the cytoplasm (Fig. 22C). The smooth endoplasmic reticulum fills the cells, which tend to be very large. The Golgi complex is not prominent in these cells and in a few cases profiles suggesting autophagic engulfment of the Golgi complex have been observed (Figs. 22D,23). Cells with an intermediate morphology are also present. In these the cisternae of endoplasmic reticulum are swollen but not 'beaded', a few ribosomes are present on the reticular membranes, and the Golgi complex is conspicuous. The functional significance of the variability in Golgi complex and endoplasmic reticulum morphology is not known.

In all cells, there is a moderate number of small mitochondria, often associated with pools of glycogen. Small dense bodies which appear to be secondary lysosomes or residual bodies are occasionally seen in the perikarya, but they are unusually rare in these nerve cells.

Type 2 sensory cell dendrites form a discrete patch in the minor canal epithelium near the blind end (Figs. 15,24,25). Each dendrite bears one (or rarely two) cilium and a ring of microvilli. A very stout striated rootlet extends downward into the cytoplasm from the basal body of each cilium and a smaller but also quite well-developed accessory striated rootlet extends at an angle into the

cytoplasm from the lateral surface of the basal body. A basal foot has not been observed. As in Type 1 sensory cells, many large tubular mitochondria are present within the dendrites. The dendrites are connected to each other apically by junctional complexes consisting of desmosomes and septate junctions. There are a few coated pits and vesicles at or near the apical surface and numerous small clear vesicles are present, concentrated in the apical region of the cytoplasm. The dendrites contain a large number of microtubules. The dendrites have been traced to their cell bodies only in *Tetrastemma*, but cells in a similar position and with a similar morphology are also present in *Paranemertes* and *Amphiporus*, and it is assumed that these are Type 2 sensory cell bodies in these two species. The somata are located near the medial surface of the cerebral organ at a level just posterior to the blind end of the canal. They have an ultrastructure similar to that of Type 1 sensory cells, and in these cells also, the endoplasmic reticulum and Golgi complex exhibit a variable morphology. The nucleus of these cells is distinguishable from that of Type 1 sensory cells in being slightly larger and having less clearly demarcated areas of hetero- and euchromatin (Fig. 23). The axons of Type 1 and Type 2 sensory cells join to form the cerebral organ nerve (Figs. 26,27). The axons are very slender, seldom exceeding 1 μm in diameter. They contain many microtubules, small mitochondria and small clear vesicles.

*6) Gland cells;*

In both Type A and Type B gland cells the nuclei are very large, and contain a prominent nucleolus (Figs. 28,29). Most of the nucleoplasm is in the euchromatic form. A well-developed rough endoplasmic reticulum and Golgi complex, as well as large numbers of secretion granules, are prominent features of gland cells. The rough endoplasmic reticulum occupies large areas of the cell that are free of secretion granules. Several well-developed Golgi complexes occupy an area in the vicinity of the nucleus in each cell. Most of the Golgi lamellae contain dark material, similar in electron density to the material in secretion granules, and these appear to release vesicles into the cytoplasm (Fig. 29A). Newly released Golgi vesicles containing dense material are 150 to 200 nm in diameter. In small areas of each complex, the Golgi lamellae are more tightly packed and contain an electron lucent material. Vesicles appear to be released from these areas as well. These are very small (74 to 110 nm in diameter) and have clear contents. Small clear vesicles in the vicinity of the Golgi sometimes have a bristle coating on their cytoplasmic surface (Fig. 29B). Thus the Golgi complex in gland cells produces at least two, and possibly three kinds of vesicles which can be distinguished morphologically. Usually, profiles of three or more separate stacks of Golgi lamellae are seen arranged in a circle, with the releasing face to the inside. The circle is surrounded by rough endoplasmic reticulum, which is

separated from the Golgi by a narrow band of cytoplasm containing many clear vesicles of very small diameter (Fig. 29B). Within the circle, newly released Golgi vesicles, condensing vacuoles, and a few mature secretion granules are present. The condensing vacuoles are of variable size, with the largest being slightly larger than mature secretion granules. The material within the vacuoles is less electron dense than that in either dense vesicles newly released from the Golgi or mature secretion granules. Also, there is an electron lucent halo separating the vacuole contents from the vacuole membrane. Though the vacuoles are circular, there are sometimes small bumps in the vacuole membrane which appear to result from fusion of Golgi-produced vesicles with the vacuoles. The secretion granules are 600 nm to 1 $\mu$m in diameter, with electron dense contents (Figs. 29A,30). In Type A gland cells the granule contents tend to be of uniform electron density, and the granules are spherical (Figs. 29A,30A). In Type B gland cells, the contents are of uneven electron density and sometimes contain light crystalline structures (Fig. 29A). Type B granules are of variable shape, either spherical or lobular. The shape of the granules - spherical in Type A and irregular in Type B - suggests that the granule contents are of different consistencies, probably fluid in Type A and semisolid in Type B gland cells.

As described above from histological preparations, Type 2 vesicular material occurs in some gland cells in isolated

vacuoles of variable size. This material will be described with the material in Type 2 vesicular cells.

The gland cell processes are slender with relatively clear cytoplasm (Fig. 31). They are 1 to 2 μm in diameter, widening at the apical end. Type A processes bear a single cilium (Fig. 31B). Mature secretion granules are always present within the processes. Each process contains a cylinder of microtubules oriented parallel to the long axis of the process and situated just within the cell membrane. Microtubules are closely associated with the secretion granules in the processes and in the cell body, sometimes extending into the circle formed by the Golgi complex. Release of secretion granules has not been observed, but it is assumed that release involves exocytosis by fusion of the granule membrane, as intact granules are very rarely seen in the canal lumen. In addition to mature secretion granules, the gland cell processes contain a small number of clear vesicles, and coated pits and vesicles are occasionally present in and near the apical membrane (Fig. 31A). These are probably involved in recycling the granule membrane incorporated into the plasma membrane during exocytosis (Silverstein *et al*, 1977).

*7) Type 1 vesicular cells;*

Type 1 vesicular cells are ciliated, bearing 10 to 14 cilia arranged characteristically in a ring at the cell periphery. The basal body of each cilium bears a basal foot and an accessory striated rootlet extending laterally in

opposite directions into the cytoplasm, and a single long slender striated rootlet directed basally. Type 1 vesicular cells are characterised by the presence of many vesicles of variable morphology which comprise the vesicular material (Fig. 32). The vesicles can be subdivided into five size classes, each with characteristic contents.

1) Small vesicles, 120 to 150 nm in diameter, containing a material of light, uniform electron density which lines the inner face of the vesicle membrane leaving the central core of the vesicle clear (Fig. 33). Some of the vesicles have a bristle coating on their cytoplasmic surface, and the material in these tends to be slightly darker and to fill up the entire vesicle, although this is not always the case.

2) Vesicles 400 nm to 1.4 μm in diameter containing very electron dense, heterogeneous material (Fig. 32,34,35).

3) Vesicles 600 nm to 1.2 μm in diameter containing material of a density which is intermediate between that of material in sizes 2 and 4 vesicles (Fig. 32).

4) Vesicles 1.3 to 1.7 μm in diameter, some of irregular contour, containing light flocculent material (Figs. 32,34).

5) Vacuoles 1.5 to >5 μm in diameter containing aggregations of size 2 vesicles (Figs. 32,34,35).

Size 1 vesicles fill the apical cytoplasm, and coated pits of approximately the same size are present in the surface membrane. The number of vesicles in this size class decreases abruptly toward the mid-region of the cell. Size 2, 3 and 4 vesicles occupy the mid-region (Fig. 32), with

the larger, lighter vesicles (size 4) situated nearest the apical part of the cell, adjacent to size 1 vesicles, the smaller darker vesicles (size 2) situated nearest the basal part of the cell and the others (size 3) in between. Size 3 vesicles are also present in the basal region of the cell, either singly, or aggregated into vacuoles (size 5). In *Paranemertes* these vacuoles are relatively few, whereas in *Tetrastemma* and *Amphiporus* they are abundant.

In addition to the vesicular material just described, the cells contain various organelles, including many stacks of Golgi lamellae which produce a distinctly different, sixth class of vesicle, less than 100 nm in diameter (Fig. 34A). The Golgi complexes, and the vesicles they release, are present in the mid-region of the cells among size 2, 3 and 4 vesicles (Fig. 32). Rough endoplasmic reticulum is present as individual long cisternae surrounding the nucleus and adjacent to the cell membrane throughout the cell except in the apical region containing size 1 vesicles. In *Paranemertes* and *Amphiporus*, but not in *Tetrastemma*, the bases of the adjacent vesicular cells are strongly interdigitated, and bundles of microfilaments are evident in the interdigitations. The base of the vesicular cell epithelium rests on the connective tissue capsule of the cerebral organ, and adjacent to this in the region of Type 1 vesicular cells are muscles of the head and cephalic glands. There is no indication of any exchange of material through the base of the cells.

*8) Type 2 vesicular cells and Type 2 vesicular material;*

Type 2 vesicular cells can be sub-divided into epithelial cells, which occupy a relatively small segment of the canal epithelium at the blind end (Fig. 36), and subepithelial cells, which are located subjacently, posterior to the blind end of the canal (Fig. 37). Individual subepithelial Type 2 vesicular cells are occasionally seen in areas remote from the end of the canal. The apical surface of epithelial Type 2 vesicular cells bears cilia and microvilli (Fig. 36). The cilia and their striated rootlets are oriented apparently haphazardly, lying at any angle to the long axis of the cells. In some regions the apical surface lacks specializations and is heavily coated with a coarse granular material. The apical cytoplasm is filled with clear vesicles, 120-150 nm in diameter. A few large clear vesicles are also present in this region. The vesicular material visible at the light microscopic level consists of many large vacuoles containing a heterogeneous dense material. A relatively sparse rough endoplasmic reticulum is scattered throughout the cytoplasm and several Golgi complexes producing small (<100nm) vesicles are present in the mid-region of the cells.

Subepithelial Type 2 vesicular cells (Fig. 37) are very large. They have a nucleus of irregular shape and a few mitochondria, but generally lack organelles and have very little cytoplasm. The vesicular material in these cells differs from that in epithelial Type 2 vesicular cells in

that it is less electron dense, and although usually contained in membrane-bounded vacuoles, is sometimes dispersed in the cells, as if some of the vacuoles had burst. It is possible that the vesicular material is sensitive to slight changes in osmotic pressure, and that it is amplified by slightly hypo-osmotic media. Large open spaces are present in the cells, also suggesting cellular disruption. In some areas, intact dense granules of approximately the same size as secretion granules in gland cells, but of lower electron density, can be resolved (Figs. 37C,37D).

Type 2 vesicular material is also contained within gland cells, where it apparently arises through lysosomal breakdown of secretion granules. Configurations are seen which span the range between fully formed, intact secretion granules, and dense bodies which can be identified as Type 2 vesicular material (Fig. 38). In Type A cells, and to a lesser extent in Type B cells, an initial stage in this breakdown is aggregation of secretion granules into large globules (Fig. 39). Although usually this degradation of gland cell contents involves the secretion granules exclusively, in some cases all of the synthetic apparatus, including endoplasmic reticulum, Golgi complex and mitochondria, is incorporated into lysosomes along with the secretion granules (Fig. 40). These cells then lose their glandular characteristics and take on the appearance of Type 2 vesicular cells. It is possible that all subepithelial

Type 2 vesicular cells represent gland cells that have undergone autolysis of the secretion product and synthetic apparatus.

Type 2 vesicular material, either intra- or extracellular, is occasionally observed surrounded by gland cells, among gland cells near the connective tissue capsule of the cerebral organ, among bundles of axons in the proximal part of the cerebral organ nerve or among sensory cell bodies (Fig. 41).

*9) Subepithelial support cells;*

Subepithelial support cells are multipolar cells whose processes insinuate themselves among gland cells, sensory cells, and axons in the cerebral organ nerve (Figs. 26A,27,42,43). The nuclei of these cells are slightly elongate, with an irregular contour. They are characteristic in being smaller and more electron dense than other nuclei in the cerebral organs. The cytoplasm also tends to be of a higher electron density than the cytoplasm of axons or gland cells, thus their processes are conspicuous and easily traced. The cells contain a few scattered cisternae of rough endoplasmic reticulum, a poorly developed Golgi complex and a small number of mitochondria. Dense bodies, which appear to be either secondary lysosomes or residual bodies, are common, both in the perinuclear cytoplasm and in the processes. The processes contain bundles of microfilaments.

Subepithelial support cells, when associated with sensory cell bodies and axons, should be regarded as glial

cells, although glial cells cannot be distinguished as a separate cell type in the cerebral organs, since the processes of a single support cell can be associated with both gland cells and nerve cells (Fig. 43).

**Relationship of the cerebral organs and the vascular system**

In *Tetrastemma*, the cephalic blood vessel does not lie adjacent to the cerebral organs, being separated from them anteriorly by some of the cephalic nerves, and posteriorly by the cerebral ganglia (Fig. 3). In *Paranemertes* and *Amphiporus* the cephalic blood vessel lies adjacent to the posterior part of the cerebral organ, from which it is separated by a layer of connective tissue 10 to >30 μm thick (Fig. 42C). The thickness of connective tissue separating the two depends to some extent on the extent of contraction of the worm. Most commonly, Type B gland cells, or subepithelial support cell processes surrounding Type B gland cells, lie closest to the connective tissue capsule of the cerebral organ in the vicinity of the blood vessel. Occasionally, cells containing Type 2 vesicular material are present next to the connective tissue capsule in the vicinity of the blood vessel, but these occur with equal frequency in areas remote from the blood vessel. There is no apparent specialization of the connective tissue capsule, or of the plasmalemma of cells in the part of the cerebral organ adjacent to the cephalic blood vessel. Cells have not been observed straddling the connective tissue capsule of

the cerebral organ, and vesicular material has not been observed outside the organ.

**B. Discussion**

Based on the analysis of structure provided by this study, the following model of cerebral organ function, applying to *Paranemertes*, *Tetrastemma* and *Amphiporus*, is proposed.

1) The cerebral organs are chemoreceptors.

2) The function of gland cells is to secrete a mucous coating over the dendrites. The function of epithelial vesicular cells is to remove the mucus after it has passed over the dendrites. It is envisaged that while the cerebral organs are active, there is a constant turnover of mucus across the sensory epithelia.

3) The canal epithelium is specialized to allow efficient exchange of sea water through the organ, and the direction of flow is such that gland cell outlets are upstream from sensory epithelia, and vesicular cells are downstream.

4) The rate of flow through the canal, and the rate of mucous turnover affects the sensitivity of the cerebral organs, and may be under nervous control.

5) During periods of low cerebral organ activity, the amount of secretion product in gland cells is regulated by crinophagy - selective lysosomal breakdown of secretion granules - and through this process, gland cells are sometimes transformed into subepithelial Type 2 vesicular

cells.

6) The cerebral organs in these three species are not endocrine glands.

In the discussion that follows, the interpretation of ultrastucture underlying this model will be presented, and at the same time, the results obtained in this study will be compared with those of earlier workers, in particular those of Ling (1969a). The points to be discussed are:

1) Flow in the cephalic canal.

2) The fine structure of sensory cells and their modal sensitivity.

3) Innervation of the cerebral organ and control of activity.

4) The fine structure of gland cells.

5) The fine structure of vesicular cells and vesicular material.

6) The relationship of the cerebral organs with the central nervous and vascular systems.

**Flow in the cephalic canal**

The cephalic canal is apparently divided into two channels, designated the major and minor canals. In *Lineus*, this division is more extensive than that seen in the hoplonemertean species studied (Ling, 1969a and b; Ferraris, 1979a;), consisting throughout of a solid septum formed by the specialized, expanded cilia of lappet cells. In *Amphiporus*, *Paranemertes* and *Tetrastemma* lappet cells are

present only in a short segment of the canal near the blind end, but a separation of the rest of the canal into two channels is effected by ciliated cells with particularly well-developed striated rootlet complexes, though lacking specialization of the ciliary shaft. In addition, the cells lining the major and minor canals are differentiated into epithelia which have clearly distinct histological characteristics.

The same cell types similarly arranged are found in the major and minor canals of the hoplo- and heteronemerteans studied so far. Thus there are ciliated cells lining the minor canal, a large sensory epithelium lining much of the major canal, and vesicular tissue present at the blind end of the canal. One difference between the two orders is in the location of the secretion of the gland cells. In *Lineus* the gland cells processes are found at the first and second bends of the major canal. In *Paranemertes*, *Tetrastemma* and *Amphiporus* the processes of Type A gland cells are situated along the middle of the minor canal and those of Type B gland cells are near the end of the major canal. A second difference is the presence of Type 1 vesicular cells along the anterior part of the major canal in *Amphiporus*, *Tetrastemma* and *Paranemertes* (on a small branch of the major canal in the latter species). Type 1 vesicular cells have not been described in *Lineus*.

It is reasonable to assume that the direction of flow is opposite in the two channels of the canal, and as

discussed below, the orientation of ciliary axonemes and basal feet suggests that this is the case. In *Lineus*, there is a complete division of the canal into two channels by a ciliary septum, whereas in the hoplonemerteans studied the division is not complete. Lappet cells are present only near the blind end of the canal, presumably at the point where the flow changes direction. Although along most of the canal the fluid layers may be in contact, it is unlikely that there is a problem of turbulence caused by the two opposing streams, since the viscosity of sea water (described by Reynold's Number; Alexander, 1968; Cromer, 1977) in a canal of such dimensions would be relatively high, and therefore little or no mixing would be likely to occur, particularly at high flow velocities.

Ling (1970) has suggested that the direction of flow in the cephalic canal of *Lineus* is inward through the major canal and outward through the minor canal, based on the orientation of basal feet of the cilia lining the canal. Gibbons (1961) was the first to report that the direction of the effective stroke of cilia is towards fibrils number 5 and 6 in the axoneme, and that the position of the 5 and 6 fibrils is correlated with the position of the basal foot on the basal body. It is also known that in mechanoreceptive cilia, the stimulus applied in the direction of orientation of the basal foot and of the 5 and 6 fibrils is the depolarising stimulus (Flock and Duvall, 1965; Barber, 1968, 1974). In addition, in certain protozoan cilia able to

change their direction of beat, the orientation of ciliary axonemes is random (Okajima, 1953; Sleigh, 1960) and there are no basal feet on the basal bodies (Wessenberg, 1966). In one case, it was found that the cilia beat towards fibre 1 (Afzelius, 1961), but in all other examples reported the direction of the effective stroke is towards fibres 5 and 6, and the basal foot (Satir, 1965).

Ling's conclusion, that cilia in the cephalic canal of *Lineus* beat towards the basal foot, is supported by the evidence which he presents regarding the direction of movement of gland cell secretion in the canal (Ling, 1970). If his interpretation is correct, then in *Lineus* the current flows in through the major canal passed the gland cell outlets, across the sensory epithelium to the vesicular cells at the blind end, and out again through the minor canal.

The direction of flow in the cephalic canal of *Paranemertes* is illustrated in Figure 44. The same applies to *Tetrastemma* and *Amphiporus*. In these species the direction of flow is opposite to that in the cerebral organs of *Lineus*, but the relationship of gland cells, sensory cells and vesicular cells with respect to the direction of flow is similar. In the hoplonemerteans studied the orientation of basal feet, which corresponds to the position of the 5 and 6 microtubule pairs in the ciliary axoneme, is towards the blind end in cells of the minor canal, and towards the open end in cells of the major canal. If the

effective stroke is towards the basal feet in the hoplonemerteans, then current in the minor canal flows passed the different cell types in this order: Type A gland cell processes, Type 2 sensory cell dendrites, Type 2 vesicular cells. In the major canal it flows passed the cell types in this order: passed Type B gland cell processes, Type 1 sensory cell dendrites, Type 1 vesicular cells and to the outside.

**The fine structure of sensory cells and modal sensitivity**

In the cerebral organs of *Lineus*, as in the hoplonemerteans of this study, there is an extensive sensory epithelium along the major canal, consisting of sensory cell dendrites and their associated support cells arranged in a honeycombed pattern. Although Ling (1969a) reports that there are at least two types of bipolar cells in *Lineus*, he describes only one type of dendrite. In the hoplonemerteans there is an additional group of sensory cells (Type 2) whose dendrites have a distinctly different morphology and constitute a separate sensory area on the minor canal near the blind end. Type 1 sensory cell dendrites bear numerous slender microvilli which form a network, extending among the support cell cilia. Type 2 dendrites bear a single cilium encircled by a ring of microvilli which are stouter and straighter than those of Type 1 dendrites and do not form a network. The dendrites in *Lineus* are similar to the Type 2 dendrites of hoplonemerteans in that they also bear a

cilium, but they lack microvilli (Ling, 1969a).

Although the modality to which the sensory elements are sensitive cannot be established on the basis of morphological evidence alone, it is possible to make some predictions based on dendritic morphology and the relationship of cells within the cerebral organs.

Ling (1969a) discussed the function of the cerebral organs in *Lineus*, and presented some evidence in favour of photoreceptive, mechanoreceptive and chemoreceptive functions. Ling (1969a) and Willmer (1970) reported that light alters the activity of gland and vesicular cells in the cerebral organs. Ling suggested the lappet cells as possible photoreceptors, citing the loss of motility in the cilia, and dilatation of the ciliary membranes as features similar to a developmental stage of rods and cones in vertebrates and therefore possibly indicative of photosensitivity. There is no evidence that the lappet cells form synapses onto other nerve cells, however, and they possess no other features characteristic of sensory cells. In any case, the function of lappet cells appears to be in the division of the canal into two channels, and this function accounts for the specialized features of the cilia.

It is unlikely that the bipolar cell dendrites are photoreceptors either. The dendrites in the cerebral organs lack the great amplification of membrane surface area which is almost invariably associated with specific light sensitivity. In addition, the association of the dendrites

with mucus secreting cells, and the specialization of the canal for efficient circulation of sea water through the organ, do not have significance within the context of a photoreceptive function. The change in gland and vesicular cell activity in response to light, observed by Ling (1969a) and Willmer (1970), can perhaps be accounted for by a change in behavioural activity of the worm under different light regimes, which may entail an increase or a decrease in cerebral organ activity. Alternatively, since many cells not specifically photoreceptive are nevertheless weakly responsive to light (Millott, 1968), it is possible that a general sensitivity to light in the cells of the cerebral organs may affect the level of cerebral organ activity.

Ling (1969a) has also argued in favour of a mechanoreceptive function in the cerebral organs of *Lineus*, citing the presence of three right angled bends in the canal, and a "cupula" [1] among the canal cilia as evidence supporting this view. Although the complex geometry of the cerebral organ canal in some species is intriguing, it is unlikely that it gives orientational information to the worms, since sensory cell dendrites are not present along the full length of the canal involved in the three bends. The dendrites are situated along a segment of the canal between the second bend and the blind end, and are therefore present in both horizontal planes but not in the vertical

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[1] "Cupula" designates a ciliary structure in molluscan statocysts, but in vertebrates it refers to a secreted material. Ling uses the word in the latter sense.

plane. In the hoplonemerteans the geometry of the canal is less complex and both Type 1 and Type 2 dendrites are present in only one plane.

The morphology of Type 2 dendrites in the hoplonemerteans, and of the dendrites in *Lineus*, give no definitive clue to their modality, since both mechano- and chemoreceptors are commonly ciliated. However, the morphology of Type 1 dendrites which form the main sensory epithelium in hoplonemerteans (Type 1 dendrites) suggests a chemoreceptive modality. The vast majority of mechanoreceptive dendrites are ciliated (Barber, 1974) while these are unciliated, and the structure of Type 1 dendrites is very similar to that of other known or presumed chemoreceptors (Bannister, 1968; Welsch and Storch, 1969; Graziedei, 1973; Wright, 1974).

A chemoreceptive function of the cerebral organs in both hoplo- and heteronemerteans is suggested by the association of sensory cells with mucus secreting cells. Such an association is common in multicellular chemoreceptors, including the amphids of nematodes (Storch and Reimann, 1973; Ward *et al*, 1975; Batson, 1978), the nuchal organs of polychaetes (Whittle and Zahid, 1974; West, 1978), the gastropod osphradium (Benjamin and Peat, 1971; Crisp, 1973), the cerebral organs of sipunculans (Åkeson, 1958), and many chemoreceptors of arthropods (Altner and Prillinger, 1980) and vertebrates (Graziedei, 1973; Boeckh, 1981). A number of possible functions of mucus at gustatory

and olfactory surfaces have been suggested (Bannister, 1974). There is increasing evidence that mucous coatings may play an active role in transduction of the chemical stimulus. Mucus probably acts as a solvent of stimulant molecules (Boeckh, 1981), and may be responsible in some measure for receptor specificity, acting as a selective filter of odorant molecules (Den Otter, 1981). It may affect the rate at which compounds are transported, producing a gradient of activity along the sensory epithelium (Mozell, 1966, 1970; Bannister, 1974; Bostock, 1974). In addition, a direct role in transduction has been suggested by Bannister (1974), who proposed that competition for anionic binding sites might cause the displacement of inorganic cations from mucopolysaccharide molecules, initiating electrical changes at the receptor membranes. In the nemerteans, the association between gland cells, dendrites, and vesicular cells may be an adaptation for rapid turnover of mucus across the receptor epithelium. If mucus is involved in the transduction process, sensitivity of the dendrites may be a function of the rate of mucous turnover.

The division of the cephalic canal into an incurrent and an excurrent channel is a specialization which allows an efficient circulation of sea water through the organ. Such efficiency would not be required for mechanoreception; it is not likely that mechanical stimuli would be transmitted through sea water circulating in the canal. In a chemoreceptor, on the other hand, the dendrites must come

into direct contact with the external medium, and their sensitivity would depend in part on how rapidly the medium could be sampled. Additionally, the position of the cephalic canal openings - into the cephalic grooves on the ventrolateral surface of the head - is ideal for sampling the substrate over which the worm crawls.

Type 1 and Type 2 dendrites in the hoplonemerteans studied have distinct structures, and therefore probably also have distinct modal sensitivities, although both types are associated with gland and vesicular cells in the same way. Because of this association, it seems likely that both types are chemosensory, but it is possible that they are sensitive to different classes of molecules. Bürger (1895) and Dewoletzky (1887) have suggested that the cerebral organs may be sensitive to water quality, ie. the concentration of dissolved gasses, while Kipke (1932) and Reisinger (1926) (and this study) have presented evidence that they are responsible for prey detection. Further resolution of the problem of modal sensitivity of the cerebral organs cannot be achieved with morphological data, and will depend on obtaining experimental information about cerebral organ function.

**Innervation of the cerebral organs and control of activity**

In multicellular sense organs the sensitivity of the receptors is often modifiable, being controlled by efferent input to the receptors (Flock, 1967; Keverne, 1982). For

example, in vertebrate olfactory organs, recent evidence suggests that efferent pathways may influence incoming olfactory information by selective disinhibition. The effect of this input on behaviour has been shown in rats: The discharge from olfactory receptors is enhanced by food odours in rats that are hungry, but not in satiated rats, and the enhancement is abolished by section of the efferent fibres (Keherne, 1982).

Although the receptors in the cerebral organs are apparently not innervated, there is circumstantial evidence from behavioural studies (Reisinger, 1926; and this study) which suggests that the level of activity in the organs may be variable. It is possible that cerebral organ sensitivity may be controlled, not by alteration of the receptor threshold, but by alteration of the degree of stimulus access to the receptors through control of flow rate in the cephalic canal. Although no evidence of nervous input to the cerebral organs in the form of chemical synapses has been observed, there is a nerve fibre present among the ciliated cells of the minor canal which may be responsible for the control of ciliary beat frequency in these cells. It is probable that this fibre innervates the ciliated cells, as there is no other more distal cell type with which it could be associated. The great rarity of the fibre would account for the fact that its synapse has not been observed. The fibre may form junctions with only a few ciliated cells which act as pacemakers, mechanically entraining the beat

frequency of other cilia, thus controlling flow rate in the canal, and ultimately determining cerebral organ sensitivity.

**The fine structure of gland cells and of vesicular material within gland cells**

The gland cells in the cerebral organs are exocrine, and produce a neutral mucoprotein substance which is released into the cephalic canal. Gland cell ultrastucture is characteristic of cells specialized for production and storage of membrane bound secretion granules destined to be released exocytotically. The cell cytoplasm is almost filled with secretion granules or with the organelles responsible for their production. The rough endoplasmic reticulum and Golgi apparatus are very well developed, occupying large areas of the cell, and precursor granules of various sizes and densities are present near the concave face of the Golgi. High synthetic activity is further indicated by the morphology of gland cell nuclei (Fawcett, 1981), which are large, with very little darkly-stained nucleoplasm and with a prominent nucleolus.

Type 2 vesicular material in gland cells is resolved with the electron microscope as dense bodies containing secretion granules in varying degrees of lysosomal degradation. Although the definitive identification of active lysosomes depends on the histochemical identification of acid hydrolase activity, these dense bodies are typical

of lysosomes (Miller and Palade, 1964; Pitt, 1975), and fit no other interpretation. The autophagy is usually selective, involving only the secretion granules, but in some cases the organelles involved in the synthesis of secretion product are also incorporated into lysosomes. In the latter cases, the gland cells lose their glandular characteristics and are apparently transformed into subepithelial Type 2 vesicular cells. Presumably, these gland cells lose their processes and have no contact with the canal, although this has not been established.

Autophagy of the secretion product in gland cells, or crinophagy (deDuve, 1969), has been reported in vertebrate and invertebrate glandular tissues (Farquhar and Rinehart, 1954; Smith and Farquhar, 1966; Orci *et al*, 1968; Hopkins, 1969; Perrelet, *et al*, 1971; Wendelaar Bonga, 1971; Melmed *et al*, 1973; Farquhar, 1974; Patzelt *et al*, 1977; Garreau de Loubresse, 1980), and is known mainly from vertebrate endocrine glands. Circumstances leading to crinophagy, and the steps involved in the process, have been analysed experimentally in the anterior pituitary gland of rats (Smith and Farquhar, 1966). The anterior pituitary consists of several different kinds of hormone producing secretory cells. In any of the cell types, if the target organ is removed, or the demand for secretion is lowered in some other way, the inhibition of secretion is immediately recognizable within the secretory cells by an increase in the number of crinophagic lysosomes which appear (Farquhar

and Rinehart, 1954; Farquhar, 1974). Crinophagy of secretory granules also occurs in normally secreting cells, and the level of breakdown increases when secretion is inhibited. The lytic enzymes are provided by the Golgi complex, which releases primary lysosomes in some regions, while still releasing putative secretory granules in others.

Crinophagy has also been studied in exocrine glands where the demand for secretion varies. In crustacean shell glands, crinophagy occurs at a low level, even at times when the demand for secretion is high (Garreau de Loubresse, 1980). When secretion is inhibited, the cells become overloaded and the level of crinophagy rises. Autoradiographic labelling shows that older secretion granules are broken down more readily than newly synthesized granules. There is a continual synthesis of protein in the cells, regardless of their state of overloading, although the level of synthesis decreases when the cells are overloaded, perhaps due to overcrowding of the synthetic organelles. In late stages of crinophagic degradation, organelles involved in synthesis may also become incorporated into lysosomes and these cellular constituents are also digested.

Crinophagy is thought to exist as a regulatory mechanism in these systems (Smith and Farquhar, 1966; Garreau de Loubresse, 1980): the number of secretory granules available for release to the exterior will depend upon the balance between the level of synthesis and the

level of breakdown in the cell, but both processes are in action all of the time. A second function of crinophagy is believed to be in the solubilization of a stored secretion product prior to its use. In the mammalian thyroid gland, thyroglobulin is synthesised and stored in the follicular lumen of thyroid cells. When the active hormone thyroxine is required, the thyroglobulin is reincorporated through endocytosis, and degraded in lysosomes, which then release the active hormone (Wollman, 1969). A similar role has been ascribed to lysosomes in the synthesis of erythropoietin (Libbin *et al*, 1974). Crinophagy is also involved in dedifferentiation of gland cells prior to their destruction, or to a change in the type of secretion granule produced, as occurs in pancreatic islet cells of rats (Orci *et al*, 1968). Finally, in some cases, crinophagy is believed to represent a general response of gland cells to stress, rather than a specific response for disposal of secretory material (Holtzman, 1976).

The present study provides morphological evidence that crinophagy is available as a cellular mechanism in the gland cells of the cerebral organ, and that it is one source of Type 2 vesicular material. Crinophagy in gland cells is not involved in further processing of secretory material prior to its release into the canal, as the secretory processes contain only intact, Golgi-produced secretory granules. It most likely exists as a regulatory mechanism controlling the amount of secretion present in the cells according to a

variable demand for secretion in the cephalic canal.

**Type 1 vesicular cells**

Type 1 vesicular cells occupy a large segment of the major canal epithelium in the anterior part of the cerebral organ in *Tetrastemma*, *Paranemertes* and *Amphiporus*. They are situated downstream from Type B gland cells and Type 1 sensory cell dendrites. They were overlooked in *Amphiporus* by Ferraris (1979a), and have not been described in *Lineus* (Ling, 1969a, 1970; Ferraris, 1979a). These cells appear to be involved in the removal and degradation of material from the cephalic canal. The morphology of the Type 1 vesicular cell epithelium, including the appearance and organization of vesicles in the cells, is remarkably similar to the morphology of the convoluted tubules in the mammalian kidney, which exhibit a classic example of an endocytic, lysosomal epithelium (Maunsbach, 1969).

Type 1 vesicular cells are characterised by the presence of a large number of vesicles of variable morphology. Vesicles comprising the vesicular material (this does not include Golgi produced vesicles) can be divided into five size classes, each with a characteristic morphology.

Size 1 vesicles fill the apical cytoplasm, and include some coated pits and vesicles at and near the apical surface. Coated vesicles are known to be involved in the selective uptake of material into cells. The coating

material is a lattice-forming protein, clathrin (Pearse, 1976), which may mark specific receptor sites on the membrane and may act as a scaffolding in vesicle formation. Uptake by coated vesicles is a receptor mediated process which allows cells to interiorise a large volume of a specific solute without ingesting a correspondingly large volume of solution (Silverstein *et al*; 1977). Once inside the cell the vesicle sheds the clathrin coat which is then recycled back to the surface membrane, unless the vesicles are involved in trans-epithelial transport (Wild, 1980). The usual fate of vesicles once they have lost their coat is fusion with lysosomes (Wild, 1980). Size 2, 3 and 4 vesicles are present in reverse order in the mid-region of the cell, with the larger, size four, vesicles nearest the apical cytoplasm, and size 2 vesicles situated basally.

