CONTRIBUTIONS TO HYDROMEDUSAN NEUROETHOLOGY
FROM A STUDY ON TWO OLINDIADS:
GONIONEMUS VERTENS (Agassiz, 1865) and *EPERETMUS TYPUS* (Bigelow, 1915)

by

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Frontispiece: An adult medusa of *Gonionemus vertens*. Photo by Jack Dietrich.

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Abstract

The behavioural repetoires of the two olindiad hydromedusae *Gonionemus vertens* and *Eperetmus typus* were tabulated, extracellular recordings of events associated with their behaviour were made, and an ultrastructural survey of all their tissues was carried out. Many of the control mechanisms of their behaviours are similar to those hypothesised for other hydromedusae, as summarized by Spencer & Schwab (1982), but some differences are present:

1) Both species lack the excitable exumbrella and endoderm which in other hydromedusae are involved in the mediation of a protective "crumpling" response. The behavioural pattern is absent in *G. vertens* but in *E. typus* it is present and is mediated by neuronal pathways in the bell margin and radii. The structure and distribution of gap junctions and septate junctions in the tissues of *G. vertens* are similar to those reported for hydromedusae with excitable epithelia, so these characteristics of the intercellular junctions appear independent of excitability.

2) Turning during swimming makes use of a unique subumbrellar sphincter muscle.

3) Nerves are present in the swimming muscle of the subumbrella and velum of both species. Circular conduction of the swimming event in the subumbrella of *G. vertens* is

more rapid and more sensitive to excess Mg^{++} ions than is radial conduction, suggesting the event is propagated by neuronal conduction in a circular direction and myoid conduction in a radial direction. No capacity for independent spontaneous activity of the muscle sheets was observed.

4) In tentacles of *G. vertens* the events associated with local activity are distinct from those associated with coordinated activity both in shape and conduction parameters. This suggests double innervation of the tentacle muscles is present to permit both local and coordinated contractions.

5) Coordinated contractions of the tentacles are far less common in *G. vertens* than in *E. typus* and most other anthomedusae. "Cryptic" tentacle pulses are more common in *G. vertens*, indicating that the behavioural difference is due to electrical activity in the epitheliomuscular cells of the tentacle ectoderm that does not elicit visible contractions.

6) Feeding of *G. vertens* relies on a number of possible "coordinating" mechanisms and a large amount of local activity. Pointing appears to involve coordination by the "activation" of local pacemakers; sensory input seems to coordinate the swimming component of feeding; and enhanced general "excitation levels" in the animal are reflected by

overall increased tentacle activity and "coordination" of radial muscle contractions.

7) Somersaulting is unique to *G. vertens*, but there is no evidence for unique musculature or nervous structures. This behavioural pattern probably relies on the shape and density of the medusa's bell.

8) Tentacle anchoring in *G. vertens* seems to result from a coordinated control of secretion of an adhesive. Anchor detachment is apparently not centrally coordinated.

Other morphological characters of these two species that have not been reported in hydromedusae before include mesogleal spurs and supportive cells at the base of the velum, possible paddle cilia on the velum and gonads of *G. vertens*, ciliated chambers in the exumbrellar bell margin of *E. typus*, and supportive cells between the "motor giants" of the inner nerve ring of *E. typus*. These observations suggest that hydromedusae harbour far greater morphological and neurophysiological diversity than is commonly recognized.

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Introduction and General Background

Great advances have been made in recent years in deciphering the neurophysiological organization and the control of behaviour in hydromedusae (see Anderson, 1980; Spencer & Schwab, 1982 for reviews). This work has, however, been carried out almost exclusively with anthomedusan preparations (Spencer & Schwab, 1982), though important contributions from other hydromedusan groups have been, and are being, made (Kerfoot, 1980; Spencer, 1975; Roberts & Mackie, 1980). The questions that come to mind in attempting to construct a more general understanding of organization of behaviour in the hydromedusae are:

1) How well do the current hypotheses actually describe the organization of other hydromedusae? Results from the leptomedusan *Aequorea victoria* (Kerfoot, 1980) and the trachymedusan *Aglantha digitale* (Mackie & Meech, in press; Weber *et al.*, 1983) suggest there may be some major differences between members of the group.

2) Is a generalized hypothesis of hydromedusan organizaton at all possible? Some workers think so (e.g. Spencer & Schwab, 1982), but an answer to this question should have a wider taxonomic base than is currently available. A resolution to the apparent incongruity (Spencer & Schwab, 1982) between the neurophysiological organization of hydrozoan medusae and that of their polyps should also be

included in any such answer.

In this study, I have primarily investigated the neuromuscular organization and physiology underlying the behaviour of the olindiad hydromedusa *Gonionemus vertens* (Agassiz, 1865). The goal of this investigation was to re-analyse current hypotheses of hydromedusan neuroethology with a slightly greater taxonomic base and from a fresh perspective, keeping the above questions in mind.

The family Olindiadidae is a group of quite distinct hydromedusae in the order Limnomedusae (Arai & Brinckmann-Voss, 1980; Russell, 1953). *Gonionemus* is perhaps the most widely known medusa of this group. It was studied by pioneering coelenterate workers (Yerkes, 1902a, 1902b; Yerkes & Ayer, 1903; Hyde, 1902), whose observations were incorporated into early treatises on nervous systems (Jennings, 1906; von Uexküll, 1909; Parker, 1919), and subsequently had a disproportionate influence on common views of hydromedusan organization and behaviour by their inclusion in many further textbooks (e.g. Barnes, 1974; Kaestner, 1967; Meglitsch, 1972). The medusa is, however, quite different from the anthomedusae dominating present investigations in aspects of both behaviour and neuromuscular organization. It appears to be a species whose re-examination could potentially be very fruitful for expanding theories of hydromedusan neuroethology.

To aid my interpretations of control mechanisms of behaviour of *G. vertens*, I have studied a second olindiad medusa concurrently, *Eperetmus typus* (Bigelow, 1915). This medusa is superficially quite similar to *G. vertens* but possesses some very different behavioural traits. I thought comparisons with this species may be more enlightening than the comparisons with more distantly related species more commonly made in studies such as this.

A brief description of the gross morphology of the olindiad medusae and a review of current hypotheses of hydromedusan neuroethology follows to aid in the presentation of the rest of the thesis.

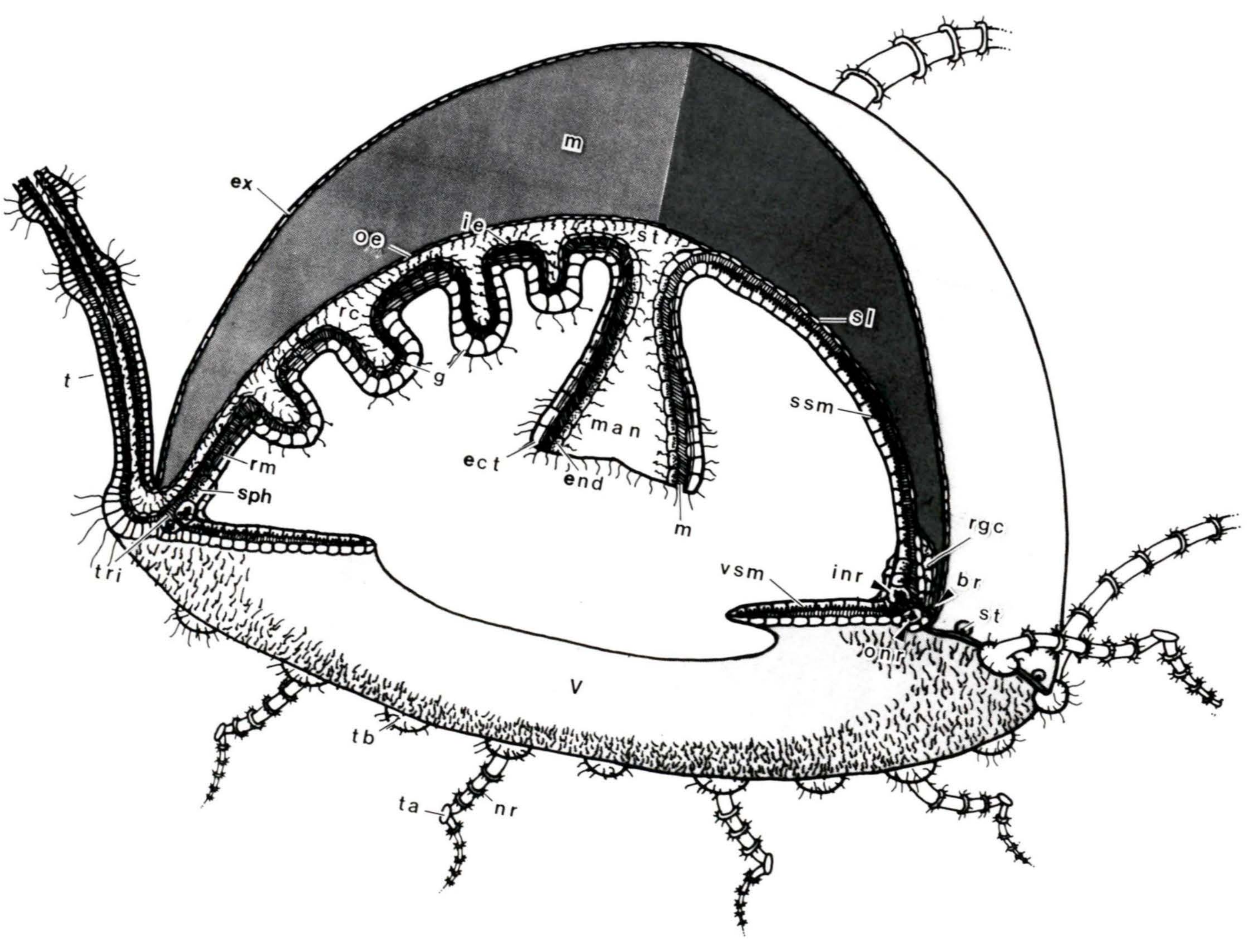
A) *General body plan*

Gonionemus vertens (fig.1 & frontispiece) is a medium-sized hydromedusa with a maximum bell diameter of about 2.5 cm. The hemispherical bell consists of a firm mesoglea thickest at the bell apex and tapered toward the bell margin. The outer surface is encased by the exumbrellar ectoderm, while the inner wall is lined by the subumbrellar ectoderm. Within the mesoglea four endodermal radial canals extend from the bell apex to its margin, each with an overlying convoluted ridge of gonad. At the bell margin a simple, broad, endodermal ring canal encircles the bell opening. A thin endodermal subumbrellar lamella extends throughout the bell within the mesoglea between the radial canals and the ring canal. It remains close to the subumbrellar ectoderm throughout.

Seventy to one hundred and thirty hollow tentacles emerge from the lower margin of the bell, the endoderm of the ring canal sending branches into each. Larger tentacles are reflexed back over the bell while smaller tentacles are oriented more laterally. The tentacles have a beaded appearance, formed by annular ectodermal swellings bearing nematocysts. Each tentacle possesses a sub-terminal adhesive pad, used in anchoring the animal to the substrate. These "anchors" create a sharp bend close to

Figure 1: Diagramatic representation of *Gonionemus vertens*

br: cellular bridge between endoderm and exumbrella
ect: ectoderm
end: endoderm
ex: exumbrella
g: gonad
ie: inner endoderm
inr: inner nerve ring
m: mesoglea
man: manubrium
nr: nematocyst ring
oe: outer endoderm
onr: outer nerve ring
rc: radial canal
rgc: ring canal
rm: radial muscle
sph: sphincter muscle
sl: subumbrellar lamella
ssm: subumbrella swimming muscle
st: statocyst
t: tentacle
ta: tentacle anchor
tb: tentacle bulb
tri: tri-radius
v: velum
vsm: velar swimming muscle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the tips of all the tentacles, alluded to in the animal's generic name. At the base of each tentacle is a tentacle bulb, consisting of a swelling of exumbrellar tissue of the bell margin and an outpocketing of the ring canal endoderm as it joins the tentacle endoderm. No ocelli are present.

At the bell margin a velum extends inwards as a tissue "shelf", occluding approximately 4/5 of the bell opening. This "shelf" consists of two layers of ectodermal cells, one subumbrellar and one exumbrellar, separated by a thin mesogleal sheet. As the mesoglea of the velum enters the bell margin and merges with the bell margin mesoglea, it separates the subumbrella and exumbrella of the bell. This region has been referred to as the "tri-radius" (Spencer & Schwab, 1982), and is the site of concentrations of nervous tissue in the exumbrella, subumbrella, and endoderm. These are termed the outer, inner, and endodermal nerve rings respectively.

Both the outer and inner nerve rings are embedded in ridges of epithelial tissue. The ridge containing the outer nerve ring consists largely of developing cnidoblasts, and where tentacles are present, forms the tentacle bulbs. Smaller nerve bundles and nerve tracts are also present in this ridge, and some ridge tissue together with neuronal tissue protrudes into the overlying mesoglea of the bell between each pair of tentacles to form statocysts. The ridge containing the inner nerve ring consists of epithelial cells and a band of circularly oriented smooth muscle, which I have called the sphincter muscle.

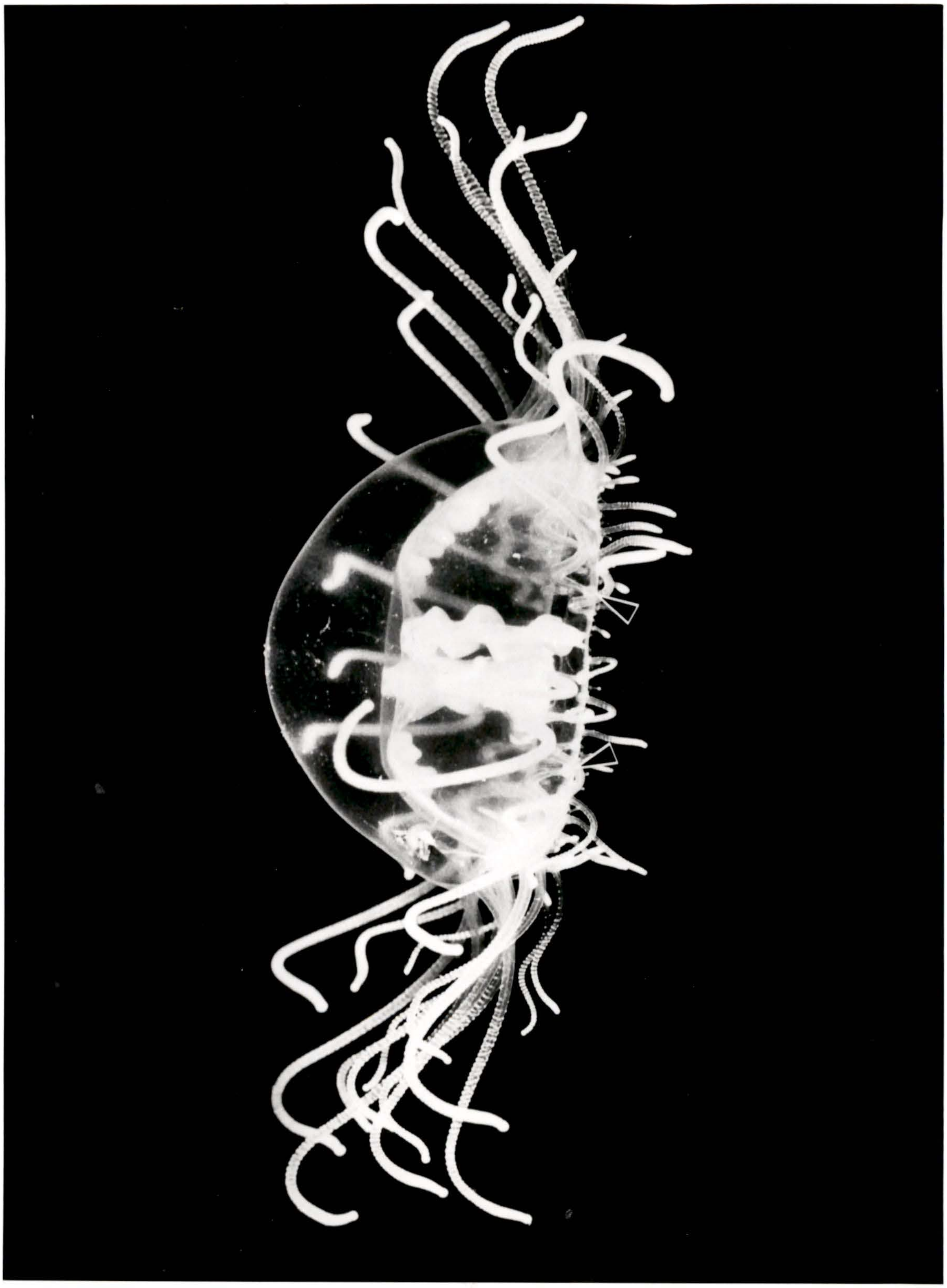
A stout, muscular manubrium hangs from the apex of the bell. It is approximately one half as long as the height of the bell, and consists of a small basal stomach, a short column, and, surrounding the terminal mouth, four flexible, frilly "lips". Each of the four corners of the stomach leads into one of the radial canals.

The subumbrellar ectoderm of the bell consists largely of a circularly-oriented striated muscle sheet, the swimming muscle, which extends over the whole inside wall of the bell where it is not occupied by the manubrium, gonad ridges, or bell margin tissue. In addition, two bundles of radially-oriented smooth muscle run the length of each radial canal on either side of the base of the gonad ridge, coalescing into a single cord where gonad tissue is absent close to the bell apex and bell margin.

In summary, muscular tissue in *G. vertens* consists of the manubrium, radial muscles, the sphincter muscle, the swimming muscle, the tentacles, and the velum; overt concentrations of neuronal tissues are present in the bell margin as the inner, outer, marginal, and endodermal nerve rings; and the only discrete macroscopic sensory structures are the statocysts. Other tissue types are the bell exumbrella, the gonads, and the endoderm of the radial canals, the ring canal, the stomach, and the subumbrellar lamella.

Eperetmus typus (Bigelow, 1915) (fig. 2) differs from *G. vertens* on a gross scale only in its generally larger size (maximum bell diameter of 4.5 cm (Arai & Brinckman-Voss, 1980)), paler pigmentation, centripetal pouches off the ring canal, the absence of tentacle anchors, the presence of pronounced terminal knobs on the tentacles, and the presence of cnidoblast-rich cleft-like pads in place of tentacle bulbs.

Figure 2: An adult medusa of *Eperetmus typus*. Note the similarity to *G. vertens* except for pronounced swollen tentacle tips, and nematocyst-rich clefts at the base of tentacles (arrows) in lieu of tentacle bulbs. Tentacle anchors are absent. Photo courtesy of K. Larson (x3.5).



B) Behaviour

The potential for both local activity of individual effectors and coordinated activity involving a number of effectors has long been recognized in hydromedusae (Ebbecke, 1957; Passano, 1963; Leonard, 1982) and hydropolyps (e.g. Josephson, 1974b). The local activities have, however, been largely set aside in neuroethological analyses. Most of the coordinated behaviour consists of clearly recognizable, stereotyped, behavioural patterns which appear to be present in some form in the behavioural repertoire of all hydromedusae (Spencer & Schwab, 1982).* It is these stereotyped behavioural patterns and their related electrophysiology that have dominated hydromedusan neuroethology.

The behavioural patterns considered in the present work are crumpling, feeding, somersaulting, swimming, tentacle posture maintenance, and tentacle anchoring (fig. 3). Together with local activity of the various effectors,

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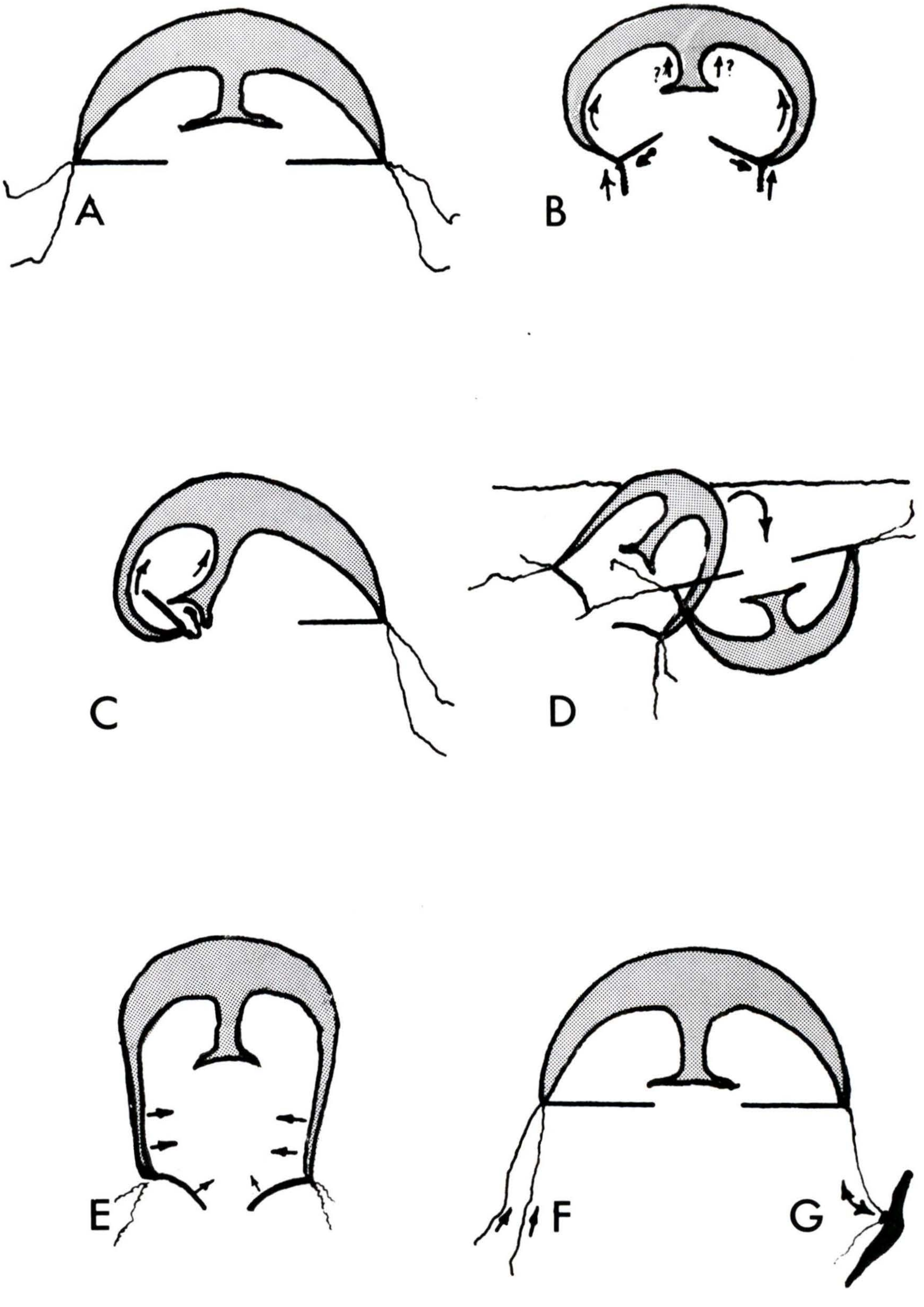
* Behaviour patterns are defined as a "probablistic sequence of behavioural acts, single behavioural acts, or simultaneous clusters of behavioural acts" (Drummond, 1981), where a behavioural act is defined as a behavioural unit "which cannot be broken down further into components which occur independently". Stereotyped behaviour patterns, or "fixed behaviour (action) patterns" are such sequences with a high probability of occurring.

Figure 3: Hydromedusan behaviour patterns considered in the present thesis.

A) rest position
B) crumpling
C) feeding (pointing only is shown)
D) sommersaulting
E) swimming
F) tentacle posture maintenance
G) tentacle anchoring and detachment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these encompass all of the observed behavioural repertoires of both *G. vertens* and *E. typus*. A brief historical review of studies on each of the behavioural patterns follows.

1) Crumpling:

Crumpling is a term coined by Hyman (1940) to describe a "protective behaviour" of many hydromedusae in which the medusa "folds into the smallest possible compass". The behavioural pattern was earlier described by Romanes (1878) as "muscular spasms" of the swimming muscle, later shown to be an erroneous interpretation. Since Romanes and Hyman, crumpling has been studied in a number of hydromedusae (see Spencer & Schwab, 1982 for review). In anthomedusae, where the response has been best studied, the behaviour typically consists of a simultaneous contraction of all radial muscles, tentacles, manubrium, and sphincter muscle (when present), velar twitches, and an inhibition of swimming. The contractions involved are typically of relatively long duration in comparison to those seen in other behaviours. In *Stomotoca atra* the behavioural pattern can be elicited and mediated by epithelial conduction pathways alone (Mackie, 1975), while in others, such as *Sarsia tubulosa* (Mackie *et al.*, 1967) and *Polyorchis penicillatus* (King & Spencer, 1981) at least some contribution by neuronal elements is required to achieve the full response. In the limnomedusa *Proboscidactyla flavicirrata* crumpling appears to be mediated primarily by epithelial pathways (Spencer, 1975), but seems to require input from tentacle nerves as well to produce a full response. In *S. atra*, *P. flavicirrata*, and *P. penicillatus* crumpling is graded, while in *S. tubulosa* it is ungraded, a phenomenon attributed by Passano *et al.* (1967) to a synergistic interaction between neuronal and epithelial excitation.

The absence of crumpling in a number of hydromedusae has been attributed or correlated in the various species to the lack of appropriate effectors (C.L. Singla, pers.comm.), insufficient flexibility of the medusa's bell (Hyman, 1949), or an inexcitable exumbrella (Spencer & Schwab, 1982). *G. vertens* is a hydromedusa for which the absence of crumpling has been correlated with an inexcitable exumbrella (Mackie, 1975). *Eperetmus typus*, on the other hand, has been reported to exhibit "an especially vivid example" of crumpling (Mackie & Mackie, 1963). A comparison of these two species could thus be very useful in elucidating the control of crumpling and the role of epithelial conduction.

2) *Feeding*:

I have used the term "feeding" to refer to the behavioural patterns associated with the transfer of captured prey from the tentacles to the manubrium (Spencer & Schwab, 1982). Yerkes (1902) has considered this "food-taking" and Hyman (1940) termed it the "feeding response". In most hydromedusae so far studied, this activity involves some degree of "pointing": a term coined by Romanes (1877) and currently used to describe the combination of the contraction of a number of tentacles (normally carrying prey), a local involution of the associated portion of the bell margin, and an outward complementary flexion of the manubrium (Mackie & Singla, 1975; Spencer & Schwab, 1982). In some species this is associated with an inhibition of swimming (Hyman, 1940; Horridge, 1955b; Hernandez-Nicaise & Passano, 1967), and can be characterised by a high threshold to mechanical and electrical stimulation and a relatively long delay between stimulation and response (Mackie, 1975; Romanes 1878).

For *G. vertens*, the "feeding reaction" described by Yerkes (1902) includes pointing in response to pieces of various kinds of meat, fish extract, and gelatine, and, to some food extracts, a swimming response in addition to pointing.

3) *Somersaulting*:

"Somersaulting" is a behavioural pattern unique to *G. vertens*, referred to by Agassiz in the medusa's specific name (Russell, 1953). The behaviour consists of an inversion of the medusa as it strikes the water surface, normally followed by cessation of swimming and a period of passive sinking with tentacles extended. Yerkes (1902), Perkins (1903), and subsequent workers have considered this a "fishing" behaviour. Yerkes (1902) suggested that the inversion is elicited by stimulation of the exposed portion of the bell by light, while Perkins (1903) proposed a chemical stimulation of the exposed bell is responsible for the behaviour. Morse (1906) rejected both hypotheses and suggested that the bell simply lost its balance as it protruded out of the water and turned over.

As a behavioural pattern unique to *G. vertens* somersaulting is of interest because it suggests that there may be unique morphological characteristics or control mechanisms present in this species.

4) *Swimming*:

Swimming is the most obvious behavioural pattern in hydromedusae, the simplest for analysis, and the most studied. Investigations, pioneered by Romanes (1877) and most recently reviewed by Spencer & Satterlie (1982), have been primarily concerned with the neuronal control of swimming. In addition, Gladfelter (1972, 1973) has analysed the functional morphology of swimming, and Daniel (1983) has studied the behaviour from a biomechanical point of view. *G. vertens* has been used to some extent in all of these approaches, and two characteristics have been reported which are controversial with regard to swimming in other hydromedusae. These are the capacity for spontaneous swimming contractions in the absence of the bell margin (Yerkes,1902b; Wolfe, 1928) and a capacity for asymmetric contraction and unequal contraction rates in the swimming bell during turning (Gladfelter, 1973). Both have tacitly been attributed to the presence of a nerve net in the swimming musculature reported by Hyde (1902) or have been treated with skepticism.

5) *Tentacle posture control*:

Tentacle posture in many hydromedusae is controlled by spontaneous, synchronous contractions of the tentacles occurring intermittently and/or just before swimming. In some species these contractions consist only of synchronous twitches of the tentacle tips, but in all species studied the coordination has been shown to depend on a conduction system and pacemakers in the bell margin (Spencer &

Schwab, 1982). Agassiz (1865) mentions coordinated tentacle contractions in association with swimming in *G. vertens*, but it is not mentioned by Yerkes (1902) or later workers. Mackie & Mackie (1963), on the other hand, report a marked tentacle coordination in association with swimming in *E. typus*, so these two species may provide an informative contrast for this behavioural pattern as well.

6) *Tentacle anchoring*:

Attachment by the anchor pads of the tentacles is important to the mode of life of *G. vertens* (Agassiz, 1863; unpub.obs.) and a number of other olindiad and anthomedusan species (Honegger, 1984). To date, this function has been largely thought to involve some form of specific coordinated activity. For example, Agassiz's (1863) descriptions of the habits of *G. vertens* suggest a coordinated control of anchor detachment, Singla (1977) predicts neuronal control of detachment in *G. vertens* on the basis of morphological evidence, Honegger (1984) predicts the same for the olindiad *Vallentinia gabriella*, and Spencer & Schwab (1982) suggest anchoring and detachment of the adhesive pads may be a "fixed behavioural pattern". These conjectures are tested in the present work.

Materials and Methods

Specimens of *Gonionemus vertens* were collected with SCUBA or hand-dipped at Snug Harbor, San Juan Island, Wash., USA, and at Ogden Point and Saxe Point in Victoria, B.C., Canada from May to September in 1980-1984. *Eperetmus typus* specimens were hand-dipped at Friday Harbor, San Juan Island in September, 1981, and at Sooke Harbour, Sooke, B.C. in June and July of 1982 and 1984. Individuals of both species were maintained in the laboratory for up to one month in 12 ^{o}C recirculating seawater and in bowls of sea-water at 4 ^{o}C in a refrigerator. The techniques I used for behavioural, electrophysiological, and histological observations are as follows:

A) *Behaviour*

I observed the behaviour of the medusae in the laboratory in aquaria and finger bowls (at 11-15 ^{o}C) with the aid of a dissecting microscope, where required. I also observed *G. vertens* in the field with the aid of SCUBA.

I investigated the local activity of the various effectors using three criteria:

a) the degree of autonomy from the bell margin,

b) the presence or absence of nervous tissue, and

c) the capacity for myoid or epithelial conduction.

I did this by monitoring spontaneous activity of whole effectors or portions of excised effectors and documenting the effects of "excess" magnesium ions (one part 6.7% solution of $MgCl_2$ in distilled water to two parts sea water). I considered activities maintained in the absence of the bell margin to originate within the effector itself, a capacity for spontaneous activity to indicate the presence of nervous tissue (Mackie, 1970), and insensitivity of an electrically elicited response to excess magnesium ions as indicative of neuroid conduction.

I used cinematography to analyze swimming, turning, and somersaulting. Animals were filmed in a cooled aquarium with a 16mm Bolex camera at 64 frames per second using Eastman black and white negative film 7224. Analysis of cinematographic records was done from tracings of projected frames.

Electrical stimulation, when I used it, was applied with suction electrodes as for electrophysiological observations. Mechanical stimuli were applied with wood, glass or metal probes, while I applied chemical stimuli (fresh water, sea water, 1 M HCl, 1 M KCl, food extracts) with the aid of pasteur pipettes.

Other techniques that I used are described together with the appropriate results.

B) Electrophysiology

For electrophysiological recordings, I pinned dissected animals in Sylgard-lined plastic petri dishes containing sea-water at 13-16 ^{o}C. These dissections, unless otherwise indicated, consisted of a bisection or quadrisection of the specimen; each section was then used individually. "Emarginate" preparations were prepared in the same manner, except the bell margins were cut off as well. I performed some additional operations as well; these are described with their results.

I made all extracellular recordings using plastic suction electrodes. Events were displayed on a Grass 79C polgraph with EEG AC-coupled amplifiers and on a Tektronix 5103N storage oscilloscope using the Grass amplifiers as pre-amplifiers. Electrical stimulation was applied with suction electrodes and low resistance wire electrodes, using a Grass S44 stimulator and a stimulation isolation unit as the stimulus source. The stimuli consisted of single or repeated square impulses of short duration (0.1 - 2 ms.), generally just over threshold requirements. Intracellular recordings were made with a WPI pre-amplifier and 40-60 MΩ glass micropipettes.

Chemical stimuli and mechanical stimuli were applied in the same fashion as for behavioural observations. Excess magnesium ions were also used, again at a concentration of 2.3% in 2/3 strength normal sea-water (diluted with distilled water).

I also monitored swimming and electrical activity in the tentacles of whole animals suspended in aquaria as for the investigation of somersaulting (see fig. 5), except the suspending thread was strung through the center of the apical bell mesoglea rather than through an excentric position and was made taut. Swimming of individuals tethered by this thread in an aquarium was monitored by a Grass movement transducer attached to the thread while suction electrodes attached to individual tentacles monitored tentacle activity. Light and temperature (13-15 ^{o}C) remained constant throughout the recording periods.

C) *Histology*

I anesthetized the medusae for 30 minutes in a 6.7% solution of magnesium chloride diluted with an equal volume of sea water prior to fixation. Tissue for electron and light microscopy was fixed in 2.5% glutaraldehyde in Millonig's phosphate buffer at room temperature, rinsed in the same buffer, and postfixed in 1% osmium tetroxide in phosphate buffer at 4 ^{o}C, followed by a distilled water rinse. The tissues were then carried through a graded series of alcohols and propylene oxide prior to embedding. For low magnification electron microscopy tissue was embedded in Carolsfeld's aqueous epon (appendix A); for higher magnification electron microscopy and light microscopy tissue was embedded in epon following Luft's (1961) recipe. Thin sections were mounted on uncoated grids, mesh sizes 75-200, stained with uranyl acetate and lead citrate (Venable & Coggeshall, 1965), and examined in a Philips EM 300. Thick sections were stained with Richardson's stain (Richardson *et al.*, 1960) for light microscopy.

For lanthanum impregnated tissues, I added lanthanum nitrate to the glutaraldehyde and osmium fixatives and intervening rinse to make 1% solutions. Glucose was added to all the lanthanum-containing solutions to a final concentration of 3% to counteract osmotic stresses (C.L. Singla, pers. comm.). I stained these tissues *en bloc* with 1.5% uranyl acetate in the 50% alcohol dehydration step. Further dehydration and embedding was carried out as described above. The sections were viewed without further staining.

For scanning electron microscopy, some of the tissue fixed as described above without lanthanum was taken through an alcohol/amyl acetate series, critical point dried and gold coated. The tissue was examined on a JEOL 35 scanning electron microscope.

Results:

A) *Behaviour*

Jennings (1906) stressed the importance of compiling an initial catalogue of movement capabilities of an animal prior to an analysis of its behaviour. Past studies of hydromedusan neuroethology have, however, emphasised only coordinated whole animal behaviour. To avoid bias in the interpretation of my results, which I feel may be introduced by the more common approach, I have followed Jenning's recommendation and have presented my behavioural observations in two sections. The first of these reports observations on local activities of muscular effectors and the second reports observations on coordinated behavioural patterns.

1) *Local activity*:

a) *Manubrium*: The manubrial column is capable of flexing in all directions, shortening, and slow peristaltic contracting, while the manubrium lips are capable of extremely variable "exploratory" movements and slow peristaltic activity. Bending and shortening of the manubrial column generally have a rapid onset and long duration while activity of the manubrial lips is more continuous. Typical spontaneous activity of the manubrium is a rapid flexing in one direction, followed by "exploration" by the manubrial lips, and then rapid bending in another direction. Peristaltic activity of both the column and lips is used in engulfing and swallowing prey.

To a large extent directional activity of the manubrium appears to be totally independent of events elsewhere in the animal, although some correlation is apparent during "pointing" (see discussion of feeding). Excised manubria continued to behave "normally", and portions of ectodermal manubrium tissue less than 1 mm^2 demonstrated a continued capacity for spontaneous activity. A contribution by injury currents to activity in these small tissue pieces cannot, however, be ruled out.

In *E. typus* the manubrial column is also capable of flexing and shortening and the lips also show variable "exploratory" movements. Spontaneous activity of the manubrium is generally independent of activity elsewhere in the animal, but directed responses are present during feeding.

Excess magnesium ions reversibly abolished all spontaneous activity in both species.

b) Radial muscle: I observed only simple contractions of slow onset, long duration, and gradual relaxation in the radial muscles of *G. vertens*. In an intact animal this activity tends to curl the margin inward and plays a role both in feeding and in moving an anchored animal about on the substratum. Generally, the whole length of intact or excised radial muscle associated with a single radius contracts as a unit, but I was unable to demonstrate that both bands of the muscle tissue are active during these contractions. Smaller portions of the radial band sometimes contracted independently of the remainder of the muscle, and excised portions as small as 1 mm^2 continued to exhibit spontaneous activity without clear rhythmicity.

I was able to elicit radial muscle activity by mechanical stimulii applied to the bell margin or tentacles close to the respective radial canal base, and by electrical stimulation of the adjacent gonad tissue, the radial muscle, the nerve ring regions of the bell margin, or the tentacles. Generally more than one radial muscle was active during spontaneous or elicited acivity, but I could not demonstrate direct coordination between muscles of different radii.

The radial muscles of *E. typus* are capable of graded, local activity, as in *G. vertens*, but also exhibit a more rapid, ungraded contraction associated with crumpling. Muscles of each radius tend to act as a single muscular unit, as in *G. vertens*.

All spontaneous activity and responsivity to stimulation was reversibly abolished by excess magnesium ions in both species.

c) *Sphincter muscle*: The sphincter muscle of *G. vertens* appears capable only of slow contractions comparable to those of the radial muscle. I did not observe twitches or faster contractions on the scale of swimming muscle contractions. Functionally the sphincter muscle often appeared as four portions acting in conjunction with the immediately adjacent radial muscle, but independently of each other. Like the radial muscles, it is important in feeding and in movement of anchored individuals. Stimuli which induce radial muscle activity also induce sphincter muscle activity, but the sphincter muscle can also respond alone. Severing the radial muscle adjacent to the bell margin prevents that radial muscle from responding to stimuli to the bell margin or tentacles, but the response by the sphincter muscle remains unaltered. Generally, the sphincter muscle of approximately one half of each quadrant adjacent to a given radial muscle will act with that radial muscle as a single unit in response to a given stimulus or during spontaneous activity. If the bell margin was severed, however, the isolated portion of the sphincter muscle responded with an adjacent unit. The sphincter muscle of a quadrant with the bell margin severed from both adjacent radial muscles responded to stimuli applied to the bell margin as a single unit rather than two.

The sphincter muscle of *E. typus* is capable of the same movements as that of *G. vertens*, but also contracts more rapidly with crumpling. Portions of the sphincter muscle in this species also tend to act with the adjacent radial muscle, but I did not investigate the extent of functional partitioning as fully as in *G. vertens*.

Excess magnesium ions reversibly abolished all spontaneous activity and responsivity of the sphincter muscle in both species.

d) Swimming muscle: I observed only a rapid, circularly coordinated contraction in the swimming muscle. This is the contraction responsible for swimming. In the absence of bell margin tissue all capacity for spontaneous activity by this muscle was lost, contrary to Yerkes' (1902) and Wolfe's (1928) findings. An emarginate preparation responded to mechanical stimulation of wound edges or electrical stimulation at any point on its surface with a single, or more rarely with a double, contraction of the whole muscle, but I did not observe the rhythmic spontaneous activity of emarginate bells reported by Wolfe (1928).

Coordination within the swimming muscle is dependent on the continuity of the subumbrellar ectoderm, as demonstrated by cutting or scraping through this tissue to isolate portions of an emarginate muscle sheet. Such portions of swimming muscle responded to stimuli independent of activity elsewhere. Conduction continued, however, through strips of tissue less than 1 mm wide. In an intact animal, coordination over a cut in the muscle sheet was re-established within 3 hours.

Conduction does not occur through gonad tissue. If cuts are made in the subumbrellar tissue leading from a gonad ridge to both the apical and bottom edge of an emarginate preparation, the subumbrellar muscle sheets on either side of this ridge act independently of each other.

I also observed only "swimming" contractions in the swimming muscle of *E. typus*. In this species the muscle is dependent on the bell margin for spontaneous activity, as well, and is functionally partitioned by the gonad ridges.

Excess magnesium ions reversibly abolished spontaneous activity of the bell margin within five minutes in *G. vertens*. The spread of contractions elicited by electrical stimulation was initially only slowed by the excess $MgCl_2$, but within 30 min all capacity of the swimming muscle to contract was reversibly abolished. The effect of magnesium on the swimming muscle of *E. typus* was similar.

e) *Tentacles*: In *G. vertens*, tentacle contractions are important in feeding, and, in anchored animals, in movement over a substrate. Spontaneous activity not directly associated with these functions is common as well. I observed four distinct types of contractions in the tentacles of intact animals.

The most common type of contraction in freshly collected animals was a graded, relatively slow, "coiled" contraction of various portions of tentacles or of whole tentacles. This appears equivalent to the "corkscrew" contraction described by Yerkes (1902). He reported it could only be elicited by fish extract and "motile touch", but I found that this type of contraction also ocurred spontaneously and could be elicited by any mild mechanical stimulus. I did not observe corkscrew contractions of isolated tentacles as reported by Yerkes (1902).

In response to more extreme mechanical stimulation and all electrical stimulation, a second contraction type occurred which differs from "corkscrew" contractions only in being linear rather than coiled. This type of contraction replaced the "corkscrew" contractions in animals that had been in captivity longer than approximately three days, even though the individuals showed otherwise normal behaviour and growth.

A third but infrequent type of spontaneous contraction was a rapid, complete, linear contraction of single tentacles. I could not determine natural stimuli responsible for this contraction, and could not reliably elicit it.

The fourth type of contraction was a rapid partial contraction associated with coordinated activity of the tentacles. Primarily the tentacle tips exhibited this contraction; however more of each tentacle became involved if the coordinated events were close in succession.

The duration of elicited tentacle contractions typically could be related to the strength or duration of stimulation. Tentacles from fresh animals would, however, maintain contractions for maximum periods of only 2-3 s, despite continued electrical stimulation.

Relaxation of tentacles is slow and uniform, but may, at times, be interrupted by contractile twitches. No evidence of circular muscle activity was visible during relaxation. Relaxation could not be induced by electrical stimulation of endodermal tissue, as could be expected if circular endodermal muscle were involved in relaxation.

Excised tentacles and portions of tentacles demonstrated a continued capacity for spontaneous activity and responsiveness to stimulation. Only the graded linear contractions were observed in these tentacles.

The tentacles of *E. typus* are comparable to those of *G. vertens* in that they are capable of both local and coordinated contractions and activity when severed from the bell. I did not, however, observe "corkscrew" contractions or particularly rapid local contractions in the tentacles of this species, though rapid contractions were associated with crumpling.

Bathing tentacles of both species in excess magnesium ions limited spontaneous activity and responses to stimulation to progressively more local contractions, and within 30 min totally abolished both reversibly.

f) Velum: In the velum of *G. vertens* I observed only rapid contractions that were coordinated with swimming contractions. Spontaneous activity in the absence of the bell margin was not present, although responsiveness to electrical stimulation remained. No capacity for strictly localized activity was observed. The spread of elicited contractions was initially slowed and eventually abolished reversibly by excess magnesium ions.

Circularly coordinated total contraction of the velum is involved in swimming in *E. typus* as well, and requires the presence of the bell margin for spontaneous occurrence. In contrast to *G. vertens*, however, local twitches are also present, both in conjunction with and independent of crumpling. I did not elucidate the morphological basis of these twitches or their relationships to other movements. As in the velum of *G. vertens*, elicited events in the velum of *E. typus* were initially slowed and then abolished by excess magnesium ions, as were the spontaneous twitches.

2) *Behavioural patterns*:

a) Crumpling: Crumpling in *E. typus* involves constriction and involution of the bell margin, tentacle shortening, velar twitches, and swimming inhibition. The behaviour is long-lasting (4.5-5.5 s) and well coordinated. Given appropriate stimuli all of the muscular components show independent activity as well, but only during the crumpling behaviour, however, are they coordinated with one another for long periods and associated with swimming inhibition.

Unlike other crumpling hydromedusae *E. typus* shows no evidence of excitable exumbrellar or endodermal epithelia. Neither mechanical nor electrical stimuli to the exumbrella, the subumbrellar lamella, radial canal endoderm, stomach endoderm or endoderm of the ring canal centripetal pouches produced discernible behavioural responses. Crumpling could be elicited only by mechanical stimulation to the bell margin or electrical stimulation at locations adjacent to the bases of tentacles, also on the bell margin. Single electrical stimuli (50-70 V x 0.1 ms) in this region were sufficient to elicit crumpling, even during vigorous swimming.

Cutting the bell margin in two opposite locations of an otherwise intact animal isolated the two halves with respect to each other's crumpling activity (even though swimming synchrony was not lost). Cutting radial muscle bands and nerves close to the bell margin (leaving radial endoderm intact) also prevented the respective radial muscle complements from taking part in the crumpling.

G. vertens shows many of the components of crumpling (see especially the "synchronous" radial muscle contraction of feeding); however these are not closely coordinated with each other nor associated with swimming inhibition. Mechanical agitation of the bell margin in the same manner as that which elicits crumpling in *E.*

typus usually elicited swimming in *G. vertens*. At times contractions of radial and sphincter muscles occurred as well, with a short and variable latency, but this activity was confined to the vicinity of the stimulation site, and the contractions lasted only 2-3 s (vs. 4.5-5.5 s of a crumpling contraction in *E. typus*). Stronger stimulation at times elicited contraction of the musculature of more than a single radius, but these contractions were not closely coordinated with one another and were shortlived. Swimming was not inhibited by the radial and sphincter muscle contractions.

Electrical stimuli applied to the base of a tentacle bulb, the stimulus location which in *E. typus* elicits crumpling, in *G. vertens* elicit local sphincter muscle and radial muscle contractions and increase local tentacle activity. To reliably elicit the sphincter and radial muscle contractions in *G. vertens* required, however, stimuli of at least 150v and 0.1ms at 10 pulses per second (vs. single shocks of 50-70 v x 0.1 ms to produce crumpling in *E. typus* with comparable suction electrodes). With these stimulus parameters the responses began almost immediately, but if stimulation was continued for longer than 2-2.5 s fatigue set in before stimulation stopped. Tentacle contractions may not be elicited directly by such stimulation, since there was not a one-to-one relationship between stimuli and tentacle twitches. Cutting the radial muscles and nerves of a radius close to the region of stimulation effectively abolished the participation of that radial muscle in the response, suggesting that the excitation is carried by radial nerves. If the stimulation was continued for several minutes, the contractions of the tentacles would summate gradually to the point where all the tentacles were reflexed into the bell and writhing around. This writhing activity of the tentacles was not coordinated and continued for several minutes after cessation of stimulation.

b) *Feeding*: I observed responses by *G. vertens* to live euphausiids (*Euphausia pacifica*) and brine shrimp (*Artemia salina*), freshly thawed portions of brine shrimp, and crude extracts of euphausiid, brine shrimp, and herring (*Clupea harengus*) tissue. The extracts were prepared by grinding freshly thawed portions of the appropriate tissue in sea water and decanting the supernatant.

i) *Live prey*: Live euphausiids encountering distal portions of tentacles of an anchored *G. vertens* typically adhered to the contacted tentacles but continued to move about, dragging these tentacles along. During this activity other tentacles were also contacted and became attached to the prey. The tentacle anchor pads did not participate in prey capture. 1 to 3 s after the initial encounter, the tentacles attached to the prey contracted rapidly and completely (in fresh animals with "corkscrew" contractions), thus bringing the prey close to the bell margin. Other tentacles encountered through this manoeuvre also contracted. The sphincter muscle in the immediate vicinity and the closest one, or two radial muscles (depending on the size and activity of the prey) then also contracted asynchronously and maintained contraction for 4-6 seconds. The effect of the radial and sphincter muscle contractions coupled with asynchronous periodic relaxations and contractions of the basal portions of the tentacles brought the prey close to the bell opening, and eventually completely into the bell cavity. During this time the manubrium was quite active, flexing initially towards the point of activity on the margin, but subsequently in apparently random directions, with the manubrial lips continually exhibiting "exploratory" movements. If, during this activity, the manubrium came in contact with the prey, the manubrial lips proceeded to engulf it and tear it away from the tentacles. Swallowing of the prey followed by gradual peristaltic contractions.

Except when small prey was involved, the feeding sequence rarely culminated so simply. More commonly the medusa commenced swimming when the prey was either struggling at the bell opening or at the bell margin. One to five swimming contractions occurred, but these only occasionally dislodged the individual from the substrate. The swimming bout tended to reposition the prey under the bell opening, after which the feeding sequence continued. At times such swimming bouts were followed closely by "synchronous" contractions of all radial muscles and the sphincter muscle, though upon close examination these conractions could be seen to actually be up to 0.5 s apart. If the individual was still anchored, these served to protrude the manubrium out of the bell opening and bring it in contact with the prey. The "synchronous" contractions lasted 4-8 s, and commonly were repeated a number of times at irregular intervals, interspersed with further swimming bouts. During such a feeding sequence the remaining tentacles did not participate directly in the behavioural pattern, but did show an increased tendency towards synchronous tip contractions. Once the prey was completely engulfed by the manubrium, tentacles became fully extended again and little or no swimming, sphincter or radial muscle activity was present.

If the medusa became dislodged due to the swimming bouts, or was not anchored when the prey was caught, prey handling was generally less effective. The behavioural sequence remained basically the same, but prey loss was more frequent.

Contractions of single tentacles and groups of tentacles, and radial and swimming muscles periodically occurred spontaneously as well without stimulation by potential food particles. The "synchronous" contraction of the radial muscles of an anchored animal, when seen in the absence of overt stimulation, caused the manubrium to be applied to the substrate, where the manubrial lips spread out and "snuffle" around.

ii) dead meat: A piece of recently thawed brine shrimp placed on the tips of a few tentacles of an attached or recumbent *G. vertens*, elicited a similar response to that described above. Generally the "prey" was manoeuvred directly into the bell cavity where it was engulfed and swallowed by the manubrium without the aid of swimming bouts. Such prey was completely swallowed within about 4 min of the initial presentation.

In an individual with radial canals (together with neuronal and muscular components) severed close to the bell margin the radial muscles did not become involved in feeding sequences. Tentacle and sphincter muscle contractions occurred as before, and the "prey" was manoeuvered to the bell opening. After this first presentation of the prey to the bell mouth the manubrium became very active, periodically flexing as before, but only in apparently random directions. Once the prey entered the bell cavity and the manubrium came in contact with it, it was engulfed and swallowed as described earlier. Swimming bouts, however, were more frequent during this sequence, so time from initial presentation to swallowing was about 10 min.

Severing single radial canals (with muscle bands) close to the bell margin prevented the severed radial muscles alone from participating in feeding and prevented the manubrium from "pointing" to bell margin locations close to their bases. Severing radial canals close to the manubrium base also prevented manubrial pointing to the same region of the bell margin, but did not prevent the radial muscle from participating. Leaving the outer endoderm of the radial canal intact during these operations did not alter their effects.

iii) food extracts: Coarse euphausiid, brine shrimp, and herring extracts applied to tentacle tips elicited immediate contraction of these tentacles ("corkscrew" contractions in fresh animals), pointing, and, with a short latency, a bout of swimming. Often tentacle, radial and sphincter muscles remained contracted during these swims. The animal then relaxed and sank with the tentacles extended. For approximately 4 min afterwards, however, the tentacles showed an increased frequency of coordinated tip contractions. Neither sea-water, fresh water, 1M KCl, nor dilute HCl were effective in evoking this response, nor were mechanical or electrical stimuli. All these stimuli elicited either tentacle contractions alone, or tentacle contractions and swimming bouts, but never full pointing responses. Applications of smaller amounts of extract, or application to tentacle tips isolated from the rest of the animal by a vaseline bridge, resulted in "pointing" without a swimming bout. Increasing concentrations of extracts in the bath water, on the other hand, resulted in increased contractile activity in all tentacles (asynchronous and synchronous) and increased swimming activity.

c) *Somersault*: Observations in the lab and in the field indicated that under natural conditions the somersaulting behaviour occurs only at the surface of the water. Collisions with solid obstacles and salinity or temperature discontinuity layers in the water column did not induce the behaviour.

Cinematography indicates that asymmetric distortions of the bell margin (as seen in normal turns, see description of swimming) are not present during somersaulting. Instead, an asymmetric distortion of both the exumbrella and subumbrella occurs at the point of contact with the water surface (fig. 4).

