THE STRUCTURE OF, AND PRELIMINARY ELECTRO-PHYSIOLOGICAL INVESTIGATION UPON, CILIATED RECEPTORS IN THE GIANT SCALLOP, *Placopecten Magellanicus* (GMELIN)

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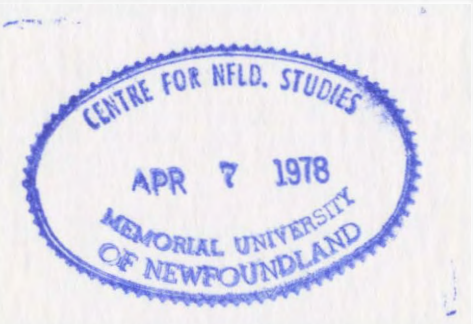

The structure of, and preliminary electrophysiological investigation upon, ciliated receptors in the Giant scallop, *Placopecten magellanicus* (Gmelin)

by

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ABSTRACT

Studies have been carried out on the regeneration of the excised mantle eyes of the scallop Placopecten magellanicus (Gmelin). Many of the experiments performed by Butcher (1930) were repeated. The tentacles and the mantle cavity of the scallop have been examined for the occurrence of ciliated sense organs. When found these have been described anatomically. Preliminary physiological investigations have been made on the sense organs from the long tentacles.

Results from the regeneration studies were negative. Two reasons have been suggested to explain the failure of these experiments, the water temperature was too low thus preventing regeneration, the species used in the present study is different from that used by Butcher.

Examination of the tentacles has revealed a number of ciliated structures. A ciliated papilla found on both the long tentacles, in large numbers, and on the short tentacles, in small numbers has been extensively examined. Anatomical investigations in conjunction with preliminary physiological investigations suggest that the papillae subserve a sensory function and possibly respond to mechanical stimuli. A preliminary investigation of other ciliated structures on the short tentacles and the abdominal sense organ have been made. A chemosensory function is

suggested for the structure of the receptors on short tentacles. The abdominal sense organ has no known function; several hypotheses are offered.

A brief histological study of the anatomy of the circumpallial nerve has been made. The nerve displays a structure similar to that of the visceral ganglion.

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LIST OF ABBREVIATIONS

A	Axon
APN	Anterior Pallial Nerve
B	Bath
BL	Basal Lamina
bb	basal body
bf	basal foot
bp	basal plate
C	Cilium
CC	Cerebral Connective
CG	Cerebral Ganglion
CPC	Cerebropedal Connective
CPN	Circumpallial Nerve
CPV	Circumpallial Blood Vessel
CVC	Circumvisceral Connective
Ca	Camera
Cc	Ciliated cell
Cf	Collagen fibre
Cp	Ciliated papilla
Cs	Collagen sheath
D	Digitimer
E	Eye
G	Golgi apparatus
GC	Ganglion Cell
Is	Isolated stimulator
LT	Long Tentacle
M	Mitochondrion
MC	Macrocilium
MP	Membranous Pad
Ma	Mantle
Mc	Mucous cell
Mg	Mucous granule
Mi	Microvesicle
Mt	Microtubule
Mv	Microvilli
mlb	multilamellar body
mvb	multivesicular body
N	Nucleus
Nm	Nuclear membrane
n	nucleolus
O	Oscilloscope
ON	Optic Nerve
PG	Pedal Ganglion
PPN	Post Pallial Nerve
Pa	Preamplifier
Pg	Pigment granule
R	Root
Rer	Rough endoplasmic reticulum

ST	Short Tentacle
Sc	Support cell
Sd	Septate desmosome
TN	Tentacular Nerve
V	Velum
VG	Visceral Ganglion
VN	Velar Nerve
VT	Velar Tentacle

INTRODUCTION

1. Purpose of Study

The epithelia of Lamellibranch molluscs have been shown to be rich in ciliated structures and cells. In the Pectinidae, cilia have been shown to occur in specialized structures on the tentacles, within the mantle cavity, and in the eyes. The ciliated retina of the eyes has been extensively studied by a large number of authors, Barber, Evans and Land (1967), Dakin (1910a, 1928), Miller (1958), Patten (1887). Barber (1974) reviewed the widespread occurrence of cilia in sensory systems and it has been suggested that the cilia may be important in the transduction of stimuli in the many sensory systems in which they occur.

In order to attempt to clarify the importance of cilia in the transduction of visual stimuli in the ciliated retina of the eye of P. magellanicus, it was decided to attempt to repeat a study carried out by Butcher (1930) on the regeneration of eyes in a related genus, Pecten. It was hoped to be able to follow the regeneration at the ultrastructural level, and physiologically, and to determine what relationship there was between the regeneration of cilia and the renewal of electrical activity in the retina.

Dakin (1909) suggested that the tentacles, which bear

a number of cilia, might be sensory. Since the size and abundance of the two types of tentacles is so disparate it was decided to examine both the long and the short tentacles to determine if any difference existed in the ciliated structures on their epithelia, and to describe the anatomy of the structures. Furthermore it was decided to attempt a physiological investigation into the stimuli to which the sense organs responded.

Eisig (1887) and List (1902), described a discrete paired ciliated structure in the mantle cavity of various Lamellibranchs, adjacent to the anus. White (1937) demonstrated that this structure was single in the Pectinidae, and was characterised by cilia whose length was in excess of 100 µm. None of the histological data were presented photographically or as diagrams. Therefore in order to elucidate the structure of this suggested sense organ it was decided to make an attempt to clarify its structure at both the light and ultrastructural level.

2. Biology

The Giant scallop, Placopecten magellanicus (Gmelin), is widely distributed along the east coast of North America. Its geographical and vertical distribution have been well established by a number of authors (Merril, 1959; Posgay, 1957; Squires, 1962). Its southern range extends as far south as Cape Cod, while scallop beds have been discovered

as far north as Pistolet Bay in Labrador (Merril, 1959). The inability to acclimatize to high temperatures appears to limit its southern range, and also its shallowest depth (Dickie, 1958; Naidu, 1970). Scallops have been found at depths of between 2m - 210m. Dickie (1958) has shown experimentally that the upper temperature tolerance is no greater than 25°C. The optimum temperature for growth and spawning lies in the range of 10 - 15°C. Its most northern range, and its deepest depth, were originally thought to be dependent upon this 10°C minimum needed for spawning to occur. However, spawning has actually been observed to occur under more rigorous temperature conditions, so the limitations for its most northern range, or deepest depth, have yet to be determined.

Medcof and Bourne (1962) have shown that the Giant scallop will tolerate salinities of 20‰. However, it should be noted that full open water salinities of 35‰ invariably occur at depths greater than 3m in Newfoundland coastal waters, and that salinities as low as 20‰ are unlikely to be encountered.

The scallop is found commonly in shallow, sandy, and silty bays around the coast of Newfoundland, and is the largest of the Lamellibranch molluscs found in these waters. Since the bays in which they are found so admirably fit the ecological parameters required by these animals, they are abundant and frequently occur in large beds, which are

easily accessible to the SCUBA diver for collection purposes.

3. Historical Review

The gross anatomy and embryology, as well as a variety of behavioural aspects have been well described by a number of authors. The most definitive account was the monograph of Drew (1906), who described the anatomy, embryology and behaviour of the Giant scallop. Very few changes have had to be made to the anatomical description since that time, though certain discrepancies have been noted in the account of the nervous system. Further studies on the embryology have been carried out in this species, initially by Borden (1928), and later by Merril and Posgay (1967), while Culliney (1974) studied the development from fertilized egg to settled spat. This latter paper included a study on the parameters that caused the spat to settle.

Although the anatomy of the nervous system and ganglia has been adequately studied by both Drew (1906), in his general description, and Dakin (1910), in his more specific description of the visceral ganglion, little has been done on the large number of sense organs, which probably exist around the mantle peripheri. The visceral ganglion of the Lamellibranchs is generally larger, and functionally more important, than the rather diminutive cerebral ganglia. Molluscs have adapted in a manner such that the functions normally assumed by the cephalic region, have been sublimated to the mantle peripheri, a position more suited for the

reception of stimuli in their sedentary niche. Consequently this has given rise to the greater importance of the visceral ganglion. The ganglion is a multi-lobed body, which in certain species is asymmetrical due to the more numerous neurons in the right lobe, a direct consequence of the greater number of eyes arranged on the right, or upper, mantle lobe (Dakin, 1910).

The anatomy of Pecten eyes, which are very similar to those of Placopecten, was first described by Patten (1887). In this description he indicated that there was a possibility that two retinae might be present. Dakin (1910a, 1928) described in detail the anatomy of the eye and included an exhaustive description of the two retinae. He also suggested that one of the retinae, the distal retina, was ciliated, and the other one was not. Miller (1958) in a study on the ultrastructure of the retinae showed the presence of cilia in the retinae, and demonstrated that the cilia formed whorls of concentric membranes, which he suggested to be the site of transduction effected in much the same way as the vertebrate rod cell. These results were later confirmed in a more comprehensive ultrastructural study by Barber, Evans and Land (1967). Land (1965, 1966a) studied the unusual mechanism of image formation in the eye, which is formed by a spherical reflecting mirror, that is situated at the back of the eye. Further studies on these structures and a theory of how they function were made by Huxley (1968) and

Land (1966b).

Behavioural studies on species of Pecten were carried out by Buddenbrock and Moller-Racke (1953), Dakin (1909), Gutsell (1930), and Uexküll (1912). These researchers demonstrated that the animals responded to angular motion and also to a decrease in light intensity. In fact it was shown that one species would respond to 0.3% diminution in the intensity of illumination. These studies also demonstrated that the eyes were sensitive to contrast, and when placed in a tank that contained a portion with a darkened background the scallop would extend its long tentacles towards the darkened area. If the darkened area was then moved the tentacles would track the motion. This was demonstrated to be a localized response, the tentacles were effector organs for stimuli being received by the eyes. The circumpallial nerve was suggested as being the site of integration.

Direct physiological evidence from the eyes was presented by Hartline (1938), who showed that the two retinae were functionally different in their physiological response. The distal retina was demonstrated to respond to a decrease in illumination, a phenomenon termed the "off" response. The proximal retina was shown to cease to respond when the light source was removed. Land (1966a) further investigated the behavioural implications of Buddenbrock's and Moller-Racke's work. He showed that the

ciliated distal retina was responsible for the "off" response. Cronly-Dillon (1965) studied the spectral sensitivity of the eye although his results are of little use as he was unaware that the image formation was by an interference reflecting system. Gorman and McReynolds (1969) expanded our knowledge on this topic, and in other papers demonstrated that the distal retina responded in a similar fashion to the vertebrate rod cells, so illumination induced hyperpolarization in the retinal cells (McReynolds and Gorman, 1970a, 1970b, 1974).

Although there is much now known concerning the anatomy and electrophysiology of the eye, only one study has been made on the development of the organ. Butcher (1930) followed the regeneration of eyes in specimens of Pecten whose eyes had been excised. The study was carried out by histological sectioning of the regenerating eye from a callous to the fully developed state. The results suggested that a fully functional eye was restored and that this eye was a primary receptor. No electrophysiological evidence was presented as to whether these redeveloped eyes were functional or not. No ultrastructural study has since been carried out to determine the sequence of tissue regeneration, and in particular the regeneration of the distal retina with regard to the pattern of development of the cilia.

As has already been stated the functions normally assumed by the cephalic region have in the Lamellibranchs,

been assumed by the visceral ganglion. Thus the mantle rim is the foremost region in the reception of stimuli relating to the environment. One would expect to find tactile, visual, chemical and vibration receptors at the mantle rim. The incidence of visual receptors has already been discussed.

The mantle edge of many Lamellibranch species is covered by a number of tentacles, which vary in both size and shape. In the scallop the arrangement of the tentacles was first described by Eisig (1887), and subsequently by List (1902). Both these authors noted that two types of tentacles existed and that they could be differentiated by their size and position on the mantle rim. The tentacles were termed the long and the short tentacles. The long tentacles were found on the same lobe as the eyes, the ophthalmic lobe, while the short tentacles were found on the outermost lobe, the periostracal lobe. The long tentacles were more extensible than the short tentacles, and were not as numerous. Both authors suggested that the tentacles had a sensory function. Drew (1906) and Dakin (1910) concurred with this postulate. Dakin (1909) showed that ciliated cells occurred on the tentacles. These ciliated cells were linked via a nerve network, which ultimately joined with the tentacular nerve. It was also noted that the mantle rim and the tentacles were richly supplied with nerves, and Drew and Dakin suggested that the tentacles might have a chemosensory function. Uexküll (1912) suggested

that a tactile function was probable, and also showed that scallops responded to movements of starfish in adjacent aquaria by extending their tentacles towards the starfish. Gutsell (1930) readily demonstrated tactile sensibility through stimulation of isolated segments of the mantle bearing tentacles with a jet of water. Unfortunately he was unable to determine if the tentacles were also sensitive to chemical stimuli.

Behavioural work by Buddenbrock and Moller-Racke (1953) showed that the long tentacles, which are extended to areas of low light intensities, do not do so in response to a chemical stimulus. What modality they did respond to was not determined. The size and the great mobility of the long tentacles and the fact that the behavioural evidence showed that they were directed towards low light intensities suggested that they would have sensory receptors, either mechano- or chemo-receptors, in their epithelia.

German histologists late in the last century discovered paired ciliated structures adjacent to the anus in the mantle cavity in a number of Lamellibranchs. Theile (1889) first described this structure in Arca. Eisig (1887) also described it in his review. The structure was described as a flap of tissue upon which rested a number of hair cells. List (1902) described this structure in certain species of the Mytilidae. The abdominal sense organ, as it was termed by List, consisted of a pad of columnar ciliated cells

attached to a basal lamina. The bases of the cells were drawn out into axons thus indicating that the ciliated cells were primary receptors. White (1937), in a histological study, stated that in the Pectinidae the structure was similar but that the organ was a single flap of tissue and not paired as in the case of the other groups studied. He showed that the cilia were extremely long and suggested that the cells had a chemo-sensory function.

A number of different functions have been suggested for the abdominal sense organ. Theile (1889) and Eisig (1887) both suggested that it might respond to vibrations set up in the water around the animal, functioning in a similar fashion to the lateral line organ of certain fish. Teleologically one can argue that this function is highly unlikely. The stimulus would have to pass the sensitive mantle rim without stimulating it prior to reception at the abdominal sense organ a fact which is hard to believe. Dakin was unable to show any alteration in behaviour when the abdominal sense organ was stimulated, or when it was completely removed. White (1937) suggested that it might possess a function similar to that of the osphradium, which is a structure found in Gasteropods and Lamellibranchs. The osphradium has been commonly described as being a chemo-receptor although it has been shown to be an extremely sensitive osmoreceptor in Aplysia (Stinnakre and Tauc, 1969). Dakin (1910) showed that the osphradium of the Pectinidae

is small though well supplied with neuronal input. Later studies (Setna, 1930) suggested that the osphradium of Pecten extended down the gill axes. White (1937) argued that since these two organs are so close together they would be unlikely to have a similar function.

MATERIALS AND METHODS

1. Collection and Maintenance of Scallops

All specimens of P. magellanicus were collected by SCUBA divers in depths from 10 - 20 m. The majority of collections were made in Salmonier Arm, two other collections for smaller individuals being made in North Harbour, both locations being in Newfoundland.

Those specimens being utilized for the study on the sense organs were maintained in shallow wet benches in the Marine Sciences Research Laboratory (MSRL) in Logy Bay.

The scallops used in the regrowth study were maintained under a variety of conditions. Twenty individuals were numbered and 18 of these had their eyes removed from a number of different portions of the upper or lower mantle. These individuals were then placed in a series of scallop trays fabricated from netting and wire, which were suspended from an array at approximately 10 m depth in Logy Bay. The scallops were placed in the water in September 1973 and the entire array was removed from the water in April 1974. The first two scallops were removed from the array on the 4th of October 1973 and others were retrieved at approximately two week intervals. A second group of twenty scallops, from which some or all of the eyes had been removed from the upper or lower, or both mantles, were returned to their

site of collection in Salmonier Arm. They were retained on the bottom at approximately 10 m in a herring-mesh cage. The cage was placed in position in April 1974, and scallops were then collected at one month intervals until December 1974 when the remaining scallops were released.

2. Preparation of Tissues for Anatomical Investigation

a) Histological sections

All samples of eyes, tentacles, and abdominal sense organs destined for wax embedding, were fixed in Bouin's fixative for 24 hours at room temperature. The tissue was then washed in several changes of 50% ethanol until the odour of picric acid was removed from the tissue. The samples were dehydrated through an alcohol series according to the following schedule: 20 minutes in 70%, 80%, 95% ethanol followed by three changes in absolute ethanol over one hour. The tissue was then cleared in three changes of xylene for a total of one hour. Following the clearing, the samples were infiltrated with a Paraplast embedding medium (Fisher Scientific) and xylene for 24 hours at 58°C followed by several changes of pure Paraplast for 24 hours at 58°C.

Sections of 7 to 10 µm thickness were cut on an American Optical 820 Spencer microtome. The sections were affixed to the slides with a 1% solution of egg albumin, and allowed to flatten overnight on a slide warming tray. De-waxing and rehydration of the sections was through a

standard xylene and ethanol graded series, to water.

The tentacles, eyes, and abdominal sense organs were stained in Mallory-Heidenhein's 1,2,3 rapid process stain (Cason, 1950). Permanent mounts were made using Adam's Histoclad mounting fluid, with "0" thickness cover slips. Sections were viewed with a Zeiss Photomicroscope II. Photographs were made using Kodak Panatomic X black and white film using a red filter, (Kodak Wratten gelatin filter #25), at initial magnifications from x10 to x400.

b) Epon sections for electron and light microscopy

(i) Fixation

It is well known that the fixation of invertebrate tissue, in particular molluscan tissue, has not yet been perfected or standardized, and so a variety of fixatives and buffers were tried, only one of which proved to be satisfactory. The fixatives used were as follows:

Dalton's chrome osmium fixative. The proportions used were as follows: Equal volumes of a 4% potassium dichromate solution, adjusted to pH 7.2 with potassium hydroxide, and a 3.4% sodium chloride solution, were mixed together, and an equal volume of this solution and a solution of 2% osmium tetroxide in distilled water were mixed to make the final solution for the fixative (Dalton, 1955).

Karnovsky's fixative. This was made up in Sorensen's phosphate buffer at pH 7.3, and in sea water. A number of

combinations of fixative from five parts of buffer to the full strength fixative were used (Karnovsky, 1965).

1% osmium tetroxide in Millonig's phosphate buffer at pH 7.2 (Millonig, 1961).

2.5% glutaraldehyde in Millonig's phosphate buffer at pH 7.2.

2.5% glutaraldehyde in sea water buffered at pH 7.2.

Of all these fixatives, initial fixing in 2.5% glutaraldehyde in Millonig's buffer proved the most acceptable in the preservation of cilia and of membrane-bound structures. Therefore this fixative was employed at all times, the samples being fixed for two hours at room temperature, then washed in buffer until the odour of the fixative was not detectable, which could take up to 24 hours at room temperature. The specimens were than post-fixed in 1% osmium tetroxide in Millonig's buffer at pH 7.2 for two hours.

(ii) Removal of tissue and embedding.

The eyes were removed from the optic lobe of the mantle edge by carefully cutting the eye-bearing tentacle close to the mantle edge. The capsule surrounding the eye was nicked with a razor blade in order to facilitate the entry of the fixative. The whole eye and the tentacle was then immersed in the fixative. The removal and fixation of the short and long tentacles and the abdominal sense organ was

similar to that of the eyes. Care was taken to remove the tentacles as close to the mantle as possible. Some sections of the mantle rim were removed with their incumbent eyes and tentacles. These samples were prepared for scanning electron microscopy. After post-fixation the samples were transferred directly to 35% ethanol without washing. Dehydration was effected by a graded ethanol series from 35% to 95% ethanol with final dehydration for one hour in frequent changes of absolute ethanol dried with silica gel. Subsequently the tissues were immersed in propylene oxide for one hour with frequent changes of fluid.

The samples were then infiltrated with a mixture of equal volumes of propylene oxide and Epon 812 (plus DDSA and NMA) without its hardener, for 1 hour, this mixture being replaced by the complete medium Epon 812 plus DDSA, NMA and DMP 30, and then placed in an oven at 60°C overnight (Luft, 1961). Some large specimens were infiltrated under vacuum in a vacuum dessicator in order to improve infiltration.