A reasonable interpretation of these profiles is as follows: Size 1 vesicles, which bring material from the canal lumen into the cell, fuse with each other and with primary lysosomes from the Golgi complex, giving rise to size 4 vesicles, which are fairly large and contain light flocculent material. As lysosomal degradation proceeds within size 4 vesicles, the vesicles shrink and their contents are condensed, giving rise to size 3 vesicles. As degradation is completed, size 3 vesicles shrink, their contents become very electron dense, and the result is the production of size 2 vesicles, which are residual bodies. Residual bodies accumulate at the base of the cell and may

aggregate into large (size 5) vacuoles. There is no evident pathway for expulsion of residual bodies at the base of the cells, since the cerebral organ in this region is surrounded by muscle tissue and cephalic glands, and is remote from the vascular system. Residual bodies have occasionally been observed in the apical region of the cell, however, and it is possible that under certain circumstances they are extruded at the apical surface.

**Type 2 vesicular cells and vesicular material**

Ling (1970) has demonstrated that the vesicular cells in the cerebral organs of *Lineus* (corresponding to Type 2 vesicular cells in this study) actively take up gland cell secretion from the cephalic canal by phagocytosis. According to the orientation of cilia in the canal, the vesicular cells are situated downstream from the gland cells. The surface of the vesicular cells is irregular and infolded, and some profiles suggest phagosomes in the process of formation. Ling (1970) observes gland cell secretion in the segment of the canal between the gland cell processes and the vesicular cells, and also within the pits in the vesicular cell membrane. In worms kept for a period of time in 1 to 2% Trypan Blue, he observes accumulation of the dye in the vacuoles of vesicular cells.

Once inside the cell, the phagosomes apparently fuse with clear microvesicles from the Golgi complex in a process similar to lysosomal breakdown, and the neutral mucoprotein

ingested by the cells is converted to acid mucopolysaccharide which accumulates in large vacuoles at the base of the cells. Ling (1970) suggests that the fate of this material in vacuoles is engulfment by basal cells, which then migrate across the connective tissue capsule of the cerebral organ into the vascular system.

In the hoplonemerteans of this study the vesicular cells in the posterior part of the cerebral organs (Type 2 vesicular cells) have an ultrastructure similar to the vesicular cells in *Lineus*, and are probably also endocytic cells. Type 2 vesicular cells are situated downstream from Type A gland cells. They appear to remove material from the canal in coated vesicles or in larger phagocytic vesicles. The material appears to be processed subsequently in the cells by fusion with primary lysosomes produced by the Golgi complex, and converted to acid mucopolysaccharide. The lysosomal nature of this process can be inferred from the cellular ultrastructure, which suggests a sequence of endocytosis and lysosomal breakdown.

The fate of Type 2 vesicular material in the hoplonemerteans studied is not clear. There is no indication of bulk transfer out of the cerebral organs via diapedesis of basal cells as Ling (1970) reports in *Lineus*. The subepithelial support cells described in this study almost certainly correspond to the basal cells described by Ling (1970), but these do not appear to leave the cerebral organs. Also, considering their obvious supportive function,

and their association with gland cells, sensory cells and vesicular cells, it seems unlikely that they would undergo a great deal of movement. It is possible that the vesicular material is recycled within the cerebral organs, and degraded mucoprotein units are re-utilised by gland cells.

Type 1 and Type 2 vesicular cell epithelia both present morphological features which suggest endocytic and lysosomal functions, but there are some differences between the two epithelia. The surface area exposed to the canal is much greater in Type 1 than in Type 2 vesicular cells, and in Type 1 vesicular cells, the number of vesicles in the apical cytoplasm, both coated and uncoated, is much greater, suggesting a higher level of endocytic activity. If the vesicular cells are indeed involved in uptake of gland cell secretion, as appears to be the case, the difference in the extent and level of activity of the two epithelia may be accounted for by the difference in amount of secretion product involved: There are many fewer Type A than Type B gland cells, and according to the pattern of flow in the canal suggested above, Type 2 vesicular cells are situated downstream from Type A gland cells, while Type 1 vesicular cells are situated downstream from Type B gland cells. There is, however, more Type 2 than Type 1 vesicular material in the cerebral organs. This may be accounted for by the fact that Type 2 vesicular material is also produced by direct breakdown of secretion granules within gland cells.

In both Type 1 and Type 2 vesicular cells the amount of vesicular material present varies within and between species. Among the different species, the amount of Type 1 vesicular material is greater in *Amphiporus* and *Tetrastemma* than in *Paranemertes*, and the amount of Type 2 vesicular material is greatest in *Amphiporus*, slightly less in *Tetrastemma* and still less in *Paranemertes*. Two possible explanations for this variation can be suggested. Firstly, some variation in the amount of vesicular material in different species may be accounted for by the level of activity of the cerebral organs at the time of fixation. The level of cerebral organ activity may vary depending on the behaviour in which the worm is engaged. As *Paranemertes* is a species active out of water, the activity of the cerebral organs may be suppressed while the worms are submerged, in which case the morphology presented in this study would be that associated with a low level of activity. Conversely, as *Tetrastemma* is active while submerged (see appendix), the morphology of the cerebral organs in this species may represent that associated with a higher level of activity. This may also apply to *Amphiporus*, however, little is known of the natural history of this species. Secondly, the amount of material can vary with the osmolarity of the fixative (see Chapter VI). Although the same fixative was used for all three species, the osmolarity of sea water to which each species is adapted probably differs. For example, the normal osmolarity of sea water in Puget Sound is 32 ppt, while in

St. Andrews Bay it is 34 ppt (Laverack and Blackler, 1974). Also, salinity in the microhabitat of the worms may differ. If the osmolarity to which each species is adapted differs, the relative osmolarity of the fixative to which each species was exposed would also differ.

**Relationship of the cerebral organs with the central nervous and vascular systems**

Although the anatomical relationship of the cerebral organs with the central nervous and vascular systems differs in *Amphiporus*, *Tetrastemma* and *Paranemertes*, the structure of the cerebral organs in the three species is essentially the same. This suggests that there is no special functional significance, either in the closer association of the cerebral organs with the cerebral ganglia in *Tetrastemma*, or in the closer association of the cerebral organs with the cephalic blood vessel in *Paranemertes* and *Amphiporus*.

In *Tetrastemma* the cerebral organs are juxtaposed to the dorsal cerebral ganglia, while in *Paranemertes* and *Amphiporus* they are fully separate, situated well anterior to the cerebral ganglia. However, the functional intimacy of association of the cerebral organs with the rest of the nervous system is probably the same in all three species. Regardless of the location of the cerebral organs with respect to the cerebral ganglia, the connection between the two is restricted to the cerebral organ nerve, which in all cases is a bundle of fibres made up of the axons of Type 1

and Type 2 bipolar sensory cells, with a very small component contributed by the nerve fibre observed among the ciliated cells.

Similarly, with regard to the vascular system, the differences in anatomical relationships between the cerebral organs and the cephalic blood vessel are not reflected in cerebral organ histology. In *Tetrastemma* the cephalic blood vessel passes adjacent to the anterior medial part of the cerebral organ, coming closest to the ciliated cells of the minor canal and to Type A gland cells. In *Paranemertes* and *Amphiporus* the blood vessel passes medial to the posterior part of the cerebral organ, and Type B gland cell tissue is the most closely associated with the vascular system. If there were a functional association between the cerebral organs and the vascular system, one would expect either, that the histology of the cerebral organs in the area nearest the cephalic blood vessel would exhibit some specialized feature common to all three species, or, given the unlikely possibility of a functional difference between *Tetrastemma*, where the blood vessel is relatively remote, and *Paranemertes* and *Amphiporus*, where it is not, that the structure of the posterior, glandular area of the cerebral organ in contact with the blood vessel in the latter two species would differ from the posterior glandular area of *Tetrastemma* . There is, however, no common factor in the relationship of the cerebral organs with the vascular system in the three species, and there is no difference in

structure between the posterior glandular area in *Amphiporus* and *Paranemertes*, and that in *Tetrastemma*.

The histological organization of the cerebral organs in *Amphiporus* and *Paranemertes* bears no relationship to the vascular system. The cerebral organs are approximately bilaterally symmetrical about an anterior-posterior axis which bissects the cephalic canal (Fig. 5), but the relationship of the cerebral organs with the cephalic blood vessel is asymmetrical. It passes adjacent to the Type B gland cells on one side of the organ, but has no association with those of the other side. Moreover, all cells in the cerebral organ have a recognizable polarity with respect to the cephalic canal except subepithelial support cells, which are multipolar, and subepithelial Type 2 vesicular cells, which occur mainly in the area posterior to the blind end of the canal, surrounded by Type B gland cells. These latter cells are occasionally observed near the connective tissue capsule of the cerebral organ, but they can occur at any point along the capsule, not just in the region nearest the blood vessel. None of the cells in the cerebral organ are polarised with respect to the cephalic blood vessel.

The fine structure of the cerebral organs in the vicinity of the blood vessel gives no indication that material might be exchanged between the cerebral organs and the vascular system. Transfer of secretion from endocrine glands to the vascular system is generally recognizable at an ultrastructural level by exocytotic figures along the

gland cell membrane nearest the blood vessel (Rhodin, 1974). These are not observed in either vesicular cells or gland cells along the connective tissue capsule of the cerebral organs. The processes of subepithelial support cells are often interposed between gland cells and the connective tissue capsule, but they are not observed straddling the capsule, and vesicular material is not observed outside the cerebral organs.

The relationship of the cerebral organs with the vascular system has often been considered of prime importance in interpretations of cerebral organ function, and has contributed to the idea that the cerebral organs may be endocrine glands. However, the anatomical evidence obtained in this study suggests that there is no direct functional relationship between the cerebral organs and the vascular system, and that the cerebral organs in these species are therefore unlikely to have an endocrine function.

## IV. Behavioural evidence for a chemoreceptive function of the cerebral organs

### A. Introduction

Unlike most nemerteans, *Paranemertes* is active at low tide on the exposed mudflat, and its behaviour is therefore accessible for study, either in the field or under closely simulated field conditions in the laboratory. In this study, the behaviour of *Paranemertes* was observed on artificial mudflats in the laboratory to determine whether any stereotyped or predictable behaviour patterns existed which could be used in an analysis of cerebral organ function. The ability of *Paranemertes* to home following its mucous trail, and a characteristic searching behaviour elicited by trails of the preferred prey species, *Platynereis bicanaliculata*, were found to be suitable for analysis. The behaviour of *Paranemertes* with and without cerebral organs was compared. Homing behaviour was not affected by cerebral organ removal, but the ability to respond to prey trails was abolished.

### B. Results

*Paranemertes* lives in temporary burrows on mudflats over a wide range in the intertidal, concentrated at the mid-tide level. The worms become active at low tide, emerging from the mud to forage on the surface. The distance travelled varies from only a few centimetres for worms that explore the area around the burrow without leaving it, to as

much as two metres. While crawling, *Paranemertes* pauses occasionally, making exploratory movements with the head, or sometimes remaining stationary for about five to ten minutes with the head buried in the mud. *Paranemertes* is occasionally observed everting its proboscis and capturing prey. After foraging, a worm either burrows in a new place or returns to the original burrow by following its mucous trail. These observations largely agree with the results obtained by Roe (1976) in a study of the ecology of *Paranemertes* at Snug Harbor, although in this study the worms homed less frequently.

Worms without cerebral organs and sham-operated worms behaved in the same way as intact worms on the artificial mudflats in that they also remained buried in the mud at high tide, foraged at low tide and could 'home' following their mucous trails.

**The detection of prey;**

The response of *Paranemertes* to prey was tested with the trails of adult polychaetes from three known prey species (Roe, 1970,1976): *Platynereis bicanaliculata*, *Armandia brevis* and *Podarke pugettensis*. Prey trails were made by dragging an intact, living polychaete lightly across the mud a short distance in front of a crawling nemertean. Some nemerteans were tested while foraging during low tide on the artificial mudflat or in the field, and others were taken from the group kept in running sea water, placed on a

surface of wet mud, and tested after a few minutes of crawling. These different test situations did not appear to affect the response. *Paranemertes* did not respond to an arc in the mud traced by a probe or a conspecific.

*Paranemertes* responded consistently to the trail of *Platynereis*, the preferred prey species (Roe, 1970), in a manner which indicated prey recognition ($n=51$; $P<.01$). *Paranemertes* would follow the trail back and forth repeatedly, spending at least minutes and often until the end of the low tide period searching for the prey. While the prey trail was followed, the head was turned from side to side continuously on the mud surface and occasionally in the air, extended off the trail a few centimetres, retracted and re-extended in a different direction. In most cases, the proboscis was everted when the trail was first contacted, and again but with decreasing frequency while it was followed. *Paranemertes* was incapable of recognising direction in the prey trail.

Occasionally, *Paranemertes* crawled through a small puddle on the mudflat in which a prey trail began. Once, it detected the trail from a distance of about 3 cm and altered its course towards the trail, then spent time searching for the prey in the characteristic way. Other times the trail was ignored, even if it was contacted directly. It is possible that *Paranemertes*, although capable of distance chemoreception, simply does not usually respond to prey which it detects while underwater. According to Roe (1976),

*Paranemertes* elicits an escape response from nereid prey, and this response is more effective if the nereid is underwater.

Contact with the trail of *Platynereis* elicited searching behaviour in intact (n=51; P<.01) and sham-operated worms (n=13; P<.01), but not in worms without cerebral organs (n=25; P<.01). When a *Platynereis* trail was contacted by worms without cerebral organs, it was acknowledged by a pause or it was ignored altogether. Although *Paranemertes* without cerebral organs sometimes responded to the presence of the trail, perhaps as a chemical change in the substrate, they did not respond to it as an indication of prey. They would, however, eat dead or injured *Platynereis* presented to them.

The response of intact *Paranemertes* to a *Platynereis* trail was inhibited by prior contact of a conspecific with the trail: if *Paranemertes* was allowed to explore one half of a *Platynereis* trail and was then removed, a second *Paranemertes* responded to the untouched half of the trail, but discontinued searching when they reached previously explored trail (n=14; P=.006). This behaviour is clearly adaptive, since it reduces the likelihood of *Paranemertes* searching for prey that has probably already been removed. It was not possible to determine whether the cerebral organs were responsible for this recognition of the presence of a conspecific, since worms without cerebral organs did not respond to prey trails in any case.

The trails of *Armandia* and *Podarke* did not usually elicit searching behaviour. *Paranemertes* would eat an injured *Armandia* presented to it, but rarely responded to *Armandia*'s trail (n=8; P>.05). *Armandia* has a strong swim-flip escape response, and is eaten mainly in the summer when it is sluggish from the heat, and when numbers of *Platynereis* are low (Roe, 1976). It may not be energetically advantageous for *Paranemertes* to search for *Armandia*, even though it will attack an *Armandia* when it encounters one. Contact with *Podarke* or its trail elicited an avoidance reaction, or no response at all (n=13; P<.01). This is probably due to the large size of *Podarke* used in these experiments. *Paranemertes* will eat juvenile *Podarke* but tends to avoid adults (Roe, 1976). Because the trails of *Armandia* and *Podarke* do not elicit searching behaviour reliably, the role of the cerebral organs in detecting the trails of these species could not be determined by direct experiment. However, the difference in foraging behaviour of worms with and without cerebral organs (described below) suggests that the cerebral organs are responsible for detecting these prey species as well as *Platynereis*.

Six observations of proboscis eversion, presumably at potential prey, were made during study of the worms foraging on artificial mudflats. In all cases, *Paranemertes* remained at the spot of proboscis eversion with its anterior end in the mud for about five to ten minutes, and presumably, swallowed the prey. In only two cases was the prey actually

seen, and in both of these it was *Armandia brevis*. *Armandia* would struggle violently during the attack and come out of the mud as a result of the struggle. Also, a foraging worm would occasionally pause for five to ten minutes with its head buried in the mud. It is likely that *Paranemertes* was feeding at these times, even though proboscis eversion was not observed.

The observations of apparent feeding and of attacks on prey were restricted to intact or sham-operated worms, indicating that worms without cerebral organs were probably foraging unsuccessfully. *Paranemertes* without cerebral organs were never observed feeding, unless injured prey was presented to them, and then only after several minutes of direct contact of the prey with the head.

**Mucous trail following;**

*Paranemertes* produces a mucous trail while crawling on the mud, and after foraging, sometimes follows this trail back to its burrow (Roe, 1976 and this study). The trail is a continuous strand of mucus which is clearly visible on the mud surface (Fig. 45), and is strong enough to withstand moderate manipulation. Worm densities at Snug Harbor and on the artificial mudflats were such that *Paranemertes* trails intersected frequently, and homing worms often encountered at least one intersection of their own trail with that of a conspecific's. *Paranemertes* crawled through such intersections on its own trail with no apparent response,

whether crawling forwards or backwards, indicating that much of trail following probably relies on mechanical rather than chemical cues.

The trails were manipulated in a variety of ways so that the trail-locating abilities of intact and operated worms could be compared. If the mucous trail was displaced without being broken, the homing worm invariably followed the mucous strand without any evidence of searching behaviour, and ignored the groove which marked the original path ($n=6$; $P=.016$). If a short piece was cut out of the trail and removed, a worm crawling head first would stop at the point of disruption and search at the broken end, turning its head from side to side as it extended it back and forth over the surface, until it rejoined the trail and continued its journey ($n=8$; $P=.004$). If the underlying groove in the mud was undisturbed, recovery of the trail was much quicker, and searching movements centred on the groove. If the disruption was encountered while the worm was crawling backwards, it would turn around and search for the trail with its head. In cases where the trails of two worms were disrupted at their intersection, the homing worm was apparently unable to distinguish its own trail from the conspecific's ($n=10$; $P>.05$; the variance of this response was large, however, and the sample size was probably too small to detect a significant difference).

Trail-following ability was not affected by removal of the cerebral organs, even in cases where chemical, rather

than mechanical cues were almost certainly required, for example when trails were relocated after minor breaks (n=5; P=.031).

**C. Discussion**

*Paranemertes* responded with a characteristic searching behaviour to the trail of *Platynereis*, and this response was abolished by removal of the cerebral organs. The consistent response of *Paranemertes* to *Platynereis* trails has made it possible to assess the role of the cerebral organs in prey detection, and to demonstrate their chemoreceptive function in this species.

The results reported here support the conclusion reached in Chapter III that the cerebral organs are chemoreceptors, and indicate that in *Paranemertes* they are specifically involved in prey recognition. The conclusion is also supported by the behavioural observations of Reisinger (1926) and Kipke (1932) on *Prostoma*, a freshwater nemertean thought to be closely related to *Tetrastemma* (Gibson, 1972). Reisinger (1926) observed an intensification of ciliary beating in the cephalic canal in the presence of food, and Kipke (1932) observed an increased sensitivity to food following regeneration of the cerebral organs in this species.

The role of the cerebral organs in behaviour has not been studied in other groups. In the lineids, the cerebral organs differ anatomically from those of the hoplonemerteans

studied, in having neurosecretory cells and a much more intimate association with the vascular system. These anatomical features must have functional significance, and may indicate a functional capacity of the cerebral organs which is not present in the hoplonemerteans.

## V. Behavioural physiology of *Paranemertes*

### A. Introduction

The behavioural physiology of nemerteans has been the subject of only a few studies, perhaps because on the one hand, their repertoire of stereotyped behaviours is rather limited, and on the other, the central nervous system is relatively inaccessible, being partly or wholly embedded in muscle tissue. The currently available information on this subject comes exclusively from the behavioural analysis of locomotion (Eggers, 1924, 1935; Friedrich, 1933; Corrêa, 1953a, b), and there are as yet no published accounts of the electrophysiological analysis of behaviour in nemerteans. During the course of this study I have had a brief opportunity to work on the electrophysiology of *Paranemertes*, using extracellular suction electrodes to record from the lateral nerve cords, proboscis and cerebral organ nerve [2]. Although this study was directed mainly at obtaining a basic understanding of the neuromuscular system in *Paranemertes*, some preliminary results relating to the function of the cerebral organs were also obtained, and are presented here, along with a brief description of some physiological properties of the neuromuscular system.

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[2]Dr. Harold Koopowitz was the first to record from the lateral nerve cords of *Paranemertes*. He very kindly drew my attention to the preparation, and allowed me to use his recording apparatus so that I could continue the study which he began.

## B. Results and Discussion

Among nemerteans, *Paranemertes* (Fig. 46) is a good subject for electrophysiological study because it is large, and its brain and lateral nerve cords are readily accessible, being situated within the muscle layers of the body wall in a spongy parenchyma ventral to the rhynchocoel, which can be opened by a middorsal incision in the body wall.

### Recording from the lateral nerve cords

In the unstimulated lateral nerve cord there is a great deal of spontaneous activity of no apparent pattern, which alternates with periods of quiescence (Fig. 47A). Some of this activity is clearly associated with contractions of the body-wall musculature. As decapitate worms are normally behaviourally inactive, this activity of the musculature is probably an artifact provoked by the pins through the body wall, and possibly also by the recording electrode. The amount of spontaneous activity is greater in decapitate preparations and in the isolated lateral nerve cord (Fig. 47B) than in preparations with brain connections intact.

Electrical stimulation of the lateral nerve cords of capitate and decapitate preparations elicits a series of small potentials followed by a large compound potential (Fig. 48A) which is associated with a contraction of the bodywall musculature (Figs. 48C,48D). The amplitude of the compound potential tends to increase with increasing

stimulus intensity (Fig. 48A), but also varies considerably with stimuli of constant amplitude and duration. This variation cannot obviously be ascribed to fatigue or facilitation. In preparations bathed in isotonic $MgCl_2$, the number of small potentials increases with increasing stimulus intensity, but the compound potential and associated muscle contraction are abolished (Fig. 48B). This suggests that the compound potential is a post-synaptic event, possibly generated in the neuropil of the lateral nerve cords. The response recorded from the musculature to stimulation of the lateral nerve cord is greater to paired than to single stimuli, indicating the existence of neuromuscular facilitation (Fig 49).

Tactile stimuli applied to the body wall of capitate preparations in ipsi-and contralateral positions anterior and posterior to the recording electrodes elicited a burst of spikes in the lateral nerve cord (Fig. 50). Posteriorly applied stimuli were generally more effective in eliciting a response than those applied anteriorly, but there was no difference in the response to ipsi- and contralateral stimulation. The size of the response was reduced in preparations in which the brain commissures had been severed, but the relative size of the response to ipsi- and contralateral stimulation remained the same, indicating that there are extensive peripheral connections between the lateral nerve cords. This has been demonstrated in several other species by Friedrich (1933). As with spontaneous

activity, elicited activity is very complex, and it is not possible to discern any predictable pattern in either.

Chemical and tactile stimuli were applied to the intact head of *Paranemertes* and the response was recorded in the lateral nerve cord. Since the nerves of *Paranemertes* are directly sensitive to chemical stimuli such as prey extract (prepared by grinding up whole prey), the exposed nerve cord was isolated from the intact head as illustrated in Figure 51A. Whole, live *Podarke pugettensis* placed near the head sometimes elicited a burst of activity in the lateral nerve cord (Fig. 52A). Although *Platynereis bicanaliculata* is the preferred prey species, small specimens of *Podarke* are taken in the summer when *Platynereis* is unavailable. (*Platynereis* was unavailable at the time of these experiments, but a piece of previously frozen *Platynereis* was used to prepare a prey extract.) Drops of prey extract from *Podarke* or *Platynereis* applied to the head elicited bursts of activity from the lateral nerve cords. Prey extract from either species provided a more effective stimulus than did whole, live *Podarke* (Fig. 52B). This is not surprising, since prey extract administered to the head of a freely crawling *Paranemertes* was a more effective stimulus than intact prey, and usually caused proboscis eversion, whether the worm was submerged or out of the water.

Activity recorded in the lateral nerve cord in response to a stimulus applied to the head is probably associated with locomotion, proboscis eversion or both, and in the case

of *Podarke*, might be associated with attack or avoidance. The response of *Paranemertes* to *Podarke* in the field is ambiguous - although *Paranemertes* will take *Podarke* when *Platynereis* is unavailable, it eats small individuals but avoids large ones. Roe (1976) suggests that large *Podarke* may produce a toxic substance in the skin which causes this avoidance. The stronger response obtained using prey extracts may be accounted for by the fact that *Paranemertes* is more likely to attack an injured *Podarke* or other prey, and will do so even when submerged. Intact live prey are usually overlooked if *Paranemertes* is submerged. Whether or not the cerebral organs are involved in mediating responses obtained in the lateral nerve cords to stimulation of the head, has not yet been determined. Recordings from the cerebral organ nerve (described below) indicate that the organs are sensitive to chemical stimuli, however, behavioural studies (Chapter IV) suggest that the cerebral organs are responsible for the recognition of intact prey, but that in the absence of the cerebral organs other receptors are able to initiate a response to injured prey or to prey extract.

**Recording from the cerebral organ nerve**

The cerebral organ nerve in *Paranemertes* is 15 to 20 μm in diameter, and is embedded in the head musculature. Through careful dissection, the nerve can be exposed, and recordings obtained using small tip diameter suction

electrodes. As when recording from the lateral nerve cords, it is essential that the stimuli - applied to the opening of the cephalic canal - be separated from the cerebral organ nerve, because the nerve is directly sensitive to prey extract. Accordingly, the head with the central nervous system and cerebral organs exposed, was pinned onto a piece of Tygon tubing over a small hole in the tubing which was positioned beneath the opening to the cephalic canal, as illustrated in Figure 51B. Stimuli were applied in the chamber enclosed by the tubing. The preparation lasts for up to twenty minutes.

Mechanical, chemical, osmotic, photic and thermal stimuli were applied. Vibrations were generated by tapping the side of the dish lightly, by blowing on the surface of the water with a pipette or by gently blowing bubbles in the water near the opening of the cerebral organ canal. Chemical stimuli consisted of drops of prey extract prepared from *Platynereis* or *Podarke*. Drops of concentrated sea water or distilled water were applied near the canal opening to determine the response to an osmotic change. Responses to temperature were tested by pipetting heated or chilled sea water into the bath near the opening of the cerebral organ. Responses to light were tested simply by turning the room lights on and off at various intervals.

In the unstimulated cerebral organ there is little or no spontaneous activity in the nerve. No response was recorded to light, temperature or weak mechanical stimuli.

Responses of variable amplitude and duration were obtained from prey extract and distilled water (Fig. 53) indicating a sensitivity of the cerebral organs to these chemical stimuli. The responses to distilled water and prey extract were recognizable simply as activity in the nerve: as with responses recorded from the lateral nerve cords, the duration and amplitude of the activity elicited were not consistent.

The preliminary results reported here provide some useful information about the neuromuscular system of *Paranemertes*, but serve mainly to demonstrate the feasibility of this approach to the study of nemertean behaviour. It is hoped that in the future, electrophysiological techniques will be used to answer some of the many outstanding questions about the behavioural physiology of nemerteans.

## VI. The role of the cerebral organs in osmotic stress tolerance

### A. Introduction

The cerebral organs are thought to play a neurosecretory or an endocrine (non-nervous) role in osmotic stress tolerance, however, some of the evidence supporting this interpretation of cerebral organ function is equivocal. This problem has been investigated by Lechenault (1965), who studied the effects of cerebral organ and cerebral ganglion removal on volume regulatory ability in *Lineus*, and by Ling (1970) and Ferraris (1979a), who observed cytological changes in cerebral organ gland cells and vesicular cells, and movement of acid mucopolysaccharide from the cerebral organs to the vascular system during hypo-osmotic stress in *Lineus*, and possibly also in *Amphiporus* (Ferraris, 1979a).

Ling (1970) and Ferraris (1979a) reported that the amount of material in cerebral organ gland cells increased in concentrated sea water (interpreted as decreased secretory activity) and decreased in dilute sea water (interpreted as increased secretory activity); also, that the staining properties of vesicular material changed during exposure to dilute sea water, and the amount of material increased. These cytological changes cannot be regarded as reliable indicators of cerebral organ activity for two reasons. Firstly, exposure to 50% or 150% sea water is likely to impose an osmotic stress on the tissue and may

cause changes in the histological appearance of gland cells which suggest an increase or decrease in the amount of material present, but which are unrelated to secretory activity. In any case, the amount of secretory material which accumulates in gland cells is affected by the rate of synthesis, release, and autophagic breakdown of secretory product, and is therefore not always a reliable indicator of secretory activity (Highnam, 1965; Wendelaar Bonga, 1971). For example, in active cells, if the rate of synthesis exceeds the rate of secretion, the cells will appear full, even though they may be discharging a large volume of material. The histological appearance of gland cells is determined by the relative rates of synthesis, and release or breakdown of material, and these rates cannot be determined histologically (Highnam, 1965; Wendelaar Bonga, 1971).

Lechenault (1965) demonstrated that the cerebral organs or the cerebral ganglia, but not both, are required in long term volume regulation of *Lineus*. Although his results support a neurosecretory role of the cerebral organs in osmotic stress tolerance, they argue against an endocrine role: if the cerebral organs act in a neurosecretory capacity, volume regulation might occur in spite of their removal - neurosecretory material from the cerebral organs could be stored in a neurohaemal area in the brain and released under central nervous control. However, if the cerebral organs act in an endocrine capacity, their removal

should abolish the worm's ability to regulate volume, even if the cerebral ganglia remain intact. Thus the conclusions arrived at from Lechenault's experiments contradict those of Ling (1970) and Ferraris (1979a).

The anatomical evidence presented in this study suggests that in *Paranemertes*, *Tetrastemma* and *Amphiporus* the association of the cerebral organs with the vascular system does not have a special significance, and that the cerebral organs are not endocrine glands: As explained in Chapter III, the cellular structure and organization of the cerebral organs with respect to the vascular system is asymmetrical and unpolarised, and at the interface between the two there is no morphological indication of transfer of material.

To determine whether the anatomical relationship of the cerebral organs with the vascular system changes during salinity stress, the effect of exposure to 75% and 50% sea water on the cytology of the cerebral organs was examined in *Paranemertes* [3]. The effect of dilute sea water on cerebral organ cytology was compared with that of hypo-osmotic fixative on the cytology of worms from normal sea water, as a control experiment designed to show the cytological appearance of a passive response to osmotic change. In

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[3]Although *Amphiporus* would have been a preferable choice for these experiments, since it would have allowed a direct comparison with the results of Ferraris (1979a), this species could not be obtained at the time this part of the study was done. Use of *Paranemertes* was felt to be justified, since the histology and ultrastructure of the cerebral organs, and the relationship with the vascular system are very similar in the two species.

addition, the role of the cerebral organs in salinity stress tolerance was examined by comparing the volume regulatory ability of *Paranemertes* with and without cerebral organs.

## B. Results

### The effect of hypo-osmotic stress on cerebral organ cytology

To determine the cytological effect of exposure to dilute sea water, the histology of the cerebral organs has been compared, 1) in worms exposed to 75% and 50% sea water and fixed in glutaraldehyde of equivalently reduced osmolarity (730 mosm and 480 mosm), 2) in worms from normal sea water fixed in hypo-osmotic glutaraldehyde (730 mosm and 480 mosm), and 3) in worms from 100% sea water fixed in glutaraldehyde of approximately equivalent osmolarity (970 mosm). The histology of the cerebral organs of *Paranemertes* under normal conditions has been described in Chapter III, and is illustrated in Figures 8 and 11.

#### *1) The effect of dilute sea water*

In worms exposed to 75% sea water, only slight changes were apparent in the cytology of gland cells and vesicular cells, and there was no visible effect on other cells of the cerebral organ. The amount of dark granular material in Type 1 vesicular cells increased. The maximum increase was already apparent in worms fixed after one hour (Fig. 54A), and subsequently the amount decreased towards normal. Type 2 vesicular material was more dispersed than in worms from

normal sea water and the amount of material was greater. There was little change in the structure of gland cells after one hour in 75% sea water, but the secretion granules stained more intensely, and after six hours exposure, the gland cell contents were depleted in some areas and the amount of vesicular material within gland cells had increased (Figs. 54B,54C).

In worms exposed to 50% sea water the changes in cytology of the cerebral organs were quite marked and some cell damage was apparent. After one hour of exposure, all of the cells appeared swollen and many contained small clear vacuoles. The amount of dense material in Type 1 vesicular cells increased, though not as much as in worms from 75% sea water, perhaps because the cells were damaged and some of the material may have been leached out. There was greater variation than normal in the amount of secretion product in gland cells, and after six hours, the contents of gland cells were greatly depleted (Fig. 54D). The amount of Type 2 vesicular material in gland cells was greater, and the number of subepithelial Type 2 vesicular cells increased.

After 24 hours, all cells that had undergone change had returned to normal or near-normal in worms from 75% sea water (Fig. 54E), and some recovery was apparent in all cells in the cerebral organs of worms from 50% sea water. No evidence of movement of gland cell or vesicular cell contents across the connective tissue capsule of the cerebral organ was observed, and the relationship of the

cerebral organ with the cephalic blood vessel did not change.

*2) The effect of hypo-osmotic fixative*

The effect of fixation in 730 mosm glutaraldehyde, was most apparent in Type 1 vesicular cells, where the amount of dark granular material increased (Fig. 55A), as also happened in dilute sea water. Type 2 vesicular material also increased slightly, and the material was aggregated into smaller globules and appeared more dispersed. There was no perceptible change in the structure of sensory cells or gland cells after this treatment, but as in dilute sea water, the gland-cell product stained more intensely and the nuclei were more prominent.

Fixation in 480 mosm glutaraldehyde caused considerable change in the appearance of cells, and in general, the cells appeared more damaged than after exposure of the living worm to 50% sea water. This is probably a reflection of the fact that in living tissue cellular mechanisms are available which lessen the impact of the osmotic stress. In Type 1 vesicular cells there was an increase in the number of small darkly staining vesicles, and the cells appeared to be damaged, showing empty spaces within and between cells (Fig. 55B). The amount of Type 2 vesicular material increased, and the material within subepithelial Type 2 vesicular cells was present in a large, very loosely compartmentalised pool, as if the vacuoles and the cells had burst (Fig. 55C,55D). In gland cells there was greater variation than normal in the

amount of secretion product present (Fig. 55D), some cells containing only a few scattered granules, others containing large globules of coalesced secretion product. In general, the number of secretion granules inside gland cells appeared to be reduced, and in some areas the cell membranes appeared to have ruptured. In all regions of the cerebral organ, there were large areas in which the cells appeared swollen and vacuolated.