The "somersault" behaviour consists of two components: a bell inversion followed by a period of swimming inhibition and an extension of all tentacles. I was able to artificially induce repetitive inversions in the water column by tethering an *G. vertens* individual by a loose horizontal thread strung through the upper bell mesoglea (fig. 5a). Such an animal inverted reliably whenever it reached the upper limits of its tether (fig. 5b). With the majority of inversions, swimming would cease and tentacles were extended, as occurs in natural somersaults. Under both natural and experimental conditions, however, very active individuals at times failed to relax after inverting.

d) Swimming: Cinematography indicated that swimming in *G. vertens* consists of a circularly synchronous contraction of the subumbrellar swimming muscle, coordinated with contraction of the velum and, possibly, the sphincter muscle. Progression of the muscular contraction up the subumbrellar muscle sheet effects the power stroke of the swim, while subsequent relaxation of the swimming muscle, together with a gradual relaxation of the margin, results in the recovery stroke (figs. 6 & 7). Full relaxation of the bell margin did not always take place before a subsequent swimming contraction. This phenomenon was usually associated with a higher frequency of swimming contractions but was not very marked. The power stroke initiates rapid acceleration, while the start of relaxation during the recovery stroke results in marked decelleration. After this initial decelleration, the animal continues to drift forward at variable velocities (fig. 7).

Velum contractions appear synchronous with the swimming muscle contractions, maintaining a small bell opening throughout the power stroke.

As elucidated from cinematography (fig. 8), the side of the bell on the inside of an upcoming turn starts contracting first, but major acceleration and actual turning is not initiated until the opposite side of the bell also starts contracting. The major part of the turn is executed during this latter stage and the maximum velocity is attained (fig. 9). As the second half of the bell appears to contract more quickly than the first, the final portion of the swimming contraction is synchronous and contributes little to the turn. The side of the bell that initiated the swim appears to relax at a slower rate and less completely than the opposite side, making the recovery stroke look asymmetric as well. At times, relaxation of this portion of the bell margin is far from complete before the subsequent swim is initiated (fig. 8 & 9). Another major part of the turn occurs just prior to, or during, the initial relaxation, and final turning occurs with the development of asymmetry of the relaxation. It is likely the asymmetries of the bell observed during turning are due to sphincter muscle contractions rather than activity of the swimming muscle (see below), but this could not be determined directly from the cinematographic records. A series of surgical experiments were, therefore, performed to investigate how turning is performed in *G. vertens* (fig. 10).

Removal of all or half of the velum did not eliminate the capacity to turn (fig. 10a & b); and delaying excitation of one half of the swimming muscle by cutting through the swimming muscle sheet adjacent to the ring canal around one half of the medusa's bell and extending the cuts up to the base of the gonad ridges along three radii did not interfere with the capacity to turn in any direction (fig. 10c). A tonic contraction of the bell margin, which simulates a sphincter muscle contraction, did, however, result in the medusa swimming primarily in circles with the contracted portion on the inside of the turns (fig. 10d). This tonic contraction of the bell margin resulted when the wound of the operation of fig. 10c was healing. Conduction across the wound was re-established within 30 min of the operation (as determined by electrophysiological recordings), but the tonic contraction remained for up to a day or longer.

Normal swimming in *E. typus* appears similar to that in *G. vertens*, except for a lower maximum velocity (fig. 11). As in *G. vertens*, the bell margin is the first to contract and the last to relax, appearing to maintain a slightly different state of contraction than the rest of the bell. In addition to normal swimming, *E. typus* occasionally shows "escape" swims in response to tactile stimulation of the margin, in lieu of crumpling (fig. 12). During this "escape" swim a second swimming contraction follows closely on the first, prior to the full relaxation of the bell, thus resulting in a greater maximum velocity than in normal swimming (fig. 12b). The portion of the bell at the margin which shows a different state of contraction from the rest of the bell during swimming contractions appears relatively wider in this medusa than in *G. vertens* (figs. 11a, 12a), perhaps indicating the presence of the wider sphincter muscle. In both species, contractions of the radial muscles were at times elicited by the same stimuli as elicited swimming, but such contractions did not interfere with the swimming response.

e) *Tentacle posture control*: In *G. vertens*, coordinated tentacle contractions occur; however there is considerable variation in their frequency both within and between individuals, and they could not be reliably elicited. Feeding, the presence of food extracts, post-feeding relaxation, continuous swimming, or repeated strong electrical stimulation of the bell margin commonly all caused an increased frequency of synchronous contractions. In some individuals which showed a great deal of coordinated tentacle activity, synchronous contractions could be elicited by single shocks to the bell margin, but in other animals this was not possible. I did not observe reliably occurring synchronous tentacle contractions prior to swimming as reported by Agassiz (1865).

Even though most of the activity seen in the tentacles is local, tentacles of an individual are generally of approximately equivalent extension. This coordination in posture is dependent on the integrity of a conduction pathway in the bell margin, since a medusa with two diametrically opposed cuts through the bell margin will commonly have tentacles extended to different degrees in the two bell halves.

E. typus, in contrast to *G. vertens*, shows a marked propensity for coordinated tentacle activity. Local activity occurs, but most of the activity is synchronous. Continuous twitching of tentacle tips in the absence of other behaviours and marked coordinated contractions during swimming are conspicuous characteristics of the species (see also Mackie & Mackie, 1963). Synchronous contractions of tentacle tips in *E. typus* could be elicited by single shocks to the bell margin at all times. Coordination of tentacle activity and posture is interrupted in *E. typus* by cutting the bell margin as in *G. vertens*.

f) Tentacle anchoring: Observations of *G. vertens* in the field and in the laboratory provided no evidence of a central control mechanism for anchor detachment. Rather, animals usually appeared to "struggle" to free themselves from attachment by vigorous swimming. Individual tentacles, however, appeared able to become detached more easily in resting animals.

Anchor attachment was also not associated with a distinctive behavioural pattern. Contact with a substrate was sufficient to effect attachment. There was some evidence, however, for control of the receptiveness of the anchor pads to attachment, either coincident with, or caused by, tentacle relaxation. As described above, individuals with two cuts through the bell margin can have two "populations" of tentacles of different tonus. While swimming, fully extended tentacles of such individuals would attach onto a substrate upon contact, while the more contracted tentacles did not. Likewise, anchor pads of relaxed tentacles attached more readily to a glass probe than those of contracted tentacles. The tentacle anchors did not appear to change in shape during tentacle contractions.

In animals recovering from anesthesia induced by excess magnesium ions, the majority of the tentacle pads were less likely to stick to a glass probe than in fully recovered, relaxed animals. Tentacle pads which were attached before being exposed to excess magnesium ions, however, remained attached. If forcibly detached these remained quite sticky for a time, but were incapable of effectively re-anchoring while under anesthesia, suggesting the capacity to attach may require secretion of fresh adhesive material. Muscular activity did not appear to be required to maintain attachment, since the anchors remained attached in excess $MgCl_2$ ions. Tentacles removed from the animal were not observed to anchor.

Figure 4: A bell inversion during somersaulting of *G. vertens* traced from a cinematographic record. Tracings represent outlines of the subumbrella taken at intervals of 0.0935 s. No asymmetric contraction of the bell margin, typical of normal turning, is evident. A distortion at the point of contact with the water surface can be observed instead (thick arrow). Other arrows indicate the direction of motion.

WS: water surface

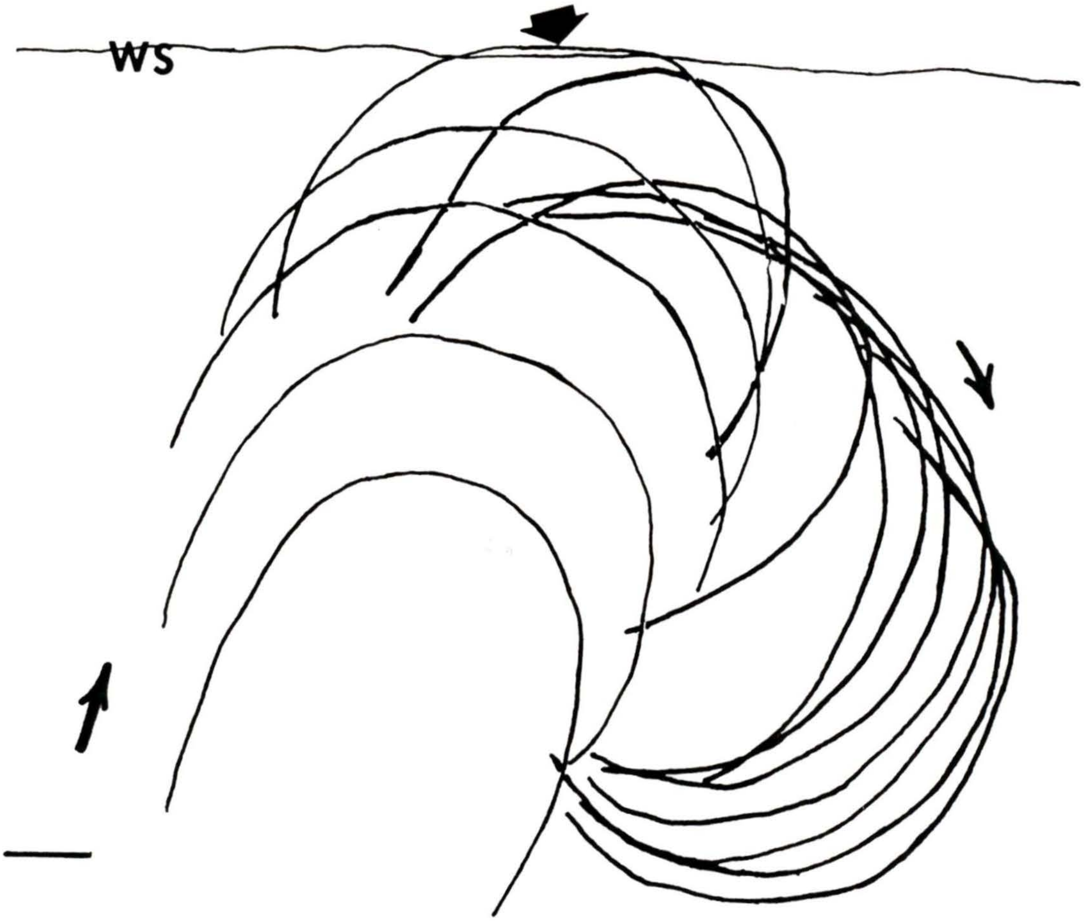
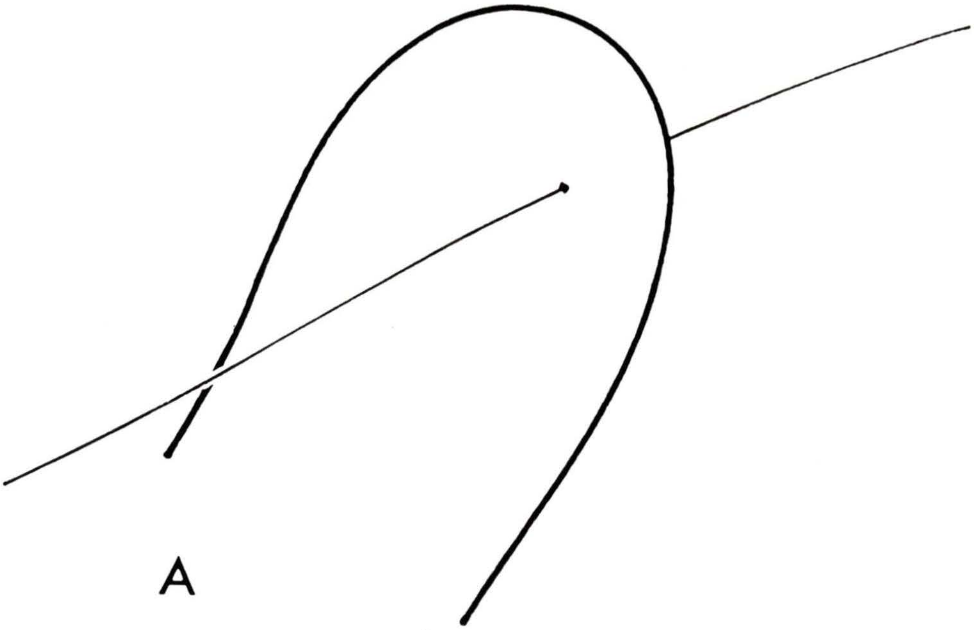

WS

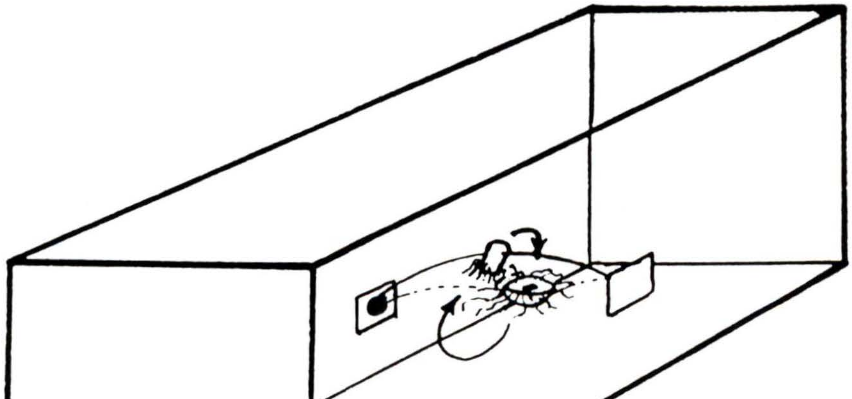
Figure 5: The experimental apparatus used to induce repeated inversions of *G. vertens* in the water column.

A) The position of the tether in the upper mesoglea of the medusa's swimming bell.

B) A *G. vertens* individual tethered by the thread through the swimming bell in an aquarium. With the thread oriented horizontally and left quite slack, the medusa will swim in a circle, inverting regularly at the upper limits of the tether.



A



B

Figure 6: Tracings from a cinematographic record of normal swimming contractions during a swimming bout of *G. vertens*. See the text for a discussion. Solid outlines are tracings taken at 0.0625 s intervals, dashed outlines represent positions midway through these intervals. The scale bar represents 2 mm.

A) Tracings of subumbrellar outlines during swimming.

B) Tracing of the subumbrella at rest.

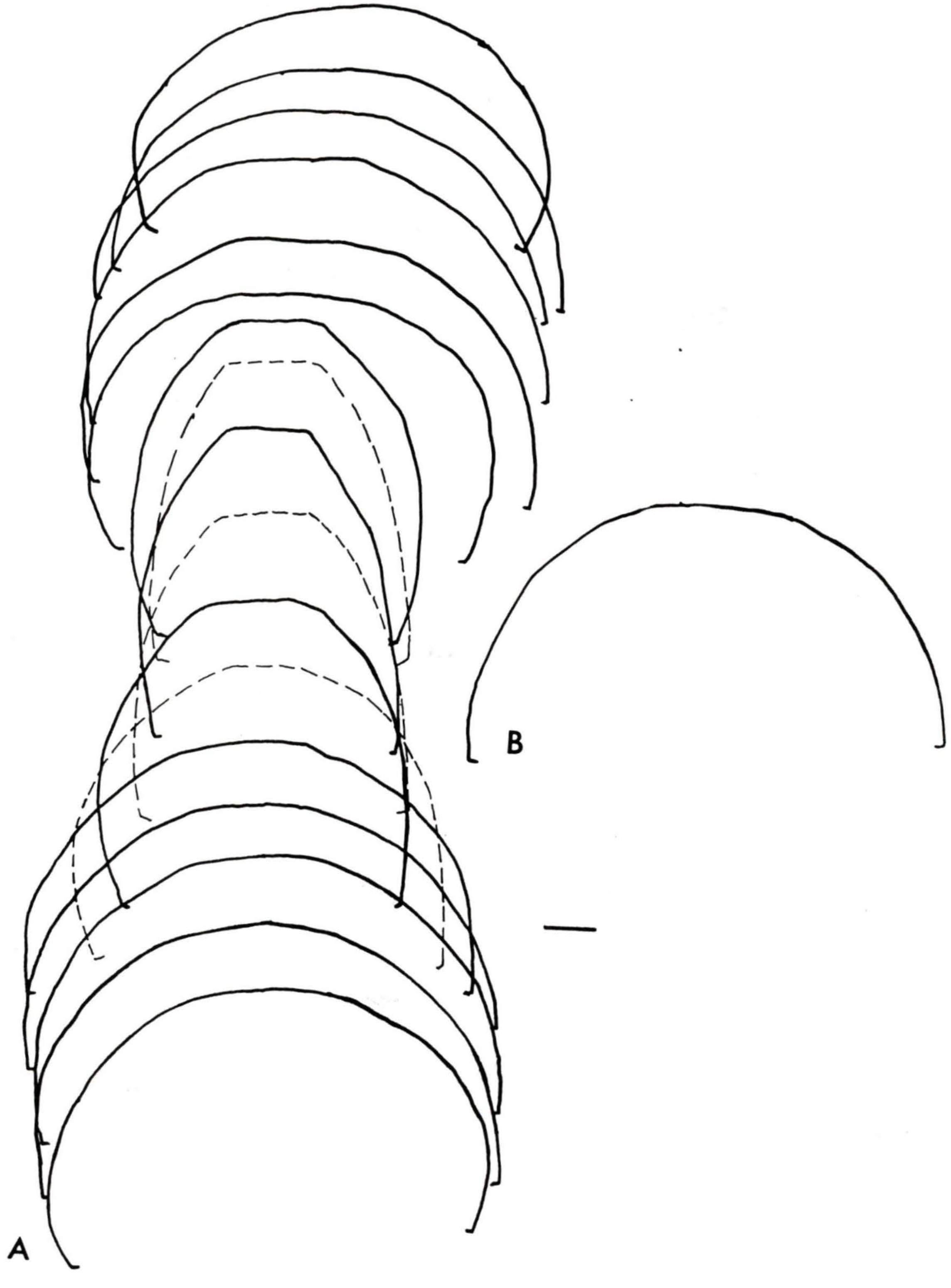

B
A

Figure 7: Plot of subumbrellar bell diameters and velocity of *G. vertens* during a swimming bout. The bell diameters were measured at the bell margin and at a point 1/2 the distance between the bell margin and the apex of the subumbrella.

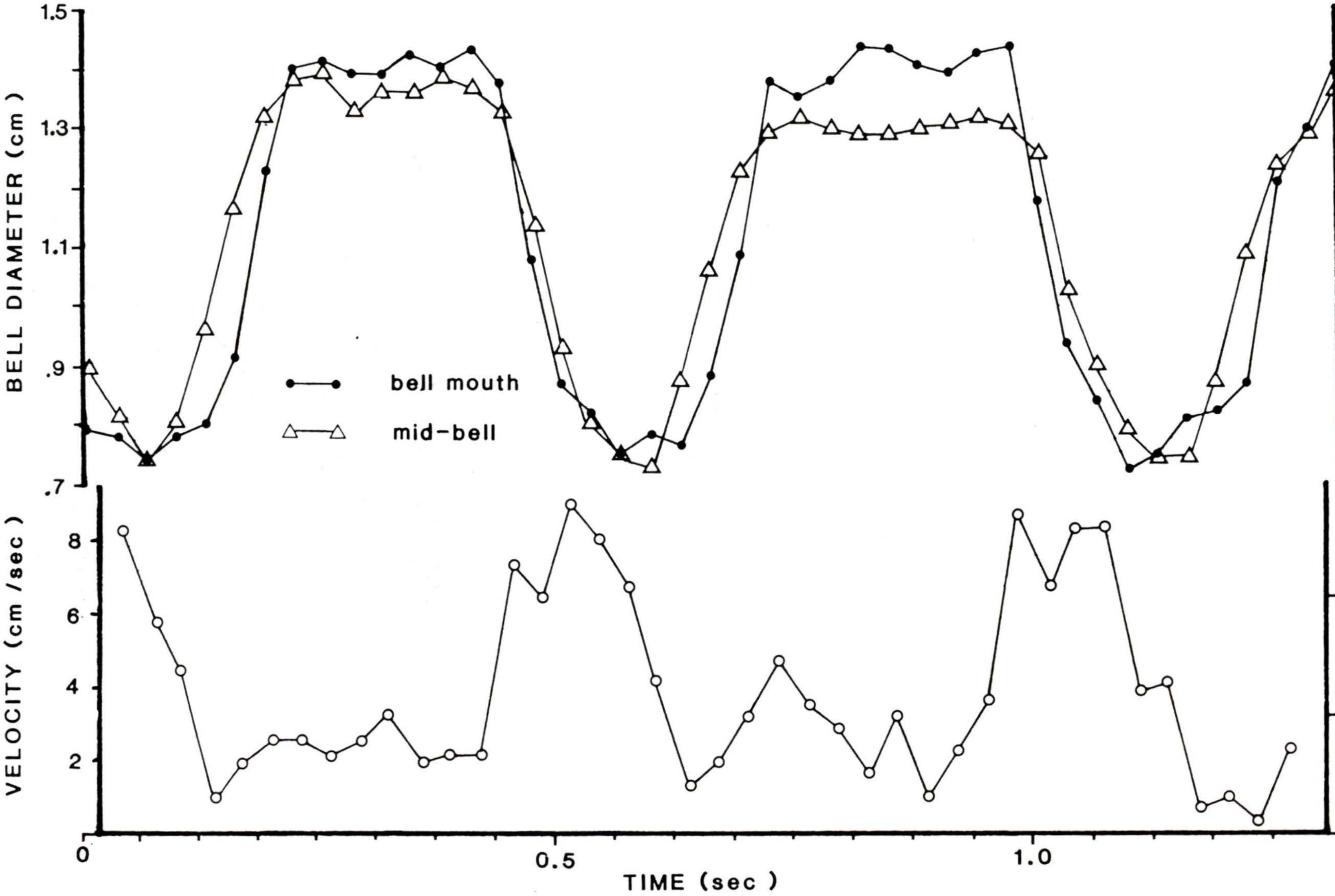

1.5
1.3
1.1
.9
.7
BELL DIAMETER (cm)
● bell mouth
△ mid-bell
8
6
4
2
VELOCITY (cm /sec)
0
0.5
1.0
TIME (sec)

Figure 8: Tracings from a cinematographic record of *G. vertens* while turning. The bell margin commences contracting asymmetrically at the start of a turn (open arrows) and relaxes asymmetrically at the end of the contraction as well (solid arrow). Tracings were taken at intervals of 0.047 s. The scale bar represents 2 mm.

Figure 9: Graph of the bell diameter, velocity, and change in direction of the swimming bell of *G. vertens* during turning. The bell diameter measured was the diameter of the subumbrella at the mouth of the bell, and the direction was taken as the orientation of the bell margin. The bell margin does not relax fully between swimming contractions due to the maintained asymmetrical contraction of the bell margin.

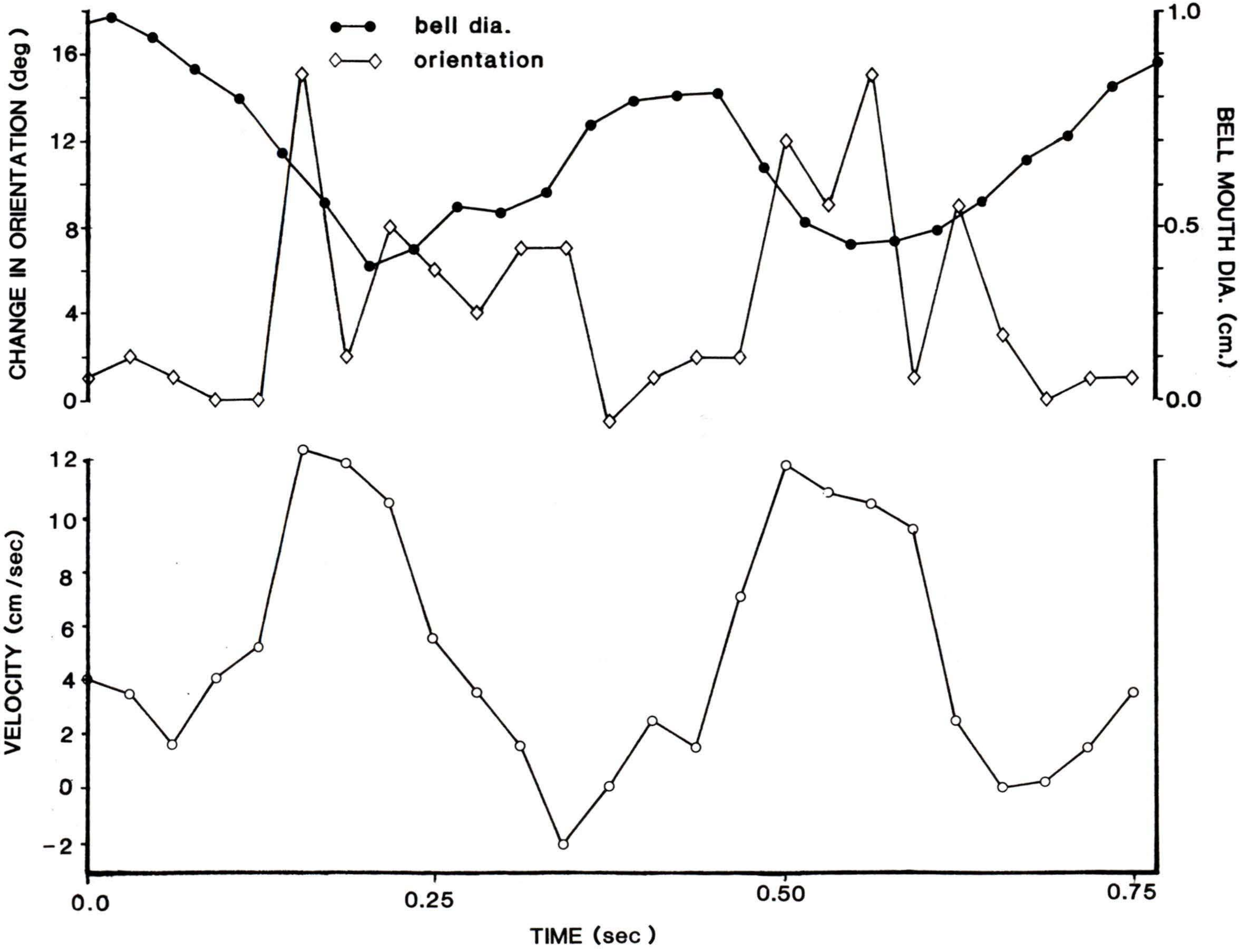

bell dia.
orientation
CHANGE IN ORIENTATION (deg)
0
4
8
12
16
BELL MOUTH DIA. (cm.)
0.0
0.5
1.0
VELOCITY (cm/sec)
-2
0
2
4
6
8
10
12
0.0
0.25
0.50
0.75
TIME (sec)

Figure 10: The effects of surgical operations on potential contributors to turning on the observed directions of motion in *G. vertens*:

A) removal of the whole velum has no effect;

B) removal of 1/2 of the velum has no effect;

C) cutting through the swimming muscle of one half of the medusa forcing excitation of this portion of the swimming muscle to come through the apex of the bell (i.e. causing an asymmetric initiation of the swimming contractions) has no effect.

D) an induced chronic contraction adjacent to the margin of the bell permits motion in one direction only.

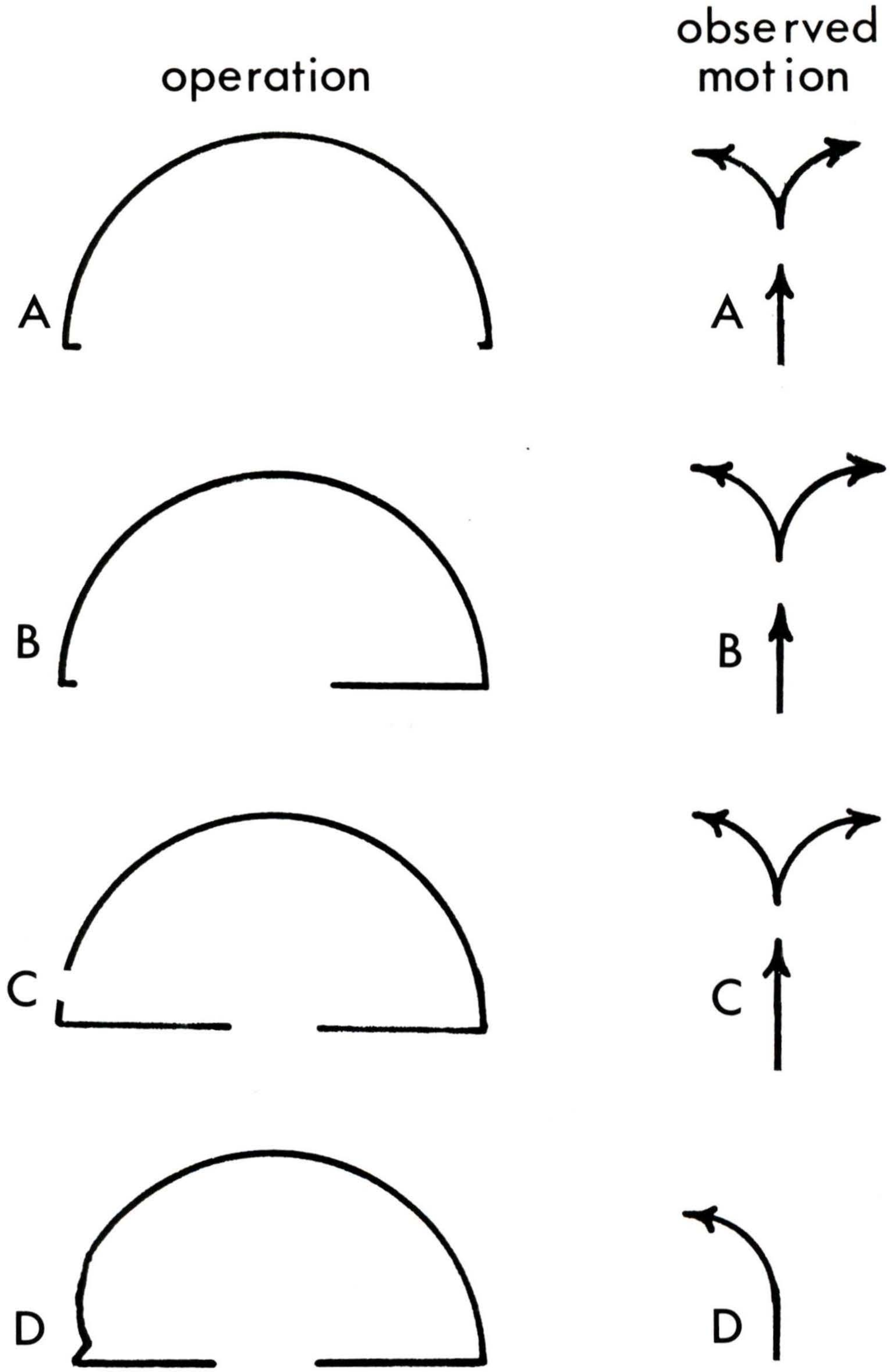

operation
observed motion
A
A
B
B
C
C
D
D

Figure 11: A normal swimming contraction of *E. typus*.

A) Tracings of the subumbrellar outline from a cinematographic record at intervals of 0.0935s. The bell portion that maintains a slightly different degree of contraction at the bell margin (arrow) is wider than that in *G. vertens*. The scale bar represents 2 mm.

B) A graph of the swimming bell diameter and velocity during this swim. The bell diameter was measured at the bell margin and at a point 1/2 the distance between the bell margin and apex of the subumbrella.

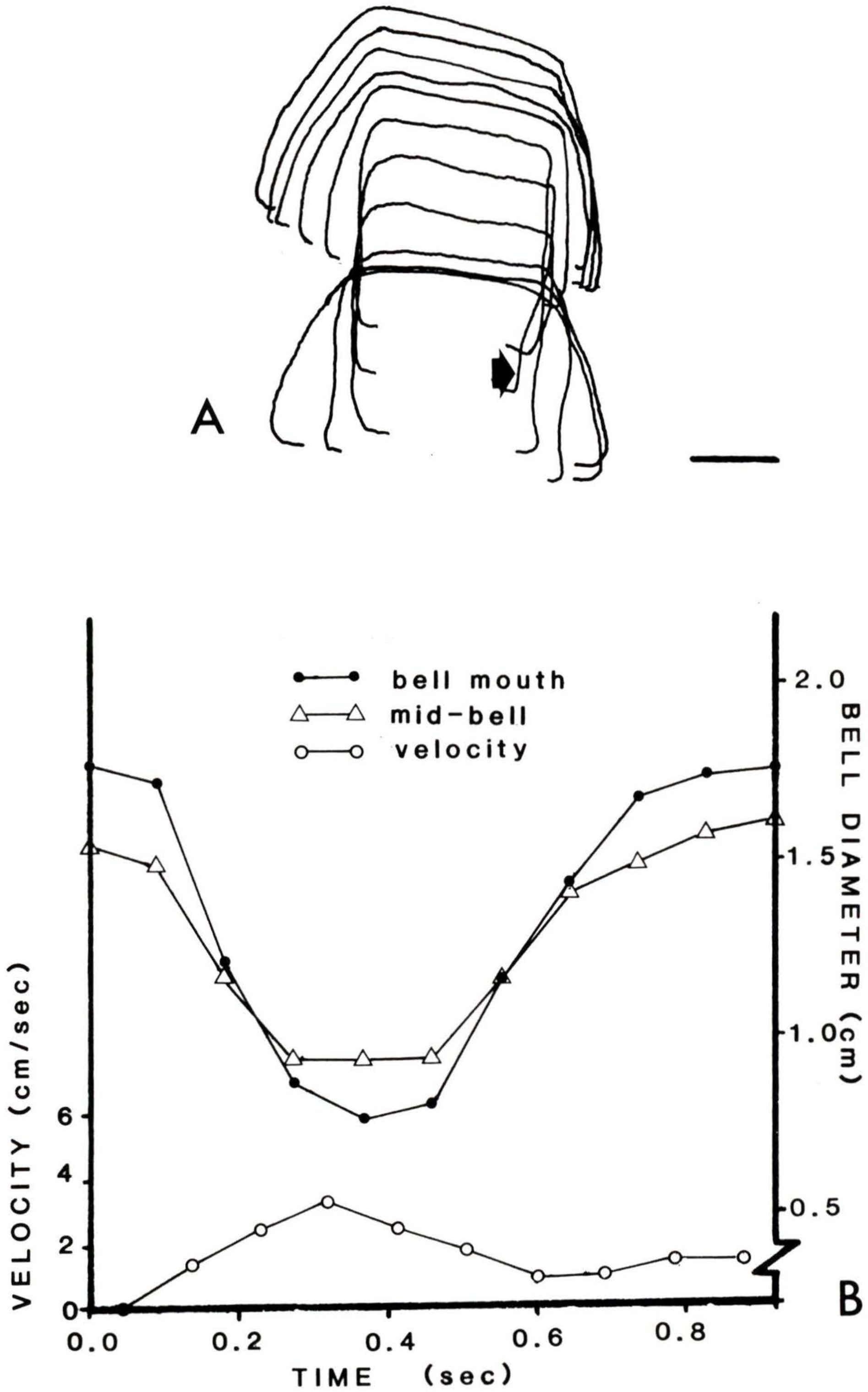
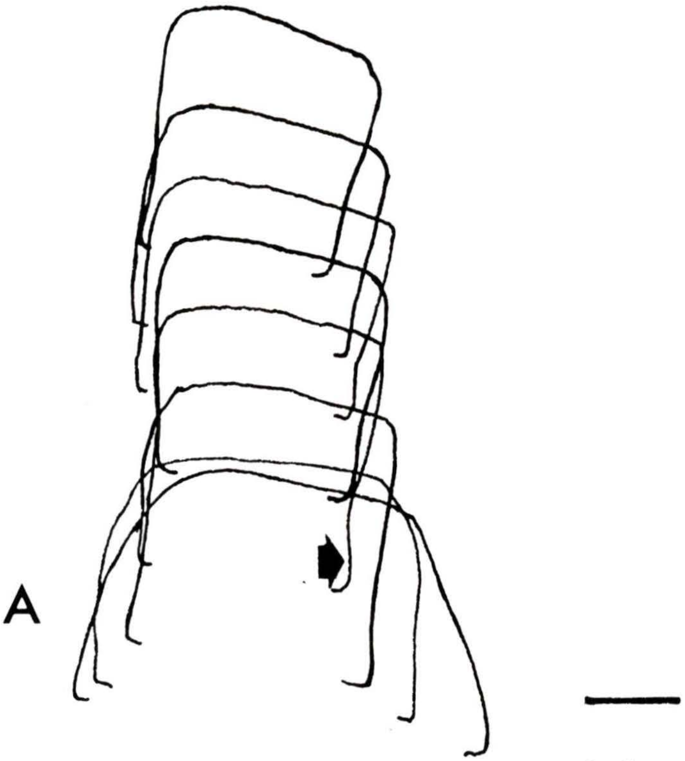
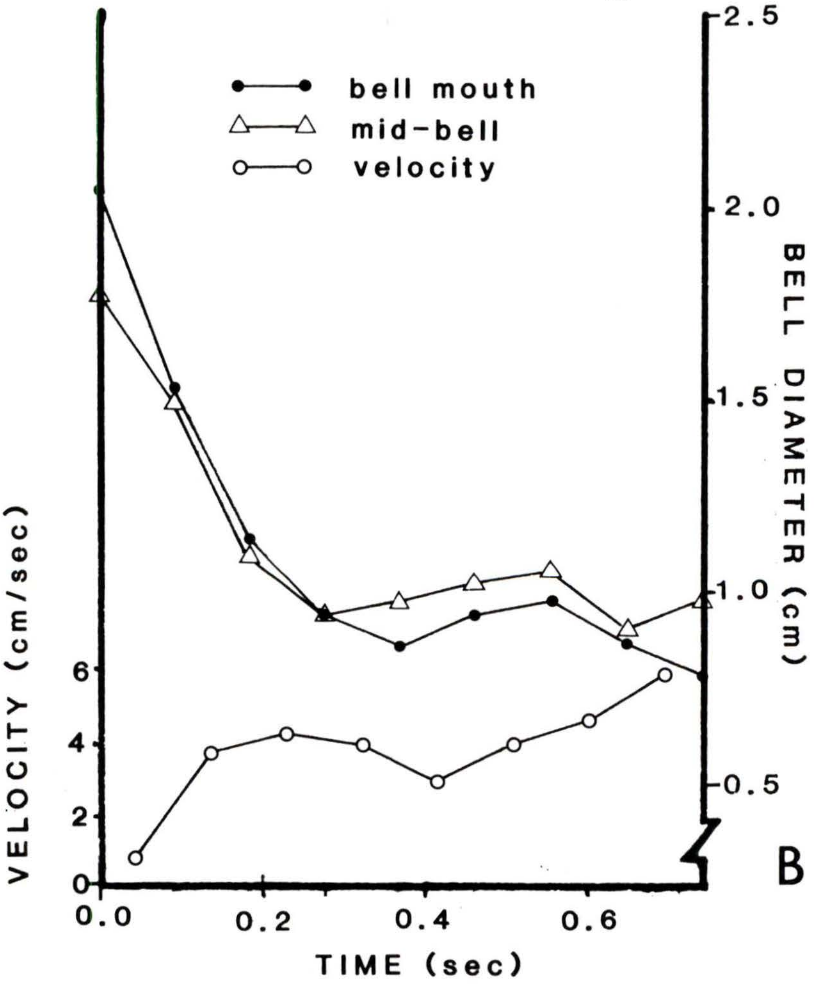

A
B
bell mouth
mid-bell
velocity
VELOCITY (cm/sec)
0
2
4
6
BELL DIAMETER (cm)
0.5
1.0
1.5
2.0
TIME (sec)
0.0
0.2
0.4
0.6
0.8

Figure 12: Two bell contractions during "escape" swimming of *E. typus*.

A) Tracings of the subumbrellar outline from a cinematographic record at intervals of 0.094s.

B) A graph of the swimming bell diameter and velocity during this escape swim. The bell diameter was measured at the bell margin and at a point half the distance between the bell margin and apex of the subumbrella.


A


bell mouth
mid-bell
velocity
VELOCITY (cm/sec)
0
2
4
6
BELL DIAMETER (cm)
0.5
1.0
1.5
2.0
2.5
TIME (sec)
0.0
0.2
0.4
0.6
B

B) *Electrophysiology*

I have presented my electrophysiological results in two sections (as in Part A) for local activities and for coordinated behavioural patterns. In the first I have described events as they occur in the various effectors and their relationship to local activities, and in the second I have described events associated with coordination of the activities of these effectors.

1) *Local activities*:

a) *Manubrium*: I was able to record no spontaneous through-conducted events with extracellular electrodes from the manubria of *G. vertens* and *E. typus*. Both electrical and mechanical stimuli applied to the manubrium ectoderm elicited local contractions extending 1-1.5 mm, but no through-conducted events could be recorded, though behavioural observations indicate such communication is present in the column. Stimuli to the endoderm evoked neither electrical nor behavioural responses.

b) *Radial muscle*: I was also not able to record electrical correlates of radial or sphincter muscle contractions of *G. vertens*. Coordinating events were not detectable for synchronous or sequential contractions of radial muscles, though recordings were made from all endoderm types, the radial muscle bands, the subumbrellar lamella, and the bell margin.

In one *E. typus* preparation I was able to record bursts of biphasic events corresponding to local contractions of the radial muscles with a suction electrode placed over a radial muscle band (fig. 13). The recorded events decreased in size from about 0.07 mV to about 0.016 mV during a burst, but also appeared sporadically as single 0.05-0.06 mV events. Bursts of events corresponded to clearly visible radial contractions, while the single events corresponded to barely discernible twitches. I recorded no events corresponding to tentacle pulses (see description of tentacle electrophysiology) from this position, but 0.01 mV events coincident with swims were evident.

c) Sphincter muscle: I was unable to record electrical correlates of sphincter muscle activity during either local activity or swimming contractions of either species.

d) Swimming muscle: An electrical correlate of swimming contractions, the swimming pulse (SP), was recorded from the swimming muscle, bell margin, and velum in *G. vertens*. In AC-coupled extracellular recordings from the subumbrellar muscle sheet the SP was a compound event 0.17-0.65 mV in amplitude and 100-180 ms in duration (fig. 14). Shape and amplitude varied considerably with position, shape, and tip diameter of the recording electrode and, of course, with electronic filtering used in amplification, but generally were comparable to events recorded from swimming muscles of other hydromedusae (Spencer & Schwab, 1982). Adjacent to the gonad ridge 0.02-0.03 mV pre-pulse events (pre-swim pulses) could occasionally be recorded as well (fig. 14).

In the absence of the bell margin spontaneous SP's were abolished, but the events could still be elicited by electrical stimulation of the muscle sheet, mechanical stimulation of wound edges in the muscle sheet, or by exposing the preparation to air. Conduction of spontaneous and elicited SP's was blocked by the gonad ridges and by cuts in the subumbrellar muscle sheet.

In an emarginate preparation conduction velocities of elicited SP's was 14.5 ± 3.96 cm/s (n=35) in a radial direction and 26.3 ± 8.1 cm/s (n=13) in a circular direction. No distinct differences associated with regions of the bell were detectable. Excess magnesium ions slowed and eventually abolished SP conduction in emarginate and whole preparations. Slowed conduction in a circular direction was approximately 8.18 ± 0.75 cm/s (n=10), while radial conduction velocity was 11.5 ± 3.2 cm/s (n=10).

In *E. typus* as in *G. vertens*, the extracellularly recorded SP varied considerably in shape. Primarily, however, it consisted of an initial biphasic event 0.38-0.56 mV in amplitude (peak-to-peak) followed by a broader 0.02-0.05 mV after-potential. During bouts of swimming contractions, SP's commonly overlapped so the "after-potential" was lost and the peak-to-peak amplitude of subsequent contraction pulses was reduced (fig. 15).

In addition to SP's, 0.014-0.02 mV events coincident with TP's could be recorded from the subumbrella, at least in the vicinity of endodermal pockets of the ring canal (fig. 15). I did not investigate the extent of conduction of these events into the remainder of the subumbrella, though it could provicde further insights into the function of TP's.

e) *Tentacles*: Three major types of spontaneous electrical events were recorded extracellularly from the tentacles: tentacle contraction pulses (TCP's) and fast tentacle contraction pulses (fTCP's) associated with independent contractions of individual tentacles, and tentacle pulses (TP's) associated with coordinated tentacle activity (fig. 16). In addition, events 0.005 mV and less in amplitude not clearly associated with other events or activity were recorded at times (fig. 17). No distinctive electrical activity associated with corkscrew, as compared to linear, contractions was recorded and no events associated with relaxation.

The most common events were TCP's, conducted spontaneously in either direction in a tentacle at a velocity of 10.1 ± 4.9 cm/s (n=32). These generally consisted of an initial positive-going event, a second larger negative-going event, and a final slower positive event (fig. 16b). Relative sizes of portions of the event and total size varied considerably in a single recording and with location along a tentacle's length (figs. 16 & 17). Total amplitude ranged from 0.05 to 4.2 mV. Polarity of the event could be reversed by increasing the suction in the recording electrode (fig. 17).

Individual TCP's could be correlated with single muscular twitches. Commonly these events occurred in bursts associated with a prolonged contraction of a tentacle portion (figs. 16 & 17). During such bursts the recorded events initially increased in size and then maintain a close to maximal size until the end of the burst (fig. 17b). Changes in size occurred in all three components of the TCP, but primarily in the first two. I was not able, however, to discern the functional signifigance of the individual components. Events lacking the initial depolarization were present in some bursts, but again I could not discern an associated behavioural difference. TCP's occurring independently of bursts varied in size, again related to the length of tentacle involved in the muscular twitch. TCP's were not conducted beyond individual tentacles (fig. 16) and commonly were not conducted throughout the length of a single tentacle (fig. 17). Any portion of a tentacle appears capable of initiating a burst of TCP's which then spreads to adjoining regions. At times two concurrent TCP rhythms appeared in single recordings (fig. 17c). The capacity of tentacle tissue to produce TCP's was not abolished by detaching it from the rest of the animal.

The fast tentacle contraction pulse (fTCP) was associated with a rapid, ungraded contraction of a whole or partial tentacle. As was the normal TCP, this event was limited to single tentacles and could be observed in isolated tentacles. It was distinguishable from other TCP's primarily by its large size relative to concurrent TCP's. In extracellular recordings it appeared as a large complex event, 0.1-4.0 mV in amplitude, occurring either during a train of TCP's or independently. The event was quite rare and could not be reliably elicited, and so was not studied further.

The third type of spontaneous event recorded from the tentacles was the tentacle pulse (TP). It was associated at all times with the presence of TP's in the bell margin (see description of tentacle coordination), and, if coordinated tentacle contractions were present, it was correlated with these. The spontaneous event was conducted only from the base to tip in the tentacles, at a velocity of 17.8 ± 2.0 cm/s (n=12). Amplitudes ranged from 0.1 to 2 mV, but at a single recording site varied by only 5-10% (range/mean). In tentacles detached from the animal no comparable spontaneous TP's were present.

In extracellular recordings, the TP appeared as a compound event commonly smaller than TCP's and of opposite net polarity (with low suction in the recording electrode) (fig. 16). It typically consisted of a small biphasic component with fast rise and fall times followed by a larger, broader, monopolar portion (fig. 17b). Size and shape varied again from preparation to preparation and with differences in electrode position and tip characteristics, at times appearing either as a simple monopolar (fig. 16) or biphasic (fig. 26) event. Suction in the recording electrode did not affect shape or polarity as it did TCP's (fig. 17a). I could not distinguish differences between TP's associated with coordinated contractions and those for which behavioural correlates could not be detected ("cryptic" events) (see also description of tentacle coordination). TP's and TCP's at times occurred simultaneously and summed in electrophysiological records (fig. 16), but generally their occurence appeared independent of one another (see also fig. 27). Both were conducted over a simple strip of ectoderm of partly severed tentacles.

Continued electrical stimulation of a tentacle resulted in a recurrent "fatigue" of the contractile response (fig. 18). Spontaneous bursts of TCP's still occurred during such fatigue, and at such times the stimulus appeared more effective, suggesting a cyclical change in the threshold to elicitation of TCP's is present. As expected, increasing the stimulus strength was also effective in postponing the fatigue (fig. 20b).

Cyclical spontaneous TCP activity could also be seen in a tentacle of an individual recovering from magnesium-induced anesthesia (fig. 19a), prior to recovery of the TP and swimming rhythms. Comparable, though independent, rhythms of cryptic events could be recorded from the inner and outer nerve ring regions at the same time (fig. 19b & c), indicating that pacemaker activity without muscular activity could be expected at this level of anesthesia.

I recorded two types of events from *E. typus* tentacles: TCP's associated with independent contractions and TP's associated with coordinated contractions (fig. 20). Both were variable in amplitude and shape and could often only be reliably distinguished on the basis of through-conduction and disparity in concurrent size. Spontaneous conduction velocities were obtained only for TP's (10.2 ± 0.4 cm/s (n=8)), since spontaneous TCP's were more difficult to localize. Both events continued to conduct past a cut leaving only a tiny strip of connecting ectoderm. Pre-pulses usually were only clearly distinguishable in TP's recorded from the tentacle bases, so in much of the tentacle the two pulse types were indistinguishable on the basis of shape. The pre-pulses of the tentacle bases, no doubt comparable to pre-tentacle pulses (pTP's) recorded in other species, also become visible throughout the tentacle during fatigue of spontaneously occurring TP's (fig. 20c).

Spontaneous TCP's were conducted in either direction in the tentacles as simple mono- or bi- polar events, .08-.3

mV in amplitude, but did not necessarily spread throughout a whole tentacle. As in *G. vertens*, the size of the electrical event reflected the relative proportion of tentacle participating in the contraction and trains of TCP's represented continued or maintained tentacle contractions. TCP's could still be recorded from isolated tentacles.

All spontaneous TP's I observed in *E. typus* were conducted from the base to the tip in the tentacles. In general they were monopolar or biphasic events of variable amplitude (0.06-1.5 mV) similar in shape to TCP's, but in tentacle bases appeared bimodal or of longer duration due to the presence of relatively large pTP's. TP's varied up to 70% in amplitude (range/mean) in a given recording situation, compared to 30% in *G. vertens*. In a group of spontaneous TP's, the size of the second portion of the event decreased progressively (fig. 20c), commonly associated with a reduction in the magnitude of the contractile twitch. Severing a tentacle from a medusa reduced the amount of spontaneous activity recorded from that tentacle, but due to the difficulty in distinguishing TP's from TCP's, it was unclear if TP's were really absent.

Single TP-like events that were through-conducted into all the tentacles could be evoked by electrical stimulation of any location on a tentacle or of the outer nerve ring region in a location that was effective in eliciting crumpling. This event was conducted at a velocity of 13.4 ± 3.2 cm/s (n=8) in the tentacles. Repeated stimulation resulted in cyclical fatigue of the response as seen in *G. vertens*, but in this case the pre-pulse failed too (fig. 21). This phenomenon in *E. typus* differed from that in *G. vertens* as well in that the initial response was relatively large compared to the following responses. As in *G. vertens*, some facilitation in amplitude of these later responses could, nevertheless, still be present. Regions adjacent to the stimulation site tended to give larger responses to the first two or three stimuli, rather than only the first one.

f) Velum: I recorded velar swimming pulses (vSP's), pre-swim pulses (pSP's), and small cryptic events from the velum of *G. vertens*. vSP's corresponded to contractions of the circular muscle coincident with the swimming contractions of the rest of the bell. As did the swim pulse of the swimming bell, the vSP varied considerably in shape and amplitude according to the recording parameters and could not be clearly distinguished from SP's elsewhere by these characteristics (fig. 14). The presence of pSP's did, however, generally distinguish vSP's from SP's in the swimming bell.

Spontaneous vSP's were abolished by removal of the bell margin, but the events could still be elicited by electrical stimulation. Such elicted vSP's in isolated vela had a radial conduction velocity of 4.46 ± 0.3 (n=6) cm/s and a circular conduction velocity of 3.84 ± 0.4 (n=8) cm/s. In the intact animal circular conduction velocities were about 42.1 cm/s. Excess magnesium ions slowed and eventually abolished conduction of elicited SP's in the velum.

A variety of small electrical events, 0.05 mV in amplitude and smaller, independent of SP's, were also recorded from the velum. Some of these appeared to occur coincidentally with TP's in adjacent tentacles (fig. 22a). Electrical stimulation of the outer nerve ring region evoked cryptic subthreshold responses in the velum with a circular conduction velocity of 16 cm/s which became distinguishable when the swimming response failed (fig. 22b). I could not demonstrate any association of these elicited "subthreshold" events with spontaneous events, but a TP-like event was elicited in the tentacle simultaneously, so the events may be somehow related to the TP's.

The vSP in *E. typus* appeared similar to SP's recorded from the subumbrellar muscle (fig. 23). As in the subumbrella, contractions of the velum tended to overlap resulting in a quite complex train of recorded events during a swimming burst (fig. 23b).

Events in addition to vSP's could be recorded from the velum of *E. typus* as well (fig. 23a). The largest of these, regular biphasic events of up to 0.08 mV amplitude (peak-peak), appeared coincident with TP's recorded from the outer nerve ring. Smaller variable events could also be recorded, but I could not determine their significance.

