

THE BEHAVIOUR AND NEUROMUSCULAR SYSTEM OF *GONACTINIA* *PROLIFERA*, A SWIMMING SEA-ANEMONE

By ELAINE A. ROBSON

Department of Zoology, University of Cambridge, and

**Department of Zoology, University of Reading*

(Received 30 March 1971)

INTRODUCTION

A few sea-anemones are able to swim. In *Stomphia coccinea* and at least one species of *Actinostola* swimming consists of sharp flexions of the column due to parietobasilar contractions (Yentsch & Pierce, 1955; literature reviewed in Robson, 1966, and in Ross, 1967; Ellis, Ross & Sutton, 1969). *Bolocerooides* lashes the tentacles synchronously (Komori, 1931; see Josephson & March, 1966) and *Gonactinia*, which is unrelated to it and much smaller, swims in a similar way (Prouho, 1891; see Robson, 1966). Swimming has a relatively high electrical threshold and fatigues rather rapidly. It seems to depend upon the excitation of a pacemaker system distributed equireadially at the circumference. In *Stomphia* it is possible that pacemaker activity may be associated with a population of multipolar nerve cells in the column (Robson, 1963).

The significance of swimming behaviour is not entirely clear. It follows appropriate sensory and electrical stimuli and possibly represents an escape reaction from predators (and see Ross, 1967).

The chief co-ordinated sequences of short-term behaviour in *Gonactinia* are walking and swimming, and catching and swallowing prey. These have been studied to ascertain the parts played by reflex and intrinsic activity, if possible in terms of the neuromuscular elements concerned. Neither form of locomotion can be regarded as a chain of reflexes, for in swimming the tentacle movements follow excitation of a pacemaker, and walking too seems to depend on intrinsic activity as yet undefined. Reflexes are important in feeding behaviour. All short-term patterns of behaviour, however, may be thought of as occurring in the context of long-term phases of activity (Batham & Pantin, 1950*a, b*).

When the contractions of different muscles are studied striking differences emerge between ectodermal and endodermal elements. Although the endodermal neuromuscular system appears to be similar to that of other anemones, the ectodermal system responds in quite unusual ways, and the neuromuscular arrangements in this anemone appear to require a new kind of explanation. The ectodermal muscle plays a large part in movements of the tentacles and often of the column. It is so well developed, however, in few other anemones (including, though, *Bolocerooides*; see Carlgren, 1942) and its properties in *Gonactinia* appear to be exceptional. The model

* Present address.

suggested for *Gonactinia* can be applied to *Bolocerooides* and also to *Stomphia*, where the muscle in question is endodermal.

Gonactinia prolifera is an unusually lively anemone about the size of a large *Hydra*. It is almost unique in multiplying by transverse division (e.g. Fig. 8), a process which will be referred to as strobilation without implying any homology with Scyphozoa (Sars, 1835; Blochmann & Hilger, 1888; Carlgren, 1904; see Schmidt, 1970). In dividing specimens the behaviour of adult and differentiating tentacles can be compared, giving some insight into the development of swimming and other responses.

Useful comparison can be made with *Protanthea simplex*, the only related species (family Gonactiniidae), since the anemones have many features in common. In consequence of well-developed ectodermal muscle both use the tentacles very efficiently to catch zooplankton and to creep about. *Protanthea* does not swim and *Gonactinia*'s ability to perform repeated tentacle strokes probably lies in relatively small differences in the properties of the ectodermal nervous system.

MATERIAL AND METHODS

Gonactinia prolifera (M. Sars) has been found from Scandinavia to the Mediterranean at depths probably between 5 and 100 m (see Carlgren, 1949). Specimens were obtained from the Gullmarsfjord within a depth of 15–26 m, on stones, shells and *Flustra*, and on loose algae such as *Laminaria*, *Fucus*, *Demarestia*, *Ceramium* and *Brogartella*. They need clean plankton-bearing water and are not found in muddy samples. In this habitat the salinity varies little from 34‰ and annual temperature extremes are within 0 to 20 °C (Swedmark, 1966). The anemones survived well in aquaria with flowing sea water at 14–17 °C. Observations were made in still water at temperatures within 10 to 15 °C, a range within which responses appear to be normal.

Fresh specimens are characteristically salmon-pink owing to carotenoids derived from crustacean prey. Little is known about the biology of these anemones but their normal food supply must be abundant because in aquarium conditions they become pale even when fed with *Artemia* nauplii and they do not produce new strobilae. The largest specimens seen were 6 mm high with the crown of tentacles 16 mm in diameter and one may suppose that in natural conditions rates of growth and division are governed mainly by the food supply. In September, when the present material was collected, both *Gonactinia* and *Protanthea* may have ripe gonads and the two anemones probably have similar life-histories (Nyholm, 1959). In *Gonactinia* gametes are often produced by strobilating specimens, hence sexual and asexual reproduction are not exclusive modes of differentiation (Blochmann & Hilger, 1888).

In this study direct observation has been used a good deal, supplemented by analysis of ciné film taken with a Bolex H. 16 camera. The frequency of swimming strokes was recorded also by using a tapping key and kymograph. For electrical stimulation shocks from an AEL Stimulator 104A were delivered using chlorided silver wires in glass tubes or plastic suction electrodes filled with sea water. Details of procedures are given later as needed. Standard histological methods were employed.

THE NEUROMUSCULAR SYSTEM

Relevant features of general anatomy are shown in Fig. 1 A, B, C. There are two cycles of tentacles, usually 16 in all, and a corresponding number of mesenteries, eight of which join the pharynx. Below the pharynx the mesenteries remain narrow but they fan out basally and the main septa almost reach the centre of the pedal disk.

It is not possible to construct a 'wiring diagram' of the neuromuscular and sensory systems from present anatomical facts. The general histology resembles that of *Protanthea* (Carlgren, 1893), but the anemone is so small that details of the neuromuscular system are resolved with difficulty by the light microscope. Vital staining with methylene blue and Batham-Holmes's silver method (Batham, Pantin & Robson, 1960) have revealed something of the ectodermal nerve cells and sense cells but as yet very little about these elements in the endoderm. An incomplete description nevertheless affords some basis for discussion.

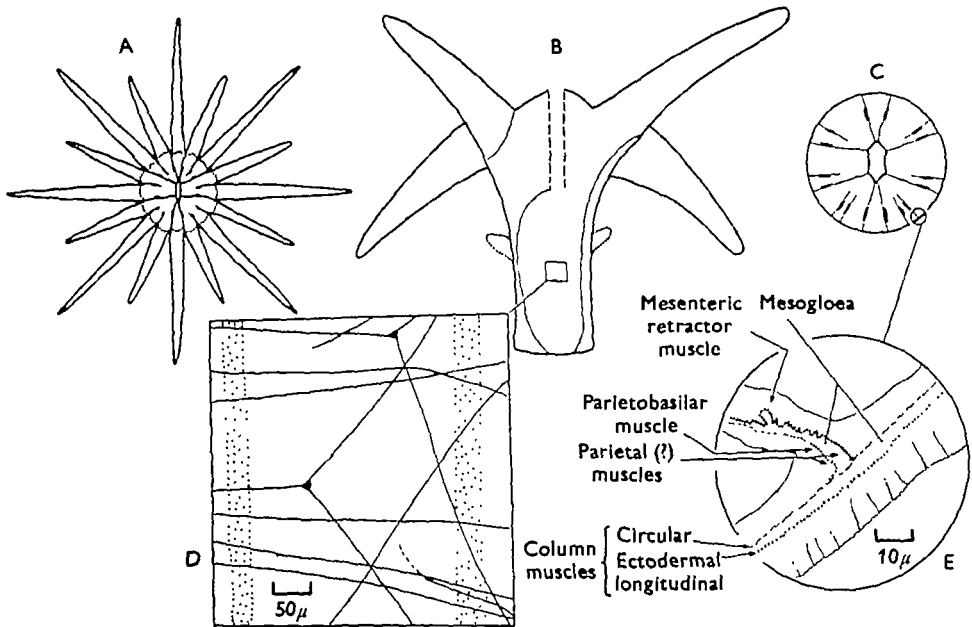


Fig. 1. Structure of *Gonactinia*. A, Diagram of the crown from above, with positions of mesenteric retractor muscles indicated. B, Diagram of side view showing outlines of tentacles (including strobila) and primary and secondary mesenteries. C, Diagram of transverse section through pharynx showing arrangement of mesenteries and retractor muscles. D, Drawing of part of a preparation of the column showing the ectodermal nerve-net. Area as indicated in B, with positions of two mesenteries stippled. Batham-Holmes silver method. E, Drawing of transverse section of column and part of a secondary mesentery to show the arrangement of muscles. Area as indicated in C.

Ectoderm

There are stout longitudinal muscle fibres over the column, tentacles and oral disc (Fig. 1 E). At the base of the column the muscle fibres go to the edge of the pedal disk, which lacks ectodermal muscle, while at the top they run onto the tentacles. The radial muscle of the oral disc is also continuous with the longitudinal muscle of

the tentacles. The muscles of the column and tentacle bases are able to give strong twitch-like contractions, and the largest muscle fibres correspondingly occur here. In the column, for example, they are sometimes more than $700\ \mu\text{m}$ long and over $1\ \mu\text{m}$ wide. In the distal part of the tentacles and in the oral disk they are shorter, perhaps only $250\text{--}300\ \mu\text{m}$ long, and finer.

In view of the interesting physiological properties of the ectodermal neuromuscular system and the interpretations suggested later it is a pity that more cannot be said here about the muscle itself. The fibres are unstriated and tapering, each with an adjacent central nucleus, that is to say, they do not form a musculo-epithelium. It is not clear to what extent they form a muscle-field, i.e. a physiologically continuous smooth muscle (Batham & Pantin, 1951; see p. 634).

The presence of nerve cells associated with ectodermal muscle was reported by Carlgren (1893). Successful silver preparations have shown up this ectodermal nerve-net overlying the muscle fibres in the column and oral disc (Fig. 1 D). It consists of bipolar and tripolar nerve cells, the former resembling those of *Metridium* on a small scale (Pantin, 1952; Batham, Pantin & Robson, 1960). The cell bodies are about $10\ \mu\text{m}$ wide, with processes usually less than $0.5\ \mu\text{m}$ wide whose terminations cannot be seen. For the size of the anemone these neurites are quite long, some over $1\ \text{mm}$.

Multipolar cells have sometimes stained in methylene blue preparations. They are up to $12\ \mu\text{m}$ in diameter, with from 3 to 5 fine processes sometimes more than $300\ \mu\text{m}$ long. These cells have been seen on the oral disk, tentacles and upper column and they could be numerous since on one occasion seven were counted in an area $130\ \mu\text{m}$ across (a density of perhaps $500/\text{mm}^2$).

Methylene blue also reveals sense cells scattered over the column and crown, with some concentration at the tips of tentacles. The cell body is about $10\ \mu\text{m}$ high and has a cilium of at least the same length, and one or two fine neurites which can usually be traced for about $50\ \mu\text{m}$. In the tentacles sense cells are usually unipolar and centripetal as in other anemones (Groselj, 1909). The column has mixed unipolar and bipolar sense cells without special orientation, whose numbers in some preparations correspond to a density of $5000\text{--}10000/\text{mm}^2$.

The innervation of the ectodermal muscle is as yet unknown since the fine processes of nerve cells and sense cells taper to disappearance in light microscope preparations.