**Volume regulation**

The ability of *Paranemertes* to regulate volume in 75% and 50% sea water was measured in intact worms and in worms without cerebral organs, using weight as an indication of volume. The results are illustrated in Figure 56.

All worms gained weight in dilute sea water compared to controls in 100% sea water (Kruskal-Wallis; $P<0.001$). The weight of worms in dilute sea water rose sharply in the first hour then reached a plateau. After 12 hours, all worms in 75% and in 100% sea water, both operated and intact, began to lose weight. Worms in 50% sea water continued to gain weight after 12 hours, but began losing weight after 24 hours in the dilute sea water. Worms in 100% sea water also lost weight over the 60 hour period, and the amount of weight lost by worms in dilute sea water was not significantly different from the amount lost by controls in 100% sea water. Worms with cerebral organs removed gained more weight than intact worms in 75% sea water, but they

also lost more weight during the latter part of the experiment.

Because operated worms gained more weight than intact worms, a second experiment was performed using sham-operated worms to determine whether the difference in response was due to the absence of the cerebral organs or to the stress of the operation. The results are illustrated in Figure 57. As in the first experiment, all worms in 75% sea water gained weight compared with controls kept in 100% sea water (Kruskal-Wallis; $P<0.001$). In the first hour, both intact and sham-operated worms gained less weight than worms with cerebral organs removed (Mann Whitney; $P<0.05$), but after the first hour, and for the remainder of the experiment, the weights of sham-operated worms were the same as those of worms without cerebral organs, and both were consistently higher than those of intact worms. After 48 hours in 75% sea water, the weight of worms from all groups had decreased toward the original weight, however, as in the first experiment, this weight loss was also apparent in control worms of all three groups. None of the worms lost weight significantly compared with controls in 100% sea water.

## C. Discussion

### The effect of hypo-osmotic stress on cerebral organ cytology

In *Paranemertes* exposed to dilute sea water the cytology of the cerebral organs changed, and the change was

most apparent in gland cells and vesicular cells. The changes observed were similar to those occurring in *Lineus* and *Amphiporus* under similar conditions. It cannot be concluded, however, based on these cytological changes, that the cerebral organs are structures specifically involved in coping with osmotic stress. A number of factors could account for the observed changes. The osmotic stress causes massive secretion from cephalic glands and epidermal mucous glands, clearly as a protective response, and the same may be induced in cells of the cerebral organs. The osmotic change may cause an increase in the sensory activity of the cerebral organs, and thus an increase in gland and vesicular cell activity. Alternatively, the cytological changes may not reflect changes in activity at all, but may result from direct osmotic effects on the cells. For example, osmotic pressure may explode secretion granules and cause aggregation and lysosomal breakdown of secretion material within gland cells, resulting in an increase in the amount of Type 2 vesicular material, and it may force intact secretion granules out of the cells. It is clear from the cytological effects of hypo-osmotic fixative demonstrated in this study, that gland and vesicular cell morphology changes passively in response to osmotic forces. The relative contribution of any or all of these factors to the observed changes in cytology cannot be determined histologically.

In addition to cytological change in gland cells and vesicular cells, Ling (1970) and Ferraris (1979a) observed

Ferraris (1979a) observed histological profiles which may indicate transfer of a small amount of vesicular material from the cerebral organs to the vascular system in *Amphiporus* exposed to dilute sea water. She reported that "Occasionally, at 0.5 and 3 hours vesicles having the same staining affinity and appearance as the green-blue vesicles observed in vesicular and basal cells, were found in the tissue between the cerebral organ and the blood vessel" (Ferraris, 1979a, p. 443). Considering the structure of the cerebral organs in *Amphiporus*, the lack of anatomical specializations suggesting a functional relationship between the cerebral organs and the vascular system, and the small amount of material involved, it seems unlikely that the transfer of vesicular material to the cephalic blood vessel, would constitute an endocrine response. Perhaps, as suggested for *Lineus*, vesicular material that is produced in large quantities during hypo-osmotic stress is disposed of in the vascular system.

**Volume regulation**

Oglesby (1981) distinguished two types of volume regulation during exposure of an organism to hetero-osmotic media. The traditional concept of volume regulation in hypo-osmotic media refers to the gradual restoration of the original volume following the maximum increase. A second type of regulation is that which limits water influx throughout the period of exposure and results in a lower

volume maximum. The former will be referred to as long term volume regulation and the latter as short term volume regulation. Measurement of the limitation of water uptake which constitutes short term volume regulation generally requires comparison of an observed volume increase with the potential increase in a true osmometer, calculated from the solute and water content of worms in normal sea water (Machin, 1975; Oglesby, 1981). Although in this study the solute content of *Paranemertes* has not been measured, it is clear that short term volume regulation is occurring during exposure to 75% sea water, because intact worms gain less weight than operated worms. The reduction of regulatory capacity in operated worms may be due to increased permeability of the body wall at the point of wounding, or to some general reduction of homeostatic capacity due to the stress of the operations. Among operated worms, the reason for greater initial rate of weight gain in worms without cerebral organs than in sham-operated worms may be the greater severity of the former operation. Removal of the cerebral organs probably causes some brain damage, and thus might affect central nervous control of volume regulation. The cerebral organs are not involved in short term volume regulation in *Paranemertes*, since both groups of operated worms gain the same amount of weight.

Lechenault (1965), in a study of volume regulation in *Lineus*, did not consider whether short term volume regulation occurred, and his data bearing on this aspect are

puzzling: In *Lineus ruber* subjected to 30 ppt sea water (Lechenault, 1965; Fig. 1), all worms gained the same amount of weight, but the two groups of operated worms gained weight much more slowly (18 hours) than the group of normal worms (3 hours). After 3 hours in 30 ppt sea water, the weight of intact worms had increased by 11%, whereas that of operated worms had increased by only 3%. Comparing the two species, *L. viridis* in 28 ppt sea water with both cerebral organs and cerebral ganglia (but with the cephalic region inverted) gained less weight than unoperated *L. ruber* in 30 ppt sea water. *L. viridis* without cerebral organs or cerebral ganglia gained a great deal more weight after three hours than did *L. ruber* - much more than would be expected from the differences (28 ppt as opposed to 30 ppt) in concentration of sea water. This would be a surprising species difference, as *L. ruber* and *L. viridis* are very closely related, and may constitute a single species (Cantell, 1975). It is difficult to explain these early weight changes in terms of simple variables, and the possibility that other factors are influencing the results cannot be evaluated, as no data are presented from controls in 100% sea water and no statistical description of the data is given.

Long term volume regulation during exposure to hypo-osmotic sea water occurs in *Lineus* (Lechenault, 1965) but not in *Paranemertes*. *Paranemertes* gained weight when exposed to 75% and 50% sea water. Although this was followed

by a decrease in weight in both operated and intact worms, controls from normal sea water showed the same amount of weight loss over the same time period. Intact *Lineus ruber* gained weight in dilute sea water, reaching a maximum at 3 hours, and recovering to normal weight after 72 hours (Lechenault, 1965). Through various ablation and grafting techniques it was found that the water efflux did not occur if both the cerebral organs and the cerebral ganglia were missing, and that regulatory capacity was restored if either the cerebral organs or the cerebral ganglia were re-implanted (Lechenault, 1965). If the cerebral organs do play a role in long term volume regulation in lineids, as these experiments suggest, such a function may be unique to this group, and may be correlated with the presence of neurosecretory cells in the cerebral organs of lineids, and their absence in other groups. It is, however, unlikely that the control of long term volume regulation is a primary function of the cerebral organs in the lineids, since its usefulness in ecological terms is limited. On the shore, intertidal non-estuarine nemerteans can be exposed to reduced salinity for only as long as the low tide period, a maximum of less than 12 hours, and the worms may reduce this time behaviourally.

## VII. Summary and Conclusions

1) The histology and fine structure of the cerebral organs have been described in three species of monostiliferous hoplonemerteans: *Paranemertes peregrina*, *Tetrastemma candidum* and *Amphiporus lactifloreus*. The structure of the cerebral organs in these three species is essentially the same, and suggests a chemoreceptive function.

2) The cephalic canal is divided into two channels, the major and minor canals - by specialized ciliated cells along most of its length, and by lappet cells along a short segment near the blind end. According to the orientation of ciliary axonemes and basal feet in cells of the canal epithelium, the minor canal is an incurrent channel and the major canal is an excurrent channel.

3) The cell types present in the cerebral organs are as follows:

a) Ciliated cells: Cells lining the minor canal, including barrier-forming ciliated cells which have very well-developed striated rootlet complexes and separate the major and minor canal epithelia.

b) Lappet cells: Cells of the minor canal epithelium near the blind end of the canal which form a septum of cilia and microvilli separating the major and minor canals.

c) Ciliated support cells: Cells of the major canal epithelium which are associated with Type 1 sensory cell dendrites.

d) Type 1 sensory cells: Bipolar cells comprising the main nervous component of the cerebral organs. The dendrites of these cells, together with ciliated support cells, form an extensive sensory epithelium along the major canal from about the middle of the canal to near the blind end.

e) Type 2 sensory cells: A second group of bipolar sensory cells whose dendrites form a small sensory epithelium on the minor canal near the blind end.

f) Type A gland cells: Mucoprotein secreting cells situated in the mid-region of the cerebral organs on the ventral, medial and dorsal surfaces, with processes extending to the canal lumen amoung the ciliated cells of the minor canal.

g) Type B gland cells: Mucoprotein secreting cells forming several large lobes surrounding and posterior to the blind end of the canal, with processes extending to the lateral fork of the canal at the blind end.

h) Type 1 vesicular cells: Cells forming an extensive endocytic/lysosomal epithelium along the major canal from near the canal opening to the beginning of the Type 1 sensory epithelium.

i) Type 2 vesicular cells: Epithelial Type 2 vesicular cells form a small endocytic/lysosomal epithelium on the minor canal at the blind end. Subepithelial Type 2 vesicular cells are situated in a group just posterior to the blind end of the canal; they contain large amounts of Type 2 vesicular material (a heterogeneous material, largely acid

mucopolysaccharide, contained in vacuoles), and have very little cytoplasm and few organelles. A few of these cells are situated individually among gland cells. Some or all of these subepithelial cells may arise through transformation of gland cells by autophagic breakdown of secretion product and synthetic organelles.

j) Subepithelial support cells: Multipolar cells comprising the cellular connective tissue of the cerebral organs. These cells extend processes among gland cells, sensory cells and subepithelial Type 2 vesicular cells.

4) The rate of flow through the cerebral organs is thought to be controlled by an efferent nerve fibre which is present among the ciliated cells lining the minor canal. It is suggested that this fibre controls the rate of ciliary beat in a few cells which act as pacemakers, their cilia mechanically entraining the beat frequency of other cilia in the canal. The sensitivity of dendrites may be controlled by the rate of flow through the canal.

5) The direction of flow in the canal is such that gland cells are upstream from sensory epithelia, and epithelial vesicular cells are downstream. It is proposed that the function of gland cells is to provide a mucous coating over the dendrites, and that the function of epithelial vesicular cells is to remove the mucus after it has passed over the dendrites.

6) Vesicular material is produced through lysosomal breakdown of the secretion product of gland cells. In

epithelial vesicular cells (Type 1 and 2), vesicular material arises through breakdown of material ingested from the canal lumen. In gland cells it arises through direct lysosomal breakdown of secretion granules. This crinophagy may be a mechanism for regulating the amount of secretion product in gland cells, according to a variable demand for secretion in the canal. In some cases, lysosomal breakdown within gland cells involves the entire synthetic apparatus in addition to the secretion granules. The result of this breakdown is the transformation of gland cells into subepithelial Type 2 vesicular cells.

All types of vesicular material increase in amount when the cerebral organs are exposed to hypo-osmotic media, partly as a result of passive osmotic forces. In epithelial vesicular cells, this may also be due to an increase in the amount of secretion product taken in from the canal. In gland cells, it may be due to an increase in the rate of incorporation of secretion granules into lysosomes because of osmotic damage to the secretion granules.

7) In *Paranemertes* and *Amphiporus*, the cephalic blood vessel passes close to the posterior glandular region of the cerebral organs. However, there is no morphological indication that the cerebral organs are specialized to produce and transfer material to the vascular system. Therefore it is thought that the cerebral organs in these species, and in *Tetrastemma*, where there is no association with the vascular system, are not endocrine glands.

8) The behaviour of *Paranemertes* with and without cerebral organs has been studied and compared. Intact *Paranemertes* respond to the trails of *Platynereis bicanaliculata*, the preferred prey species, with a characteristic searching behaviour. This behaviour is abolished by removal of the cerebral organs. Intact *Paranemertes* are capable of homing following their mucous trails, and can relocate their trails after minor breaks. This behaviour is not affected by removal of the cerebral organs. It is concluded that the cerebral organs are chemoreceptors involved in prey detection.

9) Extracellular recordings have been obtained from the lateral nerve cord and the cerebral organ nerve of *Paranemertes*. Responses recorded in the cerebral organ nerve indicate that the cerebral organs are sensitive to distilled water and prey extract, but not to light, temperature or vibration,

10) The ability of *Paranemertes* to regulate volume in dilute sea water was determined by measuring weight changes of worms kept in 75% and 50% sea water. *Paranemertes* is capable of short term volume regulation, which involves the limitation of water uptake throughout the period of exposure to dilute sea water. The worms are not capable of long term volume regulation: although worms lose some of the weight gained in dilute sea water, control worms in 100% sea water lose the same amount of weight. The ability to regulate volume is reduced in worms without cerebral organs, however,

this reduction also occurs in sham-operated worms, indicating that the reduction in volume regulatory capacity is caused by the stress of the operations, and not by the loss of some factor from the cerebral organs. It is concluded that the cerebral organs in *Paranemertes* are not involved in volume regulation.

## Appendix I: Natural history of *Tetrastemma candidum*

### A. Introduction

Over the course of this study, *Tetrastemma candidum* was collected at intervals from January 1978 through May 1980 from the East Rocks, St. Andrews Bay. Some interesting details of the natural history of this species have come to light, and are reported in this appendix.

### B. Results and Discussion

In St. Andrews Bay, algal cover on the shore is sparse in the spring, increases rapidly over the summer months to a maximum in the fall, then declines over the winter, due to rough weather and frost which remove old growth, and low light and low temperatures which slow new growth. A clearly recognizable zonation is evident in the distribution of algae on the rocks, with *Fucus serratus*, *F. vesiculosus*, *F. spiralis* and *Ascophyllum* sp. occupying successive bands from the lower to the upper shore.

In St. Andrews Bay, *Tetrastemma candidum* occurs most commonly on *Fucus vesiculosus*, but in the fall, when numbers of *Tetrastemma* are highest and algal cover is at a maximum, *Tetrastemma* is also common on *Ascophyllum* sp. It is almost never found on *F. serratus* or *F. spiralis*, regardless of the time of year, though the latter species is situated between *F. vesiculosus* and *Ascophyllum* sp. on the shore.

From careful observations of *F. vesiculosus* in the field, and attempts to collect *Tetrastemma* directly from the algae, it became apparent that the worms are absent from the surface of the plant during low tide. To determine the location of the worms at low tide, a sample of alga was brought back to the laboratory and separated into two fractions, one containing the bladders, the other containing the remainder of the algae, and the number of worms emerging from each sample was recorded. All worms emerged from the bladder fraction, and the total number of worms collected was greater than that expected from a non-fractionated sample of the same size, suggesting that some worms remain in the bladders during the normal collecting procedure (described in Chapter II). The infection rate of *F. vesiculosus* bladders, determined from fractionated samples, was 1.3%. Worms would begin to appear on the surface of the algae almost immediately after immersion in sea water, and would continue to appear over a period of about two days. These observations suggest that the worms are generally active during high tide, when they forage on the surface of the algae, and that they crawl into the bladders at low tide, but that not all worms are active at every high tide.

**Behaviour and feeding**

While emerging from the algae in the laboratory, *Tetrastemma* was occasionally observed everting its proboscis at small unidentified crustacea present on or near the

surface of the algae. A variety of potential prey species was offered to *Tetrastemma* in small aquaria without algae. These included *Harmothoe imbricata*, *Eulalia* sp., *Perinereis cultrifera*, *Marinogammarus* sp., *Idotea* sp., unidentified copepods, and unidentified crab zoeae. The prey was offered by simply leaving it in the bowl with the worms, as *Tetrastemma* avoided anything that was presented to it directly. Of the potential prey offered, *Tetrastemma* took copepods, crab zoeae, gammarids and *Idotea* sp. Small crustacea were captured with the proboscis and apparently swallowed whole. Larger prey species were paralysed with the proboscis, the soft parts were subsequently ingested and the empty exoskeleton was left. When eating the large *Idotea* sp., *Tetrastemma* would crawl inside the exoskeleton to ingest the soft parts. *Tetrastemma* did not take non-crustacean prey. This species apparently specializes on crustaceans but shows no preference within the class.

**Phototaxis**

Both adults and juveniles of *Tetrastemma candidum* are strongly positively phototaxic, a very unusual trait in a nemertean. In the laboratory, worms newly placed in aquaria crawl around restlessly in apparently random direction for one to two hours, then come to rest at the lighted end of the aquarium. Once the initial restlessness subsides, the worms remain stationary for hours unless disturbed. If the aquarium is turned around, or the direction of the light is

reversed, however, the worms immediately begin to crawl towards the light, and once again come to rest at the lighted end. The ecological significance of this behaviour is not known, but it may function in keeping the worms on the distal fronds of the algae.

**Reproduction and development**

*Tetrastemma* is dioecious and sexually dimorphic, with females being approximately twice the width of males and of a different colour (Fig. 58). The females are about 2.5 mm in diameter. The ripe ovaries are conspicuous as a bright yellowish-white line on either side of the middorsal line, and the body wall is coloured bright, almost fluorescent, green. The males are slender (about 1 mm in diameter) and the ripe testes are visible through the body wall as two white lines, one on either side of the middorsal line. The colour of the body wall varies in males, and can be yellow, pink, orange or dull green.

Numbers of *Tetrastemma* are highest in the summer and fall, decline over the winter, and are replenished in the late spring when juveniles hatch. Juveniles grow very rapidly over the summer and have nearly reached adult size by the fall. At this time of the year, and throughout the winter, all of the worms appear to be in the same size class (taking into account sexual dimorphism), thus it is likely that *Tetrastemma* is an annual species. McIntosh (1873-74) reports that *Tetrastemma candidum* reproduces twice a year.

Although I did not observe ripe adults in the field except in the spring in either year of sampling, it is possible that in some years, worms that hatch in the spring become ripe, and spawn before the winter.

In 1979, 12 females spawned in the laboratory between late April and mid-May. The cocoons produced were kept in fingerbowls at room temperature (15 - 17°C), and the time course of development was followed from spawning to hatching. At the time of spawning, the male and female together secreted a parchment-like tubular cocoon into which the female released the eggs. The male left the cocoon during or immediately after spawning by the female, and all of the eggs were fertilized in either case. The female remained in the cocoon and exhibited a characteristic brooding behaviour throughout the 8 to 11 days required for development of the eggs. Brooding involved the generation of peristaltic waves by contraction of the body wall musculature, and occasional turning around within the cocoon to face the opposite direction. If the cocoon was disturbed with a probe, the female would sometimes evert her proboscis at the probe and sometimes ignore the disturbance, however brooding females were much more likely to evert their proboscides at a disturbance of this kind than were non-brooding worms. Two females were removed from their cocoons about five days after fertilization, and released approximately 5 cm away. One of the females returned to the cocoon, and remained until the juveniles hatched; the other

did not return, but development of the embryos in the untended cocoon proceeded apparently normally.

I was not able to determine where on the algae cocoons are produced in the field. Large amounts of algae were carefully examined for cocoons during the spawning season in 1979, and no cocoons were found on the surface of the algae. A small sample (200) of bladders was examined for cocoons, and none were found (only two worms were found, both male). It is unlikely that cocoons are produced off the algae, and since none were found on the surface, it is probable that they are produced in the bladders. The brooding behaviour of females may be necessary to aerate the eggs, as well as to defend them.

**Development**

The fertilised eggs undergo holoblastic, spiral cleavage, with macro- and micromeres being approximately the same size. The embryos develop into a spherical, ciliated blastula in 36 hours. After gastrulation, the embryo gradually elongates and doubles on itself within the egg membranes. The eyespots appear at 7 to 9 days, and the juveniles hatch and crawl away in 8 to 11 days. The main developmental events are summarised in Table II.

# Figures and Figure Legends

Figure 1:

Diagram illustrating the histological organization of the cerebral organs in *Paranemertes*. The top of the figure is anterior. (Subepithelial support cells are not illustrated in this diagram or in Figures 2 and 3.)

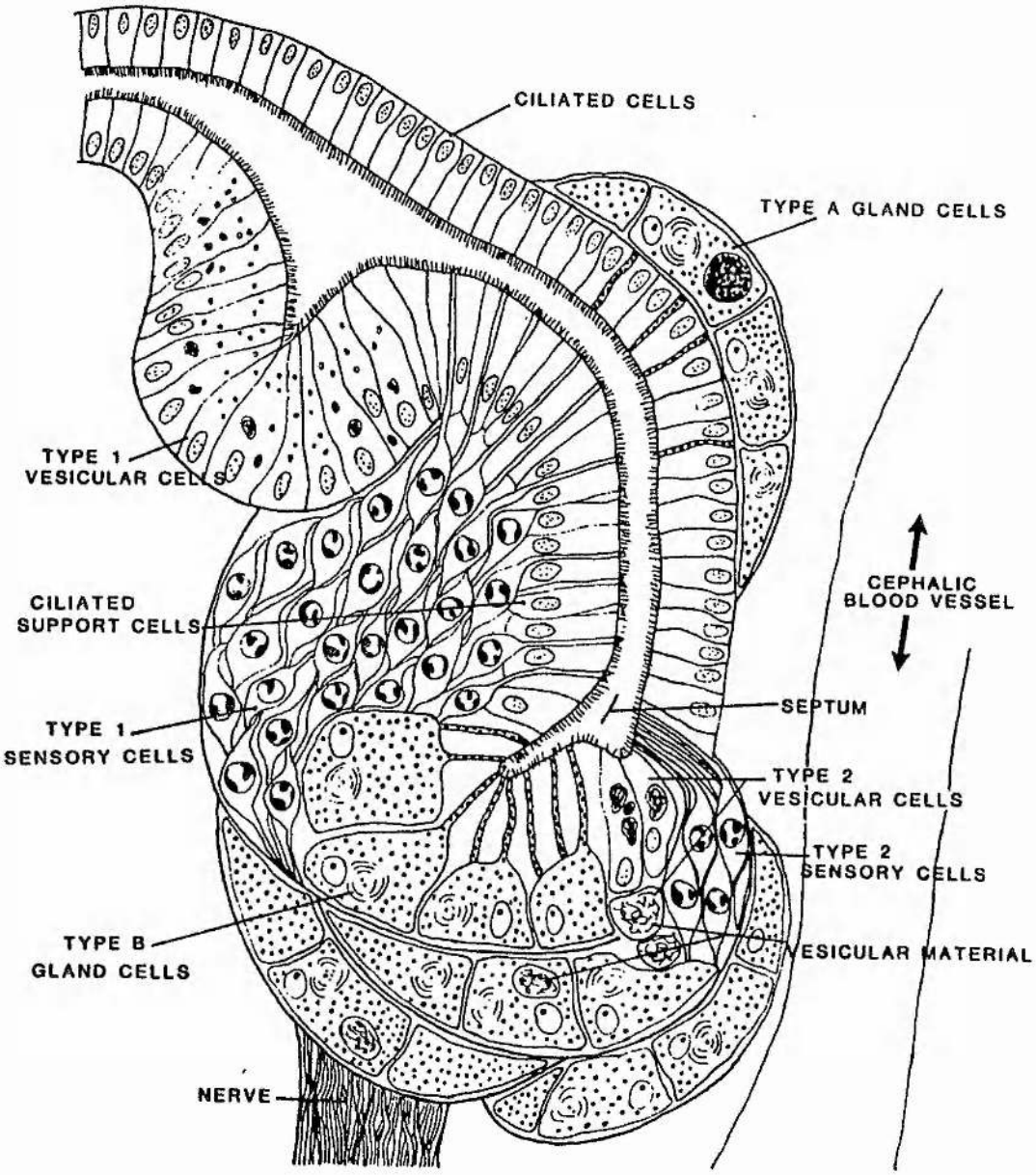

CILIATED CELLS
TYPE A GLAND CELLS
TYPE 1
VESICULAR CELLS
CILIATED
SUPPORT CELLS
CEPHALIC
BLOOD VESSEL
SEPTUM
TYPE 1
SENSORY CELLS
TYPE 2
VESICULAR CELLS
TYPE 2
SENSORY CELLS
TYPE B
GLAND CELLS
VESICULAR MATERIAL
NERVE

Figure 2:

Diagram illustrating the histological organization of the cerebral organs in *Amphiporus*. The top of the figure is anterior.

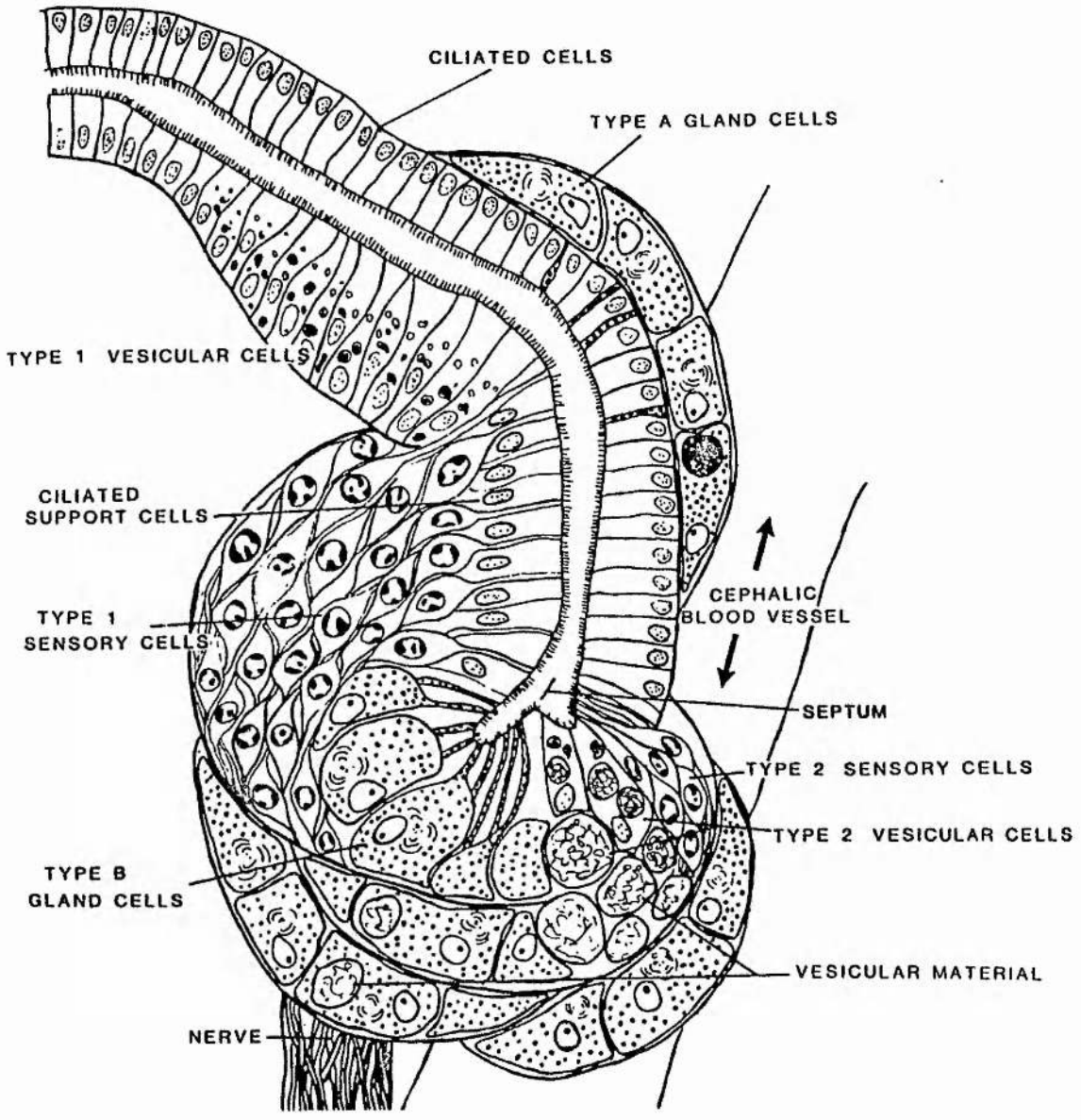

CILIATED CELLS
TYPE A GLAND CELLS
TYPE 1 VESICULAR CELLS
CILIATED SUPPORT CELLS
TYPE 1 SENSORY CELLS
CEPHALIC BLOOD VESSEL
SEPTUM
TYPE 2 SENSORY CELLS
TYPE 2 VESICULAR CELLS
TYPE B GLAND CELLS
VESICULAR MATERIAL
NERVE

Figure 3:

Diagram illustrating the histological organization of the cerebral organs in *Tetrastemma*. The top of the figure is anterior.

