'SOME ASPECTS OF THE FINE STRUCTURE OF THE SENSE ORGANS AND NEURO ENDOCRINE SYSTEM OF ARCHAEOGASTROPODS'

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Thesis submitted for the degree of Ph.D.

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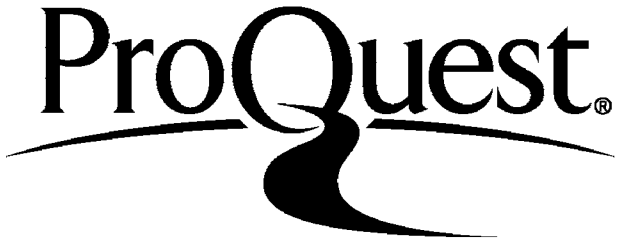

ABSTRACT

The investigations reported in this thesis have been carried out primarily on British representatives of the Archaeogastropoda. The work falls into two parts:- the first relates to the fine structure of epithelial sensory cells and the second to the fine structure and function of the juxtaganglionar organ, a putative endocrine gland.

Observations on the sensory cells are primarily concerned with those found in the cephalic and epipodial tentacles, and the epipodial sense organs. The general organisation and innervation of these structures is described together with the ultrastructure of their sensory cells. The species examined most extensively were *Gibbula umbilicalis* (da Costa) and *Emarginula reticulata* Sowerby. Much similarity was observed between these species, particularly with regard to the epipodial sense organs. Similar structures were also found on the mantle edge bordering the shell slit of *E. reticulata*. Members of the Patellacea were examined briefly for comparison.

Fine structural observations on the juxtaganglionar organ were carried out on *G. umbilicalis*. The organ was found to possess many features characteristic of an endocrine gland and to show similarity to the dorsal bodies of pulmonates. Animals were collected at monthly intervals throughout one year in order to investigate the possibility of seasonal variation in the activity of the organ. Such variation was observed in females and was shown to correlate with the reproductive cycle. Males were not examined in this respect. The juxtaganglionar organ of an opisthobranch, *Aplysia punctata* Cuvier, was examined for comparison. It was found to exhibit further similarity to the pulmonate dorsal bodies.

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GENERAL INTRODUCTION

This study concerns two aspects of archaeogastropod fine structure and is presented as two separate parts.

The first part relates to the general structure and organisation of epithelial sense organs, their innervation and relation to the central nervous system, and to the ultrastructure of the sensory receptor cells contained within them. The study is comparative and concerns representatives of three archaeogastropod superfamilies.

The second part concerns the juxtaganglionar organ, a putative endocrine gland associated with the cerebral ganglion. This study was conducted with the intention of providing further information on the fine structure of the organ and the extent to which it is associated with the cerebral ganglion. It was hoped that this would enable more informed comments to be made regarding the possible homology of this structure with the dorsal bodies of pulmonates. The majority of the work was conducted on *Gibbula umbilicalis*, but the organ of the opisthobranch *Aplysia punctata* was examined for additional comparison.

Although ostensibly the two parts of this thesis are separate it was hoped that there would be an underlying theme. This concerned the possibility of an association between sensory input and the endocrine system, mediated by neurosecretory cells. Investigations on the possible presence of neurosecretory cells within the cerebral ganglion

however, were largely unprofitable and inconclusive. Studies were therefore directed towards either end of the system.

The net result is that the work has been reported in the two parts mentioned above. The possible link between the two, in the area of neurosecretion, is a field that clearly merits further investigation. The difficulties involved in the study of this phenomenon within the Archaeogastropoda, indicate the need for a more concentrated study.

P A R T I

SENSE ORGANS

C H A P T E R 1

INTRODUCTION

It is well known that the body wall of soft-bodied invertebrates is richly supplied with nerves and commonly bears sensory structures (Bullock & Horridge, 1965). These may be distributed over much of the surface or be grouped together in particular areas, sometimes forming discrete sense organs.

Within the Mollusca these highly sensitive regions are numerous. They include the cephalic appendages of gastropods (tentacles and rhinophores), lips, mantle edge, siphon tip, pneumostome, margins of the foot, osphradium, the arms and suckers of cephalopods and the tegmentum of the shell valves of chitons (aesthetes).

The sensory equipment of prosobranchs, though it cannot be compared in its acuity with that of the cephalopods, includes such structures as the cephalic tentacles, eyes, statocysts, osphradium and certain elaborations of the external surface which are presumed to be primarily sensory in function, for example, the epipodium and epipodial tentacles, cephalic lappets, metapodial and pallial tentacles. Further areas may have additional roles as sense organs even though their main function is of another nature, for example, the mantle edge and siphon (tip). The distribution of many of these elaborations within the prosobranchs however, is not uniform. This is particularly so in the Archaeogastropoda.

The Caenogastropoda (meso- and neogastropods; Cox, 1960; Fretter, 1979) although not investigated in this study are more uniform in this respect. They possess well developed eyes and cephalic tentacles, frequently a siphon, but only rarely such structures as metapodial tentacles (e.g. *Lacuna* and *Nassarius*), and even less frequently pallial tentacles (e.g. *Rissoa*).

The Archaeogastropoda likewise possess eyes and cephalic tentacles, although the former are sometimes poorly developed or reduced, as in *Lepeta* and *Propilidium*. With regard to other sensory regions however, there is considerable variation within the order. The most major differences for obvious reasons occur between the various taxa. These differences are examined below. The classification system used is that of Fretter and Graham (1976, 1977, 1978) and is given in Table 1. Fig. 1 shows diagrammatically the occurrence and distribution of the sensory structures under consideration in the major archaeogastropod subdivisions.

The greatest distinction occurs between the Rhipidoglossa, with, in general, a well developed epipodium and only occasional pallial tentacles, and the Patellacea (Docoglossa), which never possess an epipodial fold or tentacles, but have a well developed mantle fringe often bearing numerous pallial tentacles. There is much variation however, within both of these groups.

RHIPIDOGLOSSA

Throughout the Rhipidoglossa the cephalic tentacles

TABLE 1

Classification used in text* (based on Fretter & Graham, 1976, 1977, 1978).

ORDER:	ARCHAEOGASTROPODA (Diotocardia)		
Superfamily:	Pleurotomariacea	Zeugobranchia	Rhipidoglossa
Family:	Pleurotomariidae		
	Scissurellidae		
	Haliotidae		
Superfamily:	Fissurellacea		
Family:	Fissurellidae		
Superfamily:	Trochacea		
Family:	Trochidae		
	Turbinidae		
	Phasianellidae		
	Skeneidae		
Superfamily:	Patellacea		Docoglossa
Family:	Acmaeidae		
	Patellidae		
	Lepetidae		

The Neritacea are not now regarded as true members of the Archaeogastropoda (Fretter & Graham, 1978).

Family:	Trochidae
Subfamily:	Margaritinae
	Solariellinae
	Gibbulinae
	Monodontinae
	Calliostomatinae

* Only groups with British representatives are given with the exception of the Pleurotomariidae.

FIGURE 1

Distribution of sense organs in the Archaeogastropoda. Sense organs on the head and foot are shown on the main figure. The shell and sense organs associated with the mantle are shown on the overlay.

Pleurotomariidae - epipodium poorly developed, only cephalic tentacles present. Probably no discrete sense organs on mantle edge.

Scissurellidae - epipodial tentacles possibly of two types papillate and non-papillate. Pallial tentacles present in shell slit.

Haliotidae - epipodium hypertrophied, numerous papillate epipodial tentacles and epipodial sense organs. Pallial tentacles project from holes in shell.

Trochidae - papillate epipodial tentacles and epipodial sense organs. Tentacles less numerous and sense organs sometimes absent in more advanced genera. Mantle with no discrete sense organs.

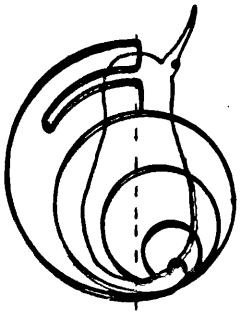
Fissurellidae - epipodium well developed with non-papillate tentacles. Epipodial sense organs numerous, occurring on tentacles. Pallial sense organs (previously tentacles) in shell slit.

Patellidae - epipodium absent, replaced by elaboration of mantle edge in the form of pallial tentacles.

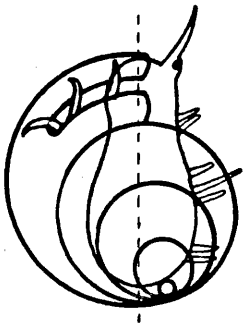
Epipodial and pallial sense organs shown as small circles.

FIGURE 1

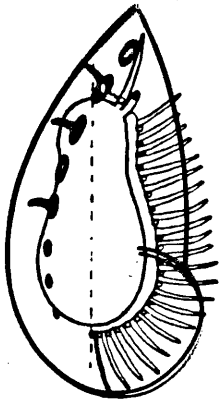
Pleurotomariacea



Pleurotomariidae

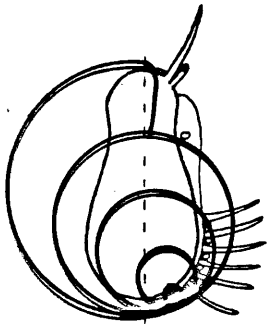


Scissurellidae

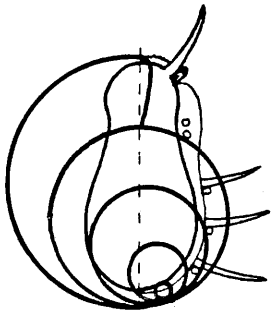


Haliotidae

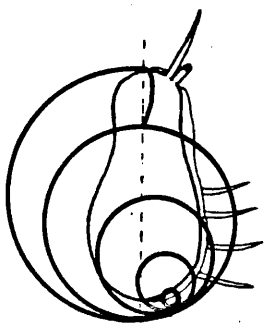
Trochacea



Margaritinae

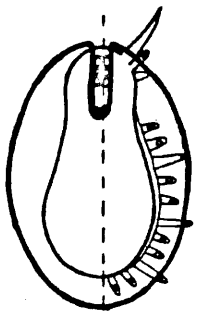


Trochidae
Gibbulinae



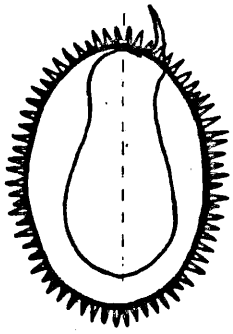
Calliostomatinae

Fissurellacea



Fissurellidae

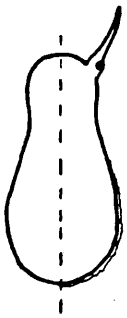
Patellacea



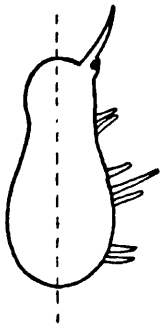
Patellidae

FIGURE 1

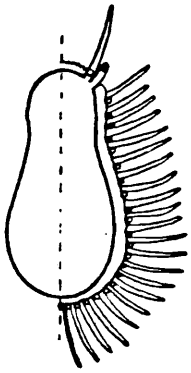
Pleurotomariacea



Pleurotomariidae

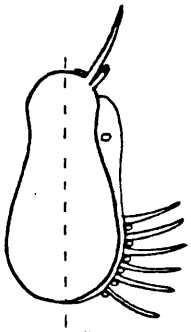


Scissurellidae

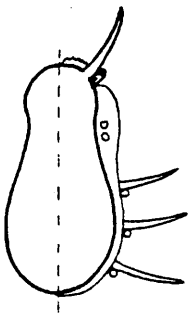


Haliotidae

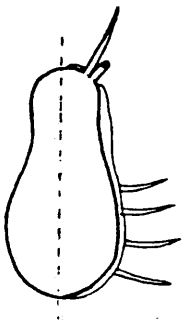
Trochacea



Margaritinae

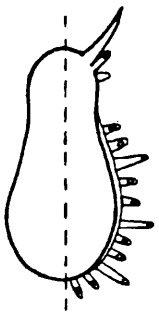


Trochidae
Gibbulinae



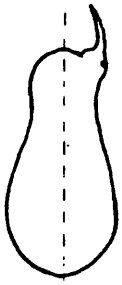
Calliostomatinae

Fissurellacea



Fissurellidae

Patellacea



Patellidae

and eyes are basically similar (though there is a trend toward the constriction and closing off of the optic vesicle). It is in the degree and elaboration of the epipodium that most variation occurs.

This structure (the epipodium) is generally regarded as a primitive feature (Fretter & Graham, 1962) and it is best developed in the zeugobranchs (Pleurotomariacea and Fissurellacea, Fretter & Graham, 1976). These animals also show other primitive features such as the presence of a slit or series of holes in the shell, relating to the mantle cavity (Yonge, 1947). Even within this group, however, there are noticeable differences.

The Haliotidae have perhaps the most elaborate epipodium, comprising in *Haliotis tuberculata* L., two folds from which project papillate tentacles and an intermediate lobed area (Crofts, 1929). The whole structure is paired and extends from just behind the eyestalks to the posterior limit of the foot, joining in the midline.

Compared with the Haliotidae, the Fissurellidae have a somewhat less well developed epipodium in the form of a low ridge with fewer, shorter tentacles (Boutan, 1885; Ziegenhorn & Thiem, 1926; Odhner, 1932).

The epipodium of the Scissurellidae is also smaller than that of the Haliotidae, but unlike the Fissurellidae the tentacles are long and of two distinct types, some papillate and others smooth (Vayssière, 1894; Bourne, 1910; Fretter & Graham, 1976). The Pleurotomariidae have an

even smaller epipodium which bears no tentacles. It is simply a low ridge with a number of small papillae (Dall, 1889; Bouvier & Fischer, 1898, 1899, 1902; Woodward, 1901; Fretter, 1964, 1966; Fretter & Graham, 1976).

The epipodium of the Trochacea is generally more uniform than that of the zeugobranchs. All families show a similar epipodial plan of anterior neck lobes and a posterior fringe bearing tentacles and epipodial sense organs, beneath the operculum. It has been stated that the Turbinidae could almost entirely satisfactorily be described as members of the Trochidae with a calcareous operculum (Graham, 1965). This applies not only to the epipodium but to the soft parts in general.

The trochids (Trochidae) show a trend involving the reduction of the epipodium accompanied by the development of other presumably more advanced features including a labial spout, glandular lips to the right kidney opening, a tightly coiled shell and the loss of the umbilicus except in larval stages (Deshpande, 1957; Fretter & Graham, 1962).

The most primitive subfamily of British trochids are the Margaritinae. These are small, possess six pairs of epipodial tentacles and as many or more epipodial sense organs (Fretter & Graham, 1977). The most advanced British subfamily, the Calliostomatinae are larger, but possess only four (occasionally five) pairs of tentacles and no epipodial sense organs. The Gibbulinae and Monodontinae appear to be intermediate. Little is known of the Solariellinae but they

are thought to be similar to the Margaritinae.

Although the epipodium is regarded as a primitive feature and it is at its most elaborate in certain members of the Pleurotomariacea, there is no evidence to suggest that this was the type of epipodium possessed by the earliest gastropods. It is generally thought that the Pleuromariidae are the most primitive of all recent gastropods and that theirs is the most basic structure (Fretter, 1979). It may therefore be suggested that the early gastropods had a poorly developed epipodium and that later certain archae-gastropods (in particular the Halioditae) have exploited this preadaptation to suit their mode of life. The possession of such a structure may not therefore, in itself be a primitive feature, but an adaptation to a rock clinging mode of life.

Of special interest in this study are the cephalic and epipodial tentacles and the epipodial sense organs. Throughout the Rhipidoglossa the cephalic tentacles appear to be similar. They are generally highly mobile and are covered with numerous small papillae, though the size of the latter may be variable. The epipodial tentacles however, seem to be of two sorts. Those of the Trochacea and Haliotidae are slender, highly extensile, papillate structures resembling the cephalic tentacles. Those of the Fissurellacea are not papillate and are much shorter and less extensile (Boutan, 1885; Ziegenhorn & Thiem, 1926; Odhner, 1932). Forbes and Hanley (1849-53) and Jeffreys (1862-69) stated

that in British species these tentacles are alternately long and short and describe them as cirri. The Scissurellidae may possess both types of epipodial tentacle or possibly a third type which is long and extensile but bears no papillae (Vayssière, 1894; Bourne, 1910; Fretter & Graham, 1976).

The occurrence of the epipodial sense organs within the Rhipidoglossa is irregular and worthy of comment. The earliest description of these organs that has been found was that of Forbes and Hanley (1849-53) in certain species of trochid. Their shape and location is variable. They have not been noted in the Pleurotomariidae and there is little evidence for their presence in the Scissurellidae. In the Fissurellidae they are well developed and occur on the ventral surfaces of the epipodial tentacles (Odhner, 1932; Fretter & Graham, 1962, 1976). They are likewise well developed and even more numerous in the Haliotidae, lying ventrally at the bases of the epipodial tentacles. In this group they also occur at the bases of the cephalic tentacles, possibly a unique situation. Crofts (1929) called these structures sub-tentacular organs and observed that they were composed of columnar cells, taller than the surrounding epithelium, with nuclei arranged in two rows and in general resembled the osphradium. This is one of the few descriptions of their structure.

When found in the Trochacea they usually occur near the bases of the epipodial tentacles and under the neck lobes. In both locations they are often raised on a short stalk (Frank, 1914; Fretter & Graham, 1977).

Elaborations of the edge of the mantle skirt, with the exception of slight papillation are not common in the Rhipidoglossa. They are however, found in some fissurellids, such as *Diodora* (Fretter & Graham, 1962, 1976). In contrast the mantle edge bordering the shell slit or hole(s) of zeugobranchs often bears specializations, notably tentacles or large papillae. Definite tentacles in this region have only been observed in the Haliotidae and Scissurellidae (Crofts, 1929; Yonge, 1947; Fretter & Graham, 1976), but papillae appear to be present in all species including the pleurotomariids (Fretter, 1964).

PATELLACEA (Docoglossa)

In comparison with the Rhipidoglossa the sensory elaborations of the external surface of the Patellacea are few and simple. All species possess cephalic tentacles and most possess eyes. The tentacles however, are usually shorter, stouter structures than those of the Rhipidoglossa, are not papillate and are much less mobile. They are relatively longer and more mobile in the Acmaeidae than the Patellidae and Lepetidae. The former is also the more primitive group (Davis & Fleure, 1903; Yonge, 1960). The eye is a simple pit in the surface epithelium at the base of the tentacle. There is no discrete optic tentacle as is found in most of the Rhipidoglossa.

The epipodium is absent and this, at least in the Patellidae is believed to have left space for the development of pallial gills (Fretter & Graham, 1962). In association with these gills is a subpallial sensory streak (Yonge, 1947;

Bullock & Horridge, 1965). This is present on both sides of the animal and curves over the anterior end of the shell muscle into the nuchal cavity. Its length varies with the species. There is evidence that it may be continuous with the osphradium and that it is only present in young individuals, *Patella vulgata* L. $<$ 20mm in length (Fretter & Graham, 1976). The descriptions of this structure in the literature however, are vague and confusing, little detail of its internal organisation is given. Similar structures may be present in the Polyplacophora (Yonge, 1939) and also in *Haliotis* (Crofts, 1929). Yonge (1947) pointed out that this was not an indication of close relationship between the Patellidae and Haliotidae.

In the Acmaeidae and Patellidae the epipodium is replaced by specializations of the mantle skirt. These take the form of numerous small tentacles, the pallial tentacles, which are generally accepted to be the functional counterparts of the epipodial tentacles. Although they are small they can extend beyond the limits of the shell into the external environment. The Lepetidae have not been studied extensively, but Yonge (1960) stated that *Lepeta concentrica* Middendorff, possesses minute pallial tentacles. Fretter and Graham (1976) however, did not observe them in British species. The sublittoral habit and mode of life of the Lepetidae seem to have resulted in a general reduction of their sensory equipment.

Having discussed the location and occurrence of

the various sensory specializations of the Archaeogastropoda, mention must now be made of their suspected function and the modalities to which they may be sensitive.

The cephalic and papillate epipodial tentacles are almost certainly the seat of a well developed tactile sensitivity. They are used by the animal as a guide to its movements through the environment in which it lives. They are kept in continuous movement when animals are active, the main direction being up and down and their motion is timed with the locomotor waves which pass along the foot (Fretter & Graham, 1962). The non-papillate epipodial tentacles may have a different function. Boutan (1885) was of the opinion that in *Fissurella* these structures were primarily respiratory in function because of their proximity to the lateral pedal artery and their apparent lack of innervation. This last observation however, is incorrect and in all cases these tentacles are innervated by nerves arising from the pedal cords (Ziegenhorn & Thiem, 1926; Odhner, 1932). Their exact function remains obscure, though it may be that they are simply stalks to raise the epipodial sense organs above the general body surface. A degree of tactile sensitivity in the tentacles themselves however, seems likely.

An additional epipodial tentacle is sometimes found behind the right eyestalk, e.g. in *Diodora*, *Emarginula* and *Puncturella* (Yonge, 1947; Fretter & Graham, 1976). This, though present in both sexes has been assumed to act as a penis (Dall, 1889; Odhner, 1932). A similar structure

the right postoptic tentacle is found in many other rhipidoglossans (Fretter & Graham, 1977). Bourne (1910) stated that in *Scissurella* that of the male is spatulate and more abundantly provided with gland cells than that of the female.

The function of the epipodial sense organs is also uncertain. Though they are often noted in the literature, little is mentioned of their role in the living animal except to say that it is unknown. Bullock and Horridge (1965) describe them, together with the osphradium and the lateral sensory strip of the patellids, as respiroreceptors. As such they may be presumed to test the respiratory water current in some way and may be chemically or mechanically sensitive, or both. This however is not altogether consistent with their location.

There has been little speculation as to the precise function of other epipodial structures. Burdon-Jones and Desai (1960) believed that the left neck lobe of trochids acts to some degree like an inhalant siphon. Its fringed margin may act as a coarse filter for suspended material and also test the inhalant water current. This is supported by the studies of Fretter (1975) on the filter feeding trochid *Umbonium vestiarium* (L.).

The docoglossan pallial tentacles, like the rhipidoglossan epipodial tentacles are thought to respond to tactile stimuli. There is nevertheless, little evidence to suggest that they are only mechanically sensitive. On

the contrary, Arnold (1957) has shown that the cephalic tentacles and pallial tentacles (or at least some part of the mantle edge) of *Patella vulgata* are responsive to changes in salinity. Similarly he has shown (Arnold, 1972) that other littoral species such as *Littorina littorea* (L.) and *Gibbula cineraria* (L.) respond to salinity changes. He did not however, localise any particularly sensitive areas in these last species. An additional sensitivity attributed to patellid pallial tentacles is involved in the homing response part of which may be mediated by chemical recognition of the mucous trail laid on the outward journey (for literature see Newell, 1979).

Most of the studies cited above have been concerned mainly with general morphology and internal anatomy. Descriptions of the sensory structures are usually brief and give little detail of their internal organisation and innervation. Additional studies have been undertaken which relate specifically to the nervous system (Bouvier, 1887; Thiele, 1890; Gilchrist, 1897; Retzius, 1900). These were generally comparative and dealt with the nervous system as a whole. They gave little detail beyond simple innervation of the sensory structures.

In this last respect it is interesting to note the controversy which occurred at the turn of the century as to whether the epipodium was related to the foot or to the mantle, its location lying between the two. There was no doubt that it was innervated by nerves arising from the pedal cords,

but exponents of the idea that it was pallial suggested that the dorsal part of the pedal cord (demarcated by a shallow groove) was in fact related to the pleural ganglion (Wegmann, 1884; Lacaze-Duthiers, 1885; Boutan, 1885). Other workers held that the pedal cords related entirely to the pedal ganglion and therefore that the epipodium was a pedal structure (Spengel, 1881; Haller, 1884; Pelseneer, 1888a, 1888b, 1890, 1891; Bourne, 1910). This latter view is now that which is accepted and it is believed that the dorsal part of the pedal cord is primarily sensory and the ventral part primarily motor (Bullock & Horridge, 1965).

Several studies have been concerned essentially with the structure of the sensory cells (Boll, 1869; Flemming 1884; Lacaze-Duthiers, 1886; Merton, 1920; Schulz, 1937; Demal, 1955). They have demonstrated, usually using silver staining methods, the comparatively abundant supply of nerves to the body wall of molluscs. In all cases they reported that the sensory cells were primary receptor cells (i.e. a nervous receptor cell, Bullock & Horridge, 1965). There is no indication, even in special sense organs that non-nervous receptor cells (formerly secondary receptors) are involved. In most cases the cell body of the sensory cell lies, within, immediately beneath, or very near the epithelium. The distal processes of these cells were often found to end in a brush-like structure, hence the term 'Pinzelzellen' (brush cells) (Crisp, 1971). The central, axonal, processes of the cells usually extend to the central nervous system, especially in the less advanced forms,

i.e. most prosobranchs. In the stylommatophoran pulmonates however, there may be accessory ganglia within the sense organs, such as the tentacular ganglion, which may allow some peripheral integration (Bullock & Horridge, 1965).

In these early studies several types of primary sensory cell were distinguished particularly in the opisthobranchs (Merton, 1920) and pulmonates (Schulz, 1937). These distinctions were based mainly on the arrangement and degree of branching of the distal, dendritic, process and its general location. In many cases the cells are scattered throughout the body wall, but in certain areas there may be clusters of cells, often of one type, in patches or placodes of specialized sensory epithelium, for example the osphradium of prosobranchs, the ommatophore of pulmonates and Hancock's organ of opisthobranchs. The epipopidal sense organs may represent a further example.

In recent years, work on the sense organs and sensory cells has been more extensive and has usually involved electron microscopy. Particular attention has been given to the pulmonates (Rogers, 1971; Zylstra, 1972; Wright, 1974a, 1974b; Wondrak, 1975; Kataoka, 1976; Benedeczky, 1977, 1979; Kulkarni, 1977; Hernádi & Benedeczky, 1978; Zylstra, Boer & Sminia, 1978; Jones & Saleuddin, 1978; Saleuddin, 1979) and cephalopods (Graziadei, 1964; Barber, 1966; Barber & Wright, 1969a; Woodhams & Messenger, 1974; Emery, 1975, 1976). Less attention has been paid to the opisthobranchs (Bonar, 1978; Emery & Audesirk, 1978; Kress, 1981) and prosobranchs (Storch & Welsch, 1969; Crisp, 1971,

1973, 1981; Phillips, 1977, 1979). Studies have also been carried out on bivalves (Barber & Dilly, 1969; Moueza & Frenkiel, 1976, Moir, 1977a, 1977b; Owen & McCrae, 1979).

The main aim of the above investigations has been to examine the ultrastructure of the sensory cells in the hope of establishing the modality to which they are sensitive. At this level different types of receptor may be distinguished by the presence or absence of cilia (kinocilia and stereocilia), the number and arrangement of these structures and the organisation of the basal body and its rootlets. Unfortunately however, as yet no reliable morphological criteria have been found which distinguish receptors for one modality from those for another (Crisp, 1971; Owen & McCrae, 1979) with the possible exception of photoreceptors in the eyes. Indications of function and sensitivity therefore, can only be gained by comparison with the structure of sensory cells from other species (not necessarily molluscan), the function of which is more clearly understood.

Additional information may perhaps be gained from electrophysiological and behavioural studies, but unfortunately these are rarely linked with ultrastructural observations. Phillips (1975) however, noted that the mantle edge of *Acmaea (Notoacmea) scutum* Eschscholtz was sensitive to chemical, photic and mechanical stimulation. He was nevertheless only able to find two morphologically distinct types of receptor (Phillips, 1979). Similar observations have been reported by Crisp (1971, 1972, 1976) for *Hinia reticulata* (L.) *(Nassarius reticulatus* (L.)). It is clear therefore that even with

such studies, attributing a particular sensitivity to a particular sensory receptor must be done with caution. The task may be made easier if a structure contains only one type of receptor.

The purpose of this study has been to examine the fine structure of certain epithelial sense organs and the ultrastructure of the sensory cells they contain in British Trochacea, particular attention being paid to the cephalic and epipodial tentacles and epipodial sense organs. At the start of the investigation the only published electron microscope observation on these sense organs was that of Andrews (Fretter & Graham, 1977). This was essentially a preliminary examination and was that which led to the investigations reported here. During the course of the work, however, the study of Crisp (1981) on the epithelial sensory structures of trochids was published. This was found to overlap with certain aspects of the present work and consequently the investigation was extended to include species belonging to other archaeogastropod groups (superfamilies), namely the Fissurellacea and Patellacea. The results are divided into three sections, each relating to one superfamily. These are further divided into subsections describing the general distribution, innervation, fine structure and ultrastructure of the sense organs.

Section 1 concerns members of the Trochacea and concentrates on the cephalic and epipodial tentacles and epipodial sense organs. Most of the work was carried out on *Gibbula umbilicalis* (da Costa), but specimens of

G. cineraria (L.) and *Monodonta lineata* (da Costa) were also examined. Differences where observed are noted. During the study it was found that epithelial receptors are not restricted to specialised sensory regions, but occur in varying numbers over a large part of the external surface. These areas were examined briefly using scanning electron microscopy.

Specimens of the Indo-Pacific trochid, *Umbonium vestiarium* (L.), were examined for comparison as this species has a mode of life strikingly different from the British species (Fretter, 1975). It is classified in the subfamily Umboniinae and occurs, often in very large numbers, in the surface layers of mudflats and sandy beaches where it obtains food by filter-feeding. This is in sharp contrast to British trochids which are in general intolerant of silty environments, (with the possible exception of *Solariella amabilis* (Jeffreys) which may also possess a ciliary food collecting mechanism; Fretter & Graham, 1977).

The observations reported in Section 2 concern the fissurellacean, *Emarginula reticulata* Sowerby. As with the trochids the structures of most interest were the cephalic and epipodial tentacles and epipodial sense organs. During scanning electron microscopical examination however, structures showing obvious similarity to the epipodial sense organs were observed on the mantle edge bordering the shell slit. The study was therefore extended to include this region.

The studies on patellacean species are given in

Section 3 and involve *Patella vulgata* L. and *Acmaea testudinalis* Müller. They were examined mainly for comparison with the preceeding species and the observations concern largely only the ultrastructure of the tentacular receptors (cephalic and pallial).

The general anatomy of the nervous system and innervation of the sense organs of *P. vulgata* has been described previously by Bouvier (1887); Gibson (1887); Pelseneer (1898-1899); Davis and Fleure (1903); Fretter and Graham (1962) and Choquet and Lemaire (1969), and that of *A. testudinalis* by Bouvier (1887); Willcox (1898, 1906) and Fretter and Graham (1962). Apart from a brief description of the innervation of the cephalic tentacles this aspect is not considered here.

The pallial tentacles of *A. testudinalis* have not been examined. A description of them has been given by Phillips (1977, 1979) for a closely related species (or subspecies; Fretter & Graham, 1976), *Acmaea scutum*.

C H A P T E R 2

MATERIALS AND METHODS

SPECIMENS

Gibbula umbilicalis, *G. cineraria* and *Patella vulgata* were collected at various localities on the Dyfed and Gower coasts in South Wales. *Monodonta lineata* was obtained only from the Dyfed coast.

Emarginula reticulata and *Acmaea testudinalis* were obtained from the University of London Marine Station, Millport, Isle of Cumbrae, Scotland. Attempts were made to obtain other living fissurellaceans, but these were unsuccessful, even *E. reticulata* was only available in small numbers.

Umbonium vestiarium was kindly provided by Dr. V. Fretter. The specimens were collected from Bardargah Bay, near Bushehr, Persian Gulf and were preserved in 70% alcohol.

With the exception of *E. reticulata* all living specimens were maintained in tanks containing artificial sea water (Instant Ocean), linked to a tidal machine (see Jennings, in preparation). They were exposed to a tidal regime approximating that of mean tide level (50% emersion). Even at low tide, however, the tanks never drained completely to accommodate those species which normally occur lower on the shore, namely *G. cineraria* and *A. testudinalis*. Rocks were placed in the tanks as a substratum. *E. reticulata* was kept in standard continuous flow aquarium tanks containing rocks with encrusting red sponges.

The majority of animals were used soon after arrival, but specimens of *G. umbilicalis* were frequently kept for longer periods. Attempts were made to feed these animals

using calcium alginate with complan and dried nettle (see Appendix 1) - a food known to be eaten by *Littorina littorea* (Arason, personal communication) and other caenogastropods and freshwater pulmonates (Andrews, personal communication). There was however, no evidence that this was taken by *G. umbilicalis*. Animals nevertheless survived well for two months or more.

ROUTINE HISTOLOGY

For routine histological studies tissues were fixed in one of the following fixative mixtures:-
Heidenhain's Susa (Pantin, 1946)
Stieve's fluid (Humason, 1979)
Bouin's fluid (Pantin, 1946)
The last was used most often.

After fixation tissues were dehydrated in alcohol, cleared and infiltrated and embedded in paraffin wax. Chloroform was used as the clearing agent as it was found to cause less hardening than xylene. Sectioning was generally more satisfactory when tissues were embedded in Fibrowax (Lamb & Co.). A vacuum oven was used for infiltration of large specimens.

Sections 6-8µm thick were cut on a Beck rotary microtome and stained using either Heidenhain's iron haematoxylin or Heidenhain's Azan (Pantin, 1946). Sections of tissue fixed in Susa or Stieve were treated with Lugol's iodine to remove mercuric deposits. Photomicrographs were taken on a Ziess Photomicroscope II.

DEMONSTRATION OF THE NERVOUS SYSTEM

For demonstration of the nervous system and detailed innervation of the sensory structures whole mount specimens were stained using Owen's modification of Liang's method for nerve fibres (Liang, 1947; Owen, 1959). This method was found to be quick and easily repeatable, although thorough washing in distilled water is necessary to remove all the SO_2 water prior to counterstaining, otherwise preparations fade rapidly with time. Whilst rendering most nerves clearly visible, this method failed to demonstrate the finest nerve branches.

For this last purpose attempts were made to stain thick (15µm) wax sections using the silver methods of Rowell (1963) and Albrecht (1962). Neither of these methods, however, produced satisfactory results.

DARK-FIELD MICROSCOPY

Living tissue was examined under dark-field illumination using a Ziess Photomicroscope II. This was used to investigate details of surface ciliation without fixation.

ELECTRON MICROSCOPY

The following fixation methods were used in electron microscopy:-

Method 1

Primary fixation at $4^{o}C$ for 2½ hours in 5% glutaraldehyde in 0.1M Sorensen's phosphate buffer, pH 7.2 with 14% sucrose.

Postfixation after two rinses in buffer, in 1% osmium tetroxide in the same buffer for 1 hour at room temperature, followed by a further two rinses in buffer before dehydration.

Method 2

As Method 1, but with 1.5% NaCl in place of sucrose.

Method 3

As Method 1, but with 0.1M cacodylate buffer, pH 7.2, in place of phosphate buffer.

Method 4

As Method 2, but with 0.1M cacodylate buffer, pH 7.2, in place of phosphate buffer.

Method 5

Primary fixation for 1 hour at room temperature in 3% glutaraldehyde in 0.1M cacodylate buffer, pH 7.0 with 25% sucrose and 0.5% $CaCl_2$.

Postfixation for 1 hour at room temperature (without rinsing in buffer) in 1% osmium tetroxide in the same buffer (Coggeshall, 1971).

Method 6

Combined glutaraldehyde (2.5%) and osmium tetroxide (1%) in 0.1M cacodylate buffer, pH 7.4 with 8.6% sucrose. Stock fixatives were kept separate, cooled to $0^{o}C$ and mixed, one part glutaraldehyde to two parts osmium tetroxide, immediately before use. Fixed for 1 hour in an ice bath and washed in ice cold buffer (Hirsch & Fedorko, 1968).

Method 7

Parducz fixative (Phillips, 1977). Six parts osmium tetroxide (2%) to one part saturated aqueous mercuric chloride.

Fixation for 2 hours at 4°C.

SCANNING ELECTRON MICROSCOPY

Methods 1 and 7 were used for scanning electron microscopy. After fixation tissues were dehydrated in a graded ethanol series and critical point dried either directly from absolute ethanol or via absolute acetone, in a Polaron 3000, CO_2 critical point drying apparatus. Once dry, tissues were mounted on stubs with silver paste or sticky tabs and coated with gold-palladium in a Polaron E 5100, cooled diode sputter coating apparatus. Specimens were examined in Cambridge Instruments S4-10 and S600 scanning electron microscopes.

Fixation Method 1 produced results superior to those of Method 7. Specimens fixed by the latter method often had strands of dried mucus on the surface. Consequently, after initial trials, Method 7 was not used again.

Animals were usually narcotised in 7.5% $MgCl_2$ diluted with an equal volume of sea water before fixation.

TRANSMISSION ELECTRON MICROSCOPY

All methods, except Method 7, were used for transmission electron microscopy. After fixation tissues were dehydrated in a graded ethanol series and embedded, via propylene oxide, in TAAB or Spurr resin. Thick (0.5µm) and thin (silver-gold) sections were cut using glass knives on a Cambridge Instruments, Huxley Ultramicrotome Mark II. Thick sections were mounted on glass slides for light microscopy and stained in 1% toluidine blue in borax (Dawes,

1971). Thin sections were collected on uncoated grids and stained in Watson's uranyl acetate (Dawes, 1971) and lead citrate (Reynolds, 1963). They were examined in AEI EM 6B, AEI Corinth 275 and Zeiss EM 109, electron microscopes.

With the exception of Method 5 all the fixation schemes used produced satisfactory results. Methods 1-4 utilized buffers with either sucrose or NaCl to raise the osmolarity from approximately 200 mOsm to 650-700 mOsm, i.e. approximately two thirds of the blood osmolarity as recommended by Bone and Denton (1971). All osmolarities were measured using a Knauer Halbmikro milliosmometer.

Methods 1 and 6 produced slightly more even fixation than 2, 3 and 4. However, because of the more complex procedure of Method 6 it was not used extensively. The most frequently used method was Method 1. Tissues fixed using Method 5, particularly epithelia, were highly electron-dense, even with reduced staining, and showed little definition.

BEHAVIOURAL EXPERIMENTS

Preliminary experiments were undertaken on the response of *G. umbilicalis* to a potential food source and to try to establish whether this aspect was worth more thorough investigation. For this purpose a transparent perspex Y maze was constructed such that a slow, constant flow of sea water passed down both arms and out of the stalk of the Y. In one arm a small piece of freshly collected alga encrusted rock was placed as a potential food source (Fretter & Graham, 1977). An animal, previously starved for

48 hours or more, was then placed at the base of the stalk and its behaviour observed. (Care was taken to ensure that the rate of water flow was the same in both arms.) In no case was there any obvious movement into the arm containing the food. In most cases the animal remained inactive at the base of the stalk. The response did not differ when water flow was stopped.

The overall impression gained from these experiments was that these animals, unlike carnivorous caenogastropods (Crisp, 1971), have no distinct feeding response. They appear, when active, to rasp the substratum continually. The activity in turn may be regulated by the tidal cycle (Newell, 1979). As a result this aspect was not investigated further.

C H A P T E R 3

RESULTS

SECTION 1

Trochacea

1.1. Distribution of Sense Organs

Gibbula umbilicalis

G. umbilicalis possesses two prominent cephalic tentacles arising dorso-laterally from the head (Plate 1a). These are highly mobile and except for a furrow along the dorsal surface, are covered with numerous very small papillae. Their general colour is pale with dark transverse bands. Lateral to each tentacle is a short, stout eye stalk, slightly drawn out laterally. The dark opening of the eye lies dorso-laterally near the apex of the eye stalk. A short slender postoptic tentacle (sub-ocular peduncle, Crisp, 1981) projects ventrally from the base of the right eye stalk.

Medio-dorsal to each cephalic tentacle is a cephalic lappet bearing 10-15 papillae on its anterior edge. These lappets extend towards the mid line but do not meet. A similar papillate ridge lies along the postero-lateral edge of the snout. The lips are large and radially lobed.

Large neck lobes arise posterior to each eye stalk, that on the right is continuous with the eye stalk. These are broad, flat structures formed by the anterior part of the epipodium. During life they curl to form the ventral portions of the inhalant and exhalant (left and right respectively) openings to the mantle cavity. The left neck lobe (Plate 1b) bears 20-30 short finger-like projections

(digits) on its free margin. Some of these, usually five, are slightly larger than the others. The free margin of the right neck lobe is smooth and its dorsal surface possesses cilia which beat in an outward direction carrying mucus-bound particulate material to the margin where it falls away from the body. Ventral to each neck lobe, where the anterior of the foot joins the lobe is an epipodial sense organ. These are raised above the body surface on a short stalk and usually possess some white pigmentation.

Continuous with the neck lobes are the epipodial folds. These border the dorsal margin of the foot and join posteriorly in the mid line, for the most part they are in close contact with the operculum. The edge of the fold is irregular and three papillate epipodial tentacles arise ventrally out of short sheaths on each side. Dorsal to each epipodial tentacle the epipodial fold is formed into two rounded lobes. The most anterior tentacle on each side lies between the neck lobe and the operculum, the others under the operculum. An epipodial sense organ is associated with the base of each tentacle, lying just posterior to the anterior one and ventral to the other two, often projecting on a short stalk from the tentacle sheath. The pigmentation of the epipodial tentacles is similar to that of the cephalic ones, though usually less dense. In addition to the dorsal, papilla-free furrow, the epipodial tentacles also have a ventral one.

The edge of the sole and the ventral portions of the sides of the foot also bear small papillae.

Gibbula cineraria

The sensory regions of *G. cineraria* exhibit only minor differences from those of *G. umbilicalis*.

The cephalic lappets are broader, possess more papillae (15-20) and extend over the base of the cephalic tentacles. Likewise the postero-lateral ridges on the snout are better developed. The eye stalks are longer and more slender, the left is markedly concave ventrally, resembling that of *Umbonium vestiarium* (see later P.133). The neck lobes are generally thinner, less rigid structures and the digits of the left neck lobe are slightly longer and more uniform in length. The epipodial tentacles have less well developed sheaths.

Monodonta lineata

In *M. lineata* the cephalic lappets are small and without definite papillae. The eye stalks are flattened dorsally and the opening of the eye lies at the apex. The right post-optic tentacle is poorly developed. The digits of the left neck lobe, though similar in number are alternately longer and shorter, some 2-3 times longer than the short ones. A small digit is occasionally present on the ventral surface of the right neck lobe.

There are one or two and rarely three, epipodial sense organs at the base of each epipodial tentacle and, infrequently, some interspersed between them.

The above descriptions are only general. It must be stated that there is considerable individual variation within all the species. This is especially true of the epipodial sense organs, where two small ones may often occur in the place of one larger one or *vice versa*. The right postoptic tentacle may be absent or even bifid, possibly as a result of damage. Animals have also been found with one too few, or one too many, epipodial tentacles on one or both sides (Plate 2a). The pigmentation of the sensory structures and the general body colouration is also variable, but usually *M. lineata* is more densely and brightly pigmented that *G. cineraria*. *G. umbilicalis* is intermediate.

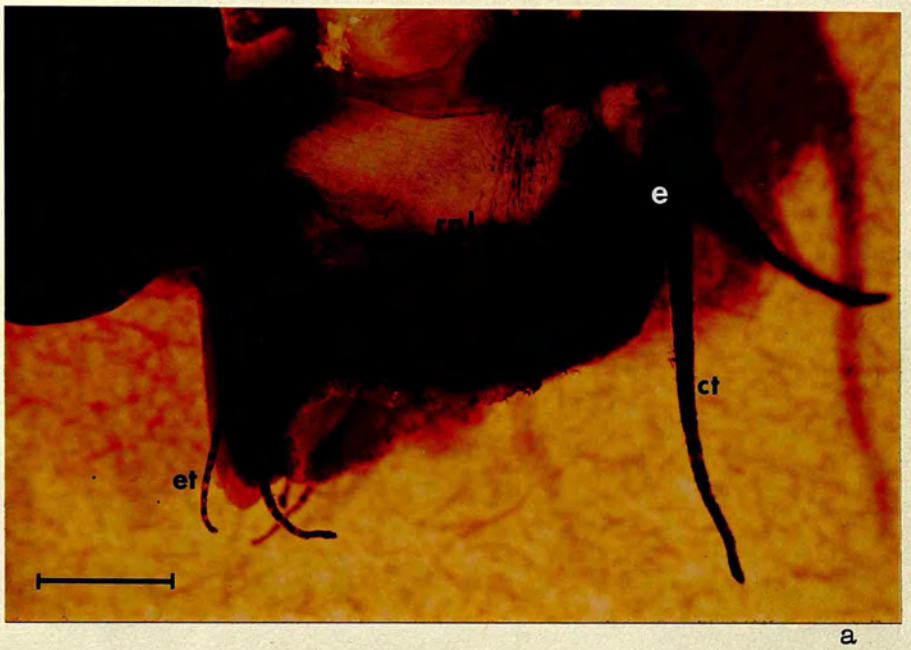
PLATE 1

a) *Gibbula umbilicalis*, female removed from shell viewed from the right showing cephalic and epipodial tentacles, right eye and right neck lobe.
Bar = 2.0mm.

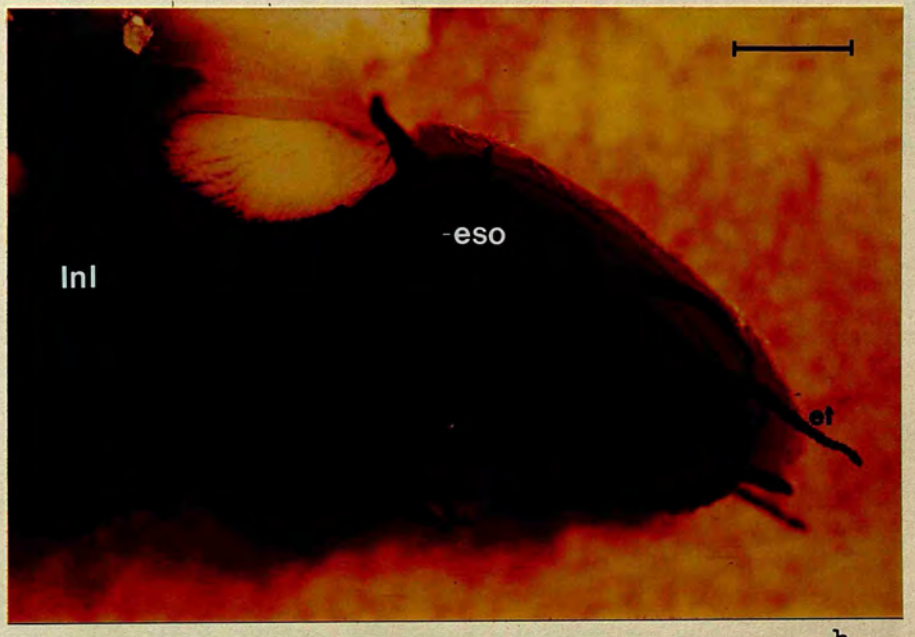
b) *Gibbula umbilicalis*, animal removed from shell viewed from the left. Middle and posterior of the foot showing left neck lobe, epipodial tentacles, epipodial fold and epipodial sense organs.
Bar = 1.0mm.

ct - cephalic tentacle
e - eye
ef - epipodial fold
eso - epipodial sense organ
et - epipodial tentacle
lnl - left neck lobe
rnl - right neck lobe

PLATE 1



a



b

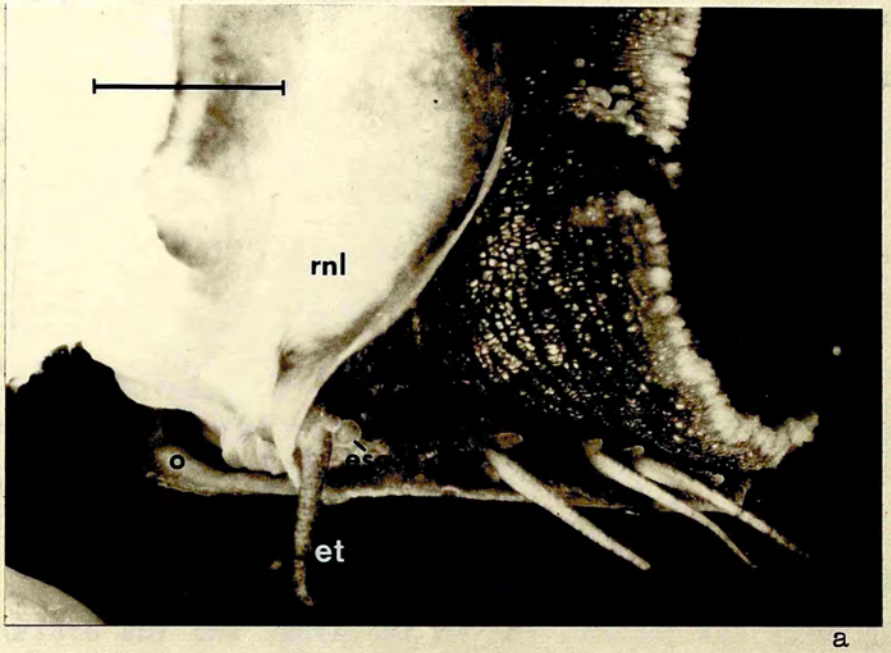
PLATE 2

a) *Monodonta lineata*, animal removed from shell viewed from the right. Atypical specimen with four epipodial tentacles.
Bar = 2.0mm.

b) *Gibbula umbilicalis*, upturned living specimen in initial stages of emergence. The operculum is raised and the tentacles extended.
Bar = 2.0mm.

ct - cephalic tentacle
eso - epipodial sense organ
et - epipodial tentacle
o - operculum
rnl - right neck lobe

PLATE 2



a

b

1.2. Use of the Tentacles During Life

When completely withdrawn into the shell the only part of the animal visible is the operculum. Upon extension however, the operculum is raised slightly and the cephalic and epipodial tentacles are protruded around the edge. If the shell is lying aperture down, the tentacles almost immediately contact the substratum and if undisturbed the animal emerges further until the sole of the foot is completely extended on the ground. During this process the tentacles are continually in motion, frequently touching the substratum and the sides of the shell.

If the shell is inverted with the aperture uppermost (Plate 2b) the tentacles do not contact any firm objects other than the shell and are moved rapidly in all directions, apparently in search of the substratum. This process continues with further extension of the animal and the foot is extended over the sides of the shell in order to locate and grip a firm object. At this time the slightest mechanical disturbance or sudden change in light intensity causes the animal to withdraw, though this is usually only for a short period. If left inverted with no firm objects nearby or sudden water currents to move the shell, the animals in general do not seem capable of righting themselves and eventually withdraw completely.

Under normal conditions the animals move around actively and the tentacles are kept extended. The cephalic tentacles often touch the substratum in front of the animal

and the epipodial ones that at the side. They do not contract if they touch a stationary object, but do so rapidly if touched by a moving object or if pinched.

The location of the epipodial sense organs renders them difficult to examine in an undisturbed living animal as they lie between the foot and the epipodial fold, under the body whorl of the shell. There is little evidence that they are actively moved around, though the stalk does contract slightly when touched, especially that of the ones under the neck lobes. They were never seen in contact with the substratum.

1.3. The Nervous System and Innervation of Sense Organs

G. umbilicalis, like all archaeogastropods has a primitive hypoathroid nervous system (Fretter & Graham, 1962). The cerebral ganglia are elongate, connected by a long cerebral commissure and are situated in the narrow haemocoelic cavity (the dorsal cephalic sinus) between the body wall and the anterior oesophagus. The pleural ganglia lie in close proximity to the pedal ganglia and both of these are connected to the cerebral ganglia by long connectives (Fig. 2a). The pedal ganglia are drawn out into cords with numerous commissures.

More advanced features include the development of sub- and supra-oesophageal ganglia (although these are only slight swellings) and the presence of a branchial (osphradial) ganglion associated with the ctenidium.

The sensory regions of the head are mainly innervated by nerves arising from the cerebral ganglia. Three nerves arise from the anterior margin of each ganglion, two supplying the dorsal areas of the snout and lips and the other innervating the cephalic lappets (Fig. 2b). The ventral areas of the snout and lips are innervated by nerves from the labial ganglia. Each cephalic tentacle is supplied by a tentacular nerve arising dorso-laterally from the cerebral ganglia. Close to this lies the optic nerve which passes along the eye stalk to the eye. At the origin of the right optic nerve, a fine nerve arises and passes into the right postoptic tentacle.

Each neck lobe is innervated by a nerve arising dorsally from the pedal ganglia. This nerve divides into two distally, one branch supplying the lobe itself and the other the epipodial sense organ beneath it. Every digit of the left neck lobe receives a branch from the main lobe nerve.

The epipodial tentacles are innervated by nerves originating from the dorsal surface of the pedal cords. These nerves also divide distally, just below the epipodium, one branch supplying the tentacle, the other the sense organ at its base. Additional nerves arise from the pedal cords, some run to the epipodial fold, but the majority pass ventrally into the bulk of the foot.

Whole mount preparations of material stained by the modified Liang's technique (see Materials and Methods) have shown that the more detailed innervation of the cephalic and epipodial tentacles is similar. Within the tentacle the main nerve (tentacular nerve) passes centrally, directly to the tip, tapering as it progresses. At regular and frequent intervals very fine branches are given off radially (Fig 3a; radial nerves), running to the epithelium. In this region however, they become too fine to be seen using this method. These branches are thought to be the sensory nerves innervating the papillae on the surface. Motor nerves innervating the tentacular muscles must also be present, but were likewise too fine to be seen.

The epipodial sense organ nerve, after its origin from the nerve innervating the tentacle or neck lobe,

passes directly into the sense organ (Fig. 3b). Once inside the organ very fine side branches arise and pass to the epithelium of the stalk, but the bulk of the nerve runs distally to the tip where it branches extensively just below the specialised epithelium of this region.

Attempts to obtain further information on the exact course of the finest nerve branches using silver stained sections were not succeessful.

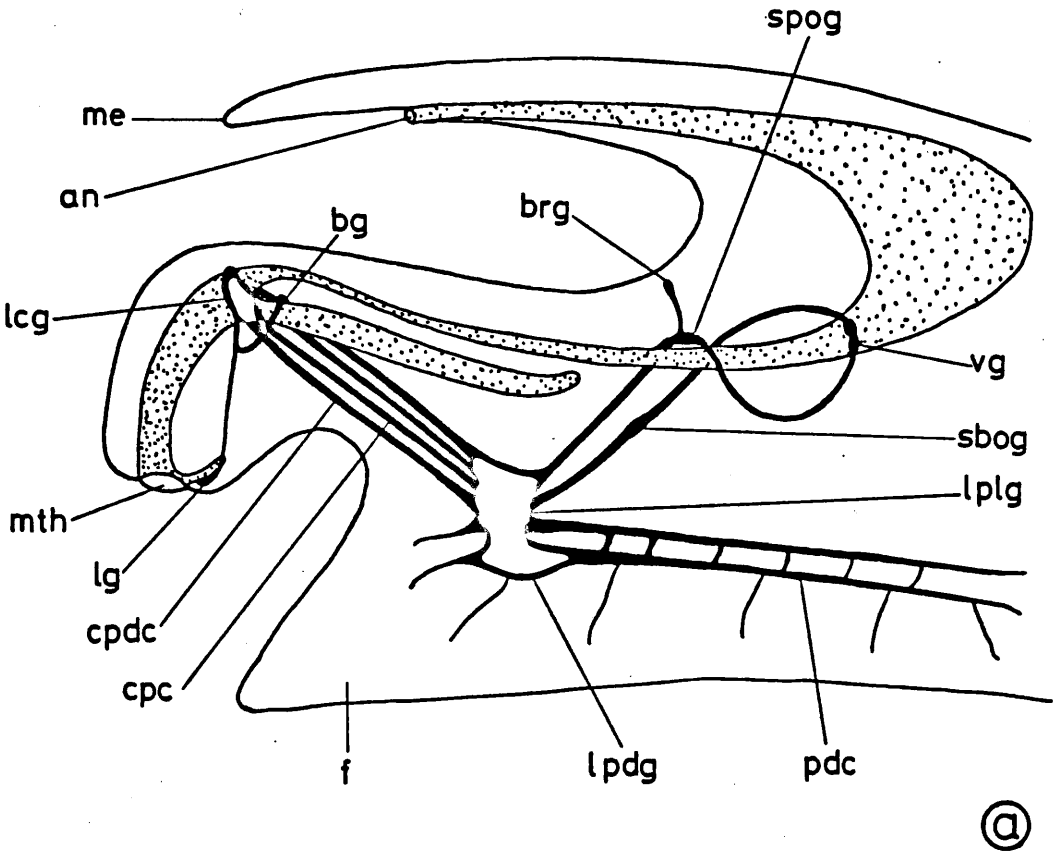
FIGURE 2

a) Lateral view of hypoathroid nervous system of *Gibbula umbilicalis*. (Adapted from Fretter & Graham, 1962).

b) *Gibbula umbilicalis*, dorsal view of right half of head showing nerves arising from the cerebral ganglion.

an - anus
bc - buccal connective
bg - buccal ganglion
brg - branchial ganglion
cc - cerebral commissure
cg - cerebral ganglion
cl - cephalic lappet
cln - cephalic lappet nerve
cpc - cerebropleural connective
cpdc - cerebropedal connective
ct - cephalic tentacle
e - eye
f - foot
l - lip
lcg - left cerebral ganglion
lg - labial ganglion
ln - labial nerves
lpdg - left pedal ganglion
lplg - left pleural ganglion
me - mantle edge
mth - mouth
on - optic nerve
pdc - pedal cord
rpot - right postoptic tentacle
rpotn - right postoptic tentacle nerve
sbog - suboesophageal ganglion
snt - snout
spog - supraoesophageal ganglion
tn - tentacular nerve
vg - visceral ganglion

FIGURE 2


spog
me
an
bg
brg
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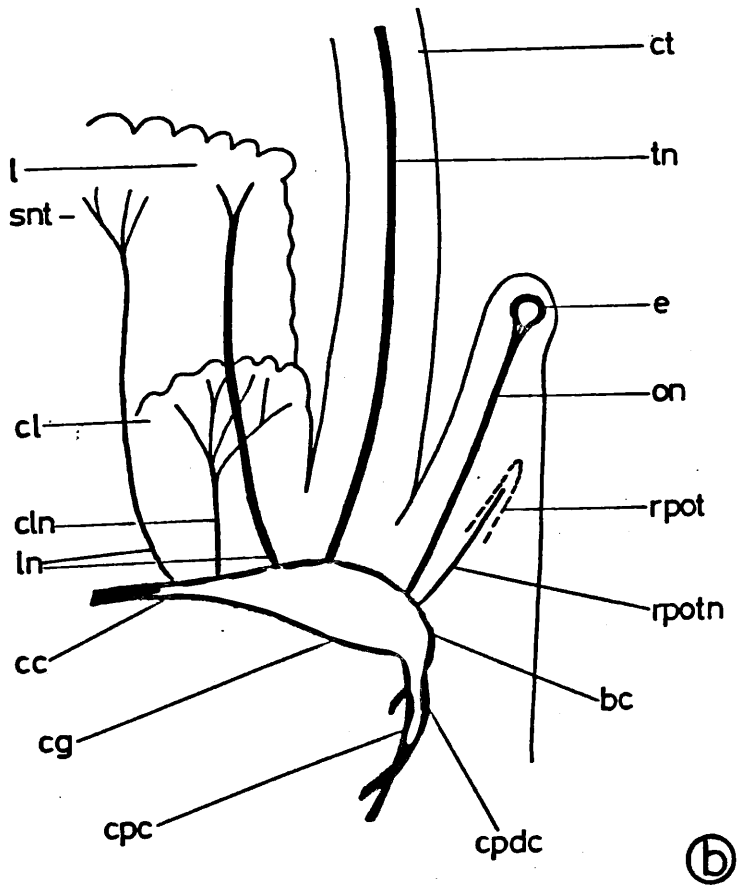
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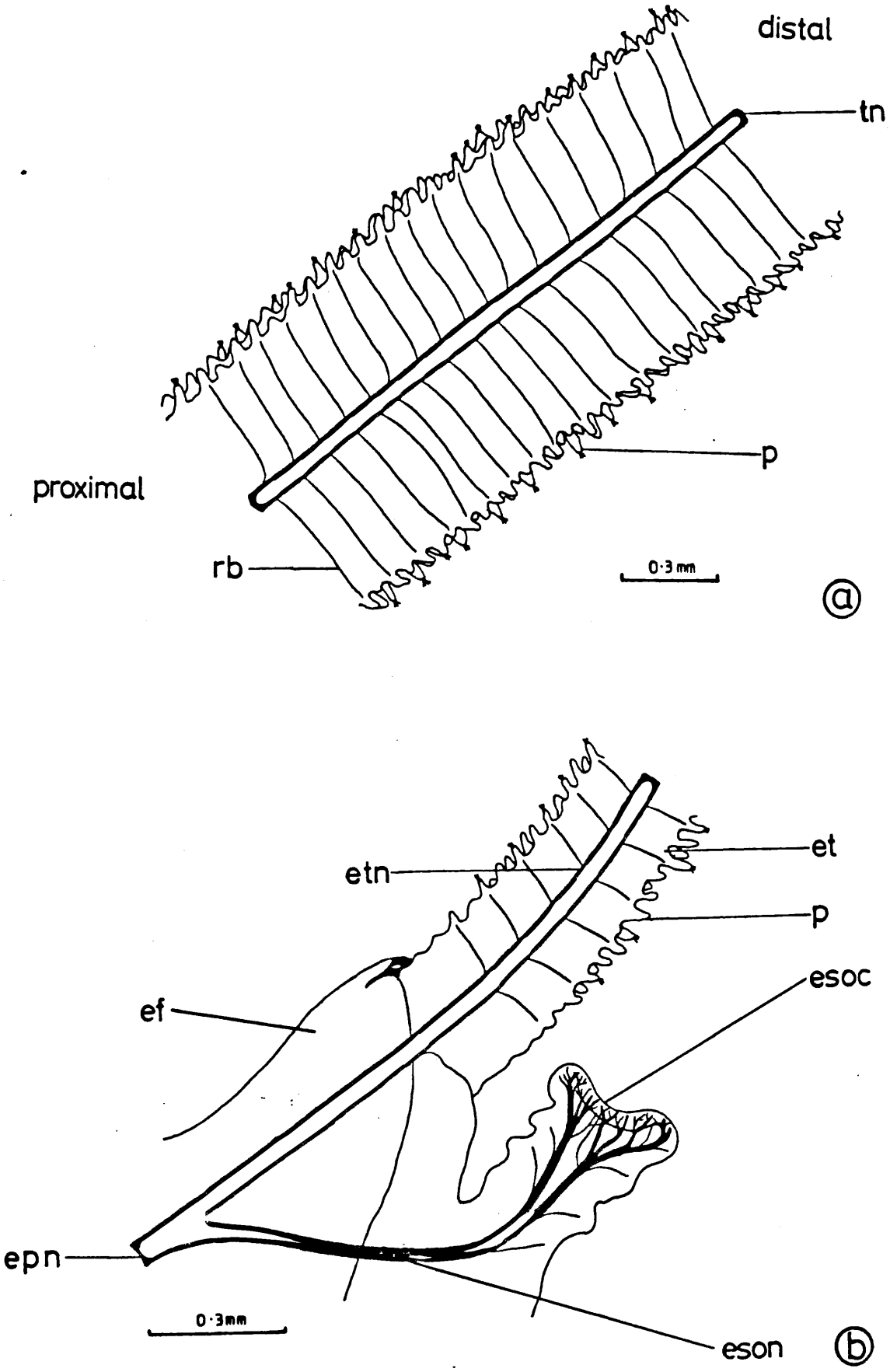
FIGURE 3

a) *Gibbula umbilicalis*, innervation of part of cephalic tentacle. Drawn from whole mount preparation stained using the modified Liang's technique.

b) *Gibbula umbilicalis*, innervation of epipodial sense organ and part of epipodial ~~sense organ~~ tentacle. The epipodial nerve arises from the pedal cord. Drawn from whole mount preparation stained using the modified Liang's technique.

ef - epipodial fold
epn - epipodial nerve
esoc - epipodial sense organ cupule
eson - epipodial sense organ nerve
et - epipodial tentacle
etn - epipodial tentacle nerve
p - papilla
rb - radial branch of tentacular nerve
tn - tentacular nerve

FIGURE 3



1.4. Internal Structure of the Tentacles and Epipodial Sense Organs

Cephalic Tentacles

In cross section the cephalic tentacles appear approximately circular in shape with a highly irregular margin (Plate 3a). The tentacular nerve runs centrally, though slightly displaced ventrally and is surrounded by a perineural sheath. The nerve is similar throughout its length, showing no concentration of cell bodies which might indicate the presence of a tentacular ganglion (Plate 3b). There are occasional nuclei within it, but these are thought to be those of glial cells. The radial branches are not visible in paraffin wax sections, but thick resin sections reveal that they divide just below the epithelium and pass into the bases of the papillae. Each radial branch supplies only a small number of papillae.

Dorsal to the tentacular nerve is a core of radial muscle fibres from which fibres arise and pass to the surrounding epithelium. Dorsal to this core is a haemocoelic space which is continuous basally with the dorsal cephalic sinus. Like the tentacular nerve, this tapers distally and disappears approximately 2/3 of the way along the tentacle.

Among the radial muscle fibres lie ovoid blocks of longitudinal muscles forming a ring around the haemocoel, radial muscle core and tentacular nerve. These muscles originate in a thickened region of the body wall latero-

dorsal to the cerebral ganglion and run the length of the tentacle progressively decreasing in size.

Between the ring of longitudinal muscle blocks and the surrounding epithelium is a layer comprising mainly connective tissue, the ends of the radial muscle fibres and smaller fibres supporting the epithelium. The connective tissue is thicker beneath the dorsal furrow, especially basally and may act as a flexible rod to strengthen the tentacle.