(iii) Sectioning and staining

Epon sections of this embedded material for both light and electron microscopy were obtained on a Reichert Ultramicrotome II. Thick sections for light microscopy of between 0.5 to 1.0 μm were cut on glass knives made with a LKB Knife Breaker. Thin sections, approximately 30 to 40 nm thick, which had silver interference colours, were obtained

with an E.I. Dupont diamond knife.

Thick sections for comparison with the histological sections, were stained with 1% toluidine blue in distilled water at pH 11.1 (Trump, Smuckler, and Benolitt, 1961). Permanent mounts of the sections were made with Adam's Histoclad under #0 thickness cover slips.

Thin sections mounted on uncoated copper grids were stained in a saturated solution of uranyl acetate in distilled water (Watson, 1958), washed in distilled water, then stained in a 1% solution of lead citrate after Venable and Coggeshall (1965), then washed in distilled water and dried. In both cases the stains were filtered through millipore filters mounted on syringes to ensure that they were free of solid contaminants. The sections were viewed under a Zeiss EM9S transmission electron microscope with an accelerating voltage of 60 kV. Photographs of the sections were made on Kodak thick base estar plates 6.25 cm square.

c) Scanning electron microscopy

Those samples being prepared for scanning electron microscopy were fixed as previously described. However immediately prior to post-fixation the samples were acid etched in 1% hydrochloric acid according to the method of Budelmann, Barber and West (1973). The purpose of this treatment was to remove the mucopolysaccharide coat found covering the epithelia of most marine invertebrates. The samples were then post-fixed in osmium tetroxide and

following this dehydrated to 70% ethanol if they were to be stored.

The majority of long tentacles contracted on being immersed in the fixative. In order to overcome this problem certain samples were immersed in 0.1 mM EGTA in filtered sea water, for 30 mins. prior to fixation. EGTA is a calcium chelating agent which prevents regenerative release of calcium ions within muscle cells thus effectively preventing contraction (Ford and Podolsky, 1970).

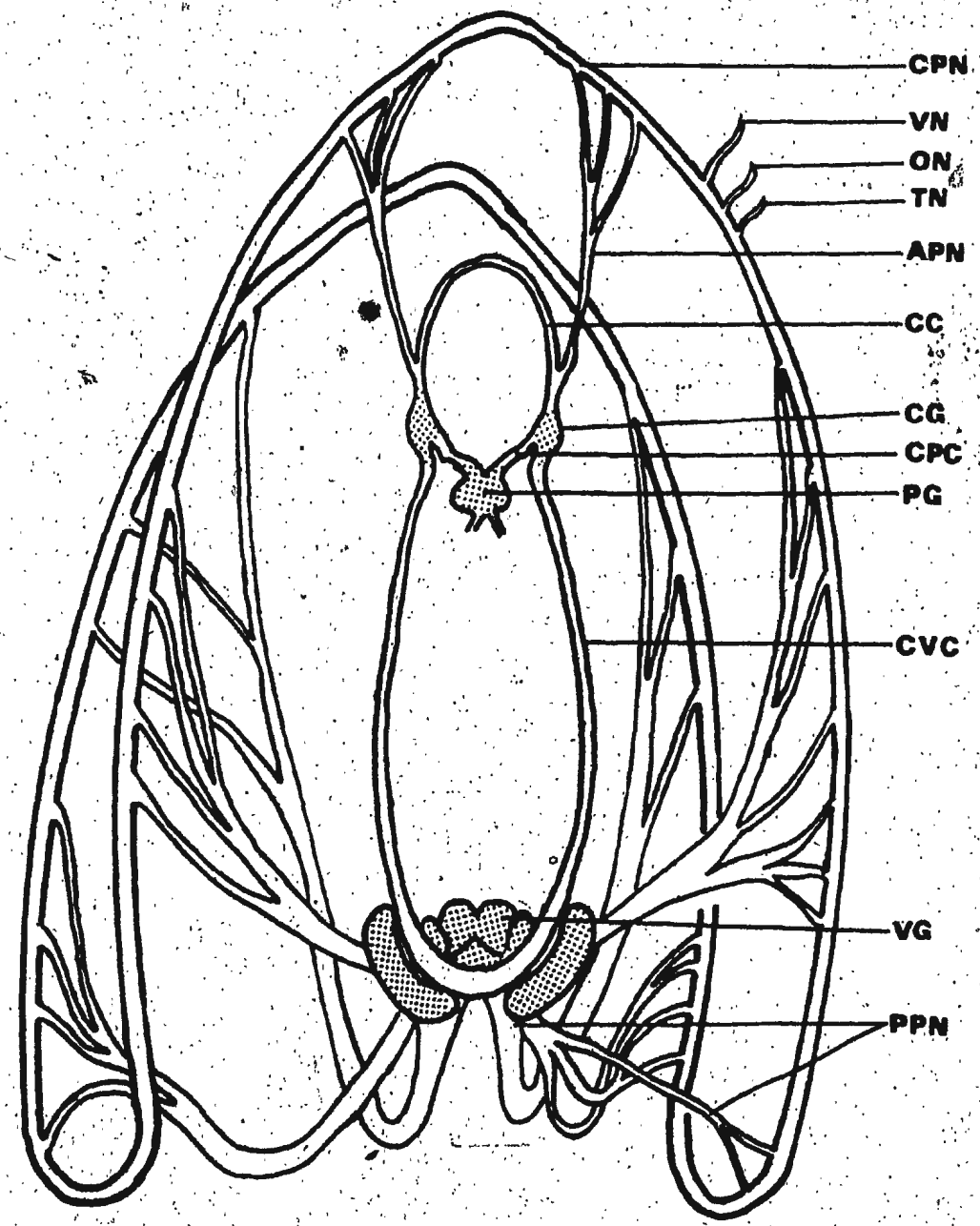
The samples were then freeze-dried, those which were stored in 70% ethanol being rehydrated prior to freeze-drying. To minimize ice crystal damage and to promote more rapid cooling, they were placed in a quenching fluid, Freon 12 (dichlorodifluoromethane), maintained just above its melting point of -158^{o}C by liquid nitrogen. Once in the quenching fluid the samples froze and were then transferred to liquid nitrogen and via this to a Speedivac-Pearse Tissue Drier. The ice was removed under a vacuum of approximately 0.03 Torr at -60^{o}C for eight hours.

The dried samples were mounted on aluminium stubs with silver conducting paint (Ladd Research Industries) allowed to dry and then placed in an Edward's Vacuum Coating Unit (Model E12E) and coated with a thin layer of gold approximately 20 - 30 nm thick, an even coating being ensured by the use of a planetary rotation unit (Budelmann et al, 1973).

The coated samples were viewed with a Cambridge

Fig. 1.

Plan view of the central and peripheral nervous system of P. magellanicus.


CPN
VN
ON
TN
APN
CC
CG
CPC
PG
CVC
VG
PPN

Stereoscan Mark 2A Scanning Electron Microscope, operating at accelerating voltages from 4.5 to 10 kV. Photographs of the specimens were recorded on Tri X film by a 35 mm Exacta camera.

3. Dissections and Electrophysiology

a) Preparation of sample

In order to carry out the electrophysiological experiments the tentacular nerve, which arises as a branch from, or feeds into the circumpallial nerve, must be exposed. This dissection was carried out as follows. A scallop was opened by carefully separating the adductor muscle from its point of attachment on the upper or right mantle. A small segment of the upper mantle from the anterior side (right side) near the ears of the valves, bearing one or two long tentacles, a few eyes, many small tentacles and the velum, was dissected free, and pinned onto the dissecting dish with insect pins. An incision was made along the circumpallial blood vessel, which lies superior to the circumpallial nerve (see Fig. 1). The circumpallial nerve is surrounded by a thick collagen sheath. The tentacular nerve is also surrounded by a collagen sheath and is embedded in the surrounding muscle tissue, hence is extremely hard to see unless followed out from the circumpallial nerve. In order to ascertain the exact destination of this nerve it became necessary to dissect the nerve as far as possible to its periphery. This was facilitated in

the initial stages by removal of the collagen and muscle by enzymatic digestion. The method employed was a modified method of that used by Rojkind, Portales and Cid (1974) to separate out rat liver cells. A 50/50 mixture of collagenase and hyaluronidase (Sigma) each made up to 30 mg/100 ml in filtered sea water was poured on the preparation and left for periods of up to two hours. The tentacular nerve was then followed out from the circum-pallial nerve, the remaining tissue being dissected away with dissecting needles ground to a scalpel edge.

In later experiments, after the pathway of the nerve had been elucidated, the tissue surrounding the nerve was removed solely with the dissecting needles. In order to minimize the occurrence of spurious electrical activity due to muscle contractions and activity from other nerves, care was taken to remove any extraneous tissue in the form of the short tentacles, eyes and the velum.

All experiments were conducted in a controlled temperature chamber fabricated from laminated plexiglass sheets. The chamber was 11.5 cm in diameter and was 3.5 cm deep. The temperature of the chamber was controlled by sea water passing through a coil of glass tubing. Since the experiments were all performed in the winter months when water temperatures seldom rose above $4^{o}C$ the system prevented the temperature rising above $10^{o}C$. A 21.0 cm long silver/silver chloride bath electrode was grounded to

Fig. 2.

Layout of equipment used during electrophysiological experiments.


Ca
O
Pa
B
In
D

the table. A small dissecting platform filled with Sylgard 184 encapsulating resin (Dow Corning) was mounted in the center of the chamber. The bath was placed on a small raised table, which was mounted on a steel plate supported on tennis balls in order to damp out vibration. The dissecting platform was illuminated indirectly from beneath by light reflected from a mirror. A tripod stand supported a Wild dissecting microscope over the dissecting table. All the apparatus on the steel plate was grounded to the oscilloscope module (see Fig. 2).

A number of different electrodes were employed both for recording and for stimulation until a satisfactory type could be found. Stimulating electrodes made from teflon coated 35 gauge silver wire, from which a small amount of insulation had been removed, were found to be satisfactory. Recording electrodes made from machine drawn glass capillary tubing and filled with filtered sea water, from a syringe to which was attached a millipore filter and a 20 gauge needle, were found to be suitable. The tip diameter was in the order of 1 - 2 μm with a resistance of 5 - 10 MΩ. The electrode was held by a silver/silver chloride contact, which was connected to the preamplifier.

b) Stimulation

Several physical methods were employed to stimulate the tentacles to attempt to ascertain to what modality they responded.

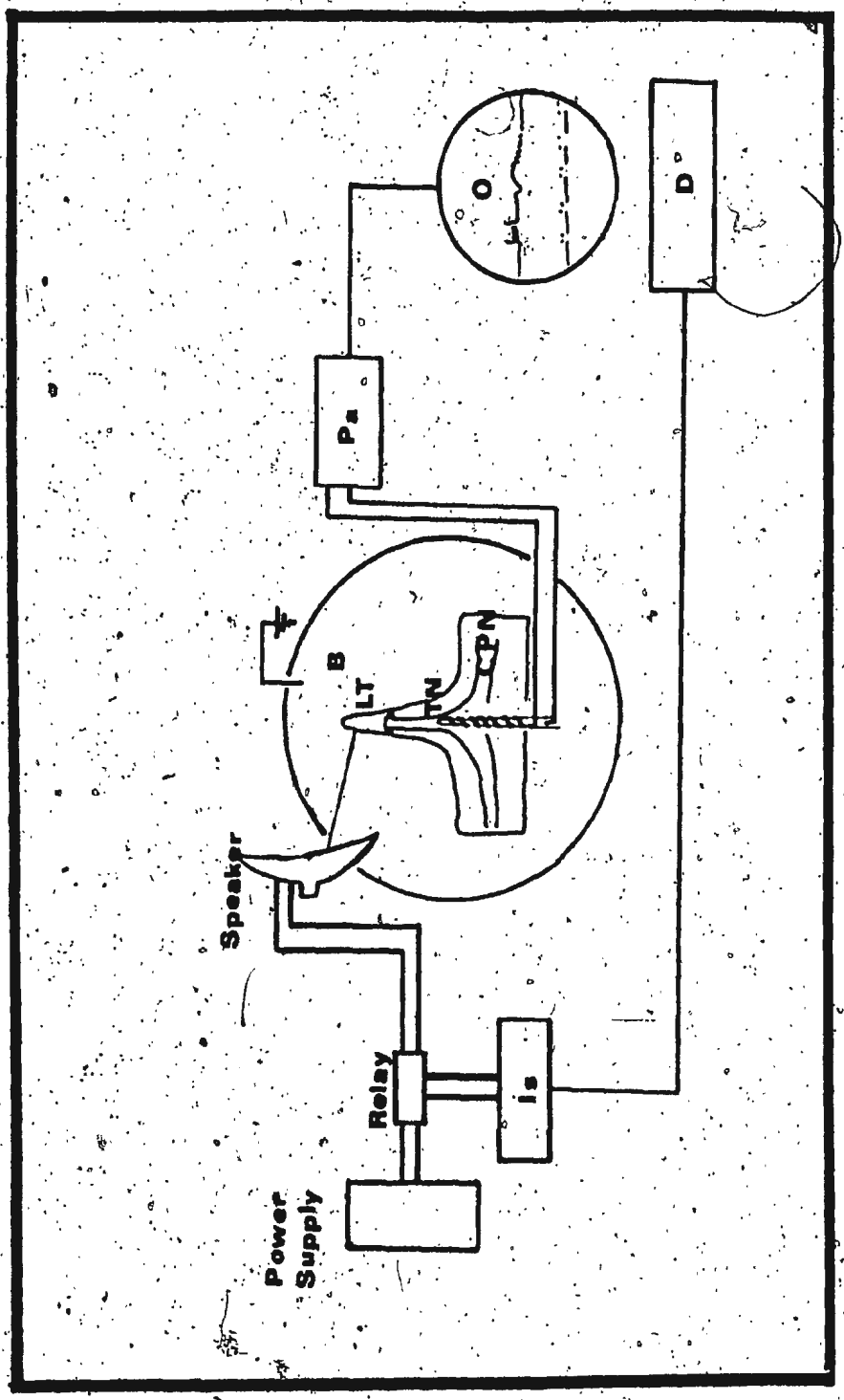
Electrical stimuli were provided by a Device's Isolated Stimulator Type 2533. The voltage output could be varied from 0 V - 100 V D.C. The duration of the pulse was also variable and ranged from 0.1 msec to one second. The stimulator was either triggered manually or by a Digitimer Type 3290 (Devices Ltd.) with a predetermined delay synchronised with the sweep of the oscilloscope beam.

Hyperosmotic stimuli were provided by crystals of sodium chloride placed in close proximity to the tentacle tip.

Two types of mechanical stimuli were used. The first, a localized mechanical stimulus, was provided by an oscillating 35 gauge silver wire. The oscillations were effected by attaching the silver wire to the voice coil of an eight ohm speaker which was powered by a Hewlett Packer Square wave generator, the frequency of which was set at 150 Hz. The magnitude of the stimulus could be altered by varying the amplitude of the square wave pulse. The duration of this stimulus was controlled through a relay powered by a Device's Isolated stimulator. The stimulus could therefore be synchronised automatically through the Digitimer such that it could be administered with a given delay after the beam was triggered. The duration of the stimulus could be varied according to the pulse width set on the isolated stimulator. The duration was varied between 20 msecs and one second. The second mechanical

Fig. 3.

Block diagram of the recording lay out.


O
D
Pa
+
B
LT
LPN
Speaker
Relay
Is
Power
Supply

stimulus was provided by a stream of water directed by a polyethylene tube connected to a 5 ml syringe. The tubing was stapled to the dissecting dish 5 mm from the tip of the tentacle. There are two disadvantages to this last method, the first being that the intensity of the stimulus cannot be accurately gauged and the second being that the stimulus is not localized.

c) Recording

The recording electrode was introduced into the nerve bundle via a slit in the collagen sheath adjacent to the point of conjunction of the tentacular and circumpallial nerves. The signals were recorded differentially and preamplified by a type P15 AC Preamplifier (Grass Instruments), amplified on a Type 3A3 dual trace differential amplifier, which was AC coupled, and displayed on a Type RM565 oscilloscope. The oscilloscope was triggered externally by a Digitimer Type 3290 (Devices Ltd,) (see Fig. 3). Traces were recorded on Kodak 2495 RAR thin base estar film contained in a continuous recording camera Type PC2A (Nihon Kohden Kogyo Co. Ltd.). The film was developed according to Kodak specifications at 95°C for 1.5 mins., in one to one D19 Developer.

RESULTS

1. Eyes

No experiment that was performed for the regeneration study was successful. Although experimental procedure as described by Butcher (1930) was repeated, regeneration could not be induced.

Because the animals were maintained under a variety of conditions, and were also returned to the site from which they were collected, the possibility that regeneration was prevented by factors due to their maintenance may be stated to be improbable. Observations indicated that the feeding behaviour of the animals was unaffected after removal of the eyes, and many faeces or pseudo-faeces were collected in the depressions in the substrate in which the animals were commonly found. Therefore it seems unlikely that the failure of regeneration was caused by the lack of food.

One sample of what appeared to be a partially regenerated eye was obtained from an individual maintained under natural conditions. This was the only eye that was approximately the same in appearance as the regenerated eyes in the studies carried out by Butcher (1930). This sample was removed from the ears of the mantle, the position at which, according to Butcher (1930) and Dakin

Fig. 4.

Mean monthly surface sea water temperatures for Logy Bay, Newfoundland (Steele, 1975), and Woods Hole, Massachusetts (1911) (Sumner, Osburn and Cole, 1911).