2) *Behavioural patterns*:

a) *Crumpling*: I recorded no spontaneous or elicited electrical events from the bell exumbrella, subumbrellar lamella, ring and radial canal endoderm, or stomach endoderm of *G. vertens* individuals 0.4-2.5cm in bell diameter. All of these are tissues apparently involved in the coordination of crumpling in other hydromedusae (Spencer & Schwab, 1982). An electrical stimulus applied to the base of tentacle bulbs (effective in evoking crumpling in *E.typus*) commonly elicited TP-like events on a one-for-one basis in *G. vertens* (see description of velum electrophysiology), and, upon repeated stimulation, also increased local tentacle activity, local radial and sphincter muscle contractions and eventually also induced swimming bursts. However, I was not able to record electrical events coordinating these activities.

In *E. typus* a very distinct crumpling behaviour is present. It consists of an inhibition of swimming, velar twitches, and a maintained simultaneous contraction of the tentacles, and radial and sphincter muscles. But, as in *G. vertens*, no electrical events coordinating these activites could be recorded extracellularly from the bell exumbrella or endodermal tissues. In addition, the behaviour could only be elicited by mechanical or electrical stimulation of the outer bell margin. The exumbrella and endodermal tissues appeared insensitive to mechanical, electrical, and chemical stimuli. I found no evidence of a large epithelial event associated with crumpling.

During a crumple, the swimming rhythm was disrupted, but TP rhythms were only slightly depressed, if affected at all (fig. 24). In crumples induced by electrical stimulation, electrical activity was present in the velum at initiation, but these events were not clearly reproducible (fig. 25). Events similar to small TP's were also present during the early stages of a crumple.

At the start of a "spontaneous" crumple, tentacles contracted maximally and synchronously with the radial and sphincter muscles. They would, however, start relaxing earlier than these other muscles, but were kept slightly contracted by muscular twitches associated with TP's that occurred later during the crumple (see fig. 25). There was no evidence that events other than TP's are present in the tentacles during crumpling. Electrical stimuli to the bell margin effective in eliciting crumpling also elicited TP-like events which were propagated into all the tentacles (fig. 26). The stimulus threshold required to elicit this event, however, was far lower than that required for crumpling, and the event did not change its form once the crumpling threshold was reached (fig. 26b).

b) *Feeding*: Shrimp extract applied to the tip of a tentacle of *G. vertens* induced a train of TCP's distinctive in that the initial events were of large amplitude rather than facilitating gradually (fig. 27a, compare fig. 16). In the adjacent outer nerve ring region a series of 0.001-0.002 mV events was present reflecting TCP activity in a neighbouring tentacle. I did not record events directly correlated with sphincter and radial muscle activity during pointing. TP rhythms appeared to be unaffected.

In *E. typus* the application of shrimp extract to a tentacle increased TCP activity as well (fig. 27b). I made no recordings from other regions of the bell during feeding in this species.

c) *Swimming coordination*: Events 0.006-0.011 mV in size occurred at a relatively constant time period prior to SP's recorded from the inner and outer nerve ring regions of *G. vertens* (fig. 14). These no doubt are analogous to pre-swim pulses (pSP's) reported for other hydromedusae and shown to represent activity of "swimming giants" of the inner nerve ring (Satterlie & Spencer, 1980). I was not able to isolate these pulses from SP's with excess magnesium ions, as has been done in some of the other species (e.g. Mackie, 1975). pSP-SP latency were variable to some extent, and pSP's at times occur independently of or in pairs before SP's. Conduction of elicited swims in the bell margin was about 29.6 ± 9.8 (n=8) cm/s, comparable to conduction velocities of pSP's in other hydromedusae (see Spencer & Satterlie, 1983). Other than the pSP, the shape of the swimming event recorded from the inner nerve ring region was not different from those recorded elsewhere in the bell (fig. 14). I was not able to glean any evidence of sphincter muscle activity during normal swimming from these records.

I did not investigate the neurophysiological organization of swimming in *E. typus*.

d) Somersault: I made no recordings of electrical correlates associated with bell inversions or subsequent inhibition of swimming during "somersaulting" of *G. vertens*.

e) Tentacle coordination: Regular coordinated movements of tentacles of *G. vertens* are not the rule.

Coordinated electrical events, the TP's described earlier, were, however, recorded throughout all tentacles and when coordinated twitches of the tentacles did occur, they normally coincided with these TP's.

The event in the bell margin corresponding to TP's and associated with their coordination and initiation generally appeared similar to the TP's of the tentacles. It consisted of a small, rapid, biphasic event followed by a larger monophasic peak of longer duration (fig. 16c). Shape and size varied, again depending on recording parameters, occurring at times as simple monophasic events or as small biphasic events (fig. 28). Amplitudes ranged from 0.03-0.16 mV but were relatively constant within a single recording situation (< 5% variation). Attempts to separate the waveform into discrete components with excess magnesium ions were unsuccessful. Putative post-synaptic potentials coincident with TP's were recorded, however, from a non-spiking epithelial cell in the outer nerve ring region (resting potential -52 mV) suggesting an epithelial component may be present in the extracellular event. Conduction of spontaneous TP's in the bell margin was approximately 24 cm/s.

Cuts through the nerve ring regions interrupted conduction of the TP's in the bell margin, so neither the subumbrellar epithelium nor the velum appear capable of effectively propagating the events. Bell portions separated by cuts through the margin possessed different TP rhythms (fig. 29b), indicating that all portions of the bell margin are capable of initiating TP's.

The majority of spontaneous and elicited coordinated tentacle contractions were associated with TP's, but not necessarily in conjunction with changes in pulse amplitude (fig. 28a). TP's appeared to occur independently of other events (fig. 28b, 33a), but some ambiguity still remained in distinguishing changes in shape associated with coordinated activity. Shape and size of recorded TP's were quite variable from record to record and it was difficult to reliably induce coordinated tentacle activity, so I was not able to distinguish differences in pulse form of TP's associated with their effectiveness to produce visible contractions. Coordinated tentacle contractions also occurred occasionally without TP's (fig. 28b) and small "cryptic" events presently of unknown function were present in both outer nerve ring and tentacle recordings (fig. 29). It is thus also possible that tentacle coordination in *G. vertens* may involve mechanisms in addition to, or other than, that reflected by TP's.

No correlation between the occurrence of TP's and SP's was evident in either pinned out preparations or in restrained whole animals. In a continuous recording session of 70 min. with a suspended whole animal, swimming contractions and TP's occurring within sequential 2 min. periods showed a non-significant correlation coefficient (r) of 0.2, suggesting very little interaction.

E. typus, in contrast to *G. vertens*, does display a marked degree of coordinated tentacle activity. TP's are conducted throughout the tentacles and bell margin (see description of tentacle electrophysiology) and appear responsible for this coordinated activity. In extracellular records, the TP in the outer nerve ring consisted of a monophasic event of short duration and up to 0.15 mV in amplitude (the pre-tentacle pulse (pTP)) followed by a second 0.03 mV event of longer duration and opposite polarity (fig. 30a). Peak-to-peak amplitude varied in continuous recordings, but not necessarily in conjunction with the amplitude of TP's recorded from the tentacles (fig. 30a). Correlates of TP's were also recorded from the subumbrellar epithelium and velum (see description of swimming muscle and velum neurophysiology), but neither tissue was effective in propogating TP's beyond a cut in the bell margin.

Coordinated tentacle contractions with TP's could be elicited by electrical stimulation of a tentacle or of the outer bell margin, as described earlier. I observed no other forms of coordination of tentacle activity in *E. typus*, and recorded no instance of TP's without concomittant contractions. Excess magnesium ions abolished both spontaneous and elicited coordinated and local activity.

Swimming contractions are often accompanied by coordinated tentacle contractions in *E. typus*, as described earlier by Mackie & Mackie (1963). When observed here, however, the timing between TP's and SP's was variable and the TP's generally occurred during the final portion of the SP's (fig. 31) rather than prior to them, as is the case in some other hydromedusae (see Spencer & Schwab, 1982). During continuous swimming the TP rhythm fatigued repetitively, comparable to what was observed during continuous electrical elicitation (fig. 31).

f) Tentacle anchoring: I recorded no electrical correlates overtly corresponding to coordination of tentacle anchoring.

Figure 13: Radial muscle contraction pulses in *E. typus*. Top trace is a recording from a tentacle base showing both swimming (small arrows) and tentacle activity while the bottom trace is from an electrode placed over the radial muscle band. The large arrow indicates a burst of radial muscle contraction pulses. Events concurrent with swims are also present (●), but none with TP's (■).

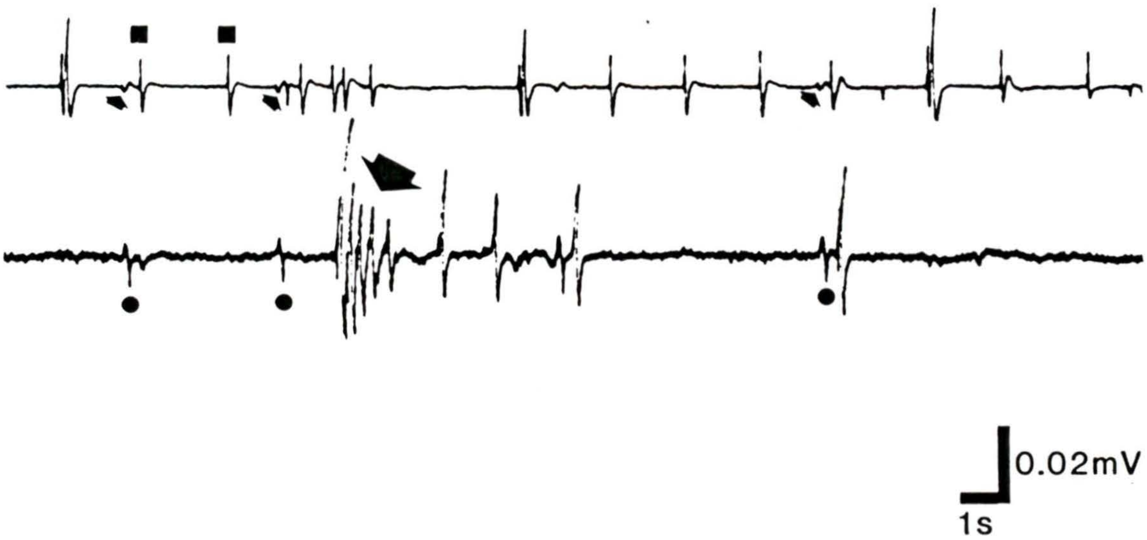

0.02mV
1s

Figure 14: Shape of the swimming pulse of *G. vertens* in AC-coupled extracellular records.

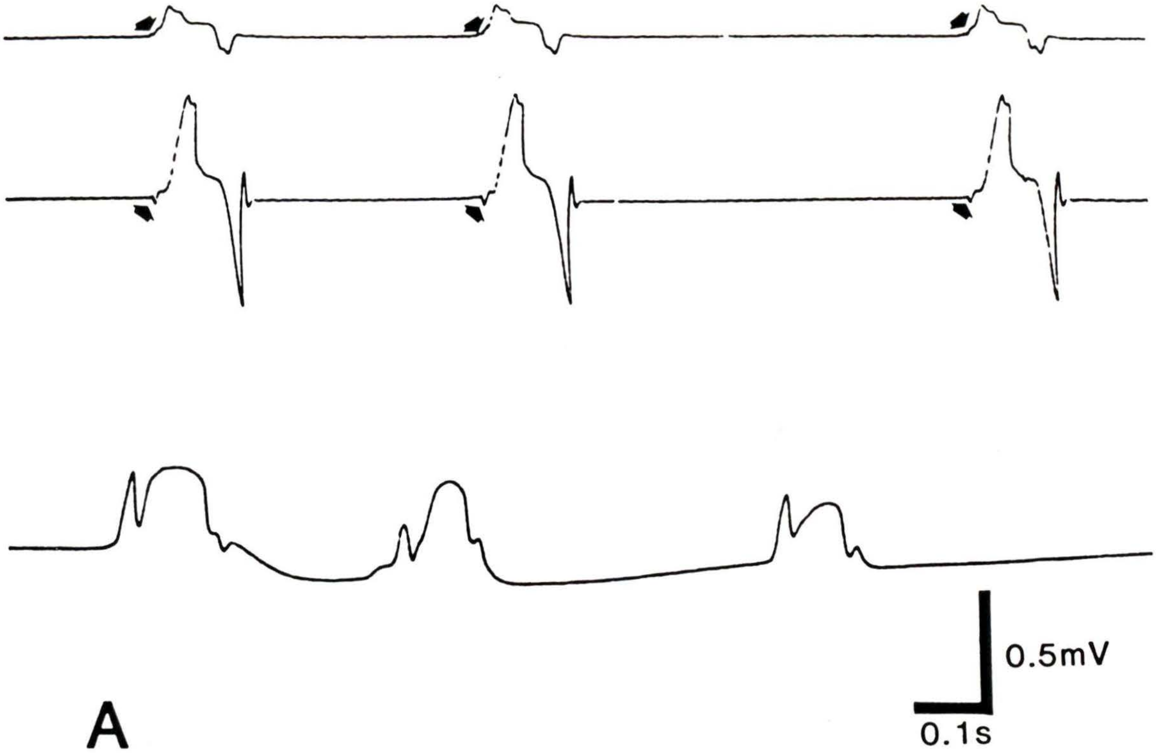
A) Chart records from the base of the gonad ridge (top trace), the velum (middle trace), and the swimming muscle sheet (bottom trace). Pre-swim pulses (pSP's) can be recorded from both the base of the gonad ridge and the velum (arrows).

B) An oscillograph of a pSP recorded from the bell margin (arrow). The large event following the pSP is the initial portion of a swimming pulse.

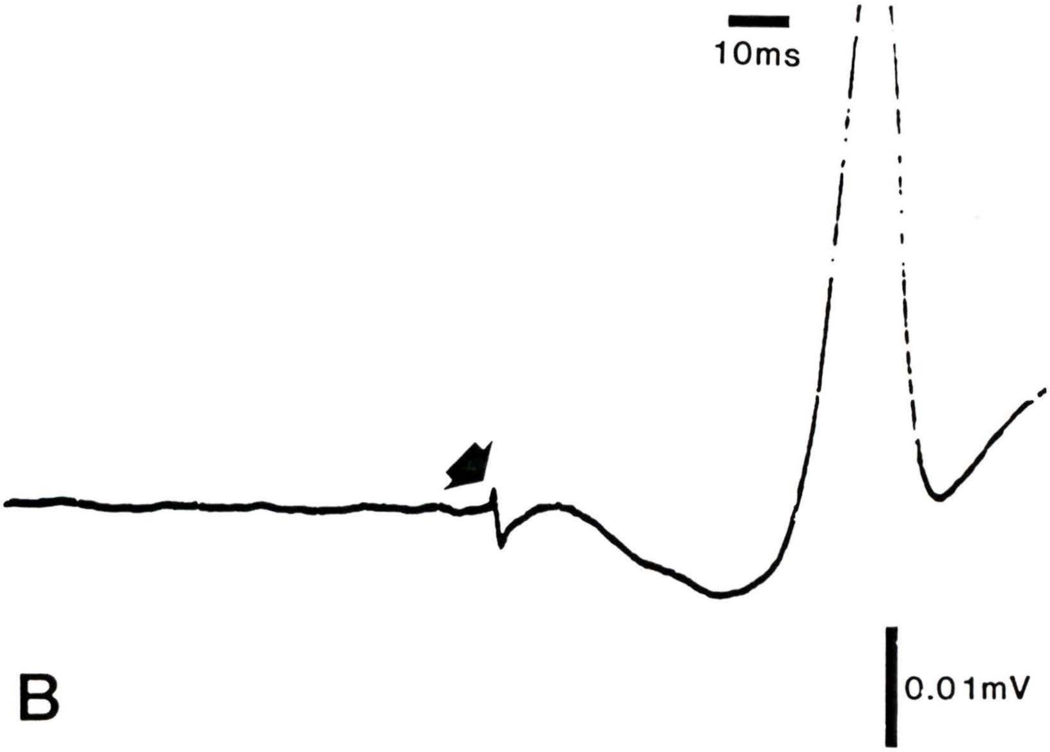
B) Recorded from the velum.

C) Recorded from the swimming muscle.

Note that in A) and B) pre-pulses are present (arrows) while none can be reliably distinguished in C).



A



B

Figure 15: Events recorded from the subumbrella of *E. typus* adjacent to the bell margin. SP's (●) following one another closely show a reduction in amplitude and a loss of the final portions of the waveform. Cryptic events can also be recorded from at least portions of the subumbrella, the largest of which (■) are coincident with TP's. The smaller events (arrows) are not coincident with other events.

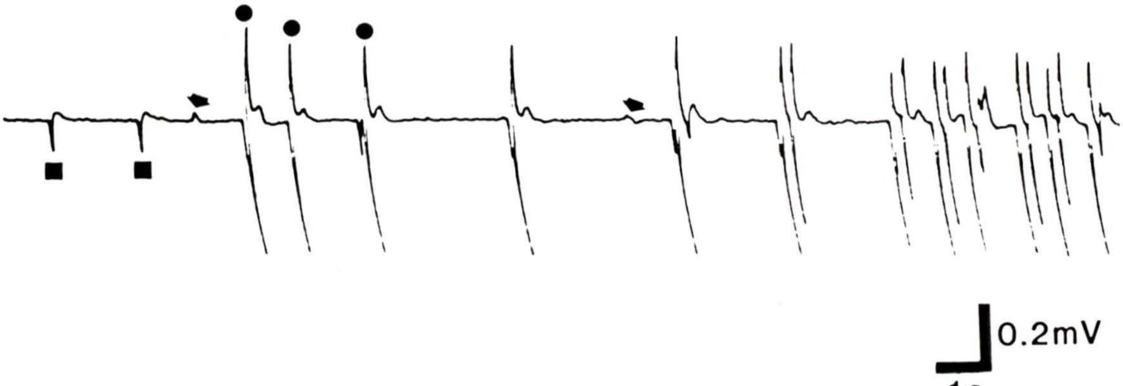

0.2mV
1s

Figure 16: Events recorded extracellularly from the tentacles of *G. vertens*.

A) Tentacle pulses (TP's:■) are through-conducted to all tentacles while tentacle contraction pulses (TCP's:▲) and fast tentacle contraction pulses (fTCP's:✱) are not. TCP's commonly appear in bursts of events (large arrow). The tracings are simultaneous records from two different tentacles.

B) Waveform of TP's (■) and TCP's (▲) in the tentacle. The two types of events will summate in the record (large arrow) but otherwise appear to occur independently. Note the presence of a pre-pulse in the TP's (small arrows) which is absent in the TCP's.

C) A TP of the outer nerve ring. Note the similarity to those recorded from the tentacle.

D) The fTCP (✱) as recorded from the mid-portion of a tentacle compared to TP's (■) and TCP's (▲).

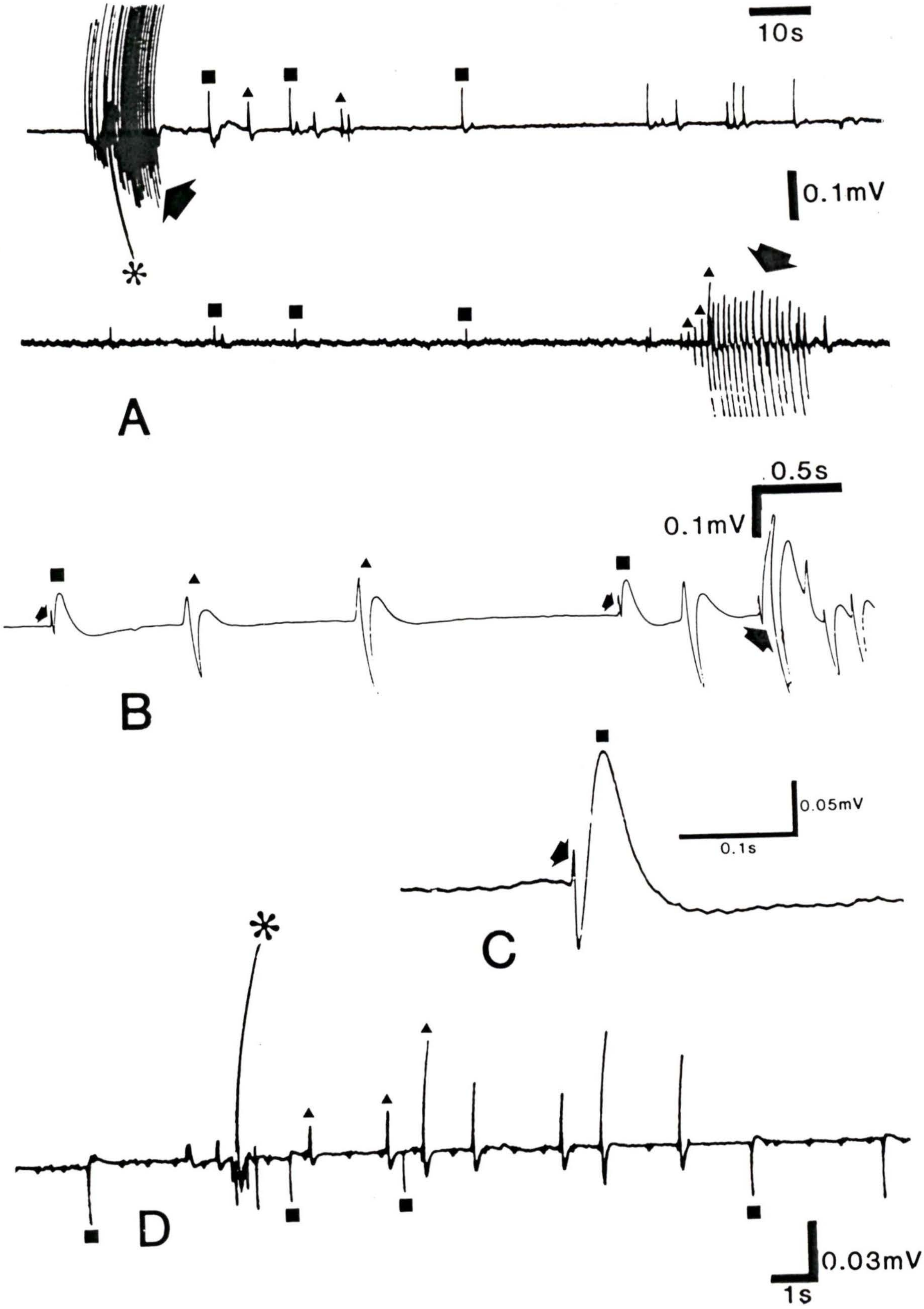

10s
0.1mV
*
A
0.5s
0.1mV
B
0.05mV
0.1s
C
*
D
0.03mV
1s

Figure 17: Variation in the TCP's of *G. vertens* :

A) Variation of TCP's along a tentacle's length and with increased suction in the recording electrode. The top trace is from the base of a tentacle while the second and third traces are from the middle and tip of the same tentacle respectively. The arrow marks the time when increased suction is applied to the electrode at mid-tentacle. Note that the net polarity of TCP's is reversed while that of TP's (■) remains the same. All events other than the TP's are small cryptic events or TCP's. Two concurrent TCP rhythms (open arrow) occur commonly (see C below).

B) Variation in TCP's during a burst. Total amplitude of the events commonly increases at the start of a burst. Note that some of the TCP's lack the initial portion of the waveform (arrow), which is of unknown significance. Note also the low-amplitude cryptic events (small arrows).

C) Two concurrent TCP rhythms recorded with a single electrode. The arrow indicates a second rhythm starting up while a first is still present.

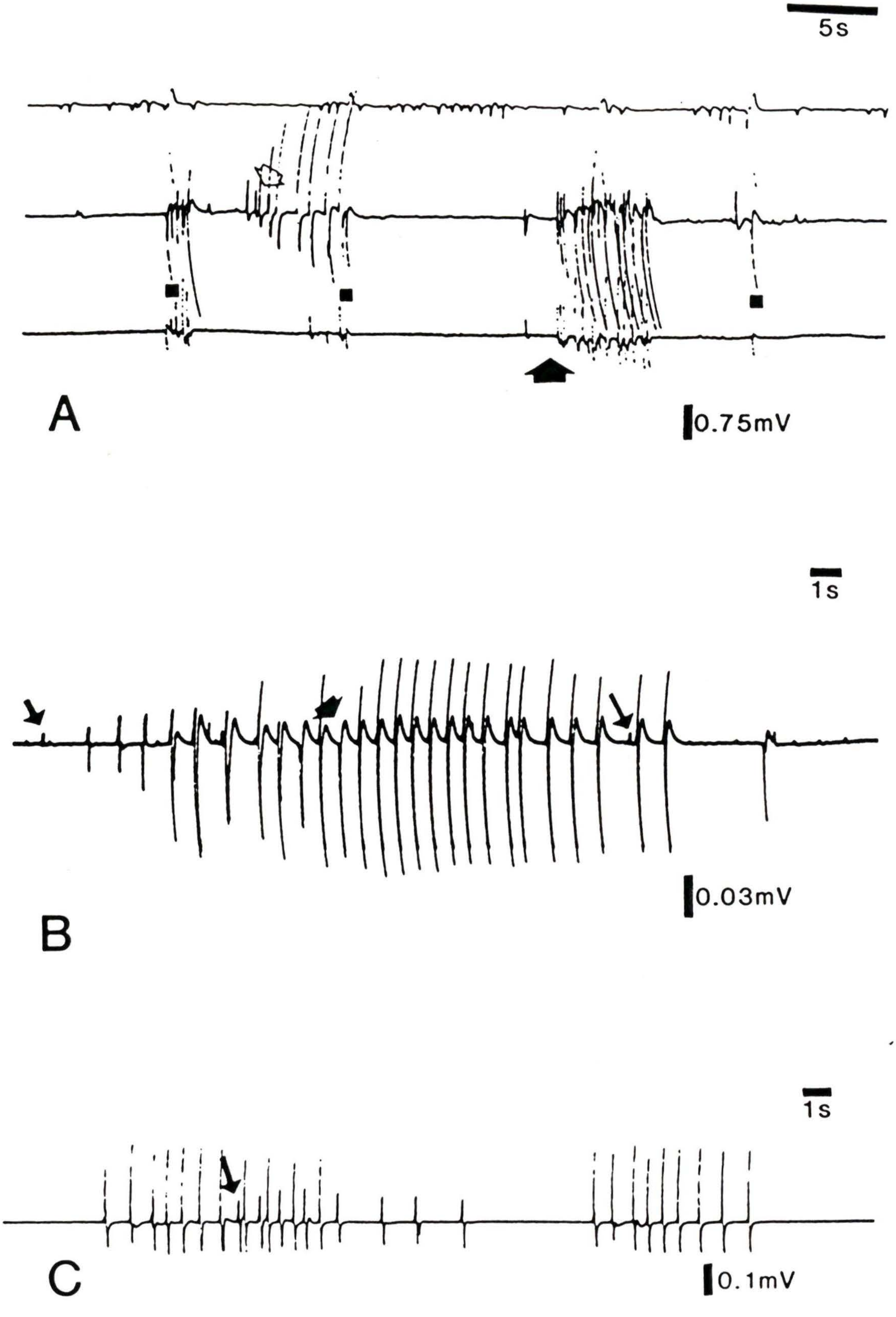

5s
A
0.75mV
1s
B
0.03mV
1s
C
0.1mV

Figure 18: Recurrent "fatigue" in a tentacle of *G. vertens* in response to continued electrical stimulation.

A) The pattern of "fatigue" as recorded from two points along the stimulated tentacle. "Spontaneous" bursts of TCP's occur as well (arrows), during which time the stimuli are more effective. The regular low amplitude events in the lower trace indicate stimuli.

B) A series of superimposed records of the tentacle response at mid-tentacle while "fatiguing" to stimuli at 1pps of 3 volts (top trace) and 5 volts (bottom trace) (1ms duration). The contractile event (▲) fails, but the "TP" (■) remains throughout. Fatigue sets in less rapidly with the greater stimulus (arrow).

In both A) and B) stimuli were applied at mid-tentacle and recordings made from the tip (top trace) and base (bottom trace).

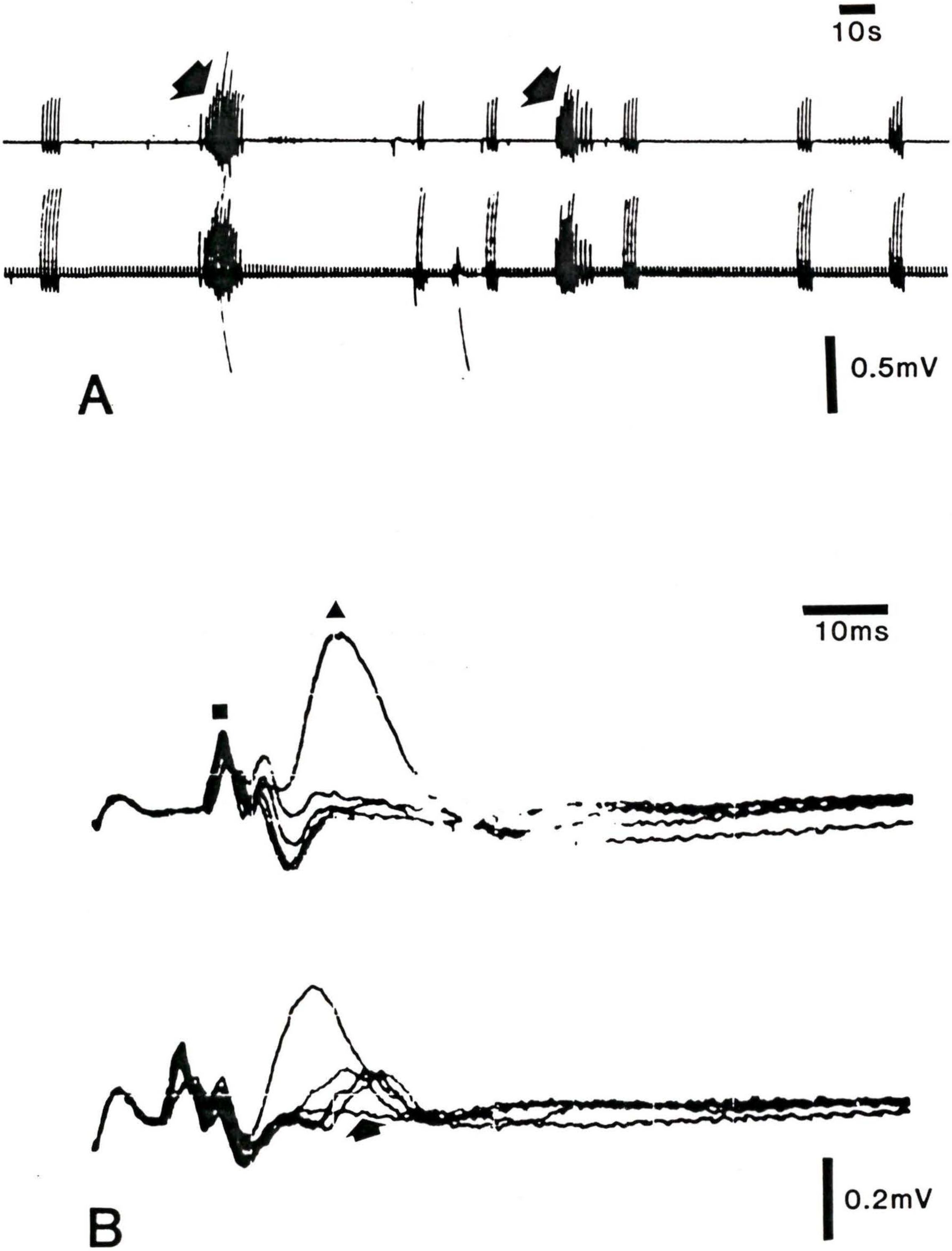
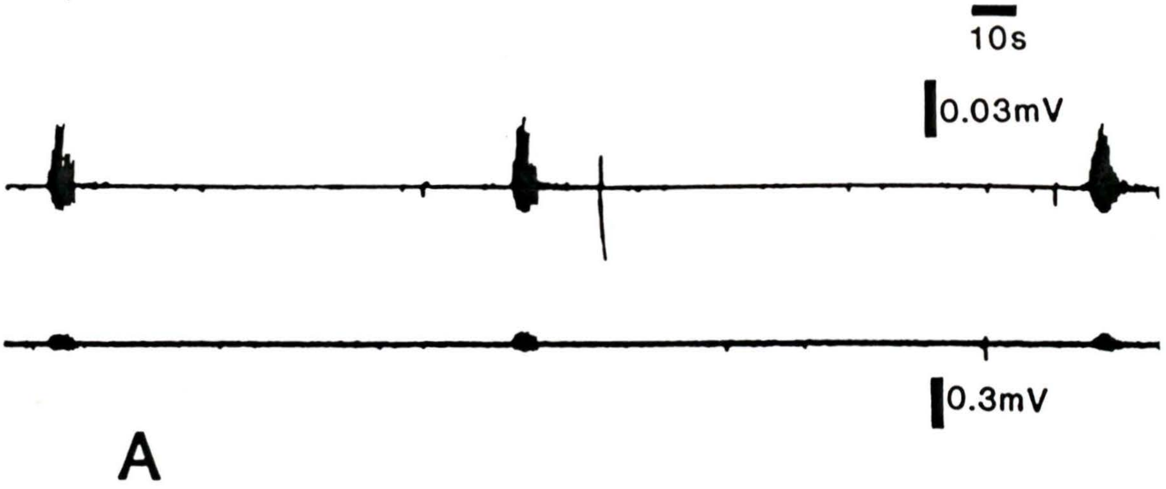

10s
0.5mV
A
▲
■
10ms
0.2mV
B

Figure 19: Cyclical activity in a tentacle and in the inner nerve ring of *G. vertens* during recovery from magnesium-induced anesthesia.

A) Record from the tip (top trace) and base (bottom trace) of a tentacle.

B) Record from an inner nerve ring site.


10s
0.03mV
0.3mV
A


10s
0.05mV
B

Figure 20: Events recorded from tentacles of *E. typus*

A) A comparison of the occurrence of TP's (■) and TCP's (▲) as recorded from two tentacles.

B) A comparison of shape and size of TP's (■) and TCP's (▲) recorded along a single tentacle. Bottom two traces are from the middle and tip of a single tentacle respectively while the top trace is from a second tentacle.

C) Recordings from the base (top record), middle (second record), and tip (bottom trace) of a tentacle during a spontaneous TP burst. Note the invariant pTP's (arrows) at all recording locations.

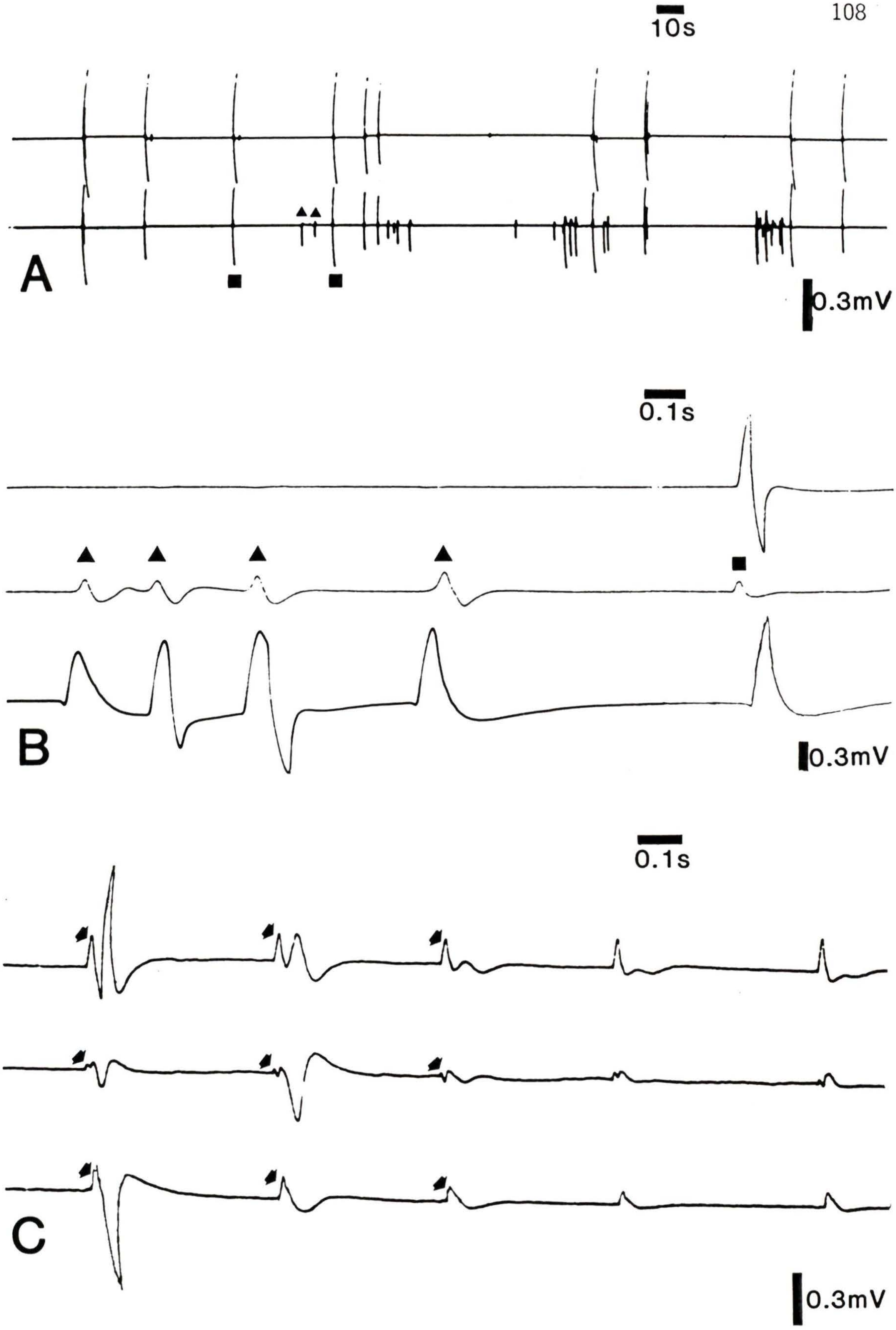

10s
A
0.3mV
0.1s
B
0.3mV
0.1s
C
0.3mV

Figure 21: Cyclical fatigue of electrically elicited TP's in *E. typus*. Stimuli at 1 pps. were applied to the basal portion of a tentacle and continued throughout the record. The regular lower amplitude events in each record are stimulus artefacts.

A) Recordings from a distant tentacle (top trace) and mid-portion (middle trace) and tip (bottom trace) of the stimulated tentacle.

B) Superimposed traces of responses leading up to the fatigue in the stimulated tentacle (top trace from mid-tentacle, bottom trace from the tentacle tip). The initial reduction in amplitude of the response occurs mostly in the contraction pulse, but final failure includes failure of the pre-pulse (arrow).

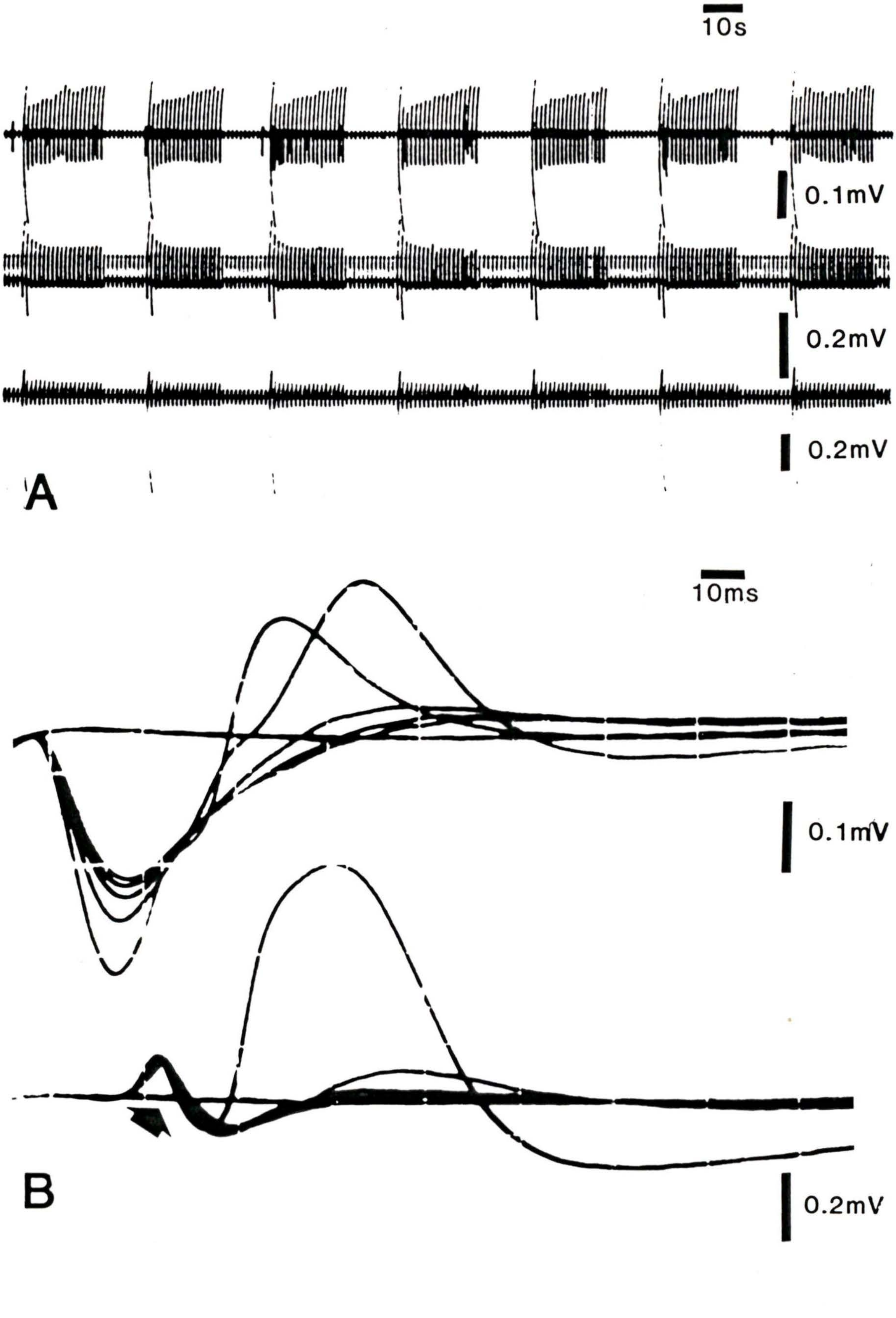
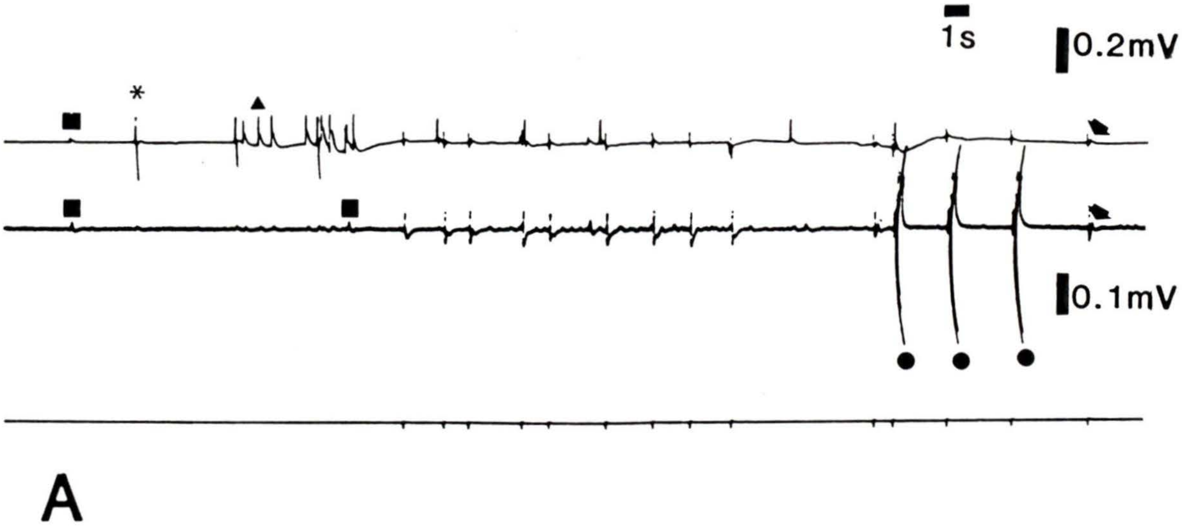

10s
0.1mV
0.2mV
0.2mV
A
10ms
0.1mV
B
0.2mV

Figure 22: Events recorded from the velum of G. *vertens*

A) Of the spontaneous small events, some are coincident with TP's (■) in a tentacle. Electrical stimuli applied to the outer nerve ring can elicit swims (●) but also elicit events in both the tentacle and velum (arrows), which are evident when the swimming response fails. The top trace is from a tentacle recording showing TP's (■), fTCP's (✱), and TCP's (▲), the middle trace is a recording from the subumbrellar side of the velum, and the bottom trace indicates stimuli.

B) The low-amplitude elicited event (arrow) from outer nerve ring stimulation as recorded from two points on the velum. vSP's (●) can be elicited as well by equivalent stimuli. A spontaneous cryptic event is also shown (◆).


1s
0.2mV
*
0.1mV
A

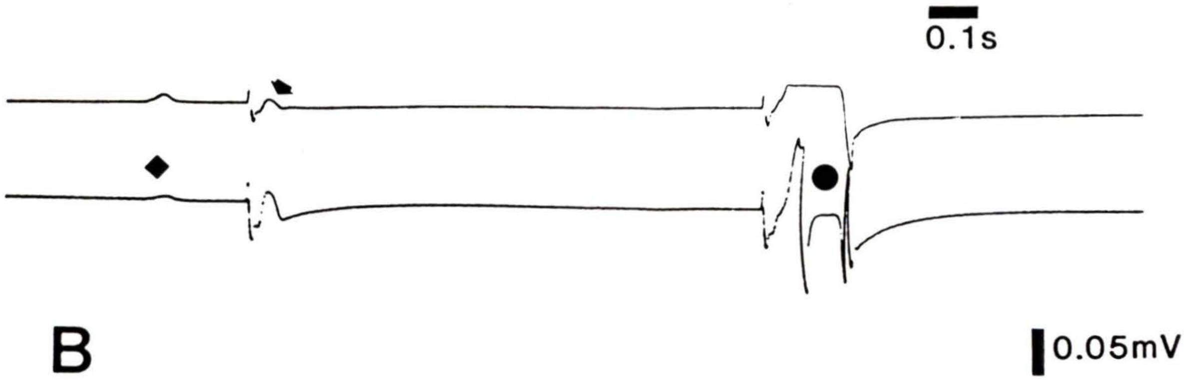

0.1s
B
0.05mV

Figure 23: Events recorded from the velum of *E. typus*

A) Velar swim pulses (vSP's; ●) and smaller amplitude events recorded from a location adjacent to the base of the velum. The largest of these latter events (■) are coincident with TP's.

B) A train of closely apposed vSP's showing summation of events.

C) The waveform of single vSP's (●) in a chart record.

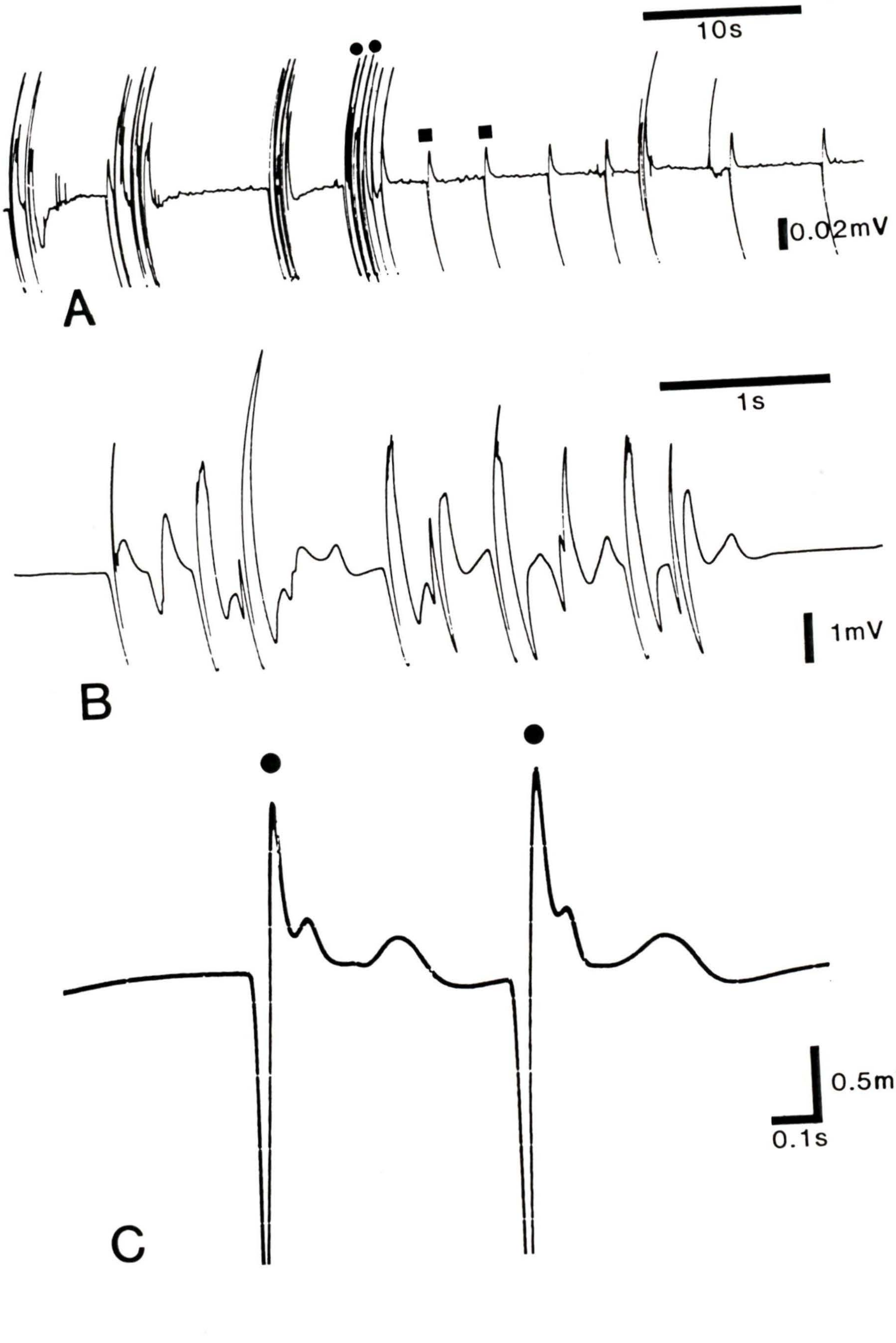

10s
0.02mV
A
1s
1mV
B
0.5mV
0.1s
C

Figure 24: Electrical events during a mechanically elicited crumple of *E. typus* recorded from the outer nerve ring, velum, tentacle, and subumbrella. SP's (●) cease during the crumple but some TP's (■) are present at the start of the behaviour (arrows) and normal TP rhythms return before swimming recommences. No large epithelial events are present in any of these tissues during crumpling.

A) Recordings from the outer nerve ring (top trace) and the velum (middle trace) The solid bar in the bottom trace indicates the start of a crumple which continues for the remainder of the record.

B) Recordings from a tentacle (top trace) and the subumbrella (middle trace). The solid bar indicates the duration of the crumple.