The outermost layer of this connective tissue layer is for the most part highly irregular in outline and is covered by a basal lamina upon which lies the epithelium with its numerous papillae. The epithelial cells are mostly columnar and may contain large amounts of pigment, those in the dorsal furrow however, are almost cuboidal. The detailed structure and arrangement of the epithelium, papillae and sensory cells will be discussed later. No mucous cells, epithelial or subepithelial have been observed throughout the tentacles in *G. umbilicalis* though epithelial ones have occasionally been seen in *M. lineata*.

Epipodial Tentacles

The epipodial tentacles are essentially smaller, more slender versions of the cephalic ones, and the nerves and muscles are arranged in an identical way. The haemocoelic space arises from the pedal sinus and the longitudinal muscle blocks originate in the musculature of the foot. The

papillae are concentrated on the sides of the tentacles as there is both a dorsal and a ventral papilla-free furrow.

Epipodial Sense Organs

Each epipodial sense organ can be divided into two distinct portions, the stalk which raises it above the general body surface and the sensory region itself which usually forms a small depression or cupule lined by a sensory epithelium at the distal end of the stalk.

The stalk is round to oval in cross section and comprises mostly connective tissue and small muscle fibres. The majority of the latter are randomly arranged, but some of the larger ones run longitudinally. The epipodial sense organ nerve lies centrally at the base of the stalk, but passes to the periphery as it branches beneath the sensory cupule.

The sides of the stalk are ridged transversely (in fixed tissue) and are covered by a columnar epithelium, 10-15µm high with a brush border. Between these cells lie numerous gland cells of two types. Type A closely resembles the familiar goblet-like mucous cell, its contents occupying most of its volume and staining red with azocarmine. Some of these cells also show γ-metachromasia with toluidine blue, possibly when the secretion is ready for release. Type B gland cells resemble the epithelial cells in shape, but their cytoplasm is granular and stains orthochromatically with toluidine blue. Also within the epithelium are small

groups of cells bearing short cilia, though little cellular detail is apparent at this level.

Large subepithelial gland cells lie beneath the epithelium and also occasionally below the epithelium of the sensory cupule. Their secretory product is structurally similar to that of the type A cells, but is more dense and never shows metachromasia with toluidine blue.

The epithelium of the sensory cupule is a pseudostratified columnar epithelium with a brush border and is markedly taller than the surrounding epithelium (20-25μm high). It lies on a thickened basal lamina supported by fine connective tissue (Plate 4a). Long, fine cilia up to 80μm in length also project from this epithelium and form a mat in the cupule. Details of the fine structure of this epithelium will be discussed later (P. 95). The branches of the epipodial sense organ nerve which innervate the epithelium are too fine to be seen clearly in paraffin wax sections, but ~~thin~~ resin sections show them to be numerous immediately below the basal lamina and also that they are usually associated with granular glial cells. On rare occasions they may be seen to pass through the basal lamina into the epithelium (Plate 4b). No gland cells have been observed within this sensory epithelium.

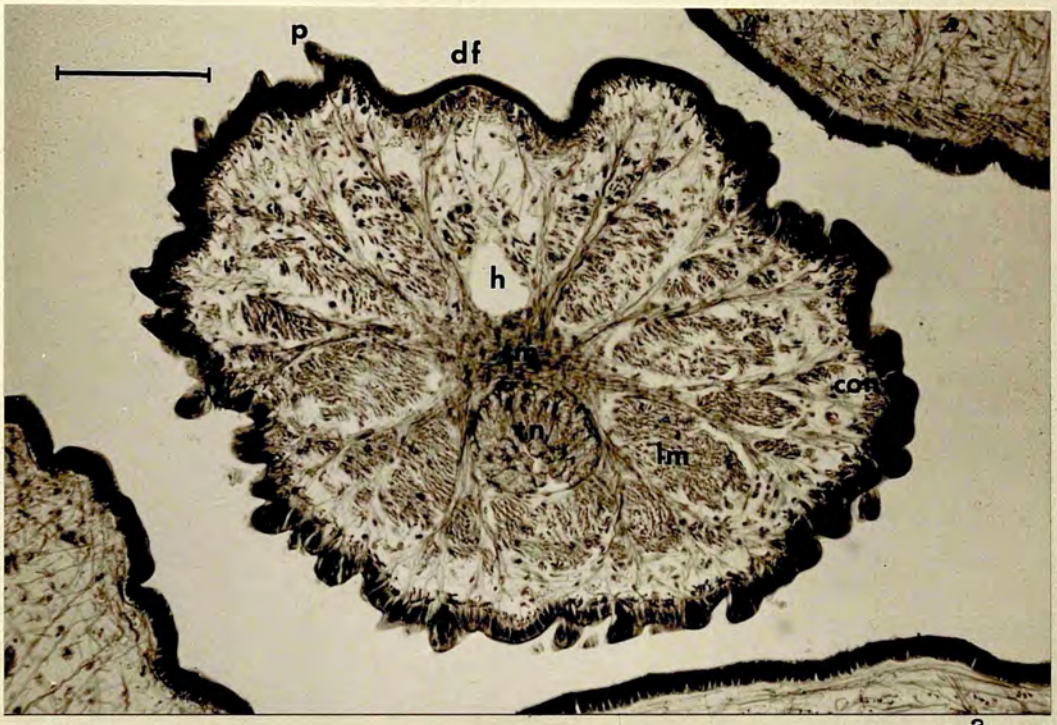
PLATE 3

a) *Gibbula umbilicalis*, transverse paraffin wax section through the cephalic tentacle, approximately halfway along its length. Heidenhain's iron haematoxylin. Bar = 100µm.

b) *Gibbula umbilicalis*, longitudinal paraffin wax section through the cephalic tentacle showing the origin of the tentacular nerve from the cerebral ganglion. Heidenhain's Azan. Bar = 200µm.

b - buccal mass
cg - cerebral ganglion
con - connective tissue
df - dorsal papilla-free furrow
e - eye
h - haemocoelic space
lm - longitudinal muscle
p - papilla
rm - radial muscle core
tn - tentacular nerve

PLATE 3



a

b

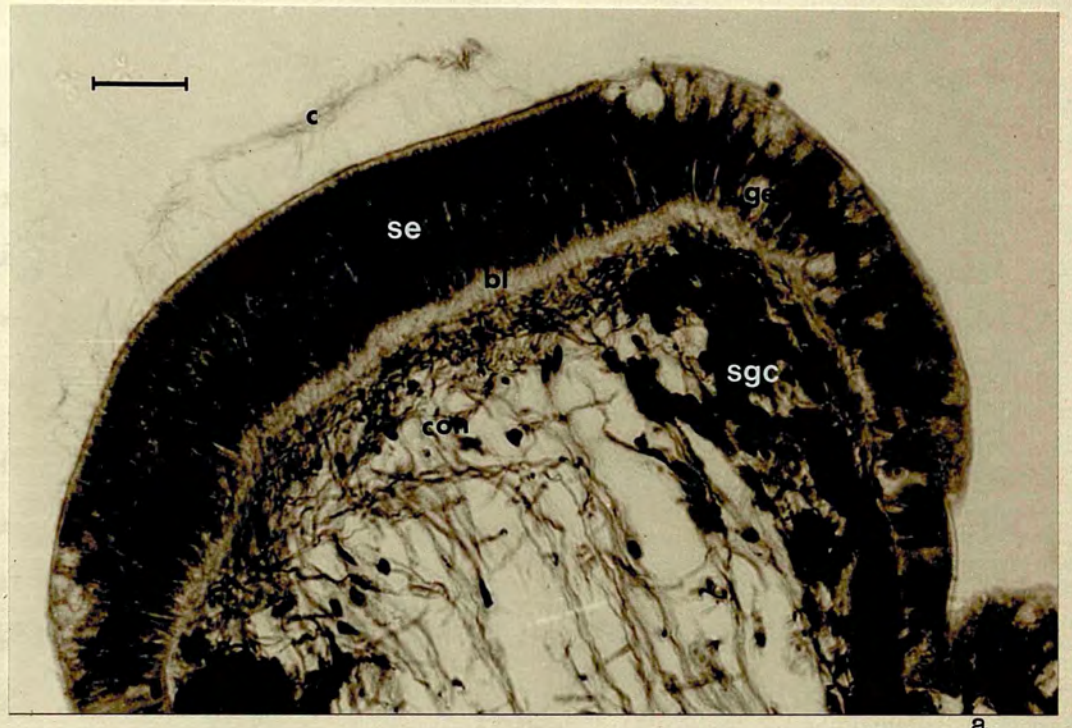
PLATE 4

a) *Gibbula umbilicalis*, longitudinal paraffin wax section through epipodial sense organ. Heidenhain's iron haematoxylin.
Bar = 20µm.

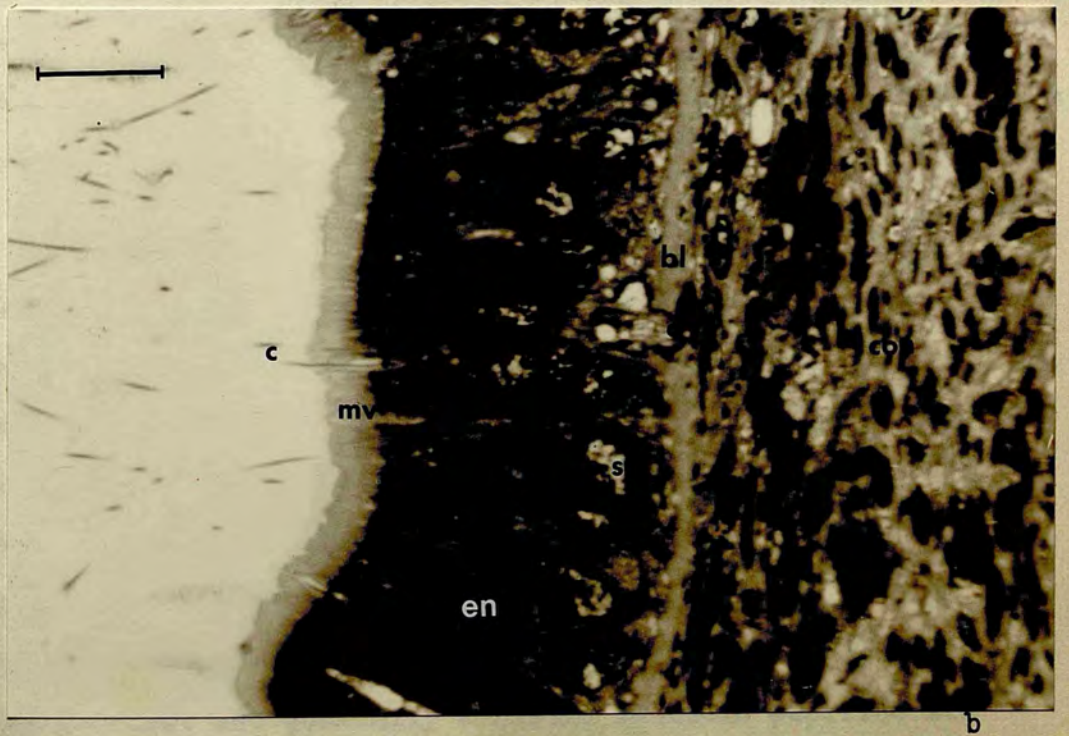
b) *Gibbula umbilicalis*, longitudinal thick resin section (0.5µm) through the sensory epithelium of the epipodial sense organ showing a group of axons penetrating the basal lamina. Toluidine blue.
Bar = 10µm.

a - group of axons penetrating basal lamina
bl - basal lamina
c - cilia
con - connective tissue
en - nucleus of supporting epithelial cell
ge - glanduar epithelium on rim of sense organ
mv - microvilli
se - sensory epithelium
sgc - subepithelial gland cells
sn - nucleus of sensory cell

PLATE 4



a



b

1.5. Fine Structure of the Tentacular Papillae

The fine structure and organisation of the papillae and sensory cells of the cephalic and epipodial tentacles are essentially similar and are therefore discussed together. Any observed differences are noted. A three dimensional reconstruction is shown in Fig. 4.

Fixed tentacles, even after relaxation with $MgCl_2$ show some degree of contraction and the surface is thrown into many shallow transverse ridges, these are most obvious in the non-papillate furrows (Plates 5 & 6). In the cephalic tentacles they are more pronounced distally indicating that this region of the tentacle may be capable of a greater percentage increase in length. This is supported by the presence of a collagenous supporting rod in the basal region. This however, is not so in the epipodial tentacles where the basal portion also bears pronounced ridges (Plate 6a & b).

The papillae are borne on the crests of these ridges, usually one row of papillae per ridge, but basally on the cephalic tentacles 2-3 rows per ridge (Plate 5). The length of the papillae is variable, short ones irregularly interspersed amongst longer ones (Plate 7a). This is unlikely to be due to differences in the state of contraction, as there are no muscle fibres within them (see below). Usually however, the papillae at the base of the tentacle are the shortest and generally cephalic tentacle papillae are longer than those on the epipodial tentacles.

The shape of each papilla is that of a truncated cone, slightly distended basally. They are covered in a layer of microvilli and bear an apical crown of short, stout cilia, 1-2μm in length (Plate 7b). These number 100-200 on the cephalic tentacle papillae, but usually < 100 on the epipodial tentacle papillae. Within this crown there is often a central dome of microvilli from which 2-3 shorter cilia project (0.5μm long). The outermost cilia usually project radially over a flange-like ring of microvilli which surrounds the crown.

These cilia are sensory and originate from the terminations of the sensory dendrites within the papillae. Internally the papillae comprise only sensory cells and unspecialised epithelial cells. The latter are flattened cells and make up the walls of the cone, leaving a central core which is filled with the sensory cells.

The sensory cells are simple bipolar neurones with the cell body (perikaryon) located within the epithelium. Each cell has a distal process, the dendrite, which runs to the tip of the papilla and a proximal process, the axon, which passes to the tentacular nerve (Fig. 4.). Within each papilla there may be 8-20 such cells with their perikarya grouped in the slightly expanded basal region.

The dendrites, on leaving the perikaryon, are roughly circular in cross section (Plate 8a), but distally, just below the ciliated crown they expand and wrap around each other rather like the leaves of a leek (Plate 8b & c & Fig. 5.). There is usually one, sometimes two, central

cells which remain circular, but the outer ones become progressively more and more C-shaped. In some sections an additional circular dendrite may be seen at the edge of the main group. This may originate from a distinct type of cell, but was only observed on a small number of occasions. It is not thought to be present in all papillae.

The axons, after their origin from the perikaryon, pass through the basal lamina underlying the epithelium and into the connective tissue below (Plate 9 & Fig. 4). In this area they lie in groups surrounded by glial cells containing ovoid electron-dense granules (Plate 8d). The basal lamina does not lie flat under the papillae, but projects slightly into the base where the axons emerge.

Structurally all the sensory cells appear to be similar and in contrast to the epithelial cells their cytoplasm is poor in organelles. The distal margin of the dendrite which is exposed to the exterior bears both cilia and microvilli. The latter are shorter than those on the surrounding epithelial cells and the tip therefore appears sunken (Plate 10a). The number of cilia per cell varies, the more expanded outer ones bearing the most. The central circular cell (s) probably bears the 2-3 shorter cilia mentioned earlier.

Internally the cilia have the normal 9+2 arrangement of microtubules throughout their length (Plate 10a & b). The basal body has a striated rootlet which extends inwards but has no basal foot. The orientation of the central pair

of microtubules shows no distinct pattern either linear or radial and appears to be random. Occasional bulbous swellings were noted on the cilia, but these are thought to be artifacts (Ehlers & Ehlers, 1978). The apical junctions between adjacent sensory cells are similar to those between the epithelial cells (see below) though the terminal web of microfilaments is poorly developed or absent. There is no interdigitation of the lateral membranes.

Cytoplasmic inclusions in the dendrites comprise only small electron-lucent vesicles, microtubules, occasional bundles of microfilaments and long narrow mitochondria (Plate 10c). The latter are particularly abundant in the expanded distal region, just beneath the cilia (Plate 9).

The perikaryon contains an irregularly shaped nucleus, but this is essentially oval, its long axis running parallel to that of the papilla. The cytoplasm distal to the nucleus contains Golgi bodies, small and large vesicles of varying electron-density, mitochondria, multivesicular bodies and, infrequently, large lamellate bodies (Plates 9 & 10d). The basal cytoplasm is similar and the cell body tapers rapidly to form the axon which almost immediately penetrates the basal lamina. Initially the axon may contain mitochondria and large vesicles, but for most of its length it contains only small vesicles and microtubules.

The unspecialised epithelial cells forming the walls of the papillae are flatter than the cells covering the rest of the tentacle surface, but have a similar internal

structure. The plasma membranes of adjacent cells interdigitate extensively especially in the mid to apical region (Plate 10c), and immediately below the apical surface the cells are joined by zonulae adhaerentes with many associated microfilaments and frequently septate desmosomes. The microfilaments form a dense terminal web under the apical membrane which bears numerous microvilli. The latter seldom branch, are 1-2μm long and are supported by microfilaments arising from the terminal web. They are clothed in a glycocalyx comprising a mass of very fine filamentous material (Plate 10c). This sometimes appears thicker at the tips of the microvilli, possibly providing extra support and gives them the appearance of having dilated ends (Plate 10a).

The nucleus is irregular in shape, contains one or two nucleoli and lies approximately in the centre of the cell. In addition to the usual cell organelles (Golgi bodies, mitochondria, ER, and ribosomes), large clear vesicles, small electron-lucent vesicles, multivesicular bodies, dense lamellate bodies and pigment granules occur, especially in the apical cytoplasm. The pigment granules were usually either present in large numbers or not at all and the cells containing them often occur in groups. Basally the cells (except those in the papillae) lie on a basal lamina 0.2μm thick, attached by hemidesmosomes. This in turn overlies a network of collagen fibres and small muscle fibres.

Examination of living tissue under dark-field illumination has shown that when tentacles are contracted

the papillae lie at an angle to the main axis, their apices pointing towards the tentacle tip (Plate 11a). In extended tentacles they lie almost perpendicular to the main axis (Plate 11b). The ciliary crown appears as a refractile band at the tip of each papilla. No movement was observed in these and likewise the papillae themselves showed no independent movement. The pigment in the epithelial cells appeared orange/brown in these preparations and was never present in large amounts in the papillae.

FIGURE 4

Gibbula umbilicalis. A stereogram of a tentacular papilla.

a - axon

bl - basal lamina

c - cilium

con - connective tissue

d - dendrite

ec - epithelial cell

pec - pigmented epithelial cell

scb - sensory cell body

FIGURE 4

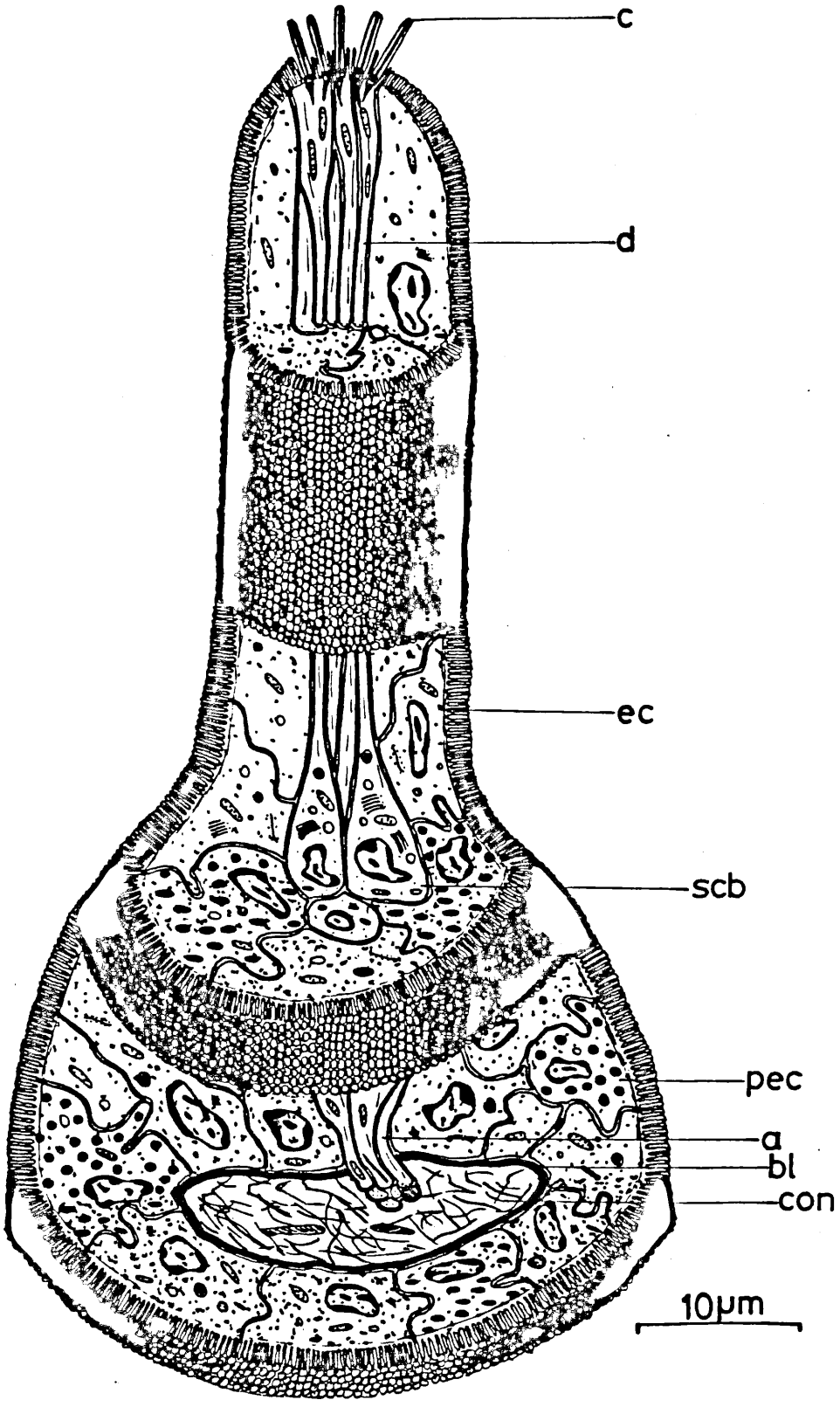


FIGURE 5

Gibbula umbilicalis. A diagrammatic representation of a section through a papilla near its tip showing the leek leaf arrangement of the distal parts of the dendrites.

bb - basal body of cilium

d - dendrite

ec - epithelial cell

FIGURE 5

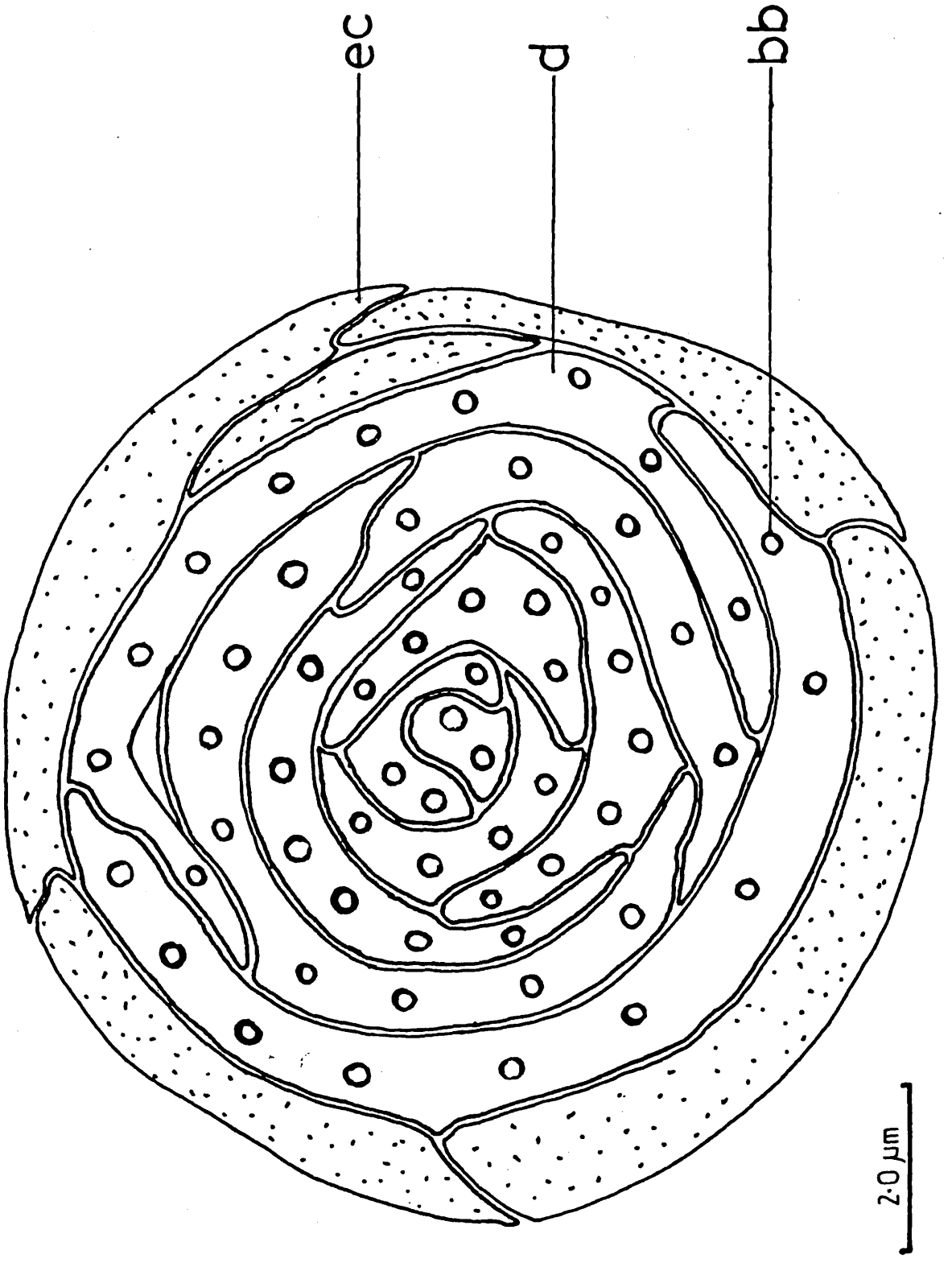


PLATE 5

Gibbula umbilicalis, scanning electron micrograph of the proximal half of the left cephalic tentacle and its origin between the cephalic lappet and eye stalk. Ectoparasitic protozoa are abundant in many areas. Bar = 200µm.

cl - cephalic lappet

df - dorsal papilla-free furrow

e - eye

ep - ectoparasitic protozoa

l - lips

PLATE 5

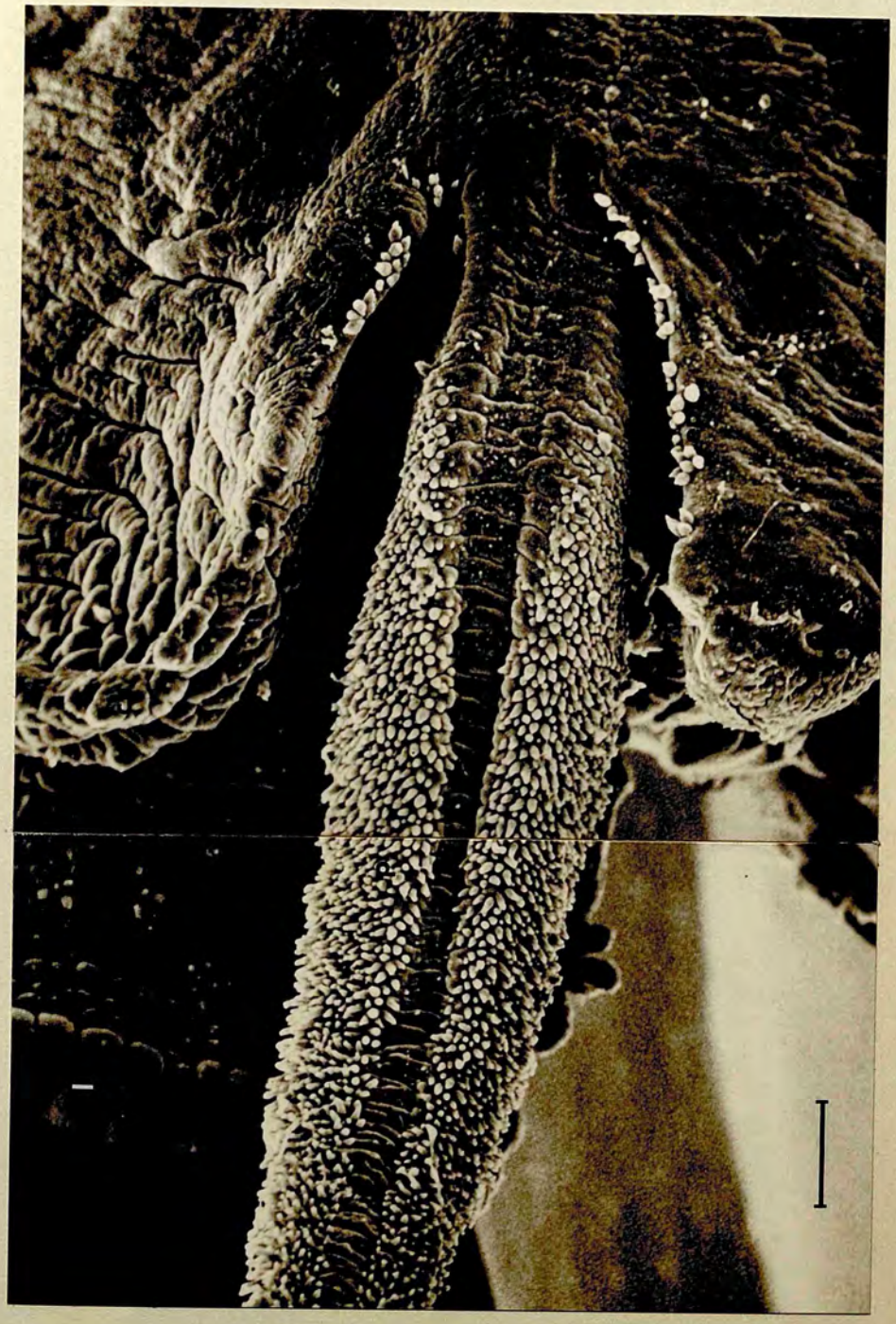


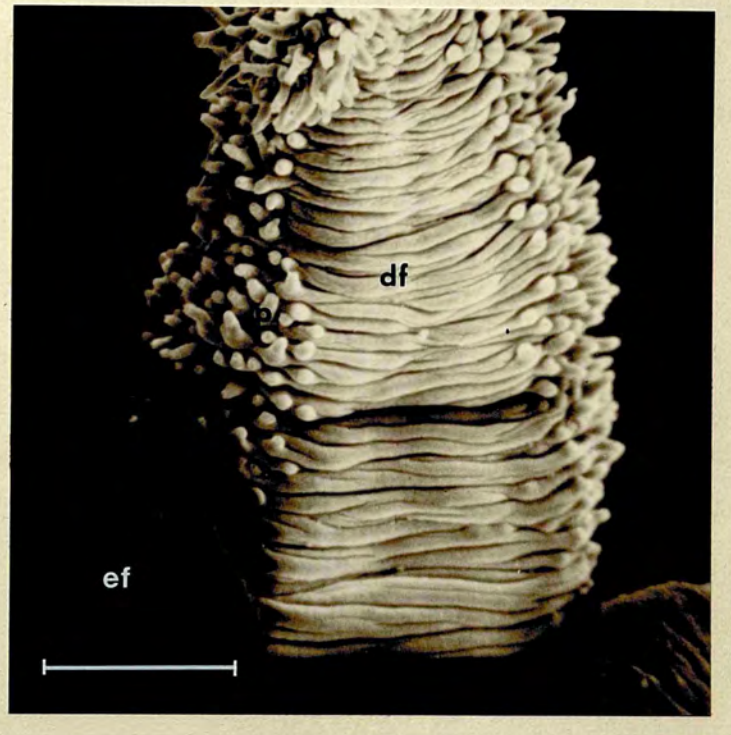
PLATE 6

a) *Gibbula umbilicalis*, dorsal view of epipodial tentacle showing dorsal papilla-free furrow and rounded lobes of epipodial fold.
Bar = 0.1mm.

b) *Gibbula umbilicalis*, ventral view of anterior epipodial tentacle showing ventral papilla-free furrow and part of the epipodial sense organ.
Bar = 0.1mm.

df - dorsal papilla-free furrow
ef - epipodial fold
eso - epipodial sense organ
p - papilla
vf - ventral papilla-free furrow

PLATE 6



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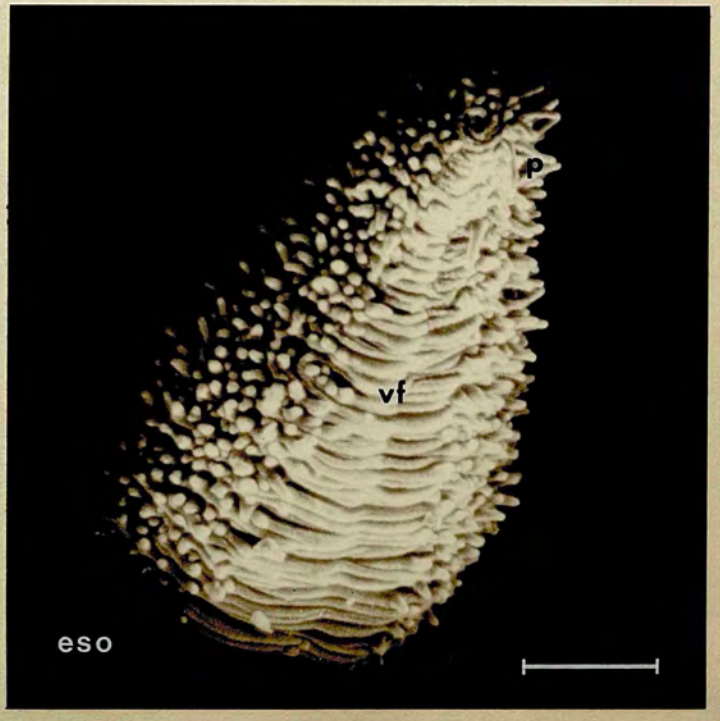


b

PLATE 7

a) *Gibbula umbilicalis*, papillae on cephalic tentacle. Bar = 20µm.

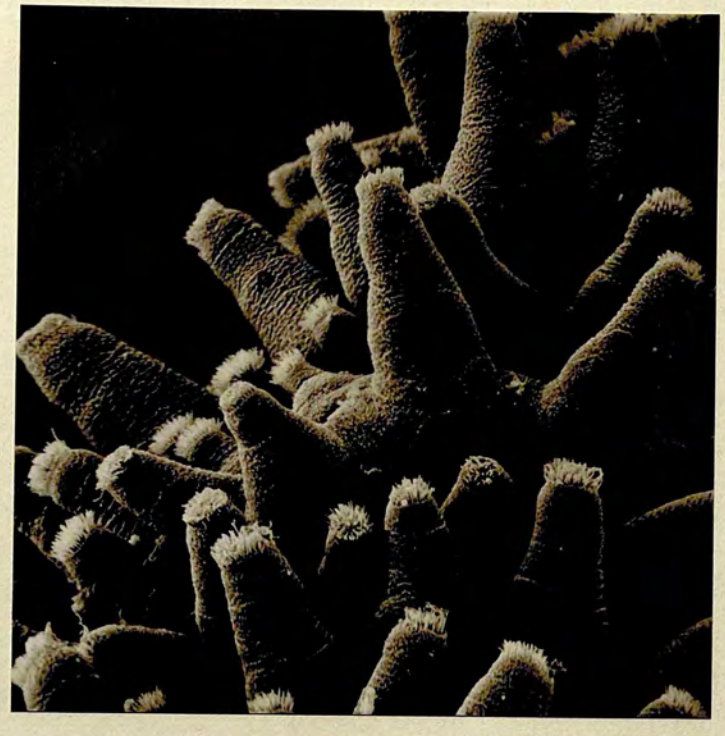
b) *Gibbula umbilicalis*, papilla from epipodial tentacle at high magnification showing apical crown of cilia. Note the three shorter cilia in the centre (▲). Bar = 1.0µm.

c - cilia

mv - microvilli

p - papilla

PLATE 7



a

b

PLATE 8

a) *Gibbula umbilicalis*, transverse section through middle of papilla showing central group of circular dendrites surrounded by epithelia cells.
Bar = 2.0µm

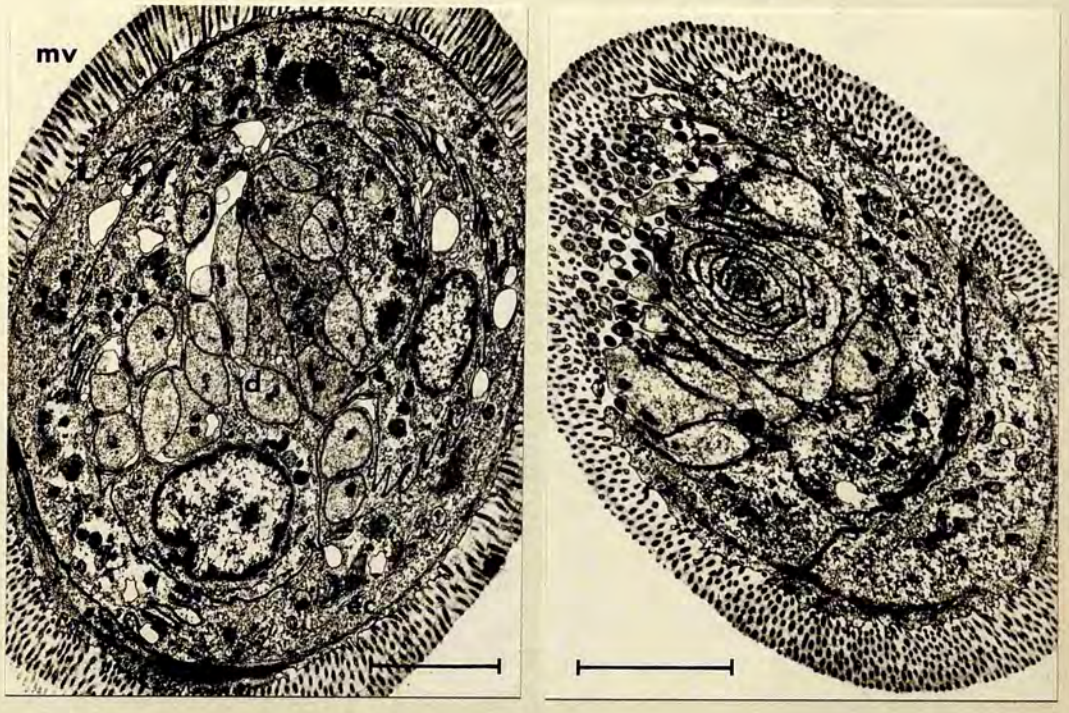
b) *Gibbula umbilicalis*, oblique section of tip of papilla showing dendrites wrapped around each other like the leaves of a leek.
Bar = 3.0µm.

c) *Gibbula umbilicalis*, as above but higher magnification. Note circular central and peripheral dendrites (▲).
Bar = 1.0µm.

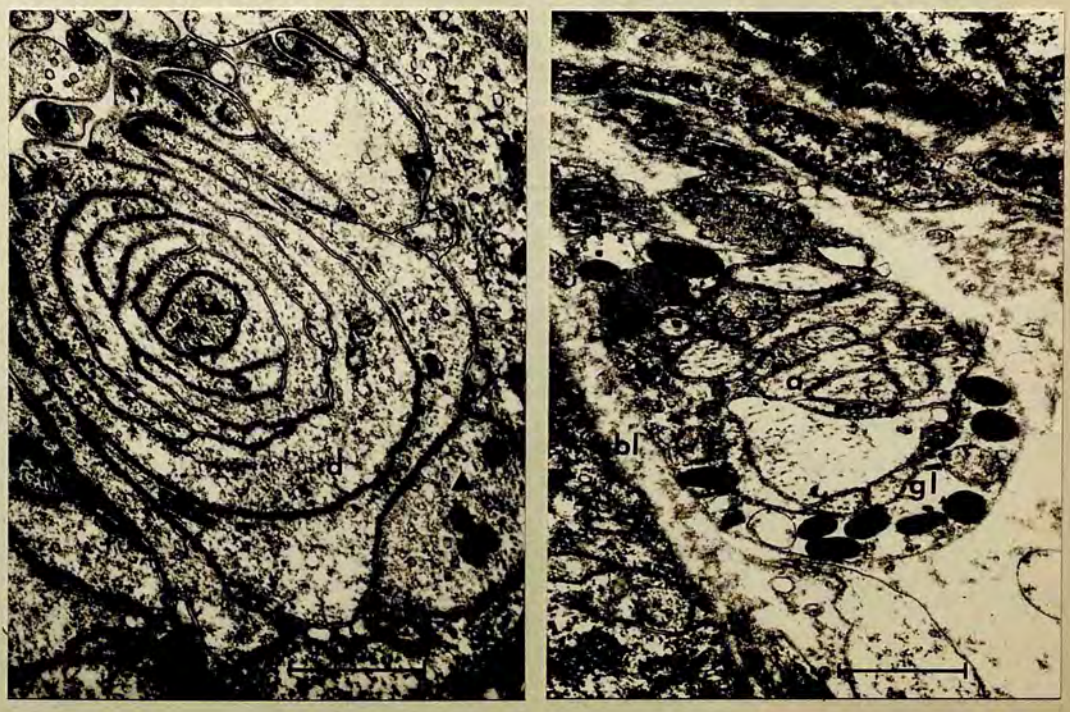
d) *Gibbula umbilicalis*, a group of axons from one papilla lying beneath the basal lamina. Note association with granular glial cell process.
Bar = 1.0µm.

a - axon
bl - basal lamina
c - cilia
d - dendrite
ec - epithelial cell
gl - glial cell process
mv - microvilli

PLATE 8



a b



c d

PLATE 9

Gibbula umbilicalis, composite longitudinal section through cephalic tentacle papilla. Note axon penetrating the basal lamina of the epithelium.

Bar = 5.0µm.

a - axon

bl - basal lamina

c - cilia

d - dendrite

sn - sensory cell nucleus

PLATE 9.

PLATE 10

a) *Gibbula umbilicalis*, approximately longitudinal section of the tip of a papilla showing cilia and microvilli arising from the apical portion of the dendrites.
Bar = 1.0µm.

b) *Gibbula umbilicalis*, transverse section of cilia in apical crown of papilla. (9 + 2 configuration).
Bar = 0.25µm.

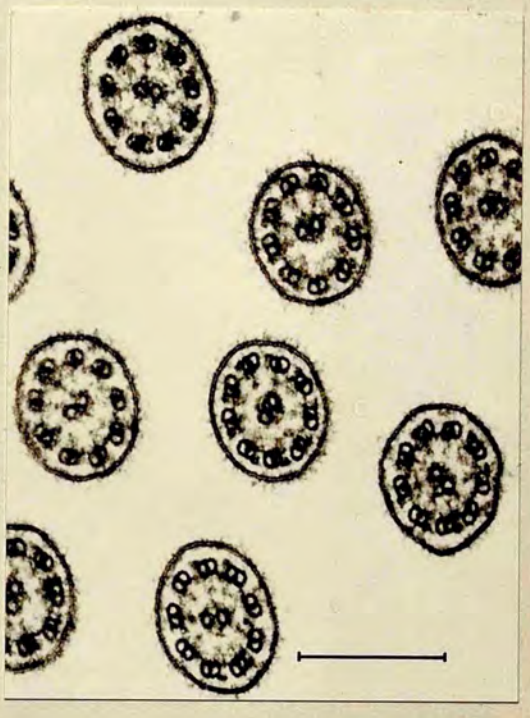
c) *Gibbula umbilicalis*, longitudinal section of papilla showing microtubules in dendrites (arrow), interdigitating cell membranes of epithelial cells and well developed glycocalyx surrounding the microvilli.
Bar = 1.0µm.

d) *Gibbula umbilicalis*, distal portion of sensory cell body containing vesicles of variable size and Golgi bodies.
Bar = 1.0µm.

bb - basal body of cilium
c - cilium
d - dendrite
g - Golgi body
gx - glycocalyx around microvilli
i - interdigitations of epithelial cell membranes
r - rootlet of cilium
tw - terminal web of microfilaments
za - zonula adhaerens

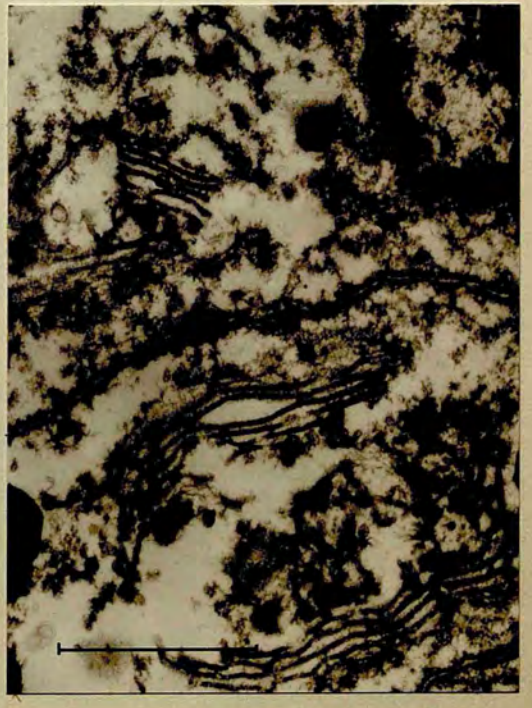
PLATE 10

a



b

c



d

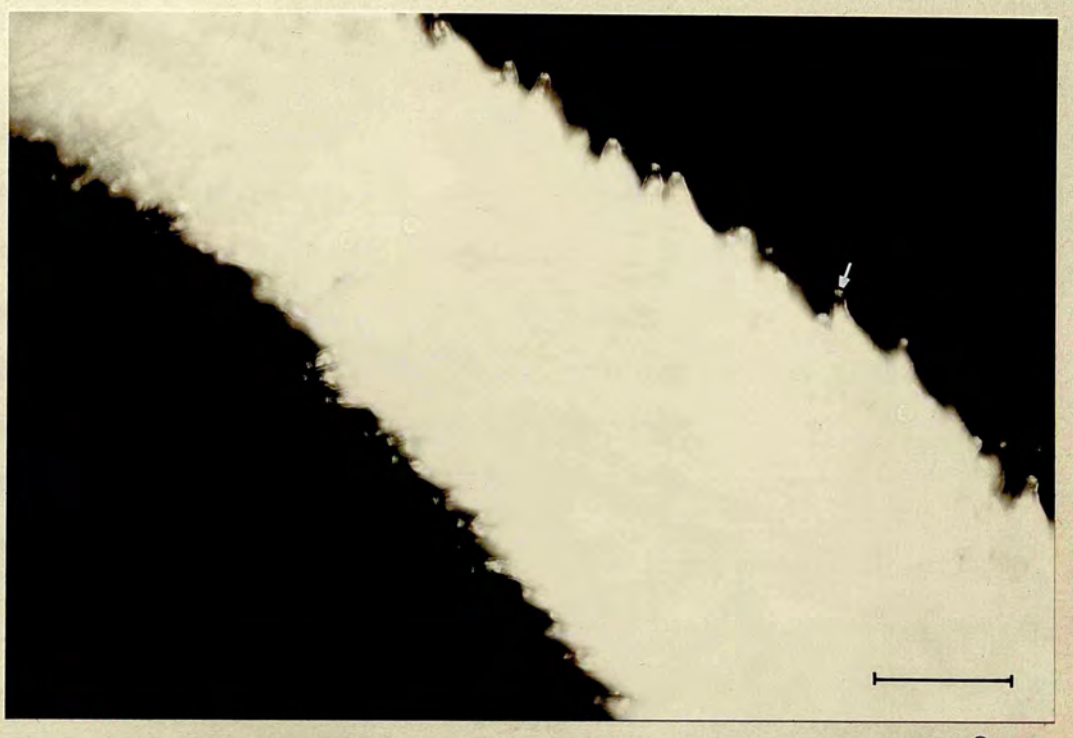
PLATE 11

a) *Gibbula umbilicalis*, epipodial tentacle viewed under dark-field illumination. Tentacle contracted with papillae lying at an angle to the surface.
Bar = 0.1mm.

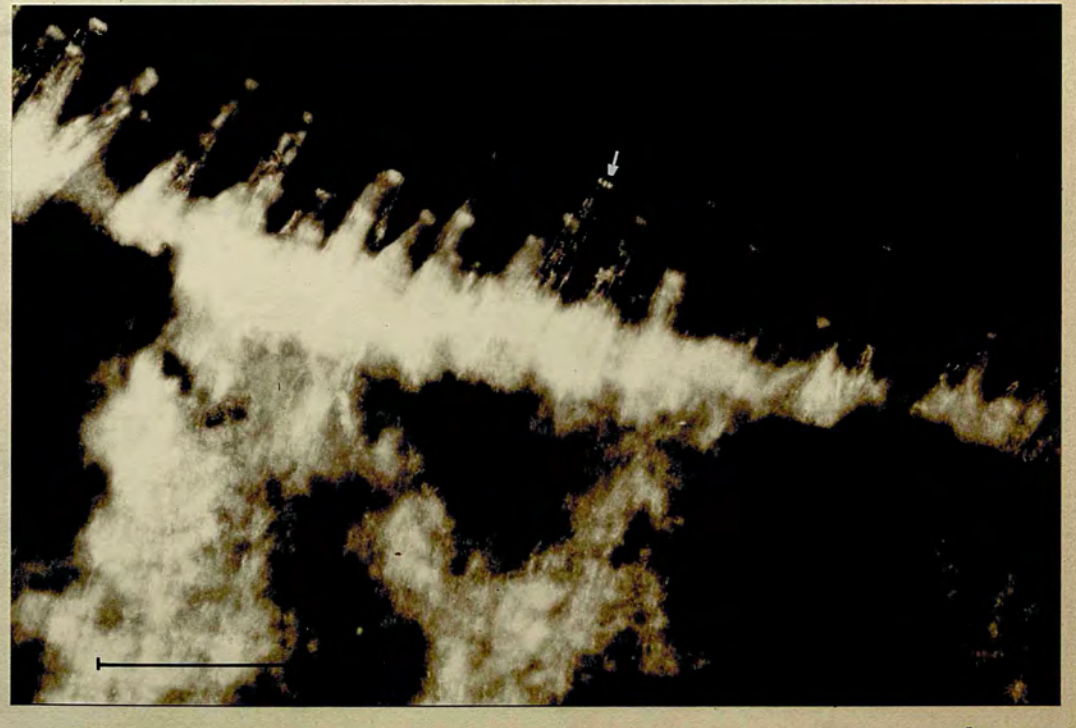
b) *Gibbula umbilicalis*, cephalic tentacle viewed under dark-field illumination. Tentacle expanded with papillae more nearly perpendicular to the surface.
Bar = 0.1mm.

arrows = refractile crown of cilia at the tip of the papillae.

PLATE 11



a



b

1.6. The Fine Structure of the Epipodial Sense Organs

A three dimensional reconstruction of an epipodial sense organ is shown in Fig 7. Typically they are cup-shaped structures 200µm in diameter, raised on a ridged stalk. The central depression, the sensory cupule, is usually round or slightly ovoid, rarely exceeding 100µm in diameter (Plate 12a). More unusual forms are encountered however, where the cupule is long and narrow or not depressed at all, but more dome shaped (Plate 12b). These variants are particularly common under the neck lobes.

The sensory cilia seen in routine sections appear as a tangled mass under the scanning electron microscope. This however is thought to be an artefact caused, most probably, during critical point drying. When living tissue is examined under dark-field illumination the cilia project outward and are held straight and rigid, extending 40-50µm above the rim of the organ (Plate 13a). They show no motility. Measurements of their length are difficult to make but estimates suggest that many may reach 70-80µm in length.

These long cilia arise singly from gaps in the general covering of microvilli (Plate 14a) and occasionally large microvillus-like structures can be seen in the space around the base of the cilium. They project only slightly, if at all, above the normal microvilli and are shown in thin resin sections to form a ring around the base of the cilium.

The rim and stalk of the organ are covered with numerous tufts of short cilia 1-2μm long (Plates 14b & 15a & b). They are more abundant on the former. Each tuft originates from a gap in the covering of microvilli and may possess 40-60 cilia. Numerous smaller tufts also occur, usually with $<$ 10 cilia and some with only a single short cilium (Plate 15a). The distinction between the cupule and the rim is not always clearly defined and the tufts of short cilia are often seen interspersed amongst the long ones in the transitional zone (Plate 15b). Infrequently the openings of gland cells are observed between the ciliary tufts, but there is no particular association between the two.

The general appearance of the tufts, particularly the larger ones is very similar to the apical crowns of cilia on the tentacular papillae. Under dark-field illumination the former are seen as refractile bands just above the surface and show no motility (Plate 13b). The few shorter cilia often present in the papillary crowns, have not been observed in these tufts.

In thin resin sections of the specialised epithelium of the sensory cupule two cell types were revealed. Firstly the presumed sensory cells which bear the long cilia and secondly non-sensory supporting cells (Plate 16a). The structural arrangement of these two cell types is complex. Good transverse sections of this epithelium have not been obtained, but examination of longitudinal and oblique sections has enabled diagrammatic representations of transverse sections to be constructed (Fig 6a & b). The apical region

of each sensory cell is narrow and almost circular in cross section (1-2μm in diameter), they are evenly distributed and rarely lie in contact with each other (Fig 6a). The regions between them are filled by the supporting cells, each one partly encircling several sensory cells. There is considerable interdigitation of lateral membranes between the supporting cells, but little or none between these and the sensory cells.

This arrangement persists except in the basal region where the perikarya of the sensory cells lie. Here the cells increase in diameter considerably (4.5-5.5μm) and often lie in contact with one another leaving only small spaces between them (Fig 6b). Consequently the supporting cells become narrower in this region and are attached to the basal lamina only by a slender stalk. It is this arrangement that produces the pseudostratification seen in light microscopy, with two marked bands of nuclei visible in longitudinal sections (Plate 4b, 16a & Fig. 7). The nuclei of the sensory cells are basal and those of the supporting cells medial. The basal lamina upon which this epithelium rests is thickened (up to 1.0μm) and is composed of a network of fine collagen fibres (Plate 17c).

The sensory cells, like those in the tentacular papillae, are simple bipolar neurons with an intra-epithelial perikaryon. The dendrite is short (10-12μm long), while the axon is long and passes into the nerve of the epipodial sense organ (Fig. 7). Each sensory cell has a pronounced indentation in its apical margin, from which projects a single long cilium (Plate 16b & c). Around this, but still

within the depression, arise nine specialised microvilli (stereocilia) (Plate 17a & b). The walls of the depression are steep and are developed distally into nine thickened microvilli. Transverse and slightly oblique sections through this region reveal an arrangement characteristic of these cells with a single central kinocilium surrounded by a ring of nine stereocilia and a further ring of nine thick microvilli (Plate 17b). For both of these ring structures nine is undoubtedly the most common number of elements, but infrequently other numbers have been observed, notably eight. Rarely the central kinocilium is absent, though this is believed to result from damage.

The kinocilium is approximately 0.25μm in diameter and appears to possess the standard 9+2 arrangement of subfibrils for most of its length, though this is never sharply defined. Neither striated rootlets nor basal feet have been observed to extend from the ciliary basal body. Associated with this however, are numerous small (50nm diameter), non-membrane bound, electron-dense structures (Plates 16c & 17a). These may be groups of microfilaments cut in transverse section. They are linked to each other and to the basal body by further microfilaments, an arrangement particularly evident beneath the membrane of the apical depression, lateral to the basal body (Plate 17a arrows).

The stereocilia are almost triangular in cross section (sides approx. 0.15μm long) and possess a concentration of electron-dense material at each corner (Plate 17b). This occurs on both sides of the cell membrane, but externally

it is more filamentous and often connects the stereocilia with each other, with the kinocilium and with the thickened microvilli. The filaments appear to be similar to the filaments of the glycocalyx surrounding the microvilli of the supporting cells.

The thickened microvilli forming the outermost ring are circular in cross section (0.2-0.25μm in diameter) with homogeneous contents. Both these and the stereocilia are 2.5-3.0μm long, similar to that of the normal microvilli. As accessory centriole is often found 1.0-1.5μm below the basal body. This does not show a consistent orientation to the basal body.

The remainder of the dendrite contains only mitochondria, microtubules and occasional small electron-lucent vesicles. The distinction between the dendrite and the apical cytoplasm of the perikaryon is not clear though the latter in addition to the above organelles may contain large electron-dense, heterogeneous granules and Golgi bodies. The nucleus is highly irregular in shape, often appearing in several isolated pieces and the chromatin is densely clumped in comparison to that of the supporting cell nuclei. Below the nucleus there is little cytoplasm before the cell narrows to form the axon. This does not pass directly through the basal lamina, but runs for a short distance above it. In so doing the axons from different cells (10-20) form into a group and pass through the basal lamina collectively at an indentation (Plate 17d). However, sections actually showing the penetration of the basal lamina

are rare. Microtubules, mitochondria and small electron-lucent vesicles (50nm in diameter) are the only inclusions found in the axons (Plate 17c). In general they seem poorly preserved. This together with the fact that the axons do not penetrate the basal lamina directly renders the basal region of the epithelium somewhat confused in appearance.

Within and below the basal lamina the small groups of axons are surrounded by glial cells similar to those around the axons of the tentacular papillae. These small groups join to form larger nerves (Plate 18a & b, Fig 7), which eventually form the epipodial sense organ nerve. These larger nerves have glial cells around and within them. The dark granules which they contain are an aid to recognition of nervous tissue, especially the finest branches. The length of the axons is not known, though it seems likely that they run to the pedal ganglion (Bullock & Horridge, 1965).

The cytoplasm of the supporting cells is relatively rich in organelles and generally appears to be well fixed. Apically they bear long, occasionally branched, microvilli 2.5-3.0μm long (Plate 16a & b), clothed in a glycocalyx which projects a further 0.5μm from the surface. The inter-cellular junctions are typically zonulae adhaerentes, like those in the tentacles but the terminal web of microfilaments is poorly developed. The apical cytoplasm is dense and contains numerous Golgi bodies, mitochondria, small vesicles, bundles of microfilaments and large electron-dense granules with a heterogeneous content (Plate 19a). The nucleus is

generally ovoid, contains one or two nucleoli and lies medially. The basal regions of the cells are very narrow and contain little other than bundles of microfilaments. These may help to support this otherwise weak portion of the cell. They are attached to the basal lamina by hemi-desmosomes.

. The non-sensory epithelial cells of the rim and stalk of the epipodial sense organ are similar to the supporting cells in the sensory cupule. They are however, not so tall (10-15μm compared with 20-25μm) and the microvilli are not as long, rarely reaching 2.5μm in length. In addition they do not contain the basal bundles of microfilaments. Frequently interspersed between them are the type A and B gland cells seen in optical microscopy (Plate 18c & d). The type A cells are almost totally filled with a heterogeneous mass of electron-lucent secretion. The cytoplasm is dense and the nucleus is small and basal. The type B cells possess a larger nucleus which is likewise basal, but the secretory product is electron-dense and contained in isolated granules within the cytoplasm.

Sections through the tufts of cilia seen in scanning electron microscopy show that these are borne on bipolar neurons with intra-epithelial nuclei and that the ciliary structure is similar to that in the tentacular papillae (Plate 19b). Sections through the cell (s) bearing the smaller groups of cilia were infrequent, but there is evidence to suggest that these may represent a distinct cell type. This is indicated by the different structure of the ciliary

basal body which bears a long rootlet extending well into the cytoplasm and may also possess a basal foot (Plate 20a & b).

The subepithelial gland cells appear to contain a third type of secretory product which although similar to that of the type A cells was considerably more electron-dense (Plate 18b). Also noted beneath the epithelium were groups of ciliated cells. These were only seen on one occasion and were observed as a group of four cells (10-15μm long) near the rim of the organ. Certain areas of the cell margin were highly irregular and bore numerous cilia projecting in many directions (Plate 19c & d). They were not noticeably orientated towards the epithelium. Most of the cilia examined had the normal 9+2 subfibril structure and were surrounded in a fine collagenous material. A 9+0 arrangement was however, occasionally seen. The cytoplasm contains large numbers of highly cristate mitochondria and a spherical to ovoid nucleus. In areas where the cell margin is smooth, it is in contact with glial-like cells. This suggests that these cells may be nervous and even sensory in nature.

FIGURE 6

a) *Gibbula umbilicalis*, transverse section through apical region of the specialised epithelium of the epipodial sense organ cupule.

b) *Gibbula umbilicalis*, transverse section through basal region of the specialised epithelium of the epipodial sense organ cupule.

bb - basal body of kinocilium

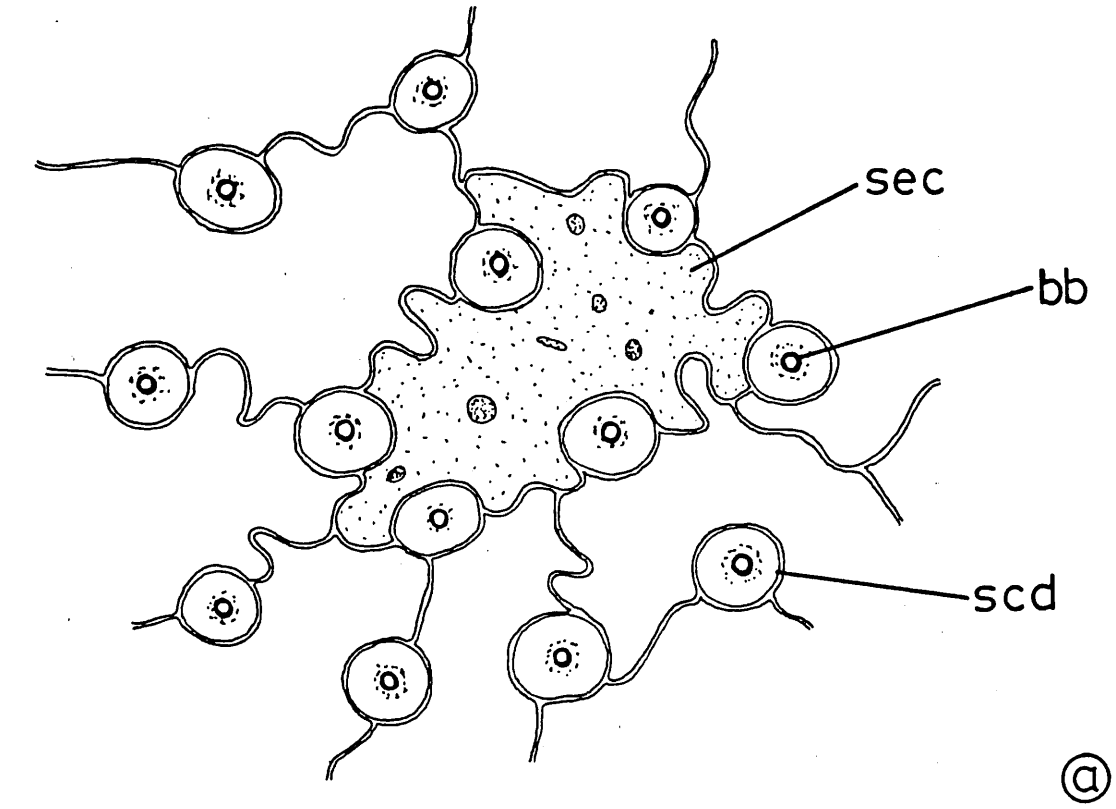
mf - microfilaments

scd - sensory cell dendrite

sec - supporting epithelial cell

scb - sensory cell body

FIGURE 6



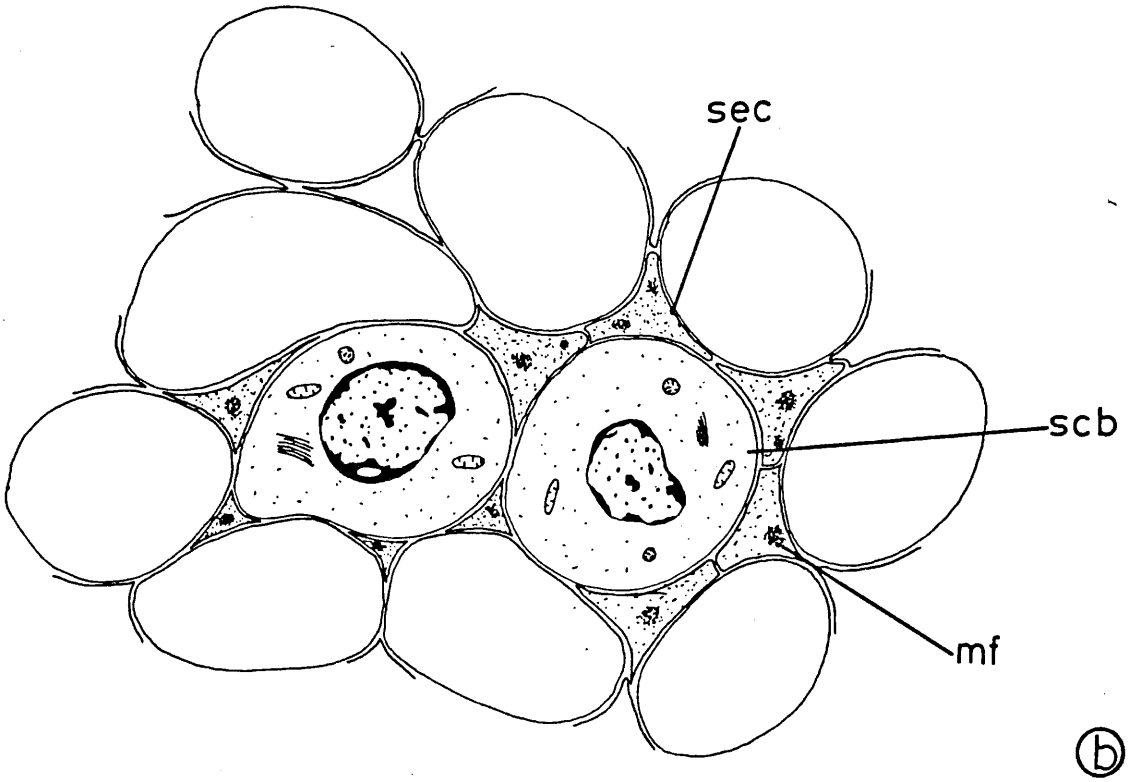


FIGURE 7

Gibbula umbilicalis, a stereogram of the epipodial sense organ and stalk.