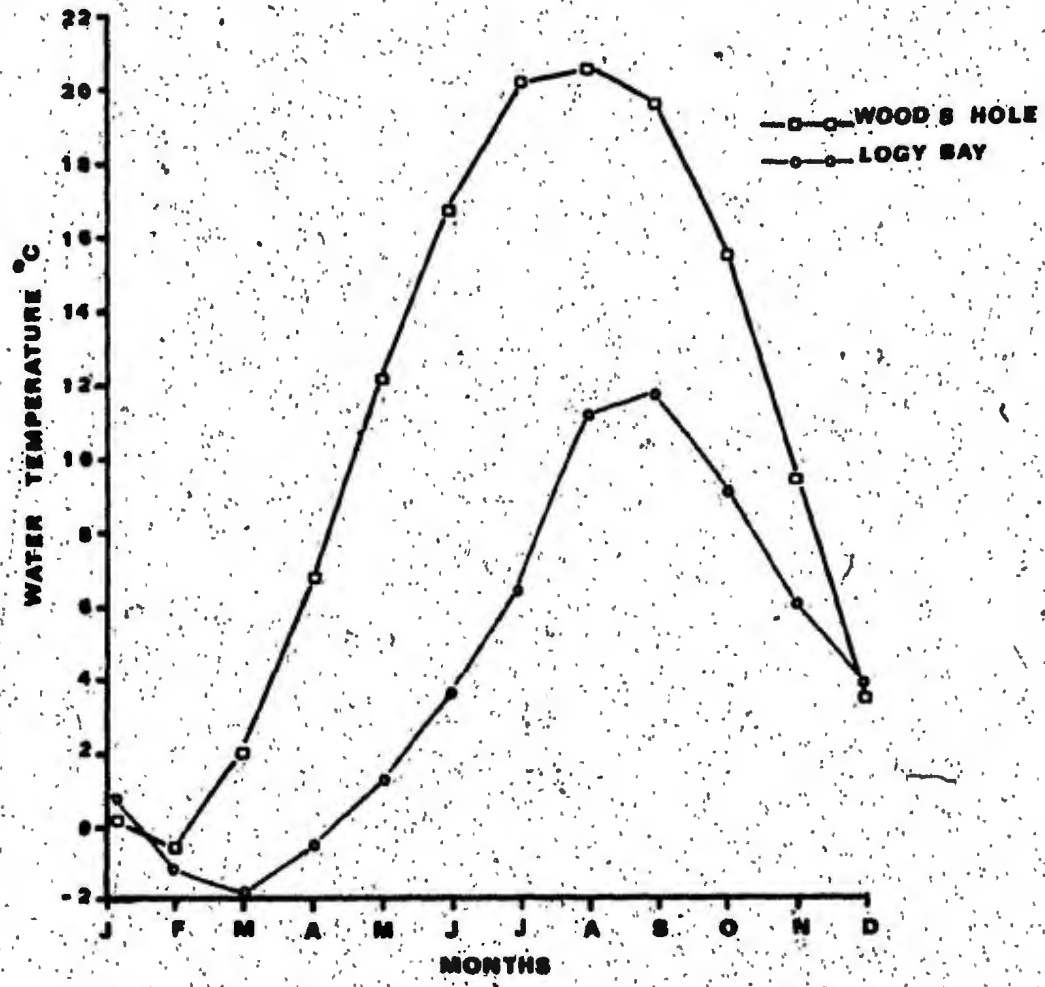
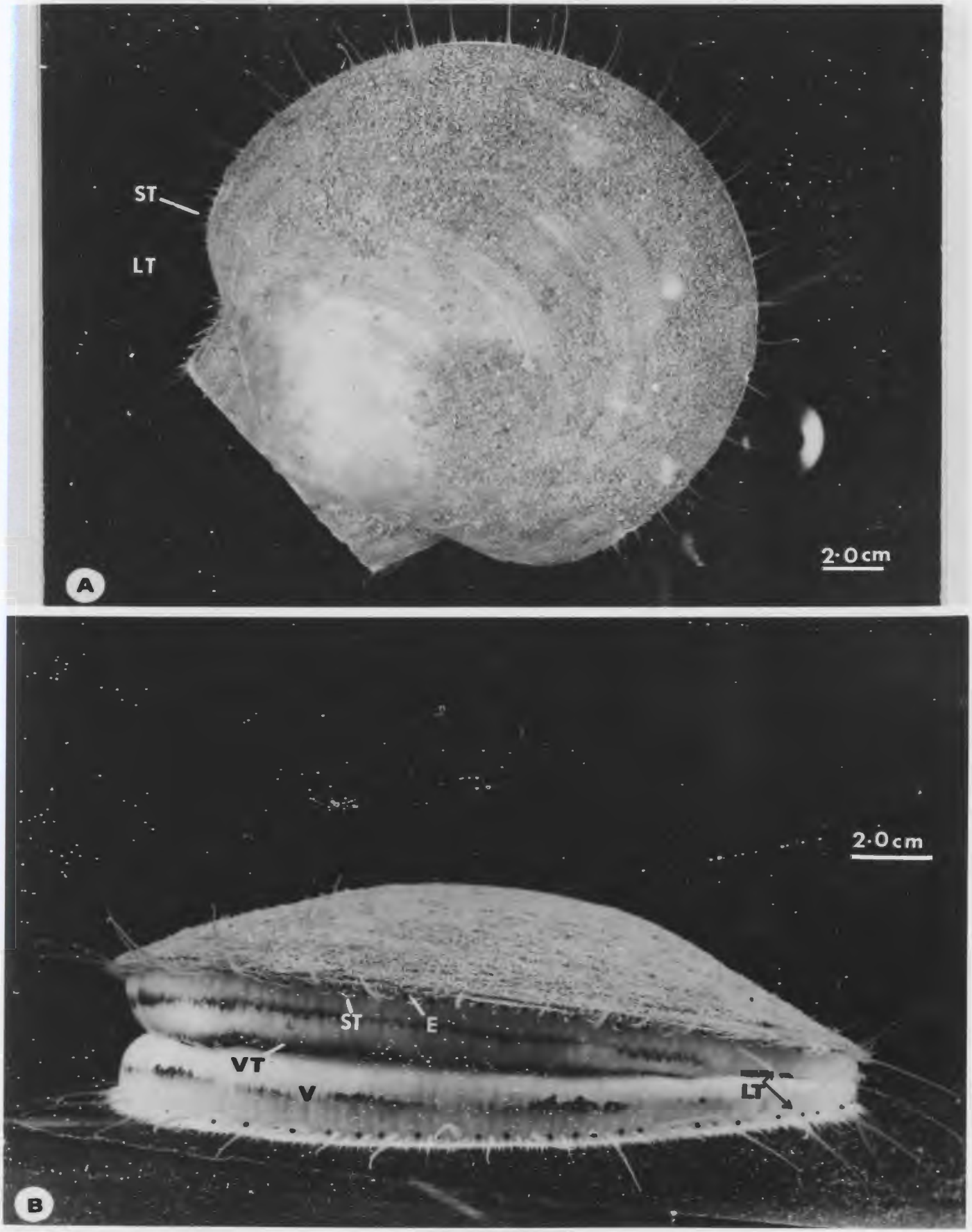

WOOD S HOLE
LOGY BAY
WATER TEMPERATURE °C
22
20
18
16
14
12
10
8
6
4
2
0
-2
J
F
M
A
M
J
J
A
S
O
N
D
MONTHS

Fig. 5.

Fig. 5A. Vertical view of P. magellanicus showing long and short tentacles extended.

Fig. 5B. View of P. magellanicus showing arrangement of eyes, tentacles and velum.


ST
LT
2·0cm
A
2·0cm
ST
E
VT
V
LT
B

(1928), the most rapid growth of the eyes is supposed to occur. When sections of this eye were examined under the transmission electron microscope, the retinal structure appeared to be very disorganized, and there is an equal probability that this is a degenerating or nonfunctional eye rather than a regenerating one.

It is notable, however, that there is a significant difference between the sea water temperatures at Woods Hole and those at Salmonier Arm and Logy Bay, Newfoundland. The optimum growth conditions only occur in Logy Bay for periods of approximately 3 - 4 months, whilst in Woods Hole these temperatures are maintained for periods up to 8 months (Fig. 4).

2. Long Tentacles

The long tentacles are highly extensible and may vary in size from approximately 5 mm to 60 mm. Under normal undisturbed conditions they radiate out from the mantle like the fingers of a hand (Figs. 5A and 5B).

Examination of the long tentacles under the binocular dissecting microscope revealed that they bore a number of papillose-like structures. These projections can only be seen when the tentacles are extended. When contracted the papillae are displayed as a number of tightly opposed annuli, which become broader to the base of the tentacle.

Examination with the scanning electron microscope corroborated and extended the results obtained with the

Fig. 6.

Scanning electron microscope photographs of the long tentacles.

Fig. 6A. Low power SEM of distal third of long tentacle showing many papillae. Arrow shows papillae (x200).

Fig. 6B. Low power view of broken tip of the tentacle. Arrow shows papilla radiating out from tentacle. Note peduncular stalk at base of papillae (x450).

Fig. 6C. Ciliated papillae. Arrow shows cilia at base of papillae. (Tissue soaked in 1.0 mM EGTA prior to fixation) (x1900).

Fig. 6D. Ciliated papillae. Note cellular lip at apex of the papillae. The difference in appearance of the epithelia of 6C and E from 6D is due to the prolonged acid etching of the former (x1827).

Fig. 6E. High power view of papilla, showing large number of cilia (x4750).

Fig. 6F. Proximal portion of tentacle near base. Papillae have been replaced by pads of ciliated cells (x5000).

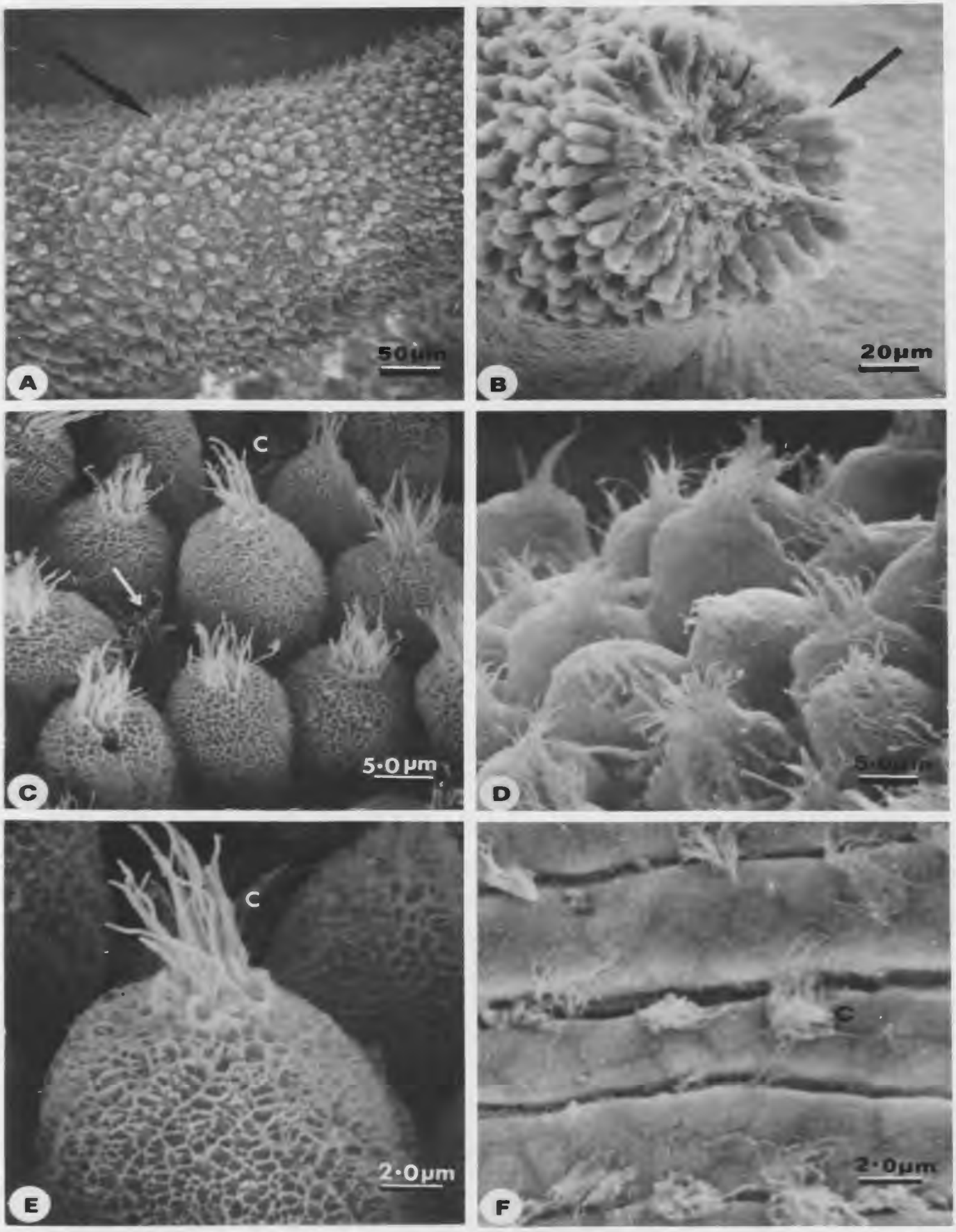
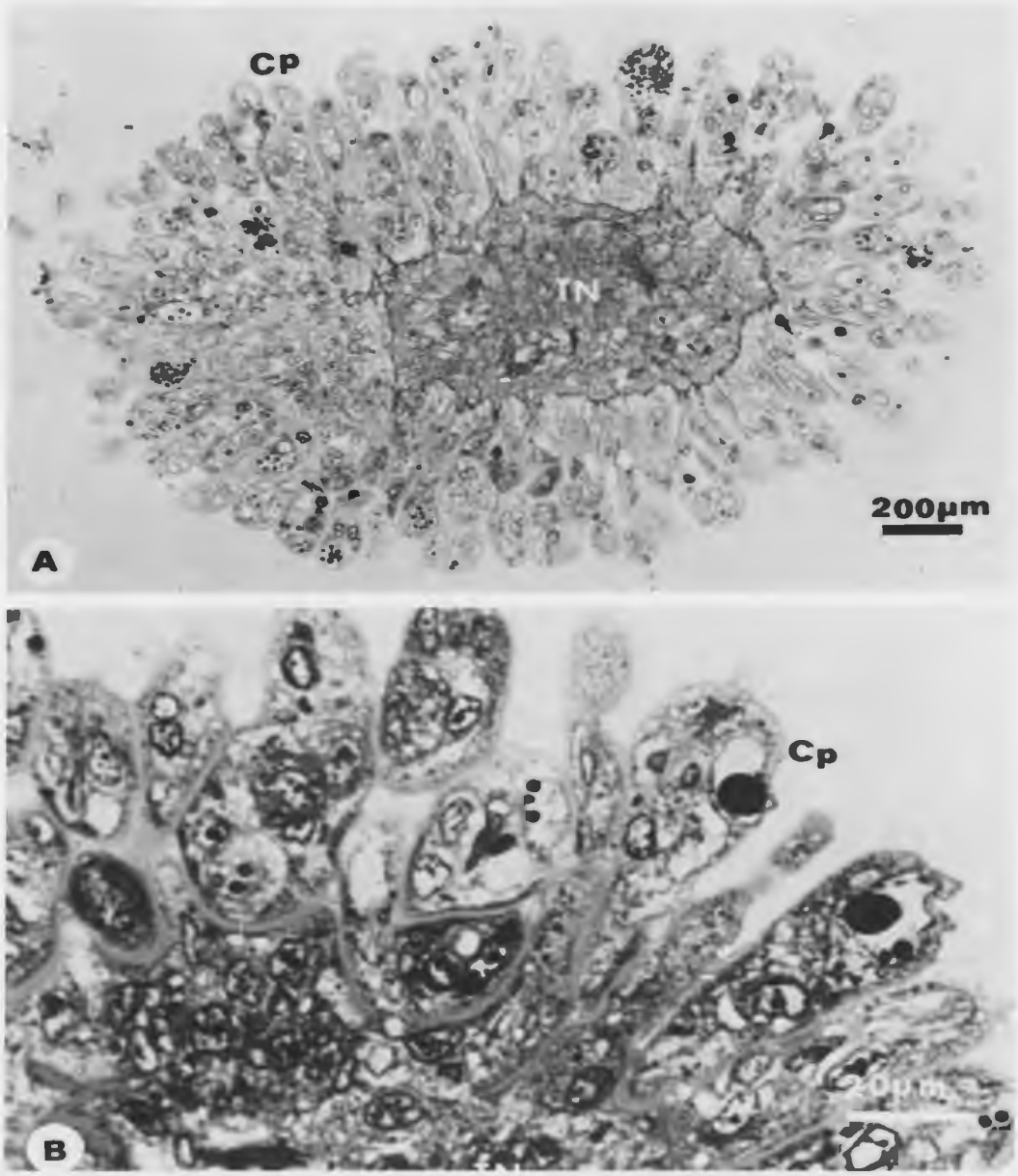

50µm
A
20µm
B
C
5·0µm
C
D
C
2·0µm
E
C
2·0µm
F

Fig. 7.

Light micrographs of Epon embedded sections of long tentacle. Sections 1 μm thick, stained with 1% Toluidine blue at pH 11.1.

Fig. 7A. Low power of whole tentacle tip in cross section (x45).

Fig. 7B. High power of portion of tentacle tip in cross section showing ciliated papillae (x690).


CP
TN
200µm
A
Cp
B

dissecting microscope. It was confirmed that the papillae were confined to the distal third of the tentacle, and that the papillae were very numerous there being approximately $30/mm^2$ of tissue.

The papillae radiate out from the central column of the tentacle to which they are attached by a narrow peduncular stalk at their proximal end (Fig. 6A). These papillae bear many cilia at their apices.

The cilia arise from a central depression and vary from 8 - 10 μm in length (Fig. 6E). The difference in appearance between the epithelia of the papillae in Figs. 6A, B, C, E and 6D and F probably reflects the amount of damage occurring when the samples were acid etched.

There is a strong demarcation between the distal third of the tentacle, which bears the papillae (Figs. 6A-E), and the proximal two thirds, which bear numerous multi-ciliated groups of cells running at 90° to the long axis of the tentacle on the apices of the annuli (Fig. 6F).

Light microscopic examination of thick, epon embedded, sections of the tentacles, showed that the papillae radiated out from the long axis of the tentacle. The central portion of the tentacle is a column of muscle and connective tissue within which is contained the tentacular nerve (Fig. 7A). Depending upon the level at which the section is cut, the dimensions of the central column of the tentacle are approximately 140 x 90 μm. The total

Fig. 8.

Composite diagram of the sensory papilla, with multiciliated cells at base.

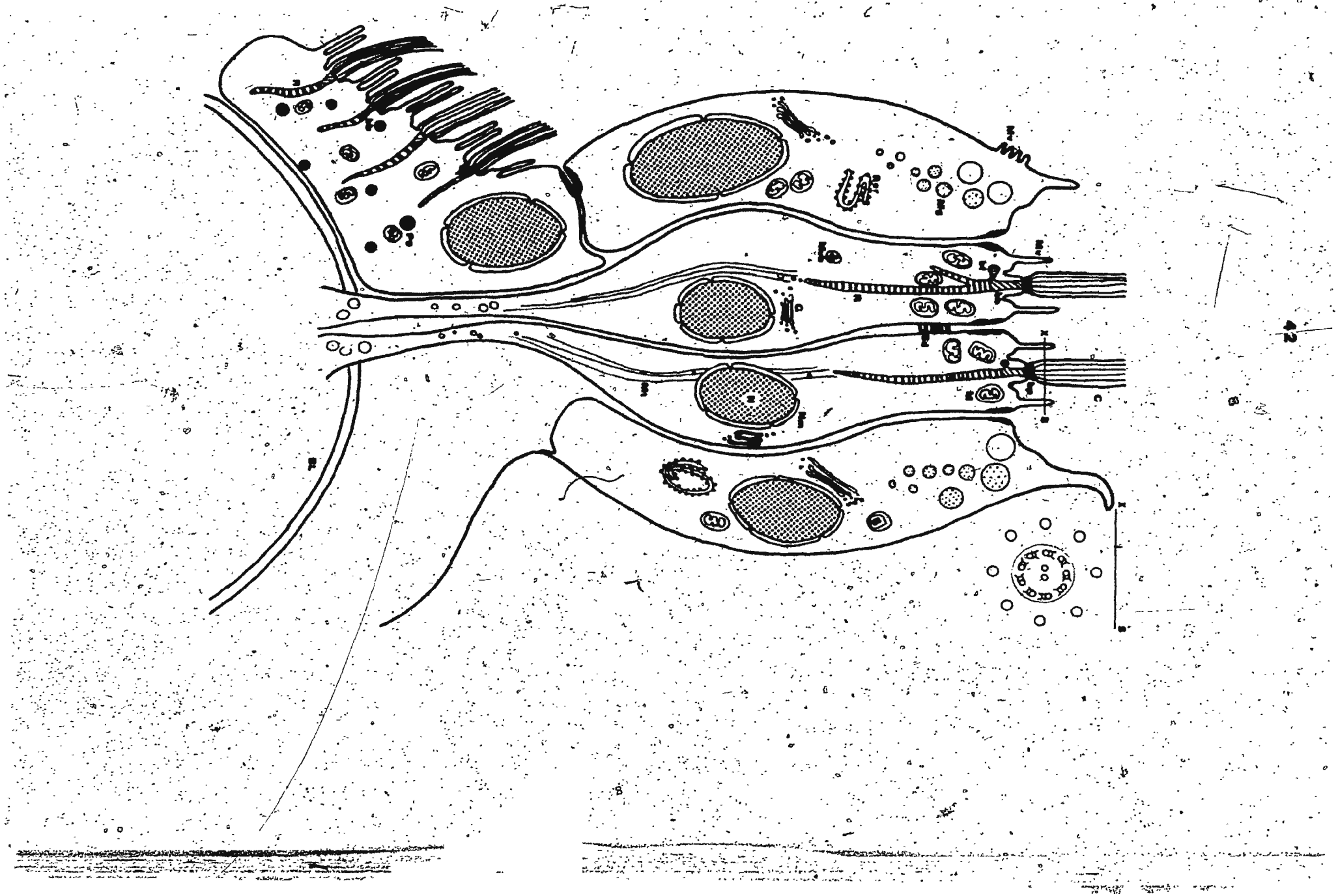
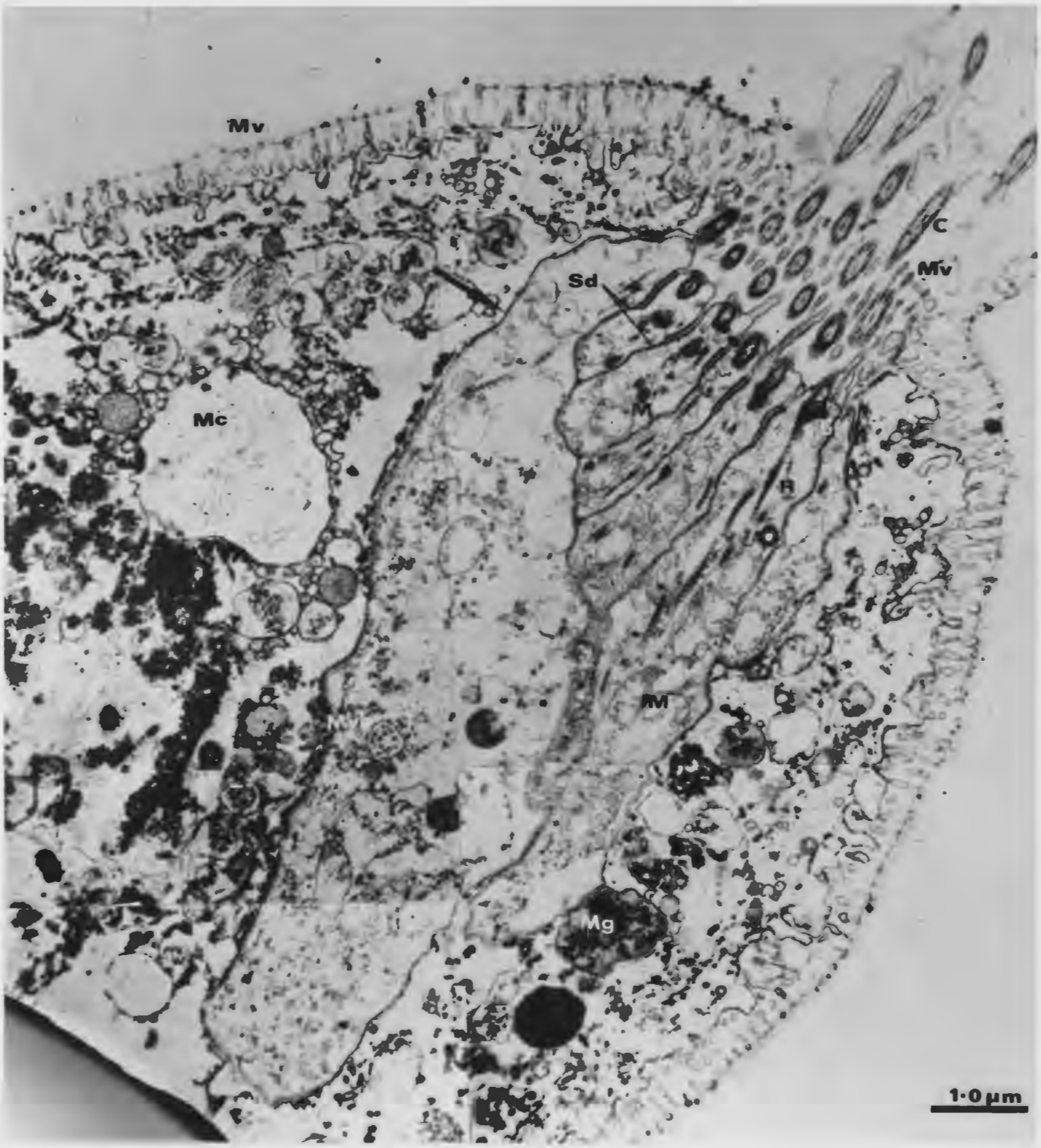


Fig. 9.

