

Coordination in a Diphyid Siphonophore

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The behaviour of Chelophyses has been analysed from the point of view of coordination between stem and nectophores, and an electron microscope study of the effectors and conducting elements has been carried out.

Coordination between the stem and anterior nectophore involves two pathways, one epithelial and the other nervous. The nervous link consists of a bundle of small neurites and a single giant axon. There is some evidence that this mediates rapid escape behaviour. After the nerves have been cut, coordination is maintained *via* the epithelial route. Impulses can jump from epithelial cells into nerves but the transmission process is unclear. Neuro-epithelial transmission involves conventional synapses.

As in physonectid siphonophores, the stem has two nervous systems each with its own giant fibre, and a slow system, the endodermal epithelium. In the nectophore, marginal nerve centres generate a swimming rhythm. Conduction in the subumbrellar muscle is myoid. The exumbrellar epithelium and the subumbrellar endoderm are conducting tissues.

Histological study reveals synapses in the predicted locations and gives details of myo-epithelial organization and nervous layout. Novel histological features include elements resembling steroid-secreting cells, which ensheathe nerves and are innervated by them, and innervated giant non-nervous cells lying between the nerve ring and the hydroecium. The subumbrellar muscle cells are shown to have sarcolemmal invaginations reminiscent of the *t*-tubule system of vertebrate muscle.

INTRODUCTION

This paper is the second of two dealing with coordination of activities in siphonophores. The problem is of interest in that the siphonophores are probably the most complex and highly evolved animal colonies, the pelagic tunicates not excepted, and because the mechanisms of coordination within the colony are probably versions of the same mechanisms involved in

coordination within individuals. The companion paper (Mackie, 1978) may be referred to for references to recent work on coelenterate colonies generally. Coordination in pelagic tunicates offers remarkable parallels to what is emerging in siphonophores. The subject is treated in reviews by Anderson (1980) and Bone and Mackie (1982).

The companion paper on physonectid siphonophores stresses interactions between the stem, gastrozooids, palpons, tentacles and bracts. The nectophores of physonects are small and tend to autotomize. The present study of *Chelophyses*, a calycophore, was begun largely in order to clarify nectophore-stem interactions. It also seemed desirable to carry out a fairly detailed ultrastructural survey of a siphonophore stressing those action systems involved in behaviour, since this has not previously been done, and because the information provided is essential for realistic interpretation of the physiological results.

Several recent papers are highly relevant. Bassot *et al.* (1978) deal with epithelially mediated responses in *Hippopodius*, a slow moving and possibly primitive calycophore offering interesting points of comparison with the more advanced diphyids such as *Chelophyses*. Chain *et al.* (1981) and Bone (1981) have studied the electrophysiology of the striated muscle sheet in *Chelophyses*, and Bone and Truman (1982) have looked at swimming biomechanics in the same species. Finally, Chain (1981) has studied the electrophysiology of gastrozooids in the physonect *Agalma*.

MATERIALS AND METHODS

Specimens of *Chelophyses appendiculata* Eschscholtz were obtained from plankton hauls in the Rade de Villefranche at depths between 75 m and the surface during the months of April and May 1977. The species is obtainable, often in abundance, throughout most of the year. Specimens were kept in a cold room at 14°C. Observations of behaviour were made in a 50 l tank illuminated from the sides and with a dark background. As deterioration in the laboratory is rapid, specimens were used for fixation and experiments within a few hours of capture. Some additional observations on other siphonophores are included in the section on electrophysiology. The species concerned are: *Hippopodius hippopus* Forskål, *Abylopsis tetragona* Otto and *Sulculeolaria quadrivalvis* Blainville.

For electron microscopy, specimens were fixed in 3% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) with added NaCl (0.3 M) to bring the osmolarity to that of sea water. Samples were postfixed in 1% osmium tetroxide in cacodylate buffer. After dehydration, material was embedded in Spurr's resin. Thin sections were stained with uranyl acetate and lead citrate.

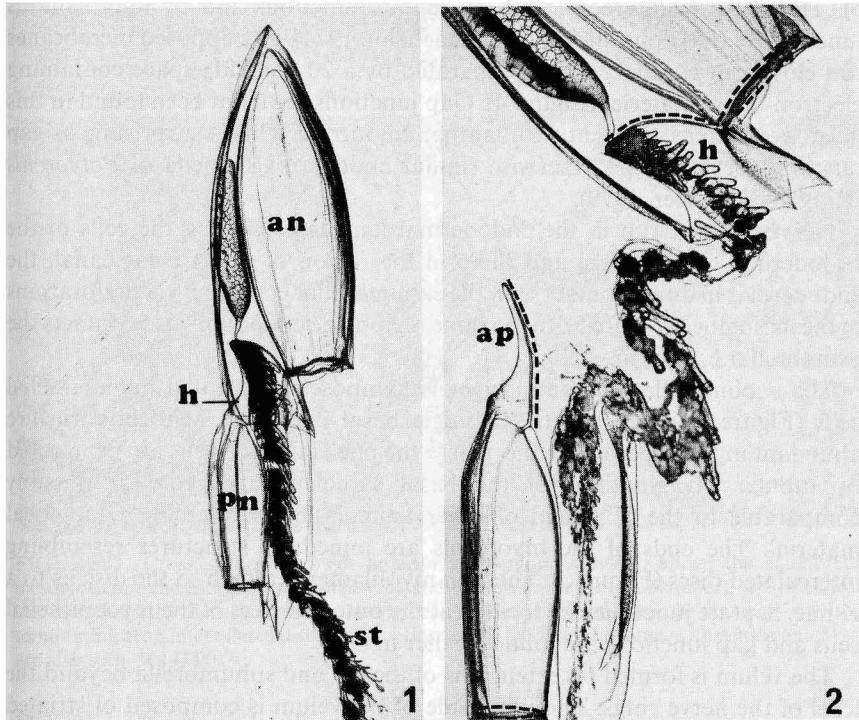
Electrophysiological recordings were made using plastic suction electrodes, with display on a Tektronix storage oscilloscope. Photographic records were made with a Polaroid camera. Electrical stimuli were delivered through plastic or wire electrodes, using a Grass S44 stimulator.

Histology and ultrastructure

The following account deals with the stem and nectophores, especially with those aspects of the histology most relevant to behaviour.

(a) Nectophores

Adult colonies of *Chelophysa appendiculata* have two superimposed nectophores, one anterior and one posterior (Figures 1, 2). The stem arises from the



FIGURES 1, 2 Specimen of *Chelophysa* intact (Figure 1) and after separation of the two nectophores (Figure 2). The stem has been partially pulled from its sheltered position in the hydroecial groove of the posterior nectophore in Figure 2. The broken lines show the location of nerve pathways in the nectophores. an: anterior nectophore; ap: apical peduncle of posterior nectophore; h: hydroecium; pn: posterior nectophore; st: stem. Figure 1 is magnified $\times 4$, Figure 2 $\times 8$.

apex of the hydroecial cavity of the anterior nectophore and runs back in the hydroecial groove of the posterior nectophore. The posterior, but not the anterior nectophore is *caducous*, and *replaceable*. The basic histological organization of the two nectophores is similar.

The exumbrellar epithelium is a uniform sheet of cells one cell thick as described in *Hippopodius* (Bassot *et al.*, 1978). The cells lack myofibrils, and nerves are not present among them. The cells are thin (about 6 μm) and flat. They possess numerous vesicles containing granular material, presumably mucus, and are joined together at their outer edges by septate junctions. Gap junctions are found at inner contact points between adjacent membranes.

The endodermal lamella is a sheet of cells spanning the spaces between the radial canals and ring canal. It is separated from the subumbrellar ectoderm layer by a thin sheet of electron-dense mesogloea (Figure 3). The flattened cells of the lamella lack myofibrils except in the vicinity of the radial canals (Figure 5). The cells are interconnected by septate junctions and by long sinuous junctions of the "continuous" type (Staehelin, 1974). The apposed membranes run closely in parallel, and are separated by a 20 nm wide space containing electron-dense material (Figure 4). Gap junctions have not been found in this layer, even after treatment with lanthanum nitrate. This is surprising, as gap junctions occur in the otherwise similar endodermal lamella of *Polyorchis* (King and Spencer, 1979).

Nerves do not run in the endodermal lamella or among the cells of the endodermal canals. Here and there in the region of the circular canal, the endoderm is in direct contact with the subumbrellar ectoderm via perforations in the mesogloea (Figure 5). In the same region, the endoderm also contacts the exumbrellar ectoderm.

The subumbrellar ectoderm layer is composed of striated myoepithelial cells (Figure 3). Each cell has a single basal myofibril. Mitochondria are abundant in the superficial zone, where the nuclei lie. The cells are penetrated by tubular invaginations of the basal sarcolemma (Figure 5) possibly comparable to the T system of other animals, but containing mesogloal material. The ends of the myofibrils are joined by structures resembling intercalated discs (Figure 3). The thin myofilaments attach to the disc as to a Z-line. Septate junctions are located at the outer borders of the myoepithelial cells and gap junctions are found further in.

The velum is formed by extensions of the ex- and subumbrella beyond the level of the nerve rings. The inner side of the velum is composed of striated epitheliomyocytes, whose myofibrils run circularly. The outer side consists of smooth epitheliomyocytes, orientated radially.