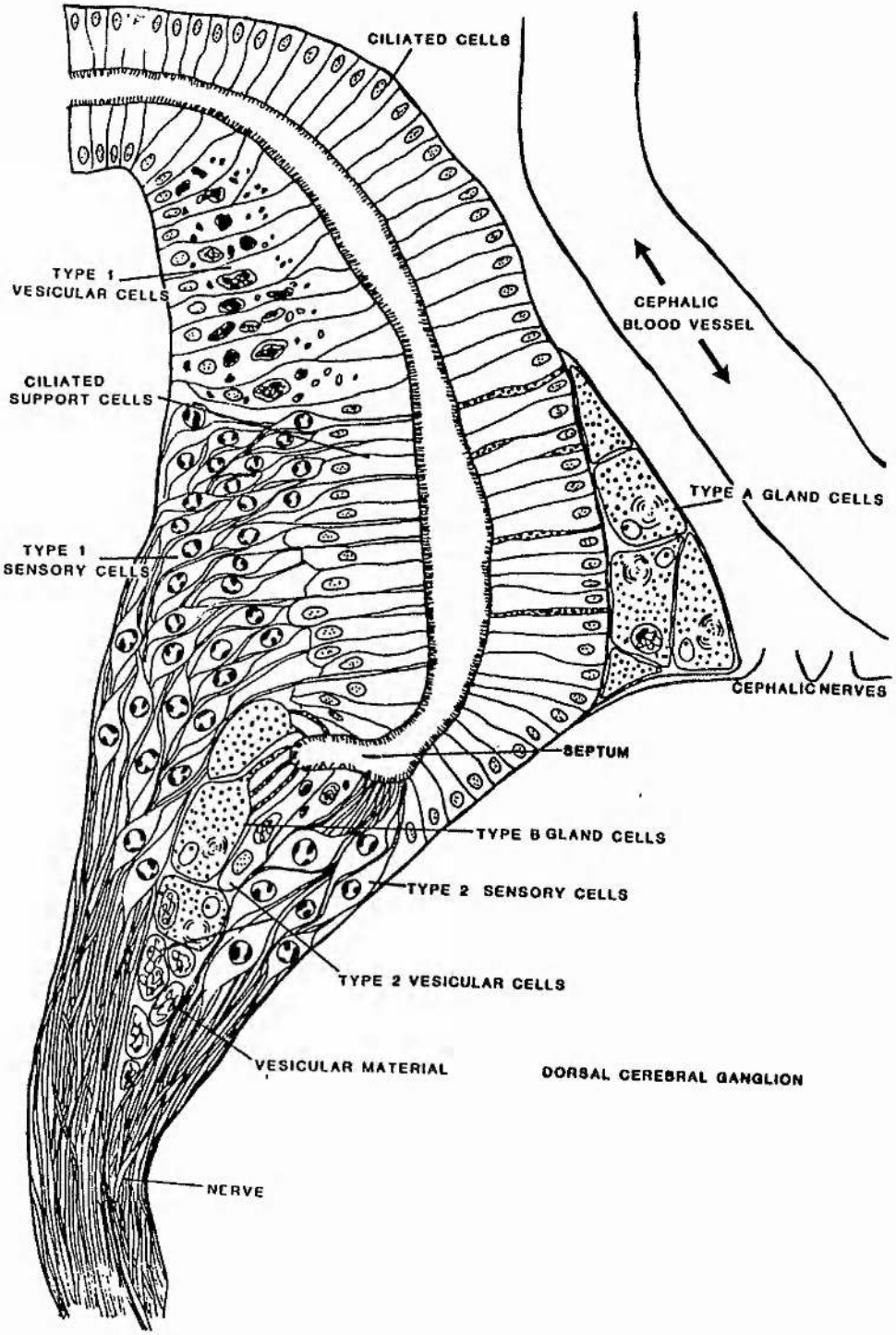
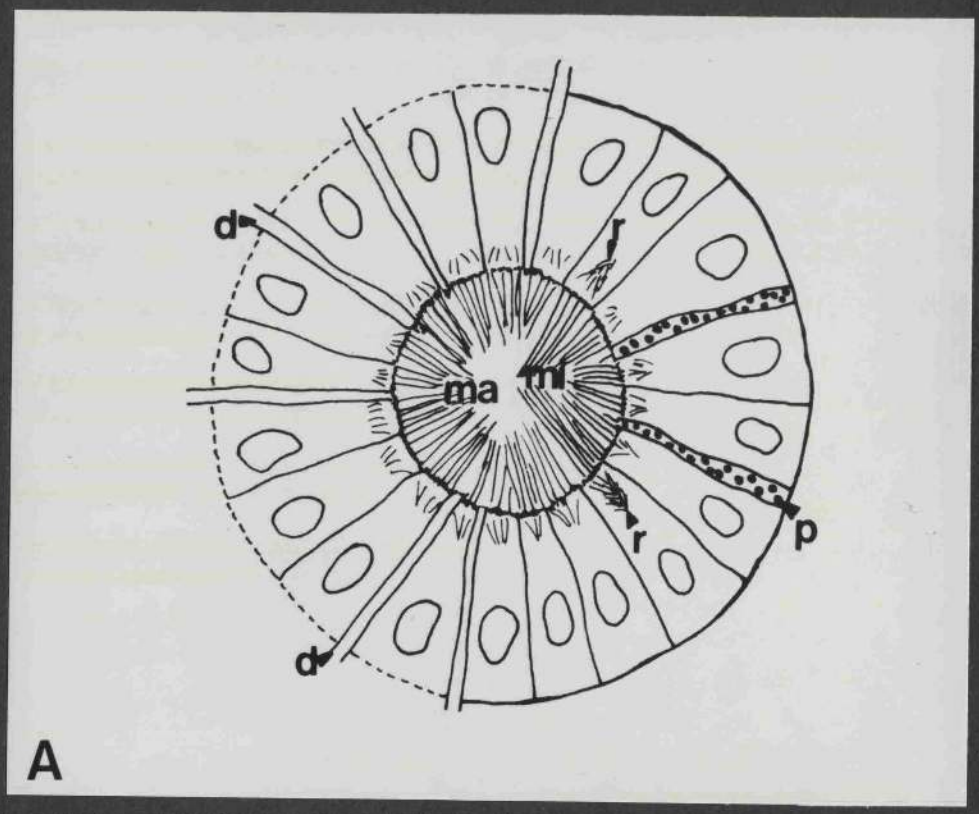

CILIATED CELLS
TYPE 1
VESICULAR CELLS
CILIATED
SUPPORT CELLS
TYPE 1
SENSORY CELLS
CEPHALIC
BLOOD VESSEL
TYPE A GLAND CELLS
CEPHALIC NERVES
SEPTUM
TYPE B GLAND CELLS
TYPE 2 SENSORY CELLS
TYPE 2 VESICULAR CELLS
VESICULAR MATERIAL
DORSAL CEREBRAL GANGLION
NERVE

Figure 4:

A. Diagram of a cross section through the cephalic canal in the mid-region of the cerebral organ illustrating the division of the canal into two channels; d=dendrites of Type 1 sensory cells; ma=major canal; mi=minor canal; p=process of Type A gland cell; r=well-developed striated rootlet complexes of cells separating the major and minor canal epithelia;

B. *Paranemertes*; cross section through the posterior part of the cerebral organ at the level of the canal septum; V=Type 2 vesicular material; ma=major canal; mi=minor canal; large arrowhead=septum; small arrowheads=Type B gland cell processes. Scale bar=20$\mu$m.


d
ma
mi
r
p
d
A

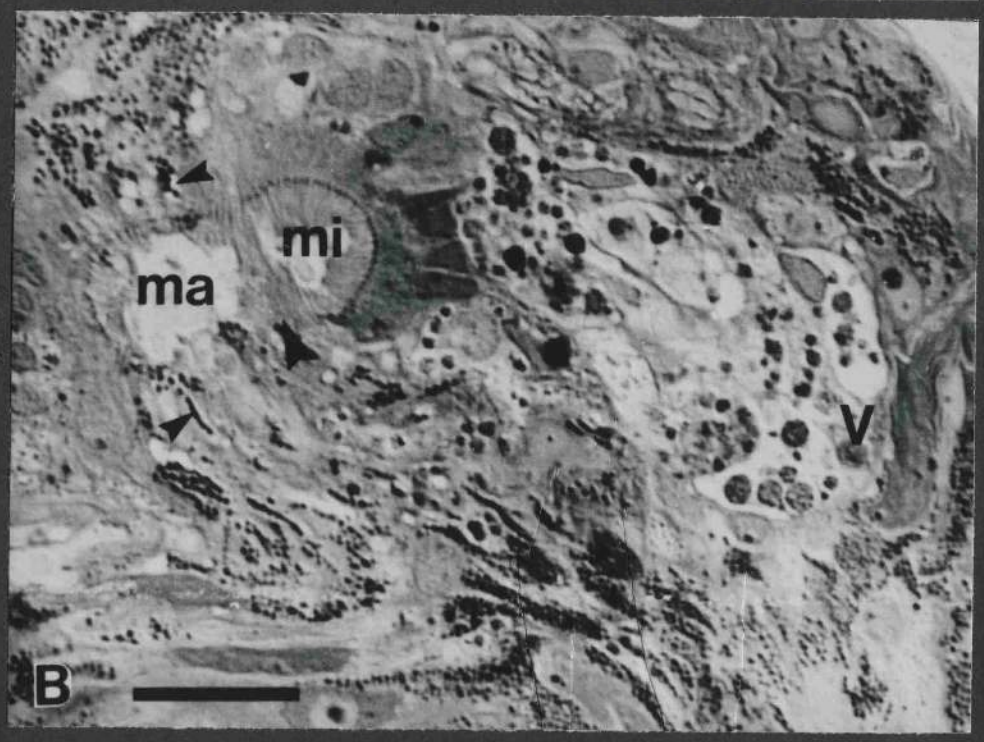

mi
ma
V
B

Figure 5: *Amphiporus*;

A. Section through the mid-region of the cerebral organ showing Type 1 sensory cells (1) and Type A gland cells (g); c=ciliated cells; s=epithelium made up of ciliated support cells and Type 1 sensory cell dendrites; arrowheads=large globules of coalescing secretion granules in gland cells. Note that the cerebral organ is approximately bilaterally symmetrical about an axis that bisects the cephalic canal. Scale bar=50 μm.

B. Cross section through the cerebral organ posterior to the blind end of the canal; 1=Type 1 sensory cells; 2=Type 2 sensory cells; vp=subepithelial Type 2 vesicular cell; arrowhead=coalescing secretion granules and Type 2 vesicular material in a gland cell. asterisk=position of blood vessel. Scale bar=50μm. Although this section is posterior to the blind end of the canal, there is nevertheless a recognizable bilateral symmetry about an axis which is approximately indicated by the arrows; note that the position of the blood vessel with respect to the cerebral organ is asymmetrical.

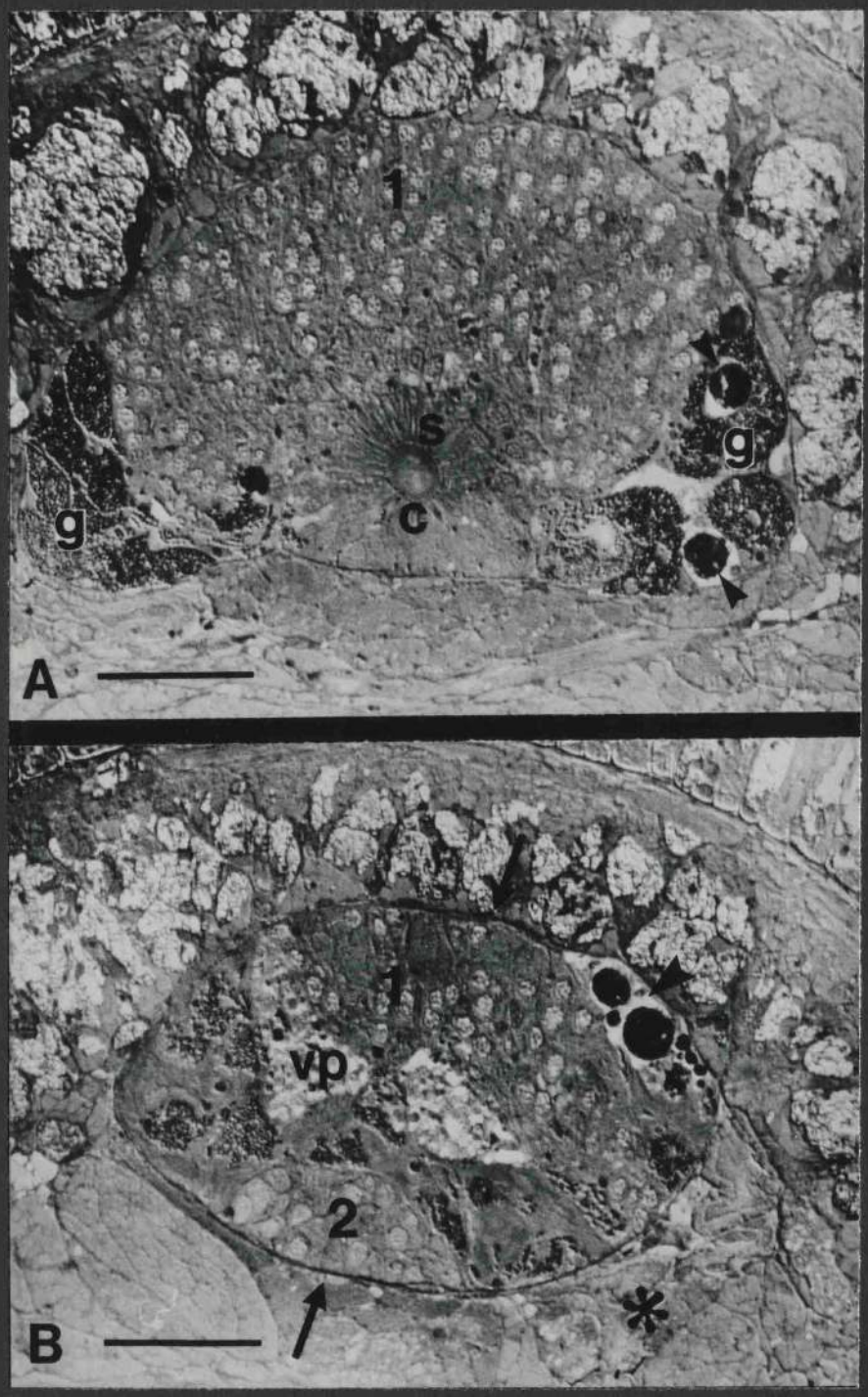

1
s
g
g
c
A
1
vp
2
B

Figure 6: *Tetrastemma* ; Horizontal section through the cerebral organ. The canal opens to the exterior near the top of the micrograph but out of the plane of section. Type 1 vesicular cells (V1) are in the anterior part of the cerebral organ surrounding the canal laterally, dorsally and ventrally. The presence of heterogeneous, darkly staining material in vacuoles is characteristic of these cells. In the middle of the cerebral organ the canal is surrounded laterally, dorsally and ventrally by Type 1 sensory cell dendrites and their associated support cells (SP). The cell bodies of Type 1 sensory cells (S1) form the bulk of the cerebral organ in the mid region. Type A gland cells (not shown) form a lobe on the dorsal, ventral and medial surface of the cerebral organ in the mid-region. Processes of these cells (arrowheads) extend to the lumen among the ciliated cells (C) lining the minor canal. Type B gland cells are mainly located in dorsal and ventral lobes (above and below the plane of section) posterior to the blind end of the canal. The canal bifurcates slightly at its blind end. The processes of Type B gland cells (G) extend to the lateral fork. (S2)=Type 2 sensory cell bodies; (CG)=dorsal cerebral ganglion. Scale bar=20$\mu$m.

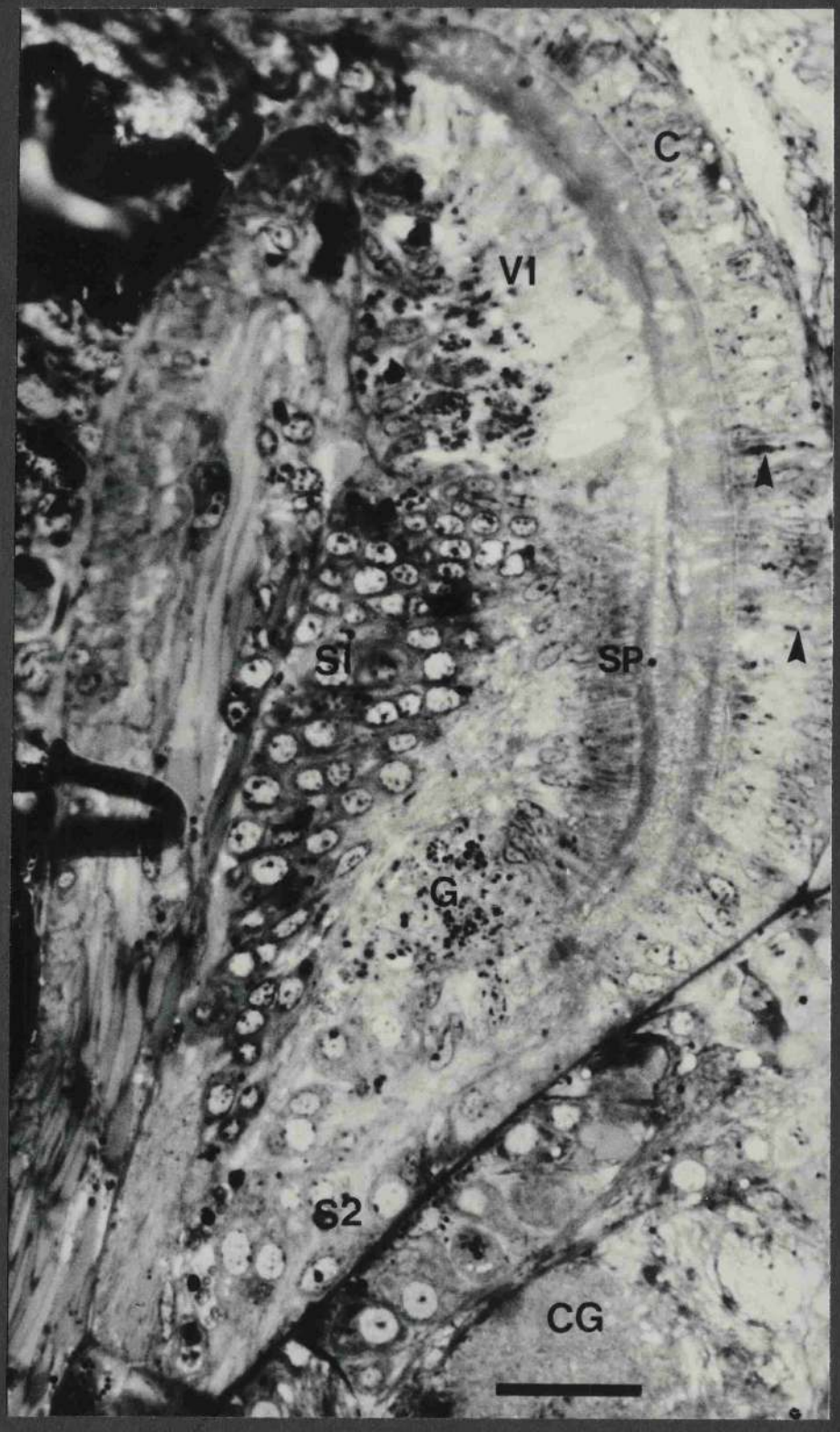

C
V1
SI
SP
G
S2
CG

Figure 7: *Tetrastemma*;

A. Cross section through the cerebral organ posterior to the blind end of the canal; V=subepithelial Type 2 vesicular cells; S2=Type 2 sensory cell bodies. Scale bar=40μm.

B. Horizontal section through the posterior part of the cerebral organ; S1=Type 1 sensory cells; S2=Type 2 sensory cells; D=Type 2 sensory cell dendrites; G=Type B gland cell processes; V=Type 2 vesicular material in or among Type B gland cells. Scale bar=20μm.

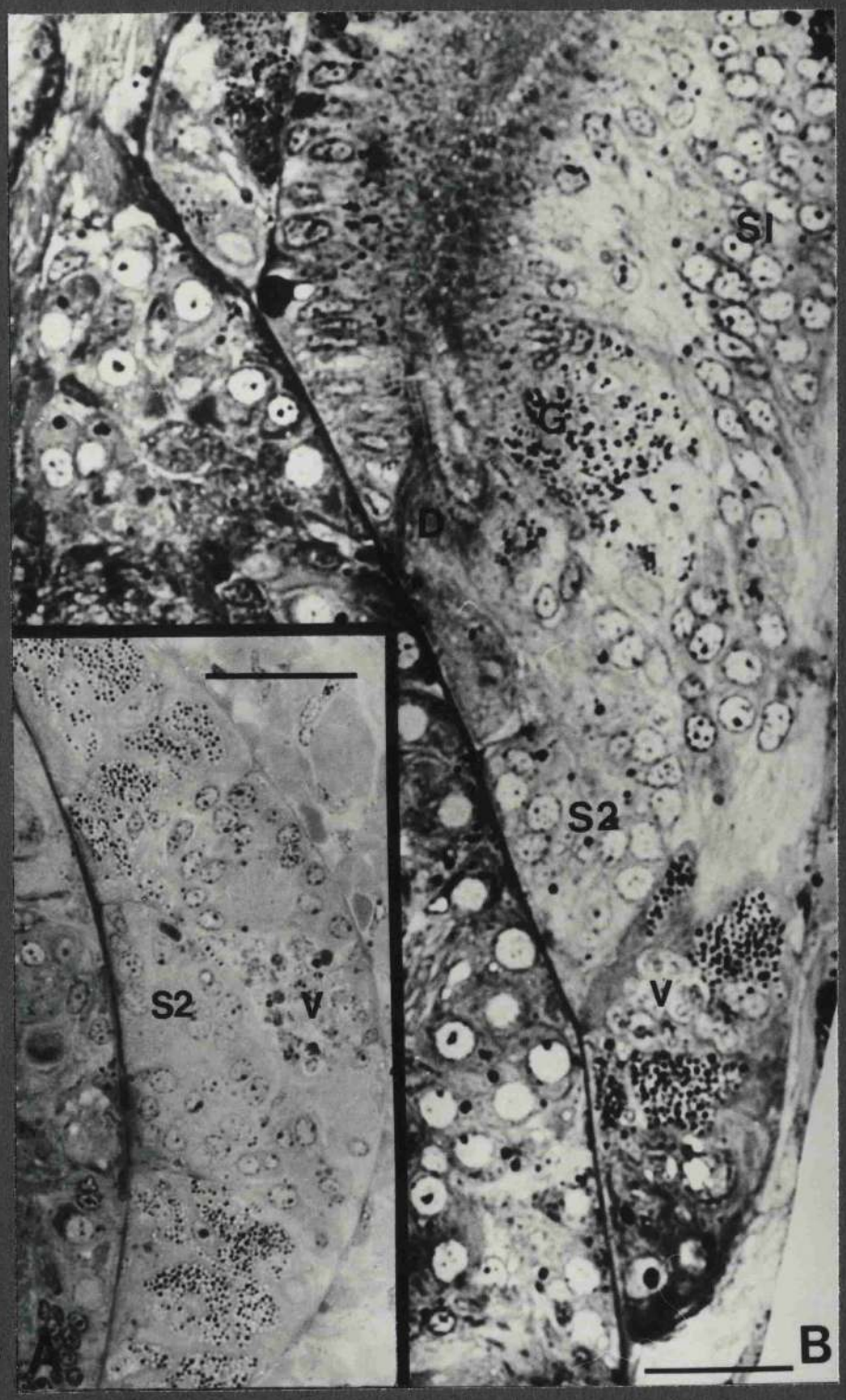

S1
D
S2
V
S2
V
A
B

Figure 8: *Paranemertes*; cross section through the cerebral organ posterior to the blind end of the canal;

A. V=Type 2 vesicular material; S1=Type 1 sensory cell somata and axons; S2=Type 2 sensory cell somata and axons; arrowheads=subepithelial support cells among Type B gland cells; asterisk=cephalic blood vessel; Type 2 vesicular material is sometimes observed in non-glandular areas - in this case, some material is present (arrows) among the axons of Type 2 sensory cells. Scale bar=50μm.

B. Type B gland cells; there is a variable amount of secretion product present in each of the three cells indicated; arrowheads indicate Type 2 vesicular material in gland cell #2 and near the connective tissue capsule of the cerebral organ; Scale bar=10μm.

C. Subepithelial Type 2 vesicular cells (V) among Type B gland cells. Scale bar=10μm.

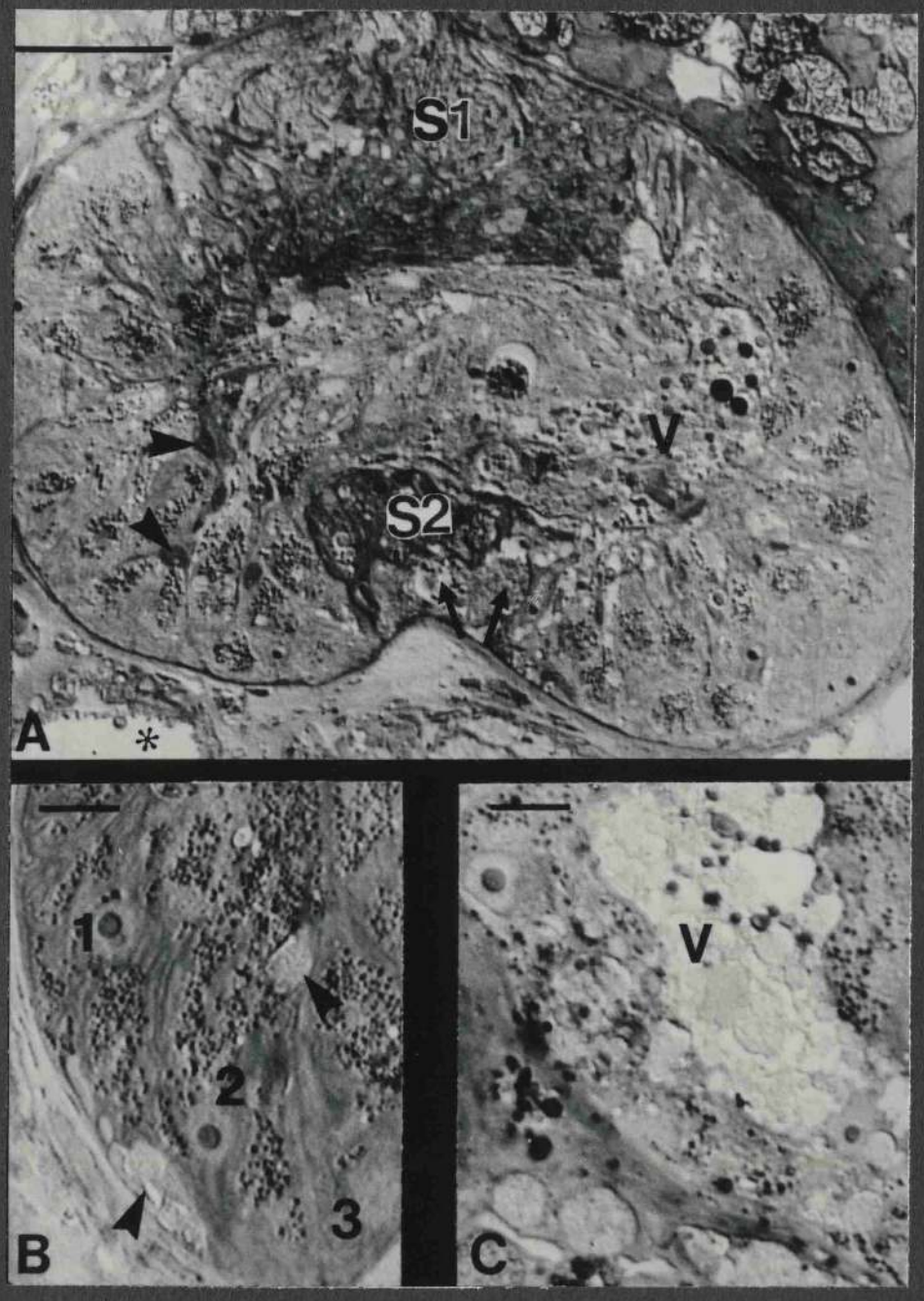

S1
V
S2
A
*
1
2
3
B
V
C

Figure 9: *Tetrastemma*; gland cells and processes;

A. Cross section through the mid-region of the cerebral organ showing Type A gland cells and their processes (arrowheads); Type A gland cell processes empty into the minor canal; Scale bar=30μm.

B. Cross section through the posterior part of the cerebral organ showing Type B gland cells (g) and their processes (arrowheads); Type B processes empty into the major canal; c=dorsal cerebral ganglion. Scale bar=25μm.

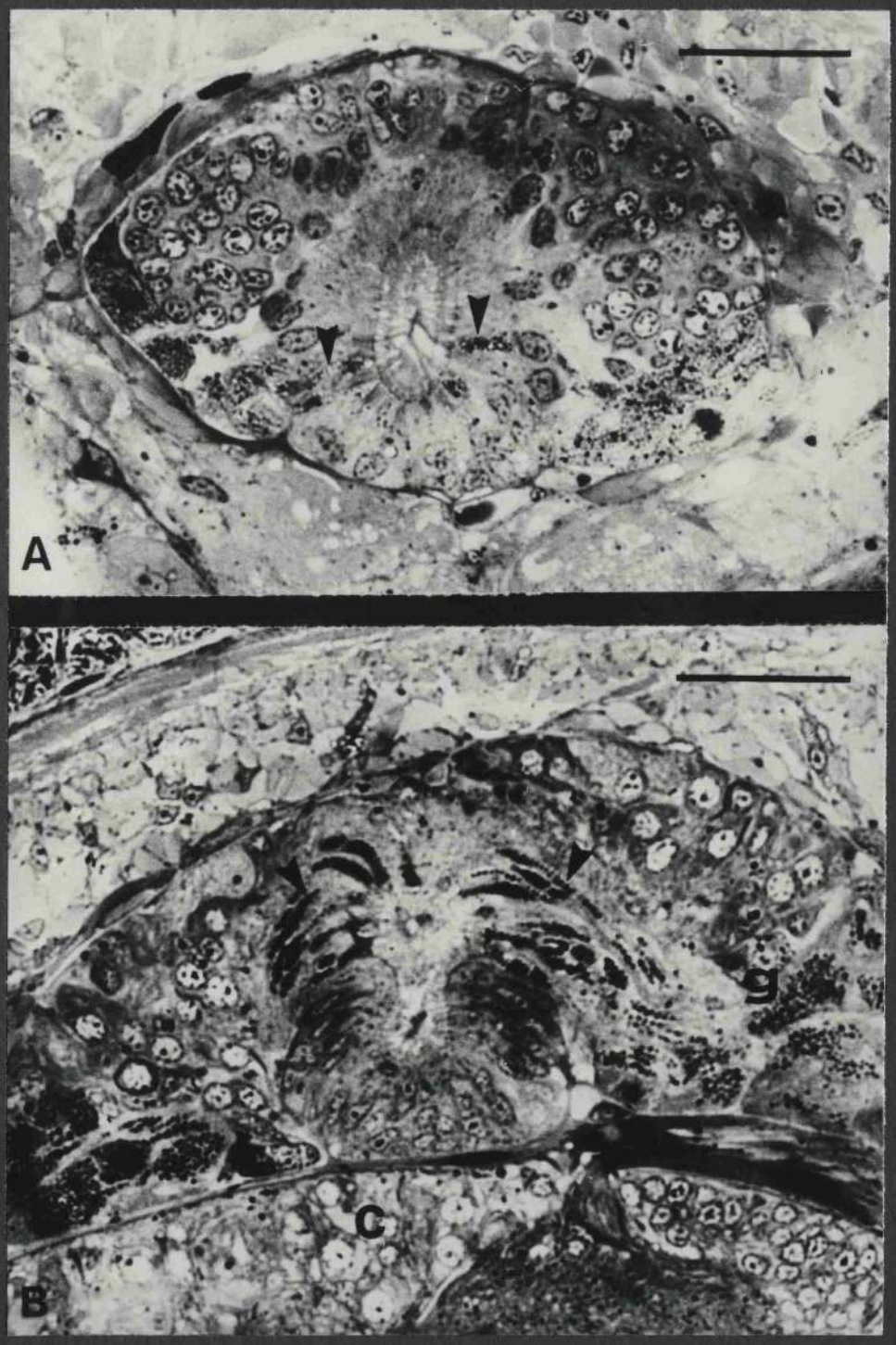

A
B
C

Figure 10: *Amphiporus*;

A. Cross section through the cerebral organs showing Type A gland cells and their processes (arrowheads); S=Type 1 sensory cell bodies; V=Type 1 vesicular cells; Scale bar=50μm.

B. Cross section through the end of the minor canal, anterior to the end of the major canal, showing Type B gland cell processes (arrowheads) extending to the major canal; V=Type 2 vesicular cells (at the end of the minor canal). Scale bar=20μm.

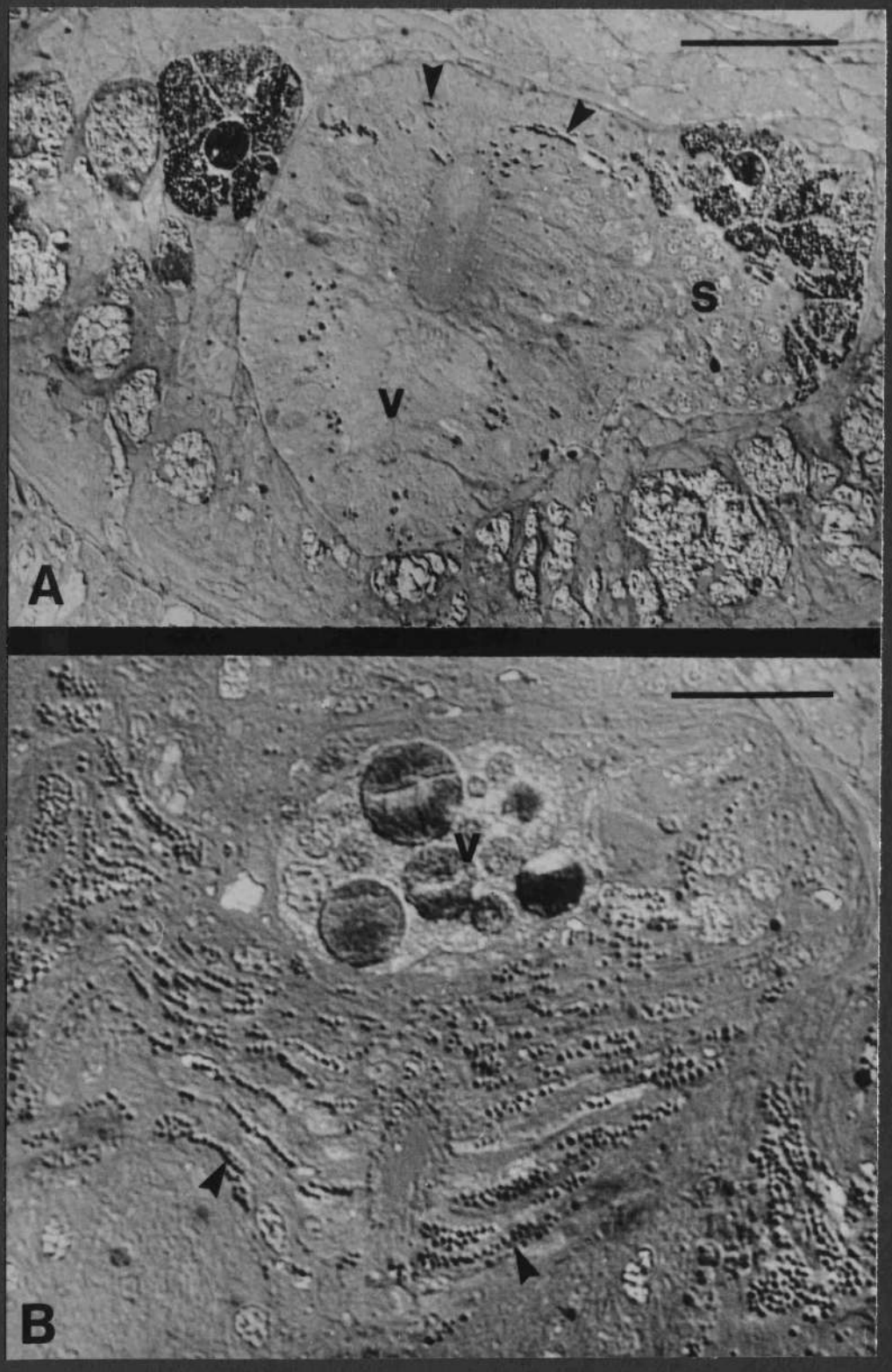

S
V
A
V
B

Figure 11: Type 1 vesicular cells; arrowheads indicate vesicular material;

A. *Amphiporus* ; Scale bar=20μm.

B, C and D. *Paranemertes*; B. Scale bar=20μm. C. and D. Scale bar=50μm.

Note that in *Amphiporus* there is a greater amount of dark granular material in large aggregations; also note in Figures B - D the variation in amount of dark granular material in different individuals of *Paranemertes*.

