NEUROMUSCULAR PROPERTIES OF MESENTERIES FROM THE SEA-ANEMONE METRIDIUM

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INTRODUCTION

An attempt has been made to identify electrical correlates of behaviour in Metridium senile (L.), a sea-anemone whose neuromuscular physiology and structure are already familiar (Parker, 1919; Hall & Pantin, 1937; Batham & Pantin, 1950 a, b, c; 1951; 1954; Pantin, 1952, 1965 b; Batham, Pantin & Robson, 1960; Robson, 1961; Ross, 1960 a, b). In a recent paper Josephson (1966) described electrical potentials associated with contractions of the sphincter muscle and tentacles in the tropical anemone Calliactis polypus. Using preparations of isolated mesenteries from Metridium we have been able to confirm and to extend these results. Our conclusions agree with those of Pickens (1968) for the burrowing anemone Calliactis praelongus (Carlsgren).

In Metridium a through-conduction system extends throughout the mesenteries and column. Electrical stimuli given to the column at 1 per sec. cause twitch-like contractions of the retractor muscles of the mesenteries, of most of the tentacles and of the radial muscle of the oral disk (Hall & Pantin, 1937). Excitation of the through-conduction system is all-or-none and other muscles such as those of the column respond at different frequencies of stimulation. In this anemone excitation is conducted faster in mesenteries than in other regions, attaining 100 cm/sec. or more at 18°C. (Robson, 1961). These fast pathways correspond to a richly developed nerve-net on the retractor surface of the mesenteries (Pantin, 1952; Batham et al. 1960).

The longitudinal retractor muscles show three types of response to electrical stimulation (Batham & Pantin, 1954; Pantin, 1965 b):

(1) Quick, twitch-like contractions of the whole muscle ensue at stimulus frequencies between about 3 per sec. and 1 in 3 sec. They require two shocks and show facilitation attributed to the neuromuscular junctions (Pantin, 1935 a, 1952). There is a delay between stimulus and contraction of 50–80 msec. which could be largely conduction time, and maximum tension is reached a few tenths of a second later (Batham & Pantin, 1954; Grimstone, Horne, Pantin & Robson, 1958).

(2) Slow contractions are evoked by trains of stimuli at lower frequencies, down to about 1 in 15 sec., and they may also follow quick contractions. They often arise spontaneously, and they vary considerably in latency, speed and amplitude. Maximum tension is reached only after 1–3 min., and relaxation may take more than twice as long. Slow contractions may involve the whole muscle or may remain local. Since the

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threshold of electrical stimulation is the same as for quick contractions, it is clear that
the slow contractions can be initiated by excitation in the through-conduction
system, but as suggested by Pantin (e.g. 1965a) they may be propagated in the
muscle itself.

(3) Direct local contractions of the retractor muscle field near the stimulating
electrode have been observed after single shocks at intensities about twice the threshold
of the through-conduction system (Pantin, 1965b).

Retractor muscles show fivefold extensibility and attain maximal resting length only
when the anemone is fully expanded. Normally coelenteric pressure is 4–6 mm. H2O,
with an isometric ceiling of 11–16 mm. H2O for the column muscle but very much
higher for the retractor muscle (Batham & Pantin, 1950a). Maximum isometric
tension for the circular muscle of the column is 40 kg./cm.².

The structure of mesenteries of Metridium has been described in detail (Batham &
Pantin, 1951; Pantin, 1952; Grimstone et al. 1958; Batham et al. 1960). On one side is
transverse muscle with a sparse nerve-net. The other is occupied mainly by the
longitudinal retractor muscle. This consists of a smooth muscle-field whose fibres are
of one kind, approximately 0.5–1 μ in diameter and up to 2 mm long when extended.
Adjacent off-retractor fibres are thinner and shorter. All the muscle fibres are part of
musculo-epithelial cells (Hertwig & Hertwig, 1879).

Over the whole longitudinal muscle-field runs a well-developed nerve-net of bipolar
cells. From end to end these cells are 1–10 mm long. For most of their length the
neurites are commonly between 1 and 2 μ in diameter, although some are wider and
may occasionally attain 5 μ. They adhere to one another but do not fuse, and make
contact also with the fine processes of sense cells. Nerve processes end over the muscle
field as expanded terminations that are up to 10 μ wide and span several muscle fibres.
It is possible that contractions may be initiated from these morphological neuromuscular
junctions. On the other hand, while muscle fibres greatly outnumber nerve cells most
of them are traversed by one or more neurites. In one sample forty-nine nerve cells
were counted in an area of about 6 mm.² which must have contained several thousand
retractor muscle fibres (Batham et al. 1960). There is no histological evidence for
double innervation which might be correlated with the existence of quick and slow
contractions.