Endoderm

The endodermal muscles are like those of other anemones with the conspicuous absence of a marginal sphincter and of basilar muscles in the pedal disk (Table 1). In contrast to the ectoderm all the muscle fibres seem to be musculo-epithelial.

Circular muscle is found throughout the column (fibres $100\text{--}150\ \mu\text{m}$ long), tentacles ($50\text{--}100\ \mu\text{m}$), oral and pedal disks and pharynx, and the mouth can be pursed by a weak sphincter outside the lips. Each mesentery has three or possibly five muscles: the retractor, which can contract fast, has fibres nearly as stout as those of the ectodermal longitudinal muscle of the column; on the opposite face is transverse muscle (which in the eight primary septa opens the pharynx); and there is also the parieto-basilar muscle. Near the pedal disk the retractor and parietobasilar fan out, but not necessarily in exact correspondence on the two faces of the mesentery. It is difficult to see this, and the action of these muscles in locomotion needs to be clarified (p. 625).

Table 1. *Distribution of muscles in Gonactinia*

Part	Ectodermal muscle	Rate of contraction*	Function	Endodermal muscle	Rate of contraction*	Function
Column	Longitudinal	Seems variable from twitch (0.3 sec) to slow	Bends column	Circular	Slow	Elongates column
Tentacle	Longitudinal <i>which comprises</i> (i) Basal, (a) oral (b) aboral (ii) Distal	Slow (2-10 secs; 0.5 mm/sec) (i) Twitch, seems variable (duration 0.2-0.7 sec) (ii) twitch, seems variable (duration 0.2 sec or more) Probably slow	General shortening (e.g. walking) (a) inflexion, (b) abflexion Bends or shortens tentacle Opens mouth	Circular	Slow	Elongates tentacle
Oral disk	Radial		Opens mouth	Circular (includes oral sphincter)	Slow	Constricts disk and mouth
Pharynx	Longitudinal	Slow	Shortens pharynx; peristalsis	Circular	Slow	Peristalsis
Mesenteries	None	—	—	Retractors	Twitch (duration 0.2 sec plus; ? 7 mm/sec) also slow (duration e.g. 2 sec)	Shorten column (and possibly bend)
				Transverse	Slow	Open pharynx
				Parieto-basilar	Slow (? also twitch)	Bending; pedal movements
				(? Parietals)	(Slow)	(Would shorten and bend column)
				Marginal filaments	Slow	Envelop prey
Pedal disk	Absent	—	—	Circular	Slow (duration e.g. 1-2 sec)	Peristaltic action when detached; pedal disk becomes concave

* From ciné film at 18 frames/sec.

From mid-pharyngeal level downwards, transverse sections show groups of longitudinal muscle fibres situated parietally on both sides of the septa. Whether or not these can be considered to form distinct parietal muscles as in many other anemones, they probably contribute to slow shortening of the column.

Until now most attempts to stain endodermal nerve cells and sense cells have failed, but as the retractor muscles give quick facilitated contractions the presence at least of a mesenteric nerve-net seems probable (p. 624).

Functions of muscles

Table 1 lists recognizable muscles and their functions.

The tentacles are able to bend axially (flexion) towards the mouth or out towards the column, and these movements will be termed *inflexion* and *abflexion* (Fig. 10A). They are due to contraction of the proximal longitudinal muscle of the tentacles on the oral and aboral surfaces respectively. The quick twitches performed by these basal muscles distinguish them functionally from distal regions (p. 628).

CNIDAE

Cnidae are included here because without them the capture of prey and locomotion would be impossible, and it is necessary to understand the part they play in the animal's behaviour. According to Carlgren (1940) five kinds are present: microbasic *p*-mastigophores and amastigophores (penicilli), atrichs, basitrichs, and spirocysts. With the exception of mesenteric filaments most of the cnidae are ectodermal. The tentacles and especially their tips are well supplied, and also the lips and pharynx. They are found all over the oral disk and column, and the pedal disk has some spirocysts and penicilli but it is not clear when these are used.

As in *Protanthea* the tentacles adhere in walking by means of spirocysts if not atrichs as well (Nyholm, 1959). These cnidae discharge very readily and are used both to anchor the tentacles and to arrest prey. The discharged thread is very sticky and behaves as though it were elastic, so that a captured nauplius of *Artemia* struggles against the equivalent of stretchable flypaper. During walking the tentacles leave 'footprints' of discharged spirocysts.

Microbasic amastigophores, on the other hand, contain toxin and are used mainly to stun prey and perhaps in defence (not studied here). If *Artemia* nauplii are pierced by the shafts of only one or two of these cnidae they may stop swimming almost at once, their movements becoming feeble and then ceasing. Penicilli also discharge in the absence of prey since some are found in tentacular 'footprints'. Their mechanical threshold seems higher than for spirocysts, and they are most effective against nauplii which charge into a tentacle at speed; such prey seems to be immobilized at once, whereas nauplii which cause less impact continue to move for longer after they are caught. Prey which is still struggling when swallowed is dispatched by penicilli of the lips and pharynx or mesenteric filaments.

Discharged cnidae are extruded, and the timing of this process affects the duration of tentacle adhesion. Until the tentacles adhering to prey or to the substrate are released some force is needed to pull them away. Yanagita (1959) found rapid extrusion of undischarged penicilli from acontia of *Diadumene* to be an ion-dependent process

Requiring chloride. In *Gonactinia nauplii* arrested by the tentacles may drop into the mouth after 10 sec or less (p. 618) but tentacles adhering to the ground during walking usually remain attached for much longer (p. 619).

Although cnidoblasts are independent effectors in the sense that they are receptors which also react mechanically (Parker, 1919; Pantin, 1942), their threshold is influenced by environmental factors. Synaptic junctions between cnidoblasts and neurites are reported from electron-microscope studies (Westfall, 1970). In *Calliactis* the threshold of discharge of tentacular cnidae used in adhesion is lower when the pedal disk is free than when it is attached (Davenport, Ross & Sutton, 1961). McFarlane's important discovery (1969*a, b*) that pulses arise in an ectodermal conducting system when the tentacles are in contact with a *Buccinum* shell and that they coordinate detachment of the pedal disk is probably very relevant. In *Stomphia* the tentacles are unreactive during swimming but regain their normal responsiveness as the anemone re-attaches (Ross & Sutton, 1964*b*), and similarly the cnidae of the pedal disk discharge much more readily during resettling than during swimming (Ellis *et al.* 1969). In *Gonactinia* there are probably similar effects as the tentacles fail to adhere to a glass rod during swimming and walking.

OBSERVED SEQUENCES OF BEHAVIOUR

Feeding

Gonactinia probably feeds on small Crustacea such as copepods and other zooplankton. Feeding behaviour has been studied using *Artemia nauplii*, which are about 1 mm long when newly hatched.

Prey is captured with the tentacles, taken to the mouth and swallowed. Moving objects which bump into any part of the anemone may be caught for the tentacles sweep out 180° and the outer ones reach the base of the column. The tentacles themselves are so sensitive to vibration that even a nauplius swimming past an extended tentacle without touching it causes it to bend locally, increasing the chances of capture (p. 627). Nauplii which strike the oral surface of the crown (i.e. tentacles and oral disk) cause inflexion of one or more tentacles and are usually swallowed within 30 sec. Any which swim into the tip of a tentacle, however, reach the mouth almost at once for the tentacle then curves precisely towards it (Fig. 7A, B). Nauplii which strike the column are usually retrieved very accurately by abflexion of one or more tentacles, or, if just below the crown, by the combined operation of two or three tentacles which curl and abfect locally. The column itself has cnidae but it is not clear whether these are able to arrest moving prey.

Once living prey of suitable size has touched a tentacle it is almost certain to be caught. An adequate stimulus such as given by motile organisms is necessary and with dead nauplii the tentacles hardly respond. The tentacular response consists of a localized contraction plus the discharge of cnidae on contact, by means of which prey immediately adheres and is stunned. *Artemia nauplii* are at once anchored or enmeshed by spirocysts; and if also penetrated by one or more penicilli (microbasic amastigophores) they are stunned within seconds. Of these cnidae spirocysts appear to have the lower mechanical threshold; in *Diadumene* they discharge most readily in the

presence of crustacean body fluid (Williams, 1968). Prey adheres to tentacles until it is released by extrusion of the discharged cnidae between 10 sec and a minute later, by which time the tentacles have usually conveyed it to the mouth (Fig. 7C). Should the muscular response of tentacles fail, the moribund captured nauplii fall off the crown instead and are lost. This may happen in bright light, or outside the temperature range 9–15 °C, or when the sensory threshold is raised by previous swimming, walking or prolonged feeding.

The responses of tentacles are those which would be expected following simple mechanical stimuli, and so are those of the oral disk enabling food to be swallowed. After capture by the tentacles prey usually reaches the mouth by means of the cilia and muscles of the oral disk. Localized radial contraction opens the mouth towards the adjacent food or the tentacles involved with it (this is a reflex which often follows flexion of tentacles, p. 631), and on contact with large prey the mouth may continue to stretch until it is as wide as the oral disk, which takes some time. Small prey, however, is engulfed within 10 sec. Passage through the pharynx into the coelenteron occupies 1 or 2 min. Food is carried down by pharyngeal cilia, but only if the pharynx opens can objects accepted by the mouth be finally swallowed. This happens when the transverse muscles of the mesenteries contract, apparently following mechanical stimulation of the pharynx. Once prey is within the pharynx the mouth is closed by a circular contraction which purses the lips and then spreads half-way down the pharynx. Swallowed nauplii reach the coelenteron and are enveloped by the free borders of mesenteries. The mesenteric filaments carry out contact digestion and the nauplii are completely dispersed within a few hours. If an inedible object such as filter paper is swallowed, however, it is rejected within minutes.

This kind of feeding behaviour can be interpreted as a chain of reflexes, in which the responses to chemical as well as to mechanical stimuli enable prey to be caught and swallowed. Mechanical responses alone would be insufficient (p. 627), in the first place because few cnidae discharge to pure mechanical stimuli (Pantin, 1942). Food juices may lower sensory thresholds generally, since unfed *Gonactinia*, for example, become more lively with the tentacle tips moving about instead of extending motionless, but they may also have a specific effect. The radial muscles of the oral disk contract in response to chemical as well as to mechanical stimuli, and a sea-water extract of *Artemia* nauplii causes the mouth to open (see Pantin & Pantin, 1943; Ross, 1964; Lindstedt, Muscatine & Lenhoff, 1968). This picture is an oversimplification, nevertheless, since in *Metridium* food extracts affect the pattern of inherent activity (Pantin, 1950). McFarlane (1970) finds that preparatory feeding behaviour in *Tealia* is associated with pulses in an ectodermal conducting system (p. 617).

With respect to mechanical responses (p. 627), each step in catching and swallowing is determined by the location and intensity of recent stimuli, causing contraction of particular muscles. As a result, feeding behaviour is adaptable and efficient. The more a victim struggles the greater the number of tentacles which become involved in retaining it. A large number of nauplii may be caught in succession by different tentacles in a surprisingly short time.

Walking

Walking is very efficient in *Gonactinia* and anemones can move several centimetres with ease. When dropped upside down they may creep using the tentacles, but this has not been observed as a normal method of locomotion. Usually the anemone loops about like a caterpillar, spanning several millimetres at a time. A step consists of a sequence of contractions of different muscles and takes at least $\frac{1}{2}$ min and usually longer to complete. It appears to be more than a simple chain of reflexes since if walking is interrupted it is usually resumed later. It is inhibited by bright light.