Transmission electron microscope (TEM) montage of a ciliated papilla. Arrow shows ciliated sense cell bearing a cilium at its apex. The ciliated cells are within a capsule formed by the mucous cells, and have a fine granular cytoplasm. Section stained with uranyl acetate and lead citrate (x16250).


Mv
Sd
C
Mv
Mc
M
R
M
Mg
1·0µm

cross-section of the tentacle is approximately 300 x 140 um.

It was found that certain of the papillae bear cilia, while others have no ciliated cells but contain cells replete with granules (Fig. 7B). The cells of the papillae are born on a fibrous basal lamina that is approximately 1.0 μm thick. This lamina encloses the central muscle column. The ciliated papillae have a length of 50 to 60 μm, and have an average diameter of 12.5 μm at their widest point. The nuclei of the cells are in the lower portion of their cell bodies. The peduncular region may be seen to contain a number of fibres. In order to clarify the following results, a drawing of the complete structure may be seen in Fig. 8.

Results obtained from ultrathin sections viewed with the transmission electron microscope concurred with those obtained via light and scanning electron microscopy. Transverse sections of the tips of the long tentacles show the papillae in longitudinal section. These can be seen to bear ciliated cells (Fig. 9). The ciliated cells are born within a capsule, which is approximately 7.0 μm across and 20 μm long. The capsule of cells is recessed 0.5 μm from the lip of the papilla. The lip or rim of the papilla bears a cellular projection 0.125 μm long. The ciliated cells are approximately 12.0 μm long, and are 0.5 μm at their narrowest point and 2.75 μm at their widest point. These dimensions will vary depending upon the angle at

Fig. 10.

Transmission electron micrograph of a ciliated papilla. The section passes through almost the entire length of a ciliated sense cell. Section stained with uranyl acetate and lead citrate. (x10800).

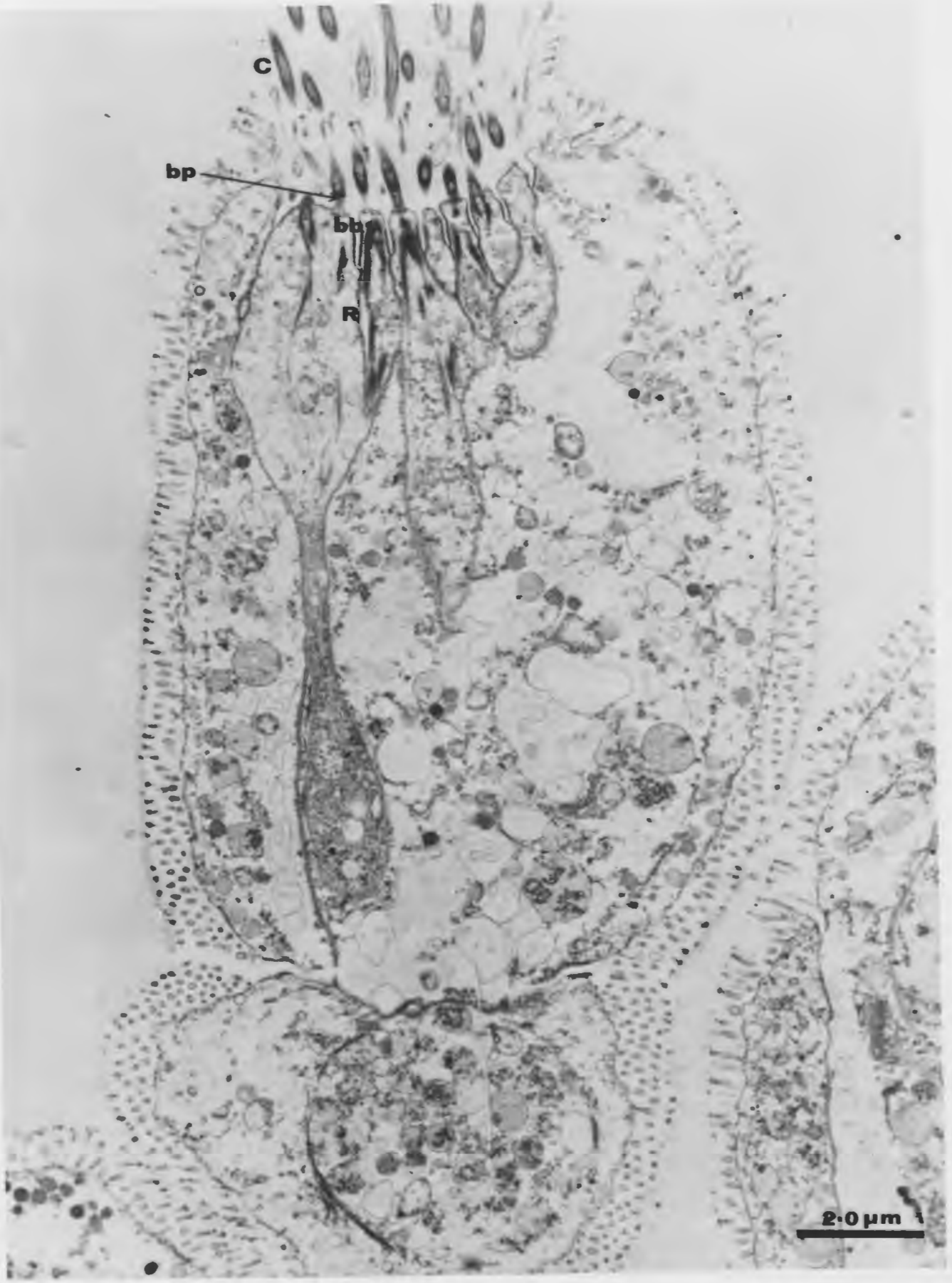
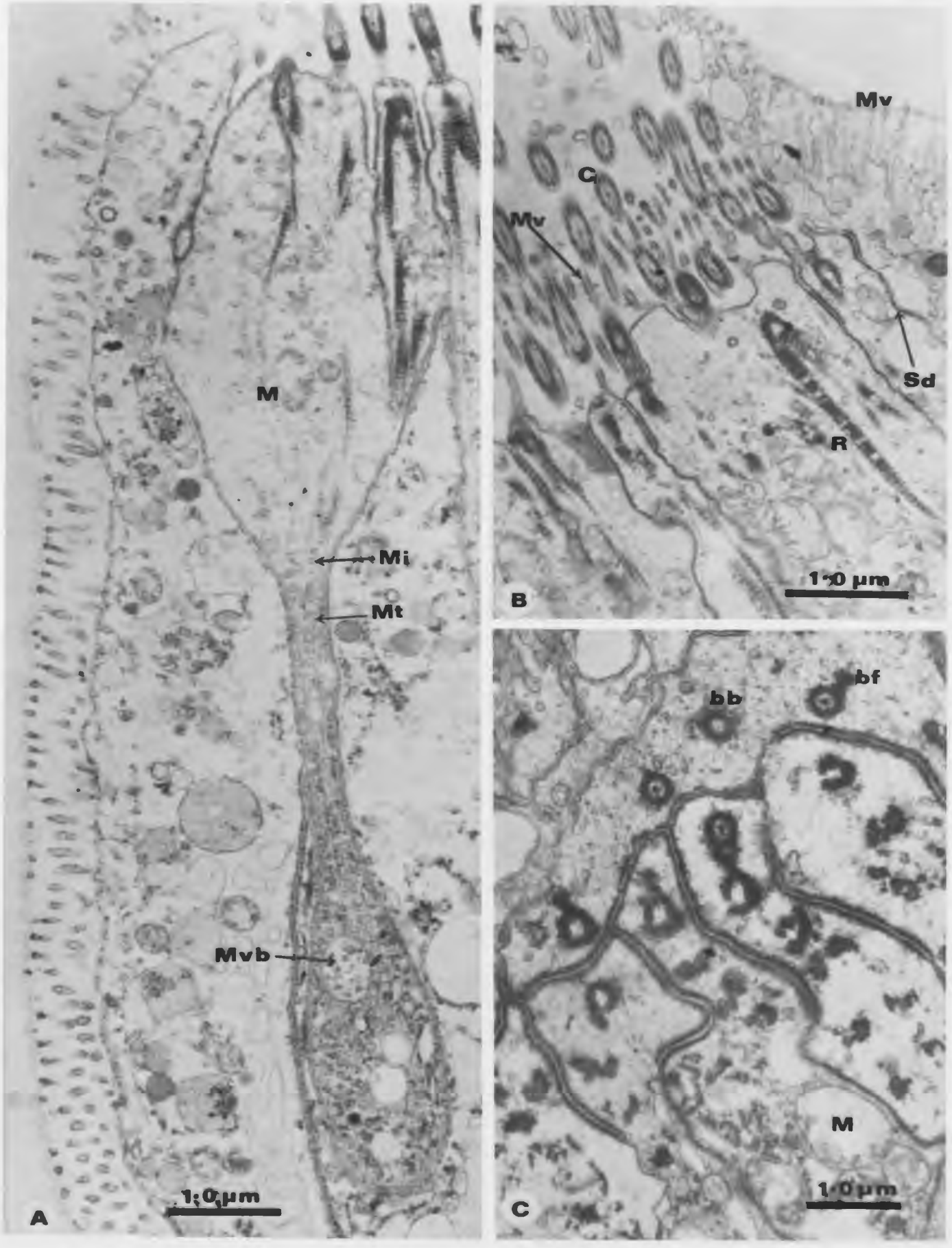

C
bp
bb
R
2·0 µm

Fig. 11.

Fig. 11A. Magnification of longitudinal section of ciliated sense cell from Fig. 10. The proximal portion of the cell bears microtubules between which are microvesicles. Electron-opaque vesicles occur at the base of the cells. Note multivesicular body (x17800).

Fig. 11B. Oblique TEM section of the apex of a ciliated papilla. More than one cilium arises from the apex of each cell. Multiple roots occur, as do septate desmosomes (x19125).

Fig. 11C. TEM cross section at basal body level. Basal feet arise from the basal bodies at a number of different angles (x15300).

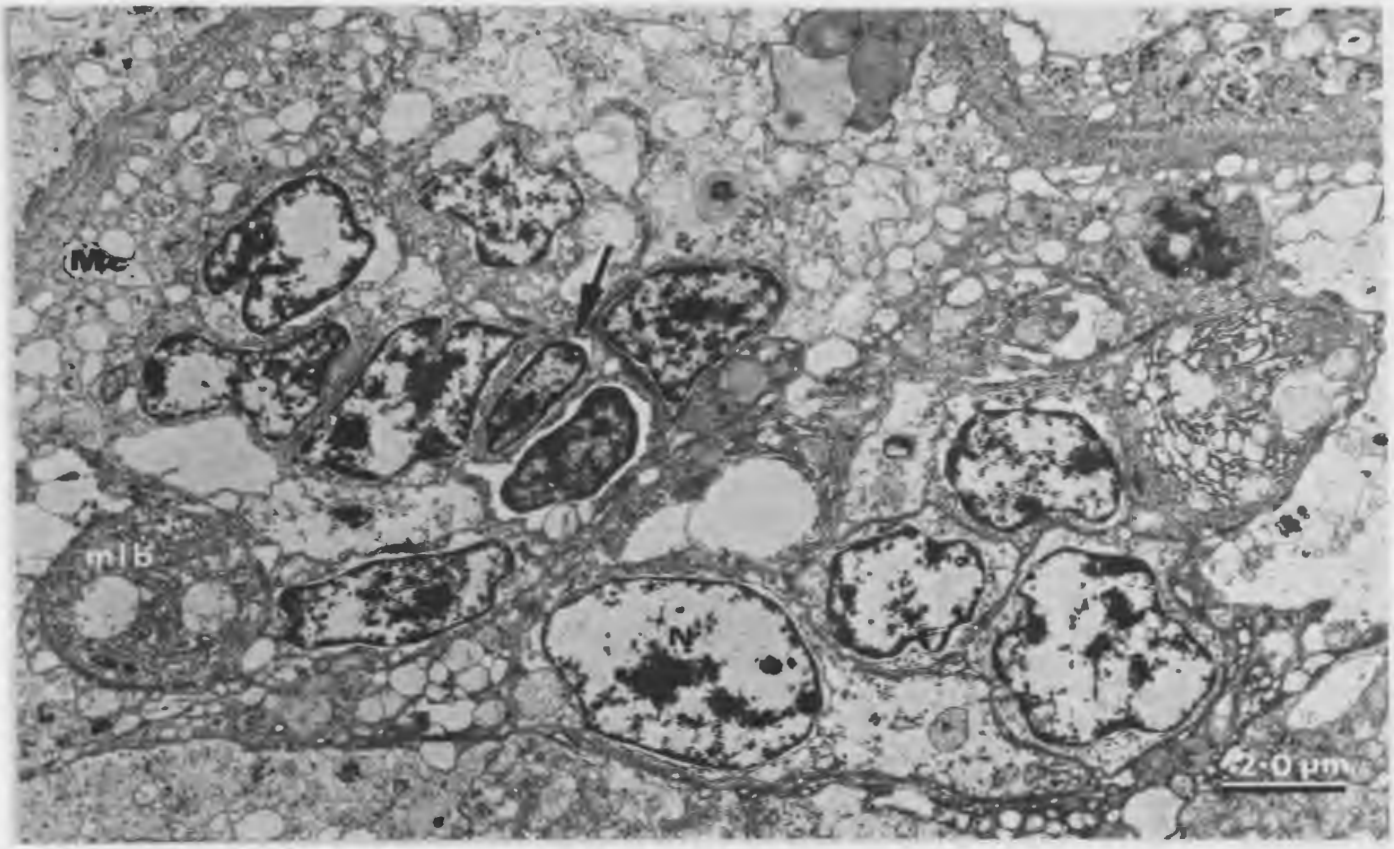

M
Mi
Mt
Mvb
1·0µm
A
Mv
C
Mv
Sd
R
1·0 µm
B
bf
bb
M
1·0µm
C

which the sections were taken. The cells surrounding the capsule are much larger than the ciliated cells being at least 5 μm in diameter and 20 μm long. They contain numerous vacuoles and granules. There are at least two supporting cells per papilla. The exterior of the papilla is covered by microvilli 2.0 μm long, which are embedded in an electron-dense substance that is probably a mucopolysaccharide.

The cytoplasm of the ciliated cells is comparatively electron-dense and is more uniform than that of the surrounding supporting cells. These cells contain many organelles, the most noticeable of which are the cilia at the apices of the cells. The cilia arise from clearly defined electron-dense basal bodies. The cilia are supported on a boss at the apex of the cells (Fig. 9). An electron-dense basal plate lies 0.3 μm from the distal end of the cells, and the peripheral axonemes of the cilia arise from this plate. The basal bodies give rise to striated roots, which may run almost the whole length of the cell. In some sections (Figs. 10 and 11A) microtubules may be seen to be closely associated with the proximal end of the roots. Microvesicles are adjacent to the microtubules and form electron-dense granules at the base of the cell (Fig. 11A). Multivesicular bodies also occur in this latter region. Other organelles are scattered throughout the cytoplasm of these cells. Mitochondria are numerous

Fig. 12.

Cross section of base of papilla at level of nuclei showing that four or five ciliated cells are associated with at least one mucous cell (x6800).


Mc
mlb
N
2·0 µm

in the apical region of the cells (Figs. 9 - 11C), while long septate desmosomes occur between adjacent cells in this region also (Figs. 9 and 11B).

When examined in cross-section the cilia have a normal axonemal complement of 9+2 microtubules. However none of the central pairs of tubules in adjacent cilia have a common alignment. Transverse sections have demonstrated that there are as many as five cilia per cell in the papillae, and that in certain sections basal feet are attached to the basal bodies (Fig. 11C). The basal feet do not have a common alignment and consequently the cilia probably do not beat in a metachronal manner.

Sections towards the base of the papillae elucidate the structure of the capsule (Fig. 12). The capsule is composed of two bundles of four or five cells each cell associated with a supporting or mucous cell. The nuclei of the ciliated cells are smaller than the nuclei of the supporting or mucous cells and contain much dense chromatin. The chromatin of the supporting cell nuclei is more diffuse than that in the ciliated cells. Highly vacuolated areas partially encircle the capsule. These correspond to the supporting cells, which in the longitudinal sections may be seen to flank the capsule (Fig. 9).

When tissue that had been relaxed in EGTA prior to fixation was examined under the scanning electron microscope, cilia could be seen to arise from the base of the

Fig. 13.