The two nerve rings lie in the usual place at the base of the velum, separated by a thin mesogloal layer. The inner ring is reduced to a bundle 3–5 μm thick lying against the mesogloea and covered by thickened striated epithelio-

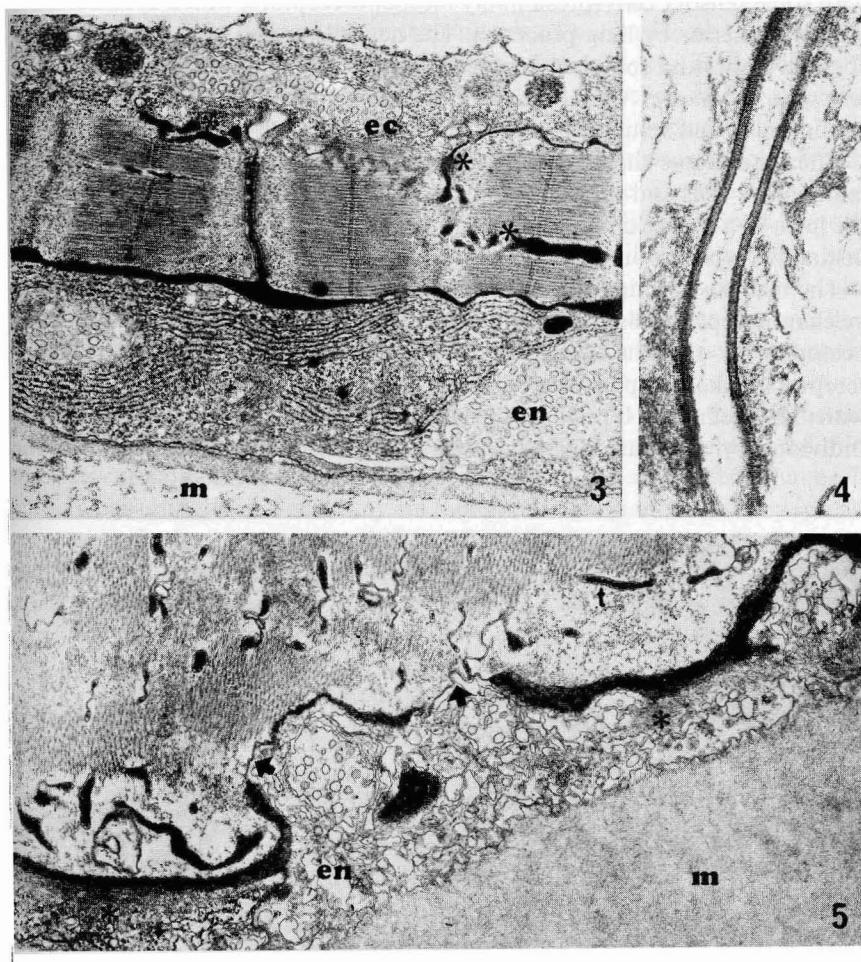


FIGURE 3 Cross section through anterior nectophore showing, above the exumbrellar mesogloea (m), the endodermal lamella (en), and the subumbrellar ectoderm (ec), separated from the endoderm by densely-staining mesogloea. In the ectoderm, muscle filaments are seen terminating at an intercalated disc. Invaginations of the basal sarcolemma, with dense contents (*) are also seen. $\times 14,000$.

FIGURE 4 Endodermal lamella : part of the sinuous intercellular junctional region near a canal. $\times 40,000$.

FIGURE 5 Section close to the circular canal showing interruptions in the mesogloea (arrows) between the ectoderm and the endoderm (en). Small aggregations of smooth myofibrils (*) are seen in the endoderm. m : mesogloea of exumbrella. $\times 17,000$.

myocytes (Figure 6). It consists of a chain of a few bipolar neurons, along with some sensory cells (presumably mechanoreceptors) which communicate with the exterior by long processes. The neurites are all of similar thickness (2–3 μm diam.) and contain many microtubules. Along their length, they make *en passant* synapses with other neurons or with myoepithelial cells of the subumbrella and velum (Figures 6, 7).

The outer nerve ring is much more complex than the inner. It consists of many nerve cells and they are arranged in a complex network rather than in the form of a simple chain. Groups of them are ensheathed by processes of a distinctive type of non-nervous epithelial cell.

The neurons forming the outer nerve ring include both interneurons and mechanoreceptor cells (Figure 8). The latter lie flush with the surface of the nectophores—thus the nerve ring is not entirely sub-epithelial. The mechanoreceptors make synapses with some of the non-nervous cells on the ring, and with interneurons, but they also receive synapses from interneurons. Individual synapses are always polarized in one direction. The neurites of the

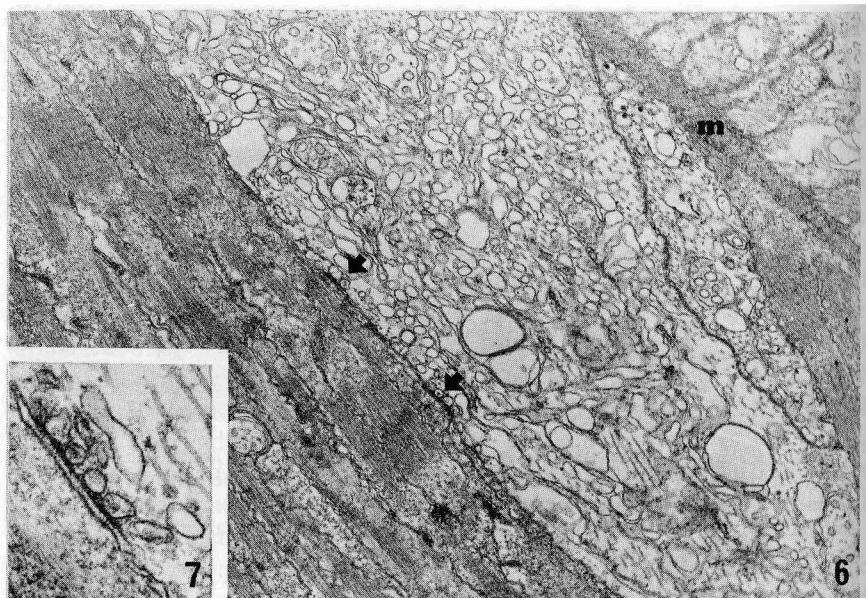


FIGURE 6 Section of the inner nerve ring of the anterior nectophore. Note the small number of neurites (two, in the picture) and neuromuscular synapses (arrows). $\times 14,000$.

FIGURE 7 Synapse between a neurite and a non-nervous cell process in the outer nerve ring. $\times 40,000$.

outer ring are quite variable in diameter. They make synapses along their length with each other and with adjacent epithelial cells.

The non-nervous cells of the outer ring are as numerous as the neurons. They are ultrastructurally quite distinct from other cell types. They are absent from the inner nerve ring, and from the tissues of the velum and exumbrella bordering the outer ring. These cells are epithelial, forming part of the surface of the nectophore, but their bases are drawn out into elaborate folds which insinuate themselves into the nervous tissue (Figure 8). The nucleus is lobulated (Figure 9). The cytoplasm contains a richly developed smooth endoplasmic reticulum in the form of vesicles and small cisternae. Here and there mitochondria and rough endoplasmic reticulum are also seen. The cells are richly innervated, receiving synapses from adjacent neurites (Figure 10).

A final noteworthy feature of the outer nerve ring in *Chelophyses*, seen only in the anterior nectophore, is a pair of symmetrically placed structures which emerge from the nerve ring, pass through the notch dividing the hydroecial lobes and end in the hydroecium (Figure 11). After examining thick and thin sections cut along the full extent of these structures, we can confirm that each of them has a single nucleus; they are therefore single giant cells. They spread out and lie flush with the nectophore surface where they abut upon the outer nerve ring. Their basal regions are complexly folded, interdigitating with extensions of the mesogloea. Their cytoplasm is characterized by elongated saccules orientated along the main axis of the cell. The saccules often contain small membrane-bounded vesicles. In the hydroecial notch each of these two structures ends in a bulbous expansion containing the nucleus (Figure 11). In this region, the two giant cells are richly innervated.

(b) Stem

The stem is a long, hollow bilayered stolon whose lumen is continuous with the cavities of the attached zooids, and with the hydroecial canal of the anterior nectophore and the pedicular canal of the posterior nectophore.

The ectodermal layer is made up of myoepithelial cells whose contractile portions are so well developed that early authors thought that they constituted a separate layer. However, developmental observations show that this complexity is only superficial. At its inner end, each ectoderm cell forms longitudinal extensions, numbering between one and twenty according to the age of the cell and containing smooth muscle fibres. At the same time, the mesogloea adjacent to the ectoderm forms longitudinal crests and furrows. The result is that the contractile extensions of any given cell become separated from one another either by mesogloal outgrowths or by infiltration of processes from neighbouring cells. In longitudinal sections, the stem muscle acquires a stratified appearance (Figure 12).

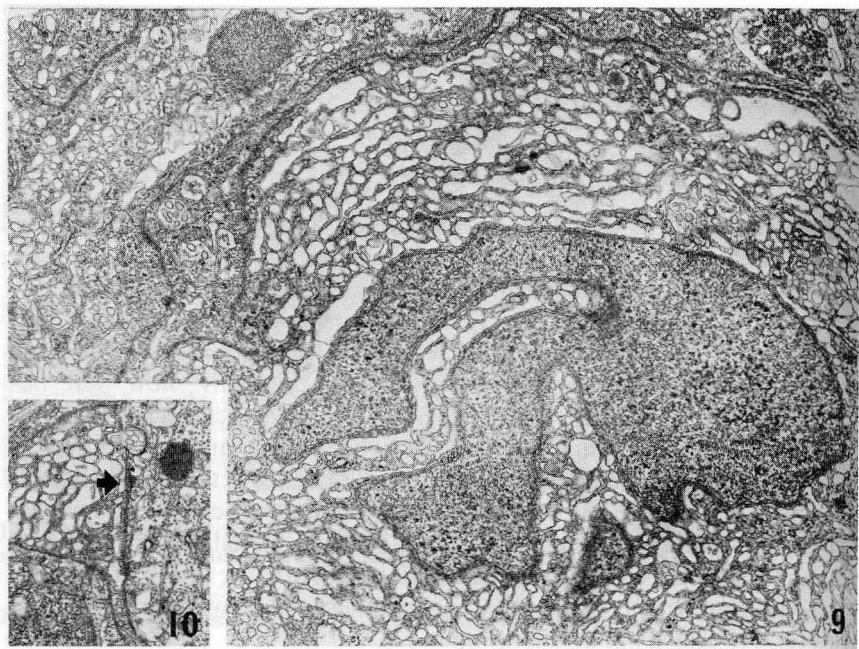
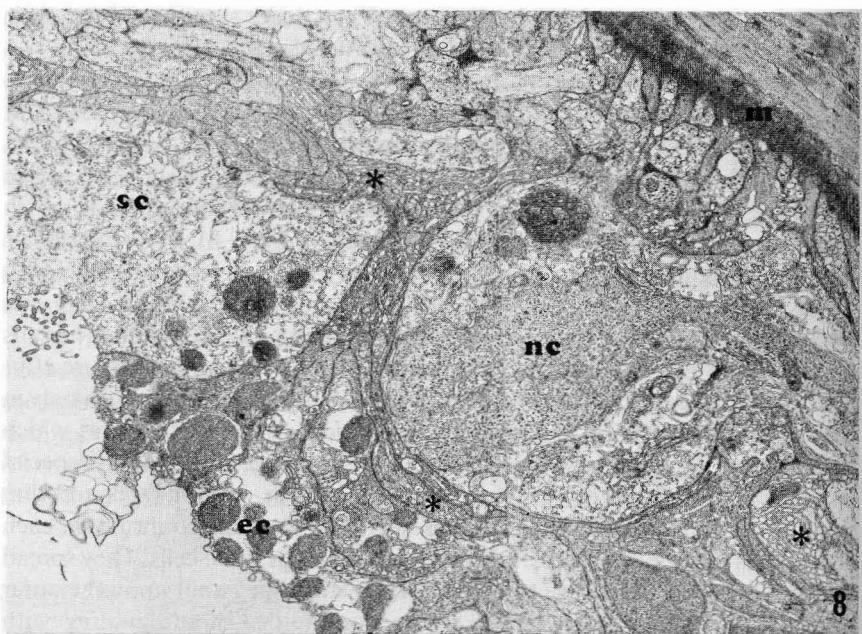