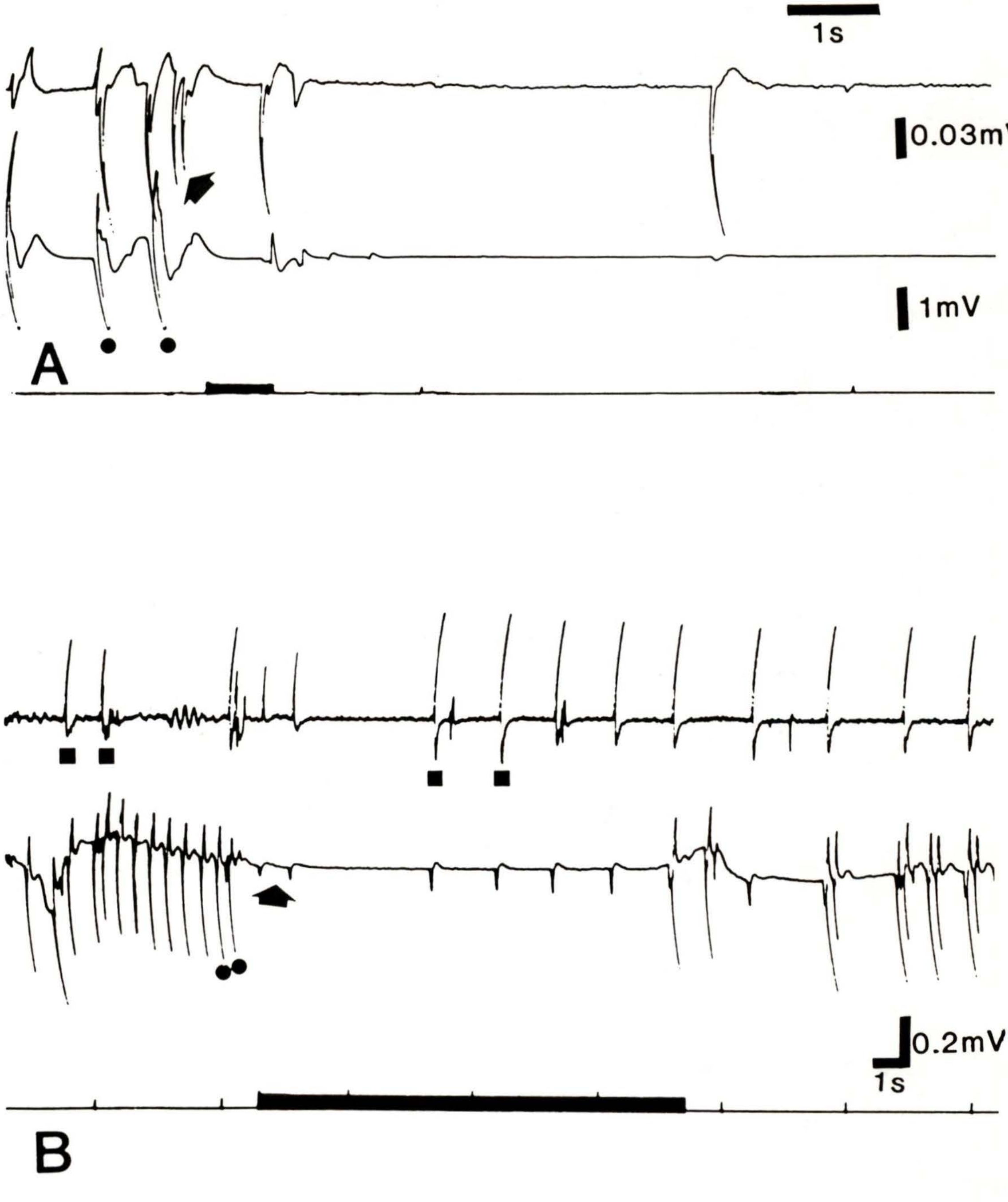
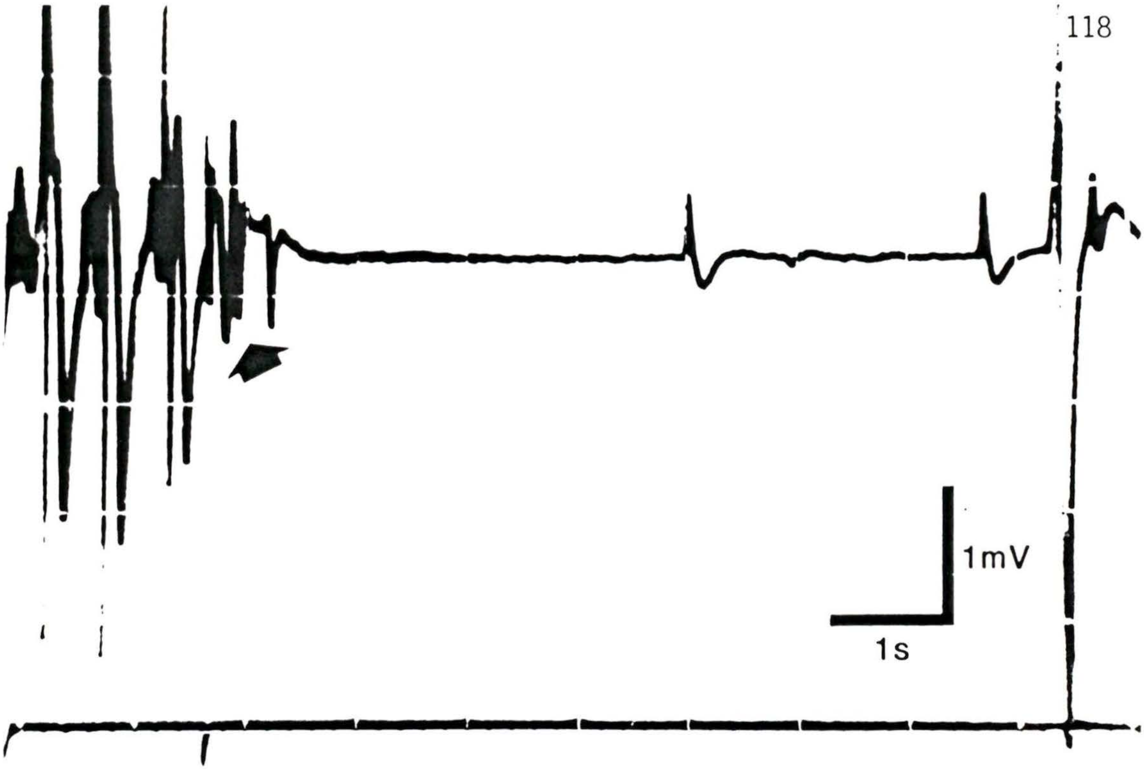

1s
0.03mV
1mV
A
0.2mV
1s
B

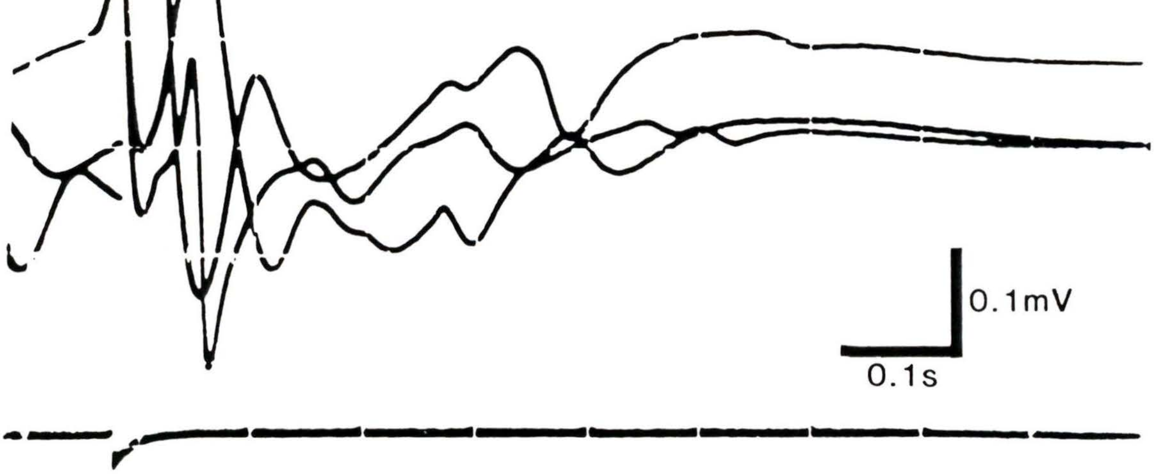
Figure 25: Electrically elicited crumpling in *E. typus* as recorded from the velum. Stimulation was applied to the outer nerve ring region. The bottom trace in both records represents a stimulus monitor.

A) Events still occur in the velum immediately after the application of a stimulus (arrow).

B) Three superimposed elicited crumples. The events at the start of the crumples do not occur at a constant latency to the stimulus.



A



B

Figure 26: Responses in a tentacle to 10, 50, and 70 volt stimuli (1 ms duration) applied to the outer nerve ring region of *E. typus*. A TP (■) is invariably elicited, but crumpling only occurs in response to the 50 and 70 volt stimuli. No difference in the form of the TP is evident when crumpling is elicited. Further TP's or TCP's occur commonly after such stimulii (arrow) but not at a constant latency from the stimulus.

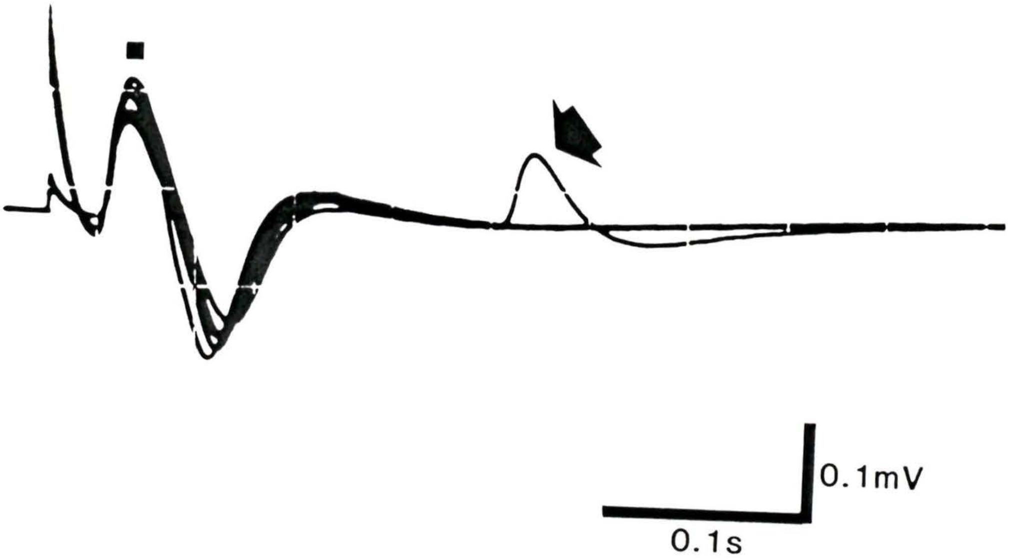
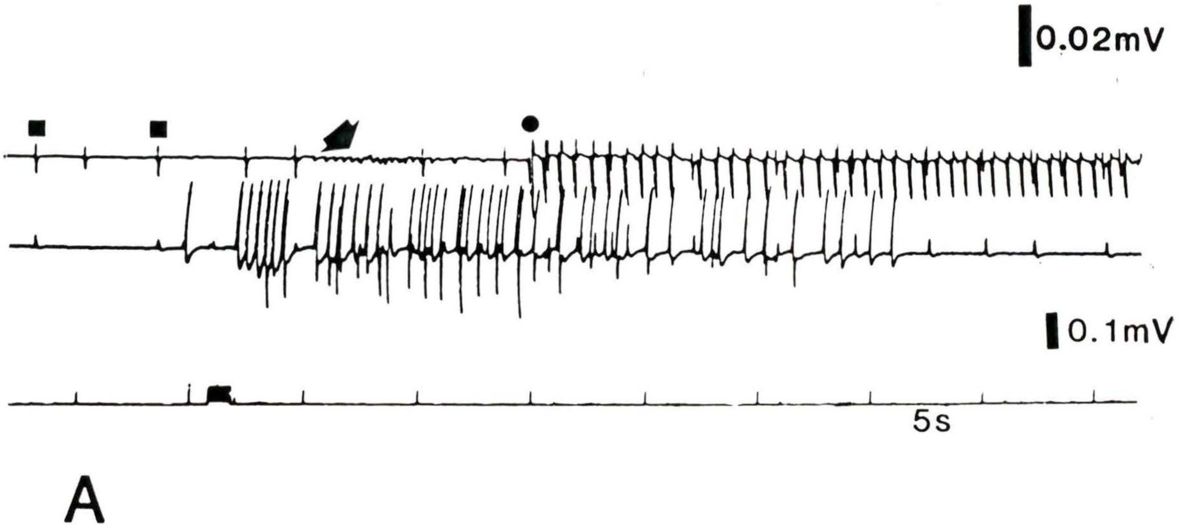

0.1mV
0.1s

Figure 27: Electrical activity in response to crude shrimp extract applied to tentacle tips in *G. vertens* and *E. typus*. The solid bars in the time monitor indicate times of extract application. Each division of the time scales represents 5 seconds.

A) The response in *G. vertens*. A train of large TCP's is initiated immediately in the tentacle (middle trace) and after some time (with variable latency) a train of swimming contractions also starts. The top trace is a record from a nearby outer nerve ring location showing TP's (■), low amplitude events (arrow), and SP's (●).

B) The response in *E. typus*. TCP's occur as short bursts (▲) but an effect on the TP (■) rhythm is not evident. Both records are from the same tentacle, the lower trace being from the tentacle tip.


0.02mV
0.1mV
5s
A

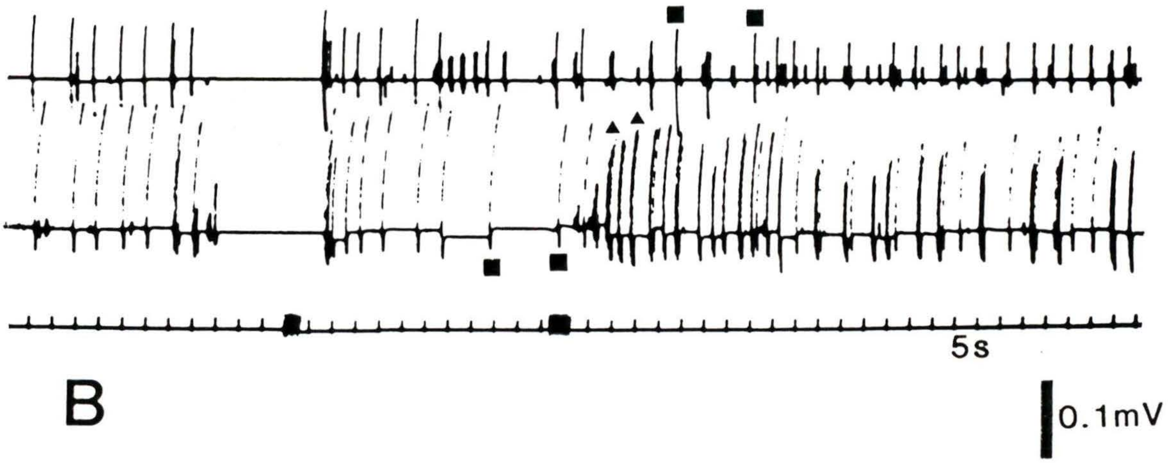
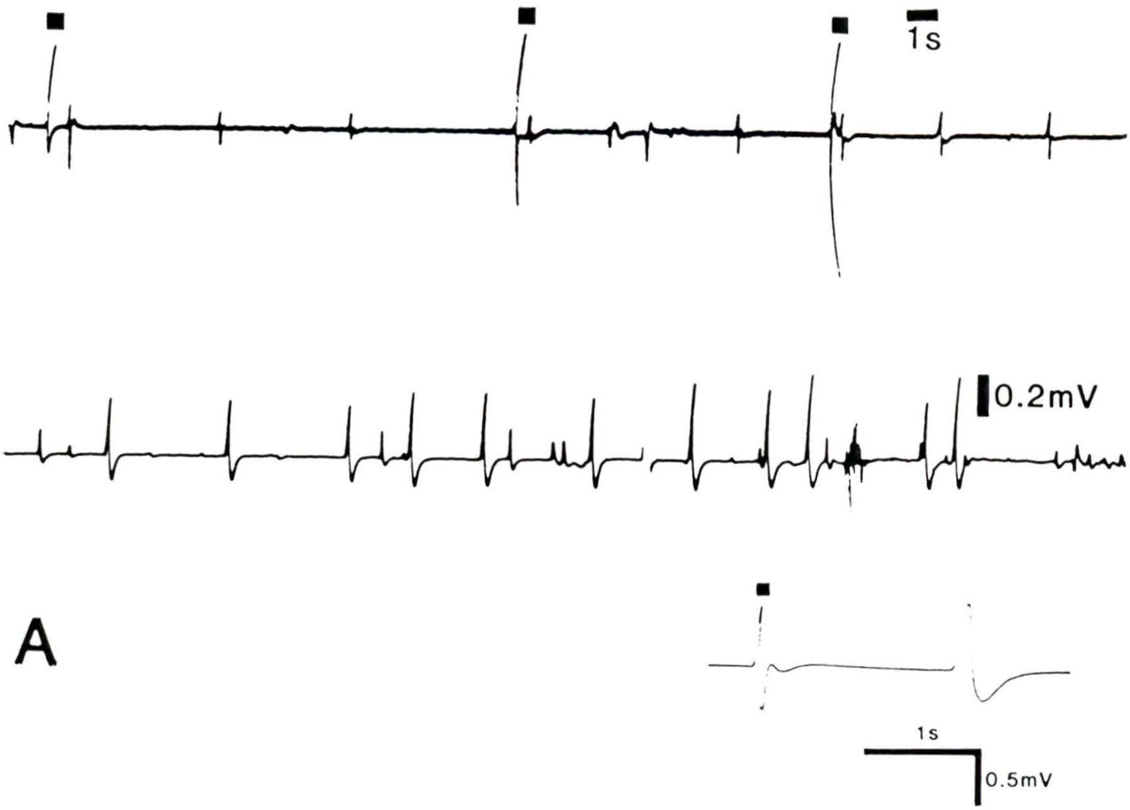

5s
B
0.1mV

Figure 28: Tentacle coordination in *G. vertens*

A) Tentacle contractions can be coordinated by TP's (■) of differing or constant amplitude in the extracellular record. The top trace was recorded from the middle of one tentacle showing TP's of differing amplitude, while the second trace is from a second tentacle with TP's of constant amplitude. The inset shows that TP's in the first tentacle do not trigger TCP's: the second event shown is a TCP. Coordinated contractions occurred with each TP.

B) Coordinated tentacle contractions can occur without a 1:1 correlation with TP's. The trains of coordinated events in these records were induced by applying sudden suction to the outer nerve ring region at a distant location. The top trace is a record from the outer nerve ring while the bottom two traces are from two different tentacles. The bursts of coordinated non-TP events (arrows) all had contractions associated with them, while TP's (■) occurring shortly afterwards did not.


1s
0.2mV
A
1s
0.5mV

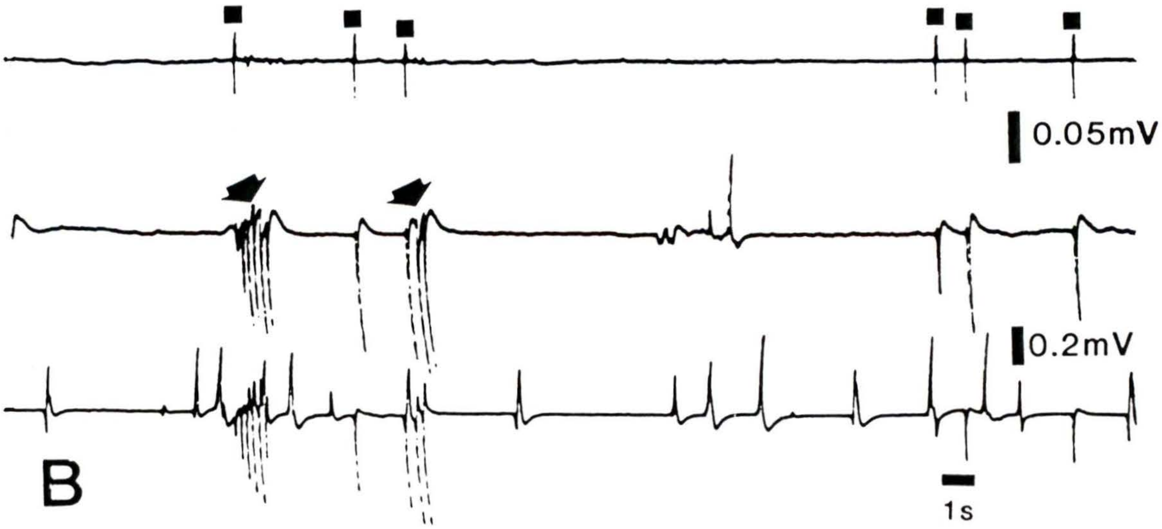
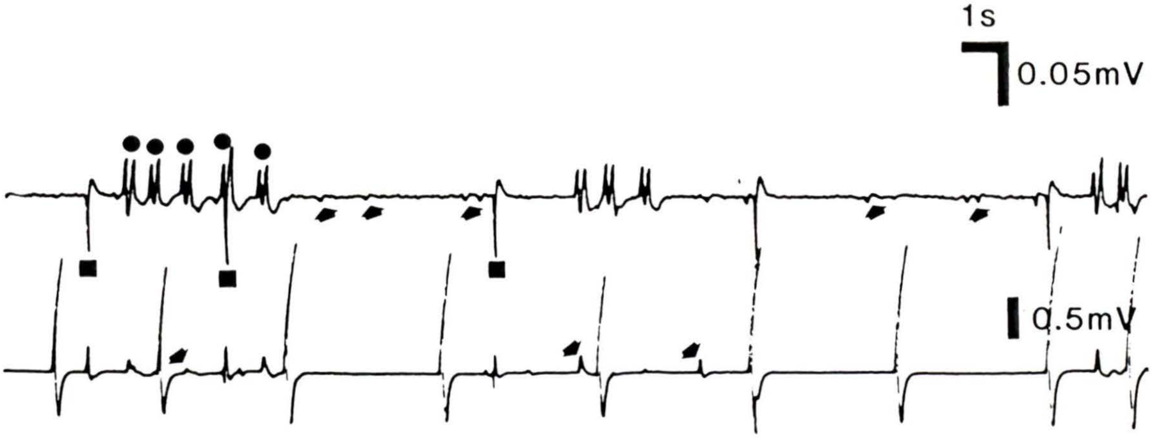

0.05mV
0.2mV
B
1s

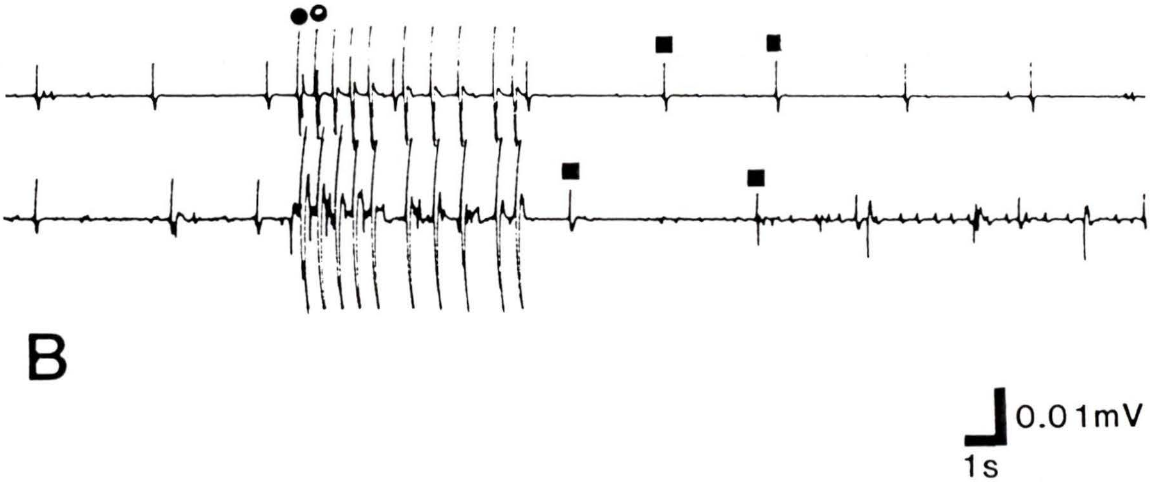
Figure 29: The relationship between the TP's and other events in the bell margin in *G. vertens*.

A) TP's (■) occur without a constant relationship to other events. The top trace is a recording from the outer nerve ring region while the bottom trace is from a tentacle. Other small cryptic events (arrows), present both in the bell margin and in the tentacles, do not show clear affinities to other events as well.

B) The propagation of TP's (■) in the bell margin is interrupted by cuts through the nerve rings, while SP (●) conduction is not, suggesting that TP's are only conducted by bell margin tissue. The records are from outer nerve ring locations on either side of a cut through the bell margin leaving most of the velum intact.



A

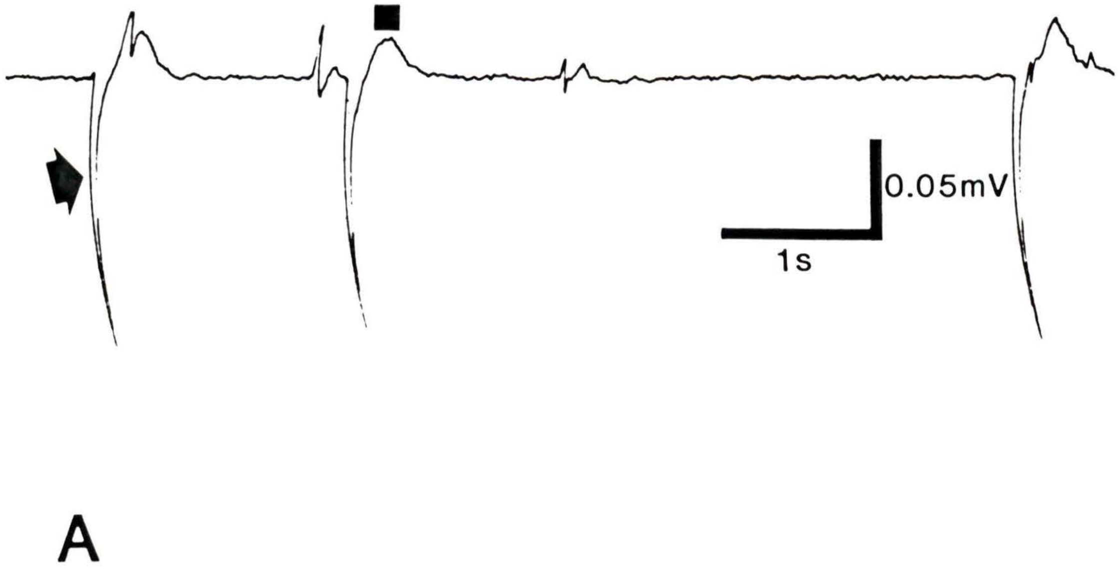


B

Figure 30: The TP in the outer nerve ring of *E. typus*.

A) TP's from the outer nerve ring at high chart speed. The pTP (arrow) is relatively larger than the after-potential (■), in contrast to the situation in *G. vertens*.

B) Variation is present in the amplitude of the TP's (■) recorded from the outer nerve ring (top trace), but this variation is not coincident with that seen in TP's of an adjacent tentacle (bottom trace).


0.05mV
1s
A

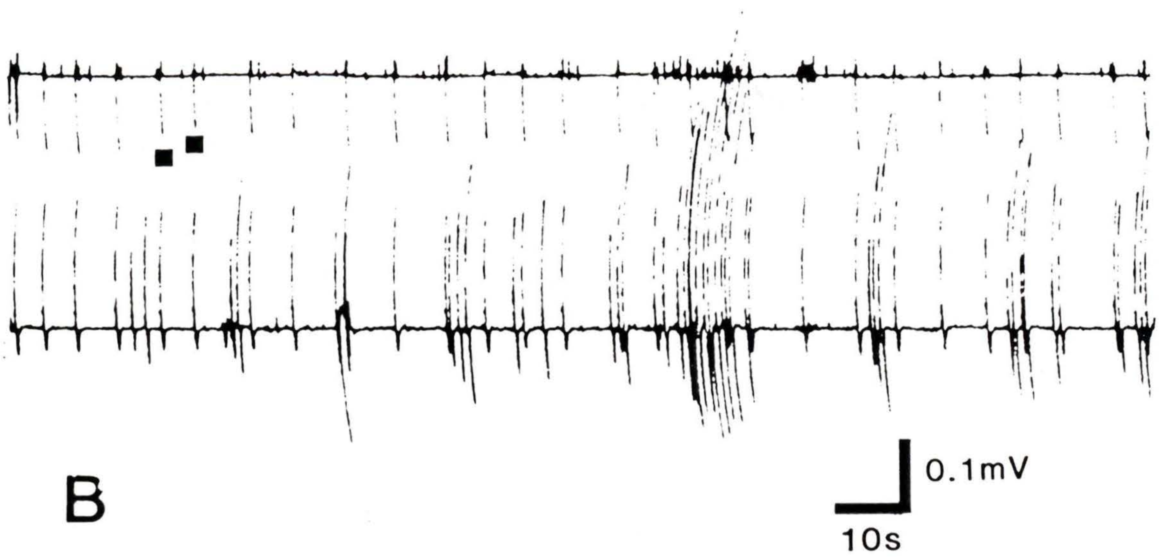
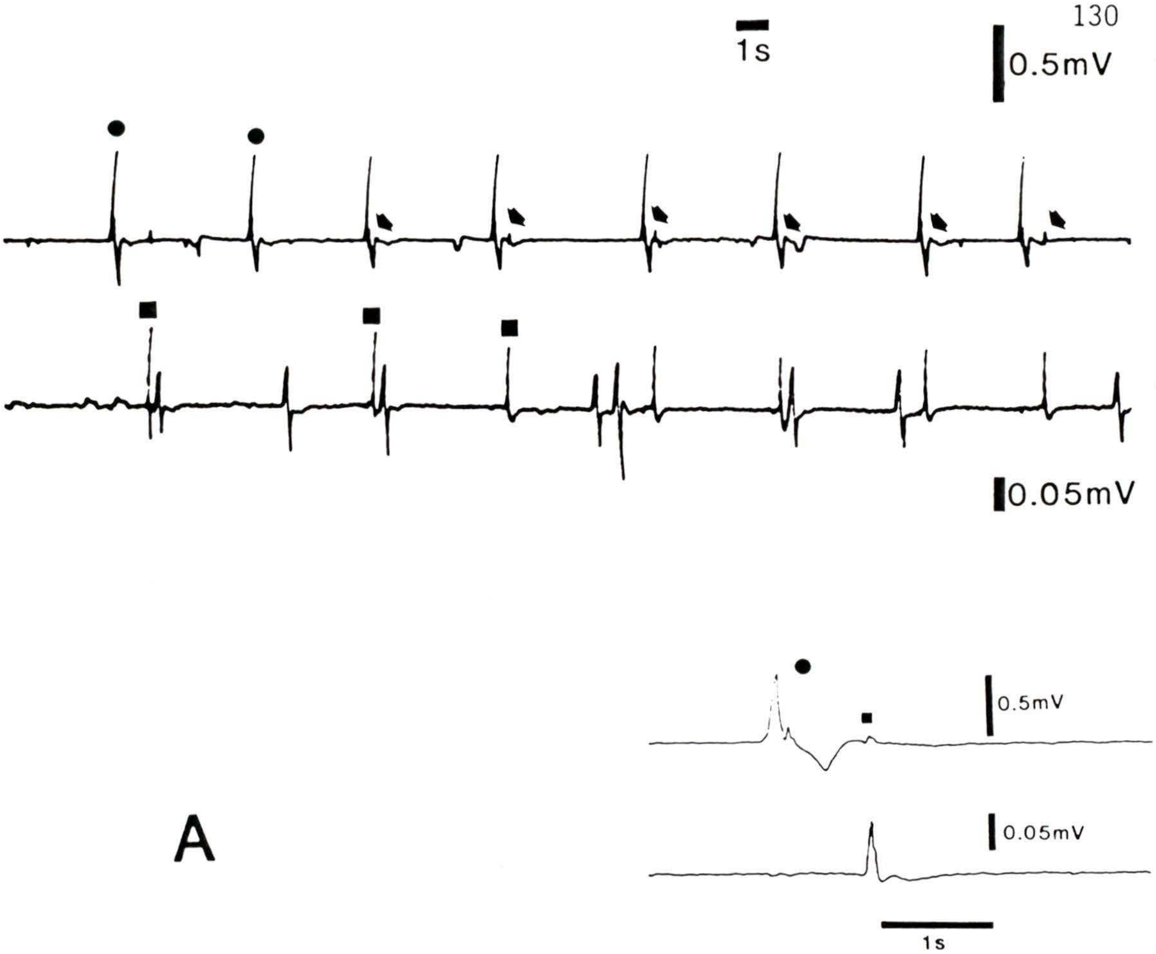

0.1mV
10s
B

Figure 31: The relationship between TP's and SP's in *E. typus*.

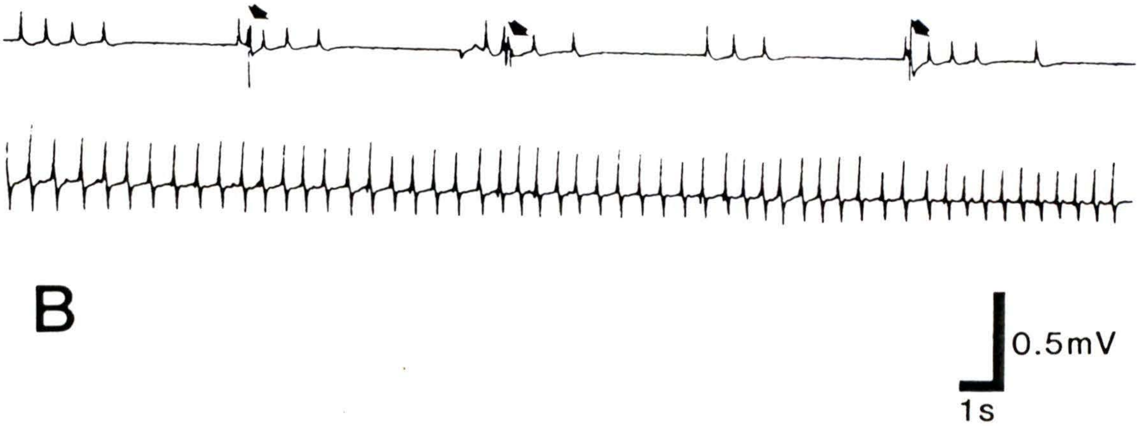
A) TP's (■) often occur coincidentally with SP's (●) as indicated by the arrows, but not at a constant latency.

When the events do occur together, the TP's generally appear during the latter portion or after the swimming event (inset). The top record is from the inner nerve ring while the bottom record is from a tentacle.

B) Repetitive fatigue of TP's during continuous swimming. The top record is from a tentacle and the bottom record is from the inner nerve ring. Other than a few TCP's (arrows), the events recorded from the tentacle are all TP's. All the large events recorded from the inner nerve ring are SP's.



A



B

C) *Histology*

I have presented my histological observations in the two sections of muscular effectors and non-muscular bell portions, as well as segregating the observations on the two species.

1) *Gonionemus vertens*:

a) *Muscular effectors*:

i) *Manubrium*: The manubrium consists of a pendulous, square column terminating distally in extensible manubrial lips and proximally in a small stomach. The endoderm of the column consists of tall, vacuolated, secretory cells, while the ectoderm consists of relatively short cells spread over numerous pronounced mesogleal ridges. Smooth muscle fibres are present in both tissues. In the endoderm these run in a circular direction, while in the ectoderm they run primarily in a longitudinal direction (fig. 34c). Trans-mesogleal bridges containing transversely oriented fibres are present as well (fig. 34d), as reported earlier in the manubrium of *Aequorea victoria* (Kerfoot, 1980). Nerve axons have been found amongst the basal processes of both ectodermal and endodermal cells (figs. 32e, f), the endodermal nerves containing dense-cored vesicles typical of endodermal nerves elsewhere in the animal. I have observed no synapses in the manubrium.

ii) Radial muscle: The radial muscles consist of two bands of radially oriented smooth muscle myocytes running along each radial canal. Well developed mesogleal ridges at the base of the gonad ridges (fig. 33) support these myocytes. Where gonad tissue is absent, the bands coalesce into a single cord mounted on a short, rugose mesogleal pedestal. As this cord approaches the bell margin it gradually flattens out and merges, by re-orientation of the muscle fibres, into the sphincter muscle bands (see sphincter muscle description).

The radial myocytes possess non-muscular asymmetric somata exposed to the exterior and three to five muscle fibre "feet". Mitochondria, irregular vacuoles, some endoplasmic reticulum, an irregular nucleus, Golgi, and large membrane-bounded granules associated with the cell surface are typical of the non-muscular cytoplasm (fig. 34a). Gap junctions are present among the somatic portions of the radial myocytes (fig. 34a), but are quite rare. Apical junctions lack pronounced septate desmosomes, though some intercellular amorphous material and occasional septa are present.

The muscle fibre "feet" of the radial myocytes contain unordered arrays of thin and thick myofilaments typical of coelenterate smooth muscle (Chapman, 1974). Muscle fibres of each myocyte interdigitate with fibres of adjacent cells as they run along the mesogleal ridges. Desmosomes between the muscle fibres are common (fig. 34), but I observed no gap junctions.

Bundles of up to thirty nerve axons, 0.7-1.5 µm in diameter, are present among the radial myocytes, mostly sandwiched between the muscle filaments and apical soma. Mitochondria and occasional 118-150 nm dense-core vesicles are typical of these axons (fig. 34c). I observed one neuro-neuronal synapse (fig. 34d), but no neuromuscular synapses or neuron somata. All radial nerve elements observed were associated with radial musculature. Some large bundles near the adjacent swimming muscle (fig. 35a) may, however, be analogous to the radial nerves referred to in *Polyorchis penicillatus* (Spencer, 1979).

At the edges of the radial canals, swimming myocytes and radial myocytes are adjacent to one another (fig. 35a). In these regions some of both radial and swimming myofilaments become oriented at right angles to the normal orientation, so both circularly and radially oriented myofilaments are present in the same cells (fig. 35b & c). Chapman (1974) described similar "cross-layered" myocytes in the subumbrella of *Obelia* medusae and the velum of *Muggiaea atlantica*. I observed no myocyte containing both smooth and striated myofilaments. I also found no gap junctions between swimming and radial myocytes.

iii) Sphincter muscle: The sphincter muscle appears to be a circular continuation of the radial muscle bands close to the bell margin (fig. 36). It consists of a narrow band of myocytes similar to radial myocytes adjacent to the inner nerve ring on one side and on the other to swimming myocytes (figs. 37a & 43). This band of myocytes is covered by the same epithelial sheath that extends over the inner nerve ring (fig. 37a) and is continuous with the swimming myocytes. The radial muscle cord appears to bifurcate as it approaches the bell margin, each half then extending under the surface epithelium to form the sphincter muscle (fig. 36).

As in the radial myocytes, the sphincter myocytes possess distinct non-muscular asymmetric somata and three to five, sometimes quite widely dispersed, smooth muscle feet running along mesogleal ridges. The somata contain cellular inclusions comparable to those of radial myocytes, including the large granules typical of ectodermal surfaces. In addition, in some individuals, membrane-bound, rod-like, closely striated organelles of unknown origin and function are present (fig. 37d). Desmosomes between muscle fibres are common, but gap junctions (fig. 37b) are very rare. I observed no gap junctions between the somata of the sphincter myocytes, or between sphincter myocytes and other cell types.

Small bundles of 0.5-0.7 µm nerves are dispersed amongst the myocytes (fig. 37a & b), primarily located between the cell soma and muscle fibre regions. I found no synapses or neuron somata. In some "axons" profiles of filaments resembling myofilaments were present (fig. 37c), but it is not clear what their signifigance is.

iv) Swimming muscle: The subumbrellar swimming muscle sheet consists of a single layer of striated muscle cells (fig. 38a & c). The sheet extends over all of the subumbrellar surface except at the radial canals where gonadal tissue is present, under the base of the manubrium, and at the bell margin. Where gonadal tissue is absent the swimming muscle extends under the radial muscle mass through trans-mesogleal pathways. The swimming myocytes in an animal of 2 cm bell diameter were 15.5 µm tall over most of the subumbrella, but up to 50 µm tall over the ring canal. Of this height, 8-10 µm adjacent to the mesoglea are taken up by circularly oriented myofilaments. The nucleus, extensive endoplasmic reticulum, Golgi, vacuoles and various granules, are present in the remainder of the cells (fig. 38). Mitochondria, present less densely in other portions of the cells, are concentrated at the top of the muscle feet.

The myofilament-containing portion of each myocyte is split into several thin and tall parallel muscle fibres up to 80 µm long (fig. 38a). The fibres run around the bell in an unpolarized fashion. Fibres of adjacent cells interdigitate with each other, so the total effect is a series of parallel "sheets" of muscle tissue, as described by Fraser (1932). At the ring canal these are distributed over mesogleal ridges (fig. 38a).

The muscle fibres possess a distinct ortho-striated organization of the myofilaments, mostly coincident with that of adjacent fibres (fig. 38b). Desmosomes between adjacent muscle fibres (fig. 38b) are common, while gap junctions are rare. In contrast, gap junctions between epithelial portions of the myocytes (fig. 38d) are common, as illustrated by the gap junction distribution in three 111 µm long cross-sections of the subumbrellar muscle sheet I observed with the electron microscope. Thirty gap junctions were detected in the distal portion of the cells while only three were detected between muscle fibres. I did not observe gap junctions between the swimming myocytes and sphincter myocytes, radial myocytes, or epithelial sheath cells.

Apical junctions between cells lack well developed septate desmosomes, though occasional septa are present (fig. 38c).

Circularly oriented single axons (fig. 38b) and bundles of 1-2.5 µm diameter axons are present at the top of the muscle feet. Neuron somata occur in the vicinity of the bell margin (fig. 38a), but I observed neither synapses nor interneuronal gap junctions.

v) *Tentacles*: The tentacles of *G. vertens* are characteristically beaded in appearance (fig. 39a), caused by the alternation of nematocyst-bearing ridges with bands of thinner ectoderm (fig. 39). Ridge ectoderm is 60-120 µm thick while non-ridge ectoderm is 12-20 µm thick. In both regions the ectoderm is muscular, each cell possessing numerous, longitudinally oriented, smooth muscle feet. In the ridge region vacuoles just distal to the muscular feet (also present in the non-ridge regions (fig. 39c)) are greatly distended, nematocysts are present amongst the apical portions of the epithelial cells, and the mesoglea forms numerous ridges to which the nematocysts are anchored and between which lie the muscle fibres (fig. 39). The anchoring of nematocysts in G. *vertens* has been described previously (Westfall, 1970a) and my result supports the earlier findings (figs. 39b & d): a long cord of supportive rods extends from the nematocyst to a location close to the mesoglea. Here, via microfilaments, it forms desmosomes with narrow epitheliomuscular processes (fig. 40a). These in turn form hemi-desmosomes to the mesogleal outcroppings.

Epithelial nuclei are present both in the apical regions of the tentacle ridges (fig. 39d) and amongst the muscle fibres (fig. 39b), but I could not determine if these indicate the presence of a true bilayer of ectodermal cells.

Gap junctions are present in the ridge and non-ridge epithelia amongst apical portions of cells (fig. 40d). I observed no gap junctions between cnidocytes and other epithelial cells. Apical desmosomes are present, but do not appear septate (fig. 40b). Desmosomes between muscle fibres are common (fig. 40b).

Bundles of seven to twenty-six 0.3-0.5 µm nerve axons are present at the apices of muscle fibre groups (figs. 39, 40a), but no neuron somata were observed. Neuro-neuronal and neuromuscular synapses were observed with 100-115 nm relatively clear and 40-130 nm dense-core synaptic vesicles (fig. 40c & e). I did not see the synapses to cnidoblasts reported earlier by Westfall (1970b).

The endoderm of the tentacles of *G. vertens* consists of ciliated cells, 60-120 µm tall, surrounding a central chamber (fig. 39b). The cells consist primarily of very large vacuoles, 50-110 µm tall by 5-10 µm wide, which limit the cytoplasm to apical and basal masses connected to each other by very thin sheets of tissue (figs. 31b & 41a). Mitochondria, granules, and smaller vacuoles are distributed throughout the cytoplasm, but nuclei, digestive vacuoles, and endoplasmic reticulum are generally limited to the apical portions and interconnecting tissue sheets. The basal portions of the cells contain amorphous, finely granular material (fig. 41a) and longitudinally-oriented filaments (fig. 41d). Gap junctions are present within the tissue sheets (fig. 41c) and between apical portions of the cells. Apical junctions between the cells contain only infrequent septa (fig. 41b).

At the bases of tentacle bulbs, muscle fibres and associated gap junctions are present in the ectoderm (fig. 42a). In the same region muscle fibres are present in the ring canal endoderm and the fine granular basal substance is present in the tentacle endoderm (fig. 42c). Cellular bridges between ectoderm and endoderm with gap junctions are also present here (fig. 42b). Tentacle nerves appear to originate from the marginal nerve ring and other peripheral nerve bundles.

vi) Velum: The velum consists of two layers of cells separated by a thin layer of mesoglea (fig.47). The inner subumbrellar cell layer is made up of striated myocytes similar to those of the subumbrellar muscle sheet. Circularly oriented axons, 0.6-1.7 µm in diameter, are present in some of these cells at the top of the muscle feet. These occur both singly and in small bundles, and appear to run radially into the velum from the inner nerve ring (fig. 43). I have observed neither synapses nor neuron somata in the velum. Gap junctions and desmosomes are distributed as in the subumbrellar muscle sheet, but gap junctions amongst the muscle fibres (fig. 44c) are more common. Distinct apical septate desmosomes are generally absent, though irregular intercellular depositions and occasional septa are present (fig. 44b).

The exumbrellar surface of the velum consists of a non-muscular epithelium of squamous cells, 6-12 µm tall and 12-15 µm wide. Deep clefts are present in the epithelium, at times leaving only a very thin portion of cytoplasm adjacent to the mesoglea (fig. 43). Gap junctions are common between the cells. In addition, interdigitating cell processes, 0.05-1.4 µm in diameter, are common, and gap junctions between these and the cells surrounding them are numerous (fig. 45b). Apical cell junctions again generally lack distinct septate desmosomes (fig. 44).

Sparse putative nerve axons, 0.6-1.8 µm in diameter (fig. 45b), are present amongst the basal portions of some cells, but I observed neither synapses nor neuron somata. Adjacent to the bell margin, the exumbrellar surface of the velum is ciliated (fig. 45c), and these ciliated cells also contain tracts of parallel microtubules (fig. 45a). The cilia in fixed material possess sub-terminal swellings (fig. 45c), but I did not determine if these cilia were actual paddle cilia or if the swellings were fixation artifacts. The ciliated cells form gap junctions both with adjacent cells and with interdigitating processes.

At the base of the velum, a mesogleal ridge is present on the exumbrellar side. Cells associated with this ridge possess a central core of densely packed microtubules which extend from the mesoglea to the cell surface (fig. 43b). At a corresponding location on the subumbrellar side of the velum a deep cleft is present, and a shallow cellular band provides the only contact between the bell margin and the velum.

b) Non-muscular bell portions:

i) Bell margin: The bell margin is a distinctive region of *G. vertens* characterised by elaborations of subumbrellar, exumbrellar, and endodermal tissues, as well as being the origin of the tentacles and velum (fig. 46).

In the subumbrella the sphincter muscle and inner nerve ring are pressed together close to the tri-radius in a tissue ridge covered by a sheath of cells (fig. 46). This epithelial sheath is continuous with the swimming muscle sheet and the subumbrellar epithelium of the velum. Processes from the epithelial cells extend down amongst the nerves and nerve bundles of the inner nerve ring to terminate at the mesoglea of the velum base (fig. 47). Gap junctions are present between the sheath cells (fig. 45), but I observed none between sheath cells and subumbrellar swimming myocytes or velar myocytes. I also observed no septate desmosomes in this epithelial sheath. Putative ciliated sensory cells containing tracts of parallel microtubules are also present amongst these epithelial cells.

The inner nerve ring usually contains the profiles of two to three large axons 12-15 by 7-9 µm in cross-sectional diameter. Presumably these are comparable to the "swimming motor giants" (Spencer & Satterlie, 1980) of other hydromedusae. In addition, there are approximately 200 smaller axons, 0.8-3.5 μm in diameter, present as a somewhat diffuse bundle spread along the mesoglea of the velum base (fig. 47a). Cell somata for the smaller axons appear to lie scattered in the vicinity of the "giants", while somata of the "giants" are not spatially segregated from the location of their axons. Processes from the smaller axons run both into the velum and across the mesoglea to the outer nerve ring (fig. 48). The "giants" are often observed in close apposition to each other (fig. 47b), and at times extend through the epithelial sheath to the outer surface.

Small mitochondria, some endoplasmic reticulum, neurotubules, and sometimes quite numerous 169-243 nm dense-core vesicles are present in the inner nerve ring axons. In the somata Golgi and nuclei are also present (fig. 47a).

I observed no neuro-neuronal, neuromuscular, or neuroepithelial synapses associated with the inner nerve ring, but Spencer & Satterlie (1983) report a neuroepithelial synapse between a swimming motor giant and an epithelial sheath cell in this species. I saw no gap junctions between nerve cells or between nerve cells and other cells.

A ridge of specialized tissue is also formed by the exumbrella at the bell margin. An epithelial sheath encloses this ridge as well, but this ridge is ciliated and contains a number of nerve rings, cnidoblasts, and epithelial tissue (figs. 46 & 48).

The sheath cells send processes into the ridge tissue, as do those of the subumbrellar bell margin. At their apices the cells are joined by septate desmosomes (fig. 49). Gap junctions commonly occur adjacent to these apical junctions and within the ridge mass the cells form extensive gap junctions with cnidoblasts (fig. 49b). The epithelial cell somata contain large numbers of free ribosomes, whereas the adjacent cnidoblasts contain RER (fig. 49). I found no gap junctions between cnidoblasts. Trans-mesogleal processes of epithelial cells with gap junctions to endodermal cells are present (fig. 49c).

Most of the nerve tissue lies in a very compact cord, up to 25 µm in diameter, consisting of approximately 600 nerve fibres. It lies immediately adjacent to the tri-radius, and is probably comparable to the outer nerve ring of other hydromedusae (Spencer & Schwab, 1982)(fig. 48a). Somata of the nerve axons appear to lie primarily in the surrounding tissue (figs. 48a & 50a). The majority of the axons are 0.3-2.0 µm in diameter, though generally two or three 2.4-5.5 µm axons are also present towards the base of the velum. In any given cross section, approximately 10% of the axon profiles contain concentrations of 140-170 nm dense-core vesicles which may be neurosecretory (Singla, 1978) (fig. 48). Some nerve fibres cross the mesoglea of the velum base to the inner nerve ring, accompanied by epithelial processes (fig. 48a). Cellular bridges to a bundle of endodermal nerves within the mesoglea of the triradius also occur occasionally (fig. 50c).

In addition to the outer nerve ring, there is a smaller bundle of fifteen to twenty 0.6-1.5 µm diameter nerve fibres, probably corresponding to Hyde's (1902) "marginal nerve ring", lying close to the bell side of the bell margin tissue (figs. 46 & 48b). A number of other small nerve tracts and single axons are dispersed throughout the ridge as well (fig. 48). These appear to come from neuron somata in the ridge tissue, form pathways between the nerve rings, supply the statocysts (figs. 44 & 48c), lead into the tentacles, or serve putative sensory cells (fig. 49). Neuro-epithelial (fig. 50b) and neuro-neuronal (fig. 50b & c) synapses are present in these processes, but I found no synapses or gap junctions within the outer nerve ring itself.

Separating the exumbrellar margin tissue from the bell proper is a 0.4-1.7 µm wide band of tissue forming a cellular bridge between the bell exumbrella and the ring canal endoderm. This tissue encloses the statocysts (where present), forms the microvillar lining of the statocyst chambers, and envelops the inner side of the tentacle bases (fig. 52). The cells giving rise to this bridge at the junction between the exumbrella proper and the bell margin tissue possess a central core of microtubules (fig. 52a) comparable to that seen in cells at the base of the velum. I found no gap junctions within the bridge or between bridge cells and ectodermal margin tissue.

ii) *Endoderm*: I observed three structurally distinct types of endoderm in the bell of *G. vertens*: the "inner" endoderm, the "outer" endoderm and the subumbrellar lamella (fig. 33).

The "inner" endoderm, orange in live specimens, lines the gonad chambers and the subumbrellar side of radial and ring canals, while the black, "outer" endoderm lines the exumbrellar side of radial and ring canals. The subumbrellar lamella, present throughout the bell where endodermal canals are absent, is a thin, colourless, sheet of cells embedded in the mesoglea beneath the subumbrellar ectoderm.

Cells of the inner endoderm (fig. 53a) are approximately 20 μm tall by 4 μm wide. A large central nucleus, Golgi, numerous mitochondria, extensive endoplasmic reticulum, digestive vacuoles, miscellaneous electron lucent vacuoles, numerous free granules, cilia, and microvilli are present. In some cells of the radial canal bundles of axial microtubules are also present (fig. 53d). Intercellular borders are highly, though irregularly, convoluted. Gap junctions are abundant along their full length (fig. 53a & b), and septate desmosomes, often enclosing interdigitating cellular strands as described by Hand & Gobel (1975) and King & Spencer (1980) (fig. 54a), are present at their apices.

Impregnated with lanthanum and viewed *en face* (fig. 54b), the septate desmosomes appear as an expanse of dark, impregnated intracellular space with the septa extending through it as parallel 4 nm electron lucent lines. The septa appear long and gently curved, indicating that the junction is of the "*Hydra*" type (Staehelin, 1974) described by Hand and Gobel (1972) and Wood (1959). In conventionally stained preparations the intercellular space in these junctions is about 11 nm wide and septa, ocurring at a periodicity of 8-11.5 nm, are about 4 nm thick.

Lanthanum-filled gap junctions, viewed transversely, have an 11 nm central layer of lanthanum (fig. 54c). These junctions are about 24 nm in overall width. Electron lucent bridges, 7.6-9.8 nm wide, interrupt the lanthanum impregnation with a periodicity of about 8-11 nm. Within some of these clear bridges a central 2.3-2.9 nm pore into which lanthanum has penetrated is visible (inset of fig. 54c). Viewed *en face* a hexagonal arrangement of junctional sub-units, as described by Hand & Gobel (1972), Peracchia (1973), and King & Spencer (1980), is visible (fig. 54d). Center to center distances of the sub-units is about 9.8-10.8 nm.

Nerves of 0.4-1.0 µm diameter are present scattered amongst the bases of inner endoderm cells of the radial and ring canals (fig. 53) and gonad chamber (fig. 58b). In addition, there are more concentrated bundles adjacent to the tri-radius of the bell margin (fig. 55a) and opposite the radial muscles in the radial canal (fig. 55d). Bundles of six to twelve 0.5-1.3 µm diameter irregular axons are typical of these regions (fig. 55b). Vesicles, 283-337 by 200 nm in size, with excentric 95-147 nm dense cores (fig. 55b) are commonly present, and, as they appear not to be present in nerves of any other tissues, may be diagnostic of endodermal nerves. Singla (1978) reports similar vesicles in endodermal nerves of *Polyorchis penicillatus*. A bundle of eight to fifteen nerve axons is generally embedded in the mesoglea of the tri-radius (fig. 55a), also with these vacuoles. This bundle appears to originate from the endoderm and makes occasional contact with tissue of the exumbrellar margin (fig. 50d) (see bell margin description). No direct contacts with outer nerve ring neurons have been observed so far.