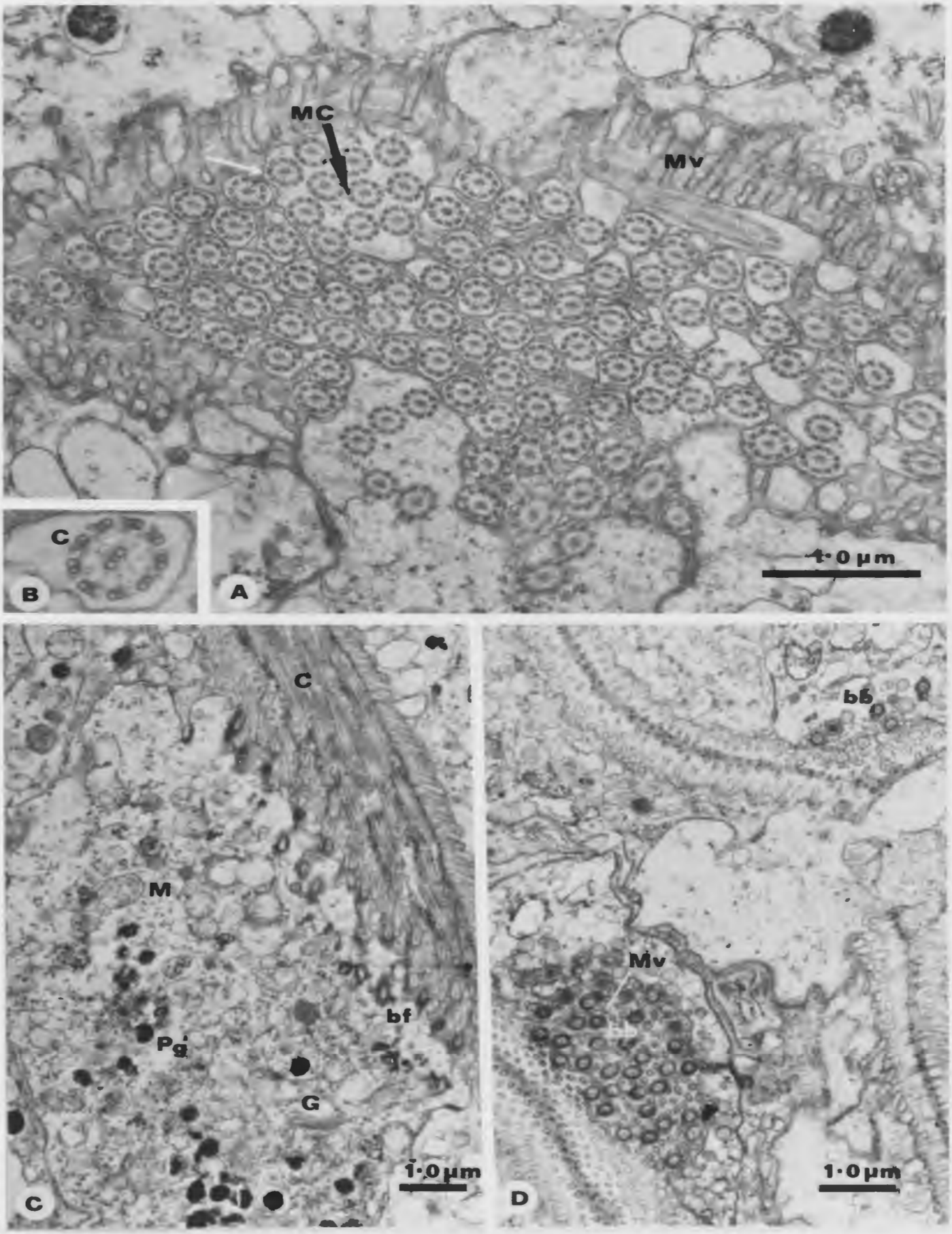
Cross sections of multiciliated cells at the base of the papillae.

Fig. 13A. Cross section of a multiciliated cell showing numerous cilia with 9+2 sub-structure. White arrow denotes single membrane around macrocilium (x24862).

Fig. 13B. Inset of high power TEM of a cross section of a cilium (x46375).

Fig. 13C. Longitudinal section of multi-ciliated cell. The cytoplasm of the cell is characterised by numerous pigment granules, Golgi apparatuses, and vacuoles. (x10400).

Fig. 13D. Cross section of multiciliated cell at level of basal body showing each cilium surrounded by microvilli (x12900).


MC
Mv
C
B
A
1·0 µm
C
M
Pg
bf
G
1·0µm
C
bb
Mv
1·0µm
D

papillae (Fig. 6C). Figures 13A and 13D are cross sections of the base of the papillae containing one of these ciliated cells. As with cilia of the papillae, these cells have cilia with the normal complement of 9+2 microtubules and each cilium is surrounded by a ring of microvilli projecting from a cellular rim. The cilia run between the papillae towards the peripheri of the tentacle (Fig. 13C). However unlike the cilia of the papillae the central tubules of the adjacent cilia do have a common alignment. Macrocilia have also been observed and these contain from two to eleven cilia with normal axonemal complements within a single ciliary membrane (Fig. 13A). These cilia may be seen to arise from cells approximately 10.6 μm in diameter. These cells contain many pigment granules, mitochondria, and Golgi apparatuses.

No tissue can unequivocably be described as nervous tissue, although a number of different methods were employed to attempt to investigate this possibility. However, the presence of microtubules in the base of the cells suggests that the proximal end of the cells become an axon. The diameter of the base of the cells of approximately 0.35 μm is similar to that described for axons in primary chemoreceptors (Laverack, 1974). The problem with determining whether the tissue is nervous or not is that much of the tissue appears vacuolar as one would expect nervous tissue to appear. This will have to

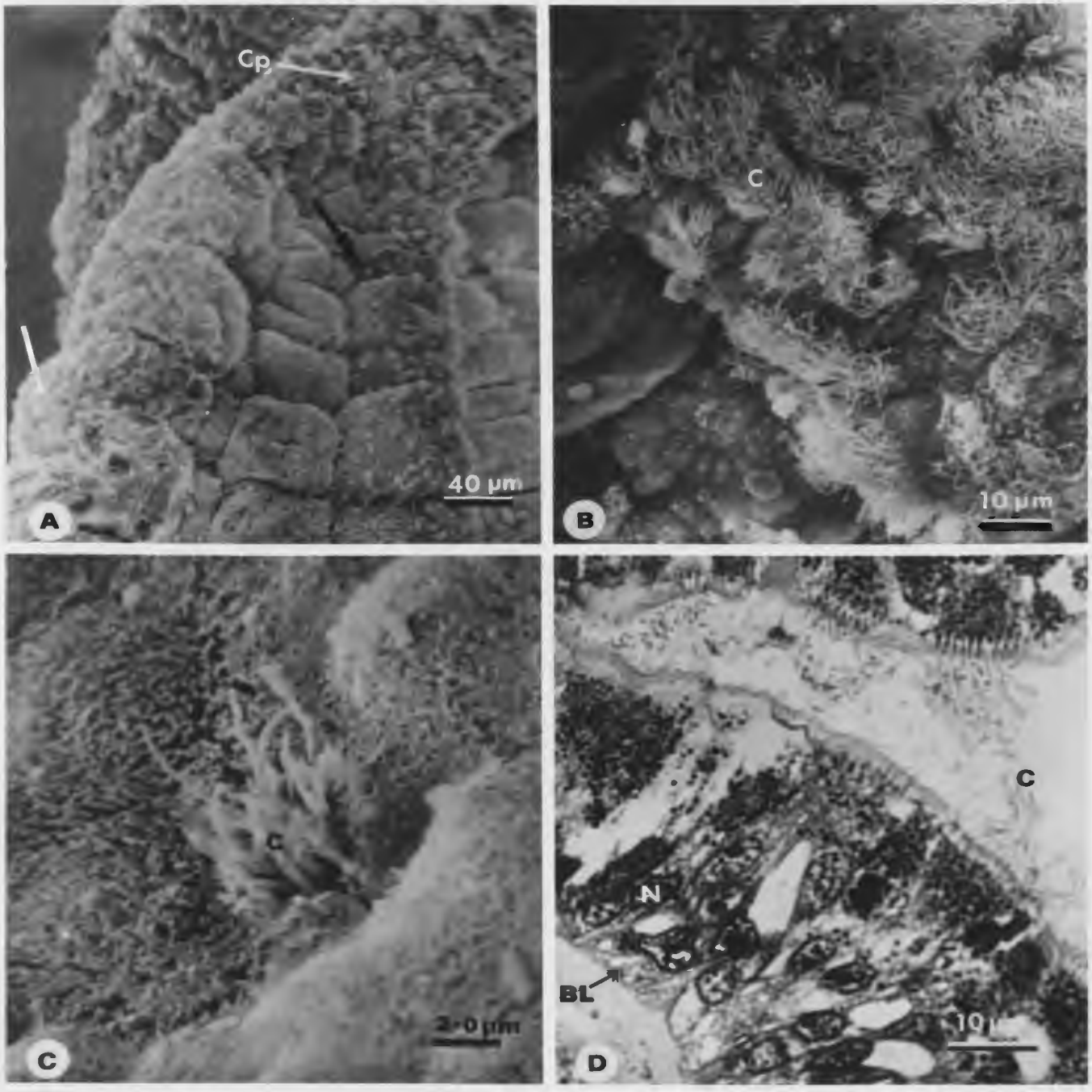
Fig. 14.

Fig. 14A. SEM photograph of the apical portion of a short tentacle. White arrow shows ciliated pad cells on convex portion of the tentacle, black arrow shows ciliated pits on concave portion of the tentacle. Ciliated papillae are arranged in the extreme tip of the tentacle (x250).

Fig. 14B. SEM of ciliated pads of cells (x970).

Fig. 14C. SEM of a ciliated pit. Cilia may be seen to arise from the centre of the pit (x4850).

Fig. 14D. Light micrograph of a 1 µm thick Epon section of the ciliated pads of the short tentacles (x560). Section stained with 1% Toluidine blue at pH 11.1.


Cp
40 µm
A
C
10 µm
B
C
2·0 µm
C
C
N
BL
10 µ
D

be clarified by histological staining techniques.

3. Short Tentacles

Some ciliated cells have been observed on the short tentacles. A preliminary investigation of their structure was made. The study was not extensive since it was decided to concentrate upon investigating the ciliated cells of the long tentacles.

The short tentacles are approximately 10 mm long when extended (Fig. 5B). They are spatulate in shape, their ventral surface being concave, and in many individuals the distal portion of this surface may be darkly pigmented.

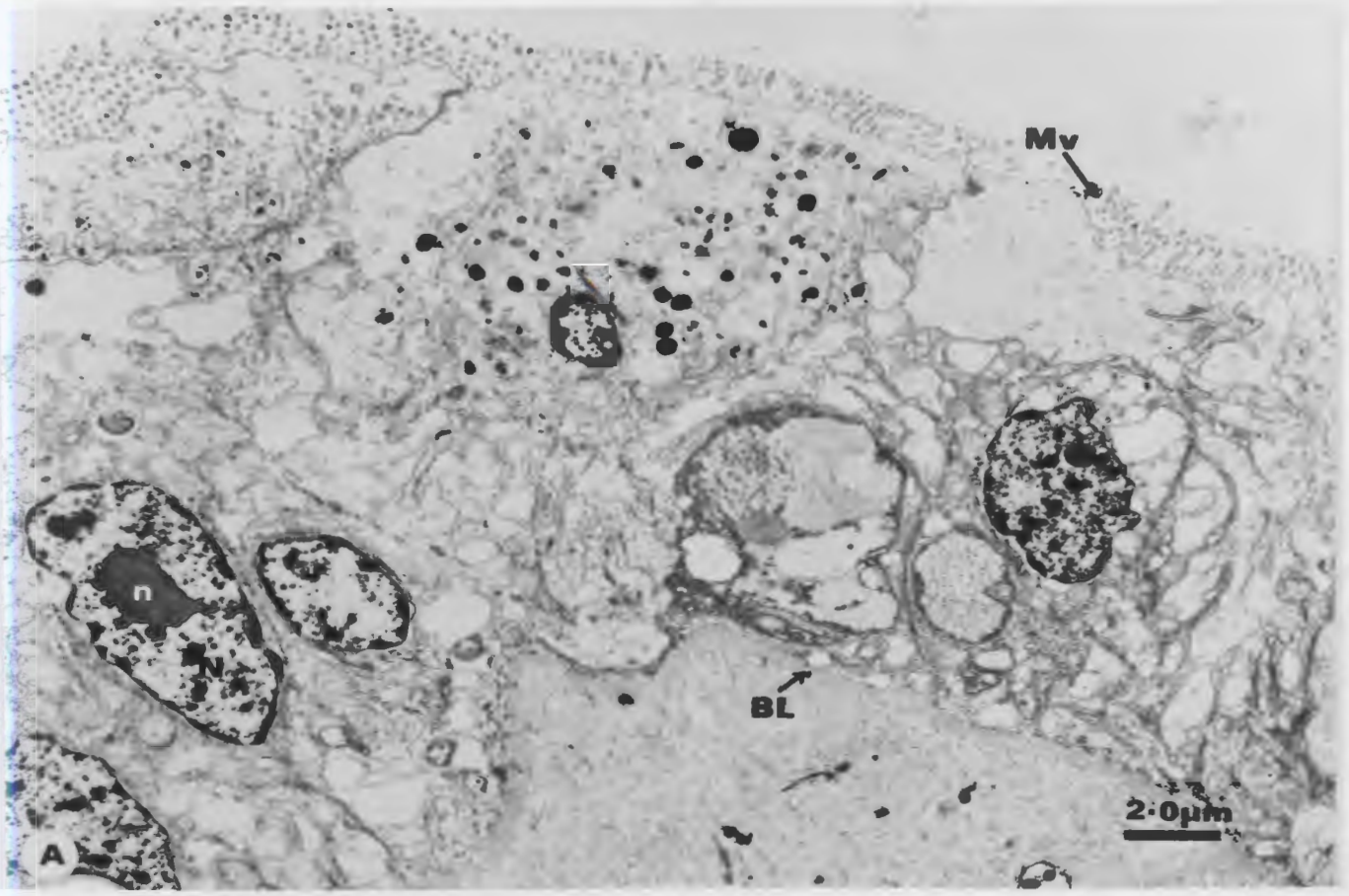
When examined under the binocular dissecting microscope, papillae, similar to those described on the long tentacles, were observed. These were confined to the very tip of the tentacle and did not cover the distal third of the tentacle as was seen to occur on the long tentacles. More detailed examination of the tip revealed that the papillae were indeed similar to those previously described (Fig. 14A).

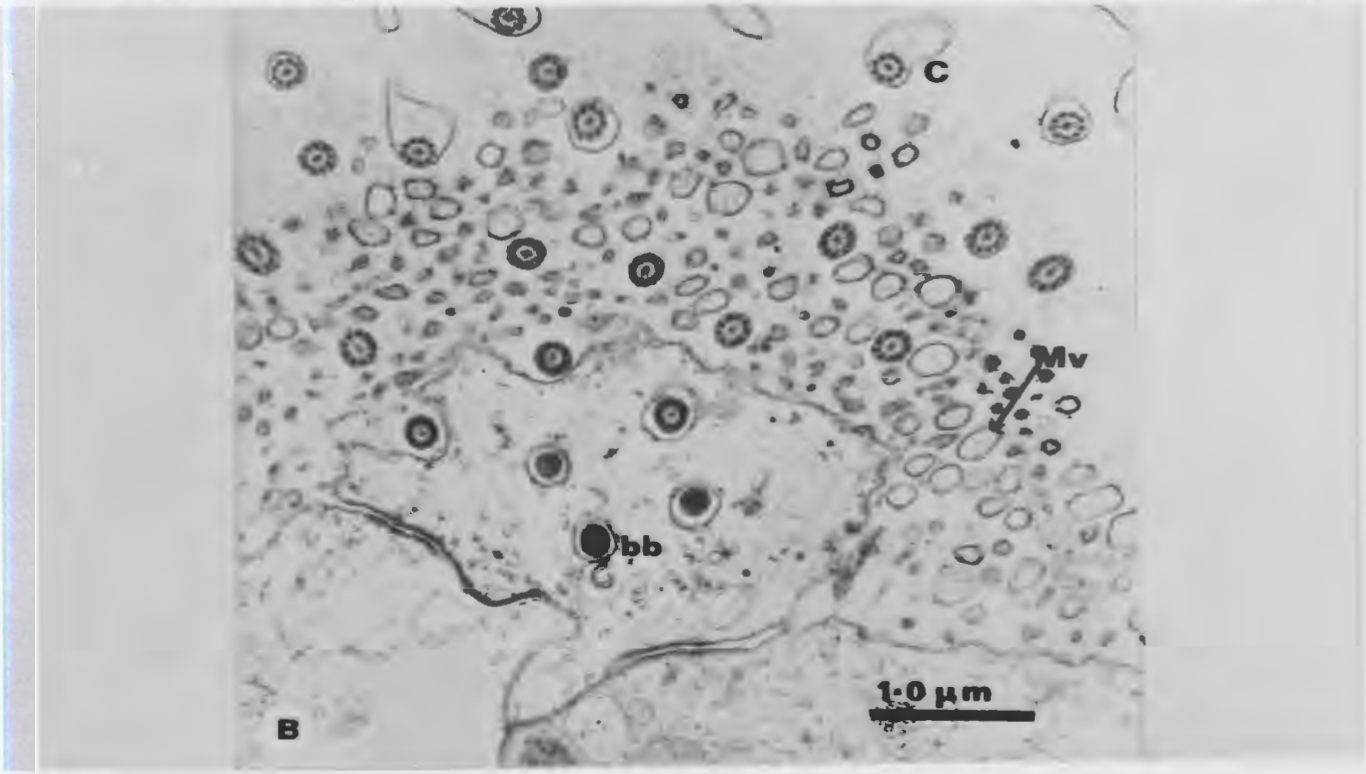
Examination of the ventral convex portion of the tentacle revealed further ciliated structures. The most common of these were found on all surfaces of the tentacles below the tip and consisted of a cell or a number of cells each of which bore a tuft of cilia. The cilia were approximately 10 μm long. These tufts of cilia resembled those found at the base of the long tentacles.

Fig. 15.

Fig. 15A. Low power TEM photograph of a section of short tentacle. The entire surface is covered by numerous microvilli. The section passes obliquely through the top of a ciliated pad cell (x600).

Fig. 15B. Oblique section through the apex of a ciliated pad cell (x19200).


Mv
n
N
BL
2·0µm
A


C
Mv
bb
1·0 µm
B

The second structure consisted of a number of ciliated cells that formed a pit in the surface of the tentacle. The pit-like structures could only be observed on the ridges of the ventral surface of the tentacle (Fig. 14A). The length of cilia arising from the pits was similar to those of the ciliated tufts, being in the range of 8 - 10 μm long. The diameter of the pit was approximately 0.4-0.5 μm.

Epon sections 1.0 μm thick were examined under the light microscope and confirmed certain of the results obtained with the scanning electron microscope. The tufts of cilia may be seen to arise from a single cell (Fig. 14D). The dimensions of these cells varied from 8 to 9 μm in diameter and they were approximately 14 μm long. Numerous cilia could be observed at the apices of these cells. The cells were heavily pigmented thus obscuring the nucleus.

The ciliated cells that formed the pits were not observed under the light microscope.

a) <u>"Tufted" cells</u>

Fig. 15B is an electronmicrograph of an ultrathin oblique section through a tufted cell. The section passes through the cell at the level of the basal bodies. The cilia have a normal axonemal complement of 9+2 microtubules. Each cilium is surrounded by a number of microvilli of various diameters. The microvilli are 2.0 μm long and arise from a shoulder around the cilium. The cilia arise from the pit formed by this structure. The cilia have

Type II basal bodies and striated roots. There are many mitochondria surrounding the base of each ciliary root near the apex of the cell.

b) Pit cells

The pit is composed of more than one cell and each of these cells gives rise to a number of cilia. The cilia have the normal 9+2 complement of microtubules. The cells are much smaller than the tufted cells and vary between 2 to 3 μm in diameter at their apex. Long microvilli cover the surface of the cells forming a thick array in the central pit.

The epidermis of the short tentacles is supported on a basal lamina 0.3 μm thick. The basal lamina is attached to a central column of muscle. Other cell types may be observed in the epidermis. These include large mucous cells 10.0 μm wide by 11.0 μm in length, containing much rough endoplasmic reticulum, Golgi apparatuses, and a number of pigment granules (Fig. 15A).

4. Abdominal Sense Organ

The abdominal sense organ which was first described by the German histologists can easily be observed with the naked eye, being slightly yellowish in colour in contrast to the white of the mantle tissue. The organ lies on an elevated ridge of tissue, that runs from a point of insertion inferior to the anus, to a point of attachment

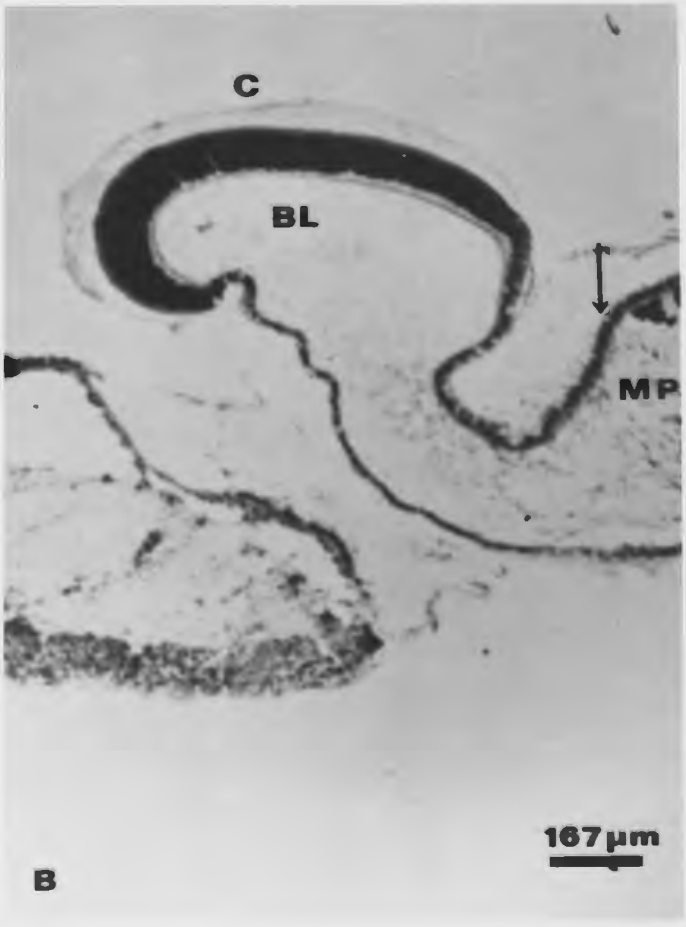
Fig. 16.