FIGURE 8 Portion of the outer nerve ring of the anterior nectophore. Neurites are seen lying near the mesogloea (m). A sensory cell (sc) and an interneuron (nc) enveloped by processes of non-nervous cells (*) are shown. $\times 5300$.

FIGURE 9 Detail of a non-nervous cell in the outer nerve ring. Note the lobulated nucleus, and the richly developed smooth endoplasmic reticulum. Rough endoplasmic reticulum is visible under the cell membrane. $\times 13,000$.

FIGURE 10 A neurite makes a synapse upon a non-nervous cell similar to the one shown in Figure 13. $\times 12,000$.

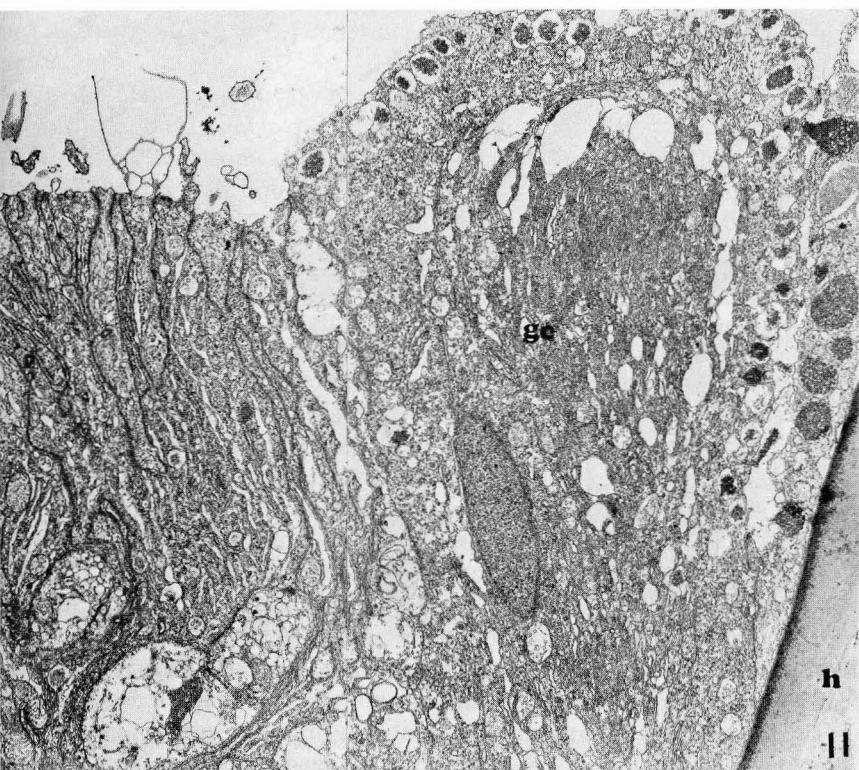


FIGURE 11 Section passing through the deepest part of the hydroecial notch (h), showing the bulbous extremity (gc) of one of the two giant cells which emerge from the outer nerve ring. A nucleus is present in it. $\times 11,000$.

The ectodermal cells are interconnected by septate junctions at their outer edges and by desmosomes in their contractile regions (Figure 13). Gap junctions have been observed both in the outer and inner regions (Figures 14, 15).

The endoderm of the stem is less elaborate than the ectoderm. It appears to function essentially as a conduit for passage of food materials through the colony, a process brought about by ciliary beating and peristaltic contractions. The basal parts of the cells contain a few smooth muscle fibres running

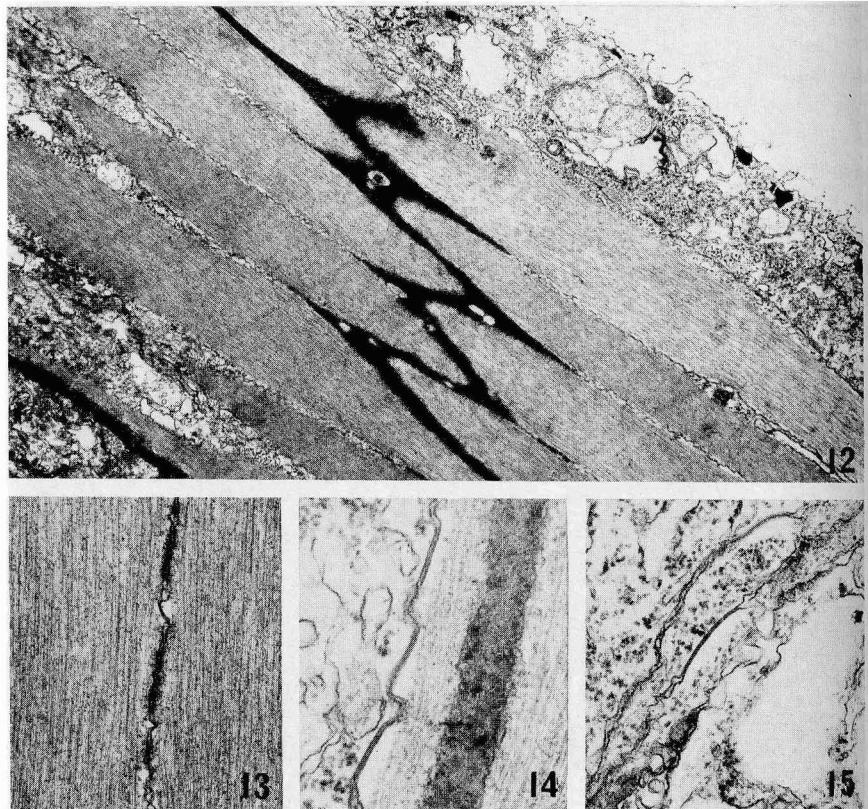


FIGURE 12 Longitudinal section of the stem showing stratified appearance of the well developed smooth muscle tissue. $\times 8000$.

FIGURE 13 Desmosomes between contractile processes of endodermal epitheliomyocytes. $\times 18,000$.

FIGURE 14 Gap junctions between contractile processes of epitheliomyocytes. $\times 40,500$.

FIGURE 15 A gap junction between superficial parts of epitheliomyocytes. $\times 32,500$.

circularly. Septate junctions join the outer edges of the cells, but are also seen in deeper regions of contact. Sinuous junctions, possibly of the continuous type, are also seen. They exhibit a very consistent intercellular separation of 30 nm, with dense material in the gaps. Occasionally, here and there, we have observed perforations in the mesogloea allowing the two cell layers to make direct contact.

Earlier studies on the innervation of the stem have dealt mainly with physonects. Korotneff (1884), Schneider (1898) and Mackie (1973, 1976b) have all described two superimposed giant axons running the whole length of the stem in Agalmidae and Forskaliidae. These cells lie in the ectoderm and are connected with a superficial nerve plexus composed of smaller neurites arranged in a network. According to C. L. Singla (cited by Mackie, 1978) the physonectid endoderm lacks nerve elements.

In the stem of the calycophore *Chelophys* we have found comparable nerve structures in the corresponding regions: a pair of giant axons in the ectoderm and a superficial plexus of small neurons. Nerves are also absent from the endoderm.

The two giant axons are both smaller than their counterparts in physonects, measuring about 10 μm in diameter, in keeping with the fact that the stems are much thinner. Neuromuscular synapses occur along the length of the giant axons. Serial thick sections show that the giant axons never give off branches to the zooids attached to the stem. Both interneurons and mechanoreceptor cells contribute processes to the superficial plexus.

(c) *Connections between the stem and nerve rings*

In physonects, the nerve rings of the nectophore are linked to the stem by nerves crossing the exumbrella. In view of the possibility that similar connections exist in calycophores we have examined the junctional tissues in *Chelophys* by electron microscopy, concentrating the search in the neighbourhood of the hydrocial canal, which would represent the shortest route between stem and nerve rings in the anterior nectophore, and along the peduncular canal in the posterior (Figures 1, 2).

A giant axon 8 μm thick (Figure 16) has been found in the hydroecium of the anterior nectophore. It lies in the ectoderm, running along beside the hydrocial canal for its full length. Smaller neurites are also seen running in bundles alongside the giant. Serial sections show that the giant axon and neurites both originate at the stem attachment point at the apex of the hydroecium and run right through to the nectophore nerve ring. On leaving the hydroecial cavity they pass through the notch which divides the two lobes of the basal lamina of the hydroecium. The hydrocial canal also uses this notch, and the two non-nervous giant cells (Figure 11) issuing from the outer nerve ring end in this region.