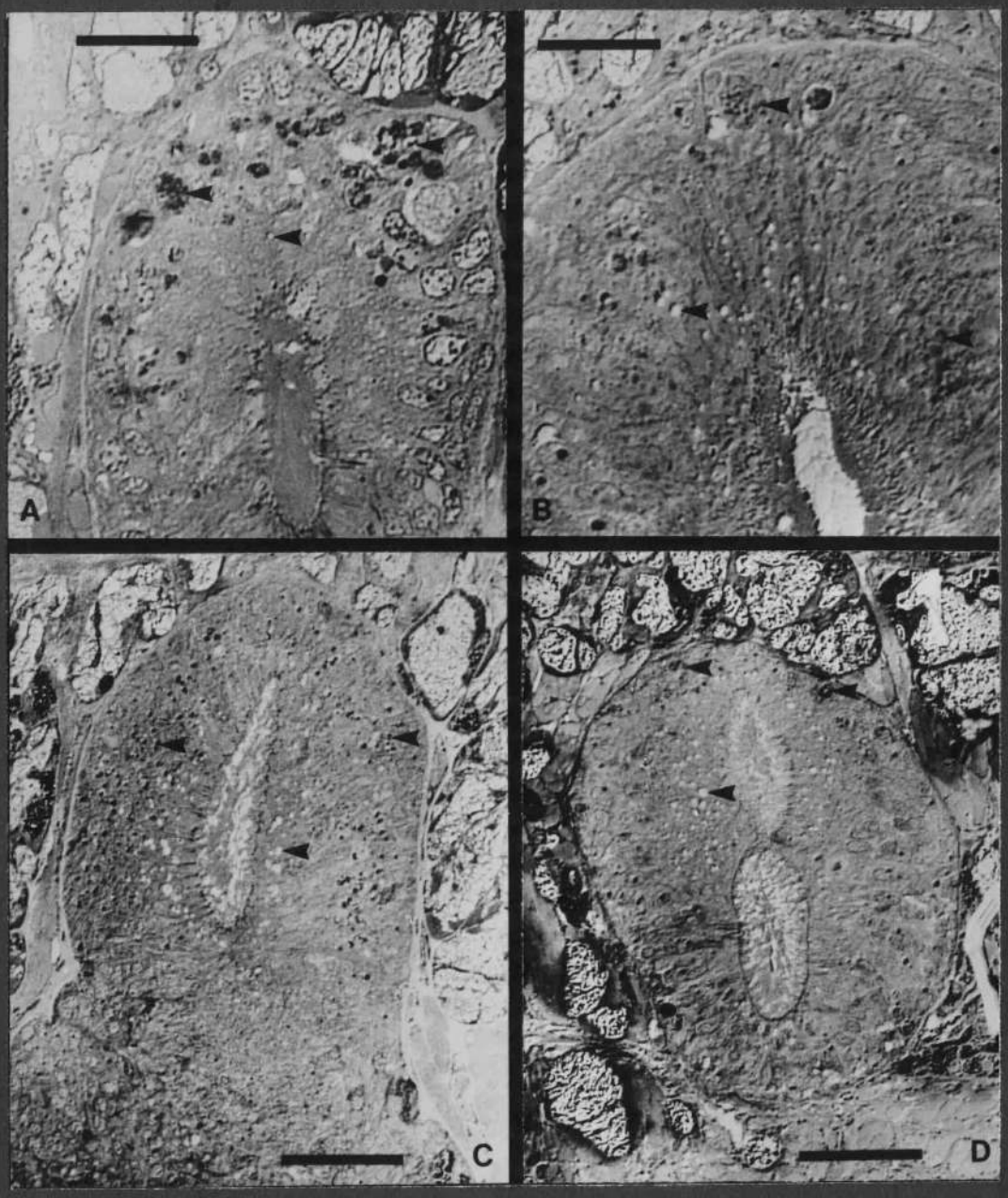

A
B
C
D

Figure 12: *Tetrastemma*;

A. Cross section through both cerebral organs at the level of Type 1 vesicular cells; o=ocellus; co=cerebral organ; p=proboscis; asterisk=position of blood vessel. Scale bar=75μm.

B. Type 1 vesicular cells (V1) and ciliated cells (C); arrowheads=striated rootlet complexes of cells separating the epithelia of the major (open circle) and minor (closed circle) canals; asterisk=blood vessel. Scale bar=10μm.

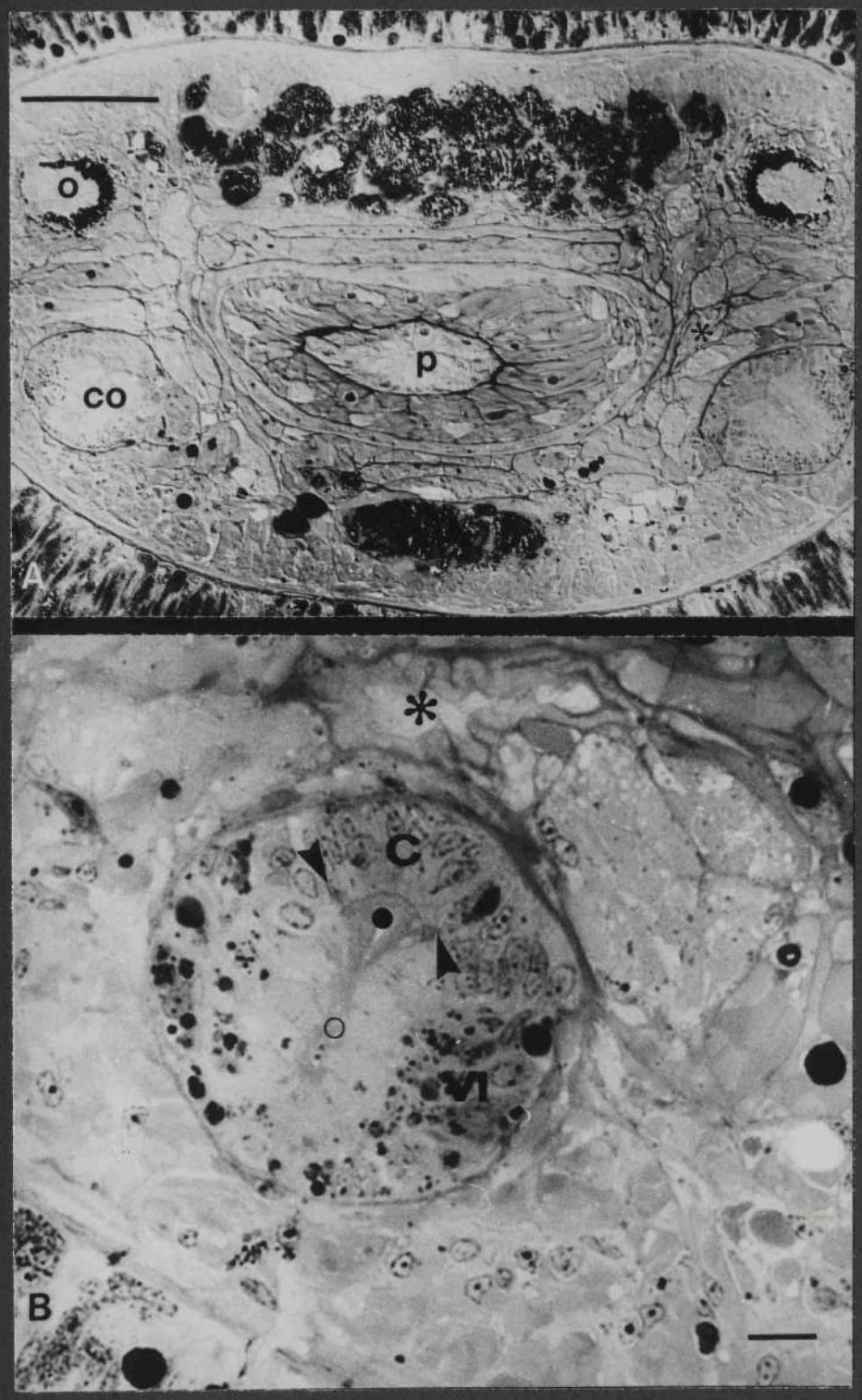

o
p
co
A
*
C
VI
B

Figure 13:

A. Diagram of a cross section through the shaft of a cilium (from Gibbons, 1961) showing the numbering system given to the nine axial microtubule pairs; according to Gibbons (1961) the basal foot corresponds in position to fibrils 5 and 6, and indicates the direction of the effective stroke. B, C and D. *Tetrastemma*; oblique sections through ciliated cells showing the orientation of ciliary axonemes relative to the basal foot (open arrows) and accessory striated rootlet (solid arrows); arrowheads indicate examples of cilia in which the pattern of axial tubules is clear and the 5 and 6 fibrils can be identified. Scale bars=1$\mu$m.

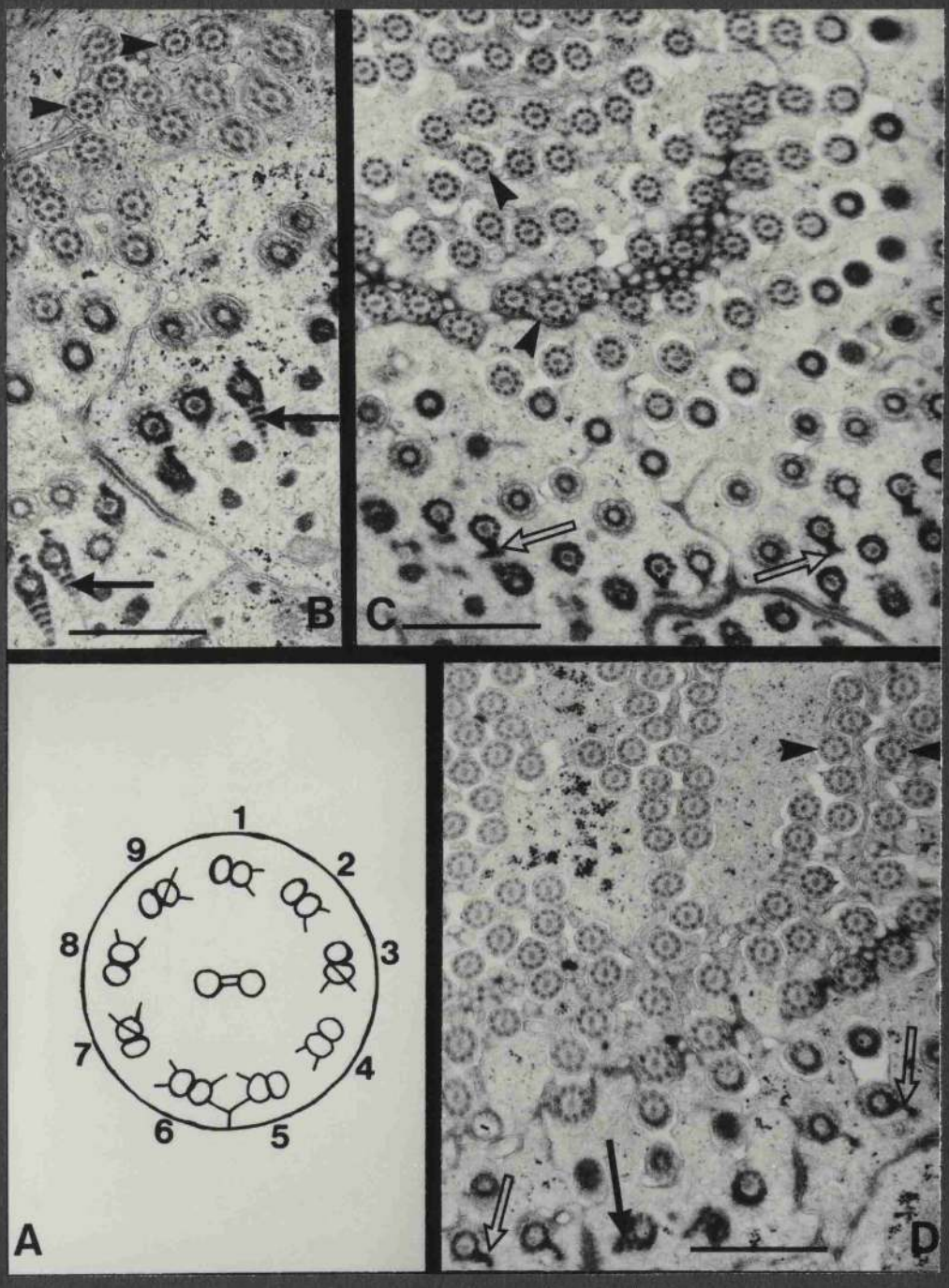

1
2
3
4
5
6
7
8
9
A
B
C
D

Figure 14: *Tetrastemma*;

A. Ciliated cells lining the minor canal; R=rootlet complex of cell separating the major and minor canal epithelia; C=ciliated cell. Scale bar=2μm.

B. Higher magnification of cell with well-developed rootlet complex (R) in A; open arrow=basal foot; solid arrow=accessory striated rootlet; n=nucleus of ciliated cell. Scale bar=1μm.

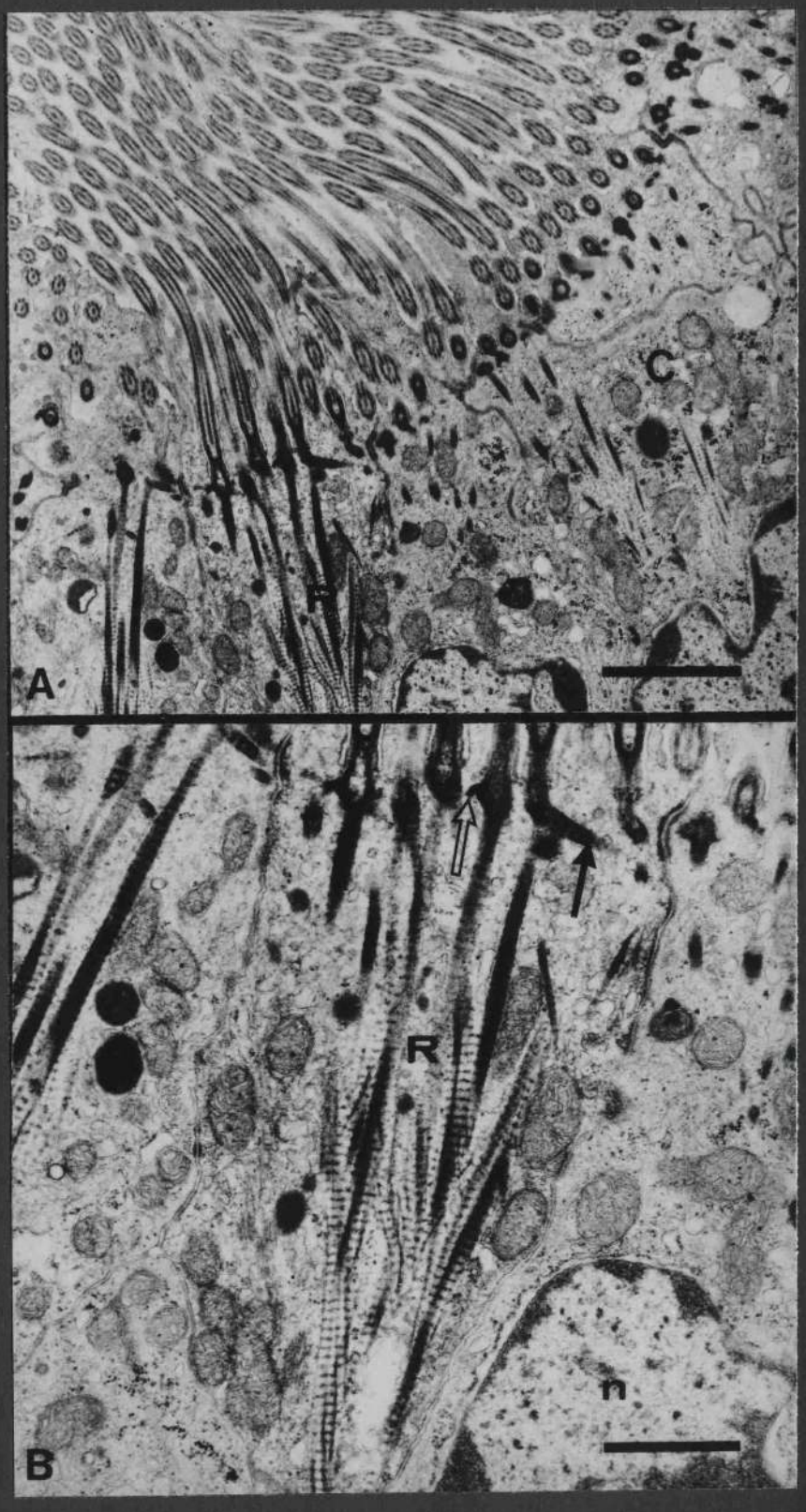

C
A
R
n
B

Figure 15: *Amphiporus*; lappet cells near the blind end of the canal. The cilia and microvilli of lappet cells produce a septum (S) which separates the major (MA) and minor (MI) canals; Arrows indicate Type 2 sensory cell dendrites; these form part of a continuous sensory epithelium, the bulk of which is not included in the section. Scale bar=3$\mu$m.

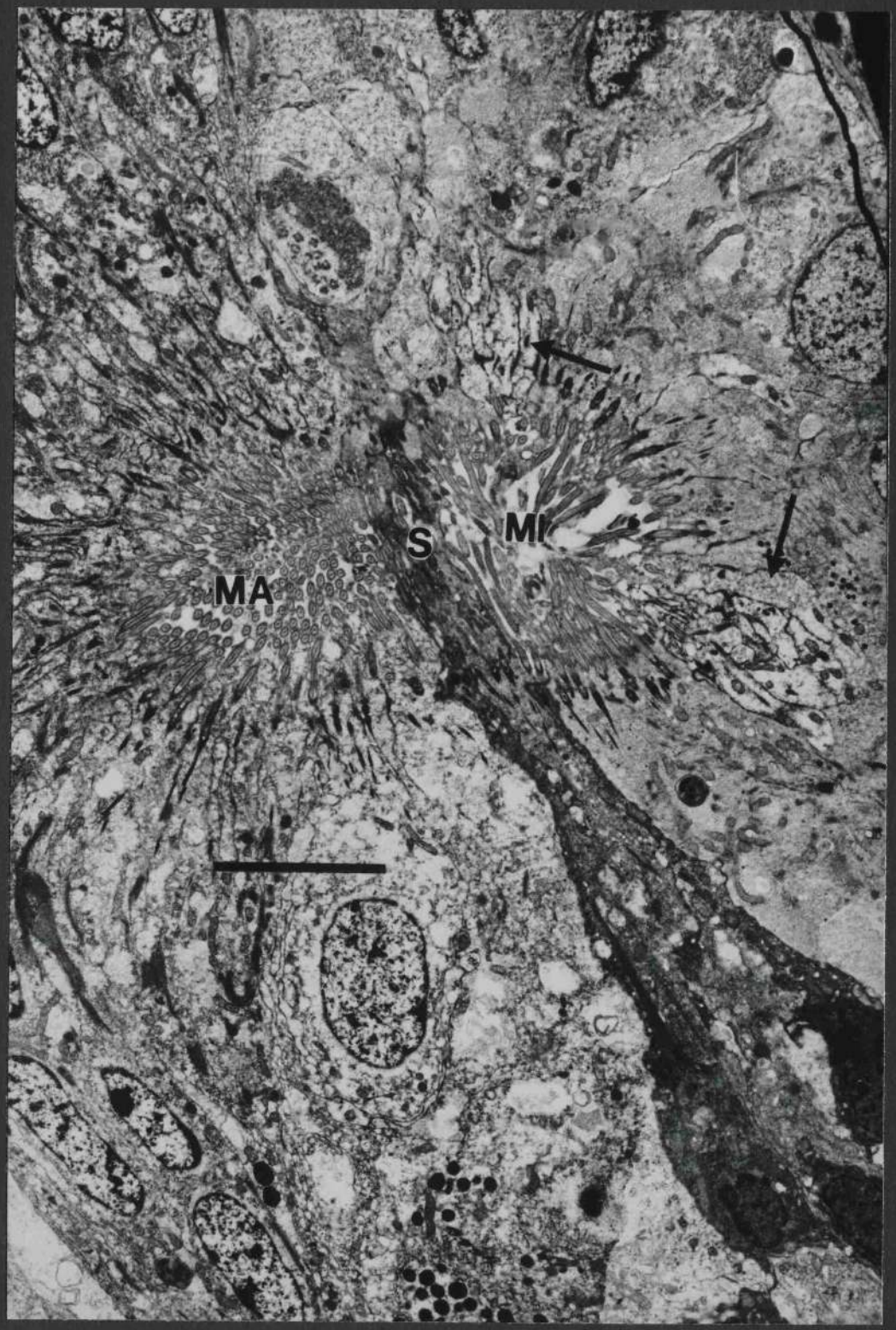

MA
S
MI

Figure 16: Nerve fibre containing small dense vesicles. The fibre is present among the ciliated cells of the minor canal epithelium; arrowheads indicate fibres or bundles of fibres; g=glycogen pool; ct=connective tissue capsule of the cerebral organ;

A. *Paranemertes*. Scale bar=2μm.

B. *Tetrastemma*. Scale bar=0.5μm.

C. *Amphiporus*. Scale bar=0.5μm.

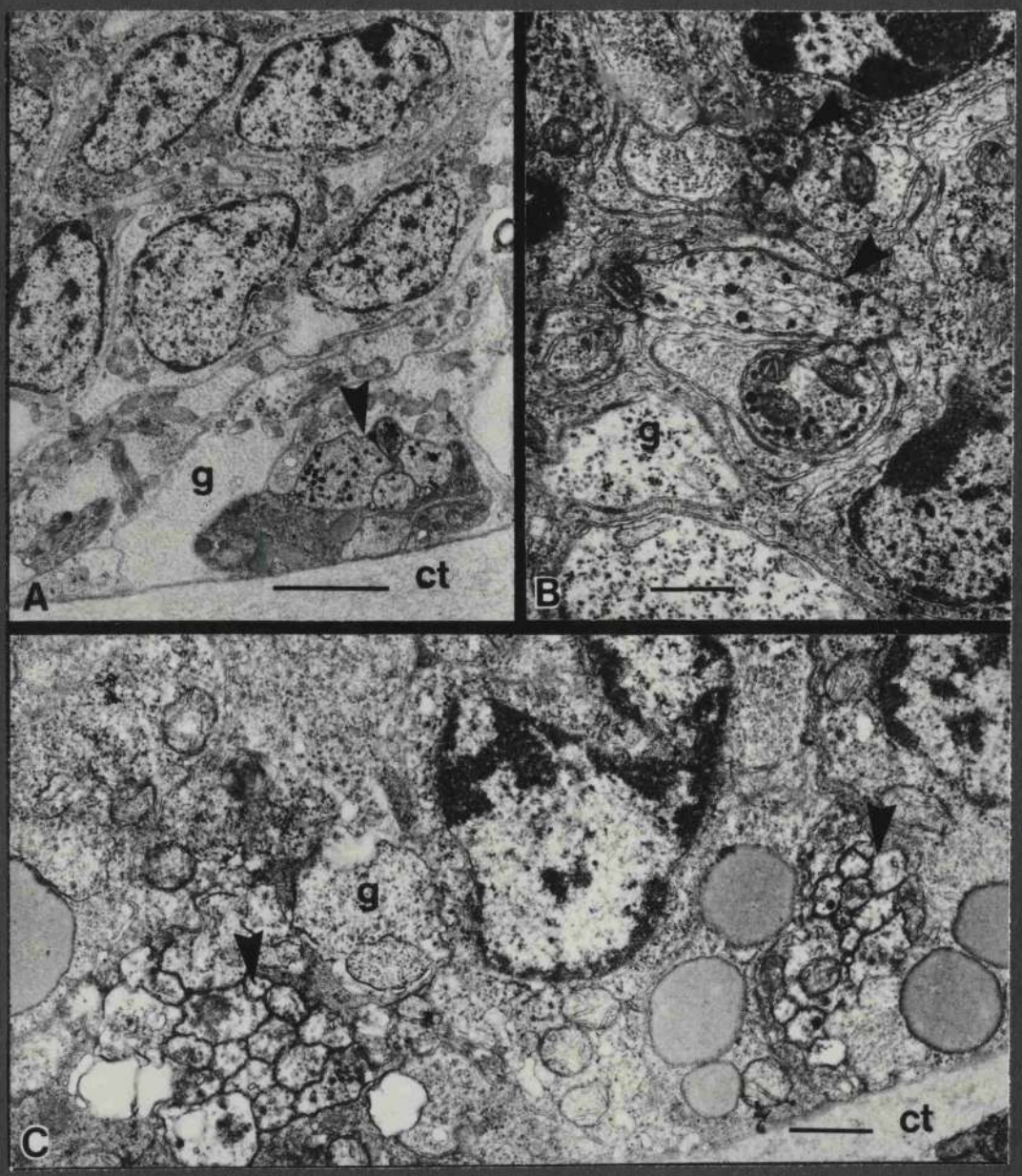

g
ct
A
g
B
g
ct
C

Figure 17: *Tetrastemma*; cross section through the cephalic canal showing the sensory epithelium made up of Type 1 sensory cell dendrites (some are indicated by arrowheads) and ciliated support cells. Ciliated support cells are strongly reinforced by bundles of microfilaments (inset; scale bar=500nm). The sensory-support cell epithelium lines the major canal (ma), and ciliated cells line the minor canal (mi). Arrows are in cells separating the major and minor canal epithelia, and indicate the approximate line of separation between the major and minor canals. The well-developed striated rootlet complexes of these barrier-forming cells are not visible in this section. Scale bar=3μm.

ma
mi

Figure 18: *Tetrastemma*; horizontal section through the cephalic canal at the same level as Figure 17. s=network of microvilli of Type 1 sensory cell dendrites (asterisks) on the major canal; c=cilia of ciliated cells lining the minor canal; note that the basal feet (arrowheads) of cilia on opposite sides of the canal point in opposite directions; n=nucleus of ciliated support cell. Scale bar=2μm.

Figure 19: *Tetrastemma*; Type 1 sensory cells and ciliated support cells (s). d=Type 1 sensory cell dendrites;
A. Section through the long axis of the support cells and dendrites; mv=microvilli of dendrites. Scale bar=750 nm.
B. Section through the apical ends of the support cells and dendrites; r=striated rootlet of support cell. Scale bar=500 nm.

p
p
p
p
s
s
A
BV

Figure 20: Type 1 sensory cell dendrites;

A and B. *Paranemertes*; note microtubules and vesicles in the dendrites; some of the vesicles are coated (arrowheads). Scale bars=300 nm.

C. *Amphiporus*; oblique section through the cephalic canal; s=ciliated support cells; asterisks=dendrites. Scale bar=2$\mu$m.

C
S
S
S
A
B

Figure 21: *Tetrastemma*;

A. Type 1 sensory cell bodies (sb) below the sensory-support cell epithelium (d); arrowheads indicate large dense bodies whose contents resemble Type 1 vesicular material. Scale bar=5μm.

B. Type 1 sensory cell bodies. Scale bar=1.5μm.

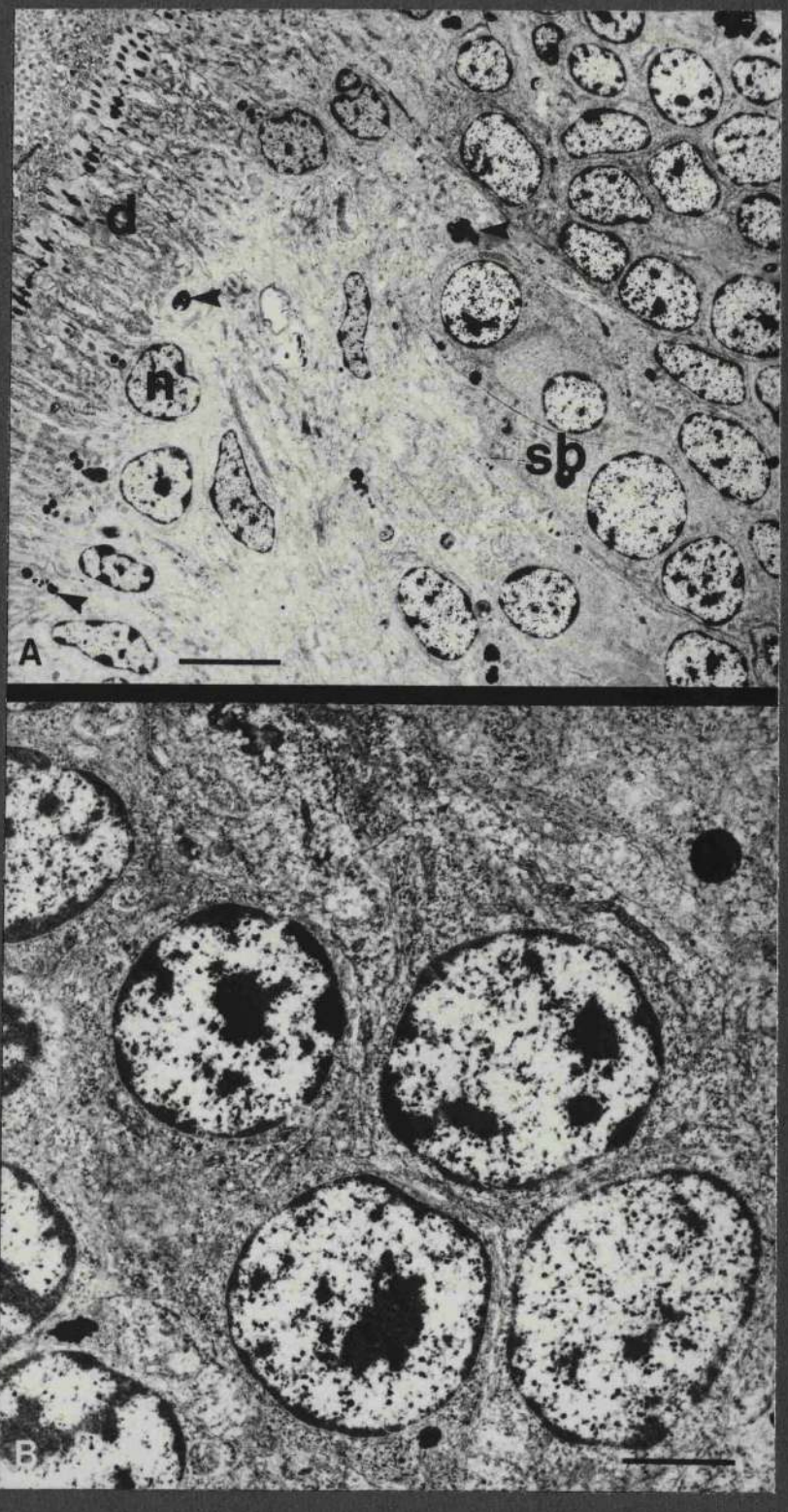

d
h
sb
A
B

Figure 22: *Amphiporus*; Type 1 sensory cell bodies; n=Type 1 sensory cell nucleus; arrowheads indicate processes of subepithelial support cells (=glial cells);

A. Cell bodies with little cytoplasm and poorly developed Golgi complex and endoplasmic reticulum. Scale bar=2$\mu$m.

B. Cell with large Golgi complex (g), many free ribosomes (r) and some smooth endoplasmic reticulum (s). Scale bar=1.5$\mu$m.

C. Greatly amplified smooth endoplasmic reticulum (s); p=glycogen pool. Scale bar=2$\mu$m.

D. Myelin figure (f) which may represent a degenerating Golgi complex. Scale bar=2$\mu$m.

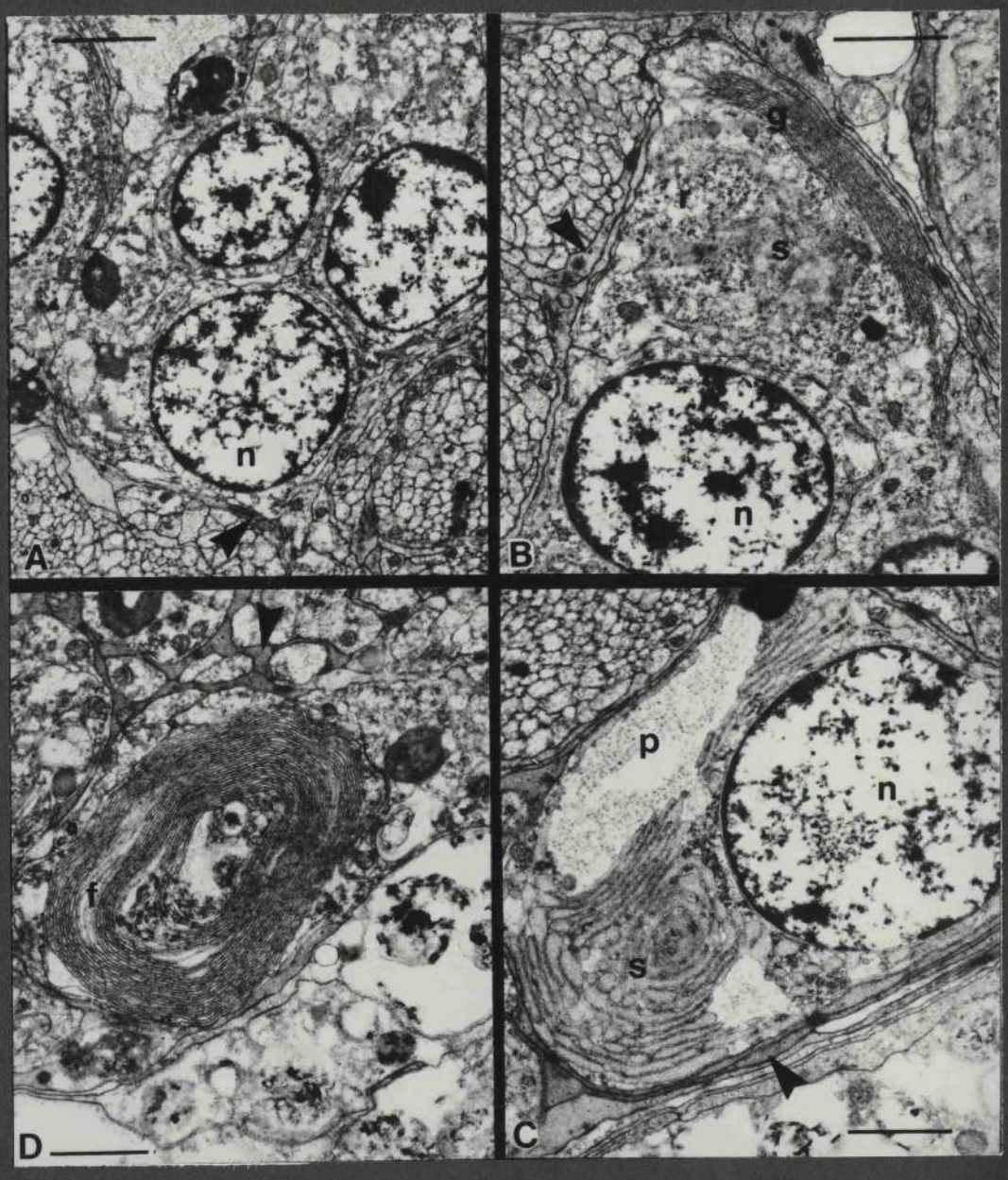

A
n
B
g
i
s
n
D
f
C
p
n
s

Figure 23: *Tetrastemma*;

A. Type 1 (S1) and Type 2 (S2) sensory cell bodies; note the greatly amplified smooth endoplasmic reticulum (s) and apparently deteriorating Golgi complex (g) in the Type 1 sensory cell; v=Type 2 vesicular material. Scale bar=3μm.