MATERIAL AND METHODS

Recently collected specimens of Metridium from 4 to 9 cm in pedal diameter were
obtained from the Marine Biological Laboratories, Woods Hole. Anemones were
maintained in natural or artificial sea water (Instant Ocean supplied commercially) at
temperatures between 13° and 18° C. Perfect mesenteries were dissected from anemones
anaesthetized with magnesium chloride as described by Batham et al. (1960), and were
arranged for recording with the retractor surface uppermost as shown in Fig. 1. One
end of the mesentry was pinned to a wax-filled Petri dish while the other end was
pinned to a small wax plate able to move freely in a horizontal plane. By means of a
short piece of capillary tubing the wax plate could pivot on the vertical arm of a bent
glass lever whose proximal end was fastened to a short piece of watchspring held in a
clamp. The lever swung easily and exerted a tension of about 1·5 g. on the preparation.
The Petri dish was set in a recessed plastic block with a smear of lanoline, the block
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itself being cemented to the bottom of a 2 l. crystallizing bowl. The bowl was filled with sea water cooled to 14–16° C.

In these conditions mesenteries might remain viable for 1–2 days without tearing. Recovery from the effects of magnesium chloride was rapid: preparations responded to stimuli after 20–30 min. and exhibited normal responses and spontaneous slow contractions within a few hours. The preparations assumed a resting length of about one third of their full extension in the intact anemone, and shortened by almost 50% when fully contracted.

Recording and stimulating electrodes were made from drawn plastic tubing (polyethylene or polyvinyl chloride) and had internal diameters of 0·2–0·4 mm. The electrodes

were filled with sea water and were held against the mesentery by slight suction. Electrical contact with the sea water in the electrodes was made with chlorided silver wires. Other chlorided silver wires placed in the sea water surrounding the preparation provided indifferent electrodes for recording and stimulation. Potentials were amplified with conventional capacitor-coupled amplifiers and displayed on an oscilloscope or pen writer. Mechanical recordings were made with a transducer (RCA 5734) connected to the compliant lever to which the mesentery was attached.

Electrical stimuli were pulses of 1 msec. duration, and intervals of 3–5 min. were usually allowed between positive tests to ensure recovery. To avoid stimulating the responding muscle directly shocks were sometimes delivered off the preparation. For this purpose, the adjacent paired mesentery was left attached to the fixed end of the preparation by a slip of pedal disk, forming a tag on which the stimulating electrode could be placed.

Fig. 1. A mesentery set up for mechanical and electrical recording (see text).
RESULTS

Mechanical responses to electrical stimulation

Various characteristics of quick and slow contractions reported previously have been confirmed and are illustrated in Fig. 2.

Quick contractions follow two or more stimuli and the staircase resulting from a train of shocks shows summation and facilitation (Fig. 2a, b). In these experiments effective intervals for paired stimuli were between 0.15 and 2 sec. The size of a twitch depends on the interval between stimuli (see Figs. 5, 6). It may also possibly depend on the tone of the muscle, but as the resting length of the preparation varies the relation is not clear-cut. Figure 2d-f illustrates this. Figures 2d and e are consecutive responses to paired stimuli 0.4 sec. apart given during relaxation and at the peak of a spontaneous slow contraction. The latter shows how twitches given during increased tension are often smaller: although the reduced twitch shown in Fig. 2f was seen shortly afterwards when the preparation had relaxed.

The minimum latency between stimulus and twitch is about 50 msec. as in whole anemones. The initial rate of shortening is probably of the order of 1 cm/sec. After long trains of stimuli the tension produced by quick contractions approaches the maximum of which the muscle is capable (see Batham & Pantin, 1950a) but that of a single twitch is usually between one-fifth and one-fiftieth of the isometric value.

How slow contractions are initiated and propagated are questions of particular
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interest. They may arise spontaneously, and may involve part or all of the muscle-field. They are initiated by repetitive stimuli at low frequencies such as 1 in 5 sec. and in addition they almost invariably follow quick contractions. Their variable latency and amplitude are illustrated by the examples shown in Fig. 2c–f. The tension reached is usually much greater than that attained by a single twitch. Speed of contraction, also variable, is at most one-tenth of that attainable in quick contractions. In our experiments induced slow contractions were often faster than spontaneous ones.