A - type A gland cell

B - type B gland cell

bl - basal lamina

c - cilium (kinocilium)

con - connective tissue

cr - ciliary rosette

eson - epipodial sense organ nerve

mcsc - monociliary sensory cell

secc - subepithelial ciliated cell

sgc - subepithelial gland cell

FIGURE 7

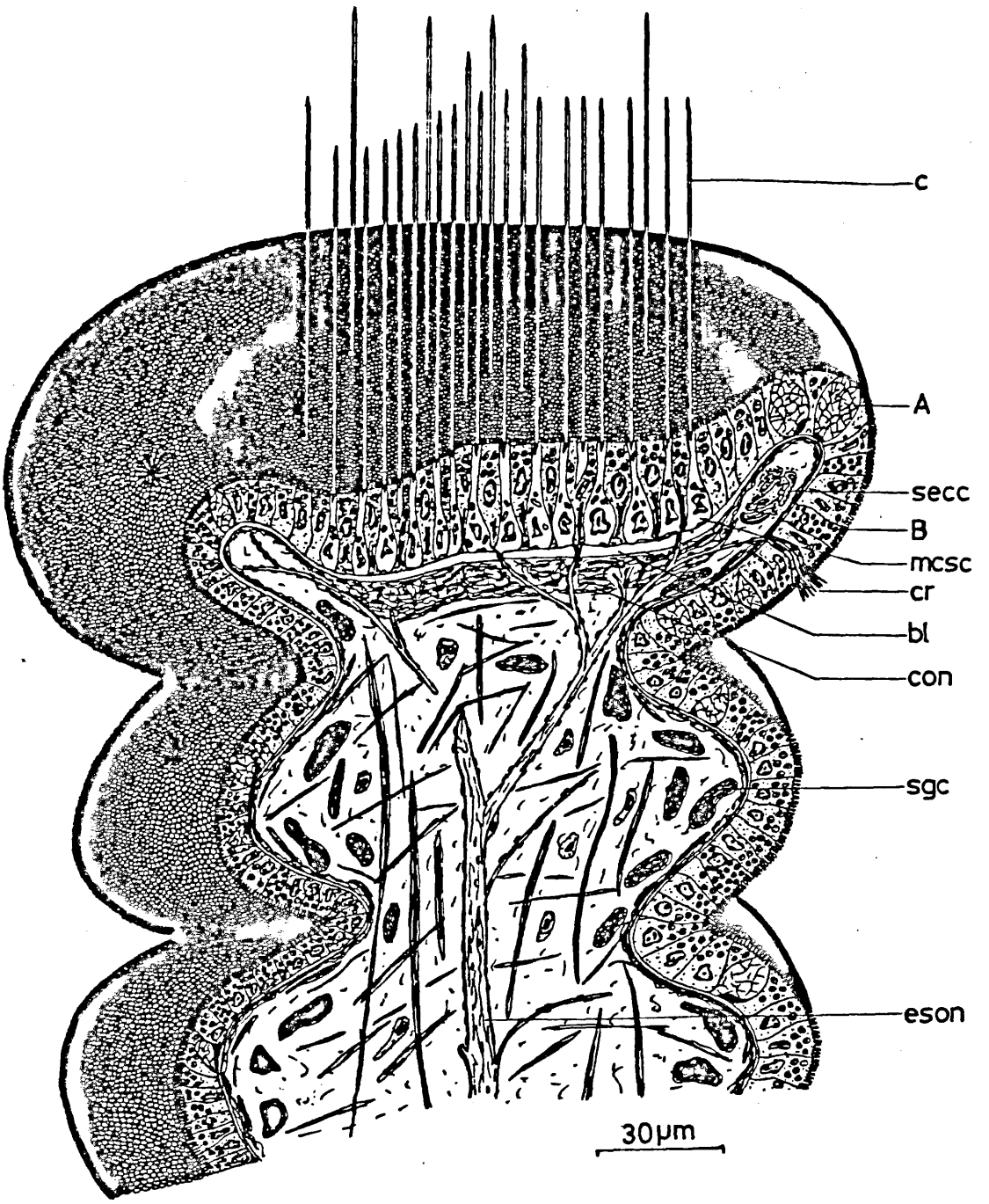


PLATE 12

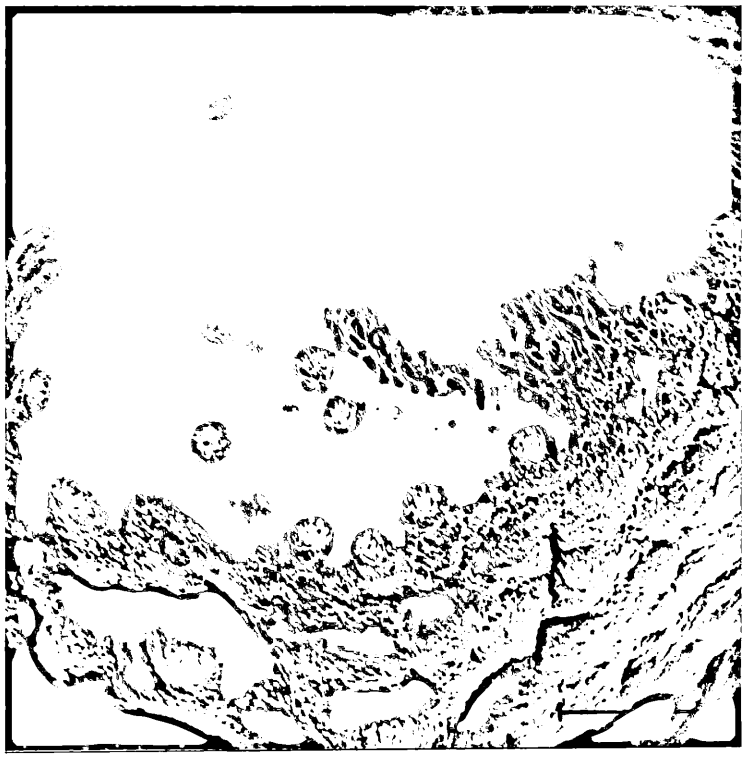
a) *Gibbula umbilicalis*, epipodial sense organ posterior to anterior epipodial tentacle, note depressed central cupule filled with tangled mass of cilia.
Bar = 100µm.

b) *Gibbula umbilicalis*, epipodial sense organ under left neck lobe, central portion with cilia not sunken.
Bar = 40µm.

c - cilia
ef - epipodial fold
eso - epipodial sense organ
et - epipodial tentacle

PLATE 12

a



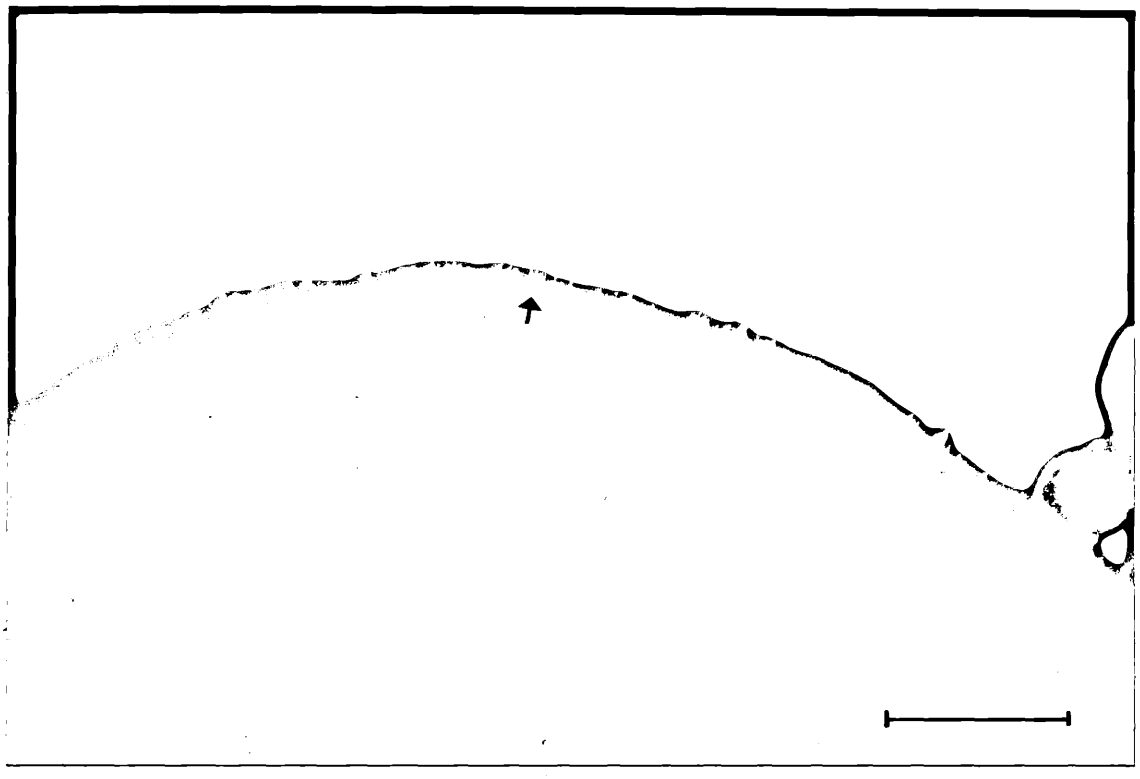
b

PLATE 13

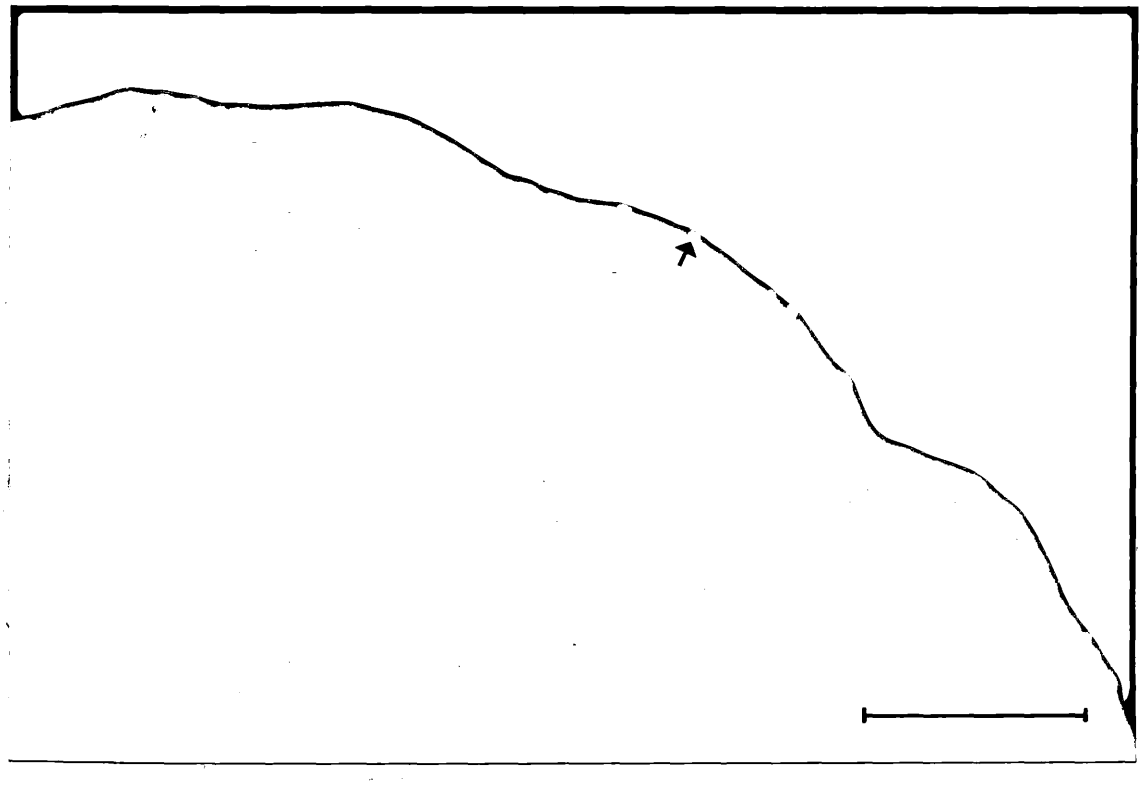
a) *Gibbula umbilicalis*, side view of epipodial tentacle under dark-field illumination. Long sensory cilia (arrow) project straight and rigid above the rim of the organ.
Bar = 25µm.

b) *Gibbula umbilicalis*, rim of epipodial sense organ under dark-field illumination. Tufts of cilia appear as refractile bands (arrow).
Bar = 50µm.

PLATE 13



a



b

PLATE 14

a) *Gibbula umbilicalis*, high magnification electron micrograph of long cilia (kinocilia) projecting from the cupule of the epipodial sense organ. Note large microvillus-like structures visible around the base of some kinocilia (arrow).
Bar = 4.0µm.

b) *Gibbula umbilicalis*, epipodial sense organ. Note tufts of short cilia on the rim and stalk of the organ (arrow). Gland cell openings are also frequent.
Bar = 50µm.

go - gland cell opening
k - kinocilia (um)

PLATE 14

a

b

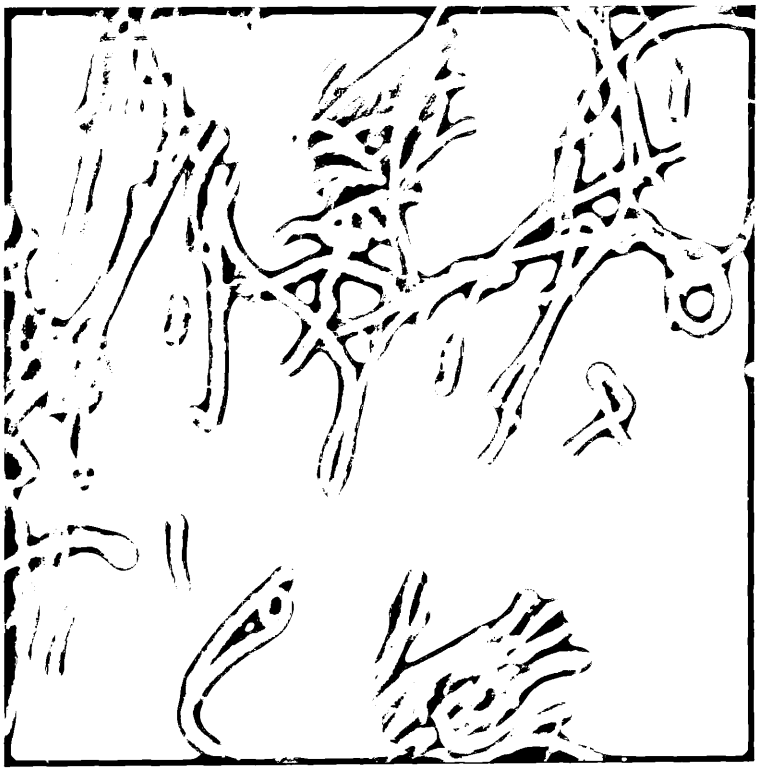
PLATE 15

a) *Gibbula umbilicalis*, part of the rim of an epipodial sense organ bearing one large tuft of cilia and several smaller ones.
Bar = 5.0µm.

b) *Gibbula umbilicalis*, transitional zone of epipodial sense organ between the rim and the cupule, note the presence of both tufts of short cilia and longer single cilia. The holes in the covering of microvilli are thought to relate to monociliary cells which have lost their cilium.
Bar = 5.0µm.

PLATE 15

a



b

PLATE 16

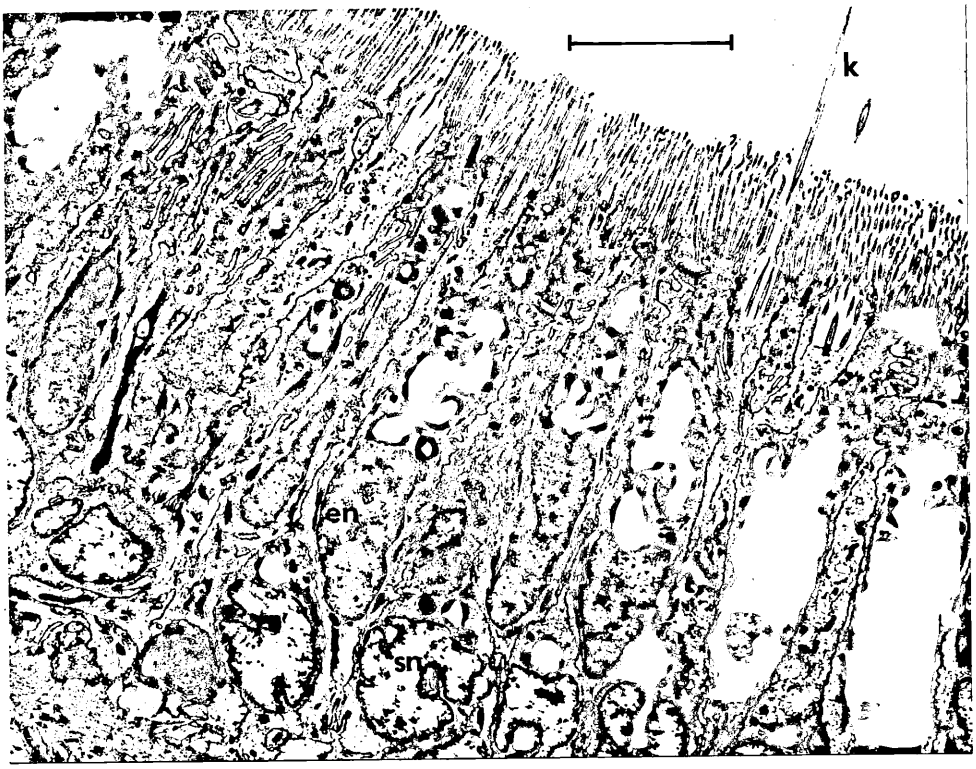
a) *Gibbula umbilicalis*, longitudinal section through the sensory epithelium of the epipodial sense organ cupule. Note pseudostratification with basal, high contrast nuclei of epithelial cells. The apical cytoplasm of the epithelial cells also shows numerous large electron-dense granules.
Bar = 5.0µm.

b) *Gibbula umbilicalis*, distal region of monociliary cell in epipodial sense organ. A single kinocilium projects from a depression in the apical border. This is surrounded by a ring of stereocilia and a ring of thickened microvilli.
Bar = 2.0µm.

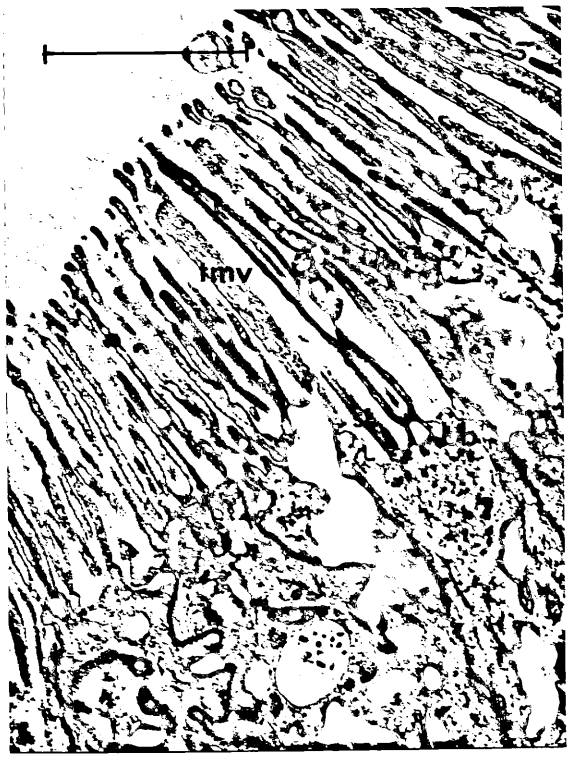
c) *Gibbula umbilicalis*, cell body on dendrite of monociliary cell showing few cytoplasmic inclusions. Note electron-dense material associated with the base of the kinocilium (arrow).
Bar = 4.0µm.

bb - basal body of kinocilium
d - dendrite
en - epithelial cell nucleus
k - kinocilium
sn - sensory cell nucleus
st - stereocilium
t_{mv} - thickened microvilli

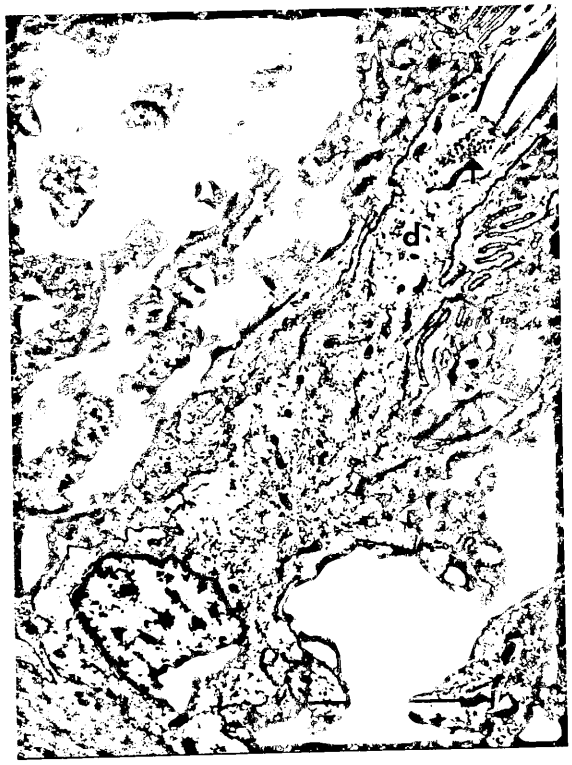
PLATE 16



a



b



c

PLATE 17

a) *Gibbula umbilicalis*, high magnification electron micrograph of the origin of the kinocilium from a monociliary cell in the epipodial sense organ. Note electron-dense material lateral to the basal body (arrow).
Bar = 0.5µm.

b) *Gibbula umbilicalis*, transverse section through monociliary cell at the level of the brush border showing rings of stereocilia and thickened microvilli.
Bar = 0.5µm.

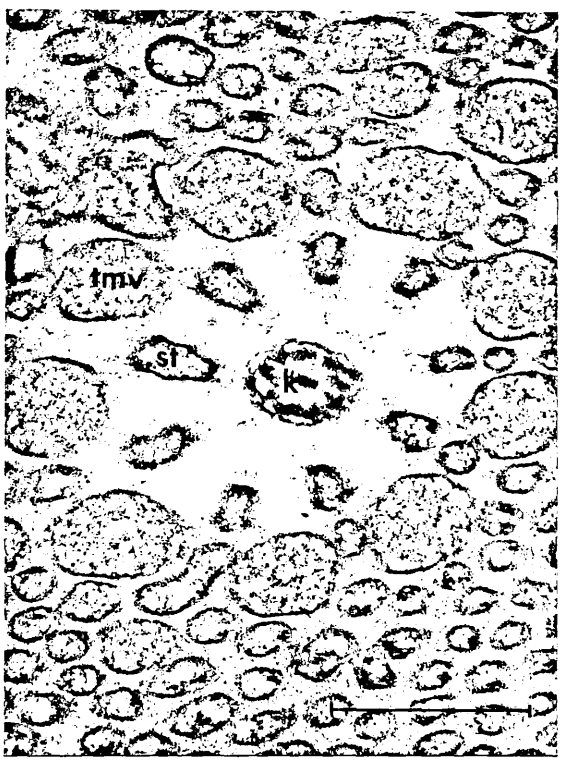
c) *Gibbula umbilicalis*, section through monociliary cell axon immediately above the thick basal lamina, note electron-lucent vesicles.
Bar = 0.5µm.

d) *Gibbula umbilicalis*, group of axons passing through an indentation of the basal lamina, note associated pigmented glial cell processes.
Bar = 2.0µm.

a - axon
bl - basal lamina
k - kinocilium
tmv - thickened microvilli
st - sterocilium

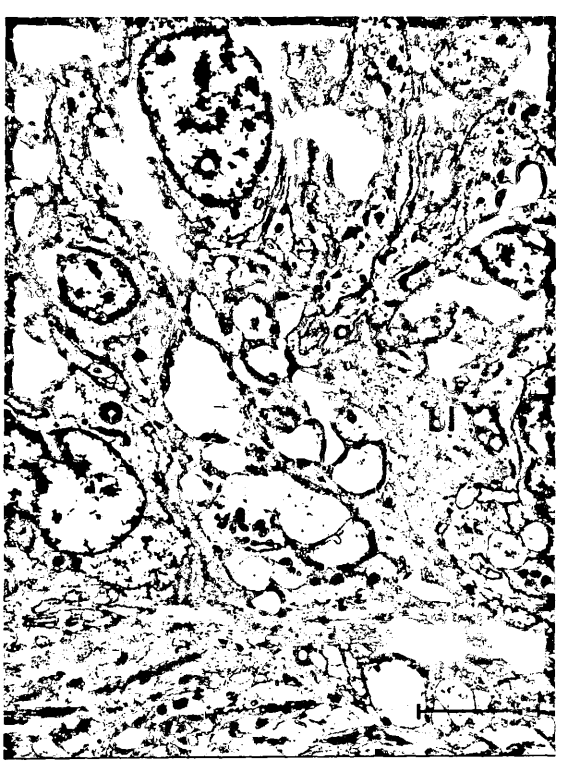
PLATE 17

a



b

c



d

PLATE 18

a) *Gibbula umbilicalis*, a group of axons underlying the sensory cupule of the epipodial sense organ. Note surrounding layer of pigmented glial cell processes.
Bar = 3.0µm.

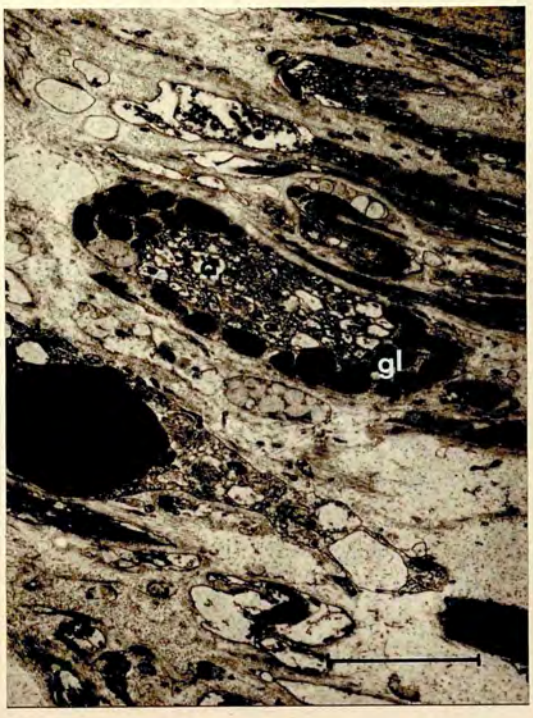
b) *Gibbula umbilicalis*, a larger group of axons lying deeper within the epipodial sense organ. Glial cell perikarya lie within the group. Note also part of two subepithelial gland cells.
Bar = 5.0µm.

c) *Gibbula umbilicalis*, type A epithelial cell on the rim of the epipodial sense organ.
Bar = 5.0µm.

d) *Gibbula umbilicalis*, type B epithelial gland cell on the stalk of the epipodial sense organ.
Bar = 4.0µm.

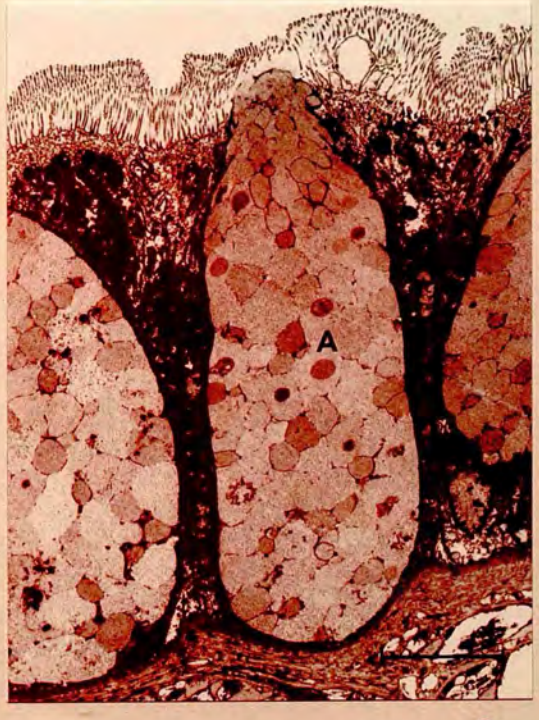
A - type A epithelial gland cell
a - axons
B - type B epithelial gland cell
gl - glial cell process
sgc - subepithelial gland cell

PLATE 18



a

b



c

d

PLATE 19

a) *Gibbula umbilicalis*, supporting epithelial cell of epipodial sense organ cupule. Apical cytoplasm containing large heterogeneous, electron-dense granules, mitochondria, Golgi bodies, microfilaments and small vesicles.
Bar = 2.0µm.

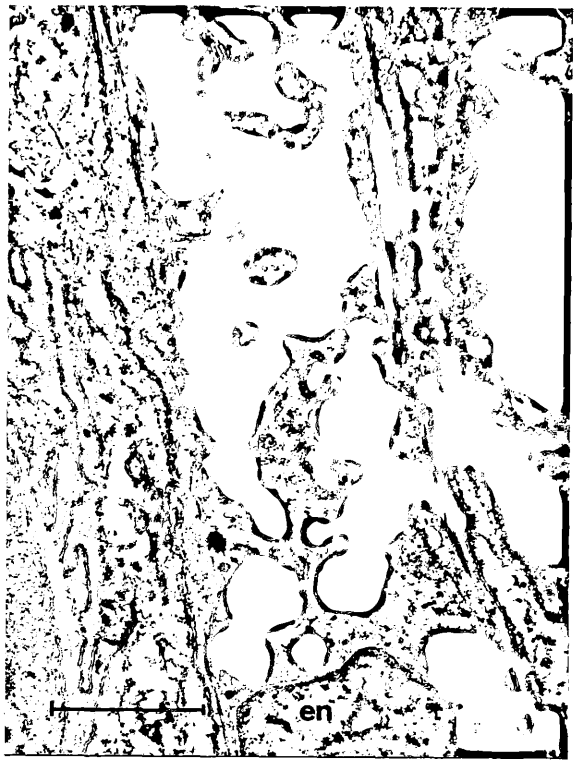
b) *Gibbula umbilicalis*, oblique section through multiciliary tuft on the rim of the epipodial sense organ. Their structure is similar to that of the papilliary tufts on the tentacles.
Bar = 2.0µm.

c) *Gibbula umbilicalis*, subepithelial ciliated cell found under the rim of the epipodial sense organ. The ciliated areas of the cell membrane are highly irregular in outline. Note granular glial cell process associated non-ciliated region.
Bar = 4.0µm.

d) *Gibbula umbilicalis*, part of subepithelial ciliated cell in epipodial sense organ. Note numerous highly cristate mitochondria. Most cilia have the 9 + 2 subfibril structure but in some the central pair are absent giving a 9 + 0 arrangement (arrow).
Bar = 2.0µm.

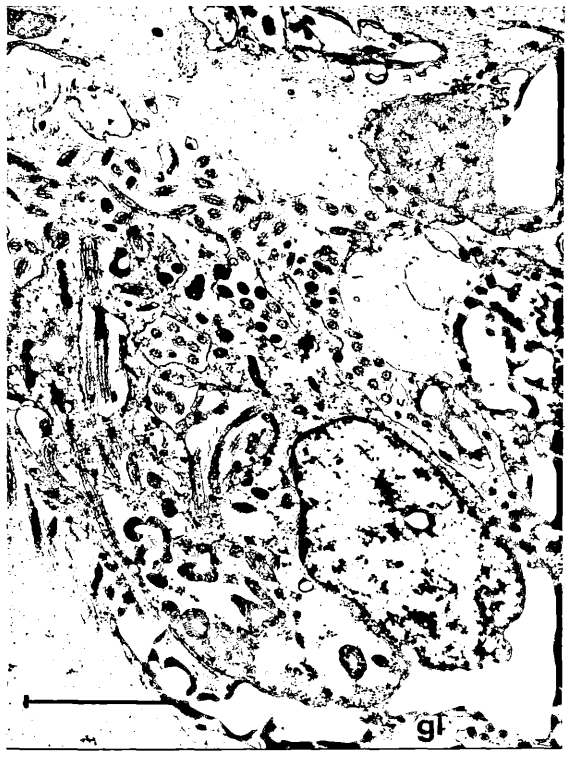
c - cilia
d - dendrite
m - mitochondria
en - epithelial cell nucleus
gl - glial cell process

PLATE 19

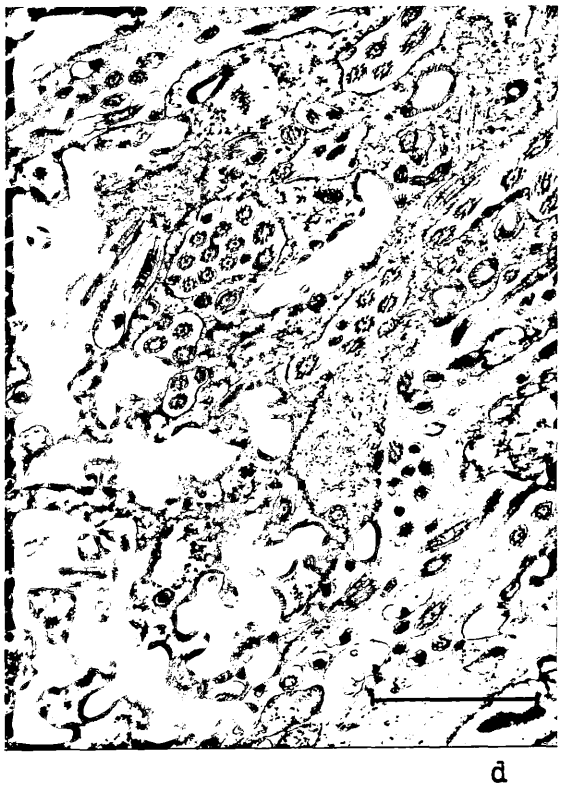


a

b



c



d

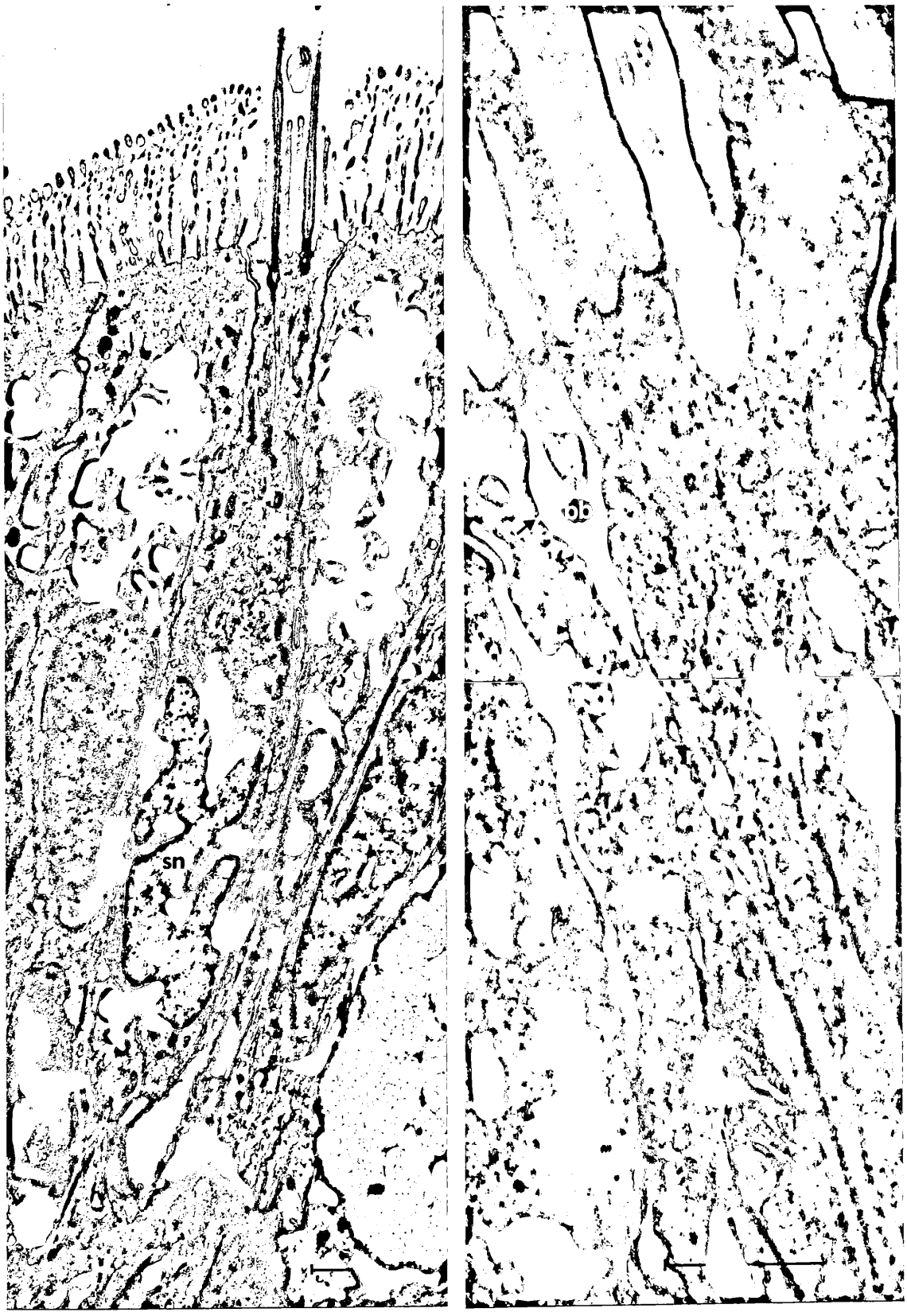
PLATE 20

a) *Gibbula umbilicalis*, longitudinal section through unusual sensory cell on the rim of the epipodial sense organ.
Bar = 2.0µm.

b) *Gibbula umbilicalis*, higher magnification of the ciliary basal body and long rootlet of the above cell. Note the thickening of the basal body suggestive of a basal foot (arrow).
Bar = 0.5µm.

bb - basal body of cilium
r - rootlet of cilium
sn - sensory cell nucleus

PLATE 20



a b

1.7. Sensory Receptors on Other Regions of the Body Surface

These regions of the body surface were examined using scanning electron microscopy alone. There is little direct evidence that the structures described are sensory, but their similarity with some of the receptors already described suggests this probability.

The eye, eye stalk and right postoptic tentacle

The opening to the eye appears on the surface as a hole approximately 60µm in diameter, at the end of the eye stalk on its flat dorso-lateral face (Plate 21a), the lateral edges of which are developed into lobed ridges. The ventral and medial faces are round and only gently lobed. The dorsal and lateral lobed ridges together with the rounded apex of the stalk are covered in numerous squat papillae, each bearing a crown of short cilia (Plate 21b). The ventral and medial regions and in particular the flat dorso-lateral face do not bear such papillae, but possess only a small number of ciliary tufts not raised above the surface.

The right postoptic tentacle is a short tapering structure about 1/3-1/2 the length of the eye stalk. Its surface is slightly uneven and scattered over it are papillae similar to those on the apex of the eye stalk, though less densely packed together. It bears no motile cilia.

The neck lobes

The dorsal surface of the right neck lobe is

covered anteriorly with a dense mat of cilia 4-5μm in length (Plate 22a). Posteriorly this is interrupted, first by isolated areas and then larger areas without cilia until the most posterior region, where it is devoid of cilia altogether. These cilia are motile and beat towards the free margin in the living animal. The ventral surface of the lobe is covered with rounded lobules each bearing a small number of ciliary tufts.

Unlike the right neck lobe the dorsal surface of the left lobe is largely free of motile cilia, the only areas bearing such cilia are the dorsal surfaces of the 4-5 large digits (Plate 23). Most of the dorsal surface of the lobe is devoid of all cilia. The tips and sides of all the digits bear well developed papillae with ciliary crowns. The ventral surface is similar to that of the right lobe.

The cephalic lappets, snout ridges and the ventral margins of the sides of the foot all bear well developed papillae similar to those on the left neck lobe digits. The mantle edge bears two distinct folds, the inner (ventral to the periostracal groove) possesses papillae like those on the eye stalk. The sides of the foot, the head, snout, epipodial fold and lips all bear ciliary tufts. These may or may not be raised above the surface on papillae.

Many of the animals examined possessed ectoparasitic Protozoa on their surface (probably Ciliaphora: Peritricha). These parasites were particularly common on the epipodium as a whole and on the head (Plates 5 & 22b).

PLATE 21

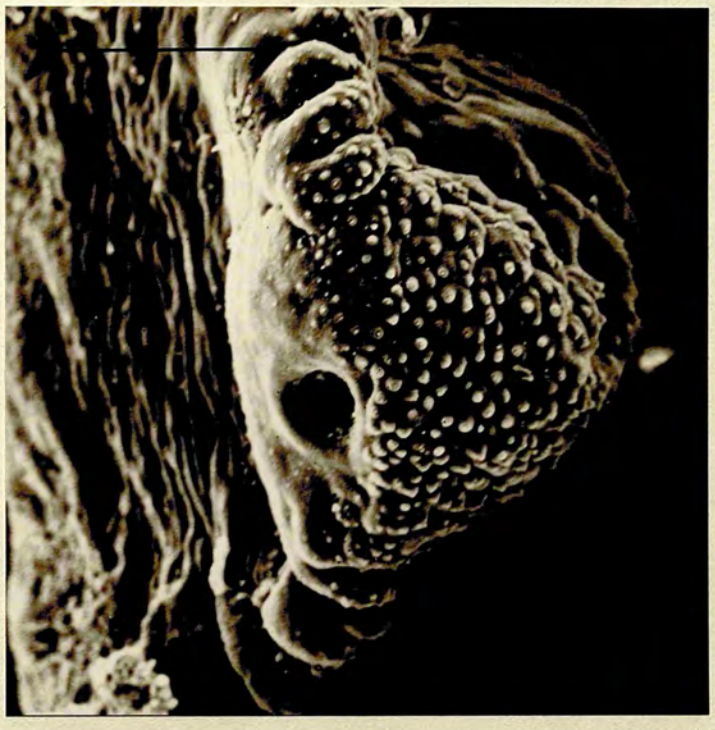
a) *Gibbula umbilicalis*, left eye showing numerous squat papillae on the rounded apical surface. Note circular opening of the eye (optic vesicle) on the dorso-lateral face.

Bar = 0.2mm.

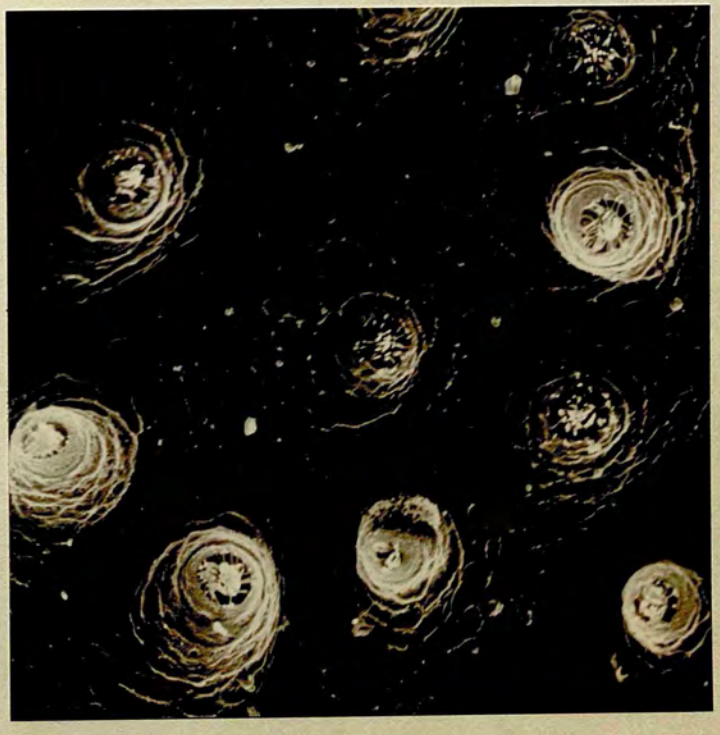
b) *Gibbula umbilicalis*, higher magnification of the above papillae showing crowns of short cilia.

Bar = 20µm.

PLATE 21



a



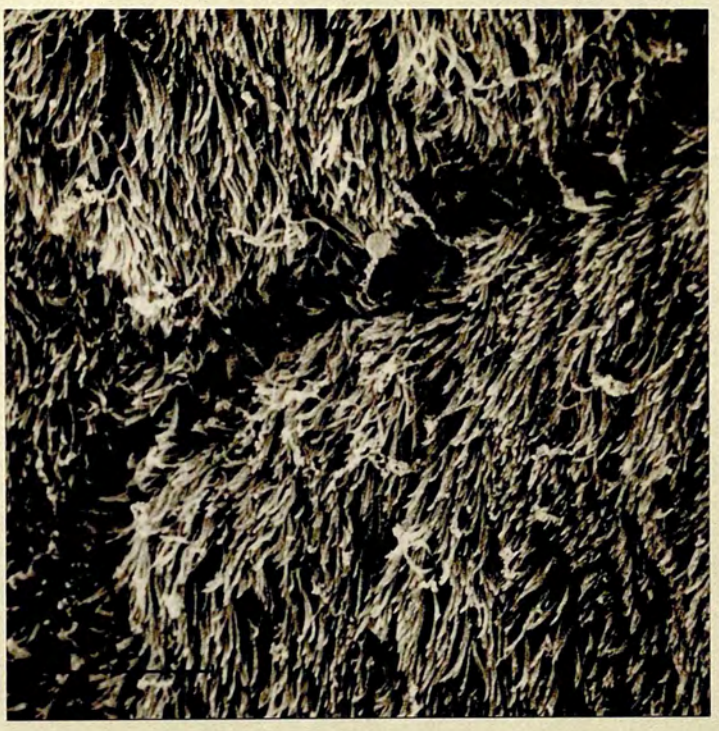
b

PLATE 22

a) *Gibbula umbilicalis*, dorsal surface of right neck lobe (anterior region) covered with a dense mat of motile cilia.
Bar = 10µm.

b) *Gibbula umbilicalis*, ectoparasitic protozoan on the side of the foot.
Bar = 20µm.

PLATE 22



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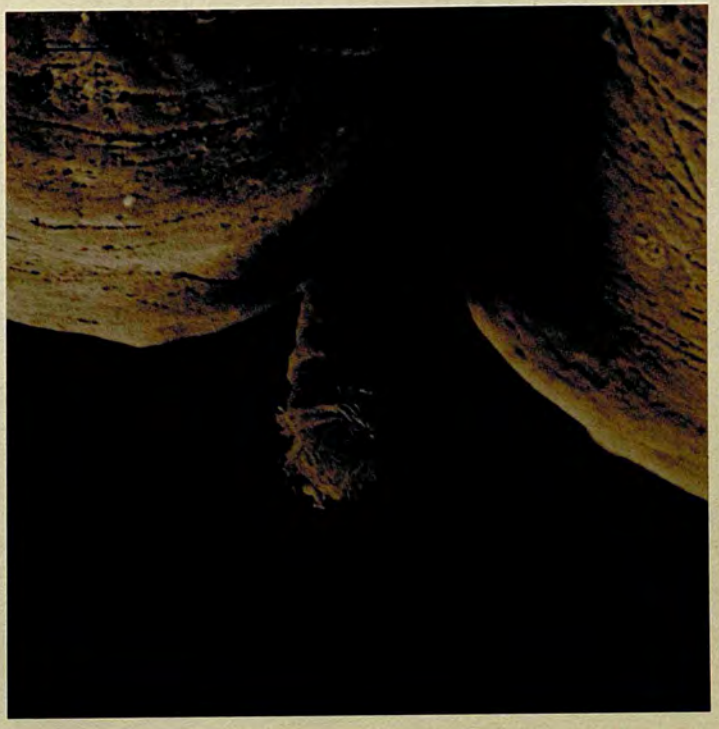


b

PLATE 23

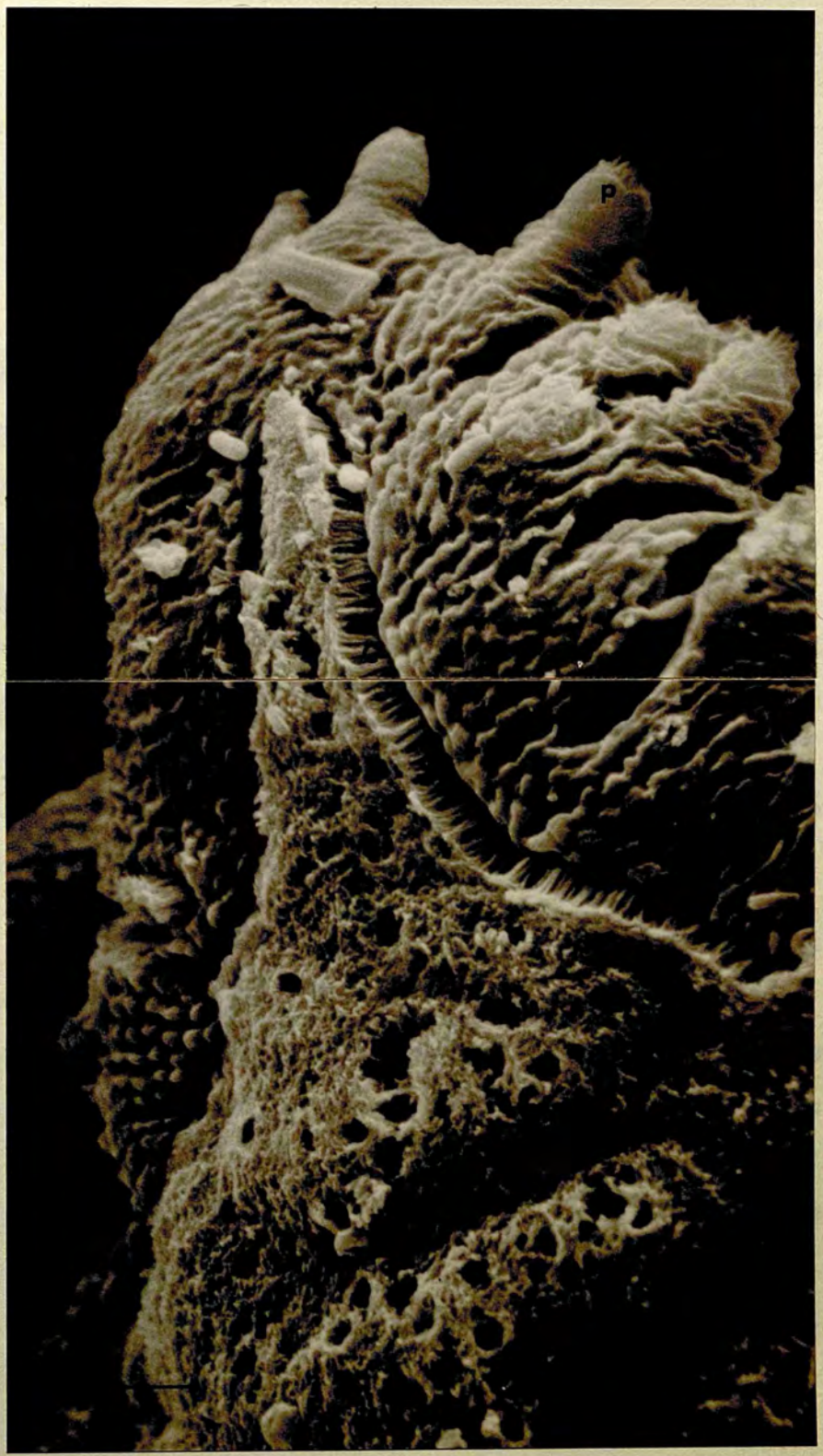
Gibbula umbilicalis, dorsal surface of one large digit on the left neck lobe showing ciliated surface and marginal papillae. The holes in the covering of cilia may represent gland cell openings.

Bar = 10µm.

c - cilia

p - papilla

PLATE 23



1.8. *Umbonium vestiarium*

U. vestiarium possesses the same basic structure as the other trochids but shows considerable specialisation associated with its filter-feeding mode of life (Fretter, 1975).

The head is broad, comprising mainly the eye stalks and their laterally extended bases (Fig. 8). The opening of the eye lies at the apex of the eye stalk. The anterior margin of the head is concave and without lappets. The finely papillate cephalic tentacles are unequal in size, the larger, right tentacle arises from a small pit just median to the right eye stalk, the left from the base of the left eye stalk. The snout is very small and lies concealed under the head, its sides bear long papillae. There is no right postoptic tentacle but a small papilla under the left eye stalk.

The neck lobes arise ventral to the eye stalks and are both greatly enlarged. They roll to form the inhalent and exhalent siphons of the mantle cavity. The left neck lobe is fused to the posterior half of the eye stalk from which it runs medially, and then ventrally, laterally and dorsally, at this point lying close to its origin with the eye stalk again. The tube thus formed is the inhalent siphon. Its free edge is elaborated in the form of branched tentacles which project across the opening. From the siphon the lobe continues posteriorly to the first epipodial tentacle, its free margin bearing only a small

number of digits. The right lobe is arranged in a similar manner though it is not fused with its eye stalk and its free margin is smooth. There are no epipodial sense organs under either lobe.

There are four, slender, papillate epipodial tentacles on each side, two under the operculum and two between this and the neck lobe. They are not sheathed basally and project directly from the epipodial fringe. There is a small epipodial sense organ between the anterior tentacles and one ventral to each of the posterior three.

The fixation of the specimens of *U. vestiarium* was adequate for general morphology but detailed examination using scanning electron microscopy revealed poor preservation of all ciliary structures. However possible sensory structures could be identified as circular or oval blemishes in the covering of microvilli which was well fixed (Plate 24a).

Like *G. umbilicalis* the head-foot complex appears to possess large numbers of ciliary tufts. In contrast however these are rarely raised above the surface on papillae (Plate 24a). Definite papillae were only observed on the tentacles and rim of the epipodial sense organs (Plate 24b & c). The latter were usually small and poorly defined. The long cilia seen in the British species never remained in these specimens.

The region of particular interest and that which is particularly developed toward a filter feeding mode of life is the left neck lobe. In the animals examined

the siphon was withdrawn and its free edge deeply folded. At intervals branched projections were seen to arise from this edge almost totally occluding the lumen (Plate 25a). The more distal parts of these projections bore large numbers of elongate papillae with occasional cilia remaining (Plate 25b arrow). The preservation of these presumably delicate structures however, was poor and little detail apparent.

The right neck lobe was essentially an enlarged version of that of *G. umbilicalis*.

FIGURE 8

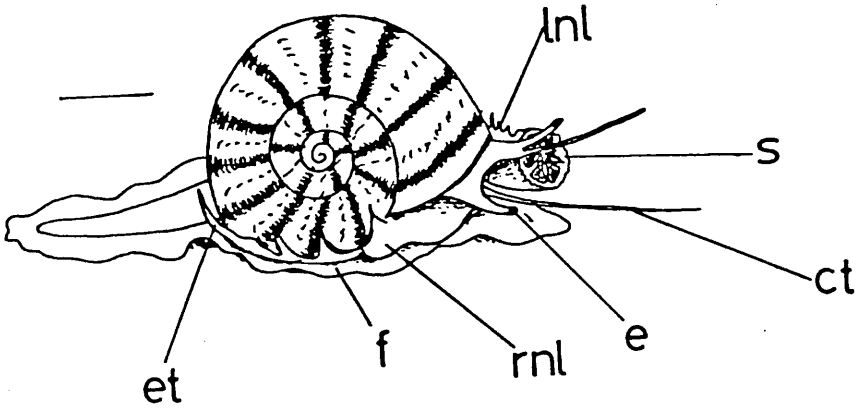
a) *Umbonium vestiarium*, living animal creeping over substratum.
Bar = 3.0mm.

b) *Umbonium vestiarium*, ventro-lateral view of head and anterior part of foot. The right neck lobe is reflected against the eye stalk.
Bar = 0.6mm.

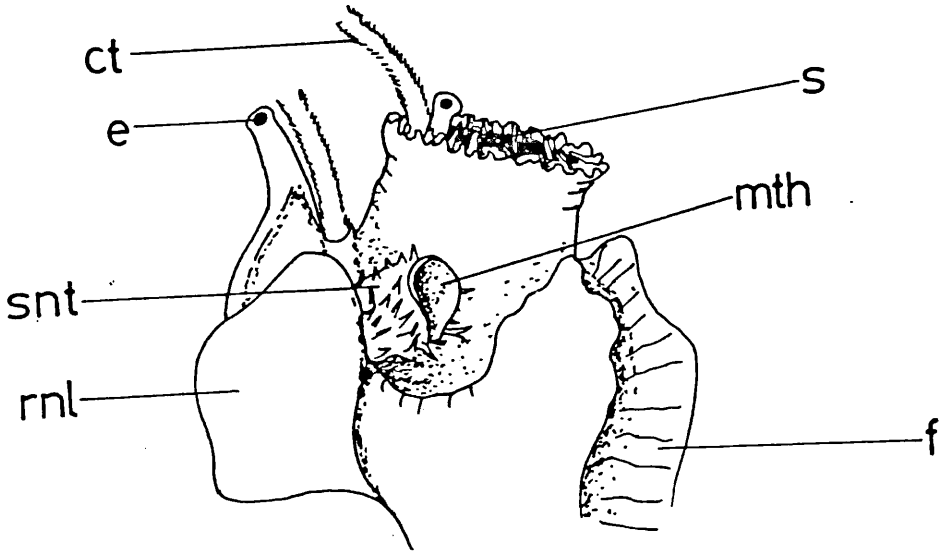
a and b redrawn from Fretter (1965) as living animals were not examined personally.

ct - cephalic tentacle
e - eye
et - epipodial tentacle
f - foot
lnl - left neck lobe
mth - mouth
rnl - right neck lobe
s - siphon
snt - snout

FIGURE 8



(a)



(b)

PLATE 24

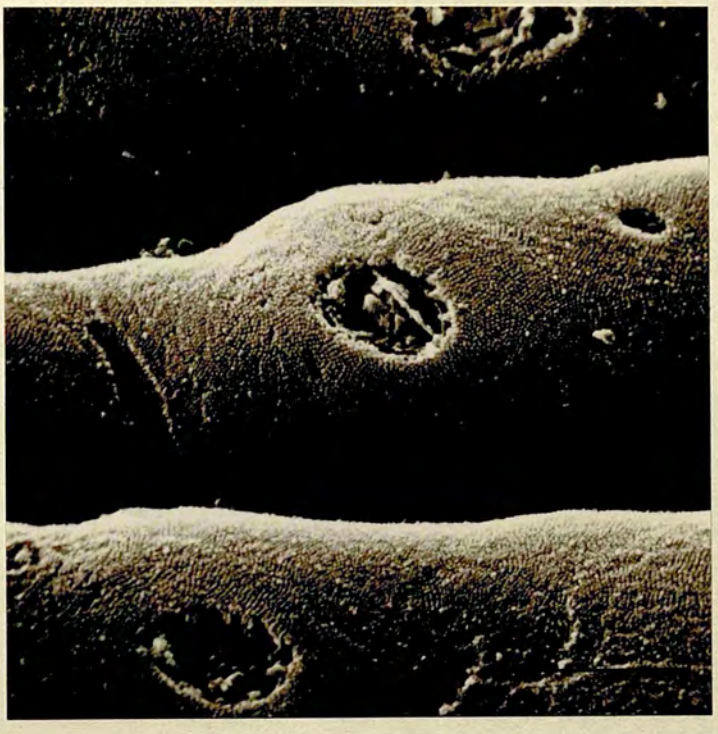
a) *Umbonium vestiarium*, possible sensory structures on the posterior of the left neck lobe. Cilia are thought to have been lost due to poor fixation.
Bar = 5.0µm.

b) *Umbonium vestiarium*, papillae on the cephalic tentacle with evidence of apical ciliary crown.
Bar = 10µm.

c) *Umbonium vestiarium*, epipodial sense organ and part of epipodial tentacle. Note small papilla on the rim of the epipodial sense organ. The long cilia of the sensory cupule have been lost.
Bar = 50µm.

eso - epipodial sense organ
et - epipodial tentacle
p - papilla

PLATE 24



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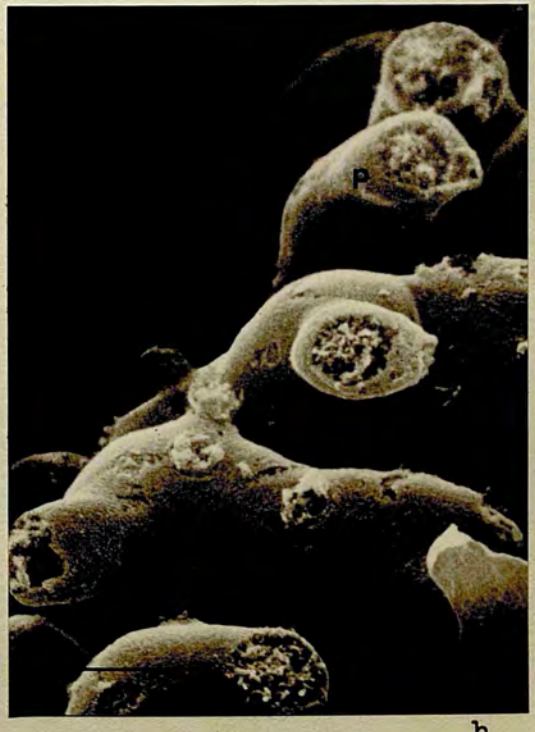


b

c

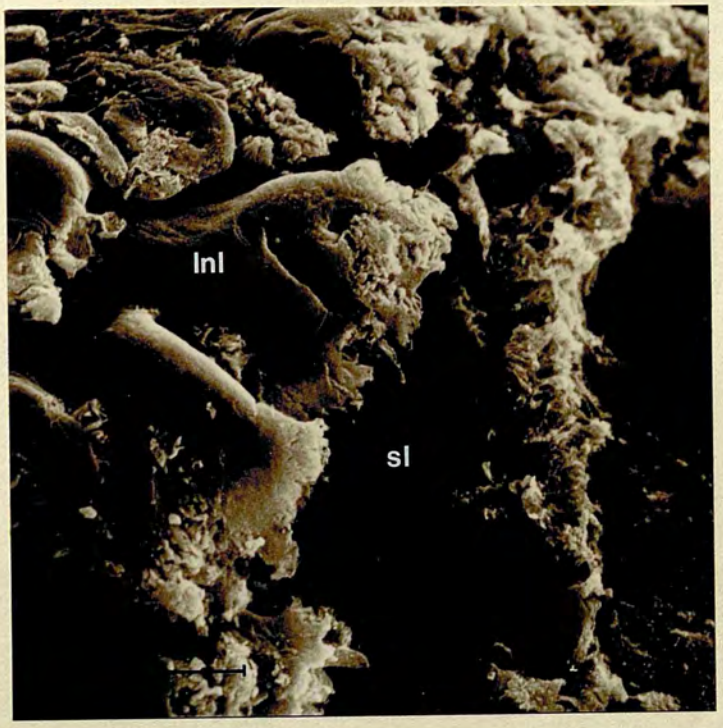
PLATE 25

a) *Umbonium vestiarium*, siphonal part of the left neck lobe showing branched projections extending into the lumen of the inhalant siphon.
Bar = 100µm.

b) *Umbonium vestiarium*, elongate papillae on the projections of the left neck lobe. Note the few remaining cilia (arrow).
Bar = 4.0µm.

lnl - left neck lobe
p - papilla
sl - siphonal lumen

PLATE 25



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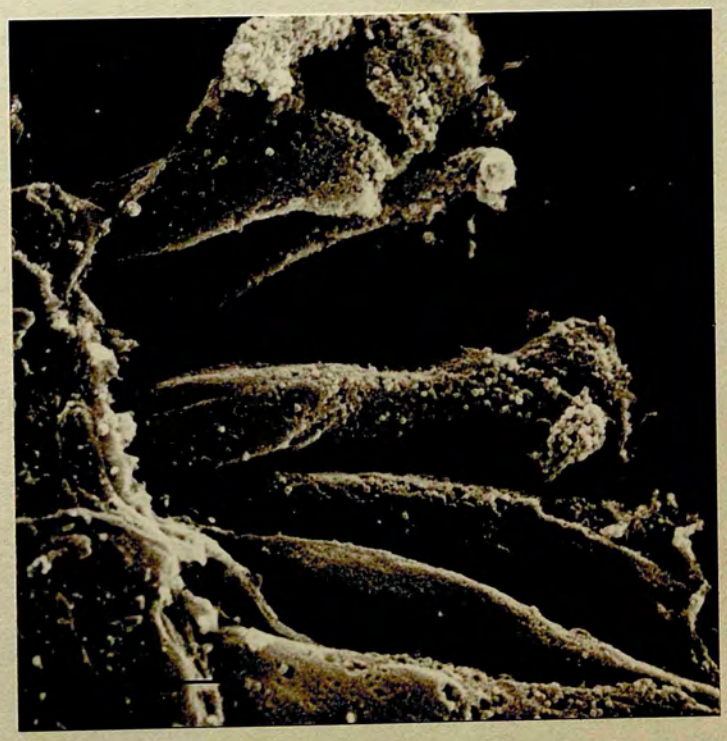


b

SECTION 2

Fissurellacea

(*Emarginula reticulata*)

2.1. Distribution of Sense Organs

The cephalic tentacles of *E. reticulata* are well developed and arise from small bosses on the sides of the head above the base of the snout (Plate 26a & b). When extended they are slender tapering (subulate) structures up to 4-5mm long in a large animal (shell length 11-12mm). They are white, like most of the animal and show no distinct papillation. In an active animal (Plate 26b) they are extended well under the anterior margin of the shell and are clearly visible. Though capable of independent movement they are not nearly as mobile and flexible as the tentacles of *G. umbilicalis*. Most movement seems to be brought about by movement of the head as a whole. They are very often in contact with the substratum in front of the shell.