B. Higher magnification of the Type 1 sensory cell in A. Scale bar=1μm.

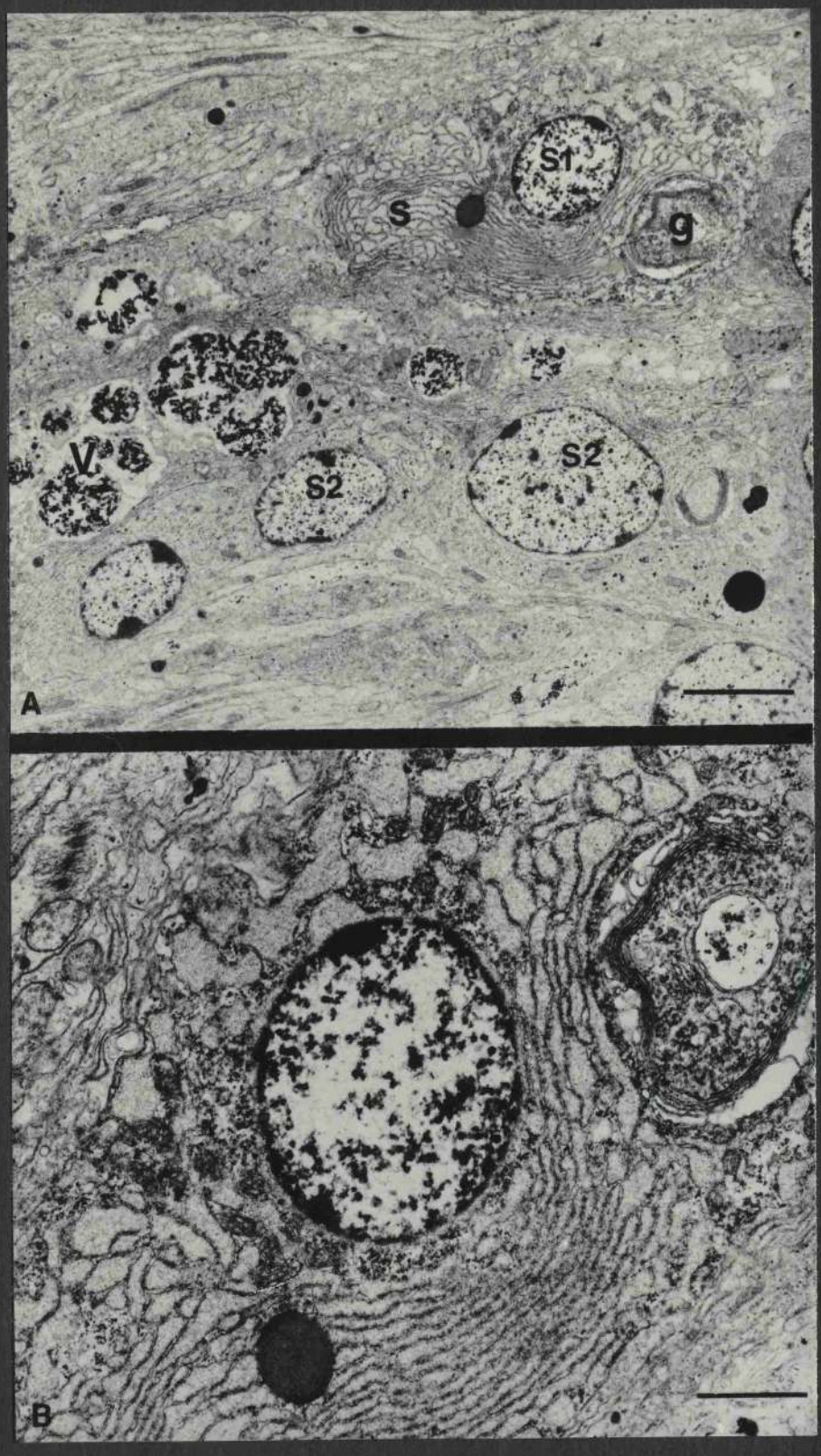

S1
S
g
V
S2
S2
A
B

Figure 24: Type 2 sensory cell dendrites;

A. *Tetrastemma candidum*

B. *T. melanocephalum*. Scale bars=250 nm.

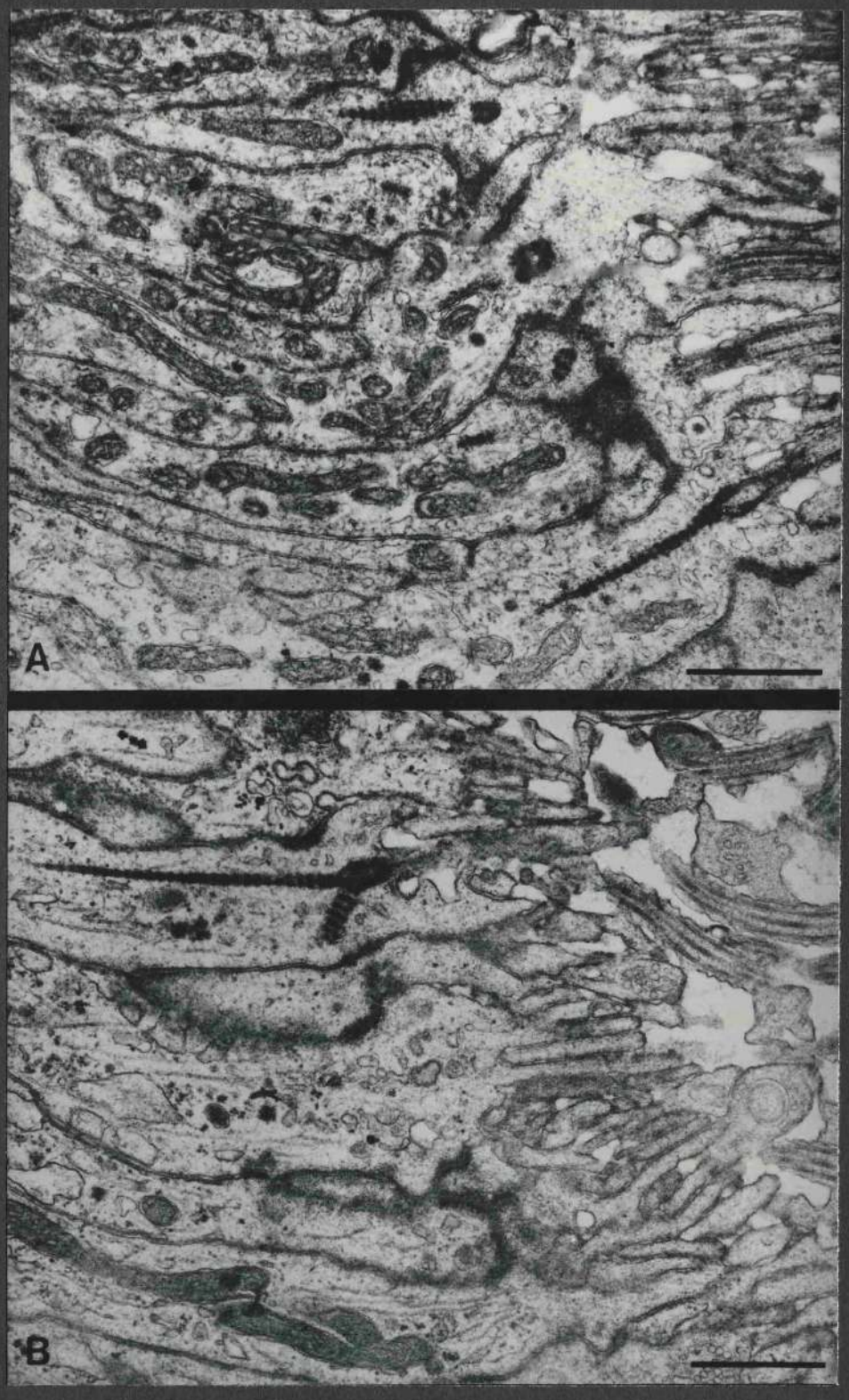

A
B

Figure 25: *Paranemertes*; Type 2 sensory cell dendrites;

A. Scale bar=1.5μm.

B. Scale bar=500 nm.

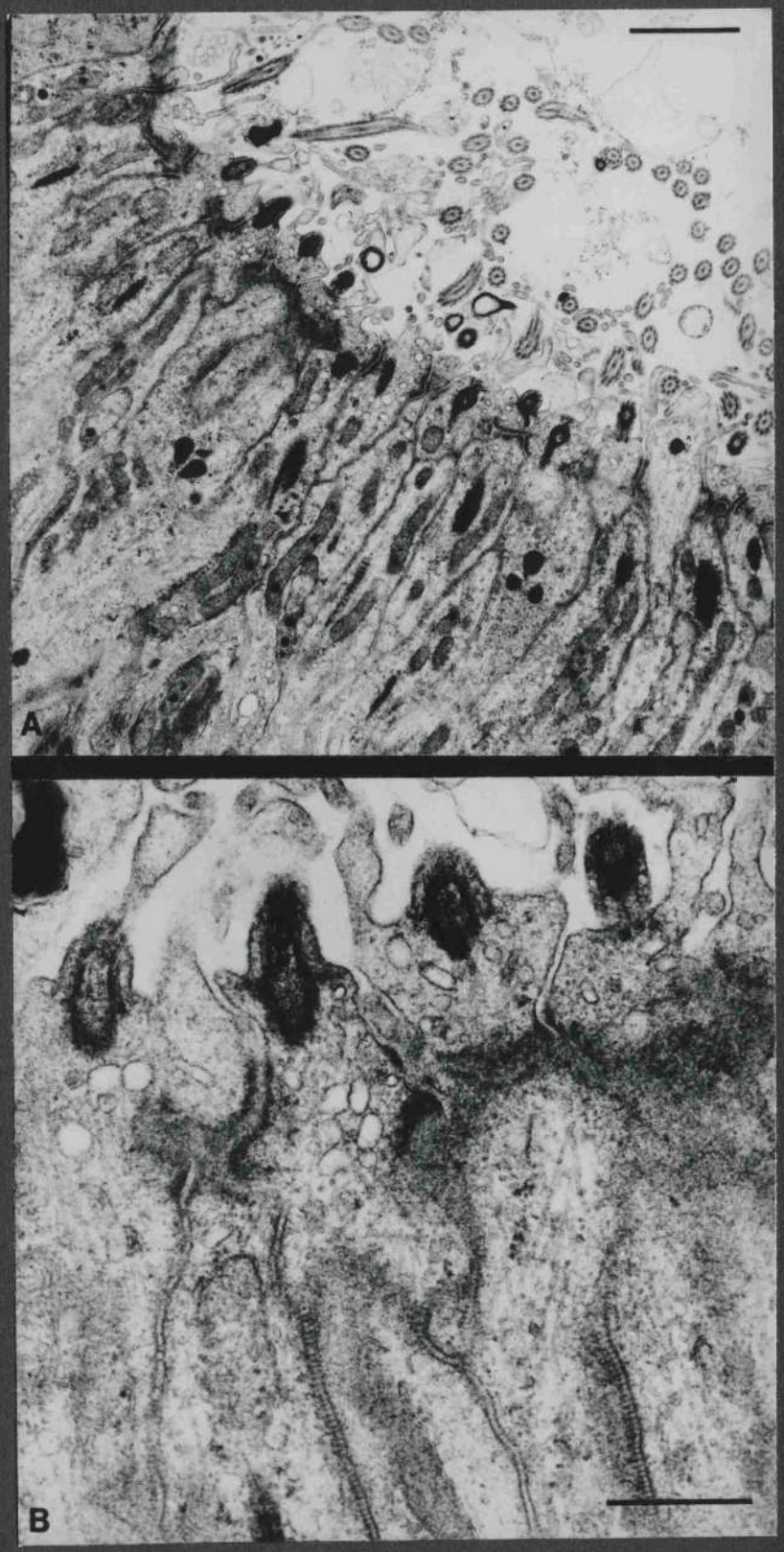

A
B

Figure 26: *Tetrastemma*;

A. Cross section through part of the cerebral organ nerve; arrowheads=subepithelial support cell processes. Scale bar=1.5μm.

B. Light micrograph of a horizontal section through the cerebral organ nerve showing the contribution of Type 1 (1) and Type 2 (2) sensory cells; V=Type 2 vesicular material. Scale bar=20μm.

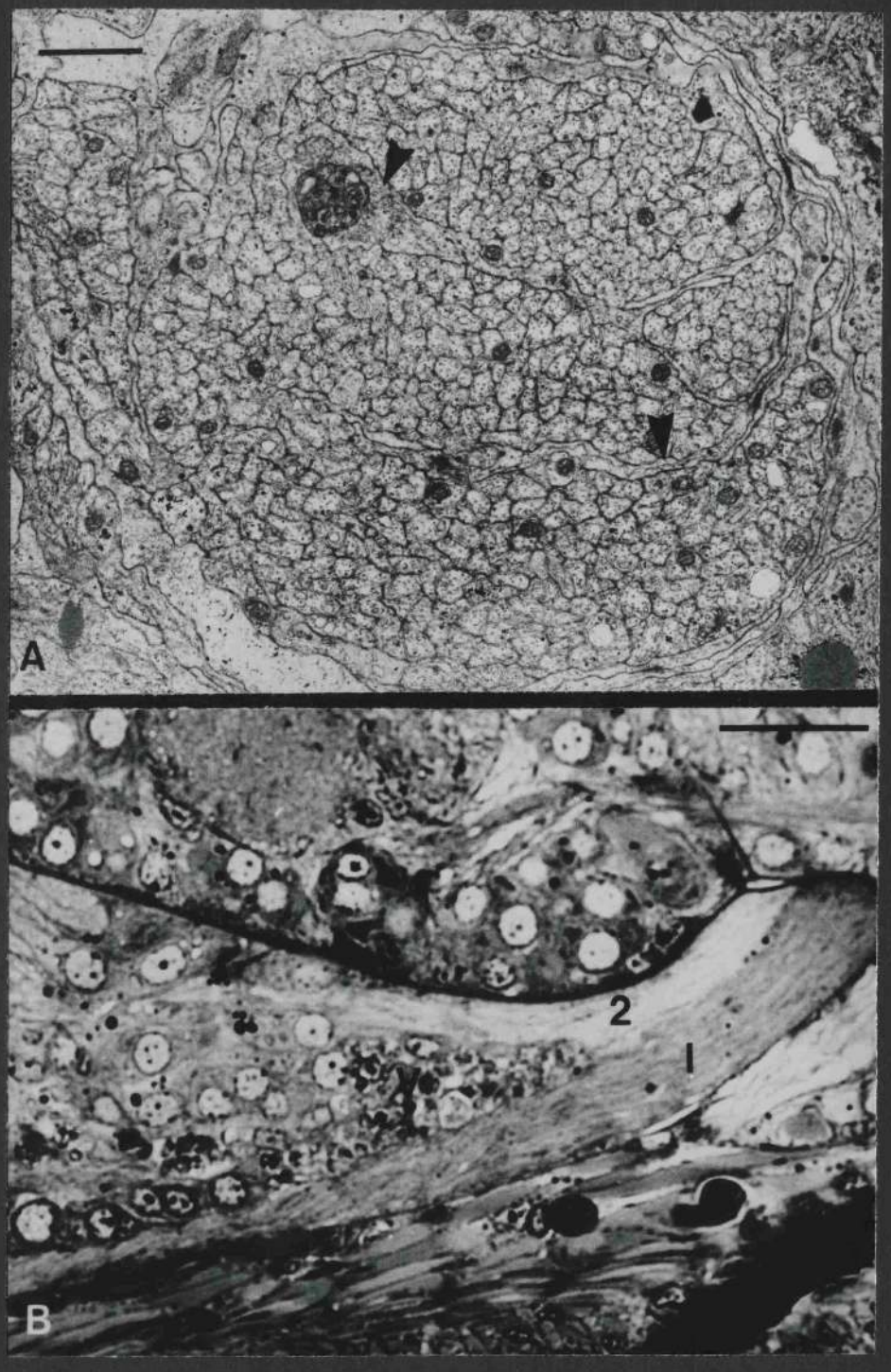

A
2
1
B

Figure 27: *Paranemertes*;

Cross section through part of the cerebral organ nerve. n=nuclei of subepithelial support cells; Scale bar=5μm.

u
u

Figure 28: *Tetrastemma*;

Type B gland cell; er=rough endoplasmic reticulum; g=Golgi complex; N=nucleolus. Scale bar=1.5μm.

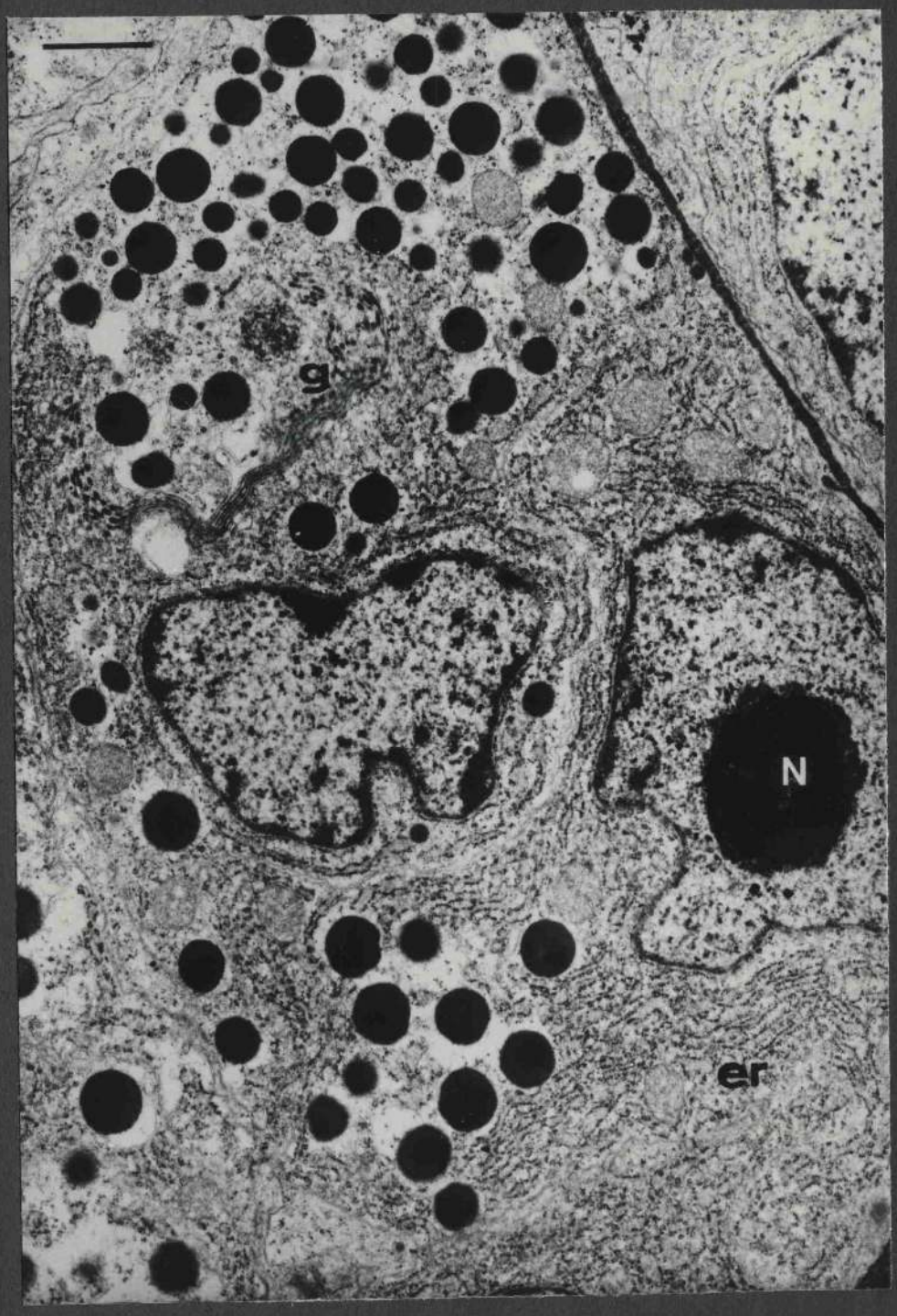

g
N
er

Figure 29: *Paranemertes*; synthesis of secretion granules in gland cells;

A. Type A gland cell; arrows indicate vesicles containing dense material which appear to be in the process of being released by the Golgi complex; c=condensing vacuole; n=gland cell nucleus; f=microfilament bundles inside sub=epithelial support cells - the membranes separating these processes from the gland cell are not visible because of the angle of section. Scale bar=2.5μm.

B. Type B gland cell; er=rough endoplasmic reticulum; sv=zone containing small clear vesicles which separates the endoplasmic reticulum from the Golgi complex; c=condensing vacuoles; large arrowheads=coated vesicles; small arrowheads=clear, uncoated vesicles within the area surrounded by Golgi complex. Scale bar=2μm.

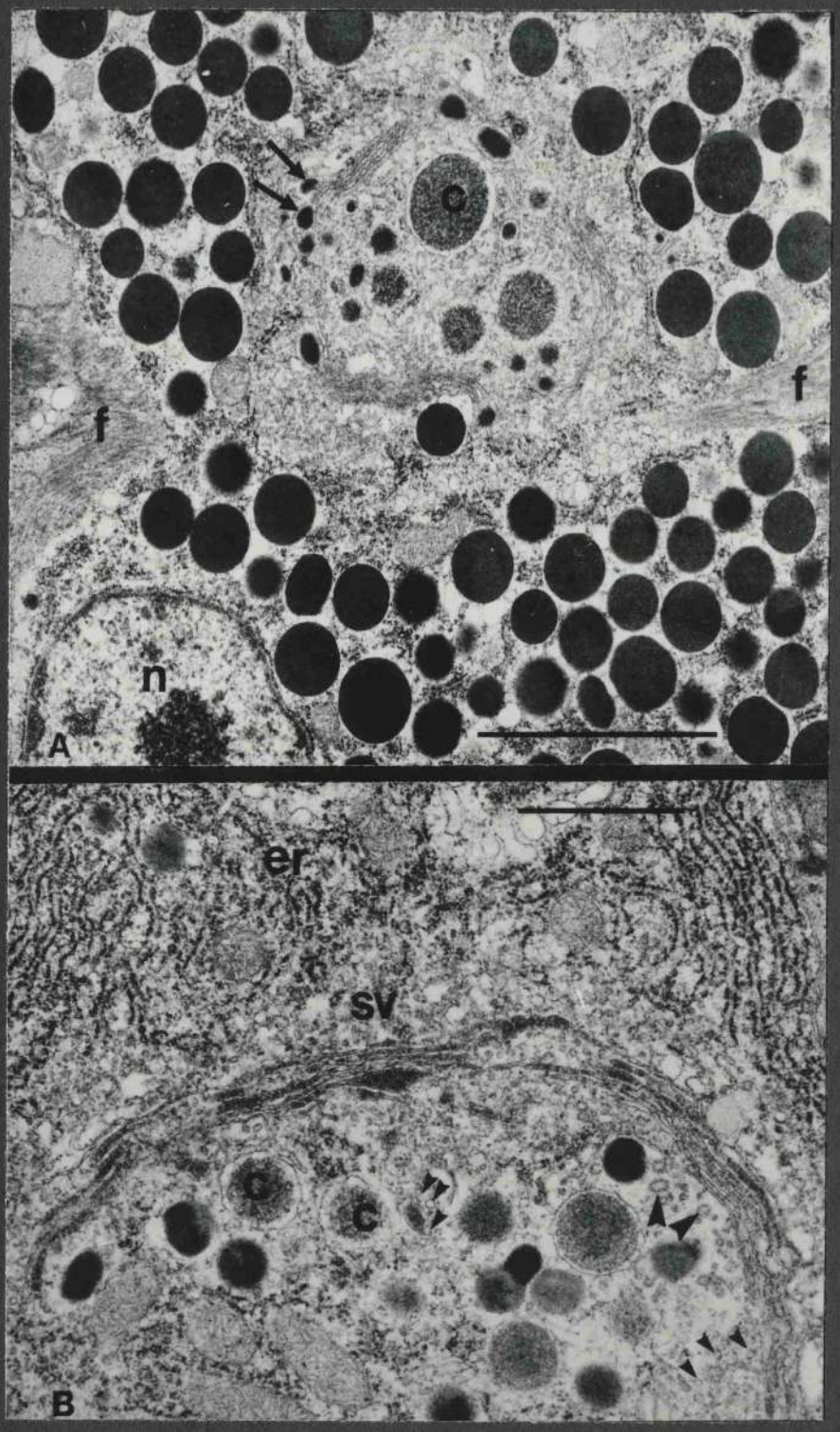

c
f
f
n
A
er
sv
c
c
B

Figure 30: Mature secretion granules in gland cells;

A. *Amphiporus*; secretion granules in Type A (A) and Type B (B) gland cells; note the difference in morphology of secretion granules in the two types of gland cell; V=Type 2 vesicular material; f=microfilaments in subepithelial support cell process; arrow=Type B granule containing crystalline structure. Scale bar=1.5μm.

B. *Tetrastemma*; Type A gland cells containing mature secretion granules; arrowhead=area of synthesis; Scale bar=1.5μm.

V
B
A
A
B

Figure 31: Gland cell processes;

A. *Paranemertes*; process of Type B gland cell showing coated pits at the apical surface. Scale bar=350 nm.

B and C. *Tetrastemma*;

B. Type A gland cell process showing the cilium; r=rootlet of the cilium; g=secretion granule. Scale bar=1$\mu$m.

C. Type A gland cell processes among ciliated cells of minor canal. Scale bar=3$\mu$m.

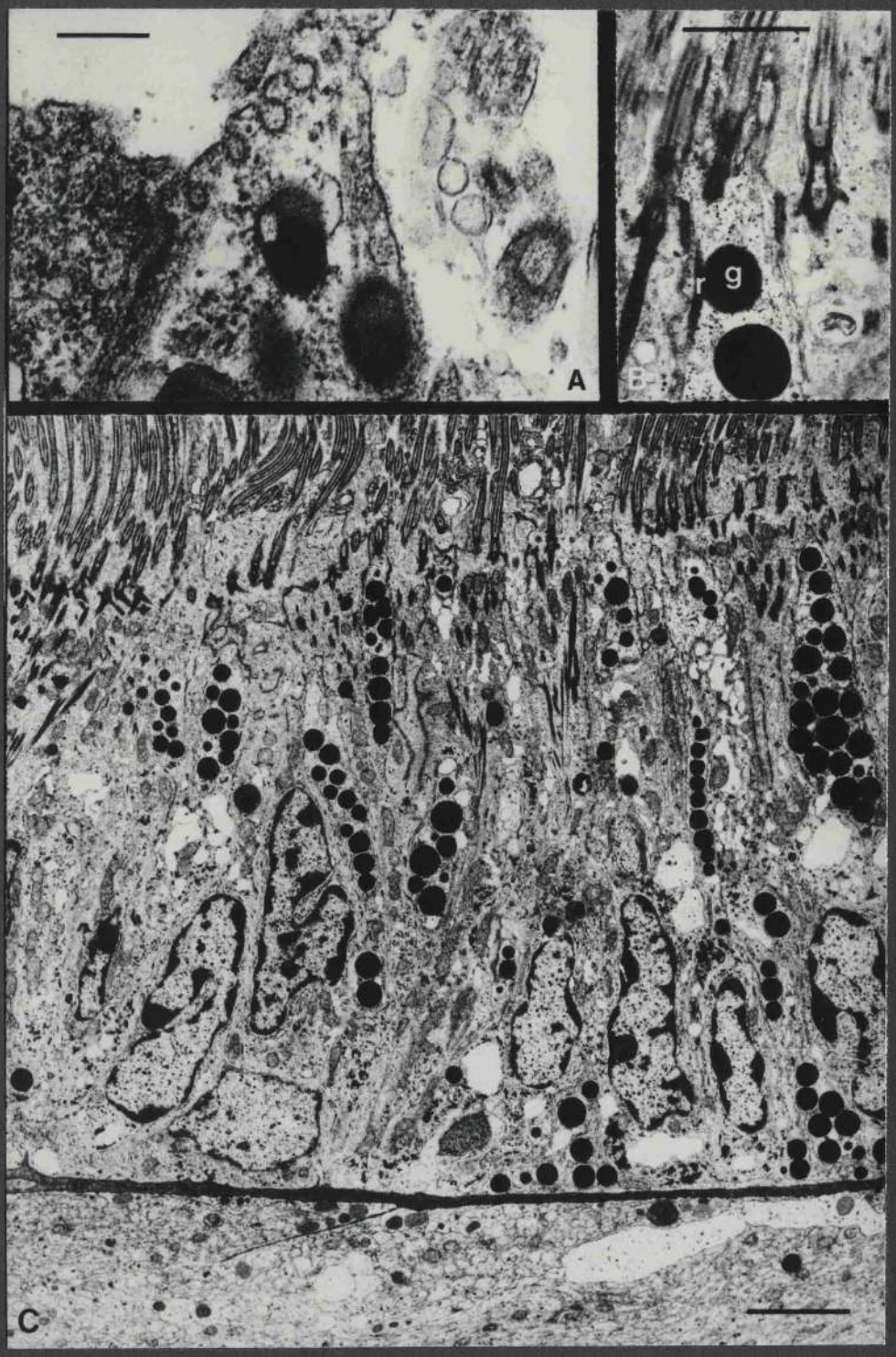

A
r
g
B
C

Figure 32: *Tetrastemma*;

Type 1 vesicular cells; L=cilia in the canal lumen; ER=rough endoplasmic reticulum; G=region of Golgi complexes; b=connective tissue capsule of the cerebral organ. The vesicular material consists of a large number of vesicles of different sizes; size 1 vesicles fill the apical cytoplasm (1), and are too small to be visible at this magnification; size 2 - 5 vesicles are indicated by numbers. In the upper left corner of the figure, the beginning of the Type 1 sensory cell - ciliated support cell epithelium is visible. Scale bar=4$\mu$m.

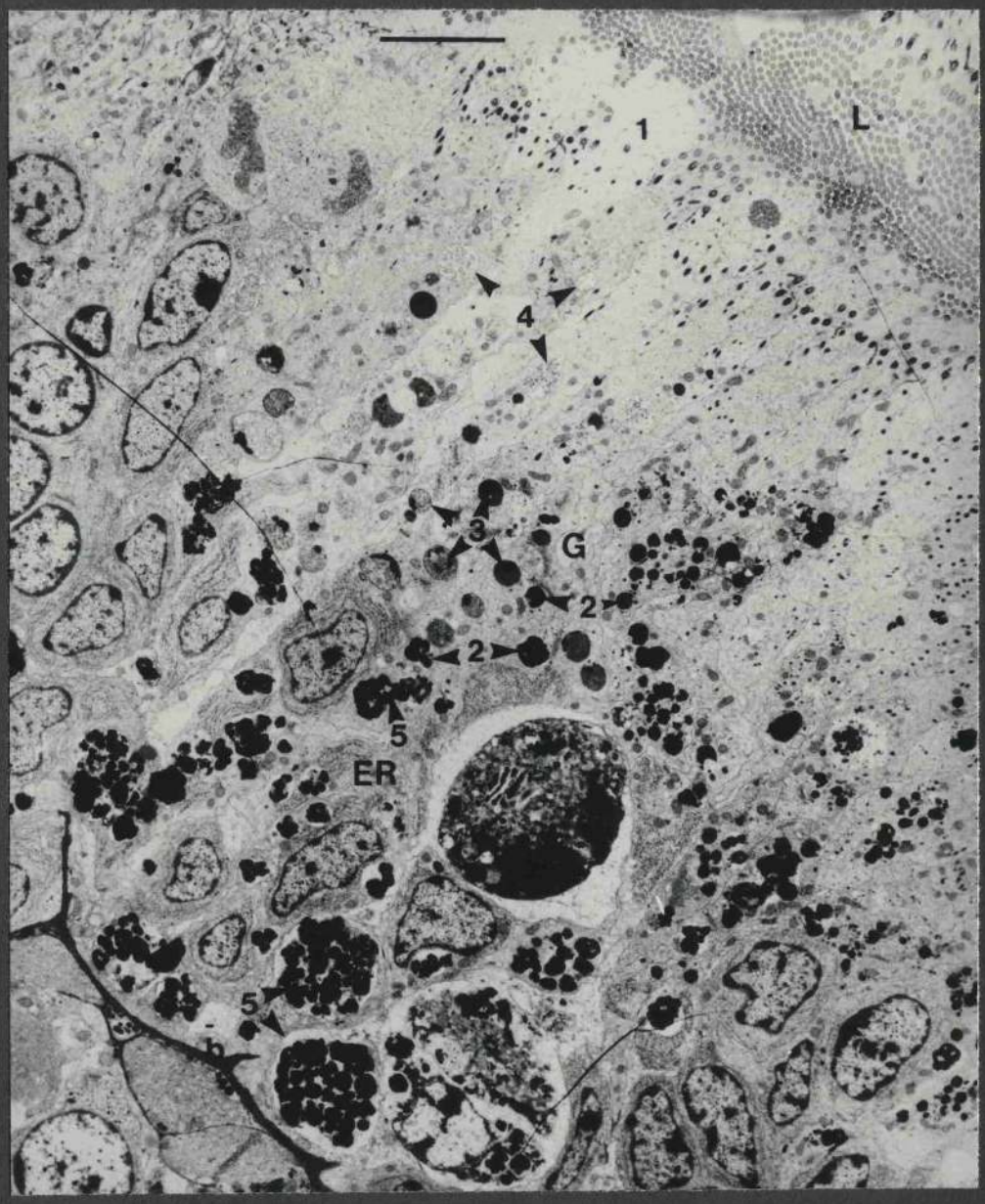

1
L
4
3
G
2
2
5
ER
5
b

Figure 33: Type 1 vesicular cells; Apical cytoplasm containing size 1 vesicles; arrowheads=coated vesicles;

A. *Paranemertes*. Scale bar=500 nm.

B. *Tetrastemma candidum*; asterisks=coated pits. Scale bar=200 nm.

C. *T. melanocephalum*. Scale bar=1μm.

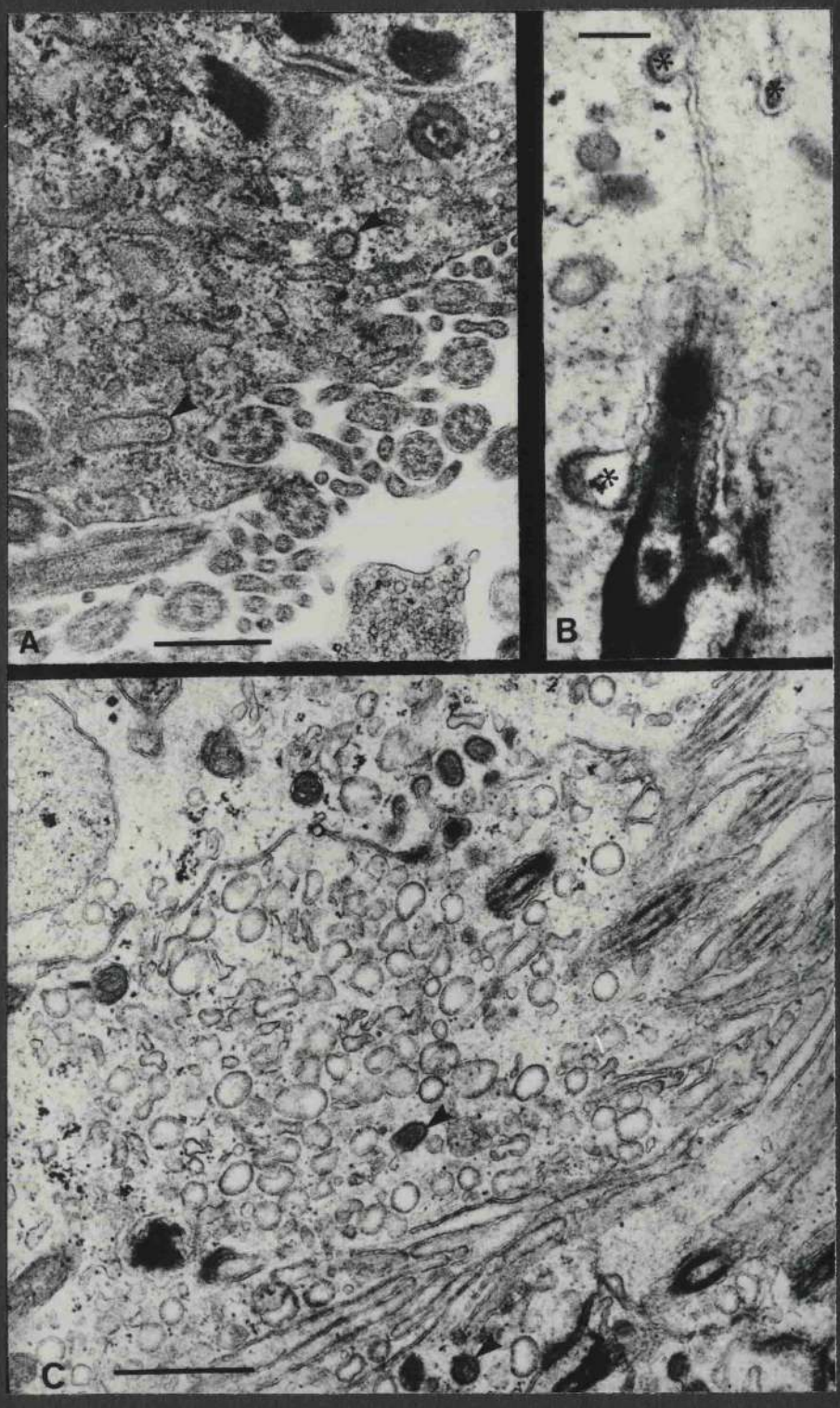

A
B
C

Figure 34: *Tetrastemma*; Type 1 vesicular cells;

A. Golgi complex (g) in the mid-region of the cells near size 3 and size 4 vesicles. Scale bar=1μm.

B. Small vesicles (size 2) with dense, heterogeneous contents, and larger vacuoles (size 5) containing aggregations of size 2 vesicles. Scale bar=1μm.