Direct local contractions of the muscle field near a stimulating electrode were not examined in these experiments.

![Fig. 3. Electrical response to one shock, interpreted as a nerve-net potential. (a) One recording electrode, (b) another preparation, two recording electrodes aligned with stimulating electrode. Lower trace illustrates how profile changes with recording site.](image)

**Electrical responses**

Electrical stimuli give rise to two types of electrical potential. In this account they will be referred to as nerve-net impulses and muscle action potentials but it should be emphasized that this interpretation is a provisional one adopted in the light of evidence at present available. This identification is not intended to preclude other interpretations should they later seem more appropriate.

A single shock excites an all-or-none compound pulse, regarded as a nerve-net impulse (Figs. 3, 7). It is irregular and its form depends on the recording site (Fig. 3b) and possibly on the relative position on the mesentery of the stimulating electrode. Total duration is a few milliseconds and the jagged components, which summate, last 1–3 msec. The maximum amplitude recorded was almost 0.4 mV.

Although these putative nerve-net impulses are quite reproducible the recording site sometimes deteriorates in a matter of minutes. There is little evidence for facilitation but subthreshold stimuli may summate, since near threshold an impulse may appear only after the second of two shocks. Thresholds were found to be remarkably similar in preparations from different anemones.

Conduction speed varies with the length of the preparation. That is to say, the actual conduction time between any two recording sites is constant whatever the state of muscular tone. Conduction velocities measured from the partially contracted mesenteries ranged approximately from 10 to 40 cm/sec. When the conduction times are referred to the resting dimensions of the original anemone, however, most estimates
for longitudinal conduction speed are in the range 70–120 cm./sec. and so correspond to that of the through-conduction system (Robson, 1961). In a transverse direction a few similar estimates vary from 30 to 300 cm./sec.: these figures are uncorrected in that they refer to distances measured on the preparation and not to its resting dimensions but they suggest that the system is not necessarily isotropic.

Fig. 4. Electrical responses interpreted as muscle action potentials, from three preparations. (a) Response following the second of two shocks 1 sec. apart. (b) Electrical and mechanical responses following a pair of shocks 0-4 sec. apart; the potential (upper trace) precedes the quick contraction (lower trace). The sweep starts with the second stimulus. (c) Correlated electrical responses (top traces) and quick contractions (lower trace) following six shocks at 1 sec. interval. The last shock was ineffective. Potentials show initial facilitation then decline slightly.

The second type of pulse, regarded as a muscle action potential, very rarely follows single shocks but appears after subsequent stimuli, provided that the interval is between about 0-15 and 2 sec. (Fig. 4a). This is a smooth graded potential 50–100 msec. in duration, whose major component is usually positive and whose size is inversely
related to the stimulus interval (Fig. 5). The maximum amplitude recorded was 1.2 mV. The size of potentials is independent of the distance between stimulating and recording electrodes. It is difficult to interpret these potentials as movement artifacts since they entirely precede the quick mechanical response (Fig. 4b). The latency between

![Graph](image1.png)

Fig. 5. Effects of facilitation. The sizes of both electrical and mechanical responses depend on interval between stimuli. Responses of one preparation to trains of six stimuli. ▽, Height of mechanical response to 3rd shock; △, to 4th shock; ▼, of electrical response to 3rd shock; ▲, to 4th shock.

![Graph](image2.png)

Fig. 6. Facilitation in electrical and mechanical records from a preparation receiving trains of twenty shocks at 1 per sec. and 2 per sec. Potentials recorded from two sites are shown.

electrical and mechanical events decreases when the distance between stimulating and recording electrodes is increased, but the minimum value was not ascertained. The muscle action potentials do not summate but show some facilitation, which seems independent of mechanical events (Fig. 4c, 6): their size increases in a train of stimuli only up to the third or fourth shock and then declines slightly although mechanical facilitation may still be increasing. Figure 6a suggests that it may rise again if the train is prolonged.
Fig. 7. Potentials following a pair of shocks 1 sec. apart. Top trace: first shock causes a nerve-net impulse. Lower trace: second shock is followed by an identical nerve-net component, preceding a muscle action potential.