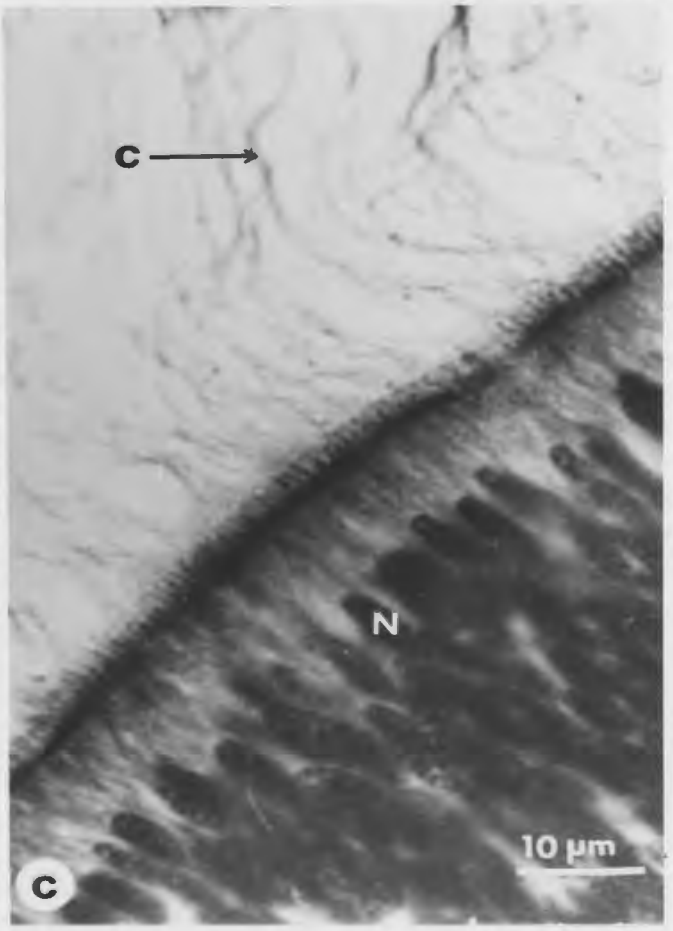
Fig. 16A. Whole mount of the abdominal sense organ viewed with the binocular dissecting microscope. The bulbous end of the sense organ is pointed towards the anus, the tapered portion is orientated towards the free edge of the mantle. The sense organ is mounted on a membrane (x48).

Fig. 16B. A light micrograph of a wax section of the abdominal sense organ. Section is transverse to the long axis of the sense organ. Arrow indicates cilia, which are commonly found covering the mantle (x60).

Fig. 16C. High power light micrograph of the distal portion of the sensory epithelium. Note the length of the cilia, and the dark band at base of the cilia, which represents the basal bodies of the cilia (x1400). Sections 16B and 16C stained in Mallory-Heidenhein's rapid process one step stain. Sections are 7 μm thick.


C
MP
208 µm
A


C
BL
MP
167 µm
B


C
N
10 µm
C

on the left mantle. The position of the organ is such that it lies at approximately ninety degrees to the direction of the exhalent current sea water flow.

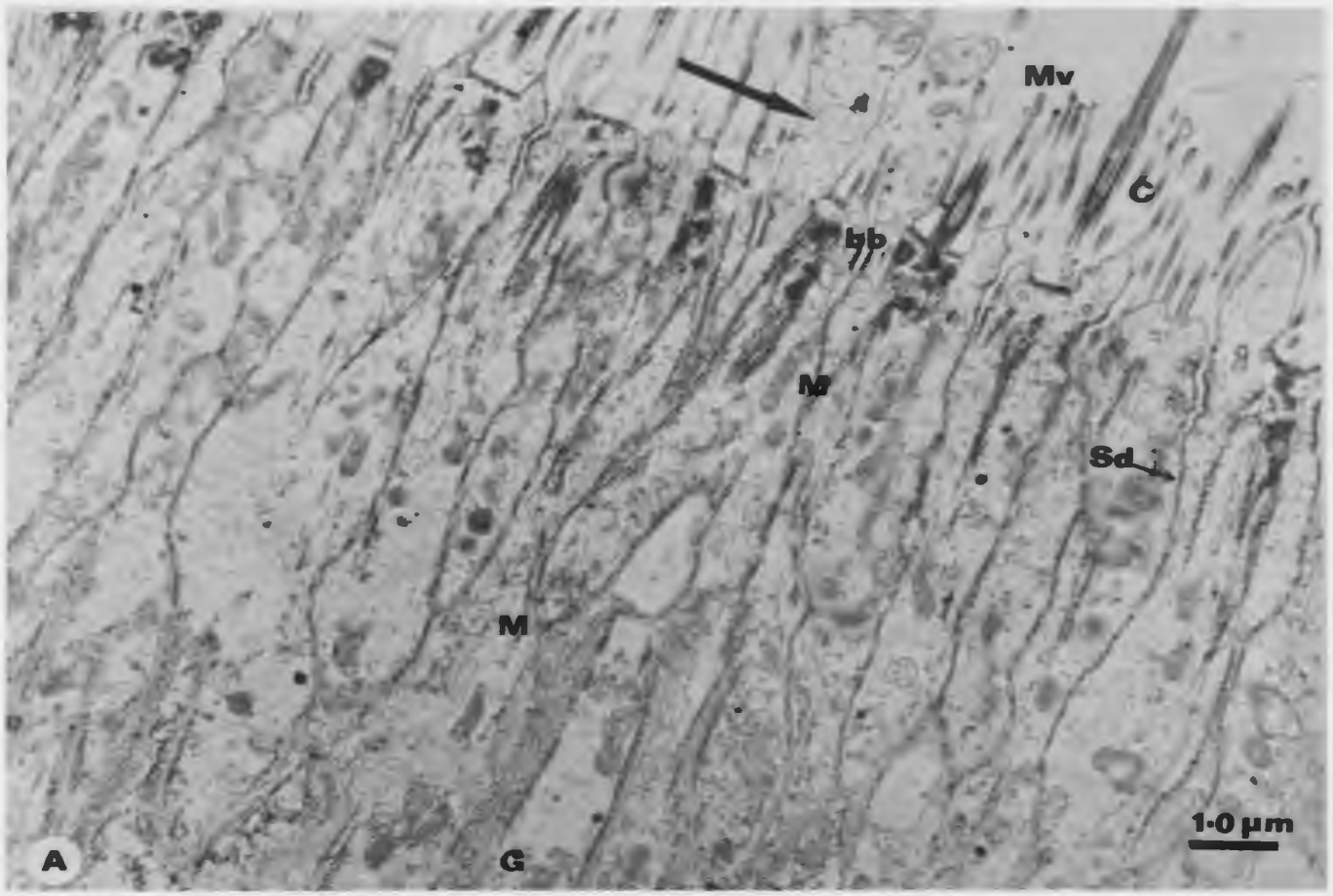
The organ is a ciliated pad of tissue, approximately 3 mm long and 0.5 mm wide at its widest point. It tapers in size from the raised bulbous portion proximal to the anus, to the point of attachment at the left mantle. When viewed under the binocular dissecting microscope, long cilia may be observed, which appear as an opalescent halo that covers the free edge of the organ (Fig. 16A). Dissection of the base of the organ and its surrounding tissue, demonstrated that it was innervated by the post pallial nerve and by a number of nerves arising from the free edge of the mantle.

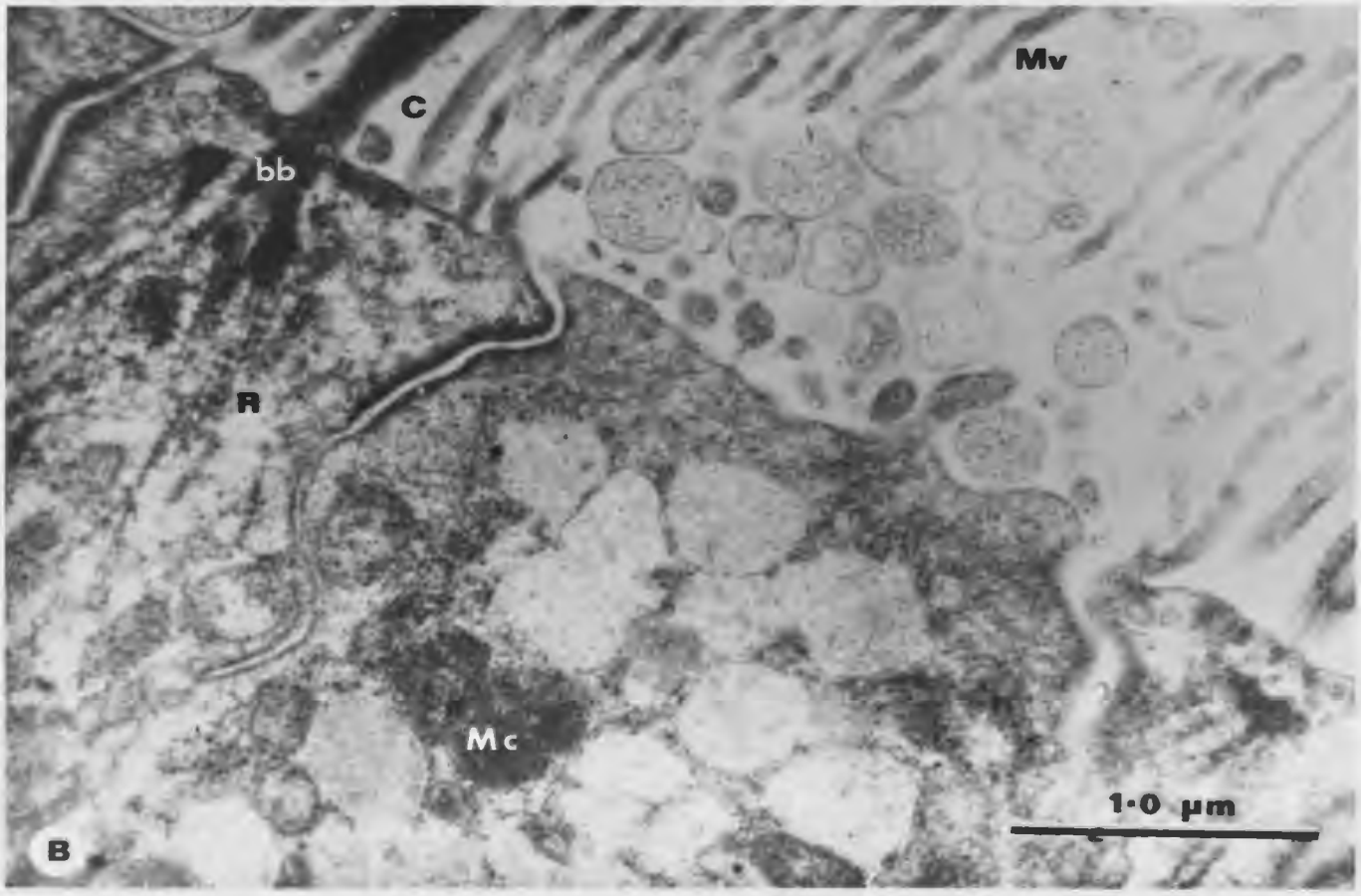
Thick sections of wax and epon embedded tissue confirmed the previously described findings. The tissue can be divided into several different regions (Figs. 16B and 16C). The total width of the organ, from the densely stained band of tissue that runs around the apical periphery, to the basal lamina, is between 125 - 130 μm. The densely stained apical band is on an average 1.0 - 1.5 μm thick. The cellular portion of the organ consists of an apical region without nuclei, which is 10 - 12 μm thick, and a nuclear region which is 100 - 110 μm thick. The cells are columnar and the previously described long cilia arise from their apices. These cilia are 60 - 70 μm long, the

Fig. 17.

Fig. 17A. Longitudinal section of abdominal sense organ cells. Each cell bears a single cilium, surrounded by a double row of microvilli. Arrow shows a much enlarged cellular projection filled with vacuoles (x9600).

Fig. 17B. High magnification of the apex of the sensory epithelium of the abdominal sense organ. A mucous cell is flanked on either side by a ciliated cell. Multiple roots arise from the basal bodies (x34285).


Mv
C
bb
M
Sd
M
1·0 µm
A
G


Mv
C
bb
R
Mc
1·0 µm
B

length varying, depending upon whether the measurements were taken from a wax-embedded section or an epon section, the greater degree of shrinkage occurring in wax embedded sections. Towards the mantle end of the organ, the cilia are considerably shorter and resemble those commonly occurring over the majority of the mantle surface (Fig. 16B). The cells are mounted on a layer of loose fibrous tissue, resembling parenchyma; with few nuclei, and this is in turn attached to the basal lamina. The fibrous tissue is 8 - 10 μm thick and the basal lamina is 3 - 4 μm thick.

Ultrathin transverse sections were examined under the transmission electron microscope. Two distinct cell types could be seen. The first type consisted of cells that contain many large mucous droplets (Figs. 17A and 17B), which are flanked on either side by more numerous and smaller cells each bearing a single cilium. A possible third cell type of intermediate size, and bearing numerous cilia, was also observed.

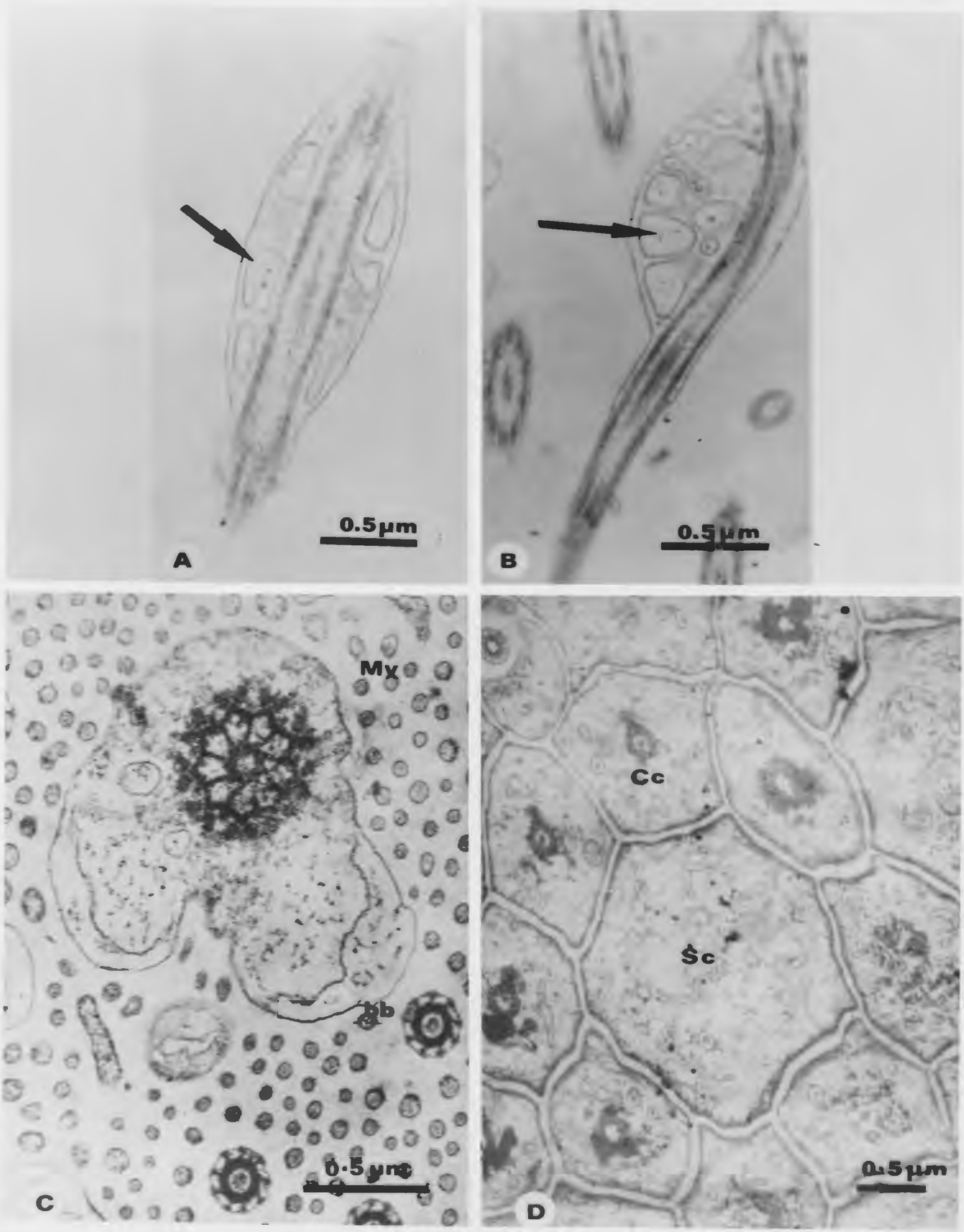
The cells with a single cilium are fairly uniform in size, their diameter varying from 0.8 - 1.4 μm. However their total length is unknown. At the apex of each cell is a cellular projection 0.3 - 1.0 μm in length. A double row of microvilli that are 1.0 - 2.7 μm long arise from the free edge of this projection. The projection may contain elaborate membrane whorls, and in these cases they are much enlarged, being approximately 1 μm across and up to 2.5 μm

Fig. 18.

Fig. 18A and 18B. Longitudinal section through the ciliary shaft of cilium from the abdominal sense organ showing the enlargement of the ciliary membrane. Arrow shows vacuoles within ciliary membrane (Fig. 18A x33000, Fig. 18B x35100)

Fig. 18C. Transverse section through the basal body region of the cilia. Each cilium is surrounded by a double row of microvilli (x38680).

Fig. 18D. Transverse section of a supporting or mucous cell surrounded by eight ciliated cells (x23800).


0.5µm
A
0.5µm
B
Mx
Xb
0.5µm
C
Cc
Sc
0.5µm
D

long or greater. The cytoplasm within the projection is less electron-dense than that of the parent cell, but contains a number of saccular inclusions. Elaboration into tightly concentric membranes also occurs in the microvilli.

The circum-apical projection surrounds an indentation at the center of the cell which is 0.01 μm in depth, and in which is contained a boss from which arises a cilium (Fig. 17A). The cilia have elongated basal bodies 1.1 - 1.7 μm long, which are characterized by two extremely electron-dense vertical bands 160 nm apart and corresponding to the peripheral axonemes. These are connected to an equally electron-dense basal plate. The cytoplasmic material between the peripheral axonemes is more electron-dense than the shaft of the cilium. The length and position of the basal bodies corresponds to the densely stained peripheral band observed in the thick sections. Basal feet may also be seen at the level at which roots are given off (Fig. 17A).

Longitudinal sections of the ciliary shafts and tips revealed that certain cilia were surrounded by sac-like swellings of numerous shapes and sizes (Figs 18A and 18B). The cilia are normally between 200 and 250 nm in diameter except where they are swollen, in which case they may be as much as three times as broad. The sacs on the cilia contain a number of clear or slightly granular vacuoles which vary from 20 nm to 150 nm in diameter. Cross-sections

of cilia near their tips revealed that they had the normal 9+2 axonemal microtubular complement and peripheral tails. The ciliary membrane, in those cilia which have sacs, is modified on one side into the sac, and is connected to the ciliary shaft by a narrow duct. The central fibrils of a single row of cilia have a common orientation, but the central fibrils in parallel rows do not have a common alignment. Sections taken at the level of the basal bodies showed that the cilia are surrounded by a double row of microvilli.