4
g
3
A
2
5
5
B

Figure 35: *Tetrastemma*; Type 1 vesicular cells;

A. Small residual bodies (size 2 vesicles) in the basal region of the cells; er=endoplasmic reticulum. Scale bar=1$\mu$m.

B. Large vacuoles (size 5) containing aggregations of size 2 vesicles. Scale bar=1$\mu$m.

a
A
1e

Figure 36: *Paranemertes*;

A. Type 2 vesicular cell containing many large vacuoles filled with heterogeneous dense material; S2=Type 2 sensory cell bodies; arrowheads=processes of subepithelial support cells. Scale bar=4μm.

B. Higher magnification of the apical cytoplasm of the Type 2 vesicular cell in A. The cilia and striated rootlets are characteristically haphazardly arranged; note that the apical cytoplasm is filled with small clear vesicles; g=Golgi complex. Scale bar=1μm.

S2
S2
A
g
g
B

Figure 37: *Amphiporus*; Subepithelial Type 2 vesicular cells; A and B. Type 2 vesicular cells filled with vesicular material; there is very little cytoplasm in these cells, and the empty spaces suggest that the cells may be swollen; N=nuclei of Type 2 vesicular cells; arrowheads=processes of subepithelial support cells.

A. Scale bar=3μm.

B. Scale bar=1μm.

C. Type 2 vesicular material in which there appear to be incompletely degraded secretion granules (arrows) similar to those in gland cells. Scale bar=2.5μm.

D. Higher magnification of the granules (arrows) in C. The intact membrane around these granules is one of several indications that this kind of cell arises through transformation of a gland cell into a vesicular cell by breakdown of secretion granules and synthetic apparatus (see also Figures 38, 39 and 40). Scale bar=1μm.

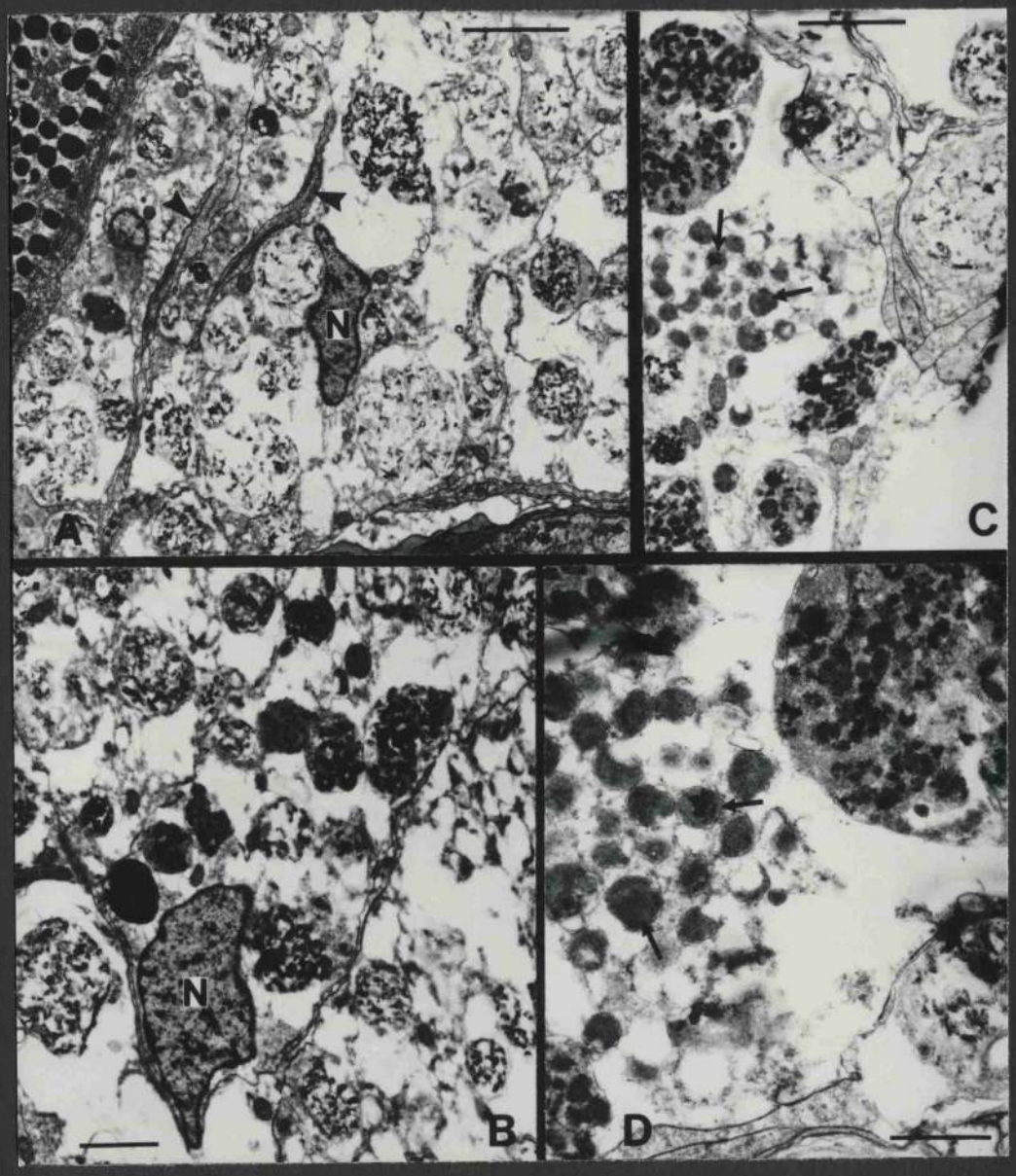

A
N
C
B
N
D

Figure 38: *Tetrastemma*; Type 2 vesicular material within gland cells. Scale bars=1.5μm.

A. Small patch of Type 2 vesicular material (asterisk) in a gland cell.

B. Degradation of secretion granules to Type 2 vesicular material within a lysosome;

C. Further stage of breakdown of secretion granules to Type 2 vesicular material.

A
B
C

Figure 39: *Paranemertes*; degradation of secretion granules to Type 2 vesicular material within gland cells;
A. In Type A gland cells the initial stage in degradation of secretion granules is often the formation of a large globule of coalescing secretion granules (arrow). Scale bar=3μm.
B. In Type B gland cells degradation of secretion granules is indicated by the appearance of lysosome-like dense bodies (arrow). Scale bar=1.5μm.

A
B

Figure 40: *Amphiporus*; Type 2 vesicular material within gland cells;

A. Type B gland cell in which the secretion granules and much of the synthetic apparatus is being degraded in lysosomes; N=nucleus of gland cell. Scale bar=3$\mu$m.

B. Higher magnification of part of the cell in A, showing lysosomes containing mitochondria, rough endoplasmic reticulum and secretion granules. Scale bar=750 nm.

B
A
N

Figure 41: *Paranemertes*

A. Type 2 vesicular cell near the connective tissue capsule of the cerebral organ (arrowheads). Scale bar=1μm.

B and C. Extracellular Type 2 vesicular material surrounded by gland cells.

B. Scale bar=3μm.

C. Scale bar=500 nm.

A
B
C

Figure 42: *Paranemertes*;

A. Subepithelial support cell processes (arrowheads) surrounding a Type 2 vesicular cell near the connective tissue capsule of the cerebral organ. Scale bar=1μm.

B. Nucleus of a subepithelial support cell and support cell process (arrowheads) containing dense vesicles. Scale bar=2μm.

C. Cephalic blood vessel (CV) adjacent to the cerebral organ; arrowheads indicate processes of subepithelial support cells; N=gland cell nucleus. Scale bar=5μm.

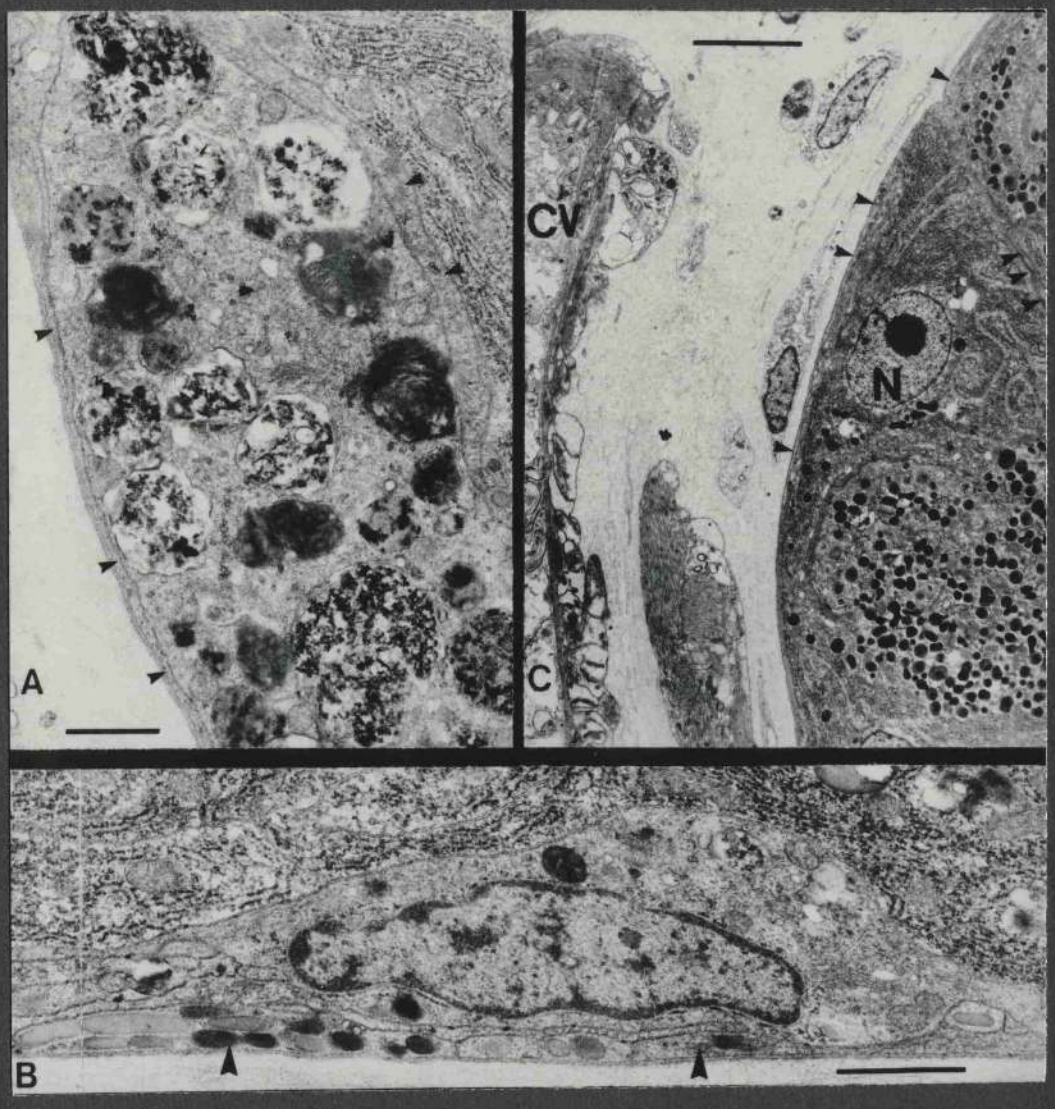

A
CV
N
C
B

Figure 43: *Paranemertes*;

Subepithelial support cell processes (arrowheads) associated with gland cells (G) sensory cells (S) and vesicular cells (V). Scale bar=5μm.

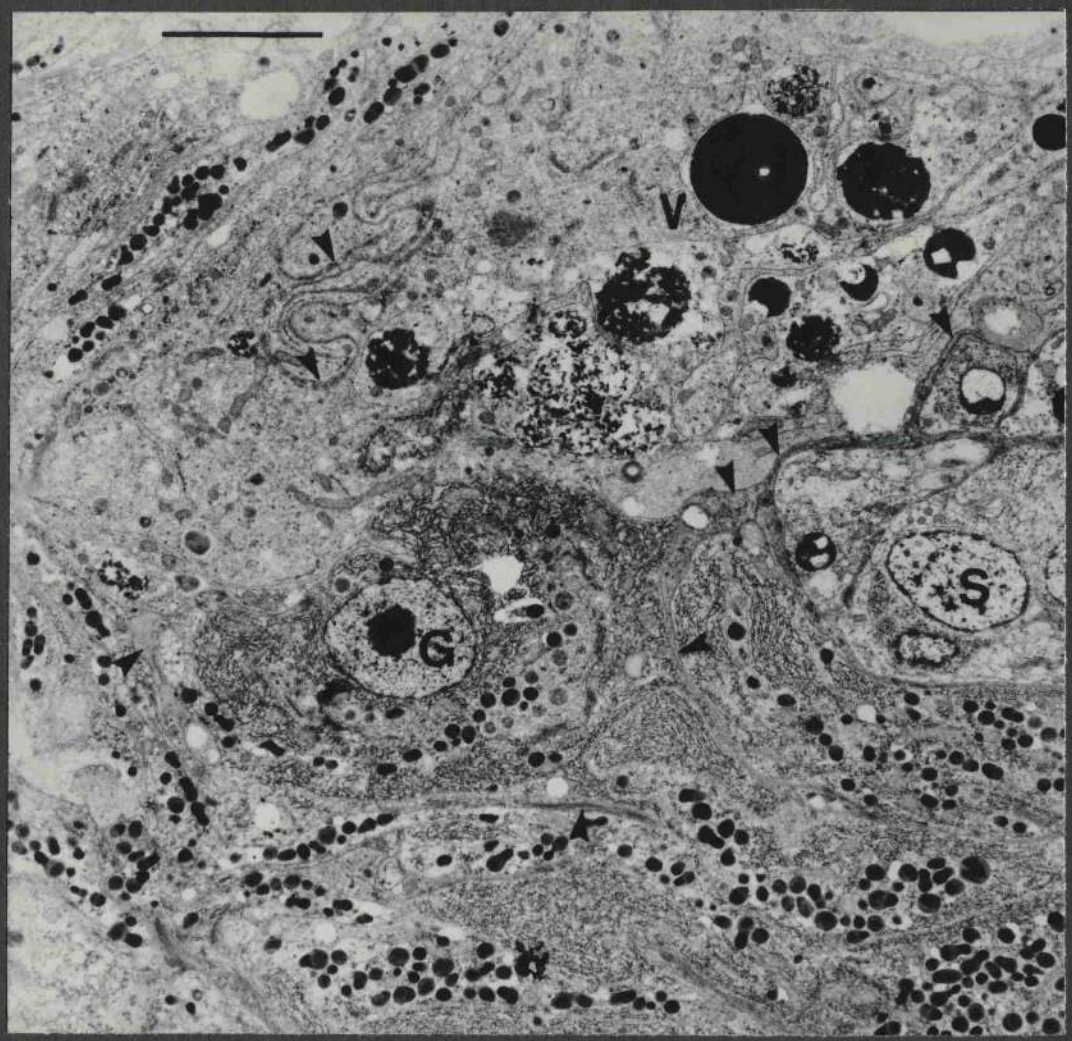

V
G
S

Figure 44: Diagram of the cerebral organ of *Paranemertes* illustrating the direction of flow through the cephalic canal based on the orientation of ciliary basal feet in cells lining the canal.

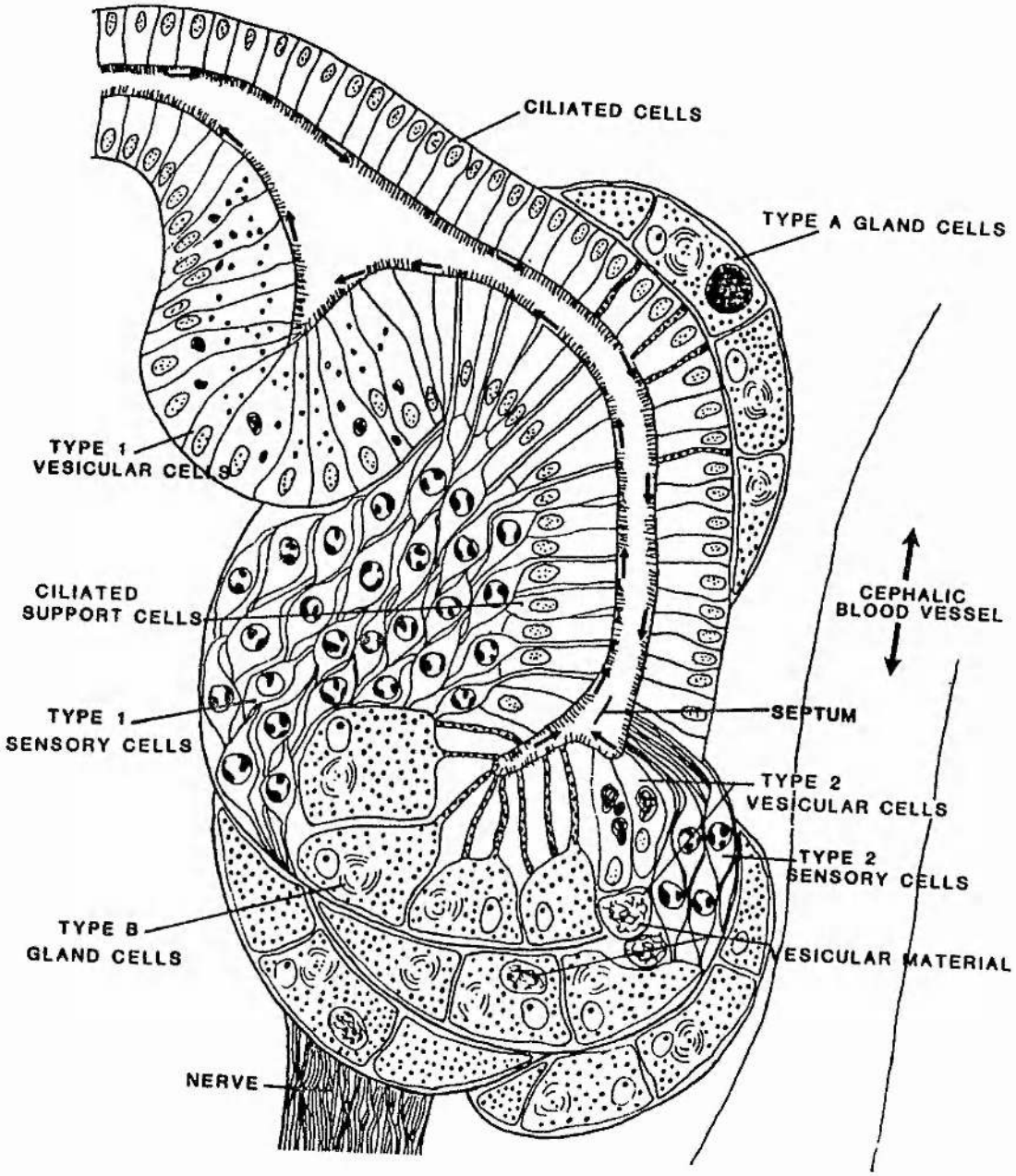

CILIATED CELLS
TYPE A GLAND CELLS
TYPE 1
VESICULAR CELLS
CILIATED
SUPPORT CELLS
CEPHALIC
BLOOD VESSEL
TYPE 1
SENSORY CELLS
SEPTUM
TYPE 2
VESICULAR CELLS
TYPE 2
SENSORY CELLS
TYPE B
GLAND CELLS
VESICULAR MATERIAL
NERVE

Figure 45: The mucous trail of *Paranemertes*.

Figure 46:

A. Ventral view of the head of *Paranemertes*. Arrows indicate the position of opening of the cephalic canals.

B. Dissection of the head of *Paranemertes*. The left cerebral organ (triangle), cerebral organ nerve (arrows) and dorsal cerebral ganglion (CG) are indicated. The cerebral organ nerve is about 20 μm in diameter.


A

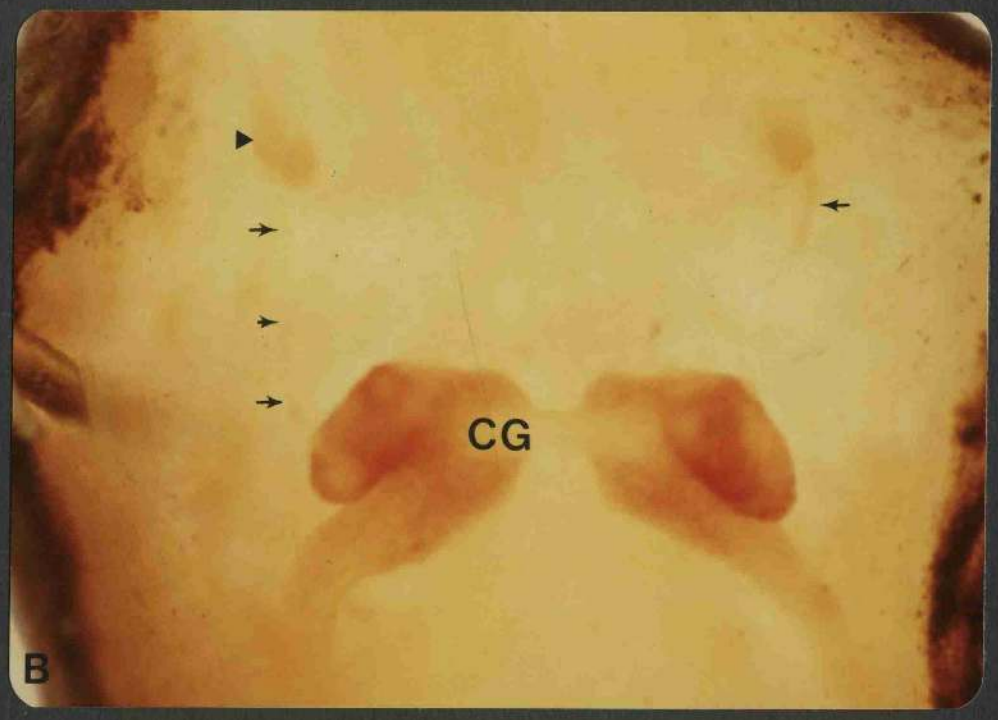

CG
B

Figure 47: Spontaneous activity recorded from the lateral nerve cords of *Paranemertes*.

A. Recordings from the exposed lateral nerve cord; connections with the brain intact.

B. Recordings from the isolated lateral nerve cord.

Vertical scale=10 μV; horizontal scale=10 sec.

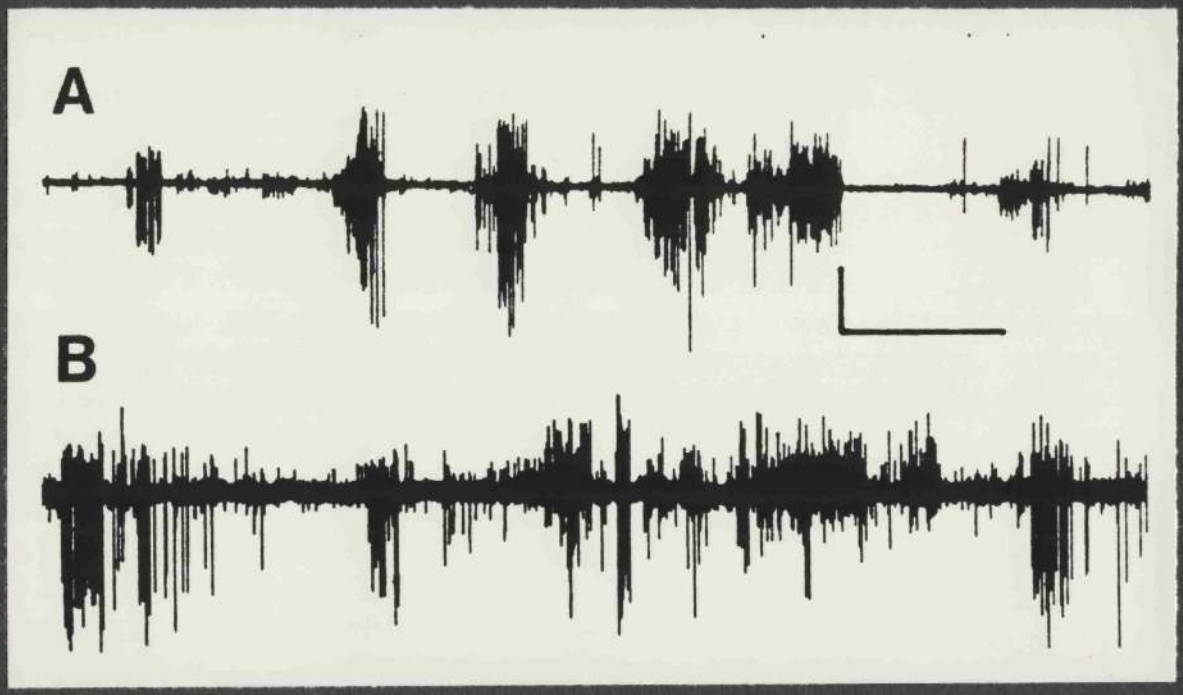

A
B

Figure 48: Responses to electrical stimulation in *Paranemertes*.

A. Change in amplitude of the compound potential with increasing stimulus intensity. Stimulating and recording electrodes on the lateral nerve cord. Vertical scale=200 μV; horizontal scale=10 msec.

B. Responses to stimulation of a preparation in $MgCl_2$. Stimulating and recording electrodes on the lateral nerve cord. Vertical scale=20 μV; horizontal scale=10 sec.

C. Recordings from the lateral nerve cord (upper trace) and adjacent muscles (lower trace) in response to a 35 V, 1 msec duration stimulus of the lateral nerve cord. Vertical scale=200 μV; horizontal scale=10 msec.

D. Recording from the bodywall musculature during stimulation of the lateral nerve cord (upper trace) and stimulation of the muscles directly (lower trace). Scale is the same as in A and C.

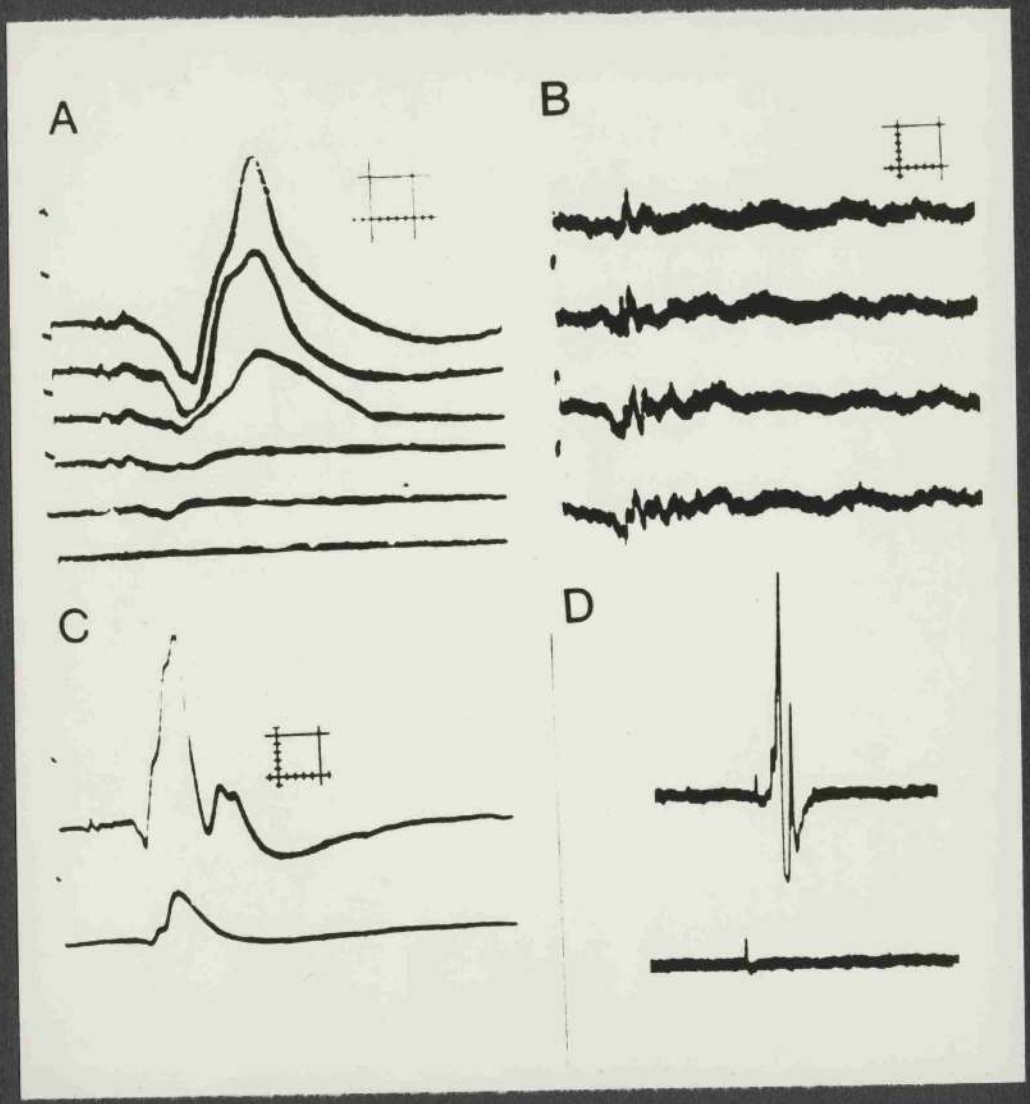

A
B
C
D

Figure 49: Neuromuscular facilitation in *Paranemertes*.

Vertical scale=10 μV; horizontalscale=10 sec.

A. Response to two shocks administered 500 msec apart. Stimulating electrode on the lateral nerve cord, recording electrode on the bodywall muscles.

B. Responses to alternating twin (arrows) and single (triangles) pulses; twin pulses were 20 msec apart, and stimuli were administered at 1 sec intervals.