Fig. 8. Electrical responses to a pair of shocks: superimposed traces from three preparations. (a, c) Shock interval 1 sec. (b) Shock interval 0.5 sec.
Threshold and conduction velocity are identical with those of nerve-net impulses. These potentials are not correlated with the onset of slow contractions and their conduction time is again independent of the degree of contraction of the preparation. It was possible to record them consistently from one site for several hours, but it was usually necessary to change the position of recording electrodes more often. The degree of variation at two recording sites is illustrated in Fig. 6.

Fig. 9. Electrical responses to five shocks 1 sec. apart, from two recording sites: pairs of traces above and below have different scales. Top traces are superimposed and show consistent nerve-net component, initial facilitation of muscle action potentials and constant conduction velocity.

The relation between presumed nerve-net impulses and muscle action potentials is shown by the response to paired shocks (Figs. 7, 8). Each stimulus of the pair is followed by a nerve-net impulse, whose form on both occasions is identical. The second shock is followed also by a muscle action potential propagated at the same speed. The nerve-net impulse clearly precedes the muscle potential in Figs. 7 and 8a. In Fig. 8b and c, however, the muscle potential appears to begin before the nerve-net impulse although the latter remains distinct. The following explanation assumes that the second nerve-net impulse initiates the muscle action potential. As these events approach the recording electrode, depolarization of remote muscle may be detected before the nerve-net in the vicinity of the electrode is depolarized; so with external electrodes the nerve-net impulse may appear to be superimposed on the earlier part of the muscle potential. Allowing for records in which nerve-net impulses do not show at all (e.g. Fig. 4a), it can be maintained that these potentials precede, and do not follow, the muscle action potentials.

Figure 9 summarizes much of the preceding account; in superimposed responses to
a train of five stimuli at 1 per sec. the nerve-net components remain distinct, and it can be seen that the muscle potentials show little facilitation after the third shock.

*The refractory period*

It has not proved possible to demonstrate that there are two different refractory periods.

Figure 10 shows an incomplete curve for the threshold of response to the second of two shocks. It shows the threshold of test stimuli delivered at given intervals after a conditioning shock at twice threshold strength. At intervals of less than 2 sec. any response to the test shock consisted of both types of potential.

![Fig. 10. Approximate threshold of response to second stimulus (from one preparation: see text).](image)

The following experiment suggests that the refractory period of the nerve-net is longer than that of the muscle. Two stimulating electrodes several millimetres apart were used to give pairs of stimuli, one shock from each electrode. The stimulus interval was progressively reduced. Recording from two sites aligned between the stimulating electrodes, no combination was found at which the second shock produced a nerve-net impulse without a muscle action potential at a recording electrode. Both types of potential appeared, or failed to appear, together. This would not be expected if the refractory period of the nerve-net were shorter than that of the muscle, in which case even if the muscle were refractory at a recording site a nerve-net impulse should be detectable before the muscle recovered; so the converse is provisionally assumed to be true (Bullock, 1943).

During the refractory period the system behaves as though conduction were slower. This is seen as an increased latency of response to a second stimulus at short intervals after the first. Figure 11a shows how nerve-net impulses following two shocks 0.5 sec. apart have identical latencies. In Fig. 11b the response to a second shock now 0.38 sec.
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after the first is greatly delayed. That the increased latency applies to both the nerve-net impulse and the muscle action potential is confirmed in Fig. 12.

The relation between latency of response to a second shock whose intensity is just

![Fig. 11](image1.png)

**Fig. 11.** Electrical response to a pair of shocks: superimposed traces showing how latency increases in the relative refractory period. Nerve-net impulses marked △. (a) Interval 0.5 sec. (b) Interval 0.38 sec.

![Fig. 12](image2.png)

**Fig. 12.** Electrical response to two shocks 0.165 sec. apart. Shocks initiate superimposed traces from two recording electrodes. In $R_1$ (nearest the stimulating electrode) the first shock is followed by a nerve-net impulse, and the second by a similar impulse plus a muscle action potential but both are delayed. $R_2$ (furthest from the stimulating electrode) shows similar increased latency but most of the muscle action potential is off screen to the right.
above threshold and the interval between stimuli is shown in Fig. 13. It can be seen that the latency increases greatly as the interval is decreased. This phenomenon is seen only with stimuli near threshold; at short stimulus intervals the latency is reduced to almost normal values by increasing the intensity of stimulation. The increased latency might be due to a delay in the initiation of a conducted event or to conduction velocity changes near the stimulating electrode. It is not caused by an overall change in conduction velocity; the delay between the appearance of events at two recording sites is approximately equal for the first and second shocks of a pair, indicating nearly constant conduction time between the recording sites (Fig. 12, 13).