Immediately posterior to each cephalic tentacle is an eye stalk with which it is fused basally. This may be up to 0.8mm long and bears a distinct black eye at its tip. Behind the right eye stalk is a well developed post-optic tentacle up to 1.2mm long. This is constricted in its middle region, but expanded and slightly flattened distally. It is capable of some change in shape, but shows little other motility. Animals of both sexes were examined, but no obvious differences in this structure were noted. There are no cephalic lappets and the entire head, including the snout is smooth and devoid of any marked papillation. The lips are radially lobed and have a distinct orange/brown pigmentation. There is a pronounced neck which is smooth and

bears no neck lobes.

Posterior to this is the epipodial fold in the form of a low ridge encircling the foot approximately 1/2 - 2/3 of the way between the sole and the attachment of the shell muscle. It is most pronounced postero-laterally. From this fold project a variable number of epipodial tentacles (Plate 26a), usually from 20-26, depending on the size of the animal, distributed equally along the two sides of the foot.

These tentacles are white in colour and fall into two distinct types: short (1.0mm) and not extensile, and long (2-3mm) extensile. The anterior 3-4 tentacles on each side are usually short, these are followed posteriorly by the longer tentacles usually with two short ones between each. The arrangement of alternate long and short tentacles described by Forbes and Hanley (1849-53) and Jeffreys (1862-69) was not observed. The number of long tentacles rarely exceeds three per side. These features are clearly visible in ventral view (Plate 26a).

The short tentacles are dorso-ventrally flattened and triangular in outline, curling over ventrally at the tip. They seem almost incapable of independent movement. On the ventral surface of the distal portion which is usually hidden from view, is a small orange/brown pigmented region, the epipodial sense organ.

The longer tentacles are more mobile and capable of considerable extension. When fully extended they can reach

beyond the edge of the shell and contact the substratum. They taper evenly toward the somewhat truncated apex which bears a faintly pigmented epipodial sense organ.

A single additional tentacle arises in the midline posteriorly on the metapodium, between the epipodial fold and the sole. This metapodial tentacle has not previously been described. It is usually less than 1mm in length, rounded distally and unlike the other tentacles is pale orange in colour (Plate 26a). It is capable of very little movement.

The edge of the mantle skirt is devoid of distinct papillae or tentacles. It comprises three folds, the outer, middle and inner pallial folds, but little detail can be seen at this level. In the region of the shell slit however, it is considerably modified and is produced into a siphon (Plate 26b). This lies near the blind (posterior) end of the slit and is a semi-transparent, extremely delicate structure which is withdrawn rapidly if the animal is disturbed.

Anterior and posterior to this lie several unpigmented papilla-like structures (Plate 26b). These are the structures termed pallial tentacles by Yonge (1947). Examination at higher magnifications however reveals that they are not tentacular in appearance, but closely re emble the epipodial sense organs (internally and externally). They may therefore more appropriately be called pallial sense organs. They are small and scarcely visible at this level, usually there are four on each side of the slit, three pairs anterior and one pair posterior to the siphon.

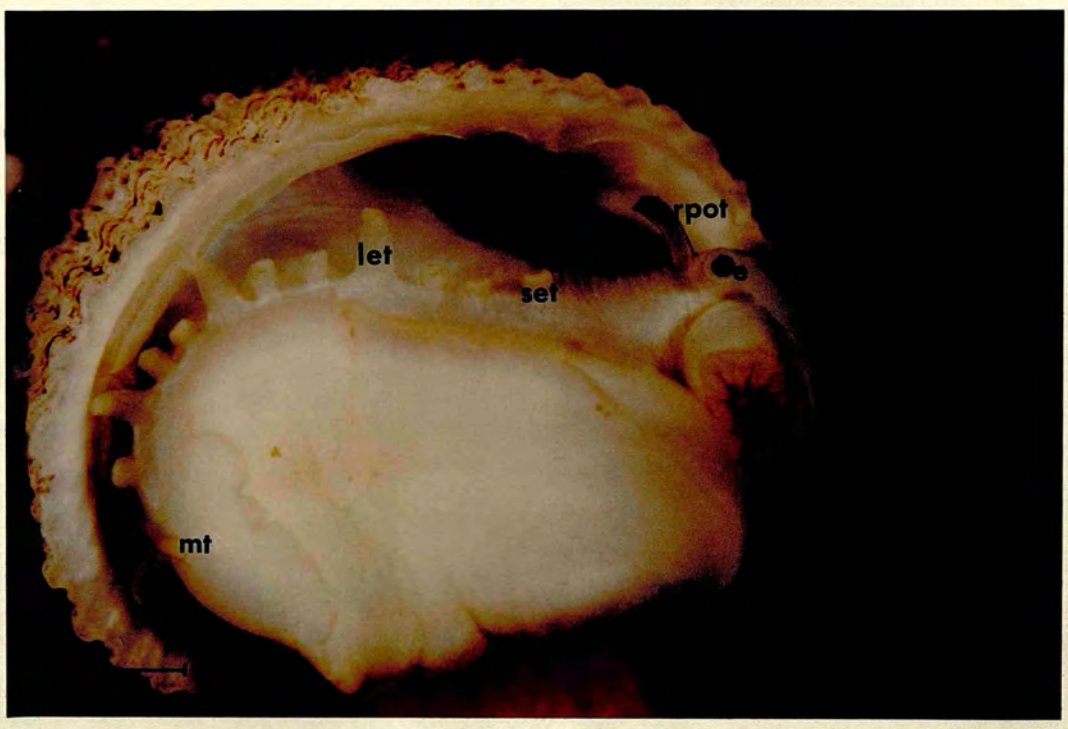
PLATE 26

a) *Emarginula reticulata*, ventral view animal (shell upturned) showing cephalic tentacles, eyes, right postoptic tentacle, long and short epipodial tentacles, and metapodial tentacle.
Bar = 2.0mm.

b) *Emarginula reticulata*, living animal actively crawling on rock surface. The shell is raised anteriorly exposing the head. The cephalic tentacles are extended well beyond the limits of the shell. Note also the extended siphon and pallial sense organs, anterior and posterior to this.
Bar = 2.0mm.

ct - cephalic tentacle
e - eye
let - long epipodial tentacle
mt - metapodial tentacle
pso - pallial sense organ
rpot - right postoptic tentacle
s - siphon
set - short epipodial tentacle

PLATE 26



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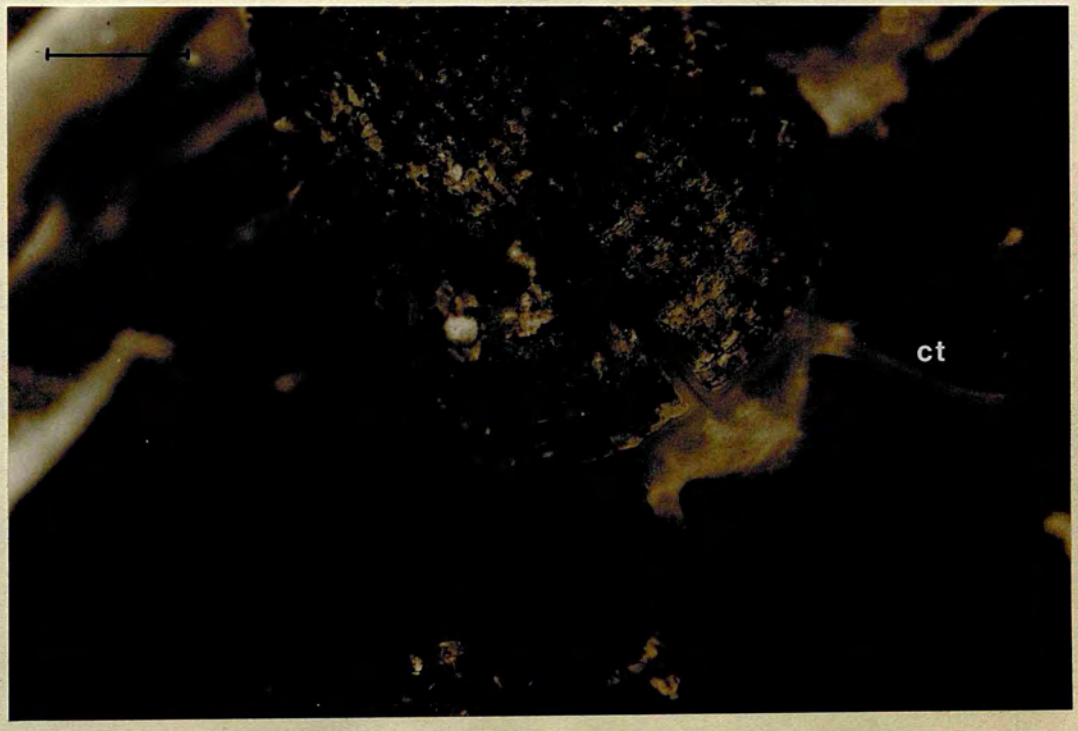


b

2.2. Nervous System and Innervation of Sense Organs

The nervous system and innervation of the sense organs was demonstrated using the modified Liang's technique (see Materials and Methods). Serial paraffin wax sections were used to a lesser extent. Particular attention was paid to the innervation of the head and its sensory appendages, the foot and epipodial sense organs, and the mantle skirt, especially that bordering the shell slit.

The general organisation of the nervous system is similar to that of the trochids in that it is hypoathroid. The assumption of secondary bilateral symmetry and the limpet-like habit however, have increased the relative importance of the edge of the mantle skirt as a sensory area which is reflected in the hypertrophy of its innervation.

The innervation of the cephalic appendages is shown in Fig. 9a. Though essentially similar to that of the trochids, there are notable differences. There is only one major nerve to the snout and the cephalic lappet and its nerve are absent. The tentacular nerve may split in two basally, the two branches extending in close proximity to the tip. Very fine radial branches could occasionally be distinguished running to the epithelium. The nerve innervating the right postoptic tentacle appears to arise from the optic nerve near its origin, not from the cerebral ganglion.

The innervation of the foot and epipodial tentacles is shown in Fig. 9b. The paired pleural and pedal

ganglia form a large suboesophageal mass. The pleural ganglia form the antero-lateral portions of this mass, but are not clearly separable from the pedal ganglia. Two long pedal cords extend posteriorly, connected at intervals by commissures, some markedly thicker than others. The pedal cords give off laterally, numerous small branches, which penetrate the tissue of the foot; for clarity, only those innervating the epipodium are shown in Fig. 9b. The majority of the nerves not shown innervate the ventral part of the foot, but a small number extend to the narrow area dorsal to the epipodial fold.

Some of the nerves innervating the epipodium are unbranched, whilst others may divide before reaching it. The branches thus formed however, are restricted to the epipodium. Each epipodial tentacle has a single central nerve which may originate directly from the pedal cord, or from a branch of a nerve from the pedal cord, or may be formed by an anastomosis of two such branches. The innervation of the long and short tentacles is similar in this respect. The pattern of innervation is not identical in each half of the foot and in addition it seems likely that considerable variation exists between individuals.

There is however, a difference in the distribution of nerves in the short and long tentacles. In the short ones, the central nerve sends out very fine branches to the epithelium, starting at the base of the tentacle. The main trunk of the nerve however, passes to the epipodial sense organ on its ventral surface, below which it divides

repeatedly. Few nerves run to the tentacle tip. In the long tentacles, branches running to the epithelium are rare basally and the nerve appears to run directly to the sense organ at the tentacle tip. Occasionally it may divide in the middle region and fuse again distally. Under the sense organ the nerve branches extensively as in the short tentacles. It is therefore evident that in both types of epipodial tentacle most of the nerve fibres innervate the epipodial sense organ.

The mantle skirt is innervated by the internal and external pallial nerves arising from the paired branchial and pleural ganglia respectively. Peripherally these nerves branch and anastomose extensively forming a ring of nervous tissue, the pallial nerve ring, which completely encircles the mantle skirt. The margins of the slit and the anterior edge of the mantle skirt are innervated by branches of the internal pallial nerve and the remainder by the external pallial nerve. The pallial nerve ring gives off many small peripheral nerves throughout its length. Most of these enter the middle and inner mantle folds, very few have been seen to pass into the outer fold. The detailed innervation of the area bordering the shell slit is shown in Fig. 10.

At the posterior, blind end of the slit the pallial nerve ring gives rise to two nerves, one on each side of the midline which innervate the posterior pair of pallial sense organs. In the siphonal region a further nerve arises (one on each side of the slit) which branches and passes

into the thin walls of the siphon. The pallial sense organs anterior to the siphon (usually three on each side) are innervated by nerves arising from the ring, as are the areas between them. The most anterior region is similar to the remainder of the skirt edge. The nerves supplying both the anterior and posterior pallial sense organs branch repeatedly under the distal epithelium.

FIGURE 9

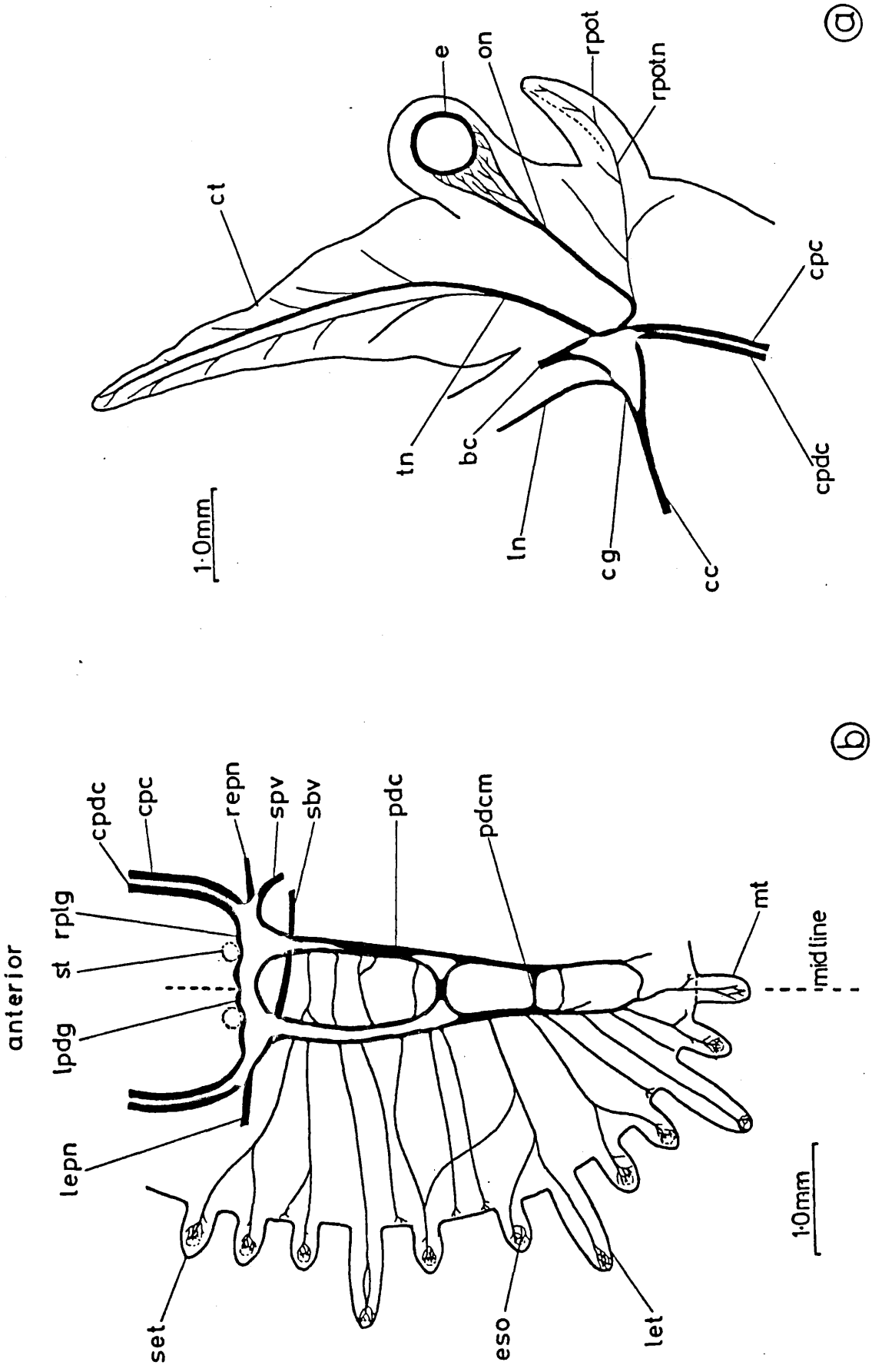
a) *Emarginula reticulata*, dorsal view of right half of head showing innervation of cephalic tentacle, eye and right postoptic tentacle.

b) *Emarginula reticulata*, dorsal view of the nervous system of the foot. Only the epipodial nerves of the left side are shown.

Both figures are drawn from whole mount preparations stained using the modified Liang's technique.

bc - buccal connective
cc - cerebral commissure
cg - cerebral ganglion
cpc - cerebropleural connective
cpdc - cerebropedal connective
ct - cephalic tentacle
e - eye
eso - epipodial sense organ
lepn - left external pallial nerve
let - long epipodial tentacle
ln - labial nerve
lpdg - left pedal ganglion
lplg - left pleural ganglion
mt - metapodial tentacle
on - optic nerve
pdc - pedal cord
pdcm - pedal commissure
repn - right external pallial nerve
rplg - right pleural ganglia
rpot - right postoptic tentacle
rpotn - right postoptic tentacle nerve
sbv - suboesophageal visceral loop
set - short epipodial tentacle
spv - supraoesophageal visceral loop
st - statocyst
tn - tentacular nerve

FIGURE 9


a
e
on
rpot
rpotn
ct
cpc
tn
bc
cpdc
ln
cg
cc
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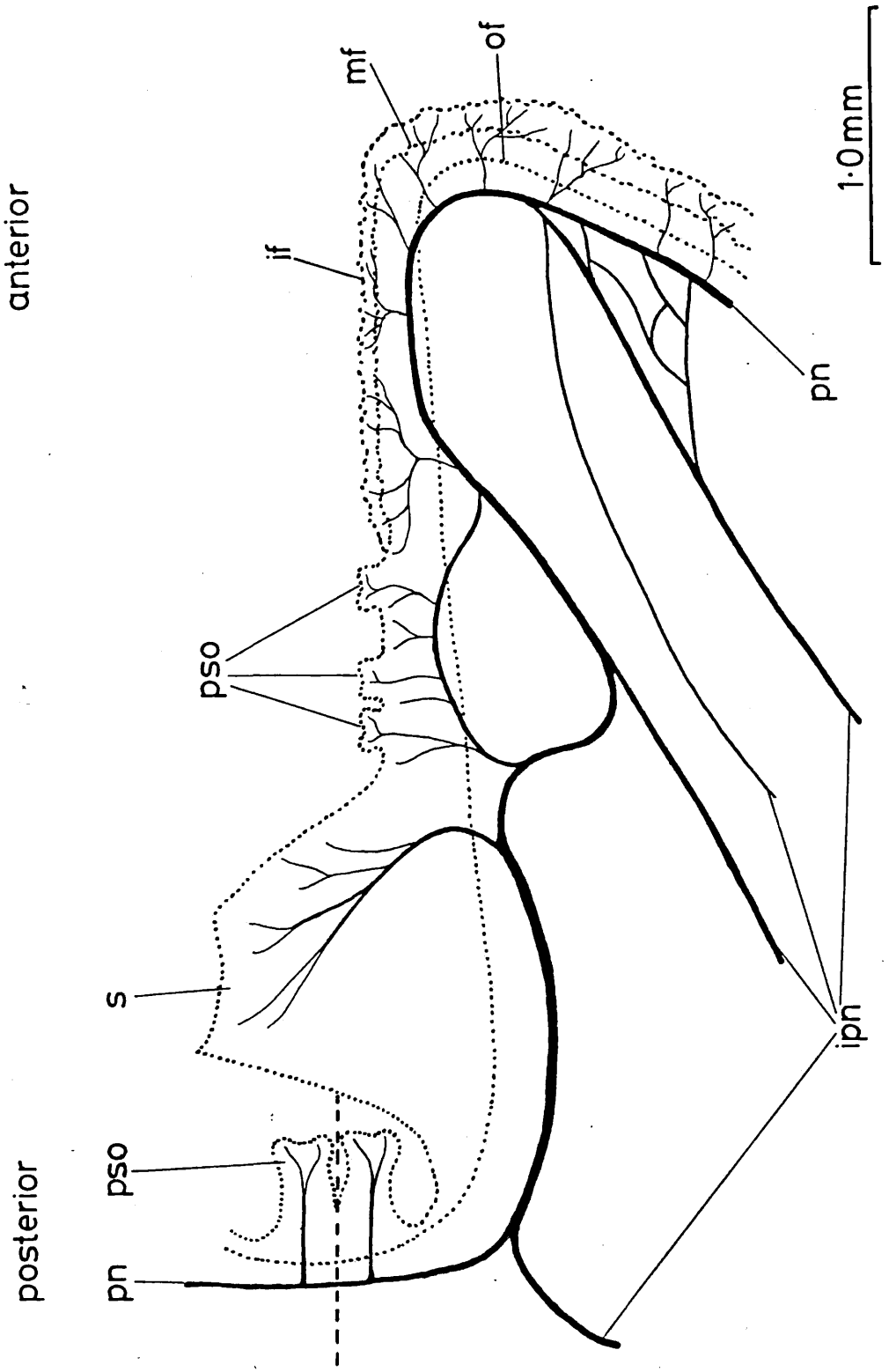
b
anterior
cpdc
cpc
repn
spv
sbv
pdc
pdcm
mt
midline
st
rplg
lpdg
lepn
set
eso
let
1·0mm

FIGURE 10

Emarginula reticulata, innervation of the mantle edge bordering the shell slit. Drawn from whole mount preparation stained using the modified Liang's technique.

if - inner mantle fold
ipn - internal pallial nerve
mf - middle mantle fold
of - outer mantle fold
pn - pallial nerve ring
pso - pallial sense organ
s - siphon

FIGURE 10



2.3. Fine Structure of Cephalic Tentacles

In many respects the cephalic tentacles of *E. reticulata* are similar to those of the trochids. Although in the living animal they bear no obvious papillation, in the scanning electron microscope they can be seen to bear numerous small tubercules or papillae over most of the surface (Plate 27a). These tend to be smaller basally and are absent in the dorsal region. The internal organisation of the tentacles is also similar to that of the trochids (Plate 27c).

The tentacular nerve, although it may be in two parts is surrounded by a core of muscle fibres, particularly thick dorsally. From this core, radial muscle fibres pass toward the epithelium and interspersed between them are blocks of longitudinal fibres. The latter are smaller, but more abundant than in *G. umbilicalis*. Peripheral to these muscles is a broad layer of connective tissue and small muscle fibres over which lies the epithelium. The latter is composed of low columnar cells and because of the reduced size of the papillae is more regular in outline. The longitudinal muscles originate in the body wall latero-dorsal to the cerebral ganglion. The dorsal cephalic sinus extends only into the more basal region.

The papillae appear merely as bulbous swellings in the epithelium (Plate 27b) and each bears a small tuft of short cilia at its apex (Plate 28a). These cilia project 1-2μm above the general covering of microvilli and are usually

10-20, rarely 30 in number. An additional longer cilium (extending 4-5μm above the surface) was frequently seen to arise at the edge of the tuft (Plate 28b). Gland cell openings were occasionally seen between the papillae, but they showed no regular orientation with respect to them.

Transverse thin resin sections through the tips of the papillae show the sensory cells which bear the cilia to be arranged in the familiar leek leaf pattern (Plate 29a). There are rarely more than 10 of these cells per papilla, each bearing 2-6 cilia. Those in the centre possess fewer than those at the edge. The cell bodies lie within the epithelium and are easily distinguished from the remaining epithelial cells by their relatively electron-dense cytoplasm and nuclei (Plate 29b). From the distal part of the cell body a dendrite 6-9μm long extends to the epithelial surface where the cilia originate. At the base an axon arises and passes toward the basal lamina underlying the epithelium (Plate 29c). This is presumed to penetrate the basal lamina and together with axons from other cells form the radial branches of the tentacular nerve. The actual penetration of the basal lamina however has not been seen.

The cilia at the dendrite tip have the normal 9+2 subfibril complement and a basal body with 1-2 short rootlets (approximately 0.6μm long) (Plate 30a). No basal foot was observed and the central pair of subfibrils of different cilia showed no particular orientation. In addition to cilia the cells also possess a small number of microvilli. Neighbouring cells are joined by zonulae adhaerentes and

septate desmosomes. Below the ciliary basal bodies, but still within the leek leaf structure, the dendrites contain elongate mitochondria whose long axes lie parallel to the long axis of the dendrite. This region and the dendrite in general also contain microfilaments, microtubules and small clear vesicles. The latter may arise by pinocytosis from the bases of the microvilli.

The cell body lies near the base of the epithelium and contains an essentially ovoid nucleus. The cytoplasm contains several Golgi bodies, and often vesicles of varying electron density, particularly apical to the nucleus. Most organelles however, are difficult to distinguish because of the electron-density of the cytoplasm. Basal to the nucleus the cell body tapers rapidly to form the axon. There are few organelles in this region. Although, as stated earlier, axonal penetration of the basal lamina has not been observed, numerous small groups of axons were seen in the connective tissue immediately below the epithelium (Plate 30b). These groups often contain 12 or more individual axons and may therefore be presumed to originate from more than one papilla. Each group is always partly if not completely surrounded by a glial cell process containing many ovoid electron-dense granules and occasional mitochondria. The axons themselves contain only mitochondria, microtubules and infrequently small electron-dense vesicles.

The single longer cilium seen to arise from some papillae is thought to originate from a cell type structurally very similar to the cells in the pallial and epipodial

sense organs (Plate 30c). This is the only section obtained through this region of the cell, but the typical arrangement of a central kinocilium surrounded by two rings of firstly stereocilia and secondly thickened microvilli is clearly visible. The kinocilium shows little internal subfibril structure, but this is not thought to be typical. The stereocilia are clothed in a well developed glycocalyx.

Longitudinal sections have not been obtained through cells of this type so little can be said regarding the structure of the basal apparatus. In some transverse sections however, an additional circular dendrite at the edge of the leek leaf was observed (Plate 30d). This contains electron-dense material similar to that seen in the epipodial sense organ receptors (Plate 30d arrow).

PLATE 27

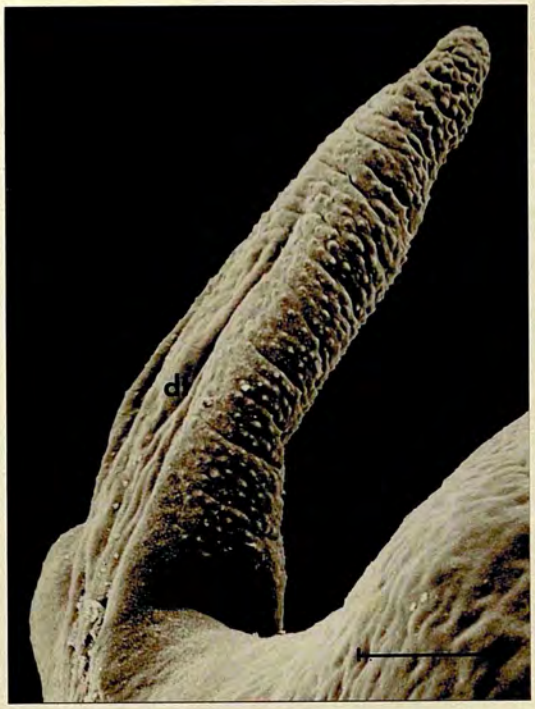
a) *Emarginula reticulata*, dorso-lateral view of left cephalic tentacle showing numerous small papillae. Bar = 200µm.

b) *Emarginula reticulata*, papillae of cephalic tentacle. Bar = 20µm.

c) *Emarginula reticulata*, transverse thick resin section of the basal part of the cephalic tentacle. Note the central core of radial muscle fibres, the approximately central tentacular nerve, the ring of longitudinal muscle blocks, the broad layer of connective tissue, and the dorsal furrow. Toluidine Blue. Bar = 50µm.

con - connective tissue
df - dorsal papilla-free furrow
h - haemocoelic space
lm - longitudinal muscle
p - papilla
rb - radial branch of tentacular nerve
rm - radial muscle core
tn - tentacular nerve

PLATE 27



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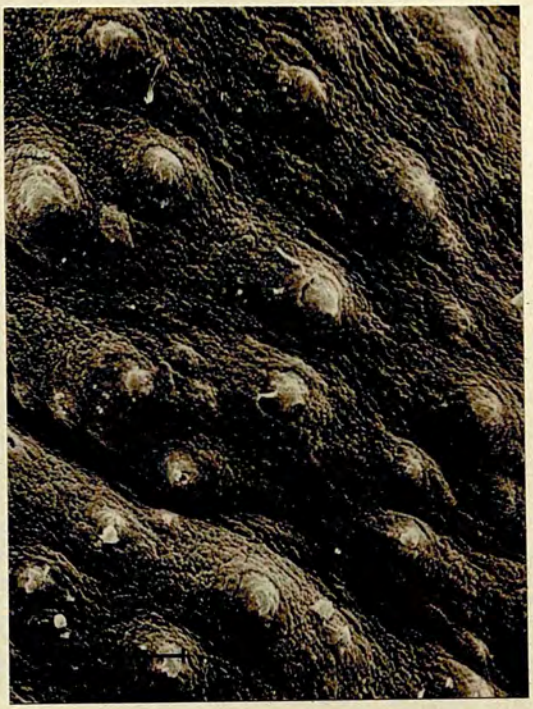
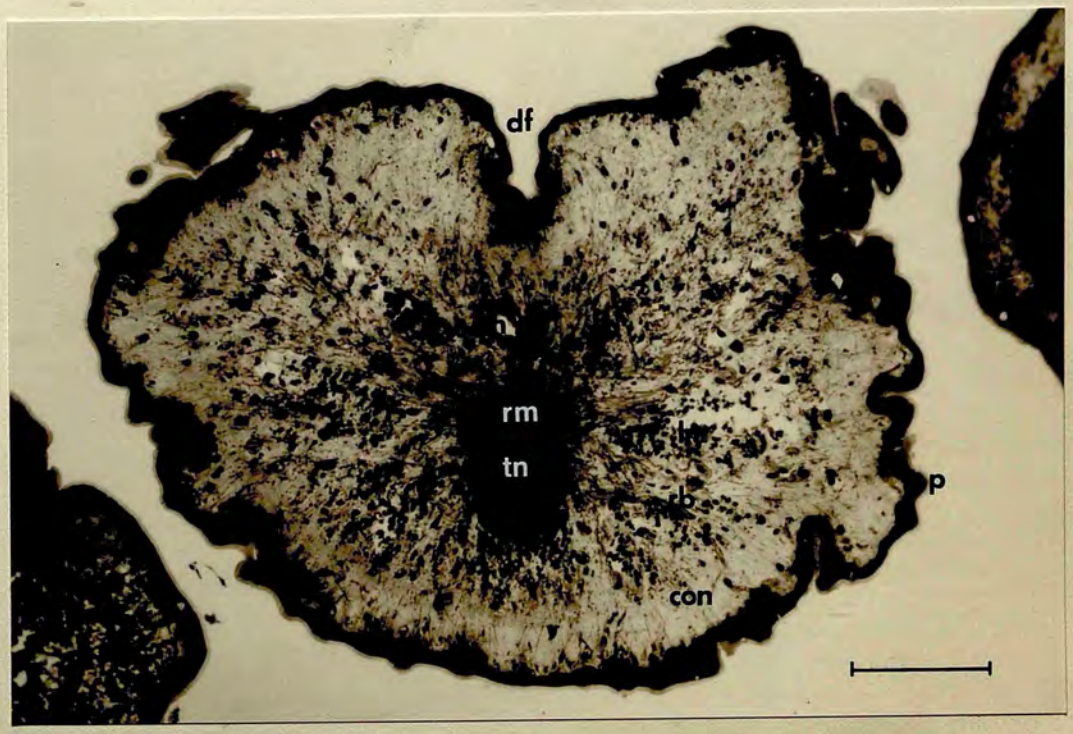


b



c

PLATE 28

a) *Emarginula reticulata*, high magnification of typical cephalic tentacle papilla bearing a small tuft of short cilia.
Bar = 4.0µm.

b) *Emarginula reticulata*, as above but with a single longer cilium in the tuft.
Bar = 4.0µm.

PLATE 28

a

b

PLATE 29

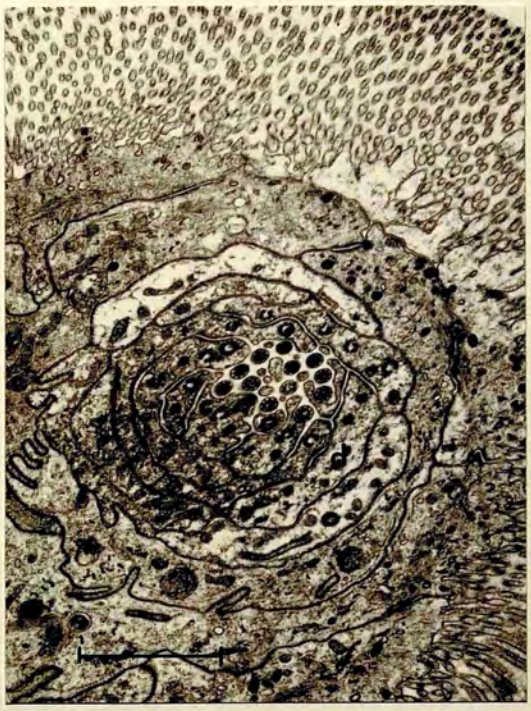
a) *Emarginula reticulata*, transverse to oblique section through the tip of cephalic tentacle papilla. Note leek leaf pattern of dendrites.
Bar = 1.0µm.

b) *Emarginula reticulata*, longitudinal section through sensory cells of a papilla.
Bar = 2.0µm.

c) *Emarginula reticulata*, basal constriction of sensory cell perikaryon forming the axon which passes to the basal lamina.
Bar = 1.0µm.

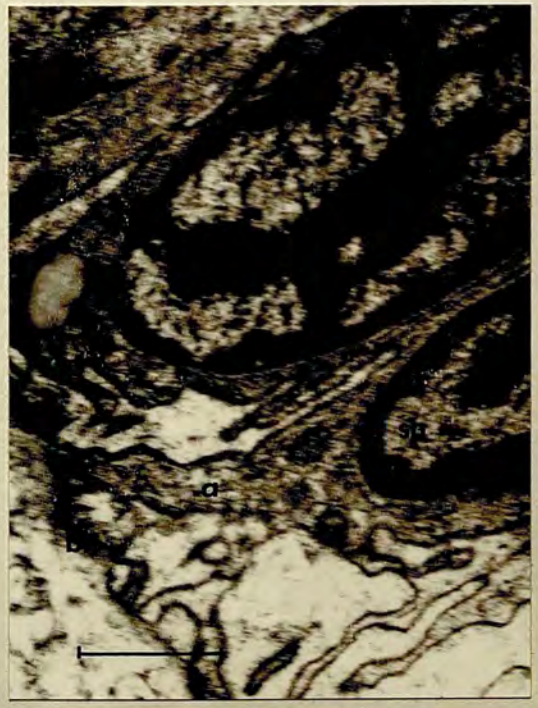
a - axon
bl - basal lamina
d - dendrite
sn - sensory cell nucleus

PLATE 29



a

b



c

PLATE 30

a) *Emarginula reticulata*, distal portion of dendrite in cephalic tentacle papilla showing cilia, basal bodies and short rootlets.
Bar = 1.0µm.

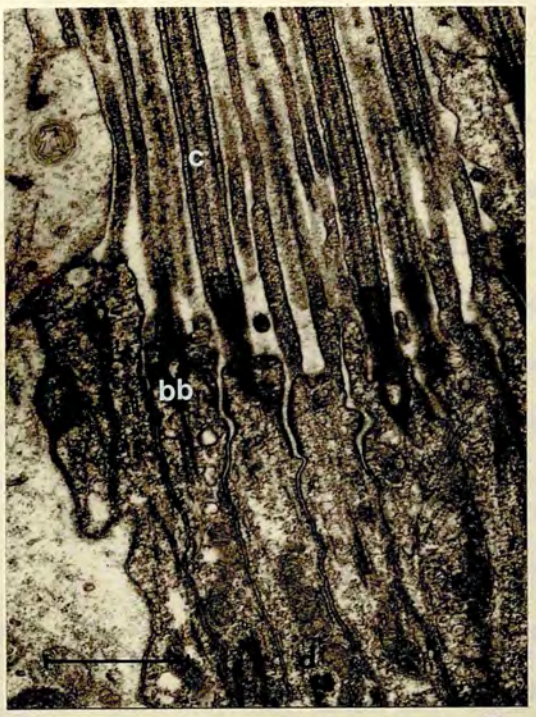
b) *Emarginula reticulata*, a group of sensory cell axons in the subepithelial connective tissue of the cephalic tentacle. Note pigmented envelope of glial cell process.
Bar = 1.0µm.

c) *Emarginula reticulata*, oblique section through distal portion of dendrite thought to give rise to the single long cilium seen in some cephalic tentacle papillae. Note similarity to monociliary sense cells in epipodial and pallial sense organs.
Bar = 1.0µm.

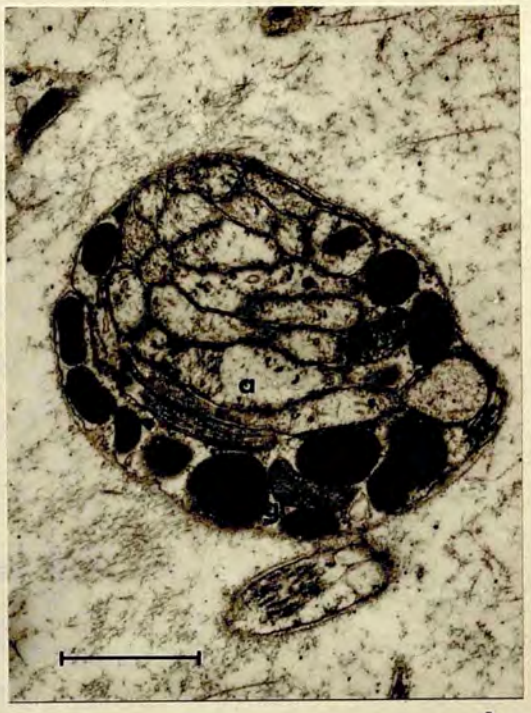
d) *Emarginula reticulata*, transverse section through distal part of cephalic tentacle papilla. Note circular dendrite with electron-dense granular material (arrow).
Bar = 1.0µm.

a - axon
bb - basal body of cilium
c - cilium
d - dendrite
gl - glial cell process
k - kinocilium
r - rootlet of cilium
st - stereocilium
t_{mv} - thickened microvilli

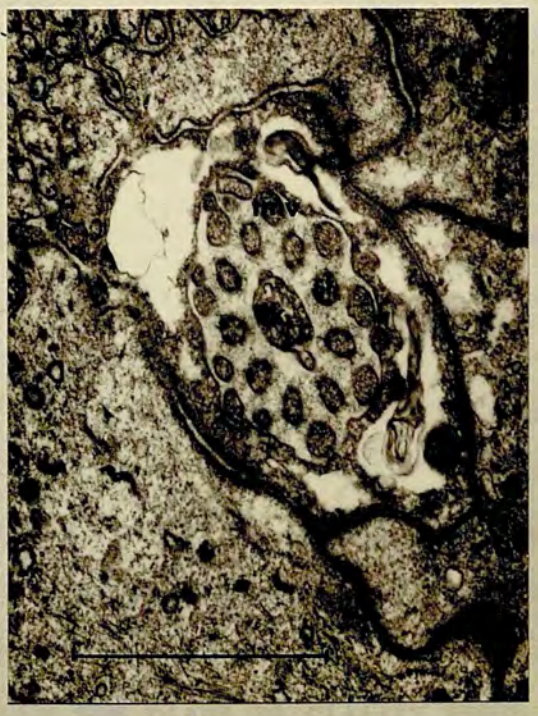
PLATE 30



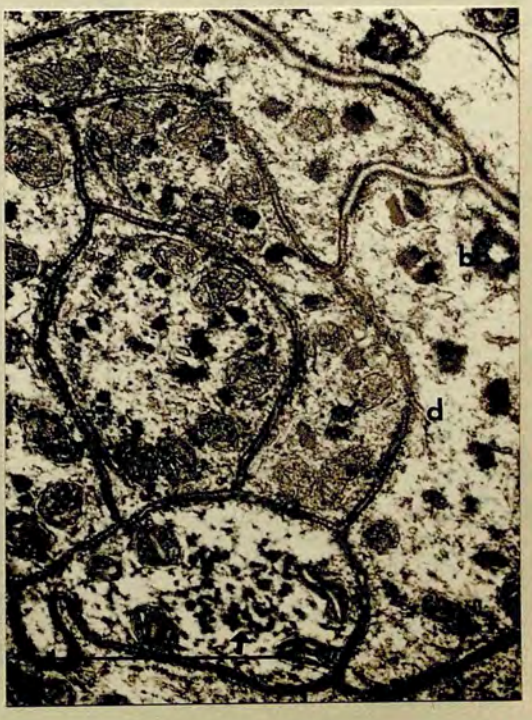
a



b



c



d

2.4. Fine Structure of the Epipodial Tentacles and Sense Organs

The fine structure of the epipodial sense organs of both the long and short epipodial tentacles is similar; no differences apart from their location within the tentacle have been observed. However, before their structure is described mention must be made of the organisation of the tentacles on which they are borne as these show pronounced differences.

The short epipodial tentacles are squat structures, dorso-ventrally flattened particularly in the distal region (Plate 31a). The latter portion is frequently curled over ventrally so that the epipodial sense organ is obscured or partly obscured. Basally they are almost round in cross section and there is usually a slight constriction where they arise from the epipodial fold (Plate 31b).

The tip and sides bear large numbers of short cilia 4-5μm in length (Plate 32a). These are not thought to be sensory and when viewed under darkfield illumination are seen to be motile. The direction of beat is toward the tentacle tip. They are borne on the free margin of the epithelial cells which in addition bear microvilli (Plate 32b). The ciliary basal bodies possess rootlets (not obvious in this section) and a pronounced basal foot. The latter all appear to be orientated in one direction, i.e. in line with the plane of ciliary bend.

Although not often clearly visible the position of the epipodial sense organ is indicated by a dense tuft of long cilia. In some cases the tentacle tip is less extensively folded over and the sense organ can be seen as a rounded cupule, 30-40μm in diameter, filled with the cilia previously mentioned (Plate 33a). This is bounded proximally and laterally by a distinct lip. As in the trochids, living tissue showed that the cilia normally project outward from the cupule and are non-motile. The remainder of the tentacle surface is relatively smooth and with the exception of the area immediately proximal to the sense organ, shows no folds or signs of contraction. Small tufts of short cilia (Plate 33b) (2-3μm long) and the openings of gland cells, occur frequently. The latter are particularly abundant near the motile lateral and apical cilia. The small tufts of cilia are thought to be sensory and to be borne on receptor cells whose axons form the fine lateral branches of the main tentacle nerve seen in preparations stained in the modified Liang's technique.

Most of the epithelium is almost cuboidal (approximately 5-8μm high) and bears only microvilli on its surface (Plate 34a). The lateral and apical ciliated epithelial cells are taller (approximately 10-12μm). The epithelium of the sensory cupule is quite distinct, being very tall (25-30μm) and possessing two distinct layers of nuclei. The remainder of the tentacle comprises the central nerve (not visible in Plate 34a) and connective tissue containing only a small number of muscle fibres. The latter however, are longitudinal and appear to be directed towards the sense organ (Plate 34a arrow). In this position their

contraction may pull the sense organ inwards and cause the tentacle tip to curl over, possibly to protect it. This correlates with the folded appearance of the area proximal to the sense organ in many tentacles, while the general paucity of muscle fibres correlates with the smooth appearance of the remainder of the surface and the lack of motility observed in living animals.

In contrast the long epipodial tentacles are characterised (in fixed tissue), by their ridged and contracted appearance (Plate 35a & b). The sense organ is always clearly visible at the tip and appears slightly displaced dorsally with a thickened rim (lip) ventrally and laterally. The cupule is similar in size to that on the short tentacles and again in living tissue the cilia project outward and are non-motile.

The long tentacles possess no motile cilia and the entire surface except the sense organ cupule is covered by a shallow columnar epithelium bearing only microvilli (Plate 34b). Gland cells are infrequent and ciliary tufts occur primarily near the tip. This correlates with the absence of branches from the main nerve in the basal regions of the tentacles. The specialised epithelium of the sense organ cupule is like that in the short tentacles.

The most striking feature of these tentacles in comparison with the short ones, is the increased amount of musculature (Plate 34b). Centrally, around the nerve is a ring of transverse muscle fibres which is in turn surrounded by a layer of well developed longitudinal fibres. The latter

extend basally into the musculature of the foot. This increase in musculature together with their contracted appearance correlates well with their observed motility in living animals.

From the descriptions of the external appearance of the epipodial sense organs already given, it is clear that these structures are very similar to those of the trochids. This similarity is not only superficial, but is also evident at the ultrastructural level.

The longitudinal paraffin wax sections of the tentacles (Plate 34a & b) have already shown that the epithelium of the cupule is tall (up to 30μm) and that it is pseudostratified, possessing two distinct layers of nuclei. The outer layer is that of the supporting cells and the inner, less precisely defined, is that of the sensory cells. This is particularly clear in Plate 34a, although the sensory cilia are scarcely visible. Plate 34b shows more distinctly that the covering layer of microvilli is much thicker in the cupule.

Thin resin sections (Plate 36a) show the nuclei of the supporting cells, irregular to ovoid in shape, lying in the apical half of the cells forming a precise layer. The nuclei of the sensory cells are more regular in shape than those of *G. umbilicalis* and occupy a broad band. Oblique sections through this epithelium show that the relationship of the supporting and sensory cells is identical to that in the trochid (Plate 36b).

Each sensory cell possesses a single long kinocilium arising from a depression in its apical border (Plate 36c). This has the normal 9+2 complement of subfibrils and is surrounded by a ring of nine stereocilia, triangular in cross-section, interconnected by a filamentous glycocalyx (Plate 37a). The additional ring of nine thickened microvilli seen in trochids is also present, but was never so well defined (Plate 37b). Neither the stereocilia nor the thickened microvilli are as long as the general covering of normal microvilli and they are not visible externally (Plate 37c). Occasional cells were seen in which the kinocilium appears to be absent, probably as a result of damage (Plate 36b arrow).

The ciliary basal body lacks both rootlets and a basal foot, but possesses an aggregation of electron-dense material linking it to the bases of the stereocilia and to an accessory centriole nearby (Plates 36c & 37d). The latter showed no particular orientation with regard to the basal body. Immediately below this region is an area containing long mitochondria, microtubules and occasional multivesicular bodies.

The cell body is elongate and apically there is no clear distinction between it and the dendrite (Plate 38a). The apical cytoplasm contains granules of variable electron-density, mitochondria and Golgi bodies. As in the trochids the basal portion of the perikaryon and the origin of the axon are poorly demarcated. Small groups of axons however, have been observed to pass through the basal lamina and

to be associated with glial cells (Plate 38b). The basal lamina is less well developed than that of *G. umbilicalis* (0.5µm thick) and the groups of axons tend to be smaller, but more numerous.

The supporting cells (Plates 36a & 38a) bear numerous long microvilli (3-4µm) projecting from their free margin, and contain large heterogeneous granules, Golgi bodies, mitochondria and multivesicular bodies in their apical cytoplasm. Basally they are extremely narrow and contain only bundles of microfilaments originating in hemidesmosomes between the basal plasma membrane and the basal lamina (Plate 38a). In many respects these cells are similar to the normal tentacular epithelial cells (Plate 38c). Both cell types contain small electron-lucent vesicles (100nm in diameter) in their apical cytoplasm. These appear to arise by pinocytosis from the apical plasma membrane.

The right postoptic tentacle and the metapodial tentacle have been examined briefly using scanning electron microscopy and paraffin wax sections. They show little similarity with the epipodial tentacles and do not possess epipodial sense organs. The right post-optic tentacle is cylindrical basally, but dilated or flattened dorso-ventrally at its apex. It is extensively ciliated dorsally and possesses numerous gland cells in the epithelium. Although it is innervated, no putative sensory cells were observed on its surface. Those cilia present are thought to be motile. It shows little similarity with the right post-optic tentacle of *G. umbilicalis*.

The metapodial tentacle lies ventral to the epipodial fold and is dorso-ventrally flattened throughout its length. Its apex is smoothly rounded. Like the right postoptic tentacle its dorsal surface is covered with motile cilia and contains many gland cells. Occasional tufts of cilia, possibly sensory, were seen on its ventral surface.

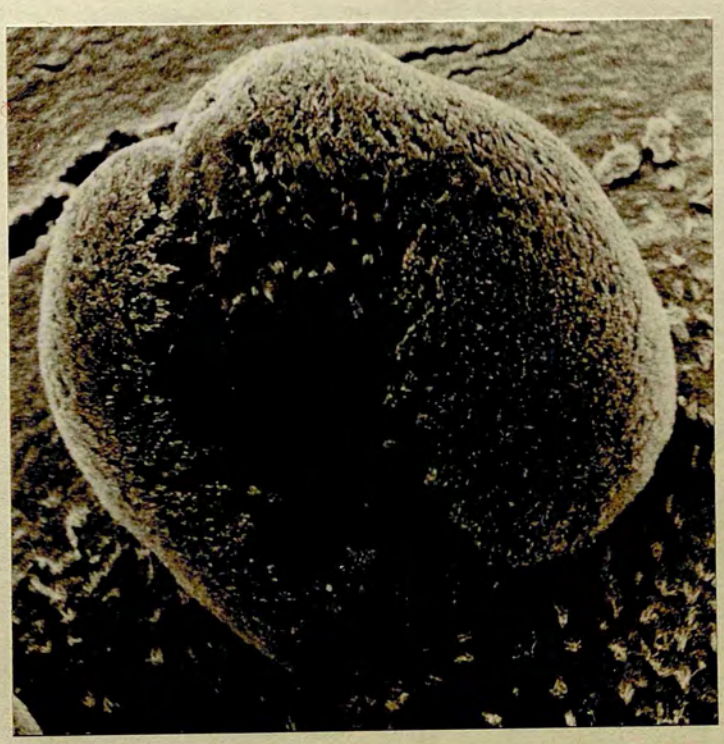
PLATE 31

a) *Emarginula reticulata*, apical and ventral surfaces of a short epipodial tentacle. The tip is folded over ventrally obscuring the epipodial sense organ.
Bar = 100µm.

b) *Emarginula reticulata*, dorsal view of a short epipodial tentacle. Note lateral ciliation.
Bar = 100µm.

PLATE 31

a



b

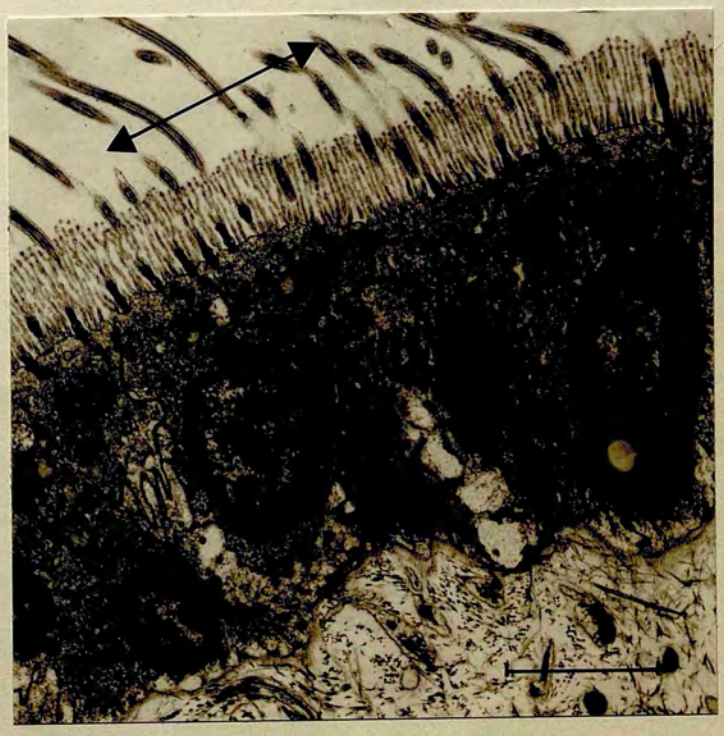
PLATE 32

a) *Emarginula reticulata*, motile cilia on dorso-lateral surface of a short epipodial tentacle. Note opening of discharged gland cell (arrow).
Bar = 4.0µm.

b) *Emarginula reticulata*, longitudinal section through the ciliated epithelial cells shown above. Note basal foot on ciliary basal bodies (arrow). The plane of ciliary beat is shown by the double-ended arrow.
Bar = 3.0µm.

PLATE 32

a



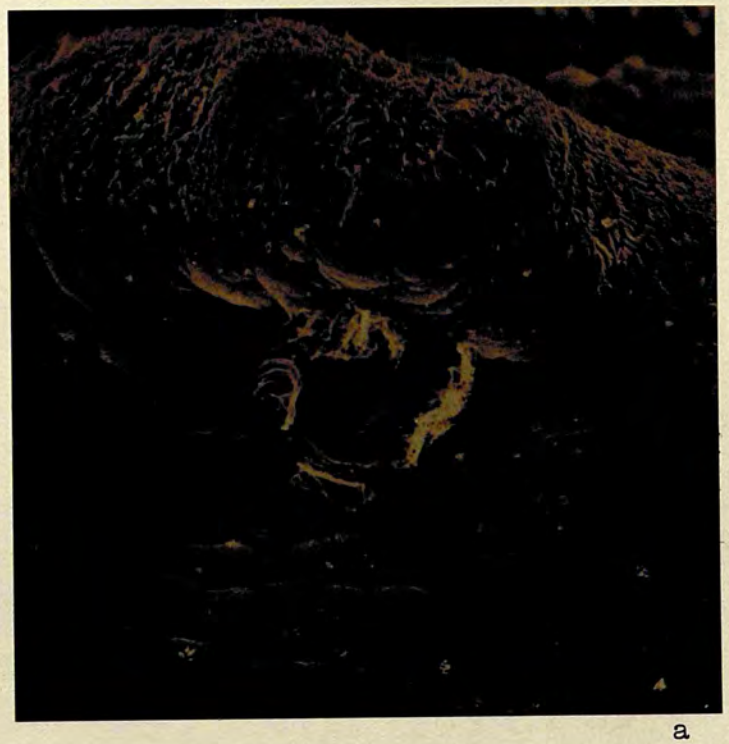
b

PLATE 33

a) *Emarginula reticulata*, ventral view of short epipodial tentacle showing position of epipodial sense organ.
Bar = 50µm.

b) *Emarginula reticulata*, tuft of short cilia on the ventral surface of a short epipodial tentacle, possibly sensory.
Bar = 2.0µm.

PLATE 33



a

b

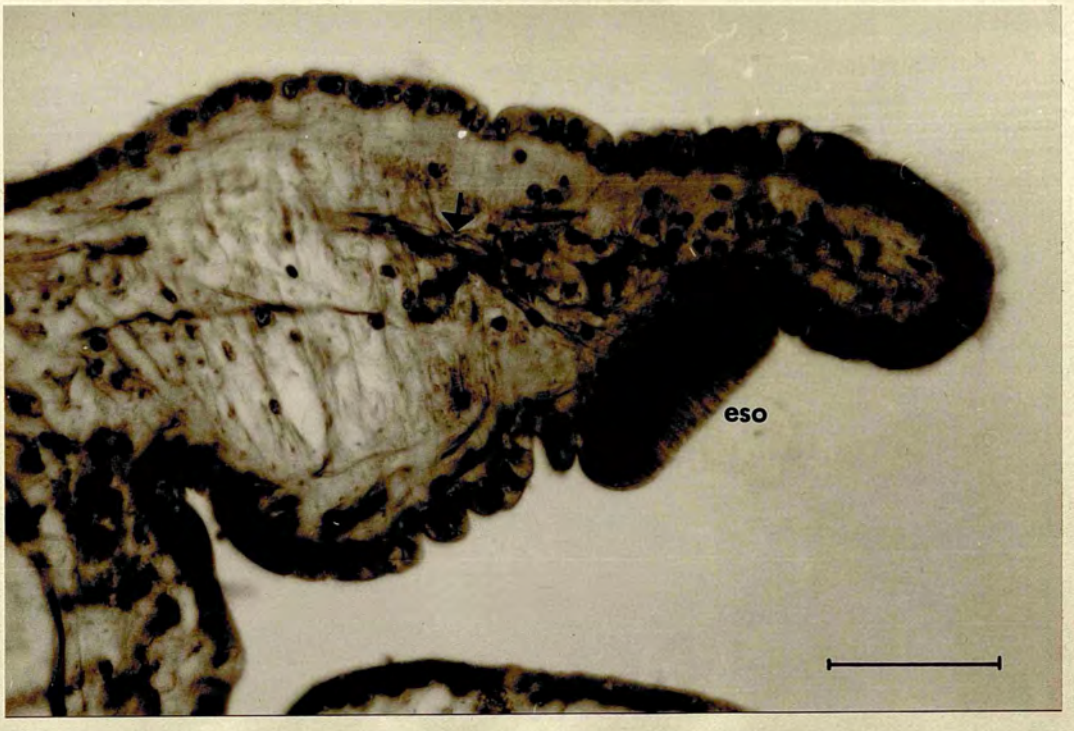
PLATE 34

a) *Emarginula reticulata*, longitudinal section through a short epipodial tentacle. Note specialised pseudo-stratified epithelium of the epipodial sense organ, ciliated epithelium of the tentacle tip and the small number of muscle fibres directed towards the epipodial sense organ (arrow).
Bar = 50µm.

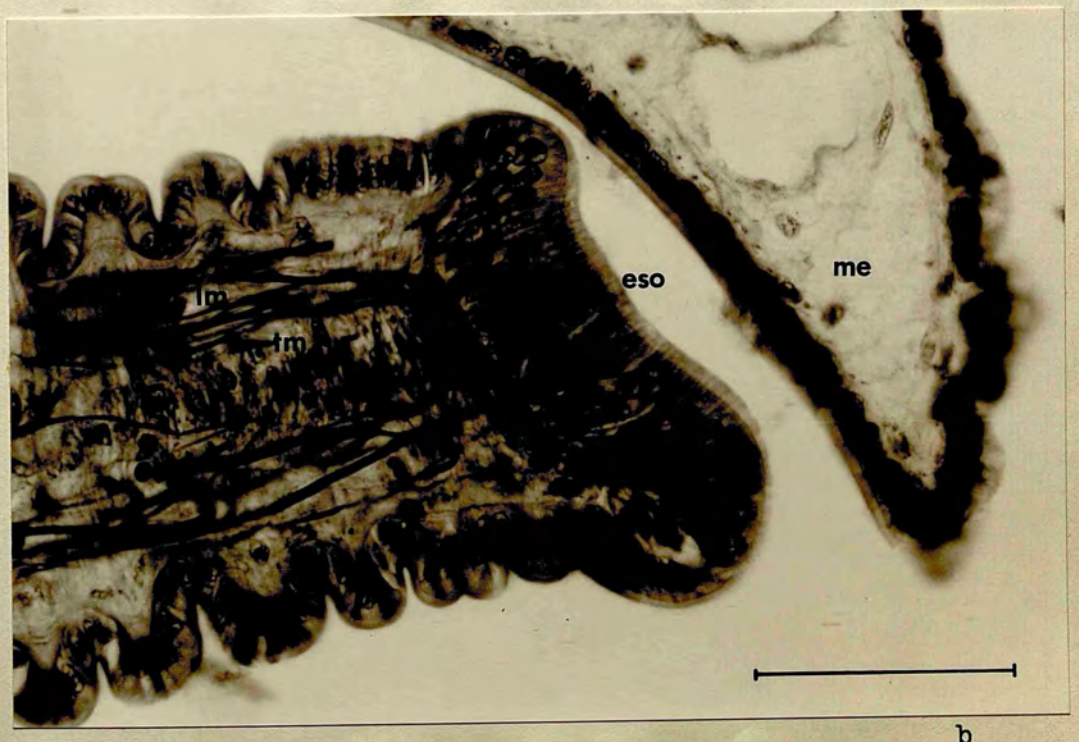
b) *Emarginula reticulata*, longitudinal section through distal part of a long epipodial tentacle. Note pseudo-stratified epithelium of the epipodial sense organ at the tentacle tip, abundance of muscle fibres (longitudinal and transverse), and the contracted appearance of the lateral epithelium.
Bar = 50µm.

eso - epipodial sense organ
lm - longitudinal muscle
me - mantle edge
tm - transverse muscle

PLATE 34



a



b

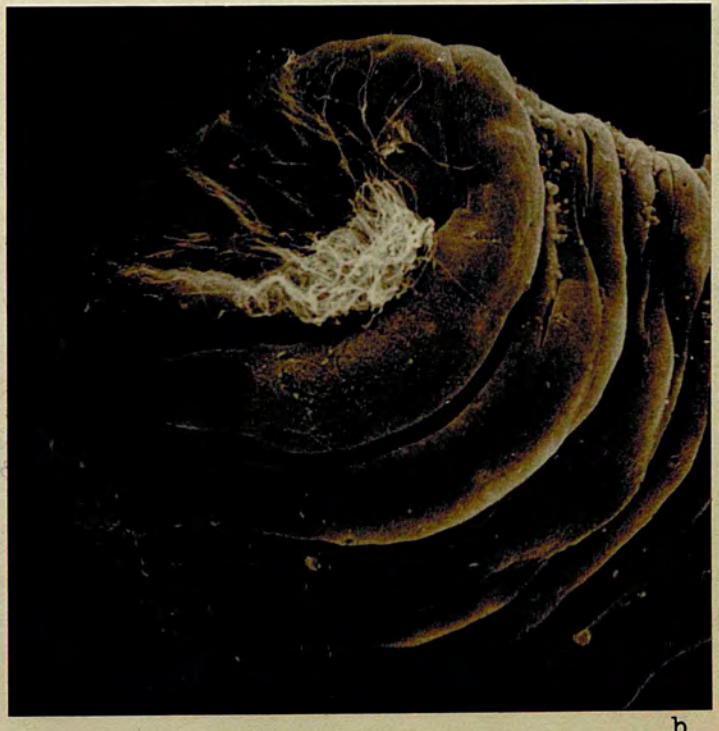
PLATE 35

a) *Emarginula reticulata*, dorsal view of long epipodial tentacle. Note apically placed epipodial sense organ and contracted appearance of the tentacle itself. Bar = 0.1mm.

b) *Emarginula reticulata*, dorsal view of epipodial sense organ at the tip of a long epipodial tentacle. Note slight displacement of the organ dorsally. Bar = 50µm.

PLATE 35

a



b

PLATE 36

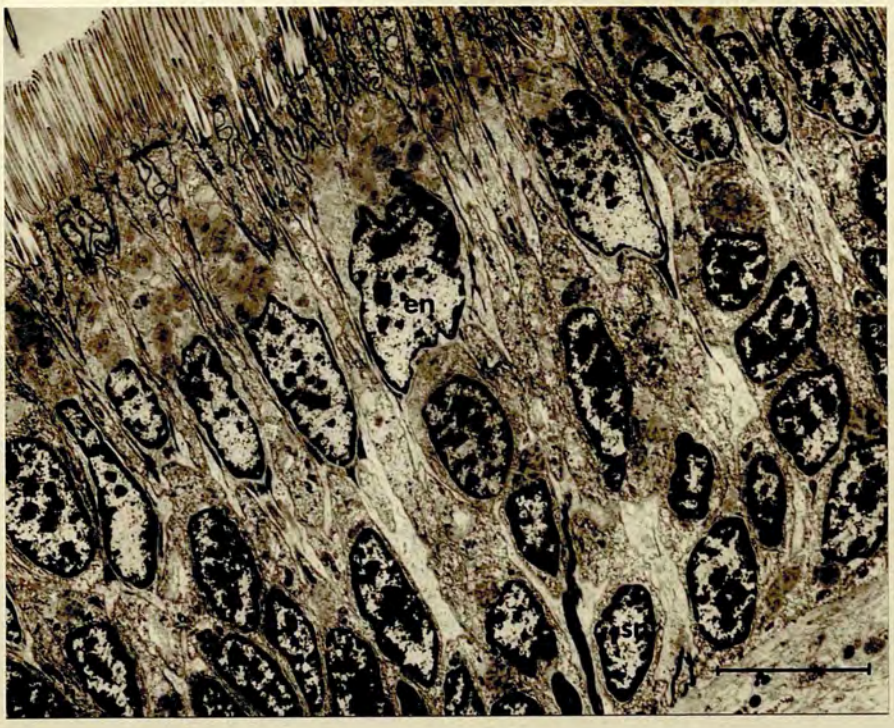
a) *Emarginula reticulata*, longitudinal section through the pseudostratified epithelium of the epipodial sense organ. Note median layer of epithelial cell nuclei and the broader basal band of sensory cell nuclei.
Bar = 5.0µm.

b) *Emarginula reticulata*, oblique section through the epithelial margin of the epipodial sense organ. Note circular dendrites with a single kinocilium surrounded by a ring of nine stereocilia. An outer ring of nine thickened microvilli is also present but is not clear. Occasional cells have lost the kinocilium (arrow).
Bar = 2.0µm.