Numerous striated roots, which run down the length of the cell for a distance of some 10 to 15 μm, ending at the nucleated portion of the cells, arise from the basal bodies (Fig. 17A). There may be as few as one, or as many as eight roots per cilium. Septate desmosomes lie between the shoulders of the apices of the cells (Fig. 17A).

Mitochondria may be observed in large numbers in the non-nuclear portion of the cells. Golgi apparatuses are frequently present in close proximity to the nuclei (Fig. 17B) but little granular endoplasmic reticulum is present. Tightly apposed whorls of membranes are common throughout the cytoplasm of the cells (Fig. 17A).

Cross-sections demonstrate that between eight and twelve ciliated cells surround each mucous cell (Fig. 18C). The mucous cells are larger than the ciliated cells being 2.6 to 5.0 μm in diameter, but having an apical diameter

of 2.0 μm. Longitudinal sections show that the upper 5 μm of the cells usually contains a number of clear vacuoles, whilst the remainder of the cell is packed with large electron-opaque mucous grains of various sizes up to 1.0 μm in diameter. Secreted mucous droplets occur outside the apex of the mucous cell. The cytoplasm of the mucous cells contains much rough and smooth endoplasmic reticulum and also Golgi apparatuses (Fig. 17B).

Multi-ciliated cells have only been observed in cross-sections of the organ. These cells are intermediate in size between the mucous and single-ciliated cells, being approximately 2.0 μm in diameter. The cilia from these cells do not have surrounding double rows of microvilli but appear as a single clump of cilia with no definite spatial arrangement. As many as 20 roots may be found in these cells, which arise from complex basal bodies whose size is three times that of a normal basal body. Fig. 18B is such a structure taken near the level of the basal plate and is 0.75 μm across. The cytoplasm of these cells is more uniformly electron-dense than that of the mucous cells.

5. Histological Sections

The circumpallial nerve, as has previously been stated, arises from both the visceral and cerebral ganglia and runs around the mantle rim. Branches to the eyes, tentacles, and to the velum, are given off at appropriate intervals.

Fig. 19.

Fig. 19A. 7 µm thick transverse section of the mantle margin. The circumpallial nerve lies inferior to the circumpallial blood vessel. Tissue stained in Mallory-Heidenhein's rapid process one step stain (x55).

Fig. 19B. 7 µm thick transverse section of mantle margin. Tentacular nerve arises from the circumpallial nerve. Collagen fibres from the collagen sheath around the tentacular nerve run towards the tentacle peripheri (x200).

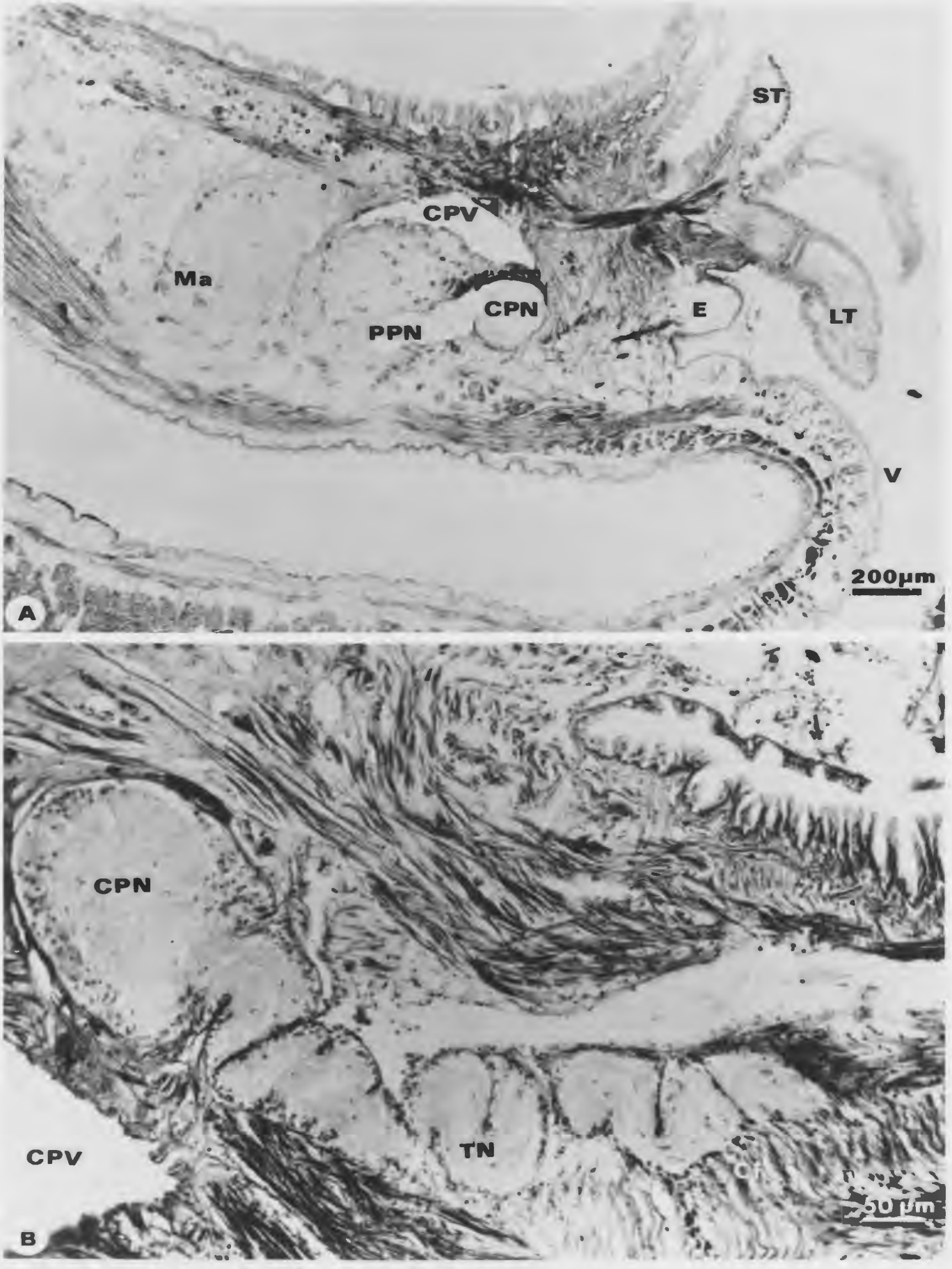
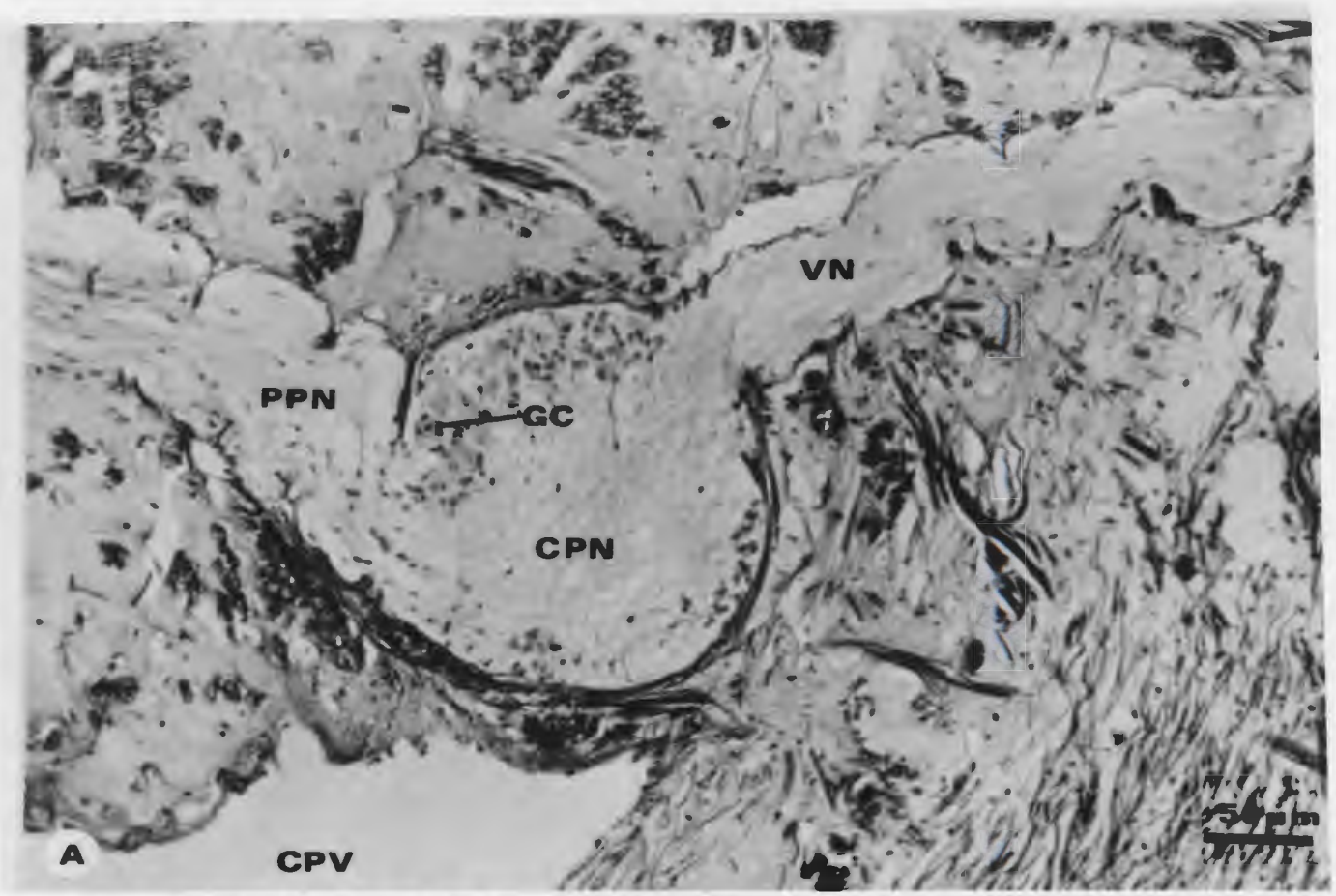
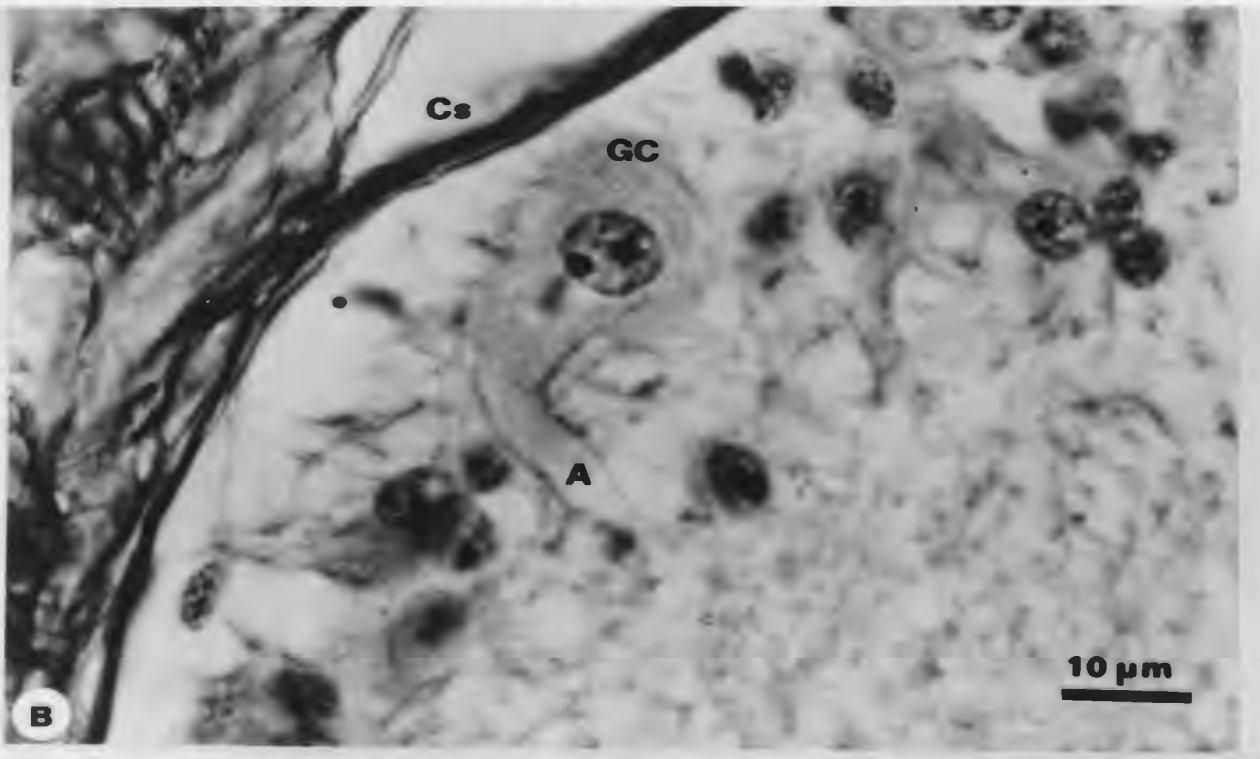

ST
CPV
Ma
CPN
PPN
E
LT
V
200µm
A
CPN
CPV
TN
B

Fig. 20.

Fig. 20A. Transverse section showing the point of origin of the post pallial and velar nerves with a large group of cell bodies (x240).

Fig. 20B. High magnification of ganglion cell body from which an axon is arising (x1400).


VN
PPN
GC
CPN
A
CPV


Cs
GC
A
10 µm
B

This nerve is also innervated from the visceral ganglion directly via the pallial nerve at a number of points throughout its length. This observation is contrary to that which was stated by Dakin (1910a), who said that the pallial nerves directly innervated the eyes. Fig. 19A is a low power light micrograph of the mantle rim. The circumpallial nerve is seen in cross-sectional view and lies immediately below the circumpallial blood vessel. The circumpallial nerve is 225 µm in diameter and is surrounded by a collagen sheath 2 µm thick. Cell bodies occur frequently and are in their greatest concentrations at those points where there are junctions of the nerves from either the peripheri or the visceral ganglion via the post pallial nerve (Figs. 19B and 20A). Cells as large as 14 µm in diameter may be seen to give off an axon that may be as large as 4.0 µm in diameter. Smaller cells also occur and some of these also give rise to axons (Fig. 20B).

The tentacular nerve runs in between sheets of muscle from where it leaves the circumpallial nerve to the tip of the tentacle. At the base of the tentacle the nerve is much folded and is 100 - 175 µm in diameter whilst at its tip the path of the nerve is more direct and the nerve is some 50 µm smaller. This nerve too is surrounded by a collagenous sheath, 1.5 µm thick. At the distal portion of the tentacle the nerve is supported in the haemocoelic space by a number of bridges of collagen which run to the

tentacular margin (Fig. 20B). The tentacular nerve also contains a number of cell bodies, which are smaller than those found in the circumpallial nerve. No axons have been seen to rise from these cells and therefore it is suggested that they may be either glial cells or cells associated with the collagen sheath.

6. Electrophysiology

a) Hyperosmotic stimuli

Stimuli provided by sodium chloride crystals proved ineffective in producing a response. The high local osmolarity around the tentacle effectively destroyed the structure of the tentacle, thus rendering it unable to respond to either osmotic or non-specific chemical stimuli.

b) Mechanical stimuli

Mechanical stimuli appeared to elicit a response. However, the amplitude of the responses was very small and hard to separate from the noise level. Indeed it is hard to decide whether the responses were artifacts or were genuine.

Of the two methods employed, namely stimulation by vibration, and stimulation with a water jet, the latter proved the most effective. A possible reason for this was that the first method only stimulated a small portion of the sensory epithelium while the water jet stimulated the whole tentacle. The probability that recordings were being made from only a few fibres, or a single fibre, was

Fig. 21.

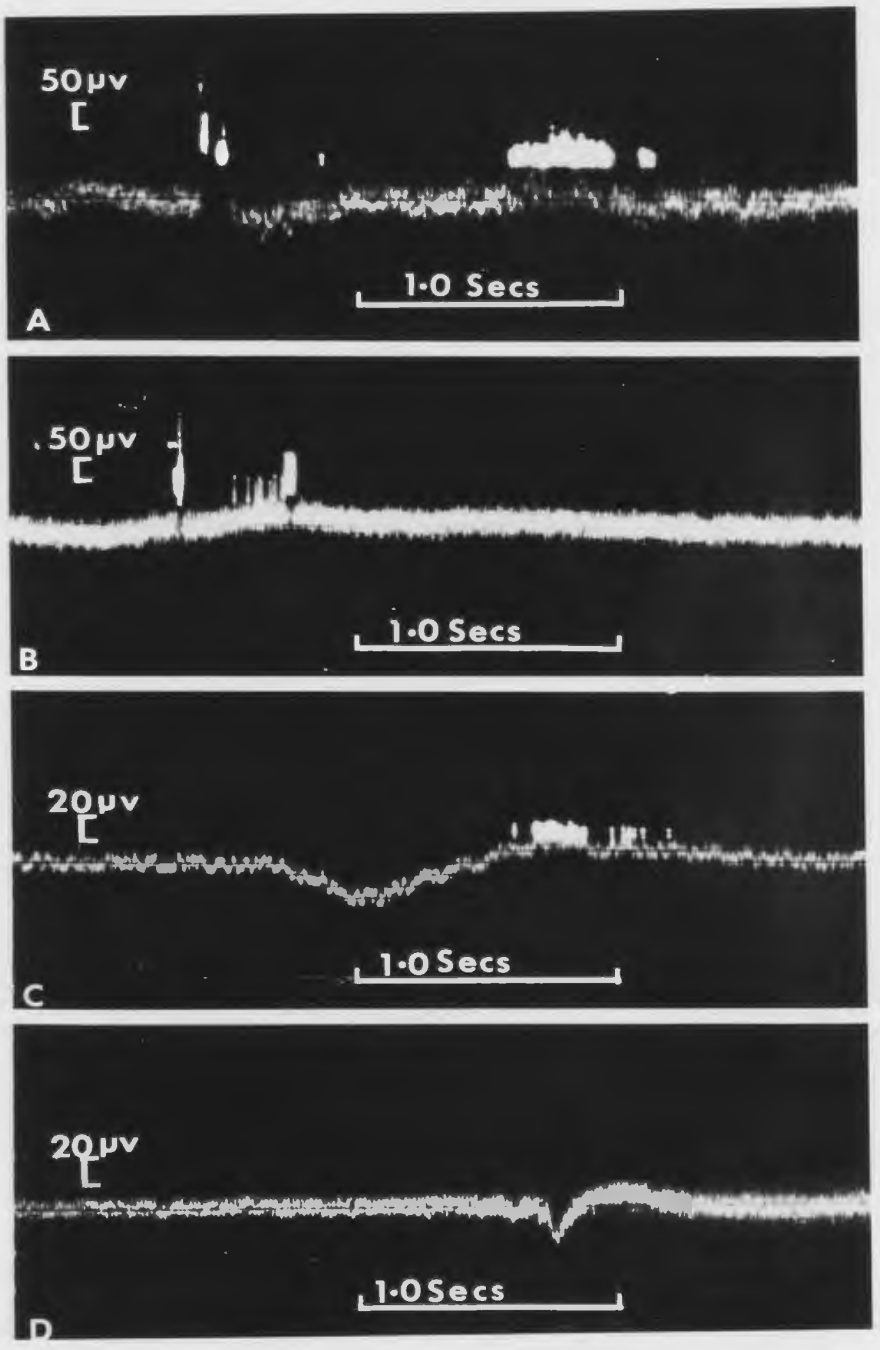
Fig. 21A. Electrophysiological recording of electrical activity in the tentacular nerve after mechanical stimulation of the tentacle. Duration of the stimulus is 100 msec.

Fig. 21B. Repeat of 21A with recording electrode removed. Stimulus duration is 500 msec.

Fig. 21C. Recording of electrical activity in the tentacular nerve after mechanical stimulation with a jet of sea water. Duration of the stimulus may only be estimated.

Fig. 21D. Repeat of 21C with recording electrode removed.

For all experiments the frequency filters on the preamplifier were set as follows. The high filter was set to 3 kHz and the low filter to 0.3 Hz.


50μv
1·0 Secs
A
50μv
1·0 Secs
B
20μv
1·0 Secs
C
20μv
1·0 Secs
D

high. Consequently, when using the more localised loud speaker method, the changes were small that the recording electrode was in, or near the axons of the sensory structures that were being stimulated.