Successful means of attachment are important as the anemones are exposed to water currents and may live on loose algae. The tentacles adhere by means of cnidae but these do not appear to be necessary for adhesion of the pedal disk since none were observed at former sites of attachment (but see Ellis *et al.* 1969).

The sequence observed in a typical step is described below and illustrated in Figs. 2 and 3.

(1) Before walking, an expanded anemone leans at an angle to the substrate so that a few tentacles touch it. This position often follows mechanical stimulation of the column (p. 632). The tentacles may be active and appear to 'search' the ground ahead.

(2) A step begins with slow inflexion of all the tentacles and they and the column stretch out parallel to the substrate. It is not clear which muscles contract in slow inflexions of this kind.

(3) One of the tentacles, usually a primary one, adheres at the tip and now shortens fully.

(4) At about the time of tentacle adhesion the pedal disk detaches and as the tentacle shortens the column is lifted over several millimeters. Release of the pedal disk could involve mesenteric muscles but there is no decrease in diameter until later.

(5) The column is now shortening slowly by means of the mesenteric retractor muscles; the ectodermal longitudinal muscle does not seem to be concerned. Non-strobilating and strobilating specimens behave identically.

(6) Meanwhile the pedal disk becomes concave due to a circular contraction which proceeds outwards to the rim. The anemone is now balanced over a stubby tentacle like a one-armed acrobat. The rest of the crown is maximally extended by coelenteric fluid transferred from the contracted column, and these tentacles are non-adhesive and inert. Fluid is not seen to escape from the mouth.

(7) At this stage the column, much shortened, bends exactly towards the contracted tentacle; the muscles involved are again more probably mesenteric than ectodermal.

(8) The concave side of the bent column begins to relax and the pedal disk flattens and expands. Expansion of the foot as the mesenteric muscles relax is caused by a circular contraction which affects the lower column.

(9) The pedal surface suddenly becomes adhesive. It re-attaches just next to the contracted tentacle, which is now released from the substrate.

(10) The column re-extends, with an increase in circular tone, and the crown regains its normal dimensions. The tentacle used for stepping is usually re-extended by a circular contraction now or a few minutes later. When a second step follows another

tentacle is used. If walking is vigorous an upright position is not resumed between steps.

(11) The direction of stepping is usually away from stimuli to the column such as contact with a glass rod or suction electrode (pp. 626, 632) but walking need not continue in a straight line.

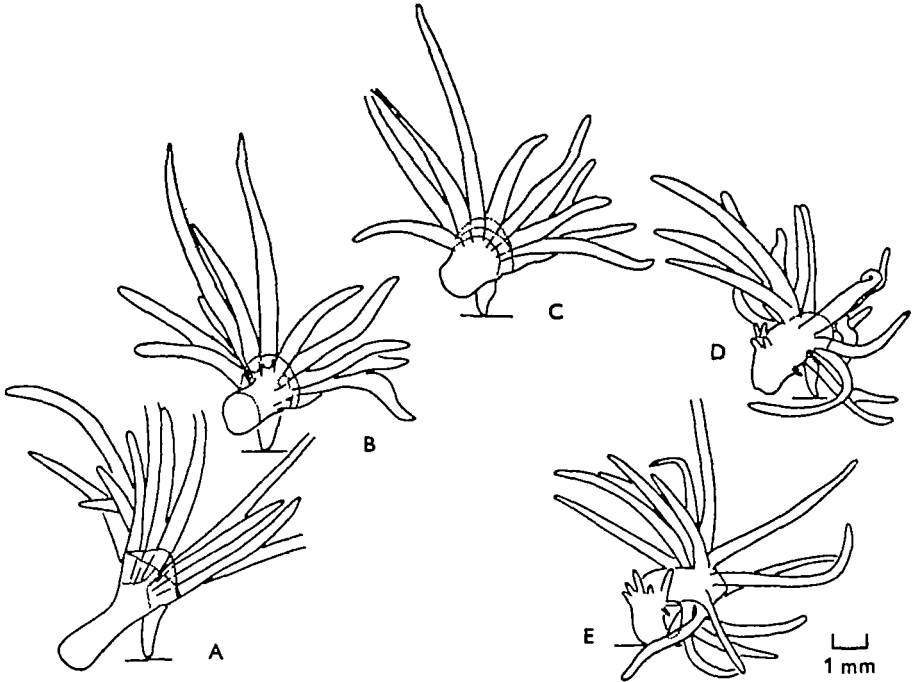


Fig. 2. Walking. Drawings from ciné film to illustrate the progress of a step. A, B, C and D, E form a sequence but are from two different specimens.

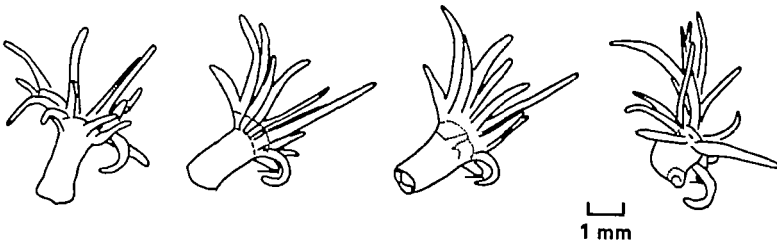


Fig. 3. Walking. Drawings from ciné film of another specimen showing detachment and most of a step.

Strobilating specimens seem able to walk normally up to the time of division. During a step old and new tentacles move in a concerted manner even though the new crown may behave independently when swimming (p. 627). The initial slow inflexion (item 2 above) affects the new crown slightly before the main tentacles, so that some kind of excitation must pass upwards from the pedal disk at this stage.

Walking is such a well-integrated and persistent activity that it is difficult to interpret as a chain of reflexes, although each phase of a step is reflex-like and pre-

dictable. Since it is more easily interrupted than stopped, walking could perhaps be described as a pattern of co-ordinated reflexes which is capable of temporary autonomy. Furthermore, there must be co-ordination between the ectodermal and endodermal muscles involved; these include chiefly the longitudinal muscle of tentacles and radial muscle of the oral disk (ectodermal), and the retractors and probably parietobasilar muscles of mesenteries and much of the circular muscle (endodermal). Conducting pathways between at least some of these muscles presumably exist.

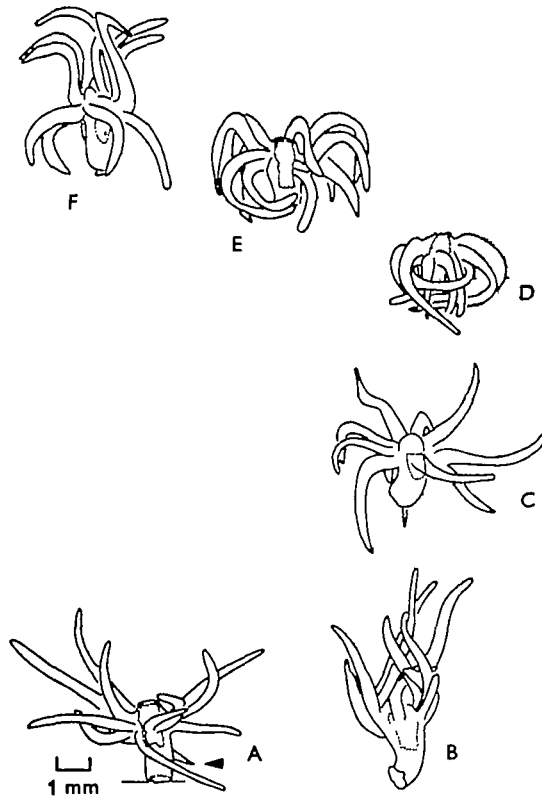


Fig. 4. Swimming. A-F are drawings from ciné film of the initial reaction to *Coryphella* (arrow shows where the nudibranch made contact with the column about $2\frac{1}{2}$ sec earlier) plus the first swimming stroke. A, Abflexion caused by stimulus. B, Complete inflexion. C, D, Complete abflexion. E, F, Inflexion of inner tentacles, with inflexion of outer tentacles beginning at F. Detachment between A and B.

Swimming

Swimming is a relatively short burst of activity in response to mechanical, chemical and electrical stimuli (Fig. 4). Observations suggest that swimming may result whenever the crown or column are stimulated with intensity sufficient to excite an ectodermal pacemaker system. Stimuli inadequate to provoke swimming sometimes cause walking instead, and anemones often walk about after swimming, but the relation between the two modes of locomotion is not fully clear.

The pedal disk detaches and as swimming begins the column often shortens maximally. The tentacles row the anemone forwards with well-defined, synchronous

strokes and their profile suggests that only the ectodermal longitudinal muscle at the tentacle bases is contracting. Swimming bouts are short and last for only 10–30 strokes, usually about 20 at 12–14 °C. Stroke frequency is at first greater than 1/sec (in the limit 2/sec) but it declines by about half before swimming stops. The system fatigues easily and swimming can be elicited only three or four times before the anemone becomes unresponsive. Latency before the first stroke depends to some extent on the intensity of stimulation but it is far too variable for swimming to be regarded as a simple reflex.

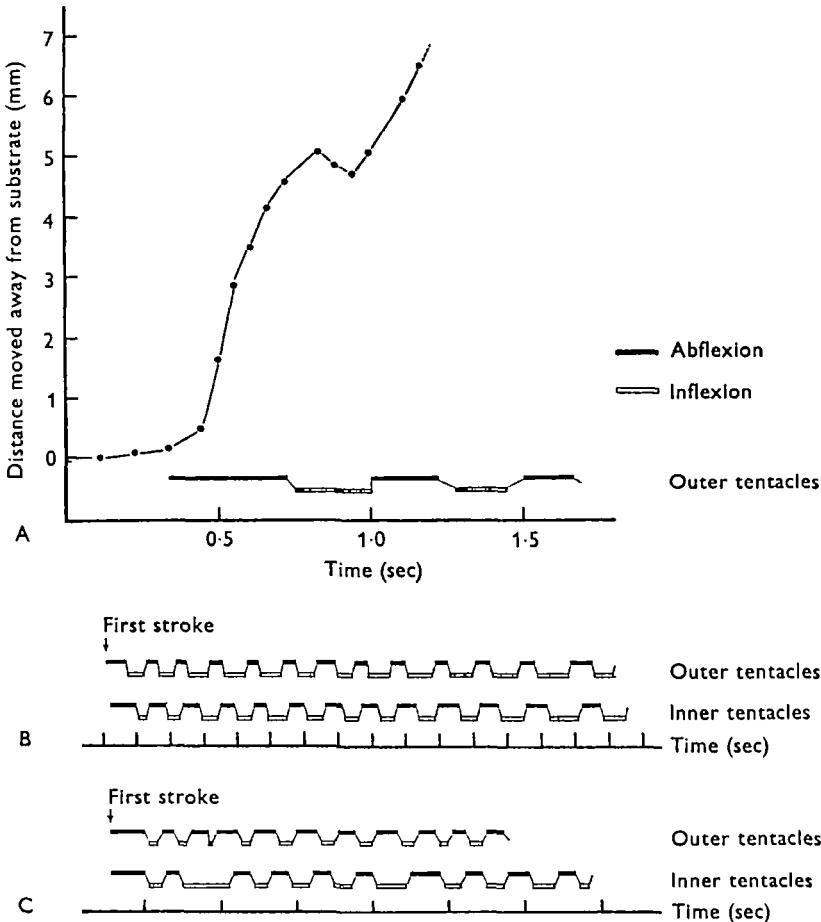


Fig. 5. Swimming records from ciné film (taken at 16 or 18 frames/sec) representing three different specimens following contact of tentacles with *Coryphella*. A, Distance moved vertically upwards during the first stroke and part of the second. The specimen was about 3 mm high, with outer tentacles 5 mm long, and achieved a maximum speed of 2 cm/sec during the first stroke. B, swimming sequence showing how the outer tentacles normally lead. The final strokes are not recorded. C, Abnormal sequence showing irregular strokes of the inner tentacles, probably due to refractory conducting pathways. In both cycles of tentacles strokes continue for 1 nger than shown. Note that time scale differs from B.