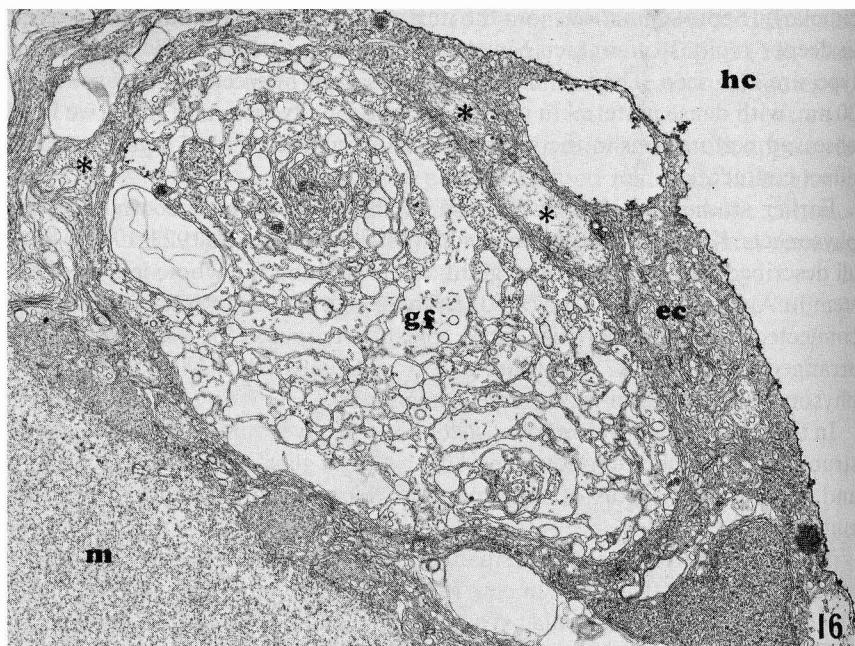


FIGURE 16 Section across the hydroecial wall of the anterior nectophore, midway up from the margin. A single giant fibre (gf) is seen along with smaller neurites (*). ec: ectodermal cell; hc: hydroecial cavity; m: mesogloea. $\times 8500$.

The giant axon running in the hydroecial ectoderm has the same diameter as the two giant axons of the stem and it is possible but unlikely that it represents the continuation of one of them. We have not observed neuro-effector synapses along its length but there are numerous synapses between the small neurites running up the hydroecium, and between them and the non-nervous giant cells.

In the posterior nectophore we have found neurons in the ectoderm of the apical peduncle, running along beside the peduncular canal (Figure 17). The neurites, few of which exceed $1.5 \mu\text{m}$ in diameter, run singly or more often in small groups and make numerous synapses among themselves and with adjacent non-nervous cells. A giant axon is never present.

Serial sections along the nectophore have failed to reveal any nerve elements in the extensive region between the peduncle and the margin, where the usual two nerve rings are present. Special attention was paid to the tissues adjacent to the central canal and to the wall of the hydroecial groove, which would represent the most direct pathway for any nerves going to the margin, but results were negative.

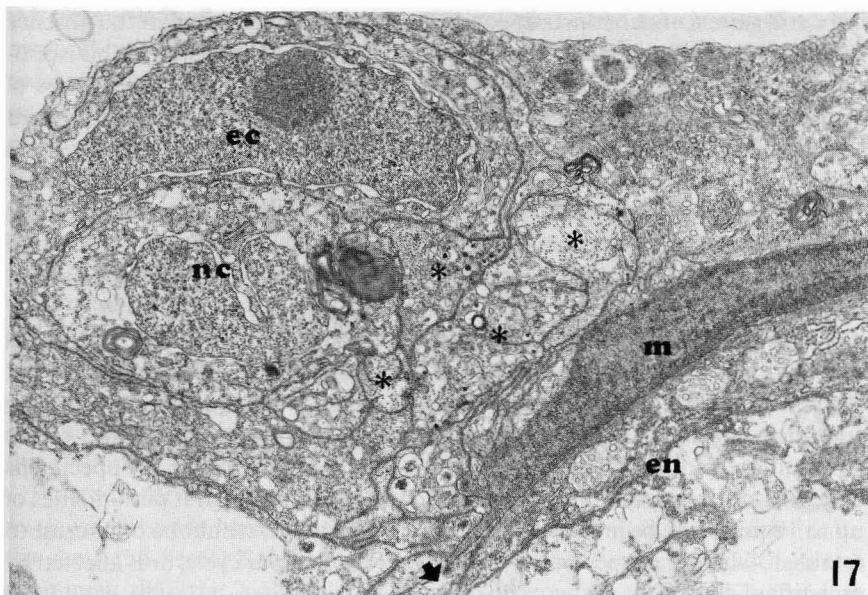


FIGURE 17 Group of neurites (*) lying over the peduncular canal of the posterior ectophore. The ectoderm (ec) and endoderm (en) are in direct contact through a perforation (arrow) in the mesogloea (m). $\times 9000$.

At the base of the peduncle in the region where the innervation ends, the mesogloea is interrupted, allowing the ectoderm and endoderm to communicate directly (Figure 17).

Behaviour

Chelophyses is a hardy species and can be kept in good condition for lengthy periods in the lab at temperatures 4–6°C above that of their natural environment. Specimens caught in a plankton net are often intact and unblemished except for the loss of the terminal part of the stem. The stem is a strobilating structure which eventually breaks up into free subcolonies (eudoxoids) so loss of the hind regions does not constitute a serious injury.

Most of the specimens observed in the lab were slightly heavier than water and sank slowly to the bottom of the tank. The ectophores are buoyant structures (Jacobs, 1937) and float to the surface after detachment from the stem. The large anterior ectophore is the most buoyant part and the colony hangs more or less vertically in the water with this ectophore uppermost. The more mature eudoxoids are fairly buoyant and may provide sufficient flotation to maintain the end of the stem at some angle other than the vertical.

In still water, under constant conditions of illumination and in the absence of mechanical disturbance, specimens, with few exceptions, exhibit spontaneous activity. They suddenly start to dart around the tank by a series of rapid swimming contractions and with the stem somewhat shortened. Toward the end of this swimming bout, and as momentum decreases, the stem relaxes and the tentacles and tentillae swirl out like a cloak around it. Some species swim in an arc and the tentacles spread out in the "veronica" pattern described for *Muggiaeae* (Mackie and Boag, 1963). During the ensuing period of quiescence the extended filaments drift apart and the regularity of the pattern is gradually lost.

These spontaneous swimming bursts occur every few minutes, sometimes with considerable regularity. In nature, the pattern is probably highly regular, for collision with solid objects would be rare. In an aquarium, swimming specimens strike the walls of the tank, which leads to prolongation of the swimming episode, and disruption of the overall rhythm. Many specimens observed swam approximately every two or three minutes, but periodicities of up to 10 min have been observed. It is not known, but would be of interest to establish, whether the periodicity varies over the 24 hour cycle, or is affected by nutritional state.

Spontaneous activity is inherent in both nectophores. The frequency is usually higher in the anterior nectophore, but as soon as one nectophore starts to swim, the other usually joins in. The posterior nectophore often continues to swim after the anterior has stopped, leading to a slower, less violent kind of locomotion, during which the stem is often seen to be well extended and trailing outstretched behind. In some specimens swimming by means of the posterior nectophore was more often seen than swimming with both. Bone and Truman (1982) suggest that activity of the posterior nectophore alone may serve to counteract the tendency to sink in the water column. Detached nectophores show spontaneous activity cycles similar to those seen in intact specimens. It appears that spontaneity is inherent in the swimming centres of both nectophores.

Bone and Truman (1982) show that during escape swimming both nectophores may contract together at frequencies up to 8 Hz. At frequencies higher than about 4–5 Hz (produced artificially by stimulation of the anterior nerve rings) the posterior nectophore fails to follow the beating of the anterior on a one to one basis. When the two are coordinated, the anterior leads the posterior by about 30 ms. It is not possible to be sure that coordination is physiological, i.e., involves a conducted signal, or mechanical. The locomotory centres of the two nectophores are not connected by nervous pathways. On the other hand the close coupling suggests a physiological mechanism.

Siphonophores are acutely sensitive to touch and, being covered all over with excitable epithelia, are sensitive in regions where there are no nerves

(Mackie, 1965). Both nectophores of *Chelophyses* are touch sensitive and respond by escape swimming bursts. At the same time, the stem contracts. A specimen was observed which sank slowly to the bottom of the tank after swimming; each time the posterior nectophore touched the floor of the tank, a new swimming episode was provoked. This resulted in the production of an artificial rhythm. Swimming bursts are prolonged by collisions, which stimulate the exumbrellar epithelium. The duration of escape swimming episodes is quite variable, depending perhaps on the interval since previous activity, or on the animal's general physiological state. Some jaded specimens which had been in the tank for a long time failed to swim when stimulated, but instead showed only flicks in the velar region of the anterior nectophore, which represent contractions of the radial muscle fibres (see below).

The stem responds to stimulation by contracting locally, or over its whole length. Stem stimulation usually leads to swimming in fresh specimens, but this response becomes hard to evoke in jaded specimens.

Water-borne vibrations readily cause swimming. The receptors for this response have not been identified, but there are sensory cilia projecting from the stem and nectophore margins (see above). These structures, though less prominent than the "palpocils" of some hydrozoans (Schulze, 1871, Tardent and Schmidt, 1972) appear well placed to pick up vibratile stimuli, especially those of the velum, a diaphragm-like structure which quivers at the slightest tremor in the tank. The stem may respond to vibration by contracting, even if the nectophore remains passive.

The effects of light have not been systematically explored. We merely note that after a period of adaptation to dim light, sudden illumination may trigger swimming. Reduction in light intensity has no effect. Photoexcitability has been reported in both nervous subsystems of the stem of a physonectid siphonophore (Mackie, 1976b), so nerves may also be the photoreceptors in *Chelophyses*. To determine the longer-term effects of ambient light level on the regulation of spontaneous activity calls for a new study using more specimens and better controlled conditions than were available at the time of the present work.