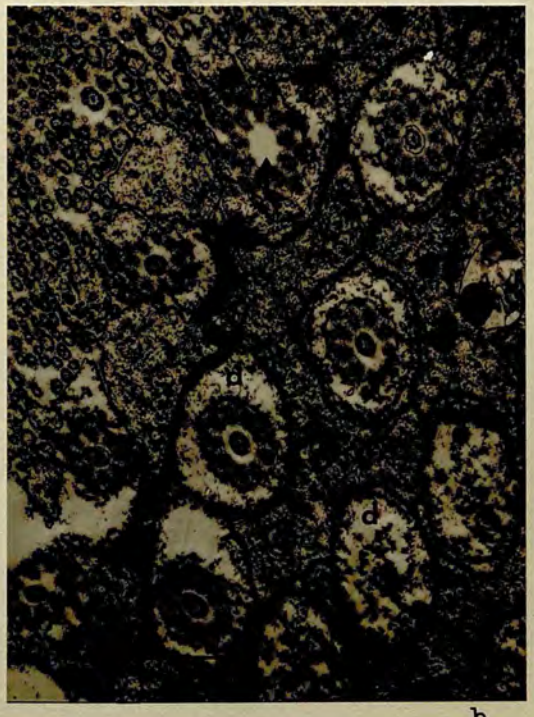
c) *Emarginula reticulata*, longitudinal section through distal part of monociliary cell dendrite. Note stereocilia and accumulation of electron-dense material around the basal body of the kinocilium.
Bar = 1.0µm.

bb - basal body of kinocilium
d - dendrite
en - epithelial cell nucleus
k - kinocilium
sn - sensory cell nucleus
st - stereocilium

PLATE 36



a



b

c

PLATE 37

a) *Emarginula reticulata*, transverse section through distal part of monociliary cell dendrite in epipodial sense organ. Note the central single kinocilium, the triangular stereocilia inter-connected by a well developed glycocalyx, and the basal parts of the thickened microvilli.
Bar = 0.5µm.

b) *Emarginula reticulata*, transverse section through the brush border of epipodial sense organ showing kinocilium, nine stereocilia and nine thickened microvilli.
Bar = 0.5µm.

c) *Emarginula reticulata*, high magnification of the surface of epipodial sense organ showing kinocilia projecting from holes in the surface. Note the stereocilia and thickened microvilli do not project above the general covering of microvilli.
Bar = 2.0µm.

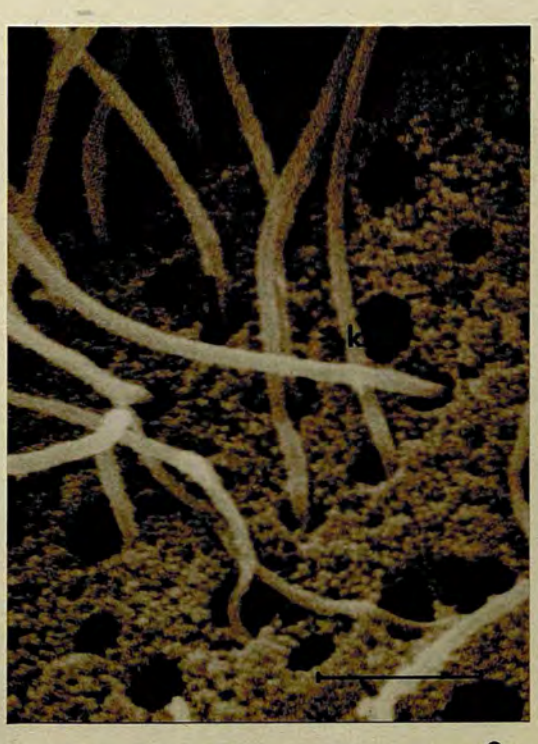
d) *Emarginula reticulata*, longitudinal section of distal part of two monociliary cell dendrites. Note the presence of an accessory centriole in each and the differential orientation of these with regard to the basal body.
Bar = 2.0µm

ac - accessory centriole
d - dendrite
gx - glycocalyx
k - kinocilium
st - stereocilium
tmv - thickened microvillus

PLATE 37

a

b



c

d

PLATE 38

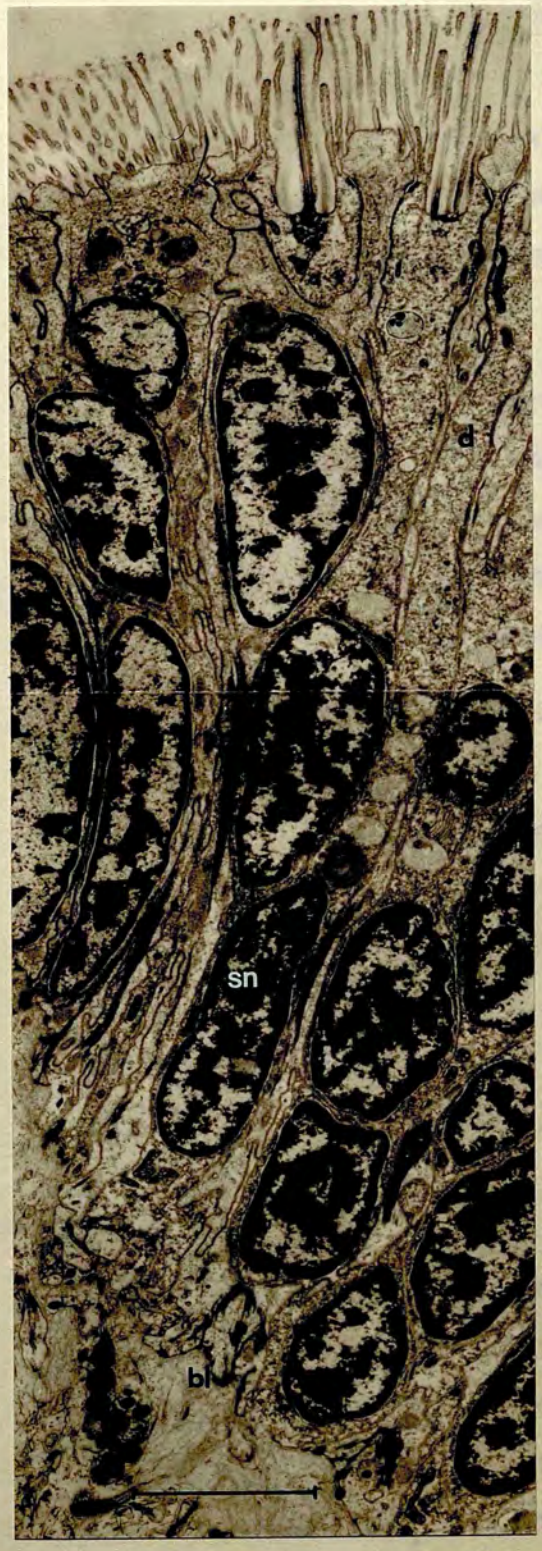
a) *Emarginula reticulata*, longitudinal section through sensory epithelium of epipodial sense organ showing perikaryon and dendrite of one monociliary sense cell.
Bar = 3.0µm.

b) *Emarginula reticulata*, axons of monociliary cells penetrating the basal lamina of epipodial sense organ. Note pigmented glial cell process.
Bar = 1.0µm.

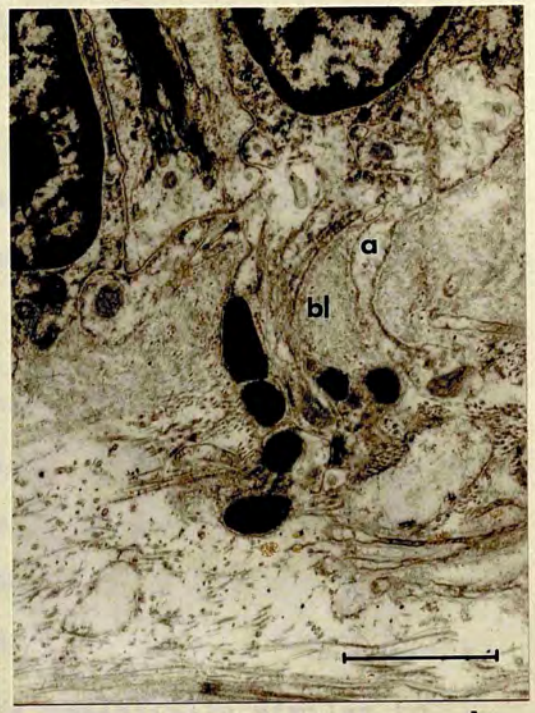
c) *Emarginula reticulata*, unspecialised epithelial cell of epipodial tentacle.
Bar = 2.0µm.

a - axon
bl - basal lamina
d - dendrite
sn - sensory cell nucleus

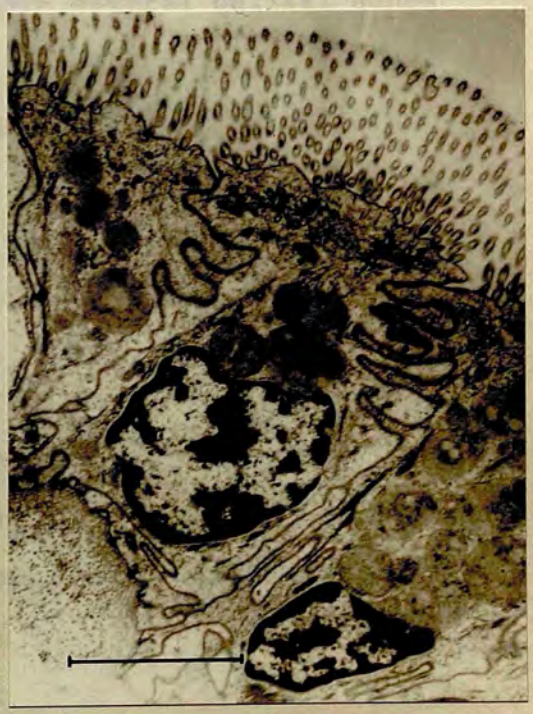
PLATE 38



a



b



c

2.5. Structure of the Mantle Edge bordering the Shell Slit and the Ultrastructure of the Pallial Sense Organs

The mantle edge bordering the shell slit has been examined primarily using scanning electron microscopy, only the pallial sense organs have been examined under transmission electron microscopy.

In this region the mantle edge can be divided into two identical halves corresponding to the right and left sides of the shell slit. These halves join posteriorly in the midline at the rounded end of the slit. Each half can be further divided into four distinct regions :- (Plate 39 & Fig. 11).

1. The anterior region, which is essentially similar to the remainder of the mantle edge
2. The sensory region, which bears several pallial sense organs
3. The siphonal region, through which the exhalent water current passes
4. The posterior region, which is blind ending and bears a further pallial sense organ.

The anterior region

Like the rest of the mantle edge this region is composed of three folds (Plate 40a). The outer and middle folds remain unchanged as they curve round the anterior of the shell and pass into the slit. The outer fold is a thin layer of tissue (15µm thick) which curls outward and in the living animal lies against the smooth inner surface

of the shell. The middle fold is much thicker (50μm) and is ridged transversely (Plate 40b). The inner mantle fold however, does show some modification in the slit. Elsewhere it has a broad outer face covered with numerous rounded tubercules and is separated from the middle fold by a deep cleft. In the slit this face becomes much reduced and the two folds lie much closer together (Plate 41a). They are structurally similar though the inner one is more deeply ridged.

Associated with both these folds are two types of ciliary structure (Plate 41b). Firstly there are tufts of short cilia (one arrow) raised above the surface on small bosses on top of the ridges. Secondly there are larger tufts of longer cilia (5-6μm long - two arrows) which lie mostly between the ridges and also on the inner face of the inner mantle fold. These are not thought to be sensory in function and resemble tufts of cilia on the floor of the mantle cavity, sides of the foot and ctenidia. They may function in the removal of particulate material. The tufts of short cilia in many ways resemble those seen on the cephalic tentacles. They are 1.5-2.0μm long and from 10-20 in number. Furthermore, in many cases a single long cilium (up to 20μm long) also occurs within the tuft.

The sensory region

This region lies immediately posterior to the anterior region. The outer mantle fold remains unchanged, but middle and inner folds fuse, forming a single broad

fold. This extends for approximately 0.5mm from the anterior region to the origin of the siphon. Borne on this fold are several cup-shaped pallial sense organs (Plate 42a) which show an obvious similarity to the epipodial sense organs particularly those of *Gibbula*. It is for this reason that these structures have been called pallial sense organs, rather than papillae or tentacles as they have been described previously (Ziegenhorn & Thiem, 1926; Yonge, 1947). They are slightly smaller than the epipodial sense organ of *G. umbilicalis*, measuring rarely more than 150µm in diameter. The central sensory cupule is approximately 50µm in diameter and is filled with the familiar mass of long fine kinocilia (Plate 42b).

The rim of each organ bears many tufts of short cilia but unlike those of the trochid the latter are borne on bosses and contain fewer cilia (Plate 42b). They are identical to the tufts on the inner and outer folds of the anterior region and often possess an additional long cilium. They are not restricted to the rim of the organs, but also occur in the areas between them, though usually in fewer numbers. In these latter areas tufts of longer cilia not raised above the surface also occur (Plate 43a). The openings of gland cells are frequent over much of the surface, particularly on the sense organ rims.

Siphonal region

The walls of the siphon are formed from an outward extension of the fold on which the pallial sense organs lie. In fixed tissue it is collapsed and curls over into the mantle

cavity. The outer face is distinctly lobed and bears both raised tufts of short cilia and groups of longer cilia (Plate 44a). The former lie on the lobes whilst the latter project between them. The free edge of the siphon bears a row of even longer cilia (10µm) projecting outward (Plate 44b). The inner wall is smooth and devoid of ciliation.

The posterior region

A pair of pallial sense organs (one from each half of the slit) arise from the region between the siphon and the outer mantle fold at the blind end of the slit (Plate 43b) They are identical to the other pallial sense organs except that they are raised further above the surface on distinct stalks.

Ultrastructure of the sensory epithelium of the pallial sense organs

The similarity between the pallial and epipodial sense organs is not only apparent on examination of the surface, but is also evident in thin resin sections.

The epithelium is tall (25µm) in contrast to the surrounding epithelium ($<$ 10µm) and again it possesses two distinct layers of nuclei (Plate 45a). The structural arrangement of these cells in relation to one another is identical to that in the epipodial sense organs (Plate 45b).

In order to avoid repetition a detailed description of the sensory cells is not given. That they represent a

similar cell type to that found in the epipodial sense organs is evident from the brief description given below.

Each sensory cell has a depression in its apical margin out of which arises a single long kinocilium (Plate 45c). This is surrounded by the familiar rings of stereocilia and thickened microvilli (nine in each ring) both of which are shorter than the normal microvilli and have a well developed glycocalyx. The kinocilium has no basal rootlet or foot, but again the basal body is associated with an electron-dense network of microfilaments and an accessory centriole. The bulbous swelling at the base of the kinocilium in Plate 45c is not a regular feature. The sensory cell body contains an ovoid nucleus, Golgi bodies, multivesicular bodies, large heterogeneous vesicles and mitochondria (Plate 45d).

Small groups of axons surrounded by glial cells are numerous beneath the basal lamina of the cupule, but none were observed actually passing through it.

FIGURE 11

Emarginula reticulata, diagrammatic representation of the mantle edge bordering the shell slit. The siphon is extended as in an active animal.

if - inner mantle fold

mc - mantle cavity

mf - middle mantle fold

of - outer mantle fold

pso - pallial sense organ

s - siphon

FIGURE 11

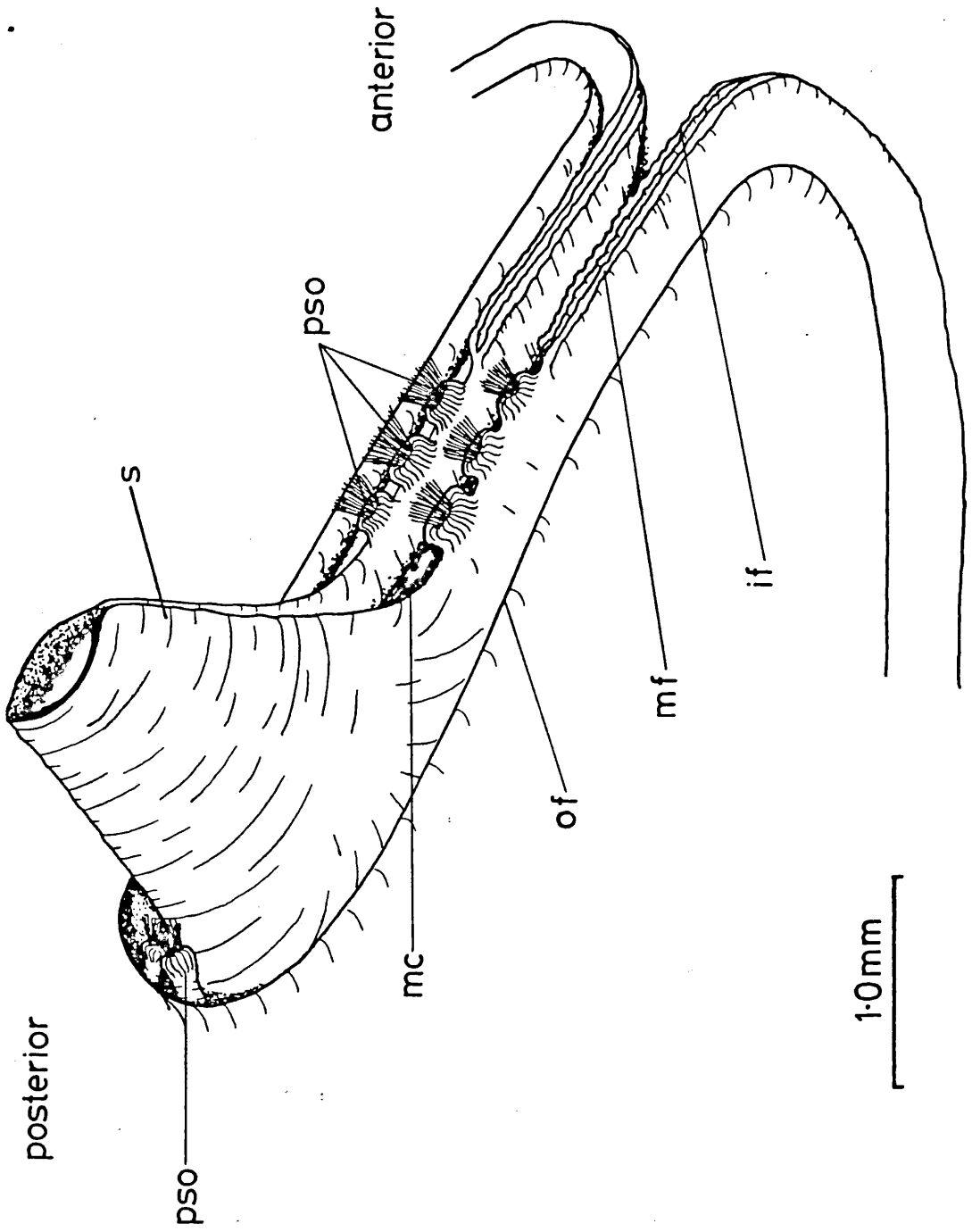


PLATE 39

Emarginula reticulata, mantle edge bordering the right side of the shell slit showing the four distinct regions. Bar = 200µm.

1 - anterior region

2 - sensory region

3 - siphonal region

4 - posterior region

PLATE 39

PLATE 40

a) *Emarginula reticulata*, anterior region of mantle edge bordering the left side of the shell slit. Note inner, middle and outer mantle folds. Bar = 300µm.

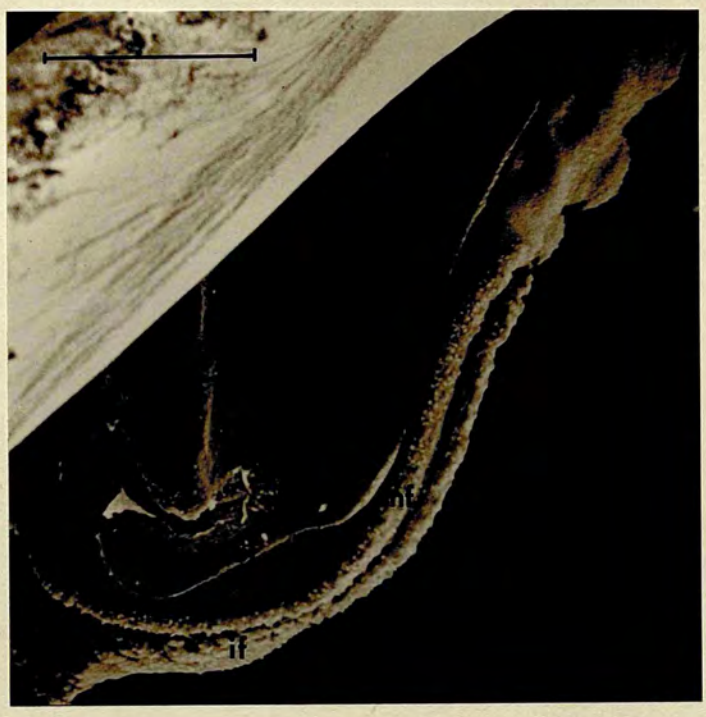
b) *Emarginula reticulata*, junction of anterior region of mantle edge bordering the left side of the shell slit with the general mantle edge. Note expanded inner mantle fold of general mantle edge. Bar = 100µm.

if - inner mantle fold

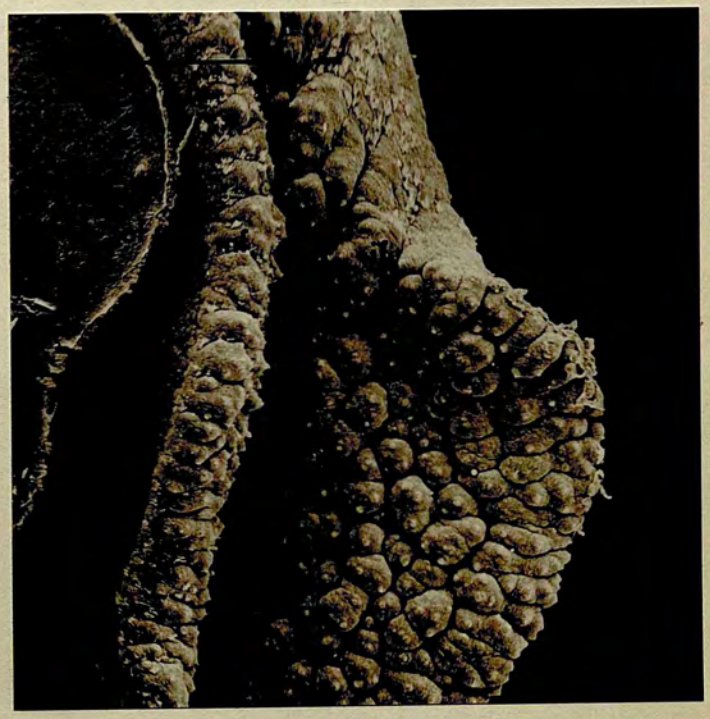
mf - middle mantle fold

of - outer mantle fold

PLATE 40



a



b

PLATE 41

a) *Emarginula reticulata*, anterior region of mantle edge bordering the shell slit. Note reduction in size of the inner mantle fold.
Bar = 50µm.

b) *Emarginula reticulata*, middle mantle fold in anterior region of mantle edge bordering the shell slit. Note small bosses with tufts of short cilia on the ridges (one arrow), and larger tufts of larger cilia between the ridges (two arrows). Some of the smaller tufts also possess a single very long cilium.
Bar = 20µm.

if - inner mantle fold

mf - middle mantle fold

PLATE 41

a

b

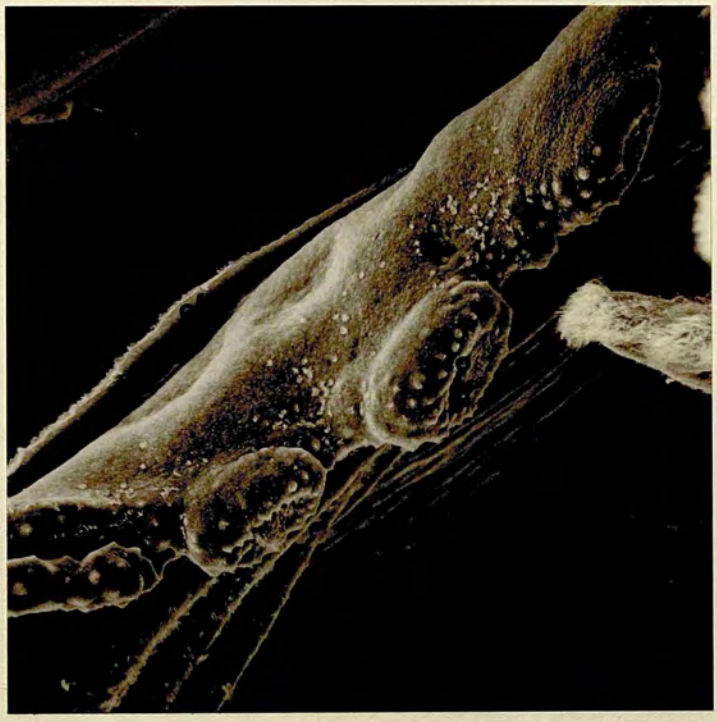
PLATE 42

a) *Emarginula reticulata*, sensory region of mantle edge bordering the shell slit showing three pallial sense organs.
Bar = 100µm.

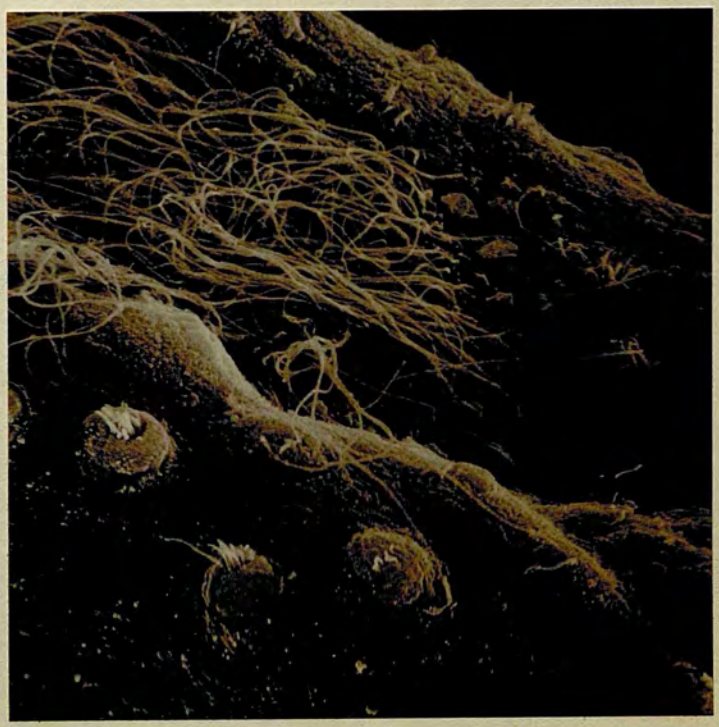
b) *Emarginula reticulata*, pallial sense organ with cupule filled with a mass of long cilia. Note also small ciliated bosses on the rim and the openings of gland cells.
Bar = 10µm.

of - outer mantle fold
pso - pallial sense organ

PLATE 42



a



b

PLATE 43

a) *Emarginula reticulata*, portion of mantle surface between the pallial sense organs bearing ciliated bosses and a tuft of longer cilia not raised above the surface. One of the bosses possesses an additional long cilium. Bar = 4.0µm.

b) *Emarginula reticulata*, posterior region of mantle edge bordering the shell slit bearing two stalked pallial sense organs. Part of the siphon is also shown. Bar = 100µm.

pso - pallial sense organ

s - siphon

PLATE 43

a

b

PLATE 44

a) *Emarginula reticulata*, lobed outer surface of siphon bearing ciliated bosses on the lobes and tufts of longer cilia between the lobes.
Bar = 10µm.

b) *Emarginula reticulata*, free edge of the siphon bearing a continuous row of long cilia.
Bar = 10µm.

PLATE 44

a

b

PLATE 45

a) *Emarginula reticulata*, longitudinal section through the pseudostratified sensory epithelium of a pallial sense organ. Note median epithelial cell nuclei and basal sensory cell nuclei.
Bar = 5.0µm.

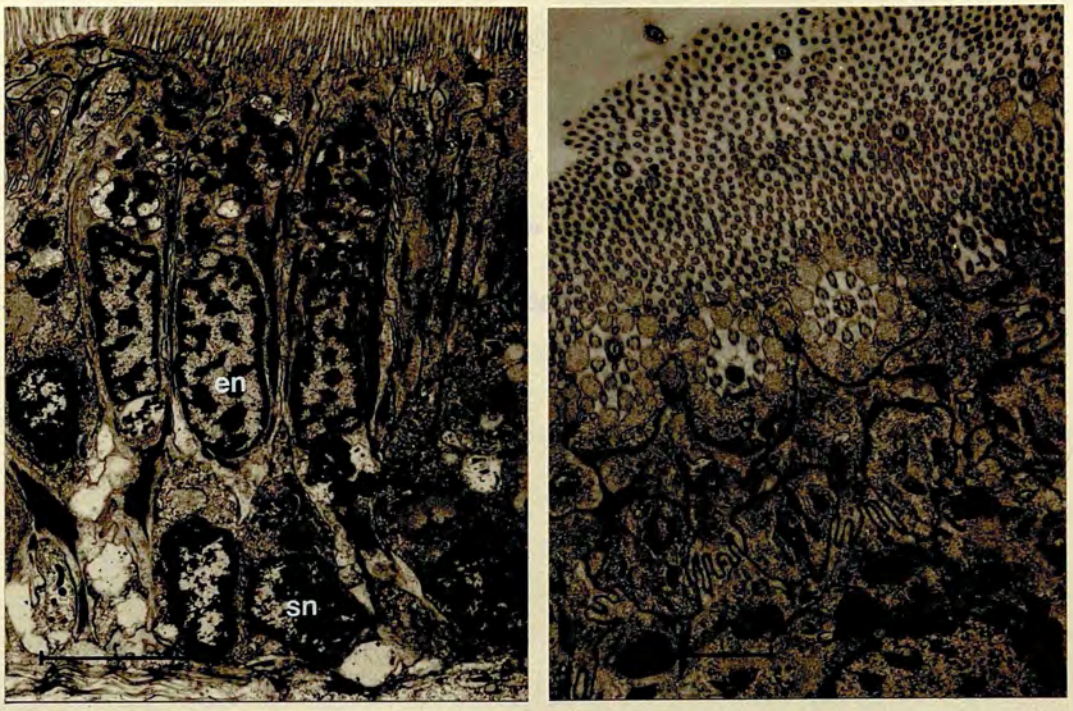
b) *Emarginula reticulata*, oblique section through sensory epithelium of pallial sense organ. Note circular sense cell dendrites each bearing a single central kinocilium surrounded (basally) by rings of nine stereocilia and nine thickened microvilli.
Bar = 2.0µm.

c) *Emarginula reticulata*, longitudinal section of monociliary dendrite tip in pallial sense organ. Note accessory centriole and accumulation of electron-dense material associated with the basal body of the kinocilium. The bulbous swelling at the base of the kinocilium is not a regular feature.
Bar = 0.5µm.

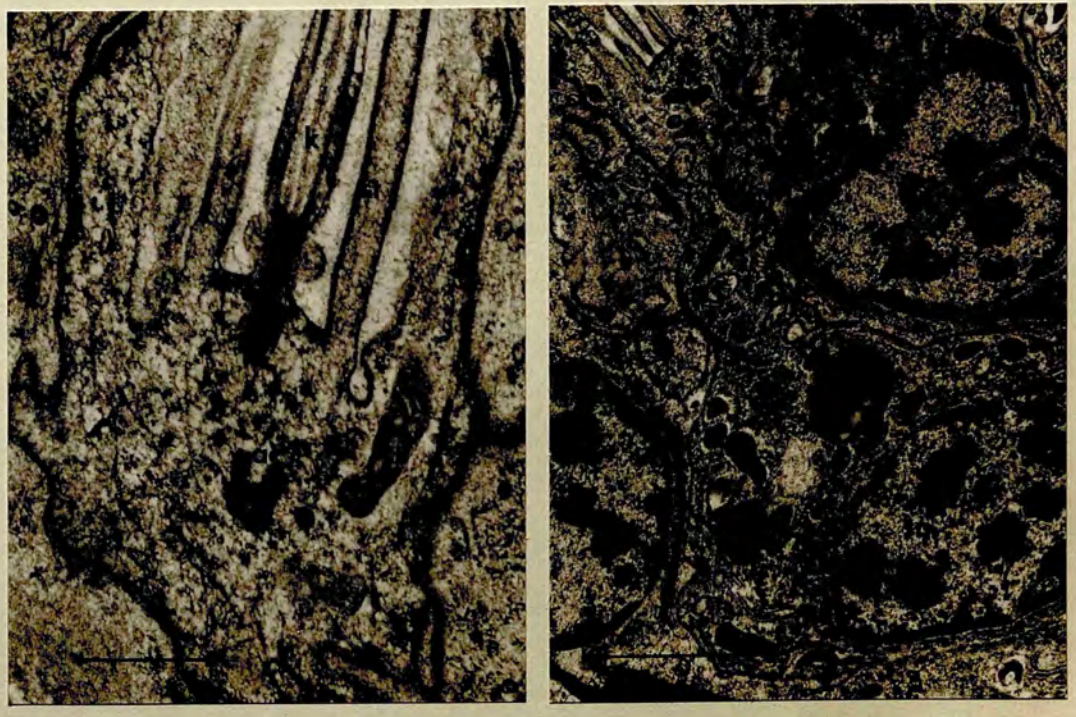
d) *Emarginula reticulata*, longitudinal section of perikaryon and dendrite of monociliary cell in pallial sense organ.
Bar = 2.0µm.

ac - accessory centriole
d - dendrite
en - epithelial cell nucleus
k - kinocilium
sn - sensory cell nucleus
st - stereocilium

PLATE 45



a b



c d

SECTION 3

Patellacea

3.1. *Patella vulgata*

Cephalic tentacles

The cephalic tentacles of *P. vulgata* have been divided into two distinct regions, (Choquet & Lemaire, 1969), a basal part, the 'bourrelet' (pad or cushion) which lies above the cerebral ganglion and contains the eye, and the remainder, the 'filament tentaculaire' which is extensile and constitutes the tentacle proper. It is the latter region that has been investigated in this study.

The innervation of the cephalic tentacles is shown in Fig. 12a after Choquet & Lemaire (1969). (Examination of material stained using the modified Liang's technique is in agreement with this). Unlike the preceding species the tentacular nerve of *P. vulgata* divides basally into two branches which pass toward the edge of the tentacle. More distally these branches divide further. The result is the formation of a ring of small nerves lying midway between the epithelium and the tentacle core (Fig 12b). The latter is composed only of connective tissue and transverse muscle fibres. Surrounding the ring of nerves is a ring of longitudinal muscle made up of many separate blocks.

Numerous radial muscle fibres arise from the central core and pass into the connective tissue underlying the epithelium, between the nerves and blocks of longitudinal muscle. Very fine nerves arise from the elements of the tentacular nerve and pass towards the epithelium.

Scanning electron microscopy of the tentacular filament shows the surface to be extensively ridged both longitudinally and transversely (Plate 46a). Much of this however, may be due to the contracted state of the tentacle (despite relaxation) or to shrinkage during preservation. It may be that the surface is almost smooth when the tentacle is fully extended. The exposed surfaces of the ridges bear numerous patches of short cilia (Plate 46b). These patches are large (usually $> 20\mu m$ in diameter) in comparison with the papillary tufts of the preceding species ($< 10\mu m$ in diameter) and frequently contain more than 200 cilia (plate 47a). Their size however, is variable. The cilia are approximately $5\mu m$ in length but extend only $2-3\mu m$ above the surrounding microvilli. Their tips are often overturned. Such patches were the only ciliary structures found on the tentacle surface. Gland cell openings were infrequently observed.

Thin resin sections show that the cilia are borne on groups of primary sensory cells with subepithelial cell bodies. The latter lie in connective tissue beneath muscle fibres which underly the epithelium (Fig. 13).

Each cell body contains an ovoid nucleus, well developed Golgi bodies, mitochondria, granules of varying size and electron-density and multivesicular bodies (Plate 47b). They may also be in contact with processes of glial cells containing large highly electron-dense granules. The cell bodies lie in groups and all the dendrites arising from one such group are thought to belong to the same ciliary patch.

For much of their length the dendrites contain only microtubules, mitochondria and occasional electron-dense granules (Plates 47c &48a). Within the epithelium however, having passed through the basal lamina they broaden and bear the cilia seen in the scanning electron microscope on their free margin (Plate 48b). Each cilium possesses 2-3 well developed striated rootlets (periodicity of banding approximately 50nm) which may extend as much as 5µm into the cell (Plate 48c). No distinct basal feet were observed. Basally the ciliary filaments show signs of the normal 9+2 sub-fibril arrangement (Plate 48d), but this is lost before they leave the layer of microvilli and the contents become homogeneously electron-dense. These were the only type of cilia observed within the patches. Transverse sections through the epithelium were not obtained and therefore the number of dendrites comprising the group and the number of cilia that each possessed is not known. The bundles of dendrites below the epithelium however, often contained more than fifteen elements.

Pallial tentacles

The pallial tentacles, although highly variable in length are all thought to be similar in structure. Typically they arise from a pit in the lower surface of the mantle edge and taper smoothly to a rounded apex (Plate 49a). Their surface is smooth, broken only by small tufts of cilia (Plate 49b) particularly near the tip. The cilia are approximately 5µm long, extending 2-3µm above the covering of

microvilli. Although these tufts are considerably smaller than the patches on the cephalic tentacles (often with as few as 10 cilia) the cells which bear them are very similar. They have subepithelial cell bodies which lie in groups in the connective tissue below the epithelium (Plate 50a). Dendrites containing mainly mitochondria and microtubules extend from these and pass through the basal lamina of the epithelium (Plate 50b). At their free margin they dilate and bear the cilia. These are identical to the cilia on the cephalic tentacles, possessing well developed striated rootlets from the basal body (no basal feet) (Plate 50c), and the filament having a homogeneous electron-dense content for much of its length (Plate 50d).

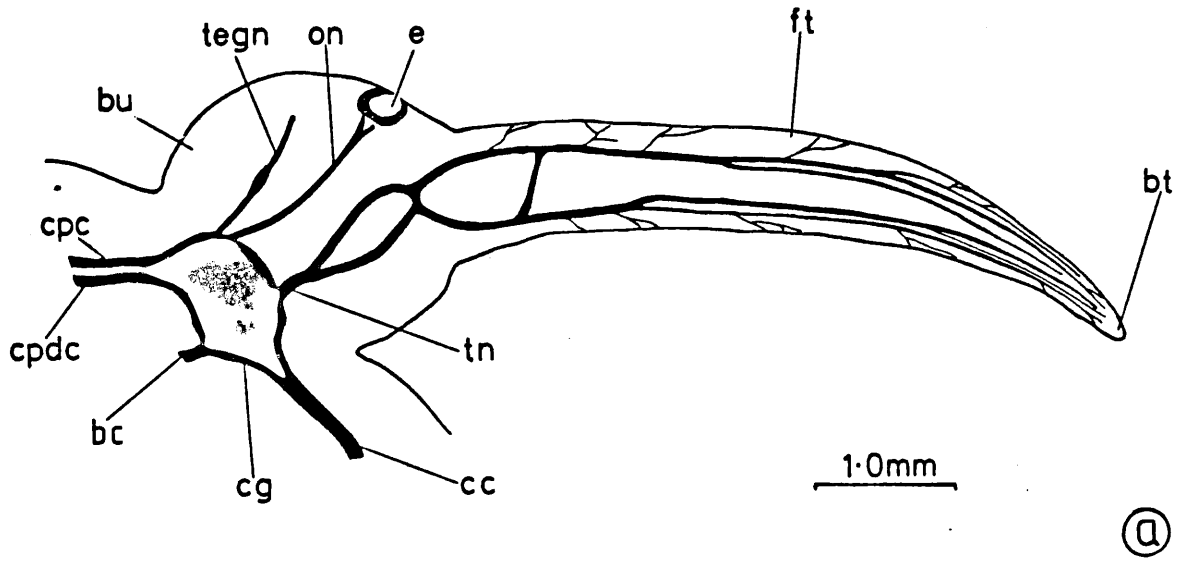
FIGURE 12

a) *Patella vulgata*, cerebral ganglion and innervation of the cephalic tentacles and eye. After Choquet and Lemaire (1969), confirmed by whole mount preparations stained using the modified Liang's technique.

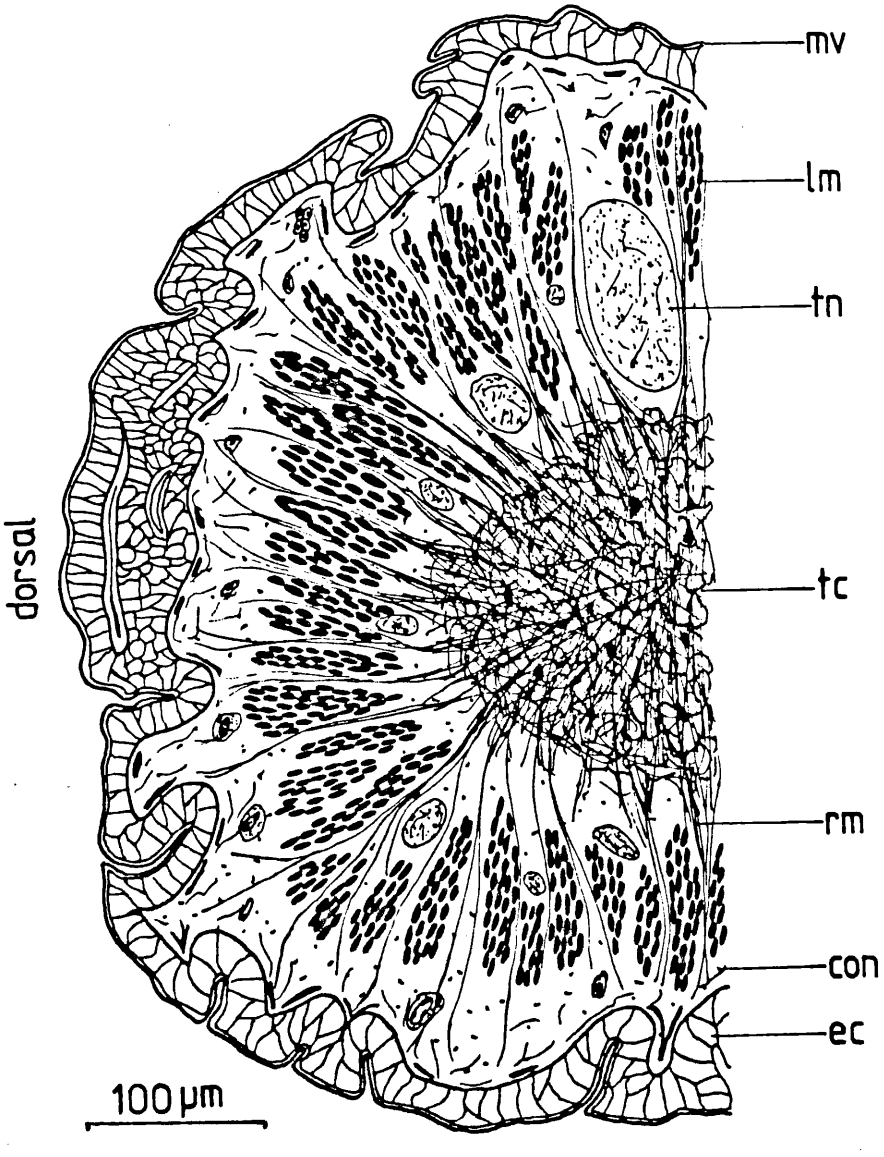
b) *Patella vulgata*, diagram of a transverse section through the filament of the cephalic tentacle. Only the dorsal half is shown, the ventral half is similar.

bc - buccal connective
bt - 'bouton terminale'
bu - 'bourrelet'
cc - cerebral commissure
cg - cerebral ganglion
con - connective tissue
cpc - cerebropleural connective
cpdc - cerebropedal connective
e - eye
ec - epithelial cell
ft - 'filament tentaculaire'
lm - longitudinal muscle
mv - microvilli
on - optic nerve
rm - radial muscle
tc - tentacle core of transverse muscle
tegn - tegumentary nerve
tn - tentacular nerve

FIGURE 12


tegn
on
e
ft
bu
bt
cpc
cpdc
tn
bc
cg
cc
1·0mm

(a)


mv
lm
tn
tc
rm
con
ec
dorsal
100 µm

(b)

FIGURE 13

Patella vulgata, diagram of a group of subepithelial sensory cell bodies in the cephalic tentacle. Dendrites pass through the basal lamina into the epithelium where they expand and bear cilia on their apical margin.

a - axon
bl - basal lamina
c - cilium
con - connective tissue
d - dendrite
ec - epithelial cell
gl - glial cell process
scb - sensory cell body

FIGURE 13

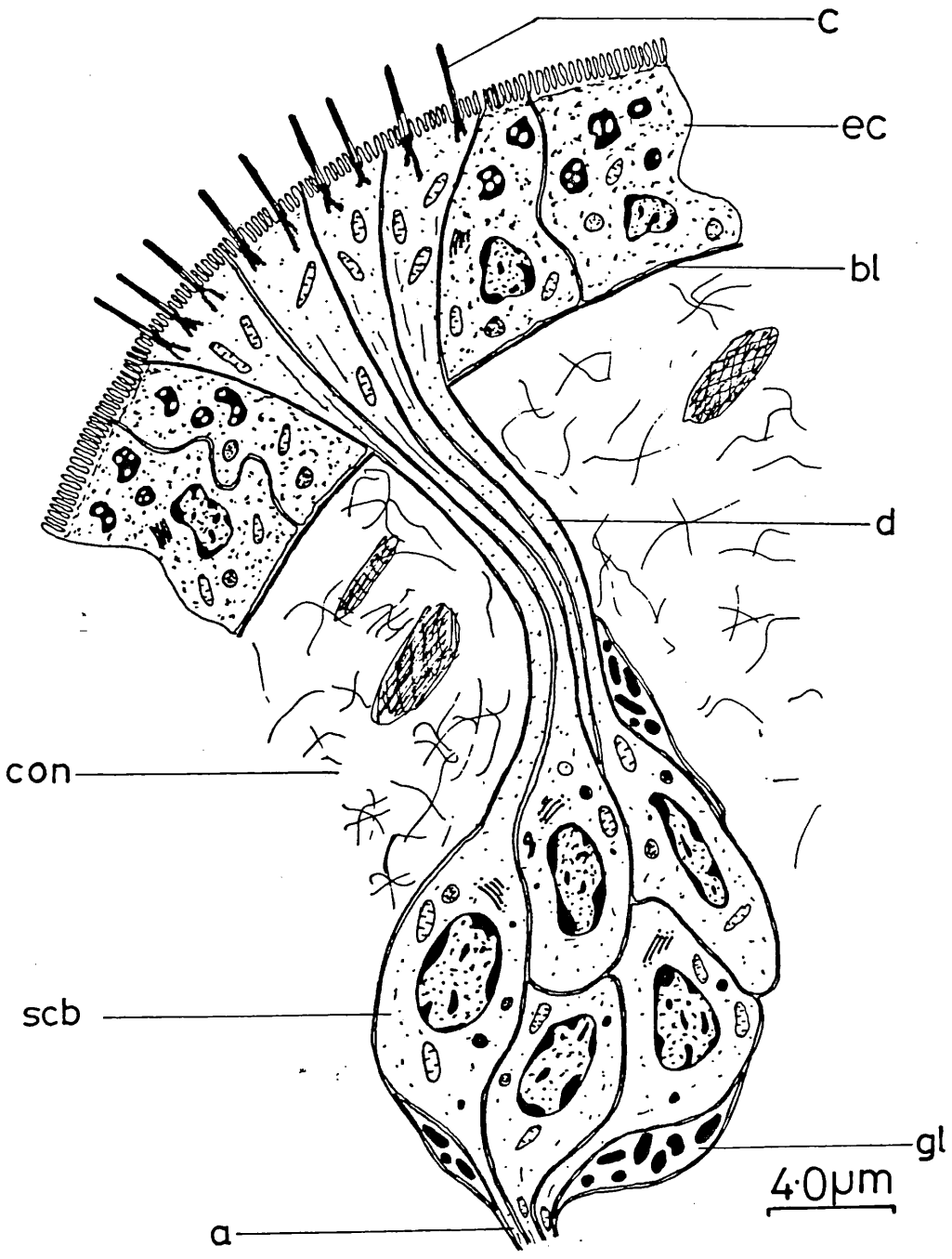


PLATE 46

a) *Patella vulgata*, antero-ventral view of left side of head showing division of the cephalic tentacle into the 'bourrelet' and 'filament tentaculaire'.
Bar = 1.0mm.

b) *Patella vulgata*, ridged surface of the tentacular filament with numerous ciliary patches.
Bar = 40µm.

bu - bourrelet
ft - filament tentaculaire
l - lips

PLATE 46

a

b

PLATE 47

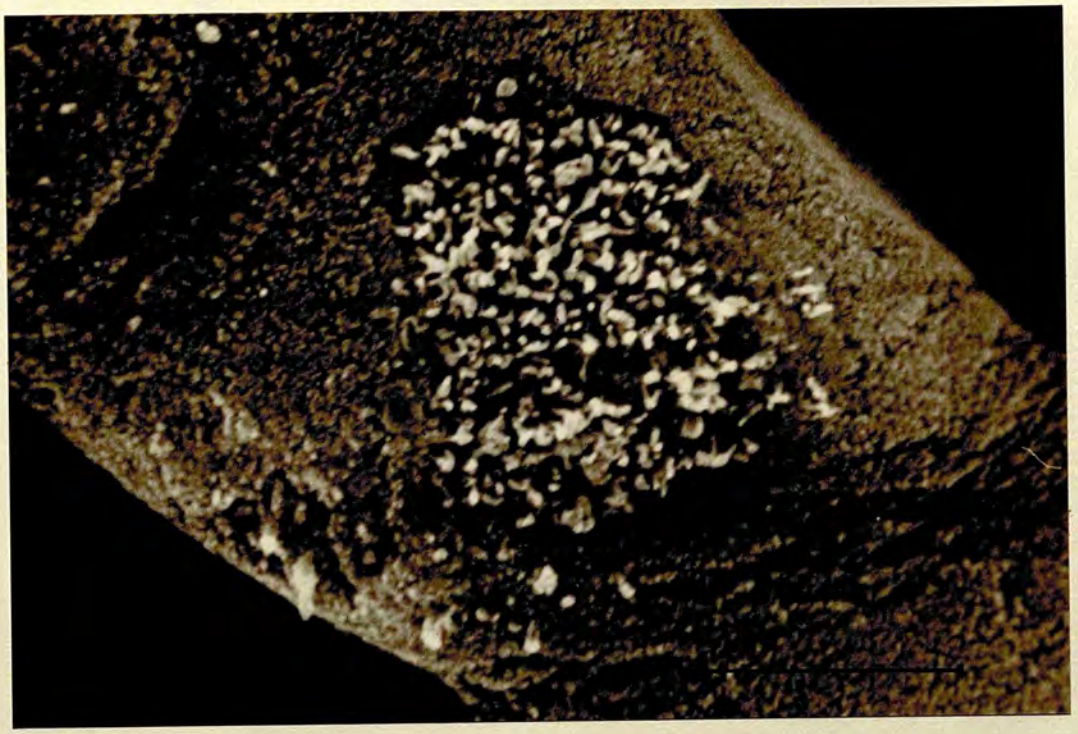
a) *Patella vulgata*, patch of cilia on filament of cephalic tentacle. Such patches usually contain many more cilia than the papillae of the preceeding species examined.
Bar = 10µm.

b) *Patella vulgata*, subepithelial cell bodies of sensory cells in filament of cephalic tentacle. Note mitochondria and Golgi bodies.
Bar = 1.0µm.

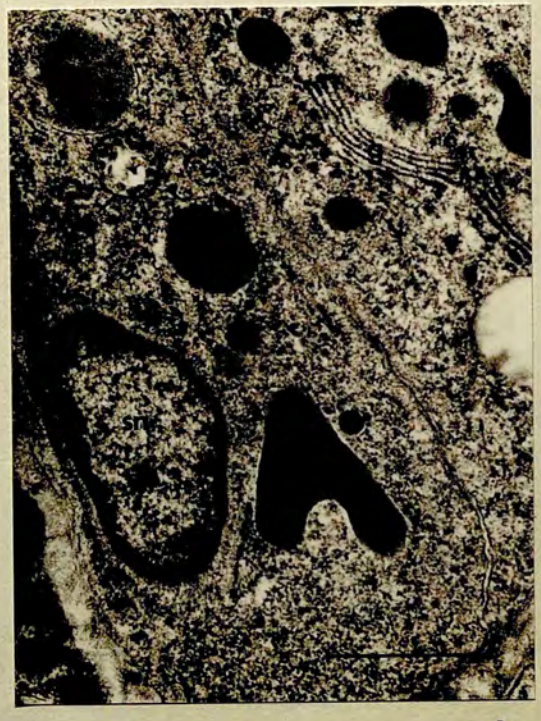
c) *Patella vulgata*, group of sensory cell dendrites penetrating the basal lamina in the cephalic tentacle filament. Note association with pigmented glial cell processes.
Bar = 2.0µm.

bl - basal lamina
d - dendrite
g - Golgi body
gl - glial cell process
sn - sensory cell nucleus

PLATE 47



a



b

c

PLATE 48

a) *Patella vulgata*, a group of sensory dendrites below the epithelium of the cephalic tentacle. Note mitochondria and microtubules.
Bar = 1.0µm.

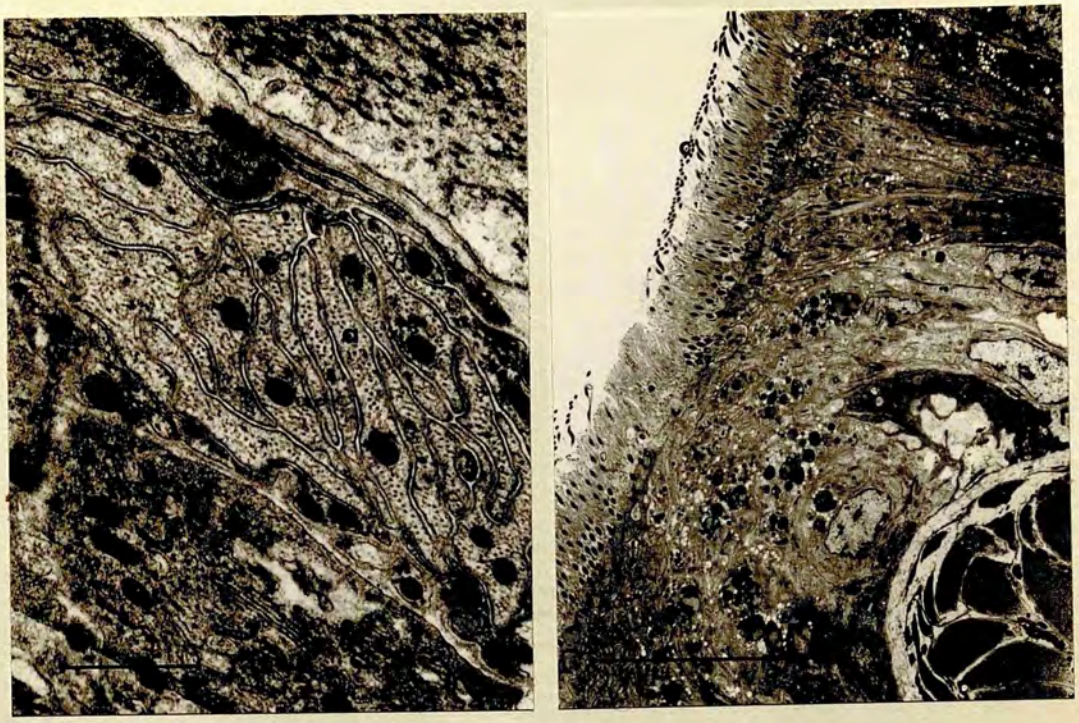
b) *Patella vulgata*, longitudinal section of ciliated patch on cephalic tentacle.
Bar = 10µm.

c) *Patella vulgata*, expanded apical portion of sensory dendrite in ciliated patch of cephalic tentacle. Note well developed striated rootlets attached to basal body.
Bar = 1.0µm.

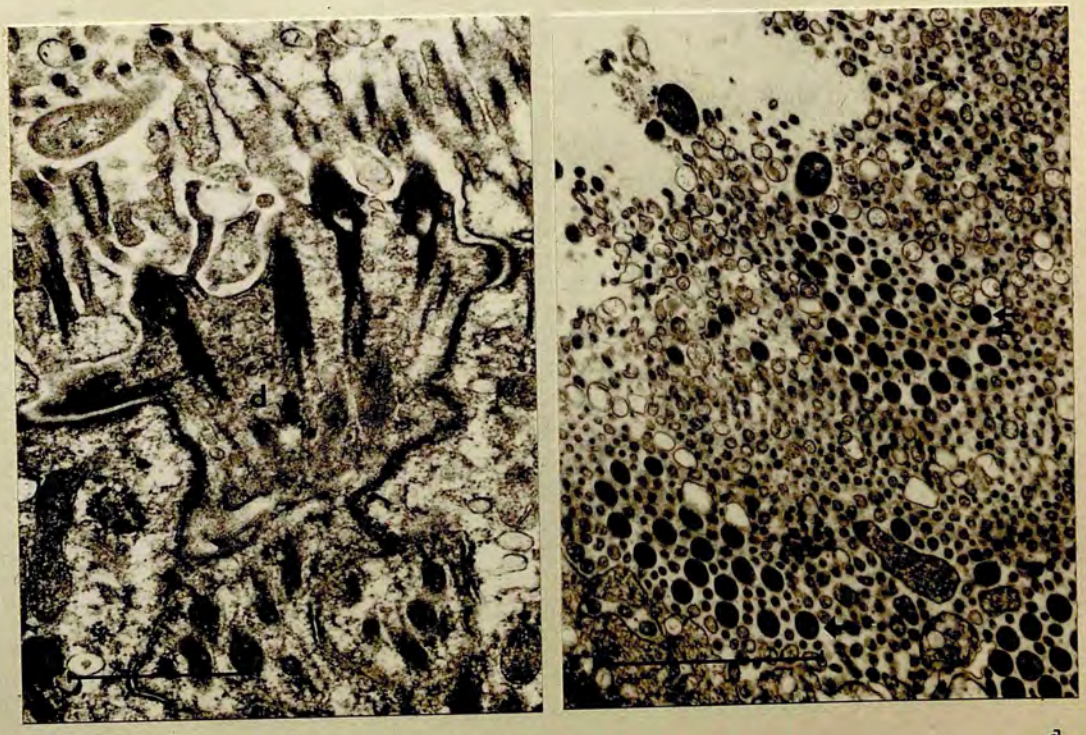
d) *Patella vulgata*, oblique section through brush border of ciliated patch on cephalic tentacle. Proximally the cilia show evidence of the normal 9 + 2 subfibril arrangement (one arrow), but distally they become homogeneously electron-dense (two arrows).
Bar = 2.0µm.

a - axon
c - cilia
d - dendrite
r - rootlet

PLATE 48



a b



c d

PLATE 49

a) *Patella vulgata*, ventral view of pallial tentacle.
Bar = 100µm.

b) *Patella vulgata*, small tuft of cilia on surface of pallial tentacle.
Bar = 2.0µm.

PLATE 49

a

b

PLATE 50

a) *Patella vulgata*, a group of subepithelial sensory cell bodies lying in the connective tissue of the pallial tentacle.
Bar = 2.0µm.

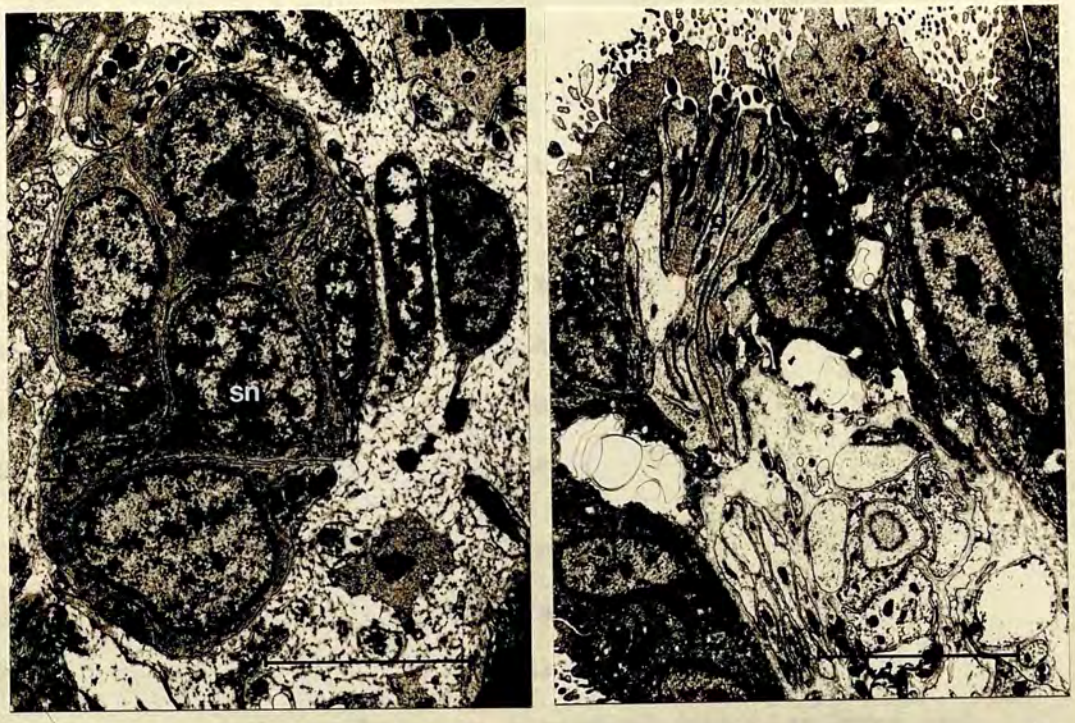
b) *Patella vulgata*, dendrites of sensory cells in pallial tentacles passing through the epithelium.
Bar = 4.0µm.

c) *Patella vulgata*, distal portion of dendrite in pallial tentacle sensory cell. Note long striated rootlets.
Bar = 1.0µm.

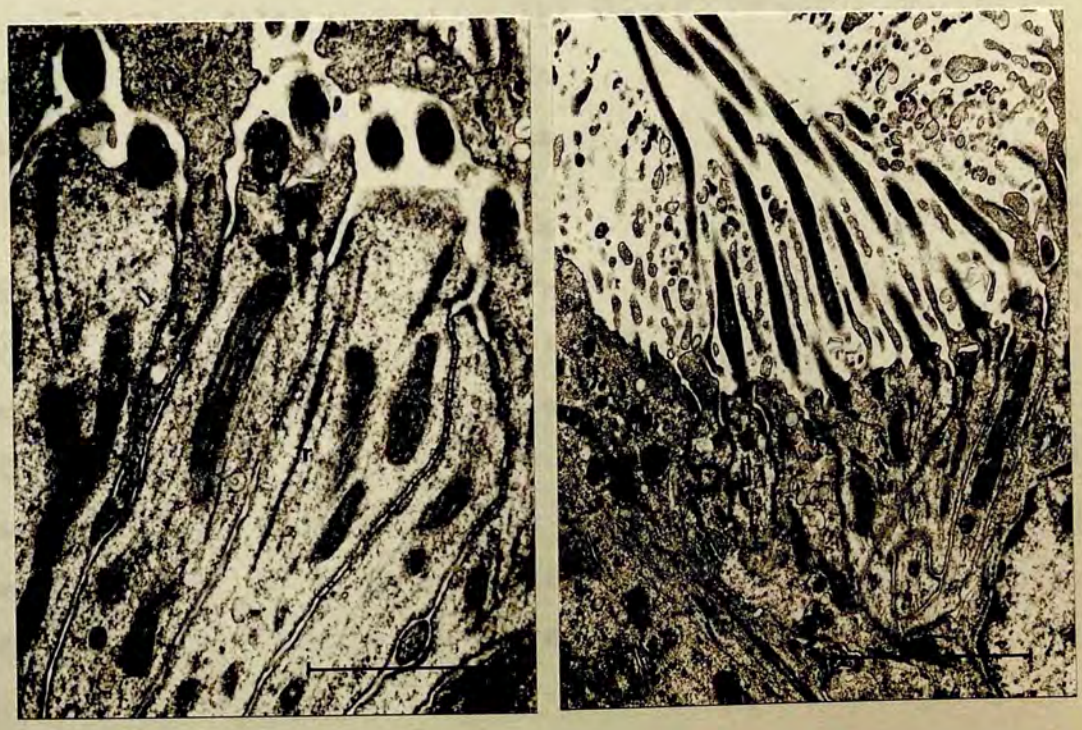
d) *Patella vulgata*, cilia borne on free surface of dendrite in pallial tentacle sensory cell. Distally the cilia have a homogeneous electron-dense content.
Bar = 2.0µm.

d - dendrite
r - rootlet
sn - sensory cell nucleus

PLATE 50



a b



c d

3.2. *Acmaea testudinalis*

Cephalic tentacles

Like the cephalic tentacles of *P. vulgata* those of *A. testudinalis* may be divided into two portions, a basal part equivalent to the bourrelet, which contains the eye and a distal part, the tentacular filament. This distinction however is not as clearly defined as it is in *P. vulgata* and the tentacle as a whole is more slender.