In each swimming stroke the tentacles complete a sequence of abflexion and inflexion, corresponding to propulsion and recovery respectively. During abflexion the anemone moves forward at average speeds up to a few mm per second, while during

Inflexion it remains stationary or falls (Fig. 5 A). Maximum speed during a stroke is 10–20 mm/sec. The outer cycle of tentacles usually leads the inner throughout (Fig. 5 B) and at the end of a long bout this becomes more obvious and the inner ring may drop out of the last stroke or two. Anomalous responses may occur in which the two cycles are quite out of phase or in which asynchronous activity results from lack of coordination round the crown, occasionally with tentacles beating intermittently or showing a kind of tetanic fibrillation (Fig. 5 C and p. 635). In a vigorous response all tentacles abflex to the stimulus and the anemone bends and shortens and appears to leap forwards with the first stroke. Weaker stimuli often cause slow inflexion as in walking, followed by a pause before strokes begin. The sweep of tentacle movements at first is incomplete because although abflexion is maximal the tentacles return only to a horizontal position, but complete inflexion normally ensues. At the end of strong abflexions there may be slight axial rotation as the anemone moves forward. Towards the end of swimming the amplitude of strokes is less than at the beginning.

After swimming movements cease the tentacles are inert to mechanical stimuli and fail to adhere (p. 617). If the column shortened as swimming began it now relaxes, and the pedal disk, hitherto concave, expands and re-attaches.

To what extent swimming occurs in nature is hard to determine. As reported by Prouho (1891), some anemones respond to rough mechanical stimulation with a glass rod by swimming rather than walking away. But this was seen only in lively specimens when they had not long been in the aquarium. It seems more significant that certain aeolid nudibranchs secrete material which provokes swimming on contact. The following, for example, are very effective: *Coryphella rufibranchalis*, *Aeolidia papillosa*, and two others not yet identified but resembling *Facelina* sp. and *Favorinus albidus*. Detached cerata also cause swimming. Those of *Coryphella*, for example, held in forceps cause a vigorous reaction and swimming on contact with either column or crown. The nature of the active material is not known but it appears to be labile since forceps used for handling nudibranchs cause swimming on contact but soon lose this property. The above species of nudibranchs feed on coelenterates, but observation shows that it is not their cnidosacs which provide the effective stimulus. A few anemones swam after contact with *Hero formosa* and *Dendronotus frondosus*. *Duvaucelia plebeia* caused a sharp response but no swimming. Other opisthobranchs, tried because they were available, produced little or no reaction: *Polycera quadrilineata*, *Doto coronata*, *Codlina obvelata* and young *Aplysia*. Other kinds of animal collected from the same substrates as *Gonactinia*, including small echinoderms and Crustacea, failed to cause swimming.

Swimming in *Gonactinia* can be termed an escape reaction but the nudibranchs tested apparently do not prey on it. *Aeolidia*, for example, feeds on anemones but it is found in different habitats. *Coryphella* and those referred to as *Facelina* and *Favorinus* occur among algae yielding *Gonactinia* but prey on hydroids (Miller, 1961; Thompson, 1964). In the commonest of these, *Coryphella*, the cerata contained nematocysts of hydroid origin only (possibly including *Tubularia*), and in laboratory tests this nudibranch did not attack *Gonactinia*. And although the anemone itself may swim away, *Coryphella* and other nudibranchs are clearly not immune from being 'stung' when held in contact with its tentacles.

Stomphia coccinea also swims on contact with *Aeolidia* and in that case the effective

material is secreted mainly by the pedal glands and withstands dialysis and boiling (Robson, 1961). In preliminary experiments with *Gonactinia*, however, the most effective contact stimuli involved cerata not pedal mucus. Owing to the scarcity of *Aeolidia* it has not yet been possible to repeat an unsuccessful attempt to see whether a tissue-free extract in seawater would cause swimming in *Gonactinia*.

ANALYSIS OF RESPONSES

As the results of electrical and mechanical stimulation have suggested a new model to account for the properties of the ectodermal neuromuscular system they are described in some detail.

Electrical stimulation

The observations are preliminary since no recording was attempted. Fairly consistent responses were obtained using suction electrodes of internal diameter up to 200 μm , and despite the small size of the anemone it is possible to infer something about the contractions of different muscles and the basis of co-ordination between them. In the first instance the ectodermal and endodermal muscle systems are functionally distinct and have different electrical thresholds.

Electrical stimulation of the column may cause any of the following: retraction, detachment, elongation (muscles endodermal); abflexion of tentacles, flexion of the column, inflexion of tentacles (muscles ectodermal); and partial or complete sequences of walking or swimming. These are considered in turn.

Endodermal muscles

The endodermal neuromuscular system is basically like that of other anemones.

Retraction. As in *Metridium* and *Calliactis* the retractor muscles give a rapid, facilitated response showing through-conduction and are also able to contract slowly (Table 1). The column may shorten by about half. Electrical stimulation of the column, oral disk or tentacles usually elicits reflex twitches of the eight muscles together. Stimuli from an electrode placed on the lower third of the column, however, were sometimes ineffective.

It is assumed that the retractor muscles are excited by through-conducting paths in the nervous system for the following reasons. Above threshold there is no response to one shock, while a second causes uniform quick contraction so long as the interval is within the range approximately 0.2–2.0 sec (duration 1 msec). Each further shock is followed by a distinct twitch. Judged by eye the amplitude of contraction is not affected by stimulus strength but depends on frequency (Pantin, 1935*a*). Latency is short.

There is some variation, but this might not be unexpected if the units of the through-conducting nerve-net (not yet seen: p. 616) were smaller and fewer than in *Calliactis*. In some specimens more than two shocks may be needed initially. In others the through-conduction system becomes refractory during trains of stimuli, with contractions dropping out in a manner rare in *Calliactis*. Another difference is that mechanical stimulation of the column may provoke twitches of nearby retractor muscles only, suggesting that pathways linking the retractors are not always through-conducting. Finally, after-discharge in the through-conduction system of *Gonactinia* is rare.

Slow contractions of the retractor muscles, i.e. complete shortening which takes a few seconds as during a walking step, were not examined. In the retractors of *Metridium* slow contractions often follow quick ones, giving a compound response, and they are elicited by trains of shocks at intervals in the range 3–20 sec (Robson & Josephson, 1969).

Detachment. Detachment is a local reaction of the ectoderm of the pedal disk, usually involving the whole circumference; but dynamic aspects of the process are still obscure (Ellis *et al.* 1969; McFarlane, 1969*b*). It is probably assisted by the parietobasilar muscles, for when these contract synchronously they appear to pull away the pedal disk from the substrate. In *Gonactinia* such a reflex may follow electrical stimulation of the base of the column and a twitch of the lower parts of the septa has been observed. The position of the stimulating electrode seems to be important since when it is placed on the lower third of the column stimuli which would cause retraction if delivered higher up often fail to do so, whereas the pedal disk detaches (i.e. with parietobasilar contraction evident). Contraction of parietobasilar is followed by that of the pedal circular muscle and usually by slow shortening of the retractors. The detached pedal disk becomes concave (Figs. 2, 3). The behaviour of isolated pedal halves stimulated electrically seems identical. In the light of MacFarlane's work on *Calliactis* (1969*a, b*) it is clear that more information about thresholds and other parameters is needed.

Re-attachment is preceded by the contraction of pedal circular muscle passing on to the lower part of the column, and the pedal surface expands characteristically. It is now very adhesive and normally regains contact with the substrate as the retractors relax.

Elongation. Elongation is brought about by slow contraction of the endodermal circular muscle of the column, and this occasionally follows electrical stimuli. Circular contractions usually spread but sometimes a narrow ring marks the position of the electrode.

Circular contractions usually succeed contractions of other muscles, as for example: in the column, after shortening of retractors and perhaps parietobasilar (or parietals if present); in the pedal disk, after parietobasilar contraction; in the tentacles, following slow shortening; and in the oral disk and pharynx, after contraction of transverse septal muscles. The reciprocal activity of these muscles involves inhibition in a manner not fully understood (Batham & Pantin, 1954; Ewer, 1960) and means that electrical stimulation of whole animals often has compound results.

Ectodermal muscles

Most of the ectodermal responses to electrical stimuli are quite different.

Single shocks to the column may cause twitch-like contraction of the longitudinal muscle or of the muscles which flex the tentacles or both, and these muscles appear to be excited via the same conducting system. The size of a contraction near the electrode and the extent to which distant muscles are involved depend on the intensity of the stimulus and may also show facilitation. Nevertheless, contractions of both column and tentacles are usually confined to the radius of the stimulating electrode (ipsiradial); that is to say, the response spreads longitudinally rather than round the circumference, suggesting that the conducting system is non-isotropic.

Ectodermal responses fatigue, and with repeated stimuli the threshold rises or vanishes regardless of electrode position. This threshold varies among different specimens, but initially it is often lower than that of the endodermal retractors, and it is easy to show that the two sets of longitudinal muscles are excited separately. Unlike retraction, the response of a given specimen to single shocks has quite variable latency. There is a tendency for the ectodermal conducting system to show after-discharge and during swimming it probably acts as a pacemaker.

Flexion of tentacles. When the stimulating electrode is placed anywhere on the column a weak shock produces slight abflexion of the tentacle directly above. Increasing the stimulus intensity brings larger abflexions and the response spreads round the crown until all tentacles are involved. Near threshold two shocks 0.5 or 1 sec apart cause two abflexions of the ipsiradial tentacle, the second larger than the first. The latency of response may vary from sometimes very short to an appreciable delay (i.e. judged by eye). The delay must be attributed to the conducting system since the muscles are unaffected and it is difficult to regard abflexion as a straightforward reflex (p. 634). In some specimens two or three abflexions of a tentacle follow a single stimulus. Others fail consistently to respond to single electric shocks while remaining exceedingly sensitive to vibration so that tapping the bench at once causes the tentacles to move (p. 627).

Unless the response is relatively weak, abflexion produced by stimulating the column is usually followed by inflexion. Stimuli to the column never produce inflexion alone whereas shocks to the oral disk may cause inflexion of individual tentacles. All of this agrees with the results of mechanical stimulation (p. 631).

Flexion of column. Especially if the electrode is located on the lower half of the column, one or more shocks often cause contraction of the longitudinal muscle, i.e. flexion of the column. It is usually a twitch in the neighbourhood of the electrode (ipsiradial) so that the anemone bends towards it. Amplitude usually depends on strength of stimulus, and the area of muscle involved looks proportionately larger or smaller. Flexion is sometimes diametrically away from the electrode (contraradial), as in the case of mechanical stimuli (p. 632).