Physiology

(a) Nectophores

1) *Conducting epithelia* The exumbrellar epithelium of *Chelophyses* is an excitable cell sheet which conducts impulses at c.50 cm/sec with a refractory period of 5 ms (Mackie 1965 and Figure 18A). Repetitive firing occurs with strong stimulation. The impulses are through-conducted without decrement or polarity across the whole surface. They can be elicited by quite delicate

tactile stimulation as well as by shocks. As first shown by Spencer (1975) in a jellyfish the conduction time of exumbrellar impulses increases with successive stimuli at high frequency. This is shown in Figure 18B for stimulation at 50 Hz. Latency decreased abruptly after the first shock (labelled 1) and then increased steadily (2–15).

The subumbrellar endoderm (endodermal lamella and canals) is a conducting tissue in *Chelophyes*, as in numerous medusae and some other siphonophores (Mackie, 1976a; Bassot *et al.*, 1978). Surgical experiments similar to those carried out on other species show that impulses can pass from exumbrellar ectoderm to subumbrellar endoderm, doubtless by way of the cell bridges seen by electron microscopy (p. 5 above). There is also a direct route from the endoderm to the stem, which might be the hydroecial canal but, because the ectoderm and endoderm are coupled both at the nectophore end and at the stem end of this canal, it is impossible to be sure that the epithelial pathway is not purely ectodermal. Cutting the canal does not block the pathway, so it is not purely endodermal. Whether the canal is a pathway or not, there is only one effective epithelial pathway from nectophore to stem, and it is shown as ectodermal in Figure 22 for simplicity.

2) *Muscular responses* As noted above, the nectophore of *Chelophyes* has two sets of muscles, both ectodermal, the circular striated muscle (swimming muscle) and the radial smooth fibres of the velum (radial muscle). The radial muscle appears to be the homologue of the Claus fibres of physonects such as *Nanomia* whose contractions deform the velum and thus deflect the water jet during swimming, bringing about a change in the direction of locomotion

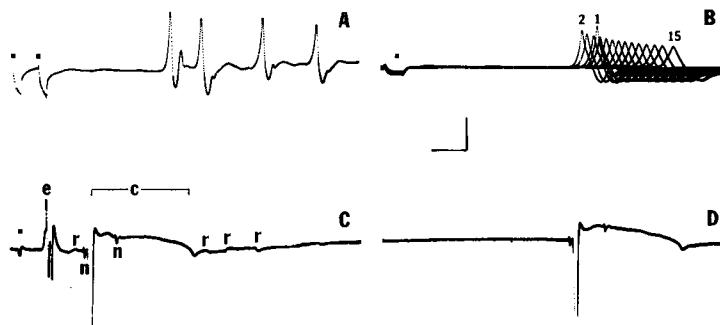


FIGURE 18 Nectophore of *Chelophyes*: exumbrellar conduction and swimming. A. Two shocks (■) 5 ms apart each evoked an epithelial impulse in the exumbrellar epithelium. The preparation continued to fire spontaneously. B. Epithelial responses to 15 stimuli at 50 Hz superimposed. After the second impulse, there is a progressive increase in conduction time. C. Recording from velum near marginal nerve ring, stimulating on exumbrella. The arriving epithelial impulse (e) evokes a composite swim (see text). c: circular muscle; n: swimming motoneurons; r: radial muscle. D. For comparison with C, a simple swim. Scale. Vertical and horizontal bars represent: (A) 0.5 mv, 5 ms; (B) 0.5 mv, 2 ms; (C, D): 0.5 mv, 20 ms.

(Mackie, 1964). Calycocephorans have not been observed to use their radial muscles in this way, but this requires further study, preferably with the aid of cinematographic records.

Contractions of the swimming muscle are accompanied by distinctive potentials having durations of up to 40–50 ms, and showing a pronounced plateau or its derivative in extracellular recordings. Intracellular recordings of these events are now available, and the wave form of the action potential, its ionic basis and the membrane constants of the myoepithelium have been systematically investigated (Chain, 1979; Chain *et al.*, 1981; Bone, 1981). In recordings from near the margin, nervous triggering events can be picked up. These are equivalent to the pre-swim pulses (PSPs) described by jellyfish workers (first by Ohtsu and Yoshida, 1973). Propagation across the muscle sheet is myoid, as deduced by Chun (1882) from his observation that nerves are absent.

Radial muscle contractions have smaller, less distinctive electrical correlates consisting of a series of small potentials with somewhat variable wave forms.

An epithelial impulse propagated across the exumbrella arrives at the margin and may cause either a) a contraction of the radial muscle alone, b) a contraction of the radial muscle accompanied by a swim (circular contraction) or c) the same, but with subsequent spontaneous swimming unaccompanied by radial contractions. There are thus two sorts of swimming, the simple swim in which the circular muscles alone contract, and the composite swim in which both muscle systems are active. In Figure 18C a composite swim is illustrated. The electrode was placed on the velum near the nerve ring so that it picked up the potentials from both muscle systems as well as from the nerve ring (PSPs) along with the arriving epithelial impulse. In Figure 18D one of the subsequent spontaneous swims is shown. By mentally subtracting this event from the record in Figure 18C we are left with the epithelial impulse (*e*) and a ripple of small potentials (*r*) representing radial muscle contractions. The PSPs in these two records are small (100 μ v) events which immediately precede each swim. Similar events may also occur during a swim, as in *Stomotoca* (Mackie, 1975). Figure 21A (lower line) shows a case where the arriving epithelial impulse evoked only radial muscle activity. Composite and simple swims are again shown in a naturally occurring sequence in Figure 21C; this record clearly shows how the *r* potentials disfigure and complicate the pure wave form of the simple swim potential. The electrode in this example was not near enough to the nerve ring to pick up PSPs.

In all cases observed (with one significant exception noted below, p. 26) the radial response begins immediately upon arrival of the epithelial signal but the swim itself is delayed. This delay is rather variable, but it falls within the range 15–40 ms. The interpretation of these observations may be sought in the fact that the radial muscle is part of a layer which is directly continuous with the

conducting epithelium which carries the excitatory signal. Thus the signal can pass directly to the muscle and initiate a response with little delay. The swimming muscle on the other hand is excited indirectly via the nervous system (as evidenced by the PSP) so the epithelial impulse must first enter the nervous system in order to excite the swimming neurons, which then excite the muscles. The fact that a single epithelial pulse can trigger a long burst of swimming also fits the supposition that activation of the muscles is neuronally mediated, since there is abundant evidence that medusan swimming rhythms are generated by neuronal pacemakers.

It may be appropriate to comment briefly on an earlier recording from *Chelophyes* (Mackie, 1965, Figure 12) which though technically crude provided the first electrophysiological evidence to be obtained for "pure" epithelial conduction in any animal, in the shape of a potential (*P*1) interpreted as an exumbrellar impulse. The recording was from the velum and was made with a glass capillary electrode pushed into the epithelium (it is not however an intracellular recording). The event labelled *P*1 is almost certainly (as claimed) an epithelial impulse, recorded at rather low amplitude. There then follows a flurry of potentials intermingled with a biphasic deflection of large amplitude. This undoubtedly corresponds to what is here termed a composite swim: the flurry of small potentials would represent radial muscle contractions and the large event a circular contraction. Recordings alluded to in the text (p. 450) which showed *P*1 without after events were from points on the exumbrella too far away from the margin to allow muscle potentials to be picked up. Thus, the record does indeed demonstrate epithelial conduction, but it also shows two sorts of muscle activity.

(b) Stem

Recordings from the stem with suction electrodes show the electrical correlates of three conduction systems, all of which can be excited by single shocks delivered through another suction electrode on the stem (Figures 19A, B). All three systems are through-conducted along the whole length of the stem. Following the usage adopted for physonectid siphonophores we will refer to the systems as the two nervous systems (*n*1, *n*2) and the slow (*s*) endodermal epithelial system. Potentials associated with muscular twitches (*t*) in the ectodermal myoepithelium are also recorded, but these are not conducted along the stem. They are local potentials produced by input from nerves.

*n*1 and *n*2 are usually easily distinguishable in *Chelophyes* (Figures 19A, B) because one system tends to conduct faster than the other. However, the two systems may have very similar conduction velocities and their potentials may thus be superimposed, giving the appearance of a single event (Figure 19C). In one such preparation (Figure 19D) the event could be resolved into its two

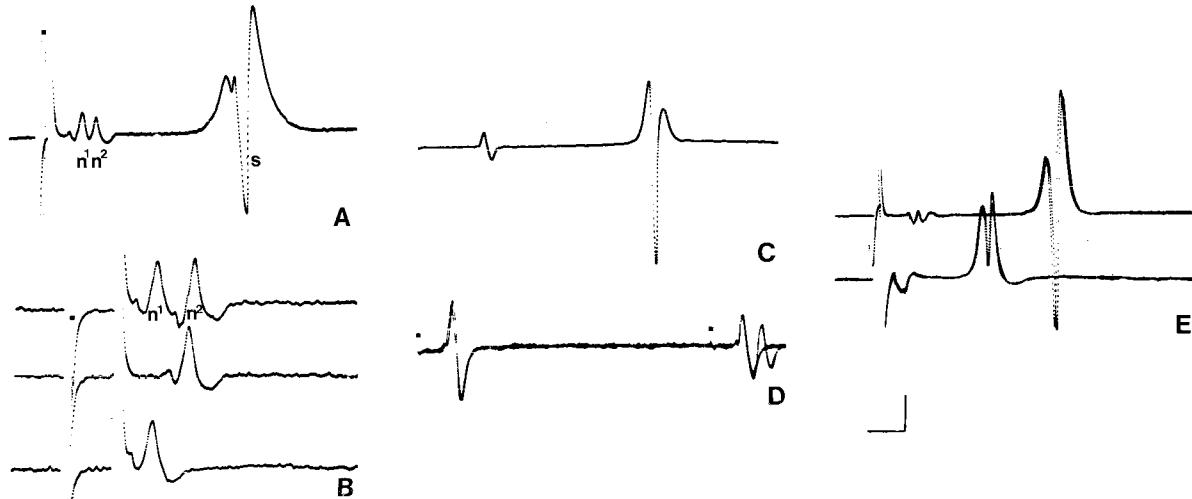


FIGURE 19 *Chelophyes* stem conduction systems. A. A single shock evokes all three potentials, n^1 , n^2 and s . B. The same preparation, showing independence of n^1 and n^2 . C. Another preparation in which n^1 and n^2 are indistinguishable owing to their similar conduction velocities. D. Similar preparation, two sweeps superimposed, showing separation of n^1 and n^2 on second shock given 40 ms after the first. n^2 failed to fire on one occasion. s did not fire. E. Preparation used for estimating conduction velocities, two recording electrodes in a line along the stem.