The internal structure of the filament and its innervation is essentially the same as that of *P. vulgata* with the tentacular nerve dividing basally to form a ring of smaller nerves lying between a central muscular core and the epithelium. The surface (in fixed material) is thrown into shallow folds throughout its length and bears numerous ciliary patches (Plate 51a). The latter are variable in size, but tend to be larger basally.

In nearly all respects the sensory cells which bear the cilia resemble those of *P. vulgata*. They are primary receptors with the cell bodies lying in groups amongst the subepithelial muscle fibres (Plate 51b). The cilia, though larger than those of *P. vulgata* (up to 8μm) have a homogeneous electron-dense content for most of their length and possess well developed striated rootlets extending into the cytoplasm from the basal body. No basal feet were observed (Plate 51c).

PLATE 51

a) *Acmaea testudinalis*, ridged surface of cephalic tentacle bearing patches of sensory cilia.
Bar = 10µm.

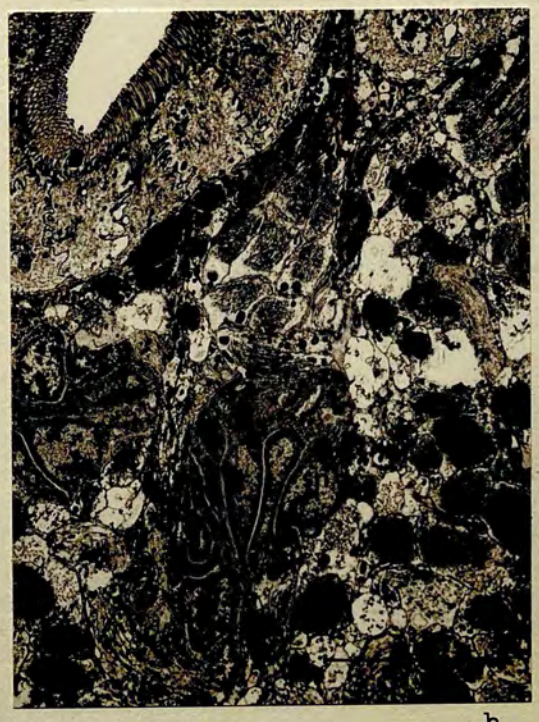
b) *Acmaea testudinalis*, group of sensory cell bodies lying amongst subepithelial muscle fibres in the cephalic tentacle.
Bar = 10µm.

c) *Acmaea testudinalis*, ciliated apical surface of sensory dendrite in cephalic tentacle. Note homogeneous electron-dense content of cilia and long striated rootlets.
Bar = 2.0µm.

c - cilia
r - rootlet
sn - sensory cell nucleus

PLATE 51

a



b

c

C H A P T E R 4

DISCUSSION

The species examined most extensively in this study, the trochid *Gibbula umbilicalis* and the fissurellid *Emarginula reticulata*, evidently possess a large number of sensory cells in their body wall. These are widespread, but are particularly abundant in those areas in intimate contact with the environment, namely the tentacles, mantle edge, sides of the foot, epipodial folds, lappets and neck lobes if present. They also occur in discrete patches of specialised sensory epithelium as in the epipodial and pallial sense organs. These observations are in agreement with those on other gastropods (see Introduction) and soft-bodied invertebrates in general (Bullock & Horridge, 1965).

Much similarity was noted between the two species even though they belong to different superfamilies. The structure and possible functions of the receptors of both will, as far as possible, be discussed together. Comparison is made with the patellacean species *Patella vulgata* and *Acmaea testudinalis*. The observations on *G. umbilicalis* correlate well with those of Crisp (1981) on other trochids. It is clear from her studies and those reported here on *G. cineraria* and *Monodonta lineata* that all members of the Trochidae and probably the Trochacea (Graham, 1965) are similar in respect of their epithelial sensory specialisations.

As stated in the Introduction numerous different types of epithelial sensory cells have been described in the literature. These are usually classified according to whether they are primary or secondary receptors and whether they possess cilia, stereocilia or microvilli or a combination

thereof. Further distinctions are frequently made amongst ciliated receptors, depending on the structure of the basal body. Some have been found in which the basal body has both a foot and rootlets, a foot and no rootlets, rootlets and no foot or neither foot nor rootlets. Similarly there may be variation in the length and number of cilia, whether or not the apical plasma membrane is infolded to form a pit, and the number and length of rootlets if present. Zylstra (1972) used such characters to distinguish six different types of receptor in the epidermis of *Lymnaea stagnalis* L.

Unciliated epithelial receptors have been found in molluscs (Zylstra, 1972; Wright, 1974b and Benedeczky, 1977), although they are rare. In vertebrates they are often associated with chemoreception (Zylstra, 1972; Barber, 1974; Wright 1974b), but additional roles including mechanoreception have been suggested in invertebrates (Navoni, 1973). Such receptors however, were not found in this study.

Whilst different types of receptor can be recognised by electron microscopy it is impossible to determine their function by this means. It has been stated several times in the literature (Crisp, 1971; Knapp & Mill, 1971; Wright, 1974b; Benedeczky, 1977; Owen & McCrae, 1979) that there is no reliable morphological means of determining the modality to which a particular receptor type is sensitive. This is to say that there are no structural features which are entirely characteristic of any of the three most important groups of

sensory cells, namely mechanoreceptors, chemoreceptors and photoreceptors (Laverack, 1968). Laverack however, does believe cilia to be a basic feature of mechanoreceptors.

Inferences relating to receptor function may nevertheless be drawn by comparison with receptors of more certain function in other species. Such receptors often occur in distinct sense organs whose function is well known, such as statocysts, vertebrate acoustico-lateralis systems and olfactory epithelia. Additional evidence may also be derived from receptor location and behavioural and physiological experiments on living animals or tissue. Such considerations must therefore be taken into account when suggesting possible functions for the receptor cells described here.

The cephalic tentacles of both *G. umbilicalis* and *E. reticulata* share the same basic structure of a central nerve and muscular core surrounded by radial muscle strands and groups of longitudinal muscle fibres. Differences do exist, but these are minor and mainly relate to the tentacular nerve which bifurcates basally (though still remaining central) in *E. reticulata*, and the papillae which are relatively larger and more abundant in *G. umbilicalis*. This same basic structural plan is also found in *Haliotis* (Crofts, 1929) and it is tempting to suggest that it is shared by all members of the Pleurotomariacea, Fissurellacea and Trochacea.

G. umbilicalis, unlike *E reticulata*, possesses epipodial tentacles which are essentially smaller versions

of the cephalic ones. Again minor differences in the relative size, abundance and distribution of the papillae were noted, but these are thought to be of little significance. Crofts (1929) has shown that this is also true in *Haliotis*. The epipodial tentacles of *E. reticulata* and Fissurellacea in general however, are structurally quite distinct from the cephalic (Boutan, 1885; Ziegenhorn & Thiem, 1926; Odhner, 1932; Fretter & Graham, 1962, 1976). These will be discussed later (see P.247) in relation to the epipodial sense organs.

Each papilla on those tentacles which bear them, contains a group of ciliated, bipolar primary sensory cells, the terminal, dendritic portions of which are arranged in a characteristic leek leaf fashion. Although in *G. umbilicalis* no morphological distinction could be made between these receptors, two distinct types were found in *E. reticulata*, one multiciliary, the other monociliary. It is quite possible that some papillae in *G. umbilicalis* possess, in addition to multiciliary cells, another, possibly monociliary, cell outside the main group.

As primary receptors these cells are typical of epithelial receptors of invertebrates. Storch and Welsch (1969) reported the presence of non-nervous (secondary) receptor cells in nine of the eleven prosobranch species they examined, but this observation is unusual and has since been questioned by other authors (Crisp, 1971; Zylstra, 1972).

The intra-epithelial cell body of the cells is frequently seen in other genera; *Octopus* (Graziadei, 1964), *Nautilus* (Barber & Wright, 1969a), *Vaginulus* (Renzoni, 1968),

Cardium (Barber & Wright, 1969b), *Lima* (Owen & McCrae, 1979), *Placopecten* (Moir, 1977b) and *Donax* (Moueza & Frenkiel, 1976). However, primary receptors with subepithelial cell bodies occur with almost equal regularity: *Lymnaea* and *Biomphalaria* (Zylstra, 1972), *Helix* (Hernádi & Benedeczky, 1978), *Phestilla* (Bonar, 1978), *Aplysia* (Emery & Audesirk, 1978), *Hinia* (Crisp, 1971) and *Acmaea* (Phillips, 1979).

In both *G. umbilicalis* and *E. reticulata* the cilia of the multiciliary receptors possessed only short rootlets extending from the basal body and no basal foot. The monociliary receptors will be discussed later (see P. 247), in relation to the epipodial and pallial sense organs.

Comparison between these cells, particularly the distal portion of the dendrite and its cilia reveals similarity with receptors in organs of the genera listed :-

Octopus	- statocyst	Barber (1966)
Helix	- ommatophore	Wondrak (1975)
Helix	- lip	Benedeczky (1977)
Helix	- foot epidermis	Hernádi & Benedeczky (1978)
Arion	- tentacle	Wright (1974b)
Aplysia	- tentacles & rhinophores	Emery & Audesirk (1978)
Hinia	- tentacle	Crisp (1971)
Pecten	- statocyst	Barber & Dilly (1969)
Placopecten	- tentacles (pallial)	Moir (1977b)
Lima	- tentacles (pallial)	Owen & McCrae (1979)
Lumbricus	- epidermis	Knapp & Mill (1971)

Some of the above, particularly those located in

the statocysts, are almost certainly mechanoreceptors, whilst others, notably those in the tentacle tip of pulmonates, are thought to be chemoreceptors (Wright, 1974b). Such a general comparison appears therefore to be of little use, but the high degree of similarity shown with the putative mechanoreceptors found by Moir (1977b) and Owen and McCrae (1979) merits further discussion. Both of these investigations relate to the highly extensile pallial tentacles of pectinid bivalves.

The pallial tentacles of *Placopecten magellanicus* (Gmelin) (Moir, 1977b), although not similar in internal organisation, show an immediate similarity in external appearance. They are densely covered apically by numerous papillae radiating from the surface, each of which bears a tuft of non-motile cilia projecting from its tip. Similar tufts were also reported from the basal region, but here they were not raised on papillae. The cilia are borne on groups of primary sense cells (Type II cells) whose cell bodies lie within the epithelium of the papilla. Each cell bears several cilia, but unlike those examined here, the basal bodies have a foot and long rootlets.

In *Lima hians* (Gmelin) (Owen & McCrae, 1979) the pallial tentacles, although they do not bear papillae, contain three types of sensory cell, one of which shows much similarity to the multiciliated cells described here. Typically 4-6 of these cells are grouped together in a Type B complex and are seen externally as a tuft of cilia on the surface. Again the cells are primary receptors with intra-

epithelial cell bodies, but the most striking similarity is the leek leaf arrangement of the dendrite tips. As with *P. magellanicus* however, the basal bodies of the cilia bear well developed rootlets extending deep into the cytoplasm and an obvious basal foot. The latter, together with the two central axonemal subfibrils are orientated in a characteristic fashion, radiating from the centre of the cell cluster. The multiciliary receptors of both *G. umbilicalis* and *E. reticulata* possess only short rootlets, no basal foot and the central axonemal subfibrils appear to be randomly orientated. Also worthy of note is the fact that in approximately one in ten of these type B complexes in *L. hians* a single extra receptor was found. This is structurally very similar to the epipodial sense organ receptors and the extra receptor occasionally seen in the tentacles of *E. reticulata*.

The significance of the orientation of the basal feet and central axonemal subfibrils was noted by Gibbons (1961). He stated that in motile cilia the direction of beat lies in the same plane as the basal foot and at right angles to the central subfibrils. This observation has since been adapted by Barber (1974) who suggested that in non-motile sensory cilia, displacement of the cilia in the plane of beat (if they had been motile) would cause excitation of the cell. Owen and McCrae (1979) were therefore of the opinion that in *L. hians*, displacement of a tuft in any direction would cause at least some of the cilia to bend in the excitatory plane.

This argument however, cannot strictly be applied

to the ciliary tufts of *G. umbilicalis* and *E. reticulata* as firstly there are no basal feet and secondly the central axonemal subfibrils show no particular orientation. Crisp (1981) stated that there was little need for accurate directional sensitivity in such receptors, as the nervous system of these animals was not likely to be capable of computing the relative positions of almost infinitely flexible parts. This may also apply to *P. magellanicus* where no orientation was noted either. The need for such radial orientation in *L. hians* is not clear, but may relate to the relatively low number of cilia in each tuft or to mucous cells often associated with them. *P. magellanicus, G. umbilicalis* and *E.reticulata* all appear to possess a greater number of cilia in each tuft and even with their random orientation, displacement of the tuft in any direction is still likely to excite some of the cells.

The similarity shown with the receptors of *P. magellanicus* and *L. hians* thus suggests a mechano-sensitive function for these multiciliated cells. This is in accordance with the view of Fretter and Graham (1962) who stated that there can be little doubt that the cephalic tentacles of prosobranchs in general are primarily sensitive to tactile stimulation. The almost identical structure of the epipodial tentacles of *G. umbilicalis* indicates that this is also true in their case.

Observations on the behaviour of living animals and the extreme mobility of the tentacles, particularly in trochid, suggests that their main function is to provide

information relating to the topography of the animal's immediate surroundings. Such mobility would be less necessary for chemoreception. This is especially noticeable when animals are upturned. In both species the cephalic tentacles, and the epipodial tentacles of *G. umbilicalis* appear to search for a firm object which the animal may use to right itself. Burke (1964) was also of the opinion that the chief function of both cephalic and epipodial tentacles of the trochid *Tegula funebralis* (Adams) was that of mechanoreception. Putative mechanoreceptors have been noted in the tentacles of the prosobranch *Hinia reticulata* (Crisp, 1971), and Charles (1966) observed a high degree of tactile sensitivity in the tentacles and where present, rhinophores of many gastropods.

On the above evidence it seems probable that these multiciliary tentacular receptors respond to mechanical stimulation. It is therefore suggested that they be considered mechanoreceptors, which collectively are multidirectionally sensitive. A mechanoreceptive function was also the suggestion of Crisp (1981).

The presence of a second type of receptor, the monociliary receptor, in the tentacles of *E. reticulata* and the possibility of such a receptor in *G. umbilicalis*, indicates however, that mechanoreception may not be the only sensitivity of these structures. It is interesting to note that Burke (1964) attributed a certain degree of chemosensitivity to the tentacles of *T. funebralis* and Feder (1963, 1967) has shown that several trochid species (including *Gibbula*) respond characteristically when the tentacles

(cephalic and epipodial) are touched by predatory starfish. Crisp (1971) also suggested this possibility in *H. reticulata*. It will be discussed further in relation to the epipodial and pallial sense organs.

Scanning electron microscopy of other regions of the external epithelium in both species indicates the presence of sensory cells which superficially resemble the multiciliary tentacular receptors, in many areas. These may or may not be raised above the surface on papillae and are usually much less abundant than on the tentacles. The internal structure of these cells was only examined on the rim of the epipodial sense organs of *G. umbilicalis*. Although there may be considerable variation in the number of cilia per group, the similarity with the tentacular receptors is maintained internally. The variation in ciliary number may simply reflect the number of cells present in each group - the more cilia - the more cells.

It is therefore suggested that these cells are also mechanoreceptive and that they provide the general body surface with a degree of tactile sensitivity. As in the cephalic tentacles, a proportion of these tufts in *E. reticulata* possessed an additional longer cilium, particularly those on the mantle edge.

The specimens of *Umbonium vestiarium* examined indicate that the sensory specialisations of the external epithelium of this species are essentially the same as those of the other trochids. It is evident however, that the

neck lobes, particularly the left one, are considerably adapted to suit its mode of life. Both are hypertrophied and form comparatively well developed inhalant and exhalant siphons relating to the mantle cavity. The digits of the left lobe have become greatly branched and extend into the inhalant siphon, but unfortunately the poor state of preservation of the surface cilia rendered the specimens unsuitable for more detailed examination. Nevertheless, evidence of cilia (probably sensory cilia) was found in many areas including the left neck lobe. The last structure may be expected, together with the osphradium, to provide information on the inhalant water current and the nature and amount of suspended material reaching the gills. It may therefore possess both mechano- and chemoreceptors. Suitably preserved specimens will merit further investigation.

The epipodial tentacles of *E. reticulata*, not being papillate, are clearly distinct from those of the trochids. They are of two types and the epipodial sense organ is actually part of their structure. The two types are easily distinguishable at low magnifications by their shape, extensibility and relative position of the sense organ. It seems probable that the major function of both types is to raise the sense organ above the body surface. In the case of the short tentacles, into the pallial groove, and the long tentacles, under the shell into the external environment. The cilia present on the short tentacles are motile and possess basal feet in line with the direction of beat in accordance with the observations of Gibbons (1961).

This direction of beat is apical and may aid in the removal of particulate material which accumulates on the body surface (Yonge, 1947). Their absence from the long tentacles indicates that they are unlikely to be of importance in the functioning of the sense organ. The presence of two types of epipodial tentacle has been observed previously in fissurellids, (Forbes & Hanley, 1849-53; Jeffreys, 1962-69; Yonge, 1947) but the differential extensibility and positioning of the sense organ was not noted.

The other tentacle types found in *E. reticulata*, namely the right postoptic and metapodial tentacles, were not examined ultrastructurally. It has been suggested (Dall, 1889; Odhner, 1932) that the former may function as a penis and Bourne (1910) noted that it was larger and more abundantly supplied with gland cells in males of *Incissura (Scissurella) lytteltonensis*. Yonge (1947) however, pointed out that in *E. reticulata* and other similar species, not only is copulation impossible, but sperm would have to pass between the ctenidial filaments against the respiratory current in order to reach the tentacle. Both tentacles possess motile cilia on their dorsal surface and are highly glandular. They may thus assist in the removal of particulate material. In *G. umbilicalis* the structure of the right postoptic tentacle is that of a minute cephalic tentacle. It possesses no motile cilia and is not obviously glandular.

Observations on the mantle edge bordering the shell slit of *E. reticulata* indicate that this is a highly specialised region. Whilst the siphon is primarily related to directing the exhalant respiratory current, the areas immediately anterior and posterior to it possess the pallial sense organs. These together with the epipodial sense organs of this species and *G. umbilicalis* appear identical in structure. They represent a discrete type of sense organ, easily recognisable at the ultrastructural level and seem to be present in many rhipidoglossans (see Introduction). The pallial sense organs, as such, have not been described previously, but similar structures also occur in *Puncturella noachina* (L.) (personal observation) and so called 'pallial tentacles' have been noted in many of the Fissurellacea (Ziegenhorn & Thiem, 1926; Odhner, 1932; Yonge, 1947). These structures however, should not be confused with the pallial tentacles associated with the shell slit/holes of *Scissurella* and *Haliotis* respectively (Crofts, 1929; Fretter & Graham, 1962, 1976) or furthermore with those on the mantle edge of the Patellacea (Fretter & Graham, 1976). These are clearly quite distinct structures.

Typically the pallial and epipodial sense organs are composed of a depressed central portion (the sensory cupule) lined by a deep, pseudostratified epithelium and surrounded by a raised rim containing ciliary tufts and gland cells. These ciliary tufts have already been discussed. The sensory cells found within the cupule are all monociliary and are identical with cells found in association with some groups of multiciliary cells occurring elsewhere in

E. reticulata. They are characterised by the complex organisation of microvilli and stereocilia occurring at the dendrite tip. Crisp (1981) did not comment on the outer ring of thick microvilli in *G. cineraria* which are evident, though not clear, in her electron-micrographs. The method of fixation may significantly affect their appearance. She noted that the accessory centriole was always orientated at 90° to the basal body of the kinocilium, but other orientations were found in the present study. Their significance is not known.

The stereocilia of these cells are somewhat intermediate between ordinary microvilli and the typical stereocilia of the vertebrate acoustico-lateralis system. Indeed Crisp (1981) referred to them as specialised microvilli. The term stereocilia was used here to avoid confusion with the thick microvilli. It should be noted however, that although they contain electron-dense material associated with their membrane and their bases, they do not possess axial filaments or distinct rootlets extending into the cytoplasm - features present in typical stereocilia (Barber, 1974, Welsch & Storch, 1976).

The general appearance of these monociliary cells is similar to that of the collar cells (cyrtocytes or choanocytes) of sponges (Porifera) (for literature see Nørrevang & Wingstrand, 1970; Dorsett, 1976). Such cells were initially thought to be characteristic of the Porifera, but recent studies indicate that choanocyte-like cells are present in most of the large metazoan groups and that they may represent a fundamental cell

type (Nørrevang & Wingstrand, 1970; Welsch & Storch, 1976). They may be defined as cells possessing a single long flagellum (kinocilium) surrounded by a collar of microvilli (sterocilia) arranged in a perfect circle. Between individual microvilli there is a mucous substance (glycocalyx) such that a mucous membrane is formed between and inside the microvilli (Nørrevang & Wingstrand, 1970). Many also possess electron dense, filamentous material linking the ciliary basal body to the stereocilia/microvilli and accessory centriole when present.

The function of this cell type, however, is not uniform. In sponges and choanoflagellates they are active in food collection by endocytosis, but in many groups they are modified as flame cells, solenocytes or sensory receptors.

Cells with this structure and a sensory function have been reported in coelenterates (Tardent & Schmid, 1972; Lyons, 1973; Peteya, 1975; Westfall & Sims, 1978; Hundgen & Biela, 1982), platyhelminths (MacRae, 1967), priapulids and sipunculids (Moritz & Storch, 1970, 1971), annelids (Knapp & Mill, 1971), molluscs (Moir, 1977a; Owen & McCrae, 1979), echinoderms (Laverack, 1968 ; Nørrevang & Wingstrand, 1970), chaetognaths (Bone & Pulsford, 1978), urochordates (Bone & Ryan, 1978) and cephalochordates (Bone & Best, 1978).

A feature peculiar to the cells reported in the prsent study is the additional ring of thickened microvilli surrounding the stereocilia. Such an arrangement has not been observed in other cells. Nørrevang & Wingstrand (1970) however, noted that in echinoderms the stereocilia may be modified into

longitudinal ridges or radial lamellae, but stated that this is simply a modification of an obvious basic plan. Such is likely to be the case here.

In almost all the reports above, the cells were thought to be mechanoreceptors. MacRae (1967), Knapp & Mill (1971) and Moir (1977a) suggested a chemoreceptive function, but with little certainty. Barber (1974) also concluded, by comparison with other mechanoreceptors, that these cells were mechanosensitive. Bone & Ryan (1978) compared the cells of tunicates with cells in the vertebrate acoustico-lateralis system.

As stated earlier, Owen & McCrae (1979) found that such receptors were associated with approximately one in ten of their type B complexes in the pallial tentacles of *L. hians*. They suggested a mechanoreceptive function based on the complex arrangement of filaments linked with the basal body of the kinocilium. The arrangement observed here is not as complex as that of *L. hians*, but is nevertheless clearly specialised.

On the above evidence therefore it would seem probable that the monociliary receptors of the species examined here are mechanoreceptors. The obvious structural differences between them and the multiciliary receptors however, suggest that they may be sensitive to a different type of mechanical stimulation, possibly vibration (Owen & McCrae, 1979), or particulate material in suspension (water turbidity) as distinct from simple touch reception. The pallial sense organs are well situated to respond to vibration and may be important in siphon withdrawal. The situation of the

epipodial sense organs however, is not so clear. Neither vibration nor turbidity sensitivity is entirely consistent with their location. Little of the inhalant water current entering the mantle cavity of either species can pass by them (Yonge, 1947). Furthermore whilst the trochids (in general) are intolerant of silty conditions (Fretter & Graham, 1977), *E. reticulata*, which possess many more of these organs, is often found in conditions of high silt (Fretter & Graham, 1976). A role in the assessment of water turbidity would suggest the reverse (unless the suspended material is used as a food source which is not so in *E. reticulata*).

On these considerations therefore the possibility of other sensitivities must be examined. These centre on chemoreception and indeed the structure of these organs as a whole (a deep, pseudostratified epithelium) is not unlike that of the osphradium (Bailey & Benjamin, 1968; Welsch & Storch, 1969; Benjamin & Peat, 1971; Crisp, 1973) and chemoreceptive organs in general (Welsch & Storch, 1976). In addition the presence of numerous mucous cells associated with them is supportive of chemoreception. It is widely accepted that mucus is important in the adsorption of odorant molecules (Barber, 1974; Laverack, 1974).

On the basis of location the epipodial sense organs are ideally situated to function as osmoreceptors (monitoring the osmotic pressure of the external medium). In *G. umbilicalis*, when the animal is exposed at low tide, a small volume of water is held around it, between the sides of the foot, the substratum and the shell. The epipodial sense organs are

surrounded in this water and thus well placed to monitor changes in its salinity that may occur during wet weather. Arnold (1972) has shown that this species responds to changes in salinity, and personal observations on the shore indicate that during wet weather animals are less abundant on the upper surface of rocks. Again however, this argument is not so readily applicable to *E. reticulata*, for whilst water is held in the pallial groove during exposure, this species normally lives sublittorally to 265m. (Fretter & Graham, 1976). It is therefore unlikely ever to encounter reduced salinity. (It is not found in estuarine environments). Occasional specimens may occur up to LWST (10% emersion), but even then they are generally found on the undersides of boulders and overhangs. The abundance of these organs in *E. reticulata* (and other species which are rarely, if ever, exposed e.g. *Diodora* and *Haliotis*) indicates that they are unlikely to function as osmoreceptors.

Two other possible functions, again associated with chemoreception relate firstly to the nature of the substratum as a potential food source (largely detritus in the case of *G. umbilicalis* and detritus and sponges, in *E. reticulata*, (Fretter & Graham, 1976, 1977)) and secondly to the avoidance of potential predators.

Little or no information is available on the first of these possibilities, particularly concerning detritivores. Carnivorous species, e.g. *Hinia*, have a quite distinct feeding response and clearly recognize the presence of food (Kohn, 1961; Crisp, 1971). Preliminary experiments with *G. umbilicalis*

were unsuccessful as, when active, animals appeared to rasp the surface continually. No distinct feeding response was ever observed. With such species this aspect may be difficult to investigate.

More information is available concerning escape responses towards predators. Such responses are common among molluscs in general (Feder, 1967; Mackie & Grant, 1974). Within the Archaeogastropoda they have been recorded in the following genera:- *Haliotis, Emarginula, Fissurella, Diodora, Gibbula, Monodonta, Cantharidus, Calliostoma, Tegula, Melagraphia, Zedilonia, Acmaea, Patella* and *Patina* (Bullock, 1953; Clark, 1958; Feder, 1963, 1967; Burke, 1964; Margolin, 1964a, b; Yarnall, 1964; Phillips & Chiarappa, 1980). The predators involved are usually asteroid echinoderms or other gastropod molluscs, but Phillips and Chiarappa (1980) showed that *Acmaea scutum* responds to predatory flatworms.

In some cases the responses are initiated on contact with the predator, but instances of distance reception and responses to extracts have been recorded (Bullock, 1953; Feder, 1963; Burke, 1964; Feder & Arvidsson, 1967). The site of such distance reception is not known, although Szal (1971) noted the presence of numerous organs (bursicles) on the ctenidia of *Tegula funebralis*, which he suggested were sensitive to predatory starfish.

The responses to contact with starfish may be mediated through much of the body surface, but particularly the tentacles, sides of the foot and mantle edge. The

occurrence of monociliary receptors on the cephalic tentacles and mantle edge of *E. reticulata* correlates with this and as already stated such cells may also occur in *G. umbilicalis*. It is tempting to suggest that the cells in the epipodial sense organs being less accessible (except those on the long tentacles of *E. reticulata*) may be responsible for distance reception. Such a capability however, has not been clearly shown although personal observations indicate that it is probable.

It is evident from the above arguments that no one modality can be assigned to this monociliary receptor with any degree of certainty. Whilst ultrastructural features and comparison with receptors in other species indicate mechano-reception, their location, association with mucous cells and behavioural aspects are suggestive of chemoreception. The situation is further complicated by the fact that potential functions in one species or location may not be so appropriate in the other species or to another location. The most promising way to resolve the problem may be by the use of electro-physiological experiments. The epipodial sense organs having a high concentration of a single sensory cell type in their cupule may lend themselves particularly well to such investigations.

It is nevertheless, clear from the abundance of this type of sense organ that they are of considerable importance to the animals. This is particularly true of limpet-like forms - *E. reticulata* may have over thirty such

organs in total and large specimens of *Haliotis* a great many more (Crofts, 1929). Their significance to the conispiral forms like the trochids may be less as some, e.g. *Calliostoma* have none (Fretter & Graham, 1977). In this last respect it is interesting to note that Feder (1967) found that the response of *Calliostoma zizyphinium* (L.) to starfish, though still present, was much less vigorous than that of *Gibbula* spp. It may be that these organs are of particular significance to sublittoral species with a limpet-like mode of life. An explanation of this however, is not readily apparent.

It is nonetheless evident that not all the possible functions suggested above can be carried out by this one cell type. It is probable that some relate to other sensory cell types, if indeed the animals are sensitive to them at all. Some such cells were observed, but only on rare occasions, others may remain to be described, particularly in areas not examined here. The multiciliated cells lying beneath the epipodial sense organ of *G. umbilicalis* may be an example, but because of their sparse distribution little can be said of their structure, particularly whether or not they communicate with the exterior. Cells of similar structure have been described in *Octopus* (Emery, 1976). They were thought to be olfactory in function.

The investigations involving the patellacean species *P. vulgata* and *A. testudinalis* were not extensive and as has been stated were primarily undertaken for comparative purposes. Whilst there is much similarity

between these two species there are notable differences between them and the rhipidoglossan forms. As stated in the Introduction the Patellacea possess no epipodial tentacles or epipodial sense organs. The epipodium as a whole is replaced by a series of pallial tentacles on the mantle edge, but as yet there appears to be no discrete counterpart for the epipodial sense organs. In this last respect however it would be interesting to examine the ultrastructure of the lateral sensory strip.

The internal organisation of the cephalic tentacles of both species is quite distinct from that of the rhipidoglossan species. The tentacular nerve branches repeatedly near the base and forms a ring of separate nerves around a muscular core. There are no papillae and when extended the surface is probably smooth. The sensory specializations on these tentacles take the form of much larger patches of cilia not borne on any form of projection. The receptor cells bearing the cilia are primary sensory cells, but their cell bodies are subepithelial. The ciliary basal bodies bear 2-3 well-developed, striated rootlets extending deep into the dendrite. The ciliary filaments possess an indication of subfibrils basally, but for most of their length they contain only a homogeneous electron-dense material. These sensory cells are therefore clearly distinct from all those discussed previously.

The pallial tentacles of *P. vulgata* contain sensory cells similar to those on the cephalic tentacles although they are usually found in smaller groups. The

pallial tentacles of *A. testudinalis* were not examined, but as stated in the Introduction those of a closely related species *Acmaea scutum* have been studied by Phillips (1979). Unlike the pallial tentacles of *P. vulgata* the ciliated dendrites in those of *A. scutum* are concentrated at the tip, forming a large tuft. In addition the dendrites are long and run the length of the tentacle to a cluster of cell bodies near the base. In all other respects however, the cells are similar to those of *P. vulgata* and possess cilia with an electron-dense content.

This type of cilium seems to be rare in molluscs, but Phillips (1979) showed that its appearance was unlikely to be an artefact of fixation and drew comparison with the eulaterofrontal cilia on the gill of *Mytilus edulis* L. (Owen, 1974). He suggested that the electron-dense material contributed to the rigidity of the structure. Such considerations indicate a mechanoreceptive role for these cells, although Phillips (1979) did not suggest any definite function.

Phillips (1979) noted a second type of sensory cell in these tentacles which possesses cilia with the normal 9 + 2 complement of subfibrils. This cell type accounted for less than ten per cent of the total and might be revealed in *P. vulgata* and *A. testudinalis* on more thorough investigation.

Although tactile sensitivity seems likely for the more numerous cell type this is by no means certain. Phillips (1975) has shown, using electrophysiological techniques, that the pallial margin as a whole is capable of distance

and contact chemoreception, mechanoreception and photoreception. The presence of only two types of sensory cell therefore suggests that one or both has multiple sensory capability. Alternatively there may be other cell types which were not observed. Similar results were reported by Crisp (1971, 1972, 1976) using *Hinia*.

The overall impression gained by the examination of *P. vulgata* and *A. testudinalis* is that they, and probably patellaceans in general, differ markedly from the rhipidoglossan species studied, in terms of their epithelial sensory specializations. This has interesting phylogenetic implications in the Archaeogastropoda.

In terms of the structure of the cephalic tentacles and the presence or absence of epipodial/pallial sense organs there appears to be a clear distinction between the Pleurotomariacea, Fissurellacea and Trochacea on the one hand and the Patellacea on the other. The first three groups contain species which possess epipodial sense organs and the general morphology of their cephalic tentacles is based on the same plan. The structure of the cephalic tentacles of the Patellacea however, is distinct and they do not possess epipodial sense organs. It is tempting to suggest that the latter organs are present in all rhipodoglossan species (with the probable exception of the Neritacea) and that they may be characteristic of the above groups. Their loss in some genera, e.g. *Calliostoma*, may have occurred secondarily. Szal (1971) also indicated that chemosensitive

ctenidial bursicles may be found in all zeugobranchs and trochaceans but not in patellaceans, including those with ctenidia.

There is further distinction within the rhipidoglossan groups between the Pleurotomariacea and Trochacea, and the Fissurellacea. The first two possess epipodial tentacles which differ from the cephalic ones in size only, while those of the latter group are structurally quite different. It may also be found that pallial sense organs occur only in fissurellaceans, but as yet the pleurotomariaceans have not been examined in this respect. The link between the Pleurotomariacea and Trochacea is further supported by aspects of their internal organisation. Both possess a spiral caecum in the stomach and a left kidney in the form of a papillary sac (Fretter & Graham, 1962; Andrews, 1981). Whether the left kidney of the pleurotomariaceans also contains a nephridial gland remains to be investigated. It must however, be emphasised that with the exception of the Haliotidae, the Pleurotomariacea have not been studied extensively, particularly with regard to their sense organs. Comments concerning the group may well be biased toward the haliotids. Nonetheless it is evident that the epipodium of the Pleurotomariidae and Scissurellidae, especially the former, is poorly developed. It is only in forms with a limpet-like mode of life that this structure has become very well developed and in *Haliotis* the animal is completely encircled by a sensory fringe. In this instance there are obvious parallels with the mantle edge of patellids, but the two are clearly different structures and represent an example

of convergent evolution.

These observations indicate a close phylogenetic relationship between the Pleurotomariacea, Trochacea and Fissurellacea, particularly between the first two. It may be suggested that these groups represent one line of archaeogastropod evolution and the Patellacea another, separation occurring early in their evolution from the gastropod stock (bellerophontids). Additionally the Fissurellacea may be a group derived from, or related to, early pleurotomariaceans, but distinct from the lines leading to the Haliotidae and Trochacea.

These conclusions fit well with the generally accepted views on prosobranch phylogeny (Cox, 1960; Fretter & Graham, 1962; Morton, 1963; Fretter, 1979; Andrews 1981 & in preparation; Salvini Plawen, personal communication). They are not in complete agreement with those of Golikov and Starobogatov (1975) who suggested that the Trochacea should be grouped with the Monotacardia (Caenogastropoda) and Neritacea in the Pectinibranchia.

It must be emphasised however, that phylogenetic relationships are often highly complex. A wide range of anatomical features must therefore be examined before meaningful conclusions can be drawn (Purchon, 1978). The observations described here may nevertheless, be of use as part of such a multi-system approach to the problem of prosobranch phylogeny.

SUMMARY (Part I)

The cephalic and epipodial tentacles of *Gibbula umbilicalis* (and probably trochaceans in general) and the cephalic tentacles of *Emarginula reticulata* are essentially similar. They are slender, papillate structures with a similar internal organisation. The papillae each contain a group of multiciliary primary receptor cells with intra-epithelial cell bodies. The distal portion of the dendrites show a characteristic leek leaf arrangement and the apical surface bears several short non-motile cilia. The ultra-structure of these cells is discussed in relation to that of sensory cells in other groups. This, combined with the use of the tentacles during life is indicative of a mechano-receptive role. It is suggested that this type of receptor functions as a multi-directional mechanoreceptor. The epipodial tentacles of *E. reticulata* are of two types, long and short, both of which are structurally quite distinct from the cephalic ones. The epipodial sense organ forms an integral part of their structure.

Multiciliary primary receptors similar to those in the tentacular papillae occur in varying concentrations over much of the body surface, particularly in *G. umbilicalis* e.g., cephalic lappets, eye stalks, neck lobes, sides of the foot, epipodium and mantle edge. These may or may not be raised above the surface on papillae. They are thought to provide the general body surface with a degree of tactile sensitivity.

The mantle edge bordering the shell slit of *E. reticulata* is a highly specialised region possessing a well

developed siphon and discrete sense organs, the pallial sense organs, structurally identical to the epipodial sense organs. These structures are not tentacular and should not be described as pallial tentacles.

The epipodial sense organs of both species are essentially identical. Together with the pallial sense organs of *E. reticulata* they form a distinct, easily recognisable type of sense organ. The central, usually depressed, region, the sensory cupule, has a highly specialised pseudostratified epithelium. This contains only one type of sensory cell, a monociliary primary receptor with an intra-epithelial cell body. The dendrite tip possesses a single long kinocilium surrounded by rings of stereocilia and thickened microvilli. They show much similarity to the collar cells (choanocytes) of the Porifera. Cells of a similar type are also thought to be associated with some of the multi-ciliary receptors in *E. reticulata*. Various aspects of mechano- and chemoreception are discussed with regard to this cell type. None however, can be suggested with any certainty.

It seems probable that additional sensory cell types may occur in both species, notably the putatively sensory, multiciliated cells occurring beneath the epithelium of the epipodial sense organ rim. These, however, were seen only rarely and their function is not known.

The patellacean species examined show marked differences from the rhipidoglossan species. The epipodium is lacking being replaced by elaboration of the mantle edge.

The internal structure of the cephalic tentacles and the ultrastructure of the sensory cells are quite distinct. The tentacular nerve branches basally forming a ring of nerves around a central core of transverse muscle fibres and connective tissue. The sensory cells, although multiciliary primary receptors, have subepithelial cell bodies and possess cilia with an electron-dense content. These appear on the surface as large patches and are never raised on papillae. The sensory cells of the pallial tentacles are essentially similar to those of the cephalic ones.

The similarities between the trochacean and fissurellacean species and the differences exhibited between these and the patellacean species are discussed in conjunction with what is known about other members of the Archaeogastropoda. Within the order the greatest degree of similarity seems to be between the Haliotidae and Trochidae (probably Trochacea). The Fissurellidae, although generally similar to the Haliotidae and Trochidae, show differences in epipodial tentacle structure. The Patellacea in all respects appear quite distinct.

P A R T II

JUXTAGANGLIONAR ORGAN

C H A P T E R 5

INTRODUCTION

Since the first description of neurosecretion in a mollusc (Scharrer, 1935) numerous reports of this phenomenon have been published and the neuroendocrinology of molluscs has been the subject of frequent reviews (Gabe, 1966; Simpson, Bern & Nishioka, 1966a; Martoja, 1968, 1972; Tombes, 1970; Joosse, 1972, 1978, 1979; Golding, 1974; Boer & Joosse, 1975; Highnam & Hill, 1977; Le Breton, 1979). From these it is clear that hormones play an integral role in the physiology of these animals.

Neurosecretory cells have been reported in all groups (with the exception of the Monoplacophora) and appear to be of numerous different types. Non-nervous, endocrine glands however, are much less common. A small number of possible endocrine structures has been described, particularly in the gastropods and cephalopods, but few have been investigated thoroughly.

In recent years, however, the dorsal bodies of pulmonates have been the subject of extensive examination, notably in the Department of Biology at the Free University in Amsterdam, Holland. These organs are now known to be endocrine glands which produce a hormone controlling certain aspects of the reproductive cycle. Dorsal bodies are not found in prosobranch or opisthobranch gastropods. Instead some members of these groups have been found to possess a putative endocrine organ, the juxtaganglionar organ (JGO) in

a similar position to that of the dorsal bodies (adjacent to the cerebral ganglia). It has been suggested several times in the literature (Joosse, 1972, 1979; Highnam & Hill, 1977; Nolte, 1978) that these two structures (the dorsal bodies and the JGO) may be homologous and therefore that the JGO may have a role in the control of reproduction. This organ, however, has only been described in a relatively small number of species and has not been examined extensively. Information concerning its structure and function is therefore limited, what little is known is summarised below.

The JGO was first described in the viviparous opisthobranch, *Hydromyles globulosa* Rang, by Martoja in 1965 (Martoja, 1965a). Since then it has been found in several other opisthobranch species, *Aplysia punctata* Cuv. (Martoja, 1965b), *Aplysia rosea* Rathke, *Dolabrifera virescens* (Risso) (cited as *Aplysiella*) and *Glossodoris tricolor* (Cantraine), (Vicente, 1966). A similar structure has also been described in certain archaeogastropods, *Diodora mamillata* (Risso), *Patella lusitanica* Gmelin, *Monodonta turbinata* (Born) (cited as *Trochocochlea*) (Martoja, 1965c), *Haliotis* (Miller & Nishioka, after Joosse, 1972) and *Patella vulgata* L. (Choquet & Lemaire, 1969). These represent members of all four archaeogastropod superfamilies. However, it has been looked for in vain in the more advanced prosobranchs (caenogastropods) (Martoja, 1972).

Typically the JGO is a paired structure lying in close association with the cerebral ganglia. In most species it lies on the posterior or dorsal surfaces of the ganglia, but in *Glossodoris* it is ventral. Histologically Martoja

observed it to be composed of groups of glandular cells, interspersed with connective tissue. The cells are polyhedral with spherical to ovoid nuclei and finely granular cytoplasm. No connections between it and the cerebral ganglia have been noted, the perineurium appears to be intact. Similarly no ducts have been seen to leave the organ.

In addition Martoja (1965a, 1965b) noted that the extent of the organ varies seasonally and that this correlates with the reproductive cycle. The organ is largest when the gametes are beginning to mature, but by the time of gamete release it undergoes a considerable degree of atrophy. This was particularly noticeable in the incubatory phase of *Hydromyles* (Martoja, 1972).

These observations combined with the fact that the organ appears to be little developed in immature animals has led to the suggestion that it is an endocrine organ in control or partly in control of the reproductive cycle. Initial suggestions indicated that it is linked specifically with hermaphroditism, either simultaneous (*Aplysia*) or successive (*Hydromyles* and *Patella*), but its presence in dioecious (gonochoristic) species does not support this (*Haliotis*, *Diodora* and *Monodonta*).

Vicente (1966) and Choquet (1971) have both provided evidence for a gonadotrophic factor produced by the cerebral ganglia of *Aplysia* and *Patella* respectively. Vicente believed that the JGO may act as a neurohaemal/storage organ for the release of this factor. This however, does not

correlate with the presence of the intrinsic glandular cells noted by Martoja.

As yet there have been no ultrastructural or specific experimental studies conducted on the JGO to provide further information on its function. The work of Vicente and of Choquet has involved some extirpation/re-implantation and organ culture experiments, but these have not treated the JGO as an entity distinct from the cerebral ganglia.

The aim of the second part of this thesis is to examine the fine structure of the JGO. The species used is the archaeogastropod *Gibbula umbilicalis* as this species is common intertidally and was found to possess a well defined and easily accessible JGO. The study also includes an investigation into the possibility of seasonal changes in the activity of the organ which might relate to the reproductive cycle. Although the course of the reproductive cycle has been established by Underwood (1972), there is evidence that the exact timing of the cycle varies with locality and from year to year (Williams, 1964; Underwood, 1972). It was decided therefore that the cycle of the animals collected for this study should be determined. The JGO of *Aplysia punctata* Cuv. was examined briefly for comparison of prosobranch and opisthobranch organs. Experimental studies were not attempted, but it is hoped that these might be a logical extension to the work.

The JGO has not previously been described in *G. umbilicalis*. It was first noticed during dissections relating to Part I of the thesis. Its significance was not appreciated

immediately, but examination of the literature indicated the merit of further investigation.

Due to the possibility of homology between the JGO and the pulmonate dorsal bodies it is necessary to comment, briefly, on what is known of the structure and function of the latter. Much of the examination of the JGO has been conducted with this in mind.

The first extensive study on the dorsal bodies was carried out by Joosse (1964), using the basommatophoran snail, *Lymnaea stagnalis* L.. He showed the dorsal bodies to be present in this species as two isolated groups of cells, the medio-dorsal and latero-dorsal bodies, attached to the perineurium of each cerebral ganglion. The cells within the organs were found to be pear-shaped, forming an outer cortex of cell bodies and an inner medulla of cell processes, and to lie in groups separated by radial strands of connective tissue. The overall size of the bodies increases throughout life, but examination at different times of the year indicated a marked increase in cellular activity in the spring. This is revealed by an increase in nuclear and cytoplasmic volume. The decrease in volume at times of lower activity is compensated for by increase in the size of the inter-cellular spaces (lumina).

Joosse (1964) also performed extirpation experiments involving the dorsal bodies and showed that they have a profound effect on the production of egg masses. This was the first real evidence of an endocrine role in the regulation of reproduction for the dorsal bodies.

An electron microscope study, primarily on the medio-dorsal bodies, was conducted by Boer, Slot and van Andel (1968), using four species of basommatophoran snail. They noted large numbers of mitochondria in the cell bodies and an abundance of small, membrane-bound secretory granules (80-90nm in diameter) in the cell processes. This study however, did not include an examination of the dorsal bodies at different times of the year. Instead it concentrated on the relationship between the dorsal body cells and the underlying neurosecretory cells in the cerebral ganglion. It was found that in certain genera, namely *Planorbarius*, *Australorbis* (*Biomphalaria*) and *Ancylus*, there is close contact between dorsal body cell processes and cerebral neurosecretory cells, through the perineurium. Such contact however, was not observed in *Lymnaea*. The authors believed these contacts to be of little functional significance and to be related to the embryological origin of the dorsal bodies from perineural elements.

In recent years investigations on the dorsal bodies have been frequent. Experimental evidence clearly indicates that they produce a hormone, the dorsal body hormone, which regulates (stimulates) vitellogenesis and growth, differentiation and synthetic activity in the female accessory sex organs (Joosse & Geraerts, 1969; Geraerts & Algera, 1972, 1976; Geraerts & Joosse, 1975; Veldhuyzen & Cuperus, 1976). These studies have been concerned with basommatophoran species, the Stylommatophora have been less extensively investigated. Their dorsal bodies, nevertheless, have been shown to have a similar function (Wijdenes & Runham, 1976; Saleuddin, Wilson,

Khan & Jones, 1980; Minnen & Sokolove, 1983; Minnen, Wijdenes & Sokolove, 1983). The location and organisation of stylommatophoran dorsal bodies, however, is more variable than those of the Basommatophora. In *Succinea putris* (L.) they remain as distinct organs, but in most other species they are more diffuse and the cells are dispersed in the thick layer of connective tissue surrounding the cerebral ganglia (Nolte, 1978; Wijdenes & Vincent, 1981). In *Limax maximus* L. isolated cells may also be found in the connective tissue around the suboesophageal ganglia (Minnen, Wijdenes & Sokolove, 1983).

One feature of the stylommatophoran dorsal bodies apparently not shared with those of the Basommatophora is the presence of neurosecretory innervation. Nolte (1978, 1983), Wijdenes and Vincent (1981) and Wijdenes, Vincent, Griffond and Gomot (1983) have shown that neurosecretory axons originating from the cerebral commissure make an "en passent" synapse-like contact with the dorsal body cells. Wijdenes (1981) and Wijdenes, Vincent, Griffond and Gomot (1983) believed that these axons arise, in *Helix*, from the cerebral green cells which are homologous with the growth hormone producing light geen cell of *Lymnaea* (Geraerts, 1976a). They postulated an antagonism between growth and reproduction and suggested an inhibitory control of the dorsal body cells by the cerebral green cells. In this respect they drew comparison with the nervous inhibition known to control the optic glands of cephalopods (for literature see Wells & Wells, 1977). The basommatophoran dorsal bodies are not thought to be under direct nervous control, but may be regulated (inhibited) by a hormone from the lateral lobes of the cerebral ganglia,

(Geraerts, 1976b; Roubos, Geraerts, Boerrigter & van Kampen, 1980). It is interesting to note however, that contacts observed by Boer, Slot and van Andel (1968) in certain basommatophoran species were with the light green cells (medio-dorsal cells).

In both the Basommatophora and Stylommatophora the dorsal bodies are not thought to influence the male part of the reproductive system. This is thought to be under the control of a separate system (Geraerts, 1976b; Joosse, 1979; Wijdenes, 1981). This fact, if the dorsal bodies and JGO are truly homologous, is particularly interesting in relation to the possible role of the latter in males of gonochoristic species such as *G. umbilicalis*.

Information relating to the chemical nature of the dorsal body hormone is limited. Ebberink, Loenhout, Geraerts, Hogenes and Hoogland (1983) believed it to be a peptide and are at present examining its purification and characterisation. Krusch, Schoenmakers, Voogt and Nolte (1979) however, considered that the ultrastructural features of the dorsal body cells, viz. large numbers of mitochondria and lipid droplets is more characteristic of steroid synthesising cells and have shown the presence of 3B-hydroxysteroid dehydrogenase (3B-HSD) in the dorsal body. Nolte (1983) has proposed a working hypothesis that the dorsal bodies produce a steroid hormone which is bound to protein for storage. 3B-HSD activity, nevertheless, could not be demonstrated in *Lymnaea stagnalis* (Boer, Slot & van Andel, 1968).

The dorsal bodies, however, are not the only structures involved in the control of reproduction in pulmonates. The system is complex and involves several other neuroendocrine/endocrine structures, notably neurosecretory cells in the cerebral ganglion (caudo-dorsal cells of *Lymnaea*) and its lateral lobe when present. Endocrine roles are also evident for the gonad of the Stylommatophora (Laviolette, 1954; Runham, Bailey & Laryea, 1973; Gomot, 1974, 1976; Wijdenes, 1981) and possibly the cephalic (optic) tentacles (Pelluet & Lane, 1961; Gottfried & Dorfman, 1970; Wattez, 1973, 1975, 1976, 1978; Bierbauer, 1974, 1977; Takeda, 1983).

The involvement of the cephalic appendages in the control of reproduction is also of interest in the prosobranchs and opisthobranchs. Choquet (1965, 1967, 1971) and Choquet and Lemaire (1969) reported that the cephalic tentacles of *Patella vulgata* have an inhibitory effect on spermatogenesis. Vicente (1966) however, indicated that gametogenesis was stimulated by the rhinophores of *Aplysia rosea*. Nevertheless in no gastropod has the exact source of the tentacular factor been established. Lane (1962, 1963, 1964a, b) proposed that the collar cells associated with the tentacular ganglion of pulmonates were modified neurons of a neurosecretory nature. Rogers (1969), however, on ultrastructural evidence considered this unlikely. The role of the tentacles remains to be established more clearly, so far the results of experimental studies are often contradictory (Gerearts & Joosse, 1975; Highnam & Hill, 1977; Bride & Griffond, 1981). No possible collar cell equivalents were seen in the tentacles of any of the species examined in Part I of this thesis.

Only certain aspects of the endocrinology of reproduction in opisthobranchs and prosobranchs have been examined in any detail. The ovulation hormone-producing cells associated with the abdominal ganglion of *Aplysia* have been the subject of much study, particularly with regard to their electrophysiology (Strumwasser, Kaczmarek, Chiu, Heller, Jennings & Viele, 1980). In this last respect they may be compared to the caudo-dorsal cells of *Lymnaea* (Vlieger, Kits, Maat & Lodder, 1980). Other aspects of opisthobranch reproductive control, however, are poorly understood.

In the prosobranchs, only the highly specialised hermaphrodite species *Crepidula fornicata* Phil and *Calyptraea sinensis* (L.) have been investigated in any detail. These species and particularly the differentiation and dedifferentiation of the male external genital tract, have been extensively examined by Lubet, Strieff and P. & S. Le Gall (for literature see Le Gall & Strieff, 1975; Le Breton, 1979; Le Gall & Féral, 1982). This process is complex and involves hormones produced by the pleural and right pedal ganglia. In contrast the control of the gonad and internal accessory sex organs may be more simple. The cerebral ganglia/cerebropleural connectives are thought to produce a hormone(s) which stimulates vitellogenesis, spermatogenesis and growth and differentiation of both male and female internal accessory sex organs. The exact source of this hormone is not known and as yet a JGO/DB has not been found in these species. The control of reproduction in these species appears to be further complicated, however, by additional factors needed to bring about sex change.

Some investigations have been carried out on

gonochoristic species, but these have again concentrated on the male external genital tract and its differentiation (Strieff & Le Breton, 1970; Le Gall, Griffond & Strieff, 1974; Féral, 1978, 1980). Ram (1977, 1983) and Ram, Ram & Davis (1982) however, have shown that nervous system extracts may induce the laying of egg capsules in *Busycon*.

The control of gametogenesis and development of the accessory sex organs in the opisthobranchs and prosobranchs seems in general to be poorly understood in comparison to that in the pulmonates. While some investigations have been conducted on prosobranchs, the source of potential regulating hormones has not been established. The possible homology of the JGO and the dorsal bodies of pulmonates may therefore be of considerable significance, particularly as the function of the latter in the control of the above processes is comparatively, well understood.

C H A P T E R 6

MATERIALS AND METHODS

SPECIMENS

For general anatomical studies on the JGO specimens of *Gibbula umbilicalis* were collected from various localities on the Dyfed coast, South Wales. They were kept in tanks containing artificial sea water linked to a tide machine as described in Part I.

For studies on the seasonal variation in activity of the JGO animals were sampled at four-weekly intervals throughout one year from St. Non's Bay, St. Davids, Dyfed (grid ref. SM 752 242). All specimens were mature (shell width of more than 10mm, Underwood, 1972) and were collected from the same position on the shore at approximately mean tide level. They were kept for a maximum of three days before use in tidal tanks synchronised with the natural tidal cycle.

Specimens of *Aplysia punctata* were obtained in March 1981 from the Marine Biological Association of the United Kingdom, The Laboratory, Citadel Hill, Plymouth. They were maintained in standard continuous flow aquarium tanks.

ROUTINE HISTOLOGY

The methods used in routine histology were essentially the same as those described in Part I. Material was fixed in sea water Bouin's, or Stieve's fixative and stained by one of the following procedures:-

a) Heidenhain's iron haematoxylin.

b) Heidenhain's Azan.

c) Delafield's haematoxylin and eosin.

SELECTIVE STAINING

Selective staining techniques were used for two purposes:-

1. To investigate the presence of neurosecretion in the cerebral nervous tissue, particularly that underlying the JGO, and/or the presence of neurosecretory axons within the JGO.

2. To obtain information on the nature of the secretion of the JGO cells.

The following techniques were employed:-

Neurosecretion

a) Heidenhain's Azan (Pantin, 1946)

This stain is not specific for neurosecretion and was used primarily as a general microanatomical stain. Much neurosecretory material however, is acidophilic before any pre-treatment (Gabe, 1966), and readily takes up the azocarmine of this stain. The method has therefore been used frequently during investigations on neurosecretion, particularly when difficulty is experienced in obtaining basophilia after oxidation (Panov, 1980).

b) Aldehyde Fuchsin (Cameron & Steele, 1959)

Aldehyde fuchsin (paraldehyde fuchsin) and chrome haematoxylin-phloxin have been used extensively for the histological demonstration of neurosecretion. The former was used in this study due to its more rapid and simple method. Gabe (1966) stated that affinity for aldehyde fuchsin exactly paralleled that for chrome haematoxylin, although Panov (1980) indicated that the former may be

more selective, at least in insects.

Sumner (1965) believed that the method operates by acidified permanganate oxidation of cystine (thought to be an abundant amino acid in neurosecretion) to cysteic acid. The aldehyde fuchsin then reacts ionically with the sulphonic acid radicals thus produced. Unfortunately, however, aldehyde fuchsin is not specific for such groups and may also show affinity for other acid radicals (phosphate and carbonyl) and aldehyde groups (Gabe, 1966), though affinity with the latter may be non-ionic. Furthermore, sulphonic acid radicals may be produced by oxidation of other tissue components and may also be present without oxidation (sulphated acid muco-polysaccharides).

This indicates that the results obtained using aldehyde fuchsin must be treated with caution. Positive reactions may be given by lipofuscins, melanin and gliosomes - elements common in nervous tissue, and many other tissue components (Gabe, 1966; Simpson, Bern & Nishioka, 1966a; Panov, 1980) as well as by neurosecretion.

c) Alcian blue/Alcian yellow (Wijdenes, 1981)
This method, introduced initially without prior oxidation for polysaccharide differentiation (Ravetto, 1964), has frequently been used for the demonstration of neuro-secretory material, particularly in molluscs (Wendelaar Bonga, 1970; Wijdenes, 1981). Like the preceding method it relies upon the oxidation of sulphur containing amino acids. Wendelaar Bonga (1970) stated that the S-S bonds

of cystine and the SH groups of cysteine were oxidised to form strongly acidic SO_3H groups (sulphonic acid). These then react with the alcian dye at lower pH (blue). The oxidation also produces weakly acidic carboxyl groups from hydoxyl and aldehyde groups, which stain with the dye at higher pH (yellow). This method may therefore enable different types of neurosecretory material to be distinguished on the relative proportions of strong and weak acid groups. Cells stain in varying shades of blue, green and yellow. Additional differentiation may be possible by the use of an acidic counter stain (phloxin).

Again, however, this method is not entirely specific for neurosecretory material. Concentrations of any proteins rich in cystine and/or cysteine may be stained and substances originally rich in SO_3H groups and carboxyl groups, e.g. acid mucopolysaccharides will also produce a positive reaction.

The method of Wijdenes (1981) is very similar to that of Wendelaar Bonga (1970), but overcomes the problem of alcian yellow insolubility by the use of N,N-dimethyl-formamide (Sigma).

Nature of the JGO Secretion

d) Periodic acid - Schiff reaction for carbohydrate (1,2 glycol groups) with diastase controls (Humason, 1979).

e) Bromophenol blue and mercuric bromophenol blue for proteins (amino groups), (Mazia, Brewer & Alfert, 1953).

f) Deamination for blocking amino groups -
Van Slyke's nitrous acid reagent (Humason, 1979).

All tissue was fixed in sea water Bouin's fluid except that stained in Alcian blue - Alcian yellow which was fixed in Stieve's fixative.

ELECTRON MICROSCOPY

The fixation methods used for electron microscopy have been given in Part I.

Scanning electron microscopy was not used extensively in this study; only fixation method 1. was employed.

The JGO/cerebral ganglion of *G. umbilicalis* was initially fixed for transmission electron microscopy using method 1. This method, however, produced variable results with this tissue and membrane preservation was generally poor. Consequently other methods were employed, notably methods 3, 5. and 6. None of these however, produced results that were consistently better than those obtained using method 1. The problem of obtaining good membrane preservation, particularly in the JGO, was never satisfactorily overcome.

Procedures subsequent to fixation were the same as those described in Part I.

SEASONAL EXAMINATION OF JGO AND GONAD

Initially it was decided that samples of ten mature animals (5 male and 5 female) would be collected at intervals of four weeks throughout one year. The gonad would

then be examined using paraffin wax sections to assess the maturity of each individual and the JGO would be examined ultrastructurally for quantitative evidence of changes in cellular activity which might correlate with this. It was thought that this evidence might take the form of changes in cell and/or nuclear size, the numbers of secretory granules and mitochondria, the extent of the endoplasmic reticulum, the abundance of Golgi bodies and the numbers of granules in the process of release (Joosse, 1964; Martoja, 1965a; Wedelaar Bonga, 1971a, b; Berlind, 1977; Roubos, Geraerts, Boerrigter & van Kampen, 1980). It soon became apparent, however, that such a morphometric investigation could not be accomplished in the time available, particularly considering the enormity of quantitatively assessing the above parameters and statistically analysing the data obtained. As a result, a more qualitative approach was adopted, and fewer animals were examined. The only parameters assessed quantitatively were cell and nuclear size. (All ten animals, however, were fixed and embedded as this presented little extra work and may prove useful if the study is continued.)

It was also decided that, in the first instance, only females would be examined. The reason for this was that the work of Underwood (1972) has shown that the maturity of females can be determined more easily and accurately than that of males. In the latter the state of sperm maturity cannot be assessed accurately in paraffin wax sections and all stages of sperm maturation are present in the gonad throughout the year. The variable factors appear to be the total numbers of sperm and the relative proportions of the

various stages. Underwood (1972) and Williams (1964), however, have both shown that the male cycle is synchronous with that of the female. This may be of considerable importance if males are examined in the future.

Gonad

The maturity of the gonad of two females, a and b, from each sample was assessed and the results plotted graphically to show the course of the reproductive cycle. This assessment was made on 8µm paraffin wax sections, fixed in seawater Bouin's and stained in Heidenhain's Azan. The number of mature and immature oocytes (those with and those without a gelatinous coat respectively, Underwood, 1972) per field of view at 200 times magnification were counted. This was done for four fields of view per animal, each in a different portion of the gonad. These four counts were then pooled and the results calculated as the percentage mature oocytes of the total number of oocytes.

Juxtaganglionar Organ

The JGO of one of each pair of animals at certain points in the reproductive cycle was then examined ultrastructurally. Particular attention was paid to animals at the following stages in the cycle:- (see Fig. 16)

W - after the major burst of spawning

X - in the middle of the resting phase

Y - at the onset of gamete maturation

Z - at maximum gonad maturity, prior to spawning

Animals at several intermediate stages were also studied.

The quantitative assessment of cell and nuclear size was made, as follows, in terms of the area of these structures in electron micrographs. Thin resin sections were cut through the thickest part of the JGO (i.e. at the junction of the cerebral ganglion and cerebral commissure) and were photographed at 3000 times magnification on a Zeiss EM 109 electron microscope (actual magnification 3,210 X, after calibration). The outlines of five of the largest cells and nuclei were then traced onto high quality tracing paper of uniform weight/cm^2. These were then cut out and weighed and the figures converted into actual measurements in μm^2.

C H A P T E R 7

RESULTS

SECTION 1

The Juxtaganglionar Organ of *Gibbula umbilicalis*

1.1. Gross Morphology

The JGO of mature *Gibbula umbilicalis* is a small paired structure lying on the posterior face of each cerebral ganglion and the cerebral commissure. It is largest in the region where these join (Plate 52a). Medially it extends as a narrow strip along the posterior edge of the commissure and on rare occasions joins with its counterpart from the opposite side in the midline. In this region it can sometimes be seen without dissection through the thin body wall covering the dorsal part of the head. Laterally it follows the edge of each ganglia, ending approximately at the origin of the cerebropleural and cerebropedal connectives.

The organ is found in both sexes and also in immature animals. It is smaller in the latter, though it is not known whether this is in proportion to the smaller size of the animal as a whole. There is considerable individual variation in size, shape and colour of the organ. In some specimens it is smooth and flat, while in others it is more lobed, though it never occurs in isolated patches. The colour ranges from cream/yellow to yellow/orange. These differences show no obvious link with either the time of year or the sex and no clearly visible change in relative size of the organ as a whole was observed at any time.

The tissue of the organ is not compact and is very easily damaged during dissection. Though it lies in contact with the cerebral ganglion and cerebral commissure, it is not firmly attached to them. Posteriorly it is connected to the body wall and dorsal surface of the anterior oesophagus

by thin connective tissue strands. There are no discrete blood vessels associated with either the JGO or the nervous tissue, though both are bathed in the blood (haemolymph) of the dorsal cephalic sinus which arises from the anterior aorta via the cephalopedal sinus (Fretter & Graham, 1962).

The cerebral ganglia show no white or bluish areas which often indicate the presence of neurosecretory cells, (Gabe, 1966; Andrews, 1968; Joosse, 1978). Their general colour is cream/white but with numerous randomly distributed small maroon pigment cells, particularly on the dorsal surface. These are much less frequent on the cerebral commissure.

A JGO of similar appearance also occurs in *G. cineraria*, *Monodonta lineata* and *Calliostoma zizyphinum*.

PLATE 52

a) *Gibbula umbilicalis*, female with body wall of right side of head and neck removed to show the cerebral ganglion (spotted with pigment cells), cerebral commissure passing over the anterior oesophagus, and JGO, the thin yellow strip along the posterior face of the ganglion and commissure.
Bar = 1.5mm.

b) *Gibbula umbilicalis*, part of a parasagittal paraffin wax section through the head showing JGO attached to the cerebral ganglion. Note perineurium separating JGO from cerebral ganglion (arrow). Heidenhain's Azan.
Bar = 0.1mm.

ae - anterior oesophagus
bw - body wall
cc - cerebral commissure
cg - cerebral ganglion
ct - cephalic tentacle
ds - dorsal cephalic sinus
e - eye

PLATE 52

a

b

1.2. Fine Structure

In paraffin wax sections the JGO appears as a mass of glandular cells interspersed with strands of connective tissue (Plate 52b). It is semi-circular to triangular in cross section and is separated from the nervous tissue by the perineurium. The cells are not arranged in groups and bear no obvious processes. The connective tissue is not organised into radial strands.