The threshold for column flexion is higher than for abflexion of the tentacles. This could be explained if muscle near the electrode responded directly but had a higher threshold than the ectodermal conducting system which transmits excitation to the tentacles. Flexion away from the electrode would then follow when the conducting system alone was excited and excitation was conveyed round the column as well as to the crown. How excitation proceeding round the column brings about a contraction at the opposite diameter is a problem discussed later (p. 634), but the evidence that it does so is consistent and is supported by the results of mechanical stimulation.

Complex responses: walking and swimming

After repeated shocks anemones often walk away from the electrode. The threshold for walking varies in different specimens and some are much livelier than others. The stimulus parameters have not yet been studied, but it can at least be said that electrical stimulation increases any tendency to locomotion. The indirect effects of such stimulation might last for some time, perhaps for hours.

More can be said about swimming as a response to electrical stimulation of the column. Some anemones swim more readily than others, often according to size, but when tested nearly all will do so. Since the response fatigues after testing two or three times and the anemone usually pulls itself off the electrode as it swims, it is not easy to obtain repeatable results. All observations suggest, however, that there is an electrically excitable pacemaker responsible for the repetition of swimming strokes below whose threshold no swimming occurs.

Swimming strokes are repeated flexions of the tentacles, and the electrical threshold for such contractions is sometimes less than that for retraction (p. 626). The threshold for swimming, however, always exceeds that for retraction. Single shocks do not excite swimming unless of very high intensity. With two or more shocks frequency and number matter more than intensity. For example, at twice the threshold for retraction 10 shocks of 1 or 2 msec at 10/sec nearly always produce swimming, while shocks at 1/sec are seldom effective unless the specimen is one which is liable to swim after single large shocks. Similarly pairs of shocks are most effective at short intervals. There was not enough data to construct any kind of strength-duration curve (Ross & Sutton, 1964*a*), nor is it clear to what extent the duration and vigour of the response depend on the stimulus. In general, weaker stimuli are followed by fewer strokes and tentacles beat with lower frequency and amplitude.

It is possible to drive the pacemaker in a swimming specimen but only if shocks are given at about the rate of natural swimming strokes. When swimming anemones are given rapid trains of shocks, e.g. at 10 or 20/sec for 1 sec the tentacle strokes are inhibited, slowing down and usually stopping with hardly any recovery. Strong mechanical stimuli have the same effect.

Although it can be influenced by external stimulation the swimming pacemaker is autonomous. In strobilating anemones when the young crown of tentacles shows independent swimming strokes although they are fewer and less frequent than those of the main crown they are usually quite out of phase with them. Further observations bear out that the pacemaker must be diffuse, i.e. radially equivalent, and that it is probably ectodermal and located partly or entirely in the upper column. Two anemones were bisected vertically almost to the base and were later stimulated with an electrode on one half of the divided column. The two halves of the crown 'swam' similarly but not synchronously. In some anemones vigorous swimming strokes are exhibited without pedal detachment. Consistent with this, transected upper halves give almost normal responses when stimulated electrically as do the pieces resulting from a further cut across the crown, although the strokes are weak owing to low hydrostatic pressure in the coelenteron. Since there is no indication that isolated tentacles can perform repeated swimming movements as in *Bolocerooides* (Josephson & March, 1966) the pacemaker may be concentrated rather in their vicinity.

Mechanical stimulation

Gonactinia is extremely sensitive to mechanical disturbance, and merely tapping the dish or introducing an object at the water surface causes inflexion or abflexion of all the tentacles. The tentacles bend individually towards local sources of vibration such as small Crustacea swimming past. With repetition these responses fade.

Mechanical stimuli of increasing intensity (such as prodding) provoke graded

local contractions which spread and are eventually superseded by sequences of co-ordinated behaviour such as walking and occasionally swimming away (p. 623). The purposive appearance of such behaviour is unusually convincing. Mechanical thresholds are usually influenced by factors such as local muscular tone and the animal's state of activity.

The effects of mechanical stimuli applied by hand with a fine, newly drawn glass rod were surprising, but nevertheless consistent with the results of electrical stimulation. Most of the stimuli given were weak or moderate and elicited ectodermal responses only, consisting of relatively fast, twitch-like contractions according to the following pattern. The weakest stimulus to a tentacle or to the column sets up propagated excitation such that it bends diametrically away from the point of stimulus (contraradial). Slightly stronger stimuli cause bending exactly towards the stimulus;

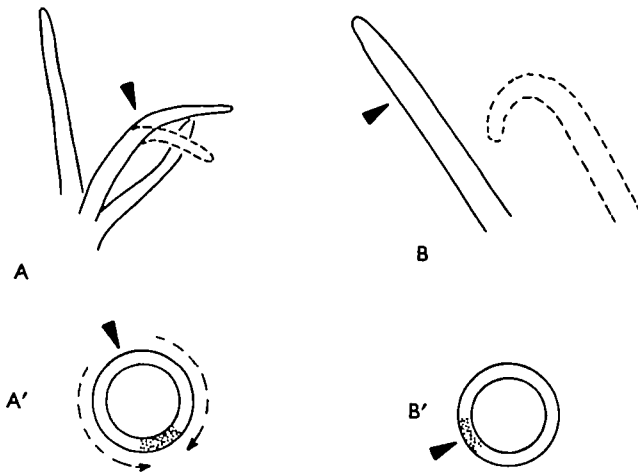


Fig. 6. Responses of tentacles to a light touch with a glass rod. A, Contraradial; B, ipsiradial response. A' and B', diagrams representing tentacles in transverse section to show the position of responding longitudinal muscle (stippled) in relation to the stimulus.

that is to say, the muscle now responds with a graded local contraction at the point of contact (ipsiradial). In this respect the radial muscle of the oral disk is similar. With greater intensity or after several stimuli the response spreads peripherally, for example one tentacle may react and then several or all and flexion of the column may increase.

It is convenient to list these results according to the location of stimuli.

Tentacles

A distinction has already been made between quick and slow contractions (p. 616). Before any twitch-like responses develop the young tentacles of strobilating or regenerating specimens are able to give slow, propagated contractions. When tested with a glass rod the youngest responding tentacles (one quarter or less of adult length) show only a general shortening of the tentacle touched. At a later stage the same stimulus causes abflexion of all the new tentacles together (p. 632), but only later still can the growing tentacles produce localized fast responses. By that stage they also begin to wave about or twitch independently and they are able to capture nauplii and to 'swim' with infrequent strokes (p. 627).

In adult tentacles maximal slow shortening occurs only when they are attached to the substrate during walking (p. 619) or after repeated rough handling, although local shortening occurs when they adhere to prey. Such contractions are usually propagated from the tip of the tentacle and last for several seconds or are maintained, and they are comparable in speed to slow contractions of the mesenteric retractor muscles (p. 625). Slow contractions appear to affect individual tentacles only and not to spread round the crown.

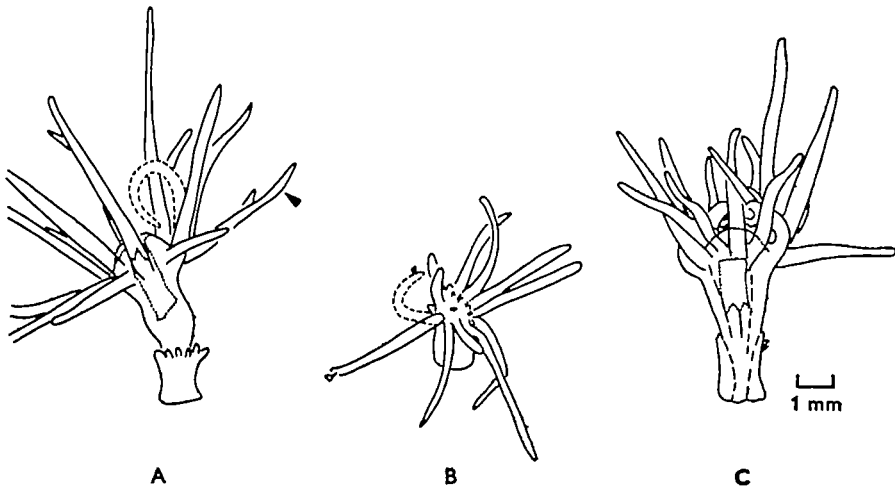


Fig. 7. A, Mechanical stimulation; contraradial bending and inflexion of one tentacle touched with a glass rod. B, Catching prey; curling and inflexion of a tentacle after a nauplius adheres near the tip; adjacent tentacles are regenerating. C, Feeding; several tentacles curled over the oral disk after conveying food to the mouth.

In order to describe the fast reactions of tentacles (i.e. apparently fast or twitch-like contractions) it is necessary to distinguish between distal and proximal regions (p. 616). The distal half of a tentacle can bend in all directions. The proximal half, however, bends either towards or away from the mouth and hardly moves laterally at all. These movements, termed inflexion and abflexion (Fig. 10A), are particularly well defined when the basal muscle contracts and the whole extended tentacle flicks sharply in or out. The effects of stimulus location and intensity will be considered next.

The distal part of a tentacle responds to a light touch anywhere by bending away locally (Fig. 6A). When the object adheres due to discharged cnidae (p. 616), however, bending is towards the stimulus (Fig. 6B). In the case of the highly adhesive tip of the tentacle, it usually shortens as well, becoming opaque. Whatever the direction of local bending, after any but the weakest stimuli the tentacle then inflexes sharply from the base (Figs. 7A, 8). The amplitude both of local bending and of inflexion depends on the strength of the stimulus. As this increases, inflexion spreads to all neighbouring tentacles (Figs. 8, 9A).

When the proximal half of a tentacle is touched the response depends on where it is stimulated. Touching the oral surface causes inflexion, and if the stimulus is strong enough adjacent tentacles of both cycles respond too. Touching the aboral surface, however, causes abflexion (Fig. 10B). With stronger stimuli abflexion spreads peripherally, but there is a new distinction between the inner and outer tentacles:

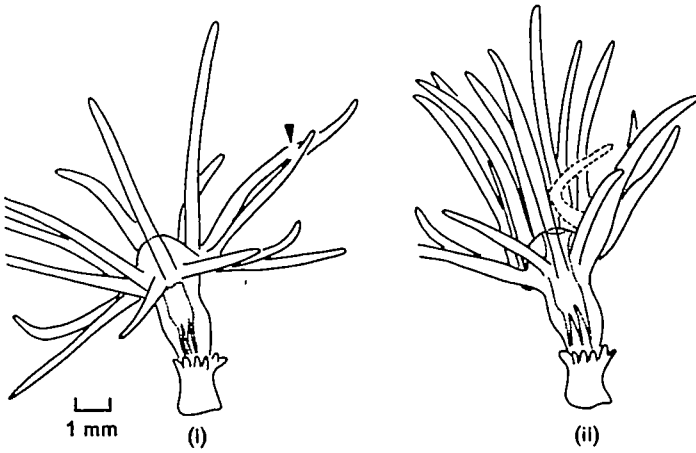


Fig. 8. Mechanical stimulation. (i) Before touching one tentacle distally with a glass rod as indicated. (ii) Response: the tentacle bent away and inflected, plus inflexion of the whole crown.