Figures 10 and 13 show that the onset of the refractory period is detectable at intervals of about 0.4 sec. The absolute refractory period was not determined, but there was some evidence that at the shortest effective intervals the muscle action potential became truncated before failing to appear. This is of some interest as a comparable reduction in size of the mechanical response occurs in the sphincter muscle of Calliactis parasitica at stimulus intervals between the value for maximal facilitation and the absolute refractory period (Pantin, 1935a).

The effects of magnesium chloride

The progressive effects of Mg$^{2+}$ ions on this preparation have not been examined, but the recovery of mesenteries from anaesthesia in MgCl$_2$-sea water follows a predictable sequence. When set up in sea water as shown in Fig. 1, the anaesthetized mesentery is stretched to the maximal resting length it could have in the anemone. Within an hour a working length of about one third of this is assumed, and spontaneous slow contractions ensue. Electrical and mechanical responses to electrical stimuli
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reappear together, usually after about 15 min. in sea water, during which neither can be detected. The threshold is at first very high but it falls rapidly and it may be almost normal within 30 min. of return to sea water. In some preparations conduction speed of the potentials seems normal from the moment they reappear; in others, it is perhaps half as fast as usual, and takes a few hours to recover. The height of both types of potential and their refractory periods are usually depressed but all parameters are normal after 6 hr. in sea water.

Electrical responses of the oral disk and tentacles

Records were obtained from a few oral disk preparations pinned out on wax with the crown uppermost. Single shocks evoke small irregular potentials somewhat larger than 0.1 mV., which appear to be equivalent to those interpreted as nerve-net impulses in mesentery preparations. Their speed of conduction referred to resting dimensions (as conduction time is again independent of tone) is about 50–100 cm./sec. Samples were too few to indicate whether there was a consistent difference between radial and tangential measurements. If the second shock of a pair follows at intervals between 0.15 and 2 sec. it causes a small twitch of the ectodermal radial muscle. A corresponding smooth slow potential is seen which can again be interpreted as a muscle action potential. Its size, like that of the twitch, depends on the interval between stimuli. The two types of potential again have one threshold and with regard to refractory period and latency show the same properties as their presumed homologues in mesenteries.

Tentacles also show quick longitudinal contractions after the second of two stimuli. The electrical response again shows two components with one threshold; an electrode over a tentacle, for example, records a small brief pulse after each stimulus (less than 0.1 mV.) which would be a nerve-net impulse. After a second shock it precedes a muscle potential (e.g. 0.2 mV., duration 0.4 sec.), and in this case it is possible that it may show facilitation.

In whole anemones these responses of the disk and tentacles can be elicited by shocks applied anywhere. Similar electrical responses could not be recorded from the general surface of the column, which lacks the ectodermal nerve-net and muscle field present in the crown.

Presumed nerve-net impulses are conducted from one mesentery to another across an intervening sector of oral or pedal disk. Impulses also pass from the mesentery to the ectoderm of the crown. When mesenteries are stimulated potentials can be recorded from the oral disk, and twitches of the disk and tentacles follow as expected.

INTERPRETATION OF RESULTS

There is strong evidence that in Metridium as in Calliactis the quick withdrawal reflex is excited by a through-conducting nerve-net (references on p. 151). It is suggested that brief compound pulses recorded with external electrodes represent impulses in this through-conduction system. They precede graded potentials which are associated with rapid facilitated contractions and are interpreted as muscle action potentials. These are reported from the mesenteric retractor muscles and ectodermal muscle of the disk and tentacles, and they would be expected in the sphincter, although
not examined there, since it also performs quick contractions. Other interpretations of these results may now be considered.

Two types of potential have been recorded but there appears to be one threshold, one conduction speed and one refractory period. It is possible to account for the results in terms of a single conducting system (A) which excites a responding system (B). System A, to which the apparent threshold and refractory period belong, would initiate excitation in system B. Excitation of B always precedes a quick contraction. The refractory period of B must be the same as or less than in A.

The response of system B follows the conduction of excitation in system A so closely that it can be interpreted as a locally triggered response. While it is of course possible that excitation initiated in system B may be propagated there independently it is not necessary to suppose that this is the case.