The gland cells appear to be all of one type and are estimated to comprise 80-90% of the organ. One such cell is represented diagrammatically in Fig. 14. This is a composite drawing based on many electron micrographs. All the features shown were frequently observed, but never all in one section.

Typically these cells are very variable in shape, but even at maximum size rarely exceed 25μm in any one dimension. Every cell is almost, if not completely, surrounded by a fine basal lamina (< 50nm thick). Thus each is separated from its neighbour by two layers of basal lamina (Plate 53a). Between these is a blood space of variable width which communicates peripherally with the dorsal cephalic sinus (Plate 53b, c). The organ is therefore penetrated extensively by blood space.

The most characteristic feature of the cells is the presence of numerous small, electron-dense, membrane-bound secretory granules (Fig. 14; Plate 54a), 100-200nm in diameter. Associated with these are large numbers of

mitochondria and an extensive network of rough endoplasmic reticulum (RER) (Plate 54b, c). The mitochondria possess well developed septate cristae (Threadgold, 1976) and frequently, dense intra-mitochondrial granules. These three types of organelle (granules, mitochondria and RER) are usually localised in separate regions of the cell. The RER may sometimes envelope a cluster of mitochondria. Occasional granules, however, occur throughout the cell.

Other cytoplasmic structures include bundles of microfilaments, microtubules, lamellate bodies, free ribosomes, occasional small clear vesicles, large electron-dense droplets and infrequently lipid droplets (Plate 54d). Golgi bodies were rarely observed though this may be due to the difficulty experienced in obtaining good membrane preservation (see Materials and Methods).

The cells possess a small number of cilia arising singly or occasionally in pairs from invaginations of the plasma membrane (Plate 55a). Their exact length has not been determined, but they extend well into the intercellular space. They possess the normal 9 + 2 arrangement of sub-fibrils (Plate 55b). Motility, however, is unlikely as they too are enclosed within the basal lamina.

Part of the cell surface is frequently thrown into quite extensive processes which interdigitate with those of neighbouring cells (Fig. 14; Plate 53a). These processes contain for the most part only secretory granules, lamellate bodies, cup-shaped vesicles and microtubules (Plate 55c, d). The secretory granules appear to be released from the processes,

but this was rarely observed (Plate 55d, 56a). The mechanism for release seems to be simple exocytosis (merocrine secretion), but the empty omega-shaped profiles of the cell membrane usually associated with this type of secretion were never observed. Again poor membrane preservation may account for this. The cup-shaped vesicles and lamellate bodies (whorls) may represent examples of membrane sequestration (Buma & Roubos, 1983a).

The connective tissue within the organ is composed of several cell types, the most abundant of which is the pore cell (Plate 56b). These are relatively large cells (usually greater than 25μm in diameter) which occur primarily near the periphery of the organ. They are characterised by specialisations of the plasma membrane in the form of invaginations (pores) bridged by small cytoplasmic processes linked to each other by thin diaphragms (Plate 56c). The invaginations are coated with a fine filamentous layer, similar in texture to the basal lamina. The cytoplasm is mostly electron-lucent, containing large electron-dense bodies and numerous coated vesicles at the periphery associated with the invaginations. The overall appearance of these cells is similar to that in the cells of the ganglionar capsule of pulmonates (Plummer, 1966; Newman, Kerkut & Walker, 1968), and to the fibrocytes described by Nicaise (1973). Plummer (1966) suggested a role in the production of collagenous material and so they may in this case be responsible for the production and maintenance of the basal lamina. This cell type, however, is common in the connective tissue throughout the body of many gastropods and bivalves and in

some instances has been thought to produce blood pigment (Sminia & Boer, 1973; Welsch & Storch, 1976).

A second type of connective tissue cell with long flattened cytoplasmic processes occurs on the surface of the organ. Collectively these cells form a network over the surface of the organ and may therefore provide support (Plate 56d).

Other cell types occurring in the connective tissue include pigment cells and small muscle fibres. The latter are frequent, but show no particular orientation. They are distributed randomly and may assist circulation of haemolymph through the organ. Small groups of axons were infrequently observed but were never seen to contain neurosecretory granules. They are thought to be associated with the muscle fibres. No 'synapse-like' structures were observed with the glandular cells (re. Nolte, 1978; Wijdenes & Vincent, 1981).

The above observations are based primarily on females. Males, however, were also examined and were found to possess a JGO of similar ultrastructural appearance. Plate 58b is that of a male.

FIGURE 14

Gibbula umbilicalis, diagrammatic representation of a single JGO cell showing features commonly observed in electron micrographs.

bl - basal lamina
c - cilium
ibs - intercellular blood space
m - mitochondrion
mf - microfilament bundle
mt - microtubule
n - nucleus
rer - rough endoplasmic reticulum
sg - secretory granule

FIGURE 14

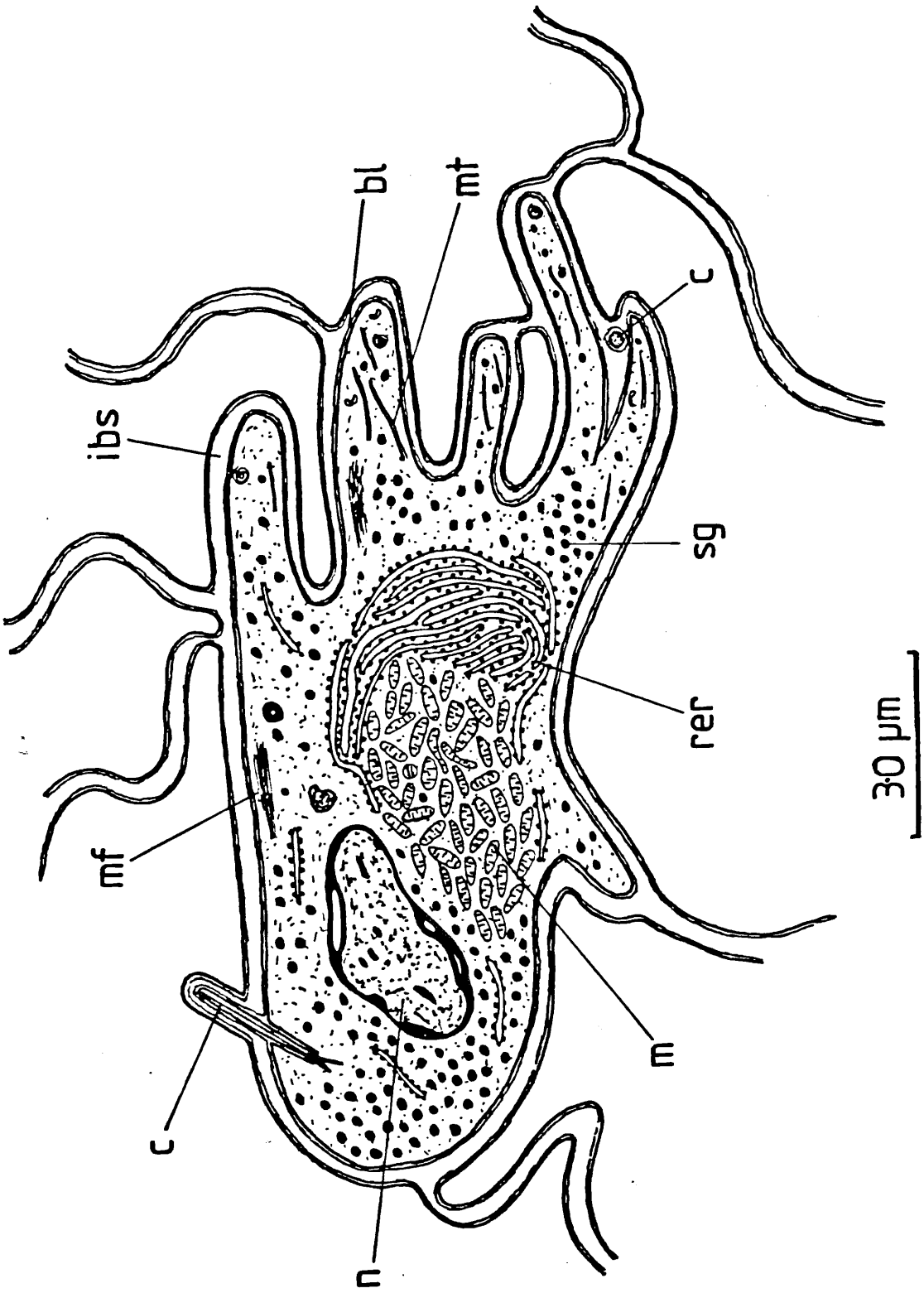


PLATE 53

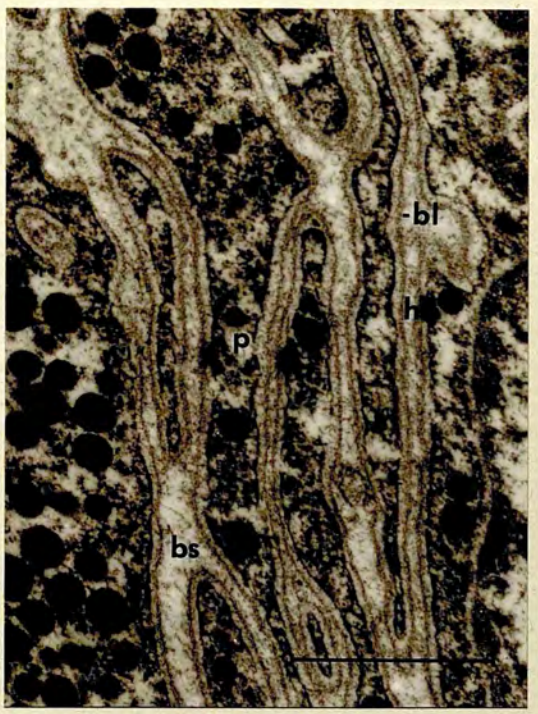
a) *Gibbula umbilicalis*, JGO, interdigitating cell processes showing the surrounding basal lamina. The space between these is a blood space. Hemidesmosomes connect the basal lamina to the plasma membrane.
Bar = 1.0µm.

b) *Gibbula umbilicalis*, part of the edge of the JGO showing the basal lamina passing into the organ between adjacent peripheral cells.
Bar = 1.0µm.

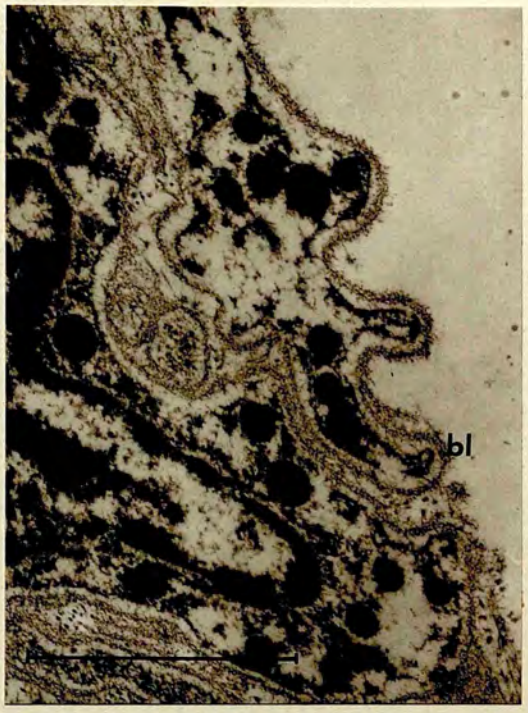
c) *Gibbula umbilicalis*, scanning electron micrograph showing holes in the surface of the JGO. These are thought to connect the intercellular blood space with the dorsal cephalic sinus.
Bar = 20µm.

bl - basal lamina
bs - blood space
h - hemidesmosome
p - process of JGO cell

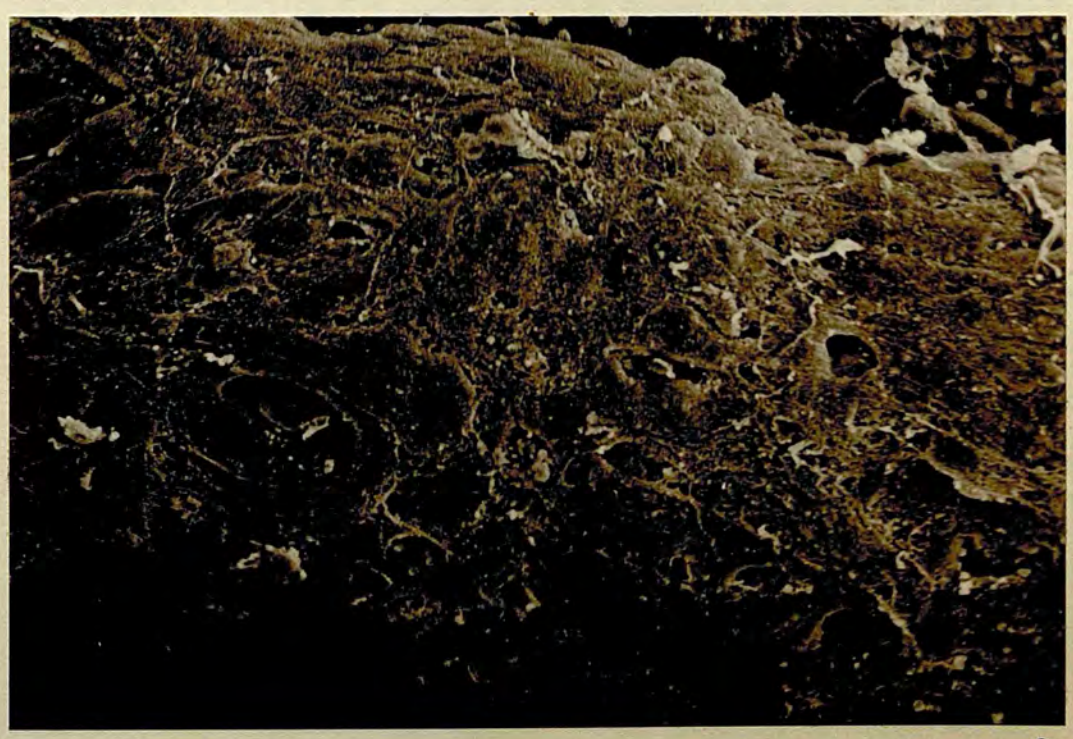
PLATE 53



a



b



c

PLATE 54

a) *Gibbula umbilicalis*, glandular cell of JGO showing electron-dense secretory granules, mitochondria and RER.
Bar = 2.0µm.

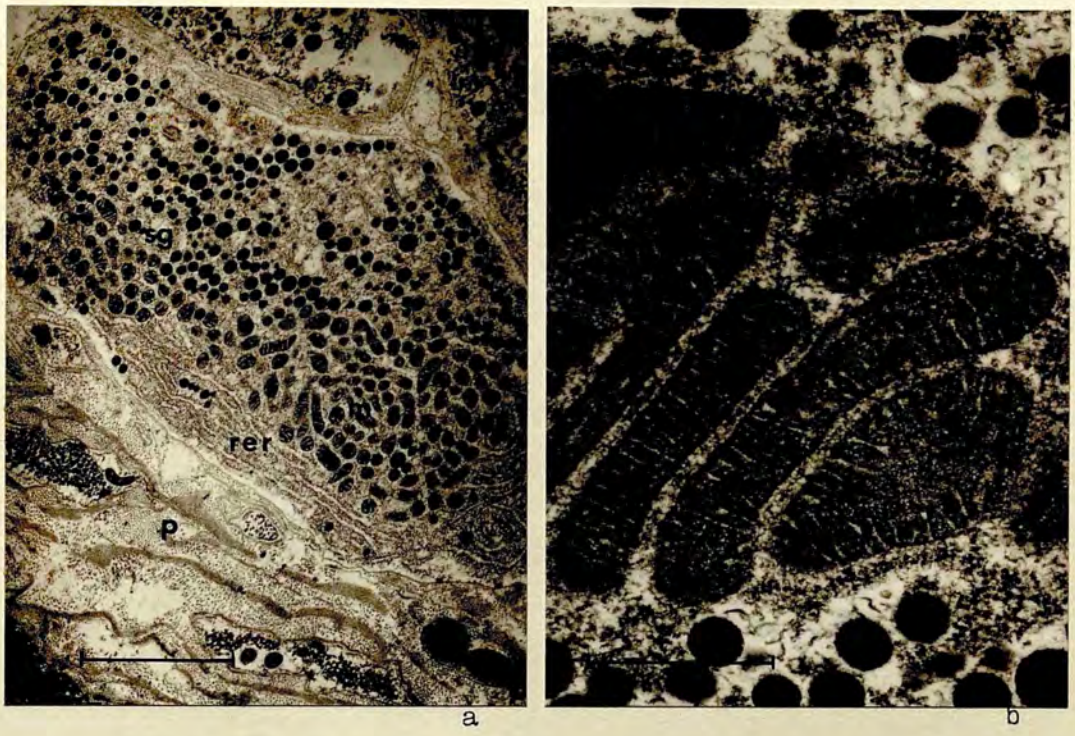
b) *Gibbula umbilicalis*, highly cristate mitochondria of JGO cell.
Bar = 0.5µm.

c) *Gibbula umbilicalis*, RER of JGO cell.
Bar = 0.5µm.

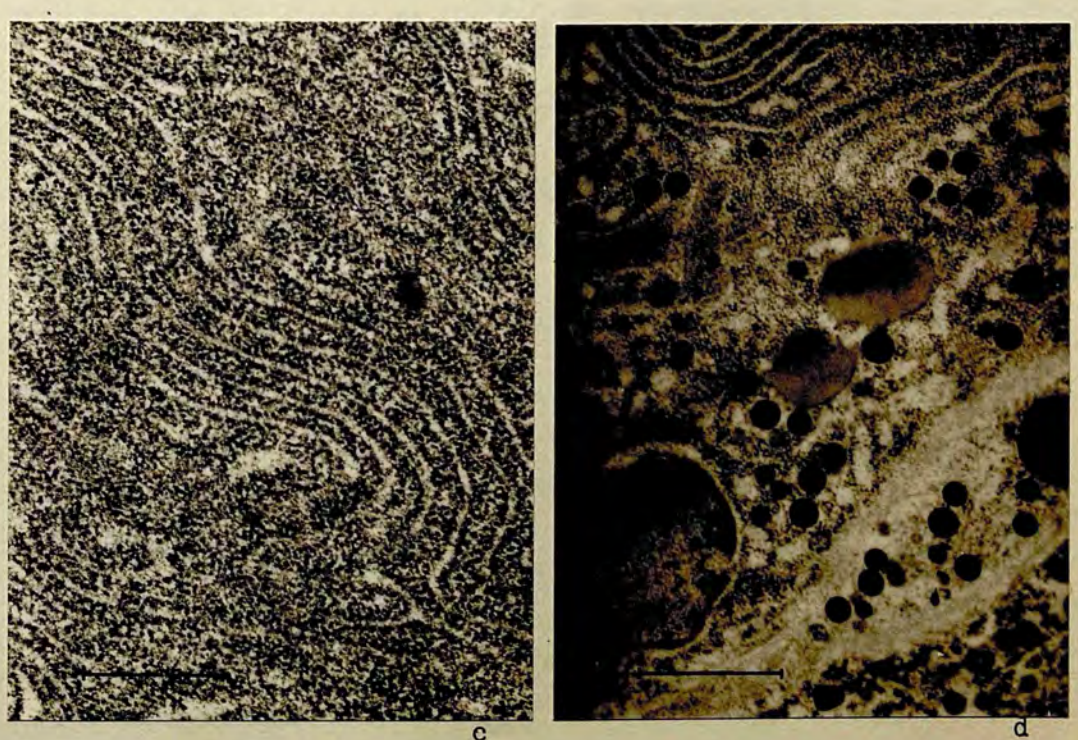
d) *Gibbula umbilicalis*, lipid droplets in JGO cell. These were only rarely observed.
Bar = 1.0µm.

l - lipid droplet
m - mitochondria
p - perineurium
rer - rough endoplasmic reticulum
sg - secretory granule

PLATE 54



a b



c d

PLATE 55

a) *Gibbula umbilicalis*, cilium arising from an invagination of the cell membrane of a JGO cell.
Bar = 0.5µm.

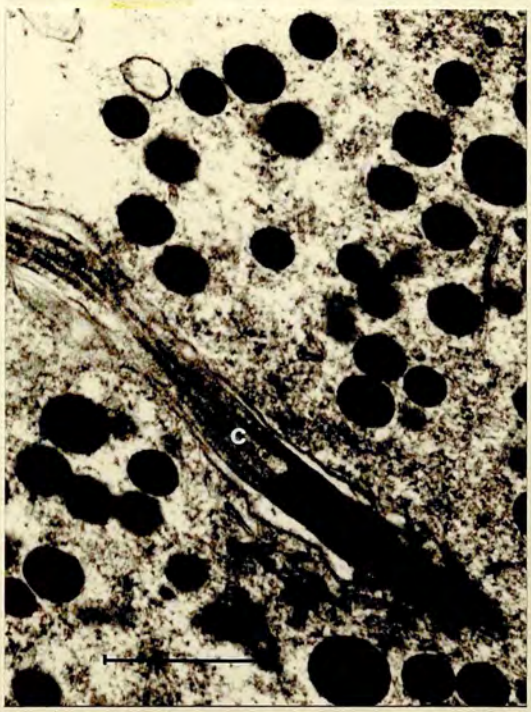
b) *Gibbula umbilicalis*, transverse section through two JGO cell cilia showing the 9 + 2 subfibril arrangement and the covering basal lamina. The ciliary membrane is poorly preserved.
Bar = 0.2µm.

c) *Gibbula umbilicalis*, oblique section through JGO cell process showing secretory granules, microtubules and a cup-shaped vesicle.
Bar = 0.3µm.

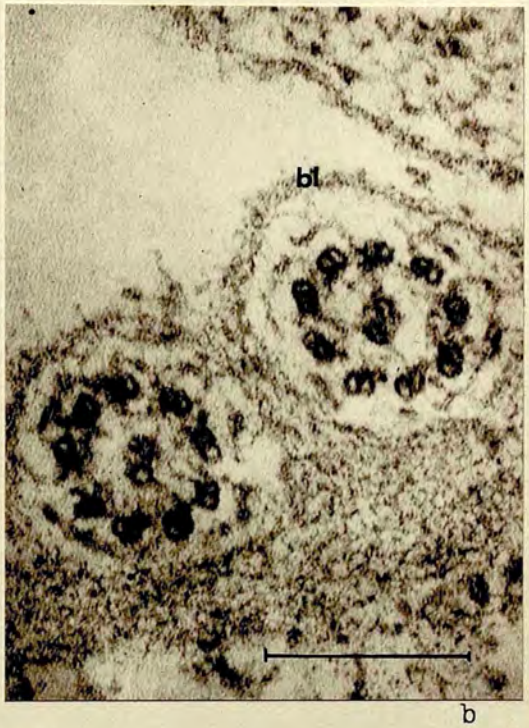
d) *Gibbula umbilicalis*, part of JGO cell process showing lamellate body and secretory granules, one of which may be undergoing release (arrow).
Bar = 0.3µm.

bl - basal lamina
c - cilium
cv - cup-shaped vesicle
lb - lamellate body
mt - microtubules

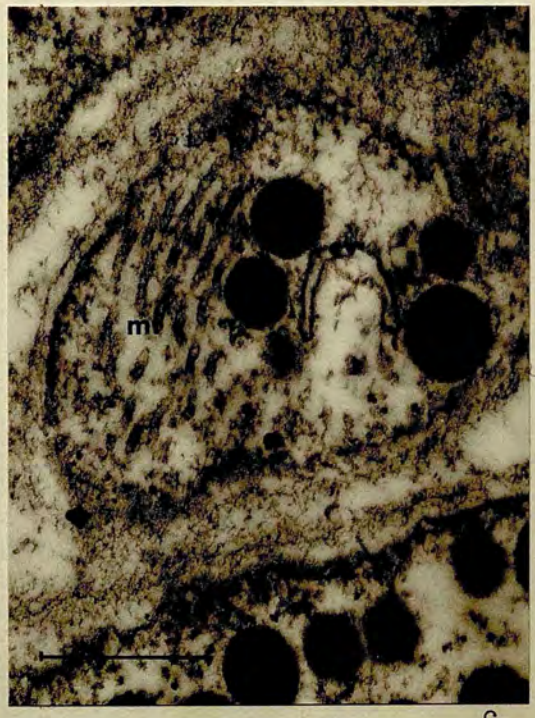
PLATE 55



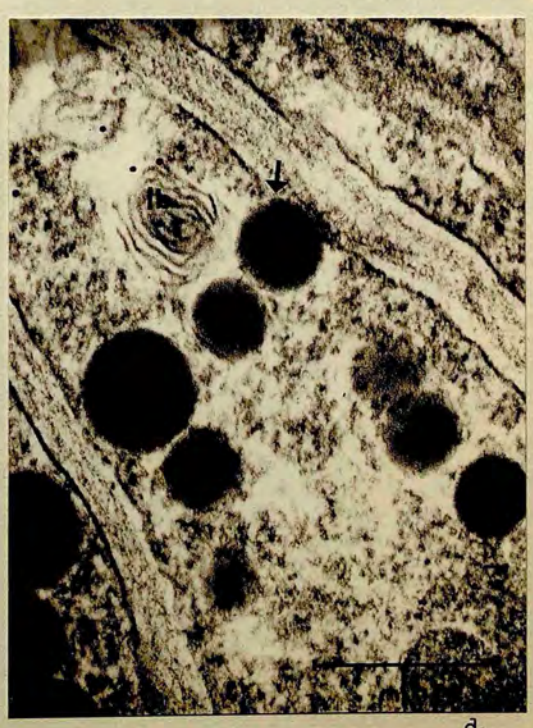
a



b



c



d

PLATE 56

a) *Gibbula umbilicalis*, exocytotic release of secretory granule from JGO cell process. Merocrine secretion.
Bar = 0.2µm

b) *Gibbula umbilicalis*, connective tissue pore cell in JGO.
Bar = 2.0µm.

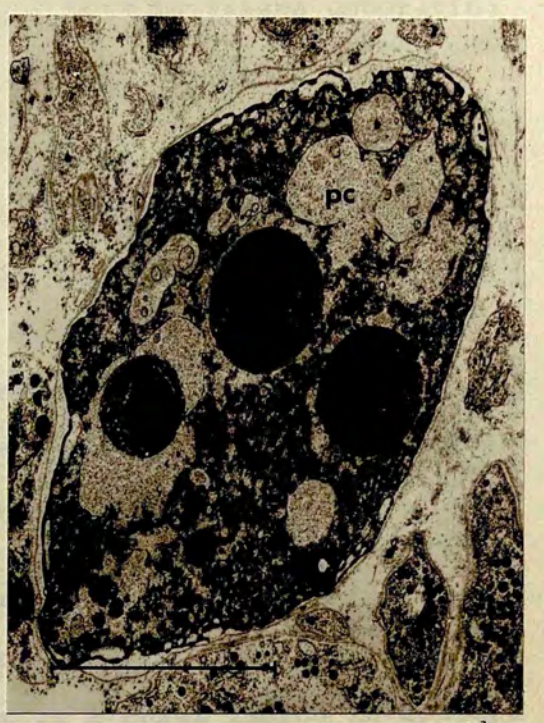
c) *Gibbula umbilicalis*, part of pore cell showing specialisation of the plasma membrane. The filamentous material coating the invaginations is similar in texture to the basal lamina.
Bar = 0.5µm.

d) *Gibbula umbilicalis*, flattened connective tissue cell found on the surface of the JGO.
Bar = 2.0µm.

bl - basal lamina
cv - coated vesicle
f - flattened connective tissue cell
fc - filamentous coat of invagination
pc - pore cell

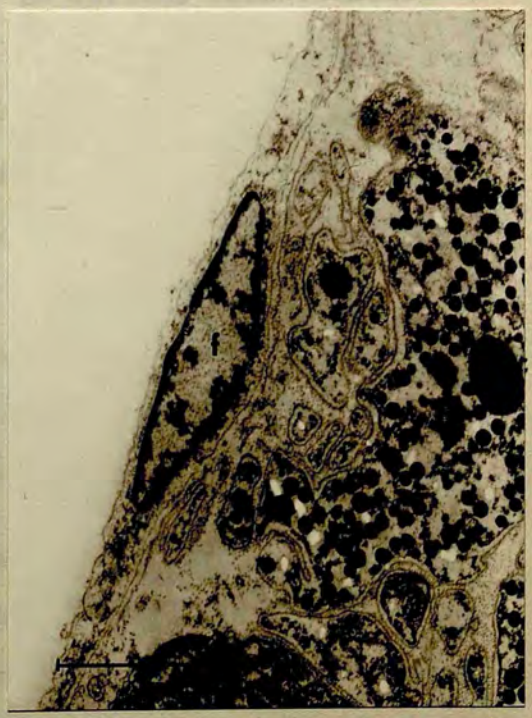
PLATE 56

a



b

c



d

1.3. The Structure of the Central Nervous Tissue underlying the JGO

Particular attention has been paid to the portions of the perineurium and nervous tissue lying beneath the JGO in order to examine the possibility of some sort of contact between the two. The tissue was studied for evidence of neurosecretory perikarya, neurosecretory "end feet" (Golding & Whittle, 1974, 1975) and/or the processes of neurosecretory cells passing through the perineurium.

The perineurium surrounding most of the central nervous system is 5-7μm thick and is composed of three layers (Plate 57a). The innermost layer is a basal lamina in contact with the nerve cells below, next is a layer of flattened bundles of collagen fibres lying in various orientations. The third and much the thickest layer comprises small muscle fibres embedded in a loose collagenous matrix. Beneath the JGO however, the perineurium is much thinner, usually 2μm or less (Plate 57b). This is largely due to the almost total disappearance of the thick outer layer which appears to fuse with the lateral margins of the JGO giving the appearance that the JGO arises out of this layer. The inner layers of the perineurium remain unchanged beneath the JGO.

The structure of the cerebral ganglion beneath the JGO does not differ from that elsewhere. The peripheral layer of neuronal perikarya is variable in thickness, in places as much as 7-8 cells deep (Plate 57c). These are all of similar size (rarely exceeding 16μm in diameter), but show considerable variation in appearance indicating the probability of several

different cell types. There is, however, no evidence that any are neurosecretory; granules when present are small (80nm or less in diameter) and probably represent granules of neurotransmitter (Plate 57d). Glial cell processes are abundant between the neuronal perikarya; they contain dense bundles of microfilaments and oval to rod-shaped electron-dense inclusions. They extend to the edge of the ganglion where they are joined to the inner layer of the perineurium by hemidesmosomes (Plate 58a). Occasional pigment cells containing large, heterogeneous, electron-dense granules are also found just beneath the perineurium. These probably account for the maroon speckling of the ganglion seen under the dissecting microscope.

There are no neuronal perikarya in the cerebral commissure lying beneath the JGO; the neuropile extends to the perineurium (Plate 58b). Electron-dense glial cell bodies are frequent, their long thin processes ramifying into the depth of the neuropile.

In neither the cerebral ganglion nor commissure was there any evidence of neurosecretory axons abutting the perineurium or passing through it into the JGO. The only evidence of possible neurosecretion was the presence of electron-dense granules, 100-200nm in diameter, in some axons within the neuropile of the ganglion (Plate 58c).

In two sections, however, cells (the internal JGO cells) identical to the glandular cells of the JGO were found within the cerebral ganglion (Plates 58d, 59a). In one case the cell body appeared to give rise to a process which

penetrated the perineurium and passed into the JGO (Plate 59a). Unfortunately the preservation of the tissue in this section is poor. The cell body within the nervous tissue is in close contact with glial cell processes and it is thought that these too, traverse the perineurium, together with the glandular cell processes. On several occasions bundles of glial cell processes surrounding a glandular cell process were seen within the JGO. The individual elements of these bundles are not or only partly separated from each other by basal lamina, providing further evidence that they originate outside the JGO (Plate 59b). This arrangement is drawn diagrammatically in Fig. 15. Further examination is needed to establish whether or not the internal JGO cells are in intimate contact with neurons.

PLATE 57

a) *Gibbula umbilicalis*, perineurium surrounding the cerebral ganglion. This is composed of three layers:- an inner basal lamina (arrow), a medial layer of collagen fibre bundles, and an outer collagenous matrix containing small muscle fibres.
Bar = 2.0µm.

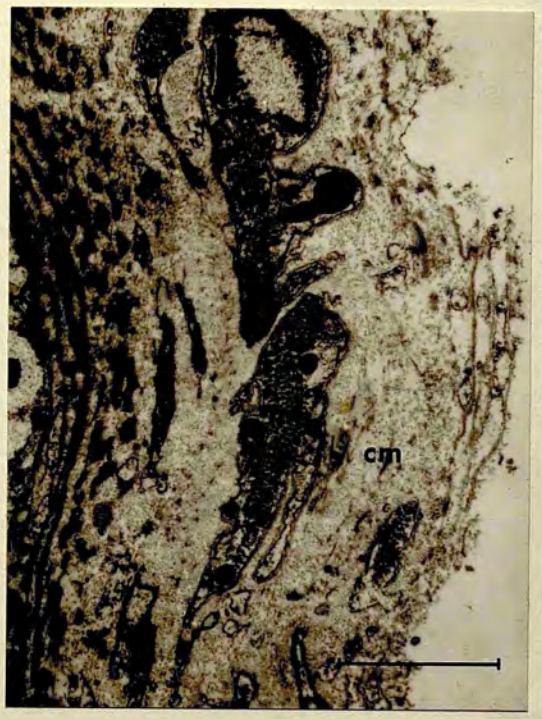
b) *Gibbula umbilicalis*, the periphery of the JGO showing the reduction in the outer perineural layer.
Bar = 5.0µm.

c) *Gibbula umbilicalis*, a portion of the cerebral ganglion underlying the JGO. Numerous perikarya are present but none appear to contain neurosecretory granules. The large electron-dense inclusions may represent lipofuscin.
Bar = 10µm.

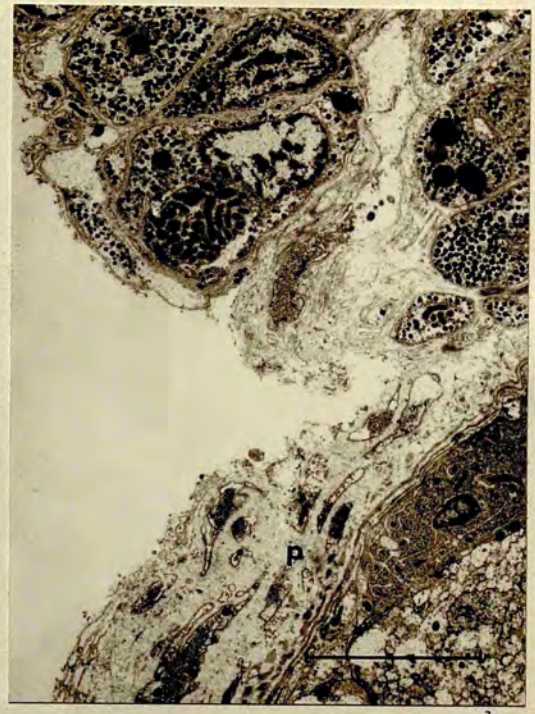
d) *Gibbula umbilicalis*, granular neuronal perikaryon in cerebral ganglion underlying the JGO. The granules are small (80nm or less in diameter) and probably represent neurotransmitter.
Bar = 4.0µm.

cf - collagen fibres
cg - cerebral ganglion
cm - collagenous matrix
jgo - juxtaganglionar organ
mf - muscle fibre
p - perineurium

PLATE 57



a



b

c

d

PLATE 58

a) *Gibbula umbilicalis*, edge of cerebral ganglion underlying the JGO, showing glial cell processes between the neuronal perikarya. These processes frequently contain bundles of microfilaments and electron-dense inclusions. Bar = 1.0µm.

b) *Gibbula umbilicalis*, portion of the cerebral commissure underlying the JGO. Note electron-dense glial cells with long processes extending into the neuropile. (Male specimen).
Bar = 10µm.

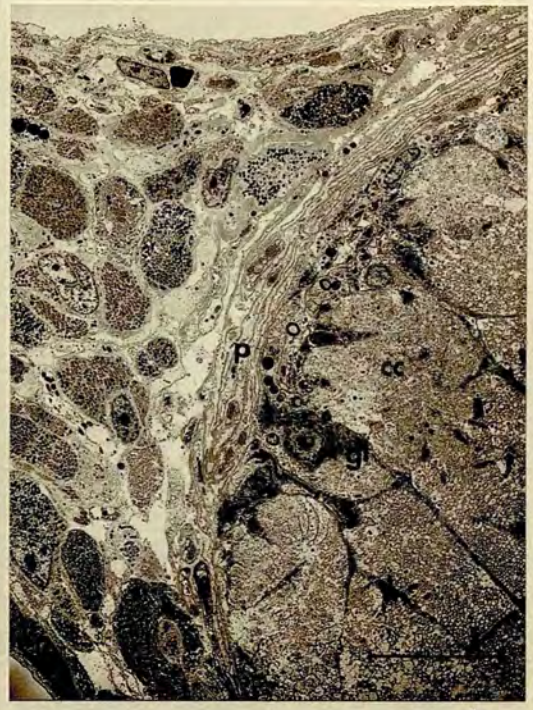
c) *Gibbula umbilicalis*, neuropile of cerebral ganglion with a possible neurosecretory axon.
Bar = 1.0µm.

d) *Gibbula umbilicalis*, internal JGO cell abutting the perineurium under the JGO.
Bar = 3.0µm.

cc - cerebral commissure
gl - glial cell
h - hemidesmosome
i - internal JGO cell
n - neuronal perikaryon
ns - neurosecretion (possible)
p - perineurium

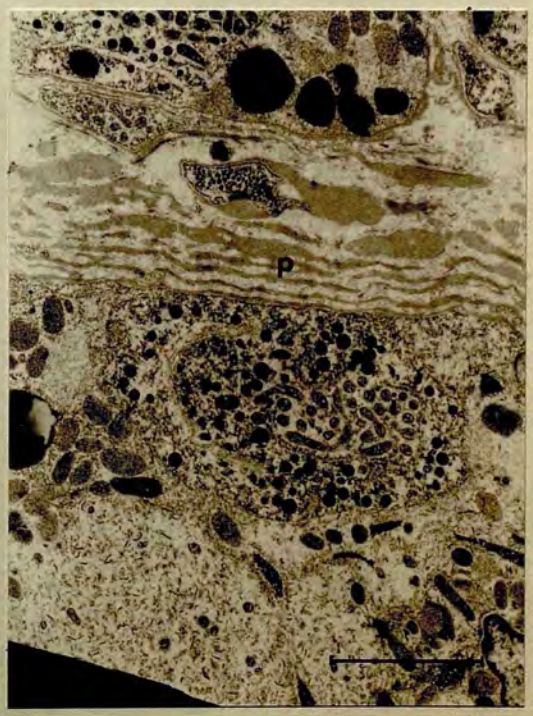
PLATE 58

a



b

c



d

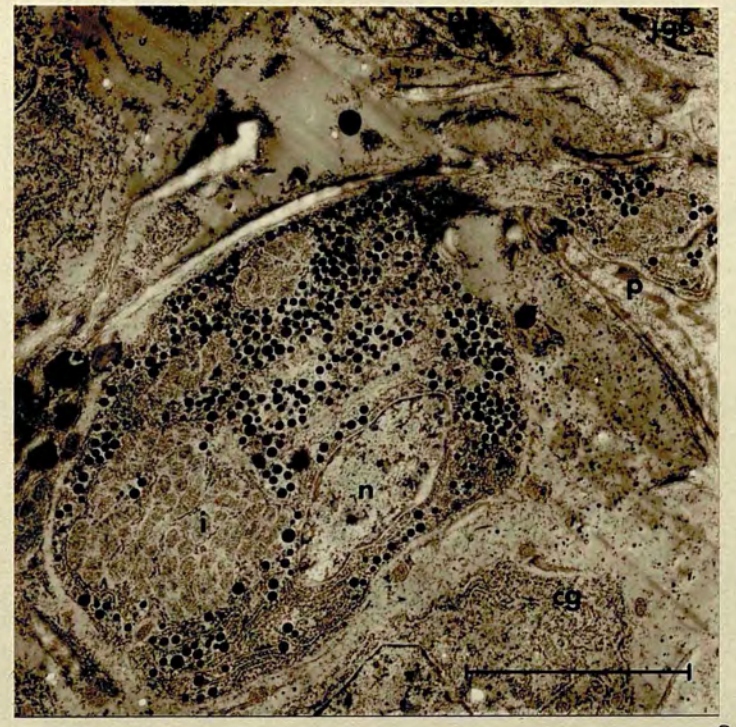
PLATE 59

a) *Gibbula umbilicalis*, an internal JGO cell with evidence of a process (arrow) passing through the perineurium in to the JGO. Unfortunately the preservation of the tissue is poor.
Bar = 5.0µm.

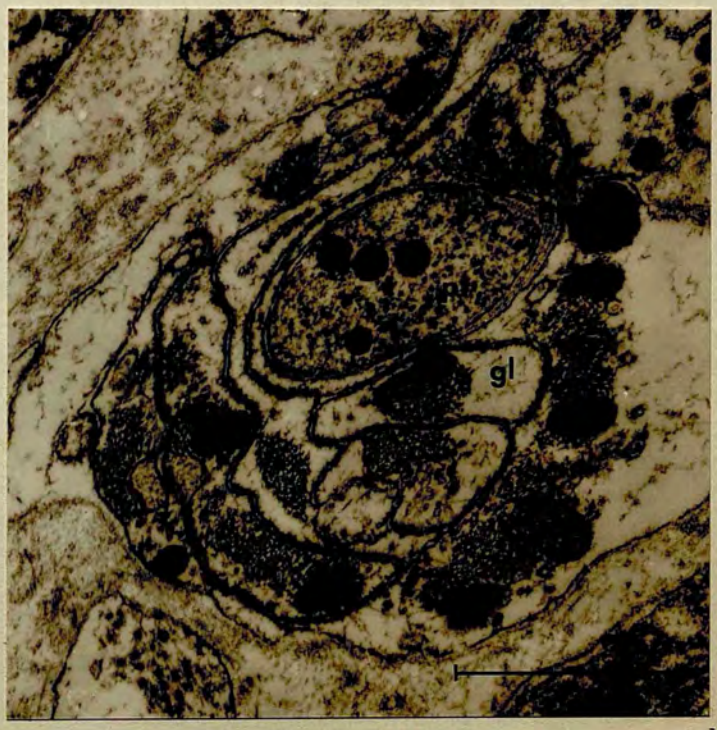
b) *Gibbula umbilicalis*, a JGO cell process (possibly an internal JGO cell) surrounded by glial cell processes. Note basal lamina surrounding only part of the JGO cell process. Glial cells are recognised by microfilament bundles and large electron-dense inclusions.
Bar = 1.0µm

bl - basal lamina
cg - cerebral ganglion
gl - glial cell process
i - internal JGO cell
jgo - juxtaganglionar organ
mf - microfilaments
n - nucleus of internal JGO cell
p - perineurium
pr - process of internal JGO cell.

PLATE 59



a



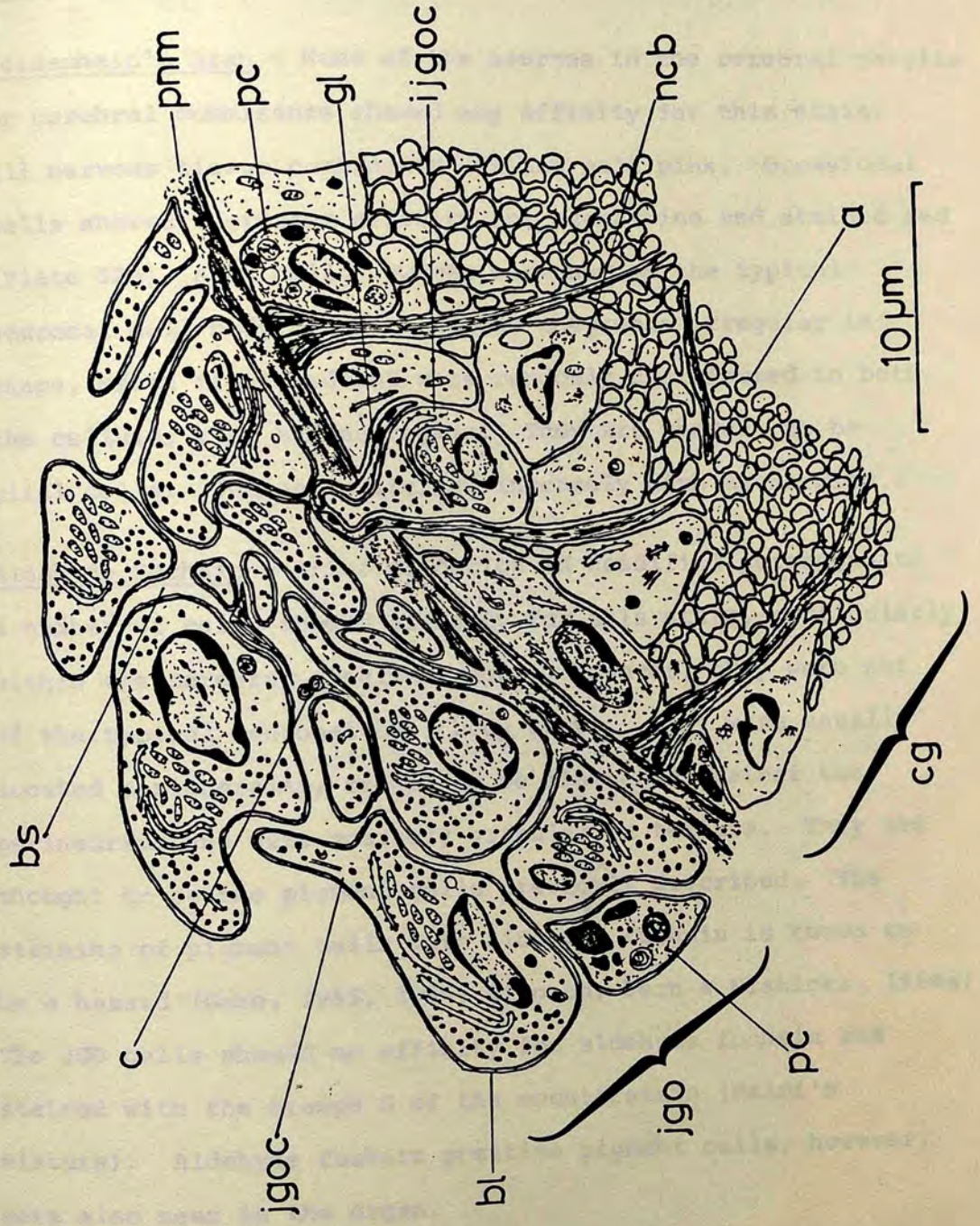
b

FIGURE 15

Gibbula umbilicalis, diagrammatic representation of part of the JGO and underlying cerebral ganglion. An internal JGO cell is shown giving rise to a process which, together with glial cell processes, passes through the perineurium into the JGO.

a - axon
bl - basal lamina
bs - blood space
c - cilium
cg - cerebral ganglion
gl - glial cell process
ijgoc - internal juxtaganglionar cell
jgo - juxtaganglionar organ
jgoc - juxtaganglionar cell
ncb - neuronal cell body
pc - pigment cell
pnm - perineurium

FIGURE 15



1.4. Selective Staining

Neurosecretion

Heidenhain's Azan - None of the neurons in the cerebral ganglia or cerebral commissure showed any affinity for this stain. All nervous tissue components stained pale pink. Occasional cells showed a greater affinity for azocarmine and stained red (Plate 52b), such cells however were not of the typical neuronal cell body shape. Instead they were irregular in shape, often flattened and were randomly distributed in both the cellular rind and neuropile. They are thought to be glial cells. JGO cells stained intensely with azocarmine.

Aldehyde Fuchsin - After oxidation in acidified permanganate a number of cells showed affinity for this stain, particularly within the cerebral ganglia. Again, however, they were not of the typical neuronal cell body shape. They were usually located peripherally, tended to be flattened against the perineurium and were coarsely granular in texture. They are thought to be the pigment cells previously described. The staining of pigment cells with aldehyde fuchsin is known to be a hazard (Gabe, 1965, 1966; Simpson, Bern & Nishioka, 1966a). The JGO cells showed no affinity for aldehyde fuchsin and stained with the orange G of the counterstain (Halmi's mixture). Aldehyde fuchsin positive pigment cells, however, were also seen in the organ.

Alcian blue/Alcian yellow (ABAY) - Positive staining with ABAY after oxidation in acid permanganate closely paralleled that of aldehyde fuchsin. Within the cerebral ganglia,

coarsely granular pigment cells were frequent usually at the periphery. These stained green indicating the presence of both sulphated and carboxyl radicals. Similar cells were also seen in the JGO, these may possibly include the pore cells seen in electron microscopy. The glandular cells of the JGO stained only with the counterstain (phloxine, 1% aqueous). Unlike the preceding stain, however, a great many tissue components throughout the body were stained in various shades of blue, green and yellow. The most noticeable were mucous cells (some of which also stained with aldehyde fuchsin) and connective tissue components, including the perineurium. This is due to the presence of sulphated and carboxylated acid mucopolysaccharides (Bancroft, 1975). They may be expected to stain without prior oxidation (Ravetto, 1964).

Nature of JGO Secretion

The results of the selective staining techniques employed together with the general staining affinity of the JGO are given below :

P.A.S.	-
Mercuric bromophenol blue (MBPB)	+++
Bromophenol blue (BPB)	+++
Deamination and BPB	+
Azocarmine	+++
Eosin	++
Orange G	++
Iron Haematoxylin	++

(+++ = strongly positive; ++ = positive; + = weakly positive; - = negative)

The strongly positive reactions obtained using the MBPB and BPB techniques and the fact that this was substantially reduced by prior deamination, indicates the presence of a large amount of proteinaceous material (NH_2- groups, Runham, 1961). That the reaction persists without mercury is indicative of a basic protein (Mazia, Brewer & Alfert, 1953). This is further supported by the fact that the tissue stains with the acidic dyes azocarmine, eosin and orange G. The deep staining of the tissue with Heidenhain's iron haematoxylin is also indicative of an abundance of proteinaceous material (Humason, 1979). The negative reaction with P.A.S. indicates little or no carbohydrate.

These results correlate well with those of Martoja (1965b) on the JGO of *Aplysia* which likewise indicated the presence of a basic protein. They also correlate with the ultrastructural appearance of the cells which is similar to that of protein secreting cells in general.

Histochemical tests designed to demonstrate the occurrence of lipid were not performed since electron microscopy revealed only minute quantities of lipid-like material. Martoja did not identify any in the JGO of *Aplysia*.

1.5. Seasonal Variation in the Activity of the JGO and Gonad

Gonad

The results of the seasonal examination of the gonad are given in Table 2 and are represented graphically in Fig. 16. The reproductive cycle can be divided into three distinct phases:-

1) The resting phase from December to early April when the gonad contains few mature oocytes (less than 10%) and appears as a loose, brown, sack-like structure. At this time the digestive gland is large and occupies most of the upper whorls of the visceral hump. The sexes can only be distinguished by examination of the right kidney opening (the urinogenital pore) which is bordered by glandular lips in the female, but is simple in the male.

2) The maturation phase from late April to early August when there is a rapid rise in the percentage of mature oocytes which reaches a peak at 70-80% at the beginning of August. At this time the gonad is green in the female (cream in the male) and almost totally envelops the much reduced digestive gland.

3) The spawning phase from late August to November. The percentage of mature oocytes is variable during this period, possibly because there is more than one burst of spawning. By the end of November, however, the percentage is similar to that of the resting phase.

These results, considering the small number of animals used correlate well with those of Underwood (1972). It is interesting to note, however, that the maturation phase

commenced approximately one month earlier (March) in animals collected in Plymouth.

A small number of animals collected for this study were found to be heavily infested with cercariae larvae of a digenean trematode. They showed no external signs of parasitism and were not noticeably larger than uninfected snails (cf. Cheng, Sullivan, Howland, Jones & Moran, 1983; Joosse & van Elk, 1983). Internally the digestive gland and gonad particularly were almost filled with trematode larvae. They are thought to comprise less than 10% of the population. Similar observations have been reported by Fretter and Graham (1962) and Underwood (1972). Infected animals were not used in this study.

JGO

Examination of the JGO at various times during the year revealed no immediately apparent differences in the ultrastructural features of the cells. At no time was there an obvious increase or decrease in the numbers of secretory granules and/or mitochondria per unit area. Golgi bodies and evidence of granule release were always scarce.

This ultrastructural examination however did reveal marked changes in the size (area in electron micrographs) of the JGO cells and their nuclei during the year, (Table 3 and Fig. 17a, b). The raw data are given in Appendix 2. Figure 18 shows both cellular and nuclear area in relation to the state of the gonad of the particular individuals examined.

It is clear from this that there was a pronounced

increase in both cell and nuclear area during the spawning phase in August/September 1980. This persisted through the resting phase of the gonad during the winter and reached a peak in April/May 1981 at the onset of maturation of the gonad. During the course of maturation both parameters decreased rapidly to reach a minimum in June/July, 1981 before beginning to increase again at the end of July.

Increases in cell and nuclear size are usually associated and frequently indicate an increase in cellular activity, particularly in secretory cells (Gabe & Arvy, 1961; Threadgold, 1976). It is therefore suggested that the JGO has a period of increasing activity commencing at the end of the spawning phase (autumn), extending over the gonad resting phase (winter and early spring) (Plate 60a), and ending once maturation has begun (late spring). This is followed by a much shorter period of reduced activity (Plate 60b) during maximum gonad maturity and spawning (summer). The increase in JGO activity after the summer resting phase appears to occur earlier in 1981 than in 1980. This may be due to changes in the environmental conditions from one year to the next. An additional sample from August 1981 may have proved useful in order to determine whether or not the relatively high figure for July 1981 really does represent the onset of increased JGO activity.

The suggested rise in activity may be expected to involve an increase in the amount of RER and an elevation of the number of mitochondria and secretory granules (and active Golgi bodies). As already stated, however, this was not

obvious. The frequency of these organelles, although not assessed quantitatively, appeared to remain constant. This may in part be explained by examination of Figure 19 which shows changes in cytoplasmic area during the year (calculated from cell area and nucleus area). This shows that cytoplasmic area is greatly elevated by the end of the active phase of the JGO. Such an increase in cytoplasmic area without a concomitant decrease in organelle density (per unit area) must therefore indicate an overall increase in organelle numbers.

As part of this study the JGO of a juvenile female (shell width 8.0mm) was also examined. The specimen was collected in March 1981 and the gonad was small with no histological evidence of mature oocytes. Its sex was established by examination of the urinogenital pore. Structurally, the JGO cells were similar to those of adult females (Plate 60c, d), but were markedly smaller than the adult examined at this time of year, suggesting that the organ was inactive. The mean cell and nuclear areas are given in Table 3 and are also plotted on the graphs in Figure 17a, b (J).

In analysing these results, however, it must be remembered that the figures for each sample are based on the cells of only one animal. Measurements of cell and nucleus area were taken simply to provide some quantitative evidence of a trend observed on qualitative examination. They should not be taken as proof of variation in JGO activity, but merely as an indication.

TABLE 2

RESULTS OF QUANTITATIVE ASSESSMENT OF GONAD MATURITY (OVARY)

SAMPLE	DATE	SPECIMEN	TOTAL NUMBER OF OOCYTES	NUMBER OF MATURE OOCYTES	NUMBER OF IMMATURE OOCYTES	% MATURE OOCYTES	MEAN % MATURE OOCYTES
1	25. 8.80	a	235	116	119	49.4	48.8
		b	285	137	148	48.1*	
2	22. 9.80	a	433	62	371	14.3*	14.5
		b	356	52	304	14.6	
3	19.10.80	a	289	131	158	45.3	36.8
		b	397	112	285	28.2	
4	15.11.80	a	477	102	375	21.4*	14.4
		b	496	36	460	7.3	
5	13.12.80	a	327	0	327	0.0	6.3
		b	372	47	325	12.6	
6	10. 1.81	a	374	19	355	5.1*	5.4
		b	442	25	417	5.6	
8	8. 3.81	a	354	43	311	11.9	8.6
		b	473	25	448	5.3	
9	4. 4.81	a	427	17	410	4.0*	2.4
		b	360	3	357	0.8	
10	10. 5.81	a	346	113	233	32.7*	32.8
		b	420	138	282	32.9	
11	31. 5.81	a	251	161	90	64.1	56.8
		b	281	139	142	49.5*	
12	28. 6.81	a	299	165	134	55.2	56.0
		b	308	175	133	56.8*	
13	25. 7.81	a	245	177	68	72.2	73.4
		b	231	172	59	74.5*	

* animal used for ultrastructural examination of the JGO.

TABLE 3

AREA OF CELLS AND NUCLEI IN JUXTAGANGLIONAR ORGAN AT DIFFERENT TIMES OF YEAR.

SAMPLE	DATE	SPECIMEN	NUCLEAR AREA (μm^2) MEAN (n=5)	NUCLEAR AREA (μm^2) S.E.M.	CELL AREA (μm^2) MEAN (n=5)	CELL AREA (μm^2) S.E.M.	% MATURE OOCYTES
1	25. 8.80	b	7.6	0.5	29.5	1.6	48.1
2	22. 9.80	a	9.8	0.6	54.8	5.3	14.3
4	15.11.80	a	18.0	2.0	85.0	3.4	21.4
6	10. 1.81	a	18.1	1.3	78.9	6.6	5.1
9	4. 4.81	a	20.2	2.4	113.4	14.0	4.0
10	10. 5.81	a	21.1	2.3	109.2	5.1	32.7
11	31. 5.81	b	10.4	0.4	67.6	10.8	49.5
12	28. 6.81	b	7.7	0.7	45.2	2.7	56.8
13	25. 7.81	b	14.7	0.4	66.7	7.6	74.5
JUVENILE	8. 3.81	-	5.3	0.7	33.3	2.1	0.0

FIGURE 16

Gibbula umbilicalis, graph showing female reproductive cycle during one year. Each point represents the average of two animals.

W - end of major burst of spawning

X - middle of resting phase

Y - onset of maturation phase

Z - maximum gonad maturity

FIGURE 16

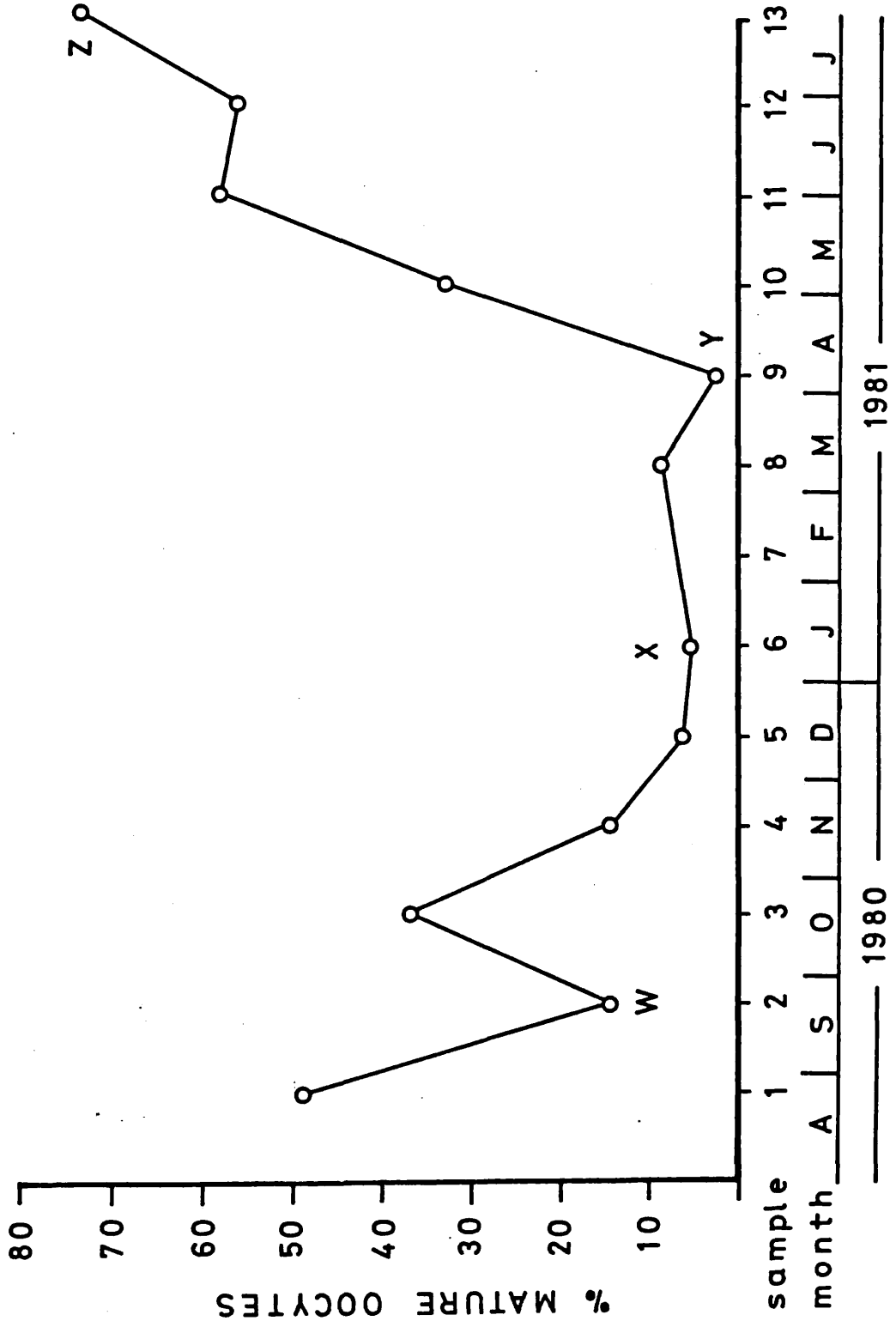

% MATURE OOCYTES
80
70
60
50
40
30
20
10
W
X
Y
Z
sample
month
A
S
O
N
D
J
F
M
A
M
J
J
1
2
3
4
5
6
7
8
9
10
11
12
13
1980
1981

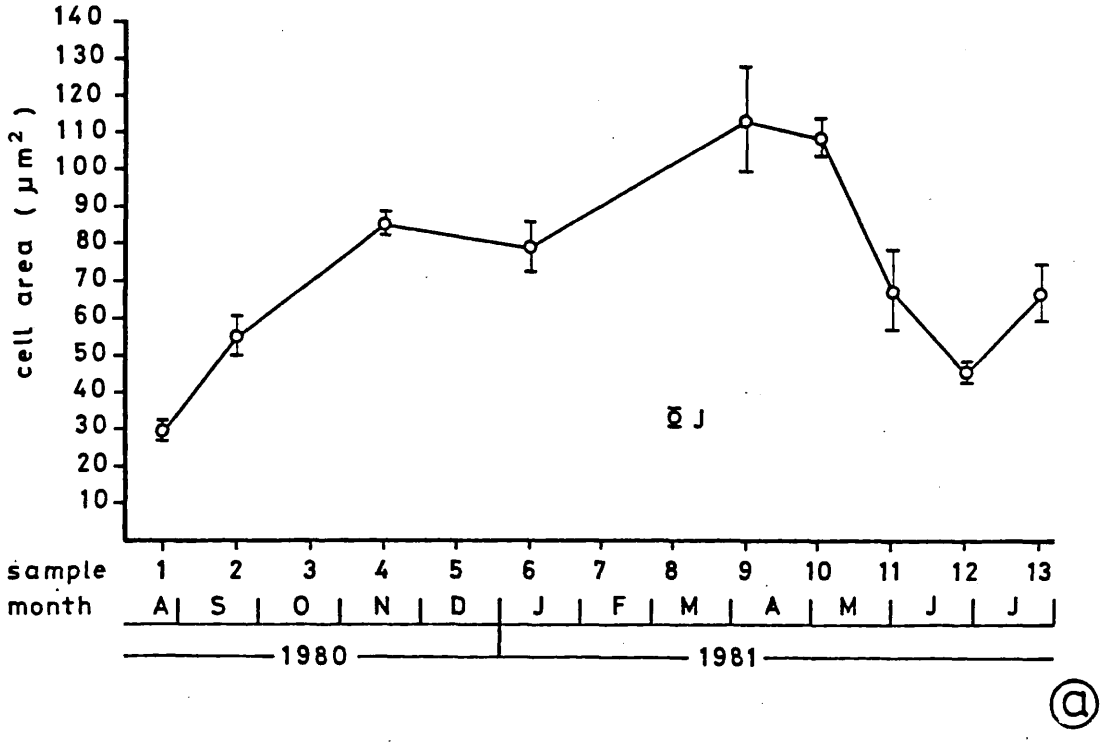
FIGURE 17

a) *Gibbula umbilicalis*, seasonal variation in area of JGO cells.

b) *Gibbula umbilicalis*, seasonal variation in area of nuclei of JGO cells.