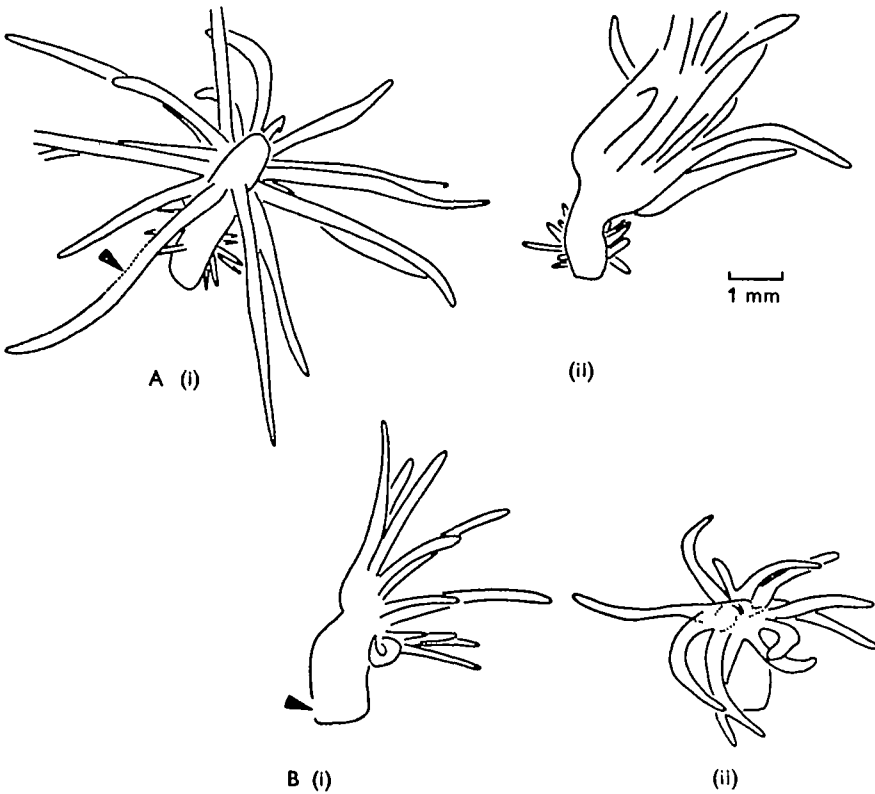


Fig. 9. Mechanical stimulation. A, Maximal inflexion of all tentacles caused by a strong stimulus to one tentacle. (i) Extended, (ii) response (including the young crown). B, Abflexion of all tentacles caused by a strong stimulus to the column. (i) Before stimulation, (ii) response (includes some ipsiradial contraction of the column).

Excitation spreads from stimulated outer tentacles to all of the others, but from stimulated inner tentacles to tentacles of the inner cycle only. After quite strong stimuli abflexion of the stimulated tentacle is followed by flexion, the tentacle bending sharply outwards and then in as if it were catching prey or swimming (pp. 618, 622).

The apparently polarized spread of excitation from stimuli given to the proximal aboral surface of tentacles is difficult to explain (p. 636). It is not invariable, for some exceptional specimens showed inflexion wherever the proximal surface was stimulated while in others when one tentacle was touched all responded.

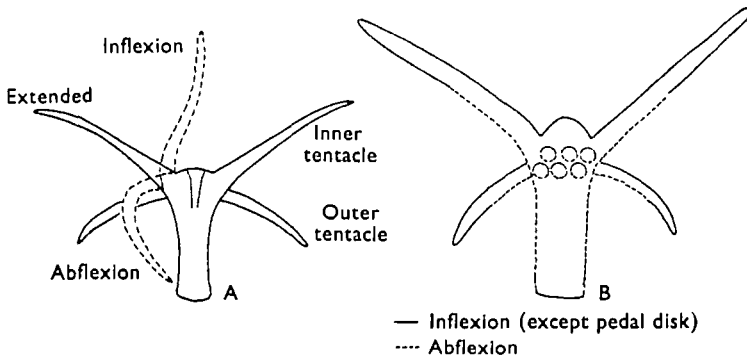


Fig. 10. Flexion of tentacles. A, Diagram to illustrate terms used. B, Diagram summarizing the effects of light mechanical stimulation. Broken line indicates the area within which touch with a glass rod causes ipsiradial abflexion; continuous line (except for the pedal disk) shows where the same stimulus causes inflexion.

It is possible that inflexion and abflexion are mutually exclusive. In a specimen which had one tentacle held inflected across the disk, the tentacle base was touched on the aboral side with a glass rod. This caused abflexion of the two neighbouring tentacles but not of the stimulated one. The possible role of inhibition is discussed on page 636.

Oral disk

When the oral disk is touched one or more nearby tentacles inflect sharply (cf. electrical stimulation, p. 636). As often occurs when prey is caught, strong stimuli cause inflexion of all the tentacles. There is in addition a graded local contraction of the disk muscle, and the mouth opens radially towards the point of stimulus. Similarly, inflexion caused by mechanical stimulation of the tentacles is commonly followed by mouth opening (p. 618). The mouth opens symmetrically when all radii are involved.

Oral disk reflexes are fast, and very precise in a radial direction. Excitation spreads round the circumference more readily when stimuli are applied to the oral disk than to tentacles, but it would be consistent with observations if tangential pathways required facilitation (p. 636).

The column

The effects of mechanical and electrical stimulation are similar and the radial localization of most responses is again remarkably exact. A weak stimulus applied anywhere on the column provokes sharp abflexion of the tentacle at that radius. As stimuli are increased nearby tentacles respond first, and then the whole set (Fig. 9B).

With moderate stimuli the ectodermal longitudinal muscle contracts locally as well. Exactly as described for tentacles, the column usually twitches towards the glass rod, but it may bend away by contraction of the muscle diametrically opposite. One specimen bent first towards the stimulus and then away (cf. tentacles, p. 631). After a twitch the column recovers or else leans slowly away from the point of stimulus and the anemone may then walk. Sharp stimuli to tentacles may also be followed by a twitch of the column, usually at the opposite radius so that the animal again bends away. Touching the top of the column, however, may cause abflexion of contralateral tentacles.

Strobilating specimens respond predictably to such stimuli. Touching the column near the base causes abflexion of new tentacles, usually followed by the main crown, whereas a stimulus above the new crown usually causes inflexion of these tentacles.

Strong stimuli to the base of the column cause retraction and the anemone shortens suddenly. Ciné-film records suggest that latency is then shorter and contraction faster than in twitches of the ectodermal longitudinal muscle. After repeated retractor contractions the column may not relax for several minutes and walking usually ensues.

General shortening of the column is achieved mainly by the mesenteric muscles and it is difficult to observe whether the ectodermal longitudinal muscle is concerned as well. In theory it should be able to give slow symmetrical contractions but good evidence for this was not seen.

Pedal disk

Stimuli to the basal third of the column provoke detachment of the pedal disk and walking, especially if repeated (p. 626). As noted previously the epithelial mechanism of detachment is not yet clear (p. 625), but detachment can spread across the pedal disk and in itself involves no decrease in diameter. In this anemone there are no basilar muscles and so pedal movements must be due to the action of circular and parietobasilar muscles. Touching the free surface of the pedal disk usually causes localized contractions.

DISCUSSION

Present understanding of the neuromuscular mechanisms of sea anemones is based almost entirely on studies of *Calliactis* and *Metridium*. These originated with the work of Parker (1919) on *Metridium* and with Pantin's classic experiments on *Calliactis* (1935*a, b, c, d*, further references, 1965). The cardinal points of experiments are substantiated by histological findings (Pantin, 1952; Batham *et al.* 1960; Robson, 1965).

The familiar analysis of the closure reflex in *Calliactis*, supported by histological work on *Metridium* and *Calliactis* and more recently by the results of electrophysiological recording (Josephson, 1966; Robson & Josephson, 1969), is as follows. The sphincter muscle is supplied by fast through-conducting tracts of the nervous system running up the mesenteries, and it contracts in rapid steps showing neuromuscular facilitation. The through-conduction tracts are a specialized part of the general nerve-net consisting of numerous bipolar nerve cells. The main sensory input is from large numbers of endodermal receptors in the angles of the mesenteries, whose processes make contact with those of the nerve cells. Motor output is most probably via the expanded endings of nerve processes overlying the muscle fibres (Pantin, 1952).

Muscles able to give quick facilitated contractions can also contract slowly. Pantin's explanation for this (1965), with which later work agrees, is that facilitated contractions are initiated from all of the neuromuscular junctions, while slow contractions are initiated at a few only and are propagated in the muscle. The former require two or more shocks at intervals of 0.3–3 sec, the latter perhaps five or more at intervals of 3–15 sec. As yet the neuromuscular transmitter has not been identified nor has the ultrastructure of these neuromuscular junctions been described.

Outside the through-conduction paths mediating total reflexes the general nerve-net is characterized by interneural facilitation. Typical muscles show only slow contractions, often with reciprocal inhibition (Batham & Pantin, 1954; Ewer, 1960). But electrophysiological recording has so far failed to illuminate these common features.

As mentioned earlier, McFarlane (1969*a, b*; 1970) has discovered two new slowly conducting systems one of which is ectodermal (SS 1) and mediates detachment in *Calliactis* and preparatory feeding behaviour in *Tealia*. The interpretation of motor pathways, however, has not yet been completed.

Table 2. *Ectodermal responses following electrical or mechanical stimulation of the column*

	Feature	Conclusion
1	Muscular response is fast and accurately localised	Ectodermal muscle gives twitch-like contractions which are not propagated
2	Stimulus may cause distant muscular response only	Ectodermal conducting system present (speed under 10 cm/sec)
3	Either (a) ipsiradial localization of this response (tentacle abflexion) or else (b) contraradial (column flexion)	Suggests non-isotropic properties possibly of the conducting system (a) but more probably of the responding system (b)
4	Stimulus may cause local muscular response at place stimulated: usually distant response as well	Muscle is excited locally, perhaps directly, and the threshold for this is distinct, probably higher than that of the conducting system
5	With increasing stimulation the distant response spreads peripherally (i.e. round the crown)	Conduction facilitated round the circumference
6	Amplitude of distant ipsiradial contraction (abflexion) graded according to stimulus intensity and may show facilitation. Latency variable. After-discharge may occur. Threshold labile	Impulses in conducting system do not have all-or-none effect on response. Responding system must consist of two elements, perhaps muscle plus intermediate units from which motor impulses arise. Conducting system may not be through-conducting
7	Abflexion of one tentacle caused by a stimulus of moderate strength may be followed by inflexion; i.e. for tentacles an ipsiradial – contraradial sequence	Tentacles can perform strokes used in swimming independently, as in feeding, hence swimming is not a highly specialized phenomenon
8	Intensive stimulation excites synchronous repetition of the whole peripheral response; interval range of swimming strokes 0.5–2.0 sec	Facilitation allows interaction of responding elements, i.e. of intermediate units, giving rise to sustained rhythmical discharge (pacemaker activity)

It is easy to see that within this framework the endodermal neuromuscular system of *Gonactinia* presents nothing unusual. The properties of the mesenteric and circular muscles are such that even with little histological information to hand it can be

assumed that a mesenteric through-conducting system supplies the retractor muscles and that allowing for a smaller scale the kind of organization differs little from that in *Metridium*.

The ectodermal arrangements in *Gonactinia*, on the other hand, simply cannot be interpreted in this way. The nature of the difficulties and a possible solution will be described next (see Table 2).