It appears reasonable to suppose that system B could be the muscle because there is little alternative. What we have interpreted as muscle action potentials in _Metridium_ have also been recorded from the sphincter region and tentacles of _Calliactis polypus_ (Josephson, 1966) and from the retractor muscles of _Calliactis praelongus_ (Pickens, 1968), where they are related to mechanical events in the same way. On this basis all actinian muscles showing quick facilitated contractions should exhibit this type of graded potential.

Conducting system A could be either the nerve-net, as suggested here, or the epithelium or the muscle-field. While it is difficult to maintain that it also is the muscle-field, the possibility that what we have interpreted as nerve-net impulses are conducted in the epithelium of the mesentery cannot be ruled out, especially as the retractor muscle fibres are all part of musculo-epithelial cells. It is not possible to settle the question by removing the epithelium experimentally as these tissues do not lend themselves to microdissection. On the other hand our results suggest that the system has a non-homogeneous distribution, whereas on morphological grounds at least the epithelium does not appear to be non-uniform in any corresponding way.

The following properties of nerve-net impulses agree with the provisional suggestion that they are conducted in the nerve-net. They are irregular, consisting of short components (1–3 msec.) which summate when synchronous and vary with the recording site. The action of Mg$^{2+}$ ions is rapid and reversible. Again, these potentials occur in areas of _Metridium_ possessing a well-developed nerve-net (e.g. the retractor surface of mesenteries and the crown). They almost certainly represent activity in the through-conduction system, which is equated with the nerve-net in both _Metridium_ and _Calliactis_ on very strong grounds (Pantin, 1952). Non-isotropic conduction might be correlated with some orientation of nerve processes.

A less simple explanation is that activity in the nerve-net itself was not detected (i.e. below 0.01 mV. in amplitude) and our records might represent secondary excitation of the overlying epithelium.

The attribution of these potentials to the nervous system does not explain how they are initiated at the stimulating electrode or exactly how they are propagated in the nerve-net, since these problems are intractable without intracellular recording techniques. It is, however, fully consistent with the idea that quick contractions are initiated at points over the whole muscle-field, with facilitation localized at neuromuscular junctions (Pantin, 1935 a, b).
DISCUSSION

Future work will show to what extent the electrophysiological properties of other Anthozoa resemble those of Calliactis polypus, Metridium senile and also Calamactis praelongus and support the premise that quick withdrawal responses in anemones involve regionally differentiated parts of the neuromuscular system specialized for rapid through-conduction and quick facilitated contraction (Pantin, 1952).

The provisional identification of nerve-net impulses and muscle action potentials in Metridium accords with the properties of similar elements in Scyphomedusae (Horridge, 1953; Passano, 1965). These jellyfish have two subumbrella nerve-nets, termed the giant-fibre net and the diffuse net. The first is composed of bipolar nerve cells resembling those of Metridium mesenteries in shape and size (Horridge, 1954). In Aurelia, Cassiopea and Cyanea potentials in this giant-fibre net are compound pulses whose components appear to be of less than 5 msec. duration. They are conducted at 45–50 cm./sec. at normal temperatures. Each giant-fibre impulse causes a contraction of the subumbrella swimming muscle preceded by a graded muscle action potential; but here the refractory period of the muscle exceeds that of the nerve-net and limits the rate at which the bell pulsates. The motor impulses which control swimming arise in the marginal ganglia. In the diffuse nerve-net excitation is conducted more slowly. In Aurelia it causes a wave of contraction of the marginal tentacles: in Cassiopea these impulses facilitate the response of the swimming muscle to giant fibre impulses. The diffuse net also influences discharge of the marginal ganglia (see also Romanes, 1885; Bullock & Horridge, 1965).

Electrophysiological evidence thus supports the conclusion that through-conducted responses are co-ordinated by the nervous system in anemones and Scyphomedusae, but this generalization no longer applies to Hydrozoa. Non-nervous conduction has been demonstrated very clearly in the swimming bells of siphonophores and hydro-medusae, where conducting epithelia appear to be the rule rather than the exception. They co-ordinate all-or-none protective movements and swimming and may be excited either directly or via the nervous system; swimming, for example, is controlled by pacemakers in the marginal nerve rings. It is probable that conducting epithelia in turn influence activity in the nervous system (Mackie & Passano, 1968). There may be parallel development of through-conduction in epithelial and nervous pathways, each controlling different responses, as in the siphonophore Nanomia (Mackie, 1964).