Scale. Vertical and horizontal bars represent: (A): 0.5 mv, 2 ms; (B): 0.2 mv, 1 ms; (C): 0.5 mv, 5 ms; (D): 0.2 mv, 5 ms; (E): 0.5 mv, 5 ms.

(lower amplitude) components by giving a second shock immediately after the first. It is not clear why conduction time for one of the systems increased and the other did not. Initiation delay must be taken into account as well as conduction velocity. The most reliable values for conduction velocities are presumably those obtained by measuring the intervals between potentials recorded sequentially by two recording electrodes, e.g. Figure 19E. However this requires a fairly long piece of stem. The actual velocity values obtained from the experiment in Figure 19E were 0.96, 0.80 and 0.24 m.s^{-1} for n_1 , n_2 and s respectively. The n values are rather low compared with other specimens of *Chelophyes* but the relationship $n_1/n_2 = 1.2$ is typical. Comparative values for three calyphophores are given in Table I.

Both n_1 and n_2 show spontaneous activity and function quite independently of one another. Both can fire repeatedly at high frequencies and have refractory periods of about 5 ms at 20°C .

The s system has a long refractory period (20 ms) and conducts slowly (0.2 - 0.3 m.s^{-1}). However, conduction time decreases with repeated stimulation. In physonectids there appear to be two reasons for this: firstly, the s spike has a long after-depolarization so that a second spike produced soon after the first reaches threshold more quickly. Secondly, the epithelium is electrically coupled to the ectodermal myoepithelium and depolarizations of the latter caused by n input invade the endoderm and again significantly reduce spike initiation latency and so increase conduction velocity. This effect, whereby s potentials are carried along at a rate approaching the conduction velocity of the stem nerves instead of at their own unassisted (very slow) velocity has been termed the piggyback effect (Mackie, 1976b) and will be referred to further below.

The stem of most calyphophores is inconveniently small for microelectrode work so the validity of this picture, which comes from work on physonects, has not been verified directly. There can be little doubt, however, that the

TABLE I

Representative conduction velocities (V) in metres per second for nerves (n) and epithelial (s) stem systems in three calyphophoran siphonophores, with data for two physonects from Mackie (1978) for comparison

Species	Vn^1	Vn^2	V_s	Temp. $^\circ\text{C}$	Giant fibres
Sub-order Calyphophora					
<i>Chelophyes</i>	1.1	0.9	0.25	22	present
<i>Sulculeolaria</i>	1.7	1.3	0.3	23	assumed present
<i>Hippopodius</i>	0.5	0.3	0.2	22	absent
Sub-order Physonectae					
<i>Forskalia</i>	3.7	2.0	0.4	20	present
<i>Nanomia</i>	2.7	1.5	0.3	14	present

arrangement is closely similar in the two suborders. Some intracellular recordings from *Sulculeolaria* fit the physonect model precisely, showing a resting potential in the ectoderm of -80 mv and small junctional potentials (15 mv) associated with *n* input and larger junctional potentials (20 mv) representing coupling potentials from *s* spikes in the endoderm. The fast twitch response of the stem muscles can be confidently discussed in the same terms as in physonects and, while no work has been done on slow contractions in calycophores, the physonect model, which relates them to *s* input, may well apply too.

Muscle twitch potentials may accompany even single *n* events and where *n* frequency is high the twitch potentials sum and facilitate achieving large complex depolarizations which overshadow and obscure the causative nerve events. In several experiments, addition of 1 part of isotonic magnesium chloride to four or five parts of sea water (1:4 or 1:5 Mg/SW) was used to reduce the amplitude of the twitch response.

Where, in the physonects, all evidence to date implicates *n*₁ and *n*₂ equally in twitch generation, there are signs that this is not always so in calycophores. In most records from *Hippopodius* (Figure 20A) twitch potentials follow *n*₁ only, or are larger after *n*₁ than after *n*₂. In *Chelophyses* on the other hand, *n*₂ seems to have the greater neuromuscular excitation role (Figure 20B). Both these records illustrate the facilitation of twitch potential amplitude with a second *n* event even where only one *n* system may be involved. Figure 20B also demonstrates reduced *s* latency following larger twitch potentials, the piggyback phenomenon.

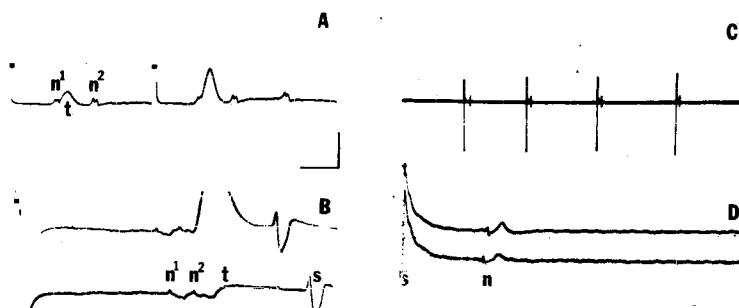


FIGURE 20 Extracellular recordings from the stem, three species. A. *Hippopodius*. Muscle twitch potentials (*t*) follow *n*₁, not *n*₂. Note facilitation of twitch response after second shock. B. *Chelophyses*, in 1:5 Mg/SW. The two traces were evoked by shocks about 1 second apart. Muscle twitch potentials (*t*) are associated with *n*₂, and show facilitation on second shock (upper). Note decrease in *S* conduction time on second sweep. C. *Abylopsis*. Series of *S* potentials, each of which triggered an *n* event about 40 ms later. D. *Hippopodius*. Oscilloscope set to trigger on rising slope of *s* potential. In two successive sweeps (and consistently in others in this series) an *n* event follows the *s* potential 40 ms later.

Scale. Vertical and horizontal bars represent: (A): 1 mv, 20 ms; (B): 0.5 mv, 5 ms; (C): 1 mv, 0.2 ms; (D): 0.5 mv, 15 ms.

In physonects there is no evidence that *s* events ever directly or indirectly affect the patterns of activity seen in the two nervous systems but in all of the calycoophores observed *s* events have an obvious triggering role. Many *s* potentials are followed after 30–60 ms by an *n* potential, either *n*1 or *n*2, sometimes both (Figure 20C, D). Triggered *n* events can contribute to and enhance large scale muscle twitches. There are thus the makings of a positive feedback loop, since *s* events can probably be generated by large *n*-induced muscle twitches, judging by the findings on physonects.

In an attempt to localize the site of interaction between the *s* and *n* system a *Hippopodius* stem was cut up into short segments. Triggering was observed at all levels, so the two conduction systems must be presumed to interface frequently along the stem. The mechanism and sites of interaction remain unknown, and are ignored in the already complicated Figure 22.

(c) *Communication between the anterior nectophore and the stem*

The marginal nerves of the anterior nectophore are connected to the nerves in the stem by a nerve tract which runs through the hydroecial ectoderm as described above. In addition to this presumed conduction route the ectoderms of the two regions are directly connected, as are the endodermal epithelia and either or both of these epithelial pathways might, in theory, function as a conduction route. The problem is to decide which are the actual conduction routes. Paradoxically the most obvious route, the nerve tract, has been the hardest to associate with a specific function.

Stimulation of the exumbrellar epithelium evokes propagated impulses which on reaching the stem evoke activity in all three conduction systems (Figures 21A, B). Cutting the hydroecial nerve and hydroecial endoderm canal does not block transmission of these responses, proving that an ectodermal epithelial event is sufficient to generate activity both in the nervous and non-nervous conduction systems of the stem. Presumably this takes place at the root of the stem.

It is possible to insert a recording electrode into the hydroecium and to record from the very root of the stem where it joins the nectophore. Nervous events recorded from this region show only small muscle twitch potentials compared with recordings from other parts of the stem. The arrival of an epithelial impulse from the nectophore epithelium is marked by a large potential at the stem root, which presumably represents, at least in part, the epithelial impulse itself. Each large depolarization is followed by a ripple of *n* events. In Figure 21B two such epithelial depolarizations were recorded, because the exumbrellar epithelium fired twice. Further along the stem (upper channel) the large epithelial events are still present but now they have assumed the unmistakable characteristics of *s* potentials. The time interval between them has increased, because the first potential is being carried piggyback by a

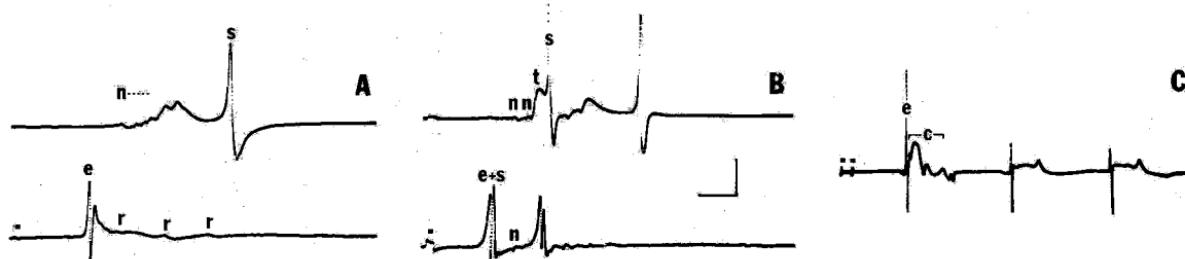


FIGURE 21 *Chelophyses*, nectophore-stem interactions. These records are discussed in detail in the text. A. Upper electrode several millimetres down the stem, lower on nectophore margin. A stimulus to the exumbrellar evoked an epithelial pulse (*e*) which caused radial contractions (*r*) in the velum and *n* and *s* events in the stem. B. Lower electrode at stem root, upper further down stem, shock on exumbrella. The large signals recorded at the stem root are considered to be combined ectodermal and endodermal (*e* + *s*) depolarizations. C. Recording on the velum, stimulating on the stem (two shocks). Excitation arrived at the nectophore in the form of an epithelial (*e*) impulse and a composite swim, followed by spontaneous swims. *c*: circular component of composite swim (see Figure 30c).