Neuron somata can be observed both in the tri-radius region (fig. 55a) and in the radial canal (fig. 55d). A putative synapse between a ring canal neuron soma and the surrounding epithelial cell has also been observed (fig. 55c).

The outer endoderm is similar to the inner endoderm, but is generally more electron dense and not as thick (fig. 56). The cells generally measure 8-12 μm in height and 4.5-7.0 μm in width. The distribution and structure of septate desmosomes and gap junctions is comparable to those of the inner endoderm, but I did not observe nerves or microtubule bundles. A shallow layer of putative circular myofilaments, absent in most of the inner endoderm, is present close to the mesoglea in at least some parts of the outer endoderm of the radial canals (fig. 56d).

The subumbrellar lamella contains similar cellular inclusions as the other endoderm types, except that it lacks digestive vacuoles. It also differs from these others in that it is sandwiched by mesoglea to form a unicellular tissue layer of a fairly uniform 1.3-1.9 µm thickness in close apposition to the subumbrellar ectoderm (fig. 57a). The cells are 4-12 µm long. Gap junctions and septate desmosomes are present throughout, without clear order (fig. 57b). Cellular bridges to the subumbrellar ectoderm are common, but no associated gap junctions have yet been observed. Putative nerve axons have been observed in junctional regions with the radial canals (fig. 57c), but not elsewhere.

The junctions between inner and outer endoderm are characterised by a deep intercellular cleft (fig. 58a & b). Gap junctions are present in the remaining narrow region of direct apposition (fig. 58c).

At the contact region between subumbrellar lamella and radial canal numerous desmosomes and microfilament bundles (and possibly neurons) are found (fig. 59). Gap junctions to radial canal cells (possibly both inner and outer endoderm cells) and between putative nerve axons are present.

Lastly, the endodermal cell giving rise to the transmesogleal bridge to the exumbrella also posesses gap junctions with both inner and outer endoderm cells (fig. 60).

iii) Exumbrella: Over the whole of the bell proper the exumbrella consists of a uniform layer of squamous cells interspersed with scattered cnidocytes. The squamous cells are 9-12 µm wide and 2-3 µm tall. They possess a short, microvillar border and an associated mucous investment (fig. 61a). I observed no cilia. The cytoplasm is electron dense and granular, at times with many vacuoles. An elongate nucleus, some SER, mitochondria, membrane-bound 0.6-1.0 µm granules typical of all ectodermal cell surfaces, and occasional massive bundles of vertically oriented parallel microtubules (fig. 59a) are also present. Cnidocytes contain nematocysts and possess cnidocils (fig. 61d) in addition to these inclusions.

The border between most cells is irregularly convoluted with apical septate junctions only occasionally present or indistinct (fig. 61b). Gap junctions (fig. 61c) are present with moderate frequency but with no apparent preferred location. The border between cnidocytes and adjacent exumbrellar cells, by comparison, is quite regularly convoluted (fig. 61d), with septate junctions present sporadically throughout (fig. 61e). I saw no gap junctions between established cnidocytes and neighbouring cells or between migrating cnidocytes and adjacent cells.

I found no nerves in the exumbrella of the bell proper.

iv) Gonads: I did not investigate the structure of gonadal tissue extensively, but some knowledge of its histology is important in establishing putative conduction pathways. Ectodermal tissue contains the sexual tissue, and, in mature animals, is quite extensive. Its outer surface is densely ciliated with putative paddle cilia (fig. 62a). As with the cilia of the velum, these may be fixation artifacts. The gametogenic tissue, interspersed with epithelial cell processes, contains many free ribosomes (fig. 62b,c). Gap junctions are abundant in this tissue (fig. 62c), and are also present in the region of apposition with radial myocytes (fig. 62b), though perhaps not directly between gonadal tissue and the myocytes. Cellular contact with endodermal tissue is also quite common (fig. 62d), but I observed no gap junctions. I have not observed nerves in gametogenic tissue, but they are present in the underlying endoderm (fig. 62b).

2) *Eperetmus typus*:

On a histological level, *E. typus* differs from *G. vertens* in having a wider sphincter muscle (fig. 63, 66a); much fewer myofilaments in the swimming muscle (fig. 63c &

d); a less abrupt junction between sphincter and swimming muscle (fig. 63a); a more massive basal tissue in the tentacle endoderm (fig. 63); nerves and radially oriented smooth muscle fibres and a lack of cilia on the exumbrellar side of the velum (fig. 65); a more dispersed inner nerve ring (fig. 66a); microfilament containing processes between some inner nerve ring swimming giants (fig. 66c); microfilament (rather than microtubule) bundles at either side of the exumbrellar bell margin; unique blind chambers in the exumbrellar bell margin containing apparent modified cilia (fig. 67a & c); and communicating pores between the statocyst chambers and the exterior (fig. 68).

As in *G. vertens* the sphincter muscle is subumbrellar, innervated, and covered by an epithelial sheath (fig. 63b); the swimming muscle is innervated (fig. 63d); nerve bundles and longitudinal muscles are present in the tentacle ectoderm (fig. 68); striated musculature and nerve bundles are present in the subumbrellar velum surface; the inner nerve ring is covered and inundated with processes from an epithelial sheath; three to four large diameter (11-14 mμ) "swimming motor giants" and small diameter (0.5-1.5 mμ) axon bundles are present in the inner nerve ring (fig. 66); the outer nerve ring consists primarily of a compact mass of 500-600 small diameter (0.24-3.0 mμ) nerve axons and four to five larger axons of 4-7 μm diameter; an endodermal nerve ring is present and a bundle of endodermal nerves is embedded in the tri-radius mesoglea; putative supportive cells (though somewhat different) are present at the velum base in association with a mesogleal spur at the velum base and adjacent to the junction with the exumbrella proper; two types of ring canal endoderm can be distinguished; and a cellular bridge at the bell margin from the exumbrella to the endoderm is present (fig. 67b).

I have observed gap junctions amongst the epithelial sheath cells over the inner nerve ring, but I did not investigate their distribution elsewhere. Likewise, I did not investigate the distribution of apical junctions and synapses.

Figure 32: The manubrium of *G. vertens* (A: SEM
B: light micrograph; C,D,E, & F: TEM).

A) A manubrium with two lips removed (x50).

B) A cross section of one corner of the manubrium corner. The mesoglea appears most important in establishing the square shape of the column. No marked muscular specialization is evident (x100).

C) A portion of a cross-section of the column wall. Longitudinal smooth muscles are present in the ectoderm and circular smooth muscles are present in the endoderm (x5,920).

D) A trans-mesogleal process in the column with transverse muscle fibres extending from the ectoderm to the endoderm (x4,100).

E) A nerve axon amongst ectodermal myocyte processes of the column (x16,610).

F) A nerve axon adjacent to the mesoglea in the endoderm of the column (x12,770).

cl: manubrium column
ect: ectoderm
end: endoderm
li: manubrium lip
m: mesoglea
n: nerve axon
rc: radial canal
s: stomach

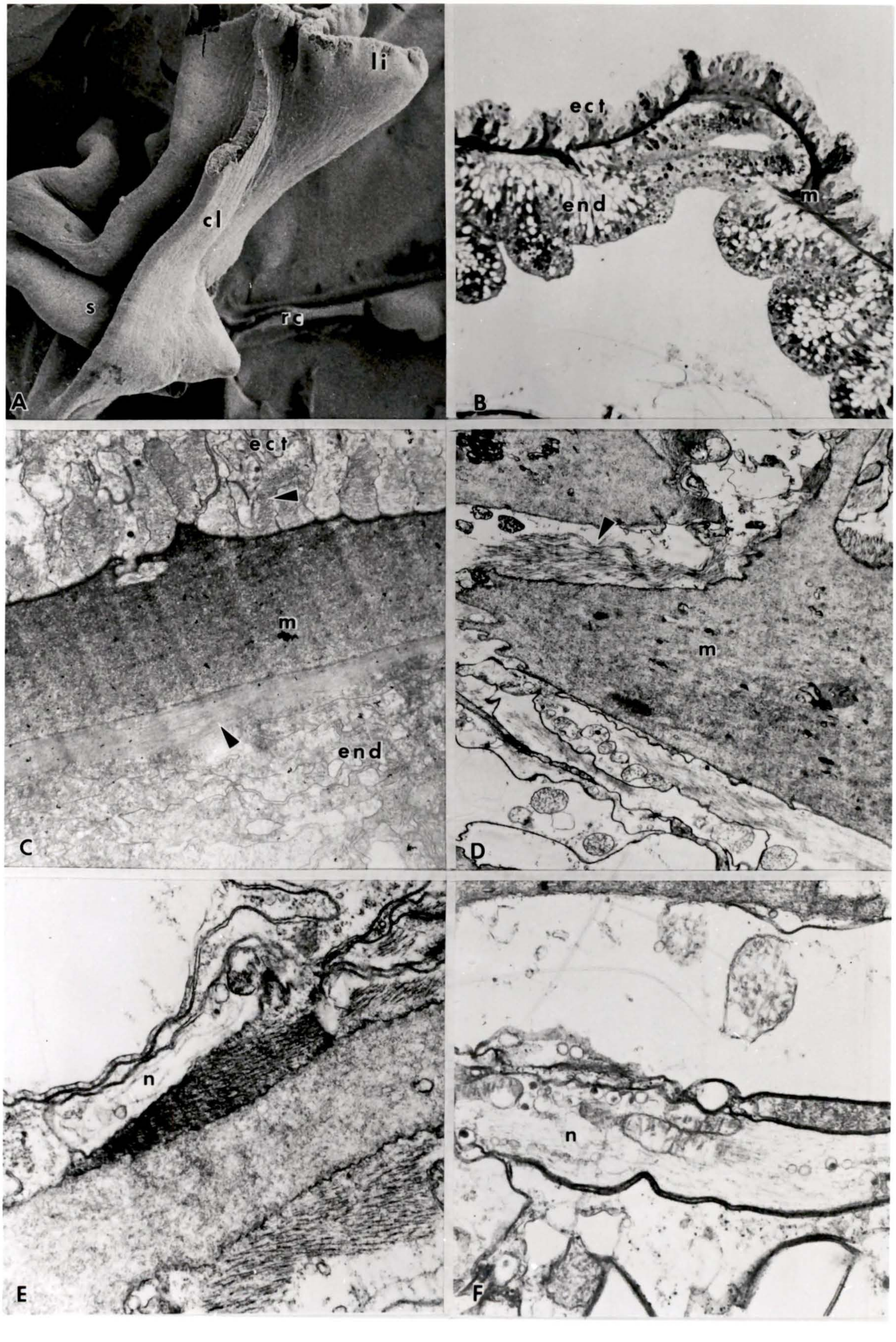

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Figure 33: A cross-section through part of a radial canal of *G. vertens*. A radial muscle band is present at the base of the gonad tissue ridge. Three types of endoderm are present in this region: the inner endoderm, the outer endoderm, and the subumbrellar lamella (light micrograph, x40).

g: gonad tissue
ie: inner endoderm
m: mesoglea
oe: outer endoderm
rm: radial muscle
rc: radial canal
sw: swimming muscle
sub: subumbrellar lamella


g
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m

Figure 34: The radial muscle of *G. vertens* (TEM).

A) A cross-section of myocytes. Note smooth muscle feet, nerve bundles, surface granules, desmosomes amongst muscle feet, and gap junction between myocytes (arrow and inset) (x31,120).

B) An apical junction amongst radial myocytes. Only a few septa are evident (arrows) (x103,320).

C) A representative portion of a nerve bundle amongst radial myocytes (x18,700).

D) A neuro-neuronal synapse amongst nerves of the radial muscle (arrow) (x41,534).

ds:	desmosome
gj:	gap junction
mf:	muscular foot
nb:	nerve bundle
nu:	nucleus
sg:	surface granule

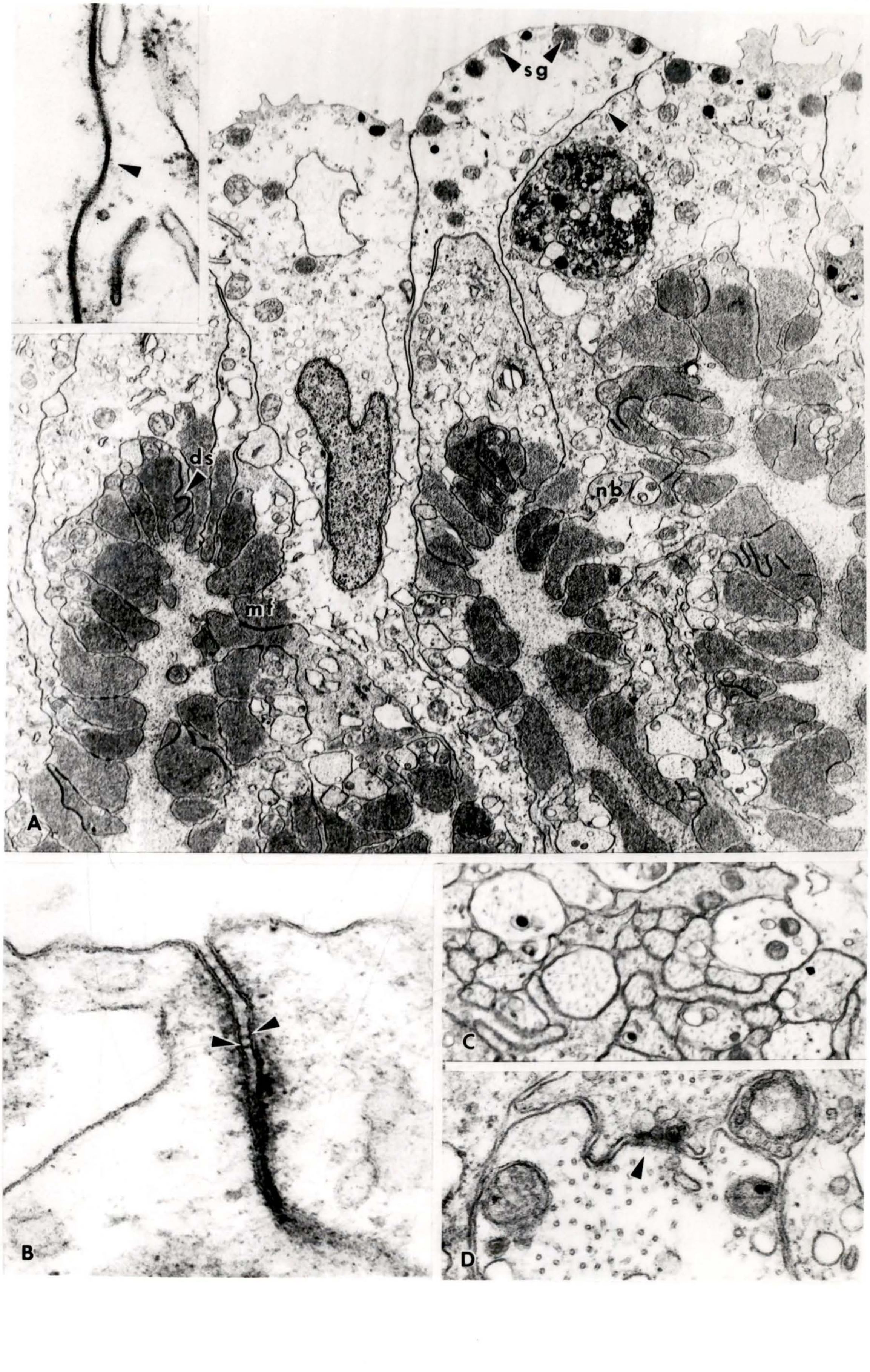
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Figure 35: Radial and swimming muscle at the edge of a radial canal in *G. vertens* (TEM).

A) The apposition of radial and swimming muscle tissues in a radial muscle cord. The two types of muscle fibres appear to intermingle quite readily, and some of the myofilaments in each type of muscle become oriented in the direction of the other type (see b & c below). No discrete radial nerve bundle is present, but large nerve bundles in association with the radial muscle (arrow) may be analogous. Note also the presence of endodermal nerve bundles in the adjacent radial canal endoderm (arrows). (x13,060)

B) Circular and radial smooth muscle myofilaments in a radial muscle myocyte (x22,230).

C) Circular and radial striated myofilaments in a swimming muscle myocyte (x66,070).

m: mesoglea
rc: radial canal endoderm
rm: radial muscle
sw: swim muscle

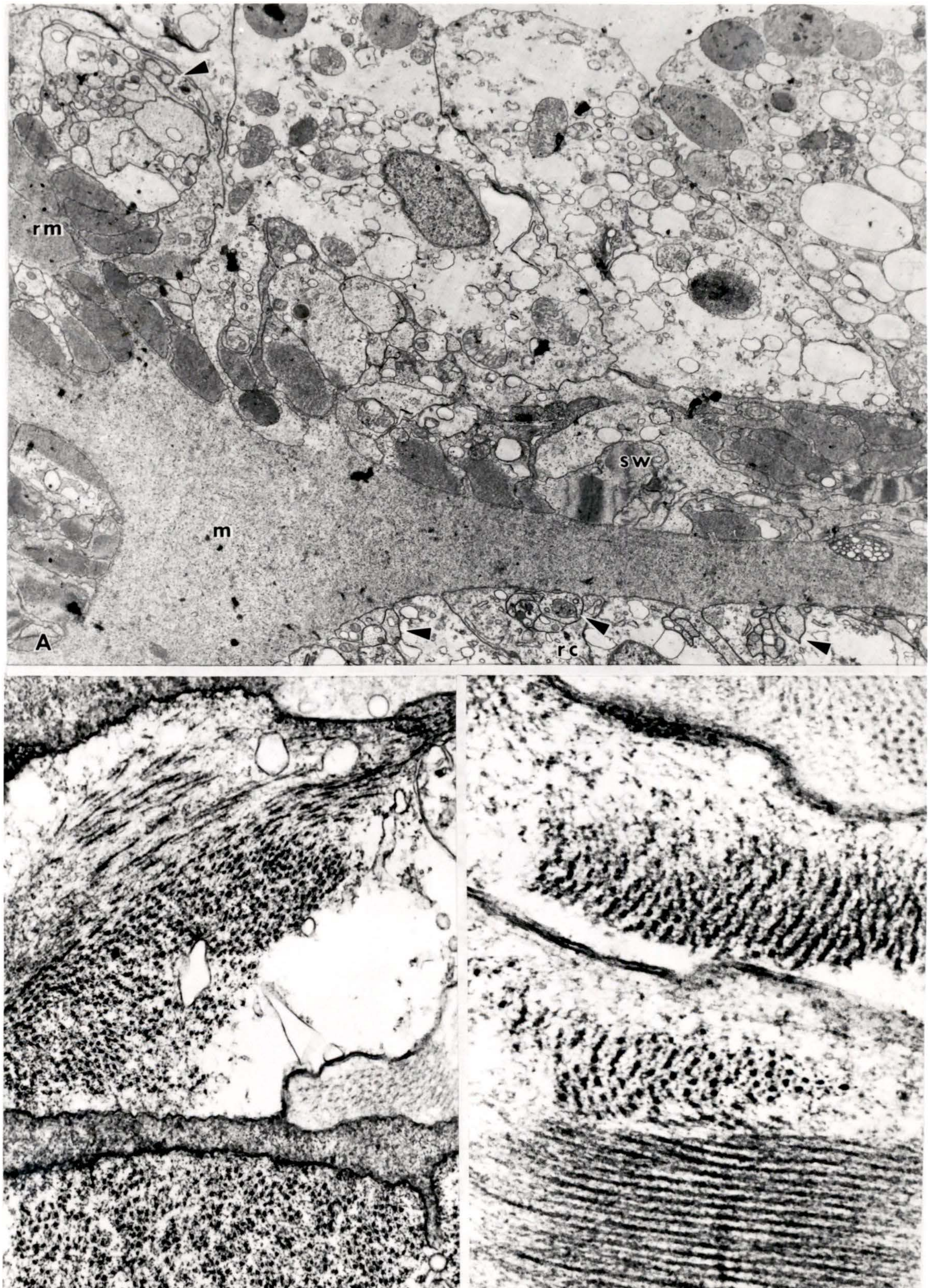
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Figure 36: A radial muscle cord of *G. vertens* at the bell margin showing its junction with the sphincter muscle (SEM, x450).

bm: bell margin
g: gonad
rm: radial muscle cord


g
rm
x
bm

Figure 37: The sphincter muscle of *G. vertens* (TEM).

A) A cross-section of the sphincter muscle showing its location between the inner nerve ring and the swimming muscle. Note the well spaced muscle feet on mesogleal ridges, desmosomes, nerve bundles (arrows), and the large granules similar to surface granules of other ectodermal cells (aqueous epon) (x2,860).

B) A gap junction (arrow) between sphincter myocytes (x199,260).

C) A "nerve" process amongst sphincter myocytes containing myofilament-like structures (arrow) (x30,442).

D) Striated rodlets found in sphincter myocytes of some individuals (x79,290).

inr: inner nerve ring
sph: sphincter muscle
sw: swimming muscle

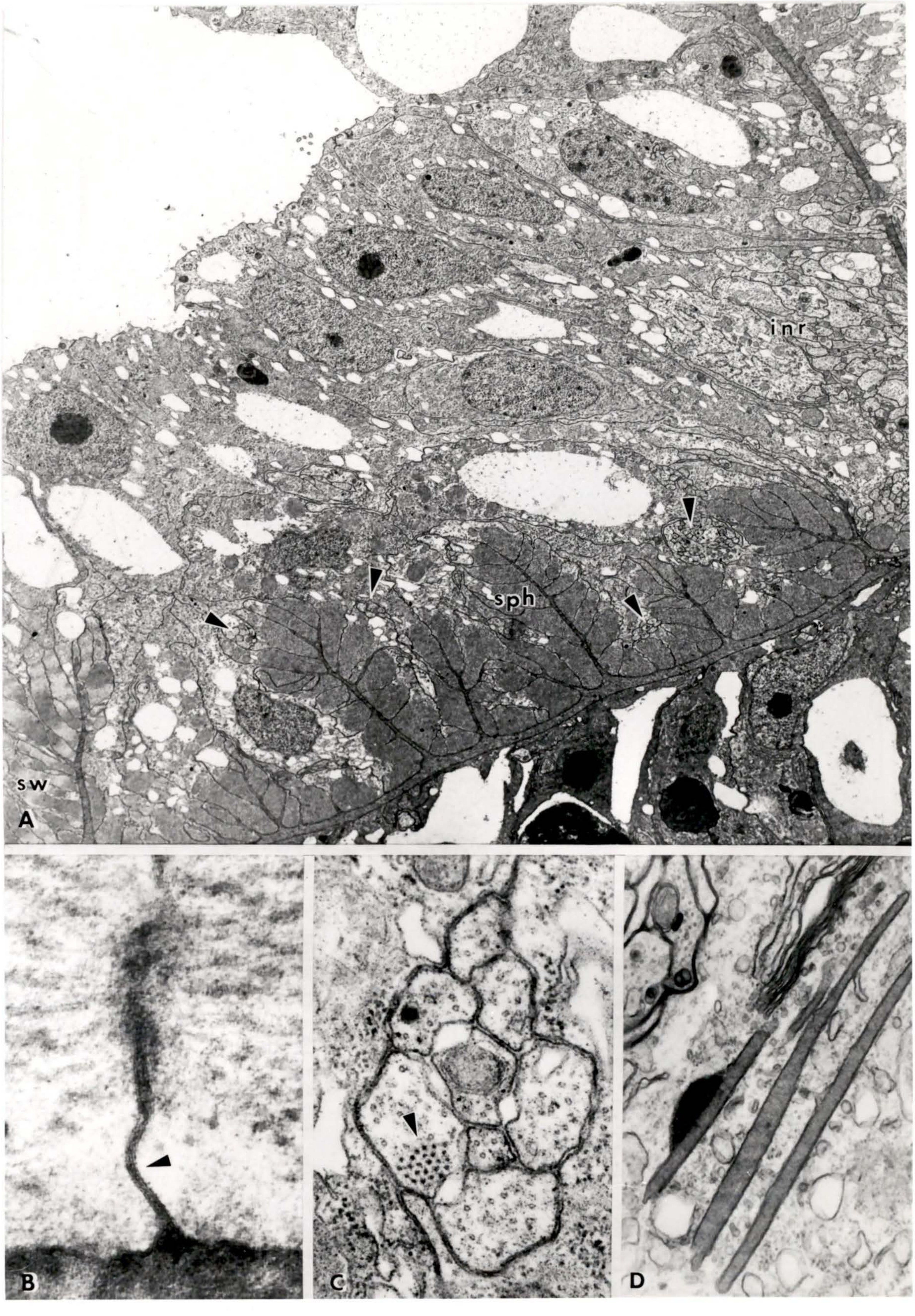
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Figure 38: The swimming muscle of *G. vertens* (TEM).

A) A radial cross-section of swimmimg myocytes close to the bell margin. Striated muscle feet, numerous mitochondria, and surface granules are present in the myocytes. Mesogleal ridges are present in the swimming muscle adjacent to the bell margin. Note also the large neuron soma (x4,820).

B) A circularly oriented cross-section of the swimming muscle in the bell proper. Striations of adjacent muscle fibres are generally in register. Desmosomes between muscle fibres are present (arrow & inset). Note also the circularly oriented nerve process (x6,690).

C) An apical junction between swimming myocytes. Amorphous intercellular material is present, but no discrete septate desmosomes (x120,540).

D) A gap junction adjacent to the cell apex between swimming myocytes (x97,420).

mf: muscle fibre
mit: mitochondria
m: mesoglea
n: nerve axon
ns: nerve soma
sg: surface granules
sw: swimming myocyte

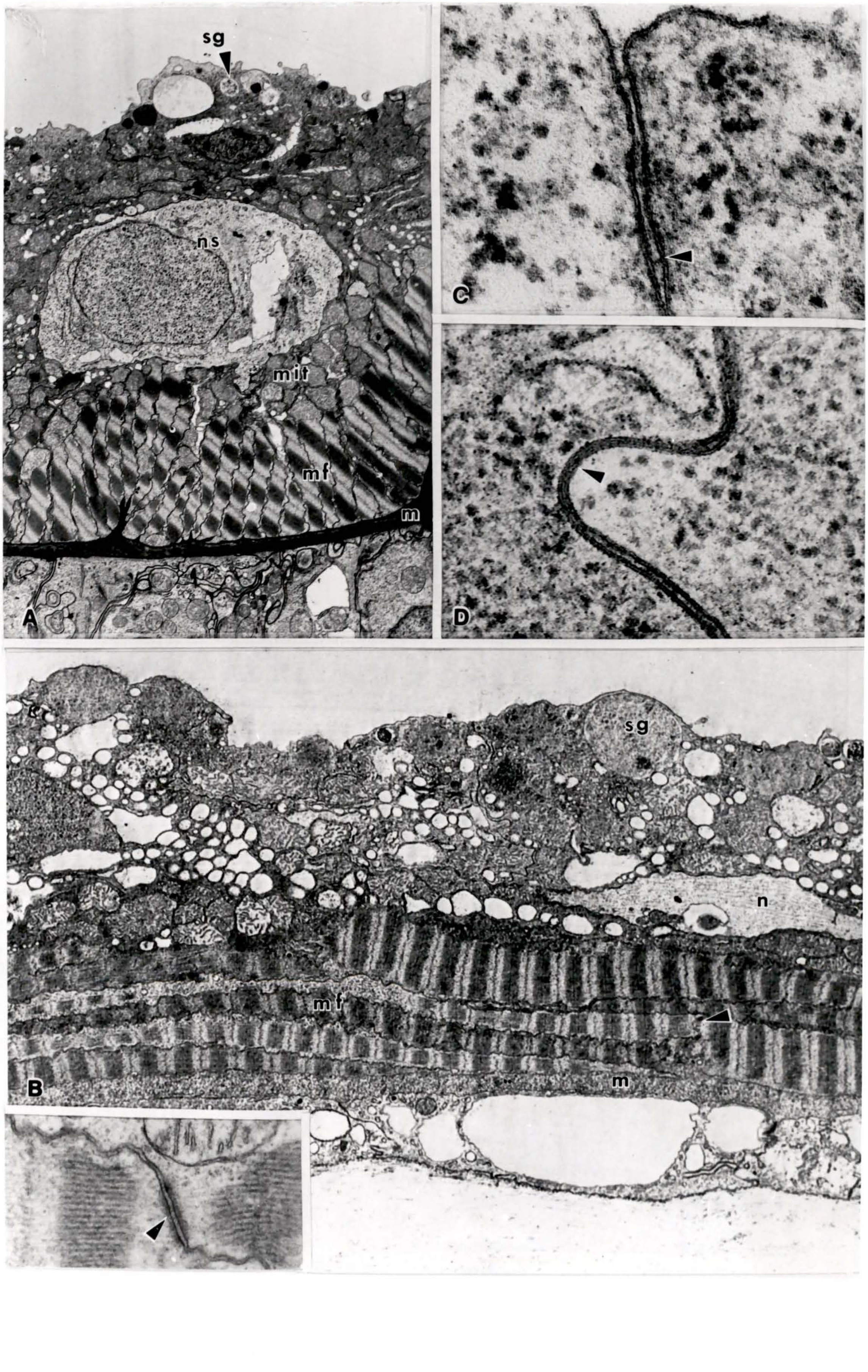
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Figure 39: The tentacle ectoderm of G. *vertens* (A: SEM, B: light micrograph, C,D, & E: TEM).

A) The tentacle surface showing a nematocyst-bearing ridge and adjacent regions. The arrow indicates a cnidocil (x740).

B) A cross-section through a tentacle. In the ridge region the nematocysts lie at the surface over a layer of large vacuoles. The ectodermal musculature in these ridges is concentrated between mesogleal outcroppings (arrow). Nematocysts are absent in the non-ridge region (asterisk), the mesoglea is smooth, and the musculature is more uniformly distributed. The endoderm consists primarily of thin sheets surrounding large vacuoles as they extend from the central chamber to the mesoglea (x220).

C) The ectoderm in a non-ridge region. Vacuolation is still present, though not as pronounced as in the ridges. Nerve bundles are present amongst muscular feet (arrow) (x5,470).

D) An apical portion of a tentacle ridge. The large vacuoles separate the ectoderm into surface and basal cytoplasm connected by thin sheets of tissue. The nematocysts are imbedded within the surface portion but send bundles of connective rods (arrow) to the mesoglea (x2,220).

E) A basal portion of ridge ectoderm. The apices of the outcroppings are the site of nematocyst anchoring complexes. Muscle feet are located between the mesogleal structures and nerve bundles (arrows) are clustered around their apices (x13,260).

end: endoderm
mf: muscular feet
m: mesogleal ridge
nem: nematocyst
na: nematocyst anchoring complex
ri: tentacle ridge
v: vacuole

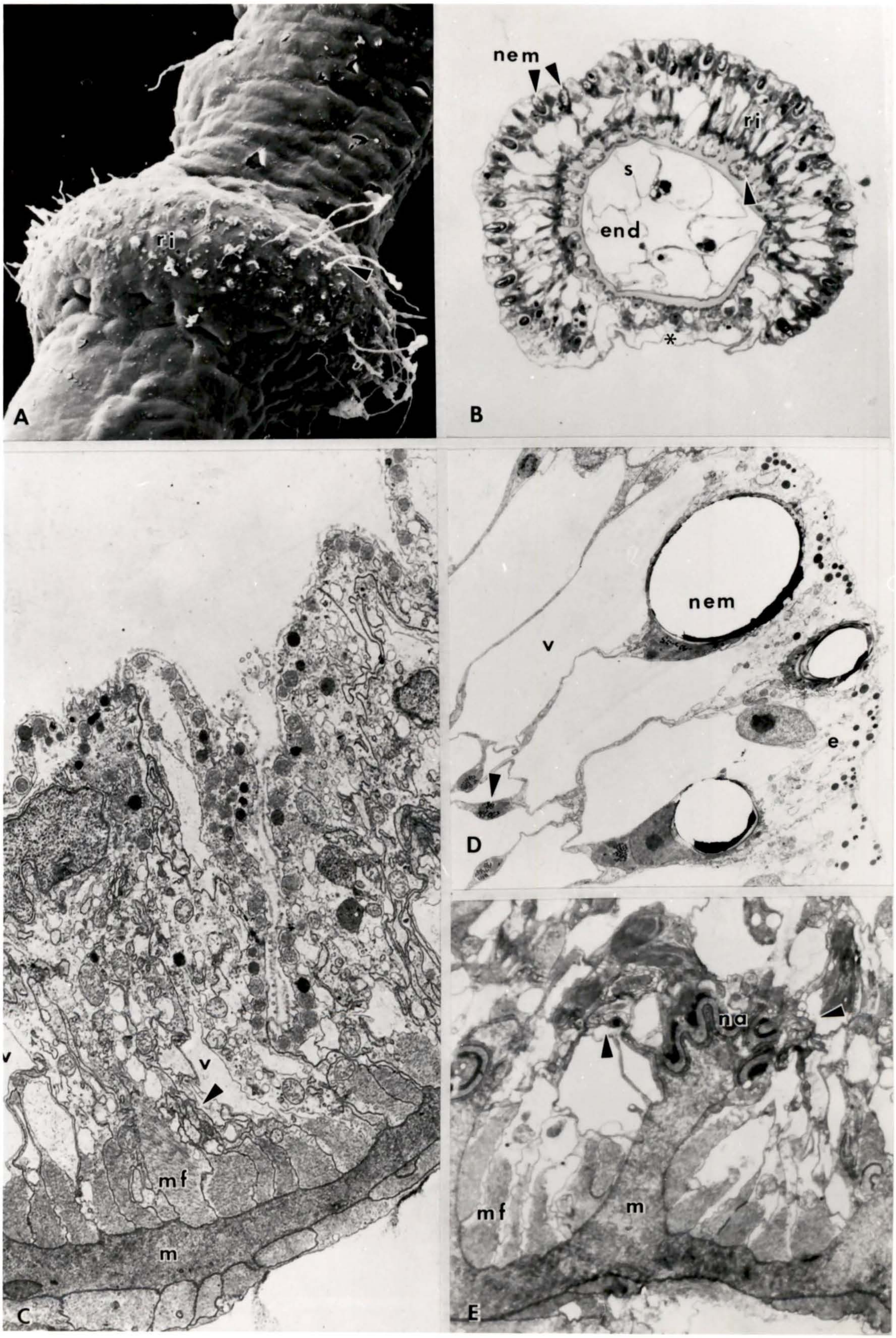
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Figure 40: Nerves and intercellular junctions of the tentacle ectoderm in *G. vertens* (TEM).

A) A nematocyst anchoring complex and nerve bundle in ridge ectoderm (x18,490).

B) An apical junction between ridge cells. Only occasional septa are evident (arrow) (x70,180).

C) A neuromuscular synapse (x26,790).

D) A gap junction adjacent to the cell surface (x54,120).

E) A neuro-neuronal synapse (x49,790).

F) A desmosome between muscle feet (x36,801).

na: nematocyst anchor

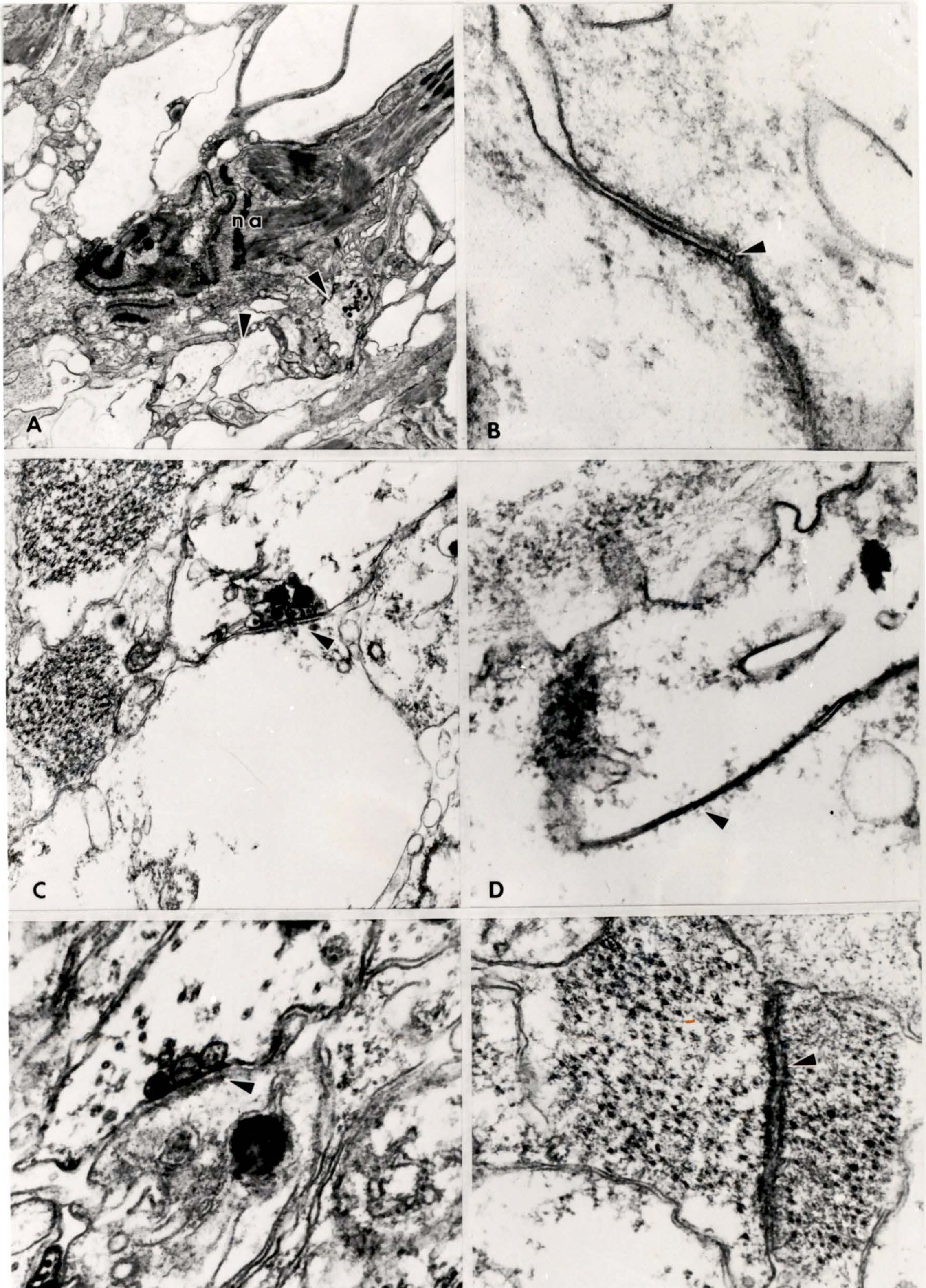
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Figure 41: The tentacle endoderm of *G. vertens* (TEM).

A) A cell profile in a longitudinal section. Note the cilium (arrow), large vacuoles, and granular basal cytoplasm. Nuclei and digestive vacuoles (not shown) are normally present in apical and intervacuolar cytoplasm (x6,890).

B) An apical junction between endodermal cells. Some intercellular material appears to be present, but distinct septate desmosomes cannot be distinguished (x48,930).

C) A gap junction (arrow) within an inter-vacuolar process (x39,850).

D) Longitudinally oriented microfilaments are present in the granular basal cytoplasm (x23,030).

ci: cilium
bc: basal granular cytoplasm
lu: lumen of tentacle
m: mesoglea
v: vacuole

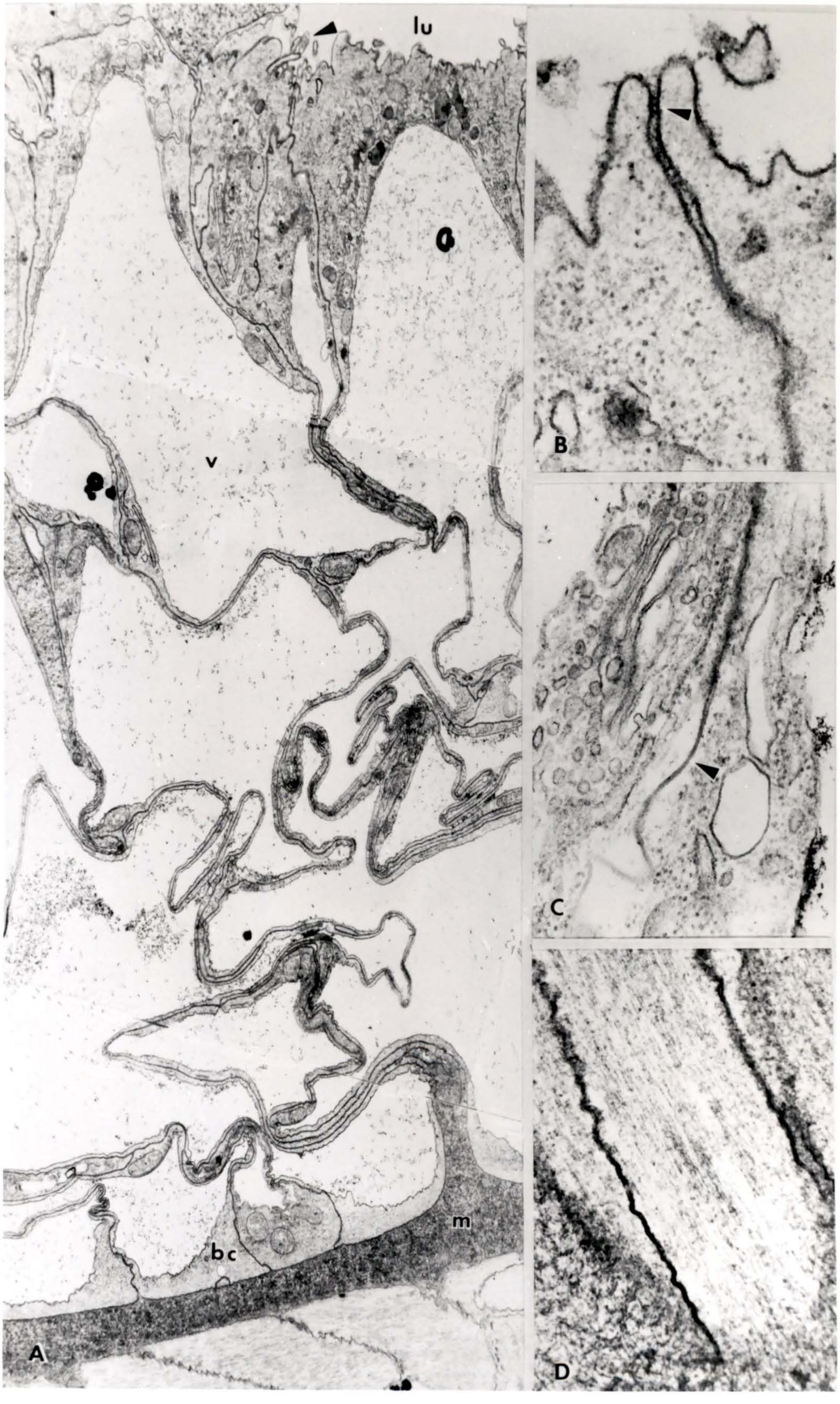
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Figure 42: The origin of tentacle tissues in the bell margin of *G. vertens* (TEM).

A) Muscle fibres are present in the ectoderm of a tentacle bulb adjacent to the mesoglea. Note the vacuolated endoderm typical of tentacles. Gap junctions amongst muscular tissue are present (inset and arrows) (x107,630).

B) A trans-mesogleal process with a gap junction between ectoderm and endoderm in a tentacle bulb (x86,100).

C) The junction of tentacle endoderm with ring canal endoderm. Myofilaments are present in the ring canal endoderm and typical granular cytoplasm is present in the base of the tentacle endoderm (arrow & inset) (x5,410; inset x27,060).

dv: digestive vacuole
m: mesoglea
mf: myofilaments
rc: ring canal
lu: tentacle canal
v: vacuoles

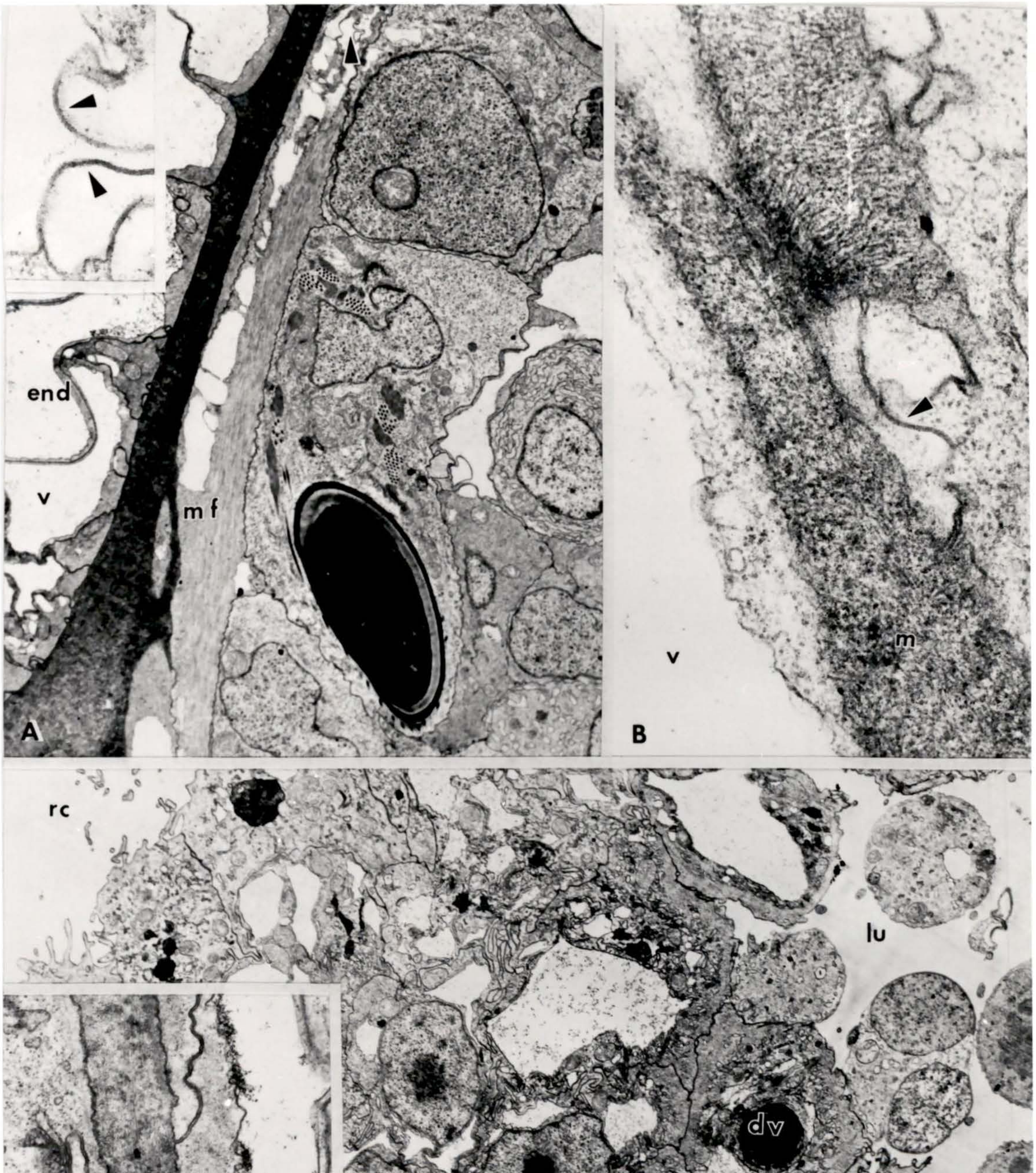
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Figure 43: The velum of *G. vertens* (TEM).

A) A radial cross-section at the base of the velum. Muscle fibres are present on the subumbrellar side of the velum but are absent on the exumbrellar side. The muscle fibres are circular in orientation and are striated. Nerve axons (arrows) are present amongst them and appear to run into the velum from the inner nerve ring. Deep clefts are present in cells of the exumbrellar side and on the subumbrellar side of the velum base (asterisks). A mesogleal ridge is present on the exumbrellar side of the velum base opposite the subumbrellar cleft. The large vacuoles evident in the subumbrellar epithelium are not present in all preparations, and are of unknown significance (aqueous epon) (x2,140).

B) Cells containing axial bundles of microtubules are present in association with the mesogleal ridge at the base of the velum's exumbrellar side (x24,650).

inr: inner nerve ring
m: mesoglea
mt: microtubule core
sw: subumbrellar muscle
v: vacuole
ms: mesogleal spur
n: nerve axons
sep: septate divisions
sg: surface granules
sr: striated musculature
sub: subumbrella

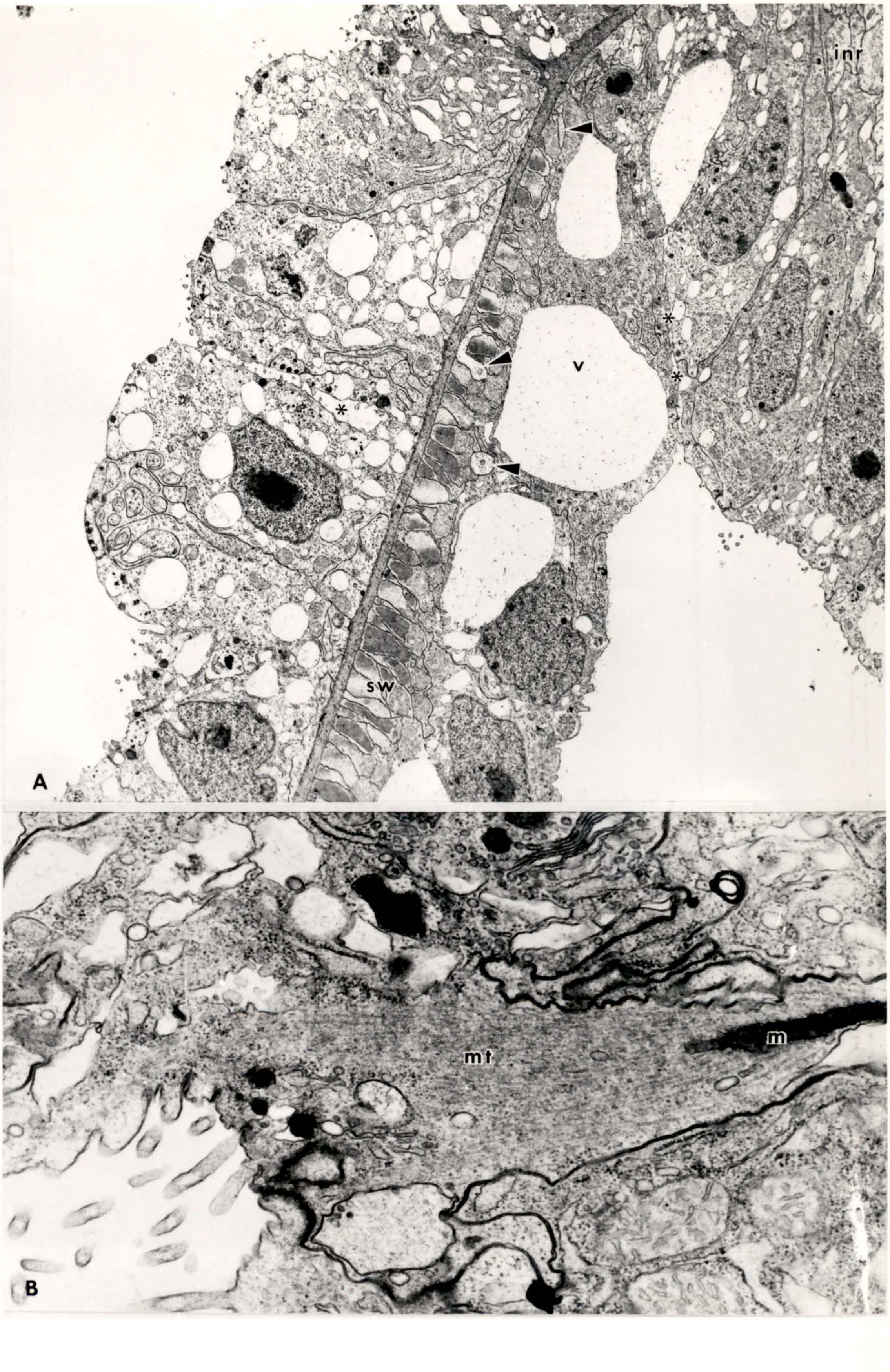
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Figure 44: Intercellular junctions in the velum of *G. vertens* (TEM).

A) An apical junction in the exumbrellar epithelium of the velum. Intercellular material is present, but discrete septate desmosomes cannot be resolved. The asterisk marks the apex of the intercellular border (aqueous epon) (x110,700).

B) A gap junction (arrow) between exumbrellar cells of the velum (aqueous epon) (x87,945).

C) An apical junction between subumbrellar cells of the velum. The apex of the intercellular border is marked by an asterisk. Septum-like intercellular material is present (arrow) but no clear septate desmosomes (x87,945).

D) A gap junction (arrow) between muscular feet of the velum subumbrella (x87,945).