The vibration method only proved effective when the tentacle was stimulated for a minimum period of one second. However, when activity occurred (Figs. 21A and 21B) it was always preceeded by a deflection of the trace, which may be caused by movement of the tentacle. A burst of firing occurred on the portion of the trace that rose above the mean noise level. The spike amplitudes varied from preparation to preparation but remained constant in a given experimental situation. The amplitudes were between 20 and 40 microvolts. These results are comparable to the amplitude of the responses achieved by Land (1966a) in his physiological study of the electrophysiological responses recorded from the optic nerve of Pecten. The duration of the response was not prolonged and continued for periods up to 300 msecs.

Using a water jet as a source of stimulation was more successful. A trace of the response of the sense organs when stimulated by this means is shown in Fig. 21C. Fig. 21D is a repeat of the same experiment, with the electrode removed from the nerve and placed on the tentacle in roughly the same position as when the electrode was in the nerve. The pattern of the response is similar to that of

the earlier experiment but the movement artifact is more pronounced due to the fact that the tentacle could not be firmly anchored to the dish without causing damage to the epithelium. However, the magnitude of the artifact may be used to estimate the strength of the stimulus though this is obviously not quantitative.

The amplitude of the response varied as before and was between 10 μV and 35 μV. The duration of the response was dependant on the strength of the stimulus, and lasted up to 900 msecs.

The ability of the tentacle to respond to the stimulus declined after repeated applications of the water jet over a short period. In order to elicit the response the tentacle had to be rested for a period of some 30 mins. The duration of the response also declined over the period of repeated application of the stimulus.

DISCUSSION

The structures of numerous organs thought to be sensory have been described in various species of vertebrate and invertebrate animals. Apart from certain visual and acousticovestibular systems, there is often little direct physiological evidence to support the contention that the described structures are indeed sensory. Their classification as sense organs has often been made by the circumstantial evidence of their particular structure, their position, or by behavioural criteria. These can indicate if the structure is a sense organ or not even though electrophysiological data is not available.

In order for a structure to be a functional sense organ it must be located in the animal in such a way that sensory reception might easily be effected. The relative exposure of a structure will suggest the type of stimulus to which it might respond. A ciliated sense organ located in a pit would not make a good mechano-receptor, and in such a case it would be more advantageous for reception if the organ were exposed.

While most sense organs contain sensory cells that have a specialized structure often bearing cilia, there are unspecialized cells that respond to selective stimuli, for example the epithelial cells of the siphon of the surf clam

Spisula that are sensitive to touch (Mellon, 1972). Apart from most invertebrate visual receptors, and certain other sense organs, the majority of sensory receptor cells have some sort of ciliated structure (Barber, 1974).

Though cilia appear to play an important part in transduction of stimuli, they need not be modified, and in many sensory systems the ciliary structure is similar to that of motile cilia. In some systems ciliary modifications may occur, for example mechanoreceptors may have long stiff cilia (Laverack, 1968). Modified basal bodies may also occur, an example of this is the basal body of a vibration receptor in Leucothea, a ctenophore, in which the basal body and the roots are thought to have become modified into a single electron dense sphere (Horridge, 1965a, 1966). Other modifications include the loss or addition of microtubules to the axoneme of the cilium. Many examples of loss of the central pairs of tubules exist among non-motile sensory cilia (see Barber, 1974).

Various other organelles are common in sense organs. Mitochondria occur near the distal end of the cells whilst the nuclei together with endoplasmic reticulum occur in the proximal portions of the cells. Perinuclear Golgi apparatuses are common in sense cells (Coggeshall, 1971). At the base of the cells there are few organelles although microtubules are common in the region where the axon arises.

The failure of the experiments on the regeneration of

the eyes are of interest because they are in contrast with the success of similar investigations by Butcher (1930). As was stated in the materials and methods, the experiments were performed in the same manner as those of Butcher. Since the animals were maintained under natural conditions it seems improbable that environmental factors, for example light and lack of food, were responsible for the failure of regeneration. However, as was pointed out in the results, the sea temperatures in Newfoundland are extremely low for two thirds of the year. It is significant that when samples from the mantle rim were examined histologically, degeneration of any of the tissue remaining after the eyes had been removed had not started. A similar problem occurs in the regeneration of nervous tissue in the Crustacea, and Fahrenbach (1974 pers. comm.) attributes the problem solely to low water temperatures. Therefore it seems conceivable that the failure to regenerate may be the result of the low temperature conditions in local waters.

However, while Butcher was working on a similar species, Pecten gibbus borealis, his description of the animal in the text makes it unclear as to which species he utilized for his experiments. There are two scallops commonly found in the Woods Hole region (Abbott, 1974), P. magellanicus (Gmelin), and Argopecten irradians irradians (Lamark, 1819), and the juveniles of these two species are

not clearly distinct from one another. Argopecten irradians irradians has had a number of synonyms, Aquipecten irradians (borealis)(Say) and two other synonyms which Gutsell 1930 stated were, Pecten irradians (Lamark) and Pecten gibbus (Linné). Unfortunately Pecten gibbus is also a synonym for Argopecten gibbus (Walter, 1969) a related species with a more southerly distribution. Butcher has further confused the problem in his literature review by differentiating between Pecten gibbus borealis and Pecten irradians. The most northerly range of Argopecten gibbus is North Carolina (Allen and Costello, 1972) and it is not reported to be indigenous to Woods Hole.

However, it should be noted that Dall (1898) failed to separate the more northerly form from that of Florida and Jamaica, and employed Pecten gibbus with irradians as a subspecies for the Florida zone. I suspect that Butcher denoted the more northerly species as a further subspecies of Pecten gibbus with the subspecific name of borealis.

The embryology and anatomy of A. irradians irradians is similar to that of P. magellanicus (Sastry, 1965). Therefore, a priori, one would expect regeneration to be similar in related species. Scarsso and Iraldi (1973) have shown that regeneration of eyes in Helix aspersa and Cryptomphallus aspersa occurred and was similar in both species. However, Bullock and Horridge (1965) have stated

that regeneration of nervous and sensory systems in closely related species may differ. It is therefore conceivable that the failure to regenerate may be due to the fact that a different species was used for the experiments. Although this is a possibility, it is more probable that the failure was due to low water temperatures.

As was discussed in the introduction, the tentacles of scallops have long been implicated in the reception of stimuli both for reasons of position and behaviour. It has frequently been suggested that they possessed sites for the reception of tactile stimuli (Buddenbrock and Möller-Racke, 1953; Dakin, 1909; Drew, 1906, 1907). This theory has recently received support from the study of Thomas and Gruffydd (1971) who demonstrated that a tactile as well as chemical stimulus was required to induce the escape response of a bay scallop. This response was swimming away from a number of different predatory starfish. A tactile stimulus was insufficient to induce swimming although it did cause the scallops to withdraw their tentacles and close the valves of their shells. This suggested that the tentacles are indeed in possession of touch receptors.

The structural results previously described show that the ciliated cells at the distal tips of the long tentacles are probably ciliated sense organs. The end organs are discrete but prominent structures that are well located for the potential reception of both tactile and chemical

stimuli. The number of ciliated cells is small as are the cells themselves. It is not known whether or not the cilia are motile. If they are motile they do not beat in a metachronal fashion, as the basal feet and central microtubules of adjacent cilia do not have the common alignment that would be required for such a mode of beating to occur (Gibbons, 1961). It is interesting to note that the cilia, which arise from the cells at the bases of the end organs, do so from cells that are much larger than the cells of the end organs. These cilia do have a common alignment of their central tubules and in all probability are motile and beat metachronally. Multiple cilia also arise from these cells and other authors have shown that macrocilia are involved in the movement of mucous in the olfactory organs of certain Gadoid fish (Lowe, 1974), and also in the movement of water in the ctenophore Beroë (Horridge, 1965b). A similar function is probable for the macrocilia of the long tentacles. However it should be noted that certain authors consider these macrocilia to be degenerating cilia (Thornhill, 1972). They have also been considered to be sensory (Barber and Wright, 1969a; Wilson and Westermann, 1967).

Barber (1966, 1968) showed that the basal feet of the cilia in the sensory cells in the statocyst of the cephalopod mollusc Octopus all have a common alignment, and Budelmann and Wolff (personal communication) confirmed

that transduction of the stimulus occurs when the cilia are bent towards their basal feet. If this situation holds true in the end organs, in which the basal feet are arranged at a number of different angles to one another, then the organ would be able to respond to stimuli from a number of different directions. However it should be noted that septate desmosomes exist between adjacent cells in the end organ. Loewenstein and Kanno (1964) and Kanno and Loewenstein (1966) have shown that transport of macromolecules between cells in Drosophila salivary gland is greatly enhanced in the region of the septate desmosomes. Ito and Loewenstein (1969) demonstrated that the passage of macromolecules may be effected through an area of contact as small as 1 μm^2 of contact between the cells. Furthermore Potter, Furshpan and Lennox (1966) have shown that tight junctions are sites of low resistance between squid embryo and its yolk cells. Although the structure of tight junctions and septate desmosomes is different the fact that cytoplasmic bridges appear to exist within the desmosomes suggests that these too are sites of low intercell resistance. Since the resistivity of cytoplasm is approximately ten times lower than the membrane resistance, the cable properties of the cytoplasm is such that a potential of sufficient magnitude could pass electrotonically to all the cells in the papillae. Although directional sensitivity is lost, the level of sensitivity of the organ

is enhanced, since the stimulation of one cell will generate a response an order of magnitude greater by the number of cells in the organ. The response therefore may be summated at the sense organ.

Though tentative, the electrophysiological results support the hypothesis that the end organs are touch receptors. There are problems associated with interpreting the electrophysiological results. While the traces demonstrate the likely occurrence of electrical activity, the site at which the activity is generated is contentious. The distal third of the long tentacles, unlike the short tentacles, is exclusively covered by the ciliated papillae. The ciliated cells at the bases of the papillae almost certainly bear motile cilia. Since the only portion of the tentacle stimulated by vibration was the tip, the results suggested that the responses are derived from the tip, possibly from the papillae. A similar pattern follows when one examines the results from the second mechanical stimulus, the water jet. It is interesting to note that the stimulus to which the tentacles best responded, the water jet, was the same stimulus used by Gutsell (1930) in his attempts to discover the modality to which the long tentacles responded. Unfortunately the stimulus is broad, and because the whole tentacle is stimulated it becomes impossible to attribute the response to the activity of any particular structure. Furthermore since the magnitude of

the stimulus is large and causes a large movement artifact, it is conceivable that the activity that occurs may be due to action potentials generated by the tip of the electrode touching the membranes of the surrounding axons, and thus generating spurious action potentials. This problem can only be solved by further study.

Assuming that the results are due to sensory activity, the response adapts rapidly to the stimulus, and repeated stimulation will eventually elicit no response. Furthermore it required a period of up to half an hour for the sensitivity to return. Fuortes (1971) has shown that vertebrate touch receptors also rapidly adapt to a stimulus of constant intensity and that the response to the stimulus is transitory. Since the scallop end organs respond in a similar manner, this provides more evidence to support the theory that they are mechano-receptors.

To sum up the discussion on the long tentacles, it can be stated that three types of ciliated structure or cell may be seen to occur in the epithelia of the long tentacles. Of these three one is a discrete ciliated structure or papilla. Large numbers of these papillae occur on the distal third of the long tentacles. The structure of these papillae has been described, and when considered in conjunction with their location the evidence suggests that they are ciliated sense organs. Electrophysiological studies performed on the papillae of the long tentacles,

while preliminary, tentatively suggest that the papillae may be receptors, and that they may respond to mechanical stimuli.

The positional and structural factors already described are also true for the other sense organs. Of the structures described for the short tentacles one is the same as found on the long tentacles, the other, the ciliated cells in the pit, are unique to the short tentacles. This latter structure is similar to the proposed chemo-receptors found on the tentacles of the cockle Cardium by Barber and Wright (1969b). Thomas and Gruffydd (1971) showed that a chemical stimulus was essential to induce the escape response and it is possible that these ciliated organs are responsible in part for this response.

The abdominal sense organ poses an interesting problem. Dakin (1910) showed that when the organ was ablated no behavioural modification occurred when the animal was stimulated with a number of different modalities. The functions it has been most commonly assumed to carry out are vibration detection and olfaction. Since the osphradium of this mollusc is diminutive it is possible that the abdominal sense organ has an olfactory function. It might be pointed out that the so-called "olfactory organ" of cephalopod molluscs has presented a similar problem with no definite modality of response having yet been determined

(see Barber and Wright, 1969a; Watkinson, 1909). The ciliary modifications found in the abdominal sense organ remind one of those described in the frog olfactory receptors (Reese, 1965). Furthermore the cells of the abdominal sense organ appear as bipolar neurons with the cilia at their dendritic portion much as do the cells in the frog olfactory epithelium. One might argue however that the cilia are not responsible for the reception of stimuli, and this function is effected by the modified whorls of microvilli that occur at the apex of each cell. Microvillous arrays have also been shown to occur in some of the gasteropod osphradia (Crisp, 1973; Laverack, 1974; Welsch and Storch, 1969). It is quite possible that the abdominal sense organ subserves two functions that of chemoreception and perhaps also the reception of mechanical stimuli. Bailey and Laverack (1963) and Storch and Welsch (1969), have shown that the osphradium of some marine gasteropods respond to osmotic as well as to mechanical stimuli. However it has been suggested that the means by which mechanical stimulation is effected is due to distortion of the cell membrane and that the method is similar when osmotic stimuli occur. This hypothesis is supported by physiological evidence as Ottoson (1965) has demonstrated that activity in the frog muscle spindle can be altered by osmotic stimuli acting on the sensory bulbs of the spindle.

The following hypotheses are therefore suggested for the function of the abdominal sense organ. There are two possible functions, the first being chemosensory, the second being mechanical.

The chemosensory function is possible for the following reasons. The osphradium which is commonly assumed to be a chemoreceptor in Lamellibranch molluscs is small. The abdominal sense organ possesses both positional and structural criteria necessary for a chemoreceptor. That is it presents a large epithelial area directly to the flow of water around the mantle. Structurally it has both the cilia which occur in many chemosensory systems and modified microvilli which have been shown in the marine gasteropods to be functional in the reception of chemical stimuli.

The second type of function that it might subserve is that of a mechanoreceptor. Hartnoll (1967) has shown that P. maximus orients in a unidirectional current flow in such a manner that their ciliary currents are assisted by the tidal currents. It is possible that the abdominal sense organ may perform this function, thus fulfilling the function proposed by the early German histologists.

Although the visceral ganglion of the scallop is large, and the mantle is richly supplied with nerves from it, the behavioural investigations have demonstrated that the sense organs of the eyes and the tentacles, appear to

be able to function as isolated units when the nerves connecting the mantle rim to the ganglion are removed. Consequently it has been suggested that the circumpallial nerve is in fact a much elongated ganglion. Certainly the histological evidence suggests this, the structure of the nerve is reminiscent of that of the visceral ganglion, with large cell bodies giving rise to axons and the presence of numerous smaller cells scattered throughout the bundles of axons. In terms of energy saved the integration of many of the stimuli at the mantle rim would be advantageous. A similar, though more complex system, has been described in the cephalopod molluscs by Graziadei (1965a, 1965b). This author has shown that integration of tactile stimuli, derived from the ciliated touch receptors in the suckers of these molluscs, occur at large encapsulated cell bodies that lie immediately inferior to the suckers. The suckers are connected to each other by a lateral nerve network and the majority of integration occurs at these encapsulated cells. Thus, only extreme stimuli, which require movements of the whole animal, would reach the central ganglia. Similarly with the scallop, those stimuli that do not require movements of the animal as a whole, may be integrated at the mantle margin within the circumpallial nerve.

One may only speculate on how transduction of stimuli occurs in these sense organs. In his review of cilia in

sense organs Barber (1974), presented a number of alternative theories of transduction in mechano-receptors of which he suggested that the Piezo-electric theory and the deformation theory were the most likely. In the former case the mechanism for transduction is via the static electric currents which are generated on distortion of the mucopolysaccharide film which is thought to coat the cilia (Fukada, 1974). The deformation theory suggests that ion permeability is altered on distortion of the cell membrane by movement of the cilia. The product of this is the production of a generator potential in the excitable portion of the cell. It is notable that in all the sense organs described here the epithelium is completely covered by a mucous coat. In the end organs each unit is associated with a mucous-producing cell. Perhaps this has some significance in the transduction of the stimuli.

SUMMARY AND CONCLUSIONS

The eye regeneration experiments carried out by Butcher in 1930 were repeated on the local species of scallop, P. magellanicus. The results from these experiments were negative. Two reasons have been suggested to attempt to explain the failure of these experiments. The first possibility is that the species used by Butcher was different from that used in this series of experiments, and that this affects the re-growth. Secondly, the water temperatures may have been too cold for regeneration to occur, credence may be given to this theory by the fact that degeneration of the sites from which the eyes had been removed had not been initiated for periods of up to one and one half years.

The structure of the long tentacles has been extensively studied. They bear ciliated papillae which radiate out from the long axis of the tentacle at 90°. The papillae occupy the distal one third of the long tentacles and are numerous, there being some 30/mm^2 of epithelial tissue. These papillae may also be observed on the apex of the short tentacles. The papillae are 50 to 60 µm long and are attached to a basal lamina on the central column of the tentacle, and the exterior of them is covered by numerous short microvilli. There are some 10 to 15 sensory cells

per papilla, associated with two or more mucous or supporting cells. The ciliated cells bear up to five cilia at their apices, and the base of each cilium is surrounded by a single row of microvilli. The cilia are unmodified and bear the normal number of microtubules, 9+2 per axoneme. The central pair of tubules have no common alignment in adjacent cilia, therefore it is unlikely that the cilia beat metachronally. The proximal portions of the ciliated cells bear many microtubules suggesting that the bases of these cells become axons. However, no definite link has been observed between the tentacular nerve and the papillae.

Ciliated cells occur at the base of the papillae and on the proximal two thirds of the tentacle. These ciliated cells bear many cilia, which have a normal axonemal complement of 9+2 microtubules. The central pairs of tubules have a common alignment and therefore possibly beat in a metachronal fashion. These cells also bear macrocilia, which are composed of from two to fourteen 9+2 microtubular substructures that are bound within a single membrane, with the central tubules having a common alignment.

Electrophysiological experiments were preliminary, however the results suggested that the tentacle tip responded to mechanical stimuli. Due to the nature of the stimuli it was not possible to determine the exact structure from which the response was derived.

Two other types of ciliated cells were observed on the

short tentacles, as well as papillae similar to those described on the long tentacles. The first cell type has many cilia per cell and the cells form a pad, situated on the convex portion of the tentacle. The second type of cell bears few cilia and the cells form a pit in the concave portion of the tentacle. The cilia of both cell types are surrounded by a single ring of microvilli, and have a standard 9+2 array of microtubules. The microvilli surrounding the pit cilia are long and fill the lumen of the pit. This second structure has the appearance of a sense organ.

The abdominal sense organ is a ciliated pad of tissue some 3 mm long and 0.5 mm wide situated within the mantle cavity of the scallop. The sensory epithelium is 125 to 130 μm thick, and rests on the basal lamina, which is pierced by a number of nerves from the post pallial nerve and the mantle margin. The sensory epithelium is composed of a number of ciliated cells bearing a single cilium. The cilia are 60 μm long and have a normal axonemal complement. The cells are arranged in rows and the central tubules of a single row have a common alignment. However the cilia of adjacent rows do not have a common alignment of the central tubules. A number of ciliated cells are associated with a mucous cell. The apices of the cilia may be enlarged to form sacs contained within which are a number of vacuoles. Although no nervous tissue has been observed, histologists

working on the abdominal sense organ of the Mytilidae have shown that the bases of the cells become axons.

A brief histological study of the nervous supply of the mantle margin and the tentacles demonstrated that the circumpallial nerve has the appearance of a large ganglion, and cell bodies occur in it frequently.

In conclusion it is suggested that a number of ciliated sense organs have been discovered in the peripheri and within the mantle. The structure of the papillae in conjunction with the preliminary physiological results suggest that they are mechanoreceptors. Further investigations are required to demonstrate that there is a direct connection between the papillae and the tentacular nerve. Further physiological investigations of a similar nature would serve little purpose as the amount of information which would be gained from this type of study would not warrant the amount of time that would be required to adequately complete the study. It is suggested that further studies be carried out to elucidate the structure of the pit cells of the short tentacles, and the abdominal sense organ.

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