Scale. Vertical and horizontal bars represent: (A) 2 mv (upper), 0.5 mv (lower), 10 ms; (B) 2 mv (upper), 1 mv (lower), 10 ms; (C) 1 mv, 50 ms.

large twitch depolarization. The second has fallen off its carrier potential and is therefore propagating more slowly than the first. The *n* events, being more rapidly conducted, now precede the *s* potentials.

A point of special interest in this and similar recordings concerns the apparent direct transformation of an ectodermal epithelial impulse into an endodermal one (*s* potential). The impulse arrives from the ectoderm of the nectophore, triggers *n* events and simultaneously propagates down the stem endoderm as an *s* potential, frequently with piggyback assistance from ectodermal twitch depolarizations. Production of *n* events in these circumstances must involve an epithelio-neural triggering step of the type seen at the nectophore margin. Production of the *s* potential might also be indirect, but this is unlikely since conducting epithelia are only known to excite one another by direct current flow from cell to cell. We propose therefore that the ectoderm and endoderm are tightly coupled at the stem root (more so than in other regions) so that ectodermal impulses can cross into the endoderm by direct current flow. Impulses do not continue down the stem ectoderm: this epithelium is incapable of propagative electrogenesis in any siphonophore.

It might be expected that, if ectodermal impulses can cross freely into the endoderm at the stem root, the reverse process could also occur, but there is no evidence that this can happen. *s* events never invade the exumbrellar epithelium. It would appear then that impulse traffic is one-way at this interface. A similar rectifying step between two conducting epithelia occurs at the margin of *Hippopodius* (Bassot *et al.*, 1978). The fact that impulses do not cross this interface does not mean that *s* activity in the endoderm would not be picked up as coupling potentials in the ectoderm and contribute to ectodermal depolarizations, as in other parts of the stem.

Treatment with 1:4 Mg/SW does not block the nectophore/stem interactions described above except in so far as it reduces the amplitude of twitch depolarizations and so abolishes any piggyback effects on *s* conduction.

Stimulation of the stem strong enough to evoke a flurry of nervous events and muscle twitches in the stem is followed by a contraction of the radial muscles in the nectophore margin and one or a few series of swimming contractions. The stem *s* system need not be activated for this to happen. This response is not abolished by cutting the hydroecial canal and nerve tract, proving that excitation can pass to the nectophore margin from the stem via the exumbrellar epithelium. Patterns of *n* events that fail to produce substantial stem twitches do not result in firing of the exumbrellar epithelium, and this becomes more marked in jaded specimens.

On the assumption that translation of stem activity into exumbrellar impulses is mediated by nerve induced depolarization of the ectoderm in the transitional region, it would be expected that magnesium, which is a fairly reliable blocker of chemical synapses in coelenterates, would interfere more

with passage of excitation in this direction than in the reverse direction, from nectophore to stem, where the critical step probably does not involve a chemical transmitter. Epithelia have never been observed to synapse directly on to nerves in coelenterates but are believed to excite them electrically (Mackie, 1975; Spencer, 1981). While various observations suggest that this prediction may prove to be correct, the critical experiments required to test it properly were not carried out.

The role of the hydroecial nerve tract has not been established with certainty, but there are good clues as to its function, not least of which is the evidence from physonects, where a comparable nerve mediates escape swimming (Mackie, 1964). We know that there is an alternate epithelial pathway, capable of functioning after the nerve has been cut. The presence of a giant fibre in the nerve tract again speaks for some role in relation to escape. Some of our results tend to confirm this picture. In Figure 21C for example, despite the slow sweep speed, it can be seen that the epithelial pulse arrives *only just before* (c.5 ms) the circular swimming muscle fires, implying that the swimming pacemakers had already been activated by impulses arriving via a non-epithelial, fast route, for when the entire response is epithelially mediated, delays of 15–40 ms are seen. The obvious candidate for a fast pathway is the hydroecial nerve tract, its giant fibre in particular. As shown by the electron microscope investigation, other nerves run along beside the giant, so other swimming activities may also be neurally coordinated. These suppositions need to be tested further however.

(d) *Coordination of activities involving the posterior nectophore*

Such activities were not systematically studied in this investigation. Observations by Bone and Truman (1982) suggest that swimming of the two nectophores may at times be coordinated, at times independent. The absence of a nerve tract connecting the nectophore margin and the stem means that any coordination must involve the equivalent of the epithelial route which we know to be present in the anterior nectophore. The nerves entering the posterior nectophore from the stem do not extend beyond the apical peduncle, but, before they end, they make numerous synapses with adjacent epithelial cells (see earlier, p. 12). These could be sites where nerve impulses jump into the exumbrellar epithelium. The latter is probably in electrical communication with the endoderm, for connections between the two cell layers occur at the base of the peduncle, and again at the margin. Thus, as in the anterior nectophore, there is probably a single effective epithelial pathway, but it involves both ectoderm and endoderm layers. The situation in the posterior nectophore of *Chelophyses* would appear to accord very closely with that described for the nectophores of *Hippopodius*, where connecting nerves are also lacking (Bassot *et al.*, 1978).

DISCUSSION

Considering first the histological findings, the nectophores of *Chelophyses* show the basic medusan locomotory apparatus modified in some novel ways. The simplicity of the inner nerve ring is a predictable feature, given that there is only one effector system (the striated muscle sheet) in the subumbrella. In striking contrast is the complexity of the outer ring and here we find a new type of sheathing epithelial cell characterized by abundant SER, somewhat resembling steroid secreting cells in vertebrates and, like them, innervated. It would be interesting to compare the appearance of these cells before and after prolonged nervous stimulation. Two giant non-nervous cells run from the outer nerve ring toward the hydroecium. They too are innervated, as are some enigmatic cells lying next to them. Nothing remotely comparable to these features has been reported in other medusans.

The neurons and mechanoreceptors of the outer ring are similar to those described for medusae. The receptors both synapse with, and receive synapses from, nerve cells, as in the ocellus of *Nemopsis* (Yamamoto and Yoshida, 1980). This suggests that the sensitivity level or range can be adjusted, but no experimental evidence is available.

The layout of the nectophore epithelia presents few surprises. Connections exist between the ectoderm and endoderm at the margin, as required by the physiological findings. In contrast to *Hippopodius*, *Chelophyses* lacks sub-umbrellar radial muscle and cannot curl in its margin. Radial smooth muscle occurs in the velum only, and the physiological recordings show that these fibres contract along with the circular striated system during some swims. Unfortunately the existence of two sorts of swimming contractions has not been verified by direct observation of the behaviour, and we cannot say for sure what is the effect of adding a radial component to the swim. Two possibilities are (a) that radial contractions, by antagonizing contractions of the circular muscles lining the inner face of the velum, help maintain the shape of the velar orifice during expulsion of water, so producing a narrower, faster jet and better thrust, and (b) that, if asymmetrical, the radial contractions might deflect the water jet sideways as in physonects, allowing the animal to alter course. Further study using cinematographic analysis and pressure transducers as in Bone and Truman's (1982) investigation should lead to clarification of these points.

The striated muscle sheet is one of the most specialized coelenterate muscles, possessing not only structures resembling intercalated discs, but also a system of sarcolemmal invaginations reminiscent of the transverse tubular (*t*) system of muscles in higher animals. However the *t* tubes do not associate internally with *sr* or other vesicular components in any consistent way. Such elements are not present in abundance. In view of these observations, and because Ca does

not enter the muscle from the outside during activation, or not over most of its surface, Bone (1981) suggests that the *t* tubes are Ca storage sites. If so, the cell membrane of the *t* tubes would have to be capable of admitting Ca along with Na when excited. There are a number of precedents for cells having different conductances in different areas of the cell membrane (e.g., Naitoh and Eckert, 1974). The densely staining material lying within the *t* tubes might have some special Ca^{2+} binding affinity.

Turning to the stem we find a microscopic structure similar in most respects to that of physonects: a strongly developed longitudinal muscle layer with innervating nerves, and a weakly developed circular muscle epithelium in the endoderm, lacking nerves. Our investigation has allowed us to elucidate how the complex interdigitating array of myofibrils arises during growth. The partitioning of this thick mass of muscle by mesogloea intrusions is presumably significant in terms of mechanical support, but it might also be related to functional separation of individual motor blocks within the system. Nutrient transfer from the endoderm would also necessarily involve mesogloea pathways, so well developed mesogloea intrusions would be expected. We have been able to distinguish histologically two giant axons in the stem whose presence accounts for the high conduction velocities found in physiological experiments. The axons are smaller than their counterparts in physonects but similar in appearance. The associated nerve plexus cannot be visually resolved into two sub-systems, although evidence from experiments on *Nanomia* shows that there must be two such systems in the physonects.

The only previous evidence for nervous connections between nectophore and stem in a calycothoracan is a brief mention in Mackie (1964) referring to *Muggiae atlantica*. By contrast, *Hippopodius* and related prayids lack such connections. In *Chelophyses* and *Muggiae*, the nerve passes through a deep notch in the hydroecial mouth plate. Other diphycids having this feature are *Lensia*, *Sulculeolaria* and *Eudoxoides*. It may be that they also have hydroecial nerves, and that *Diphyes* and *Dimophyses* which lack the notch, lack the nerve. It has been suggested above that a primary function of the nerve is to decrease conduction time between stem and nectophore in escape behaviour, and we have found the nerve to contain a single giant fibre. Forms lacking the nerve would be expected to show sluggish responses. The tract also contains small nerve fibres, some of which innervate the enigmatic non-nervous cells of the notch region. The tract may therefore be implicated in several different integrative roles.