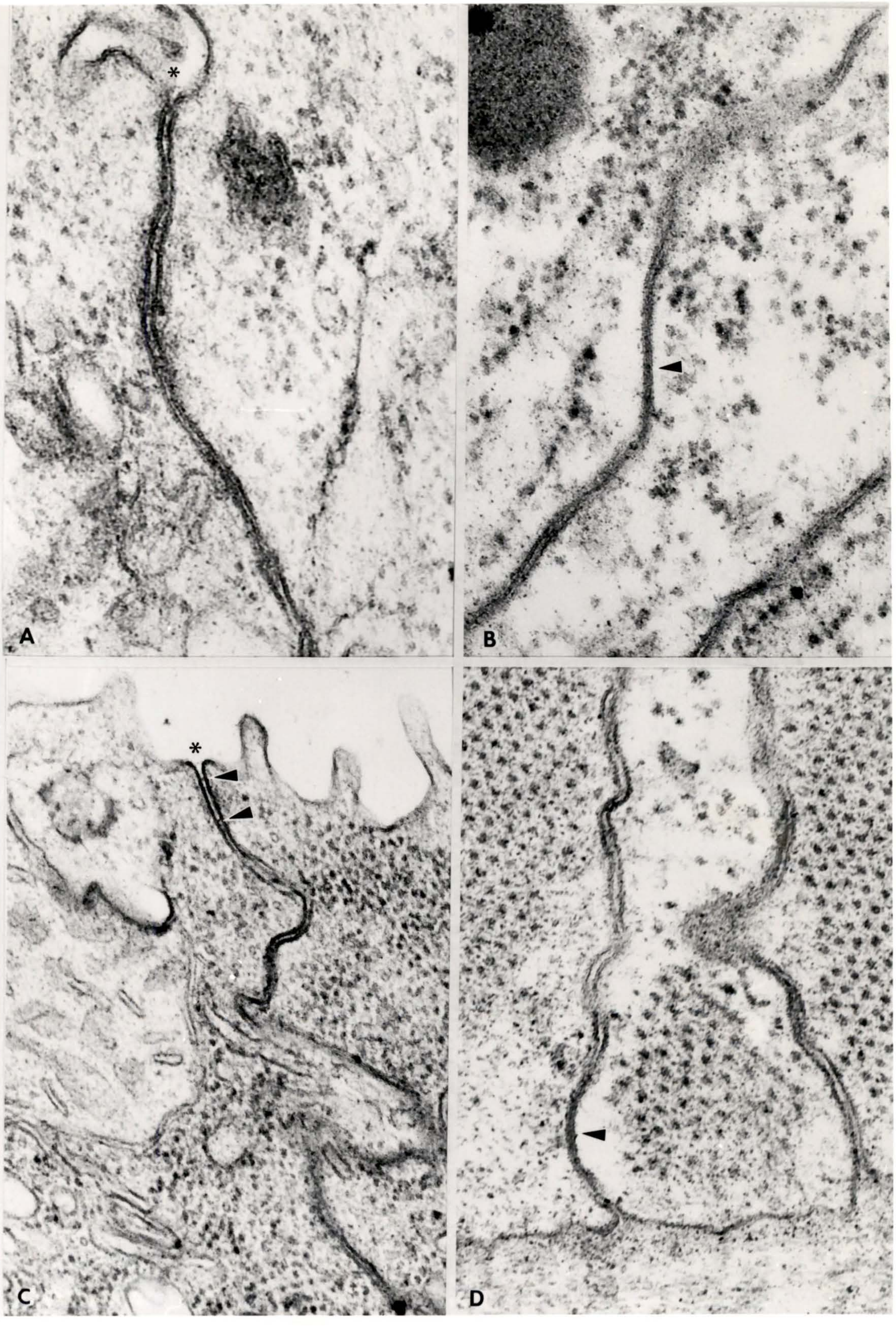
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Figure 45: Ciliated cells on the exumbrellar side of the velum of *G. vertens* (A & B: TEM; C: SEM).

A) A radial cross-section of a ciliated cell. Vertically oriented parallel microtubules and interdigitating processes are present (x21,286).

B) Gap junctions to interdigitating processes are present (arrow), as are putative nerve processes in some cells (x43,283).

C) The exumbrellar side of the velum adjacent to its base. The cilia present possess subterminal swellings (arrow) (x8,486).

ci: cilium
ip: interdigitating process
mt: microtubules
n: nerve axon

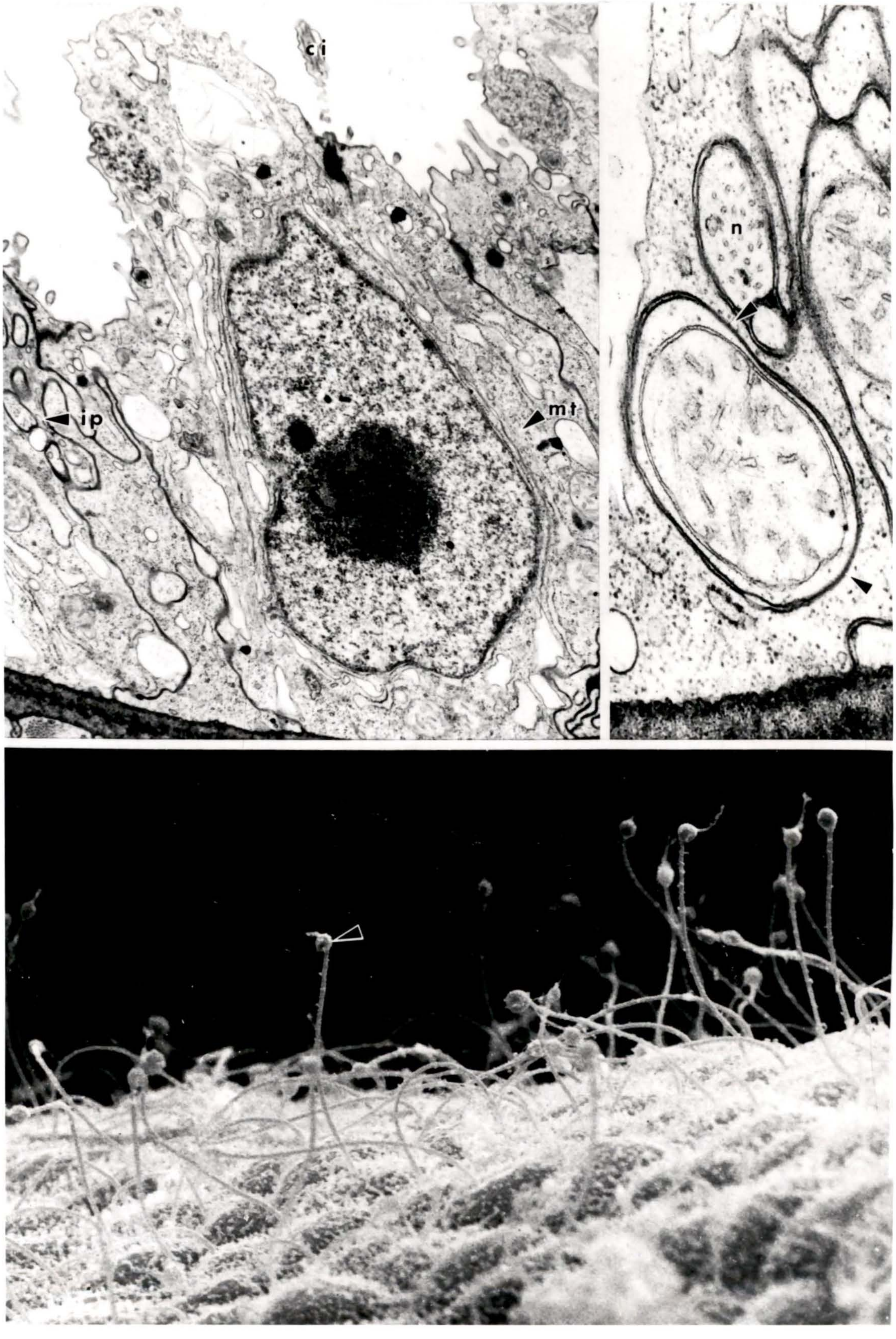
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Figure 46: A cross-section of the bell margin of *G. vertens* (aqueous epon).(TEM)

The mesogleal core of the velum extends to the bell margin to form the tri-radius. On the subumbrellar side of this mesogleal structure are the inner nerve ring and sphincter muscle, together covered by an epithelial sheath, while on the exumbrellar side are an outer nerve ring and scattered smaller nerve bundles, including a "marginal nerve ring", and a mass of epithelial and cnidoblast tissues. The exumbrellar tissue also extends into the tentacle bulbs and into the statocysts. The statocysts are supported over the ring canal by a mesogleal pedestal projecting over the ring canal (x1,840).

es: epithelial sheath
inr: inner nerve ring
m: mesoglea
mnr: marginal nerve ring
mp: mesogleal pedestal
onr: outer nerve ring
rc: ring canal
sph: sphincter muscle
st: statocyst chamber
sw: swimming muscle
tb: tentacle bulb
tr: tri-radius
v: velum

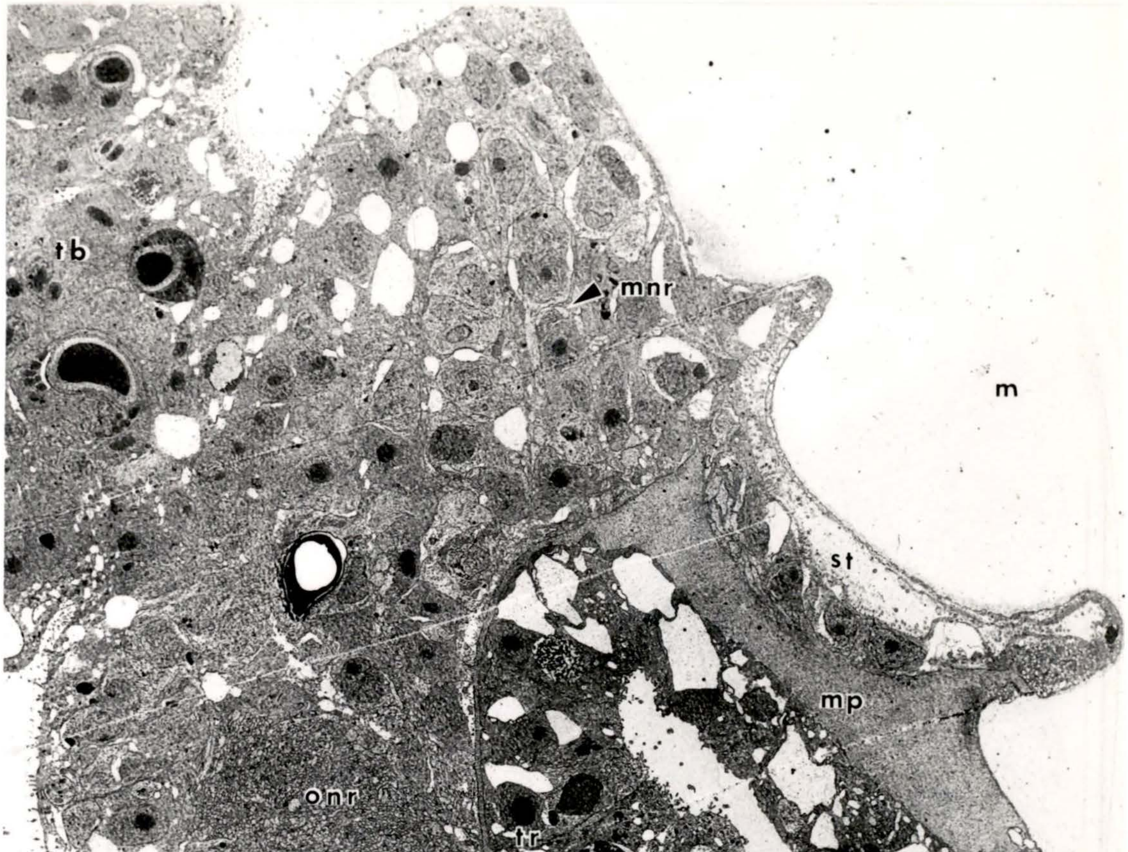
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Figure 47: The inner nerve ring of *G. vertens* (TEM).

A) A cross section of the inner nerve ring. Note the epithelial sheath, large "swimming motor giants", and the smaller diameter axons. The soma of small diameter axons lie back from the axon region. Epithelial cells send processes down amongst the nerve axons (arrows). Gap junctions between epithelial cells have been observed (double arrow & inset) (x11,020; inset x141,066).

B) A light micrograph of a ciliated cell (arrow) in the epithelial sheath over the inner nerve ring (x1,400).

C) A TEM of a ciliated cell in the epithelial sheath. Note the tracts of microtubules (arrows) (x12,054).

es: epithelial sheath
inr: inner nerve ring
m: mesoglea
ns: neuron soma
sg: swimming motor giant

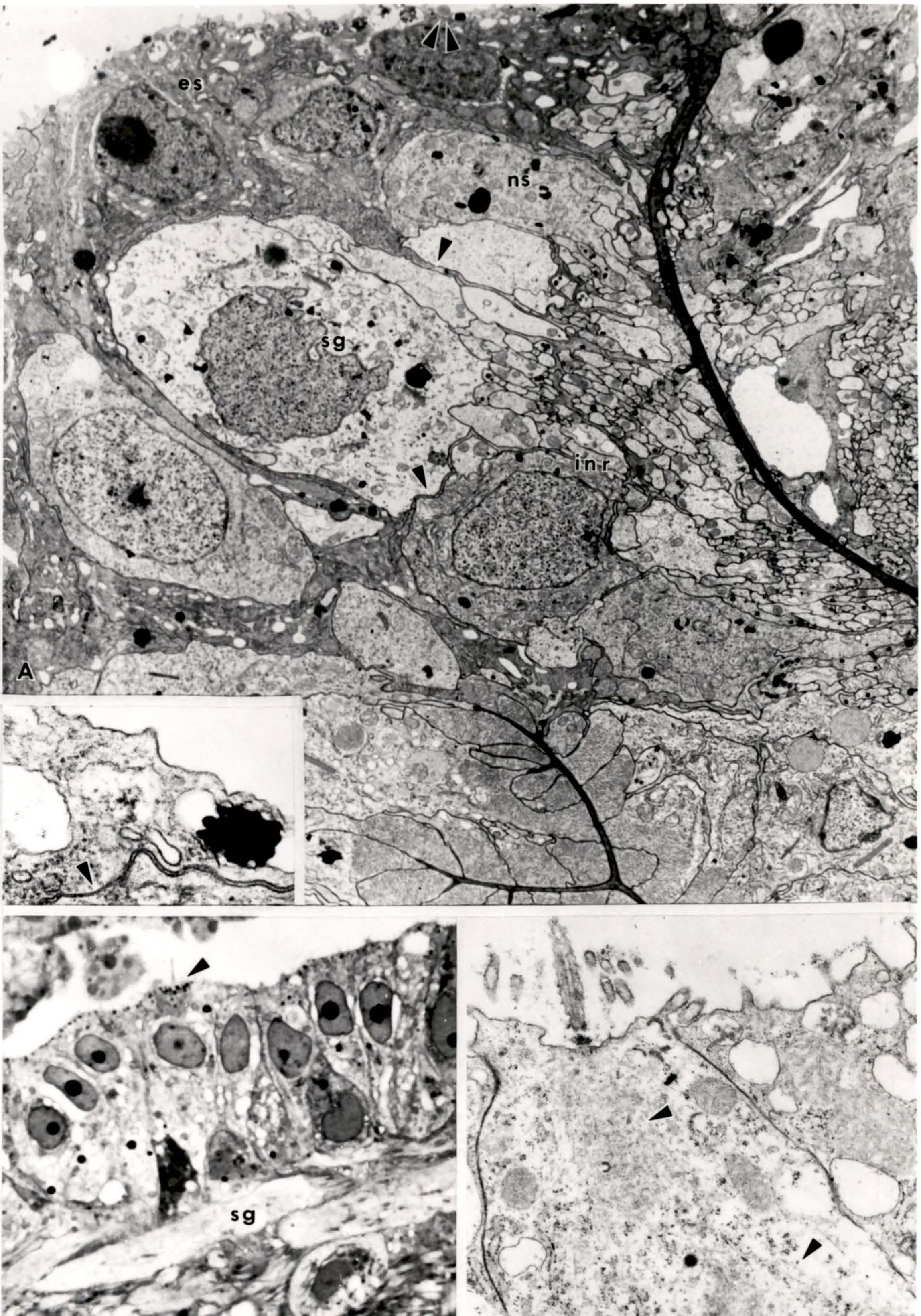
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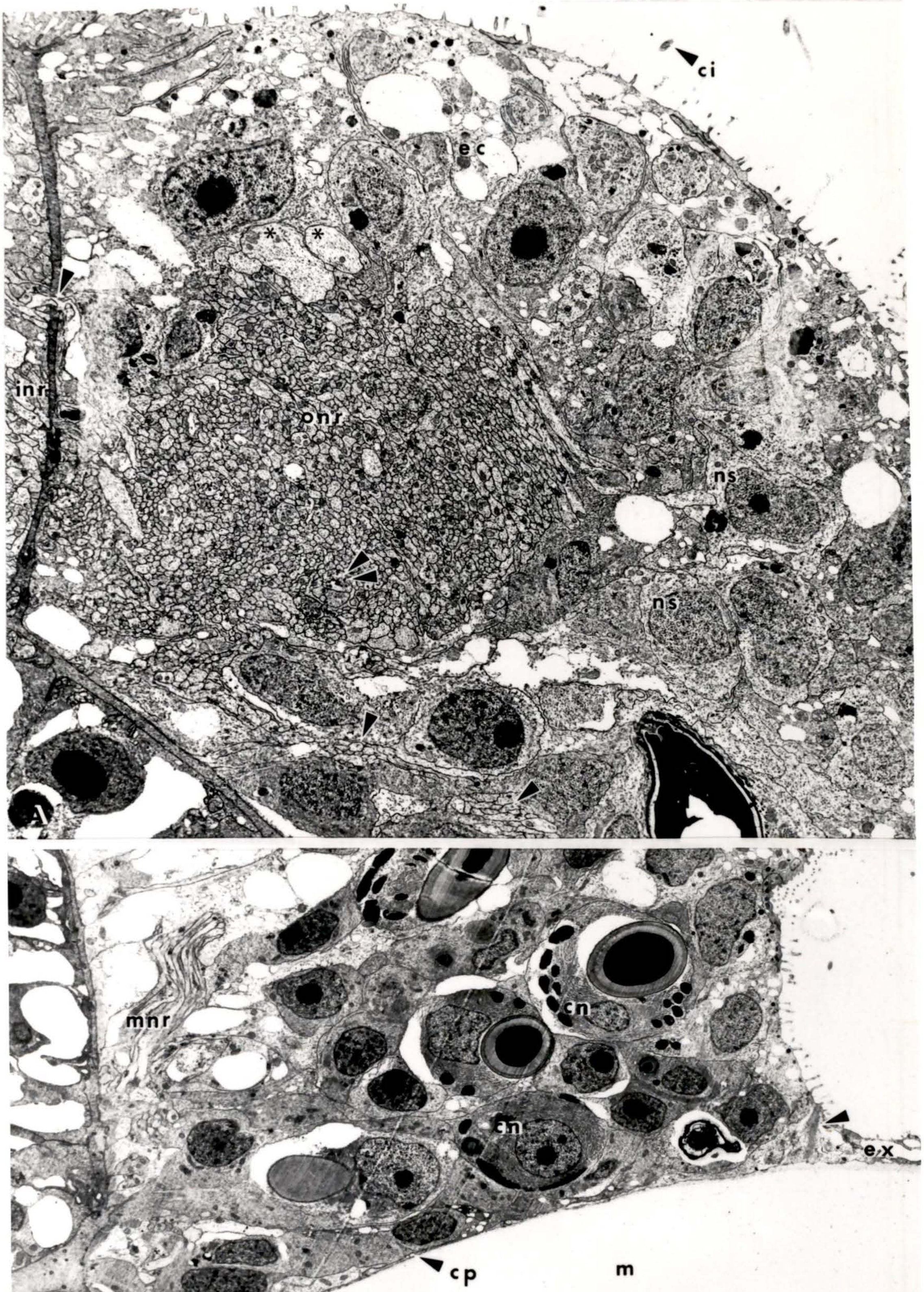
Figure 48: The exumbrellar bell margin of G. *vertens* (aqueous epon TEM).

A) A cross-section of the exumbrellar tissue ridge adjacent to the tri-radius. The outer nerve ring and neuron somata lie within a mass of epithelial and cnidoblast tissue. Note the nerve tracts into the surrounding tissue and to the inner nerve ring (arrows). The axons of the outer nerve ring are generally of small diameter, but a few larger axons are also present (asterisks). Neuron somata appear to lie within the surrounding tissue. Some of the axons (double arrows) contain numerous neurosecretory granules. (x2,890; aqueous epon)

B) A cross-section of the exumbrellar tissue ridge adjacent to the bell showing epithelial and cnidoblast tissues and the marginal nerve ring. The cell at the junction of the bell exumbrella ("exumbrella proper") and bell margin possess a microtubular core and extend into a cellular bridge to the endoderm. (x1,990)

ci:	cilium
cn:	cnidoblast
cp:	trans-mesogleal cell process
ep:	epithelial cells
ex:	exumbrella proper
inr:	inner nerve ring
mnr:	marginal nerve ring
ns:	neuron soma
onr:	outer nerve ring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cn
ex
cp
m
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Figure 49: Epithelial tissue of the exumbrellar bell margin in *G. vertens* (TEM).

A) An apical portion with epithelial cells and cnidoblasts. Note the cilium, microvilli, and surface granules. Apical junctions possess septate desmosomes (inset). (x22,600; inset x119,000)

B) Extensive gap junctions are present between the cnidoblast and epithelial cell processes of fig.18(a). (x130,600)

C) A gap junction in a cellular process extending from bell margin ectoderm to the endoderm. (x210,000)

ci: cilium
cn: cnidoblast
m: mesoglea
mv: microvilli
sg: surface granule

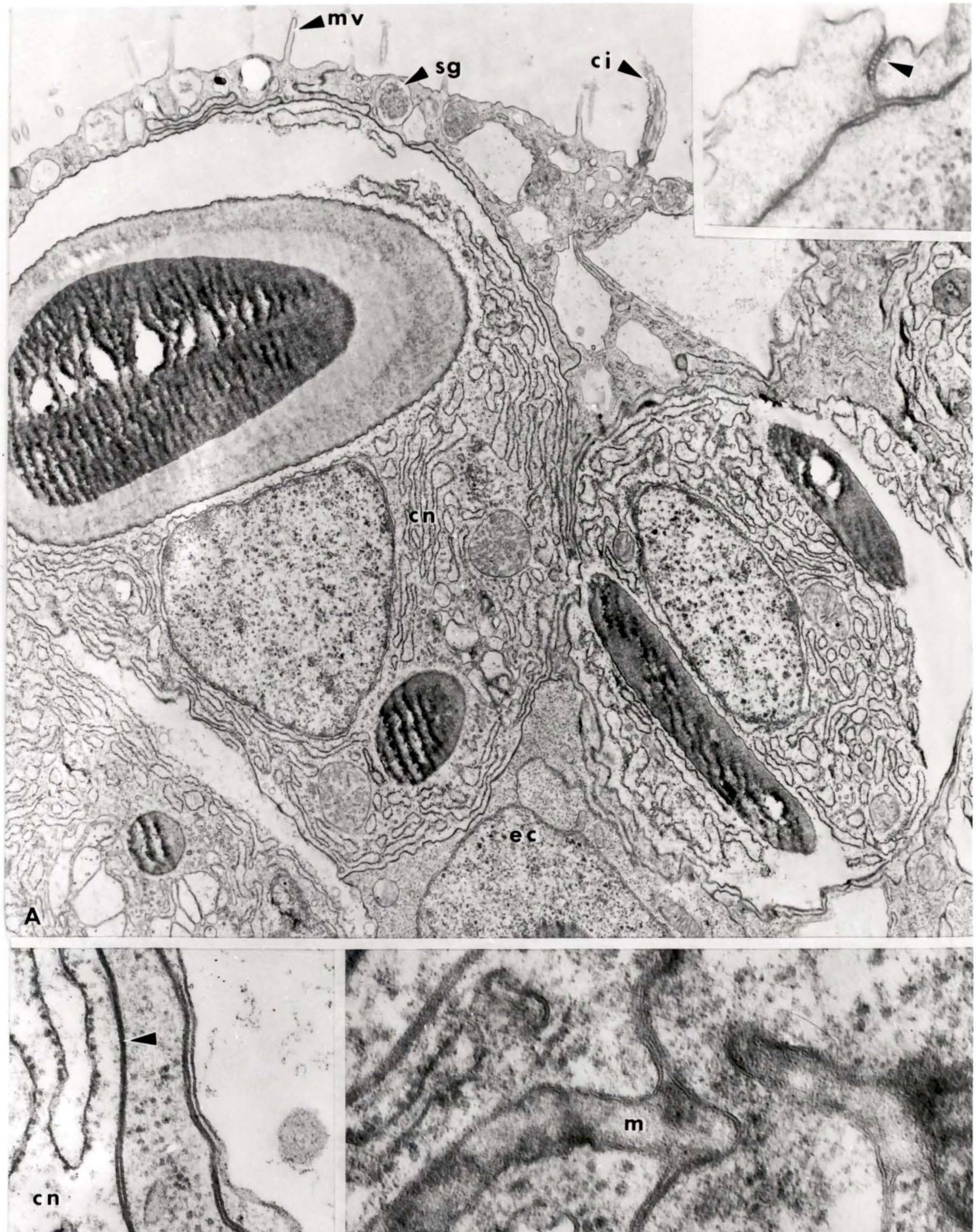
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Figure 50: Nerve processes in the exumbrellar bell margin of *G. vertens* (TEM).

A) A neuron soma within epithelial tissue of the bell margin. Note also the other nerve tracts leaving the outer nerve ring (arrows). (x1,500)

B) A nerve axon within epithelial tissue of the bell margin between the outer nerve ring and the bell proper: a synapse to an epithelial cell (arrow & inset) and from a putative neuron soma onto the axon (double arrow) are present. (x2,890; inset x26,200)

C) A synapse amongst marginal nerve ring neurons running into the tentacle bulb. (x13,060)

D) Communication between endodermal processes and the ectoderm in the tri-radius (x5,790).

end: endoderm
ex: exumbrellar tissue of bell margin
ns: neuron soma
sub: subumbrella

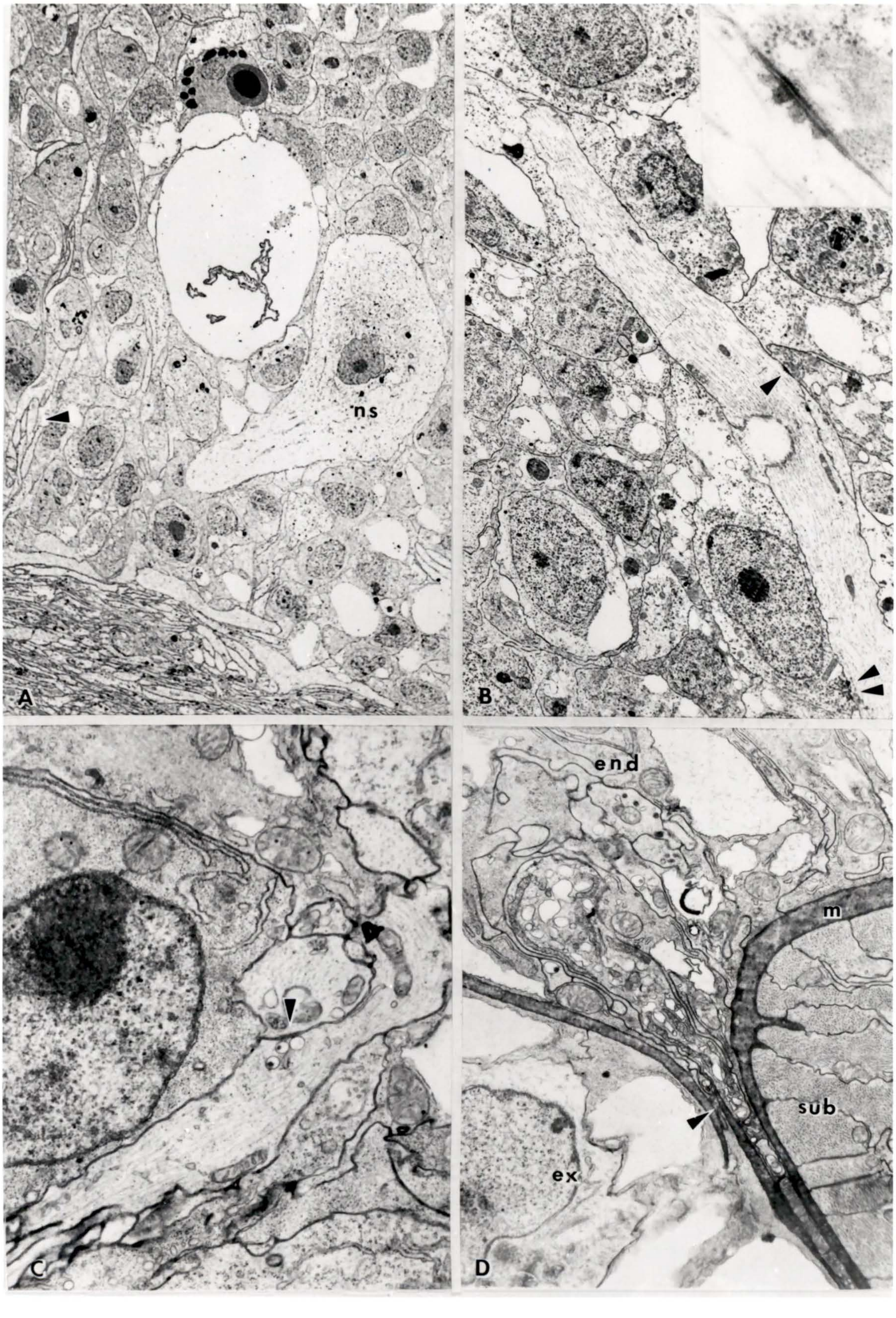
ns
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Figure 51: Sensory cells in the exumbrellar bell margin of *G. vertens*. These cells communicate with the exterior, possess cilia, and contain extensive tracts of microtubules (inset). Note also the approaching axon (aqueous epon; TEM) (x3,870; inset x22,730).

ci: cilium
n: nerve axon
sc: sensory cell

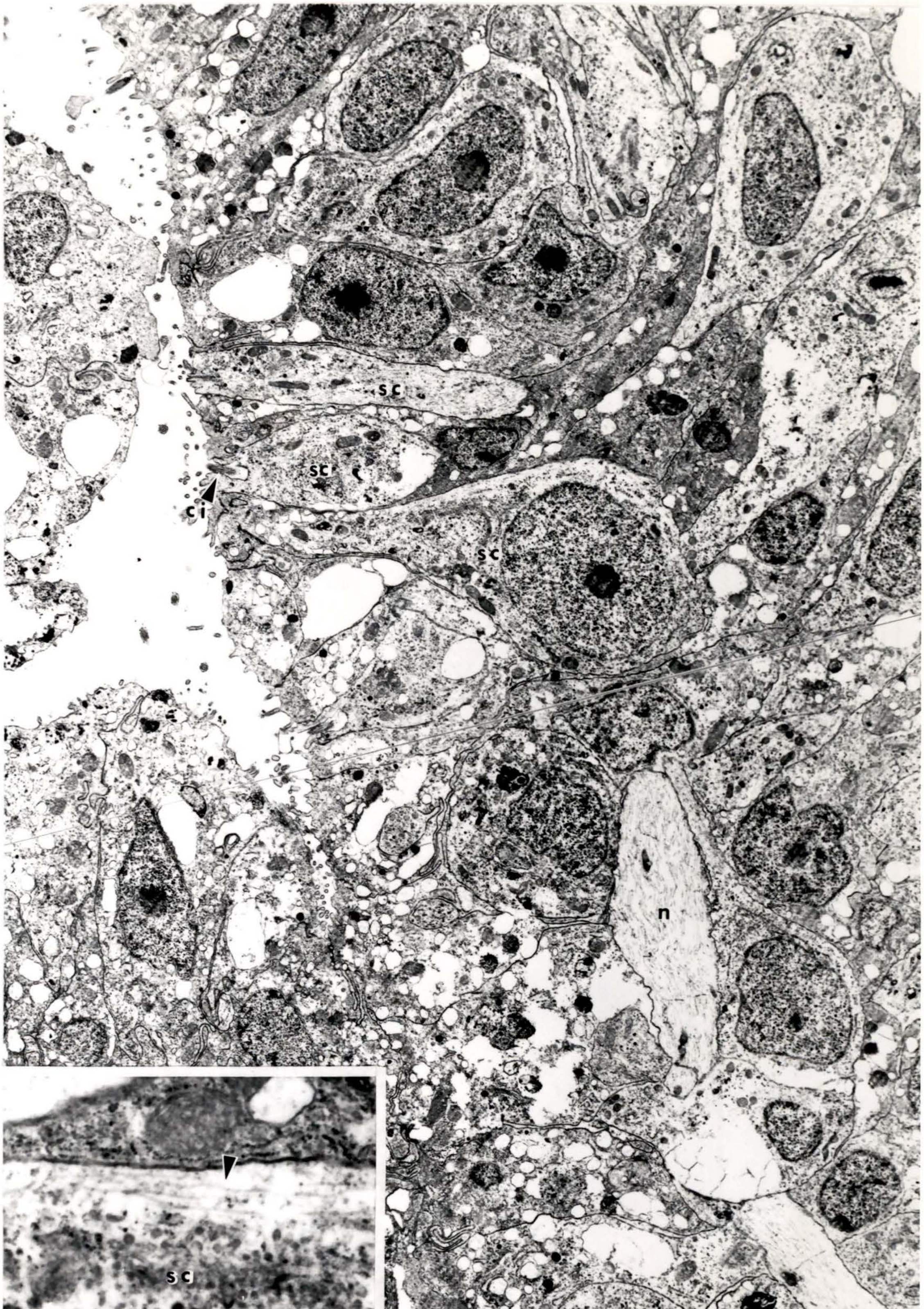
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Figure 52: The cellular bridge from exumbrella proper to the endoderm at the bell margin of *G. vertens* (TEM).

A) The origin of the bridge at the junction of the bell exumbrella and the bell margin. The cell giving rise to the bridge contains an axial core of microtubules (arrow & inset). (x11,190; inset x24,800)

B) The bridge passes by exumbrellar bell margin tissue as a thin process (arrow).(x8,590)

C) The bridge encloses bases of the tentacles (see text). (x1,990)

D) The bridge also encases the mesogleal pedestals of the statocysts (see text) (x5,327).

E) At the statocyst chamber the bridge tissue forms the chamber's lining with a shallow microvillous border (arrow). (x4,520; inset x2,240).

m:	bell mesoglea
mnr:	marginal nerve ring
rc:	ring canal
stc:	statocyst chamber
stm:	statocyst mesoglea
ten:	tentacle endoderm

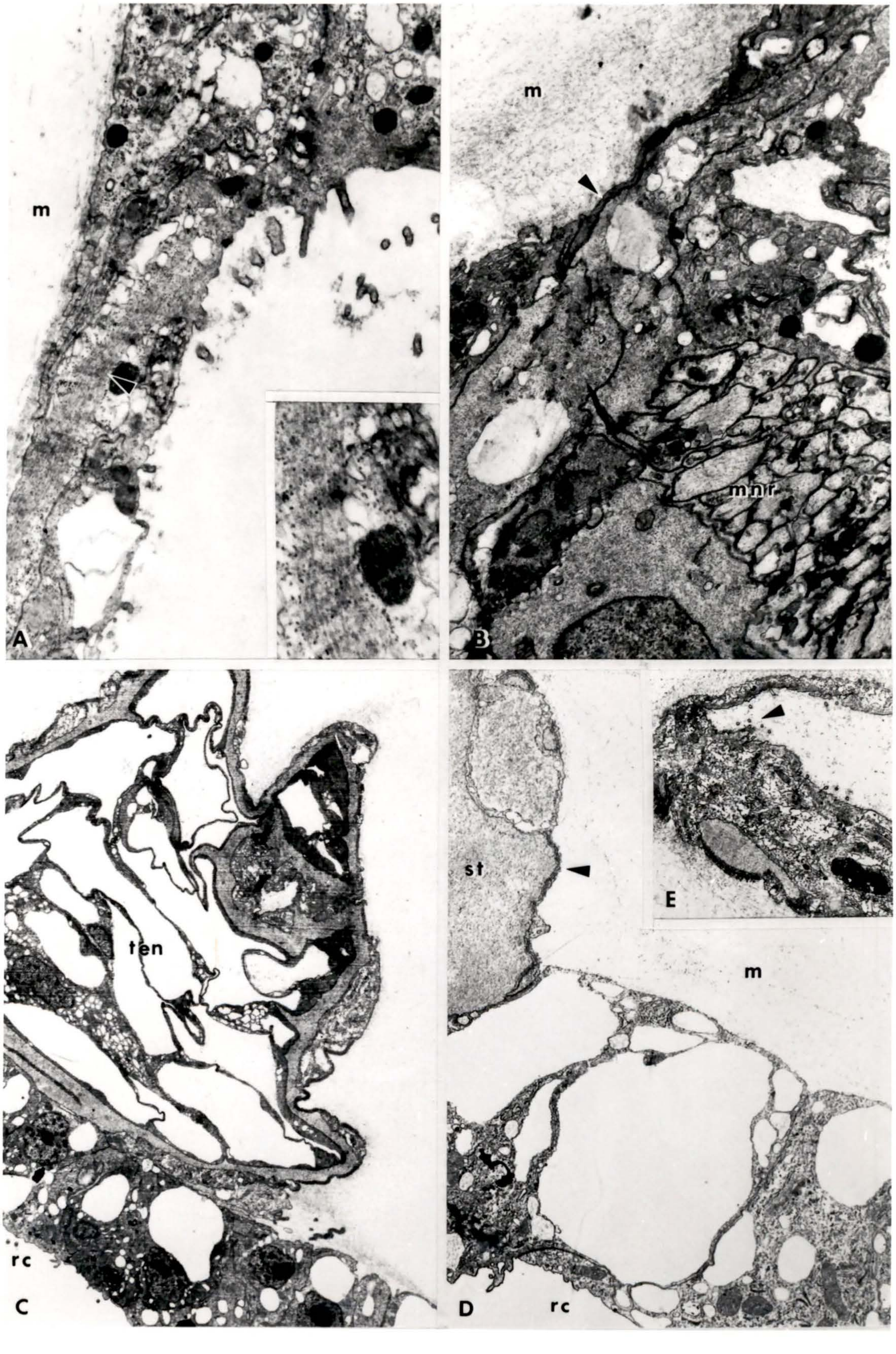
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Figure 53: The inner endoderm of *G. vertens* (TEM).

A) The inner endoderm of a radial canal showing microvilli, cilia, extensive interdigitation amongst cells, digestive and other vacuoles, central nucleii, golgi, mitochondria, microtubular tract in some cells (asterisk), and occasional nerve axons (arrow). (x5,560)

B) A gap junction from fig.21a. (x69,400)

C) A nerve axon pair in close proximity to fig. 21a. (x29,930)

D) Microtubules in an inner endoderm cell of 21a and an adjacent single axon. (x17,480)

ci: cilium
dv: digestive vacuole
m: mesoglea
n: nerve axon
rc: ring canal
rm: radial muscle

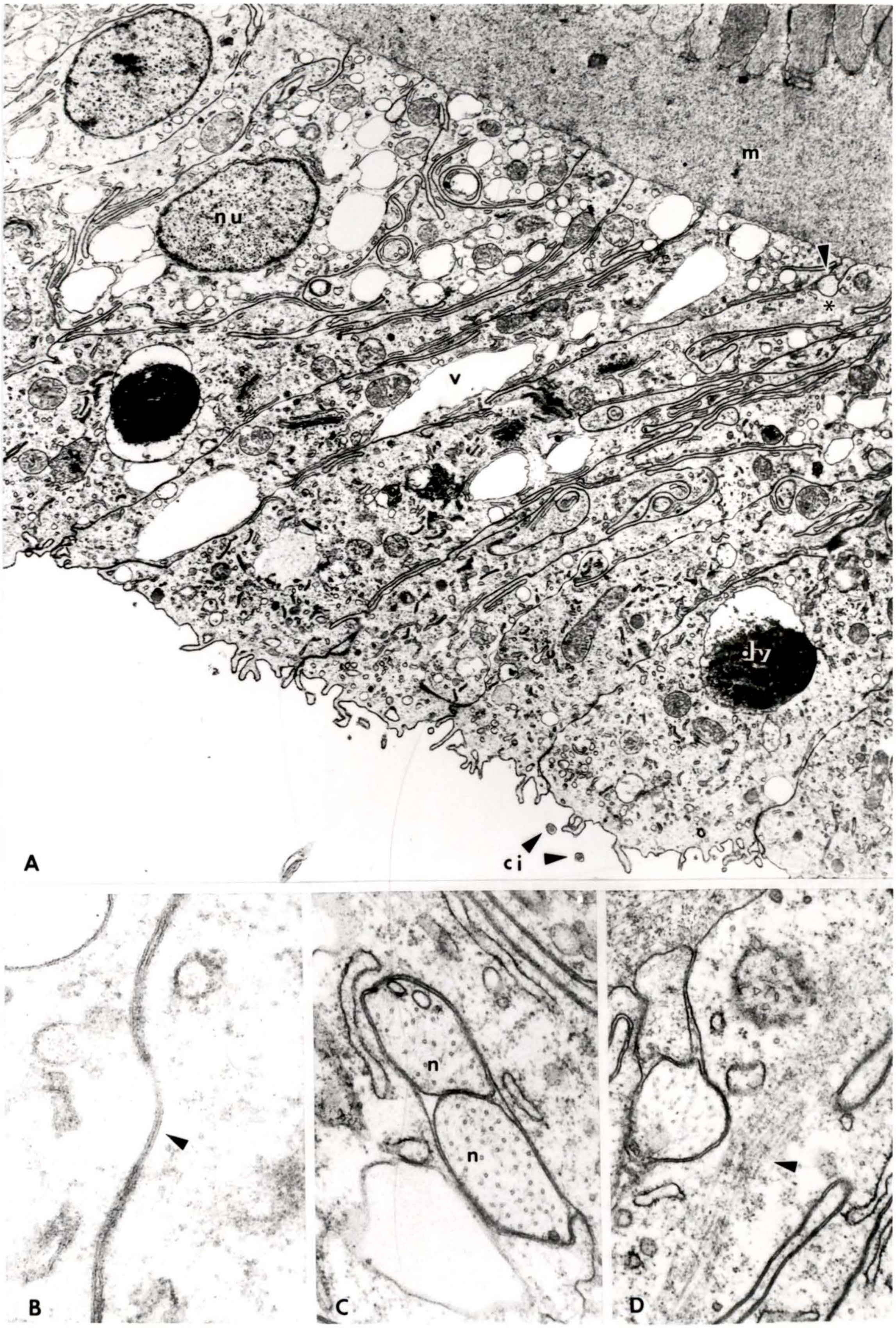
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Figure 54: Inter-cellular junctions in the inner endoderm of *G. vertens* (TEM).

A) An apical septate desmosome. Intercellular septa and interdigitating cellular processes as described by King & Spencer (1980) and Hand & Gobel (1972) are present. Note also the adjacent gap junction (x41,880).

B) An *en face* view of a lanthanum-impregnated septate desmosome. Septa, appearing as clear lines (Hand & Gobel, 1972), are interspersed with regions of lanthanum-filled intercellular space (x86,590).

C) A transverse section of a lanthanum-impregnated gap junction. The junction appears periodic, as described by King & Spencer (1980) for *Polyorchis penicillatus*. Electron-lucent intercellular bridges (arrows) alternate with portions of lanthanum-filled intercellular space. Intercellular channels are visible as thin opaque lines in the center of some electron lucent bridges (inset) (x211,870; inset x333,120).

D) An array of hexagonal junctional complexes in an *en face* section of a gap junction (x153,688).

gj: gap junction
ip: interdigitating process

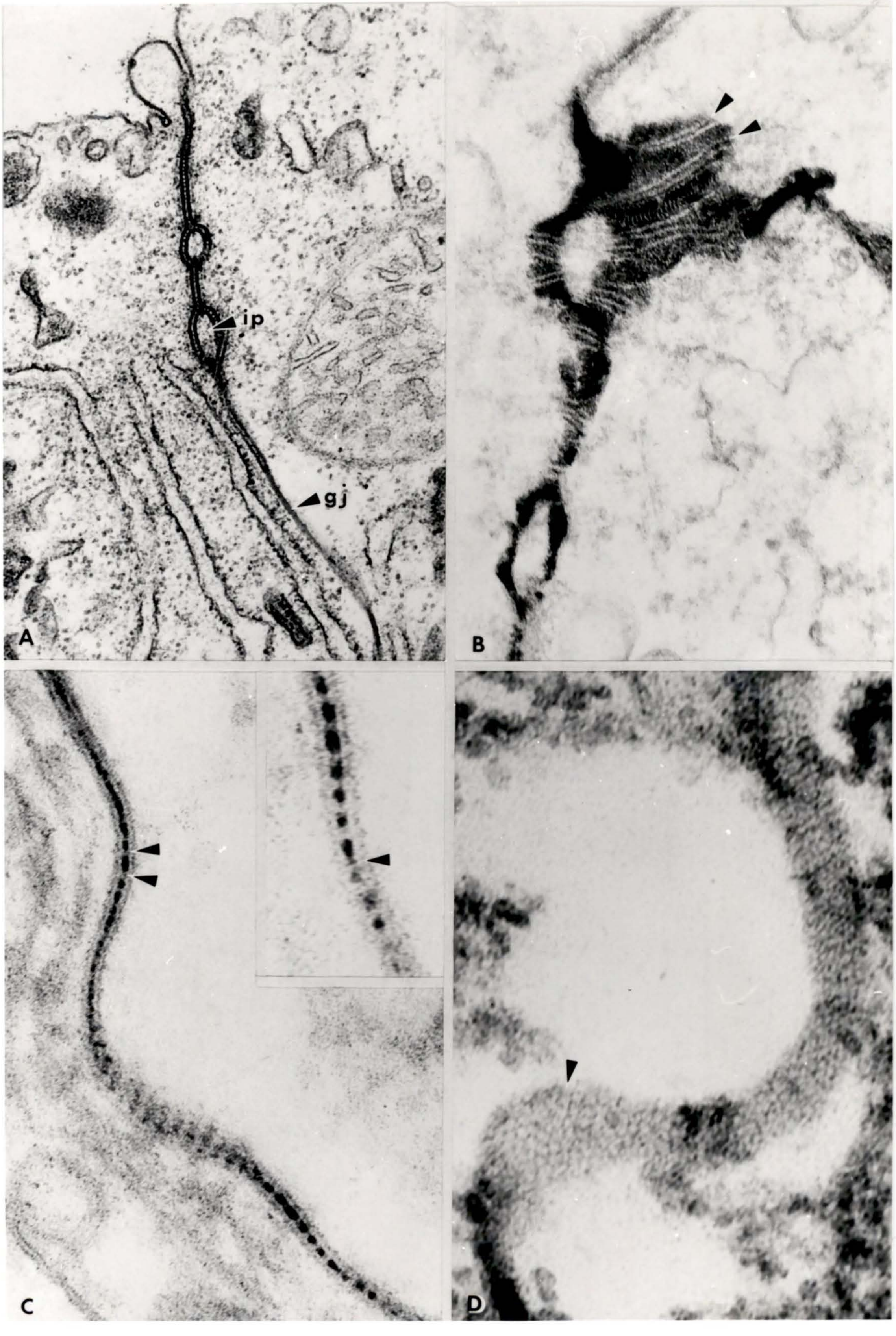
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Figure 55: Nerves in the inner endoderm of *G. vertens* (TEM).

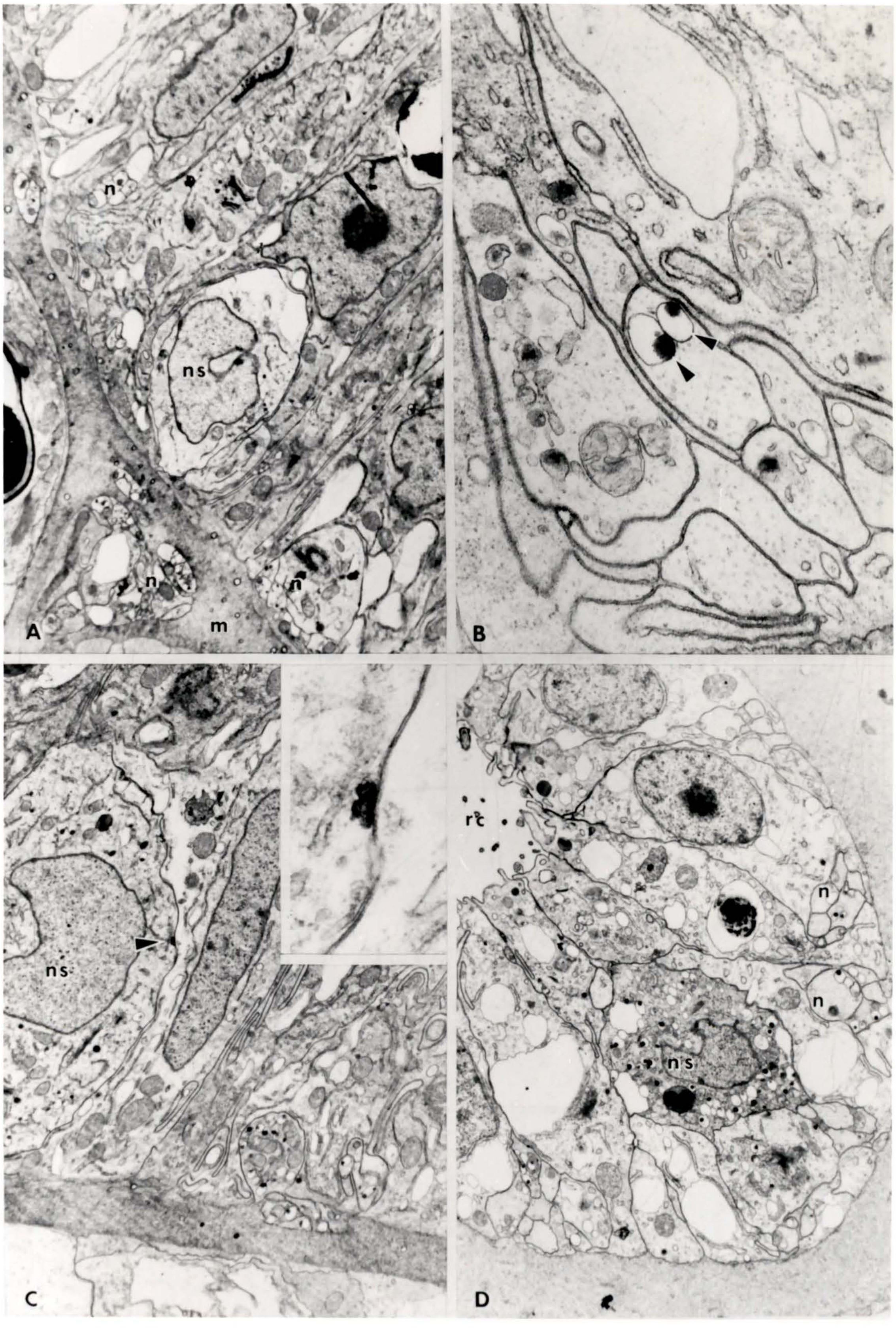
A) Nerve bundles at, and in, the tri-radius of the bell margin. Note also the neuron soma (x10,824).

B) A "typical" nerve bundle of the ring canal. Note the characteristic vesicles with excentric concretions (arrow). (x25,200)

C) A synapse between a ring canal neuronal soma and an adjacent epithelial cell (arrow & inset). (x6,080; inset x34,300)

D) Nerve bundles in a radial canal. Note the neuron soma. (x6,200)

m: mesoglea
n: nerve axons & bundles
ns: neuron soma
rc: radial canal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rc
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D

Figure 56: The outer endoderm of *G. vertens* (TEM).

A) The outer endoderm of the ring canal. Cilia, microvilli, gap junctions (arrows), apical septate desmosomes, as well as cell inclusions comparable to those of the inner endoderm are present (x13,224).

B) Septate desmosomes and interdigitating processes are present at the apices of outer endoderm cells (x57,340).

C) A gap junction (arrow) in the area of fig. 24(a) (x59,530).