The main question is how the ectodermal muscle is excited. This muscle is unusual in that contractions do not seem to be readily propagated within it compared, for example, to endodermal muscles of *Metridium*. Histologically there is no musculo-epithelium, i.e. the muscle fibres and epithelial cells are separate. It seems possible that the pronounced radial localization of all ectodermal responses might reflect some feature in the arrangement of the muscle, such as, for instance, a predominance of longitudinally rather than laterally placed junctions between muscle fibres. Further evidence is needed here.

As to the identification of possible conducting systems in the ectoderm, incomplete information is fortunately of use. As silver preparations show a nerve net at any rate over the column (p. 614) it can be supposed that the elements which might conduct ectodermal excitation comprise this nerve net, the muscle fibres and the epithelium. From its appearance in relation to the size of the animal, it seems reasonable to suppose that the nerve net acts as the chief ectodermal conducting system. Nothing of its general aspect, however, would lead one to expect the non-isotropic responses which are observed.

An interesting point arises in relation to the small size of this anemone. Its lively behaviour does not imply the presence of particularly fast conducting systems, since with the short distances involved conduction speeds only of about 5 cm/sec would be needed to account for transmission times. From ciné-film records the rate of ectodermal conduction in the column is of this order.

Information is needed about motor innervation but this is particularly difficult to obtain in coelenterates, owing mainly to sampling difficulties when the problem is tackled by means of electron microscopy. There is, however, a preliminary histological clue as to the nature of motor pathways in the presence of the multipolar nerve cells which have so far been seen in the upper part of the column, the oral disk and proximally in the tentacles. They have not yet been observed in silver preparations, but in a few methylene blue preparations they seemed to stain almost exclusively. It is suggested that they may have an intermediary motor function.

The following assumptions are made without evidence: that multipolar elements corresponding to these cells can excite the ectodermal muscle locally and that they can interact with one another. Excitation reaches them via the ectodermal conducting system, i.e. the general nerve net, and they are best considered as forming part of it (Pantin & Vianna Dias, 1959). It is suggested that when the multipolar units discharge the underlying muscle contracts, and that the amplitude of response depends perhaps on frequency but more probably on the number of cells firing. Latency of response then varies with the lability of these units, i.e. with their threshold for discharge, and with the stimulus. It is supposed that a multipolar unit may fire when excitation reaches it via more than one of its neurites. This would explain how the column or a tentacle may contract diametrically away from the source of stimulus,

as the contraradial multipolar units would fire when excitation reached them simultaneously from two directions (Fig. 11).

If it is also assumed that interneural facilitation is an important controlling factor, this model accounts for a number of features of the ectodermal neuromuscular system in *Gonactinia*. Stimuli which provoke graded local contractions would excite the muscle either directly or via multipolar units locally. Since distant responses are usually excited as well, the threshold for local contractions is higher than that of the conducting system (general nerve-net). Larger stimuli, either electrical or mechanical,

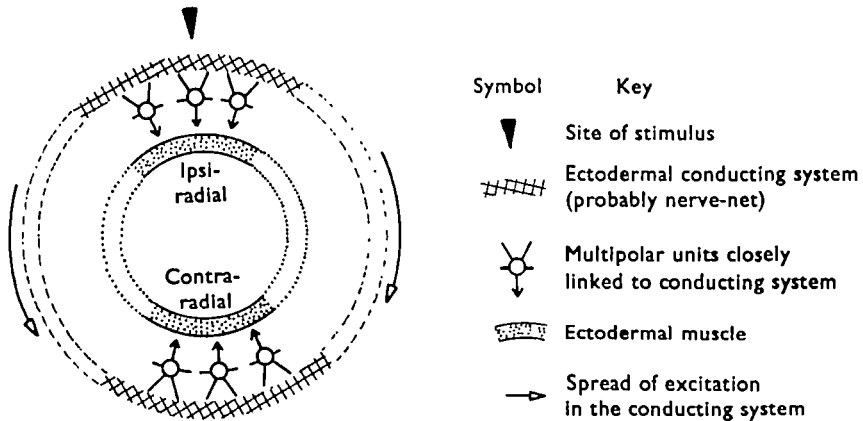


Fig. 11. Model to explain some properties of the ectodermal neuromuscular system in *Gonactinia* (see text). The concentric arrangement refers to that of the column or tentacles in transverse section, but the symbols do not denote the actual structure of elements represented.

increase the amplitude of distant responses probably by exciting more of the nerve-net, and hence more of the multipolar units. These elements are independently excitable but interact with each other. They show a tendency to after-discharge, and when a sufficient number are involved their activity is self-sustaining. The discharging elements fatigue readily and the threshold of ectodermal responses is correspondingly labile.

It is probably the refractory period of the ectodermal nerve net which limits the rate of swimming strokes; their frequency falls within a narrow range, with intervals normally between 0.5 and 2.0 sec. The pacemaker system can be driven by electrical stimuli only when these are applied at natural frequencies (p. 627) and it is fairly easily inhibited. Synergy of the tentacle strokes during swimming depends on the peripheral conducting paths remaining open; when tentacles beat irregularly or out of phase this appears to be due to local or transient refractoriness of the nerve-net (Fig. 5C).

Refractoriness or locally high electrical thresholds are partly attributable to the small size of the nerve cells, most neurites being less than $1 \mu\text{m}$ in diameter. Normal sensory stimuli may succeed much better than electrical stimulation. A specimen from which no sign of ectodermal response can be provoked by electrical stimulation reacts in the liveliest way to a slight tap on the bench. In *Calliactis* the intensity of a mechanical stimulus applied at a single point affects the number and frequency of impulses set up

in the nerve net (Passano & Pantin, 1955), and a similar relation may be assumed in *Gonactinia*. In that case intense sensory stimulation, either localized or general, would be expected to excite most of the multipolar units and to provoke swimming, which is what happens. The evidence from electrical stimulation is that a few shocks at brief intervals are most effective in causing swimming (p. 627), which also leads one to suppose that successful chemical or mechanical stimuli would induce rapid trains of sensory impulses. It has been suggested that anemones adapt less rapidly to chemical than to mechanical stimuli (Pantin & Pantin, 1943), and indeed in *Gonactinia* quite massive mechanical stimuli are usually needed to cause swimming, although contact of a single cerata of *Coryphella* with one tentacle may suffice. In conclusion, then, in natural conditions the anemone would swim away from any stimulus which set up adequate sensory disturbance, and it appears less surprising that contact with animals which may be ecologically irrelevant has this effect (see, for example, Mackie, 1970).

The ectodermal nervous system and its connexions probably show regional differentiation, although little can be said about this at present. Some explanation is needed for the differential responses of tentacles to mechanical stimuli according to their location (Fig. 10B). The crown further presents a difficult problem of apparently polarized connexions. In swimming and other activities abflexion is followed by inflexion and this order appears not to be reversible, i.e. inflexion is not followed by automatic abflexion (a sequence which would appear to be of little use). In swimming the outer tentacles move before the inner ones suggesting that excitation causing abflexion spreads from the upper column to one cycle, then to the other. In accordance with this, if the outer tentacles are touched with a glass rod a stimulus to the proximal aboral surface causes both circles to respond. But it will be recalled that when the stimulus is applied to the corresponding area of an inner tentacle, only the inner cycle responds (p. 630). When stimuli are applied which result in inflexion no difference between inner and outer tentacles can be discerned. Possibly there is some functional or developmental distinction between the nerve-net of the column and of the oral disk, and some anomaly arises in the zone between them. Or there may perhaps be a parallel with *Calliactis*, where in the oral disk and tentacles conduction occurs much more readily in a centrifugal than in a centripetal direction (Pantin, 1935c).

Features requiring elucidation are the apparently non-isotropic conduction and the possible role of interneural facilitation. They are omitted from Fig. 11 because little more can be said about them until more information is available. With regard to inhibition, the observation recorded on page 631 suggested that prolonged flexion of a tentacle might prevent abflexion. Possible explanations, however, include refraction as well as inhibition either within the nerve-net or of postulated motor units, and the same alternatives hold in the case of stimuli which interfere with swimming strokes. For the moment it does not seem necessary to postulate reciprocal inhibition to account for the normal inflexion-abflexion sequence.

Nothing has been said of how ectodermal and endodermal systems interact in the animal's normal behaviour. In other anemones the oral disk is an area where ectodermal and endodermal parts of the nervous system interconnect (Batham, 1965), and in *Calliactis* tentacles as well as sphincter respond to excitation of the through-conduction system (Pantin, 1935a; Josephson, 1966). In *Gonactinia*, however, excitation of the through-conduction system leaves ectodermal muscles unaffected. It is possible that

Ectodermal and endodermal responses may be less closely linked, in the sense that fewer inter-connecting physiological pathways may be available than in anemones such as *Calliactis*, but the evidence for such a statement is circumstantial. The reflex-like ectodermal movements involved in catching and swallowing prey are followed by the contraction of endodermal muscles, but feeding behaviour stops when interrupted. In swimming the ectodermal and endodermal components may sometimes be separated for detachment and shortening of the column do not invariably occur. In walking, however, the action of endodermal muscles is essentially coordinated with that of ectodermal elements. What, if any, is the seat of persistent excitation here, and how is walking related to longer-term activity? Also, if McFarlane's slow conduction systems (1969*a, b*; 1970) occur generally in sea anemones, what part would they play in the behaviour of *Gonactinia*?

It may be asked whether the model suggested for the ectodermal neuromuscular system of *Gonactinia* might apply elsewhere. It can in fact be transferred intact to the other swimming anemones, *Stomphia* and *Actinostola* (Robson, 1966) and *Bolocerooides* (Josephson & March, 1966). In *Stomphia* ipsilateral and contralateral contractions of parietobasilar muscles are seen during swimming and sometimes after stimulating the column, besides which the endodermal nerve-net of the column comprises large multipolar nerve cells. It would be supposed that firing of these multipolar units caused local parietobasilar contraction. *Bolocerooides* in many ways resembles *Gonactinia* except that pacemaker elements (which would be the present multipolar units) localized at the base of the tentacles are more excitable. It appears to swim more readily than *Gonactinia* on mechanical stimulation.

Concerning non-swimming anemones, the behaviour of *Protanthea*, the only species related to *Gonactinia*, is similar in other respects, and in one specimen contraradial contractions (a particular feature explained by the model) occasionally followed an ipsiradial twitch of the column after mechanical stimulation. Further observations are needed. There is also an old statement by Fleure & Walton (1906) 'that when one side of the base of *Tealia* is irritated, the opposite side often contracts rapidly'. In most anemones, however, any responses which show ipsilateral or contralateral orientation to stimuli are slow, and it is not always certain how they are mediated. There are records, for example, of anemones walking away from persistent stimuli to the base, but the interpretation of this is not straightforward because the animals' phasic activity is affected (e.g. Batham & Pantin, 1950*a, b*). In *Metridium* bending of the column is due to parietal contractions, but these may be inhibited by contraction of the circular muscle. Hence in some circumstances a contralateral reaction may be a consequence of ipsilateral inhibition (Batham & Pantin, 1954; see Ewer, 1960). The complex interactions of slowly responding systems counsel caution, and without much evidence to the contrary it is best to regard the state of affairs in *Gonactinia* and other swimming anemones as exceptional.

SUMMARY

1. In *Gonactinia* well-developed ectodermal muscle and nerve-net extend over the column and crown and play an important part in the anemone's behaviour.
2. Common sequences of behaviour are described. Feeding is a series of reflex

contractions of different muscles by means of which plankton is caught and swallowed. Walking, in the form of brief looping steps, differs markedly in that it continues after interruptions. Anemones also swim with rapid tentacle strokes after contact with certain nudibranch molluscs, strong mechanical disturbance or electrical stimulation.