Turning now to the physiological results, the main action systems and coordination pathways are summarized in Figure 22. Some of the these have been inferred rather indirectly but still inspire sufficient confidence to be included; others are directly demonstrable. Intracellular recordings are needed to confirm several points. It is encouraging that past models derived

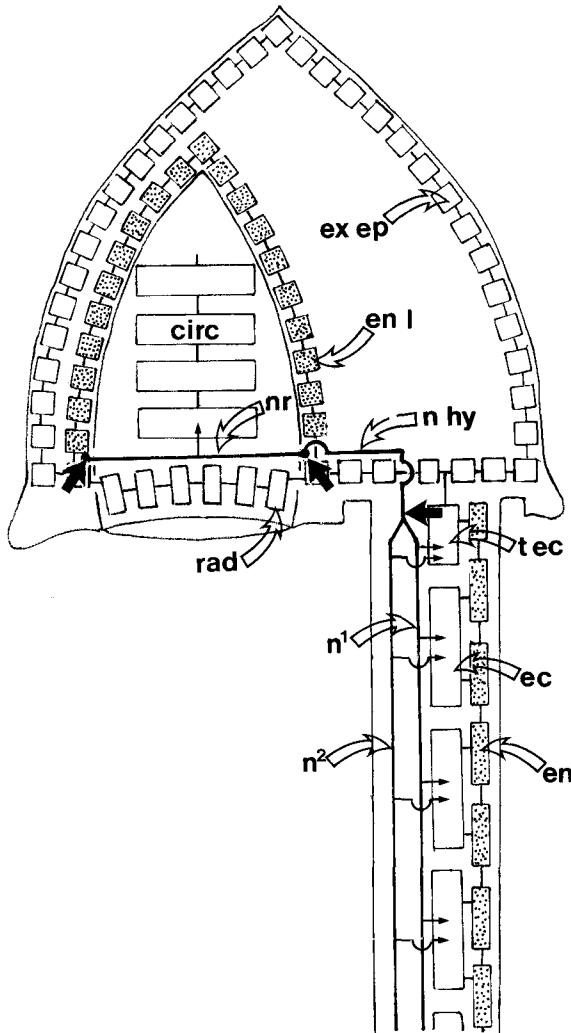


FIGURE 22 Principal action systems and coordination pathways in and between the anterior nectophore and stem of *Chelophyes*. Large black arrows symbolize epithelial-neural excitation steps, small ones neuro-epithelial. Lines between excitable units represent coupling. No attempt is made to distinguish degrees of coupling, threshold differences etc at the various junctions. The existence of a pathway does not mean that it is active in all circumstances.

Stimulation of the exumbrellar epithelium (ex ep) evokes impulses which propagate to the margin where they excite nerves in the nerve ring (nr), which excite the circular swimming muscle (circ). Epithelial impulses also propagate directly to the radial muscles (r) of the velum, causing them to contract during swimming.

On reaching the stem, epithelial impulses pass directly to ectoderm cells in a transitional zone (tec) which has the property, when excited, of exciting the stem nervous systems (n^1 , n^2). Depolarizations of the transitional ectoderm also pass directly to the endoderm (en) where they propagate as s events.

from extracellular recordings have proved to be fairly realistic when tested by intracellular recordings (see for instance Chain, 1981). Comparison of Figure 22 with a similar scheme for interactions between stem, gastrozooids, tentacles, bracts and palpons in physonects (Mackie, 1978, Figure 10) will show many points of resemblance. Our present findings tend to draw the two suborders closer together rather than revealing profound differences. The organization of the stem systems is virtually identical. Coupling lines replace two headed arrows between the two cell layers in the present figure as the interactions observed are almost certainly mediated electrically. The ability of *s* impulses to trigger nervous events in calycophores is a novel feature which the diagram makes no attempt to explain. In the interests of simplicity only those systems which are reasonably well understood are included.

As noted earlier, there is only one physiologically recognizable epithelial pathway between the stem and the nectophore although the two cell layers may participate. For simplicity, Figure 22 shows a single pathway.

The posterior nectophore is omitted. Coordination of responses between it and the stem may be assumed to occur as in the anterior nectophore, but with the omission of a direct nervous link.

In siphonophores, as in other hydrozoans, spontaneity nearly always seems to be a nervous property. Both stem nervous sub-systems and the nerve rings of the nectophores contain impulse generators producing characteristic output patterns. Epithelia, both simple and myo-, show little if any intrinsic rhythmicity with one possible exception, the stem endoderm, where *s* events have been reported to occur spontaneously (Spencer, 1971; Mackie, 1976). The evidence however is inconclusive.

Conducting epithelia are obviously very important in *Chelophyses*. Those of the nectophore feed into the nervous system where they evoke swimming, rather than the crumpling responses seen in free medusae. The crumpling musculature is absent. A parallel exists here with the trachyline medusa *Aglantha* which has also lost its crumpling effectors (Mackie, 1980). In *Aglantha* however epithelial impulses appear to inhibit the swimming pacemakers rather than exciting them as in *Chelophyses*, and this is also true in other medusae, including *Polyorchis*, where Spencer (1981) recorded inhibitory hyperpolarizations from within the swimming motoneurons.

Throughout the length of the stem, activity in *n1* and *n2* causes twitch depolarizations in the ectodermal muscles (ec) but impulses do not propagate within this tissue. Two-way interactions occur between the ectoderm and the endoderm (Mackie, 1976).

Activity in the stem nerves leads to twitch depolarizations in the transitional ectoderm which, if sufficiently large, propagate to the exumbrellar epithelium as exumbrellar impulses. On reaching the nectophore margin they cause swimming as before.

The hydrocial nerve (*n hy*) is implicated as an alternate, but faster, pathway mediating escape swimming responses following stem stimulation.

Whether, when hit by or colliding with a foreign body, the best strategy is to swim or to stop swimming will doubtless vary from one species to another. *Chelophyes*, with its extraordinary powers of locomotion (Bone and Truman, 1982) escapes by swimming, as does *Aglantha*; others protect themselves by stopping swimming and by crumpling.

In all cases where epithelial excitation causes a response in nerves, whether to excite or to inhibit them, the evidence indicates an electrical transmission mode. Such responses are not affected by magnesium levels sufficient to block chemical junctions in the same animal. Epithelioneural translation steps are vitally important in two places in *Chelophyes*, at the nectophore margin and the stem root (Figure 22, heavy black arrows). The persistence of coordination after the hydroseal nerve has been cut bespeaks the ability of nerves to excite epithelia and to be excited by them. In physonects, similar translation zones probably occur in equivalent places, but they have only been demonstrated at the bract-stem interface (Mackie, 1978). Here, as in the transitional ectoderm of the stem root in *Chelophyes*, the translation process is not a simple, all or none affair. The coupling is quite labile, and the threshold for passage across

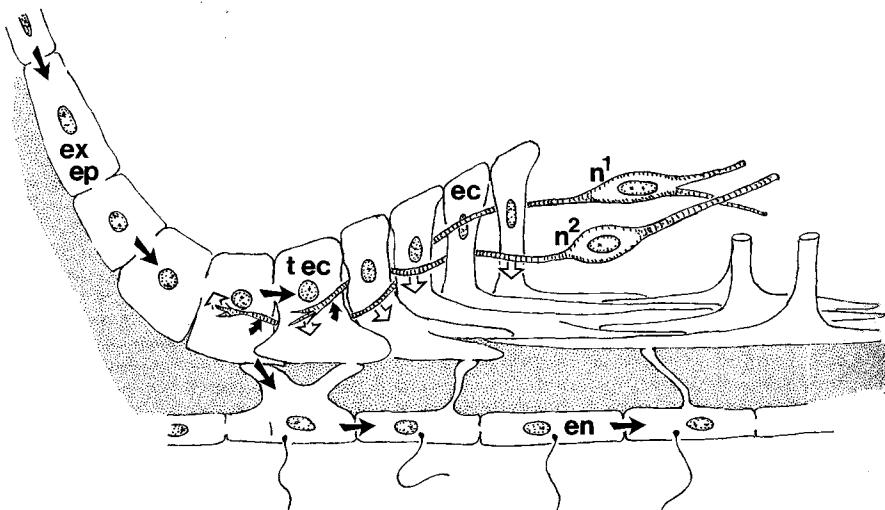


FIGURE 23 Proposed interactions in and around the transitional zone at the base of the stem, graphically portrayed.

Propagated depolarizations (large black arrows) pass from the exumbrellar epithelium (ex ep) and enter the transitional ectoderm (tec), and endoderm (en) where they continue as *s* events. They also excite nerves (*n*¹, *n*²) by an unknown mechanism (small black arrows). From then on down the stem the mature ectodermal myoepithelial cells (ec) are excited conventionally through chemical synapses (outline arrows), although there is secondary input in the form of coupling potentials from the endoderm, which sends bridging processes across the mesogloea to join the ectoderm.

Excitation can pass in the reverse direction (to the exumbrellar epithelium) when nerves sufficiently depolarize the transitional ectoderm.

the interface may be high. At the nectophore margin by contrast, a single epithelial impulse will suffice to excite the swimming pacemakers in a fresh specimen. Analysis of the mechanisms involved in these epithelioneural translations is now one of the more pressing needs in coelenterate neurophysiology. Remarkably similar problems face tunicate workers although the mediation may here be synaptic (see review by Anderson, 1980).

Neuro-epithelial excitation steps are easier to explain than epithelioneural. They involve chemical synapses and are affected by magnesium. The present EM study has revealed synapses in the required places including the important interface between the peduncular nerve and the exumbrellar epithelium in the posterior nectophore.

The transitional ectoderm at the stem root is a critical region having special properties as described. It is shown in Figure 22 as a truncated version of the symbol for stem ectoderm on the grounds that it probably consists of immature cells in the growth zone of the stem. The special interactions occurring in the tissues of this region would be lost or altered as differentiation proceeds and the cells get carried down the stem; in particular, the tight coupling between the ectoderm cells which allows impulses to spread between them is lost, and their ability when depolarized to excite nerves in the locality.

Figure 23 is an attempt to portray the interactions occurring in this critical transitional zone.

A point of importance in any future study of the behaviour of *Chelophyses* would be the elucidation of the role or roles of the hydrocial nerve. It is unlikely that it merely duplicates the function of the epithelial link, or merely provides a faster pathway. Careful comparison of the behaviour of *Chelophyses* before and after sectioning of the nerve should clarify matters.

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