D) Putative myofilaments (arrow) in the base of a radial canal outer endoderm cell (x59,780).

ci:	cilium
dv:	digestive vacuole
ip:	interdigitating process
m:	mesoglea
rc:	ring canal or radial canal
v:	vacuole

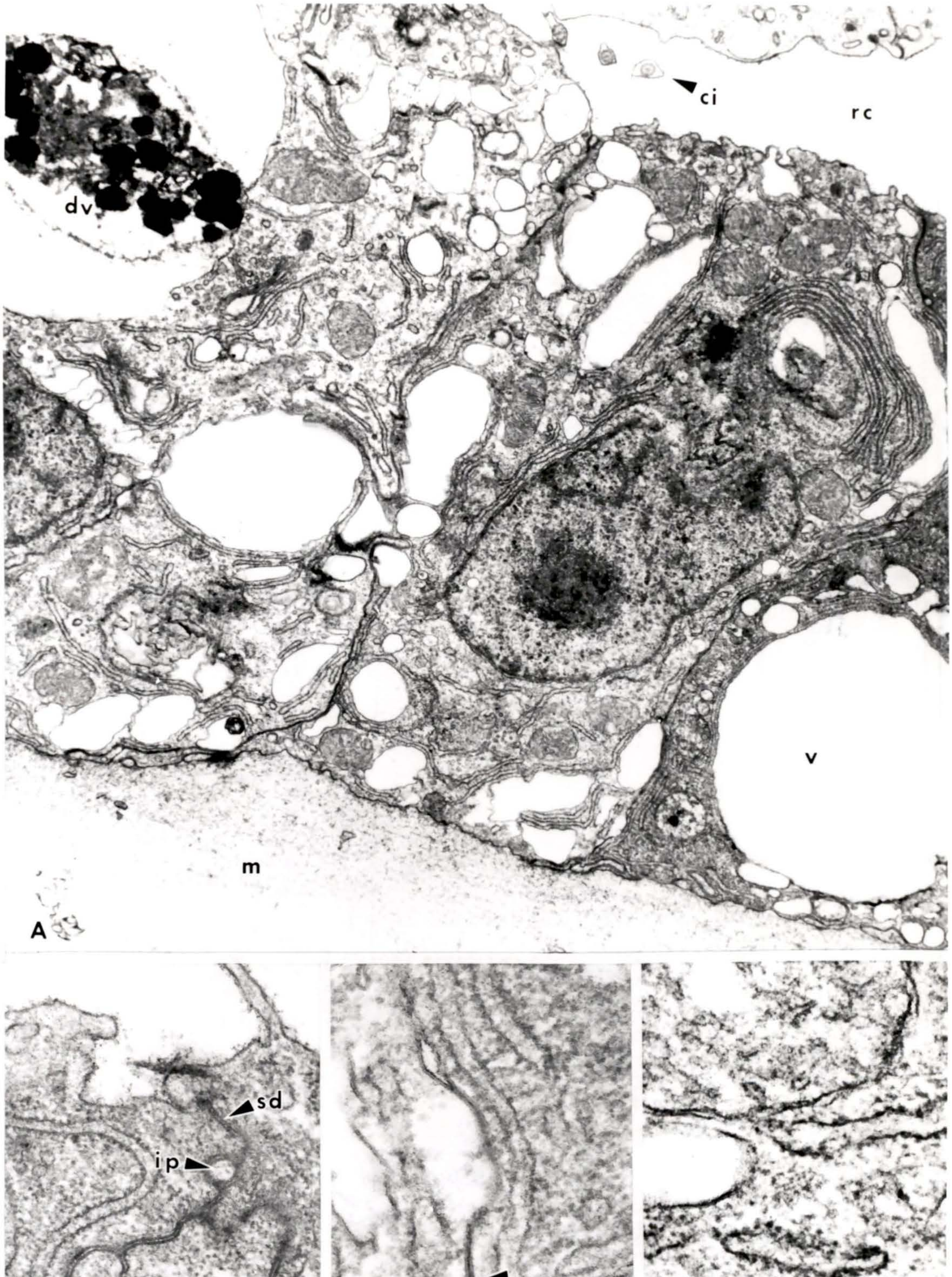
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Figure 57: The subumbrellar lamella of *G.vertens* (TEM).

A) Cellular inclusions, other than the absence of digestive vacuoles, are comparable to those of other endoderm tissues of the bell. Trans-mesogleal cellular bridges to the ectoderm are present (arrow) (x14,770).

B) Cell junctions within the subumbrellar lamella. Both extensive gap junctions and septate desmosomes are present (55,490).

C) A putative nerve axon in the subumbrellar lamella in the vicinity of the junction to a radial canal (see text). Note the vesicles with excentric cores (arrows) (x22,110).

cb:	cellular bridge
gj:	gap junction
m:	mesoglea
n:	nerve axon
sd:	septate desmosome
sl:	subumbrellar lamella
sw:	ectodermal swimming myocyte

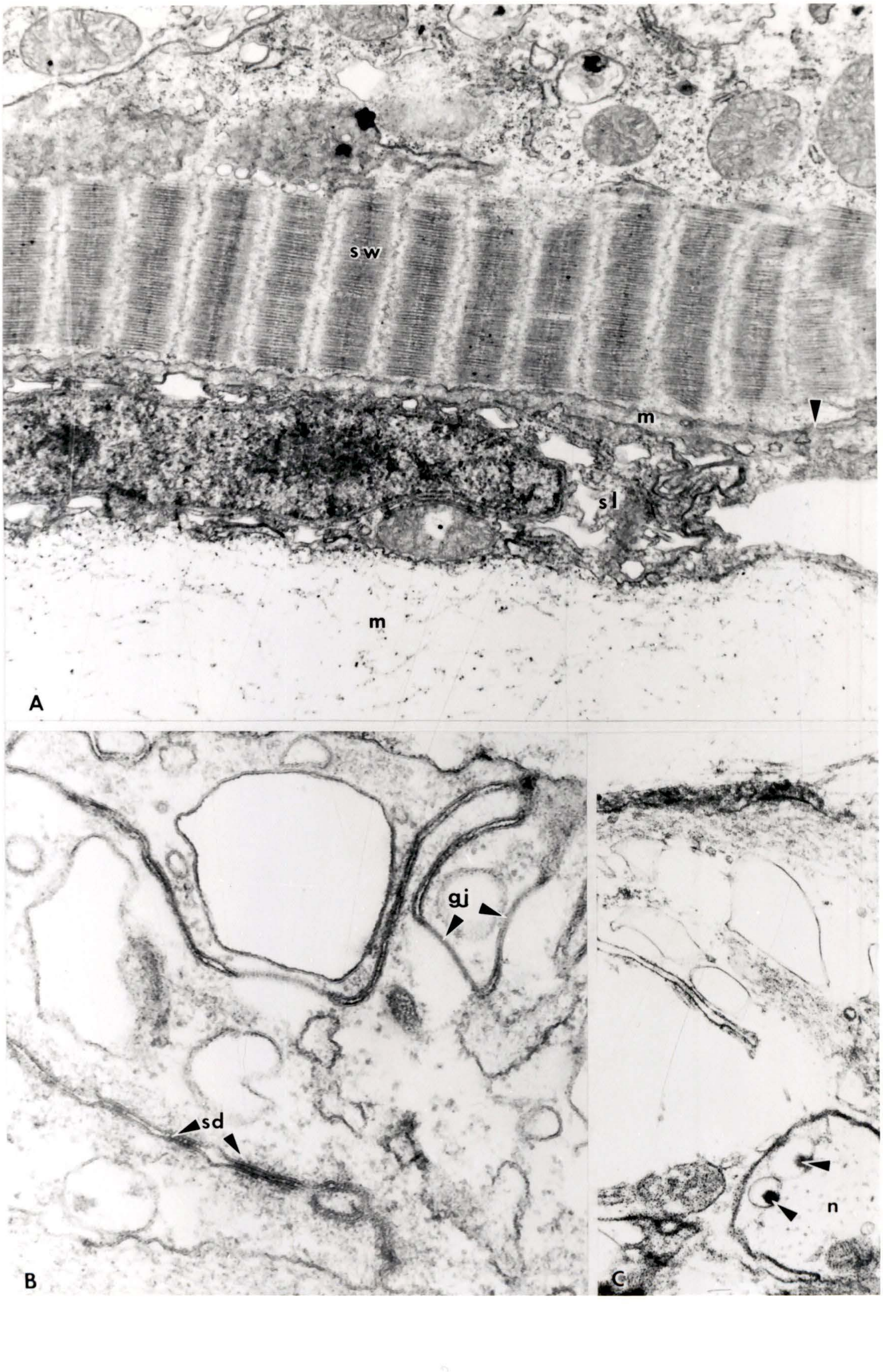
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Figure 58: The junction of inner and outer endoderms in the ring canal of *G. vertens* (TEM).

A) The two endoderm types appear of quite different density. A deep intercellular cleft containing mucous and cilia are present at their junction (asterisk) (x8,437).

B) The cleft of fig.28a. Note the cilium (arrow), dense intercellular substance, and reflexed microvilli (x33,932).

C) A gap junction (arrow) between inner and outer endoderm cells adjacent to the intercellular cleft and apical junction (x81,180).

ci: cilium
cl: cleft
ie: inner endoderm
oe: outer endoderm
rc: ring canal

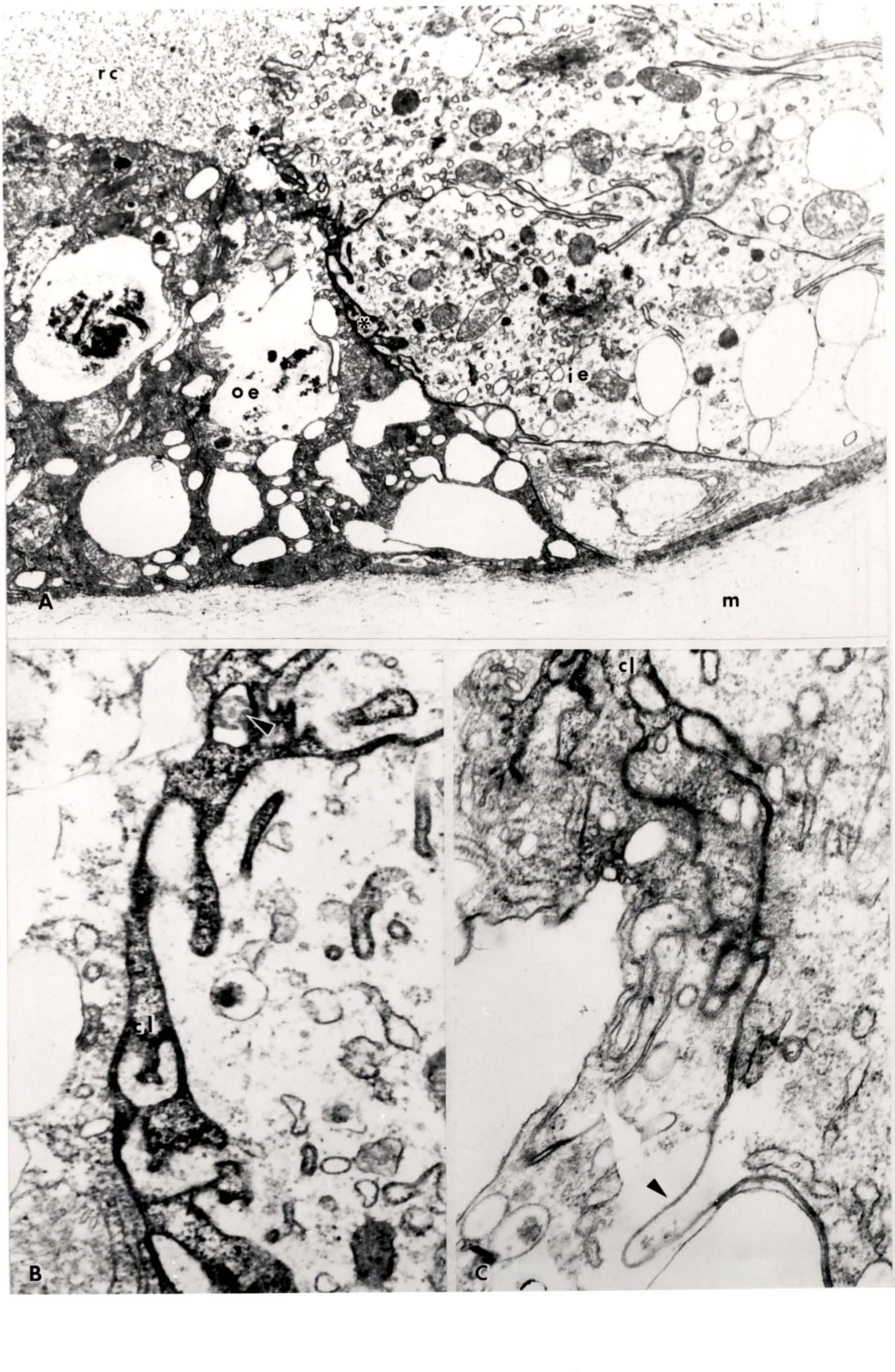
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Figure 59: The junction of the subumbrellar lamella to radial canal endoderm in *G. vertens* (TEM).

A) A low magnification micrograph of the junctional region. Note the mesogleal processes, vacuolation, and putative myofilaments (arrow & inset) (x14,051; inset x69,190).

B) Gap junctions to the ring canal endoderm from the endoderm are present (arrows and inset). Note also the extensive hemi-desmosomes formed by subumbrellar lamella cells to the mesoglea (x14,640; inset x49,250).

gj: gap junction
hd: hemi-desmosome
m: mesoglea
rc: radial canal

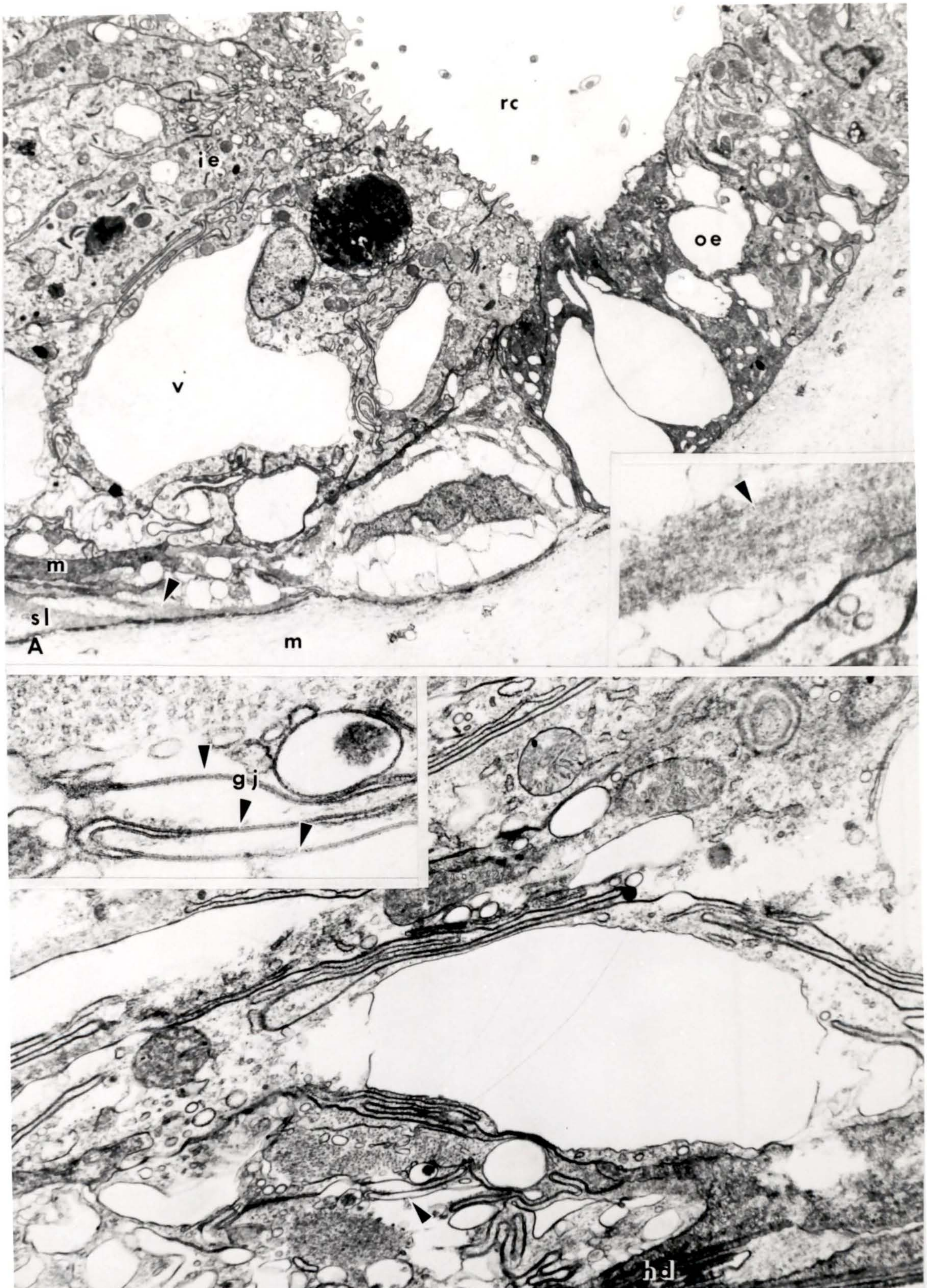
rc
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Figure 60: The junction of the cellular bridge to the exumbrella with ring canal endoderm in *G. vertens*. (TEM).

A) The junction between the cellular bridge and ring canal endoderm. In the absence of statocysts, the origin of the cellular bridge coincides with the junction of inner and outer endoderm (arrow). A mesogleal spur, around which runs the cellular bridge, extends from the bell margin to this point (x2,585).

B) An endodermal cell giving rise to the cellular bridge. Gap junctions (arrows) are present both to the outer and inner endoderm cells (x25,977).

cb: cellular bridge
ie: inner endoderm
m: mesoglea
ms: mesogleal spur
oe: outer endoderm
rc: ring canal

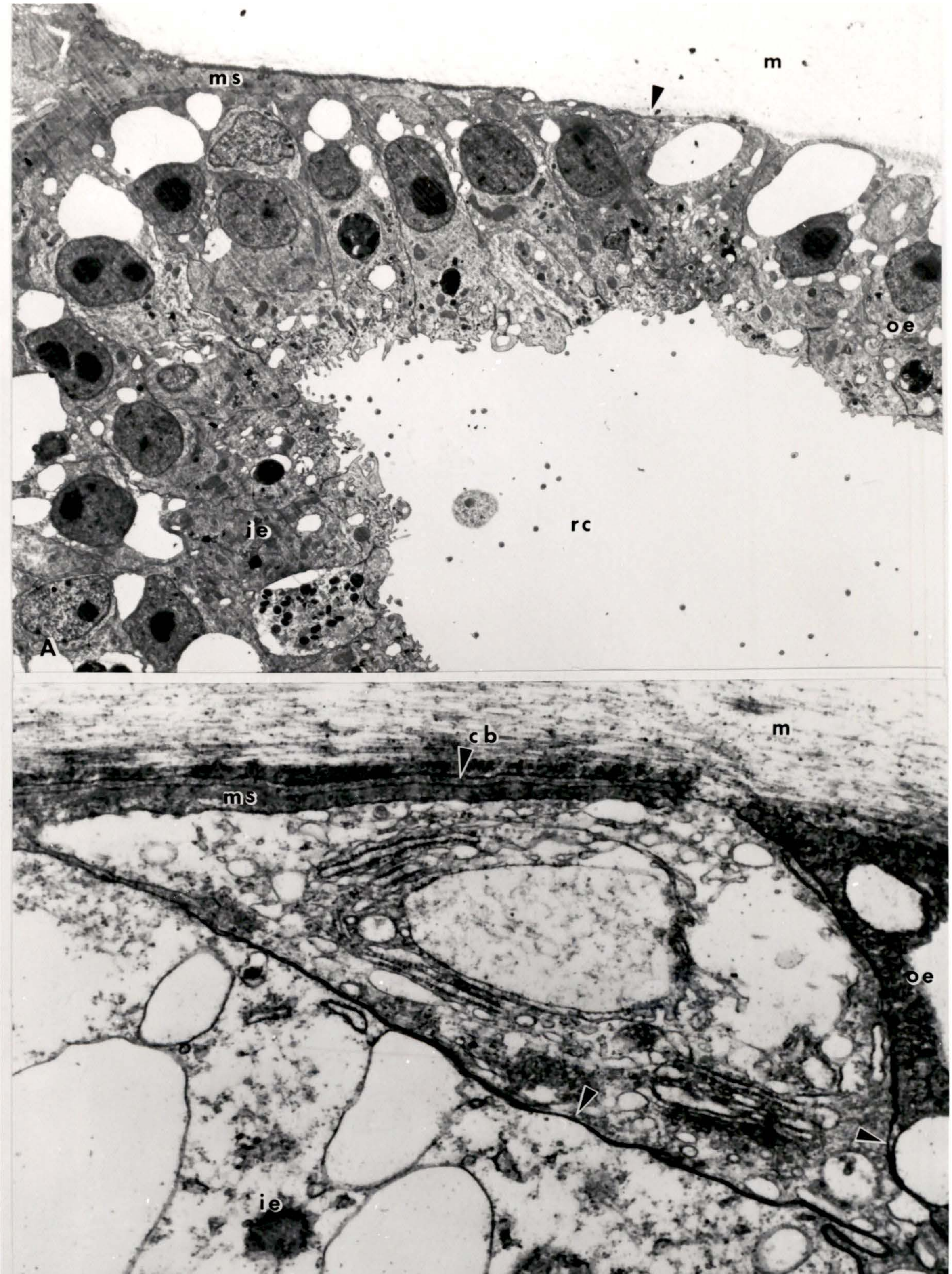
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Figure 61: The exumbrella of *G. vertens* (TEM).

A) A low magnification micrograph showing microvilli, mucous investment, interdigitation between cells, a microtubular column, surface granules, and vacuoles (x8,364).

B) An apical junction between exumbrellar cells. Distinct septate desmosomes are not present (x49,250).

C) A gap junction between exumbrellar cells close to their bases (x73,870).

D) A nematocyst in the exumbrella. Note the regularly reflexed interdigitation with an adjacent cell (arrow) (x4,550).

E) The reflexed interdigitation of fig.31d. Intercellular material appears similar to that seen in many apical junctions (arrow) (x112,300).

id:	interdigitation
mt:	microtubules
mu:	glycocalyx
ne:	nematocyst
sg:	surface granule
v:	vacuole

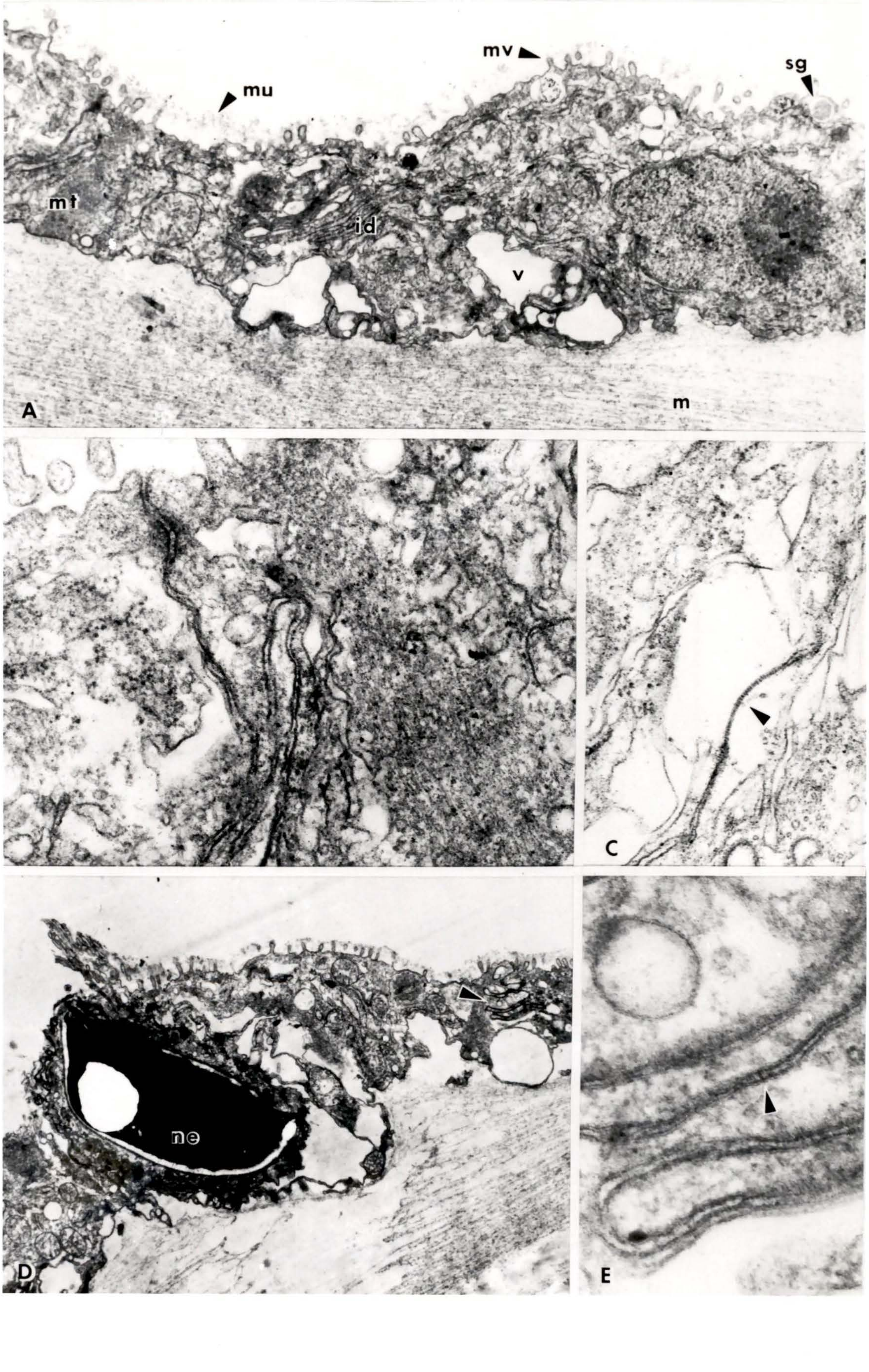
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Figure 62: Gonad tissue of *G. vertens* (TEM).

A) The gonad surface. Cilia with sub-terminal swellings (arrows) are present (x4,910).

B) The junction between a radial muscle and gametogenic tissue. Gap junctions are present in this region (arrow and inset). Note also the adjacent endodermal nerve axons (arrow) (x9,815; inset x78,523).

C) Gap junctions amongst gametogenic cells (x147,750).

D) A trans-mesogleal process between gonad ectoderm and endoderm (x52,250).

ci: cilium
g: gametogenic tissue
gj: gap junction
n: nerve axon
rm: radial myocyte

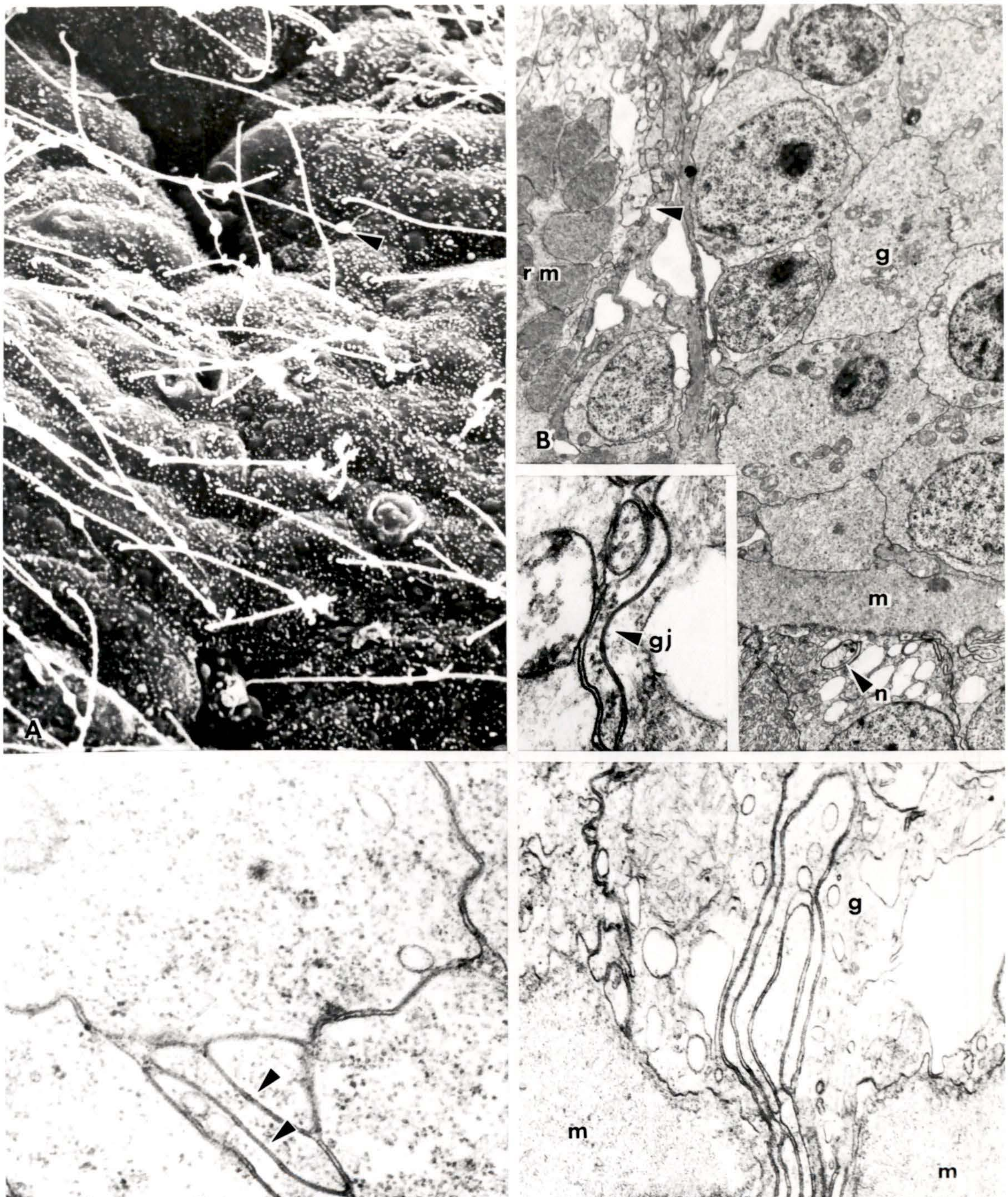
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Figure 63: Sphincter and swimming muscles of *E. typus* (A: SEM, B, C & D: TEM).

A) The radial muscle cord approaching the bell margin. Note that termination of the cord occurs short of the bell margin itself, indicating the presence of a wider sphincter muscle than is present in *G. vertens* (compare fig.6) (x180).

B) A radial section of sphincter myocytes. Note the epithelial sheath and nerve bundles (arrows) (x3,480).

C) The apposition of sphincter and swimming muscle. An intervening non-muscular epithelium appears to be present (x1,830).

D) A nerve axon among swimming myocytes. Note also that the myofilament complement of the swimming muscle is far less well developed than that of *G. vertens* (compare fig.10) (x9,790).

es: epithelial sheath
m: mesoglea
mf: myofilaments
n: nerve
rm: radial muscle cord
sph: sphincter muscle
sw: swimming muscle
vel: velum

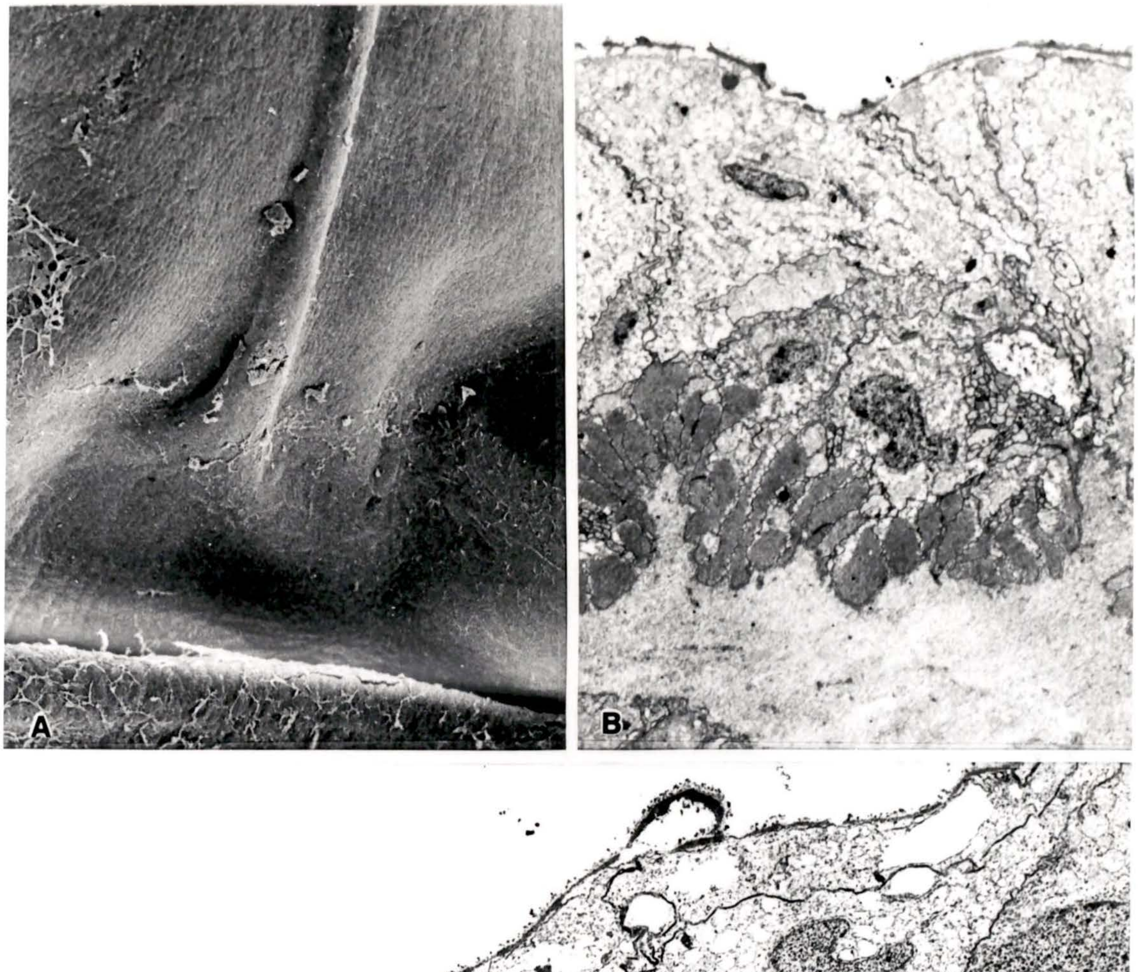
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Figure 64: An oblique section of a tentacle wall of *E. typus*. Longitudinal smooth muscle and nerve bundles are present in the ectoderm. A pronounced basal mass is also present in the endoderm (TEM, x4,670).

ect: ectoderm
end: endoderm
m: mesoglea
mf: myofilaments
n: nerves

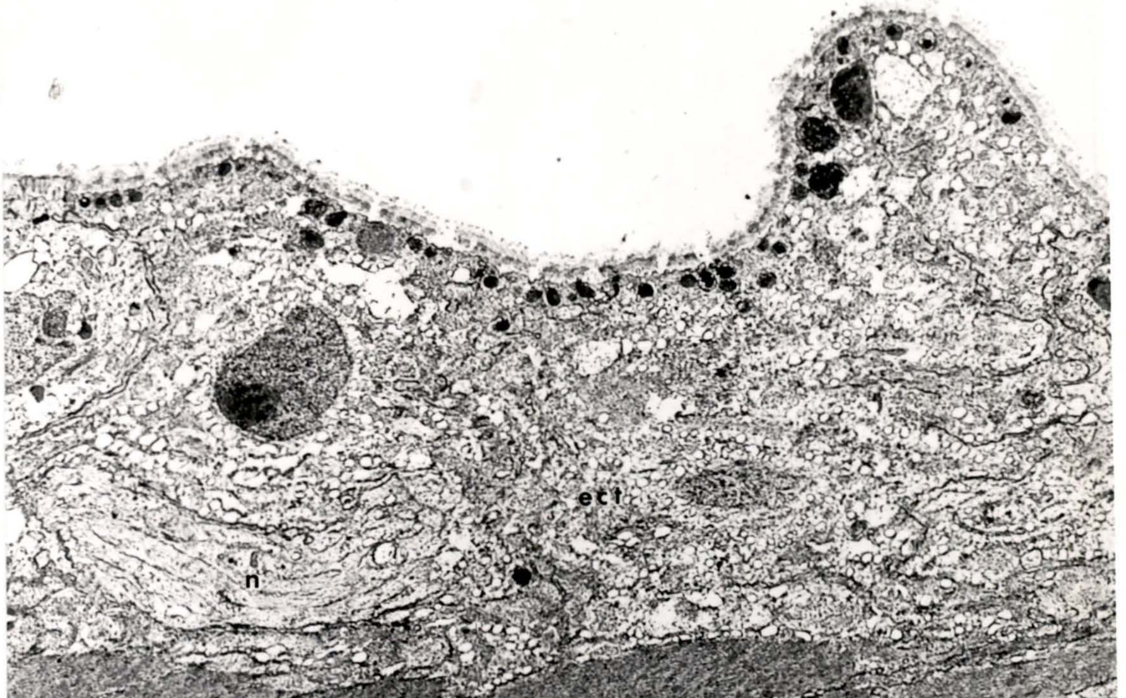
ect
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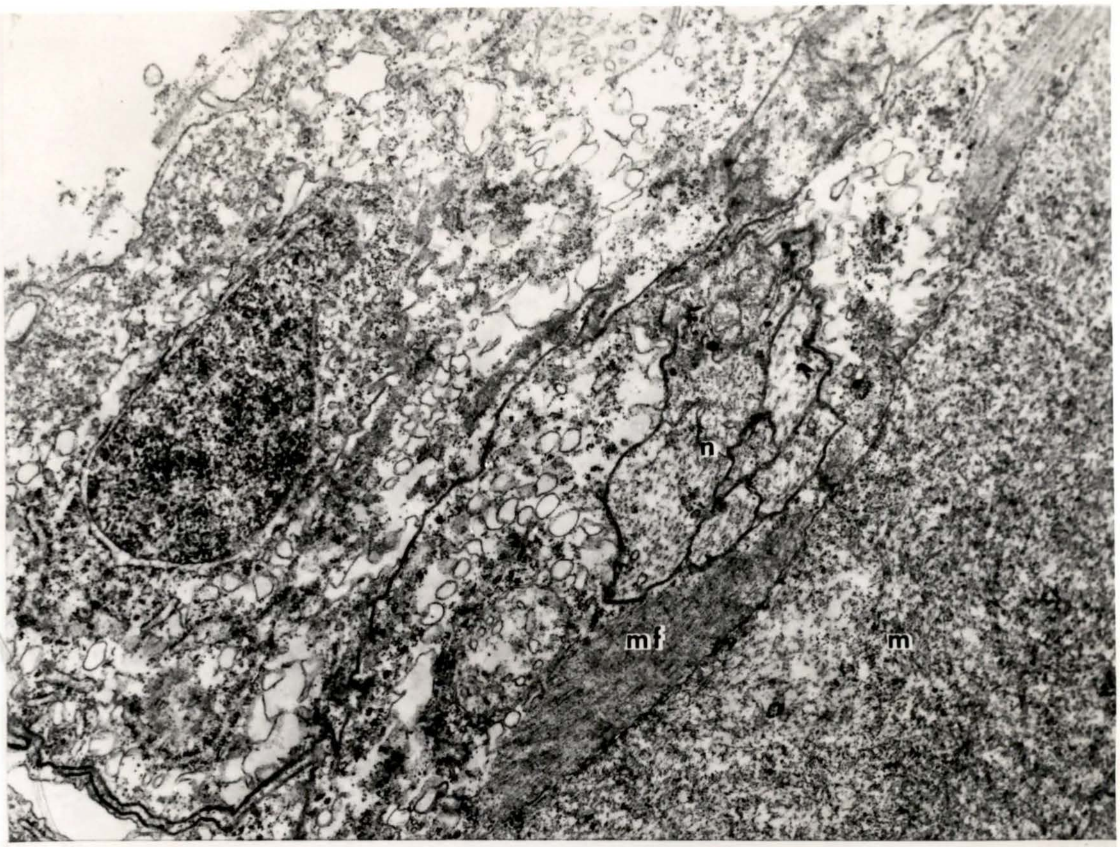
Figure 65: Radial sections of the velum of *E. typus* (TEM).

A) Nerve bundles and smooth, radially oriented muscle are present on the exumbrellar side (x15,645).

B) The subumbrellar side contains nerve bundles and circular striated muculature (x9,126).

m: mesoglea
mf: myofilaments
n: nerve bundle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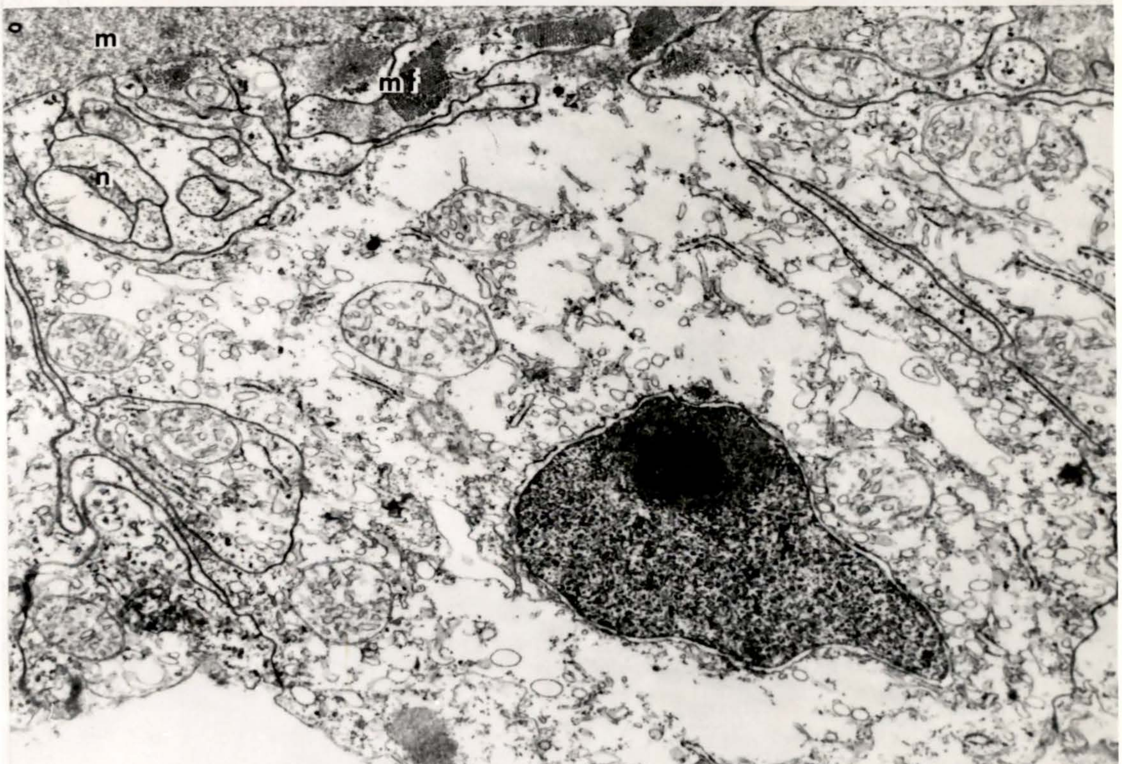
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Figure 66: The bell margin and inner nerve ring of *E. typus* (A: light micrograph, B: TEM).

A) Note the large "swimming motor giants" dispersed adjacent to the velum base (arrows), the epithelial sheath over inner nerve ring and adjacent sphincter muscle, the outer nerve ring, the ciliated chamber, the statocyst, and emerging tentacle canal (x1,640).

B) The inner nerve ring. Note again the epithelial sheath with numerous processes amongst inner nerve ring neurons. Bundles of smaller diameter axons are also present (arrows), and a pronounced bundle of endodermal nerves is present in the tri-radius mesoglea (x3,360).

C)Cell processes between the "swimming giants" containing axial cores of microfilaments (x5,820).

ch: ciliated chamber
en: endodermal nerves
es: epithelial sheath
inr: inner nerve ring
onr: outer nerve ring
sg: swimming motor giants
sph: sphincter muscle
ten: tentacle canal
v: velum

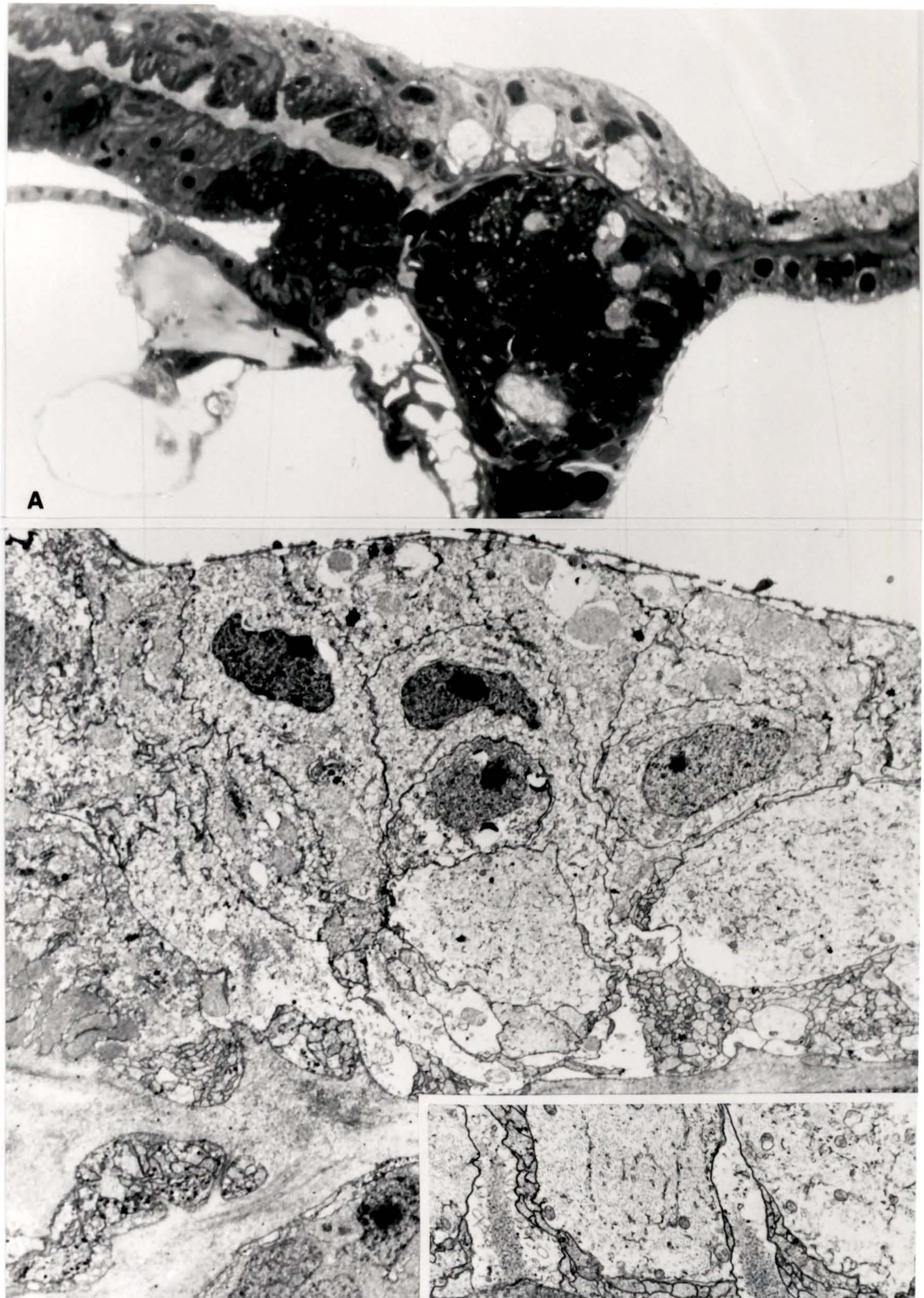
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Figure 67: The exumbrellar bell margin of *E. typus* (A & B: TEM, C: light micrograph).