Conclusive evidence for epithelial conduction is that here it occurs in the absence of a nerve-net. The exumbrellar surface of a hydrozoan swimming bell consists of simple epithelium and is devoid of nerve cells. Touch or electrical stimuli cause a motor response: the radial muscle of the velum and subumbrella margin contracts in siphonophores (e.g. Hippopodius, Chelophyes), while in hydromedusae (e.g. Sarsia, Phialidium) a general protective response involves tentacles and manubrium as well as subumbrellar radial muscle. In various hydromedusae studied (Mackie & Passano, unpublished) epithelial potentials are propagated over the exumbrella at 15–35 cm./sec., last 5–15 msec. and are up to 2 mV. in size. Recorded with fine suction electrodes, the potentials have a simple biphasic form, less jagged than the nerve-net impulses of Metridium. In siphonophores they are conducted at 24–50 cm./sec. The absolute

Non-nervous conduction also co-ordinates swimming contractions of the sub-umbrellar circular muscle, a striated musculo-epithelium often lacking direct innervation. In *Sarsia* complex potentials lasting up to 0.3 sec. and 2.4 mV. in size are propagated over the muscle at 5–8.5 cm./sec.

A special feature in medusae such as *Sarsia* is the endodermal lamella, a single layer of cells persisting between the radial canals and isolated within mesogloea except for cellular processes in contact with the ectoderm. The lamella is excitable and co-ordinates contractions of the manubrium and tentacles.

There is reason to believe that epithelial conduction may be common also among hydroids. At present the best evidence comes from *Hydra*, where large pulses preceding longitudinal contractions are undoubtedly of epithelial origin (Josephson & Macklin, 1967). They last 0.1–0.2 sec. and are conducted at 3–8 cm./sec. In *Tubularia* three conducting systems have been found in the stalk, one the seat of pulses similar to those regarded as epithelial in *Hydra* (Josephson & Mackie, 1965). While the possibility that propagation involves nerve elements cannot be excluded in either case, in *Cordylophora*, however, no nerve processes have been found in stolons known to conduct (Josephson, 1961; Jha & Mackie, 1967).

Although epithelial conduction has played a significant part in the evolution of behaviour patterns within the Hydrozoa, and is now well known in ctenophores (Horridge, 1966), it has not yet been demonstrated in other Cnidaria. The possible role of epithelial conduction in the evolution of motor pathways and receptor systems among coelenterates raises questions which are clearly of general interest.

In *Metridium* much of the animal’s behaviour consists of slow contractions for which electrical correlates have not yet been found. Complex phases of activity such as locomotion involve several different sets of muscles and there may be reciprocal inhibition, as for example between circular and parietal elements of the column (Batham & Pantin, 1954). How is this activity co-ordinated? In no species are the role of the nervous system and its interaction with other elements completely understood, but in sea anemones there is much to be learned about the integration of different components of behaviour and it is to be hoped that electrophysiological recording may prove to be one of the means of doing so.

**SUMMARY**

1. A method is described for a simultaneous electrical and mechanical recording from isolated mesenteries of the sea-anemone *Metridium senile* (L.).

2. The retractor muscle gives quick and slow contractions. Shock intervals of 0.15–2 sec. produce facilitated twitches on all stimuli after the first, usually followed by a slow contraction. Trains of stimuli at lower frequencies are followed by slow contractions only. Slow contractions may also arise spontaneously.

3. Two types of potential were found using external suction electrodes, provisionally interpreted as nerve-net impulses and muscle action potentials. The first is an all-or-none compound pulse, whose brief components summate (1–3 msec., height up to 0.4 mV.). Conduction speed in an expanded anemone would be 70–120 cm./sec. at 14–16° C., corresponding to that of the through-conduction system. The second always
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precedes a twitch and is a smooth, graded potential lasting 50–100 msec. Its size, up to 1.2 mV., is inversely related to stimulus interval, but unlike the quick mechanical response it shows but slight initial facilitation. A muscle potential follows a nerve-net impulse and is propagated at the same speed. The refractory period of the nerve-net probably exceeds that of the muscle.

Potentials showing similar features have been recorded from the oral disk and tentacles.

These results support the suggestion that the sites of facilitation and initiation of contractions are neuromuscular junctions throughout the retractor muscle. Quick contractions are co-ordinated by the nerve-net, whereas it is possible that slow contractions, however initiated, may at least in part be propagated in the muscle itself.

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