3. Swimming is attributed to temporary excitation of a diffuse ectodermal pace-maker possibly situated in the upper column.

4. From the results of electrical and mechanical stimulation it is concluded that the endodermal neuromuscular system resembles that of other anemones but that the properties of the ectodermal neuromuscular system require a new explanation. The size and spread of responses to electric shocks vary with intensity, latency is variable and there is a tendency to after-discharge. There is precise radial localization, for example touching a tentacle or the column causes it to bend towards or away from the stimulus.

5. A model to explain these and other features includes multipolar nerve cells closely linked to the nerve-net which would act as intermediate motor units, causing local contraction of the ectodermal muscle. This scheme can be applied to other swimming anemones but there is no evidence that it holds for sea anemones generally.

This work could not have been carried out without much generous help received at Kristinebergs Zoological Station and I would like to express my grateful thanks to the Director and to all members of the laboratory. I wish to thank Mr L. Erdős for loans of photographic equipment, and Dr C. Bliding, Dr H. Lemche and Dr K. W. Ockelmann for valuable taxonomic advice. Grants from the E. M. Musgrave Fund, the Research Board of the University of Reading and the Science Research Council are gratefully acknowledged.

REFERENCES

- BATHAM, E. J. (1965). The neural architecture of the sea anemone *Mimetricidium cryptum*. *Am. Zool.* **5**, 395-502.
- BATHAM, E. J. & PANTIN, C. F. A. (1950a). Inherent activity in the sea-anemone, *Metridium senile* (L.). *J. exp. Biol.* **27**, 290-301.
- BATHAM, E. J. & PANTIN, C. F. A. (1950b). Phases of activity in the sea-anemone, *Metridium senile* (L.), and their relation to external stimuli. *J. exp. Biol.* **27**, 377-99.
- BATHAM, E. J. & PANTIN, C. F. A. (1951). The organization of the muscular system of *Metridium senile*. *Q. J. microsc. Sci.* **92**, 27-54.
- BATHAM, E. J. & PANTIN, C. F. A. (1954). Slow contraction and its relation to spontaneous activity in the sea-anemone *Metridium senile* (L.). *J. exp. Biol.* **31**, 84-103.
- BATHAM, E. J., PANTIN, C. F. A. & ROBSON, E. A. (1960). The nerve-net of the sea-anemone *Metridium senile*: the mesenteries and the column. *Q. J. microsc. Sci.* **101**, 487-510.
- BLOCHMANN, F. & HILGER, C. (1888). Über *Gonactinia prolifera* Sars, eine durch Quertheilung sich vermehrende Actinie. *Morph. Jb.* **13**, 385-401.
- CARLGRÉN, O. (1893). Studien über Nordische Actinien. I. *K. svenska Vetensk-Akad. Handl., Stockh.* **25** (10).
- CARLGRÉN, O. (1904). Bemerkungen über die Querteilung der *Gonactinia prolifera*. *Zool. Anz.* **27**, 545-7.
- CARLGRÉN, O. (1940). A contribution to the knowledge of the structure and distribution of the cnidae in the Anthozoa. *Acta Univ. Lund, N.F. Avd.* **2**, 36 (3).
- CARLGRÉN, O. (1942). Actiniaria. II. *Dan. Ingolf-Exped.* **5** (12).
- CARLGRÉN, O. (1949). A survey of the Ptychodactiaria, Corallimorpharia and Actiniaria. *K. svenska Vetensk-Akad. Handl., Stockh.* (4) **1**, 1-121.
- DAVENPORT, D., ROSS, D. M. & SUTTON, L. (1961). On the remote control of nematocyst-discharge in the attachment of *Calliactis parasitica* to shells of hermit crabs. *Vie Milieu* **12**, 197-209.
- ELLIS, V. L., ROSS, D. M. & SUTTON, L. (1969). The pedal disk of the swimming sea anemone *Stomphia coccinea* during detachment, swimming and resettlement. *Can. J. Zool.* **47**, 333-42.
- EWER, D. W. (1960). Inhibition and rhythmic activity of the circular muscles of *Calliactis parasitica* (Couch). *J. exp. Biol.* **37**, 812-31.

- FLEURE, H. J. & WALTON, C. L. (1906). Notes on the habits of some sea anemones. *Zool. Anz.* **31**, 212-20.
- GROSSELJ, P. (1909). Untersuchungen über das Nervensystem der Aktinien. *Arb. zool. Inst. Univ. Wien* **17**, 269-308.
- JOSEPHSON, R. K. (1966). Neuromuscular transmission in a sea anemone. *J. exp. Biol.* **45**, 305-19.
- JOSEPHSON, R. K. & MARCH, S. C. (1966). The swimming performance of the sea-anemone *Bolocerooides*. *J. exp. Biol.* **44**, 493-506.
- KOMORI, S. (1931). Einige bemerkungen über die Biologie der Aktinie *Bolocerooides*. *Mem. Coll. Sci. Kyoto Univ. B* **7**, 55-62.
- LINSTEDT, K. J., MUSCATINE, L. & LENHOFF, H. M. (1968). Valine activation of feeding in the sea anemone *Bolocerooides*. *Comp. Biochem. Physiol.* **26**, 567-72.
- MACKIE, A. M. (1970). Avoidance reactions of marine invertebrates to either steroid glycosides of starfish or synthetic surface-active agents. *J. exp. mar. Biol. Ecol.* **5**, 63-9.
- McFARLANE, I. D. (1969*a*). Two slow conduction systems in the sea anemone *Calliactis parasitica*. *J. exp. Biol.* **51**, 377-85.
- McFARLANE, I. D. (1969*b*). Co-ordination of pedal-disk detachment in the sea anemone *Calliactis parasitica*. *J. exp. Biol.* **51**, 387-96.
- McFARLANE, I. D. (1970). Control of preparatory feeding behaviour in the sea anemone *Tealia felina*. *J. exp. Biol.* **53**, 211-20.
- MILLER, M. C. (1961). Distribution and food of the nudibranchiate Mollusca of the south of the Isle of Man. *J. anim. Ecol.* **30**, 95-116.
- NYHOLM, K. G. (1959). On the development of the primitive actinian *Protanthea simplex* Carlgren. *Zool. Bidr. Upps.* **33**, 69-77.
- PANTIN, C. F. A. (1935*a*). The nerve-net of the Actinozoa. I. Facilitation. *J. exp. Biol.* **12**, 119-38.
- PANTIN, C. F. A. (1935*b*). The nerve-net of the Actinozoa. II. Plane of the nerve-net. *J. exp. Biol.* **12**, 139-55.
- PANTIN, C. F. A. (1935*c*). The nerve-net of the Actinozoa. III. Latency and after-discharge. *J. exp. Biol.* **12**, 156-64.
- PANTIN, C. F. A. (1935*d*). The nerve-net of the Actinozoa. IV. Facilitation and the 'staircase'. *J. exp. Biol.* **12**, 389-96.
- PANTIN, C. F. A. (1942). The excitation of nematocysts. *J. exp. Biol.* **19**, 294-310.
- PANTIN, C. F. A. (1950). Behaviour patterns in lower invertebrates. *Symp. Soc. exp. Biol.* **4**, 175-95.
- PANTIN, C. F. A. (1952). The elementary nervous system. *Proc. Roy. Soc. Lond. B* **140**, 147-68.
- PANTIN, C. F. A. (1965). Capabilities of the coelenterate behaviour machine. *Am. Zool.* **5**, 581-9.
- PANTIN, C. F. A. & PANTIN, A. M. P. (1943). The stimulus to feeding in *Anemonia sulcata*. *J. exp. Biol.* **20**, 6-13.
- PANTIN, C. F. A. & VIANNA DIAS, M. (1952). Rhythm and afterdischarge in medusae. *Ann. Acad. bras. Sci.* **24**, 335-49.
- PARKER, G. H. (1919). *The Elementary Nervous System*. Philadelphia and London: J. B. Lippincott.
- PASSANO, L. M. & PANTIN, C. F. A. (1955). Mechanical stimulation in the sea-anemone *Calliactis parasitica*. *Proc. Roy. Soc. Lond. B* **143**, 226-38.
- PROUHO, H. (1891). Observations sur la *Gonactinia prolifera* (Sars) draguée dans la Méditerranée. *Archs Zool. exp. gén.* **9**, 247-54.
- ROBSON, E. A. (1961). The swimming response and its pacemaker system in the anemone *Stomphia coccinea*. *J. exp. Biol.* **38**, 685-94.
- ROBSON, E. A. (1963). The nerve-net of a swimming anemone, *Stomphia coccinea*. *Q. J. microsc. Sci.* **104**, 535-49.
- ROBSON, E. A. (1965). Some aspects of the structure of the nervous system in the anemone *Calliactis*. *Am. Zool.* **5**, 403-10.
- ROBSON, E. S. (1966). Swimming in Actiniaria. *Symp. zool. Soc. Lond.* **16**, 333-59.
- ROBSON, E. A. & JOSEPHSON, R. K. J. (1969). Neuromuscular properties of mesenteries from the sea-anemone *Metridium*. *J. exp. Biol.* **50**, 151-68.
- ROSS, D. M. (1964). The behaviour of sessile coelenterates in relation to some conditioning experiments. *Anim. Behav.*, Suppl. **1**, pp. 43-52.
- ROSS, D. M. (1967). Behavioural and ecological relationships between sea anemones and other invertebrates. *Oceanogr. mar. Biol.* **5**, 291-316.
- ROSS, D. M. & SUTTON, L. (1964*a*). The swimming response of the sea anemone *Stomphia coccinea* to electrical stimulation. *J. exp. Biol.* **41**, 735-49.
- ROSS, D. M. & SUTTON, L. (1964*b*). Inhibition of the swimming response by food and of nematocyst discharge during swimming in the sea anemone *Stomphia coccinea*. *J. exp. Biol.* **41**, 751-7.
- SARS, M. (1835). *Beskrivelser og Iagttagelser over nogle moerkelige eller nye i Havet veg den Bergenske Kyst levende Dyr*. Bergen.
- SCHMIDT, H. (1970). *Anthopleura stellula* (Actiniaria, Actiniidae) and its reproduction by transverse fission. *Mar. Biol.* **5**, 245-55.

- SWEDMARK, B. (1966). La Station Zoologique de Kristineberg, Fiskebäckskil (Suède). *Cahiers Biol. mar.* **7**, 101-6.
- THOMPSON, T. E. (1964). Grazing and the life cycles of British nudibranchs. *Symp. Br. Ecol. Soc.* **4**, 275-97.
- WESTFALL, J. A. (1970). The nematocyte complex in a hydromedusan, *Gomionemus vertens*. *Z. Zellforsch. mikrosk. Anat.* **110**, 457-70.
- WILLIAMS, R. B. (1968). Control of the discharge of cnidae in *Diadumene luciae* (Verill). *Nature, Lond.* **219**, 959.
- YANAGITA, T. M. (1959). Physiological mechanism of nematocyst responses in sea-anemone. VII. Extrusion of resting cnidae - its nature and its possible bearing on the normal nettling response. *J. exp. Biol.* **36**, 478-94.
- YENTSCH, C. S. & PIERCE, D. C. (1955). A 'swimming' anemone from Puget Sound. *Science, N. Y.* **122**, 1231-3.