A) The outer nerve ring is made up primarily of small diameter axons with a few larger axons close to the velum base (arrows) (x150). An epithelial sheath with processes amongst the nerve ring neurons (double arrow), supportive cells at the velum base (with hemidesmosomes to the mesoglea(asterisks)) and at the junction to the bell exumbrella (top right-hand corner of plate and fig. 38b), and a unique chamber containing modified cilia (inset) are also present. (x2,530; inset x16,360)

B) The supportive cells at the junction of the bell margin with the bell exumbrella. Note that microfilaments are present in these cells and those at the base of the velum (fig.38a). Note also the start of the cellular bridge to the endoderm. (x16,360)

C) A cross section of the bell margin containing three adjacent ciliated chambers (arrows).

ch: ciliated chamber
ci: cilium
ep: cellular bridge
es: epithelial sheath
ex: bell exumbrella
onr: outer nerve ring
v: velum

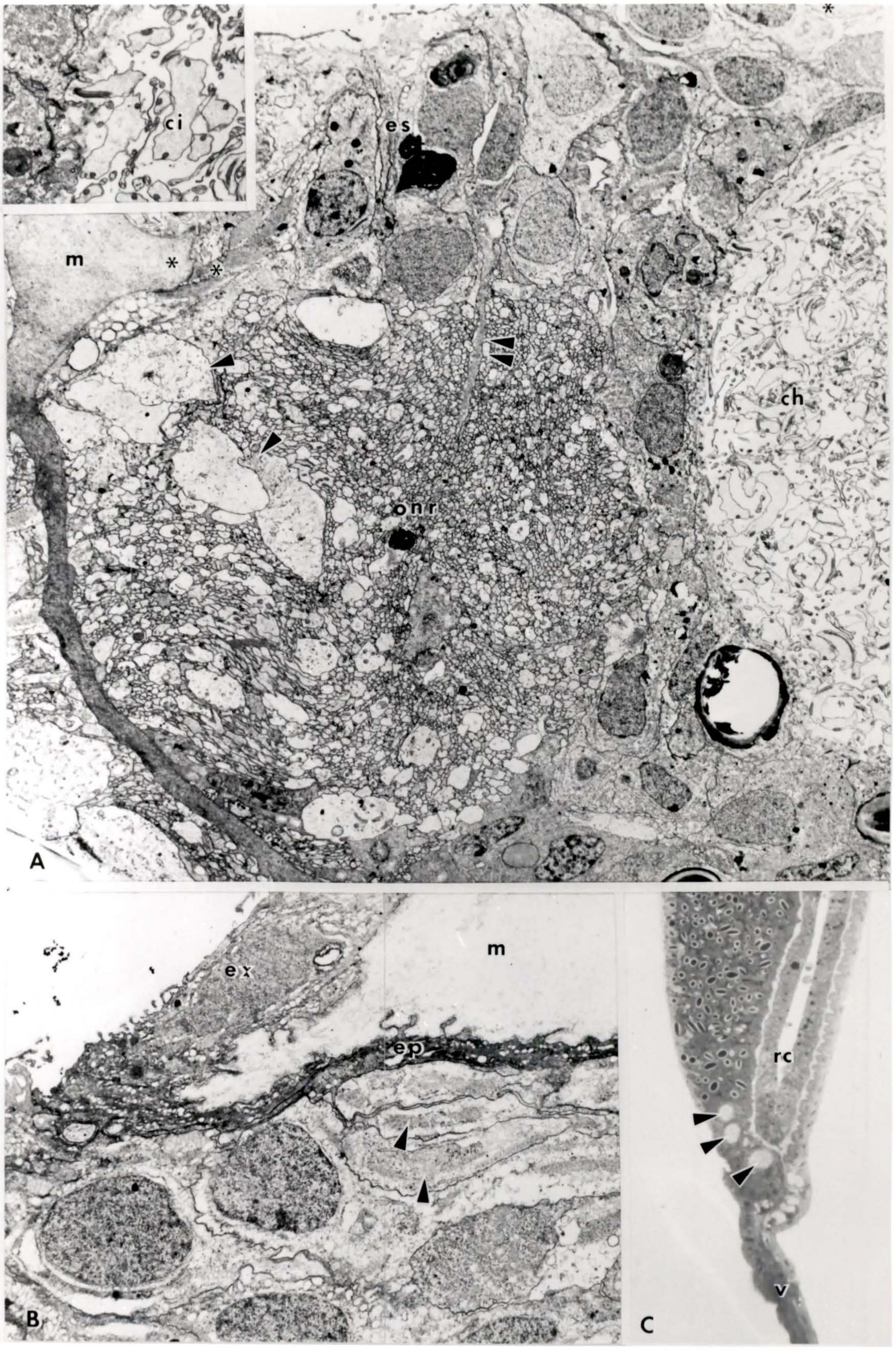
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Figure 68: Communication of the statocyst chamber with the exterior in *E. typus*. Note also the close apposition of the ciliated chamber to the outer nerve ring. (TEM, x3,170)

m: bell mesoglea
ch: ciliated chamber
onr: outer nerve ring
stc: statocyst chamber

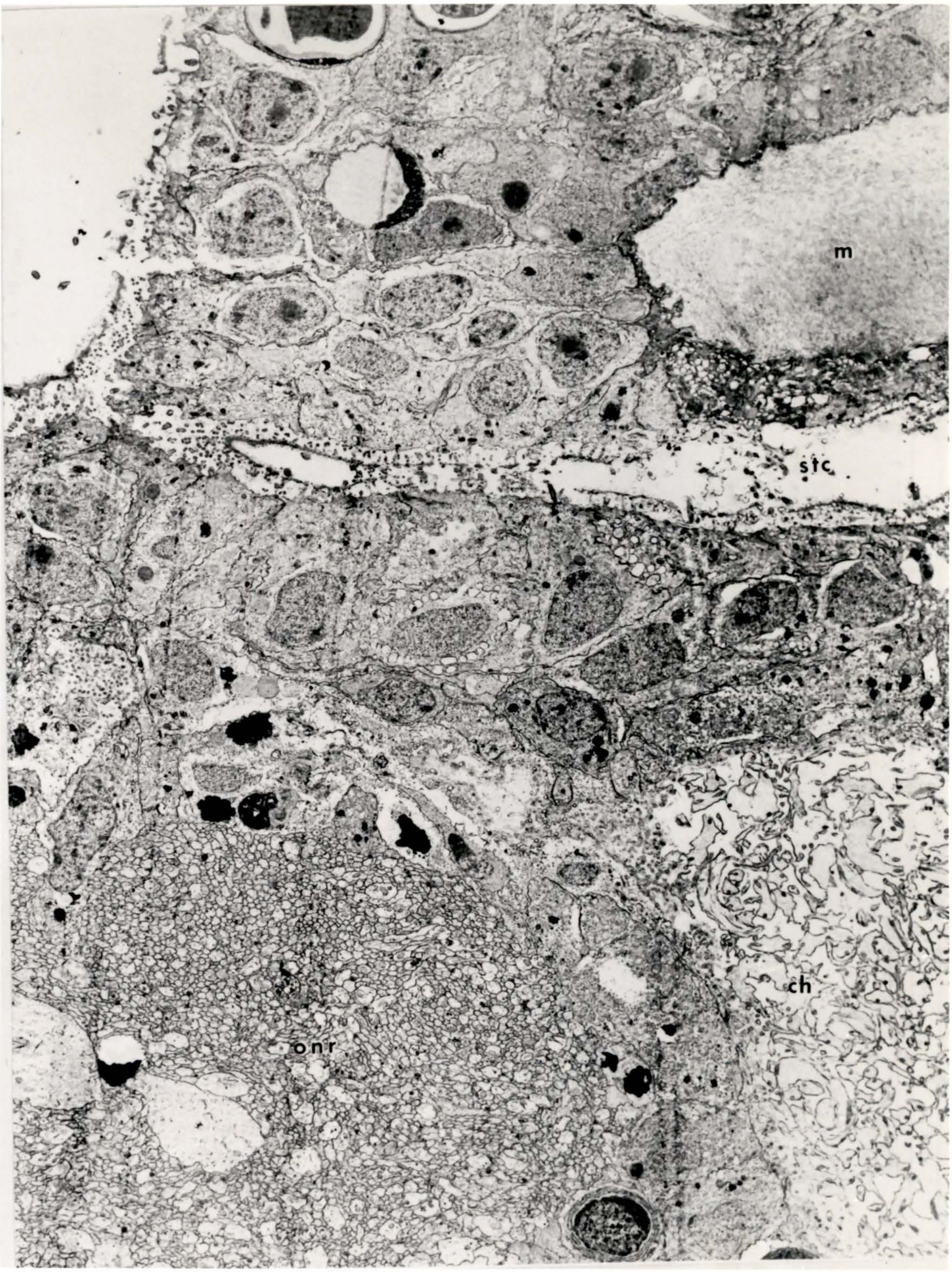
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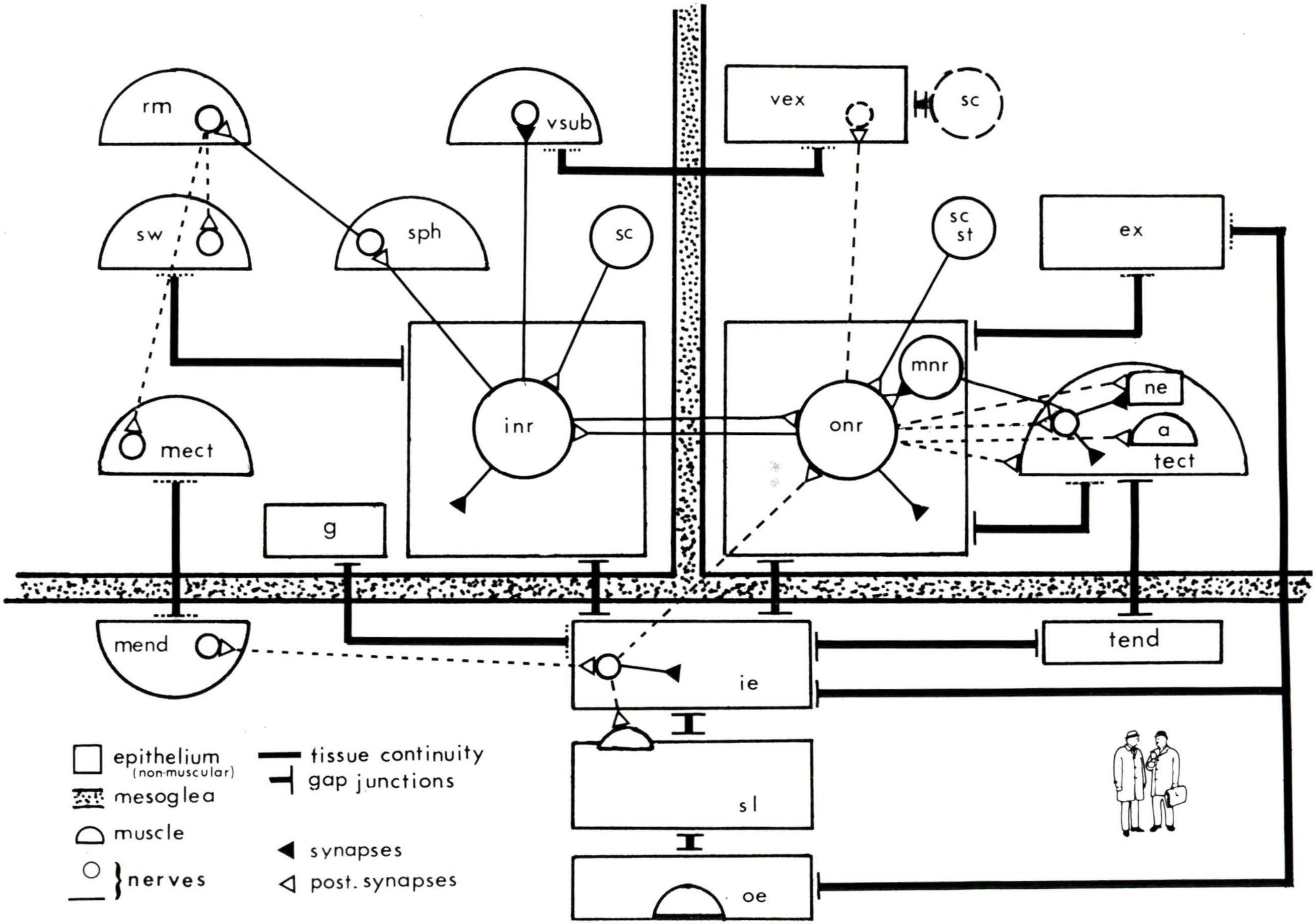
Figure 69: A schematic representation of *G. vertens* showing observed and postulated (post. & dashed lines) epithelial and neuronal connections between tissues. The representation is based on structural observations, hypotheses from behavioural and electrophysiological observations, and on comparisons with other hydromedusae.

a: tentacle anchors
ex: bell exumbrella
g: gonad
ie: inner endoderm
inr: inner nerve ring
mect: manubrium ectoderm
mend: manubrium endoderm
mnr: marginal nerve ring
nc: nematocysts
oe: outer endoderm
onr: outer nerve ring
rm: radial muscle
sc: sensory cells
sl: subumbrellar lamella
sph: sphincter muscle
st: statocyst
sw: swim muscle
tect: tentacle ectoderm
tend: tentacel endoderm
vex: velum exumbrella
vsub: velum subumbrella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mend
ie
tend
sl
oe
epithelium
(non-muscular)
mesoglea
muscle
nerves
tissue continuity
gap junctions
synapses
post. synapses

Discussion

A number of hypotheses for the neurophysiological control of local activities of effectors and behavioural patterns of *G. vertens* and *E. typus* can be made based on my behavioural observations. These are supported to some extent by the morphological and electrophysiological characteristics I observed and by comparisons with other species. My interpretations are as follows:

A) *Local activities*:

1) Nerve tissue with diffuse pacemaker capacity is present throughout the manubrium, radial, sphincter, and tentacle musculature, since in all cases even small pieces of isolated tissue are capable of spontaneous activity and so far only nerve tissue has been shown to be spontaneously active in coelenterates. Similar autonomy of manubrial and tentacle activity has previously been reported in *G. vertens*, by Yerkes (1902). I also observed neurites in all of these tissues in *G. vertens* (fig. 73), and all I examined in *E. typus* and recorded muscle potentials originating from local tissue in tentacles of both *G. vertens* and *E. typus* (figs. 16 & 22) and from the radial muscle of *E. typus* (fig. 13). The cyclical TCP activity during recovery from magnesium anesthesia (fig. 21) and the

cyclical variation in the threshold of tentacle responses to electrical stimulation (figs. 20 & 23) both also suggest some form of pacemaker is present in this tissue.

2) Conduction pathways for local activities are present in the manubrium, radial muscles, sphincter muscle, and tentacles but they differ in the extent to which they spread excitation within each effector and in the extent to which they spread excitation to other effectors. These are characteristics comparable to those of the "pacemaker systems" Josephson (1974b) described in *Tubularia*. My results indicate that the following types of conduction pathways are present in the various effectors and effector combinations:

Through-conduction of conduction of excitation appears to be minimal in the manubrial lips, as indicated by the lack of coordinated activity. The manubrial column, on the other hand, does appear to have some through-conduction in longitudinal tracts, as shown by the rapid flexing in discrete directions. I found no histological evidence, however, of through-conducting pathways or of specialized longitudinal tracts of ectodermal muscle in the manubrium column; nor is electrophysiological evidence for the organization of the manubrium available yet to test these hypotheses. Peristalsis of the manubrium is likely to involve the circular endodermal muscles, perhaps aided by the trans-mesogleal muscular processes, but coordination, if present, was not studied.

The capacity for strictly local activity is present in the radial and sphincter musculature, but it occurs less commonly than coordinated activity of whole "units" of sphincter muscle and radial muscle associated with each of the four radii. A through-conducting pathway thus appears present within these units. Communication between the four units appears minimal, but can be demonstrated by cutting through the sphincter muscle adjacent to a radial muscle; the sphincter muscle remnant then does not act independently, but rather is coordinated with the adjacent unit. I was only able to make recordings from the radial muscle of *E. typus*, but these recordings offer some clues as to the nature of the conduction system within that muscle. As in the tentacles, contraction pulses occur both singly and in bursts, associated with twitches and more extensive contractions respectively. This indicates the same pathways are used for both strictly local activity and for activity of whole radial muscle bands. The bursts of events observed differ from TCP bursts, however, in that rather than facilitating, they decline in amplitude. This "de-facilitation" is also evident in records from radial muscles of *Stomotoca atra* (Mackie, 1975). The phenomenon may indicate that the initial twitch of a contraction (corresponding to the initial event of a burst) is conducted throughout the muscle unit immediately rather than incrementally. In comparison, a tentacle contraction may start in only a portion of the tentacle and entrain neighbouring regions, and the amplitude of the contraction pulses facilitates with repetition (see below). A single conduction system in the radial and sphincter muscles thus appears capable of both limited spread and through-conduction. The conduction system is probably represented by the observed nerves because it is sensitive to excess magnesium ions, there are very few gap junctions between radial myocytes, and nerve tracts are present in the appropriate locations (fig. 38), so the conduction system probably is represented by the observed nerves.

Localized activity is most common in the tentacles, but excitation can spread to neighbouring regions within a single tentacle in an incremental fashion, depending on the strength or duration of stimulation. This type of conduction has been described by Passano (1976) as hydrozoan "incremental" conduction. Some labile blockage of conduction also can occur, however, since portions of a tentacle can contract quite vigorously without entraining adjacent portions. Yerkes' (1902) observations of the behaviour of tentacles of *Gonionemus murbachii* are equivalent to mine, and the behaviour of tentacles of *Aequorea victoria* (Kerfoot, 1980), *Polyorchis penicillatus* (Spencer, 1978), and *Proboscidactyla flavicirrata* (Spencer, 1975) is similar, suggesting that the behavioural control of local tentacle activity is similar in all of these species. The nature of these control systems, however, stills remains to be discovered. My thesis has provided some insights into these mysteries:

Electrical events corresponding to muscular twitches were recorded from the tentacles of both olindiads, and as twitches summed to produce prolonged contractions, bursts of these tentacle contraction pulses (TCP's) occurred. Facilitation in amplitude of the extracellular electrical events occurs during a burst, apparently related to the strength of the twitch and distance it is conducted. Such facilitation of extracellular events appears to be common amongst many coelenterate smooth muscle recordings (Pantin, 1935, 1952; Spencer, 1971; Mackie, 1975; Kerfoot, 1980). Spencer (1971) suggests the size of epithelial pulses reflects the proportion of underlying cells which are active, so it is most likely that facilitation in these tentacles represents activity in a progressively greater proportion of the cellular processes underlying the suction electrode. Two independent TCP rhythms can occur concurrently at single recording sites on tentacles of *G.*

vertens, so variable proportions of muscle fibres could be active at one time in a given cross-section of a tentacle. It remains to be seen if the incremental conduction in *G. vertens* and *E. typus* tentacles is mediated by facilitaion of neuromuscular junctions, greater activity in single cells, or by neuroid conduction. It also remains to be seen how such conduction can, at times, be blocked to produce more localized contractions.

The fast tentacle contraction pulses (fTCP's) of *G. vertens* correspond to the largest observed twitches of the tentacles, but are limited to individual tentacles or even portions of individual tentacles, as is other local activity. It thus is most likely that fTCP's are singly occuring TCP's of maximal size. Somehow the TCP conduction system must also be capable of such rapid, immediate, through-conduction and excitation.

Corkscrew contractions of the tentacles of *G. vertens* appear to be a a general response to mild stimulation rather than a feeding-specific reaction as suggested by Yerkes (1902) and reported for some other species (Kerfoot, 1980; Nägel, 1894). Different portions of a tentacle cross-section appear capable of independent activity, as discussed above, so it is likely that a subtle feature of the organization or excitation of the tentacle muscle produces the corkscrew contractions. No morphological or electrophysiological evidence, however, is yet available to test this hypothesis.

Conduction of the contraction pulses is sensitive to excess magnesium ions and occasional pre-pulses can be observed, so the nerves I have seen in the tentacles no doubt represent the conduction system. In contrast, Ohtsu (1980) suggests that the TCP's of *Spirocodon salatrix* are conducted too slowly to be neuronally mediated.

3) An active role of nerves in the propogation of the contraction pulse in the swimming musculature of the olindiads is suggested by its sensitivity to excess magnesium ions in behavioural experiments. Circularly oriented nerves in the swimming muscle of *G. vertens* have been observed both with methylene blue staining (Hyde, 1902), and with electron microscopy (fig. 42), and the rapid, magnesium-sensitive conduction of the swimming pulse in the circular direction, indicates that these nerves are indeed active in propogating the swimming contraction. The lack of autonomous spontaneity and the incapacity for strictly local activity would indicate, however, that unlike the nerve tissue in other musculature of these species, these nerves lack pacemaker capacity and represent only through-conducting pathways. The occasional multiple

swimming contractions in response to single stimuli of an emarginate *G. vertens* can probably be attributed to the duration of the stimuli and consequent multiple excitation, rather than indicating a capacity for spontaneous activity, as has been suggested previously (Yerkes, 1902; Wolfe, 1928).

The capacity to conduct around wounds, the ability to reestablish effective conduction across a wound within 3 hrs, and the insensitivity of radial conduction of the swimming pulse to excess magnesium ions all suggest that myoid conduction is also present within the muscle sheet. Gap junctions, correlated with neuroid conduction in other animals (Mackie, *et al.*, 1984), are present between the swimming myocytes, as would be expected. In comparison, excitation of the swimming muscle in most Anthomedusae is spread by myoid conduction alone throughout the swimming bell (Satterlie & Spencer, 1982), while in *Aequorea victoria* (Kerfoot, 1980) and *Aglantha digitale* (G.O.Mackie & C.H.Singla, pers.comm.) nervous conduction is implicated as well, as in *G. vertens* and *E. typus*. The innervated swimming muscle appears to be a primitive condition in hydromedusae, superseded by the anthomedusan nerve-free muscle sheet, as evidenced by a "remnant" neuron network in the bell margin of *Polyorchis penicillatus* postsynaptic to swimming "giants" (Spencer & Satterlie, 1983). The organization of these olindiads may represent an intermediate condition in which the muscle sheet is no longer capable of local responses, as it is in Leptomedusae (Kerfoot, 1980), but still makes use of neuronal conduction.

As in other hydromedusae, the velum of the olindiads appears to involve more complex control mechanisms than those of the subumbrellar swimming muscle. Distribution of the swimming contraction in the velum of *G. vertens* also appears mediated by a nerve net, as indicated by the pSP's, the sensitivity to magnesium ions, and the ultrastructural identification of nerve processes. No evidence of myoid conduction was found, but the exceptionally slow radial conduction observed in isolated vela (presumably significant in ensuring coincident contraction of the swimming bell and velum) suggests that some specialized radial conduction mechanism is present.

In addition to swimming activity, smaller conducted events are present in the vela of both species. Mackie (1975) correlated small potentials in the velum of *Stomotoca atra*, comparable to those elicited by stimulation of the bell margin in *G. vertens*, with radial fiber contractions. Asymmetric contractions of velar radial fibres have been implicated in turning of a number of other anthomedusae (Gladfelter, 1972) and in *Geryonia proboscidactyla* (Horridge, 1955a). In the olindiads, however, the velum appears not to be significant in turning (fig. 10) and in *G. vertens* velar radial fibres appear absent. In these species the larger of the velar events appears to be related to tentacle pulses (TP's), and in *E. typus* also at times to radial twitches, but their functional signifigance is not clear. In both species nerves or other potentially conducting tissues are present in these areas.

B) Behavioural patterns:

1) Crumpling:

In other hydromedusae, including the only other limnomedusan investigated, *Proboscidactyla flavicirrata* (Spencer, 1975), crumpling has been considered intimately associated with excitable epithelia. As reported earlier by Yerkes (1902), both crumpling and excitability of the exumbrella are absent in *G. vertens*, and my results suggest that endodermal bell tissues are also inexcitable. Thus, upon initial inspection, the correlation between excitable epithelia and crumpling seems borne out in *G. vertens*. In *E. typus*, however, where the same tissues appear to be inexcitable as in *G. vertens*, crumpling is present. Local activity in appropriate effectors is present in *G.vertens*, even in reponse to stimuli comparable to those effective in producing crumpling in *E. typus*, but coordinated activity, all-or-none sustained contraction, and an associated inhibition of swimming are absent. The crumple of *E. typus* depends on the integrity of the bell margin, is an all-or-none response to single stimuli (as is the neuronally-enhanced crumple of *Sarsia tubulosa* (Mackie, 1975)), and is sensitive to excess magnesium ions: all evidence that this crumple is entirely neuronally mediated. By analogy then, the lack of crumpling in *G. vertens* may be attributed to the absence of an appropriate neuronal mechanism rather than the lack of excitability in the exumbrella and endoderm.

Spencer & Schwab (1982) suggest that pre-tentacle pulses (pTP's) are involved in the mediation of crumpling in *Sarsia tubulosa* and *Polyorchis penicillatus*, but in *E. typus*, even though tentacle pulses (TP's) are coincident with the initiation of crumpling, they do not appear sufficient to produce the full crumpling response (fig. 28), nor do they have the same long-lasting effect on tentacles as a crumple does. Stimulation of the bell margin of *G. vertens* at sites which elicit crumpling in *E. typus* also elicit TP's in this species, suggesting that the

TP pathway may be superimposed on a crumpling pathway in the olindiads but is not directly involved in the behaviour. Overall activity levels of tentacle, swimming, radial, and sphincter muscles can also be enhanced by stimulation of the bell margin of *G. vertens*, but the mechanism involved has so far remained hidden. It is not clear if this mechanism is related to the control mechanism of crumpling in *E. typus*, but it does appear an "elusive" neuronal crumpling coordinating system is missing or poorly developed in *G. vertens*.

The lack of excitability in the exumbrella and endoderm of the olindiads does not appear to be of signifigance to the crumpling behaviour. Conducting epithelia have, however, been quite important in recent theories for the evolution of nervous systems (Spencer & Schwab, 1982; Mackie, 1970), so it is of interest why tissues that are excitable in other species are not so in these olindiads. The characters most likely to be associated with excitability are membrane properties, which I did not investigate. One could also, however, hope to detect a gross morphological character in the exumbrella or endoderm associated with their "sensory" capacity and King & Spencer (1980) have suggested the distribution of gap junctions and septate junctions in excitable epithelia is determined by their electrotonic function. I investigated these characters but was not able to detect any criteria by which excitability could be distinguished, as outlined below.

The exumbrella of *G. vertens* appears structurally similar to the excitable exumbrella of *Euphysa pacifica* (Josephson & Schwab, 1979) and the endoderm is also similar to that seen in other hydromedusae. I found distinct inner and outer endoderm types in *G. vertens* and *E. typus* which have not been reported before for other hydromedusae, but such a distinction appears likely in at least *P. penicillatus* (M.G.King, pers.comm.) and *Aequorea victoria* (Kerfoot, 1980) as well. The endoderm types are different on the basis of cytoplasmic ground substance, stature, innervation, and putative musculature, but until more work is done on other species the signifigance of these differences will remain unclear.

The distribution of intercellular junctions I have found in *G. vertens*, as summarised in Table 1, suggests that gap junctions are present in all tissues, albeit at variable densities. The distribution of these gap junctions within the tissues also appears different, to some extent, to that reported by other workers. In *P. penicillatus* (Spencer, 1979) and *Stomotoca atra* (Mackie & Singla, 1975) gap junctions are situated primarily amongst the myoid portions of the swimming myocytes, while in *G. vertens* more are

Table 1: Distribution of intercellular junctions in the tissues of *G. vertens*.

	GAP JUNCTIONS[1]	APICAL JUNCTIONS[2]	DESMOSOMES	NERVE TISSUES		
				axons	somata	synapses[3]
MUSCLES						
MANUBRIUM						
ectoderm	*	*	+	+	*	*
endoderm	*	*	+	+	*	*
RADIAL MUSCLE	present	C	+	+	-	n-n
SPHINCTER MUSCLE	rare	-	+	+	-	-
SWIM MUSCLE	common	D	+	+	+	-
TENTACLE	common					
ectoderm	common	D	+	+	-	n-n,n-m,n-nem[4]
endoderm	common	C	-	-	-	-
VELUM						
exumbrella	common	C	-	putative	-	-
subumbrella	common	C	+	+	-	-
OTHER						
BELL ENDODERM						
inner endoderm	abundant	A,B	-	+	+	n-e
outer endoderm	abundant	A	-	-	-	-
subumbrellar lamella	abundant	A,B	+	putative	-	-
BELL MARGIN						
exumbrella	common	A,B	-	+	+	n-n,n-e
subumbrella	present	-	-	+	+	n-e[5]
cellular bridge	*	-	-	-	-	-
BELL EXUMBRELLA	common	A,B,C	-	-	-	-
GONAD	common	*	-	-	-	-

[1] gap junctions per cell profile in thin sections
abundant = 3.0
common = 0.5 - 3.0
present = 0.1 - 0.5
rare = 0.1

[2] A = septate desmosomes encircling cytoplasmic strands (fig. 23)
B = septate desmosomes without cytoplasmic strands
C = apical junctions with only occasional septa
D = apical junctions without visible septa

[3] n-n = neuro-neuronal
n-m = neuro-muscular
n-e = neuro-epithelial

[4] Westfall, 1970

[5] Satterlie & Spencer, 1982

\+ present
\- not found
* insufficient data

present in the somatic portions; and gap junctions in the endodermal canals of *P. penicillatus* are concentrated amongst the basal portions of the cells (King & Spencer, 1980), while in *G. vertens* they appear to be distributed evenly. King & Spencer (1980) suggest the position of gap junctions in *P. penicillatus* endoderm serves to enhance the conductile capacity of the tissue. It seems unlikely, however, that such a factor would render the tissues of *G. vertens* inexcitable, since in both the excitable exumbrella of *Euphysa japonica* (Josephson & Schwab, 1979) and the excitable endoderm of *Stomotoca atra* (C.L.Singla, pers.comm.) gap junctions adjacent to apical junctions are common.

Gap junctions have been found in *G. vertens* at most of the tissue appositions expected of hydromedusae with excitable epithelia (fig. 41). I predict that with further scrutiny gap junctions could also be found in the remaining regions: i.e., within the marginal trans-mesogleal bridge between the radial muscles and endoderm, and between swimming myocytes and the epithelial sheath of the inner nerve ring. Electrical coupling between all of these tissues thus appears feasible on a morphological basis.

Comparison with other hydromedusae (Table 2 & 3) indicates that the gap junctions present in the endoderm of

G. vertens are also structurally similar to those found in excitable coelenterate tissue and to those found in other animals, though some differences may be present. The intercellular gap in non-junctional membrane regions in *G. vertens* appears narrower (Table 2) than reported or observed in other hydromedusae and the gap of gap junctions and the width of lanthanum-impregnated gap junctions appear large (Table 3). The present survey technique, however, has not been sufficiently rigorous to substantiate differences of the observed magnitude and Staehelin (1974) has suggested that the width of lanthanum impregnation in gap junctions may vary considerably. The 8.5 nm junctional intercellular space reported for gap junctions in the excitable exumbrella of *P. penicillatus* (Singla, 1978) indicates that junctional gaps of the width found in *G. vertens* should not preclude excitability.

In general, it appears that neither gap junction distribution nor structure in *G. vertens* tissues provide clues to the nature of the excitablility in other epithelia.

King & Spencer (1980) also suggest that septate desmosomes may enhance the conductile capacity of a tissue layer by increasing the apical resistance of intercellular contact. The excitable exumbrella of *Euphysa japonica*,

Table 2: Comparisons of widths of gap junctions and non-junctional membrane regions in G. vertens with values taken from the literature.

	NORMAL MEMBRANE APPOSITION WIDTH (nm)		GAP JUNCTION WIDTH (nm)	
	total	gap	total	gap
Gonionemus[1] (present work)	33.7±1.8	10.1±0.6	21.2±1.1	5.1±0.9
Gonionemus tentacle (Westfall, 1970)	33*	17.5*	-	-
Stomotoca endoderm (Mackie & Singla, 1975)	34*	11*	22*	-
Eutonina exumbrella (Josephson & Schwab, 1979)	46*	19*	19* (15)	5* (5.5)
Polyorchis endoderm	30*	13*	20*	5.6*
exumbrella (Singla, 1978)	-	-	-	8.5
Polyorchis endoderm (Spencer, 1979)	35*	12*	17*	5*
Polyorchis endoderm (King & Spencer, 1980)	-	-	15-17	4-5
Hydra (Hand & Gobel, 1972)	38*	19*	22*	5* (3-4)
hydromedusae (Satterlie & Spencer, 1982)	-	20-25	-	3-5
animals (Staehelin, 1974)	32-42	20-30	13-19	2-3

[1] averaged over all tissues (n=30)

* estimated from published micrographs

however, lacks specialized apical junctions all together (Josephson & Schwab, 1979), and the the septate desmosomes of *G. vertens* are distributed in a fashion that could be attributed to functions of of cell adhesion, support, and/or metabolic "sealing" requirements (Staehelin, 1974) rather than an electrophysiological function, but still have a similar distribution to that found in *P. penicillatus* (Spencer, 1979; King & Spencer, 1980). Other apical junctions in *G. vertens* generally also contain some form of intercellular deposition, at times even in a septate arrangement less pronounced than that in septate desmosomes (Table 1), so septate junctions may represent just one end of a continuum of apical junction structure rather than being an isolated phenomenon. In any case, it does not seem likely that neurophysiological characteristics of *G. vertens* can be related to septate desmosome distribution.

As I suggested earlier, excitability in these hydromedusan epithelia seems to depend on structures or physiology which I was not able to distinguish in my work. The characteristics of intercellular junctions which I studied most likely are associated with other functions, such as metabolic transfer (Loewenstein, 1981) or cellular support or sealing (Staehelin, 1974). The lack of discrete sensory structures in an excitable exumbrella has been

Table 3: Comparison of lanthanum-impregnated gap junctions of *G. vertens* with those in the literature.

Table 3: Structural characteristics of lanthanum- impregnated endodermal junctions of G. vertens as compared to relevant values reported in the literature.

	TOTAL WIDTH (nm)	"GAP" WIDTH (nm)	LUCENT BRIDGE WIDTH (nm)	'PORE' DIAMETER (nm)	HEXAGONAL UNIT PACKING (nm) (center-center)
Gonionemus (present work)	23.5	11.1	7.6-9.8	2.3-2.9	9.8-10.8
Polyorchis (King & Spencer, 1979)	16-18	7.5-8.5	6.0-7.5	2	10.0-11.5
Hydra (Hand & Gobel, 1972)	22.4	7.5	-	2	9.5-11.0
animals (Staehelin, 1974)	13-19	6-7	-	1.5	9-10

reported earlier (Josephson & Schwab, 1979), but I can now say that other gross distinguishing structures of excitability are also absent.

The question still remains, however, whether the inexcitability of the epithelia observed in *G. vertens* and *E. typus* is a primitive or derived condition in hydromedusan evolution. This is of interest for three reasons: 1) the observed distribution of intercellular junctions in the olindiads may have been set in the past in epithelia that were excitable, 2) insight into the control mechanisms of crumpling may be provided by such knowledge, and 3) since anthozoans and scyphozoans seem capable of meatbolic transfer in the absence of gap junctions (Mackie *et al.*, 1984) the original function of the junctions may have been something other than metabolic transfer. Taxonomic relationships within hydromedusan groups are inadequately known (Russell, 1953) to be useful in answering this question, so as an alternative I have considered the adaptive signifigance of inexcitability. To *G. vertens* an excitable exumbrella and crumpling could well be detrimental, since in its benthic habitat it would potentially be continually crumpled. The sympatric anthomedusan *Cladonema californicum* does, however, possess an excitable exumbrella and does crumple (pers. obs.), though it could be argued that this medusa's smaller size would reduce the selective pressure against crumpling. No selective advantage to an inexcitable exumbrella in *E. typus* can, however, be deduced on the basis of present knowledge of its habits and no evidence is available to suggest this species has historically gone through a benthic stage comparable to that of *G. vertens*. The most parsimonious interpretation with the present evidence is, then, that the inexcitable exumbrella (and endoderm?) of the two olindiads is a primitive condition. This in turn suggests that: 1) the distribution of intercellular junctions reflects mostly functions other than electrophysiological ones; 2) contrary to current thought (Mackie, 1975; Spencer & Schwab, 1982) the neuronal component of crumpling is the primitive control mechanism, while the purely neuroid mediation of the response observed in *Stomotoca atra* (Mackie, 1975) is a secondary development (anthomedusae appear to have specialized in the development of neuroid and myoid conduction in nerve-free epithelia both for crumpling and swimming, which is reflected in theories of hydromedusan neuroethology (Spencer & Schwab, 1982)); and 3) gap junctions appear to have evolved "for" functions other than electrical conduction.

2) *Feeding*:

The observations on feeding in *G. vertens* indicates the presence of a "pointing" excitation mechanism from the tentacles to the sphincter and radial muscles and the manubrium. Such a mechanism has also been suggested for *Stomotoca atra* (Mackie, 1975) and *Proboscidactyla flavicirrata* (Spencer, 1975), and has been demonstrated in *Aequorea victoria* (Kerfoot, 1980). A pronounced latency between stimulation and excitation and between tentacle activity and radial muscle activity is typical in all these species, a delay Mackie (1975) suggested may be attributable to activation of local pacemakers. Activity of the muscular effectors during feeding in *G. vertens* is comparable to local activities observed at other times, and the electrophysiological responses in tentacles recieving food stimuli in both olindiads appears to be responses by the local TCP systems only (fig. 29). As suggested by Mackie (1975) for *Stomotoca atra*, pointing in these species appears to utilize local action systems coordinated by a pointing excitation mechanism as well. The "activation" delay in *G. vertens*, however, is minimized by stimulation with food extracts, suggesting that in this species at least part of the delay is regulated by sensory input. Distinctive "feeding potentials" have been recorded in tentacles of *Aequorea victoria* (Kerfoot, 1980), *Proboscidactyla flavicirrata* (Spencer, 1971), and *Sarsia tubulosa* (Passano *et al*., 1967

Hirnandez-Nicaise & Passano, 1967), but were not recorded in these olindiads, and no other events associated with the coordination of pointing could be recorded, so again the presence of a hidden coordinating mechanism is indicated.

The swimming component of feeding in *G. vertens*, also reported by Yerkes (1902), appears to be elicited independently of the pointing response, since isolating the stimulation from the bell margin can evoke pointing alone. Inhibition of swimming by the pointing response as reported for *Aequorea forskalea* (Horridge, 1955b), may, in fact, also be present in *G. vertens*, since the swimming response appears to be greater when pointing is prevented by cutting the appropriate radius.

Coordination of the "synchronous" radial muscle contractions observed during feeding requires further work, but it, too, appears to be independent of pointing. It is tempting to consider this behaviour a modified crumple, but the lack of absolute synchrony is unusual. Alternatively it could be induced by an "activation" mechanism and/or levels of central "excitation" in the rest of the animal, as reflected by the increased frequency of coordinated tentacle contractions. The polyp of *G. vertens* has also been shown to become more active in response to food extracts (Perkins, 1903) and the tentacles of *Sarsia* *tubulosa* show a feeding-specific change in tonus in response to food extract (in this case a greater extension) (Henschel, 1935) suggesting coordinated "excitation" levels may be of importance to feeding in other hydromedusae as well.

3) *Somersault*:

My results indicate that inversion during somersaulting results from a purely physical interaction of the bell with the water surface, as suggested earlier by Morse (1906), since an asymmetric contraction of the swimming muscle hypothesised by Yerkes (1902) and Perkins (1908) was not observed (fig. 4). A swimming inhibition during the latter part of somersaulting appears to be induced by the inversion process, as shown by the behaviour of tethered individuals (fig. 5). Murbach (1903) and Thomas (1921) investigated the statocysts of *Gonionemus murbachii*, but were not able to distinguish a clear function, and in *Aglantha digitale* (Mackie, 1980) and *Aequorea victoria* (Singla, 1972) statocysts have been demonstrated to effect turning and righting respectively, but it is not clear if these functions are performed through an inhibitory influence. Statocysts are nevertheless the most likely candidates for eliciting the inhibition during somersaulting of *G. vertens*, but I did not test this.

4) *Swimming*:

Swimming in *G. vertens* and *E. typus* appears to be controlled by neuronal entities in the bell margin, as in other hydromedusae (Spencer & Schwab, 1982), since swimming spontaneity is lost by removal of the bell margin and is sensitive to excess magnesium ions. The giant axons I observed in the inner nerve ring (fig. 51) are probably responsible for this in the olindiads as in other hydromedusae, since events comparable to the pre-swim pulses (pSP's), considered to indicate activity in these neurons, can also be recorded from the bell margin of *G. vertens* (fig. 14). From these motor "giants", excitation probably spreads into the swimming muscle, through the subumbrellar nerve net described by Hyde (1902) and by myoid conduction, as discussed earlier. Radial nerves of the radii may provide connections between the inner nerve ring and the subumbrellar nerve net, as indicated by the presence of pSP's at the base of the gonad ridges in *G. vertens* and the small events coincident with swimming in the radial muscle of *E. typus*. The gonad tissue contains numerous gap junctions but appears to be inexcitable and will not conduct the swimming event.

I have found little evidence to suggest the sphincter muscle of the olindiad species contracts during normal swimming, as might have been predicted by its location between the inner nerve ring and the swimming muscle. A delayed or slow relaxation of the bell margin, as would be predicted if the smooth musculature of the sphincter muscle were active during swimming contractions, occurs to some extent in both *G. vertens* and *E. typus*, but is not very pronounced and is not effective in delaying decelleration (figs. 7 & 11). The bell margin tends to relax last during swimming in both *Aequorea victoria* and *P. penicillatus* as well (unpub. obs.), but both lack the subumbrellar sphincter muscle. It is thus likely, though not clearly evident in the cinematographic results, that the sphincter muscle is simply dragged along by the rest of the bell during these swimming contractions and delays in relaxation of the bell margin are more a function of the swimming muscle.

During escape swimming the bell margin of *E. typus* remains contracted between swimming contractions to a far greater extent, with the predicted effect on velocity during swimming (fig. 12). Swim pulses commonly overlap in electrophysiological records (figs. 15 & 25), and the time between the bell contractions during the escape swim is much shorter than in normal swimming. Escape swimming thus probably results from a more rapid swim initiation rate than normal. *Sarsia tubulosa* has been reported to maintain a contracted margin during swimming bouts as well (Leonard, 1980), but lacks a subumbrellar sphincter muscle. It does not seem likely the sphincter muscle is active in escape swimming of *E. typus* either.

In contrast, turning does seem to depend primarily on sphincter muscle contractions in swimming of both *G. vertens* and *E. typus*. Gladfelter (1973) proposed a turning mechanism for *G. vertens* which involves an asymmetric contraction of the velum and the swimming muscle and unequal contraction rates of opposite sides of the swimming bell. My cinematographic records are comparable to Gladfelter's for both *G. vertens* and *E. typus*, but I believe they are misleading. I propose that when the swimming muscle appears to be contracting asymmetrically and at unequal rates, it is actually not contracting at all, but is being dragged along by a local sphincter muscle contraction. As evidence against Gladfelter's proposed scheme, I found that asymmetric contractions of neither the velum nor the swimming muscle are capable of inducing turning in *G. vertens* (fig. 10); and a capacity for unequal contraction rates in different portions of the swimming bell at the same time has never been demonstrated in hydromedusae; and radial velum muscles, which have been implicated in turning of some anthomedusae (Gladfelter, 1973), are absent in *G. vertens*. On the other hand, a simulated tonic contraction of one portion of the sphincter muscle does induce turning; the contractions of the bell margin involved in turning appear to be local and of long time course, which is more typical of the sphincter muscle than of the swimming muscle; and acceleration and turning do not commence in a turn until the time I hypothesise the swimming muscle starts contracting (fig. 9). It is thus most likely that turning depends on activity in the subumbrellar sphincter muscle in these olindiads, even though it is a markedly different turning mechanism than that proposed for other hydromedusae.

Another difference between the organization of the swimming system of the two olindiads and that reported for anthomedusae is the relationship between pre-tentacle pulses (pTP's) and the pre-swimming pulses (pSP's). In the anthomedusae and *Proboscidactyla flavicirrata* it appears pTP's can excite the pSP neurons (Spencer, 1971 Passano, 1975; Anderson & Mackie, 1977; S. Arkett, pers. comm.), but in *G. vertens* as in *Aequorea victoria* (Kerfoot, 1980), the two rhythms appear quite independent (fig. 33), and in *E. typus* pSP's or SP's appear to elicit pTP's rather than the reverse (fig.35). The variability in latency between SP's and pTP's in *E. typus* suggests that this elicitation depends more on a secondary sensory stimulation, as suggested for *Geryonia proboscidactyla* by Horridge (1955a), than on a direct neuronal interaction. pTP's may have an influence on, or reflect, "l'humeur" (Passano *et al.*, 1967) of *G. vertens* and *E. typus* individuals, as suggested by their ubiquitous appearance, but the potential controlling influence on swimming rhthyms attributed to them in *Sarsia tubulosa* by Leonard (1982) does not appear to be present in the olindiads.

5) *Tentacle coordination*:

As in other hydromedusae that have been investigated (as reviewed by Spencer & Schwab, 1982), some coordinated postural control of the tentacles is present in both olindiads and appears to be mediated by a conduction pathway in the bell margin. The coordinated contractions implicated in this postural maintenance are, however, much more frequent in *E. typus* than in *G. vertens*. Gross neuromuscular organization of the tentacles and outer nerve ring appears to be similar in the two species, as indicated by my ultrastructural observations and by staining with a FMRFamide antibody (Mackie & Stell, in prep.), and so provides no indication of possible bases of the behavioural difference. Electrophysiological recordings, on the other hand, have produced some clues.

Coordinated contractions of the tentacles in the olindiads are almost always associated with the presence of tentacle pulses (TP's) (fig. 32) which are through-conducted into all the tentacles via the bell margin, as reported for other hydromedusae (Spencer & Schwab, 1982). The olindiad TP's consist of a pre-tentacle pulse (pTP) followed by a slower time course second event, as reported for at least outer nerve ring recordings from these other hydromedusae (Spencer & Schwab, 1982). Mackie (1975), Spencer (1979), and Ohtsu (1980) have suggested that the pTP represents neuronal activity while the second event represents activity in surrounding epithelial tissues or muscle. The intracellular recording of pTP-related events I made from an epithelial cell of the exumbrellar bell margin of *G. vertens* and the observation of neuro-epithelial synapses in this area suggest the TP of this species has a similar cellular basis in the bell margin. It may be reasonable to extend this interpretation to *E. typus* as well, since both components are present in the TP of the bell margin of this species too.

Greater controversy, however, exists about the identity of the TP within the tentacle. Mackie (1975) and Spencer and Schwab (1982) suggest that TCP's and TP's are identical events other than that the latter are through-conducted while the first are not. For at least *Aequorea victoria* (Kerfoot, 1980) and *Proboscidactyla flavicirrata* (Spencer, 1971), however, there is evidence that the TP (pTP in

Kerfoot's terminology) and the TCP are distinct events. Ohtsu (1980) also reports that four types of pulses occur in the bases of *Spirocodon salatrix* tentacles, one of which is the TCP and another the QSCP, which is comparable to the TP (Ohtsu, 1980). In *G. vertens* the TP and TCP are quite clearly distinct events, as evidenced by their independent rhythms and different shape, conduction velocity, and sensitivity to suction in the suction electrode (fig. 16). Both appear, nevertheless, to be conducted in the same ectodermal tissue, and in *E. typus* the distinction in shape is at times less evident. The TP of *G. vertens* appears sufficient on its own to produce a tentacle contraction when coordinated tentacle contractions occurs (fig. 32), rather than needing to trigger a TCP, as Ohtsu (1980) suggests in *Spirocodon salatrix*. Though less clear, the TP appears to produce contractions in tentacles of *E. typus* on its own as well. I also did not find distinct types of musculature that could be associated with local, as compared to coordinated, activity of the tentacles, and these have never been reported in other hydromedusae. It seems most likely, then, that the distinction between the TP's and TCP's is due to conduction of the neuronal component in separate nerve tracts, as suggested by Kerfoot (1980) for *Aequorea victoria*, but that the second portion of the TP's occurs in the same cells that produce TCP's.

Ohtsu (1980) also reports that the second portion of QSCP's is equivalent to another event which can occur independently.

The behavioural difference in tentacle control of the two olindiads is associated with the absence, in *G. vertens*, of a visible behavioural correlate of a large proportion of TP's in the tentacles. Such "cryptic" TP's are also present in at least *Proboscidactyla flavicirrata* (Spencer, 1971), *Cladonema radiatum*, *Geryonia proboscidactyla*, and *Phialidium hemisphaericum* (Passano, 1965) and *Spirocodon salatrix* (Ohtsu, 1980). Ohtsu (1980) suggests that during "cryptic" TP's events are occurring in the epitheliomuscular cells but do not produce visible contractions. Based on my evidence, this appears to be true for *G. vertens* also, but more work is needed to substantiate this hypothesis and to understand how the elicitation of contractions by TP's is turned on and off.

6) *Tentacle anchoring*:

Anchors of tentacles of *G. vertens* isolated from the bell are no longer functional, and the capacity of tentacles to anchor in a coordinated manner depends on the continuity of the bell margin, so anchoring seems to be under the control of a mechanism mediated by the bell margin. The presence of nerve tissue indicates that this control may be mediated by neuronal pathways, as suggested by Singla (1972). This mechanism may involve either control of secretion of adhesive substance and/or control of neuronally mediated muscle activity, but the muscular activity, if present, does not appear to be needed to maintain attachment. Coordinated detachment, however, appears to be generally effected by brute force. Detachment of the anchors is thus likely to be under purely local control rather than a "fixed behaviour pattern" as suggested by Spencer & Schwab (1982), while attachment may be a behavioural pattern with secretory cells as the sole effectors.

Conclusion

My analysis of the neuroethology of *G. vertens* and *E. typus* suggests that some modifications of current "anthomedusan" hypotheses of behavioural control are required to make them applicable to these olindiads.

The first modification which I would suggest is that epithelial conduction is not as critical to behavioural control as thought by some authors (e.g. Josephson, 1974; Spencer & Schwab, 1982). The two olindiads studied appear to have behavioural repetoires similar to those of other hydromedusae even though they appear to lack this characteristic. The excitable epithelia typical of the anthomedusae appear to make use of the conductile potential of gap junctions more fully, but this appears to be an evolutionarily more advanced capacity, and the distribution of gap junctions and septate junctions seems to be related to functions other than electrical conduction.

The second modification to current hypotheses which I would suggest is that the control of local activities of effectors can be quite distinct from the control of behavioural patterns involving a number of effectors. Through-conducting pathways of the bell margin may be only one of the potential coordinating systems, and does not necessarily typify behavioural control in hydromedusae.

Blocks of smooth muscle capable of independent local activities appear to be the basic functional unit in hydromedusan behaviour as they are in many hydropolyps (Josephson & Mackie, 1965; Josephson, 1974b; Spencer & Schwab, 1982) and as suggested by Passano (1963). As typified by the olindiads, these smooth muscles contain nerve tissues with a diffuse pacemaker capacity and a conduction system ranging from incremental to through-conducting. Whole animal behaviour appears to arise from coordinating systems superimposed on these individual effectors. The swimming muscle could be a highly specialized one of these muscle blocks, having gained striation but lost the capacity for local spontaneity.

Coordinated behaviours initiated by pacemakers of the bell margin and mediated by through-conducting pathways are some of the most clearly defined behaviours, have lent themselves well to investigation, and form the basis of hypotheses for hydromedusan behavioural control. I have found suggestive evidence, however, that coordination during feeding involves activation of local pacemaker systems and independent responses to sensory input. I have demonstrated as well that, at least in the tentacles of *G. vertens*, activity coordinated and initiated by pacemakers of the bell margin is projected onto an effector independently of local control mechanisms. I suggest, therefore, that it is insufficient to base general hypotheses of hydromedusan organization of behavioural control on the coordinating systems of the bell margin alone; consideration should be given to the, as yet elusive, alternate coordinating mechanisms and control mechanisms of local activity.

Beyond these criticisms of current hypotheses, my results indicate that there may be even greater morphological and physiological diversity amongst hydromedusae than commonly recognized. The subumbrellar sphincter muscle, the supportive structures at the base of the velum, possible paddle cilia on the velum and gonads and, in *E. typus*, the ciliated chambers adjacent to the outer nerve ring and supportive cells between the inner nerve ring neurons have all never been seen before in hydromedusae (other than, perhaps by Hertwig & Hertwig (1878)). Mackie and co-workers have also recently found marked morphological and neurophysiological nuances in the trachymedusa *Aglantha digitale* (Mackie, 1980; Mackie, 1984; Mackie & Meech, 1985; Roberts & Mackie, 1980; Singla, 1978). This diversity emphasises that generalizations of hydromedusan organisation based on only a few groups can very easily lead to misinterpretations, so to make these generalisations the investigation of more hydromedusan groups is clearly required. My analyses of behavioural patterns have, however, all also profited greatly from comparisons between two medusae of the same family. So, beyond expanding the taxonomic base of the hypotheses by the study of a wide range of hydromedusae, I would recommend closely related species be studied as well.

In summary, a unifying hypothesis for the organization of hydromedusan and perhaps hydrozoan, neuroethology appear within grasp, conceptually, if not technically. Current theories are addressing some difficult, very basic questions (reviewed in Josephson, 1974a and Spencer & Schwab, 1982), mostly addressed at deciphering the activity and role of individual cells and cell types in stereotyped behaviours. I believe, however, that they require some modification to accomodate other hydromedusae such as olindiads, and that they represent only a portion of what is really the essence of hydrozoan neuroethology.

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Appendix A: Carolsfeld's "aqueous" epon.

Standard embedding epoxies are traditionally maintained as anhydrous as possible. In the present work, however, it was found that 6-8 drops of distilled water added to 25 ml. of epon 812 or Polybed epoxy mixtures just prior to the addition of DMP-30 actually resulted in an epoxy of superior sectioning qualities. With this "aqueous" epon it was possible to reliably and easily cut silver sections up to 650 μm^2 in size with a glass knife. As with standard epon mixtures, the hardness of the epoxy and its sectioning characteristics can be altered by manipulating the NMA/DDSA ratio, but with the present tissue, Luft's (1961) starting point formulation appeared satisfactory.

The "aqueous" epon does, however, possess some peculiar characterisitics. Thick sections are difficult to work with because the epon is quite sticky and takes up Richardson's stain. It was found, however, that a small wire loop or an inverted cover slip are effective in lifting sections from the knife boat, and Richardson's could be extracted from the epon of stained sections by reheating them in water. To stain thin sections with uranyl acetate a special technique is also required. As in normal staining methods, the grids are placed on drops of uranyl acetate, but in this case the sections should face upwards. The preparation is then covered and heated in a 60 ^{o}C oven for approximately 3 hrs. A petri-dish lined with Para-film was found most suitable for this procedure. Subsequent staining with lead citrate can be carried out in the normal manner. As an alternative, *en bloc* staining was found very effective in staining the tissue with uranyl acetate.

The "aqueous" epon also appears more sensitive to heating in the electron beam than is normal epon, so use of a cooled column in the electron microscope is advantageous. Even so, at high magnification material tends to appear somewhat "grainy", but at low magnification images can be exceptionally crisp. In the present work, figures 41a, 47a, 48a, 48b, 50, 52, and 55 are of material embedded in "aqueous" epon, while the remainder are of conventionally prepared tissues.

Appendix B: Hydromedusan Behaviour and Mechanisms of Evolution

As a secondary goal in this thesis, I wish to address the question of whether morphological changes in evolution accurately reflect selective forces. Paleontological evidence for evolutionary changes commonly only consists of morphological characters, but selective forces that may have shaped those changes can only have acted on a complex of structure and function ("phenotypic complex") as it is presented to the environment. Conventional evolutionary theory suggests all morphological changes are accompanied by a change in the phenotypic complex and are closely allied to selective forces, but Boucot (1983) and Brooks (1984) have more recently suggested phenotypic complexes ("functions" in their terminology) are conserved during evolution in the face of continuing morphological change, i.e. morphological changes are independent of selective forces.

Passano (1982) has suggested that coelenterates may be one of the few animals in which differences in behaviour may be related to unitary changes in the underlying mechanisms, due to their relative simplicity. Taken in an evolutionary context, this suggests that interspecific differences in coelenterate behaviour may be used to demonstrate the relationships between morphological, physiological, and behavioural evolution with the above question in mind. To do so, action systems would be considered the evolving units, with morphology represented by the structure and distribution of the muscles, nerves, and intercellular junctions; physiology represented by the electrophysiology; and phenotypic complexes represented by the behavioural patterns. Hydromedusae possess a series of probably the most distinctive behavioural patterns of the coelenterates, so it occurred to me that as a result of a comparative study of hydromedusan neuroethology, such as my thesis, differences in the representatives of morphology, physiology, and phenotypic complex could well be tabulated. Some indications might then be gleaned on whether morphological and physiological changes have occurred independently of each other and/or independently of the phenotypic complex in behavioural evolution of these hydromedusae. A clue as to how closely morphological changes are linked to selective pressures may then be derived.

I have presented such a tabulation in Table 4, using my results for *G. vertens* and *E. typus* and published results (Spencer, 1979; Spencer & Schwab, 1982) for *Polyorchis penicillatus*, which I have considered a representative anthomedusan.

Table 4: Hypothesised nature of characters underlying behavioural differences between three hydromedusae.

Behavioural Pattern	Polyorchis	Gonionemus	Eperetmus
crumpling	-	e	-
sumersault	-	m	-
swimming (normal)	-	-	-
swimming (escape)	-	-	e
turning	m,e	-	-
tentacle anchoring	-	m,e	-
tentacle coordination	-	e	-

e = electrophysiological difference
m = morphological difference
\- = no behavioural difference

Of the behavioural patterns considered, the absence of crumpling in *G. vertens* appears correlated with a neurophysiological difference; somersaulting is dependent on morphological characteristics; the normal swimming behaviour is essentially the same for all three species; "escape" swimming in *E. typus* depends on a neurophysiological characteristic; turning in the olindiads is distinct from that in *P. penicillatus* because of both morphological and neurophysiological differences; tentacle coordination is less pronounced in *G. vertens* because of a neurophysiological difference; and tentacle anchoring is present in *G. vertens* due to both morphological and neurophysiological characteristics.

A physiological difference that has no overt effect on the form of behavioural patterns is epithelial conduction in the endoderm and exumbrella of *P. penicillatus* while morphological differences that have no overt effect on the form of behavioural patterns are the nerves in the swimming muscle and velum of the olindiads, and the absence of radial muscle fibres in the velum of *G. vertens*.

The first thing to note from these results is that, in this selection of hydromedusae, differences in behavioural patterns can be correlated, crudely, with unitary differences in morphology of neurophysiology as was postulated by Passano (1982). The comparisons indicate that neurophysiological and morphological differences as defined here, can occur independently of one another or together in producing behavioural differences. Both neurophysiological and morphological differences are also present without correlates in the form of behavioural patterns, but all of those I found could be thought to be under other selective pressure as well: the excitable exumbrella has a sensory function and epithelial conduction for crumpling and swimming may be a more economical or efficient manner of spreading excitation. Not enough is known about the function of radial muscles in the velum to evaluate the significance of their absence in *G. vertens*.

Evolution of behaviour in hydromedusae thus substantiates something we may already intuitively know: morphological characters alone are not enough to describe the selective pressures that may have acted or may be acting on them. Morphological changes in excess of functional changes, as suggested by Boucot (1983) and Brooks (1984), could, however, not be clearly demonstrated.

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