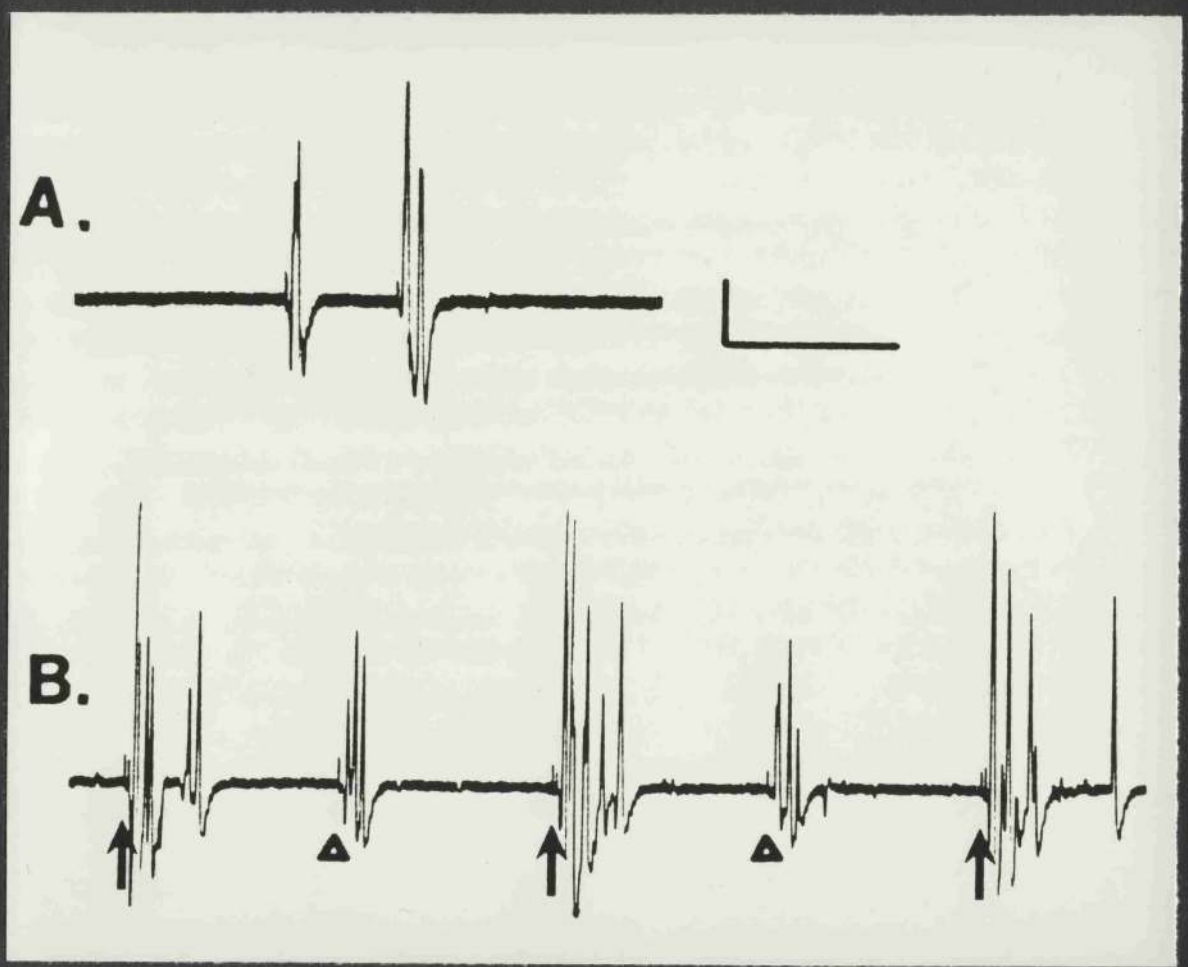

A.
B.

Figure 50: Responses to tactile stimuli recorded in the lateral nerve cord of *Paranemertes*.

Vertical scale=10 μV; horizontal scale=10 sec.

A. Responses from a preparation with an intact nervous system.

B. Responses from a preparation where the brain commissures had been cut.

C. Responses from a decapitated worm.

CA=contralateral anterior stimulation;

LA=ipsilateral anterior stimulation;

CP=contralateral posterior stimulation;

IP=ipsilateral posterior stimulation.

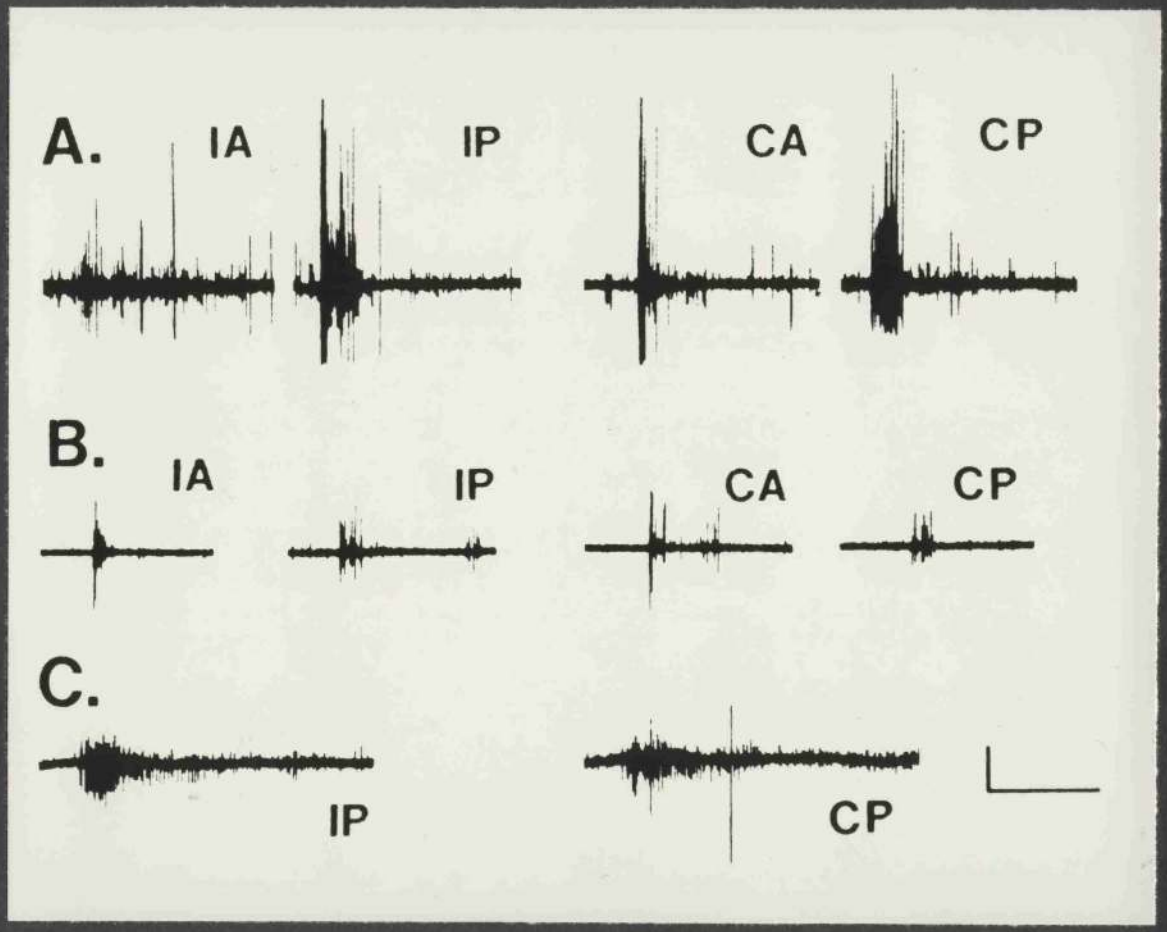
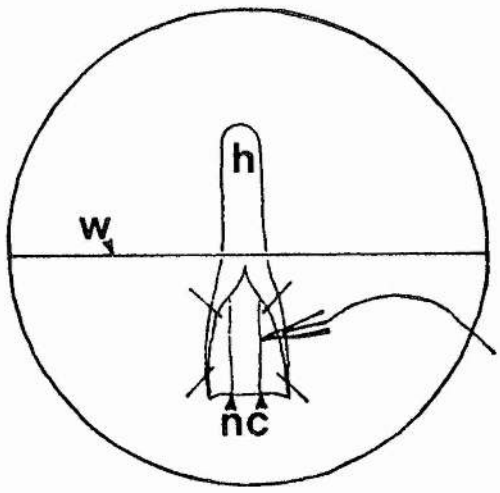

A.
IA
IP
CA
CP
B.
IA
IP
CA
CP
C.
IP
CP

Figure 51:

A. Diagram of the preparation used to isolate the exposed lateral nerve cords (nc) from the intact head (h) of the worm. The barrier (w) is a piece of dental wax sealed to the Sylgard base in the dish with vaseline; there is a small notch cut in the wax where the worm passes under it.

B. Diagram of the preparation used to isolate the exposed cerebral organ nerve from the opening to the cephalic canal. Stimuli were applied inside the chamber (t) enclosed by a piece of tygon tubing which was pinned and sealed with vaseline to the Sylgard base in the dish; the dissected head, with cerebral organ nerve exposed, is pinned over a hole in the tubing such that the cephalic canal opens into the chamber (t); the arrowhead indicates the position of opening of the cephalic canal.

A




B

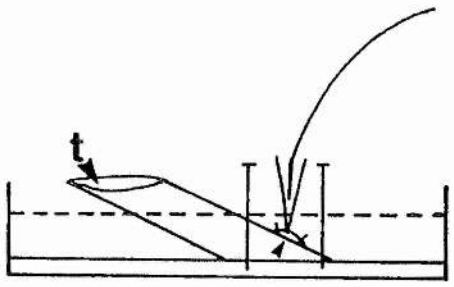


Figure 52: Responses of the lateral nerve cord to stimuli delivered to the undissected head of *Paranemertes*. Vertical scale=50μV; horizontal scale=12 sec.

A. Response to a drop of *Podarke pugettensis* extract. The two traces are continuous.

B. Response to contact with a live *Podarke*. Curved arrows delimit the time of contact.

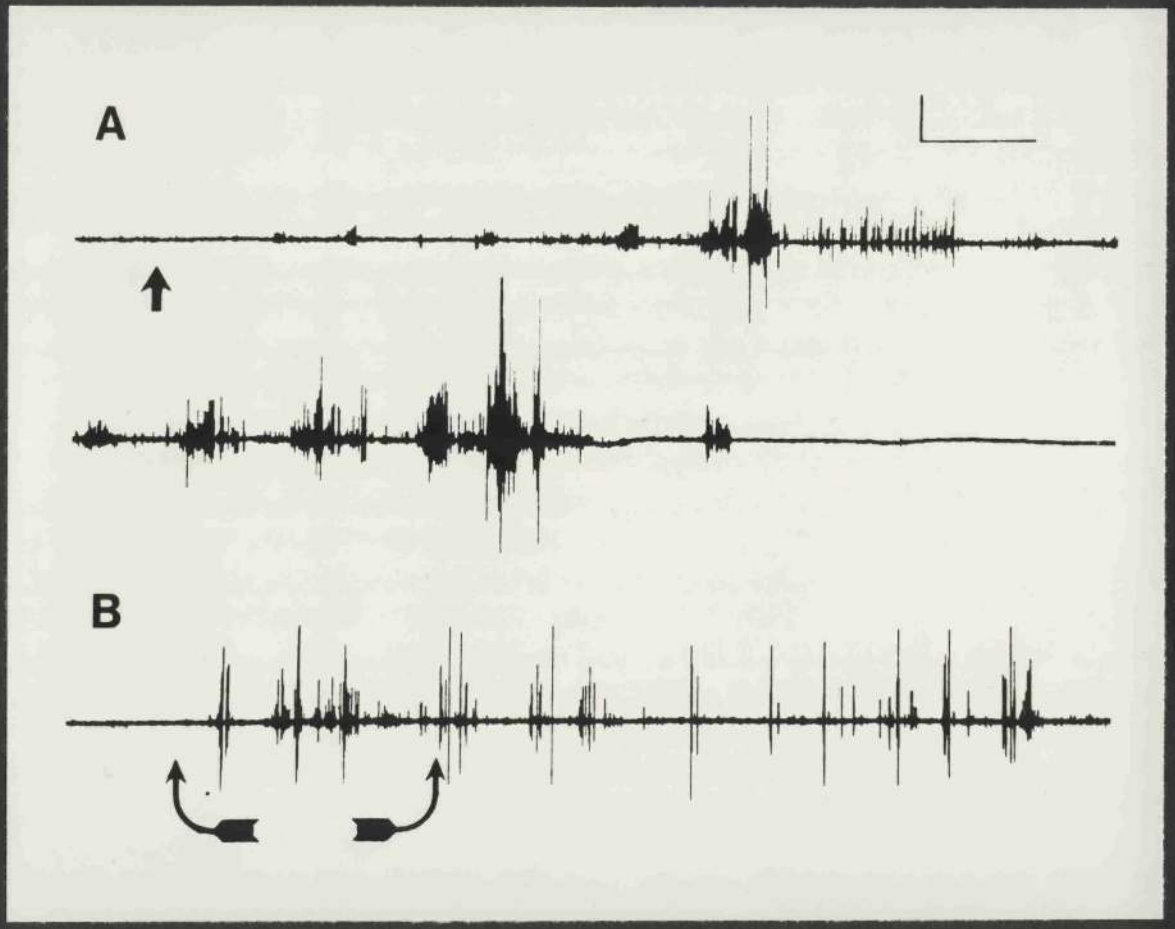

A
B

Figure 53: Recordings from the cerebral organ nerve of *Paranemertes*.

Vertical scale=20 μV; horizontal scale=10 sec.

A. No response was recorded to a small amount (about 1 ml) of sea water.

B. Response to distilled water (about 0.5 ml).

C. Response to prey extract (about 0.5 ml).

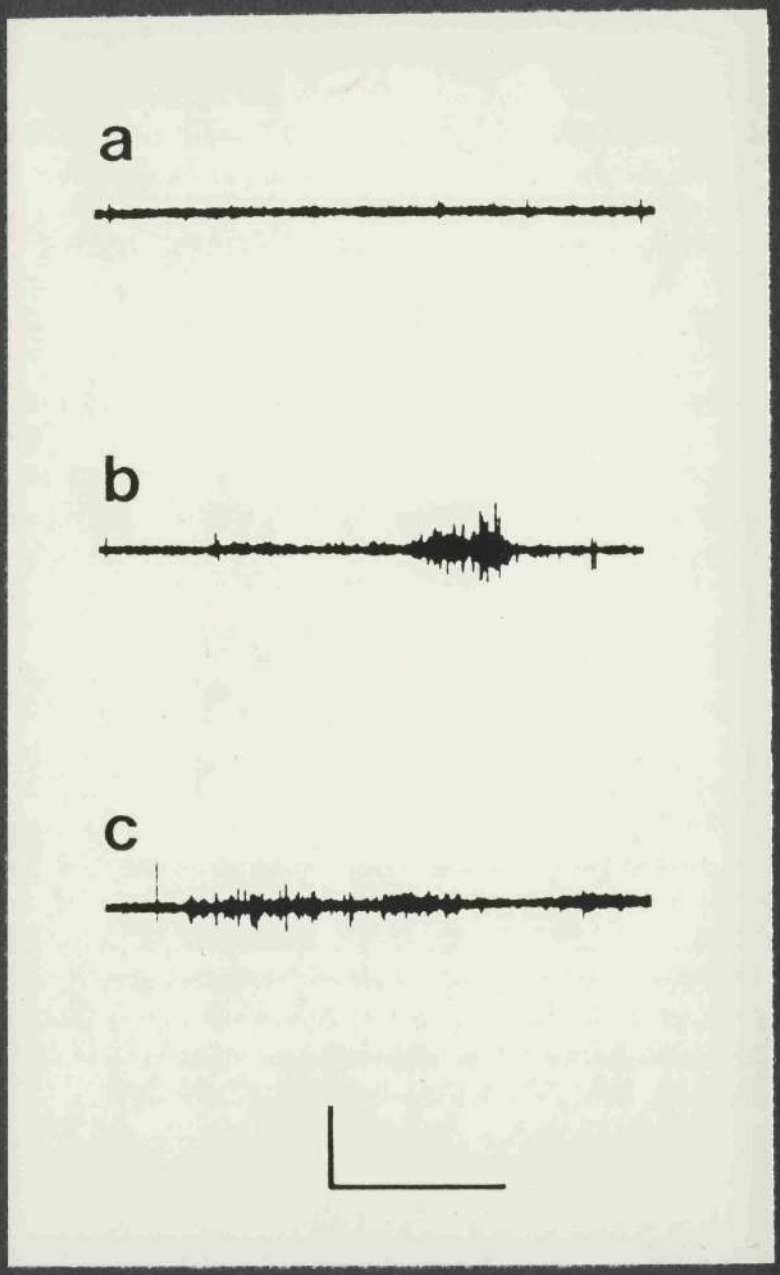

a
b
c

Figure 54: The effect of immersion in dilute sea water on the cytology of the cerebral organs of *Paranemertes*.

A. Type 1 vesicular cells fixed after exposure to 75% sea water for one hour; mi=minor canal; ma=major canal; arrowheads indicate dark granular vesicular material. Scale bar=20 μm.

B. and C. Type B gland cells after exposure to 75% sea water for six hours; asterisks indicate vesicular material within gland cells; arrowhead indicates a globule of coalesced secretion product. Scale bar=8 μm.

D. Type B gland cells (g) and Type 2 vesicular material (asterisks), after exposure to 50% sea water for six hours; cg=cephalic glands. Scale bar=8 μm.

E. Cross section through the posterior part of the cerebral organ, fixed after 24 hours exposure to 75% sea water; c=canal; g=Type B gland cells; asterisk indicates Type 2 vesicular material; arrowheads indicate the connective tissue capsule of the cerebral organ. Scale bar=20 μm.

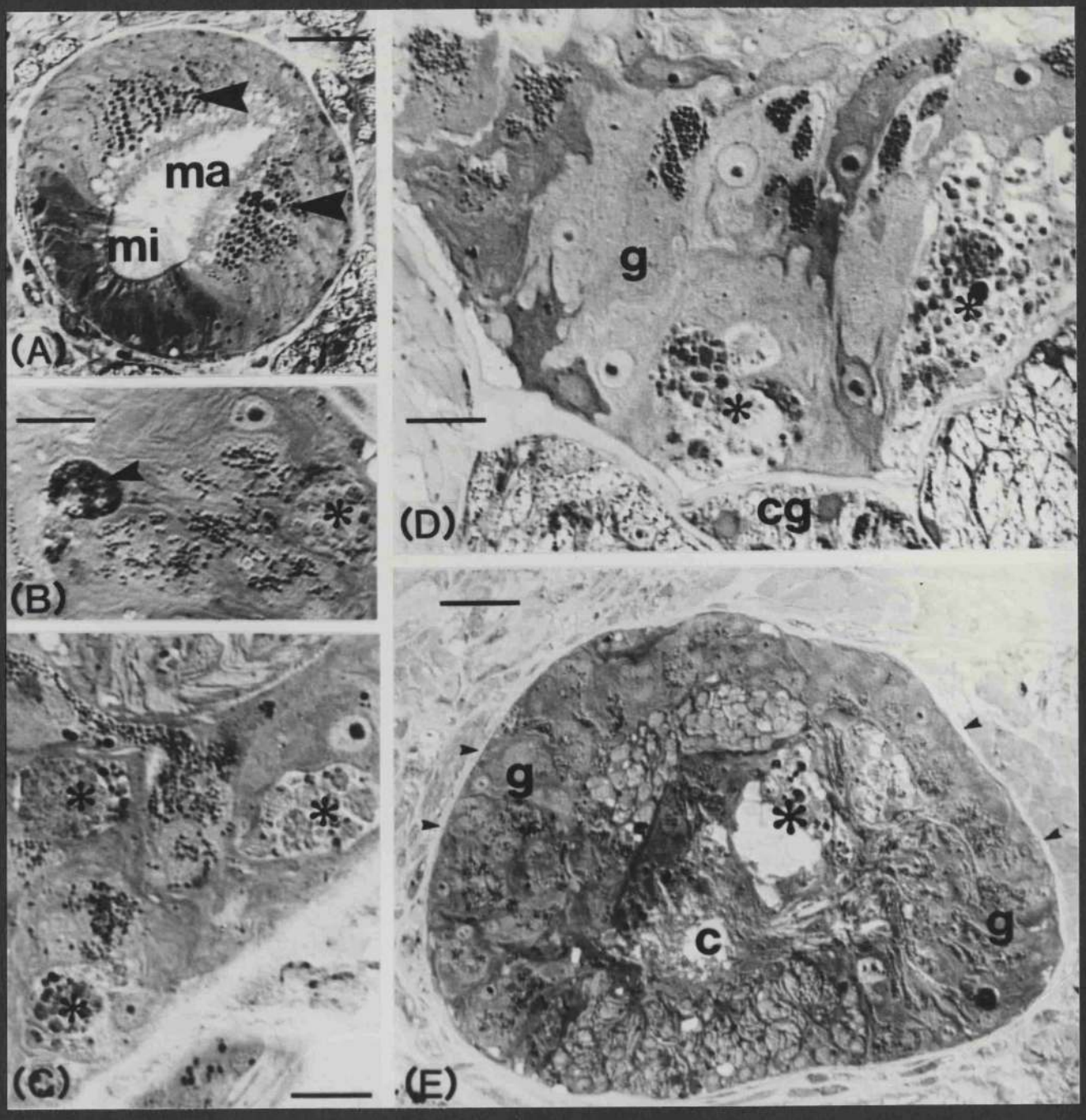

ma
mi
(A)
(B)
(C)
g
cg
(D)
g
c
g
(E)

Figure 55: The effect of hypo-osmotic fixative on the cytology of the cerebral organs of *Paranemertes*.

A. Type 1 vesicular cells afer fixation in glutaraldehyde of 730 mosm; ma=major canal; mi=minor canal; arrowheads indicate dark granular vesicular material. Scale bar= 20 μm.

B. Type 1 vesicular cells after fixation in glutaraldehyde of 480 mosm; C=canal; arrowhead indicates a damaged cell. Scale bar=8 μm.

C. Type 2 vesicular material (asterisks) after fixation in glutaraldehyde of 480 mosm; larger arrowheads indicate subepithelial Type 2 vesicular cell nuclei; smaller arrowheads indicate Type B gland cell processes. Scale bar=8 μm.

D. Cross section through the posterior part of the cerebral organ after fixation in glutaraldehyde of 480 mosm; scb=sensory cell bodies; cg=cephalic glands; bv=cephalic blood vessel; larger arrowheads indicate Type B gland cell processes; smaller arrowheads indicate connective tissue capsule of the cerebral organ; open triangle indicates an apparently normal Type B gland cell between two damaged gland cells; asterisk indicates Type 2 vesicular material. Scale bar=20 μm.

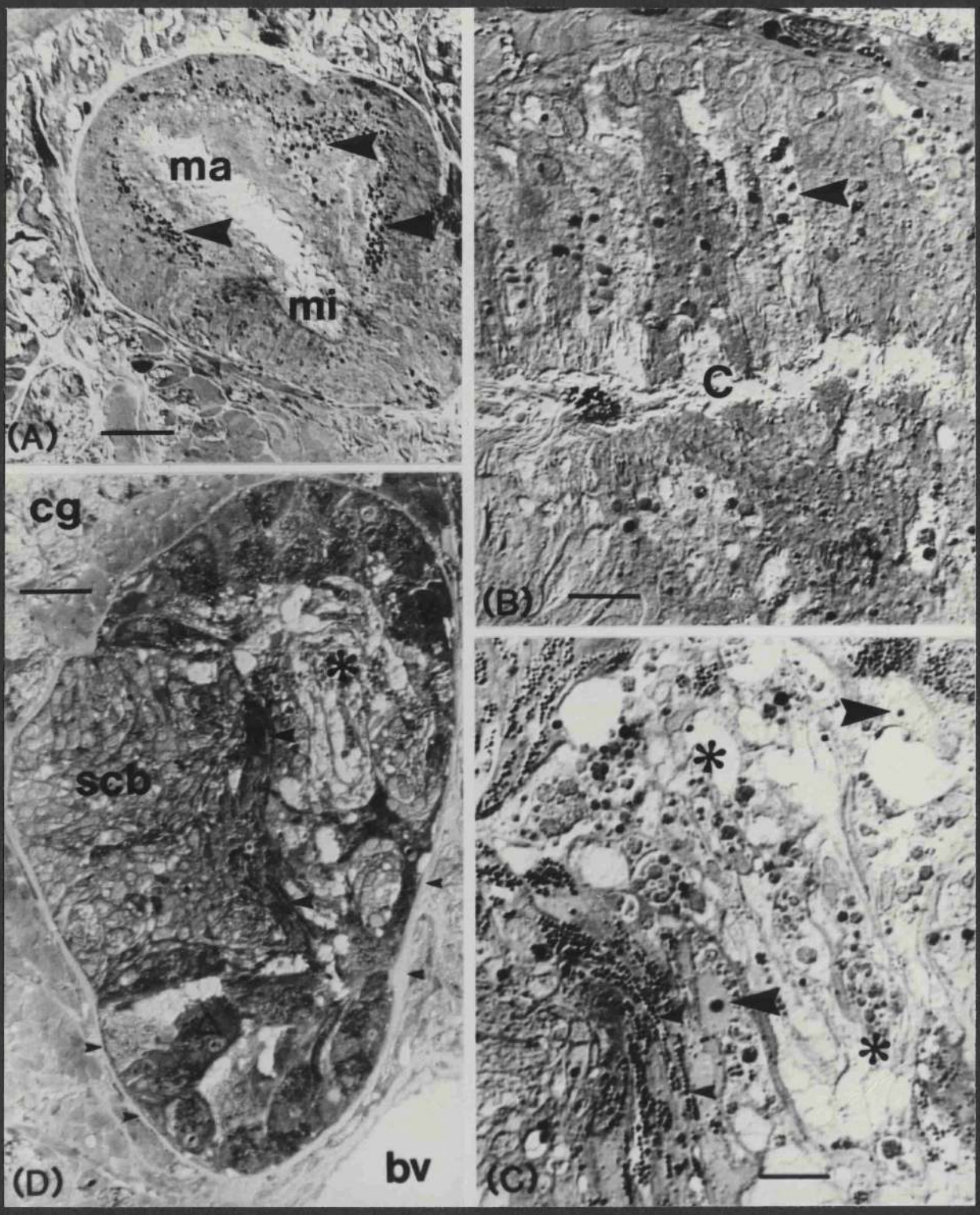

ma
mi
(A)
C
(B)
cg
scb
(D)
bv
(C)

Figure 56: Percent weight change in *Paranemertes* exposed to 75%, 50% or 100% sea water over a 60 hr period. Vertical bars indicate standard deviations; standard deviations for worms in 100% sea water were slightly less.

A. Intact worms in 75% sea water (upper plot) and 100% sea water (lower plot).

B. Worms without cerebral organs in 75% sea water (upper plot) and 100% sea water (lower plot).

C. Intact worms in 50% sea water (upper plot) and 100% sea water (lower plot).

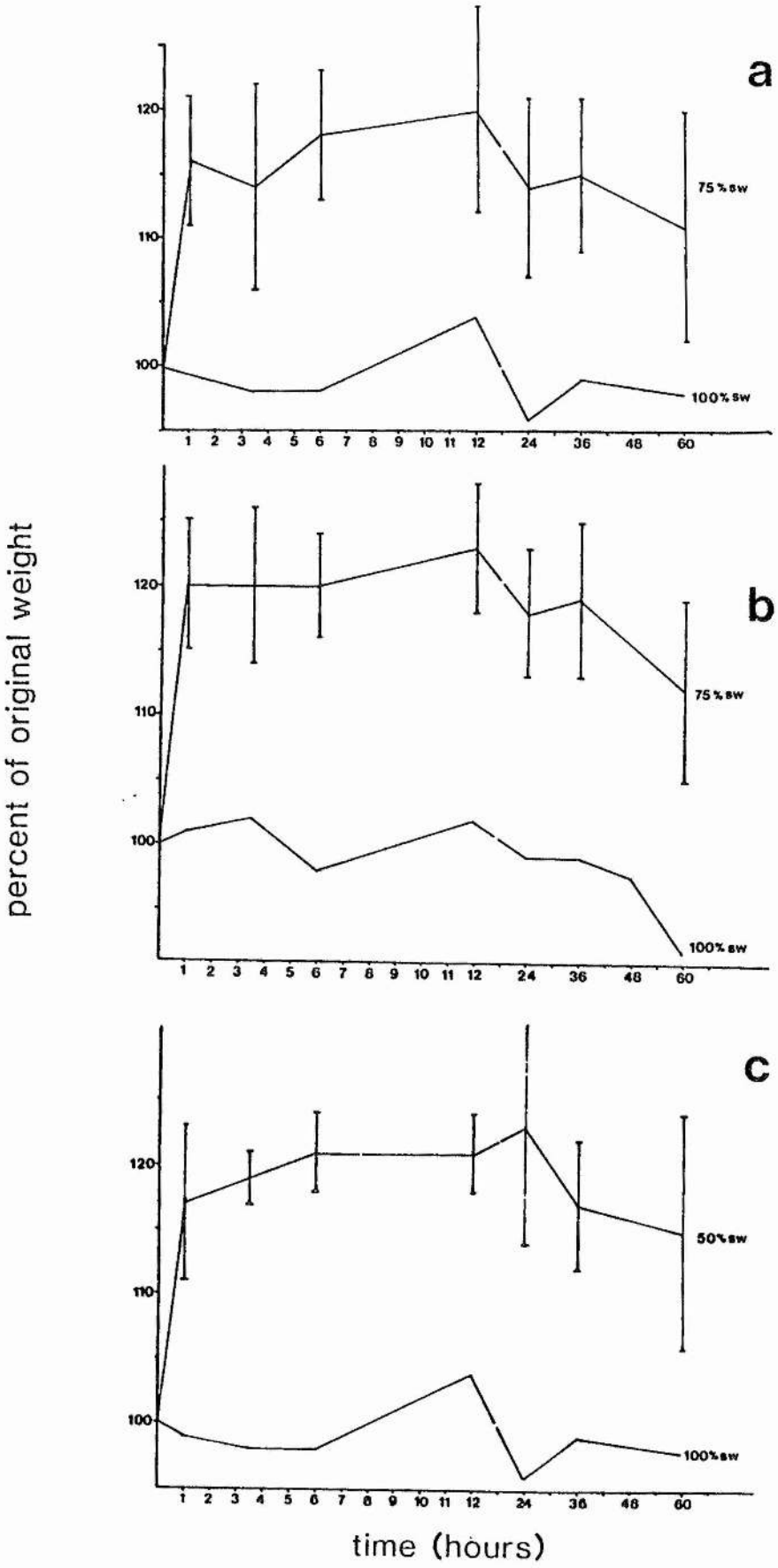

a
b
c
120
110
100
75% sw
100% sw
50% sw
1 2 3 4 5 6 7 8 9 10 11 12 24 36 48 60
percent of original weight
time (hours)

Figure 57: Percent weight change of *Paranemertes* exposed to 75% or 100% sea water over a 48 hr period. Vertical bars indicate 95% confidence intervals.

A. Intact worms.

B. Sham-operated worms.

C. Worms with cerebral organs removed.

D. Percent weight change of *Paranemertes* exposed to 75% sea water for 48 hr (data from A, B and C).

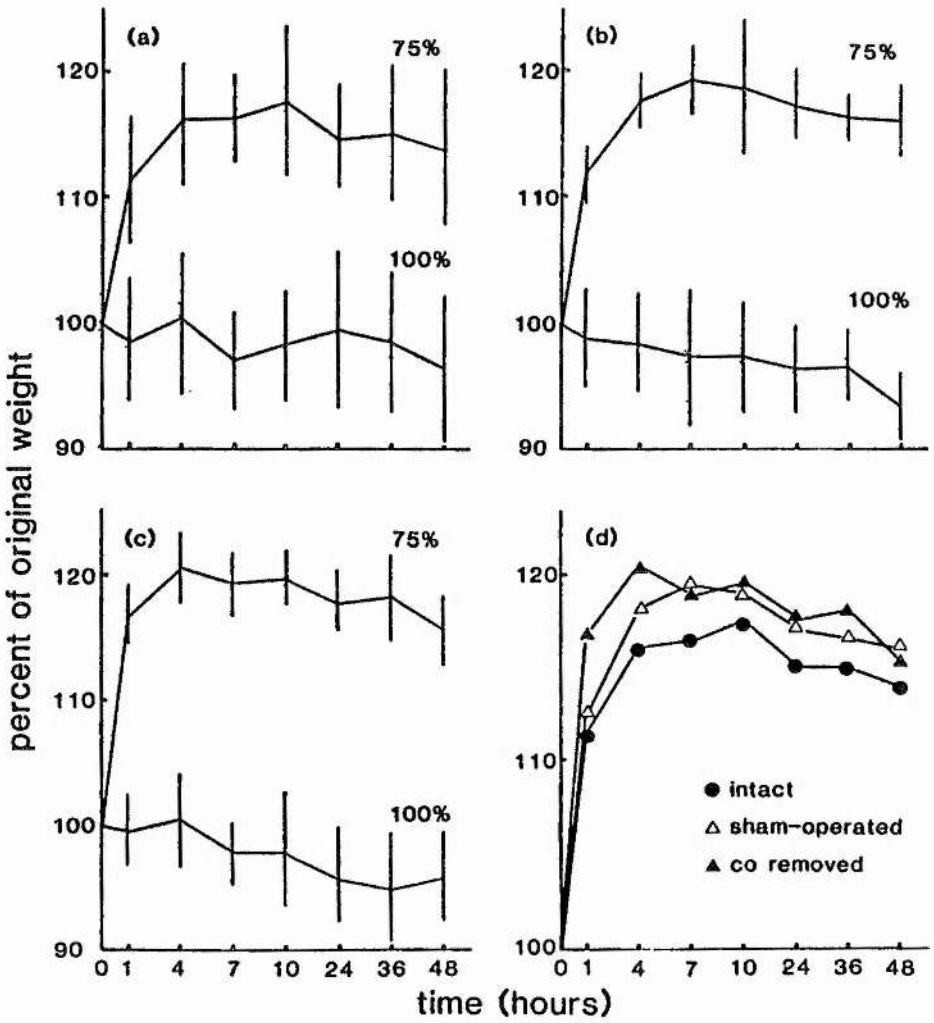

(a)
75%
100%
(b)
75%
100%
(c)
75%
100%
(d)
intact
sham-operated
co removed
120
110
100
90
0 1 4 7 10 24 36 48
percent of original weight
time (hours)

Figure 58:

A. Male (M) and female (f) *Tetrastemma candidum*; p=proboscis; o=ocelli.

B. Ventral view of the head, showing the position of the cephalic canal openings (arrows) on the anterior cephalic grooves.


m
o
f
p
A


n
B

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