Each point represents 5 cells or nuclei from one individual. Bars are standard error bars.
J = juvenile specimen

FIGURE 17


cell area (µm²)
140
130
120
110
100
90
80
70
60
50
40
30
20
10
J
sample 1 2 3 4 5 6 7 8 9 10 11 12 13
month A S O N D J F M A M J J
1980
1981
a

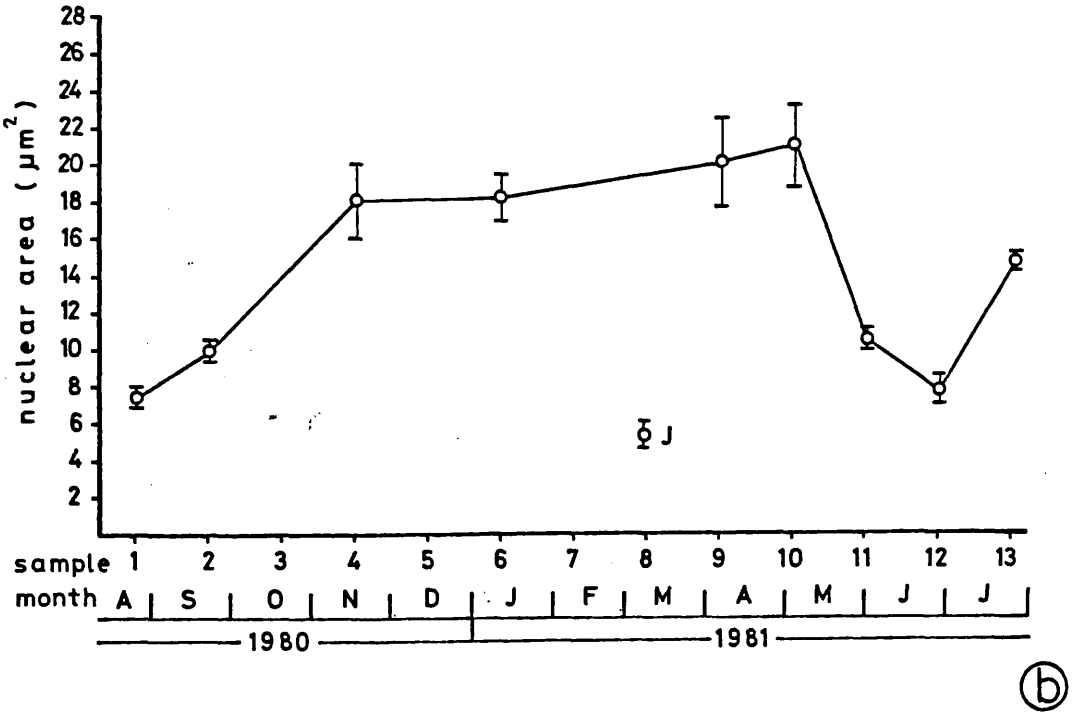

nuclear area (µm²)
28
26
24
22
20
18
16
14
12
10
8
6
4
2
J
sample 1 2 3 4 5 6 7 8 9 10 11 12 13
month A S O N D J F M A M J J
1980
1981
b

FIGURE 18

Gibbula umbilicalis, graph showing seasonal variation in area of JGO cells and nuclei in relation to the reproductive maturity of the individuals examined.

J = juvenile specimen

FIGURE 18

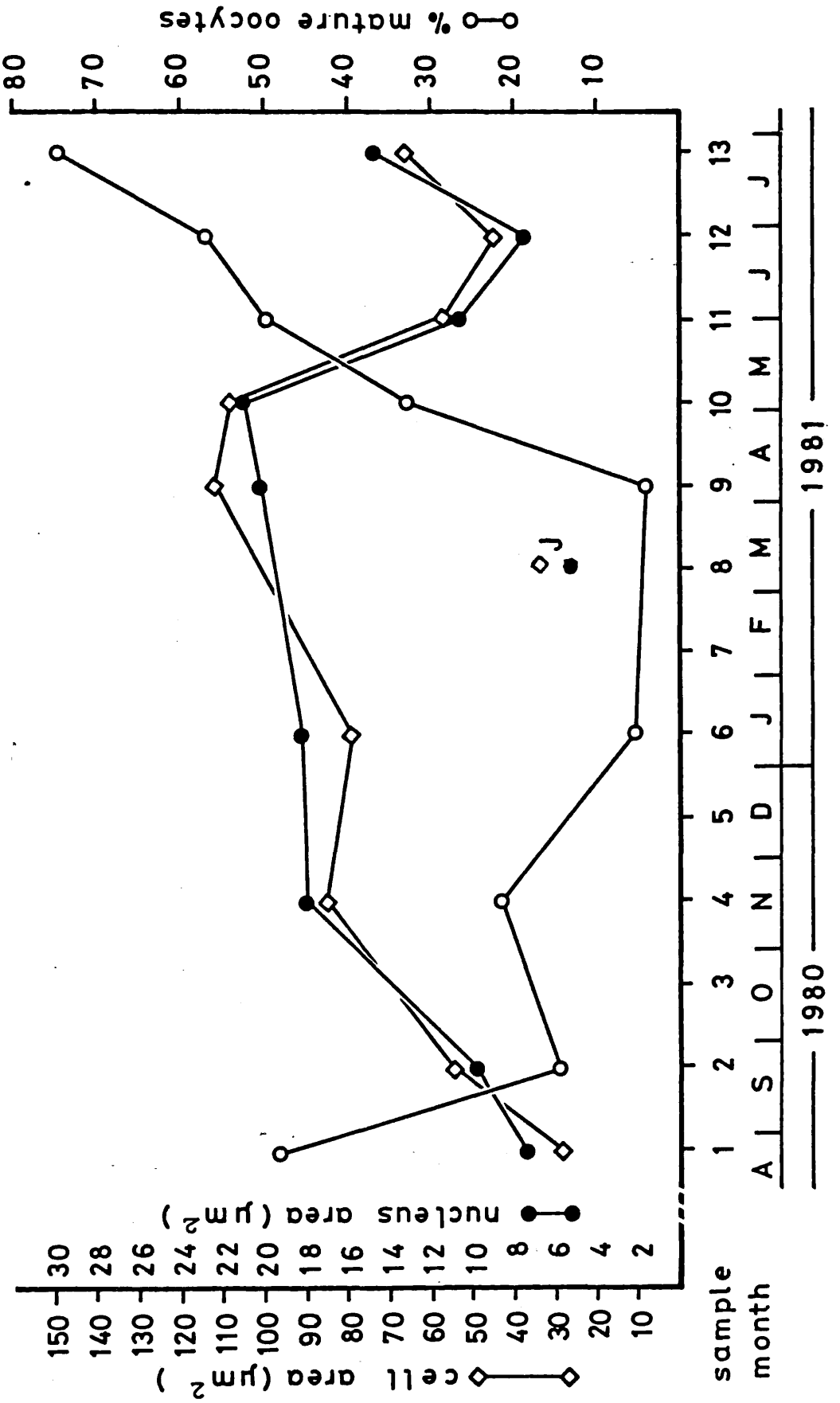

% mature oocytes
cell area (μm²)
nucleus area (μm²)
sample month
A S O N D J F M A M J J
1980
1981

FIGURE 19

Gibbula umbilicalis, graph of seasonal variation in cytoplasmic area (calculated from cell area - nuclear area) of JGO cells in relation to the reproductive maturity of the individuals examined.

J = juvenile specimen

FIGURE 19

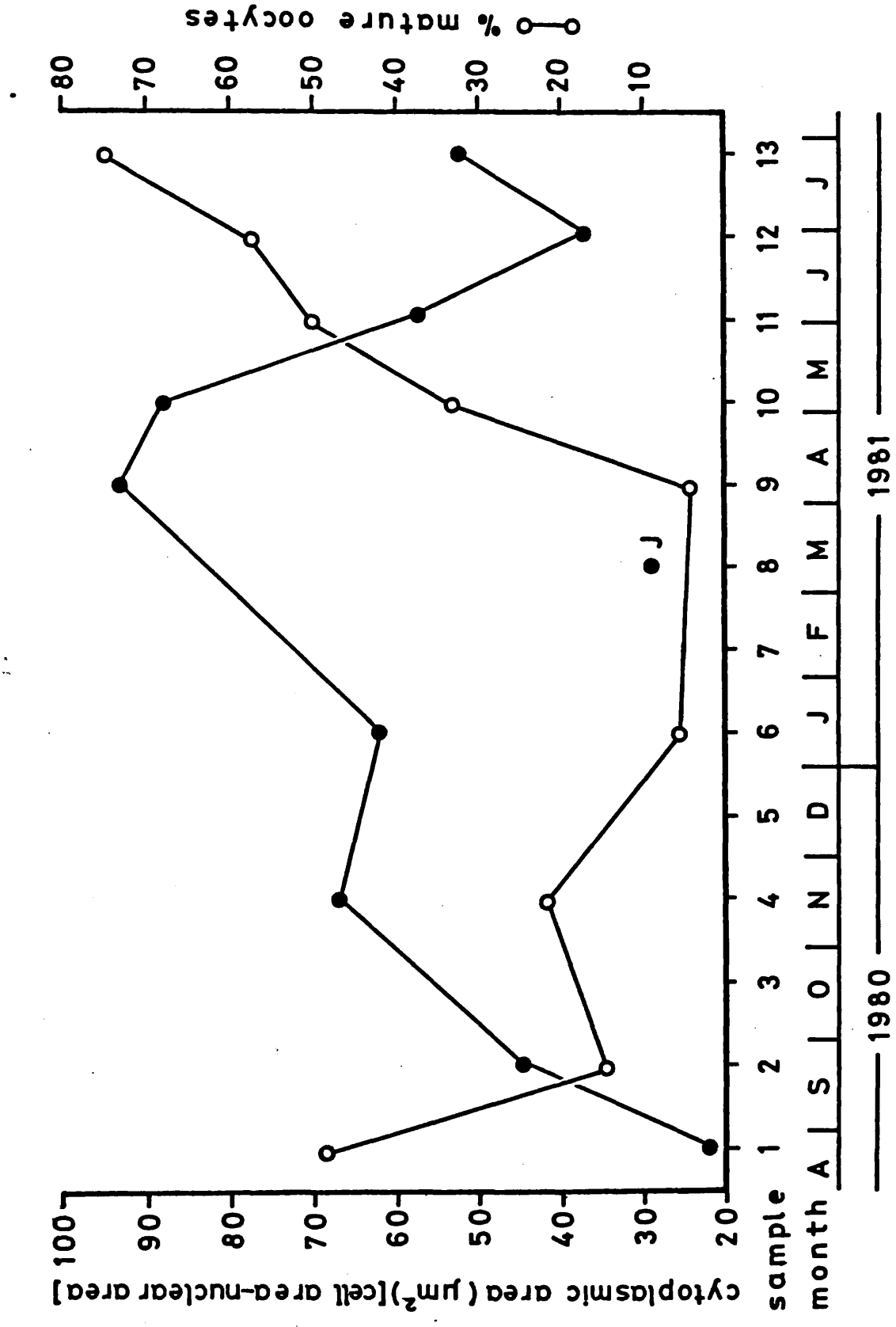

% mature oocytes
80
70
60
50
40
30
20
10
cytoplasmic area (μm²)[cell area-nuclear area]
100
90
80
70
60
50
40
30
20
sample
1
2
3
4
5
6
7
8
9
10
11
12
13
month
A
S
O
N
D
J
F
M
A
M
J
J
1980
1981
J

PLATE 60

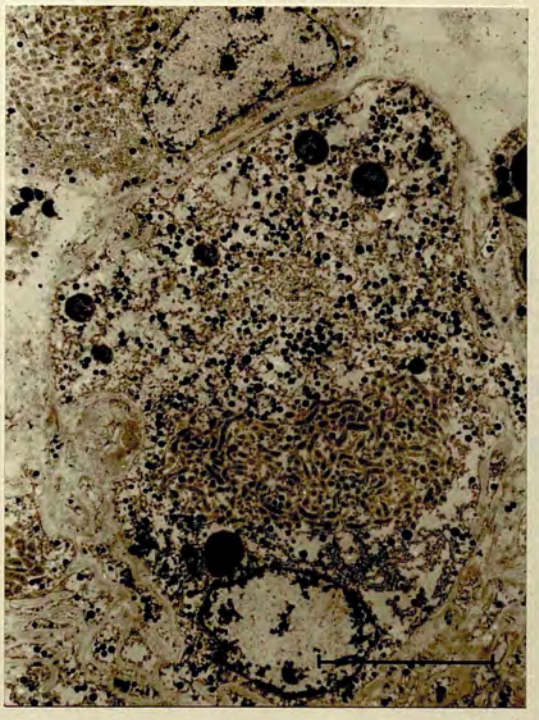
a) *Gibbula umbilicalis*, large cell from thin resin section of JGO during its active phase, (Sample 9, April 1981). Bar = 5.0µm.

b) *Gibbula umbilicalis*, large cell from thin resin section of JGO during its resting phase, (Sample 1, August 1980). Bar = 5.0µm.

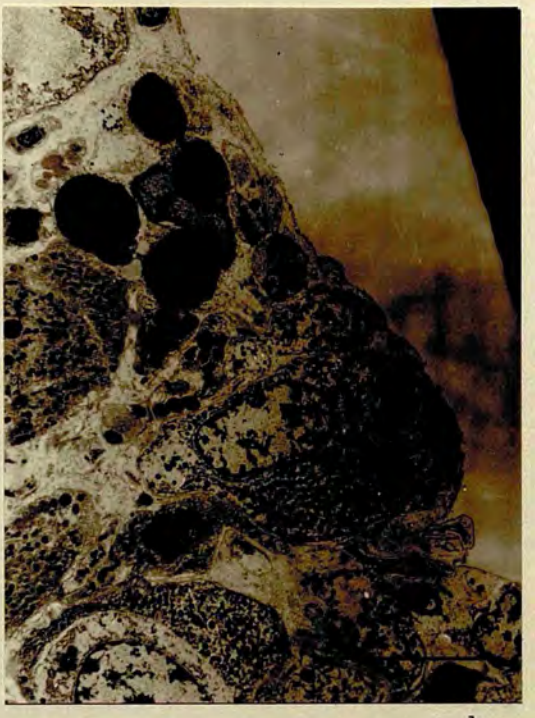
c) *Gibbula umbilicalis*, JGO of juvenile specimen. Bar = 5.0µm.

d) *Gibbula umbilicalis*, JGO of juvenile specimen. Bar = 5.0µm.

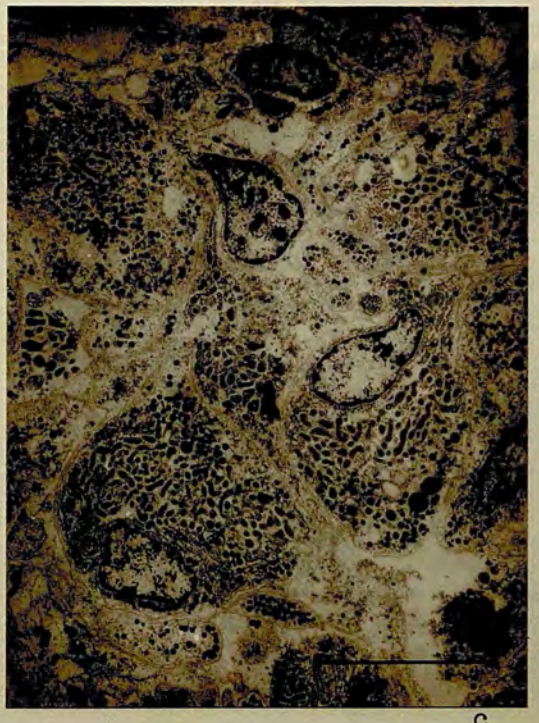
PLATE 60



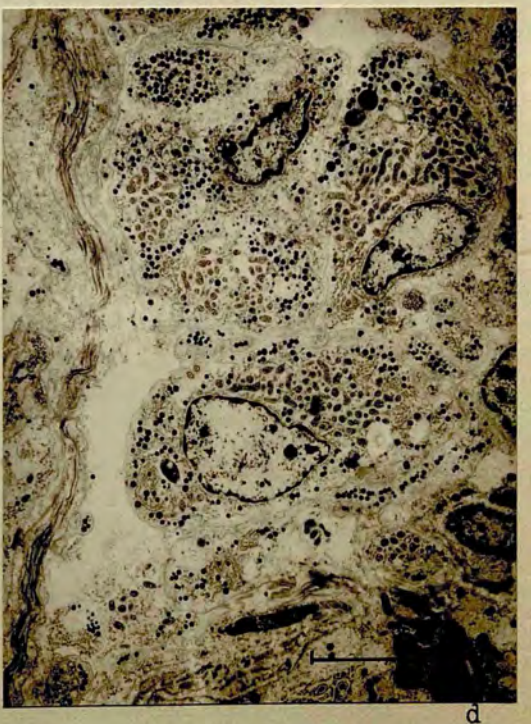
a



b



c



d

SECTION 2

The Juxtaganglionar Organ of *Aplysia punctata*

HISTOLOGY

Observations on paraffin wax sections of the JGO of *Aplysia punctata* correlate well with those of Martoja (1965b). It is composed of small groups of cells lying on the postero-dorsal face of each cerebral ganglion, in close contact with the perineurium (Plate 61a). The cytoplasm stains intensely with azocarmine, but the layers of cells noted by Martoja were not observed. In addition, cells of similar size and staining affinity occur frequently within the cerebral ganglion, especially in the medio-dorsal region (Plate 61b). These were not noted by Martoja and as in *Gibbula umbilicalis* will be called the internal JGO cells.

ULTRASTRUCTURE

The groups of cells seen in light microscopy are clearly visible under the electron microscope (Plate 62a, c). They are usually composed of 4-6 cells and are separated from one another by narrow blood spaces. Like that of *G. umbilicalis* the organ as a whole seems to lie within the outer layer of the perineurium, but in this species the ganglia are covered by additional layers of connective tissue which separate the JGO from the cephalic haemocoel. These connective tissue layers are, however, penetrated by numerous pores which are thought to allow the passage of haemolymph into the region surrounding the JGO (Plate 62b). As in the trochid, there is a basal lamina surrounding every cell within the organ, though in some groups cells were occasionally observed in membrane to membrane contact.

The JGO cells are variable in shape, in these

specimens rarely exceeding 16μm in any dimension. They possess an irregularly shaped nucleus with a prominent nucleolus and cytoplasmic processes which interdigitate with those of other cells (Plate 62d). The cytoplasm contains numerous mitochondria scattered throughout, but the most striking feature of these cells, particularly in comparison with those of *G. umbilicalis*, is the almost complete lack of electron-dense secretory granules and RER (at least at this time of year). Occasional granules (or droplets) with a diameter of 500-600nm occur, but none with a diameter of 100-200nm were seen. The more distal parts of the cell processes however, were observed to contain a small number of granules approximately 70nm in diameter (Plate 63a). These small granules were occasionally seen within the cell body, particularly near the Golgi body from which they appear to arise (Plate 63b). (Golgi bodies were more commonly observed in this species, possibly due to the superior fixation of the tissue.) Cilia frequently arise from invaginations in the plasma membrane and project into the intercellular space. They have the normal 9 + 2 subfibril complement (Plate 63a).

The internal JGO cells are in most respects identical with those in the JGO itself and occur in larger numbers than in *G. umbilicalis* (Plate 63c). They lie in groups in close proximity to the perineurium. Apart from their location they may be distinguished by the fact that they are never surrounded by a basal lamina. They bear cytoplasmic processes and cilia which project into the intercellular spaces within the ganglion (Plate 64a). The processes are directed towards the perineurium and like their counterparts outside the ganglion

contain small granules (Plate 64b). There is also evidence that these processes penetrate the perineurium and pass into the JGO (Plate 64c).

In regions where there are no internal JGO cells the very large neuronal perikarya directly abut the perineurium beneath the JGO together with what are presumed to be the processes of glial cells (Plate 65d). These perikarya may contain numerous granules up to 200-300nm in diameter and of variable electron-density (possibly neurosecretion). No evidence of intimate contact ('synapse-like' contacts) was observed between nervous tissue and JGO cells, internal or otherwise.

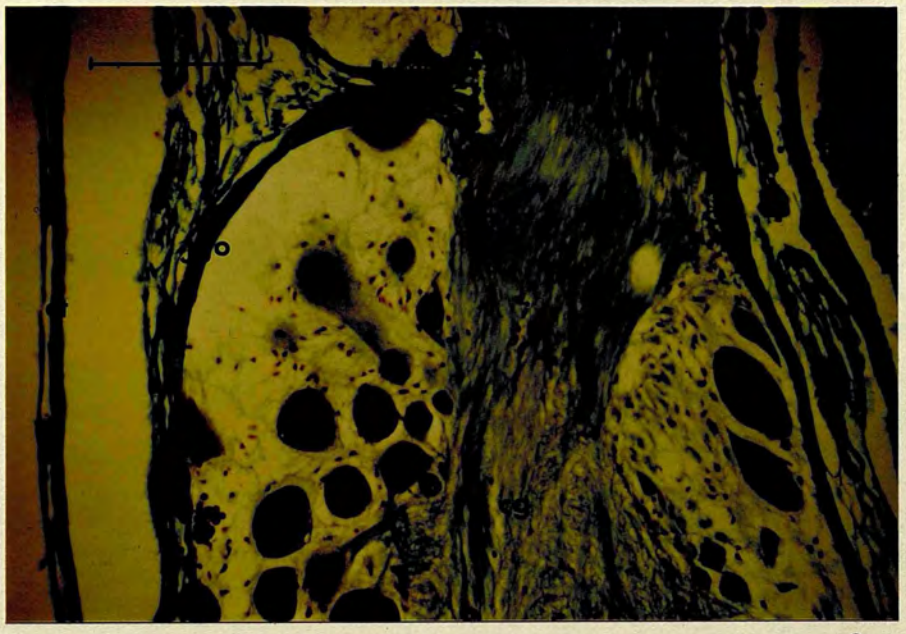
PLATE 61

a) *Aplysia punctata*, paraffin wax section of part of the cerebral ganglion and commissure showing location of JGO. Heidenhain's Azan.
Bar = 200µm.

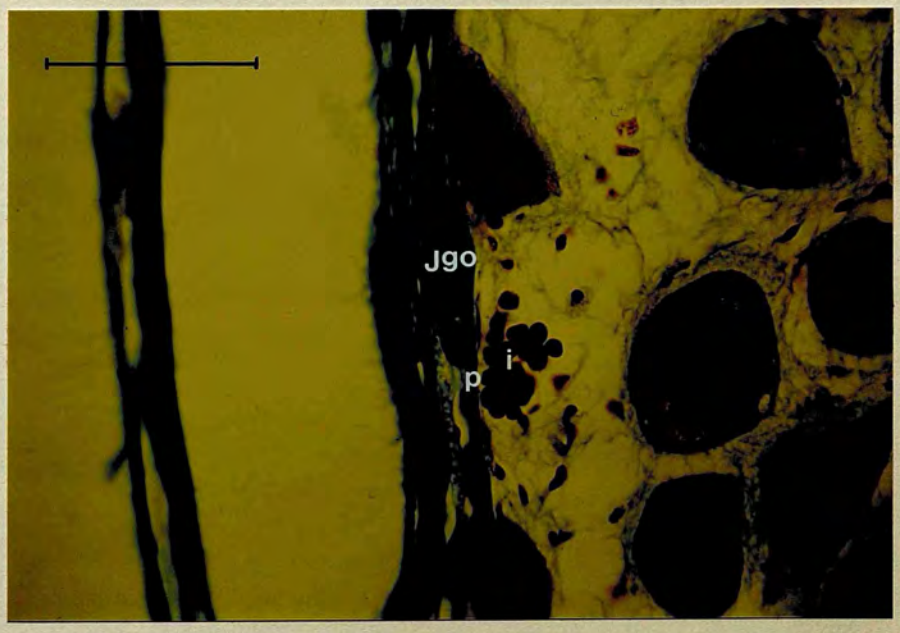
b) *Aplysia punctata*, part of the above section enlarged to show internal JGO cells. Heidenhain's Azan.
Bar = 100µm.

cc - cerebral commissure
cg - cerebral ganglion
ct - connective tissue sheath
i - internal JGO cells
jgo - juxtaganglionar organ
p - perineurium

PLATE 61



a



b

PLATE 62

a) *Aplysia punctata*, low magnification electron micrograph of the JGO showing grouping of cells. Note obvious lack of secretory granules when compared to the JGO of *Gibbula umbilicalis*.
Bar = 10µm.

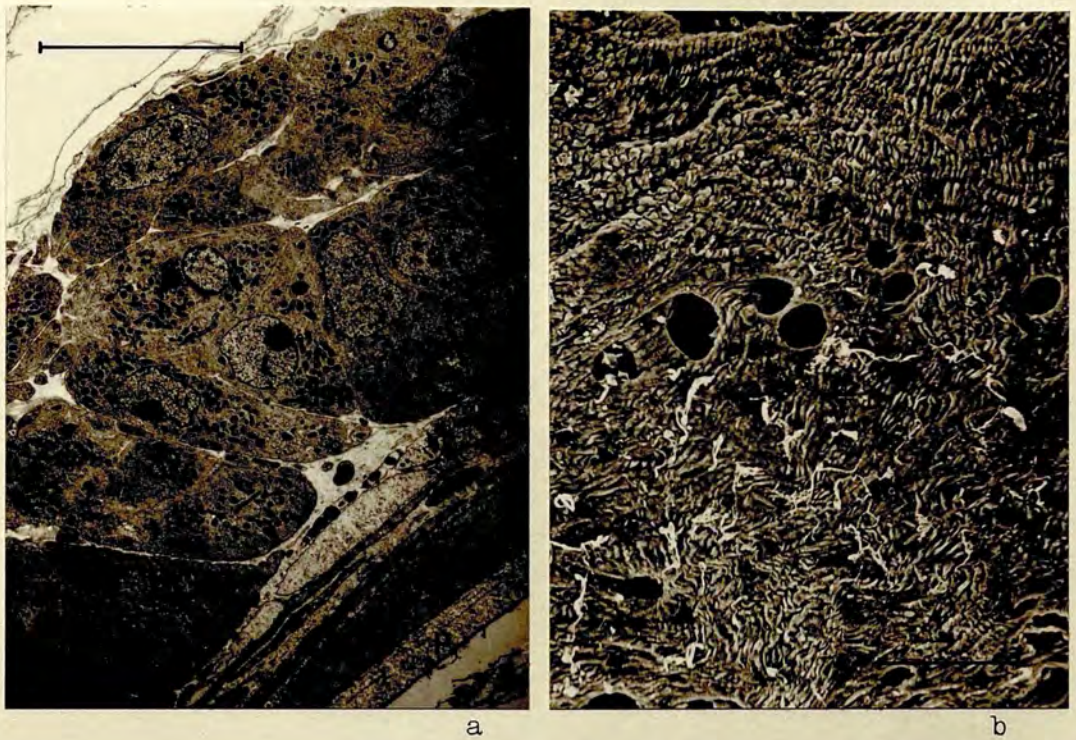
b) *Aplysia punctata*, scanning electron micrograph of dorsal surface of the cerebral ganglion. Note pores in connective tissue sheath.
Bar = 100µm.

c) *Aplysia punctata*, portion of perineurium with JGO cells on both sides.
Bar = 5.0µm

d) *Aplysia punctata*, interdigitating cell processes in the JGO. Note surrounding basal lamina.
Bar = 1.0µm.

bl - basal lamina
cg - cerebral ganglion
i - internal JGO cell
jgo - juxtaganglionar organ
p - perineurium
pr - process of JGO cell

PLATE 62



a b

c d

PLATE 63

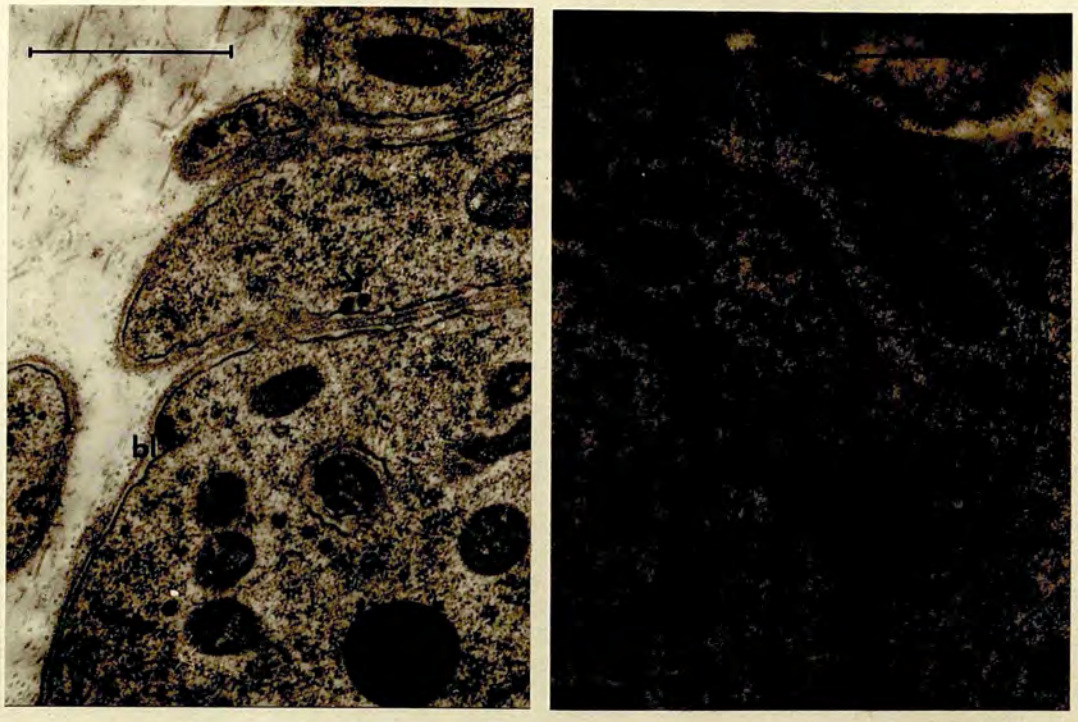
a) *Aplysia punctata*, the edge of a group of JGO cells. Note transverse section of cilium in an invagination of the cell membrane and small electron-dense secretory granules (arrow).
Bar = 1.0µm.

b) *Aplysia punctata*, JGO cell with mitochondria and Golgi body. Note small granules associated with the Golgi body (arrow).
Bar = 1.0µm.

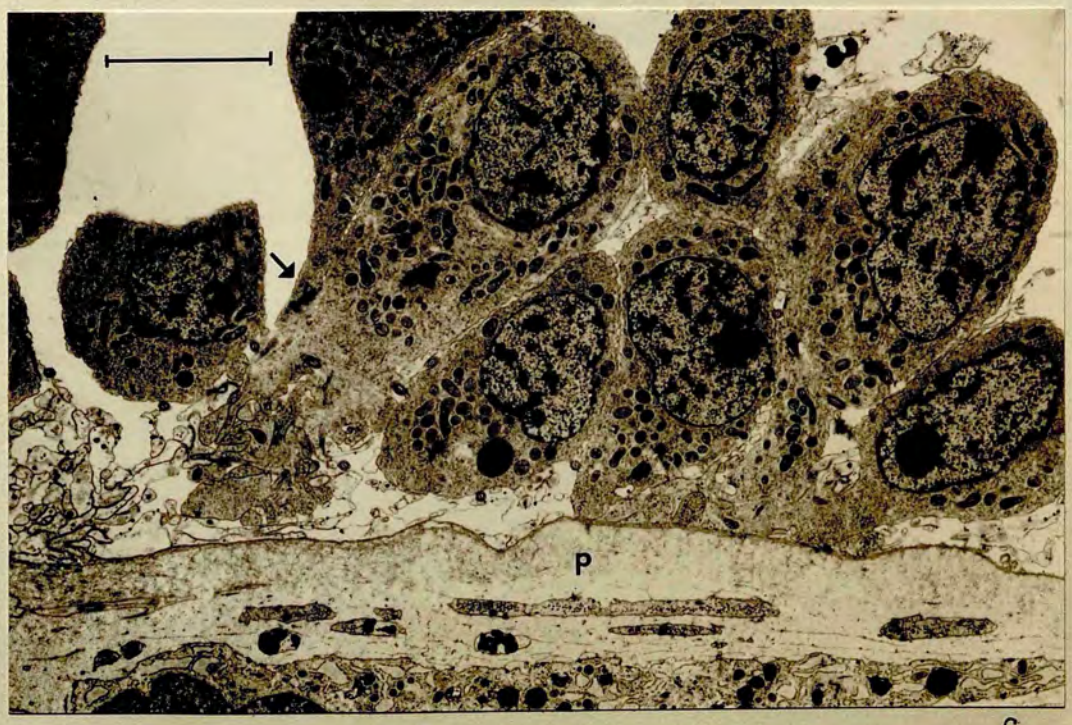
c) *Aplysia punctata*, group of internal JGO cells. Note processes directed toward the perineurium (arrow). The large spaces are thought to result from shrinking during preservation.
Bar = 5.0µm.

bl - basal lamina
c - cilium
g - Golgi body
m - mitochondrion
p - perineurium

PLATE 63



a b



c

PLATE 64

a) *Aplysia punctata*, internal JGO cells. Note cilia associated with the cells and the absence of a basal lamina.
Bar = 2.0µm.

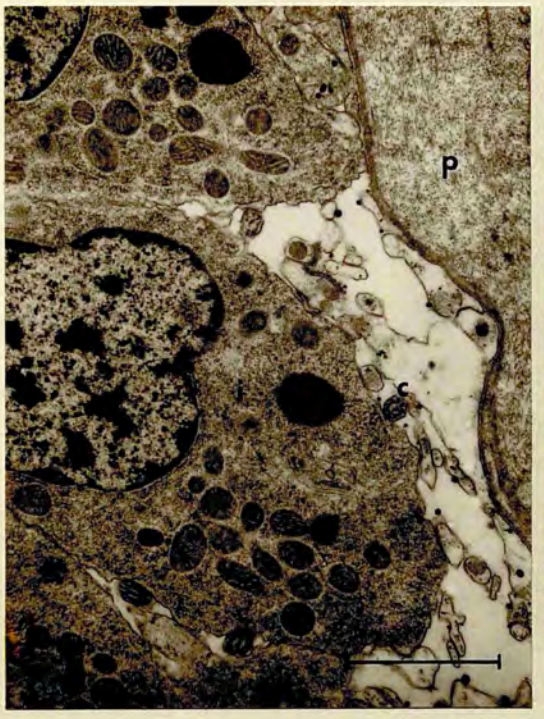
b) *Aplysia punctata*, granule laden processes of internal JGO cells underlying the perineurium.
Bar = 1.0µm.

c) *Aplysia punctata*, processes from (presumed) internal JGO cells passing through the perineurium. Glial cell processes surround the internal JGO cell.
Bar = 2.0µm.

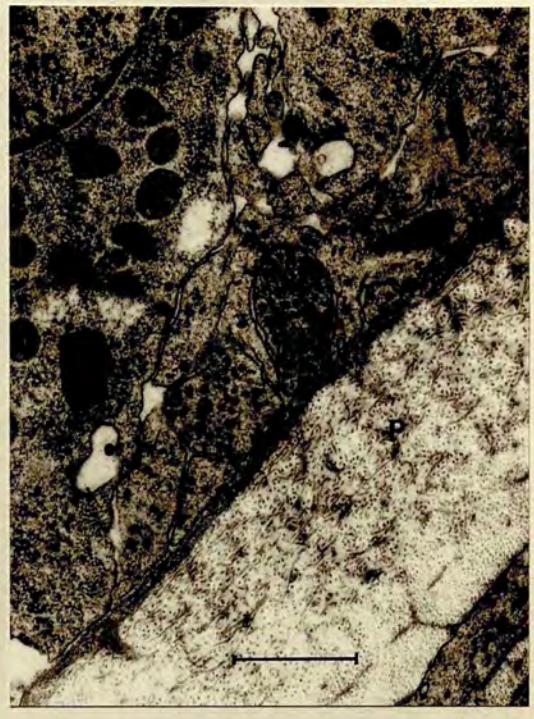
d) *Aplysia punctata*, part of a large neuronal perikaryon within the cerebral ganglion abutting the perineurium in a region where internal JGO cells are absent. The cytoplasm contains numerous granules of varying electron-density, possibly neurosecretion. Glial cell processes interdigitate with the plasma membrane.
Bar = 2.0µm.

c - cilium
i - internal JGO cell
n - neuronal perikaryon
p - perineurium
pr - process of internal JGO cell

PLATE 64

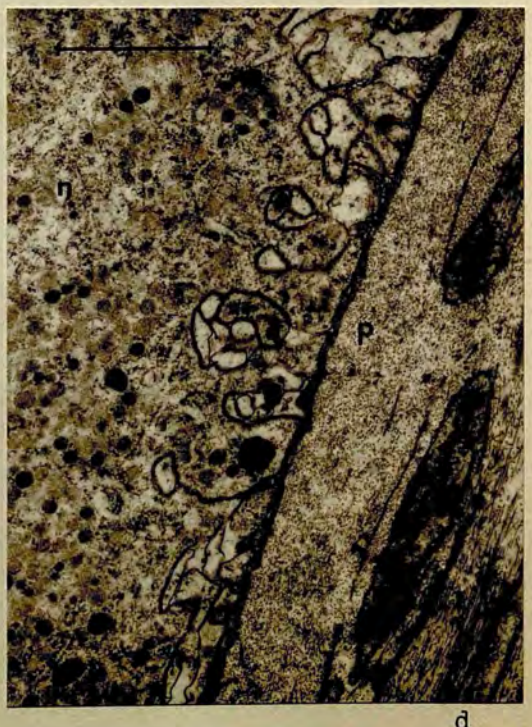


a



b

c



d

C H A P T E R 8

DISCUSSION

It is evident from these results that the JGO of *Gibbula umbilicalis* possesses many of the characteristics of an epithelial endocrine organ. It is composed primarily of glandular cells with interdigitating processes, it has no secretory ducts, it is extensively penetrated by blood space lined by basal lamina, and there are indications of cyclical variation in activity (Tombes, 1970; Barrington, 1975; Highnam & Hill, 1977). As such it may be taken as a non-nervous structure similar to the corpora allata and thoracic glands of insects, the Y-organs and androgenic glands of crustaceans and also the epithelial endocrine glands of vertebrates.

The presence of large numbers of highly cristate mitochondria indicates that the cells are capable of considerable activity. The abundant RER suggests that this takes the form of protein synthesis, the end-product of which is packaged into the numerous, electron-dense, membrane bound secretory granules, 100-200nm in diameter. Granules of this size-range are frequently seen in endocrine cells (Pearse, 1969, 1971). Secretion release occurs by simple exocytosis (merocrine secretion) from the cell processes.

The results of selective staining techniques and the general tinctorial affinity of the tissue indicate the presence of considerable proteinaceous material of a basic

nature. This is supported by the ultrastructural appearance of the cells which shows much similarity with that of the peptide secreting cells of the mammalian adenohypophysis (Young, 1965; Foster, 1971) and with peptide secreting cells in general (Pearse, 1968, 1969, 1971; Gould, 1978). The scarcity of Golgi bodies is surprising in protein secreting cells, but this is thought to be due to the difficulty experienced in obtaining good membrane preservation.

The presence of cilia on the JGO cells is unusual, but is not unknown in other endocrine structures, e.g. the mammalian adenohypophysis (Young, 1965; Dingemans, 1969; Foster, 1971; Heap, Lederis & Neumann, 1971). It is not known whether they are motile and aid in the circulation of haemolymph, though the surrounding basal lamina would severely limit movement. Their presence may simply reflect the epithelioid nature of the tissue.

The organ appears to be poorly innervated, although axons were occasionally seen. These are believed to relate to the small muscle fibres present in the connective tissue. No evidence of neurosecretion was found either histochemically or ultrastructurally either in the JGO or abutting the perineurium beneath it. Synapse-like structures between neurons and gland cells were never observed.

The occurrence of glandular cells (the internal JGO cells) within the cerebral ganglion is an uncommon feature, although Clark (1955) has indicated in nephthyd polychaetes that glandular epidermal cells exist in posterior lobes of the brain. The possible functional significance

of these cells is discussed later in relation to the pulmonate DB. Their presence, however, indicates that the possibility of a nervous origin of the organ cannot be excluded, although evidence from studies on the DB would indicate a postembryonic origin from perineural elements.

Cyclical variation in cell and nuclear area is taken to reflect changes in cellular activity. Gland cells in general are thought to increase in size with increasing activity (Gabe & Arvy, 1961; Threadgold, 1976). Similar observations have been reported in other endocrine organs e.g. the pulmonate DB (Joosse, 1964) and the vertebrate pituitary (Iwasawa & Kera, 1982; Jacobi, Lloyd & Mears, 1982; Young & Ball, 1983). In this case the changes are seasonal and appear to be linked with the reproductive cycle. Cell and nuclear area and hence activity rise steadily over the winter during the gonad resting phase reaching a peak just after the onset of maturation in the spring. A short period of reduced activity then follows before the start of the next cycle.

As stated in the Results however, the observations concerning variations in cellular activity are based on very few animals (one per sample). They can therefore only be used to give an indication of the actual situation. Furthermore they concern only females, no information was obtained with regard to cyclical activity of the JGO in males.

Nevertheless these results are consistent with those of Martoja (1965a, b) on *Hydromyles globulosa* and *Aplysia punctata*. She noted that JGO was largest immediately prior

to maturation and that by gamete release it had undergone considerable atrophy. It was this that led her to suggest that the JGO had a role in the stimulation of maturation. It is tempting to draw the same conclusions from the results presented here. It must, however, be stated that whilst variation in cell size may indicate variations in cell activity they give no indication concerning secretion release. This last aspect is of considerable importance in determining the function of the JGO and is discussed later.

General Features of the JGO of *Aplysia punctata*

Examination of the JGO of *Aplysia punctata* reveals an overall similarity with that of *Gibbula umbilicalis*. The structure lies within the outer layer of the perineurium, is composed of ciliated glandular cells rich in mitochondria and is poorly innervated. There are JGO cells within the cerebral ganglion (comparatively large in number) with no apparent contact with neurosecretory cells, but with evidence of processes passing through the perineurium into the JGO itself.

There are, however, clear distinctions, notably the similar size and scarcity of the secretory granules and the less abundant RER. In part this may be due to the month of examination (March), which was at a time when the gonad was reaching maximal maturity. (In fact, animals were seen to copulate and spawn in aquarium tanks.) The results obtained here for *G. umbilicalis* and the observations of Martoja (1965b) suggest that at this time the JGO would be inactive. This however, cannot explain the smaller size of the granules in *A. punctata*. Furthermore, even when inactive the JGO cells of *G. umbilicalis* always contained an appreciable number of granules, both in the cell body and in the cytoplasmic processes.

Possible Homology with the Pulmonate Dorsal Body

The general features exhibited by the JGO of both species do not support the initial hypothesis by Vicente (1966) that the organ is a neurohaemal-storage organ similar to the crustacean sinus gland. The latter is composed primarily of the terminations of neurosecretory cells originating in the brain and X-organ (Highnam & Hill, 1977), while the JGO contains mostly intrinsic glandular cells. (Recent evidence suggests that there may be rare intrinsic glandular cells in the sinus gland (May & Golding, 1983).

Instead, the suggestion of Martoja (1965a, b, c, 1972) and Joosse (1972, 1979) that the JGO is an endocrine organ in its own right, possibly representing the prosobranch and opisthobranch homologue of the pulmonate DB seems more acceptable. Indeed, the two organs show considerable structural similarity.

Table 4 is a comparison between the JGO and DB, based on the observations described here for the JGO of *G. umbilicalis* and those of Joosse (1964) and Boer, Slot & van Andel, (1968) for the DB of basommatophoran pulmonates.

It is evident from this that whilst there may be an overall similarity, differences do exist between the JGO and DB. These, however, are perhaps not surprising in such widely separated groups of gastropods, since there is considerable variation in DB fine structure between basommatophoran and stylommatophoran pulmonates.

The differences between the JGO and DB in much more distantly related gastropods would not seem incompatible with possible homology. Indeed, considering the variation shown within the pulmonates one might expect additional differences in a group as far removed as the archaeogastropods.

The JGO of *A. punctata* has features in common with that of the trochid and with the DB. This might suggest a common origin of the organs, possibly related to the archaeogastropods and is further indication of homology. Like that of the trochid it possesses glandular cells with a proteinaceous content (Martoja, 1965b) and additionally JGO cells which occur in the cerebral ganglion. Like the DB, however, the cells tend to be grouped and the granules they contain are small (70nm in diameter).

TABLE 4

COMPARISON BETWEEN THE JGO OF *Gibbula umbilicalis* AND THE DB OF BASOMMATOPHORAN PULMONATES (Joosse, 1964; Boer *et al.*, 1968)

SIMILARITIES

1. The association of the structure with the cerebral ganglia.
2. The location within the perineurium.
3. The occurrence of gland cells interspersed with connective tissue.
4. The lack of any secretory ducts.
5. The penetration of the tissue by blood space.
6. The occurrence of glandular tissue in the cerebral ganglia (not *Lymnaea stagnalis*).
7. The seasonal variation in the size of the cells and their nuclei and its apparent link with the reproductive cycle.

DIFFERENCES

1. DB cells are organised in groups, separated by radial strands of connective tissue.
2. The DB has a medulla of cell processes all directed towards the cerebral ganglion and a cortex of cell bodies (not in Stylommatophora).
3. DB elements in the nervous tissue (when they occur) are processes of cells not cell bodies as in the JGO.

Continued

4. DB secretory granules are 60-90nm in diameter and tend to be concentrated in cytoplasmic processes. JGO granules are 100-200nm in diameter and show no particular concentration in the cytoplasmic processes.
5. Mitochondria of the DB cells have tubular cristae, those of the JGO cells have septate cristae.
6. The DB cells may have a conspicuous SER.

Relationship of the JGO with the Nervous System

A great deal of the morphological variation in the DB of pulmonates is concerned with the degree to which it is associated with the nervous system. Boer, Slot and van Andel (1968) have shown that the DB in certain basommatophoran species, viz. *Australorbis glabratus* (Say) and *Planorbarius corneus* (L.) a specialised area, the disc, lies between the DB (medio-dorsal body) and the cerebral ganglion. In this region DB cell processes and neuronal processes may be in close contact, not separated by basal lamina. This also occurs in *Ancylus fluviatilis* Müller, but to a lesser extent, and also in *Helisoma* (Simpson, Bern & Nishioka, 1966b). In other species such as *Lymnaea stagnalis*, such intimate contact was apparently absent.

It was this lack of uniformity even within one order, combined with the fact that the neuronal elements involved were derived from neurosecretory cells (the light green cells) known to have a neurohaemal area in the median lip nerve, that led Boer, Slot and van Andel (1968) to suggest that DB-cerebral ganglion contact was of little functional significance. They believed that it reflected the embryological origin of the DB from elements of the perineurium.

The Stylommatophora with their generally much more dispersed DB (sometimes extending around the suboesophageal ganglia (Minnen, Wijdenes & Sokolove, 1983), were originally believed not to show any form of DB-cerebral ganglion contact. Recently, however, it has been shown that nerves arising

from the cerebral commissure branch out amongst the DB cells, frequently making 'en passant', synapse-like contact with them (Nolte, 1978, 1983; Wijdenes & Vincent, 1981; Wijdenes, Vincent, Griffond & Gomot, 1983). These nerves are thought to originate from neurosecretory growth hormone producing cells (homologous with the light green cells of basommatophorans) in the cerebral ganglia. Wijdenes (1981) postulated that the antagonism between growth and reproduction may have its morphological basis in an inhibiting innervation of the DB by neuroendocrine growth hormone cells. In contrast, control of the DB in basommatophoran species is thought to be connected with the lateral lobes of the cerebral ganglion (absent in Stylommatophora) (Roubos, Geraerts, Boerrigter & van Kampen, 1980). It is interesting to note, however, that Wendelaar Bonga (1970) found neurosecretory axons of the light green type in the DB of *L. stagnalis*.

No evidence of a similar neurosecretory innervation of the gland cells was found in the JGO of either species examined here. The occurrence of JGO cells within the cerebral ganglion is a form of gland cell - nervous tissue contact not found in pulmonates. Although no synapse-like structures were observed between internal JGO cells and either neuronal perikarya or axons, the presence of these cells is noteworthy. In the light of the more recent work on pulmonates discussed above, it would seem incautious to dismiss them as being of little functional significance, simply resulting from the origin of the tissue (which in this case is not known). If some form of control is exerted on the JGO cells, however, it seems unlikely that only those

cells within the cerebral ganglion are involved as these only form a small percentage of the total number of JGO cells (in *G. umbilicalis*).

The whole aspect of JGO cell-nerve cell contact/communication clearly merits further investigation. The failure to demonstrate neurosecretion by selective staining techniques in this study should not be taken to indicate its absence. It is known that the neurosecretory material of lower prosobranchs has little affinity for the stains used in classical methods (Simpson, Bern & Nishioka, 1966a) and may retain its acidophilia even after oxidation (Gabe, 1966). Furthermore the neurosecretory cells of archaeogastropods, in contrast to those of opisthobranchs and pulmonates are small, differ little in size from ordinary neurons and tend to be dispersed throughout the nervous system (Gabe, 1966; Martoja, 1972). These combined factors make the study of neurosecretion difficult in this group.

At the ultrastructural level, however, Simpson (after Simpson, Bern & Nishioka, 1966a) found elementary granules in some perikarya in the cerebral ganglion of *Calliostoma*, none of which stained differentially with so called 'neurosecretory stains'. Although no such perikarya were observed in *G. umbilicalis*, granules of an appropriate size (100-200nm in diameter) were seen in some axons in the neuropile of the cerebral ganglia. It would seem probable therefore that neurosecretion, although not demonstrated with the limited number of techniques used here, does occur in this species.

Nature of the JGO Secretion

One of the more significant differences between the JGO and DB listed in Table 4 concerns the size of the secretory granules, 100-200nm in diameter in *G. umbilicalis* compared with 60-90nm in the pulmonates. Linked with this are other variations in cytoplasmic components, notably a significant amount of SER and little RER, the presence of frequent lipid droplets and mitochondria with circular or tubular cristae in DB cells (Boer, Slot & van Andel, 1968; Krusch, Schoenmakers, Voogt & Nolte, 1979; Wijdenes & Vincent, 1981; Nolte, 1983). Lipid droplets were very rarely observed in the JGO.

These differences may relate to differences in the chemical nature of the secretion (hormone?) produced by the cells. At present, however, there is some uncertainty concerning this aspect. Krusch, Schoenmakers, Voogt and Nolte (1979) and Nolte (1983) working on stylommatophoran species consider the ultrastructural features of the DB cells to indicate the synthesis of steroid material, notably SER, mitochondria with tubular cristae, lipid droplets and few, small secretory granules. Nolte (1983) has compared the DB cells with the steroid secreting cells of the mammalian adrenal cortex and Krusch, Schoenmakers, Voogt and Nolte (1979) have demonstrated in the DB enzymes necessary for the synthesis of steroid hormones (3B-hydroxysteroid dehydrogenase). Ebberink, Loenhout, Geraerts, Hogenes and Hoogland (1983), however, believe that the DB cells produce a proteinaceous hormone and have partially purified a biologically active peptide

from the DB of the basommatophoran snail *Lymnaea stagnalis*. Nolte (1983) noted a high level of labelled amino acid uptake in the DB and ultrastructural evidence of protein synthesis, but believed that this related to the production of enzymes for steroid synthesis and a binding protein for steroid storage. It seems highly improbable, however, that an organ whose function is similar in the two pulmonate orders should produce in one a steroid (Stylommatophora) and in the other a peptide (Basommatophora).

The ultrastructural features of the JGO, particularly in *G. umbilicalis*, clearly indicate the synthesis of protein. This is supported by the results of histochemical tests. There is very little evidence of steroid synthesis. Lipid droplets are rare and may well result from autophagy of excess secretion and/or membrane reclamation. Such droplets are frequently found in protein secreting cells and may be an important reserve of membrane lipids (Smith & Farquhar, 1966; Båge & Fernholm, 1975).

The biologically active peptide produced by the DB of *L. stagnalis* has an isoelectric point of approximately 4.0 (Ebberink, Loenhout, Geraerts, Hogenes & Hoogland, 1983) and is therefore acidic. The general staining affinity of the JGO of *G. umbilicalis*, however, indicates the presence of basic protein (though this may be a carrier protein). A difference in the type of protein (carrier) produced by the cells may therefore account for the difference in granule size. Nevertheless, it must be emphasised that the selective staining techniques employed here were not extensive. Additionally, tissues were fixed in Bouin's fluid

which, due to the presence of picric acid, may render proteins acidophilic (Baker, 1958). The DB of *L. stagnalis* stain weakly in both acidic and basic dyes (Joosse, 1964).

Further evidence that DB hormone is a peptide has been given recently by Minnen and Sokolove (1984). These authors have partially purified a low molecular weight protein which appears to be concentrated in the DB of the stylommatophoran *Limax maximus*. This substance (GAL-SF) stimulates galactogen synthesis in the albumen gland and may represent the DB hormone.

Seasonal Changes in Activity and the Function of the JGO

A feature of particular interest shared by the JGO and DB is seasonal variation in cell and nuclear size (area). Joosse (1964) has shown that in *Lymnaea stagnalis* the DB cells and nuclei increase in size (and therefore activity) during the winter and early spring and then suddenly decrease during early summer. Nolte (1983), however, could find no evidence of such seasonal changes in DB activity in *Helix pomatia*, although there was an indication of a diurnal activity rhythm. The results obtained here indicate that the situation in *Gibbula umbilicalis* resembles that in *L. stagnalis*.

In *G. umbilicalis* the suggested changes in JGO activity closely parallel changes in the female reproductive cycle, (the female part of the ovotestis of *L. stagnalis* shows a constant picture throughout the year). Activity in terms of cell and nucleus area progressively increases during the winter months reaching a peak just after the onset of gonad maturation in the spring. During maturation, there is a sharp reduction in activity and by the time maximal gonad maturity is reached JGO activity is near minimal.

The view of Martoja (1965b) that the JGO is involved in the stimulation of maturation (or part thereof) would be further supported by evidence of secretion release during the maturation phase. Unfortunately observations of granule release were rare. It is possible that synthesis of secretion gradually increases during the winter and early spring and that it is stored during this period. Release

may then occur during the maturation phase stimulating part of this process, coincident with the reduction in cell area. The few occasions when granule release was observed were in early August 1980 when the gonad would be expected to be at or near maximal maturity. The gradual release of secretion over an extended period would account for the scarcity of release profiles in electron micrographs. The TARI method of Buma and Roubos (1983b) for the demonstration of exocytotic release of secretion may be useful in future studies.

Changes in cell size noted in the JGO of *G. umbilicalis* were not recognised externally as changes in the overall size of the organ. Martoja (1965a) stated that variation in JGO size was obvious in *Hydromyles globulosa*. In *G. umbilicalis*, however, the organ is a somewhat spreading structure. Its exact shape is variable so that small changes in size would probably be undetectable. Alternatively, decrease in cell size may be compensated for by increase in the volume of intercellular space as in the DB of *L. stagnalis* (Joosse, 1964). Such changes were not noted here at the electron microscope level; they may be more obvious in light microscopy.

In the light of what is now known about DB function in pulmonates, one may postulate that the JGO secretion exerts its influence on maturation by the stimulation of vitellogenesis. Other aspects of DB hormone function such as stimulation of growth, differentiation and synthetic activity of the female accessory sex organs may occur in *Aplysia punctata*, do not apply in a trochid such as *G. umbilicalis* as, with the exception of small glandular lips on the female urinogenital pore, these structures are absent.

Martoja (1965b) noted that in immature specimens of *A. punctata* the JGO was poorly developed, supporting the view that it has a role in maturation. This may also be true of *G. umbilicalis* in which a juvenile was found to have small JGO cells at a time when adult JGO cells were large.

It must be emphasised again, however, that the results obtained and the suggestions given above with regard to function, concern only females (or the female part of the reproductive system in *A. punctata*). Although in *G. umbilicalis* the JGO of the male is structurally similar there is no information regarding its seasonal appearance. In pulmonates the DB are not thought to influence the male part of the genital tract. This is thought to be controlled by a separate system involving in the Basommatophora the lateral lobes of the cerebral ganglion (Geraerts, 1976b; Joosse, 1979; Wijdenes, 1981) and in the Stylommatophora the cerebral ganglion and gonad (Joosse, 1979; McCrone & Sokolove, 1979; McCrone, Minnen & Sokolove, 1981; Wijdenes, 1981; Sokolove & Minnen, 1983). A similar system(s) may be found in the hermaphrodite *A. punctata*, but this seems unlikely in the gonochoristic species *G. umbilicalis*. One would not expect males to have a well developed but functionless JGO. The function of the JGO in males of such gonochoristic species therefore merits further investigation.

The possibility that the organ has a stimulatory influence on both male and female systems is not unlikely. The hormone produced by the optic glands of cephalopods is known to stimulate reproductive development in both sexes (Wells & Wells, 1959, 1972, 1977). In males it stimulates

multiplication of spermatogonia, the production of spermatophores and the growth of the genital duct.

Experimental investigations on the endocrine control of reproduction in prosobranch gastropods have concentrated on the development of the male accessory sex organs. A JGO/DB-like structure does not appear to be involved. There is, however, some evidence to suggest that factors originating from the cerebral ganglion influence maturation and the sexual cycle. Choquet (1971) proposed the presence of three factors in *Patella vulgata*, a tentacular inhibitory factor, a cerebral mitogenic factor, and a cerebral vitellogenic factor. A cerebral vitellogenic factor is also thought to be present in *Calyptraea sinensis* and *Crepidula fornicata* (Le Gall & Strieff, 1975).

Highnam & Hill (1977) raised the possibility that in *P. vulgata* the vitellogenic factor is produced not by the cerebral ganglion but by the JGO which was not isolated from the ganglion in Choquet's experiments. This argument cannot be extended to *Calyptraea* or *Crepidula* as no JGO or possible DB homologue has been identified in either species. The existence of such a structure has, nevertheless, been postulated by Joosse (1972) and Wijdenes (1981).

Conclusive evidence as to the function of the JGO can only be obtained using experimental techniques such as extract injection, extirpation/reimplantation and organ culture. These techniques have been used frequently on molluscan material and have provided much of the information now available concerning DB function. The feasibility of

operative techniques involving a somewhat spreading organ (at least compared with the DB of *L. stagnalis*) in a small species such as *G. umbilicalis* remains to be established. A larger species such as *Monodonta lineata* may prove more useful.

An appropriate starting point might be organ culture and simple homogenised extracts such as used by Bentley and Olive (1982). Media for the culture of marine prosobranch tissues have been used successfully by other workers (Wolff & Haffen, 1952; Strieff, 1967; Le Breton, 1969; Le Gall, 1974). Such experiments may provide useful information before more complex techniques are contemplated, e.g. the uptake of labelled material by the gonad and in the case of *A. punctata*, accessory sex organs (Goudsmit, 1975; Wijdenes, Elk & Joosse, 1981; Minnen, Wijdenes & Sokolove, 1983; Sokolove, Melrose, Gordon & O'Neill, 1983; Sokolove & Minnen, 1983). If a reliable indication of function can be established further experiments may be conducted concerning the biochemical nature of the JGO hormone (?) using purification and bioassay techniques.

The possibility remains, however, that the JGO is not homologous with the DB and that it influences another aspect(s) of the animal's physiology - if it is an endocrine organ (Nolte & Machemer-Röhnisch, 1966). The apparent correlation between its activity and the reproductive cycle may be coincidental.

Nevertheless, even with this in mind it is clear that the JGO should be regarded as a potential endocrine structure. At present it seems most likely that it is

homologous with the DB and therefore, if structural homology can be taken to indicate functional homology, that it functions in the stimulation of part of the reproductive process.

The apparent absence of the JGO in caenogastropods, from which pulmonates and opisthobranchs may be derived, warrants further investigation, particularly into the less specialised gonochoristic species. Those of special interest might be those derived from the procerithiacean stock (heterogastropods) which are thought to be close to the origins of opisthobranchs and pulmonates and which themselves may be derived from archaeogastropods (Fretter, 1979). The presence of the structure in all four archaeogastropod superfamilies (Pleurotomariacea, Fissurellacea, Trochacea and Patellacea) suggests it is likely to have been ancestral to all gastropods.

On a broader scale, it is interesting to note that Vicente (1970) and Vicente and Gasquet (1970) identified a structure similar to the JGO in chitons. They named this the juxtacommissural organ since polyplacophorans have no discrete ganglia. Although little detail is known of this organ it is tempting to speculate that it might represent another example of homology embracing amphineurans, prosobranchs, opisthobranchs and pulmonates, and which may even include the cephalopods.

SUMMARY (Part II)

The JGO of *Gibbula umbilicalis* has been examined both histologically and ultrastructurally. It is suggested that the tissue is an endocrine gland involved in the production of a proteinaceous secretion. No evidence of a neurohaemal function was found.

The organ is composed primarily of intrinsic glandular cells with abundant RER, mitochondria and electron-dense secretory granules 100-200nm in diameter. Lipid-like inclusions were rare. Indications of variation in the area of the cells and nuclei in electron micrographs are thought to imply variations in cellular activity. Females only were examined in this respect. Such variation appears to be linked with the reproductive cycle. Activity progressively increases during the gonad resting phase (autumn and winter) and reaches a peak at the onset of maturation in the spring. A short period of reduced activity follows before the onset of the next cycle. Secretion release is thought to occur during gonad maturation. These observations correlate well with the previous suggestion that the organ is involved in the stimulation of maturation.

No evidence of neurosecretion was found in or near the JGO. The possibility of neurosecretomotor innervation seems unlikely. The phenomenon of neurosecretion in this species, however, is thought to merit further study.

The ultrastructural features of the JGO support the hypothesis that it is homologous with the pulmonate

dorsal bodies. Differences between the two, however, are apparent; notably in the size of the secretory granules and the presence of JGO cells in the cerebral ganglia. Nevertheless, these are not thought to be incompatible with homology particularly considering the distant relation of the two groups and the differences in dorsal body structure exhibited within the pulmonates.

The JGO of an opisthobranch, *Aplysia punctata* was examined for comparison. This was found to possess features common to both the trochid JGO and the dorsal bodies, viz. JGO cells within the cerebral ganglion, but smaller secretory granules.

The possible function of the JGO is discussed in the light of what is known concerning dorsal body function. Homology would suggest a role in the stimulation of vitellogenesis in females. The function of the organ in males is less evident, but a role in the stimulation of spermatogenesis may be indicated by the dual effect of the cephalopod optic gland hormone.

The reported presence of a JGO in members of all four archaeogastropod superfamilies and the possibility of a similar structure in polyplacophorans, suggests that the organ is of primitive origin. Its apparent absence in the more advanced prosobranchs (caenogastropods) is surprising and needs further study.

APPENDIX 1

CALCIUM ALGINATE - food for snails

Method

To 1 litre water at 60°C, stirred on an electric stirrer, add 10 grams dried nettle powder and 10 grams complan. Then add 10 grams sodium alginate powder very slowly and continue stirring until the mixture is homogeneous and has thickened. Pour a thin layer of the mixture into a dry developing dish or such like and cover with calcium chloride solution (50 grams per litre). Cut gelatinous sheet into strips and keep under water in a refrigerator until needed.

Dried milk, dried egg etc. may be used instead of nettle and complan.

APPENDIX 2

MEASUREMENTS OF CELL AND NUCLEAR AREA

		Cell area (μm^2)	Nuclear area (μm^2)
Sample 1		30.9	8.3
(25.8.80)		33.9	6.3
Specimen B		24.3	8.6
		30.0	8.3
		28.4	6.3
	Mean	29.5	7.6
	S.E.M.	1.6	0.5
Sample 2		50.3	8.1
(22.9.80)		45.8	10.9
Specimen A		68.5	8.6
		66.5	10.6
		42.9	10.9
	Mean	54.8	9.8
	S.E.M.	5.3	0.6
Sample 4		82.0	18.6
(15.11.80)		98.0	18.7
Specimen A		84.8	16.7
		82.9	24.3
		77.5	11.6
	Mean	85.0	18.0
	S.E.M.	3.4	2.0

Continued

		Cell area (μm^2)	Nuclear area (μm^2)
Sample 6		71.3	15.5
(10.1.81)		62.3	27.3
Specimen A		72.4	17.6
		97.4	24.5
		91.1	15.9
	Mean	78.9	20.2
	S.E.M.	6.6	2.4
Sample 9		107.3	15.5
(4.4.81)		144.5	27.3
Specinen A		85.4	17.6
		82.5	24.5
		147.3	15.9
	Mean	113.4	20.2
	S.E.M.	14.0	2.4
Sample 10		126.4	27.3
(10.5.81)		95.4	17.9
Specimen A		106.5	20.3
		113.1	25.1
		104.6	14.9
	Mean	109.2	21.1
	S.E.M.	5.1	2.3

Continued

		Cell area (μm^2)	Nuclear area (μm^2)
Sample 11		98.7	10.6
(31.5.81)		68.9	10.8
Specimen B		50.6	11.3
		37.9	9.2
		82.1	10.0
	Mean	67.6	10.4
	S.E.M.	10.8	0.4
Sample 12		41.0	7.2
(28.6.81)		48.4	6.4
Specimen B		54.3	7.2
		40.0	10.6
		42.3	7.0
	Mean	45.2	7.7
	S.E.M.	2.7	0.7
Sample 13		62.5	16.3
(25.7.81)		65.4	14.3
Specimen B		95.8	14.2
		52.7	14.4
		57.3	14.3
	Mean	66.7	14.7
	S.E.M.	7.6	0.4

Continued

		Cell area (μm^2)	Nuclear area (μm^2)
Juvenile		36.8	7.7
(8.3.81)		30.3	5.4
		39.7	5.1
		26.1	4.3
		30.9	3.7
	Mean	33.3	5.3
	S.E.M.	2.1	0.7

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To my parents

(at last)